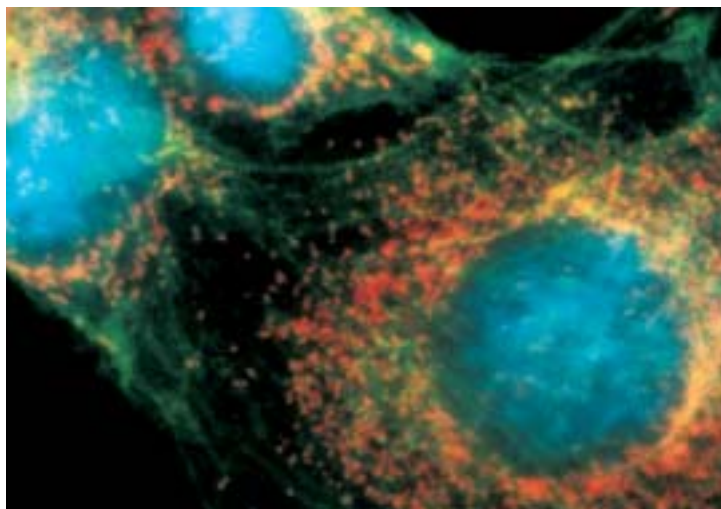


Rays of Hope

Spectroscopy as an Emerging Tool for Cancer Diagnostics and Monitoring

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MOLECULAR PROBES

Vibrational and fluorescence spectroscopy, laser light-scattering techniques, and other optics-based approaches are gaining momentum in the quest for early detection and more effective treatment of cancer.

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This year, more than a half-million people will die from the most-feared of human diseases — cancer. The American Cancer Society's Cancer Facts and Figures 2003 (1) lists this and many more grim statistics. For example, more than 1.3 million others will be diagnosed with a form of the disease in 2003, an annual rate of new diagnoses that officials expect to double by 2050, thanks to our aging population. In all, nearly nine million living Americans are currently either coping with or have a history of cancer. Heart disease may claim more lives. Sudden outbreaks of infectious disease tend to command more headlines. But it seems as if no other disease touches, transforms, and torments as many lives as cancer does.

Perhaps one of the most frustrating things about cancer is its stubbornness. The sequencing of the human genome, the advent of biotechnology, the discovery of new surgical techniques — none of these innovations, nor the vast sums of money spent to develop them, have yet brought cancer within the crosshairs of modern research. And the treatments of choice — chemotherapy, radi-

ation, invasive surgeries — often seem more devastating than the disease itself. Even the vital act of biopsy can, in some cases, pose a traumatic experience for the patient.

We all know that much about cancer — whether you've had it, whether you've known someone who has, or whether you just plain fear it. But many people are discovering something new about cancer that certain spectroscopists have known for some time. They're discovering that tumors, like everything else in our physical world, are simply objects composed of matter. Thus, it's only a matter of time before spectroscopists perfect the art of detecting, measuring, and understanding what makes cancer cells tick. They're not there yet, but they're getting close.

Spectroscopy is not and will never be a cure for cancer, but the National Cancer Institute (NCI) is taking a hard look at how optical technologies can change the way the disease is characterized, studied, and treated. As illustrated by NCI's 2004 federal budget request (2), there is a growing acknowledgement that measurement technologies such as spectroscopy and microimaging have an important and growing role to play in the early diagnosis of cancer, the examination of tumors, and even the treatment of certain types of the disease.

The government's more focused approach to funding cancer research based on spectroscopic diagnosis, biopsy, and characterization comes as good — if somewhat belated — news to researchers who have spent decades honing these techniques. Around the world, several academic research groups, private enterprises, and government laboratories have been using spectroscopic and related instrumentation to investigate the mysteries of the cancer cell.

This article, though far from an exhaustive summary, reports on some of today's most intriguing cancer research based on spectroscopic technology. It also touches on some of the opportunities, challenges, and priorities facing this still-emerging field.

DOLLAR SIGNS: FUNDING AND GOVERNMENT PRIORITIES

Funding opportunities for cancer research are distributed across the full spectrum of government, industrial, and philanthropic sources. But in terms of federal funding, NCI — one of the National Institutes of Health (NIH) — is a major indicator of trends and opportunities.

NCI's \$5.9-billion budget request for fiscal year 2004 (2) identifies numerous priorities that would suggest a growing appreciation for optical technologies in cancer research. The agency has targeted the following general categories for its research initiatives: genes and the environment; signatures of the cancer cell and its microenvironment; molecular targets of prevention and treatment; cancer imaging and molecular sensing; cancer communications; and the interface of aging and cancer. From this list, it is clear that the agency is targeting molecular-level research, powered by imaging and characterization, as the best strategy for advancing the discovery and development of new prevention and treatment methods.

NCI states (2) that investments in imaging technology during the past 25 years have dramatically improved patient care. Imaging has permitted earlier detection, more precise therapies and surgeries, and heightened ability to monitor biological processes without biopsy.

"Indeed, more imaging resources are devoted to the study and treatment of cancer than to any other disease," states the NCI web site (<http://plan.cancer.gov>). "And, in many ways, the needs of cancer research and treatment drive the direction of imaging research. As we learn more about the molecular basis for cancer, this level of sustained investment in cancer imaging becomes even more necessary and productive."

Of course, there are many instrumental methods of clinical imaging,

and conventional optical spectroscopy is not currently as widely used in routine diagnostics as many believe it could be. However, NCI is expressing the desire to identify new microimaging and functional imaging technologies to fully exploit the discoveries generated through genomics and proteomics.

To further its objective to expand the discovery, design, and development of novel imaging agents and diagnostics, NCI has prioritized a range of projects to fuel research in the area. The agency has requested nearly \$35 million to pursue this goal with projects such as a \$5.3-million effort to establish a research resource based on data banks of standardized digital image and spectroscopic data associated with known clinical outcomes. Other NCI priorities include a \$14-million objective to increase clinical trials of imaging methods and technologies. If approved, this plan would set aside \$10 million to initiate multiinstitutional clinical evaluations of various spectroscopic and imaging technologies for the early detection of various cancers and for the monitoring of tumor response to therapy. As the financial supporters of cancer research increasingly target their efforts at the molecular level, spectroscopic technology could have a major role to play in fulfilling these general objectives.

SIGNS OF LIFE: A BIOMEDICAL SPECTROSCOPY COMMUNITY FORMS

Optical biopsy. Biodiagnostics. Biovibrational spectroscopy. The field emerging from the application of spectroscopy to biomedical measurement challenges is in search of a name that sticks. However, definitively naming this diverse range of research pursuits may be premature. There is still a long way to go before scientists have racked up the sufficient number of success stories on which to base a new discipline that is also viable in the commercial marketplace.

Nonetheless, one of the earliest signs that an area of research is beginning to heat up is the sudden appearance of conferences and publications — and, often, fledgling entrepreneurial ventures spun out from academic or military research.

Biomedical spectroscopy based on vibrational or optical techniques has spawned all of these. The public acceptance of magnetic resonance imaging (MRI) — spectroscopy's look-alike cousin — for diagnostics and screening surely has focused the search for an alternative approach for the biomedical market that exploits the strengths of the ultraviolet (UV) or infrared (IR) wavelengths.

Like-minded scientists engaged in this search tend to seek one another out to compare notes, and several conferences have emerged on the scene to facilitate this exchange. For example, in September 2003 at the University of Nottingham (United Kingdom), the Royal Society of Chemistry presented a Faraday Discussion conference (3) entitled, "Applications of Spectroscopy to Biomedical Problems." Running the better part of three days, this event began with an overview of the field, followed by an international collage of research spanning the range of vibrational spectroscopic — Fourier transform-infrared (FT-IR), Raman, circular dichroism, FT-IR microspectroscopy — as well as fluorescence, various magnetic resonance, and mass spectrometric methods. Researchers disclosed results from studies of salivary gland tumors, epithelial cancer cells in whole tissues and in tissue cultures, and prostate cancer, in addition to new observations of cancer cell behavior and general improvements in early detection.

The Nottingham conference follows closely on the heels of another United Kingdom-based event, the Second International Conference on Biomedical Spectroscopy (4), which was held July 5–8, 2003, in London. Similar in interdisciplinary scope to the aforementioned conference, this event focused more broadly on issues such as biomedical and clinical science, biochemistry and biophysics, biomaterials and biosensors, proteomics and pharmaceuticals, and biotechnology and biomedical engineering.

SPIE, the international society for optical engineering (Bellingham, WA), has conducted a biomedical optics conference since 1985 (5). BIOS 2003, held in January 2003 as part of SPIE's Photonics West conference and trade show,

included spectroscopy-oriented programs on manipulation and analysis of biomolecules, cells, and tissues; optical diagnostics and sensing; multiphoton microscopy in the biomedical sciences; and spectral imaging instrumentation, applications, and analysis. Other recent offerings have included programs on "least-invasive diagnostics," with conference tracks on optical biopsy, bio-

medical vibrational spectroscopy, and other symposia combining spectroscopy specialists with cancer experts.

Spectroscopic investigation of biological molecules is a long-established field of study. However, these gatherings, and the publications they spawn, may be signs of a growing momentum in the application of that accumulated knowledge toward the real-world goal of medical

diagnostic equipment suitable for use by caregivers in the clinical setting.

SIGNS OF DISEASE: SPECTROSCOPY IN CANCER DIAGNOSIS

Many spectroscopists feel strongly that IR techniques can spot cancers that slip by other techniques (6). Especially when used in conjunction with IR microscopy, the technique has been used to identify and map functional groups in tissues, using vibrational modes as an intrinsic contrast mechanism. Some have shown that IR microspectroscopy is a high-sensitivity technique for observing changes in cellular biochemistry and variations in tissue architecture. IR as a nonsubjective verification technique complementary to conventional histological examinations of tumor tissue is just one of many practical applications envisioned. But the goal of IR researchers has not been limited to detecting tumors. Many groups use IR to monitor the biological processes resulting from chemotherapy and other treatments, to provide pathologists with results more rapidly — often in real time — so that surgeries and surgical biopsies are as quick and easy as possible for the patients. This makes sense not only from a patient-care perspective but also from an economic standpoint; minimized surgeries take less of the surgical team's time, and they enable faster recovery times and shorter hospital stays.

IR has been found to be promising for these types of applications as well as for diagnostic work. For example, researchers have tried to show IR spectroscopy's potential for assessing the effects of chemotherapy more rapidly than the standard techniques of flow cytometry and DNA ladder formation, without the need for reagents. IR was compared against these accepted methods in the study of apoptosis in leukemic cells. Other groups around the world have reported a range of applications using IR microspectroscopy for such studies as the biopsy of human colon and bladder sections, the detailed study of single eukaryotic cells, and multiple ways of studying breast tumor tissue.

Raman spectroscopy is also of growing interest to many researchers because, although it is based on an inherently

weak signal, it is highly specific. Raman spectra can reveal subtle changes in tissues and cells, theoretically permitting earlier diagnosis and treatment. In addition, Raman enjoys a key advantage over other optical techniques in its relative indifference to water, a key component of biological tissues. A tutorial (7) on the use of Raman in these and other applications was written by Brian T. Fisher of

the University of Florida's (Gainesville) biomedical engineering and physiology program.

Raman has also been investigated as an in vitro diagnostic tool for detection of cancers of the breast, brain, colon, bladder, uterus, ovary, and cervix. Raman can zero in on important biological markers including metabolites such as citrate and glycogen, phospholipids, glucose, and

DNA content. Adapting the technique for in vivo diagnostics has, however, remained a challenge, due in part to the interference arising from the silica optical fibers and elements used in fiber-optic-based instrumentation. Efforts to improve the performance of Raman in vivo have focused on overcoming the limitations of the optical fiber materials, but other factors are also at work. The transferability of spectra among instruments, the creation of useful spectral databases for biological samples, and the development of on-line signal analysis techniques aimed at tissue classification all remain as hurdles before Raman can realize its potential as an in vivo diagnostic tool (7).

Other techniques — some new, some well established — are being evaluated anew with an eye toward early detection of cancer cells. The in situ detection of precancerous conditions based on quantitative optical imaging and spectroscopy was reported (8) by a group from the University of Texas at Austin. These researchers are developing apparatuses based on fluorescence spectroscopy, reflectance spectroscopy using polarized and non-polarized light, 3-D real-time fiberoptic confocal imaging, and optical coherence tomography. Their devices are based on low-power light sources, fiber optics, and semiconductor detectors to provide painless, noninvasive, and instantaneous measurements. The group focuses on curable precursors to epithelial cancer in the cervix, ovaries, and oral cavity. They report that information similar to histological analysis can potentially be obtained in real time, saving patients the pain and expense of a biopsy.

SIGNS OF HOPE: SHINING LIGHTS IN CANCER DIAGNOSTICS

As the field of biomedical spectroscopy mushrooms, more and more research groups are adding diagnostic projects on to their agendas. In addition to cancer, groups are focusing on a range of other conditions such as diabetes and heart disease. A few of these groups are highlighted here.

Spectroscopic diagnosis owes much to Michael S. Feld (9), who essentially laid the groundwork for the field in

1985 by showing that fluorescence could be used to diagnose atherosclerosis. Feld is a professor of physics and director of the George R. Harrison Spectroscopy Laboratory at the Massachusetts Institute of Technology (MIT, Cambridge). He has directed MIT's Laser Biomedical Research Center since founding it in 1985.

Feld's group has pioneered several diagnostic applications of spectroscopy. In 1991, he showed that Raman spectroscopy can be used for histochemical analysis. In 1998, his group developed the technique of light-scattering spectroscopy for measuring the size distribution of epithelial cell nuclei to characterize precancerous change. The group developed another new technique in 2001, the method of trimodal spectroscopy. This clinical technique combines fluorescence and reflectance for spectral diagnosis. Feld and his co-workers are currently exploring methods of low-coherence optical interferometry to measure nanometer length changes and small-scale dynamical processes in biological systems, with the aim of studying fractal structure and nonlinear dynamics in tissues, cells, and nuclei.

The quest for better imaging options to diagnose breast cancer at its earliest stages has inspired Bruce Tromberg (10) to create a device based on frequency domain diffuse optical spectroscopy. Tromberg is director of the Laser Microbeam and Medical Program at the University of California-Irvine's Beckman Laser Institute. His hand held scanner reportedly points out physiological changes in breast tissue by detecting changes in water, fat, and hemoglobin concentrations. The experimental device is said to be sensitive to subtle changes such as cellular growth and proliferation of the blood vessels surrounding a tumor — changes not always visible to other techniques such as mammography, ultrasound, or MRI.

Overcoming the weaknesses of mammography is a major goal of biomedical spectroscopists. Maryann Fitzmaurice, of the Department of Pathology at Case Western Reserve University (11), is part of a multiinstitutional collaborative group — a group that includes Michael Feld and others from MIT — working on new

technologies for the spectroscopic diagnosis of disease in human tissue. Incorporating fluorescence, light scattering, and Raman into a range of programs, Fitzmaurice and her colleagues have studied microcalcifications in the diagnosis of breast cancer using Raman spectroscopy. Microcalcifications are among the most common abnormalities detected with mammography, but fewer than 10% of suspect microcalcifications are found to be associated with malignancy upon follow-up biopsy — a false-positive that leads to costly and unnecessary surgery. The group uses a system that first locates microcalcifications within individual breast ducts using phase-contrast microscopy and then collects a Raman spectrum from these formations using confocal microspectroscopy. Principal component analysis helps the researchers detect subtle chemical differences in the microcalcifications that correlate with the underlying breast disease.

Catching cancer early is one goal of biospectroscopists. Another is to make cancer surgery, when required, as rapid and inexpensive as possible. In certain breast-conserving tumor-removal surgeries, the surgeon removes the malignant tissue along with a small margin of surrounding normal tissue. This specimen is subject to intraoperative surgical pathology to determine if all malignancy has been removed, which adds time and expense to the surgery. Nirmala Ramanujam (12), a biomedical engineering professor at the University of Wisconsin-Madison, seeks a spectroscopic solution that can do the work of the pathologist in real-time and noninvasively. Ramanujam's group is developing an optical method based on fluorescence and diffuse reflectance spectroscopy in the UV/Vis region to guide the surgical excision of breast tumors. The method can provide sensitive and specific detection of tumor margins with submillimeter spatial resolution.

Optical imaging and microscopy are at the root of Vadim Backman's work at Northwestern University (Evanston, IL [13]). Backman is looking for new minimally invasive optical diagnosis and imaging approaches to detect early cancers. He employs light-scattering spectroscopy to analyze nano- and microar-

chitectures of living tissues, and he also delves into the fractal organization of cells and tissues.

Near-infrared (NIR) spectroscopy has been studied heavily for its potential in the analysis of blood glucose, but relatively few groups have yet employed NIR for detection of malignancy. At Texas A&M University (College Station [14]), researchers at the Optical Biosensing Laboratory are using NIR in the 2.0–2.5- μm region because it allows them to obtain in vivo results using fiberoptics with no interference from the presence of water.

Other optical techniques involving laser-induced fluorescence or light scattering have made headlines in recent months. Perhaps not spectroscopic in the truest sense, some of these techniques share the same goals and much of the same equipment. For example, the *New York Times* recently reported (15) on a laser-based "instant pathology" system developed by Watt W. Webb and co-workers at Cornell University (Ithaca, NY). This system, the newspaper reported, capitalizes on the ability of certain biological substances, such as amino acids and collagen, to luminesce at distinctive wavelengths when exposed to laser light in a two- or three-photon microscopy method. Webb's system skips the need for a pathologist by biopsying living tissue at the single-cell level, revealing unusual shapes or changes in cells and their connecting tissue. The technique may make an effective complement to MRI, which has far greater penetrative powers but yields lower-resolution measurements. The Cornell researchers told the *Times* that their method could be used to verify suspicious regions in the body found by MRI or conventional endoscopy.

A team of Oregon researchers (16) is developing a low-cost approach to skin-cancer diagnostics using the principles of polarized light scattering and simple equipment acquired at a nearby discount electronics store. Steve Jacques and graduate researcher Jessica Ramella-Roman, with the OGI School of Science & Engineering at Oregon Health & Science University (Portland) and the Oregon Medical Laser Center (Portland), hope to create effective op-

tical biopsy and surgical methods for skin cancers. Their light-scattering approach is designed to overcome the natural, deep-layer scattering of photons in skin that can obscure the signals from cancerous cells nearer the surface.

Jacques and Ramella-Roman have developed a system based on two polarized filters; it takes two simultaneous measurements of vertically and horizontally polarized light, and a computer is used to subtract the deeply scattered signals from the signals of interest closer to the surface. One goal is to devise a commercial instrument that is easy to use and priced within the means of practicing dermatologists. Jacques had proven that the technique can work on more sophisticated equipment, but wanted to demonstrate that it could also be built cheaply — their apparatus includes two off-the-shelf digital web cameras, a gutted computer-game player, and some standard optical equipment, all reconfigured for work with patients. The team is now planning to build a slightly more expensive model that could give clearer images yet, but would still be affordable for practitioners.

Max Diem, a long-time leader in the field of biological spectroscopy, has developed an active research program aimed at developing potential biomedical applications of vibrational spectroscopy (17). His group, at City University of New York's Hunter College, balances their advanced research in vibrational circular dichroism spectroscopy with a program aimed at applying IR microspectroscopy to study human cells and tissues. The Diem group uses the latest microspectroscopic techniques to acquire the spectral signatures of entire cells and tissue sections, and has found these signatures to be good indicators of a cell's state of health, differentiation, and maturation. This work has broad potential in the automatic screening for, and monitoring of, diseases such as cancer.

This review can only touch upon a few of the groups performing novel studies aimed at cancer diagnostics and monitoring — another sign that the field is rapidly expanding.

CONCLUSION

With cancer firmly entrenched as one of the nation's top killers, spectroscopists have every reason to direct their research toward its prevention, diagnosis, and cure. While many researchers focus on the use of optical measurement technologies in the identification of new drugs and drug targets, or on the fundamental behavior of biological molecules, a growing number of others are working on the other end of the disease continuum. Spectroscopists such as those profiled here are helping to give us the best possible chance to fight this killer by catching it early, diagnosing it accurately, and treating it effectively.

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