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

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Optical Clearing of Tissue

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We use this protocol and it's working

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21925

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

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DMSO Sigma Aldrich Catalog #472301

OptiPrep™ Density Gradient Medium Sigma Aldrich Catalog #D1556)

2-Propanol (99.5 %) Millipore Sigma Catalog #278475

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 H2O

<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

Preparation of SBiP buffer: 500ml

*** Mix SBiP at 4°C till fully dissolved, kept at 4°C.

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 stirring can help to dissolve faster)

References:

Economo, Clark, et al., A platform 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 $1.22\ \mu\text{m} \times 1.22\ \mu\text{m} \times 8\ \mu\text{m}$ on Z1 with a 5x objective.