



A New Federal Institute Focuses on Biomedical Imaging & Bioengineering

Donna J. Dean and Brenda J. Korte

Innovative projects in fluorescent imaging and other areas of optics and photonics are funded by the National Institute of Biomedical Imaging and Bioengineering (NIBIB), the newest component of the National Institutes of Health. Convergence of research in the physical sciences with the life sciences is a key focus of NIBIB.

Research and training opportunities highlight NIBIB's role in defining new horizons for cross-cutting work that can reshape biology and medicine.

Throughout history, technological progress has paved the way for major advances in medical science. In the late 17th century, the development of the microscope provided the first glimpse of tiny living structures such as bacteria, cells and capillaries. In the late 19th century, physicists began to harness the power of X-rays, first to visualize internal organs and tissues and later to analyze the structure of biological molecules such as DNA. The expertise of electrical engineers half a century ago led to the first cardiac pacemakers, while at the same time invention of the laser set the stage for laser surgery and innovative imaging tools.

More recently, advances in bioimaging and bioengineering are helping to change our views on everything from how the brain develops to how to treat cancer. In recognition of the remarkable progress in these fields and their potential for reshaping biology and medicine, the Congress of the United States legislated the establishment of a new institute at the National Institutes of Health (NIH): the National Institute of Biomedical Imaging and Bioengineering (NIBIB). As the first component of NIH to be primarily concerned with technology, NIBIB provides a base at NIH for researchers with training in fields such as physics, engineering, materials science and computer science, as well as the life sciences. By encouraging these investigators to submit grant applications, the goal is to promote cutting-edge, multidisciplinary research that might not otherwise receive NIH funding. The tools developed with NIBIB support are expected to have far-reaching applications throughout biology and medicine and to foster new approaches for detecting, treating and preventing disease.

Table 1 shows the research areas that NIBIB is interested in promoting. Most provide rich opportunities for researchers in optics and photonics. NIBIB has a particular interest in developing optical imaging technologies, which have the potential for being safer than imaging tools based on X-rays or ion beams. They also have the potential to be less expensive and more portable than other techniques and therefore could be used by a wider range of healthcare facilities.

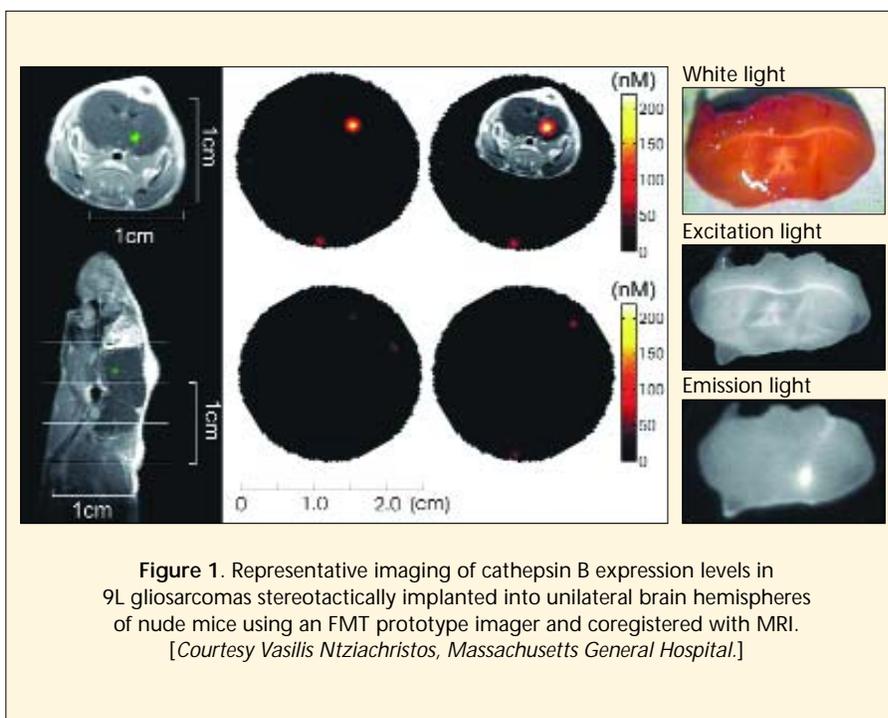


Figure 1. Representative imaging of cathepsin B expression levels in 9L gliosarcomas stereotactically implanted into unilateral brain hemispheres of nude mice using an FMT prototype imager and coregistered with MRI. [Courtesy Vasilis Ntziachristos, Massachusetts General Hospital.]

Current NIBIB research funding opportunities, which can be viewed at www.nibib.nih.gov/research/investigators.htm, include several program announcements and requests for applications that might interest specialists in optics and photonics. One program announcement, titled Novel Technologies for In Vivo Imaging, invites applications for the development of innovative imaging technologies, particularly those that have the potential to provide insight into genetic or molecular disease mechanisms. The goal of these technologies is to provide cellular and molecular information similar to that currently provided by histological or in vitro microarray techniques. Another example is the program announcement titled Systems and Methods for Small Animal Imaging, which calls for the development of devices that can noninvasively image small animals, thereby allowing scientists to study biological processes in living animals.

In addition to providing research funding, NIBIB offers many training programs that may interest those planning to pursue careers in optics and photonics. The institute offers pre- and post-doctoral training opportunities, as well as a Mentored Quantitative Research Career

NIBIB Future Research Directions

- Biosensors
- Biomaterials & Tissue Engineering
- Drug & Gene Delivery Systems/Devices
- Medical Devices & Implant Science
- Micro- & Nanotechnology
- Imaging at Cellular/Molecular Level
- Image-Guided Interventions
- Bioinformatics
- New & Multiple Imaging Modalities

Table 1. The future plans of the National Institute of Biomedical Imaging and Bioengineering include diverse opportunities for researchers in optics and photonics.

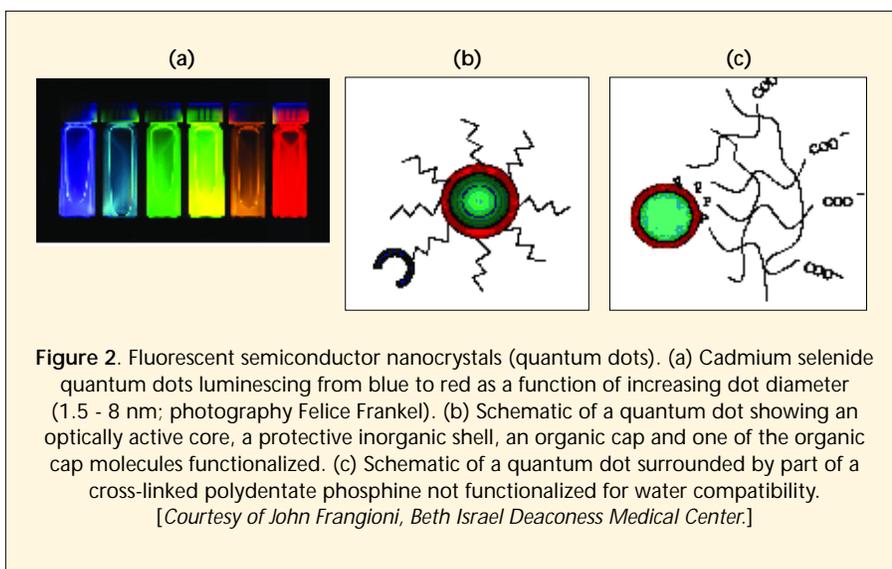


Figure 2. Fluorescent semiconductor nanocrystals (quantum dots). (a) Cadmium selenide quantum dots luminescing from blue to red as a function of increasing dot diameter (1.5 - 8 nm; photography Felice Frankel). (b) Schematic of a quantum dot showing an optically active core, a protective inorganic shell, an organic cap and one of the organic cap molecules functionalized. (c) Schematic of a quantum dot surrounded by part of a cross-linked polydentate phosphine not functionalized for water compatibility. [Courtesy of John Frangioni, Beth Israel Deaconess Medical Center.]

Development Award, which encourages pursuit of research careers in bioengineering or biomedical imaging. Also, in collaboration with the National Science Foundation (NSF), NIBIB co-funds several biophotonics awards and supports the NIH-NSF Bioengineering and Bioinformatics Summer Institutes Program. In this 10-week summer program, undergraduate and graduate students gain interdisciplinary education and research experiences in bioengineering or bioinformatics. Another training initiative under development will provide research training for medical residents at academic centers with NIH-sponsored research. More information about NIBIB's emerging training programs can be found at <http://www.nibib.nih.gov/training/students.htm>.

New imaging methods

NIBIB currently funds several innovative projects in fluorescent imaging and other areas of optics and photonics. At the Massachusetts General Hospital in Boston, Vasilis Ntziachristos has developed a prototype scanner for three-dimensional visualization of fluorescent probes, or fluorophores, in small animals. The device—called a fluorescence-mediated molecular tomography (FMT) imaging system—is being used to study enzymatic activity and gene expression in animal models of cancer, atherosclerosis and other diseases (Fig. 1). The animals are administered molecular “smart”

probes that become fluorescent only after a particular molecular interaction has occurred, such as a tumor-related enzyme cleaving the probe from a larger molecule. The probes are designed to fluoresce in response to near infrared (NIR) light, which penetrates deeper into tissue than visible light. After being administered the probes, the animal is placed in the FMT device, which contains arrays of NIR light emitters and detectors arranged at multiple angles. Using advanced computer algorithms, the device converts the detected light into an image of a tumor or other structure targeted by the probe. Because the light is scattered as it passes through the tissue, the spatial resolution of the NIR images is relatively low. However, by merging NIR images with those produced through magnetic resonance imaging (MRI) or other high-resolution techniques, the precise location of the structure can be seen in relation to the rest of the body.

Using the FMT imaging system, researchers can detect fluorophores at a threshold as low as 10^{-15} moles and at depths up to 15 centimeters, which is substantially better than the few millimeters obtainable through reflectance imaging, the more common method of whole-animal fluorescent imaging. Unlike FMT, reflectance imaging uses only a single emitter and detector.

An advantage of the FMT imaging system over the major forms of medical imaging—such as X-ray imaging, com-

puted tomography, conventional MRI and ultrasonography—is that FMT targets molecular events while the others typically provide images of structure. Thus FMT can identify disease-related abnormalities before anatomical or physiological changes become apparent. Similarly, FMT might be used to monitor how therapies affect cellular function, even before effects to physiology or structure can be detected.

Besides being used to visualize molecular events in animal organs, fluorescence imaging might also enable the study of molecular events within cells. With NIBIB support, Bennett Goldberg at Boston University is developing a new method of fluorescence imaging called spectral self-interference fluorescence microscopy, which shows promise of providing resolution of a few nanometers. By comparison, standard light microscopes cannot discern two objects closer together than about 200 nm in the focal plane. Higher resolution is possible with techniques such as electron or ion microscopy, but these involve killing and freezing the cells or organisms. When fully developed, spectral self-interference fluorescence microscopy may be the first technique to provide high-resolution functional images within living cells.

So far, Goldberg has been able to obtain one-dimensional resolution of 5 nm. In his experiments, fluorophores are bound to a silicon dioxide surface which sits atop a silicon wafer. The fluorophores are illuminated with laser light and the resulting fluorescent emission spreads in all directions, including down to the silicon wafer. The silicon dioxide/silicon interface acts as a mirror, reflecting the light upwards, where it interferes with the light coming down from the probes. Depending on the distance between the probe and the reflecting surface, the ascending and descending light waves may be in phase and therefore additive, or out of phase and subtractive. This self-interference intensifies some wavelengths and diminishes others, creating a light spectrum that varies with the distance between the probe and the reflecting surface. The resulting spectrum can therefore be analyzed to measure the height of the probes and different heights can be color-coded to create an image.

Goldberg is currently working on a technique to obtain three-dimensional resolution, which will be used to study how proteins are transported to sites within bacterial cells. Protein transport is critical to many bacterial functions, including cell division and the ability to infect host tissues.

New imaging agents

The goal of fluorescent imaging is to detect structures in the body using harmless light. Reaching this goal has become a distinct possibility with the development of quantum dots, which are nanometer-sized fluorescent crystals composed of semiconductor combinations (Fig. 2). Quantum dots have many advantages over the fluorescent dyes and proteins currently used for imaging. Since emission frequency varies with the diameter of the crystal, quantum dots can be manufactured to produce light in any color of the rainbow. Furthermore, only one wavelength of light is needed to illuminate all the different colored dots, which would enable simultaneous tracking of many different molecules or cells within the body. Once illuminated, quantum dots shine approximately 1,000 times longer than most fluorescent dyes—in some cases, emitting light for months at a time. A dot that stays lit for years is not out of the realm of possibility. Targeting dots to particular cells is also easier than with fluorescent dyes because the dots can be coated with reactive chemical groups that will bind multiple ligands that attach to the target cells. In contrast, each fluorescent dye molecule can usually handle only one ligand, which makes detecting small numbers of target cells difficult.

To see fluorophores a substantial distance within the body, the emission wavelength must be in the near-infrared wavelength range of 700 to 900 nm. Within this wavelength window, or “tissue window,” light passing through the tissue is less likely to be absorbed or scattered. To create quantum dots that emit in the NIR window, NIBIB researchers John Frangioni at Beth Israel Deaconess Medical Center in Boston and Mounji Bawendi at the Massachusetts Institute of Technology in Cambridge have synthesized dots composed of

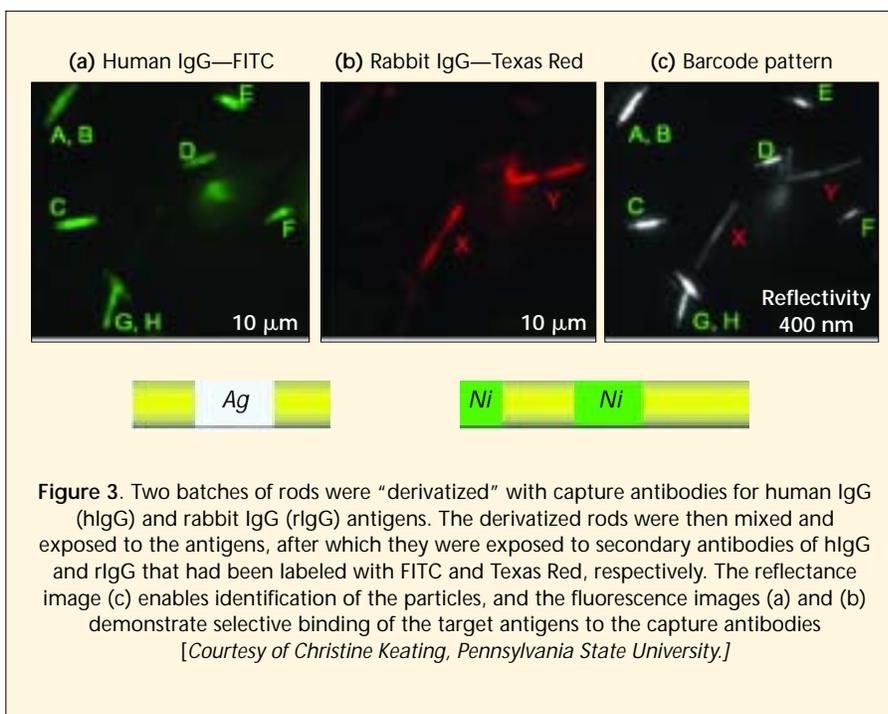


Figure 3. Two batches of rods were “derivatized” with capture antibodies for human IgG (hIgG) and rabbit IgG (rIgG) antigens. The derivatized rods were then mixed and exposed to the antigens, after which they were exposed to secondary antibodies of hIgG and rIgG that had been labeled with FITC and Texas Red, respectively. The reflectance image (c) enables identification of the particles, and the fluorescence images (a) and (b) demonstrate selective binding of the target antigens to the capture antibodies
[Courtesy of Christine Keating, Pennsylvania State University.]

cadmium telluride cores and cadmium selenide shells. Dots with optimal photonic properties will be covered with cancer-specific ligands, which will allow the dots to attach to cancerous tissues in animals.

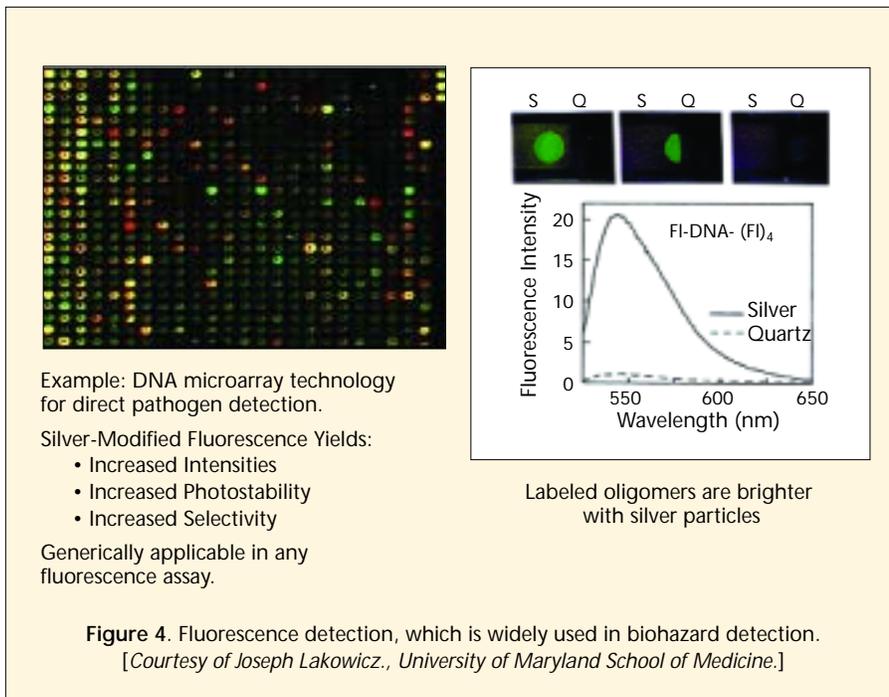
Other tiny particles that will likely revolutionize the way that scientists label biological structures are metallic bar codes. These are cylindrically shaped rods, 30–200 nm in width and 0.4–4 μm in length, composed of layers of different metals, such as gold, silver and platinum. Because different metals reflect light to different degrees, the striping pattern produced by the metal layers can be observed through conventional optical microscopes (Fig. 3). Just like the bar codes on soup cans and other consumer items that are used to track retail store inventories, metallic bar codes could be used by scientists to monitor large numbers of molecules, each of which would have a bar code attached to it. So far, bar codes have been synthesized with as many as seven different metals and 13 distinguishable stripes, making the number of potential bar codes virtually unlimited.

Metallic bar codes are the creation of NIBIB research collaborators Christine Keating and Michael Natan, along with

their research groups at the Pennsylvania State University in University Park. Using bar codes, Keating and Natan have developed assays for molecules that have immense variability, such as DNA and antibodies. The assays created to date rely on observing both the bar code patterns with conventional optical microscopy and the fluorescent tags with fluorescence microscopy. But Keating and Natan are working on an approach that would dispense with the fluorescent tags and simply rely on optical readout. Once this technique is developed, molecular assays of biological processes and systems may become nearly as simple as ringing up food items at the grocery store.

New methods for enhancing fluorescence

Scientists sometimes need to detect agents that are present in minute quantities, and this can be problematic. If the agent is a microorganism, a unique genetic sequence in the organism can be amplified using polymerase chain reaction (PCR), a technique that employs many cycles of DNA synthesis to produce multiple copies of the sequence for use in assays. But the usefulness of PCR is limited by the fact that it is cumbersome and expensive. An alternative approach relies



With its emphasis on cross-cutting research and applications, NIBIB is uniquely positioned at NIH to help guide biomedical imaging and bioengineering projects to fruition.

on fluorescence-based assays in which the agent of interest is labeled with a fluorophore; the fluorophore can be illuminated and the resulting fluorescence detected with a sensor. NIBIB grantees are working on several methods for increasing fluorophore intensity so as to allow for detection of minute quantities of agents, perhaps even down to the level of single molecules.

To enhance fluorescence, Joseph Lakowicz at the University of Maryland School of Medicine in Baltimore places fluorophores near islands of silver particles. The interaction between the fluorescent light and the electric field surrounding the free electrons on the surfaces of the silver particles produces several unusual effects on fluorescent emission. It enhances the light intensity by a factor of 1,000 or more, which makes even ordinarily nonfluorescent molecules, such as DNA, fluorescent (Fig. 4). The light is also oriented in one direction, rather than spreading out in all directions, which makes it possible to

orient the light toward the sensor. Using these properties of metal-enhanced fluorescence, Lakowicz is developing sensitive assays for bioterrorism-related pathogens, such as *Yersinia pestis*, the bacterium that causes bubonic and pneumonic plague. He is also employing this technique to improve immunoassays for prostate specific antigen, an enzyme that is increased in the blood of men with prostate cancer. Lakowicz's colleague Zygmunt Gryczynski is attempting to apply these concepts to create fluorophore-metallic colloid conjugates for use as novel in vivo imaging agents.

At the University of Utah in Salt Lake City, Steven Blair is taking a different approach to enhancing fluorescence. He places fluorophore-labeled compounds inside tiny apertures that have been etched in a gold film deposited on a transparent quartz substrate. The apertures are 100 nm in diameter, which is less than the wavelength of the fluorescent light. Because of limited space, the light cannot move laterally within the

aperture; therefore, light that ordinarily would travel sideways is forced through the aperture, enhancing the fluorescent emission. Proximity of the fluorophore to a metal—in this case, gold—also appears to contribute to the increased light intensity, which totals about 30 times more than normal. Blair hopes to produce DNA microarrays in which single-stranded DNA segments representing different genes are placed in the apertures, followed by a solution of fluorophore-labeled DNA or mRNA segments. Only those labeled segments that are complementary to the DNA segments in the apertures would stick to the array. Shining light on the gold side of the array would light up the fluorophores sitting inside the apertures, and the resulting light could be detected on the quartz side. The beauty of this approach is that it virtually eliminates unwanted fluorescence from unbound species.

What the future holds

The six projects described here are just a sampling of the innovative technologies that NIBIB is helping to create. All six combine research in the physical sciences with research in the life sciences, and all six could have applications throughout the worlds of biology and medicine. With its emphasis on cross-cutting research and applications, NIBIB is uniquely positioned at NIH to help guide these and similar projects to fruition.

It will be interesting to see where these projects lead and what new research horizons will emerge. It is hoped that they will enhance public health and lead to discoveries that are just as influential as those spawned by the discovery of the microscope over three centuries ago.

Acknowledgment

The NIBIB would like to acknowledge the contributions to this article made by Cheryl A. Fee and Equals Three Communications staff.

Donna J. Dean (deand@nibib.nih.gov) is deputy director of the National Institute of Biomedical Imaging and Bioengineering (NIBIB) at the National Institutes of Health, Department of Health and Human Services in Bethesda, Md. Brenda J. Korte (kortebr@mail.nih.gov) is a health scientist administrator in the NIBIB Division of Discovery Science and Technology. Visit the NIBIB Website www.nibib.nih.gov for more information about the institute.