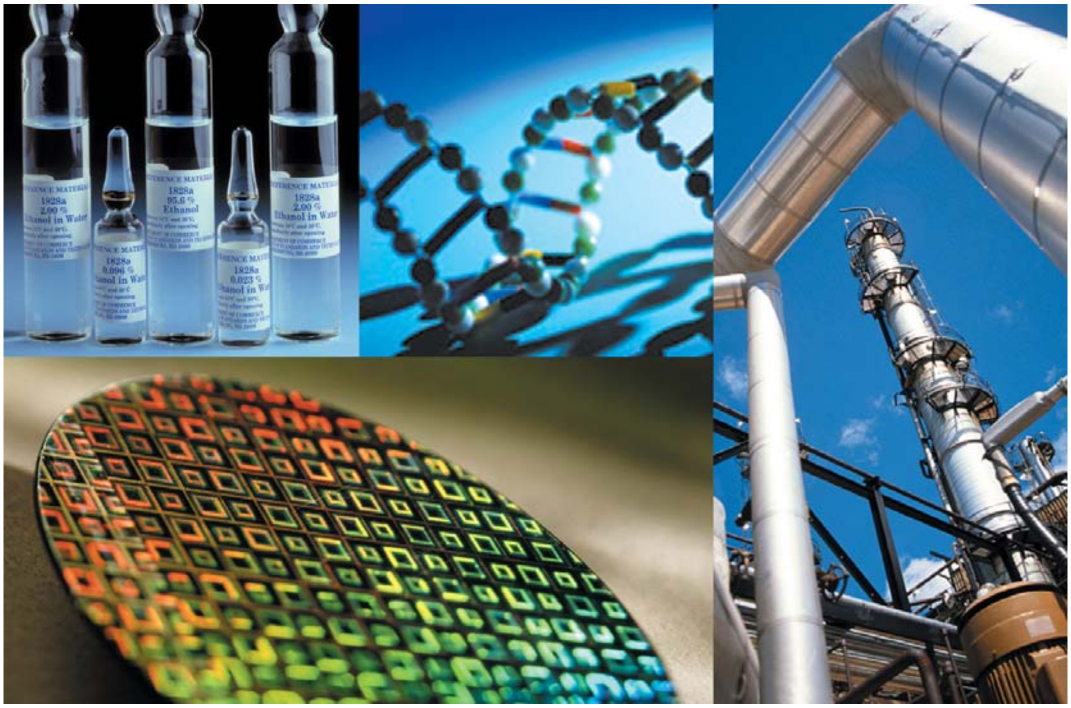
# Chemical Science and Technology Laboratory

## Annual Report – FY2003



NIST

**National Institute of Standards and Technology**
Technology Administration, U.S. Department of Commerce

**NISTIR 7084**

# NISTIR 7084

# Chemical Science and Technology Laboratory Annual Report - FY2003

William F. Koch, Acting Director
Chemical Science and Technology Laboratory

February 2004

U. S. DEPARTMENT OF COMMERCE
Donald L. Evans, Secretary

TECHNOLOGY ADMINISTRATION
Philip J. Bond, Under Secretary for Technology

NATIONAL INSTITUTE OF STANDARDS AND TECHNOLOGY
Arden L. Bement, Jr., Director

Compiled and Edited by William F. Koch, Eric B. Steel, Ellyn S. Beary, Joseph M. Conny, and Carolyn C. Schwennsen. Cover design by Ann L. Leith.
For information contact NIST/CSTL
Telephone: 301-975-8300
Facsimile: 301-975-3845
E-Mail: cstl@nist.gov
Web Sites: www.nist.gov
www.cstl.nist.gov

February 2004

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# Chemical Science and Technology Laboratory

Welcome to the Annual Report of the Chemical Science and Technology Laboratory for fiscal year 2003. CSTL is one of the seven discipline-based laboratories at the National Institute of Standards and Technology. My name is Bill Koch and I am the Acting Director for CSTL. I have been serving in this capacity since July 2003, when Dr. Hratch Semerjian was appointed the Deputy Director for NIST. CSTL has been fortunate to have had Dr. Semerjian as its Director for over 10 years, and I am grateful to him for his insights and visionary leadership that left CSTL a well-trimmed ship on a true course; focused, strong and with boundless opportunities. It has been my privilege to take over the helm, and to move forward with the creative energy that has always characterized CSTL.

This past year has been one of change for CSTL. In addition to the changes in leadership that I just mentioned, there has been the resultant cascade of changes throughout our operating unit. I am most pleased at the ease and facility with which CSTL achieved these major changes, a testament to the depth of its leadership and its dynamic succession planning. Over the course of these leadership changes, the concept of teaming to take full advantage of individual strengths has developed significantly. I am proud of CSTL's Division Chiefs and our group leaders for their dedication, resolve, and creativity. But most of all it has been my honor to lead an outstanding research staff. It is due to their efforts that CSTL is recognized as the world's leading metrology laboratory in chemical measurements. Today, CSTL has the most comprehensive array of chemical, physical, and engineering measurement capabilities of any place in the world. Our activities, products, and services in the national and international metrology community have favorably positioned our Nation in the highly competitive global market place – and CSTL's entire staff is to be applauded. Our staff clearly manifests our six core values – Excellence, Leadership in Metrology, Responsiveness, Objectivity, Respect, and Organizational Commitment.

We have been challenged in recent years with flat and even decreasing budgets. Therefore, it has become all the more important to rigorously set our priorities and to focus our efforts on projects and programs that will create the largest return for our Nation. We must continue to be proactive in communicating the breadth and depth of our capabilities as well as the quality and impact of our work. Over the past year we have continued to strengthen our interactions with industry and other stakeholders to assess their needs, and to help us prioritize our work. We are grateful for the candid input and ideas these colleagues have shared with us.

While maintaining our focus on core mission responsibilities of measurement science and standards, we are expanding our programs in Nanotechnology, Biosciences and Health, Data and Informatics, and Homeland Security. It is my pleasure to invite you to look through the main body of this report, which provides a snapshot of CSTL activities and accomplishments during fiscal year 2003. The dynamic nature of our programs can hardly be captured in a single document, but I hope that what follows will give you a flavor of our work and capabilities, and will entice you to make full use of our measurement services, and to seek out opportunities for collaborations and partnerships with us.

William F. Koch, Acting Director
Chemical and Science Technology Laboratory

**National Institute of Standards and Technology** **NIST**

*...working with industry to develop and apply technology, measurements and standards*

**http://www.nist.gov**

***Arden L. Bement, Jr., Director***
***Hratch Semerjian, Deputy Director***

From automated teller machines and atomic clocks to mammograms and semiconductors, innumerable products and services rely in some way on technology, measurement, and standards provided by the National Institute of Standards and Technology. NIST is a non-regulatory federal agency within the U.S. Commerce Department's Technology Administration. Founded in 1901, NIST's mission is to develop and promote measurements, standards, and technology to enhance productivity, facilitate trade, and improve the quality of life. NIST has an operating budget of about $864 million and employs about 3,000 scientists, engineers, technicians, and support and administrative personnel. About 1,600 guest researchers complement the staff. In addition, NIST partners with 2,000 manufacturing specialists and staff at affiliated centers around the country. NIST carries out its mission in four cooperative programs:

- ***The Measurement and Standards Laboratories*** provide technical leadership for vital components of the nation's technology infrastructure needed by U.S. industry to continually improve its products and services. NIST's seven discipline-based Measurement and Standards Laboratories work at all stages of the pipeline from advancing basic science and pioneering new measurement methods to the development of standard test methods, materials, and data to ensure the quality of commercial products. The seven NIST Laboratories are: Electronics and Electrical Engineering Laboratory (EEEL), Manufacturing Engineering Laboratory (MEL), Chemical Science and Technology Laboratory (CSTL), Physics Laboratory (PL), Materials Science and Engineering Laboratory (MSEL), Building and Fire Research Laboratory (BFRL), and Information Technology Laboratory (ITL).

- ***The Advanced Technology Program (ATP)*** bridges the gap between the research lab and the market place, stimulating prosperity through innovation. Through partnerships with the private sector, ATP's early stage investment is accelerating the development of innovative technologies that promise significant commercial payoffs and widespread benefits for the nation.

- ***The Manufacturing Extension Partnership (MEP)*** was established in 1989 as the first federally funded extension centers to help small manufacturers improve their capabilities and performance, which is a necessity for survival in the global marketplace. Today, the Manufacturing Extension Partnership is a nationwide network of more than 400 not-for-profit centers and field offices. The centers, serving all 50 States and Puerto Rico, are linkied together through NIST. MEP makes it possible for even the smallest firms to tap into the expertise of knowledgeable manufacturing and business specialists all over the U.S.

- ***The Baldrige Quality Program*** is an outreach program associated with the Malcolm Baldrige National Quality Award that promotes performance excellence among U.S. manufacturers, service companies, educational institutions, and health care providers; conducts outreach programs and manages the annual Malcolm Baldrige National Quality Award which recognizes performance excellence and quality achievement.

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TA
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# Chemical
# Science and
# Technology
# Laboratory

***Acting Director***
***William F. Koch***

***Acting Deputy Director***
***Eric B. Steel***

## A. Overview

The Chemical Science and Technology Laboratory (CSTL) of the National Institute of Standards and Technology (NIST) is the United States' reference laboratory for chemical measurements. CSTL is entrusted with developing, maintaining, advancing, and enabling the chemical measurement system for the U.S., thereby enhancing U.S. industry's productivity and competitiveness, assuring equity in trade, and improving public health, safety, and environmental quality. With current world events, the need for accurate and timely chemical and biological measurements is more important than ever. CSTL is ready to meet these new challenges.

Today as part of the Commerce Department's Technology Administration, NIST's vision is to be the global leader in measurement and enabling technology, and delivering outstanding value to the nation. NIST provides scientific leadership for the Nation's measurement and standards infrastructure and ensures the availability of essential reference data and measurement capabilities. To discharge these responsibilities, NIST maintains expertise in a broad range of science and technology areas. The Chemical Science and Technology Laboratory is responsible for measurements, data, and standards in chemical, biochemical, and chemical engineering sciences. Building on a hundred-year history of technical excellence, today's CSTL has the most comprehensive array of chemical, physical, and engineering measurement capabilities and expertise of any group worldwide working in chemical science and technology.

**NIST's Vision**
**Preeminent Performance:** ***Future economic competitiveness, national security, and public well-being will be shaped by revolutionary developments in the biosciences, nanoscience, and information and knowledge management – a transformation enabled by NIST's unique measurements, standards, and enabling technologies and services.***

**NIST's Mission**
***… is to develop and promote measurement, standards, and technology to enhance productivity, facilitate trade, and improve the quality of life.***

**CSTL's Vision** *is to be a world-class research laboratory that is recognized by the nation as the primary source for the chemical, biochemical, and chemical engineering measurements, data, models, and reference standards that are required to enhance U.S. industrial competitiveness in the world market.*

**CSTL's Mission** *is to serve as the nation's Reference Laboratory providing the chemical measurement infrastructure to:*

- *enhance U.S. industry's productivity and competitiveness;*
- *assure equity in trade; and*
- *improve public health, safety, and environmental quality.*

CSTL’s programs have been guided by a formal Strategic Plan for more than a decade. Periodically, CSTL’s Strategic Plan is reassessed and updated to respond to National priorities, changing customer needs, and the rapid evolution of science and technology. This process assures continued alignment with the similarly evolving NIST 2010 strategic plan. Although the details of the strategic planning process have varied over the years, its essential characteristics — **customer-focus** and **openness to all stakeholders** — have been preserved. CSTL’s Strategic Plan focuses on achievement of three **strategic goals**:

***Measurement Standards:*** *Establish CSTL as the pinnacle of the national traceability and international comparability structure for measurements in chemistry, chemical engineering, and biotechnology, and provide the fundamental basis of the nation’s measurement system.* This objective is achieved by:

- Developing and demonstrating international comparability for chemical and physical measurements,
- Supporting and strengthening the traceability structure in the U.S., and
- Supporting and strengthening voluntary standards organizations.

***Chemical and Process Information:*** *Assure that U.S. industry has access to accurate and reliable data and predictive models to determine the chemical and physical properties of materials and processes.* This objective is realized by:

- Developing benchmark data for the properties of important substances, classes of substances, and systems;
- Developing data collections, data prediction methods, and models to meet high-priority industrial and national needs;
- Contributing to the development of consensus standards for key properties, substances, and processes; and
- Developing procedures or protocols for data access, facilitating data exchange, and disseminating formatted data.

***Measurement Science:*** *Anticipate and address next-generation measurement needs of the Nation.* This final goal is achieved by:

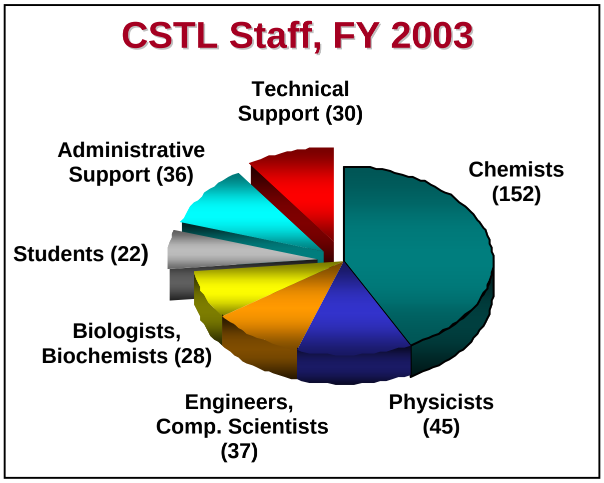
- Maintaining a strong and cutting-edge research program to support the Nation’s measurement and standards infrastructure, and
- Establishing new measurement capabilities to support new and advanced technology development and dissemination.

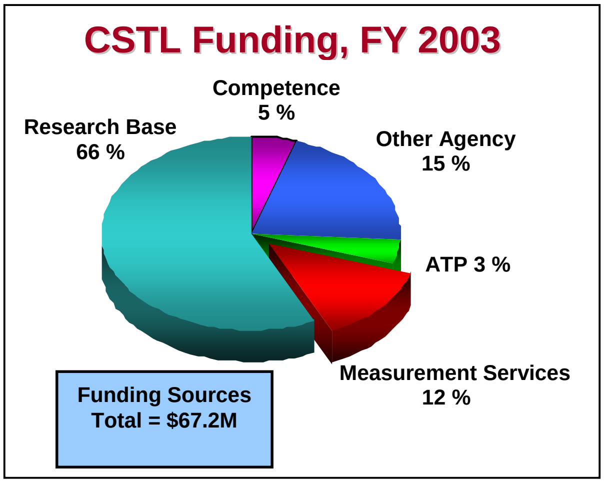
## *Organizational Structure and Resources*

CSTL is organized to reflect the technical expertise that is the foundation of our technical programs and allows us to accomplish our mission. The Laboratory consists of five Divisions: the Biotechnology Division, the Process Measurements Division, the Surface and Microanalysis Science Division, the Physical and Chemical Properties Division, and the Analytical Chemistry Division. Each Division employs a group structure organized to achieve synergy and critical mass in its technical program areas.

While the divisional structure has remained stable over the past year, there have been significant changes in leadership, and these are reflected in the organization chart below. Since July 2003 Hratch Semerjian, CSTL's director of 11 years, began serving as Deputy Director of NIST, and Bill Koch, CSTL's Deputy Director, was named Acting Director of CSTL. The vacated position is now held by Eric Steel, a Group Leader from the Surface and Microanalysis Science Division. John Small moved into the vacated Group Leader position. Unrelated to this cascaded change in leadership is the appointment of Fred Schwartz as Group Leader of the Structural Biology Group in the Biotechnology Division, a position vacated by Gary Gilliland. The facility with which CSTL achieved these major changes is a testament to its successful succession planning. In addition the concept of teaming to take full advantage of individual strengths has developed significantly.

To achieve its goals, CSTL maintains an experienced, well-educated professional staff. The full-time permanent staff numbered 265 in FY03, in addition to 85 temporary and part-time employees. Also, there were 237 guest researchers working closely with CSTL staff in various aspects of the research program. The technical capabilities of CSTL staff are extensive; they hold degrees in chemistry, physics, engineering, biology, and computer science. A capable technical support staff augments the professional staff. Approximately 80 % of the technical staff has PhD degrees.





CSTL's physical facilities are located at the major NIST sites in Gaithersburg, Maryland and Boulder, Colorado, as well as at the Center for Advanced Research in Biotechnology (CARB) in Rockville, Maryland and the Hollings Marine Laboratory (HML) in Charleston, South Carolina. The Physical and Chemical Properties Division has operations located at the NIST sites in Gaithersburg and Boulder. Biotechnology Division staff work closely with that of the University of Maryland Biotechnology Institute located at CARB. The Analytical Chemistry Division is the primary liaison with HML, having staff located in Charleston. HML is a cooperative research facility involving NIST, the National Oceanic and Atmospheric Administration, the South Carolina Department of Natural Resources, the University of Charleston, and the Medical University of South Carolina.

The Analytical Chemistry and Biotechnology Divisions have now been located in the Advanced Chemical Sciences Laboratory (ACSL) for over four years, the first new research facility on the NIST Gaithersburg Campus in over thirty years. Although ACSL adds significantly to CSTL's research capabilities, it does not meet all facility needs of our program.

**Hollings Marine Laboratory**

NIST is proceeding with implementing its facilities master plan, the current focus of which is the Advanced Measurements Laboratory (AML). CSTL is scheduled to move into the AML during spring of 2004. This new facility will accommodate the Surface and Microanalysis Science Division, as well as the Pressure and Temperature activities in the Process Measurements Division. Attention is now being directed to improving the facilities in Gaithersburg and Boulder not located in the ACSL and AML.

## *CSTL Strategic Directions*

In a recent review of our strategic plan, the CSTL leadership defined five major thrusts areas. These thrusts define our unique role and channel our core competencies to address existing and anticipated stakeholder needs, and cross-cut organizational lines.

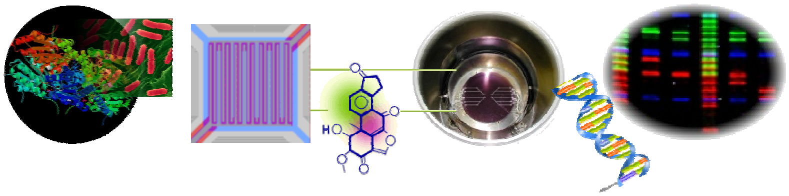
**CSTL core competencies are the scientific expertise and infrastructure in measurement science, measurement standards, and properties data that provide the stability and agility to meet our customers' needs. CSTL views these competencies as critical to the future viability of our organization. They are dynamic and relevant, enabling new as well as promoting change in existing technologies, and leave CSTL and NIST poised to meet both current and next generation measurement and standards needs.**

1. Nanometrology: *for chemical characterization, device characterization, and processes at the sub-micrometer scale*
2. Properties Information Infrastructure: *technologies for generation, evaluation, and dissemination of chemical and physical property information*
3. Biometrology: *for biochemically- or cell-derived materials, devices, or processes*
4. Process Metrology: *for chemical engineering processes*
5. Chemical Metrology: *for the chemical characterization of materials and complex mixtures*

These are, in name and perhaps emphasis, a slight departure from the five thrust areas that CSTL identified and invested in since 1997: Biotechnology, Nanotechnology, Healthcare, Data and Informatics, and International Measurement Standards. We have chosen to focus on the metrological aspects of these emerging and changing areas to more clearly describe to our staff the specific role CSTL should play. NIST has also renamed its Programmatic Strategic Focus Areas (SFAs) to emphasize the metrological role of NIST. It is fair to say that Nanometrology Information/Knowledge Management, and Biosystems are infrastructural, while Homeland Security and Heath are more applied areas.

***NIST Programmatic SFAs***
**Homeland Security**
**Nanometrology/Nanotechnology**
**Biosystems and Health**
**Information/Knowledge Management**

It is critically important to ensure that CSTL programs and projects are dynamic and flexible enough to respond to national and international technology trends, and the changing needs of our customers and stakeholders. CSTL has several mechanisms in place that facilitate the development of new projects at the Division, Laboratory and NIST levels. Each year, CSTL Management reviews its portfolio of programs and projects, and considers proposals for high priority projects. Priority is given to new projects that closely align with NIST and CSTL strategic goals.



Selection of new projects for reprogramming and identification of projects to be phased out require specific criteria. CSTL has used the following criteria for its decision-making:

- Industrial Need – Magnitude and immediacy of industrial need
- Match to Mission – CSTL provides the Nation's chemical measurement infrastructure
- Making a Difference – Is the CSTL contribution critical for success?
- Nature and Size of Impact – Measure of anticipated impact relative to investment (rate of return)
- Timely Quality Output – The ability to respond in a timely fashion with high-quality output
- Science/Technology Opportunity – Recent scientific and technological advances present new opportunities

To provide our staff with stimulus for innovative ideas and new areas of research, CSTL continues its tradition of one-year funding for small seed projects that evaluate the feasibility of new technologies and measurement methods. In FY2003, CSTL funded 11 such projects, following a rigorous internal peer review process. Progress reports for these projects are included in Section C of this Annual Report.

## *CSTL Programs*

CSTL is a multifaceted, synergistic organization with a unique customer base. Perhaps the most remarkable aspect of the CSTL customer-base is its breadth. The primary customers we serve are: industry; federal, state, and local government agencies; standards and industrial trade organizations; and the academic and scientific communities.

U.S. industry is the largest consumer of our products and services. These customers come from established industrial sectors, such as the chemical manufacturers, and emerging industries, such as biotechnology and nanotechnology. The products and services they rely on are as varied as Standard Reference Materials (SRMs), Standard Reference Data (SRDs), calibration services, and novel measurement methods. Reference materials and calibrations provide traceability to the International System of Units (SI), which is essential to fair trade, improved reliability of measurements, and regulatory compliance. Data compilations facilitate modeling of chemical processes and rational product design. New measurement techniques ensure improved product quality and process efficiency, and in turn enhance competitiveness.

Federal, state, and local governments are another important consumer of our products and services. These agencies use our products and services in ways similar to the commercial sector, but in different arenas. For example, calibrations, evaluated data, and reference materials are used to ensure the reliability of environmental monitoring programs. Carefully characterized physiological samples lend credibility to forensics tests. Measurement technologies developed and perfected in CSTL verify compliance with international treaties. Other national laboratories exploit the expertise and techniques developed here in discharging their mandates.

A final group that uses our expertise includes trade organizations, standards committees, and the academic and scientific community. These groups rely on CSTL's expertise for advice and guidance in establishing practical standards and uniform protocols. The academic and scientific communities rely on the data produced and compiled by our staff as touchstones for their own research.

**CSTL's programs** are customer-based and serve to focus our activities. The agility of our technical staff permits CSTL to respond in a timely manner to changes in customer needs within each industry segment, changes in priorities among industries as well as National priorities. CSTL has identified 10 programmatic areas aligned with industrial segments and National priorities, and these are highlighted in Section B of this report. It is clear that those projects listed contribute to strengthening the metrological infrastructure of each industry sector. Affirmation and, if needed, modification of this structure is made yearly based on needs assessments and stakeholder input. Any modification is a reflection of CSTL's current level of activity with specific industries, and captures changes in priority both internal and external.

1. **Automotive and Aerospace**
2. **Biomaterials**
3. **Pharmaceuticals and Biomanufacturing**
4. **Chemical and Allied Products**
5. **Energy and Environmental Technologies**
6. **Food and Nutritional Products**
7. **Forensics and Homeland Security**
8. **Health and Medical Technologies**
9. **Industrial and Analytical Instruments and Services**
10. **Microelectronics**

It is also beneficial to articulate CSTL projects by activities that cross-cut industry sectors. Therefore, CSTL has three cross-cutting programs that are clearly aligned with CSTL's three strategic goals.

1. **Measurement Standards**
2. **Data and Informatics**
3. **Technologies for Future Measurements and Standards**

## *Measures of Success*

CSTL employs many mechanisms to capture feedback on the quality, relevance and impact of our programs and activities. In its mandated review of NIST programs, the National Research Council (NRC) Board on Assessment (BoA) Panel for CSTL provides a rigorous peer review of the quality of our technical activities. The Panel is comprised of technical experts from industry, academia, and national laboratories covering the broad range of disciplines corresponding to CSTL program areas. Direct customer feedback on the quality of our work, external recognition of our technical staff, the leadership roles of our staff in standards activities, and the performance of CSTL in Key Comparisons conducted among other countries' National Metrology Institutes, under the auspices of CIPM Consultative Committees, provide further direct evidence for the quality and relevance of our technical programs. Our performance in CIPM Key Comparisons in a broad range of measurement fields, where our performance is compared to the best laboratories of the world, is an excellent measure of our capabilities and an opportunity to demonstrate our global leadership.

CSTL also organizes many workshops and meetings to assess the needs of specific industry segments, and to prioritize these needs to address the highest impact areas. Workshops, which bring together experts from NIST, industry, academia, and other national agencies, are a particularly useful and cost-effective means of developing competent technical strategies for meeting the NIST mission. CSTL interactions with other federal agencies also provide us with invaluable knowledge on measurements, standards and data needs that may result from promulgation of new regulations, or new more sensitive and selective measurement methods that may be needed to improve the science base needed for decision making. Direct customer and stakeholder feedback on the relevance of our current work and anticipated measurement needs of industry is also sought out through ongoing interactions. This information is used to help develop programs in appropriate directions and assure that CSTL programs are effectively meeting the needs of our customers.

Other direct indicators of the relevance of our work are CSTL outputs, such as new calibration services, SRMs, SRDs, publications, invited talks, patents, licenses, the number of companies using our calibration services, the number of institutions who purchase our Standard Reference Materials (SRMs) in the U.S. and abroad, and the number of institutions who purchase or license our Standard Reference Data (SRD) products, or download our web-based databases. The quality of Measurement Service delivery is another important performance measure for our customers. We constantly monitor and strive to improve the turnaround time of our calibration services. We have been successful in reducing the certification times of our SRMs (to less than two years), and improving our prioritization process to reduce out-of-stock items. CSTL is also making special efforts to expand our web-based data dissemination efforts, and to provide more standardized formatting and single-portal access to all CSTL databases.

Some of the CSTL outputs and interactions in FY2003 are summarized in the following table.

***Selected CSTL Outputs – FY 2003***

| Div. | Pubs. [1] | Talks | Committees [2] | Seminars | Conferences | CRADAs | Patents Issued | SRMs /RMs [3] | SRDs | Cals. [4] |
|---|---|---|---|---|---|---|---|---|---|---|
| 830 | 1 | 17 | 28 | 8 | 4 | 0 | 0 | 0 | 0 | 0 |
| 831 | 85 | 98 | 42 | 5 | 21 | 1 | 2 | 3 | 1 | 0 |
| 836 | 33 | 72 | 96 | 15 | 5 | 1 | 4 | 0 | 0 | 1140 |
| 837 | 43 | 93 | 74 | 6 | 12 | 2 | 0 | 1 | 4 | 0 |
| 838 | 82 | 114 | 100 | 16 | 1 | 0 | 1 | 0 | 17 | 2 |
| 839 | 88 | 178 | 127 | 20 | 11 | 0 | 0 | 67 | 1 | 359 |
| **Total** | **332** | **572** | **468** | **70** | **64** | **4** | **7** | **71** | **22** | **1501** |

[1] Publications appearing in print in FY2003. Another 223 manuscripts have been submitted for publication.
[2] Committee totals include 64 editorships and TRC
[3] CRADA's signed this year only
[4] Includes four licensed. There are a total of 38 active patents.
[5] SRMs/RMs (Certificates issued)
[6] Calibrations were performed for over 160 customers

***Division Key:***

| | | | |
|---|---|---|---|
| 830 | Laboratory Office | 837 | Surface and Microanalysis Science Division |
| 831 | Biotechnology Division | 838 | Physical and Chemical Properties Division |
| 836 | Process Measurements Division | 839 | Analytical Chemistry Division |

SRM – Standard Reference Material

SRD – Standard Reference Data

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Lastly, economic impact studies, conducted by outside experts, investigate the impact of CSTL products and services on the U.S. economy, thereby providing a quantitative tool for measuring the influence and reach of our programs.

Evaluations of the economic impact of NIST's metrology programs in specific technical areas are carried out through NIST-commissioned studies, performed by external contractors. These studies provide both qualitative assessments and quantitative estimates of the economic impacts resulting from the several categories of technology infrastructure that NIST provides to U.S. industry. Quantitative estimates are provided either as benefit-to-cost ratios or as rates of return to the nation (social rate of return). The results of these impact assessments not only respond to the need to measure and analyze current and past performance but also contribute to future strategic planning. In FY2003, we completed a study of the impact of measurement uncertainties on healthcare costs, described in some detail immediately below. In addition, we summarize some of the results of earlier impact studies, with updated information on subsequent activities and benefits accrued since the publication of each report.

### *Standards for Calcium Testing:*

NIST and the Mayo Clinic in Rochester MN jointly commissioned a study to evaluate the impact of clinical laboratory measurement variations on both patient care and healthcare costs. The issue of measurement variability is accentuated in recently developed integrated healthcare networks, which were designed to help reduce clinical practice variation. In this study mathematical models were designed and data analyzed from over 89,000 patients receiving calcium tests at the Mayo Clinic between 1998-1999. This data analysis was done in order to evaluate the impact of laboratory variation on total healthcare costs to help illustrate the importance of traceable standards and robust reference methods. Based on interviews with laboratory managers and equipment manufacturers, it was determined that calibration error has the potential to cause a bias of 0.1 mg/dL to 0.5 mg/dL in up to 15 % of the calcium tests. The study revealed that patients with elevated or borderline calcium levels resulted in increased heath care costs of up to $31 per patient for an analytical bias of 0.1 mg/dL and up to $89 per patient when the bias was 0.5 mg/dL. In the U.S., with approximately 3.55 million patients per year receiving serum calcium tests that are affected by systematic bias, the potential economic impacts range from $60 million to $198.8 million for analytical biases of 0.1 mg/dL and 0.5 mg/dL, respectively.

**This is the first of a three-part study, and the impact of measurement uncertainty on PSA measurements is underway, to be followed by a similar study for uncertainties related to cholesterol measurements.**

### *NTRM Gas- Mixture Standards:*

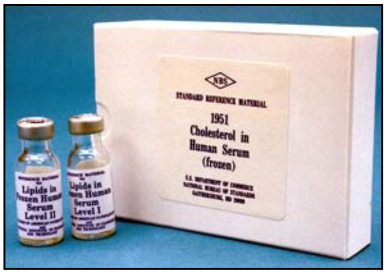
The NIST Traceable Reference Materials (NTRM) program was created to help address the problem of increasing needs for reference materials with a well-defined linkage to national standards. An NTRM is a commercially produced reference material with a well-defined traceability to existing NIST standards for chemical measurements. This traceability linkage is established via criteria and protocols defined by NIST and tailored to meet the needs of the metrological community to be served. The NTRM concept has been implemented in the gas standards area to allow NIST to respond to increasing demands for high quality reference materials needed to implement the "Emissions Trading" provisions of the Clean Air Act Amendment of 1990.

The NTRM program was created by NIST in collaboration with the U.S.

Environmental Protection Agency (EPA) and the specialty gas companies (SGCs) to increase the availability of NIST-certified reference materials. Under the program, SGCs follow NIST technical guidance to manufacture SRM-equivalent standards and submit these standards to NIST for certification. Once certified, the NTRMs are the functional equivalent of SRMs and are used to assay the large volume of secondary reference standards demanded by consumers to meet regulatory requirements. For this impact study, SGCs were surveyed, as well as end users such as electric utilities, transportation equipment firms, petrochemical firms, commercial laboratories, and government agencies. Since NTRM program inception in 1992, 15 specialty gas companies have worked with NIST to certify over 8600 NTRM cylinders of gas mixtures that have been used to produce approximately 500,000 NIST-traceable gas standards for end users, with a market value of about $140 million. Over the past decade, this process has been and continues to be an integral component of the high accuracy reference gas supply chain. According to the economic impact study published in August 2002, the social rate of return of this program is 225 %, with a benefit-to-cost ratio estimated to be 24 to 1, and a net present value of $56 million.

**Currently NIST is working with the SGCs to reduce the uncertainty in the NTRMs. This is driven by market demand for a “better product”; improvement in bottom-line profit by avoiding redundant analyses; and desire to anticipate future changes in regulatory requirements particularly as they relate to emissions trading.**

### *Cholesterol Standards:*



Chemical metrology is at the heart of accurate medical diagnosis and the development of measures to improve our health and ensure long life. In the U.S. more than one trillion dollars are spent each year on health care, which is about 14 % of the U.S. GDP. More than 20 % of these expenditures are for measurements.

Over the last three decades, NIST, in cooperation with the College of American Pathologists (CAP), has developed a series of highly accurate and precise methods for a number of clinically important serum constituents, including cholesterol. These methods are recognized by the international clinical laboratory community as "definitive" and have been used to certify a series of cholesterol SRMs. The first pure crystalline cholesterol (SRM 911) was introduced in 1967. Using the definitive method, serum cholesterol SRMs were developed in 1981 (SRM 909) and again in 1988 (SRMs 1951 and 1952). These SRMs have led to a steady decrease in the measurement uncertainty from 18 % relative in 1969 to about 6 % in 1999. The economic consequences of NIST’s Cholesterol Standards Program are experienced at several levels of the supply chain from manufacturers, to network laboratories, to clinical laboratories that ultimately deliver medical services to the consumer. The results of this study indicate that NIST has played an important economic role in support of a national effort to monitor, measure, and control cholesterol levels, thereby contributing to the reduced level of heart disease. The economic impact study estimates a benefit-to-cost ratio of 4.5, and a social rate of return of 154 %. The net present value was calculated to be more than $3.5 million. This report was published in September of 2000, and the timeline extended from 1986 to 1999.

***Recent Developments:*** NIST Cholesterol SRMs remain an important part of the traceability chain for clinical laboratories and manufacturers of cholesterol measurement kits. Approximately 500 SRM units were sold per year over the past three years.

**Cholesterol SRMs have continued to play a critical role in the steady decrease in the measurement uncertainty from 18 % relative in 1969 to about 6 % in 1999, and 3 % today.**

***Beyond Cholesterol:*** These Cholesterol SRMs, along with NIST’s suite of clinical standards, also serve as important “high order” reference materials, providing measurement traceability for in vitro diagnostic (IVD) products. This level of traceability is required for IVD products to be imported into European markets, as specified in the EU IVD Directive that went into effect in December of 2003. Since U.S.-based companies supply 60 % of the IVD products sold in Europe, it is critical to U.S. industry that NIST maintain and expand SRMs that provide the required traceability. Specifically, the need for manufacturers to provide traceability statements to their customers and to calculate uncertainty for their device calibrators has its origins in this IVD Directive. In order to help manufacturers meet the implementation deadline, NIST's CSTL and ITL collaborated with industry representatives to develop and offer a widely accessible and broadly disseminated web cast workshop to provide the tools needed by the industry to perform uncertainty calculations for their calibrators and controls. The uncertainty workshop was presented before a live audience on the NIST Gaithersburg campus and was also simultaneously web cast around the world and viewed by over 150 participants. It was made available on the web for future viewing in CSTL archives. Since its availability, nearly 1000 clips from the workshop have been replayed.

### *Sulfur in Fossil Fuels:*

The sulfur content of fossil fuels is one of the most important intrinsic factors that determine fuel prices. The accurate determination of the sulfur concentration in fossil fuels is required as a result of environmental regulation that places increasingly lower limits on their sulfur content and the imposition of large fines for non-compliance. At every stage in the process (mining, transportation, buying and selling, and combustion) the sulfur content of both oil and coal must be determined in order to meet buyer and seller specifications that are dictated in large part by government environmental regulations. The efficient and cost effective movement of coal and oil from the mine and well to power plants and refineries requires precise and accurate determination of sulfur content in two or more laboratories. For equity in trade and the efficient production of energy, it is mandatory that instrumentation in these laboratories be calibrated using accurate standards.

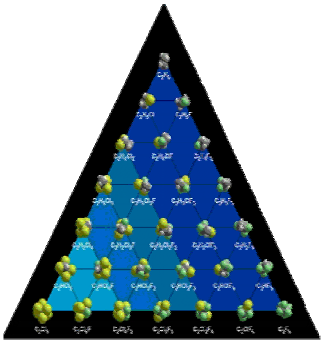
NIST has certified the sulfur content in about 30 coal and fuel oil SRMs to an accuracy of better than ± 0.1 % relative, using isotope dilution mass spectrometry. These SRMs provide industry with the primary calibration materials needed for instrumentation used in routine measurements. SRMs also provide industry with a strong traceability link to NIST for such measurements, whether they be for setting the price of fuel or for demonstrating compliance with environmental regulations. Surveyed industry representatives indicated that NIST SRMs have decreased the level of uncertainty associated with their measurements of sulfur content. This reduction has led to economic benefits throughout the supply chain. Included in the measures of economic benefits are improvements in product quality, production efficiency, and reductions in transaction costs and sulfur emissions to the environment. This study, published in February 2000 estimates a benefit-to-cost ratio of 113, and a social rate of return of 1,056 %. The Net Present Value was calculated to be more than $400 million. The time period studied was from 1984 to 1999.

***Recent Developments:*** The petroleum refining industry faces a June 2006 deadline to comply with stringent limitations on the sulfur content of highway diesel fuel. By this date, most refiners must meet a 15 µg/g or parts per million (ppm) standard for at least 80 % of the highway diesel fuel produced, with a 500 µg/g (ppm) cap on the remaining 20 % of their production. By 2010, all highway diesel fuel must meet a 15 µg/g (ppm) cap. In addition, the industry expects that the EPA will propose similar regulations for the non-road diesel fueled

***A new twist on existing standards:***
**New environmental regulations have capped Hg emissions at 26 tons starting in 2010 and reduced to 15 tons by 2018. There has been value-added to NIST's eight natural coal SRMs that were originally certified only for S and now have been reanalyzed and certified for Hg. These new certifications will take on significant importance since they will be used increasingly by the electric power industry to ensure compliance with these new regulations.**

vehicles. The benefits to the industry in the 2000 Economic Impact report were derived from improved production efficiency in producing diesel fuel that complied with the 500 μg/g (ppm) sulfur cap. The new cap of 15 μg/g (ppm) sulfur puts additional constraints on the industry that translate to higher potential cost avoidance by the use of SRM standards. In December 2003, NIST released a new low-level diesel fuel SRM with a sulfur concentration of 11 μg/g that will meet the new need of the industry. In addition, a kerosene SRM (SRM 1616b) is under development with sulfur levels at approximately 8 μg/g, the lowest level of any Certified Reference Material available worldwide. This material is being used in a NIST-led pilot study now underway under the auspices of the *Comité International des Poids et Mesures* (CIPM).

### ***Alternative Refrigerants:***



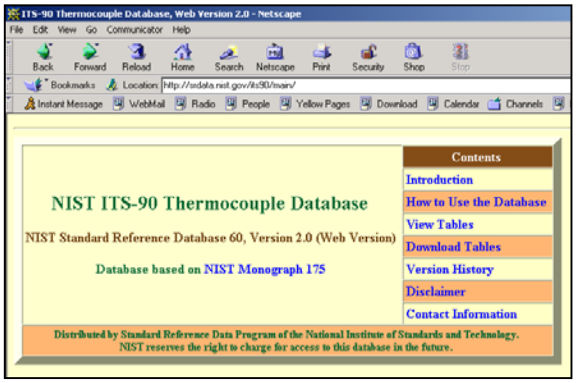
Occasionally, an accelerated R&D program must be undertaken to respond to industry needs that are constrained by set deadlines. With the timetable imposed by the Montreal Protocol of 1987 as an incentive to develop new alternatives to CFCs, NIST engaged in research that would allow industry to make the switch to alternative refrigerants in a timely and economical fashion. NIST began by identifying the basic requirements for new refrigerants according to the new rules, and then started research on determining the physical properties of such candidate alternatives. NIST's most effective form of information dissemination has been the REFPROP program, a computer software package that is available through NIST's Standard Reference Data Program. The REFPROP program enables manufacturers and users of alternative refrigerants to model the behavior of refrigerant mixtures in their respective manufacturing processes, a key method in developing CFC replacements. A comparison of industry benefits with the funding stream of NIST's research program estimated a social rate of return of at least 433 %, and a benefit-to-cost ratio of 4 to 1. This report was published in January 1998, and the period studied was from 1987 to 1996.

***Recent Developments:*** The NIST REFPROP database has been updated several times since the economic assessment, and version 7.0 was released in August 2002. This database continues to be the primary technology transfer vehicle for the continuing work of CSTL on refrigerants. The latest version includes additional fluids and mixtures, improved property models, increased calculational speed, and an enhanced user interface with additional properties and calculation and plotting options. Its position as the *de facto* standard in the refrigeration industry is stronger than ever. In addition to direct use by engineers in industry (it is the number two seller among the databases distributed by the NIST Standard Reference Data Program), it provides the core property routines for the CYCLE-D and REFLEAK databases distributed by NIST as well as packages distributed by several refrigerant and equipment manufacturers to their customers. The *de facto* status will likely become official in the coming year: a draft ISO standard on refrigerant properties specifies the same models (and thus the same property values) as REFPROP. Although REFPROP is not explicitly specified in the standard, the ISO Working Group recognized the high quality and wide use of REFPROP and adopted the same models. A new title for version 7 of REFPROP (Reference Fluid Thermodynamic and Transport Properties) with "Reference Fluid" replacing the "Refrigerant" of earlier versions indicates our direction to provide a single database would encompass refrigerants, cryogenic fluids, hydrocarbons, and simple inorganics. At this time, Version 7.1 of NIST REFROP is scheduled for release in the summer of 2004, and would incorporate NIST14 capabilities. Plans to incorporate the NIST12 capabilities will follow.

**DuPont phased out their own "Refrigerant Expert" package in 1999, and pointed their customers to REFPROP and CYCLE-D, and REFPROP has been licensed for use with the ASPEN process simulation package.**

### Thermocouples:

The NIST thermometry program includes both calibration services and research on thermocouples. Thermocouples are among the most commonly used sensors for monitoring and control of manufacturing processes. The annual sales of thermocouple products sold by the U.S. thermocouple industry (suppliers of wire and thermocouple assemblies) into the U.S. market are approximately $280 million. The incorporation of these devices into higher levels of product structures across a broad base of domestic industries affects a much larger portion of the manufacturing sector, estimated to be on the order of $80 billion.



NIST ITS-90 Thermocouple Database

NIST Standard Reference Database 60, Version 2.0 (Web Version)

Database based on NIST Monograph 175

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Introduction
How to Use the Database
View Tables
Download Tables
Version History
Disclaimer
Contact Information

Distributed by Standard Reference Data Program of the National Institute of Standards and Technology.
NIST reserves the right to charge for access to this database in the future.

Benefits were estimated based on surveys and interviews of the thermocouple industry and did not include the much larger, though diffuse, community of device users. Participants were asked to estimate the additional expenses that would have been incurred if NIST were to cease to provide primary calibration services. NIST expenditures included research on the fundamental and infrastructural aspects of thermocouple principles, measurement, and test methods as well as calibration services. This study, published in July 1997, conservatively estimated the social rate of return to be 32 %, and a benefit-to-cost ratio of 2.95.

**Now available online at**
**http://srdata.nist.gov/its90/main/**

***Recent Developments:*** Currently, the ITS-90 relies on the fundamentally more accurate high temperature platinum resistance thermometer to define the temperature scale in the range of 631 °C to 1064 °C. In this range, prior to the introduction of the ITS-90, less accurate type S (platinum-rhodium vs. platinum) thermocouples were used to define the temperature scale. Work at NIST based on the new scale definition led to new reference functions with improved accuracy for both type S and R thermocouples. Even more importantly, the new definition of the ITS-90 enabled the determination at NIST of highly accurate reference functions for pure element thermocouples, such as gold versus platinum and platinum versus palladium. In the range from 0 °C to 1000 °C, gold versus platinum thermocouples provide an order of magnitude more accuracy than the previous type S thermocouples commonly used as reference standards in industry.

**Based on the NIST research, gold versus platinum thermocouples are now readily available to the user community from several U.S. manufacturers.**

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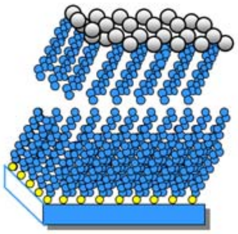
The next sections of this Report will delve into each of CSTL's 13 programs individually, highlighting major activities and accomplishments in these areas for FY2003. Following the highlights of the 10 customer-focused programs, this Report addresses the three goal-aligned programs. Most of the details of the projects listed in the Measurement Standards, and Data and Informatics Sections are presented in the context of their impacted industry sector, and a reference is supplied to provide a more coherent view. CSTL, however, also works on the cutting-edge of metrology and develops tools to anticipate next generation needs. These tools are not necessarily identified with a particular industry sector, and therefore are presented here in the program title: Technologies for Future Measurements and Standards, which reflects CSTL's third strategic goal.

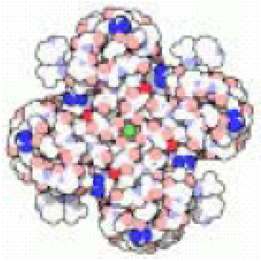
Technical details of these activities can be found on CSTL's website or by contacting the principle investigators listed in the Report.

***http://www.cstl.nist.gov/***

# B.  CSTL Programs


Automotive and Aerospace


Biomaterials


Pharmaceuticals and Biomanufacturing


Chemical and Allied Products

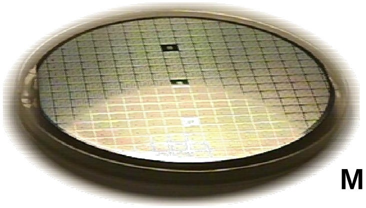

Energy and Environmental Technologies

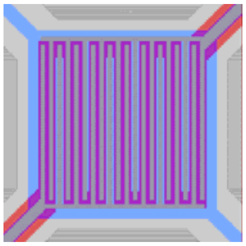

Food and Nutrition

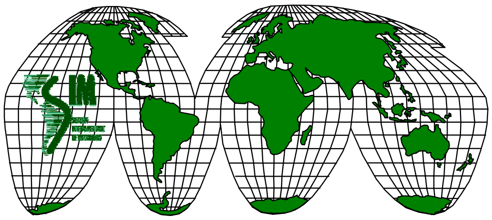

Forensics and Homeland Security


Health and Medical Technologies


Industrial and Analytical Instruments and Services


Microelectronics


Technologies for Future Measurements and Standards



Measurement Standards

Data and Informatics

# 1. Automotive and Aerospace (Transportation)

CSTL provides standards for emissions testing and flow measurements to help the transportation industry meet regulatory requirements. CSTL also works with the industry to provide physical and chemical property data that can be used in sophisticated computer models for next generation combustion engine design, and provides a new generation of chemical standards and reference data to support advanced materials such as super alloys for high technology jet engines.

**When California Low Emissions Vehicle (LEV11) regulation is fully implemented in 2010 it is estimated that smog-forming emissions in the Los Angeles area will be reduced by 57 tons per day, while the statewide reduction will be 155 tons per day.**

## *New Gas Standards for Calibrating Instrumentation Used for Measuring Emissions from Next Generation Low Emission Vehicles*

***W.J. Thorn III and W.D. Dorko (839)***

Stakeholders in the American Industry-Government Emissions Research (AIGER) group are working together to facilitate the automobile industry meeting more stringent 2003 Federal Tier II and California LEV II emission regulations. AIGER members include the U.S. EPA, California Air Resources Board (CARB), General Motors, Ford, and Daimler-Chrysler. NIST and the U.S. Motor Vehicles Manufacturers have worked together since 1975 to develop sixty gaseous Standard Reference Materials, which are the Nation's benchmarks against which all U.S. EPA mandated fuel economy and mobile source emission measurements must be traceable by federal law. In 1998, NIST worked with a Specialty Gas contractor to blend two cylinders each of five low NO standards at concentrations ranging from of 0.5 µmol/mol to 1.25 µmol/mol. The ten new standards were prepared employing a NIST-developed cylinder pretreatment, a NIST SRM 2629a as the parent NO source and a diluent balance gas of specially scrubbed (active metal getter) nitrogen ($O_2 < 2$ nmol/mol).

***CSTL researcher developed a novel pretreatment process that passivates gas cylinder walls allowing the long-term storage of low level NO gas permitting the commercial production of this high priority standard.***

AMERICAN INDUSTRY / GOVERNMENT EMISSIONS RESEARCH
AIGER

**The low NO standards have been analyzed periodically against NIST dynamic permeation standards and have exhibited excellent NO conc. stability (<2 % relative) for approx. five years. See example below:**

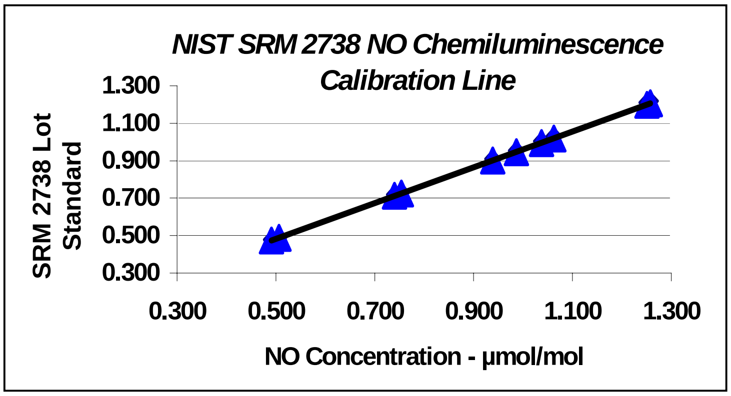
***Stability of Low NO Standard (Nominal Concentration 0.500 µmol/mol)***

| ***Year*** | ***Sample 1*** | ***Sample 2*** |
|---|---|---|
| **1999** | **0.499** | **0.507** |
| **2000** | **0.502** | **0.512** |
| **2001** | **0.500** | **0.514** |
| **2002** | **0.501** | **0.516** |
| **2003** | **0.491** | **0.506** |

Newer vehicles produce lower levels of these pollutants because their engines employ fuel injection whose air-to-fuel ratio is optimized by an on-board computer, and their emissions are further reduced by more efficient catalytic converters. During testing the exhaust levels are diluted by the use of constant volume sampling bags or by new mini-diluter technology. AIGER stakeholders have identified the need for significantly lower NIST gas standards containing carbon monoxide, hydrocarbons and nitric oxide; with the completion of SRM 2737 (0.5 µmol/mol) and SRM 2738 (1.0 µmol/mol) nitric oxide in nitrogen being their highest priority. NIST responded with thirteen cylinders at nominally the same concentration levels of SRM 2737 and SRM 2738. These were recently analyzed by NIST and returned to

AIGER members for their interim use − until the new SRMs become available in March 2004.

NIST assigned NO and $NO_X$ values to the NIST low NO standards by two different methods. Shown is the NO calibration curve in which the assigned NO concentration is plotted on the x-axis, and the ratio of the measured value to the SRM 2738 lot standard is plotted on the y-axis. All candidate SRM mixtures at 0.5 µmol/mol and 1.0 µmol/mol were analyzed in December, 2002, which was sixteen months after blending. All cylinders are stable and all contain 0.2 µmol/mol nitrogen dioxide.



The project has technically resolved NIST's low NO SRM stability problems, provided AIGER members with interim standards to work with, and will soon provide much needed lower concentration NIST certified NO SRMs in the near future; and thus help facilitate vehicle manufacturer's in meeting U.S. EPA and CARB's current and future lower emission regulations. In addition, AIGER and NIST are discussing technical issues to upgrade the NTRM protocol to meet mobile source accuracy and stability criteria for emission measurements. The NTRM "prime" (NTRM') addendum to NIST SP 260-126 has been submitted to AIGER for comment.

**A rollout of the new NTRM' gas standards are scheduled at a meeting between AIGER, specialty gas companies and NIST to be held in parallel with the March 2004 Pittsburgh Conference meeting in Chicago.**

### *Fourier-Transform Microwave Spectroscopy (FTMW) Critically Evaluated for Real-time Measurements of Automotive Exhaust Emissions*

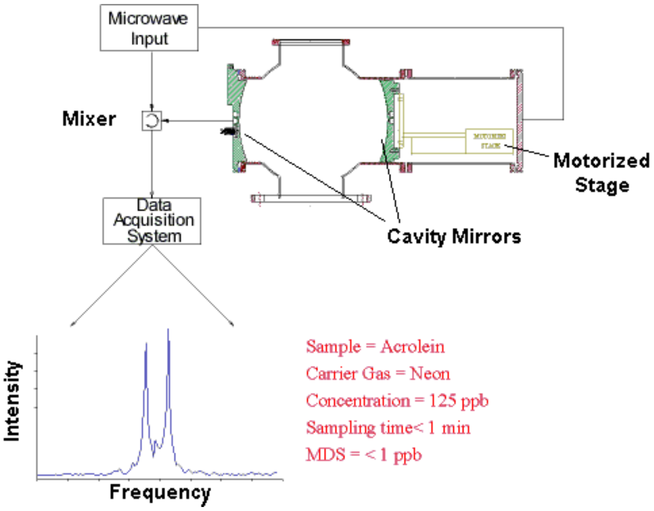
***R.R. Bousquet and P.M. Chu (839)***

Fourier-transform microwave (FTMW) spectroscopy is being investigated as a means for quantitative real-time measurements of automobile exhaust emissions. The principal goal of the present program is to critically evaluate the technique's potential as a sensitive, reliable, and robust tool for quantitative measurements of trace gases in complex gaseous mixtures for real-time analysis of automobile exhaust emissions, environmental monitoring, process control, and homeland security applications.

***FTMW spectroscopy combines the high spectral resolution of microwave spectroscopy and the high sensitivity of molecular beam and cavity-enhanced methods creating a technique that can provide unambiguous identification of vapor phase analytes that possess permanent electric dipole moments.***

**In FY 03, additional effort was focused on characterizing the factors that determine the overall sensitivity of the detection system and upgrading the software and hardware to enhance the signal to noise.**

Current work has focused on improving the sensitivity of the detection system and characterizing the effects of species such as water and carbon dioxide present in the sample gas. Continued efforts to improve the signal stability and reproducibility pushed the stability to approximately 1 % over 24 h. Further development continues in a number of areas including: use of low noise preamplifiers in the initial signal processing, implementation of a dual phase modulation/detection system, improvements of the cavity mode quality, and addition of adjustable antenna for optimization of the microwave radiation transmission at specific analyte transition frequencies. Finally, detection limits ranging from 0.1 µmol/mol to 10 µmol/mol have been realized for several analytes in pure nitrogen matrices. Furthermore, minimal changes in the detection limits were observed for the addition of ambient levels of oxygen, carbon dioxide and water to the sample matrix suggesting that this is a viable technique for real-time ambient monitoring.



**Schematic diagram of FTMW system, and sample of spectral output.**

Accurate quantitative measurements of trace species in bulk gases are becoming more vital to both science and industry with detection requirements to the low part per billion regions and beyond. Analytical methods that exhibit high chemical selectivity and sensitivity are particularly advantageous for the analysis of complex matrices. Through this research we hope to demonstrate the potential of FTMW spectroscopy to help meet these measurement needs.

**Improvements planned for FY 2004 should push the detection limits of the FTMW spectrometer to the nmol/mol to pmol/mol region. Additionally, to facilitate the deployment of the FTMW spectrometer in field and laboratory applications, higher-pressure measurements will be investigated.**

### *Properties of Rocket Propellant (RP-1)*

***J. W. Magee, D. G. Friend, T. J Bruno, M. L. Huber, E. W. Lemmon, A. Laesecke, R. A. Perkins, and J. A. Widegren (838); I. M. Abdulagatov (Russian Academy of Sciences)***

The National Aeronautics and Space Administration (NASA) has, for decades, used a kerosene blend, designated RP-1, as a rocket propellant. Presently, NASA and its contractors are designing advanced rocket engines that will combust RP-1 fuel. While reliable thermophysical properties are essential for this purpose, present-day models are either based on a pure component surrogate compound (dodecane) or on estimation methods that are grounded in very limited data. To address the issues surrounding the accuracy of thermophysical properties of RP-1, new measurements and models are needed.

***CSTL researchers are supplying NASA with validated property information at high temperatures and pressures for the development of a Rocket Engine Prototype planned for future launch vehicles.***

NATIONAL AERONAUTICS AND SPACE ADMINISTRATION

Prior to the work reported here, published data were limited to near atmospheric pressure and temperatures to less than about 373 K. A measurement program is underway that is intended to provide high quality data in order to support the advanced model development. Measurements of chemical composition, stability, density, heat capacity, viscosity and thermal conductivity are being conducted in a range of temperatures up to 700 K and pressures to 60 MPa. The first compositional studies, based largely on a gas chromatography - mass spectrometry (GC-MS) method, have identified key components and classes of chemicals found in a sample of RP-1. Initial measurements of density and viscosity have been completed, and advanced models, based on both published data and these new measurements, are under development.

A GC-MS method was used to chemically characterize a sample of RP-1 supplied by NASA. As expected, the ion chromatogram showed that RP-1 is a complex liquid fuel that consists of significantly more than 100 components. When the best resolved chromatogram peaks were individually integrated and interpreted, a report was prepared that identifies the key constituents of this mixture. From this report, an initial slate of compounds has been selected for a surrogate mixture to be used for modeling thermophysical properties. The selection was based on the predominance in RP-1 and the variety of chemical structures to represent key classes of compounds identified in the complex fuel. An experimental program was initiated to measure important thermodynamic (density and heat capacity) and transport (viscosity and thermal conductivity) properties of RP-1 in ranges of temperature from 300 K to 700 K and pressure from 0.1 MPa to 60 MPa. The first measurements reported were the density and viscosity. Modeling studies have concentrated on the pure compounds comprising the surrogate mixture. Short equations of state and models for the transport properties have been developed for each of these constituents of RP-1, and a preliminary Helmholtz energy mixture model has been developed that is based using a set of predicted interaction parameters. Similarly, a preliminary mixture model has been nearly completed for the transport properties of RP-1.

**In FY 03, density and viscosity measurements have been reported in ranges of temperature from 300 K to 700 K and pressures from 0.1 MPa to 60 MPa.**

### *Properties and Processes for Cryogenic Refrigeration*

***R. Radebaugh, P. Bradley, and M. Lewis (838); J. Gary and A. O'Gallagher (ITL); E. Luo, (Chinese Academy of Sciences).***

Cryocoolers are required for many technology areas, including the cooling of infrared sensors for surveillance and atmospheric studies, the cooling of superconducting electronics, magnets, and power systems, the cooling of cryopumps for clean vacuums in semiconductor fabrication processes, the liquefaction of industrial gas, and many other existing and potential applications. This research aims to develop measurement and modeling techniques for evaluating and improving performance of cryocoolers and their components; to develop new and improved refrigeration and heat transfer processes below about 230 K; and to provide an electronic database on cryogenic material properties.

***CSTL researchers have developed test measurements that led to obtaining temperatures of about 17 K in a small two-stage pulse tube refrigerator that we designed to achieve 15 K. This prototype is the first step in the development of a system for subcooling liquid hydrogen to 15 K for use in the next generation space vehicle.***

CSTL researchers worked with Sierra Lobo on the refrigeration system for supercooling liquid hydrogen for the NASA's next generation space vehicle. We collaborated with scientists at Sierra Lobo, a small company doing research in cyrogenic technologies, to investigate the technology of densifying hydrogen Next steps would involve scaling the overall system to much larger sizes if NASA chooses to develop this approach.

With a medical device company, we completed measurements on the performance of a simple pulse tube refrigerator for the cooling of dermatological pens to about 183 K. Such temperatures are higher than typical operating temperature of pulse tube refrigerators, and although our models predicted the performance well, issues with the compressor have hampered some necessary measurements.

**The use of these technologies has been hampered because of problems with existing cryocoolers, including short lifetimes, inefficiency, high cost, and excessive vibration. Metrology needs to be developed to characterize losses, and models are needed to optimize the design of such systems. Material properties at cryogenic temperatures are required for the design of cryogenic equipment.**

We have carried out measurements of the complex fluid dynamic impedance of small inertance tubes and compared them with two types of models. Our measurements revealed that typical laminar flow elements have large errors in the measurement of oscillating flows, whereas hot-wire anemometers do not. This year, a large (500 W at 30 K) prototype pulse tube neon liquefier was developed, and extensive tests have now been completed to measure losses in both the regenerator and the pulse tube. The pulse tube effectiveness was found to be even better than expected, but losses in the regenerator were higher than expected. Measurements indicated that temperature non-uniformities exist which may be a result of non-uniform flow; we have not yet been able to pinpoint the reason for such behavior. Solving this problem is very important for large pulse tube refrigerators being considered for cooling of power applications of superconductors (motors, generators, transformers, and fault current limiters).

In FY 03, we have provided a wide range of industries with new tools to improve the performance of cryocoolers, particularly those operating at temperatures below about 50 K and above 150 K. Our model for inertance tubes led to the publishing of design curves for their optimization, and the model results in more efficient cryocoolers. Our work on two-stage pulse tubes at 30 K and below will aid the superconductor industry in the development of superconducting systems that can be marketed to a larger customer base and improve the electrical infrastructure of the U.S. Our materials properties database is now receiving considerable attention and is useful to all industries dealing with cryogenic temperatures. An expanding customer base is requiring our models, data, and technical expertise in the development of cryocoolers for an ever-increasing range of applications. To improve efficiency of these coolers, we are continuing the development of a test apparatus to measure nearly all types of losses in cryocoolers and to measure how they are influenced by various parameters.

***Dissemination of data and information:***

- **Papers were presented at the Cryogenic Engineering Conference on the results of our measurements and modeling of inertance tube behavior.**
- **A book chapter on “Refrigeration for Superconductors” has been developed for a special IEEE Proceedings on *Electronic and Large Scale Applications of Superconductivity,* covering the fundamentals of refrigeration for both small and large superconducting systems.**
- **Database on the properties of cryogenic materials to be expanded.**

## *Benchmark Data on Liquid Fire Suppressants for Use in Aircraft*

***C. Presser and B. Johnson (836); C.T. Avedisian (Cornell University); G. Papadopoulos (Dantec Dynamics); J. Hewson (Sandia National Laboratory); D. Keyser (NavAir); P. Disimile and J. Tucker (46th Air Force Test Wing)***

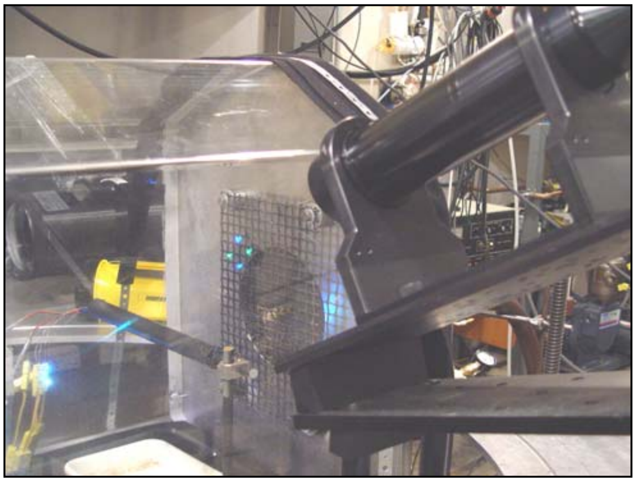
***An accurate representation of droplet transport is important in predicting the success of fire suppression in obstructed spaces in aircraft using fire suppressant that have high boiling points.***

The new generation of non-ozone-depleting Halon fire suppressants include chemicals that have higher boiling point temperatures (i.e., >330 K) and that exist in the liquid phase under high-pressure release or in ambient conditions. Release of these agents in a confined and cluttered space such as ***aircraft dry bays*** and nacelles results in the dispersal of droplets that either travel along ballistic trajectories, move with the convecting flow, or impact upon nearby solid obstacles. An accurate representation of droplet transport is crucial for the numerical modeling of fire suppression in obstructed spaces in aircraft.

**This project will provide a parametric data set to validate the VULCAN computational fluid dynamics fire physics code for spray-clutter interactive environments, which is under development at Sandia National Laboratories.**

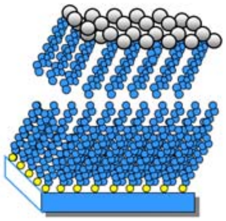
To better understand the physics of droplet transport around and behind solid objects, an experimental arrangement was set up to impose controlled grid-generated turbulence on the air stream. Experimental results from this facility provide new experimental data for a well-characterized, homogenous, droplet-laden turbulent flow field around prescribed obstacles to provide data for flow model validation.

The agents considered in the investigation were 3M™ fire protection fluorocarbons HFE-7100 and HFE-7000 with boiling points of 334 K and 307 K, respectively, in addition to water. Phase Doppler measurements were used to obtain droplet size and velocity distributions in a droplet-laden homogenous turbulent flow field around a horizontal, 32 mm diameter cylinder serving as an obstacle. These measurements help elucidate the effects of different physical properties of the agent on droplet transport, as well as the effect of droplet size on impact and coating of the cylinder surface, rebound into the free stream, vaporization near the cylinder as it is heated, and entrainment behind the cylinder. The results indicated that, unlike water, HFE7000 and HFE7100 vaporized readily without any droplet accumulation and dripping from the cylinder. Droplet size is correlated with boiling point. Downstream in the wake of the cylinder, a distribution of smaller size droplets (generally, less than 30 μm) were entrained in the recirculation zone. Comparison of results for the unheated and heated cylinder with water found that near the heated cylinder surface, droplet vaporization resulted in smaller mean droplet sizes, relative to the unheated case.



**Apparatus showing phase Doppler transmitter (right) and horizontal cylinder (lower left) within Plexiglas® chamber.**

# 2. Biomaterials



The biotechnology industry is making significant advances in the areas of biomolecular research for use in new environmentally friendly materials and process technologies. CSTL supports these efforts by developing measurement methods, standards and tools for quality control, as well as standards for calibration of specialized bio-based equipment. In addition, advances in tissue engineering continue to grow rapidly requiring standards to assure the integrity of tissue-engineered products during development, storage or shipment.

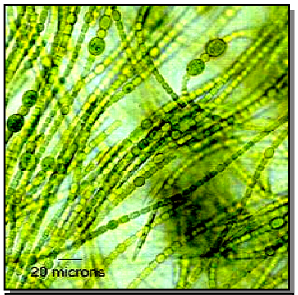
**The total market for the regeneration and repair of tissues and organs is estimated to be $25 billion worldwide.**

## *Desiccation Tolerance of Genomic DNA of the Cyanobacterium Nostoc Commune*

***H. Rodriguez (831), M. Birincioglu (Guest Researcher); P. Jaruga (CARB, UMBI); B. Shirkey, S.C. Smith, D.J. Wright, and M. Potts (Virginia Tech Center for Genomics and Virginia Tech, Blacksburg, VA)***

Desiccation is likely to have been an important determinant in the evolution of primitive cells. Today, desiccation remains the most acute environmental stress suffered by living cells. It is known that some cells remain desiccated for extended periods of time (decades or more), however, the mechanisms that enable a cell to survive as it enters and leaves the air-dried state are unknown. The Cyanobacteria was selected for this work since it is ecologically important as well as a phylogenetically diverse assemblage of prokaryotes, thus offering an excellent model system for the study of nitrogen fixation, photosynthesis, and cell differentiation. *Nostoc commune* has a cosmopolitan distribution and is of particular interest in that it has a marked capacity for desiccation tolerance.

***Water is indispensable for the maintenance of cell integrity and function. The removal of water from a cell causes such severe perturbation that the cell may die within seconds of its exposure to the atmosphere.***



**Cyanobacteria, *Nostoc commune***

This work demonstrated the stability of desiccated DNA *in vivo*. Key physiological processes and metabolic networks that contribute to desiccation tolerance using functional genomics and proteomics have been identified. This work is complimentary to the ongoing research in CSTL in the field of biomarkers of tissue engineering. It broadens the work at NIST by directly applying the biomarkers developed for the field of tissue engineering to the field of cell desiccation tolerance and cryopreservation. Understanding these processes will aid the development of better long-term storage methods of tissue-engineered medical products.

**Complete technical results are reported in *Nucleic Acids Research*, 2003 vol 31, No 12.**

**DNA from *Nostoc commune* ENG1996 colonies desiccated for four years at time of assay or rehydrated with distilled water for time periods before isolation of the DNA for RAPD-PCR assay. Lanes from left to right: from dry to rehydration for 5 min, 30 min, 1 h, 3 h, 24 h.**

## *Comparison of Reagents for Shape Analysis of Fixed Cells by Automated Fluorescence Microscopy*

***J.T. Elliott, A. Tona, and A.L. Plant (831)***

Morphology analysis of cells in culture is commonly employed by pathologist and cell biologist to characterize cell behavior. For example, cell morphology can be a marker for cell phenotype or aberrant cell behavior. In addition, cell size and shape have been implicated as potentiators of intracellular signaling events. Automated microscopy and image analysis can provide quantitative information about the size and shape of cultured cells, but it requires that the edge of a cell can be clearly identified. Generating adequate contrast at the edge of thin well-spread cells cultured on solid substrates can be challenging with traditional staining techniques. Therefore we conducted a comparative analysis of six fluorescein-based probes for their effectiveness as whole cell stains for automated morphology analysis of fixed cells. The identification of a high-contrast and robust staining procedure for fixed cells will be useful to any research groups that use cell morphology to address how culture conditions, extracellular matrix modification and biomaterial substrates influence cellular behavior.

***CSTL researchers have identified a fluorescent cell stain to generate high intensity contrast at the periphery of fixed cells, thus improving the quality of cell morphology measurements made by automated microscopy and image analysis routines.***

The results of this study indicate that formaldehyde fixed cells stained with fluorescein-5-maleimide exhibited the highest contrast at the cell edge and required 25-fold shorter exposure times than cells stained with 5-chloromethyl fluorescein diacetate (CMFDA, Cell Tracker Green), a commercially available whole cell stain. In addition, the average area determination of cells stained with fluorescein-5-maleimide was less sensitive to changes in the image analysis parameters (threshold value) that are used to identify cell objects.

**The stain described here is used routinely in our laboratory for morphological evaluation of cultured cells on biomaterials. We have recently begun to combine this stain with other cell stains to improve the level of quantitative information that can be gathered from cells in culture.**

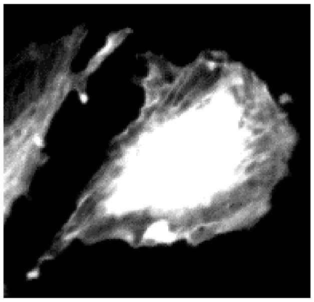
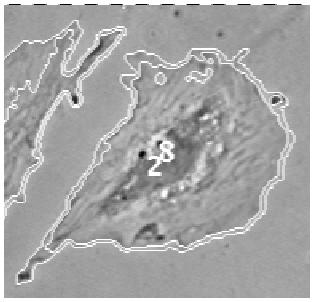


**Figure to left shows vascular smooth muscle cells stained with fluorescein-5-maleimide, the brightest stain identified in this study. The high contrast exhibited at the periphery of the stained cells facilitates automated object detection with image analysis software. Figure to right shows the outline of a cell that was identified by automated image analysis.**



## *Characterization and Control of Gel-Fluid Phase Transitions in Supported Phospholipid Monolayer Films*

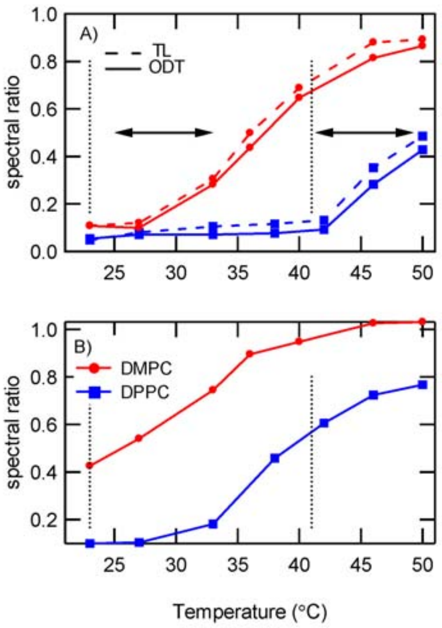
***L.J. Richter (837); C.S.-C. Yang, J.C. Stephenson, and K.A. Briggman (PL)***

The physical structure (gel or fluid phase) of lipid domains in membranes influences cellular signaling and other biological function, and hence must be characterized for both model and biological systems. All cells are surrounded by plasma membranes based on lipid bilayers. There is increasing evidence that the physical structure of the lipid membrane has important physiological implications. Lipid microdomains consisting of high phase transition temperature lipids and cholesterol have been implicated in the function of signaling proteins. Conventional calorimetery cannot be done on planar model systems, due to the low surface area. Thus, very little is known of the thermal phase behavior of lipids in supported structures. In order to address this knowledge gap, CSTL researchers are validating optical techniques for the characterization of the thermal phase of supported lipid constructs.

***Hydrated supported phospholipid structures (Langmuir-Blodgett films, supported bilayers, etc.) have been widely studied as model systems for biological membranes. Supported monolayers and bilayers may also be used as platforms for novel biosensors and biomaterials.***

Vibrationally-resonant sum frequency generation (VR-SFG) is a non-linear optical probe uniquely suited for the *in-situ* characterization of planar interfaces, as the signal is symmetry forbidden in centrosymmetric media. This selection rule also makes VR-SFG ideally suited to the study of the gel-fluid phase transition in phospholipids, as the methylene CH stretch spectral features in the gel phase are forbidden by the all-trans symmetry of the alkane chains, while these features are allowed in the disordered chain fluid phase.

In a typical structure of a hybrid monolayer (HBM) a lipid monolayer formed on a hydrophobic film on a solid substrate. Independent control over both the lipid layer and the hydrophobic film may be used to optimize the HBM for specific applications. The VR-SFG spectra of HBMs formed with two different phospholipids are shown in the figure: Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and Dimyristoyl-sn-glycero-3-phosphocholine (DMPC), with 14 and 12 methylene groups per alkane chain, respectively. The lipid chains were perdeuterated to provide contrast against the protonated support. The top graph depicts the ratio of the thermal phase dependent methylene CD stretch feature to an internal standard, the CD stretch signal from the terminal methyl groups, for the phospholipids on a well ordered octadecanethiol (ODT) on gold support. The inflection in the step-like increase in the ratio indicates the temperature of the gel-fluid phase transition $T_M$ in the two films. As expected, the longer chain DPPC has a higher $T_M$ than the shorter DMPC.



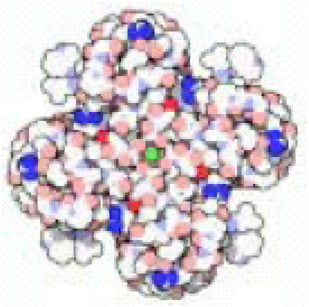
**Scaled fluid phase spectral intensity for HBMs formed with DMPC (red) and DPPC (blue). Vertical Lines mark the $T_M$ for unsupported vesicles. (Top) HBM support is a pure ODT or TL film. Arrows indicate shift in $T_M$; (Below) HBM support is mixed TL/MCH film. $T_M$ HBM ≈ $T_M$ vesicles.**

**VR-SFG has been established as a diagnostic for the thermal phase of supported bilayers. It was discovered that $T_M$ for the most commonly formed HBMs is significantly different from that of bilayers in vesicles. This change in $T_M$ could affect the biomimetic characteristics of HBMs. A procedure for controlling $T_M$ via modification of support was developed.**

The $T_M$ of HBMs formed on the ODT support is uniformly shifted by about 10 °C to higher temperature from the $T_M$ of vesicles. This perturbation of $T_M$ is also observed for HBMs on a support formed from a well-ordered thiolated lipid (TL) film of Dipalmitoyl-sn-glycero-3-phosphothioethanol. It was discovered (data in graph B) that $T_M$ can be controlled by selectively incorporating a small hydrophilic molecule, mercaptohexanol (MCH), into the TL film, introducing slight structural disorder and weak polar forces. HBMs formed on supports fabricated with a 1:2 mole ratio of MCH to TL in the film-forming solution have $T_M$ essentially identical to that of vesicles.

***Next Steps:*** **The ability of VR-SFG to characterize the thermal phase of supported lipid films and the ability to control the phase transition via modifications of the support will be used to study the influence of lipid phase on the insertion and structure of small membrane bound proteins and peptides.**

# 3. Pharmaceuticals and Biomanufacturing



**Increased demand for new products has replaced price as the primary force in the U.S. pharmaceutical industry's overall growth.**

CSTL provides a breadth of resources that support the pharmaceutical industry including reference data as well as artifact standards such as optical filters and fluorescence standards used for instrument calibration. CSTL measurements and standards facilitate the drug discovery process, help optimize production of new pharmaceuticals, and ensure quality control in manufacturing processes. In addition, CSTL's fundamental work in enzyme characterization promotes the transition to biomanufacturing leading to more environmentally sustainable manufacturing.

### *Bioseparations - Separation of Different Topological Forms of Circular DNA*

***K.D. Cole (831)***

***Improved methods for the production, characterization, and purification of circular DNA are needed to meet the needs of the pharmaceutical, biotechnology, and molecular biology communities.***

The DNA from many microorganisms and cellular organelles is circular. Plasmids are important examples of circular DNA that are widely used to clone genes and express foreign proteins. We are studying the separation of circular DNA to improve methods for the analysis and the isolation of large amounts of plasmid DNA. Circular DNA can be selectively retained in gels by electric fields by a process termed electrophoretic trapping. Our efforts have been to characterize the process, develop models, and attempt to exploit electrophoretic trapping for improved analytical and preparative methods for circular DNA. The migration of circular DNA can be completely arrested in gels when the electric field strength reaches a critical value.

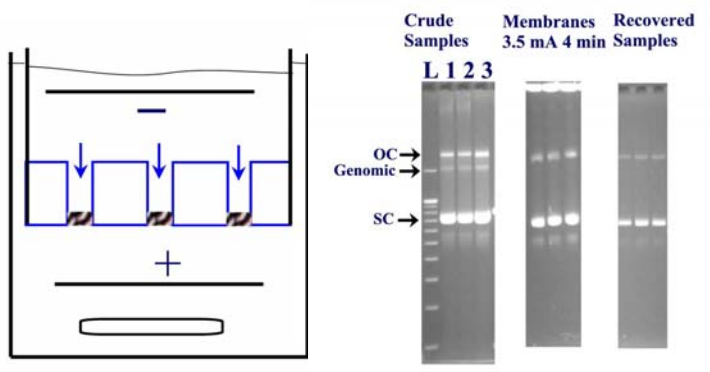
**The factors that determine the formation of the electrophoretic traps in gels are not well understood. A better understanding of these factors would allow us to control their formation and to design better gels for the selective separation of circular DNA.**

CSTL researchers have studied the effects of the gel structure and the topological form of the DNA on trapping. The concentration of polymer (agarose) used to form the gel has a large effect on the formation of the "traps" for circular DNA.

**"The influence of agarose concentration in gels on the electrophoretic trapping of circular DNA," Cole, K. D.; Åkerman, B., *Separation Sci. Technol.* 38, 2121-2136 (2003).**

The electric field strength for the onset of trapping, the apparent trap density, and the release characteristics of circles from traps were studied using both direct current and field inversion gel electrophoresis (FIGE) experiments. The average distances before 13 kbp (kilobase pairs) open circles were trapped were ≈ 80 μm in 0.25 % gels, 180 μm in 1 % gels, and greater than 500 μm in 2.5 % gels. The higher trap density and longer trap length in low concentration gels explains the experimental observations that under some conditions DNA circles migrate faster in higher concentration gels compared to their migration in lower concentration gels. Lower concentration gels had a higher density of traps and the traps were more heterogeneous when compared to traps formed in gels formed with higher concentrations of agarose.

An apparatus was designed for the electrophoretic capture and recovery of circular DNA in thin layers (membranes). Rapid separations were done by the use of a low conductivity buffer and high electric field strengths. Two methods that specifically retain circular DNA in the membranes are demonstrated using the supercoiled and open circular forms of two plasmids with sizes of 4.4 kbp and 13 kbp. Electrophoretic trapping (by an impalement mechanism) in agarose gel-filled membranes used electric field strength to immobilize circular DNA in the membranes. These membranes and methods should be scaleable to process large number of samples or to prepare larger amounts of pure circular DNA by increasing the area of the membranes. The apparatus facilitates the rapid screening of a large number of trapping materials and methods for recovery of DNA.



**Schematic diagrams of the apparatus used for electrophoretic capture in thin layers and gel electrophoresis of DNA purified using the apparatus showing the location of supercoiled (SC) and open circular (OC) plasmid DNA. The recovered plasmid DNA is free of genomic (host) DNA.**

**"An apparatus for electro-phoretic trapping of circular DNA in thin layers," Cole, K. D. Biotechnol. *Applied Biochem.* 37, 251-257 (2003).**

These studies permit the more rational design of gels for the separation of circular DNA. The gels can be designed or "tuned" for a particular analytical or preparative separation and thus give an improved separation of the circular DNA. We plan to extend this research to use fluorescence microscopy in order to observe individual circular DNA molecules, tagged with fluorescent dyes, as they separate in gels. This will provide insight into the mechanism of separation and thus allow us to design improved separations.

**Both the data and the apparatus can be used by researchers to prepare highly pure circular DNA in a matter of minutes.**

## *Develop Imaging Tools to Characterize Fluorescence from Fluorophores Immobilized on Surfaces*

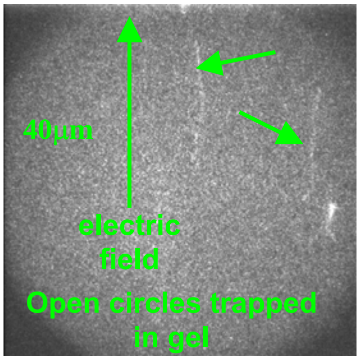
***A. Gaigalas, K.D. Cole, and L. Wang (831)***

A large variety of new fluorescent reagents have been developed for the measurement of biological targets either in solution or on surfaces. These fluorescent dyes have to a large extent replaced the use of more dangerous and less convenient radioisotopes. However, the use of fluorescent reagents to detect biological targets on surfaces is not without their drawbacks, including the photobleaching of the fluorophore, fluorescence quenching of the light due to interactions in the microscopic environment on the surface, and background subtraction. The research described here aims to improve the limits of detection of targets and to increase the quality of the data obtained.

***Imaging of fluorescent signals from surfaces has a number of important applications in biotechnology, including the detection of cell types, viruses, and measurement of target molecules. Advances in genomics and proteomics have made larger scale screening techniques possible.***

CSTL researchers are measuring the process of photobleaching in solution and the effect of protective additives on the rates of bleaching. A fluorescent microscope has been set up to image targets including DNA molecules, viruses, bacteria, and microarrays. We are starting measurements of these targets and the evaluation of the images using commercial software programs. We are in the process of improving the microscope to improve the quality of the images obtained. These preliminary experiments have also indicated the importance of the sample preparation (labeling with the fluorophore) and the surface environment on the quality of the measurements obtained.

**Effective addictives help to prevent fluorophores from photodegrading and photobleaching. This serves to increase the limit of detection and the overall contrast to the auto-fluorescence background. In turn, this enhances and makes possible applications such as virus and bacteria counting and the imaging of individual microarray spots.**



**Imaging of bacterial artificial chromosomes (220,000 base pairs) during agarose gel electrophoresis.**

We plan to continue measurements of photobleaching for different fluorescent reagents and the effect of stabilizing additives. The effect of different surfaces and substrates on fluorescence quenching of fluorophores and background will be examined.

**"A frequency-domain technique for studying photodegradation process of fluorophores," Gaigalas, A. K.; Wang, L.; Vogt, R. F., *Photochem. Photobiol*. 76, 22-28 (2002);**
**"Photodegradation of fluorescein in solutions containing n-propyl gallate," Gaigalas, A. K.; Wang, L.; Cole, K. D.; Humphries, E., in review.**

## *Enzyme-Catalyzed Synthesis of Optically Active Drug Intermediate Compounds Using Supercritical Fluids as the Solvent*

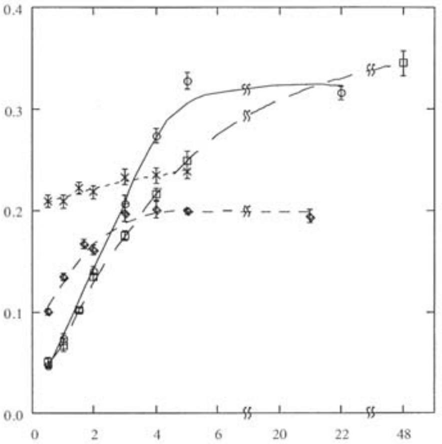
***Y.B. Tewari (831); K.W. Phinney (839); T. Ihara (AIST, Japan)***

***In the pharmaceutical industry, enzyme-catalyzed reactions are used for the synthesis of chiral drug intermediates. Because these reactions are generally carried out in organic solvents, the generation of large volumes of organic waste is of concern.***

Recently, supercritical carbon dioxide ($SCCO_2$) has been used as an alternative solvent for biochemical reactions and has been shown to have several advantages over commonly used organic solvents. Specifically, carbon dioxide is non-toxic, non-flammable, available in abundance, and is environmentally friendly and recyclable. The low viscosity and high diffusivity of $SCCO_2$ serve to provide favorable mass transfer properties. In this work, a reaction vessel was designed, fabricated, and tested to study enzyme-catalyzed reactions in $SCCO_2$ media. The equilibrium constants of the lipase-catalyzed transesterification reaction (benzyl alcohol + butyl acetate = benzyl acetate + 1-butanol) were studied in $SCCO_2$ . The equilibrium constant of this reaction in $SCCO_2$ is similar to those obtained in

**The enzyme used in this study was a lipase from *Candida antarctica*. (Structure from PDB)**

n-hexane, toluene, and other non-aqueous solvents. These results also show that the rate of reaction in $SCCO_2$ media is higher than those in organic solvents.



**Reaction quotient vs time in different solvent systems. The solvents used were n-hexane (O), toluene (☐), carbon dioxide (×), and the system without solvent (◇).**

This is the first equilibrium study that has been performed in supercritical carbon dioxide. We have demonstrated that supercritical carbon dioxide provides kinetic and environmentally friendly advantages for carrying out enzyme-catalyzed reactions. This work provides comparisons between enzyme-catalyzed reactions in traditional non-aqueous solvent systems and in supercritical carbon dioxide. The data will facilitate implementation of supercritical carbon dioxide as a solvent for biocatalysis in the biotechnology and pharmaceutical industries.

Alternatives to organic solvents are important for reducing the levels of residual solvents in pharmaceutical products and for reducing the environmental burden resulting from solvent waste.

Studies of additional enzyme-catalyzed reactions in supercritical carbon dioxide are planned. These investigations will focus on reaction systems of industrial significance and relevance.

**"A Thermodynamic and Time Course Study of the Lipase-Catalyzed Transesterification of Benzyl Alcohol and Butyl Acetate in Supercritical Carbon Dioxide Media," Tewari, Y. B.; Ihara, T.; Phinney, K. W.; Mayhew, M. P., submitted to *J. Mol. Catal. B: Enzymatic*.**

## *Critical Evaluation of Capillary Electrophoresis for the Determination of Enantiomeric Composition of Ephedra Alkaloids*

***K.W. Phinney and L.C. Sander (839); T. Ihara (AIST, Japan)***

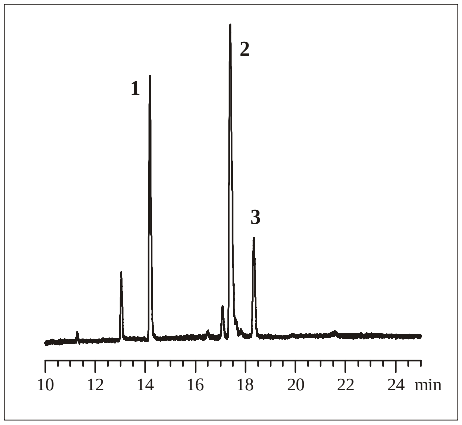
Ephedrine and pseudoephedrine are two of the major alkaloids found in ephedra, but these compounds are also found in traditional prescription and over-the-counter medications. Both compounds exist as pairs of enantiomers, but only (-)-ephedrine and (+)-pseudoephedrine are found in ephedra plants. Detection of (+)-ephedrine, (±)-ephedrine, or (-)-pseudoephedrine in a product containing ephedra would suggest that the content had been altered through the addition of synthetic alkaloids. Hence, the enantiomeric composition of these compounds is important in verifying the natural (plant) origin of the alkaloids.

***Dietary supplements containing ephedra have generated considerable interest because of their proposed ability to promote weight loss and increase athletic performance. However, ephedra has also been associated with adverse health effects.***

**Ephedra plant**

The high efficiency and ease of method development in capillary electrophoresis (CE) make CE attractive for enantioselective separations. However, CE has not been widely used for the

**The CE methods developed by CSTL researchers provided slightly different selectivity, thereby reducing the likelihood of undetected peak overlap. The use of a high sensitivity UV detection cell dramatically improved detection capabilities by increasing peak areas and signal-to-noise ratios.**



**Analysis of a solid oral dosage form of a dietary supplement. Peaks are: internal standard (1), (-)-ephedrine (2), and (+)-pseudoephedrine (3).**

determination of enantiomeric purity because the sensitivity of the technique is viewed as being inferior to that of liquid chromatography (LC). In this work, three CE methods were developed for the separation of ephedrine and pseudoephedrine enantiomers found in plant material, plant extracts, and dietary supplements containing ephedra. Neutral cyclodextrins or mixtures of neutral and charged cyclodextrins were used as the chiral additive to achieve enantioselective separations. Sample preparation involved a simple methanol extraction, and a high sensitivity UV detection cell allowed detection and quantification of these analytes in a variety of complex matrices. The identity of the enantiomers was confirmed through a standard addition method. This work has demonstrated that CE can be used to determine the enantiomeric composition of chiral analytes in complex matrices. The resulting methodology provides new measurement capabilities that complement existing LC and LC/mass spectrometry methods. The analysis of ephedra-containing materials is important from a regulatory standpoint to confirm the natural origin of the ephedra alkaloids and to determine the levels of ephedrine and related alkaloids in dietary supplements.

**In FY 03 five Standard Reference Materials (SRMs) containing ephedra (SRMs 3240-3244) were certified.**

### *Use of SRM-Based Column Performance Tests to Guide LC Column Selection in the Pharmaceutical Industry.*

***L.C. Sander and S.A. Wise (839)***

***Reversed-phase liquid chromatography (LC) is a mature analytical technique, however, analytical methods are often developed by trial and error, without full understanding of the fundamental mechanisms that make separations possible.***

Chromatographic columns provide the basis for chemical measurements in reversed-phase liquid chromatography (LC). Columns from different manufacturers or different manufacturing lots may exhibit a broad range of properties even though the columns are nominally similar (e.g., $C_{18}$ columns). Such differences in retention behavior provide opportunities for method development and optimization; however, variability in LC column manufacture may invalidate existing methods and necessitate method redevelopment. Between-column reproducibility is an important aspect that must be considered for the implementation of methods for routine use. Changes that occur in column performance with usage can also directly affect system suitability. In 2002, the U.S Pharmacopeia announced the formation of a Working Group on HPLC Columns. This group was charged with “creating categories in order to subclassify the USP designation L1 for $C_{18}$ (octadecylsilane) HPLC columns.” Several classification schemes are currently under consideration in this effort, including approaches based on Standard Reference Material column performance tests.

A clear need exists to distinguish among the hundreds of commercial LC columns that are available to the analyst. Details of column preparation and other descriptive information are usually considered proprietary by manufacturers; however, performance based tests provide a way of characterizing column properties without disclosing such trade secrets. The availability of unbiased tests for the assessment of LC column performance are required to facilitate: 1) the development and optimization of chromatographic methods by providing a rational basis for column selection, 2) evaluation of changes in column performance that occur over the life of the column; 3) evaluation of the suitability of columns for specific applications; and 4) characterization and classification of column properties to permit selection of similar columns among multiple manufacturers.

Three Standard Reference Materials have been developed specifically to address the needs of column characterization. SRM 869a Column Selectivity Test Mixture, SRM 870 Column Performance Test Mixture, and SRM 877 Chiral Selectivity Test Mixture offer independent tests to assess column effectiveness for the separation of compounds based on differences in solute shape (“shape selectivity”), general aspects of column performance (efficiency, retentiveness, activity toward bases and metal chelators), and column effectiveness for the separation of various classes of optically active compounds (“chiral selectivity”). The U. S. Pharmacopeia is currently considering the usage of SRM 869a and SRM 870 to augment their L1 designation for $C_{18}$ columns.

**The availability of SRMs to support column characterization will greatly facilitate column selection in pharmaceutical and clinical applications. Utilization of these SRMs by column manufacturers will result in the production of more reproducible LC columns, which in turn will improve the ruggedness of methods for routine use.**

Although SRMs 869a, 870, and 877 provide considerable information about column performance, other tests may be required to characterize recently introduced columns with new bonding chemistries. Several classification schemes have been considered, and an approach by Lloyd Snyder and John Dolan (LC Resources) has considerable merit. This approach uses 10 carefully selected solutes to compare column selectivities. An algorithm has been developed to calculate a single numerical descriptor, which provides an indication of the relative similarity of two columns. On this basis, decisions can be made concerning column choice in method development by selection of a column that is similar to one used in an existing method, or alternatively, selection of a dissimilar column to one that failed to provide adequate separation. A large body of data already exists for commercial columns characterized by Snyder and Dolan (≈ 150 columns to date). Future work at NIST may be directed towards the development of an SRM for LC column classification based on this Snyder/Dolan approach.

### *New Glass Filter Reference Materials for Validating the Performance of UV/visible Spectrophotometers in the High Absorbance Range*

***J.C. Travis, M.V. Smith, and G.W. Kramer (839)***

SRM 2930 is a set of three neutral density glass filters and a blank used to verify the accuracy of the transmittance/absorbance scales of molecular absorption spectrometers (spectrophotometers), and are individually certified at five visible wavelengths using the Reference Spectrophotometer located at NIST. The 0.1 %, 0.3 % and 92 % transmitting filters of SRM 2930 bracket and thus extends the range of transmittance coverage of SRM 930 (10 %, 20 % and 30 % transmitting) and SRM 1930 (1 %, 3 %, and 50 % transmitting). The extension of absorbance coverage to three absorbance units permits a dynamic range of three orders of magnitude in chemical concentration, reducing the need to dilute highly absorbing samples for spectrophotometric analysis and avoiding needless handling and additional uncertainty. With the 92 % transmitting filter (plain glass)

***The new filters standards will permit pharmaceutical and chemical producers to validate and quantify a product or constituent without an intermediate dilution step, subject to introducing needless bias and uncertainty. The existence of the new SRM also adds a traceability link for standards producers to provide a similar product and leverage NIST’s measurement capability.***

included in the new set, the total neutral density glass coverage of the three SRMs is now consistent with the transmittance calibration range supported by most NMIs who support transmittance scales. The nine neutral density glass filters of the new set of three coupled with the older SRMs provide coverage of absorbance consistent with chemical needs and with the measurement capabilities of modern instruments. The software of the NIST Reference Spectrophotometer (HAS-II) was extended to include a stepwise procedure to extend the range of qualification of the instrument to three absorbance units. The initial 12 sets of SRM 2930 have been assembled and initial stability, homogeneity, and uncertainty determination measurements have been made.

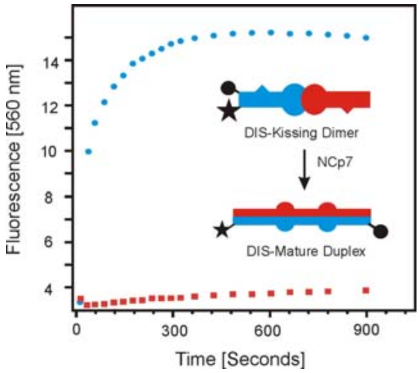
**The filters will be available for sale in FY 04. Production of these filters will not be considered continuous, and renewal will be on a case-by-case basis. Secondary producers will be encouraged to use these filters to provide traceability. Recertification services will be provided for a few years, after which all SRM absorbance filters may be recertified through the NIST Calibration service for continued traceability support.**

## *A Fluorescence Assay Designed to Screen for Inhibitors of HIV-1 Nucleocapsid Chaperoned Maturation of the Dimerization Initiation Site*

***J.P. Marino (831)***

RNA-based drug discovery in the pharmaceutical industry requires general approaches for detecting and quantifying nucleic acid-protein interactions that can be used as high-throughput screens (HTS). As an extension of our studies of the dimerization and maturation of the HIV-1 Dimerization Initiation Site (DIS) Stem-loop, we have developed a fluorescence assay to detect NCp7 catalyzed DIS maturation. Our assay uses a molecular beacon approach to detect hairpin strand exchange associated with NCp7 refolding of the DIS kissing dimer. One DIS hairpin is labeled with a fluorescent dye on the 5'-end and the static quencher, dabcyl, on the 3'-end. Upon interaction with the second DIS hairpin to form the kissing dimer complex, the fluorescence emission of the dye remains highly quenched and static. In the absence of inhibitor, NCp7 catalyzed maturation of DIS kissing dimer, that is associated with strand exchange between the two hairpin stems, results in a burst of fluorescence from the reported dye as it no longer in close proximity to the quenching agent in the mature duplex form (Fig. 1). This fluorescence assay can form the basis for a HTS screen to identify novel inhibitors of the NCp7 mediated maturation of the DIS dimer.

***The development of specific inhibitors of retroviral protein-RNA complexes is of significant interest to the pharmaceutical industry since these complexes provide potential novel drug targets. A key roadblock to the realization of these goals is the availability of rapid and sensitive assays to measure and quantify the RNA interactions.***



**Example of inhibition of NCp7 catalyzed DIS maturation by Neomycin at a concentration of 1 μM, as assayed using our molecular beacon detection approach. Fluorescence after addition of NCp7 to the DIS kissing dimer in the absence (blue) and presence (red) of Neomycin.**

Measurement technology developed in this study will have an impact on the biotechnology and pharmaceutical industries. The research is also responsive to the Advanced Technology Program's interest in projects directed at developing novel approaches for manipulating protein-nucleic acid interactions for possible therapeutic benefits or medical diagnostic purposes.

Through collaboration with researchers at the HIV-1 Drug Resistance Program at the National Institute of Cancer, we plan to apply our methods to screen large public domain libraries of low molecular weight compounds. Inhibitors of DIS and/or NCp7 identified in the HTS screen will then be optimized using rational design strategies based on NMR structural analysis.

**A fluorescence method for rapidly screening and quantifying HIV-1 NCp7 chaperoned DIS maturation has been developed. This method has been validated as a screening tool using aminoglycoside antibiotics, like Neomycin, which have been shown to inhibit NCp7 activity**

### *Structure of Pseudomonas aeruginosa PhzD, an Isochorismatase from the Chorismate Biosynthetic Pathway*

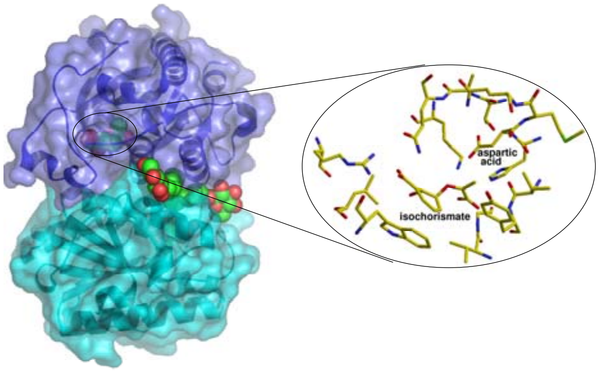
***J.E Ladner (831); E. Eisenstein (831/UMBI); J. Parsons (UMBI)***

This study provides a description of the enzymes involved in the chorismate metabolic pathway by solving their three-dimensional structures, modeling the mechanisms of the chemical transformations, and mapping pathway control nodes involved in the biocatalytic conversion of glucose to aromatic hydrocarbons. Only when the detailed three-dimensional structures are known for the enzymes can the precise enzymatic mechanisms and relationships between the structure and the physical properties be predicted. Most recently, the structure of the product of gene phzD from *Pseudomonas aeruginosa* has been solved. The phenazines are biologically active (antibacterial, antifungal, antitumor) aromatic products synthesized mainly by *Pseudomonas* and *Streptomyces* as part of their chorismate pathways.

***Industrial production of drugs, new biopolymers and indigo dyes, can be facilitated by increased understanding of the aromatic amino acid synthesis pathway. The increased ability to manipulate this pathway through metabolic and protein engineering will save non-renewable, petroleum-based feedstock chemicals.***

**The enzyme on the right (stick model) is part of the pathway that synthesizes a bioreactive molecule that has antimicrobial properties. The labelled amino acid, aspartic acid, initiates the reaction by acting like a general acid in the protonation of the ether oxygen of the substrate. The determination of the crystal structure with the substrate provides the basis for hypothesizing how the enzyme works and thus can provide clues as to how we can control or alter its action.**

**PhzD isochorismatase**
**from *Pseudomonas aeruginosa***



**The work this year has yielded a better understanding of the chorismate pathway. We have solved the structure of the product of gene phzD and are pursuing both biochemical and crystallographic methods to determine its role and substrate**

The synthesis of the aromatic biologically active molecules is a very important area of study because these pathways do not exist in animals. They exist solely in bacteria, fungi, and higher plants. The elucidation of these pathways can provide pharmaceutical researchers with a better understanding for the amplification or inhibition of the production of these molecules.

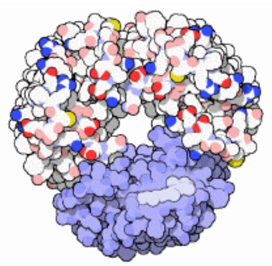
## *Theoretical Protocols for Calculating Structural, Spectroscopic, and Reaction Properties of Enzyme Active Sites*

***M. Krauss (831); Y.S. Lee (NIH)***

The reaction path and the electronic structure of enzyme active sites are analyzed to explore the common electronic character of enzyme catalysis. The methodology can be applied to the same or analogous enzymes within families of organisms or between different families to discern catalytic motifs and functional properties of active site residues. The very large size of the enzyme systems requires a means of dividing them into a reactive region treated quantum mechanically and the protein and solvent environment that is treated classically. Two flavors of coupling the quantum region to the classical are used in this research. In one, the quantum region interacts with effective fragment potentials (EFP) that represent the electrostatic, polarization, and repulsive interaction. The EFP are fitted to the quantum interactions for model systems and produces a consistent interaction across the quantum/classical interface. Classical and quantum calculations that determine the qualitatively correct reaction path are initiated using crystal structures with bound analog inhibitors. The reaction path can then be used for virtual screening of inhibitors for drug development. The ability to calculate the substrate reaction path for homologous enzymes from different types of cell organisms and simple mutants provides a flexibility and directness in the theoretical virtual screen that should make it very competitive with most experimental programs.

***Applications are to a variety of enzyme types chosen for their importance in validating the methodology and for applications to industrial, military, and medical problems.***

Both the QM/MM (Quantum Mechanics/Molecular Mechanics) and EFP codes are part of a protocol for studying enzyme behavior. Crystal structures with bound analog inhibitors are used to initiate classical and quantum calculations that determine the qualitatively correct reaction path. With the reaction path in hand, optimization and dynamics of the entire protein/ligand system with ab initio QM/MM can be considered. Also, spectroscopic properties are calculated from both the EFP and QM/MM data.



**Bacteriorhodopsin (bR) is a naturally occurring transmembrane protein that converts light energy into metabolic energy. Structural image from PDB.**

In bacteriorhodopsin, proton transfer from the cyctoplasm to the extracellular side is initiated from protonated asp96 in the cytoplasmic region towards the deprotonated Schiff base. This occurs in the transition from the late M photocycle state to the N state. A quantum mechanics/molecular mechanics (QM/MM) model is constructed from the bacteriorhodopsin E204Q mutant crystal structure. Structural transformation occurs in the M state allowing a channel to form between the asp96 and Schiff base that permits a water chain between these groups. Since a part of this channel is lined with hydrophobic residues, there has been a question on the mechanism of proton transfer in a hydrophobic channel. Using the energy optimized structures and the molecular dynamics trajectories, the entire vibrational spectrum of bacteriorhodopsin has been calculated to compare with experimental observations.

***Ab initio* dynamics using the CHARMM/GAMESS methodology was used to calculate the transfer of the proton through a hydrophobic channel, a new concept that is described in a paper submitted to the *Journal of the American Chemical Society*.**

Determination of a qualitatively correct reaction path is required before a QM/MM calculation can be undertaken. The structures of the reaction path in the enzyme active site can be used to design high-throughput screens for assessing reactant, transition state, and product analogues for lead compounds for drugs. The ability to generally calculate vibrational spectra for hydrogen bond networks bound in proteins will provide an important tool for analyzing the function of these systems.

## *The MIRG 2002 Study: Assembly State, Thermodynamic, and Kinetic Analysis of an Enzyme/Inhibitor Interaction*

***F. Schwarz (831), E. Eisenstein (831,UMBI); D. Myszka and Y. Abdiche (Univ. of Utah); F. Arisaka (Tokoyo Institute of Tech.); O. Byron (Univ. of Glasgow); P. Hensley and J. Thomson (Pfizer, Inc.; C. Lombardo (The Burnham Institute), W. Stafford (Boston Biom. Res. Institute) and M. Doyle (Bristol-Myers Squibb)***

***The analytical technologies in the biotechnology industry often utilized to measure biomolecular interactions include analytical ultracentrifugation (AUC), isothermal titration calorimetry (ITC), and surface plasmon resonance (SPR).***

The pharmaceutical industry has put increased effort into the discovery and development of protein-based therpeutics and reagents ("biologics") that exhibit highly specific interactions with their biological targets. Fully characterizing the interactions involving biomolecules requires information on the assembly state, affinity, kinetics, and thermodynamics associated with complex formation. In order to evaluate the capabilities and validate the results from industrial, academic, and government laboratories to implement analytical ultracentrifugation (AUC), isothermal titration calorimetry (ITC), and surface plasmon resonance (SPR), the Molecular Interactions Research Group (MIRG) of the Association of Biomolecular Resource Facilities (ABRF) developed a standardized model system and distributed it to a panel of scientists employing these methods. The Association of Biomolecular Resource Facilities that initiated this study is a non-profit standards organization sponsored by 33 companies and consisting of about 800 members from industry, academia, and government.

**www.ABRF.org**

The Association of Biomolecular Resource Facilities

The model system consisted of a well-characterized enzyme-inhibitor pair, namely bovine carbonic anhydrase II (CA II) and 4-carboxybenzenesulfonamide (CBS). Accordingly, samples of the CBS-CAII model system were distributed to a panel of scientists. Study participants were asked to measure one or more of the following: 1) the molecular mass, homogeneity, and assembly state of CAII by AUC; 2) the affinity and thermodynamics for complex formation by ITC; and 3) the affinity, kinetics, and thermodynamics of complex formation by SPR. The results of these measurements were evaluated.

**Carbonic Anhydrase II**

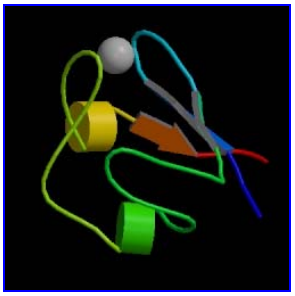
**Samples were distributed to a total of 55 industrial, academic, and governmental laboratories that consisted of 7 AUC, 12 ITC, and 36 SPR laboratories. The results of the *study showed excellent agreement to within 10 % on the binding affinities and within 2 % on the binding enthalpies* between the ITC and SPR laboratories. The AUC results on the assembly state of the enzyme were in agreement with the ITC results on the stiochiometry of the reaction.**

## *A Metric of Amino Acid Exchangeability Based on Experimental Data*

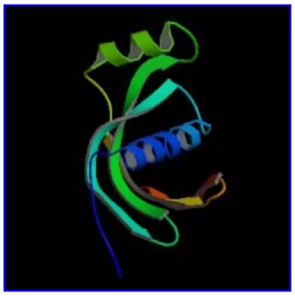
***A. Stoltzfus (831); L.Y. Yampolsky (CARB/UMBI)***

***Comparative sequence analysis is a fundamental part of computational genomics, necessary for genome annotation and functional prediction in bioinformatics.***

The significance of amino acid sequence differences between proteins is often interpreted by means of a classification of amino acids (e.g., polar vs. non-polar), or by some continuous measure of amino acid distance. Available measures based on observed propensities of evolutionary change are undesirable because they include confounding mutational effects; physicochemical measures are uncertain because they rely on some simplified model of the role of amino acids in proteins. What is needed is a measure that reliably reflects this role, without the confounding effects of mutation.



**Methionine is one of the essential amino acids and must be provided by the diet.**



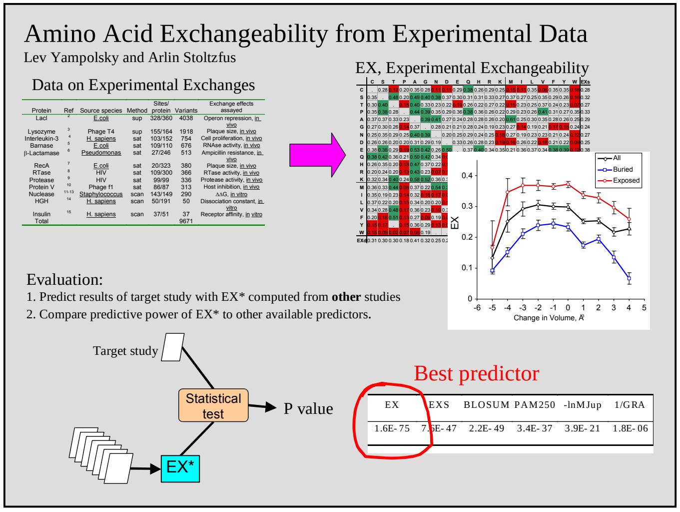
**Cysteine is an amino acid found in many proteins throughout the body.**

Comparative analysis of protein sequences relies on interpreting differences that arise primarily by the evolutionary replacement of one amino acid by another, without the confounding effects of mutation. How will such changes alter protein activity? Probabilities of evolutionary change reflect, not only effects on the protein, but also rates of mutation between codons. For example, the low rate of evolutionary interchange of Methionine and Cysteine might be due to incompatibility at the protein level, but is more likely explained by the genetic code, which dictates that three separate mutations are needed to go from a Methionine codon to a Cysteine codon. Effective comparative sequence analysis relies on the ability to separate these two factors: the exchangeability of amino acids and the mutational exchangeability of codons.

Prior work on this project resulted in the identification of systematic data on the effects of switching amino acids in proteins; the development of a statistical method for transforming the results from different studies to a common scale; the derivation of a measure called the “experimental exchangeability”, the mean effect $EX_{ij}$ of replacing amino acid i with amino acid j, based on data from 9671 experimental amino acid exchanges from 12 selected studies; and a test for evaluating the power of an exchangeability metric, which was used to show that the method for computing EX works. Also this year, the second phase of the project has begun, with two demonstration projects to assess the utility of EX in protein sequence analysis. ***The first project addresses the disease-causing effects of amino acid changes in human genes.*** For each type of amino acid change, the observed disease-causing propensity is computed from data on known disease mutants and SNPs (single-nucleotide polymorphisms). EX outperforms other possible metrics of severity, accounting for 46 % of the variance in the logarithm of disease-causing potential.

**The first phase of the project was completed this year, marked by the submission of a manuscript titled “Amino acid exchangeability from experimental data" *Genetics*, in review. In addition, EX matrix was made available to researchers in the field of protein sequence analysis.**

Future plans include more extensive modeling and analysis both of protein mutants and of evolution, and the modification of methods of sequence analysis to take advantage of an asymmetric measure of pure exchangeability with statistical weights. The evolutionary analysis will determine the relative contributions of mutational and selective factors in accounting for patterns in the evolution of proteins and of protein-coding genes. The analysis of mutants will apply simple models for predicting frequencies of disease-causing alleles, and will address the long-term independence of mutational and selective factors, as well as relate activity effects to thermodynamic effects on stability of mutants.



Amino Acid Exchangeability from Experimental Data

Lev Yampolsky and Arlin Stoltzfus

Data on Experimental Exchanges

| Protein | Ref | Source species | Method | Sites/ protein | Variants | Exchange effects assayed |
|---|---|---|---|---|---|---|
| LacI | 2 | E.coli | sup | 328/360 | 4038 | Operon repression, in vivo |
| Lysozyme | 3 | Phage T4 | sup | 155/164 | 1918 | Plaque size, in vivo |
| Interleukin-3 | 4 | H. sapiens | sat | 103/152 | 754 | Cell proliferation, in vivo |
| Barnase | 5 | E.coli | sat | 109/110 | 676 | RNAse activity, in vivo |
| β-Lactamase | 6 | Pseudomonas | sat | 27/246 | 513 | Ampicillin resistance, in vivo |
| RecA | 7 | E.coli | sat | 20/323 | 380 | Plaque size, in vivo |
| RTase | 8 | HIV | sat | 109/300 | 366 | RTase activity, in vivo |
| Protease | 9 | HIV | sat | 99/99 | 336 | Protease activity, in vivo |
| Protein V | 10 | Phage f1 | sat | 86/87 | 313 | Host inhibition, in vivo |
| Nuclease | 11-13 | Staphylococcus | scan | 143/149 | 290 | ΔΔG, in vitro |
| HGH | 14 | H. sapiens | scan | 50/191 | 50 | Dissociation constant, in vitro |
| Insulin | 15 | H. sapiens | scan | 37/51 | 37 | Receptor affinity, in vitro |
| Total | | | | | 9671 | |

EX, Experimental Exchangeability

Evaluation:

1. Predict results of target study with EX* computed from **other** studies
2. Compare predictive power of EX* to other available predictors.

Best predictor

| EX | EXS | BLOSUM | PAM250 | -lnMJup | 1/GRA |
|---|---|---|---|---|---|
| 1.6E-75 | 7.6E-47 | 2.2E-49 | 3.4E-37 | 3.9E-21 | 1.8E-06 |

## *Biological Macromolecule Crystallization Database*

***G.L. Gilliland, M. Tung, T.N. Bhat, and J.E. Ladner (831)***

***The BMCD was designed as a tool to assist x-ray crystallographers in the development of protocols to crystallize biological macromolecules, those that have previously been crystallized, and those that have not.***

The NIST/CARB Biological Macromolecule Crystallization Data (BMCD) contains crystal data and crystallization conditions for biological macromolecules. The BMCD includes the crystallization data for peptides, proteins, protein-protein complexes, nucleic acids, nucleic acid-nucleic acid complexes, protein-nucleic acid complexes, and viruses for which diffraction quality crystals have been obtained and reported in the literature. Besides collecting data from the literature, data can be acquired from other resources such as the Protein Data Bank (PDB). The mapping between BMCD data model and mmCIF (macromolecular Crystallographic Information File) representation of data of PDB entries resulted in a new and expanded data representation for the BMCD. This new representation of data gives a consistent way to carry out data exchange between the BMCD and other structural biology resources, and hence provides a new avenue for BMCD data acquisition. This new representation of data is not only compatible with the earlier BMCD data conventions, but also with the PDB data. The data model simplifies and reduced the number of many-to-many relationships making the database much easier for data management. For example, tables for depositors and auditing information have been added. With this new data model, data to be added to the BMCD can be extracted from the PDB mmCIF data set using a Star (CIF) Perl API.

**http://www.nist.gov/srd/biotech.htm**

This new data model for the BMCD has resulted in improved data management capabilities. In addition, data to be added to the BMCD can be extracted from the PDB mmCIF data set using a Star (CIF) Perl API. Since a significant fraction (15 % to 20 %) of the PDB contains comprehensive crystallization information, the BMCD can be significantly expanded using the new model.

**The PDB resource contains more than 18,000 crystals for which data can be extracted.**

Even tapping into the wealth of information in the PDB, much of the crystallization data required to reproduce the experiments will still be lacking from the majority of entries. The missing information includes the crystallization method, crystal growth temperature and pH, and chemical additives. Rather than collecting this information from the literature, which is very time consuming, a data mining approach using data clustering will be carried out. Data clustering will be based on the characterization of crystals and molecules, using such data items as unit cell parameters and macromolecular or protein properties. A new BMCD website will be created that will deliver the results of clustering rather than just the individual entry information, and will also allow more complex queries.

***Future Plans:*** **A new generation data model called a "Data Mart" will be employed with the BMCD website to provide new ways of analyzing and querying the underlying data.**

## *Predicting Vibrational Spectra of Dissolved Salts*

***L. Wang (831); K.K. Irikura (838)***

*Details provided in the* **Exploratory Research** *Section*

## *Structure to Function: Using a Structural Genomics Approach*

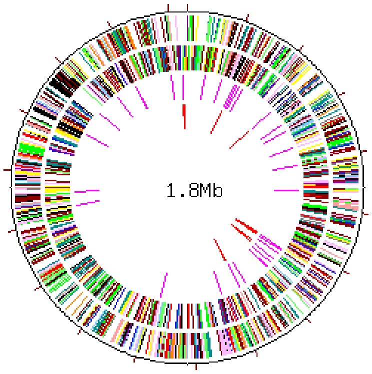
***G.L. Gilliland and J.E. Ladner (831); A. Teplyakov, G. Obmolova, O. Herzberg, J. Orban, and J. Moult (UMBI); A.J. Howard (Illinois Institute of Technology).***

With large-scale genomic sequencing efforts meeting with success, post-genomics research efforts are focusing on functional assignment of gene products. One approach is the use of structural data determined by crystallographic or NMR methods. In the work described here, CSTL researchers and collaborators have elucidated or are in the process of determining more than forty protein structures.

***Research involving the large-scale structure determination of hypothetical proteins from Haemopilus influenzae or homologs from other organisms has been underway to assess how structural information can influence functional assignment.***

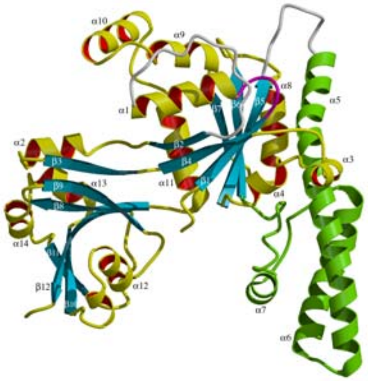
Many of the gene products of completely sequenced organisms are "hypothetical", that is they cannot be related to any previously characterized proteins, and so are of unknown structure and function. In an effort to address this issue, an interactive website, entitled "Structure 2 Function" was developed collaboratively by NIST, UMBI, and the Industrial Macromolecular Crystallography Association. Structure 2 Function (S2F) is a structural genomics project that aims to solve structures of these poorly characterized proteins by using X-ray crystallography and protein NMR techniques. More than 30 scientists participate in the compilation of this structural data, and are experts in NMR, protein purification, gene cloning, crystallization, bioinformatics and proteomics. The project status is updated daily.

**http://s2f.carb.nist.gov/**



**Schematic representation of the Haemophilus influenzae KW20 Rd genome.**

The initial set of targets was selected from the first completely sequenced bacterial genome of the *Haemophilus influenzae*. Structures of hypothetical proteins are being used to guide further studies and narrow the field of such studies for ultimately determining protein function.



**The folding pattern of the three-domain is shown as a ribbon representation of the polypeptide backbone of the polymer. The secondary structure elements, α-helices and β-strands, are indicated.**

As an example, a specific protein (YchF) from Haemophilus influenzae was cloned, expressed, and the crystal structure determined. The polypeptide chain is folded into three domains having a crab like structure. The crab-like three-domain structure suggests where the binding site for double-stranded nucleic acid is located. Fluorescence measurements have demonstrated the ability of this protein to bind a nucleic acid duplex, and have also shown the specific binding of GTP (guanosine 5'-triphosphate). Taken together with other experimental data and given the universal phyletic distribution of this family of proteins, these results suggest that YchF may be part of a ribonucleoprotein complex and may function as a GTP-dependent translation factor.

This project involving a structural genomics approach has demonstrated that working on a number of proteins in parallel, chosen on the basis of a unifying scientific theme, is a powerful strategy.

A large body of structural information has been obtained for hypothetical bacterial proteins while methods development to accomplish large-scale structure determination are on going. In addition to aiding in functional assignment, this effort is identifying new protein folds and a number of possible new targets for drug development.

***Future Plans:*** **The approach developed in this project will focus next on alternative splicing, a new and important scientific discovery, resulting from the analysis of the genomic scale sequence information on higher eukaryotes.**

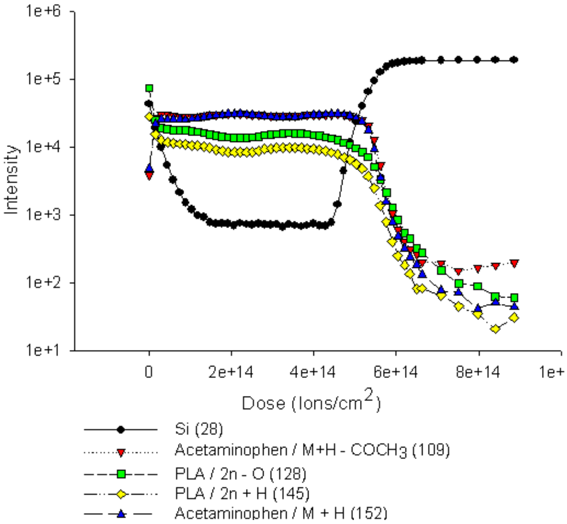
## *Cluster SIMS Depth Profiling of a Polymer Drug Delivery System*

***C.M. Mahoney, S. Roberson, and G.J. Gillen (837)***

***CSTL researchers developed a cluster SIMS technique to aid pharmaceutical companies in the development of new drug delivery systems, including complex controlled release formulations, which are highly dependent upon the homogeneity and diffusivity of the materials involved.***

Secondary Ion Mass Spectrometry (SIMS) has already proven to be a powerful tool in the spatial analysis of drug delivery materials. However, with the advent of cluster primary ion beams, such as $SF_5^+$, the ability to determine the distribution of a drug as a function of depth becomes feasible for the first time. With SIMS, the distribution of both drugs and excipients within a drug delivery system can be determined with a high degree of spatial resolution ($<1 \mu m$) and sensitivity (as low as ppm) when compared to other analytical methods (such as Raman or IR).

The behavior of a series of poly(lactic) acid (PLA) films doped with varying concentrations of the model drug 4-acetamidophenol, under $SF_5^+$ primary ion bombardment, was investigated in order to determine whether the distribution of the drug could be measured as a function of depth. The figure shows a typical depth profile of a PLA film containing 20 % by weight 4-acetamidophenol (≈ 200 nm) spun cast onto a Si wafer, and shows that the secondary ion intensities associated with both polymer and drug moieties remain stable with increasing $SF_5^+$ primary ion dose. These secondary ion intensities remain constant until reaching the PLA / Si interface, at which point both PLA and 4-acetamidophenol intensities drop, corresponding to an increase in Si secondary ion intensity. The polymer film / Si interface was surprisingly well defined (≈ 10 nm to 15 nm depth resolution) which may imply that sputter-induced topography formation was not a significant limitation. Several other biodegradable materials including polyglycolic acid, polylactic glycolic acid, polycaprolactone and polyethylene glycol were also depth profiled, with similar results to PLA. These results suggest that the common main chain structural unit of these polymers, R-CO-O-R, facilitates beam-induced bond cleavage and damage removal.



**SIMS depth profile of a PLA film containing 20 % by weight 4-acetamidophenol (≈ 200 nm) spun cast onto a Si wafer. The film was analyzed using $SF_5^+$ primary ion bombardment.**

Future plans involve utilizing the signal enhancement obtained with cluster primary ion beams for improvements in secondary ion imaging of drug delivery systems. Once this is achieved, we will explore the possibility for 3D molecular characterization of these systems. The utilization of SIMS for quantification of drugs in complex drug delivery matrices is also an important part of our future research plans. Collaborations with key pharmaceutical manufacturers will be initiated so that we can utilize the cluster SIMS capability for applied problem solving and to help with the characterization and development of new drug delivery materials.

**These results indicate that in these particular model systems, the distribution of the drug as a function of depth can be monitored when applying cluster SIMS.**

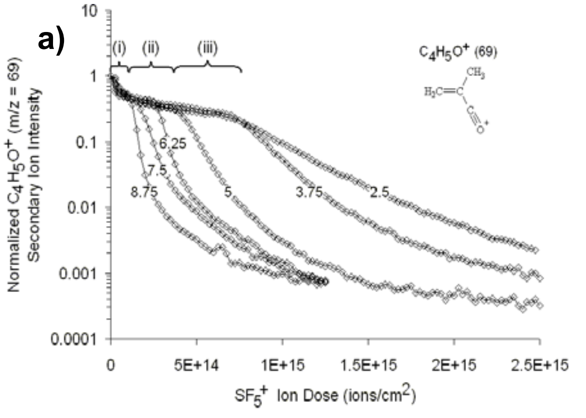
## *Impact Energy Dependence of $SF_5^+$ Ion Beam Damage of PMMA Films Studied using ToF-SIMS*

***M.S. Wagner (837)***

Recent advances in instrumentation for Secondary Ion Mass Spectrometry (SIMS) have focused on the application of polyatomic primary ion sources for enhancing the molecular secondary ion signals of organic materials. A few studies have also noted that some polymers, in particular poly(methylmethacrylate) (PMMA), display the unusual characteristic of maintaining their molecular ion signals after extended bombardment with polyatomic ion beams. In this study, the damage of ≈ 70 nm thick spin-cast films of PMMA by $SF_5^+$ with impact energies from 2.5 – 8.75 keV was studied using Time-of-Flight (ToF) SIMS. Sputtering of the polymer using $SF_5^+$ was alternated with collection of static positive and negative ion ToF-SIMS spectra to assess the damage created by $SF_5^+$ ion bombardment in the ion dose range of 2.5 x $10^{13}$ to 5 x $10^{14}$ $SF_5^+$ ions/cm$^2$. All beam energies displayed maintenance of the characteristic molecular ion signals for PMMA despite extended $SF_5^+$ bombardment. An increase in the $SF_5^+$ bombardment energy increased the sputter rate of the polymer without increasing the accumulation of beam-induced damage. Additionally, neither fluorocarbon cations nor anions were observed in the ToF-SIMS spectra of the damaged PMMA films.

***CSTL researchers studied the effect of $SF_5^+$ impact energy in the range of 2.5 keV to 8.75 keV on the damage of PMMA thin films.***

The figure below shows that as the $SF_5^+$ primary ion dose increased, the $C_4H_5O^+$ secondary ion intensity could be described by three regions. Region (i) is characterized by an initial intensity decrease followed by region (ii) that shows a quasi-stabilization of the secondary ion intensity. After this extended stable regime, region (iii) is characterized by a rapid decay in $C_4H_5O^+$ secondary ion intensity as the $Si^+$ secondary ion intensity increased, indicating that the polymer film had been sputtered through and the silicon substrate had been exposed. However, the slope



**The (a) $C_4H_5O^+$ (m/z = 69) and (b) $Si^+$ (m/z = 28) secondary ion intensity (normalized to its initial intensity for each damage profile) as a function of $SF_5^+$ ion dose for impact energies between 2.5 keV and 8.75 keV. The $SF_5^+$ impact energy is marked on each of the damage profiles. The $C_4H_5O^+$ secondary ion is characteristic for the PMMA film while the $Si^+$ secondary ion is characteristic for the silicon substrate. The three regions of the damage profiles are marked in (a) for the 5 keV $SF_5^+$ damage profile.**

of region (ii) in the damage profiles did not increase as a function of $SF_5^+$ impact energy, suggesting that changing the impact energy did not change the rate of damage accumulation in the polymer film. These results suggest that the highest impact energy available would be optimal for molecular depth profiling of polymer films.

The measurements reported here have demonstrated that the degree of damage accumulated by the PMMA film was not affected by the $SF_5^+$ impact energy. However, increasing impact energy increased the sputter rate. These results have provided optimized conditions for depth profiling of polymer samples, with the anticipation of aiding in the development of polymeric biomaterials and drug delivery vehicles.

# 4. Chemical and Allied Products

CSTL provides reference data, theoretical models, and artifact standards that are important to the chemical industry. These standards are used for process design and quality control, and help ensure traceability of field measurements to reliable, world-recognized standards.

**Often referred to as the keystone industry because nearly every sector of the manufacturing economy uses its products, the U.S. chemical industry ranks among the world's leading industries, accounting for an estimated one-quarter of total world chemical production.**

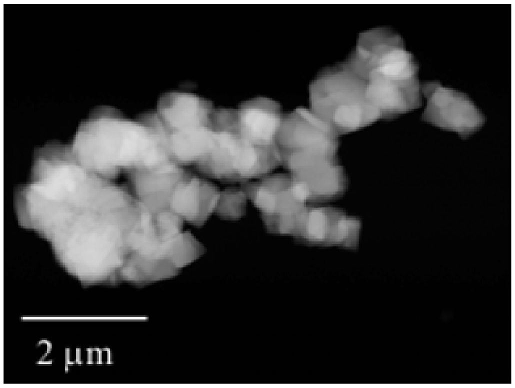
## *Zeolite Reference Materials: Chemical Properties, Structural Parameters, and Particle Size Distribution*

***S. Turner, R.A. Fletcher, E.S. Windsor, and R.R. Cavanagh (837); J.R. Sieber, T.W. Vetter, R.L. Zeisler, and D.A. Becker (839); B.H. Toby and J. Kelly (MSEL); S.D. Leigh (ITL); M. Davis (Caltech); S. Yang and A. Navrotsky (Univ. California at Davis)***

***The need for NIST–characterized zeolite reference materials was the consensus of an NSF-sponsored meeting that included representatives from academia and industry.***

Zeolites encompass a large class of hydrated silicate materials that have structures containing channels and cages of various dimensions. Both synthetic and natural zeolites have widespread industrial applications in petroleum refining, chemical manufacturing, gas separation and dehydration, environmental cleanup, and as ingredients in detergents. Despite their widespread use over the past three to four decades, no materials with characterized physical parameters have been available for intercomparison studies.

Three zeolites were studied: faujasite, Linde Type A, and ZSM-5. To deal with their hydroscopic nature and its effect on chemical and structural characterization, materials were handled in a humidity-controlled environment with minimal exposure to ambient environmental conditions. Major elements were determined by x-ray fluorescence analysis, gravimetry, and instrumental neutron activation analysis (INAA), while trace element concentrations were determined by INAA. Structural parameters were characterized using synchroton x-ray diffraction and neutron diffraction. Particle size distributions were characterized by optical and sedimentation techniques.



**Dark field scanning transmission electron microscopy image of faujasite zeolite.**

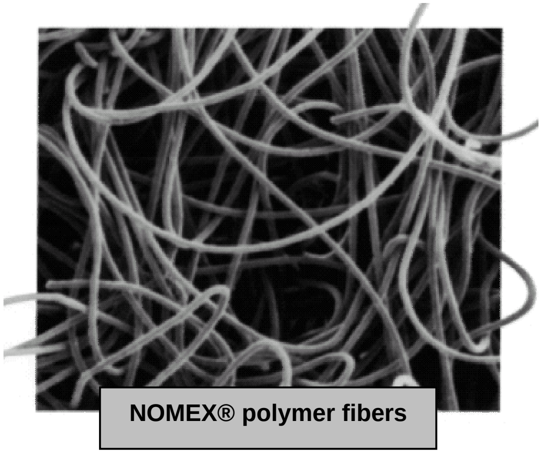
Reference and information values related to the chemistry, structure and particle size distributions have been obtained. The materials will provide a basis for an intercomparison of zeolite measurements between analytical laboratories. They will also be available to customers as NIST Standard Reference Materials.

# *Characterization of Spatial Variations in Nomex® Fiber Crystallinity with Confocal Raman Microscopy*

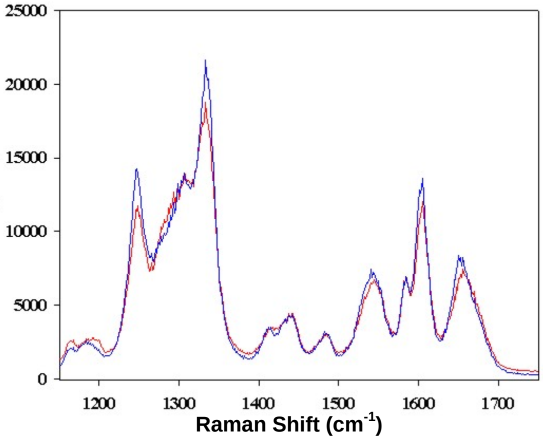
***C.A. Michaels, D.B. Klinedinst, E.S. Windsor (837); and D.B. Chase (DuPont Co.)***

***CSTL scientists used Confocal Raman microscopy to image the variation in crystallinity for DuPont Nomex® fibers used for protective clothing.***

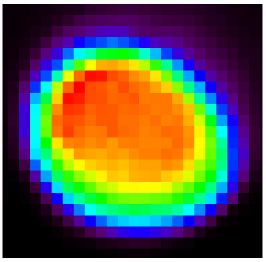
Confocal Raman microscopy has been used to study spatial variations in crystallinity for spun Nomex® polymer fibers provided by collaborators at DuPont. The spinning process is thought to yield a "skin" region on the outside of the fiber with crystallinity differing from that of the bulk. Because of its impact on the material performance properties there is significant interest in probing the skin properties as a function of processing parameters. The subtle dependence of the Raman spectra of this material on crystallinity is exploited to allow imaging of crystallinity changes across polished fiber cross sections. Images acquired with 1 μm$^2$ lateral resolution reveal no detectable skin, thus setting an upper limit on its size for the fibers studied. Ongoing efforts will focus on higher resolution imaging of these materials and those prepared under varying processing conditions. These aramid fibers are widely used in materials for their fire retardant properties, in applications ranging from protective apparel for firefighters to composite aircraft parts. The degree of crystallinity of these fibers has a significant effect on aspects of material performance including mechanical strength, chemical resistance and dyeability, and can be modified through variations in processing conditions. It has been shown that changes in the fiber crystallinity result in subtle changes in the Raman spectra, thus providing a non-destructive means for measuring this property with spatial resolution of approximately 1 μm. Raman imaging of the fiber skin requires a sample preparation method that allows measurement of the fiber cross section. Several approaches have been used, including polishing of epoxy mounted fibers and microtome sectioning of epoxy mounted fibers. Analysis shows that the epoxy matrix yields no significant Raman signal and thus does not interfere with the measurement. As configured, the microscope probes a lateral sample area of 1 μm$^2$ with a sampling depth approximately 5 μm. Careful analysis of the variation of aramid band intensities across this fiber cross-section yields no evidence for the existence of the postulated skin. This measurement does not preclude the presence of a skin smaller than the probe volume; however it does set an upper limit on its size for this type of fiber. Measurements aimed at refining this limit are ongoing and are also being extended to fibers prepared under different processing condition.



**NOMEX® polymer fibers**



**Normalized Raman spectra of high (blue) and low (red) crystalline content Nomex® fiber acquired with 785 nm excitation.**



**32 x 32 μm Raman image of a polished Nomex® fiber cross section mounted in an epoxy matrix plotted on a color scale ranging from black (minimum intensity) to red (maximum intensity)**

## *Advanced Experimental Capabilities for Fluid Properties Measurements*

***R.A. Perkins, A. Laesecke, J. Magee, M. McLinden, S. Outcalt, and J. Scott (838); S. Altintas (PREP Postdoc); A. Abdulagatov (Russian Academy of Sciences); C. Lösch (Rubotherm)***

***Advances in measurement technology enable new and improved measurements of the thermophysical properties of gases, liquids, and supercritical fluids.***

The need for measurement standards as well as data needs of the chemical, energy, refrigeration, and aerospace industries continues to drive the development of new measurement devices for the thermophysical properties of fluids. Unique apparatus are under development in CSTL for improved measurements of density, sound speed, critical points, and phase equilibrium. In addition to high accuracy, the goals in this effort are for rapid measurement, a small sample size for rare and/or hazardous substances, and materials for construction that will withstand corrosive fluids.

***Density*. A two-sinker densimeter (photo at left) demonstrated a reproducibility of 0.0002 kg m-3, representing <1 part per million for liquids and about 20 parts per million for gases at 1 MPa.**
***Critical Points*. Under development is a short-residence-time flow apparatus to rapidly measure the critical temperature up to 1000 K and pressure up to 10 MPa for small fluid samples with an uncertainty of 0.08 K and 0.01 MPa.**
***Sound Speed*. A cylindrical sound speed apparatus, based on the pulse-echo technique, has been developed for liquid measurements from 90 K to 310 K. Tests on liquid propane verified that the uncertainty in sound speed is less than 0.05 %.**
***Phase Equilibria*. A new apparatus was demonstrated using propane to measure the phase equilibrium where multiple liquid phases coexist with the vapor phase. This apparatus operates from 200 K to 400 K and utilizes a moveable capillary line to sample each phase.**

An apparatus developed along these lines has been used to establish several *de-facto* standards for the thermophysical properties of fluids. These standards allow the calibration and performance verification of devices used to measure the thermophysical properties of fluids. Data measured with these apparatus along with evaluated literature data are used in NIST databases such as REFPROP, which provides convenient access to the most accurate property data available.

**Sinkers and magnetic suspension coupling at the heart of the densimeter. The electromagnet at the top hangs from a balance and levitates the permanent magnet below it. The permanent magnet and sinkers are normally contained within a pressure vessel. The two sinkers, a balance, the magnetic suspension coupling, and a mechanism to pick up each sinker constitute the density measuring system.**

## *International Standards for Refrigerant Properties*

***M.O. McLinden and E.W. Lemmon (838)***

Knowledge of the thermophysical properties of any fluid in a thermodynamic cycle is required to evaluate the energy efficiency, capacity, etc. Standards exist for determining and reporting the performance of air-conditioning systems at standard ratings conditions. However, differences among multiple property formulations lead to different performance ratings. This is especially problematic in international trade involving refrigerants and was the prime motivation for ISO to develop a standard for refrigerant properties.

***A new ISO standard for the thermodynamic properties of 14 key refrigerants (10 pure fluids and four refrigerant blends) has been completed and is currently in review by the international community.***

The new ISO Standard, entitled "Refrigerant Properties," has progressed to the status of a Draft International Standard in just over three years. The ISO Working Group first had to decide on the scope of the Standard (including which properties and which fluids to include). The group then had to determine how to define the properties (*e.g.* in terms of experimental data, published equations of state or other models, or some sanctioned computer package). NIST staff were the lead authors of the Standard as well as key technical experts in the Working Group (WG) discussions. The widespread use of the NIST REFPROP database was acknowledged in the WG discussions.

**This new Standard, if accepted, will define the properties used by the air-conditioning and refrigeration industry for the performance rating of their equipment. It will remove the long-standing potential for contention and confusion arising from different manufacturers using different property formulations.**

The properties for the refrigerants R12, R22, R32, R123, R125, R134a, R143a, R152a, R717 (ammonia), and R744 (carbon dioxide) and the refrigerant blends R404A, R407C, R410A, and R507A were felt to be sufficiently well known to be included. Older refrigerants, such as the CFC R12, were included for comparison with the newer HFCs, such as R134a. All of the key thermodynamic properties, in single-phase states and along the saturation boundary, are included. The Standard defines the properties with tables and by defining the equations used to generate those tables. The equations of state and mixture model are from the published literature, and, for completeness, all numerical coefficients are also given.

ISO International Organization for Standardization

Although the Standard does not specify any particular computer package, the models specified in the Standard are the same ones used in REFPROP. Thus, REFPROP will be compliant with the new standard; this will elevate the current status of REFPROP as a *de facto* standard to one with a true international sanction. This is a fitting acknowledgment of the 15 years of effort, which NIST has devoted to the properties of the "alternative" refrigerants.

### *The Industrial Fluid Property Simulation Challenge*

***R. Mountain, A. Chaka, R. Johnson, D. Friend, J. Magee, L. Watts, T. Bruno, R. Chirico, A. Laesecke, E. Lemmon, and M. McLinden (838); J. Olson, J. Moore, and D. Frurip (Dow Chemical); M. Schiller (Dupont); J. Golab (BP); F. Case (Colgate-Palmolive); R. Ross (3M); P. Kolar (Mitsubishi Chemical)***

The demand for accurate physical property data far exceeds our capacity to supply it experimentally. This is especially true for unusual, novel, or toxic materials and mixtures, or state points far from ambient where it can be challenging or expensive to obtain experimental data in a timely manner.

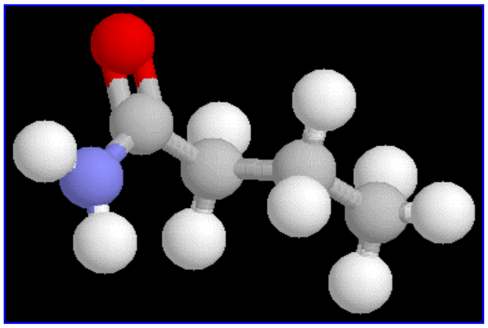
**Accurate physical property data in chemical product and process design are often inaccessible from experiments. Important data include the solubility of oxygen in fluids at elevated temperatures or pressures and the viscosity and thermal conductivity of fluids at elevated pressures.**

Molecular simulations hold great promise to meet this growing need for property data. Methods based on molecular dynamics and Monte Carlo algorithms in particular have substantial potential for predicting thermal and transport properties of dense fluids. It is not clear, however, that this approach is suitably mature for industrial applications. The First Industrial Fluid Properties

Simulation Challenge was organized by NIST and collaborators from industry (Dow Chemical, DuPont, Colgate-Palmolive, and BP) to assess the capabilities of various simulation techniques. The challenge was sponsored by a committee of the American Institute of Chemical Engineers to predict certain liquid properties for specified conditions.

***The Industrial Fluid Properties Simulation Challenges are organized by NIST and several companies (Dow Chemical, DuPont, Colgate-Palmolive, BP, and now 3M and Mitsubishi Chemical) to assess the current capability of molecular simulations to make useful predictions.***

The First Challenge revealed that existing simulation methods for predicting the shear viscosity may be too inefficient, prompting an interest in improving sampling methods. Detailed reports on the first Challenge will be published in a special issue of Fluid Phase Equilibria. The Second Challenge was opened in September 2003 and will run until September 2004. The challenge has generated interest and support in academia and industrial R&D for improving force laws and for improving the efficiency of simulation sampling methods.



***Topics for the Second Challenge:***

- **Vapor pressure and heat of vaporization for acetone and butyramide (shown in figure) at elevated temperatures**
- **Henry's Law constants for gases in ethanol at elevated temperatures**
- **Heat of mixing for butylamine with heptane**

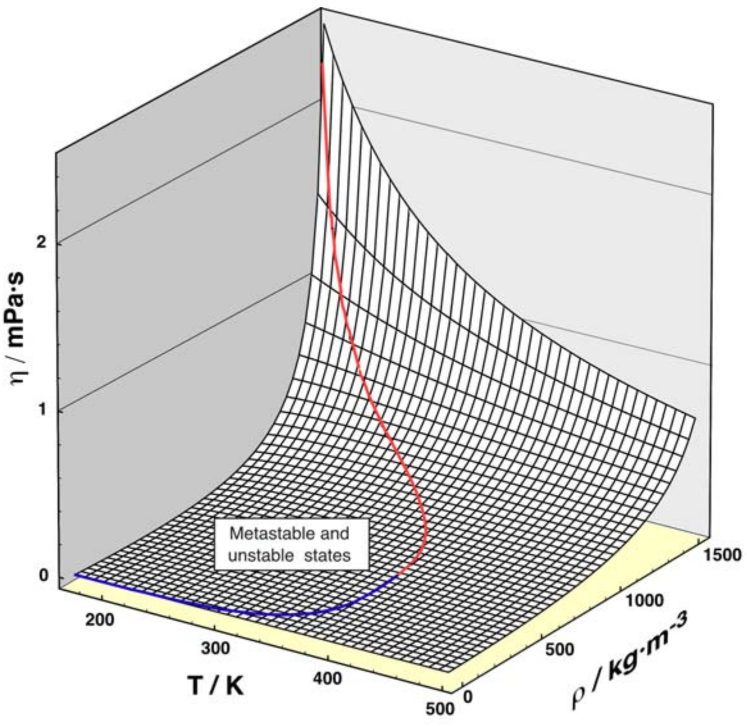
**http://www.cstl.nist.gov/FluidSimulationChallenge**

## *Transport Property Models for Refrigerants*
### *M.L. Huber, A. Laesecke, and R. Perkins (838)*

Transport properties, such as viscosity and thermal conductivity, are important in the design of chemical processing equipment for various industrial sectors including the petroleum refining, transportation, energy, and refrigeration. The NIST computer program REFPROP (NIST REFerence fluid PROPerties) has become the global *de-facto* database standard for modeling refrigerant properties. We have developed new models and correlations that improve the representation of the viscosity and thermal conductivity surfaces for refrigerant fluids and have coded them into REFPROP.

***A combined correlational/predictive model was used to predict transport properties for the many fluids of industrial importance that lack wide-ranging property data. We then coded the models in REFPROP, the NIST computer program and database for thermodymamic and transport properties of fluids.***

The method of extended corresponding states (ECS) was the modeling approach that proved successful in providing viscosity and thermal conductivities for fluids that lack wide-ranging and accurate data. This modeling method applies a technique in which one obtains estimates of the properties of a fluid of interest by mapping onto the properties of a well-characterized fluid known as the "reference fluid". We developed a high-accuracy correlation for the viscosity surface of R134a (figure at right with viscosity along z-axis), an important fluid used in many applications including automobile air conditioning units, supermarket display cases, and in health care, as a propellant for asthma inhalers. R134a is also important as a reference fluid for ECS models to predict properties of similar, but less well-characterized halocarbon fluids. The new surface represents the viscosity to within 2 % along the liquid saturation boundary and 0.5 % in the dilute vapor region. (The blue/red line in the figure indicates the transition from vapor (blue) to liquid (red) at the critical point.) We also developed a high-accuracy correlation for the viscosity surface of R125, another important refrigerant fluid that is a component of refrigerant blends such as R407C and R410A that may be used as replacements for R22. We then applied the ECS model to 17 additional pure halocarbon fluids, and developed improved surfaces for calculating the viscosity and the thermal conductivity of these fluids.

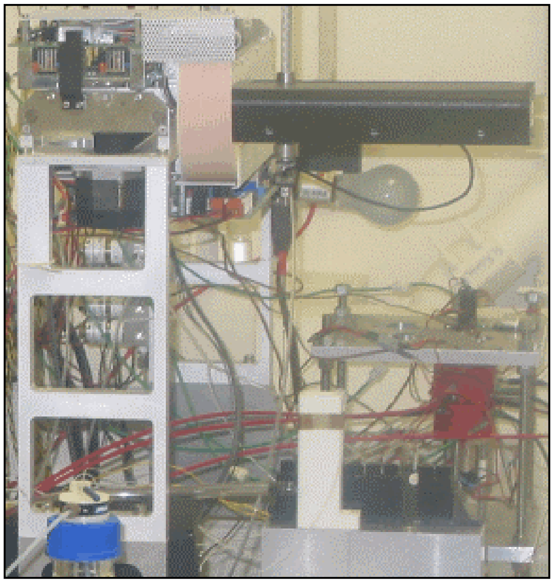


## *High-Throughput Liquid Transport Properties Measurements in Membranes and Films*

***C. Muzny, S. Outcalt, J. Scott, and L. Watts (838); R. Hedden (854)***

Measurements of diffusion and sorption for specific liquids and gases in thin layer films are important for characterizing membranes and films according to their transport properties. These measurements provide a means to delineate design criteria for mass separating agents such as membranes and polymeric adsorbents by compiling the effects on transport behavior from both chemical and structural subgroups in the material.

***Although polymeric and inorganic materials are used in membrane and adsorptive separation processes, the lack of predictive capabilities for the transport properties of mixtures in any selected material is a significant barrier to the optimum use of existing materials and development of new materials.***



A major product of this project is an automated high-throughput apparatus for measuring the diffusivity and solubility of a variety of test components in a wide variety of membranes. The technique employs sixteen test cells that are temperature controlled individually and that can be loaded with different solvents, test solutions, and membranes. The figure below shows the robotic system for the apparatus.

A critical component of this method is to have easily detectable, standard test compounds that probe different physical and chemical characteristics of the membranes. The testing of this instrument using standard test fluorophores has continued. The molecular diffusivity of G4 PAMAM dendrimers that are used as test fluorophores was characterized via fluorescence correlation spectroscopy. This novel technique provides the fundamental measurement needed for membrane transport characterization of a molecular species. With the instrument operating in a continuous test and data production mode, numerous operational problems were identified. One issue, fluorescence stability, was largely addressed by testing a range of buffer solutions in order to control the pH. Automation issues have also been solved, and the instrument has now been operating stably for periods of up to one month. Final launch of the web-based database for membrane transport data produced by this instrument is the next major goal.

**The availability of data and predictive methods from this effort will impact industries that use membrane-based separations including water purification, pharmaceuticals, and chemical manufacturing.**

### *Measurements, Modeling, and Data for Pressure-Driven Membrane Separations*

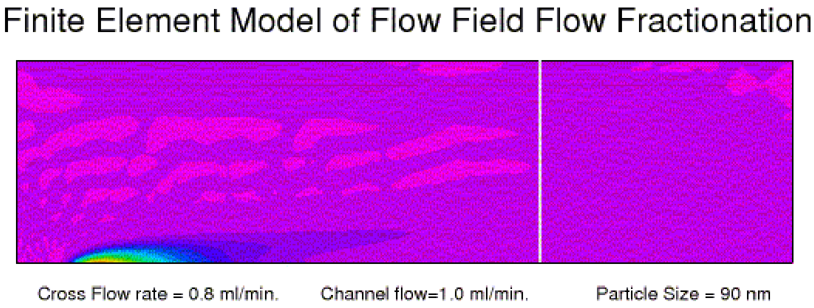
***C. Muzny, H. Sun, and J. Moon (838); J. Cho (Univ. of Colorado); M. Chapman (U.S. Bureau of Reclamation); and J. Ranville (Colorado School of Mines)***

The accepted physical property used industrially to characterize membranes is the pore size distribution or molecular weight cutoff. Since the concept of pore size distribution has no industrial standard, we have developed a set of standard membranes with highly regular pore structures for use in the development of standard membrane characterization methods. These are inorganic membranes with pores in the 10 nm to 100 nm size range and with very narrow pore diameter distributions.

***The ultimate goal of this work is to provide a standard test mixture for testing membrane fouling along with standard procedures for evaluating membrane performance.***

**The standard test solutions and procedures resulting from this work will provide industry with a uniform method for pressure-driven membrane separation performance testing.**

The liquid-liquid porosimetry apparatus for physical characterization of membranes that we previously developed has been used as the principle characterization method for these standard membranes, although field-emission scanning electron microscopy has also been used for pore size verification. Membrane testing has also included a fouling study using uniformly sized polystyrene beads as a model foulant. By looking at both monodisperse and polydisperse foulant solutions, we have been able to test the validity of a standard fouling model on particles in the 10 nm to 100 nm size range. Characterization of these fouling solutions has also included field flow fractionation, and correlations between the results of the field flow fractionation and flux reduction continue to be studied. As a final test of the overall goal of providing standards for pressure driven membrane performance testing, a small-scale membrane module test bed is now in service and being used to test the performance of spiral wound, reverse osmosis modules under the standard conditions.



**The flow field flow fractionation technique is being used as an idealized system for the modeling of fouling during pressure driven membrane separations.**

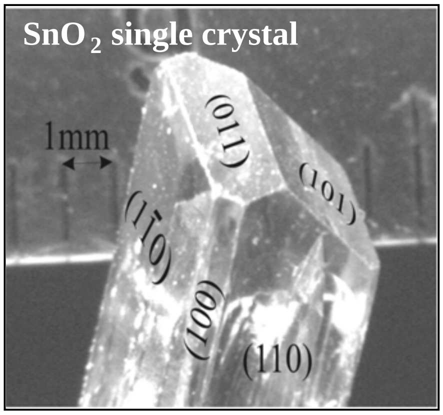
## *Surface Oxygen Chemistry of the Gas-Sensing Material $SnO_2$ (101)*

### *A.M. Chaka (838); U. Diebold, and M. Batzill (Tulane University)*

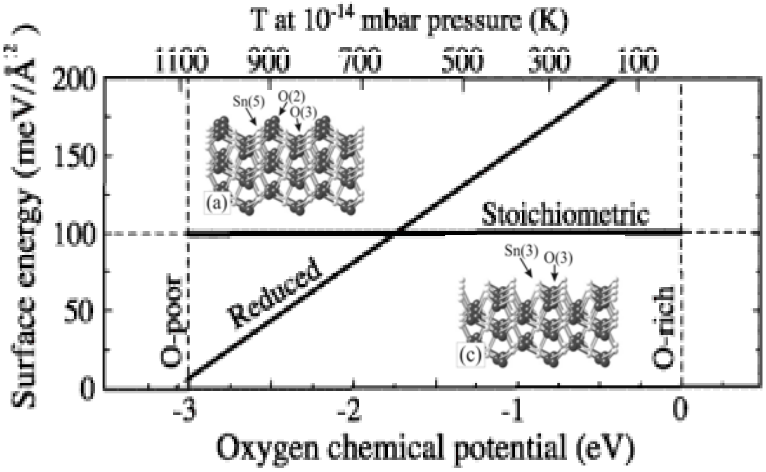
Stannic oxide ($SnO_2$) is a widely used material for gas-sensing devices that are based on metal-oxides. As an oxidation catalyst, $SnO_2$ is sensitive to the adsorption of oxidizing and reducing gases. A change in the electrical resistance of polycrystalline $SnO_2$ films occurs if the composition of the ambient gas changes. Improving the sensitivity and chemical differentiability of metal oxide sensors has been difficult due to the complexity of the polycrystalline films and poor understanding of the mechanisms responsible for performance.

***Understanding the fundamental mechanisms of gas sensors at the atomic level can lead to the design of more sensitive and robust sensors with greater chemical differentiation.***

It is not known which crystalline faces of polycrystalling $SnO_2$ are responsible for sensor activity, nor what the mechanisms are. During the past year experimental observations a $SnO_2$ single crystal using various microscopic and spectroscopic techniques (e.g., scanning tunneling microscopy and low energy loss diffraction) have identified the (101) surface as one which undergoes significant changes at elevated temperatures in the presence of low $O_2$ pressure. The crystalline surface changes dramatically yet reversibly when the $O_2$ partial pressure is varied around $10^{-12}$ Pa while heating from 550 K to 680 K.



First principles modeling identified a facile oxidation/reduction reaction as oxygen occupied or vacated bridging sites on the surface. The stoichiometric $Sn^{4+}O_2$ surface is stable only at high oxygen chemical potential. As $pO_2$ is decreased below a threshold pressure, a $Sn^{2+}O$ bulk termination becomes the most favored thermodynamically (figure below). These two surfaces interconvert without reconstruction by occupying and vacating bridging oxygen sites. The calculated differences in free energy between the two surfaces predict a temperature threshold of 680 K at which the interconversion occurs, which is consistent with experimental results. This variability of the surface composition is possible because of the dual valency of Sn ($Sn^{2+}$ or $Sn^{4+}$) and is essential for the application of this material in gas-sensing devices.



Future plans call for calculating the band structure and density of states at the material's surface. These calculations will link the differences in surface stoichiometry and oxidation state with observed changes in conductivity as a function of oxygen partial pressure and temperature. Additional crystal faces will be investigated ((110), (100) and (001)), as well as adsorption of CO and NO for sensor application.

## *The NIST WebBook* and the IUPAC/NIST Chemical Identifier - Standards and Delivery Mechanisms for NIST Chemical Reference Data*

***P. Linstrom, D. Tchekhovskoi, S. Stein, and G. Mallard (838)***

The NIST Chemistry WebBook remains one of the most used resources for chemical and physical property data. Usage has continued to grow at about 10 % to 20 % per year, currently over 650,000 distinct IP addresses access the WebBook per year. We continue to see a very high rate of return users indicating a level of satisfaction with the WebBook. Usage continues to grow for all of the components of the WebBook.

***Committees of IUPAC (Steve Stein, Chairman) and ASTM (Peter Linstrom, pivotal member) have pursued INChI and the web-based standards for chemical information communication.***

**Finding the enormous amount of thermochemical data (heats of formation, entropies, heat capacities, heats of reaction) as well as thermophysical property data (vapor pressure, viscosity, boiling points, melting points, etc) that are available to the technical community is time consuming and expensive.**

A major part of this project is to find and evaluate thermochemical and thermophysical property data - with a major emphasis in the evaluation process being the deployment of automated tools for that evaluation. The second part is to make this and other data such as infrared (IR), ultraviolet (UV) and mass spectra available and easy to access. The identification and communication of this information has required the development, testing and implementation of algorithms for extracting a unique chemical identifier from the chemical structure.

More effort has gone into acquiring data and a major donation of infrared spectral data from the Dow Chemical Company was received this year. In addition, the work on the IUPAC/NIST Chemical Identifier (INChI) has continued. CSTL scientists continue to work on the INChI along with data-exchange standards for chemical data, which is an essential part of making the WebBook a leading part of the next generation of electronic chemical information.

During 2003 the eighth edition of the NIST Chemistry WebBook* was released. In addition to an enhanced profile of prediction tools, a major increase in the infrared spectra was made with the release of the digitized Coblentz Society infrared spectral data. This data had only been available in printed form and the release of the data in fully digitized electronic form represents a major addition to the resources of the WebBook. Perhaps as a result of the increase in IR data, the IR usage increased more than the overall usage. The finalization of the donation of over 50,000 IR spectra by The Dow Chemical Company to NIST culminated a multiyear effort. Historically the large collections of data that are present in the chemical company records have not been available to the public. The decision by Dow to donate the data and the decision to make it available through NIST and the WebBook represent a major vote of confidence for the WebBook.

***http://webbook.nist.gov/chemistry**

## *Predicting Vibrational Spectra of Dissolved Salts*

***L. Wang (831) and K. K. Irikura (838)***

Details provided in the ***Exploratory Research*** Section.

## *Thermophysical Properties and a Data Retrieval System for Ionic Liquids*

***J. W. Magee, M. Frenkel, R. D. Chirico, Q. Dong, A. Laesecke, M. O. McLinden, R. A. Perkins, J. A. Widegren, D. G. Archer (838); and G. J. Kabo (Belarusian State U., Belarus)***

***Ionic liquids have been proposed as solvents for Green Processing. To provide U.S. industry with the knowledge base to exploit these solvents, we have begun to study the physical properties of ionic liquids, and to develop both standardized systems and a comprehensive data retrieval system.***

Ionic liquids are a class of organic salts that are liquid at or near room temperature. They are generally composed of a large asymmetric organic cation and either organic or inorganic anion. As such, they are non-volatile and non-flammable. Therefore, they have been proposed as solvents for *Green Processing*. The physical properties of ionic liquids may be tuned, for example by altering the hydrophobicity, and this affects solubility, reaction rate, and selectivity. In spite of the advantages, fundamental data on their physical and chemical properties are scarce and a knowledge base of standardized systems is needed that would lead to a comprehensive database for U.S. industry to exploit these solvents.

**CSTL has played a key role in recently approved projects under the auspices of the International Union of Pure and Applied Chemistry (IUPAC) that will provide recommendations of standardized systems for data and will develop a comprehensive database for ionic liquids.**

To achieve these goals and reach an international consensus, two IUPAC projects have been initiated with CSTL involvement. The first project, *Thermodynamics of Ionic Liquids, Ionic Liquid Mixtures, and the Development of Standardized Systems* (2002-005-1-100), addresses the concern that currently there are no internationally adopted standards for reporting thermodynamic data for ionic liquids. To address this issue, a standardization mechanism for reporting thermodynamic data for ionic liquids could be accomplished by an expansion of the Guided Data Capture software developed by the NIST Thermodynamics Research Center. These issues were discussed by members of the project's task group, which included CSTL staff, at a workshop of the 17th IUPAC Conference on Chemical Thermodynamics (Rostock, Germany). Outcomes of this workshop were reported in a recent issue of the *Journal of Chemical and Engineering Data*. The second IUPAC project, *Ionic Liquids Database* (2003-020-2-100), presents a vision for a distributed-access data retrieval system for ionic liquids and their mixtures that encompasses chemical structures, solvent properties, ionic liquids in synthesis, reviews, reactions and catalysis, manufacturer information, benchmark properties and models, and thermophysical and thermochemical data.

In addition to the IUPAC projects, NIST continues to provide benchmark physical properties measurements for selected ionic liquids. For the archetypal ionic liquid, 1-butyl-3-methylimidazolium hexafluorophosphate, we published the first reports of thermodynamic properties for the ideal state at temperatures to 1500 K and high-accuracy thermodynamic properties in condensed states (crystal, glass and liquid) covering a range of temperatures from 5 to 550 K.

### Publications

- **Preface to Special Section: Papers Presented at the Workshop on Ionic Liquids, ICCT, Rostock, Germany, July 28 to August 2, 2002. J. W. Magee, *J. Chem. Eng. Data 2003, 48,* 445.**
- **Thermodynamic Properties of 1-Butyl-3-methylimidazolium Hexafluorophosphate in the Ideal Gas State. Y.U. Paulechka, G.J. Kabo, A.V. Blokhin, O.A Vydrov, J.W. Magee, and M. Frenkel, *J. Chem. Eng. Data 2003, 48,* 457-462.**
- **Thermodynamic Properties of 1-Butyl-3-methylimidazolium Hexafluorophosphate in the Condensed State, G. J. Kabo, A. V. Blohkin, Y. U. Paulechka, A. G. Kabo, M. P. Shymanovich, J. W. Magee, *J. Chem. Eng. Data 2004, in press.***

## *Evaluated Molecular Structures and Vibrational Frequencies for C1-C2 Chlorocarbons*

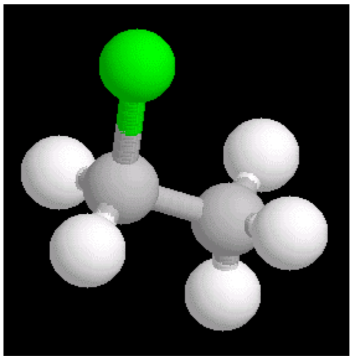
***D.R. Burgess, Jr. and J.A. Manion (838)***

***This work is a compilation of gas-phase molecular geometry and vibrational frequency data for all industrially important $C_1$ and $C_2$ chlorocarbons, including radicals.***

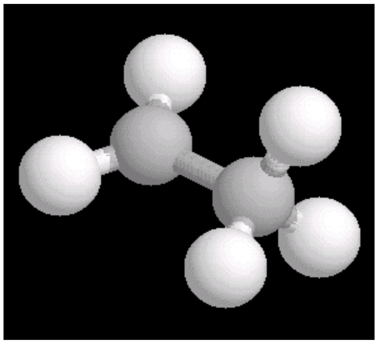
Chlorinated hydrocarbons are used throughout the chemical industry, both as end products and as base chemicals in the production of refrigerants, plastics, pesticides, solvents, and other products. Reliable molecular property data are needed to derive accurate thermodynamic information for use in models of industrial production, to better understand the fates of chlorinated compounds in the environment, and to improve waste destruction methodologies.

- **Models for predicting the thermal decomposition of chlorinated hydrocarbons in waste destruction are very dependent on accurate thermochemical functions for stable and metastable chlorinated hydrocarbon species.**
- **Thermochemical properties such as the equilibrium constant for formation strongly depend upon specific molecular properties such as the molecular vibrational frequencies.**

We recently reviewed the experimental information on the enthalpies of formation of the stable $C_1$ and $C_2$ closed-shell chlorinated hydrocarbons. Presently, molecular geometry and vibrational frequency data for $C_1$ and $C_2$ chlorocarbons have been compiled and evaluated. The available experimental data were supplemented with *ab initio* and density functional theory calculations both from the literature and from our own work. Comparisons between experimental and calculated data were made to verify the experimental data, to validate the calculated values, and to provide theoretical values in the absence of experimental data. We have attempted to correct the theoretical geometrical parameters and vibrational frequencies for systematic differences by benchmarking the calculations using similar molecules with reliable experimental data. Based on the evaluated experimental data, supplemented with those from calculations, recommended values for the molecular structure and vibrational frequencies (including torsion and inversion modes) are given for each of the molecules.



The $C_1$ chlorocarbons include the chlorine-substituted methanes, methyl radicals, methylenes, and the chloromethylidyne radical. The $C_2$ chlorocarbons include the chlorine-substituted ethanes (figure left), ethyl radicals (figure right), ethenes, ethenyl radicals, ethynes, and the chloroethynyl radical. We also include data for the unsubstituted, reference compounds (*e.g.*, methane, methyl radical, ethane, ethyl radical).

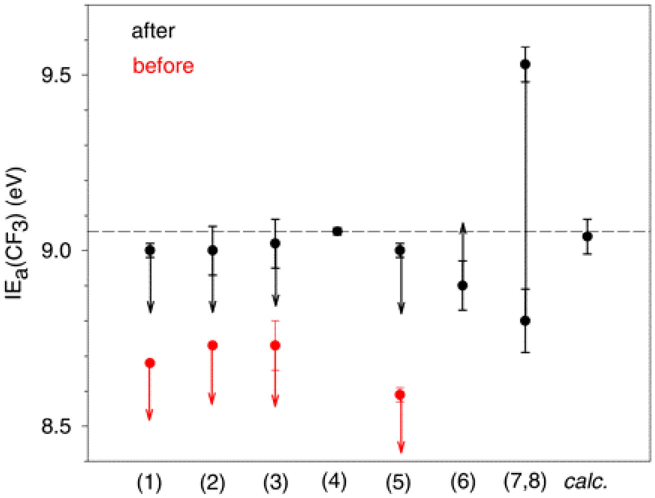


## *Thermodynamic Interpretation of Low-Pressure Ion-Molecule Reactions*

***K K. Irikura (838)***

***Thermochemical data are used to determine the feasibility and the thermal requirements of chemical reactions. Part of the quality assurance for thermodynamic data compilations such as the NIST WebBook involves understanding the proper procedures for analyzing experimental data.***

Gas-phase studies of reactions between ions and molecules have been a rich source of thermochemical data for more than 30 years. Indeed, they are a major part of the NIST Chemistry WebBook. Surprisingly, there are two competing procedures for extracting thermodynamic data from the experimental observations. However, only one of these procedures can be correct. In practice, the two procedures usually give similar results, so the fundamental confusion in interpretation has been neglected. However, for some types of reaction (those with large entropy changes) the two methods of analysis give widely different results.



The procedures for extracting thermochemical data are based on an interpretation of enthalpy change or free energy change. In both reactions above, the "enthalpy" interpretation infers that DH is less than or equal to zero, while the "free energy" interpretation infers that DH is less than or equal to zero. From the Gibbs equation, results in the thermochemistry differ by TDS, which is large when DS is large. The figure below shows the inferred ionization energy (y-axis) of CF3 from Eq. 1. The red experimental points (before) were obtained using the "enthalpy" interpretation. The points themselves are limiting values from experiments, whereby other values would be in the direction of the arrows and, hence, tend to be smaller. If they are later interpreted by the "free energy" method (after), the red limiting data points move upward (corresponding black limiting points) and become consistent with other experimental and theoretical results.

This work (*J. Am. Chem. Soc., 121,* 7689-7695, 1999) has stimulated at least one additional experimental study and an opposing opinion. To reply, in preparation is an additional publication with additional evidence and arguments in favor of one interpretation.

## *Evaluation of Free Radical Standard Potentials*

***P. Neta and R. E. Huie (838)***

The standard electrode potential of a substance is a fundamental thermodynamic quantity that represents the tendency for a molecule to gain or lose electrons. It is widely used for predicting the course of chemical reactions. Although standard electrode potentials have been extensively evaluated and tabulated for stable species, the situation is much less satisfactory for reactive intermediates such as free radicals. The explosive growth in biochemical research exploring one-electron oxidations and reductions highlights the need for further evaluation of standard potentials for free radicals. Toward this effort, an IUPAC Task Group that included CSTL representatives was formed with the objectives of updating the 1989 standard electrode potential compilations and developing a consistent set of recommended potentials for a wide range of organic and inorganic radicals in aqueous solutions.

***Standard electrode potentials for a broad set of organic and inorganic free radicals are being compiled and evaluated as part of an international collaboration under the auspices of IUPAC.***

**IUPAC**

The first major phase of this activity, data compilation and evaluation for the major organic and inorganic radicals, has been largely completed. Standard potentials for a number of organic radicals derived from phenols, anilines, indols, and related antioxidants were evaluated based on various equilibrium studies and recommendations presented to the Task Group. As well, standard potentials were derived for the solvated electron and the hydrogen atom, which are particularly important since they form an anchor point for the reducing end of the electrode potential scale. Other members of the IUPAC Task Group outside of CSTL have dealt with a range of other organic and inorganic free radicals, including the important hydroxyl radical, which anchors the oxidizing end of the electrode potential scale. The planned CSTL contribution to this IUPAC effort includes an extension to aromatic organochalcogens and organoradicals derived by oxidation and the inorganic P, As, Sb, Bi radicals. We plan also to work with the Task Group to optimize the standard potential scale and establish a NIST web site for the dissemination of these results.

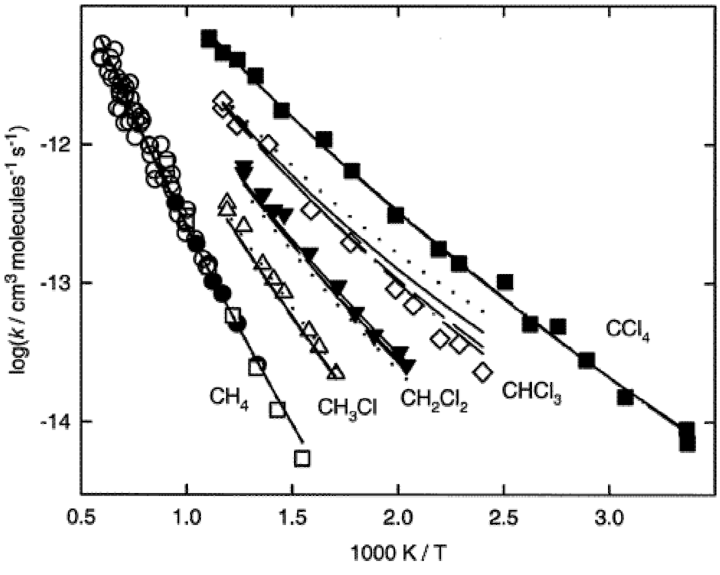
**This activity is expected to have an immediate impact in the increasingly important field of physiological free radical chemistry. It is also expected to have an impact on the development of advanced oxidation technologies, which are typically based on free radical chemistry, and antioxidants for use in stabilizing organic systems such as polymers.**

## *Isodesmic Reactions for Transition States (IRTS)*

***V.D. Knyazev (838)***

***The isodesmic computational approach used here is based on combining accurate experimental knowledge of reactivity in one or several reactions of the same class with computational information based on quantum chemistry.***

The isodesmic approach to studying thermochemistry relies on the conservation of bonds in reactions, with the same number of each type of bond (single, double, triple) occurring in both products and reactants. The technique of isodesmic reactions for transition states (IRTS) has been developed and validated for calculation of reaction barriers and rate constants. This computational approach is based on combining accurate experimental knowledge of reactivity in one or several reactions of the same class with computational information based on quantum chemistry. The IRTS technique has been shown to yield the energy barriers and rate constants for abstraction reactions of two classes (H + chloroalkanes and Cl + halomethanes) with a high degree of accuracy. For example, average deviations between calculated and experimental rate constants for seven H + chloroalkane reactions considered are 17 % to 24 %, depending on the quantum chemical method used. In contrast, a non-isodesmic quantum mechanical approach typically results in a several-fold difference between calculated and experimental rate constants. Accuracy in reaction barrier calculations at the level of a few kJ mol$^{-1}$ has been demonstrated for the Cl + halomethanes reaction.



**The figure shows calculations of the rate constant for several hydrogen abstraction reactions as compared with experimental data (symbols). Different line styles correspond to different levels of quantum chemical theory used. *J. Phys. Chem. A, 106,* 11603-11615 (2002).**

The results will have an impact on the field of chemical kinetics by providing a predictive computational tool for a wide range of chemical reactive systems such as those used in materials chemistry, nanotechnology, polymer chemistry, biotechnology, and soot/pollutant formation. We plan to extend the field of application of the IRTS technique to reactions involving large molecules relevant to polymer chemistry, nanotechnology, and biochemistry. We also intend to develop an IRTS tool for exploring reactivities in going from small to large molecular systems.

# 5. Energy and Environmental Technologies

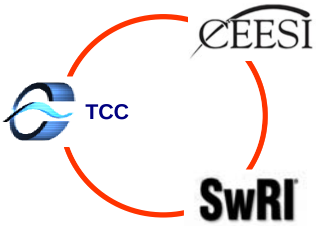
Many CSTL activities impact the energy industry sector, including reference data and models, fuel SRMs, metering standards, and flow calibrations. NIST's accurate and reliable flow standards and databases are critical to this industry both for process optimization and for use in custody transfer applications. CSTL also provides standards that allow this industry to verify compliance with environmental regulations. The Environmental Technologies industry sector, part of an estimated global market of $530 billion, depends on NIST/CSTL for world recognized artifact standards as well as reference data and models to demonstrate compliance with environmental regulations, and for reliable decision-making for environmental remediation, waste disposal, emissions trading and other applications.

## *Natural Gas Flow: North American Laboratory Comparison Project*

***P. I. Espina (836) and W. F. Guthrie (ITL)***

The natural gas industry requested that NIST develop a round robin testing program to ensure the quality of measurements in the three major natural gas flow calibration facilities in North America: namely, the Colorado Engineering Experiment Station, Inc. (CEESI), Ventura, IA, TransCanada Calibrations Ltd. (TCC), Winnipeg, Manitoba, Canada, and Southwest Research Institute (SwRI) Metering Research Facility, San Antonio, TX.

***In 2004, 750 billion cubic meters of natural gas will be consumed in the USA (24 % of our energy needs). Consumption is projected to increase by 50 % by the year 2025. Metrology is necessary to assure proper custody transfer of the resource in the North American natural gas industry.***

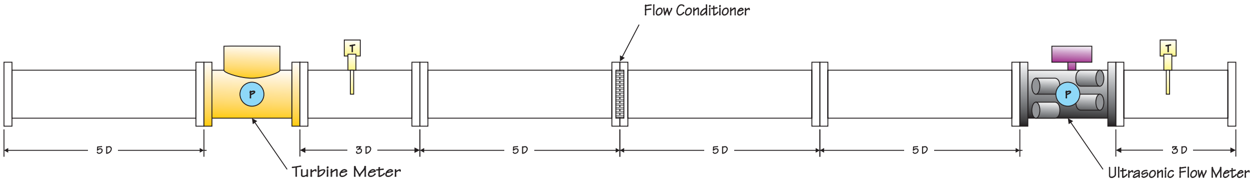


With the support of Daniel Industries (a flow meter manufacturer in Houston, TX), NIST developed the transfer standard package used in this laboratory comparison (schematic shown), one of the largest transfer standards ever used in a NIST calibration program. The transfer standard was composed of a turbine meter separated from a multi-path ultrasonic flow meter by a flow conditioner. The transfer standard had a diameter of 300 mm, a length of 9.7 m, and a mass of 4,000 kg.

The transfer standard package, which has volumetric flow range from 726 $m^3\ h^{-1}$ to 6,169 $m^3\ h^{-1}$, was instrumented with a NIST-developed sensor package that included pressure, temperature, and frequency instrumentation controlled by a laptop computer.

**Sensor Package**

After agreement among the stakeholders, the transfer standard was extensively tested prior to the start of the comparison to assess and quantify its repeatability, with hysteresis effects, its day-to-day, flow-no flow-flow, and mount-dismount-mount reproducibilities, and its sensitivity to flow profile effects. With this knowledge, the transfer standard was used to compare the performance of the laboratories by testing on 5 occasions: SwRI (Oct. 2002) – CEESI (Nov. 2002) – CEESI (May 2003) – SwRI (May 2003) – TCC (Aug. 2003). In addition, the package was tested for pressure effects at SwRI, which was the only laboratory capable of varying line pressure. The results will be used to (i) quantify the level of comparability among calibration laboratories to improve future performance, (ii) guide future laboratory comparison projects, and (iii) guide the upcoming CIPM Key Comparison on natural gas flow being led by PTB/Germany and NMi/Netherlands.



**Schematic of Transfer Standard (flow from left to right)**

## *High-Temperature Reference Correlations for the Solubility of Gases in Water*

### *A.H. Harvey (838); R. Fernández-Prini (CNEA, Argentina); J.L. Alvarez (CNEA)*

**CSTL researchers provide reference correlations for solubility of important gases in water at high temperatures. These standards will find use in numerous areas of high-temperature aqueous physical chemistry, particularly in the steam power industry and in geochemical applications.**

In the steam power industry, it is important to know the distribution of solutes such as $O_2$ and $CO_2$ between liquid water and steam at high temperatures. The solubility of gases in water is an important parameter in the design of many industrial and environmental processes. These quantities are also important in geochemical modeling, particularly for common geologic gases such as $CH_4$, $CO_2$, and $H_2S$. For all of these applications, it is useful to have reliable, evaluated data in the form of a correlation covering the entire temperature range of interest, which extends to near the critical temperature of water.

CSTL researchers took advantage of two relatively recent advances to produce state-of-the-art correlations. First, our collaborators in Argentina have developed improved methods for reducing experimental data to the thermodynamic quantities of interest (the Henry's constant and the vapor-liquid distribution constant). Second, previous work at NIST has produced theoretical constraints on the high-temperature behavior of these properties as the solvent critical point is approached. This leads to improved functional forms that better describe the high-temperature data and have superior extrapolation behavior for systems where the range of high-temperature data is limited.

**New reference correlations for the Henry's constant and the vapor-liquid distribution constant have been published:**
**Fernández-Prini, Alvarez and Harvey, *J. Phys. Chem. Ref. Data* 32, 903-916 (2003).**

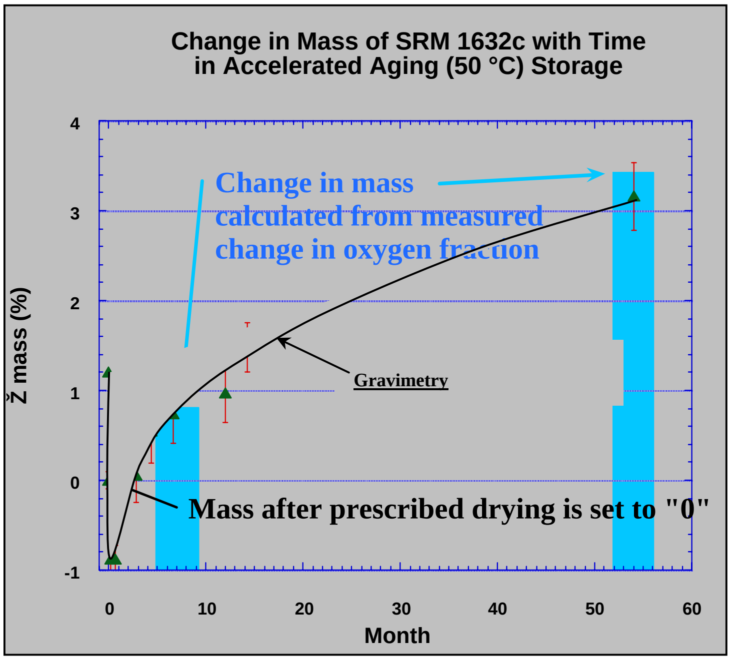
We conducted a thorough literature search and found usable high-temperature data for 14 solutes in ordinary water and 7 solutes in heavy water. We evaluated the data, converted them to the appropriate thermodynamic quantities, and produced correlations for the Henry's constant and vapor-liquid distribution constant, with both correlations based on the same final data set. The correlations developed in this work have been incorporated into an official guideline that will be adopted by the International Association for the Properties of Water and Steam at its 2004 meeting.

## *Influence of Oxygen on the Shelf Life of Coal SRMs*

***R. Zeisler (839) and W.D. James (Texas A&M)***

In 1997 users of SRM® 1632b (Trace Elements in Coal), issued in 1985, reported a change in the measured values of the carbon mass fraction with the new values being about 1.6 % lower than previously measured. Additional evidence of change was provided by trace element data in this material, which indicated approximately the same change in mass fraction values for a number of elements determined by INAA, although the new measurements were still within the bounds of uncertainty given in the certificate. While a change in carbon content through loss of $CO_2$ may have been a plausible explanation for lower than previously measured carbon values, the measured decrease of a larger number of elemental content values may be explained by a gain of matrix mass, such as by irreversible oxidation and therefore increased matrix mass. To directly measure mass fractions of oxygen in coal samples, a fast (14-MeV) neutron activation analysis (NAA) procedure was employed. The procedure was applied to test samples of SRM 1632c Trace Elements in Coal (Bituminous).

***CSTL scientist and collaborators validated the long-term stability of a coal SRMs under proper storage. Samples were found stable during the more than four-year observation, except for the ones stored open at higher temperature.***



Twenty-six samples each were selected and compared for three storage conditions: (1) in liquid nitrogen vapor and (2) at room temperature in the original containers packaged under argon, and (3) at 50 °C and open to air to accelerate aging. The 14-MeV NAA is an effective procedure for the direct determination of oxygen. It demonstrated sufficient precision and accuracy to determine a relative change of about 5 % or more, in the oxygen mass fraction of about 11 %, which is equivalent to about 0.5 % of the total mass. To evaluate the sample stability, test points were set at 6 and 54 months for the oxygen determination, more frequent gravimetric tests were carried out for the samples in the open 50 °C storage. The gain in mass and in oxygen for the latter samples is illustrated in the figure above. All gain in mass can be explained by the gain in oxygen content. No change in oxygen content was measured in the other storage conditions. The measurements give assurance that properly packaged coal SRMs, protected from oxygen and heat, are stable during their shelf life.

## *Determination of Hg in Crude Oils in the Department of Energy Strategic Petroleum Reserve by ID-CV-ICPMS*

***W.R. Kelly, S.E. Long, and J.L. Mann (839)***

The Clean Air Act as amended in 1990 required the U.S. Environmental Protection Agency (EPA) to submit a report to Congress on Hg emissions to the atmosphere. The EPA responded with the Mercury Study Report to Congress that identified

***CSTL researchers have developed a highly accurate technique for measuring mercury in crude oil. Prior to this work, Hg in this matrix has been a major analytical challenge.***

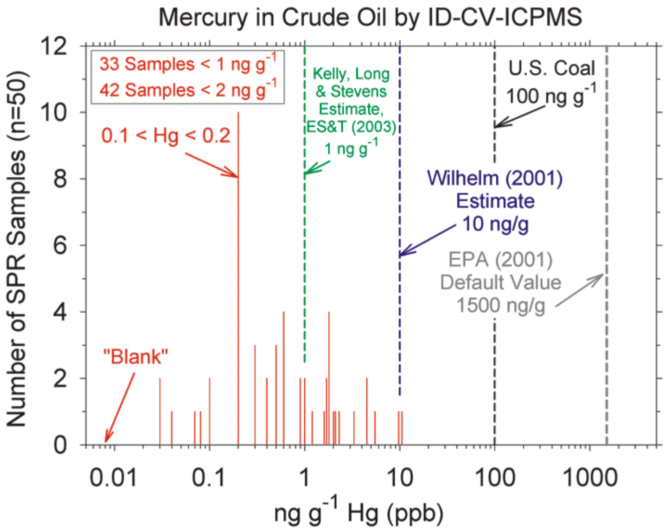
a number of sources of Hg emissions to the atmosphere and quantified those emissions with the best available estimates. However, no estimates were given for crude oil consumption because of the lack of reliable data. It is well established that coal is a major source of Hg emissions to the atmosphere. The Hg content of coal combusted in the US is about 100 ng g$^{-1}$. The EPA, concerned with the well-known toxic behavior of Hg, now requires reporting of Hg emissions from companies that process more than 10 pounds of Hg per year, and this reporting requirement may be a prelude to regulation of Hg emissions. If the mean content of Hg in crude is 10 ng g$^{-1}$, as commonly believed, then all refineries would be over the limit.

**Given the fact that the magnitude of crude oil consumed in the US is equal to that of coal, many scientists have speculated that crude oil could be a major unknown component to the global Hg cycle.**

**DOE's Strategic Petroleum Reserve**

Isotope dilution cold vapor inductively coupled plasma mass spectrometry (ID-CV-ICPMS), a highly accurate analytical method developed at NIST was used in this study**.** The high accuracy of this technique is superior to existing techniques because of three unique capabilities: 1) very low and controllable blanks which average 3 ± 2 pg (± 1s), 2) high sensitivity of the ICPMS, and 3) a digestion method using only high purity nitric acid in closed system (Carius tube) which prevents loss of the highly volatile Hg and prevents contamination of the sample.

The Hg concentration determined in almost 100 different crude oils from the U.S. Strategic Petroleum Reserve (SPR) by ID-CV-ICPMS, populate a new database and allow for more strategic decision-making. The Hg concentration ranged from 0.02 ng g$^{-1}$ to 10 ng g$^{-1}$ (figure) with about 80 % of the samples falling below 2 ng g$^{-1}$, much lower than previously reported.



**These measurements constitute the first and only high-accuracy database on Hg in crude oils indicates that the Hg concentration is about an order of magnitude lower than currently believed.**

### *Absorption Coefficient Measurements of Aerosol Particle Agglomerates*

***C. Presser (836); J. Conny (837); A. Nazarian (SAIC)***

*Details provided in the* ***Exploratory Research*** *section*

## *A Question of Accuracy: How Does Thermal-Optical Analysis for Climatically Important Black Carbon in Atmospheric Soot Behave Optically?*

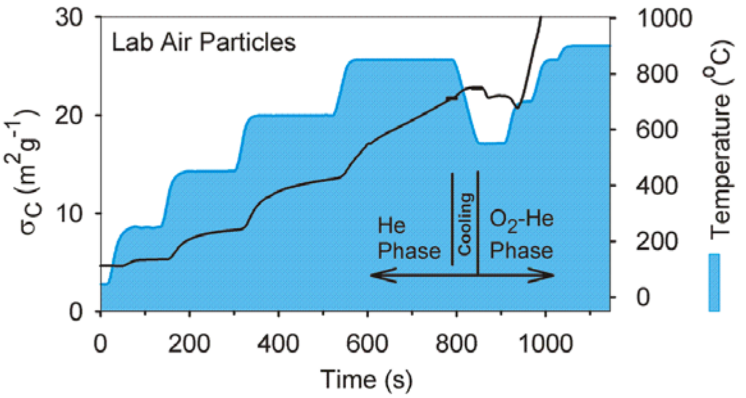
***J. M. Conny (837)***

Refractory black carbon (BC) in atmospheric particulate matter presents a formidable measurement challenge for climate change researchers. Optical methods for determining particulate carbon mass require prior knowledge of the absorption and scattering cross sections, the latter which varies widely with the chemical composition of particles. Thermal-optical analysis (TOA) measures light-absorbing refractory carbon mass but does not require prior knowledge of the optical cross sections. However, TOA incorporates critical assumptions about the optical behavior of organic carbon (OC) and BC. This work tests these assumptions and pursues an approach to optimizing TOA for accuracy through an assessment of how the absorptivity of particulate carbon behaves during analysis.

***CSTL researchers test critical assumptions regarding the optical behavior of Thermal-optical analysis (TOA), which impact its accuracy. This is critical since TOA is a principal method in the U.S. for measuring refractory carbon in atmospheric soot.***

**TOA has an essential optical component; therefore, it plays an important role in climate change studies for determining the mass of light-absorbing aerosol carbon.**

In TOA's light transmission method (TOT), refractory BC is distinguished from OC through a protocol of thermal desorption accompanied by pyrolysis in helium and thermal oxidation in a He-$O_2$ flow. As carbon evolves from a filter sample it is detected by flame ionization detection. Pyrolysis of OC generates char, which is indicated by the attenuation of 670 nm laser light. BC is differentiated from OC when the attenuated laser signal returns to the level prior to the initiation of the heating protocol, indicating that an amount of carbon equivalent to the original OC component has been removed from the filter sample and only the carbon mass equivalent to BC remains.



Accuracy in TOT is hindered by the positive bias from unpyrolyzed OC measured as BC and by the negative bias from the unaccountable oxidation of BC at high temperatures during analysis. TOT assumes that the absorptivity of the pyrolized OC remains constant at high temperatures and that BC and pyrolized OC have the same absorptivity. In this work, the attenuation due to particulate carbon absorption was isolated from the total attenuation, which included scattering by non-carbonaceous material. Particulate carbon absorptivity was then calculated by dividing the laser attenuation due to carbon by the carbon mass which gives the apparent specific absorption cross section ($\sigma_C$). The figure shows how $\sigma_C$ typically varies during the course of analysis. Assessment of $\sigma_C$ at critical points in TOT provides a robust approach for adjusting TOT's temperature protocol (blue area in figure) to insure consistency in TOT's optical behavior and to achieve accuracy in atmospheric BC measurement.

## Structure and Reactivity of the Hydrated Hematite (0001) Surface

**A.M. Chaka (838); T. Trainor, P. Eng, and M. Newville (Univ. Chicago); G. Waychunas (Lawrence Berkeley National Lab); J. Catalano and G.E. Brown (Stanford Univ.)**

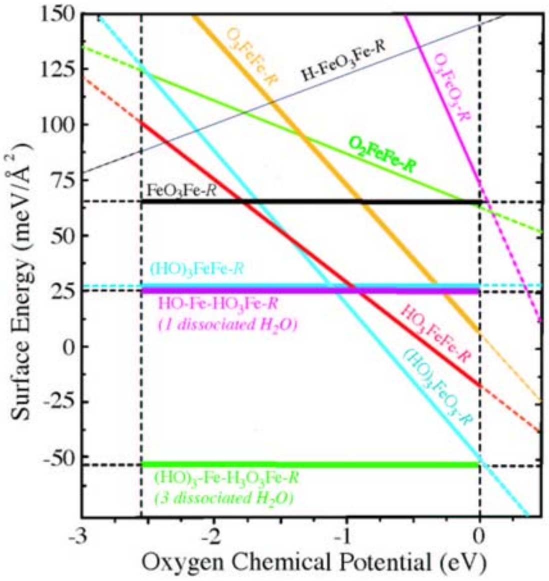
Reactions at metal-oxide surfaces are of great significance in the chemistry of contaminants in aquifers and soils, as well as industrial/technological processes involving catalysis, microelectronics, and passivation of metals. Hematite (α-$Fe_2O_3$) and the isostructural corundum (α-$Al_2O_3$) are among the most common metal oxides in industry and in soils, but exhibit drastically different chemistries for reasons that are not understood.

***An understanding of metal oxide structure and chemistry in a realistic environment at the nanoscale will help in the development of robust predictive models for creating catalysts, coatings, sensors, microelectronic components, and corrosion inhibitors, as well as in efforts to remediate polluted soils, ground water, and surface water.***

The types of surface functional groups exposed at metal-oxide/water interfaces are likely to differ substantially from models based on ideal surface terminations of the bulk crystal and should result in dramatic differences in reactivity compared with clean or dehydroxylated surfaces. The combination of theoretical and experimental work performed in a wet environment has provided the first evidence for the fundamental mechanisms involved in the early stages of hydrolysis of iron atoms in iron oxide. Using crystal truncation rod diffraction (CTR) and *ab initio* thermodynamics (first principles density functional theory (DFT) electronic structure calculations linked to finite temperature and pressure thermodynamics), it was determined that the stable hydrated hematite (0001) surface consists of two domains – one with Fe-rich regions terminated by singly coordinated hydroxyls, and one with oxygen-rich regions terminated by doubly and triply coordinated hydroxyls. The presence of singly coordinated hydroxyls on hydrated α-$Fe_2O_3$(0001) and their absence on hydrated α-$Al_2O_3$(0001) explain the higher reactivity of the former. DFT calculations indicate that water reacts with hematite both heterolytically and homolytically at a threshold water pressure orders of magnitude lower on α-$Fe_2O_3$(0001) than on α-$Al_2O_3$(0001). The electronic structure of corundum does not support the homolytic dissociation mechanism of water.

**Knowing how the structure and reactivity of a metal oxide surface change upon exposure to water enables better design and control of surface properties for industrial conditions.**

Phase diagrams of the surface free energies calculated as a function of water vapor pressure and in equilibrium with liquid water (see figure), as well as surface relaxations, were used to identify the structure of the two domains observed in the CTR experiment on the hydrated hematite surface and to account for the difference in reactivity between Fe and Al oxide.



**Hematite (0001) Surface Energy Phase Diagram in Equilibrium with $O_2$ and $H_2O$**

## *Chemical Kinetic Database for PAH formation for Heptane Combustion*

***V. Babushok and W. Tsang (838)***

A new approach has been developed that extends the possible use of molecular simulations to include polycyclic aromatic hydrocarbon (PAH)/Soot formation processes. In combination with the capability of existing databases to cover oxidations, this represents at least in principle the complete picture for the combustion with heptane as a fuel.

***CSTL researchers build a kinetic database for heptane combustion that can be used to simulate both the oxidative properties (ignition delay, flame velocities) as well as the PAH/Soot forming propensities of the system.***

In studying the combustion of heptane, particular emphasis was placed on the beta C-C bond scission reaction and isomerization of the radical intermediates in the decomposition. These include, the four heptyl and 2 pentyl radicals as well as all the 1-olefinyl radicals, 1-butenyl-3, 1-butenyl-4, 1-pentenyl-3, 1-pentenyl-4 1-pentenyl-5, 1-hexenyl-3, 1-hexenyl-4, 1-hexenyl-5, and 1-hexenyl-6.

The effects of energy transfer leading to pressure dependencies have also been considered. These reactions lead to the small, unsaturated compounds and radicals that are the inputs to existing PAH/Soot models. The model was validated against existing data on ignition delays (from OH) and flame velocities. Some problems on existing databases for the latter have been identified and modifications have been made to fit results under rich conditions.

**Existing mechanisms for the combustion of heptane have been extended to include the reactions involved in the formation of the precursors of PAH/Soot. Cracking reactions of heptane were also involved.**

Our approach is being used by engineers at Wright-Patterson Air Force Base to model the impact of the use of nitromethane additives on soot formation. Future plans are two-fold. First, a large extension of the present work is planned to cover an assortment of the components of a real fuel mixture that can serve as a surrogate, which was a focus of discussion at the workshop “Combustion Simulation Databases for Real Transportation Fuels“ sponsored by NIST in FY03. Second, the optimization and extension of existing databases are planned so that current data and concepts are included.

## *OH Kinetics – Evaluation of Data and Experimental Techniques*

***V.L. Orkin and M.J. Kurylo (838)***

Reactions of the hydroxyl radical in the gas phase are central to atmospheric chemistry and combustion processes. We have been engaged for a number of years in carrying out accurate and precise kinetic determinations, and also in developing computational procedures for estimating these values. One of the major reasons for carrying out these studies is the important role of OH in establishing the atmospheric lifetime of chemical compounds, which is the key parameter in estimating environmental impact due to their emission to the atmosphere, ozone depletion potentials (ODPs) and global warming potentials (GWPs).

***CSTL researchers critically reviewed the determination of lifetimes for chemicals emitted into the atmosphere has been, with particular reference to measurement methods and techniques for hydroxyl radical kinetics and procedures for estimating the ozone depletion potential and global warming potential.***

Over the past several years, we have improved the flash photolysis-resonance fluorescence (FP-RF) technique sufficiently to be able to obtain a precision of better than 3 % in measurements of the kinetics of OH reactions over the temperature interval of atmospheric interest, and have applied this capability to determine more accurate and reliable atmospheric lifetimes. In the past year, we carried out an extensive review of the determination of atmospheric lifetimes, with particular reference to measurement methods and techniques for OH kinetics and procedures for estimating the ODP and GWP. The review covered all aspects of atmospheric lifetimes, from the basic concept of an atmospheric loading, removal processes, sources and estimates of OH, and how atmospheric lifetimes are determined and used. The major part involved a critical review of the accuracy and precision of all commonly utilized techniques and a thorough analysis of uncertainties and its proper treatment. Finally, the evaluation of OH reaction rate constant data was discussed in detail. The review treated in great detail areas that are often glossed over, such as uncertainty analysis. Thus, it is expected to be relied upon heavily in subsequent data analysis efforts. Although discussion was particularly aimed at OH kinetics, the review is clearly applicable to kinetic measurements of other radicals and will have an impact well beyond its immediate target.

**M.J. Kurylo and V.L. Orkin "Determination of Atmospheric Lifetimes via the Measurement of OH Radical Kinetics," *Chem. Rev.*, *103,* 5049-5076 (2003).**

The accurate measurements of OH rate constant over the wide temperature range using FP-RF technique will be continued for selected reactions. Firstly, reliable data are needed for particular compounds of industrial interest. Secondly, a number of new studies of OH reactions are being done using a relative rate techniques at room temperature, which require reliable and accurate absolute data for the rate constants of reference reactions. Thirdly, accurate measurements of temperature dependences when being combined with the higher temperature measurements create an experimental basis to support developing calculation tools for predicting the reactivity of new chemicals.

### Collaboratory for Multi-Scale Chemical Science

***T. C. Allison (838); L. Rahn, (Sandia National Laboratory)***

This project is part of the DOE's National Collaboratories Program which funds initiatives to create collaborative infrastructure in a variety of scientific fields. The purpose of the Collaboratory for Multi-Scale Chemical Science (CMCS) project is to enable groups of scientists to work together more productively; collect relevant data and archive it in a searchable manner; provide an alternate venue for scientific publication; foster communication between scientists in different disciplines; and reduce the time and effort required to attack large problems.

***CSTL scientists will continue to work with DOE on CMCS for another one to three years depending on funding.***

The CMCS project is creating a collaborative environment for research in the chemical science areas. This environment is centered around a web portal. It was necessary to create the infrastructure that would provide useful tools to create, collect, analyze and share data. It must also offer the means to annotate data, creating new and open formats for various data and its associated pedigree. Finally, it must establish a framework in which scientists from different disciplines working on problems with dramatically different length scales can work in a meaningful and productive fashion.

To that end, a simple object access protocol (SOAP) service was established which makes a large database of chemical names, synonyms, formulae, and CAS numbers accessible within the

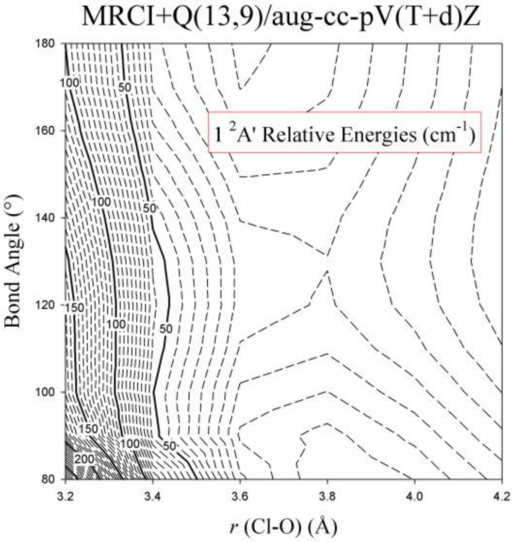
CMCS portal. Critical components of the CMCS infrastructure related to searching were extensively modified to enhance response time and make the infrastructure extensible to larger data collections. A set of recommendations for attaching pedigree information to chemical data was developed. At the present time a great deal of the infrastructure has been created and the focus is shifting toward demonstration of the capability of the CMCS framework in addressing real scientific problems. Near term activities will include the development of more powerful searching techniques, development of "universal" formats for relevant types of data, creating translators for legacy data, and further incorporation of NIST data accessible through the CMCS portal.

### *Weakly-bound Isomers of the ClOO Free Radical*

***K.K. Irikura (838)***

The principal gas-phase cycle for the catalytic destruction of stratospheric ozone involves the chloroperoxyl radical, ClOO. Surprisingly little is known about this important molecule. For example, its structure has not been determined experimentally. One recent experiment, however, suggests the existence of an isomer of ClOO that is very weakly bound. The atmospheric implications of this finding, and even its veracity, were unknown.

***A recent experiment provided evidence for the existence of a weakly-bound form of the ClOO radical, which is important in the stratospheric destruction of ozone. The primary goal of this work was to verify or deny the existence of such an isomer.***



Recent theoretical work here at NIST has confirmed the experimental result and determined that the Cl-O bond in this isomer is 20 times weaker than in the stable form of ClOO. The potential energy functions for three of five excited valence states of ClOO have been mapped. The figure shows a contour plot of the potential energy function. Furthermore, the experimental inference of weak binding has been corroborated for two of the three excited states that were mapped for potential energy. The secondary goal of this project is to characterize the new states for spectroscopic identification. Further calculations are underway to characterize the weakly-bound states sufficiently well for their implications in atmospheric chemistry to be determined and for the experimental spectrum to be assigned.

In addition to their potential environmental impact, these results are theoretically important for two reasons. First, this may be representative of a more general feature of the gas-phase chemistry of oxygen. Second, the type of electronic structure represented by this radical isomer is especially challenging for popular methods of quantum chemistry, thus providing a good test case for the development of better methods.

Detailed dynamics calculations must be done to determine whether the new forms of ClOO are important in atmospheric chemistry. Spectroscopic simulations should also be done for comparison with the experimental observation. Related systems, such as ONOO, should also be investigated to learn if weakly-bound isomers are a general feature of the gas-phase chemistry of oxygen.

**This study on ClOO chemistry provides important data for global ozone models to predict the impact of the ClOO radical in the upper atmosphere.**

## *SRMs for Air Particulate Matter Less Than 2.5 µm ($PM_{2.5}$)*

***R. Zeisler, J.R. Kucklick, B.J. Porter, D.L. Poster, M.M Schantz, R.O. Spatz, and S.A. Wise (839)***

***In cooperation with USEPA, NIST is establishing benchmark SRMs for monitoring elemental, organic carbon, and other species in air particulate matter that will be used to evaluate analytical methods, help to provide measurement traceability, and improve inter-laboratory comparability of PM measurements.***

The Environmental Protection Agency (EPA) issued new national ambient air quality standards in 1997 for air particulate matter (PM). The new standards will allow regulation of the respirable PM fraction, that is, PM <2.5 µm ($PM_{2.5}$ or fine PM). The new standards accompany existing standards for PM <10 µm ($PM_{10}$). Research recommendations have already been made by the NRC at the request of Congress and EPA to focus on evaluating the types of particles that cause detrimental health effects. To support compositional analyses and other investigations on the fine PM, quality assurance materials are necessary. Since few appropriate reference materials are available, NIST and EPA have an agreement to develop, certify, and issue a suite of SRMs that will be used for the evaluation of analytical methods to provide national measurement traceability and to improve inter-laboratory comparability of $PM_{2.5}$ measurements.

**Newly released SRM 2783, air particulate on polycarbonate filters, is intended for elemental analysis using X-ray fluorescence, neutron activation analysis, and other analytical techniques.**

SRM 2783 provides 0.5 mg of fine PM on a polycarbonate filter membrane with certified mass loadings for 24 toxic and environmentally important elements. The material for the SRM was collected in Vienna, Austria as total suspended PM and then processed by the International Atomic Energy Agency to resemble a $PM_{2.5}$ distribution.

**Cyclone sampler (left) and filter carriers (right) for collecting large amounts of PM.**

For organic compounds in $PM_{2.5}$, we are in the process of collecting a 200 g bulk sample of fine PM at a site in Baltimore, MD with a high-volume sampler (figure). Because the collection, preparation, and certification of this SRM will require 2 to 3 years, an interim reference material was prepared from a 20 g batch of $PM_{2.5}$ collected earlier at the Baltimore site during different sampling periods in 1998-99 and 2001. This interim reference material has been characterized at NIST for polycyclic aromatic hydrocarbons (PAHs) and nitro-substituted PAHs and has been distributed to 18 laboratories as part of a NIST/EPA interlaboratory comparison for the determination of organic compounds. Based on these results NIST will value assign analyte concentrations and make the material available to the broader community.

**The results of this intercomparison will be used with NIST measurements to assign concentration values for the compounds of interest. The interim reference material will then be made available to laboratories involved in EPA's $PM_{2.5}$ research programs.**

# *Development and Evaluation of Analytical Method for Nitro-substituted Polycyclic Aromatic Hydrocarbons in Atmospheric Particulate Matter*

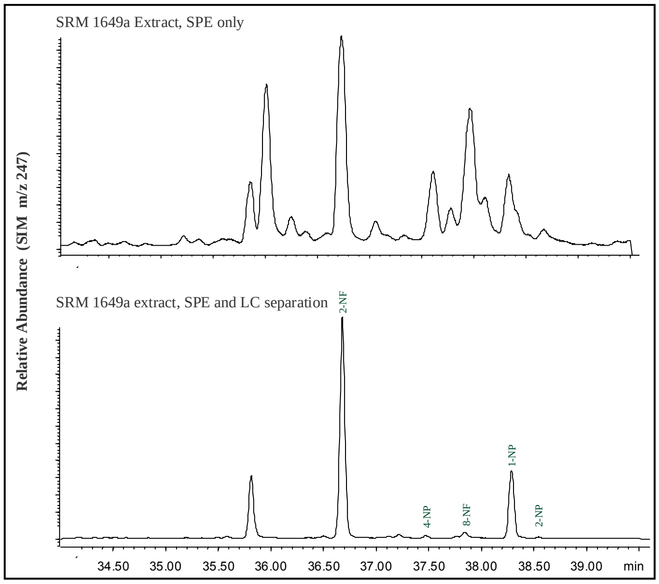
***M.M. Schantz, H.A. Bamford, and S.A. Wise (839)***

***Using a selective fractionation and analytical method, nitro-PAH concentrations were determined in existing NIST SRMs of atmospheric particulate matter as well as a more recent collection of fine atmospheric particles. These newly analyzed materials will aid in assuring the accuracy of results from monitoring studies of nitro-PAHs in ambient air.***

Nitrated PAHs (nitro-polycyclic aromatic hydrocarbons) are often present on ambient atmospheric particles from either direct sources, such as diesel and gasoline exhaust or gas-phase reactions of PAHs with nitrogen oxides. An analytical method has been developed using pressurized fluid extraction followed by isolation of the nitro-PAH fraction from the parent PAH fraction using normal-phase liquid chromatography (LC) with final analysis using gas chromatography/mass spectrometry in the negative chemical ionization mode. Using this method, 28 mononitro- and dinitro-PAHs were quantified in two NIST air particulate reference materials, SRM 1648 Urban Particulate Matter and SRM 1649a Urban Dust. In addition, nitro-PAH determinations were made on a fine air particulate matter (particle size <2.5 µm) that was collected in the late 1990s as part of the NIST/EPA effort to develop a fine particulate matter reference material.

**While nitro-PAHs are present in air particulate samples in the low ng $g^{-1}$ range, they are persistent in the environment and have been shown to be more mutagenic and carcinogenic in some cases than the parent PAHs.**

The normal-phase LC fractionation step was critical for the correct determination of nitro-PAH concentrations in air particulate samples. The figure compares the GC/MS chromatogram of the nitrofluoranthene and nitropyrene molecular ion (m/z of 247) before and after extracts of SRM 1649a were fractionated using the LC method. Prior to LC fractionation (top chromatogram), there are a large number of interfering peaks; whereas, after LC fractionation, quantitative analysis of the nitrofluoranthenes and nitropyrenes is possible. In addition, the use of a moderately polar GC column resulted in the separation and quantification of some isomers that co-elute on the typically used non-polar GC columns. For example, when using a non-polar GC column the most prominent peak above, 2-nitrofluoranthene, which is prevalent in ambient air, co-elutes with 3-nitrofluoranthene, which is prevalent in diesel emissions.



## *Developing Methods for the Determination of Past-Use and Current-Use Persistent Organic Pollutants in Standard Reference Materials*

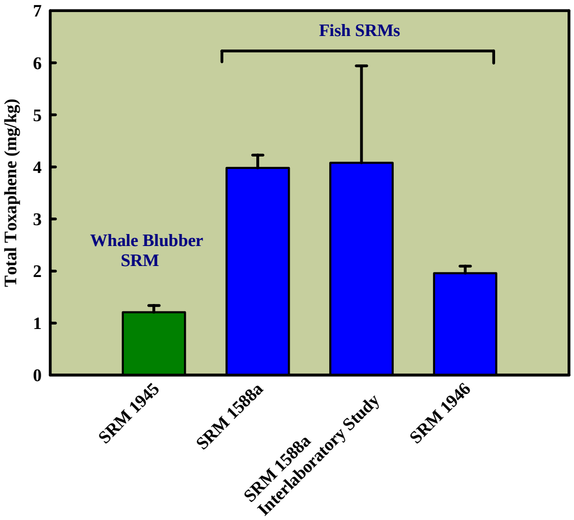
***J.R. Kucklick, M.M. Schantz, S.A. VanderPol, K.J. Tuerk, and S.A. Wise (839)***

***NIST has developed methods for a wide range of persistent organic pollutants, including PCBs and chlorinated pesticides, for assigning concentrations in environmental matrix SRMs. The compounds studied here include the banned pesticide toxaphene, polychlorinated naphthalenes (PCNs), which are mainly used in the electrical industry, and polybrominated diphenyl ethers (PBDEs), which are currently used as flame-retardants.***

For many persistent organic pollutants (POPs) such as polychlorinated biphenyl (PCB) congeners and chlorinated pesticides, well-established measurement methods and reference materials are available for several different matrices. However, for some pollutants such as toxaphene, polychlorinated naphthalenes (PCNs), and polybrominated diphenyl ethers (PBDEs), established analytical methods or natural-matrix certified reference materials are lacking because these compounds are either more challenging to measure or they have only recently emerged as compounds of environmental concern. For example, toxaphene is listed in the Stockholm Convention on POPs for elimination, while PCNs are candidates for elimination. PBDEs are a class of fire retardant compounds whose concentrations are increasing exponentially in humans and are being extensively monitored and investigated. To meet the need for reference materials, we have developed methods and provided reference values for these compounds in selected SRMs.

**Reference values for the PCNs, toxaphene, and the PBDEs on relevant environmental matrix SRMs will provide laboratories a tool to help assess data quality. SRM 1944 and SRM 1649a were chosen as interlaboratory comparison materials for the Second International Intercomparison Exercise for the PCNs, which is administered both by NIST and Environment Canada.**

Toxaphene and selected toxaphene congeners were measured in SRM 1945 (Organics in Whale Blubber), SRM 1946 (Lake Superior Fish Tissue), and SRM 1588a (Organics in Cod Liver Oil) using gas chromatography/mass spectrometry with negative chemical ionization (GC/MS-NCI). Average total toxaphene values (figure) for SRM 1588a were in excellent agreement with a prior interlaboratory study using this material. Average values in SRM 1946 were at the Food and Drug Administration's Consumption Advisory Limit (2 mg kg$^{-1}$), while toxaphene levels in SRM 1945 were about 50 % of this value. The five major PBDE congeners present in most biological samples were also measured in SRMs 1588a and 1945 using GC/MS with electron impact ionization. The levels are in a useful range for routine environmental analysis and agreed well with measurements made on these two materials by other laboratories. PCN congeners were measured in selected abiotic SRMs including SRM 1944 New York/New Jersey Waterway Sediment, SRM 1649a Urban Dust, and SRM 1941b Organic in Marine Sediment. PCN Levels were determined in the SRMs using GC/MS-NCI following isolation using a liquid chromatography technique that isolates planar compounds.



## *Expansion of NIST's Environmental Specimen Banking Activities*

***P.R. Becker, R.S. Pugh, S.S. Vander Pol, J.R. Kucklick, R.D. Day, S.J. Christopher, B.J. Porter, M.B. Ellisor, and S.A. Wise (839)***

***NIST maintains the National Biomonitoring Specimen Bank (NBSB) both on the NIST Gaithersburg campus and at the recently completed Hollings Marine Laboratory in Charleston, South Carolina. Established in 1979, the NBSB is a cryogenic environmental specimen bank that stores specimens such as human livers, human diet samples, fish tissues, mussels, oysters, and marine sediments collected as part of several monitoring and research programs supported by a number of federal agencies.***

With the completion of its new cryogenic specimen banking facility at the Hollings Marine Laboratory in Charleston, SC, NIST is expanding its capability to support monitoring and research on marine environmental health issues. The banking of marine mammal tissues (blubber, kidney and liver) is a major effort; however, additional programs have been added including the banking of bird eggs and feathers and marine mammal blood. All of these programs have been established at the request of other agency sponsors, i.e., National Oceanic and Atmospheric Administration (NOAA), U.S. Geological Survey (USGS), and U.S. Fish and Wildlife Service (USFWS), and with the requirement that NIST develop collection and banking protocols for the new matrices.

Current projects at the Hollings Marine Laboratory include the National Marine Mammal Tissue Bank sponsored by the NOAA National Marine Fisheries Service and USGS. In 1999, NIST established egg collection and banking protocols and began to archive the contents of seabird eggs for the Seabird Tissue and Archival Project (STAMP). This project is a collaborative effort among NIST, USGS, and USFWS to monitor contaminants in eggs of seabirds of the Alaska Maritime National Wildlife Refuge. In 2002, NIST began banking blood for NOAA's Marine Mammal Health and Stranding Response Program. Collection and storage protocols were developed for whole blood, plasma, and serum as part of health assessment studies on populations of bottlenose dolphins in the Gulf of Mexico and on the US Atlantic coast. Marine mammal blood is now being banked at the HML facility. The latest addition to banking has been an effort begun in 2003 to bank peregrine falcon eggs and feathers for the USFWS.


Hollings Marine Laboratory, Charleston SC

The STAMP egg collection has resulted in the banking of contents from 321 eggs representing three species of seabirds (common and thick-billed murres, and black-legged kittiwakes) from colonies in Alaska. NIST recently analyzed banked tissues from 47 white-sided dolphins from the Atlantic coast and 15 rough-toothed dolphins from the Gulf of Mexico for persistent organic contaminants and heavy metals. The contents of 67 murre eggs from colonies in Alaska have also been analyzed for persistent organic contaminants and mercury.

**The largest number of specimens in the NBSB are from marine mammals with Gaithersburg and Charleston together containing 2,182 tissue specimens from 784 individual animals representing 35 species from the Atlantic and Pacific oceans, Gulf of Mexico, and Alaska.**

## *SRMs for Contaminants in Marine Sediment and Aquatic Species Tissue*

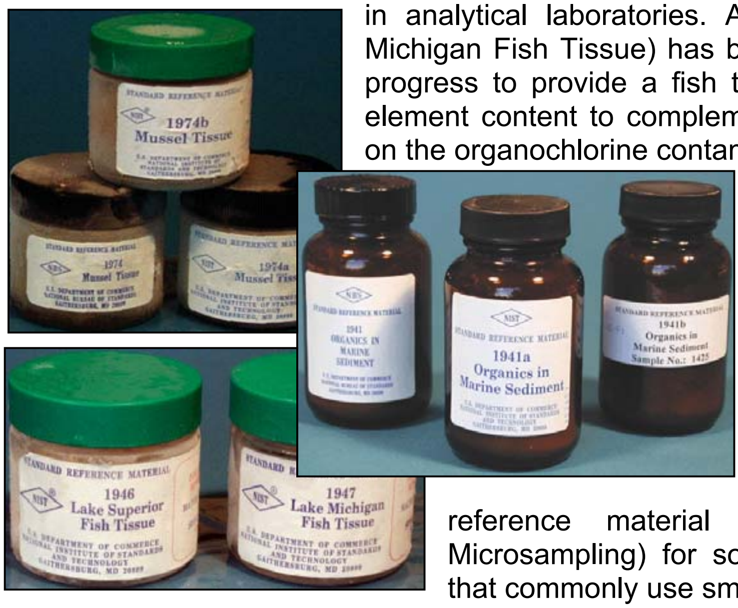
***S.A. Wise, S.J. Christopher, R. Demiralp Oflaz, R.R. Greenberg, J.R. Kucklick, S.E. Long, E.A. Mackey, B.J. Porter, D.L. Poster, M.M. Schantz, and R. Zeisler (839)***

The certification of several sediment and tissue matrix SRMs has been completed in the past year including two renewals of previous SRMs and two new materials. Both marine sediment SRM 1941b and mussel tissue SRM 1974b have certified concentrations for 60 polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), and chlorinated pesticides compared to only 11 and 9 certified constituents in the original sediment and mussel tissue SRMs issued in 1989 and 1990, respectively.

***Natural matrix SRMs consisting of sediment and aquatic species tissue have recently been developed to address the need for natural matrix SRMs in assuring quality of environmental measurements for inorganic and organic contaminants. These materials include marine sediment (SRMs 1941b, 2702 and 2703), mussel tissue (SRM 1974b) and fish tissue (SRM 1946 and SRM 1947).***

**SRM 1941b Organics in Marine Sediment and SRM 1974b Organics in Mussel Tissue (*Mytilus edulis*) are the third issue of these popular marine matrix SRMs for organic contaminants, which have found widespread use in a number of marine monitoring programs supported by the Environmental Protection Agency (EPA) and the National Oceanic and Atmospheric Administration (NOAA).**

A new marine matrix SRM, 2702 Inorganics in Marine Sediment, was issued last year along with SRM 1946 Lake Superior Fish Tissue. SRM 1946 is the first NIST fish tissue SRM and is intended for both the environmental measurement community with certified values assigned for 30 PCBs, 15 chlorinated pesticides, total mercury, and methylmercury, and the food and nutrition measurement community with certified values assigned for proximates and 13 fatty acids. Both SRM 1946 and SRM 1974b are issued as frozen tissue homogenates rather than freeze-dried tissues to provide a matrix similar to that typically analyzed in analytical laboratories. A second fish tissue (SRM 1947 Lake Michigan Fish Tissue) has been prepared and measurements are in progress to provide a fish tissue SRM with emphasis on the trace element content to complement SRM 1946, which focuses primarily on the organochlorine contaminants.



SRM 2702, which replaces the popular SRM 2704 Buffalo River Sediment, has certified values for 25 elements, reference values for 8 elements, and information value for 11 elements covering all of the priority pollutant elements. In addition, the stock material used for SRM 2702 is also being used to develop the first reference material (SRM 2703 Marine Sediment for Microsampling) for solid sampling direct analysis techniques that commonly use small analytical test portions.

## Detoxification of PCB-Contaminated Materials using Electron Beam Technology and Ultraviolet Radiation

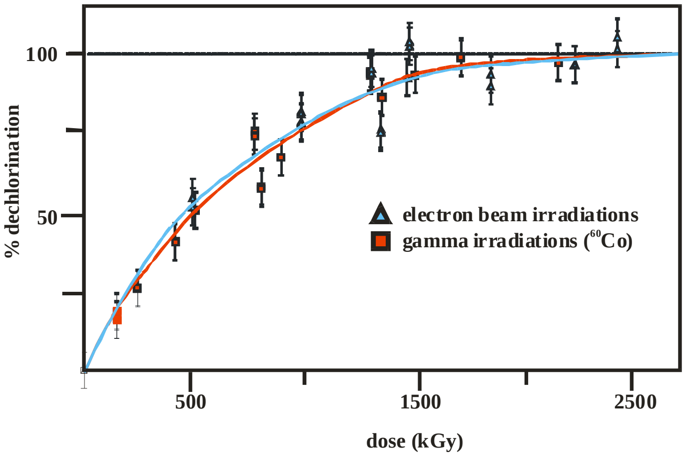
***D.L. Poster (839); P. Neta and R.E. Huie (Div. 838); O. Kantoglu, J. Silverman, and M. Al-Sheikhly (Univ. of Maryland)***

***For several years, CSTL has collaborated with the University of Maryland on studies of the radiolytic and photolytic dechlorination of polychlorinated biphenyls (PCBs). New results show that aqueous-based additives enhance the dechlorination process.***

The widespread use of chlorinated organic compounds such as polychlorinated biphenyls (PCBs) in a range of applications challenges the environment because of the toxicity of these compounds, their persistence in the environment, and the deleterious effect of their bioaccumulation in organisms and aquatic sediments. Reduction or removal of chlorinated organic contaminants from environmental systems is complex. Most materials that contain chlorinated species are incinerated. This method, however, suffers from two disadvantages: the medium is incinerated along with the contaminants, and some contaminants may be converted to other toxics such as dioxins. Degradation of chlorinated species by radiolytic or photolytic methods potentially overcomes these disadvantages. However, when applied to organic-rich compartments such as sediment, the process can be inefficient. Other constituents in sediment compete or react with secondary electrons and organic radicals produced during radiolysis. We have investigated procedures to suppress these competing processes while at the same time enhancing the dechlorination yield.

**The feasibility of electron beam treatment for PCB-contaminated systems has been demonstrated and initial design parameters for possible scale-up have been established.**

In earlier work, PCBs in water and transformer oil were successfully dechlorinated using electron beam and gamma irradiation technology (figure), but more recently, we have moved to the more complex matrix of marine sediment. The use of aqueous-based additives (e.g., alcohol solutions and triethylamine) to enhance dechlorination processes with electron beam and ultra-violet technologies has effectively degraded PCBs and a metabolite of the chlorinated pesticide DDT (DDE) in marine sediment (SRM 1944). In the electron beam irradiated samples, the concentrations of 29 PCB congeners decreased with dose (83 % at the highest dose). Photolysis alone led to little dechlorination, but photolysis with added base led to about 60 % dechlorination. Current investigations focus on the dechlorination of contaminants in the presence of food-grade surfactants. Initial results suggest that these additives enhance the electron beam and ultra-violet induced destruction of PCBs in marine sediment.



## Measurement of Ultra Low Sulfur in Fuel Products for Next Generation Internal Combustion Systems

***R.D. Vocke and W.R. Kelly (839)***

Fuel sulfur is the principal poison affecting the sophisticated catalytic converters and on-board diagnostic systems that are central to reducing pollution from advanced internal combustion technologies. As the requirements for reducing these emissions from internal combustion engines become more stringent, extremely efficient and long-lived after-treatment systems are being nationally and internationally mandated by regulatory actions. Through a staged process, petroleum producers are seeking to produce gasoline and diesel fuels that are functionally "sulfur-free" by the end of this decade. In support of these efforts, we have produced two new low sulfur fuel SRMs and one near-zero sulfur fuel SRM that will help producers meet current and evolving regulatory limits.

***The United States has called for a limit of 15 μg g$^{-1}$ sulfur in diesel fuel by 2006 and a limit of 30 μg g$^{-1}$ S in gasoline by 2007. SRM 2723a and SRM 2299 should fully meet metrology needs for sulfur regulation of diesel fuel and gasoline, respectively, at these levels.***

We have completed the characterization of the sulfur content of two low-S fuels and one near-zero-S fuel. Certificates have been issued for SRM 2299, Sulfur in Gasoline (Reformulated), and SRM 2723a, Sulfur in Diesel Fuel Oil, which have sulfur mass fractions of 13.6 μg g$^{-1}$ ± 1.5 μg g$^{-1}$ and 11.0 mg kg$^{-1}$ ± 1.1 mg kg$^{-1}$, respectively. Depending on the applicable national emission standard, near-zero or sulfur-free means a maximum S concentration between 5 μg/g and 10 μg/g. Such near-zero sulfur levels pose significant analytical challenges. SRM 2298, Sulfur in Gasoline (High Octane), was however, certified for a sulfur mass fraction of 4.7 μg g$^{-1}$ ± 1.3 μg g$^{-1}$. Capitalizing on recently improved analytical capabilities stemming from lower sulfur blanks and improved blank control, we are completing work on another near-zero sulfur fuel SRM (Sulfur in Kerosene) whose relative total uncertainty has been reduced by more than an order of magnitude from that reported for SRM 2298. In addition to developing sulfur SRMs, we are the pilot institution for an International Committee of Weights and Measures-Consultative Committee on the Quantity of Material (CCQM) Key Comparison and Pilot Study aimed at assessing participating national metrology institutes' ability to provide accurate measurements of low and very low sulfur diesel fuels. We anticipate that, at the conclusion of the CCQM Key comparison and Pilot Study, we will have another diesel fuel SRM with sulfur mass fraction near 40 μg g$^{-1}$.

**Nitrogen oxides and non-methane organic gases are the primary emissions from internal combustion engines that impact ozone and particulate matter pollution. To reduce these emissions, "after-treatment" systems to further remove these engine gases are being nationally and internationally mandated by regulatory action. Sulfur is the most deleterious "poison" to these systems and impedes the implementation of all major air pollution reduction strategies in the transportation sector.**

### *International Infrastructure for Traceability in Atmospheric Ozone Measurements*

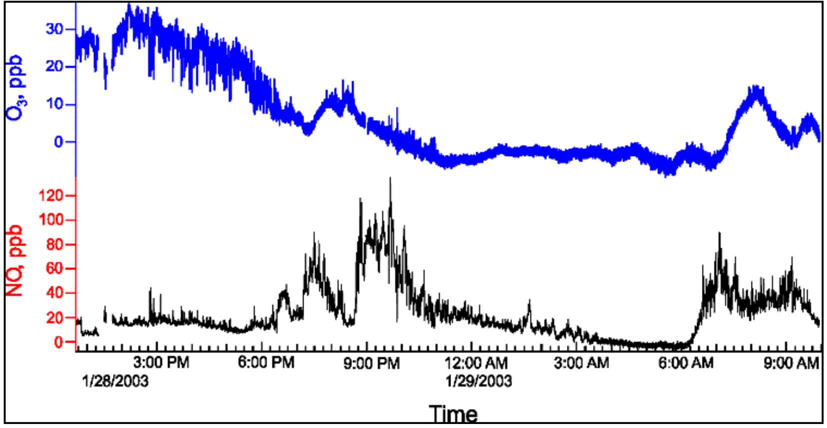
***P.M. Chu and J.E. Norris (839)***

NIST is pursuing several efforts to support the growing national and international interest in the comparability and SI-traceability of global $O_3$ measurements. NIST is partnering with BIPM to develop advanced primary $O_3$ reference standards and to share the dissemination of secondary or transfer standards. The basic project plan includes 1) Transfer the current $O_3$ "national reference photometer" technology to BIPM. 2) Assess the performance of the current standard reference photometer (SRP) with respect to current and future $O_3$ measurement requirements. 3) Develop a primary reference standard with capabilities of SI-traceability verification.

***The concentration of ozone ($O_3$) in the atmosphere remains a significant issue from the perspectives of health, science, and politics. Collaboration with BIPM along with 19 other National Measurement Institutes and the development of the advanced primary ozone reference standard will substantially improve the SI-traceability of $O_3$ measurements.***

**At tropospheric levels, $O_3$ is a health concern and contributes to global climate change as a greenhouse gas, while stratospheric $O_3$ protects earth from harmful UV radiation. Since 1983, NIST has provided the infrastructure for the calibration and traceability of $O_3$ measurements through the distribution of Standard Reference Photometers (SRPs) based on UV photometry to US Environmental Protection Agency (EPA) facilities and 16 laboratories abroad.**

Over the past two years, NIST has been working extensively with BIPM to transfer the responsibility of international $O_3$ measurement traceability to BIPM. This effort has included the construction of five new SRPs, including extensive training of BIPM staff during the construction process. This work completes a major portion of NIST's collaboration with BIPM to transfer the current SRP capabilities to BIPM. As an initial effort to evaluate the current level of international comparability of $O_3$ standards, NIST is participating, along with 19 other National Metrology Institutes in the CCQM-P28 pilot study, led by BIPM. During FY 2003, a gas phase titration experiment was performed to check the consistency of the current SRP $O_3$ measurement capability with NIST NO gravimetric standards and an $NO_2$ permeation calibration system. The results indicate the $O_3$, NO and $NO_2$ standards agree to within 1 %. Under NIST's Small Business Innovative Research (SBIR) Phase II funding, Aerodyne Research Inc. is developing a quantum cascade laser system to serve as NIST's authoritative $O_3$ reference standard. Aerodyne's successful Phase I results have demonstrated that this new technology will significantly extend NIST's measurement capabilities for $O_3$, NO and $NO_2$.



**Demonstration of real-time quantum cascade laser measurements of ambient O3 and NO by Aerodyne Research Inc., SBIR Phase I funding.**

### *Development and Measurement of Heavy Hydrocarbon ($C_6$-$C_{16}$) Gas Standards*

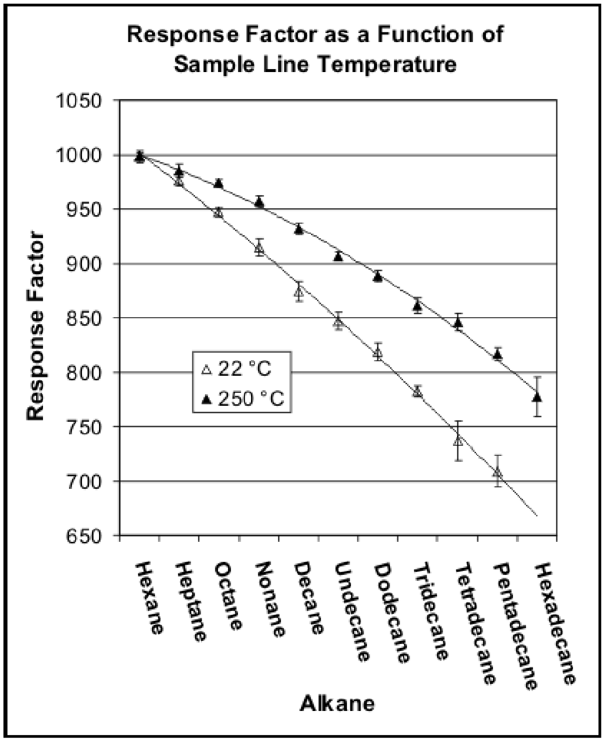
***G.C. Rhoderick (839)***

***CSTL researchers have developed a number of primary gravimetric standards for heavy alkanes to address measurement needs of state and federal agencies that monitor ambient air for compounds related to diesel exhaust.***

Due to the health risk associated with long-term exposure to diesel exhaust from vehicles and stationary equipment, it was requested that NIST develop measurement standards containing heavy ($C_{10}$ – $C_{16}$) alkanes, which could then be used to assure measurement quality in air monitoring and exhaust studies. In response, a number of primary gravimetric standards were developed and analyzed by gas chromatography (GC) with a flame-ionization detector (FID).

While the development of $C_6$-$C_{10}$ compounds as standards was highly successful, $C_{11}$-$C_{16}$ compounds proved more challenging because of difficulties in retrieving these higher-boiling compounds from gas mixture cylinder for analysis. Standards were prepared gravimetrically and intercompared using GC-FID. Taking into account safety issues, system temperatures were elevated to the maximum levels including the FID at 350 °C and the sample transfer system from the gas cylinder to the GC column at 250 °C. GC-FID results agreed very well with the gravimetric values, differing within ± 0.6 % for all hydrocarbons. However, analyses using varying system temperatures exhibited major differences. GC-FID response factors should be the same for each hydrocarbon regardless of system temperature since the FID signal is proportional to the number of carbon atoms detected per unit time. The figure, however, shows that except for hexane, response factors at 250 °C are larger than at 22 °C. These results suggest that the heavier hydrocarbons absorbed within the regulator and the sample transfer line from the cylinder to the GC. However, results show a consistent response for an individual hydrocarbon.

**The results of this work indicate that if the efficiency of sample transfer from the cylinder to the GC remains constant, one should be able to use a heavy alkane standard to determine an unknown provided that the carbon number of the standard alkane is the same as the carbon number of the sample alkane.**



## *Global Climate Change Measurement Needs Development of Gas Mixture Standards to Support*

***R.C. Myers and G.C. Rhoderick (839)***

Primary gas standards for $CH_4$, $CO_2$, $CF_4$, $N_2O$, and $SF_6$ were developed to address quality assurance in measurements of climate change gases. A number of specific drivers for this work can be identified. First, there was a need to replenish the NIST in-house inventory of $CH_4$ and $CO_2$ primary standards for supporting several NIST SRMs containing $CH_4$ and $CO_2$, which are frequently used by the atmospheric research community. A total of 22 gravimetrically prepared primary standards of $CH_4$ in air, many of which were prepared this past year, now exist and are used to define the NIST primary calibration methane scale for laboratory measurements and traceability. This suite of primary standards range in concentration from 0.8 μmol $mol^{-1}$ to 10 μmol $mol^{-1}$.

***Primary standard were developed for several radiatively-active atmospheric gases: methane ($CH_4$), carbon dioxide ($CO_2$), tetrafluoromethane ($CF_4$), nitrous oxide ($N_2O$), and sulfur hexafluoride ($SF_6$).***

Second, primary standards were needed to support the NIST FTIR Spectral Database. Three standards each of $CF_4$ and $N_2O$ were prepared for analysis by FTIR to determine band strength intensities, which are to be archived in the database. The database is then typically used as a reference data standard to determine chemical species concentrations in unknown gas samples and for measurements in real time.

**A number of additional NIST primary standard materials will likely be developed for species that contribute to global warming, possibly leading to the development of new SRMs to support national ambient monitoring of those species.**

Third, primary gas standards were needed for NIST's participation in comparison studies with other national metrology institutes (NMIs). One study with BIPM's Consultative Committee for Amount of Substance, CCQM-P41 Greenhouse Gases, involves several NMIs and laboratories from the World Meteorological Organization in an effort to evaluate and improve the preparation and measurement capability of gravimetrically prepared $CH_4$ and $CO_2$ mixtures. Two primary gas standards, $CH_4$ in air and $CO_2$ in air were submitted by NIST for evaluation. A second BIPM-CCQM study, CCQM-K15, involves $CF_4$ and $SF_6$. Here, a suite of three standards containing $CF_4$ and $SF_6$ were prepared in order to assign values to the CCQM-K15 sample. The third study is a comparison of three NIST primary standards of $CH_4$ in air with primary standards prepared by the Climate Modeling and Diagnostics Laboratory of the National Oceanic and Atmospheric Administration.

# 6. Food and Nutritional Products

CSTL supports the food industry by providing reference measurements and reference standards for quality assurance and to help ensure compliance with the nutritional labeling regulations. CSTL provides robust metrological traceability for nutrients in food products, contaminants and adulterants in food products, detection of genetic modifications in food products, and chemical composition and contaminants in dietary supplements/nutraceuticals. More than 50 % of the U.S. population uses dietary supplements, accounting for roughly $10 billion in sales every year.

**The U.S. processed food and beverage industry is a major participant in the global market. Almost half of the world's top 50 food processing firms are headquartered in the U.S.**

## *Development of Analytical Methods and SRMs for Chemical Characterization of Botanical Dietary Supplements*

***S.A. Wise, T.A. Butler, S.E. Long, M.C. Mildner, E.A. Mackey, K.E. Murphy, K.W. Phinney, B.J. Porter, L.C. Sander, M.B. Satterfield, K.E. Sharpless, L.J. Wood, and L.L. Yu (839); T. Ihara (National Metrology Institute of Japan, AIST).***

***Taxonomically authentic botanical reference materials with assigned values for active and/or marker compounds are needed for quality assurance of analytical measurements associated with the manufacturing process and for the verification of manufacturers' label claims for the dietary supplement industry. Assigned values for contaminants and adulterants are also necessary to address public health and safety concerns.***

In 2001 NIST, the Food and Drug Administration (FDA), and the National Institutes of Health's Office of Dietary Supplements (NIH-ODS) initiated a multi-year program to develop Standard Reference Materials (SRMs) and analytical methods for a number of botanical and botanical-containing dietary supplements; common examples include St. John's wort, ephedra, ginkgo, and saw palmetto. The goal of this collaborative program is to provide SRMs for eight to ten different botanical dietary supplements over a six-year period. Potential health risks may result from contamination (e.g., pesticides, heavy metals), adulteration (presence of unlabeled foreign materials including pharmaceuticals), or variability in product composition (e.g., changes in levels of active constituents). Secondly, product quality and consistency must be maintained, through verification of dietary supplement label claims. These needs can be addressed by the development of analytical methods and reference materials to support chemical measurements for dietary supplements. FDA and NIH-ODS identified Ephedra-containing materials were identified as the highest priority by. A suite of ephedra-based materials has been prepared consisting of authentic ephedra plant material. These materials have been ground and sieved to 80 mesh, as necessary, blended, packaged, and radiation sterilized for distribution as SRMs. Qualitative and quantitative chemical characterization of these five materials is nearing completion. The six ephedrine alkaloids, the primary active markers in ephedra, have been measured at NIST using several analytical methods (i.e., liquid chromatography with UV absorbance detection (LC-UV), LC/mass spectrometry (MS), LC/tandem MS (MS/MS), and capillary electrophoresis (CE-UV) to assign concentration values. Measurements are being done for contaminants such as toxic metals (As, Cd, Hg, and Pb) using inductively

***Ephedra sinica***

coupled plasma MS (ICP-MS) and instrumental neutron activation analysis (INAA). Collaborating laboratories including FDA, National Research Council Canada, and ChromaDex are providing additional analyses of the ephedra SRM suite. An LC and thin-layer chromatography (TLC) profile or "fingerprint" of the plant material will also be provided for use as a reference for comparison to other similar materials.

**NEW SRMS PRODUCED IN FY 03**

**SRM 3240 *Ephedra sinica* Stapf (aerial parts of authentic plant)**
**SRM 3241 *Ephedra sinica* Stapf (dried native extract)**
**SRM 3242 *Ephedra sinica* Stapf (dried commercial extract)**
**(dried extracts either natural or fortified to contain 8 % total ephedrine alkaloids)**
**SRM 3243 Ephedra-Containing Powdered Solid Oral Dosage Form (finished product)**
**SRM 3244 Ephedra-Containing Protein Powder (finished product)**

The next high priority botanical materials for development of SRMs are: G*inkgo biloba*, St. John's wort, saw palmetto, and green tea. The ginkgo materials (plant, extract, and solid oral dosage form) have been obtained and the preparation (grinding, blending, and bottling) of this SRM suite is in progress. Acquisition of the St. John's wort, saw palmetto, and green tea are in progress and will be completed in early 2004. NIH-ODS also plans to expand the scope of this project to include multivitamin and multielement supplements. The development of a multivitamin and multielement dietary supplement SRM, with values assigned for the 35 vitamins and elements listed on the supplement label, will be a major effort in the next two years.

**The availability of SRMs with certified concentrations of active/marker constituents and contaminants provides the measurement tools necessary to assess the quality of dietary supplements.**

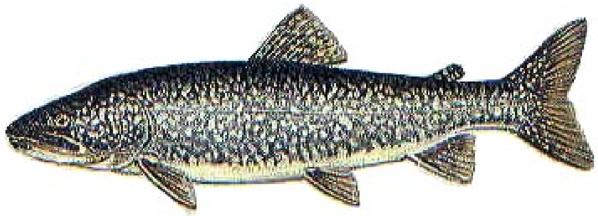
### *Certification of SRM 1946 Lake Superior Fish Tissue – Frozen Fish Tissue for Environmental Contaminants and Food Constituent Measurements*

***S.A. Wise, S.J. Christopher, J.R. Kucklick, S.E. Long, E.A. Mackey, C.S. Phinney, B.J. Porter, R.S. Pugh, D.L. Poster, M. Rearick, KE. Sharpless, and M.M. Schantz (839)***

NIST natural matrix SRMs for organic and inorganic contaminants in marine tissue are necessary for the development and validation of analytical procedures and to provide quality assurance of chemical measurements in the marine environmental and food measurement communities.

***SRM 1946 Lake Superior Fish Tissue, is the first fish tissue SRM, and is intended to address the needs of both the environmental contaminants and food measurement communities.***

SRM 1946 Lake Superior Fish is a fresh frozen tissue homogenate prepared from trout collected in Lake Superior and was developed primarily for use in the determination of organic contaminants with certified and reference concentrations for 42 polychlorinated biphenyls (PCBs) and 17 chlorinated pesticides, which is the largest number of organochlorine compounds with values assigned in any natural matrix SRM or in any certified reference material (CRM) from other national or international sources. SRM 1946 has values assigned for 13 PCB congeners that have not been previously measured in any natural matrix SRMs. This is the first natural matrix SRM with certified values for the three non-ortho-substituted PCB congeners, which are present at 10 to 100 times lower concentrations than the other PCB congeners measured, but they are considered to be the most toxic congeners with respect to mammalian cells and biological systems. The increased number of PCB congeners with assigned values in this SRM was a result of improvements in the analytical methodology used for



these measurements, particularly for the non-ortho-substituted PCB congeners, and the methylmercury.

Because SRM 1946 is comprised of edible fish tissue, the food chemistry community can also use this material for measurements that are necessary to support nutritional labeling requirements. SRM 1946 is characterized for nutrient concentrations including proximates (fat, protein, carbohydrates, ash, and solids), 25 individual fatty acids (the most fatty acids measured in any SRM), and 12 nutritive minerals and trace elements. The individual fatty acids that are certified in SRM 1946 include the nutritionally important omega-3 fatty acids, which have not been certified previously in a natural matrix SRM.

**The development of SRM 1946, with certified and reference values for over 100 chemical constituents, including 3 coplanar PCB congeners and the omega-3 fatty acids, represented a significant technical challenge. This SRM is the only CRM of its kind in the world and will serve as an invaluable quality assurance tool for quality assurance of contaminant measurement programs related to the marine environment on local, regional, and continental scales.**

Of particular importance in both the environmental contaminants and food measurement communities is the determination of total mercury and the more toxic mercury species, methylmercury. Both of these environmentally relevant species are characterized in SRM 1946. This is the first certification of these compounds in a fresh fish tissue homogenate CRM. The certified concentration of methylmercury in SRM 1946 is approximately at the level proposed by the U.S. EPA for freshwater and estuarine water quality criterion (0.3 mg/kg fish). Thus this SRM will be valuable in assessing methods used to monitor methylmercury levels in fish to determine allowable fish consumption advisories.

A companion fish tissue material, SRM 1947 Lake Michigan Fish Tissue has been prepared and measurements are in progress to provide a fish tissue SRM with emphasis on the trace element content to complement SRM 1946, which focuses primarily on the organochlorine contaminants. SRM 1947 will be completed in FY04.

***Publications:*** ***"Determination of Polychlorinated Biphenyl Congeners and Chlorinated Pesticides in a Fish Tissue Standard Reference Material,"*** **Poster, D.L., Kucklick, J.R., Schantz, M.M., Porter, B.J., Leigh, S.D., and Wise, S.A. Anal. *Bioanal. Chem.*, 375, 223-241 (2003).**

***"Determination of Non-ortho Polychlorinated Biphenyls in Environmental Standard Reference Materials,"*** **Brubaker, Jr. W.W., Schantz, M.M., and Wise, S.A., *Fresenius'J. Anal. Chem.*, 367, 401-406 (2000).**

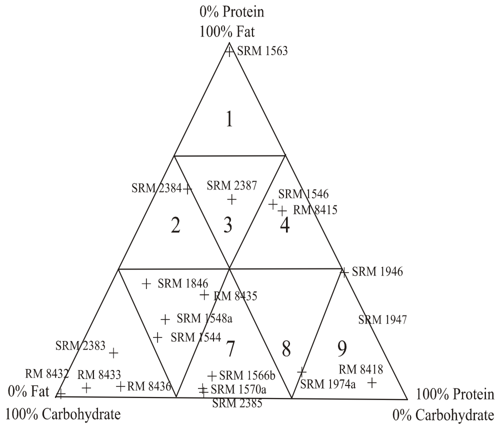
***"Determination of Methylmercury and Butyltin Compounds in Marine Biota and Sediments using Microwave-Assisted Acid Extraction/Digestion, Solid-Phase Microextraction, and Gas Chromatography with Atomic Emission Spectrometric Detection,"*** **Tutschku, S., Schantz, M.M., and Wise, S.A., *Anal. Chem.*, 74, 4694-4701 (2002).**

### *A Complementary Suite of Food-Matrix SRMs to Support Industry Compliance with Nutritional Labeling Requirements*

***K.E. Sharpless, J. Brown-Thomas, C.S. Phinney, B.J. Porter, and L.J. Wood (839)***

***CSTL researchers develop reference materials and analytical methods to underpin measurements made for compliance with nutritional labeling laws, provide traceability for food exports needed for acceptance in many foreign markets, and improve the accuracy of information that is provided on product labels to assist consumers in making sound dietary choices.***

The composition of a food-matrix SRM should be matched closely to that of the test sample being analyzed; a fat-protein-carbohydrate food composition model developed by AOAC International is a useful way to do that. NIST has been working with other federal agencies and the food industry over the past several years to provide a suite of SRMs within this model to address nutrition-labeling issues. Two new food-matrix SRMs were completed this year: SRM 2385 Slurried Spinach and SRM 2387 Peanut Butter. Now, there are food-matrix reference materials with values assigned for nutrient concentrations available within all sectors. Ideally, these materials will improve the accuracy of nutrition information provided on product labels. In the figure, all of the food matrix SRMs are shown in their respective positions in the AOAC triangle.



Now that materials are available within or on the borders of all sectors, this particular aspect of NIST's food and nutrition program can be expected to decrease. Focus will then shift to more specialized analytes (e.g., individual amino acids, allergens, mycotoxins) in foods, as well as to dietary supplements.

### *Keeping Tin Cans Lead-Free*

***J.R. Sieber, K.E. Murphy, and S.E. Long (839)***

When steel mills manufacture tin-plated sheet steel for food cans, they must test the tin and the electroplating bath for lead content. If lead is allowed to accumulate in the tin or the bath, it will become part of the tin coating and may leach into food stored in cans. In recent years, the allowable Pb content of tin plate has been reduced in recognition of potential health risks in products used for foods. Steel mills typically purchase Sn anodes according to ASTM B 339 Standard Specification for Pig Tin, with a restriction of 50 µg $g^{-1}$ Pb maximum. Residual amounts of anode tin are typically remelted on-site to form new anodes. Incoming and remelted tin anodes are analyzed by atomic emission spectrometry to meet material acceptance and process control requirements.

***Tinplate is a high volume commodity made by many US and foreign steel companies. About 3 million tons of tinplate are manufactured and shipped from US steel producers annually, at a typical selling value of $800 per ton during 1999. Container manufacturers purchase much of the tinplate to make products such as food and beverage cans.***

SRM 1727 Anode Tin was developed in cooperation with ASTM International to provide a tool for improving the accuracy of Pb determinations at trace levels in tin, tinplate products, and plating bath solutions. SRM 1727 is certified for Pb content on the basis of analyses by isotope-dilution inductively-coupled plasma mass spectrometry. Information values are provided for ten additional elements. SRM 1727 is sold in solid form (a block 30 mm on each side) for spectrometric analysis and may be chipped for use with chemical methods of analysis.

**SRM 1727 will provide a means for validation of the accuracy of analytical determinations of Pb at trace levels enabling these industries to control their tinplate lines more economically and provide safer products.**

## *Plasmid-Based Standard for Quantitative Real Time Polymerase Chain Reaction (PCR) Measurements*

***M.J. Holden and J.R. Blasic, Jr. (831)***

CSTL researchers are investigating the utility of a synthetic DNA construct for the validation of measurement protocols, equipment, personnel and varied laboratory practices used in the quantitation of specific DNA sequences. This cross-platform material will provide information on the comparability of different platforms and detection probe types.

***Quantitative Real Time PCR is a powerful tool for the detection and quantitation of specific DNA sequences. It has broad applications in areas including: biotech crop measurements, health care, pharmaceutical development, bio-warfare agent detection, as well as basic research.***

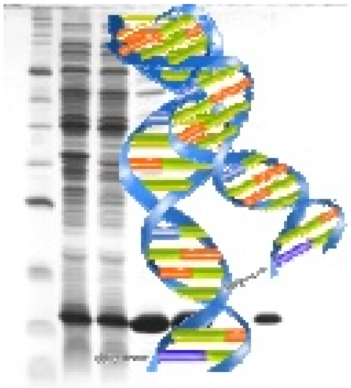
The material for this study and future standard was designed from the ground up so that it could be used with different types of protocols, instrumentation and probes. It was also designed so that it resembled no known gene. The designed DNA sequence (200 base pairs) was synthesized by a self-assembly process and ligated into a plasmid. The plasmid was sequenced, propagated in bacteria, isolated and purified on cesium chloride gradients. PCR protocols were designed and assays conducted to verify the suitability of the material for this purpose. Studies were conducted to determine the recoverability and stability of the plasmid material after lyophilization.

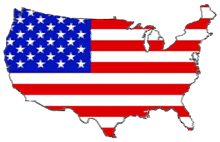
**The material designed by CSTL researchers is being used for the validation of measurement protocols, equipment, personnel and varied laboratory practices applied to the measurement of specific DNA sequences.**

A round-robin intercomparison pilot study was set up by the Comité Consultatif pour la Quantité del Matière (CCQM) under the auspices of the Comité International des Poids et Mesures (CIPM) and the Bureau International des Poids and Mesures (BIPM). The CCQM has the participation of 14 laboratories from around the world including national measurement institutions from 11 countries and the European Union. Multiple sets of lyophilized DNA materials were shipped to the participants. Test materials were prepared so that participants in the study could produce a standard curve of known DNA concentration and then analyze and report on the values of DNA samples of unknown concentrations. The participants could select their instrumentation and probe type and were free to design whatever measurement protocol they deemed suitable. Several of the laboratories utilized more than one probe type and instrument. Very recently, the intercomparison participants returned their data and detailed information on how the studies were conducted. Analysis of the data is now underway.

The results of this study will provide basic information on the comparability of instrument and detection platforms for quantitative real time PCR. It will also shed light on sources of error and point to ways to improve and standardize methods. The study will lead to the improvement of the design of the material itself and clarify the utility of this type of material as a standard for monitoring the quality of quantitative PCR measurements. It is likely that the plasmid material will be redesigned to accommodate detection platforms that have been recently developed.

# 7. Forensics and Homeland Security





CSTL has provided DNA standards for human identification since 1992; however, over the past year the effort has focused on new methods of analysis to respond to the need to analyze extremely degraded samples. CSTL has also had a long history of assessing standards needs and developing new measurement technologies for crime scene investigations in which handguns or explosives are used. The forensic community both for quality assurance and to provide legally defensible analyses uses reference standards. CSTL is also active in the development of microsensor arrays and other measurement technologies for the identification of explosives, as well as chemical and biochemical weapons. CBRNE (Chemical, Biological, Radiation, Nuclear, Explosives) related measurements, methods, and standards is a major new area of expansion for CSTL.

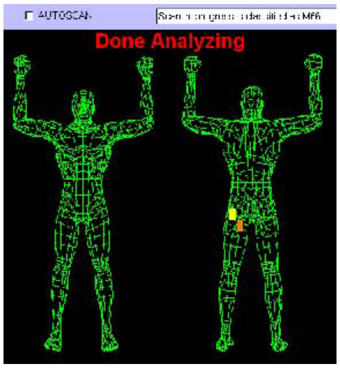
## *Particle Removal Rates from Planar and Cloth Surfaces; A Critical Step for Providing Airport Security*

***R.A. Fletcher, G.J. Gillen (837), and E. Ferguson (SURF Student)***

In a portal detection system, an object or person is subjected to an air jet array that dislodges and transports explosive particles to an ion mobility spectrometer for identification and detection. The ability to remove particles from various surfaces is the critical first step in successful detection of trace explosive residues. CSTL researchers are developing the methodology to determine particle removal rates from various surfaces likely to be encountered in an airport screening environment. We are interested in the conditions necessary to optimize remove of particles of various sizes and compositions.

***CSTL researchers are assisting the Transportation Security Administration (TSA) in the evaluation of next generation walk-through portal explosive screening devices to support security checkpoint screening at US airports.***

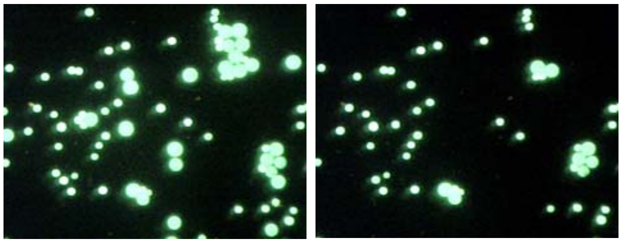
**Commercial walk-through portal for explosive detection.**



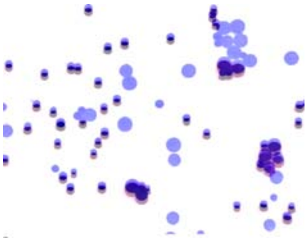
Done Analyzing

To date we have assembled a system to test gas jet removal of particles from surfaces. With this system, we utilize monodisperse fluorescent polymer spheres ranging from 1 μm to 45 μm in diameter as test particles distributed on two materials; polycarbonate surfaces and muslin cloth. In this initial set of experiments, we are using spherical particles in an effort to verify our experimental approach before studying more complex particles (high explosives) and surfaces. After particles are dispersed on the substrate, optical images of the sample surface are taken with a fluorescence microscope using 488 nm laser illumination. A high velocity, computer controlled, nitrogen gas jet is used to remove the particles from the surface. Micrographs are taken before and after exposure of selected areas of the surface to the gas jet. Particle removal rates from the respective surfaces are determined by particle counting implemented through image processing and analysis. The micrographs shown in the figure at right illustrate typical before and after images and subsequent identification of the particles removed by the air jet exposure. Below is a plot of the particle removal efficiency as a function of jet pressure for particles of different sizes on a polycarbonate surface. Here the measured jet pressure relative to ambient lab room pressure ($P_{jet}/P_{atm}$) is plotted against the percentage loss of particles. The pressure ratio is proportional to the gas velocity (and other fluid dynamic parameters) and for $P_{jet}/P_{atm}$ < ca 1.8, the jet is subsonic. The figure demonstrates that the particle removal efficiency is strongly dependent on particle size. Particles in the range of 6 µm to 10 µm in diameter and smaller (1 µm particle data not shown) are tightly bound to the surface and are not effectively removed by our pulsed air jet system. Since the size of explosive particles in trace residues (such as fingerprints) appears to be in the micrometer and smaller range, further optimization of the air jet removal process is needed for effective sampling of these materials.

**Micrograph of 20 µm and 40 µm Fluorescent Spheres on Polycarbonate Before and After Air Jet**

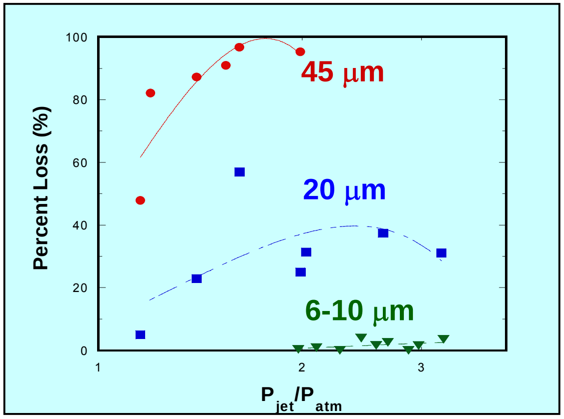


**Before** **After**



**An overlay of these two images (inset) demonstrates identification of removed particles (light blue).**

The work described here may help improve the design of next-generation explosive detectors by defining optimal parameters for air jet removal of particles. The metrology capabilities that we have implemented will also be used to test new and improved methods for particle removal including the use of ionized air and $CO_2$ gas jet systems. As next steps we plan to increase the reliability of the measurement by automating both data acquisition and image analysis.



**Plot of particle loss from polycarbonate filters for 45 µm (circles), 20 µm (squares) and 6 µm to 10 µm polystyrene spheres (triangles) as a function of gas jet pressure relative to ambient pressure. A quadratic fit was made to the data.**

## *Particle Size Characteristics of Trace High Explosives: Pentaerythritoltetranitrate (PETN) and Cyclotrimethylenetrinitramine (RDX)*

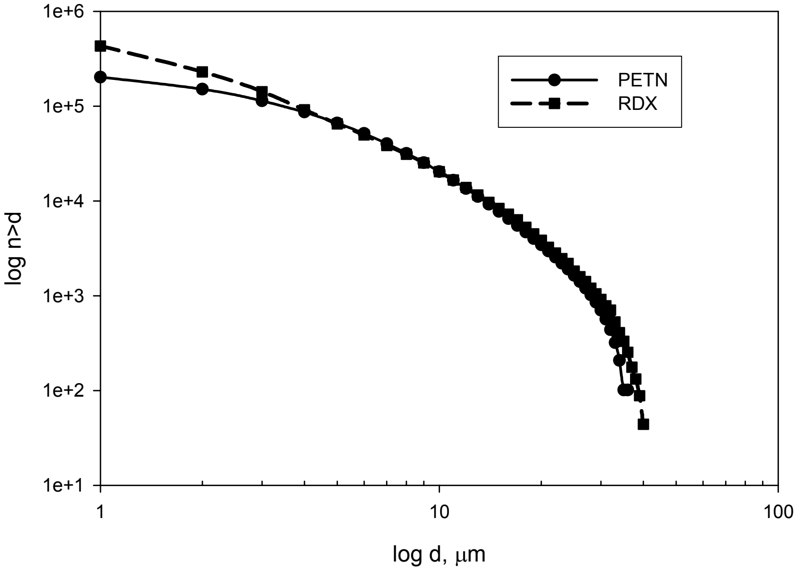
***J.R. Verkouteren (837)***

***CSTL researchers in collaboration with the Transportation Security Administration (TSA), the NIST Office of Law Enforcement Standards (OLES), and the NIST Advanced Technology Program (ATP), are investigating particle size in explosive traces to develop effective methods and materials for testing and calibrating trace explosives detectors.***

The need to screen for explosives at airports and other points of entry has resulted in the deployment of thousands of trace explosives detectors and created a critical need for effective calibration of these detectors. The detection of traces of high explosives is often dependent on collection of explosive particles, particularly for low vapor pressure explosives such as RDX and PETN. Of fundamental importance in determining the effectiveness of any particle collection scheme is the size and distribution of particles expected in the traces left by handling explosives.

Particle collection can be accomplished by many methods, including swiping of surfaces, agitation with air jets, etc. The ability to model particle size in explosive traces was tested by measuring the particle size distributions produced by fragmentation of crystals of RDX and PETN. These particle size distributions were found to deviate from a power law (linear under a log transformation), probably due to a tendency of the explosives, particularly PETN, to smear rather than fragment. The data indicate that more small particles are expected to result from fragmentation of RDX compared to PETN, which matches observations of particle sizes in fingerprints produced from C-4 (a compound containing RDX) and Semtex-A (a compound containing PETN).

To simulate the particle size produced during handling of explosive compounds, crystals of RDX and PETN were removed from the explosive compounds C-4 and Semtex-A, respectively, and crushed to produce populations of particles that were sized using image analysis and scanning electron microscopy. In addition, fingerprints with the two compounds were simulated by handling C-4 and Semtex-A and successively pressing a finger on a series of glass slides for analysis by polarized light microscopy (PLM). The figure shows that the deviation is more pronounced for PETN, with fewer numbers of smaller particles expected than for RDX. These data agree with PLM observation of the fingerprints, as more small particles of RDX are observed, compared with PETN, although the RDX particles tend to be bound by the plastic matrix. Tenth generation fingerprints of Semtex-A contain many wide (up to 100 µm diameter) thin plates of PETN, consistent with a smearing process.



**Plot of the particle size distributions of RDX and PETN particles determined by scanning electron microscopy. The size distributions deviate from the expected power law (linear under a log transformation) resulting from a fragmentation process probably due to the tendency of the explosives, particularly PETN, to smear rather than crush.**

**The results obtained from this work will be used to guide improvements in explosive particle sampling procedures for trace explosive detection equipment.**

Working with TSA, OLES, ATP, and with first responder organizations, our results will be applied to the development of test materials and methods used to determine the effectiveness of trace explosive detectors. Knowledge of the particle size of explosives in trace residues, particularly as they differ for different compounds, will guide the development of challenge samples and sampling schemes. The ultimate goal is to improve detection of explosives residues and therefore support Homeland Security efforts.

### *Adsorption of Energetic Materials (Explosives) on Surfaces*

***T.J. Bruno (838); K.E. Miller (Univ. of Denver)***

***Because of the critical need to detect the presence of explosives, residues, taggants, etc., in a variety of venues (public places, military installations, hostile locations abroad), several highly sensitive detectors have been developed. Before these devices can be used in most such applications, their performance must be certified.***

The development of surrogate soil stationary phases for gas chromatography was pioneered at NIST for the evaluation of contaminant adsorptive interactions in the environment. We have now extended our approach to the liquid phase by producing heat-treated spray dried soil surrogates, and have applied these surrogates to the study of energetic materials (explosives), residues and taggants. The application of our adsorption metrology to energetic materials is important because of the increased emphasis on homeland security. Several new detectors have been developed for in-the-field detection of explosives, but these devices cannot be used until their performance is certified. Certification cannot be done without an explicit knowledge of the vapor phase concentration of the explosive being measured. Since surfaces play a significant role in determining how much material will exist in the vapor phase, an understanding of the adsorptive interactions is critical. The chromatographic approaches that we have developed are particularly apt in providing that understanding.

Following our earlier development of a method to prepare clay soil surrogates (suitable for gas chromatographic adsorption enthalpy measurements), we have now extended the technique to the liquid phase. This was done by preparing a surface by spray-drying a clay suspension to produce 8 µm to 10 µm spherical particles that were subsequently heat-treated at 500 °C. This material was then slurry- packed in an HPLC column configuration. Measurements were done on a series of substituted benzenes (including hetero-substituted benzenes such as RDX) to determine the partition coefficient, retention factor and enthalpy of adsorption, $\Delta H_{ads}$, in the presence of water. Our measurements led us to a predictive correlation of the interaction on the basis of substituents on the main ring. Our correlation allowed us to conclude that the interaction of substituted benzenes on clay is remarkably similar to that on porous graphitic carbon. At the same time, we noted dramatic differences when the interactions were compared on an organic-like surface such as octadecyl silane. In addition to the development of this predictive correlation, we have measured the enthalpy of adsorption of energetic materials on the clay surface in the presence of water. We found, for example, that the $\Delta H_{ads}$ of RDX is 30 % higher than that of benzene on the clay. We have also completed measurements of the enthalpy of solution, $\Delta H_{sol}$, of a number of energetic materials in a polymer, polydimethylsiloxane. Thus, the $\Delta H_{sol}$ of TNB, TNT, and RDX were measured to be 57.9 kJ/mol, 59.5 kJ/mol, and 62.4 kJ/mol, respectively. It is interesting to note that these values are approximately 20 kJ/mol lower than the enthalpy of vaporization, $\Delta H_{vap}$, for these compounds.

**This work has demonstrated the importance of surfaces when considering the free concentration of energetic materials that need to be detected in the field, and the need to have a predictive capability for a variety of compounds. Our observations of the similarity of the clay surface with graphitic carbon, and the enthalpy difference between the bulk solid and absorbed explosives will advance our ability to certify new detection devices.**

### *Development of SEM-EDS Analysis Procedures for C, N And O to Enable Characterization of Explosives Particles, Polymeric And Organic Materials*

***J.T. Armstrong and J.A. Small (837)***

Details provided in the ***Exploratory Research*** Section

### *Determination of Traces of Fissionable Materials Using Delayed Neutron Activation Analysis*

***R.M. Lindstrom, E.A. Mackey, and G.P. Lamaze (839)***

***An important tool in nuclear forensics is the collection and analysis of "swipe" samples at sites where materials of interest may be, or may have been at one time. Tiny traces of fissionable uranium or plutonium can be left behind whenever these materials are handled or transported.***

Detection and measurement of small traces of fissionable uranium and plutonium can be done by delayed neutron activation analysis. The method is intrinsically specific to nuclear fission, the sensitivity is excellent, and the procedure is simple, rapid, and readily automated for high throughput.

Delayed Neutron Activation Analysis is being established at NIST for the measurement of small quantities of fissionable nuclides such as $^{235}$U and $^{239}$Pu. After a brief neutron irradiation, the sample is placed quickly into a neutron detector array and the neutron emission rate measured and compared with that of a standard. The method is well-tested, rapid, specific, matrix independent, nondestructive, and sensitive. The system being built at NIST is calculated to have a detection limit for either of these species about 10 pg, based on a straightforward extrapolation from published practice. The analysis time is less than 2 min per sample.

**IAEA inspector collecting a swipe sample from process equipment in a nuclear fuel fabrication facility in 1994 (*Anal. Chem., 74,* 28A, 2002).**

Preliminary tests have been performed using uranium standards prepared by depositing solutions containing known amounts of uranium onto filter papers, irradiating, and counting on a detection system with one $^{3}$He detector surrounded by hydrogenous moderator. Two shielding configurations were tested, one using water as the moderator and the other using polyethylene. Based on these results and a review of the literature, a final detection system was designed. This consists of ten neutron detectors in a 30 x 30 cm cylindrical moderator of polyethylene, lined with 2 cm of lead to absorb gamma radiation. The design of the manual system incorporates the existing pneumatic rabbit assembly for irradiation control. Incorporated in its design is the ability to unload a sample from the receiver after irradiation with compressed air. This feature will be used to move the sample rapidly to the neutron detector through a polyethylene flight tube.

In FY 04, we plan to improve sample throughput, the transfer system can be readily automated with computer control. It has been demonstrated elsewhere that $^{233}$U, $^{235}$U, and $^{239}$Pu can be distinguished by the relative yields of delayed neutron precursors with different half-lives, and of several fission products. We plan to add a gamma-ray detector into the neutron moderator to exploit this signature.

**This research will provide the Nation with a readily accessible, rapid means of measuring traces of fissionable U and Pu in samples of forensic interest. In addition, the specificity and sensitivity of this method of analysis will be put to use in certifying trace uranium in Standard Reference Materials.**

### *Mapping the Protective Antigen Binding Site on the Anthrax Toxin Receptor*

***K.D. Ridge (831)***

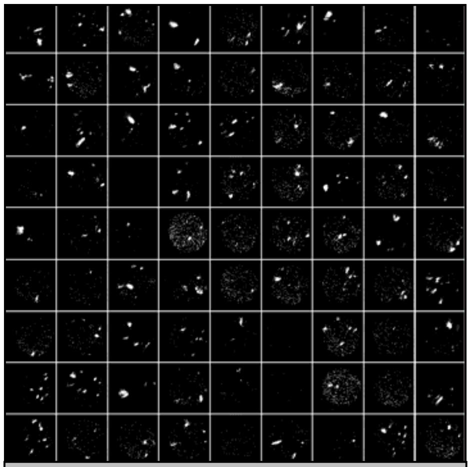
Details provided in the ***Exploratory Research*** section.

## *Automated Analysis of Organic Particles Using Cluster SIMS*

***G.J. Gillen (837)***

***CSTL researchers explored the uses of SIMS particle searching, combined with energetic cluster ion bombardment to rapidly characterize the distribution of explosive and narcotic particles on various surfaces to support continued development of trace detection screening technologies for homeland security.***

Trace analysis of explosive and narcotic residues is important for homeland security, customs, forensic, law enforcement, and environmental applications. Key issues of research for these applications include the nature of the trace residues to be sampled (particle size distribution of explosives and narcotics in fingerprints, for example), the efficiency of various types of particle collection systems, and the development and characterization of organic particle standard reference materials. Each of the areas requires the use of analytical techniques that can rapidly and uniquely identify micrometer-sized explosive/narcotic particles among a background of ambient particles. We are evaluating secondary ion mass spectrometry (SIMS) for this application. Automated imaging analysis of <u>organic particles</u> by SIMS has not previously been feasible due to the sensitivity limitations imposed by low molecular secondary ion yields, degradation of the sample by the primary ion beam and the prohibitively long acquisition times that are required to survey multiple fields using low primary dose SIMS conditions. However, recent research suggests that the use Cluster SIMS, which provides large enhancements in molecular ion yield and a reduction in beam-induced damage may overcome the previously mentioned limitations, making organic particle searching feasible. In the present study, we explore the uses of SIMS particle searching, combined with energetic cluster ion bombardment using $SF_5^+$ or $C_8^-$ primary ions, to rapidly characterize the distribution of explosive and narcotic particles on various surfaces to support continued development of trace detection screening technologies for homeland security. Characteristic SIMS mass spectra were obtained from particles (a few micrometers in diameter) of various pure explosives and narcotics dispersed on silicon wafers using either $SF_5^+$ or $C_x^-$ (x=1-10) primary ion bombardment. As expected, cluster bombardment provided large enhancements in molecular secondary ion signals compared to monoatomic bombardment under identical conditions. For example, $C_8^-$ bombardment gave characteristic molecular ion signals for explosives that were 300 to 1000 times greater than for $C_1^-$ bombardment under identical conditions. All of the samples examined also exhibited significant reductions in beam-induced damage under cluster bombardment. The combination of high secondary ion yields and reduction in beam-induced damage allows us to image the distribution of organic particles while achieving high count rates and short acquisition times. SIMS particle searching is currently being successfully employed in our laboratory to determine the distribution of explosive particles in fingerprints, the efficiency of different types of explosive particle collection filters and to study the release of particles during the sampling process.



**Mosaic of secondary ion microscope images of acetaminophen (m/z = 152). Image acquisition time 20 s. $SF_5^+$ primary ion bombardment with beam dose during acquisition of $2.3 \times 10^{13}$ ions/cm$^2$.**

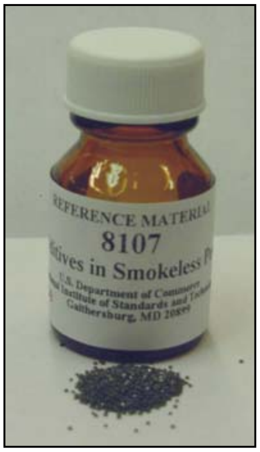
**Cluster beam SIMS offers increased secondary ion signals and the capability for minimizing beam-induced damage and therefore allows for the rapid characterization of organic particles on surfaces.**

## *Certification of NIST Reference Material Supporting Improvised Explosive Device Measurements*

***W.A. MacCrehan and M. Bedner (839)***

***Smokeless powder is a blend of nitrocellulose and a series of additives including the propellant nitroglycerin (NG) and stabilizers such as diphenylamine (DPA) and ethyl centralite (EC, N, N'-diethyl, N, N'-diphenylamine) along with N-nitrosodiphenylamine (NnDPA) that forms during aging of DPA-stabilized nitrocellulose.***

As part of their investigations of explosives crime, national, state and local forensic laboratories measure the additives in smokeless powder (gunpowder). US military laboratories also measure the chemical composition of such propellant powders to determine the stability of munitions. Improvised explosives devices (IEDs), such as pipe bombs, most commonly have smokeless powder as the active filler ingredient. When investigating an explosives incident, forensic investigators collect residues of the incompletely burned powder from the crime scene. As part of the evidence investigation process in the forensic laboratory, the physical appearance and chemical composition of the powder are carefully measured. This permits a categorization of the residue powder for comparison to samples of known powders. The resulting information can often be used to link residues to powders recently purchased by or collected in evidence from the perpetrator.



To help assure the quality of forensic measurements of evidence such as explosives residues, the forensic community has a certification program for laboratory measurements, called ASCLD/LAB (American Society of Crime Lab Directors/Laboratory Accreditation Board). As part of a five-year certification process, laboratory methods and operator proficiency are tested with challenge samples of known properties. Currently, there are no test or reference materials to support explosives measurements. To address the need for forensic explosives reference materials, the NIST Office of Law Enforcement Standards (OLES, in conjunction with the National Institute of Justice) and the NIST SRMP have sponsored the development of RM 8107 Additives in Smokeless Powder. In addition to the need of forensic laboratories, precise measurement of the chemical composition of smokeless propellants is critical to the US military community as a means of assuring the safety of munitions. Certification of NIST RM 8107 will promote accurate forensic and military smokeless powder measurements.

**In 2004 NIST will release RM 8107, Additives in Smokeless Powder thus providing a reference material to support the quality of forensic and military smokeless powder measurements.**

RM 8107 consists of a rifle-type ball powder. A major powder manufacturer, Primex Technologies, provided 4 kg of rifle powder from a single, carefully blended manufacturing batch. For the certification measurements of the four additives, an ultrasonic solvent extraction method was developed to recover the analytes. Two separation techniques were used to determine the NG, DPA, NnDPA, and EC content: micellar capillary electrophoresis and liquid chromatography (LC). The mean values obtained by the two techniques were in good agreement. Since the LC technique provided lower uncertainty in the measurements, it is being used for the certification measurements.

With the development of this smokeless powder reference material, forensic laboratories will have a reliable material for the development and validation of measurement methods for improvised explosives devices. The reference material may also be used as a proficiency challenge sample to test operator and laboratory performance in explosives measurements as part of laboratory accreditation activities. In addition, US military laboratories can use the additive certified reference values in the NIST reference material to assure accurate monitoring of munitions stability.

The smokeless powder RM 8107 was developed to help assure the quality of ‘low explosives’ type measurements. However, ‘high explosives’ such as TNT and RDX (ingredients of military and plastic explosives) are often used in terrorist explosive devices. In conjunction with NIST’s OLES and the DHS’s Office of Domestic Preparedness, we plan to develop a reference material for these high explosives. A solid matrix material will be coated with commonly used military explosives. The goal is to develop a particulate explosives material that can be used to test explosives detection equipment used for airport security screening, evaluation of suspicious packages by first responders, and forensic laboratory post-blast investigations.

## *Refractive Index Measurements of FBI Liquid for Forensic Glass Particles*

***J.R. Verkouteren (837)***

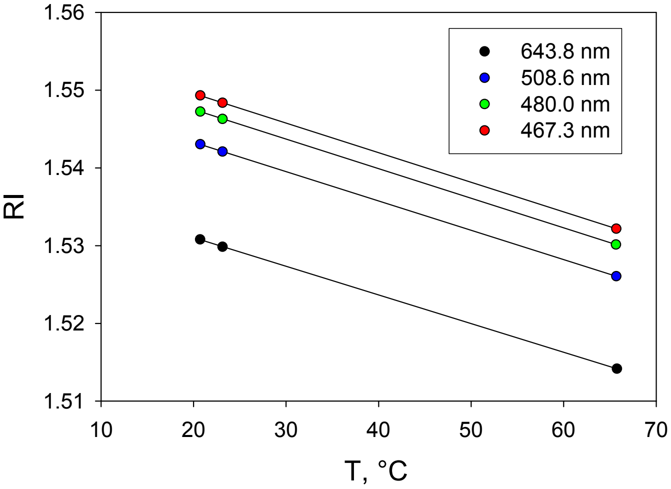
The FBI uses a light-microscope-based heating stage technique for refractive index measurements, and disseminates reference materials and training to hundreds of crime labs throughout the country. The measurement technique requires that the glass particles be immersed in a liquid, the refractive indices of which are known for wavelengths spanning the visible range, and for temperatures ranging from ambient to 100 °C. The success of litigation often hinges on the accuracy of these measurements; therefore the FBI requested calibration of their immersion liquid through the Office of Law Enforcement Standards (OLES) using a highly precise technique developed in CSTL.

***Microscopic glass particles play a major role in forensic analysis aimed at linking individuals to crime scenes. Refractive index can be used to prove a common source of glass particles, and is readily measured from microscopic particles using relatively inexpensive equipment.***

The refractive indices of the FBI liquid were measured at four wavelengths in the visible and at three temperatures ranging from 20 °C to 65 °C using the method of minimum deviation (see figure). The data were modeled to provide equations to calculate refractive index at any temperature from 0 °C to 100 °C and at any wavelength in the visible. The uncertainties are an order of magnitude larger than the level requested by the FBI owing to limitations of the liquid itself. The FBI liquid is very viscous and optical homogeneity was compromised, probably as a result of persistent temperature gradients on the order of a few hundredths of a degree C. As the light microscope technique used by the FBI depends on temperature stability of the same order for optimum refractive index accuracy, our results indicate that this liquid is probably not the best choice for a reference liquid.

**Crime labs throughout the country collecting refractive index data on glass particles must know the accuracy of the technique in order to support their results in the courtroom. Our work with the FBI will help to establish the uncertainties of the method by working to establish appropriate reference materials.**

The work described here established the need for a new immersion liquid. Therefore, NIST's Physics Laboratory, in collaboration with CSTL will produce a glass standard reference material as a calibration material for the immersion technique. With both a glass SRM and a calibrated liquid, crime laboratories will be able to determine the uncertainties of the refractive index immersion technique.



**Plot of the refractive indices of the FBI immersion liquid provided to NIST for calibration. The sample was measured at 4 wavelengths in the visible and at 3 temperatures ranging from 20 °C to 65 °C using the method of minimum deviation.**

## *NIST Support of the CDC Chemical Counter-Terrorism Laboratory Network*

***K.E. Murphy, M.M. Schantz, B.A. Benner, Jr., T.A. Butler (839)***

***NIST has developed an improved method for the determination of cyanide concentrations in whole blood samples and is collaborating with the Emergency Response and Air Toxicants Branch of the Centers for Disease Control and Prevention (CDC) to help provide quality assurance and measurement proficiency standards for the "Chemical Counter-Terrorism Laboratory Network" (CTLN).***

Cyanide is considered a likely candidate for use in chemical terrorism because it is easy to obtain and has been used in previous attacks. Cyanide is analytically challenging, and because it is unstable, blood-based standards are not commercially available. NIST developed an improved method for the determination of cyanide in whole blood based on headspace gas chromatography with mass spectrometric detection (GC/MS), employing isotopically labeled cyanide ($K^{13}C^{15}N$) as the internal standard. Use of the isotopically labeled internal standard coupled with GC/MS resulted in decreased analysis times and improved accuracy and precision. The improved method has been adapted by CDC for transfer to state laboratories within the CTLN and is being used to provide value assignment and stability testing data for a pool of cyanide-spiked blood samples produced by CDC for the CTLN.

At the start of this project, NIST did not have an in-house method for the determination of cyanide. A method based on headspace analysis with GC/MS detection was implemented and improved by the use of an isotopically labeled internal standard. An interagency agreement with CDC has been established to evaluate the content and stability of cyanide in spiked, frozen human whole blood. NIST has started a series of periodic analyses that will proceed over the upcoming year to provide value assignment and monitor the stability of the cyanide concentrations in the proficiency standards.

**A commercial supplier has prepared proficiency standards containing different levels of cyanide and approximately 700 vials per level have been packaged. Preliminary results indicate that the cyanide concentrations in all levels of the frozen proficiency standards have remained stable for the past two months.**

It is imperative that measurements made in response to a chemical terrorism event be both rapid and accurate. There are currently no standards to underpin the accuracy of blood cyanide measurements. NIST's value assignment and stability monitoring of these blood based cyanide proficiency standards will enhance CDC and state public health preparedness in the event of a chemical terrorism incident involving cyanide.

***Future Plans:*** **NIST will monitor the stability of cyanide in the frozen human whole blood proficiency standards over the upcoming year and will continue to work closely with CDC to meet the needs of the CTLN, which has grown from the original five members to include laboratories in 46 states across the U.S.**

## *Cell-Based Sensors for Screening Toxins*

***L. Locascio and J. Travis (839); A. Suggs, B.J. Love, and N.G Love (Virginia Polytechnic and State Univ.)***

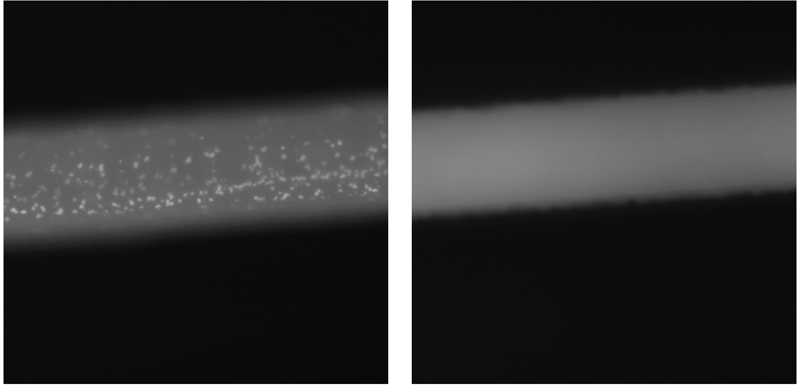
This collaborative effort to develop a cell-based sensor has focused on the detection of electrophilic chemicals in water since for many years Love's group has studied the negative biological response of bacterial cells to this class of toxins. In the presence of electrophilic toxins, *E. coli* cells rapidly efflux a large amount of potassium into the surrounding media, a response that contributes to bacterial deflocculation and ultimately bioreactor failure. Prototype bioreactor devices are designed as microfluidic chips containing immobilized bacteria with integrated optodes to detect potassium efflux upon exposure to electrophiles. We are targeting the device for the detection of toxins in wastewater streams but also hope to extend this work to screen for toxins in drinking water.

***CSTL researchers and collaborators work to develop a cell-based microfluidic biosensor that can serve as an early warning system for the detection of chemicals in the wastewater that may cause bioreactor failure.***

Contamination of the bioreactor, the heart of the wastewater treatment plant, from chemical dumping can have a devastating impact on wastewater processing. In fact, system shut-down caused by bioreactor failure often results in the diversion of large amounts of raw untreated sewage to rivers and streams with an obviously detrimental effect. Several different classes of chemicals, including electrophilic toxins, feeding into water treatment plants have been known to cause bioreactor degradation leading to system processing failures. Recent results from Love's group have led to the hypothesis that sludge deflocculation or biofilm detachment, a mechanism by which the bioreactor can fail, occurs through the activation of the glutathione-gated $K^+$ efflux (GGKE) system stimulated by electrophilic toxins. Therefore, prior to biofilm detachment, bacterial cells expel large amounts of potassium from inside of the cell to the outside. In this collaborative effort, we have designed a cell-based microfluidic biosensor to detect the presence of electrophilic toxins in water streams entering wastewater treatment plants in an effort to prevent failure of the bioreactor. The approach is to immobilize bacterial cells in a microfluidic system and monitor cell response, in particular potassium efflux, as the water flows through a small bed of immobilized cells. In essence, we have created a micro-bioreactor. The behavior of our micro-bioreactor is then used to predict the behavior of the process bioreactor when exposed to the same water supply. A negative response in the microsystem can be used to decide when to divert part of the incoming stream to prevent process failure. The microbioreactor has several key components including an immobilized bed of live bacterial cells (*Escherichia coli* K-12); an optode film that is responsive to potassium ions; an optical detection unit; and microfluidic channels to move cell media and the water samples through the cells and past the optode film.

**In FY 03, we focused our attention on two aspects of this project:**
**(1) cell immobilization in polymer microchannels, and (2) development of a miniaturized optical detection component.**

Love's group hypothesized that cell adhesion to an abiotic polymeric surface would be influenced by several factors including the chemical characteristics of the bacterial membranes; the physiological state of the cells at the time of adhesion; and the characteristics of the polymer material used to fabricate microfluidic channels. Earlier work, published in 1987 by another research group in the *Journal of Microbiology*, was performed to determine the characteristics of the bacterial membrane, specifically the relative hydrophobicity of *E. coli* K-12 cells in different growth media, that could affect cell adhesion. Experiments showed that cells grown in an N-limited medium (20:1 C:N) exhibited greater cell surface hydrophobicity than a balanced C:N media supporting previous findings published by a research group in the Journal of Applied Microbiology in 1999. Based on these results, polymers were chosen that were expected to enhance cell adhesion and these were tested in cell binding experiments. *E. coli* cells were also immobilized onto polymer surfaces coated with polyelectrolyte multilayers. It was determined that the cells adhered better to uncoated polyethylene terephthalate glycol (PETG) surfaces than to PETG surfaces coated with polyelectrolyte (see figure).



**Fluorescence image of PETG microchannels after exposure to *E. coli* cells. Fluorescent dots indicate the presence of live cells. A) Uncoated PETG channel, and B) channel coated with polystyrene sulfonate.**

The detection system was miniaturized to make the device more suitable as a prototype field device. A microfluidic device was mounted on a small holder and a miniaturized light source was coupled to the channel using fiber optics. Light was collected through fiber optics into a hand-held spectrometer (Ocean Optics) coupled to a laptop computer for data processing. This device is now being evaluated to measure the optode film response in the presence of varying concentrations of potassium. We hope to expand this work to the development of a microfluidic biosensor for detection of chemical and biological toxins in drinking water.

**The development of a hand-held device for in-situ monitoring of environmental toxins in water streams could provide a reliable early warning system can have a profound environmental and financial impact by detecting the problem before failure occurs.**

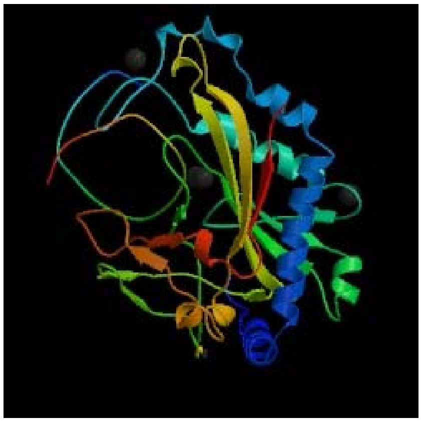
### *Standards for Detection of Biological Threat Agents*

***K.D. Cole, L. Wang, A. Gaigalas, and D. Hancock (831)***

CSTL scientists are characterizing surrogates that can safely be used for the testing of detection devices, remediation technologies, and personnel training. There are many instances where these surrogates are preferable to the use of the actual bacteria, viruses, and toxins. We have identified several surrogates and are in the process of developing methods to fully characterize them. We will use these well-characterized surrogates as model systems to study and improve the instruments and devices used for the detection of biological threats. If necessary, follow-up work will be performed at BSL-3

***CSTL researchers are working with the Department of Homeland Security (DHS) and other government agencies to determine the best ways that NIST can meet the needs for data, measurements, and reference materials for the detection of biological threat agents.***

laboratories that are able to handle the live agents. Additionally, the detection of biological threats is done from environmental samples that represent serious challenges because of their complex and variable compositions and the possibility of interferences.



**_Bacillus globigii_, crystal structure from PDB**

To date, we have identified several surrogates for the biological threat agents that we can safely use in our laboratories. The surrogates include a spore-forming bacteria (Bacillus globigii), an RNA virus (the bacteriophage MS2), and the Ricin A chain. Detection of biological threats currently depends upon either recognition by antibodies or detection of specific nucleic acid sequences. We are in the process of developing improved sensitivity and reliability of the assays. The methods we are studying include classical microbiological techniques, flow cytometry of fluorescent-labeled surrogates, fluorescence microscopy, and DNA based assays such as PCR.

CSTL scientists will continue to build strengths to characterize these surrogates and identify new ones. The devices and instruments need to be tested using realistic surrogates and from complex environmental samples. NIST can help assure national security by helping to test these devices and proving the reliability of both data and measurements. The surrogates will be used to study the devices and instruments used to detect existing as well as new emerging threats. These complex organisms and biological molecules will require sophisticated techniques to characterize them. New devices and instruments that are being developed will also need well-characterized surrogates to determine their performance.

**The development of methodologies to characterize surrogates for biological threats is important and challenging because of the needs of accuracy, sensitivity, and reliability. It is essentially certain that well-characterized surrogates will be used for standardizing the devices and instruments used for the detection and measurement of these threats.**

## *Creation and Characterization of SRM 2395—A Human Y-Chromosome DNA Profiling Standard*

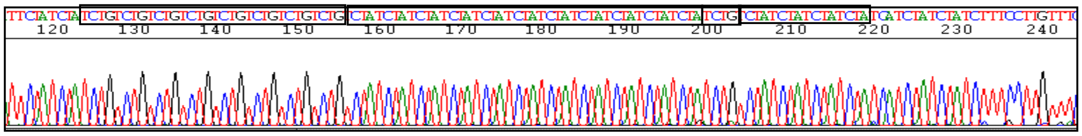
***J.M. Butler, R. Schoske, P.M. Vallone, J.W. Redman, and M.C. Kline (831)***

The Y-chromosome is passed largely unchanged from father to son and contains genes that endow the recipient with male characteristics. Forensic DNA testing laboratories, genetic genealogy companies, and academic groups studying human history perform Y-chromosome testing by examining a number of genetic markers. The ability to obtain consistent and reliable results between laboratories that share information through DNA databases is critical. To address this need, NIST has developed a human Y-chromosome DNA profiling standard, SRM 2395, that can be used to check the accuracy of analytical methods used to measure Y chromosome variation at common short tandem repeats (Y-STR) and single nucleotide polymorphism (Y-SNP) markers. One unit of SRM 2395 consists of six frozen tubes of DNA, each of which contains approximately 100 ng of well-characterized extracted human genomic DNA. There are five male samples (Components A-E) and one

***SRM 2395 has been produced with multiple male DNA samples that have been characterized at common Y-chromosome short tandem repeat and single nucleotide polymorphism markers to enable calibration of results obtained with either research assays or commercial kits.***

**SRM 2395 is being used already in genetic genealogy companies such as Relative Genetics to calibrate their results. New Y-STR typing kits from ReliaGene Technologies and Promega Corporation have used our SRM to verify the accuracy of their assay.**

female sample (Component F) packaged in a single box. The female specimen may be used as a negative control for Y-chromosome specific assays. The Certificate of Analysis lists typing results for 31 Y-STR and 42 Y-SNP markers commonly used in Y-chromosome testing. DNA sequencing has been performed on 22 of the 31 Y-STRs to permit an even more extensive characterization of those markers.



**Sequencing results for the Y-STR marker DYS390 on SRM 2395 component E. The sequence indicates that there are 24 repeats present with the motif of [TCTG]$_8$ [TCTA]$_{11}$ [TCTG]$_1$ [TCTA]$_4$**

## *Evaluation and Automation of Single Nucleotide Polymorphism Typing (SNP) Methodologies for Mitochondrial DNA (mtDNA)*

***M.C. Kline, J.W. Redman, P.M. Vallone, and J.M. Butler (831)***

Procedures for analysis of mtDNA are laborious and expensive because they typically involve DNA sequencing of more than 600 base pairs in the control region across hypervariable region I (HVI) and hypervariable region II (HVII). Therefore, techniques for rapid screening of samples, particularly to weed out ones that do not match, will enable more samples to be run by forensic DNA laboratories in a timely and cost-effective manner.

***Mitochondrial DNA (mtDNA) is used in human identity testing for mass disaster reconstruction, missing person investigations, and forensic casework involving highly degraded DNA samples.***

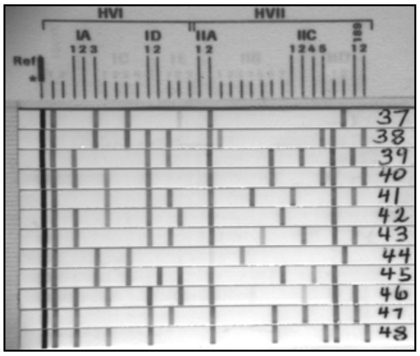
**NIST provided beta-testing of a commercial mtDNA system, and automation of the process. The commercial system offers forensic laboratories doing human identification a rapid test for screening samples that may or may not require labor-intensive full sequencing.**

Roche Molecular Systems has developed a system that probes ten sites in HVI and HVII. Their manual process of hybridization and detection was adapted to an automated format at NIST as part of beta-testing their product. In addition, we ran 666 U.S. population samples to evaluate the power of discrimination for the mtDNA LINEAR ARRAY test. The 666 NIST samples resulted in 282 different types, of which 185 were seen only once in our population. These unique samples represent 65.6 % of the types seen and 27.8 % of the people. Several groupings of samples seem to represent specific ethnic groups. Fifty-one samples were determined to be mitotype 1111111111 by LINEAR ARRAYs. After SNP typing the 51 samples could be sub-typed to 12 different SNP types with four samples being unique.

An automated procedure for processing the mtDNA LINEAR ARRAYS using a Tecan/Profiblot hybridization robot is under development. Analysis of the mtDNA mitotypes/haplotypes in 128 Hispanic, 252 African American, and 286 Caucasian population samples doubled the available population data on U.S. samples. We discovered that the pH of the washing buffer is critical to obtaining successful results, and this information is now included in the technical manual for this product.

**LINEAR ARRAYS allow laboratories without labor-intensive sequencing capabilities to rapidly screen mtDNA samples**

A paper describing the automated LINEAR ARRAY processing is in preparation. We are in the process of developing new SNP assays to increase the power of discrimination for mtDNA.



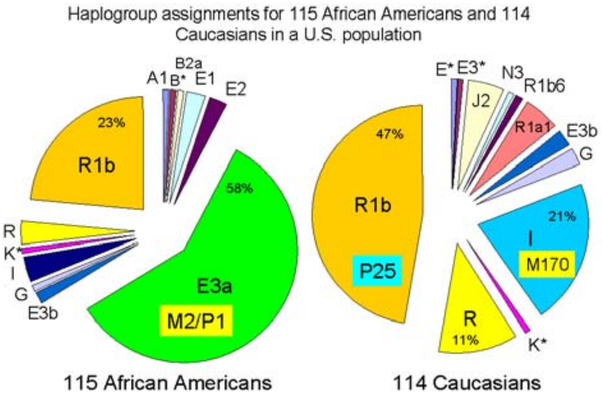
**This figure shows the mtDNA LINEAR ARRAY results for 12 different DNA samples. These samples were processed in an automated fashion in a method developed at NIST as part of beta-testing this Roche Molecular Systems product.**

## *Typing Single Nucleotide Polymorphisms Located in the Y Chromosome*

***P.M. Vallone and J.M. Butler (831)***

A single nucleotide polymorphism or SNP is a single base change in the human genome. A SNP is predicted to occur every 1000 bases or so in the human genome. There is interest in typing SNPs for human identity (forensic) purposes. More specifically, SNPs located on the Y chromosome are attractive typing candidates due to their low mutation rate and male specificity. The fact that the Y chromosome is only found in males makes genetic markers located upon it useful in mixed male-female sample (as found in a sexual assault crime). The low mutation rate of the Y chromosome SNPs implies potential for determining the geographical origin of an individual.

***To date, few studies have been performed evaluating the frequency of known Y chromosome SNPs in U.S. populations. By evaluating the frequency of 50 well-characterized SNP sites in over 200 U.S. population samples we provide a useful database of DNA typing information.***



**This figure represents the distribution of various Y chromosome haplogroups (Hg) for 2 U.S. populations. Hg R1b represents the majority of the Caucasian samples while 58 % of the African American samples are in Hg E3a. Note there is an admixture of 23 % of Hg R1b (major Caucasian Hg) observed in the U.S. African American population.**

Here, we used a commercial DNA typing kit and custom assays developed in-house to determine the frequency of 50 Y SNPs in 2 U.S. populations (African American and Caucasian). The two assay methodologies, hybridization and primer extension, indicated full concordance for typing eight overlapping SNP sites. The overall success rate for typing that samples was over 99 % for either methodology. A pie chart summary of the data is illustrated in the figure. SNP frequency distributions for the 50 genetics markers typed indicated groupings that were unique to each population. 47 % and 23 % of the Caucasian and African American samples fell into haplogroup R1b respectively. 58 % of the African American samples were found to be in the haplogroup E3a. It is of interest to note that none of the Caucasian samples fell into haplogroup E3a making it a population specific grouping. This information is useful in determining the forensic utility of using Y-SNP in forensic investigations.

CSTL researchers have provided forensic scientists with population data on newly discovered genetic markers. This work supports companies who have an interest in developing new forensic testing kits. By providing typing data for the 50 Y-SNPs in a U.S. population a company can select the appropriate markers that will be most beneficial for human identity testing.

**CSTL researchers have completed one of the first studies involving Y-SNPs frequencies in U.S. populations. The results of this study have been submitted for publication to the *Journal of Forensic Science*. This work was presented at the International Society of Forensic Genetics, Arcachon, France (Sept 2003).**

# 8. Health and Medical Technologies

CSTL provides quality assurance for the healthcare industry by providing the appropriate chemical and physical reference standards, developing reference methods and databases, and also by working closely with the clinical and medical community to transfer the accuracy of the highest level of metrology to high-throughput clinical laboratory measurements. Measurements are responsible for up to 15% of the $1.3 trillion annual costs of healthcare in the U.S. A significant portion (25% to 30%) of these measurements are performed for non-diagnostic reasons, such as re-tests, error prevention, and detection.

**Healthcare costs amount to ~ 14% of the GDP, an estimated $1.5 trillion. By 2007, healthcare spending as % of GDP is projected to reach over 16%.**

## *HIV Protease Structural Database*

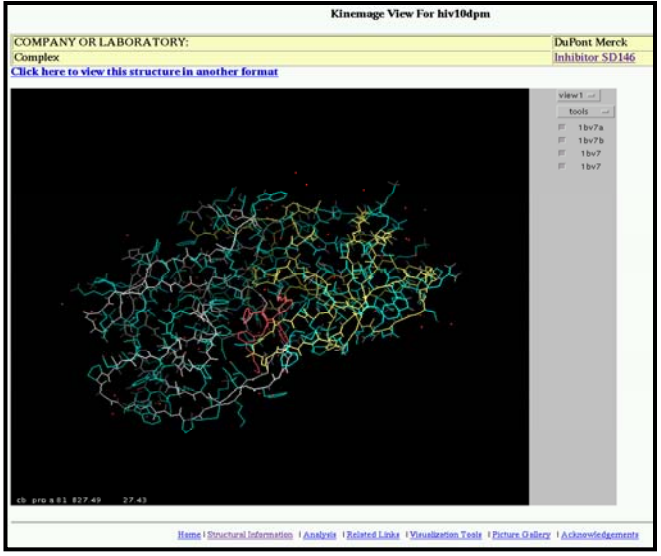
***T.N. Bhat, G.L. Gilliland, V. Ravichandran, and M.D. Prasanna (831); J. Vondrasek and A. Wlodawer (NCI/NIH)***

The HIV Protease Database (HIVdb) contains experimentally determined three-dimensional structures of human immunodeficiency virus type 1 (HIV-1), human immunodeficiency virus type 2 (HIV-2), and simian immunodeficiency virus (SIV) proteases and their complexes with inhibitors or products of substrate cleavage. The structural holdings of the HIVdb are from two sources, the Protein Data Bank and from academic, government and industrial laboratories that have not deposited the coordinates in the Protein Data Bank.

***Developed in collaboration with NIH, the HIV Protease Database is a web-based archive for HIV protease structural information located at*** **http://srdata.nist.gov/hivdb.**

**Recently cited in Science's NetWatch ("Databases: HIV's Achilles Heel," *Science, 300,* 1349, 2003), the HIVdb provides macromolecular structural data on HIV and related proteases as well as data analysis tools for scientists engaged in research and drug development in the fight against AIDS.**

The HIVdb permits the user to customize the query to retrieve the desired information. The query interface provides the option to select entries by the year in which the structures have been solved, resolution range, R Factor, strain in which the proteases were isolated from, inhibitor type, company or laboratory in which the structures were solved, virus type and citation or depositor author. In addition, the users have the option of dynamically structuring the report page that is generated. Future efforts will focus on converting the web server language from Active Server pages (ASP) to Java-based server languages for the option of incorporating readily available software.



Kinemage View For hiv10dpm

COMPANY OR LABORATORY: DuPont Merck

Complex: Inhibitor SD146

## *Standards Requirements for Systems Biology Approaches to Health Care: Mitochondrial Proteomics*

***G.B. Vásquez and P.E. Barker (831); S. Zullo (ATP/NIST)***

The health care community, particularly that involved with clinical diagnostics, is moving towards a systems biology or proteomic approach to disease detection and diagnosis. A broad set of human health issues relate to mitochondrial function, from the biology of cancer development to issues relating to homeland security that include bioterrorism and chemical toxicities. Mitochondrial proteins are encoded by both nuclear and mitochondrial genes, which makes the human mitochondrial proteome a good candidate for an integrated systems biology/proteomics model system. The human mitochondrion is central to basic life functions for the generation of cellular energy, and as such is the site of key components of the biosynthetic pathways, cellular decision points leading to apoptosis (programmed cell death), and other important functions. The mitochondrion represents a discrete subcellular organelle with a non-nuclear genome that is comprised of about 1000 or more different protein species with tissue-specific features.

***Currently there is great hope in the utility of mitochondrial proteomics in health care, especially the areas of pharmacology, clinical diagnostics, and cancer and heart disease treatments. A NIST-sponsored workshop on standards for proteomics, held on September 17-18, 2002, reviewed the needs of the mitochondrial proteomics community in model systems, methods, and data. Following the workshop was a genesis of proteomics efforts by NIST to meet the standards requirements that were identified.***

A workshop to review of the standards needs of the mitochondrial proteomics communities, held on September 17-18, 2002, resulted in a road map for NIST's efforts in proteomics. This document outlines the how NIST should address the needs of the evolving proteomics community. This has also led to NIST's involvement in international proteomics standards planning through the Human Proteomics Organization (HUPO). Additionally, a laboratory in the Biotechnology Division was tailored for proteomics standardization work.

**The focus of this workshop was to provide:**

- **assessment of the mitochondrion as an integrated model for systems biology studies,**
- **assessment of emerging proteomics technologies,**
- **identification of the standards needs for proteomic applications in the clinical diagnostics industry, and**
- **guidance for determination of appropriate data elements (Common Data Elements, CDEs) for health care proteomics.**

**OVERVIEW OF CSTL ACTIVITIES IN MITOCHONDRIAL PROTEOMICS STANDARDS AND DATA**

- **Development of an XML library for 2-D polyacrylamide gel electrophoresis (2-D PAGE) for the storage and querying of proteomics 2-D PAGE data. (Veerasamy Ravichandran, MEL, and XML community)**
- **MitoAnalyzer, a program that allows the user to determine how a polymorphism affects the resulting protein in human mitochondrial DNA (http://www.cstl.nist.gov/biotech/strbase/mitoanalyzer.html). (Barbara C. Levin)**
  - **Human Mitochondrial Protein Database, which provides comprehensive data on mitochondrial and human nuclear encoded proteins involved in mitochondrial biogenesis and function. (http://bioinfo.nist.gov:8080/examples/servlets/index.html). (Veerasamy Ravichandran)**

***The later works resulted in a follow-up meeting to the workshop, "Mitochondrial Proteomics: Data Issues and Standards", held at NIST, November 5-7, 2003.***

## *Substrate Specificities and Kinetics of Action of DNA Glycosylases Involved in Cellular Repair of Oxidative DNA damage*

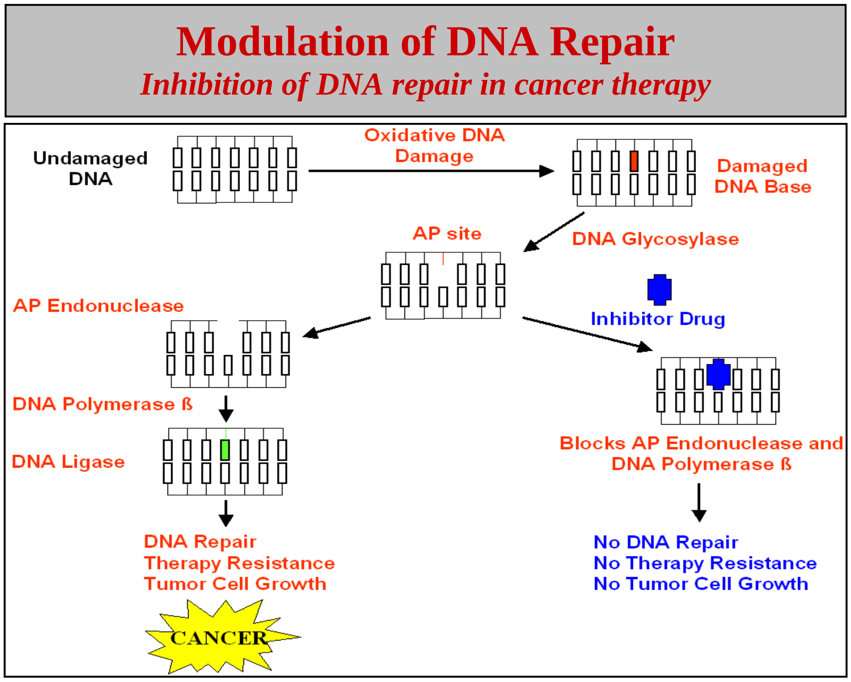
***M. Dizdar (831)***

***A new approach has been developed to measure how well the cell repairs oxidative DNA damage. This approach involves a study of substrate specificities and kinetics of action of the DNA glycosylase repair enzyme.***

Oxidative DNA damage is generated by free radicals formed in living cells by normal metabolism and exogenous sources such as ionizing radiations and carcinogenic compounds. Numerous products are formed in DNA by oxidative damage. Most DNA oxidative products are cytotoxic and mutagenic with deleterious biological consequences. In living cells, repair systems exist to repair damaged DNA to sustain the genetic integrity and to prevent genomic instability, which may lead to the onset and progression of cancer and other diseases. Deficiencies in DNA repair with elevated genomic instability are associated with a number of diseases. Oxidative DNA damage is mainly repaired by base-excision repair involving repair enzymes called DNA glycosylases. In our studies, we used gas chromatography/isotope-dilution mass spectrometry (GC/IDMS) because of its ability to simultaneously measure multiple modified bases in damaged DNA. Our studies showed that extensive variability exists in the substrate specificities of numerous DNA glycosylases.

**GC/IDMS enables the precise determination of substrate specificities of DNA glycosylases by identifying the modified bases that are or are not removed from damaged DNA by a given enzyme.**

These measurements lead to a better understanding of DNA repair mechanisms, thereby enabling the development of methods for prevention of diseases and development of new therapeutic approaches. This includes the improvement of cancer therapies by inhibiting DNA repair in drug- or radiation-resistant tumors by altering the expression of DNA repair genes (which generate the DNA glycosylases and AP endonucleases shown in the figure). By coupling these altered expression patterns with DNA repair inhibitors that show strong selectivity in killing tumor cells in comparison to normal cells, new approaches to curing solid-tumor cancers are being developed.


Modulation of DNA Repair

*Inhibition of DNA repair in cancer therapy*

## *DNA Damage by the Antitumor Drug 3-Amino-1,2,4-benzotriazine 1,4-dioxide (Tirapazamine)*

***P. Jaruga (831, UMBC), M. Birincioglu, H. Rodriguez, and M. Dizdar (831); G. Chowdhury and K. Gates (Univ. of Missouri)***

*Details provided in the* ***Exploratory Research*** *Section.*

## *Human Mitochondrial Protein Database: A Resource for Human Mitochondrial Proteomics*

***V. Ravichandran, P.E. Barker, G.B. Vasquez, T.N. Bhat, and G.L. Gilliland (831); S.J. Zull (ATP)***

*Details provided in the **Exploratory Research** Section.*

## *DNA Repeat Element Mutation Frequency Measurements of the Polypurine/pyrmidine Tract Contained in the PKD1 Gene of Polycystic Kidney Disease*

***J.P. Jakupciak and C.D. O'Connell (831); A. Bacolla and R.D. Wells (TAMU)***

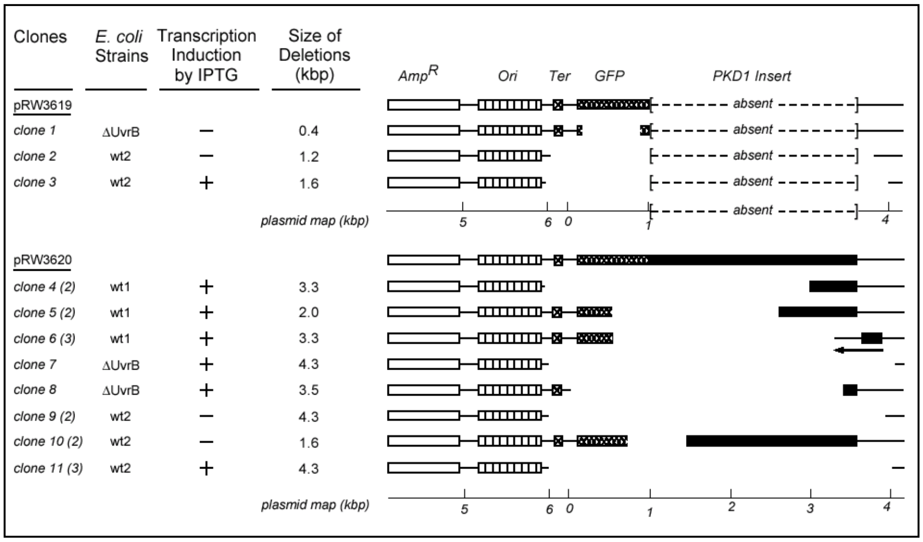
***More than 10% of the human genome is composed of interspersed repetitive elements. Already more than a dozen human diseases have been associated with trinucleotide repeat elements. Therefore, identifying repeat elements that disrupt or contribute to gene dysfunction will significantly increase our knowledge of human disease.***

The presence of repeated sequences is a fundamental feature of genomes. Repeat elements are the simplest form of regularity and analyzing repeats gives first clues to discovering new biological phenomena in the same way as repeated words give a starting point to deciphering a script written in an unknown language. The human genome consists of repeating DNA patterns of various sizes, from very small to very large. Although the functions of these repeating regions are not well understood, they appear important for understanding the expression, regulation and evolution of DNA. In eukaryotic genomes, tandem repeats are involved in various regulation mechanisms that are still being discovered. Repeat elements are involved in human neurological disorders, such as fragile X syndrome.

**Instability of polypurine/pyrimidine repeats has also been shown to be associated with polycystic kidney disease (PKD). We sought to measure the mutation frequency of the polypurine/pyrimidine tract present in PKD and characterize the repeat element instability.**

Our studies will provide fundamental measurements for researchers in industry and academia investigating DNA mutation(s) associated with human disease and supports efforts to understand the function of repeat elements in the human genome. Characterization of repeat elements will provide insights into human predisposition and susceptibility for disease.

Our measurements support the concept that non B-DNA structures, formed by the repeat elements, are located at the mutation sites. Overall, the results indicate that human disease is associated with DNA mutations (break points) that coincide with non B-DNA conformations. This work provides the first detailed insight regarding DNA structural conformations and their function at DNA break points. The results of this study have been submitted to *Science*.



**Measurement of the break points of 16 long deletion events**

## *High-Throughput Analysis of Telomerase by Capillary Electrophoresis and Real-Time PCR*

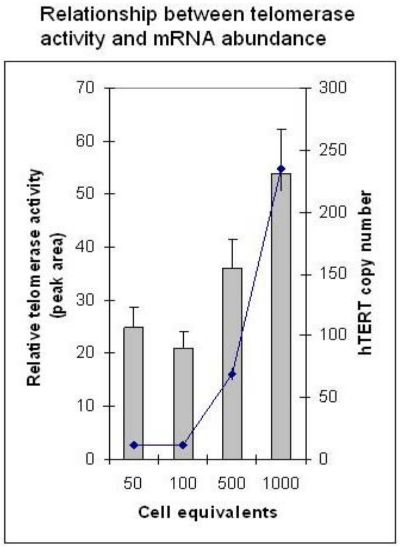
***D. Atha and J. Jakupciak (831); W. Wang (NCI/NIH)***

Clinical studies show a direct association between telomerase activity and cells that are cancerous. The enzyme telomerase is expressed in 85-90 % of all human cancers, but not in normal tissues. Currently, telomerase assays measure the small amount of enzyme activity in tissue lysates, urine or blood serum. The most commonly used system for the detection and quantification of telomerase activity (TA) is the PCR-based assay known as the telomerase repeat amplification protocol or TRAP assay.

***The use of telomerase as a biomarker for cancer has been hindered because of a lack of high-throughput methods, which are essential for large cohort studies. We are developing robotic high-throughput capillary electrophoresis and real-time PCR-based (gene expression) methods for the analysis of telomerase.***

**The TRAP assay was successfully transferred from the manual to a robotic platform with results that were consistent in sensitivity and reproducibility. The sample handling system of robot-assisted-TRAP will streamline validation of telomerase with increased sensitivity, reproducibility and automation over previous low-throughput methods.**

Most of the TRAP assay systems use a slab-gel based electrophoresis system to size and semi-quantify the PCR products. We have improved the sensitivity and reproducibility of this system using capillary electrophoresis. However, such methods are still low-throughput. We have developed a high-throughput system or RApidTRAP (robot-assisted-TRAP). The MWG SE 4204 robot was used to generate TRAP/PCR samples for analysis using an ABI 3100 16-capillary array instrument. TA measurements were made using this method on (50 to 500) cells per reaction isolated from tumor cell extracts with a percent standard deviation (CV%) of 20. This was about half the variation obtained using the slab-gel based method. Measurement of hTERT mRNA was made using specific primers and probes on a LightCycler in the range of (10 to 7000) cells / reaction. The percent standard deviation (CV %) of these samples ranged from 0.21 to 4.47, which is a variation of about 2 to 40 RNA molecules per 1000. This is well within the criteria for differentiating between cancer and normal cells / tissue.



**Comparison of hTERT mRNA and Telomerase Activity (TA). The relationship between the relative telomerase activity (shown in bars) and the amount of cellular hTERT mRNA ($\tau$). The amount of cellular hTERT mRNA (copy number) was measured in the A549 cells in comparison to the telomerase activity (total peak area) obtained from automated TRAP/PCR measurements.**

## *Silicon Nanoparticles as DNA Labels*

***V. Reipa and L. Wang (831)***

*Details provided in the* ***Exploratory Research*** *Section.*

# *Semiconductor Nanocrystal Probes for Human Metaphase Chromosomes*

***P.E. Barker and Y. Xiao (831)***

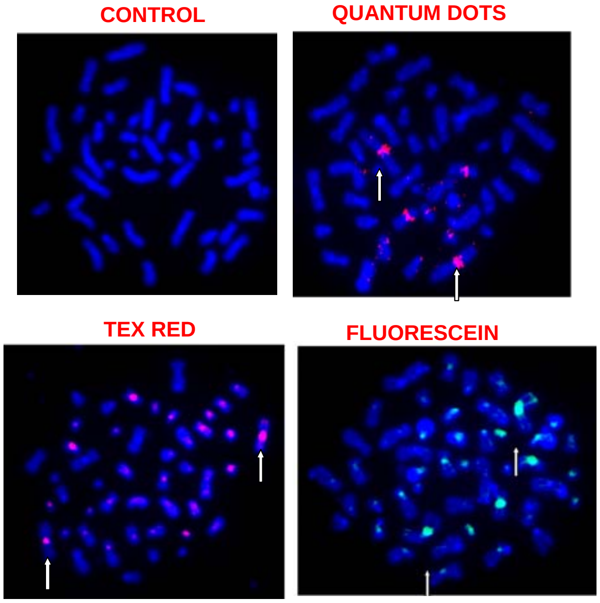
About one third of breast tumors have amplification of the gene for the tyrosine kinase receptor HER2/neu. In contrast to the other breast cancer patients, HER2 overexpressor tumors can be specifically targeted by the first rational design drug, Herceptin (trastuzumab), a humanized mouse monoclonal antibody that targets cells with high expression of the HER2 gene. Clinical testing for HER2 overexpressors follows two approaches, FISH (fluorescence in situ hybridization), a gene test measuring the HER2 gene at the DNA level, and an IHC (immunohistochemical) test that measures the amount of HER2 protein expressed on the cell surface. Most FISH tests employ organic fluorescent tags.

***This work demonstrates the physical advantages of quantum dots as labels for human metaphase chromosomes. The purpose is to provide researchers and clinical laboratories in industry and academia with alternative, stable and high luminosity fluorophores for measuring gene expression associated with medical and oncology diagnostic tests.***

**The IHC and FISH tests do not always select the same patients for trastuzumab therapy for treating breast HER2 overexpressor tumors. In the process of developing a standard cell analyte with known HER2 levels, alternative fluorophores were investigated in a model system.**

Recently, an inorganic tag of the binary compound CdSe, has become available. CdSe and other similar quantum dot labels are much more stable on exposure to light, and are brighter than conventional organic fluors. The CdSe labeled DNA probes were directly compared with fluorescein- and Texas Red-labeled probes, and proved to be superior for quantitative measurements of the type necessary for HER2 gene analysis. It was demonstrated that DNA probes used for FISH testing could be detected with quantum dot labels, and such a strategy may be more quantitative for medical testing than prior approaches.

**FISH FOR PB6 CELLS LABELING WITH QUANTUM DOTS, TEX RED AND FLUORESCEIN (HUMAN TOTAL DNA AS A PROBE)**



**Specificity of fluorophore-streptavidin detection of biotinylated total human DNA in metaphase chromosomes.**
**Bar in panel C is 10 microns.**
**(A) Control (no fluorophore),**
**(B) Streptavidin-Qdot 605 detection of chromosome 1q12 region (vertical and horizontal arrows),**
**(C) TexasRed-streptavidin detection of biotinylated DNA hybridized to 1q12 (vertical arrows)**
**(D) FITC-streptavidin detection of 1q12 sites (vertical arrows).**

## *Temperature Gradient Focusing for Biological Assays in Microchannel Systems*

***K.M. Balss, D.J. Ross, and M.J. Tarlov (836); H. Begley (863); K.G. Olsen (Loyola College)***

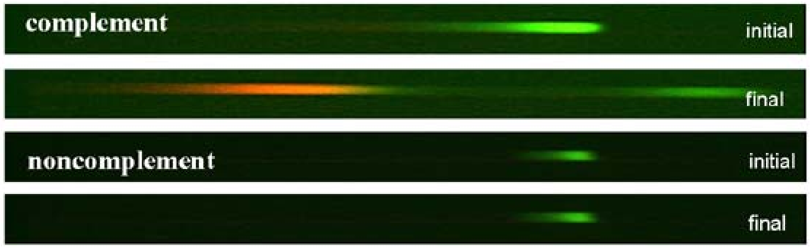
Temperature gradient focusing (TGF) offers three significant advantages for capillary or microfluidic assays. First, targets present at trace levels can be concentrated before the assay reaction to enhance sensitivity. Second, because TGF focuses targets to a fixed point, they can be held stationary for mixing with a recognition probe transported by bulk flow. The facile mixing of reactants shortens analysis time. Third, probe-target binding partners formed in the assay are focused and concentrated at a point spatially distinct from that of the focused targets resulting in sensitive, low-background detection.

***A fast and simple nucleic acid assay was designed to provide proof-of-concept for temperature gradient focusing (TGF) in capillary or lab-on-a-chip systems. The TGF approach described here using peptide nucleic acids to recognize other nucleic acids through hybridization could be extended to other biological assays such as measurement of protein/protein, nucleic acid/protein, or drug/target binding.***

**Two powerful features of TGF were demonstrated in this work:**

- **the ability to concentrate charged analytes**
- **the ability to hold analytes stationary in a microfluidic channel for mixing and subsequent binding with a probe molecule by bulk flow of the probe through the analyte zone.**

To demonstrate proof-of-concept, an assay for DNA was developed using peptide nucleic acids (PNAs). PNA molecules contain the same nucleobases as DNA and, thus, exhibit similar base-pairing properties as DNA. However, unlike negatively charged DNA molecules, PNAs are neutral. In the TGF assay, the PNA molecules serve as molecular recognition probes for a specific single-strand DNA (ssDNA) sequence. The assay involves three steps. First, the negatively charged ssDNA targets, the analyte molecules, are focused and concentrated at a predetermined point in the capillary. Second, fluorescently labeled PNA is then introduced in the capillary and carried by bulk flow of the buffer through the focused ssDNA. Third, if PNA/DNA hybridization occurs (i.e., the ssDNA is complementary to the PNA) the negatively charged ssDNA/PNA duplex focuses to a different location from that of the ssDNA because of its different eletrophoretic mobility and a fluorescent band is observed. If the ssDNA is not complementary to the PNA, a focused fluorescent band is not observed because the neutral PNA is not focused by TGF.



**Fluorescence micrographs of focused DNA and PNA/DNA in a microcapillary. In the experiment the ssDNA is first focused by TGF. PNA is then introduced into the channel by bulk flow while the zone of concentrated ssDNA is held stationary by TGF. The ssDNA is labeled with a green fluorescent dye, while the PNA is labeled with an orange fluorescent dye. The top two fluorescence images are those obtained after focusing of the perfect ssDNA complement (initial) and then 15 min after introduction of the PNA (final). The bottom two images are control experiments where ssDNA target that is non-complementary to the PNA is first focused (initial) and then the PNA is flowed for 27 minutes (final). Because the neutral PNA does not bind to the ssDNA, a focused orange spot is not observed in this case. Each image is ≈ 2 mm long. In these experiments the ssDNA targets were fluorescently labeled to confirm that the PNA/DNA duplexes are focused to a different location. In a real assay, it would not be necessary to label the ssDNA targets.**

# *Micellar Affinity Gradient Focusing*

***D.J. Ross and K.M. Balss (836); W.N. Vreeland and P.B. Howell (839)***

***A new focusing method, micellar affinity gradient focusing (MAGF) was invented to meet the need for effective pre-concentration techniques in microfluidics. This method is the first electrokinetic focusing method that provides the ability to concentrate and separate analytes based upon properties other than electrophoretic mobility or isoelectric point.***

**Initial experiments indicate that MAGF can provide a simple method for focusing classes of analytes – such as neutral analytes – that cannot be electrophoretically focused by other means.**

Micellar affinity gradient focusing (MAGF) is a new method for the focusing and concentrating hydrophobic and neutral analytes in microfluidic channels or capillaries. MAGF works by creating a gradient in the retention of the analyte into the micelles as illustrated in Fig. 1. On one side of the retention factor gradient, the analyte is strongly partitioned into and moves with the micellar phase. On the other side, the analyte is weakly partitioned into the micellar phase so that it moves with the mobile phase (buffer). Using charged micelles and the combined application of an electric field and a pressure gradient, the micelles can be made to move from the region of high retention to the region of low retention while the mobile phase moves from the region of low retention to the region of high retention. When this is done, the net velocity of the analyte will be positive on one side of the gradient and negative on the other side and zero at some point in the middle. Analyte will then move towards the zero-velocity point from both directions and be focused and concentrated there. Different analytes, with different affinities for the micellar phase, will be focused at different points along the channel, so that the method provides a focusing mode analog to micellar electrokinetic chromatography (MEKC) separation. Unlike previously described focusing separation methods (such as isoelectric focusing (IEF), electric field gradient focusing (EFGF), or temperature gradient focusing (TGF)), MAGF provides for separation of analytes based on properties – such as hydrophobicity – that are not related to the electrophoretic mobility. Consequently, it can be used to focus neutral and zwitterionic molecules that cannot be focused by previously described methods (Fig. 2).

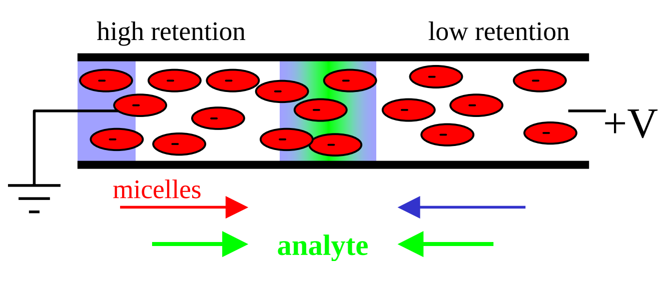


**Fig. 1. Schematic of micellar affinity gradient focusing in a microchannel or capillary.**

**Fig. 2. Micellar gradient focusing and separation of two zwiterionic fluorescent dyes, rhodamine B (red) and rhodamine 110 (green). For scale, the image is 2 mm long.**

## *Microanalytical Technologies to Support NIH Measurement Programs*

***L.E. Locascio (839); M. Gaitan and D. Reyes (812); N. Morgan and P. Smith (NIH/OD/ORS); T. Pohida (NIH/CIT); T. Phillips (NIH/OD/ORS); E. Perruccio (NIH/NEI) and P. Becerra (NIH/NEI)***

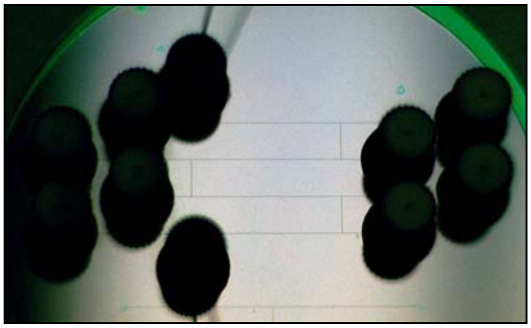
***We recently established collaborations with NIH for the purpose of enhancing medical research using microfluidics and microengineering. There are two ongoing projects with teams at NIH whose research involves:***

- ***the immune response to Herpes Papilloma Virus (HPV)***
- ***vision-related diseases involving defective neuronal differentiation or cell survival***

***For the first project, we are developing chip-based microfluidic devices for use in multi-analyte immunoaffinity capture and detection of proteins related to HPV in cervical secretions. The second project involves patterned immobilization of mammalian retinal cells using microfluidic channels to guide their growth and facilitate observation of their behavior under different conditions and treatments.***

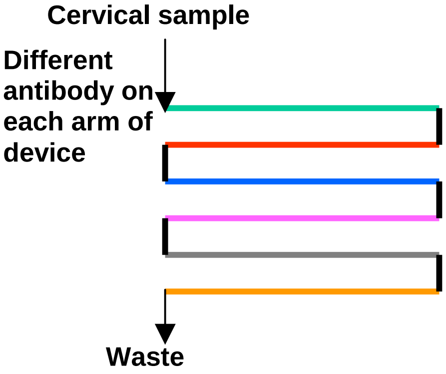
The purpose of this collaborative effort is to fabricate microsystems based on microelectromechanical systems (MEMS) and microfluidics to support and improve the measurement capabilities of medical researchers at the NIH laboratories.

*Project 1. Human Papilloma Virus (HPV)*. The first project involves an epidemiological study of the immune response to HPV, for which the simultaneous isolation of multiple proteins from microliter samples of cervical secretions is required. For this purpose, we are developing a microfluidic system capable of multi-analyte detection in a single small volume sample by performing multiple sequential heterogeneous immunoassays on chip. Microfluidic channels designed in a serpentine pattern are fabricated at NIST using silicon and glass substrates as shown in the figure. Different antibodies are then covalently immobilized to each separate arm of the chip so that the system is capable of measuring a number of analytes equal to the number of arms. The channel device architecture has several advantages over existing array technology including the fact that the device is reusable, and the captured proteins can be extracted after measurement with their biological activity intact. We are designing the system so that the basic methodology can be applied to many different applications relevant to clinical and biological research at both institutions.



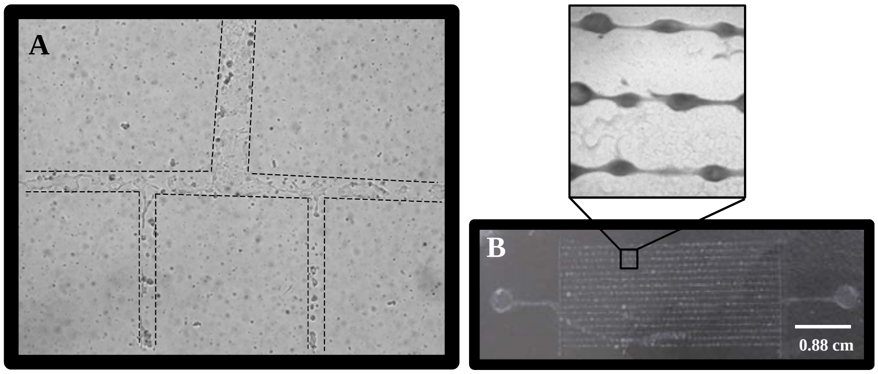
**Microfabricated silicon/glass device with 4 arms for antibody capture.**

*Project 2. Vision-related disease.* There exist a number of techniques that have been used for patterning cells on surfaces. The strategy often used to adhere single (mammalian) cells involves immobilization of extracellular proteins onto the surface for further adhesion of the cells by interaction with these proteins. In this work, immobilization of rat retinal epithelial cells is accomplished using micropatterned polyelectrolyte multilayer (PEM)-coated surfaces. PEMs were deposited in discrete lines using a (poly)dimethylsiloxane (PDMS) microfluidic network on top of a flat PDMS slab. The layers were formed by sequentially flowing the polyions throughout the microfluidic network. Retinal cells, seeded on flat PEMs/PDMS surfaces



**Schematic diagram of device with 6 arms for measurement of 6 analytes.**

adhered and grew on the PEM areas preferentially as shown in the figure at right. Cells were allowed to grow for up to two weeks showing the pattern delineated by the PEMs. We believe this approach could provide a useful and simple tool to pattern single cells or cell networks in specific locations to monitor neural activity of retinal cells as they are exposed to different stimuli.



**A) Overnight growth (~18hr) of retinal cells on poly(ethyleneimine). Cell growth is observed on the polycation surface areas (within the dotted black lines). B) Two weeks cells' growth on poly(ethyleneimine). Cells are covering all the polycationic area.**

## *Development of Reference Methods and Reference Materials for Clinical Diagnostic Markers*

***M.J. Welch, D.M. Bunk, M. Satterfield, L.T. Sniegoski, and S.S-C. Tai (839)***

NIST has a long-standing effort to promote accuracy in health-related measurements through development of reference methods and SRMs. Certification measurements have now been completed for SRM 2921 Cardiac Troponin I, an important marker for heart attacks. (See report titled "Development of SRM 2921, Human Cardiac Troponin Complex: A Primary Calibrator for Assays Used to Diagnose Heart Attacks.")

***NIST is committed to promoting accuracy and providing measurement traceability to the U.S. in vitro diagnostic (IVD) industry through development of reference methods and SRMs. To meet new regulatory requirements and help the US IVD industry compete globally, new reference methods for disease markers and metabolites are being developed here and in collaboration with other national metrology institutes. In addition, new SRMs are being developed for disease markers and important existing clinical SRMs are being renewed.***

Method development has been completed for triiodothyronine (T3) a marker for thyroid function, and participation has begun in an international round-robin comparison for thyroid markers. (See report titled "Development of Reference Methods and Participation in International Round-Robins for Determination of Thyroid Markers.") Work has begun on a new SRM for homocysteine (HCY) and tetrahydrofolic acid (FOL) in serum. This new material will have three levels: (high HCY, low FOL; normal HCY and FOL; and low HCY, high FOL). HCY is considered a risk factor for heart disease and other diseases associated with oxidative damage, while FOL is a strong antioxidant, which is known to reduce the risk of neural defects in fetuses and is

***Activities Completed or Underway***

- **New methods SRMs in serum for**
  - **cardiac troponin I**
  - **homocysteine and folate**
- **Methods development and participation in inter-comparisons for thyroid hormones in serum**
- **Collaborative reference method development for C-reactive protein with NMIs in UK and Germany**
- **Renewal of existing clinical frozen serum SRMs for:**
  - **cholesterol and triglycerides**
  - **electrolytes**
  - **glucose**

believed to counteract the effects of homocysteine. The new material will be ready for certification measurements in late 2003.

Research has begun on development of a reference method for another risk factor for heart disease, C-reactive protein (CRP). Modest increases in CRP have been linked to arteriosclerosis and the increased risk of heart attacks. NIST, working with scientists at the Laboratory of the Government Chemist (LGC) in the UK and the Physikalisch-Technische Bundesanstalt (PTB) in Germany, is planning a proteomics approach to isolating characteristic peptides from CRP for measurement by liquid chromatography/mass spectrometry. Measurements have begun for the certification of new lots of SRM 1951b Lipids in Fresh-frozen Human Serum, SRM 956b Electrolytes in Frozen Human Serum, and SRM 965a Glucose in Frozen Human Serum, three of the most used clinical SRMs.

### *Development of SRM 2921, Human Cardiac Troponin Complex: A Primary Calibrator for Assays Used to Diagnose Heart Attacks*

***D.M. Bunk and M.J. Welch (839)***

After evaluation of six different candidate reference materials using two round-robin studies, a single reference material has been chosen and used to prepare SRM 2921, the first reference material for cTnI measurements. More than 20 different commercial cTnI assays, made by 12 different manufacturers from the United States, Europe, and Japan were used to evaluate the human cardiac troponin complex chosen for SRM 2921. SRM 2921 will be used by assay manufacturers developing new assays and for validating calibration materials for their current assays.

***The clinical measurement of serum cardiac troponin I (cTnI) has become an important tool in the diagnosis of acute myocardial infarction and myocardial damage. Unfortunately, a ten-fold difference in measured concentrations between assays is common, but as much as a hundred-fold difference in response has also been observed. To address the need for standardization of cTnI assays, NIST, the American Association for Clinical Chemistry (AACC), and the International Federation for Clinical Chemistry (IFCC) have worked together to develop SRM 2921, human cardiac troponin complex.***

The certification of SRM 2921 was accomplished through a combination of analytical methods, both qualitative and quantitative. The values of the concentration of each of the three troponin subunits, including cTnI, were determined using amino acid analysis coupled with a chromatographic separation. The structure of the cTnI protein was evaluated using mass spectrometry coupled with chromatography to determine the extent of post-translational modifications such as phosphorylation and degradation of the protein chain.

Data from the second round-robin demonstrated that the use of one common reference material could reduce interassay variability to 10-15 %, a substantial improvement compared to the 130 % variability observed in the first round-robin. A third round-robin study will be carried out to determine fully the ability of SRM 2921 to harmonize the cTnI assay results from patient samples and to establish its commutability. Future work will also include the development of a reference method for cTnI in serum.

**In a recent issue of *Clinical Laboratory News*, the selection of a material to be used as a primary cTnI standard was touted as an "important milestone" in the standardization of clinical cTnI assays.**

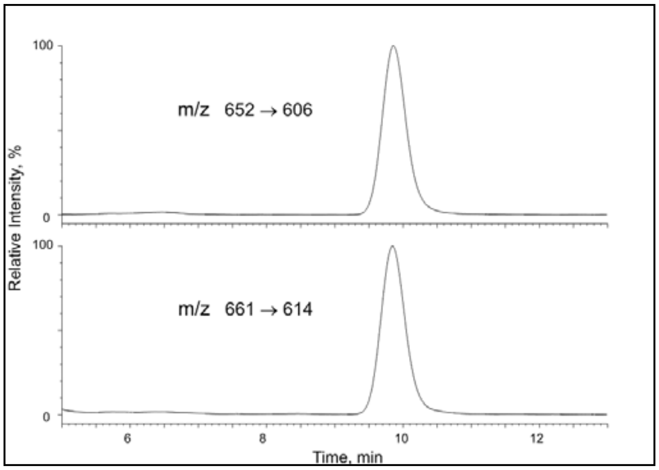
## *Development of Reference Methods and Participation in International Round-Robins for the Determination of Thyroid Markers*

***S. S-C. Tai, D.M. Bunk, and M.J. Welch (839)***

***In the past year, NIST has completed development of a reference method for the determination of serum total triiodothyronine (T3), a marker for thyroid function. This method is based upon isotope dilution combined with liquid chromatography and tandem mass spectrometry (LC/MS/MS). NIST has also participated in international round-robin comparisons of reference methods for the determination of thyroid markers, total thyroxine (T4) and total T3.***

A new reference method based upon isotope dilution combined with LC/MS/MS has been developed for serum T3, a marker for thyroid function. Development will begin in FY 04 for a human serum-based SRM for thyroid markers, T4 and T3, and other important hormones including cortisol, estradiol, progesterone, and testosterone.

The major challenges of serum T3 measurement are the low concentration of T3 normally found in serum, and the degradation of serum T4 into T3 during sample preparation. Because the concentration of serum T4 is approximately 100-fold higher than that of T3, even slight degradation of T4 into T3 can have a strong influence on the measured serum T3 values. The T4 degradation in this reference method was minimized to a small percentage, and can be corrected for in the T3 measurement process. This method was critically evaluated and found to produce results of high precision and accuracy for this low level hormone. This LC/MS/MS method for T3 along with LC/MS/MS method for T4 developed at NIST was used in international round-robin comparisons with excellent agreement with laboratories in Europe.



**LC/MS/MS ion chromatogram for serum T3 marker**

**The reference methods base on isotope dilution LC/MS/MS developed here and the SRM that will be used to certify these methods will provide critical traceability to the in vitro diagnostics industry and will help improve the reliability of routine clinical measurements.**

## *Improved Methodology for Determination of Arsenic in Body Fluids*

***E.A. Mackey and R. Zeisler (839)***

***To quantify the lowest levels of arsenic typically found in body fluids, detection limits for instrumental neutron activation analysis (INAA) must be reduced by about an order of magnitude. In this work, improvements in the INAA detection limits for As in biological samples were investigated using improved detection systems and simple radiochemical procedures.***

The current INAA detection limit for As in typical body fluids is generally between 1 ng $g^{-1}$ and 10 ng $g^{-1}$. The Na, P, and Br in the sample, however, elevate the baseline level of radiation next to, and under, the As peak of interest. Therefore, the wide As detection limit range depends upon the amount of background produced by matrix elements such as Br, P, and Na.

Two approaches for decreasing As detection limits were investigated. The first approach involved the use of a dissolution procedure to volatilize Br. Samples were digested with nitric acid and water and heated to near dryness. This was followed with heating with additional nitric acid and perchloric acids, again until near dryness. The digested material was diluted and counted using a conventional $\gamma$-ray spectrometry system consisting of a germanium detector and associated electronics. The $\gamma$-ray spectra from these digests were compared with spectra from the intact irradiated samples, acquired prior to digestion. Results indicated that the Br-to-As ratio was reduced by a factor of two using this approach. However, this reduction is not sufficient to permit quantification of As at the low levels present in materials such as human urine supernatant (SRM 2670a).

**State-of-the-art $\gamma$-ray spectrometry as well as simple radiochemistry can improve INAA detection limits for As in human body fluids. This work demonstrates that the use of more advanced electronic signal-processing systems can improve the limit of quantitation for As in biological fluids by a factor 4 or more.**

The second approach involved use of electronics capable of processing high count-rates without degraded resolution so that counting could be started earlier in the decay. The combination of a "zero dead-time" spectrometer with the latest gamma-ray spectrometry software has permitted this technical improvement. The As-to-Br ratio in spectra acquired using this system was up to a factor of 4 higher than obtained using a conventional spectrometry system and peaks were well resolved. Using this system the limit of quantitation for a 12 h count of human urine supernatant is about 1 ng $g^{-1}$.

A simple volatilization procedure did not yield sufficient improvement to detect levels of As present in fractions of biological fluids. Use of a more sophisticated detection system was successful but required longer counting times, thereby limiting throughput. Additional work will involve investigating the utility of simple radiochemical separation procedures in conjunction with the improved detection system.

### *Reference Methods and SRMs for Toxic Species in Body Fluids*

***S.E. Long, R.D. Vocke, L.L. Yu, K.E. Murphy, T.A. Butler, and E.A. Mackey (839)***

***Clinical assessment of the impact of toxic species on human health depends upon the quality of chemical measurements and the availability of suitable reference materials. Toxic species that are currently receiving scrutiny in the most common clinical matrices, blood and urine, present considerable measurement challenges since concentration levels are often close to the limit of quantitation by traditional methods. To address these challenges, we are developing new analytical methods with improved sensitivity and capability for toxic elements and cyanide in blood and urine. The methods have been used to augment the range of reference materials available from NIST.***

We have developed new analytical methods for the determination of trace and ultra-trace levels of toxic elements and cyanide in blood and urine. A species-specific method has been developed to accurately determine levels of inorganic mercury in whole blood. The method is based on selective reduction of the inorganic mercury component, which is combined with an ultra-sensitive isotope dilution vapor-generation ICP-MS measurement. The organic mercury present in the sample is not detected. The method has been applied to the determination of inorganic mercury in the elevated level of SRM 966 (Toxic Metals in Bovine Blood). This has been used to upgrade the SRM certificate value for inorganic mercury from a reference value to a certified value, which should be a valuable enhancement for the user community.

Certification of a replacement freeze-dried urine reference material (SRM 2670a, Toxic Elements in Urine) has been completed, mostly using a measurement approach based on isotope dilution combined with the high sensitivity of sector field inductively coupled plasma - mass spectrometry. The new material represents a considerable technical advancement compared with its predecessor, SRM 2670, having ten elements certified at the natural background level and fourteen elements certified at the elevated (spiked) level. Many of these certification measurements were made at the ultra-trace concentration level, which were possible as a result of the availability of new high-quality cleanroom facilities.

**A new instrument system featuring a combined liquid chromatography – inductively coupled plasma mass spectrometry system (LC-ICPMS) with a dynamic reaction cell for reducing spectral interferences has been installed, and will be used to investigate the role of toxic species in human nutrition and disease.**

We have also developed an improved method for the determination of cyanide in whole blood based on headspace gas chromatography with mass spectrometric detection (GC/MS), and employing $K^{13}C^{15}N$ as the internal standard. Cyanide (CN) is a very fast acting poison, which is rapidly absorbed in the blood and inhibits the cellular utilization of oxygen. Death can result from a blood CN concentration as little as 2.5 µg/mL. Despite its known hazard, CN is extensively employed in a number of chemical processes. Its presence in tobacco smoke and certain foods represent important health issues. The determination of cyanide is analytically challenging, and because it is unstable blood-based standards are not commercially available. The new analytical method is being used to provide value assignment and stability testing data for a pool of baseline and cyanide-spiked blood samples produced by the Emergency Response and Air Toxicants Branch of the Centers for Disease Control and Prevention (CDC).

**LC-ICPMS with dynamic reaction cell**

### *Radiochemical Method for Benchmarking Silicon Measurements Made During Clinical Investigations*

***R. Zeisler and R.L. Paul (839)***

***The role of silicon in diseases and disorders has yet to be fully elucidated. This is mainly due to analytical difficulties associated with the low-level determination of silicon in biological materials, such as high and variable blank values or analyte losses. This work demonstrates that the use of a radiochemical neutron activation analysis (RNAA) procedure, involving the NIST reactor, can provide detection limits for Si in biological materials that are fit for value assignment of Si in benchmark SRMs.***

In recent years, the role of trace concentrations of silicon in biological systems, namely in the human body, has become of increasing clinical concern and research interest. Association of elevated silicon levels in blood components and various tissues in patients with chronic renal failure and in women with silicon breast prosthesis was reported, as well as co-localization of silicon with aluminum in senile plaques in the brain tissue of patients with Alzheimer’s disease.

The analytical difficulties associated with the determination of Si at low levels in biological materials were documented in a recent

**It was concluded that the challenging task of accurate low-level determination of Si in biological materials can be accomplished with the employment of the inherently suitable properties of RNAA.**

intercomparison on the element determination in pork liver and pork filet samples. Used in this study were various modes of electrothermal atomic absorption spectrometry, inductively coupled plasma optical emission spectrometry, and direct wavelength dispersive X-ray fluorescence analysis. Severe discrepancies were found among results of these techniques, especially at the few μg g$^{-1}$ level. In order to be able to improve the performance of routinely applicable analytical techniques, there is a need to develop biological SRMs with certified values of silicon. The development of a new radiochemical neutron activation analysis (RNAA) method is a critical step towards the development of appropriate benchmark SRMs.

The new RNAA method, utilizing the $^{30}$Si(n, $\gamma$)$^{31}$Si nuclear reaction with thermal neutrons, was developed for low-level determination of Si in biological materials. The radiochemical separation consists of an alkaline-oxidative decomposition followed by distillation of $SiF_4$. Nuclear interferences, namely that of the $^{31}$P(n,p)$^{31}$Si with fast neutrons, have been examined and found negligible only when irradiation is carried out in an extremely well-thermalized neutron spectrum, such as available at the NIST reactor. The RNAA procedure yields excellent radiochemical purity of the separated fractions, which allows the measurement of the $\beta^-$ activity of the $^{31}$Si by liquid scintillation counting. Interference-free detection limits achieved were 150 μg g$^{-1}$ for irradiation of 200-mg samples in the NIST reactor RT-4 channel for 30 minutes, and 0.5 μg g$^{-1}$ for irradiation of 500-mg samples in the RT-5 channel for 10 h. Initial values have been established for SRM 1549 and SRM 1577b. Future plans call for implementing and refining the RNAA method for assigning values of the Si content in clinically relevant SRMs such as SRM 1598a.

## *Standards for Fluorescence Microarray Analyses*

***G.W. Kramer (839); A.G. Gaigalas (831); P.C. DeRose (839)***

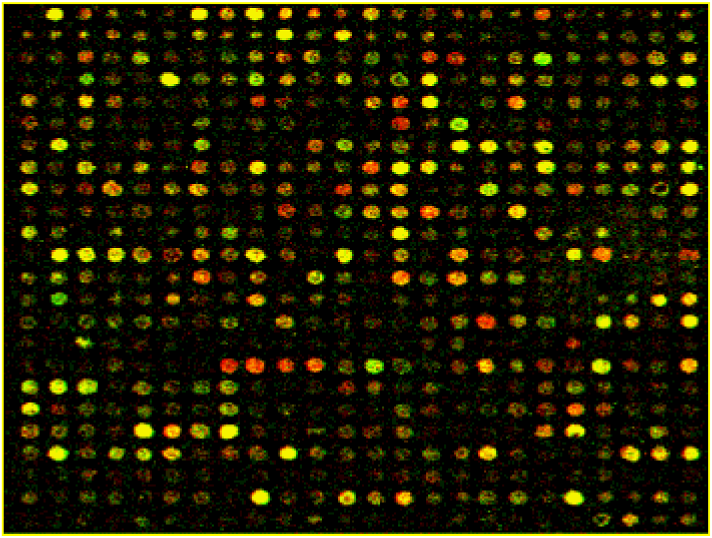
***U.S. manufacturers of microarray scanners have asked NIST to develop measurement standards for fluorescent microarray analyses. Specifications for a series of physical artifacts have been developed during several NIST workshops and monthly teleconferences. Work continues in finding suitable fluorescent materials, identifying appropriate fabrication methods, and creating instrumentation for certifying the fluorescence of such standards.***

**If artifact standards for microarray assays can be created and certified, then assay quantitation can be improved, results will be intercomparable, and the measurements can be made traceable ultimately back to the SI. The purpose of this project is to develop such standards in collaboration with the manufacturers of microarray readers**

DNA microarrays, also known as DNA or gene chips, have become important tools for gene expression analyses and are poised to revolutionize clinical diagnostics and enable personalized medical care—where treatment can be individually tailored to a specific person through genetics-based diagnoses. The related protein microarrays show great promise for pharmaceutical drug discovery research as well as clinical diagnostic tools. For microarrays, single-stranded genetic or protein material (probes) is bound in an array to a surface the size of a standard microscope slide permitting tens of thousands of molecular reactions to be tracked in parallel when an analyte solution is washed over the array. To accommodate this many sites in such a small area, the individual spots of genetic material or protein must be very small, commonly on the order of 10 μm to 100 μm in diameter. Target DNA or protein in the analyte solution are labeled with a fluorescent dye and allowed to interact with the probes bound on the surface of the array. A favorable interaction leads to fluorescence signal from the appropriate spots. A device called an array scanner or reader detects which spots in the array fluoresce and how much.

Today, there is no consensus method for microarray assays; there are multiple technologies for fabricating microarrays ranging from nano-pipetting to pin-printing to photolithography, and there are several types of scanners. The method variations and uncertainties are so great currently that the biological information obtained from microarray assays is often a function of the method and equipment used, and comparisons of results generated by differing technologies is not possible.

Prompted by a request from the scanner industry for standards development by NIST, a workshop on fluorescence standards for microarray assays co-sponsored by Agilent Technologies and NIST's Biotechnology and Analytical Chemistry Divisions was held on December 10, 2002. Attended by representatives from the major array reader manufacturers, its purpose was to develop technical specifications for fluorescence intensity, uniformity, and detection limit standards for the calibrating and validating microarray readers. Several parameters such as the excitation and emission wavelengths of the fluorescent tags seemed to be common across the industry; however, there were diverging views on other specifications such as spot size and the form factor of the array.



**Example of microarray readout**

Following the initial technical workshop, the participants worked out many issues concerning the types of standards, intensity levels, form factors, etc. in teleconferences. It was agreed that unpatterned artifacts for each of two colors (similar to the dyes Cy-3 and Cy-5 that are commonly used now) would be developed. One set of artifacts with fluorescence intensities in the mid-to-high range would serve as uniformity/homogeneity standards and to measure signal-to-noise ratios for bright features, while a second set of materials would be developed with low fluorescence levels to serve as detection limit standards and to measure signal-to-noise ratios for dim features. To avoid having to accommodate separate sets of standards to serve the two predominant form factors (25 mm by 75 mm microscope slide and ≈ 15 mm square), it was agreed that the artifact would be made oversize with two active areas that could be separated by breaking the glass along a scribed line.

A second technical workshop was held at NIST on May 14, 2003 to come to agreement on many of the physical factors and to begin the search for appropriate fluorescent materials and application processes to fabricate the standards. Through monthly teleconferences, the group continues to refine parameters and to search for suitable materials.

***NIST sponsored workshops:***

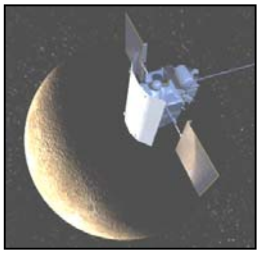
- **December 10, 2002: a workshop on fluorescence standards for microarray assays co-sponsored by Agilent Technologies and CSTL.**
- **May 14, 2003: workshop to begin the search for and fabrication of appropriate fluorescent materials for standards.**

# 9. Industrial and Analytical Instruments and Services

Instrument manufacturers depend on NIST/CSTL for physical and chemical standards, as well as NIST databases, for instrument calibration to provide traceability to national standards to the end users of their products and instruments. CSTL work supports both mature analytical applications, as well as the newest technologies such as high-throughput screening, and cutting edge research for next-generation semiconductor manufacturing.

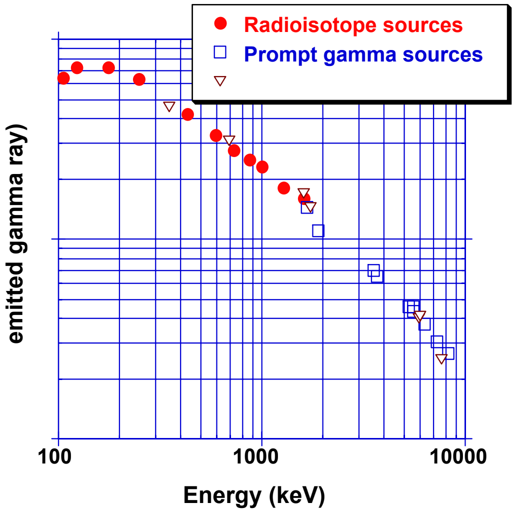
## *Calibration of a Gamma-ray Detector for a Mission to the Planet Mercury*

***R.L. Paul, G.P. Lamaze, and R.M. Lindstrom (839); E.A. Rhodes (Johns Hopkins Univ.)***



The NASA MESSENGER spacecraft, designed and built by scientists and engineers at the Johns Hopkins University Applied Physics Laboratory, will become the first mission to orbit the planet Mercury. Once in orbit, a gamma-ray spectrometer aboard MESSENGER will map the gamma-ray emission spectra over the planet resulting from capture of cosmic ray induced neutrons by nuclei of elements in the planet's crust. Analysis of these spectra will allow determination of the composition of Mercury's crust, which will shed light on the evolution of this planet and our solar system. In order to get an accurate assay map, the gamma-ray detector must be calibrated for efficiency vs. energy over the spatial orientation angles associated with the orbits around the planet. This calibration is usually performed using calibrated radioisotope sources. However, radioisotope sources have weak or nonexistent gamma-ray emission at energies above 3 MeV, while the gamma-ray energies of importance for planetary assay extend up to energies of around 9 MeV. An additional problem facing JHU scientists was that the germanium detector must be cooled by a small cryocooler that has an estimated lifetime not much longer than the mission. Therefore, the calibration measurement could not be allowed to consume much time. Because prompt gamma-rays span the energies needed for the calibration, the only viable method for obtaining a timely and accurate calibration of this detector was to use gamma-rays produced by neutron capture of a target in a high intensity neutron beam. Measurements were made using the unique beam facilities of the NIST Center for Neutron Research. Targets made of NaCl and Cr, irradiated at the NG0 beam port, provided the necessary gamma rays for calibration of the MESSENGER detector, which was rotated to the angular orientations required for the space flight. In order to obtain an absolute efficiency calibration, radioisotope sources of $^{56}$Co, $^{228}$Th, and $^{226}$Ra were calibrated at NIST and were measured in the same configurations as the targets. The spectrometer has been loaded aboard the spacecraft. This calibration procedure will allow MESSENGER to yield a relatively accurate compositional map of Mercury, and will likely set a new standard for space-flight gamma-ray spectrometers.

***CSTL scientists developed the calibration procedure for the Messenger Spacecraft that meets the time and energy parameters required by the mission engineers.***

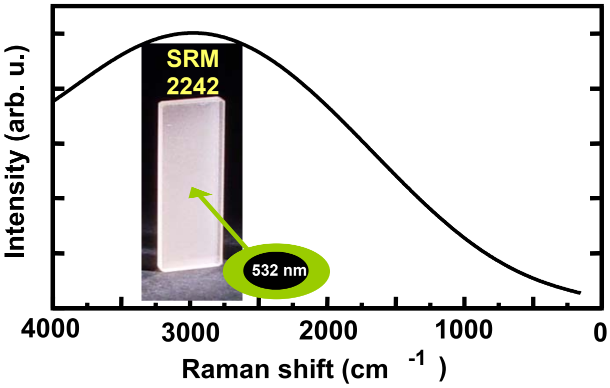


**An efficiency calibration determined using radioisotope sources of $^{56}$Co, $^{228}$Th, and $^{226}$Ra measured in the same configurations as the targets.**

## *Standards for Raman Spectroscopy*

***W.S. Hurst and J.E. Maslar (836); S.J. Choquette (839); E.S. Etz (837)***

***CSTL scientists are providing chemical and pharmaceutical industries with an inexpensive and easily implemented means for calibration of the Raman spectral intensity.***



Raman spectroscopy is now finding its place in the industrial environment for process measurements and quality control. NIST standards will promote the acceptance of Raman spectroscopy as a tool for process control in the chemical and pharmaceutical industries and will provide a means for instrument qualification as required by regulatory agencies such as the FDA. The project critically evaluates existing approaches and develops new methods and associated standards that will provide for calibration of the intensity of Raman spectral data. Intensity calibration is needed to make process-control Raman measurements "instrument-independent," to enable analysis of spectral data for unknown mixtures, and to quantify instrument performance and stability as needed by U.S. industries. CSTL scientists are developing a series of fluorescent glasses that will become available as Standard Reference Materials (SRMs) and will provide instrument calibration. SRM 2241, a glass suitable for use with laser excitation at 785 nm, was made available last year. A new fluorescent glass with stable properties suitable for use with laser excitation at 532 nm, SRM 2242, will be available in early 2004 and will supply the glass artifact along with a curve expressing its relative irradiance as a function of the Raman Shift in wavenumbers. SRM 2243, for 488 nm and 514 nm laser excitation, is under development and expected to be available in late 2004. It has been examined by members of the ASTM E13.08 for user advice on the magnitude of the intensity response, in order to establish the final fluorophore concentration. While the final fluorophore concentration has been determined, additional NIST work is needed to minimize laser-bleaching effects. In response to requests by members of ASTM E13.08 for NIST to include in its suite of materials for intensity calibration one for use at 633 nm, NIST will develop and complete a SRM for 633 nm in the next two years.

- **SRM 2242, a manganese-oxide bearing glass suitable for use with 532 nm laser excitation has been developed and will be available in early 2004.**
- **This will be followed by SRM 2243 for 488 nm and 514 nm expanding the series and enabling this technique's use to be expanded to the pharmaceutical, chemical, and forensics communities.**

## *Fluorescence Standards Suite for Spectral Correction of Fluorometers*

***P.C. DeRose, D.H. Blackburn, and G.W. Kramer (839)***

***CSTL researchers are working to prepare, characterize and certify a set of fluorescent glass SRMs that will enable spectral emission to be calibrated on fluorometers in the near UV and visible regions of the spectrum.***

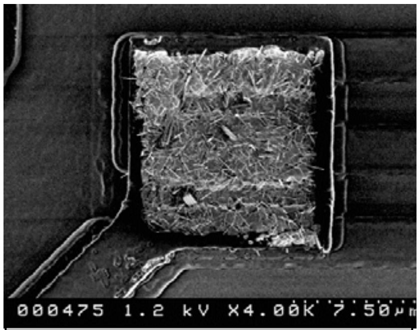
Luminescence measurements have become the detection methods of choice for new clinical and biochemical assays, and related high-throughput techniques, due to their extraordinary selectivity and sensitivity. These new analytical methods are becoming increasingly quantitative, requiring standards to calibrate the luminescence measuring instruments that they utilize and aid in the validation of the methods. Ideally, users would like to employ the same organic dye probes used for analyte detection as standards for fluorescence intensity and spectral correction. Unfortunately, organic dyes photobleach quickly, do not have long shelf lives in solution, have environment dependent fluorescence and are expensive to produce at high purity. After studying the characteristics of the different types of fluorescent materials, we found metal oxide doped (MOD) glasses to be the best choice for use as fluorescence standards for spectral correction. These glasses are photostable, robust, relatively inexpensive and can be made to suit most detection formats. Our research-grade fluorometer is being used to characterize the spectral characteristics of a series of MOD glasses to determine which will have the correct spectral shape and intensity for appropriate standards. A UV light chamber and a laser are also being used to irradiate the candidate glasses over a timed period. The fluorescence intensity of each glass is measured before and after irradiation to determine its rate of photobleaching.

**The certified $U_3O_8$ and $MnO_2$ glasses can be used in combination with SRM 936, a quinine sulfate dihydrate; a blue spectral emissivity standard that is the only standard presently sold for spectral correction of fluorescence**

## *Nanowires on Microhotplates*

***R.E. Cavicchi and C. Montgomery (836); B. Nikoobakht and S. Stranick (837)***

***CSTL research represents the first formation of nanowires on the electrical contact of a micromachined structure. Vapor phase transport of ZnO powder in argon was used to deposit ZnO nanowires on a microhotplate prepared with an ultrathin film of gold metal islands.***



**Growth of ZnO nanowires on microhotplate.**

Sensing devices based on nanowires and nanorods hold great promise for applications in chemical sensing. Small size implies that a greater fraction of atoms of these nanomaterials lie near the surface, where interaction with the environment can provide measureable changes in the electrical and optical properties. Of particular interest is the concept of a “nanolaser,” where the dimension of the optical element is small compared to the wavelength of light. Lasing has only been observed in nanorods by optical pumping for a few systems, and only once by the much more practical electrical-driven pumping for a device geometry that is suitable only for demonstrations. There has been much excitement recently over the development of growth methods for nanowires for optical materials. Based on techniques used to prepare carbon nanotubes, these methods involve the use of finely dispersed metal catalysts that promote the growth of crystals in one dimension from a vapor that passes over them. This work represents the first formation of nanowires on the electrical contact of a micromachined structure. Vapor phase transport of ZnO powder in argon was used to deposit ZnO nanowires on a microhotplate prepared with an ultrathin film of gold metal islands. Growth temperature was 1000 °C. Nanowires of average diameter 50 nm were observed on the tungsten-based contact pads of the microhotplate. The growth was selective to the contact pads. This shows the compatibility of nanowire growth with MEMS (microelectromechanical systems) devices and establishes a mode for producing electrical contacts.

**ZnO nanowire structures can be grown selectively on the contact pads of a microhotplate or similar thermally-agile MEMS structure.**

## *Hot Electron Chemicurrents Produced in Chemical Processes at Surfaces: A Theoretical Model*

***J.W. Gadzuk (837)***

Understanding the mechanisms of energy dissipation in surface reaction dynamics is necessary for useful predictive modeling of processes such as those that occur in vapor phase deposition and fabrication processes on surfaces. Newly discovered phenomena in which small but measurable currents are produced when molecules interact with metal surfaces has provided the empirical basis for a new generation of detectors in which the chemical event itself generates the hot electrons that are detected. The purpose of this theoretical work is to provide the foundation for some aspects of the underlying physics that are operative in these hot electron-based devices.

***CSTL researchers have discovered small but detectible currents produced when molecules interact with metal surfaces.***

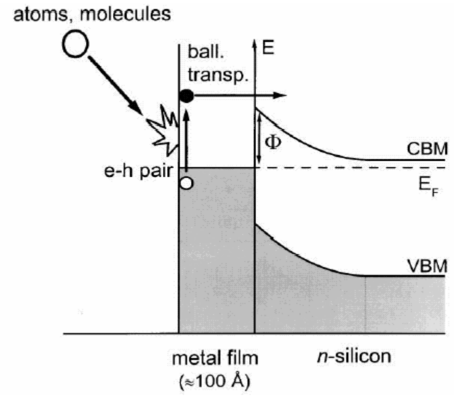


**Fig. 1. Chemically induced hot-electron detector.**

The crucial role of substrate electronic excitations in limiting the reaction rate of chemically selective atomic and molecular surface processes such as sticking/adsorption/dissociation/inelastic scattering has long been debated. Recent experiments carried out at the University of California at Santa Barbara have detected hot electrons produced when various gases were adsorbed on a thin metal film that formed a Schottky barrier with a Si substrate upon which the film was deposited, as illustrated in the figure to the left. A theoretical model for the electronically non-adiabatic effects has been developed that accounts for the observed initial hot electron production and subsequent detection as a “chemicurrent”, roughly ≈ (0.001 to 0.01) electrons per incident strongly-interacting adsorbate. This is based upon analogies with electron-hole pair shakeup processes that occur in the dynamic response of metallic systems to transient localized perturbations first addressed within the context of core level spectroscopies of solids.

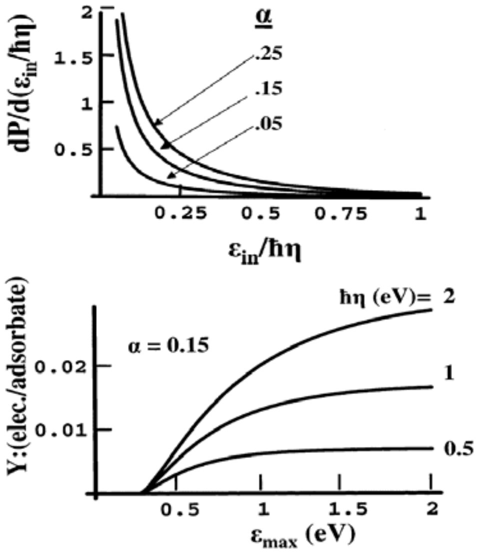


**Fig. 2. (Top) Hot electron energy distribution. (Bottom) Chemicurrent yield vs. chemisorption energy for labeled incident energies.**

The theory of chemicurrents includes a twofold thrust describing: i) the hot electron production, symbolized by the starburst in Fig. 1; and ii) the solid-state detector characteristics of the metal film and Schottky barrier also shown in Fig.1. A flux of reactants characterized by some incident energy $\nabla\eta$ and an interaction strength parameter $\alpha$ (simply related to the charge transfer between the reactant and the surface) creates the same distribution of excited electrons as occurs in core state spectroscopies in which the core electron is excited to a kinetic energy $\nabla\eta$. The theoretical predictions for the hot electron energy distribution as a function of scaled energy which follow from previously developed and confirmed spectroscopy theory, are shown in Fig. 2 (top), for events characterized by charge transfer in the not-unreasonable range ≈ 0.25 to 1.0 electrons $(0.05<\alpha<0.25)$. Using these excited state distributions in conjunction with theory for the thin film detector, the predicted chemicurrent or electron yield per incident

**This technology “…may usher in a new generation of chemical detectors.” *(NSF)***

adsorbate is shown in Fig. 2 (bottom) as a function of (essentially) the chemisorption energy, for various incident energies. The predicted yields of about 0.01 electrons/adsorbate are in excellent agreement with the observed yields for appropriate adsorbates such as O, H, and $NO_2$.

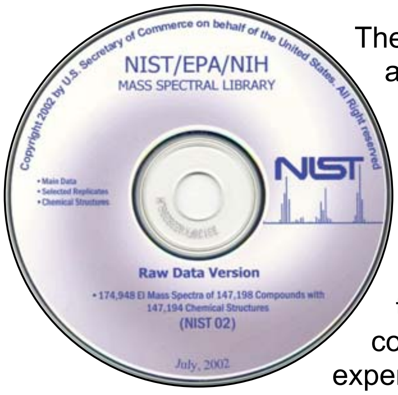
This theoretical work highlights some of the underlying system parameters that affect hot electron production as a result of a gas/surface reaction, and results are indeed consistent with the salient features of the experimental work.

### *The NIST Mass Spectral Library - Improving the Evaluation*

***S.E. Stein, A.I. Mikaia, C.L. Clifton, J.K. Klassen (838); and Y.A. Mirokhin (Contractor)***

Since the latest version of the NIST Mass Spectral Database (NIST02) release, the focus has been on tools and methods to improve the evaluation of the data in preparation for future releases. Ion-thermochemistry data are being used to rank probability of ion occurrence. Work is continuing on both the development of standards for Gas Chromatograph/Mass Spectral (GC/MS) libraries. As always, we continue to work closely with the instrument manufacturers and the user community to define the needs for the MS Database.

***CSTL researchers work to provide mass spectrometrists with a comprehensive, reliable library of mass spectral 'fingerprints' to assist the task of compound identification using the method of GC/MS.***



The new version of the MS Interpreter takes into account experimental and estimated heats of formation for ions. In addition, it finds multiple paths for ion decomposition. As a result of these improvements, a far larger fraction of the peaks in a typical mass spectrum are being identified. While the Interpreter is being developed as a tool for evaluation of NIST MS data, it is also a useful tool for quickly understanding a new mass spectrum. A candidate molecule can be drawn and the Interpreter will take the structure and compare it to the data from the unknown. While such analysis does not constitute a proof of the structure, it can reduce the effort of the experimenter considerably. In addition to tools to better evaluate the data currently in the Database, some changes in the direction of new data acquired have been made. The NIST MS Database has had fewer of the important derivative spectra than it should. These spectra are of compounds that are not easy to analyze by conventional GC/MS. Therefore, a derivative is made, which is easier to analyze. The largest groups of these derivatives are methyl esters, which are well represented in the database, and trimethylsilyl esters, which are underrepresented in the database. The emphasis on the addition of these derivatives as well as the continuing addition of spectra of newly discovered drugs, drug metabolites, and natural products will insure that the NIST MS Database meets the needs of the GC/MS community.

The GC/MS instrument is one of the most widely used tools for analysis of organic compounds. The efforts at NIST to provide both the best data and tested methods for accessing that data have led to a wide acceptance of the NIST MS Database and the NIST search software as the best combination of tools to use for MS analysis. With over 2,500 new libraries installed on instruments each year and a large number of upgrades to the new version of the Database, the higher quality and increased coverage of the new version will continue to improve search results in a large number of laboratories.

**Progress is being made towards the next release that will include new evaluation tools and an ongoing examination of the structure and naming of compounds.**

## *The NIST Retention Index Database*

***S.E. Stein, P. Linstrom, and W.G. Mallard (838)***

***The use of retention data provides a far greater degree of confidence in the identification than mass spectra or retention index alone would be capable of providing.***

One of the major problems in the use of gas chromatography combined with mass spectrometry in compound identification is the similarity in mass spectra of homologous series. This problem has traditionally been solved by the use of retention index data (a measure of the time a compound elutes from the chromatographic column), but data and prediction for retention indices have not been widely available. The collection and evaluation of the retention index data will provide a new tool for the identification of organic compounds. The initial release of the database (expected early in FY 2004) will contain data for 9000 chemical compounds along with complete literature references. During 2003 the first release of the database was prepared. Researchers are checking the release to confirm that all structures and references are present. In addition, the first stage of the collection of data for the second release has begun. Data for over 14,000 compounds are expected to be present with the second release. In addition, a scheme for using group additivity methods for predicting retention index data has been developed. The prediction scheme is used to find outliers in the data and allow for data checking with minimal resources. In addition, the prediction scheme will be available as a part of the WebBook.

**There is a very substantial body of data that has been identified and is in the process of being digitized and analyzed for inclusion in the next version.**

The broad question of confidence in identification using modern analytical methods remains important. The use of retention data - which is an orthogonal set of data to mass spectral data - provides a far greater degree of confidence in the identification than either mass spectra or retention index alone would be capable of providing. This additional confidence is used in NIST deconvolution software to determine a match factor that takes into account all that we know about an identification. Retention data has been used in certain very narrow areas - particularly in natural products or petroleum analysis, but the lack of high quality data sets has made it difficult to use retention data for a broad scope of problems.

## *Automatic Mass-Spectral Deconvolution and Identification Software (AMDIS)*

***W.G. Mallard, S.E. Stein (838); and O.V. Toropov (Contractor)***

The Automatic Mass-spectral Deconvolution and Identification Software (AMDIS) was originally designed to detect chemical weapons in violation of the Chemical Weapons Convention while at the same time not reveal confidential information that was not relevant to the Convention. The same fundamental algorithms would clearly be of use in automating the analysis of data taken in gas chromatography/mass spectrometry (GC/MS) in an industrial environment. In these cases the role of AMDIS is to provide faster, unbiased, and more statistically testable results - thus allowing higher quality analysis. The major efforts over the last year have been focused on assisting the instrument companies in using AMDIS in conjunction with their instrument software.

***CSTL scientists work to extend the utility of the AMDIS software and insure that it is available to the widest range of users.***

During 2003 presentations were made to several industrial users at their sites with an emphasis on using AMDIS in solving their problems. In addition, detailed presentations were made to two major instrument manufactures to aid in their integration of AMDIS into their instruments. On-going activity includes changes in library handling to allow for more records in the libraries and new modes of searching. In all cases these changes have come about from direct requests from the users or the instrument companies.

**AMDIS provides the first broadly available method for automating GC/MS analysis.**

AMDIS offers a tool for finding, with high confidence, components in complex mixtures that would otherwise be impossible. In addition, AMDIS can significantly increase the productivity of analysts in the laboratory by doing the first pass analysis in minutes without direct intervention, rather than hours - with full involvement of the analyst. AMDIS can also aid in providing statistically valid confidence measures for analysis. There is a growing awareness in the GC/MS community of the utility of AMDIS.

## *Determination of Hydrogen in High-Purity Niobium for Particle Accelerators*

***R.L. Paul (839), H.H. Chen-Mayer (PL), and G.R. Myneni (Jefferson Laboratory)***

***CSTL researchers have used prompt gamma-ray activation analysis (PGAA) and neutron incoherent scattering to study the effects of these two processes on the hydrogen content of the niobium.***

Niobium RF superconducting cavities are fast becoming the basic accelerator structures of a new breed of particle accelerators. It is suspected that the presence of interstitial elements in the niobium used to build these cavities may result in degradation of mechanical, thermal, and electrical properties. Prior to use, the high-purity niobium is chemically polished (treated with acid) to remove surface imperfections that could degrade the superconducting properties. This chemical polishing is believed to introduce trace hydrogen, while vacuum heating may be used to remove it. Hydrogen measurements were made at the Center for Neutron Research using two methods: cold neutron prompt gamma-ray activation analysis (PGAA), which measures gamma-rays emitted upon neutron capture; and neutron incoherent scattering (NIS), which measures neutrons scattered from the sample. The latter technique takes advantage of the anomalously high neutron scattering cross section for hydrogen. The samples were then acid treated with applied voltage for (0.5 to 5) min, and again analyzed. The measurements indicated no change in H concentration after the acid treatment. To see if more rigorous treatment could induce changes, two samples were again vacuum degassed at 800 °C for 8 h, and one sample was again acid treated for 5 min. Both techniques measured a significant decrease for the degassed sample, and a significant increase for the acid treated sample, indicating that both processes can affect the H content of the niobium. One possible explanation for the observed results is that heat treatment at 800 °C causes removal of strongly bound hydrogen, thereby activating sites where hydrogen can be picked up by the niobium upon subsequent acid treatment.

The results of this and future research will lead to newer and better ways to manufacture and dehydrogenate high purity niobium. The ultimate benefit will be a significant decrease in the cost of building accelerators to study fundamental physics. Since many more accelerators are planned for the future, the studies we are doing on niobium have a wide impact on the entire landscape of accelerator related science and technology.

## *Characterization of the New Thermal Neutron Prompt Gamma Ray Activation Analysis Instrument*

***E.A. Mackey, R.M. Lindstrom, H.H. Chen Mayer, G.P. Lamaze (839); D.L. Anderson (USDA); P.J. Liposky (NCNR)***

A new thermal neutron prompt $\gamma$-ray activation analysis (PGAA) instrument was built at the NIST Center for Neutron Research. It replaced the original PGAA instrument that was built in the late 1970's. This new instrument provides greater sensitivities, better detection limits, and much lower background radiation. The new instrument is also easier and safer to assemble for use.

***CSTL researchers have a new thermal neutron prompt $\gamma$-ray activation instrument for use that was designed to provide greater sensitivities, better detection limits, and much lower background radiation.***

A sapphire filter was placed in the neutron beam shutter assembly to reduce the fast neutron and low energy $\gamma$-ray components of the beam. The fast neutron component of the beam was reduced by a factor of five and the low-energy background $\gamma$-radiation by factors of 5 to 10. A new external beam tube, sample chamber, beam stop, and support structure were built and a new detection system installed. The new beam tube is made of two cylindrical aluminum sections lined with a lithiated polymer. The sample chamber was also fabricated from aluminum and lined with lithiated polymer, and may be evacuated to minimize the number of neutrons scattered and absorbed by air. The beam tube and sample chamber assembly is suspended from the aluminum support structure. The detection system consists of a 40 % efficient germanium detector (resolution 2.0 keV at 1332.5 keV) and bismuth germanate Compton suppressor. The detection system shield consists of lead surrounded by borated and lithiated polyethylene, placed on a table attached to the support structure. The new, more compact beam stop is welded to the support structure. Captured gamma-ray photopeaks from H, B, C, N, Na, Al, Fe, Ge, I, and Pb in the background spectrum were either of lower intensity or eliminated with the new PGAA instrument. The improved detection system, positioned closer to the sample, increased element sensitivity by 5 % to 50 %.

**PGAA limits of detection have been greatly reduced compared with those of the original instrument due to reduced $\gamma$-ray backgrounds and increased element sensitivities.**

## *Outreach Activities in Thermometry*

***D.C. Ripple, K.M. Garrity, C.W. Meyer, G.F. Strouse, W.L. Tew, and C.D. Vaughn (836)***

The CSTL Thermometry Group strives to maintain world leadership in thermometry, and to provide our users with all of the tools to attain traceability to NIST standards. Research activities alone cannot achieve this goal. Outreach activities by the Thermometry Group provide visibility of our work, a mechanism for training and education of users, and a forum for cooperation and exchange of ideas with other scientists. By sponsoring conferences and presenting workshops, the CSTL Thermometry Group educates industrial users and fosters communication within the field of thermometry. In FY2003, the Thermometry Group sponsored the premier conference on thermometry, the 8th International Temperature Symposium. The proceedings of these Symposia are valued for their documentation of the state of the art in thermometry. We also provided training to industry, through a workshop for the ASTM committee on Petroleum Products and through workshops at the NIST campus in Gaithersburg. Because of

***In October 2002, CSTL scientists sponsored the International Temperature Symposium, which is held once a decade.***

our outreach activities, the Thermometry Group is world-renowned as a source of thermometry expertise. Numerous non-NIST laboratories have attained a level of competence in thermometry that is commensurate with many foreign National Metrology Institutes, partly because of ready accessibility to methods established at NIST.

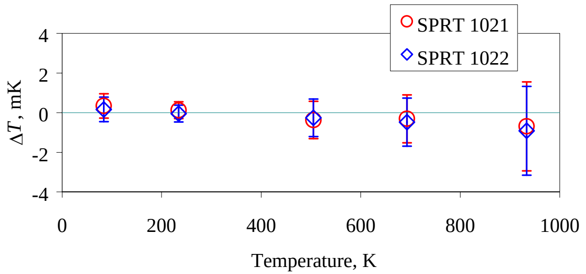
### *Proficiency Testing and Measurement Assurance for the International Temperature Scale of 1990 (ITS-90)*

***G.F. Strouse and D.C. Ripple (836)***

***CSTL scientists have developed proficiency testing that provides the concrete evidence necessary for objective evaluation of a laboratory's capabilities.***

**The design and implementation of the NIST proficiency testing program in thermometry assists industry in achieving accreditation and international acceptance, and furthers confidence in the quality of industrial measurements.**

An increasing number of companies are seeking accreditation through NIST's National Voluntary Laboratory Accreditation Program (NVLAP) for ITS-90 Standard Platinum Resistance Thermometer (SPRT) calibration services with uncertainty claims that are smaller than those of many National Metrology Institutes (NMIs). In order for NVLAP to grant accreditation to those companies and for those companies to gain acceptance in the global marketplace, validation of the submitted uncertainty claims is critical. For that purpose, the CSTL Thermometry Group developed a tiered proficiency-testing program and service that is designed with several levels of proficiency testing that are commensurate with the submitted uncertainty claims. At the lowest uncertainties, the level of scrutiny is comparable to that which an NMI undergoes for international acceptance. The newly developed symbiotic relationship between the CSTL Thermometry Group and NVLAP in the arena of proficiency testing for ITS-90 SPRT calibration services will improve the international acceptance of NVLAP accreditations and uncertainty claims by industry.



**Sample results of a proficiency test. $\Delta T$ is equal to the participant calibration results minus the NIST results; error bars indicate the combined expanded uncertainty ($k$=2) of the comparison.**

A selection matrix, with specific cutoff values based on an analysis of the uncertainties of numerous NMIs, is used in conjunction with the submitted uncertainty claims to determine the appropriate proficiency test. The proficiency tests range from calibration of a single SPRT (large uncertainty claims) to a combination of a measurement assurance program (three SPRTs) and the direct comparison of fixed-point cells (NMI level uncertainty claims). A prototype NVLAP proficiency test was performed with a U.S. company to test the new service, with results shown. The new program, created and implemented within the CSTL Thermometry Group includes protocols and report forms designed to maximize confidence in the results and transparency of the interpretation. CSTL continues to be the only laboratory capable of meeting the proficiency test and measurement assurance demands of the U.S. temperature community.

## *A New Noninvasive Approach to the Measurement of Phase Equilibrium*

***T.J. Bruno (838) and W.C. Andersen (FDA)***

***CSTL scientists developed a noninvasive method to measure the phase behavior of mixtures crucial for optimizing large and small-scale chemical separations.***

Accurately describing the phase behavior of mixtures, including those that are reactive, corrosive or toxic, is crucial for optimizing large and small-scale chemical separations in the chemical and petrochemical industries. CSTL scientists have developed a new noninvasive approach incorporating infrared spectroscopy for composition determination and a vapor entraining magnetic rotor that eliminates the need for recirculation pumps. The new apparatus measures phase equilibrium without disturbing the mixture being measured. The new apparatus also permits observation and quantitation of any intermolecular complexation, and the novel metrology eliminates the need to withdraw any mixture samples from the measurement vessel. The instrument has been used for the phase equilibrium measurement of two systems: carbon dioxide + butane (to assess the capabilities of the instrument) and R-134a + polyolester lubricant (required for process optimization in the refrigeration/air conditioning industry). Currently, the apparatus is being used for the measurement of amine containing mixtures, important industrially yet challenging because of intermolecular association.

**The new apparatus, designed and constructed by CSTL scientists, measures phase equilibrium without disturbing the mixture being measured and has been used for the following systems: $CO_2$ + butane; R-134a + polyolester lubricant; and amine containing mixtures.**

Two unique and novel features provide the noninvasive approach. First, the composition measurement is based upon mid-range infrared spectroscopy (FTIR), with probes coupled to an equilibrium vessel with fiber optic cables. The compositions of the vapor and liquid phases of a mixture at equilibrium are determined from the spectra, independently and nearly simultaneously, at a particular temperature and pressure. Moreover, any intermolecular complexation can be observed and measured by the spectra. Second, the approach to equilibrium is driven by a new vapor entraining magnetic rotor that eliminates the need for recirculation pumps. The novel metrology eliminates the need to withdraw mixture samples from the measurement vessel for any reason. Measurements on the refrigerant + lubricant mixture were needed by the U.S. Department of Energy, and previous measurements using conventional instruments were unsuccessful. The new approach made the measurement possible.

## *Measurement Standards for Thermal Analysis*

***D.G. Archer (838)***

***CSTL scientists provide leadership in the development of the measurement infrastructure for TA methods.***

Thermal Analysis (TA) is a family of measurement methods that follow a characteristic property (heat, mass, permittivity (dielectric), modulus, or others) of a material as temperature is varied. Thermal analysis methods are widely used in research and in production analysis in industries including pharmaceutical, polymer, chemical, aerospace, semiconductor and safety engineering. The widespread use of TA methods requires a measurement infrastructure that includes calibration and standard test methods. This infrastructure requires at least two components: consensus documentary standards and reference materials for calibration of TA instruments and for validation of standard test methods.

This year we created and managed an Interlaboratory Test Study to determine the precision of this test method and to explore some of the confounding factors that affect interlaboratory precision. Volunteer laboratories were enlisted; materials were selected and tested at NIST and then distributed, with directions, to the volunteer laboratories. The study was conducted in two parts. One group was given a sample of 1-phenyl-1H-tetrazole-5-thiol, a thermally unstable mercaptan. The second group was given portions of a commercial sample of 2-butanone peroxide, a thermally unstable free-radical initiator used in the polymer industry. The results from 15 volunteer laboratories have been collected. These results will be analyzed statistically and the appropriate research report will be completed. Also, the temperature and enthalpy of fusion of a sample of bismuth have been completed with high-temperature adiabatic calorimetry. These measurements will be the basis of the certification of SRM 2235, bismuth for calibration of differential scanning calorimetry.

**ASTM International Standard E967 Standard Practice for Temperature Calibration of Differential Scanning Calorimetry and Differential Thermal Analyzers was revised and approved by consensus.**

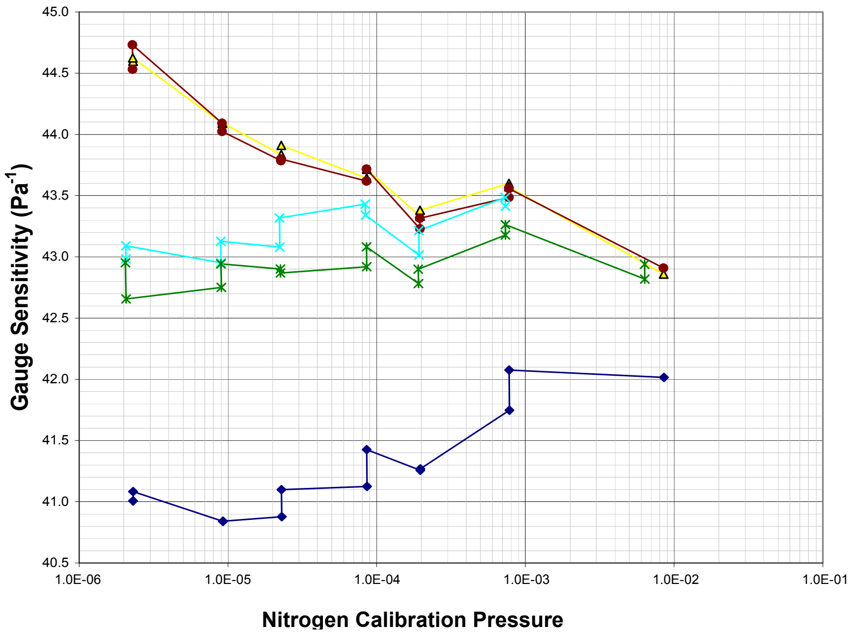
## *Improved Vacuum Transfer Standards – Ionization Gauges*

***P.J. Abbott (836); P. Mohan (NPL India)***

CSTL's goal is to disseminate NIST's realization of the Pascal at high vacuum levels directly to our customers and into readily available commercial gauges. However, commercial vacuum transfer gauges are unstable and hamper efforts to effectively disseminate NIST's uncertainties to our customers. In recent years, a new and improved hot cathode ionization gauge has become commercially available. The sole manufacturer claims improved stability over that of a conventional Bayard-Alpert (BA) ionization gauge by up to a factor of 10, thereby making it a superior candidate as a high-vacuum transfer standard below $10^{-4}$ Pa. However, in the course of two recent rounds of calibrations at NIST, several of these gauges have shown far worse stability (calibration shifts as large as 10 % at the lowest measurable pressures) under certain operating conditions than is reasonable to expect from the manufacturer's literature. Furthermore, the response of the gauge seems to depend strongly on its history of gas exposure. Our work confirms the history dependence of the gauge

***CSTL investigators have documented the effect that ionic pumping has on the stability of Bayard-Alpert (BA) gauges which will lead to reduced uncertainty in high vacuum metrology.***

**The gauge response prior to exposure and after exposure differed by up to 10% at $10^{-6}$ Pa of nitrogen, over three times higher than manufacturer expectations.**



**The change in gauge response as a result of ionic pumping. The gauge was conditioned at $10^{-2}$ Pa for 3 h on August 14, 2003. The before and after conditioning data differ by as much as 10 % at the lowest calibration pressures.**

response and identifies ionic pumping of gas into the gauge walls as the source of the instability. It also suggests a simple method of stabilizing the gauge's response. We have established that ionic pumping of gas by components internal to the gauges is responsible for the shifts in calibration that have been observed. The pumping speed of the gauge was found to be greatest just after a high temperature bake, a common procedure used to achieve high vacuum. It was also found that the gauge could not pump gas indefinitely, but rather became saturated after exposure to a pressure of $10^{-2}$ Pa for three hours.

Understanding the effect of ionic pumping on the stability of these gauges will lead to reduced uncertainty in high vacuum metrology, important to industrial process control, secondary laboratory calibrations, and international comparisons of high vacuum standards. Additionally, these results provide important information to the manufacturer for mechanical re-design and/or optimizing operating voltages of the gauge to minimize ionic pumping.

### *Materials Evaluation and Database Tabulation for Fundamental Parameters and Algorithms Used For Quantitative Electron Probe Microanalysis*

***J.T. Armstrong (837)***

**_CSTL scientists have regularized, standardized, and improved the accuracy of EPMA data reduction to be comparable to the level of measurement precision._**

In recent years, much work has been done to better understand the physical processes underlying electron probe microanalysis (EPMA) and improve the correction procedures employed for quantitative analysis. Today there are literally dozens of different combinations of physical parameters and correction schemes in use by the active laboratories involved in quantitative EPMA, and contained in the software packages of the various commercial manufacturers of x-ray analysis instrumentation. Carelessness has to an extent settled into the EPMA community, in that exactly *which* parameters and algorithms have been employed to calculate elemental concentrations is seldom reported (nor is the raw data typically published). Different implementations of reputedly the same correction procedure can yield significantly different results – either because of errors in the programming or undocumented changes/simplifications in equations and/or parameters. In some commercial systems, the corrections employed in the software are proprietary information and unknown to the user. As a result it has become impossible to directly compare analytical results from different laboratories without adding a significant uncertainty due to the lack of knowledge of how the data has been manipulated. Few laboratories and no current commercial systems make any attempt to quantify the magnitude of this uncertainty in their error budget, although there is abundant evidence that it is often considerably larger than the reported uncertainties in the measurements themselves. This work is part of an ongoing effort to attempt to regularize, standardize and improve the accuracy of EPMA data reduction to be comparable to the level of measurement precision.

**The parameters tabulated and evaluated were incorporated into the NISTZAF and TRYZAF correction programs and are available in text and spreadsheet files and have evaluative comparisons with previously used data.**

To that end, we have tabulated new sets of the fundamental parameters of use in quantitative EPMA, based on the most recent experimental measurements and theoretical developments, including data extracted from and traceable to the NIST Physics Laboratory Physical Reference Database. In many cases, these values are significantly different from values currently in common use by the EPMA community. The parameters tabulated and evaluated during the past year were incorporated into the NISTZAF* and TRYZAF* correction programs. They include ICRU tabulated mean ionization potentials, the mass absorption coefficients for K-alpha, L-alpha, L-beta, and M-alpha lines between 100 eV and 50 keV derived from the NIST XCOM and NIST

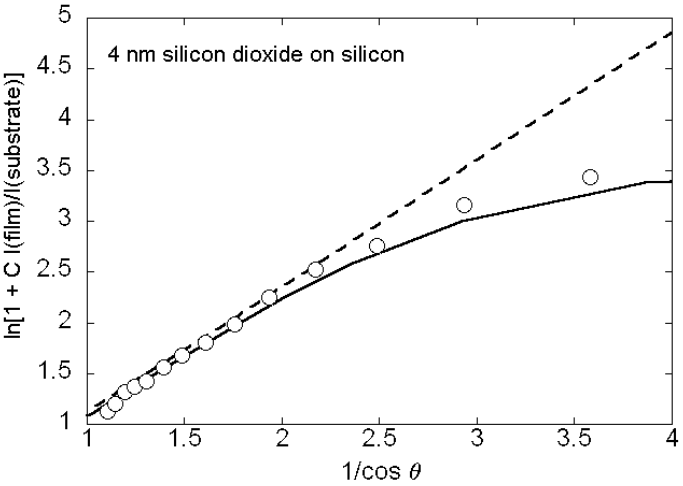
FFAST databases, the fluorescence yields compiled most recently by Hubbell, and the new x-ray line and edge energies of Deslattes. These tables are available in text and spreadsheet files and have evaluative comparisons with previously used data. We have demonstrated that accurate correction algorithms exist to predict the variation of x-ray intensity as a function of beam energy. These methods can be used to accurately determine particle and layered specimen composition (and thickness) without the need to independently standardize at each beam energy. They can also assist in improving the accuracy of commercially popular "standardless analysis" procedures.

***NISTZAF and TRYZAF allow the analyst to calculate concentrations and factors (atomic number (Z), absorption (A), and fluorescence (F)) based on the k ratios and instrumental parameters.**

## *Development of a New Database for the Simulation of Electron Spectra for Surface Analysis (SESSA)*

***C.J. Powell; W.S.M. Werner and W. Smekal (Contractors)***

A new NIST database has been developed for applications in Auger-electron spectroscopy (AES) and X-ray photoelectron spectroscopy (XPS), the two techniques most commonly used for surface analysis. The new database provides data for many parameters needed for the quantitative interpretation of AES and XPS spectra. The parameters include: differential inverse inelastic mean free paths, total inelastic mean free paths, differential elastic-scattering cross sections, total elastic-scattering cross sections, and transport cross sections for both AES and XPS; Auger-electron lineshapes, Auger-electron backscattering factors, electron-impact ionization cross sections, and fluorescence yields for AES; and photoionization cross sections, photoionization asymmetry parameters, and photoelectron lineshapes for XPS. It also simulates spectra for multi-layered thin-film samples (with layer compositions and thicknesses specified by the user) and measurement conditions specified by the user. The simulated spectra can then be compared with experimental measurements, and the film thicknesses and compositions adjusted to find maximum consistency between the simulated and measured spectra. Most quantitative analyses by AES and XPS have been previously based on the implicit assumption that the specimen is homogeneous over the sampling depth for the measurements or that the sample morphology is limited to one of several relatively simple morphology classes (and for which the effects of elastic scattering were neglected).



**Plots of a function of Si 2p photoelectron intensities (I) for a 4 nm $SiO_2$ film on a Si substrate as a function of 1/cos$\theta$, where $\theta$ is the electron emission angle with respect to the surface normal. The open circles show experimental measurements, the solid line shows results from SESSA, and the dashed line shows the result obtained if elastic scattering is neglected.**

**SESSA will enable improvements in the accuracy of quantitative AES and XPS analyses of multi-layered structures.**

## *Evaluation of Electron Elastic-Scattering Cross Sections*

***C.J. Powell; A. Jablonski (Polish Institute of Physical Chemistry); F. Salvat (Univ. of Barcelona)***

***CSTL scientists examined the extent to which three quantities derived from Differential Cross Sections (DCS) varied depending on whether they were obtained from the TFD or DHF potential.***

Differential cross sections for the elastic scattering of electrons by atoms are needed for modeling the transport of electrons in materials by Monte Carlo simulations and other means. Such transport calculations are needed to improve the accuracy of surface analyses, bulk analyses, and thin-film analyses of inhomogeneous samples by the techniques of Auger-electron spectroscopy (AES), X-ray photoelectron spectroscopy (XPS), electron-probe microanalysis (EPMA), and analytical electron microscopy (AEM). The cross sections are also needed for similar transport calculations in other applications including radiation dosimetry, radiation therapy, radiation processing, radiation protection, and electron-beam lithography. Since cross sections have been measured only for a limited number of atoms and electron energies, it is necessary to make use of calculated cross sections in the transport calculations.

**The atomic differential cross sections were empirically useful for simulations of electron transport in solids for electron energies above about 300 eV.**

An extensive analysis was made of differential cross sections (DCSs) for the elastic scattering of electrons by neutral atoms that were derived from two commonly used atomic potentials: the Thomas-Fermi-Dirac (TFD) potential and the Dirac-Hartree-Fock (DHF) potential. DCSs from the latter potential are believed to be more accurate. We compared DCSs for six atoms (H, Al, Ni, Ag, Au, and Cm) at four energies (100 eV, 500 eV, 1000 eV, and 10 000 eV) from two NIST databases in which DCSs had been obtained from the TFD and DHF potentials. While the DCSs from the two potentials had similar shapes and magnitudes, there could be pronounced deviations (up to 70 %) for small scattering angles for Al, Ag, Au, and Cm. In addition, there were differences of up to 400% at scattering angles for which there were deep minima in the DCSs; at other angles, the differences were typically less than 20 %. The DCS differences decreased with increasing electron energy. DCSs calculated from the two potentials were compared with measured DCSs for six atoms (He, Ne, Ar, Kr, Xe, and Hg) at energies between 50 eV and 3 keV. For Ar, the atom for which measured DCSs are available over the largest energy range, there was good agreement between the measured DCSs and those calculated from the TFD and DHF potentials at 2 keV and 3 keV, but the experimental DCSs agreed better with the DCSs from the DHF potential at lower energies. A similar trend was found for the other atoms. At energies less than about 1 keV, there were increasing differences between the measured DCSs and the DCSs from the DHF potential. These differences were attributed to the neglect of absorption and polarizability effects in the calculations.

We also examined the extent to which three quantities derived from DCSs varied depending on whether the DCSs were obtained from the TFD or DHF potential. First, we compared calculated and measured elastic-backscattered intensities for thin films of Au on a Ni substrate with different measurement conditions, but it was not clear whether DCSs from the TFD or DHF potential should be preferred. Second, we compared electron inelastic mean free paths (IMFPs) derived from relative and absolute measurements by elastic-peak electron spectroscopy and from analyses with DCSs obtained from the TFD and DHF potentials. In four examples, for a variety of materials and measurement conditions, we found differences between the IMFPs from the TFD and DHF potentials ranging from 1.3 % to 17.1 %. Third, we compared mean escape depths for two photoelectron lines and two Auger-electron lines in solid Au obtained using DCSs from the TFD and DHF potentials. These mean escape depths varied from 4.3 % at 70 eV to 0.5 % at 2016 eV at normal electron emission, and became smaller with increasing emission angle.

Although measured DCSs for atoms could differ from DCSs calculated from the DHF potential by up to a factor of two for electron energies less than 1 keV, we found that the atomic DCSs were empirically useful for simulations of electron transport in solids for electron energies above about 300 eV. This unexpected result appears to be due to the smaller absorption correction (i.e., smaller total inelastic scattering cross sections) in the calculated DCSs for solids than for the corresponding atoms. The atomic DCSs could also be useful for energies down to at least 200 eV if relative measurements were made.

## *Development of Critical Benchmark Standards and Methods for the Metals Industry*

***J.R. Sieber, A.F. Marlow, M.R. Winchester, L.L. Yu, T.A. Butler (839); R.M. Lindstrom (837); M.P. Cronise, and C.N. Fales (TS)***

***CSTL researchers in collaboration with industry have produces several new SRMs that will benefit the customers in aerospace, automotive, semiconductor, and construction industries.***

Metals Industry laboratories need assistance with certified reference materials, improved analytical methods and uncertainty estimates. NIST and ASTM International have a long-standing partnership to develop SRMs and analytical methods. Collaboration is even more important now that NIST is implementing a quality system in compliance with ISO/IEC 17025, *General requirements for the competence of testing and calibration laboratories*. To meet the challenges of industry-wide compliance with ISO/IEC 17025, it is crucial to have effective industry standard test methods that make efficient use of certified reference materials. This fiscal year, the effort on behalf of the metals industry was diversified to include six SRMs for metals and alloys, including: SRM 2426, Galvalume®, Al-Zn Alloy; SRM 2696, Silica Fume; SRM 2453, H in Titanium; two SRMs for related materials, and participation in the development of a new ASTM International Standard Method of Test for Ni-base alloys. The new test method for Ni-Co alloys is of critical importance for alloy production control and product quality. With the NIST input, the new method provides options for calibration of X-ray fluorescence (XRF) with fewer reference materials. All of these accomplishments benefit the titanium, steel and aluminium segments of the metals industry, the petroleum industry, the mining industry and their customers in aerospace, automotive, semiconductor, and construction industries. None of this could be accomplished without the expertise and close cooperation of leading metals producers.

The NIST contribution to a new ASTM XRF method for Ni-base alloys gives industry labs the option of using a full fundamental parameters (FP) calibration. FP methods require fewer calibration standards than conventional XRF methods for calibration of matrix corrections. This is one way to reduce demand for reference materials while concurrently broadening the scope of the method. The wide range of reference materials developed this year, demonstrates the breadth of the US metals industry, its suppliers, and its customers. It also demonstrates the need to renew existing SRMs that have gone out of stock. Feedback from ASTM International makes it clear that the industry requires a steady supply of both new and existing SRMs to maintain analytical methods that satisfy its own and its customers' high expectations for product quality.

**These accomplishments benefit the titanium, steel and aluminium segments of the metals industry, the petroleum industry, the mining industry and their customers in aerospace, automotive, semiconductor, and construction industries.**

## *SpectroML—An Extensible Markup Language for UV-Visible Spectroscopy Data*

***A.D. Nguyen, A. Arslan, M.V. Smith, J.C.Travis, and G.W. Kramer (839)***

The interchange and storage of analytical chemistry data has long been hampered by multiple, incompatible data formats. Over the past 15 years, several different interchange formats have been developed that allow data generated by different vendor systems of a given technique to be exchanged. For example, using JCAMP-DX, users of most commercial FT-infrared and NMR systems can interchange their respective data. However, in the last few years, the emergence of platform and application independence through the Internet has advanced the possibilities for data interchange beyond the capabilities of these protocols.

***CSTL scientists developed an XML-based mechanism for instrument-to-instrument, instrument-to-application, and application-to-application data interchange called SpectroML.***

**AnIML is based on a hierarchical model that calls for the initial development of a core markup language containing the elements common to all applicable analytical techniques.**

Today's analytical chemists need not only to interchange data generated within a specific technique, they need to interchange, import, export, store, and combine all their data from multiple sources at multiple sites. Users are now no longer content with just having "pictures" of their data (i.e., data plots). They need real access to their data from within a report itself—they want to be able to expand plots, re-integrate peaks, overlay spectra, etc. without having to revert to the instrument or computer system that generated the original data. And, they don't want to have to purchase, learn, or even load proprietary programs to do this. In industrial settings, experimental data belong to and must be accessible to the entire organization, not just the laboratory personnel that generate it. Proprietary data formats and existing interchange programs simply cannot fill such corporate needs for data accessibility.

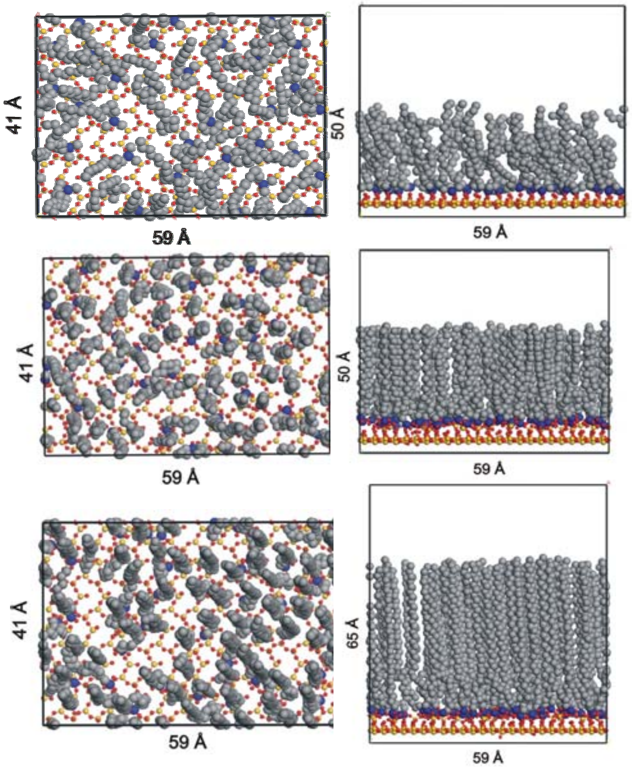
We have created an extensible markup language (XML) for UV/visible data called SpectroML and are using it to interchange data between our spectrometers and our optical filters database. SpectroML is now an integral component in certification and recertification process for optical filter SRMs. We are working with the ASTM Subcommittee E13.15 on Analytical Data Management, representatives from instrument companies, and the IUPAC committee responsible for JCAMP-DX to create a unified XML-based approach for interchanging molecular spectrometry and chromatography. Based in part on SpectroML, this effort is being called the Analytical Information Markup Language (AnIML). The development of AnIML as a universal molecular spectrometry and chromatography mark up language will revolutionize data interchange, eliminate long standing barriers to data exchange, enable new data manipulation software, facilitate new web-based notions for data usage, and permit the development of realistic schemes for long-term data archival. When coupled with parallel efforts in developing XML approaches to the manipulation of other chemical data, it will pave the way for the fully electronic chemistry data archive, where any known chemical fact or information is but a simple query away on your wireless Personal Digital Assistant from anywhere in the world.

## *Molecular Simulation of Alkylsilane Stationary Phases in Liquid Chromatography*

***K.A. Lippa and L.C. Sander (839); R.D. Mountain (838)***

***CSTL scientists found that alkylsilane chain structural analyses are consistent with previous experimental observations.***

Shape-selective polymeric alkylsilane stationary phases (typically $C_{18}$ chains) are routinely employed over the more common monomeric alkylsilane stationary phases in reversed-phase liquid chromatography (RPLC) to improve the separation of geometric isomers of constrained solutes, such as polycyclic aromatic hydrocarbons, carotenoids, steroids and polychlorinated biphenyls. Critical to describing such shape-selectivity processes in RPLC is the elucidation of the molecular-level structural features of the alkylsilane stationary phase that promote such separations. Previous molecular dynamic simulation models that illustrate the structure and dynamics of alkyl chromatographic stationary phases have been focused on only monomeric-type materials. We have investigated the molecular dynamics of chromatographic models that represent both monomeric and polymeric stationary phases with alkylsilane lengths, surface coverages, bonding chemistries and temperature conditions that are typical of actual materials prepared in the laboratory.



**Top and side view snapshots of molecular dynamic simulated LC phases at experimentally relevant surface coverages (μmol/m$^2$). Alkylsilane chains are displayed in gray (C), blue (Si) and red (O). The silica surface is displayed in red (O) and yellow (Si). Hydrogen is not displayed for clarity.**

The resultant structural features of the alkylsilane chains (i.e., length, conformational order, *gauche* dihedral angle defects) of our simulation models were related to the observed shape-selectivity properties of corresponding LC stationary phases. The structural characterization of these computational models are consistent with previous experimental observations: 1) alkyl chain order increases with increased surface coverage; 2) monomeric and polymeric phases with similar surface coverages give rise to similar degrees of alkyl chain order; 3) longer chain length phases exhibit significantly more order than $C_{18}$ phases; and 4) alkyl chain ordering is significantly enhanced at lower temperature. In addition, a significant portion of the alkyl chain closest to the silica substrate surface is disordered and the opposite end is most ordered.

**The application of molecular dynamic simulation techniques for the design of future RPLC phases will enable the analyst to intelligently design surface-modified materials that possess specific structural characteristics that will result in a particular retention mechanism.**

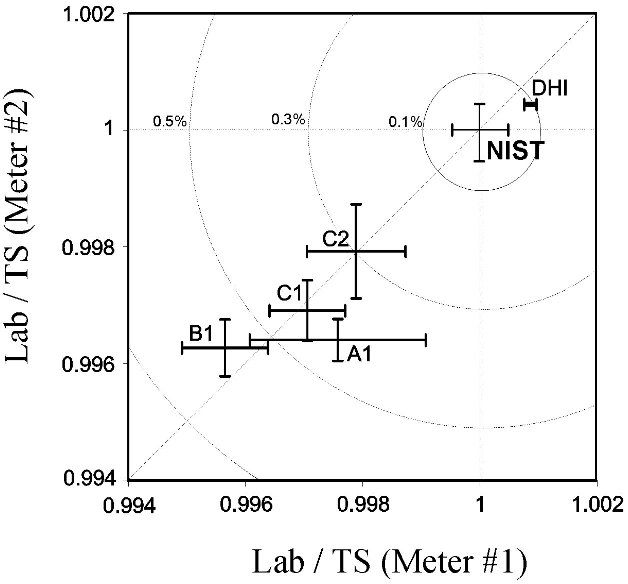
For simulation models that represent RPLC columns that have been characterized as highly shape-selective (through use of the SRM 869a Column Selectivity Test Mixture), a significant region of alkyl chain order with primarily *trans* dihedral angle conformations is observed. This is consistent with the view that the alkyl chains comprising the polymeric stationary phase contain a series of well-defined and rigid slots in which shape-constrained solutes can penetrate and hence be selectively retained. The results of this recent simulation work have promoted the synthesis of low- to moderate-surface coverage polymeric alkylsilane RPLC stationary phases that are anticipated to possess unique shape-selectivity behavior.

## *Low Gas Flow Measurement Assurance Program*

***J.D. Wright and G.L. Kline***

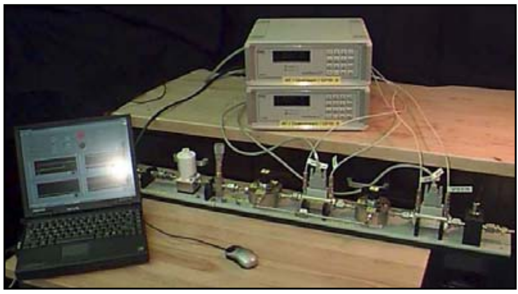
***CSTL scientists compared the DoD and DHI labs with each other and to the NIST standards with the purpose of evaluating the Molbloc laminar flowmeters for calibration stability, gas specie effects, and suitability as working or reference standards to calibrate other flowmeters.***

A Measurement Assurance Program (MAP) ended in September 2003 that compared the gas flow standards of Department of Defense (DoD) laboratories to each other and to the national gas flow standards at NIST. Two sets of four laminar flowmeters (Molblocs manufactured by DH Instruments) were used as the transfer standards (TS) during the MAP. The 8 transfer standards were calibrated between 0.04 and 30 L/min on more than 10 occasions at NIST, over the 2-year period of the MAP, and were calibrated by the participating labs as well. The participating labs were the Air Force Primary Standards Laboratory, the Army Primary Standards Laboratory, the Marine Corps Logistics Laboratory, and DH Instruments in Phoenix, Arizona.



**A Youden plot for the 1 L/min comparison data, all labs.**

The uncertainty of mass flow measurements made with the transfer standard (including 2-year reproducibility) were 0.13% to 0.23% on flow range of these data. Differences between individual labs and NIST were as large as 3%, but in general, values of 0.6% or less were observed. The flow differences at one of the tested flows (1 L/min) are graphically presented to the left. In this plot, made with data collected independently from two flowmeters at the same flow, separation between labs (A, B, C, etc.) along the diagonal represents systematic differences. The NIST reference value at (1,1) has error bars proportional to the 2-year reproducibility of the transfer standard. The transfer standard reproducibility in each lab is shown with error bars as well. The differences between labs were generally within the uncertainty expectations (0.16% to 0.55%), but in several cases, changes in the participants' facilities or procedures were recommended that would improve their calibration results. The DoD participants used piston provers, and significant differences in data scatter were discovered depending on the design of their provers.



**The transfer standard with its laptop data acquisition computer.**

The comparison showed where uncertainty improvements could be made, demonstrated flow measurement proficiency, and established flow traceability from the U. S. national standards to the participants. This improves the accuracy of flow measurements made by end users and assures that the participating labs' uncertainty specifications are met under all the actual, dynamic conditions of measurement.

**Our DoD sponsors will make the transfer standard available for other laboratories to use in their efforts to maintain proficiency and traceability to NIST.**

# 10. Microelectronics

CSTL supports the microelectronics industry by providing the necessary physical standards for process control of parameters such as temperature and humidity. CSTL also provides reference materials to facilitate quality control in manufacturing, as well as providing reliable kinetic and thermophysical property data to facilitate process modeling, to meet critical industry needs. CSTL research remains on the cutting edge in order to provide dramatically improved measurement tools to keep advanced U.S. microelectronics manufacturing globally competitive.

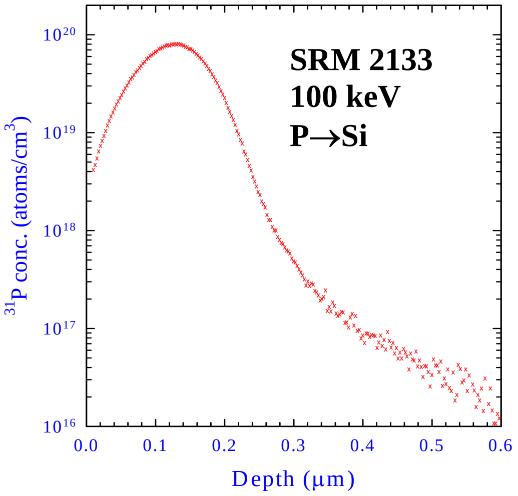
## *Development of Phosphorus Ion Implant in Silicon Depth Profile Standard*

***D. Simons (837), R. Paul (839), W. Guthrie and J. Lu (898)***

This project provided the U.S. semiconductor industry with a means to measure the implanted dose of phosphorus in silicon with low uncertainty. The shrinking size of microelectronic devices based on silicon technology has lead to narrower tolerance requirements for implanted doses of electrically active dopant species, and more attention toward confirmation of electrical models of device performance. Both of these developments require accurate determination of dose values by secondary ion mass spectrometry (SIMS), the analytical method of choice for dopant measurements. Previously NIST had developed and issued Standard Reference Materials for calibration of SIMS measurements of implanted doses of boron (SRM 2137) and arsenic (SRM 2134) in silicon. The specific need for a certified reference material for phosphorus, the third major dopant in silicon, was demonstrated by an interlaboratory round-robin exercise that showed nearly a factor of two disparity in measured dose values among participating laboratories when they used their own in-house phosphorus reference materials. That need has now been satisfied through the availability to the U.S. semiconductor industry of SRM 2133 – Phosphorus Implant in Silicon Depth Profile Standard, issued in April, 2003.

**The phosphorus dose in SRM 2133 was certified by radiochemical neutron activation analysis with an expanded relative uncertainty of 1.7 %.**

All known sources of uncertainty were considered, including a small spatial heterogeneity among samples. SRM 2133 should meet the needs of the U.S. semiconductor industry for many years based on the projections of the latest edition of the International Technology Roadmap for Semiconductors.

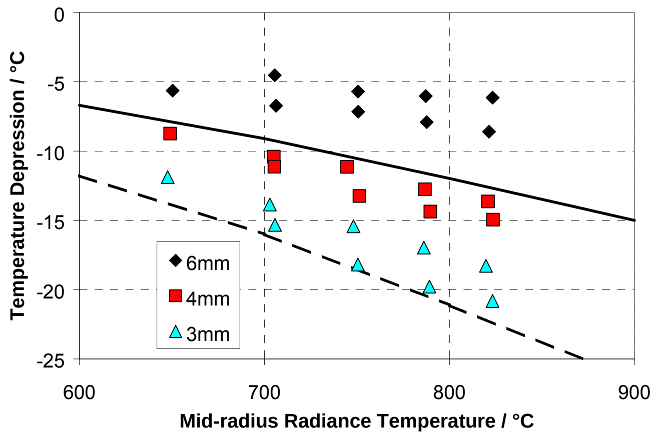


## *Proximity Effects of Lightpipe Thermometers in Rapid Thermal Processing Tools*

***K. G. Kreider and W. A. Kimes (836); D. P. DeWitt, B. K. Tsai (PL)***

***Rapid thermal processing (RTP) is used for thermal oxidation and dopant annealing of silicon wafers. Accurate temperature measurements from commonly used lightpipe radiation thermometers (LPRTs) are critical to this process.***

Lightpipe radiation thermometers (LPRTs) are used as temperature-monitoring sensors in rapid thermal processing (RTP) tools for semiconductor fabrication. These LPRTs can yield accurate temperature measurements when they are calibrated *in situ* or calibrated *ex situ* and used with a suitable model to correct for surface emissivity and chamber irradiation effects. Wafer temperature measurements are frequently performed in a highly reflecting chamber to obtain a near-unity effective emissivity of the wafer. However, the sapphire lightpipe tip has a low reflectivity (high absorptivity) that enhances radiation heat transfer from the target region. This low reflectivity causes a depression in the wafer temperature, degrading the thermal uniformity necessary for fabrication of high-quality semiconductor devices. This project was initiated to quantify the effect of lightpipe proximity to the wafer temperature measurement and to provide a model for use with commercial tools.



**Temperature depression caused by an LPRT at the indicated distances from a wafer. Symbols are measured values. The solid and dashed lines represent values from the model for proximities of 6 and 3 mm respectively.**

To minimize disturbances in the reflectivity of the RTP chamber, small, 2 mm diameter, sapphire lightpipes are often the temperature sensor of choice. Our experiments were performed to measure and model the effect of LPRT proximity on the wafer temperature in the NIST RTP test bed. We measured the spectral radiance temperature with the center lightpipe and compared these measurements with the three LPRTs at the mid-radius of the wafer and the thin-film thermocouple (TFTC) junctions of a NIST calibration wafer. Depressions in the wafer temperature up to 25 °C with the lightpipe at 2 mm spacing were observed. A finite-element radiation model of the wafer-chamber-lightpipe was developed to predict the temperature depression as a function of the lightpipe proximity distance and the chamber-wafer separation. The experimental results were compared with those from a model that accounts for lightpipe geometry and radiative properties, wafer emissivity and chamber cold plate reflectivity.

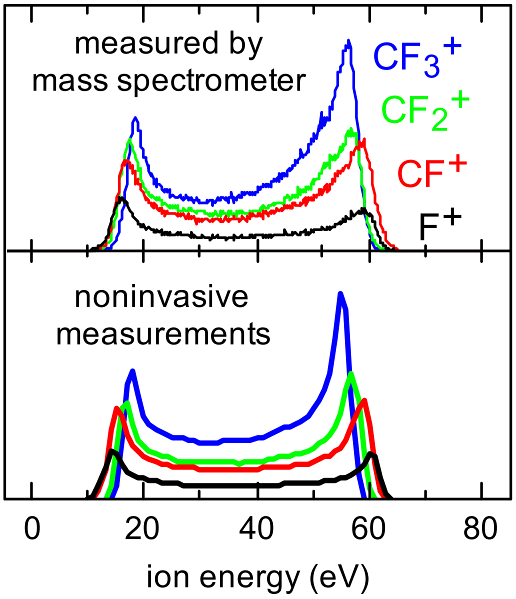
**Technical results are reported in *"Effects of Lightpipe Proximity on Si Wafer Temperature in Rapid Thermal Processing Tools"* AIP Conference Proceedings on Characterization and Metrology for ULSI Technology, 2003.**

# *Development of Plasma Process Monitoring and Diagnostic Techniques for the Semiconductor Industry*

***M. Sobolewski and K. Steffens (836)***

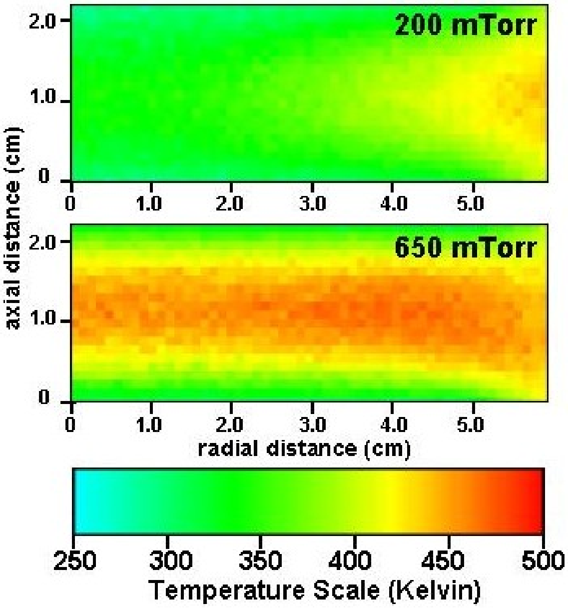
Over a third of the steps in the fabrication of microelectronic devices use plasma deposition and etching approaches for surface treatment and thin film deposition. Plasma models and diagnostics are now being implemented in process monitors and controllers to improve processing flexibility and reliability. Plasma processing reactors have historically been designed and operated using empirical methods alone, but continued evolution of these tools requires a much greater reliance on process and reactor modeling. To obtain more reliable predictions of the spatial uniformity, chemistry, and electrical properties of processing plasmas, further progress in model development and validation is required. Also, to enable improvements in process control, a need exists to develop sensors that are compatible with the manufacturing environment.

***Model-based plasma process design and control is an important need identified in the* International Technology Roadmap for Semiconductors**



**Ion energy distributions measured by the noninvasive, model-based technique (bottom) and by a mass spectrometer (top).**

One major area of activity has been the development and testing of a model-based technique for in-situ, real-time monitoring of ion energy distributions and total ion flux. The technique relies on radio frequency (rf) current and voltage measurements made outside of a plasma reactor, and thus it is well-suited for process monitoring applications in manufacturing where the use of invasive probes to measure ion energy or flux is not practical. Recently, tests of the technique were performed in argon and $CF_4$ discharges. The tests included a complete sensitivity analysis which quantified the dependence of ion energies and other outputs on all required input parameters, allowing us to rigorously determine the uncertainties in the outputs. Also, recent experiments have demonstrated the application of the technique to real-time monitoring of process drift in an inductively coupled plasma reactor. The drift occurs due to the deposition of a conductive layer on the surface of the dielectric window adjacent to the inductive source. Changes in the conductive layer produce large changes in ion flux and ion energy which are accurately monitored in real-time by the model-based technique.

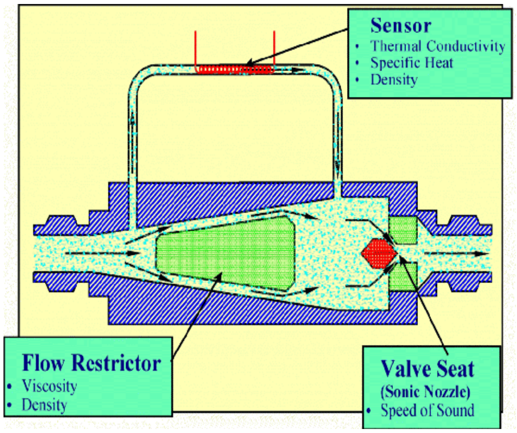


**Temperature maps of $CF_4$ plasmas.**

As model-based reactor design and process development become increasingly utilized, species density measurements and gas temperatures can provide important input and validation for plasma modelers. Spatial variations in plasma temperature can cause spatial differences in gas density and reaction rates and can complicate the interpretation of gas density measurements. The planar laser-induced fluorescence (PLIF) technique not only provides two-dimensional (2-D) density maps of important fluorocarbon radical species in dielectric etching plasmas, it also has been extended to enable 2-D temperature mapping in fluorocarbon plasmas. Using PLIF of the CF radical, temperature maps have been measured in $CF_4$ plasmas as a function of pressure and power. Temperature has been observed to vary spatially by up to 150 K in these plasmas. Temperature increases with pressure and power and is lowest near the cooled electrode surfaces. In addition, the presence of a silicon wafer was found to increase the temperature throughout the plasma, especially when heat transfer between the wafer and the cooled electrode surface was poor.

## *Thermophysical Properties of Gases used in Semiconductor Processing*

***J. J. Hurly, K.A. Gillis, and M.R. Moldover (836)***



The semiconductor industry requires accurate process gas property information to develop and control manufacturing processes such as gas control in chemical vapor deposition processes and the use of mass flow controllers. CSTL responds to these data needs by measuring and publishing the thermophysical properties of semiconductor process gases. These measurements exploit novel, accurate, NIST-developed acoustic techniques. The measured properties include the speed-of-sound, ideal-gas heat-capacity, density (equation of state), viscosity, and thermal conductivity. Representatives of the semiconductor industry have identified the process gases, "surrogate" gases, and the binary mixtures of process and carrier gases of highest priority. They also established targets for the accuracy of the thermophysical property data that are needed to model manufacturing processes and to control the process quality. Specific areas that will benefit from this work are chemical vapor deposition (CVD) processes and the calibration of mass flow controllers (MFCs) using surrogate gases. As results are acquired, NIST disseminates them via the internet at the URL http://properties.nist.gov/semiprop. The database includes the heat capacity at constant pressure, thermal conductivity, viscosity, and virial coefficients $B(T)$ and $C(T)$ that determine the pressure-density-temperature relation. The diffusion coefficients for gaseous mixtures of process gases and carrier gases will also be included.

Gases and Conditions for Viscosity Data.

| Gas | Temperature Range (K) | Maximum Pressure, MPa |
|---|---|---|
| He | 298 | 3.3 |
| Ar | 200 - 375 | 3.3 |
| $N_2$ | 298 | 3.3 |
| $C_3H_8$ | 225 - 375 | 0.9 |
| $SF_6$ | 298 | 1.8 |
| $CF_4$ | 200 - 375 | 3.3 |
| $C_2F_6$ | 225 - 375 | 2.8 |
| $N_2O$ | 225 - 375 | 3.4 |
| $NF_3$ | 225 - 375 | 3.4 |

This year a new viscosity measurement system for hazardous gases was completed and used to measure the viscosity of 9 gases. The acoustic viscometer also determines the speed of sound in the test gas. We used this data to determine the ideal-gas heat-capacity to within 0.1 % and virial coefficients that reflect each gas's non-ideality. From the virial coefficients, we developed an equation of state that predicts the gas's densities to within 0.1 %.

## *Electron Probe Characterization of $KNbO_3$ and $NaNbO_3$ Single Crystals for use as Quantitative Microanalysis Reference Materials*

***R. B. Marinenko (837), Z. Samardžija, S. Bernik, B. Malič, M. Čeh (Jožef Stefan" Institute, Jamova 39, SI-1000 Ljubljana, Slovenia)***

Alkali niobates are being used as optoelectronics components due to their distinctive electro-optical, nonlinear optical properties. Many of the niobates are chemically complex and require accurate chemical reference materials to aid in determining their "as processed" composition. One area of specific interest is reference standards for electron probe microanalysis (EPMA) of alkali niobates with the general formula $K_{1-X}Na_XNbO_3$, a ferroelectric material with perovskite structure. The ceramic material $K_{0.5}Na_{0.5}NbO_3$ (KNN), a solid solution of the two alkali niobates, is of major interest because of its high piezoelectric coefficients and moderate dielectric permitivity. Complicated fabrication can lead to undesirable multiphase ceramics; therefore, a careful characterization of the product is required on the micrometer scale to verify that the desired composition has been produced.

| | % Mass Fraction (1 $\sigma$ uncertaity in parentheses) | | | | | |
|---|---|---|---|---|---|---|
| | $KNbO_3$ | | | $NaNbO_3$ (0.30 % mf Ca) | | |
| | K | Nb | O (stoic.) | Na | Nb | O (stoic) |
| EPMA-WDS | 21.56 (0.14) | 51.54 (0.16) | 26.90 | 14.08 (0.08) | 56.53 (0.20) | 29.09 |
| Nominal | 21.72 | 51.61 | 26.67 | 14.03 | 56.69 | 29.28 |
| KNN Anal. | 9.97 (0.28) | 19.98 (0.31) | 60.31 (0.41) | 9.74 (0.26) | Form. $K_{0.50}Na_{0.49}Nb_{1.00}O_3$ | |

Potassium and sodium niobate single crystals were evaluated with the electron probe microanalysis (EPMA) using wavelength dispersive spectrometers (WDS). Alkali metal migration that usually occurs in materials exposed to the electron beam, was insignificant. The microheterogeneity determined from line traverses and random points was minimal with all analysis points being less than the expanded 3 $\sigma$ uncertainty predicted from the x-ray counting statistics for each element. Quantitative analysis was also in agreement with the expected stoichiometry of the crystals although a Ca impurity of 0.3 % mass fraction was found in the $NaNbO_3$ specimen.

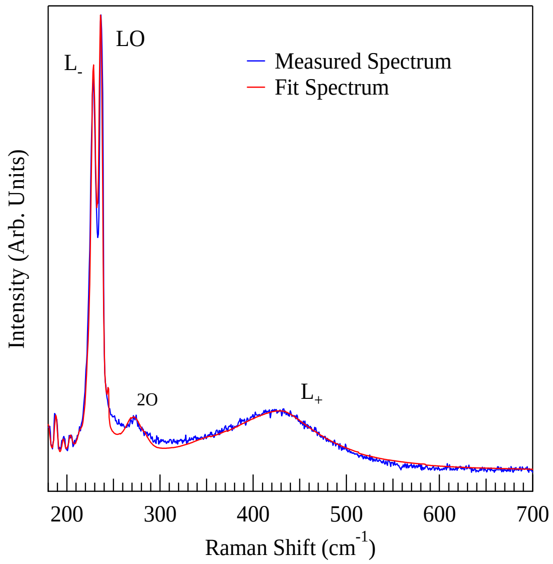
The $KNbO_3$ and $NaNbO_3$ single crystals tested appear to be excellent reference materials for characterizing KNN ceramics with EPMA on the micrometer scale. In addition, because no migration of the alkali elements were observed when these specimens were tested with a high-energy electron beam, these materials will make excellent K and Na reference standards for the microanalysis of other alkali-containing materials.

## *Non-Contact Free Carrier Density Measurements for Compound Semiconductors*

***J. E. Maslar and W. S. Hurst (836)***

Transport of free electrical carriers is central to the operation of all optoelectronic devices and reliable measurement of the carrier properties is critical. Hall or capacitance-voltage measurements are traditionally used to obtain this information, but require electrical contact. This precludes the use of these techniques *in situ* during growth or processing and, typically, even on actual device layers. Raman spectroscopy, as an optical technique that can be used for transport property determination, does not suffer from these limitations. In addition, it is non-destructive, spatially resolved, and can be applied to a specific buried layer, which is sometimes a problem for traditional electrical measurements. A number of issues are central to determining the accuracy and precision of this method, including the semiconductor material under investigation, the measurement system parameters, and the Raman spectral model used to fit the measured spectra. NIST is systematically addressing such issues. The results of this investigation should facilitate the utilization of Raman spectroscopy for spatially resolved, off-line characterization as well as process monitoring and control during film growth and subsequent patterning processes.

***Accurate in situ methods are needed to quantitatively determine the electrical carriers in optoelectronic components***



**The measured Raman spectrum for n-type GaSb. Fitting the data with a Raman spectral model results in a determination of carrier concentration of about 6 x $10^{18}$ $cm^{-3}$**

Raman spectra of n-type doped GaSb, p-type GaSb, n-type GaInAsSb, and n-type GaN were recorded to determine the carrier density. Modeling of the Raman spectra from the different materials also requires different spectral models. The spectral models for the Sb-based materials are based on a zinc blend crystal structure and account for conduction band non-parabolicity, absorption of excitation radiation, and included multiple carrier types (due to multiple conduction band minima being occupied). The computer code for modeling the Sb-based materials has been written and all n-type Sb-based material spectra we have obtained have been fit. These results have been compared to electrical measurements. From this comparison, it was determined that more sophisticated spectral models should be developed. The spectral models for the N-based materials are based on a wurtzite crystal structure and do not need to account for absorption or multiple carrier types.

## *Neutron Transmutation Doping of Compound Semiconductors*

***R.M. Lindstrom (839)***

The addition of small amounts of selected elements to silicon and other materials controls the semiconducting nature of the material. Typically these elements are implanted at a high energy then annealed to more evenly distribute the dopant. Doping of semiconductors can also be accomplished by using neutron capture, followed by beta decay, to transmute elements. New compounds are being explored, to exploit the experience gained in industry with silicon.

***The ability to dope GaN uniformly would be as useful for tuning the semiconductor properties of this promising material.***

Transmutation of silicon by nuclear reactions has become well established for the production of high-power rectifiers and other electronic devices. In this application, phosphorus is created by the capture of neutrons by $^{31}$Si to make $^{32}$Si, which decays to $^{32}$P. Because silicon is transparent to neutrons, the phosphorus dopant is created uniformly through a large Si crystal.

The same process as used for elemental silicon is applicable to 1:1 compound semiconductors. Recently, neutron transmutation doping (NTD) has been explored in two additional systems. In collaboration with North Texas State University and the NIST Center for Neutron Research, samples of $Hg_{0.8}Cd_{0.2}Te$ films on $Cd_{0.95}Zn_{0.05}Te$ substrates have been subjected to neutron irradiation. The goal is to transmute both Hg to Au and Te to As. A first experiment showed that transmutation is feasible. The results of this experiment were reported at the Narrow Bandgap Conference in 2003. To study the effects of irradiation quantitatively, additional samples have been exposed to a pure thermal neutron flux over a range of fluences.

Gallium nitride is of great current interest for high-temperature devices. The opportunity exists for uniform doping of this material, by transmuting both $^{69}$Ga and $^{71}$Ge to the corresponding Ge isotopes. In collaboration with IEEL, a sample of bulk GaN was obtained from ATMI, Inc. and irradiated with $1.0 \times 10^{18}$ neutrons/cm$^2$, for a calculated uniform doping of $2.5 \times 10^{16}$ atoms Ge/g Ga, or $1.3 \times 10^{17}$ atoms Ge/cm$^3$. This material is has been returned to ATMI for characterization.

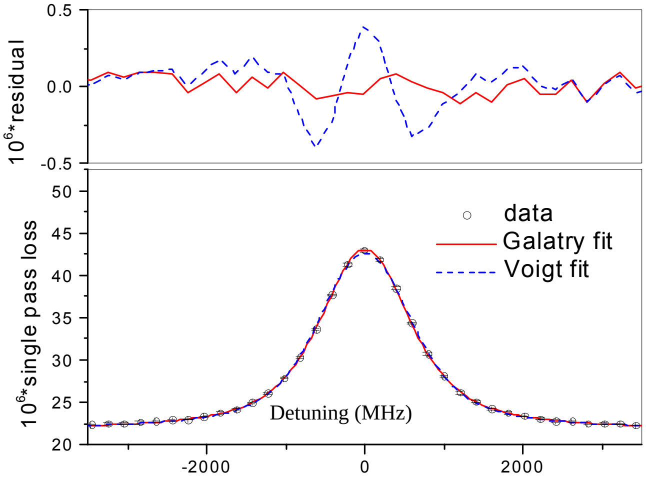
## *High-Resolution Cavity Ring-Down Spectroscopy (CRDS) Measurements of Water Vapor*

***J.T. Hodges and J.G. Cormier (836)***

CRDS is being developed as a primary measurement standard enabling quantitative on-line measurements of water vapor at trace concentrations ≈$10^{10}$ molecules cm$^{-3}$ to $10^{13}$ molecules cm$^{-3}$, a domain where gravimetric techniques are inapplicable. This research has applications in semiconductor processing, as well as trace gas monitoring and analysis, and atmospheric research. It also supports a broad class of spectroscopic techniques that are based upon the absorption of electromagnetic radiation by water vapor.

***Trace amounts of water vapor occurring in bulk source gases or de-adsorbed from system surfaces can adversely effect important processes related to the growth and manufacturing of semiconductors, photonic devices and other micro- and nano-scale solid state systems.***

In FY 2003, two sets of CRDS experiments covering different spectral ranges were implemented. In the first system, a CW diode laser and a custom high-resolution CRDS apparatus were used to measure absolute line strengths, and line shapes for water vapor transitions in the near-IR. A long-term reproducibility of better than 0.3 % and relative uncertainty less than 2 % in the determination of line strength was demonstrated. (See figure for a typical spectrum.) The effects of pressure broadening and collisional narrowing of the line shape were quantified and a spectral resolution on the order of 1 MHz was achieved, illustrating the high spectral resolution of the CRDS method. The second CRDS experiment considered the water vapor absorption continuum in the mid-IR spectral range. The temperature dependence of the continuum absorption coefficient was measured from –15 °C to 20 °C. This work exploited the high precision of the mid-IR CRDS technique and access to NIST standards of humidity generation and measurement. A detection limit of 30 pmol mol$^{-1}$ was demonstrated. Finally, to



**Measured spectrum of $H_2O$ absorption transition, CRDS measurements and best-fit theoretical line shapes (bottom), and fit residuals (top).**

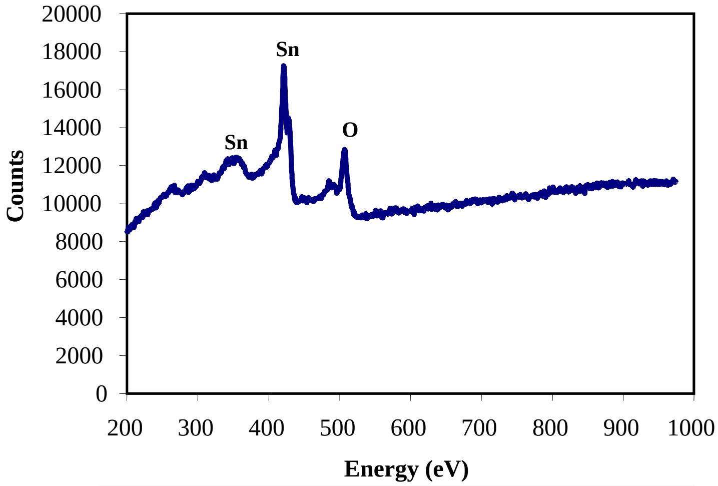
complement the mid-IR CRDS studies of the water vapor continuum, similar CRDS measurements of the water vapor continuum were also begun using the near-IR CRDS system. These studies suggest that near-IR CRDS measurements of the water vapor continuum may answer some important scientific questions regarding the role of water dimers in the radiation absorption by atmospheric water vapor.

### *Identification of Contaminants in Surface Films by Scanning Auger Microscopy*

***S.A. Wight (837)***

**_CSTL researchers use scanning Auger microscopy to identify contaminants on the surfaces of two technologically important materials for the semiconductor industry._**

The surface specificity of Auger microscopy is ideally suited to identify contaminant films and particles on surfaces. In the electronics industry, as devices continue to shrink, scanning Auger microscopy is being more widely utilized to identify contaminates. NIST's analytical scanning Auger microscope (ASAM) has been applied to two specific measurement challenges. 1) It was used to identify the contaminant precipitates in tin-copper electrodeposited films on copper substrates. These films are an important step towards lead-free solders for the microelectronics industry. 2) The ASAM was used to identify and provide a maximum thickness for the contamination areas on a copper plate used for the proposed new NIST capacitance standard based on the charge of the electron.



**Auger spectrum of contaminant particles on Sn-Cu film.**

The contaminate particles in the tin-copper electrodeposited films were identified as tin oxide precipitates by scanning Auger microscopy. Prior to analysis, the area of interest was carefully cleaned, by low energy ion etching, to remove the ubiquitous organic contamination that otherwise masks the composition of the particles. In the cleaning process it was necessary to strike a critical balance between removing the oxygen and organic contaminants that had adsorbed onto the surface during transport in air and not removing the particles before they are analyzed.

The contamination films on the copper plate were identified as mixed hydrocarbon and oxide with a thickness of $\leq 3$ nanometers. The thickness was determined by argon ion sputtering with a calibrated etch rate for different lengths of time until the carbon and oxygen peaks were completely removed from the Auger spectrum. This was repeated at multiple locations on the surface of the copper plate to assess the variability and determine the maximum contamination film thickness. The Auger results have provided the NIST researchers with the feedback they needed to continue development of important new technologies in the field of microelectronics.

**Recently, we have measured transition line shapes and strengths near a wavelength of 940 nm and the temperature dependence of the water continuum absorption at a wavelength of 10 µm.**

# 11. Measurement Standards

### *… anchoring the Nation's measurement infrastructure and supporting mature and emerging technologies*

NIST provides a wide variety of measurement, standards, and data services and programs to help U.S. industry improve its international competitiveness, commercialize new technology, and achieve total quality in all facets of business operations. CSTL maintains and disseminates measurement standards for chemical, biomolecular, and chemical engineering measurements by developing Standard Reference Materials (SRM®s), Standard Reference Data (SRD), and by providing Calibration Services. CSTL partners with NIST Technology Services to help disseminate the standards to NIST customers.

With a significant role in NIST's Calibration Services, CSTL provides calibrations for volumetric test measures, pressure and vacuum gauges, thermometers, hydrometers, humidity measuring instruments, fluid flow meters and air speed instruments and optical filters. These calibrations help customers achieve the highest measurement quality and productivity. In addition, instrumentation manufacturers and other users rely on our calibration services to provide traceability to U.S. national measurement standards. In FY 2003, CSTL completed 1,501 calibrations with 180 customers served. The Process Measurements Division and the Analytical Chemistry Division were responsible for the large majority of calibrations within CSTL in FY 2003.

### *Physical Standards Development*

**Examples of Physical Standards research activities in CSTL:**

**Temperature – *Primary acoustic thermometry, Methods and devices to disseminate ITS-90***

**Pressure – *Intrinsic pressure standards in the range of 0.3 MPa to 5 MPa***

**Vacuum - *Next-generation vacuum gauging technology***

**Flow – *New volumetric liquid flow standards reduces uncertainty by 10-fold***

**Humidity - *Rapid, low-cost methods for calibration of permeation tube generators***

SRMs provide scientists and engineers in industrial and academic research with internationally accepted standards for critical technical decision-making. NIST pioneered, and continues to lead, in the development of certified reference materials used for quality assurance. Traditionally, SRMs have been the primary tools that NIST provides to the user community for achieving chemical measurement quality assurance and traceability to national standards. Currently, NIST catalogs nearly 1,440 SRMs; in 2003 NIST sold approximately 31,000 SRM units to about 6,500 unique customers. Approximately 24,000 of the units sold were from the ≈ 700 different types of materials that are certified for chemical composition. CSTL provides technical leadership for most of the chemical and compositional standards produced by NIST. SRMs are used for three main purposes: to help develop accurate methods of analysis (reference methods); to calibrate measurement systems; and to assure the long-term adequacy and integrity of measurement

### *Chemical Standards Development*

**Examples of new reference materials in production from CSTL:**

**Health - *Human cardiac troponin complex, toxic elements in urine***

**Food - *Ephedra standards, nutrients in peanut butter, and slurried spinach***

**Environmental - *Contaminants in fish tissue and marine sediment, atmospheric PM2.5,***

**Chemicals - *Low carbon steel, zeolites***

**Commodities - *Aluminum alloys,* electronic scrap artifact**

**Forensics/Homeland Defense - *Smokeless gun powder, drugs of abuse, Y-chromosome DNA***

quality assurance programs. NIST SRMs also legally constitute part of the National Measurement System infrastructure of the United States and, as such, are essential transfer mechanisms for national as well as international measurement traceability.

NTRM

To meet the growing need for traceable standards, CSTL pioneered the NIST Traceable Reference Materials (NTRM) program. An NTRM is a commercially produced reference material with a well-defined traceability linkage to existing NIST primary standards. The first NTRM program in gas-mixture reference materials was developed in 1992 for the compressed gas industry. The high availability of gas-mixture reference materials as NTRMs has significantly aided vehicle emissions testing in the U.S. and has become the cornerstone of emissions trading in the fossil-fueled electric power industry. In 2003 137 gas SRMs were recertified for 14 Specialty gas companies. Convinced of the success of the NTRM program, CSTL is examining this model as well as other mechanisms to reach markets where a high demand for NIST traceability exists.

**A study conducted by RTI International documented the success of the gas-mixture NTRM program, estimating that the "net benefits" of the program projected through 2007 will be $50M to $63M with a social rate of return of about 225%.**

One of these mechanisms by which CSTL meets the need for additional traceable standards is intrinsic standards development. Intrinsic standards can reduce the need for labor-intensive artifact standard production and repeated costly inter-laboratory measurement comparisons. A paradigm is envisioned in which appropriate SRMs are not supported in perpetuity, but are produced through enough cycles to establish the material as an intrinsic standard or transfer the continued production to the private sector with an acceptable traceability strategy. A specific step in this direction is the imminent publication of internationally determined band positions of dilute acidic holmium oxide solution as an intrinsic wavelength standard for calibration in spectrophotometry. In a related development, CSTL researchers have developed an algorithm to implement holmium oxide solution wavelength calibration and a proposed extended use of the material as a universal simultaneous standard for both wavelength and absorbance. Additional details are provided in the following highlight.

**Intrinsic standards "based on well characterized laws of physics, fundamental constants of nature, or invariant properties of materials" (ANSI/NCSL Z540) have many uses in physical and chemical metrology.**

## *Development and Dissemination of Intrinsic Standards for Chemical Measurements*

***P.M. Chu, D.L. Duewer, M.L. Salit, and J.C. Travis***

***Intrinsic standards enable National Metrology Institutes (NMIs) to concentrate efforts on metrology rather than artifact production or calibration, and offer end-users of commercial standards ready access to traceability through data.***

Three separate efforts within the CSTL report significant progress in the development of intrinsic standards. (1) Samples of SRM 2034 (dilute holmium oxide solution sealed into fused silica cuvettes) were distributed to fifteen NMIs. Results from these laboratories, NIST/PL, and our own reference instruments will form the basis for an internationally supported intrinsic wavelength standard for UV/visible chemical spectrophotometry. (2) In collaboration with the NIST Physics Laboratory (NIST/PL), we have prepared the first of three manuscripts reporting improved spectral line positions for mercury and argon atomic lines in low-pressure discharges. These positions serve as secondary length standards and support higher accuracy in the determination of atomic line positions in such analytical plasmas as the Inductively Coupled Plasma (ICP). (3) SRD 79 Quantitative Infrared Database is an on-going project providing quality-assured quantitative infrared absorption reference data to support open-path Fourier transform infrared (FT-IR) measurements, such as those described in EPA Method TO-16. New spectra based on gravimetrically prepared samples are added to the database as they are acquired. Comparisons with Pacific Northwest National Laboratories continue to play a key role in validating the data.

**Economy of scale encourages users to purchase the physical realization of an intrinsic standard (an artifact transfer standard) rather than constructing it themselves. Thus, the production of artifact standards devolves from the NMI to the commercial sector, which is better suited to production.**

### *The NIST Gravimetric Hygrometer: A Primary Humidity Standard*

***C. W. Meyer, G.E. Scace, J.T. Hodges (836);***
***J. Valencia (Guest Researcher, CICATA, Mexico)***

The NIST gravimetric hygrometer (GH) hygrometer is a primary humidity standard that determines the amount of water in a gas sample by measuring the mass fraction of the water. This is accomplished by trapping the water in collection tubes containing desiccant; the collection tubes are weighed before and after trapping, and the mass difference is due to the trapped water. The mass of the dried gas sample is determined by volume measurements using interferometric measurements of piston displacement (see figure), in combination with gas density calculations. Performance optimization and uncertainty evaluation are accomplished through both control tests and measurement of the humidity from a well-characterized generator.

***NIST has a primary working standard for humidity that can validate the performance of the generators used for calibrating customer hygrometers. This helps assure NIST customers that its humidity standards and calibrations are maintained at the levels needed by industry.***

**NIST humidity calibrations are performed by generating samples of gas with well-characterized humidities, which are then supplied to customer hygrometers. To insure the highest quality calibrations, the performance of these generators should be periodically tested by measurement of the generated humidity using a primary humidity standard. The GH was built to fulfill this function.**

**Prover tubes with pistons used for the determination of the sample volume.**

The GH is now operational and its uncertainties are currently being evaluated and minimized. Comparisons with the NIST Low Frost Point Generator and 2-Pressure Generator have determined its present agreement with these generators to be within 0.4 % of water mass fraction. The comparisons have also shown the present day-to-day reproducibility of GH measurements to be within 0.3 %. Control tests have identified methods for further reduction of the measurement uncertainties. Specifically, tests have been performed to determine the water collection capacity of the collection tubes and to accurately account for changes of gas mass in the collection tubes. Additional tests have been designed to determine the amount of residual water in the gas after it passes through the collection tubes.

## *Elucidation of Systematic Effects in Primary pH Measurements*

***K.W. Pratt (839)***

***CSTL supports the certification of seven pH SRM buffers covering a range of roughly pH 1.7 to 12.4.***

SRMs provide the primary benchmarks to establish the traceability of pH measurements. International recognition of these SRMs is supported by NIST participation in Key Comparisons (KC), administered by the Conseuil consultatif pour la quantité de matière (CCQM), which is described in more detail in the ***International Measurement Standards*** section of this chapter. NIST performance in prior pH KCs has been sufficient to ensure international recognition of the pH SRM suite. However, the reproducibility of the NIST primary pH measurements in these KCs and in prior SRM certifications has been degraded by larger-than-expected Type A uncertainties (“scatter”) of the measurement replication in the primary pH measurements. The primary measurement yields the standard pH values, designated pH(S), of the given buffer. The Type A uncertainties in these pH(S) values have been larger than expected, based on analysis of the set of expected Type B (systematic) components of the overall uncertainty in the primary pH(S) measurement. This state of affairs suggested that an additional, uncontrolled factor, hitherto not considered in the uncertainty analysis, limited the reproducibility of primary pH metrology as realized at NIST. A fundamental study was initiated to elucidate this unknown factor. Transient mixed potential phenomena arise within Ag|AgCl electrodes, used in the primary measurement, following their transfer from the storage solution (0.01 mol $kg^{-1}$ HCl) to the pH buffer being certified. These transient phenomena decay to a negligible level if the electrodes are stored in the given buffer solution for at least 12 h prior to performing the primary measurement. This pre-equilibration step has been incorporated into the NIST procedure for primary pH measurements. The results of the fundamental study into the transient behavior of Ag|AgCl reference electrodes provided new information regarding the systematic uncertainties and behavior of Ag|AgCl electrodes in primary pH measurements. These fundamental studies will be continued, yielding explanations of transient phenomena in Ag|AgCl electrodes based on modern electrochemical theory. The quality of primary pH measurements obtained at NIST now equals or exceeds that of other NMIs that currently perform similar measurements. Based on these pH SRM certifications, it is expected that the NIST performance in future CCQM pH KCs, as evaluated by the Type A uncertainties that are attained, will surpass the corresponding “best” level attained by the set of NMIs participating in the given CCQM pH KC.

**Changes in the NIST protocol for primary measurements of pH(S) have already yielded significant improvements in the uncertainty obtained in certifications of pH SRMs.**

# *Selected Chemical and Physical Standards Activities in FY 2003*

*(Described in more detail elsewhere in this report)*

## *Automotive and Aerospace*

### *New Gas Standards for Calibrating Instrumentation used for Measuring Emissions from Next Generation Low Emission Vehicles*

***W.J. Thorn III and W.D. Dorko (839)***

## *Pharmaceuticals and Biomanufacturing*

### *Use of SRM Based Column Performance Tests to Guide LC Column Selection in the Pharmaceutical Industry*

***L.C. Sander and S.A. Wise (839)***

### *New Glass Filter Reference Materials for Validating the Performance of UV/visible Spectrophotometers in the High Absorbance Range*

***J.C. Travis, M.V. Smith, and G.W. Kramer (839)***

## *Chemical and Allied Products*

### *Zeolite Reference Materials: Chemical Properties, Structural Parameters, and Particle Size Distribution*

***S. Turner, R.A. Fletcher, E.S. Windsor, and R.R. Cavanagh (837); J.R. Sieber, T.W. Vetter, R.L. Zeisler, and D.A. Becker (839); B.H. Toby and J. Kelly (MSEL); S.D. Leigh (ITL); M. Davis (Caltech); S. Yang and A. Navrotsky (Univ. California at Davis)***

### *International Standards for Refrigerant Properties*

***M.O. McLinden and E.W. Lemmon (838)***

### *Measurements, Modeling, and Data for Pressure-Driven Membrane Separations*

***C. Muzny, H. Sun, and J. Moon (838); J. Cho (U. of Colorado); M. Chapman (U.S. Bureau of Reclamation); and J. Ranville (Colorado School of Mines)***

## *Energy and Environmental Technologies*

### *Natural Gas Flow: North American Laboratory Comparison Project*

***P. I. Espina (836) and W. F. Guthrie (ITL)***

### *Influence of Oxygen on the Shelf Life of Coal SRMs*

***R. Zeisler (839) and W.D. James (Texas A&M)***

### *SRMs for Air Particulate Matter Less Than 2.5 µm (PM$_{2.5}$)*

***R. Zeisler, J.R. Kucklick, B.J. Porter, D.L. Poster, M.M Schantz, R.O. Spatz, and S.A. Wise (839)***

### *Developing Methods for the Determination of Past-Use and Current-Use Persistent Organic Pollutants in Standard Reference Materials*

***J.R. Kucklick, M.M. Schantz, S.A. VanderPol, K.J. Tuerk, and S.A. Wise (839)***

### *SRMs for Contaminants in Marine Sediment and Aquatic Species Tissue*

***S.A. Wise, S.J. Christopher, R. Demiralp Oflaz, R.R. Greenberg, J.R. Kucklick, S.E. Long, E.A. Mackey, B.J. Porter, D.L. Poster, M.M. Schantz, and R. Zeisler (839)***

***International Infrastructure for Traceability in Atmospheric Ozone Measurements***
***P.M. Chu and J.E. Norris (839)***

***Development and Measurement of Heavy Hydrocarbon ($C_6$-$C_{16}$) Gas Standards***
***G.C. Rhoderick (839)***

## *Food and Nutrition*

***Development of Analytical Methods and SRMs for Chemical Characterization of Botanical Dietary Supplements***
***S.A. Wise, T.A. Butler, S.E. Long, M.C. Mildner, E.A. Mackey, K.E. Murphy, K.W. Phinney, B.J. Porter, L.C. Sander, M.B. Satterfield, K.E. Sharpless, L.J. Wood, and L.L. Yu (839); T. Ihara (National Metrology Institute of Japan, AIST).***

***Certification of SRM 1946 Lake Superior Fish Tissue – Frozen Fish Tissue for Environmental Contaminants and Food Constituent Measurements***
***S.A. Wise, S.J. Christopher, J.R. Kucklick, S.E. Long, E.A. Mackey, C.S. Phinney, B.J. Porter, R.S. Pugh, D.L. Poster, M. Rearick, KE. Sharpless, and M.M. Schantz (839)***

***A Complementary Suite of Food-Matrix SRMs to Support Industry Compliance with Nutritional Labeling Requirements***
***K.E. Sharpless, J. Brown-Thomas, C.S. Phinney, B.J. Porter, and L.J. Wood (839)***

***Keeping Tin Cans Lead-Free***
***J.R. Sieber, K.E. Murphy, and S.E. Long (839)***

## *Forensics and Homeland Security*

***Certification of NIST Reference Material Supporting Improvised Explosive Device Measurements***
***W.A. MacCrehan and M. Bedner (839)***

***Standards for Detection of Biological Threat Agents***
***K. D. Cole, L. Wang, A. Gaigalas, and D. Hancock (831)***

## *Health and Medical Technologies*

***Standards Requirements for Systems Biology Approaches to Health Care: Mitochondrial Proteomics***
***Gregory B. Vásquez and Peter E. Barker (831); Steven Zullo (ATP/NIST)***

***Development of Reference Methods and Reference Materials for Clinical Diagnostic Markers***
***M.J. Welch, D.M. Bunk, M. Satterfield, L.T. Sniegoski, and S.S-C. Tai (839)***

***Development of SRM 2921, Human Cardiac Troponin Complex: A Primary Calibrator for Assays Used to Diagnose Heart Attacks***
***D.M. Bunk and M.J. Welch (839)***

***Development of Reference Methods and Participation in International Round-Robins for the Determination of Thyroid Markers***
***S. S-C. Tai, D.M. Bunk, and M.J. Welch (839)***

***Reference Methods and SRMs for Toxic Species in Body Fluids***
***S.E. Long, R.D. Vocke, L.L. Yu, K.E. Murphy, T.A. Butler, and E.A. Mackey (839)***

***Standards for Fluorescence Microarray Analyses***
***G.W. Kramer (839); A.G. Gaigalas (831); P.C. DeRose (839)***

### *Industrial and Analytical Instruments and Services*

**Standards for Raman Spectroscopy**
***W.S. Hurst, J.E. Maslar (836); S.J. Choquette (839); and E.S. Etz (837)***

***Fluorescence Standards Suite for Spectral Correction of Fluorometers***
***P.C. DeRose, D.H. Blackburn, and G.W. Kramer (839)***

***Improved Vacuum Transfer Standards – Ionization Gauges***
***P.J. Abbott (836); P. Mohan (NPL India)***

**Development of Critical Benchmark Standards and Methods for the Metals Industry**
***J.R. Sieber, A.F. Marlow, M.R. Winchester, L.L. Yu, T.A. Butler (839); R.M. Lindstrom (837); M.P. Cronise, and C.N. Fales (TS)***

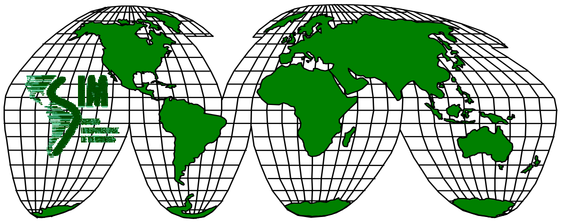
***Elucidation of Systematic Effects in Primary pH Measurements***
***K.W. Pratt (839)***

### *Microelectronics*

***Electron Probe Characterization of*** $KNbO_3$ ***and*** $NaNbO_3$ ***Single Crystals for use as Quantitative Microanalysis Reference Materials***
***R. B. Marinenko (837), Z. Samardžija, S. Bernik, B. Malič and M. Čeh, (Jožef Stefan Institute, Slovenia)***

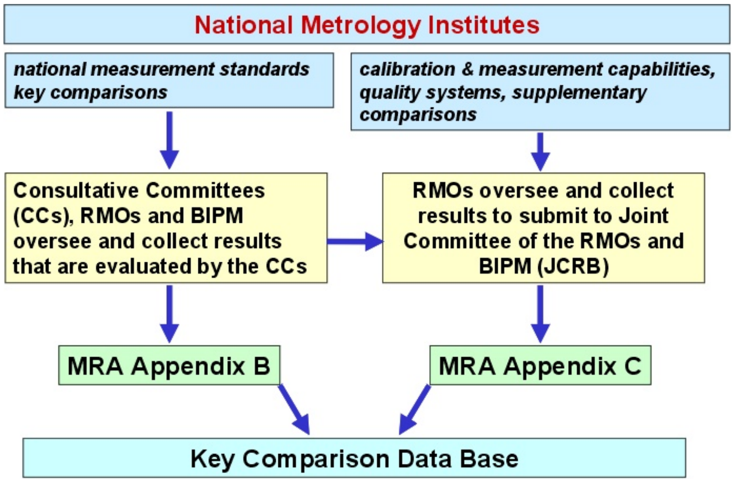
***Development of Phosphorus Ion Implant in Silicon Depth Profile Standard***
***D. Simons (837), R. Paul (839), W. Guthrie and J. Lu (ITL)***

## *International Measurement Standards*



Increased requirements for quality systems documentation for trade and effective decision-making regarding the health and safety of the U.S. population have increased the need for demonstrating "traceability to NIST" and establishing a more formal means for documenting measurement comparability with standards laboratories of other nations and/or regions.

NIST is the U.S. National Metrology Institute (NMI) and the agency of the U.S. Government responsible for U.S. efforts under the Treaty of the Metre. The Committee International des Poids et Mesures (CIPM), and its various consultative committees, organizes comparisons of national measurement standards. After review, the results of these comparisons are recorded in the BIPM database (Appendix B). In addition, coordination of similar efforts with Regional Metrology Organizations (RMOs) extend the comparison efforts to as many participants as practicable**.**



***http://kcdb.bipm.org/BIPM-KCDB/***

In October 1999, NIST signed the CIPM Mutual Recognition Arrangement (MRA).

Since 1999 CSTL has systematically compared U.S. national measurement standards to establish degrees of equivalence of U.S. national measurement standard with those of other NMIs. These international activities add value to NIST standards and services, particularly for our customers involved in international trade. These MRA-related activities guarantee recognition of U.S. standards by U.S. trading partners.

**The objectives of the MRA are:**

- **To establish the degree of equivalence of national measurement standards maintained by NMIs;**
- **To provide for the mutual recognition of calibration and measurement certificates (CMCs) issued by NMIs; and**
- **Thereby to provide governments and other parties with a secure technical foundation for the wider agreements related to international trade, commerce, and regulatory affairs.**

For implementation of this MRA, the signatory NMIs agreed to:

1. Declare and document their calibration and measurement capabilities (CMCs) [Appendix C] ***(http://kcdb.bipm.org/AppendixC/default.asp)***
2. Participate in relevant international comparisons to support their CMCs [Appendix B] ***(http://kcdb.bipm.fr/BIPM/KCDB/; http://icdb.nist.gov )***
3. Implement and document the existence of a system for assuring the quality of the measurement services provided.

Regional Metrology Organizations (RMOs) play an important role in the MRA. They have the responsibility for carrying out key comparisons within their regions. They also carry out supplementary comparisons and other actions to support mutual confidence in the validity of calibration and measurement certificates through the Joint Committee of the RMOs and the BIPM (JCRB). They are also responsible for the entries into the BIPM key comparisons database for the calibration and measurement capabilities of their member NMIs.

**In order to most effectively address the unique needs of all 32 countries within SIM, CSTL has initially focused the SIM program on training and capability assessment rather than participation in MRA-driven KCs. During the past three years, 16 intercomparison exercises were carried out to assess the proficiency of SIM NMIs and/or their designated laboratories. Four additional exercises are planned for 2004.**

Systema Interamericano de Metrologia (SIM) is the RMO that includes the United States. The metrology organizations that make up SIM are known as: NORAMET (North America), CAMET (Central America), CARIMET (Carribean), ANDIMET (Andes Region), and SURAMET (South America). Regional cooperation leads to a wider harmonization of measurements and standards, facilitates free flow of trade, and is a necessary first step to globalization. NIST and National Research Council (NRC) Canada link SIM countries to the BIPM, and as such, provide greater opportunities for international trade and commerce. CSTL staff provides leadership for SIM by chairing the Chemical Metrology Working Group of SIM, and by serving as the U.S. respresentative to the JCRB, in order to assure the effective, fair, and metrologically sound implementation of the MRA.

## *CIPM Consultative Committees (CCs)*

The CIPM has set up a number of Consultative Committees, which bring together the world's experts in their specified fields as advisers on scientific and technical matters. Among the tasks of these Committees are the detailed consideration of advances in physics that directly influence metrology, the preparation of Recommendations for discussion at the CIPM, the identification, planning and execution of key comparisons of national measurement standards, and the provision of advice to the CIPM on the scientific work in the laboratories of the BIPM.

**GLOSSARY**

| | |
|---|---|
| **BIPM** | **Bureau International des Poids et Mesures** |
| **CIPM** | **Committee International des Poids et Mesures** |
| **CC** | **Consultative Committees** |
| **CCM** | **Consultative Committees for Mass** |
| **CCQM** | **Consultative Committee on the Quantity of Material** |
| **CCT** | **Consultative Committees for Temperature** |
| **JCRB** | **Joint Committee of the RMOs and BIPM** |
| **KC** | **Key Comparisons** |
| **KCRV** | **Key Comparison Reference Values** |
| **MRA** | **Mutual Recognition Arrangement/Agreement** |
| **RMO** | **Regional Metrology Organizations** |
| **SIM** | **Systema Interamericano de Metrologia** |

### *CIPM Consultative Committees for Temperature (CCT) and Mass (CCM)*

The Process Measurements Division has significant participation in the CC's for Temperature (CCT) and Mass (CCM). We have lead or participated in many Key Comparisons in the past several years. In thermometry, these efforts have resulted in establishing equivalence levels over the ITS-90 range from 14 K to 1235 K. Key Comparisons organized by the CCM include Division activities in both the pressure and vacuum and the flow project areas. In the pressure and vacuum project the Division leads or participates in Key Comparisons that cover the pressure range $3 \times 10^{-6}$ Pa to 500 MPa. NIST has piloted three CCM Key Comparisons in the last several years, completing two in FY02 that demonstrated general equivalence among the participants, revealed no systematic bias between alternative realizations of the

Pascal, were the only CCM Key Comparisons completed on schedule, and set the standard for the manner in which Key Comparisons should be conducted. In addition, CSTL researcher G. E. Mattingly continues to chair the CCM's Working Group for Fluid Flow (WGFF). Through FY 03, this working group has progressed applying its strategy to produce Key Comparisons (KCs) in seven different flow measurement areas: water, hydrocarbon liquids, low-pressure air, high-pressure and high-flow natural gas, high pressure nitrogen or air, air speed, and liquid volume. In each of the seven flow areas, different NMIs have accepted the responsibility to produce transfer standards and the test procedures needed to compare the respective flow standards of the participating NMIs. One of these - the Korea Research Institute of Standards and Science (KRISS) has completed this work in water flow and with approvals from the CCM, has officially started the working group's first KC. This KRISS program is expected to set precedents that should advance the state-of-the-art in conducting all future flow laboratory comparisons. The Working Group for Fluid Flow chair continues to work closely with the NIST Statistical Engineering Division to develop sound, statistically based test procedure designs and supporting analysis methods applicable to all KCs, and to assess the metrological linkage techniques that will be required to connect the results of the KCs to the associated Regional Metrology Organization (RMO) tests that will follow the KCs to metrologically link all of the participating NMIs.

**NIST's Quality Manuals for calibrations are basically equivalent to ISO 17025 Requirements for the Competence of Testing and Calibration Laboratories and its calibration services only needed relatively minor upgrades to comply with the NIST-wide objectives of ISO17025 equivalency by December 2003.**

NIST will pilot the KC for the low-pressure gas flow. Recent advances in U.S. standards and procedures (described elsewhere in this report - see citation below) are expected to materially improve the performance-level of this KC. Characterization of the transfer package will begin in FY04.

### *Natural Gas Flow: North American Laboratory Comparison Project*

***P. I. Espina and W. F. Guthrie (898)***

Details provided in the **Energy and Environmental Technologies** section.

## *Consultative Committee on the Quantity of Material (CCQM)*

The Consultative Committee on the Quantity of Material (CCQM) has seven working groups: (1) Gas Analysis, (2) Organic Analysis, (3) Inorganic Analysis, (4) Electrochemistry (5) Biometrology, (6) Surface Analysis and, (7) Key Comparisons. These working groups are responsible for selecting and overseeing the operation of key comparisons that address chemical measurement-related issues important for international trade, environmental, health, and safety-related decision making. CSTL staff are active in all seven and has provided formal leadership for the Organic Analysis, Biometrology and Key Comparisons Working Groups. During the past five years, approximately 96 comparison studies have or are being conducted under the auspices of the CCQM. CSTL's Analytical Chemistry Division (ACD) has participated in 75 of these, serving as Coordinating Laboratory in 31. Over 25 additional studies are planned over the next two years and NIST has already committed to coordinate at least seven of these. In FY03, the comparisons in which CSTL participated were for the determination of a variety of organic and inorganic components in matrices such as gaseous mixtures, sediments, food, water, fuel, and serum.

**NIST's Quality Manual for Chemical Measurements summarizes and formalizes policies and approaches for addressing quality-related issues concerning the services that it provides is being updated to assure appropriate compliance with ISO/EC 17025 and ISO Guide 34.**

Participation in relevant key comparisons (documented in MRA Appendix B) serve to benchmark the claims made in the Calibrations and Measurements Capabilities (documented in MRA Appendix C). CSTL's Analytical Chemistry Division has led the critical review of CMC data for Appendix C in the BIPM Database. SIM has approximately 1140 CMC entries scheduled for inclusion in the BIPM database. By the beginning of 2004, the BIPM database included approximately 3000 CMCs for chemistry, with about 1000 from NIST.

## Selected CCQM KCs Completed in FY 2003

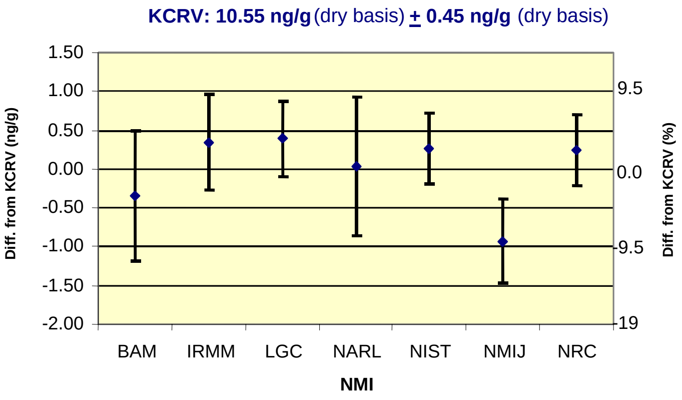
### Results of CCQM-K25 Key Comparison for Polychlorinated Biphenyl Congeners in Sediment

***M.M. Schantz and S.A. Wise (839)***

Polychlorinated biphenyls (PCBs) consist of 209 possible congeners depending on the substitution of the chlorine atoms around the biphenyl molecule. PCBs have been widely used as industrial fluids, flame retardants, diluents, hydraulic fluids, and dielectric fluids for capacitors and transformers. As a class of compounds, they are environmentally stable and tend to bioaccumulate. Of the 209 possible congeners, approximately 150 congeners have been reported in the environment. A CCQM key comparison for PCB congeners in sediment was conducted among nine laboratories, with NIST serving as the coordinating laboratory. All but one of the participant laboratories used gas chromatography/mass spectrometry (GC/MS) with carbon-13 labeled PCB congeners as internal standards/surrogates (isotope dilution (ID)-GC/MS).

***This Key Comparison demonstrated a high level of equivalence in capabilities of the participating national metrology institutes to successfully measure PCB congeners 28, 101, 105, 153, and 170 in a moderately contaminated sediment using ID-GC/MS.***

Five PCB congeners were chosen: PCB 28, PCB 101, PCB 105, PCB 153, and PCB 170. PCB 28 (2,4,4'-trichlorobiphenyl) is volatile and under certain conditions coelutes with PCB 31 (2,4",5-Trichlorobiphenyl). PCB 101 (2,2',4,5,5'-pentachlorobiphenyl) has the potential coelution with a minor congener, PCB 90 (2,2',3,4',5-pentachlorobiphenyl). PCB 153 (2,2',4,4',5,5'-hexachlorobiphenyl) is typically one of the most abundant congeners and potentially coelutes with PCB 132 (2,2',3,3',4,6'-hexachlorobiphenyl). PCB 105 (2,3,3',4,4'-pentachlorobiphenyl) is a congener with a lower concentration than the other congeners selected and which may change elution order with PCB 132 depending on the analytical conditions. Finally, PCB 170 (2,2',3,3',4,4',5-heptachlorobiphenyl) is one of the less volatile congeners, is typically found at lower concentrations, and can potentially coelute with PCB 190 (2,3,3',4,4',5,6-heptachlorobiphenyl). The ability of a laboratory to measure these five congeners should indicate their ability to measure the suite of 150 PCB congeners found in sediments.



**CCQM K25 PCB 105 Equivalence**

The uncertainties of the key comparison reference values (KCRVs) ranged from 2 % to 5 % indicating excellent agreement among the participants. The agreement among laboratories and comparison to the KCRV is shown in the figure for PCB 105. Similar degrees of equivalence were demonstrated for the other four congeners.

## *Results From the CCQM-K14 Key Comparison for Calcium in Serum*

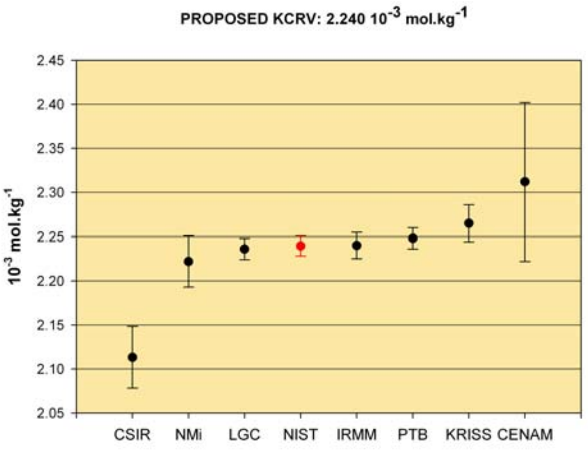
***S.E. Long and K.E. Murphy (839)***

Under the auspices of the CCQM Inorganic Analytical Working Group, the CCQM-K14 Key Comparison addressed the need for better measurement methodology for routine clinical measurements of total calcium in blood serum. It is evident that the current measurement system is unsatisfactory from a clinical diagnostic standpoint, and the resulting impact on health-care costs may be considerable.

***The Analytical Chemistry Division achieved excellent agreement with other national metrology institutes in a CCQM Key Comparison of calcium in blood serum (K14). The calcium determination was by isotope dilution inductively coupled plasma – mass spectrometry. Measurements were nearly identical to the key comparison reference value chosen for this Key Comparison.***

**We have demonstrated the veracity of the analytical measurement system employed here in this Key Comparison for this calcium in blood serum. Such comparisons provide documented performance for the NIST quality system.**

Following the successful participation by the Analytical Chemistry Division in the pilot study during FY01, calcium was determined in an un-modified frozen human serum, which was distributed by European Commission's Institute for Reference Materials and Measurements (IRMM), the coordinating institute for this study. Eight other national metrology institutes also participated. Calcium was determined using a method employing isotope dilution "cold-plasma" inductively coupled plasma – mass spectrometry (ICP-MS). The result ((2.2395 ± 0.0115) mmol.kg$^{-1}$) was in excellent agreement with the other laboratories and also the proposed value for the key comparison reference value, which was (2.240 ± 0.008) mmol.kg$^{-1}$. The analytical data from the key comparison are shown below. It is interesting to note that there has been a shift from using thermal ionization mass spectrometry (TIMS) for making such measurements. The shift to ICP-MS has been made possible by the advent of systems for reducing or eliminating spectral interferences. Of the nine participants in the study, only two used TIMS. The remainder used a combination of high-resolution sector field, collision cell and cold-plasma ICP-MS, while one laboratory used ICP optical emission spectrometry.



**CCQM K14: Calcium in Serum**
**Results for 8 NMIs (NIST shown in red)**

## *Results from International Comparisons for Selected Components in Natural Gas*

***G.C. Rhoderick and F.R. Guenther***

NIST participated in the CCQM-K16 Natural Gas Key Comparison, for which the final report was released in October of 2002. The study evaluated the degree of equivalence among national metrology institutes (NMIs) for the given measurand in natural gas. Unlike past comparisons, this natural gas included heavier hydrocarbons and was designed to emulate real natural gas at both high and low calorific value.

NIST completed the analysis of the low calorific value natural gas standard in FY03 and released the report to the coordinating laboratory. The table below lists the NIST results of the comparison.

***NIST's results show excellent agreement with the gravimetric key comparison reference values. A pilot study similar to the key comparison was organized by NIST for the SIM countries. Results of this pilot study on natural reveal much larger deviations than those of the CCQM key comparison.***

**Agreement between NIST value and Preparation Value (given as % mol mol$^{-1}$)**

| Compound | Reference Value | Reference Value Uncertainty* | NIST Value | NIST Value Uncertainty* | Absolute Difference $\Delta_x$ | Relative Difference $\Delta_{x/x}$ |
|---|---|---|---|---|---|---|
| **Methane** | **81.6826** | **0.0027** | **81.77** | **0.46** | **0.087** | **0.11 %** |
| **Nitrogen** | **12.1205** | **0.0007** | **12.11** | **0.16** | **-0.010** | **-0.09 %** |
| **$CO_2$** | **4.0446** | **0.0005** | **4.055** | **0.026** | **0.010** | **0.26 %** |
| **Helium** | **0.50813** | **0.00016** | **0.5158** | **0.0026** | **0.0077** | **1.51 %** |
| **Ethane** | **0.7489** | **0.0002** | **0.7493** | **0.0046** | **0.0004** | **0.05 %** |
| **Propane** | **0.29144** | **0.00010** | **0.2905** | **0.0026** | **-0.0009** | **-0.32 %** |
| **iso-Butane** | **0.20165** | **0.00019** | **0.2034** | **0.0019** | **0.0018** | **0.87 %** |
| **n-Butane** | **0.20260** | **0.00007** | **0.2024** | **0.0018** | **-0.0002** | **-0.10 %** |
| **iso-Pentane** | **0.04861** | **0.00011** | **0.0483** | **0.0006** | **-0.00031** | **-0.64 %** |
| **n-Pentane** | **0.04982** | **0.00011** | **0.0500** | **0.0007** | **0.00018** | **0.36 %** |
| **neo-Pentane** | **0.04972** | **0.00006** | **0.0495** | **0.0007** | **-0.0002** | **-0.44 %** |
| **n-Hexane** | **0.05061** | **0.00011** | **0.0502** | **0.0008** | **-0.0004** | **-0.81 %** |

* Expanded uncertainty $U = ku_c$, with the combined standard uncertainty, $u_c$, determined from experiment and a coverage factor $k = 2$. The true value for the analyte amount-of-substance fraction is asserted to lie in the interval defined by the NIST value ± $U$ with a level of confidence of approximately 95 %.

A similar study was organized by NIST as a pilot for the SIM countries. Since this was a pilot study, the results will not be made available publicly. All the participating labs submitted methane values that were within ± 1.0 % of the certified concentration. However, the agreement among labs for all of the remaining compounds was poor. One lab was consistently within ± 1.5 % of the certified values with the exception of carbon dioxide (4.6 %) and n-butane (1.7 %). A SIM pilot study on automobile emission gases, piloted by NIST, will be completed by March 2004. A new SIM pilot study on industrial gas emission (sulfur dioxide) piloted by NIST will be initiated in late FY2004.

**The CCQM-K16 comparisons were designed to evaluate the capabilities of NMIs to certify natural gas standards. They are intended to provide evidence for calibration and measurement capabilities listed by each institute in the BIPM Mutual Recognition Arrangement Database.**

## *Joint Committee on Traceability in Laboratory Medicine (JCTLM)*

**The JCTLM created two working groups:**

I. **Reference Materials and Reference Procedures**
II. **Reference Laboratory Networks**

Under the auspices of BIPM, the Joint Committee on Traceability in Laboratory Medicine (JCTLM) was created in June 2002 to provide a worldwide platform for promoting and guiding the internationally recognized and accepted equivalence of measurements in laboratory medicine and the traceability of these measurements to appropriate standards. The committee is charged with facilitating the identification of the "higher-order" reference measurement procedures and certified reference materials that are currently available which provide the traceability of values assigned to calibrators and control materials for *in vitro* diagnostic devices (IVD).

***One requirement of the recently enacted European Directive 98/79/EC on in vitro diagnostic medical devices is that "the traceability of values assigned to calibrators and control materials ... must be assured through available reference measurement procedures and/or reference materials of higher order."***

At present, neither reference materials nor reference methods are available for more than 300 different chemical and biochemical species that are measured in medical laboratories using IVDs. Reference methods and/or materials exist for only about 30 worldwide. The US IVD industry has asked NIST to work with our counterparts in Europe and the Asia-Pacific to provide the reference materials and methods of "higher order" that are urgently needed to comply with the requirements of the EU IVD Directive.

To facilitate the identification of "higher order" reference methods and reference materials that are currently available, the Joint Committee on Traceability in Laboratory Medicine (JCTLM) was created at a meeting held at the BIPM in early June 2002. Three meetings were held this year to review nominations for more than 80 Reference Methods and 435 Reference Materials in eight analyte categories.

***JCTLM Working Group I* chaired by NIST and IRMM is charged with establishing a process for *identifying, reviewing against agreed upon criteria, and publishing a List of "higher order" Certified Reference Materials and Reference Measurement Procedures* required for IVD industry compliance with the EC IVD Directive.**

Based on this work and input from medical professionals and IVD manufacturers, JCTLM Working Group I (Reference Materials and Reference Procedures) will also establish a list of highest priority needs for new reference procedures and reference materials. NIST, EU and AP Laboratories have also begun a dialogue to share the workload in developing and maintaining the more than 100 standards that are needed for compliance by the IVD industry with the EU IVDD. NIST has been asked to provide about 40 % of the standards based on our current capabilities and U.S. market share.

Staff in CSTL's Analytical Chemistry Division and Biotechnology Divisions are developing the "higher-order" reference materials and methods for the US IVD industry to comply with the requirements of the EU IVD Directive. These include the following health status markers:

| Health Status Marker | Disease State |
|---|---|
| Cardiac Troponin-I | Heart attack, damage |
| Cadmium and Mercury | Toxic metal poisoning |
| Folates | Neural tube defects |
| HER-2 Neu | Breast cancer |
| Homocysteine | Heart disease risk |
| Glycated Hemoglobin | Diabetes status |
| Prostate-Specific Antigen | Prostate cancer |
| Triiodothyronine and TSH | Thyroid function |
| Trinucleotide Repeat | Mental retardation |
| Speciated Iron | Hemochromatosis, anemia |

In 2003, work was completed for standards for Toxic Metals in Urine, Cardiac Troponin-I and Electrolytes in Human Serum. Work will continue on the high priority list presented above. SRMs will be completed for Homocystein, Folates, Thyroid Markers in 2004. Work will also begin on standards for gene expression including RNA standards and a fluorescence standard for microarray scanning devices. JCTLM Review Teams for Blood gases, Blood Groupings, Microbial Serology, Non-Electrolyte metals and Vitamins will be established for providing lists of "higher order" methods and materials by December 2004.

## *Strategic Bilateral Collaborations and Intercomparisons with NMIs*

***W.E. May, F.R. Guenther, S.A. Wise, and R.R. Greenberg (839)***

In addition to these global and regional activities, we are also establishing a limited number of strategic bilateral collaborations and intercomparisons with NMIs around the world. For example, our collaboration with the Netherlands Measurement Institute (NMi) for determining the equivalence of primary gas standards has resulted in a formal "Declaration of Equivalence" that is recognized by the U.S. EPA and European environmental regulatory bodies as documenting the equivalence between seven NIST and NMi primary gas mixture suites. Our formal agreement with NRC-Canada (via the NAFTA Treaty) for cooperation in marine environmental studies has fostered collaborations between the U.S. and Canada in the certification of a several certified reference materials important for trade and environmental decision-making. We have recently signed a Cooperative Arrangement with NIMC (AIST Japan) for collaborative efforts in the area of pure volatile organic compound standards. These highly pure reference compounds will serve as primary references for many of our gas mixture SRMs and our quantitative reference database (SRD-79). Several additional strategic bilateral arrangements with other National Metrology Institutes /Standards laboratories are being discussed.

**CSTL's Analytical Chemistry Division continues to work with CITAC (Cooperation in International Traceability in Analytical Chemistry) to establish practical, yet metrologically sound, vertical traceability links between the NMIs and chemical testing labs in the various countries and regions around the world.**

通商産業省 工業技術院
Agency of Industrial Science and Technology
Ministry of International Trade and Industry Japan

## 12. Data and Informatics

***… assuring that U.S. Industry has access to accurate and reliable data and predictive models***

One of CSTL's goals is to assure that U.S. industry has access to accurate and reliable data and predictive models to determine the chemical and physical properties of materials and processes. CSTL's data and informatics activities impact all industry sectors from biotechnology and microelectronics to energy and instrument manufacturers. Versatile interactive databases provide easy access to high quality NIST data. Many databases are now available via the World Wide Web. The NIST ***Standard Reference Database*** (SRD) series has grown to over 50 electronic databases in chemistry, physics, materials, building and fire research, software recognition, and electronics. Through this program CSTL provides SRDs for Analytical Chemistry, Atomic and Molecular Physics, Biotechnology, Chemical and Crystal Structure, Chemical Kinetics, Industrial Fluids and Chemical Engineering, Materials Properties, Surface Data, and Thermodynamics and Thermochemistry. A few of the highlights in the area of Data and Informatics are described below, and a full listing of activities, with references to the appropriate program section, is also provided.

**CSTL continues to be a prominent source of SRD products at NIST. In FY 2003, 4,225 SRD units from CSTL were sold by NIST. Among these, 734 units were sold directly to customers and 3,491 units were sold through distributors.**

In a number of data areas, including structural biology, thermodynamics, and kinetics, NIST researchers are working with other scientists and organizations to establish data standards and more rapid methods of data entry. One example is the ***Thermodynamics Research Center*** (additional TRC details are provided in this section), which is working with several journals to have the thermodynamic data from accepted articles, go directly into the TRC database entry system through an electronic process. This assures that customers have the most up-to-date and complete information possible. The creation of data transfer and traceability standards is another key area of development. These standards remove barriers to the sharing of information and allow researchers to analyze results and collaborate in new ways. Another key concept is the establishment of the pedigree of data, in which enough information is retained to easily trace results and assign uncertainties to measured values, thus answering the vital question: "How good is that number?".

The ***NIST Chemistry WebBook,*** remains one of the most used resources for chemical and physical property data. The numbers of users, between 10000 and 20000 per week, and the variety of users, in industry, government and academia is a clear indication of the success of the WebBook. The fraction of returning users, typically between 45 to 55%, is a good indication that the user community feels that the resource is valuable. During 2003, the eighth edition of the NIST Chemistry WebBook was released. More effort has gone into acquiring data and a major donation of infrared spectral data from the Dow Chemical Company was received this year. In addition, the work on the IUPAC/NIST Chemical Identifier (INChI) has continued. The work on the INChI, along with work on data-exchange standards for chemical data is an essential part of making the WebBook a leading part of the next generation of electronic chemical information. In addition to an enhanced profile of prediction tools, a major increase in the infrared spectra was made with the release of the digitized Coblentz Society infrared spectral

**Usage of the WebBook has continued to grow at a rate of up to 20% per year, currently over 650,000 distinct IP addresses access the WebBook per year.**

**http://webbook.nist.gov/chemistry**

data. This data had only been available in printed form and the release of the data in fully digitized electronic form represents a major addition to the resources of the WebBook.

The WebBook is also a tool to aid future evaluation projects both at NIST and in collaboration with outside organizations. It is difficult to overstate the possible impact of the ongoing work on developing standard protocols for transmission of chemical data. The need for such standards has only grown as the use of the Internet in electronic commerce has grown. This need has been acknowledged by the large number of commercial as well as governmental entities participating in the IUPAC and ASTM committee work.

CSTL also engages the broader community through conferences and seminar activities. CSTL's Physical and Chemical Properties Division organized and co-sponsored ***The 15th Symposium on Thermophysical Properties*** held in Boulder, CO from June 22 to 27, 2003. The community involved in thermophysical properties represents a diverse group of those involve in their measurement, prediction, simulation, and application. The researchers and practitioners in the field comprise physicists, chemists, biologists, materials scientists, chemical engineers, mechanical engineers and others who may seldom have an opportunity to learn of the advances and impediments faced by their colleagues.

**The Symposium on Thermophysical Properties is a well-established series held roughly every three years since 1959, and is recognized as one of the premier international conferences associated with the theoretical, experimental, simulation, and applied aspects of the thermophysical properties of gases, liquids, and solids, including biological systems.**

The conference attracted about 450 participants from more than 40 countries, with about half from outside the United States. The authorship of more than 660 technical contributions – oral presentations, posters, and software exhibitions – comprised more than 1500 individuals from 49 countries. The conference consisted of 117 sessions including two plenary sessions, and was organized by CSTL staff and other members of the international community of experts. There were specialized and more general focus areas devoted to properties of various classes of materials; properties for process design and environmental applications; techniques, including molecule simulation and novel experiments; phenomena; and tools for specialized thermophysical property problems. Planning is underway for the 16th Symposium, which will be held in 2006 in conjunction with the International Conference of Chemical Thermodynamics.

## Selected Data Activities for FY 2003

### Thermodynamics Research Center (TRC) Comprehensive Program on Critical Data Evaluation

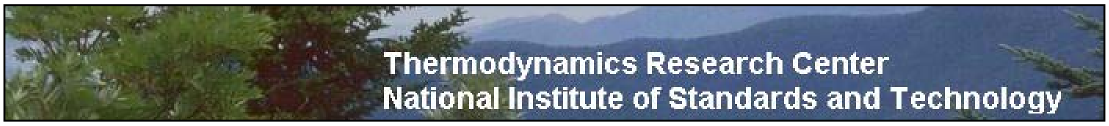
***M. Frenkel, R. D. Chirico, G. R. Hardin, R. A. Stevenson (838);***
***Q. Dong, X. Yan, R. C. Wilhoit, X. Hong (Contractors); V. V. Diky, (Guest Researcher)***

***Traditional methods for thermophysical data evaluation fail in light of the unprecedented growth in data availability (almost doubling every 10 years). Therefore, CSTL researchers are designing a dynamic system of generating evaluated data for industrial and scientific applications.***

The extraordinary growth of available data requires efficient new methods and computer tools for collection, critical evaluation, mining, quality control, management, and dissemination of thermophysical, thermochemical, and transport property data. The TRC concept of "dynamic" data evaluation requires large electronic databases capable of storing essentially all experimental data with detailed descriptions of the relevant metadata and uncertainties. The combination of these electronic databases with artificial intelligence (expert-system) software will allow the dynamic generation of evaluated recommended data as needed. This concept contrasts sharply with static compilations, which must be initiated far in advance of need. Dynamic compilations dramatically reduce the effort and costs associated with data programs.

**TRC efforts in the development of new XML-based technology for thermodynamic data communications (ThermoML) have been recognized in the establishment of the IUPAC project "XML-based IUPAC Standard for Experimental and Critically Evaluated Thermodynamic Property Data Storage and Capture". It is anticipated that ThermoML will become a prototype of this IUPAC standard.**

Significant advances were made in the TRC Data Entry Facility (to support mass-scale data entry), Guided Data Capture (GDC) software (to enhance in-house and external data entry), and ThermoML (an XML-based approach for international data collection and management). Full-scale cooperation between the Journal of Chemical and Engineering Data and NIST has been established in direct data communication, quality assurance, processing, and distribution, based on the global use of GDC and ThermoML. The algorithms for the implementations of the Dynamic Data Evaluation concept for pure organic compounds were fully developed in 2003 and incorporated into the framework of the ThermoData Engine software currently under development at TRC. Moreover, the targeted goal of 300,000 data points per year has been achieved at the TRC Data Entry Facility (for mass-scale data entry) with strict adherence to quality assurance. This resulted in the capture of all relevant experimental data published between 1993 and 2003 in 5 principal journals (Journal of Chemical and Engineering Data, Journal of Chemical Thermodynamics, International Journal of Thermophysics, Fluid Phase Equilibria, and Thermochimica Acta) using the TRC information systems infrastructure. To inform customers of TRC's progress in dynamic data generation and evaluation, the TRC Consortium Annual Workshop was held in FY 2003.



### *NIST Computational Chemistry Comparison and Benchmark Database (CCCBDB)*

***R. D. Johnson III (838)***

***CSTL researchers have developed a database that allows users to compare thermochemical properties from one species to another.***

As computers become faster, modeling becomes a more valuable tool. Quantum mechanics can be used to predict thermochemical properties. However there are many approximations in quantum mechanical calculations and the uncertainty in the predictions is not well established. The NIST Computational Chemistry Comparison and Benchmark Database (CCCBDB), recently updated to version 9, is a website and database which allows users to compare thermochemical properties determined by experiment or by quantum chemical calculations.

**The CCCBDB serves an average of 27000 web pages per month and over 10000 distinct hosts. As well as its stated purpose, the CCCBDB is also used as a source of benchmark experimental data.**

**http://srdata.nist.gov/cccbdb/**

By compiling benchmark experimental and theoretical (from quantum mechanics) results, the CCCBDB can illustrate the uncertainty in the theory by allowing comparison between theory and experiment. The CCCBDB also provides developers of new computational methods a set of benchmark experimental data to test the new methods. The experimental and calculated data includes enthalpies of formation, entropies, heat capacities, geometries (bond lengths, angles, rotational constants), vibrational frequencies, and dipole moments.

Data and calculations for several new species have been added in response to user requests. The species include diborane ($B_2H_6$), formamide ($CHONH_2$), and a selection of substituted aromatics, toluene ($C_6H_5CH_3$), aniline ($C_6H_5NH_2$), phenol ($C_6H_5OH$), chlorobenzene ($C_6H_5Cl$), styrene ($C_6H_5C_2H_3$), and naphthalene ($C_{10}H_8$). New web pages are being added continuously to allow more comparisons and east of data retrieval. The number of calculations on all molecules continues to grow as does the use.

## *Descriptive Terms of Particle Appearance for Search and Classification*

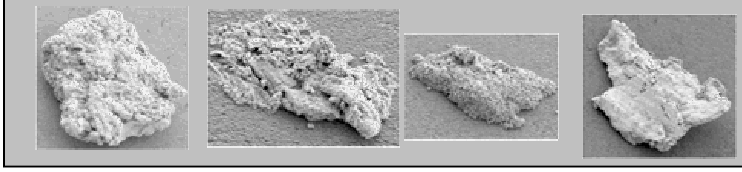
***D. S. Bright (838)***

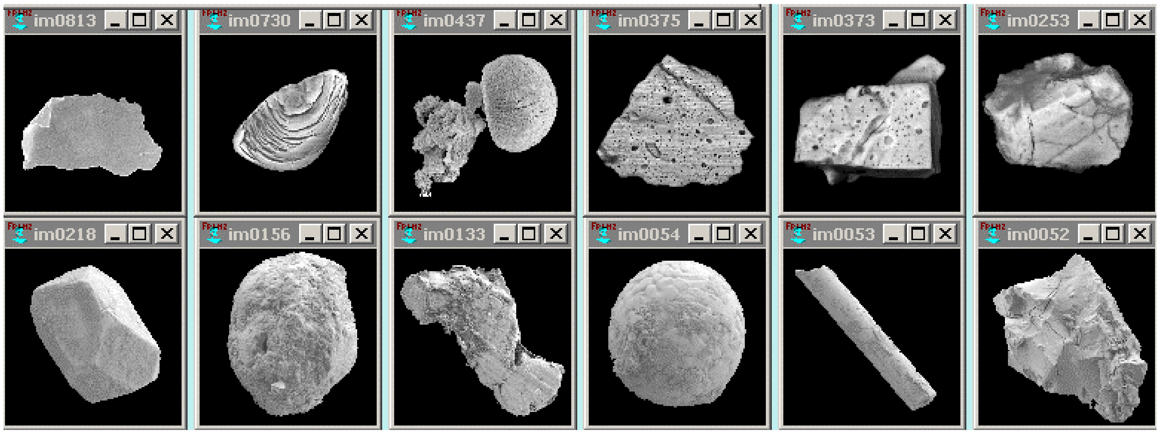
It is very tedious to visually search an image collection containing thousands of particles. Typically, one particle is in mind and the user wants to find other particles that look similar. The images need to be indexed or described, in order to search them automatically. We are working on numerical, computer-generated descriptors for these images, such as aspect ratio, roundness, and others with more obscure meaning.

***Morphology is important for particle classification and for determining particle origin and chemical composition. The CSTL developed database will make searching the visual collection easier and more accurate.***

Since the images in our trial data set are so visually distinctive, we thought that common sense visual terms, such as “round”, “smooth”, or “sharp edged” might make useful descriptors that would perform better and be more easily understood than the numerical descriptors. To this end, we made a glossary of 70 terms (see sample shown), with a few pictures, and a brief explanation with links to other terms. We also assigned these terms to a collection of 1600 particle images, and developed software to search the collection using the terms.

**Flattened oblate, thicker than flake. Not bent …**





**Typical scanning electron micrographs of particles from the collection of 1600 images.**

# *Data Activities in FY 2003*
## *(Described in more detail elsewhere in this report.)*

### *Automotive and Aerospace (Transportation)*

***Benchmark Data on Liquid Fire Suppressants for Use in Aircraft***
***C. Presser and B. Johnson (836); C.T. Avedisian (Cornell University); G. Papadopoulos (Dantec Dynamics); J. Hewson (Sandia National Laboratory); D. Keyser (NavAir); P. Disimile and J. Tucker (46th Air Force Test Wing)***

### *Pharmaceuticals and Biomanufacturing*

***A Metric of Amino Acid Exchangeability Based on Experimental Data***
***A. Stoltzfus (831), L. Y. Yampolsky (CARB/UMBI)***

***Biological Macromolecule Crystallization Database***
***G.L. Gilliland, M. Tung, T.N. Bhat, and J.E. Ladner (831)***

***Structure to Function: Using a Structural Genomics Approach***
***G. L. Gilliland, J.E. Ladner (831); A. Teplyakov, G. Obmolova, O. Herzberg, J. Orban, John Moult (UMBI); and Andrew J. Howard (Illinois Institute of Technology).***

### *Chemical and Allied Products*

***International Standards for Refrigerant Properties***
***M.O. McLinden and E.W. Lemmon (838)***

***The Industrial Fluid Property Simulation Challenge***
***R. Mountain, A. Chaka, R. Johnson, D. Friend, J. Magee, L. Watts, T. Bruno, R. Chirico, A. Laesecke, E. Lemmon, and M. McLinden (838); J. Olson, J. Moore, and D. Frurip (Dow Chemical); M. Schiller (Dupont); J. Golab (BP); F. Case (Colgate-Palmolive); R. Ross (3M); P. Kolar (Mitsubishi Chemical)***

***Transport Property Models for Refrigerants***
***M.L. Huber, A. Laesecke, and R. Perkins (838)***

***Measurements, Modeling, and Data for Pressure-Driven Membrane Separations***
***C. Muzny, H. Sun, and J. Moon (838); J. Cho (University of Colorado); M. Chapman (U.S. Bureau of Reclamation); and J. Ranville (Colorado School of Mines)***

***Evaluated Molecular Structures and Vibrational Frequencies for C1-C2 Chlorocarbons***
***D.R. Burgess, Jr. and J.A. Manion (838)***

***Thermodynamic Interpretation of Low-pressure Ion-molecule Reactions***
***K. K. Irikura (838)***

***Evaluation of Free Radical Standard Potentials***
***P. Neta and R. E. Huie (838)***

## *Energy and Environmental Technologies*

***Natural Gas Flow: North American Laboratory Comparison Project***
***P. I. Espina (836) and W. F. Guthrie (898)***

***High-Temperature Reference Correlations for the Solubility of Gases in Water***
***A.H. Harvey (838); R. Fernández-Prini (CNEA, Argentina); J.L. Alvarez (CNEA)***

***A Question of Accuracy: How Does Thermal-Optical Analysis for Climatically Important Black Carbon in Atmospheric Soot Behave Optically?***
***J. M. Conny (837)***

***Chemical Kinetic Database for PAH formation for Heptane Combustion***
***V. Babushok and W. Tsang (838)***

***OH Kinetics – Evaluation of Data and Experimental Techniques***
***V.L. Orkin and M.J. Kurylo (838)***

***Thermodynamics Reserch Center (TRC) Comprehensive Program on Critical Data Evaluation***
***M. Frenkel, R.D. Chirico, Q. Dong, R.A. Stevenson, and G.R. Hardin(838); V.V. Diky (Guest Researcher); X. Yan, R.C. Wilhoit, and X. Hong (Contractors)***

***Weakly-bound Isomers of the ClOO Free Radical***
***K.K. Irikura (838)***

***Expansion of NIST's Environmental Specimen Banking Activities***
***P.R. Becker, R.S. Pugh, S.S. Vander Pol, J.R. Kucklick, R.D. Day, S.J. Christopher, B.J. Porter, M.B. Ellisor, and S.A. Wise (839)***

## *Forensics and Homeland Security*

***Standards for Detection of Biological Threat Agents***
***K. D. Cole, L. Wang, A. Gaigalas, and D. Hancock (831)***

## *Health and Medical Technologies*

***HIV Protease Structural Database***
***T.N. Bhat, G.L. Gilliland, V. Ravichandran, and M. D. Prasanna (831); J. Vondrasek (NCI/NIH); A. Wlodawer (NCI/NIH)***

***Human Mitochondrial Protein Database: A Resource for Human Mitochondrial Proteomics***
***V. Ravichandran, P. E. Barker, G. B. Vasquez, T.N. Bhat, G.L. Gilliland (831), S. J. Zull (ATP)***

***Standards Requirements for Systems Biology Approaches to Health Care: Mitochondrial Proteomics***
***Gregory B. Vásquez and Peter E. Barker (831); Steven Zullo (ATP/NIST)***

## *Industrial and Analytical Instruments and Services*

***Materials Evaluation and Database Tabulation for Fundamental Parameters and Algorithms Used For Quantitative Electron Probe Microanalysis***
***J. T. Armstrong (837)***

***Development of a New Database for the Simulation of Electron Spectra for Surface Analysis (SESSA)***
***C. J. Powell; W. S. M. Werner (Contractor); W. Smekal (Contractor)***

***Evaluation of Electron Elastic-Scattering Cross Sections***
***C. J. Powell; A. Jablonski (Polish Institute of Physical Chemistry); F. Salvat (University of Barcelona)***
***SpectroML—An Extensible Markup Language for UV-Visible Spectroscopy Data***
***A.D. Nguyen, A. Arslan, M.V. Smith, J.C.Travis, and G.W. Kramer (839)***

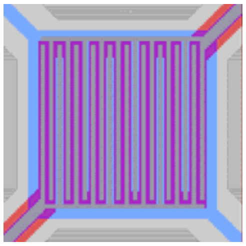
***Molecular Simulation of Alkylsilane Stationary Phases in Liquid Chromatography***
***K.A. Lippa, L.C. Sander, and R.D. Mountain (838)***

## *Microelectronics*

***Thermophysical Properties of Gases used in Semiconductor Processing***
***J. J. Hurly, K.A. Gillis, and M.R. Moldover***

# 13. Technologies for Future Measurements and Standards

### *… anticipating and addressing next generation measurement needs of the nation*



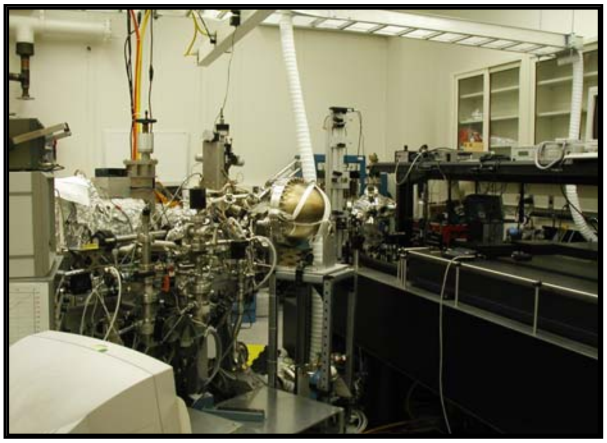
CSTL maintains a strong basic research program in broad aspects of measurement science that positions us with the capability to provide the required measurements, standards, and data to support future technology development. New measurement techniques are important for a host of industries including biotechnology, healthcare, environmental technologies, and the chemical process industry. Many of our activities, such as protein crystallography and materials microanalysis, are at the leading edge of science and technology.

## *Molecular Electronics Metrology*

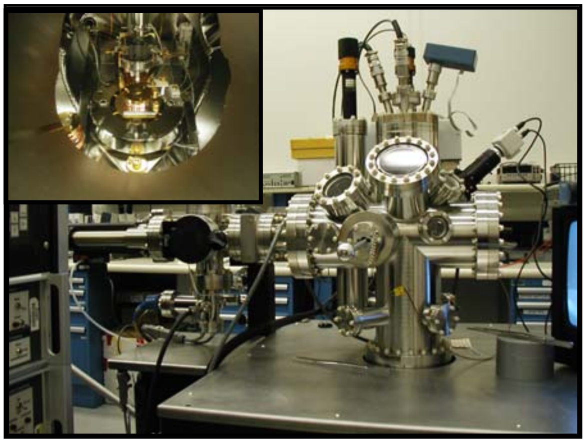
***J. D Batteas, J.C. Garno, and S. W. Robey, L.J. Richter (837); C.A. Gonzalez (838), C.A. Hacker (EEEL); C. D. Zangmeister and R. D. van Zee (836)***

***Molecular Electronics ("moletronics") is a field that many predict will have important technological impacts on the computational and communication systems of the future. In these systems, molecules perform the functions of electronic components.***

The drive to increase electronic device performance, with the associated push to ever-smaller device dimensions, has led industry observers to conclude that silicon-based technology will reach a point of diminishing gains in the near future. This, in turn, has generated interest in alternative technologies based, for instance, on single-electron devices and molecular components. It is hoped that the tremendous flexibility available with organic synthetic chemistry and self-assembly techniques can be harnessed to produce non-linear devices analogous to silicon-based diodes and transistors, but comprised of single or small numbers of molecules. The CSTL team of researchers in molecular electronics is integrating a range of techniques that will provide key information on electronic structure and electron transport in candidate molecular electronic systems.



**Photoemission system used to study molecular electronic structure.**



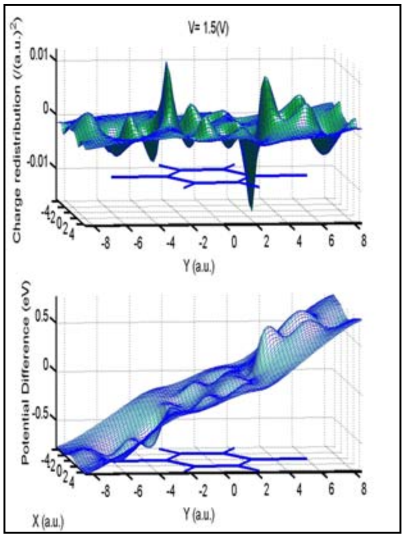
**Atomic-Force/Scanned-Tunneling Microscope used for film characterization studies.**

This work will develop measurement techniques and expertise necessary to understand electronic structure and transport found in molecular systems comprised of organic thin films and small ensembles. The overall goal is to reliably provide experimental details which, when coupled with theoretical input, will help to elucidate the physical mechanisms that produce device function in molecular based systems. This work is also aimed at providing the requisite measurement protocols for such systems.

The methodologies being employed include two-photon photoemission, which accesses unoccupied electronic levels and tracks electron relaxation effects, scanned probed microscopies, which can characterize electron transport down to the single molecule level and afford means of manipulating matter on the nanometer scale, as well as theoretical modeling of electronic states and transport properties to better elucidate the mechanisms involved in such transport function.

### ***Theoretical Studies of Electron Transport in Molecular Wires*.**

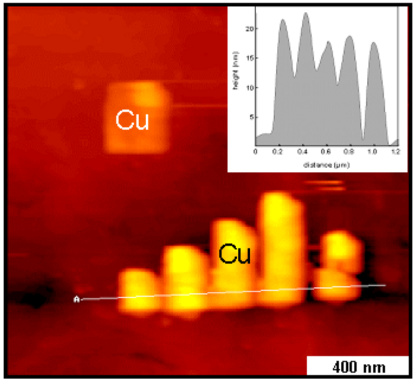
We have continued our study of the possible mechanisms governing electron transport in molecular wires. Particular attention has been focused to the electrostatics at the molecule-metal interface, which has been found to affect significantly the transport properties of the system. In order to properly describe the electrostatics at the interface, a novel algorithm has been developed that efficiently computes the electrostatic potential by solving the Poisson equation at each cycle of the self-consistent field iteration based on *ab initio* electronic structure calculations. A result of this calculation is shown in the figure to the right. This algorithm has been used to study the possible sources that lead to asymmetric current-voltage (I-V) curves in atomic wires as well as in a series of organic thiolates connected to gold electrodes. In order to develop efficient and robust models to gather a qualitative picture of electron conductance through molecular bridges, a simple algorithm to compute the first-order expansion of the system's Green function projected only onto fragments of the isolated molecule (no electrodes are included) has been implemented. This simple model, called the Green Function Condensed-to-Fragments model (GFCF) was found to be very useful in studying electronic transport properties of molecular bridges where the contact to the electrodes does not seem to play an important role in determining the shape of I-V curve. This approach has been used to compute I-V characteristics of the molecule 2'amino-4,4'-di(ethynylphenyl)-5'-nitro-1-benzenethiolate ($NH_2$-$NO_2$-OPE), found to exhibit "negative-differential resistance" (NDR) behavior at 60 K. The results of the calculations are in good agreement with the experimental I-V curves. Calculations based on the same model predicted that the fluorinated derivative 4,4'-di(ethynylphenyl)-2'-fluoro-1-benzenethiolate (F-OPE) was also a good candidate for NDR behavior. Chemists in the Nanotechnology Group at Kraft Foods worked on the synthesis of the molecule and very recently, scientists in NIST's Surface and Microanalysis Sciences Division (837), have made I-V measurements that qualitatively agree with the predictions of this model. This is the first time that a theoretical prediction of the behavior of molecular bridges upon electrification has prompted experimental work leading to its validation. These results indicate the potential of theoretical tools in the rational design of electronic devices at the nano-scale.



**Charge Distribution and Electrostatic Potential profiles for benzene di-thiolate attached to gold electrodes.**

***SPM Directed Device Fabrication*.** Precisely engineered nanostructures provide a means for the exploration of chemical reactions under spatially well-defined and controlled environments. Although not yet practical for high throughput applications and manufacturing, scanning probe lithography studies provide fundamental information on tip-surface interactions, structures, and properties at the level of nanometers.

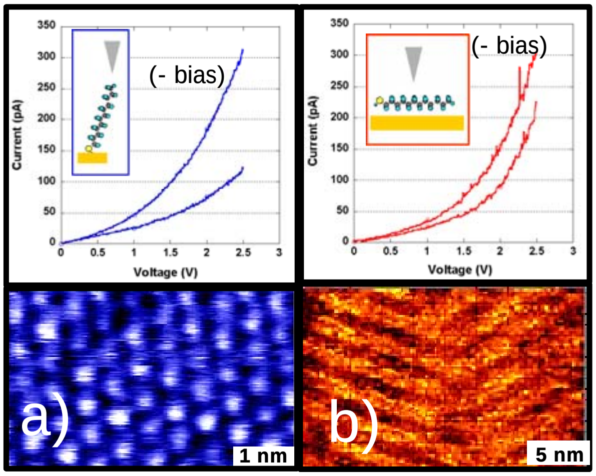
Under the support of ATP, nanometer-sized patterns of copper were constructed at dimensions ranging from 100 nm to 400 nm via AFM-based lithography (see figure). The writing density and size of the patterns affect copper growth. Varying solution chemistry parameters, such as the immersion intervals and the concentration of metal salts, can control pattern sizes. Copper grows beyond the edges of the pattern boundaries. An effective resist was found using mercaptoundecanol, with very high selectivity observed, even at the nanometer scale. Longer chain length alcohols ($C_{11}$ vs. $C_6$) were more effective as resists. Preliminary results using silanized tip coatings are promising for inhibiting copper deposition on AFM tips during *in situ* experiments. Future work will investigate charge transport with copper overlayers on SAMS using conductive probe AFM measurements at a metal-molecule-metal interface.



**Pattterned Cu contacts on the SAM surface. Patterns range from 150 nm to 400 nm and are ≈20 nm thick.**

### *Orientational Effects on Electron Tunneling in Dodecanethiol.*

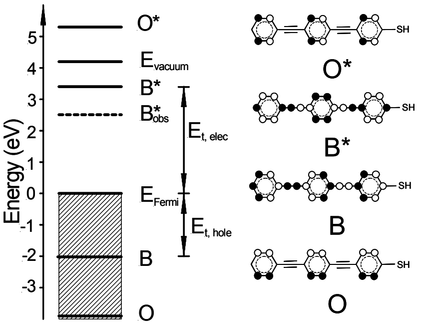
Obtaining a detailed understanding of electronic transport properties in molecules requires the ability to correlate structure and transport mechanisms. Here we are utilizing dodecanethiol as a “standard” test molecule to provide reference I-V measurements. This molecule affords a means of probing the impact of molecular orientation on tunneling behavior. Self-assembled monolayers of dodecanethiol have been prepared with regions of densely packed molecules whose molecular axis is oriented ≈30° from the surface normal right next to regions of lower density molecules which are arranged with the molecular axis parallel to the surface. Asymmetry in the tunneling I-V curves show that, in the case of the standing up phase, the tunneling behavior is rectified by the asymmetry of the transport junctions, while in the laying down molecule, this asymmetry is almost completely removed (see figure to right) and may be explained by differences in metal surface potentials of the tip and sample surface.



**Orientation dependence on tunneling behavior in dodecanethiol showing asymmetric I-V behavior in molecules standing up on the surface (a) vs. more symmetric behavior in molecules laying down (b).**

***Spectroscopic Measurements.*** The configuration of molecules within and the electronic structure of electrically-active molecules are believed to be governing factors in molecular conductance. To investigate whether these molecular properties are related to the test-structure measurements, ultrafast laser spectroscopies have been used to study the geometry and electronic structure of moletronic films. The electronic structure of these films was studied using one- and two-photon photoemission.

Specifically,oligo(para-phenylene-ethynylene) thiolate chemisorbed on gold surfaces was studied. Within 5 eV of the Fermi level, four states were observed, two



**Energy-level diagram for OPE chemisorbed on Au.**

occupied (2.0 eV and 4.0 eV below the Fermi level–B/B*) and two and two unoccupied (3.2 eV and 5.3 eV above the Fermi level). This information is shown schematically in the energy level diagram. The two states closest to the Fermi level are assigned to a pi-conjugated molecular orbital along the backbone of the molecule, and the other two are assigned to molecular orbitals formed by the "ortho" carbons. (See schematic representation of these orbitals in the figure.) From this assignment, the hole- and electron-barriers can be determined. These are, respectively, 2.0 eV and 3.4 eV. The charge transport gap can also be estimated. It is between 3.7 eV and 5.4 eV, depending on the criteria used to determine the onset of transport.

This work combines for the first time detailed theory and molecular scale measurements that can provide a basis for the complete understanding of electronic structure and transport effects in molecular electronic systems. The foundation that these results provide not only benefits the development of molecular electronics applications, but also affords a groundwork for the investigation of molecular based optoelectronic device applications.

**This project provides detailed insight into the complex behavior of electronic transport through molecules.**

***Future Plans:*** On the horizon are experiments aimed at probing the electrical behavior of ensembles of molecules based on patterning to afford a means of assessing molecular function in device level measurements, as well as scaling conductance properties in molecular ensembles. Future work will be aimed at correlating compression effects on the tunneling properties as investigated by conducting probe atomic force microscopy. The results from these experiments will be compared to theoretical models and to the performance of device-prototypes fabricated and tested by our collaborators in NIST's Semiconductor Electronics Division in EEEL. The photoemission work will systematically investigate the effects of chemical substitute on the molecular orbitals. Theoretical modeling of the transport properties and the valence structure of these electrically-active compounds is also underway.

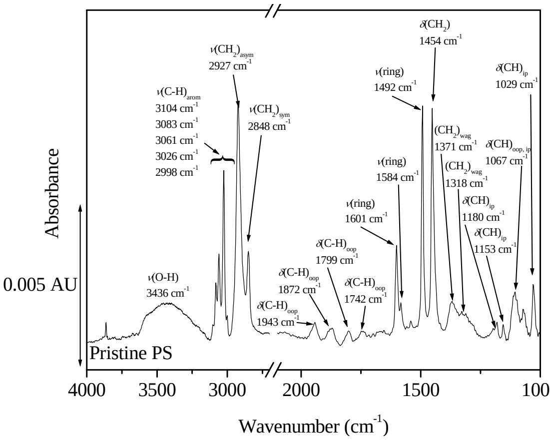
### *Microfluidics and the Microanalytical Laboratory*

***L. Locascio, A. Henry, W. Vreeland (839); D. Ross (836); M. Gaitan (EEEL)***

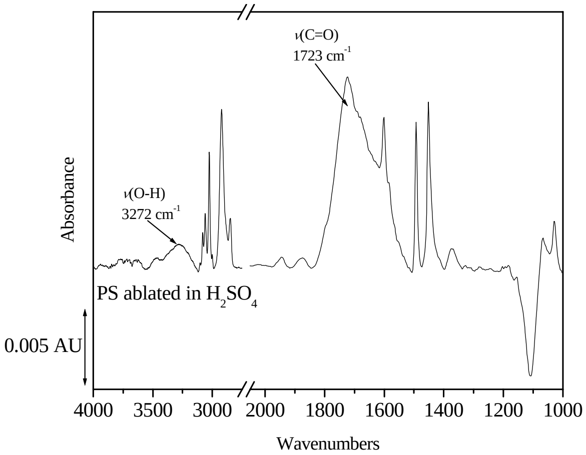
The Microanalytical Laboratory project was established as a competence project in 1998 and has just completed its last year of funding. The work performed in this project is collaborative between the Analytical Chemistry and Process Measurements Divisions in CSTL and the Semiconductor Electronics Division in EEEL, and involves the development of new techniques to improve measurement capabilities in microsystems based on microfluidic technology.

***The goal of this competence project is to advance the knowledge and understanding of Lab-on-a-Chip devices based on microfluidics technology in order to promote higher performance and improved measurements in these miniaturized analytical systems.***

To date, most of the separations performed using microfluidic systems utilize electroosmotic pumping for fluid flow and separate analytes on the basis of their electrophoretic mobilities. While this mode of separation is a useful one for many bioseparations, scenarios arise in which it is necessary to separate neutral analytes. For these separations, it is necessary to incorporate a stationary phase in the microchannel; the analytes can be flowed through the microchannel using electroosmotic pumping but the basis of the separation is the interaction of the analytes with the stationary phase. This mode of separation science is known as capillary electrochromatography (CEC) and is the hybridization of capillary electrophoresis and high-performance liquid chromatography. With CEC, different species interact with the stationary phase for different periods of time; thus, a separation of the neutral species occurs. This year, we have focused some of our effort on the development of microchip-based CEC ($\mu$CEC) devices fabricated using polymer laser ablation.

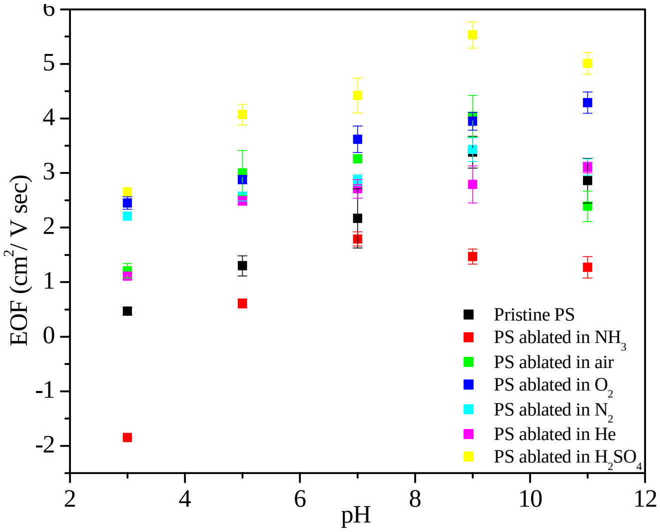


**Transmission infrared spectrum at Brewster's angle of poly(styrene) spin-coated in Si.**



**Transmission infrared spectrum at Brewster's angle of poly(styrene) spin-coated in Si and ablated under $H_2SO_4$ vapor.**

The laser ablation of polymeric materials results in a microchannel surface that is significantly rougher than embossed microchannels. Additionally, the chemical species present on ablated surfaces are much different than those present on a pristine or embossed polymer substrate, and can be modified by changing the atmosphere under which the channel is ablated. The figures show the transmission infrared spectra at Brewster's angle of poly(styrene) (PS) spin-coated on Si, and PS ablated in an atmosphere of $H_2SO_4$ vapor. The two spectra are quite different with the $H_2SO_4$-ablated PS spectrum showing bands indicative of carboxylic acid species suggesting that this channel would have a higher density of negative charges at pH values > ~4. Because electroosmotic flow (EOF) rates increase with increasing surface charge density, we expect a higher EOF in carboxylate-terminated PS surfaces than in their pristine counterparts.



**Measurement of EOF using current monitoring method in PS microchannels fabricated by laser ablation in various atmospheres.**

The figure shows the dependence of EOF on pH for microchannels ablated under a variety of atmospheres.

The microchannel ablated in $H_2SO_4$ supports the highest EOF, indicating higher density of charged moieties on this microchannel surface. While the presence of charged species on microchannel surfaces is essential to drive EOF, it is also important for the covalent addition of stationary phases to the microchannel surfaces for $\mu$CEC separations. We have modified $H_2SO_4$ ablated microchannels to link various stationary phases suitable for $\mu$CEC, and experiments are now underway to use those stationary phases in the first PS-based device.

In this project, we strive to produce critical fundamental data on flow control and manipulation that underpins device performance in these miniaturized flow systems. This work is comprehensive involving microflow measurement, temperature measurement, flow modeling, chemical surface modification, and materials characterization. We are continuing collaborative work with CFD Research Corporation (initiated last year) on the development of microflow models for polymer microchannels that will be ultimately applied to predict and optimize results of separations performed in polymer-based systems. In the past 5 years of this competence building project, we have produced approximately 50 publications with 8 patents/invention disclosures. In the next year, we plan to become more involved in the newly formed standards activities groups to support microtechnologies. We also will initiate a new effort with the National Institute of Justice for the development of microsystems based for DNA forensic analysis. Our research efforts in the development of novel analytical methods for microchemical separation and detection will continue to be a major part of our program.

We are also continuing the development of temperature gradient focusing methods for microanalytical separation and pre-concentration. This year, the method was extended to the separation and concentration of neutral and chiral analytes.

### *Temperature Gradient Focusing for Biological Assays in Microchannel Systems*

***K.M. Balss, D.J. Ross, M.J. Tarlov (836); H. Begley (BFRL), K.G. Olsen (Loyola College)***

See ***Health and Medical Technologies*** for details.

Our work continues to involve several active collaborations both internally (EEEL, PL, CSTL, and MSEL) and externally (Virginia Tech, Cornell, Stanford, Clemson, CFD Research Corporation, University of North Carolina, University of Maryland. For example:

**In FY 03, we have developed essential collaborations for the propagation of fundamental data and measurement methods. We have also developed several key collaborations for the development of systems that have application to NIST strategic focus areas including Homeland Defense, Nanotechnology and Health Care.**

### *Cell-based Sensors for Screening Toxins*

***L. Locascio, J. Travis (839);***
***Suggs, B.J. Love, N.G Love (Virginia Polytechnic and State University)***

See ***Forensics and Homeland Security*** for details.

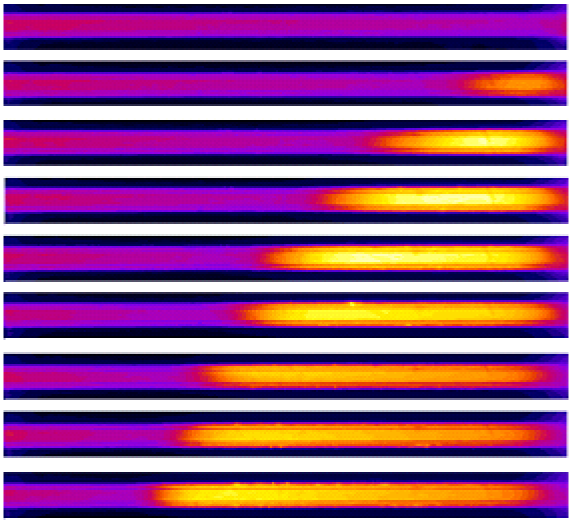
In FY 03, we developed methods to enhance mixing in microfluidic formats, and also introduced methods for mixing based on selective release of reagents from liposomes see details below.

### *Microfluidics: Rapid Mixing in Microfluidic Channels*

***W. Vreeland and L.E. Locascio (839)***

***CSTL researchers have employed lipid vesicles to mix and deliver reagents in microfluidics systems with exquisite rapidity and control, and have demonstrated reagent mixing approximately five times faster than standard passive mixing.***

The liposome is a spherical structure composed of a phospholipid bilayer membrane that encapsulates a volume of intravesicular aqueous solution. Liposomes are bathed in an external aqueous solution and are generally ~100 nm to ~1 μm in diameter. The ability to encapsulate a species of interest inside liposomes renders that species inert to chemicals residing outside of the membrane. We have developed a bio-inspired liposome system that allows for the controlled introduction of polar species into a microfluidic system through the modulation of temperature using thermally triggerable liposomes. Thermally triggered liposomes take advantage of the dramatically increased bilayer permeability near the lipid chain melting transition temperature ($T_m$). Thus at a controlled temperature, a thermally triggerable liposome will release its contents into the extravesicular microfluidic space, allowing for precise delivery of agents to specific regions in the microfluidic environment.



This phenomenon is shown in the figure, where a solution of thermally triggerable liposomes (composition 97 % dipalmitoylphosphatidylcholine + 3% cholesterol) encapsulating self-quenched fluorescent sulforhodamine B flow through a microchannel with a lateral temperature gradient.

**False-color micrographs of the fluorescence intensity of a solution of 97 mol% DPPC, 3 mol% cholesterol liposomes encapsulating self-quenched 100 mM sulforhodamine B in 0.5 M Tris buffer flowing through a polycarbonate microfluidic channel under different applied temperature gradients of 20°C-40°C to 40°C-80°C over 2 mm distance at a flow rate of 5 µL / hour.**

The left end of the channel is thermostated at 20 °C and the right side is thermostated at temperatures ranging from 40 °C to 80 °C in 5 °C increments; please note that due to the low thermal conductivity of polycarbonate, the magnitude of temperature gradient in the channel is slightly less than the nominal temperatures at the termini. As the liposomes flow from the lower temperature region of the channel to the higher temperature region, they pass through the $T_m$ that is specific for this formulation of liposomes (36 ˚C in this demonstration). At the $T_m$, the liposomes release the fluorescent dye into the extravesicular space causing an increase in fluorescence at that point and downstream in the channel in all panels except the first, where the temperature gradient does not pass through the $T_m$ of this liposome formulation. Thus, the fluorescent dye, which was initially segregated from the extravesicular space, is effectively “mixed” into the microfluidic environment. Because the liposomes are dispersed throughout the channel, mixing upon release from the liposomes is inherently quite fast. Measurement of the profiles in the figure show the dye goes from “unmixed” to “mixed” state in approximately 200 µm under the experimental conditions shown here, whereas standard diffusive mixing would require almost 9 cm. Since this “mixing” technique is controlled by the $T_m$ of the liposome formulation, rather than the fluid mechanical properties of the channel, this technique should be less sensitive to different operational flow rates than traditional microfabricated mixers. Additionally, because the liposome formulation dictates the $T_m$, mixed populations of liposomes can be used to control a series of sequential reactions where the temperature in the channel determines reaction timing and sequence.

**This work is the first demonstration of a mixing strategy in microfluidic systems that is not dependent on the operational flow-rate. This system is ideally suited for biological analyses as the liposome itself mimics the natural environment of the biological cell.**

All other techniques for enhanced mixing efficiency in microfluidic systems operate optimally in certain fluid mechanical regimes; our liposome-based system, however, depends on the heat transfer and thermodynamic parameters of microfluidic device and thus is limited by a different set of criteria. This is crucial to our effort to design and demonstrate microfluidic systems that are both robust and efficient. Future work on this project will focus on using liposomes to act as packages to study individual biomolecules, such as single-molecule proteins. Since the permeability of the lipid membrane can be easily modulated through temperature control, various species can be presented to the biomolecule in a controlled fashion to study the effects of that agent on individual, single biomolecules.

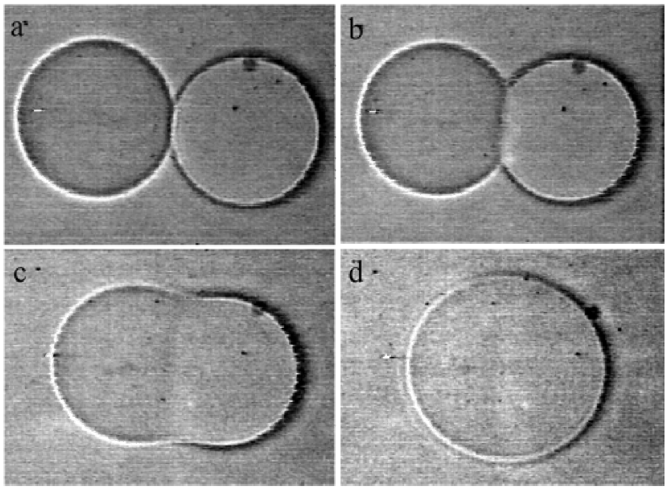
## *Nanobiotechnology: Nanofluidics and Nanoscale Chemical Reactions*

***L.E. Locascio, W. Vreeland, K. Brazhnik (839); R. Kishore, S. Kulin, K. Helmerson (PL)***

This project focuses on the development of nanoscale structures to facilitate the performance of ultra-small volume chemical reactions and separations. The nanoscale structures that we are designing are composed most often of phospholipid molecules and are self-assembled spherical or tubular structures with diameters ranging from tens to hundreds of nanometers. We have demonstrated the use of these nanometer structures for controlled chemical reactions using picoliters of reagents.

***The work described here is associated with the Single Molecule Manipulation and Measurement competence program whose purpose is to study the behavior of biomolecules one molecule at a time to elucidate the differences that make them uniquely beneficial or detrimental.***

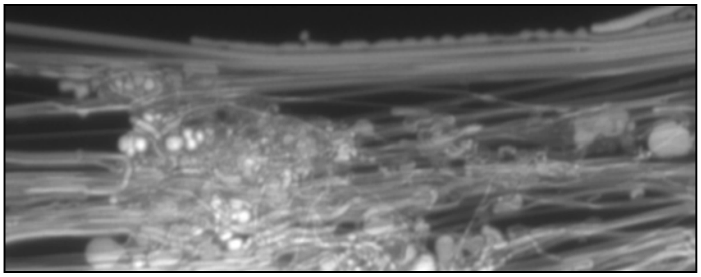
Due to their amphiphilic nature, when phospholipid molecules are dispersed in water they self-assemble into bilayer membranes to form structures called liposomes that are often spherical and encapsulate an aqueous internal volume. Liposomes range in size from 50 nm to 10's of micrometers encapsulating volumes that are measured in attoliters to picoliters.



**a.Two liposomes brought into contact using optical tweezers.**
**b. Laser pulse at the interface initiates fusion of the two membranes.**
**c. C, D. Fusion is completed in the next 2 successive video frames.**

Water-soluble molecules, including DNA, RNA, proteins and small ionic molecules, can be readily incorporated into the liposomes upon formation. In this work, we use liposomes to sequester very small amounts of reagents in discrete packages to control their reaction. The liposomes are trapped and manipulated in a microenvironment using optical tweezers. Liposomes containing two different reagents that are brought into contact using the optical tweezers do not bind or fuse, therefore their contents cannot react. However, if the liposome membrane is exposed to a short UV laser pulse while in contact with another liposome, the membrane destabilizes and the two liposomes fuse together to form a single liposome structure as shown in the figure above. During fusion, the contents of the two liposomes react immediately. The time scale of liposome fusion events varies in length from milliseconds to seconds and is most likely dependent on the lamellarity of the liposome membrane.

**Phospholipid tubes formed in a microchannel under conditions of high shear under flow. Tubes are oriented in a direction parallel to the long axis of the microchannel.**



Phospholipids can be forced to form non-spherical structures under certain conditions. We recently reported the formation of phospholipid tubes in a microfluidic device under conditions of high shear. Above the phase transition temperature of the phospholipid, lipid hydration in the microchannels resulted in self-assembly of lipids into tubes with diameters of several hundred nanometers to a few micrometers and lengths of up to several centimeters. In general, tubes aligned parallel to the lateral dimension of the microchannel. Elasticity of tubes was assessed via secondary manipulation with optical tweezers and with an optical scalpel. It was determined that lipid tubes formed and stored in a microfluidic setup became mechanically rigid after a few days of storage at room temperature or after a month of storage at 4 °C.

The work described here is critical to our efforts in the development of new tools for observing and characterizing single molecule behavior. The characterization of single biomolecules, rather than the study of ensembles of biomolecules, is an important topic in the field of biology since it has been elucidated that the presence and behavior of the biological outlier or the mutant version of the biomolecule can facilitate amplification of that species resulting in catastrophic consequences as highlighted in recent reports on prions. Although our efforts in this area are new, we have made considerable progress toward packaging single molecules, performing controlled reactions with a few molecules, and designing nanochannels through which we will attempt to manipulate and characterize single DNA molecules. Future work will involve further characterization of the lipid nanotubes to determine stability and lifetime. In the next few years of the competence program, we plan to pursue the incorporation of lipid nanotubes into higher-level microsystems and apply these toward the study of single DNA and RNA molecules.

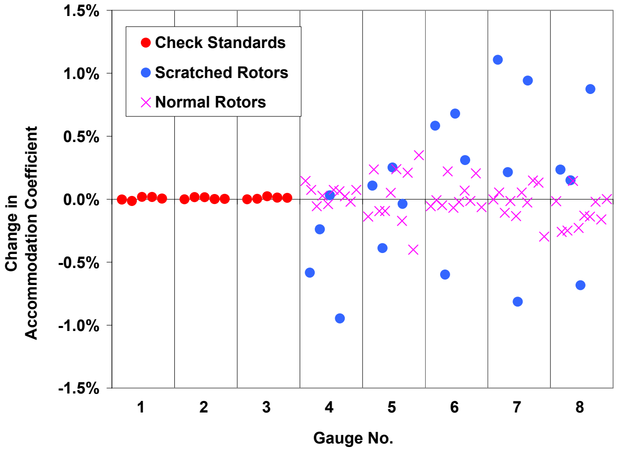
**Nanowire devices will provide multiple options as building blocks for sensors, miniaturized measurement platforms, and as advanced scatter probe structures, enabling true nanometer spectroscopy. This methodology will provide an inexpensive and straightforward approach to the synthesis of sensing devices with multiple functionalities that can be integrated to form state-of-the-art measurement systems.**

### *Improved Vacuum Transfer Standards – Spinning Rotor Gauge Stability Studies*

***R. F. Chang (836)***

NIST/CSTL seeks to disseminate its realization of the Pascal at high vacuum levels directly to our customers and into readily available commercial gauges. However, commercial vacuum transfer gauges are not sufficiently stable to effectively disseminate NIST's measurement uncertainties to our customers. Understanding the sources of these instabilities is important to gauge users, as well as to primary standard laboratories that rely upon high stability for maintaining their Quality Systems and for satisfactory performance as transfer standards

***The spinning rotor gauge has become the transfer standard of choice for vacuum calibrations from $10^{-4}$ to 1 Pa due to good calibration stability (changes < 0.5%/year). While internal NIST SRGs exhibit better long-term stability, some calibration customer gauges have shown inexplicably larger shifts.***



Earlier we had observed that de-mounting and re-mounting of the SRG suspension head may cause an average shift of ~ 0.1% in accommodation coefficient (which is directly tied to calibration stability), σ, and in some case as large as 0.4%. However, this did not fully explain observations the 1% and larger shifts in customer calibrations. In FY03, we investigated the effects of various handling procedures that affect surface properties of the rotor, such as their rinsing in cleaning solution, weighing and measuring, mounting and inspecting under a microscope, and even rolling and shaking of the rotor in a thimble to simulating transit between laboratories. Surprisingly, these procedures shifted σ only ~0.25% on average, although customer shifts as large as 0.8% were observed. These and other factors suggest that the large observed shifts in σ are partly a manifestation of a rotational-axis-dependence of σ. Inspection of customer rotors revealed many with non-uniform blemishes (*e.g.*, scratches, corrosion), and some with polar orientations. A series of experiments using deliberately longitudinally-scratched rotors showed an average shift in σ of 0.5%. Shift values ranged from -1% to 1%, as shown in the figure, when the suspension head was de-mounted and re-mounted. Each new suspension has a different rotational axis for the rotor. The extent of the resulting variability is close to that observed from calibration customers. Three check standards, undisturbed between calibrations, were calibrated along with the scratched rotors. The check standards reproduced σ each time as expected. The data of normal rotors, which have some blemishes but without deep longitudinal scratches, are also shown for comparison.

**Results suggest a few areas of exploration for improved stability of SRGs. For instance, we need to explore the manufacturing processes of the rotor such that a preferred rotational axis may be established, and the surface is mechanically and chemically hardened to resist corrosion and scratches. Choice of soft materials for the thimble or treatment of its inner surface may also aid in reducing the probability of scratches on the rotor due to sudden loss of suspension.**

We have completed a first-ever, comprehensive investigation into the causes of calibration instability of SRGs, and the findings have refuted many long-held beliefs. Our results can significantly affect the manufacturing processes as well as routine operations and maintenance of SRGs. There are implications for new material selections as well as input into the next generation of SRGs, underway at both NIST and the Forschungszentrum-Jülich in Germany.

### *Johnson Noise Thermometry*

***W. L. Tew (836); S. W. Nam and S. Benz (EEEL)***

***Johnson Noise Thermometry (JNT) is a primary technique that is well suited to certain demanding application environments and as a high-temperature contact method for thermodynamic determinations relative to the International Temperature Scale of 1990.***

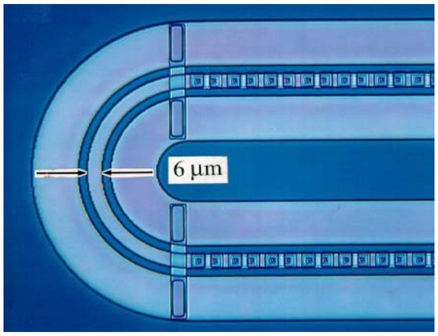
JNT is the only established thermodynamic method in contact thermometry which remains practical for applications above 800 K. Johnson noise temperature probes can be designed to sample the thermal noise from noble-metal alloy resistance elements up to at least 1800 K; such elements are relatively insensitive to changes caused by thermal strains or diffusion of impurities. The NIST JNT technique represents an important innovation by applying Josephson-based voltage standards to create an intrinsically calculable noise source, the Quantized Voltage

Noise Source (QVNS). This approach conveys key operational advantages over conventional JNT methods already in use at other National Metrology Institutes (NMIs), and allows a fixed-point-independent method of measuring SI temperatures.

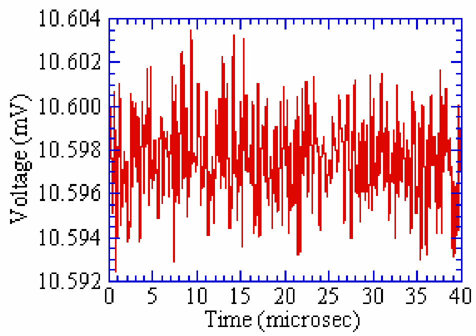
**The new NIST technical approach is being developed to help move JNT into wider use in both the industrial and metrology domains.**

For NIST temperature metrology, the new JNT system is competitive with radiation-based methods for $T$<1235 K. For industrial applications, the NIST method could be tailored to the use of solid-state noise references for those application environments where thermometric stability is critical and serviceability is limited, such as power generation plants and chemical processing applications. This will benefit all users of temperature instrumentation who are engaged in accuracy-critical applications limited by artifact thermometer performance.

New circuit designs of the Josephson QVNS have dramatically reduced the common mode voltage error measured by the Johnson noise measurement electronics. This has enabled a significant improvement in the demonstrated accuracy of new NIST methodologies. The new accuracy benchmarks are relative to ITS-90 assignments for the verification test fixed points: 1) the gallium triple point (GaTP, 302.916 K) ; and 2) the water triple point (WTP, 273.16 K). These fixed points establish ITS-90 temperatures that are compared to the JNT-derived temperatures. The latest data demonstrate an uncertainty of 250 μK/K in the absolute mode and 100 μK/K in the relative mode for the $T$(GaTP)/$T$(WTP) ratio. These results are within the current combined estimated uncertainties for those measurements. For the relative mode data, the agreement with the ITS-90 value is within the JNT statistical precision alone as estimated by the available data.



**Magnified image of a section of a Nb-PdAu-Nb Josephson array.**



**A pseudo-noise trace as produced by the QVNS with a 1.6 kHz repetition rate**

Construction has begun on a low-noise comparison furnace for use between 273 K and 934 K that will enable comparisons of JNT-based temperatures with ITS-90 over that range. The comparison furnace will be installed in the Division 836 electrically shielded room facility. A second set of digital correlator electronics has been built to allow two systems to be operated, one in Boulder and the other in Gaithersburg.

The achievement of an uncertainty of 0.001 % in the relative mode will be competitive for temperature metrology applications above ~700 K. New thermodynamic data in the overlapping ranges of contact and radiation-based thermometry between 700 K and 1235 K would be a significant input for the next international temperature scale. In certain industrial areas, JNT systems based on solid-state noise references could eventually reduce the cost-of-operation of any process that is limited by the calibration stability of artifact-based thermometers. Here 0.1 % to 0.01 % uncertainties are sufficient, depending on the specific application.

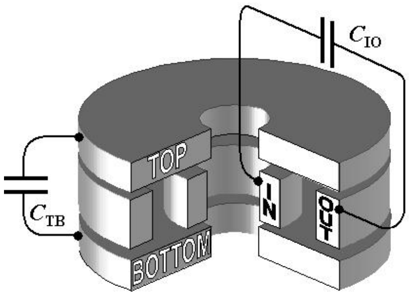
CSTL researchers plan to use the JNT prototype system with a QVNS, to determine the differences between JNT-determined temperatures and temperatures on the ITS-90 over the range of the comparison furnace (273 K and 933 K). EEEL researchers will continue to refine the QVNS and development of a custom programmable arbitrary bit stream generator, a key component to improving noise and other waveform synthesis capabilities.

## *Atomic Standard of Pressure*

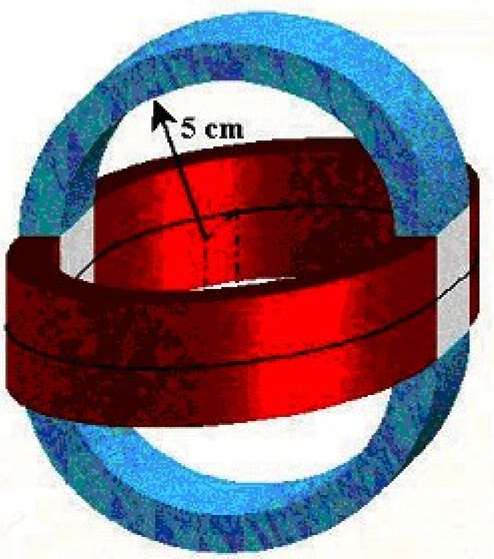
### *M. Moldover and J. Schmidt (836); K. Szalewicz (U. Delaware); Y. Wang (EEEL)*

CSTL researchers are developing a novel primary standard for pressure in the range 0.3 MPa to 5 MPa. The new standard will determine the pressure $p(\varepsilon,T)$ by measuring and calculating the dielectric constant $\varepsilon(p,T)$ of helium with extraordinary accuracy. The uncertainties from electrical and temperature measurements will be smaller than the uncertainty of existing pressure standards (piston gages).

This standard is being developed to: (1) replace artifact-based pressure standards with a standard based on calculable properties of a pure substance: $\varepsilon(p,T)$ of helium gas and; (2) reduce the uncertainty of pressure standards in the range 300 kPa to 5 MPa. Below 300 kPa, the primary pressure standard at NIST is a mercury manometer. Above 300 kPa, the pressure standards are commercially manufactured piston-cylinder sets. These sets are complicated artifacts. In operation, the cylinder and piston deform significantly and the piston rotates continuously to insure gas lubrication. Because of these complications, piston-cylinder sets are calibrated against the primary-standard mercury manometer below 300 kPa and their performance is extrapolated to higher pressures using numerical models of the coupled gas flow and elastic distortions. Piston-cylinder sets exhibit a poorly-understood specie and gas flow dependencies. Thus, the extrapolation is not fully trusted and it cannot be checked with existing technologies. When $\varepsilon(p,T)$ of helium becomes the pressure standard, it will be possible to test models of piston-cylinder sets and to reduce their uncertainty.



**Section of cross capacitor and components.**



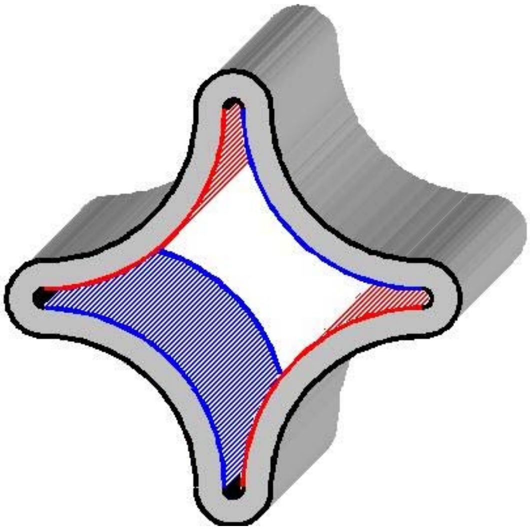
**Quasi-Spherical Microwave Cavity. The cavity is assembled from hollow, copper, quasi-hemispheres. Each quasi-hemisphere has the shape of two quadrants of a sphere (radius = 5 cm) with 0.01 cm wide cylindrical extensions (exaggerated in the sketch). The extensions split degenerate microwave frequencies.**

Dielectric constant measurements are being improved by drawing on NIST's expertise in electrical metrology. Using that expertise, we developed a novel, doughnut-shaped, four-electrode, cross capacitor. In comparison with conventional capacitors, cross capacitors are more stable and less subject to surface contamination (oxides, adsorbed water, or films of oil). Toroidal capacitors have an additional advantage; there are no "end effects" to complicate measurements. During FY02/03, a toroidal cross capacitor and a rod-shaped cross capacitor were used to measure the dielectric constant of helium in the range 0 to 7 MPa. The measured values of $\varepsilon(p,T)$ from the cross capacitors of differing designs were consistent with the theoretical values and with each other within an uncertainty of $2\times10^{-7}$ which corresponds to a relative pressure uncertainty of $5\times10^{-5}$ which is approximately an order of magnitude larger than current pressure standard uncertainties.

The theory for $\varepsilon(p,T)$ is being advanced at the University of Delaware, and a concept for a more stable cross capacitor is shown in the figure. This capacitor is made from a single, long, sapphire crystal. The crystal is edge-grown, with a hollow, star-shaped cross section. It has four surfaces that are coated with thin electrodes. We expect that the single-crystal sapphire capacitor will be more stable than conventional cross capacitors assembled from many metal and insulating parts. To advance capacitance bridge technology an order of magnitude, NIST has let a SBIR contract to a bridge manufacturer. In parallel, NIST's EEEL is developing the technology to test such a bridge when it is delivered. It is not certain that capacitance bridges can achieve the desired performance at a reasonable price. Thus, CSTL is exploring the alternative of making very accurate measurements of $\varepsilon(p,T)$ of helium at microwave frequencies. Our approach uses quasi-spherical microwave cavities. This concept grew out of CSTL's long experience in developing and using of spherical cavities for acoustic thermometry.

**To reduce the uncertainty a factor of ten, we are simultaneously attacking the problem on four fronts:**

1. **improve the theory for $\varepsilon(p,T)$ and the theory for the virial coefficients of helium,**
2. **manufacture cross capacitors with greater stability and larger capacitances,**
3. **obtain a more accurate capacitance bridge for measuring $\varepsilon(p,T)$ at audio frequencies,**
4. **measure $\varepsilon(p,T)$ with high resolution at microwave frequencies.**



If successful, this program will revolutionize pressure standards and greatly improve capacitance bridges. Already, CSTL has used cross capacitors to measure $\varepsilon(p,T)$ of the primary constituents of natural gas including methane, ethane, propane, nitrogen, carbon dioxide, and argon. The measurements span the 0°C to 50°C temperature range and extend to 7 MPa. In this range, they are more accurate than any previous measurements and provide reference data for use in metering natural gas.

**Sketch of single crystal sapphire cross capacitor. Thin-film electrodes are colored blue and red. The prototype is 25 cm long and 2 cm across the largest "diameter".**

## *Improving Limits of Detection in Microanalysis with High Speed Energy Dispersive X-Ray Spectrometry (silicon drift detector EDS)*

***D. E. Newbury (837)***

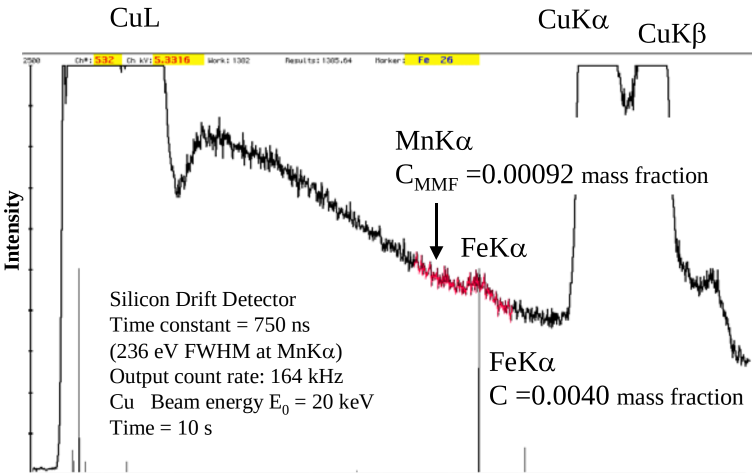
***The performance of a new silicon drift detector (SDD) EDS capable of much higher count rates has been evaluated in terms of the $C_{MMF}$ attainable. Trace element constituents (concentration < 0.01 mass fraction) have been detected in less than 10 seconds of spectrum accumulation time.***

A critical issue in many applications of electron beam x-ray microanalysis is achieving a low value of the concentration limit of detection, expressed as $C_{MMF}$ (minimum mass fraction). The formula for $C_{MMF}$ incorporates with equal weight two critical spectrometry terms, the peak counting rate, P, and the spectral peak-to-background ratio, P/B. The P/B is a direct consequence of the physics of generation of characteristic and continuum (bremsstrahlung) x-rays and the resolution of the spectrometer. For a given target composition and beam energy, the measured P/B is effectively limited by the choice of the spectrometer. Thus, the high-resolution wavelength dispersive spectrometer (WDS) provides a better (lower) value of $C_{MMF}$ than the conventional silicon energy dispersive spectrometer (Si-EDS) for equivalent peak counting rates on a particular peak. However, when many trace constituents must be evaluated at each analyzed point, the capability of an energy dispersive spectrometer to simultaneously evaluate the entire excited energy range of x-rays provides a substantial time advantage over the WDS, which must be scanned sequentially over this range with several diffractor changes. In this case, to compensate for the inherently poorer P/B of the EDS, it becomes advantageous to increase the peak counting rate, P. Conventional Si-EDS systems are limited to total spectrum output count rates of approximately 25 kHz.

Radiant Detector Technologies, LLC

The silicon drift detector (SDD) energy dispersive x-ray spectrometer, developed by Radiant Detectors LLC of Northridge, CA under NIST SBIR grants, is capable of a much higher counting rate than the conventional Si-EDS. In an SDD, the collection of the charge deposited during photoelectric capture of an x-ray photon is much faster due to (1) a shaped internal electrical field created by a lithographically applied electrode pattern on the back surface; (2) the reduced thickness dimension of the SDD (300 □m compared to 3 mm for Si-EDS); and (3) the higher temperature of operation (250 K vs 77 K for Si-EDS) for faster charge carrier drift velocities. Total spectrum counting rates in excess of 500 kHz have been achieved, limited by the digitization of the data stream not the SDD detector performance.

The SDD can achieve $C_{MMF}$ values of below $10^{-3}$ mass fraction in short spectrum accumulation times, e.g., 10 s. The figure shows an example of a 10 s spectrum of copper recorded at a total spectrum output count rate of 164 kHz (input count rate ~300 kHz).
Two examples of trace measurements are shown: the detection of iron at a level of 0.0040 mass fraction, and a $C_{MMF}$ value established for manganese at 0.00092 mass fraction. For a focused beam of 20 keV electrons incident on copper, the total sampled mass corresponds to 16 picograms of copper. Within this matrix mass, the trace iron constituent corresponds to the detection of 63 femtograms, while the concentration limit of detection for manganese corresponds to 14 femtograms.



**Copper target excited with 20 keV electrons and measured with a Radiant Detectors LLC SDD x-ray detector. The spectral regions for manganese (Mn K-shell) and iron (Fe K-shell) are indicated.**

An operational SDD detector has been successfully demonstrated in the spectrum acquisition mode to significantly lower the limit of detection in point beam analysis. However, the real promise of the SDD lies in high-speed x-ray mapping. X-ray mapping with the conventional EDS, while widely used, is subject to great time penalties, running to hours, if the analyst wishes to map minor constituents (0.01 to 0.1 mass fraction) or even longer for trace constituents (< 0.01 mass fraction). The SDD has the capability, in principle, to achieve efficient and effective x-ray spectrum imaging, in

**The conventional monolithic semiconductor EDS device was invented in the U.S. in the 1960s, and U.S. manufacturers dominated the market until the 1980s, when foreign competitors gained a significant market share. The SDD is now emerging as a "killer application" which threatens to sweep away virtually the entire market for conventional monolithic semiconductor EDS devices since the SDD doesn't require constant liquid nitrogen service and the SDD has been demonstrated to be capable of both higher resolution at long time constant (125 eV for SDD instead of 129 eV at MnKαfor Si-EDS) as well as much higher count rates at short time constant (500 kHz to 1 MHz for SDD compared to 25 kHz for monolithic Si-EDS**

which an entire x-ray spectrum is saved at each picture element (pixel) of a map with a short dwell time, e.g., 100 ms. X-ray spectrum imaging can greatly improve the utility of mapping by recording comprehensive "flash" maps requiring 10 minutes or less to accumulate but still capable of mapping minor and trace level constituents. The current limitation to this process is the high rate digitization that is required. Existing digital pulse processing systems become unstable at approximately 200 - 400 kHz when the full spectrum must be sampled with a short integration time (e.g. 10 ms). More research is planned in this area to extend the maximum digitization rate to higher values.

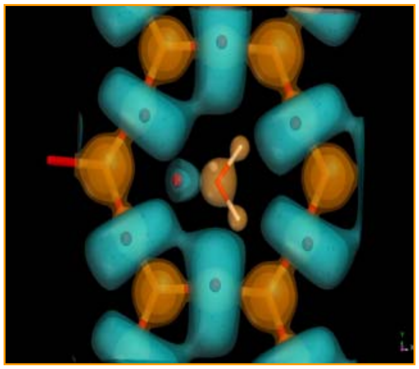
The SDD can have other impacts throughout x-ray spectrometry. A separate SBIR grant to Radiant Detectors LLC has demonstrated that the SDD can serve as the detector for x-rays in a diffractometer, providing distinct advantages over the conventional gas detector by eliminating the need for a monochromator, since the energy resolution of the EDS is sufficient to distinguish the $CuK\alpha$ and $CuK\beta$ x-rays from a copper target used to scatter off the crystalline target. A closely related application is the replacement with an SDD of the gas flow proportional counter in a wavelength-dispersive x-ray spectrometer (WDS) used on the electron microprobe. By using the SDD as a high-speed counter, the WDS would become compatible with medium vacuum operation ($10^{-6}$ Pa). The energy resolution of the SDD will also permit easy separation of different photon energies diffracted to the same Bragg angle, $n \lambda = 2d \sin \theta$ where n is the integer order, $\lambda$is the x-ray wavelength, d is the crystal plane spacing, and $\theta$ is the Bragg angle. When the product $n \lambda$ is the same for two different photon energies, both are recorded at the same diffraction angle. The SDD would show these components as two well-separated peaks.

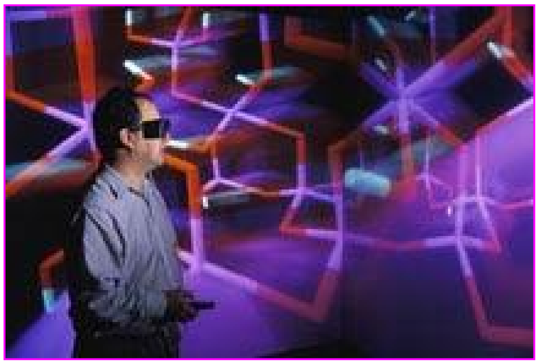
## *New Smart Gel Mechanism Elucidation from Immersive Visualization of Molecular Simulation*

***C.A. Gonzalez (838); S. Satterfield (ITL); Y. Simon-Manso (Universidad de Chile); Y. Aray (Instituto Venezolano de Investigaciones Cientificas); Manuel Marquez-Sanchez (KRAFT Foods, Inc.)***

***Scientists in industry are currently working on the application of "shake gels" in the manufacture of novel biological fluids, food thickening agents, drug delivery materials, cosmetics, and sensors.***

"Shake gels" are a class of materials resulting from an aqueous solution of a clay (Laponite) and an associative polymer such as polyethylene oxide (PEO). These watery mixtures of clays and polymers, firm up into gels when shaken, and then relax again to the liquid phase after some time has passed, however the process is complex and as yet unknown. The main objective of this work is to elucidate the mechanisms governing the gelling processes in "shake gels", and to provide reliable predictive tools to guide experimentalists in the rational design of novel "shake gel" systems tailored to different industrial applications. In particular, we are interested in understanding the nature of the interactions between the different components of the "shake gel" (water, laponite and PEO) and the effects of the shaking in the gelling process. To achieve this goal the information obtained from theoretical calculations were analyzed with a 3-D immersive visualization engine available at ITL, which allows scientists to immerse themselves among giant-sized molecules whose behavior can be seen and understood in minutes instead of the weeks required using traditional techniques. The work described here will develop the necessary theoretical infrastructure to aid in the elucidation of the complicated mechanisms involved in the formation of "shake gels".





Making use of a novel algorithm developed at the Instituto Venezolano de Investigaciones Cientificas to determine the topology of the electrostatic potential of molecules interacting with surfaces, we were able to determine the mechanisms by which PEO interacts with the clay surface and water in order to produce the gel. According to this mechanism, it is the polymer's oxygen atoms, instead of its hydrogen atoms as previously thought, that attach to the clay. In addition, electrical charges affect the binding process, resulting in water binding to clay surfaces in a perpendicular arrangement, which is believed to help create the firmness of the gel. Our results also indicate that the polymer size is important. Thus, our calculations predict that larger or smaller polymers cannot undergo the conformational changes necessary to optimize the binding to the clay. These predictions are in very good agreement with experimental findings that "shake gels" have only been observed with solutions of PEO.

**This research provides important mechanistic details about the electrostatic interactions between water, PEO and Laponite not previously known. For the first time, detailed quantum chemical calculations and atomistic simulations are integrated in order to gain a more complete picture of the physical and chemical properties of these gels.**

The next step of this program will involve a systematic study of the role of the "shaking" and temperature in the gelling process. Once a better picture of the role of these two factors is obtained, the models will be used to guide experiments in the synthesis of other "shake gels" with different chemical and physical properties that might be useful for industrial applications (for example: optical properties, viscosity, etc.).

### *Universal Validation Standard for UV/visible Spectrometry*

***D.L. Duewer and J.C. Travis***

*Details provided in the* ***Exploratory Research*** *section.*

### *Growth of Oriented ZnO Nanowires on Sapphire and Gallium Nitride*

***Babak Nikoobakht and Stephan J. Stranick (837)***

*Details provided in the* ***Exploratory Research*** *section.*

### *Progress Towards Development of an Improved Method of Phase Identification by Selected Area Electron Diffraction*

***S. Turner, D.S. Bright (837); and V.L. Karen, (MSEL)***

*Details provided in the* ***Exploratory Research*** *section.*

# C. Exploratory Research

Since 1999, the CSTL laboratory director has invited all technical staff to submit Exploratory Research Proposals. The insights gained from these proposals have become an integral part of our strategic planning, and a mechanism to engage all staff scientists in this important process. Scientists are encouraged to begin to discuss ideas among themselves, and where appropriate, combine the ideas into a single proposal. Over the past five years 67 exploratory research projects have been funded, and CSTL has invested close to $5M. More importantly, we have been able to provide seed money for critical areas of research such as new approaches to modeling, measurement methods, or data development and dissemination, as well as novel mechanisms to provide reliable and timely measurement traceability to our customers. The work described in the following pages is a result of the research from FY 2003 funded projects.

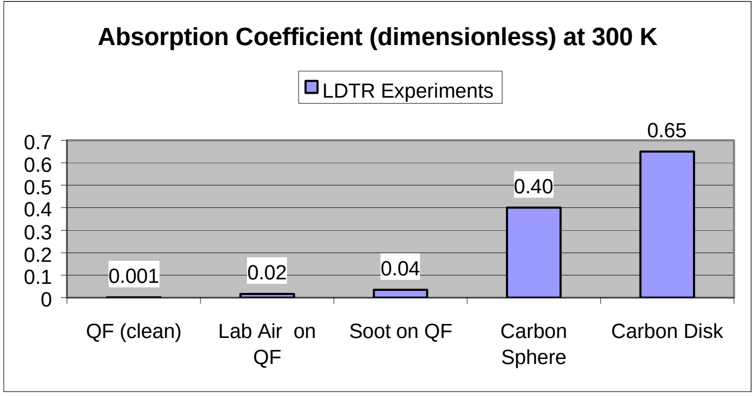
## *Absorption Coefficient Measurements of Aerosol Particle Agglomerates*

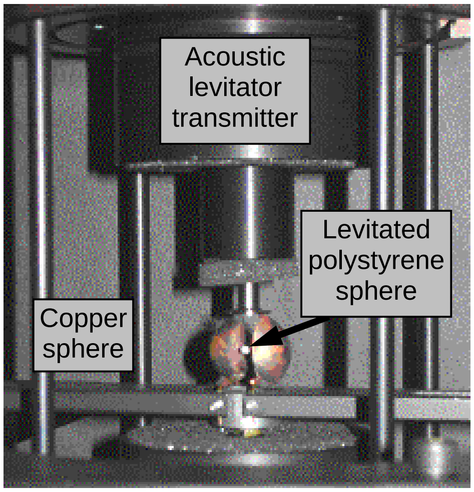
***C. Presser (836); J. Conny (837); A. Nazarian (SAIC)***

***CSTL Program Area: Energy and Environmental Technologies***

***Currently, the largest uncertainty in predicting the change in Earth's global average temperatures over time is due to inadequate knowledge of the optical properties of atmospheric aerosols such as soot and cloud condensation nuclei.***

Greenhouse effects associated with climate change may be influenced strongly by the chemical and physical properties of aerosol particles, i.e., particulate matter (PM), in the atmosphere. Although a variety of methods are used to measure atmospheric aerosol black carbon mass, those based on thermal-optical analysis (TOA) are widely used in the US. TOA methodology makes critical and untested assumptions about the thermal and optical behavior of PM on a quartz fiber collection substrate, as well as the instrument-produced byproducts of pyrolysis. In response, we have developed a new approach to characterizing the optical behavior of soot PM, which combines a laser-driven thermal reactor (LDTR), an acoustic levitator, and Stokes/anti-Stokes Raman spectroscopy as a well-controlled thermal environment for the non-intrusive determination of thermal-physical and chemical-kinetic properties.





LDTR measurements were performed to determine the effect of carbon shape and size on its absorption characteristics. Results were obtained for a spherical and disk-shape agglomeration of compacted particles. This information is important for characterizing particles when their surface is not always spherical and well-predicted by theory. Measurements were carried out for different average particle temperatures, up to 1200 K, to determine the heat transfer characteristics of cleaned and baked quartz fiber filters (QF), which serve as the substrate for PM collection. Using a direct laser heating approach, absorption coefficient results were obtained at different sample temperatures (from ambient to 1180 K) and gas pressures (at 75 Torr and 750 Torr) for cleaned filters, indoor air particles, and soot from an acetylene-fuel flame (above figure). It was observed that the absorption coefficient increased with temperature for the cleaned filters, whereas the absorption coefficient decreased for the filter exposed to indoor air particles and soot. Significant progress was made in integrating Raman spectroscopy diagnostics and the acoustic levitator, which is designed to circumvent the use of a substrate for particles in the LDTR. New reactor copper spheres were fabricated to allow for levitation of soot particles inside the sphere, and to provide optical access for the Raman measurements (figure above).

***Next Steps:*** We plan to use this technique to develop a unique database that includes, in addition to soot absorption coefficient, other optical and physical properties for soot, other representative samples of particulate matter, and multiphase and multicomponent liquid droplets that are representative of cloud condensation nuclei characteristics. These data will be used to provide input information for climate change models, and to improve the performance of optically based devices used to monitor particulate matter in the environment.

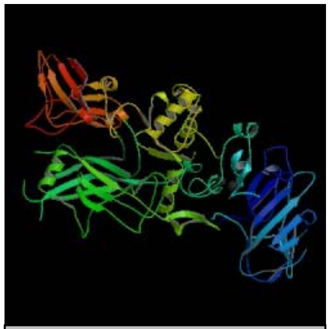
### *Mapping the Protective Antigen Binding Site on the Anthrax Toxin Receptor*

***Kevin D Ridge (831)***

***CSTL Program Area: Forensics and Homeland Security***

Anthrax is a disease caused by the gram-positive, spore forming bacterium *Bacillus anthracis*. Humans are typically incidental hosts through ingestion of contaminated foods of animal origin, various animal products, and more recently, malicious exposure. Because of the heightened public awareness and concern surrounding anthrax, there is an acute need to understand the molecular mechanisms governing the intoxication process and to apply this knowledge to the development of new detection methods and therapeutic strategies that confront this disease. The major contributing factor to the pathogenesis of *Bacillus anthracis* is a secreted, three-component toxin. The three proteins comprising the toxin are the lethal factor (LF), the edema factor (EF), and the protective antigen (PA). Although the mechanism of anthrax intoxication is not entirely clear, some discrete steps have been delineated through a variety of experimental approaches. Initially, PA binds to a cell surface integral membrane protein, the anthrax toxin receptor (ATR), and is cleaved by a cell surface protease generating a truncated carboxyl-terminal form of the protein (PA63). PA63 possesses the inherent ability to self assemble and the oligomeric form of the protein is able to bind EF or LF with high affinity. The resulting complexes are then internalized where they exert their toxic effects.

***CSTL researchers are unraveling the mysteries of anthrax intoxication to allow for early detection and subsequent intervention protocols responding to toxin exposure.***



***Bacillus anthracis*, protective antigen (PA) Source: PDB**

A comprehensive understanding of the molecular mechanisms governing the pathogenesis of *Bacillus anthracis* requires quantitative information about the interaction of PA with its host cell membrane bound receptor, ATR. As the binding of PA to ATR can be considered as the first step in the intoxication process, it represents a potentially important target site for early detection and therapeutic intervention. However, since little is currently known about this interaction at the molecular level, mapping of the PA binding site on ATR is of intense interest to the scientific community as well as those charged with protecting public health. Mapping the PA binding site(s) on ATR is necessary for advancing our understanding of these biological macromolecules and their mechanistic role in the pathogenesis of Bacillus anthracis. Clearly, knowledge of the critical “establishment” stages of anthrax intoxication should provide decisive targets for the early detection of anthrax and subsequent intervention protocols responding to toxin exposure.

**Next Steps:** The availability of the ATR gene enables a systematic study of the PA binding site. Edward Eisenstein of UMBI/CARB, who is investigating PA63 assembly, has constructed a synthetic PA gene. With these proteins in hand, detailed biochemical and biophysical measurements of the ATR/PA interaction are likely to provide a quantitative description of the anthrax intoxication process

**Mapping the Protective Antigen (PA) binding site(s) on the Anthrax Toxin Receptor (ATR) is necessary for advancing our understanding of these biological macromolecules and their role in the pathogenesis of *Bacillus anthracis*.**

## *Development Of SEM-EDS Analysis Procedures For C, N And O To Enable Characterization Of Explosives Particles, Polymeric And Organic Materials*

***J.T. Armstrong and J.A. Small***

***CSTL Program Area: Forensics and Homeland Security***

Procedures were developed for quantitative phase identification of organic particles and surface layers using energy dispersive x-ray analysis (EDS) in a scanning electron microscope (SEM) to determine C, N, and O relative concentrations as well as those of heavier elements. Tests of these procedures on gunpowder grains and explosives microparticles showed that, although radiation damage induces partial dehydration in these materials, repeatable steady-state residual compositions are left that can be used for accurate phase identification.

***CSTL scientists have determined that accurate EDS analysis or phase fingerprinting of C, N, and O will open a vast potential range of applications for analytical scanning electron microscopy in many areas.***

Using several commercial thin window EDS analyzers, we found that C, N, and O can be routinely measured with detection limits of better than 0.5 % for C and O and ~1 % for N in the presence of major C. Measurement precisions of ~0.5 % (absolute) can be attained for these elements. Although some commercial software packages were found to fail in attempts to separate peaks from backgrounds for these elements, sequential simplex in NIST's DTSA program was found to work satisfactorily. Optimum correction procedures for these elements were identified and tested on standard (inorganic) multilayer specimens (insulating organic particles need to be coated with a conducting material, we chose Au). Results showed comparable accuracy to that obtained for elements like Na, Mg, Ga, Ge, and As (commonly analyzed elements having low energy x-ray lines). The major problem found in analyzing organic materials was, as expected, radiation damage and decomposition under electron bombardment. Procedures were tested that minimized this damage, but none were found so far that completely eliminate it. We found, however, that the degree of decomposition during analysis could be controlled, resulting in repeatable, distinctive residue compositions – even for materials as volatile as RDX explosive. The data suggest that by use of controlled conditions, “fingerprint” C-N-O EDS spectra can be obtained from analysis of organic particles and thin layers that could be used to identify their initial phase composition. (See figures 1 and 2 on the next page.)

**Procedures were tested that minimized the radiation damage and decomposition under electron bombardment expected when analyzing organic materials.**

Accurate EDS analysis or phase fingerprinting of C, N, and O will open a vast potential range of applications for analytical scanning electron microscopy that could potentially double its usage or more in areas as important and disparate as forensics and homeland security, nanotechnology, microelectronics, pharmaceutical analysis, corrosion research, failure analysis, and environmental sample analysis.

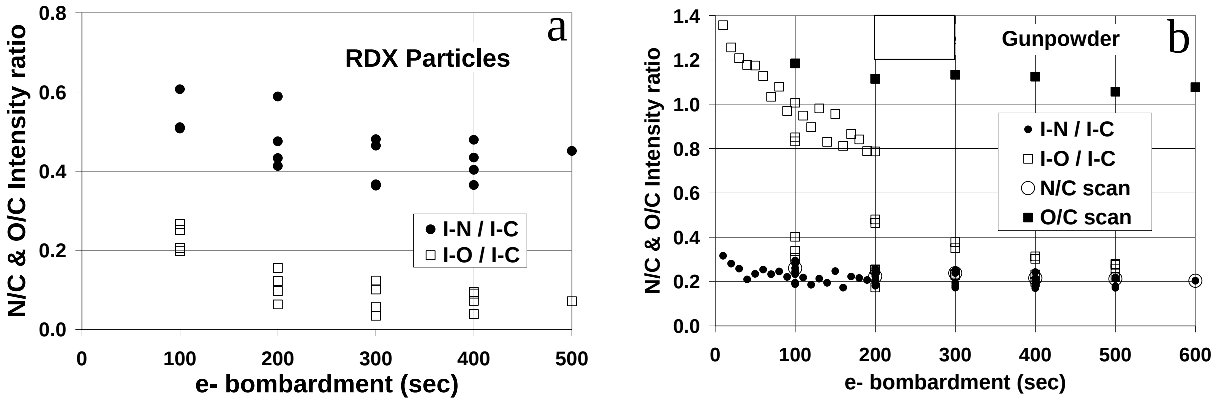


**Fig. 1. Decrease in relative intensities of nitrogen and oxygen with respect to carbon as a function of electron bombardment time for RDX (a) and gunpowder (b). Trends are determined by a series of replicate analyses in the same spot. In both materials, a distinctive steady state composition of residual C-N-O is obtained.**

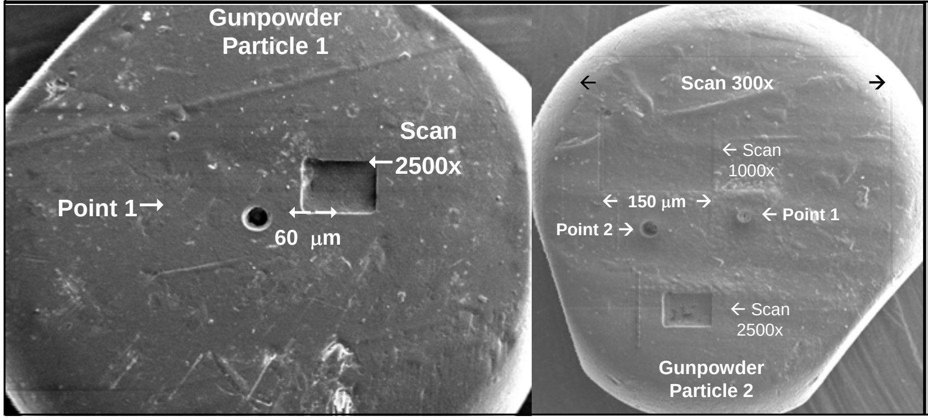


**Fig. 2. SEM images of gunpowder particles. Note visible radiation damage inflicted by the electron beam during analysis. Minimal damage is seen for the 'whole particle' 300x scan in Fig. 2b; however, even here significant oxygen loss occurred (Fig. 1b).**

***Next Steps:*** We propose ramping up this project to develop experience with a wider range of organic materials and a wider range of beam dose conditions. We also propose testing whether cryoanalysis would significantly improve sample stability measurement reproducibility.

### *DNA Damage by the Antitumor Drug 3-Amino-1,2,4-benzotriazine 1,4-dioxide (Tirapazamine)*

***M. Birincioglu, H. Rodriguez, M. Dizdar (831); P. Jaruga (UMBC, MD); and G. Chowdhury and K. Gates (University of Missouri)***

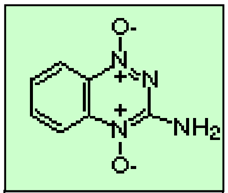
***CSTL Program Area: Health and Medical Technologies***

Hypoxia is a typical feature of cancerous tumors. Drugs activated in hypoxia have wide potential applications, particularly in overcoming the resistance of hypoxic tumor cells to chemotherapy. Key features of such drugs include redox properties activated by reductase enzymes such as flavoproteins. In hypoxic tumor cells, the enzymatically reduced drug and/or its intermediates lead to formation of reactive hydroxyl radicals that effectively damage DNA of tumor cells, resulting in destruction of tumors. Tirapazamine is such a bioreductively activated DNA-damaging agent that selectively kills the hypoxic cells found in solid tumors.

***CSTL results provide researchers with expertise in the measurement of oxidative DNA damage.***

This compound shows great clinical promise and is currently being examined in a variety of clinical trials, including several phase III studies. It is well established that DNA is an important cellular target for tirapazamine; however, the structural nature of the DNA damage inflicted by this drug remains poorly understood. As part of an effort to understand the chemical events responsible for the hypoxia-selective cytotoxicity of tirapazamine, the studies reported here were designed to characterize this drug's ability to damage DNA. Here, we used gas chromatography/mass spectrometry and liquid chromatography/mass spectrometry to characterize and quantify oxidative DNA damage mediated by tirapazamine. A multiplicity of modified DNA bases were identified and quantified. It appears that the hypoxic conditions, under which tirapazamine operates, along with the unique chemical properties of the drug, yield a unique variety of DNA damage that includes poorly repaired, potentially cytotoxic DNA base lesions that block DNA transcription and replication. Overall, the results indicate that DNA base damage may contribute to the biological effects of tirapazamine *in vivo*. The results also provide researchers in industry and academia with expertise in the measurement of oxidative DNA damage, this work supports the efforts to develop methodologies to test new anticancer drugs similar in chemical structure to tirapazamine and to help elucidate the mechanism of action of hypoxia-selective antitumor drugs and their efficacy to destroy tumor cells, but not normal cells.

**This work provides the first detailed insight regarding the structural identity and quantities of the DNA lesions caused by this new anticancer drug.**



**Tirapazamine**

**Next Steps:** This study will be extended to the characterization of DNA damage by this drug *in vivo*, and by other structurally similar hypoxia-selective antitumor drugs.

## *Human Mitochondrial Protein Database: A Resource for Human Mitochondrial Proteomics*

***V. Ravichandran, P. E. Barker, G. B. Vasquez, T.N. Bhat, G.L. Gilliland (831); S. J. Zull (ATP)***

***CSTL Program Area: Health and Medical Technologies***

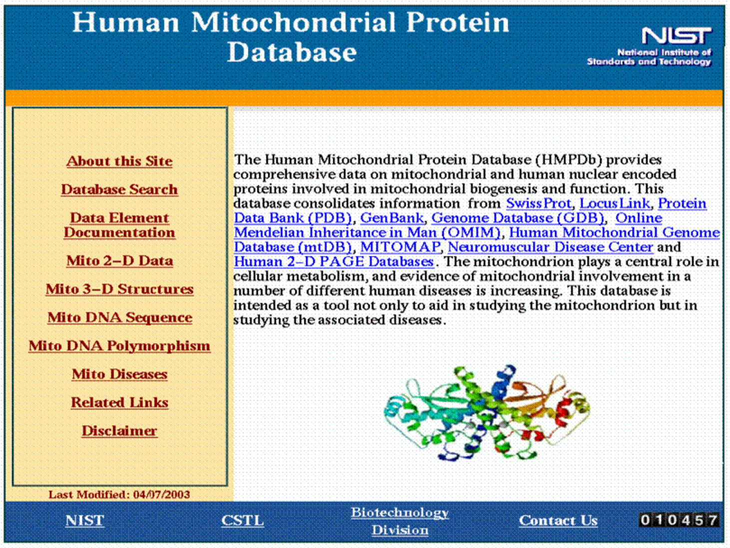
A publicly available web-based resource, the Human Mitochondrial Protein Database (HMPD), has been developed to unify data from a broad collection of related resources that provides information about human nuclear and mitochondrial-encoded proteins. Our database is designed to address the problem presented by the increasing amount, complexity, and types of proteomics data available in public databases. At this point, no systematically established correlations between the different types of data have been established that allows meaningful comparisons. One of the primary reasons for this is the lack of data standards. Despite this, a wealth of data exists and is readily available from numerous web resources that are applicable to proteomics and have direct relevance to identifying proteins and assigning function. However, serious problems with the interactions between these resources arise because of problems with data exchange and interoperability. Although public proteomics data resources are highly informative, individually, the collection of available content would have more utility if provided in a standard and centralized context and indexed in a robust manner for a specialized area. The HMPD currently consolidates information from many publicly available sources.

***The Human Mitochondrial Protein Database (HMPD), a centralized database for mitochondrial proteins, has been developed as an instrument for standardizing proteomics data using the mitochondrion as a model system.***

A customizable interface has been developed to permit complex queries that include the name of the protein, tissue, mitochondrial compartment, chromosome number, molecular weight range, pI range, and keywords. Users can also restrict the data being searched to 2-D PAGE images, Locus links, the Genome Database, OMIM, or RefSeq information. To further narrow the search, selected results are grouped with gene name, Protein name and SwissProt ID, where the SwissProt ID is linked with detailed information for a protein of selection.

**The HMPD web site, http://bioinfo.nist.gov:8080/examples/servlets/index.html, was recently cited in *Science's* NetWatch ("RESOURCES: Probing the Cell's Powerhouse," *Science, 300*, 713, 2003).**

A graphical tool was developed to visualize the human mitochondrial DNA sequences (16,569 bp). A mouseover feature has been added to include the annotated information. In addition, a polymorphism check tool has been implemented to dynamically compare the change in mtDNA changed between two populations, and a standard reference data set for two-dimensional gel electrophoresis has been implemented.



Human Mitochondrial Protein Database

NIST National Institute of Standards and Technology

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The Human Mitochondrial Protein Database (HMPDb) provides comprehensive data on mitochondrial and human nuclear encoded proteins involved in mitochondrial biogenesis and function. This database consolidates information from SwissProt, LocusLink, Protein Data Bank (PDB), GenBank, Genome Database (GDB), Online Mendelian Inheritance in Man (OMIM), Human Mitochondrial Genome Database (mtDB), MITOMAP, Neuromuscular Disease Center and Human 2–D PAGE Databases. The mitochondrion plays a central role in cellular metabolism, and evidence of mitochondrial involvement in a number of different human diseases is increasing. This database is intended as a tool not only to aid in studying the mitochondrion but in studying the associated diseases.

Last Modified: 04/07/2003

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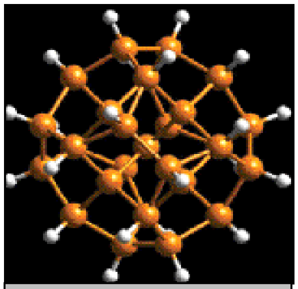
***Next Steps:*** Future plans include developing this web site as a standard reference data for mitochondrial proteomics. Experimental data from areas that impact proteomics will be archived and incorporated into the database (HMPDB). Data definitions and standards dictionaries will be developed to facilitate data acquisition, display, and exchange.

## *Silicon Nanoparticles as DNA Labels*

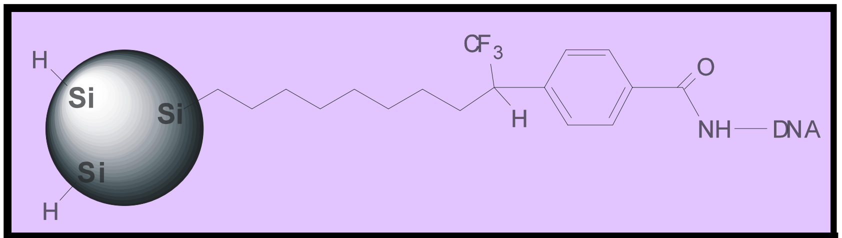
***Vytas Reipa and L. Wang (831)***

***CSTL Program Area:*** ***Health and Medical Technologies***

DNA hybridization assays are increasingly applied for pharmacogenomic research and drug discovery, infectious and genetic disease and cancer diagnostics. Micro analytical devices, high-throughput simultaneous test arrays, capillary electrophoretic analysis and dipstick devices have presented new challenges and requirements for nucleic acid detection, and fluorescent methods currently dominate in many of these applications. The continuing quest for improved labels for these assays has produced a series of candidate labels including genes encoding enzymes, quantum dots, and new examples of the fluorophore (e.g. cyanine dyes) and enzyme class of labels. Organic fluorophores, either covalently linked or intercalated to DNA are subject to photo degradation, environmental sensitivity, which may limit their widespread application in high light intensity methodologies, such as DNA micro arrays. Manufacturing of such chips also requires rigorous quantitation of the amount of surface attached oligonucleotides, which is currently problematic using intercalating organic dyes. We explored the applicability of highly luminescent silicon nanoparticles for DNA tagging in aqueous solutions. The silicon nanoparticles possess superior properties, such as high photo-stability and low toxicity, which are crucial for applications of luminescence (fluorescence)-based assays in clinical diagnosis.



***1 nm diameter Hydrogen capped Si particle*** ***(Biophotonics International).***



**Oligonucleotide-conjugated silicon nanoparticles**

The multiple chemical steps for obtaining oligonucleotide-conjugated silicon nanoparticles include an electrochemical etching step and two photo-induced reaction steps followed by a DNA labeling step that allows the formation of a carboxamide bond between silicon nanoparticle and oligonucleotide. The procedure enhances aqueous solubility of hydrophobic silicon nanoparticles, which is a key requirement for the effective use of nanoparticles in biological applications. We successfully conjugated 1 to 2 nm diameter silicon nanoparticles to a 5'-amino modified oligonucleotide whose sequence is from rat tubulin genome. Under UV excitation, photo-luminescence of the conjugates is dominated by two blue bands (400 nm and 450 nm maximal). The quantum yield of oligonucleotide-conjugated nanoparticles that were coated with a layer of 1-octene was determined to be 0.08 as measured against quinine sulfate in 0.1 M $HClO_4$ as a reference standard. We have demonstrated the feasibility of the present coupling chemistry to obtain silicon nanoparticle-labeled nucleotides.

**The research will facilitate the development of alternatives to organic fluorophores which will provide superior performance when used in biological applications.**

It is expected that the alternatives will be superior in the following respects: high luminescence quantum yield, high photo-stability, low toxicity, and size-dependent luminescence. These properties are critically important for biological applications such as DNA sequencing, microarrays for high-throughput screening, flow cytometry measurements, and fluorescence in-situ hybridization.

***Next Steps:*** Size exclusion chromatography separation and the use of a longer carbon chain will likely enhance the luminescence quantum efficiency of the nanoparticles, which is critical for applications such as fluorescence in-situ hybridization in chromosomes.

## *Predicting Vibrational Spectra of Dissolved Salts*

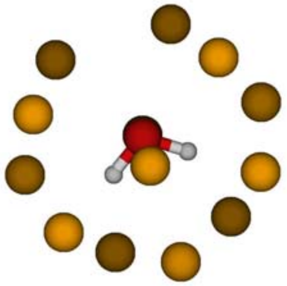
***LiLi Wang (831); K. K. Irikura (838)***

***CSTL Program Area: Pharmaceuticals and Biomanufacturing***

***CSTL scientists use vibrational spectroscopy to identify compounds by comparing observed spectra with those of candidate compounds. Despite the ubiquity of ions throughout chemistry, to date there are no reliable theoretical procedures for predicting their vibrational spectra in solution.***

Aqueous ions are commonplace and important in biology, geochemistry, many manufacturing processes, and elsewhere. Vibrational spectroscopy (infrared, Raman, and near-infrared) is often used to analyze or monitor such systems. For example, most biological molecules are ions, and vibrational spectroscopy is important for identifying their structures and interactions. For chemical analysis, molecules can only be identified by spectroscopy if the spectra of candidate molecules can be predicted in some way, for comparison. Modern theoretical methods can now predict gas-phase spectra with useful precision, but predicting condensed-phase spectra is more challenging. Ions are especially difficult because they interact strongly with the solvent or matrix, often causing dramatic changes in the spectrum. Although significant progress has been achieved in modeling solvation, the primary emphasis has been on energetics, with a secondary emphasis on molecular structure. The performance of solvation models in predicting vibrational spectra using quantum chemistry has received little attention.

To date, the most popular, inexpensive continuum models have been found inadequate, and have failed rigorous testing. Consequently, computations with explicit solvent molecules are now underway. Two reasonable electronic-structure models have been identified and compared for the prototype ion $H_2O^+$ solvated by neon, which was chosen as the initial, simple case for which benchmark experimental data are available. Increasingly large computations still have not reached the bulk limit.



**A sample structure for $H_2O^+(Ne)_{13}$**

**Reliable theory will permit observed spectra to be assigned with confidence, thus providing the necessary interpretation of the experimental data. The current limited success is essential for aqueous frequency predictions.**

***Next Steps:*** Ion-solvent clusters are mechanically “soft”, with multiple, competing structures, and the corresponding effect upon the vibrational spectrum remains to be investigated. Simulated-annealing approaches will also be applied to find the most stable structure for each cluster. Both procedures will help in reaching the bulk limit, a major goal of this project.

### *Trace Nanoanalysis Using Hyperspectral Imaging*

***J. H.J. Scott and J. M.Conny (837)***

***CSTL Program Area: Technology for Future Measurement***

***CSTL Researchers develop tools and utilities for exploring the application of chemometrics to trace nanoanalysis in the analytical electron microscope (AEM) and electron microprobe; and apply multivariate data analysis strategies developed for bulk analytical methods to chemical analysis at high spatial resolution in the analytical electron microscope.***

The recent introduction of multispectral and hyperspectral imaging techniques to analytical electron microscopy and electron probe microanalysis has led to an explosion in the amount of chemical data available from each sample. Thousands of channels of spectral information can be saved for each of millions of pixels on the specimen, and the challenge is to find techniques that can exploit this wealth of raw data. Following the lead of analytical chemists working with non-microscopic methods, this work attempts to apply multivariate statistical tools and chemometrics to meet this challenge. The goal is to provide a new suite of analysis tools for the microanalysts in government labs, academia, and the industrial sector. The NIST/NIH Desktop Spectrum Analyzer (DTSA) used for testing new statistical approaches and validating methods such as Principal Components Regression (PCR) and Partial Least Squares (PLS) for multivariate calibration. Software implementing these algorithms (in Matlab) was linked to DTSA output via a series of scripts. This allows ideas borrowed from chemometrics (such as multivariate curve resolution, multivariate image analysis, and inverse calibration methods) to be applied to current problems in microanalysis.

In FY 03, these researchers accomplished the following:

- Implemented a set of scripts for pipelining data from the NIST/NIH Desktop Spectrum Analyzer package (DTSA) to the PLS toolbox in Matlab for multivariate analysis
- Developed an environment for testing the viability of existing methods in chemometrics in the context of hyperspectral data acquisition in the AEM and electron microprobe
- Imported synthetic Fe-Ni-Cr spectral data from DTSA into Matlab to evaluate Principal Components Regression (PCR) and Partial Least Squares (PLS) as potential methods for multivariate quantitative microanalysis
- Imported synthetic datasets for exploring non-linear spectral pre-treatments to optimize multivariate calibration using energy-dispersive x-ray (EDS) data
- Used multivariate curve resolution to resolve mixed experimental spectra into “pure components” without *a priori* chemical knowledge of the system; investigated numerical convergence problems

**These tools and utilities may permit leveraging of existing expertise in chemometrics to enhance performance of chemical measurement tools such as energy-dispersive x-ray spectrometry.**

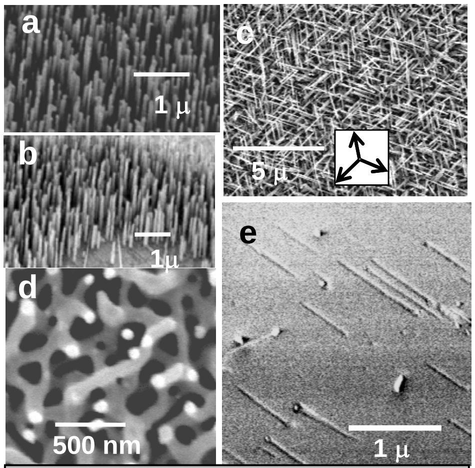
***Next Steps:*** Researchers plan to investigate current topics being explored in analytical chemistry, chemometrics, and multivariate analysis and apply them to problems of ultramicroanalysis.

## *Growth of Oriented ZnO Nanowires on Sapphire and Gallium Nitride*

***Babak Nikoobakht and Stephan J. Stranick (837)***

***CSTL Program Area: Technologies for Future Measurement***

***CSTL researchers develop novel methods to grow and manipulate semiconductor nanowires that have unique properties and make them well suited for applications in opto-electronics and as potential building blocks of future measurement platforms.***



**Electron micrograph of a) and b) normal NWs on c-plane sapphire and GaN c) 60˚ tilted NWs on c-plane sapphire d) NWs on ZnO network (top view) e) horizontal NWs on a-plane sapphire 1µm.**

Nano-light sources and detectors can be realized by engineering a nanowire with a diode junction/functionality. This along with other nano-device functionality could enable the development of sensor arrays capable of performing quantitative chemical measurements. These methodologies, when further refined, could be employed to generate novel opto-electronic devices for use in sensor and proximal probe applications. In FY 03, CSTL scientists grew ZnO nanowires (NWs) on sapphire and GaN surfaces via a vapor-phase transport process using Au thin film as catalyst. We have been able to successfully control the growth direction of individual ZnO NWs and the morphology of their ensemble. We present the first examples of vertical and 60º-tilted NWs on c-plane sapphire, vertical NWs on GaN substrate and unidirectional growth of horizontal NWs on a-plane sapphire. In the case of normal NWs on sapphire and GaN, XRD analysis shows that single-crystal *wurtzite* NWs grow in the [0001] direction normal to the basal c-plane, which proves that a-plane sapphire is not essential for growth of vertical ZnO NWs. We have found that the thickness of the Au-film and pre-growth annealing of Au/sapphire substrate are critical in controlling the morphology of the nanowires. Utilizing the above factors, two new morphologies have been selectively synthesized. AFM and SEM studies show that in the absence of annealing, Au films with feature heights between 1 to 10 nm result in NWs 60º tilted with respect to the surface, whereas pre-annealed Au films with heights below ~6 nm result in growth of NWs in the surface normal direction. For the annealed Au films thicker than 6 ± 2 nm, a network of ZnO is formed followed by the growth of NWs in the surface normal direction. We have attributed the normal and tilted growth directions to the surface concentration of O and Al ions on sapphire. In order to form horizontal NWs, results show that the substrate needs to be a-plane and instead of an Au film as catalyst, scattered Au droplets with diameter less than 15 nm must be present on the surface. In this case NWs all grow in (110) direction on the basal plane of the a-plane sapphire. The findings of this study have been submitted for publication in *Advanced Materials* and *Journal of Physical Chemistry B*.

**Nanowire devices will provide multiple options as building blocks for sensors, miniaturized measurement platforms, and as advanced scatter probe structures, enabling true nanometer spectroscopy. This methodology will provide an inexpensive and straightforward approach to the synthesis of sensing devices with multiple functionalities that can be integrated to form state-of-the-art measurement systems.**

***Next Steps:*** Our preliminary studies have focused on the growth and manipulation of semiconductor nanowire. These methodologies will be further refined and then employed to generate novel opto-electronic devices for use in sensor and proximal probe applications.

## *Progress Towards Development of an Improved Method of Phase Identification by Selected Area Electron Diffraction*

***S. Turner, D.S. Bright (837); and V.L. Karen, (MSEL)***

***CSTL Program Area: Technologies for Future Measurement***

***CSTL researchers explore the possibility of improved phase identification by transmission electron microscopy by applying electron diffraction data obtained from a double-tilt, rotate sample holder to reduced cell and phase matching programs.***

Selected area electron diffraction (SAED) is a very useful technique for identification of materials examined by transmission electron microscopy. In combination with chemical information, the structural data obtained by SAED greatly narrows the range of possible phases. SAED has been limited, however, in that information is generally obtained for the sample in only one orientation. Currently, if SAED is obtained for more than one orientation its use is limited because it has been difficult to relate the 3-D information in the diffraction patterns. The recent availability of a double-tilt, rotate sample holder with improved manipulation capabilities along with the availability of reduced cell and phase identification programs developed at NIST suggested the possibility of an improved method of phase identification. Ideally, structural data collected from more than one orientation could be obtained, the geometric relationships determined, reduced unit cells derived and the phase identified from the reduced cells. In this work, the feasibility of this approach was investigated.

In FY 03, initial steps were made to interface the electron microscope diffraction data from multiple orientations with a reduced cell program for phase identification. Over twenty diffraction patterns and their angular relationships were obtained from a single tetragonal crystal. Diffraction patterns were calculated for the material and a comparison of the calculated and experimental interzone angles showed good agreement. Additionally, diffraction patterns for a cubic material were modeled. Application of the acquired and modeled data to the reduced cell program resulted in unexpected challenges. We have solved some of the problems and can now generate reduced volumes that in some cases are consistent with identification of the analyzed phase. We will continue to address problems in the interfacing of multiple diffraction patterns with a reduced cell and the associated phase identification program. More work needs to be done to overcome problems associated with interfacing the experimental data to the programs and to evaluate the effect of uncertainty in the data on phase determination.

***Next Steps:*** Work will continue on efforts to address problems in the interfacing of multiple diffraction patterns with a reduced cell and the associated phase identification program. More work needs to be done to overcome problems associated with interfacing the experimental data to the programs and to evaluate the effect of uncertainty in the data on phase determination.

## *Universal Validation Standard for UV/visible Spectrometry*

***D.L. Duewer and J.C. Travis***

***CSTL Program Area: Technologies for Future Measurement***

The traditional suite of UV/visible absorption reference materials designed for research grade instruments has fallen short of meeting the traceability requirements of quality systems associated with “factory floor” instruments. The goal of this proposal was to develop a universal standard that would serve as a replacement for the entire suite of current NIST SRMs (Standard Reference Materials) for chemical spectrometry in the UV/visible spectral region. This would be possible due to the development of a novel whole-spectrum fitting algorithm. This algorithm provides the core functionality for establishing the UV/visible absorption spectra of holmium oxide solution (such as NIST SRM 2034) as an intrinsic standard for the total performance validation of chemical spectrophotometers.

The declaration of dilute holmium oxide solution as an intrinsic wavelength standard was underway at the time the proposal was developed, and is reported elsewhere. Data from the study that declared dilute holmium oxide solution as an intrinsic wavelength standard were used in the successful development of an algorithm to fit the entire (certified) intrinsic spectrum to the spectrum of properly prepared holmium oxide solution taken on an instrument to be validated. The algorithm accounts for the wavelength bias, the absorbance bias, and – most significantly – the spectral bandwidth of the recording instrument. A significant breakthrough in algorithm development was the approach by Statistical Engineering Division collaborators to perform a fit that actually extracts the experimental instrument function ("slit function") in addition to the other expected bias data.

**The universal standard will provide simultaneous wavelength calibration and validation of the absorbance scale and spectral bandwidth specification for practical instruments with a single artifact, data acquisition, and automated data analysis.**

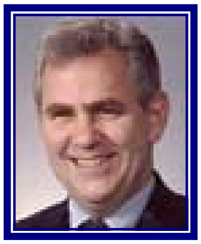
The universal standard will provide simultaneous wavelength calibration and validation of the absorbance scale and spectral bandwidth specification for practical instruments with a single artifact, data acquisition, and automated data analysis. In addition to making life easier for the end user, traceability support will be almost entirely outsourced from NIST. The attention of CSTL can then be turned to emerging measurement technologies while this mature technology is supported by the intrinsic standard.

***Next Steps:*** Work is continuing on the certification of 4% (w/v) holmium oxide dissolved in 10% (v/v) perchloric acid as an intrinsic wavelength standard. With modest additional investment of resources, we will demonstrate that this material is suitable for use as an intrinsic standard for both wavelength and absorbance when spectral baseline artifacts (arising from differences in the surface reflectance of individual cuvettes and minor organic contamination of solvents and cuvettes) are eliminated experimentally. However, we believe that it is possible to mathematically account for these artifacts within the spectral-fit software. This algorithmic development would greatly simplify the necessary experimental burden on the end-user and would enable routine use of the intrinsic standard to meet most chemical traceability requirements.

# D. Staff Recognition

Our staff is CSTL's greatest resource. We are proud of all of them and their accomplishments, both at work and in their communities. In FY 2003, the following staff were formally recognized for their achievements.

**John Butler** (831) received the Scientific Prize from the International Society of Forensic Genetics for his contributions to the field of rapid and accurate DNA typing for forensics and human identification.



**Rich Cavanagh** (837) was elected a Fellow of the American Vacuum Society for his sustained and outstanding contributions in areas of interest to the AVS.

**Bill Dorko** (839) received the Department of Commerce Silver Medal for the development and implementation of the NIST Traceable Reference Materials (NTRM) Program for gas mixture standards.

**Carol Driver** (838) received the CSTL Secretarial Achievement Award for her outstanding skills and providing extraordinary service to the staff and programs of CSTL.

**David L. Duewer** (839) received the Edward Bennett Rosa Award for his innovative use of visualization techniques to summarize and communicate complex statistical information. The Edward Bennett Rosa Award is granted for outstanding achievement in or contributions to the development of meaningful and significant engineering, scientific, or documentary standards.

**John J. Hurly** and **Keith A. Gillis** (836) were awarded the Department of Commerce Bronze Medal for their work with semiconductor process design and the metering of semiconductor process gases, needs documented in the 1999 Semiconductor Industry Association (SIA) International Technology Roadmap for Semiconductors.

**Robert Greenberg, Richard Lindstrom** (839) and **David Simons** (837) received the Judson C. French Award for their development and critical evaluation of an instrumental neutron activation analysis method for the determination of arsenic in silicon and transferring this technology to the semiconductor industry's chip fabrication lines.

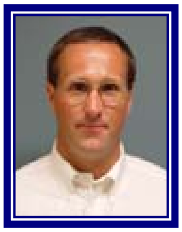
**Frank Guenther** (839) received the Department of Commerce Silver Medal for the development and implementation of the NIST Traceable Reference Materials (NTRM) Program for gas mixture standards. He was also awarded the Safety Award for Superior Accomplishment for the development and implementation of an efficient process for inventorying and cataloging chemicals used within the Analytical Chemistry Division of CSTL.

**Donna Kimball** (839) received the Eugene Casson Crittenden Award for her continued energy, leadership skills, and organizational talents which she used to co-organize and coordinate the NIST "Take Our Sons and Daughters to Work Day" Program. She also managed all logistical aspects of the NIST Centennial Symposium at the 2001 Pittsburgh Conference.

**Dick Lindstrom** (839) received the Department of Commerce Silver Medal for the development and delivery of cutting-edge nuclear technology to support U.S. nonproliferation efforts.

**Gerald Mitchell (839)** was awarded the Safety Award for Superior Accomplishment for the development and implementation of an efficient process for inventorying and cataloging chemicals used within the Analytical Chemistry Division of CSTL.

**Ray Radebaugh** (838) has been honored with the Department of Commerce Gold Medal for his outstanding contributions to the successful transfer of cryocooler technology to U.S. industry for a broad range of applications including military surveillance, space exploration, health care, and high performance electronics.



**David Ross** (836) was named the Sigma Xi Young Scientist of the year for 2002-2003 for his seminal research in the area of microfluidic systems.

**Jennifer Scott** (838) is the winner of the NIST Boulder Building Tomorrow's Workplace Award (BTW) for her leadership role in encouraging mentoring activities and in establishing a positive environment for recognizing the potential of strengthening the effectiveness of the organization by making use of the differences in the workforce personnel

The CSTL Technical Achievement Award went to the team responsible for the certification of SRM 1946, "Lake Superior Fish Tissue." **Michele Schantz** and **Stephen Wise** (839) were the project coordinators. Contributing analysts were **Wayne Brubaker, Jr., Steven Christopher, John Kucklick, Stephen Long, Elizabeth Mackey, Curtis Phinney, Barbara Porter, Dianne Poster, Rebecca Pugh, Michael Rearick, Katherine Sharpless,** and **Silke Tutschku.**

**Anneke Sengers**, scientist emeritus, was selected as the North American recipient of the 2003 Women in Science Awards presented by the cosmetics company L'Oreal and the international organization UNESCO.

**Steve Stranick** (837) received the ACS Analytical Chemistry Division Arthur F. Findeis Award for his contributions to the fields of analytical chemistry including his development of a novel microscope that combines the high spatial resolution of near-field scanning optical microscopy with the quantitative chemical probe capabilities of infrared spectroscopy.

**Wing Tsang** (838) was appointed to the position of NIST Fellow. Dr. Tsang is recognized for his world-class accomplishments and leadership in the area of chemical kinetics.

**Marc Salit** and **Greg Turk** (839) were awarded the Department of Commerce Bronze Medal for for their work on an innovative, chemometric-based approach to correct for low frequency noise, or drift, that has improved the precision of chemical measurements by up to 20-fold.

**John D. Wright** (836) received the Department of Commerce Bronze Medal for designing, assembling, testing, and publishing a definitive description of an extraordinary, new gas-flow standard.

# The CSTL Divisions

**CSTL's laboratory activities** are primarily located at the NIST headquarters site in Gaithersburg, MD. We also have research activities in NIST laboratories in Boulder, CO, and at the Center for Advanced Research in Biotechnology (CARB) in Rockville, MD. CARB is a collaborative effort of NIST, the University of Maryland, and Montgomery County, MD.

## Chemical Science and Technology Laboratory

Dr. William F. Koch, Acting Director
(301) 975-8301
william.koch@nist.gov

Eric Steel, Acting Deputy Director
(301) 975-8300
eric.steel@nist.gov

## Biotechnology Division

Dr. Vince L. Vilker, Chief
(301) 975-2629
vincent.vilker@nist.gov

- *DNA Technologies*
- *Bioprocess*
- *Structural Biology*
- *Biomolecular Materials*

## Process Measurements Division

Dr. James R. Whetstone, Chief
(301) 975-2600
james.whetstone@nist.gov

- *Fluid Flow*
- *Fluid Science*
- *Process Sensing*
- *Thermometry*
- *Pressure and Vacuum*
- *Thermal and Reactive Processes*

## Surface and Microanalysis Science Division

Dr. Richard R. Cavanagh, Chief
(301) 975-2368
richard.cavanagh@nist.gov

- *Microanalysis Research*
- *Surface and Interface Research*
- *Analytical Microscopy*

## Physical and Chemical Properties Division

Dr. Gregory J. Rosasco, Chief
(301) 975-2483
gregory.rosasco@nist.gov

- *Experimental Kinetics and Thermodynamics*
- *Chemical Reference Data and Modeling*
- *Computational Chemistry*
- *Experimental Properties of Fluids*
- *Cryogenic Technologies*

## Analytical Chemistry Division

Dr. Willie E. May, Chief
(301) 975-3108
willie.may@nist.gov

- *Spectrochemical Methods*
- *Organic Analytical Methods*
- *Gas Metrology and Classical Methods*
- *Molecular Spectroscopy and Microfluidic Methods*
- *Nuclear Methods*

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February 2004