

# SEM User Guide

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## Introduction

The TM3000 Scanning Electron Microscope (SEM) captures surface images of a specimen in a vacuum chamber by scanning an electron beam over a sample. An Electron gun produces an electron beam which is concentrated into a fine beam by passing through a series of electromagnetic coils and condenser and objective lens. Electrons bombard the specimen and secondary and backscattered electrons are formed. These electrons are detected by a secondary electron detector which produces an image of the surface of the specimen. Figure 1 below is from Hitachi's 'Let us Familiarize Ourselves with the SEM!' document to illustrate generally how a SEM works. For more information regarding how a SEM works refer to [SEM Vendor Manual](#) and [Let Us Familiarize Ourselves with SEM!](#).

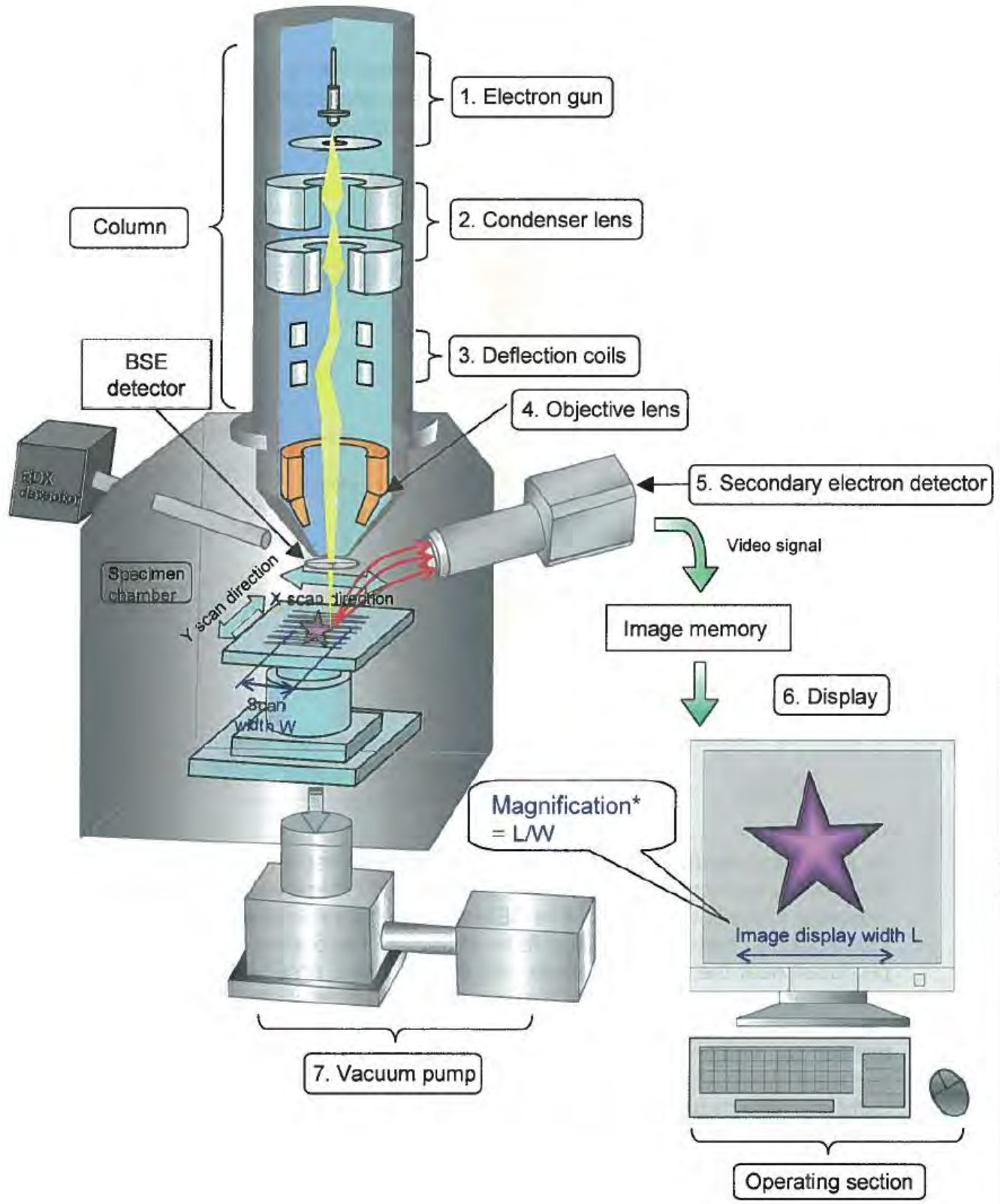


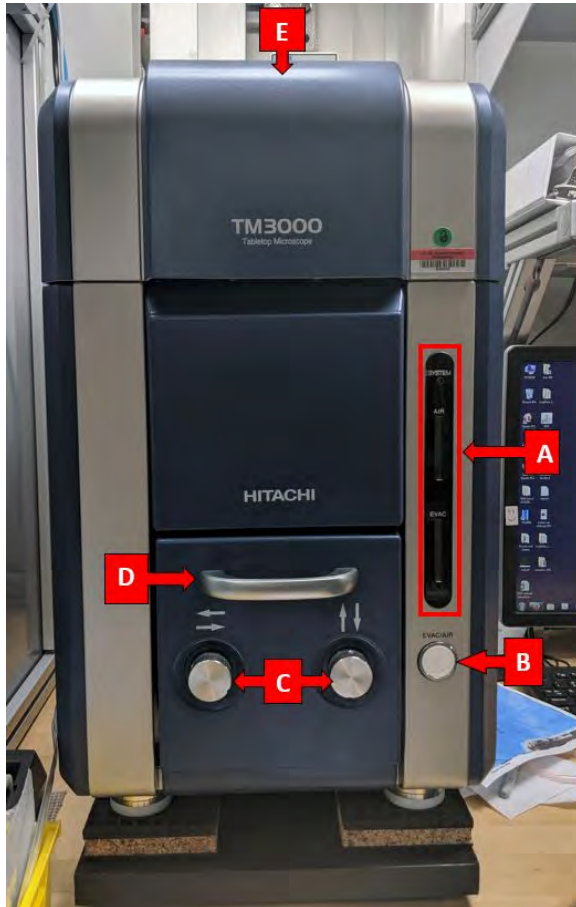
Figure 1. Image taken from 'Let Us Familiarize Ourselves with the SEM!' by Hitachi. Illustrates general parts and introduction to how SEM produces an image.

## Apparatus and Materials

## Scanning Electron Microscope Setup

### TM3000 Microscope

The TM3000 is a tabletop scanning electron microscope (Fig XX). The microscope rests on a series of vibration reducing pads to minimize shipboard vibration. The SEM operates under a vacuum and has an external diaphragm pump to bring the system down to an initial vacuum and then an internal Turbo Molecular Pump brings the system down to its final vacuum state. Power is supplied to the SEM via an Uninterrupted Power Source (UPS) located next to the workstation computer.



The microscope has the following features:

**A. Status indicator bar:** Three indicators on bar. From top to bottom:

- **System:** Indicates if the system is on or off. A solid green light indicates system is on.
- **Air:** A yellow light that indicates if the chambers are infilling with air. A flashing yellow light means the system is in the process of filling chambers with air. A solid yellow light indicates the chambers are filled with air.
- **Evac:** A blue light that indicates if the chambers are under vacuum. A flashing blue light means the system is in the process of evacuating air and going down to vacuum. A solid blue light indicates the chambers reached the final vacuum state.

**B. Evac/Air Button:** Sends command to the microscope to either fill chambers with air or evacuate chambers. The button does the command opposite to its current state.

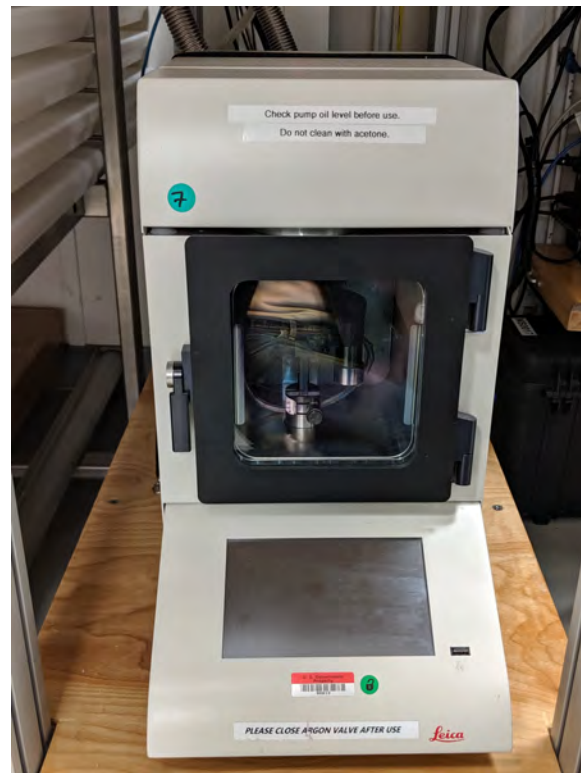
**C. X and Y Stage Knobs:** These knobs move the sample stage in the X and y direction. This allows user to change the field of vision in the imaging software.

## Sputter Coater

Sputter coating is performed using ionized argon to create a plasma. The argon ions are accelerated by high voltage and directed toward the source via a magnet where they collide with the target and displace surface atoms. Due to this collision the surface atoms are directed toward the area below the target and coat the sample. This coating process can be set to directional or diffuse (which provides more even coating on a bigger surface and is better for fissured samples), depending on the process pressure. This also influences the coating rate (diffuse means slower rate) and the grain size (directional means finer grains).

The Leica EM ACE200 coating system includes the following main functional units:

- -Vacuum chamber
- -Touch screen control panel
- -Sample stage with 18 positions for 1/2" SEM stubs
- -Removable internal shielding, shutter, and door
- -Carbon source and sputter targets
- -Vacuum pump
- -Sample stage



### Vacuum pump

The vacuum pump brings the Sputter Coater sample chamber into a vacuum state. The pump is connected to the sputter coater via the flexible metal hosing (Figure XX). Routine maintenance is needed on the vacuum pump.

D. **Sample chamber Pull out Handle:** Pull on the handle to access the sample stage area.

E. **Top Cover:** Pull open the cover to access the electron gun. Only necessary during certain maintenance procedures.

**\*CAUTION:** The microscope is not meant to be used during high sea states. If used the image quality will deteriorate and a user may not be able to focus an image

## Diaphragm Pump

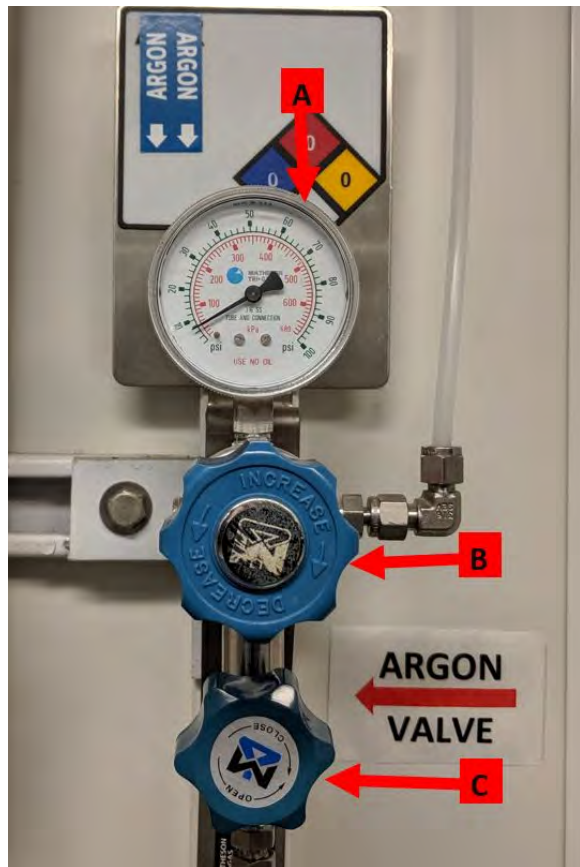
The diaphragm pump takes the SEM down to an initial vacuum state and then the internal SEM Turbo Molecular Pump take the system down to its final vacuum state. The diaphragm pump is connected to the SEM through a 'power and signal cable' on the back of the SEM. According to the manufacturer the pump is good for 720 hours and at that point should be serviced or replaced.

Pumping Speed: 1 m<sup>3</sup>/h



## Gas Gauges

The sputter coater fills the sample chamber with argon supplied by an Argon gas bottle from the upper tween deck. The line is fed through the ship to Core Deck. The figure below is the Argon setup for the Sputter Coater. There is one pressure gauge (A) and upper valve (B) and lower valve (C). The pressure gauge reads the pressure of the gas in the line. The upper valve adjusts the pressure of gas going into the Sputter Coater. The pressure should be between (). The lower valve either opens or closes the gas line to the sputter coater.



## Sample Preparation

Samples can be imaged untreated, sputter coated, or with silver paint. The purpose of treating the sample is to reduce the charge on the sample. Charge on the sample attenuates the electron beam and produces fuzzy images that make it difficult to identify structures or features on the sample. It is recommended to first image an untreated sample and if too much charge is detected in the image, apply a treatment and then re-image.

## All Samples

A sample can be loaded into the SEM in a variety of ways. Three things are needed to assemble a sample mount are listed below:

1. A mount to put in the SEM
2. A piece of adhesive to stick to the stub and to a sample
3. Sample material/glass/filter paper containing a sample

**NOTE:** All sample must be dry before putting into the SEM. A wet sample under vacuum will explode and result in loss of sample and get material all over the sample chamber

The tables below list the various options of how to assemble a mount.

Sample Mount	Adhesive Option	Mounting Medium	Pros /Cons	Sample Mount	Adhesive	Mounting Medium	Pros /Cons	Sample Mount	Adhesive	Mounting Medium	Pros /Cons
Small Stub (Screw on)	Carbon Dot	Square Cover Glass	Very unlikely to get cover glass off stub	Large Stub (Screw on)	Carbon Dot	Square Cover Glass	Very unlikely to get cover glass off stub	Adapters			
		Circle Cover Glass	Very unlikely to get cover glass off stub			Circle Cover Glass	Very unlikely to get cover glass off stub				

		Sample directly on	Works well but must place sample on dot			Sample directly on	Works well but must place sample on dot
	Double Sided Tape	Square Cover Glass	Can remove cover glass from stub		Double Sided Tape	Square Cover Glass	Can remove cover glass from stub
		Circle Cover Glass	Can remove cover glass from stub. Same size as the stub. Not on inventory if used talk to ALO for ordering more			Circle Cover Glass	Can remove cover glass from stub. Not on inventory if used talk to ALO for ordering more
		Sample directly on	Tape not anti-static so sample could have a lot of charge			Sample directly on	Tape not anti-static so sample could have a lot of charge

Once the sample has been mounted you may proceed to [Loading Sample](#) or to [Additional Sample Treatments](#).

## Additional Sample Treatments

### Sputter Coating Treatment

The Leica EM ACE200 Sputter Coater coats samples with a thin layer of gold palladium or carbon to reduce charge on a sample and increase the signal to noise ratio, producing clearer images when observed in the SEM. Material can be processed as long as it is not sensitive to vacuum, argon plasma, or the heat generated during carbon coating. Sputter coating is not required for all samples. Images up to a magnification of 4000x are of high quality without any sputter coating. For detailed observations in magnifications greater than 4000x and for very delicate fossils, sputter coating before using the SEM can be used to potentially receive higher quality images.

Possible Targets

- Gold Palladium
- Carbon Thread
- The instrument can use other metal targets but these are currently not supplied to the ship

### Argon Gas Supply

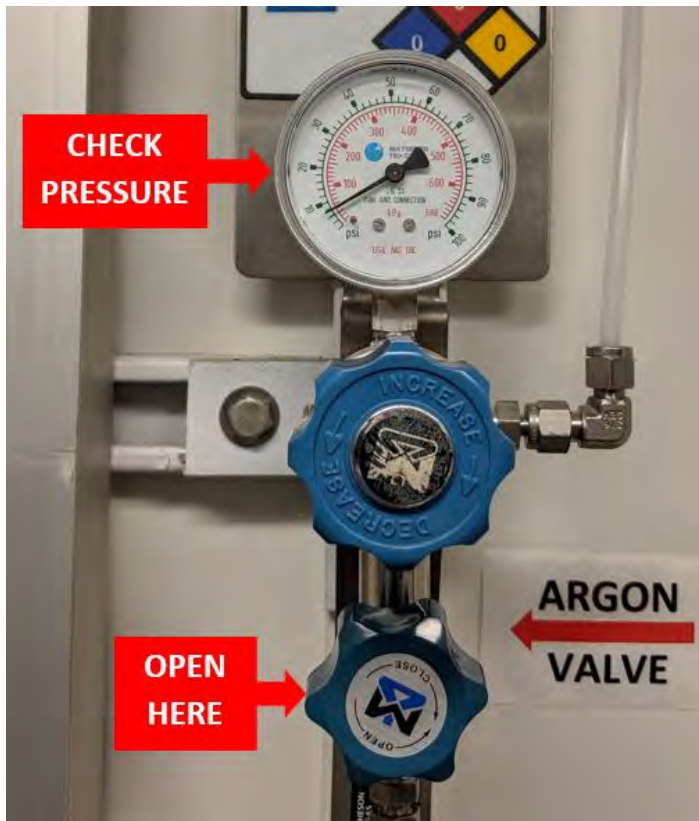
The working gas (argon) must be supplied under a pressure of ~500 mbar ±100 mbar (5.6—8.4 psi above atmosphere). The gas should be at least 99.99% pure.

**Caution!** Do not use a standard two-stage regulator for the sputter coater unless it is equipped with a low-pressure delivery-side gauge. Most regulators don't effectively pick up pressure until 10 or even 15 psi or higher. It may be necessary to put a second low-pressure-range regulator downline from the main line regulator. Make sure you have the right tool for the job!

### Sputtering a Sample

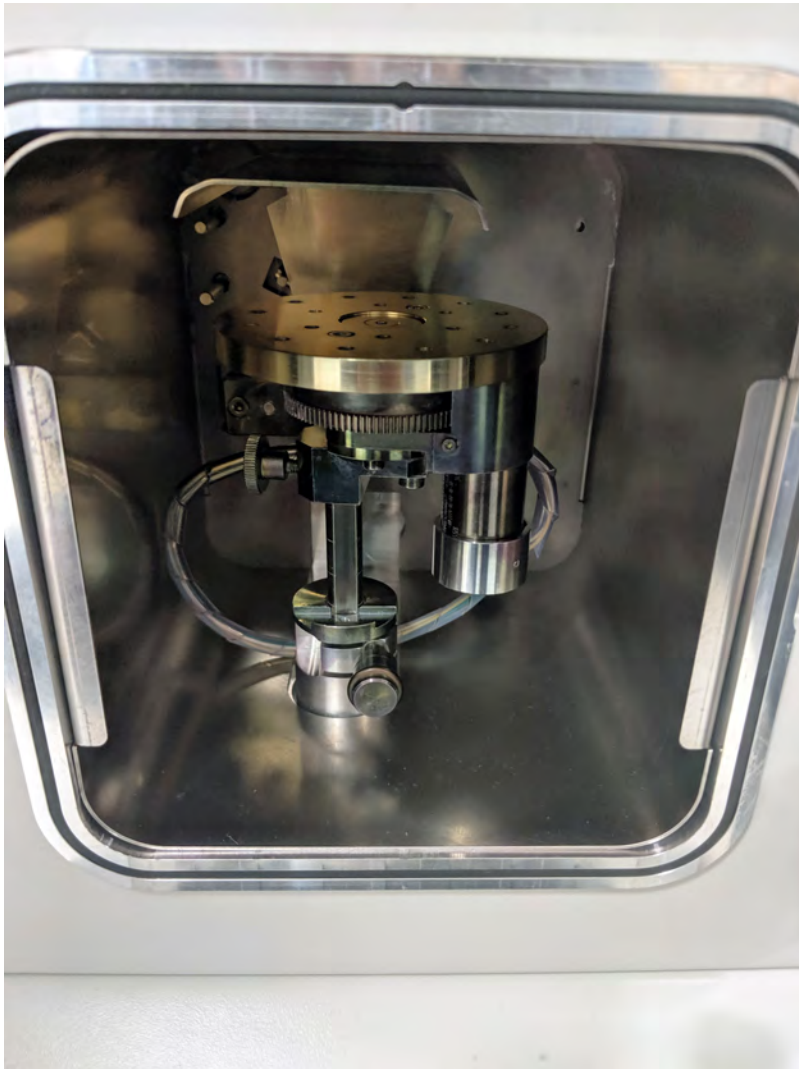
#### Starting Measurement

1. Check the Argon levels on the regulator and confirm the level is between 5.6 - 8.4 psi. Turn the lower knob to the **open** position.



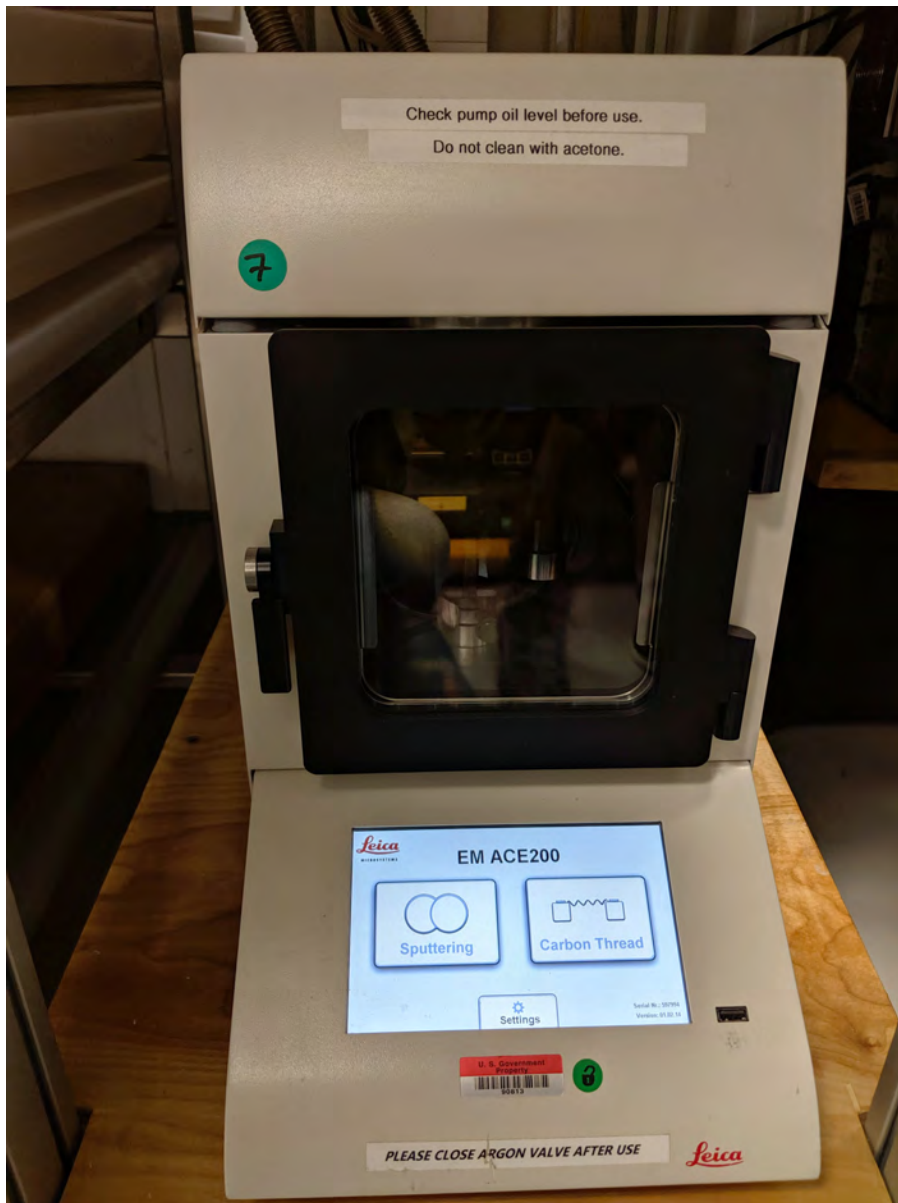
2. Open the sample chamber door.

3. Wearing gloves, place sample(s) on stage. Do not put sample(s) in the center of the stage or material will not land on the sample.



3. Check that the vacuum pump behind the Sputter Coater is turned on.
4. Turn on the main power supply to the instrument and make sure the chamber door and cover are closed.
5. Select the **Sputtering** button.





6. Press the **Pump** button to start pump-down. It can take up to 5 minutes to reach vacuum.



7. During pump-down, set the process parameters:

- **Target material:** select from a drop-down list; the instrument automatically chooses parameter settings. Typically set to **Gold Palladium**
- **Directional mode:** intended for relatively flat samples; base vacuum is  $4 \times 10^{-2}$  mbar for this mode.
- **Diffuse mode:** intended for more topographic samples; base vacuum is  $8 \times 10^{-2}$  mbar for this mode.
- **Sputter current/purge cycles:** automatically set when target is selected. Change under the **Setup** button.
- **Process termination time:** Adjusts the time spent coating samples. Time is typically between 12sec - 30sec. Select the **Clock** icon then use the +/- buttons to select a time in seconds.
- **Rotation speed:** press the **Rotation** button, check **Rotation On**, and use the +/- buttons to set the speed.
- **Vent after process:** If selected, will automatically vent the chamber after the sample(s) have been sputtered.

8. Press the **Start** button (which then becomes the **Stop** button). The Start button can be pressed while the Sputter Coater is still achieving vacuum. Below is the sputtering sequence:

- Pump until base vacuum is reached.
- Stabilize the plasma.
- Pre-sputter, if target requires it (to clean oxidation from the target and to enable a stable sputter rate).
- Start the sputter processing by opening the shutter and starting rotation (if activated).
- Terminate sputtering by time.

- Close the shutter.
- Display results of the process.
- Vent or stay under vacuum, per settings.

9. When sputtering complete press **Vent** to release the vacuum. Then the door will open and the samples can be removed.

**Note:** if the vacuum cannot stabilize, make sure the argon supply valve is open and pressure is on the line. **What happens when pump can't stabilize?**

## Silver Paint Treatment

Silver paint allows a path for electrons to discharge from the sample.

### Prepare Sample

1. Dab a very small amount of paint on the side of sample and extending to the edge of the sample stub. The painted sample area will obscure surface features so it is important to use very little on the sample.

## Loading Sample in SEM

### Start Microscope

1. On the back of the instrument make sure the main power breaker is in the 'On' position
2. Flip the 'On' switch on the right side of the microscope



3. The SEM will flash the yellow 'Air' and blue 'Evac' status indicator lights (Fig XX) and then resume the last operation it was under when turned off, likely the Evac command.



## Screw on Stub

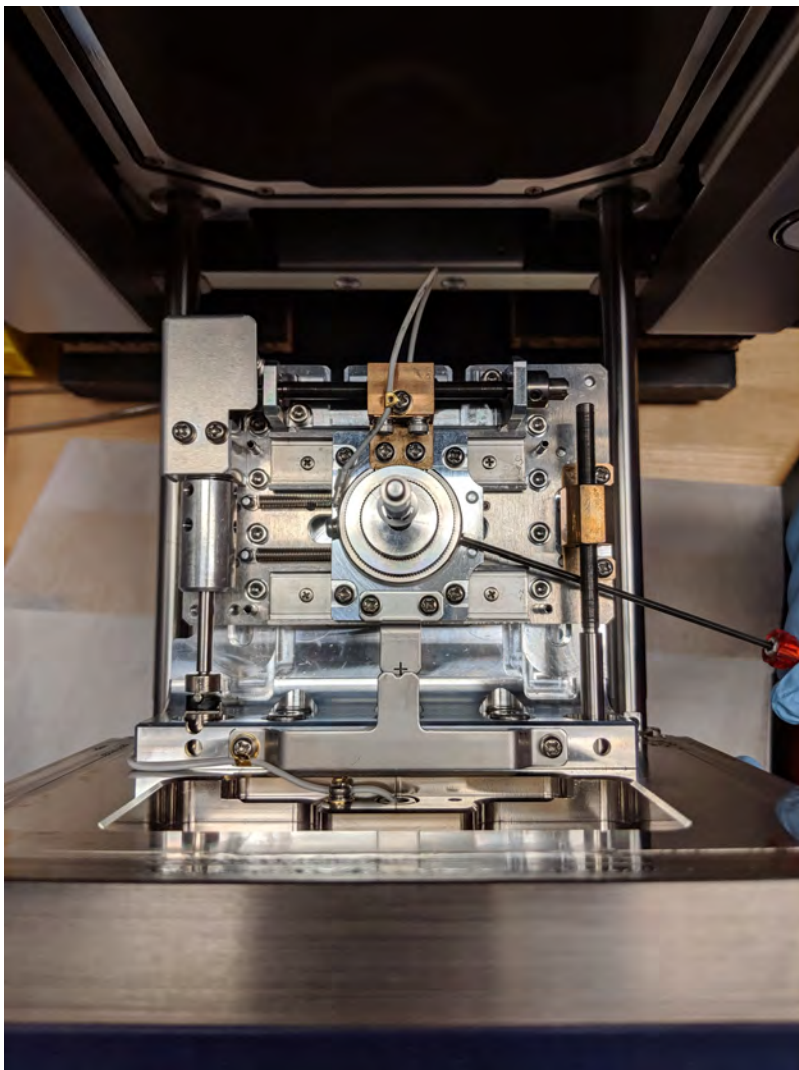
### Adjust Height of Sample Assembly

1. Make sure the **Air** status bar is solid yellow. This indicates the chamber is not in a vacuum state and the chamber can be opened. If not, press the **E vac/Air** button to refill the chamber with air. During the fill process the status bar will flash yellow. When the chamber is full of air and safe to open, the status bar will change to a solid yellow.

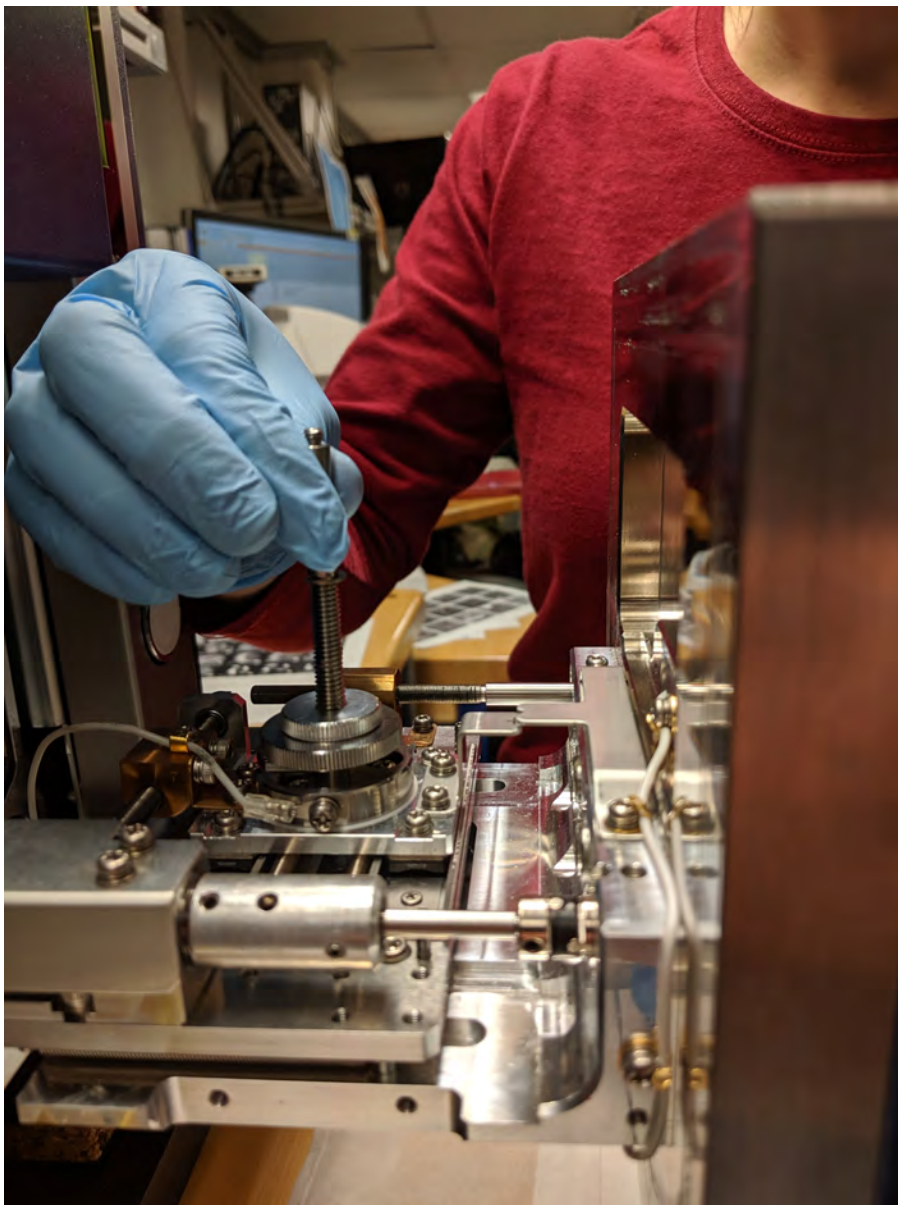


2. Grab the handle and open the door.

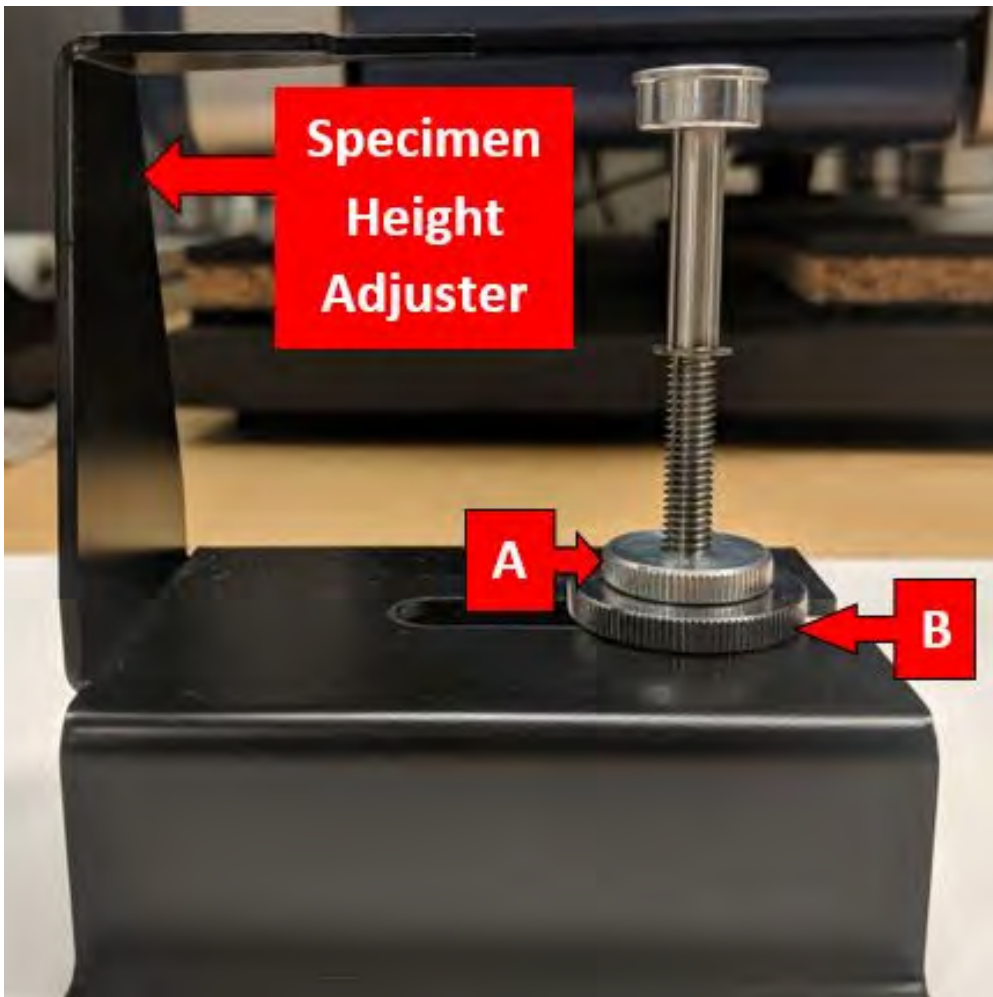
3. Using the red Hex Key, turn the set screw about half a turn to loosen the sample stage (Fig XX). Do not unscrew too much or the set screw could fall out.



4. Pull out the sample stage (Fig XX).

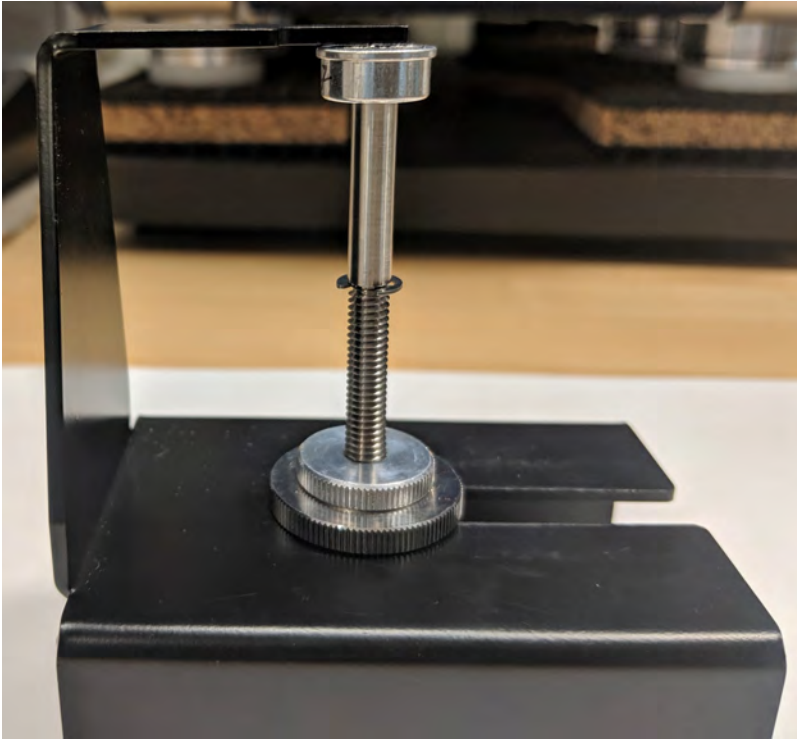


5. Place the sample stage in the black Specimen Height Adjuster tool (Fig XX). The height of sample screw can be adjusted via the two lock nuts, A and B, in the figure below.



6. The goal is to have the stub/sample 0.5mm - 1.0mm below the black bar. This bar represents the height of the door seal. If the stub is touching the black bar on the height adjuster then it will hit the door seal. If the height is too low the sample will be outside of the focusing circle for the electron beam and the image will be unable to focus.

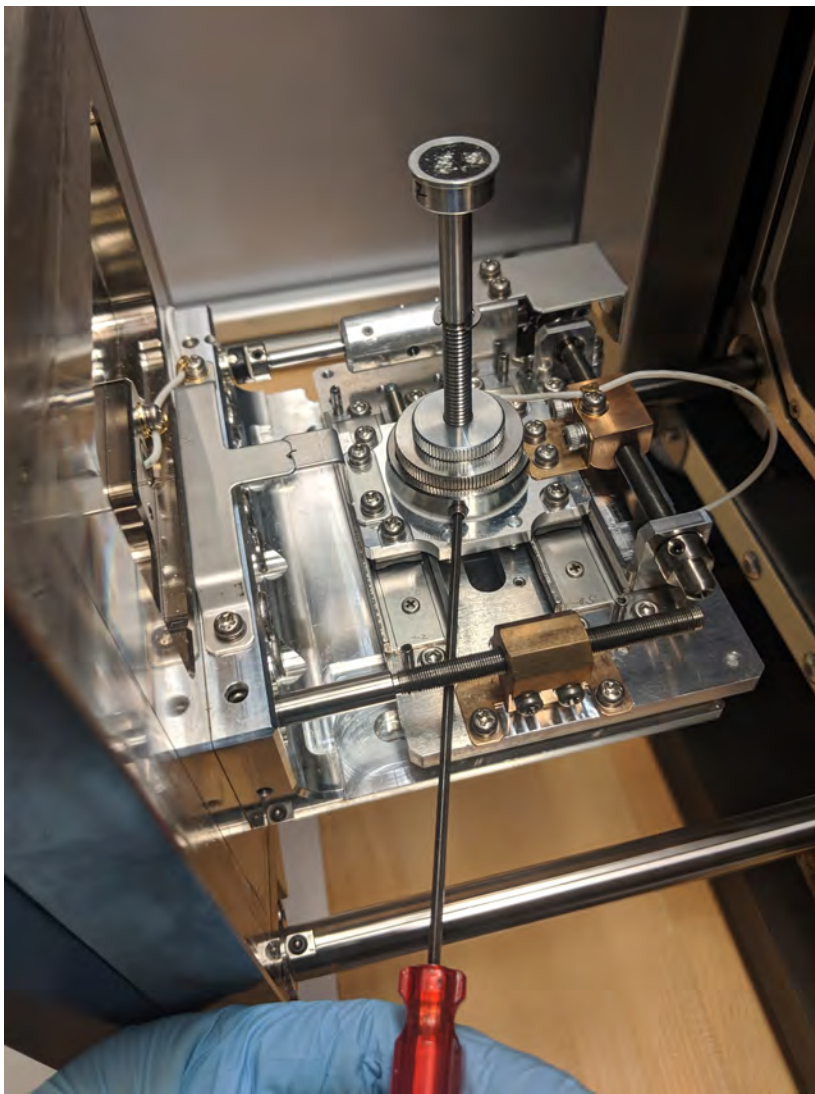
- a. Loosen Locknut A
- b. Turn the threaded sample screw to raise or lower the height.
- c. When the sample stub is just below the black bar, retighten locknut A.
- d. Double check the height is good.



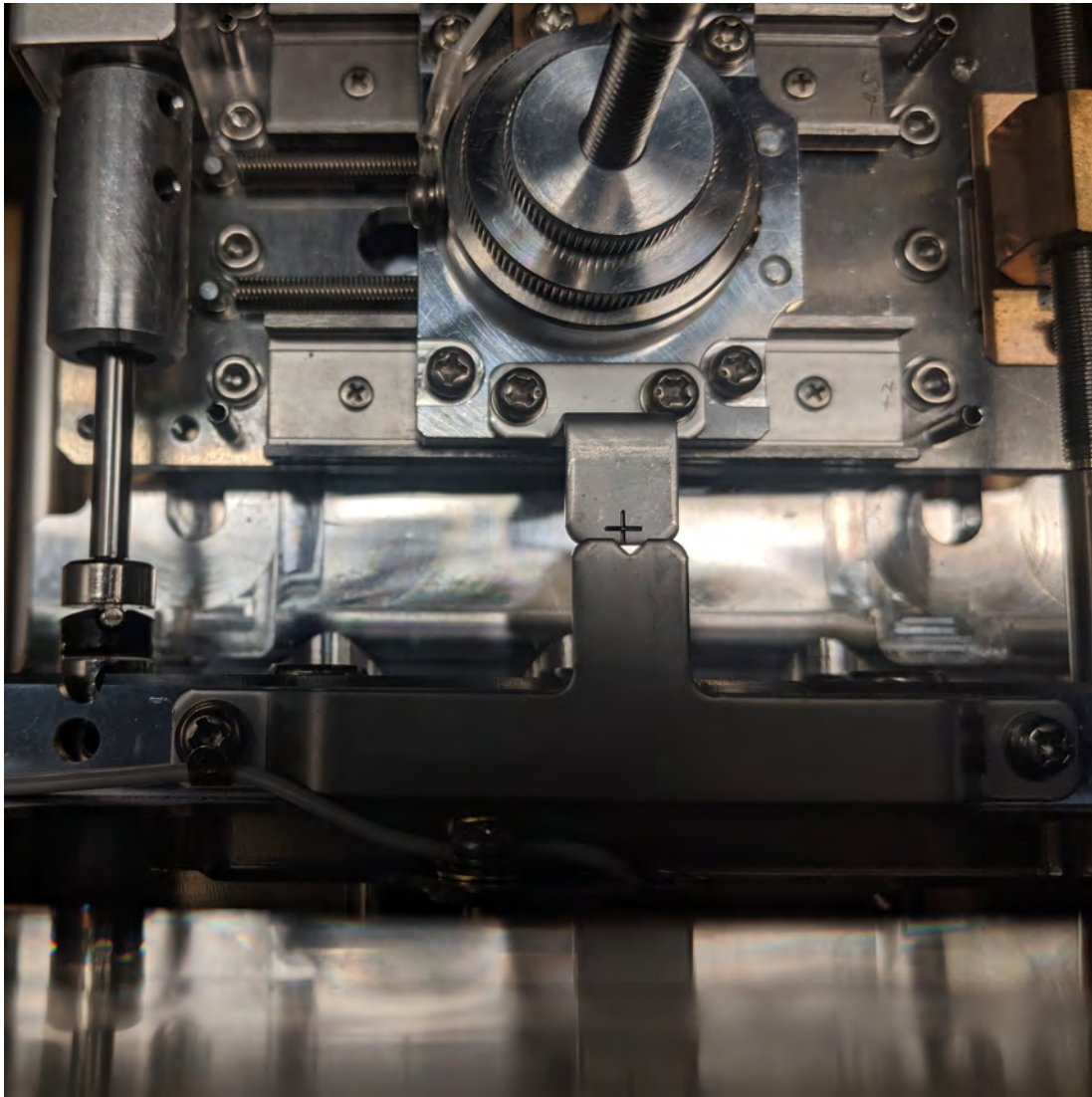
### **Put in SEM**

1. Open the door to the sample chamber
2. Put the sample assembly back in the hole.
3. Tighten the set screw using the hex key (Fig XX). Hand tight is sufficient.





4. Look through the v plate in the chamber. With the X and Y Stage Knobs on the door, adjust the sample stage position so the cross-hairs on the stage aligns in the V (Fig XX).



4. Close the door.

5. Push the **Evac/Air** button to put the chamber into a vacuum. Press on the door for a few moments to help get the vacuum started. It will take several minutes to get the chamber down to vacuum.

## Capture Image

While the SEM is drawing down the vacuum the image capture software can be opened.

## Open Software

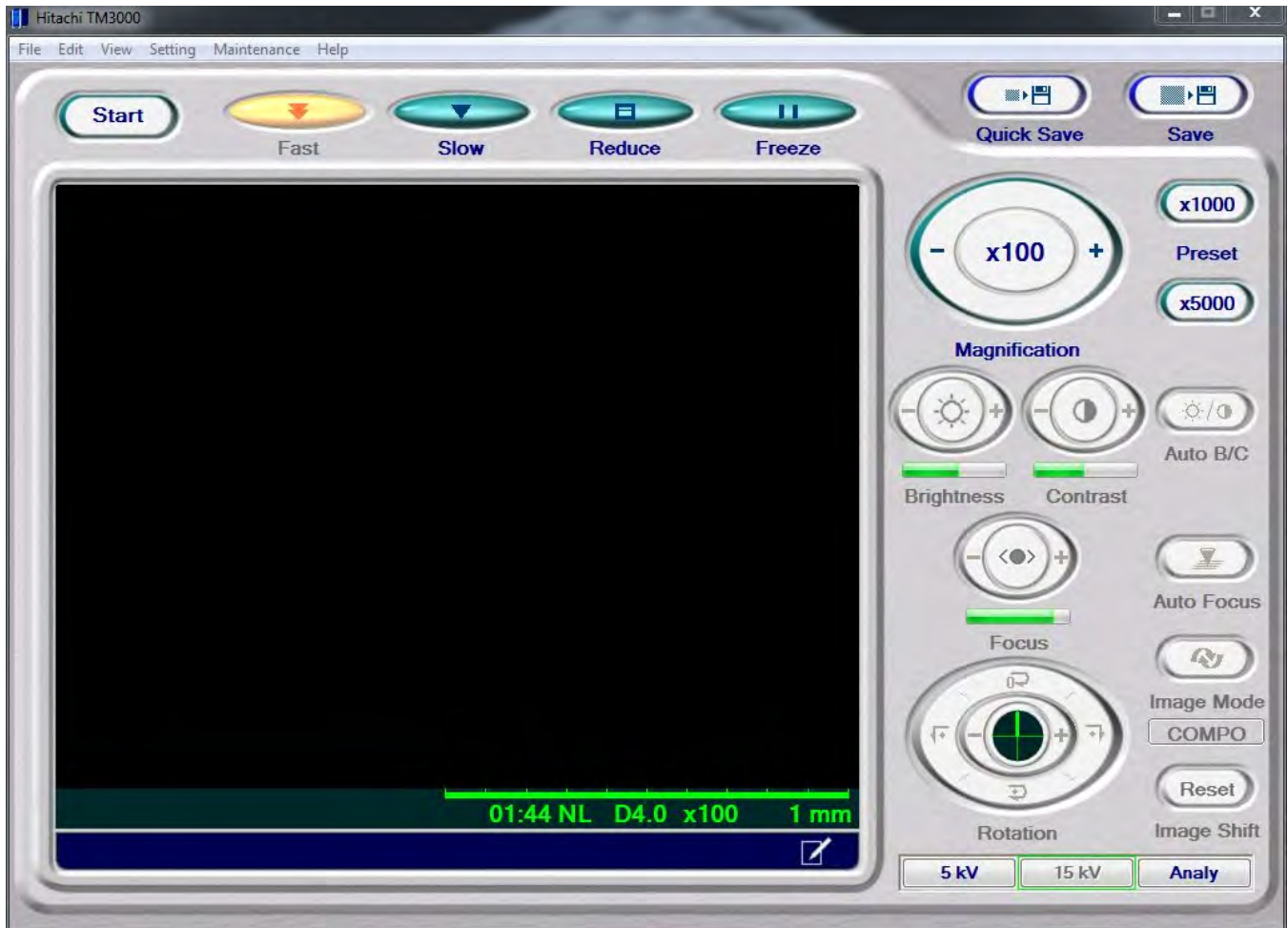
1. Click the TM3000 software icon on the desktop



2. While the SEM pumps down to vacuum the software will display this status screen.



3. When the machine is fully under vacuum the start up screen opens. Note the screen will appear black at first. The electron beam gun is not activated until a user presses Start.



Below are the tools available in the software:

- **Start:** Turns on the high voltage which generates an electron beam. Must press start to generate an image.
- **Fast:** Image updates quickly. Useful to select 'Fast' while changing Field of View.
- **Slow:** Image updates slowly. If in calm sea state, can produce a higher quality image.
- **Reduce:** Takes just the center portion of the screen to update.
- **Freeze:** Stops updating the image and preserves the image as it was at the time of pressing Freeze.
- **Magnification:** Control to increase or decrease magnification. Ranges from x40 to x30,000.
- **Auto B/C:** Click this to automatically adjust the brightness and contrast of the image.
- **Auto Focus:** Click this to automatically focus the image.
- **Image Mode:**
- **5 kV / 15 kV / Analy:** Sets the beam voltage between either 5kV, 15kV, or **Analy**. **Not clear what Analy mode does.**
- **Quick Save:** Saves the current image on the screen. Captures a lower resolution image of 640x520 pixels.
- **Save:** Re-captures the image and saves it at a higher resolution image of 1280x1040 pixels.

3. Press **Start**.

## Naming and Saving Conventions

Naming convention for SEM files:

1. EXP number
2. Site
3. Hole
4. Core
5. Section
6. Sample
7. Taxa/specimen/piece
8. No. 1, 2, 3...

E.g.

349\_U1431A\_1H\_1\_10-11cm\_Emiliana\_huxleyi\_1,  
349\_U1431A\_1H\_1\_10-11cm\_Emiliana\_huxleyi\_2,  
349\_U1431A\_1H\_1\_10-11cm\_Emiliana\_huxleyi\_3, ...

Storage convention for SEM files on the local PC:

1. SEM
2. Site
3. Hole
4. Sample

E.g.

Folder SEM  
Folder 1431  
Folder 1341A  
Folder U1341A\_1\_H\_1\_1-11cm (all files for this SEM sample)

NOTE: All files need to be moved to USERVOL and DATA1 by the end of the expedition.

## Uploading Data

Data upload requires a two softwares used in the order below:

1. SEMUploader to convert files into a format readable in the database
2. MegaUploaderTron (MUT) to upload files into the database.

Two file types are generated by the SEM:

- **.tif** : This file is the image taken by the software.
- **.txt** : This file contains the metadata for the image. This file includes parameters under which the image was taken, instrument specifics, filename, timestamp, and directory.

The SEMUploader uses these two files to generate a new **.SEM** file. This is the only file uploaded to LORE and contains callouts to the .tif and .txt file.

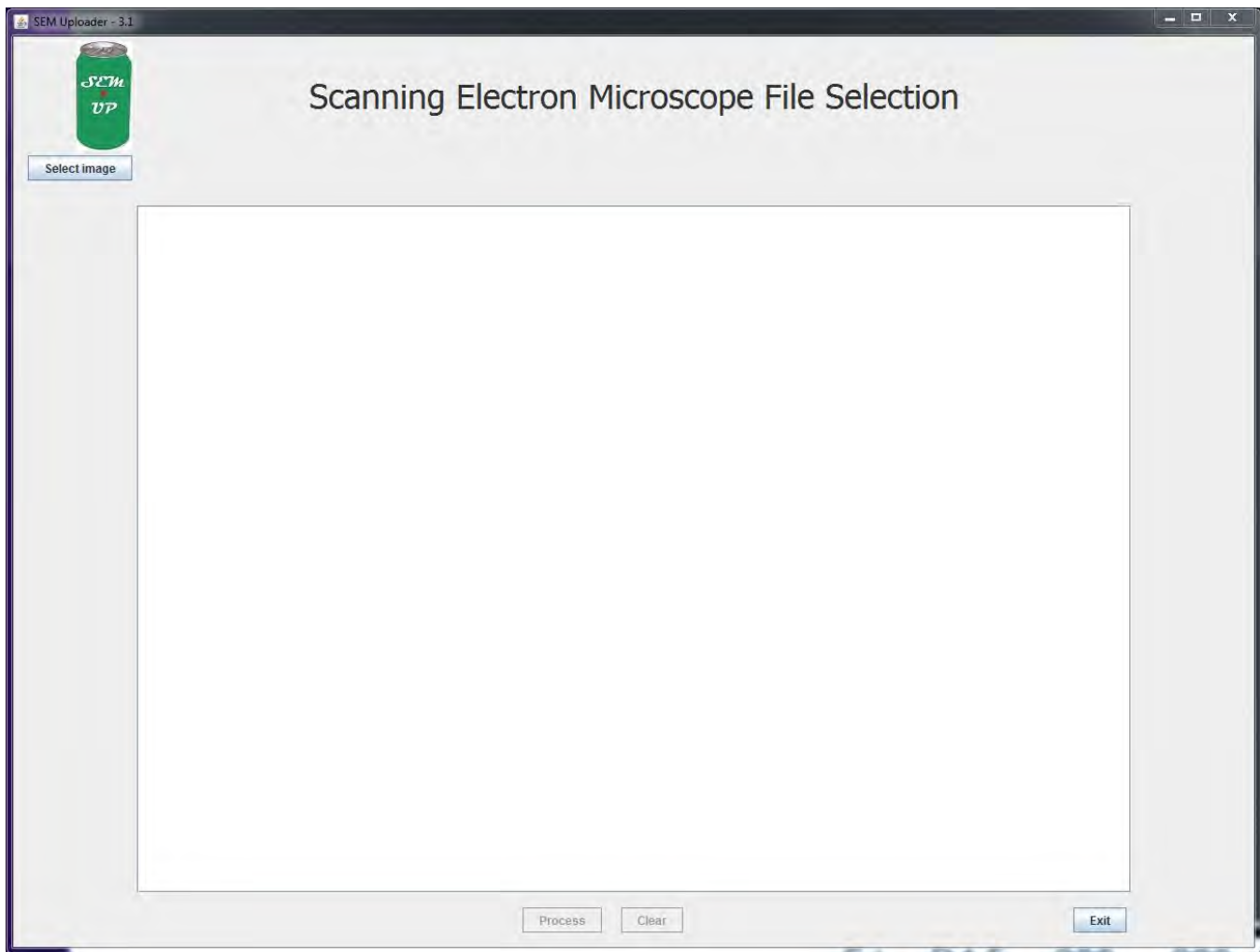
## SEMUploader

The SEM Uploader can be configured to list the expedition specific sub-lists with the help of the developers. For information on how to see the expedition sublists in the SEM Uploader please refer to the section [Add Expedition Specific Lists to SEM Uploader](#)

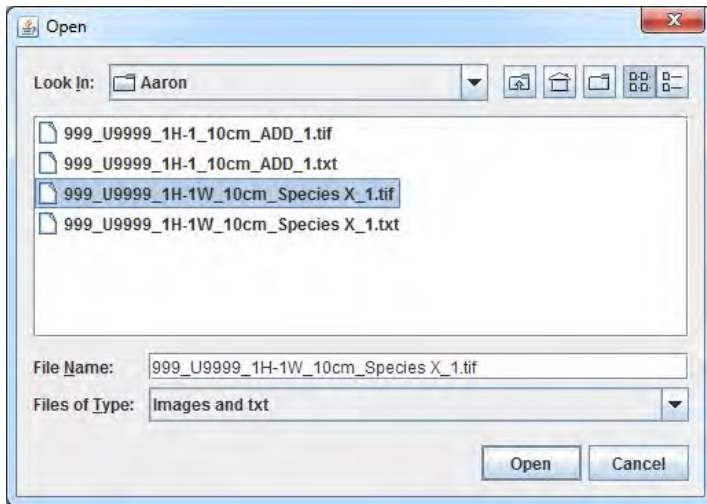
1. Open **SEMUploader** (Figure XX)



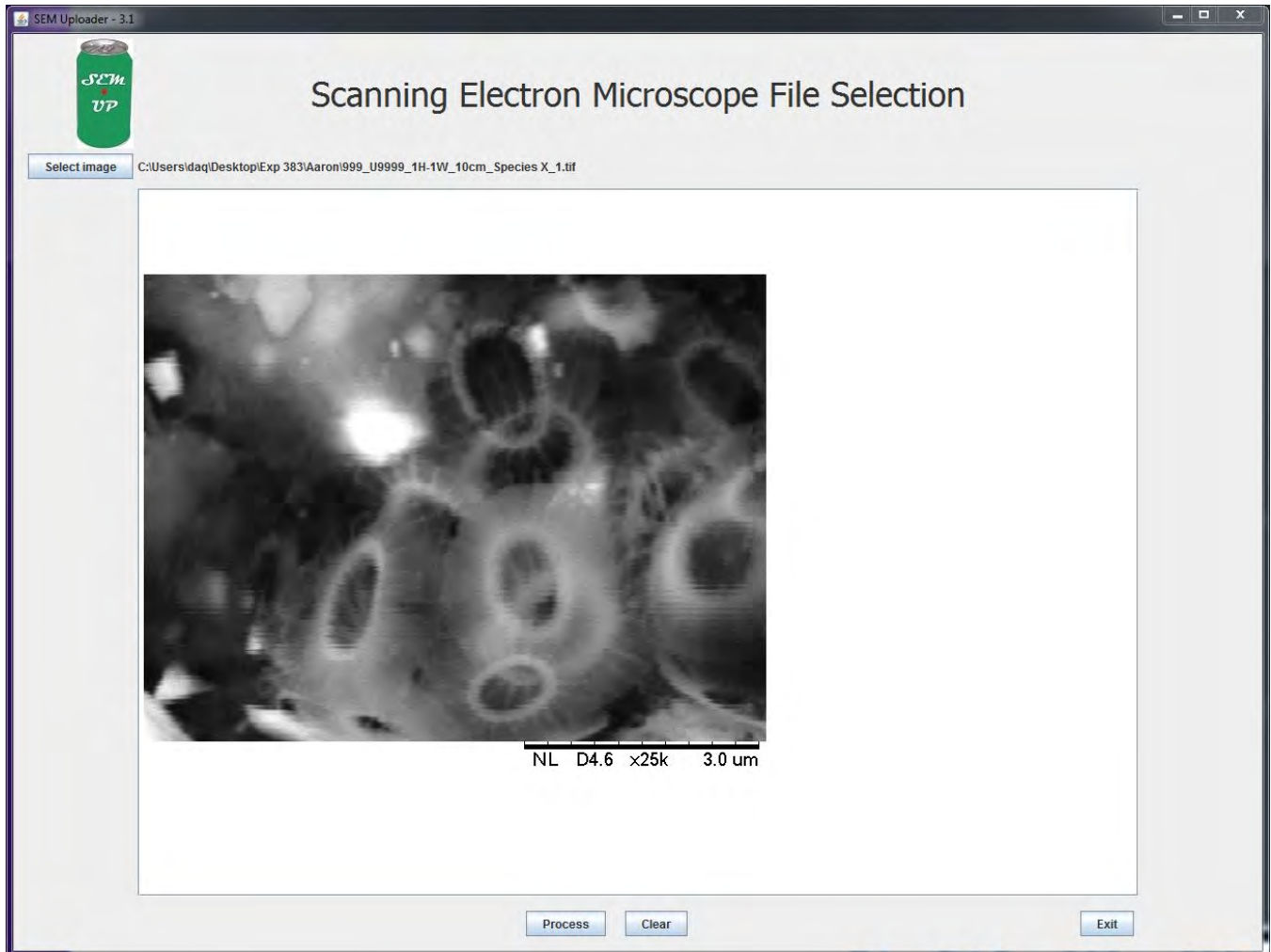
2. The Main Window opens up (Figure XX). Click **Select Image** in the upper left corner.



3. Navigate to the images and select the desired .tif file.

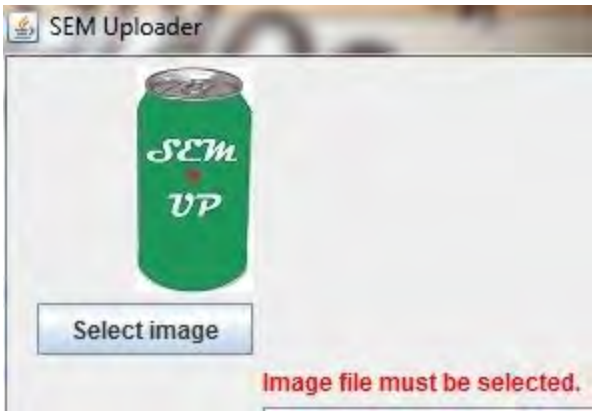


4. The image populates the screen. Click **Process** to move forward. OR click **Exit** if you decide to upload later. **CLEAR** will clear out the image from the selection. The user can select another image right away if the one chosen is not the one that they wanted to upload.

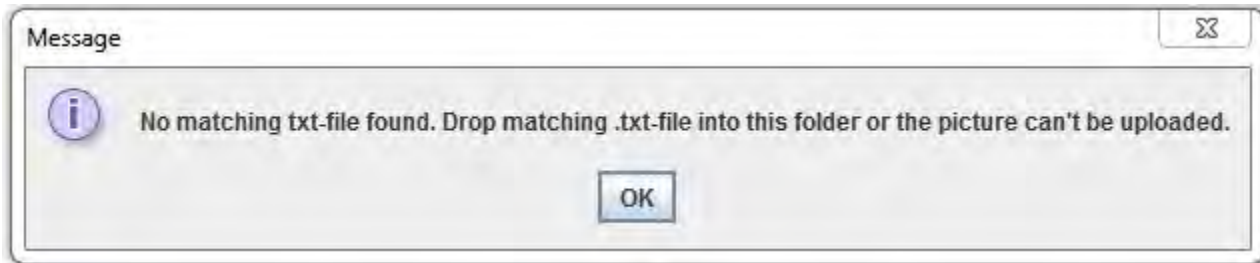


At this point a user could encounter two potential errors:

- If the user selects a file that is not a .tif file the message below will appear (Fig XX). To resolve click on the appropriate file type




- If the user selects a file that does not have a corresponding .txt file the message below will appear (Fig XX)



5. After clicking **Process** the sample information window appears. In this window we can assign a sample to the image and text files. The image can be assigned to a sample id either through the **Hierarchy** tab or the **Text Id** Tab. Use the Hierarchy entry by clicking through the appropriate Expedition, Site, Hole, Core, and Section. The samples off the section will pop up after the other fields have been entered. To use the Text Id entry type in the Text Id of the Sample and Click **Validate Text Id**.

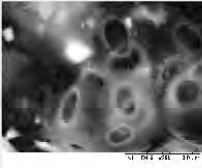
SEM Uploader - 3.1



## Scanning Electron Microscope File Selection

Image File: C:\Users\daq\Desktop\Exp 383\Aaron\999\_U9999\_1H-1W\_10cm\_Species X\_1.tif  
 Txt File: C:\Users\daq\Desktop\Exp 383\Aaron\999\_U9999\_1H-1W\_10cm\_Species X\_1.txt

Instrument: **TM3000**      Date taken: **5/30/2019 20:53:13**  
 Signal name: **BSE COMPO**      Magnification: **25000**  
 Working distance: **6100 um**      Filament current: **1850 mA**  
 Accelerating voltage: **15000 Volt**



Hierarchy    Text Id

Expedition	Site	Hole	Core	Section
999				
383				
QAQC				

Text Id: \_\_\_\_\_      Label Id: \_\_\_\_\_      Sample selected: \_\_\_\_\_

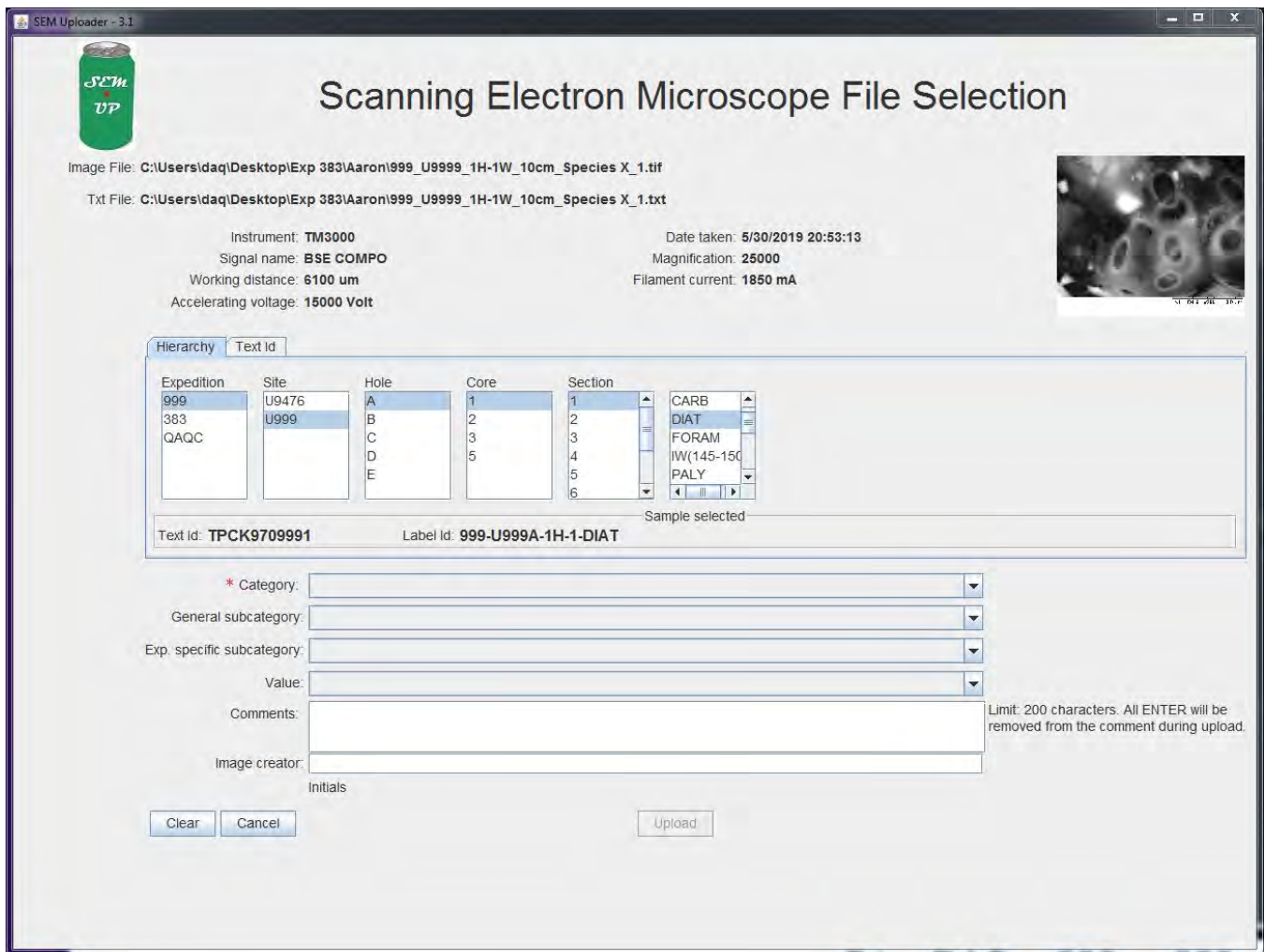
\* Category:    
 General subcategory:    
 Exp. specific subcategory:    
 Value:    
 Comments:   
 Image creator:   
 Initials:

Limit: 200 characters. All ENTER will be removed from the comment during upload.

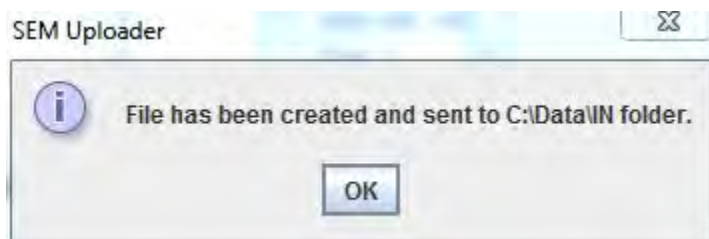
6. After the sample has been selected go through the drop down lists below. Only the **Category** Field is required. Below the categories are described:





- **Category:**
- **General Subcategory**
- **Exp. Specific subcategory**
- **Value**
- **Comments**
- **Image Creator**

7. Click **Upload**. This creates the new .SEM file that will upload into the database. A message box pops up indicating the file has been created.



## MUT Upload

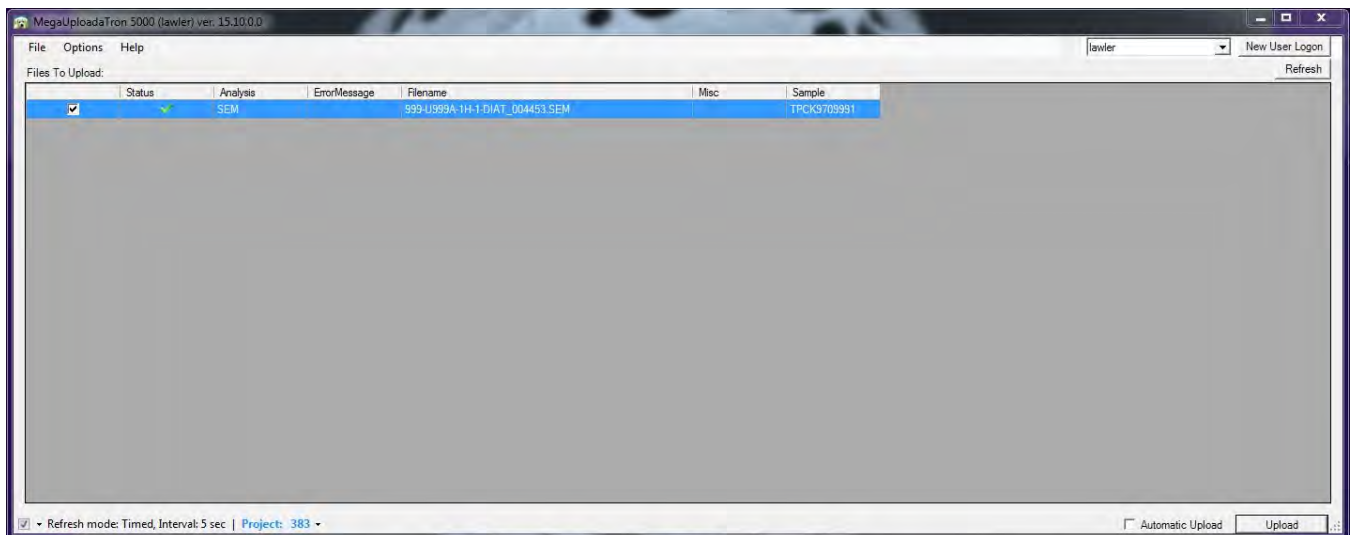
1. Click on the MUT icon to open the software



2. Login to MUT with your LIMS applications username and password.



3. The files should populate when MUT opens.



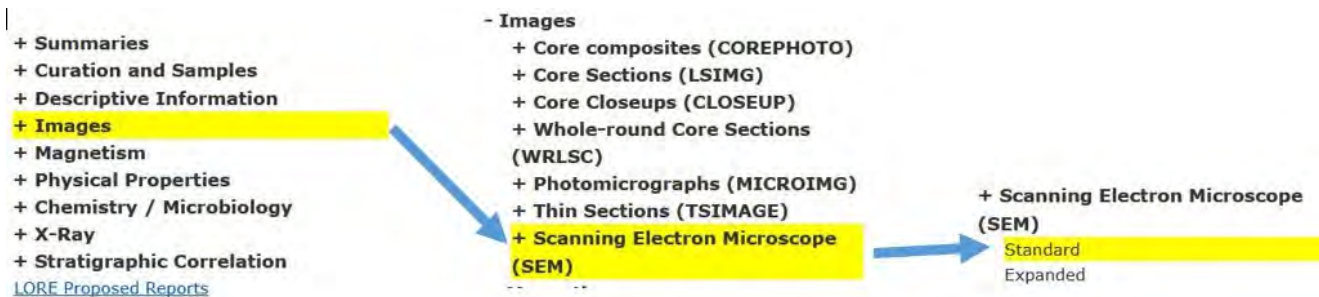
4. Click **Upload**.

The software can be left open in the background with the **Automatic Upload** box checked.

## LIMS Integration

Images and metadata can be accessed in LORE.

1. Open LORE in a web browser
2. On the left side of the screen expand Images > Scanning Electron Microscope > select either Standard or Expanded (Figure XX).



Below is a table describing the information uploaded to LORE.

Analysis	Components	Definition
Scanning Electron Microscope (SEM)	Exp	expedition number
	Site	site number
	Hole	hole number
	Core	core number
	Type	type indicates the coring tool used to recover the core (typical types are F, H, R, X).
	Sect	section number
	A/W	archive (A) or working (W) section half.
	Top offset on section (cm)	position of the SEM sample measured relative to the top of the section or section half.
	Bottom offset on section (cm)	position of the SEM sample measured relative to the top of the section or section half.
	Top depth CSF-A (m)	location of the SEM sample expressed relative to the top of the hole.
	Bottom depth CSF-A (m)	location of the SEM sample expressed relative to the top of the hole.
	Top depth [CSF-B] (m)	location of the SEM sample relative to the top of a hole. The location is presented in a scale selected by the science party or the report user.
	Bottom depth [CSF-B] (m)	location of the SEM sample relative to the top of a hole. The location is presented in a scale selected by the science party or the report user.
	Image link	link to URL of JPG, PNG, or TIF version of SEM image.
	Image filename	filename of SEM image provided for identification purposes.
	Image Metadata	link to URL of TXT metadata file of image.
	Category	Category of the specimen pictured in the image.
	Observable	Specific category describing the pictured specimen
	Magnification	user setting on the SEM when the picture was taken.
	Timestamp (UTC)	point in time at which the SEM picture was uploaded.
Instrument	SEM Scanning Electron Microscope (currently Hitachi TM3000).	
Text ID	automatically generated unique database identifier for a sample, visible on printed labels.	
Test No	unique number associated with the instrument measurement steps that produced these data.	
Comments	uploader's notes about the sample or image or specimen on the image.	

## Trouble Shooting

### Poor Image Quality

A variety of environmental, sample preparation, and instrument conditions can result in poor image quality. Please refer the possible causes listed below:

Environmental	Problem	Possible Cause	Solution

Sample Preparation	Problem	Possible Cause	Solution

Instrument Conditions	Problem	Possible Cause	Solution
	Receive error message indicating filament 'burned out' and no image is produced	Filament has burned out	Replace filament. See Replace the Filament below

## Maintenance

### SEM

#### Add Expedition Specific Lists to SEM Uploader

If the expedition has sub lists for the parameters listed below, the sub list name can be given to the developers:

- Fossil (fossil\_group)
- Lithology (principal\_lithology)
- Composition (composition)
- Texture (texture, sorting, porosity)
- Alteration (alteration)
- Structure (structure\_group, structure)

The sub list name is what is defined in the sub list excel workbook and entered in the config sheet (ie "princ\_lith\_354" or "macrofossil\_356"). These sub value lists equal the expedition specific subcategory in the SEM Uploader. Adding them to the table that creates the drop-down lists in the SEM Uploader will result in the same values in the "Value" drop-down (Fig XX) in that program as they appear in DESClogik in the description column.



11. Replace the head, gently tighten the fastening screws, connect the cables, and close the cover.

Also note that there is an ACE200 youtube video where the loading of the thread can be seen at <http://www.youtube.com/watch?v=yKd1IyKIDT8>).

## 3D Imaging

1. In the TM3000 software, display the specimen to be scanned as you would for collecting 2D images. To collect the set of four images and text files for generating a 3D image:

File > 3D- Image Capture...

Four obliquely illuminated images will be collected at the cardinal quadrants (N, E, S, W). You will be prompted to save this in a particular folder. To organize your files, follow a filing and naming convention as in acquiring 2D SEM images (i.e. Expedition > Site > Hole > Core > Section > Interval > Sample).

1. Open 3D-Viewer
  - a. Import the obliquely illuminated images collected in Step 1.

File > Automatic Import > (select the folder images and text files)

Once the images are imported, you can explore the available functions and buttons to extract 3D parameters of the model. You can define vertical, horizontal or free-hand transects across the image, and the relief profile will be displayed in the sub-window at the bottom. Parameters such as surface roughness will also be displayed.

1.
  - a. To create a 3D model:

Analyze > Open 3D view

Notes:

1. Attempts to create 3D models of sputter coated specimens were unsuccessful, possibly due to the distortion in the apparent contrast of the obliquely illuminated images.
2. In step 1, when acquiring the 3D images, the TM3000 software often complains of the specimen being out of focus. We did not have time to investigate the optimum distance ideal for this 3D imaging routine.