Optical clearing of tissue samples using CLARITY

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Traditional tissue staining

Time consuming
Results in loss of spatial resolution
Not optimal for study of protein expression in whole tissue blocks
What is CLARITY?
Why is CLARITY important?

Reduces light scattering
Improves reagent penetration
Experiment timeline

Perfusions (1 day) -> Hydrogel embedding (1 day) -> Lipid removal (22 days)

Tissue homogenization (1 day) -> Imaging (3 days)
Mouse perfusions, and Hydrogel embedding
Lipid Removal

- SDS solution with Boric acid with deionized water
- Acts like a detergent that removes lipids
- Tissue clearing lasts from day to weeks depending on tissue size.
Mouse brain after 22 days of clearing
Mouse caecum after 22 days of clearing
Mouse liver after 22 days of clearing
Tissue Homogenization using TDE

Mouse Kidney after 22 days of clearing
Multi-photon microscopy
Mouse brain with 2-photon microscopy

Red: Blood vessels
Green: Macrophages
Mouse brain with 2-photon microscopy

- Blood vessels
- Macrophages
Depth projection of mouse brain

1.5 mm
Mouse caecum with 2-photon microscopy
Challenges encountered

Using a shorter tube to dispense agarose solution

Added heating lamp to keep solution at around 37°C

Agarose polymerization
Conclusions

• Lipids limit two photon microscopy.
• CLARITY removes lipids without disrupting other components of tissue.
• CLARITY is a solution to lipid induced light scattering in two photon microscopy.
• CLARITY will be used in the study of inflammation in the brain.
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