BEGINNERS SAFETY MANUAL FOR JEOL JSM-6360LV SCANNING ELECTRON MICROSCOPE

Produced by Melvin

Last updated Jun 2025

Changelog

03 Jun 2025	Changelog added to this document. New Troubleshooting step “Vacuum pump does not start up when EVAC button is pressed” added to this document.

1. Safety Guidelines

Please adhere to the safety guidelines for your own safety and health. When in doubt, always approach the Bioimaging facility for assistance.

2. Training

Use of confocal microscopy and the online booking system is authorized by facility staff only. All users must attend the confocal training session conducted by the Bioimaging facility staff before they are allowed to access the confocal system.

2.1 To arrange a training session:

- Please email Bioimaging Facility (bioimaging@tll.org.sg) for a training session.
- Fill in the necessary particulars in the form here: http://microscopy.tll.org.sg/pages/conf_training_form.html (E.g. Lab, PI, sample, and a brief description of your project)
- We will help you identify the most appropriate microscope for your imaging needs.
- We will then arrange training session according to your availability within a week.
- Training sessions usually last 1 - 3 hours depending on users’ previous experience.
- Users receiving training are highly encouraged to bring their own samples so that staff can adjust trainings to your imaging needs, but if not possible, staff have their own samples that they can work with.

Once you have completed the training session, we will grant you access to both the confocal PC and the online booking system. Please keep in mind that only bioimaging facility members are allowed to conduct the training. None of your lab members are allowed to conduct the training for you.

If you need a refresher or some specific advice on anything microscopy related, please approach any member of the Bioimaging Facility for assistance/help.

We also offer training on the various types of image analysis and image processing software available here at TLL, including ImageJ/Fiji, Huygens and Imaris.

3. Online Booking and System Access

- Booking of all Light microscopes prior to use is **COMPULSORY** through microscopy resource booking via TLL intranet (*https://intranet.tll.org.sg/App/tll_intranet/booking_searches*)
- Users are only allowed to book the confocal system that they have received training on. If they wish to book other confocal system, they have to receive a separate training.
- Users are entitled to advance bookings of up to 2 weeks. They are advised to plan their experiments accordingly to avoid any disappointments.
- During Office Hours (*Weekdays from 8.30am to 6pm*)
  - Each user is entitled to a **MAXIMUM of 2 bookings** per system per week.
  - Users who are trained on multiple systems are entitled to a maximum of 3 bookings per week, but it has to be shared across the systems that they have received training on.
  - Each booking must not exceed 3 hours.
  - If users have utilized all their entitled bookings for the week, **"24Hr Rule"** can be applied where they can book the system in less than 24 hours in advance according to its availability. If extra slots are booked within the 24-hour period, a note of **"24Hr Rule"** should be made in the booking description.
- During Non - Office Hours (*Weekdays after 6pm, Sat, Sun & Public holidays*)
  - If users require more slots in a particular week, they can book on weekdays during non-peak hours (*after 6pm*), on weekends (*Sat and Sun*) and on public holidays.
  - If extra time is required, bookings can be extended out of peak hours *(E.g. 3 – 7pm).*
- Bookings exceeding these limits are subjected to cancellation without prior warning.
- Simultaneous bookings of two or more different systems are not allowed. Multiple bookings for the same system on the same day during office hours is strictly prohibited.
- If users cannot attend a booked session for any reason, it is **COMPULSORY** that they cancel their booking through TLL intranet and email (*confocal@tll.org.sg*) to announce the availability. If they are the last users for the day, they need to check if the system has been switched off completely.
- If users are swapping a session with another user, they must change the booking details accordingly.
- Under any circumstances, users are not allowed to make bookings on behalf of other people. Users who have received training but are yet to gain access to microscopy resource booking may approach TLL Bioimaging department for booking assistance if they need to use it urgently.

- If a user fails to show up within the first 30 minutes of their booking, the slot is forfeited and is free for any user to use it.
- If any users violate any of these Booking rules, users will be subjected to the 3 strikes policy.

1st offence	A warning will be issued along with the reminder of the rules.
2nd offence	A second warning will be issued and your respective PI will be notified.
3rd offence	Banned from using any of the facility's microscope for 2 weeks.

3.1 Acknowledgements

If you use the TLL Microscopy and Imaging facility and/or have been trained or assisted any of the bioimaging facility members in your research, then this should be acknowledged appropriately in your publications and presentations.

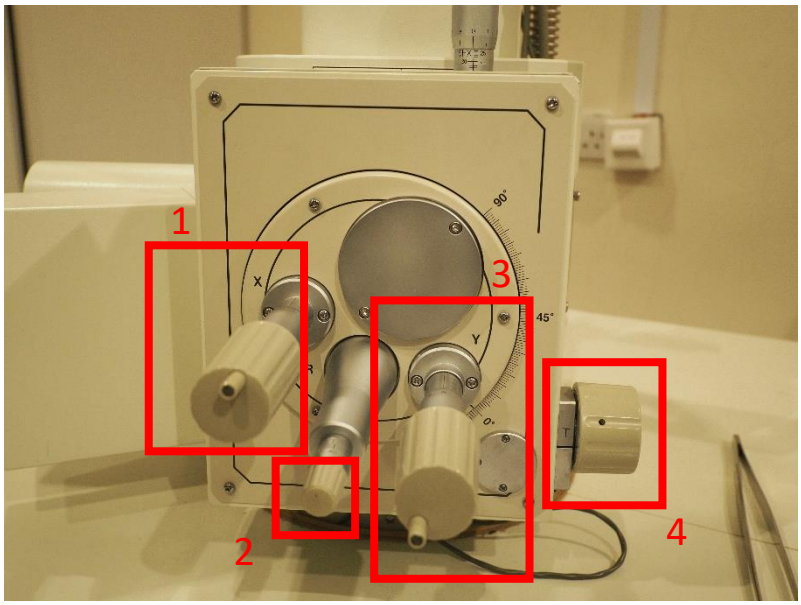
4. Operation Procedures

Every microscopy system in TLL Bioimaging Facility has its specific instructional manual which are found in every microscopy room. Strictly adhere to the correct order of operation for all system. Failure to do so will result in disciplinary action from the facility. Any issues encountered during the operation of the system are advised to seek help from the Bioimaging facility.

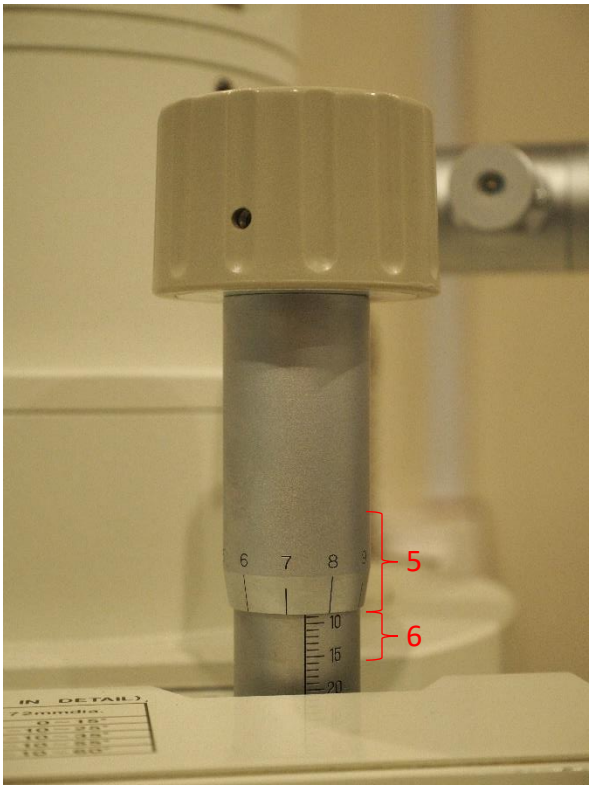
Modification, exchange or removal of components beyond this operational manual is strictly prohibited and is only carried out by the manufacturer, Bioimaging facility or by experienced users approved by bioimaging facility.

If the SEM is not on when you plan to use it, ***do not attempt to switch it on yourself****. Inform Bioimaging facility and let them switch the SEM on for you.* ***This includes starting up the JEOL Scanning Electron Microscope software****.*

4.1 Stage controls



1. **Stage X controller:** Moves the stage left/right
2. **Stage rotation:** Rotates the sample on the XY plane.
3. **Stage Y controller:** Moves the stage forwards/backwards
4. **Stage tilt:** Tilts the stage. **Keep the tilt at 0°. Do not use or it will cause damage to the detector.**



5. **Stage height:** Indicates decimal position of the stage height in increments of 0.1 mm.
6. **Stage height:** Indicates stage height in increments of 1.0 mm.

To read stage height, look at the marking at **(6)** to get the whole number value of the stage height, then look at the marking at **(5)** to get the first decimal place of the stage height. In the picture given above, this screw gauge is showing a stage height of around 9.75mm.

4.2 Loading sample into microscope

1. Drop the stage to more than 25.0mm using the stage screw knob. If your sample protrudes above the level of the standard sample holder, drop the stage lower.
2. Press, hold and release the VENT button on the microscope. A sound can be heard, and the VENT button light starts blinking.
3. Check that tilt is set to 0°.
4. Pull the stage tray out by holding the face on both sides. It is held in purely by vacuum pressure and weak magnetism.
5. Load sample into the holder by clipping the sample holder into the disk in the stage. Align the flat side of the sample holder to the line on the stage, and face the rounded end of the sample holder away from the line on the stage.
6. Look into the chamber to see that the internal elements are in good condition, then push the stage in carefully, ensuring that your sample does not impact the objective (shown in picture below).

7. Once the stage is pushed into position, keep your hand pushed on the stage, then press and hold the EVAC button until the light starts blinking, then wait for the light to stop blinking (around 1½ minutes).

※ *Keep sample chamber under vacuum when not in use.*

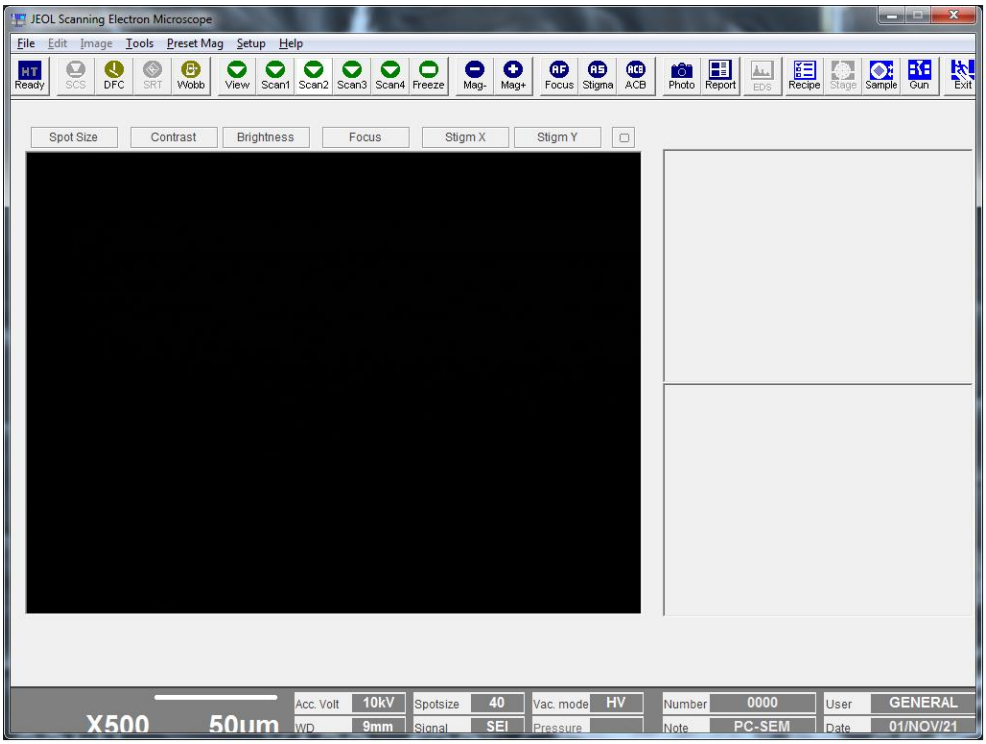
4.3 Loading liquid nitrogen frozen samples

Time is of the essence when preparing samples using liquid nitrogen for viewing inside the SEM. The sample needs to be transferred from the liquid nitrogen straight to the sample chamber in as little time as possible to minimise formation of ice crystals that can affect imaging experience.

1. Lower the stage to more than 30.0mm using the stage screw knob.
2. Wear the cryo glove on your left hand.
3. Mount the sample on the sample holder.
4. Vent the sample chamber and pull out the sample holder.
5. Clear any obstacles that may come between the liquid nitrogen tank and the sample holder.
6. Place the sample and the sample holder onto the provided ladle and lower them into the liquid nitrogen while holding the ladle with your right hand. The sample and sample holder should be fully submerged into the liquid nitrogen.
7. The liquid nitrogen will bubble violently. Wait for the bubbling to die down.
8. Once the bubbling has died down, quickly and carefully lift the ladle, use your gloved left hand to move the sample holder and sample to the stage, then carefully push the stage in.
9. Press the EVAC button to start evacuating the sample chamber.

4.4 Sample viewing

Once you have loaded the sample into the microscope, move to the computer terminal and you will see this menu.

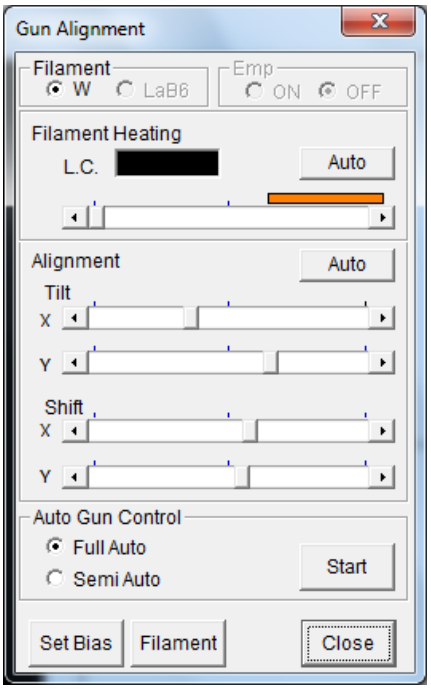


Top toolbar settings:

- **HT Ready/Wait:** When at **HT Ready**, press the button to power on the filament. When at **HT Wait**, wait for the status to change to **HT Ready** before pressing it.
- **Scan#:** Sets scanning speed during live view. The smaller the number, the faster it scans, at the cost of live view detail. **SCAN1** is the fastest, but uses a cropped frame to attain maximum scanning speed. **This does not affect the acquisition detail.**
- **Freeze:** Freezes the live view frame.
- **Mag-/Mag+:** Changes the magnification on the microscope. Recommend using the physical control panel instead
- **Focus:** Auto-focuses the objective.
- **ACB:** Auto Contrast/Brightness. Press this to let the microscope automatically adjust contrast and brightness.
- **Photo:** Takes a picture of the sample at the current set magnification, focus, and contrast/brightness. This uses a fixed scan speed that is as fast as **SCAN4.**
- **Gun:** Opens the electron gun menu.

Bottom toolbar settings:

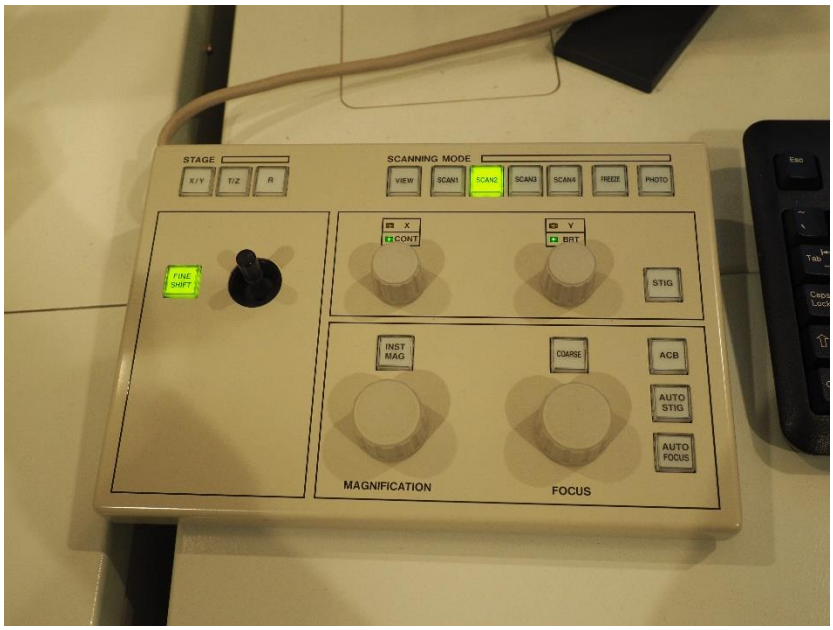
- **Acc. Volt:** Sets the voltage of the electron gun. 10kV or less recommended for biological samples.
- **WD:** Working distance. 9-10mm recommended for most work.
- **Spotsize:** Sets scanning electron beam spot size. Smaller spot size has more detail, but more noise. Larger spot size has less detail, but picture is smoother. Recommend not more than 50 on standard SEI mode.
- **Signal:** Selects the detector used on the SEM
- **Vac. Mode:** Sets High Vacuum (HV) or Low Vacuum (LV).
- **Pressure:** (only appears on LV mode) Sets chamber pressure.



Once you have arrived in this mode, press the **Gun** button on the top toolbar to bring up the electron gun parameters.

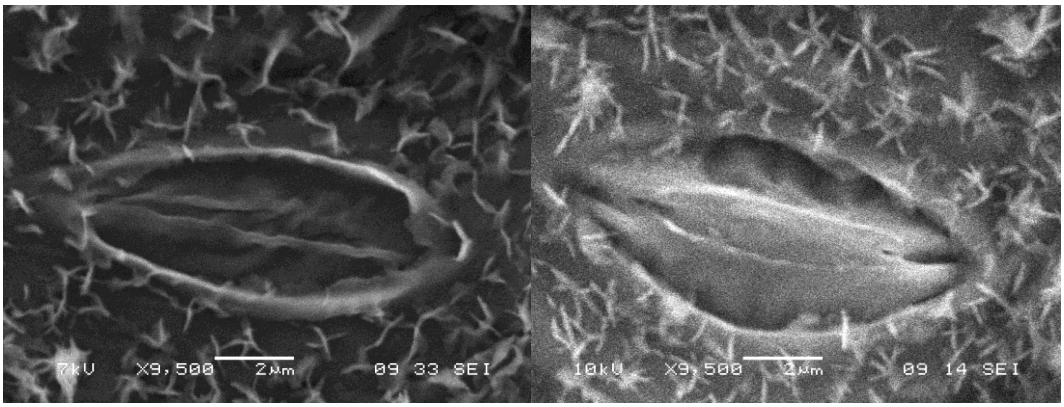
Look at **Filament heating** and check that the slider is at its lowest. Press the **HT Ready/Wait** button, press the **SCAN1** or **SCAN2** on the control console, then carefully move the slider to the right until you see that the screen turns grey/white and you see some faint images of your sample. **Do not move the slider to the orange region.**

4.5 Acquiring Images



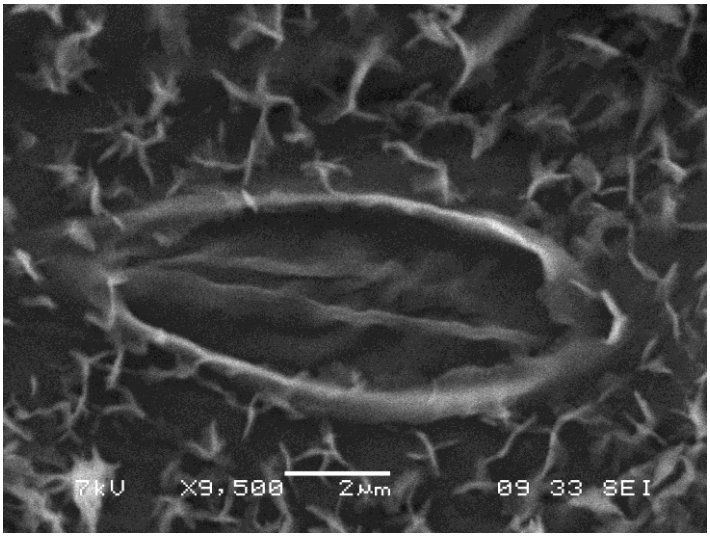
1. Set the WD to 9 or 10mm
2. Press **ACB (Auto Contrast-Brightness)** to show a relatively visible image of your sample.
3. **Carefully** rotate the stage z-height knob until your sample shows up in the screen.
    - ※ If you are using the standard sample holder, the stage z-height indicated on the screw gauge will be around the same as the working distance indicated on the computer monitor when the sample is in focus.
    - ※ If you are using a sample holder that protrudes out from the loader (most notably the thick cylinder slugs), the correct screw gauge height will be more than the indicated working distance by the height that protrudes out from the edges of the sample loader.
    - ※ It is better to use a sample holder that protrudes out from the loader than to use a sample holder that sinks into the loader.
4. Once you have reached a certain focus level and the sample is reasonably clear, you can press **ACB (Auto Contrast-Brightness) again** to automatically set contrast and brightness. You can manually control the contrast and brightness afterwards using the upper two knobs on the control console.
5. You can use the **Focus** knob to bring your sample to sharper focus.
6. Magnification can be set using the **Magnification** knob.
7. Set the Accel voltage.
8. Spot size can be set on the computer terminal. The spot size is the size of the scanning spot used by the microscope. Larger spot size (left) means higher beam power but lower fine detail. Smaller spot size (right) means fine details can be seen

more easily but you will see more noise in your image.



9. Once you have found the correct place to take a picture, press **Photo** on either the control console or the top toolbar in the control software. The screen will slowly scan the frame until it is complete, and the picture will freeze.
10. Two buttons will appear: **Save** and **Cancel**. Press **Save** to save the photo as a TIFF. Once you are done, press **Cancel** to return to the last live view mode that you were using before you took the photo.

4.6 Interpreting your photo



All pictures taken with the SEM will have a series of figures that show the microscope settings when the picture was taken. Using the picture above:

- **7kV:** Accelerating voltage
- **×9,500:** Magnification factor
- **2µm:** Scale. Scale bar is above this figure.
- **09:** Working distance (mm)
- **33:** Spot size
- **SEI:** Detector used. In this case this is the standard detector.

4.7 Unloading your sample

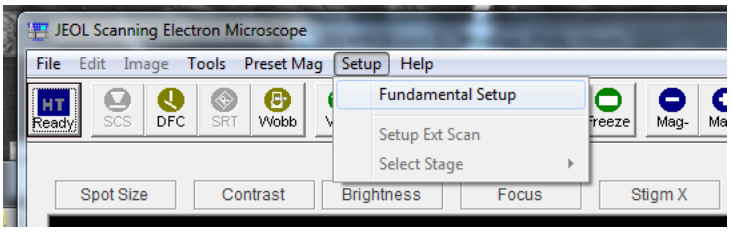
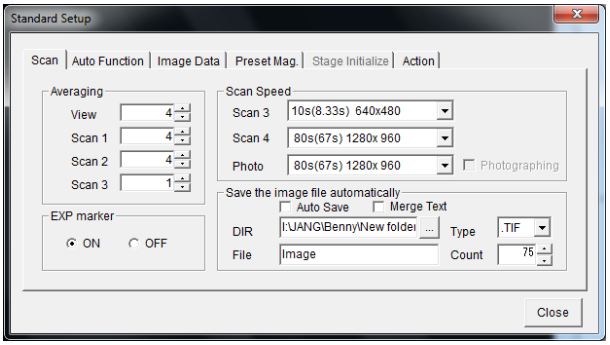
Before closing your session, remember to remove your sample from the sample chamber.

1. Drop the stage height to more than 25.0mm using the stage screw knob.
2. Press the **Gun** button on the top toolbar if the window is not showing, then gradually lower the **Filament Heating** slider to the lowest.
3. Press the **HT On** button to completely switch off the filament.
4. Press the **VENT** button to equalise air pressure. The button light will flash.
5. Wait for the button light to stop flashing. (around 1 ½ min)
6. Pull out the stage tray and unclip the sample loader.
7. Look into the sample chamber to confirm that the detector is still in good condition.
8. Push the stage tray in.
9. Press the **EVAC** button.

※ *Keep sample chamber under vacuum when not in use.*

You do not need to switch off the computer or electron microscope once you are done.

5. Troubleshooting

Issue	Explanation & Remedial steps
Save button on Live View window does not work after pressing the Photo button on the control panel.	Autosave was switched on. The image was automatically saved after image acquisition was complete. To see where the image is automatically saved in, go into Setup > Fundamental Setup.  The autosave function appears in the Scan tab. Uncheck Auto Save to bring back function on the Save button, but you will need to press it every time you take an image. 

Sample chamber does not maintain vacuum pressure after the Vacuum button is pressed	The sample chamber is only held in with vacuum pressure when in use. When the sample chamber is vented, there is nothing except for friction holding the sample chamber in place. Make sure the sample chamber is held in place when the air in the sample chamber is being evacuated. 1. Pull out the sample chamber, then push the sample chamber back in. 2. While keeping your hand pushed on the sample chamber, press and hold the Vacuum button until you hear a loud pump sound. 3. After hearing the pump sound, wait 5 seconds with your hand still pushed on the sample chamber, then you can let go of the sample chamber.
Vacuum pump does not start up when EVAC button is pressed	Keep the sample chamber closed and wait for up to 30 minutes. If the vacuum pump does not start up after waiting, press the VENT button, remove your sample, and let Confocal staff know.