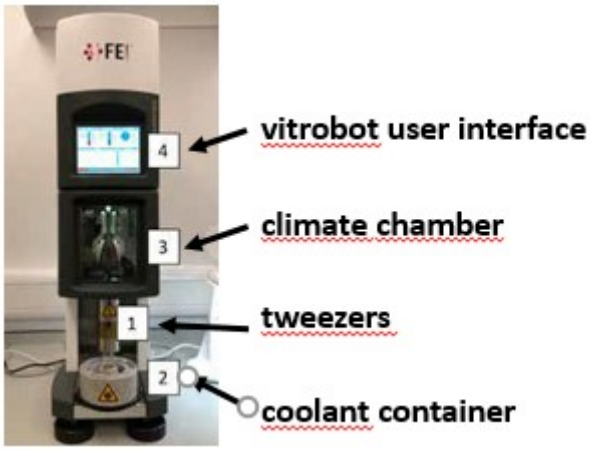
Vitrobot procedure

The VitrobotTM (Vitrification Robot) is a fully PC-controlled device for vitrification (= rapid cooling) of aqueous samples



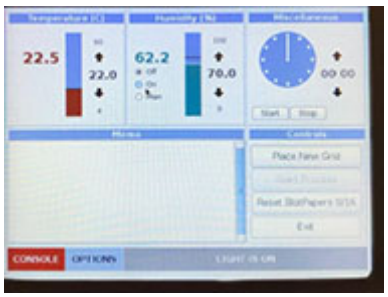
Before starting

- fill a dry 5 L tank with liquid nitrogen
- label grid boxes with the name of the experiment
- check plasma cleaner is reserved
- Assemble the necessary tools:
  - EM_grids grids are available and present in sufficient number
  - all part of the coolant container (make sure that they are dry)
  - both vitrobot tweezers, big tweezer and blot paper clipping

Starting up the vitrobot

- 1. close climate chamber's door if open
- 2. Switching on vitrobot
  - Press the hard lock switch on the right backside of the machine

- The Vitrobot User Interface page will appear after a few seconds.
  - Console page is dedicated to set humidity and temperature parameters
  - options page is dedicated to set blotting process parameters



- 3. Make sure the humidifier is operational
  - check humificator beaker state in humidity panel

Humidificator beaker ready to work

Humidificator beaker empty need to be refilled

  - if EMPTY refill it with distilled water

Use ONLY distilled water

  - a. Fill a syringe with 60 ml distilled water
  - b. inject the whole volume through the plastic tube at the bottom part of the humidifier
  - c. When removing the syringe from the tube, be careful not to tear it from its location

- 4. hermetically close the chamber
  - do the following dry Run:
    - Place new grid / continue / place ethane container
  - the white latch remains in open position after swith on the power.

Set blotting process parameters

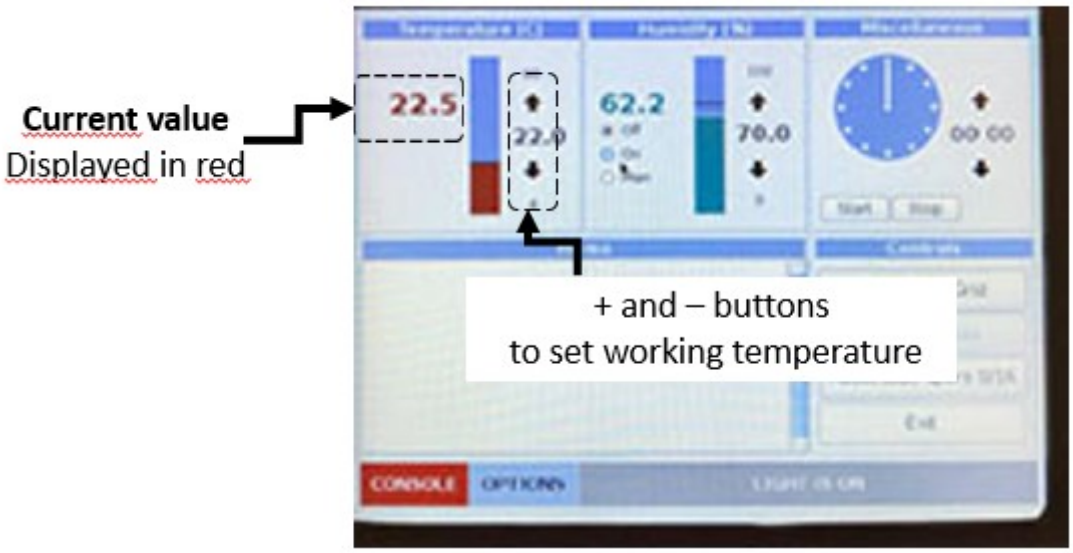
This step is done on the control panel, in the “options” page

- 1. 'blot total' needs to be '1' for blotting to occur
- 2. check 'Humidifier off during process' and 'Skip grid transfer' in the miscellaneous' side bar
- 3. Optional : set your desired blot time, force blot and waiting time (o for unsupported condition)
  - all these setting are checked at the begining of the freezing cycle

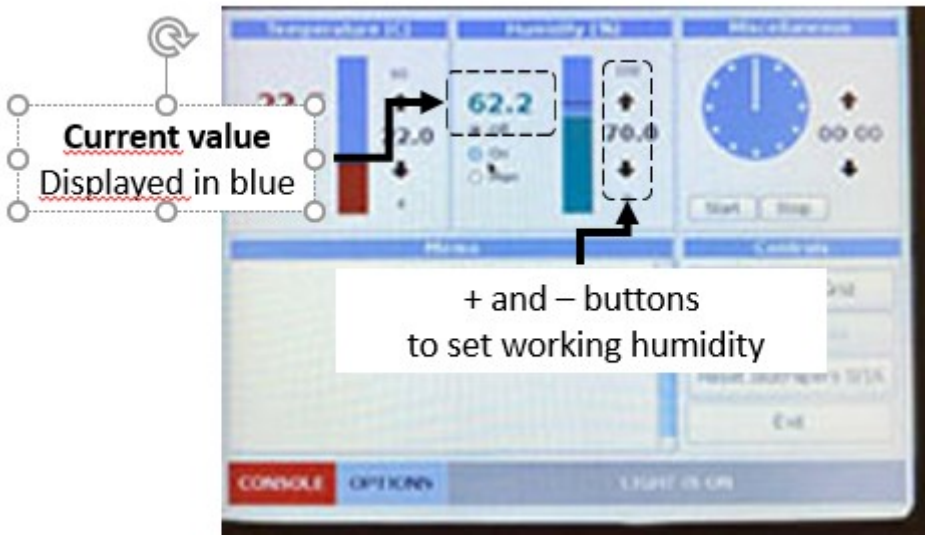
Set humidity and temperature parameters in climate chamber

This step is done on the control panel, in the “console” page

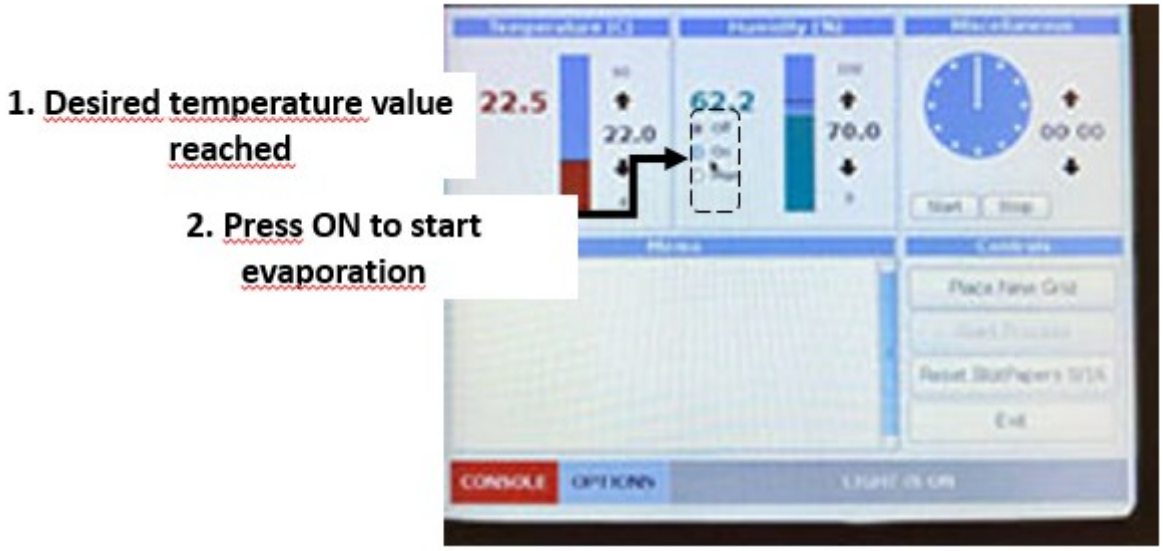
- 1. adjust desired temperature value
  - : to any value between 4 and 60°
    - the actual temperature value is displayed in red
    - set to any desired value with + and buttons in temperature panel



- 2. adjust desired humidify value
  - 96-100 % generally
    - the current humidity is displayed in blue
    - set to desired value with + and - buttons in humidify panel



- 3. wait until desired temperature is reached then enable the humidity switchbox to start
  - a. read current temperature (red value) in panel temperature
  - b. press ON/OFF switch in panel humidity



- take advantages from this waiting time to do the two following steps
  - Mounting the filter papers
  - load grids inside the plasma cleaner

Mounting the filter papers

- IMPORTANT **always wears gloves**
- place blot papers onto the blotting pads with clipping ring
- support the blotting arms during assembling to prevent damage to the arms

load grids inside the plasma cleaner

- 1. switch on plasma cleaner if not started
  - If stopped, press the hard lock switch on the backside of the machine,
  - Press CLEAR to initialyze the vaccum system

Not Initialized State
Just after swithing on

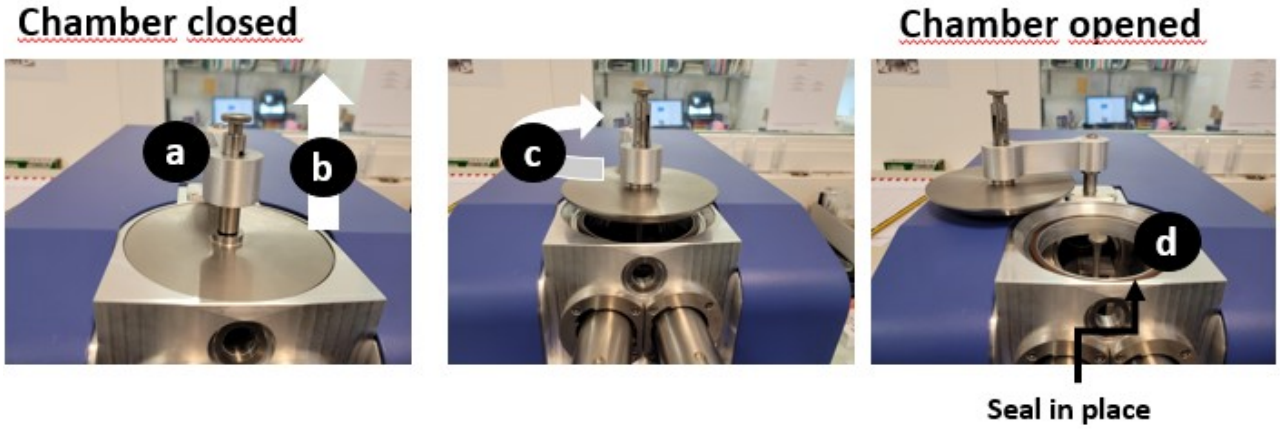
Ready State
fishione ready for use

- fishione started with chamber locked under vaccum
- READY / HIGH VACCUM messages

- 2. press Vent Lock to bring the chamber under atmospheric pressure
  - State is reached when message Lock At Atmosphere appears

From now on IMPERATIVELY **wears gloves**

- 3. Open chamber and make sure the seal remains in place



  - a. unlock the rod by pushing the button on it
  - b. pull the rod upwards to lift the cover
  - c. move the cover on the side around its axis
  - d. if necessary put back the seal in place.
    - It can either stick on the cover or come out of its channel

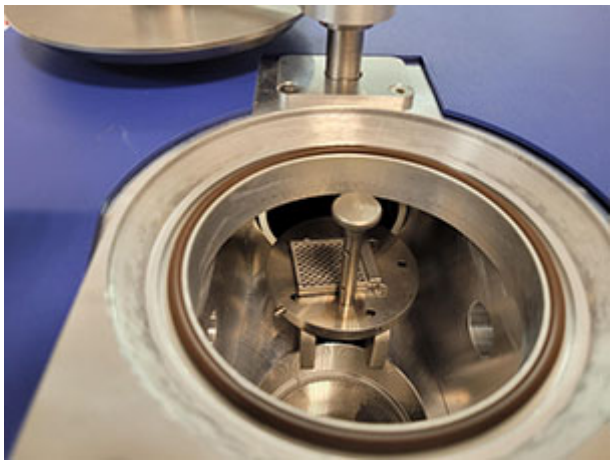
- 4. retrieve the metallic drawer and place it on the table
  - the support carrying the drawer can be taken out by lifting it by the rod.
  - Be sure to keep the table horizontal so as not to slide the drawer

  - the metallic drawer is **ALWAYS stored in the chamber**
- 5. close the door to prevent dust contamination inside the chamber
- 6. place EM_grids inside the metallic drawer
  - EM_grids with support film facing up
    - remove the cover of the metallic drawer by sliding
    - place EM_grids inside
    - generally sets of 4 to 8 **IDENTICAL** grids are loaded simultaneously
    - close the drawer by putting back in place the cover

        - put back in place the closed drawer on the support table

    - generally sets of 4 to 8 **IDENTICAL** grids are loaded simultaneously
- 7. open the door and make sure the seal remains in place
    - a. if necessary put the seal back in place
        - It can either stick to the cover or come out of its channel
    - b.clean it just by passing your finger

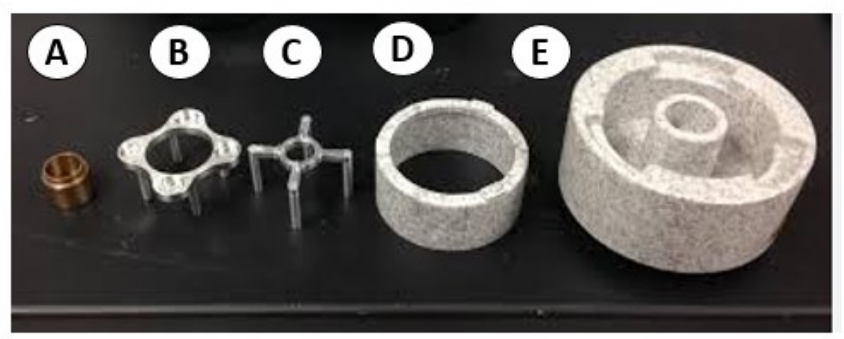
- 8. put back in place the metallic drawer inside the chamber
    - on the plateform **NOT on the inlet pump**

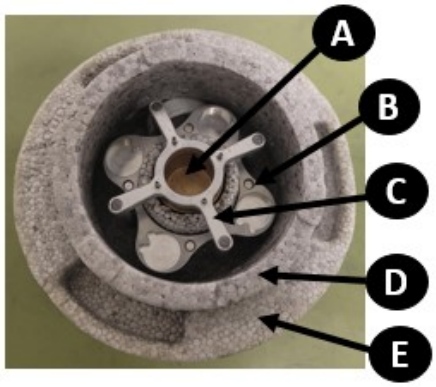


- 9. close the door
    - a. be sure the seal is in place and clean (see part.7)
    - b. move the lid around its axis to bring it over the opening of the chamber
    - c. unlock the rod by pushing the button
    - d. gently allow the lid to slide down

- 10. press VACCUM to bring the chamber under vaccum
    - State reached when message “HIGH VACCUM” appears
- 11. if not yet done on vitrobot, enable the humidity switchbox to start

Assemble different part of the coolant container as pictured below





- legende
  - A : central ethane cup
  - B : grid box plateform
  - C : spindle
  - D : Ice contamination protective sleeve (optional)
  - E : outer nitrogen container

Cooling down the coolant container

- 1. Pour LN2 into both central ethan cup (A) and outer part (E) to faster the cooling
- 2. Wait for complete evaporation of the remaining nitrogen in the central part
- 3. up to now continously fill the outer part to maintain the Nitrogen level
- 4. wait 10 to 15 min for system to equilibrate
- 5. then liquefy the ethan in the central cup already cooled to liquid nitrogen temperature
  - Ethan is liquefied by flowing a stream of gas through plastic tubing connected to an ethane flask into the central cup

- 6. let ethane start to solidify for few seconds
  - apparence of a white solid rim around the wall container indicates that ethane starts to solidify

- 7. then remove the spindle (C)
  - Solidified ethan makes stick together the spindle and the ethan cup.
  - Thawing the frozen ethan by placing the second ethan cup on the spindle for 10sec is the more careful way to remove it with a big tweezer

- 8. place grid box into the grid box platform (B)
  - make sure that the grid box is labelled with the experiment name

plasma clean grids

- 1. Grids are already loaded inside the plasma cleaner
  - if not yet done

    see part

    ### load grids inside the plasma cleaner

- 2. set desired plasma cleaning parameters :gas mixture/gas flow power
  - commonly settings 80-20-0 are used
    - The setup TABS store preset parameters
    - settings 80-20-0 corresponds to Gas mix Oxy/Ar : 80/20, power 34%
    - Gas Mix and plasma TABS show preset values

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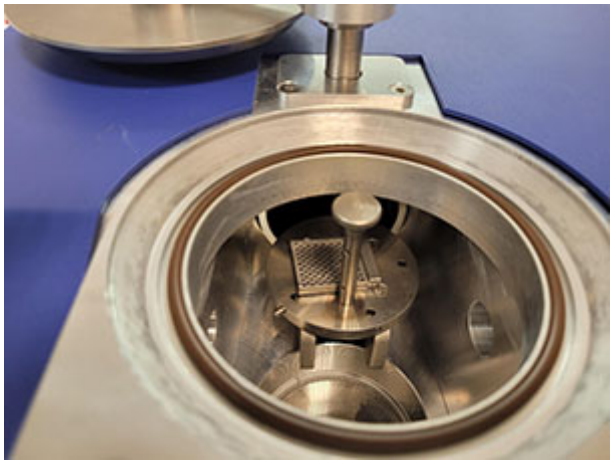
- 3. set the plasma cleaning time
  - a. use up/down arrows head selectors
  - b. press set buttom to load the time in the time display

  - commonly 90 s are used
- 4. press START to apply plasma on grids
  - The gas plasma generated can be seen through the window as a pink light
  - remaining time can be followed in the time display
- 5. press Vent Lock to bring the chamber under atmospheric pressure
  - State is reached when message Lock At Atmosphere appears
- 6. Open the chamber
- 7. Retrieve the drawer and place it on the table
  - the support carrying the drawer can be taken out by lifting it by the rod.
  - Be sure to keep the table horizontal so as not to slide the drawer

- 8. close the door to prevent dust contamination inside the chamber
- 9. place the plasma cleaned grids on a glass slide
  - remove the cover of the metallic drawer by sliding
  - place EM_grids on the edge of the glass

- 10. put the glass slide in a closed crystallizer to transfer safely grids to vitrobot bench
- 11. load or not new grids in the drawer and close it
- 12. put back in place the closed drawer on the support table
- 13. open the door and make sure the seal remains in place
  - a. if necessary put the seal back in place
    - It can either stick to the cover or come out of its channel
  - b.clean it just by passing your finger

- 14. put back in place the metallic drawer inside the chamber
  - on the plateform **NOT on the inlet pump**



- 15. close the door
  - a. be sure the seal is in place and clean (see part.7)
  - b. move the lid around its axis to bring it over the opening of the chamber
  - c. unlock the rod by pushing the button
  - d. gently allow the lid to slide down

- 10. press VACCUM to bring the chamber under vaccum
  - either grids are waiting ready for an additionnal run of freezing at vitrobot (step 4-to 15)
  - or the fishione remains on for next user
  - At the end of the day, the fishione can be switch off

Everything is ready for plunge freezing sample:

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climate chamber is equilibrated in temperature and humidity.
Filter paper are mounted
ethane is liquefied
Grids are freshly plasma cleaned
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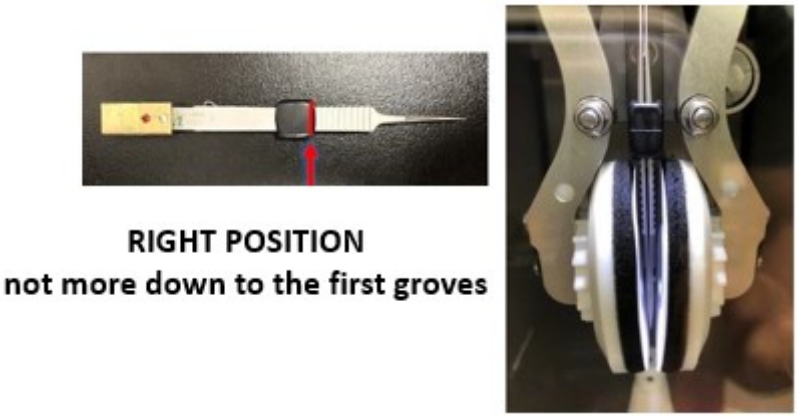
Start freezing at the vitrobot

- 1. check that the correct freezing parameters are entered
- 2. click on “place new grid” to bring in loading position the plunge rod
- 3. Lock a Glow discharged grid on tweezers
  - Grasp as little of the grid as possible with the tweezers (but enough that it will not fall out during freezing)
  - Make sure that the black clamping ring is fixed not more down to the first groves.



- 4. then fix the tweezers onto the plunge rod
  - with the support film support on the right for right-handers and on the left for left-handers
- 5. press on “continue” to bring up the tweezers into the blotting chamber
- 6. place the container onto the ethane lift
  - Always check that ethane is at right temperature
- 7. press on to lift it
- 8. click on “process”
- 9. apply sample to grid
- 10. click on “continue” to blot and plunge
- 11. carrefully detach tweezers from the plunge rod
- 12. By maintaining the grid inside the ethan cup, place the coolant container on the table
- 13. transfer grid to the grid position
- 14. Dry tweezers
- 15. then repeat sequence 1-14 for all glow discharged grids