OPTICAL IMAGING

Three-photon microscopes see through the intact skull

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The skull of the average lab mouse is about 150 µm thick. That can be hard to see through, even for the two-photon microscope. These microscopes use two photons of light to excite a fluorescent molecule; the resulting fluorescent signal lets researchers image structures like blood vessels and neurons. But tissues—bone in particular—scatter light, reducing resolution and limiting depth.

For a clearer view, researchers often turn to cranial windows, openings through the opaque bone that would otherwise get in the way of the microscope. The surgery needed is routine, but it can cause physiological perturbations to the brain.

Adding a photon results in a longer wavelength that is less prone to scattering and can be more precisely confined to a desired focal volume, explains Chris Xu, an applied physicist at Cornell University. Xu has been developing three-photon microscopy as an extension to its two-photon predecessor. He and his lab previously demonstrated its potential to image deep into the mouse hippocampus through a conventional cranial window. In their latest work, windows become optional.

"It is possible to image through the intact skull of the mouse," says first author Tianyu Wang. "With three photon, it's quite straightforward."

That could help researchers who want a less-invasive look at the brain. "There is increasing recognition that we have to look at organisms in a state as intact as possible," comments Nozomi Nishimura, a researcher at Cornell who was not involved in the current study. "This work is a great example of how technological advances can help us unravel the complexity of in vivo systems."

The team developed a protocol to expose a small area of skull and cover it with a commercially available glue. The glue, tested by their Stanford coauthors, matches the optical refractive index of bone and smooths out any surface irregularities. The result is reduced light aberration and improved resolution, Xu and Wang explain, while keeping the exposed bone protected.

They compared images of brain vasculature and calcium-labeled neurons taken through the skull with two- and three-photon microscopes. The latter had better resolution to over 500 µm. Although the glue's transparency declined somewhat over time, the researchers could repeatedly image the same neurons for over a month, with no signs of stress or damage to the animals.

Though the microscopes can be expensive, Wang hopes their results will encourage people to rethink what optical imaging is capable of. "This is a starting point to think about how we can improve," he says.

"Everybody wants to see more," laughs Xu, "To see a little bit deeper, a little bit faster, better resolution."

And a little less invasively too.

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