sulfate-latex on to a positively charged surface.

The recipe for preparing the sample is:

1) Dilute the latex particles to about 0.1% (volume fraction) in salt solution (ionic strength adjustable)
2) Put a piece of newly peeled mica or other charged plate (depending on what latex you use) in the solution for about 1 hour
3) Take the plate out and gently rinse with dionized water
4) Let the sample dry if you image in air, otherwise image it immediately in liquid.

The surface coverage can change from 10% to 55% when the ionic strength changes from 0.1 mM to 100 mM. This way you can adjust the surface coverage.

Yonghui Yuan and Chris Johnson. University of Delaware

TEM of Keratinocyte Cell Cultures:

We do a lot of TEM of keratinocyte cell cultures using this embedding cell culture plates:

1) Phosphate Buffered Saline (PBS) rinse X 3 (discard leftover down sink with bleach)
2) 1/2 strength Kamovsky's (2% fomaldehyde and 2.5% glutaraldehyde) 3 hours to overnight.
3) 0.1M Na cacodylate 15 minutes X 2
4) 1% $0s0_4$ 1 hour
5) Distilled H_20 15 minutes X 2
6) 1% aqueous Uranyl Acetate 11/2 hours
7) Dehydration: 35% EtOH > 15 minutes x2
   70% EtOH > 15 minutes x2
   95% EtOH > 15 minutes X2
   100% EtOH > 15minutes X2
   100% EtOH 30 minutes X 2
8) 3:1 EtOH:Epon 6-8 hours
9) 2:1 EtOH:Epon 2-16 hours (overnight with caps off)
10) 1:1 EIOH:Epon 6-8 hours (with caps ott)
11) 100% Epon 6-8 hours
12) Bake tor 24-46 hours in 60° C oven

NOTE: Do not use propylene oxide! It dissolves the plastic tissue culture plates. We have been using this procedure for years and don't really know what the original source is. It is protocol that works well. There may be others that work better.

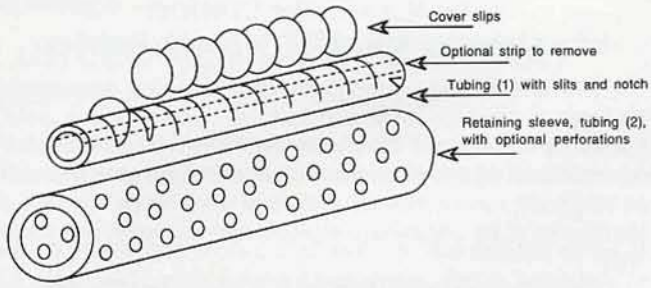
Bob Underwood, University of Washington

A Simple Cover Slip Holding Device for Staining or CPD

Materials:

Round cover slips containing attached samples to be processed.
Razor blade, gloves, and forceps.
Fixative or stain solutions. Exhaust hood, if working with fixatives.
2 sizes of CPD (critical point drying) or stain compatible, thin walled tubing (Polyethylene or teflon), diameter (1) is slightly larger than cover slip diameter, diameter (2) is slightly larger than diameter (1) so that tubing (1) containing cover slips will slide into tubing (2), length is variable depending upon application. Sleeve tubing (2) may be perforated with a small drillbit or dremel tool to facilitate liquid transfer. If using for CPD, pre-test tubing to assess com patibility with high pressure (some tubing materials will foam).
Petri or other dish to hold tubing flexed under liquid during cover slip loading.

1. Cut parallel slits 3/4 of the way through the tubing with a razor blade. An option is to remove a strip of the slit tubing along the top by cutting it lengthwise in two places as shown by the lines in the figure. Notch one end of the tubing for orientation.
2. Flex tubing to open up slits. This can be accomplished by making the tubing slightly longer than the dish and wedging it in place under fixative or other liquid after flexing.



3. Load cover slips into opened up tubing slits. The edge of each cover slip should protrude slightly to allow retrieval. Two scenarios are depicted. The first cover slip protudes further than the second. This protrusion may vary depending on the relative diameters of the cover slips and tubing available. In the first case, the optional strip should be removed to prevent damage to the sample. In the second case, the majority of the sample is within the tubing. Carefully remove tubing from dish and gently straighten to pinch and hold cover slips in place.
4. Carefully slide assembly into the larger tubing (2) (syringe body, polyethytlene straight sided rigid or teflon tubing with both ends open). The entire assembly should fit comfortably into your CPD or staining dish. The idea is to prevent the cover slips from falling out during processing.

Ed Basgall, Pennsylvania State University

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