TESCAN SEM Training Requirements and RaDar Calendar System Set-Up

- 1) All users of the SEM (and EDS) are required to receive hands-on training by Dr. Cullen. Training is held in groups of 2-4 students. Send an email to schedule training: Daniel.C.Cullen@dartmouth.edu.
- 2) Once you've received SEM training you'll need to request a RaDar account with Tescan access to make reservations.
 - a. Send one email addressed to both ccops@dartmouth.edu and Maxime.J.Guinel@dartmouth.edu (cc'ing Daniel.C.Cullen@dartmouth.edu) from your official Dartmouth email address.
 - i. Example email:

Subject: RaDar and Tescan Account Set-up Hello,

Please set-up up a RaDar account for me to use the Tescan Vega3 SEM. I've completed hands-on training on the Tescan Vega3 with Dr. Cullen. I am an/a undergraduate/graduate student/post-doc. [choose the correct classification. If you are a graduate student or post-doc you must provide an account string to be billed or an account will not be set-up]

Thank you, Jane Smith

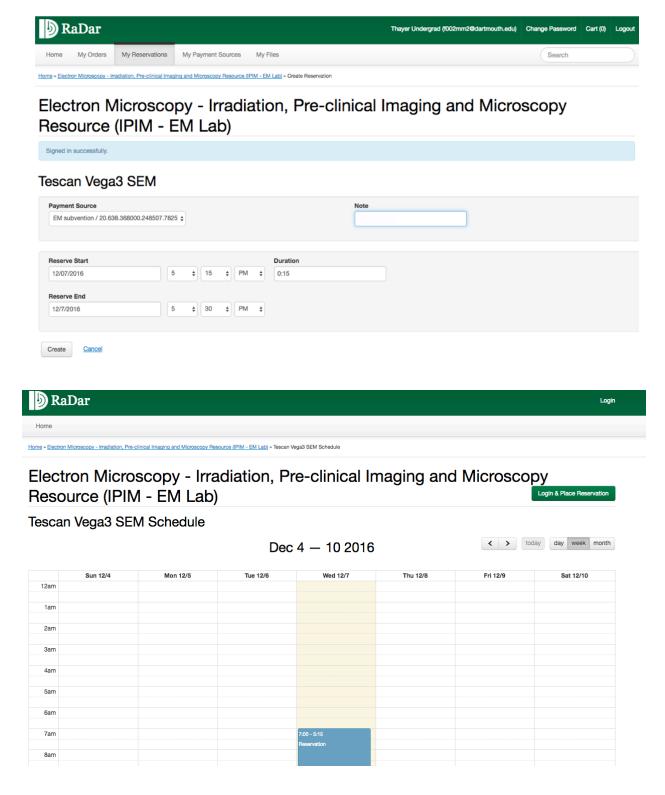
Important Notes:

If you are an undergraduate student you may receive billing invoices, but you can ignore these - you will not be billed for SEM use.

The set-up process should take 1 to 2 days and occurs during College business hours.

- 3) Once you have a RaDar account set-up with Tescan access, reserve time at: https://radar.dartmouth.edu/resources/emlab/instruments/vega3/public schedule
- 4) Rules to follow when reserving the SEM
 - a. You can have only one active reservation at a time. This gives everyone a chance to use the SEM. When you show-up to use the SEM, make your next reservation.
 - b. Standard reservations are usually 2-hours or less.
 - c. The SEM is undergraduate instructional equipment and therefore classes and undergrads have first priority using it. Graduate students may be bumped during busy periods; these generally run January through August not including term breaks. Plan your research accordingly.

Note: No modifications of any kind can be made to the SEM without express permission from Dr. Cullen and/or Prof. Levey.

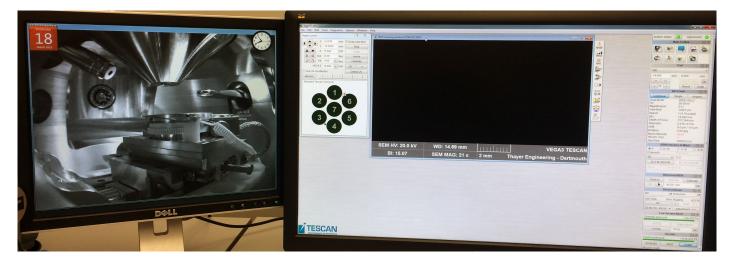


TESCAN SEM Operating Instructions

1) Start the SEM Software

- a) Sign-in to paper log-book.
- b) Start the VegaTC software if not already running.
- c) Login to the VEGA3 software using your account.
- d) Once your account opens, you should be presented with the **Scanning, Stage Control**, and video **Chamber View** windows (similar to image below).





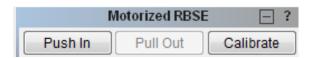
e) If the **Scanning**, **Stage Control**, or **Chamber View** windows are missing, turn them on by clicking on the **Main Toolbar** icons.



Important: Due to driver issues, the SEM chamberscope is not working on the SEM computer. When you start the SEM software you will get a camera error message. Close the message and login to the EDS computer APEX account. The chamberscope will now be visible on the EDS computer. If you do not see a camera window on the EDS computer, reboot the EDS computer and then login to the APEX account again.

2) Verify that the Backscatter and EDS detectors are fully retracted

a) The backscatter detector (**BSE**) must be in its fully retracted position. On the **Motorized RBSE** panel, **Pull Out** should be greyed out as shown below. If not, push the **Pull Out** button.



- b) The Energy Dispersive Spectroscopy (EDS) detector must be retracted to the fully retracted stop anytime the chamber door is opened or closed, or the system is actively pumping down from atmosphere. Even if you plan to use the **EDS** detector it must be retracted.
- c) If the detector is not in the fully retracted position, turn the handle clockwise to retract the detector (see the EDS detector image below).



3) Mounting Your Specimen

- a) There are numerous mounts available for you to use with your specimens.
- b) Choose the appropriate mount and wearing gloves (the orange powderless ones provided), mount your specimen onto a pin-stub, or an optional mount, at the lab bench.

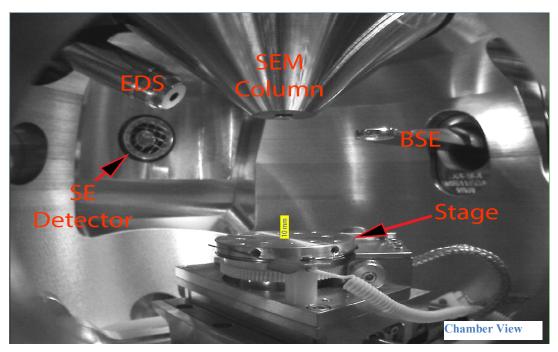


c) Gloves must be kept on until your specimen is installed in the SEM chamber and the system is pumping down.

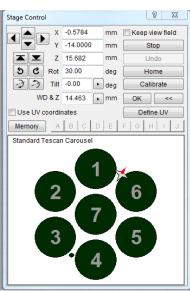
Note: If your specimen is non-conducting you will either have to coat it with gold using the Hummer 6.2 (both SE and BSE imaging modes will be available), or operate the SEM in Medium-Vacuum mode where only BSE imaging is available.SE and BSE modes are described in **Appendix A**; Medium-Vacuum mode in **Appendix C**.

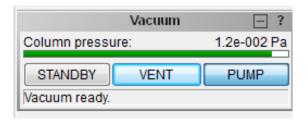
4) Vent the SEM chamber, Install your Specimen, Pump-down

a) Familiarize yourself with the location of important components in the SEM chamber using the **Chamber View** image shown below.

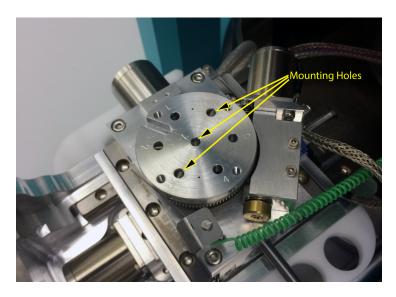


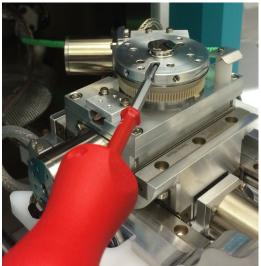
- b) Move the stage to its **Home** position.
 - i) If the **Chamber View** window shows that the stage is empty, or has a standard 10 mm or shorter specimen installed (see the yellow scale-bar in **Chamber View** image above), return the SEM stage to its home position by pressing the **Home** button on the Stage Control panel.
 - ii) If a taller than 10 mm sample is installed, or any specimen holder other than the standard SEM pin stub is installed, then manually place the stage in a safe position for venting. On the Stage Control panel (see image to right):
 - (a) Set **Z** to 50 (mm).
 - (b) Set **Tilt** to 0 (deg.).
 - (c) Set **X** to 0 (mm).
 - (d) Set **Y** to 0 (mm).
- c) Press the **VENT** button on the **Vacuum** panel (near the bottom of the Main Toolbar panels) to vent the SEM chamber. After about 30 seconds (when the nitrogen gas flow noise stops), gently pull the chamber door open.





d) Place your specimen(s) into the mounting hole(s) of the stage and tighten the set screw(s). Make the screw(s) snug, but do not over-tighten (light two-finger and thumb hold on the hex driver). Additionally, ensure that all unused mounting holes have their set screws fully inserted.



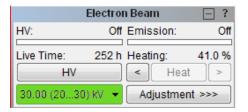


- e) Slowly push the chamber door closed while carefully watching both through the opening and on the Chamber View to ensure that your specimen does not hit anything in the chamber.
- f) Press the **PUMP** button on the **Vacuum** panel and ensure that the chamber door is closed by giving it a gentle push and then immediately trying to pull it open.
- g) Monitor the Column Pressure on the Vacuum panel until the pressure indication bar becomes green.

WAIT ONE ADDITIONAL MINUTE AFTER THE BAR TURNS GREEN TO ALLOW THE SYSTEM TO ACHIEVE A **HIGHER VACUUM.**

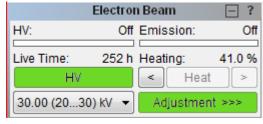
5) Turning on the Electron Beam (Energizing the High Voltage System)

a) There are four accelerating voltage indices (ranges). Select the accelerating voltage range you require from the drop-down menu under the HV (High Voltage) button on the Electron Beam panel. If you are unsure what accelerating voltage to use, start with 30kV.



Accelerating Voltage: The electron beam accelerating voltage used to analyze your specimen can be set between 200V and 30kV. The highest acceleration voltage yields the greatest signal-to-noise ratio, but there are many reasons to use a lower voltage such as:

- i. You specimens are subject to damage from the electron beam (plastics, composites, organics, etc.).
- ii. Your samples are charging (bright areas are appearing in the image where no detail can be seen), and/or your specimen image is drifting.
- iii. You are interested in the outer surface layer of your specimen rather than the bulk interior of your specimen (the higher the accelerating voltage the farther below the surface your signal is generated from).
- b) Turn on the high voltage by pressing the **HV** button on the **Electron Beam** panel.
- c) Set the Beam Intensity (BI) to 10 (a good middle value to start with) by selecting the BI button in the Scanning Window panel and then adjusting BI in the Pad panel.
- Right-click in the Scanning Window and choose
 Minimum Magnification. This is Wide Field mode.
- e) Right-click in the **Scanning Window** and choose **Auto Brightness/Contrast**.
- f) You should now have an image.





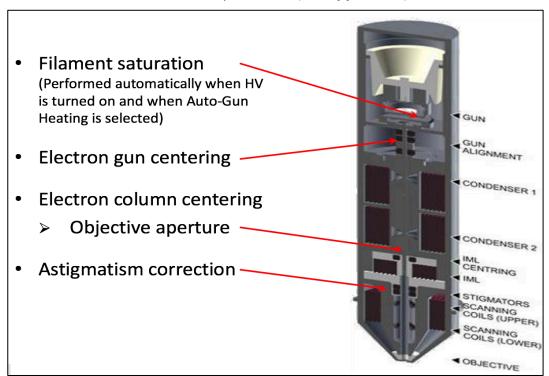
Note: The **BI** is a proxy for the diameter of the electron beam (spot size). It can be adjusted from 1 to 20.

A higher **BI** will increase the image brightness, thereby reducing the noise, but it will also reduce the resolution (sharpness) of the image. This will be particularly noticeable on higher magnification images.

g) Click on the **Speed** button in the **Scanning Window** panel (shown at right) and choose a scan speed. The higher the number the slower the scan rate and the less noise in your image, but the greater the lag when you move your specimen. For live images, speeds between 1 to 4 work well. If unsure, start with a scan speed of 3.

6) Image Optimization (Part 1)

The SEM will now need to be optimized to obtain a high-quality image. This entails setting the filament saturation point (Part 1), centering the electron gun (Part 1), and centering the electron column (Part 2). If going above ~10kx magnification, manual column centering and electron beam astigmatism correction will also need to be performed (see Appendix B).

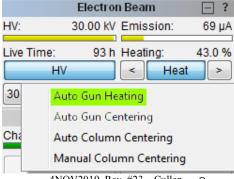




a) Automatic Filament Saturation and Gun Centering: Select the Adjustment button on the Electron Beam panel (shown at right) and choose Auto Gun Heating (which automatically also performs Auto Gun Centering) on the drop-down menu. This sets the filament

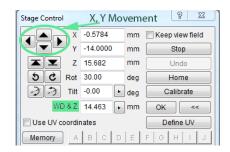
saturation point (heating) and also centers the electron beam to shoot straight down the column. (the process will take between 1 and 3 minutes).

Note: It is important to use a sample that creates good secondary electron contrast for **Auto Gun Heating** and **Centering** to work well. A sample that conducts well and has non-repeating topography (i.e. isn't featureless and doesn't have parallel machining marks) is best.



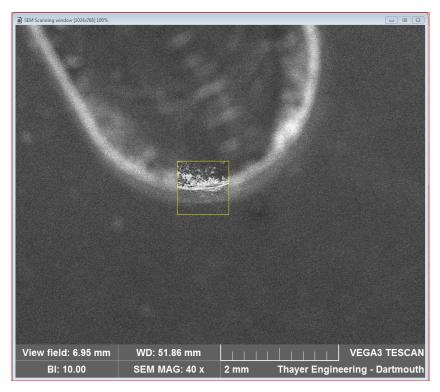
7) Changing to Resolution Mode and Focusing Your Image

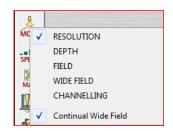
When you chose **Minimum Magnification** this puts the SEM into **Wide Field** mode. **Wide Field** mode allows multiple specimens, or all of a large specimen, to easily be seen and it puts the entire image into focus making navigation easy. While images can be collected using **Wide Field** mode the maximum magnification in this mode is quite small. To capture high quality images at high magnifications the SEM needs to be in **Resolution** mode.



- a) Click on the **Mode** button in the **Scanning Window** panel and choose **Resolution** mode (see image to right).
- b) Unless you are quite lucky, your image should now be out of focus.
- c) Find an area of varying contrast an edge or a dust particle works well

 and double-click on it with the left-hand mouse button. This will open
 up a small focusing window with a very fast refresh rate (see image
 below).





- d) On the keyboard press the W-key (labeled Focus-WD) and spin the trackball in the horizontal direction to focus the image. If you are having difficulty focusing, right-click on the image and choose Auto WD (if Auto WD is greyed out you are still in Wide Field mode).
- e) Once the image is focused, double-click outside the focusing window to close the window and put the entire image in focus.

8) Moving Your Specimen to the optimal Working Distance

a) Once you are in **Resolution** mode and in focus moving around your specimen becomes quite easy. Just click with the middle mouse button anywhere on the image to center that point in the image.

b) To get a better image you need to move your specimen closer to the SEM column (called the pole piece). Using the middle-mouse button click on the highest point on your specimen and refocus the image.

As you focus note the text information fields below the image. There is a **WD** value that changes as you focus. WD stands for Working Distance which is the distance in mm between the bottom of the pole-piece and the spot on the specimen that is being observed. It is important to note that the Working Distance is only accurate when the SEM is in **Resolution** mode and the image is in focus.

c) Once you are focused on the highest point of your specimen in **Resolution** mode you are ready to move your specimen to the standard WD used for imaging

CAUTION: You are about to move the stage. It is possible to make the stage and/or your SPECIMEN COLLIDE WITH AND DAMAGE THE POLE-PIECE, CHAMBER WALL, OR DETECTORS.

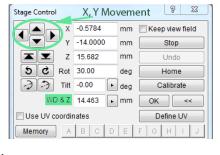
- Verify that the **Chamber View** window is open and shows no imminent contact between the stage/sample and the column, chamber wall, or detectors (see annotated **Chamber View** window on the next page).
- Watch the **Chamber View** window as the stage moves and be prepared to immediately stop the stage if contact will occur.
- The stage can be stopped by:
 - Pressing the keyboard spacebar.
 - Pressing the Return key.
 - Using the mouse to click on the large STOP STAGE button displayed in the middle of the computer screen during stage movement.

STOP STAGE

- d) Move your specimen to a **WD** of **10 mm**:
 - i) This step will cause the stage to immediately move be prepared.
 - ii) Select **10** from the **WD & Z** drop-down menu on the Stage Control panel.



Once you are at a WD of 10 mm, degauss the column by clicking on the **Degauss** button. This removes unwanted magnetism from the pole-piece.



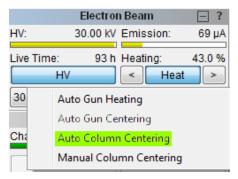
Degauss

f) Although the system tries to keep the image in focus with large **Z** changes you will likely need to refocus your image using the same steps from above. As before, when you focus **WD** will change. If **WD** is between 10 and 12 mm after you are in focus you are fine, if above 12 mm or under 10 mm, choose 10 mm again from the WD & Z dropdown and refocus your image.

9) Image Optimization (Part 2) and Magnification Changes

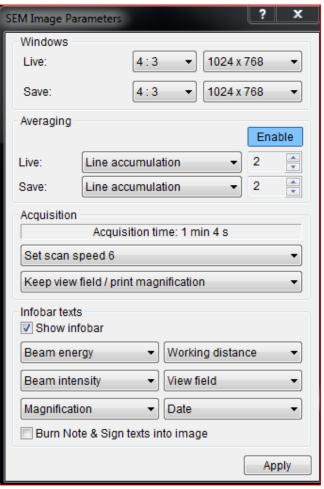
- a) To perform the second image optimization step, magnification should be between 2000x and 5000x. To change magnification:
 - i) Select the M key on the keyboard
 - ii) Spin the trackball horizontally to increase and decrease magnification
- b) Set the magnification to between 2000x and 5000x.
- c) Focus the image as best as you can.
- d) Perform **Automatic Column Centering** (Image Optimization Part 2)
 - Select the Adjustment button on the Electron Beam panel and choose Auto Column Centering on the drop-down menu (this aligns the SEM objective lens).
 - ii) The software will ask if you are using the special **ADJ specimen**. Select **Yes** as long as your sample creates good secondary electron contrast and has non-repeating topography (a metal ductile failure is ideal) **Auto Column Centering** will work fine and allow the process to complete (the process will take between 1 and 3 minutes).

Note: Image Optimization Part 1 and 2 will need to be performed for each of the HV indices that you use



10) Image Capture settings

- a) Choose SEM → Image Parameters from the menu bar (this is the only time you need go into the menus at the top of the screen when using the SEM other than to log-out).
- b) Note the settings in the Figure to the right. These are good standard settings for reports and lab notebooks. Bump the acquisition Scan Speed from 6 to 7 if you want a higher image quality, or down to 5 if a lower quality will suffice. A Scan Speed change of 1 will increase or decrease acquisition time by ~3x.
- c) The image settings necessary to collect a good image are very dependent on the specimen and SEM settings, so sometimes an acquisition Scan Speed of 5 will be more than sufficient to collect a good image, while at other times you'll need the acquisition Scan Speed to be at 7 to collect the same quality image.
- d) Microscope information, settings, and a scalebar appear at the bottom of your captured images. Most of what appears can be changed using the **Infobar Texts** selections.



11) Image Acquisition

- a) Frame the image that you want to acquire by moving the stage and adjusting the magnification.
- b) Adjust the Brightness and Contrast via:
 - i) Auto Signal (automatic brightness and contrast adjustment).
 - ii) Contrast/Brightness SE (manual adjustment) on the Scanning Window
- c) Increase the image magnification and focus the image at a higher magnification than you want for your acquisition. This ensures that your image is as well focused as possible when you collect it at the original lower magnification.
- d) Decrease the magnification back to your desired level.
- e) Press the **Image Acquire** button (this button always appears to be greyed-out, but it is active) on the bottom of the **Scanning Window** panel.



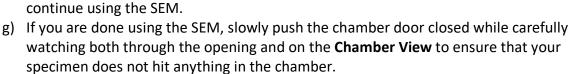
- f) Once the acquisition is complete, name the image and select a format tif or png.
- g) The image files can be found in the **Images** folder in your TESCAN user directory. Follow the **TESCAN Users** link on the desktop.

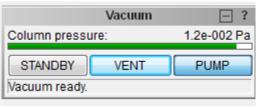
Note:

When you save SEM images two files are created, one ending in **tif**, or **png**, and another with the same name ending in **hdr**. The **hdr** file contains the SEM parameters and set-up used during the image capture. If all you want is the image you can ignore this file.

12) Specimen Exchange and/or Removal

- a) Turn off the high voltage by pressing **HV** in the **Electron Beam** panel.
- b) Verify/Retract the backscatter detector (**RBSE**).
- c) Verify/Retract the **EDS** detector.
- d) Return the stage to its initial **Home** position. As always with stage movement, be prepared to quickly stop the stage if a collision looks imminent.
 - i) Tall Specimen: If you have a tall specimen or non-standard specimen holder (as described in step 2c above):
 - (1) Enter **50** into the **Stage Control** panel **Z** field and then press OK
 - (2) When movement is complete enter **0** into the **X**, **Y**, **Rot**, and **Tilt** fields and press **OK**.
 - ii) Standard Height Specimen: If you have a standard height specimen and standard specimen holder press the **Home** button on the **Stage Control** panel.
- e) Press the **VENT** button on the **Vacuum** panel (near the bottom of the **Main Toolbar** panels) to vent the SEM chamber. After about 30 seconds, gently pull the chamber door open.
- f) Remove your specimen(s). Insert new specimen(s) starting at Step 4d if you will continue using the SEM.

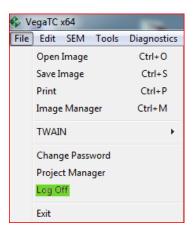




- h) Press the **PUMP** button on the **Vacuum** panel and ensure that the chamber door is closed (gently hold it in a few seconds until you see the column pressure start to decrease).
- i) Monitor the Column Pressure on the Vacuum panel until the pressure indication bar becomes green.

13) SEM Shutdown

- a) Once the pressure bars are green, log-out of the TESCAN SEM control software by choosing File in the menubar and then Log Off (see image to right).
- b) Leave the VEGA3 software at the login screen as shown below. Do not close this window as the VEGA3 software monitors the SEM vacuum system in the background as long as it is left open.
- c) Transfer your files using the ThayerFS, Thayer Dropbox, or Dropbox links on the desktop. Thumb drives are discouraged as they are malware vectors.
- d) Log your finish time in the paper logbook.
- e) ALWAYS leave the SEM powered-on and under vacuum.





Bibliography

TESCAN VEGA 3 manual and training materials.

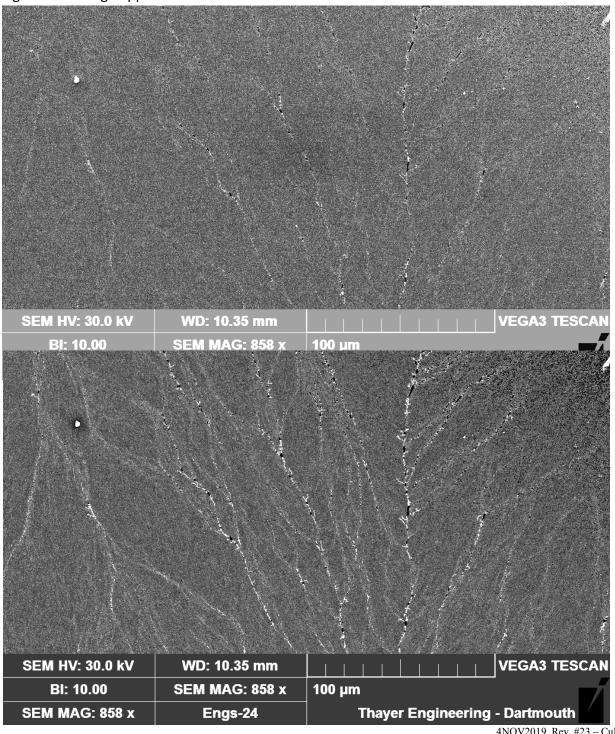
Goldstein, Joseph, et al. Scanning Electron Microscopy and X-ray Microanalysis – Third Edition, Springer Science and Business Media, LLC, 2003.

Appendix A

BSE and Combined SE/BSE Detector Operation

SE: Low energy electrons; good for revealing topography contrast.

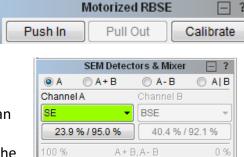
BSE: High energy electrons; good for atomic number contrast - the higher the atomic number the lighter the image appears.



An example of the two signal types can be see in the GeSn images on the previous page. The top image was taken in **SE** mode, but because the Sn dendrites are relatively flush with the Ge they do not show up very well. In the bottom image the Sn dendrites show up much better in BSE mode as Sn has a much higher atomic number than Ge.

BSE Procedure

- 1. Fully set-up the SEM according to the main SEM procedure (TESCAN SEM Instructions) through **Step 10**.
- 2. Ensure that you are focused on the highest point of your specimen and that **WD** is \geq **10 mm**. The BSE detect will take up ~5 mm of this available 10 mm distance.
- 3. Insert the BSE detector by pressing **Push In** on the Motorized RBSE panel.
- 4. Using the **SEM Detectors & Mixer** panel you should set **Channel A** to **SE** and **Channel B** to **BSE**.
- 5. Next choose **A** + **B**, **A B**, or **A**|**B**.
- a. A + B sums the SE and BSE channels together. The percentage that each channel contributes to the image can be selected using the slider at the bottom of the panel.
- b. A B takes the difference between the channels. Again, the percentage difference is adjusted using the slider.
- c. A|B presents the SE and the BSE images side-by-side in different windows. Note: Contrast and Brightness are set individually for SE and BSE images.
- 6. Continue with **Step 11** in the main SEM procedure.
- 7. When you are done examining your sample, retract the BSE detector by selecting **Pull Out** on the **Motorized RBSE** panel.



Appendix B

Manual Column Centering and Astigmatism Correction when using the SEM Above ~10kx Magnification

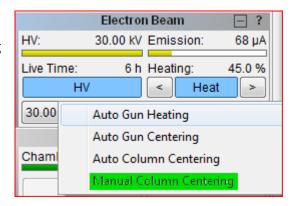
Note: All procedures below assume you are at or above 10kx magnification.

Manual Column Centering

Column centering should be performed manually at higher magnifications. Column centering can be verified by adjusting the focus (WD); if the image moves while changing focus then the column needs to be centered.

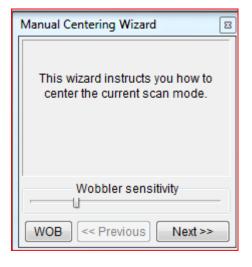
Manual Column Centering Procedure

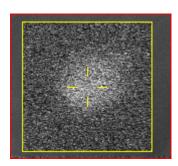
- 1. Find at an approximately spherical object in your image.
- 2. Select the Manual Column Centering function from the Adjustment button on the Electron Beam panel and the **Manual Centering Wizard** will open. Choose



Next from the first window and the Focus Window will open in the center of the scanning window, the trackball will be set to control OBJ Centering, and the wobbler will start. Adjust the trackball to cause the spherical object to go evenly in-to, and outof, focus in all directions, not smear in the vertical or horizontal direction. Once you are satisfied with the adjustment press the Finish button.

Note: There is a short video link on the desktop (**Column Centering**) that shows what the SEM image should look like during this process.







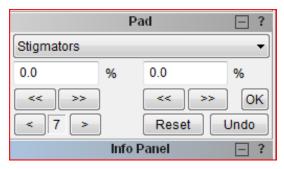
Astigmatism Correction

Stigmation adjusts the electron beam-spot cross-section to be perfectly circular so that both horizontal and vertical image elements are in focus and reduces the beam diameter thereby increasing resolution. Stigmation is only necessary above ~10kx magnification.

When the astigmatism is corrected the beam is at its smallest diameter (highest resolution) and the image shows a uniform blurring on either side of focused. See the electron beam-spot cross-section drawings and the SEM images on the next page for more details.

Astigmatism Correction Procedure

- 1. Increase your magnification to a few steps higher than your working magnification.
- 2. Find a spherical object. You will be comparing focus in orthogonal directions and need to have similar features in both directions.
- 3. Choose **WD** and focus the image as best you can.



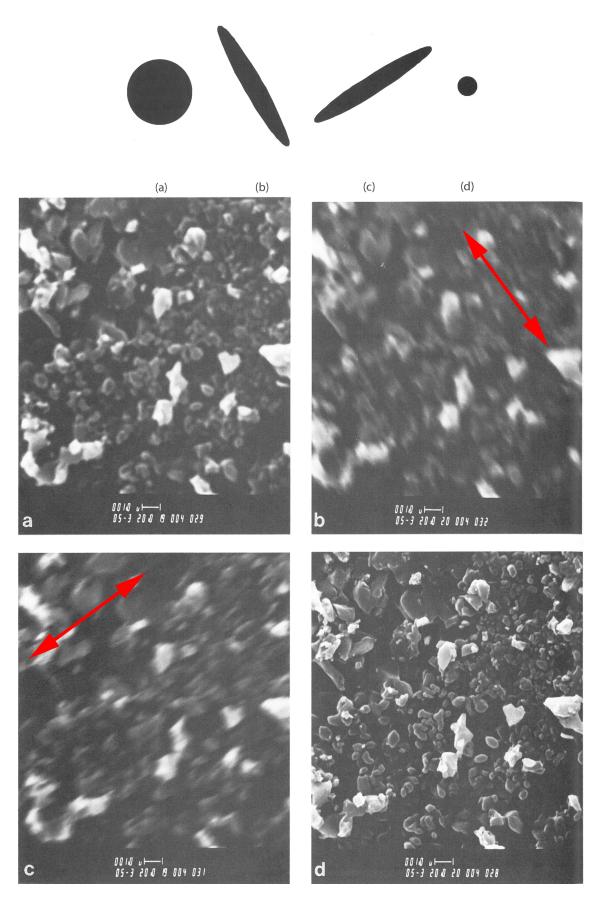
- 4. Choose **stigmators** from the info panel and use the track ball to "focus" the image – i.e. focus using stigmation control.
- 5. Change back to **WD** and repeat steps **3** and **4** until the image blurs equally on either side of focus. The astigmatism is now corrected
- 6. Over time, or if magnification is changed more than a little, astigmatism will creep back in and will have to be corrected for again.

Note: Astigmatism should be corrected near your working magnification. If the image is too blurry you may need to start at lower magnification and perform multiple focusing/astigmatism corrections.

Note: The directional blurring in images (b) and (c) is much more easily seen dynamically on the SEM as the focus is changed through the focal point.

Note: Examples of the electron-beam cross-sectional shape with associated images observed while correcting SEM astigmatism are shown on the next page. Reference the list below.

- (a) Shows an image at its best possible focus, but because the astigmatism is not correct the beam cross-sectional diameter is wide and the image appears not sharp.
- (b) Shows the image on one side of focused and because the astigmatism is still incorrect the blurring occurs non-uniformly (see red arrows).
- (c) Shows the image on the other side of focused (again with incorrect astigmatism) and the blurring occurs orthogonally to the blurring in (b).
- (d) Shows the image at the best possible focus with the astigmatism corrected. Passing back and forth through the focal point now will cause the image to uniformly blur in all directions, not directionally blur as shown in (b) and (c).



Appendix C

Medium-Vacuum Operation

In addition to the standard **High Vacuum Mode** (~0.009 Pa) the Vega-3 can operate in **Medium** Vacuum Mode (3 – 150 Pa) and Low Vacuum Mode (3 – 500 Pa). Medium Vacuum Mode (MVM) permits non-conductive samples to be imaged without being sputter-coated. SE mode is not available in MVM. You must use the BSE detector.

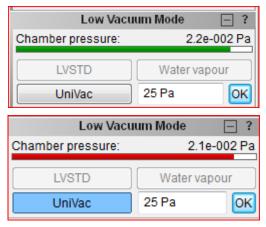
Note: Medium and low vacuum modes are used for specimens which require some small atmosphere and/or cannot be coated with Au, yet require greater than ~5keV accelerating voltage. Examples would be chocolate, dry porous bone, some dry insects etc. Biological and other specimens that require a true "wet" environment need to use an environmental SEM. Dartmouth's environmental SEM is located in the Electron Microscopy Facility at the Medical

Note: Low Vacuum Mode requires manually rearranging apertures inside the column and is therefore not available for general use. If your research requires that you operate in the 150 -500 Pa range contact Dr. Cullen, Dr. Guinel, or Prof. Levey to discuss.

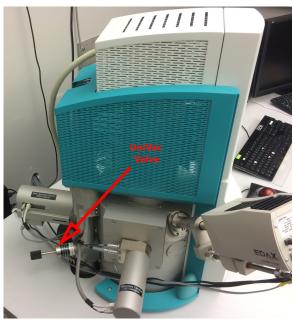
Putting the SEM into Medium Vacuum Mode

- 1. Set-up the SEM using the standard instructions above.
- 2. If on, turn-off the high voltage by pressing the **HV** button on the **Electron Beam** panel.
- 3. Press the **UniVac** button in the **Low Vacuum Mode** panel. The panel will change from a grey UniVac button with a green Chamber Pressure bar (top image) to a blue UniVac button and

a red Chamber Pressure bar (bottom image).



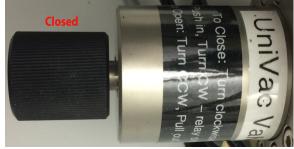
- 4. Manually close the UniVac valve located around the back left-hand side of the SEM.
 - Turn the handle clockwise.
 - b. Push in the valve handle.



- c. Turn the handle clockwise again.
- d. When the valve is properly actuated a distinct relay click can be heard.
- 5. Enter the desired Chamber Pressure into the Low Vacuum Mode panel and press the **OK** button.
- 6. Turn on the HV.
- 7. Insert the **BSE** detector and use **BSE** mode as described in **Appendix A** above.



Note: Generally, a Chamber Pressure of between 5 and 20 Pa works best. Use the lowest Chamber Pressure (highest vacuum) that you can without experiencing charging. The lower the pressure, the greater the signal-tonoise ratio.



8. To exit MVM:

- a. Turn-off the HV.
- b. Retract the BSE detector.
- c. Press the UniVac button in the Low Vacuum Mode panel.
- d. Open the **UniVac** valve.
 - i. Turn the handle counterclockwise.
 - ii. Pull out the valve handle.
 - iii. Turn the handle counter-clockwise again.
 - iv. When the valve is properly actuated a distinct relay click can be heard.

Note: Why no charging in **MVM?** In MVM gas molecules in the chamber are ionized by the electron beam creating positive and negative ions. These ions are attracted to the opposing polarity regions on the specimen (generally this will be positive ions attracted to negatively charged sample areas) and thus neutralize charge build-up on the specimen.

