

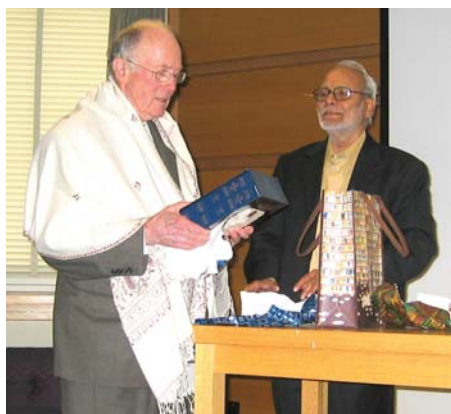


George R. Harrison Spectroscopy Laboratory Massachusetts Institute of Technology

Townes gives first Dasari Lecture

"Charles Townes is the perfect person to inaugurate the Dasari Lectures," said Harrison Spectroscopy Laboratory Director Michael Feld. That his assertion was no hyperbole became apparent as Professor Townes gave his lecture, "The fun of a physics career."

His invention of the maser and, with



Dr. Dasari presents the Bhagavad Gita to Prof. Townes at the first Dasari Lecture on Oct. 9

Arthur Schawlow, extension of the idea to the laser, created essential tools of modern laser spectroscopy. When Townes came to MIT as Provost in 1961, Ali Javan came with him. Their research on the fundamentals of laser physics and spectroscopy led to advances in understanding and technique that profoundly shaped the subsequent development of the Spec Lab.

Townes told a large and attentive audience about the high points of his remarkable career, a career in which he continues to be productive at age 92. He stressed that serendipity taught him an unusual mixture of theory, practical engineering knowledge, and experimental technique crucial to his successes. He also drew an important moral from his experiences. When leading physicists of the day, including the head of his department at Columbia University, told him he was

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New space for Spec Lab

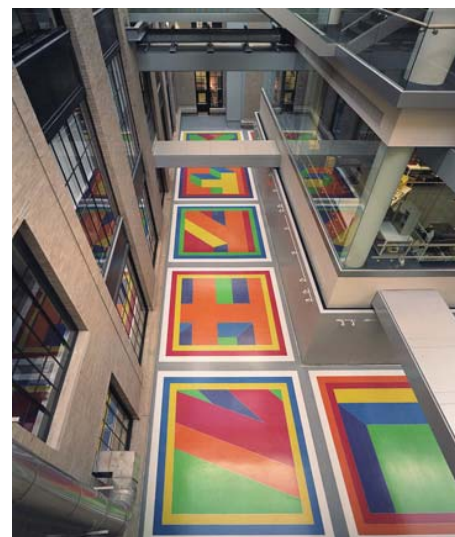
By Michael S. Feld

On October 5 MIT celebrated the completion of its two-year, more than \$50 million dollar construction project in Buildings 4, 6 and 8. The project, called PDSI because it was undertaken for the **P**hysics Department, the **D**epartment of Materials Science and Engineering, the **S**pectroscopy Laboratory, and for **I**nfrastructure, provides modern research facilities and much needed new space. The heart of the celebration was the dedication of the Green Center for Physics, which brings many physics faculty together in Bldg 6 and the newly built Bldg 6C.

For the Harrison Spectroscopy Laboratory PDSI provides a new physical plant for modern research and applications in spectroscopy. This modernized and expanded space allows the Spec Lab to continue to offer researchers the most advanced equipment of the day and to consolidate and extend its remarkable traditions of interdisciplinary research.

Since its founding in 1931 by Karl T. Compton and George R. Harrison, the Spectroscopy Laboratory has been a major center for research in spectroscopy. From the start it offered advanced facilities of outstanding capability. With its specially built vibration-isolated building and its 40-foot walk-in spectrograph, the Spectroscopy Laboratory was tailor built and equipped for high resolution spectroscopy. The world's most precise diffraction gratings were ruled here, and the famous MIT wavelength tables were compiled here. (This depression-era project kept many unemployed physicists at work!)

The Spec Lab has led in advances in theory and applications of the interactions of light with matter, the essence of spectroscopy. After he succeeded George Harrison in 1946 to become Director of the Spec Lab, Richard Lord pioneered development of Raman and infrared spectroscopy and obtained the first Raman spec-



Sol LeWitt's work of art, "Bars of Colors within Squares" surrounds the new space (MIT) 2007 Photograph by George Bouret

trum of proteins. Later during that period Ali Javan and Charles Townes brought lasers into the Spec Lab and opened the era of modern spectroscopy.

As MIT's first interdepartmental laboratory, the Spec Lab has always fostered interdisciplinary research. Since I became its director in 1976, we have striven to expand and exploit the opportunities of such research. In 1979 the National Science Foundation established a Region-

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al Instrumentation Facility in association with the Spec Lab and supported it for 26 years. The center brought in resources and attracted faculty members and has led to ongoing collaborative and multi-disciplinary research among faculty from Chemistry, Physics, Electrical Engineering, and Chemical Engineering. Since 1985, the National Institutes of Health has supported the Laser Biomedical Research Center (LBRC) in the Spec Lab. This national resource for conducting cutting edge research with lasers, light, and spectroscopy has been notably successful in fostering interdisciplinary innovative basic and applied research in biology and medicine.

The diversity of researchers in the Spec Lab is an important aspect of its interdisciplinary and collaborative work. Students and staff from many countries, backgrounds, and races stimulate and enhance the Spec Lab's environment. By nurturing diversity in a variety of ways, the Spec Lab helps MIT to increase and support more diversity in the academic and scientific and engineering professions. The Spec Lab will continue and expand its efforts in this area.

THE SPECTROGRAPH

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Assoc. Director for Scientific Coordination:

Robert W. Field

Associate Director:

Ramachandra R. Dasari

The Spectroscopy Laboratory houses two laser research resource facilities. The MIT Laser Research Facility provides shared facilities for core researchers to carry out basic laser research in the physical sciences. The MIT Laser Biomedical Research Center, a National Institutes of Health Biomedical Research Technology Center, is a resource center for laser biomedical studies. The LBRC supports core and collaborative research in technological research and development. In addition, it provides advanced laser instrumentation, along with technical and scientific support, free of charge to university, industrial, and medical researchers for publishable research projects. Call or write for further information or to receive our mailings.

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<http://web.mit.edu/spectroscopy>

There are twelve refurbished and new laboratories located on the ground floors of Bldg 6 and the new Green Building (6C). Each laboratory has been designed to meet the needs of an individual research group and to enhance the multi-disciplinary interactions among Spec Lab students and staff members from different groups and departments. These labs occupy more than 8,000 square feet and provide facilities for wet chemistry and cell-biology preparations. The labs provide the resources to:

- Study quantum dots as probes for imaging biological micro-environments
- Synthesize and characterize carbon nanotubes using Raman spectroscopy and other techniques
- Use ultra-fast two-dimensional infrared spectroscopy to probe molecular dynamics in condensed phase systems
- Use photo-acoustic spectroscopy to study thin films and materials
- Train high school students and teachers in thin-film dynamics and other areas (Outreach Laboratory)
- Study combustion dynamics
- Study stochastic gene expression and related processes (biophysics laboratory and cell preparation facility)
- Develop techniques and instruments for spectral diagnosis and imaging of disease
- Develop and apply instruments for phase and tomographic microscopy of live cells, small organisms, and tissues.

All the staff and faculty of the Spec Lab are heartened by the forward looking decision of the Institute to upgrade and modernize the Spectroscopy Laboratory. We are grateful to President Hockfield, Provost Reif, and Associate Provost Claude Canizares for their support. Dean Robert Silbey's leadership and support for the Spec Lab throughout the PDSI project is gratefully acknowledged. We especially thank the numerous benefactors who contributed to the PDSI project in general, and to the Spectroscopy Laboratory construction in particular. Our thanks go also to the many staff members of the Department of Facilities, led by John Hawes, who worked on this project.

Spec Lab professors and staff and students - especially Luis Galindo and Geoff O'Donoghue, Spec Lab engineers, and Ramachandra Dasari, the lab's Associate Director - worked hard and long to design, organize and move into the new space. All these efforts will let us work together and

build on the achievements of the past to realize wonderful opportunities for new advances in basic research and applications of spectroscopy for societal benefit.



Spec Lab creates 3D images of living cell

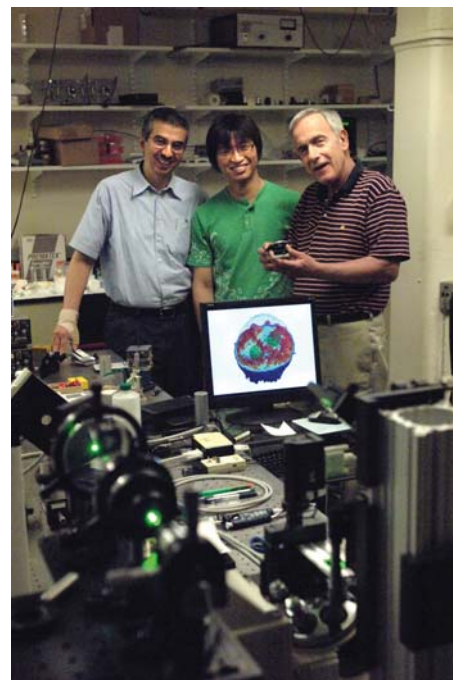
Anne Trafton, MIT News Office

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A new imaging technique developed in MIT's Harrison Spectroscopy Laboratory has allowed scientists to create the first 3D images of a living cell, using a method similar to the x-ray CT scans doctors use to see inside the body.

The technique, described in a paper published in the Aug. 12 online edition of *Nature Methods*, could be used to produce the most detailed images yet of what goes on inside a living cell without the help of fluorescent markers or other externally added contrast agents, said Michael Feld, Director of the George R. Harrison Spectroscopy Laboratory and a professor of physics.

"Accomplishing this has been my dream, and a goal of our laboratory, for several years," said Feld, senior author of the paper. "For the first time the functional activities of living cells



Asst. Prof. Dr. Kamran Badizadegan, Postdoctoral Associate Wonshik Choi, and Prof. Michael Feld display their new apparatus / Photo Donna Coveney

can be studied in their native state.”

Using the new technique, the Spec Lab team has created three-dimensional images of cervical cancer cells, showing internal cell structures. They’ve also imaged *C. elegans*, a small worm, as well as several other cell types.

The researchers based their technique on the same concept used to create three-dimensional CT (computed tomography) images of the human body, which allow doctors to diagnose and treat medical conditions. CT images are generated by combining a series of two-dimensional x-ray images taken as the x-ray source rotates around the object.

“...For the first time the functional activities of living cells can be studied in their native state...”

“You can reconstruct a 3D representation of an object from multiple images taken from multiple directions,” said Wonshik Choi, lead author of the paper and a Spectroscopy Laboratory postdoctoral associate.

Cells don’t absorb much visible light, so the researchers instead created their images by taking advantage of a property known as refractive index. Every material has a well-defined refractive index, which is a measure of how much the speed of light is reduced as it passes through the material. The higher the index, the slower the light travels.

The researchers made their measurements using a technique known as interferometry, in which a light wave passing through a cell is compared with a reference wave that doesn’t pass through it. A 2D image containing information about refractive index is thus obtained.

To create a 3D image, the researchers combined 100 two-dimensional images taken from different angles. The resulting images are essentially 3D maps of the refractive index of the cell’s organelles. The entire process took about 10 seconds, but the researchers recently reduced this time to 0.1 seconds.

The team’s image of a cervical cancer cell reveals the cell nucleus, the nucleolus and a number of smaller organelles in the cytoplasm. The researchers are currently in the process of better characterizing these organelles by combining their technique with fluorescence

microscopy and other approaches.

“One key advantage of the new technique is that it can be used to study live cells without any preparation,” said Kamran Badizadegan, principal research scientist in the Spectroscopy Laboratory and assistant professor of pathology at Harvard Medical School, and one of the authors of the paper. With essentially all other 3D imaging techniques, the samples must be fixed with chemicals, frozen, stained with dyes, metallized or otherwise processed to provide detailed structural information.

“When you fix the cells, you can’t look at their movements, and when you add external contrast agents you can never be sure you haven’t somehow interfered with normal cellular function,” said Badizadegan.

The current resolution of the new technique is about 500 nanometers, or billionths of a meter, but the team is working on improving the resolution. “We are confident that we can attain 150 nanometers, and perhaps higher resolution is possible,” Feld said. “We expect this new technique to serve as a complement to electron microscopy, which has a resolution of approximately 10 nanometers.”

Other authors on the paper are Christopher Fang-Yen, a former postdoctoral associate; graduate students Seungeun Oh and Niyom Lue; and Ramachandra Dasari, principal research scientist at the Spectroscopy Laboratory.

The research was conducted at MIT’s Laser Biomedical Research Center and funded by the National Institutes of Health and Hamamatsu Corporation. ✨

Townes, continued from page 1

wasting his time trying to make a maser, he persisted. When he set out to look for evidence of molecules in space, again important physicists told him the search was futile; again he persisted. The moral he drew: Use your own judgment to choose your research goals; then pursue them with courage, determination, and persistence.

Both Michael Feld, who was Javan’s student, and Ramachandra Dasari, who spent two years as a visiting fellow with Javan’s and Towne’s research group, recalled the years Townes was at MIT as particularly exciting. The memories they shared with the speaker and the audience gave special warmth to the occasion. “It was for me a great joy to have Charlie Townes be the first Dasari Lecturer,” said Dr. Dasari. ✨

Personality

Luis Galindo

By Mei-Hui Liu



Luis Galindo holds a probe in his optical fiber research facility

Luis Galindo attended National University of Colombia in Colombia as an undergraduate, earning a B. S. in Engineering in 1979. He came to the U. S. in 1982 and has lived in Massachusetts since then. Over the years he has earned a technical degree in Computers and Electronics in the U. S. and in Industrial Robotics in Germany. He has worked at the Spectroscopy Laboratory since 1997. Before that he traveled around the world working as a service engineer for Spectro Analytical Instruments, a German company that manufactures optical emission and x-ray spectrometers. After two years in Mexico as a service manager, he came back to the U. S. as a technical manager for Latin America and the Caribbean.

At the Spectroscopy Laboratory, Luis’s primary function is to build Raman, MMS and FastEEM probes. He has been involved in the design and development of the Raman probe for many years. He also fabricates micro optics for the Raman and MMS probes. Luis performs administrative duties and helps with mechanical, electrical, and electronic development of clinical instruments.

Luis enjoys working at the Spec Lab. He declared, “It has been a great experience, the experience of a lifetime, working here. There is not a day goes by without learning something. What I like most are the challenges that come my way and that I am able to meet. I hope it stays that way always.” Luis also enjoys spending time at home with his wife Marilyn, a native of Puerto Rico, and his 6 children (3 daughters and 3 sons), and 9 grandchildren. ✨

Research Report

Investigating the mechanism of hydrogen bond exchange in water by ultrafast two-dimensional infrared spectroscopy

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Among liquids, water is exceedingly complex to describe because its ability to form up to four hydrogen bonds results in an extended tetrahedral network of molecules that is highly structured for a liquid. Indeed, many of water's anomalous properties, such as the lower density of ice relative to the liquid and its high heats of melting and vaporization, are direct consequences of the time averaged structure of water's hydrogen bonding network. Aqueous reactivity, however, is largely dictated by the time dependent fluctuations and distortions of this network. Water has the ability to rapidly solvate nascent charge because water molecules can quickly rearrange their structure around a solute.¹ Moreover, the transport of excess protons and proton holes, which exhibit anomalously fast diffusion due to their ability to hop from one water molecule to another, is thought to be gated by the formation and breakage of hydrogen bonds.² Unfortunately, a mechanistic understanding of the dynamics of water's hydrogen bonding network is lacking largely because there are no structurally sensitive experimental techniques with the time resolution to resolve the fluctuations of water's hydrogen bond network.

To monitor water's evolving structure, we employ ultrafast infrared spectroscopy of the OH stretch of a solution of dilute HOD in D₂O. The OH stretch is significantly broadened due to a distribution of hydrogen bonding structures present in the liquid. The formation of a strong hydrogen bond results in a weakening of the OH force constant, and hence a large downshift (~ 500 cm⁻¹) of the OH stretching frequency, ω_{OH} , relative to the gas phase value of 3707 cm⁻¹ occurs. Likewise, molecules that only participate in weak or broken hydrogen bonds are only slightly red shifted (~ 100 cm⁻¹) relative to gas

phase and appear on the high frequency side of the OH lineshape. The structural sensitivity of ω_{OH} has been confirmed by recent molecular dynamics (MD) simulations showing that ω_{OH} is well correlated with the oxygen-oxygen separation of a hydrogen bonded pair of molecules.³⁻⁵ Moreover, the simulations found that ω_{OH} is best correlated with the projection onto the OH bond of the electric field of the surrounding liquid located at the proton.³ This field is due in large part to the nearest neighbor of the proton, showing that ω_{OH} is a strong probe of local structure.

Previous measurements by our group have characterized the time scales for the fluctuations and reorganization of water's hydrogen bonding network.^{6,7} The results of three-pulse echo peakshift (3PEPS) and polarization dependent pump probe measurements are displayed in Fig. 1. Both measurements show initial fast decays due to local fluctuations about the proton of HOD molecules followed by slower, long lived decays that are attributed to the global reorganization of the liquid. In the case of the 3PEPS measurement, which is primarily sensitive to spectral diffusion, an initial 60-fs decay is followed by a recurrence at 160 fs due to an underdamped O-H...O stretching motion (hydrogen bond vibration) that rapidly modulates ω_{OH} . This is followed by a 1.2-ps decay due to the global reorganization of the liquid, which includes the exchange of molecules into and out of the HOD solvation shell. Likewise, the anisotropy measurement, which describes the reorientation of HOD molecules, shows a fast 50-fs decay due to intermolecular librations (hindered rotations) followed by diffusive reorientation on a 3-ps timescale.

Although these experiments can measure the time scales for the evolution of water's structure, they cannot describe the underlying mechanism leading to these changes because they average over all hydrogen bonding environments. Two-dimensional infrared-spectroscopy measurements (2D IR) do not average over hydrogen bonding environments, and allow us to track how different hydrogen bonding environments interconvert over time. A 2D IR spectrum correlates how a molecule at an initial frequency (ω_1) evolves to a final frequency (ω_2) after a given waiting time (τ_2). By recording 2D spectra for a series of τ_2 values, we can track how water molecules initially in non hydrogen bonded configurations find hy-

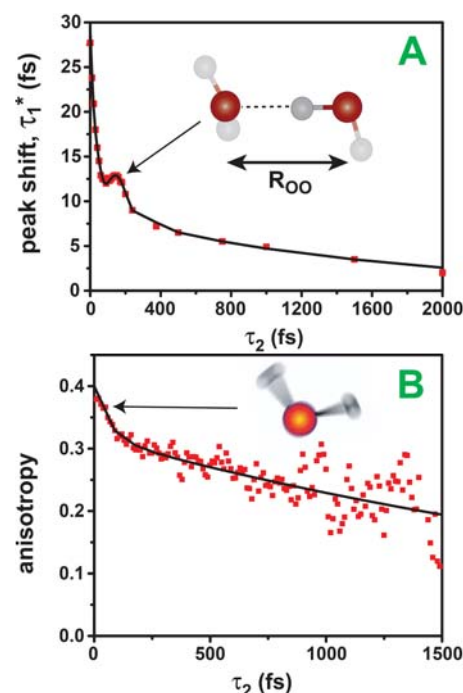


Figure 1: 1D plots showing the time scales for reorganization of water's hydrogen bonding network. A) 3PEPS measurement displaying an underdamped hydrogen bond stretch. B) Pump probe anisotropy capturing fast librational motion. From Ref. 6.

drogen bonding partners and vice versa.

In practice, a 2D IR spectrum is obtained by irradiating a sample with three input laser fields in a boxcar geometry. The signal field that is emitted into the fourth corner of the box is then measured via interferometric detection using a known reference field so that both the amplitude and phase of the signal field can be determined. In the experiments shown below, 2D IR surfaces of a $\sim 1\%$ HOD in D₂O solution flowed as a 50- μm thick liquid jet were measured using 45-fs pulses centered at 3400 cm⁻¹. These pulses support enough spectral bandwidth to span the broad OH stretching lineshape as well as part of its anharmonically shifted overtone. They were generated using a home built 3- μm OPA pumped by the output of a Femtolasers Femtopower Pro Ti:Sapphire multipass amplifier. The output of the OPA is fed into a Mach-Zehnder interferometer that splits the initial pulse into five independently controllable pulse replicas. Three of the replicas form the boxcar used to excite the sample: one acts as the reference field used to detect the signal; one acts as a tracer that follows the path traveled by the signal field; one is used for the alignment of detectors and is blocked during the experiment. Specific details regarding this setup are available elsewhere.^{8,9}

A waiting time series of 2D IR spectra

Ultrafast, continues on page 6

Tomographic phase microscopy

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Introduction

For visualizing transparent biological cells and tissues, the phase contrast microscope, with its related techniques, is the principal tool of nearly every cell biology laboratory. However, phase contrast methods are inherently qualitative and lacking in 3-D imaging capability. We describe here a novel tomographic microscopy for quantitative three-dimensional mapping of the refractive index in live cells and tissues using a phase-shifting laser-interferometric microscope with variable illumination angle.

Refractive index as an intrinsic source of contrast in light microscopy

Although most biological cells and tissues exhibit negligible absorption under visible light illumination, organelles show distinctive differences in refractive index, that amount to several percent of the mean refractive index. For this reason, in biological studies the refractive index can be a much better source of intrinsic contrast than absorption.

Local variations of refractive index in the specimen induce different phase delays from point to point in the field of view. These are used to image and visualize biological structures by such techniques as phase-contrast microscopy, differential interference-contrast (DIC) microscopy and quantitative phase microscopy.¹⁻³ These techniques are sensitive enough to easily resolve the half-radian phase delay typically induced by a single cell. However, the phase delay is proportional to the product of refractive index and path length or, more generally, the convolution of the refractive index with the point spread function of the optical system. Thus, phase microscopy techniques provide neither a 3-D image of the cell nor a 3-D map of the refractive index distribution.

One strategy for 3-D determination of refractive index is based on measurement

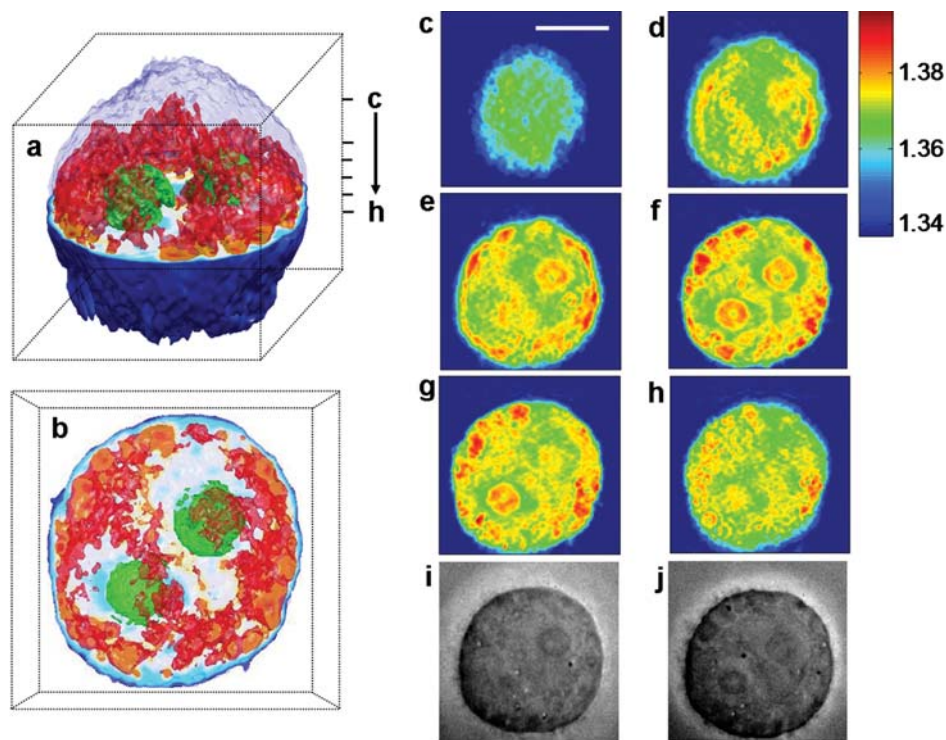


Figure 1: Refractive index tomogram of a HeLa cell. (a) 3-D rendered image of a HeLa cell. The outermost layer of the upper hemisphere of the cell is omitted to visualize the inner structure. Nucleoli are colored green and parts of cytoplasm with refractive index higher than 1.36 are colored red. The dotted box is a cube of side 20 μm. (b) Top view of (a). (c)-(h) Slices of the tomogram at heights indicated in (a). Scale bar, 10 μm. The color bar indicates the refractive index at $\lambda = 633$ nm. (i) and (j) Bright field images for objective focus corresponding to (e) and (f), respectively.

of projections of refractive index in multiple directions, in analogy to computed x-ray tomography in which the projection of absorption is measured. Projections of refractive index have been performed via a number of quantitative phase microscopy techniques, and earlier studies used beam rotation⁴ or sample rotation⁵ to form tomographic images. However, one case provided no quantitative information about the index of refraction,⁴ and the other required glycerol immersion of the sample and physical rotation of the sample in a micropipette.⁵

CT scan with biological cells and multicellular organisms

We have developed a technique for quantitative, high-resolution 3-D measurements of the refractive index of suspended or substrate-attached cells and multicellular organisms with no need for disturbing the sample or immersing it in special media.⁶

For near plane-wave illumination of a thin sample with small contrast of index of refraction, the phase of the transmitted field is to a good approximation equal to the line integral of the refractive index along the path of beam propagation. Therefore, the phase image can be interpreted simply as the projection of refractive index, analogous to the projection of absorption in x-ray tomography. If

we take many angular projection phase images over a wide range of angles, we can reconstruct a 3-D map of refractive index of the sample with an algorithm similar to that used in x-ray tomography.

In obtaining a set of angular projections of phase images, we change the direction of the illuminating beam rather than rotating the sample. This leaves the sample unperturbed during the measurement, which is critical for a biological specimen, and enables a fast dynamic study as well. A novel heterodyne laser interferometric microscopy⁷ quantitatively images the phase delay induced by the sample, and a galvanometer scanning mirror changes the direction of illumination. Illumination angles are limited to $|\theta| < 60$ degrees by the numerical aperture of condenser and objective lenses. It takes about 10 sec to cover the entire range of angles in steps of 1.2 degree.

To reconstruct a 3-D refractive index tomogram from the projection phase images, we applied a procedure based on the filtered back-projection method.⁸ A discrete inverse Radon transform was applied to every X- θ slice in the beam rotation direction, with X the coordinate in the tilt direction and θ the angle of the illuminating beam relative to the optic axis of the objective lens. An X-Z slice is re-

Tomography, continues on page 6

constructed from an X- θ slice. By merging all the X-Z slices at every pixel in the Y-direction, we can get the 3-D map of refractive index. To verify that our instrument can determine the refractive index with an accuracy of 0.001, we imaged 10- μm polystyrene beads (Polysciences #17136, $n=1.588$ at $\lambda=633$ nm). We estimate the spatial resolution of our tomography technique to be approximately 0.5 μm in the transverse (x-y) directions and 0.75 μm in the longitudinal (z) direction.

We imaged single HeLa cells in culture medium. Cells were dissociated from culture dishes and allowed to partially attach to the coverslip substrate. A 3-D tomogram of the index of refraction of a single cell (Fig. 1a,b) and x-y tomographic slices of the same cell at heights of $z = 12, 9.5, 8.5, 7.5, 6.5$ and 5.5 μm above the substrate (Fig. 1c-h) show that the index of refraction is highly inhomogeneous, varying from 1.36 to 1.40. Bright field images for objective focus corresponding to Fig. 1e-f are shown in Fig. 1i-j, respectively. There is a clear correspondence between the tomographic and bright field images in terms of cell boundary, nuclear boundary, and size and shape of the nucleoli.

Note that the refractive index of the nucleus ($n \approx 1.36$), apart from the nucleolus, is smaller than some parts of the cytoplasm ($n \approx 1.36$ -1.39) and that the refractive index of the nucleoli, $n \approx 1.38$, is larger than that of the rest of the nucleus. This is contrary to the widely cited claims that the refractive index of the nucleus as a whole is higher than that of the rest of the cell.⁹ Similar results were obtained for cultured HEK 293 cells, B35 neuroblastoma cells, and primary rat hippocampal neurons. All cells imaged contained many small cytoplasmic particles with high refractive index. These particles may be lipid droplets, lysosomes, vacuoles, or other organelles.

To demonstrate tomographic imaging of a multicellular organism, we imaged the nematode *C. elegans*. Worms

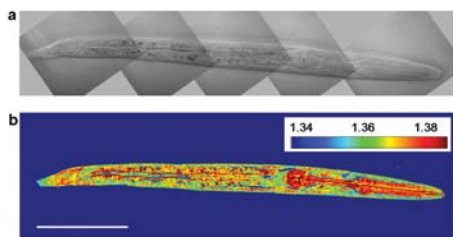


Figure 2: Bright field image (a) and slice of index tomogram (b) of the nematode *C. elegans*. Anterior is to the right. Scale bar, 50 μm . The color bar indicates the refractive index at $\lambda = 633$ nm.

were paralyzed with 10 mM sodium azide in NGM buffer and imaged in the same solution. Overlapping tomograms were created and the resulting data assembled into a mosaic (Fig 2). Several internal structures are visible, including a prominent pharynx and digestive tract.

In summary, we have developed a technique for quantitative refractive index tomography of living cells and tissues. We note that the 3-D structure mapped by tomographic phase microscopy can complement the images revealed by techniques such as hematoxylin and eosin staining. Refractive index data can be used to study light scattering properties of cells and tissues and characterize sample-induced aberrations in microscopy. Characterization and correction of such aberrations may be particularly important for modern super-resolution techniques such as STED and structured illumination microscopy. ✨

Acknowledgements

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Dresselhaus wins

2008 Buckley Award

MIT Institute Professor and Spectroscopy Laboratory core researcher **Mildred Dresselhaus** is the 2008 recipient of the Oliver E. Buckley Condensed Matter Prize from the American Physical Society. Professor Dresselhaus was cited, “for pioneering contributions to the understanding of electronic properties of materials, especially novel forms of carbon.” The prize is named after Oliver H. Buckley, an influential president of Bell Labs, and the first prize recipients were Nobel prize winners William Shockley in 1953 and John Bardeen in 1954. ✨

of HOD in D_2O is shown in Fig. 2a. Two peaks appear in the spectrum, a positive peak centered along the diagonal axis due to photobleach of the $\nu = 0 \rightarrow 1$ transition and a second anharmonically shifted negative peak due to the photoinduced

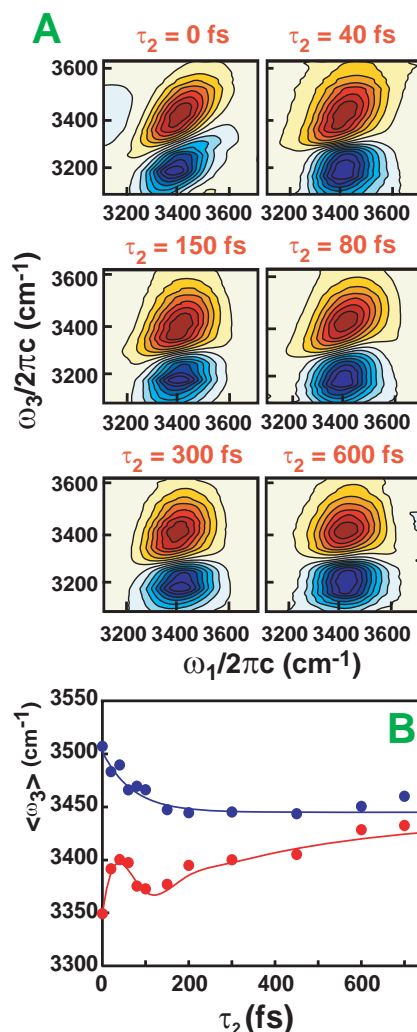


Figure 2: A) A waiting time series of 2D IR data for the OH stretch of dilute HOD in D_2O . B) The first moments of vertical slices taken at $\omega_1 = 3280$ cm^{-1} and 3530 cm^{-1} as a function of ω_2 . From Ref. 11.

absorption associated with the $\nu = 1 \rightarrow 2$ overtone transition. Various changes to the surfaces signaling the loss of frequency memory can be seen as τ_2 increases, including broadening of the surfaces along the anti-diagonal direction and a decrease of the slope of the node separating the fundamental and overtone transitions. This loss of correlation can be quantified by a number of different metrics,¹⁰ and the time scales for the loss of frequency correlation agree well with the results of our previous 3PEPS measurement.⁹

More intriguing are the frequency dependent changes to the 2D lineshape with

waiting time. Noticeably, the antidiagonal linewidth for slices taken on the high frequency (blue) side of the 2D surface are nearly twice as large as those taken at the low frequency (red) side, indicating that hydrogen bonded and non hydrogen bonded molecules experience different fluctuations.¹¹ Examining vertical slices taken on the blue (red) side of the lineshape as a function of τ_2 corresponds to watching molecules in broken or weak (strong) hydrogen bonds relax back to the band center. Fig 2b. shows the first moment for vertical slices taken at $\omega_1 = 3280 \text{ cm}^{-1}$ and 3530 cm^{-1} as a function of τ_2 . For slices taken on the red side of the lineshape, a recurrence is seen which corresponds to the hydrogen bond vibration seen in our previous 3PEPS measurement. However, slices on the blue side of the lineshape quickly relax to band center within 100 fs. This relaxation rate is faster than the characteristic time scale for the intermolecular motions of the liquid (~ 200 fs) which strongly suggests that configurations corresponding to broken hydrogen bonds are created by transient fluctuations of the liquid and do not occupy a stable minimum on water's free-energy surface. Moreover, the ~ 60 -fs decay of the first moment for ω_1 slices on the blue side suggests that it is most likely librations that shuttle water molecules in and out of hydrogen bonds.

The hypothesis that hydrogen bond rearrangement involves the concerted motion of water molecules is not a new one. It dates back to Stillinger, who coined such a mechanism as a "switching of allegiances."¹² However, there has been little experimental work to confirm this hypothesis. Evidence in support of this mechanism comes from recent MD simulation work by the Hynes group¹³ whose findings suggest that hydrogen bond exchange is initiated when a hydrogen bond acceptor becomes overcoordinated and a nearby potential hydrogen bond acceptor is undercoordinated. Once this occurs, a water molecule bound to the overcoordinated molecule can break its hydrogen bond and form a new one to the undercoordinated molecule via a large amplitude rotation. An illustration of this mechanism is displayed in Fig. 3, which shows snapshots during a hydrogen bond exchange event taken from a classical MD simulation employing the SPC/E water potential.¹⁴ As the water molecule pictured on the bottom of the figure rotates one of its OH bonds away from the mol-

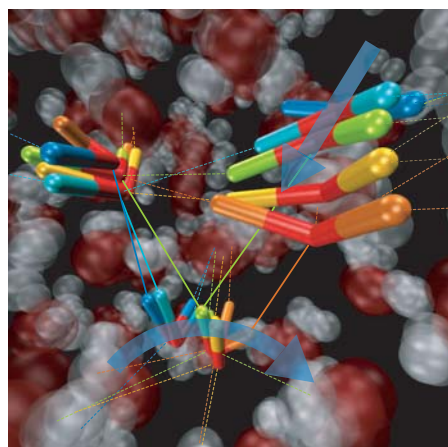


Figure 3: Snapshots from a 288-fs sequence in which a water molecule (bottom) exchanges hydrogen bonding partners (upper left: original hydrogen bond acceptor; upper right: new hydrogen bond acceptor). Hydrogen bonds are color coded, and illustrate the rotation from the initial geometry (blue) to a bifurcated state (green) and finally to a new hydrogen bonded configuration (orange). From Ref. 14.

ecule on the left, the molecule on the right slides toward the exchanging molecule to form a new hydrogen bond. The amount of time the exchanging molecule spends as a "broken hydrogen bond" is minimal.

The experiments described above allow us to gain a glimpse of water's rapidly evolving structure. The results of these experiments indicate that stable dangling hydrogen bonds are exceedingly rare in aqueous solution. This supports a picture within which water molecules that break a hydrogen bond quickly reorient to a new hydrogen bonding partner by the concerted motion of multiple molecules. Currently, we are working to further test this hypothesis by measuring a 2D IR anisotropy that will allow us to determine if there is any frequency dependence to the reorientation of water molecules. Also, we are currently exploring how the fluctuations of water's hydrogen bonding network influence the transport of protons in alkaline solution. ✨

Acknowledgements

This work was supported by the Department of Energy, the MIT Laser Research Facility, and the David and Lucile Packard Foundation.

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Dasari Spectroscopy Laboratory Lectureship



In honor of the contributions of Ramachandra Rao Dasari an endowed fund, the Dasari Spectroscopy Laboratory Lectureship, has been established at MIT. This fund will support an annual event in which a prominent scientist associated with the Spectroscopy Laboratory presents a lecture at MIT. Income from the endowment will be used for travel expenses, an honorarium, and a dinner in honor of the speaker.

Ramachandra Rao Dasari was born and educated in India. He joined the Physics faculty at the Indian Institute of Technology, Kanpur in 1962. Ramachandra came to MIT in 1966 as a fellow for two years working in the newly formed group of Charles Townes and Ali Javan. He subsequently returned to IIT-K and collaborated with Putcha Venkateswarlu to build one of the largest laser laboratories for university research in India. During his 17 years at IIT-K, Ramachandra trained a large number of Ph.D. students and established relationships between IIT-K and several national laboratories. As a physics panel

Dasari, continues on page 15

Seminar on Modern Optics and Spectroscopy

Fall Semester 2007

- September 25** Changhuei Yang, California Institute of Technology
Lighting ways in biomedicine
- October 2** David Snoke, University of Pittsburgh
Bose-Einstein condensation of polaritons in microcavities
- | | |
|------------------|--|
| October 9 | <i>1st Annual Dasari Lecture</i>
Charles Townes, University of California Berkeley
The fun of a physics career |
|------------------|--|
- October 16** Federico Capasso, Harvard University
Advances in quantum cascade lasers
- November 6** Stephen Coy, Sionex Corporation
The power of differential ion mobility
- November 13** Richard Averitt, Boston University
Active terahertz metamaterials
- November 20** Obrad Scepanovic, MIT
Multimodal spectroscopy of vulnerable plaque
- November 27** Charles Lin, Massachusetts General Hospital
In vivo cell tracking
- December 4** David De Mille, Yale University
Ultracold molecules
- December 11** Hyunbin Son, MIT
Raman spectroscopy of single-wall carbon nanotubes

Tuesdays, 12:00 - 1:00 p.m., Grier Room (34-401)

Refreshments served following the seminar.

Sponsored by the George R. Harrison Spectroscopy Laboratory, Department of Electrical Engineering and Computer Science, and School of Science, MIT

Lester Wolfe Workshop in Laser Biomedicine

Frontiers in Modern Microscopy

Tuesday, November 20, 2007, 1:00-6:00 pm
Massachusetts Institute of Technology
Grier Room 34-401
50 Vassar St, Cambridge, MA

Making light work in microscopy

Tony Wilson, Oxford University

Field-based tomographic microscopy

Wonshik Choi, Massachusetts Institute of Technology

Imaging cellular structures and molecules with X-ray tomography

Carolyn Larabell, UCSF and Lawrence Berkeley National Laboratory

Super-resolution optical microscopy with STORM

Xiaowei Zhuang, Harvard University and Howard Hughes Medical Institute

3D Optical coherence phase microscopy

Johannes de Boer, Massachusetts General Hospital

STED microscopy

Katrin Willig, Max Planck Institute for biophysical Chemistry, Göttingen

Nonlinear microscopy in local optical fields

Katrin Kneipp, Harvard University Medical School and Massachusetts General Hospital

Refreshments served at 3:30 pm

Sponsored by:

G. R. Harrison Spectroscopy Laboratory, MIT

Massachusetts General Hospital Wellman Center for Photomedicine

Harvard-MIT Division of Health Sciences and Technology and Center for the Integration of Medicine and Innovative Technology

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DASARIFEST 2007

July 14 and 15, 2007 colleagues, friends, family, and admirers celebrated Ramachandra Dasari's 75th birthday and his 27 years as Associate Director of the Spectroscopy Laboratory with a symposium, dinner, and a birthday party.



Suhasini, Ramachandra, and family



L to R: Michael Feld, Obrad Scepanovic, and Kate Bechtel empower Ramachandra with a bullhorn. Suhasini admires his spectrograph, custom-made by Seungeun Oh.



L to R: Kamran Badizadegan, Ramachandra, N.R. Desai, Ali Javan, Gabi Popescu, Alison Hearn, and Michael Feld.



Ramachandra with friend and mentor Ali Javan



Ramachandra at the symposium in his honor



Mike Otteson applauds The Spectratones: (L to R) Gajendra Singh, Seungeun Oh, Michael Feld, Obrad Scepanovic, Zoya Volynskaya, Geoff O'Donoghue, Kate Bechtel, Gabi Popescu, and Alison Hearn



Ramachandra in cap of honor sharing garlands with Suhasini



Suhasini, Lakshmi, and Ramachandra with the Putcha family



Ramachandra with symposium speakers (L to R): Abi Haka, N.R. Desai, John Thomas, Jason Motz, Obrad Scepanovic, Richard Rava, Greg Schimkaveg, Takeshi Oka, Condon Lau, and Yongkeun Park

PLEASE POST

Director's Perspective

Should *The Spectrograph* feature original scientific research?

By Michael S. Feld

Our newsletter, *The Spectrograph*, has a longstanding tradition of featuring current, original scientific research by its faculty and affiliates. These articles keep current and former colleagues, affiliates, advisors, and friends of the G. R. Harrison Spectroscopy Laboratory abreast of our most recent advances and current directions. In addition, such articles help to accomplish the dissemination of our scientific advances as mandated by NIH for resources such as our MIT Laser Biomedical Research Center. *The Spectrograph* has never explicitly or implicitly suggested that these research reports are peer-reviewed or indexed publications, and neither the Spectroscopy Laboratory nor MIT holds copyright on them. Nevertheless, one of our recent manuscripts was rejected by an Optical Society of America (OSA) publication largely on the basis of the claim that some of the research findings had been previously published in *The Spectrograph*.

I emphatically share the concerns of the OSA Board of Editors about duplicate

“...I urge the OSA to clarify its policy on prior publications and... to prevent ad hoc interpretation of its policies...”

publications and self-plagiarism, but I strongly disagree that publication of preliminary research findings in *The Spectrograph* constitutes prior or duplicate publication. The OSA clearly indicates that a “manuscript must contain significant new content not previously published or submitted elsewhere for simultaneous consideration” (http://josaa.osa.org/submit/review/ethical_guidelines.pdf, accessed on 10/21/07). Furthermore, in an open letter to colleagues, the OSA Board of Editors clearly states that “duplicate submission is the submission of substantially similar papers to more than one journal” and that “self-plagiarism is the publication of substantially similar scientific content of one’s own in the same or different journals” (http://josaa.osa.org/submit/review/plagiarism_2005.pdf, accessed on 10/21/07). *The Spectrograph* neither meets the definition of a scientific “journal” nor are “man-

uscripts” “submitted” to *The Spectrograph* for “consideration” to be published.

The science community has long struggled to find the right balance between disseminating preprints of scientific research and avoiding duplicate publication and self-plagiarism. There is nearly unanimous agreement that detailed presentation of unpublished data in scientific meetings does not constitute prior publication, but there is no consensus or clarity regarding non-peer-reviewed distribution of original data in newsletter, laboratory websites, or even well-known and respected preprint servers that have a long tradition in the field of physics. While the OSA guidelines cited above do not address these issues at all, other scientific societies take somewhat contradictory positions. The American Association for the Advancement of Science clearly warns that “posting of a paper on the Internet may be considered prior publication that could compromise the originality of the *Science* submission, although we do allow posting on not-for-profit preprint servers in many cases” (<http://www.sciencemag.org/about/authors/faq/index.dtl>, accessed on 10/21/07). While most journals such as *Science* and *Nature* accept preprint dissemination of research data in recognized preprint servers, other societies such as the American Physiological Society take a much more strict position that “widely circulated, copyrighted, or archival reports, such as the technical reports of IBM, the preliminary reports of MIT, the institute reports of the US Army, or the internal reports of NASA” all constitute prior publication (<http://www.the-aps.org/publications/journals/apsethic.htm>, accessed on 10/21/07). In contrast, the American Physical Society (APS) places the primary emphasis on peer-review and states that “manuscripts submitted to the journals must contain original work which has not been previously published in a peer-reviewed journal, and which is not currently being considered for publication elsewhere” (<http://authors.aps.org/esubs/guidelines.html> accessed on 10/21/07). Similarly, the National Academy of Sciences considers bodies of work previously published if “they have appeared in sufficient detail to allow replication, are publicly accessible with a fixed content, and have been validated by review” (PNAS, Vol. 96, Issue 8, 4215, 1999). Specifically, the PNAS editorial policies state that “a summary of work in a review, a perspective, a commentary, a

newspaper or magazine article, or wherever does not constitute prior publication.”

While recognizing that online availability of *The Spectrograph* is a concern for some editors and editorial boards, I urge the OSA to clarify its policy on prior publications and to provide sufficient detail to prevent ad hoc interpretation of its policies by individual editors or reviewers. In addition, as a Fellow of the OSA, I call on the Society not to reduce the free exchange of scientific information by limiting longstanding, traditional ways by which we disseminate and discuss preprint research data in our discipline. ✨

Laser Biomedical Research Center outside projects

The Laser Biomedical Research Center (LBRC) encourages outside projects in various areas of laser biomedicine. The facilities of the LBRC, along with technical and scientific support, are made available on a time-shared basis free-of-cost to qualified scientists, engineers and physicians throughout the United States. Researchers use the LBRC’s resources to exploit laser-based spectroscopic techniques for medical applications such as the spectral diagnosis of disease, investigation of biophysical and biochemical properties of cells and tissues, and development of novel imaging techniques. For example, ongoing collaborations are using spectroscopic instruments developed at the LBRC to optically determine elastic properties of self-assembling biological springs. In another collaborative study, researchers are assessing sampling volume and thereby the purity content of pharmaceutical tablets using integrating sphere facilities.

Outside projects can be initiated by contacting Ramachandra Dasari, Associate Director of the Spectroscopy Laboratory. Once the scope of the project is defined, a Research Project Application must be filled out. Proposals must be concise and are evaluated by members of the LBRC’s scientific staff on the basis of scientific merit, originality, potential significance and compatibility with available equipment. The review process is rapid, and applicants are promptly notified of the decisions. Participation of researchers from the small business community and from colleges, universities and hospitals that have limited research facilities is encouraged. For further details visit the website at: <http://web.mit.edu/spectroscopy/facilities/guideline.html#Application>. ✨

Spectral Lines

More than a burner

by Stephen R. Wilk

Textron Defense Systems
Cambridge, MA



Stephen R. Wilk

Several years ago I ran across a cartoon by Sidney Harris, the scientifically-oriented artist whose work appears in venues such as *American Scientist* and *Scientific American*. It shows an obviously 19th century scene of a man in a top hat addressing another man at a table. "Bunsen," says the top-hatted man, "I must tell you how excellent your study of chemical spectroscopy is, as is your pioneer work in photochemistry – but what really impresses me is that cute little burner you've come up with."

I was amazed. I have to admit that all I really knew about Bunsen was that burner – I didn't even know his full name. I'd used that natural gas burner in college and high school, and (with uncles and cousins in chemistry) I knew of it well before that. But I never gave a thought to Bunsen's real work. This cartoon was the first I'd heard of Bunsen as a spectroscopist or electrochemist.

So what is the story on Bunsen, and what does he really have to do with that burner?

Robert Wilhelm Bunsen was born in 1811 in Göttingen, Germany, the youngest of the four sons of Christian Bunsen, professor of philology at the university there. Robert studied chemistry at Holzminden and Göttingen, then toured European laboratories for three years before returning to his home town to be a lecturer at the University of Göttingen before going on to positions at Kassel, Marburg, and finally Heidelberg.

He made a false start in organic chemistry, doing work on the arsenical compound cacodyl, but soon settled in inorganic chemistry. He lost an eye due to an explosion of cacodyl cyanide. For about

ten years he was heavily involved in the study of gases. Then Bunsen's studies evolved around galvanic batteries, and he invented the Bunsen battery, which used inexpensive carbon in place of platinum or copper as the negative pole. He used this in electrochemistry, producing sodium, aluminum, and other metals from their chlorides.

His work in spectroscopy, our reason for covering his work, did not begin until comparatively late, in the 1860s. He and his younger protégé Gustav Kirchhoff observed the colors and spectra of chemical salts, heated using an alcohol flame or a burner flame. They also observed that placing such a flame in a white light led to dark absorption bands. In addition, they produced spectra in the electrical spark discharge of a Ruhmkorff coil with a small sample of the material under test applied to its electrodes. Both the light and dark bands were characteristic of the metallic part of the salt, they found, and the test was sensitive enough to require only a tiny amount of the test material although it had to be highly purified to produce only the characteristic lines. They were able to identify cesium and rubidium from extremely small samples; subsequently they isolated larger quantities of the elements by more conventional means.

Bunsen was a dedicated teacher, lecturing for 100 hours each year through seventy four semesters. He designed and built his own apparatus and was particularly skilled at glassblowing. He was reputed to have tough skin on his hands, as he used to demonstrate by holding his hand in the flame of his burner, and by removing the lids of hot crucibles with his bare hands.

Bunsen never married. The story (probably not true) was told that, while a young man at Marburg, he had proposed to a young woman and had his suit accepted, but he got so absorbed in his study of cacodyl that he neglected to visit her for several weeks and became uncertain whether or not he had actually proposed. He visited her without apologizing for his absence and re-proposed with predictable results. Years later, Kirchhoff's wife asked Bunsen why he had never married. "Heaven forbid," he replied, "When I return at night, I should find an unwashed

child on each step."

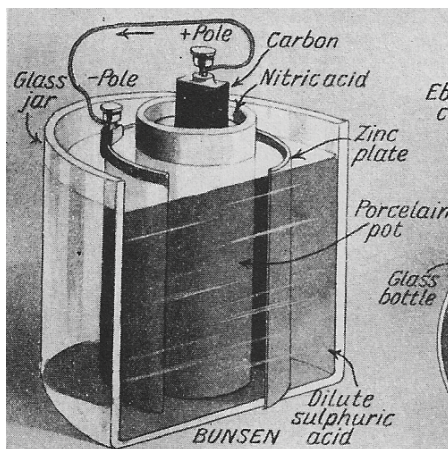
What about that famous burner? The one he used for his spectroscopic work, and with which he impressed his students by holding his hand in it, G. Gordon Liddy-like? Some people claim that he didn't invent it – that his technician did, or that he adapted it from Faraday, or that ironically in some other way he has been credited with one thing that he really didn't have much to do with. But Bunsen was reported to be a superb experimentalist, who invented the Bunsen battery, a much improved eudiometer for gas measurements, the grease-spot photometer for light measurements, the highly sensitive spectrometer he and Kirchhoff used in their measurements, an ice calorimeter, a vapor calorimeter, a thermopile, and a vacuum pump. He stressed the importance of building one's own experimental apparatus to his students and complained when they did not achieve proficiency at glassblowing.

All of which supports the notion that he improved the burner to achieve the one that now bears his name. Gas burners using the newly-available coal gas had been built by Michael Faraday in England and by Aimé Argand in Switzerland, which had a system of delivering the gas for

lighting before Germany did. Bunsen did not invent the gas burner, but I doubt if anyone ever claimed that he did. The gas system that came to operate in Germany apparently did not have the same flow rate as in England. Henry Roscoe, one of Bunsen's English students, brought a lamp called a "gauze burner" to Heidelberg, but at low flows it tended to go out. Bunsen experimented with different tube diameters, aided by his laboratory assistant, Peter Desaga, finally coming up with something very much like the present-day "Bunsen Burners", although it lacked the rotating ring that adjusted the flow of air to the flame.

But the burner Bunsen and Desaga modified did what it needed to do – it provided a colorless, sootless flame that was ideal for spectroscopic work and general laboratory applications. Bunsen himself did not patent the device. Desaga built many copies for the laboratory, then, with Bunsen's approval, he built more for

...(Bunsen) was reputed to have tough skin on his hands, as he demonstrated by holding his hand in the flame of his burner and removing the lids of hot crucibles with his bare hands...



A diagram of a bunsen battery

sale to other laboratories, calling them "Bunsen's Burners", and defending them against others who tried to file patents on the device.

Desaga's family continued in the scientific instrument business, and was still manufacturing the burners into the 1950s. The firm is still in business, but a perusal of its website fails to turn up any of the burners, many of which seem to be manufactured in Asia today. In the modern laboratory the burner uses not coal gas (made by heating coal or coke in air, producing a mixture which was about 50% hydrogen, with additions of carbon dioxide, carbon monoxide, methane, and nitrogen, the proportions depending upon the process used), but natural gas (methane, mostly, but with other gases present, including butane, propane, carbon dioxide, nitrogen, hydrogen sulfide, and even helium). That it was able to make the change is a fortuitous tribute to the man's inventiveness, guaranteeing that his name would live on, even among people unaware of his many other and arguably more significant accomplishments. ✨

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2. On the place of Bunsen and Kirchhoff in the history of spectroscopy, see William McGucken's *Nineteenth Century Spectroscopy*, Johns Hopkins Press, 1969, especially pp. 26-31.
3. The history of the Bunsen Burner gets treated at irregular intervals in *The Journal of Chemical Education*. See J. Chem. Ed. 9 1963-1969 (1932); 27(9) 514-515 (1950); 33 (1) 20-22 (1956); 77 (5) 558-559 (2000); 82 (4) 518 (2005). See also http://en.wikipedia.org/wiki/Bunsen_burner.

Dasari Lectureship, continued from page 3

member of the University Grants Commission, he helped initiate new programs, including teacher training workshops, to improve undergraduate education.

In 1979 Ramachandra, his wife Suhasini and his children moved to Canada to spend a year each at the National Research Council, Ottawa, and the University of British Columbia, Vancouver. Since 1980 Ramachandra has been Associate Director of the MIT Spectroscopy Laboratory.

Some highlights of his research are: at IIT-K, observing the first electronic spectrum of NSe and devising a new method for obtaining laser emission in copper vapor laser; at NRC, Canada, observing the Dicke narrowing in infrared transitions for

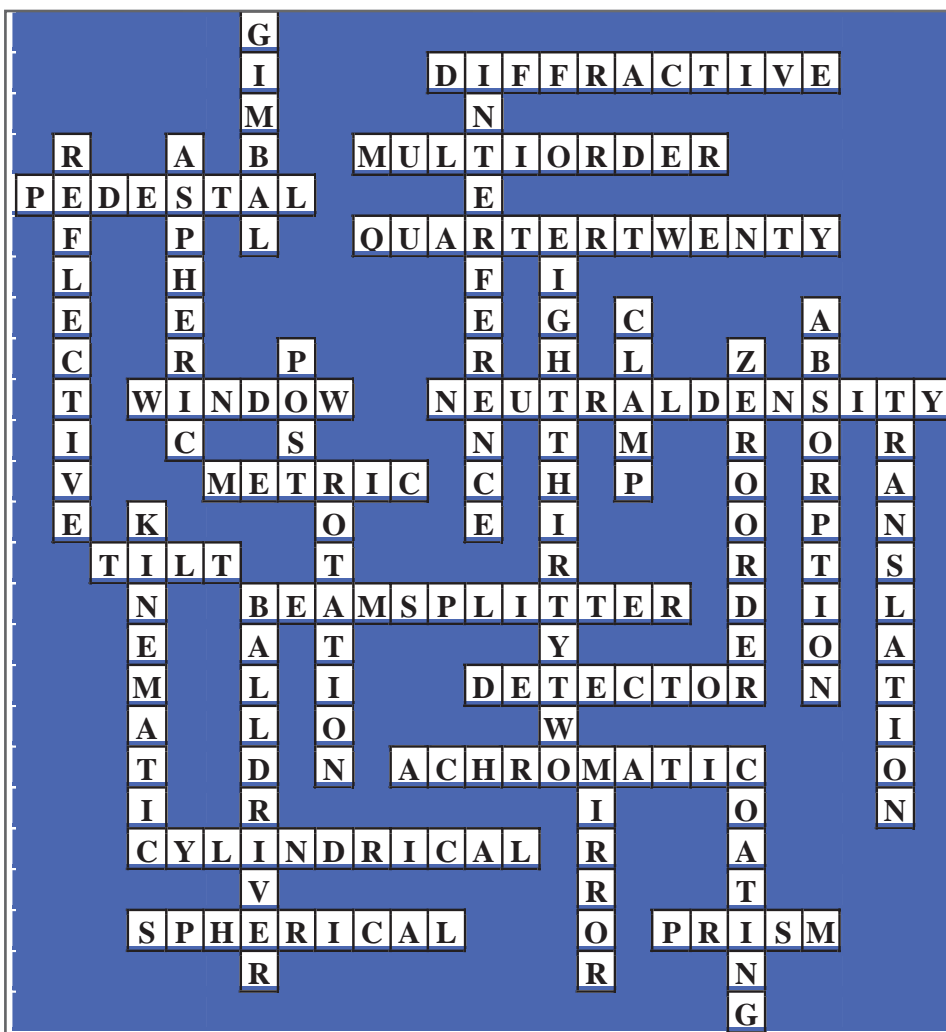
the first time; and at MIT, first measurement of laser frequencies in the far infrared; very high resolution study of N₂ laser transitions, the detection of anisotropy of gamma rays emitted from optically pumped radioactive rubidium atoms and development of the Laboratory's Raman facilities for biological and physical sciences.

His other contributions include overseeing project and facility development at the MIT Laser Biomedical Research Center, an NIH biomedical resource, and the MIT Laser Research Facility, a physical science resource. Ramachandra is a confidant to Spectroscopy Laboratory graduate students and professors, a project organizer and a troubleshooter. He is always there when needed. ✨

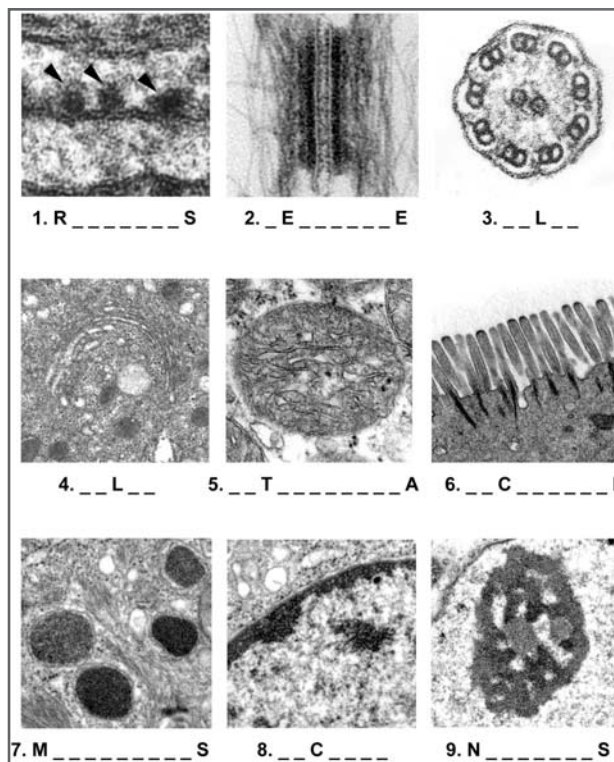
Answers to last issue's crossword

The winner of the Spring 2007 Spectrograph crossword challenge was Kate Bechtel! The winner of this issue's challenge will be published in the Spring 2008 issue of *The Spectrograph*.

Look for an interactive version of *The Spectrograph* crossword puzzle online at <http://web.mit.edu/newsoffice/2006/FamousOpticsPeople.html>



❄ The Back Page ❄



It pays to enrich your cell power!

These nine images, all transmission electron micrographs, show some classical cellular features. Magnification varies from image to image, but the features shown are all readily identifiable based on their distinctive shape and surroundings. Nevertheless, you may refer to the following clues to make sure you are on the right track.

1. These beads on a string (arrowheads) are brilliant translators of the genetic code.
2. Elmer's and Velcro are no match for this intercellular junction!
3. Their 9+2 arrangement of microtubules is reminiscent of centrosomes, but these apical membrane specializations are ideal transducers of mechanical force to or from the cell.
4. This delicate stack of membranes (crescent shaped, roughly between 11 and 4 o'clock) does as good a job in sorting, packaging and delivery as the Fed Ex!
5. Yet another precious gift from your mom!
6. All about surface area.
7. These membrane-bound structures can wreak havoc on your diffuse reflectance!
8. This giant organelle (occupying most of the image from 2 to 8 o'clock) is home to the structure shown in image 9.
9. Often considered an amorphous ball of stuff, this small organelle (~0.5-1.5 microns in size) often exhibits detailed internal structures.

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