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