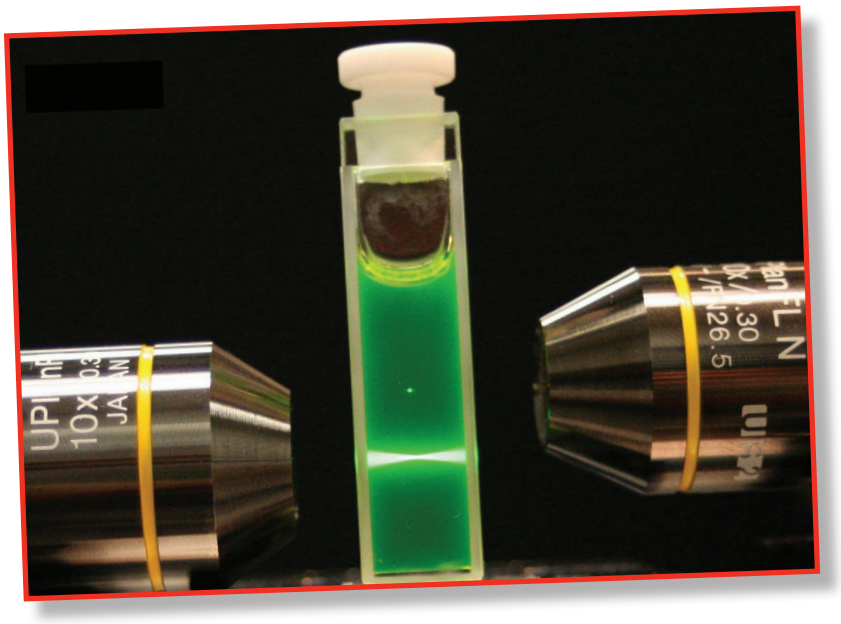

*Kevin Belfield uses two-photon fluorescence microscopy with fluorescent dyes, or probes, to image living tissue with unprecedented resolution at depths up to 2 millimeters in a variety of tissue types.*

## DETECTING EARLY-STAGE DISEASE WITH MOLECULAR TRACKING PROBES

A sudden proliferation of new blood vessels inside the body can bode well or poorly for a patient. If recovering from a wound, the faster new networks form the better. But an unusual growth spurt can also signal the presence of an aggressive cancer.

Kevin Belfield, professor and dean of NJIT's College of Science and Liberal Arts, is developing minimally invasive methods to monitor both: the pace at which wounds heal and the effectiveness of therapies that inhibit the expansion of blood vessels that feed cancerous growth. His probes, which use molecular tracking devices, aim to improve targeting selectivity, homing in exclusively on new blood vessels to detect tumors or monitor wound healing progression at a very early stage.

To heal, lesions depend on the formation of new blood vessels to deliver oxygen and nutrients that support cell growth and tissue regeneration. When the process is well regulated, scars and infections are less likely to occur.

Belfield uses two-photon fluorescence microscopy (2PFM) with fluorescent dyes, or probes (above), to image living tissue with unprecedented resolution at depths up to 2 millimeters in a variety of tissue types. The images, available in subcellular resolution in three dimensions, are captured by transmitting near-infrared light through the wound and, in the case of internal lesions, through an optical fiber. The fluorescent probes, which are injected into the bloodstream, include a biomarker-targeting peptide that binds with a specific protein associated with endothelial cells in new blood cells.

"What we're looking for is high levels of a particular integrin protein, integral to motility and adhesion, that is expressed at elevated levels in endothelial cells in new blood vessels," he says.

The images also provide qualitative information on the blood vessels themselves; chaotic vessel morphology is characteristic of tumors, for example, while vessels in healthy tissue and wounds that are healing are more orderly and symmetrical. Image analysis over time provides quantitative information on blood vessel density as a function of time, information that can be used to assess the success of growth factor, an agent designed to promote blood vessel growth.

"The ability to assess a lesion intact offers an advantage over conventional histological techniques in which the wound is biopsied and thin slices are analyzed," Belfield says, "because the gelatinous nature of wounds at early stages often results in soft, nascent tissue being removed, providing an incomplete picture of critical early stages of wound healing."

The aim of anti-angiogenic cancer therapies, which curb blood vessel expansion, is to starve a tumor (below). Without new blood vessels to feed them, they will only grow one to two millimeters in size before they run out of oxygen. Currently, anti-angiogenic agents are being explored in conjunction with other chemotherapy agents.

Belfield views 2PFM as a potentially powerful tool for the detection of early-stage cancers, including lung cancer, which is most often discovered when it is late-stage and incurable. He also views it as a possible improvement over mammography screenings, which are unable to detect tumors that are less than a centimeter in diameter, whereas 2PFM can image as little as two millimeters of muscle tissue or blood vasculature.

"Well over 50 percent of cancers are not detected until they are at stage three or four," he notes.

Belfield is collaborating with scientists at the University of Tampere in Finland on wound-healing angiogenesis and with researchers at the Sanford Burnham Prebys Discovery Institute in Orlando on blood vessel growth in tumors.

He has studied other methods for treating cancers that involve the use of light, including the deployment of cell-killing agents whose toxic power can be controlled by light, which activates the release of an acid compound that creates a pH imbalance in the cancer cells. Localizing the cell toxicity to those regions where the light detects cancer limits the collateral damage, greatly reducing harmful side effects.

"We're targeting imaging agents to biomarkers as another platform for photoreactive therapies," he says. "This will combine both the ability to image the tumor and treat it through a light-activated agent. These advances coincide with the push for precision medicine, which makes our work timely."

He added that researchers are also looking at 2PFM for use in endoscopy, colonoscopy and bronchoscopy.

"It's a way to gather critical information from any site in the body that can be reached by a thin, flexible optical fiber," adds Belfield. "There are so many applications for this technology in neuroscience as well, including the detection of plaque buildup in the brain. In time, we will have a diverse palette of very useful probes." ■

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