

The Growing Impact Of The NIR Wavelength Band To Biophotonics And In Our Lives

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An observer's report from the NIH Workshop on Optical Diagnostic and Biophotonic Methods

It is a fascinating time to be involved in optics as biological applications grow in leaps and bounds. The National Institutes of Health (NIH) has expanded its innovative “Bench-to-Bedside” funding initiative, which was developed to help speed the delivery of promising laboratory discoveries into new medical and clinical treatments, and it is driving research in optical methods. For the second time, I have attended an NIH Workshop on Optical Diagnostic and Biophotonic Methods¹, a meeting held every other year. This seventh workshop in the series, held in September, was dedicated to the work and legacy of Britton Chance and Mamoru Tamura, leaders in the development of biophotonics. The recent two-day workshop included presentations from leading researchers who are applying biophotonics to challenges in detecting, monitoring, and treating disease. Some sessions focused on specific organs, such as the brain, the eye, or the breast; other sessions centered on techniques like image-guided surgery, minimally invasive imaging, or imaging microscopic circulation of blood. All of the speakers presented compelling evidence that light-based simulation and/or detection is now having substantial impact and will continue to have even greater impact in the future on improving patient health.

I am writing this under the banner of *NIR Trends*, because much of the work in live tissue takes advantage of what is known as the NIR optical window, or therapeutic window. This is where the low relative absorbance of light by tissue molecules permits light penetration to depths of centimeters. *Figure 1* shows the spectral absorbance of hemoglobin, with and without oxygen, of melanin, and of water. The wavelength range of 650 to 950 nm is actively used in biophotonics, a band roughly bounded by the high absorbance of melanin and hemoglobin on the short end of the range and by water absorbance on the longer end. This band has been referred to as the first optical window. For some applications in this window, tissue auto-fluorescence and Rayleigh scattering limit the imaging results. A second window, from 1000 to 1350 nm, escapes the fluorescence limitation completely and takes advantage of even further-

reduced Rayleigh scattering that occurs with longer wavelengths.ⁱⁱ Absorbance by water increases strongly with wavelength, but note the dips in H₂O absorbance seen in *figure 1* at 1050 and 1310 nm. Applications such as optical coherence tomography (OCT) and small animal imaging use those center wavelengths to image structures deeper than they could at shorter wavelengths. For instance, OCT at 1050 nm enables the study of the blood vessels under the retina to understand the development of and to measure the treatment of diseases like macular degeneration. That center wavelength also enables precision measure of the optical nerve head, whose erosion is one of the earliest signs of glaucoma. High-resolution variants of OCT even enable counting individual rods and cone photoreceptors, another way of monitoring the progression of retinal diseases and the effectiveness of treatment.

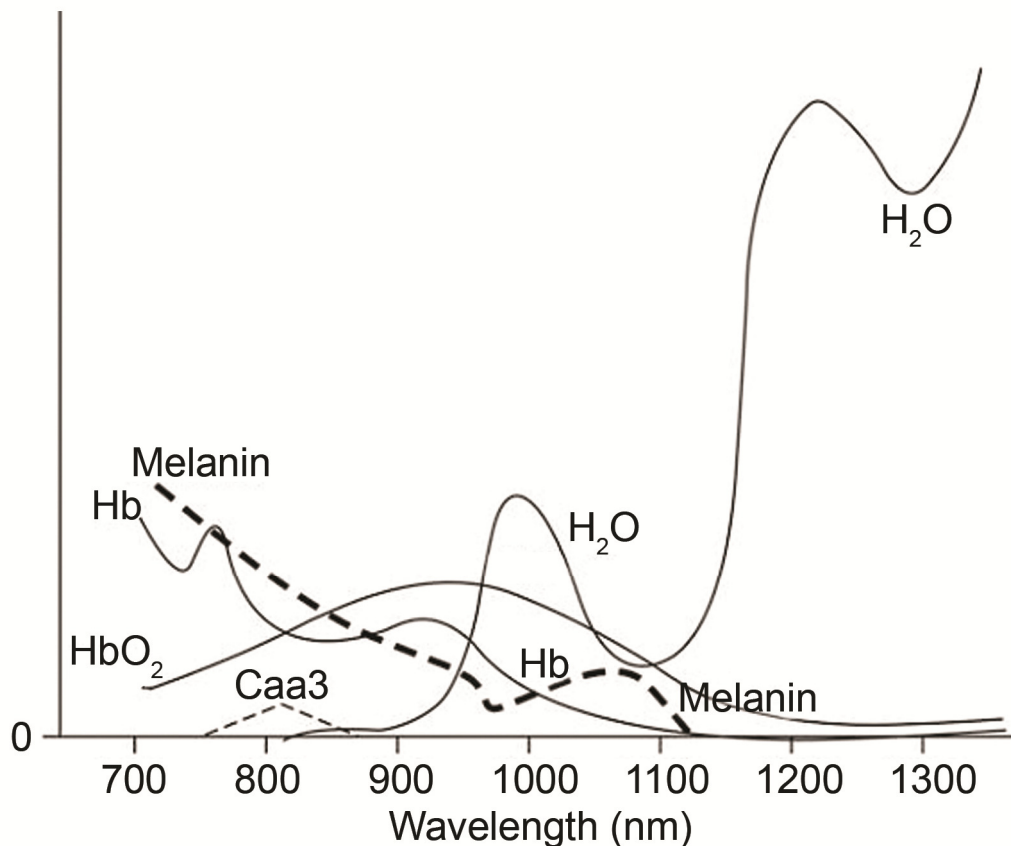


Figure 1: Relative absorbance curves for light-absorbing molecules in tissue.ⁱⁱⁱ

The small animal imaging application mentioned earlier is important to drug discovery because the effectiveness of drug treatments can be monitored in living animals and studied over time. For instance, photoluminescent taggants engineered to latch onto tumor cells permit the tumor size to be monitored externally when illuminated by laser light. The laser can be at a shorter NIR wavelength, like 808 nm, but the glow can be at a longer wavelength, such as 1310 nm. The 808 nm laser penetrates due to low absorbances, but the light is diffusely scattered. This still

provides enough excitation energy to cause emission from the taggants at their longer wavelength. Since it is glowing from within the body, the photons from the taggants only have to make it to the outside, limiting the loss to water absorbance to that of just one pass through the tissue while the reduced scattering of the longer wavelength better preserves the shapes of the tagged tumor or organ. To illustrate the phenomenon, the 58-second video ([see figure 2 video](#)) was acquired using Sensors Unlimited – Goodrich ISR Systems’ high-sensitivity, shortwave infrared (SWIR) indium gallium arsenide (InGaAs) SU320HX camera.

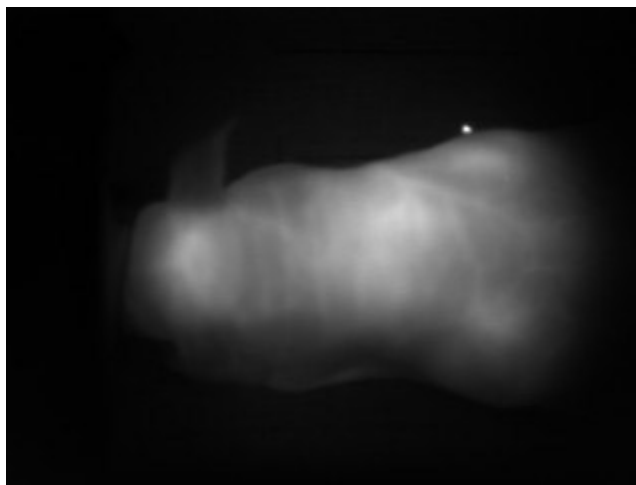


Figure 2: This 58-second [video](#) at 60 frames per second shows the uptake of nanotubes by organs and the circulatory system of an anesthetized mouse, first appearing at the three-second point in the midsection, then in the head. Toward the end of the video, the main circulatory vessels are traceable. Note the intensity flares when the mouse takes a breath. (The static image, shown above, was captured from the video 29 seconds after injection, as the map of the vasculature system is beginning to be visible.)

The photoluminescent still image in *figure 3* was captured 16 minutes after the nanotubes were injected into the small animal (mouse). The SWIR camera’s high sensitivity clearly indicates the bright spots, which are the saturated regions where high concentrations of the photoluminescent taggants have accumulated.

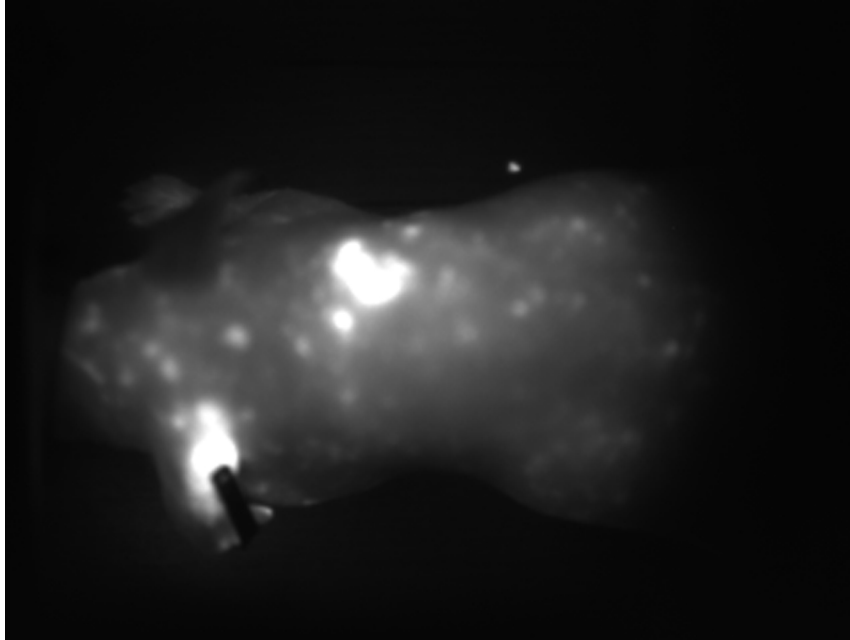


Figure 3: This is a photoluminescent image that occurs 16 minutes after the injection of nanotubes. Sensors Unlimited – Goodrich ISR Systems’ SU320HX camera clearly shows the saturated regions, indicating very high concentrations from the nanotubes that have accumulated in just a few places. (Use your display or editing software to bring up the brightness and reduce the contrast in order to see the full shape of the mouse.) For reference, note the dark rectangle on the bright area in the lower left – it is a metal identification tag clipped to the mouse’s left ear.

One of the earliest researchers to recognize the importance of the optical windows in tissue was Dr. Britton Chance^{iv}, who, in his 50 years at the University of Pennsylvania, trained many students who have each gone on to spread biophotonics concepts around the world. Dr. Chance died a year ago at the age of 97 after a remarkable career. He received his first patent at 18 and won an Olympic gold medal in sailing in 1952. In his professional work, he was an innovative researcher in the study of chemical reactions in the body, which led to the development of numerous methods and instruments, many of them optically based. I visited him in 2004 and realized very quickly that at 91 he had more energy and creativity than any other person I had ever met. To paraphrase an old song, what little he had ever forgotten was more than I ever learned in my lifetime. Most of the presenters at the NIH meeting mentioned the positive impact Dr. Chance had on their personal and research lives.

The first workshop sessions were oral presentations that focused on diseases of parts of the body like the brain, the eye, or the breast. The balance of the sessions focused on imaging methods such as minimally invasive techniques, image-guided intervention or surgery, microcirculation imaging of blood flow, and the use of molecular probes to tag cells, genetic markers, or tumors with luminescent markers for improved imaging contrast. It would be too difficult to capture fairly all of the exciting developments presented, so forgive me for highlighting just a few and recommending that you check out the abstracts at <http://spie.org/Documents/ConferencesExhibitions/NIH2011-workshop-abstract-book.pdf>.

Then look for the Journal of Biomedical Optics *Special Issue: Optical Diagnostic and Biophotonic Methods from Bench to Bedside*, to be published by SPIE. The issue for the 2009 workshop was published last year, and the new issue for the 2011 session will be published in 2012. In the meantime, I will report some highlights for you, saving two of the most impressive findings for last.

Eye Imaging

I lead off with the session focused on the eye. Dr. James Fujimoto of MIT chaired the session and presented an overview of optical coherence tomography plus an update on the technique's progress on the path from "Bench to Bedside." He reported that there were over 15 million OCT eye procedures in 2010, 20 years after his lab made key developments of the technique. He stated that the latest advances in acquisition speed, imaging processing, and deeper imaging depth at longer shortwave infrared (SWIR) wavelengths are "... dramatically improv[ing] the ability to detect small changes in retinal pathology ... improving the ability to assess treatment response, and shortening [the time to prove the efficacy of] new pharmaceuticals."

Dr. Steven Burns of Indiana University School of Ophthalmology spoke about the use of adaptive optics to improve the resolution of scanning laser ophthalmoscopes (SLO). The high-resolution images of rods and cones or of retinal vasculature showed impressive detail. His team developed a system that simultaneously shows two images: a 30-degree field of view and a 1.5-degree FOV section whose position is marked on the larger image for easy navigation. This enables the ophthalmologist to orient

SWIR-InGaAs Cameras For High-Resolution Imaging In Live Tissue



Sensors Unlimited – Goodrich ISR Systems' SU320HX area camera (left) is ideal for SWIR photoluminescence imaging, the most sensitive SWIR camera available due to its large pixel size. The higher resolution SU640HSX (right) and the GA1280J (center) area cameras are well-suited for full-field optical coherence tomography (OCT) and dental transillumination.



The new Goodrich SU-LDH2 SWIR-InGaAs digital linescan camera is a high-speed 1024-pixel linescan camera that increases the A-line rate to over 91,000 lines per second, making it the best choice for fast spectral-domain optical coherence tomography (SD-OCT) applications at 1.05 and 1.31 center wavelengths. The camera is able to capture detailed 3D volumes of the retina, the nerve head, and the choroid layer in the blink of an eye. Please visit www.sensorsinc.com for more information.

the system image to match images of the patient's eye from other sources and then quickly zoom in to examine the problem areas. The dual-FOV system images the retina at multiple levels, mapping rods and cones down to the blood flow in the microvasculature, permitting a detailed investigation of the structural components of eye disease.

Dr. Richard Rosen of The New York Eye and Ear Infirmary also showed advanced imaging of the eye with a confocal SLO system, but this one was combined with a spectral-domain OCT system running up to 100 kHz. It achieved microstructural imaging that exceeds the commercial capabilities of current, separate systems, without the expense, size, or complexity of adaptive optics. Dr. Gadi Wollstein of the University of Pittsburgh Medical Center provided perspective on the clinical use of ocular imaging devices such as OCT, confocal SLO, and scanning laser polarimetry, observing that the "Structural changes, as recorded by ocular imaging devices, have been demonstrated to precede functional changes ... which enables early detection of glaucoma." (An example would be identifying a structural change like the thinning of a layer in the optical nerve head, preceding the patient's noticing a functional problem, like loss of peripheral vision.) As mentioned in the other presentations, the repeatable aspect of the measurements provided by these high-resolution systems enable detecting changes in patients' eyes over time that would otherwise be missed. Thus, they are important advances in eye diagnostics.

Breast Diagnostics

Dr. Bruce Tromberg of the Beckman Laser Institute and Medical Clinic located at the University of California at Irvine organized the session on breast diagnostics. However, he was unable to travel, so his colleague, Dr. Albert Cerussi, gave the presentation. Dr. Cerussi talked about their joint work in the University's Diffuse Optical Spectroscopy and Imaging Lab (DOSI) and the lab's participation in a multiclinic study called American College of Radiology Imaging Network (ACRIN). This study evaluates breast cancer patients' responses to neoadjuvant chemotherapy, a method in which medicine is administered to shrink a tumor before surgery to improve patient outcomes. The primary aim of this clinical trial is to determine whether changes in a tumor's tissue optical index (TOI) in the beginning of therapy predicts the tumor's response to treatment using diffuse-optical-spectroscopic imaging (DOSI) measurements. The DOSI instrument is a bedside device with a handheld probe that obtains functional images utilizing NIR (650 to 1000 nm) absorption and scattering spectra. The TOI is a composite of the oxy/deoxy hemoglobin, water, and lipid spectral response, which provides insight into the biochemistry of tissue, whether from a tumor or the surrounding area. The long-term goal is to provide doctors with a simple bedside tool to help them make informed decisions on the type of chemotherapy to use, its duration, and the timing of surgery.

Dr. David Boas of the Massachusetts General Hospital showed images generated with a tomographic optical breast imaging system, where an array of fiber optics launch light of several key NIR wavelengths for blood-oxygen sensing, through the skin to detectors at various distances away. By reconstructing the light-scattering paths traveled, based on the time delays before detection on different sensors, a tomographic image of the breast shows where tumors

are growing. This is because the fast-growing cells of tumors command more energy and thus consume more oxygen than the surrounding normal tissue. His group is combining this optical diagnostic with X-ray digital breast tomosynthesis (DBT) to overlay structural and metabolic maps of the tissue. This is to better distinguish between healthy, benign, and cancerous tissues. The goal is to improve diagnostic sensitivity of detecting cancer over mammography's 80% while reducing that technique's false positive rate from its current levels between 20% and 30%.

Image-Guided Surgery

Also based on the NIR optical window is spatial-frequency-domain imaging, described by Dr. John Frangioni, who was the session chair for the presentations on image-guided intervention or surgery. As co-director of the Center for Molecular Imaging located at the Beth Israel Deaconess Medical Center in Boston, Dr. Frangioni has been working to develop methods to "see" tissue oxygenation over wide fields of view in real time during surgery. By illuminating the surgery field sequentially with LEDs at each of the spectral wavelengths for blood oxygen, water, and lipids, the images at each wavelength (combined in software) reveal functional information about tissue viability to surgeons as they work. He presented examples from a recent pilot study that confirmed the oxygen levels in tissue were within 10% of the readings made by a reference probe. In a first-in-human clinical trial, the new technique was combined with a NIR fluorescence imaging system called FLARE™ (also developed in his lab). The system imaged skin-flap oxygenation during reconstructive breast surgery, helping the surgeon to recognize damaged tissue and perform successful reattachment.

That lab also developed a smaller version of the fluorescence system, called the mini-FLARE™. Dr. Alex Vahrmeijer of the Image-Guided Surgery group at Leiden University Medical Center in the Netherlands described its usage with over 200 patients in 15 clinical trials. Here, LEDs excite methylene blue or indocyanine green fluorescent dyes on sequential acquisition frames of the camera to image their glow at 700 and 800 nm using exposure times between 30 and 100 milliseconds. By injecting the dyes next to tumors, lymph nodes draining the area of the tumor take up the dye. Glow picked up by the NIR camera then identifies these nodes as the sentinel nodes, the first nodes in the lymph system that would show evidence of metastases, the spread of the cancer beyond the tumor. Surgeons cut out these sentinels to have them evaluated and thus determine how extensive the patient's treatment will need to be.

Minimally Invasive Techniques

OCT made its first clinical mark with eye imaging, but is now receiving global regulatory approvals for use in diagnosing vulnerable arteriosclerosis plaque in arteries. This type of plaque causes heart attacks without warning. Imaging the inside walls of blood vessels with OCT is fast becoming the standard of care for stent placement as it enables the surgeon to be sure the walls are clear of vulnerable plaques before potentially placing a stent on top of one. Endoscopic microscopy, looking inside the body with microscopic resolution, includes the techniques of OCT, optical coherence microscopy (OCM), and confocal microscopy (CM). Dr.

Guillermo Tearney, Associate Director of the Wellman Center for Photomedicine and Professor of Pathology at Harvard Medical School, organized the Minimally Invasive Technique session. He presented an overview of the impact these techniques are having on medical diagnostics and their potential impact on patient care, mentioning that 20% to 30% of stent placements have complications due to misplacement. Globally, cardiovascular OCT systems have now been used in more than 100,000 patient examinations. Dr. Tearney also described work on a new high-resolution spectral-domain OCT (SD-OCT) system with 1-micron axial, 2-micron transverse resolution, which is capable of seeing calcium crystals on the vessel walls.

Dr. Lihong Wang, Professor of Biomedical Engineering at Washington University at St. Louis, presented exciting progress with photoacoustic tomography (PAT), a technique that uses pulsed laser light to induce thermo-expansion in tissue, creating ultrasonic waves. These sound waves travel 7 centimeters without the scattering that limits light travel in tissue to a fraction of that. Thus, by combining light stimulus with sound detection, PAT enables imaging parts of a cell, yet is able to zoom out to imaging whole organs. It responds to light absorbance, making it capable of picking up functional information like the oxygen uptake increase in the earliest stages of tumor development. PAT is a complementary technique to OCT, which images structural information.

Hitting the “bench to bedside” theme of the workshop, Dr. Steven Boppart of the University of Illinois at Urbana-Champaign described his Biophotonics Imaging Lab’s development of primary care imaging systems combining OCT with video imaging in a handheld scanner. Their aim is to develop low-cost tools that primary care doctors can employ in their outpatient exams to manage and refer patients based on quantitative data. The system enables doctors to examine the eyes, ears, oral and nasal mucosa, skin, and the cervix, but the lab is focusing first on the two diseases most frequently encountered by the physician: ear infections and diabetic damage to the retina. OCT permits quantifying bacterial biofilms in the middle ear from the outside and detecting retinal damage earlier than other methods.

Brain Diagnostics

The first two presentations of the workshop were actually the most amazing to me. First, Dr. Ed Boyden of the MIT Department of Brain and Cognitive Sciences described using NIR light to activate and silence brain activity. He displayed neuron electrical activity graphs illustrating the turning off and then turning back on of neural activity for periods of time using different colors of light in one experiment, or the stimulation of activity in other experiments. Genetic manipulation finds and isolates the genes of organisms that use light-absorbing or light-emitting abilities to thrive in their environment. This leads to creation of “optogenetic” tools by transferring the genes into key neuron cells. To enable controlling brain activity, light-sensitive DNA is added to regulate signaling activity. To detect which synapses are active, light-producing DNA is used to create voltage-sensitive fluorescent proteins that glow when a synapse is active. These tools help neuroscientists understand the brain’s functioning in concrete terms and may help to effectively and precisely treat intractable brain disorders, like Alzheimer's or Parkinson's diseases, and even to calm animals experiencing a form of post-traumatic stress disorder

(PTSD). These tools have the potential to impact our understanding of the human brain, the development of useful diagnostics, and have the potential to proactively treat addiction or currently untreatable diseases. For a video of Dr. Boyden describing the work and the future of it, check out his 18-minute presentation at the TED conference:

http://www.ted.com/talks/ed_boyden.html. (At the end of this video, he demonstrates adding light-sensitive cells to eyes of mice that have lost their natural photoreceptors, giving them the ability to see again.)

Even more dramatic was the subsequent talk given by Dr. Ron Frostig of the Department of Neurobiology and Behavior at the University of California, Irvine. Working with mice, his group has been exploring the effects of strokes and potential treatments. Blocking an artery feeding a part of the brain, they were exploring what could be done to reverse the damage. He demonstrated that stimulation of just one whisker within one to two hours of the blockage resulted in restoration of function in the associated part of the brain. Waiting more than two hours reduced the results, and the damage was permanent if one waited three hours. Trying to understand what was preventing the damage when stimulation was present, they used two optical imaging techniques, intrinsic signal optical imaging (ISOI), and laser speckle imaging (LSI). ISOI responds to changes in tissue reflectance due to processes intrinsic or wholly within the tissue, such as blood volume or the level of oxygen in the hemoglobin. LSI responds to movement, in this case to the movement of blood cells in the capillaries. In this method, the cell movement causes changes in the laser speckle pattern that system software tracks to build a map of the blood vessels' locations, the flow rate, and the flow direction. These imaging tools confirmed that the whisker stimulation caused the capillary system to modify itself by bringing fresh blood to the stimulated portion of the brain. As a final confirmation, they blocked the alternate artery feeding the area, causing irreversible damage to the tissue.

One can only conclude that if you are present when someone experiences a stroke, after you first call for help and then make sure the victim is safe while waiting for the help to arrive, you can help the person preserve brain function! Dr. Frostig confirmed that preliminary results of further experiments have shown that you don't have to stimulate a whisker – sound, touch, or visual stimuli can help. So talk to the person, stroke their limbs, show them pictures, and generally entertain and comfort them while waiting for help to arrive.

The two NIH workshops on optical diagnostics that I have attended have been a great opportunity to hear and meet the leading researchers in this rapidly developing field. The ability of these researchers to compare techniques and notes has led to cross-specialty collaborations to combine techniques for the advancement of patient care. Like many NIH-funded activities, the future of the workshop is unknown in this time of fiscal pressure to reduce the budgets. I urge those planning the Institute's funding for the next two years to maintain support for the Optical Diagnostic Workshop and the subsequent publication of the presentations. For my readers, I urge active support for the NIH funding of medical research, as it helps us all by developing cost-effective treatments for disease.

About The Author

Doug Malchow is business development manager for industrial products at Sensors Unlimited – Goodrich ISR Systems (Princeton, NJ), pioneers in the field of shortwave and near-infrared imaging based on indium gallium arsenide (InGaAs) technology. Malchow has a BS/BA in marketing from Rider University and 20 years of experience in instrumentation, imaging, and spectroscopic applications.



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<http://spie.org/Documents/ConferencesExhibitions/NIH2011-workshop-abstract-book.pdf>

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