

## Description of Deisseroth Lab Hydrogel-enabled Tissue Clearing Workshops

The Deisseroth lab offers a number of hands-on training workshops of varying length and emphases that focus primarily on the CLARITY tissue clearing protocol developed in the lab. Details of the different workshops held at Stanford University are listed below. Shorter (3-day and 2-week) workshops will be offered throughout the year. Anyone registered for these workshops will eventually be invited to participate. The extended 3-4 week workshop is offered once every summer, and participants are selected through an application process.

Due to limited lab space and equipment, a small group of participants will be invited to each workshop. The workshops are free for participants from academic institutions and national labs. However, participants are responsible for their own travel and accommodation costs.

### 1) 3-day Hydrogel-enabled Tissue Clearing Introductory Workshop

This workshop provides a complete overview of hydrogel-enabled tissue clearing, with a specific focus on the CLARITY protocol. Laboratory procedures, application strategies, and published modifications are taught using a combination of interactive lecture/discussion and hands-on demonstrations. Due to the short nature of the training, samples prepared at various stages of the protocol will be provided and participants are asked not to bring their own samples for clearing.

#### **Topics covered:**

- CLARITY processing timeline
- Hydrogel embedding
- Passive and electrophoretic tissue clearing (ETC)
- Sample mounting for confocal microscopy
- Overview of imaging techniques and data analysis strategies
- Modifications and alternative hydrogel-enabled tissue clearing techniques

### 2) 3-day Cleared Tissue Imaging and Data Analysis Workshop – Bring Your Own Sample (BYOS)

This workshop is designed for researchers that have already established a tissue clearing procedure and want to learn more about image acquisition and data analysis for their cleared tissue specimens. Participants are encouraged to bring their own sample for light-sheet imaging. The sample must already be hydrogel-embedded, cleared, stained (if necessary), and washed in PBST buffer upon arrival. Due to time and equipment constraints, **only one sample will be imaged** during the workshop. However, participants are welcome to bring more samples in order to choose the best candidate.

Researchers that have already completed the Hydrogel-enabled Tissue Clearing Introductory workshop (formerly the CLARITY workshop) are welcome to register for this workshop as well. CLARITY-processed samples will be discussed and featured, but participants are welcome to bring samples cleared using other techniques. At this time, we **cannot image samples immersed in organic solvents**.

#### **Topics covered:**

- Overview of tissue clearing strategies, modifications, troubleshooting, and microscopy methods
- Sample imaging using light-sheet microscopy (mounting, parameter set-up, data acquisition, and data post-processing on the LaVision BioTec and the home-built COLM system)
- Data handling and image analysis strategies including visualization, making movies, cell segmentation and counting using a variety of image processing software programs (Imaris, Arivis, Fiji, Vaa3D, Ilastik)

### 3) **2-week Hydrogel-enabled Tissue Clearing Intensive Workshop – BYOS**

The 2-week workshop is designed for researchers that would like to learn the steps of hydrogel-enabled tissue clearing while performing the technique on their own sample. Participants will experience both the Hydrogel-enabled Tissue Clearing Introductory Workshop (week 1) and the Cleared Tissue Imaging and Data Analysis Workshop (week 2) while implementing the CLARITY protocol on a tissue sample brought from their own lab. The results of the practice sample will foster personalized discussion of useful protocol modifications and troubleshooting that may be necessary for future samples.

#### **Sample restrictions:**

- Sample must be fixed overnight in 4% PFA (perfused if possible), then transferred to PBS buffer for storage and transport
- Sample must have some sort of fluorescent label (there will be no time for sample immunostaining); otherwise, only autofluorescence will be imaged
- Sample must be less than 3 mm thick across its shortest dimension, or able to be cut to that size after embedding (mouse brain matrices for slicing with razor blades will be provided)
- Due to time and equipment constraints, only one sample will be imaged during the workshop, but participants can bring 1-2 additional samples for clearing.

### 4) **3-4 week D-lab Extended Summer Workshop – BYOS**

The extended workshop includes training on multiple techniques developed in the Deisseroth lab – optogenetics, fiber photometry, and CLARITY. Applicants are asked to propose a mini-project using any or all of these techniques that can be accomplished within the 3-4 week timeframe. Chosen participants will receive hands-on training while working closely with one or more D-lab members to complete their proposed project. For more information on applying to the extended summer workshop, please contact Dr. Kristin Overton ([kristin.overton@stanford.edu](mailto:kristin.overton@stanford.edu)) and/or Dr. Maisie Lo ([maisielo@stanford.edu](mailto:maisielo@stanford.edu)) directly.

**To register for a workshop** or ask further questions, please send an email to the CLARITY Education Manager, Dr. Kristin Overton ([kristin.overton@stanford.edu](mailto:kristin.overton@stanford.edu)), with the following information:

- Your name
- Your institution
- Your position (graduate student, postdoc, etc.)
- Your PI's name (if applicable)
- Which CLARITY workshop(s) you would like to attend
- For a BYOS workshop, please provide a detailed description of the sample you want to bring including such information as:
  - tissue type and area of interest
  - estimated sample size or dimensions
  - clearing method used (if applicable)
  - fluorescent labelling method
  - fluorophore(s) used
  - the amount of the sample you want to image (i.e. a small section at high resolution or the whole organ/tissue sample)
  - the scale of what you want to image (cell bodies, axonal projections, i.e. desired resolution)

The sample information you provide will help us estimate the best imaging parameters for your visit and determine if any trade-offs will be necessary for the limited workshop timeframe.

- Please also indicate if you receive support through NeuroNex or NIDA