Volume 17, Number 1 ◆ Fall 2001

**Research Report**

## Spectroscopic Dection of Desease Using Tri-Modal Spectroscopy

Irene Georgakoudi
G.R. Harrison Spectroscopy Laboratory, MIT

One important area of Spectroscopy Laboratory research focuses on the development of novel spectroscopic techniques for thebiophysical characterization of tissue. Such techniques have the potential to transform the field of medical diagnosis, offering powerful new means for quantitative tissue analysis, wide area surveillance, and biopsy guidance in a non-invasive way.

Here, we describe a recently developed method for analyzing tissue spectra, called tri-modal spectroscopy or TMS. TMS is the combination of three spectroscopic techniques which characterize different aspects of tissue biochemistry, structure and morphology. The objective is to detect very early pre-cancerous changes that are not easily detected with currently available technologies.

During standard endoscopic procedures, we acquire fluorescence spectra at eleven laser excitation wavelengths between 337 and 620 nm and one white light (350-750 nm) reflectance spectrum in less than one second. Light delivery and collection is mediated through an optical fiber probe. The acquired spectra contain information about the uppermost tissue layers, where almost 90% of cancers begin.

From the recorded fluorescence and reflectance spectra, we extract three types of spectroscopic information: intrinsic fluorescence, diffuse reflectance and light scattering. Intrinsic fluorescence spectroscopy (IFS) refers to the recovery of tissue fluorescence spectra that are free of distortions introduced by tissue scattering and absorption. To remove these distortions, we combine measured fluorescence and reflectance spectra using a photon-migration-based picture [1]. The extracted intrinsic fluorescence spectra are decomposed to provide quantitative information on the biochemical tissue composition and the changes that take place in pre-cancerous tissues (Fig. 1).

The measured reflectance spectra consist mainly of photons that are scattered many times before being detected. We use a model that is based on diffusion theory to describe the diffusely reflected light and, thus, to extract information about the absorption and the reduced scattering coefficients of tissue (diffuse reflectance spectroscopy or DRS[2]). The reduced scattering coefficient depends mainly on the morphology of the connective tissue, which provides structural support for the epithelium, the most superficial tissue layer. We observe consistent changes in the

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### ✹ Also in this issue…

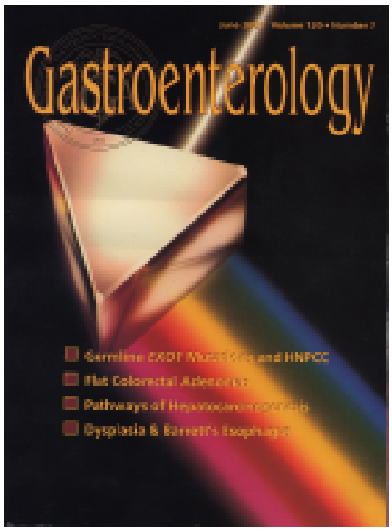


**Editorial**

## When Physicians Put Prisms on Their Journal Covers

Michael S.Feld
G.R.Harrison Spectroscopy Laboratory, MIT
Stephen F. Fulghum, Jr.
Newton Laboratories, Woburn, MA

The medical community is taking note of the field of spectral diagnosis of disease, as evidenced by the cover of the June issue of the clinical journal Gastroenterology, shown below. The issue contains an article about Spectroscopy Laboratory research demonstrating successful detection and diagnosis of invisible pre-cancer of the esophagus in patients undergoing endoscopy. The method, called tri-modal spectroscopy, combines techniques employing light scattering, diffuse reflectance and fluorescence. Similar results in the colon, the oral cavity, the bladder and the cervix have been reported

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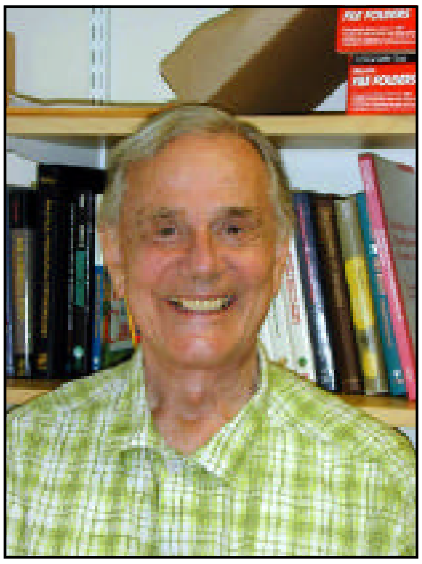
**Personalities**

## Charles W. Boone

Charles Boone grew up in West Los Angeles near the coastal city of Redondo Beach. "In those days there was no smog", he recounts, "the sand was warm, the waves thundering, and the skies cerulean blue". In his first year at Belmont High School, Chuck read "The Citadel", by A.J. Cronin, and "Arrowsmith", by Sinclair Lewis. "Both were romantic stories about a dedicated medical researcher", he says, "and they left me dreaming to be the same". Chuck majored in biology at the University of Texas, attended Harvard Medical School for two preclinical years, then transferred to the University of California Medical Center in San Francisco, where he finished his medical training and obtained a medical degree. In 1953 he completed a year of combined medical, surgical, and pediatric internship at Los Angeles City and County Hospital. Then, in pursuit of a career in medical research, Chuck took a year of graduate coursework in chemistry and mathematics at Caltech under Linus Pauling. "Those were heady days at Caltech", he says, "when the chemical structures collagen, sickle cell hemoglobin, and DNA were being worked out". He recounts how, in this pre-computer era, "sophisticated calculations derived from X-ray diffraction data were made by hand or with huge circular slide rules".

Chuck then transferred to the UCLA Medical Center, where he completed four years of residency training in pathology, becoming board certified in anatomic and clinical pathology. After another four years of graduate training to get a Ph.D. degree in biochemistry, he spent a postdoctoral year learning tissue culture methodology with Harry Eagle at the Albert Einstein College of Medicine in New York. Chuck then joined the Virus Cancer Program at the National Cancer Institute in Bethesda, Maryland, where he was chief of a cell biology section in the Laboratory of Viral Carcinogenesis. There he developed a tissue culture model of neoplastic transformation that could be used to quantitatively screen compounds for their anti-cancer effects.

In 1980, following a year refresher in surgical pathology at the Mayo Clinic, Chuck spent three years in Saudi Arabia as a diagnostic pathologist in a large tertiary care hospital. "It was a wonderful cultural adventure in the Arab world. Allah was everything," he recalls. "It was a vast eye-opener as far as alternative ways to live and think in this life." Chuck returned to the US to complete two years of training in Family and Community Medicine at the University of Maryland and the University of California at Irvine, then returned to Arabia "for another year of adventure" as a family physician attending personnel on an airbase of the Royal Saudi air force. "This time," he says, "I was lucky to visit one of the many palaces of the king of Saudi Arabia. Can you imagine a bathroom with fixtures of solid gold?"



**Charles Boone**

In 1989, Chuck returned to the National Cancer Institute to spend ten years as a program director in the Cancer Chemoprevention Program. In this capacity he implemented pioneering research in the use of computer-assisted image analysis to characterize cell and tissue structure at the micron level. Applying his knowledge of diagnostic histopathology to the measurement of precancerous human tissue (pre-invasive neoplasia), Chuck found a number of cancer-predictive structural changes within the cell nucleus that appear significantly earlier than the conventional changes ordinarily used by pathologists to diagnose cancer.

Two years ago, Chuck felt the call of Arabian culture once more, this time to Kuwait for three months as a medical supervisor in a government-sponsored weight control program. "About half of

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✹ **THE SPECTROGRAPH**

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Staff: Unhi Xu

✹ GEORGE R. HARRISON SPECTROSCOPY LABORATORY

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Associate Director for Scientific Coorination: Jeffrey I. Steinfeld

Associate Director: Ramachandra R. Dasari

The Spectroscopy Laboratory houses two laser research resource facilities. The MIT Laser Research Facility, supported by the National Science Foundation, 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, (617) 253-9774; **http://web.mit.edu/spectroscopy/www/**

**Research Report**

## Molecular Tagging Velocimetry: Combining Chemistry and Optical Spectroscopy to Study Fluid Flows

Scott E. Miller and Daniel G. Nocera
Department of Chemistry and G.R.Harrison Spectroscopy Laboratory, MIT

Research in the Nocera group focuses on the mechanisms of energy conversion in chemistry and biology. The group is well versed in synthetic methodologies spanning inorganic, organic, solid-state and biological chemistry. The coupling of time-resolved spectroscopies to the ability to make novel compounds uniquely poises us to make advances in the photocatalytic activation of small molecules, proton-coupled electron transfer, and physical and chemical sensing. A new physical sensing technique, invented by us to measure turbulence, will be described below. Before presenting this work, we will briefly describe some of our experimental capabilities.

Our spectroscopy facilities allow us to measure chemical and physical processes from the sub-picosecond to the millisecond time scale. The nanosecond system is based on a Coherent Infinity pulsed Nd:YAG laser running at 1-100 Hz. The infrared output of the YAG laser is frequency-tripled to 355 nm and used to pump an optical parametric oscillator (OPO), tunable from 450-720 nm. Typical output energies are 20-50 μJ/pulse across the visible spectrum. A nonlinear crystal allows the OPO output to be frequency doubled to the ultraviolet, further extending the range of excitation wavelengths available. Given the wide variety of compounds and materials studied, an easily tunable light source is an invaluable tool.

Two types of experiments are performed on the nanosecond system: emission lifetimes and transient absorption spectroscopy. Emission lifetimes to milliseconds are measured with a standard photomultiplier tube. Transient absorption difference spectra are obtained with the help of a gated, intensified CCD camera from Andor Technologies. A continuous wave Xe arc lamp provides the probe light, and the CCD camera detects a large portion of the spectrum (dispersed by a spectrometer) instantaneously at an arbitrary time after the laser excites the sample. To measure one transient spectrum, four 'pictures' are taken by the CCD: pump on ($I_F$), probe on ($I_0$), pump/probe on (I), pump/probe off ($I_{0F}$).

The four spectra are combined to yield a difference spectrum,

$$\Delta OD = \log [I(\nu) - I_{0F}(\nu)] / I_0(\nu)$$

and a difference spectrum corrected for fluorescence,

$$\Delta OD = \log [I(\nu) - I_F(\nu)] / [I_0(\nu) - I_{0F}(\nu)]$$

Typically, 400-4000 spectra (100-1000 sets of four) are averaged and give excellent signal-to- noise ratio down to a ΔOD of $10^{-4}$. Rates of formation and decay of transient species are determined by monitoring the change in absorption of the probe light at a particular wavelength using a photomultiplier.

Excitation on the sub-picosecond time scale is achieved using a regeneratively-amplified titanium:sapphire laser, from Coherent/B.M. Industries. The 800 nm output (800 μJ/pulse, 1 kHz, 150 fs) pumps an optical parametric amplifier (OPA), which is tunable from 460-750 nm. The 20 μJ/pulse of visible wavelength light from the OPA can again be frequency-doubled with a non-linear crystal to achieve wavelengths of 250-325 nm.

After exciting a sample with the Ti:Sapphire-pumped OPA, emission lifetimes down to tens of picoseconds are measured using a Hamamatsu C4334 Streak Camera system. This device (described in The Spectrograph, 16 (1), Spring 2000) performs time-resolved single photon counting at all wavelengths over a 100 nm window simultaneously. Streak camera detection of emission gives rapid data collection and the ability to directly compare the temporal evolution of multiple spectral features from a single experiment. Transient absorption spectra on the picosecond time scale can be obtained in a pump-probe experiment using a

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**Personalities ...** continued from page 2

adult Kuwaitis are officially obese, according to WHO standards," Chuck says.

"Dinner with Kuwaiti friends was always a great feast spread out on a huge Arabian rug that filled the room," he recalls. "We reclined on cushions like the ancient Romans, eating and chatting for hours in the welcome coolness of the evening. The food was so varied and delicious that even I, the weight-control specialist, began to gain weight."

In the summer of 2000, Chuck presented his work on computer-assisted image analysis of pre-invasive neoplasia at a scientific meeting in Hawaii that was also attended by Michael Feld, Director of the Spectroscopy Laboratory. Chuck and Michael went SCUBA diving together. "We had to throw ourselves overboard with a hundred pounds of gear and chug down 80 feet through cold thermal currents," Chuck says, "so we became friends pretty fast." Michael and Chuck began talking about the possibility of extending his cell imaging techniques to living cells and tissues, perhaps even in a clinical setting, using new optical/spectroscopic techniques developed at the LBRC that can measure the size and other parameters of living biological structures 100 times smaller than the wavelength of visible light. Early this year, Chuck joined the Spectroscopy Laboratory, where he has inspired a cell biology study of carcinogen-induced neoplastic transformation of the rat esophagus affectionately known as "Rat 1".

Chuck and his wife Alexandra celebrated their tenth year of marriage this June. They both enjoy each other's children by previous marriages, amounting to four boys and three girls in all. Chuck enjoys a life-long passion for SCUBA diving. Next Spring he plans to go diving with Alexandra's son, Alan, off the island of Guam, where Alan works as a building contractor for the military and a SCUBA instructor in his spare time. Chuck also has interests in the history of science and mathematics, classical music, and modern art. ■

**Molecular**... continued from page 3

thermoelectrically cooled CCD camera and a four-spectrum sequence similar to that of the nanosecond system. Amplified photodiodes are used for detection of single-wavelength kinetics. Time delays between the pump and probe beams are controlled by changing the path length of the pump beam using a motorized delay stage. Our picosecond system has been used to study a multitude of systems; examples from our own lab include porphyrin-based donor-acceptor systems, novel fluorophores for imaging fluid flow [1,2], chromophores for enzyme modification, and photocatalysts for the production of hydrogen. We have collaborated with other groups in the Laser Research Faculty, as well, investigating the optical properties of quantum dots (Prof. Bawendi), sensor prototypes based on conducting polymers (Prof. Swager), and a variety of human tissues (Spectroscopy Laboratory).

One especially fruitful line of research has been to define photophysical properties of molecules such that we can construct supramolecules displaying bright and long-lived luminescence[3,4]. These special active sites form the cornerstone for a new technique invented within this program to precisely describe the chaotic flow of fluids. Specifically, the technique, which we call molecular tagging velocimetry or MTV, measures the velocity flow fields of highly three-dimensional turbulent flows.

Turbulence is a fundamental phenomenon in our physical world, and optical methods are central to quantitatively measuring the motion of fluids. One of the state-of-the-art optical techniques for measuring fluid flow is particle imaging velocity or PIV. Fluid physicists seed a fluid with millions, sometimes billions, of particles depending on the volume of interest (10,000 particles per cc of fluid). A sheet of laser light illuminates a section of the flow and the reflection of light from the particles identifies their positions. A subsequent laser sheet of light records the particles' positions at a later interval. By comparing the photographs, the particles' positions are correlated with sophisticated computer algorithms, thereby reflecting the velocity of the fluid for defined groups of particles. The PIV technique is enormously powerful because it instantaneously measures the velocity of a fluid at many points - a key measurement to any fluid physicist or engineer. Nevertheless, the technique has many drawbacks arising from the need to measure the flow velocity with particles. First, a plane of light illuminates particles and subsequent illumination relies on the particles staying within the layer so that they may be illuminated at some later time. In highly three-dimensional flows, this will not be the case. Second the particles have their own inertia and they therefore may not track the flow, especially when it changes suddenly. Third, particles may not go into areas of interest. For instance, particles do not go into areas of high turbulence or in areas near surfaces.

Our idea was to replace the particle markers with molecular markers, specifically supramolecules. The requirement for the optical supramolecules for MTV measurements is that they exhibit a bright, non-quenchable luminescence, which is long-lived. Since the supramolecules are part of the flow (they are molecularly dissolved within the fluid), all the problems associated with particles are eliminated in the MTV technique. The optical supramolecules are dissolved in the fluid and a grid of laser lines is imposed upon the flow by shining a laser beam off of a grating to produce a glowing grid, defined by the luminescence from the optical supramolecule tracer. The trace must exhibit a bright, non-quenchable luminescence, which is sufficiently long lived to convect with the flow (μs to ms, depending on the fluids problem). The deformation of the grid as it moves with the flow is recorded with a CCD camera. By measuring the distance and direction each grid intersection travels and knowing

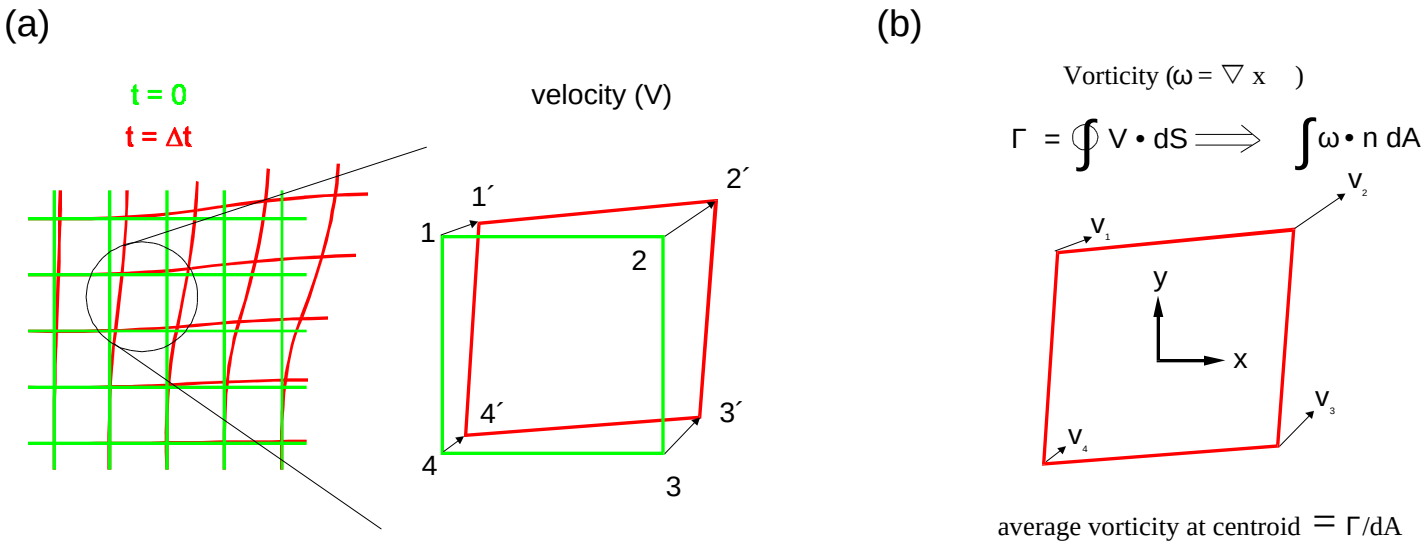


Figure 1. (a) A square grid displacing after time Δt becomes distorted. (b) The vorticity can be determined from the circulation around the grid box by using Stokes' theorem.

continued on page 5

**Molecular**... continued from page 4

the time delay between each image, the two velocity components in the grid plane may be determined by the methods described in Fig. 1 (the out-of-plane velocity can be determined with a second CCD camera). Parameters important to the fluid physicist, such as turbulence intensities, the Reynolds stress and vorticity, may be calculated.

The trick in implementing the MTV technique is that the grid must exist long enough to permit a luminescent image to move with the flow, thus providing velocimetry information. The design of successful imaging reagents requires a radiative rate in the millisecond time regime and a bright enough image to capture. Because lifetimes are long, quenching is an issue pervading most measurements. Water, oxygen and residual metals in the environment are good quenchers of luminescence. The lifetime and intensity of an excited state decrease linearly with increasing concentration of these quenchers. Accordingly, a primary challenge in the design of any successful imaging system reduces to molecular designs that minimize these quenching pathways. Unarguably, the most problematic quencher in engineering applications is oxygen, owing to is efficiency and prevalence in the environment. Oxygen quenches phosphorescent excited states by the energy transfer mechanism. The problems that oxygen brings to bear to the MTV technique can be explicitly demonstrated by considering that a tracer possessing an inherent 1 ms photoluminescence lifetime will be attenuated by $10^3$ in oxygenated solutions and reduced by over $10^4$ in air! In our work, debilitating nonradiative decay pathways of potential tracers are identified by transient laser spectroscopy and the deleterious oscillator or chemical process is then eliminated by targeted synthesis to produce successful optical supramolecule tracers. We have designed several systems that allow flows as slow as 0.2 to 1 m/s (to ensure linearity, flow displacements are measured for 1 mm) to be measured.

The special properties of the MTV technique permit us to investigate many problems of interest that previously were elusive to the fluid physicist. One important problem is the measurement of the velocimetry flow field of a rotating airfoil. With increasing attack angle, turbulence at the front or leading edge of the airfoil causes the flow to detach. This situation results in dynamic stall and the plane looses lift, thereby catastrophically affecting the flight of the aircraft. With less dire consequences, when the smooth or laminar flow over a wing becomes turbulent, the drag increases,™ thereby resulting in losses in flying efficiency. The leading edge problem is uniquely addressed by the MTV technique, owing to its high 3-D character and large range of spatial and temporal scales. Accordingly, during the past funding period, we implemented the MTV technique in the laboratories of Dr. M. M. Koochesfahani at Michigan State to experimentally examine the leading edge problem.

Figure 2 represents the first detailed picture of a flow within the boundary layer near the surface of a pitching airfoil. The color scale indicates the *quantitative* magnitude of vorticity, the fundamental parameter of turbulence; purple, blue and green indicate a clockwise flow and yellow and red indicate a counterclockwise flow. Among the many details resolved in these measurements is the occurrence of a thin flow (on the top surface of the airfoil), which is traveling in the reverse direction. It is this reverse flow, creeping backwards eruption of the boundary layer away from the wing in a highly localized manner (both spatial and temporal). These results point the way toward the design of static and active control features that can minimize the reverse flow, thus providing a road map to the design of safer and higher performance aircraft.

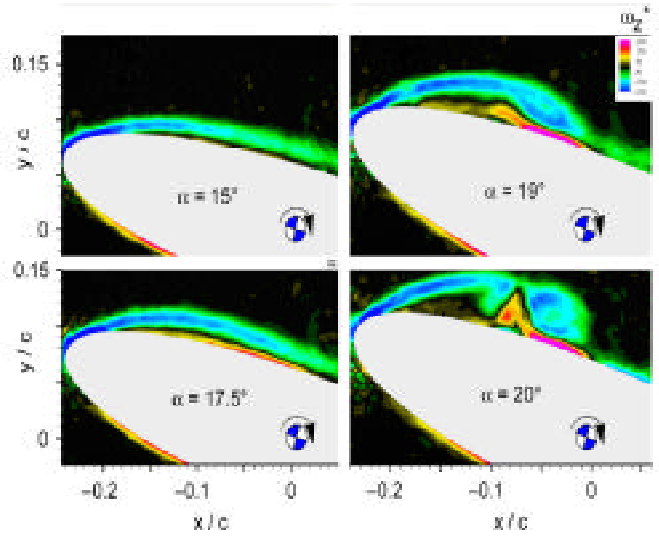


Figure 2: Evolution of the vorticity field as measured by MTV at the onset of leading edge separation on an airfoil pitching at high angles of attack. The thin flow near the surface of the airfoil at 17.5° evolves into turbulence at higher angles, causing loss of lift.

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**Lester Wolfe Workshop in Laser Biomedicine**

# Optical Methods for Detection and Treatment of Atherosclerosis

## Tuesday, December 11, 2001, 4:00 – 6:30 PM

### Massachusetts Institute of Technology, Room E25-111
### 77 Massachusetts Avenue, Cambridge

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**Optical Methods for Detection and Treatment of Atherosclerosis**
John R. Kramer, Jr., Department of Cardiology, Cleveland Clinic Foundation

**Atherosclerotic Plaque Characterization with OCT**
Brett Bouma, Wellman Laboratories, Massachusetts General Hospital

**Cardiovascular Applications Using Texaphyrins**
Wai-Fung Cheong, Pharmacyclics, Inc., Sunnyvale, CA

**Debate**
**Spectral Diagnosis of Atherosclerosis: Fluorescence or Raman**
Abigail Haka v. Jason Motz, GR Harrison Spectroscopy Laboratory, MIT
Michael S. Feld, moderator

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Refreshments served at 3:30 PM

Sponsored by G.R. Harrison Spectroscopy Laboratory, MIT
MGH Wellman Laboratories, and
Harvard-MIT Division of Health Sciences and Technology,
and CIMIT (Center for the Integration of Medicine and Innovative Technology)

**PLEASE POST**

**Seminar on**

# MODERN OPTICS AND SPECTROSCOPY

## Fall Semester 2001

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**October 16** Phillipe Guyot-Sionnest, University of Chicago
**Mid-Infrared and Electrochromic "Dyes": Semiconductor Nanocrystal Colloids and Intraband Transitions**

**October 23** Vladimir Bulovic**,** MIT
**Tailoring Exciton Behavior in Organic Optoelectronic Devices**

**October 30*** David Bensimon, Laboratoire de Physique Statistique -Ecole Normale Superieure
**The Activity of a Single Helicase on a Single DNA Molecule**

***** This lecture to be held at Rowland Institute for Science**
**100 Cambridge Parkway, Cambridge (adjacent to Longfellow Bridge) *****

**November 13** Howard Berg, Harvard University and Rowland Institute for Science
**Secrets of Bacterial Behavior Revealed by Fluorescence Resonance Energy Transfer**

**November 20** Wolfgang Ketterle, MIT
**Gaseous Bose-Einstein Condensates - a Nanokelvin Laboratory for AMO Physics**

**November 27** Adam Wax**,** MIT
**A Look Inside the Living Cell Using Light Scattering and Interferometry**

**December 4** Andrei Tokmakoff**,** MIT
**Capturing Transient Molecular Structure in Solution**

**December 11** Christopher Fang-yen, MIT
**Many Atom Dynamics and Multiple Thresholds in the Cavity QED Microlaser**

***** This lecture to be held at MIT Room (24-121) *****

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TUESDAYS, 12:00-1:00, Grier Room (34-401) [except 10/30 lecture and 12/11 lecture]

Refreshments served following the seminar

Sponsored by the George R. Harrison Spectroscopy Laboratory and the School of Science, MIT, and the Rowland Institute for Science

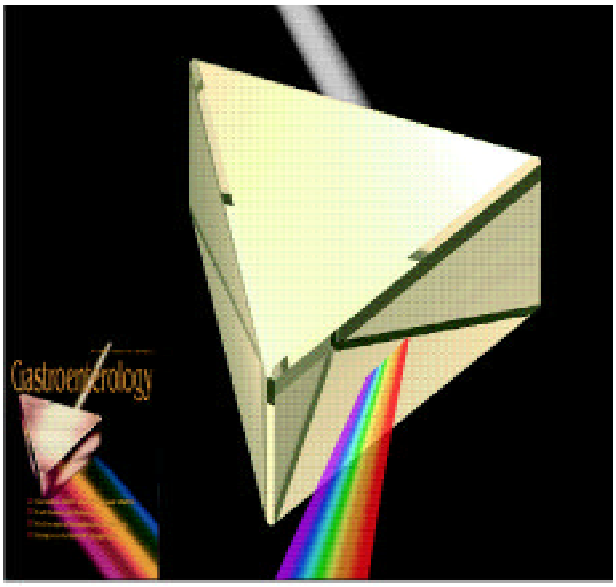
## PLEASE POST

**Editoral ...** continued from page 1

in recent issues of Nature and various medical journals. A research report about this work will be found elsewhere in this issue of The Spectrograph.

The use of spectroscopy for detecting disease has been under study by researchers in many laboratories for a number of years. Early empirical work suggested that spectroscopic signatures of various types could be statistically correlated with the presence or absence of disease in a variety of organs. (This approach has been referred to as "optical biopsy", although it might be better characterized as "optical non-biopsy", inasmuch as the goal is to make the diagnosis without tissue removal!)

As our understanding has deepened, research in the field of spectral diagnosis has moved beyond simple pattern recognition. Spectroscopic features observed from a macroscopic sample of tissue are due to its microscopic constituents. The goal of spectral diagnosis is to devise spectral techniques that enable clinically significant constituents to be identified and quantified at the microscopic level. The methods are model based rather than statistical. A growing body of work demonstrates that spectroscopic techniques can extract accurate quantitative information about tissue structure and biochemistry in this way. These features, such as the size distribution of the nuclei of cells lining the epithelial surfaces of the body, are used by pathologists in making definitive diagnoses. However, a pathology diagnosis requires tissue removal (biopsy), fixation, staining and expert microscopic evaluation, a sequence of events that is costly, labor intensive and time consuming, and, in addition, the results are largely qualitative. Spectral diagnosis has the potential to provide a powerful set of new tools for rapid, objective and accurate disease diagnosis. That is why the medical community is so interested in these new techniques.



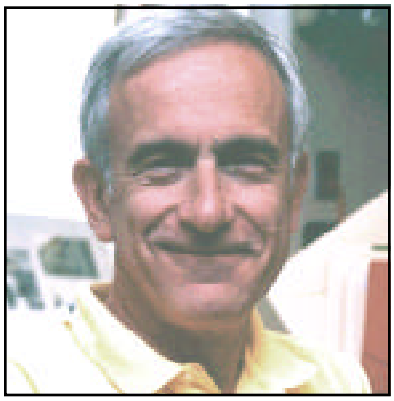

**Optician's revenge!***

We note that the illustrator has taken some liberties with the laws of optics, perhaps to convey a dramatic effect. The cover depicts a beam of white light normally incident on the back face of the prism and dispersed into an array of parallel beams of colored light exiting the front face. Readers experienced with optics will note that, in reality, a white light beam incident in this way would be totally internally reflected at the left face (near the journal binding) and then exit the front face with very little dispersion. As shown in the illustration on this page, the white beam should instead enter the back face of the prism from the upper left corner of the cover at an oblique angle. The dispersed colors would then exit the front face, directed towards the bottom edge of the cover. The colored beams would emerge in a fan of angles from a common point within the prism, with the blue beam deviating the most and the red beam the least.

The artist did, however, accurately capture one subtle feature in ways that most of us would not notice — the correct rendering of the edges of the bottom of the prism, as seen through the front face of the prism. The artist may have carefully observed an actual prism, or perhaps used an optical ray tracing program to model what the prism should look like.

Needless to say, progress in this field could not occur without close collaboration between physical scientists and physicians. The cover illustration underscores the interest of the medical community in this field.

And let us remember that it's OK to violate the laws of optics in an illustration, as long as we respect the laws of medicine in the operating room! ■



**Michael Feld**

**Steve Fulghum**

*Art work courtesy of Adam Wax

**Spectroscopic ...** continued from page 1

reduced scattering coefficient of dysplastic tissues (Fig. 2b).

A small fraction (2-5%) of the reflected photons are detected after undergoing single backscattering events. The major target particles for this type of scattering are the nuclei of epithelial cells. Changes in the shape, size and number density of cell nuclei are histopathological hallmarks of dysplasia. Analysis of the singly-backscattered light spectrum using light scattering theory (light scattering spectroscopy or LSS), provides information about the size, and number density of cell nuclei (Fig. 2c) [3,4] without tissue removal or processing.

Since IFS, DRS and LSS provide complementary information about tissue biochemistry and morphology, their combined use, i.e. TMS, can serve as an excellent tool for biophysical tissue characterization and the detection of pre-cancerous lesions. Indeed, TMS is a superior tool for the detection of dysplastic changes in Barrett's esophagus [5] and the cervix.

Presently, we are testing software that is designed to perfrom TMS analysis in 4-8 s at the time of data collection. Thus, we can test directly the potential of this tool as a real-time guide to biopsy, and, ultimately, as a tool that could, in some cases, replace biopsies.

## References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4. Backman, V. et al. Nature 406, 35-36 (2000)
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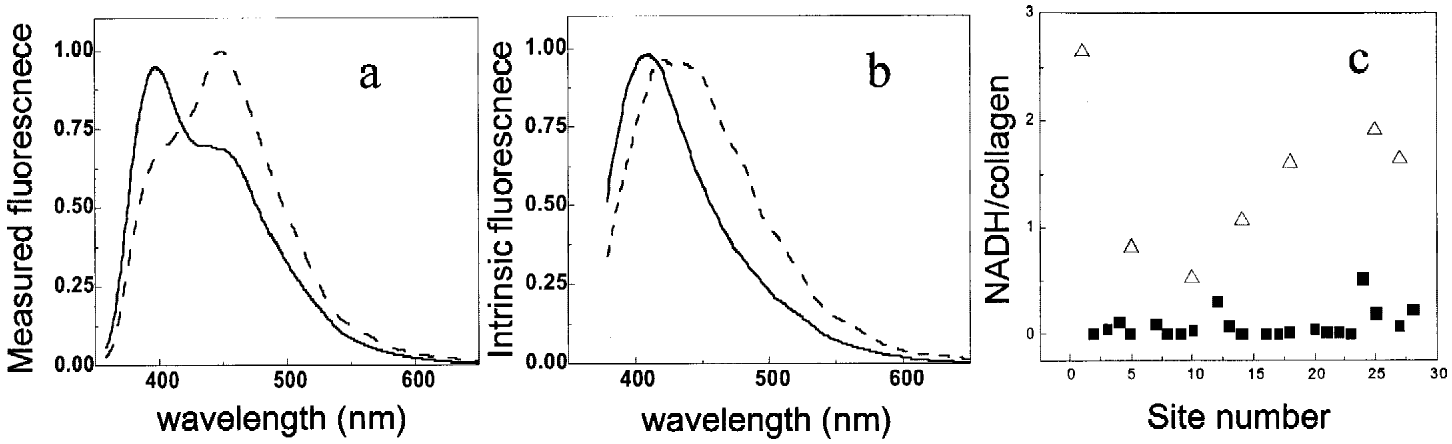



**Figure 1**: (a) Measured tissue fluorescence spectra from non-dysplastic (solid line) and dysplastic (dashed line) Barrett's esophagus tissue sites. (b) Corresponding intrinsic fluorescence spectra. (c) Ratio of NADH to collagen fluorescence to the intrinsic fluorescence spectra of non-dysplastic (squares) and dysplastic (triangles) tissues.

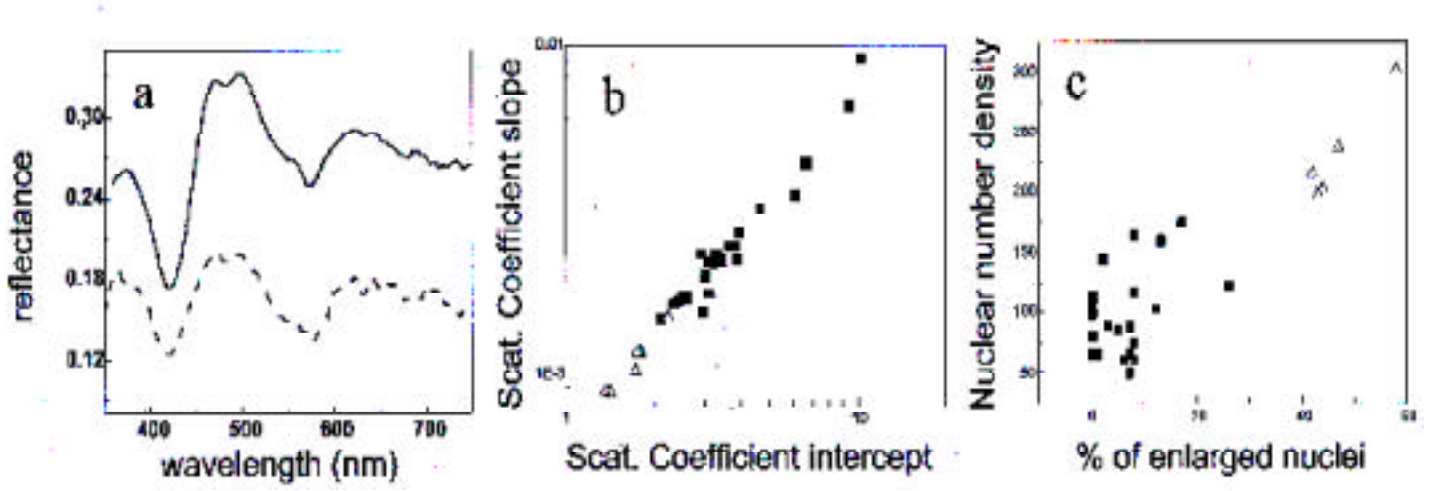



**Figure 2**: (a) Measured reflectance spectra from non-dyspalstic (solid line) and dysplastic (dashed line) Barrett's esophagus tissue sites. (b) Slope and intercept of a line describing the wavelength dependence of the reduced scattering coefficient. (c) Nuclear number density plotted vs. nuclear enlargement. Non-dysplastic: squares; dysplastic: triangles.

# Equipment Update*

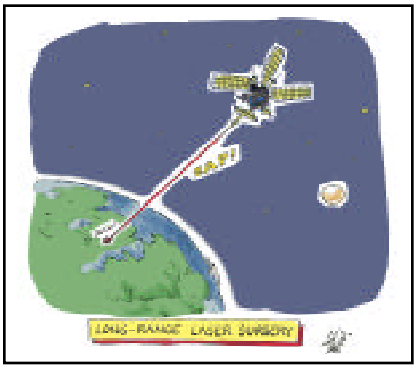
**Ultra-large Deep Depletion CCD Detector for Biomedical Applications**

The VersArray 1300BR detector is a versatile, cryogenically-cooled camera that utilizes a megapixel back-illuminated deep-depletion CCD. It is a 1340 x 1300 pixel CCD with a pixel size of 20 microns square, giving the device size of 26.8 x 26.0 mm. This detector is the first of its kind anywhere in the world and was selected by the G.R. Harrison Spectroscopy Laboratory for its superior NIR sensitivity, low-noise and wide spectral and spatial coverage. The CCD comes with proprietary anti-etaloning technology and with nearly 40% quantum efficiency at 1 micron. This detector is ideal for near infrared Raman spectroscopy, and in particular for biological and biomedical applications.

**GaN Diode Pulsed Laser at 400 nm**

The Nanostructured Materials Laboratory, directed by Professor Bawendi, is acquiring an extremely reliable pulsed laser source for fluorescence lifetime measurements. The laboratory is partially supported by the Laser Research Facility grant of the Spectroscopy Laboratory.

The unit, a GaN diode-driven pulsed laser system (PDL-800), is being acquired from Polytec PI (Auburn, MA). The laser operates at repetition rates from 2.5 to 40 MHz (in units of 2, 4, 8 and 16), with an average power of 4 mW at 40 MHz. The temporal width of the pulses is specified to be 90 ps, and the wavelength of the laser emission is 400 nm. Combined with a custom 400 nm notch filter, the laser can be used as an excitation source for time resolved single molecule fluorescence microscopy experiments. Additionally, research efforts will also aim to quantify a new method for determining the quantum yield of fluorescent materials that are not in solution phase. Such materials include, for instance, polymerfilms, microfabricated devices, etc. ■



*Spectroscopy Laboratory facilities and laboratories are available for use for approved projects. For further information, see the information box on p. 2, and ask for our Researcher's Guide.