Centre for Cellular Imaging
Sahlgrenska Academy

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NEGATIVE STAINING PROTOCOL

You will work with the following dangerous chemicals:

- staining solution of your choice:
  - **2% aq. uranyl acetate (UA)** solution. 
    Uranyl is a heavy metal and is therefore toxic but also mildly radioactive.
    The radioactivity of the 2% working solution is negligible.

    UA is also light-sensitive (forms precipitates). Keep the tubes in the dark and aliquot droplets just before staining.
  - **0.75% aq. uranyl formate**, also mildly radioactive and light-sensitive 
  - **1% aq. phosphotungstic acid (PTA)**: irritant! 
  - other:……hazard:……
- optional: 1% glutaraldehyde for postfixation (toxic and irritant) 

The main exposure risk is by solution-skin contact: wear lab coat and gloves when handling. The staining solutions are not volatile, but you can use an extractor arm if you wish.

Glutaraldehyde is volatile and you need to use an extractor arm if you postfix your grids

Keep solution and waste bottles in suitable **secondary containers** to prevent them from falling over.

Dispose of the used solutions, contaminated plasticware and filter paper in assigned waste bottles and bins.

Avoid contaminating the work surfaces!

You will need to bring with you:

- A suspension of your sample at different dilutions to establish the optimal one if it has not been done yet. **1mg/ml or 1-2 µM** is a good starting point for many samples.
- Good quality fine forceps
- Gridbox if you already have one

You will get the following from CCI EM staff:

- staining solution (please let us know a day in advance that you will need it)
- Fixative: 1% glutaraldehyde in water (buffer would crystallise on your sample when drying out)

- dH_2O
- Carbon coated grids (you also have the option to bring your own): make sure that you are able to correctly identify the filmed (coated) side of the grid
- Parafilm
- Silicone matt for grids (to be returned to staff after the staining is finished)
- Gridbox if you don't have one
- Filter paper wedges and circles
- Timer

The procedure

1. Spin down pre-filtered **uranyl acetate** (2% aquaeous solution in 2ml tubes in the fridge) to sediment any potential precipitates. **Uranyl formate** is made fresh before use and does not need to be spinned down. **PTA** stock in the fridge should be filtered immediately before use.
2. Fix a piece of parafilm down to the bench surface with water.
3. Glow discharge the grids (support film facing upwards) to make them more hydrophilic. Do this just before use as the effect wears off with time.
4. Incubate the grids (support film towards the sample drop) on droplets of your sample for 2 minutes. Just before the 2 minutes pass, aliquot droplets of uranyl acetate for staining.
5. Blot excess sample off by holding the grid vertically to a piece of filter paper. Work very quickly so that the sample does not dry out!
6. Immediately place the grid sample down on a drop of uranyl acetate for 10 seconds.
7. Blot as before and let the grids air-dry on a piece of filter paper, sample side up!
8. Your sample is ready for imaging after about at least 30 minutes of air drying but ideally longer.

You can add optional washing and fixation steps. In that case you blot off your sample as described above, place the grid immediately on a drop of 1% glutaraldehyde in water for 2 minutes, blot, incubate on 5 droplets of distilled water for a few seconds each, blot and stain with uranyl as described above. Some users wash directly after incubation on sample suspension.