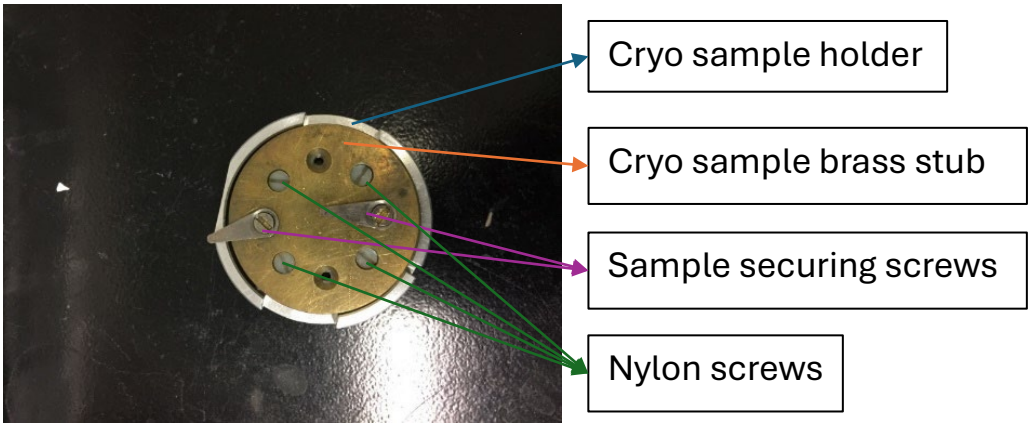
Cyro Stage Low Vacuum SEM Observation Procedure

1. **Cryo sample stage**:



The cryo sample stage has two parts: cryo sample stub and cryo sample holder. The cryo sample stub consists of a brass blank with two clips secured by screws to the upper surface, and four nylon screws screwed through the brass stub and protrude slightly from the base of the holder so that the brass stub does not contact the sample holder, to effect thermal isolation of the stub from the sample holder.

2. **Preparing the cryo sample stage**: Place the sample stage (without any sample) into a container filled with liquid nitrogen and leave it until the sample stage surface stops bubbling violently, indicating the stage is cooled to -196 °C, and ready to be loaded with samples.

3. **Preparing sample**:
   1) For bulk samples or large tissue samples, use tweezer holding the sample and dip the sample into liquid nitrogen to let sample quickly froze, then use one or both clips on the sample stage to secure the sample the stage surface by tightening the screws.
   2) For single cell sample (eg, algae), put a drop of sample on Nitrocellulose paper, then dip the paper with the sample on it into liquid nitrogen to let sample quickly frozen, then use one or both clips on the sample stage to secure Nitrocellulose paper on the stage surface by tightening the screws.

4. **Imaging**: (The observation of the sample is using Thermal Fisher Quattro S Field emission Scanning Electron Microscope)
   After loading the sample on the Cryo sample stage, quickly remove the stage from liquid nitrogen and insert it into the SEM sample chamber. Pump the SEM sample chamber down to 50-200Pa and observe the sample under Secondary electron mode. At first, the examination of the sample shows a featureless, smooth surface, which is the frozen water

in which the cells were embedded. After a minute or so, the ice sublimates, and the surface structures of the sample are revealed. This is a good time window to scan images. During scanning, the ice continues to sublimate from the interior of the sample. After a short time (about 15-20 minutes, depending on the sample), the sample surface structure collapses. Then, a new area of the sample (not collapsed yet) can move into the scanning region to continue image recording until all the sample areas are collapsed. Under Low-Vacuum SEM conditions, the sublimation of frozen hydrated samples needs fairly rapid examination and image recording.