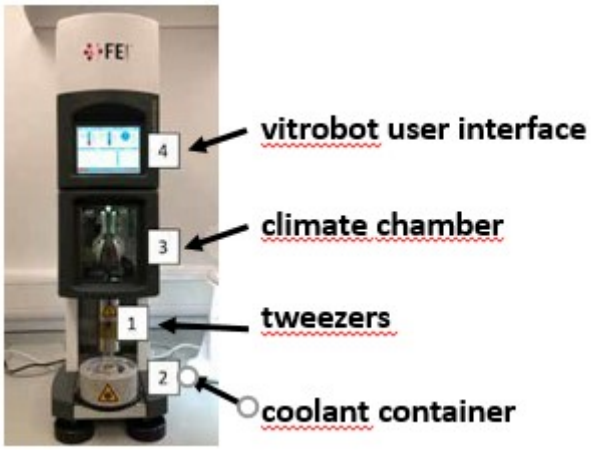
Vitrobot procedure

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



Before starting

- fill a dried 5 L tank with liquid nitrogen
- label the grid boxes
- check a number of points:
  - plasma cleaner is reserved
  - EM_grids grids are available and present in sufficient number
  - all part of the coolant container are dry
  - close climate chamber's door if open
  - Paper filter clipping ring are not missing

Starting up the vitrobot

- 1. 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

- 2. check humificator beaker state in humidity panel

Humidificator beaker ready to work

Humidificator beaker empty need to be refilled

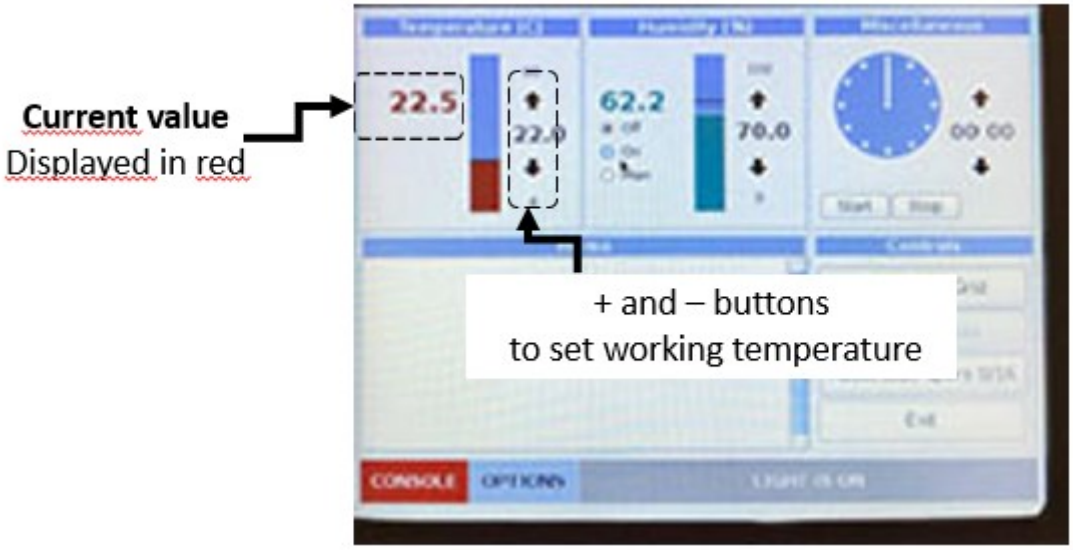
- 3. if EMPTY refill it with distilled water

Use ONLY distilled water

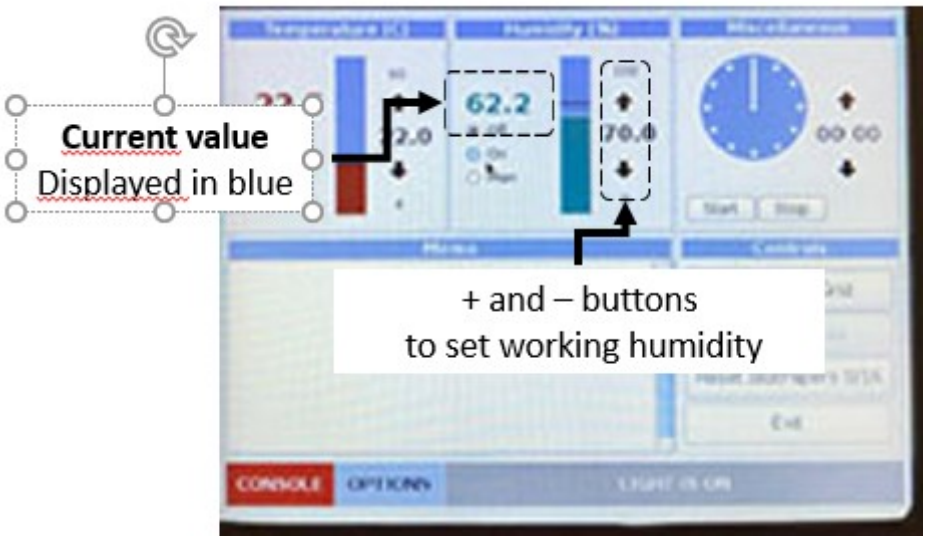
- 1. Fill a syringe with 60 ml distilled water
- 2. inject the whole volume through the plastic tube at the bottom part of the humidifier
- 3. When removing the syringe from the tube, be careful not to tear it from its location

Set humidity and temperature parameters in climate chamber

- 1. hermetically close the chamber by rotating the white latch
- 2. 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

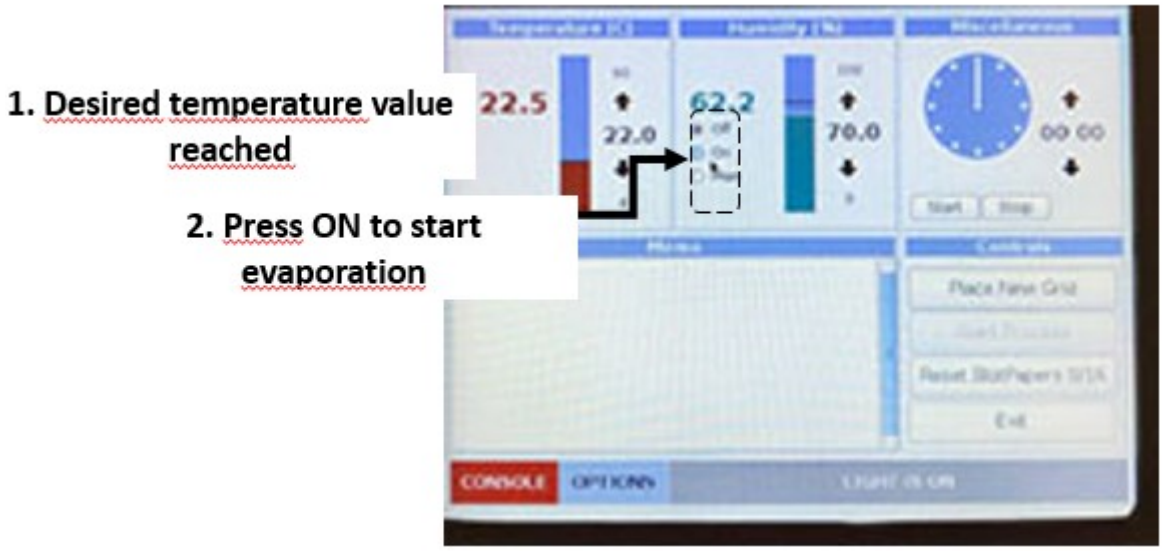


- 3. adjust desired humidify value
  - 96-100 % generally
    - the current humidity is displayed in blue
    - set to desired value with + and - buttons in humidify panel



- 4. wait until desired temperature is reached to enable the humidity switchbox to start

  press ON/OFF switch



  - take advantages from this waiting time to start next step :

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**

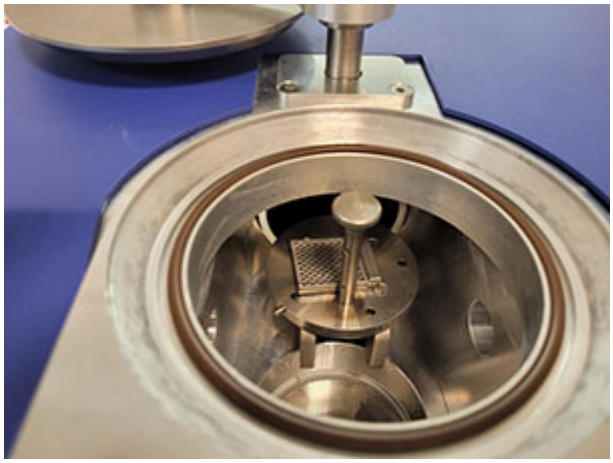
- 3. Open chamber and make sure the seal remains in place
  - a. unlock the rod by pushing the button on it
  - b. pull the rod upwards to lift the cover
  - b. move the cover on the side around its axis
  - c. if necessary put back the seal in place.
    - It can either stick on the cover or come out 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**

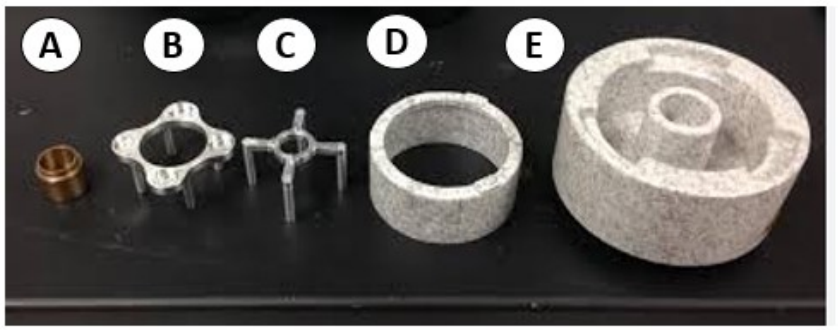


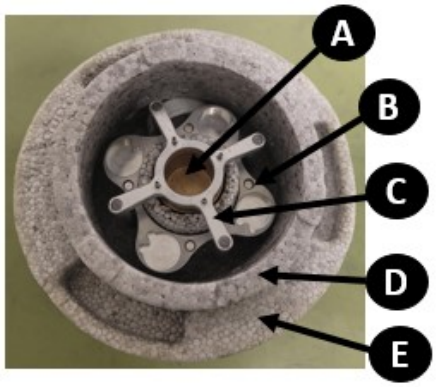
- 9. close the door

- 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

Assemble different part of the coolant container like showing below





- legende
  - A : central ethane cup
  - B : grid storage device
  - 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 and outer part 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

- 6. then remove the spindle

  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

Mounting the filter papers

- IMPORTANT **always wears gloves**
- attach the blotting papers to the the blot pad by using the with clipping ring

A way to keep the **soaking time reproducible** is do this operation always the same time before freezing

Set freezing condition

* 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 ======
```

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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
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plasma clean grids

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

    see part

    ### load grids inside the plasma cleaner

- set desired plasma cleaning parameters :gas mixture/gas flow power
  - commonly settings 80-20-0 are used
    - Gas mix: 80/20 oxy/Ar, power 34%

    * The setup TABS store preset parameters

* Gas Mix and plasma TABS show preset values

- adjust the plasma cleaning time with up/down arrows head and press set
  - commonly 90 s are used
- press START to apply plasma on grids

Preparation of the grid

- A glow discharge grids is attached to the twezzers

Start freezing

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