# CLARITY: Transparent Tissue for 3D Imaging of Neuronal Networks and Subcellular Structures

Researchers in Prof. Karl Deisseroth's laboratory have patented a revolutionary technique that can be utilized to map neural circuits in the whole brain. This technology, called CLARITY (Clear, Lipid-exchanged, Anatomically Rigid, Imaging/immunostaining compatible, Tissue hYdrogel), employs a hydrogel that preserves proteins, small peptides, small molecules, and nucleic acids in their threedimensional distribution as found in original tissue. Following a clearing procedure, CLARITY renders tissue ultrastructures highly transparent and permeable to macromolecules, enabling visualization of both three-dimensional structure and fine molecular details of intact whole tissues without mechanical sectioning. Compared to the current Scale technology, this process is much faster (2 days vs. 5 weeks) and more effective removing opaque molecules.

Immunostaining the now transparent tissue can reveal subcellular structures in their native three-dimensional context. The same tissue can then be washed and stained again for subsequent analyses. CLARITY has a wide range of applications such as high-throughput mapping and analysis of neuronal networks, mapping cellular components in whole organisms, or clinical histology of biopsies and post mortem tissue.



Images courtesy the Deisseroth Lab

**CLARITY imaging of the whole intact mouse brain.** Photographs of whole mouse brains (4 months old). (A) Before clearing (B) Cleared by the Scale method for 5 weeks (C) Cleared by the CLARITY method for two days (D) Fluorescent image of the same brain shown in C.

#### Stage of Development

Deisseroth Lab researchers regularly use CLARITY in their neural physiology research and actively maintain the CLARITY resource center.

## Applications

- Research:
  - 3-dimensional mapping of complex neural circuits with cellular resolution
  - research tool for <u>BRAIN initiative</u> to map the human brain
  - probing and mapping neural networks and other cellular components in the context of intact tissue or organisms
- Clinical histology:
  - storing, rendering, and using, and eventually actuating, the full connectivity of a human brain post mortem
  - obtaining 3D structural information from clinical biopsies such as heart, kidneys, tumors and other tissues

### Advantages

- Whole tissue analysis no sectioning
  - $\circ\,$  faster and less laborious than mechanical sectioning
  - compatible with immunostaining or molecular phenotyping that is not possible with optical sectioning
- **Integrated 3D images** reveals structure combined with fine molecular details throughout the whole tissue
- **Maintains tissue** the same brain or other tissue sample can be used for multiple rounds of staining
- Improvement over current Scale technology faster (2 days vs. 5 weeks) and more effective at removing opaque molecules

#### **Publications**

- Deisseroth, K. A., & Chung, K. (2021). U.S. Patent Application No. 16/950,640.
- Deisseroth, K. A., & Chung, K. (2020). U.S. Patent No. <u>10,545,075</u>. Washington, DC: U.S. Patent and Trademark Office.
- Hsueh, B., Burns, V. M., Pauerstein, P., Holzem, K., Ye, L., Engberg, K., ... & Deisseroth, K. (2017). <u>Pathways to clinical CLARITY: volumetric analysis of</u> <u>irregular, soft, and heterogeneous tissues in development and disease</u>. *Scientific reports,* 7(1), 1-16.
- Chung, K., Wallace, J., Kim, S. Y., Kalyanasundaram, S., Andalman, A. S., Davidson, T. J., ... & Deisseroth, K. (2013). <u>Structural and molecular</u> <u>interrogation of intact biological systems</u>. *Nature*, 497(7449), 332-337. https://doi.org/10.1038/nature12107
- White House Fact Sheet: BRAIN Initiative
- New York Times Brains as Clear as Jell-O for Scientists to Explore
- Scientific American Interview with Prof. Deisseroth
- <u>Nature See-through brains clarify connections</u>
- <u>The Verge Technique to create transparent brains could transform</u> <u>neuroscience</u>
- Inside Stanford Medicine Getting CLARITY
- <u>Stanford News</u> President Obama's new \$100 million brain research initiative taps several Stanford scientists

## Patents

- Published Application: 20150144490
- Published Application: 20170219465
- Published Application: 20210215581
- Issued: <u>10,545,075 (USA)</u>

### Innovators

- Karl Deisseroth
- Kwanghun Chung

# Licensing Contact

#### **Evan Elder**

Senior Licensing Associate

<u>Email</u>