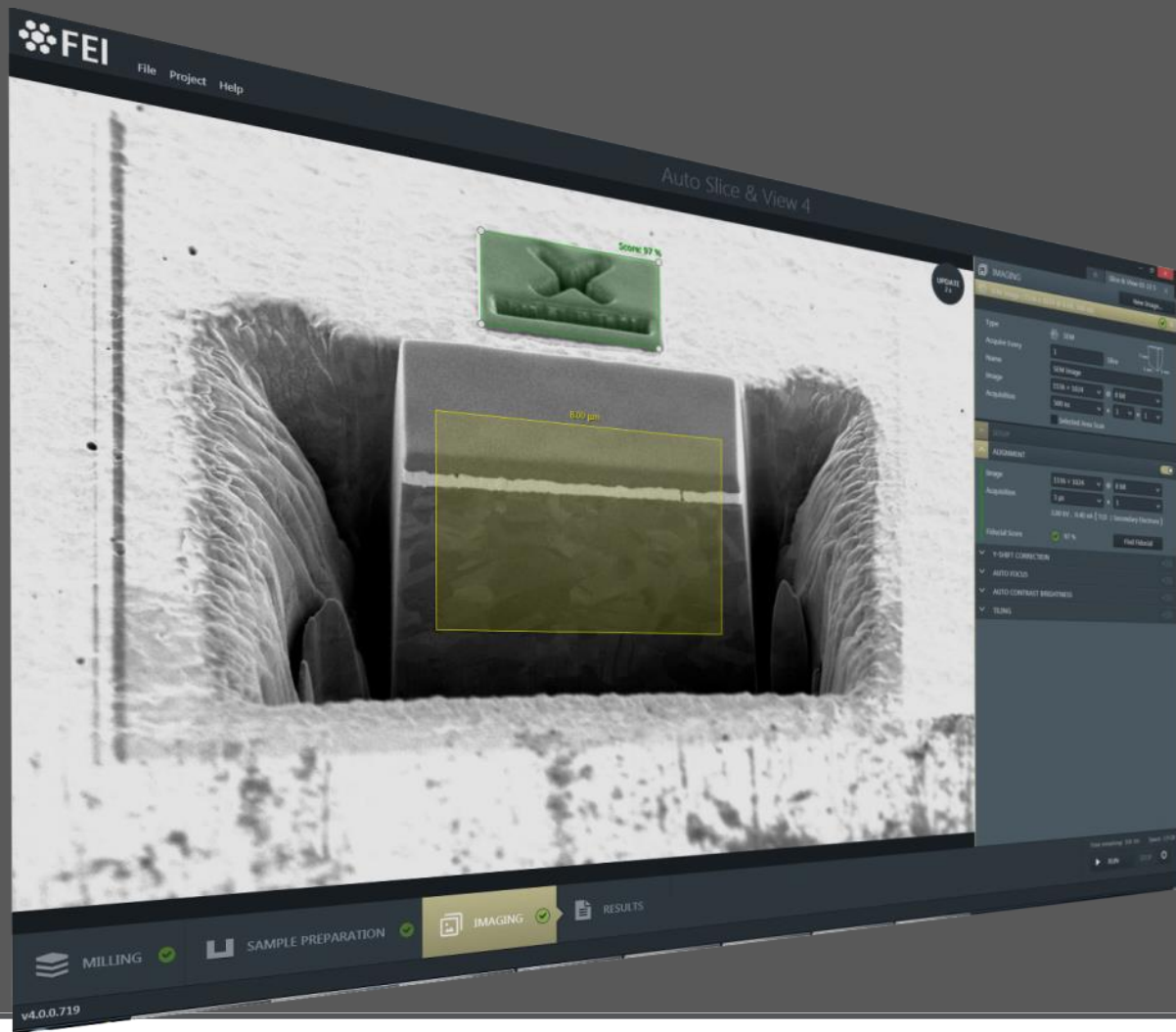




ThermoFisher
S C I E N T I F I C

Scios
ASNV4

Module 13

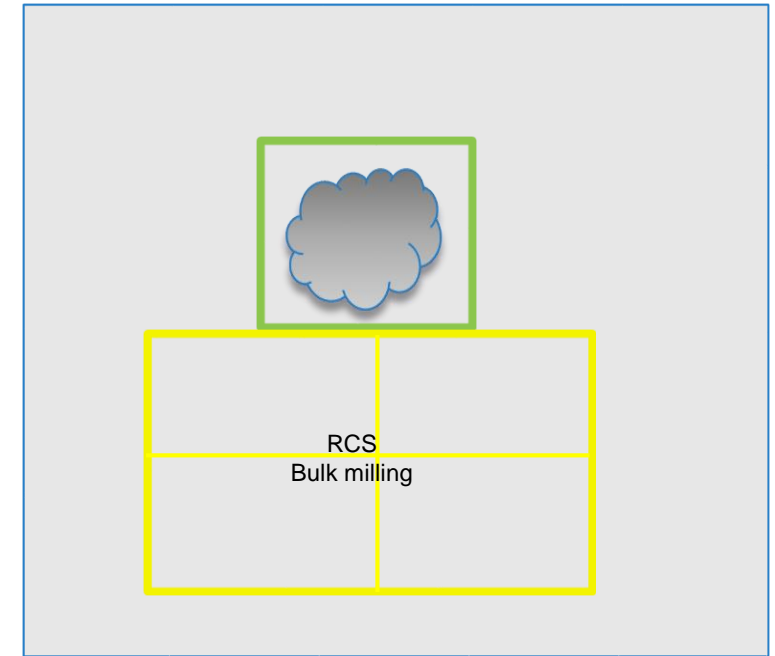
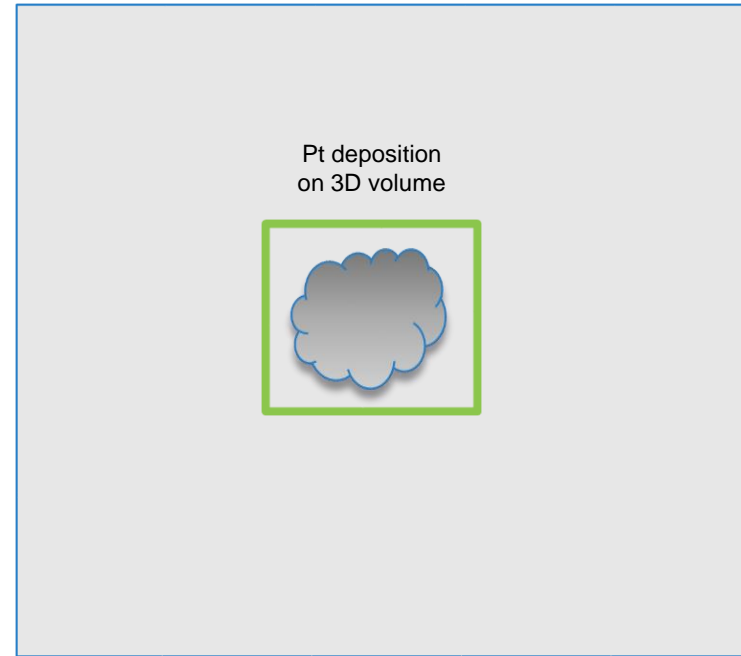


Auto Slice and View 4

- Scios pre-preparation
- Setting-up ASNV4
- Imaging set-up

ASNV pre-prep @ 52° tilt

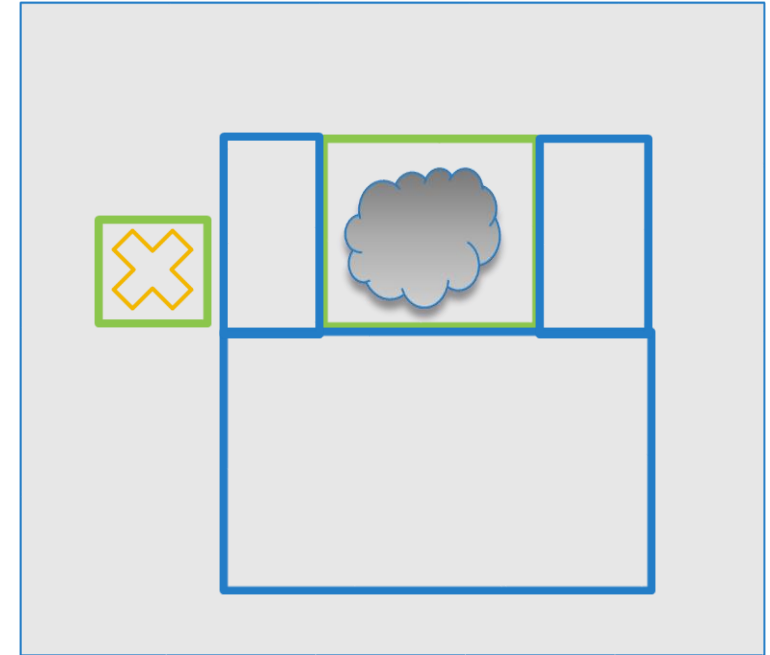
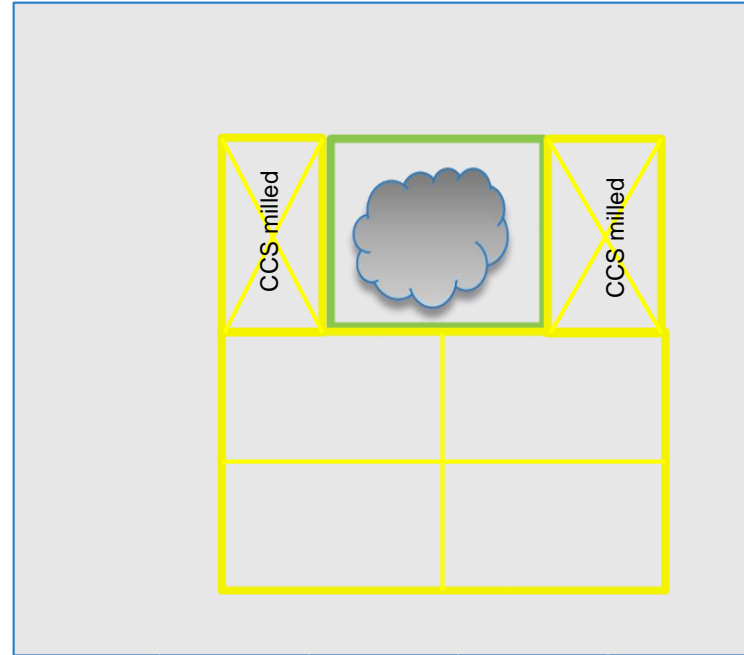
- find area of interest (link Z to WD)
- set beam coincidence point (using OptiTilt mode)
- manual preparation of sample:
 - * Pt e-depo might be helpful to find back the AOI with the FIB
 - * deposit Pt IBID layer over AOI: for example: 15x15x1um using 1nA
- Bulk milling (removing bulk material) using Regular Cross Sectioning + Si-multipass new for example: 40x20x8um using 30nA



- **FOR all coated non-conductive samples:**
- Focus on sample surface (link Z to WD)
- Bring sample to eucentric height (7mm)
- Find area of interest by using higher KV (in combination with BSE (e.g T1) imaging)

ASNV pre-prep @ 52° tilt

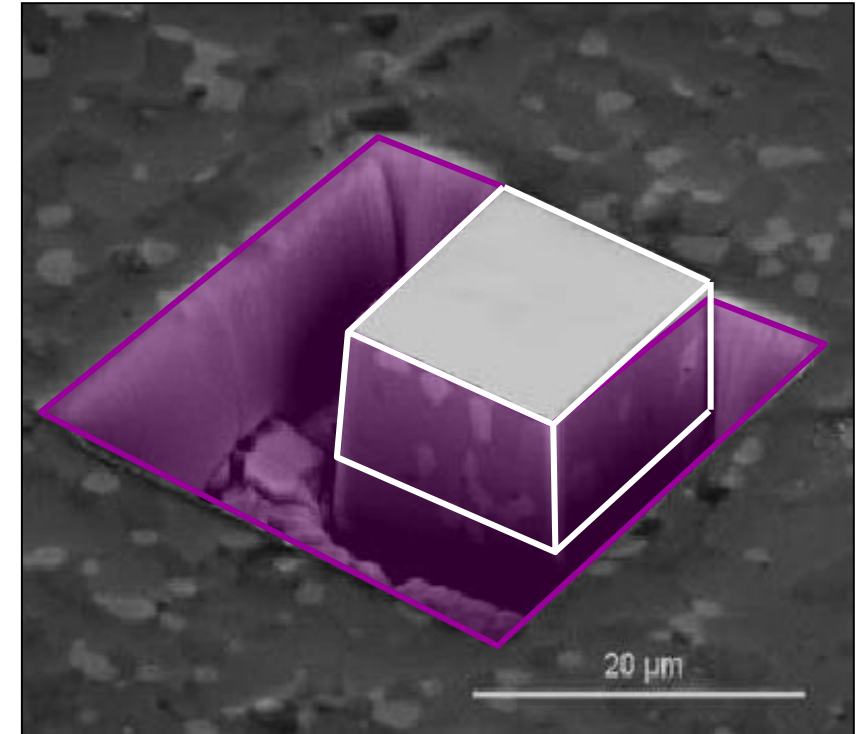
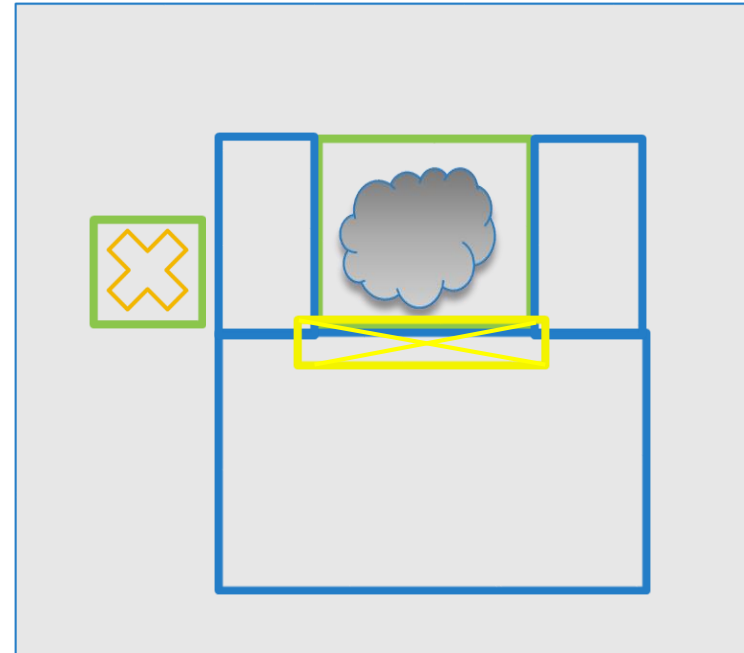
- find area of interest (link Z to WD)
- set beam coincidence point
- manual preparation of sample:
 - * Pt e-depo might be helpful to find back the AOI with the FIB
 - * deposit Pt layer over AOI:
for example: 15x15x1um using 1nA
- Bulk milling (removing bulk material) using Regular Cross Sectioning + Si-multipass new
for example: 40x20x8um using 30nA
- Mill side trenches (right and left side of Pt layer using Cleaning Cross section + Si appl. file
for example: 10x15x2 using 15nA
- Create a unique fiducial (or continue with next slide and make fiducial in ASNV4)
 - *deposit a Pt depo pad, size about 1/4 of 3D volume Pt
for example: 7x7x0.4 using 300pA Pt and *mill a cross (deep enough, Z=1um)



- Remove enough material in Y and Z to prevent shadowing (and a gradient over the images acquired during the run)

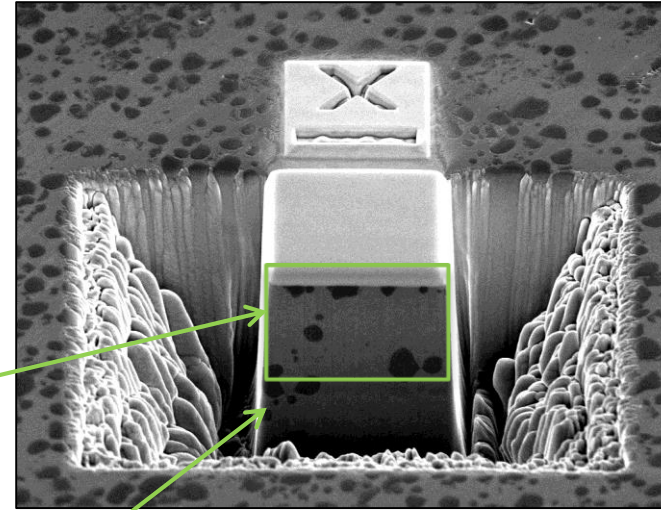
ASNV pre-prep @ 52° tilt

- find area of interest (link Z to WD)
- set beam coincidence point
- manual preparation of sample:
 - * Pt e-depo might be helpful to find back the AOI with the FIB
 - * deposit Pt layer over AOI:
for example: 15x15x1um using 1nA
- Bulk milling (removing bulk material) using Regular Cross Sectioning + Si-multipass new
for example: 40x20x8um using 30nA
- Mill side trenches (right and left side of Pt layer using Cleaning Cross section + Si appl. file
for example: 10x15x2 using 15nA
- Create a unique fiducial:
 - *deposit a Pt depo pad, size about ¼ of 3D volume Pt
 - for example: 7x7x0.4 using 300pA Pt and
 - *mill a cross (deep enough, Z=1um)
- Cleaning the cross section of AOI using CCS + Si + 3nA + tilt sample to 53.5° for example: 17x2x3um
- When CCS is finished tilt back to 52°; **sample is ready for ASNV4**

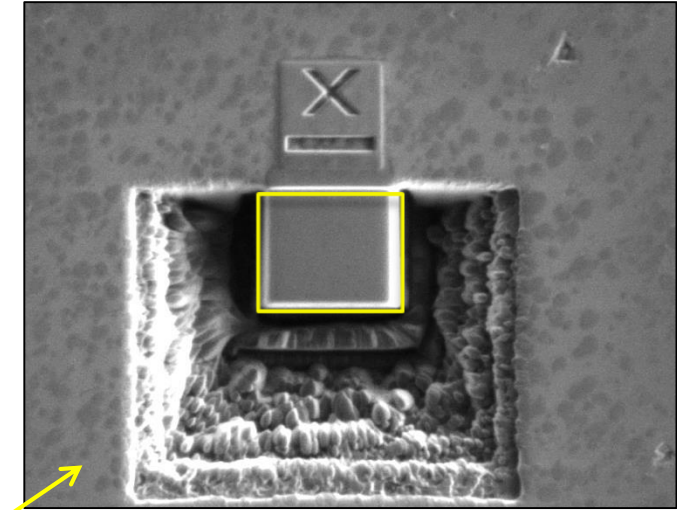


ASNV pre-prep @ 52° tilt

- (set beam coincidence point if needed = loading a existing sample)
 - select the optimal imaging conditions: OptiTilt + T1 (or T2) and optimize image (lensalign, focus, stigmat...) **link Z to WD**
 - position the imaging area in the e-beam image in the center of the screen; using stage movements. Z-slider for Y and stage movement for X.
 - focus on AOI and **link Z to WD**
 - adjust e-beam magnification, to have both the imaging area + fiducial visible in the e-beam image
 - adjust i-beam magnification to have the 3D volume + fiducial visible in the i-beam image and use beam shift to position both close to the center.
- NOTE: using OptiTilt mode effects the ion beam image.



e-beam

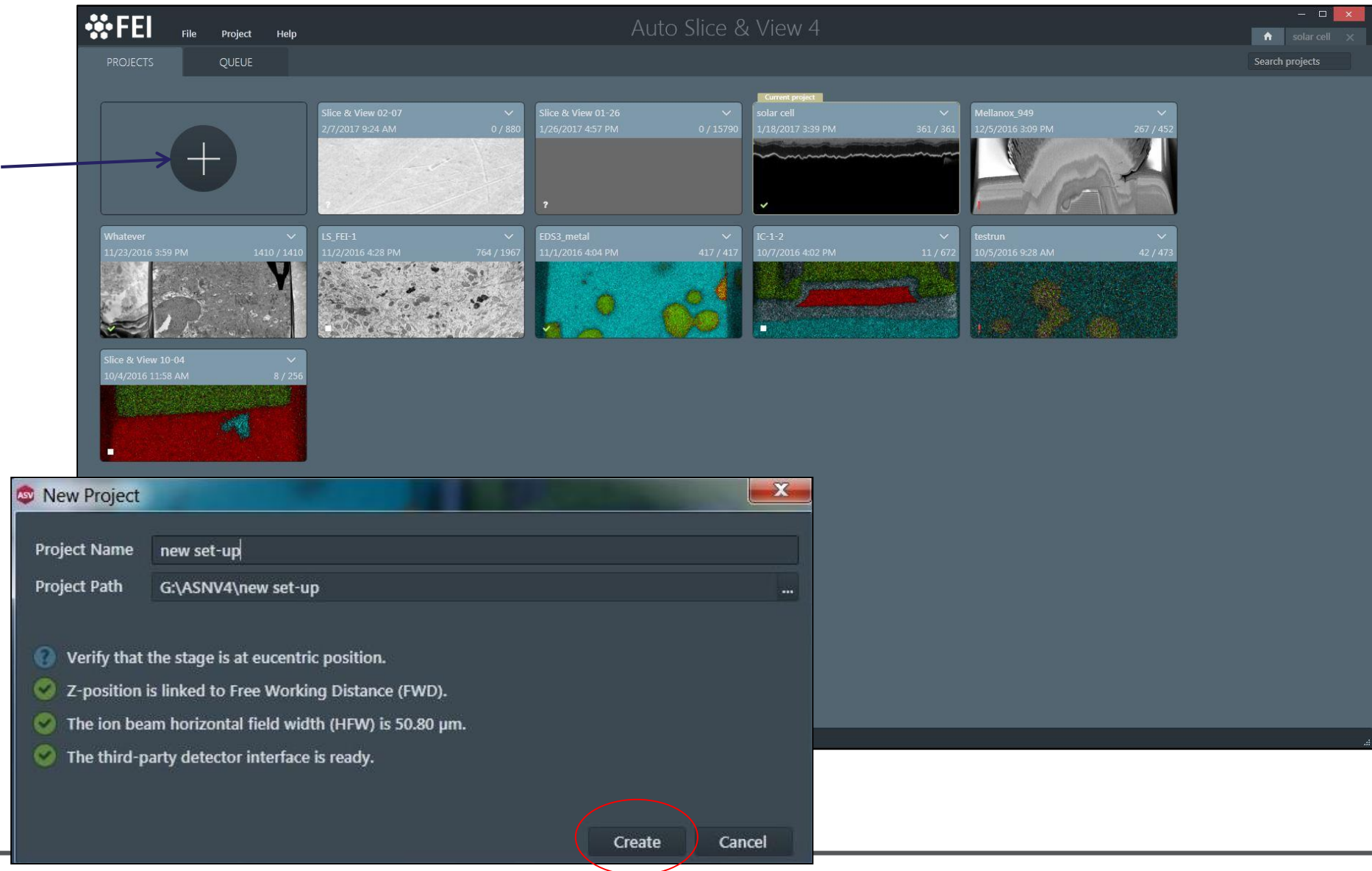


i-beam

Start Auto Slice and View 4

ASNV 4_set-up a new project

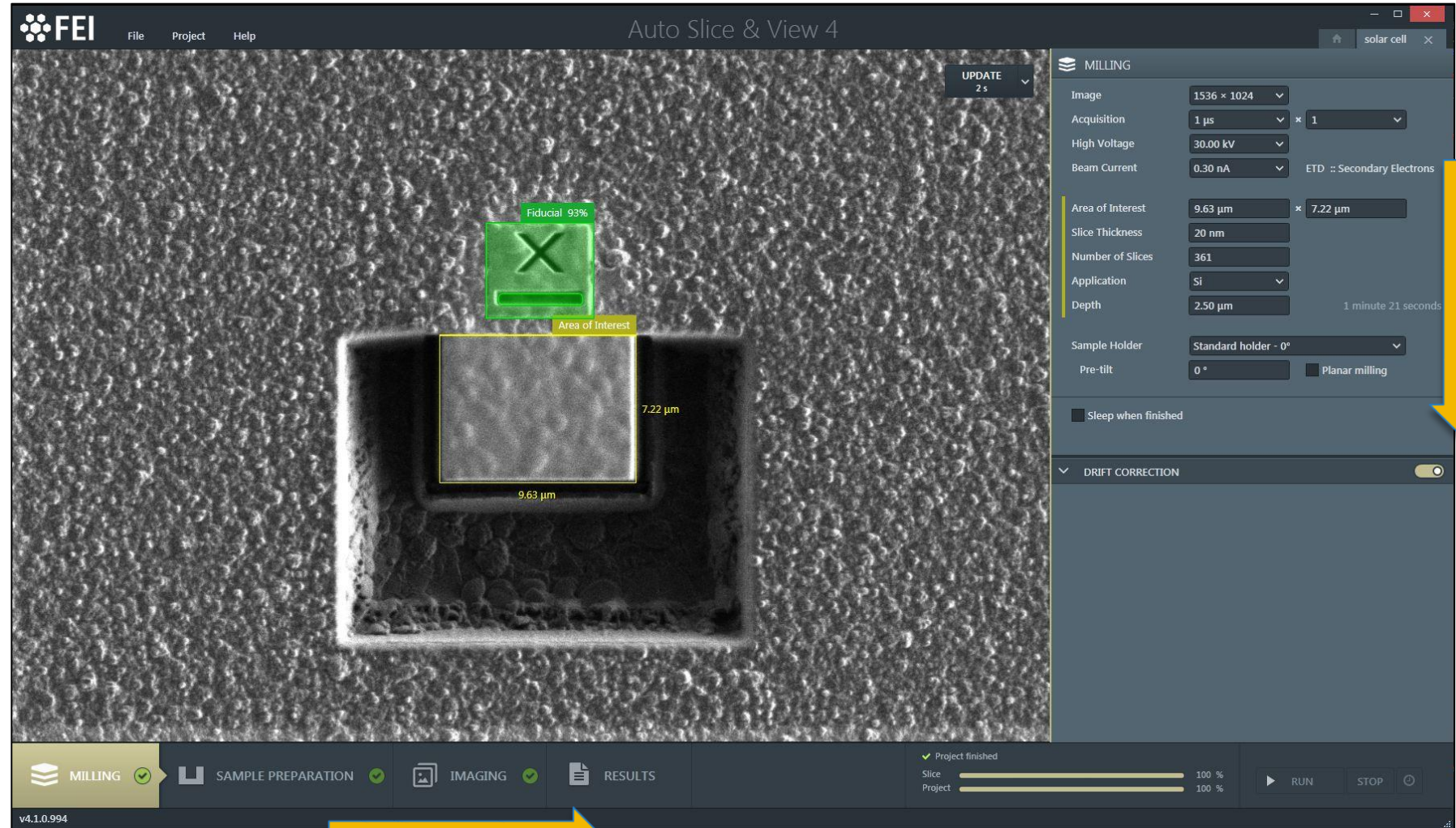
- Start ASNV4
- Click on + to create a new project
- Change Project Name
- Check Project Path
- Check the displayed microscope conditions
- Create



ASNV 4_set-up a new project

Set-up is done from left to right (bottom) and from top to bottom (RH side panel);

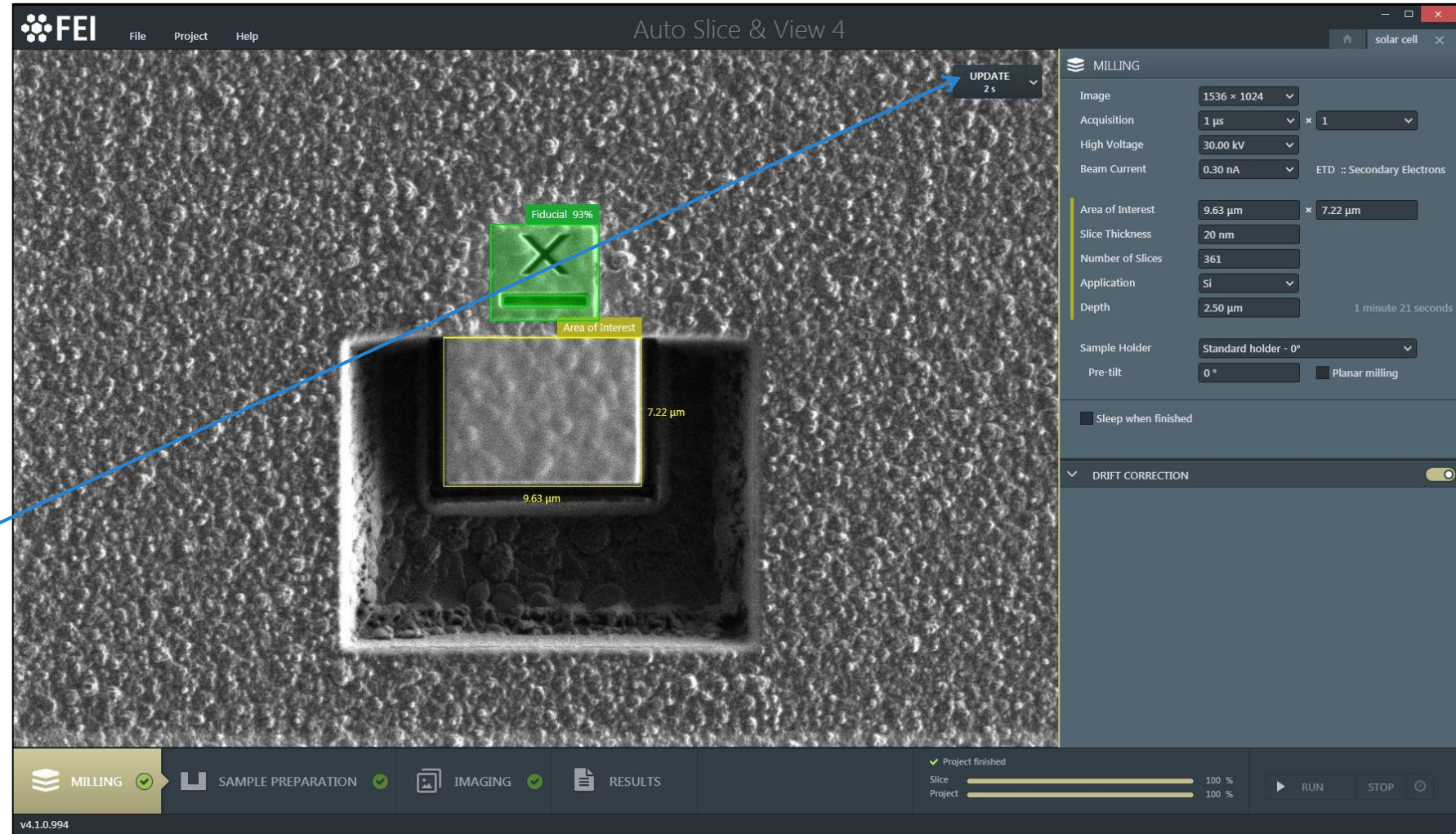
1. Milling
2. Sample preparation
3. Imaging
4. Results



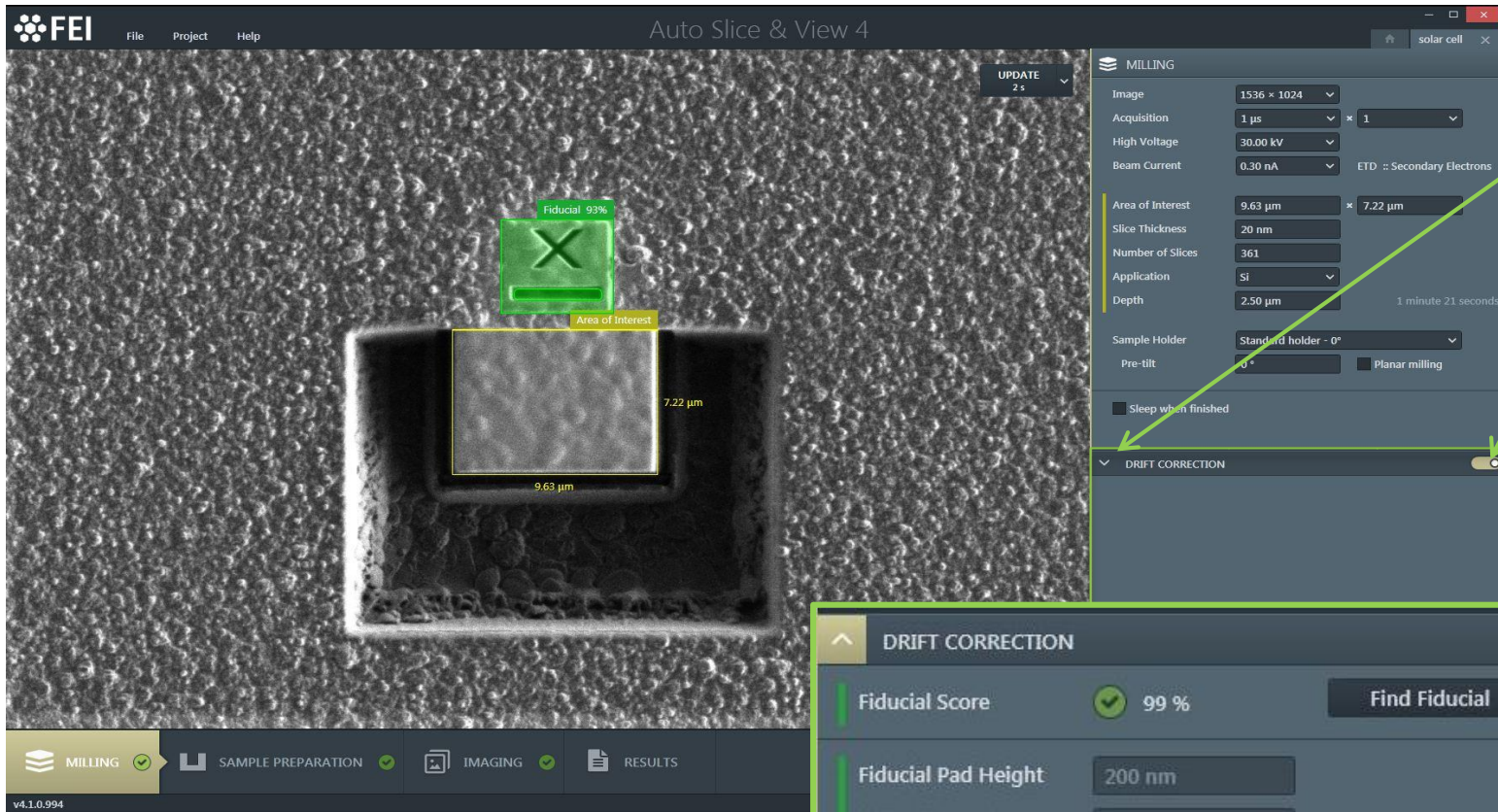
ASNV 4 set-up: Milling-1

Milling :

- define area of interest -> resize and place yellow box over 3D volume
- change FIB image acquisition conditions and milling conditions;
- All milling conditions on this page are the conditions that are used to mill the slices
- If any changes are made in the set-up the image (+ conditions) need an update



ASNV 4_set-up; Milling-2



1. if there is an existing fiducial:

- expand the drift correction box and
- activate the knob
- resize yellow box over AOI and place and resize the green box over fiducial -> find fiducial -> Fiducial Score (red box -> green)

2. if there is NO fiducial (and sample is already prepared or needs to be prepared) :

- expand drift correction and activate the knob
- resize yellow box over AOI and place and resize the green (or red) box where the fiducial should be made:
- “create fiducial”;
- fiducial will be made and matched (Fiducial score)

ASNV 4_set-up; Sample Preparation

2 scenarios for sample preparation:

1. unprepared sample; resize the blue boxes and change FIB prep conditions if needed

- press “PREPARE SAMPLE” to start the preparation

2. sample is already prepared; uncheck all boxes

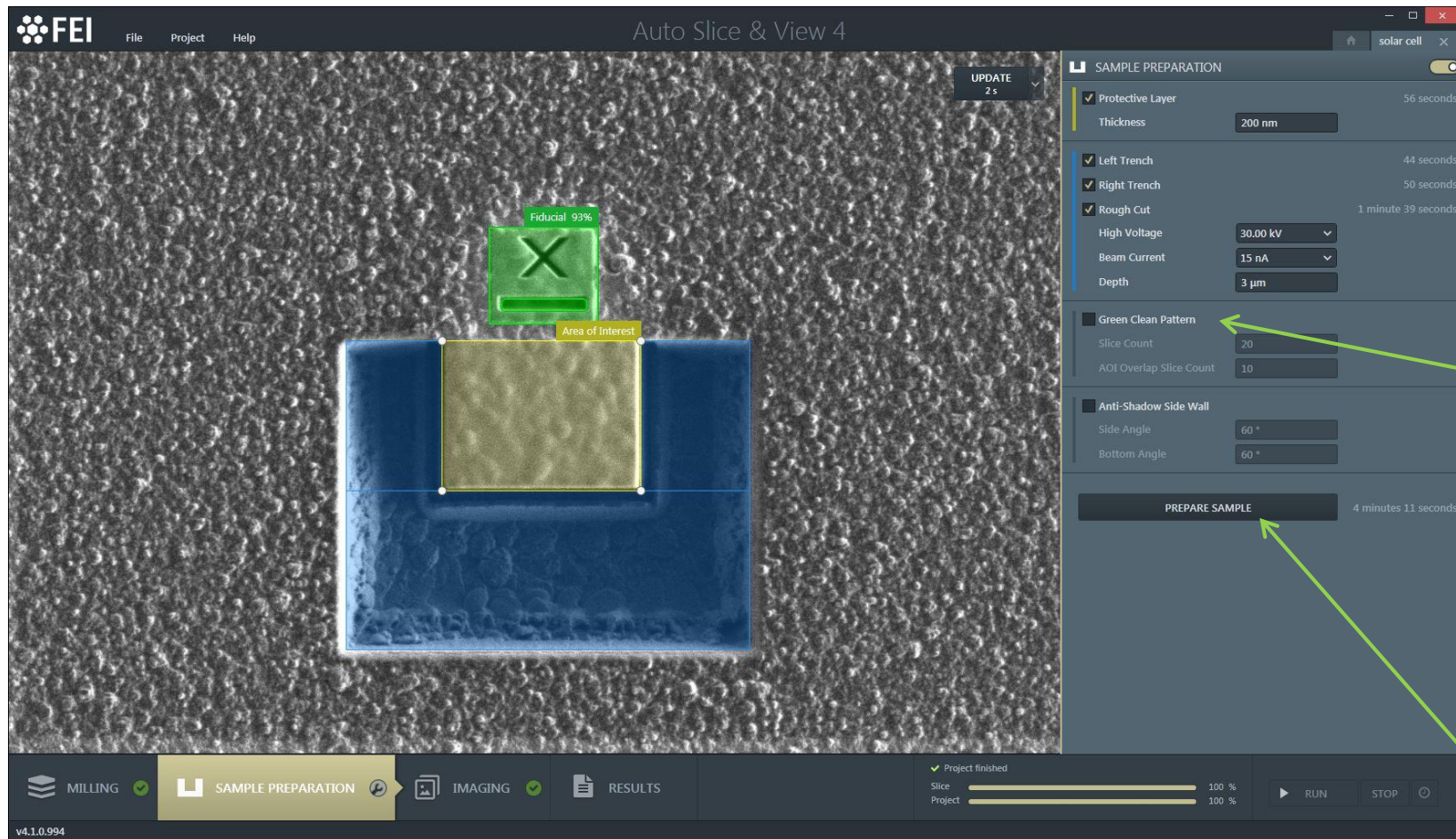
- After preparing the sample “Green Clean Pattern” (GCP) can be used to move the slicing closer to the edge (Pt-layer) of the 3D volume;

using the fiducial for pattern placement

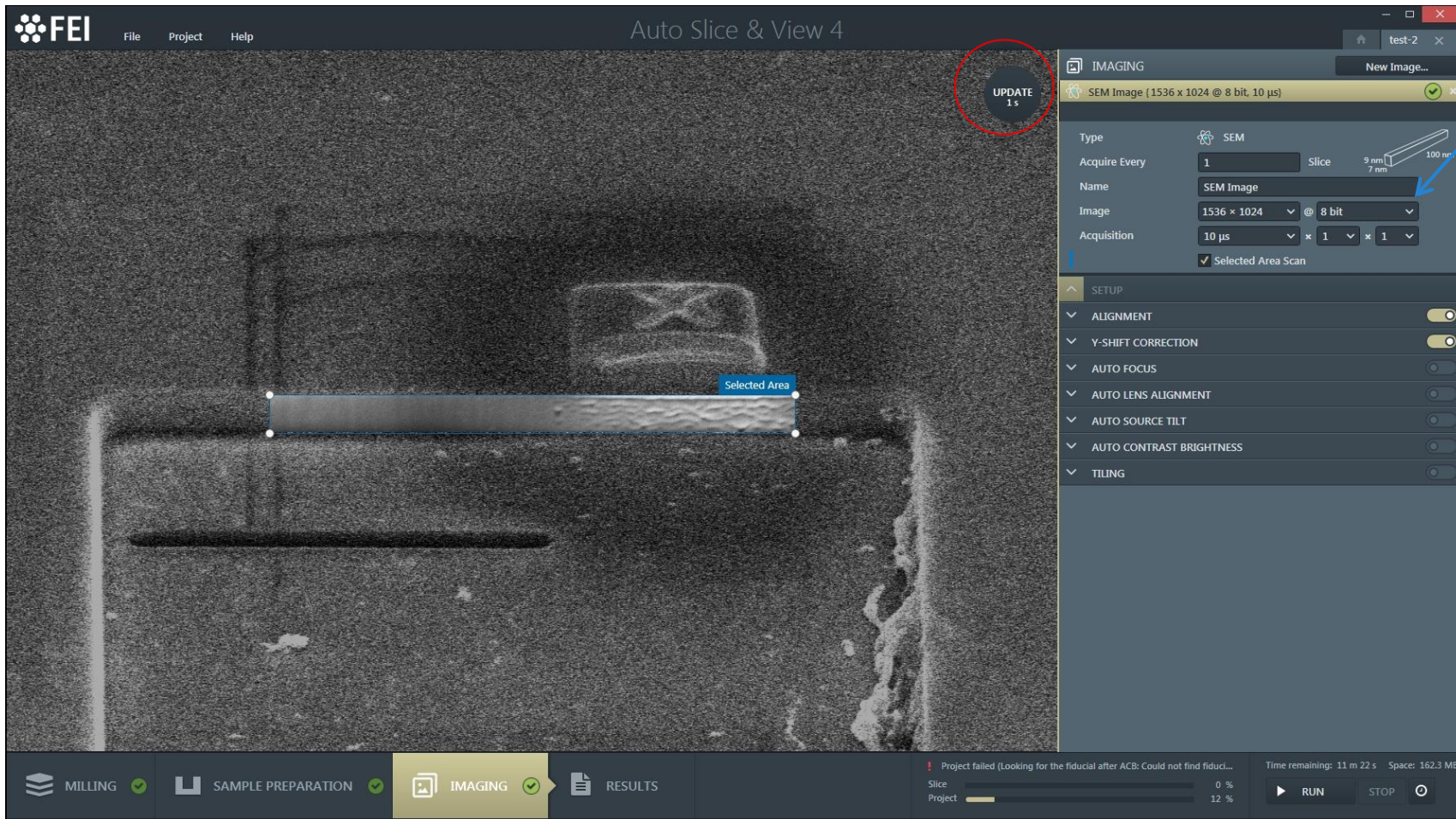
- GCP Slice count = the total amount of slices;

- AOI overlap slice count = amount of slices inside the yellow area

- press “PREPARE SAMPLE” to start the GCP

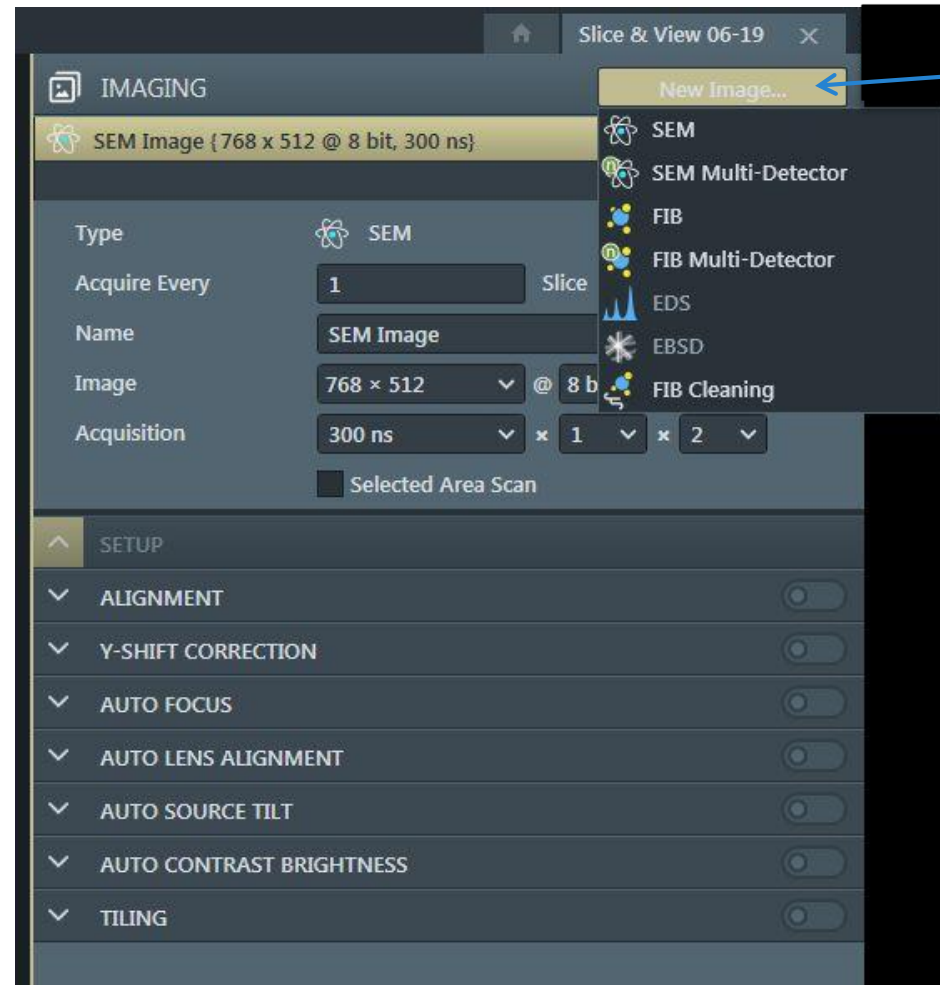


ASNV 4_set-up; Imaging-1



1. name can be changed
2. change imaging conditions; update (RH corner of image)
- # voxel size is displayed and will be changed after updating the image
4. enable alignment to use a fiducial to accurately place the imaging area
5. enable Y-shift correction; beam shift as default, for larger volumes a digital Y-shift can be used. Always needed to adjust focus during ASNV and adjust AOI to center.

ASNV 4_set-up; Imaging-2

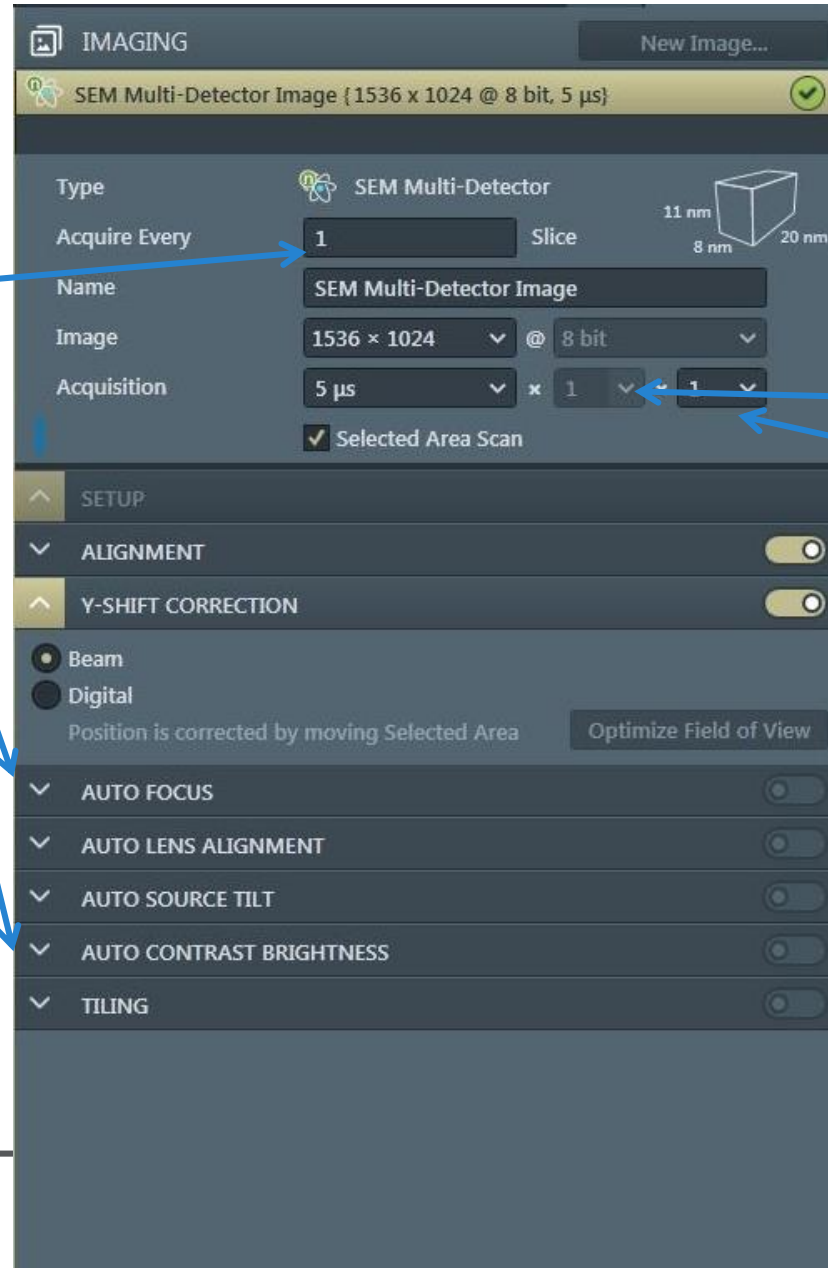
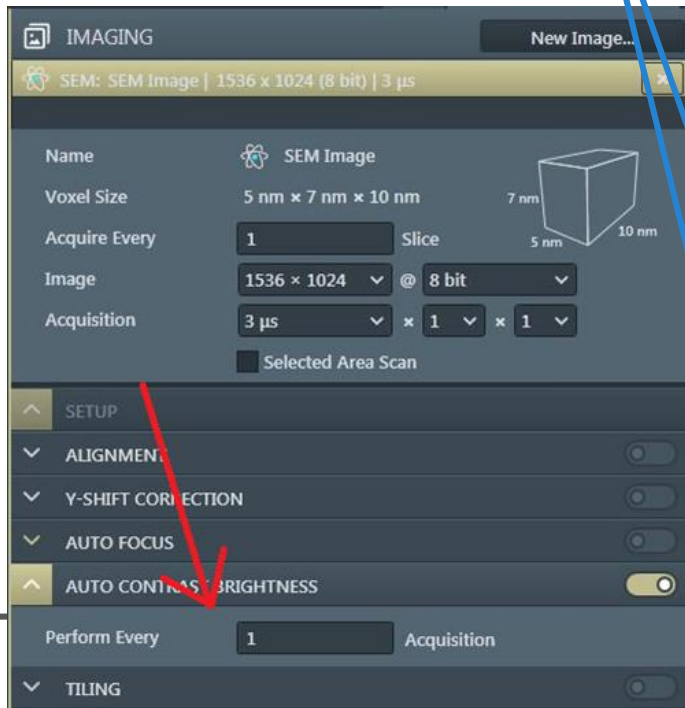


New image; after setting up an image a new image can be added to the list:

- SEM (single image)
- SEM Multi-Detector (2 to 4 images); e.g.
 - T1 (BSE), T2 (SE), T3 (SE), ETD or ICE (SE)
- FIB (single image)
- FIB Multi-Detector (2 to 3 images); e.g.
 - ETD (SE), ICE (SI), T2 (SE)
- EDS
- EBSD
- FIB cleaning; low kV cleaning

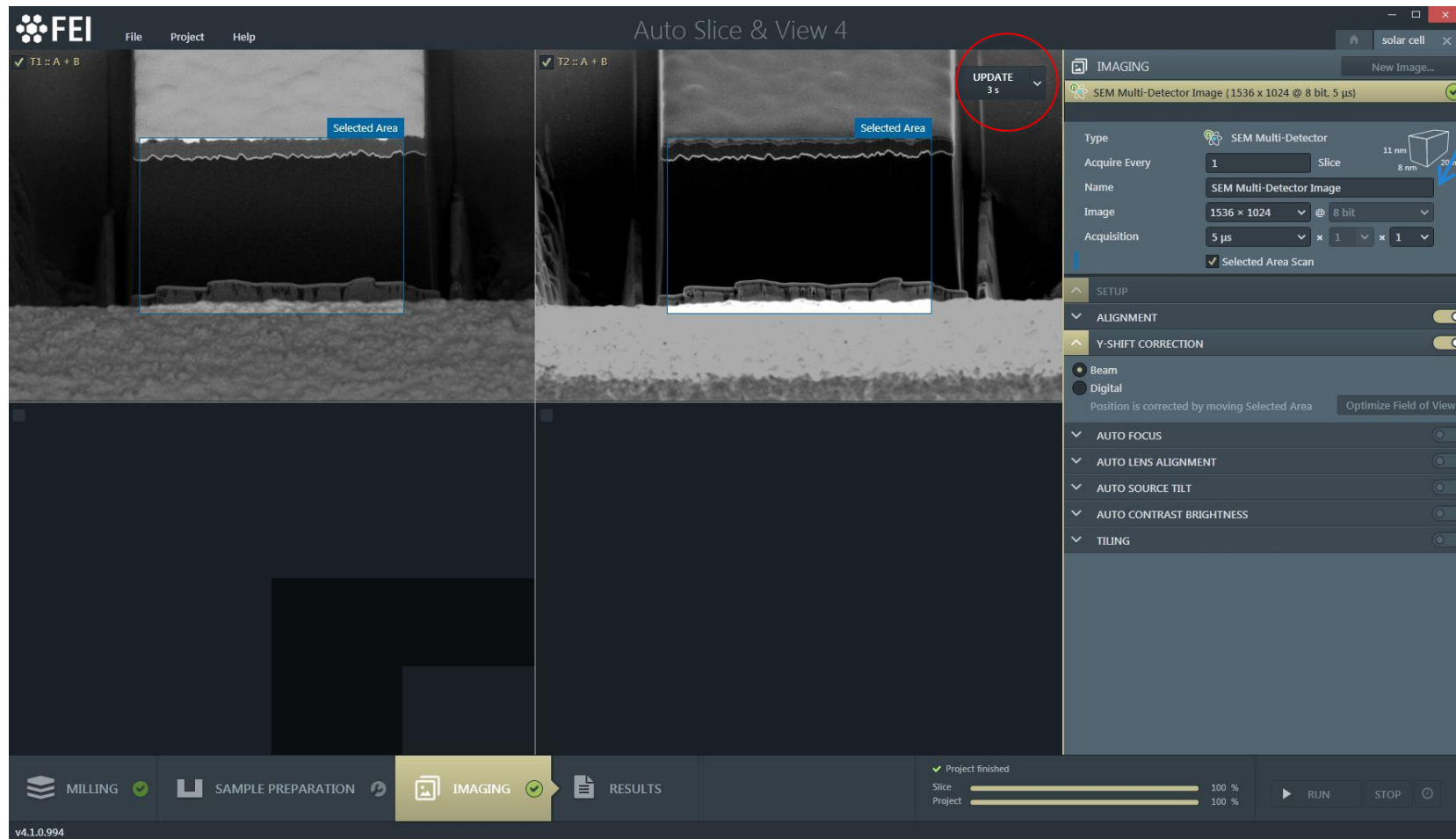
ASNV 4_set-up; Imaging-3

1. every Nth slice an image can be acquired
2. Every Nth slice AF, ACB etc. can be chosen



1. new image; after setting up an image a new image can be added to the list
2. change name
3. change imaging conditions;
line integration
frame integration (DCFI)
4. after making changes update image (RH corner of image)
5. setting-up selected area in combination with alignment.....

ASNV 4_set-up; Imaging-4_Multi detectors



1. new image;
2. multiple images; “SEM Multi Detector image”
3. change imaging conditions; focus stigmat, C/B in display 1; update (RH corner of image). Optimize only C/B for the other images and update each display.
detector name is displayed in LH corner and image set will be named after detector.
voxel size is displayed and will be changed after updating the image
All settings are done/adjusted in display 1:
4. enable alignment to use a fiducial to accurate place the imaging area
5. enable Y-shift correction; beam shift as default, for larger volumes a digital Y-shift can be used. Always needed to adjust focus during ASNV and adjust AOI to center.

ASNV on non conductive samples;

- Make sample as small as possible
- Mount sample with Carbon or Silver paint and let it dry
- Cover sample as much as possible with the conductive paint; leaving only a small area.
- After drying, sputter coat the sample.
- Prepare sample for ASNV (no fiducial); Pt deposition, bulk milling and side trenches
- Sputter coat sample again
- Make fiducial
- Clean imaging area (front of 3D volume) with Cleaning Cross Section and start ASNV SW

- NOTE: If the sample is still not stable, putting a probe needle on the sample surface close to the area of interest might help!

Milling in beam sensitive/soft material

- Lower BC (500pA and below) -> less heat -> more efficient milling
- Lower ion dose; use InP or PMMA application file
- Use neg. OL -150% + faster dwell time
- Use low dose e-beam conditions as well
- Sputter coat sample

NOTE: Temp increase during milling;

conductive material a few degrees (<10K)

non-conductive material (low thermal conductivity) high temp increase (SiO₂ ± 230K)