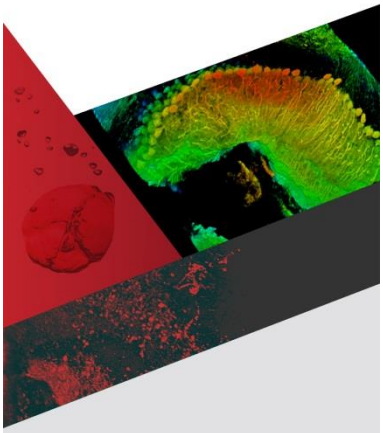
logos biosystems

application note

The X-CLARITY™ Mounting Solution: an Improved RI Matching Solution for Tissues Cleared by the X-CLARITY™ Tissue Clearing System

The X-CLARITY Tissue Clearing System is the world's first instrument of cutting edge technology that enables to transform biological tissue into a transparent state to light. The X-CLARITY Tissue Clearing System electrophoretically remove lipids, the main component of light scattering in biological tissues, from the tissue-hydrogel hybrid. The cleared tissue is labeled with appropriate probes such as fluorescent-labeled antibodies. Finally, the labeled tissue is infused and mounted in a refractive index (RI) matching medium to enhance optical clarity for 3D imaging. Here we described the newly developed RI matching medium, the X-CLARITY Mounting Solution, as an excellent optical clearing agent to provide an environment for 3D imaging with fluorescence microscopy and for prolonged preservation of fluorescence signals in the labeled tissue.



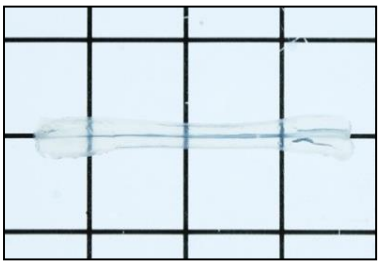
INTRODUCTION

Recently, many technologies have been developed to achieve tissue clearing for 3D volume imaging without disassembly[1]. The CLARITY technique removes lipid components, a major light scattering source, from tissues which are embedded with hydrogel as a structural alternative to lipids[3,4]. CLARITY and related techniques are recognized as a compatible method for immunolabeling of various biomolecules, which are accessible due to the porous property of tissue-hydrogel hybrids[1,5]. However, wide applications of CLARITY has been limited by drawbacks of the original do-it-yourself (DIY) design[6]. Recently, Logos Biosystems has developed the X-CLARITY Tissue Clearing System. The X-CLARITY Tissue Clearing System is a proprietarily designed instrument, which enables rapid and reproducible clearing results of various tissues due to a uniform electric current and efficient control of temperature.

The cleared tissues are ready to label with immunohistochemical stains. The labeled tissues has to be placed in an RI matching medium for 3D imaging. In the Chung's paper, a commercial mounting medium, FocusClear, was suggested as an RI matching immersion medium[3]. However, many scientists had concerns about the lack of availability of FocusClear and its high cost[6]. In this regard, alternative RI matching media have been suggested but not fully realized [5,7].

To improve the image quality, availability and reduce the cost, Logos Biosystems recently introduced an alternative RI matching medium named the X-CLARITY Mounting Solution. Here, we compare image quality and preservation of fluorescence signals in mouse brain slices in either the FocusClear or the X-CLARITY Mounting Solution.

Mouse Brain Cleared with the X-CLARITY™



Mouse Spinal cord Cleared with the X-CLARITY™

MATERIALS AND METHODS

Preparation of a Mouse Brain Tissue

A ICR mouse (12 weeks, female) was anesthetized by Avertin (Sigma, St. Louis, MO; 250 mg/kg) and perfused transcardially with ~30 mL of phosphate-buffered saline (PBS), followed by ~10 ml of 4% paraformaldehyde (PFA). The mouse brain was further incubated with 4% PFA for 24 hr at 4℃ and then incubated with Hydrogel Solution (Cat No. C13103) for 24 hr at 4℃. The polymerization of hydrogel was performed at 37℃ for 3 hr under vacuum with a prototype of the Hydrogel Polymerization System (Logos Biosystems). After polymerization of imbedded hydrogel, the brain was rinsed with the Electrophoretic Tissue Clearing Solution (Cat No. C13001) and then was cut into slices of 2 mm-thick using the Brain Matrix for mouse (TED PELLA, INC., Redding, CA), The brain slices were placed in a Mouse Brain Slice Holder (Cat No. C12004) and cleared for 2 hr by electrophoretic tissue clearing with the X-CLARITY Tissue Clearing System (Cat No. C10001) at 1.5 A, 37℃, and 30 rpm.

Post-Immunolabeling

For immunolabeling, the 2 mm-thick cleared brain slices were washed to remove SDS in 1X PBS overnight. The slices were incubated in a buffer containing two primary antibodies (1:300 α-smooth muscle actin (Abcam, Cambridge, UK) and 1:300 collagen IV (Abcam) in 1X PBS containing 6% BSA and 0.2% Triton X-100) for 1 day at 37℃ with gentle shaking, and then washed for 1 day at 37℃ in PBST (1X PBS containing 0.2% Triton X-100). The brain slices were further incubated with the Alexafluor 488-conjugated secondary antibody (1:200; for α-smooth muscle actin) for 1 day at 37℃ followed by PBST washing for 1 day. The brain slides were additionally incubated with the Cy3-conjugated secondary antibody (1:200, Jackson ImmunoResearch Laboratories, Inc., West Grove, PA; for collagen IV) for 1 day at 37℃ followed by PBST washing for 1 day.

Mounting and Imaging

After immunolabeling, each brain slice was cut along the center line into two pieces to compare two different mounting media. One half of the brain slice was incubated in the X-CLARITY Mounting Solution (Cat No. C13101) and the other half was incubated in FocusClear at room temperature for 1 day. The brain slices were placed on an Imaging Plate with a bottom of 0.17-mm thick cover glass (manufactured by Logos Biosystems). In order to detect fluorescence signal, a confocal microscope (LSM710, ZEISS, Oberkoche, Germany) equipped with a 10X Plan_Neofluar lens (NA 0.3) was used.

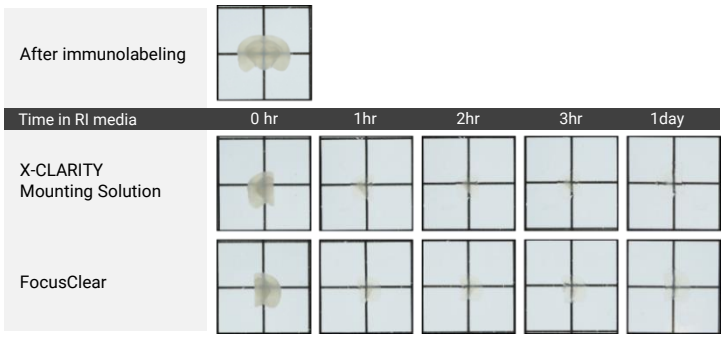
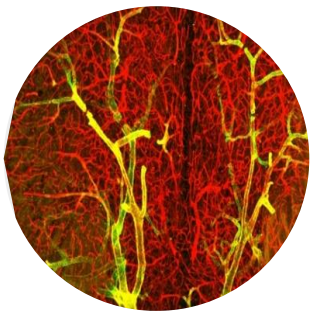
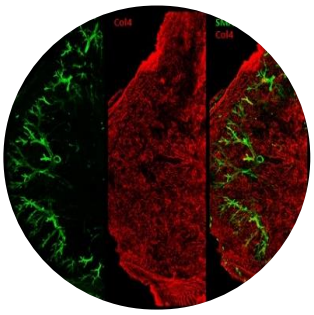


Fig 1. Comparison of cleared mouse brain slices in two different mounting media. A mouse brain slice was cleared by the X-CLARITY Tissue Clearing System and immunolabeled for α-smooth muscle actin and collagen IV. After Immunolabeling, the brain slice was cut into two pieces and immersed two different mounting media, the X-CLARITY Mounting Solution and FocusClear. Photographs were taken after indicated times of incubation (Scale: 1 square box = 1 mm^2).



Mouse Brain
Cleared with the X-CLARITY™
Blood vessels labeled with anti-SMA and the ECM labeled with anti-Collagen IV



Mouse Kidney
Cleared with the X-CLARITY™
Blood vessels labeled with anti-SMA and the ECM labeled with anti-Collagen IV

RESULTS

The 2-mm thick mouse brain slice was cleared by the X-CLARITY Tissue Clearing System and then immunolabeled as described in MATERIALS AND METHODS. After immunolabeling, the brain slice was turned into an opaque color due to lack of RI matching (Figure 1). The brain slice was vertically cut into two pieces and immersed in two different RI matching media: the X-CLARITY Mounting Solution and FocusClear, respectively. The clarity of both brain slices became evident after 1 hr of incubation in the RI matching media (Figure 1). However, there were no significant differences between two RI matching media tested at this level.

To compare the fluorescence signals from each brain slice, confocal microscopy was conducted after one day of incubation in the RI matching media under the same instrumental settings such as laser intensity power, gain, and pinhole size. As shown in Figure 2A, the green (α-smooth muscle actin) and red (collagen IV) fluorescence signals were well detected in the brain slice immersed in the X-CLARITY Mounting Solution. On the contrary, the fluorescence signals in the brain slice immersed in FocusClear was dim and difficult to read (Figure 2B). With FocusClear, high levels of laser power and gain were required to obtain similar image quality as with the X-CLARITY Mounting Solution to compensate for low signals (data not shown).

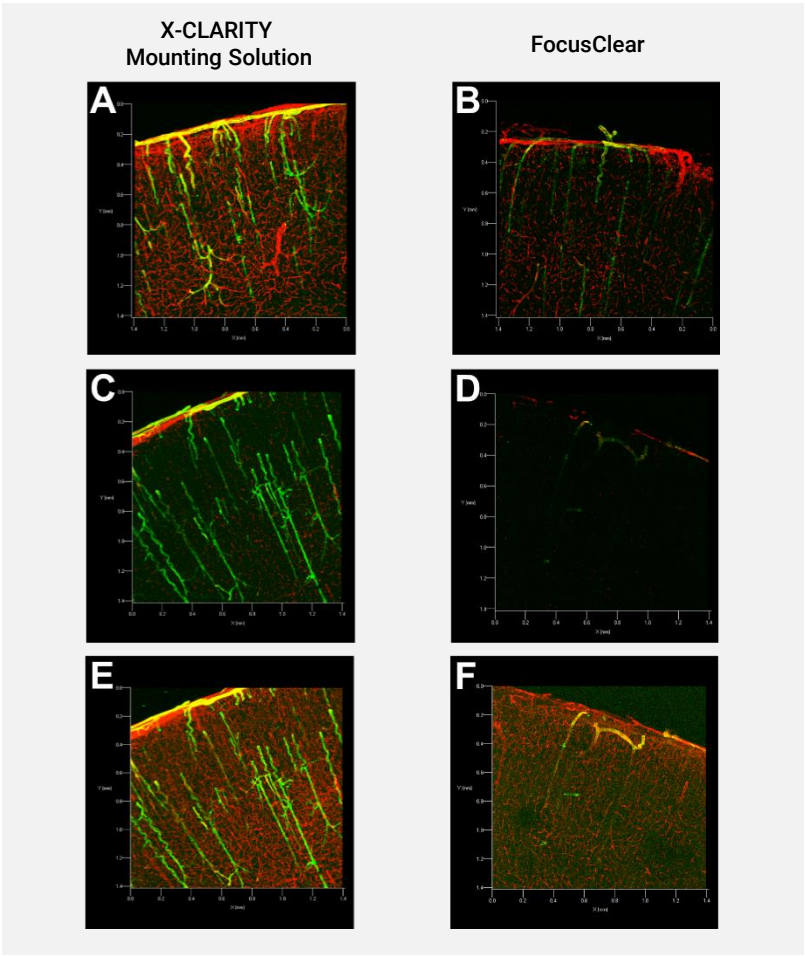
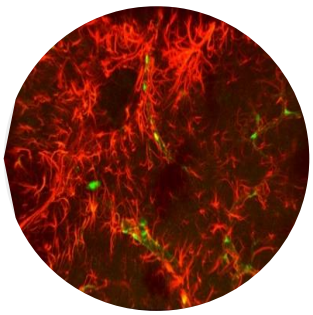


Fig 2. Confocal fluorescence microscopy of mouse brain slices. Confocal fluorescence microscopy was used to image brain slices after 1 day and 2 weeks incubation in mounting media with the same instrumental settings (A-D) or alternative instrumental settings for best imaging (E & F). Green fluorescence: α-smooth muscle actin; and red fluorescence: collagen IV.



Col1α1-GFP
Transgenic Mouse Spinal Cord
Cleared with the X-CLARITY™
Spinal cord labeled with anti-GFPA
Courtesy of Dr. Lee
University of Miami

To determine whether fluorescence signals are preserved in the tissues after prolonged incubation in the mounting media, the same brain slices were imaged after a 2-week incubation. Confocal microscopy was performed under the same instrumental settings which were used for imaging after 1 day of incubation. To our surprise, the fluorescence signals, both green and red, were not detectable in the brain slice incubated in FocusClear (Figure 2D). However, high levels of green and red fluorescence signals were detected in the brain slices incubated in the X-CLARITY Mounting Solution (Figure 2C). The instrumental settings were changed accordingly for maximum detection of fluorescence signals in each brain slice. Comparable fluorescence signals (both green and red) were obtained in the brain slice immersed in the X-CLARITY Mounting Solution (Figure 2E), while very limited green fluorescence signals were detectable in the brain slice immersed in FocusClear (Figure 2F).

Taken together, these data suggest that although there is no difference in the two mounting media in terms of optical clearing (Figure 1), there is a significant difference in detection and preservation of fluorescence signals. The X-CLARITY Mounting Solution is suitable to detect fluorescence signals in immunolabeled, cleared tissues with relatively low-level laser power. Since high-level laser power may accelerate photobleaching of fluorescence molecules especially in deep tissue 3D imaging with confocal microscopy[1,4], efficient imaging with low-level laser power is valuable for 3D volume imaging with confocal microscopy. In addition, preservation of fluorescence signals in immunolabeled tissues with the X-CLARITY Mounting Solution enables parallel processing of multiple samples for later imaging.

CONCLUSION

The X-CLARITY Mounting Solution provides: 1) efficient RI matching of cleared tissues after immunolabeling; 2) better conditions for 3D volume imaging of tissues with confocal fluorescence microscopy at low-level laser power to reduce photobleaching; and 3) preservation of comparable fluorescence signals for at least 2 weeks. Additionally, the X-CLARITY Mounting Solution is an excellent alternative to existing solutions since its price is affordable compared to the originally recognized commercial mounting medium, FocusClear[3,6].

REFERENCES

1. Richardson, D. S., Lichtman, J. W. Clarifying tissue clearing. *Cell* **162**, 246-257 (2015).
2. Hama, H., et al. ScaleS: an optical clearing palette for biological imaging. *Nat. Neurosci.* **18**, 1518-1529 (2015).
3. Chung, K., et al. Structural and molecular interrogation of intact biological systems. *Nature* **497**, 332-337 (2013).
4. Chung, K., Deisseroth, K. CLARITY for mapping the nervous systems. *Nat. Methods* **10**, 508-513 (2013).
5. Yang, B., et al. Single-cell phenotyping within transparent intact tissue through whole-body clearing. *Cell* **158**, 945-958 (2014).
6. http://forum.claritytechniques.org/.
7. Marx, V. Microscopy: seeing through tissue. *Nat. Methods* **11**, 1209-1214 (2014).

• Ordering Information

Cat #	Product	Size
C10001	X-CLARITY™ Tissue Clearing System Starter Kit - X-CLARITY™ ETC Chamber (1 ea) - X-CLARITY™ ETC Controller (1 ea) - X-CLARITY™ Pump (1 ea) - X-CLARITY™ Reservoir (1 ea) - Tissue Container (Whole Mouse Brain) (5 ea) - Container Holder for 1 Tissue Container (1 ea) - Electrophoretic Tissue Clearing Solution (12 X 1 L)	1 unit
C10101	X-CLARITY™ ETC Chamber	1 unit
C10201	X-CLARITY™ ETC Controller	1 unit
C10301	X-CLARITY™ Pump	1 unit
C10401	X-CLARITY™ Reservoir	1 unit
C12001	Tissue Container (20 units)	1 box
C12002	Container Holder for 1 Tissue Container	1 unit
C12004	Mouse Brain Slice Holder	1 unit
C12007	Whole Rat Brain Holder	1 unit
C12005	Multi-Cable	1 unit
C12101	X-CLARITY™ Reservoir Cap with Temperature Probe	1 unit
C12102	Snap-Lock Connector Tube	1 unit
C12103	Peristaltic Pump Tube	1 unit
C13001	Electrophoretic Tissue Clearing Solution	12 X 1L
C13101	X-CLARITY™ Mounting Solution	1 X 25 mL
C13102	X-CLARITY™ Mounting Solution (Value Pack)	10 X 25 mL

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