

**Supplementary Information: Legends and Figures**

**Advanced CLARITY for rapid and high-resolution imaging of  
intact tissues**

Raju Tomer<sup>1,3,4</sup>, Li Ye<sup>1,3,4</sup>, Brian Hsueh<sup>1,4</sup>, and Karl Deisseroth<sup>1,2,3,4,5</sup>

<sup>1</sup>Department of Bioengineering

<sup>2</sup>Department of Psychiatry and Behavioral Sciences

<sup>3</sup>Howard Hughes Medical Institute

<sup>4</sup>CNC Program

Stanford University, Stanford, CA

<sup>5</sup>To whom correspondence should be addressed:  
Karl Deisseroth, M.D., Ph.D.  
Department of Bioengineering  
W083 Clark Center, 318 Campus Drive  
Stanford, CA 94305  
Phone: (650) 736-4325  
deissero@stanford.edu

## Supplementary Figure Legends

**Supplementary Figure S1.** Sample transparency at key stages of the CLARITY protocol. Pictures of representative brain samples are shown during the key stages of clarification: (a) hydrogel-embedded mouse brain, (b) after lipid removal, (c) after washing of the residual SDS micelle with PBST, and (d) after 2 hours of refractive index homogenization with FocusClear at 37°C with gentle shaking.

**Supplementary Figure S2.** Summary of the control electronics framework and COLM parts. FPGA logic is used to control and synchronize various parts of the microscope.

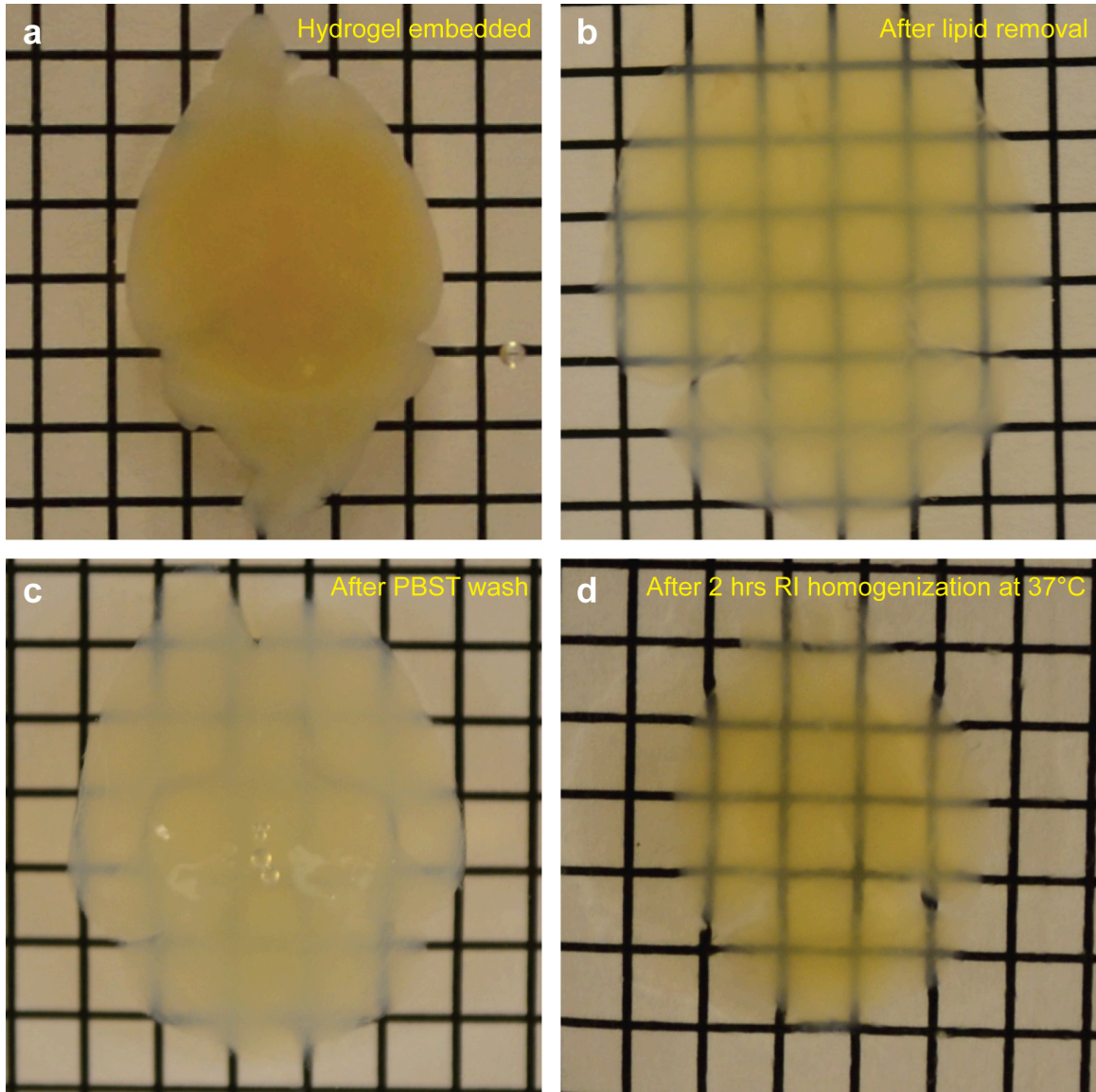
## Supplementary Movie Legends

**Supplementary Video 1.** Volume rendering of whole mouse brain volume acquired from an intact clarified Thy1-eYFP mouse brain using 10x magnification objective in COLM. The dataset was down-sampled 4 fold in each lateral pixel dimension to make the volume rendering feasible with current computing power. The entire image volume was acquired in ~4 hours.

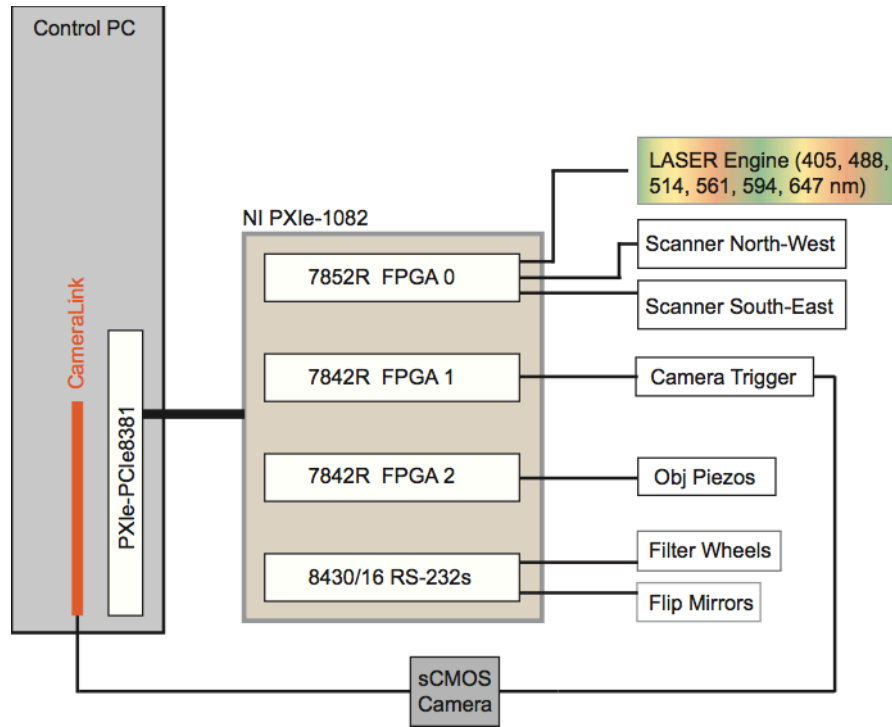
**Supplementary Video 2.** Rendering of 3.15 mm x 3.15 mm x 5.3 mm volume acquired from an intact clarified Thy1-eYFP mouse brain using the 25x magnification objective in COLM. The dataset was down-sampled 2 fold in each lateral pixel dimension. The entire image volume was acquired in ~1.5 hours.

**Supplementary Video 3.** Rendering of 1.837 mm x 0.959 x 5 mm volume acquired from an intact clarified Thy1-eYFP mouse brain using the 25x magnification objective in a confocal microscope.

### Supplementary Figure S1



## Supplementary Figure S2



COLM Parts	Vendor	Part number
LASER engine	Omicron	SOLE-6: 405, 488, 515, 561, 594, 647 nm
Camera	Hamamatsu	Orca Flash 4.0 V2
Detection Objectives	Olympus	10X/0.6 and 25X/0.95 CLARITY
Illumination Objectives	Olympus	Macro 4X/0.28 NA (Qty=2)
XYZ-theta sample stage	Physik Instrumente	Assembled from M-112K033 (Qty=3); M-116.DG
Sample chamber	Custom design	Custom design
Illumination Filter Wheels + Smart shutter	Sutter	FG-LB10-NWIQ (Qty=2)
Emission Filter Wheel	Sutter	FG-LB10-W50
Galvo XY Scanners	Cambridge Tech	6215H XY mounted (Qty=2)
Tube lens	Thorlabs	ITL200 (Qty=3)
Scan lens	Sill Optics	S4LFT0061/065 (Qty=2)
Filters	Semrock	RazorEdge long pass and band pass filters
Sample holder cuvette	Starna Cells	Quartz cuvette
Sample holder adapter	Custom	Custom
FPGA	National Instruments	7852R (Qty=2), 7842R
PXI express chassis	National Instruments	PXIe-1082
Serial card	National Instruments	8430/16
Objective piezos	Physik Instrumente	Hera stages P-622.1CD (Qty=3)