Super-resolution photoacoustic imaging could allow scientists to watch blood vessels with improved resolution

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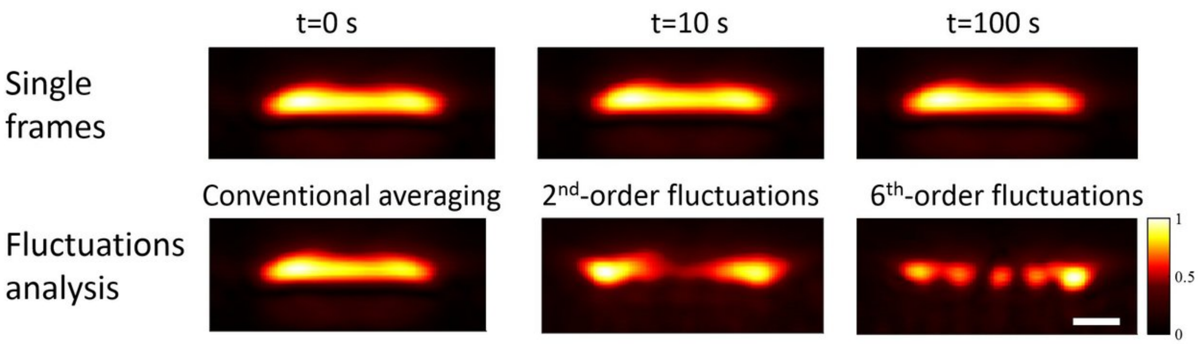

These images compare the imaging of blood flowing through five channels with various approaches. At top are single photoacoustic images from the image stack the researchers analyzed. At bottom left is the result of conventional analysis. The middle and right bottom images show the researchers' fluctuation analysis, with five channels clearly resolved in the final fluctuation analysis. Credit: Bastien Arnal (Grenoble University, Grenoble, France)

Researchers have reported an approach to photoacoustic imaging that offers vastly improved resolution, setting the stage for detailed in vivo imaging of deep tissue. The technique is based on computational improvements, so it can be performed with existing imaging hardware, and thus could provide a practical and low-cost option for improving biomedical imaging for research and diagnostics.

After further refinements, the approach could offer the opportunity to observe the minute details of processes occurring in living tissue, such as the growth of tiny blood vessels, and therefore provide insights on normal development or disease processes such as cancer.

"Our main goal is to develop a microscope that can see the microvasculature and capillary vessels," said Ori Katz, a researcher with the Hebrew University of Jerusalem, Israel, and senior author of the study. "It's important to be able to watch these grow with nearby tumors, for example."

In *Optica*, The Optical Society's (OSA) journal for high impact research, the researchers describe overcoming the acoustic diffraction limit, a barrier that previously limited the resolution obtainable with photoacoustic imaging, by exploiting signal fluctuations stemming from the natural motion of red blood cells. Such fluctuations might otherwise be considered noise or viewed as detrimental to the measurements.

"With photoacoustic imaging you can see much deeper in tissue than you can with an optical microscope, but the resolution is limited by the acoustic wavelength," Katz said. "What we have discovered is a way to obtain photoacoustic images with considerably better resolution, without any change in the hardware."

Overcoming the acoustic diffraction limit

Photoacoustic imaging combines optical illumination (which uses light waves) and ultrasound (which uses sound waves) to image biological samples in ways that would not be possible with either modality alone. Optical methods can provide excellent resolution but often only near the surface as light is highly scattered in tissue. Ultrasound can go much deeper but does not offer the same contrast as optical imaging. By integrating the two modalities, researchers have been able to overcome the drawbacks of each to advance a host of applications.

However, the imaging technique does have certain limitations. Photoacoustic imaging relies on acoustic detection, so the image resolution is determined by the acoustic wavelength. While optical microscopy, for example, can see objects on the scale of less than a micron, photoacoustic imaging is limited to tens of microns. This means that photoacoustic imaging cannot resolve small objects like microvessels or capillaries.

Katz devised the method for surpassing the acoustic diffraction limit in collaboration with Emmanuel Bossy, now at Université Grenoble Alpes in Grenoble, France. At the heart of their work is an advanced statistical analysis framework that they apply to images of red blood cells flowing through the vessels; the blood cells facilitate imaging by absorbing light at particular wavelengths. By increasing the resolution computationally, they avoided the need for any additional hardware, so the advances described can be attained using existing photoacoustic imaging systems.

Drawing inspiration from a fluorescence-based technique

The tools needed to achieve super-resolution with photoacoustic imaging were described nearly a decade ago in a work in optical microscopy with the technique super-resolution optical fluctuation imaging (SOFI). Katz and colleagues came to this work after grappling with the problem of the acoustic diffraction limit and discovered that the same mathematics used with SOFI could be used for improving photoacoustic imaging.

"Someone just needed to make the connection," Katz said. "It's the same equation—the wave equation. Mathematically, you could say it's the same problem."

In a study published in *Optica* last year, Katz and his colleagues demonstrated the ability to surpass the acoustic diffraction limit using a SOFI-inspired photoacoustic imaging technique. That work had two main limitations. First, it required the use of a long-coherence laser, not a standard part of photoacoustic imaging systems, in order to form dynamic structured interference patterns called speckle to create the signal fluctuations. Second, due to their small dimensions, the use of speckles as dynamic illumination resulted in the fluctuations having a low amplitude with respect to the mean photoacoustic signal, which in turn made it difficult to resolve the specimen in question.

In the new *Optica* study, the researchers showed that they could overcome these limitations by applying the statistical analysis framework to the inherent signal fluctuations caused by the flow of red blood cells—so the researchers didn't need to rely on coherent structured illumination—and furthermore demonstrated experimentally that they could perform super-resolution photoacoustic imaging using a conventional imaging system.

Moving toward in vivo use

The demonstration served as a proof of principle for the new technique. The researchers are now focused on developing it further, to fulfill its potential for in vivo applications.

Katz described two main challenges in reaching this goal. The first is the problem of motion artifacts. In their demonstration, the researchers imaged blood streaming through small tubes. In animal models and in humans, though, blood flow is only one of the motions they would have to consider. The technique would also need to account for the heartbeat, the changing volume of the vessels and even microscale movements of the tissue itself.

The other main challenge relates to signal levels. In recent experiments blood was the only absorber in play, but in real-world scenarios other absorbers would be present. The researchers are now working on ways to better see the signal originating from flow while suppressing any background signals.

In addition to tackling these challenges, the team is working to apply sophisticated reconstruction algorithms that will further increase the resolution and background reduction by taking into account prior information about blood flow, the imaging system response and other factors.

More information: T. Chaigne, B. Arnal, S. Vilov, E. Bossy, O. Katz, "Super-resolution photoacoustic imaging via flow induced absorption fluctuations," *Optica*, Volume 4, Issue 11, 1397-1404 (2017). DOI: 10.1364/optica.4.001397

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