and adding a coverslip, then waiting for a few minutes for the living white cells to take up the stain. Unfortunately these slides are not permanent, although their significant information can be archived photographically.

I have not tested it in cell culture, but toluidine blue makes a great supravital stain for blood and other body fluids. I use slides dipped in 0.25 to 0.5% toluidine blue (in absolute ethanol) and then dried on end at 60° C. They keep for months. After a few minutes, a complete cell differential can be done. Each kind of granulocyte is readily identified, and the basophils are often spectacular, with bright, purple granules. Mature monocytes usually have an elongated nucleus with a tiny nucleolus at each end, and small lymphocytes have either 1 or 2 somewhat purple nucleoli. Large lymphocytes have more than one nucleolus, up to 5 or 6.

1) Dissolve 0.25-0.50% toluidine blue in 100% ethanol.
2) Warm clean slides at 60° C, dip in the alcoholic stain solution, and stand on end to drain and dry. Slides are good for months.
3) Put on a *small* drop of fluid, add coverslip, and wait 2 to 5 minutes to allow for stain uptake. Preparation is not permanent because the cells are destroyed after 20-30 minutes. They are photogenic, though, if a permanent record is desired.

This method may well work on cells in tissue culture. See Millikin, P.D. 1974. "A Supravital Stain for Nucleoli in Human Lymphocytes," American Journal of Clinical Pathology 62:520-24.

Incidentally, when this stain is used for pleural or peritoneal fluid, any cancer cells may appear spectacularly large because they are not shrunken by fixation or other processing.

In the final analysis, the stain you use depends on the information you want.

Paul Millikin, Peoria, IL

Curing a False Engagement Problem in an AFM.

I have had trouble with very high deflection increase close (microns) to the specimen surface, increasing the deflection voltage by many tens of volts, causing a false engagement. One way to obtain a true engagement is by successive adjustments of the photodiode detector, but this is a tedious exercise that does not remove the large repulsive force.

The best reason that I have come up with as to the source of the problem is from long range electrostatic charge on the surface, the tip, or both. The reason (it is believed) that the repulsive force has electrostatic origins is that touching a grounded wire to the sample puck (if mounted on a metal puck), and/or a metal component of the tip holder that is connected to the probe, often (but not always) removes the repulsive force.

Specifically when using a DI fluid cell, I have found it works well to gently touch the ground wire to the wire tip clamp that protrudes from the top of the cell. Grounding the sample and tip does not always work, possibly due to bad connections. Another method that also works well, but requires a bit of time, is to mount a Polonium source (purchased from Structure Probe, but other microscopy suppliers carry them) near the sample cell. We tape the source to the side of the AFM head, and wait for the vertical deflection to reach a constant value and re-engage.

Jason Bemis, University of Pittsburgh

Is Di-amino-benzidine (DAB) Really Carcinogenic?

Some interesting questions were recently posed on the Histonet Listserver about DAB, which is used as a marker for cells. While DAB itself has not been the subject of in-depth carcinogenicity studies, it is known to be mutagenic. Further, all members of the benzidine family that have been tested have been proved to be carcinogens. In the United States, at least, all benzidine derivatives are considered carcinogens by the NTP (National Toxicology Program), and OSHA will soon make that official.

So, the question is not about DAB *per se*, but about any substance that still retains that benzidine nucleus. None of the procedures in immunohistochemistry alter that nucleus; they merely change other parts of the molecule to make it soluble, and then later insoluble. Regardless of its physical state (powder, tablet, solution or precipitate), or the manner in which it got to that state (hydrolysis, oxidation, time), the benzidine nucleus remains. Thus it is still carcinogenic.

The only proven method of destruction of the benzidine nucleus is via acid permanganate oxidation.

Richard W. Dapson, Anatech Ltd.

TEM of Cultured Cells on Coverslips

For TEM of cultured cells, we grow the cultures on "Thermanox" tissue culture coverslips (from Nalge Nunc International, 50 sterile coverslips, 13 mm diameter is catalog 174950). The coverslips can be treated with all the same chemistry as tissue, including propylene oxide and Spurr's epoxy, which are two components which solubilize polystyrene.

Coverslips are easily processed in disposable polypropylene 50 mL centrifuge tubes, useful since the cell surface will not contact the wall of these conical tubes.

1) Sink the Thermanox coverslips cell side up in freshly made Spurr's.
2) Following polymerization, remove coverslips by first sawing a small area of the epoxy/cell/substrate, then immersing in liquid nitrogen for a few seconds and prying away the substrate. The embedded cells are now on the surface of the epoxy.
3) Re-embed two fragments of the culture face to face for cross-sections, or cut the block parallel to the face for tangential sections.

We particularly like the round 13 mm Thermanox coverslips for immunocytochemistry of cultured cells since they can be floated cell side down in a drop of 100 µL antibody - gold conjugate, which conserves reagents.

If a larger culture is desired, "Permanox" culture dishes could also be used, which are equally resistant to chemicals common in TEM processing. These are also available through most EM and other suppliers.

Douglas R. Keene, Shriners Hospital Microscopy Unit

An Easily Available Specimen for Checking an AFM tip:

The simplest commercial specimen for checking out your tip may be Nuclepore filters. These contain etched nuclear particle track pits available in standard diameters ranging from 50 nm up to well over 2 micrometers.

Our original paper on the subject is archived at Los Alamos*, and published in Ultramicroscopy 37 (1991)125-129. You can also find this and two later papers on reconstructing tip shapes in the "reference papers" section of our scanned tip and electron image web page.** Updated versions of the latter two will be made available in the archives soon as well.

* http://xxx.lanl.gov/abs/cond-mat/9712003
** http://newton.umsl.edu/stei_lab/

Phil Fraundorf, University of Missouri – St. Louis