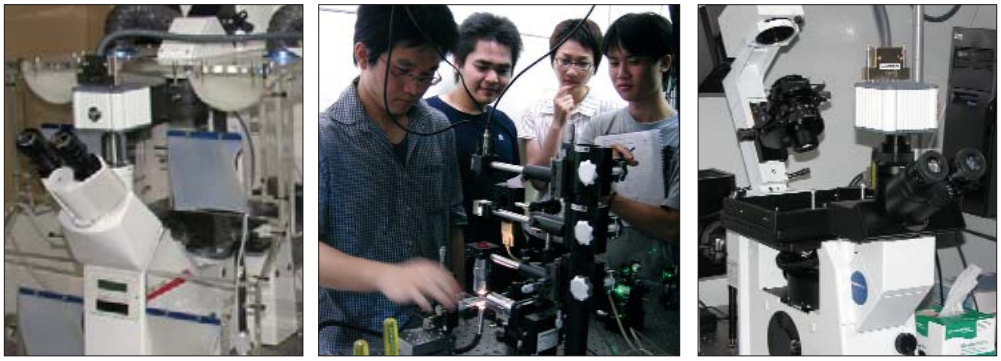

Harnessing Light for Life

An Overview of Biophotonics

Arthur E. Chiou

Biophotonics explores the interaction of light with biological materials. With myriad applications in medicine and the life sciences, the field has emerged as one of the most active frontiers of interdisciplinary research in the 21st century.

Over the past decade, optics has become well recognized as a field that plays an enabling role in a wide spectrum of modern sciences and technologies.[1] At the same time, biomedical research has greatly expanded our fundamental knowledge of the origin of diseases and their treatment.

It is perhaps not surprising, then, that following the successful integration of photonics into modern information science and technology, its role in biomedicine has begun to receive more and more attention.

Indeed, the number of researchers, meetings and publications devoted to the emerging field of biophotonics has escalated worldwide. Over the past two to three years, conferences and symposia on the topic have been held on almost every continent. At Taiwan's annual photonics conference in December 2004, for example, biophotonics was spun off for the first time as one of eight parallel sessions.

In 2002, the National Science Foundation established a Center of Biophotonics Science and Technology (CBST) in Sacramento, Calif. Faculty members at the University of California, Davis, and research staff from Lawrence Livermore National Laboratory play a key role in the center, which has an annual budget of $5.2 million for the first 10-year incubation period. Also in the United States, the National Institute of Biomedical Imaging and Bioengineering (www.nibib.nih.gov) was established in 2001 by the National Institutes of Health. It had a budget of approximately $282 million in 2004.

At the National Yang-Ming University in Taiwan, we have established a broad range of advanced optical tools and techniques at the Imaging Core, a biophotonics research core facility, over the past year and a half (Fig. 1). The facility promotes biomedical applications of photonics via educational programs, hands-on training for biomedical researchers and interdisciplinary research projects. As one of the active players in this exciting field, I have enjoyed visiting many biophotonics laboratories worldwide.

What is biophotonics?

Biophotonics encompasses the study of photonics in biomedical applications as well as that of biological materials and systems for use in photonics—although the latter is not currently a major focus of the field.

In basic life science, optical imaging and sensing at the cellular and molecular levels have played an increasing role in investigations into signal pathways associated with cellular activities in response to environmental changes. Within the realm of biophysics, applications in

Figure 1. The Imaging Core, a biophotonics research core facility, was established at the National Yang-Ming University in Taipei, Taiwan. Live cell/tissue imaging system (*left*), students in the lab (*center*) and a Raman spectro-microscope (*right*). [www.ym.edu.tw/ustymu]

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The recent development of semiconductor nano-particles (or quantum dots) as fluorescence markers has further enhanced the biomedical applications of fluorescence-based sensing and imaging techniques.

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biophotonics have enabled the study of DNA and cellular mechanics[2,3] via optical tweezers[4,5] and stretchers[3] (Fig. 2), and of cellular plasma membrane dynamics.

The main objective for biophotonics research within biomedicine is to improve disease diagnosis and therapeutics. Investigators have used many optical techniques to improve biomedical imaging and sensing—including microscopy, spectroscopy, polarimetry (or ellipsometry) and interferometry—as well as for quantitative detection of biomolecules both *in vitro* and *in vivo*. In therapeutic applications, light has been guided to interact with cells or tissues either directly (via photothermal therapy and photo-ablation such as in laser-tissue welding and laser surgery) or indirectly via light-assisted photochemical reactions such as in photodynamic therapy.[6]

According to the CBST (www.biophotonics.ucdavis.edu), current challenges for biophotonics researchers include the development of tools to enable:

- Molecular-scale imaging of living cells;
- Identification of single, abnormal cells among healthy ones;
- Enhanced understanding of DNA damage and repair;
- Sequencing of single DNA molecules; and
- Non-invasive medical diagnosis or therapy.

The following sections highlight several examples that illustrate in more detail how photonics science and technology are being harnessed for biological and biomedical applications.

Optical coherence tomography

Optical coherence tomography (OCT)—a coherent interferometric imaging technique capable of three-dimensional scanning—is moving quickly from research labs towards practical clinical applications.[7,8] It is ideally suited for non-invasive dermis- and retina-related clinical and basic studies. Recently, modified versions of OCT, such as polarization-sensitive OCT and optical Doppler tomography, have greatly expanded the technique's potential applications.

In OCT, a light beam from a broadband (or, equivalently, short coherence length) light source is split into two beams. One goes through the reference arm, and the other through the signal arm, of a Michelson interferometer. Light that is backscattered from the three-dimensional test object in the signal arm is recombined with that reflected from a mirror in the reference arm in order to generate an interference signal.

The short coherence length of the light source ensures that, of all the backscattered light, only the photons that originated from a specific layer with an optical path length equal to that of the reference arm contribute overwhelmingly to the interference signal. Optical biopsy is achieved by scanning the reflecting mirror in the reference arm along the optical axis, while the two-dimensional image in each section can be obtained by scanning either the signal beam or the test object over the transverse plane.

Polarization-sensitive OCT could be used to help researchers distinguish malignant tissues from healthy ones based on the difference in birefringence caused by the structural orientation of the tissue fibers. Optical Doppler tomography may have applications for *in vivo* mapping of the speed of blood flow; in conjunction with MRI, it could become an effective tool for cardiovascular studies.

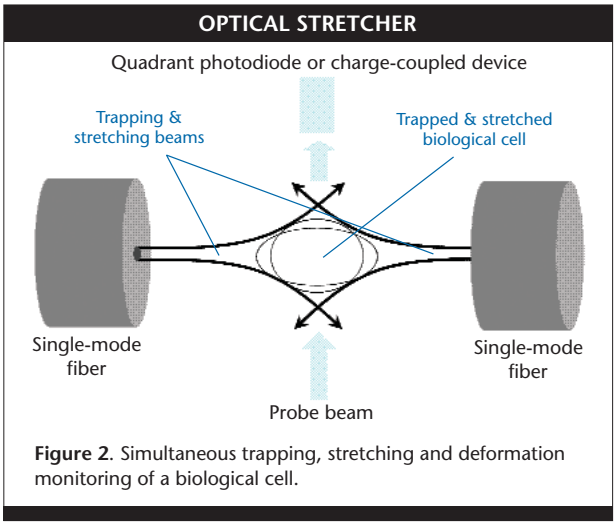

Figure 2. Simultaneous trapping, stretching and deformation monitoring of a biological cell.

Functional microscopy/spectroscopy

Historically, microscopy and spectroscopy have played critical roles in the biological and medical sciences. The discovery of cells by Robert Hooke around 1665 and of bacteria by Antoni van Leeuwenhoek in 1695 would have not been possible without the aid of their microscopes.[9]

In recent years, microscopy has evolved at an unprecedented rate, largely due to advancements in optical science. For example, new laser sources and laser lines, both continuous wave and pulsed, have become increasingly available and affordable, and some have ultra-short pulse widths on the order of a few femtoseconds.

Novel tools and techniques have also been developed to control and deliver the stream of photons precisely and cost-effectively. Moreover, tremendous improvements have been made in the sensitivity, signal-to-noise ratio and spatial and temporal resolutions of optical detection and imaging devices and systems, including single photon counting.

In addition, new methods for sample handling, delivery and tracking have been made possible through the development of micro-electromechanical systems, microfluidicity, quantum dots and fluorescence proteins.

Thanks to advances in optics, traditional three-dimensional morphological imaging has been transformed into multi-dimensional functional imaging. Researchers have added one dimension in the time domain (e.g., fluorescence life-time imaging and time-gating), two dimensions in the spectral domain (excitation spectrum vs. emission spectrum) or two dimensions in the polarization domain (polarization state of the probing beam vs. that of the reflected or scattered beam).

Modern digital technology enables scientists to fuse this optical hyper-dimensional imaging with other

modalities such as MRI (magnetic resonance imaging), PET (positron emission tomography), ultrasound and X-ray for efficient functional imaging.

Scientists have invented a wide variety of techniques in three-dimensional morphological imaging to overcome the spatial resolution limit imposed by classical diffraction theory. For example, a novel nonlinear optical imaging technique with saturated structured illumination has been demonstrated to achieve a spatial resolution on the order of 60 nm, with an illumination wavelength of 533 nm; this is better than the previous resolution limit by almost a factor of five or better.[10] The improvement is achieved essentially by deconvolution via sophisticated mathematical algorithms.

Research at Japan's Osaka University has led to another technique for overcoming the classical diffraction limit: combining the multi-photon effect with a metallic nano-tip, such as in the metallic-tip-enhanced microscopy for nonlinear Raman spectroscopy.[11] The gain in spatial resolution results from the use of a mechanical probe tip much smaller than a wavelength of light.

Taking a different approach, Stelzer and colleagues have recently demonstrated several novel microscopic imaging techniques, including multi-axis imaging microscopy,[12] differential active optical manipulation,[13] and selective-plane illumination microscopy.[14]

Nanomedicine

Nanomedicine is another excellent example of interdisciplinary study. It has emerged as an exciting research topic with important biomedical applications, and the sub-field of nano-biophotonics has been pursued intensively in recent years. Many U.S. universities have responded to a call for proposals from the National Institutes of Health for the establishment of nanomedicine centers throughout the nation.[15]

The recent development of semiconductor nano-particles (or quantum dots) as fluorescence markers has further enhanced the biomedical applications of fluorescence-based sensing and imaging techniques.[16] These nano-particles offer a number of advantages compared to conventional fluorophores. They suffer a lesser degree of photo-bleaching, for example. They also have a single broadband absorption (or excitation) spectrum, which facilitates fluorescent excitation with only one light source, and a relatively narrow fluorescence emission spectrum with a peak that can be custom-designed by controlling their size.

Researchers are pursuing studies on the biocompatibility (or toxicity) of nano-particles with living tissue and novel techniques for bio-conjugating the nano-particles for efficient delivery to specific target molecules, cells or tissues.

Another focus of nano-biophotonic research is on improving the efficiency and efficacy of drug therapy by encapsulating medications in organic nanoshells, such as liposomes or micelles[17,18] (Fig. 3). In this model, an appropriate drug is encapsulated within a nanoshell along with a contrast agent—either fluorophores or radioisotopes. The agent allows for the drug-carrying liposomes to be tracked optically or radioactively after having been injected into the body.

In this example, optical techniques are used not only for tracking the flow of the drug-carrying nanoshells but also for the drug's release once the nanoshells have been identified at their target designation. In addition, one can also use a light activation (or triggering) approach to generate the therapeutic chemical *in situ* via photochemical reactions, such as in the case of photodynamic therapy.

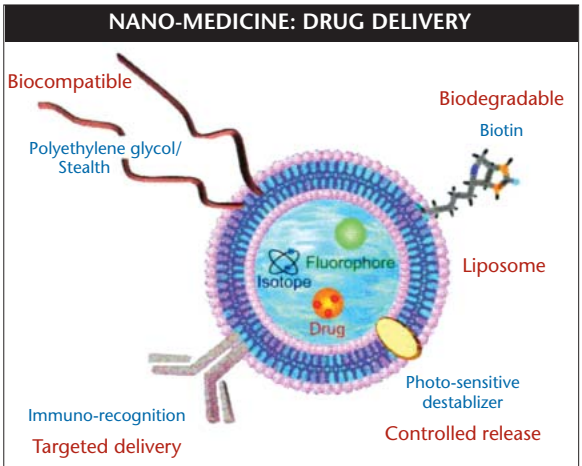

Figure 3. Nano-particles are used to deliver and track drug therapy. Medication is encapsulated in an organic nanoshell with a fluorophore as a contrast agent, which allows the drug-carrying liposome to be monitored optically. [From Ref. 18.]

Optical biosensors

Optical biosensors are capable of recognizing and capturing a specific type of target molecule; optical detection is used to measure changes in the surface caused by the binding of the molecule to its substrate—quantitatively and, ideally, in real time. The sensors have many potential applications in biomedicine as well as for environmental sensing and homeland security, including biochemical warfare.

With DNA micro-arrays,[19] which are one type of optical biosensor, a segment of a known sequence of single-stranded DNA serves as the bioreceptor for a target single-stranded DNA segment containing the complementary sequence. If the target is pre-labeled with fluorophores, researchers can detect the degree of hybridization (or matching of the two DNA strands) using known fluorescence detection techniques.

Researchers routinely fabricate high-density, two-dimensional arrays of cells, each with a known sequence of single-stranded DNA that is immobilized on its surface. The results are interpreted using a chip scanner that has one or more light sources for fluorescence excitation and an optical detector in conjunction with hardware and software to detect the fluorescence signal and to resolve its spatial location. Both the chips and the chip scanners are commercially available for high-throughput screening.[20]

Another popular class of biosensors with superb sensitivity is surface plasmon resonance (SPR) biosensors.[21-23] This tool measures any change in the optical properties of the surface where the receptors and target molecules interact; the binding causes a change in the index of refraction at the interface, which shows

up as a sensitive change in either the SPR angle or the phase of a probing laser beam encountering total internal reflection at the interface. Researchers have pursued studies to extend the single-cell SPR sensor into an array of sensors for multiple target detection.

Optical trapping and manipulation

The first experimental observation of the optical trapping and manipulation of microparticles took place in 1970.[24] Since then, the subject has received increasing attention due to its potential applications in biological, biomedical and nanoscale sciences and technologies. I have chosen to focus mainly on one important development in optical trapping and manipulation: photonics force microscopy (PFM)[25-28] based on the single-beam gradient-force trap—also known as optical tweezers.[29]

By focusing a laser beam to a diffraction-limited spot, optical tweezers offer an unprecedented way to manipulate biological samples and to generate and measure forces on the order of piconewtons —commensurate with the scale of typical molecular biological forces. More important, the use of light in optical tweezers allows experimentalists to perform physiological and *in situ* cellular studies in a less invasive manner than can be achieved by using methodologies involving mechanical probes (such as those in atomic force microscopy).

In PFM, optical tweezers are integrated with a high-speed optical tracking system to measure the three-dimensional Brownian motion of a trapped particle and map out the three-dimensional optical force field on the particle via a computational algorithm. The technique was first proposed and demonstrated by Ernst Stelzer and his co-workers at the European Molecular Biology Laboratory in Germany.

PFM's performance and functionality have been improved progressively, mainly by Stelzer's group but also by several

Medical Applications of Near-Infrared (NIR) Spectroscopy and Imaging

by Britton Chance

Near-infrared (NIR) optical devices could open new fields of medical diagnostics. NIR devices allow for non-invasive, portable imaging because they are capable of examining molecular activity at a depth of much more than a few millimeters. In biologic tissues, hemoglobin, water and fat are least absorbent in the near-infrared spectrum—which spans from about 650 to 900 nm—so NIR light can penetrate the tissue deeply.

NIR spectroscopy and imaging devices have been developed, and many are being used extensively in laboratories. For example, under the support of the Network for Translational Research in Optical Imaging at the National Institutes of Health, researchers are working on new modes of breast cancer detection. They are comparing high resolution imaging in model systems and in human breast cancer with lower resolution spectroscopic approaches for sensitivity and specificity.[1,2,3]

Like other forms of imaging, these methods require that the patient who is being examined be immobilized; in some cases, breast compression is necessary, as it is with mammography. Non-intrusive approaches are also being developed to scan the breast with handheld sensors or even lightweight, pocket-sized early warning indicators.[4]

Investigators are also looking into approaches that use LIDAR (laser radar) principles to detect signals that can penetrate tissue deeply, such as the fetal brain through the mother's abdominal tissue[5] or the adult myocardium through the chest wall.[6,7] The hope is that these technologies could one day free people from needing to have any direct contact with the electronic imaging equipment.

NIR is readily adaptable to co-image with other technologies, particularly MRI and ultrasound; in the latter case, NIR optical imaging resolution has already been increased with sound power strong enough to perturb local tissue volumes. The flexibility and adaptability of the NIR method, both in time and space, often makes it the method of choice for scientific and biological development.

By using flying spot scanning and charge-coupled device or multiple photomultiplier tube sensing emergent photons, new diagnostic tools could be developed that require no contact between sensors and patients' breast, brain or skeletal muscle. Fast gating may make immobilization of the patient non-essential, and fast computer software data analysis could make possible real-time readout images, or coupling to other devices.

NIR could be used in wearable devices for the continual monitoring of body functions, such as in head injury, stroke and epilepsy, and the occasional tracking of more slowly developing pathologies such as cancer. Another developing application is the monitoring of peripheral hypoxia, which diabetic patients must do to avoid amputation.

In all these cases, NIR is complementary to radioisotope and MRI (magnetic resonance imaging) techniques. NIR provides key indications of organ failure by providing metrics of tissue hypoxia and blood accumulation, such as in the brain or the overloaded heart.

NIR imaging is being further developed to reach higher resolution with 10^6 to 10^8 data points, but has not yet reached the level of resolution obtained by ultrasound, X-ray and MRI. However, unlike those techniques, NIR can detect biochemical and molecular beacon signals at relatively low levels, making it unnecessary to use morphological details as a diagnostic aid.

NIR is also an inexpensive technology. Most NIR devices need not cost more than a few thousand dollars at most. Although NIR still requires further development, it is a promising technology that could soon bring medical optics to a hospital or doctor's office near you.

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other research groups in different parts of the world. Current state-of-the-art PFM is capable of a temporal sampling rate in the megahertz range, a spatial resolution in the range of nanometers, and force measurements in the sub-piconewton range within an ellipsoidal trapping volume on the order of 1 μm^3 or less.

One of the biological applications of PFM is to study DNA mechanics (Fig. 4). In this technique, researchers treat a segment of double-stranded DNA (on the order of a few microns or a few tens of kilobase pairs), the surface of a polystyrene bead (diameter on the order of 1 μm or less), and the wall of the sample chamber with prescribed chemical processes such that one end of the DNA molecule can be attached to the chamber wall while the other is connected to a polystyrene bead via a chemical bond.

Optical tweezers are used to ensnare the bead and to displace it, allowing the DNA to be stretched to an extent below the limit set by either the optical trapping force or the chemical bonding at both ends of the DNA molecule. The polystyrene bead thus serves as a convenient handle for optical tweezers to hold and to stretch the DNA sample without any physical or mechanical contact.

In its steady state, the bead is expected to wander around an equilibrium position (slightly displaced from the optical axis) dictated by the balancing of the transverse gradient optical force and the elastic force of the stretched DNA molecule. The position fluctuation of the bead is a manifestation of the Brownian force acting on the bead and on the DNA molecule as well as the conformational change of the DNA molecule.

Specifically, the stretching or the contraction as well as the winding or the unwinding of the double-stranded DNA molecule is revealed by the translational and rotational motion of the bead. The former can be measured via a quadrant photodetector or any other optical position sensing detector, while the latter can also be tracked optically by using a bead with optical birefringence in conjunction with any polarization-sensitive detection scheme.

A non-spherical bead that is illuminated by a laser beam, such as an ellipsoidal bead, could also be used to generate an optical scattering signal modulated in intensity with a strong frequency component at that of the rotational frequency of the bead.

By analyzing the translational and the rotational motion of the trapped bead, researchers can detect the dynamic of the conformational change of a stretched double-stranded DNA molecule interacting with selected molecules in the surrounding buffer solution. The ultimate result is a better understanding of the relationship between the physical and biochemical properties of DNA.

STUDYING DNA MECHANICS VIA OPTICAL TWEEZERS

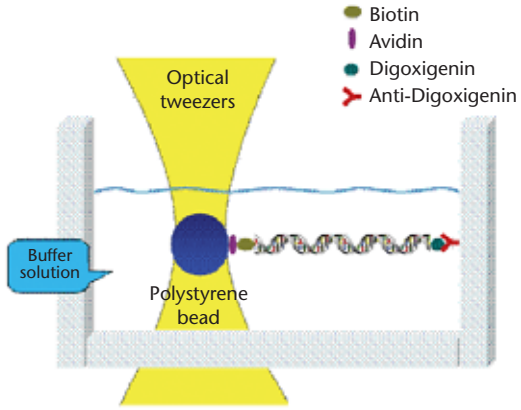

Figure 4. In photonics force microscopy, optical tweezers are integrated with an optical tracking system to measure the three-dimensional Brownian motion of a trapped particle. In this example, the tweezers are used to study DNA mechanics; they ensnare the polystrene bead and displace it, allowing the DNA to stretch and contract.

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