



MICROSCOPY

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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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Resolution and Resolving Power

A very basic point that is almost always overlooked when discussing SEM "resolution," is the difference between "resolution" and "resolving power."

An SEM might have a resolving power of 1 nm or so based on the observation of gaps between gold particles on a featureless carbon film. Fine--if ones job is to look at gold on C.

For the actual 3-D samples most everyone looks at, EVERY micrograph has a DIFFERENT resolution, closer to 10 nm, because of secondaries generated by back scattered electrons, edge effects, ...etc. If you want resolutions closer to the instrument's resolving power you have to look at thin TEM specimens without sharp edges and angles.

In our semiconductor lab we have several FESEMs advertised to have resolutions of 1 nm that can not resolve 4 and 5 nm layers in a film stack. There is no question that these instruments are superior to non-FESEMs and that today's SEMs are feature-filled and wonderfully reliable--just don't expect 1 nm resolution in real images and remember the difference between resolution and resolving power.

So if every instrument has one "resolving power" and a different "resolution" for each picture, the only test of "resolution" is to take ones bread-and-butter samples to each manufacturer and compare the resulting images. There is not any theoretical formula that will provide a more meaningful answer.

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Safe Disposal of Mercury Salts

Make sure that gloves are worn for this procedure. All containers and gloves used should be rinsed with water and the water put in the waste mercury container for next time.

All solutions containing mercury salts are collected into a jar labelled "Used Mercury Salts". We keep this in our grossing room. The jar is actually a clear glass 3 kg sodium phosphate container.

When the jar is about half full, to each 900 mL (estimate, don't measure) add 40 grams of sodium carbonate to raise the pH to 8.0 or higher and mix well.

Leave it for a while, then filter the solution and collect the precipitate into a plastic jar for disposal. Put the filter paper in the jar as well. Seal the jar very well. We coat the lid

with several layers of paraffin wax until it is about a quarter inch thick. Rinse the funnel and put the washings into the waste mercury container.

The filtrate should be clear. If it is not, repeat the process. We check by adding a little dissolved sodium carbonate to see if any more precipitate forms. If it does not, all mercury salts have been removed and the filtrate may safely be disposed of down the sink, if local regulations allow.

Pack the jar containing the precipitate inside a safety container and seal.

This procedure at the least works with B5, formol sublimate, and similar fixatives. In practice these are the two biggest problems with disposal. It should work with mercury-dichromate mixtures (Zenker type), but I have no idea what the reaction is with the dichromate.

Reference:

Crookham, J & R Dapson. Hazardous Chemicals in the Histopathology Laboratory. Anatech, Ltd.

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Cleaning Platinum Crucibles

Many years ago we regularly used platinum crucibles in many analytical chemistry procedures, and according to my analytical chemistry book, platinum can be cleaned by fusion with potassium or sodium bisulfite. I later adopted this procedure with good success to clean the platinum apertures for an RCA EML electron microscope.

Get a small ceramic crucible, put a bit of the bisulfite in it, heat it until the bisulfite melts, then drop the apertures into the melt. After a few minutes allow the melt to solidify, dissolve the bisulfite with hot water, and your apertures should be as bright and shiny as new.

If this does not work, try heating them in a mixture of equal parts of concentrated hydrofluoric acid and concentrated hydrochloric acid - using appropriate safety precautions, of course!

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Preparing Diatoms for EM

Diatoms make for beautiful specimens for both transmission and scanning electron microscopy. As well, they are studied by many people, and there is always a need for good diatom preparations for EM.

I find that the diatoms in diatomaceous earth are usually broken, and like to prepare my own from fresh specimens. The materials needed for this are a plankton net, some potassium dichromate, and 30% hydrogen peroxide. Once the diatoms are collected (plankton net tows from shoreline, or wherever), they can be cleaned using the chemicals.

1) Place the diatoms in a large beaker (and I mean large here) then pour in 15 to 20 mL of hydrogen peroxide followed by a large pinch of the potassium dichromate. The resultant reaction oxidizes the organics, leaving the glass frustules of the diatoms unharmed. It is better to be cautious with the amounts used as this is an exothermic reaction, sometimes violently so. Not that there will be an

explosion, but I've had to clean up boil overs on a number of occasions.

2) Once the reaction subsides and the solution turns a golden yellow color, it is just a matter of washing the diatoms. There are a couple of ways to do this:

a) centrifuge the diatoms, then throw out the liquid, add water, centrifuge, throw out the liquid, etc.

b) fill the large beaker with water and let the diatoms settle to the bottom (1-2 hrs), carefully pour off the fluid, and add more water, let the diatoms settle, etc. The diatoms will appear as a milky white residue in the bottom of the container. The sample may have to be concentrated to really see this). If any more information is needed please feel free to contact me.

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Preparing Powders For Electron Diffraction Studies

Questions often come up concerning the best way to prepare powders for electron diffraction analysis in the TEM. This is actually fairly simple:

1) Take an oxide powder, grind it up with mortar and pestle and take a carbon coated grid and swipe it across the fines. Two glass slides can also use to grind up the powder.

2) A number of materials can be evaporated onto a carbon coated grid or onto a cleaved NaCl sample and then float that off on water onto a grid.

3) A molybdenum wire can be smoked in air to provide crystals. If the wire is left in the smoke long enough, there will probably be enough crystal to cover a grid. Heat the wire across the terminals of an evaporator or heat with a torch to generate the white smoke. Thus MoO₃ sample will provide a good rotation calibration sample. Magnesium can also be burned to produce MgO crystals.

If there aren't sufficient particles in a single diffraction pattern to provide diffraction rings, take multiple exposures from different areas on the same piece of film.

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A Tip on Purifying Glutaraldehyde

Being an old timer, I have been around since before the advent of vials of glutaraldehyde. In the 1970s, the Merck index described a method for purifying glutaraldehyde using activated charcoal.

Basically, glutaraldehyde begins to polymerize when stored at room temperature. Since best fixation is achieved with the monomeric form, this purification protocol was designed to remove the more complex forms.

Storage under nitrogen in a sealed vial slows the polymerization, and lower concentrations degrade more slowly. Storing at 4°C is advised.

Method:

1) Mix the 25% glutaraldehyde with 1g of activated charcoal

per 100 mL of liquid.

2) Allow to stand for 30 minutes, then vacuum filter through a Whatman #1 filter.

3) Repeat three times.

4) Store at 4°C

Repeat once a year.

I have used this method for more than 30 years and consistently obtained very satisfactory fixation. It is messy, but worth the effort.

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