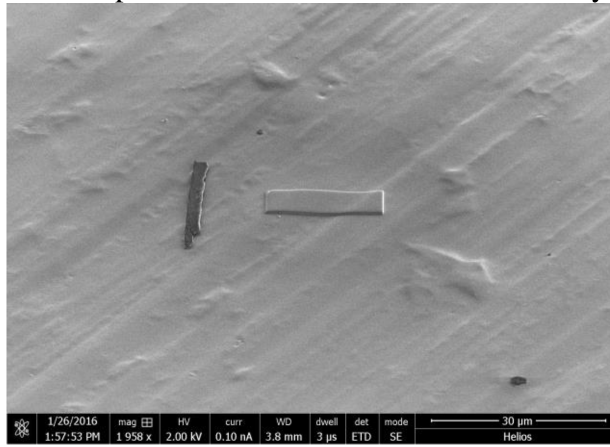
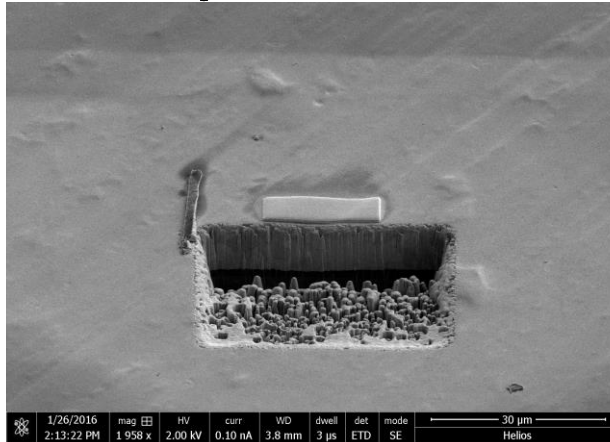
2D Image Stack Using Auto Slice & View (Advanced FIB Application):

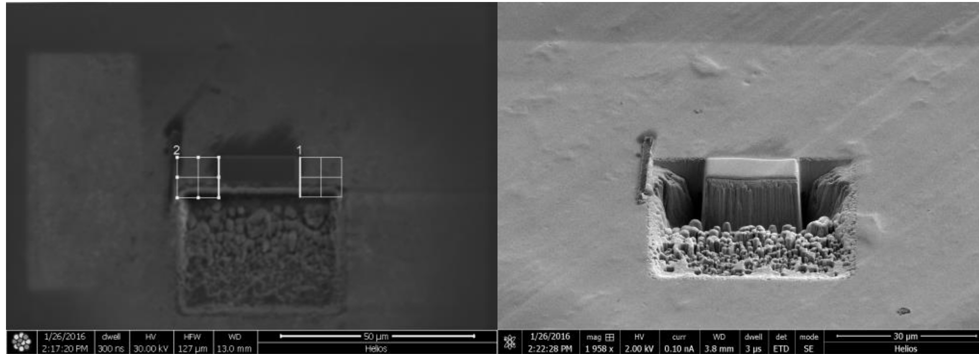
1. Bring the sample to eucentric height; follow the Ebeam and Ibeam Pt deposition steps to add a protective Pt layer on top of the sample surface. Brief procedures are described as the following:
    - For dedicated sample, an initial layer of Pt (e.g. 20 x 6 x 0.1 um) by Ebeam (Pt e-dep surface, 2 kV, 1.6 nA) at 0° stage tilt is needed before Ibeam deposition of Pt.
    - For hard sample (not so dedicated), direct Ibeam deposition (e.g. 20 x 6 x 1 um) at 52° stage tilt is fine using 0.79 nA (6 pA/um^2).
    - Make sure to insert the GIS needle before deposition starts and retract the needle after deposition. Use iSPY to monitor the deposition if needed.
    - Take a Ebeam snapshot to make sure formation of Pt layer.



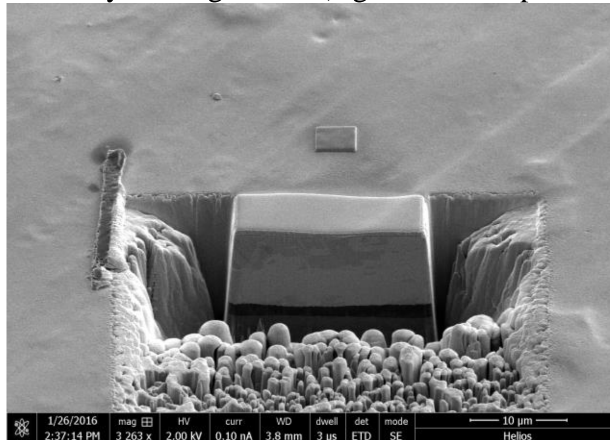
2. Mill a trench below the Pt player using regular cross section tool using the following suggestion: X= 20 um + Pt layer width; Y= Z x (1.5 or 2); Z is deep enough to show sample. (e.g. 30 kV, 21 nA or lager, 40 x 25 x 15 um, bottom to top direction)



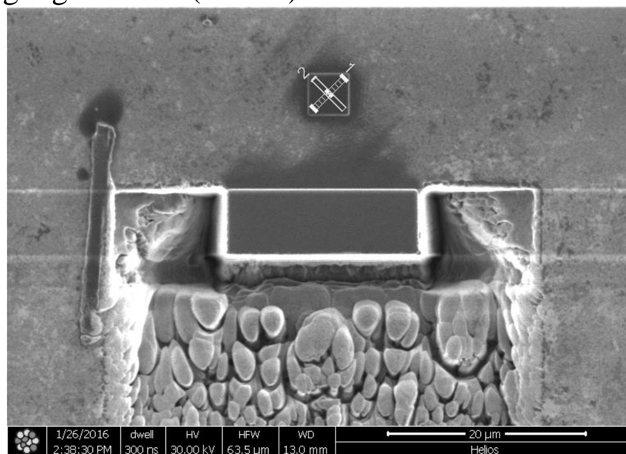
3. Mill trenches on both sides of the Pt layer (e.g. 30 kV, 21 nA or lager, 10 x 10 x 15 um): left to right direction for the one on left and right to left direction for the one on the right.



4. Make a Ibeam Fiducial marker near the Pt layer:
    - First make a Pt layer using Ibeam (e.g. 30 kV, 80 pA, 4 x 4 x 1 um).



    - Then mill a cross on the Pt layer using two rectangular cross sections, make sure the cross is as deep as possible to show enough contrast in Ibeam imaging (e.g. 5 um) using high current (2.5 nA).

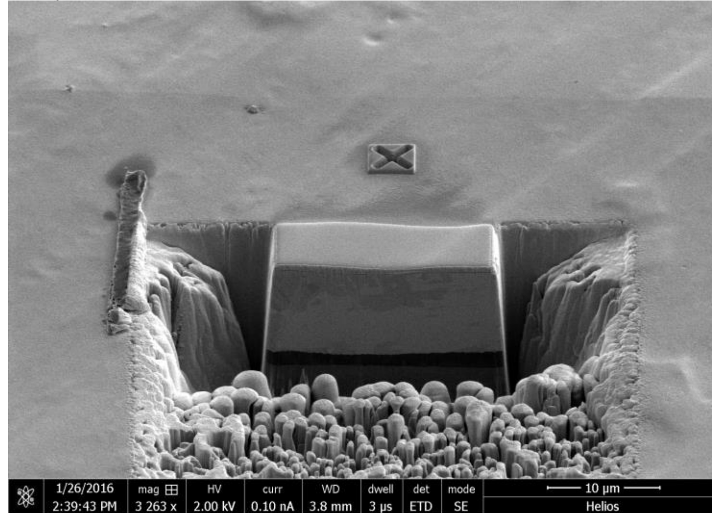


    - In UI parameter tab, save current position as milling position.

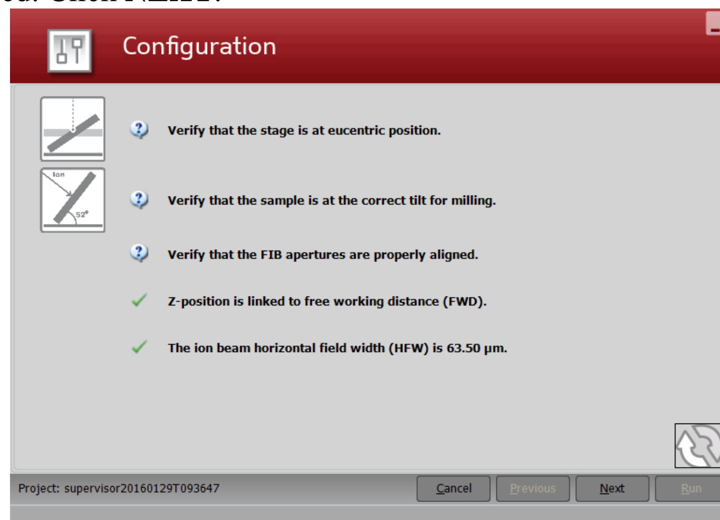
    Note: Ibeam Fiducial is used to find the correct position of AOI for slicing during data collection.

5. Clean cross section (e.g. 30 kV, 2.5 nA, 22 x 1.5 x 15 um, bottom to top direction).

6. Adjust SEM settings to observe cross section features by fine tuning focus and stigmator. For bio-samples, use Mode 2, ICD detector, 5kV, 0.4 nA, 30 us, 1536 x 1024, Contrast to Inverse, check Dynamic Focus and select Automatic (Cross Section) in Tilt Correction.

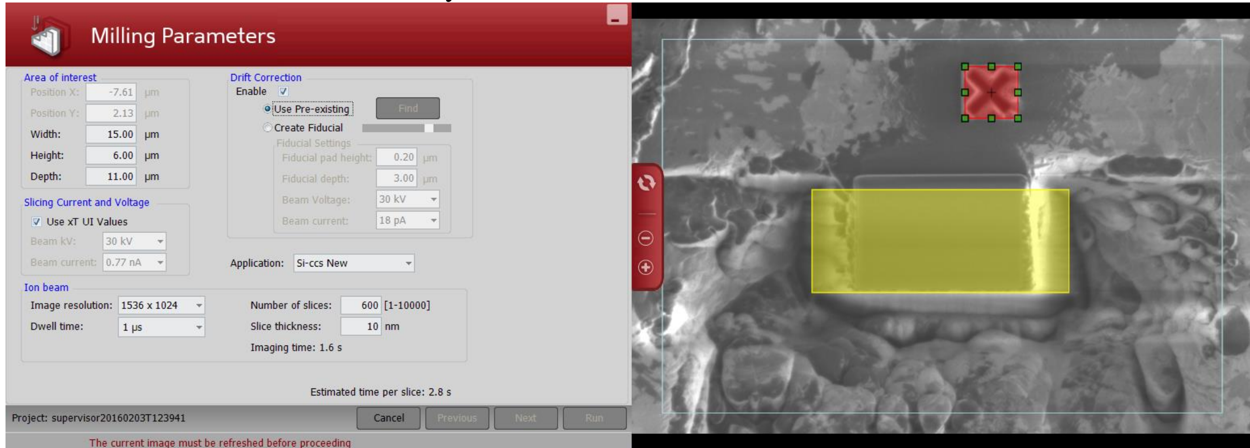


7. UI > Tools > **Auto Slice & View G3 1.5** (**ASV**):
   - Create project page: name, description, parameter file. Click **OK**.
   - Configuration page: software will check the microscope configurations (eucentric height, correct tilt angle, Ibeam current aperture properly aligned, link Z to free working distance, horizontal field width. Refresh to update the configuration if needed. Click **NEXT**.



   - Milling parameters page:
     - ❖ Choose proper slicing Ibeam parameters (30 kV, 0.79 or 2.5 nA), refresh the Ibeam image, this will be the milling position.
     - ❖ Identify Area of Interest (AOI) by adjusting the size and position of the yellow box: Width should be wider than the Pt protective layer. Depth should be the milling depth.
     - ❖ Choose Si-ccs New in Application.
     - ❖ Choose desired slice thickness.

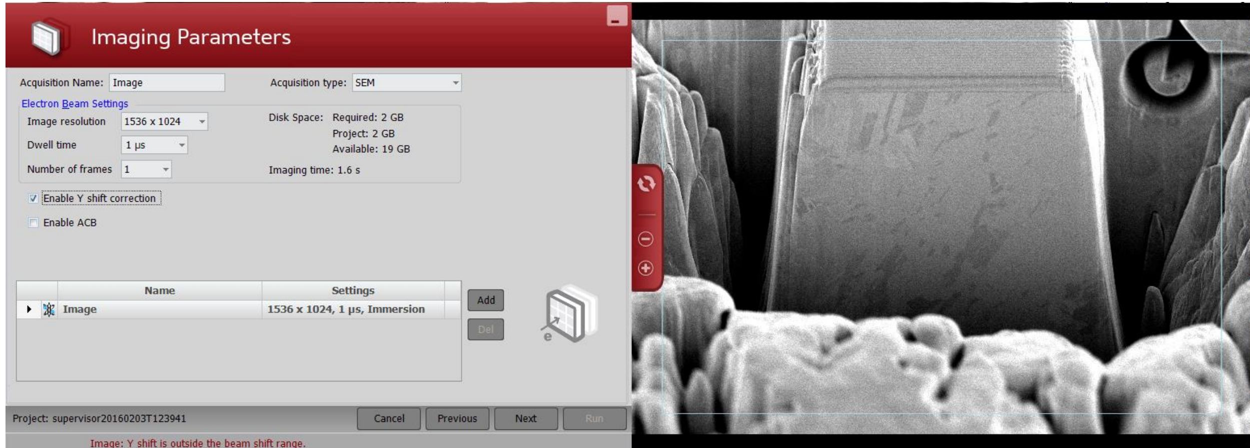
❖ Choose proper magnification to make sure AOI and Fiducial marker are within the cyan box.



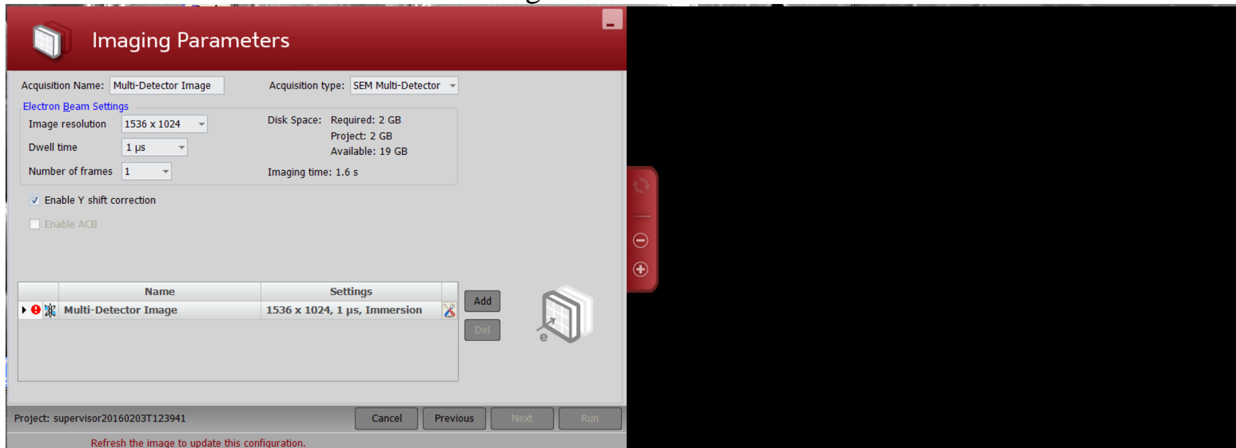
❖ Set up drift correction at last. Choose "use pre-existing" in drift correction and adjust the size and position of red box to cover the Fiducial marker. Click "find" to score Fiducial marker. The score should be as close to 1.00 as possible. If not satisfied with the score click "reset" and adjust Ibeam brightness and contrast to re-score; If satisfied with the score click **NEXT**.

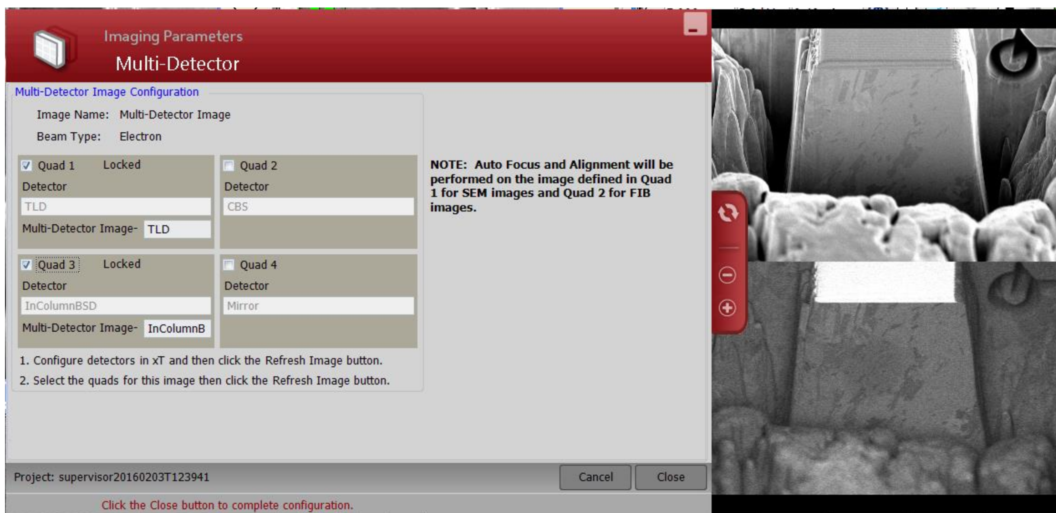
- Sample Preparation page: deselect protection layer, rough, left trench, right trench, Green Clean Pattern (This is because we have done these manually in step 1-5). Click **NEXT**.

- Imaging Parameters page: In UI and Ebeam quad choose the best imaging conditions (e.g. immersion mode 2, proper beam current, detector, SE or BSE, dynamic focus with automatic cross section in tilt correction). Do direst alignment and save current position as imaging position in UI. In ASV refresh to take an Ebeam image (Meanwhile the imaging condition is saved in ASV). Choose resolution, dwell time, enable **Y shift correction**, SEM acquisition type (SEM). Click **NEXT**.

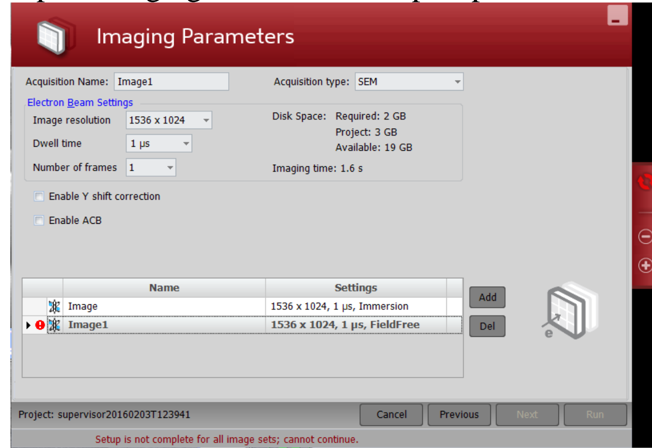


❖ (Optional) Multiple detectors: Choose SEM Multi-detector in acquisition type; use configurations toolbox to choose quads and detectors; preview multi-detector images.





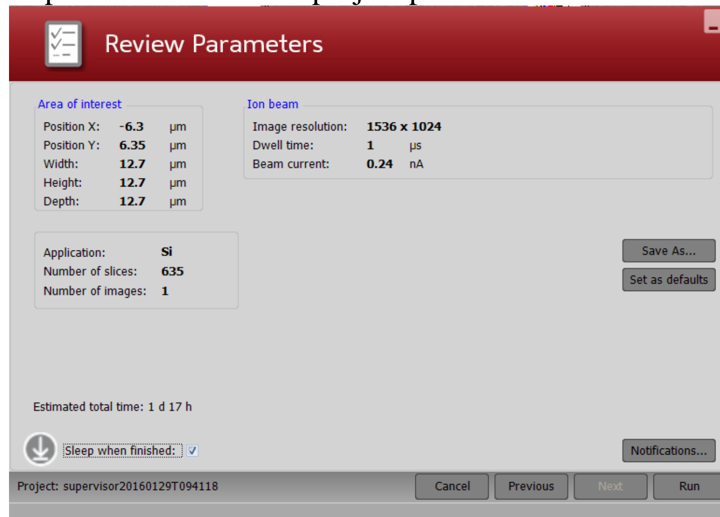
- (Optional) Multiple area: In UI, find the first image area and set up imaging condition. Use "Add" tool in ASV to add another image area. Repeat imaging conditions set up step for the second area.

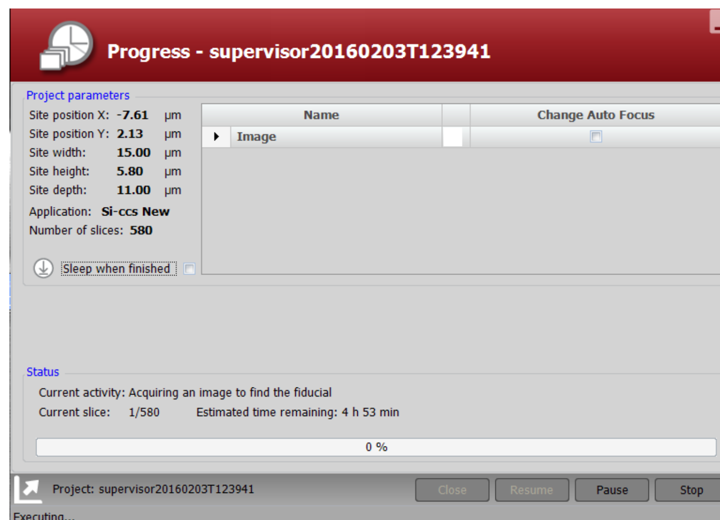


- Multidetector and Multiarea can be combined.

Note: Enable Y shift correction is for imaging condition in which Ebeam is not perpendicular to the slice surface.

- Review parameters and save project parameter. Click **RUN**.





- Click the white arrow on the lower left corner to show image stack: