Purpose: To create negative stain for future room temperature microscopy.
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Approved: Ed Eng **Date**: 2020/04/21

1. Purpose:

1.1. To float continuous carbon on a 400mesh Pd/Cu grid for negative stain.

2. Scope:

2.1. To make enough grids for the negative stain workflow, so users will have an opportunity to successfully stain their sample onto the grid.

3. Definitions

3.1. Smith grid coating trough is a device that will help in floating carbon or film onto grids, please refer to **Figure2**.

4. Responsibilities:

4.1. Open the evaporation chamber.
4.2. Place the filter paper on the evaporation stage.
4.3. Using a razor blade, split the mica into two thin sheets; depending on the thickness of the mica, you may be able to split more slices from one sheet; place the two sheets into the Cressington Carbon Evaporator (the clean sides from the inside of the mica face up).
4.4. Take two High Purity Carbon Rods, rub one end of the rod on a piece of sandpaper to achieve a purely flat side, you may use a rod sharpener to sharpen the other rod, or rub the edges against the sandpaper to a point.
4.5. Open the evaporation chamber. Take the flattened rod and insert it into the static carbon rod holder and tighten the screw securely refer to **Figure1**.
4.6. Then insert the pointed rod into the moving rod holder with the carbon rod slider spring, pull the spring back, and be sure to push the pointed rod against the flattened end on the other side before tightening refer to **Figure1**.
4.7. Close the chamber and turn on the evaporator by pressing the power switch located on the front panel. The vacuum system will engage, wait for it to reach full vacuum which may take 2-4 hours.
4.8. Press the start/stop button to charge the current throughputs, this will cause carbon evaporation. Press start/stop when the vacuum reaches 10-4 mbar, wait until it drops to zero and start again.
4.9. With each cycle, the filter paper will turn gray, gauge thickness of carbon based on the darkness of your filter paper.
4.10. Once you have the desired shade, turn off the evaporator, wait for the vacuum to shutoff and for the chamber to vent.
4.11. Open the chamber and gently remove your mica, transfer to a petri dish and place into an oven for at least 2 hours.
4.12. Cut a rectangle out of filter paper and place it onto the mesh insert of the Smith Grid casting trough refer to **Figure2**.
4.13. Fill the trough above the mesh insert with ddH2O. Be sure that the filter paper is even and lying flat on the insert.
4.14. Using tweezers, place your grids (Pd side up) in staggered rows on the filter paper.

4.15. Fit as many as you can with sufficient spacing between each grid.
4.16. Cut the mica in half. Using the tweezers, pick up the mica, carbon side facing up, and slide the mica along the slant of the Smith Grid casting trough. You will see the carbon lift off the mica and float in the water.
4.17. Maneuver the metal mesh insert so your grids are directly under the carbon film.
4.18. Slowly remove the water from the Smith Grid casting trough using the attached syringe.
4.19. Using tweezers, gently lift the filter paper with your carbon coated grids and place them on a petri dish to dry.
4.20. Once dry, you may assess the quality of the carbon film using a screening microscope to ensure that the process was successful.

5. Personal protective Equipment (PPE):

5.1. Laboratory coat
5.2. Nitrile gloves
5.3. Goggles (tinted)

6. Chemicals:

6.1. Ethanol 70%

7. Equipment

7.1. Tweezers
7.2. Whatman No. 4 Filter Paper
7.3. Grids
7.4. Petri Dish
7.5. ddH2O
7.6. Smith Grid Casting Trough
7.7. Mica
7.8. Carbon Evaporator
7.9. Double-Pointed High Purity Carbon Rods
7.10. Syringe
7.11. Razor
7.12. Oven
7.13. Scissors
7.14. Sandpaper
7.15. Hand Carbon Rod Sharpener (optional)
7.16. Ethanol
7.17. Compressed Air Duster Swap

8. Waste Disposal:

8.1. N/A

Figure 1:

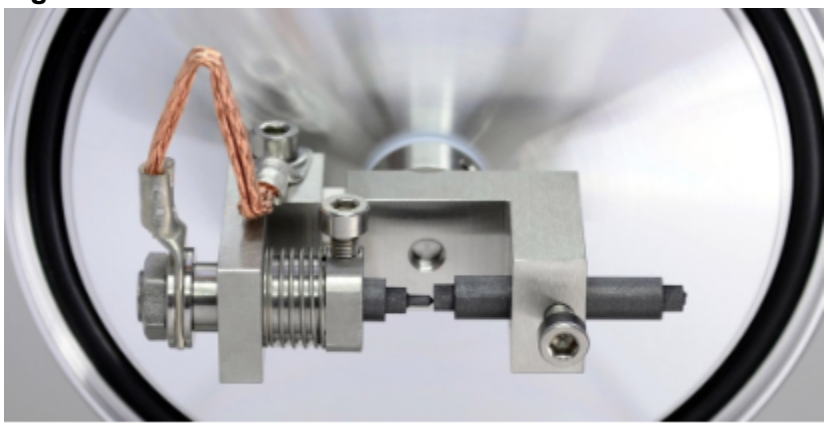

Figure 2: