Standard Operating Procedure for

Leica EM AFS2

**Training Required**

**Protective Equipment**; Gloves, safety glasses, lab coat, chemical fume hood

Prepare microfuge tubes, cold racks, ice chest, freezer packs, tools and chemicals (in labeled scintillation vials, then keep chilled) ahead and have ready and in the hood next to the AFS machine

**AFS2 Set up**

Plug in and turn on the AFS

 Choose User name and input temperature ramping program

 Or

 Choose a program that is already set up

Fill AFS with LN2

 Attach screw-on funnel to nitrogen port

 Pour LN2 into the funnel until the fill gauge reads:

 50% for 3 day program

 75% for 4 day program

 100% for longer

Start the program - May take up to 1 hour for the sample chamber to reach the desired starting temperature

Place initial solution in 20 ml scintillation vial in the AFS chamber to equilibrate

**Vitrifying samples**

Make slushy nitrogen (SN2)

 Place styrofoam cup in metal vacuum jar and fill with LN2, place top on metal jar

 Turn on the vacuum pump and open the vacuum valve

 Watch through the window, at about 4 - 5 minutes the top of the LN2 will frost over, yielding SN2

 Release the vacuum and remove the top

Vitrify Samples

 Cut samples into very small pieces (<1 x 1 x1 mm)

 Quickly plunge sample into SN2 , then drop it and plunge more samples. Repeat for each sample

 Once the frosty top layer has melted into all liquid, either top off with LN2 and re-vacuum for more samples, or continue on to next step

**Sample transfer into AFS**

Move samples to cryo-tubes

 Pour out the LN2 and all samples into shallow Styrofoam box.

 Prechill several labeled 2mL cryo-tubes in the LN2

 Using chilled forceps, transfer the samples into the cryo-tubes

**Sample transfer into AFS (continued)**

Place samples into AFS

 When AFS chamber is at the desired starting temperature, quickly dump out any remaining LN2 in the cryo-tube and place it in the rack in the AFS chamber.

Allow the sample to equilibrate, add the first solution from the scintillation vial to the cryo-tube.

Restart the program (or resume if it was paused)

**Solution Exchange**

Equilibrate the next solution in a scintillation vial in the AFS Chember

Remove old solution and replace with the new solution, swiftly to ensure there are no sudden temperature changes

**If exchanging OsO­4 in acetone at -20°**;

 Prechill the Osmium in a Styrofoam box with a -20° ice block/freezer pack.

 Swiftly move the sample cryo-vial into A) the Styrofoam box filled with freezer packs or B) an epi-rack with built-in coolant that is frozen ahead of time

Do this solution exchange in the hood.

 Place the sample vial back into the AFS after the exchange. Resume the program.

**Resin exchange and polymerization**

Remove samples from AFS, close lids, place into an epi rack (room temp) and take to resin room.

Inifltrate and then embedd in resin (check with FMIC staff for procedure if you do not have one of your own.)

## NOTES

* This whole process is extremely dependent on the samples staying frozen at all times!
* The best results will come from slow and smooth temperature ramping, but a single moment of thaw/refreeze before fixation is complete will ruin the sample.
* For every step, consider the temperature of all tools, solutions, jars and surroundings. Make sure everything is always at the correct temperature, and always minimize the time transitioning between areas (ex: LN2 to AFS and AFS to syrofoam box for OsO­4).