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scrutiny. Loubeyre says experiments using laser-shock techniques are already in the works to see if the new results can be reproduced. "The quest for metallic hydrogen is certainly not finished," he adds.

Yogendra Gupta of Washington State University agrees that confirmation is a must, but he's enthusiastic about the new results. "I think the evidence is quite strong," he says. "I'm struck by the care and rigor of the experiment and the enormous amount of theoretical work accompanying it."

In recent years many theorists have tackled the hydrogen metallization problem using density functional theory (DFT; see the article by Andrew Zangwill, *PHYSICS TODAY*, July 2015, page 34). The Sandia-Rostock group ran its own DFT calculations, and tried out two recently developed density functionals that add van der Waals interactions to more traditional implementations.

Most of the theoretical investigations agree that liquid hydrogen should undergo a first-order insulator-to-metal transition—one that involves latent heat—but they differ wildly on where the phase boundary should be. In the 1000- to 2000-K temperature range, different studies place the transition line

anywhere from 100 GPa up to 600 GPa. Says Desjarlais, "It was clear to us that good data for the transition would provide a much needed benchmark for theories."

The insulator-to-metal phase boundary traced out by the team's four shots on the Z machine stays in a narrow pressure range close to 300 GPa between 1000 K and 2000 K. Desjarlais points out that commonly used density functionals that only treat electron correlations at a local level grossly underestimate the transition pressure. He thinks the new results point to the importance of longer-range van der Waals interactions. But he's quick to add, "It also suggests that there is more work to be done, because no one functional fits our data."

Gupta thinks the results should spur theorists to look for the missing ingredient in their models. "I see this work as opening a window into things we don't understand," he says. "But that's what keeps physics going."

Sung Chang

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Imaging a mouse's brain through its skull

A modest change in the application of adaptive optics to light microscopy produces a dramatic increase in the field of view.

Biological tissues are rarely transparent. That fact has long complicated efforts by optical microscopists to resolve deeply embedded features, because the light waves used for imaging are scattered throughout the intervening tissue. The deeper the object, the more blurred the image. To ameliorate the problem, scientists in recent years have turned to adaptive optics: Using rapid, real-time analysis of a distorted-light signal, a computer-controlled deformable mirror or spatial light modulator compensates for aberrations in the optical path by reshaping the waves and thereby restoring crisp image detail.

Conventionally, the deformable mirror is placed at the microscope's so-called pupil plane—the back focal plane of the objective lens—where the mirror applies a uniform correction everywhere in the image plane. Such placement is appropriate, and provides the

best focus, when the aberrations are spatially invariant, as is the case, for instance, when a refractive-index mismatch occurs along a flat interface. However, that condition doesn't hold in turbid biological tissue; and unless the deformable mirror actively adjusts the wavefront for different scanning angles through the tissue, a position mismatch develops between distortion and compensation. The result is a good focus over a very small field of view.

Like astronomers before them, microscopists can benefit from virtually placing the wavefront modulator in the scattering layer itself, where a point-to-point correspondence exists between mirror deformations and the aberrations they correct.¹ Meng Cui and collaborators at the Janelia Research Campus of the Howard Hughes Medical Institute have now applied that scheme, known as conjugate adaptive optics, to



A restrained mouse, awaiting anesthesia and highly focused near-IR light pulses to its brain, sits under the objective lens of a microscope. The genetically engineered mouse has green fluorescent protein in its microglia—brain cells responsible for the animal's immune response to injury and to pathologies such as Alzheimer's disease and tumors; the cells fluoresce in response to intense near-IR light excitation. Collecting the fluorescence in a photodetector (not shown) from a scan of the focus through an $8000\text{-}\mu\text{m}^3$ volume of the brain maps out a three-dimensional image. Thanks to a new adaptive-optics scheme, fine details such as the threadlike branches of a single microglial cell are resolved (see inset's 2D projection). (Photograph courtesy of Meng Cui; inset adapted from ref. 2.)

brain imaging. By optically projecting a deformable mirror's corrective wavefront onto the bony outer surface of a mouse's $150\text{-}\mu\text{m}$ -thick skull, they obtained diffraction-limited images of neurons and microglia—a type of brain cell with thread-like extensions—another $50\text{ }\mu\text{m}$ under the skull,² as shown in the figure.

To resolve those cells previously, researchers had to thin the skull or remove it entirely. But noninvasive imaging is strongly preferred because microglia cells serve as the primary immune response to any kind of brain damage or infection. The Janelia group is the first to capture their dynamics through an intact skull. What's more, the unconventional placement of the deformable mirror bought them a 15-fold increase in corrected field of view—from an area of $20\text{ }\mu\text{m}^2$ to about $300\text{ }\mu\text{m}^2$.

Coming into focus

The achievements build on work Cui began three years ago to combine multiphoton fluorescence microscopy, the gold standard for *in vivo* deep-tissue imaging, with well-established wavefront engineering.³ In two-photon fluorescence microscopy, the wavelength of laser light used to excite target molecules is in the near-IR, long enough that it suffers far less scattering than visible

light and isn't strongly absorbed by hemoglobin in the blood. And because the excitation probability is extremely low, the only molecules that fluoresce are those in the tightly focused volume of tissue where the IR light intensity is highest. The two-photon technique thus reduces out-of-focus excitations that would otherwise hinder wide-field imaging.

In their new work, Cui and his colleagues installed a photodetector to pick up the fluorescence signal as it leaked out of the brain and back through the objective lens. They then iteratively changed the wavefronts of the illuminating near-IR pulses while continuously monitoring the fluorescence intensity of the signal until they found a wavefront shape that produced the strongest signal outside the brain and thus the best focus inside it. For that iterative trial-and-error process, the team divided the deformable mirror, with its 1024 independent spatial elements, or pixels, into two halves: One-half was kept fixed to provide a reference, and the other half was repeatedly changed. The procedure turns out to be remarkably fast: The total optimization time could be reduced to less than 1.5 seconds.

Allard Mosk and Ivo Vellekoop from the University of Twente in the Nether-

lands developed an earlier version of the waveshaping technique in 2007.

They focused a portion of light through a layer of white paint onto a photodetector and realized a thousandfold increase in transmission intensity after optimizing the light's wavefront (see reference 4 and *PHYSICS TODAY*, September 2008, page 20). That study underscored the power of monitoring the change in light intensity

to make the light converge to a focus, even after thousands of scattering events. Cui's implementation adapted the procedure to a microscopy context. His group scanned the laser focus through a $20\text{ }\mu\text{m} \times 20\text{ }\mu\text{m} \times 20\text{ }\mu\text{m}$ volume of brain tissue, a $1\text{-}\mu\text{m}$ -thick slice at a time, and mapped the fluorescence intensity measured at the photodetector into an image, as shown in the figure.

Fortuitously, Cui and his collaborators only had to optimize their deformable mirror occasionally. Because the skull is so stable an environment, they were able to scan the brain for hours—with each three-dimensional scan requiring just 21 seconds—to capture some cellular dynamics before re-optimizing the shape of the incident near-IR waves.

"This is a step forward, but not the end," Cui concedes about his demonstration. "We would like a still larger field of view—at least $10\,000\text{ }\mu\text{m}^2$." Ordinary adaptive optics, whether conventional or conjugate, is most effective when a single, thin aberration layer is dominant, and it breaks down when there are multiple layers. The skull has denser bone near its inner and outer surfaces than in its center, which complicates proper waveshaping. "Incorporating multiple deformable mirrors, a technique now common in astronomy, into a microscope system to account for multiple layers would be operationally extremely difficult," says Oxford University's Martin Booth. "But I'm sure someone will do it eventually."

Mark Wilson

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