

VolumeScope Set Up Checklist

1. CHECK MICROSCOPE PRECONDITIONS:

- **Make sure that the microtome is selected as accessories.**
- Make sure the Acquisition service, Processing service, Slicer server are all running. (Do not close it)

2. MICROTOME MOUNTING:

- Create a new project in MAPS and use “**Slicer Montage**” to mount the microtome.
- If “**Initialize Microtome**” failed, check the feedthrough if they are connected in the correct order. If still not work, restart the VolumeScope server from the VPC.
- Mount the ring filter (HiVac) or VS-DBS (LoVac) if the vacuum mode can be pre-determined since there is more room for installation.

3. APPROACH SAMPLE IN AIR

In this procedure the order of setup is:

1. Knife
2. Dinolite Camera
3. Sample

- “**Park Sample and Knife**”

- Mount the knife. (overtightening could bend the arm) **Make sure not touch the knife edges !!!**
- Mount the Dinolite Camera to check the eucentric position on the knife edges.

- “**Park Sample and Knife**”

- Mount the sample. (Loose the Z fixing and set up the Z position screw to zero)
- Tighten the sample at some degree using the Z fixing screw.
- Adjust the XY position with the knife edge if needed and tighten the XY fixing screws on the top.

- Press the **“Prepare Sample For Exchange”**; the sample axis position moves up to 0.6 mm.
- Move the sample up to the eucentric position by adjusting the Z-axis positioning screw. (Remember there is residual movement after tightening)
- Tighten the Z fixing screw.
- **“Park Sample”**
- Set up the cutting window. (A large cutting window is better for less debris on the block face)
- Set up backlight to observe reflection on the block face.
- Move the sample up until the backlight reflection is hardly visible anymore. (Be conservative for precious samples)
- **“Set Current”**
- **“Park”** the knife and remove the backlight.
- Set up a slicing job by putting 100 slices/slice thickness of 100 nm/cutting velocity of 0.8 mm/s.
- Stop the job after viewing debris on the knife edge.
- Remove the debris on the knife/sample using compressed air and the vacuum cleaner.
- Mount the PLA if needed. (Ring filter for HiVac, VS-DBS for lowVac)
- Document the **“Sample Axis Position”** by using snipping tool for screenshot.
- Pump the system and select the right PLA accessories.
- Wait for vacuum to stabilize. Under Hi-Vac, it usually takes 2-3 hours until the vacuum reaches down to mid-level of -6 mbar. If planning for a long job, leave the system pumping overnight is preferred. Under low-vac, wait for the pressure stabilized at the desired value.

4. APPROACH SAMPLE IN VACUUM

- Turn the beam on (ie: 1.78 kV, 100pA) and link the working distance after focus. If the stage is too far off (difficult to focus), use Standard Mode and the ETD detector. Then switch to Volumescape Usecase and T1 Detector. (WD: 6.5 mm for HiVac and 7mm for LoVac)
- Apply 135 degree **scan rotation** in the XTUI. (The cutting direction is then from left to right)

- Acquire the same image with proper horizontal field width (HFW) in quad 1 and 2; quad 3 should have mixed image of 1+2. Check the invert box for either quad 1 or quad 2.

- Compare the sample axis position before and after the pumping. In general, the after-pumping value is lower. If the after-pumping value is higher, select “Fine Approach Vacuum” panel, click “ **Decrease Next Sample Slice** ” by selecting an appropriate step size. (ie, “1um”).

- Set a reasonable amount of slices to be cut in the “**Fine Approach Vacuum**” (This procedure can speed up by using “**Sample Polishing in Air**” without imaging alternatively after each slice, not recommended for precious samples.)

- If there is a structural difference in the quad3, clicking the “**Stop After Slice**” button.

5. CUTTING ONLY JOB SETUP

- First to acquire an overview image of the whole block face in the XT UI and sent to MAPS. (Prepare the overview preferably at an energy you would like to use for acquisition.)

- Check the “**Link Box**” to link the scan rotation (bottom of MAPS UI).

SETUP OF AN ALIGNMENT TILE SET (NON-MED)

- Using the overview image, choose an area to create a VS alignment tile set, best is to place it behind a region of interest.

- In the “**Z-Stack Definition Tab**”, click “**Change Tile Set Type to Physical Only**” and type in the preferred accelerating voltage.

- In the “**Acquisition Parameters Tab**”, type in the imaging conditions and make sure select the right detector and beam current. Typical parameters for alignment tile set is summarized below:

Dwell Time	1μs	Scan Interlacing	1
Image Resolution	2048 X 1768	Drift Corrected	Unchecked
Horizontal Field Width	20.48 μm	Detector	T1
Pixel Size	10 nm	Beam Current	100 pA
Number of Frames	1	Use Case	VolumeScope
Line Integration	1	Use Reduced Area	Unchecked

- In the “**Autofunction Selection Tab**” of the “**Column Alignment Tab**”, Click “**Select All**”. (The lazy man’s trick)
- Go back to the “**Column Alignment Tab**”, Select the energy, click the “**Align**” button.
- Use “To SEM” and “From SEM” to fine tuning the focus, stigmation, contrast/brightness. (This step is needed especially the auto align by the software is failed. ie, sample is charging.)

SETUP OF DATA TILE SET

- We recommend setting up at least two Data Tile Sets. One for Overview with lower resolution, the other on the ROI with higher resolution.
- Simply right click on the alignment tile set and select “**copy grids**”, then select “**No**” to create a data tile set.
- In the “**Acquisition Parameters Tab**”, input the desired imaging parameters such as horizontal field width, pixel resolution, dwell time.
- In the “**Autofunction Selection Tab**” of the “**Data Set**”, select the autofuctions: “**Apply Alignment**”, “**Final Position Alignment**”.
- For overview tile set, **only** select “**Apply Alignment**”. Make sure check the “**Debris Analysis**”.
- Check the acquisition order of the two data sets. Make sure overview tile set run first.
- Under the “**Workflow Control**” page set up the experiment using 40 nm slices and a 0.2mm/s cutting speed. (Before starting the real job, It is better to polish the surface for couple slices (using final approach in vacuum) to remove the “damaged area” when you adjust the imaging parameters and tune the focus.)
- Go to “**Options Tab**” in the MAPS UI, select “**Turn off Beam when done**” and add “**Pump to HiVac When Done**” for LowVac job.
- Using Amira live tracker to monitor the cutting behavior. (Any missing or uneven cut, unexpected image shift, focus/stig stays consistent, the amount of debris.) Monitor at least 30 slices before leaving a long job unattended.
- Export the acquired data sets as “**Raw Acquired Images**” or “**Deconvolution Results**” (MED) in the “**Volume Rendering Tab**” after the acquisition.