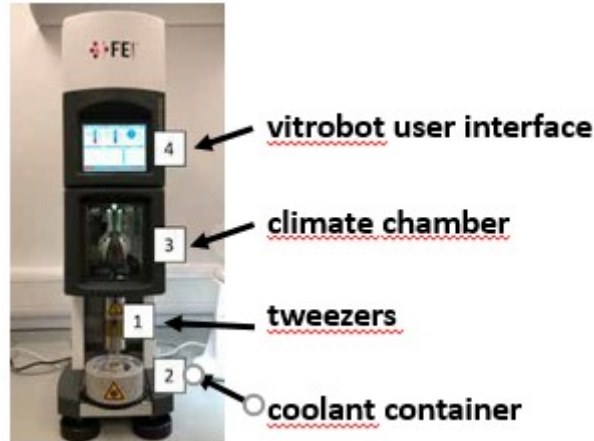


# Vitrobot procedure

The Vitrobot<sup>TM</sup> (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 several things:
  - plasma cleaner is reserved
  - EM\_grids grids are available and present in sufficient number
  - all part of the coolant container are dry
  - both vitrobot tweezers, big tweezer and blot paper clipping ring are not missing

## 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 humidicator 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

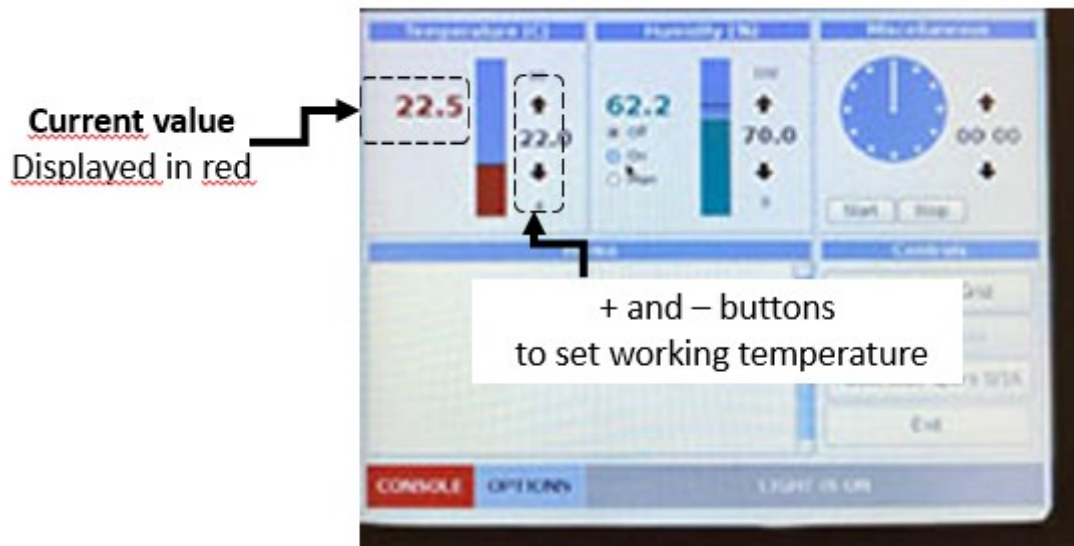
This step is done on the control panel, in the “options” page

- 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. set your desired blot time, force blot and waiting time (o for unsupported condition )

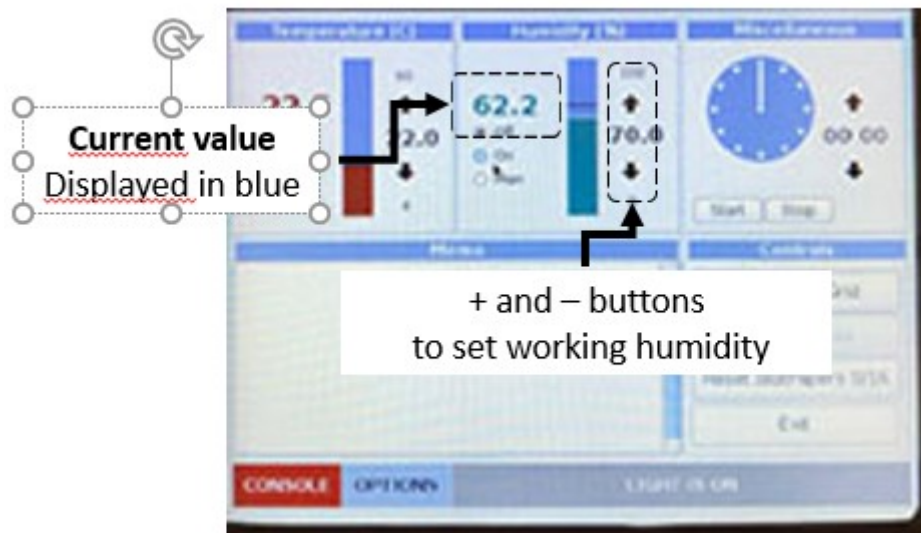
## Set humidity and temperature parameters in climate chamber

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



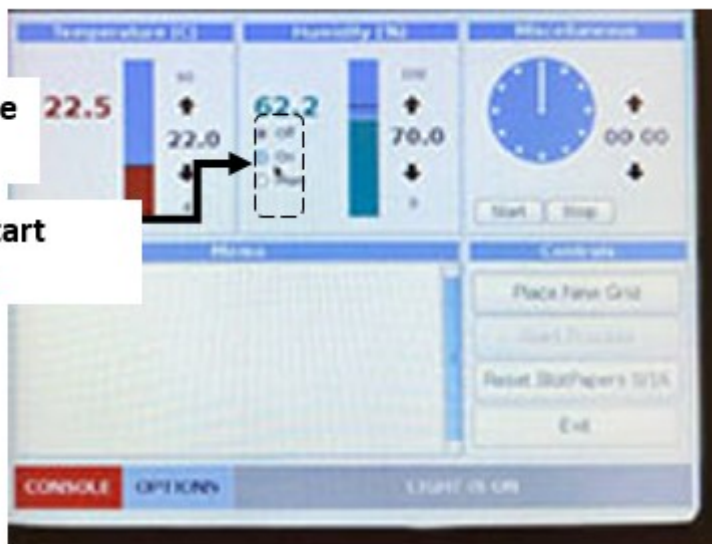
- 2. adjust desired humidity value
  - 96-100 % generally
    - the current humidity is displayed in blue
    - set to desired value with + and - buttons in humidity panel



- 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

1. Desired temperature value reached

2. Press ON to start evaporation



- 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 initialize 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

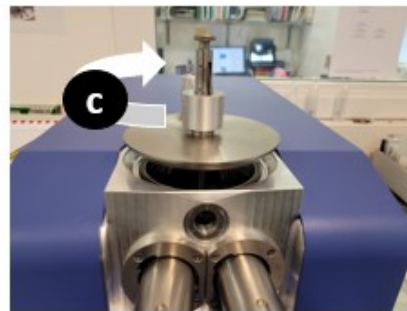
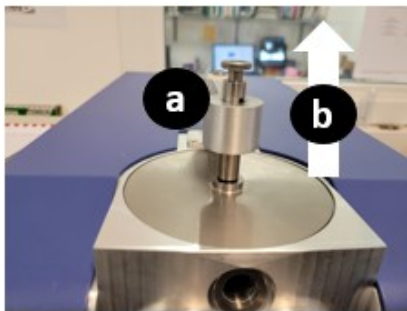


- 2. press Vent Lock to bring the chamber under atmospheric pressure
  - State is reached when message Lock At Atmosphere appears

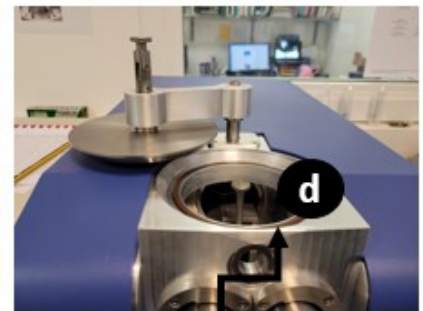
From now on IMPERATIVELY **wears gloves**

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

### Chamber closed



### Chamber opened



Seal in place

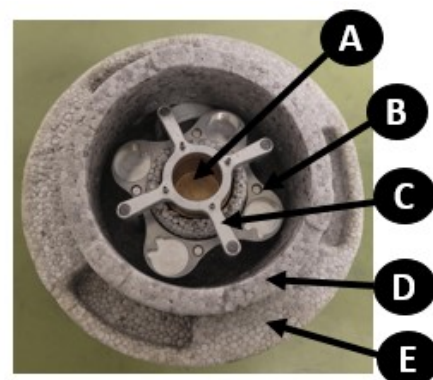
- a. unlock the rod by pushing the button on it
  - b. pull the rod upwards to lift the cover
  - c. move the cover on the side around its axis
  - d. if necessary put back the seal in place.
    - It can either stick on the cover or come out of its channel
- 4. retrieve the metallic drawer and place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

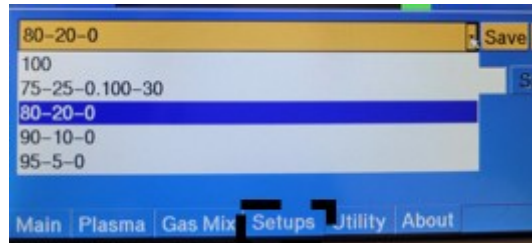
## plasma clean grids

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

see part

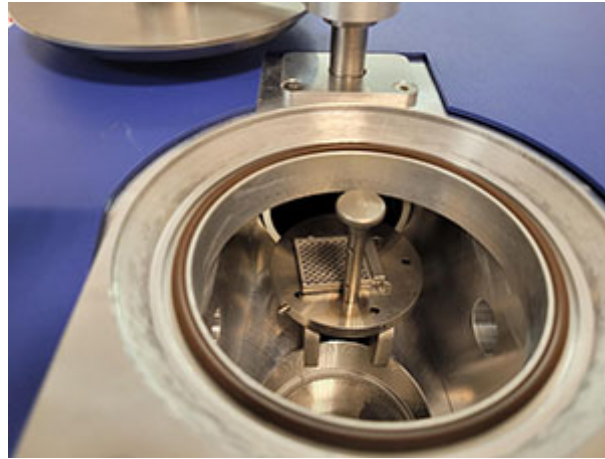
### load grids inside the plasma cleaner

- 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



- Gas Mix and plasma TABS show preset values

- 3. set the plasma cleaning time
  - a. use up/down arrows head selectors
  - b. press set button 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 platform **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

### Start freezing

- 1. set or checkcheck the blotting condition
- 1. click on “place new grid” to bring in loading position the plunge rod
- 2. Attach Vitrobot tweezers with a Glow discharged grid onto the plunge rod
- 3. press to bring up the tweezers into the blotting chamber
- 4. place the container onto the ethane lift
- 5. press to lift it
- 6. click on “process”
- 7. apply sample to grid
- 8. click on “continue” to blot and plunge
- 9. carrefully detach tweezers from the plunge rod
- 10. By maintaining the grid inside the ethan cup, place the coolant container on the table
- 11. transfer grid to the grid position
- 12. Dry tweezers then repeat

\* Now vitrobot is ready for freezingbeforeDo it when you are almost ready to freeze to keep the soaking time as reproducibile as possible

===== At this point already is ready for freezing =====

At this point already is ready for freezing:  
climate chamber is equilibrated in temperature and humidity.  
Filter paper are mounted and soaking time is controlled  
ethane is liquefied  
Grids are loaded and ready to be plasma cleaned

## Preparation of the grid

### 3. Bring the vitrobot to hermetically close the chamber

- do the following dry Run:
- A glow discharge grids is attached to the tweezers

## Start freezing

\* ++ Do it when you are almost ready to freeze to keep the soaking time as reproducible as possible|<div>

Decrire

## Mounting the filter papers, IMPERATIVELY wear gloves

- Do it when you are almost ready to freeze to keep the soaking time as reproducible as possible

Decrire

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