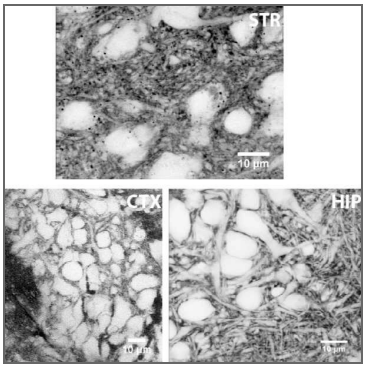

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Protocol status: Working
We use this protocol and it's working

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Mouse brain hemisphere organotypic cultures on glass coverslips

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ABSTRACT

This protocol describes preparation of organotypic hemisphere cultures prepared from mouse brain and cultured as rollerdrum cultures on glass coverslips. Having the cultures directly attached to glass coverslips facilitates imaging at very high resolution on inverted microscopes, including time lapse imaging. We specifically use these for STED microscopy based super-resolution microscopy, including super-resolution shadow imaging (SUSHI).

IMAGE ATTRIBUTION

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MATERIALS

Media and solution materials:

- MEM	Invitrogen/Gibco
- HBSS	Invitrogen/Gibco
- Glutamin 200 mM	Invitrogen
- Horse Serum	Invitrogen/Gibco
- Dest. H2O	Invitrogen/Gibco
- Kynurenic acid	Sigma
- Uridin	Sigma
- Ara-C Hydrochlorid	Sigma
- 5-Flouro-2'-deoxyuridin	Sigma
- D(+)-Glucose * H2O	VWR/Merck
- Sucrose	VWR/Merck
- CaCl2 * 2 H2O	VWR/Merck
- KCl	VWR/Merck
- KH2PO4	VWR/Merck
- MgCl2 * 6 H2O	VWR/Merck
- MgSO4 * 7 H2O	VWR/Merck
- NaCl	VWR/Merck
- NaHCO3	VWR/Merck
- Na2HPO4	VWR/Merck
- 1M HCl-	VWR/Merck
- 1M NaOH-	VWR/Merck
- Thrombin	VWR/Merck
- Chicken Plasma	Sigma (P3266-5ML)
- Poly-L-Lysine	Sigma

Cell culture ware:

- Coverslip glass 12x24 mm #1	Karl Hecht
- Cell culture tubes, flat bottom	Nunc

Prepare in advance

1 **Cutting solution:**
Sucrose 195 mM
KCl 2.5 mM
NaH_2PO_4 1.25 mM
$NaHCO_3$ 28 mM

$CaCl_2$ 0.5 mM

L-ascorbic acid 1 mM,
pyruvic acid 3 mM
glucose 7 mM
$MgCl_2$ 7 mM

Should be continuously equilibrated with 5% CO2 in oxygen (carbogen bubbling) during use.

2 **Washing solution**:
97 ml HBSS
1 ml Kynurenic acid (of 0.2M stock to reach 2 mM)
0.46 ml Glucose (11.5 mM, from 2.5M stock)
1 ml HEPES (of 2M stock to reach 20mM)
1 ml Pen/Strep (1x)
Adjust pH with 1M HCL or NaOH to 7.2, sterile filter and store at 4°C up to two days.

3 **Culturing medium:**
50 ml MEM-Medium (with Hank's salts)
20 ml HBSS
20 ml horse serum
1 ml Glutamax
2 ml B27
0.46 ml Glucose (11.5 mM final)
1 ml Sucrose (20mM final)
0.5 ml HEPES (10 mM final)
0.5 ml Pen/Strep (0.5x recommended)
3.5 mg Ascorbic acid (0.2mM final)

4 **PLL-coated glass coverslips:**
Wash/sterilize coverslips (by ethanol wash and or UV light) and place a drop of 0.01% Poly-L-lysine solution on the center part covering some 50% of the total area. Leave for 1-4 hours. Wash 3 times with dH_2O and allow to dry. Can be stored in fridge or freezer for at least 2 weeks.

5 **Thrombin stock and working solution:**
Stock solution: 0,5 g Thrombin in 50 ml dH_2O+ 50 ml HBSS solution and store in 500 µl aliquots at -20°C.

Work solution:
742,5 µl HBSS
4 µl Glucose from 2.5M stock (final 11.5 mM)
500 µl Thrombin stock

6 **Chicken plasma solution:**

Add 5 ml dH_2O per bottle using injection needle, briefly vortex, wait 5 min, sterile filter and store in 500 µl aliquots at -20°C until use.

7 **Mitosis inhibitor:**
12.1 mg of Uridine in 50 ml dH_2O
13.9 mg of Ara-C in 50 ml dH_2O
12.3 mg of 5-Fluoro-2'-deoxyuridin in 50 ml dH_2O
Mix 10ml of each solution, sterile filter and store in 500µl aliquots at -20°C *for 3-6 months*.
Use at 10 µl per culturing tube.

Preparatory steps

8 Thaw chicken plasma at 4°C, prepare thrombin working solution and keep at 4°C.

9 Disinfect surgical tools and utensils in pure ethanol and rinse off in dH_2O.

10 Turn on vibratome and set to 250 µm interval slicing.

11 Prepare cutting and dissection solutions, keep on ice. Allow culture medium to warm up (room temperature or 37°C).

12 Place culture tubes and PLL-coated coverslips at hand.

Brain dissections

13 Decapitate mouse pup and submerge the head in ice-cold cutting solution for 30 seconds. Keeping the tissue cold is of utmost importance.

14 Cut skin on scalp along midline starting caudally. Cut skull similarly and bent out skull to sides, to expose brain. Spoon out brain into ice-cold cutting solution using spatula.
The dissection should be swift and take no longer than 1 min.

15 Cut away cerebellum, and cut brain sagittally into two hemispheres.

Slicing on vibratome

16 Glue hemispheres onto slicing platform on caudal surface, so cutting starts rostrally from olfactory bulbs. Embed in low-melt agar for mechanical support.

17 Mount slice platform with hemispheres in vibratome and embed in ice-cold cutting solution. Start slicing at 250 µm interval until the region of interest is reached. Collect slices consecutively and maintain in room temperature cutting solution.

Mounting slices on glass coverslips

18 Collect the slices and place them in ice-cold wash buffer. Discard imperfect slices.

19 Using inverted Pasteur glass pipette, collect individual slices and place on PLL-coated coverslips. Remove excess medium, and add first 10 µl of chicken plasma solution, and then 10 µl of thrombin solution. Mix well around and embed slice.

20 Leave at 5 degrees C for 30 min (in fridge).

Culturing

21 Place slices on coverslips individually in roller tubes and add 0.75 ml medium to each. Maintain in rollerdrum incubator at 35 degrees C.

22 Change medium every 5-7 days. Discard cultures that detach, get infected, or do not maintain their gross structure.

23 Optionally add mitosis inhibitor on day 3-4 and remove after 12-18 hours by changing medium.