Thick Slice CLARITY for Localization of Novel Neuroactive Target Gene Products

E. C. Ping,[*] Michael C. Saul,[**] and Lisa Stubbs[**]

Parkland College, Champaign Illinois[*]

Carl R. Woese Institute for Genomic Biology, University of Illinois at Urbana-Champaign[**]

PRECS Phenotypic Plasticity Research Experience for Community College Students

Introduction

Current methods of intact tissue structural analysis are incompatible with most methods of molecular phenotyping, leading to difficulty in gaining a perspective on a tissue which includes both its structural and molecular level information.

The barrier to the visualization of intact systems is the lipid bilayers in biological tissues, which hinder our molecular probing and limit our ability to image the tissue by rendering the tissue opaque.[1] Removing them is challenging because of their importance for the structural integrity of the tissue.

The CLARITY technique is a tissue clearing method to allow for both structural and molecular analysis of a tissue sample. By attaching the biomolecules within a sample to a hydrogel matrix, we preserve the tissue's structure. From there, the unattached lipids can be drained, making the tissue optically transparent.[1]

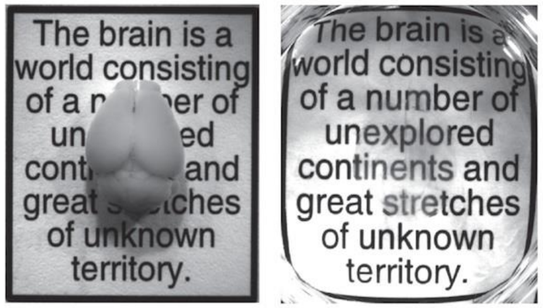


Fig. 1, Above, a mouse brain before and after CLARITY technique, by K. Chung and K. Deisseroth, Howard Hughes Medical Institute/Stanford

The now transparent tissue is permeable to light and macromolecules, and is able to undergo immunostaining and 3D imaging.

Unfortunately, whole tissue or whole body CLARITY is very expensive and time consuming, taking weeks or months to complete the process, limiting the efficiency of work that can be done.

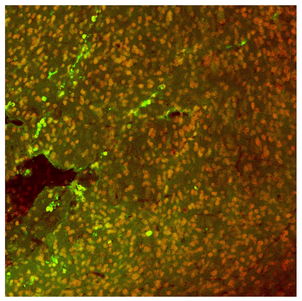
Thick Slice CLARITY

Because of the inherent limitations of thin slice immunohistochemistry (IHC) methods and the cost, both financially and in terms of time, of whole organ/body CLARITY, Xiaochen Lu developed Thick Slice CLARITY in the lab of Lisa Stubbs at the Carl R. Woese Institute for Genomic Biology at the University of Illinois at Urbana-Champaign.

Thick Slice CLARITY takes the clearing and antibody staining principles of whole organ CLARITY and applies it instead to 200µm thick slices of brain tissue.

Fig. 2, Right, GPR3 (in green) co-located with neurons (NeuN, in red) in a 200 micron thick slice of mouse brain.

This image was taken using a Zeiss LSM 700 confocal microscope after Thick Slice CLARITY techniques and antibody staining had been applied to the brain slices.



After a transcardial perfusion, mouse brains are extracted and fixed in a paraformaldehyde/acrylamide solution. The brains are then sectioned on a vibrating microtome, and the slices incubated at 37ºC for three hours, and then polymerized at 37ºC for three hours. For Thick Slice CLARITY, rather than doing clearing by electrophoretic tissue clearing (ETC) methods, passive clearing over the course of two to three days in SDS-ETC solution at 37ºC is more typical. After the lipids have been cleared from the tissue, the slices may undergo multiple rounds of antibody staining, and then may be mounted and imaged, generally via confocal microscopy.

Thick Slice CLARITY allows for the viewing of regional sections of the brain and analysis at a structural and molecular level. It has proven very useful for the localization of novel neuroactive target gene products.

Current Work

Recent research into mouse activity and exercise showed that there is a strong positive correlation between the expression levels of *Gpr3*, a gene encoding an orphan G-protein-coupled receptor involved in signal transduction, and motivation for voluntary running activity in mice.[3] Because of its density of dopamine receptors, the striatum was identified as a brain region likely to have experienced changes at a molecular level as a result of changed intrinsic exercise motivation.

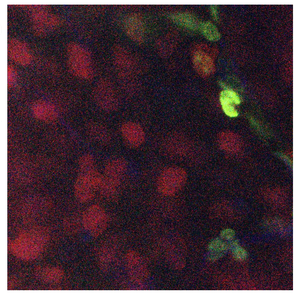


Fig. 3 Above, GPR3 (in green) co-located with neurons, identified using the pan-neuronal marker NeuN (in red).

The Thick Slice CLARITY technique, used in conjunction with standard fluorescent staining methods and imaging via confocal microscopy, has allowed for the colocalization of GPR3 with neurons in the mouse striatum, verifying the hypothesis of the area's importance. As seen on in Fig. 3, the small number of cells labeled with GPR3 reinforces standing ideas of where *Gpr3* is expressed.

Similar techniques were employed to localize NR2F1, the product of a deeply conserved autism gene, in neurons in the brain. While previously known to be existent in the human brain, its localization was unknown.

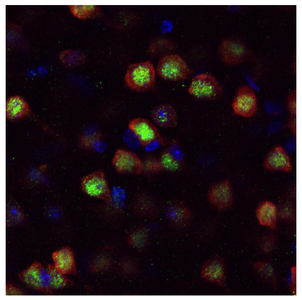


Fig 4., Above, NR2F1 (in green) localized in neurons, identified with the pan-neuronal marker NeuN (in red) and cell nuclei. .

It is important to note that NR2F1 expression, in green, is localized only in neurons, in red, and not in other cell types, such as glia, in blue. This distinction is important for work into the future.

Future Work

The localization of GPR3 with the CLARITY technique has made future work on the exercise project actionable. It will lead to further localization and other efforts, and contribute to research into the possibility of the use of pharmacological compounds to increase voluntary exercise motivation.

The localization of NR2F1 in neurons was a step towards understanding its position in the brain, and will contribute to research involved in autism and other developmental disabilities.

CLARITY itself is a fairly new technique with a lot of potential, both seen and unseen, for future purposes. **The technique has potential for mapping neural circuits and other connectomics work, for classifying and subclassifying neurons, and for protein localization in other projects**.

References

[1] K Chung and K Deisseroth. CLARITY for mapping the nervous system. *Nature Methods* (2013) 10(6): 508-513.

[2] K Chung, J Wallace, et al. Structural and molecular interrogation of intact biological systems. *Nature* (2013) doi: 10.1038/nature12107

[3] Saul, M. C., et al. (2017), High motivation for exercise is associated with altered chromatin regulators of monoamine receptor gene expression in the striatum of selectively bred mice. *Genes, Brain and Behavior*, 16: 328–341. doi:10.1111/gbb.1234

Acknowledgments

Financial support was provided by the National Science Foundation under grant #NSF REU 1559908/1559929, as part of the Phenotypic Plasticity Research Experience for Community College Students, through the University of Illinois at Urbana-Champaign Institute for Genomic Biology and Parkland College. http://precs.igb.illinois.edu/

Special thanks go to the Stubbs lab members, PRECS Pis Dr. Nathan Schroeder and Dr. C. Britt Carlson, and the technical and support staff at the Carl R. Woese Institute for Genomic Biology.

PARKLAND COLLEGE

ILLINOIS