A Quick Note on Preparing Bacteria for SEM

Philip Oshel, Microscopy Today

Questions have appeared on the microscopy listserver about how to prepare bacteria for SEM. This is an easy problem to solve, the greatest difficulty being preserving flagellae or pili. There are several references in basic EM texts, but briefly the bacteria can be treated as if they are small tissue samples, or negative staining samples for TEM. Details like of time in solutions, transition series, etc. will depend on the particular bacteria under study, and some trial and error will likely be needed. The procedures given here should give a reasonable starting point.

If the bacteria are colonies growing on agar, punch out a small sample of the colony plus agar. Trim the agar to no more than 1 mm thickness. The difficult part of this is keeping everything clean and not contaminating the colony sample.

1) Buffer wash 3 times in the buffer appropriate for the sample, 5 minutes in each solution series as follows.

2) Post-fix in Osmium. This step may be skipped if examining the samples in a low-kV instrument, although sometimes it is still needed to properly fix surface components.

3) Buffer wash to remove osmium.

4) Dehydrate through a standard EtOH series. Start at 50% or even 70%, although some specimens will require starting at 30%. Acetone can also be used.

5) Stop at the final 100% EtOH, and then dry:
Try using HMDS (hexamethyldisilizane) in a fume hood.
2:1 => 1:1 => 1:2 EtOH : HMDS (1:1 alone might do).*

6) 100% HMDS X 3. Once only might prove to be enough, but start with 3 changes with a new specimen.

7) Air dry in a fume hood for 2 hours (most likely) to overnight. Have some sort of loose cover over the specimens to keep air from blowing directly over them and dirt falling on them, but with lots of venting to allow the evaporating HMDS to escape.

If the bacteria are in fluid culture, suspend the culture and place a sample in a microfuge tube, then:

1) Spin down gently (there go the flagellae & pili).

2) Draw off the fix from the pellet, add buffer wash, suspend, let sit 5 minutes, spin gently.

3) Repeat these steps for each fluid change until the final HMDS.

4) When in final HMDS, suspend the bacteria, take a drop, and place on a prepared stub. Note: careful! you don't want to overload the bacteria on the stub, but want them spread out so that individual bacteria can be seen.

Prepared stub: take a small membrane filter of the kind that has neat round holes, not a tortuous-path filter. Bacteria tends to be lost against the background of the filter. Use filters the same size or slightly smaller than the SEM stubs. Note: a solid sheet will also work.

Sputter coat several of these filters on both sides to make conductive surfaces. Note: this can be fun, as the membranes like to fly around inside the sputter coater when the gas is let in. Stick the coated filters to stubs with silver paint. The alternative to this is to buy silver membrane filters. These are expensive, but do not require any preparation, which saves time, but is less entertaining for your labmates.

5) Dry, as above, for bacterial colonies on agar.

When the drop is dried, the next choice is to coat or not. If the bugs were osmium post-fixed, coating may not be necessary.

Note: if the bacteria in life come equipped with an extracellular coat of some kind (this is likely), then these methods will likely lose the coat, probably during dehydration. This could be avoided if an environmental or low-vacuum SEM is available. In this case, forget all the processing, just mount some cells on a stub, and stick them alive and screaming in the SEM. They may even survive the examination in the SEM, which means that if you use good sterile procedure, they could be returned to culture for future study, such as time-course experiments.

Magnetitè or other metallic or inorganic crystals in the bacteria may be detected by first examining them with low kV, say 1 kV or less. And, then again after you've gotten your images of the bugs with low kV, look at them with 10 kV or more. The lower kV will image the cell surface, and the higher kV will penetrate the cells and show where the crystals are. Since metallic elements are better sources of secondary electrons, they will show up as bright areas within the bacteria. Sputter coating will obscure this but a light enough coating will still allow sufficient beam penetration to show up metallic crystals. Also, a carbon coating can be used to give the bacteria a conductive surface. Finally, if the SEM has EDX, it could be used to pick up the x-rays from any such crystals.

These methods should generally work for most microscopic organisms, although the preparation schedules will require more adjusting. ■

* More details on drying with HMDS can be found in the literature, the University of Florida tips and tricks web site maintained by Scott Whittaker, http://www.biotech.ufl.edu/~emcl/tips.html and in "HMDS and Specimen Drying for SEM" (page 16 of the May 1997 issue of Microscopy Today).

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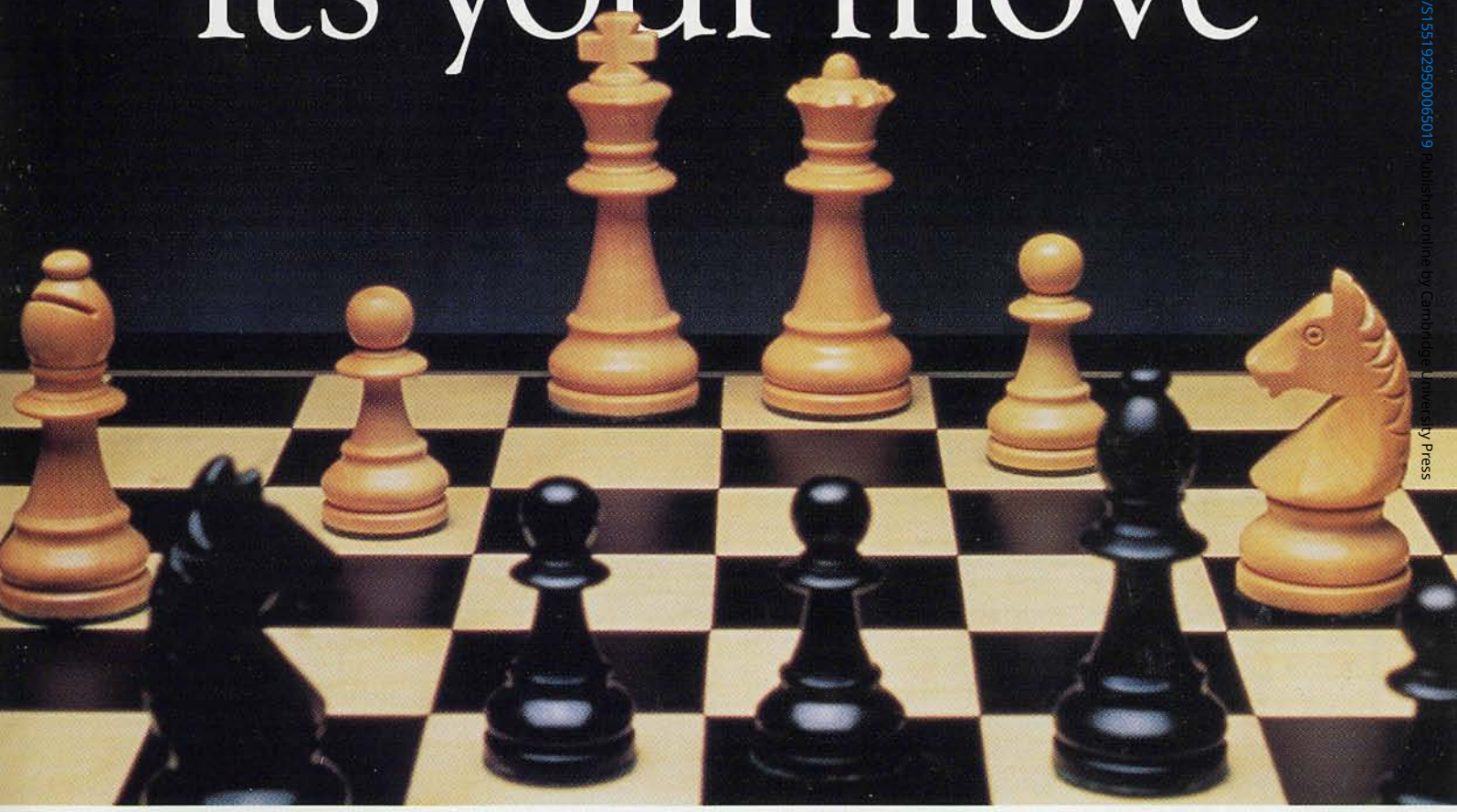

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