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Zeiss ΣIGMA VP-FE-SEM User Guide

RESEARCH AND DEVELOPMENT

Zeiss ΣIGMA VP-FE-SEM User Guide

Prepared for

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User Guide

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1.0 Procedural Section

1.1 Sample Preparation and Loading

Note: The cleanliness of the vacuum chamber is very important. It is encouraged that the investigator wear gloves while handling objects that will enter the vacuum chamber, specifically the sample carousels. It is also good practice to keep the chamber door closed as often as possible. This practice reduces the contamination rate of the chamber.

Sample preparation consists of two basics steps and will vary depending on both the nature of the analyte and the information required from the analyte

1.1.1 Support Selection

Sample supports and films are most broadly categorized by whether or not they are STEM-compatible. STEM-compatible films and grids are always slotted to allow the passage (transmission) of electrons to a detector. The most commonly used STEM-compatible support film is the lacey carbon copper grid. These grids can come equipped with a Formvar resin to enhance the stability of the lacey carbon network. The usage of STEM-compatible support films often proves convenient given that these sample mounts can also be used in normal SEM mode. The selection of an appropriate support medium for a given task is often a process of trial and error.

1.1.2 Sample Deposition

1.1.2.1 Liquid Samples

Liquid samples are deposited onto the appropriate support in a drop-by-drop fashion using a variety of tools. Given that sample supports are often large enough to house several different analytes, it is often desirable to control both the location and size of the spots deposited. The tools used to reach this end vary, but

are all fairly simple. Perhaps the most convenient instrument used to deposit spots on the support is the mechanical micropipette. The exact volume of the spot will naturally vary depending on the situation. It is advisable to test different sample volumes on a separate surface prior to deposition on a support film.

Occasionally the use of a micropipette proves to be rather unwieldy and the task requires different methodology. It has been found that simply using the disposable plastic tips of the micropipette as a spotter is a viable alternative. Glass spotters can be used to some degree of success, but have found to be a rather poor alternative to the aforementioned methods.

1.1.2.2 Solid Samples

Samples that are solid are most commonly dissolved or suspended in an appropriate solvent and mounted the same as liquid samples (Section 1.1.2.1). Samples that cannot be dissolved or suspended practically are attached to their support using either a conductive tape or a conductive paste as adhesives. It is advisable to refer to the instructions specific to the adhesive being used before utilizing them. Note that pastes and adhesives are not used when operating in STEM modes.

1.1.3 Loading the Carousel

Once prepared, sample supports are fixed to an appropriate grid carousel capable of holding several films simultaneously. These sample carousels come in many different styles and are what ultimately interface with the microscope's stage platform. All carousels are fixed to the stage via a dovetail joint. One end of the joint on the stage platform has a flat docking bar that articulates with the flat end of the grooves found on the bottom of the sample carousel. It is important to ensure that the carousel is seated correctly before continuing. This precaution will both reduce the likelihood of collision happening within the vacuum chamber and increase the ease in which samples can be located on the support carousel using the STEM Navigation tool (Section 1.3.3).

1.2 Instrument Startup

This section chronologically outlines the steps required to properly start up the instrument safely.

1.2.1 Software Startup

The microscope is controlled entirely by the *SmartSEM* software suite developed by Zeiss. The software requires users to login to an account before use. User accounts are important for both logging operator actions as well as saving personalized user interface preferences. Once opened and logged in, the software will automatically show a view of the vacuum chamber as well as the status of the instrument. The operator is encouraged to become familiar with the layout of the user interface prior to preforming any work with the instrument. The help system built into the *SmartSEM* software suite has been found to be an effective resource in this arena.

1.2.2 Gas Pressure and Vacuum Control

This microscope uses pressurized gases for both vacuum chamber valve actuation and variable pressure viewing modes. Prior to pulling a vacuum, the operator must ensure that the instrument is being supplied with compressed air at a pressure of **87 psi (116 psi, maximum)**. If the operator intends to view samples under variable pressure mode (which will not be discussed in this guide), the instrument must also be supplied with nitrogen gas at a pressure of **2.9 psi (4.4 psi, maximum)**.

Assuming the sample has been properly prepared and loaded (Section 1.1), the operator must ensure that both the vacuum chamber door is completely closed, and that the instrument is being supplied with pressurized gas before pumping down the chamber. Find the **Vac** status box at the bottom right section of the *SmartSEM* interface and select **Pump**. At this point the roughing pump will switch on and begin pulling a vacuum. Shortly thereafter, the turbo molecular pump can be heard spinning up once the roughing pump has pulled the chamber to an appropriate pressure.

The pressure of the sample chamber can be viewed under the **Vacuum** tab on the right sidebar. Pressure units can be cycled through to the user's preference by left-clicking the vacuum pressure status boxes. The microscope requires a vacuum below **0.009 Pa** before extra high tension (EHT) can be enabled, which is required to produce an image. Although the software allows enabling EHT at this pressure, better images are usually produced at lower pressures (~ 10^{-4} Pa).

1.2.3 Accelerating Voltage

The voltage used to accelerate electrons toward the sample has an important impact on the produced image. Before turning on EHT, the operator is advised to ensure that the accelerating voltage is set to a reasonably-low value (~ **2.0 kV**, ± 1.0 kV). The accelerating voltage can be changed most easily by double-clicking the **EHT** textbox at the bottom of the main camera window. When EHT is off, this textbox will show **EHT = 0.00 kV**. After checking that both the vacuum chamber pressure and accelerating voltages are at reasonable values, EHT can safely be enabled by clicking the **EHT** status box at the bottom-right of the *SmartSEM* window. If successfully enabled, the status box will change accordingly. Note that it is common practice to change EHT values in small increments (no more than ± **5 kV**) in hopes of prolonging the lifetime of the filament responsible for emitting electrons.

As a general rule, lower EHT values gives greater information about the surface of a sample with cost to resolution. Conversely, higher accelerating voltages yield higher resolutions while obfuscating details about the surface. It is also important to use lower voltages (in the absence of variable-pressure modes) on samples that conduct electrons poorly to decrease the effects of charging.

It is important to be aware of the effects accelerating voltage has on the final image. The beginner is encouraged to experiment with different EHT values while attempting to produce an image (Section 1.3). The more experienced user will also find this practice to be beneficial when working with new sample types. Once familiar with the basic process of producing an image (focusing, stigmating, etc.), practice saving images (Section 1.3.9) of the same scene at different accelerating voltage

values. Compare these images with one another and make a mental note of their differences.

1.3 Producing an Image

Note: This section assumes that the operator is working in SEM mode only and that no STEM stage or detector will be used. The operator is encouraged to begin their journey with the microscope in SEM mode since it is somewhat simpler and less hazardous then STEM mode. Please refer to section 1.4 for instructions specific to STEM imaging.

This section briefly outlines the steps required to produce a focused image of the sample in the main viewing window. Many of the procedures listed in this section will make use of both the *Zeiss SEM* keyboard and the *SEM Stage Control* board. The adjustments made using these input boards can be performed in either coarse or fine modes. To cycle between the two modes simply toggle the textbox at the bottom right of the screen that reads either **Fine** or **Coarse**, depending on the currently selected adjustment mode.

1.3.1 Detectors

The instrument comes equipped with a variety of detectors that suit different situations. For the purposes of this guide, only the TV, SE2, and STEM detectors will be covered. The data coming from the detectors are viewed in the main viewing window at the center of the *SmartSEM* program screen. At the bottom of this window can be found the currently selected detector channel, it is listed as **Signal A**. Switching detector channels can be done in a number of ways, with the simplest being via the **Camera** button on the *Zeiss SEM* keyboard.

The detector shown at startup by default is the TV detector. This channel simply shows a view of the vacuum chamber, as seen from the orifice. It is critical that the operator use this channel when making gross adjustments to the position of the sample stage (Section 1.3.2). The SE2 detector is responsible for viewing electrons generated through ionization of the sample. The electrons, called secondary electrons, are collected at a detector at an angle to the primary electron beam and are excellent at showing the topography, or surface characteristics, of a sample. The final

detector to be discussed is the STEM detector, which is composed of both dark-field and bright-field components. The STEM detector collects electrons that travel through the sample while mounted on STEM-compatible carousel and support film. Since this detector is responsible for detecting electrons that traverse through the sample, it is typically suited toward higher accelerating voltages, and thus higher resolutions.

It is important to note that while the SE2 detector can be used with or without STEM-compatible carousels and support films, the STEM detector will not function in their absence.

1.3.2 Stage Control and Working Distance Using Analog Controls

Caution: Careless use of the stage controls can cause serious damage to the instrument. Use the TV detector when making gross changes to the position of the stage and always be mindful of the stage's position relative to other objects in the vacuum chamber.

The stage is responsible for mounting and moving the sample carousel with its attached media. The stage is allowed to move through 5 degrees of freedom, all of which are most easily controlled by the two control sticks found on the *SEM Stage Control* board. Assuming the instrument was logged off correctly after its previous use, the use of these control sticks will be disabled at startup. To enable use of the control sticks, click on the **Stage** tab under the **SEM Control** group on the right side-panel. Find the checkbox that says **Joystick Disable** and uncheck it.

As previously stated, the *SEM Stage Control* board is composed of two control sticks. The narrow stick on the left of the control board is responsible for controlling both the tilt, and the vertical height (z-axis) of the stage. Changing the position of the stage in the z-axis effectively changes the working distance, or the distance between the beam aperture and the surface of the currently selected carousel. The working distance effects both the resolution and depth of field of the final image. Here, the general rule is that smaller working distances equate to higher resolution and shallower depths of field, and vice versa. Per usual, the optimum value here generally depends on the situation at hand. It is common practice, however, to simply set the working distance as small as the

operator is comfortable with. *Be sure to select the TV detector channel while making changes to the working distance.*

The larger control stick on the right is responsible for moving the stage through three degrees of freedom. As depicted on the control board, actuating the stick in the up/down-left/right directions move the stage along the corresponding x and y axes. The stick can also be rotated along its shaft to similarly rotate the stage through the axis normal to the x-y plane.

It is important to note that the velocity at which the stage moves in any of these directions is inversely proportional to the currently selected magnification level of the microscope. Put more simply, as one increases the magnification, the control sticks become more precise, moving the stage much more slowly.

1.3.3 Stage Control Using SmartSEM's Stage Navigation

It is often the case that finding the specific sample of interest is too cumbersome using the analog controls on the *SEM Stage Control* board. Here the Stage Navigation tool is a very useful aid. The **Stage Navigation** tool can be launched from the docking side-panel seen on the right of the main viewing window. Click the arrow on the panel to expand it and then double-click the item labelled **Stage Navigation**. The window that opens shows both a side and top-down view of the currently selected carousel relative to the electron beam. It is important to make sure that the carousel that is currently in use is the one selected in the **Sample Holder** dropdown near the bottom right of the window. Once properly selected, finding the right sample is as easy as double-clicking on the appropriate label in the top-down carousel view. Again, it is important to have the TV detector channel selected before moving the stage by large amounts, as is common while using the **Stage Navigation** tool.

Once the sample of interest is roughly in line of the electron beam, it is customary to revert back to using the *SEM Stage Control* board for further adjustments.

1.3.4 Scan Speed and Noise Reduction

The data coming from the currently selected detector channel on the instrument can be sampled and processed differently depending on the task at hand. The scan speed changes how fast the instrument samples data from the detector, with higher scan speeds equating to faster sampling rates (similar to a frame-rate). While exploring the sample, it is often easier to use a higher scan speed since the viewing window will update changes in the image more quickly than slower scan speeds. The catch to this is that at higher scan speeds, more image detail is lost. To investigate details more effectively, or to capture and save images of the sample, slower scan speeds are favored. The detector scan speed can most easily be changed by pressing the **+** or **–** buttons labelled **Scan Speed** on the right side of the *Zeiss SEM* keyboard. Alternatively, the scan speed parameters can be viewed and altered using the **Scanning** tab of the **SEM Control** group located on the software's side-panel.

Noise is an inherent consequence of any sensing methodology. The methods by which detector noise is handled by the software are ultimately averaging processes. For the purposes of this guide only two noise reduction procedures will be utilized: pixel averaging and line averaging. Pixel averaging is effective when the operator is actively exploring the sample, or when the sample image is changing often (e.g. during magnification of focusing). Line averaging is used when the operator is inspecting the details of an image, or when the operator wishes to capture and save an image. Changing the noise averaging mode can be done under the **Scanning** tab of the **SEM Control** group located on the software's side-panel. The second dropdown menu in the **Noise Reduction** box allows the user to select the appropriate averaging technique.

1.3.5 Reduced Raster

Many of the procedures detailed below can be performed on a small section of the viewing screen. This is advantageous when the operator wishes to use a slower scan speed (higher resolution) without having to wait for the entire frame to be scanned after making changes. To enable the reduced raster window, click the **Reduced** button directly above the **Magnification** knob on the left-hand side of the *Zeiss SEM*

Keyboard. Once enabled, a small box will appear somewhere in the viewing window. Any changes made to the scene will only take place within the perimeter of this box. The location and size of the reduced raster window can be changed by clicking and dragging around the edges of the box as desired.

1.3.6 Brightness and Contrast

The brightness and contrast of the image displayed in the main viewing window can independently be set either automatically or (after some practice) manually using the *Zeiss SEM* keyboard. Beginners are advised to ensure that the brightness and contrast levels are set automatically by the software. To enable this feature, click the **Detectors** tab under the **SEM Control** group on the right side-panel. Find the first dropdown menu in the **Signal Adjust** box and select **Auto BC = On**. Different modes can be enabled per the operator's preference using this dropdown menu.

1.3.7 Magnification and Focus

The two primary factors that affect the characteristics of the final image are the magnification and focus of the microscope. The current magnification level is shown at the bottom of the main viewing window in the textbox that reads **Mag = Y X**. Changing the magnification of the image is done using the dial labelled **Magnification** on the left-hand side of the *Zeiss SEM* keyboard. It is sometimes convenient to zoom all the way out while switching between samples (Sections 1.3.2 and 1.3.3).

The focus of the image is changed similarly by rotating the dial on the right-hand side of the keyboard labelled **Focus**. It is often advantageous to repetitively magnify and focus the microscope until the quality of the produced image begins to depreciate. Once at this point the operator should check to ensure that the electron beam is properly stigmated and that the apertures are aligned.

1.3.8 Stigmation and Focus Wobble.

Producing well-focused images, especially at higher magnifications, requires the cross-section of the electron beam to be circular. The process of circularizing the electron beam is called stigmation and it is carried out using various electromagnetic coils called stigmators. Improper beam circularization is referred to as astigmatism and can most easily be observed as directional fuzziness in the produced image. Stigmation is performed much in the same way that focusing is, except the process is done along two orthogonal axes and thus requires two knobs instead of one. To remedy astigmatism, first magnify and focus on a part of the sample that has a great degree of contrast. Once selected, locate the knobs labelled **Stigmator X** and **Stigmator Y** at the top left of the *Zeiss SEM Keyboard*. While observing areas of high contrast, rotate these stigmator knobs independently until the image appears to have no directional fuzziness. Try refocusing the image after correcting for any astigmatism and observing the results.

Often times the image will appear to move when the operator attempts to focus it. This phenomenon is referred to focus wobble and occurs when the various electron beam apertures in the focusing column are in poor alignment. To correct for focus wobble, find a part of the image that has a high amount of contrast. Focus upon the selected scene and correct for any apparent beam astigmatism. Once sufficiently focused, locate and press the button labelled **Wobble** on the top portion of the *Zeiss SEM Keyboard*. A reduced raster box will appear showing an amplification of the current focus wobble. Fixing the wobble is done using the **Aperture X** and **Aperture Y** control knobs on the top of the SEM keyboard. The goal is to use these knobs to stop the image from wobbling. If done correctly, the image will simply go in and out of focus while remaining in place. Fixing focus wobble takes a considerable amount of practice to master.

1.3.9 Saving an Image

Capturing and saving an image involves freezing the scan process on a single frame, and then exporting that frame to an image file. As previously stated (Section 1.3.4), the detector scan speed should be fairly

low and the noise reduction method should be set to line average. To freeze a frame, open the **Scanning** tab on the **SEM Control** side-panel and select **Freeze on = End Frame** from the first dropdown menu in the **Noise Reduction** box. A red dot indicator showing that the frame has been frozen will appear at the bottom right of the viewing window when the frame has been completely scanned and frozen. Once frozen, go to the **File** menu and select **Save As**. A dialogue box will open asking for the directory and filename of the image to be saved. Images can be scanned and stored at various resolutions (default, **1024 by 768 pixels**). To increase or decrease the image resolution, simply change the value of the **Store resolution = X** dropdown in the **Scanning** tab. Once the image has been exported the frame can unfrozen by selecting **Freeze on = Command** from the first dropdown menu in the **Noise Reduction** box.

1.4 STEM Mode

Imaging in STEM mode is similar in many ways to that of SEM mode. This section briefly outlines the important differences between the two techniques. It is assumed that the investigator has already loaded an appropriate stage, evacuated the vacuum chamber, and enabled EHT.

1.4.1 Stage Navigation and the STEM Detector

If the operator wishes to use the STEM detector, a STEM-compatible stage must be used. Once the appropriate stage is loaded, it is important to update the sample holder setting in the Stage Navigation tool (Section 1.3.3). Setting the correct stage type will help reduce the risk of collisions between the stage and obstacles in the vacuum chamber while simultaneously increasing the ease in which samples can be located in the viewing window.

The STEM detector is fixed to a mechanical arm that can either be fully retracted or fully extended. To utilize the STEM detector, the detector arm must be in its extended position directly below the stage. Prior to inserting the detector arm, it is imperative to move the STEM stage to what is called the ‘safe zone’. Moving the stage to the safe zone is accomplished using the **Stage Points List** tool. This tool can be found in the docking sidebar on the right-hand side of the viewing window. After

switching to the TV detector channel (Section 1.3.1), open the **Stage Points List** tool and double click the stage point labelled **$STEM_SAFE_ZONE**. The stage will move to an area in the chamber that is safe for detector arm insertion.

Once the stage is in the safe zone, the STEM detector arm can be inserted using the **STEM Control** tool. To open this tool, open the docking sidebar and double-click the tool labelled **STEM Control**. The arm can now be inserted by clicking the button labelled **STEM -> IN**. The STEM stage can now be moved into position using either the **Stage Navigation** tool (Section 1.3.3), or the **Stage Points List** tool.

Finally, the STEM detector can be displayed in the viewing window by selecting the STEM channel in the **Detectors** tab (Section 1.3.1). It is important to note that since the STEM detector is detecting electrons that are transmitted through the sample, better results are often observed at higher accelerating voltages.

1.4.2 Imaging Modes

The STEM detector is composed of both dark-field and bright-field components which are assigned labels called quadrants. The bright-field component of the detector is located directly in line with the electron beam and is responsible for detecting electrons that are either completely transmitted, or weakly deflected by the sample. This field is labelled Q1 (for quadrant 1). There are two dark-field components that form a perimeter around the bright-field component which are responsible for detecting electrons that are scattered to a certain degree with respect to the electron beam. These components are labelled Q2 and Q3.

Different quadrants, and thus different fields, can be set to three different modes independently from one another (with the modes being normal, inverted, and off). The sum of these permutations yield 27 (i.e. 3^3) distinct field modes, with several modes often being visually indistinguishable from one another. Experimentation with different combinations of field modes is encouraged given that different combinations usually lead to very different images. Changing the modes STEM detector quadrants is accomplished with the **STEM Control** tool found in the docking sidebar (Section 1.4.1).

1.5 Logging Off

Finishing a session with the microscope is roughly the reverse of the startup process. The following sections will briefly outline the steps to safely log off the instrument.

1.5.1 Stage Positioning and STEM Detector

The first steps to safely logging off the instrument involves stowing the stage and disabling the control sticks. To begin, switch the detector channel to TV (Section 1.3.1). If the STEM stage was used, move it to the stage point labelled **$STEM_SAFE_ZONE** using the Stage Points List tool found in the docking side-panel (Section 1.4.1). Once in the safe zone, retract the STEM detector by clicking the **STEM -> OUT** button found in the **STEM Control** tool found in the docking side-panel (Section 1.4.1). If a normal SEM stage was used during the session, completely lower the stage using the left control stick on the *SEM Stage Control* board. Once the stage has been stowed, the operator *must* lock the stage controls by enabling the **Joystick Disable** checkbox under the Stage tab. This helps minimize the chance of someone inadvertently moving the stage while the microscope is not in use.

1.5.2 Accelerating Voltage

After safely stowing the stage, the operator must ramp down the accelerating voltage to a low value before disabling the EHT. It is common to lower the voltage to **2.0 kV** or lower, while remembering to change in increments no larger than **5 kV**. Once the accelerating voltage is sufficiently low, EHT can be disabled by clicking the **EHT** textbox at the bottom right portion of the screen.

1.5.3 Vacuum Control and Gas Pressure

Note: If the instrument will be used again after a short period of time, or if the operator does not wish to vent the vacuum chamber, this step can be skipped.

Venting the vacuum chamber can be accomplished by clicking the **Vac** status textbox at the bottom right portion of the *Zeiss SmartSEM* window and selecting the **Vent** option. The roughing pump will promptly switch off and the vacuum chamber will begin to vent. After a short time the chamber door can be opened if the operator wishes to retrieve any samples. It is very important to minimize the amount of time that the chamber door is open in order to reduce the rate at which the vacuum chamber soils.

Finally, it is important to close the valves on the pressurized gas cylinders after venting the instrument. This prolongs the lifetime of the tanks when the microscope is not in use.

1.5.4 Closing the Software

Given that user accounts are customizable, and that user actions are logged, it is important that operators log off their account or close the *SmartSEM* software when they are finished with their session. A usage log should be kept near the instrument and be updated with information detailing the session time and general activities conducted during each user's session with the microscope.

2.0 References

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2. Robbins, Roger. *Scanning Electron Microscope Operation Zeiss Supra-40*. The University of Texas at Dallas, 2011 version.

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