National Center for CryoEM Access and Training

Standard Operating Procedures
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Purpose: To negative stain biological samples for EM workflow.
Authors: Robert Gheorghita
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1. Purpose:

1.1. To understand a biological structure or macromolecular complex through negative staining.

2. Scope:

2.1. As part of the EM workflow, negative staining with image classification technique makes it possible to work with very heterogeneous molecule populations, which are difficult or even impossible to analyze using vitrified specimens.

3. Definitions

3.1. Uranyl Acetate (UA) is a salt (pH 4-5) and is light sensitive, storage is at room temperature.
3.2. Uranyl Formate (UF) is a salt (pH 4-5) is light sensitive and storage is at -80C.
3.3. Phosphotungstic Acid (PTA) is a metal (pH 7) for samples that are pH sensitive, room temperature storage.

4. Responsibilities:

4.1. Choose a stain for your sample. Note, negative stains will have a dark background on images and the particles of interest are white.
  4.1.1. Uranyl stains are typically the first choice for staining because of its high atomic number and UA is stable for many months at RT and UF for hours-days ar RT (months at -80C).
  4.1.2. UF stain is the better choice it yields better staining due to finer grain size.
  4.1.3. PTA is to be used for samples that are pH sensitive.
4.2. Choose an appropriate buffer.
  4.2.1. Water (ddH2O) can be used for as a buffer but is sample dependent. The air water interface can break up the molecule and molecules may absorb to the carbon support in a preferred manner.
  4.2.2. Buffers that would be useful would be sample dependent, but do not use phosphate buffers as they cause metal phosphate precipitates.
  4.2.3. Glycerol and detergents may be used, but should be minimized or diluted out during wash steps.
4.3. Prepare grids.
  4.3.1. Obtain at least four negative stain carbon grids and make sure the carbon is not broken and continuous.
  4.3.2. Place grids on a grid metal holder then put onto a pedestal in the Gatan Solarus glow discharger. Use the gas recipe carbon film std H2 /O2 for 30 seconds.
  4.3.3. The grids are hydrophobic, and this will render the grids hydrophilic.
  4.3.4. Vent for a minute to open the chamber and take your grids out.
  4.3.5. Individual must log in the description of the glow discharge usage.
  4.3.6. The grids are ready for dispensing your sample and staining. As a rule of thumb use the grids within 30 minutes of glow discharging.

4.4. Prepare stain for staining.
  4.4.1. Cut a piece of parafilm (~2 inches) and lay on negative stain bench.
  4.4.2. Prepare 2x 10-20 ul drops of buffer on parafilm.
  4.4.3. Prepare 2x 10-20 ul drops of stain on parafilm.
  4.4.4. All 4 drops should be in a row
4.5. Stain your sample.
  4.5.1. Hold grid with an anticapillary forcep (negative action style tweezer) see **Figure 1.**
  4.5.2. Dispense sample, ~3ul onto grid.
  4.5.3. Time is a factor too short or long of period can affect the ability to get a good stain and see your sample.
  4.5.4. Generally, 30 second to a minute let sample stay on your grid to absorb to induce one or limited number preferred orientations.
  4.5.5. Use filter paper to wick away excess sample
  4.5.6. Apply grid to buffer drop1 and wick away excess immediately.
  4.5.7. Repeat for buffer drop2, stain drop1 and stain drop2.
  4.5.8. The amount of time the grid is on each droplet must be optimized as it is sample dependent.
  4.5.9. The last stain drop application is anywhere from 30 seconds to 1 minute.

5. Personal protective Equipment (PPE):
5.1. Laboratory coat
5.2. Nitrile gloves
5.3. Goggles

6. Chemicals:
6.1. Uranyl Formate
6.2. Uranyl Acetate
6.3. Phosphotungstic Acid
6.4. Isopropyl Alcohol 70%

7. Equipment
7.1. 1-10ul pipette
7.2. 1-200ul pipette
7.3. straight tip negative action tweezer
7.4. scissors
7.5. wax paper
7.6. Whatman #4 filter paper
7.7. carbon support film grids
7.8. 1-10ul pipet tips
7.9. 1-200ul pipet tips
7.10. grid box

8. Waste Disposal:
8.1. Uranyl Disposal fill aliquot with water rinse and dispose in a uranyl disposal designated area near the Hood.
8.2. Clean all equipment that has come in contact with stains with isopropyl alcohol 70%.
8.3. Dispose in uranyl dispose area.

8.4. Dispose all other material wax paper, filter paper, pipet tips in a hazard waste.

9. Vendors:

9.1. N/A

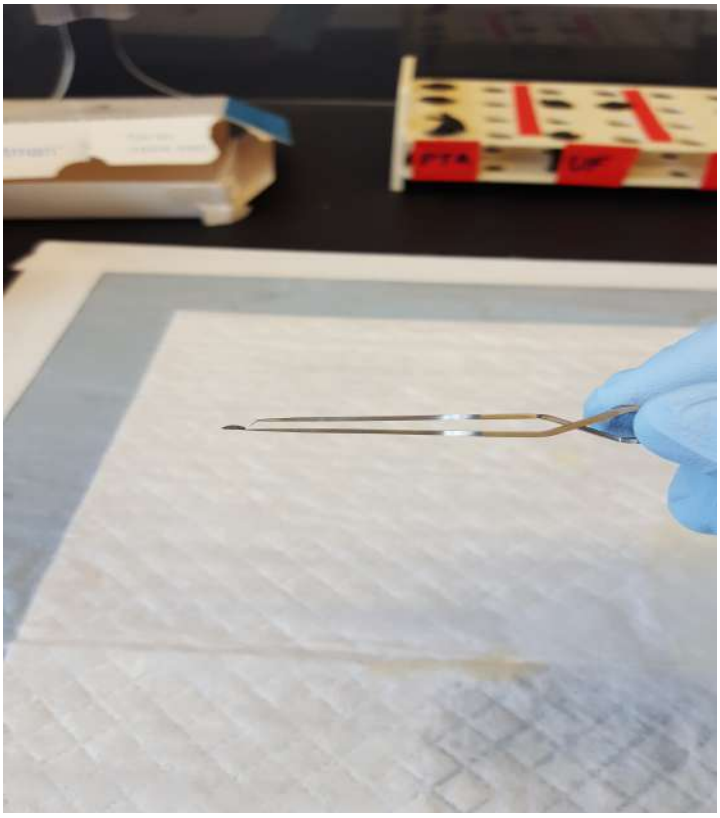

Figure 1: