IMAGING

Localization microscopy goes ultrasonic

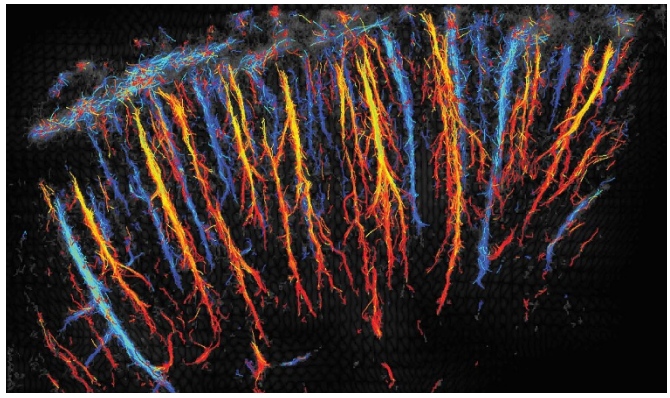
Ultrafast ultrasound microscopy allows for super-resolution ultrasound imaging of vasculature in whole organs.

Imaging with good spatiotemporal resolution in living animals at depths beyond a few millimeters remains a technically challenging task, despite advances in approaches such as optical and photoacoustic microscopy. Ultrasound imaging is noninvasive and is widely used for deep *in vivo* imaging both in basic research and in the clinic, as ultrasound waves are able to penetrate deeply into tissues. However, conventional ultrasound imaging is diffraction limited, meaning that imaging with typical ultrasound wavelengths of around 300 micrometers will achieve only ~300-micrometer resolution, which is much larger than many important biological structures such as small blood vessels.

Mickael Tanter and Olivier Couture at the Institute Langevin and their graduate student Claudia Errico sought to break the diffraction limit for ultrasound imaging. They were motivated "to prove that ultrasound imaging can solve the challenge of noninvasive microscopy deep into organs," says Tanter. To that end, they built upon single-molecule localization methods used for super-resolution fluorescence optical microscopy. Such localization methods generally involve labeling cellular structures with a photoswitchable fluorophore and then imaging the sample over multiple rounds. In each round, a small subset of the fluorophores are randomly switched on and imaged. A precise position is then determined for each fluorophore in the image. These positions from each round are overlaid, resulting in a super-resolution image of the labeled structure.

Because photoswitchable fluorophores are not useful for ultrasound imaging, Tanter and his team needed another source of contrast for their method, known as ultrafast ultrasound localization microscopy (uULM). For this, they turned to microbubbles, which are small, inert contrast agents that can be injected intravenously to label an organism's vascular system.

In uULM, ultrafast imaging, rather than photoswitching, allows individual microbubbles' signatures, or 'echoes', to be recorded. Tanter notes that the high speed is necessary "to distinguish each bubble individually even if there was a very large microbubble cloud in the imaged area." Each microbubble has a unique signature that allows it to be precisely localized and tracked over time at a rate of thousands of frames per second to generate super-resolution images. While imaging one hemisphere of a rat brain, the team recorded around one million images of microbubbles in less than three minutes. From those images, they were able to resolve blood vessels smaller than ten micrometers and nearly one centimeter deep in tissues, an unprecedented resolution at such depths.



Ultrafast ultrasound localization microscopy in the rat brain. Figure reproduced from Errico *et al.* (2015).

Recording ultrasound echoes from the microbubbles, however, was only one of many important steps toward generating super-resolution ultrasound images. Tanter recalls that a key technical challenge was being able to "separate the echoes coming from the microbubbles in vessels from echoes coming from tissues." To separate the signals, the team used a signal-processing step that allowed them to cancel out signal from the tissues, leaving them with only signal from the microbubbles.

In addition to being able to generate super-resolution ultrasound images of the vasculature in the brains of living rats, the team was also able to use uULM to measure the speed of blood flow in individual vessels over a wide range of flow rates, demonstrating the versatility of the approach and its potential utility for studying diseases that affect blood flow.

As ultrasound imaging with microbubbles is already used in the clinic, using uULM in clinical studies was a natural extension of the method, and Tanter and colleagues have already begun imaging human liver vasculature. He notes that the method may also be useful for studying microvasculature in tumors and for imaging strokes. Tanter says that future technical innovations will increase the speed of the method, allowing whole human organs to be imaged in less than five seconds. He also notes that "in neuroscience, we are combining this technique with functional ultrasound imaging in order to image the brain activity at microscopic scales, paving the way to fundamental discoveries about how our brain works."

The uULM method represents an important step forward in deep-tissue imaging, revealing the beauty of an organ's vascular organization. Tanter recalls, "We would never have imagined that these ultrasound microscopy images could be such a piece of art."

Rita Strack

RESEARCH PAPERS

Errico, C. *et al.* Ultrafast ultrasound localization microscopy for deep super-resolution vascular imaging. *Nature* **527**, 499–502 (2015).