Publications that made use of the **EPIC** facility instruments must include the following acknowledgements:

“This work made use of the EPIC facility of Northwestern University’s NUANCE Center, which has received support from the Soft and Hybrid Nanotechnology Experimental (SHyNE) Resource (NSF ECCS-1542205), the MRSEC program (NSF DMR-1720139) at the Materials Research Center, the International Institute for Nanotechnology (IIN), the Keck Foundation, and the State of Illinois, through the IIN”

Find this information online at http://www.nuance.northwestern.edu/epic/publication- acknowledgements/index.html
I. Policies and Introduction

Reservations

EPIC SPF FEI Helios FIB-SEM reservations are made using the NUCore online reservation system. Start your reservation before you begin using the instrument. When your session is complete, be sure to end your reservation in NUCore. If you need extra time on the microscope, we recommend ‘extending’ your original reservation, rather than making an additional reservation.

There is a hardware control system on the Helios FIB-SEM, so the system will not function unless you are logged in to NUcore. Tampering with or disabling the hardware control may result in revocation of your EPIC privileges.

FIB-SEM Rules

1. Please wear clean gloves when handling any components and samples that will go into the FIB-SEM
2. Do not insert any flash drives into any of the PC’s in AG97
3. Do not install any software onto any of the PC’s in AG97
4. Before you start your NUcore session and log off at the end of your NUcore session, the 3-part vacuum diagram at the bottom right in the xT microscope control software should be completely green. Contact staff immediately if this is not the case!
Saving Your Data

During your session, you may store image data to your own folder indicated with your NetID on the D:\ drive, especially when saving larger videos or images. At the end of your session, data can be migrated from the D:\ drive to the Helios folder on the EPIC_SEM drive (S:\). **Please do not forget this at before you end your session!** The SEM server is also accessible through one of the three workstation computers in the lab. You can transfer your data from the SEM server computer to a USB, etc. **You should NEVER take your data directly from any of the computers in AG97.**

II. Preliminary Sample Preparation

1. Prior to electron/ion beam exposure, samples typically need to be bulk coated with a conductive layer of sufficient thickness (typically ≥15 nm) to prevent charging during imaging (especially necessary if the specimen is insulating). Although in situ electron and ion-beam induced platinum (Pt) or carbon (C) deposition work well for protecting your microscopic region of interest (ROI) from gallium implantation and surface damage, the abovementioned bulk coating is highly recommended.
   
   a. **NOTE:** Au-Pd is not recommended for a bulk protective layer since it is known to become porous upon exposure to the ion beam

III. Start Up

1. Log in to the NUcore system and begin your reservation
2. Log into the PC’s if not already on
   
   a. Microscope PC:
      
      i. Username: supervisor
      ii. Password: heliosd413
   
   b. Support PC:
      
      i. Username: ADMIN
      ii. Password: OMNI
3. **NOTE:** There are four computer monitors for different applications on the microscope. The bottom right is the Microscope PC (see image above).
4. If not already running, open the **FEI System Control** on the desktop of the Microscope pc. The **xt microscope Server** window will open. Hit Start and wait until all the lights on the right are green (Console devices, Apertures, Motion, Imaging)
5. Start the User Interface (Start UI) once the server is running
NOTE: The username and password are the same as in step 2 above

6. Click OK if prompted to home the stage

7. Optional: To check more detailed vacuum status, switch to the Helios Vacuum Control window (see screenshot on the right). If this window is not already open, click on the desktop icon labeled “Start After Server is Running”. Every part of the diagram should be green. If not, please contact staff immediately!

8. Make sure the beam control tab on the right is selected ( ). Press Vent in the software to vent the chamber

   a. Allow chamber to vent (~5 minutes), do not force door open!
   b. NOTE: You can tell how close to the end of venting the system is by looking at the Z coordinates in the Omniprobe software (~9000-10000 µm)
   c. After venting, the vacuum diagram appears as the following:

9. Slowly slide the chamber door open (don’t let the door slam!) and start the Sample Loading procedure

10. NOTE: It is recommended to vent AFTER you have assembled your samples (Section IV), to minimize pumping down time of the chamber

11. If having problems pumping down the chamber, please see Section XII for troubleshooting

IV. Sample Loading

1. Always wear clean gloves when handling anything going into the FIB-SEM, and when utilizing supplied tools

2. The instrument stage is equipped with a universal mounting base (UMB)

   a. The UMB can hold 1 or 2 modules, which are held in place using 1 or 2 clamping bars, respectively
b. A stub module will accept 3 standard SEM pin mount stubs (Ø12.7mm x 8mm pin stub height in the triangular holes (see point 7 & 8 below)

c. A TEM row holder module will accept one TEM row holder (where the latter can hold up to 6 TEM grids; see point 8 below)

3. Extremely important! The height of your sample should never exceed 5 mm, measured from the module to the top of your sample. See the Figure below for the correct measurement (dashed line)

   a. If unsure about sample mounting, or your sample height surpasses 5 mm, please check with staff before inserting the sample!

4. Modules should be placed onto the UMB such that the side-pins on the modules fall into the fifth set of slots from either end of the UMB. This ensures that the modules have a circular soft part (appearing whitish) connecting to the screws of the outside clamping bars that will securely keep the modules in place

5. Do not excessively tighten the clamping bars (you will see the clamping bars tilt upward – this is **NOT SAFE**)

6. To check that the modules are secure in place with the clamping bar, carefully move your gloved finger up and down (micromovement) in between the clamping bar and the module. The sideview image of the UMB below shows the right clamping bar configuration on the left, and the wrong orientation on the right where the clamping bar is tilted
7. For loading one or more SEM stubs, use one module with one clamping bar (see image on the right). This is the configuration typically used for atom probe tomography (APT) preparation, preparation of cross sections, etc.

8. For TEM sample preparation (or any other targeted area lift-out of your sample to a TEM grid), you can use the configuration of loading the UMB with 2 modules and 2 clamping bars (see image on the right side).

9. Press Pump to start pumping down the chamber in the Main Control Tab (see Section V).

10. Once the Vacuum Status in the right bottom corner of the interface is completely green.

11. After pumping, the Chamber pressure should be lower than ~5e-5 Torr.

12. Note: Contact staff if it is higher or the diagram does not completely turn green.

V. Comprehensive Software/Hardware Interface Overview

1. The FEI System Control Software contains
   a. 4 image Quadrants with different imaging capabilities
   b. Drop down menus with a great number of functionalities for the stage, detectors, scanning acquisition, etc.
   c. Data bar for direct controls such as start/stop patterning, taking snapshots, setting auto contrast/brightness (ABC), etc.
For Tab details, see next page

Tabs:
1. Main Control
2. Stage Control
3. Patterning
4. Measurement/Annotation
5. Detector Settings
2. The FEI System Control Hardware includes a Control Panel with functions below
VI. Electron Beam Imaging  

a. Operation and Alignment

1. There are 4 Quadrants on the microscope UI (see Section V above). Clicking within a specific Quadrant will turn the data bar blue and activate it.

2. When Quad 1: Electron beam (E-beam) is activated, the electron logo in the top left should be highlighted blue. The lower left-hand side of this Quad in the data bar should also show the electron logo.

3. Select your desired accelerating voltage and current (most used E-beam accelerating voltage and current for sample preparation is 5.0kV; 1.4nA).

4. In the beam menu click on the Beam On button.
   a. If the system was in ‘Sleep’ mode, click ‘Wake Up’.

5. The ‘Source’ bar in the image to the right should be green.

6. To start live imaging, activate the quad and select from the toolbar; to stop the live image (pause the beam), select from the toolbar.

7. Note: If a live image is not actively being acquired for a particular quad, will appear in the upper left corner of the quad.

8. Adjust contrast and brightness if live image is difficult to see:
   a. Can be adjusted manually with brightness and contrast knobs.
   b. Can also be adjusted by selecting auto contrast/brightness from the data bar.
   c. It will usually be necessary to adjust every time a beam setting (voltage and/or current) is changed.
   d. Auto contrast/brightness can only be used during live imaging.

9. To move the stage, either:
   a. Double-click on the position you want to move the stage towards.
   b. Press & hold the middle mouse button and drag the direction you want to move towards. A yellow dot with arrow appears in the direction that you are dragging.
   c. NOTE: stage movement speed is a radial function of position, (i.e. clicking & dragging further from the direction you want to move towards will increase speed).
10. Navigate to a feature on your sample at low magnification, focus using the focus knobs on the control panel (or by right-clicking, pressing, and dragging left/right). Your sample surface should now be roughly in focus.

11. Find a microscopic feature on your sample, increase the magnification using the knob on the Control Panel to around 10000x magnification.

12. Focus and correct for astigmatism using X/Y Stigmator knobs on the FEI System Control Hardware Control Panel

   a. Focus the beam up and down with your fine focus and identify the two directions of stretching. Find the middle of the stretching and adjust one stigmator knob at a time until you achieve the clearest image

b. Advanced Alignment

   1. In the Main Control Tab, check the beam Lens Alignment under Tuning (OR drop-down menu under Beam → Lens Alignment).

   2. Select to look for motion in the image. You want your image to look stationary by left-clicking and dragging the grey square in X,Y

   3. Select Crossover button and center the source image (bright spot) using the grey square in X,Y. If you cannot see the beam, increase the brightness/contrast or run ABC

   4. After these adjustments, re-adjust your focus and stigmation at the general ROI
c. Setting Eucentric Height

1. **Extremely Important!** The **Eucentric Height** of your sample should be set **before** any milling/deposition or general tilting to high angles of your sample.

2. Click on 🏰 from the toolbar to link the Z stage position with the FWD; the icon will change to 🏰.

3. Switch to ‘Stage Control’ tab and select ‘Coordinates’

4. Follow the steps below:
   a. Find a feature (e.g. nanoparticle or contamination particle, preferably spherical in shape) at low mag (~100X) and 0˚ T (tilt) in the E-beam Quad
   b. Center the edge of this feature on the small yellow crosshair
   c. Enter the number ‘5’ next to T (tilt) and press enter to tilt the stage 5˚
   d. Activate CCD Quad 4 by left clicking. Click and hold the middle mouse button – a thick yellow bar representing the stage will appear.
   e. While holding the mouse button, drag up the mouse slowly until the center crosshair in the E-beam is centered again on your feature
   f. Return to 0˚ tilt to check that your feature does not move significantly. If it does, repeat from points a-e.
   g. Repeat a-f, at higher magnification (~20000X) for more accurately setting the Eucentric Height
   h. Tilt to higher angles (up to 52˚) until feature stays centered when stage is tilting for better accuracy setting of the Eucentric Height
   i. The Z Coordinate (FWD) should be close to 4 mm after these alignments. This is the optimum working distance (WD) for this instrument
VII. Ion Beam Imaging

Before any Ion Beam operation, check your Eucentric Height on a feature in the E-Beam (See Section VI-C above)!

a. Operation

1. Tilt the Stage to 52˚ (‘Stage Control’ Tab, select ‘Coordinates’, tilt)
2. When Quad 2: Ion beam (I-beam) is activated, the ion logo in the top left should be highlighted blue. The lower left-hand side of this Quad in the data bar should also show the ion logo
3. Select your desired accelerating voltage and current (most initial I-beam imaging is done at 30.0kV; 93pA)
4. In the beam menu click on the Beam On button
5. The ‘Source’ bar in the image to the right should be green
   a. If the I-beam was in Sleep mode, the ‘Source’ bar ramps up from red, to orange, and should end with green within several minutes
   b. If the source bar stays red for prolonged times (> 15 min), contact staff
6. To start live imaging, activate the quad and select from the toolbar; to stop the live image (pause the beam), select from the toolbar
7. Note: If a live image is not actively being acquired for a particular quad, will appear in the upper left corner of the quad.
8. Adjust contrast and brightness if live image is difficult to see:
   b. Can be adjusted manually with brightness and contrast knobs
   c. Can also be adjusted by selecting auto contrast/brightness ( ) from the data bar
   d. It will usually be necessary to adjust every time a beam setting (voltage and/or current) is changed
   e. Auto contrast/brightness can only be used during live imaging
9. Navigation using the I-beam is easiest when double-clicking to move the stage to a certain desired feature in the center small yellow crosshair
   a. You can center the same feature with the view from the E-beam by setting the so-called Coincidence (see Section VII C)
b. (Advanced) Alignment

1. Adjust Focus and (typically to a lesser degree needed) Astigmatism similar to the E-beam alignment
   a. **CAUTION!** Your sample may be sensitive to the energetic gallium (Ga+) ions, as they can mill your sample or can be incorporated near the surface of your sample. Therefore, minimum exposure to the ROI is recommended (unless sufficiently protected by Pt or C, see Section IX), e.g. by using short snapshots to focus/stigmate the I-beam
   b. Another strategy is to focus/stigmate on an area close by the ROI (preferably at the nm scale for increased accuracy)

2. Find an EPIC staff member for help if the I-beam is severely misaligned or does not show an image after turning the beam on, unpausing, and adjusting contrast/brightness

c. Setting I-beam and E-beam Coincidence

1. This will position the same ROI so that it is visible in both the E-beam and I-beam

2. Zero the beam shift for both E-beam and I-beam
   a. For E-beam: make sure you have Quad 1 selected. In the ‘Main Control’ tab, right click in the ‘Beam Shift’ square and select ‘Zero’
   b. For I-beam: make sure you have Quad 2 selected. In the ‘Main Control’ tab, right click in the ‘Beam Shift’ square and select ‘Zero’

3. Find a feature on your sample and center it by double-clicking in the I-beam (52˚ stage tilt)

4. Select the E-beam (Quad 2) and use beam shift knobs on the Hardware Control Panel to center the same feature
   a. You can also center the feature on your sample using stage movement in the E-beam first
   b. Disadvantage of this is that there is less beam shift coverage in the I-beam

VIII. Saving Images

1. Center and focus/stigmate at higher magnification than the ROI

2. Make sure that the correct window is active (i.e. E-beam vs. I-beam)

3. There are two ways to create/save images:
b. Select your own scan speed
   i. Slow down the scan using the scan speed section bar on the top of the screen
   ii. Click the pause button
   iii. **File > Save as > D:/Users (or EPIC_SEM (S:)) drive > Your NetID > Save**
   iv. Save file name as: Name_001 to start numbering your images

c. Preset Imaging conditions
   i. Go to **Scan > Preferences > Scanning**
   ii. Drag and drop the image icon (the camera) to the scan speed you want to use for all images
      a. Select speeds for screenshots (📸) and images (✍️)
   iii. On the main user interface, press the **image capture button** on the top of the screen
   iv. Save in your NetID folder on the (D:) drive (or EPIC_SEM (S:) drive)

4. **NOTE:** The capture image button is defaulted to snapshot option

IX. **Patterning (Deposition, Milling, etc)**
   a. **Before any Patterning, check your Eucentric Height on a feature in the E-Beam (See Section VI-C above)!**
   b. **Switch to ‘Patterning’ Tab,** which controls:
      i. Deposition using the E-beam or I-beam
      ii. Milling using the I-beam
      iii. Insertion of a specific Gas Injection System (GIS)
      iv. Insertion of the micromanipulator (Omniprobe)
   c. **Select and Snapshot** the desired window (E-beam or I-beam)
   d. **Set the stage tilt:**
      i. For E-beam, tilt = 0°
      ii. For I-beam, tilt = 52°
   e. Click Draw Pattern button. It turns yellow when active
   f. **Select pattern type from drop down menu:**
i. Rectangle  
ii. Regular Cross Section  
iii. Cleaning Cross Section  
iv. Circle  
v. Line  
vi. Polygon  
vii. Bitmap  
viii. Stream File (for annular milling)

g. Click and draw pattern(s) on selected ROI. See some exemplary patterns below:

![Rectangular Pattern](image1)  

![Regular Cross Section](image2)  

![Cleaning Cross Section](image3)

h. For two (or more) patterns, the patterns can be processed in serial or parallel mode.

i. Serial (serial mode) → Finishes pattern #1 and then moves to the next pattern

ii. Parallel (parallel mode) → Runs all patterns at the same time

i. The thicker/darker edge represents the finishing side of the pattern (i.e. scans bottom to top). It is recommended to finish with this line facing your ROI. To change the position of this line:

i. For Regular Cross Section → Rotate pattern 180° in advanced tab

ii. For everything else → Change Scan Direction (e.g. Bottom To Top ↔ Top to Bottom)

j. Adjust desired size parameters in patterning tab (X, Y, Z)

k. Change the Application. The application should be changed depending on what operation you want to perform (deposition vs milling). Commonly used applications and their functions are:
<table>
<thead>
<tr>
<th>Application Name</th>
<th>Application Function</th>
<th>Recommended Conditions</th>
<th>Approx. Time 15 µm x 2 µm (X,Y) Bar**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Si</td>
<td>Ion Milling</td>
<td>2-30 kV, 93 pA – 6.8 nA*</td>
<td>N/A</td>
</tr>
<tr>
<td>Pt Dep</td>
<td>I-Beam Pt Deposition</td>
<td>30 kV, 93 pA</td>
<td>1.5 µm thickness: ~15 min</td>
</tr>
<tr>
<td>Pt Dep Structure</td>
<td>e-Beam Pt Deposition</td>
<td>5 kV, ~1.4 nA</td>
<td>200 nm thickness: ~2 min</td>
</tr>
<tr>
<td>C Dep</td>
<td>I-Beam Carbon Deposition</td>
<td>30 kV, 93 pA</td>
<td>1.5 µm thickness: ~15 min</td>
</tr>
<tr>
<td>C e-Dep</td>
<td>e-Beam Carbon Deposition</td>
<td>5 kV, &gt;1.4 nA</td>
<td>200 nm thickness: &lt; 10 min</td>
</tr>
</tbody>
</table>

* We typically do not recommend using higher I-beam currents than 6.8 nA
**Estimated times for Pt or C deposition of bar may vary, as changes in current/gas flow etc. may occur

I. Prior to Deposition, **check your Eucentric Height!** If OK, you will need to insert a gas injection system (GIS):

   i. The current state of each gas injection system is indicated by ‘Warm/Cold’ in the Heat column

   ii. Double-click on ‘Cold’ to warm up the gas you wish to use

   iii. It will change from Cold to Warm when it is ready to be used

   iv. Select the check box for the gas type you wish to use to insert the GIS needle

   **NOTE:** Double check that you have properly positioned the stage at the eucentric height and are at the proper tilt angle for the type of deposition you are doing (E-beam vs. I-beam)

m. Run the selected pattern by selected the Play (播放) button in the data bar. Note: when pattern is running, the active quadrant status bar will turn **green**.
n. Optional: Pause/stop pattern by selecting the Pause or Stop ( ) to observe the progress of the pattern
   i. If using E-beam deposition, you can only stop the process to observe its progression
   ii. For I-beam deposition or milling, you can pause or stop and view live in the I-beam or take a snapshot with the E-beam

o. If inserted, remove GIS needle before moving or tilting the stage!

X. General Omniprobe Operation

1. Move to desired ROI
2. Set stage tilt is at 0° (recommended)
3. Note: double check that your ROI is correctly at the Eucentric Height otherwise you risk crashing the Omniprobe into your sample!
4. Switch to Support PC and open Autoprobe Software
5. Move to Location Storage controls:
   a. Select Eucentric High
   b. Select GOTO
      a. Wait until operation is finished
      b. Current stage position should be at 0,0, 300 (X, Y, Z)
6. Switch back to microscope PC
   a. In the Patterning tab, select insert Omniprobe
7. At this position, the tip should be centered in the E-Beam Quad and ~300 µm away from the center in the I-Beam Quad.
8. The Omniprobe can be moved using the Probe Controls section of the software
a. Switch from XY motion to Z motion (or vice versa) by clicking on the center button labeled XY (or Z)
b. Adjust the velocity of the Omniprobe depending on how far you are from the ROI. Drive carefully!
c. Only move the Omniprobe when you can see it moving in either the E-beam or I-beam Quads  
   i. Be cautious of Contrast/Brightness adjustments

9. Recommended Pt welding parameters to attach lamella to Omniprobe for TEM Sample prep:
   a. X, Y, Z: 1-2 µm, 1-2 µm, ~0.2 µm

XI. Shut Down and Sample Removal

1. Turn off BOTH beams by:
   a) hitting the yellow Beam On button with the E-beam quad selected
   b) hitting the yellow Beam On button with the I-beam quad selected
2. Double check the following settings:
   a) The stage tilt has been reset to 0˚
   b) GIS needles are retracted
   c) Omniprobe is retracted
3. Click Vent to vent the main chamber
4. Wait for the chamber to vent completely (approximately 5-8 minutes)
5. Carefully slide open the chamber door (don’t let the door slam!) and remove samples
6. Close the door and select Pump
7. Wait for the system to return to high vacuum (indicated by schematic at the bottom being green)
8. If it is a Friday and there are no users in the weekend on the NUCore calendar for the Helios, OR no usage for a day or more, please let the system go to ‘Sleep’ (Main Control Tab)
9. End your reservation on NUcore
XII. Frequently Asked Questions/Problems

1. The monitor is not powered on
   a) Begin NUcore reservation

2. The feature being milled/deposited is missing:
   a) Go to lower magnification in the E-beam window. If your feature appears at lower magnification, re-center the stage on that feature and beam shift the I-beam so that the feature is centered in the I-beam window as well
   b) Consult EPIC staff if this does not fix your problem

3. I lost my welded sample on the Omniprobe when retracting the Omniprobe during Lift-Out
   a) Retract the GIS prior to moving the Omniprobe to the Eucentric Height
   b) Do not move the stage while Lifting-Out an ROI (lamella) or when attaching it to a TEM grid

4. I pressed ‘Pump’ at the end of my session, but the chamber did not pump down for the vacuum status to turn green and vented eventually
   a) Switch to Helios Vacuum Control. Next, click ‘Actions’ (red circled in the left image above). The Actions Status/Availability box should appear as on the top right image. Double click ‘PumpChamber’ to pump down the chamber again. Watch the TMP1 value on the bottom right of the Helios Vacuum control window that should go up to 100%. If for some reason it drops down again, please retry this step
   b) Always ensure prior to leaving the room at the end of your session that the vacuum chamber is properly pumped down (= green status diagram)!

![Image of Helios Vacuum Control and Actions Status/Availability]

Happy FIBbing!