Cryo SEM Protocol (FEG)

Inserting sample

1. Check electronics are not set to standby.
2. Turn on **rotary pump** and **backing valve**.
3. Open nitrogen regulator (located on wall next to plunging station) to 10psi.
4. Open remote dewer regulator to 5psi.
5. Wait at least 10 minutes. Longer is better.
6. Cool the anticontaminator to -180˚C.
7. Fill the prep chamber and remote dewer with liquid nitrogen and wait to cool. Once the microscope has reached -180˚C adjust the temperature to -120˚C to -140˚C.
8. Place stub in cryo sledge and tighten screw to secure it.
9. Attach the sample to the stub using 50:50 of tissue tek : graphite.
10. Fill the polystyrene beaker in the plunging station with clean liquid nitrogen and cover with lid.
11. Press **backing valve** off.
12. Press **slush/vent**.
13. Attach cryo sledge to vacuum transfer device (VTD).
14. Once slush forms press **slush/vent** again to vent. Turn on lamp to help see when slush forms.
15. Place the sample quickly into the nitrogen, once it has stopped bubbling attach the VTD onto the cryo station ensuring the rod does not get immersed. Turn on **slush/vent**. While slushing hold sample so the end of the sledge is in the nitrogen but the sample is just above it.
16. As the slush forms, withdraw the sample into the VTD.
17. Close the VTD valve. Press **slush/vent**.

18. Attach the VTD to the gate valve on the prep chamber and press **load pump** while holding in place.
19. Wait around 20 seconds. Open the gate valve.
20. Advance sample into prep chamber, line up between first two dots.
21. If sample requires freeze fracturing:
    a. If it is an awkward sample with lots of charging, give sample heavy gold coating.
    b. Use the scalpel to slice off the desired section of the sample. Place knife back in holder.
22. Set the **heater** temperature. Turn on and wait for it to reach the desired temperature. Set a timer for etching time.
23. Turn the heater off. Wait for the temperature to reach below -100^{o}C before continuing.
24. Line up the stub with the dots at the end of the cold block.
25. Set the timer for sputter coating using the **raise/lower** buttons while holding down **set timer**.
26. Open the argon cylinder by turning the valve on the cylinder 4 full turns counter clockwise.

27. Press **sputter**, if it doesn't sputter press reset. The current should be 10mA- adjust the voltage dial on the electronics box if needed. If the plasma flickers, open the needle valve until stable. The plasma should be a blue, if consistently pink ask a member of bioimaging staff
28. Press **reset** and close the argon cylinder.
29. Check that the SEM stage is in the correct position for the transfer (x=35, y=25, z=25 – z height needs to be done manually) and the monitor is switched on.

30. Once the light on the ball valve turns green, open the ball valve and advance the sledge into the SEM, watching through the open ball valve, it may beep when there is contact.
31. When the sledge is in place in the stage, detach the rod by turning it a quarter turn anticlockwise.
32. Withdraw the rod back into the prep chamber and close the ball valve.

Imaging sample

- Before imaging: set up (menu bar) → recipe set up → cryo operation
- Turn HT on – if nothing comes up check **freeze** isn't on and if that doesn't work check **gun valve close** is off
- Once the sample is in wait for it to reach 10µA and 5kv
- Working distance is always 6 and then press **RDC** on the control pad and adjust the z height manually for the focus
- Use the control pad rather than the screen
  - **Quick view** for scanning mode
  - **ACB** for auto brightness and contrast
  - **Fine view** to get a clearer image
  - **Photo** to take an image

Removing sample

1. Check the HT is off. Move the stage position to x=35, y=25, z=25.
2. Open ball valve and attach VTD to sledge turning 90^{o} clockwise watching through open ball valve.

3. Retract the sledge into the VTD. Close ball valve then close gate valve.
4. Press **load pump** to vent.
5. Place VTD into cryo station and open VTD valve. Pull it back into the freezing station then turn to prevent it falling.
6. Remove VTD from cryo station and detach sledge from VTD.