User Guidelines & Standard Operating Procedure for the
Hitachi SU-1510 Scanning Electron Microscope
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DISCLAIMER

The materials contained in this document have been compiled from sources believed to be reliable and to represent the best opinions on the subject. This document is intended to serve only as a starting point for good practices and does not purport to specify minimal legal standards. No warranty, guarantee, or representation is made by Laurier as to the accuracy or sufficiency of information contained herein, and Laurier assumes no responsibility in connection therewith.
ACKNOWLEDGEMENTS

The following individuals of Laurier contributed to the writing, editing, and production of this manual: Gena Braun (Instrumentation Technician); Mihai Costea (Biology).

This manual was prepared for Laurier. Any corrections, additions or comments should be brought to the attention of the Instrumentation Technician at 519-884-0710 ext. 2361.
1. INTRODUCTION

1.1 Purpose of the Standard Operating Procedure

This standard operating procedure (SOP) is NOT a substitute for training and/or reading the appropriate manuals before use. All principle investigators and supervisors must document that training has been received by students and staff who will be using the scanning electron microscope.

A list of authorized users will be maintained by the Instrumentation Technician.

This SOP is intended to promote consistent and safe use of the Hitachi SU-1510 scanning electron microscope within the Faculty of Science. This SOP covers the potential hazards, personal protection requirements, spill and accident procedures, waste disposal considerations, and instrument operation for the Hitachi SU-1510 scanning electron microscope [henceforth referred to as the SEM].

1.2 Theoretical Background

Scanning electron microscopy uses a focused high energy electron beam to image the surface of a variety of samples and collect information on morphology and elemental composition. A scanning electron microscope is a highly versatile tool and can be used to study biological specimens, geological materials, nanoparticles, circuit boards, and many other sample types.

A SEM consists of an electron gun, focusing lenses, stage or specimen holder, and several types of detectors. The electron gun contains a heated metallic filament, usually tungsten, which provides the source of electrons. These electrons are accelerated toward an anode plate and then focused by condenser lenses and an objective lens. A deflector coil causes the focused electron beam to be scanned across the surface in a raster pattern. When the electron beam strikes the surface of the sample it causes the production of secondary electrons (SE), backscattered electrons (BSE), Auger electrons, and x-rays. SE provide topographical information, are low in energy, and are deflected toward to a Faraday cage surrounding the secondary electron detector. BSE are high in energy, produced deeper in the sample, and provide compositional information based on atomic number. BSE are not deflected by the Faraday cage and are detected by a BSE detector positioned around the objective lens. X-rays are typically detected by an energy dispersive x-ray detector (EDS) and can be used for elemental analysis. Auger electrons provide
compositional information for all elements except H and He and is based on the kinetic energy of the emitted electrons.

When a sample is hit by the high energy electron beam, a static charge will build up in the surface of the sample at that point, unless it can be conducted away. Conductive samples include semiconductor boards and other metallic samples, so the charge migrates away and do not cause a static build-up. In non-conductive samples, such as plastics and tissues, static charge will build up and create anomalies in the image. To avoid this problem, samples are coated before imaging with a very thin layer of conductive material, such as gold, using a sputter coater.

To obtain strong a strong SE signal, the SEM must operate under high vacuum to minimize scattering of the low energy SE by air molecules. As a result of the high vacuum, biological samples that contain any water quickly become desiccated when placed in the SEM, and this causes the surface structure of the specimen to change. To overcome this obstacle, samples can be dried in a critical point dryer prior to imaging, or imaged under lower vacuum using a variable pressure SEM (VPSEM or environmental SEM). Critical point drying uses liquid CO2 to dry the sample without causing the cells to collapse. A VPSEM can operate with the sample chamber under higher pressure (lower vacuum), which minimizes evaporation, and still collect BSE, which can be used for topographically information at a slightly lower resolution than that obtained using SE. SE can not be detected under higher pressure due to the possibility of a high voltage discharge. In addition, samples that are imaged at lower vacuum do not necessarily need to be sputter coated because the air in the chamber will minimize static build up (although sputter coating may still provide a better image). For imaging biological samples a pressure of 10 Pa is usually sufficient and it can be combined with a higher beam current; higher pressure will lead to lower spatial resolution and a weaker signal due to beam scattering.
1.3 SU1510 Components

The SU1510 SEM is illustrated in Figure 1-1. The electron beam is produced by a tungsten hairpin filament in the electron gun, and then accelerated by the anode at a voltage from 0.3 kV to 30 kV. The beam is focused by three electromagnetic lenses (two condenser lenses and the objective lens). The SU1510 is equipped with a secondary electron detector and a backscattered electron detector. At 30 kV and 5 mm working distance, secondary electron images can be collected with a minimum resolution of 3.0 nm (high vacuum mode) and backscattered electron images can be collected with a minimum resolution of 4.0 nm (low vacuum mode). Magnification can be set from 5x to 300,000x and vacuum can be adjusted in VP mode from 6 to 270 Pa.

Sample position can be adjusted using the knobs on the front of the chamber (Figure 1-2). Samples can be moved along the x-axis from 0 to 80 mm, along the y-axis from 0 to 40 mm, and along the z-axis (working distance) from 5 to 35 mm. If the working distance is set between 5-15 nm the sample can hit the backscatter detector; be very careful when adjusting the working distance or tilting the sample. Large samples require extra precautions. See the Protocol (Section 6) for more detail. Samples up to 153 mm in diameter and 60 mm in height can be imaged in the SEM.
1.3.1 Software Overview

The Control panel consists of a series of buttons across the top of the screen. The Operation panel is on the right side of the screen and provides a guide for imaging. All common options required for imaging can be accessed from these panels. Table 1-1 illustrates and describes the Control Panel options.

Table 1-1: Control Panel Functions (Hitachi, 2007)

<table>
<thead>
<tr>
<th>Control Panel Section</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electron Beam Control</td>
<td>When the system has reached sufficient vacuum, this section of the panel can be used to turn the accelerating voltage on and off and display the resulting emission current (under “le”).</td>
</tr>
<tr>
<td>Scan Control</td>
<td>Starts or stops scanning (Run or Freeze), and controls the speed of the scan. These controls also include the Monit button, which can be used to focus a particularly challenging specimen.</td>
</tr>
<tr>
<td>Auto/Adjustment</td>
<td>Used to automatically adjust the brightness, contrast, focus, and alignment for the image.</td>
</tr>
<tr>
<td>Magnification Control</td>
<td>Magnification can be increased by dragging the mouse to the right while positioned over this window.</td>
</tr>
<tr>
<td>Capture Control</td>
<td>Used to capture and image with a set resolution and scanning speed (if Scan Speed Link is selected, the image will be captured using the scanning speed currently in effect for observation).</td>
</tr>
<tr>
<td>Evac/Air control</td>
<td>Initiates evacuation or vents the chamber for sample access.</td>
</tr>
</tbody>
</table>
Images can be collected with a variety of scanning speeds and sizes (number of pixels). For initial imaging and searching for a sample, TV or FAST scanning is recommended. For more fine focus adjustment, a limited field of view (FOV) or SLOW1 or SLOW2 can be used. SLOW3 can be used to view the image before final image capture. Table 1-2 lists the settings for each scanning speed.

### Table 1-2: Scanning speeds and image capture options

<table>
<thead>
<tr>
<th>Scanning Speed</th>
<th>Settings</th>
</tr>
</thead>
<tbody>
<tr>
<td>TV</td>
<td>640 x 480 pixels, fast scanning</td>
</tr>
<tr>
<td>FAST</td>
<td>Full screen display, fast scanning</td>
</tr>
<tr>
<td>SLOW</td>
<td>Full screen display, slow scanning</td>
</tr>
<tr>
<td>Limited field of view (FOV)</td>
<td>320 x 240 display, fast scanning</td>
</tr>
<tr>
<td>High definition capture</td>
<td>Maximum 5120 x 3840 pixels</td>
</tr>
<tr>
<td>Integration capture</td>
<td>Maximum 2560 x 1920 pixels</td>
</tr>
</tbody>
</table>

The software provides many options for automatically adjusting the image, as follows:
- ABCC: Auto brightness and contrast
- AFC: Auto focusing
- ASF: Auto stigma and focusing (stigma causes the image to stretch and is due to an electron beam that is slightly elliptical in shape).
- AFS: Auto filament saturation (based on filament heating)
- ABA: Auto beam alignment (aligns the electron gun axis)
- ABS: Auto beam setting (combines AFS, ABA and gun bias auto alignment)
- AAA: Auto axial alignment (applies to aperture/stigma alignment)

For special focusing and image saving, the software can be used to:
- Adjust focus for tilted samples
- Improve the signal to noise ratio by averaging and pixel integration or frame integration
- Display the image as a too colour composite or in pseudo-colour
- Save the image from 640 x 480 to 5120 x 3840 pixels as a BMP, TIFF, or JPEG
2. POTENTIAL HAZARDS
The SEM operates under very high voltages. When leaving the system unattended, turn off the acceleration voltage.

Do not touch the rotary pump as it can get very hot when the SEM is in use.

If you notice anything unusual regarding instrument operating (unusual smells, sounds, etc), shut the instrument down and contact the Instrumentation Technician immediately.

3. PERSONAL PROTECTIVE EQUIPMENT
Latex or nitrile gloves must be worn to prevent contamination of the SEM. A lab coat and closed-toe and heel footwear constructed of resistant material is also required for all laboratory activities.


4. ACCIDENT PROCEDURES
All incidents must be reported to the Instrumentation Technician and if applicable, a student’s supervisor. The Instrumentation Technician will insure that all accidents, incidents and near misses involving instruments are reported as promptly as possible to the Environmental/Occupational Health and Safety (EOHS) Office via the WLU Employee Accident/Incident/Occupational Disease Report form (www.wlu.ca/eohs/forms). All incidents that result in critical injuries must be reported immediately to the EOHS Office by telephone. Additional details regarding incident reporting can be found in the WLU Accident Incident Procedure (www.wlu.ca/eohs).

5. WASTE DISPOSAL PROCEDURES
Use of the SEM itself does not result in waste; however, all WHIMIS, Department, and Health and Safety guidelines must be followed for disposal of materials used to prepare any specimens. See the WLU Laboratory Safety Manual for details.
6. **PROTOCOL**

To image at high magnification, drying (ideally with a critical point dryer) and sputter coating is recommended for all biological samples. Biological samples that contain small amounts of water can be imaged at low vacuum without drying or sputter coating. **Do NOT mount any specimens that contain significant amounts of water or oil as these will contaminate the SEM column.** Naturally conductive materials do not need to be sputter coated.

To sputter coat a dry biological sample, proceed as follows:
1. Select a specimen stub that is larger than the specimen to be imaged, and fix the specimen to the stub:
   a. If using conductive paste, use a minimal amount and make sure the paste is completely dry to avoid contaminating the vacuum system.
   b. If using double sided tape, use a minimal amount as the tape can outgas and cause image drift.
2. Check the oil in the vacuum pump and make sure it is above the minimum line and transparent.
3. Open the lid of the sputter coater and place the sample on the stub in one of the holders.
4. Open the argon cylinder and make sure the delivery pressure is set to ~ 4 psi (this should not need to be changed).
5. Turn on the sputter coater using the power switch on the back.
6. For most biological specimens, the default parameters are appropriate (25 mA, 00:02:00 H:M:S). If you need to modify these parameters, please contact the Instrumentation Technician.
7. Press START. The instrument will evacuate the air and a blue plasma should be visible. The display should read:

<table>
<thead>
<tr>
<th>Vacuum</th>
<th>Current</th>
<th>Time Remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td>1x10-1 m bar</td>
<td>25 mA</td>
<td>00:01:38 H:M:S</td>
</tr>
</tbody>
</table>

8. When the cycle is complete the specimen will be coated with a film approximately 15 nm thick.
9. Turn off the sputter coater and close the argon cylinder.

6.1 **Start-up and Sample Placement**

1. Before start-up, look around the microscope on the RIGHT side of the desk and check the oil in the vacuum pump to make sure the level is correct and the oil is transparent (if it is cloudy, it needs to be changed).
2. Check the log book for any previous problems and fill in your name, the date, and the time you start using the SEM.
3. Turn the key to ON and then START (and let go). The instrument and the computer will start up, and the software will initialize automatically.
   a. Windows username and password: sem
   b. SU1510 software: no password required
4. Make sure the stage is set as follows:
   a. X = 20
   b. Y = 20
   c. Z = 15
   d. Tilt = 0°

5. If the accelerating voltage is on, click the OFF button on the top left of the screen.

6. Vent the chamber by pressing the AIR button on the top right corner of the screen and wait for the door to open.

7. Place the specimen in the chamber:
   a. Use the round tweezers to grasp the stub and place it securely into the holder. You may have to press down on it slightly.

8. As the chamber door is pushed closed, note the position of the sample relative to the check gauge:
   a. If the sample can touch the check gauge when it is being observed, it will hit the BSE detector - DO NOT let this happen. This means the sample is set too high. Move it down and then slide the chamber door closed.
   b. If you plan to TILT the sample, please consult with the Instrumentation Technician first, and see Figure 3.5-25 in the SEM manual (pg 3.5-27).
   c. Note the maximum Z-position for this sample and do not bring the sample any higher while imaging or you may break the BSE.

9. Push the chamber back into the SEM and hold the handles to keep it shut.

10. On the Operating Panel on the far right side of the screen, select the desired conditions, set the working distance accordingly (keep in mind the maximum Z position determined in step 8c) and press Apply.
   a. The SEM High-Vacc options operate under high acceleration voltage and are recommended for dried and sputter coated samples
   b. The VP-SEM mode is a low vacuum mode and is highly recommended for biological samples that have not been dried or sputter coated. It suggests a short working distance (5 mm), and this must be set with great care to avoid hitting the BSE detector (5 mm may not be possible with larger samples; refer to step 8c).

11. Press the Evac button.
6.2 Imaging

The Operation panel is on the right side of the screen and provides a guide for imaging. Follow each step, and for more detailed assistance click on the “one-point advice” provided for more detailed assistance for each step.

1. On the Operation Guide, press the Image Adj. button
2. Press the Electron Beam ON button to turn on the high voltage.
   
   The accelerating voltage determines the wavelength and energy of the electrons in the electron beam; it is the voltage difference between the tungsten filament and anode. Theoretically, a higher accelerating voltage corresponds to a shorter electron wavelength and better resolution. However, for most biological samples (and other low atomic number materials) the higher energy electrons create increased sample/beam interaction and larger spot size and increased signal from deeper in the sample (rather than just the surface), which actually leads to decreased resolution. Therefore, for biological samples it is often best to set a lower accelerating voltage to obtain better resolution and contrast.
   
   a. You can adjust the acceleration voltage on the Cond. Tab.
      i. Select the desired accelerating voltage below “Vacc” and press the ON button.
      ii. If operating in the low vacuum mode, a higher accelerating voltage may be required.
   
   b. The filament current can also be customized on the Cond. Tab.
      i. The AFS (auto filament saturation) should be set to HIGH for high magnification, and MID for all other imaging (or LOW to maximize filament life).
      ii. Press the AFS button.
3. Press the Focus Adj button.
   
   a. Adjust the magnification to 30x first and roughly manually adjust the focus.
   
   b. Adjust the magnification to between 1000 and 5000x, and manually adjust the focus again.
   
   c. Press the AFC button to auto focus and optimize fine focus.
4. Press the Alignment button.
   
   a. Select each alignment option and move the crosshairs to limit the motion of the image and maximize the brightness.
5. Press the auto focus button again to refocus following alignment
6. Press the Astigmatism adjust button.
   
   a. Adjust the astigmatism sliders on the top left of the image to produce the sharpest image possible, or press the ASF button to automatically adjust stigma and focus.
7. Press the auto focus button again.
   
   Imaging is an iterative process: Adjust the focus, adjust the alignment options, adjust the focus again, check the alignment, etc. Focus and alignment will have to be checked each time the magnification is changed.
8. Press the B/C Adj. button.
9. Finally, capture the image at the desired size and speed settings.
   a. A slower scan rate usually provides a better image, but may show a
      smearing if the image is charging. If this occurs, the sample may
      need to be sputter coated again.
10. Save the final image in a folder under your name.
11. To remove the sample and load another sample:
   a. Turn off the accelerating voltage by pressing the OFF button on the
      top left of the screen.
   b. Turn off the vacuum by pressing the AIR button on the top right.
   c. Wait for the chamber to completely vent, and the door to
      automatically open.
   d. Remove the previous sample using tweezers.
   e. Place the next sample in the sample holder using tweezers, and
      repeat the protocol starting at Step 6 in Section 6.2.

6.3 Shutdown

1. When you are finished with the final sample, turn off the accelerating
   voltage by pressing the OFF button on the top left of the screen
2. Turn off the vacuum by pressing the AIR button on the top right.
3. Wait for the chamber to completely vent, and the door to automatically
   open.
4. Remove the sample using tweezers.
5. Slide the chamber door shut and hold it closed.
6. Press the EVAC button.
7. When the chamber has completely evacuated, close the SEM software and
   shutdown the computer.
8. When the computer is completely shutdown, turn the key to OFF.
9. Fill in the log book and indicate any problems that were encountered during
   operating.
10. Take all of your samples with you when you leave.

If you notice any problems with the instrument, please contact the
Instrumentation Technician. Users are not to make repairs. The SEM shall be
maintained and repaired by qualified persons only.
7. **Quick Reference Guide**

1. Prepare the sample and sputter coat if desired:
   a. Fix the specimen to a stub.
   b. Open the argon cylinder (delivery pressure should be set to ~ 4 psi).
   c. Turn on the sputter coater using the power switch on the back and place the prepared sample and stub in the holder.
   d. Press START.
   e. Remove the sample when the cycle is finished and turn off the sputter coater.
   f. Close the argon cylinder.
2. Fill in the log book with your name, the date, and the time.
3. Turn the key to ON and then START (and let go).
   a. Windows username and password: sem
   b. SU1510 software: no password required
12. Make sure the stage is set as follows: X = 20, Y = 20, Z = 15, Tilt = 0°
13. Press the AIR button on the top right corner of the screen.
14. Using tweezers, place the specimen in the chamber:
   a. Check the position of the sample and make sure it will not hit the BSE detector.
   b. Note the maximum
   c. **Note the maximum Z-position for this sample and do not bring the sample any higher while imaging or you may break the BSE.**
15. Push the chamber back into the SEM and hold the handles to keep it shut.
16. On the Operating Panel on the far right side of the screen, select the desired conditions, set the working distance accordingly (keep in mind the maximum Z position determined in step 8c) and press Apply.
17. Press the Evac button.
13. Press the B/C Adj. button.
14. Finally, capture the image at the desired size and speed settings.
15. Save the final image in a folder under your name.
16. When you are finished with the SEM, remove the last sample and shutdown the SEM:
   a. Turn off the accelerating voltage.
   b. Turn off the vacuum by pressing the AIR button.
   a. Remove the last sample using tweezers.
   b. Slide the chamber door shut and hold it closed.
   c. Press the EVAC button and wait for the chamber to completely evacuate.
   d. Close the SEM software and shutdown the computer.
   e. Turn the key to OFF.
11. Fill in the log book and indicate any problems that were encountered.
REFERENCES


APPENDIX 1: TRAINING FORM

Training Sign Off

By signing below, I confirm that I have:
1. Read and understood the Standard Operating Procedure for the Hitachi SU-1510 scanning electron microscope.
2. Received training on the safe use and proper care of the Hitachi SU-1510 scanning electron microscope.

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<thead>
<tr>
<th>Trainee</th>
<th>E-mail</th>
<th>Supervisor</th>
<th>Trainer</th>
<th>Date</th>
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APPENDIX 2: SEM USER LOG
<table>
<thead>
<tr>
<th>Date &amp; Time In</th>
<th>Name &amp; Extension</th>
<th>Supervisor</th>
<th>Sample Description</th>
<th>Total Time Used</th>
<th>Problems / Comments</th>
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