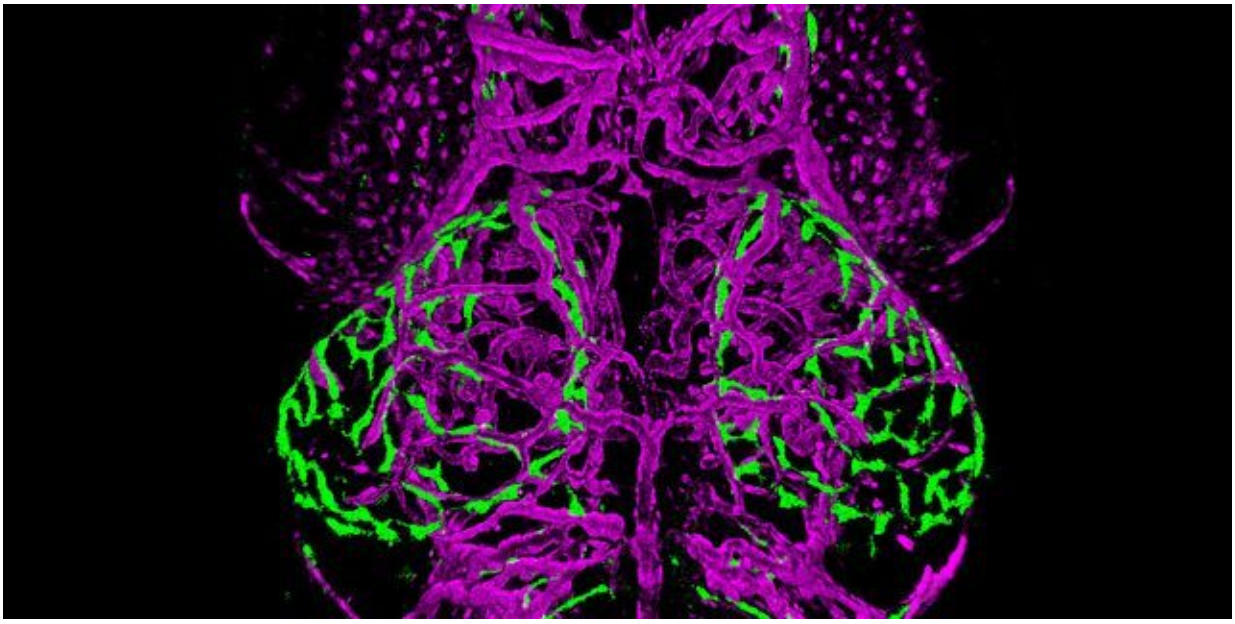
Seeing through the brain

December 1 2016, by Chinmaya Sadangi



Credit: B. Weinstein, National Institute of Child Health and Human Development, National Institutes of Health

The brain is a complex and mysterious organ, which performs many functions and the proper functioning of this precious machine is important for well-being. There are about 86 billion neurons in the brain and they interact by forming circuits. Therefore, it is critical to understand the structural organization and the neural circuits underlying brain functions. However, a major obstacle to study and understand the brain lies in the fact that it is densely composed of lipids, due to which the light is scattered and cannot penetrate inside. Thus, the high lipid

content makes it difficult to image the whole brain. The traditional method to image the brain is to slice it into thin sections (μm) followed by histological staining and imaging but this is a time-consuming, error-prone process, and there is/might be loss of information as only the surface of sections can be imaged.

However, modern approaches involve clearing the brain of the lipids and make it optically transparent so that light can pass through the brain without sectioning. There are several different clearing processes like CLARITY (Chung and Deisseroth, 2013), SWITCH (Murray et al., 2015), CUBIC (Susaki et al., 2015), 3DISCO (Ertürk et al., 2012), uDISCO (Pan et al., 2016) and many more which clear the lipids and make the brain optically transparent. With the discovery of these processes, scientists have even imaged a complete rodent (Pan et al., 2016) and its whole brain (Chung and Deisseroth, 2013; Pan et al., 2016).

The most popular and commonly used clearing method is CLARITY (Clear Lipid-exchanged Acrylamide-hybridized Rigid Imaging Tissue hYdrogel) (figure 1) which was developed at Karl Deisseroth's laboratory, who also pioneered the discovery of Optogenetics (Boyden et al., 2005). CLARITY uses SDS, a detergent to get rid of the lipids using electrical charges. Initially, the animal is perfused with formaldehyde in combination with acrylamide which in turn forms a hydrogel layer. The brain is infused with a hydrogel solution and then incubated. At the end of the process, CLARITY produces a structurally intact but transparent tissue. However, with CLARITY, multiple rounds of labeling are not possible and is limited to three rounds of immunolabeling. Also, after electrophoresis, the tissue expands from their normal size but return to its normal size after refractive index matching (Chung and Deisseroth, 2013). However, no loss of information due to the expansion and shrinkage has been reported.

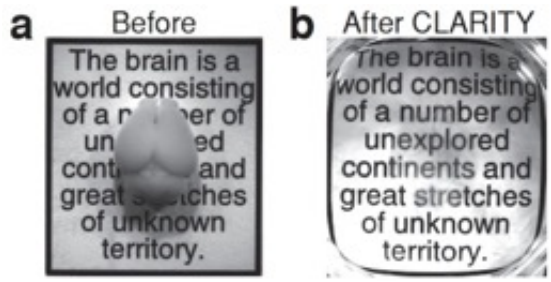


Figure 1 – Comparison between (a) normal brain and (b) cleared brain using CLARITY.

Many modifications have been made to CLARITY including passive CLARITY (Tomer et al., 2014). More recently, Kwang Chung, who is credited with the discovery of CLARITY, developed SWITCH (system wide control of interaction time and kinetics of chemicals). SWITCH provides an advantage since it allows multiple round (> 20) of labeling steps and a uniform tissue processing, thus providing access to high dimensional multi-scale information (Murray et al., 2015). Another major advantage of SWITCH is that it doesn't require the animals to undergo perfusion and can be used both in small and big animals and even human samples and SWITCH doesn't require any special equipment or reagents (Murray et al., 2015).

An alternative approach to CLARITY or SWITCH is a solvent-based method known as 3DISCO (Three-dimensional imaging of solvent-cleared organs) which removes lipids and makes the tissue optically transparent by using organic solvents like Dibenzyl Ether (DBE) and Tetrahydrofuran (THF). By using 3DISCO, the organ of interest shrinks to half of its size which is convenient for imaging rats because of their big size, and it has been reported that there is no loss of information. A modified version of 3DISCO, was recently developed by Erturk et al. and is known as uDISCO or 'ultimate DISCO'. uDISCO uses Diphenyl

Ether (DPE), instead of DBE. DPE is less prone to radical and oxidative reactions as it lacks benzylic C-H and C-O bonds (Pan et al., 2016). Fluorescence is better preserved in DPE as compared to DBE and provides a better tissue-clearing effect (Pan et al., 2016). Therefore, DPE is more suitable for clearing process compared to DBE.

With access to high resolution microscopes, like 2-photon or light sheet microscopy, it is now possible to image a complete brain within a matter of hours. A recent development in microscopy known as expansion microscopy (Chen et al., 2015) can also be used to image cleared tissues which is based on the infusion of Sodium polyacrylate into the tissue. Interestingly, this molecule is also used in baby diapers and as a result the diapers expand due to diffusion of liquid into it. Based on the same principle, expansion microscopy also expands the tissue samples by 100 folds without changing the molecular structures and it has been used to image cells and synapses in the mouse hippocampus (Chen et al., 2015) and also has been used to image RNA structure (Chen et al., 2016).

The advent of clearing techniques and advanced microscopy system has made possible to image the whole brain in pathological conditions in order to deeply understand the underlying aberrant changes occurring at the level of neural networks and connections. Recently, clearing methods have been successfully used to image brains in severe disorders such as Alzheimer's disease (Ando et al., 2014) and multiple sclerosis (Spence et al., 2014). With these techniques, it would now be possible to comprehend some of the brain secrets and to unveil how this beautiful organ works.

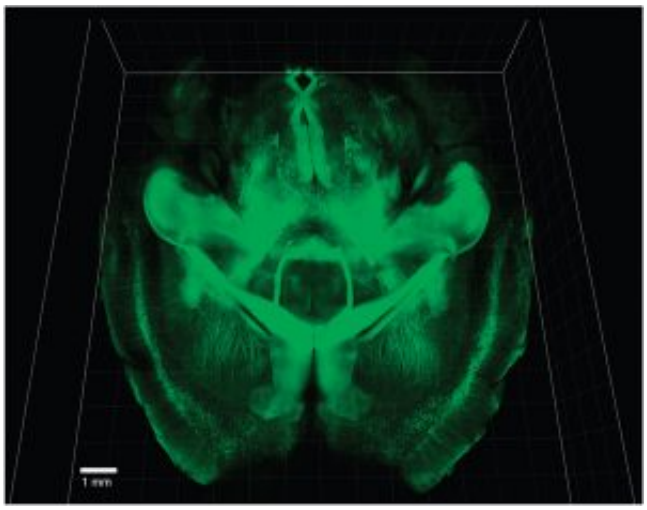


Figure 2: The nervous system projections imaged in the intact mouse brain with CLARITY. Adapted from Chung et al.

More information: Chung K, Deisseroth K. CLARITY for mapping the nervous system. *Nature Methods* 2013;10:508–13.

Murray E, Cho JH, Goodwin D, et al. Simple, Scalable Proteomic Imaging for High-Dimensional Profiling of Intact Systems. *Cell* 2015;163:1500–14.

Susaki EA, Tainaka K, Perrin D, et al. Advanced CUBIC protocols for whole-brain and whole-body clearing and imaging. *Nature Protocols* 2015;10:1709–27.

Ertürk A, Becker K, Jährling N, et al. Three-dimensional imaging of solvent-cleared organs using 3DISCO. *Nature Protocols* 2012;7:1983–95.

Pan C, Cai R, Quacquarelli FP, et al. Shrinkage-mediated imaging of entire organs and organisms using uDISCO. *Nature Methods* 2016;

Boyden ES, Zhang F, Bamberg E, et al. Millisecond-timescale, genetically targeted optical control of neural activity. Nat. Neurosci. 2005;8:1263–8.

Tomer R, Ye L, Hsueh B, et al. Advanced CLARITY for rapid and high-resolution imaging of intact tissues. *Nature Protocols* 2014;9:1682–97.

Chen F, Tillberg PW, Boyden ES. Expansion microscopy. *Science* 2015;347:543–8.

Chen F, Wassie AT, Cote AJ, et al. Nanoscale imaging of RNA with expansion microscopy. *Nature Methods* 2016;13:679–84.

Ando K, Laborde Q, Lazar A, et al. Inside Alzheimer brain with CLARITY: senile plaques, neurofibrillary tangles and axons in 3-D. *Acta Neuropathologica* 2014;128:457–9.

Spence RD, Kurth F, Itoh N, et al. Bringing CLARITY to gray matter atrophy. *Neuroimage* 2014;101:625–32.

Hama H, Hioki H, Namiki K, et al. ScaleS: an optical clearing palette for biological imaging. *Nat. Neurosci.* 2015;18:1518–29.