

Clipping Station Protocol

CEMC

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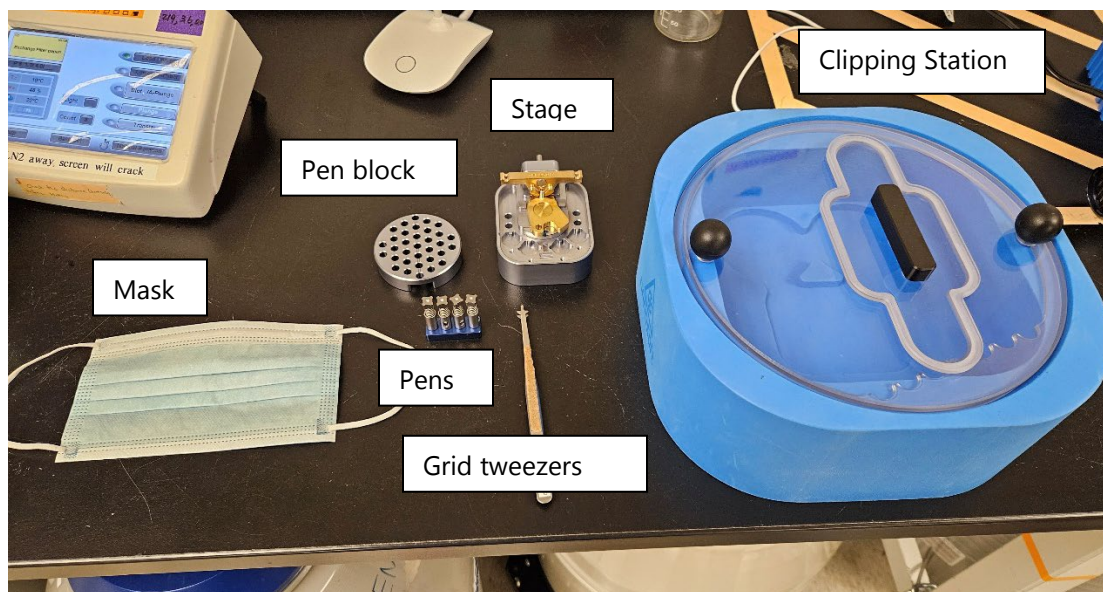
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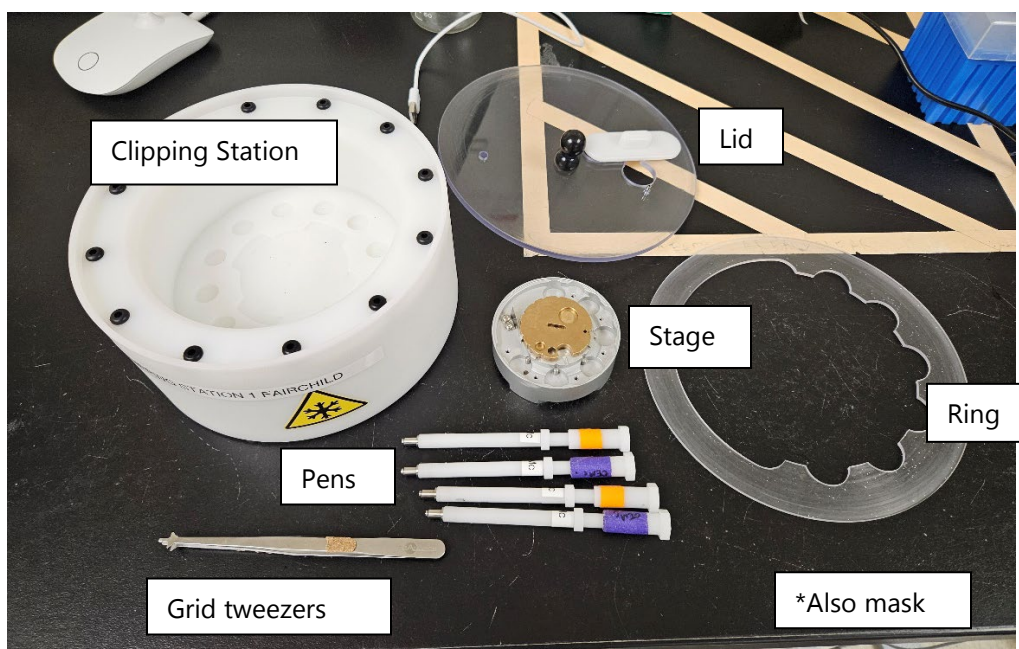
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Materials

Nanosoft Clipping Station



Thermo Clipping Station



General precautions

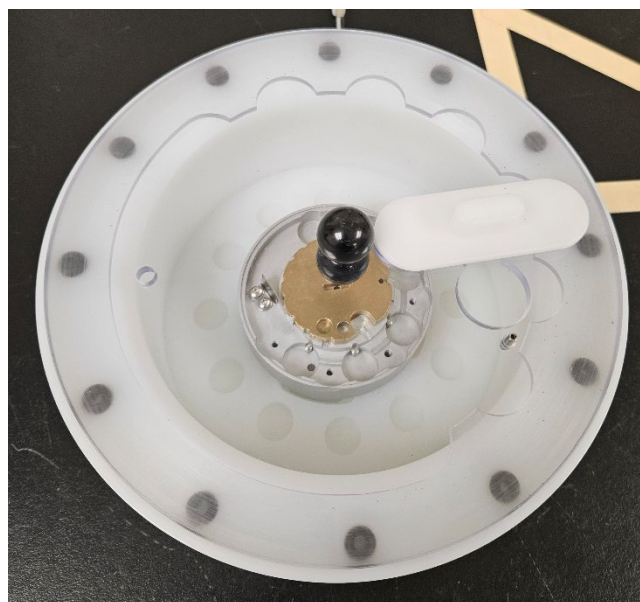
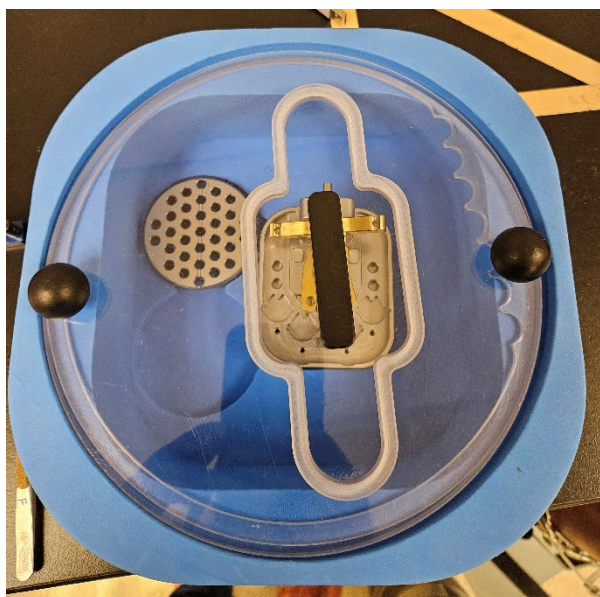
1. Make sure all equipment is completely dry before use otherwise large ice contamination will form.
2. Liquid nitrogen (LN2) is a cryogen. If left prolonged in contact with your body, it will cause cold injury to the exposed area. Long pants and close-toed shoes are necessary when using LN2. In-person training is required before handling.

About Clipping Stations and Setup

Although clipping can be done immediately after plunge freezing, it is better to give at least a day in between plunge freezing and clipping to reduce ice contamination. The time it takes to do both back-to-back increases chances of water buildup on the equipment, tools, and the LN2.

Many of the subsequent steps require you to be very near to the equipment while clipping. Therefore, wearing a facemask is recommended to avoid your breath from contaminating the sample. Also, complete the steps as quickly but confidently as possible and keep things covered as much as possible.

1. Assemble the clipping stations as shown. Make sure the plastic inner ring is between the main body of the station and the lid, which helps the lid to slid around during use.
 - a. Nanosoft station: Place the stage in the square cutout and the pen block in the circular cutout.
 - b. Thermo station: Place the stage in the center of the station into the specially formed cutout.

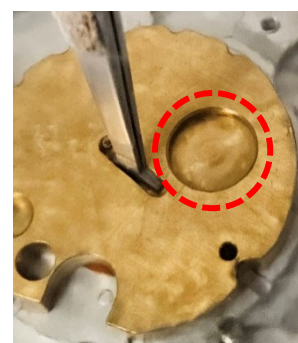


2. Fill the station with LN2 through the removeable port on the lid, covering up to the top of the brass parts. You will need to refill several times as the LN2 will evaporate. In the end, you will want there to be LN2 to cover thoroughly the silvery parts and then LN2 in the circular pits in the brass part (red circles shown right; you will be placing the rings here.) Replace the port door in between refills to avoid ice.

Nanosoft



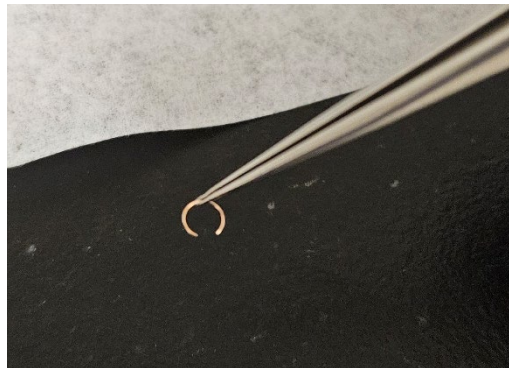
Thermo



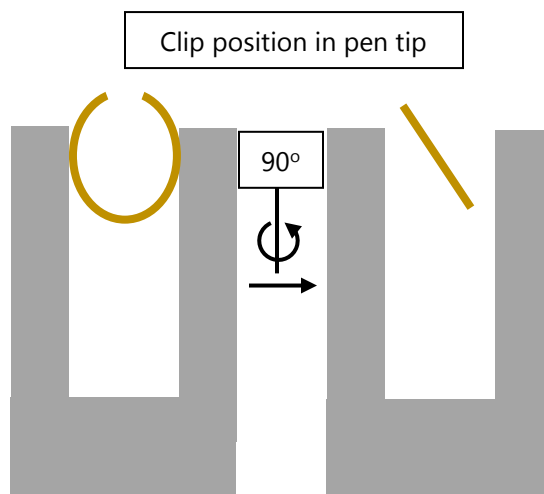
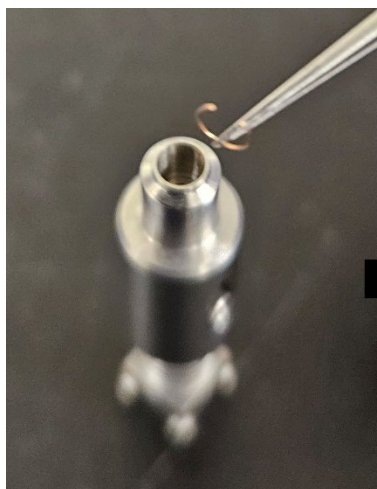
Filling the Clipping Pens

The following steps show the process for the Nanosoft pens, but the principles are the same for Nanosoft and Thermo

1. Place your C-clips on a Kimwipe. Grab the C-clip with sharp, corked tweezers by the part opposite to where the break in the clip is. Put back any C-clips you don't use.



2. Invert the pen so the tip is facing towards you. Press the C-clip into the hole of the pen tip, with the side opposite to the break going in first (the back of the "C"). Make sure the C-clip isn't sticking up but biased towards one side (cartoon below).

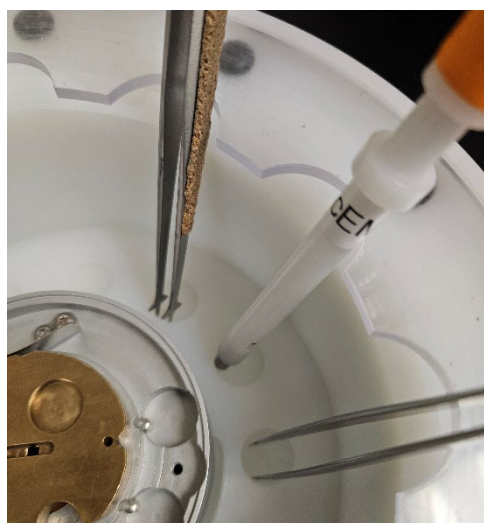
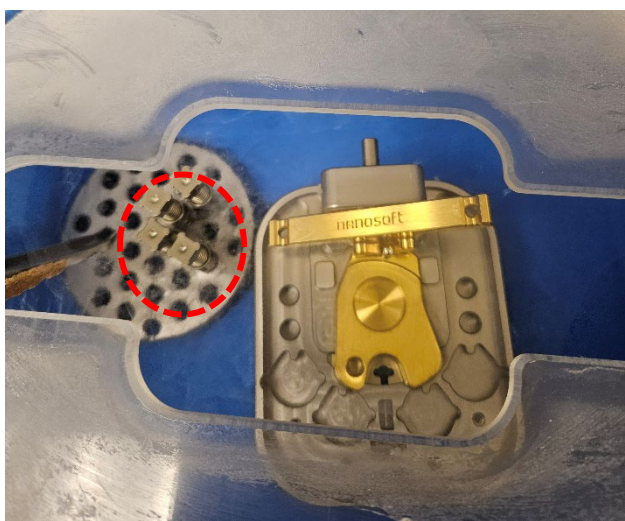


3. Hold the pen tip-down onto a clean surface (like the table), making sure the pen is perfectly perpendicular. Press down on the plunger twice. Inspect the C-clip; it should be flush on the very rim of the pen tip. Repeat as needed for each pen, one per grid.



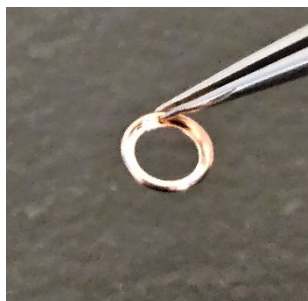
Clipping the Grids

1. Transfer the pens to the clipping station to cool them. The Nanosoft pens are designed to be picked up with the grid tweezers. Pick them up on the square sides as shown to the right. Place the Nanosoft pens (with the C-clip) into the pen block slots. The Thermo pens are picked up with your fingers. The Thermo station has circular slots towards the bottom that are designed to hold tools, plus some notches on the plastic ring to rest them into to keep them secure (Be careful—The ring and the tools have a tendency to slip and get knocked over). Both are shown below.



2. Rings have a flat bottom and a ridge that goes up. Pick up the rings by the edge and place them bottom-down into the circular pits in the brass part of the stage.

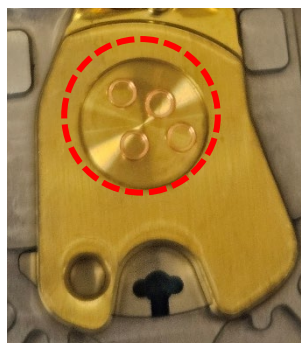
Clip side



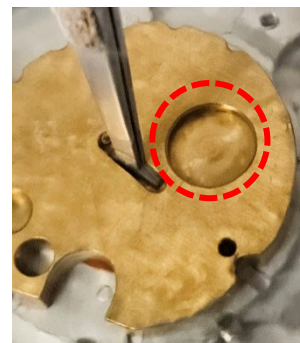
Bottom



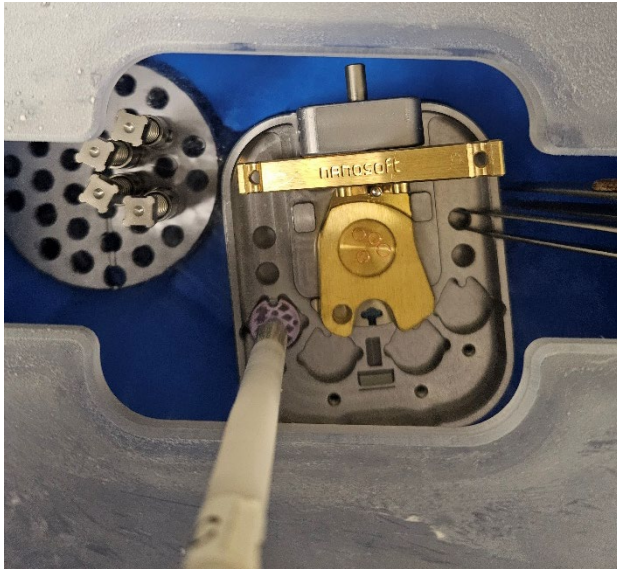
Nanosoft



Thermo

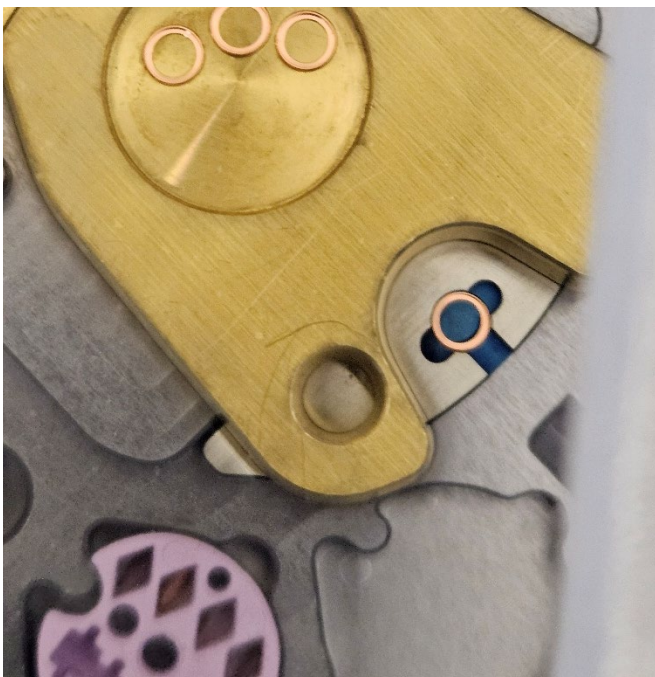


3. Place the grid box into the appropriate cutouts on the stage. Make sure that if you are using a box for unclipped grids (B01) that you have another box that accepts clipped grids (B02-04). Use the pen to unscrew the box. There is a hole by each of the box slots on the stage to screw the lids of the boxes into. The lid will overlap with the box body slightly and keep it secured. However, the grids can still be jostled out of the slot and "float away". Be mindful when the lid is not secured to the box itself.

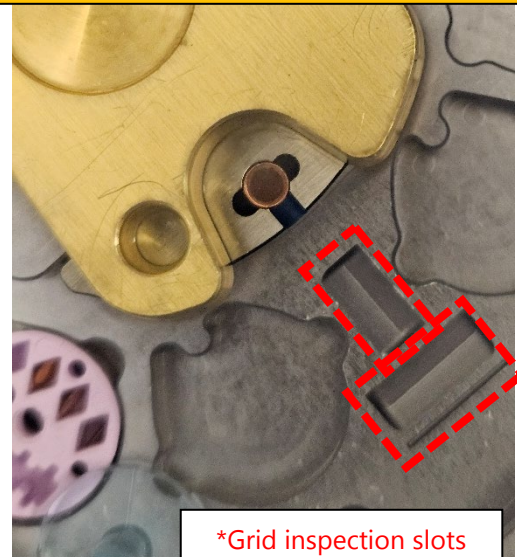


4. Take a ring and place it into the slot in front of the brass part of the stage.

5. Place your grid sample-side-down into the ring. There are little triangular slots you can use to inspect and flip the grid if needed. Visually confirm that it is well-placed.



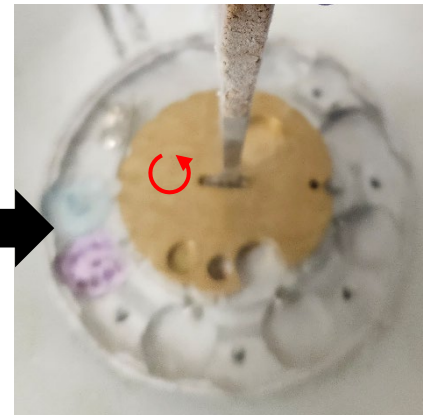
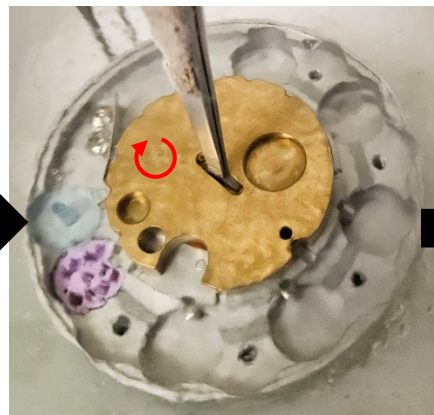
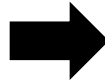
It is best to put your grids in sample-down to 1. Keep Z-height more consistent among grids and 2. Avoids damaging the sample during the clipping.



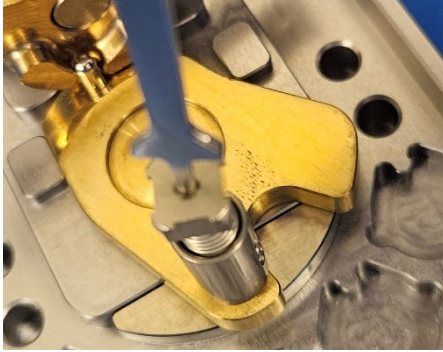
*Grid inspection slots

6. Nanosoft: Insert the grid tweezer into the ridge on the side of the seesaw and press down to place the part of the brass with the hole over the grid.

Thermo: Insert the grid tweezer into the center slot of the brass disc. Rotate counterclockwise to place the part with the hole over the grid.



7. Place a clipping pen into the hole over the grid. Press down on the plunger twice (using grid tweezer for Nanosoft, hand for Thermo) to inject the clip into the ring. Take out the pen and visually inspect that the clip is no longer in the pen and that it is in the ring. See-saw or rotate the brass part back to the starting position.



8. The grid tweezers have a completely flat tip and a tip with a little notch. The completely flat tip should contact the clip-side face of the clipped grid. The tip with the notch contacts the sample side. Using the tweezers, transfer the grid to the box, clip-side towards the front of the box (towards the notch in the box). This placement mimics how the grids are loaded into the cassette and therefore helps you keep track of the grid orientation. Seal the box and transfer it to storage once all your grids are clipped.

