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In the Event of an Emergency:
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FEI Vitrobot Mk IV Grid Plunging System
Operating Instructions

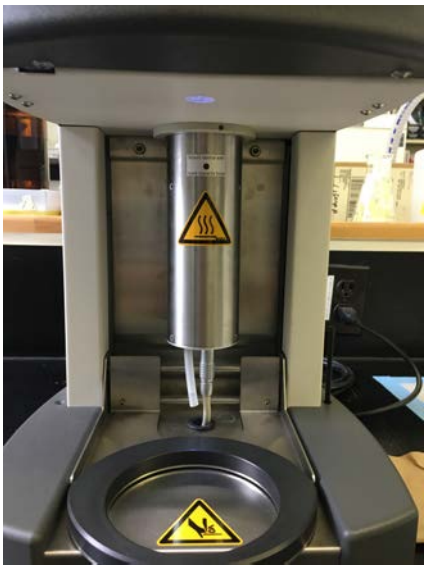
(Prepared by Aida Razi, Kaustuv Basu and Joaquin Ortega)

1. **Preparation:**
   a. Reserve your session in advance using the facility Scheduler <scheduler.campus.mcgill.ca/scheduler>
   b. Obtain the tool kit containing the tools for the Vitrobot and Sign-Out sheet from S Kelly Sears. The tool kit should contain the items shown in the image at right. If anything is missing, you must inform Dr Basu immediately.

   c. Bring the following materials:
      i. Bottle of MilliQ H_2O (deionized water)
      ii. TEM grids
      iii. rods with cryo box
      iv. 5L Dewar with liquid nitrogen (obtained from Room B25, basement of the Strathcona Anatomy & Dentistry Building).
   d. Wear protective clothing, footwear, gloves, and eyewear.

2. **Procedure**
   a. Switch on the power to the Vitrobot. The power button is located at the back of the unit. Wait until the initialization process is completed. You may follow the sequence via the Vitrobot screen.

b. Invert the humifier and carefully insert it into the base of the Vitobot by lining up the pin and pushing the humidifier upwards and rotating to the right and locking it into place. The black dot on the humidifier cylinder should be facing towards you when the humidifier is correctly in place.
c. Pour deionized water into a clean beaker and fill the syringe with water.
d. Inject ~ 40 mL of deionized through the white plastic hose located at the bottom of the humidifier.
e. Using tweezers and a Kimwipe, dry excess water around the outlets of the humidifier. Note: be careful not to touch the sensor inside the chamber.
f. Close the door to the Vitrobot chamber.
g. Using an empty syringe, remove the air from inside of the humidifier.

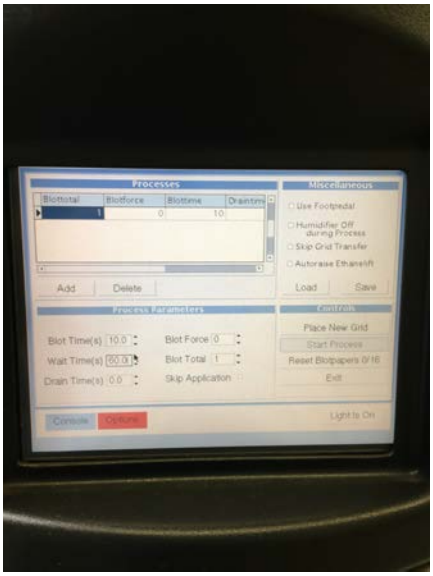


h. Using clean nitrile gloves, replace the filter paper on each blotter pad by removing the inner white plastic rings. Note: do not to mix up the rings.
i. The polished side of the filter paper faces the ring (with the rough side towards the filter pad).
j. Replace the rings on the blotting pads and with the back of the tweezers (medium size tweezers) gently push until are tightly secured on the pads.

3. Setting Up the Software

a. The Vitrobot User Interface has two pages: Console and Options.
b. On the Console page, set the desired temperature on the basis of your experiment. Use the stylus located on the right side of the unit to select items on the screen.
c. Set humidity level to 100% and press ON. Water vapor starts to fill the chamber.
d. Under the Options tab, set blotting conditions on the basis of your sample conditions:
    i. Blot time: how long the filter will blot your sample.
    ii. Blot total: Number of times blotting occurs.
    iii. Blot force: Amount of force the filter will apply to the sample.
    iv. Wait time: how long the system waits before blotting the sample.

4. **Loading the TEM grid**
   a. On the Options page, press PLACE NEW GRID => the central rod of the Vitrobot moves into position for loading the tweezers. Note: Be careful not to drop the Vitrobot tweezers (work as close to the bench surface as possible while handling the tweezers to prevent the tip bending if dropped – replacement cost is ~$1000 USD).
   b. Clamp the grid by the tweezers at the top rim. The dot on the Vitrobot tweezers dot faces towards right (dark side [carbon film] of the grid also faces the right).
   c. Secure the grid in the tweezers by sliding the clamp downward to the first or second grove.
   d. Ensure the grid is secure by gently shaking the tweezers (be careful not to drop the tweezers when doing this).
   e. Press “Continue” => tweezers with the grid move upwards into the Vitrobot chamber.



5. **Setting up the Coolant Container**
   a. Set up the Vitrobot container. Ensure the “spider” is properly sitting in the ethane container (the spider is for heat transfer - if the spider doesn’t sit properly, ethane solidifies => wax form).
   b. Place the blue grid box on the coolant container.
   c. Avoid breathing over the top of the Vitrobot liquid nitrogen to avoid ice contamination – use mask.
   d. Add liquid nitrogen to the center of the container (in the ethane cup) until the entire container is becomes and with aluminum foil.
   e. Keep adding liquid nitrogen until boiling stops in the ethane cup and the cup remains filled with liquid nitrogen.
   f. Gently blow liquid nitrogen in the ethane cup with dry nitrogen.
   g. Place the hose from the ethane cylinder in the middle cup of the container and open the regulator.
   h. Keep filling the ethane cup until the level of the ethane reaches the interface between the ethane cup and the rim of the spider.

i. After this is done, cover the top of the Vitrobot container with aluminum foil.
j. Wait for 1min for the liquid ethane to reach ~-190ºC.
k. REMOVE the spider using the large forceps. Do NOT use your fingers.
l. Place the coolant container on the platform of the Vitrobot without rotating it.
m. On the screen, press CONTINUE to raise the Vitrobot container towards the Vitrobot chamber.

6. Loading and Blotting Sample

a. Using the pipette with a new clean tip, load ~4 µL of sample.
b. Press START PROCESS to lower the tweezers with the TEM grid to enable sample loading through the side entry port.
c. Pipette the sample onto the TEM grid through the side entry port.
d. Press CONTINUE. The program will pause on the basis of the total wait time entered during set up.
e. Blotting and plunging occurs, i.e. the tweezers moves downwards of the chamber.

f. Detach tweezers from the central rod very carefully while keeping it inside of the liquid ethane (You can hold the tweezers in place by resting them on the ethane cup wall).

g. Avoid breathing on the TEM grid at all times – wear a mask.
h. The TEM grid must remain inside of the ethane all the time.
i. With one hand, hold the bottom of the tweezers and with the other hand slide the clamp to the top of the tweezers.
j. Keep pressing in the tweezers to avoid losing the TEM grid.
k. Carefully press the inside of the container until the level of liquid nitrogen and covers the grid box. Then immediately put the grid inside of the grid box.
l. Remove the inside container.
m. Place the rod inside of the liquid nitrogen. Wait 30 seconds (until boiling stops) and then place the rod on top of the grid holder box and screw it.
n. Transfer your rod into the Falcon tube or container for storage.

7. Cleaning procedure

a. Close the main valve and regulator of the ethane cylinder.
b. Remove filter paper from the blotting pads.
c. Place the Vitrobot tweezers in the box (no tweezers are loaded in the Vitrobot).
d. To turn off the Vitrobot, press EXIT button on the console screen and TURN OFF the light inside the chamber.
e. EXIT Windows and when the screen says you can turn off the computer, press the POWER button at the back of the Vitrobot.

f. Empty the humidifier
    i. Pour out water and use a syringe to remove the remaining water in the humidifier wall chamber.
g. Clean the bench.
h. Sign the logbook
i. Leave the room exactly as pictured.
j. Return the tool kit to S Kelly Sears.