

Ultra Expansion Microscopy (UExM) Adapted for Delicate Tissue Sections

The goal of this procedure is to expand samples to make it easier to see small objects, especially those near the diffraction limit for light microscopy. It is based on the protocol from Gambarotto et al., 2019 with some modifications from the ExPath protocol found on expansionmicroscopy.org and notes from the Guichard lab's updated protocol. The original protocol shows isotropic expansion of approximately four-fold, linearly, although we have not yet analyzed this for this protocol.

Materials

- Pap pen
- charged slide
- 4% paraformaldehyde in PBS (PFA)
- 30% sucrose in water
- OCT compound
- dry ice (-20°C metal block will substitute, if necessary)
- cryostat
- 1x PBS
- Monomer Fixative Solution
 - 0.7% formaldehyde
 - 10% w/v acrylamide
 - in ddH₂O
- UExM-MS (Monomer Solution)
 - 19% w/v sodium acrylate
Note: Store powdered sodium acrylate at -20°C with dessicant to prevent it from absorbing water from the air.
 - 10% w/v acrylamide
 - 0.1% BIS
 - in PBS (1x final)
 - Note: Fresh batches of UExM-MS can be checked by adding TEMED and APS to polymerize a small aliquot. If it fails to polymerize after a few minutes at room temperature, it may not have been made correctly, or reagents (such as sodium acrylate) may not be pure enough.
- metal block or other flat surface, stored at -20°C
- bucket of ice
- Parafilm
- glass slides or coverslips to use as spacers
- 10% APS in ddH₂O
- 10% TEMED in ddH₂O
- cell scraper or other blunt, straight edge for trimming gels
- slide mailer or Coppelin jar with cover
- hybridization oven that goes up to 95°C
- D (Denaturation) buffer
 - 200mM SDS
 - 200mM NaCl

- 50mM Tris
- in ddH₂O
- Note: A white precipitate may initially form. Invert and vortex until this goes away completely before using the buffer.
- many liters of ddH₂O, does not need to be sterile
- antibodies and DAPI, diluted in PBS
- glass-bottom imaging dish
- Kim wipes
- forceps

Procedure

Day 0: Fix the Sample

- 1 Fix sample in PFA and wash thoroughly in PBS.
- 2 Cryoprotect sample in 30% sucrose.

Day 1: Section and Gel the Sample

- 1 Embed sample in OCT on dry ice or -20°C metal block, ideally surrounded by dry ice.
(Note: The above steps can be done well in advance with whatever storage is best for the sample. Olfactory epithelium can be stored at 4°C at the PFA, PBS, and sucrose stages and at -80°C at the OCT stage—not indefinitely, but for more than a day.)
- 2 Draw a Pap pen border on a charged slide. Allow to dry.
- 3 Section sample on cryostat, eg. at 20µm thickness.
- 4 Pick the section up on the charged slide, within the Pap pen border.
- 5 Let the section thaw and dry for 1 minute.
- 6 Add PBS within the Pap pen border. Add it very gently and not directly to the section. The goal is to avoid lifting the section off the slide. Add just enough liquid to form a small droplet, completely covering the section.
- 7 Wash the section a total of 3 times for 5 minutes each.
- 8 Remove the PBS and add monomer fixative within the Pap pen border.
- 9 Incubate 1-5 hours at room temperature or 37°C. (I did 40 minutes RT, 30 minutes 37°C.)
- 10 Remove monomer fixative and replace with UExM-MS. Incubate at room temperature while setting up the following steps (at least 1 minute).
- 11 The following steps, steps 14-18, must be done quickly and in a chemical hood, so set up:
 - a. a cold metal block in an ice bucket. Place a sheet of Parafilm on the metal block and weigh it down, eg with stacks of glass slides, to keep it somewhat flat.
 - b. glass spacers to the desired thickness (approximately three 1-oz coverslips worked for a 20µm section). Place these on the Parafilm block such that a slide can rest on top of them, and the gel will have some room to spread.
 - c. 10% APS on ice
 - d. 10% TEMED on ice

- e. an aliquot of UExM-MS on ice. This should be 135 μ l, or approximately 0.27 μ l/mm² of the area to fill. Approximately 35 μ l is needed for a 12mm circular coverslip in the original UExM protocol, and 135 μ l UExM-MS yields 150 μ l total gel.
- 12 Add 7.5 μ l of the 10% TEMED to the 135 μ l aliquot. Mix thoroughly by pipetting.
- 13 Wipe any frost or condensation off the Parafilm between the glass spacers on the metal block.
- 14 Remove as much UExM-MS as possible from the section using a pipette. It does not need to be totally dry, but it should not drip when inverted.
- 15 Add 7.5 μ l of the 10% APS to the UExM-MS aliquot. Mix thoroughly and pipette into a droplet on the Parafilm-covered block, between the spacers.
- 16 Quickly invert the slide and place it on the gel such that it rests on the glass spacers. The gel should spread to cover the sample.
- 17 Incubate the gel on the cold block for 5 minutes.
- 18 Remove any weights and slide the Parafilm (with spacers, gel, and slide) off the metal block carefully, so as not to disrupt the gel.
- 19 Incubate the gel at room temperature overnight. (It is possible that incubating the gel at 37°C for a shorter period, such as 1 hour, will also work.)

Day 2: Denature, Expand, and Start Staining

- 1 Lightly press the slide to see that the gel is solidified. It should not ooze out.
- 2 Invert and gently peel the Parafilm off of the gel.
- 3 Ensure that the spacers are no longer on the slide. If needed, trim away excess gel, leaving very little extra space around the sample. This will make it easier to find your sample when imaging later.
- 4 Place the slide with the gel in a slide mailer filled with D buffer.
- 5 Place the slide mailer in a room temperature hybridization oven. Turn the oven on to heat up to 95°C.
- 6 Incubate in D buffer for 2-6 hours. (Overnight may be okay.)
- 7 Check the slide. If the gel is still adhered or if it is ruffled and not smooth, continue to incubate.
Safety note: Steaming hot SDS may not be great for your health. It might be better to do this in a chemical hood.
- 8 Begin expansion by transferring the gel into a beaker with approximately 500mL ddH₂O. Incubate 30 minutes at room temperature.
- 9 Repeat step 8. If needed, repeat again or let the gel sit overnight, until the gel does not seem to get any larger.
- 10 If there is lots of gel remaining that does not contain tissue sample, now is a good time to trim this off.
- 11 Transfer the gel to PBS and incubate for at least 30 minutes.
- 12 Dilute primary antibodies in PBS and incubate at 4°C overnight.
Note: If gels are large, this will require a large volume of antibody solution. To reduce the volume needed, you can seal a plastic pouch around three sides of the gel in PBS, then remove the PBS and replace it with antibody solution. Incubate this pouch on a gentle rocker in the cold room.

Day 3: Finish Staining

- 1 Transfer gel into PBS, approximately 200mL, at room temperature for 30 minutes.
- 2 Repeat step 1.
- 3 Incubate gel in a solution of fully diluted secondary antibody with DAPI at 4°C overnight. (Again, a pouch can be used, and it can be incubated on a rocker in the cold room.)

Day 4: Wash and Expand

- 1 Transfer the gel into approximately 200mL of PBS. Incubate at room temperature for 30 minutes.
- 2 Place gel in approximately 500mL of ddH₂O. Incubate at room temperature for 30 minutes.
- 3 Repeat step 2.
- 4 Place gel in ddH₂O for at least 1 hour to fully expand before imaging.

Imaging

- 1 Trim gel to fit within the glass area of the imaging dish.
- 2 Blot the gel with a Kim wipe.
- 3 Place the gel sample-side down on the glass. If you can't tell which side has the sample, try backlighting the gel as you look across the surface. See which side seems to have something embedded in it. Take your best guess, and if you have trouble getting your sample in focus later, you may need to flip the gel over and try again.
- 4 Fold a piece of Kim wipe and tear it such that forceps can be used to wipe under the edge of the gel. Repeat to remove as much water as possible. This will reduce problems with the gel sliding around during imaging or having issues with getting in focus due to limited working distance because a thin layer of water can still be many microns thick.
- 5 When the gel is where you want it for imaging, place a dry Kim wipe over the top and lightly press tap it to make the gel flush with the glass, with as few bubbles as possible.
- 6 Stretch a piece of Parafilm to fit over the gel and within the dish. Press it down to the edges to seal the gel in such that it doesn't dry out and shrink during imaging.
- 7 To find cells of interest, I recommend searching with a low-magnification objective, as this will look like an unexpanded sample with a high magnification objective. Center your cell or structure of interest, and then move to higher magnification.