New imaging technique may boost research in biology, neuroscience

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Microscopists have long sought to find a way to produce high-quality, deep-tissue imaging of living subjects in a timely fashion. Until now, they had to choose between image quality or speed when it comes to looking into the inner workings of complex biological systems.

Such a development would have a powerful impact on researchers in biology and in neuroscience, experts say. Now Dushan N. Wadduwage, a John Harvard Distinguished Science Fellow in Imaging at the FAS Center of Advanced Imaging, along with a team from MIT, detailed a new technique that would make that possible in a report in *Science Advances*.

In the paper, the team presents a new process that uses computational imaging to get high resolution images at a rate 100 to 1,000 times faster than other state-of-the-art technologies that use complex algorithms and machine learning. The method can turn a process that takes months into a matter of days.

The system, called De-scattering with Excitation Patterning (or DEEP), is believed to be the first of its kind and may one day lead to new understandings of how complicated tissue specimens, like the brain, functions because it can take images that aren't possible with other microscopes.

"Because this has the potential to actually speed up [what you can take an image of along with how fast you can do it], scientists will be able to image fast processes they haven't been able to capture before, like what happens when a neuron fires or how the signals move around in the brain," Wadduwage said. "Also, because it's technically faster, you can image a larger volume of area at one time, not just a small field of view as you would with a slower imaging system. It's like being able to look at a much larger picture, and this is very important for neuroscientists and other biologists to actually get better statistics as well as to see what's happening around the area being imaged."

The system works like many other animal imaging techniques. Near-infrared laser light is used to penetrate deep through biological tissue that scatters the light. That light excites the fluorescent molecules the researchers want to image and emit signals that the microscope captures to form an image.

There have been two main ways these types of images are taken. Point-scanning multiphoton microscopy can penetrate deep into a specimen and capture high quality images. The drawback is the process is extremely slow because the image is formed one point at a time. If the researcher is looking to capture a centimeter-sized image, for example, it can take months. It also limits studies of fast biological dynamics, such as neurons firing. The other method is call temporal focusing microscopy, which is much faster and can capture images at a wider scale but is unable to capture high resolution images at anything deeper than few millionths of a meter. The fluorescent light scatters too much, causing the image to degrade when the camera detects it.

DEEP, however, allows for fast tissue penetration at a wide scale and produces high-resolution images. The system projects a wide light into the subject as in the temporal microscopy method, but that laser light is in a specific pattern. The computational imaging algorithm knowing the initial pattern takes in the information gathered to reverse the process when it gets scattered and then reconstructs it, descattering the image. This is especially notable since it takes the reconstruction of structural features from millions of measurements to tens and hundreds. DEEP can image hundreds of microns deep through scattering tissue comparable to point-scanning techniques.

DEEP is still early years of development but is emerging from its proof-of-concept phase.

"We showed that we can image about 300 microns into the brains of live mice," Wadduwag said. "But since this is only the first demonstration, almost all aspects of the technique have room for improvement."

More information: "De-scattering with Excitation Patterning enables

rapid wide-field imaging through scattering media" *Science Advances* (2021). advances.sciencemag.org/lookup … .1126/sciadv.aay5496

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