

Tissue processing, critical point drying, sputter coating of fixed samples

Materials:

- Tissue processor (Leica EM TP)
- Critical point dryer (Leica EM CPD300)
- Sputter coater (Leica ACE200)
- Fixed samples
- Loading plate (included with EM TP)
- Sealing lid for EM Heater/cooler unit (included with EM TP)
- Baskets and basket stem (included with EM TP)
- EM vials and vial cap (included with EM TP)
- Suitable buffer (usually the same buffer that is used for fixation)
- Osmium tetroxide (2% solution) (optional)
- 30%, 50%, 70%, 80%, 90%, 100% Ethanol
- Metal tin
- Razor blade
- Tweezers/forceps
- SEM stubs
- Carbon tape

Tissue Processor

Osmium tetroxide collection:

- Osmium tetroxide is a controlled chemical, thus retrieve it from the lab management at B2. Bring the metal tin along for collection
- Place the warning sign of osmium tetroxide at the entrance door of the extraction lab, and also paste it on the fume hood while it is in used

Specimen loading:

- Place the pin through the stem in a position relative to the number of baskets to be loaded and slide on a lid
 - **Note:** 7 small baskets or 3 big and 1 small basket can be held
- Insert the baskets into the wells of the loading plate, prepare a vial filled with suitable buffer
- Load the samples into baskets
 - Take note of the position of samples. It is recommended to place the first basket at position 1 and to load the samples in clockwise order. You may use the specimen record pad (included with EM TP) if needed.
- Pick up the baskets on the stem and secure the stack of baskets with the foot (smaller diameter is facing down) by screwing it into position
- Place the basket stack in the vial filled with buffer until it is ready to be transferred to the TP
 - **Note:** Ensure that basket stack is submerged in the buffer

Reagents preparation:

- Preparation of reagents must be done in the fume hood
- To unload the carousel, raise the specimen arm to the right, then raise the lever of the H/C unit and flip it back. Unscrew the carousel and lift it up. Transport it over to the fume hood.
- Fit vials to the carousel by pushing them into the slot and turning them
 - **Note:** Carousel is numbered from 1 – 24 on the outer ring and labels are pasted on the vials. Please attach them accordingly
- Fill the reagents according to the program selected:
 - **Note:** Volume of the reagents will be depending on the stack of the baskets. It is important to ensure that the basket stack is submerged in the buffer
 - Vial cap must be used for the vial containing osmium tetroxide during transportation of the carousel from the fume hood to TP, and it should be left closed until TP lid is ready to close.
- To load the carousel, raise the specimen arm to the right, then raise the lever of the H/C unit and flip it back, and lower the carousel onto the central spindle making sure it is positioned correctly with the location pin and then screw into place gently.
 - **Note:** Check again to ensure that all the vials are fitted properly after loading the carousel
 - Although the vial containing osmium tetroxide has the cap on, use local exhaust ventilation (LEV) while loading the carousel
- Lower the H/C unit back into position and lock with the lever to the left side
- Recover the basket stacks from the vial containing the buffer and clip onto the specimen arm and lower it to the left
- Seal the H/C unit with the sealing lid
- Remove the vial cap for vial 4 and close the TP lid

Osmium tetroxide handling and disposal:

- It is compulsory for user to complete the health check-up before using the osmium tetroxide in SCELSE. Health check-up to be arranged with lab management.
- Wear blue chemical resistant secondary gloves (double gloving technique) and safety goggles while handling osmium tetroxide
- Neutralise osmium tetroxide vial or waste with corn oil, then dispose into the 500mL glass jar labelled as '**FOR OSMIUM TETROXIDE WASTE ONLY**'
- Neutralise the pipette tips or falcon tubes that came into contact with osmium tetroxide with corn oil as well before they are disposed into the yellow bin labelled as '**FOR OSMIUM TETROXIDE WASTE ONLY**'

Operation of the TP:

- Turn on the main switch on the rear
 - **Note:** Carousel will move to vial position 1 automatically if it is not at the position 1
- To select a programme, press **START**
 - **Note:** Currently only 1 program is installed. PROG01 per below:
 - Vial #1 – 3: 0.1M phosphate buffer, 10 minutes at 4°C
 - Vial #4: 1% osmium tetroxide, 2 hours at 4°C
 - Vial #5 – 7: 0.1M phosphate buffer, 10 minutes at 4°C
 - Vial #8: 30% ethanol, 15 minutes at 4°C
 - Vial #9: 50% ethanol, 15 minutes at 4°C
 - Vial #10: 70% ethanol, 15 minutes at 4°C
 - Vial #11: 80% ethanol, 15 minutes at 4°C
 - Vial #12: 90% ethanol, 15 minutes at 4°C
 - Vial #13 – 15: 100% ethanol, 15 minutes at 4°C
 - Total run time: 5 hours
 - To run with delay, move the cursor to START or END on screen, then use +/- to set the time/date for the program to begin or end at desired time. Usually, setting the END time is more convenient.
- To begin the programme, press **START** again
- When the programme is finished, basket stack remains in the last vial holding the last temperature, agitation continues until **STOP** is pressed to end the programme
- Raise the specimen arm to the right and transfer the basket stack into a fresh vial containing 100% ethanol
- Transport the vial to critical point dryer
- To unload the carousel, first press ↓ to return it to the default position, unscrew the carousel and transport it to the fumehood
 - **Note:** remember to use the vial cap for the vial containing osmium tetroxide
- Follow proper osmium tetroxide disposal (stated above), wash the vials and vial cap with lab cleaner or detergent thoroughly and air dry them on the bench

Critical Point Dryer

Operation of the CPD:

- Open the screw-on cover of the sample chamber and transfer the basket stack into the sample container
- Fill the sample container with the same exchange fluid that was used for gradual dehydration to such a level that the basket stack is completely submerged then close the screw-on cover
- Open the shut-off valve of the CO₂ tank by turning it anti-clockwise
- Turn on the main switch on the rear and touch screen after initialization
 - **Note:** Dark gray buttons can be activated, light gray buttons are inactive.
- Select the **Program**
 - **Note:** The program may vary depending on the sensitivity of the samples. Please customize your own program to fulfil your samples' need
- After selecting the program, press **OK** to go back to the main menu and press **Start**
 - **Note:** To run with delay, select **timer function**, and press the watch symbol to activate the timer function, it will turn green after activation. Set the desired time to start the program, and press **OK** and settings will be saved. By activating the timer, no programming or other changes are possible. To cancel timer settings, press **Stop**.
- Close the CO₂ tank valve when the run is completed

Sputter Coater

Mounting:

- Paste the carbon tape onto the SEM stubs
- Make a marking on the carbon tape to mark it as the starting point of sample #1
 - **Note:** For carbon conductive tape, cut it into desired shape and size, and cut one tip as a marking. For carbon conductive tap, make it marking by cutting it slightly before pasting it on the stub.
- Label the sample at the side of the stubs
- Using tweezers (or any appropriate form), gently paste the samples on the carbon tape, avoid creating air bubbles

Operation of the Sputter Coater:

- Place the stub(s) at the center of the stage (boxed by yellow tape)
- Open the shut-off valve of the argon tank by turning anti-clockwise, then open the gas-in valve (small black valve) by turning anti-clockwise
 - **Note:** Do not adjust the gas regulator. Always ensure the gas-out range is not over 0.3 barr as if the gas volume is too high, it will affect the performance of built-in valve inside the instrument which controls the gas automatically.
- Turn on the main switch on the rear and select **Sputtering**
- For air isolates samples, select **directional** mode
 - **Note:** Directional mode is intended for relatively flat samples. The vacuum for directional sputtering is 4×10^{-2} mbar. Diffuse mode is intended for more topographic samples. The vacuum for diffuse sputtering is 8×10^{-2} mbar.
- Set the sputter thickness
 - **Note:** Usually 4nm is good enough for SEM imaging. It is recommended to not coat unreasonably thick or thin
- Set the correction for quartz by gauging the distance between the sample surface and the lower reference pin, also known as 0 reference mark
 - **Note:** If the sample surface is aligned with the 0 reference mark, no correction for quartz is required; leave the value at 0 mm. If the sample surface is 10 mm below the defined 0 mark, then -10 mm has to be set for the stage height correction
- Press **Vent after process**
- Press **Start**
- Close the argon tank valve when the run is completed