✹ **Research Report**

Resonance Raman Spectroscopy of Nanotubes

M. Dresselhaus, K. Kneipp,
S.Brown, P. Corio, A. Marucci,
M.Pimenta and G. Dresselhaus

The authors are with MIT, MIT Lincoln Laboratory, and The Technical University of Berlin

Single wall carbon nanotubes (SWNT's) are cylindrical nanostructures, one carbon atom thick, about 20-50 carbon atoms around the circumference, and several microns long (Fig. 1). They have remarkable electronic properties, insofar as they can be either metallic or semiconducting, depending on the nanotube diameter and orientation of the hexagons of the structure with respect to the nanotube axis. SWNT's also exhibit remarkable Raman spectra, which can provide a wealth of information about the one dimensional (1-D) electronic density of states through a strong resonant coupling between the incident and scattered photons and the electronic transitions between the van Hove singularities in the 1-D density of states in the valence and conduction bands. Through the wide range of laser frequencies available in the Raman Laboratory of the Spectroscopy Laboratory, it has been possible to carry out resonance Raman experiments at many laser excitation energies, thereby allowing study of many novel features.

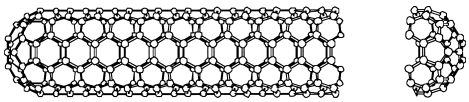

Figure 1: Structure of an armchair carbon nanotube.

Figure 2 shows the Raman spectrum of a carbon nanotube sample over a wide frequency range. The strong feature at ~155 cm^{-1} is associated with the radial breathing mode, in which every carbon atom vibrates in phase in the radial direction. Since the radial breathing frequency is proportional to

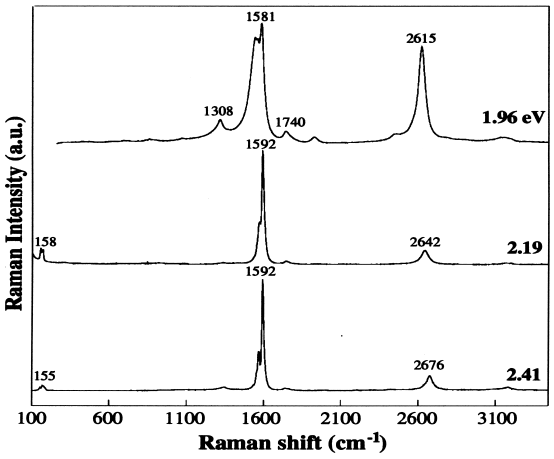

Figure 2: First and second-order Raman spectra for carbon nanotubes over the broad phonon frequency range 100-3700 cm^{-1} for E_{laser} = 1.96, 2.19, and 2.41 eV.[1]

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✹ **ALSO IN THIS ISSUE…**

✹ **Research Report**

Optical Spectroscopy to Diagnose Alzheimer's Disease

Gene Hanlon, G. R. Harrision
Spectroscopy Laboratory

This is a collaborative project with the Veteran's Affairs Hospital in Bedford, MA. Collaborators include R. Dasari, M. Feld, R. Ferrante, I. Itzkan, N. Kowall, D. Lathi, and A. McKee

Alzheimer's disease (AD) is a terminal form of dementia resulting from progressive degeneration of the neurons. Its cause is unknown. AD is the most common dementia and its social and public health impact is expanding with the expanding elderly percentage of our population. At present, definitive diagnosis is only possible by histologic examination of brain tissue under the microscope, a procedure conducted almost exclusively at autopsy. Lack of an accessible, non-invasive technique for diagnosing AD is the greatest impediment in the search for its treatment and prevention.

Recent results in the Spectroscopy Laboratory's Laser Biomedical Research Center, in collaboration with the NIH-funded Boston University Alzheimer's Disease Research Center at Bedford Veterans Affairs Hospital, indicate that near-infrared (NIR) Raman and fluorescence spectroscopy can differentiate the brain tissue of AD patients from that of normal, aged patients, and therefore may provide

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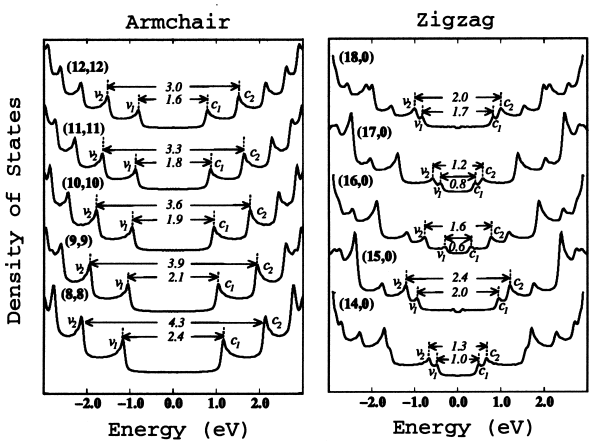

Figure 3: 1-D electronic density of states calculated with a tight binding model for (8,8), (9,9), (10,10), (11,11), and (12,12) armchair nanotubes and for (14,0), (15,0), (16,0), (17,0), and (18,0) zigzag nanotubes. [J.C. Charlier] Wave vector conserving optical transitions can occur between mirror image singularities in the 1-D electronic density of states of the valence and conduction bands of the nanotubes, i.e., v_1 -› c_1 and v_2 -› c_2, etc. These optical transitions, which are given in the figure in units of eV and have the corresponding energies of E_{11}, E_{22}, etc., are responsible for the resonance Raman effect.

THE SPECTROGRAPH

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GEORGE R. HARRISON SPECTROSCOPY LABORATORY

Director: Michael S. Feld

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Associate Director for Project Coordination: 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. More information can be found on our web site //http:web.mit.edu/spectroscopy/www/. Write or call for further information or to receive our mailings, (617) 253-4881.

the inverse nanotube diameter, the Raman effect can be used as a sensitive structural probe of nanotube samples, which have diameters on the order of ~ 1 nm. At a given laser excitation energy, only the nanotube interband transitions between singularities in the 1-D density of electronic states that are resonantly excited will contribute strongly to the Raman signal (Fig. 3). Thus, by measuring the radial breathing mode spectra for many values of laser energy, E_{laser}, we can calibrate the diameter distribution of our nanotube samples.

The major focus of our research at the Spectroscopy Laboratory has been directed to the study of tangential carbon-carbon stretch modes in the 1500-600 cm^{-1} range. By studying the spectra at many values of E_{laser} we were able to show that the Raman spectra for metallic nanotubes are characteristically different from those of semiconducting nanotubes. This is shown in Fig. 4, where the broad spectra for $1.55 < E_{laser} < 2.10$ eV are identified with metallic nanotubes, and all the other spectra in the figure are identified with semiconducting nanotubes.

At the present state of technology it is not possible to prepare carbon nanotubes samples that have only a single nanotube diameter and a single orientation of the hexagons (chirality), nor can we prepare a sample that has only metallic or semiconducting nanotubes. However, by careful selection of the laser Raman excitation energy, we have been able to carry out resonance Raman experiments to study only metallic or semiconducting nanotubes, even though the nanotube sample contains an abundance of both types. This distinction can

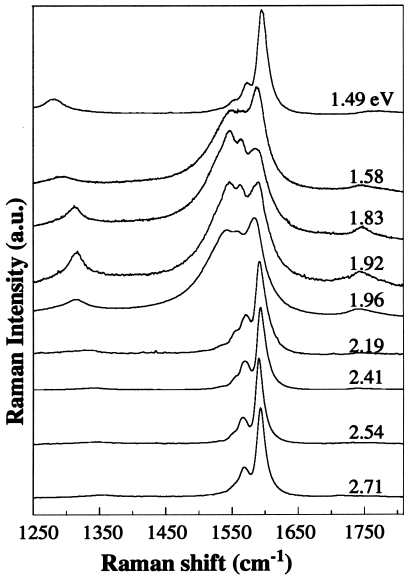

Figure 4: First-order Raman spectra for the tangential band of carbon nanotubes (1500-1650 cm^{-1}), taken for nine laser excitation energies. At lower frequencies (1300-1350 cm^{-1}) the *D*-band feature is observed, and at higher frequencies ~1750 cm^{-1} a combination mode is seen for some values of E_{laser}.

be made for two reasons. First, as shown in Fig. 4., metallic and semiconducting nanotubes have very different and distinct Raman spectra. Second, large Raman intensities are observed when the laser excitation energy is equal to the energy separation between singularities in the 1-D electron density of states in the valence and conduction bands. Due to the remarkable electronic properties of the nanotubes, the density of states for metallic and semiconducting nanotubes are very different, as shown in Fig. 3. If we now plot all possible interband transitions up to 3.0 eV for all possible nanotubes with diameters d_t in the range $d_t < 3.0$ nm, we obtain a map (Fig. 5) showing the metallic window (shaded region) for nanotubes with a specified diameter distribution. In resonant Raman scattering both the incident and scattered photons can be in resonance with an interband transition.

Additional information on nanotubes is provided by exploiting the anti-Stokes Raman peaks, which occur at the high-energy side of the excitation laser frequency. In general, according to simple Raman theory and demonstrated by many experiments, one expects the anti-Stokes spectrum to display the same Raman shifts as the Stokes spectrum, which occurs at the low energy

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Mildred S. Dresselhaus

Personalities

Mildred Dresselhaus is one of the best known professors at MIT. She holds faculty positions in both the Department of Electrical Engineering and Computer Science and the Physics Department, and she i an Institute Professor, a special honor reserved for only a few MIT faculty members who distinguish themselves amongst their peers. She was President of the American Physical Society and directed the Center for Material Science for a number of years. Millie, as she is affectionately known throughout our community, is also well-known for her very effective efforts in educating and advancing women and minority students and scientists. Her nanotube research in the Spectroscopy Laboratory is stimulating to all.

Millie was born and grew up in New York City. She received her undergraduate education at Hunter College in New York City, and then spent a year of study at Cambridge University and another year at Harvard University. After a Ph.D. and postdoc at the University of Chicago and Cornell University in the area of microwave studies of superconductivity, she arrived at MIT in 1960 as a research staff member at the Solid State Division at the MIT Lincoln Laboratory. Her move to Lincoln Laboratory coincided with her switching research fields to magneto-optic studies of semiconductors and semimetals at high magnetic fields. In 1962 she conducted the first magneto-optic studies of graphite, which elucidated many new aspects of the electronic structure of this remarkable semimetal. To distinguish between the spectra for left and right circularly polarized radiation, higher resolution was needed. So, in 1966 she joined forces with Professor Ali Javan to do the first high field magneto-optic experiment with a laser source, with Paul R. Schroeder as their jointly supervised graduate student. Paul's Ph.D. thesis not only broke new ground in magneto-optic technology, but led to a major advance in our understanding of the electronic structure of graphite as we know it today, with the first correct identification of the location of electrons and holes in the Brillouin zone.

As a result of this collaboration, Millie became acquainted with many members of Professor Javan's research group, including Michael Feld and Ramachandra Dasari, who later became directors of the Spectroscopy Laboratory. In her autobiographical material, Millie wrote that the Spectroscopy Laboratory has contributed greatly to the research capabilities of many investigators at MIT and elsewhere.

After joining the MIT faculty in 1967, first as the Abby Rockefeller Mauze visiting professor of Electrical Engineering, and then in 1968 as a permanent member of the Engineering School faculty, collaboration with the Javan group continued for a few years. Research in the Dresselhaus group shifted to many different areas of solid state physics, but including topics in carbon science and spectroscopy, such as studies of the structure and properties of carbon fibers, graphite intercalation compounds, liquid carbon activated porous carbons, carbon aerogels, carbon fullerenes and carbon nanotubes. This long history in carbon-based materials has established MIT as a world center in this research area. Many collaborators and many visitors world-wide have come to work for periods of time with the Dresselhaus group in the area of carbon science. Over the years, Raman spectroscopy has become established as a major characterization tool for carbon based materials, whether in the form of graphite, disordered carbons, porous carbons, and more recently fullerenes and carbon nanotubes. Millie writes that, "through our association with the George R. Harrison Spectroscopy Laboratory, it has been possible to break new ground in exploring the differences between the spectra for metallic and semiconducting nanotubes, differences in the Stokes and anti-Stokes spectra, identification of the characteristics of the 1D electron density of states, identification of the mechanism behind the unusual properties of the so-called zone edge D-band and its second-order G' band counterpart in sp^2 carbons and carbon nanotubes, and finally in study of the enormous enhancements in the intensity of the Raman spectra of single wall carbon nanotubes made possible by surface-enhanced Raman scattering using fractal colloidal silversubstrates in collaboration with Katrin Kneipp, Sandra D. M. Brown, P. Corio, M. A. Pimenta, A. Marucci and G. Dresselhaus."

Millie joined the MIT Department of Physics in 1983, and was named an Institute Professor at MIT in 1985. She is a member of the American Philosophical Society, the National Academy of Sciences, the National Academy of Engineering, and a Fellow of the American Academy of Arts and Sciences, the American Physical Society, the IEEE, the Materials Research Society, the Society of Women Engineers, and the American Association for the Advancement of Science. She has served as President of the American Physical Society, as Treasurer of the National Academy of Sciences, and as president of the American Association for the Advancement of Science. She is on numerous advisory committees and councils, and is a foreign member of the Japanese Academy of Engineering. She has received numerous awards, including the Killian Award in 1986, the National Medal of Science from President George Bush in 1990, and 16 honorary doctorates. She is the co-author of three books on carbon science.

Millie is featured as one of the hundred most important women in the United States in the November, 1999 issue of Ladies' Home Journal. In the article, her humanistic love of science is apparent in her reasons for speaking to her granddaughter's third-grade class: "If children don't get exposed to science, they will miss out on something that is so useful for living and for thinking about how things work." Millie showed the children how a soccer ball is a real-life model of a carbon fullerene.

Millie lives in Lexington with her husband (and research colleague) Gene, a senior scientist working at MIT's Magnet Laboratory. They have four grown children. For relaxation, Millie is an enthusiastic chamber music player, playing both violin and viola.

■

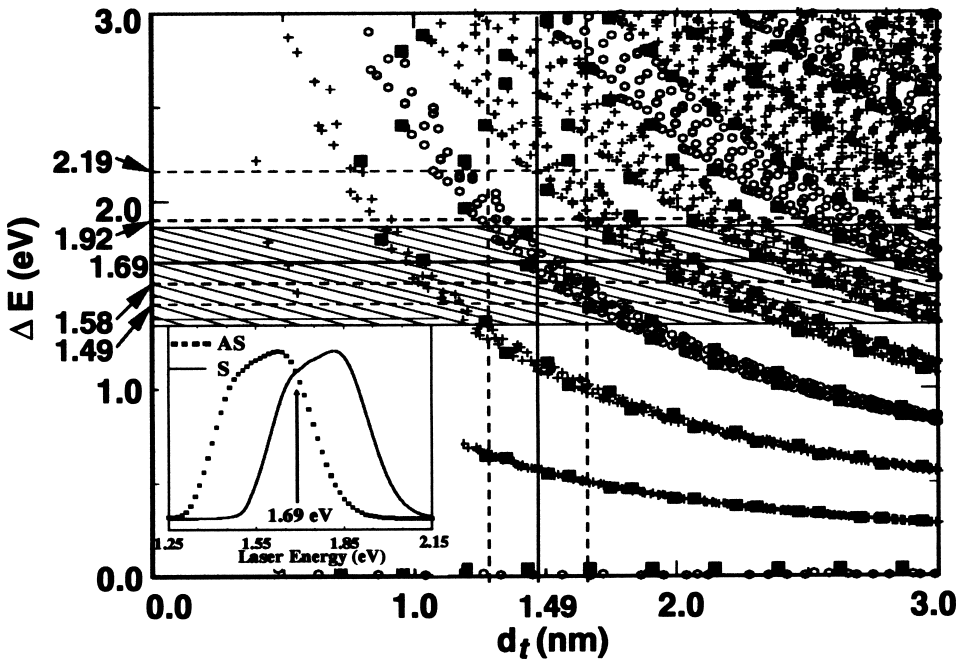

Figure 5: Calculation of the energy separations ($\Delta E = E_{ii}(d_t)$ for all carbon nanotubes vs. diameters in the range $0.7 < d_t < 3.0$ nm and $E_{ii}(d_t) < 3.0$ eV. Semiconducting and metallic nanotubes are indicated by crosses and open circles, respectively. The filled squares denote zigzag nanotubes. The dashed horizontal lines correspond to E_{laser} values at which Raman spectra were taken, and the vertical lines denote $d_t = 1.49 \pm 0.2$ nm for one of our samples. The inset is the calculated $IM(E_{laser}, d_0)$ for the resonance window for metallic nanotubes for the Stokes (solid curve) and anti-Stokes (dashed curve) scattering processes. The arrow in the inset at 1.69 eV (at the center of the metallic window) is drawn as a solid horizontal line in the main figure. The cross-hatch pattern is the E_{laser} range for which the equation predicts that metallic nanotubes in both the Stokes and anti-Stokes spectra.

side of the excitation laser. However, for SWNT's we observe Stokes/anti-Stokes frequency asymmetries. These arise from the fact that Stokes and anti Stokes scattering selectively probe metallic and semiconducting SWNT's, due to different resonance Raman scattering conditions for semiconducting and metallic nanotubes [2,3]. To select appropriate values of E_{laser} to access only metallic nanotubes in a given sample, with diameter distribution determined from measurement of the radial breathing mode frequencies, we use the plots in the inset to Fig. 5. For the range of E_{laser} common to both the Stokes (S) and anti-Stokes (AS) curves in the inset, we would expect both the Stokes and anti-Stokes spectra to exhibit contributions from metallic nanotubes, while for the lower (higher) E_{laser} values within the metallic window only the metallic nanotubes contribute to the anti-Stokes spectrum.

As a consequence, we show in Fig. 6 the Stokes (phonon emission) and anti-Stokes (phonon absorption) Raman spectra for four specially selected laser excitation energies for a nanotube sample with a diameter distribution of 1.49 ± 0.20 nm. The figure shows that at $E_{laser} = 2.19$ eV only the semiconducting nanotubes are in resonance, while at $E_{laser} = 1.58$ eV only the metallic nanotubes are in resonance for both Stokes and anti-Stokes processes.

In general, the Stokes and anti-Stokes Raman spectra for molecules and crystalline solids are the same. However, the unusual nanotube-selective resonant Raman process that is operative for carbon nanotubes can give rise to Stokes spectra that are very different from the corresponding anti-Stokes spectra for the same value of E_{laser}. This effect is shown in the spectra of Fig. 6. Here, spectra are shown at $E_{laser} = 1.92$ eV, where the Stokes spectrum is for metallic nanotubes and the anti-Stokes spectrum is for semiconducting nanotubes, whereas at $E_{laser} = 1.49$ eV, the opposite situation occurs, with the semiconducting nanotubes in resonance for the Stokes process and the metallic nanotubes in resonance for the anti-Stokes process. [2,3] A Lorentzian lineshape analysis of the Stokes and anti-Stokes spectra at $E_{laser} = 1.49$ eV (Fig. 7) shows that the Stokes spectrum contains Lorentzian components only for semiconducting nanotubes, whereas the anti-Stokes spectrum shows Lorentzian components associated only with metallic nanotubes. In contrast, at $E_{laser} = 1.92$ eV only semiconducting nanotubes contribute to the anti-Stokes spectrum, whereas the Stokes spectrum contains contributions from both metallic and semiconducting nanotubes.

Finally, SWNT's can show a strong surface-enhanced Raman effect when in contact with metallic structures of nanometer sized roughness [2]. The strong resonant enhancement of the Raman signals open up exciting

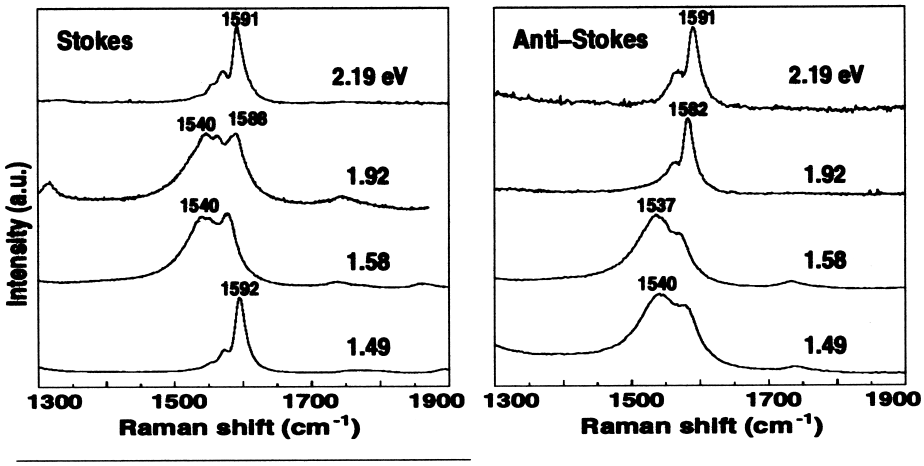

Figure 6: Stokes and anti-stokes Raman spectra for SWNT's taken at 4 different values of E_{laser}.

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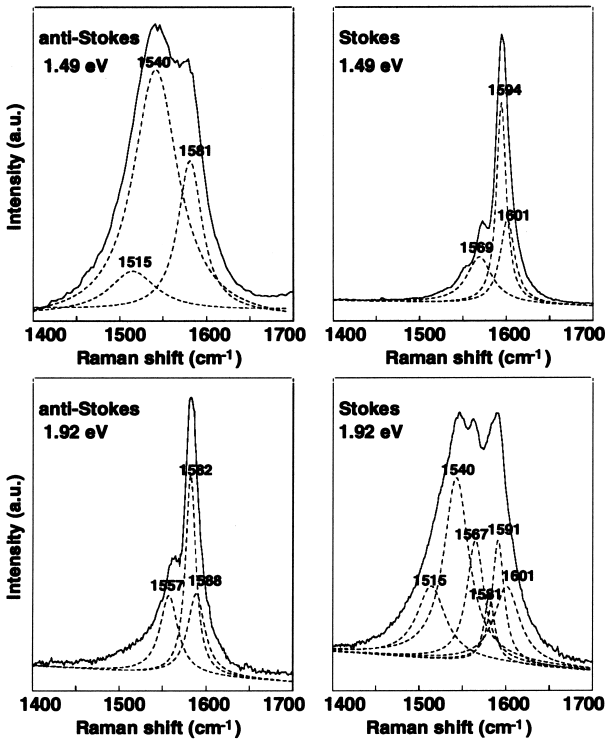

Figure 7: Lorentzian fits to the Stokes and anti-Stokes tangential

opportunities for studying the Raman spectrum of a small number and, perhaps, even single a single nanotube. In particular, it should be possible to retrieve the intrinsic properties which in the above experiments are hidden by the inhomogeneous broadening resulting from ensemble averaging. By using fractal colloidal silver particles [2] in surface-enhanced Raman scattering (SERS) experiments, similar to those reported in Spectrograph Vol. 14 #1 for single molecules detected by SERS, we have been able to observe Raman spectra with narrow linewidths from a very small number of carbon nanotubes (Fig. 8), free of the inhomogeneous line broadening generally found in normal resonant Raman scattering (~ 25 cm^{-1}). Our estimate for the natural linewidth of single wall nanotubes is ~10 cm^{-1}. The enhancement of the Raman signal by many orders of magnitude (10^{12}) through the SERS process, together with strong spatial confinement of this enhancement, should eventually allow observation of the Raman spectrum for an individual single carbon nanotube, ~1 nm in diameter.

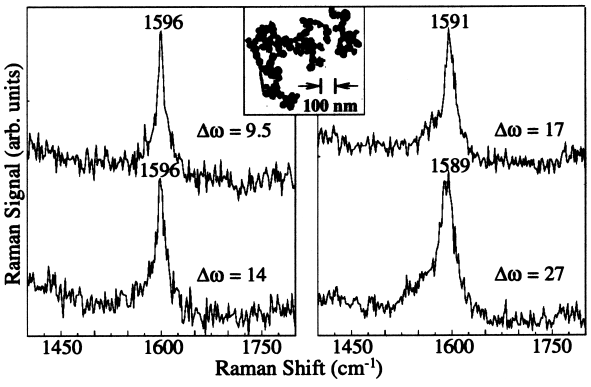

Figure 8: Typical fractal colloidal silver clusters (see inset) and selected SERS spectra of the tangential band of single wall carbon nanotubes, with linewidths as small as 9.5 cm^{-1}, collected from such a cluster area

References:

[1] S. Brown, P. Corio, A. Marucci, M. Pimenta, M. Dresselhaus, and G. Dresselhaus, "Second-Order Resonant Raman Spectra of Single-Walled Carbon Nanotubes", Phys. Rev. B 60, 1999 (In press).

[2] K. Kneipp, H. Kneipp, P. Corio, S. D. M. Brown, K. Shafer, J. Motz, L. Perelman, E. B. Hanlon, A. Marucci, G. Dresselhaus, and M. Dresselhaus, "Surface-Enhanced and Normal Stokes and Anti-Stokes Raman Spectroscopy of Single-walled Carbon Nanotubes", Phys. Rev. Lett., 1999 (In press).

[3] S. Brown, P. Corio, A. Marucci, M. Dresselhaus, M. Pimenta, and K. Kneipp, "Anti-Stokes Raman Spectra of Single-Walled Carbon Nanotubes", Phys. Rev. B Rapid Comm., 1999 (In press). ■

Alzheimer's...continued from page 1

a basis for a new diagnostic technique.

The major advantage of NIR optical techniques is their potential to provide a definitive diagnosis non-invasively, using safe, portable, low cost technology based on laser spectroscopy. The human body is translucent in the NIR region of the optical spectrum, and NIR light penetrates the adult head and can be used for non-invasive diagnostic purposes, such as cerebral oxygen monitoring. [1] The diagnostic signals observed with NIR fluorescence and Raman spectroscopy are biomolecular in origin, and therefore carry specific information about the disease. This information can be used not only to diagnose AD, but also to gain insight into the mechanisms and progression of the disease *in vivo*. Such insight would be of utmost utility in the development of pharmaceutical interventions for AD, to aid drug design, and to monitor the effects of candidate drug treatments on living patients during trials.

As a first step, we investigated whether brain tissue from AD patients could be differentiated from that of age matched non-AD controls *in vitro*. We have examined both NIR fluorescence and NIR Raman spectroscopy.

For the fluorescence study, unfixed temporal cortex specimens from AD cases and age-matched, non-AD controls were frozen at autopsy and then thawed just prior to spectral measurement. Age matched controls are especially important because the brain tissue has undergone all the changes associated with normal aging, such as diffuse amyloid deposition, arteriosclerosis, etc. - changes that must be distinguishable spectroscopically from those associated with AD if a practical diagnostic is to be realized. Spectra of intrinsic tissue fluorescence induced by 647nm light were recorded from 650 to 850 nm (Fig. 1). We used principal component analysis of the tissue spectra from 17 AD cases and 5 non-AD control cases in a calibration study to establish a diagnostic algorithm. The principal components are a set of spectral "building blocks" which can be linearly combined to accurately describe all of the tissue spectra, both normal and diseased. [2] Retrospectively applied to the calibration set, the algorithm correctly classified 23 of 24 spec-

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Future Directions of Optics in Medicine

Diagnosis, Pathology, Imaging, Therapy

Friday, November 5, 1999, 4:00-7:00 PM

Massachusetts Institute of Technology, Room 6-120
77 Massachusetts Avenue, Cambridge

Diagnosis: Mining Optical Information
Brian C. Wilson, Ontario Cancer Institute

Spectral Diagnosis: Learning from Pathology's Mistakes
Maryann Fitzmaurice, University Hospitals of Cleveland

Hide and Seek in Medical Imaging
Steven L. Jacques, Oregon Medical Laser Center

Therapy: Trouble and Opportunities for the Medical Photon
R. Rox Anderson, Wellman Laboratories of Photomedicine

Panel Discussion*
Michael S. Feld, Moderator, MIT
*Prizes will be awarded for the best audience participation!

Refreshments at 3:30 P.M.
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MODERN OPTICS AND SPECTROSCOPY

FALL SEMESTER 1999

September 21	**David E. Pritchard,** MIT Accurate Atomic Masses for Metrology and Fundamental Physics
October 5	**Tilman Pfau,** MIT Atom Optics
October 12	**Roberto Onofrio,** University of Padova Measuring Casimir and Gravitational Forces Using Micromechanical Resonators
October 19	**Kevin Lehmann,** Princeton University Spectroscopy and Dynamics in Superfluid 4He Nanodroplets
October 26	**Ernst Otten,** University of Mainz Neutrino Mass Measurement from T_2 Decay
November 2	**George Flynn,** Columbia University Order, Orientation and Chirality at the Liquid/Solid Interface; The View from a Few Billionths of an Inch
November 9	**Peter So,** MIT Modern Optical Microscopy: Catching Biology in Action
November 16	**James Anderson,** Harvard University Chemistry, Radiation and Climate: Laser Development for a New Approach
November 23	**C. Bradley Moore,** University of California at Berkeley Vibrational Spectra of Dissociating Molecules
November 30	**David S. Hall,** JILA/ University of Colorado at Boulder; Amherst College Intertwined Bose Einstein Condensates
December 7	**Deborah S. Jin,** JILA/ University of Colorado at Boulder A Quantum Degenerate Gas of Fermionic Atoms

TUESDAYS, 12:00-1:00, Marlar Lounge (37-252), Ronald E. McNair Building
For map see <http://amo.mit.edu/mos.html>
Refreshments served following the seminar
Sponsored by George R. Harrison Spectroscopy Laboratory and
Research Laboratory of Electronics,
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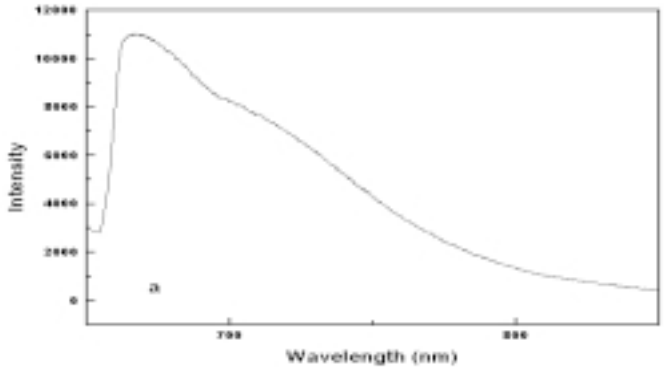

Figure 1. Brain tissue fluorescence.

imens. We then applied this algorithm to a prospective set comprised of 19 specimens from 5 AD brains and 2 non-AD control brains, none of which were included the calibration study. We found that three of the five control specimens and all AD specimens were correctly diagnosed.

The diagnostic algorithm [3] uses a linear combination of the first two principal component contributions, scores 1 and 2, to yield a numerical value, S, associated with each specimen. Positive values of S indicate a positive diagnosis for AD based on the specimen's NIR fluorescence spectrum; negative S values indicate that no AD pathology has been detected spectroscopically. In the prospective validation results, Fig. 2, open squares indicate that the specimen is from a brain with neuropathologically confirmed AD, and solid squares indicate that the specimen is from the non-AD control group. Twenty-three of 24 calibration set specimens and 17 of 19 validation set specimens were correctly diagnosed spectroscopically. [3]

In addition to differentiating diseased from control tissue, NIR fluorescence results point to the possibility of extracting other details of the tissue pathology. Among the control specimens of the calibration set, those with notable diffuse amyloid deposition showed the most negative scores, and had a distinct fluorescence lineshape. The ability to distinguish diffuse amyloid deposits from the fibrillated amyloid of senile plaques may be essential for detecting the onset of AD, since amyloid deposition occurs normally with aging, even in the absence of dementia. This analysis also revealed information contained in the fluorescence spectra regarding the pathophysiological severity of the disease. Pathology reports indicated AD severity for nine calibration set specimens. Of these, 6 of 7 "AD severe" specimens had S values above 0.04 and 2 of 2 "AD moderate" specimens had values 0.03>S>0.02. This result, though preliminary, is an important indication of the potential for monitoring disease progression and the efficacy of treatment interventions.

In a separate study, [2] NIR Raman spectra (Fig. 3) were acquired using 5 specimens from one control brain and 12 specimens from four AD brains. None of the brains used in the Raman study were used for the fluorescence study. Raman measurements were made using 830 nm excitation. Principal component analysis of the normalized, mean-centered Raman spectra followed by logistic regression analysis of the principal component scores revealed that a model based on score 2 and score 6 could properly classify this limited data set with 100% accuracy (Fig. 4).

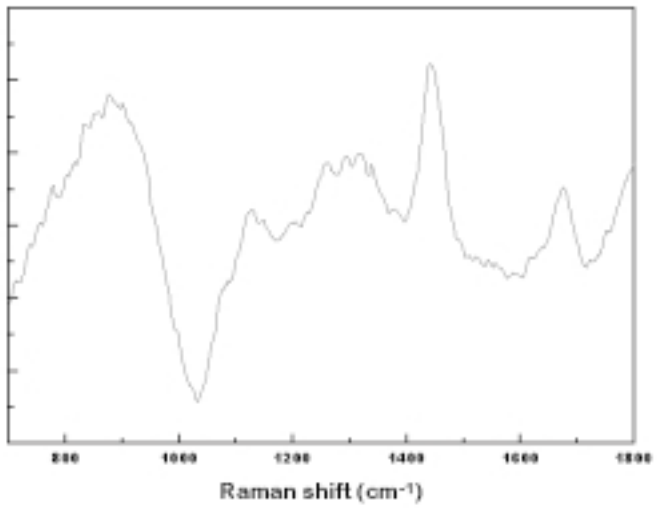

Figure 3. Brain tissue Raman spectrum.

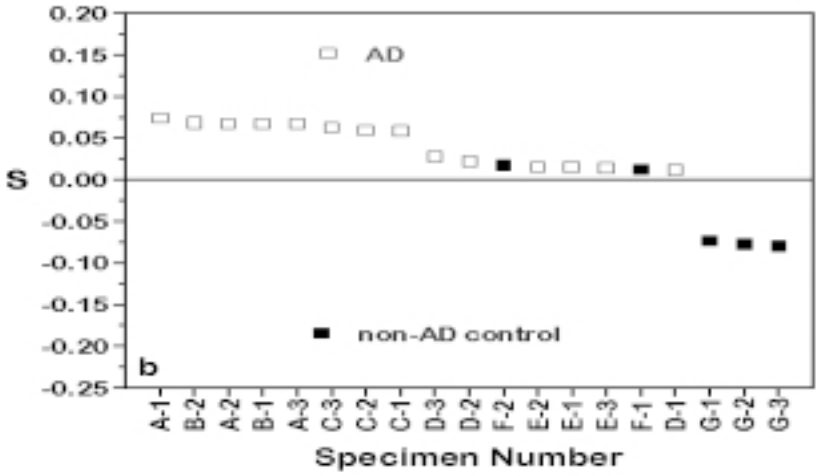

Figure 2. Validation study results.

The next step in this research is to establish that the diagnostic content of the observed NIR spectroscopic signals from brain tissue can be extracted from spectra acquired non-invasively, in situ. First, sufficient light must reach the brain to induce measurable spectroscopic signals. Second, brain tissue spectra that reach the detector, modulated by the absorption, scattering and fluorescence effects of the intervening tissue (skin, skull, dura, arachnoid and

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Alzheimer's...continued from page 8

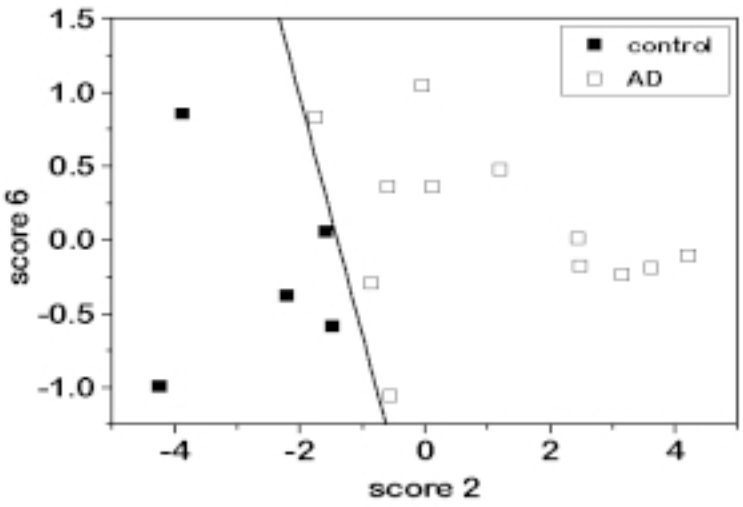

Figure 4. Raman study results.

cerebrospinal fluid), must still be analyzable for their diagnostic content. These points have been addressed in connection with non-invasive applications of NIR absorption spectroscopy. [1] Estimates based on our measurements of brain tissue spectra *in vitro* and the optical transport coefficients reported in the literature for adult head *in vivo* indicate that in the specific case of non-invasive NIR fluorescence measurements for AD diagnosis, sufficient light will reach the brain to induce measurable tissue fluorescence that is analyzable for its diagnostic content.

To evaluate potential interference from temporal bone during *in vivo* measurements, we acquired NIR fluorescence spectra of a fresh autopsy specimen under conditions identical to the brain tissue fluorescence measurements. The bone spectrum was added to that of brain tissue, and these composite spectra were analyzed using the same diagnostic algorithm used in the NIR fluorescence study. The result was that the algorithm could still distinguish between AD tissue and non-AD control tissue, and each specimen's classification was consistent with its earlier result, despite the contribution of bone fluorescence to the observed spectra.

The brunt of pathological alterations in AD occurs in association cortex, in particular the temporal lobe. The bony covering of the skull is at its thinnest over the temporal lobe, having a thickness of several millimeters at that point. It is a matter of good fortune that the brain region of interest in diagnosing AD is where the skull is thinnest and where it is the most convenient to irradiate non-invasively. To make the measurement *in vivo*, an optical fiber probe will deliver low energy laser light and collect brain tissue fluorescence. Appropriate source/detector geometry in the probe to maximize the contribution of the brain tissue fluorescence to the detected signal will be employed. [4] For these *in vivo* measurements, the optical fiber probe will be mounted in a headpiece that will position the probe at the temple and that can be worn without discomfort for the required signal acquisition time (Fig.5).

This research utilizes optical spectroscopic techniques that carry direct, molecular level information about disease. Both the excitation light used and the measured brain tissue autofluorescence and Raman spectra are at near-infrared wavelengths that can propagate through skull and overlying tissue. The results represent the first steps towards developing a clinical tool that has potential to be applied to the non-invasive diagnosis of Alzheimer's disease in living patients.

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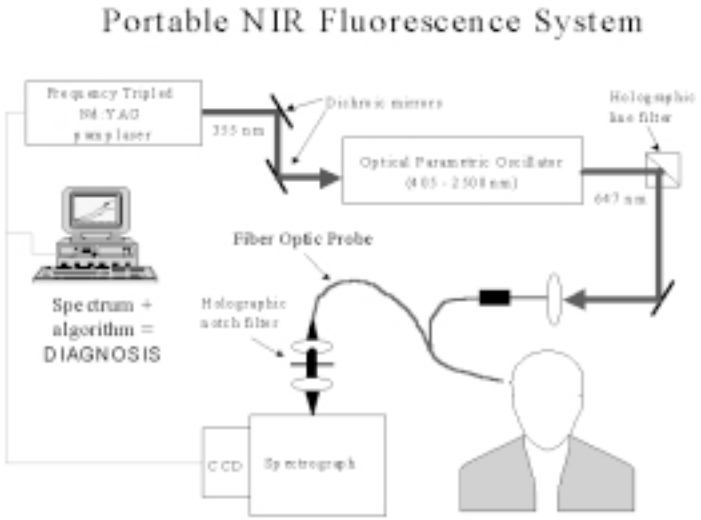

Figure 5. Portable NR Fluorescence System.

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