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4.5x Expansion of Mouse Brain tissue sections

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We use this protocol and it's working

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Abstract

This technique expands mouse brain tissue 4.5-fold in all spatial dimensions by embedding brain slices in swellable acrylamide gels. This allows enhanced resolution compared to traditional microscopy to precisely investigate subcellular targets in the brain. Here we adapted previously developed expansion protocols to reliably image enlarged Arl13b-GFP-positive cilia of striatal cholinergic interneurons in Arl13b-GFP knock-in mouse brain slices.

Materials

Reagents for homogenization buffer.

Reagents	Product No.	Vendor	Link
Sodium dodecyl sulfate (SDS)	L3771	Sigma Aldrich	https://www.sigmaaldrich.com/US/en/product/sigma/l3771
Urea	U5128	Sigma Aldrich	https://www.sigmaaldrich.com/US/en/product/sial/u5128
EDTA	ED2P	Sigma Aldrich	https://www.sigmaaldrich.com/US/en/product/sial/ed2p

Antibodies for GFP and cholinergic neuron staining.

Antibody	Product No.	Vendor	Link
anti-Choline Acetyltransferase (goat polyclonal)	AB144P-1ML	Millipore	https://www.merckmillipore.com/DE/en/product/Anti-Choline-Acetyltransferase-Antibody,MM_NF-AB144P-1ML
Anti-GFP (chicken polyclonal)	ab13970	Abcam	https://www.abcam.com/en-us/products/primary-antibodies/gfp-antibody-ab13970
Goat anti-Chicken Alexa Fluor 488	A-11039	Invitrogen	https://www.thermofisher.com/antibody/product/Goat-anti-Chicken-IgY-H-L-Secondary-Antibody-Polyclonal/A-11039
Donkey anti-Goat IgG Alexa Fluor 568	A-11057	Invitrogen	https://www.thermofisher.com/antibody/product/Donkey-anti-Goat-IgG-H-L-Cross-Adsorbed-Secondary-Antibody-Polyclonal/A-11057

Reagents needed to make gelling solution.

Reagents	Product No.	Vendor	Link
Sodium acrylate (97% purity)	408220	Sigma Aldrich	https://www.sigmaaldrich.com/US/en/product/aldrich/408220
N', N'-methylenebisacrylamide	146072	Sigma Aldrich	https://www.sigmaaldrich.com/US/en/product/sial/146072
Acrylamide (≥99%)	A8887	Sigma Aldrich	https://www.sigmaaldrich.com/US/en/product/sigma/a8887
Methacrolein	133035	Sigma Aldrich	https://www.sigmaaldrich.com/US/en/product/aldrich/133035
4-Hydroxy-TEMPO	176141	Sigma Aldrich	https://www.sigmaaldrich.com/US/en/product/aldrich/176141
Ammonium persulfate	248614	Sigma Aldrich	https://www.sigmaaldrich.com/US/en/product/sigald/248614
TEMED	1.10732	Sigma Aldrich	https://www.sigmaaldrich.com/US/en/product/mm/110732

			ls-films/sealing-film-parafilm-m/p/pc00.1

Safety warnings

[NOTE: because this chamber will be incubated at 37°C for prolonged times, it should be kept as clean as possible using 70% ethanol spray.]

[NOTE: use care when washing; in between washes, use a pipet tip to carefully remove buffer].

Ethics statement

The protocols.io team notes that research involving animals and humans must be conducted according to internationally-accepted standards and should always have prior approval from an Institutional Ethics Committee or Board.

Protocol

1 Prepare all stock solutions in ddH_2O and then prepare 10ml monomer solution.

Stock solutions in ddH_2O	To make 10 ml monomer solution:	Final concentrations:
38% Sodium acrylate (w/v)	5 ml	19% Sodium acrylate
50% Acrylamide (w/v)	2 ml	10% Acrylamide
2% N, N'-methylenebisacrylamide (w/v)	0.5 ml	0.1% N, N'-methylenebisacrylamide
10x PBS	1 ml	1x PBS
ddH_2O	1.5 ml	-

Make 500 µl aliquots of monomer solution and store at – 20°C. Thaw on ice and vortex before use.

2 Prepare 10% (v/v) TEMED in ddH_2O. Store at 4°C.

3 Prepare 10% APS (w/v) in ddH_2O. Can be stored at 4°C but loses activity with time.

4 Prepare 1% 4-hydroxy-TEMPO (w/v) in ddH2O. Solution can be aliquoted and frozen at -20°C.

5 Prepare homogenization buffer [store at room temperature to avoid precipitation].

Component	Final Concentration
Sodium dodecyl sulfate (SDS)	1% (w/v)
Urea	8 M
EDTA	25 mM
PBS (2×)	2X PBS, pH 7.5 (RT)

Prepare gelation chamber and glass slide chamber

6 Recycle a 1ml pipet tip box; cover holes with Parafilm and use tape to secure it to the box; leave some holes open. Fill the bottom of this chamber with sterile water to create a humid environment. [NOTE: because this chamber will be incubated at 37°C for prolonged times, it should be kept as clean as possible using 70% ethanol spray.]

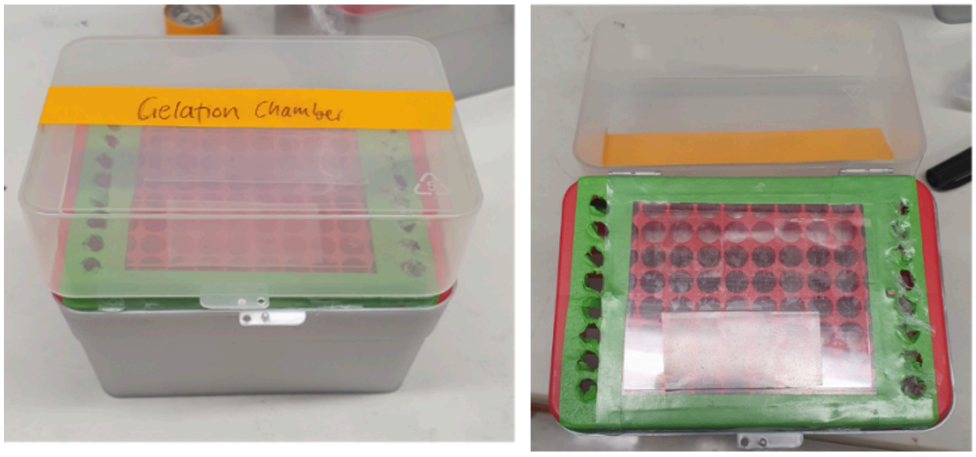

Figure 1: Gelation chamber made out of a 1 mL tip box.

7 Glass slide chambers are created by gluing 22×22mm micro coverslips to each side of the slide using Super glue (see Figure 2).

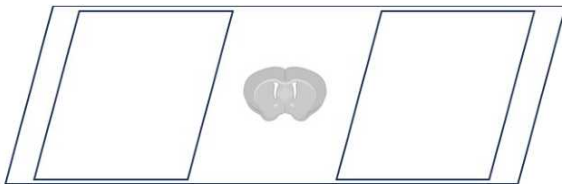

Figure 2: Schematic of brain tissue on microscope cover slide chamber prior to gelation.

Mouse brain preparation

8 Mouse brains are fixed by transcardial perfusion using 4% paraformaldehyde (PFA) in PBS.

9 Next, whole brain tissue is surgically extracted from the skull, post-fixed in 4% PFA for 24 hr and then immersed in 30% (w/v) sucrose in PBS until the tissue settled to the bottom of the tube (~48 hr).

10 Prior to cryosectioning, brains are embedded in cube-shaped plastic blocks with Tissue-Tek® OCT Compound and stored at −80°C.

11 OCT blocks are allowed to reach −20°C for ease of sectioning and 30µm slices are cut and stored at 4°C in PBS.

Gel embedding, staining and imaging

12 The gelation chamber is brought to a cold room (4°C) and cooled. Monomer solution is thawed on ice (~40 min).

13 Brain tissue sections are transferred to glass coverslip chambers using a paintbrush and washed 3 times with PBS. [NOTE: use care when washing; in between washes, use a pipet tip to carefully remove buffer]. The slice is washed one more time with PBS and left to air dry for 3 min at 4°C (in cold room).

14 Add 0.56 µl methacrolein (95% stock) and 0.56 µl, 1% 4-hydroxy-TEMPO (0.001% final) are added to 500µL monomer solution. Next, 27.7µL, 10% TEMED in ddH_2O and 27.7µL, 10% APS in ddH_2O are added and the mixture is quickly vortexed. Cover the tissue completely by carefully pipetting the mix onto the brain section. Another slide is used to cover the sample and gelation solution, while avoiding introducing bubbles to the gel.

15 The gel is left to polymerize for 30min at 4°C in the humidified gelation chamber in the cold room (Figure 1). Next, the chamber is transferred to a 37°C incubator overnight. The next day, the top glass slide is carefully removed and the gel containing the brain slice is trimmed to remove excess gel.

16 The glass slide with gel attached is next transferred to a 10cm dish containing homogenization buffer. The gel will slowly detach from the coverslip and can then be transferred to a 2 mL Eppendorf tube.

17 The tube is then filled with homogenization buffer to cover the gel completely and transferred to a Thermo-Shaker for 1.5h at 85°C.

18 The gel is next transferred to a 10cm dish and washed 3X with 10mL PBS-T (1X PBS with 1% Tween-20) for 10 min each, with gentle shaking in an orbital shaker. The buffer is then aspirated and the gel transferred to a 2 mL tube using clean gloves.

19 Primary antibody is diluted in 1mL, 2% BSA (1:1000 for anti-GFP antibody but other antigens may require 1:200) in PBS and added to the tube. Tubes are incubated with rotation for 3h or overnight at RT.

20 Gels are removed from the tubes and washed again 3X in PBS in a 10 cm dish with shaking.

21 Secondary antibody (1:2000) and DAPI (1:300) staining is carried out for 3h or overnight at RT in 2mL tubes.

22 Gels are washed 3X for 10 min each with PBS in a 10cm dish.

23 To expand the gel, wash it at least 3X in 15 mL ddH_2O for 30 min each on an orbital shaker.

24 The expanded gel is carefully cut into pieces that fit into 6-well glass bottom plates. The gels can then be imaged using a Zeiss LSM 900 with a 20x air objective/63x Oil objective.

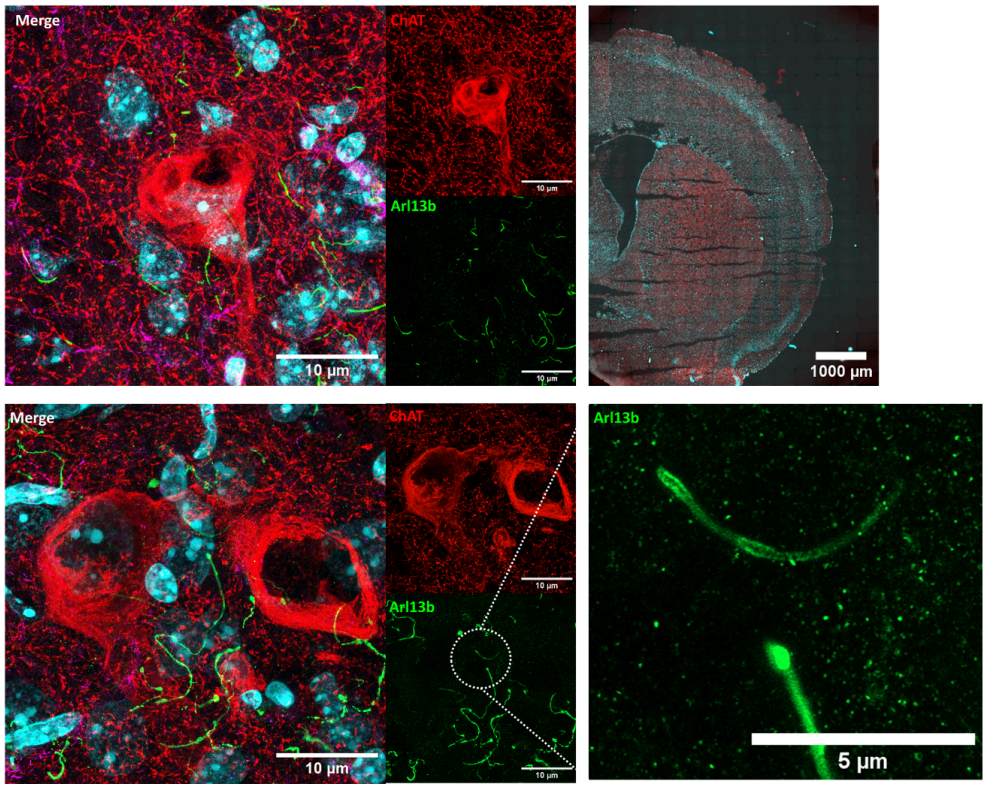

Figure 3: Expansion microscopy images of a mouse brain slice expressing Arl13b-GFP (green), stained for choline acetyltransferase (ChAT) (red) and DAPI (cyan). At left are 2 examples from the striatum. The enlarged image below right shows cilia labeled with anti-Arl13b where distinct ciliary
membranes can be detected. Above right shows a low magnification image of the brain section. NOTE THAT THESE MICE HAVE VERY STRANGE LOOKING CILIA--extremely elongated.

Protocol references

The monomer solution is adapted from Gambarotto et.al. (2019), with the implementation of the molecular anchoring strategy introduced by Kimas et.al. (2023).

Gambarotto, D., Zwettler, F.U., Le Guennec, M. et al. Imaging cellular ultrastructures using expansion microscopy (U-ExM). Nat Methods 16, 71–74 (2019). https://doi.org/10.1038/s41592-018-0238-1

Klimas, A., Gallagher, B.R., Wijesekara, P. et al. Magnify is a universal molecular anchoring strategy for expansion microscopy. Nat Biotechnol 41, 858–869 (2023). https://doi.org/10.1038/s41587-022-01546-1