Standard Operating Procedure I: SEM, BSE and STEM

ESC II, Room A119F
810 West Campus Drive
West Haven, CT 06516

Version 1.11, November 2019
> **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.
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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

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   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 to make sure** the Specimen Chamber (SC) pressure is at LE-4 Pascal. If not, stop and report to Core manager immediately.

![Image of Specimen Chamber Pressure Gauge]

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 Compressed Air and Nitrogen Pressure Gauges]

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.
3 Specimen 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.

4 **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) **Log into** the SEM computer using **FOM calendar** or **FOM Screen Lock** window below by clicking **Click here to login with NetID:**

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

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> Click **Flashing** button in the popup **Vacc** (accelerating voltage) and **Ie** (emission current) setting window:

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

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

```
> Click button
Vacc
Ie
Emission adjust
Deceleration
Beam monitor active
```

5) 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 SEM column, as highlighted in the picture below:

6) Check and make sure the PD-BSE, STEM and EDS detectors are fully retracted on the left side of SEM chamber:
5 Loading the Specimen

1) **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**.

2) 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.

3) **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.
4) Insert the specimen stage onto the exchange rod
   a) Turn the exchange rod locking knob \textit{counterclockwise} to release the rod and push the rod forward to find the fork.

   ![Diagram showing the exchange rod locking knob and red light]

   b) Turn the specimen holder \textit{lock/unlock} knob \textit{clockwise} to the \textit{unlock} position and insert the rod fork into the holes on the specimen holder. Turn the knob \textit{counterclockwise} to the \textit{lock} position and \textbf{confirm} that the holder is locked to the rod by slightly pulling the holder.

   \textbf{Warning}: \textit{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.}

5) Pull the specimen \textit{exchange rod} back into the airlock door and turn the exchange rod locking knob \textit{clockwise} to lock the rod. The \textbf{red light} on the locking knob should be \textbf{ON}.

6) \textbf{Close} and \textbf{hold} exchange chamber door with right hand and use left hand to \textbf{press} the \textit{EVAC} button on the \textit{exchange operation panel}. \textbf{Wait until} the buzzer sounds indicating the chamber is evacuated back into vacuum.
Caution: **DO NOT** hold the exchange rod to close the door as this will lead to rod bending with time.

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

8) **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.

9) 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 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**.
10) Press the CLOSE button on the exchange operation panel and wait until the buzzer sounds, indicating the sample transfer is complete.

6 Image Observation
1) Turn off small LCD from the back top left corner. Leaving the LCD on will damage the EDS detector even at retracted position.
2) 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).
3) Go to Stage tab and click Set button to set the specimen stub Size and Height
4) 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).¹
   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.

¹ Failure to follow the instruction may lead to severe damage to the lens system; the user account will be revoked and the repair fee will be charged to PI's account.
d) Click **OK** button to close the **Set Sample Size/Detectors** window above.

5) Setting imaging parameters.
   a) **Set** accelerating voltage $V_{\text{acc}}$:

   > Click inside **Electron Beam** window in highlighted rectangle region above and choose $V_{\text{acc}}$ 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 $\mu$A.

   **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.

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.

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:

Z height setting restrictions (severe damage to lens may happen with z < 5 mm):
Regular SEM: 5 – 20 mm
EDS: 11 – 20 mm
PD-BSE: 8 – 20 mm
BF-STEM: 5 – 20 mm

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.

6) Check and confirm the Specimen Chamber (SC) pressure has reached 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.

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

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

11) 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.
12) In HM mode, change the magnification and adjust imaging quality using FOCUS and STIGMA X/Y knobs on Manual Operation Panel
   a) If image drifts (swaying or heaving) during focusing:
      > 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 minimize the wobbling motion in image.

Click Stigma Align X button below and adjust STIGMA/ALIGNMENT X /Y knobs to minimize the wobbling motion in image. Repeat similar adjustment on by clicking Stigma Align Y button. (Note: reduce Magnification below 50k for this step)

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

b) If image distorts (stretching), correct astigmatism:
   > Make sure the Stigma LED on Manual Operation Panel is ON. Use the STIGMA/ALIGNMENT X/Y knobs alternating with FINE FOCUS knob to reduce distortion and obtain the sharpest image.

   c) Repeat steps a) and b) if necessary when switched to higher magnification.

13) Save image:

   a) Select the field of view, confirm image with slow scan Slow1/2 or Slow3/4.
b) Click the **Capture** button.

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

14) 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.

   ![Scan Menu](image)

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

   ![Scan Button Setting](image)

   > 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:
   > In the **Capture/Save Setting** window below, choose **Fast** capture mode with 8 frames and close the window.

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.
15) **Data save:**
   a) Click the **OFF** button to **turn off** electron beam in the **Electron Beam** window below:

   ![Electron Beam Window](image1)

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

   ![Thumbnail Column](image2)

   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** and hit **OK**.

   ![PCI Window](image3)
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 ($V_{acc}$) 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 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.

6) Resume the default Scan modes as follows:

7) Resume the default Capture mode to Slow_1280 as follows:
8) Uncheck the ON box in the Image Shift/Rotation window as follows:

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

10) Turn on small chamber LCD monitor from the top left corner on the back.
11) Put on gloves and remove samples from the specimen chamber: following the reversed order from sample loading, check Section 5 Loading the Specimen, steps 1) to 10).
12) Turn off small chamber LCD to prevent EDS detector damage from long time infrared light irradiation.
13) DO NOT close or minimize PC-SEM program
14) Use ONLY the Core USB flash drive for data transfer.
15) Log off FOM from SEM computer or user’s FOM SEM calendar. Make sure the following FOM Screen Lock window with dark background appear on the monitor to stop being continuously charged.
Note: if reporting issues, please check “Something wrong” in FOM logoff window and describe the issues in the empty box below.

8 Checklist after Experiment

1) Sign off logbook.
2) Remove ONLY samples from the stub on the specimen holder and clean the holder with Kimwipes using IPA. Do Not disassemble the specimen holder.
3) Store the specimen holder in assigned organizer box.
4) Clear the SEM work bench.

Warning: failed to follow checklist twice in one month will lead to temporary account suspension.
9 Photodiode Back Scattered Electron (PD-BSE)

1) Make sure \textbf{V}_{\text{acc}} \text{ is OFF}

2) In \textit{PC SEM} program with the sample holder at \texttt{EXC} or \texttt{HOME} position, click \texttt{Set} button in the \texttt{Stage} tab and check the \texttt{BSE} box.

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

4) Select \texttt{V}_{\text{acc}} \text{ at } 15 \text{ kV and regular } \texttt{I}_e=10 \text{ µA as the PD-BSE requires high e-beam kV.}

5) Select \texttt{Dual Screen} mode on the \textit{PC SEM} top menu bar as shown below, and choose detector on the first screen and detector the second.

6) Switch to \texttt{LM} mode and turn \texttt{V}_{\text{acc}} \text{ ON}

7) \textit{Slowly} rotate the knob shown below to move the PD-BSE detector to the measurement position. \textit{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 **SE** 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 **2** beside **PD-BSE** to activate BSE 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 adjustment
   b) Select the field of view, confirm image with slow scan or and then, click the Capture button.
   **Warning:** do not use rapid scan mode for PD-BSE imaging.

11) To quit PD-BSE detection mode:
   a) **Turn off** electron beam voltage, V<sub>acc</sub>
   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.
10 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} \leq 20 \text{ kV}** and regular **I_e=10 \mu A** 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]

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 **BIRGHTNESS** 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 \( V_{acc} \), and switch \( V_{acc} \) back to **10 kV**, and **10 uA**.

![Image of electron beam settings]

   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 and status bar](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)