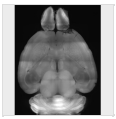

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'Uniclear' water-based brain clearing for light sheet imaging of electrode tracks

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1 Works for me dx.doi.org/10.17504/protocols.io.zndf5a6

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ABSTRACT

UniClear procedure for whole mouse brain clearing and refractive index matching. The advantages of this method are that the method is water-based and produces a mechanically robust specimen. Cleared brains can be imaged with lightsheet microscopes (e.g. Zeiss Z1 lightsheet).

MATERIALS

NAME	CATALOG #	VENDOR
DMSO	472301	Sigma Aldrich
OptiPrep™ Density Gradient Medium	D1556)	Sigma Aldrich
2-Propanol (99.5 %)	278475	Millipore Sigma

MATERIALS TEXT

2-methyl-2-butanol
https://www.sigmaaldrich.com/catalog/product/sial/152463?lang=en®ion=US

2-propanol
https://www.sigmaaldrich.com/catalog/product/sial/278475?lang=en®ion=US

Sodium dodecyl sulfate solution (SDS), 10% in H20
https://www.sigmaaldrich.com/catalog/product/sigma/71736?lang=en®ion=US

OptiPrep™ Density Gradient Medium
https://www.sigmaaldrich.com/catalog/product/sigma/d1556?lang=en®ion=US

Dimethyl sulfoxide (DMSO)
https://www.sigmaaldrich.com/catalog/product/sial/276855?lang=en®ion=US

Nycodenz
https://www.progen.com/nycodenz.html

Preparation of SBiP buffer: 500ml
*** Mix SBiP at 4C till fully dissolved, kept at 4C.
At room temperature, SBiP will get activated and emulsified for delipidation. Use each batch within a month for best effect.

- Ice cold H2O 350 ml
- 50mM Na2HPO4 2 ml
- 4% SDS (in H2O, pH7.4) 10 ml
- 2-methyl-2-butanol 80 ml
- 2-propanol 40 ml

Preparation of Opti-prep refractive index matching solution

- 60% (w%) Opti-prep solution
- 20% (w%) DMSO
- 20% (w%) PBS
- 20 mM Tris
- add overall 1.12g/ml Nycodenz (dissolve gradually by adding ~1/10th of Nycodenz powder at a time until fully mixed, heating up and stiring can help to dissolve faster)

References:

Economo, Clark, et al., A plat form for brain-wide imaging and reconstruction of individual neurons.
Elife.2016 Jan 20;5:e10566. doi: 10.7554/eLife.10566

Winnubst et al., Reconstruction of 1,000 Projection Neurons Reveals New Cell Types and Organization of Long-Range Connectivity in the Mouse Brain
Cell.2019 Sep 19;179(1):268-281.e13. doi: 10.1016/j.cell.2019.07.042. Epub 2019 Sep 5.

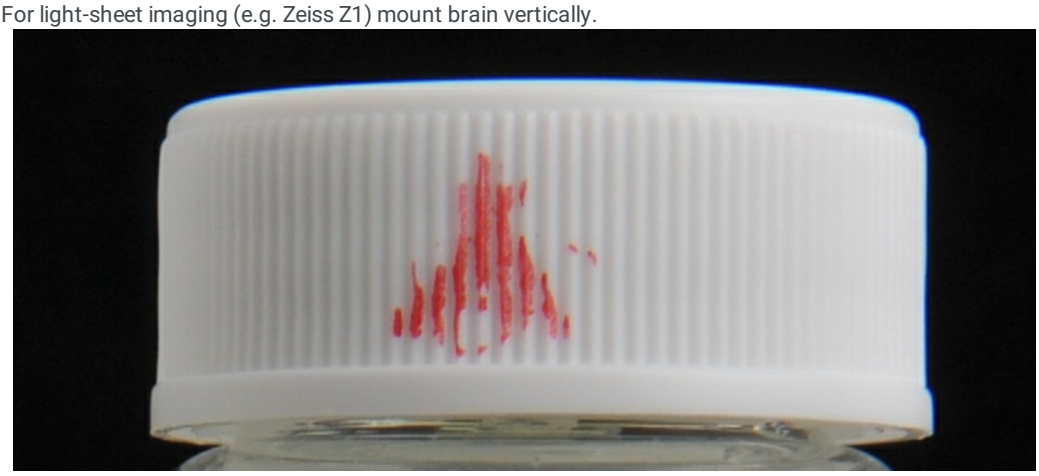
1 Perfuse mouse with cold 4% PFA. Post fix brain for at least **24:00:00** at room temperature (RT). Wash brain in PBS to remove fixative. Fixed brain samples can be kept in PBS at **4 °C** before further processing.

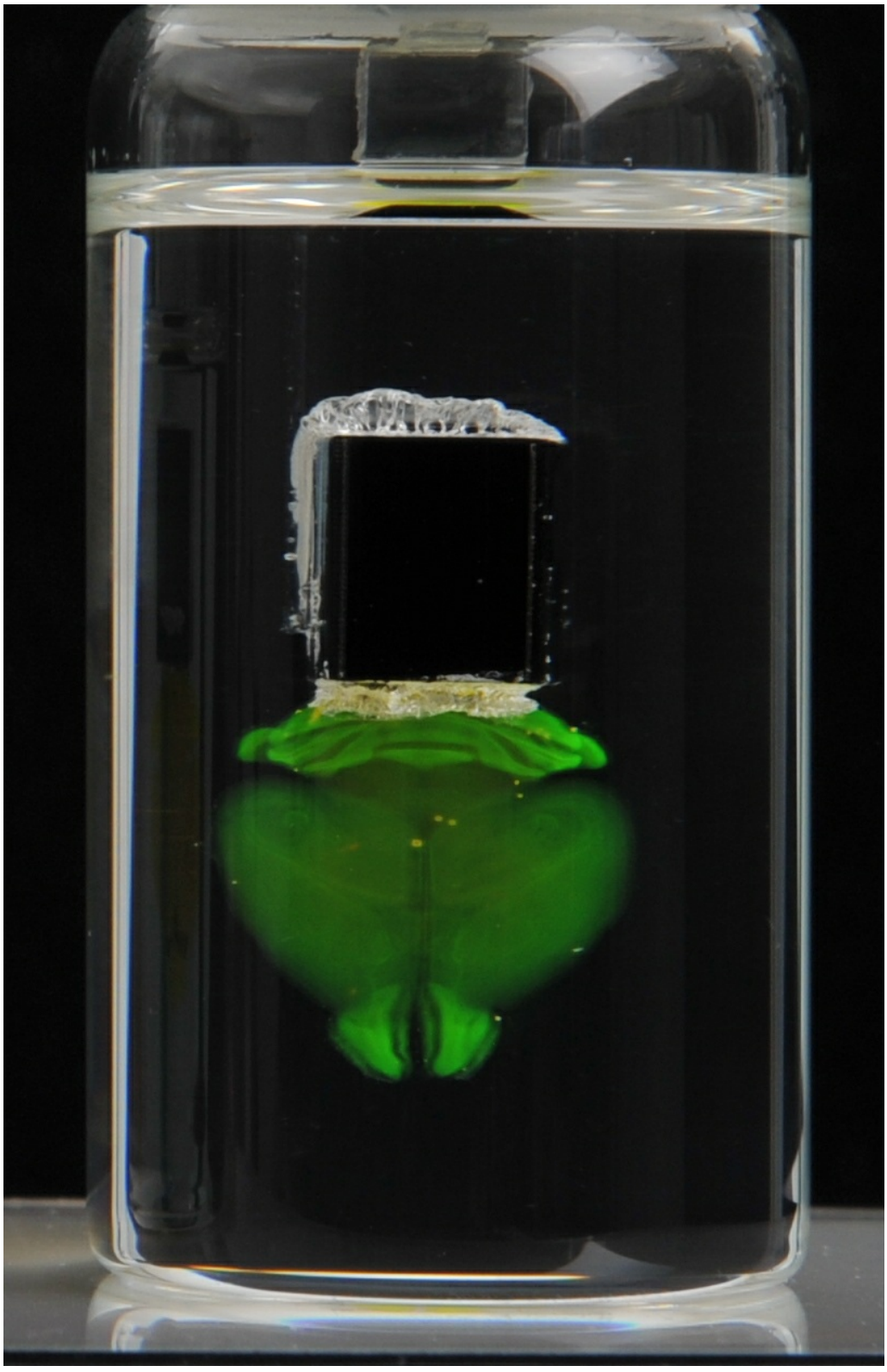
2 Delipidate brain with SBiP buffer: **20 ml** per adult mouse brain, shaking (70 RPM) at **37 °C** . On the first day: change buffer at **03:00:00** , **06:00:00** , **12:00:00** and leave over night. Starting on the second day: change SBiP buffer every **24:00:00** for consecutive 14 days , shaking (70 RPM) at **37 °C** .

3 After delipidation, wash brain in PBS for at least **12:00:00** before further processing. Sample can be stored in PBS at RT before refractive index matching.

4 Refractive index matching: first day in **20 ml** 50% PBS + 50% opti-prep mixed solution, rotating at RT for **24:00:00** . On second day transfer the brain to **40 ml** 100% opti-prep solution, rotating at RT for **24:00:00** . Index-matched brain should appear transparent.

5 For light-sheet imaging (e.g. Zeiss Z1) mount brain vertically.





Example of a mounted brain (fluorescent yellow color is DNA dye YO-PRO, which is optional). For electrode localization we use autofluorescence.

To image tissue autofluorescence use 488 nm laser. To image CM-Dil tracks use 561 nm laser. Brain can be imaged with a voxel size of 1.22 µm x 1.22 µm x 8 µm on Z1 with a 5x objective.