MICROSCOPY 101

We appreciate the response to this publication feature - and welcome all contributions. Contributions may be sent to Phil Oshel, our Technical Editor at:

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Preparing Pellets of Isolated Cells for Frozen Thin-Sectioning:

We add paraformaldehyde to the cultured monolayers, swirl for just a few minutes (about 5), scrape with a rubber policeman and pellet into a small tipped tube. We use Sarstedt #72.702. It wouldn't have to be Sarstedt, if somebody else makes them. They just need to have a very small bottom if the pellet is small. These are about 45 mm long, have an inner diameter of 4 mm, and a smaller tip, shaped like a nipple, the inside diameter of which is about 1 mm. The nipple part is about 7 mm long.

We pellet in a swinging bucket centrifuge and then microfuge to pack the cells.

We then let them fix for another hour or two and cut off the very bottom and again just above the cells, forming a log with the cells In the center that can be pushed out with a paper clip. If they stick together, fine, proceed.

If not, push them into small piles (about 0.5-1 mm) on a piece of Parafilm, drain them with filter paper cut into pie-shaped wedges using the very tip to touch the pellet gently, and coat them with cooled, still molten 1% agar. Cut away any excess agar.

Infiltrate with 3 changes of sucrose (2.3M) over about 30-60 minutes. Place onto stubs and flash freeze. This keeps the cells together, not dispersed thinly in the sucrose.

If the cells are fixed very long before pelleting, they will not like to stick together, and will disperse in the sucrose. The consistency of the cell pellet should be like cooked oatmeal. (I could make some "snotty" comment about consistencies of other substances). If they are too wet, they will disperse. and you'll have to hunt all over your grid for them. If they're too dry, the ultrastructure could be altered.

Sara E. Miller, Duke University Medical Center

Making a Monolayer of Latex Spheres for Calibrating a Scanning Probe Microscope:

A z-standard for calibrating a scanning probe microscope made be made by adsorbing latex particles onto a mica surface. The latex must be positively charged, such as amidine-latex particles, since the mica surface is negatively charged in aqueous solution. A very uniform layer of spheres may be obtained just by putting the mica in the latex solution for about an hour. The surface coverage can be adjusted by changing the ionic strength of the solution. Similarly you can also adsorb negatively charged latex particles such as

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.

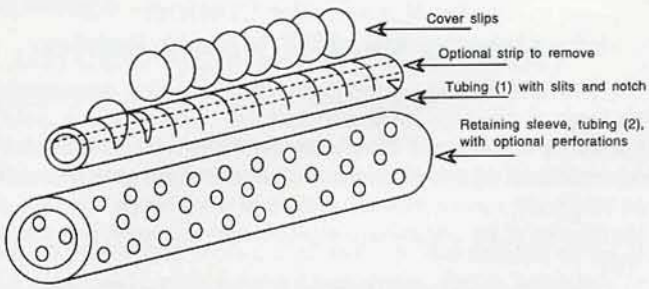
Bob Underwood, University of Washington

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 patability 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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