We appreciate the response to this publication feature and welcome all contributions. Contributions may be sent to our Technical Editor Phil Oshel, oshel1pe@cmich.edu

A Simple Cleaning Method for Penning Gauges

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The usual failure mode of cold cathode ionization gauges (CCIG) in SEM and FIB tools is dielectric deposition from hydrocarbon oils and precursor gases on electrodes of the sensor tube. Removal of these deposits fully restores the functionality of the gage. So far, I have cleaned hundreds (literally) of Edwards and Varian CCIG sensors by following this procedure:

1) Dismount the CCIG from the tool, remove the magnet, and carefully take apart the sensor tube.
2) Soak all the metal parts of the sensor tube overnight in a 50:50 solution of Micro 90™ and deionized or distilled water (Micro 90 is available from various science-supply houses). If deionized water is not available, then supermarket distilled water works. Avoid tap water.
3) In the morning, sonicate all metal parts for 10 minutes in the same solution in which they were soaking. At this point all deposits will be removed from the electrodes, resulting in nice, shiny, metal surfaces.
4) Thoroughly rinse the parts, dry them (use a hot plate or oven to speed up the drying), and put the sensor tube together.

Micro 90™ cleaning is very gentle, does not scratch electrodes, and takes a minimum of personnel time - other things can be done while the gauge is soaking or drying. Cleaning by hand-polishing of the electrodes will also work, but this takes personnel time.

A good idea would be to pre-clean one or two old gauges, which may be lying around the lab, and replace the gauge on the instrument as needed. Store the pre-cleaned gauges loosely sealed in aluminum foil in a desiccator.

Soaking in Micro 90™ is generally helpful for cleaning contamination from metal surfaces. I use this same procedure for cleaning extractor and suppressor electrodes of FIB columns, and also have tried it on the Wehnelt of a tungsten-filament SEM. With column parts, some very light polishing by 1 µm paste is usually needed in the end to remove the most stubborn deposits.

If parts are not heavily contaminated and work done carefully (gloves and tweezers), no degreasing is needed after Micro 90™, as all of the grease is removed. Wear gloves to protect hands! Micron 90™ is much stronger then regular liquid detergent and will "degrease" skin.

Caution - I never tried Micro 90™ soaking of parts with plated surfaces, chances are that plating might go away as easily as the contamination. ■

Editor's Note ...

What do you think about MT publishing detailed protocol articles, like the one by Hazelton earlier in this issue and these M-101 notes? Please send me an email with your thoughts. ...

microscopytoday@tampabay.rr.com

Negative Stains/Staining 2.5 mM Phosphotungstic Acid, 25µg/ml Bacitracin, pH 7.0

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1. Combine:
   a. 406 mg Phosphotungstic Acid {$(W_{12}0_{41})2.2H_3PO_4.48H_2O$, MW 6498.93}; and
   b. Glass redistilled water to volume 20.0 mL.
1 Stir until disolved.
2 Adjust pH to 7.0 with NaOH.
3 Add 0.625 mg Bacitracin.
4 Stir until disolved.
5 Bring to final volume 25.0 ml with glass redistilled water.
6 Filter through a 0.2 µm filter and store at 4°C.

NOTE: PTA goes into solution very readily but Bacitracin will not go into solution at low pH. The initial pH of a PTA preparation is around 2.0. Therefore, it must be raised to near 7.0 before adding Bacitracin. The operative staining molecule is the $W_{12}O_{41}$ polyanion, which may pass between that structure and WO_4. The polyanions have a reported diameter of 0.8-0.9 nm (Hayat and Miller, 1990). The anhydrous density of the stain is reported to be 4.0-4.2. The stain is very stable at 4°C, showing no signs of deterioration in the form of stain precipitate, drop in pH, or deterioration in the image produced in the electron microscope. The purpose of Bacitracin is as a surfactant, enhancing spreading. The Bacitracin molecule is smaller than that of albumin, and is, therefore, preferable as a spreading agent (Gregory and Pirie, 1973). Most samples have enough protein present, other than those in gradients, to negate the need of spreading agents. ■

1 Gregory DW and Pirie BJ. 1973. Wetting agents for biological electron microscopy. I. General considerations and negative staining. J Microsc. 99:251-5.
2 Hayat and Miller, 1990, Negative Staining

Agar Diffusion

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This procedure is a tremendous improvement upon Kellenberger's original collodion diffusion procedure, where collodion films were cast on agar and the suspension placed on the drop of film, allowed to diffuse through, and the film then floated off on water[3]. The modification was recommended by Anderson and Doane as a method for both concentrating samples onto a grid and dialysing (for lack of a better word) excess salts out. To remove excess salts allow the sample's solute to diffuse into the agar, place sterile filtered water over the sample, and allow the excess salts to diffuse into the agar. They reported that they could see an average of 5 particles/square, and that sensitivity was increased by up to 100×[1].

1. Preparation of agar plates:
   a. Prepare 1% agar (Note a) in PBS (Note b).
   b. Fill wells of a flexible microtitre plate (Note c) approximately 3/4 full with the 1% agar solution.

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c. Allow plate to cool.
d. Seal plate with microtitre plate with plate sealing tape and store at 4°C (Note d).

2. Agar diffusion:
   a. Cut enough wells off the microtitre to prepare the number of samples that must be mounted on grids for examination.
   b. Remove the sealing tape and allow excess moisture in the cup to dry out for 5 minutes if necessary.
   c. Place a formvar (Note e), coated microscope grid on the agar surface, formvar surface up. (Note f)
   d. Place 25 µl sample in the well. It is important that the grid is covered, and that the drop of suspension overlaps the grid and goes onto the agar surface (Note g).
   e. Cover the cup to prevent evaporation.
   f. Allow the sample to diffuse into the agar until the surface is almost dry.
   g. Where the salt concentration in the sample is high, such as with urine, you can remove the excess salts by adding a second drop of sterile, filtered distilled water to the sample after the first drop has diffused into the agar. If need, you can even move the grid to a new microtitre cup for this step.
3. The sample is now available for further processing as desired, followed by negative staining. Such processing may include immunological procedures. Use your favourite procedures.

Notes:

a. You may use either agar or agarose, whatever your little heart desires. It should make no difference, and agar is cheaper. However, we usually keep low melting point agarose around for making agarose cores.
b. Use any standard PBS recipe. You may also use straight water (where you wish to dialyse out salts) or Tris:Saline. Sodium Azide can be added to the agarose suspension, final concentration 0.2% to inhibit growth of contaminants.
c. Any type of microtitre plate works, flat bottom, U bottom, V bottom.
d. Anderson and Doane recommended that the plates be stored at 4°C for 4or more months. I have stored these for up to 2 years.
e. OK, no arguments here. Formvar, collodion, parlodion, etc. Use the plastic you want.
f. Anderson and Doane recommended that you make several grids for each sample. I never found this to be necessary.
g. It is not clear whether Anderson and Doane placed the drop on the grid without overlapping onto the agar, or had overlap. They reference Kelen's paper from the previous year[2], where they found problems with the drop being placed on the grid without overlap onto the agar. I never felt sufficient antibody could diffuse through the holes present in the plastic film when there was no overlap onto the agar. Rather, in my experience, this type of diffusion takes too long to complete, and I am concerned that there is a degree of evaporation, not diffusion. Evaporation introduces other problems with salts and uneven distribution. Both options are theoretically possible, and a comparative study has never been done. ■

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Microscopy AND Microanalysis

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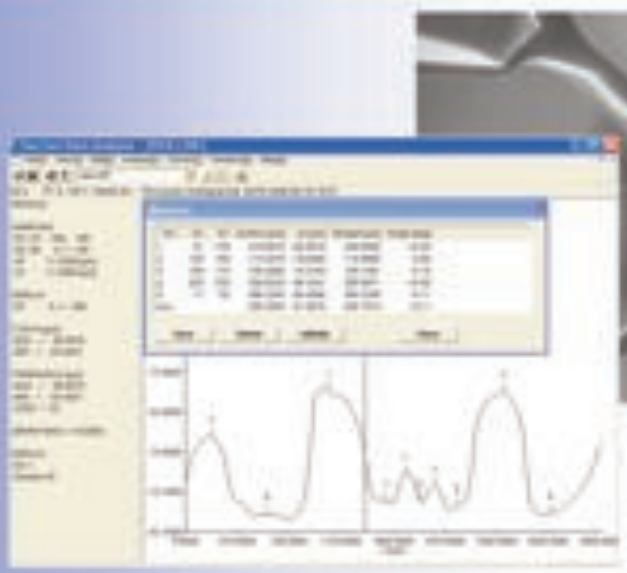

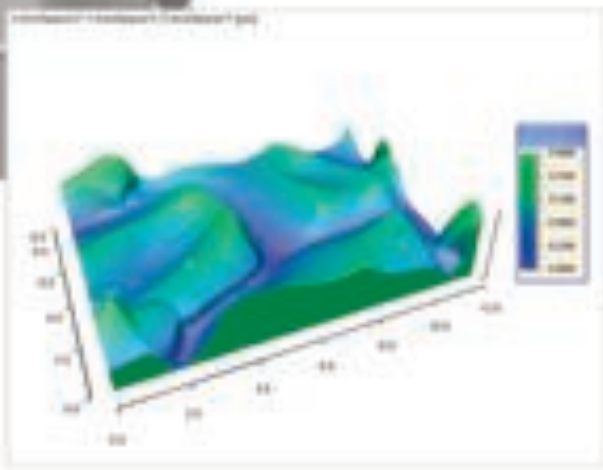

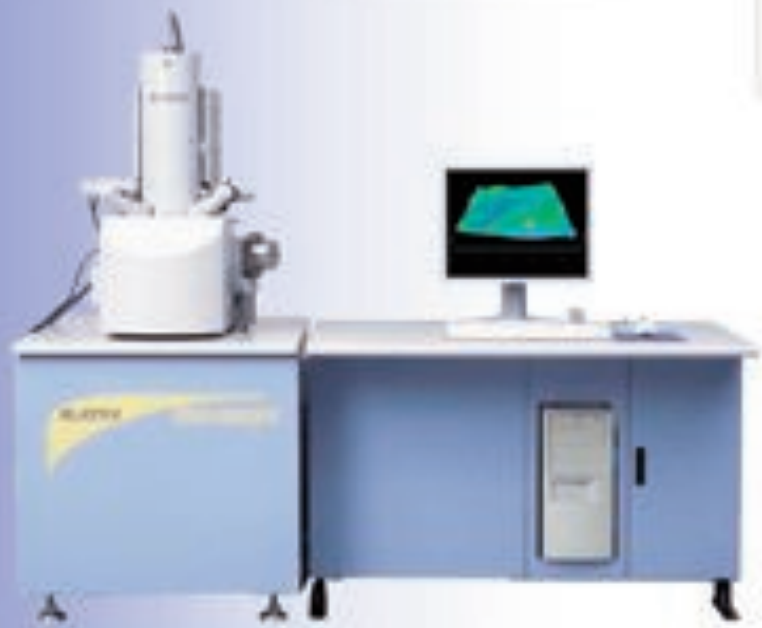

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