High-pressure freezing of cell cultures grown on sapphire disks

This tutorial will guide you through the process of cryo-fixation of adherent cells grown on 6 mm sapphire disks.

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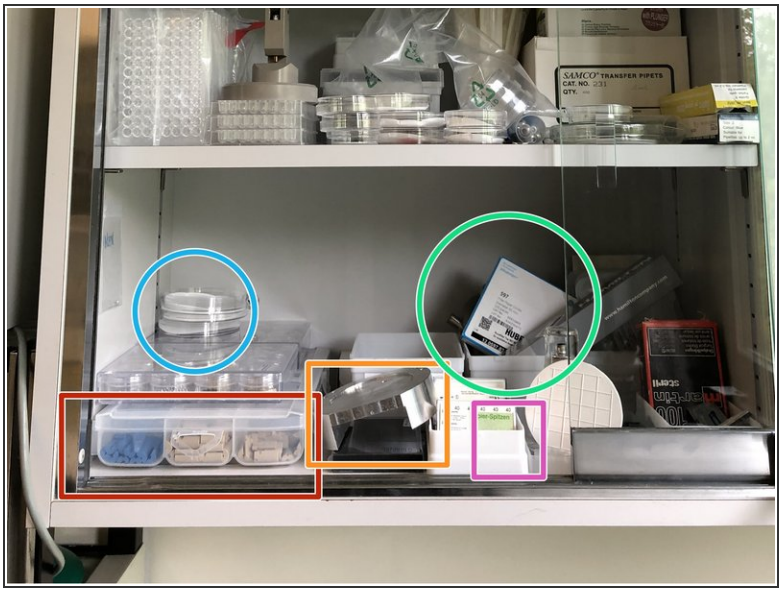
INTRODUCTION

This tutorial will guide you through the process of cryo-fixation of adherent cells grown on 6 mm sapphire disks at the Center for Microscopy and Image Analysis, UZH, Zurich.

Step 1 — High-pressure freezing of cell cultures grown on sapphire disks



Prepare your tools and parts for high-pressure freezing. In the cupboard above the machine you will find:

- the three-piece cartridge consiting of an upper cylinder, special middle plate 6 mm and lower cylinder
- spacer rings 6 mm (200 µm)
- carriers 0.1/0.2 mm
- filter paper
- mini-rack for middle plates
- metal block
- petri dishes

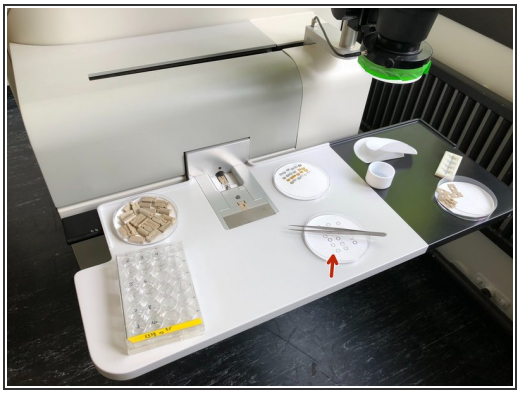
Step 2

In the cupboard on the main floor you will find:

- 2x HPF-tweezers
- 1x orange tweezer
- LN2 sample storage box
- box with 1-hexadecene Eppendorf tubes
- screwdriver for LN2 sample storage box
- EtOH glas beaker
- heating plate

Step 3

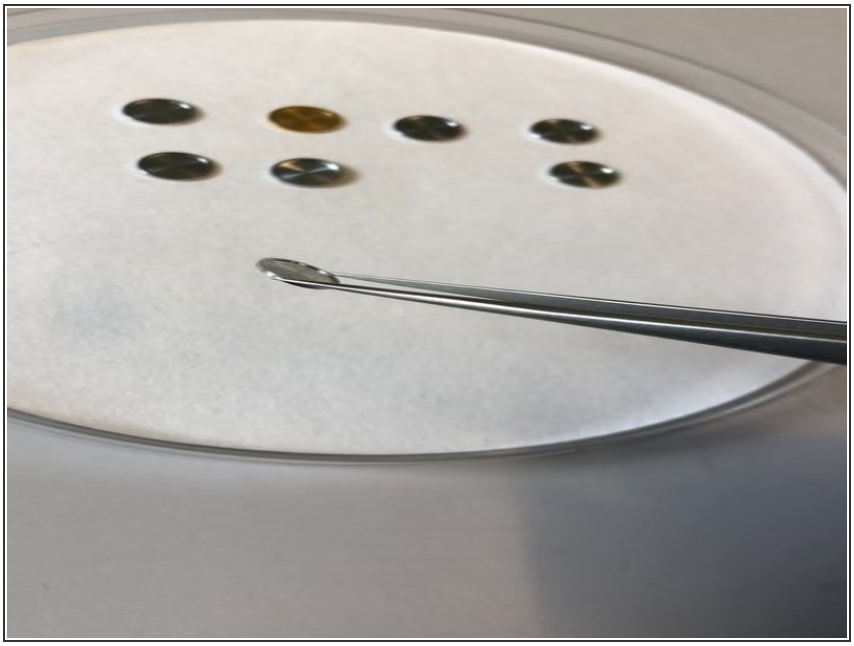




ⓘ Prepare all tools and accesories required for your cryofixation experiment.

- Check if the 200 µm spacer rings are completely flat otherwise replace them.

Step 4



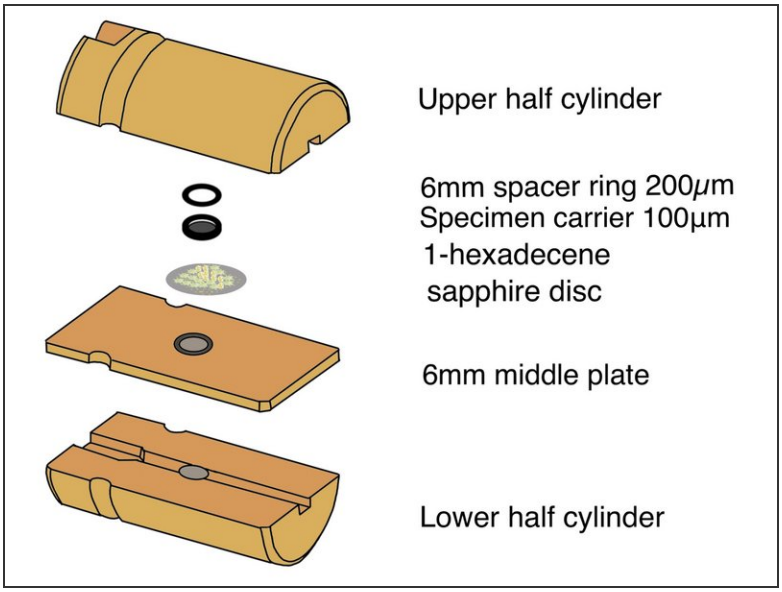
- Organize the required 100/200 cavities on a filter paper in a petri dish.

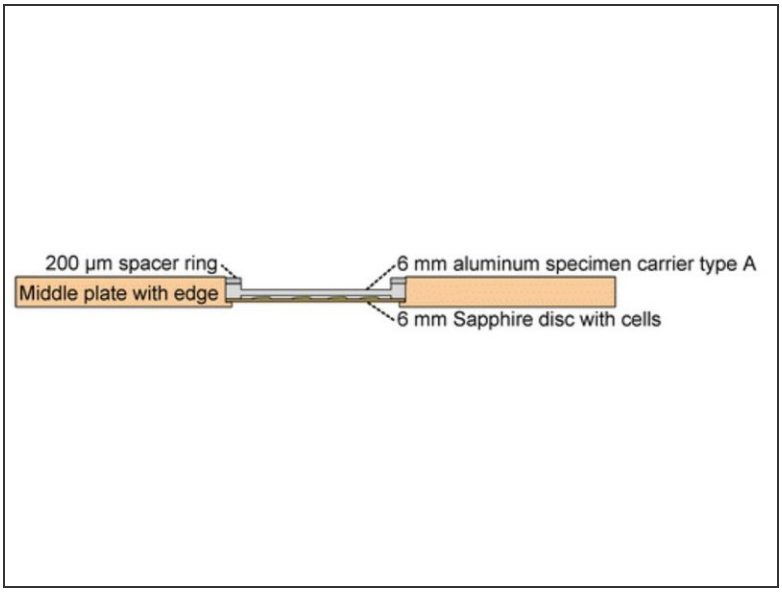
⚠ For 6 mm sapphire disks the 100 µm cavity must face the cells on the sapphire disk.

Step 5

- Insert the two-piece cartridge consisting of an upper cylinder and lower cylinder in the loading mechanism.

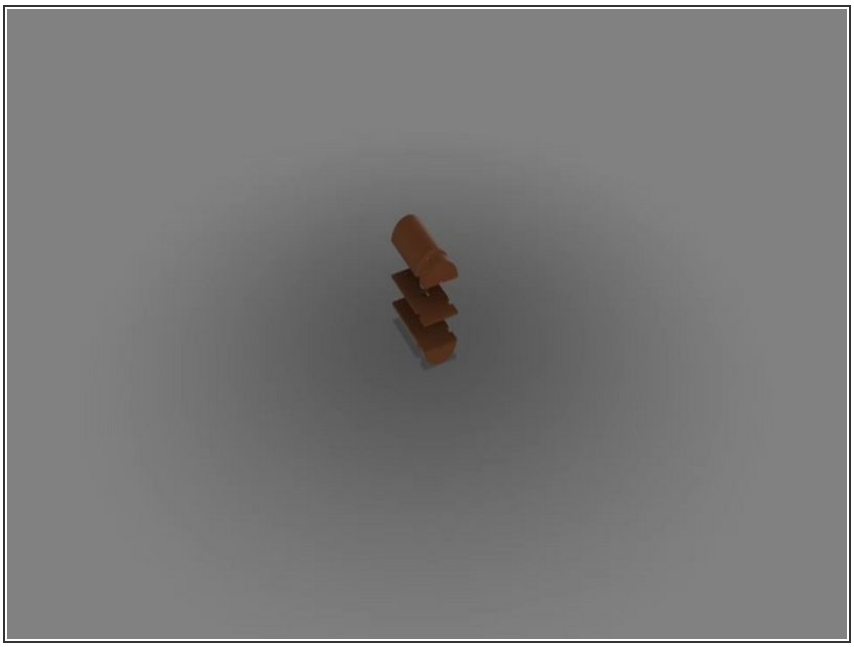
Step 6





- Overview of sandwich configuration

Step 7



- Visualization of sandwich configuration

Step 8

- Hold the sapphire at 45°.
- Align the edge within the recess of the middle plate.
- Release it.

Step 9

- Take a aluminium specimen type A (100/200 µm cavity) carrier with 100 µm cavity down and only wet this side with 1-hexadecane.

Step 10

- Add the 200 µm spacer ring on top.
- Move the middle plate to the loading station.

Step 11

- Blot excess liquid away using filter paper.

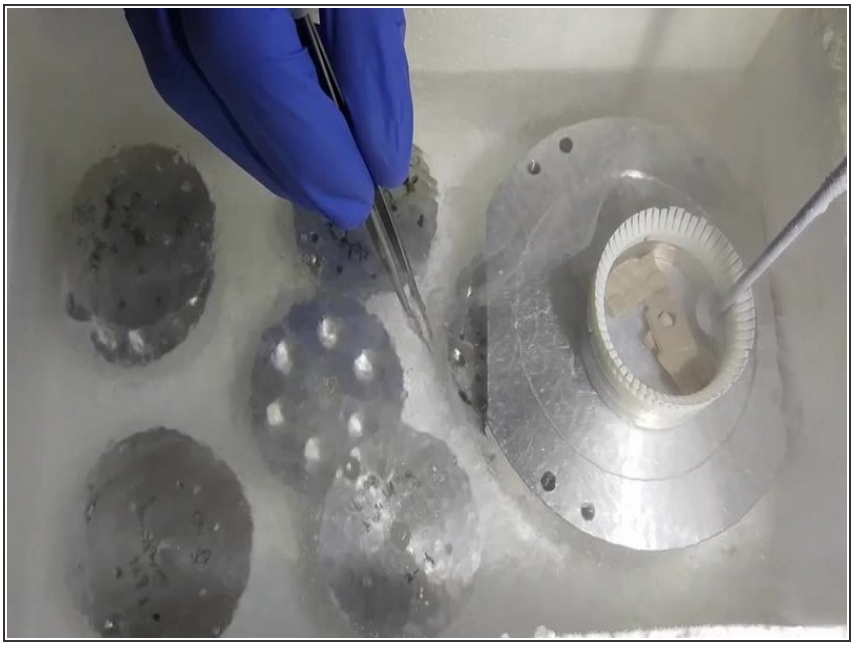
⚠ Important here is that you blot fast and put some pressure to prevent shifting of the 200 µm spacer ring.

- Flip the loading lever 180° until the process button is in position.
- Initiate freezing by pressing the button.

Step 12

- After cryofixation, the two-piece cartridge and the specimen carriers are automatically ejected into the specimen dewar and are ready for transfer.
- Open the sample dewar drawer and transfer the sample dewar to LN2 box.

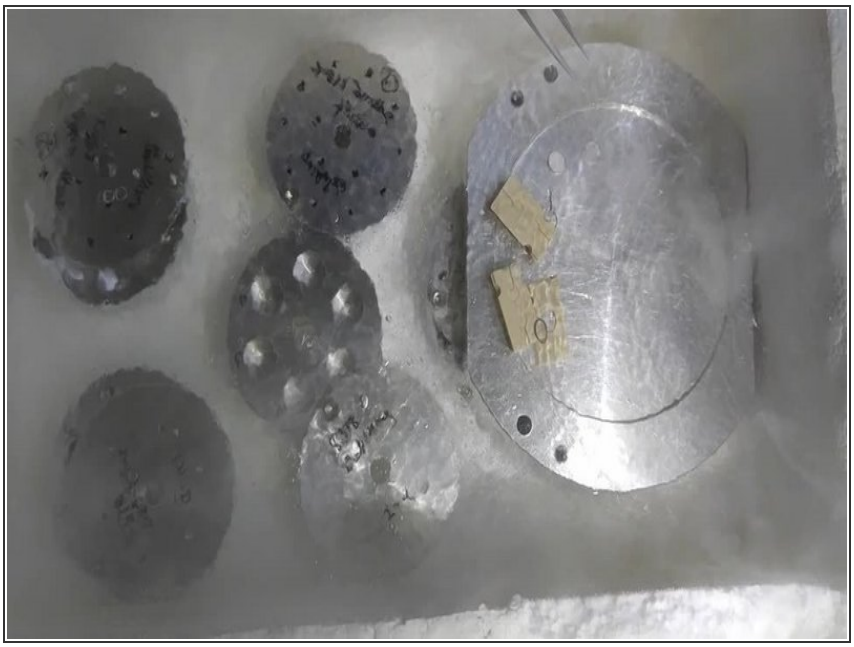
Step 13



⚠ Always pre-cool the tweezers prior to use.

- Transfer all components including your sample to the LN2 box.

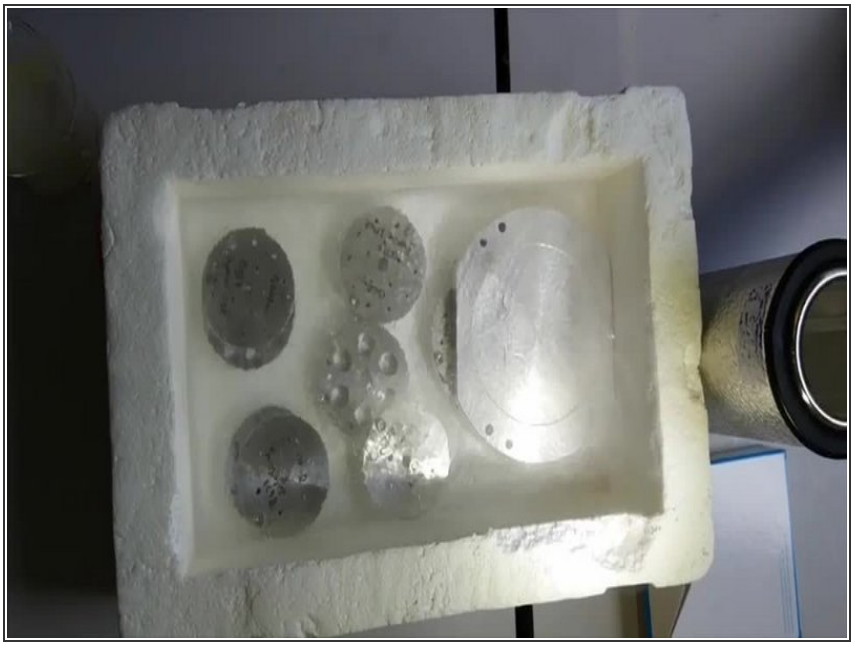
Step 14



⚠ Always pre-cool the tweezers prior to use.

- First, transfer the sapphire disk to the LN2 sample storage box.
- Then transfer the specimen carriers to a beaker filled with 70% EtOH for cleaning.

Step 15



- Place the sample dewar back into the high-pressure freezer drawer.

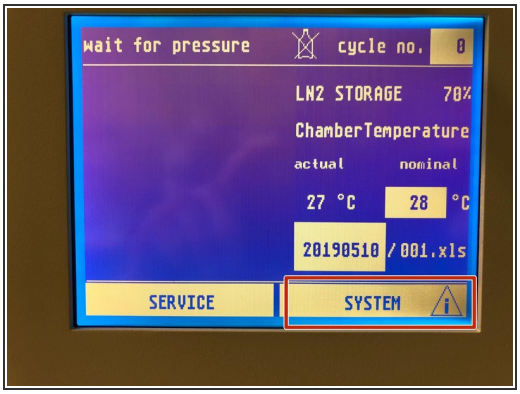
Step 16

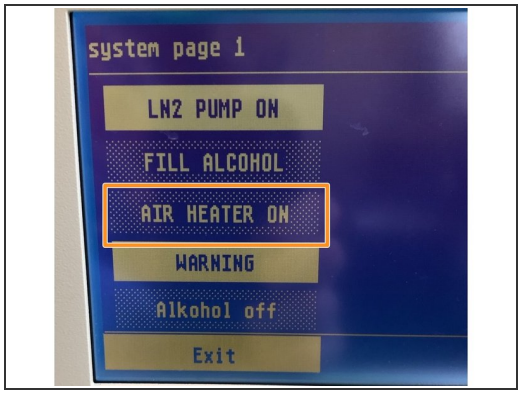
- Place specimen carriers to a hot plate for drying.

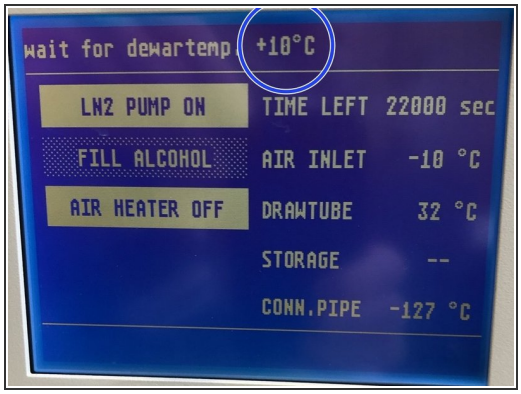
Step 17

- Once all freezing is complete the two valves at the back can be opened. Allow LN2 to drain.

Step 18







- Once the storage dewar has reached a level of below 20% select SYSTEM form the main screen.
- Select AIR HEATER ON.
- This activates the heating cycle. The 80°C heating cycle will only start once the chamber temperature warms up to 10°C.
- The bake out will automatically terminate at the end of the cycle leaving the instrument ready for the next user.

NOTE: Leave the main power to allow the heating cycle to run.

- Once the air heating cycle is started, the compressor turns on "air heater working".
- Once the cycle is complete the machine switches back to the standard screen and you can turn it off using the switch at the front.