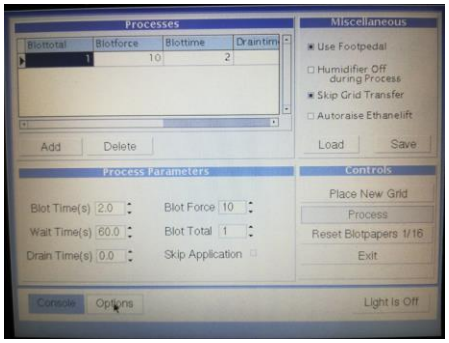
Vitrobot Mark IV Operation

Note: The vitrobot was replaced by a brand new one in March 2021. The following yellow highlighted text show the difference in operation between old and new vitrobots.

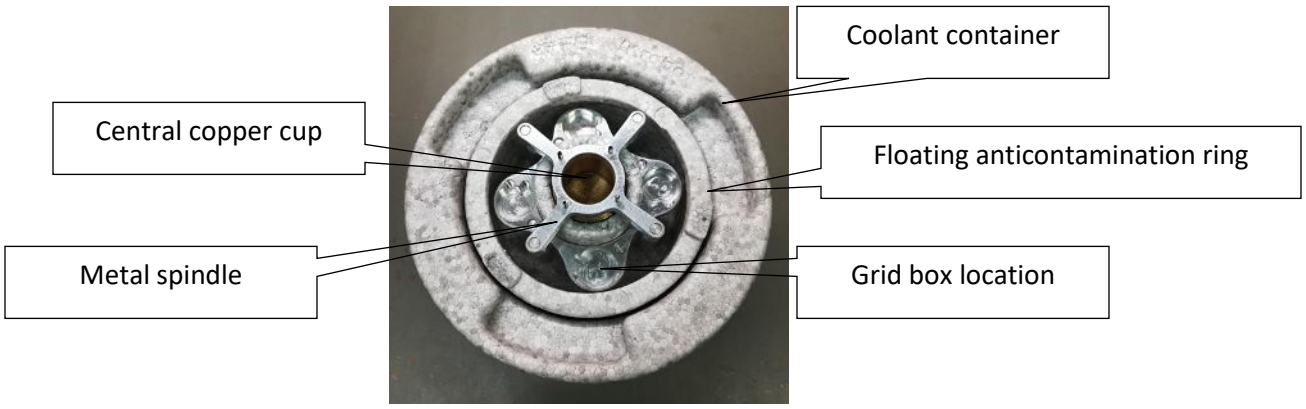
Vitrobot setup

1. Attach the power cable to water cylinder/humidifier (red dot on cable connector aligns with small red dot on the the bottom of water cylinder), connect the water cylinder/humidifier to the humidity chamber and fill the water cylinder/humidifier with disdilled water.
    a. Make sure the black dot on the cylinder is facing front, align the groove on the cylinder to the screw on the bottom of humidity chamber, push cylinder up (you may need slightly larger force) and turn right to securely water cylinder/humidifier to the humidity chamber
    b. Pull 60 ml of the water to syringe (make sure there is no air in the syringe) and connect syringe to the tube at the bottom of the water cylinder/humidifier and inject water slowly into the water cylinder/humidifier. Keep the syringe attached and pull out slowly at least 20-30 ml of air and detach syringe from water cylinder/humidifier.
2. Turn on the power switch on the back of the Vitrobot, screen remains dark for a few seconds and then shows control program starting progress. Plunge rod will extend outside of chamber once Vitrobot is fully started.
3. On the console page, set the temperature = 4°C and humidity = 100% (suggested, feel free to change according to your samles), select/check “ON” for humidifier.

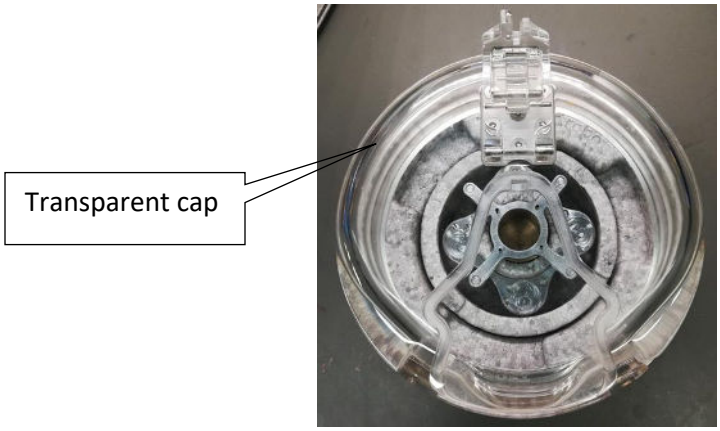


4. On the options page, set
    a. Make sure there is only one row in Processes.
    b. Set blot time = 3 sec, blot force = 4, wait time = 5 sec, drain time = 0 sec, blot total = 1 usually yields good ice thickness, but it depends on the samples and grids. Uncheck “skip application”.
    c. The blotting action is defined by time (both blot and wait) and force, all have to be optimized for each specimen and each type of grids used. Suggested starting points: **blot force 4, blot time 3 sec, wait time 5 sec** work for most of single particle samples with **glow discharged Quantifoil/C-flat/UltraAuFoil grid**; **blot force 4, blot time 3.5 sec, wait time 60 sec** for single particle samples with **glow discharged ultrathin continuous carbon grid**.

   d. Misc: recommend to check **"Use Footpadel"**, **"humidifier off during process"** and **"skip grid transfer"**
5. Wait approximately 30 mins for the system to equilibriate to the set temperature and humidity.
6. While waiting, assembly and fill the Coolant Container with LN2 (about -196°C)



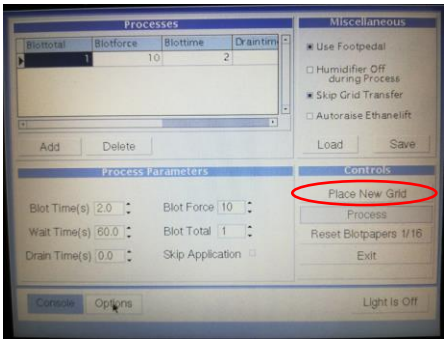
   a. Once the coolant container is chilled (not boiling violently), position the metal spindle over the central copper cup.
7. Fill central copper cup with ethane from compressed cylinder with caution (watch carefully when facility staff is doing the demo).
   a. Open the main valve of the ethane cylinder and slowly increase regulator.
   b. Control gas flow rate by regulator and slowly condense the ethane gas in the central copper cup.
   c. The liquid ethane (about -88°C to -172°C) should reach the rim of the central copper cup for optimal vitrification.
8. There is a Nanosoft transparent cap to cover the whole coolant container to keep it cold and reduce moisture condensation inside the container. This is recommended.



9. Remove the metal spindle when solid ethane begins to form (optimal temperature), the coexistence of liquid and solid ethane indicates the right temperature to proceed. If no solid, put the metal spindle back to further cool down liquid ethane; if too much solid, very slowly fill more ethane gas to warm up but do not melt solid completely.
10. Close the main valve of ethane gas cylinder.

Sample freezing

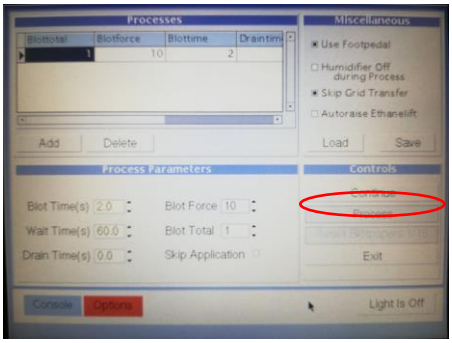
11. Change blotting pads right before freezing sample: remove the white plastic clip rings from the blotting pads. Secure pieces of fresh filter paper to blotting pads using the clip rings. **Click the reset blotting papers button**.
12. Plasma clean grids in Nanolean 1070 and use them within 30-60 mins:
    a. Place grids in 5 x 5 metal holder, keeping grid top side (the side needs to be treated, usually the film side) oriented up.
    b. Power = 70%, Gas flow = 30 sccm, time = 2mins for Au foil grid, 1min for carbon film grids, 30sec for ultrathin carbon film), 75 parts Argon, 25 parts Oxygen
    c. Keep Nanoclean1070 at high vacuum when not in use.
    d. Plasma treated grids will remain hydrophilic for several hours.
13. Press footpedal (or click Place New Grid) to move vitrobot into “Place New Grid” position (this retract the plunger rod slightly but with a few cm out of chamber for loading the tweezer).



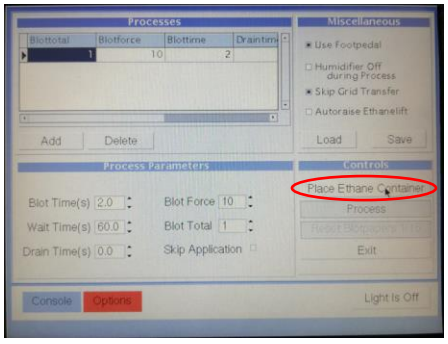
14. Pick up grid with Vitrobot tweezer, keeping track of top side of grids, move the black slider down to the first notch on the tweezer. This step locks grid by tweezer.
15. Mount the tweezer to the Vitrobot by securing them onto the plunger rod and centering with rod. You need to align grid to let plasma treated side (top side) facing either left or right side (sample can be added from either side of the climate chamber).

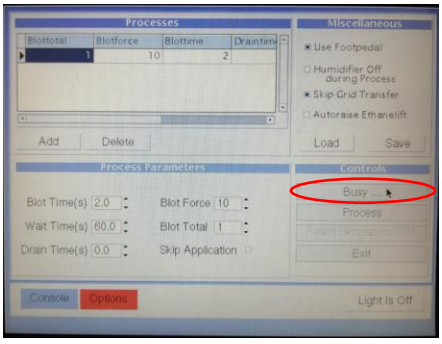
Note: Alternative is to do step 14 and 15 before step13.

16. Press footpedal (or click Continue) to retract tweezer fully into the climate chamber.



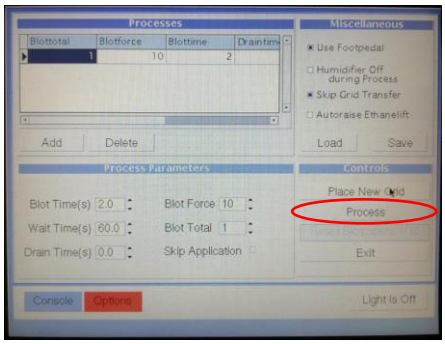
17. Place the coolant container on the platform ring.
18. Press footpedal (or click Place Ethane Container) to raise the coolant container towards



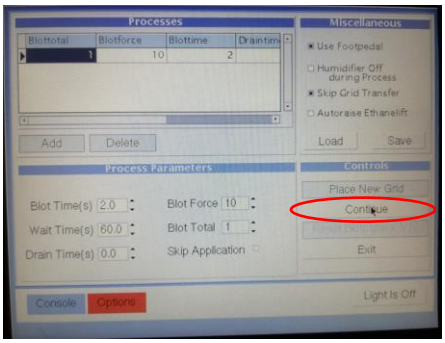


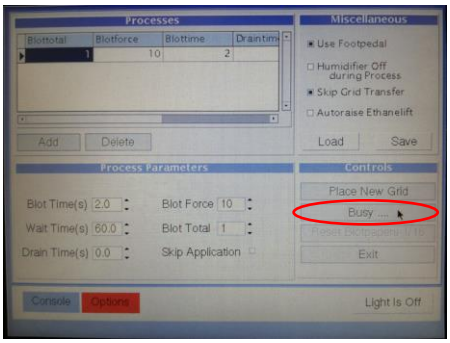
the climate chamber. Note: LN_2 may spill during this step, please step back from Vitrobot for your own safety. This step may take 5-7 sec.

19. Press footpedal (or click Process) to lower the tweezer to sample loading position.



20. Depending on the grid side orientation in Step 14, open side-entry port on the same side and pipette sample onto plasma treated side of grid.
    a. 3 ul is a good amount for a grid.
21. Press footpedal (or click Continue) to activate waiting, blotting and plunge-freezing, fill the coolant contrainer with LN2 after plunge-freezing. This step take “Wait Time” + “Blot Time”.

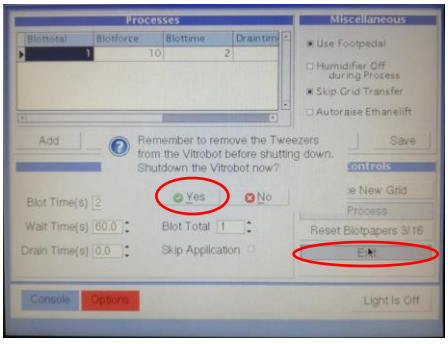




22. Transfer the grid into pre-cooled grid storage box.
    a. carefully disconnect tweezer from the plunger rod by sliding.
    b. Move the coolant container from the support ring to the bench top while keeping grid merged in the liquid ethane (this step need cooperation of both hands).
    c. Slowly lift grid out of liquid ethane and quickly dip it into LN2, gentely put grid in the storage box
23. When all grids (four per box) are placed in the storage box, tighten the screw to seal and transfer the grid box to the large storage dewar.

Instrument shutdown

24. Empty LN2 and liquid ethane from coolant contrainer to coolant waste collection container (usually Styrofoam box).
25. Make sure there are no tweezer attached to the plunger rod, Pressing the “exit” button on the console screen and follow instruction to quit the Vitrobot program (click Yes).



26. Wait until the control program is completely off (screen becomes dark/black) , **use the power switch located at the back to fully power off Vitrobot**.
27. Disconnect the humidifier (watch carefully when facility staff is doing the demo).
    a. Unplug electrical cable from the water cylinder/humidifier by pushing up the ring near the cable, holding the ring in pushed up position and pulling down the cable connector. (This is critical step, pleasewatch the demo carefully, otherwise the cable connector will be damaged)
    b. Push up and twist left to remove the water cylinder/humidifier from the Vitrobot.
    c. Empty the reservoir of water cylinder/humidifier by pouring the water from the central reservoir.
    d. Re-connect the syringe and withdraw the rest of the water from the outside reservoir.
    e. Leave the empty humidifier on the table near Votrobot and let it dry completely.

More Reading : Controlled Vitrification

1. It is highly recommended that the grid surface should be cleaned by plasma/glow discharge to make it hydrophilic.
2. The purpose of blotting is to create a 100nm vitreous ice film on the grid.
3. Temperature and Humidity (rH) matters. The bulk of specimen should be at the same temperature as the Vitrobot chamber.
4. Special care and attention should be given to the thermal equilibration of the tweezers that are holding the specimen grid. Preheating the tweezer is strongly recommended before it enters the chamber to avoid condensation on the tweezer.
5. The ethane contrainer should be filled up to the rim to prevent precooling of specimen in the cold gas before it enters liquid ethane.
6. Plunge blotted specimens into liquid ethane at aproximate -172°C, the coexistence of liquid and solid ethane indicates the right temperature to proceed.
7. Ethane residues (white ice on the surface of the grid) may protect specimen during transfer, which evaporates in the storage tank, in the airlock, and vacuum of microscope.
8. The excess of liquid ethane on the grids can be limited by the following two manipulations:
    a. Squeeze the tweezer tips together gently while removing the grid from ethane.
    b. Slowly lift the grid from ethane through ethane surface while observing how the liquid film detached from the grid.