Nanosoft Clipping Station Standard Operation Protocol

After the vitrification of samples, clipping of EM grids is an essential step to load the samples into Cryo-TEM. The clipped EM grid is called AutoGrid and you can observe a ring at the circumference of the EM grid. This ring is a combination of C-clip and C-clip ring (O-ring) which is well fitted to insert in a cassette.

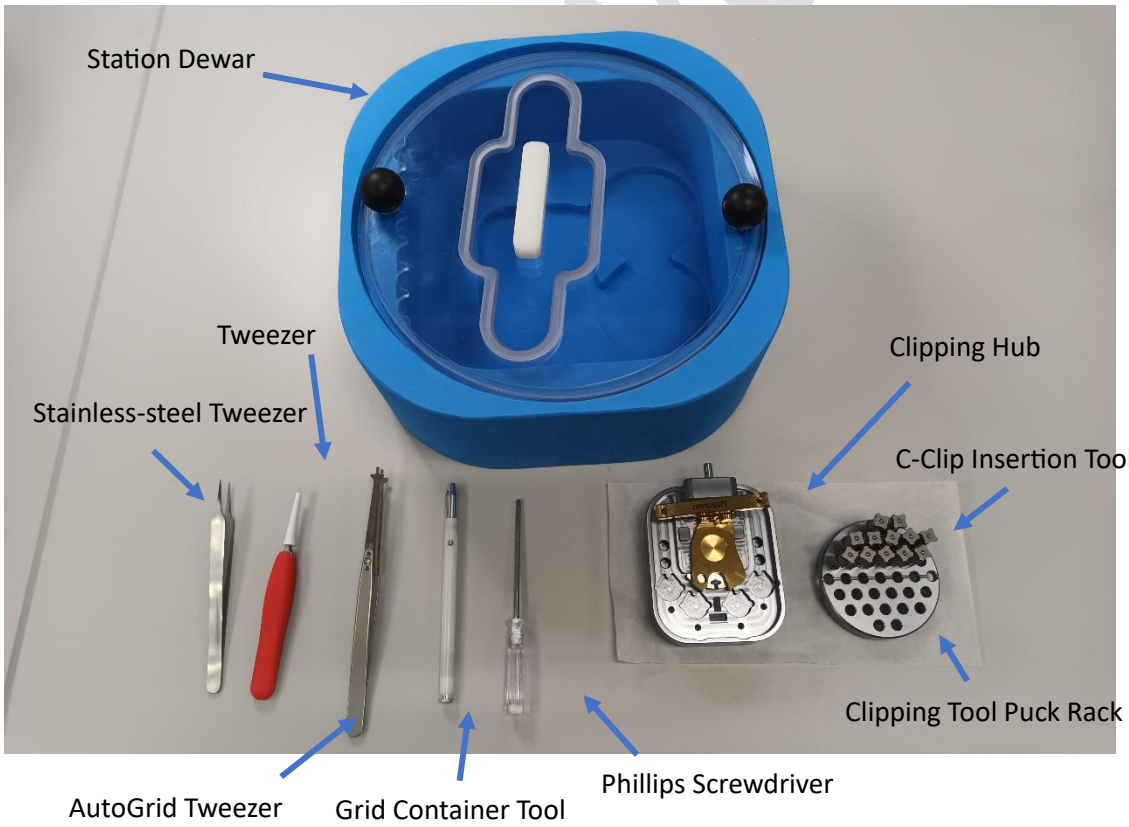
1. Preparation of Clipping Station
2. Clipping Procedures
3. Grid Storage and Clean-up

PPE (BSL-1)

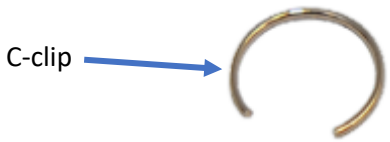
1. Laboratory Coat
2. Nitrile Gloves
3. Goggles / Safety Glasses
4. Cryogenic Gloves
5. Face Mask

1. Preparation of Clipping Station

- Get everything ready.



- Put C-clip into C-clip insertion tool using the stainless-steel tweezer and make sure the C-clip is in flat position. Place the tool on a flat hard surface and press down the trigger on the top of the tool. Check that the C-clip aligns around the rim of the tool.



Note: Remember to wear nitrile gloves or tweezers when handling the C-clip insertion tool.

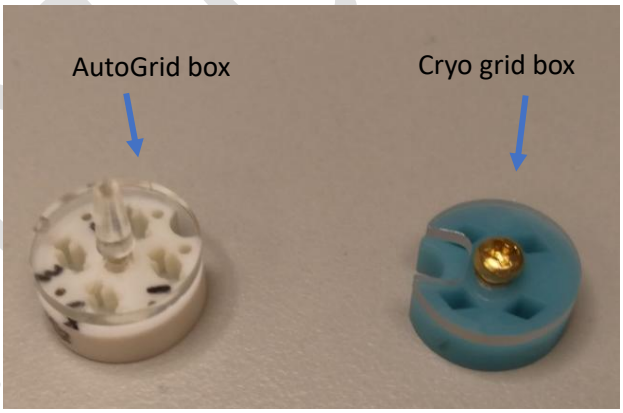
- Remove the lid on the station dewar. Put the clipping hub, C-clip insertion tool and clipping tool puck rack in the station dewar. Cool the clipping station by filling liquid nitrogen until it reaches the top of outside ring of clipping hub.

Note: Remember to wear nitrile gloves when handling the clipping hub and rack.

- Add a few drops of liquid nitrogen inside the clipping hub to observe the Leidenfrost effect to make sure the clipping hub reaches almost liquid nitrogen temperature. If the liquid nitrogen bubble is “dancing” around, it shows the temperature of clipping hub is not ready.

Optional: According to ThermoFisher’s video, no liquid nitrogen should be added inside the clipping hub after reaching the optimum temperature. All clipping procedures should be done in nitrogen vapor.

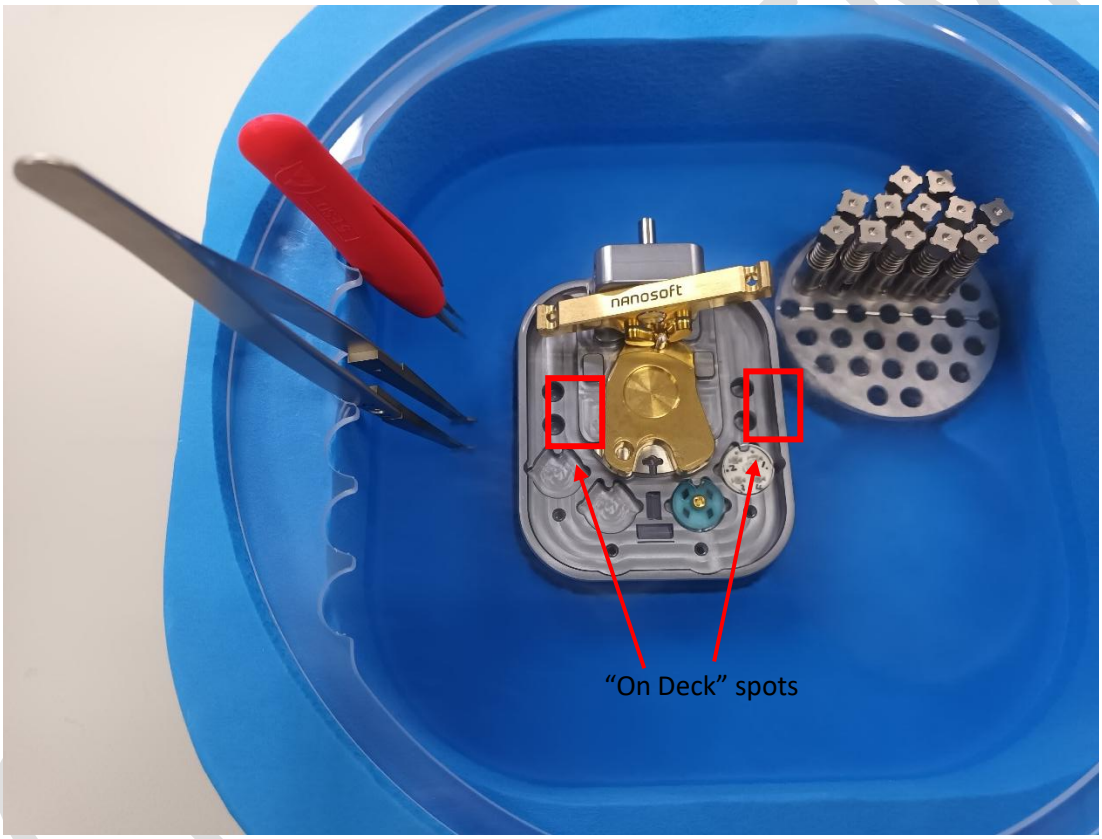
- Place AutoGrid box and transfer Cryo grid box with EM grids to the station. Cool them in liquid nitrogen before placing them in the slots of the clipping hub. Cool the Phillips screwdriver and unscrew the Cryo grid box. Use the grid container tool to unscrew the AutoGrid box.



2. *Clipping Procedures*

- Put the C-clip ring into liquid nitrogen to cool down it and then place it on the clipping hub.

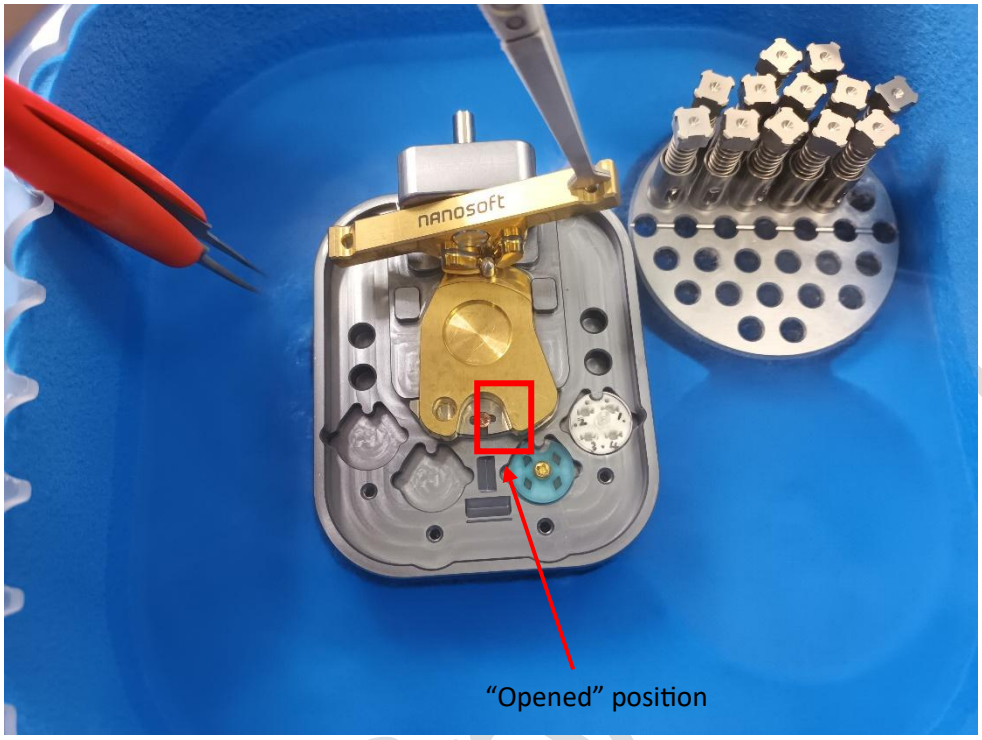
- Cool the tweezer and AutoGrid tweezer by placing them around the station dewar.



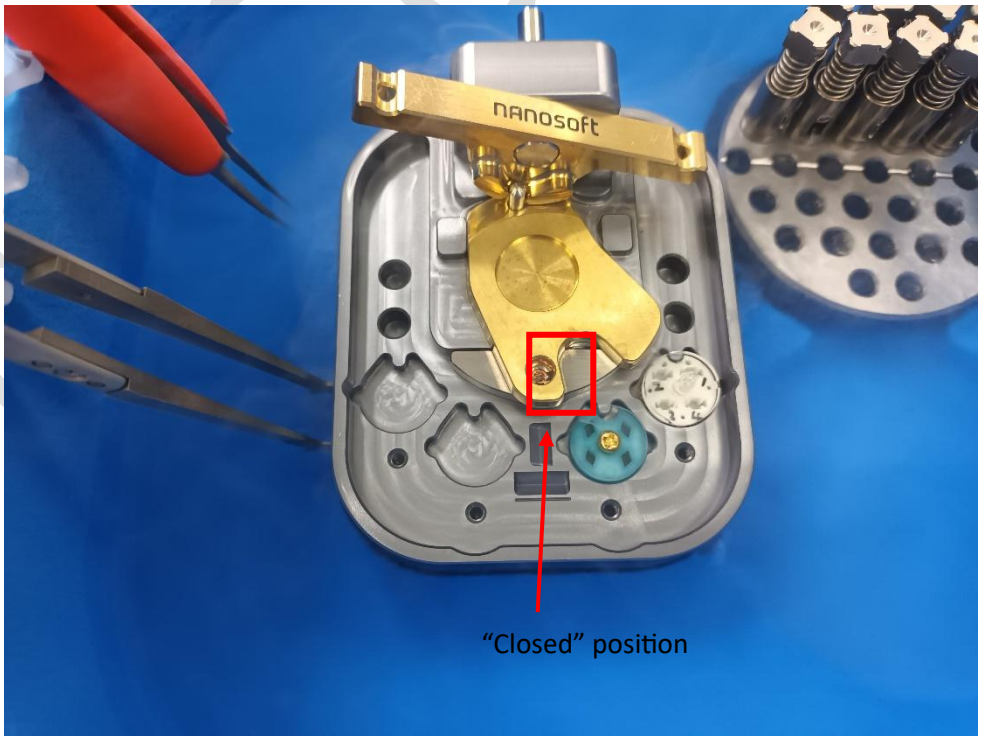
Optional: If the ice film of the EM grids is very thin, it can break easily. Therefore, it is advised to cool C-clip insertion tool in nitrogen vapor as there can be some pressure building up inside the tool which blows out most of the ice film in the tool. Especially if you have cooled it down in liquid nitrogen, there can be some liquid inside of that tool which might increase the pressure.

- The C-clip insertion tool can be placed to "on deck" spots so that the liquid nitrogen can drip out.

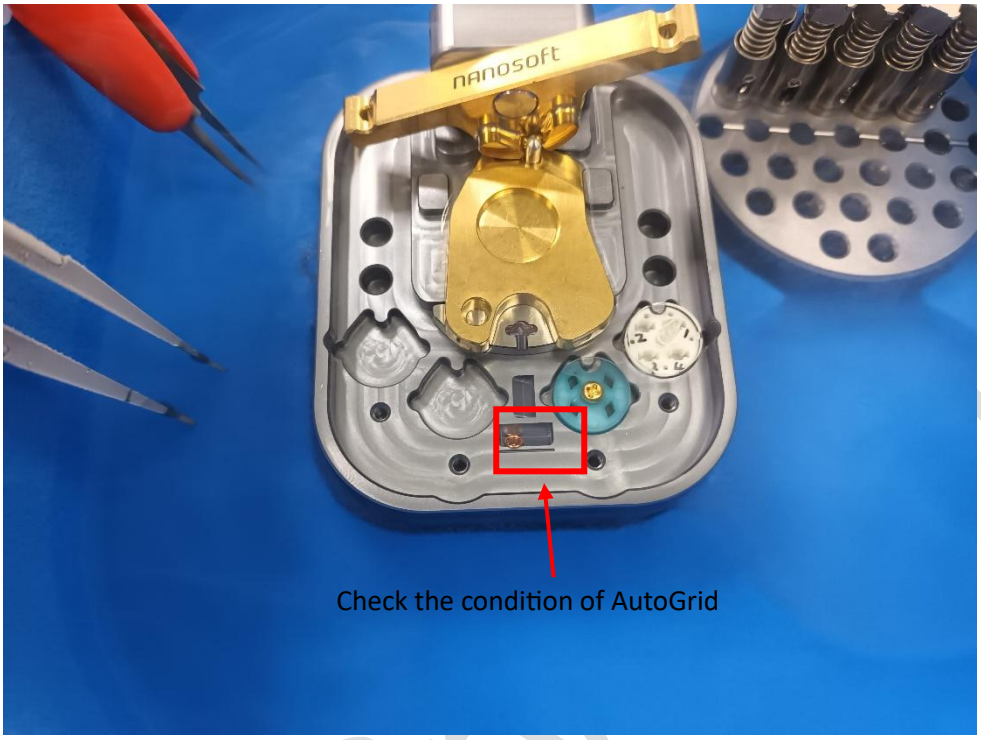
- Press the clipping hub to “Opened” position using AutoGrid tweezer and place the C-clip ring with bottom side sitting on the station. Transfer EM grid from Cryo grid box using a tweezer to the C-clip ring and the carbon side is preferred to face downward.



- Press the clipping hub to “Closed” position. Insert the C-clip insertion tool to the hole and press down the trigger on the top of the tool. The EM grid should be clipped and become AutoGrid.



- Press the clipping hub to “Opened” position. Place the AutoGrid on the clipping hub using the AutoGrid tweezer and flip it a few times. If it is not well clipped, the EM grid and C-clip will fall out. Put the AutoGrid to AutoGrid box for storage.

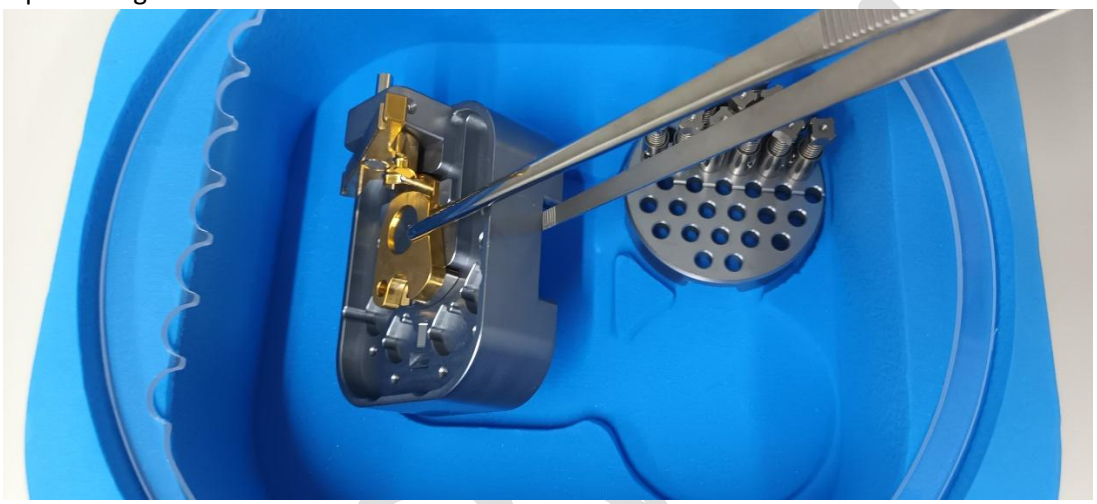


- Repeat the procedures above to clip all the grids.

3. *Grid Storage and Clean-up*

- Cool the Phillips screwdriver and screw the Cryo grid box. Use the grid container tool to screw the AutoGrid box. Put the AutoGrid box and Cryo grid box back to grid storage if applicable.

- When you have finished, use a large tweezer to remove the clipping hub and clipping tool puck rack to hot plate. Remove all the lids on the station dewar and pour the remaining liquid nitrogen.



- Place the station dewar and the lids on the table opposite to Vitrobot.

- Perform a general cleanup of the workspace with 70% ethanol and leave the room in the state which you would wish to encounter it.

- Sign on the logbook.

References

1. ThermoFisher Aquilos 2 Cryo-FIB Accessory and consumables overview Flyer.
2. Nanosoft https://www.nanosoftmaterials.com/product-page/clipping-station
3. Cryo-EM University: Sample Preparation https://www.thermofisher.com/hk/en/home/electron-microscopy/life-sciences/learning-center/cryo-em-university/sample-preparation.html

Checklist before leaving the lab:

• Place the tweezers back in the hotplate to warm and dry them.

• Place the clipping station on the table opposite to Vitrobot to ensure proper ventilation and dry.

• Tidy and clean the bench area.

• Sign the logbook.

• Remember to take all of your personal belongings with you when you leave.