Standard Operating Procedure I: SEM, BSE and STEM

ESC II, Room A119F
810 West Campus Drive
West Haven, CT 06516
> FOLLOW the SOP strictly to keep the instrument in good condition. Any violation will lead to user account suspension.
> NEVER use your own USB drive on instrument computer. Data can be transferred with the Jump Drive provided by the Core.
> NEVER surf the web on the instrument computer to minimize the risk of the computer being hacked.
> NEVER allow other users to get access to instrument computer on your reservation.
> REPORT any issues to Core director immediately so they can be fixed on time.
Table of Contents

1  Introduction............................................................................................................................. 1
2  Initial System Status Check .................................................................................................... 2
3  Starting Instrument.................................................................................................................. 3
4  Sample Preparation ................................................................................................................ 4
5  Sample Loading ...................................................................................................................... 5
6  Image Observation ................................................................................................................ 10
7  Closing SEM measurement................................................................................................... 19
8  Photodiode Back Scattered Electron (PD-BSE) ................................................................. 23
9  Scanning Transmission Electron Microscopy (STEM) ......................................................... 26
Hitachi SU8230 Standard Operating Procedure

1 Introduction

1) Instrument features:
   > Cold field emission (CFE) e-beam source: high resolution on conductive surfaces (0.8 nm on Au clusters/magnetic tape)
   > Sliding-in annular Energy Dispersive Spectroscopy (EDS) detector: high elemental mapping resolution
   > Sliding-in annular Photo Diode PD-BSE detector: high signal intensity from backscattered electron
   > Scanning Transmission Electron Microscopy (STEM) detector: high resolution compositional contrast imaging, ideal for EDS mapping

2) Location
   Materials Characterization Core
   Room A119F
   810 West Campus Drive
   West Haven, CT 06516

3) Primary Staff Contact
   Dr. Min Li
   Tel: 203-737-8270
   Email: min.li@yale.edu
   Office: ESC II, Room A119D

The Yale West Campus MCC Facilities are operated for the benefit of all researchers. If you encounter any problems with this facility, please contact the staff member listed above immediately. There is never a penalty for asking questions. If the equipment is not behaving exactly the way it should, contact a staff member.

Warning: Please follow strictly the SOP to keep the facility at good condition. We DO NOT recommend user explorations on program unless endorsed by core director.
2 Initial System Status Check

1) **Check if the small SC LED is flashing and the** Specimen Chamber (SC) pressure reads **LE-4 Pascal**. If not, stop and report to Core manager immediately.

![Image of SC LED and pressure reading](image1.png)

2) Check building supplied **Compressed Air (CA)** and **Nitrogen (N2)** pressure on the wall, make sure the CA pressure reads around **80 psi** and N2 around **120 psi**. **If both gauges read zero, contact Core Manager immediately.**

**Warning:** Operating without compressed air and nitrogen will lead to system damage.

![Image of air and nitrogen pressure gauges](image2.png)

3) Check the **LN2** dewar below and make sure it is high enough to support the measurement. A full tank can support ~1 hour so **keep checking LN2 in every half hour** to avoid pressure rise to shut down the electron beam.

**Warning:** when opening the LN2 storage tank on the ground, pull the lid slowly out of the tank. **Do Not shake the lid** which could break the foam cylinder attached to the lid.

![Image of LN2 dewar](image3.png)
3 Starting Instrument

1) **Sign in** on the logbook and put down date, usage time, sample materials, Specimen Chamber (SC) pressure, imaging modes (SEM, PD-BSE, STEM or EDS), and report any issues during measurement.

2) **Unlock** the SEM computer using **FOM calendar only**. The FOM screen lock window NetID login is not working.

3) If the **PC_SEM** program is closed, click **PC_SEM** icon on desktop, choose or type **WC MCC** as profile name and hit **OK** button to login, no password required. (If the computer is logged off, then choose the profile **PC-SEM** and type **hitachi** to login.)

4) If a flashing message in yellow “**Execute Normal Flashing**” appears on top of the imaging window:

   > Click **OK** on the popup window on the right side

   ![Popup Window](image1.png)

   > Click inside the **Electron Beam** window as highlighted in red

   ![Electron Beam](image2.png)
> Click **Flashing** button in the popup **Vacc** (accelerating voltage) and **Ie** (emission current) setting window:

![Flashing Button](image1)

> Click **Execute** button highlighted in the **Flashing** window below to flash the tip.

![Execute Button](image2)

> Click **Close** button in the popup **Vacc** and **Ie** setting window.

![Close Button](image3)

### 4 Sample Preparation

1) **Always wear gloves** for vacuum sample preparation!! **Change gloves** if touched computer keyboard and mouse.

2) **The sample for SEM needs to be completely dried!**
   a) The **powders samples** can be dripped and dried on Si substrate. Alternately, powders can be **sprinkled** on **Conducting Graphite Paint** (supplied in the Core) directly applied on the specimen stub, or on to double sided conducting carbon tape.

   **Note:**
   - **Do not press powders too hard** as it may change surface morphology.
**The Conducting Graphite Paint** is highly recommended to fix the samples especially magnetic particles for high magnification (>100 k) measurement.

b) The solid samples, large size flakes, single crystals can be fixed directly onto the sample holder using Conducting Graphite Paint.

3) Attach the specimen stub to the specimen holder; **DO NOT** overtighten the locking ring.

4) Adjust the height of specimen so that the **highest point on the sample** matches the **lower surface of the height gauge** (see photo below).

**Warning:** **Samples mounted above the dashed line will crash into the lens system or detectors** (EDS, STEM), and the **repair fee** will be charged to PI’s account.

5) **Clean sample holder:** bring the specimen stub inside the fume hood and blow off loose particles on the sample surface using the N₂ gun.

5  **Sample Loading**

1) Turn on the small LCD on the table by pressing the switch at the **top left corner** in the back. This unit includes the Specimen Chamber (SC) camera display and the camera power supply.
   > **Check and make sure** the specimen stage is at the exchange EXC position and no sample holders on the stage, as highlighted in the picture below:
   > **Check and make sure** no other detectors (PD-BSE or EDS) underneath the electron column, as highlighted in the picture below:
2) **Check and make sure** the PD-BSE, STEM and EDS detectors are **fully retracted** on the left side of SEM chamber:

3) **Check to make sure** the exchange rod is **locked**. If not, turn the exchange rod locking knob **clockwise** to lock the rod and make sure the red light on the locking knob is **ON**.

4) Wear gloves. Press the AIR button on the **Exchange Operation Panel**. Wait until the **buzzer** sounds when air introduction into the specimen exchange chamber is complete.
5) **Press highlighted corner with your thumb (preferred) or grab the handle** to open the exchange chamber door.

**Caution:** **DO NOT** hold the **exchange rod** to open the door, which will bend the rod with time and fail the sample transfer.

6) Insert the specimen stage onto the exchange rod:
   a) Turn the **exchange rod locking knob** *counterclockwise* to release the rod and push the rod forward to find the fork.
b) Turn the specimen holder lock/unlock knob clockwise to the unlock position and insert the rod fork into the holes on the specimen holder. Turn the knob counterclockwise to the lock position and confirm that the holder is locked to the rod by slightly pulling the holder. **Warning**: it is crucial that the sample holder is at the Lock position for sample transfer. Otherwise it will lead to transfer failure and parts damage on the SEM stage.

7) Pull the specimen exchange rod all the way back into the airlock door and turn the exchange rod locking knob clockwise to lock the rod. The red light on the locking knob should be ON.

8) **Close and hold** exchange chamber door with right hand and use left hand as shown below to press the EVAC button on the exchange operation panel. *Wait until* the buzzer sounds and the EVAC button LED changes to stable blue, indicating the chamber is evacuated back into a good vacuum. **Caution**: DO NOT hold the exchange rod to close the door as this will lead to rod bending with time.
9) **If it takes more than 15 mins to hear the beep**, remove the sample from the exchange chamber. It means your sample can not be accepted by our system. Please contact manager for more info.

10) Press **OPEN** button on the exchange operation panel. **Wait until** the buzzer sounds and the gate valve is open.

11) **Turn** the exchange rod locking knob **counterclockwise** to release the lock. **Push** the transfer rod forward slowly into specimen chamber until the **insertion detection lamp** on top of the exchange chamber is lit in **blue**.

**Warning:**
- **DO NOT** rotate the specimen holder knob while pushing the rod into specimen chamber. This may cause accidental switch of **Lock** position to **Unlock** on the rod leading to sample transfer failure and mechanic damage.
- **Always** hold the knob when pushing the transfer forward to prevent rod accidental sliding into specimen chamber.

12) Turn the **specimen holder knob clockwise** to **UNLOCK** position. With **left hand** holding the door, **right hand** grabs the knob and **slowly pushes against** glass window with the knuckles and pull the rod all the way to the back and turn **exchange rod locking knob clockwise** to lock the rod. The **red light** on the locking knob should be **ON**.

13) Press the **CLOSE** button on the exchange operation panel and wait until the buzzer sounds, indicating the sample transfer is complete.
14) Turn off small LCD from the back top left corner. Leaving the LCD on will damage the EDS detector even at retracted position.

6 Image Observation

1) Click the HOME button on the menu at the top right of PC-SEM window (Caution: DO NOT repeatedly click this button as this may lead to STOP button next ineffective).

2) Go to Stage tab and click Set button to set the specimen stub Size and Height

3) In the popup window below

a) Choose the Size of specimen stub one size up for safety purpose, e.g.: choose 2 inches for 1 inch specimen stub).
b) Set Height to Standard. Warning: make sure the specimen stub has been carefully aligned using height gauge.
c) Confirm that the boxes next to detectors (EDX, BSE, BF-STEM and FQ-EDX) are not checked.
d) Click OK button to close the Set Sample Size/Detectors window above.
4) Setting imaging parameters.
   a) Set accelerating voltage $V_{acc}$:

   > Click inside Electron Beam window in highlighted rectangle region above and choose $V_{acc}$ from dropdown list. (typical values: 1kV, 5 kV, 10kV or 15kV)

   **Warning:**
   > Do Not type in arbitrary numbers, as the beams from dropdown list have been calibrated by service engineer.
   > Always try small voltage first to avoid surface damage, over-charging and ebeam induced carbon deposition (black imaging box)

   b) Set emission current $I_e$:

   > Click inside Electron Beam window above to choose $I_e$ at 10 µA.
   **Note:** Consider smaller values if surface charging or carbon deposition is a concern.

   c) Click Close button to close the dropdown Electron Beam setting window.

   d) Confirm the LM (Lower Magnification mode) is active inside Magnification window below. If not, click to switch back to LM mode.
e) **Choose Rapid Scan Mode** in the window below to start with.

f) Choose the **SE(LM) (Secondary Electron Low Magnification)** detector in **Optics** tab below. If not, click and choose from dropdown list.

![Image of Optics tab with SE(LM) highlighted]

Choose Rapid Scan Mode in the window below to start with.

f) Choose the **SE(LM) (Secondary Electron Low Magnification)** detector in **Optics** tab below. If not, click and choose from dropdown list.

![Image of Optics tab with SE(LM) highlighted]

g) Set the **Probe current** to **High** in the **Operation condition** window below.  
   **Note:** high probe current is recommended at lower magnification to improve signal/noise ratio.

![Image of Operation condition window with Probe current set to High]

h) Confirm that **Cond Lens 1** is set at “5” in the **Operation condition** window above.

i) Set sample surface **Z height** (defined as the distance from the bottom of electron column to sample surface). **To avoid sample collision with detectors,** always check the table below before changing **Z height** value:

<table>
<thead>
<tr>
<th>Z height setting restrictions (severe damage to lens may happen with z &lt; 5 mm):</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regular SEM:</strong> 5 – 20 mm</td>
</tr>
<tr>
<td><strong>EDS:</strong> 11 – 20 mm</td>
</tr>
<tr>
<td><strong>PD-BSE:</strong> 8 – 20 mm</td>
</tr>
<tr>
<td><strong>BF-STEM:</strong> 5 – 20 mm</td>
</tr>
</tbody>
</table>

**Warning:**

- The default height is **8 mm**, good in most imaging cases
- The smallest Z height allowed is **5 mm**.
> Contact lab manager if Z height smaller than 5 mm is required. The sample height must be calibrated very well using the height gauge for small Z height. Otherwise the sample will crash into electron beam column.

5) Check and confirm the Specimen Chamber (SC) pressure has reached \textbf{LE-4 Pascal} on EVAC CONTROL panel as highlighted below. Never turn on the electron beam if the pressure is not ready. The electron gun chamber will be contaminated quickly.

6) Click the \textbf{ON} button to turn on beam voltage $V_{\text{acc}}$ in the \textbf{Electron Beam} window below: \textit{Note}: the process takes seconds, \textbf{please be patient} and wait till the popup window disappears.

7) Click \textbf{Contrast} on the top menu bar or \textbf{AUTO} button on the \textbf{Manual Operation Panel} below if the image is too bright or dark. The \textbf{BRIGHTNESS} and \textbf{CONTRAST} knobs can be used separately for manual adjustment.
8) Roll the track ball on the STAGE CONTROLLER to find the field of interested in LM mode:


10) Adjust focus using FOCUS COARSE and FINE knobs. Move the stage to look for the field of interest in LM mode, and then click to switch to High Magnification (HM) mode.

11) Check the Optics tab and make sure the SE mode is checked as below:

12) In HM mode, change the magnification and get sharply focused image using FOCUS, STIGMA X and STIGMA Y knobs on Manual Operation Panel.

13) If image drifts (swaying or heaving) during adjusting:
   > Click Alignment tab and click Beam Align button. The ALIGNMENT LED on the Manual Operation Panel should be flashing.
Bring the circular image to the center of the image area by adjusting X and Y knobs on the panel.

Click **Aperture Align** button below and adjust **STIGMA/ALIGNMENT X /Y** knobs on the panel to stabilize the image wobbling.

Click **Stigma Align X** button and adjust **STIGMA/ALIGNMENT X /Y** knobs to minimize the image wobbling if needed. You need to lower the magnification below 50k to observe the changes.

Click **Stigma Align Y** button and adjust **STIGMA/ALIGNMENT X /Y** knobs to minimize the image wobbling if needed. You need to lower the magnification below 50k to observe the changes.

Click **Off** on Alignment tab. Make sure the Alignment LED on **Manual Operation Panel** is OFF.

Switch back to high magnification. Readjust the **FOCUS, STIGMA X** and **STIGMA Y** knobs on **Manual Operation Panel** to get the final image before saving.
15) **Save image:**

   a) Select the field of view, confirm image with slow scan or .

   b) Click the **Capture** button .

   c) Click button next to to resume live image scan.

16) Image capture settings for **charging samples** (image distorts, streaky, drifts during scan):

   a) Choose **CSS** (Charge Suppressed Scan) mode:
      
      > Clicking on the small box in the **Scan Menu** to open the Scan Button Setting window.

      > Check the radio button next to CSS. Hit **Apply** button and **Close** in the window below:

      > The CSS Scan menu should appear as follows:
b) **If the charging is still strong on sample surface**, change the scan mode from **line** scan to **frame** mode:

> Click the small box in the **Capture** menu below:

![Capture menu](image)

> In the **Capture/Save Setting** window below, choose **Fast** capture mode with a minimum of 8 frames or more as needed and close the window.

![Capture setting](image)

c) If captured images using a) and b) still appear streaky, blurry due to charging, click **R1** button below **once** to switch to **R2** mode, wait several seconds till image contrast is acceptable and then click **Save** button to save the image.

![Image settings](image)
17) **Data save:**
   
a) Click the **OFF** button to **turn off** electron beam in the **Electron Beam** window below before data saving:

![Electron Beam Window]

b) Click **ALL** button on the bottom of the image thumbnail column below:

![Image Thumbnail Column]

c) Click **_PCI** button in above window, the collected images will be transferred into **Quartz PCI** program below. Click **File** in the menu and select **Export All...** on the dropdown menu, then **Browse** to choose **Export to Path** and **File Format**, **Export Text** (imaging parameters) and hit **OK**.

![Export All Dialogue Box]
d) Clear the thumbnail images from the left column by clicking **Remove** button.

7 Closing SEM measurement

1) Make sure the electron beam has been turned off with a **solid blue bar** appearing next to **OFF** in the **Electron Beam** window below:

2) Click inside **Electron Beam** window highlighted above and set electron beam voltage ($V_{\text{acc}}$) to 10 kV, and emission current $I_e$ to 10 µA.

3) If **Deceleration Mode** was used, uncheck the Deceleration box as shown below:

4) Switch imaging mode to **Lower Magnification (LM)** Mode

5) Click **Freeze** button to stop scanning. The **Run** button will appear after clicking.

6) Click the **EXC** button on **PC-SEM** top menu to see the specimen stage moving to the exchange position. Wait till status vertical bar next to EXC button stops flashing.

7) Resume the default Scan modes as follows:
8) Click the small box in the **Scan Menu** to open the Scan Button Setting window and change the setting back to Slow:

![Scan Button Setting Window](image)

9) **Check and resume** the default line scan mode in the **Capture**:
   > Clicking the small square next to the **Capture** as highlighted below:

![Capture Button](image)
In the **Capture/Save Setting** window below, choose **Slow/CSS** as highlighted in the window:

![Capture/Save Setting Window]

10) Uncheck the ON box in the Image Shift/Rotation window as follows:

![Image Shift/Rotation Window]

11) Check and confirm in **Stage > Set: Set Sample Size/Detectors** window below that all detector check boxes are **unchecked**.

![Set Sample Size/Detectors Window]
12) **Turn on** small chamber LCD monitor from the top left corner on the back.

13) **Put on gloves** and remove samples from the specimen chamber:
   > Press **Open** button on the exchange chamber and wait for the beep,
   > **Unlock** the transfer rod and push the rod all the way into the specimen chamber until the **blue SET light on**,
   > Turn to **Lock** position on the black knob,
   > Extract the rod all the way to the back and **lock** the rod,
   > Press the **Close** button and wait for the beep,
   > **Turn off** small chamber LCD
   > Press the **Air** button and wait for the beep,
   > Using the right hand to open the exchange chamber,
   > Turn to **Unlock** position on the black knob and extract the sample holder out from the rod,
   > Close the exchange chamber door and hold with right hand, then press the **Evac** button,
   > Release the right hand until door closes by itself.

14) **Keep** the PC-SEM window fully maximized.
15) **Upload data** to box.yale.edu, or use ONLY the Core USB flash drive for data transfer.
16) **Log off FOM** from SEM computer or user’s **FOM SEM calendar**. **Make sure** the FOM Screen Lock window below appears.
   **Note:** if reporting issues, please check “**Something wrong**” in FOM logoff window and describe the issues in the empty box.

17) **Sign off** logbook.
18) **Remove** ONLY samples from the stub on the specimen holder and **clean** the holder with Kimwipes using **IPA**. **Do Not** dissemble the sample holder.
19) **Store** the specimen holder in assigned organizer box.
20) **Clear** the SEM work bench.
8 Photodiode Back Scattered Electron (PD-BSE)

1) Make sure \textbf{Vacc} is \textbf{OFF}
2) In \textbf{PC\_SEM} program with the sample holder at \textbf{EXC} or \textbf{HOME} position, click \textbf{Set} button in the \textbf{Stage} tab and check the \textbf{BSE} box

3) \textbf{Check to make sure sample Z height is set at 8mm.}

4) Select \textbf{Vacc} at \textbf{15 kV} and regular \textbf{Ie=10 µA} as the \textbf{PD-BSE} requires high e-beam kV
5) Select \textbf{Dual Screen} mode on the \textbf{PC\_SEM} top menu bar as shown below, and choose \textbf{detector} on the first screen and \textbf{detector} the second

6) Switch to \textbf{LM} mode and turn \textbf{Vacc ON}
7) \textbf{Slowly} rotate the knob shown below to move the \textbf{PD-BSE} detector to the measurement position. \textbf{Stop} turning quickly once feel stopped.
8) **Check to make sure the small chamber LCD is OFF.** The PD-BSE is very sensitive to infrared light generated from camera inside.

9) Click \*1 beside \*2 to activate SE imaging:
   a) Find interested areas on the sample inside the annular PD-BSE detector in LM mode; adjust focus and switch to HM mode.
   b) Get a GOOD image in HM mode
   **Caution:** Always switch to SE window to adjust image quality for PD-BSE imaging

10) Click \*3 beside \*4 to activate BSE imaging:
   a) Click \*5 or AUTO button on the Manual Operation Panel to adjust the image brightness/contrast. The BRIGHTNESS and CONTRAST knobs can be used separately to do manual adjustment
   b) Select the field of view, confirm image with slow scan \*6 or \*7 and then, click the Capture button \*8.

   **Warning:** do not use \*9 rapid scan mode for PD-BSE imaging.

11) To quit PD-BSE detection mode:
   a) **Turn off** electron beam voltage, \( V_{\text{acc}} \)
   b) Switch to LM mode.
   c) Turn on the small chamber LCD
   d) **Rotate slowly to fully retract** the PD-BSE detector
   e) Click the EXC button to move the specimen stage to the exchange position. **Wait until the green status bar stops flashing** next to EXC button.
   f) Click Set button in the Stage tab shown below; uncheck BSE(PD) box, and then follow Section 7 Closing SEM measurement and 8 Checklist after Experiment to close the measurement.
9 Scanning Transmission Electron Microscopy (STEM)

**Notice:** The STEM sample holder has a standard height of **36 mm**, so no need to use **Height Gauge**.

1) Make sure **Vacc** is **OFF**.
2) Make sure the small chamber **LCD** is **OFF**.
3) In **PC_SEM** program with the sample holder at **EXC** or **HOME** position, click **Set** button in the **Stage** tab and check the **BF-STEM** (Bright Field) box as shown below:

4) Make sure the **Z height** is set at **8 mm**. **Never** change the Z height larger than 20 mm to damages the STEM detector below the stage.

5) Select **V_{acc} ≤ 20 kV** and regular **Ie=10 µA** in **STEM**

6) Select **Dual Screen** mode on the **PC_SEM** top menu bar, and choose detector for the first screen and detector the second

7) Check and make sure the **SC** chamber pressure reaches **LE-4 Pa**.
8) Switch to **LM** mode and turn on electron beam voltage **V_{acc}**.
9) **Slowly rotate the knob counterclockwisely to move the STEM detector** to the measurement position. **Stop** moving once feel stopped

![Image of STEM detector](image)

10) Click  beside  to activate SE imaging:
   a) Find interested areas on the sample in LM mode; adjust focus and switch to HM mode.
   b) Following instructions in **Section 6 Image Observation** to get a well focused image in HM mode
      **Caution:** Always switch to SE window to adjust image quality for STEM imaging

11) Click  beside  to activate STEM imaging:
   a) Click or AUTO button on the **Manual Operation Panel** to adjust the image brightness/contrast. The BRIGHTNESS and CONTRAST knobs can be used separately to do manual adjustments
   b) Select the field of view, confirm image with slow scan or  and then, click the **Capture** button  

**Notice:** do not use rapid scan mode for STEM imaging

12) To quit STEM detection mode:
   a) Turn OFF electron beam voltage $V_{ace}$, and switch $V_{ace}$ back to **10 kV**, and **10 uA**.
   b) Slowly retract the STEM detector until stopped
c) Click the **EXC** button to move the specimen stage to the exchange position. Wait until the green status bar stops flashing next to EXC button.

![EXC button image]

d) Click **Set** button in the **Stage** tab; uncheck **BF-STEM** box, and then follow **Section 7 Closing SEM measurement** and **8 Checklist after Experiment** to close the measurement.

![Stage tab image]