

Supplemental SEM Procedure for the JEOL JSM6380-LV

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Supplemental Procedure for SEM

This procedure does not replace the hands-on training with the Microscopy Center Manager or an approved representative. Appendix 1 gives you examples of your Accelerating Voltage, Spotsize, and Working distance. See Appendix 2 to see examples of the words that are in bold within the instructions.

Machine Setup

1. Turn on the JSM5000* software.
2. Turn on **IR Camera** (to be sure that nothing there is the chamber and that it is clear to open chamber door).
3. Make sure the stage **Z** is lowered to at least 25 to 30mm as indicated on the right of the **Z** stage control.
4. **Vent** the system on front panel – press and hold **VENT** until the button lights up, it will flash until the system is vented and then the light will be solid. Or you can click on **Sample** in blue in the software and click on **Vent**.
5. Open the **chamber door** by grasping it from the sides and pulling out.
6. Mount your **sample holder** on the stage. Making sure the **dovetail** on the sample holder slides firmly onto the **mount holder** and is pushed on completely to make sure center on the sample is the same as center on the stage. The large flat side of the **sample holder** should be at the top when sliding onto the **mount holder**. Making sure the **sample holder** is on correctly is very important because this helps ground \perp your sample.
7. Take a picture of your sample at this stage, what your sample looks like to the naked eye is much different than when looking through the scanning electron microscope. This will help you to better visualize where on the sample you are when looking under the electron gun.
8. Check to make sure sample is low enough that it will not make contact with the column and then push the **chamber door** closed carefully.
9. While continuing to push gently on the **chamber door** (this makes sure the vacuum pump does not pull on room air and backflow oil into the chamber) press the **EVAC** button on the front panel until it lights or **EVAC** on the **Sample** in blue in the software. Hold the door closed until you hear the pump come on.
10. Let the system pump down for 15 minutes to insure a stable environment and better imaging results
11. Set the accelerator voltage, **ACC VOLT**, **Spotsize**, and **Aperture** according *Appendix 1*. Generally you can start with an **Aperture** of 2, **5kV**, and **35 spotsize**
12. Make sure the **Magnification** is set to the lowest magnification.
13. Turn **HT** on (in the upper left corner). If the image is either too bright or too dark, press **ACB** (auto contrast brightness) to make viewing the sample easier.
14. Open the **Stage** Icon in the set of icons on the right in blue. Select **sample holder** you are using, generally one of the offerings on the top row. If using the single holder press **STAGE – INITIAL POSITION - GO** to center the sample. Remember that the image you see is rotated 90 degree clockwise in relation to where it was when putting your sample in the chamber. So the left side of your sample is now at the top when moving the stage.
15. Click on **WD** in the bottom of your screen towards the left. Select 10mm for your working distance. Ideally the working distance is between 8mm and 10mm for the best resolution and EDAX detection. Selecting 10 mm does not move the stage in the Z direction, it only sets the lens focal length to 10 mm, you will need to turn the **Z** knob

counter-clockwise to bring the stage up to meet that 10 mm focal length. If at any point the image is too bright or dark, select **ACB** along top of screen, or the two **Brightness** and **Contrast** knobs on the **manual adjustment pad**.

Preparing for Imaging

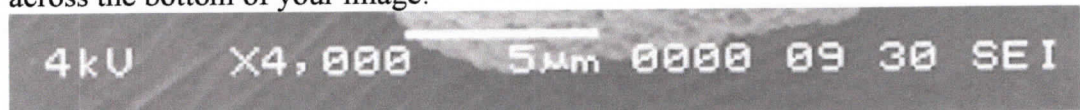
16. There are seven scan modes but generally only 4 or 5 that are used.
 - a. **Scan 1** is used for focusing your sample. It creates a small window within the **Image Display Area** that has a real-time imaging. Fine **Focus** should be done in **Scan 1** only.
 - b. **Scan 2** is the entire view of the **Image Display Area** based on your magnification. This scan is used to move around on your sample. It should never be used for fine **Focus**.
 - c. **Scan 3** is a 10 second line by line scan of your sample. This will give you a much cleaner image than **Scan 2** but is difficult to move around the sample when using it. Use **Scan 3** to get a better idea how well your image will look before committing to **Scan 4** or **Photo**.
 - d. **Scan 4** and **Photo** are for taking and saving images. **Scan 4** is a line by line scan that takes 80 seconds and gives a high resolution image. **Photo** is also a line by line scan but takes 180 seconds and gives an even higher resolution image.
17. Starting in **Scan 2**, do a coarse **Focus** on your sample using the right mouse button and clicking on **Focus**, keeping **Focus** and the right mouse button depressed while moving your mouse up and down until you achieve the best focus. (or use the knob on the **manual adjustment pad**, Page 11). Look for an area on your sample to do a fine **Focus**. Look for small distinct edges to do a fine **Focus**, soft edges or spheres do not make for good focal points. Cracks, points, or distinct holes are ideal for fine focus. Once you've chosen an area double click on it in the **Image Display Area** to center it. Use coarse focus to bring the sample into focus. (Coarse **Focus** can only be done on low magnification). Once you have chosen the object to focus on click on **Scan 1** and bring your magnification up as high as you can while still being able to see the object you are trying to focus on. Ideally you will focus at 15 times the magnification you will be imaging at. Now you will click on **Focus** using the left mouse button and keeping the mouse button depressed while you move the mouse up and down to find the best focus. **Note:** especially during fine focus, if the focus becomes better when moving in one direction but you hit the limit of focus in that direction. Let go of the mouse button at the edge of the window and then click on **Focus** with the left mouse button again and continue movement in that direction until your focus improves.
 - a. If this is the first sample you have done or if you changed the aperture you must do a **Wobble, Stigma X** and **Stigma Y** to get the best focus. *Wobble should only be attempted by someone who has been trained, ask for help from the microscopy center manager or approved representative if you need help.* Click on **Wobble** the image should appear to be breathing in and out, if it is moving side to side or up and down you will need to adjust the **X-direction** fine adjustment knob and the **Y-direction** fine adjustment knob to stop the movement. These fine adjustment knobs are located on the column along with the **Aperture selection knob**. The movements on the X & Y-fine adjustment knobs are very minor movements. If you move these knobs too much you will lose the aperture hole and the Microscopy Center Manager or a representative will need to be called to fix it.

Once you have stopped the x and y movement press **Wobble** again to close the window. Then immediately press **Lens Reset**.

- b. Now that the stigma has been corrected mechanically you will now need to refine it electronically. If your sample is really out of focus from the **Lens Reset** process, you can do a coarse **focus** followed by a fine **focus**. Once you have completed the fine **Focus** you will use the same procedure on the **Stigma X** as you do when doing **Focus**, pressing the left mouse button while clicking on either **Stigma X** or **Stigma Y**. Once the image is as good as you can get it do the **Focus** one more time. This should be performed in **Scan 1**.

18. Capturing the Image

- a. Consistency is key for research and deciding the magnification(s) in which you will taking images is crucial. Standard suggestion is to take a low magnification image, an intermediate magnification and a high magnification. Of course you can decide how many images and at what magnifications are important for your research.
- b. Frame your image as you would like to save it. Move the stage so that the object you want to image is centered horizontally and up slightly vertically (this is to make room for the image information on the bottom). **Press Scan 4 or Photo** to take the image. When the scan is complete the screen will freeze and the scanning information will be on the bottom of the **image display screen**. This will display across the bottom of your image:



Included from left to right is the voltage used, the magnification, the scale bar for the image, the number of images (if set up to record), the working distance, the spotsize, secondary electron (other option is backscatter).

19. To finish, turn **HT OFF** (top left corner).
20. Lower Z to 25 or greater.
21. Wait 10 minutes for the chamber to cool before opening the chamber, introducing cold air immediately can cause condensation on the chamber.

Other useful items

Backscatter – See page 7

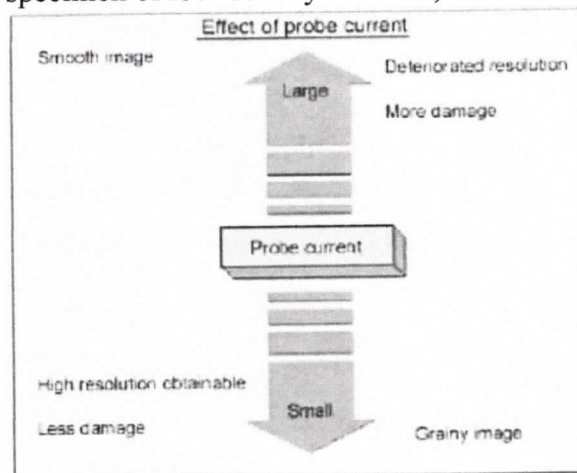
Low Vacuum Mode – See page 7

Scan Rotation – See page 11

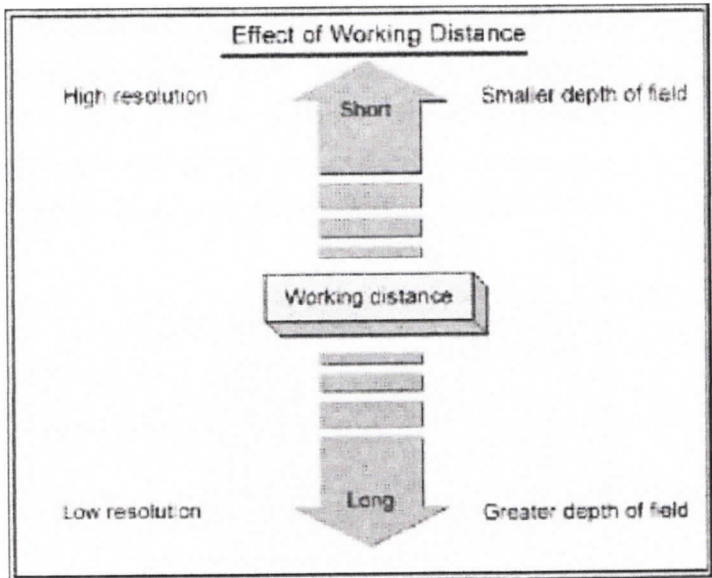
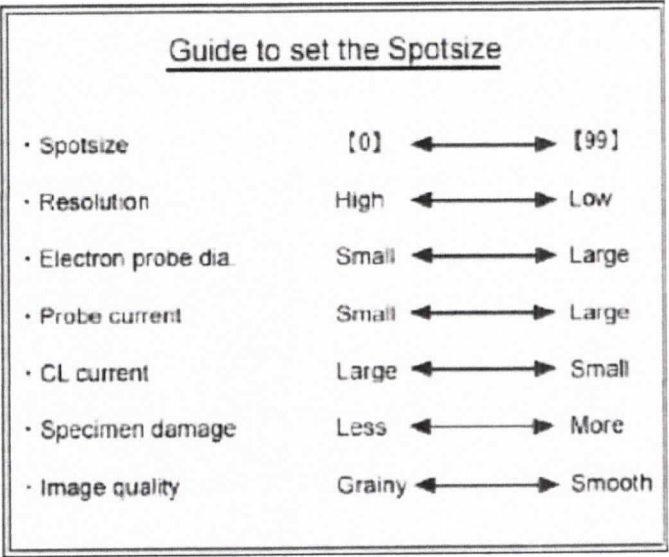
Appendix 1

Choosing Operating setup

1. Choose the correct aperture on the column for the sample you are working with. Note the beam at higher **kV** damages the aperture so for **Apertures 1 and 2** use 10**kV** or lower.
 - a. **Aperture 1** is 20 μ which gives higher resolution and a greater depth of field but can give you a much grainier image.
 - b. **Aperture 2** is 30 μ and decreases the resolution and depth of field slightly. This is the most used aperture on the machine. Use this aperture if your image using **Aperture 1** is too grainy.
 - c. **Aperture 3** is 100 μ and is used for using higher **kV** or EDS x-ray analysis. The disadvantages of the higher **kV** are; unclear surface structures, sample damage, increased charge-up, and more edge effect.
2. Selecting the **kV** and **spotsizes** is more subjective and here are a few general suggestions. When working with samples that are easily damaged by the ion beam it is suggested that you start with 5 kV and a spotsize of 30. For metals and samples that are less affected by the ion beam you can start with 10 kV or higher and again, start at a spotsize of 30.
 - a. From the JEOL Operating Manual for accelerating voltage – When you use a lower accelerating voltage, the details of microscopic structure of the specimen surface appear more clearly. When using a high accelerating voltage, the illuminating electrons can reach deeper inside the specimen and as a result unnecessary signals generated from the inside of the specimen (backscattered electrons, for example) lower the contrast, thus hiding the details of microscopic structure of the specimen surface. For this reason, especially for observing a specimen of low-density material, a low accelerating voltage is desirable.



- b. From the JEOL Operating Manual for spotsizes – As the electron probe diameter (the spotsize) becomes smaller, higher magnification and so higher resolution are obtained. However, image smoothness, that is, the S/N (signal/noise) ratio of the image, depends on the illuminating current. When you try to reduce the probe diameter, the probe current reduces as a result. Therefore, an appropriate illuminating current must be selected according to magnification, observation conditions (including accelerating voltage, tilting of specimen and others) and the specimen itself.



Appendix 2

Backscatter

Backscatter electrons differs from secondary electrons due to the high number of secondary electrons verses the lower number of backscatter electrons. For this reason the beam current for backscatter should be 5 to 10 times greater to insure adequate signal-to-noise ratio. When scanning using secondary electrons all electrons are shown equally, for this reason the image has greater resolution. When imaging in backscatter the lighter the element the darker it appears and the heavier the element the lighter it appears. This is because the heavier the element the more backscatter it produces. Multilayer metals with oxidation are good candidates for backscatter because it essentially reduces the washout of the image caused by the oxygen.

When using backscatter:

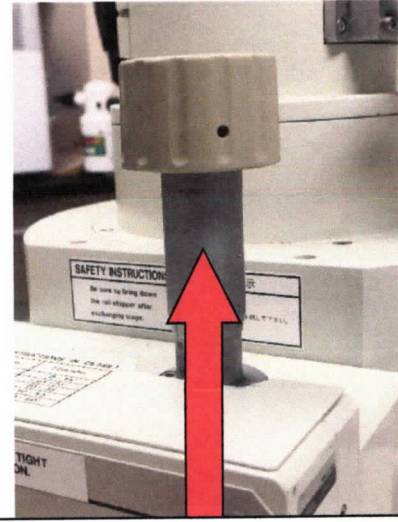
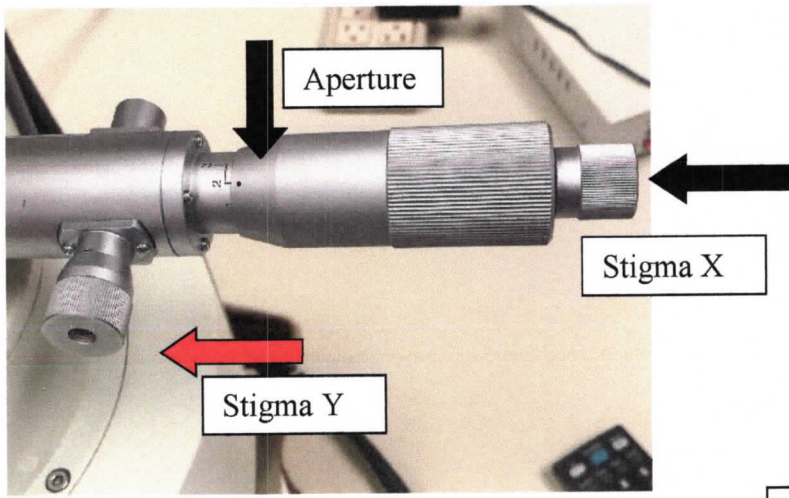
- The average **working distance** should be between 10 mm and 20 mm.
- The **spotsize** should be between 30 and 60.
- You should use **aperture 2** or 3.
- Turn off the **IR camera**. The backscatter will not work if the **IR camera** is on.
- The **signal** should be set to BEI

There are 3 modes of backscatter, Compo, Topo, and Shadow. Please get with the Microscopy Center Manager or approved representative to determine which mode is best for your sample. Any time you switch to backscatter or within backscatter modes you must press **ACB**.

Low Vacuum

Low Vacuum is best when used on samples that cannot be coated or cannot withstand the high vacuum environment. The machine is capable of running in a vacuum of 10 to 130 Pa, this low vacuum has a poor signal-to-noise ratio making imaging difficult and is suited to magnification lower than 3000x. Low vacuum assists in eliminating the effects of charge accumulation on the surface and to preserve fragile structures. Low Vacuum imaging is done using the backscatter shadow mode (BEIW).

- Set the accelerating voltage to 15kV.
- Biological specimen - pressure 50 to 70 Pa, **spotsize** 30-60.
- Dry paper, cloth, resin, etc. – pressure 20 to 30 Pa, **spotsize** 30 to 40.

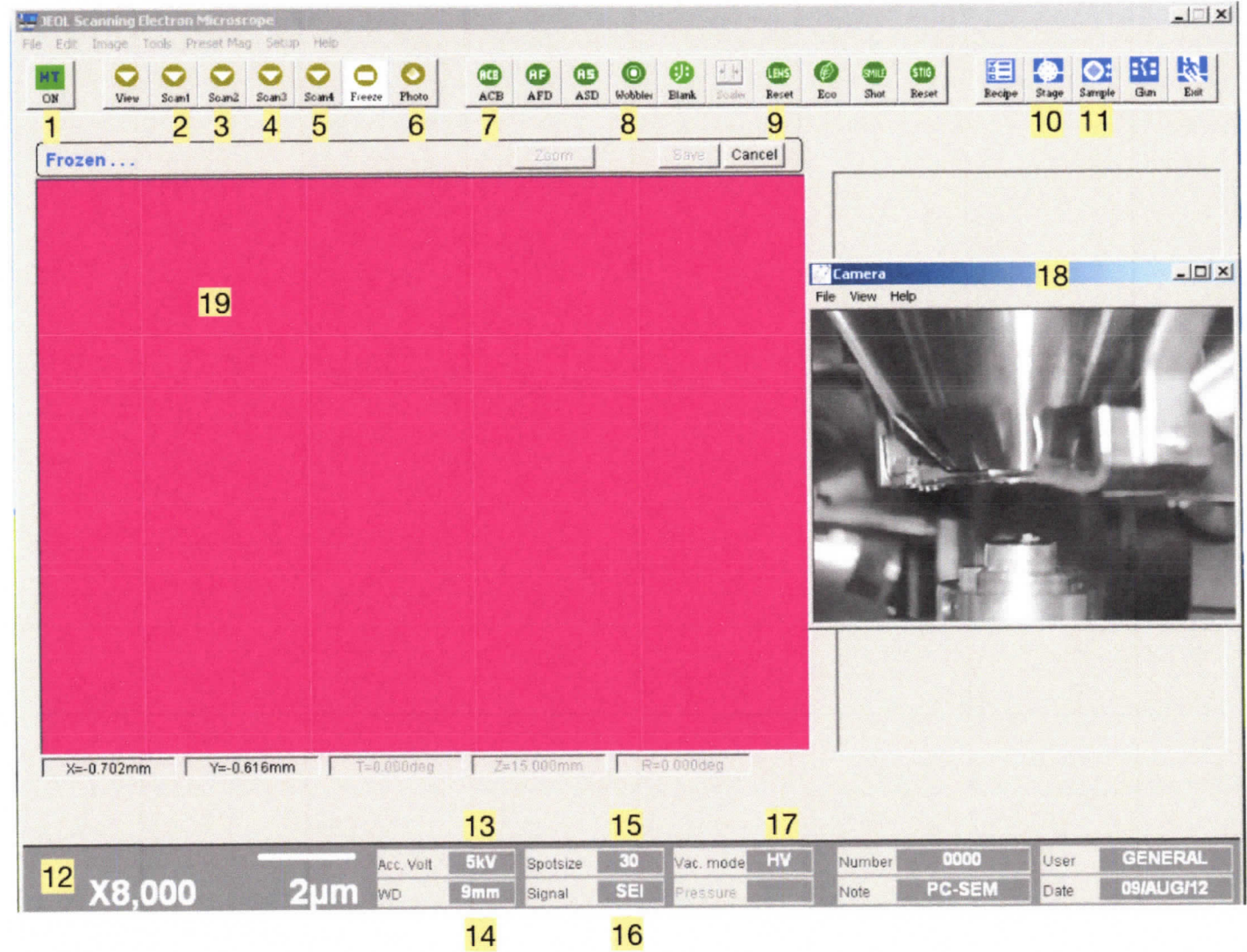


Z knob
 Clockwise lowers the stage.
 Counter clockwise raises the stage.

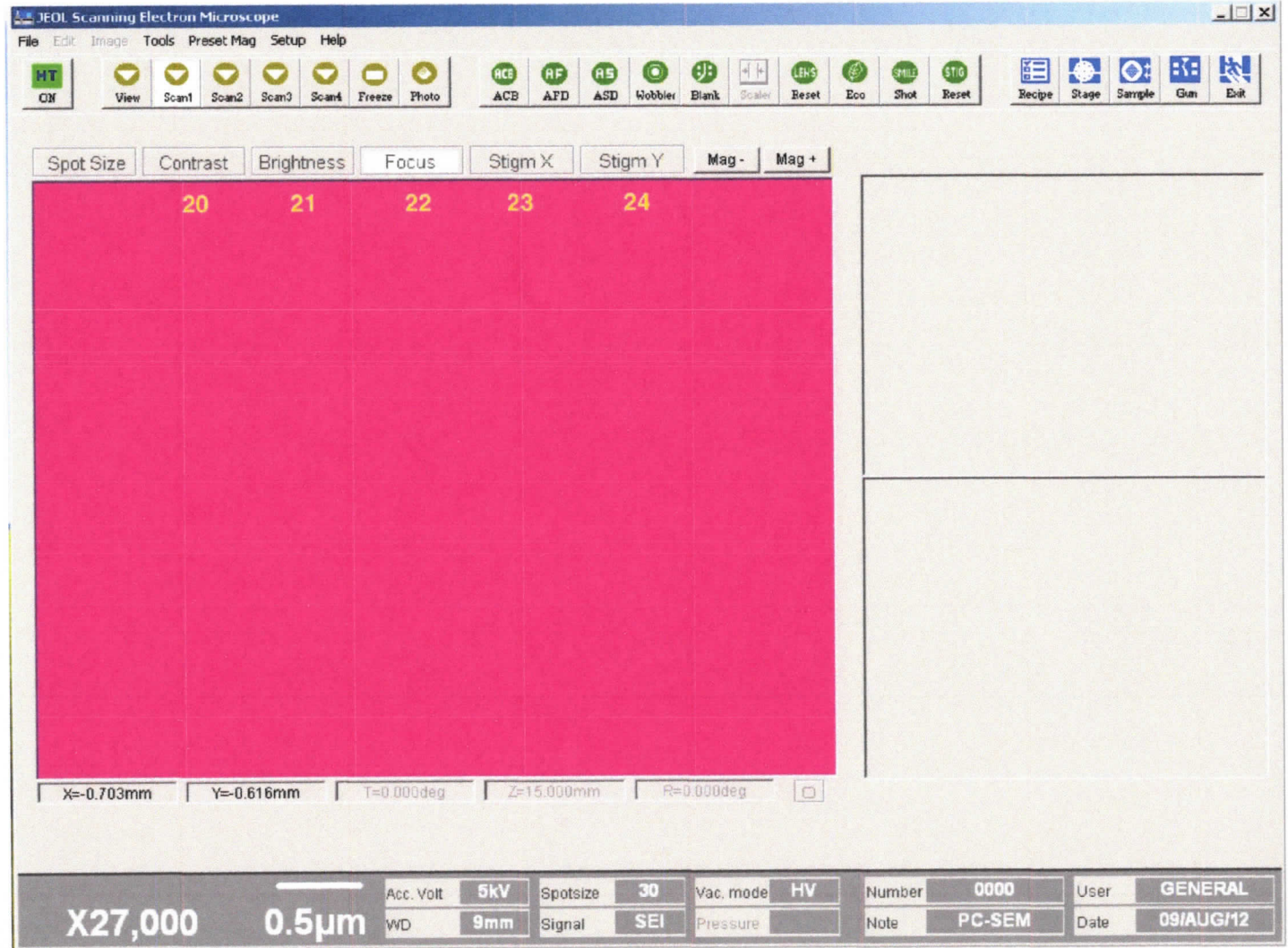


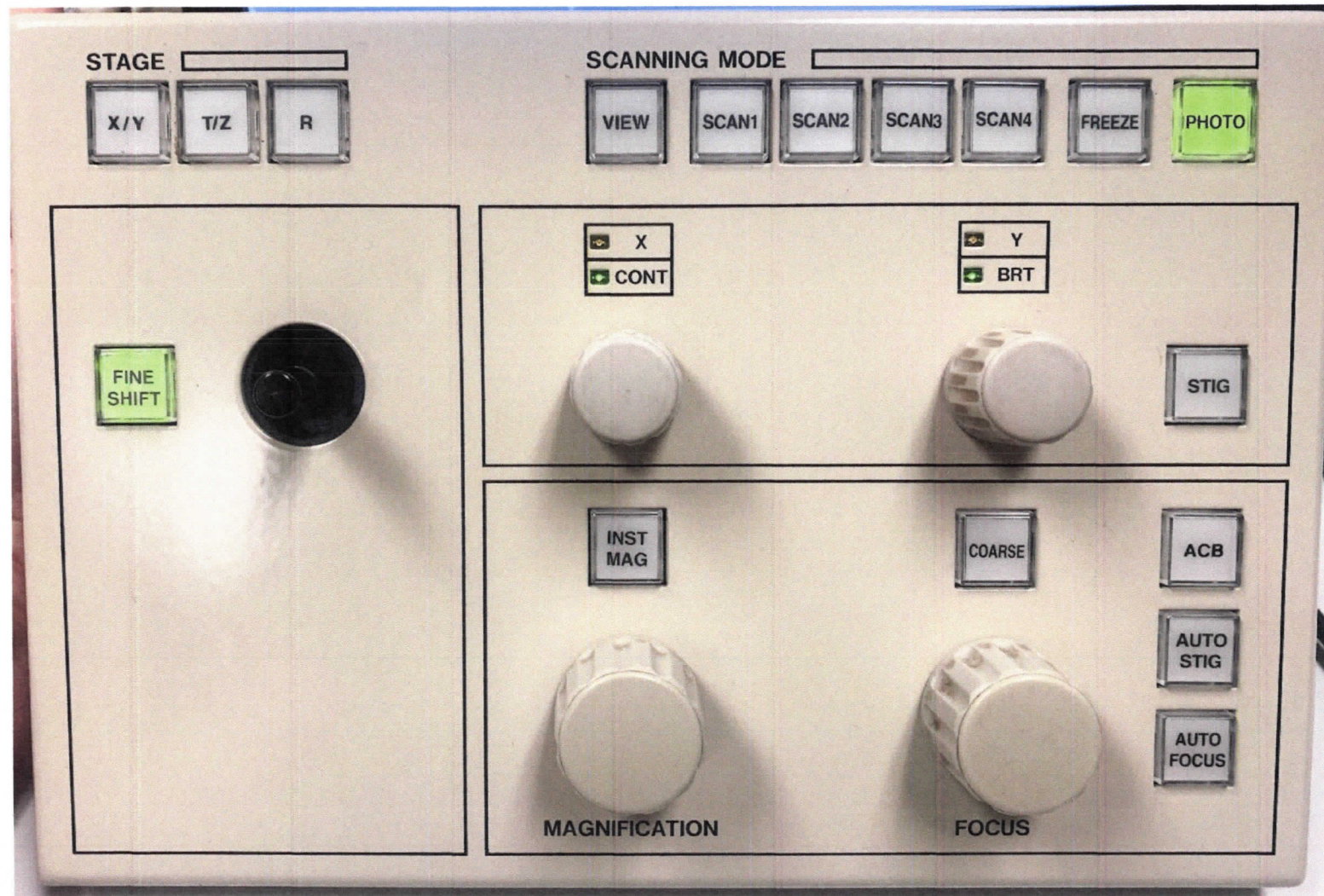
Front Panel: VENT, EVAC, & Low Vacuum

- 1 - HT On/Off
- 2 - Scan 1
- 3 - Scan 2
- 4 - Scan 3
- 5 - Scan 4
- 6 - Photo
- 7 - Auto Contrast/
Brightness
- 8 - Wobbler
- 9 - Lens Reset
- 10 - Stage
- 11 - Sample
- 12 - Magnification
- 13 - Acceleration
Voltage
- 14 - Working
Distance
- 15 - Spot Size
- 16 - Signal
- 17 - Vacuum Mode
- 18 - IR Camera
- 19 - Image Display
Area

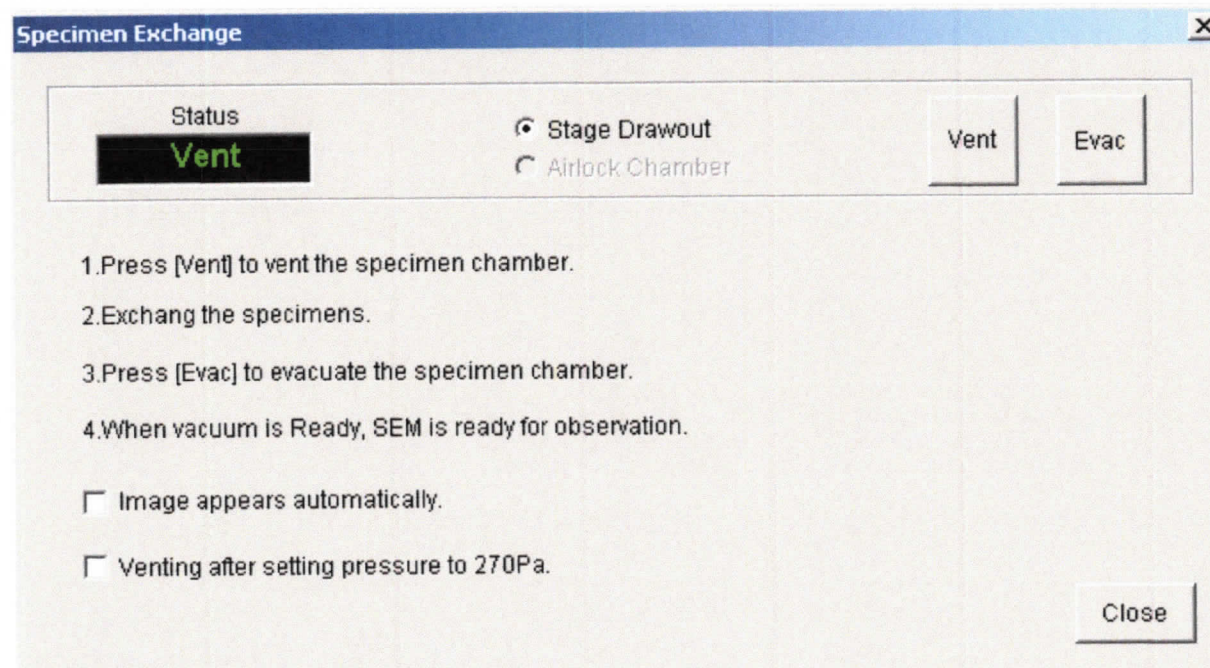


- 20 - Contrast
- 21 - Brightness
- 22 - Focus
- 23 - Stigma X
- 24 - Stigma Y

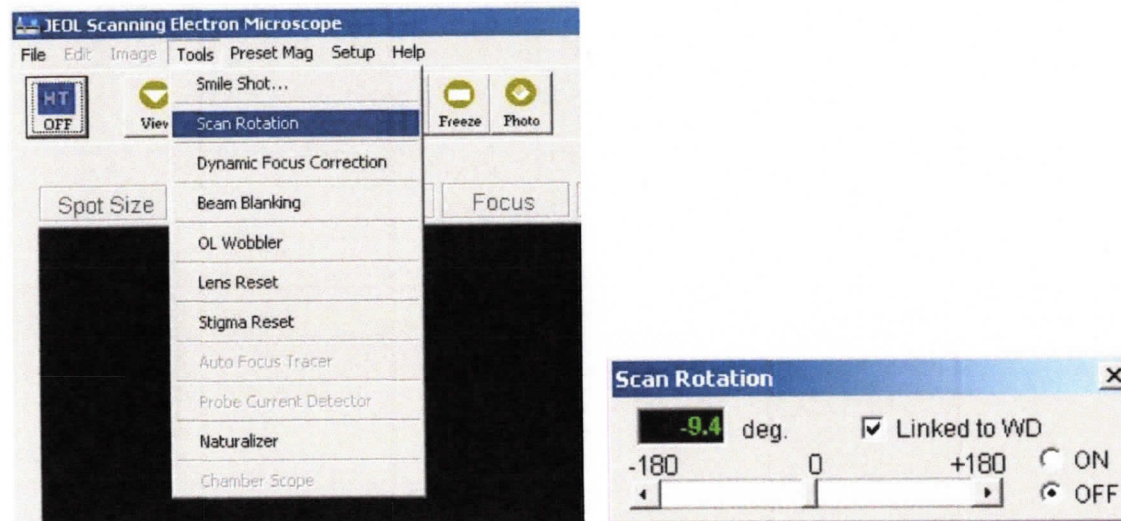




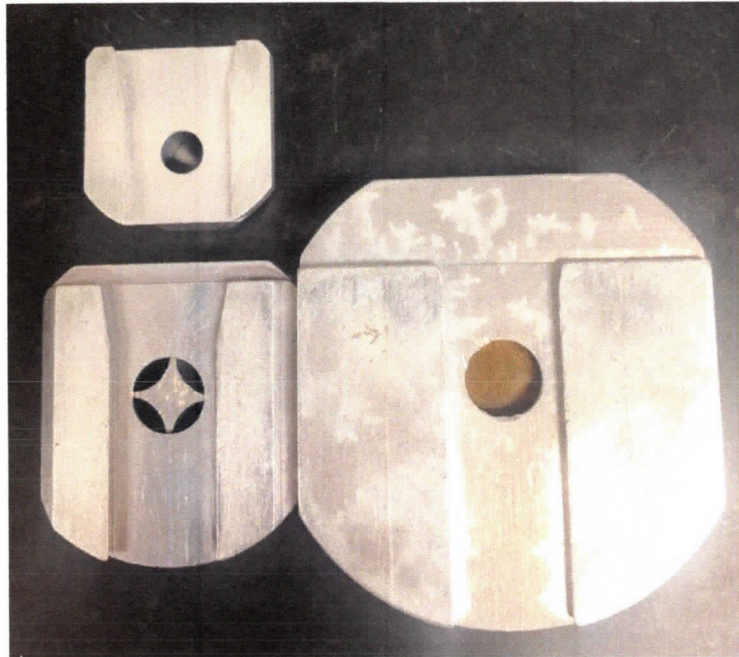
Many of the things you can do in the software can be done using the **Manual Adjustment Pad**. Several of the knobs and the joystick can have different functions based on the selection of the following buttons. When the **Course** button is lit, the knob below it is in course focus, if it is not lit, the knob functions as a fine focus. While the course focus should only be turned a limited amount, on fine focus the knob must be turned many times one direction or the other to see a difference. When the STIG button is unlit the knobs to the left are used for manual contrast and brightness, when the STIG button is lit then the knobs function as Stigma X and Stigma Y. If fine shift is lit then the joystick will only move the stage a finite amount. If instead you select the X/Y button the joystick can move the stage to its maximum allowed distance. The T/Z and R buttons are not available functions on this system.



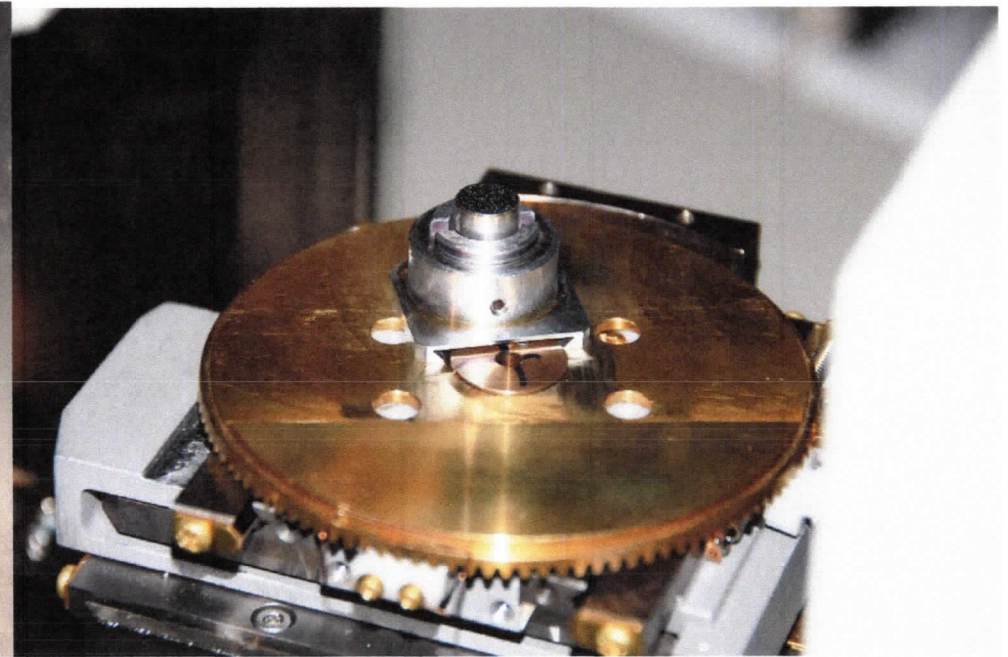
Sample popup



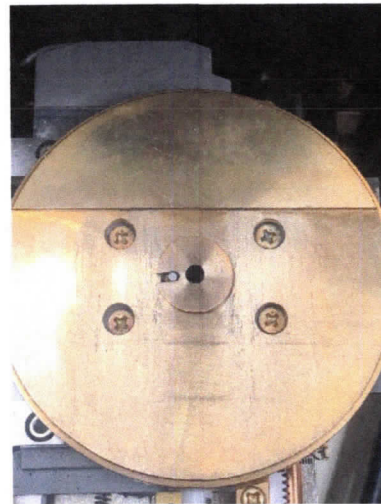
If you want all your images oriented in the same direction you can use **Tools – Scan Rotation** to orient them. Remember to turn it off when you finish because it can confuse the next person when they think they are moving to the left but because you left scan rotation on they are moving up.



Dovetail on bottom of sample holders



Stage turned for image to show how dovetail fits onto the stage.



Stage inside the SEM, the dovetail must be placed on the circular holder and the flat of the sample holder pushed toward the flat on the stage.