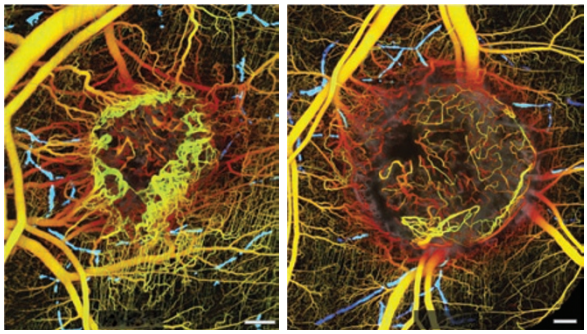
MICROSCOPY

Faster than a speeding blood cell

A new *in vivo* imaging strategy produces detailed maps of tumor microvasculature and lymphatic vessels without injected labels.

After more than a quarter-century of studying tumor biology, Massachusetts General Hospital (MGH) researcher Rakesh K. Jain had grown exasperated with the limitations of available tools for performing *in vivo* tumor imaging. "We were very frustrated about how deep we could go, and trying to compare what was going on deeper in the tumor with what was going on superficially," he says.



OFDI visualization of blood and lymphatic vessels. OFDI reveals differences in the density and structure of vasculature in an untreated tumor (left) and in one treated with an antibody that blocks angiogenic activity of VEGFR-2 (right). Lymphatic vessels, in white, are also revealed in both images. Scale bars, 500 μm. Reprinted from *Nature Medicine*.

Fortunately, things recently changed for the better, thanks to a productive collaboration with MGH imaging specialist Brett Bouma's team, who had developed an imaging technique called optical frequency–domain imaging (OFDI) (Yun *et al.*, 2003). In OFDI, a monochromatic light source transmitted through a tissue-penetrating fiberoptic probe is 'chirped', so that it rapidly steps through different wavelengths as it scans the sample; a detector then receives the 'echoes' of this light as it bounces off of tissue surfaces, enabling the computational reconstruction of the positions of those surfaces based on the wavelength of each echo.

To maximize OFDI's usefulness for the applications Jain had in mind, Bouma's team boosted its detection capabilities, making it possible to accurately measure the extremely subtle signal perturbations resulting from Doppler shifts as blood cells flow toward and away from the probe (Vakoc *et al.*, 2009). "It allows very fine sensitivity to very weak back-reflections," says Bouma. "So if we have just one or two photons scattering from a blood cell, we can detect them and characterize the velocity of the flowing blood cell as it tumbles through a capillary."

With this ultrahigh sensitivity comes great imaging speed, and Bouma and Jain could rapidly acquire tremendous amounts of data that could subsequently be translated into detailed three-dimensional vascular maps. Although Doppler OFDI does not achieve the lateral resolution of multiphoton imaging, it can penetrate much further and routinely delivers images from depths of one millimeter or greater. OFDI also enabled the researchers to quickly image considerable volumes, making it possible to reconstruct complete vascular networks from different tumors and thereby perform comparative analyses. "Breast cancer cells in the breast will respond to various therapies, but once these cells go to the brain, they may not respond," says Jain. "This technology allows us to look at what the blood vessels look like at these two sites, and they look entirely different."

OFDI also offered surprising additional benefits, including the unanticipated capability to image lymphatic vessels, which appear as regions of low light-scattering intensity. As with the blood vessel imaging, these data could be acquired purely via intrinsic signals, with no need for injection of contrast agents or fluorescent tracers. "It was a huge bonus," says Jain; "by superimposing blood and lymphatic vessels, you can see how these two networks interact with each other. We could not get this kind of insight with any other technology before this."

These advantages make OFDI a potent tool for assessing how tumors develop or respond to treatment. In one set of experiments, the researchers monitored reduction in the density of vasculature and in the length and diameter of individual blood vessels in mammary tumors from mice treated with a monoclonal antibody that targets vascular endothelial growth factor receptor 2 (VEGFR-2) over both long (imaging every 2 days) and short (imaging every 2 hours) time scales.

They could even directly distinguish healthy cells from necrotic or apoptotic ones based on changes in tissue scattering; for example, OFDI revealed how tumors treated with diphtheria toxin undergo extensive cell death within 48 hours of exposure in parallel with accompanying reduction of blood vessel length.

Both investigators describe their success as the result of highly effective collaboration between their teams and intend to continue working closely together as they prepare to move this system into the clinic. "The technology … is stable and robust," says Bouma. "Practical deployment in actual tumors in humans just requires overcoming the barriers of access and [developing] probes and catheters and endoscopes." Certain tumors may already prove amenable to OFDI imaging. "Breast cancer may be accessible with this device," says Jain, "and it's probably where the earliest impact on human cancer will come."

Michael Eisenstein

RESEARCH PAPERS

Vakoc, B.J. *et al.* Three-dimensional microscopy of the tumor microenvironment *in vivo* using optical frequency domain imaging. *Nat. Med.* **15**, 1219–1223 (2009).

Yun, S.H. *et al.* High-speed optical frequency-domain imaging. *Opt. Express* **11**, 2953–2963 (2003).