Hitachi FlexSEM 1000 User Guide / Operation Procedures

- **No food and drinks** are allowed in the room
- Remember to **"Sign in"** on the sign-up sheet
- Turn on the **ROOM IN USE** light (red light switch) when the SEM is in use
- **Wear gloves** when handling the samples and sample holders
- Ensure that you **transfer your data** into OMERO or Z: drive by the end of the imaging session

Starting the SEM

- Turn on the main switch mounted on the wall for the SEM, then turn on the power switch on the side of the SEM
- Open the FlexSEM1000 software. There is no password needed to press **Start**.
    - **Note**: It may take several minutes for the SEM to clear the chamber of air. Please wait until **Specimen exchange** button is ready

Loading the Sample

- Press the green **Specimen exchange** button once it becomes available and allow time for the SEM to fully vent the chamber with air
    - **Note**: Initial stage position should be: X = 0.000mm, Y = 0.000mm, R = 0.0deg, Z = ~23.0mm, T = 0.0deg
- Put on gloves and mount the sample on the appropriate sample holder
- Adjust the height of the specimen on the sample holder using the height gauge
    - **Note**: For the large and tilt holder, adjust the height until the gap between the height gauge and the topmost surface of the specimen is approximately 1 mm. For the cup-shaped holder, set the topmost surface of the specimen to the same position as the upper surface of the specimen holder.
- Once the specimen exchange is available, open the airlock door fully and load your sample
    - **Note**: Ensure that the sample holder is mounted properly on the stage
- Select the sample holder type on the interface
    - **Note**: For the tilt holder, stage Z direction position has to be set at 5.0mm. Warning sign will show up if it is not at 5.0mm, follow the instruction accordingly
    - For the cup holder and large holder, you will be required to select the stub size that you are using (typically either Ø26 or Ø15mm)
- Click **Next**
- Select **Capture,** and allow the stage to move the sample into the view of the camera
    - Note: Please do not insert the airlock door until the SEM map says **Freeze**
- Insert the airlock door until it is "locked" and a small gap remains in the door
- At this point, **EVAC** button will become available on the interface, and pressing it will commence evacuating the SEM, creating a vacuum environment
    - Note: While the SEM is evacuating, you may need to manually align the observation area (red circle) with the stub on the SEM map. Using the mouse, drag the center of the red circle to map onto the circumference of the stub

Saving the Image

- Select **Menu > Save condition settings > Save settings**
    - **Note**: Here, you can configure your filename, location and file type

- Check the '**Save with automatic numbering**' to avoid saving the image one by one manually after every capture
    - **Note:** Ensure to reset **auto increment** to 1 so that numbering will begin at 0001
- Check the '**Embed the data display into image**'
    - **Note**: You may customise the data display under **Menu > Save condition settings > Data display**

Finding the Desired Area

- Select **START** at the top left corner to begin the observation
    - **Note**: You may check the electron beam settings, vacuum mode settings, and detector settings under **Menu**
- Use a low magnification setting (100 – 500x) to identify the area of interest on sample, and using the **COARSE** and **FINE** knob, adjust the image just enough to bring the identified area into focus
    - **Note**: You may use either the controller or camera view to navigate the electron beam to the area of interest
    - **AUTO** focus button on the controller may be used to bring the sample into focus as well
- Activate **Scan 1** to find the area of interest on the sample
- Activate **Scan 2** to scan through the area that you have chosen
    - **Note:** Scan speed can be adjusted at the top right side of the interface, beside **Menu.** At this scanning stage, it is recommended to use **Slow2** or **Slow3**
- Activate **Scan 3** to zoom in and adjust the focus to increase fidelity of the image

Adjusting the Beam Alignment

- On controller, press **MODE** to toggle between **Stigmator** and **Alignment** mode. Pressing it again would switch between **Stigmator Align. X, Stigmator Align. Y, AFC Align** or **Off**. Alternatively, you may select **Align** on the interface and select the functions individually
- At exaggerated magnification (15,000x – 20,000x), switch to **Scan 3** mode. If the image is shifting up and down, or side to side, adjust to keep the image as still as possible using **X** and **Y** knobs on the controller, followed by **FINE** focus knob
- Repeat the same process for **Aperture align, Stigmator Align. X** and **Stigmator Align. Y**
- Ensure the alignment mode is **Off** after all the adjustments have been made. Correct astigmatism next if necessary

Correcting the Stigmatism

- Once the image does not appear to get any sharper with repeated **FINE** focus adjustments, or directionality (or streaking) is observed, it may be necessary to correct the stigmatism
- At exaggerated magnification (15,000x – 20,000x), switch to **Scan 3** mode
- Over and under focus the image until you can find the midpoint, then adjust the **X** and **Y** knobs on the controller (one axis at a time)
    - **Note:** Several repetitions of this step is required before acquiring the best results
- Once the alignment and stigmatism have been corrected and the image is as crisp as desired, proceed with adjusting the exposure of the image
    - **Note**: **AUTO** exposure button on the controller may be used
- Lastly, zoom out to the desired magnification and press **CAPT** on the controller, or the **Capture** button on the interface to take an image of the field of view on the screen

- **Note**: It is recommended to scan first using **Scan 2** at **Slow4 or 5** before capturing the image
- You may check the capture settings under **Menu**

Shutting Down

- Once imaging is completed, select the **STOP** button at the top left corner to turn off the electron beam
- Select **Specimen exchange** button once it becomes available and allow time for the SEM to fully vent the chamber with air
- Once the chamber has finished processing air, open the airlock door, and using gloves, remove the sample holder from the stage
- Insert the airlock door until it is "locked" and a small gap remains in the door
  - **Note:** If you are using the tilt holder, ensure that stage Z direction position must be reset to ~23.0mm before closing the airlock door
- Press the illuminated **EVAC/AIR** button on the front of the SEM
- Once the microscope has established a vacuum, exit the SEM software
- Turn off the power switch at the side of the SEM, and turn off the main switch mounted on the wall for the SEM
- Transfer your data, and complete the sign-up sheet