Before you begin...

- Complete the required safety training modules on UC Learning
  - Laboratory Safety Orientation (Fundamentals) 2013
  - Hazardous Waste Management
  - Compressed Gas Safety
- Submit a copy of your Training Transcript to Lab Manager
- Review the MSE SEM Policies and Regulations
- Fill out the SEM FAU Authorization Form with PI signature
- Fill out the MSE 150, 250, 309 Authorization Form with PI signature
- Receive a user name and temporary password for Faces scheduling
- Arrange a time for SEM training with Lab Manager
- Schedule a 2 hour block on Faces for your training
- Familiarize yourself with the graphical user interface (GUI) : A – D
- Familiarize yourself with SEM fundamentals: E – K
A. GUI

Menu Bar

Floating Toolbar

SEM Scanning Window

Focus Window

Info Bar

SEM HV: 5.0 kV
WD: 35.86 mm
VEGA3 TESCAN

SEM MAG: 8 x
Det: SE
10 mm
University of California Riverside
B. Floating Toolbar – 1/2

**MODE**: Opens the context menu for selecting *Displaying Modes*

**SPEED**: Opens the context menu for selecting predefined *Scan Speeds*

**MAG**: Left-click sets the *Magnification* as active function. Right-click opens context menu with predefined values of magnification.

**WD**: Left-click sets the *Focus* as active function.

**STG**: Left-click sets the *Stigmator* as active function.
B. Floating Toolbar – 2/2

**Brightness**: Left-click sets the *Brightness and Contrast* control as active function

**Auto**: Left-click starts *Automatic Brightness and Contrast*

**BI**: Left-click sets the *Beam Intensity* as active function

**Manual Column Centering**: Left-click starts the manual column centering process

**Acquire**: Left-click starts the *Image Acquisition*
C. Sidebar – 1/5

- **Opens new scanning window**

- **Active Function**

- **Current Value**

- **Changes Value Incrementally**

- **Change Sensitivity**
  - 1 = Fine, 9 = Coarse

- **Current Sensitivity**

- **Icon = Trackball is locked**
C. Sidebar – 2/5

Info Panel shows all the important parameters of the microscope, and at the same time allows a quick set-up of all the most frequently used functions.

- **Continual** button stops or starts scanning
- **Single** button starts scanning of a single frame and then stops scanning
- **Acquire** button starts the acquisition process
- **HV** button sets the High Voltage value as active function
- **Depth of Focus** shows estimated range sample surface is in focus
- **Absorb. Curr.** shows the electron current absorbed by the sample
- **Spot Size** shows the sample impinging beam size
C. Sidebar – 3/5

Detector Panel shows active detector. Electron Beam Panel controls filament heating and high voltage range.

- **SE** indicates Secondary Electron detector is active
  - **%/%** shows Brightness/Contrast
- **HV** shows high voltage value
- **Emission** shows current emitted
- **Live Time** shows total working time of filament
- **Heating** shows relative value of filament heating current in %

![Sidebar Diagram]

- **HV** turns the high voltage on and off
- **Heat** starts or stops filament heating
- **Adjustment** opens context menu
- **HV Drop Down** selects HV range
C. Sidebar – 4/5

Vacuum Panel controls the vacuum system.

- **Column Pressure** indicates the value of the pressure in the column
  - Red = Not Ready
  - Green = Ready

- **Status** shows state of vacuum
  - Venting = still venting
  - Venting finished = venting is finished and chamber can be opened
  - Pumping = still pumping
  - Vacuum ready = chamber is pumped down to sufficient vacuum
  - Vacuum off = vacuum is in standby mode

**PUMP** starts the pumping procedure (~ 3 min to complete)

**VENT** vents the microscope

**STANDBY** interrupts microscope work if necessary for a long period of time

STANDBY interrupts microscope work if necessary for a long period of time
C. Sidebar – 5/5
Nano Stage Control controls the specimen stage movement.

- **Rotation** buttons rotate the stage
- **Diagonal** stage movement
- **X-dir** stage movement
- **Y-dir** stage movement

Distance from center is proportional to magnitude of movement (large)
D. SEM Image Parameters

**Windows**
Aspect Ratio of Image = 4:3
Resolution = 1024 x 768

**Averaging**
Accumulation = Disable
(Prone to vibrational noise and drift)

**Acquisition**
Keep Actual Speed
Keep view field/print magnification

**Infobar Texts**
Show Infobar:
Beam Energy, Working Distance, View Field, Detector, Vacuum, Scan Mode
E. Accelerating Voltage – 1/2

Recommendation: Start at 5 kV and increase voltage incrementally to balance resolution to surface structures.

- High resolution
- Unclear surface structures
- More edge effect
- More charge-up
- More damage
- Clear surface structures
- Less damage
- Less charge-up
- Less edge effect
- Low resolution

30 kV

5 kV
E. Accelerating Voltage – 2/2
F. Working Distance

Recommendation: Start at \( \approx 10 \) mm and decrease WD to achieve greater resolution or increase WD to achieve greater depth of field if necessary.
G. Working Distance vs Focus (W/D) Distance

**Objective Pole Piece**

- **Over Focus**
- **In Focus**
- **Under Focus**

**Actual Working Distance** = Distance between objective pole piece and sample and can only be controlled manually with the **knob outside the chamber**

**Focus (W/D) Distance** = Distance between objective pole piece and focal point and can only be controlled by the **Focus (W/D) button**
**H. Beam Intensity – 1/3**

**Recommendation:** Decrease beam intensity until balance between resolution/grainy image and acquisition time is desired.

- **High BI:** Larger spot size for low magnification but poor resolution.
- **Low BI:** Higher resolution but grainy image – balance with higher acquisition time.

**BI = 15**

- Smooth Image
- Large
- Deteriorated resolution
- More damage

**BI = 4**

- High resolution obtainable
- Small
- Less Damage
- Grains image
H. Beam Intensity – 2/3

Accelerating voltage ↑, spot size ↓

1 kV
Spot Size: 110 nm

10 kV
Spot Size: 40 nm

5 kV
Spot Size: 54 nm

30 kV
Spot Size: 31 nm
H. Beam Intensity – 3/3

- Beam current ↓, spot size ↓

- Working distance ↓, spot size ↓

BI: 15   Spot Size: 210 nm
BI: 10   Spot Size: 63 nm
BI: 6    Spot Size: 31 nm

WD: 30 mm  Spot Size: 180 nm
WD: 15 mm  Spot Size: 110 nm
WD: 6 mm   Spot Size: 63 nm
I. SEM Chamber

- Z-dir Stage Movement Knob
- Stage Tilt Handle
- Stage Tilt Tightening Knob
- 0° Tilt Position
- Tilt Position Marker
J. Tilt (Advanced Users) - 2/2
# K. High Resolution Imaging Process Tree

<table>
<thead>
<tr>
<th>#</th>
<th>Description</th>
<th>Stage</th>
<th>Mag</th>
<th>Focus</th>
<th>Z Knob</th>
<th>Bl</th>
<th>Speed</th>
<th>Auto B/C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Center <strong>tallest part</strong> of sample in window</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>2</td>
<td>Achieve desired <strong>working distance</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Center <strong>desired sample image</strong> in window with <strong>desired Mag</strong></td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>4</td>
<td>Increase Mag to ≥ <strong>2X desired Mag</strong></td>
<td>Y</td>
<td>Y</td>
<td></td>
<td></td>
<td></td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>5</td>
<td>Beam optimization</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(if desired Mag ≥ 10 kX)</td>
<td>Y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>6</td>
<td>Achieve <strong>best focus</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>7</td>
<td>Reduce Mag back to <strong>desired Mag</strong></td>
<td>Y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>8</td>
<td>Determine optimal image conditions and acquire</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>9</td>
<td>Reduce Mag and acquire image</td>
<td>Y</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Y</td>
<td>Y</td>
</tr>
<tr>
<td>10</td>
<td><strong>Move to new sample location -&gt; Repeat #3 to #9</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
SEM Operation

I. Initiate Software
II. Sample Preparation
III. Sample Loading
IV. Turning on HV
V. Mode
VI. Beam Intensity
VII. Brightness and Contrast
VIII. Mag
IX. Focusing
X. Speed
XI. Working Distance
XII. Image Preparation
XIII. Column Centering
XIV. Stigmation Correction
XV. Image Acquisition
XVI. Saving
XVII. Sample Unloading
XVIII. Cleanup
I. Initiate Software – 1/1

1. **Record** your time-in on the **sign-in sheet**

2. Sign into Windows using provided **Username** and **Password** located on **monitor** if necessary

3. Wait for the VegaTC software to load

4. Sign into your user account with your **Username** and **Password**
II. Sample Preparation – 1/1

1. **Always wear gloves** when dealing with anything that will be placed into or in contact with the SEM
2. The specimen should be conductively fixed or glued to a specimen stub (12.5 mm specimen pin-stubs)
3. Non-conductive samples need to be coated by a conductive layer using either a carbon coater or sputter coater (see CFAMM for coater)
4. Magnetic samples will need to be fixed well by screw holder (provided by user)
5. Items located in the cabinet are available for SEM users to help prepare their samples
III. Sample Loading – 1/3

1. Click VENT to vent the microscope
2. Click Yes to confirm venting
3. Wait until “Venting finished” appears
4. Set the tilt of the specimen stage to 0° if not already set to 0° (Advanced Users only)
5. Gently pull the chamber corners toward you to open the chamber
III. Sample Loading – 2/3

6. Rotate the stage if necessary to access screw port

7. Using the provided tweezers, clamp onto the specimen stub and **blow a stream of air** over the **entire specimen stub** AWAY from the chamber using Airgun

8. Carefully insert the specimen stub into the specimen stage

9. Tighten the screw holding the specimen stub
III. Sample Loading – 3/3

10. Ensure that the sample stage is at the lowest position using Z-knob (clockwise)

11. Carefully close the chamber door by pushing it towards the chamber **CHECKING THAT THE SAMPLE DOES NOT TOUCH ANYTHING INSIDE CHAMBER**

12. Place finger against chamber door

13. Click **PUMP** to start pumping down chamber

14. Wait until bar graph shows **red** to release finger

15. Wait until the bar graph turns **green** or “**Vacuum ready**” appears (~ 3 min)
IV. Turning on HV – 1/2

1. Click the **HV Range** to choose desired HV value (pick **0 – 5 kV** as default)

2. Click **HV** to turn on the high voltage

3. Click on **HV** on the Info Panel or select **HV** in Pad Drop Down

4. Set a specific **High Voltage** in the **Pad** panel (set 5 kV as starting voltage)

5. Click **Adjustment >>>** and select **Auto Gun Heating** (required when black screen present after turning on HV)
V. Mode – 1/1

1. Click **MODE**
2. Check **Continual Wide Field** option
3. Choose desired scanning mode (default = Resolution)

<table>
<thead>
<tr>
<th>Mode</th>
<th>Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Resolution</td>
<td>High resolution&lt;br&gt;Lower depth of focus</td>
</tr>
</tbody>
</table>
| B Depth     | Good resolution  
              | Increased depth of focus            |
| C Field    | Lower resolution  
              | Large field of view  
              | High depth of focus             |
| D Wide Field | Extra large field of view            |

4. Right-click on **MAG** and select **Minimum Magnification**
VI. Beam Intensity – 1/1

1. Center the SEM window onto your desired sample using the stage control

2. Click **BI** to adjust beam intensity using the << and >>

3. Recommend **BI** of **15** to start at low mag

4. Change the sensitivity if necessary
   
   1 = Fine, 3 = Coarse
   
   **Recommended Value** = 3
VII. Brightness and Contrast – 1/1

1. Click **Auto** to auto adjust the brightness and contrast if too bright or dark as necessary

2. Click **Brightness** to manually adjust the brightness and contrast

   - **Contrast**: Hold F12 + ↔ trackball = Change only Contrast
   - **Brightness**: Hold F11 + ↑ trackball = Change only Brightness

3. Click **Tools > Histogram** to bring up the histogram (optional)

   The distribution should be centered for proper balance
VIII. Mag – 1/1

1. Click **MAG** to change the magnification

2. Turn the trackball from left to right

3. Or enter a value directly in **Pad** panel

4. Change the sensitivity if necessary

   1 = Fine, 9 = Coarse

   **Recommended Value** = 2 for Fine to 5 = Coarse
IX. Focusing – 1/1

1. Click \textbf{WD} to adjust \textit{focus distance}

2. Turn the Trackball from left to right to adjust focus

3. A \textbf{focused image} shows the \textit{actual working distance} via \textbf{WD} value

4. Change the sensitivity if necessary
   \begin{itemize}
   \item 1 = Fine, 9 = Coarse
   \item \textbf{Recommended Value} = 2 = Fine to 5 = coarse
   \end{itemize}

5. Double-left-click in the SEM scanning window to create a focus window
   \begin{itemize}
   \item Left mouse button inside = move focus window
   \item Right mouse button inside = resize window
   \item Double-left-click = remove window
   \end{itemize}

6. \textbf{WD} $\approx$ 30 mm when sample is at lowest position
X. Speed – 1/1

1. Click **SPEED** to adjust scan speed

2. Use Focus Window to determine the effect of **SPEED** and **BI** has on your image quality

Recommendation:

**SPEED** of 1 – 4 for **initial focusing**

**BI** setting should be appropriate to **MAG** value

**SPEED** of higher values looks better but takes longer to focus!

Use higher **SPEED** values of 5 – 8 when **ready to save images**
XI. Working Distance – 1/2

Use a combination of **MAG, Stage Control,** and focusing (**WD**) to first:

a. Identify and bring the **tallest position** of your sample to the **center** of SEM scanning window

b. **Increase MAG** until **distinct features** make up **majority** of window
c. Check if mode = **Resolution** or **Depth** (if not, keep increasing **MAG**)
d. Continually **increase** the **MAG** until you **CLEARLY** see focus/defocus transitions

**NOTE:** The tallest portion should be focused since this will crash into the pole-piece first as you raise the stage in the next step.

This DOES NOT have to be the desired position for your images, it is ONLY for setting the safe working distance value!
XI. Working Distance – 2/2

PROCEED WITH CAUTION AS CHANGING THE WORKING DISTANCE CAN RESULT IN DAMAGE TO THE SEM!

1. **Identify current WD** by focusing on sample
2. **Raise the specimen stage** by SLOWLY turning the Z-knob counter-clockwise
3. **Identify new WD** by focusing on sample
4. **Repeat steps 2 - 3** until desired WD is achieved
5. Click *Auto* to auto adjust the brightness and contrast if too dark when necessary
6. **SLOW DOWN WHEN YOU REACH ~ 10 mm AND DO NOT GET LESS THAN 5 mm**
XII. Image Preparation – 1/2

Imaging at \( \text{MAG} \geq 10 \text{ kX} \) requires optimization steps XIII. Column Centering and XIV. Stigmation Correction after completion of XII. Image Preparation, else skip and proceed next to XV. Image Acquisition directly.

1. Right-click on \( \text{MAG} \) and select \textit{Minimum Magnification} to see your whole sample again.

2. Identify an area of interest on your sample to image by using a combination of \( \text{MAG}, \text{Stage Control}, \) focusing (\( \text{WD} \)) , and \( \text{BI} \).
XII. Image Preparation – 2/2

3. Bring the area of interest to the **center** of SEM scanning window and to the **highest desired** magnification (e.g. Desired Mag = 10 kX)

   You will **NOT** use the **Stage Control** after this step, so **ENSURE** that the image at the Desired Mag is the one you wish to take before continuing.

4. Increase **MAG** by ≥ 2X the desired Mag using the Pad (e.g. New Mag = 20 kX, 30 kX, etc...)

   Higher **MAG** yields better results but gets more difficult to optimize.

5. Reduce **BI** if necessary to increase resolution.

6. Change scan **SPEED** to 3 or 4 to remove graininess.

7. Focus (**WD**) your sample again.

---

**Recommended Initial BI values**

<table>
<thead>
<tr>
<th>Magnification</th>
<th>Beam Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Min – 200</td>
<td>13 – 18</td>
</tr>
<tr>
<td>200 – 2000</td>
<td>8 – 12</td>
</tr>
<tr>
<td>2000 – 10k</td>
<td>7 – 10</td>
</tr>
<tr>
<td>&gt;10k</td>
<td>4 – 7</td>
</tr>
</tbody>
</table>
XIII. Column Centering – 1/2

1. Click **Manual Column Centering** button

   The Manual Centering Wizard will appear

2. Click **Next>>**

   Your image will now “wobble” in and out of focus

   If your image has any X or Y translation as it wobbles, you will need to remove it

3. Adjust the **Wobbler sensitivity** to change the extent of “wobble” if necessary
XIII. Column Centering – 2/2

4. Minimize image movement by adjusting the OBJ Centering using the trackball
   \[
   \text{X: Hold F12 + } \hspace{1cm} \text{trackball} \quad \Rightarrow \text{Change only X-movement}
   \]
   \[
   \text{Y: Hold F11 + } \quad \text{trackball} \quad \Rightarrow \text{Change only Y-movement}
   \]

   The image should remain stationary with no X or Y translation but only oscillate in/out of focus

5. Adjust the sensitivity to finely control the OBJ Centering if necessary

   \textbf{Recommended Value} = 5 first then 2

6. Check that the values are changing, else click << Previous and Next >> to reset

7. Click \textbf{Finish} when done
XIV. Stigmation Correction – 1/2

1. Click **WD** and bring **out-of-focus** first to check if any streaking occurs on **non-straight features**

When Stigmation corrected, a focused image will be come **significantly sharper**
XIV. Stigmation Correction – 2/2

2. Click **WD**, create focus window, and focus on a feature (Sensitivity = 2)

3. Set **SPEED = 4** + appropriate **BI** (see table)

4. Click the **STG** to set as active function

5. Set **Stigmator Sensitivity = 6** (slow down for accuracy near “sweet spot”)

6. Try to achieve a sharper image by adjusting the Stigmators (both X and Y)
   - **X**: Hold F12 + trackball = Change the X-component
   - **Y**: Hold F11 + trackball = Change only Y-component

5. Repeat step 1 to check if stigmation exists still

6. Repeat steps 1 – 6 until correction is complete
XV. Image Acquisition – 1/3

1. Click **WD** and achieve the **BEST** focus (Recommend sensitivity = 2)

   (Do **NOT** focus again **AFTER** this step!)

2. Click **MAG** and set back to desired magnification
   (e.g. Desired Mag = 10 kX)

3. Activate the focus window over a desired feature

   Smaller window = requires less time to refresh

   ![Example images](20_kX, 10_kX)
XV. Image Acquisition – 2/3

4. Identify maximum **Acquisition Time** for your image (e.g. 2 min) and select corresponding **Speed** (e.g. Speed 7)

5. Adjust the **Bi** until a balance between resolution is matched with graininess

6. Click **Auto** to auto adjust the brightness and contrast as you change the **Bi**

   **NOTE:** Remove focus window first else it will only adjust pixels found within focus window + change speed back to 1 for faster auto correction

7. If high resolution is desired but excessive graininess is present, increase the **Acquisition Time** (e.g. Speed 7 -> 8)

8. Repeat steps 5 – 6 until desired balance between resolution and graininess and is achieved (e.g. see next slide for examples)
XV. Image Acquisition – 3/3

Low Resolution
Low Graininess

High Resolution
High Graininess

Speed

10 8 BI 6 4
XVI. Saving – 1/1

1. Click Acquire to capture image

2. If desired, you may save information to the image file

   **Note** = the basic description

   **Sign** = the enlarged description

   **Description** = the detailed information

   **Add** = saves the Note or Sign in the list

   **Delete** = deletes the Note or Sign from the list

3. If you choose not to include any Header information, click OK

4. Choose the folder location for your images

5. Enter your file name

6. Click Save
XVII. Sample Unloading – 1/3

1. Right-click on **MAG** and select *Minimum Magnification*

2. Click **SPEED** and select **Speed 1**

3. Carefully lower the sample stage to the lowest position by turning the Z-knob clockwise

4. Set **BI** to 15

5. Click **Auto**
XVII. Sample Unloading – 2/3

6. Set **WD** to 30 mm

7. Click **HV** to turn off the high voltage

8. Click **VENT** to vent the microscope

9. Click **Yes** to confirm venting

10. Wait until **“Venting finished”** appears

11. Set the tilt of the specimen stage back to 0° if not already set to 0° (Advanced Users only)
XVII. Sample Unloading – 3/3

12. Gently pull the chamber corners toward you to open the chamber

13. Loosen the screw holding the specimen stub on the specimen stage

14. Rotate the stage if necessary to access screw port

15. Using the provided tweezers, carefully remove the specimen stub out of the specimen stage
XVIII. Cleanup – 1/1

1. Ensure that the sample stage is at the lowest position by full clockwise turns
2. Carefully close the chamber door by pushing it towards the chamber
3. Place finger against chamber door
4. Click **PUMP** to start pumping down chamber
5. Wait until bar graph shows red to release finger
6. Wait until the bar graph turns green or “**Vacuum ready**” appears (~ 3 min)
7. Open the **File** menu and select **Logoff**, click **Yes**
8. **Record** your total time used in the **Sign-in sheet**