

# Staining Grids

## Standard Procedure:

1) Take uranyl acetate (UA) and lead citrate (Pb) from refrigerator in 1-ml syringes with 0.2 um syringe filter. (If syringes need refilling, spin down UA and Pb in microcentrifuge at 10,000 rpm for 10 minutes first, then take only top part of liquid in tubes.)

2) Fill three small beakers with Millipore water and place on paper towel next to staining station.

3) Push a couple drops of stain through the filter and discard into the appropriate waste container (UA has a separate bottle, Pb can go into the waste fixative bottle).

4) Place one drop of UA per grid into a clean, disposable petri dish.

5) Float grids specimen side down on drops of UA for the appropriate time. Normal staining time is 15-20 minutes. The longer the time, the more staining occurs.

6) Toward the end of the staining time, place pellets of sodium hydroxide around the edges in a second petri dish. Push 2-3 drops of Pb through syringe filter and discard, then place one drop of Pb per grid in the dish. Cover immediately and try to avoid excessive exposure to air.

7) At the end of the staining time, pick up grids and dip 20 times in Millipore water in each of the three beakers, then place grids on drops. Standard staining times range from 2-15 minutes (longer staining times can result in precipitation). Continue to take care to avoid unnecessary exposure to air.

8) After staining, wash grids again as above. Place grids on filter paper in petri dishes, label with researcher's name, sample name(s), staining times, date, and your initials.

NOTE: Use of a pointed piece of filter paper to push grids out of the forceps will help prevent "wicking" the grids back between the forceps tips.

## Double Staining:

For hard-to-stain or low-contrast samples, such as those on Spurr's resin, follow procedure as above, but use Pb for first staining step for 2-3 minutes, then proceed with UA and a final Pb stain. This intensifies the staining effect. Remember to use sodium hydroxide pellets with Pb and do the 20 dips in each of 3 beakers of water in between steps.