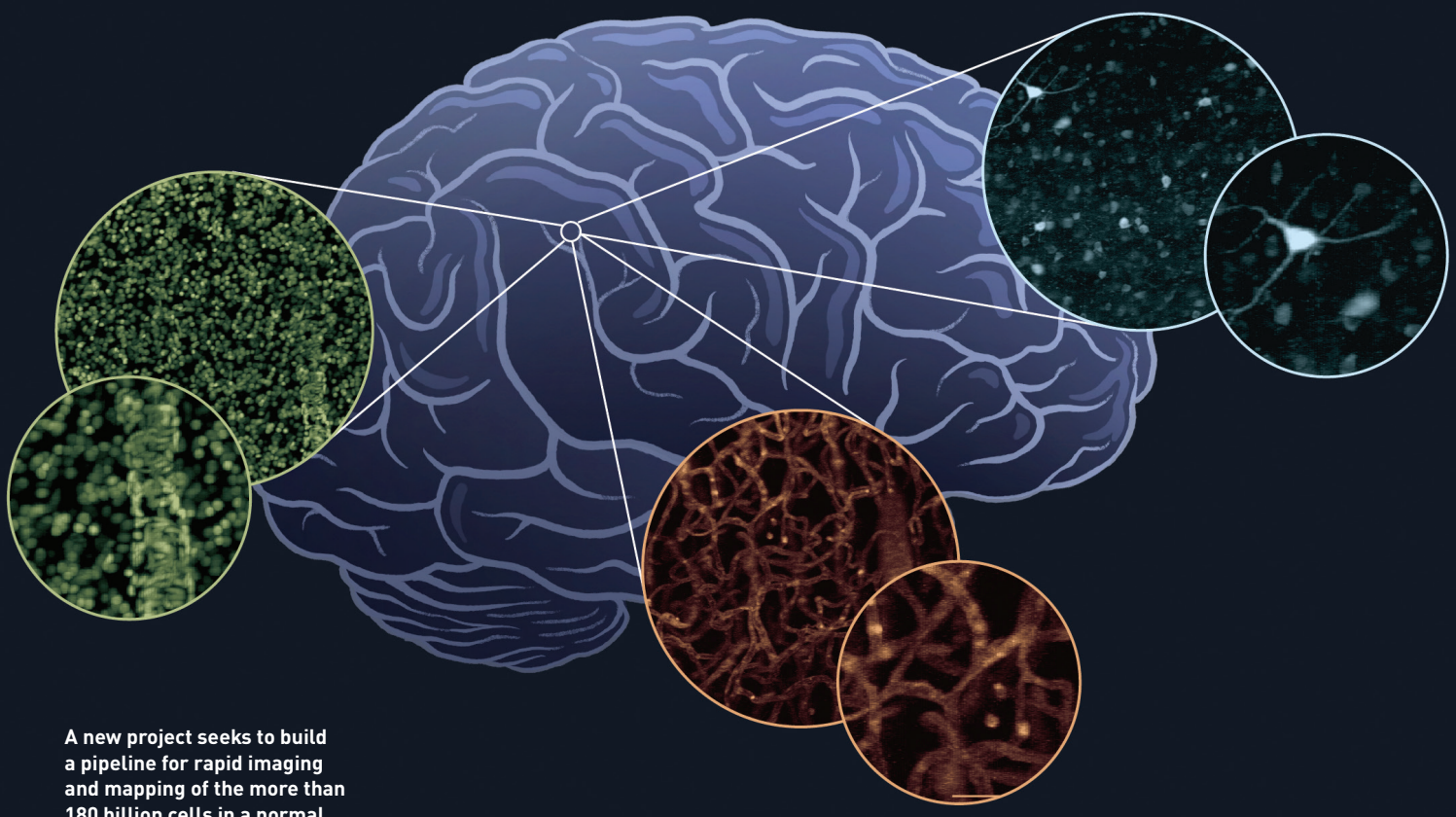


A new project seeks to build a pipeline for rapid imaging and mapping of the more than 180 billion cells in a normal human brain.

Hillman Lab / Columbia University Zuckerman Institute

IDEAS

Building a "Google Earth" for the Human Brain

A research team led by Optica Fellow Elizabeth Hillman hopes to create an imaging pipeline that can map every cell in the brain—at unprecedented speeds.

Stewart Wills

In the past decade, researchers have made incredible progress capturing detailed 3D maps of brains—the brains of mice. But the human brain, comprising more than 180 billion cells, is vastly more complex than the mouse version, with a mere 100 million. Mapping the detailed structure and organization of the tangle of cells in normal human brains—a potential boon for studies of behavior, cognition and neurodegenerative diseases—is consequently orders of magnitude slower and more difficult than in mice.

A US-based research team, led by Optica Fellow Elizabeth Hillman of Columbia University's Zuckerman Institute and colleague Zhuhao Wu of the Icahn School of Medicine at Mount Sinai, thinks it can make the job a lot faster. By combining high-speed light-sheet microscopy pioneered in Hillman's lab with progress in optically

"clearing" and fluorescently labeling opaque tissue samples—and throwing in a liberal dash of high-end computation and data science—the researchers hope to develop a system that can routinely map all of the cells in a whole human brain in the space of a week or so. And the group has secured a three-year, US$9.1 million grant from the BRAIN Initiative of the US National Institutes of Health (NIH) to try to do just that.

The work, if successful, could enable detailed, cell-by-cell mapping not just of one human brain but of hundreds—creating a database that Hillman describes as a sort of "Google Earth for the brain." OPN recently talked with Hillman to learn more about the work and where it's headed.

A need for speed

Hillman says several things converged to drive her interest in adapting her team's high-speed light-sheet microscopy platform, SCAPE (short for Swept Confocally Aligned Planar Excitation), to whole-brain imaging. One was her experience, several years before the pandemic, helping to run a course at Cold Spring Harbor Laboratories (CSHL) on imaging structure and function in the nervous system. A course module there dealt with so-called cleared tissues, in which the sample has been chemically treated to make it transparent for optical imaging, while keeping its cell-by-cell structure intact.

Hillman, who had brought a prototype of her SCAPE microscope to the course, was playing with a piece of optically cleared brain tissue during that module. She found that her system "got an image in one second that had taken another group about 20 minutes to collect." And that, she says, pushed her team toward thinking about speed as a key variable not just in imaging rapidly changing features in live animals—the sweet spot of SCAPE microscopy—but in detailed mapping of whole human brains. "The more we looked into it, the more we realized that the throughput speed of imaging these kinds of samples was becoming a huge bottleneck for people."

Courtesy of Columbia Engineering

> "We realized that the throughput speed of imaging these kinds of samples was becoming a huge bottleneck for people."
>
> *—Elizabeth Hillman*

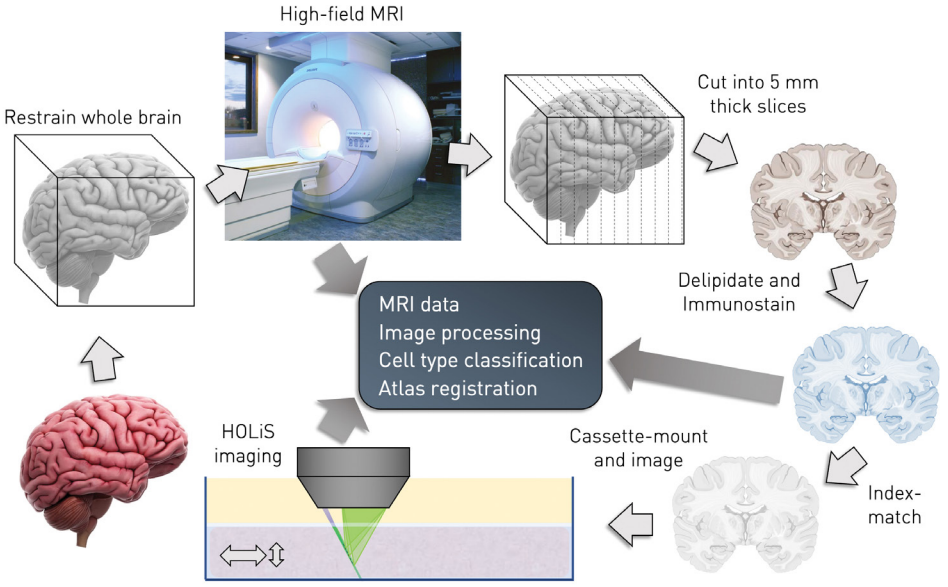
Later, during the pandemic, Hillman traveled to CSHL at the invitation of a colleague and pioneer in whole-brain imaging, Pavel Osten. There, Osten introduced her to Zhuhao Wu, who had developed multiple protocols for tissue clearing to enable the imaging of large organs. And together, Hillman says, "we came up with this crazy scheme" of imaging whole human brains—and finding "all the ways to make it as efficient as possible, as detailed as possible, to do it in … the right way to really make big progress."

Creating a pipeline

Hillman, Wu and their colleagues now have three years and US$9.1 million from NIH to turn that "crazy scheme" into a functioning system for brain mapping. Essential to success, she says, will be taking a "pipeline" approach to the entire process—and relentlessly optimizing every step.

The envisioned pipeline will begin with an MRI scan of the complete brain, to provide a baseline for cross-referencing the data against existing, coarser-grained brain atlases. Then, the brain specimen will be sliced into 5-mm-thick flat sections that will be made transparent using HuB.clear, a new iteration of Wu's tissue-clearing method specifically designed for large, intact brain slabs. Antibodies selected to bind to specific brain-cell types will next carry multicolor fluorescent dye molecules to label each brain cell in the sample slab.

The labeled brain section will then pass to Hillman's microscope. Dubbed HOLiS (for Human-brain-Optimized Light-Sheet) microscopy, the system, she explains, will operate similarly to her team's SCAPE 2.0 system, sending an oblique sheet of laser light into the sample to excite the dye molecules, and routing that excitation signal back through the same lens to be recorded by a high-speed camera. (For a tutorial on SCAPE 2.0, see OPN, April 2020, p. 22.) An exquisitely precise, constant-velocity stage will move the sample along beneath the beam to enable creation of a 3D data stack and capture of image data from each cell in the sample slab.



Schematic of the team's envisioned HOLiS (Human-brain Optimized Light Sheet) imaging pipeline for fast mapping of whole human brains.

Courtesy of E. Hillman

Finally, the copious HOLiS imaging data from the slab will be fed into sophisticated computer routines that will convert it into manageable, machine-readable form. And the process will start again on the next slab, repeating until the entire brain is mapped. Properly optimized and streamlined, Hillman says, the system should be able to image an entire brain, cell by cell—a process that would take years using conventional instruments—in the space of a week.

Sweating the details

As with any such undertaking, of course, success will hinge on mastering the details. For example, while the brains of laboratory mouse strains can be genetically engineered to express certain fluorescent molecules as the mouse develops, the labeling for human brain samples needs to be done after the fact, using the trickier techniques of immunohistochemistry. One key to this will be a "de-lipidation" step in the HuB. clear tissue-clearing protocol that strips away lipid molecules from the tissue, a bit of preconditioning that will help the dye-carrying antibodies find their targets in the sample slab.

Another early hurdle to clear will be figuring out the optimal cell-labeling strategy. Hillman explains that the team hopes the HOLiS microscope might capture as many as eight different spectral channels simultaneously, using "a full range of fluorophores" to identify different cell types. The researchers will need to determine whether to simply use one-to-one matching, with each fluorophore labeling a different cell type—or to pursue an approach that combines multiple fluorophores to create a sort of barcode. The latter approach, while more complex to implement, could potentially encode a much richer, more detailed suite of cell types. "One of the really important things we need to do in the beginning," she says, is to "figure out how far we can push that idea."

The biggest challenge, though, may lie dealing with the vast amounts of data churned out of the imaging pipeline's back end—around two petabytes of data per brain. That's more storage, Hillman notes, than "our entire institute has right now." To handle it, colleagues at the Columbia University Data Science Institute and Carnegie Mellon University will press into service a range of techniques in data sampling, deep learning, machine vision and more, to turn the data flood from the microscope into something more manageable and fit for end users.

Big job, big payoff

Refining and implementing these and other details of the pipeline adds up to a tall research order for the next three years. But if it's successful, Hillman believes, the result could enable a leap forward in studying the human brain's structure and organization.

One central goal animating the project, and the interest of the NIH BRAIN Initiative, is the need simply to understand the range of structure and cell types and their distribution in normal human brains. That's only possible, Hillman notes, with the ability to map and compare hundreds of them in a reasonable time. "The whole point of going fast here," she says, "is to commoditize this"—to make imaging of a whole human brain not just a one-off accomplishment, but a more routine undertaking.

And with comprehensive information on normal brain-cell distribution and the HOLiS-driven mapping toolkit the team is developing, it may be possible to scope out, in similar detail, the kinds of things that go wrong in specific regions of brains ravaged by disorders such as Alzheimer's disease. "It could," Hillman hopes, "unlock a whole sort of 'informatics of the human brain' piece that we just haven't had." OPN

Stewart Wills is the senior editor of OPN.