Preparation of Uranyl Formate Stain

Electron microscopy
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Rationale
This protocol describes the preparation of a 0.75% uranyl formate solution in water for use as a negative stain. The procedure is adapted from Ohi, et al's excellent 2004 paper "Negative Staining and Image Classification – Powerful Tools in Modern Electron Microscopy" which is a nice introductory overview of some of the techniques and issues in negative stain.

Materials
1. Uranyl formate: found in the heavy metals box in the EM Prep area.
2. 5M NaOH: working solutions of base are kept above the pH meter.
3. 50ml degassed/boiled ddH₂O water

Procedure
1. Weigh out 37.5 mg of uranyl formate into a 20 ml scintillation vial, and wrap with foil to keep dark.
2. Add 5 ml of degassed ddH₂O water, stirring until all of the uranyl formate has been dissolved. If chunks remain, it may be helpful to break them up by brief immersion in a sonicating bath.
3. Add drops of 5 M NaOH until the stain solution becomes slightly darker yellow (too much NaOH will precipitate the stain) and stir for another 5 min in the dark. The objective here is to titrate the pH to a reasonable extent. pH can be checked with paper strips (NOT a meter!). The issue is that the uranyl formate will precipitate out of solution as the pH rises. 4.5 is about as high as one can reasonably achieve.

Filter the solution with a 0.2 μm syringe filter into a Falcon tube wrapped with aluminum foil and add deionized water to a final volume of 5 ml. Several members of the lab are of the opinion that this solution can be frozen in aliquots at -80C until it is needed. The original paper suggests making the solution fresh for each use.