Santiago Ramon y Cajal, viewed by many as the father of neuroscience, once said that “the brain is a world consisting of a number of unexplored continents and great stretches of unknown territory”. Though much is still unknown in neuroscience, it is safe to say that those continents have been better mapped. Better still if those great stretches are transparent, made possible by a technique developed by the Deisseroth laboratory of Stanford University.

Unfortunately for researchers, brains are opaque. Optical imaging methods cannot visualise tissue at great depths because of light scattering, resulting from differences in the rate at which light travels in water and fat molecules. Single-photon microscopy can only give information from around 50µm below the brain surface, and two-photon microscopy can still only image as deep as 800 µm, preventing complete visualisation of global projection patterns and cell population positions. Researchers had to make do imaging many thin brain slices, and reconstructing 3D structures later, an approach that costs *a lot* of time and money. C’est la vie. Until CLARITY that is.

CLARITY starts with the injection of formaldehyde and hydrogels into the tissue. The formaldehyde fixes all molecules within the tissue, except fats, and the hydrogels. After heating, fats can be removed by chemical or electrical means, leaving a transparent tissue-hydrogel mesh with the tissue’s original three-dimensional structure[1]. Endogenous biomolecules such as neurotransmitters, proteins, and nucleic acids are fixed in place.

This is the key point to CLARITY, as there isn’t much point in studying a transparent blob of brain-derived molecules. Visualising the three-dimensional structure of neurons, as well as the expression and localisation of mRNA, proteins, and neurotransmitters in the context of those structures, can do much to extend understanding of brains in normal and disease state. The treated tissue is both permeable to large molecules and hardy enough to be washed, enabling multiple rounds of antibody labelling of proteins and in situ hybridisation of nucleic acids. This is plain old useful, especially for rare tissues such as stored human clinical samples. The power of generating high volumes of information about local morphology, such as synapse type, alongside global morphology and genetic data cannot be overstated.

In their seminal paper, the Deisseroth lab demonstrated the technique on the mouse brain, and sections from the frontal lobe of an autistic individual. The ability to analyse long distance neural pathways led to the discovery that neurons of that region had joined together in abnormal ladder-like patterns. Such a pattern had been suggested at by animal models of autism, but CLARITY gave a resounding result - in human tissue.

Since its inception, CLARITY has found great utility. It has been used to characterise Alzheimer’s disease pathology in three dimensions[2], processes in multiple sclerosis and anxiety disorders, and is even being evaluated for applications in cancer and autoimmune disease diagnosis. And while the technique was developed for brain tissue, it can be applied to most other tissue types provided they aren’t too fibrous or pigmented. CLARITY is therefore suited to developmental biology, uniquely positioned to better understand the three dimensional movements and interactions of cells in the embryo. It has been used with success to investigate the embryonic heart[3], and would be invaluable in research of migrating populations such as neural crest cells.

There are a number of other clearing techniques in common use. Dr Michael Kohl, early career research fellow at the University of Oxford, uses high-resolution optical microscopy to explore the coding strategies used in sensory representation and memory storage. His lab does “not use the CLARITY clearing à la Deisseroth lab for whole brains”, instead utilising “a much simpler, single

component clearing method for fixed brain slices (previously patched ex vivo, no thicker than 500 um) developed by colleagues in South Korea."

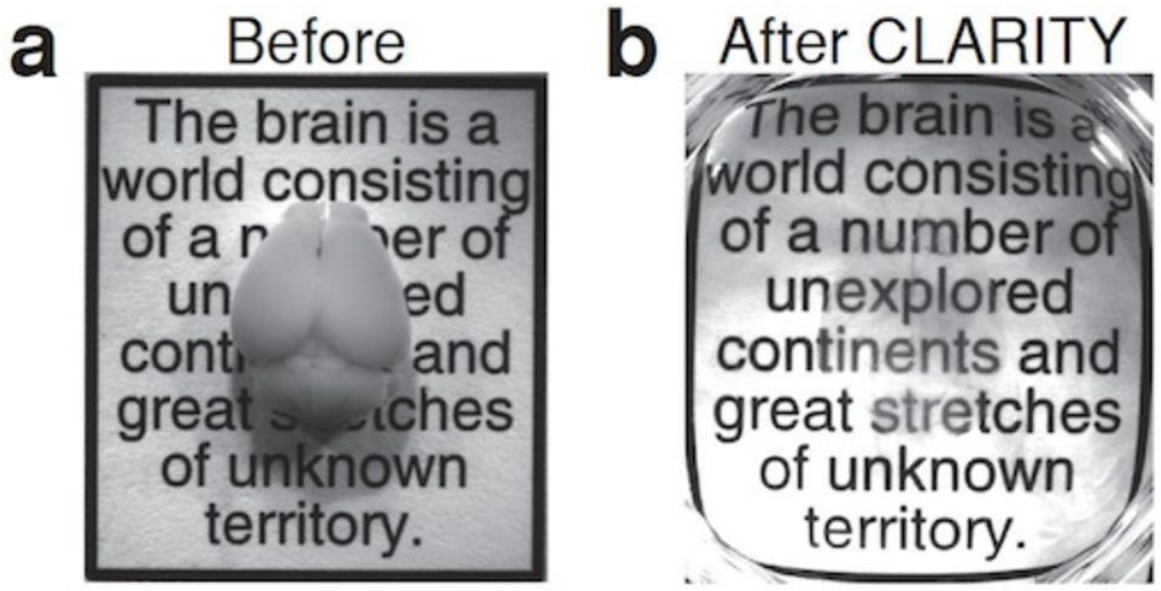
Dr Kohl told me that this method is "extremely useful as it saves us hugely laborious re-slicing of patched brain slices", and that the lab does not currently use CLARITY for the reason that it is "more involved in terms of reagents and protocol, and usually only really starts saving time and effort in the long run with some fancy confocal with special, long-working distance objectives, or sheet microscopy. Overall, it's a larger investment but definitely worth it once up and running, particularly when relating brain circuit activity across multiple areas."

There are a number of disadvantages other than start-up costs, not least that the clearing process takes four or five days with whole mouse brains, and can take weeks on larger tissue samples. All this in addition to the time taken for immunostaining in relatively thick tissue, and a ~8% protein content loss, and it is clear that CLARITY isn't perfect. Even so, it holds great promise in helping us see through the brain's mysteries.

1) Chung, K., et al. (2013). Structural and molecular interrogation of intact biological systems. Nature, doi: 10.1038/nature12107

2) Inside Alzheimer brain with CLARITY: senile plaques, neurofibrillary tangles and axons in 3-D - Kunie Ando et al, Acta Neuropathol (2014) 128:457–459:

3) Histochem Cell Biol (2016) 146:141–152 Comparison of different tissue clearing methods and 3D imaging techniques for visualization of GFP-expressing mouse embryos and embryonic hearts Hana Kolesová et al – using clarity on embryonic heart tissue



Source: The Deissoroth laboratory