ICE High Pressure Freezer
Standard Operation Protocol

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Preparation

Tools

- Cartridge (upper / lower half cylinder and middle plate)
- Tissue punch
- Cryo-storage container
- Microscope stage insert (for sapphire system)
- Cryo-box
- Sample Carrier Release
- Trisection pod holder
- Cryo-storage holder container
- Cryo-protectant, e.g. Hexadecene, sucrose (prepared by user)

Consumables

3mm Specimen system:

Sample holder middle plate	**1mm**	
	Thickness	Inner height
Carrier (A)	500um	100 / 200um
Carrier (B)	500um	300um

3mm Sapphire system

Sample holder middle plate	**850um**
	Thickness
Sapphire disc	50um
Cover ring	300um

6mm Specimen system:

Sample holder middle plate	**1mm**	
	Thickness	Inner height
Carrier (A)	500um	100 / 200um
Carrier (B)	500um	300um

6mm Sapphire system

Sample holder middle plate	**820um**
	Thickness
Sapphire disc	120um
Spacer ring	200um

System Cool Down

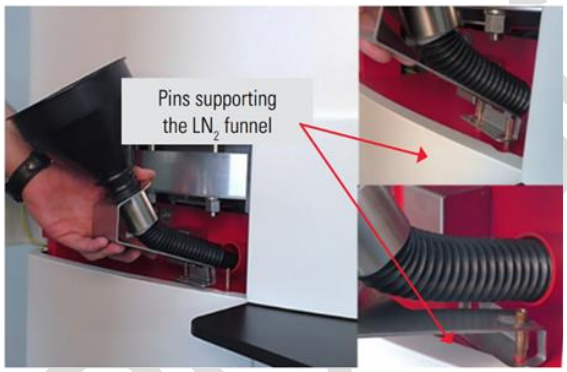
1. Turn on the main switch. The system takes around 1 minute to build up pressure and get to the main menu.
2. Assemble the sample storage dewar and lock.

3. Fill in the Dewar with LN2 (minimum required is 60%). This filling is required only at the beginning. During the experiment, LN2 will be automatically filled in the dewar.

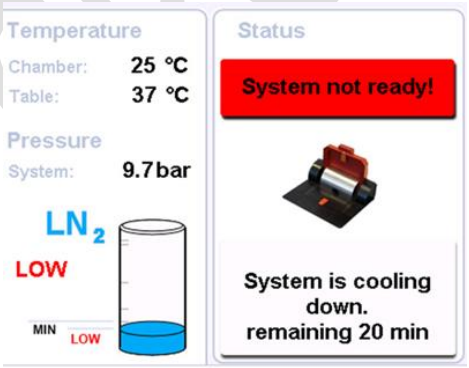
4. Put the sample storage dewar in the revolving slider. The arrow labelled “in” should point at the black point.


5. Open the front door of the instrument.

6. Put the funnel at the LN_2 inlet and fill LN_2 carefully. About 35L of LN_2 is required.



7. Fill LN_2 until the level reaches “MIN” at the screen.

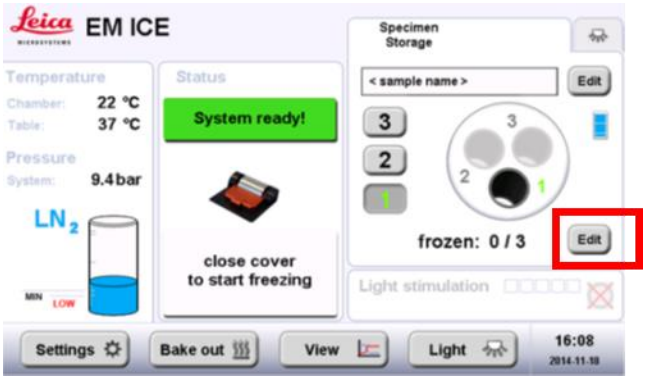


8. When the minimum level is reached, the message will state as “system is cooling down”. The system will start to count down for 20 minutes for cooling. An extra 4L of LN_2 should be added into the system.

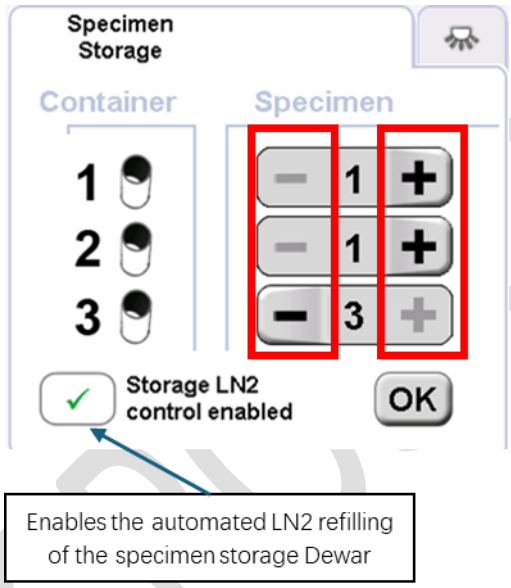
Sample Freezing

Sample Storage Setting

1. Click ***Edit*** at the main menu to set the sample storage.

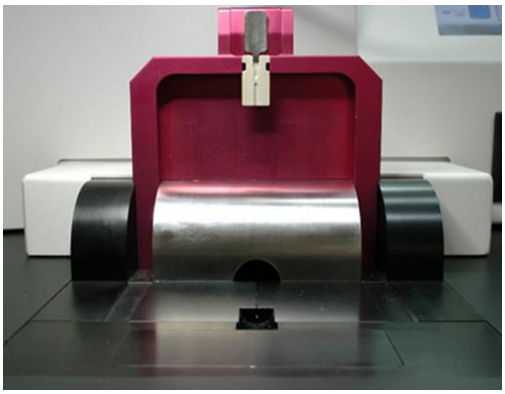
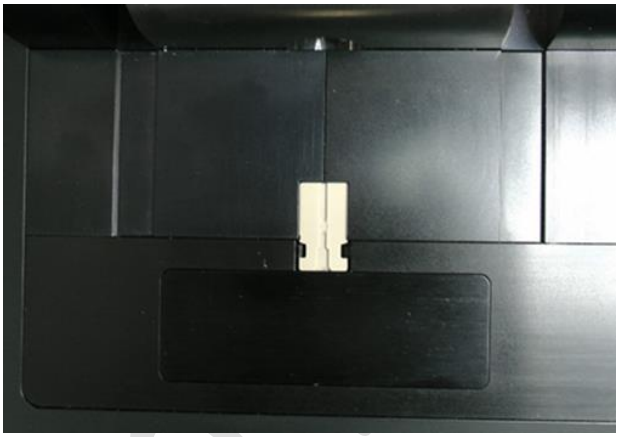


2. Set the number of samples per segment in the specimen storage Dewar using the **+** or **-** keys (Minimum: 1; Maximum: 3).



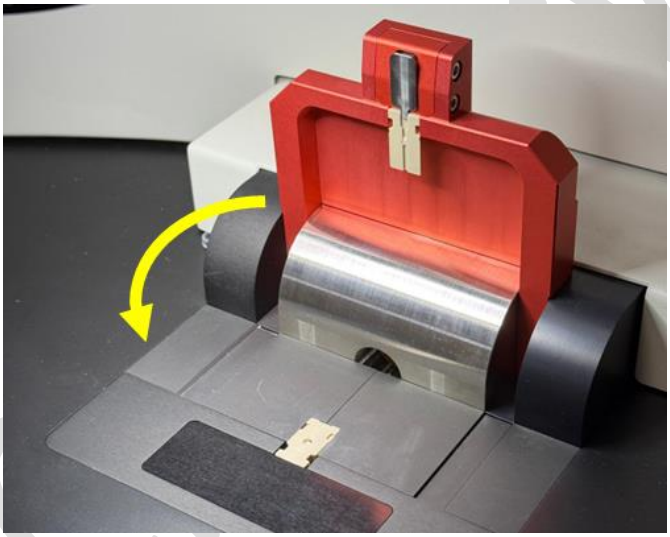
Blank Run

1. Before freezing the sample, a blank run is preferred for checking the status of the instrument.
2. Load sample cartridges in the loading station. Clamp up the upper half cylinder at the upper side and put the lower half cylinder at the lower side.

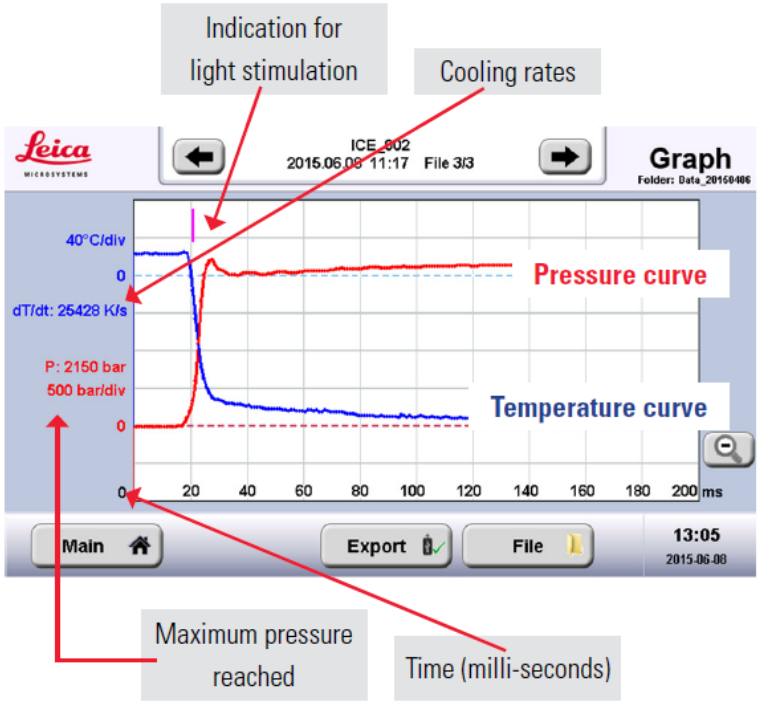



3. Put the carrier plate on the lower half cylinder. No carrier is required. Close the lid.

When the lid is closed, a loud noise will occur.



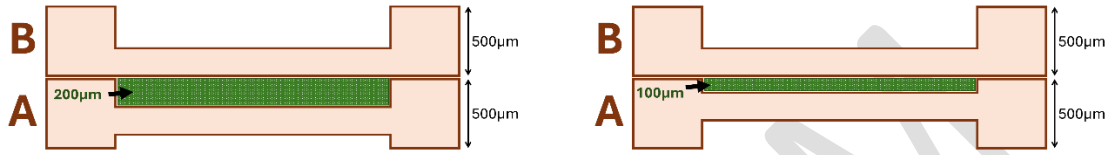
4. Check the following parameters:



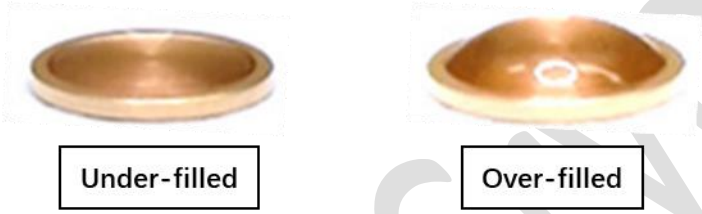
   a. dT/dt: the cooling rate must be larger than 16000 K/s
   b. The interaction between ***Temperature curve*** and ***Pressure curve*** (i.e. the cross of blue and red lines) must be within 30ms.
   c. The ***Pressure (P)*** should be around 2100 bar. Higher or lower value may still imply system problems. If the pressure is too low, try blank run again (2-3 times) and report to our staff.
5. Inform our staff when there is any problem during black run.

Sample Freezing

1. Put the new carriers in 100% Ethanol for washing.
2. Blot and dry the carriers on filter paper.
3. Load sample cartridge (2 half cylinders and carrier plate) at the loading station.
4. Choose suitable carriers or cover ring based on your sample thickness. Please note that ICE cannot ideally freeze samples with thickness over 200µm.



5. Put the sample in the middle of the carrier.
6. Fill the sample with hexadecane or sucrose. Do not under filled or overfilled. Use the microscope to check the liquid level.

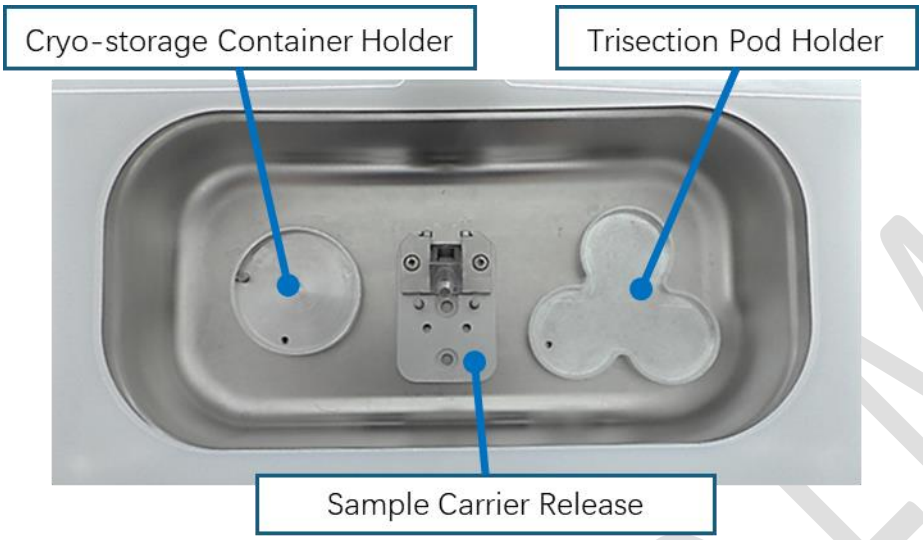


Under-filled: The air at the empty space will be expanded during the freezing process, which may damage the sample.
Overfilled: The liquid will be squeezed when pressure is applied to the sample, increasing the risk of blocking the pressure gun. In this case, the pressure cannot be settled in the system.

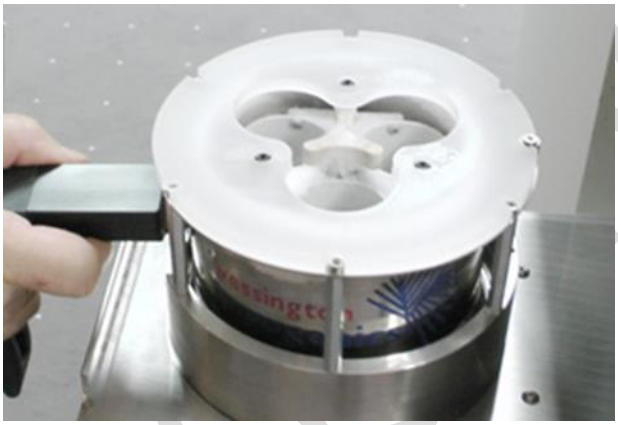
7. Put the carrier on the sample. Use filter paper to blot excess liquid.
8. Close the lid to start the freezing process.

Frozen Sample Transfer

1. Prepare the cryo-box and allow it to cool down.

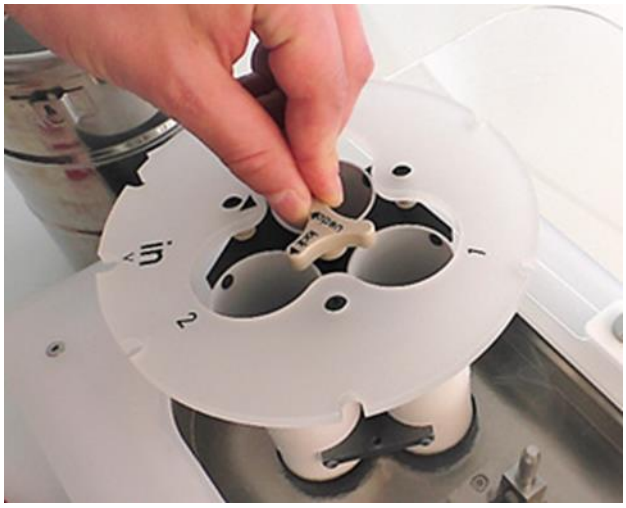


2. Remove the sample storage dewar from the slider.

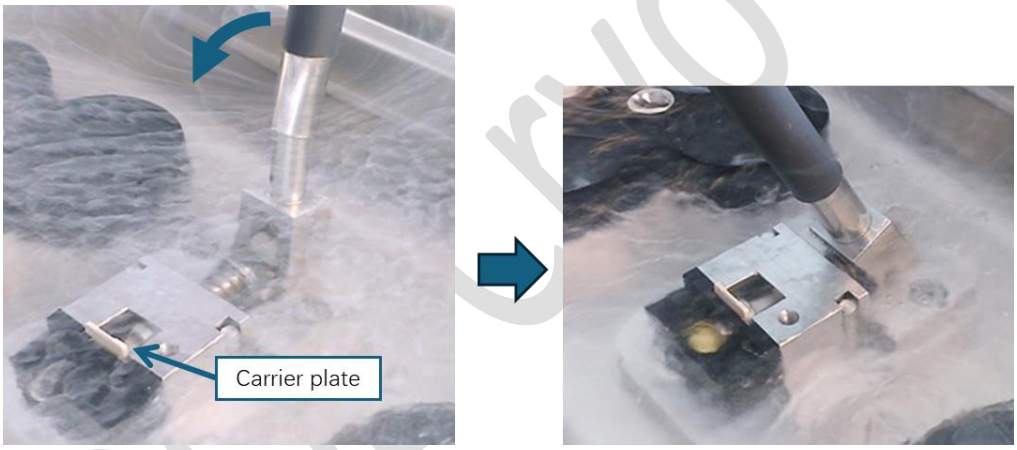


3. Transfer the segmenting insert to the trisection pod holder.

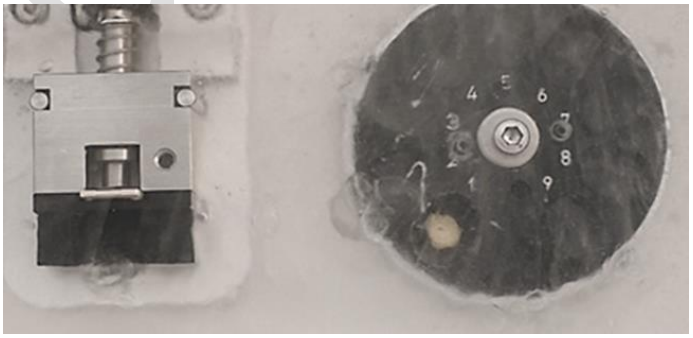
4. Unlock the bayonet locking mechanism by turning the handle to the “open” position. Remove the segmenting and leave the trisection pod at the holder.



5. If the sample is attached to the carrier plate, carefully move the lever forward and push the carrier sandwich out of the middle plate.

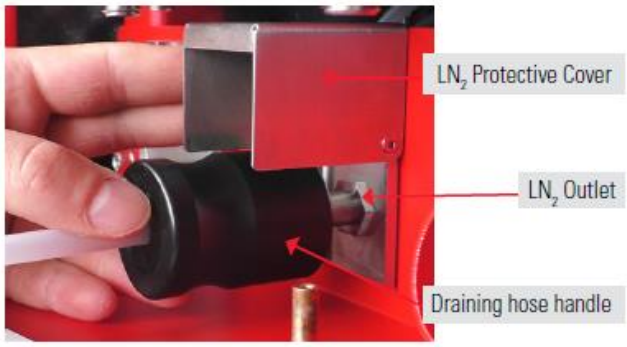


6. Push the sandwich carrier from the carrier plate into a small enclosure at the front part of the release insert. Once released from the middle plate, the carriers can be transferred into the cryo-storage container under a designated number.
7. The specimen is now ready to be transferred to a long-term storage Dewar or further processed according to the desired application.



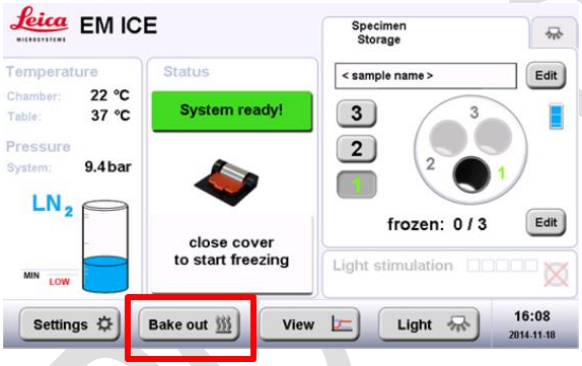
Bake Out and Shut Down

1. Drain the stored LN2 into LN2 tank (located in FIB room).



2. To initiate the LN2 draining, press ***Bake-Out*** on the main page and then ***Start***.

When the draining started, a loud noise will occur.



3. The device will begin pumping LN2 through the draining hose inside the empty Dewar. This procedure can last up to 30 minutes, depending on the starting LN2 level.

4. After draining, detach the draining hose only when the handle is warm. This can take an additional 20 minutes after the draining has ceased.
5. The Bake-out process will start automatically once the LN2 is drained. Put the Bake out sign at the ICE.
   Do not turn off the system or mains supply off during Bake-out.

6. When the process is completed, the EM ICE will automatically return to the start screen. Turn off ICE by pressing the main switch.
7. Sign the logbook.

Checklist before leaving

- Bake-out system.
- Put all the tools in the oven / on the hotplate to dry.
- Tidy and clean the bench area.
- Sign the logbook.
- Remember to take all your personal belongings with you when you leave.