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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Tips on Finding Grain Boundaries in the TEM

I spent some time working on beta silicon nitride, and I found it very time consuming, because one needs to find orientations such that two grains are in orientations that can be imaged, while at the same time the grain boundary needs to be parallel to the beam. The paper by D.R. Clark (Ultramicroscopy 4 (1979)33-44) is useful.

What I found to be helpful was to first check the image of the boundary at lower magnifications (typically X100,000) in bright field mode and tilt about an axis parallel to the image of the boundary until I attained a relatively sharp "minimum" in the width of the boundary. This is relatively fast, and it allows elimination of those grain boundaries that are way out of orientation (I was using a 30° double tilt holder).

Once I had what seemed to be a suitable grain boundary, then at that point I would check the diffraction pattern to see if there were any Kikuchi lines (from either grain) that were parallel to the grain boundary and which corresponded to reflections that could be used for high resolution TEM imaging (*i.e.*, reflections that had a large enough d-spacing). If that was the case, then I would tilt the sample along that Kikuchi line (*i.e.*, maintaining that set of reflections for that particular grain) and at the same time check the diffraction pattern from the other grain until a suitable orientation was attained for that second grain. More often than not, I had to move on to another location and start the whole operation again until all three elements (grain 1, grain 2 and the grain boundary) had the proper orientation.

In most instances, my images consisted of two beam conditions, but that was sufficient to resolve the grain boundaries which were typically about 1 to 4 nm wide. In one afternoon session I could probably end up with three to five suitable boundaries (plus coffee breaks).

Jordi Marti, Allied Signal

A Hint for Reducing Background for Immuno-TEM

Non-specific background staining is occasionally a problem with some antibodies and labeling systems. If normal washing fails to reduce the background you might want to try using a high salt buffer for washing. Phosphate buffered saline or any other recipe with NaCl in it, is probably around 150 mM (0.9 %). Try boosting the NaCl to 5X normal. This would make it 750 mM, or 4.5% by weight.

If high salt buffer is used, remember to incubate the grids in a couple of changes of regular strength (150 mM) saline before going to the next step, to get the salt back into the range of physiologic strength.

An example would be to wash the grids on five drops of high salt buffer for about 2 minutes each, followed by two drops of normal salt buffer for about 2 minutes each. Use this routine after incubating on the primary antibody or after any incubation you suspect may be contributing to background (secondary antibody, colloidal gold, etc.).

I have not found a good explanation for why this works, although it probably changes the conformation of proteins, alters their overall charge, and makes them less likely to bind to the surface of the sectioned material.

High salt concentrations are often used in biochemistry to precipitate proteins out of solution and to wash chromatography columns. Something similar is probably happening on the surface of the section, *i.e.*, only those antibody molecules which have bound specifically to antigenic sites are able to stay on the section in the presence of high salt concentrations.

Robert (Rob) Chiovefli. E. Licht Company

A Solution for Cultured Lymphocytes Missing Membranes After Preparation for TEM

This is a *very* rapid process.

The cell suspension is processed in centrifuge tubes. Spin cells at 2500 rpm for one minute, pipette off culture medium, and replace with 2.5% glutaraldehyde in 0.1M Na cacodylate and resuspend.

Immediately place tube in centrifuge and spin for 1 minute at 2000 rpm.

Each solution was changed, cells resuspended and spun immediately, therefore each solution was used for about 1.5 minutes.

Buffer wash with 0.1 M Na cacodylate
2% OsO_4 in same buffer
buffer wash (once)
10% ethanol
5% aqueous uranyl acetate
50% ethanol
75% ethanol
100% ethanol
into Spurr resin in Eppendorf centrifuge tubes and straight into oven: polymerize at 60° C.

I processed literally as fast as possible. An earlier attempt using 5 minutes at each step was an improvement but not good enough.

I suspect that the cell line is unusual in needing this short a time, but it's worth trying for other cells.

Chris Gilpin, Manchester University

If Your Older Model ISI/Topcon SEM Blows Transistors...

Some older Isi/Topcon SEMs regularly blow the transistors in the box on top of the HT tank. We think we have been able to identify and solve the problem.

The circuit inside the tank on the bias and HT side that has the 4700 pF and 2200 pF caps on it, is to filter out the back electromagnetic field from the high voltage side of the tank. We found that these same capacitors go soft, and so leak that voltage back up into the driver transistors on top of the tank. This is what causes them to blow.

It is quite simple to monitor this. If you measure the collectors of these transistors, you should measure waveforms with a peak voltage not exceeding 400 V on any of the transistors (the actual voltage will vary with bias settings, filament current and accelerating voltage). Should you find that the voltage is close to this voltage or over, then replace the relevant capacitors in the tank (C20, C21, C4, C5, *etc.*, 4700 pF 2.5 kV and 2200 pF 4 kV and 0.1 µF 630 v). This will solve the problem.

This is applicable to quite a range of their models, from the SX30, SX40, SS60, DS130, WB6, and on to the newer series of ABT 55 and 60. These are the only models we have here in South Africa. This tip may apply to more models, but we have not seen them.

Luc Harmsen, Anaspec, South Africa