

# Array tomography protocol: preparation of arrays

Tissue embedded in acrylic resin is cut into arrays of serial ultra-thin sections, which are transferred to coverslips and mounted onto glass slides in preparation for immunostaining.

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## Coverslip preparation

1. Place coverslips into a staining rack (ensure coverslips are clean and dry before use).
2. Prepare subbing solution by dissolving 1.5 g gelatin in 290 mL ultrapure water and heating to <math><60^{\circ}\text{C}</math>. Do not overheat. Dissolve 0.15 g chromium potassium sulfate in 10 ml ultrapure water. Combine the two solutions, filter and pour into a staining dish. Use fresh.
3. Immerse the coverslips in the subbing solution for 30–60 s, with gentle agitation.
4. Remove coverslips and drain off excess liquid. Leave in a dust-free place until dry.
5. The dry, subbed coverslips are stored in dust-free boxes until they are needed.

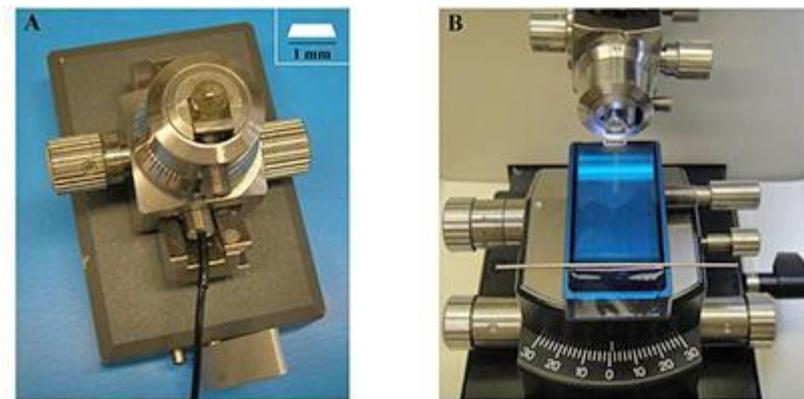
*If the coverslips will be used for more than four antibody elutions, they should be carbon-coated using a carbon-evaporator. Carbon-coating will ensure better adhesion of the sections to the coverslips. Aim for a light grey color. After carbon-coating, coverslips are very hydrophobic so leave them several days before use.*

## Preparation of arrays

1. Trim the block around the tissue to form a pyramid with a small trapezoid-shaped blockface. The trapezoid-shaped blockface is most effective when it has a width of 1 mm or less and its width is greater than its height (Figure 2A).
2. Cut semi-thin sections until you reach the tissue. Trim the block again at this point to ensure that the blockface has not become too large and the leading and trailing edge remain parallel. The Cryotrim 45 diamond knife is very good for this purpose.
3. Using a paint brush, apply diluted Weldwood contact cement (dilute with xylene~1:2) to the leading and trailing sides of the block pyramid. Blot away any excess glue with a tissue.
4. Fill the knife boat of the Jumbo Histo diamond knife with water and insert a coverslip. A stainless steel rod helps prevent the water from receding (Figure 2B).

*Carbon-coated coverslips are more hydrophobic so if using these add 0.005% Tween-20 to the water in the knife boat. Keep the water level lower to avoid it jumping onto the blockface. The Tween-20 may dissolve the glue causing ribbon breakage. If this occurs decrease Tween concentration by adding a few drops of water to the knife boat and /or add two coats of glue to the sides of the blockface.*

5. After the glue has dried (~2 min), start cutting ribbons of serial sections (60–200 nm) with the Jumbo Histo diamond knife. Thinner sections will adhere better to the coverslip.
6. When the desired length of the ribbon is achieved, carefully detach it from the knife edge using an eyelash probe. Remove the stainless steel rod and gently push the ribbon towards the coverslip so that the edge of the ribbon touches the glass at the interface of the glass and the water. The edge of the ribbon will then stick to the coverslip.
7. Using a syringe, slowly lower the water level in the knife boat until the entire ribbon sticks to the coverslip. Take out the coverslip. The position of the ribbon can be indicated using a permanent marker (but only mark the opposite side of the coverslip to the sections).
8. Lay the coverslips flat to dry before placing them on a slide warmer (~55°C) for 30 minutes. The coverslips can be stored at room temperature for at least 3 months.



**Figure 1:** Sectioning of the embedded tissue. (A) The block is trimmed to form a pyramid with a small trapezoid-shaped blockface. The width of the trapezoid should be 1 mm or less with a width greater than its height. (B) Serial sections of 60–200 nm are cut using the Jumbo Histo diamond knife. The stainless steel rod helps to prevent the water receding.

## Materials and reagents

- VWR Micro Cover Glasses (No. 1.5, 24 x 60 mm), or for quantitative studies use Bioscience Tools High Precision Glass Coverslips (CSHP - No. 1.5 - 24 x 60 mm)
- Gelatin (300 Bloom)
- Chromium potassium sulfate (chrome alum,  $K_2Cr_2(SO_4)_6 \cdot 12H_2O$ )
- Staining rack and dish
- Jumbo Histo diamond knife (Diatome)
- Cryotrim 45 diamond knife (Diatome) - optional
- Ultramicrotome
- Weldwood Contact Cemen
- Xylene
- Thin paint brush
- Slide warmer
- Eyelash probe
- Stainless steel rod
- Syringe and filter

### Additional materials and reagents for carbon-coated coverslips:

- Tween-20
- Carbon rods (e.g. Ted Pella #93010)
- Carbon evaporator (e.g. Cressington carbon-coater 308R)