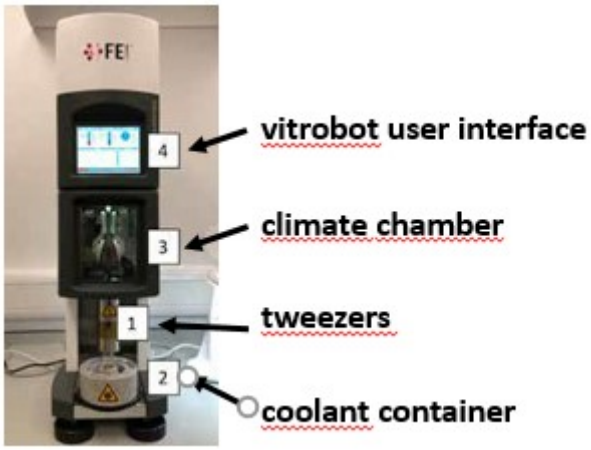
# Vitrobot procedure

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



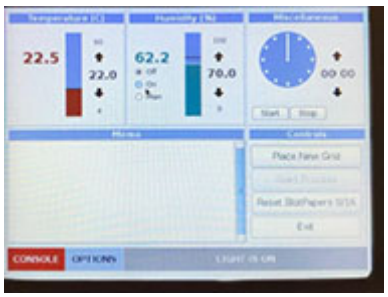

## Before starting

- fill a dry 5 L tank with liquid nitrogen
- label grid boxes with the name of the experiment
- check 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 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

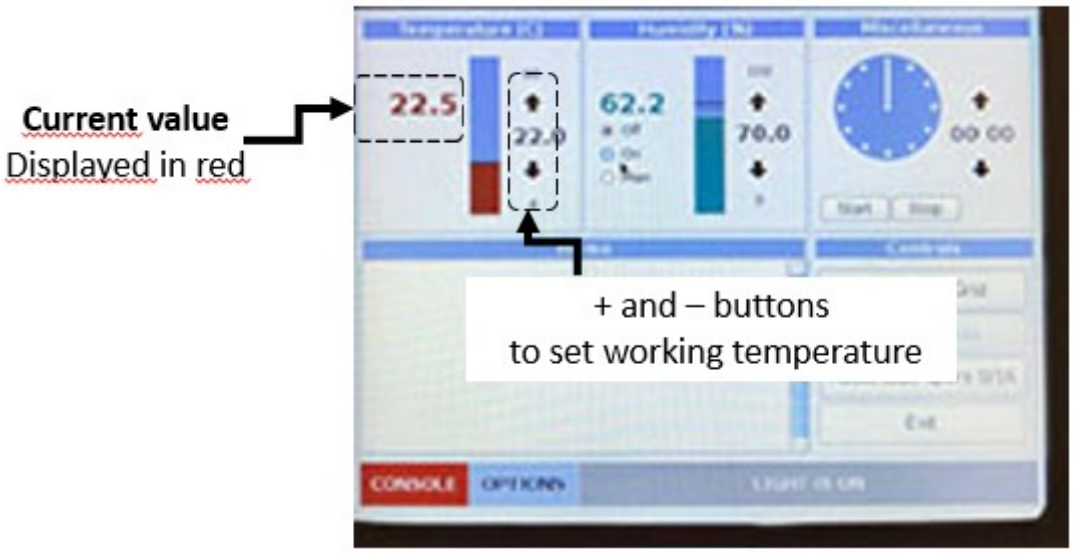
This step is done on the control panel, in the “options” page

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

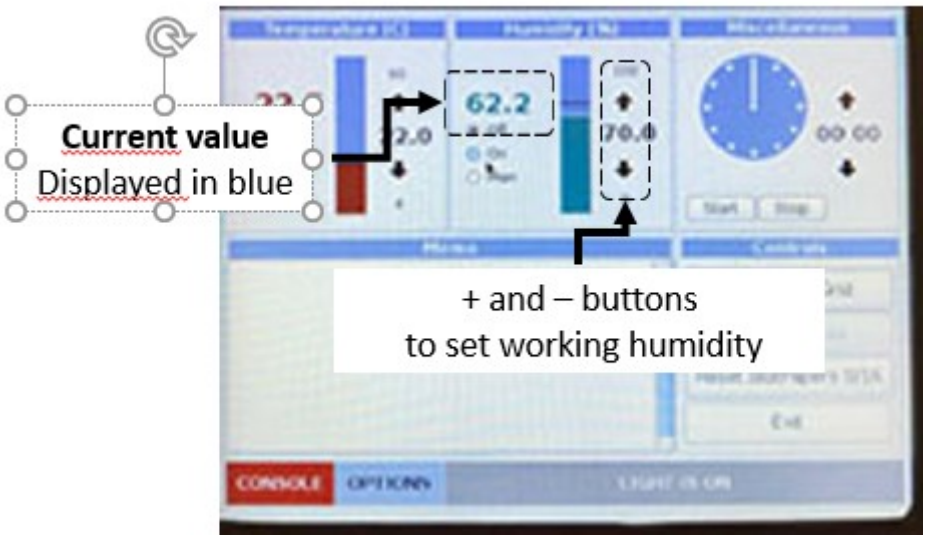
## Set humidity and temperature parameters in climate chamber

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

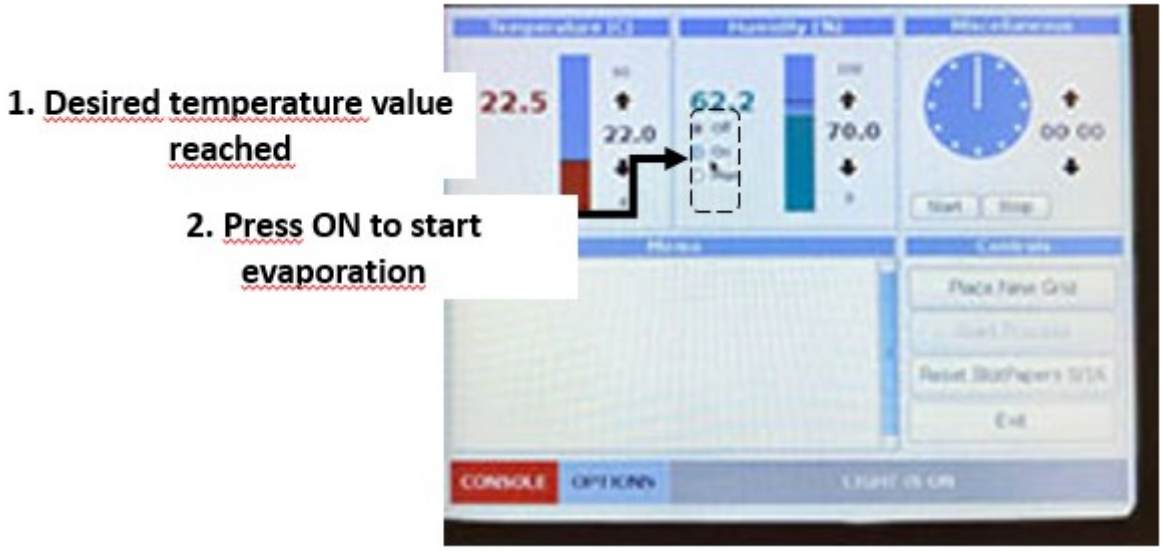
- 1. adjust desired temperature value
  - : to any value between 4 and 60°
    - the actual temperature value is displayed in red
    - set to any desired value with + and buttons in temperature panel




- 2. adjust desired 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

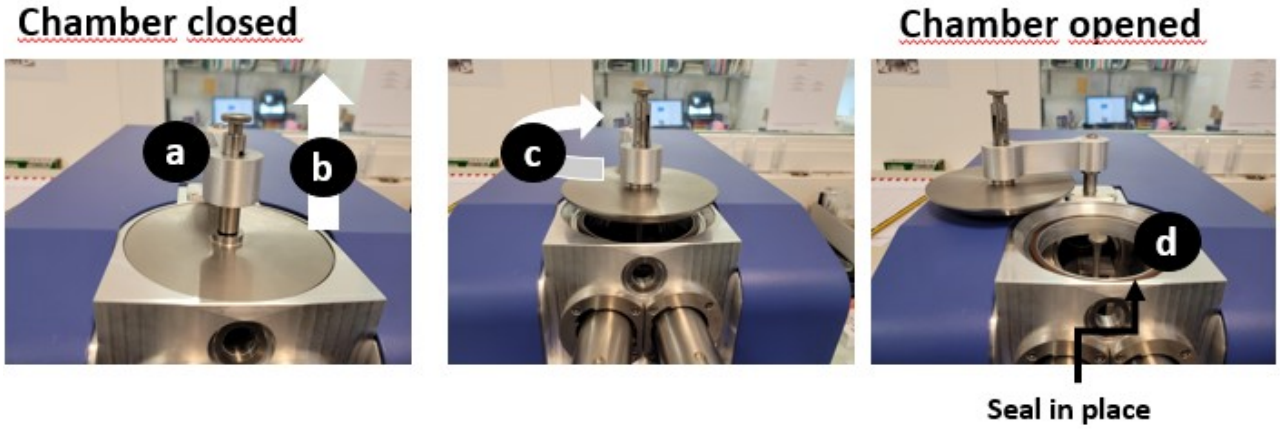

**Ready State**
fishione ready for use


- 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



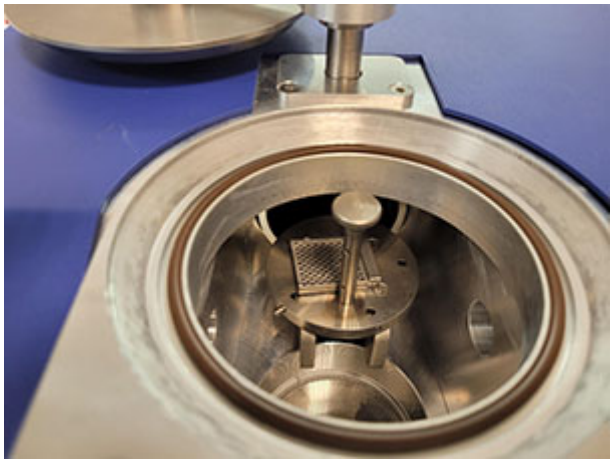

    - a. unlock the rod by pushing the button on it
    - b. pull the rod upwards to lift the cover
    - c. move the cover on the side around its axis
    - d. if necessary put back the seal in place.
        - It can either stick on the cover or come out of its channel

- 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
    - 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

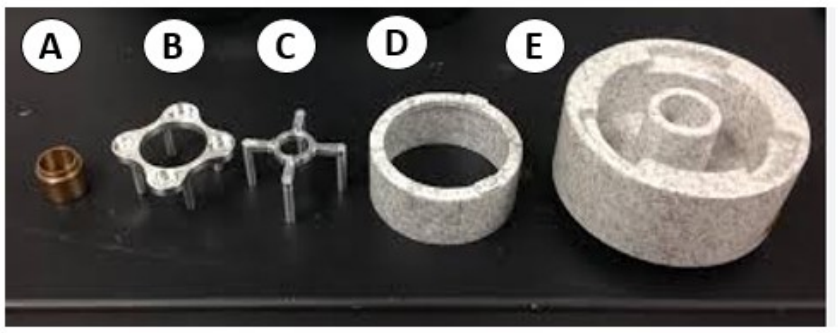
- 8. put back in place the metallic drawer inside the chamber
    - on the plateform **NOT on the inlet pump**

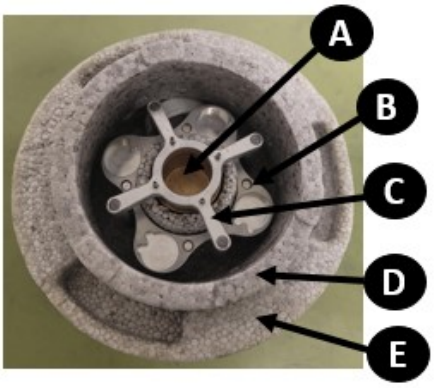


- 9. close the door
    - a. be sure the seal is in place and clean (see part.7)
    - b. move the lid around its axis to bring it over the opening of the chamber
    - c. unlock the rod by pushing the button
    - d. gently allow the lid to slide down

- 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 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. Retrieve the drawer
- 6. place the plasma cleaned grids on a glass slide

## Set blotting process parameters

## Set freezing condition

- 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 reproducible 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 twezzers

## Start freezing

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

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