Improving the view through tissues and organs

October 17 2014, by Kimm Fesenmaier

Credit: AI-generated image (disclaimer)

This summer, several undergraduate students at Caltech had the opportunity to help optimize a promising technique that can make tissues and organs—even entire organisms—transparent for study. As part of the Summer Undergraduate Research Fellowship (SURF) program, these students worked in the lab of Assistant Professor of Biology Viviana

Gradinaru, where researchers are developing such so-called clearing techniques that make it possible to peer straight through normally opaque tissues rather than seeing them only as thinly sectioned slices that have been pieced back together.

Gradinaru's group recently published a paper in the journal *Cell* describing a new approach to tissue clearing. The method they have created builds on a technique called CLARITY that Gradinaru helped develop while she was a research associate at Stanford. CLARITY allowed researchers to, for the first time, create a transparent whole-brain specimen that could then be imaged with its structural and genetic information intact.

CLARITY was specifically developed for studying the brain. But the new approach developed in Gradinaru's lab, which the team has dubbed PARS (perfusion-assisted agent release in situ), can also clear other organs, such as the kidney, as well as tissue samples, such as tumor biopsies. It can even be applied to entire organisms.

Like CLARITY, PARS involves removing the light-scattering lipids in the tissue to make samples transparent without losing the structural integrity that lipids typically provide. First the sample is infused with acrylamide monomers that are then polymerized into a hydrogel that provides structural support. Next, this tissue–hydrogel hybrid is immersed in a detergent that removes the lipids. Then the sample can be stained, often with antibodies that specifically mark cells of interest, and then immersed in RIMS (refractive index matching solution) for imaging using various optical techniques such as confocal or lightsheet microscopy.

Over the summer, Sam Wie, a junior biology major at Caltech, spent 10 weeks in the Gradinaru lab working to find a polymer that would perform better than acrylamide, which has been used in the CLARITY

hydrogel. "One of the limitations of CLARITY is that when you put the hydrogel tissue into the detergent, the higher solute concentration in the tissue causes liquid to rush into the cell. That causes the sample to swell, which could potentially damage the structure of the tissue," Wie explains. "So I tried different polymers to try to limit that swelling."

Wie was able to identify a polymer that produces, over a similar amount of time, about one-sixth of the swelling in the tissue.

"The SURF experience has been very rewarding," Wie says. "I've learned a lot of new techniques, and it's really exciting to be part of, and to try to improve, CLARITY, a method that will probably change the way that we image tissues from now on."

At another bench in Gradinaru's lab, sophomore bioengineering major Andy Kim spent the summer focusing on a different aspect of the PARS technique. While antibodies have been the most common markers used to tag cells of interest within cleared tissues, they are too large for some studies—for example, those that aim to image deeper parts of the brain, requiring them to cross the blood–brain barrier. Kim's project involved identifying smaller proteins, such as nanobodies, which target and bind to specific parts of proteins in tissues.

"While PARS is a huge improvement over CLARITY, using antibodies to stain is very expensive," Kim says. "However, some of these nanobodies can be produced easily, so if we can get them to work, it would not only help image the interior of the brain, it would also be a lot less costly."

During his SURF, Kim worked with others in the lab to identify about 30 of these smaller candidate binding proteins and tested them on PARS-cleared samples.

While Wie and Kim worked on improving the PARS technique itself, Donghun Ryu, a third SURFer in Gradinaru's lab, investigated different methods for imaging the cleared samples. Ryu is a senior electrical engineering and computer science major at the Gwangju Institute of Science and Technology (GIST) in the Republic of Korea.

Last summer Ryu completed a SURF as part of the Caltech–GIST Summer Undergraduate Research Exchange Program in the lab of Changhuei Yang, professor of electrical engineering, bioengineering, and medical engineering at Caltech. While completing that project, Ryu became interested in optogenetics, the use of light to control genes. Since optogenetics is one of Gradinaru's specialties, Yang suggested that he try a SURF in Gradinaru's lab.

This summer, Ryu was able to work with both Yang and Gradinaru, investigating a technique called Talbot microscopy to see whether it would be better for imaging thick, cleared tissues than more common techniques. Ryu was able to work on the optical system in Yang's lab while testing the samples cleared in Gradinaru's lab.

"It was a wonderful experience," Ryu says. "It was special to have the opportunity to work for two labs this summer. I remember one day when I had a meeting with both Professor Yang and Professor Gradinaru; it was really amazing to get to meet with two Caltech professors."

Gradinaru says that the SURF projects provided a learning opportunity not only for the participating students but also for her lab. "For example," she says, "Ryu strengthened the collaboration that we have with the Yang group for the BRAIN Initiative. And my lab members benefited from the chance to serve as mentors—to see what works and what can be improved when transferring scientific knowledge. These are very important skills in addition to the experimental know-how that they master."

More information: Yang, Bin and Treweek, Jennifer B. and Kulkarni, Rajan P. and Deverman, Benjamin E. and Chen, Chun-Kan and Lubeck, Eric and Shah, Sheel and Cai, Long and Gradinaru, Viviana (2014)" Single-Cell Phenotyping within Transparent Intact Tissue through Whole-Body Clearing." *Cell*, 158 (4). pp. 945-958. ISSN 0092-8674. resolver.caltech.edu/CaltechAU … S:20140801-121634734

Provided by California Institute of Technology