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Seeing Through the Human Body: Diagnostic Optical Imaging of Deep Tissue

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In 1895 the German physicist Wilhelm Conrad Roentgen discovered X-rays. The public was immediately attracted to this fundamental physics discovery by a vivid demonstration of seeing through the human body in which Roentgen displayed an image of the bones in his wife's hand.

Figure 1: One of Roentgen's earliest photographic plates showed his wife, Bertha's hand with a ring. Produced on Friday, November 8, 1895.

Thus, the first practical application of X-rays was in the area of medical imaging. Ever since, many imaging techniques have been developed to see through the human body. The evolution of X-ray techniques resulted in the development in the 1970's of X-ray computerized tomography (CT), which provides accurate, sub-mm resolution images of the human body in real time.

However, the ionizing nature of X-rays, the inability to extract biochemical information, and low contrast for certain situations restricts the applications of X-ray CT. Other techniques are required to overcome those limitations. After the discovery of X-rays, optical radiation was almost immediately considered as a natural choice of a method for seeing through the body, which is free of the many limitations of X-rays. Optical photons are non-invasive in the same way as are X-rays. In addition, they are virtually harmless (non-ionizing), carry rich spectroscopic information about the biochemical structure of the tissue, and optical devices are relatively simple and inexpensive.

In the near infrared regime (650 - 800 nm), the absorption of photons by human tissue is relatively small. The absorption length is of the order of 20 cm, and photons can penetrate deeply into the tissues. However, a significant problem is associated with the propagation of optical photons in tissue, which is related to the scattering of light. The scattering length in tissue is quite small - around 0.01 cm - and despite the fact that photons can propagate deeply, they do not travel along straight lines.

In 1929 Culter used those scattered photons in a transillumination geometry to image the female breast in a dark room. The success

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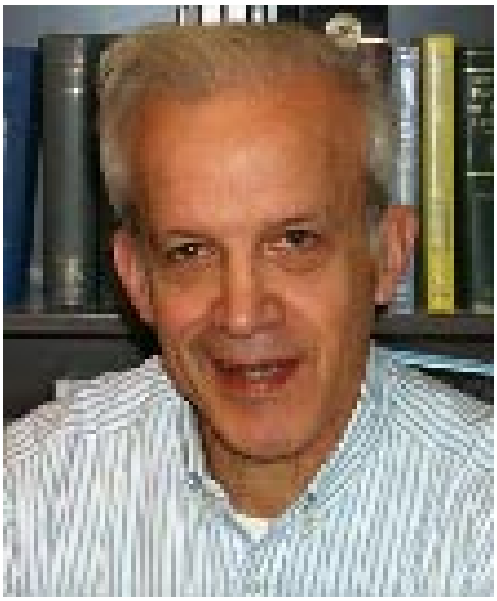

Dan Kleppner

Daniel Kleppner Selected as 2000 Lord Lecturer

Daniel Kleppner, Lester Wolfe Professor of Physics and Associate Director of the Research Laboratory of Electronics at MIT, has been selected as the ninth Richard C. Lord Lecturer. His talk, on May 2, is titled "One Hundred Years of Quantum Physics."

The Richard C. Lord Lectureship, established by the MIT Department of Chemistry and the G. R. Harrison Spectroscopy Laboratory, is an annual event to honor a scientist who has made important contributions to the field of spectroscopy. This is the first time that an MIT researcher has been selected for this honor.

An interview with Daniel Kleppner can be found on page 2. ■

Personalities: Daniel Kleppner

An Interview by Alison Hearn

Daniel Kleppner grew up in New Rochelle, New York. He recounts that like many boys in those days, he liked to play with ropes and pulleys, motors, Erector sets, crystal radios, and other such toys. In his junior year at New Rochelle High School, a remarkable teacher launched him into a career in physics. Now an experienced teacher himself, Dan believes that someone who has even a single great teacher is lucky indeed.

Dan majored in physics at Williams College and went on to a second Bachelor's degree from Cambridge University, where he studied for two years as a Fulbright fellow. He pursued graduate work at Harvard University, where he received a Physics Ph.D. in 1959. His research, under the direction of Professor Norman Ramsey, culminated in the development of the hydrogen maser. After receiving his Ph.D. degree, Dan joined the Harvard faculty as Assistant Professor of Physics. In 1966 he joined MIT faculty, where he has been ever since.

Dan's research since the mid 1970s has been devoted to Rydberg atoms and ultracold hydrogen. A Rydberg atom is any atom with an electron excited to a very high quantum state. First observed in space, he realized that with the development of tunable lasers Rydberg atoms could be made in the laboratory. Using them, he carried out pioneering experiments on the subject that has come to be called cavity quantum electrodynamics. In a series of experiments carried out in the Spectroscopy Laboratory he used Rydberg atoms to probe the connections between quantum and classical behavior, particularly the connections between quantum mechanics and classical chaos.

The second stream of Dan's research was begun in collaboration with Professor Thomas J. Greytak that started in the late 1970s and is still going strong. They were one of the first groups to pursue Bose-Einstein condensation (BEC) in an atomic gas, using atomic hydrogen. They succeeded in 1998, but a few years earlier BEC had been observed in a gas of alkali metal atoms, which are now the workhorses of the rapidly growing field of coherent atoms and gaseous quantum fluids.

Dan describes his relationship with the hydrogen atom as one of love and hate. The properties of atoms that makes them a good candidate for BEC were not known at the start of the search, and they turned out to be much less favorable in hydrogen than in the alkali metal atoms. As Dan puts it, "Nature could have been kinder to us." He has described his sometimes mixed feelings about hydrogen in a slightly metaphysical essay, "The Yin and Yang of Hydrogen," Physics Today (April 1999; pages 11-13).

Dan is quick to point to the work of his MIT colleague Wolfgang Ketterle who was one of the first to achieve BEC, and whose group is widely recognized as a world leader. . He takes what he calls grandfatherly pride in this since because Wolfgang Ketterle launched his career in cold atoms as a postdoctoral researcher with Professor Dave E. Pritchard, and David Pritchard launched his career as a graduate student of Dan.

Dan and his wife Beatrice celebrated their 40th wedding anniversary in the summer of 1998. Bea is a teacher at Beaver Country Day School, a high school where she teaches such diverse subjects as history, anthropology, psychology, and child development (complete with a childcare center at the school.) They have three children and two grandchildren, with a third on the way. Their daughter, Sofie, is a postdoc in neuroscience at UCLA. Their sons, Andrew and Paul, live nearby in Arlington and Lexington, respectively. Andrew is a web designer; Paul works in web software.

For recreation, Dan occasionally designs and builds furniture, working in a shop in his home in Belmont. He describes a wooden cradle for his granddaughter, Hannah as his chef d'oeuvre. Recently, Dan, Bea, Sofie and a few friends took coast-to-coast walk across England, hiking through the Lake District and the Yorkshire Moors. Dan also has interests in history, art and poetry that can sometimes be discerned in his essays in Physics Today. ■

THE SPECTROGRAPH

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was rather limited and the images were extremely blurred. Since then, significant efforts have been devoted to overcoming the turbidity of tissue, in order to create sharper images using optical photons. This is done using a variety of very sophisticated and clever experimental and theoretical techniques.

In principle, there are two main approaches for reducing the influence of scattering in a turbid medium such as tissue: (1) to use an experimental technique which eliminates the most scattered photons, and thus provides sharper images; and (2) to use image reconstruction techniques based on theoretical transport models which solve the inverse problem. In addition, there are hybrid techniques which combine both approaches.

The most obvious experimental strategy for sharpening images is to eliminate scattering. In 1989 Abramson and coworkers demonstrated that the so-called holographic gating technique, which detects only unscattered coherent photons, (Abramson developed this technique in 1978) can be

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used to image through tissue. However, the number of unscattered photons that can reach a detector rapidly decreases when the thickness of tissue increases. If you place a source of light on one side of your body and try to detect the unscattered photons on the other side, the reduction factor in number of photons can easily become e^{-1000}. (This factor of 1000 is based on a 10 cm thick tissue sample and 0.01 cm scattering length (10/0.01=1000).) This enormous attenuation restricts such an approach to very thin tissue samples.

In 1988 Delpy and co-workers used an optical streak camera to record images in thick tissue.[1] These experiments employed time of flight information to separate early arriving photons, which undergo few scattering events, from those that are highly scattered. The resulted images were much sharper than the traditional transillumination images. The idea of such detection is schematically illustrated in Fig. 2. Spatial resolution of a few millimeters has been achieved in reasonably thick samples by using such early arriving (but not unscattered) photons.

Streak camera detection is not the only method used to reduce the number of highly scattered photons. Alfano and co-workers have introduced several other methods for rejecting highly scattered photons, which employ fast time gating (Kerr gate) and polarization techniques.[2]

Extraction of spatial information from experiments with early arriving photons can be improved with the assistance of a theory that accurately describes their behavior. Unfortunately, the well-established diffusion approximation is often not applicable in this situation, since early arriving photons have not been fully randomized by multiple scattering events. The failure of known approximations to the radiative transport equation to accurately describe regimes which are relevant for imaging biological tissues with early arriving photons, has led to efforts to solve the radiative transport equation in a more rigorous way. Bonner and co-workers have analyzed multiple scattering phenomena using a random walk approach, in which photon transport in a turbid medium is described probabilistically.[3] This picture, combined with the concept of the "photon path", permits the introduction of Feynman path integrals in a very natural way. It has been shown by Tessendorf that the path integral approach constitutes a rigorous reformulation of the time independent transport equation, and by Perelman and co-workers that the time dependent radiative transport problem can also be rigorously reformulated.[4] This approach provides new solutions to the problem of light propagation in turbid media for early arriving photons, as well as new insights into the physical picture of the process.

One of the major problems associated with techniques based on the elimination of highly scattered photons is the significant reduction of the useful part of the signal, and thus the decrease of the signal-to-noise ratio. Because of that, in 1989 Patterson, Chance, and Wilson proposed a method which exploits the use of the diffusive photons that have undergone multiple scattering events in the media.[5] Some of this early work was carried out at the Spectroscopy Laboratory.[6] Detection in either time domain or frequency domain (up to several hundred MHz of modulation) can be employed. By placing source(s) and detector(s) at various locations, information about the spatial distribution of embedded objects can be obtained. These diffusive photons are well described by the diffusion approximation. By solving the inverse problem numerically, if not analytically, a three dimensional image can, in principle, be reconstructed.

Figure 2. Schematic diagram of a time dependent imaging experiment in a turbid medium. A short laser pulse propagates through the medium, which is in the form of a slab (lower panel). The upper curve is the transmitted signal at various times. The trade-off between spatial resolution and transmitted signal is indicated schematically by the increasing number of photon trajectories filling the sample as time progresses.

As mentioned above, a precise imaging technique using optical photons relies on both experimental design and theoretical modeling of the data. Various theoretical image reconstruction schemes have been proposed to remove the effect of turbidity and to deblur the data. The inverse algorithms often involve three components: (1) a forward model, which is based on a photon migration theory describing the propagation of photons inside the turbid medium, and can theoretically predict the signals at the detector given a conformation of the medium, (2) an object function, which compares the theoretical predictions against the experimental data, and (3) an updating scheme which, through minimizing the object function, will optimize the accuracy of the extracted image. Extension of the inverse Radon transform to the optical regime has been exploited in a heuristic manner, by modifying the CW Green's function to a form similar to that used to describe X-ray attenuation. Finite difference methods have been worked out, by expanding the solution to the diffusion equation or the transport equation on the bases of selected orthogonal eigenfunctions. Arridge, Hebden, and co-workers have applied finite element methods to complex geometries and used to model boundary effects, by numerically solving the diffusion equation directly.

We should remember, however, that techniques mentioned in this article can work only if there is a detectable difference in the optical properties of normal and abnormal tissues or, in other words, if there is enough contrast. Researchers have explored the use of various tissue properties for contrast, including absorption, scattering, fluorescence, and acoustic modulation. Both intrinsic and exogenous contrast has been exploited. For example, the increased metabolism rates in cancer cells often requires higher oxygen and blood consumption, resulting in increased absorption in cancer cells at the wavelength

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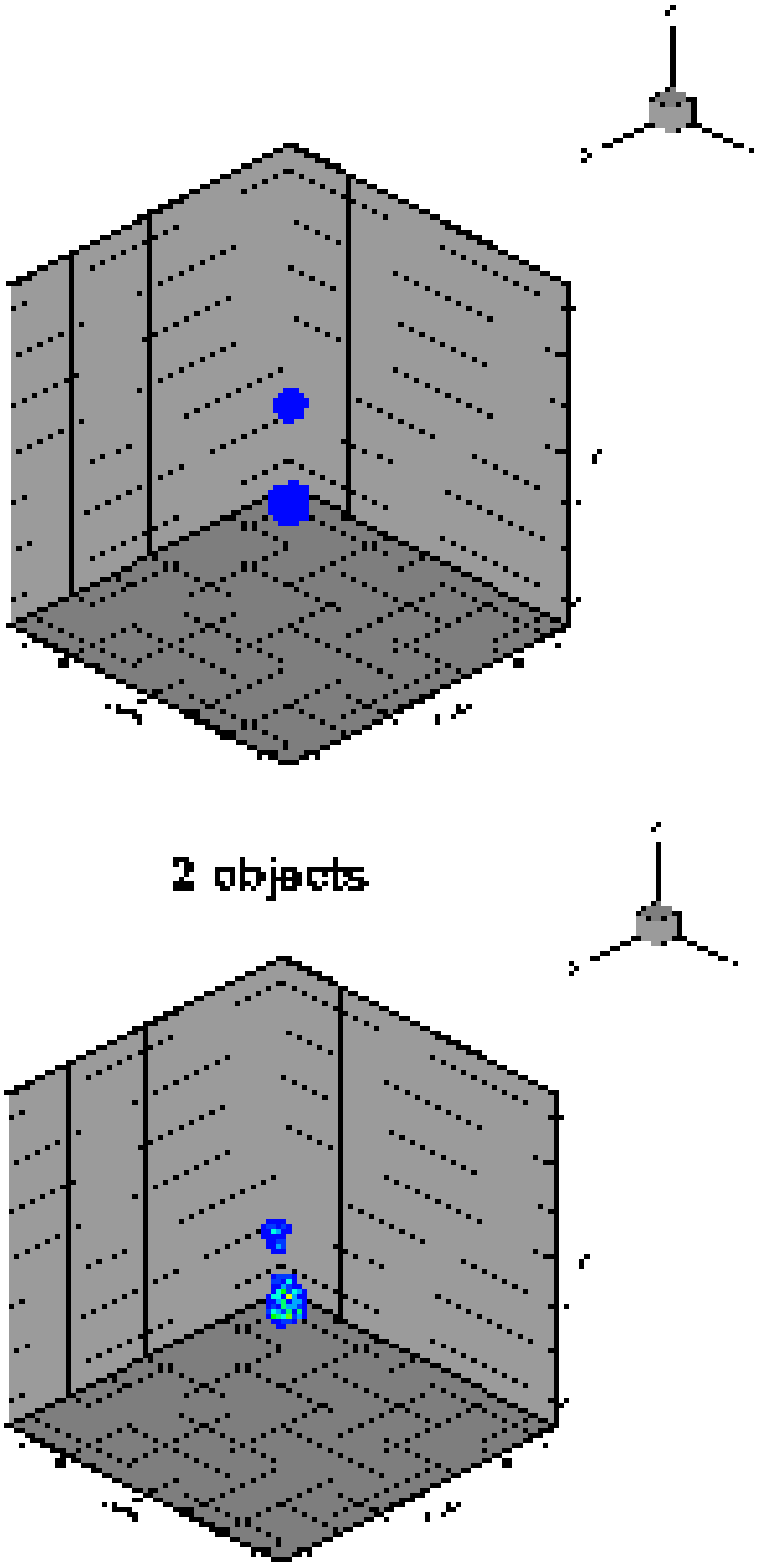

Figure 3. Reconstructed images for 2 objects embedded in a tissue-like medium (cubic glass container 6.35 cm on a side filled with scattering medium with $\mu'_s = 7.38\ cm^{-1}$ and $\mu_a = 0.03\ cm^{-1}$). The laser pulses (150 ps pulse width at 800 nm) were delivered into the medium at the front, and the time-dependent transmitted signals were collected at the back. Surface scans were conducted on the XZ and YZ planes. The early portion of the photon migration curve is used for image reconstruction. (top) exact configuration (bottom) image reconstruction with early arriving photons.

of the oxy-hemoglobin absorption peak. The increased cell density in the tumor also induces higher scattering than in normal tissues. Studies have shown that the laser-induced fluorescence of cancer cells exhibits distinct differences from those of normal tissues. Progress in contrast agent research has led to the development of biologically safe dyes with high quantum efficiencies, which can be injected into the body and selectively accumulate at the tumor sites. Fluorescence-tagged antibodies have also been used. All of these sources of contrast carry physiological information about the tissue.

The MIT Spectroscopy Laboratory has been actively involved in developing optical techniques to image thick tissue. Studies have been conducted in early time detection for enhanced spatial resolution and contrast, image reconstruction based on the extension of the algebraic reconstruction technique (ART) used in X-ray CT to the optical regime, and tests of different photon migration theories at early times. It is well known that the so-called ballistic photons, which travel along straight lines in a turbid medium without undergoing scattering, are not detectable for thick tissue. On the other hand, the diffuse photons, collected several ns after the time-of-flight, have a very high signal level. However, they are not spatially well defined. The early arriving photons at the rising edge of the photon migration curve provide high enough signal and at the same time, follow well-defined paths (often referred to as the "banana" shaped paths). Theoretically, such paths can be calculated using a point spread function (PSF), which describes the spread of photons in the medium due to scattering at a given time. By replacing the straight-line path of the X-ray CT with the appropriate PSF, the ART of X-ray CT becomes directly applicable to the optical regime. A tomographic system using early time detection has been designed and created in the Spectroscopy Laboratory, and experiments have been conducted in tissue-like scattering media with absorption and scattering properties which are similar to those of human breast tissue (Figure 3).[7]

One finding is that the conventional diffusion approximation solution to the transport equation is inadequate for a correct reconstruction of the embedded absorbers. A photon migration theory involving causality gives better results. Experiments to map out the PSF at early detection times are underway, and will result in a better understanding of propagation of early arriving photons through biological tissue, and better techniques for seeing through the human body using light.

References:

[1] D. Delpy, M Cope, P. van der Zee, S. Arridge, S. Wray, and J. Wyatt., Phys Med Biol. 33, 1433-1442, 1988.

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Future Directions of Optics in Medicine

This past November, the Spectroscopy Laboratory and the MGH Wellman Laboratories co-sponsered a workshop on future directions of optics in medicine, which generated a lot of enthusiasm and interest. Two of the speakers preapared articles summarizing their presentations. We hope you enjoy reading them.

Spectral Diagonisis: Learning from Pathology's Mistakes

Maryann Fitzmaurice

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Cleveland, OH

For the past fifty years, *in vitro* microscopic pathologic examination has been the gold standard for disease diagnosis in tissue. In that time, pathologists have encountered most of the problems and made most of the mistakes that can be made in developing a diagnostic test. Now optical spectroscopy offers the potential for real time *in vivo* tissue diagnosis, a possibility that could revolutionize the clinical diagnosis of disease and ultimately the practice of pathology. Hopefully, as spectroscopy evolves as a diagnostic tool, spectroscopists can learn from pathologists' experience in applying the common statistical measures of diagnostic test performance.

Many early optical spectroscopy diagnostic studies were small- scale proof of principle studies, intended primarily to show that optical spectroscopy could be performed *in vivo* and that the information obtained could provide the basis of a clinically useful diagnostic test. These studies have clearly shown the potential of several types of spectroscopy, including reflectance, light scattering, fluorescence and Raman spectroscopy for tissue diagnosis in a variety of clinical settings. [1,2]

Optical spectroscopy is now maturing as a diagnostic modality. And, specific diagnostic spectroscopic tests are being proposed for more extensive testing in larger scale clinical trials. In doing so, some objective measure of spectroscopic diagnostic test performance must be made as a basis for comparison with conventional diagnostic techniques.

The most common measures of diagnostic test performance used in clinical medicine are statistical and include sensitivity, specificity, predictive value and test efficiency (Table1) [3]. Ideally one would like to develop a diagnostic test with 100% sensitivity, specificity and predictive value. But in the real world this

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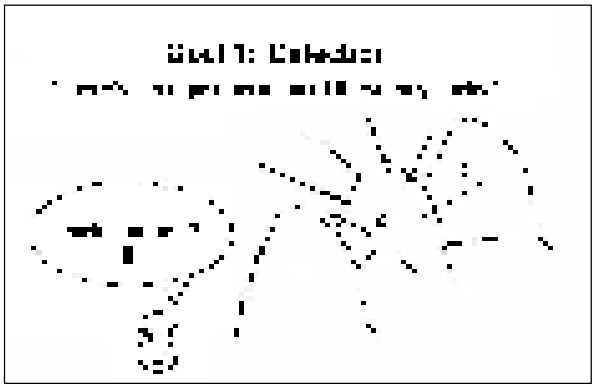
Hide and Seek in Medical Imaging with Optical Photons: Matching the technique to the Medical Condition

Steven L. Jacques

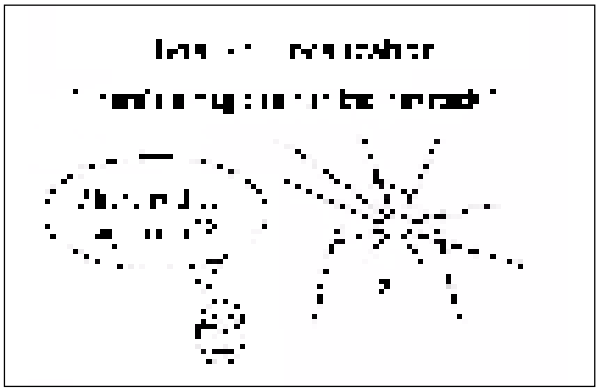
Oregon Medical Laser Center

There are three major goals in medical imaging: (1) detection, (2) localization, and (3) characterization. Detection is an initial screening tool. Localization is a medical imaging task. Characterization is often a spectroscopic fingerprint defining the constituents or nature of a lesion. (See Figs. 1)

(A)



(B)



(C)

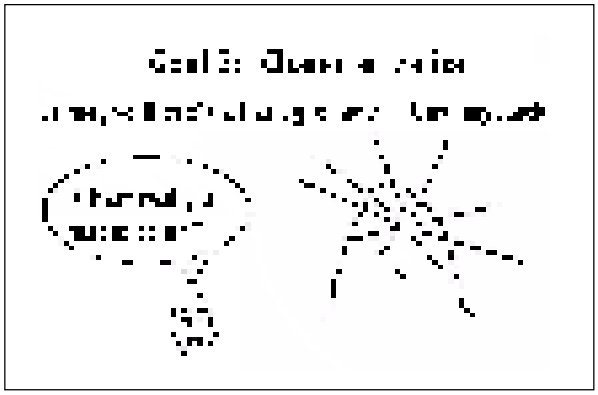


Fig. 1: Three major goals of medical imaging: (A) Detection; (B) localization; (C) characterization.

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Application of Optics to Genomics in Medicine

Tuesday, April 18, 2000, 3:30-6:30 PM

Massachusetts General Hospital
Shriners Auditorium

Problems and Opportunities for Geonomics in Medicine
Paul Matsudaira, MIT Biology Department/Whitehead Institute

Integrating Molecular Analysis into Diagnotic Medicine and Pathology
Dr. Thomas M. Baer, Arcturus Engineering, Inc.

DNA to Chips to Information
Richard Rava, Affymetrix

Panel Discussion
Michael S. Feld, Moderator, MIT

Refreshments at 3:30 P.M.
Sponsored by MIT Laser Biomedical Research Center,
MGH Wellman Laboratories, MIT Industrial Liaison Program &
Harvard-MIT Division of Health Sciences and Technology

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Seminar on
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SPRING SEMESTER 2000

February 1	**Vladan Vuletic,** Stanford University New Ways to Cool Old Atoms
February 8	**Thomas C. Killian,** National Institute of Standards and Technology An Ultracold Neutral Plasma
February 29	**Axel Görlitz,** MIT Light-Bound Matter in a New Light: From Monochromatic to Bichromatic Optical Lattices
March 7	**Mara Prentiss,** Harvard University Atom Optics on a Chip
March 14	**Jun Ye,** JILA Modern Laser Spectroscopy: Ultrasensitive, Ultrastable, and Ultrafast
March 28	**Katrin Kneipp,** Technical University Berlin Single Molecule Raman Spectroscopy: From Fiction to Fact
April 4	**Adam Wax,** MIT Wigner Phase Space Distributions: Watching Optical Ripples
April 11	**Sunney Xie,** Harvard University Single-molecule Imaging, Spectroscopy, and Dynamics of Biological Systems
April 18	**Todd Gustavson,** MIT Precision Rotation Sensing Using Atom Interferometry
May 2	Ninth Annual Richard C. Lord Lecture **Daniel Kleppner,** MIT One Hundred Years of Quantum Physics
May 9	**David Norris,** NEC Research Institute Self-assembled Photonic Crystals
May 16	**David M. Jonas,** University of Colorado at Boulder 2D FT Spectroscopy: Single Molecule Dynamics on a Femtosecond Timescale?
May 23	**John Doyle,** Harvard University Magnetic Trapping of Ultracold Neutrons
May 30	**Larry Ziegler,** Boston University Nonresonant Spectrograms: Looking Inside Femtosecond Signal Pulses

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,
Research Laboratory of Electronics, Schools of Science and Engineering,
and Industrial Liaison Program,
Massachusetts Institute of Technology and Rowland Institute for Science

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Table 1. Statistical Measures of Diagnostic Test Performance [3]

TP = true posititive (# of diseased patients correctly diagnosed)
FP = false positive (# of healthy patients misdaagnosed as diseased)
TN = true negative (# of healthy patients correctly diagnosed)
FN= false negative (# of diseased patients misdiagnosed as healthy)
Sensitivity = TP/(TP + FN)
Specificity = TN/(TN + FP)
Positive predictie value = TP/(TP + FP)
Negative Predictive value = TN/(TN + FN)
Test Efficiency = (TP + TN)/(TP + FP + TN +FN)

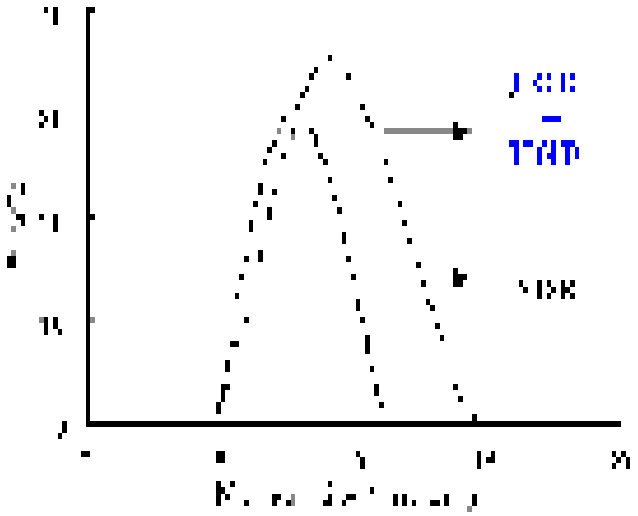

Figure 1: Overlapping populations of cell nuclei in non-dysplastic and dysplastic Barrett's epithelium studied by LSS (LGD = low grade dysplasia; HGD = high grade dysplasia; and NDB = non-dysplastic Barretts).

is for all practical purposes impossible. The reason is that for most test parameters there are overlapping populations. This was observed by Backman et al [4] in their light scattering spectroscopy (LSS) study of the size distributions of dysplastic and non-dysplastic epithelial cell nuclei in Barrett's esophagus, a precursor to adenocarcinoma of the esophagus (Figure1).

Unfortunately, with overlapping populations there is usually a trade-off between sensitivity (or positive predictive value) and specificity (or negative predictive value). When the diagnostic threshold is changed, one usually goes up and the other down. So, the real question is where to set the diagnostic threshold. Should you optimize sensitivity, specificity, or predictive value? The answer, as pathologists have learned, is that it depends upon the clinical situation.

Consider, for example, a spectroscopic technique for the *in vivo* diagnosis of Barrett's esophagus at endoscopy, such as the technique of Wallace et al [5]. Their algorithm uses as diagnostic parameters quantitative, LSS-based measures of epithelial cell nuclear enlargement and crowding, two criteria used by pathologists in the microscopic diagnosis of dysplasia. There are several ways that this type of test might be used clinically. It might be used to direct endoscopic biopsy to areas of increased likelihood of dysplasia, to be confirmed *in vitro* by conventional microscopy. Or, it might be used to make a real time *in vivo* diagnosis of dysplasia, in order to identify patients requiring annual endoscopic surveillance or to direct laser ablation therapy during the same endoscopic procedure. This type of spectroscopic diagnostic test may need to be optimized differently for use in each of these clinical situations. In fact, both the diagnostic threshold and the definitions of positive and negative results themselves may need to be different in each situation.

The two statistical measures of diagnostic performance reported most often in the medical literature are sensitivity and specificity. Yet, in the majority of clinical situations, it is sensitivity, positive predictive value and test efficiency that best reflect a diagnostic test's performance and clinical utility. When do you want high sensitivity? When the disease to be diagnosed is serious, should not be missed, and is treatable, and false positive results do not have serious adverse consequences for the patient. In this case, you want to identify every single patient with the disease for treatment or further clinical evaluation, even at the cost of misdiagnosing some healthy people as diseased.

Such might be the case for the LSS test for dysplasia in Barrett's esophagus of Wallace et al, were it to be used to direct endoscopic biopsies to be confirmed later by conventional microscopy, in order to identify patients with high grade dysplasia requiring esophagectomy. In this case, the risk of a false positive spectroscopic diagnosis is small, since the diagnosis would be confirmed microscopically. So, the goal would be to identify every possible patient with high grade dysplasia for biopsy, even if it meant biopsying some patients without high grade dysplasia. In this case only spectroscopic diagnoses of high grade dysplasia would be defined as positive. Using this definition of positive and the decision threshold shown in Figure 2A, the LSS test of Wallace et al has a sensitivity, specificity, positive predictive value and test efficiency of 100%, 86%, 29% and 89%. One might assume that a test with a positive predictive value of 29% has little diagnostic utility. But, in this case, it is the sensitivity of 100% that best reflects the test's clinical utility.

When do you want high positive predictive value? When the disease to be diagnosed is serious, should not be missed, and is treatable, and false positive results may have serious adverse consequences for the patient. In this case you want to be certain that every patient with a positive test has the disease in question, even at the risk of missing some diseased patients. Such might be the case for the LSS test for dysplasia in Barrett's esophagus of Wallace et al, were it to be used make a real-time *in vivo* diagnosis of dysplasia in order to enroll the patient in an annual endoscopic surveillance program. In this case, a false positive diagnosis of dysplasia in a patient without dysplasia could subject the patient

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to additional unnecessary endoscopic surveillance. However, all patients at risk of dysplasia, even those with indefinite findings, should be enrolled in annual surveillance. In this case, spectroscopic diagnoses of high grade dysplasia, low grade dysplasia and indefinite for dysplasia would be defined as positive. Using this definition of positive and the decision threshold shown in Figure 2B, the LSS test of Wallace et al has a sensitivity, specificity, positive predictive value and test efficiency of 88%, 94%, 78%, and 75%. In this case, the sensitivity and specificity are lower, but the positive predictive value, the value that best reflects the test's utility in this clinical situation, is substantially higher.

Test efficiency is the least well known of the statistical measures of test performance. But, it is most often the best measure of the clinical utility of a diagnostic test. In fact, experience has shown that, given the choice of several different diagnostic tests for a specific disease, with no prior knowledge of the relative performance of the tests, clinicians will usually end up using the test with the highest test efficiency. When do you want high test efficiency? When the disease to be diagnosed is serious, should not be missed, and is treatable, and false positive and false negative results are equally serious or potentially injurious to the patient. In this case, you want to be certain that the test result is accurate whether it is positive or negative. This is most often the case in clinical practice.

And, such might be the case for the LSS test for dysplasia in Barrett's esophagus of Wallace et al were it to be used to make a real time *in vivo* diagnosis in order to direct laser ablation therapy of foci of dysplasia during the same endoscopic procedure. In this case, the risk of endoscopic laser ablation of a patient without dysplasia is roughly comparable to the risk of not treating a patient with dysplasia. Spectroscopic diagnoses of low grade and high grade dysplasia would be defined as positive and lead to laser ablation, but a diagnosis of indefinite for dysplasia would not. Using this definition of positive and the decision threshold shown in Figure 2C, the LSS test of Wallace et al has a sensitivity, specificity, and positive predictive value 92%, 98%, and 85%, resp., and a test efficiency of 96%, the highest of the three scenarios.

So, in this example, the same data set was used throughout, but positive and negative results were defined differently and different decision thresholds selected for the different clinical applications of the diagnostic algorithm. This resulted in different sensitivities, positive predictive values and test efficiencies, with the most appropriate statistical measure of test performance optimized in each case.

But what about specificity and negative predictive value? As mentioned previously, specificity is one of the measures of diagnostic test performance most often reported. However, in fact, there are relatively few clinical situations in which specificity or negative predictive value is of utmost importance. When do you want high specificity? When the disease is serious but not treatable, knowledge that the disease is absent has psychological or public health value, and false positive results may have serious adverse consequences for the patient. In this case, you want to identify every single non-affected or healthy individual, even at the cost of misidentifying some diseased patients as healthy. An example would be a test to diagnose an untreatable degenerative neurologic disorder such as Alzheimer's disease, where knowledge that the patient does not have the disease is reassuring but misdiagnosis of a patient with another treatable form of dementia may deny him or her appropriate medical treatment.

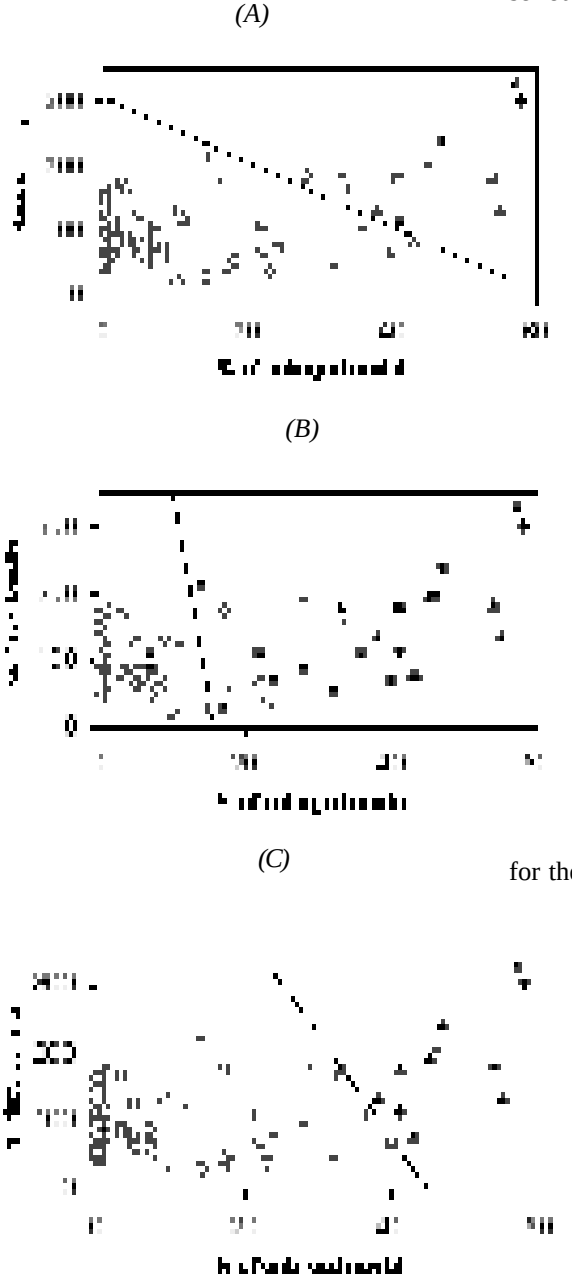

Figure 2. Endoscopic diagnosis of dysplasia in Barrett's esophagus *in vivo*, using nuclear enlargement and crowding determined by light scattering spectroscopy (**O** non-dysplastic Barrett's; ■indefinite for dysplasia (IND); (▲ low grade dysplasia (LGD); ♦high grade dysplasia (HGD)). Note: hollow symbols indicate test results defined as negative and solid symbols test results defined as positive in each scenario.

On the other hand, you want high negative predictive value when the disease is serious but not treatable, knowledge that the disease is absent has psychological or public health value, and false negative results will not have serious adverse consequences for the patient. This is the least common clinical reality. An example would be a test to identify individuals at risk of an untreatable inheritable disease, such as Huntington's chorea, for the purposes of genetic counseling. In this case, individuals who test positive could not be treated themselves, but might be counseled not to have a family in order to prevent passing on the disease to their children.

In the end, the decisions as to which statistical measure of performance to optimize and where to set the decision threshold for a specific spectroscopic diagnostic test must be made together with the appropriate clinicians, with an understanding of the relative risks and benefits to the patients and the particular population to be tested. But, in the final analysis, no matter how these factors are determined, the only thing that matters is whether or not the

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spectroscopic technique works in clinical practice. That is, whether or not it predicts the biologic endpoints of disease progression or response to therapy. So, ultimately, at some stage in the development of a spectroscopic (or any other) diagnostic test, studies with long term patient follow-up must be conducted. Since the field of optical spectroscopic tissue diagnosis is so young, few, if any, of this type of longitudinal study have been done as yet.

Optical spectroscopy is about to enter a new era of rigorous clinical testing and evaluation. As spectroscopic techniques for tissue diagnosis are tested in large-scale clinical trials, it is especially important that statistical measures of diagnostic test performance be well understood and the hard earned lessons learned by pathologists as they blazed the trail of tissue diagnosis not be forgotten.

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The many types of optical imaging can be approximately grouped into two major categories: (1) perturbation methods, and (2) hidden source methods. Perturbation methods are essentially the "ninja in woods at night" problem, in which the unseen object can only perturb the movement of light through a tissue. One example is diffuse optical tomography, in which blood absorption perturbs the diffusion of light from source to detector. Hidden source methods are the "ninja in woods at night with a candle" problem in which the object becomes the source of a signal, a much simpler imaging task. One example is the imaging of a fluorescent object. A second example is photoacoustic imaging in which a laser pulse generates sound in an absorbing object. (See Figs. 2)

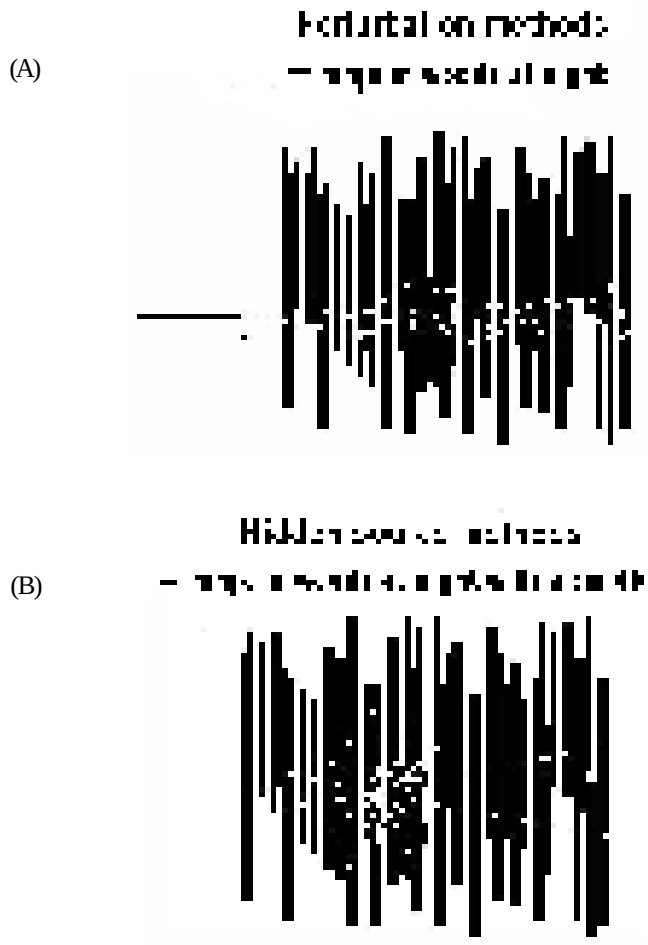

Fig. 2: Types of optical imaging: (A) Perturbation methods; (B) hidden source methods.

Perturbation methods often involve observing a small difference between the expected signal without an object and the measured signal in the presence of an object (Fig. 3A). signal-to-noise ratio can be an issue. Hidden source methods tag the object with some information such as fluorescence emission or opto-acoustic pressure waves. The background is potentially zero and the small signal is more obvious (Fig. 3B). However, one possible

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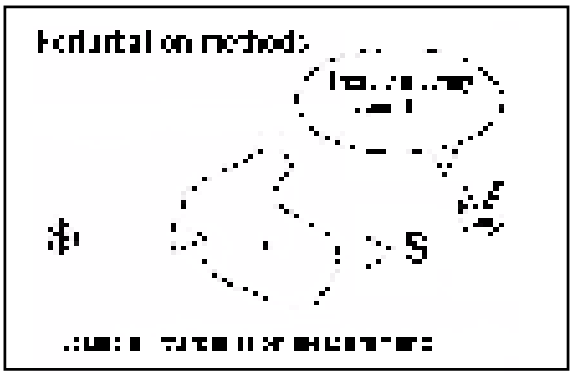
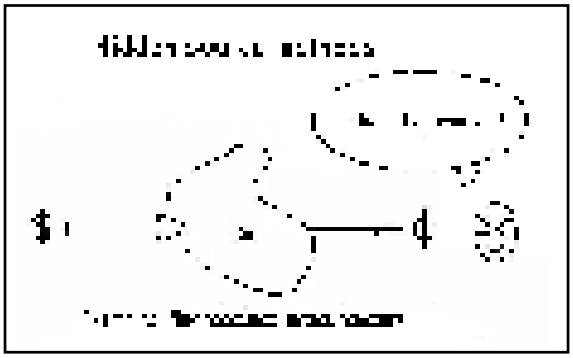
problem with hidden source methods is the amount of background signal that interferes. This "leprechaun effect" (Fig. 3C) is like the story of the captured leprechaun who was forced to tell his captor, a young boy, under which tree his pot of gold was hidden. The boy marked the tree with his neckerchief and went for a shovel. When he returned the leprechaun had marked every tree in the forest with identical neckerchiefs so the boy could not locate the pot of gold. Even if the background fluorescence, for example, is low compared to the fluorescence of an object, the sheer volume of background can still overwhelm the fluorescence from the object.

(A)



(B)



(C)

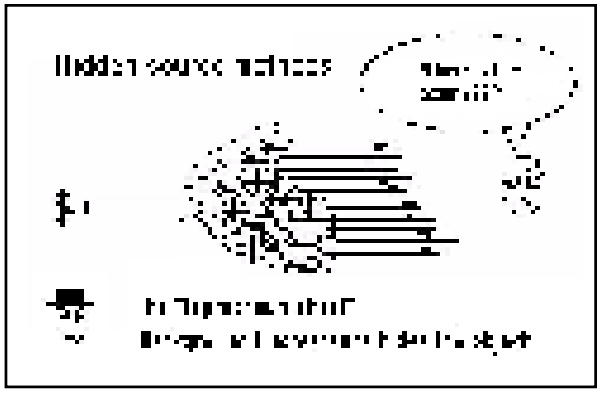


Fig. 3: Types of optical imaging: (A) Perturbation methods; (B) hidden source methods; (C) the "leprechaun effect".

Two types of lesions are usually encountered, each presenting different challenges: (1) superficial lesions, and (2) deep lesions (Fig. 4). Superficial lesions involve small volumes, but they are at the surface for immediate interaction with the delivered light. Deep lesions may involve larger volumes, but they are buried deeper within a tissue volume. Various optical techniques are often better suited for either superficial or deeply imbedded lesions.

Figure 5 lists some example optical techniques which have been classified as either

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Spectroscopy Laboratory Equipment Update

LaVision Picostar HR Intensified CCD Camera

An ultrahigh repetition rate gated intensified CCD camera system used with Coherent mode-locked Ti:sapphire laser. The main parts of the setup consist of (1) an intensified CCD camera (PicoStar HRI, LaVision), (2) a home-built far field microscope, and (3) an imaging spectrometer. A microscope objective (NA 0.6) is used for detection. The Stokes-shifted fluorescece is fed into a single stage spectrometer in Cerny-Tuner configuration for spectral analysis, or imaged onto the detector using a mirror on the spectrometer turret. The intensified CCD camera operates at 76 MHz with a programmable opening gate of 200-1000 ps, operated by high voltage pulsing electronics (HRI controller, LaVision). The CCD intensifier consists of a cooled S25 photocathode/phosphor screen/MCP detector combination with a spectral response of 400-850 nm. A lens-coupled, thermoelectrically cooled CCD chip acts as the photon integrating element within the camera head. To ensure jitter free triggering, a portion of the incident laser beam is used to operate a constant fraction discriminator (LaVision). A programmable delay generator (LaVision) is driven via the serial port of the data acquisition computer in the time window of up to 20 ns with 25 ps increments. Exposure times: 100ns-1000s; Image rate: 30frame/s; Digitization: 12bit.The personal computer with a controller card and the DaVis software (LaVision) provides data acquisition and storage.

This facility is set up with contributions from the MIT Department of Chemistry, the Center for Materials Science and Engineering and the Harrison Spectroscopy Laboratory. It is supervised by Prof. Moungi Bawendi and his associates and is used mostly by Prof. Bawendi and his collaborators to study nano-structured materials.

Hamamatsu C4334 Streak Scope Streak Camera System

A picosecond time-resolved spectroscopy system using Coherent mode-locked Ti:sapphire laser. The streak camera system consists of 3 major components: (1) the streak scope camera (Hamamatsu model C4334-01), designed to take the spectral image from the spectrograph and convert it into a two dimensional image that includes time as a second axis; (2) the imaging spectrograph (Chromex model 250is) with 3

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gratings (100 lines/mm 780nm blaze; 100 lines/mm 450 blaze; 300 lines/mm 500nm blaze) which are software selectable and tunable; and (3) the delay unit (Hamamatsu Model C1097-04) allows delays of up to 32ns in steps of 30ps for use on 1, 2 and 5ns time scales. A second delay unit (Stanford Research Systems DG535) allows for delays on time scales of 10ns and higher. The camera, spectrograph and delay generators are all controlled by the program (Hamamatsu HPD-TA) on a PC. The data can be analyzed using the same software package, which includes a fluorescence lifetime fitting module for HPD-TA (Hamamatsu TA-Fit) giving lifetimes with a resolution of ~50ps and a window up to 1ms limited by the laser repetition rate. The streak camera is capable of measuring time-resolved emission spectra over a 100nm wavelength range in real time. This makes direct comparison of kinetics of different spectral features possible

This facility is supported by the MIT Department of Chemistry and the Harrison Spectroscopy Laboratory. It is supervised by Professor Dan Nocera and his associates. At present it is used mainly by Dr. Nocera's group to study the mechanism of electron transfer in biological systems (i.e. Cytochrome C) porphyrin-based donor-acceptor systems, and by Professor Tim Swager's Group to examine the sensing mechanism of TNT sensors. ■

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a perturbative method (P) or a hidden or tagged source method (S). Understanding the class of an optical technique with its inherent strengths and weaknesses and the nature of the lesion, whether superficial or deep, is a first step toward evaluating whether an optical technique is appropriate for a particular medical condition. ■

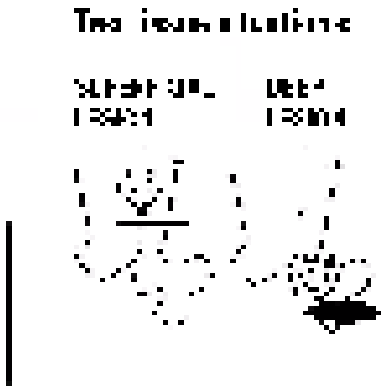

Fig. 4: Superficial objects versus deep objects.

Diffusioon Optical Tomography	P
Time gated transmission	P
Reflectance spectral imaging	P
Raman spectal imaging	S
Fluorescence spectral imaging	S
Coherence gating (Optical Coherence Tomography, OCT)	S
Photoacoustic imaging	S
Ultra-sound modulated light transmission	S

Fig. 5: Some example optical techniques classified as either a perturbation method (P) or a hidden or tagged source method (S).