Teneo Volumescope
User Operation Manual

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Overview

Teneo VolumeScope^{TM} system is the integrated solution for automated volume data acquisition based on a refined Serial Block-Face Scanning Electron Microscopy (SBF-SEM) technique. It combines mechanical (physical) and optical (virtual) slicing, which allows for extending the current in-depth resolution limit; hence high-resolution isotropic datasets can be generated. The product can be used for observation of resin embedded tissues stained with heavy metals.

The complete process of data acquisition and processing is controlled by a combination of

- FEI Teneo SEM (see the *Teneo User Operational Manual* for more details),
- FEI ultramicrotome (slicer within the UI) mounted inside the Teneo chamber (described in following chapters),
- FEI MAPS application (see the *FEI MAPS SW Application User Guide*)
- VolumeScope application embedded in FEI MAPS (see this manual).

    The FEI MAPS application is used for automated image acquisition of regions of interest. The VolumeScope application guides the user through the setup of a complete VolumeScope project using mechanical and/or optical slicing.

Note
Optical slicing is based on the MED-SEM (Multi-Energy Deconvolution SEM), which is a non-destructive technique capable of high-resolution reconstruction of the top layer of the sample. Following each mechanical cut, a series of backscattered electron images are then acquired using different accelerating voltages. Collected images serve as the input for a deconvolution algorithm that computes several subsurface layers.

Caution!
Before starting of any work on the system, please read the safety instructions within the Teneo VS safety and maintenance *chapter.*

Basic features

A typical VolumeScope project covers the defining of several regions of interest within the mechanically sliced block-face of a sample. These regions are optically sliced according to the parameters defined within the dedicated application for high resolution volume reconstruction. The dedicated interactive software guide navigates the user through the settings and operation of the product. The process utilizes several steps and it is highly automated to effectively perform time consuming experiments.

The following steps are typically a part of the job.

- A sample is placed into the ultramicrotome which is mounted on the stage of the Teneo SEM chamber. The sample is advanced towards the diamond knife to determine the correct cutting position. The surface is then polished.
- The electron column is automatically aligned to set appropriate acquisition parameters under a given set of conditions before data collection and during data acquisition to ensure optimal image quality.
- Automatic data collection is comprised of alternating mechanical cutting (a defined slice thickness is removed using a diamond knife and a fresh block-face is exposed), and electron imaging (optical slicing) on regions of interest defined on the sample surface.
- The whole process is repeated for the requested number of iterations. For the optical slicing the acquired data are automatically registered and deconvolved.
- The outputs are prepared for visualization in the dedicated software FEI Amira®.

VolumeScope System Architecture

The system uses component-based architecture. Individual components are deployed on the VolumeScope PC (VPC) and Microscope PC (MPC) to create a functional integrated solution.

Figure 1-1: *VolumeScope System Architecture*
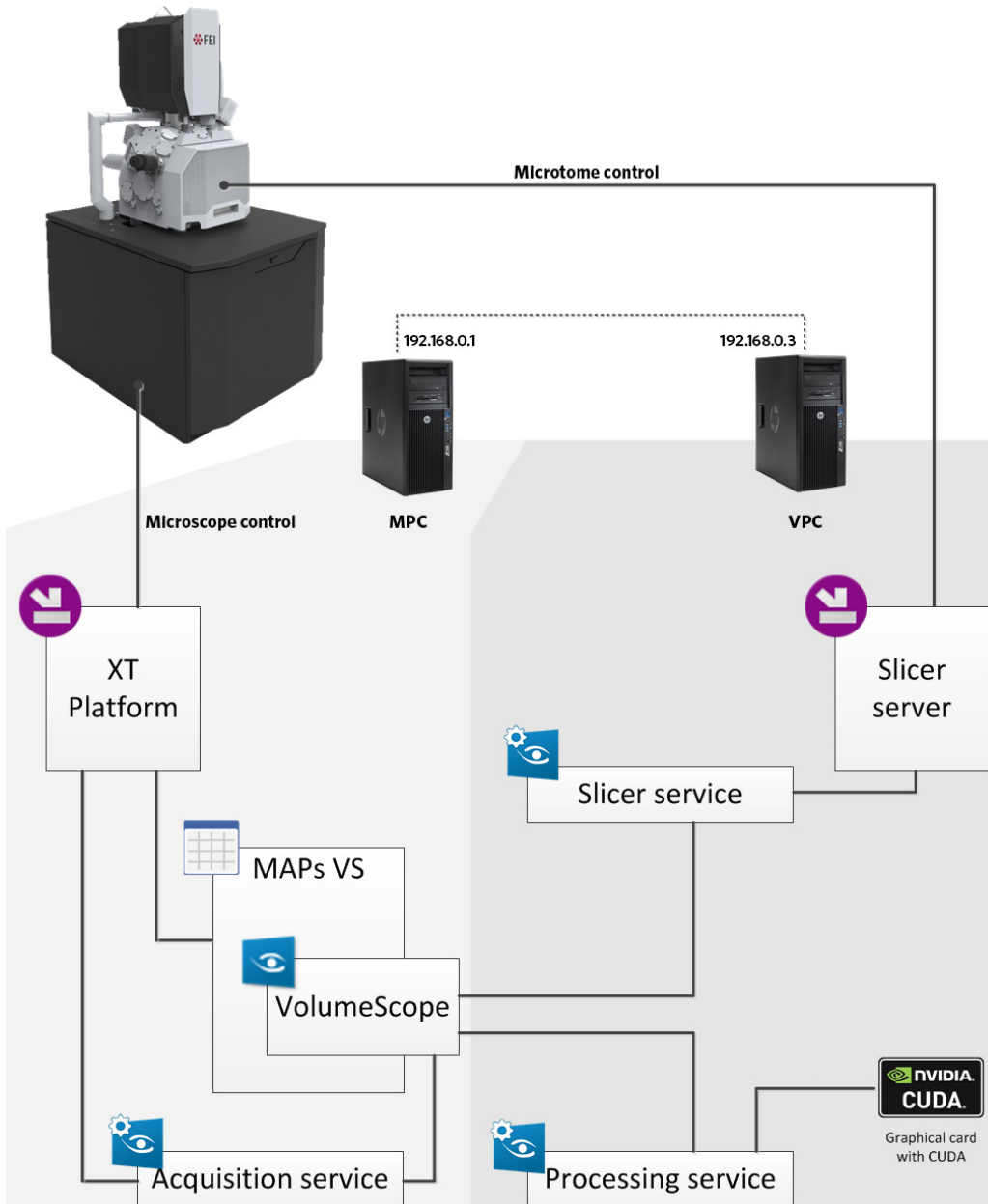
The application UI is the base point for the user to control all the other parts. The VolumeScope application communicates with three services using the computer network.

- VolumeScope Acquisition Service

    This service handles all image acquisition-related activities. It communicates with the Microscope Server to properly tune the acquisition conditions so that image quality is high enough for later processing.

- VolumeScope Processing Service

    This service is responsible for all the intensive computations, and it handles the 3D reconstruction from multiple acquired images. To be able to perform these operations, the service is deployed on the VPC, which is equipped with a high-performance graphics card that handles some of these computations.

- VolumeScope Slicer Service

    This service, together with the lightweight Slicer Server, controls all the operations performed with the slicer. Both the service and the server are deployed on a VPC to prevent unnecessary loads on the microscope system.

Getting started

Several steps must be performed before you start the VolumeScope project to ensure successful operation.

1. Start the xT microscope Server software on MPC and VPC.
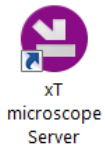
2. Make sure that the Acquisition service (MPC) is running.
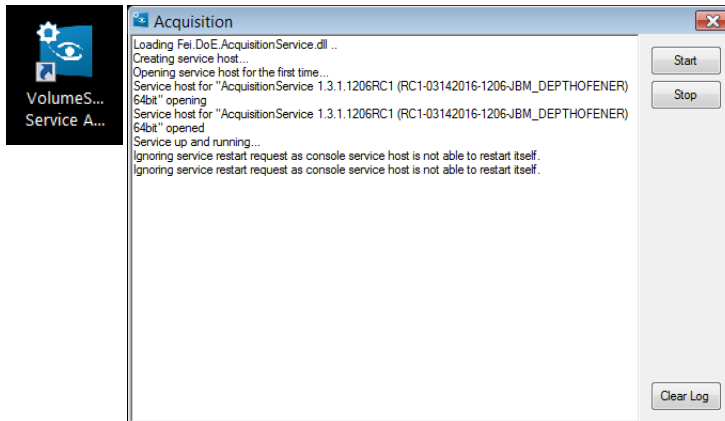
3. Make sure that the Processing and Slicer services (VPC) are running.
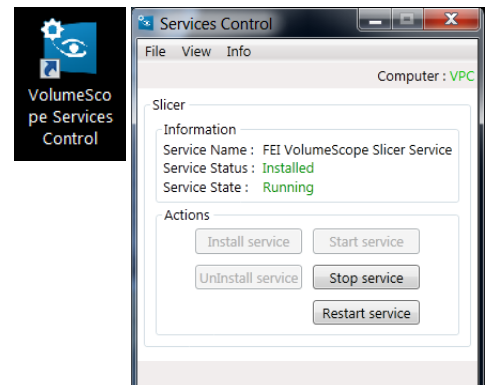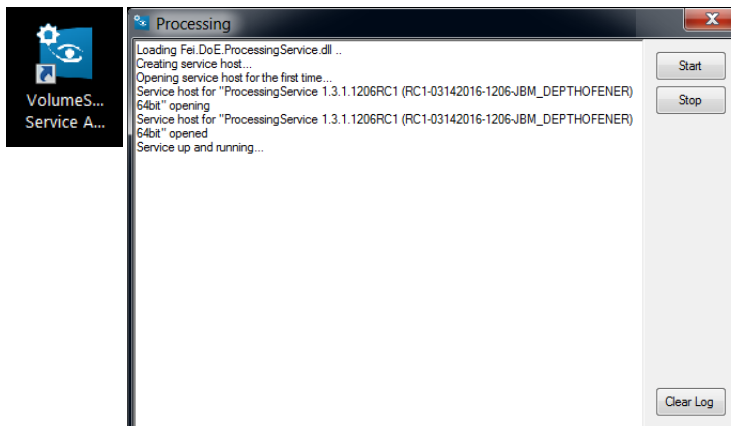
4. Start the Microscope Control (UI) software.

The microscope should be started and aligned according to the Teneo User Operational manual.

Depending on the actual system condition and settings, execute following tasks in reasonable order.

- Follow the instructions within *Ultramicrotome installation* (see below).
- Follow the instructions within *Diamond knife installation / exchange* (see below).
- Follow the instructions within *Vacuum Modes* (see below).
- Follow the instructions within *Starting system* (see Chapter 3).
- Follow the instructions within *Mechanical Slicing Setup* (see Chapter 3).

Ultramicrotome installation

Caution!
   There two are moving parts on the microtome, that can cause a finger pinch during the Mechanical slicing setup: the knife is driven by the DC motor, and the sled is driven by the piezo motor.

Caution!
   Using the Plasma Cleaner is not recommended when the VolumeScope ultramicrotome is mounted inside the microscope chamber!

Before installation of the ultramicrotome, unpack it from the transportation case (keep two antistatic PE bags for future repacking), remove the red transportation bracket, and store it, together with its screws, in the PE bag provided. When holding/manipulating the ultramicrotome body, always use its handle, if possible.

Figure 2-1:   *Ultramicrotome transportation bracket / Holding of the Ultramicrotome by the handle*
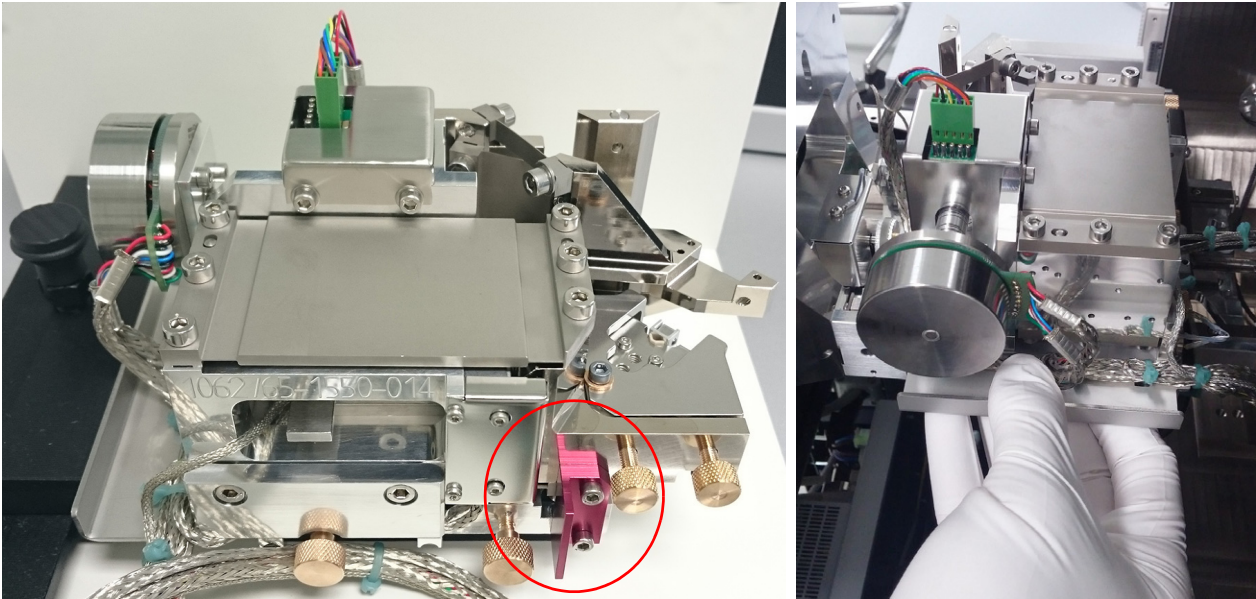
All necessary tools for the ultramicrotome installation and/or settings are available within the Ultramicrotome accessory suitcase. It includes the camera tool, eyelash with handle, diamond knife, polystyrol rods, allen keys, torque screwdriver, sample stubs, and calibration samples attached to the stub.

Figure 2-2:   *Ultramicrotome transportation case / Ultramicrotome accessory suitcase*
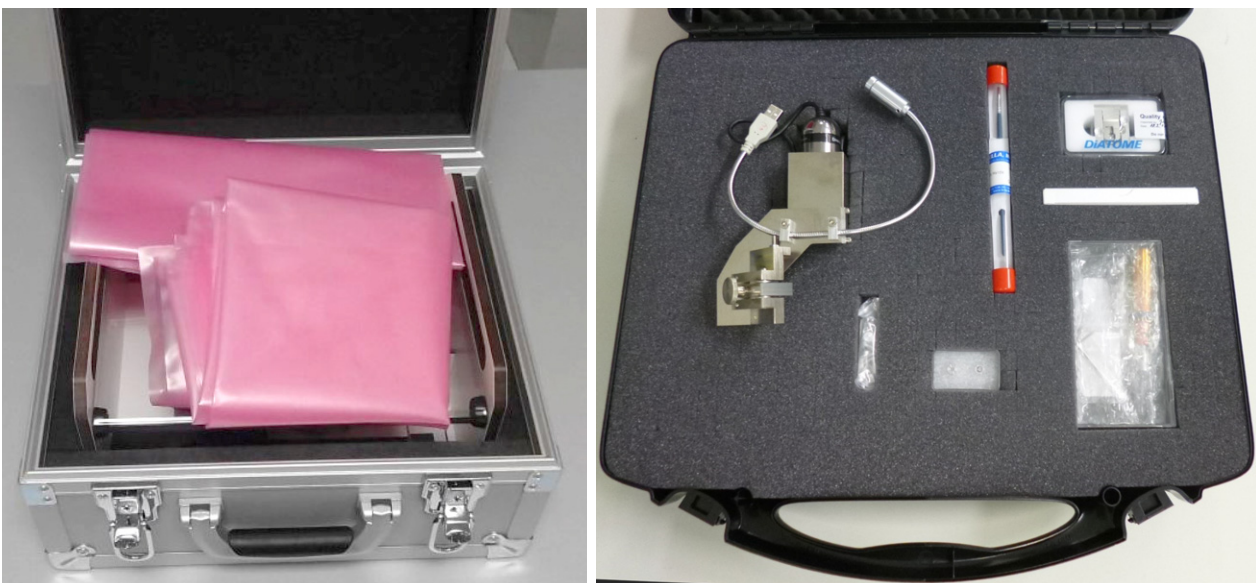
1. Follow the first three steps of the *Slicer Mounting/Unmounting* window / *Slicer Mounting* procedure.

Figure 2-3:   *Slicer Mounting/Unmounting window*
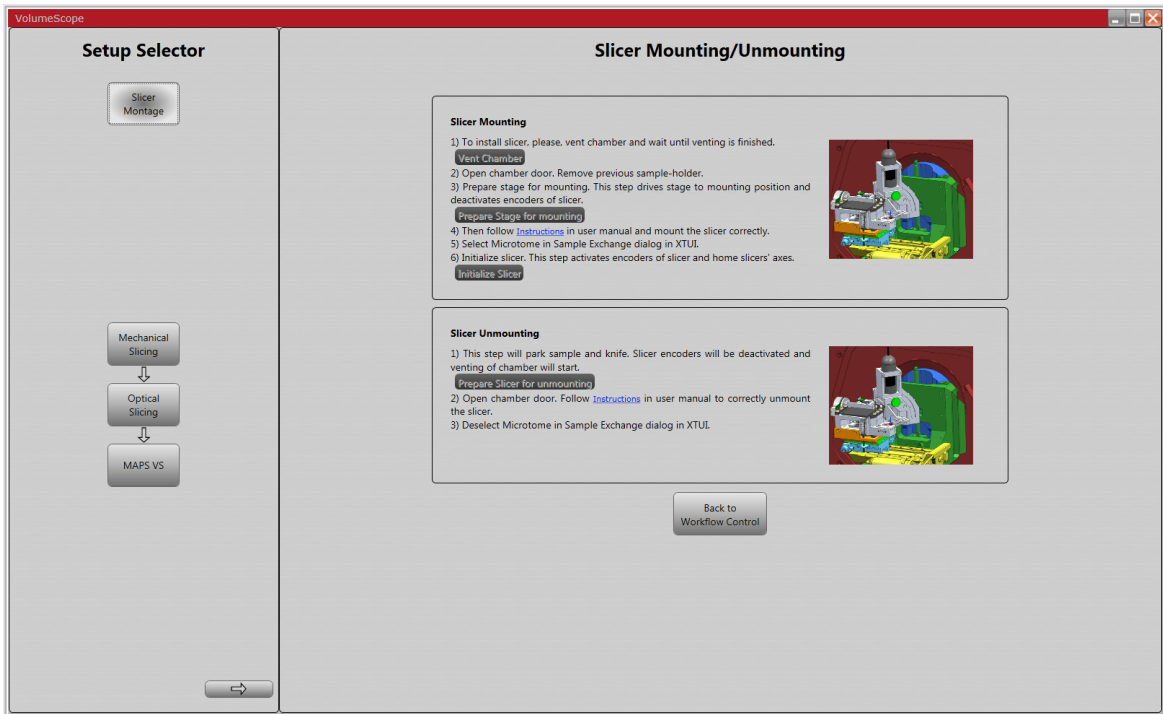
2. Slide the ultramicrotome onto the stage (secure it with two brass screws – red oval).
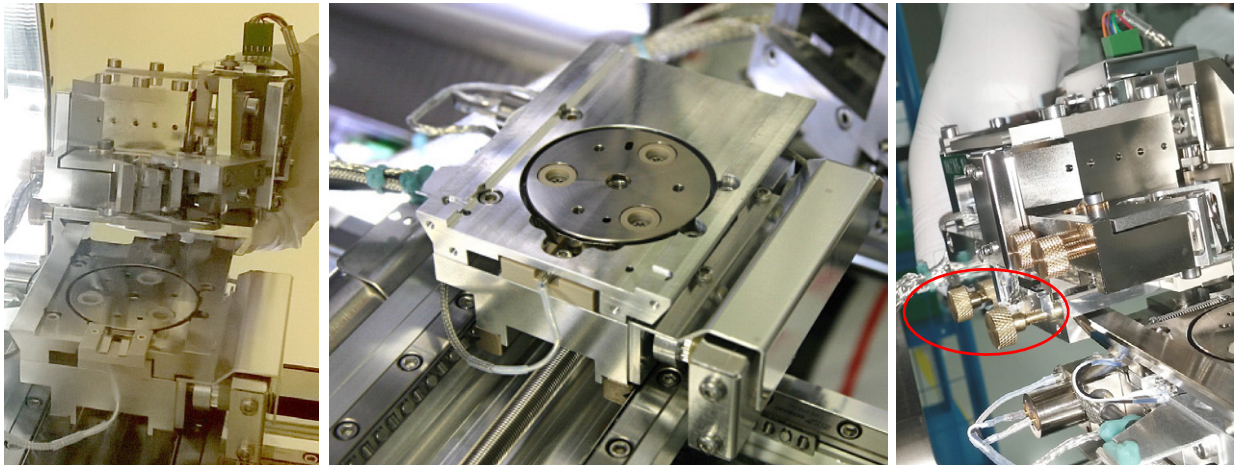
3. Connect the ultramicrotome cables to the chamber feed-through and secure them by the cable holder (two red arrows).
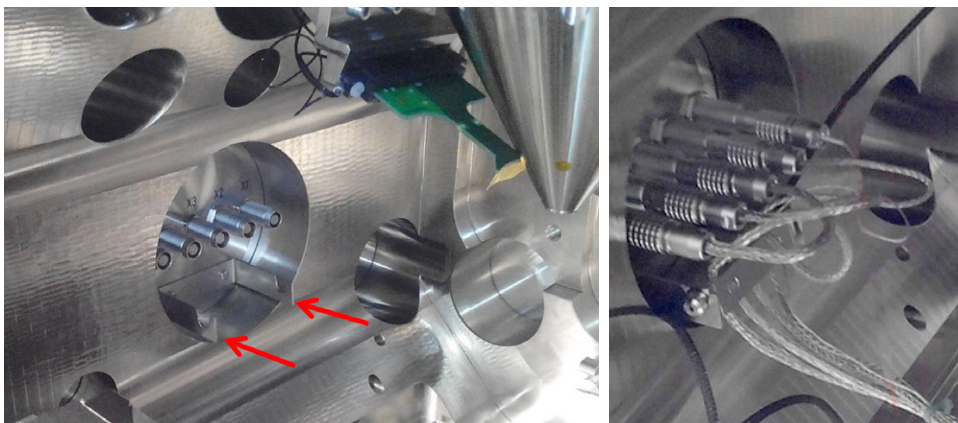
Figure 2-4: *Ultramicrotome installed on the stage*
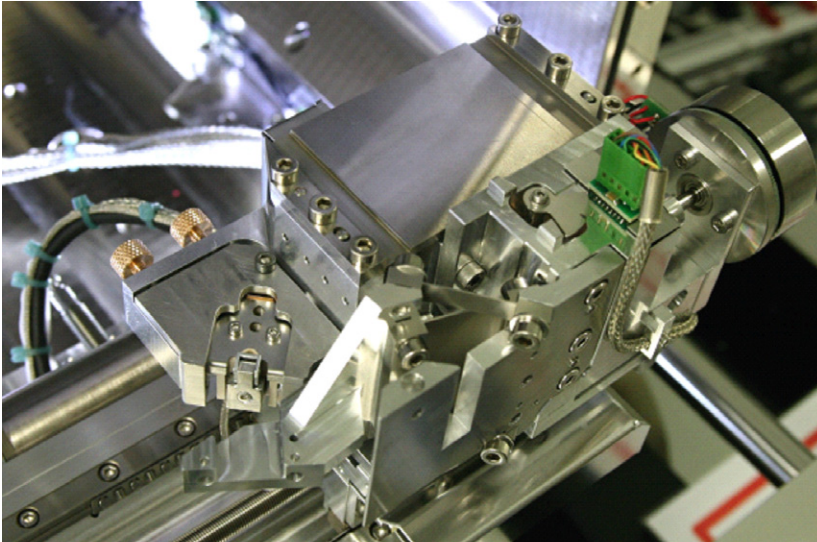
Note
  To remove the ultramicrotome follow the **Slicer Unmounting** procedure.

Diamond knife installation / exchange

Caution!
  The diamond knife should never be removed from its holder by a user! FEI cannot guarantee optimal product performance when the user removes the diamond knife from its holder. For re-sharpening of the knife (placed in its holder) send it to Diatome.

1. Loosen the two screws (red oval) on the knife holder.

2. Remove the old knife holder from the ultramicrotome.
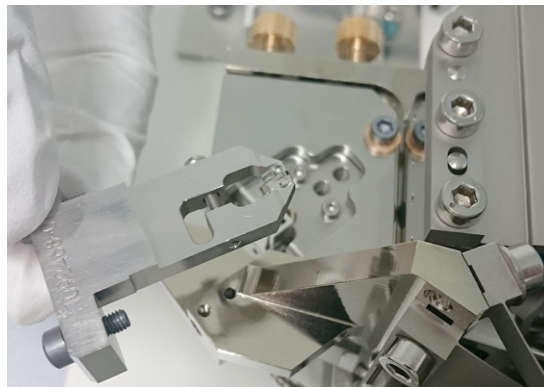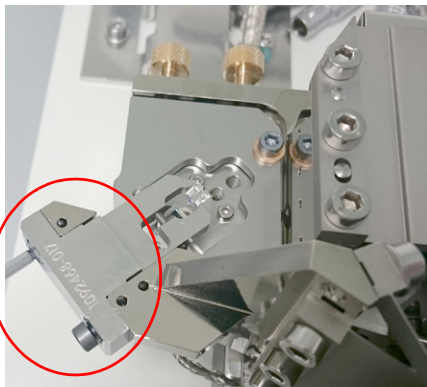
3. Unpack new diamond knife holder, release it from the protective jig (loosen two screws) and mount it to the ultramicrotome in reverse order as described above.

Note
  Lifetime of the knife depends mainly on its rate of usage, sample type, block-face size, slice thickness, etc. When the cutting quality is not satisfactory, the user can try changing relevant job parameters (cutting speed, dose etc.) and/or the trimming of the sample. If this does not help, changing the knife should be considered.
  Any debris present on the knife edge or in the surroundings of the knife should be removed before each job. We recommend that the vacuum cleaner provided be used for this purpose.
  Cleaning of the knife edge should be performed as recommended by the manufacturer.

Vacuum modes

The column settings are automatically adjusted according to the vacuum mode and the PLA accessory selected in the UI (see below). High Vacuum mode is recommended for samples that are conductive enough. For challenging charging samples HiVac and Ring Filter or LoVac mode can be used. The Low Vacuum mode is available to compensate for sample charging by admitting water vapor.

Detectors and PLA accessories

When pumping the chamber from the vented state, select the **PLA Accessories** dialogue when prompted.

- **High Vacuum** mode – **No Accessory** or **FilteRing** item (see below – the *Ring Filter Installation*).
- **Low Vacuum** mode – **VS DBS** item (see below – the *VS DBS detector Installation*).

Caution!
Be sure not to operate the microscope in Low Vacuum mode with the VolumeScope use case without the VS DBS mounted. Otherwise serious damage can occur to the system!
Before starting any lengthy acquisition in the Low Vacuum mode, ensure the water bottle is full, which should be enough for up to 14 days.

Working distances

To operate the Teneo VolumeScope select the appropriate detector in the **Detector Settings** module. It is important to observe the recommended working distances and some other conditions in relation to a selected vacuum mode and a detector / accessory.

High Vacuum

- T1 detector (VS DBS detector is not mounted!)
  WD = 8 mm – optical slicing is included in a project
  WD = 6 mm – only mechanical slicing is included in a project
  Set the T1 **Detector Settings** module / **A+B** mode
- T1 detector + Ring Filter is mounted
  WD = 6.5 mm

Low Vacuum

- VS DBS detector (must be mounted!)
  WD = 8 mm

Stage movement restrictions

When the ultramicrotome is mounted on the stage, there are some stage movement restrictions applied to the stage. This is applied when the *Sample Exchange* window / *Holder* area / *Microtome* item and the appropriate PLA Accessory (see below) are selected.

Stage module movement range

- *X-axis*: from 2.5 to 10.5 mm
- *Y-axis*: from 44.5 to 52.5 mm

When a sample is loaded to the ultramicrotome, it is expected to be find approximately in the middle of these ranges.
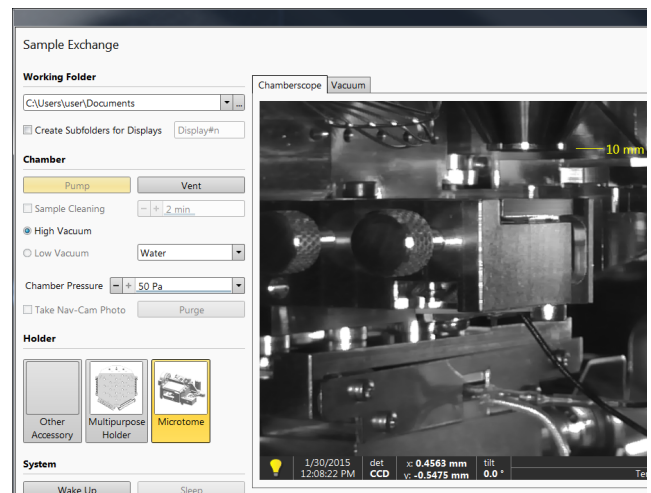
Caution!
Be sure to select these options! Otherwise the correct restrictions will not be applied.
A collision with the final lens or any mounted accessory will be possible!

Ring filter installation

This electrode is placed at the bottom of the final lens when working with charging samples in the High Vacuum mode. Its functionality is automatic after its installation and selection from the PLA Accessories dialogue.

Figure 2-5: *Ring Filter bottom / top view*
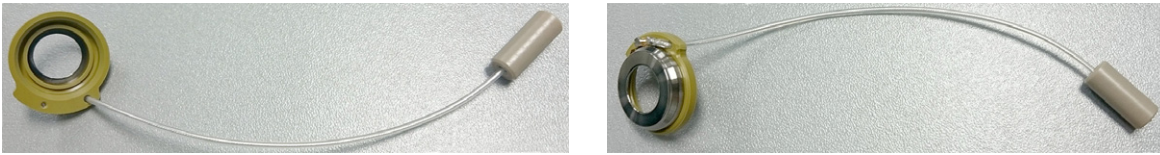
1. Click the **Vent** button to vent the microscope chamber; open the door.
2. Press the Ring filter by hand with gloves against the final lens pole and orient it so that the cable with pin faces towards the white connector. Make sure it is not tilted in relation to the final lens pole.

3. Fix the pin to the connector by insertion only.
4. Close the chamber door, select the **Vacuum** module / **High Vacuum** mode, and click the **Pump** button to pump down the chamber. The **PLA Accessories** dialogue appears.

Note
Make sure there is enough space between the ultramicrotome and detector before closing the door. If not, lower the stage until it is clear.

5. Select the **FilteRing** item to continue.
6. Set the beam **ON** and select the T1 detector from the **Detector Settings** module / **Detector** list box or from the **Detectors** menu.
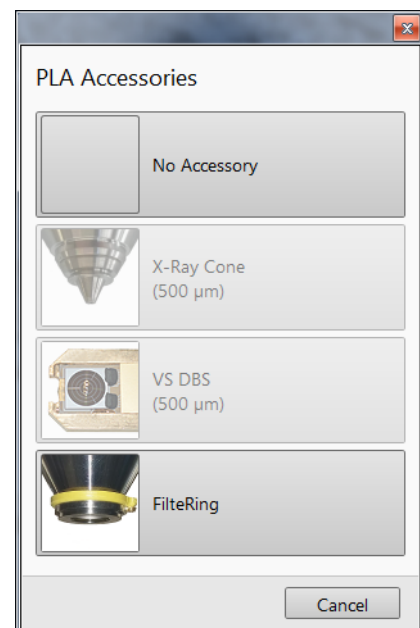
VS DBS detector installation

1. Vent the microscope chamber; open the door and remove the Ring Filter (if mounted) from the final lens pole.
2. Place the detector into the aluminum handling tool and fix it by its side clamps.
3. Press the detector against the final lens pole and orient its base so the cable is facing the left rear chamber corner.

Note
Make sure the detector is not tilted in relation to the final lens pole. This can manifest itself as a noticeable shift of the field of view towards the center of the imaging display.

4. Remove the handling tool.
5. Lead the detector cable to the left rear chamber corner and then along the left chamber side to the feed through with the Triax connector. Fix the cable to the clamps and check that it does not touch the metal mesh of the ETD detector, which is adjacent to the VS DBS detector. When the detector is not being used, it can be placed at the chamber bottom in the aluminum handling tool.

Figure 2-6: *VS DBS detector installed / Cable fixing / Chamber parked position*

Triax connector   Cable leading   Detector orientation
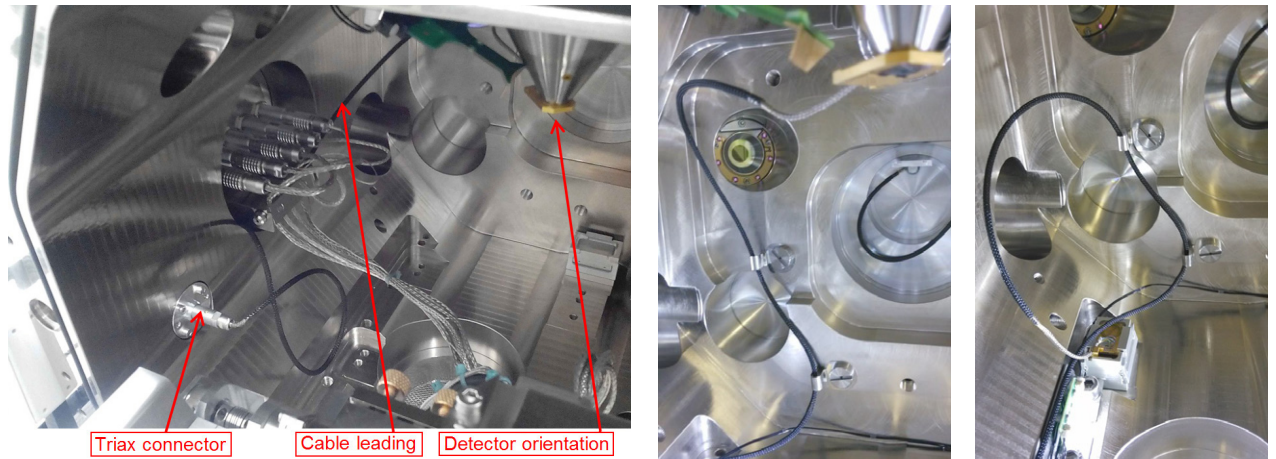
Caution!
Remove the VS DBS detector from the chamber (parked position) when the Plasma Cleaner is going to be used!

6. Close the chamber door, select the **Vacuum** module / **Low Vacuum** mode, and click the **Pump** button to pump down the chamber. The **PLA Accessories** dialogue appears.

   Make sure there is enough space between the ultramicrotome and detector before closing the door. If not, lower the stage until it is clear.

7. Select the **VS DBS** item to continue.
8. Set the beam **ON** and select the VS DBS detector from the **Detector Settings** module / **Detector** list box or from the **Detectors** menu.
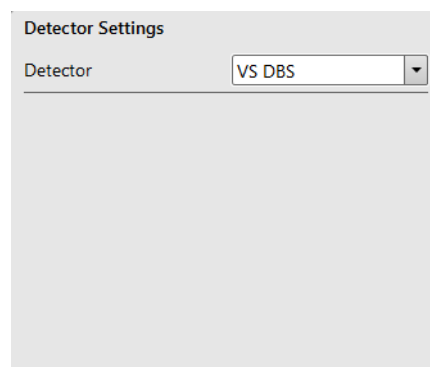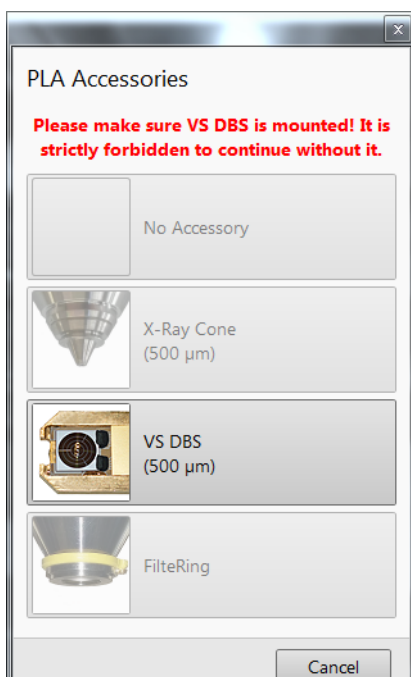
FEI MAPS

The FEI MAPS application with the VolumeScope application serves not only for the sample navigation but also controls data acquisition and processing and further extends its functionality to volume reconstructions. For more information about FEI MAPS and its usage please refer to the *FEI Maps SW Application User's Guide*.

Starting system

To start with following procedures it is necessary to have the ultramicrotome with the diamond knife installed (see Chapter 2 – *Ultramicrotome installation*).

1. Start the MAPS application from the Teneo microscope UI / ***FEI*** drop-down menu.

2. Check the connection status which is indicated by a dot in the bottom right corner of the MAPS main window. The green / red dot color indicates the application is / is not connected to the microscope.
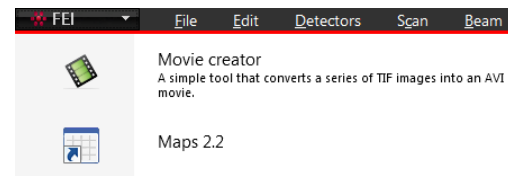
Figure 3-1:   *FEI MAPS main window*
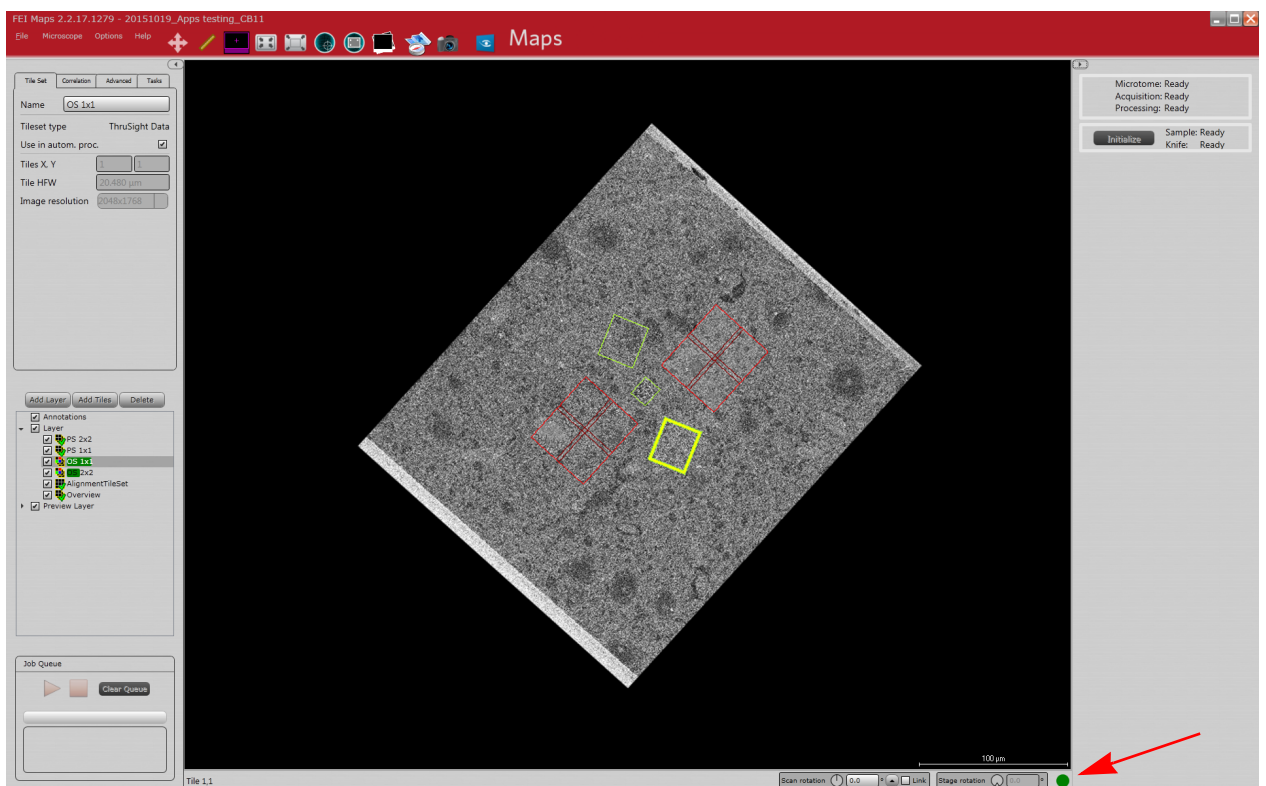
3. Check the *Microscope controls* placed on the right side of the FEI MAPS main window, where the list of items related to the VolumeScope extension can be found.

    The correct status is ***Ready***. If an item is active, status is ***Busy***. The ***Not connected*** or ***Error*** status means the item is not available and has to be checked (see Chapter 2 – *Getting started*):

- **Microtome** – informs of the availability of the ultramicrotome.
- **Acquisition / Processing** – serves for data acquisition / processing.
- **Sample** – indicates the status of the ultramicrotome Z-axis.
- **Knife** – indicates whether the knife is ready to cut.

4. Click the **Initialize** button if the **Sample** and/or **Knife** are not ready. This will home the Z-axis and the knife axis.

Creating VolumeScope project

When starting the MAPS, the user must create a new, or select an existing VolumeScope project. After that it is possible to continue with the Mechanical and Optical slicing setup.

1. The **Project History** dialog appears when the MAPS application starts. It is possible to select an existing project within the **Project History** list or to create the **New Project** by clicking the button – define the **Project Name** and the **Project Path**.
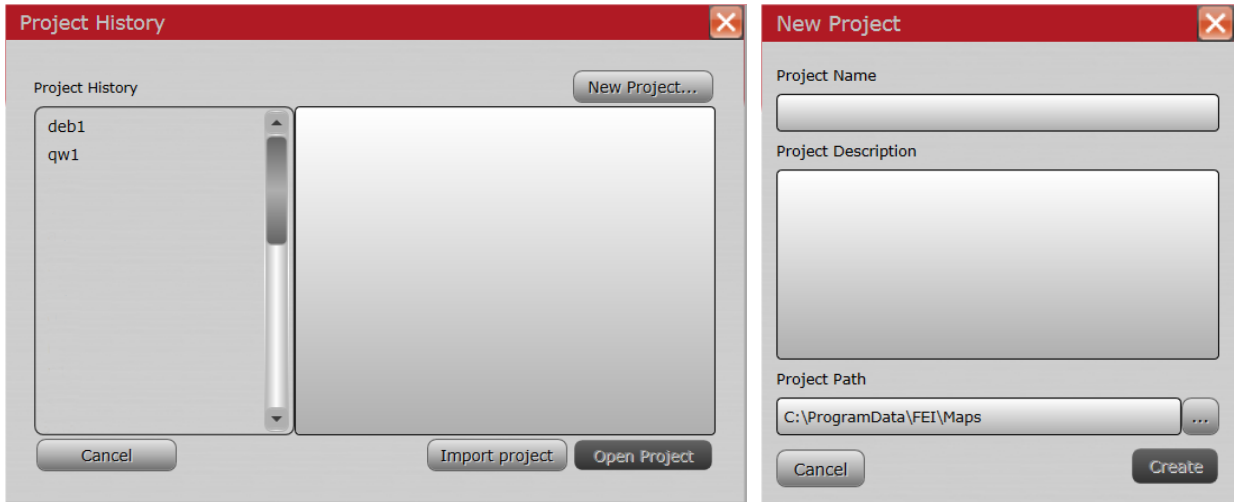
2. Complete the microtome installation (see Chapter 2) and sample approach procedures (see below).

3. Within the MAPS application acquire a low magnification overview of the sample surface from the active microscope UI display by selecting the **Microscope** menu / **Take Preview image** item or by clicking the toolbar icon.

4. Select a region of interest and create data and alignment tile sets. Click **Add tiles** button in the layer control (placed on the left side of the main window) or right-click & drag on the region of interest on the sample preview and choose **Add tiles from the selected area**. The **New Tile Layer** dialog appears – set the **Layer Type** and **Name**.
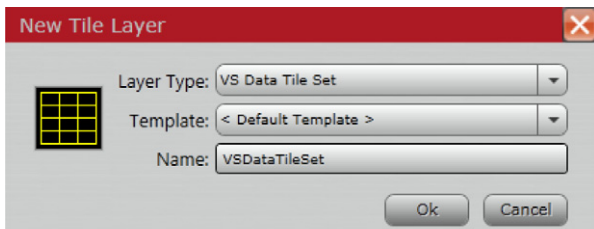
By default, all new tile sets, other than their names, have the same parameters (resolution, HFW, scanning parameters, Z-stack and other attributes.

- **VS Alignment Tile Set** – serves for the column alignment. It should be placed on a different region from the data acquisition region because of the potential radiation damage that occurs at higher electron doses.
- **VS Data Tile Set** – defines the region where the volume data will be acquired. Multiple **Data tile sets** can be selected.

  Alignment tile set should be set with the appropriate energies (HV's) for corresponding data tile sets. Selected parameters of the alignment or data tile sets are shown in MAPS UI when selecting given tile set.

- The **Tile Set** tab shows the basic properties of tile sets, which can be edited.

  The tile set **Name** / the **Tileset type** / the **Tiles X, Y** (number of tiles) / the **Tile HFW** (Horizontal Field of View) / the grabbed **Image resolution**

  The **Use in autom. proc.** check box – see the *Optical Slicing Setup / Tile Sets Definition*.

- **Correlation** tab enables users selecting of data source of images shown within the MAPS by checking the corresponding check box: *Raw acquired images / Registered acquired images / Deconvolution results*.
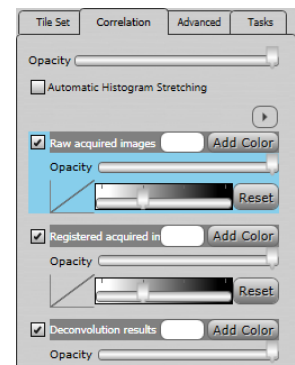
- **Advanced** tab allows definition of the neighboring tiles overlap.

  Ticking the *Use in debris analysis* check box helps to automatically distinguish a debris in images and to remove them by a knife sweep closely above the sample surface. This functionality is available for each tile set, but we advice to use it mainly for any overview tile set, covering all other tile sets. This tile set must not reach the sample edges, otherwise this can lead to an incorrect debris analysis.
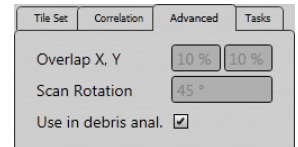
  Removing the debris is important particularly in the vicinity of areas of interest; it can influence an image quality and final usability.

- **Tasks** tab contains the list of all running MAPS processes for the specific tile set (such as tile registration, stitching, etc.).

5. Click the blue VolumeScope icon located in the FEI MAPS main window toolbar. The VolumeScope UI introductory window – VolumeScope Setup Guide appears.

6. Follow the *Z Stack Definition* and *Acquisition Parameters* settings of the *Optical Slicing Setup* workflow to set all the alignment and data tile sets properly.

   The *Project / tile sets definition example* can be found in the *Best Practices* (see Chapter 4).

7. Follow the *Optical Slicing Setup / Column Alignment* workflow and run the alignment procedure for the list of all energies (HV's) within the specific alignment tile sets.

8. When the VS project is fully defined, set the number of cuts, cutting speed etc. within the VolumeScope introductory window / *Workflow Control* area. The job can be started now.

> **Caution!**
> *After each job when venting the chamber clean the system according to the Regular cleaning described in the Teneo VolumeScope safety and maintenance chapter.*

MAPS application dedicated control

Projects complete information and detailed settings are available in the VolumeScope application. However, if this application is installed, it is possible to set some parameters for tile sets and projects directly in the MAPS application.
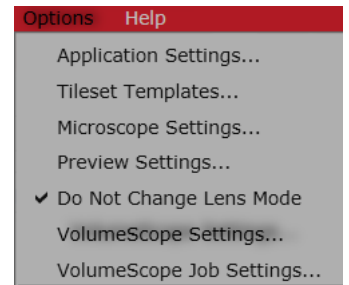
- **Tileset Templates** – the pre-set acquisition parameters can be set, stored (set new name and click the *OK* button), and loaded when defining a new tileset.
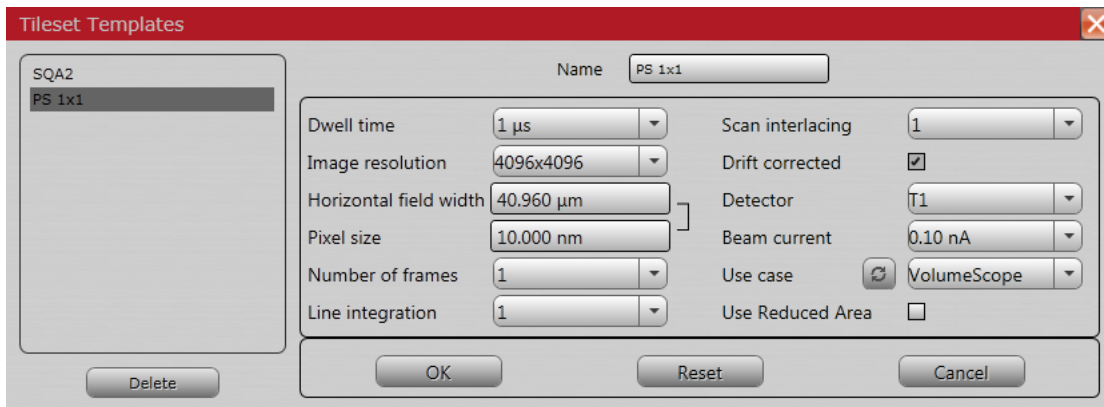
- **Volume Scope Job Settings** – the order of projects dataset acquisition can be set here (drag and drop).
- **Volume Scope Settings** – service access parameters (not set by a user), an e-mail address for notification of the project state and VolumeScope application advanced settings are accessible here.
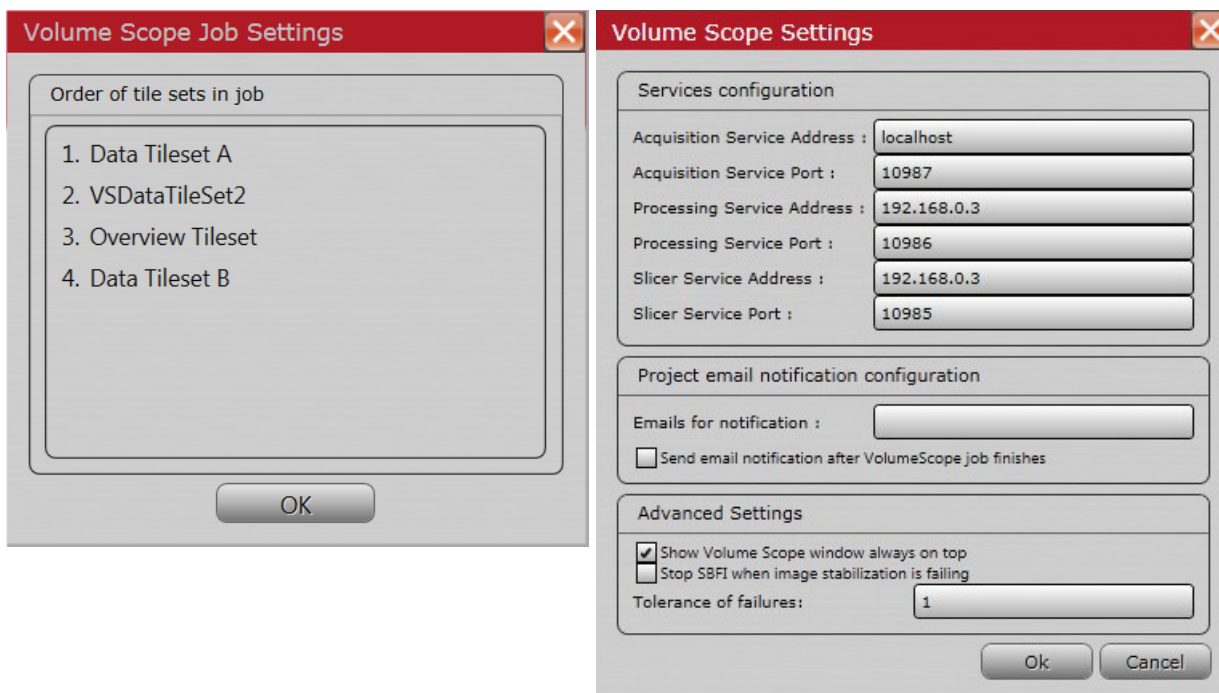
VolumeScope documentation and information about the application are also available within the MAPS application.
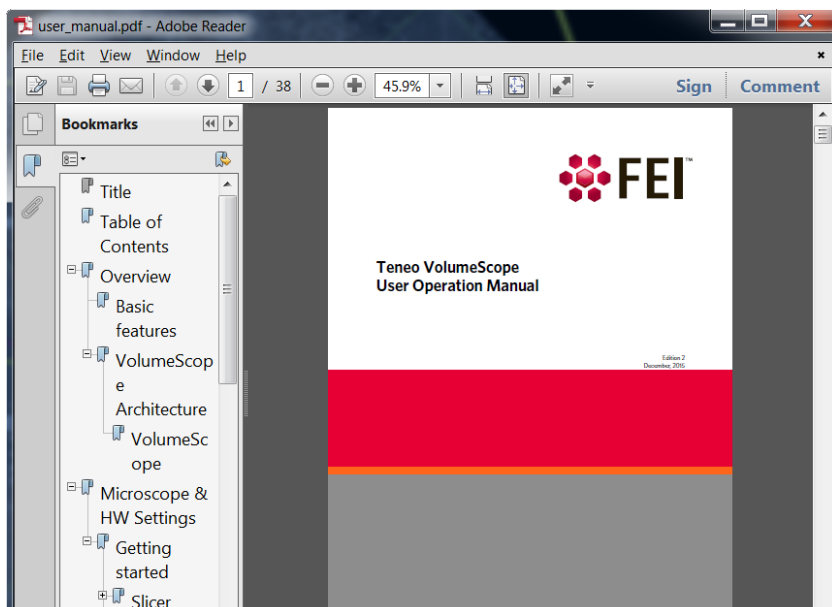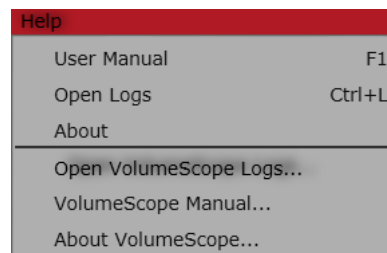
VolumeScope

The VolumeScope application enables the user to thoroughly define, start and monitor the progress of the VS project. Click the blue VolumeScope icon located in the *FEI MAPS* main window toolbar. The VolumeScope UI (*VolumeScope Setup Guide*) introductory window appears.

Figure 3-2: *VolumeScope Setup Guide window*
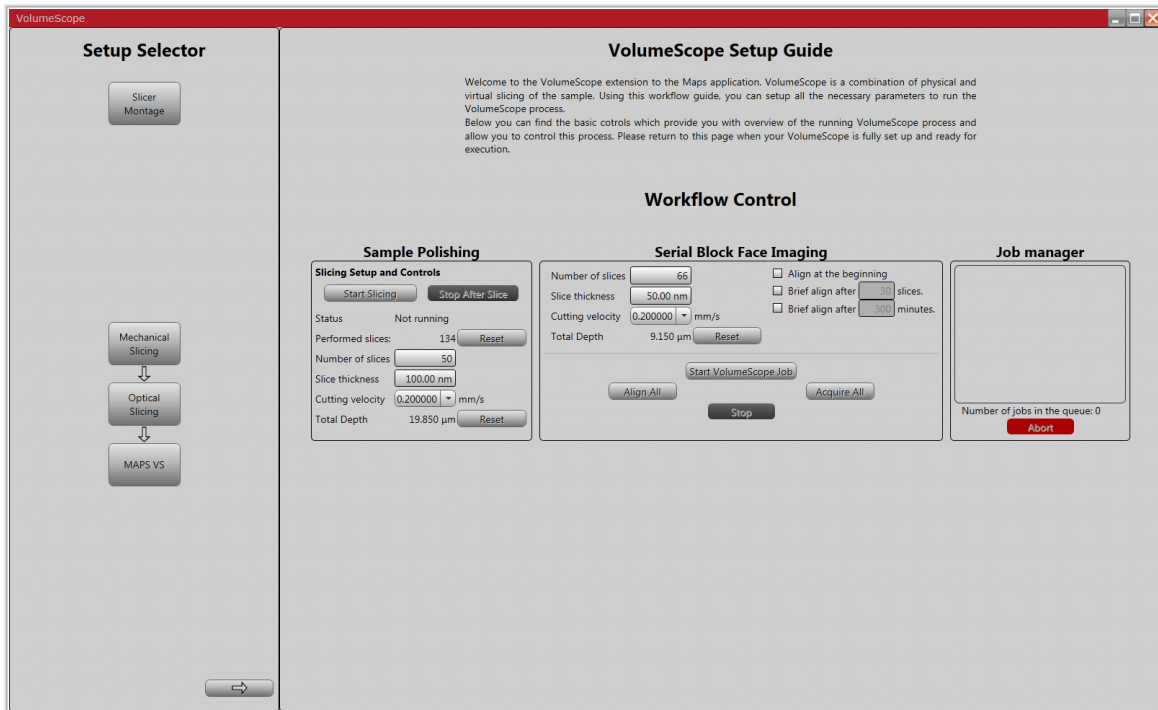
The area on the left side of the window (**Setup Selector**) allows one to navigate through the VS project. Using the workflow represented here by sequences of buttons, the user can set up all the necessary parameters to run the VolumeScope process. The header of this area changes according to a selected workflow:

- Click the **Slicer Montage** button to install the ultramicrotome on the stage (see Chapter 2).
- Click the **Mechanical Slicing** button to exchange the sample and to set up the ultramicrotome.
- Click the **Optical Slicing** button to set up the acquisition parameters and to define the optical slicing process.

The buttons with left / right arrows at the bottom lets the user to cyclically move through several workflows. Clicking the **MAPS VS** button closes the VolumeScope UI and returns the user back to the FEI MAPS application.

The area on the right side of the window shows particular options for a given step of the workflow. It changes according to the button selected on the left side area.

Workflow Control

When the VS project is fully defined (see above), the user has to return to the introductory window to start data acquisition. This area provides an overview and control of the running VS processes.

The **Sample Polishing** area lets one polish the sample surface before project initiation (see below – the *Fine Approach Vacuum*).

The **Serial Block Face Imaging** area controls the project execution.

- **Number of Slices / Slice Thickness / Cutting Velocity** – number of physical cuts / nominal thickness of the cut / cutting speed
- **Total Depth** shows an achieved depth from the last **Reset** by clicking the button.
- Checking **Align at the beginning** executes the alignment of the e-column at the beginning of the project.
- Checking **Brief align after x slices / minutes** causes the column to be re-aligned after a defined number of slices / minutes. If a time interval is defined, first a data acquisition for the given slice is finished, and then column alignment is performed.

  The list of autofunctions that are going to be performed is defined in the *Optical slicing / Column Alignment / Autofunction Selection* window / **Brief selection** list (see below). The alignment will be performed in the region defined by the Alignment tileset.
- Click the **Start VolumeScope job** button to execute the complete process.
- Click the **Align All** button to run all Alignment tile sets before the job starts if needed.
- Click the **Acquire All** button to acquire data for all tile sets without slicing – just for the actual layer.

  This is helpful to verify the correct project settings before the job starts.
- Click the **Stop** button to stop the actual project run.

The **Job manager** area reviews the status of the data acquisition and processing.

- Click the **Abort** button to stop the process(es) immediately.

  If the knife stops during the cutting action, it stays at its immediate position and has to be manually retracted (see below – the *Sample Approach Air*). To avoid sample surface damage, click the Abort button when the knife is retracted.

Slicer Montage

(see Chapter 2 – *Ultramicrotome installation*)

Mechanical Slicing Setup

With vented chamber the sample can be mounted onto the ultramicrotome. After this the sample needs to approach the knife to determine the cutting position, and finally the sample surface must be polished. The chamber is then pumped down, and the final approach procedure is repeated in a vacuum before the project starts.

Sample Exchange

This window allows the user to set the sample and the knife in their correct positions so that the approach procedure can be started. Ensure the ultramicrotome with the diamond knife is properly installed and connected, i.e. check the microscope controls within the FEI MAPS main window (see above).

Figure 3-3: *Sample Exchange window*
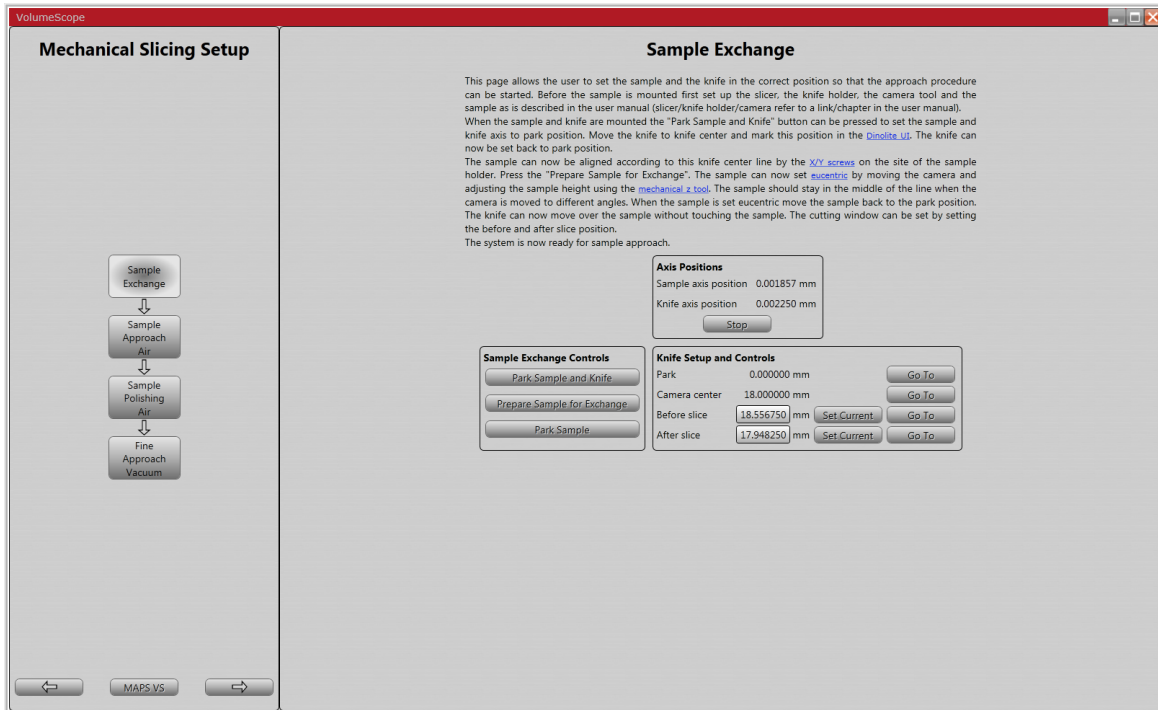
1. Place the camera tool on the ultramicrotome and fix the camera fixing screw. It might be necessary to lower the stage so that the camera tool fits. Connect the two USB cables attached to the camera tool to the VolumeScope PC to power the camera and the backlight.

Figure 3-4: *Dinolite camera tool USB cables & setting screws*

Camera USB cable

Backlight USB cable

Camera fixing screw

Positioning screw with securing nut
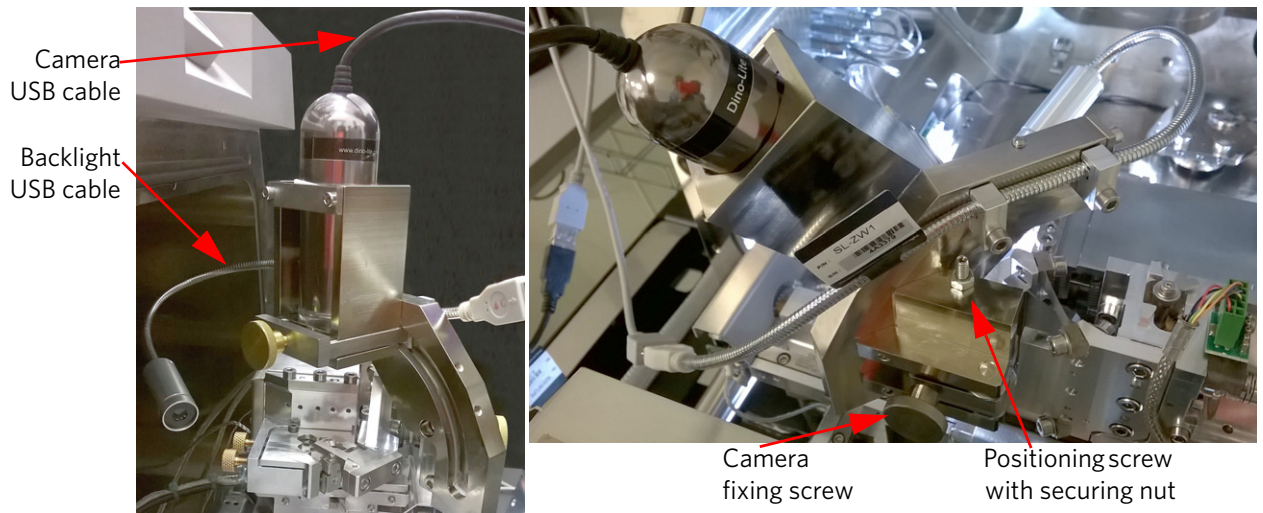
2. Start the Dinolite application, i.e. click the **DinoCapture** icon on the VolumeScope PC desktop.
3. Click the **Knife Setup and Controls** area / **Camera center** item / **Go To** button (the knife edge should be approximately at the center of the screen). Mark this position using the **Line tool** with a red line. Doing so can assist with the camera tool vs. the sample alignment.
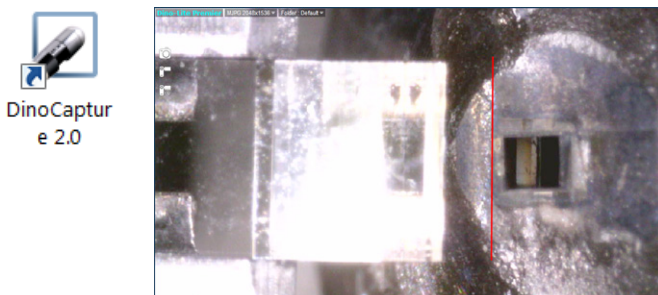
4. Verify the camera is at an eucentric position by tilting the camera. The knife-edge should be in focus for every camera angle – the edge of the knife is always at the marked position. If the camera is not at an eucentric position, change its height by adjusting the positioning screw. It is necessary to first loosen the camera fixing screw and the positioning screw fixing nut. After changing the height retighten the securing nut and fixing screw.

5. Click the **Sample Exchange Controls / Park Sample and Knife** button to move the sample and knife to a defined park position.

6. Loosen the sample holder clamp fixing screw and insert the FEI sample holder with a sample attached.

7. Mechanically lower the sample holder Z-axis to its zero position by rotating the sample Z-axis positioning screw.

Figure 3-5: *Microtome sample positioning screws*

X- / Y-axis fixing screw

X- / Y-axis positioning screws

FEI (column) alignment sample holder

Sample holder clamp fixing screw

Sample Z-axis positioning screw

FEI VS sample holder
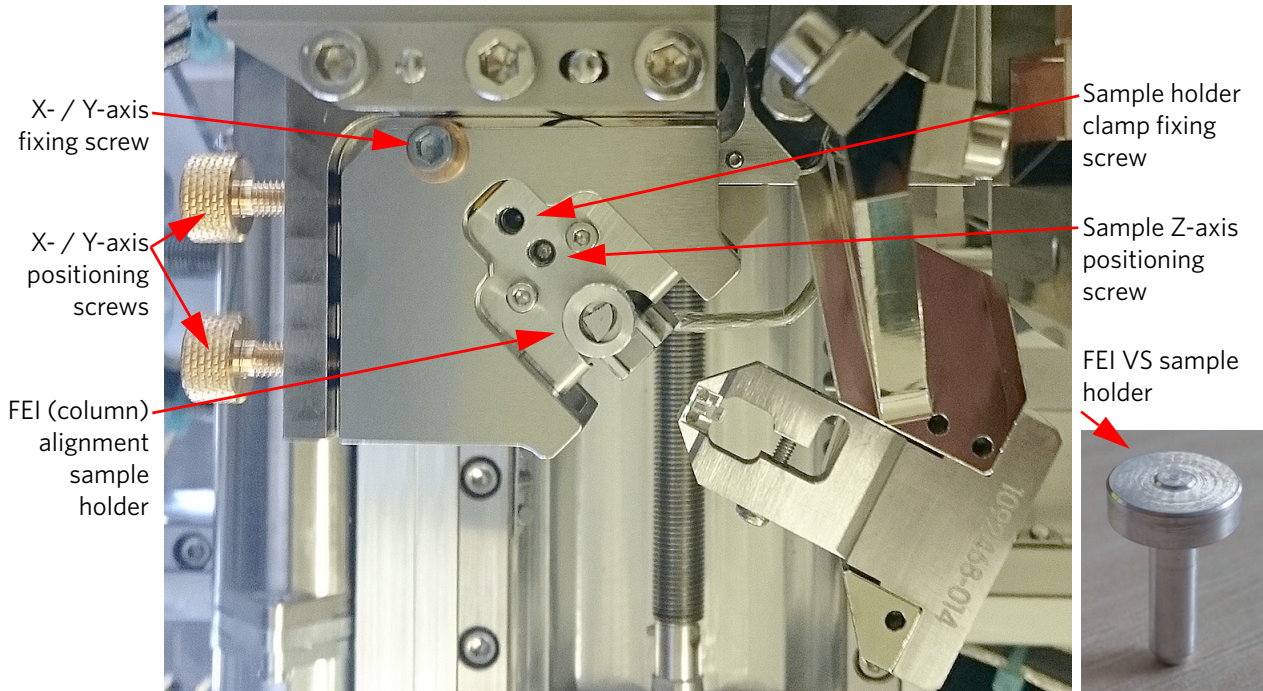
8. Loosen the Microtome X- / Y-axis fixing screw and position a sample according to the red line that you marked in the step No. 3 (the camera tool should be at 0° and the knife in the park position).
   – Manually rotate a sample so that its sides are parallel to the knife's edge.
   – Move a sample by adjusting the X- / Y-axis positioning screws so that it overlaps the red line.
   – Tighten the X- / Y-axis fixing screw.

9. Click the **Prepare Sample for Exchange** button. The sample is moved in the Z-axis direction towards the knife to the predefined position Z = 0.6 mm.

10. Verify the sample is at the eucentric position by tilting the camera from 0° to 45°: it should stay at the red line for every tilt angle. If the sample is not at the eucentric position, adjust the sample top surface (Z-axis) position. Loosen the sample holder clamp fixing screw, set the Z-axis positioning screw and tighten the fixing screw again when finished.

11. Move the sample back to the park position by clicking the **Park sample** button. The knife can now move over the sample without touching it.

Caution!
Tighten the sample holder clamp fixing screw and X- / Y-axis fixing screw by the torque screwdriver (a part of the accessory suitcase) set to the 10 cNm moment of force. It is important to fix a sample properly, because it affects cutting quality.

The system is now ready to proceed with the Sample Approach Air procedure.

Sample Approach Air

The sample can now be moved up towards the knife. The backlight at the side of the ultramicrotome provides a reflection of the knife-edge on the sample's surface. The thinner the reflection line, the closer the sample is to a cutting position. The Z-axis sample and knife position can be controlled via the elements within the **Axis Controls**, **Axis Positions**, **Sample Setup and Controls** and **Knife Setup and Controls** fields.

The **Load Cutting Parameters** button enables a user to load approaching parameters when using the same sample for a different VolumeScope project: Next sample slice / Before Slice / After Slice.

Figure 3-6: *Sample Approach Air window*
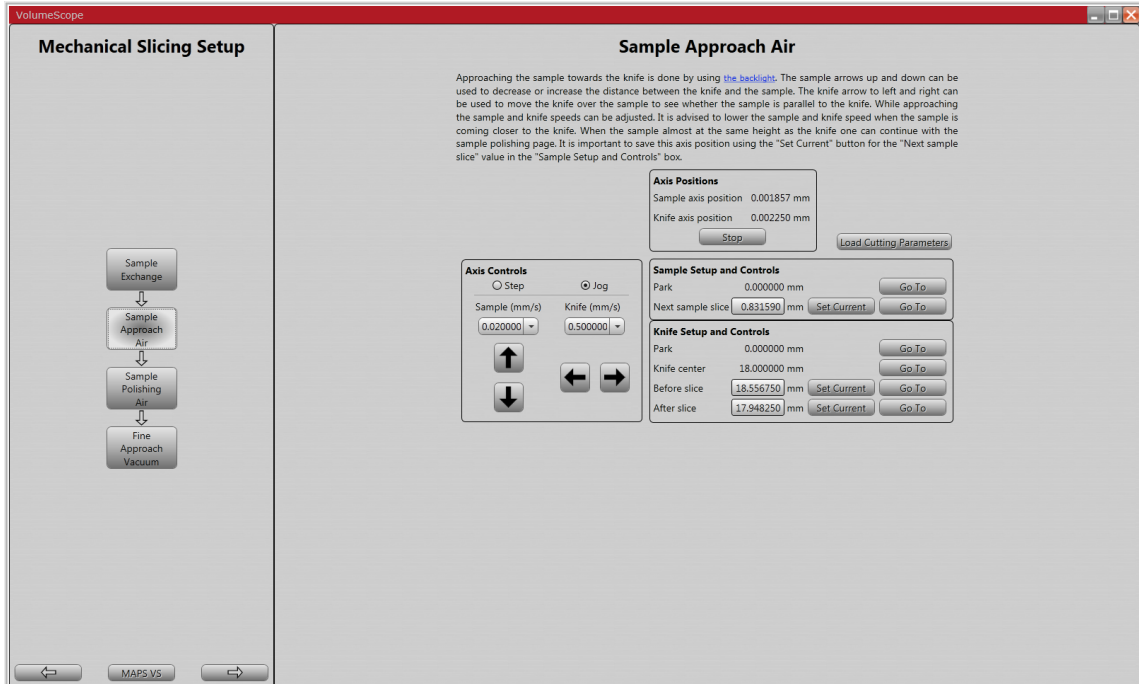
1. Set the camera to 0° and define the knife cutting limit positions.

   Move the knife across the sample to a position that is close to the block-face. Click the **Knife Setup and Controls / Before Slice / Set Current** button. Repeat this on the opposite side of the sample and click the **After Slice / Set Current** button.

1. Set the camera to about 45°. Adjust the position of the backlight to see the reflection of the knife edge on the sample surface (see figure 3-8). It may be helpful to decrease the ambient light intensity.

2. Gradually move the sample toward the knife.

   Preferably this is done in jog mode. Use the **Axis Controls** area / **Sample Up / Down** arrows to decrease or increase the distance between the knife and the sample. Adjust the **Sample / Knife (mm/s)** speed during the approach: lower the speed as you bring the sample closer to the knife.

   While approaching, use the **Axis Controls** area / **Knife Left / Right** arrows to move the knife over the sample to see whether its surface is parallel to the knife. The distance between reflection and the knife-edge should stay constant, during the knife movement. If this not the case, this distance changes according to an extent of the sample tilt.
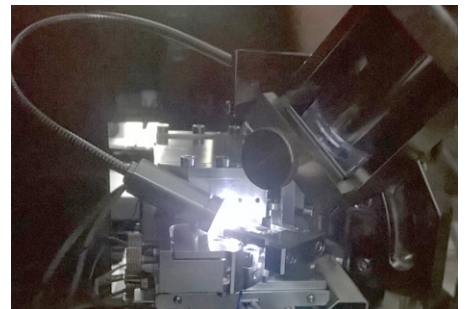
Figure 3-7: *Sample Approach Air process. Sample is further from / closer to the knife*
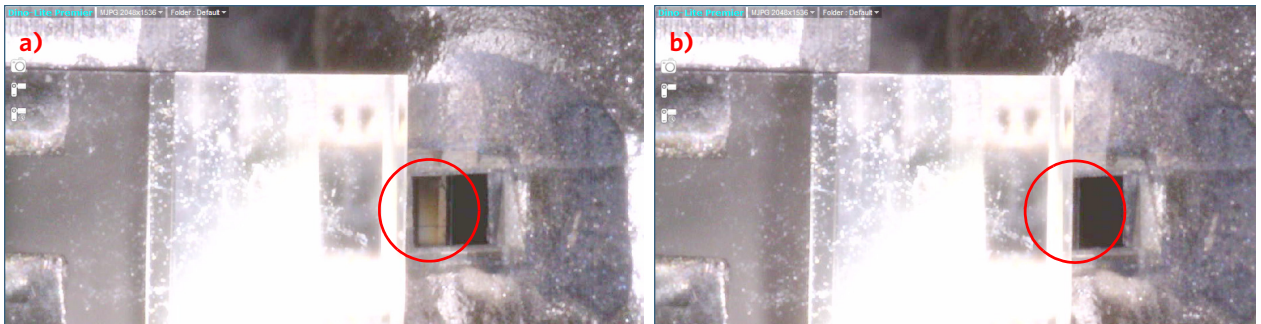
When the reflection of the knife-edge has almost disappeared, it is nearly at the same height as the knife.

3. **Important!** Save the Z-axis position by clicking the **Sample Setup and Controls** area / **Next sample slice** / **Set Current** button. Use it to start the approach.

Continue with the Sample Polishing Air window.

Sample Polishing Air

The sample is in now at the slicing position, but before pumping the chamber, the block-face needs to be polished. The slicing can be set up here. The polishing is stopped when the block-face is exposed and leveled.

Figure 3-8: *Sample Polishing Air window*
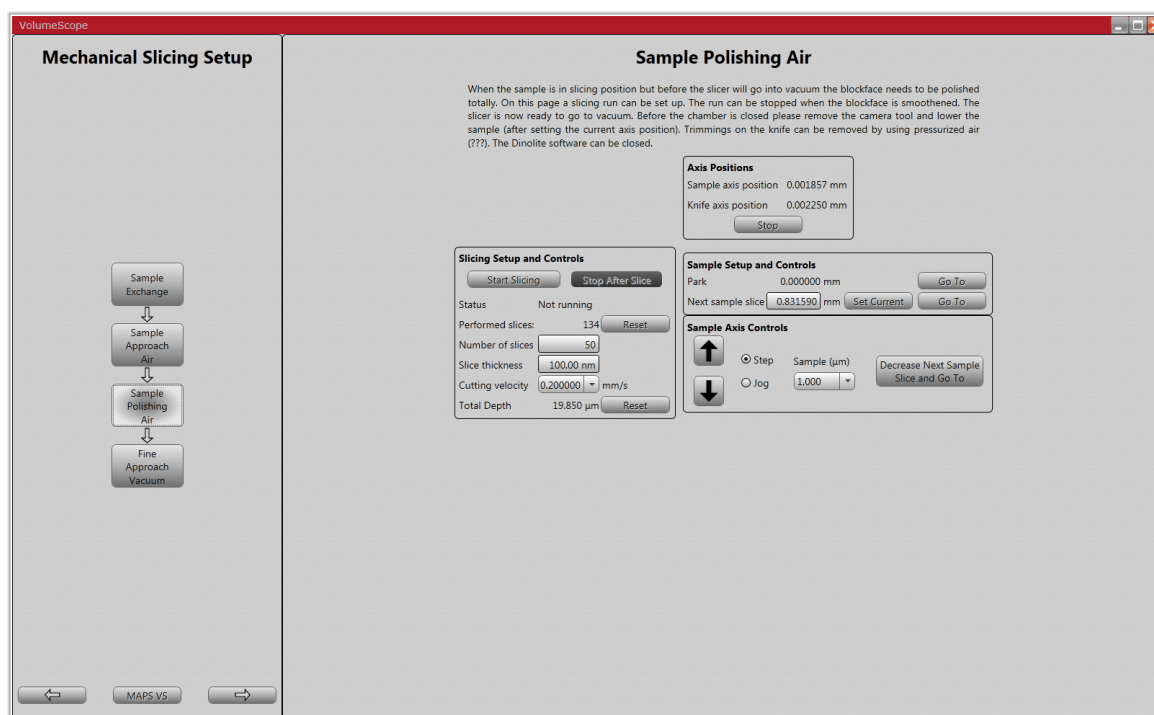
1. Optionally set the camera to 0°.
2. Set the **Slicing Setup and Controls** area / **Slice thickness** (start with e.g. 100 nm), **Number of slices** to be cut and **Cutting velocity** (e.g. 0.5 mm/s – 1 mm/s).
3. Click the **Start Slicing** button to initiate polishing. Debris will be visible on the knife surface when the knife is at slicing / cutting position and the sample is being actively polished. When the entire surface is freshly exposed, click the **Stop After Slice** button to stop the cutting.
4. Remove the camera tool and close the Dinolite application.

   If needed, remove any debris from the knife and the sample by using the vacuum cleaner provided with an appropriate adaptor (see Chapter 2 – the *Vacuum Cleaner*).
5. Before closing the chamber door and pumping down make sure there is enough room between the ultramicrotome and the final lens pole piece (use the optical display).

Pump down the chamber and continue with the Fine Approach Vacuum procedure.

Caution!
Be sure not to operate the microscope in Low Vacuum mode with the VolumeScope use case without the VS DBS mounted (see Chapter 2). Otherwise serious damage can occur to the system!

Fine Approach Vacuum

Here a final approach can be set up to make sure that the sample is at the cutting position before a VolumeScope project starts. The approach can be monitored by using the microscope UI.

Note

Because the sample Z-axis position is changed due to the vacuum the sample is lowered automatically about 1.5 μm after pumping the chamber to prevent possible accidental diamond knife collision with the sample.

Figure 3-9: *Fine Approach Vacuum window*
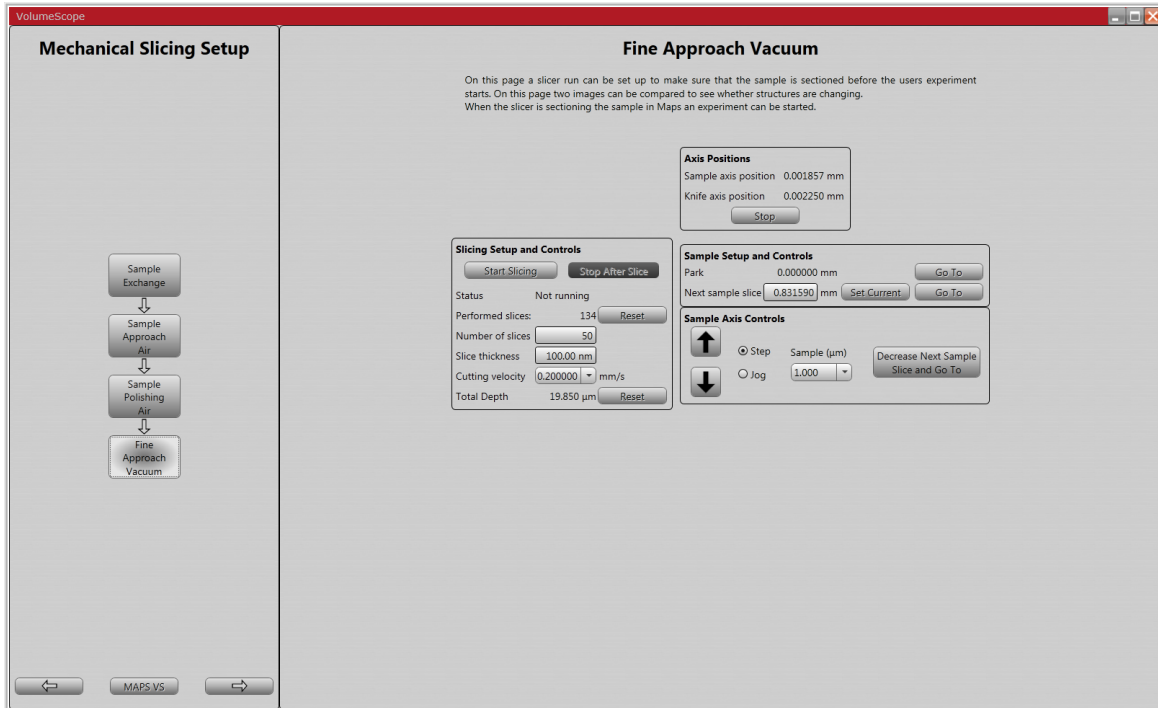
1. Go to the microscope UI and select the **VolumeScope** Use case from the toolbar drop down menu.
2. Switch the beam ON and start imaging a sample surface area suitable for the final approach.
3. Run the **Link Z to FWD** procedure by clicking the tool bar icon.
4. Set the working conditions that are safe and recommended (see Chapter 2).

Note

If the WD > 10 mm and the sample cannot be focused, it needs to be moved up a little, or the accelerating voltage has to be increased.
*If the sample is not visible in the lowest field of view, go to the **Standard** Use case and locate the sample position using the ETD detector.*

5. Set the same detector in display 1 and 2.
6. Set the **Mix 3** tab in display 3: **Progress Visualization** (or **Greyscale**) from the drop-down list. Check the **Invert** check box for **Source 1** or **Source 2** (see the *Teneo user manual*).
7. Set a reasonable amount of slices to be cut and start the fine approach (see above – the *Sample Polishing Air*). We advise starting this procedure after the vacuum is stabilized.

   During slicing, imaging is alternated between displays 1 and 2 within the microscope UI. The mixed image in display 3 remains structure-less until the cutting starts.
8. When you see a change in the display 3 image, stop cutting by clicking the **Stop After Slice** button to retract the knife after completing the cutting cycle.
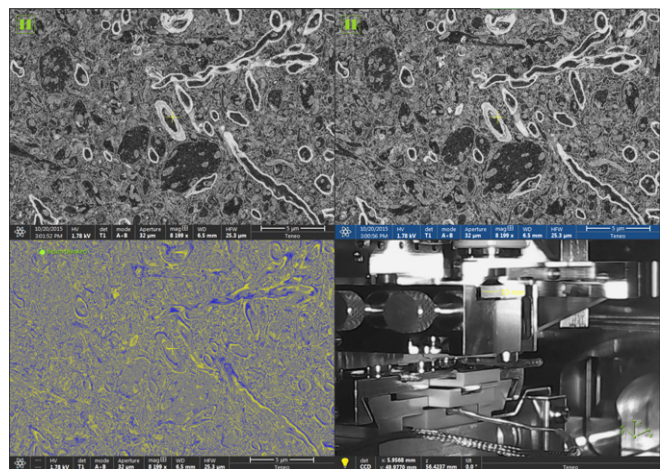
Optical Slicing Setup

When the Mechanical Slicing Setup is finished, define all the properties of the tile sets within this workflow.

The middle chain of the buttons represents the core of the workflow for optical slicing. The buttons to the right cover the needed functionality directly related to the given action in the middle chain. The buttons to the left represent advanced settings for experienced users.

Tile Sets Definition

First the areas of interest have to be defined in the FEI MAPS. There are two different kinds of tile sets. The *Alignment tiles sets* define the regions for the automatic column alignment. Any time this automatic procedure starts, the stage moves to the defined position, and a list of autofuntions outlined in the Autofunction Selection window (see below), is executed. The *Data tile sets* define the regions where the final data are going to be acquired.

The overview table informs the user of how many tile sets exist in **Total** and whether the tile sets are **Selected for automated processes** (to be a part of the VolumeScope project).

The tile sets can be selected or deselected in the FEI MAPS on the *Tile Set* tab by checking / clearing the **Use in autom. proc.** check box, which can even be changed during data acquisition.

Figure 3-10: *Tile Sets Definition window*
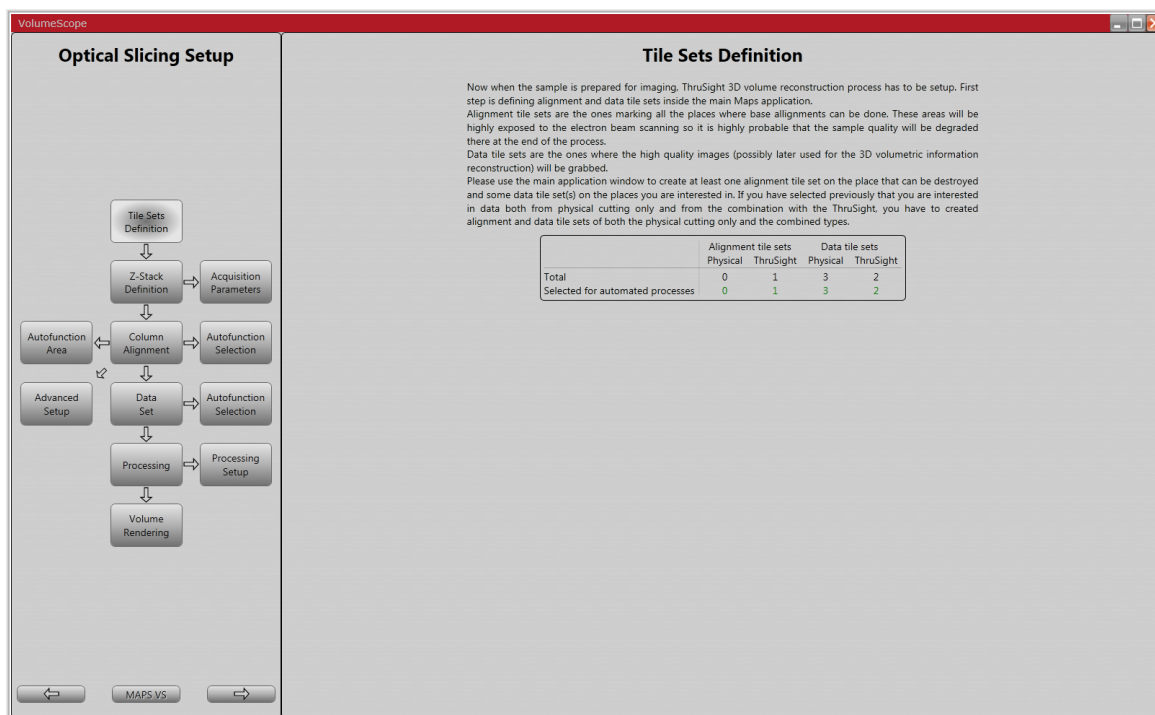
Z Stack Definition

Settings related to the sample volume to be observed are described here.

Figure 3-11: *Z-Stack Definition window*
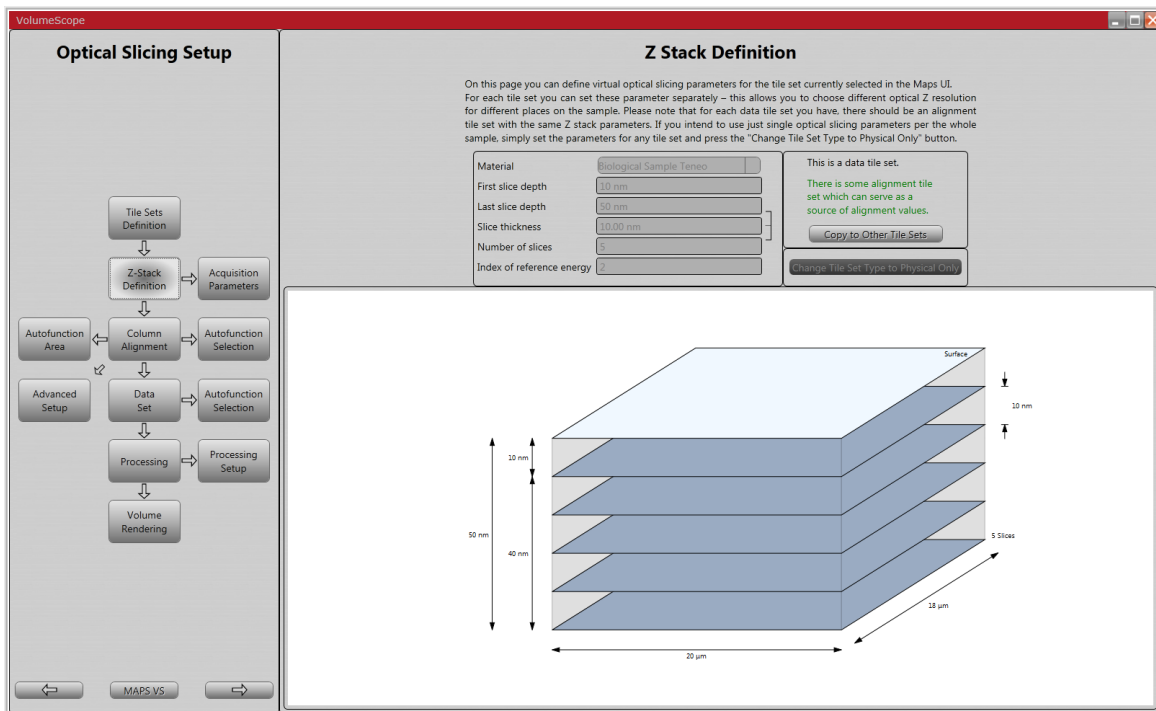
Parameters on the left side serves for defining the sample depths (primary beam energies determined by HV's) to be measured.

For mechanical slicing, click only the **Change Tile Set Type to Physical Only** button – only one image per tile at the selected accelerating voltage (HV) is acquired. The user can either enter a specific HV or can use the pull-down menu in which aligned HV are listed.

For optical slicing, the sample volume is divided into the user-defined number of optical slices, and more options are available. Each slice includes information from the given depth under the sample surface. The corresponding list of HV's is generated automatically (see below – the *Data Set*).

Click the **Copy to Other Tile Sets** button to send defined settings into the other corresponding tile sets.

Note

*The UI allows specifying the optical and physical slice thicknesses independently. This leads potentially to slice series with non-uniform slice thicknesses: Physical slices thickness is 50 nm, the **First slice depth** is 10 nm, **Last slice depth** is 50 nm and **Slice thickness** is 20 nm, respectively. This means the optical slices are at 10 nm, 30 nm, and 50 nm from the cutting surface. If the last optical slice for a particular cut is at 50 nm and the microtome removes 50 nm, the next optical slice is at 60 nm as seen from the surface of the preceding cut. Thus, the thickness of the last slice of the first cut is 20 nm and the thickness of the first slice of the second cut is 10 nm.*

Whenever the data are to be visualized in the 3D software (Amira), we recommend producing the uniform slice thicknesses (see image 3-11 as an example). Otherwise the visualization does not correspond to reality as an interpolation is included.

- *Material* – selection of the material to be observed and an appropriate calibration curve are dependent on the microscope mode. For the High Vacuum mode, select the *Biological sample Teneo*; for the Low Vacuum mode select the *Biological sample Teneo LoVac*.
- *First slice depth* – depth under the sample surface at which the first slice of the stack is located (min 10 nm).
- *Last slice depth* – depth under the sample surface at which the last slice of the stack is located (typically less then 70 nm).
- *Slice thickness* – thickness of one slice (min 1 nm).
- *Number of slices* – number of slices (min 2).

The last 3 parameters are dependent on each other. The scheme of the stack of slices is shown.

- *Index of reference energy* – optimal energy (HV) from which optical slicing starts.

Parameters on the right side provides some information related to the given tile set. The type of the selected tile set is shown. The message is in green / red color depending on whether the corresponding Alignment tile set exists / does not exist for the data tile set.

Acquisition parameters

The user can define several parameters related to image acquisition.

Figure 3-12: *Acquisition Parameters window*
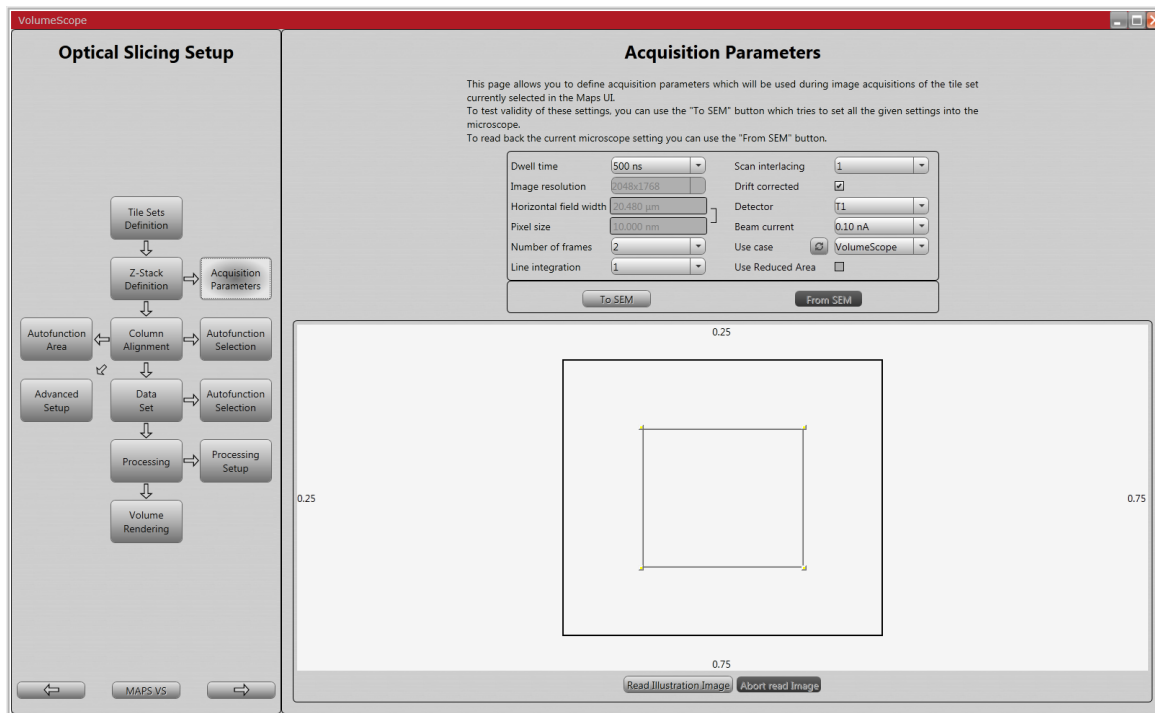
- **Dwell time** – time the beam remains on each pixel during image acquisition.
- **Image resolution** – the number of image pixels.
- **Horizontal field width** – the horizontal length of a field of view.
- **Pixel size** – calculated from the horizontal image resolution and the horizontal field width.
- **Number of frames** – the number of integrated images.
- **Line integration** – the number of scans of one image line before proceeding to the next one.
- **Scan interlacing** – the number of lines defining the size of a block into which the imaging area is divided; the beam is gradually scanned along the same lines in each block.
- **Drift corrected** – switching correction of the image drift when the integration filter is active On (checked) / Off (cleared).
- **Detector** – backscattered electron detector (T1, VS DBS, external).
- **Beam current** – the primary beam electron current.
- **Use case** – to select a microscope optical mode; by clicking the button with **circled arrows**, these parameters are send to the microscope to check its correctness.
- **Use Reduced Area** – the area defined (click & drag area borders at the bottom of the screen) from which an image is grabbed.
- **From SEM / To SEM** button – acquisitions parameters can be also read from / sent to the microscope UI. It is worth sending the adjusted parameters to the microscope to be sure they are applicable.
- **Read Illustration Image** – by clicking this button, an image is grabbed from the selected tile.
- **Abort read Image** – click this button in case image grabbing takes a long time to stop.

Column Alignment

The automatically generated list of energies determined by accelerating voltages (HV's) to be aligned, based on the Z-Stack Definition setting, is shown here. It is the input for an Alignment tile set. The obtained results are used further during an automatic job for the related Data tile sets if the *Autofunction Selection* window / *Apply Alignments* check box is selected (see below). The column is automatically aligned at HV's defined by the list, and the results are stored.

Figure 3-13: *Column Alignment window*
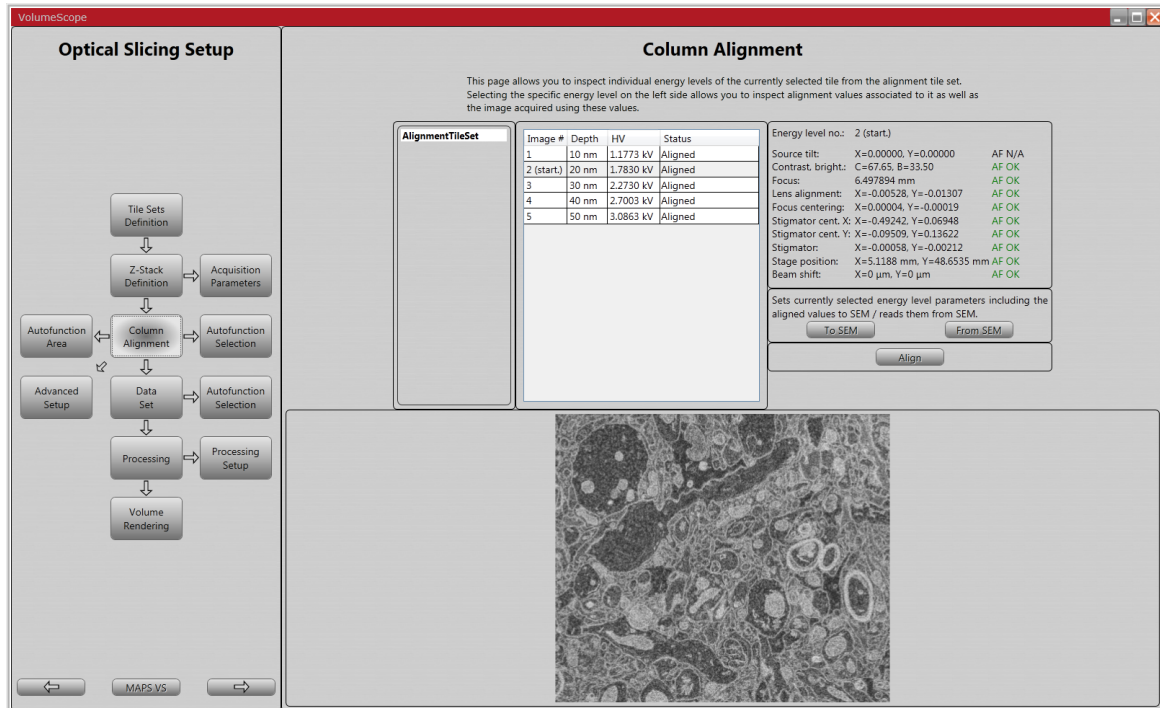
Selecting a specific HV level allows users to inspect alignment values associated with this HV as well as the image acquired (the bottom of the window). The user can adjust the microscope parameters as needed via the Autofunction Selection window (see below).

Note
> *For the alignment process we recommend choosing a different sample area than the area for the final data acquisition.*

The list of HV's to be aligned is automatically generated and shown in the middle area:

- *Image #* – the image number, ranging from 1 to M
  (M indicates the number of different HV for the given tile set).
- *Depth* – information depth.
- *HV* – accelerating voltage used for the image acquisition.
- *Status* – related to the microscope parameters adjustments for the corresponding HV.
  Aligned – all the applied autofunctions passed without error.
  Failed – some of the autofunctions failed and the image quality is poor – parameters should be adjusted manually using the *To SEM / From SEM* button (see below).
  Not aligned – none of the microscope parameters are available.
  Partly aligned – some of the microscope parameters are available; for those missing, the actual microscope settings are used.

Click the *Align / Abort* changing button to start / interrupt the selected column alignment for all HV's.

Status area in the middle part shows information for the selected HV.

- *AF aligned* – the value of the microscope parameter is available.
- *AF not aligned* – the value of the microscope parameter is not available.
- **Auto / Manual** – the parameter was acquired automatically / manually using the **From SEM** functionality (see below).

Results of the last relevant run of applied autofunctions for the given image:

- *AF OK* – the autofunction passed successfully
- *AF Failed* – the autofunction did not pass successfully; manual adjustment is needed (see below)
- *AF N/A* – the current status of a particular microscope parameter was not affected by an autofunction

The **To SEM / From SEM** buttons are used for manual adjustment of the microscope parameters.

- Clicking the **To SEM** button, the microscope parameters change according to the settings found for the selected image number / HV.
- Clicking the **From SEM** button enables reading the microscope settings for the selected image number. This can be useful when all or some of the autofunctions fail or if a user decides not to use them at all.

1. Select the desired image number and acquire an image of the desired quality within the microscope UI.
2. Click the **From SEM** button to load the microscope settings and the acquired image into the dataset.

Autofunction Selection

The list of available autofunctions is here. By default, most of the autofunctions are selected and applied during the alignment process. They are arranged in a defined way, and the use of the whole set assures the best results. Nevertheless, with a few exceptions, the user can select any combination, although some of the autofunctions are mutually dependent.

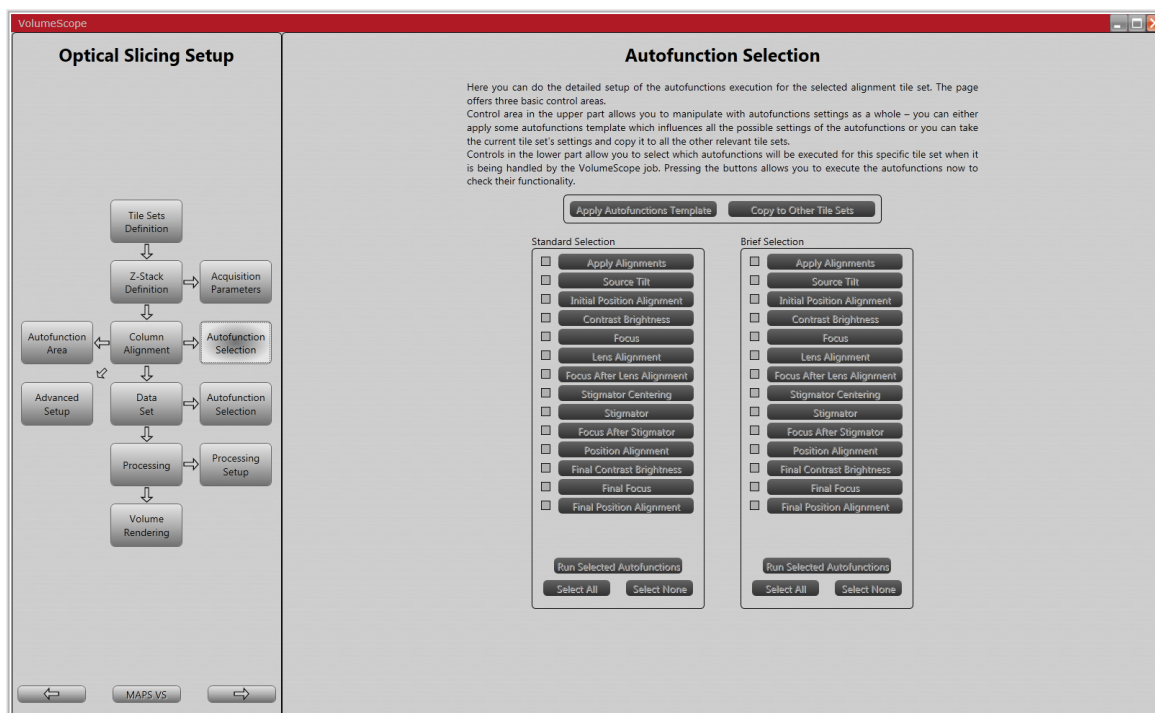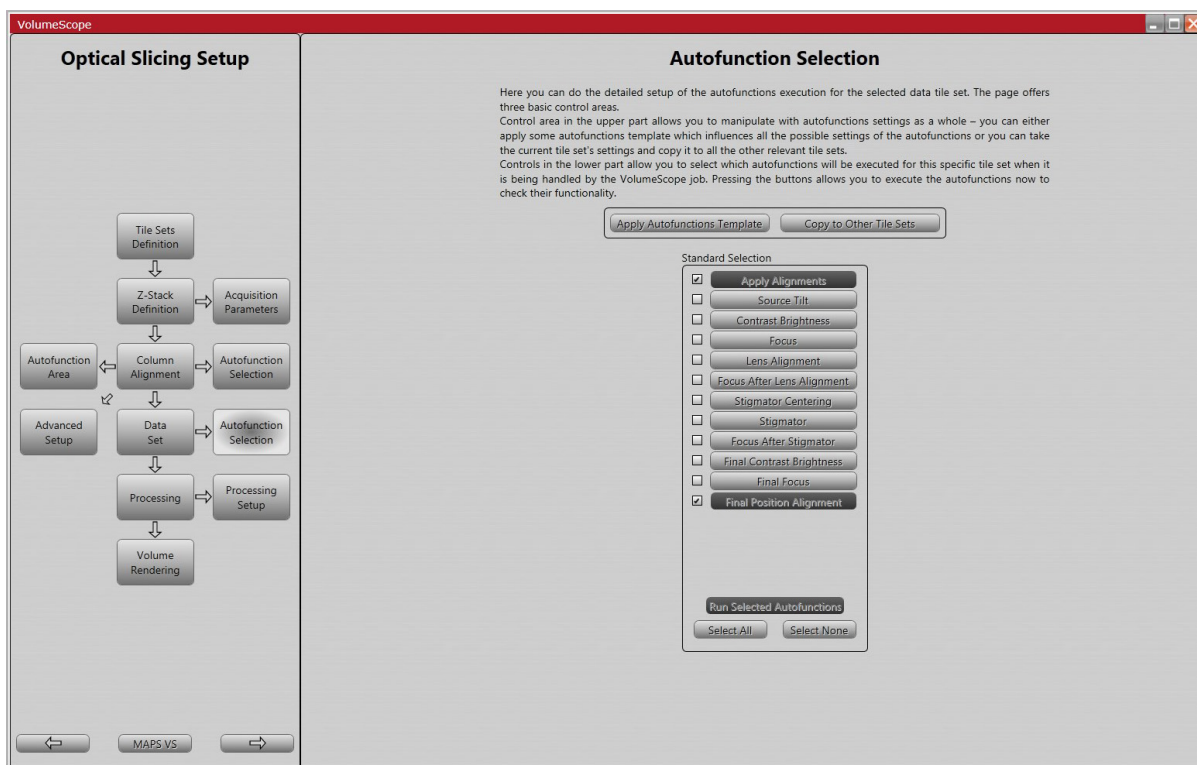**Figure 3-14:** *Column Alignment Autofunction Selection window*

Figure 3-15: *Data Set Autofunction Selection window*

The autofunction selection is indicated by a ticked check box next to the alignment button; clicking on the button directly initializes this autofunction execution. By clicking the **Run Selected Autofunctions** button, all selected autofunctions are executed. Clicking the **Select All / Select None** button selects / deselects all autofunctions. The list of available autofunctions includes the following items.

- **Apply Alignments** – the microscope settings from previous runs of microscope alignment, or data acquisition for all available autofunctions, are used. If deselected the actual microscope settings are used. Read parameters are subsequently modified by selected autofunctions.
- **Source Tilt** – assures the selected electron current is available.
- **Contrast Brightness** – optimizes the contrast and brightness of the image.
- **Focus** – focuses the electron beam to produce a sharp image.
- **Lens Alignment** – performs the lens alignment procedure.
- **Focus After Lens Alignment**
- **Stigmator Centering** – performs the stigmator centering procedure.
- **Stigmator** – compensates for any astigmatism.
- **Focus After Stigmator**
- **Final Contrast Brightness**
- **Final Focus**
- **Final Position Alignment** – this is selected by default for a data tileset in order to compensate an image drift during automated process (caused by various reasons). Compensation is achieved by shifting of the electron beam.Set the behavior within the **Maps** application > **Options** menu > **Volume Scope Settings** dialogue > **Advanced Settings** area:

– As a default, data acquisition continues even if the image stabilization fails. Ticking the **Stop SBFI when image stabilization is failing** check box forces the application to stop an acquisition. In both cases the corresponding warning message is shown to inform the user about the status and recommended action.

– The **Tolerance of failures** indicates the number of attempts to compensate the image drift between subsequent physical cuts (number 1 corresponds to two attempts). This value can be user modified.
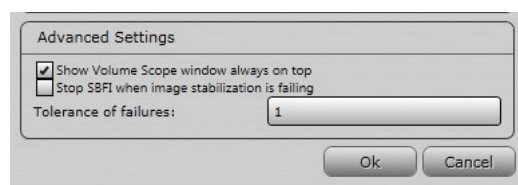
If the acquisition is not stopped after a compensation failure (default setting), the last acquired image is taken as a reference for a further compensation, regardless of the last result. This can generate data from a position different from the original. It is recommended to create a new tileset at the region of interest.
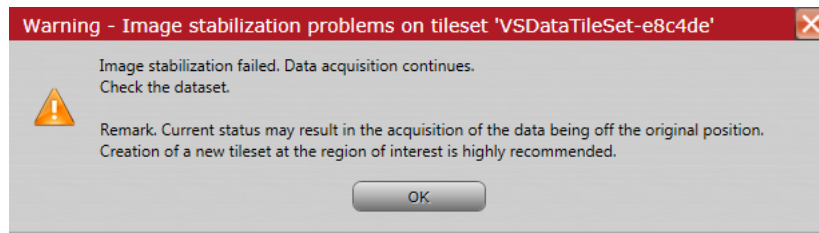
The microscope status determines which autofunctions have to be used to reach the best image quality.

The **Standard Selection** area is used for the column alignment before the project is started. The **Brief Selection** defines the set of autofunctions that are to be applied after a defined number of cuts or time period (see above – the *VolumeScope Setup Guide > Serial Block Face Imaging* box).

Autofunction performance can be influenced by changing the function's default settings. Click the **Apply Autofunctions Template** button and select the desired option. A few options for the given detectors are available. They differ in speed, and their choice should reflect the amount of available signal.

Clicking the **Copy to other tile sets** button copies all the selected settings into other related tile sets.

Auto Function

This panel enables setting of the position and shape of reduced areas for relevant auto functions. These settings are intended for experienced user (see Advanced setup below).

Figure 3-16: *Autofunction Area window*
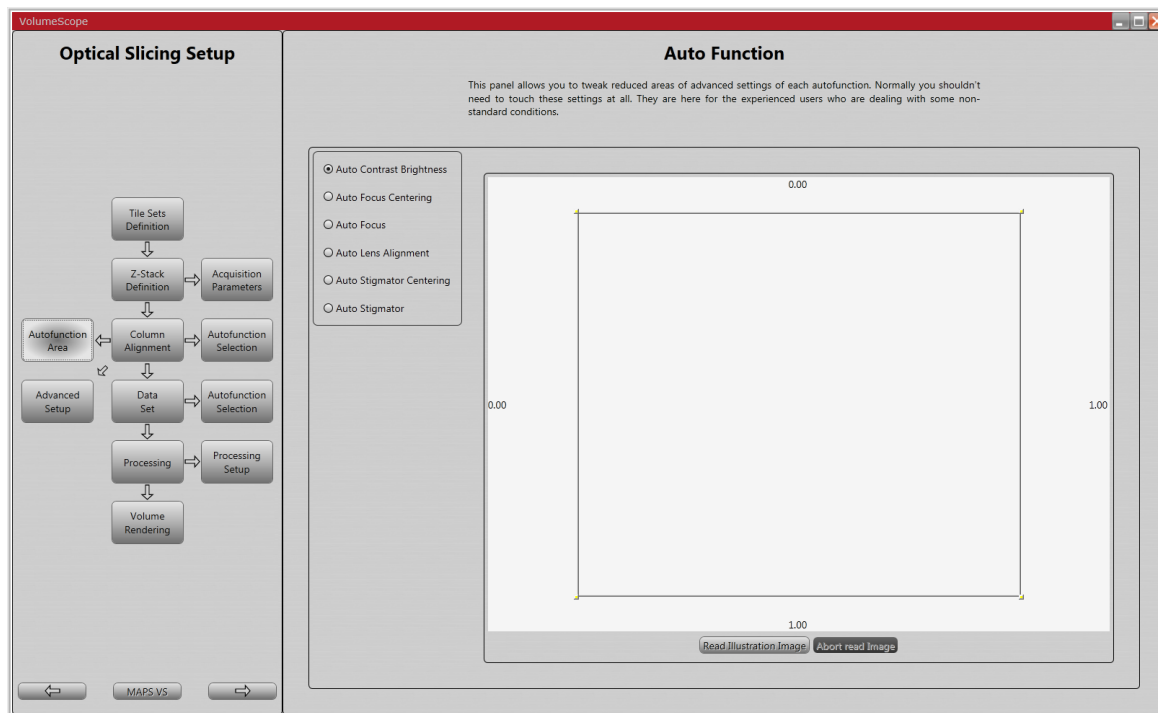
Select the radio button for the appropriate autofunction. The yellow borders indicate the position of the reduced area inside the field of view. Move and resize the borders as needed.

Advanced Setup

Selected autofunctions default settings can be fine-tuned here. They are intended for experienced users only, since unsuitable changes of these settings can lead to a deterioration of the autofunctions' functionality. If this is the case, go to Autofunction Selection (see above), click the **Apply Autofunctions Template** button, and choose one of the default settings for the given detector to restore the original settings.

Figure 3-17:   *Advanced Setup window*
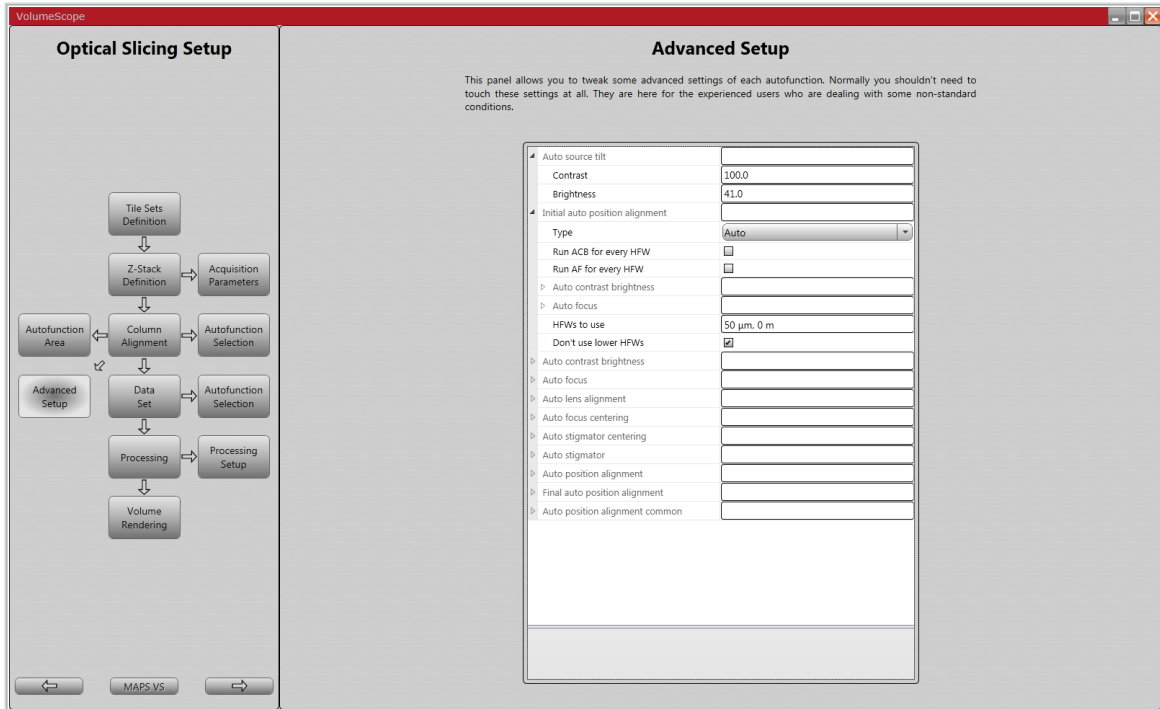
The list of autofunctions is shown. Click the arrow on the left side of the autofunction name to expand the list of available options with editable fields on the right side. Explanatory messages on the bottom provide hints about the selected items.

Data Set

This window allows users to inspect the individual energy levels (HV's) of the currently selected Data tile set. The list of HV's is based on the Z-Stack Definition.

Figure 3-18: *Data Set window*
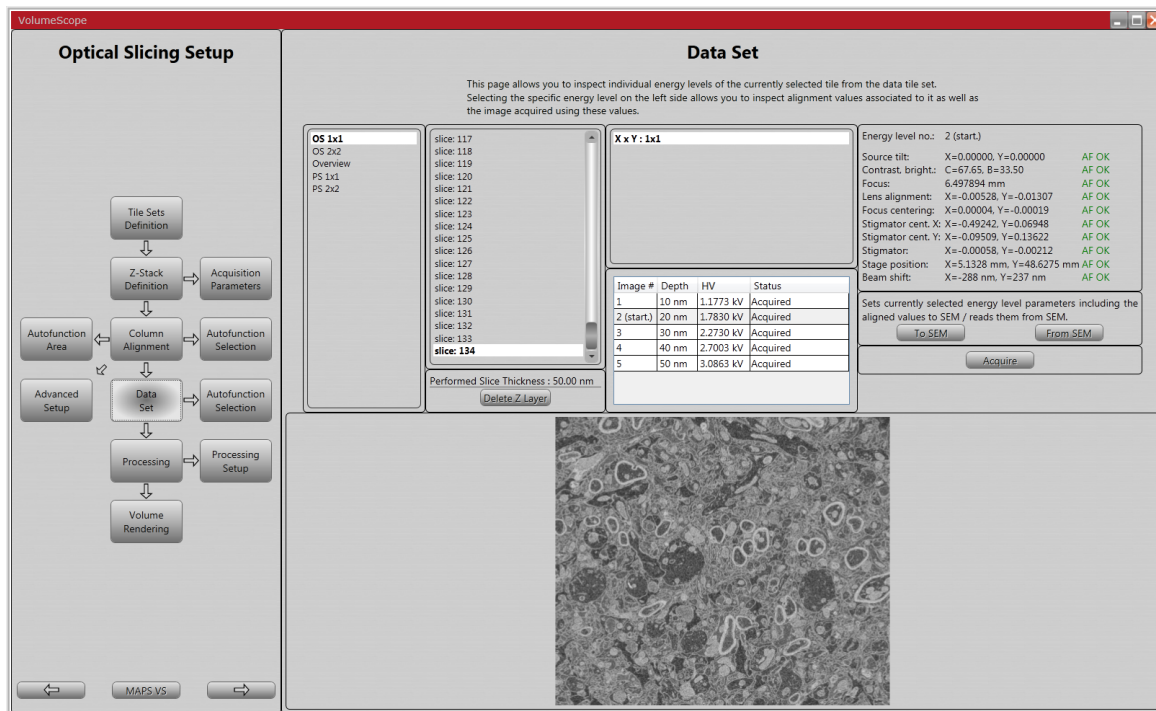
The first, leftmost Data Set window area shows the list of all the data tile sets. The second area lists acquired slices for selected data tile set. The third area shows the list of all tiles inside the selected data tile set.

The name (numbers) of the individual tiles reflects the lateral position (X × Y) of the tile inside the set – X / Y ranges from 1 to N (N indicates the number of the tile). The table below it shows the **Image #**, **HV** and **Depth** at which an image was acquired.

Click the **Performed Slice Thickness / Delete Z Layer** button to remove all data related to the given Z-layer / mechanical cut when they are not usable.

Selecting the specific tile allows inspecting all the acquired (raw) images: select a specific **Image #** level to show it at the bottom of the window. If no images have been acquired yet, no images are available, and the Status **Not acquired** is indicated. Otherwise the selected acquired images are shown.

Note
   When the image quality is poor, run the Column alignment (see above).

Processing

This window enables users to show processed images or to initiate image processing if it is not already started. Selecting the specific tile allows inspecting all processed images that are being shown on the bottom of the window (see above – the *Data Set*).

Figure 3-19: *Processing window*
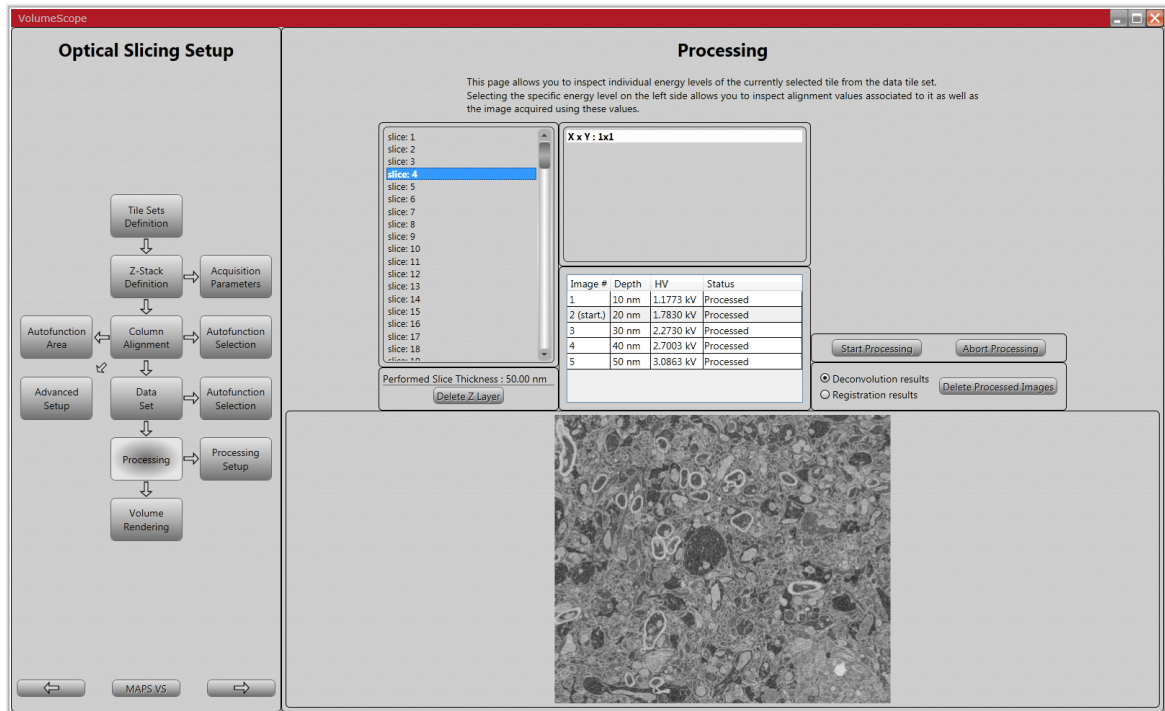
Click the **Delete Z Layer** button to remove all data related to the given Z-layer / mechanical cut.

If no images have been processed, or if there are unprocessed data remaining, click the **Start Processing** button to initiate the data procedure. The **Abort Processing** button stops the currently running process.

Switch between the radio buttons **Deconvolution results / Registration results** to see the result of the complete processing or only the registered images without deconvolution applied. Click the **Delete Processed Images** button to delete all the processed images.

Processing Setup

Settings related to the processing of the images acquired during optical slicing are listed here.

Figure 3-20: *Processing Setup window*
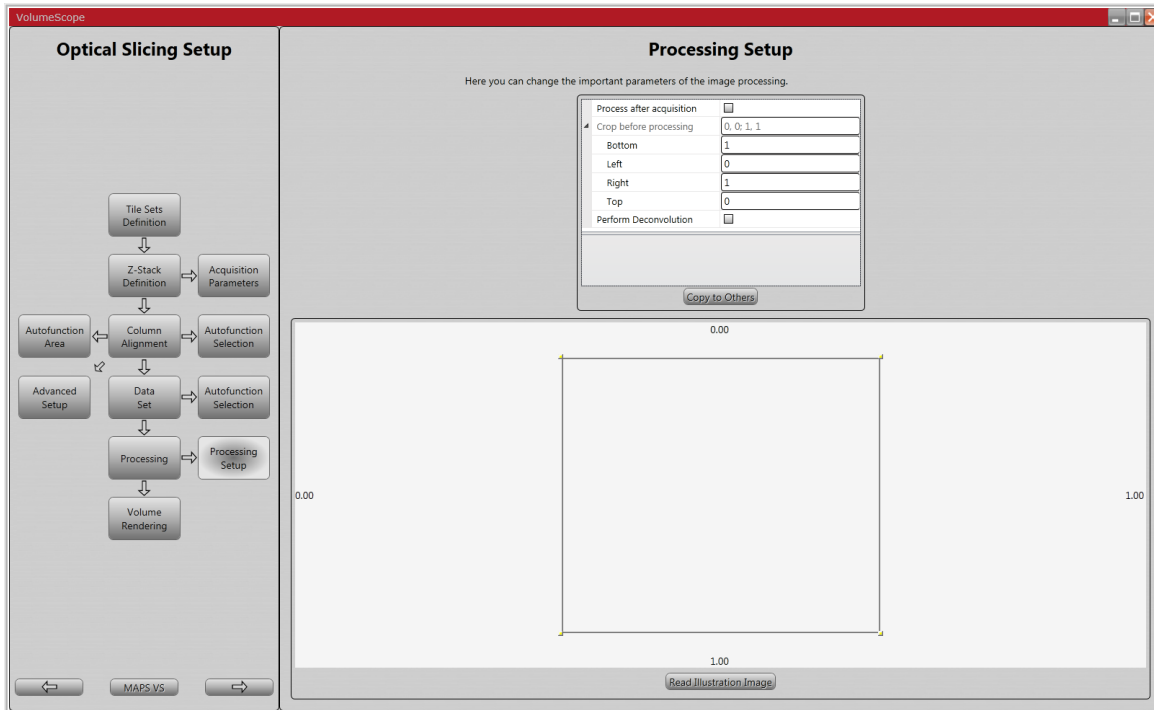
- Check the **Process after acquisition** radio button to process images immediately after acquisition.
  If cleared, the data processing has to be started manually later on (see above – the *Processing*).
- **Crop before processing** – enter relative shift values (**Bottom, Left, Right, Top**) of the square sides. This defines the region of interest to be processed. Data outside this square are cropped.
- Check the **Perform Deconvolution** radio button to process the data; if deselected, only registration is performed.
- Clicking the **Copy To Others** button propagates settings into other tile sets.
- Clicking the **Read Illustration Image** button acquires an actual microscope image.

Volume Rendering

Available images can be exported (in a .tiff format) by clicking the **Export Images** button.

Figure 3-21: *Volume Rendering window*
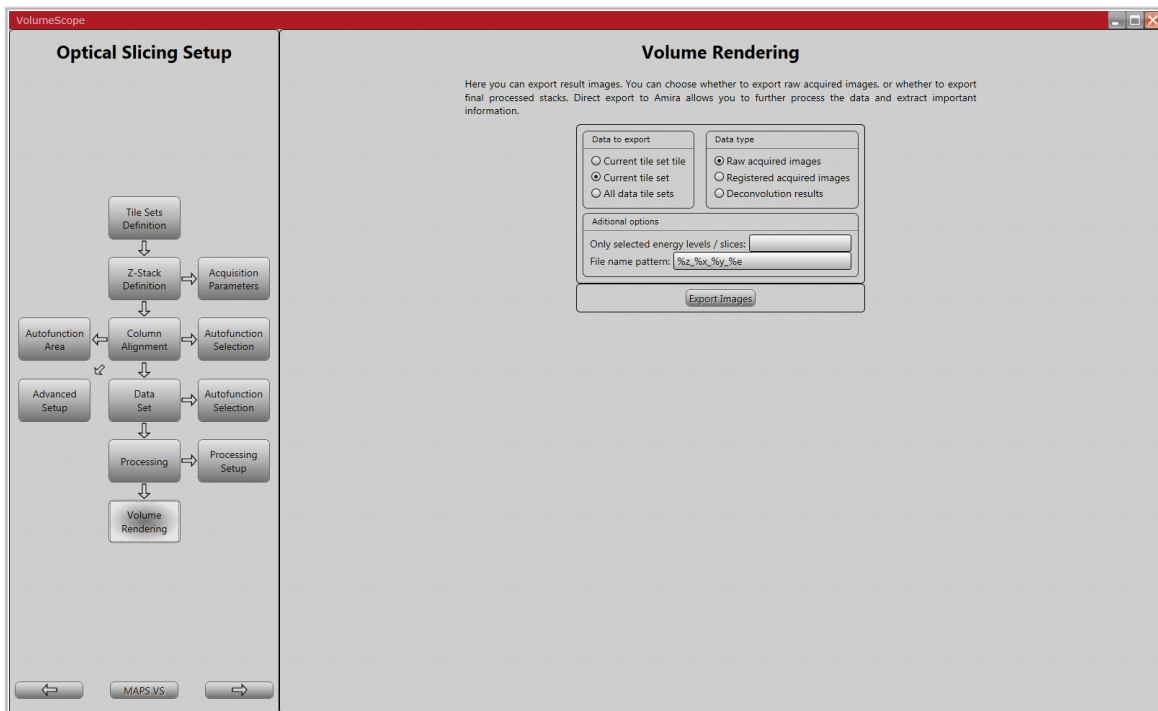
Select the **Data to export / Current tile set tile /Current tile set / All data tile sets** radio button to export the data from the actually active data tile set tile / active data tile set / all the available data tile sets.

Users can choose the **Data type** to export: **Raw acquired images** (without any processing) / **Registered acquired images** (registration only) / **Deconvolution results** (completely processed images, including deconvolution).

It may be useful to export **Only selected energy levels / slices** for all Z-layers by entering the desired image number (see above). The **File name pattern** field enables users to define names for the images to be saved (see the tooltip).

Amira application

VolumeScope data are possible to export and subsequently they can be opened within the Amira application.

There are 2 VolumeScope preset scripts within the Amira application. When the Amira script starts and any VolumeScope project is actually opened in the MAPS application, the script automatically connects to this project.

VolumeScope Live Tracker

The script is used for live visualization of data acquired by MAPS VS. Live Tracker supports only 1×1 tilesets and in case of multi-energy acquisition it always shows only 2nd energy (in most cases the reference one). To open the VolumeScope Live Tracker, click the *Project* menu / *Create Project* item. Select *Animate* and double-click the name of the script or click the *Create* button to open the module.

Figure 3-22: *Amira Live Tracker*
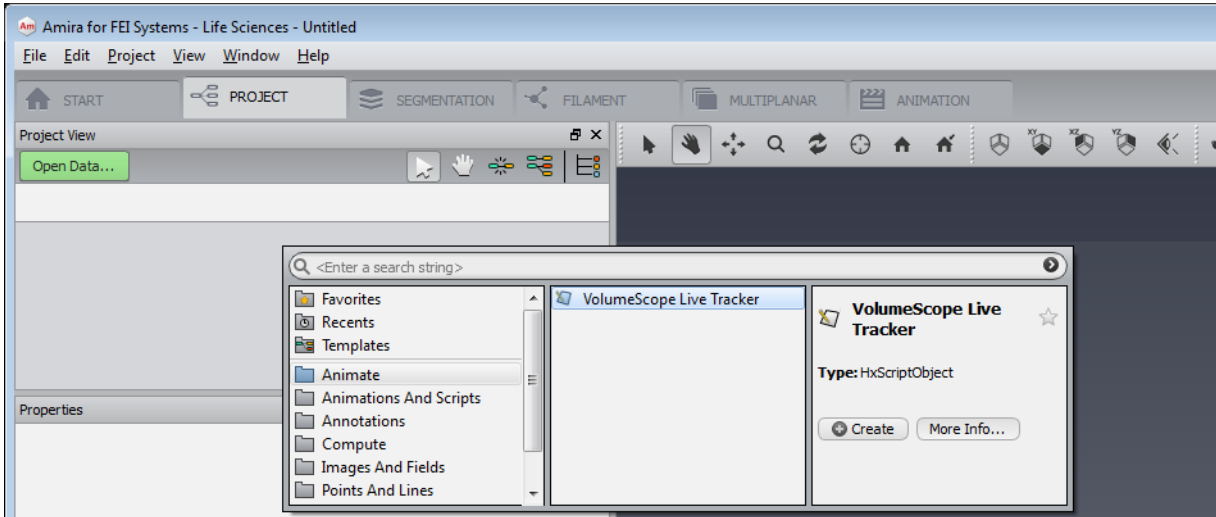
Click the *More Info* button to access the help page describing basic functionality of the module.

VolumeScope Stitcher

The script is used for stitching and basic post-processing of data acquired by MAPS VS. To open the VolumeScope Stitcher click the *Project* menu / *Create Project* item. Select *Compute* and double-click the name of the script or click the *Create* button to open the module.

Figure 3-23: *Amira Stitcher*

Click the *More Info* button to access the help page describing basic functionality of the module.
The help page is accessible also later by clicking the *?* button.

Project / tile sets definition example

- Low Vacuum or High Vacuum mode (+ Filter Ring installed)
  Slicing thickness = 50 nm / optionally run brief alignments every 150 – 200 cuts
- Physical slicing tile set (1 × 1 / 2 × 2)
  resolution = 4 096 × 3 536 / HFW = 40 μm (pixel size ~10 nm) / dwell time = 1 μs /
  accelerating voltage = 2 273 kV / beam current = 0.1 nA / number of frames = 1 / line integration = 1
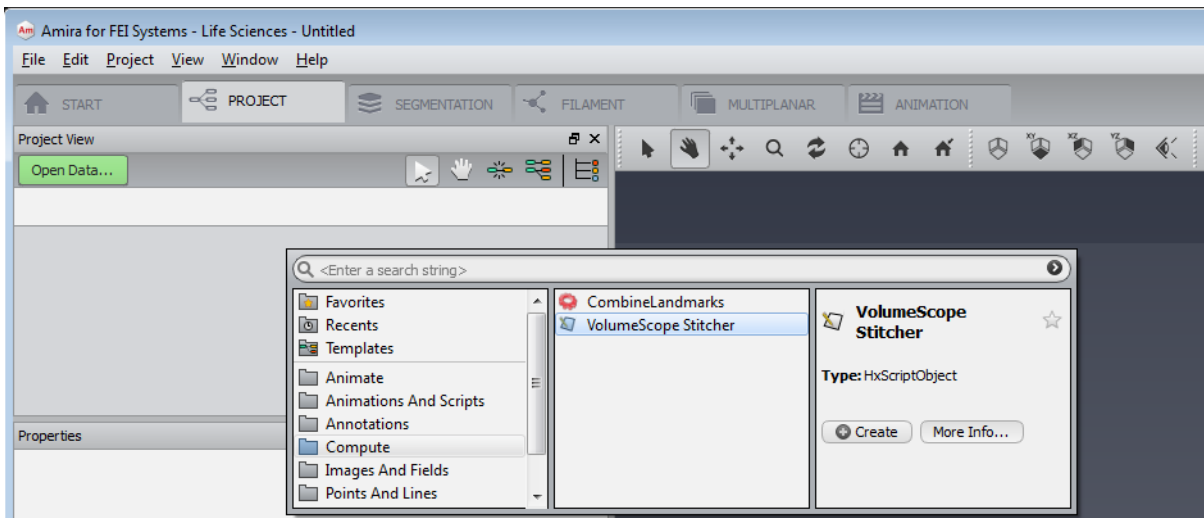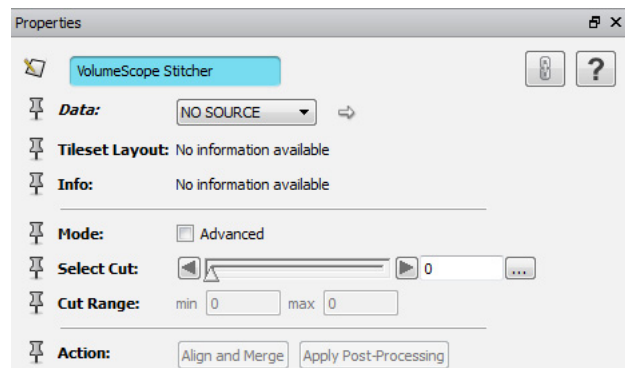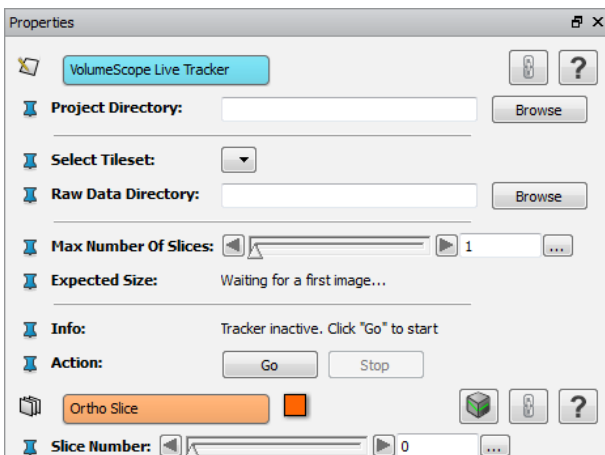- Optical slicing tile set (1 × 1 / 2 × 2)
  resolution = 2 048 × 1 768 / HFW = 20 μm (pixel size ~10 nm) / dwell time = 1 μs / 5 × 10 nm optical cuts
  (accelerating voltage = 1.18 – 3.08 kV) / beam current = 0.1 nA / number of frames = 1 / line integration = 1

Samples

- Samples should be trimmed in a rectangular shape for the best cutting results. The dimensions of the sample should be <500 μm × 500 μm (width × depth). Typically the height of the sample should not exceed 600 μm when using the FEI sample holders.
- Samples should be attached by a hard epoxy or any suitable glue to an FEI recommended sample holder. When other holders are used, FEI cannot guarantee optimal performance of the system.

Sample vs. knife positioning

- Before performing the first sample polishing, one should be aware that the sample could be slightly positioned at an angle with respect to the knife. By moving the knife back and forth over the sample when approaching it, the user can learn from the backlight reflection how the sample is orientated with respect to the knife. The quality of the backlight reflection depends on the smoothness of the block-face of the sample, the angle of the camera tool, and the position of the backlight. While approaching, it helps to play with the angle of the camera tool and the position of the backlight to find the optimal backlight reflection. The camera light should be turned off during approach, although sometimes the camera light also helps with finding the correct backlight reflection. Since the eucentric height of the sample is set to approximately 0.6 mm, the user should be aware that the cutting height should be around this value.
- A (partial) metal coat of the block face can obscure the backlight reflection.
- The sample height should be checked and eventually lowered when pumping or venting the chamber or when the instrument will be left unattended and unused for long periods of time.
- When a different sample is loaded onto the ultramicrotome, we strongly advise to create a new project in the MAPS application and adjust cutting window and next slice position according to the new sample geometry.

Sample vs. beam

- Since biological material and the resin in which it is embedded are poorly conductive, charging can occur, especially when imaging with higher energy. Higher scan speeds, different scan strategies, lower currents and different accelerating voltages can all be tried to minimize charging. In addition, the Low Vacuum mode can be used. The Low Vacuum detector should be installed according to the VolumeScope manual.
- It was determined empirically that overdosing the sample surface during data acquisition causes radiation damage. Structures like blood vessels and cell nuclei are more prone to electron beam exposition. Therefore one should try to mitigate these changes by reducing the electron dosage as much as possible. This applies also when tiling is covered within the process. Typical artefacts are then visible at the bordering areas.
  The dose D [electrons / pixel area] can be calculated as follows: $\boldsymbol{D = N_o.(N_f.I.t) / (S.e)}$, where N_f is the number of frames used for an image acquisition, I is the beam current, t is the dwell time, S is the pixel area and e is the elementary charge, N_o is the number of cuts for multiple acquisitions during optical slicing.
- A VolumeScope job can be set up according to the user's needs: thicker or thinner mechanical slices with an appropriate number of optical slices depending on the required resolution. Due to the possible damaging effects of higher beam energy on biological samples, we advise trying the best combination for a specific sample ahead of time.

Cutting

- The cutting speed during the coarse approach can be up to 1 mm/s. Slice thickness can be set to 100 nm for the coarse approach. For polishing a surface and for the actual VolumeScope job a slice thickness of < 70 nm and a cutting speed of 0.2 mm/s are recommended.
- If a VolumeScope job needs to be aborted for some reason, we recommend doing this when the knife is in its parked or retracted position.
- For safety, before starting a VolumeScope job, the **Next Slice Position** needs to be checked.

Imaging

- For a better sample imaging and handling use the Scan rotation to align sample edges rectangularly according to the monitor screen.

  In this case it is necessary to check the **MAPS** application / **Options** menu / **Prefer Scan rotation** item, and to tick the MAPS main window / *Scan rotation* area (bottom of the screen) / *Link* check box to propagate the settings, otherwise the rotation positioning readouts are not correct.
- Edges of acquired images can be out of focus when a large field of view is used (HFV > ~150 µm) together with high tile set resolution ($\geq$ 8192 × 8192). The reduced area within such a tile set or compound tile sets (M × N) can be used to prevent blurry edges and/or corners.

Other

- Before each cutting job, we advise carefully removing any debris from a sample and its surroundings for safety (according to the nature of the sample) and to prevent the adherence of debris by using of the Vacuum cleaner (see Chapter 2).
- When debris is still attached to the diamond knife after cleaning with the vacuum cleaner, or when there is a visible decrease in cutting quality, we recommend cleaning the knife using the eyelash or polystyrol rod moistened with ethanol, as recommended by the diamond knife producer (Diatome).

  Caution!
  The user must avoid touching the knife-edge and the sample block-face surface with the vacuum cleaner adaptor!
- It is good idea to execute the Fine approach vacuum procedure when:

  – A job is stopped overnight or over a weekend.

  – The chamber was vented / pumped during or after a job (because of switching vacuum mode, detector exchange, sample and/or knife cleaning from debris, etc.). Stage Z-axis direction drift during these operations is about –300 nm (pumping) / +300 nm (venting), depending on chamber door and stage movements and related manipulation.

  – The sample position drifts down in the Z-axis also during the vacuum level stabilization about tens of nm / hour. We advise to wait about 2 hours for the system to stabilize.
- Make sure the sample is at the cutting position before starting the autofunctions.

Teneo VS safety and maintenance

Before starting any work on the Teneo VS system please make sure you read this document, and the *FEI microscope systems safety manual*. Please have all of the recommended items available. Control measures are applicable for anyone exposed to the Teneo VS parts during normal operation (the computer area may also be contaminated), service, removal and refitting.

Caution! (For all people facing a contaminated instrument, including operators, instrument supervisors, FEI service engineers and third party service individuals.)
As a precaution never put yourself at unnecessary risk. Please follow the control measures outlined in this document. Should you feel at all uncomfortable about any point in this process, including questions regarding samples/practices from a particular site, please consult FEI or the local safety officer for more information and advice. FEI cannot control exactly what the operator puts into the machine; if you are working with an instrument and you feel there is a risk, please ask the local safety officer to verify it is safe to start work.

Scope

The Teneo VS system is used to remove very thin layers of a piece of material no bigger than 1 mm^3. The system consists of three main parts: an electron microscope, in-chamber microtome (referenced as the slicer) and dedicated control software. The size of the sample itself mitigates much of the risk posed by a sample. The layers (slice thickness) however are on the order of 10–100 nm and there could be about as many as 10 000 slices (debris) or more within the chamber at the moment of venting after a single run. At this moment it is possible to mobilise these slices in a locally confined area and therefore it should be considered as a fine particle hazard. The material typically used for good electron microscopy contrast is comprised of sometimes toxic resin which is very hard and often brittle; plus a biological or material specimen which may have intrinsic risks of its own (virus, prion, drugs in the case of biological specimens, or toxicity in the case of inorganic or mineral specimens). Often heavy metals or radioactive metals are added as contrast agents or fixation agents for better images.

Limits and risks

The total sample size consists of a maximum 1 mm^3 piece of resin containing a biological tissue (or materials specimen), which might have been stained with stains such as Uranyl Acetate (depleted), Lead and Osmium (all in very low amounts). Although these materials confer a very low risk of transmission (if biological) or toxicity (if material), it is useful to consider all such samples as hazardous when handling them, as the treatment process may not be complete throughout the whole sample.

The chemical treatment of the materials is in fact rendering them inert or immobilized in most cases, the material may contain intrinsic risk(s) such as:

- *Toxicity*
  Both the resin and the heavy metal stains typically used are considered toxic when inhaled, ingested or by transcutaneous routes. The total volume of the material is very small, however the Teneo VS is designed to remove nanometer layers of this material. The results of this removal are numerous thin slices of the material, which are prone to flying around, when exposed to air flow or charge.

Caution!
The Teneo VS workflow should be treated as any other hazardous dust producing workflow. The main hazard with these materials is the sections themselves especially if inhaled. Use of personal protective equipment and/ or HEPA filtered or extracted flow hood should control the risks adequately.

- *Biological* (prion, virus or bacteria)
  The processes of fixation and putting the sample into resin are modes of decontamination, so the biological risk is negligible from a typical sample preparation procedure. Usage of Teneo VS system and control measures are intended for samples from biological risk groups 1 and 2 before any chemical treatment (fixation, embedding, etc.).

Caution!
Please ensure that the samples used within the system are not from biological risk groups 3 or 4 (before any chemical treatment) as they are not covered by this document. If you feel your samples are of higher biological risk groups then 2, please contact your local FEI representative before working with these samples within the Teneo VS.

- *Radioactivity*
  The Uranyl compound stained biological material contains slightly elevated levels of radioactive materials. The radiation level does not exceed international safety levels when single samples are used. Total dose (assuming worst case conditions) is maximum 0,05 mSv per year, which is 5 % of the annual exposure limit. The radiation risk is very low and is acceptable when working with Uranyl compound stained samples.

For more information about toxicity, biological and radioactivity risks/handling refer to local legislation.

Control measures

For further reduction of residual risks posed from the sample, the following control methods are required to protect you from that residual risk. Remember this material contains potentially toxic, biohazardous and radioactive risks, the measures are designed to control all of them.

For customers and microscope operators

- Clean the instrument at the end of each use to minimize the risk to others (see *Regular cleaning*), but also to ensure optimum function of the instrument.
- Follow the *Deep cleaning* procedure when: switching from work with VolumeScope extension to normal SEM operation without VolumeScope extension, before any servicing and applications specialist(s) visit, etc. (see *Deep cleaning*).
- The person responsible for the microscope will be required to meet the FEI engineer / employee before starting any work. Completion of the *VolumeScope pre-service checklist* will be required which includes declaring, that the instrument/laboratory is safe for work. This will require an authorized signature. The laboratory supervisor must explain any relevant rules to the FEI employee and train him, or supervise him during his work all the time, when working in BioSafety Level (BSL) 2 laboratory.
- Ensure you have completed the *Quality Contamination Return* (QCR) form prior shipping of any component for maintenance. Contact service for an electronic copy if you do not have one. Ensure that the FEI engineers are informed of any relevant laboratory safety practices which are in place and any essential information regarding any samples which have been used, which pose a risk to the engineer. This should apply to those, which are in use, both in the microscope and in the microscope laboratory.

For FEI service engineers, applications specialists and third-party service individuals

- First make sure the VolumeScope pre-service checklist is completed. If you have any remaining safety questions please ensure you ask the customer before starting any work. If a part needs to be shipped to FEI or to a third party supplier, please fill in the QCR form and check that proof of decontamination is provided by the customer.

Note
The radioactive levels using the Uranyl compound stained samples are so low, that on the QCR no check needs to be placed that the part has been in contact with radioactive materials.

For all

Caution!
It is required, that you use Personal Protection Equipment (PPE – see below) during any cleaning of the system. Eye protection is optional but recommended; the dust from the resin can be an irritant, and the cleaning of the instrument involves solvents.

- Do not use compressed air, it tends to displace material randomly about the chamber and laboratory, as this serves to fragment and disperse the fragile slices of the resin.
  Compressed air can be used in the HEPA filtered flow hood. If this is not available, ensure you wear full PPE.
- Make sure there is a clear bench available for working/cleaning. This includes assembling clean parts, mounting samples, storing of transportation boxes.
  On no account must the computer desk be used for this purpose, otherwise it will become contaminated with the slices.
- All replaced parts should be cleaned before disposal/return, unless they are being sent for hazardous waste disposal through the customer/FEI site.
- Any dust can be wiped up with a wetted cloth (70 % ethanol in distilled water is fine for this purpose).
- All waste should be placed in a sealed bag to prevent dispersal of the dust.
- Do not consume and/or store food or drink in the vicinity of the microscope / cleaning area.

FEI

Caution!
All residues are considered as hazardous material and must be disposed of according to local environmental and safety requirements. Consult your local environmental agencies for decontamination and cleaning procedures (customer's responsibility when at a customers site).

Items required

- Personal Protection Equipments (PPE)
  The PPE consists of the laboratory coats, gloves, and goggles (standard BSL 2 PPE) and particle filter mask with at least level 2 protection. During any cleaning of the system anyone has to use PPE.
- Standard laboratory cleaning materials such as towels, standard vacuum cleaner, surface cleaner etc.
- Laboratory wipes (lint free cloth)
- 70 % ethanol in water in dispensing bottle (200 ml is sufficient)
- Distilled water for wiping external surfaces
- VolumeScope Vacuum cleaner with 300 nm fine particle filter (recommended type: 3M Model 497ABF)
- Separate cleaning bench at least 1 m length

Recommendation

- HEPA filtered flow hood (with negative pressure) or extraction hood for initial cleaning of the unit once removed from the microscope. Or the full PPE must be worn.

Vacuum cleaner

This equipment is used for removing debris from the contaminated surfaces of the chamber, stage, microtome with a sample, diamond knife, console, etc. Another application is to remove debris from inside the final lens pole. Each of these applications uses a different hose adaptor.

Figure 0-1:   *Vacuum cleaner set / Sample adaptor (1) and Final lens pole adaptor (2)*

Caution!
The user must avoid touching the knife-edge and the block-face surface with the Sample adaptor!

Assemble the vacuum cleaner: attach the end of the hose labeled as *To Vacuum Cleaner* to the vacuum cleaner, the other end of the hose labeled *To Accessories* attach to an appropriate adaptor and connect the power cord to a wall socket. Follow the producer's (3M) guide for the operation of the vacuum cleaner.

After cleaning the hose should be left attached to the hoover not to expose the inner part of the filter to the environment; close the end of the hose by the red plug. If the hoover hose will be dismounted, use the red plug to close the filter opening; this ensures no loose material can escape.

When you have finished the cleaning procedure, store the adaptors in a dedicated bag and keep it separately.

Do not use this cleaner for any other purpose, the capacity of the hoover is designed for the volume of material produced within the Teneo VS, using it for other cleaning applications will reduce the interval for filter replacement.

Vacuum cleaner filter replacement

For exchange use the 300 nm fine particle HEPA filter (3M filter, type 1, SV-MPF1).

Caution!
The filter must be exchanged by the FEI service engineer during an annual preventive maintenance, or after 300 cutting jobs, the parts can be ordered through FEI service. The used filter has to be packed into a sealed bag and disposed according to the local legislation rules (it is the customer's responsibility to dispose of the filter as it contains many slices).

Regular cleaning

The instrument must be cleaned down after every job when the chamber is vented. Follow the regular cleaning procedure.

Caution!
Make sure any wet waste is placed into a sealed waste container (sealable plastic bag), to prevent dust liberation on drying.
*All residues are considered as hazardous material and must be disposed of according to local environmental and safety requirements. Consult your local environmental agencies for decontamination and cleaning procedures (customer's responsibility when at a customers site).*

Caution!
The microtome and detectors are delicate instruments, care must be taken during cleaning.

1. Ensure the room is reasonably cleaned first, if it appears to be contaminated with sample material.

   This can be done with a standard HEPA vacuum cleaner (not the VolumeScope vacuum cleaner) to remove dust and debris; followed by a wipe with a wet cloth (water or 70 % ethanol in distilled water is fine) to collect any remaining debris.

2. Wipe the microscope console desk, change the gloves.
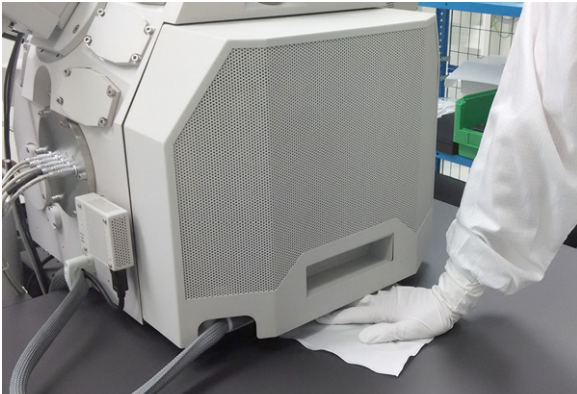
3. Wipe and clean the computer console desk taking care not to cross contaminate other work areas. Once the environment is clean, work on the microscope interior can start.

   This is to prevent contamination of the operator desk and to be able to work with reduced PPE according to local laboratory rules.

4. Click the *Vacuum* module / *Vent* button to vent the chamber. Wait until the vacuum icon turns gray, then wait another 2 minutes to allow for settling of the dust and slowly open the chamber doors to prevent stirring up the dust.

5. Use the vacuum cleaner (loose material), followed by laboratory wipes (lint free cloth and little 70 % ethanol in distilled water for persistent material) to remove any noticeable debris from the surface area of inner components.

   The majority of material will be concentrated around the slicer, however debris can collect on any surface, particularly ledges and recesses. The following images illustrate places where debris can collect.

FEI

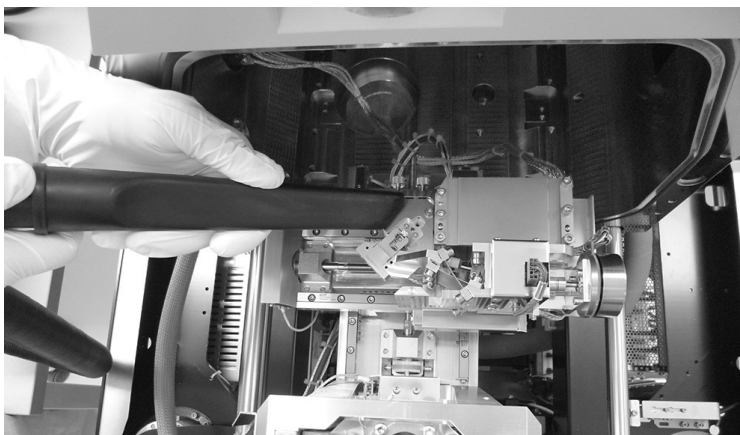**Figure O-2:** *Debris examples at: stage / sample / inner part of the chamber doors*
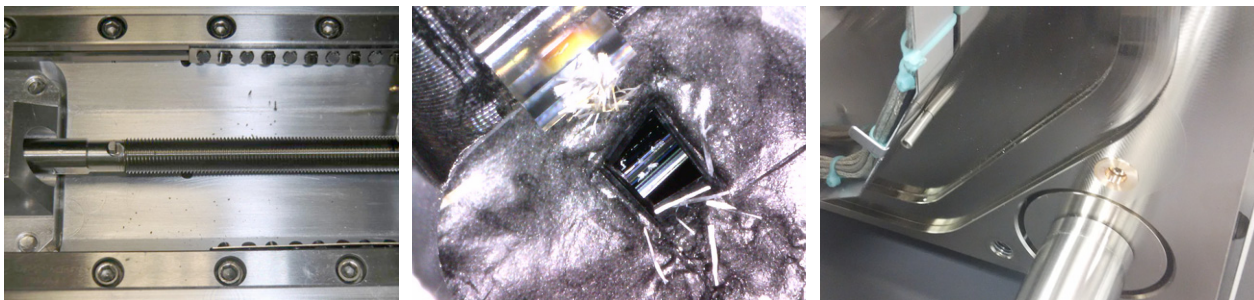
Areas to clean

Caution!
Hazardous voltages are present inside the microtome. Don't remove any cover of the microtome.

- Microtome

Clean around the knife holder and adjacent areas.

Caution!
The knife is very sharp; it can easily cut through gloves and skin. Take care when manipulating with it. Always make sure it is cleaned and placed into the storage boxes to prevent damage and injury.
The user must avoid touching the knife-edge and the block-face surface with the Sample adaptor! Although reusable, this should be treated as any other laboratory sharp for disposal, the item can be refurbished subject to adequate decontamination.
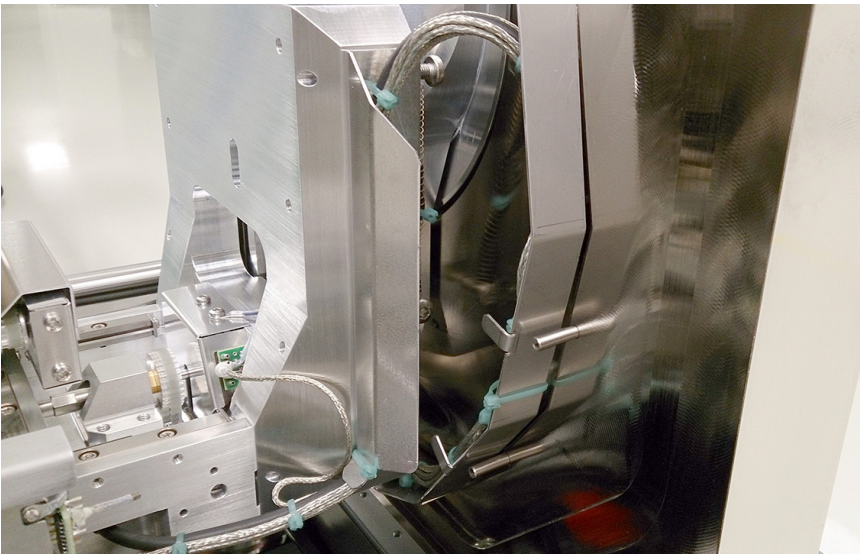
- Microtome cables

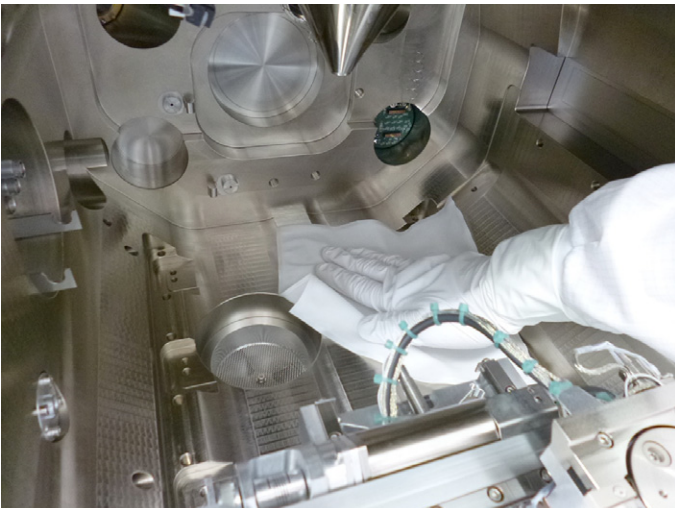The rough cable surface can hold a lot of debris, so clean it thoroughly.

- Stage

Clean accessible area around the stage with the slicer mounted (X- / Y-axis stage coordinates: 5 / 50 mm; the stage movements are limited in this case).
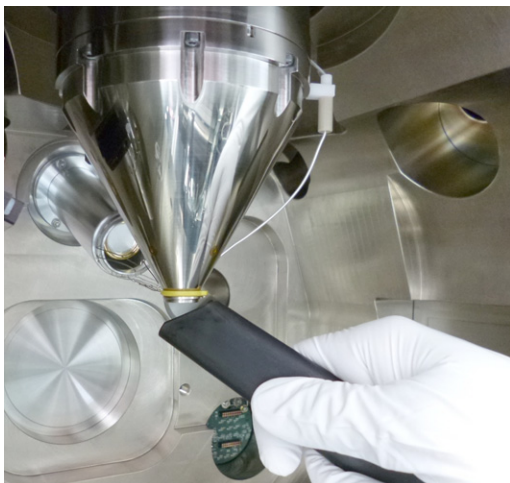
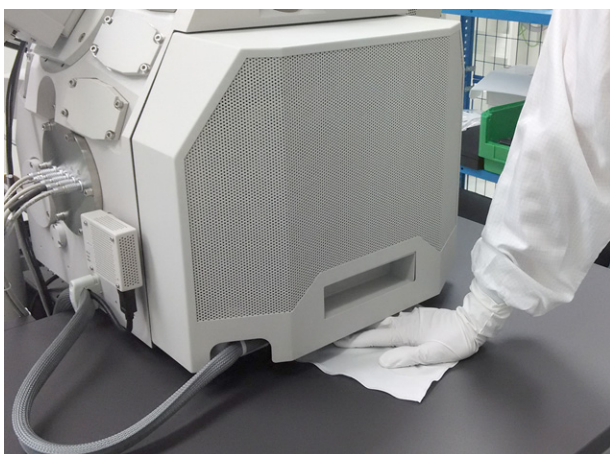- Inner part of the chamber doors
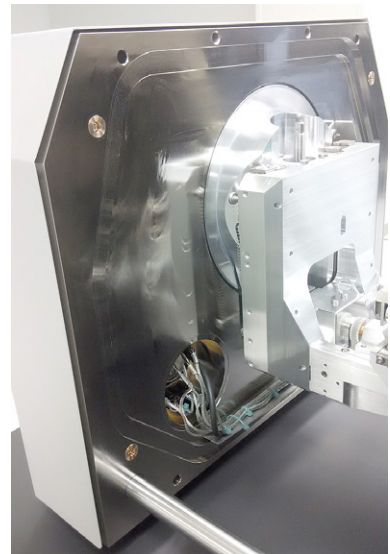
- Inner surface of the chamber

- Ring filter

- Do not clean VS DBS when installed! See below for the procedure.
- Microscope console desk around the chamber doors

- At the end of a run remember to clean the vacuum cleaner adaptor(s), which can collect material externally. It is good to wipe the adapter, hose and body of the cleaner with the wetted cloth if contamination is noticed.

Final lens cleaning

The final lens should be vacuum cleaned when imaging quality is not satisfactory. An irregular de-focus or astigmatism or contrast / brightness changes/fluctuations possibly indicate the presence of debris inside the final lens pole. To reduce these problems, follow the below procedure. This should not be used as part of the regular cleaning, but only when deterioration in performance has been identified and all other measures investigated.

6. Remove any accessory attached to the final lens pole (VS DBS, PLA, X-ray cone or Ring filter).

7. Switch the vacuum cleaner ON.

8. Attach and hold the Final lens adaptor on the pole piece and periodically cover the hole in the adaptor with a finger to produce better suction. This will improve the conditions for cleaning debris.
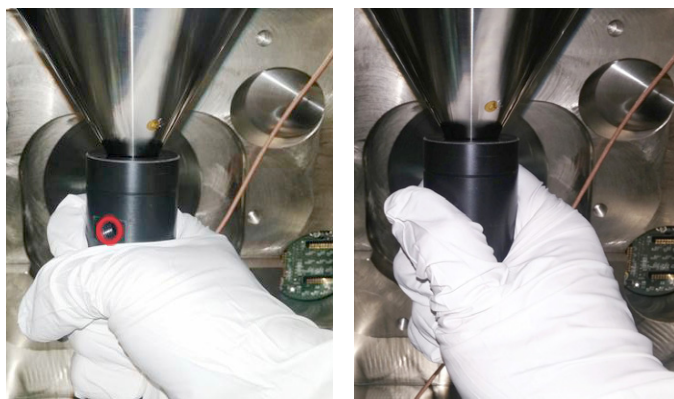
Deep cleaning

This should be performed after *Regular cleaning* when:
- Use case change (normal SEM operation without VolumeScope extension),
- Before any servicing or applications specialist(s) visit,
- Before shipping any part,
- If any significant deterioration in performance is noticed, which regular cleaning does not solve,
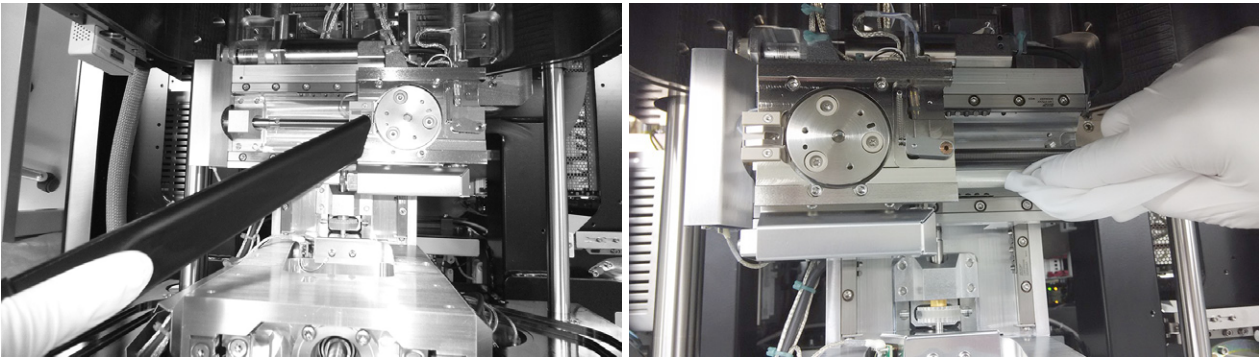- If excessive debris build up is suspected.

Remove the microtome out from the chamber, place it on the cleaning bench (see *Ultramicrotome installation*).
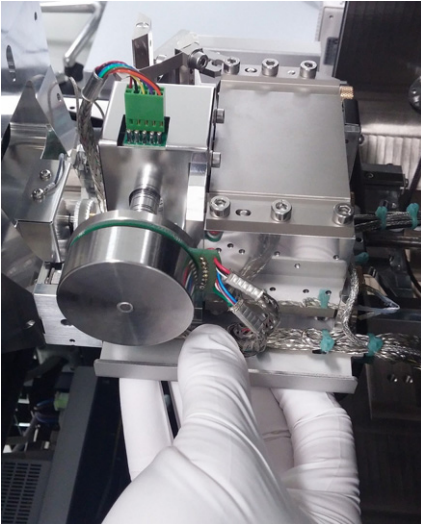
Do not use the computer or microscope console desk in order to prevent contamination of these areas!

Areas to clean
- Additional stage cleaning

Change the *Sample exchange* window / *Holder* from *Microtome* to *Other Accessory* within the microscope UI to allow full stage movements. Move the stage within the movement limits (X- / Y-axis: from -52 to +52) to reveal areas previously hidden below the slicer, and to remove the residues here. Do not install any accessories and pump down the chamber.
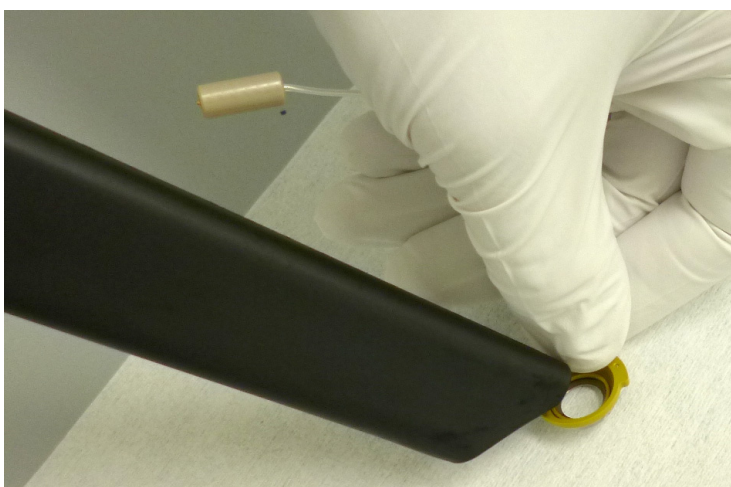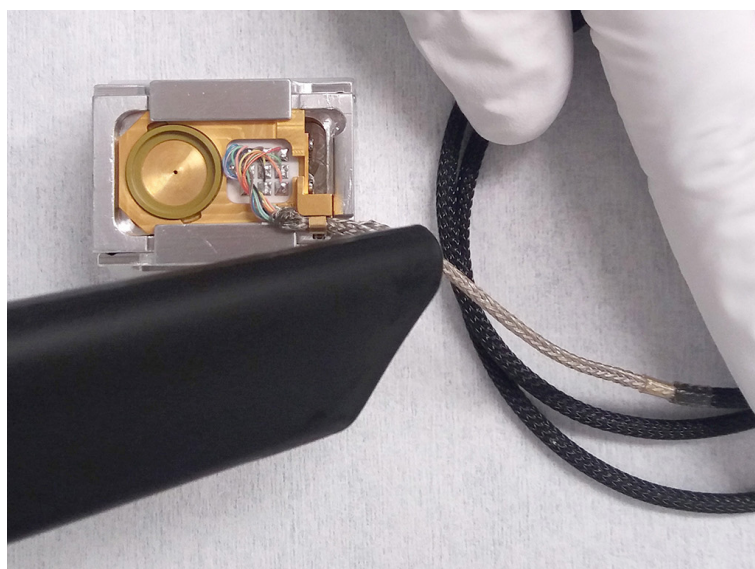
- Microtome itself
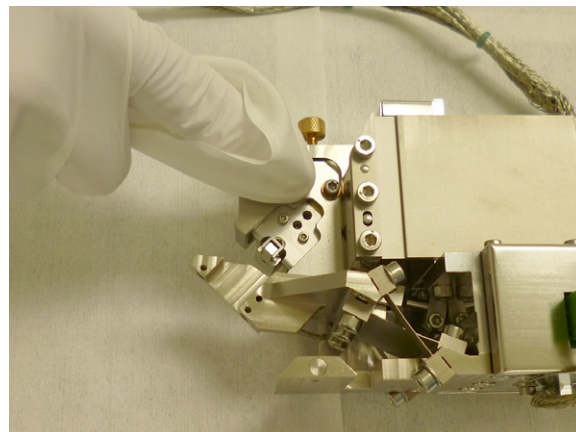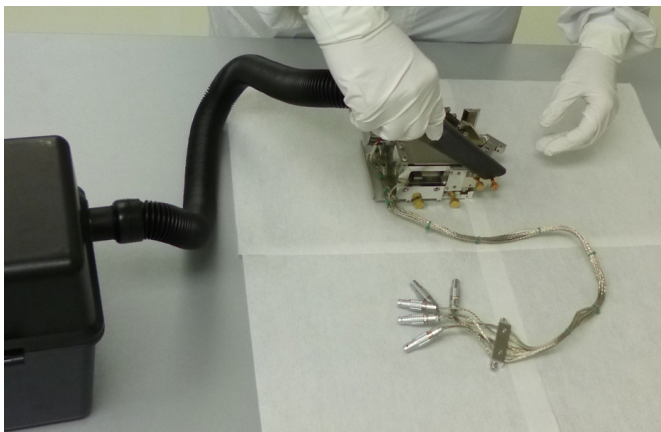
Remove the knife and the sample, store them into a dedicated boxes. Hoover the top surface of the slicer and its cables. Turn the slicer upside down also to remove any debris.

- Ring filter (removed from the chamber)

- VS DBS detector (clean only when removed from the chamber and placed in the removal/mounting tool!)

Any additional cleaning of the active surface should not be attempted! If you notice any image quality deterioration, call FEI service.

⚠️ *Caution!*
Take care not to touch the detection surface by the Sample adaptor of the hoover, as it is very sensitive!
Solvent or mechanical cleaning can severely damage the detector.

Shipping components for maintenance

Do not return any Teneo VS equipment without original packing. In the case it is not available, contact FEI service.

- Follow the *Regular cleaning* procedure and relevant parts of the *Deep cleaning* procedure according to a component intended for shipment.
  If the room is excessively dirty and you do not feel comfortable or are unsure how to proceed, contact the local supervisor/safety officer. If this is unsuccessful do not start work, take photographs and contact FEI EHS for advice.

- Ensure, that you have a cleaning bench for placing the removed parts. If there is access to a HEPA filtered flow hood please arrange it. Wear the PPE.
  Once the environment is clean, work on the microscope interior can start.

- Dismount the parts intended for shipping.
  Once the unit has been stripped and cleaned using the alcohol wipes, it should be safe to handle it without mask (as the dust inhalation risk is removed). The parts should always be handled with gloves as they may contain trace levels of contamination which are persistently absorbed on to the machine parts.

- When shipping the stage or components for repair, please ensure that the unit is as clean as possible. Equipment can be shipped as non-hazardous however components should be individually sealed in bags, and then placed in the transport box. Check local shipping regulations as the material may contain slightly elevated levels (but not exceeding safety levels) of toxic or radioactive materials. Samples and knives should not be shipped with the units. Ensure a copy of the QCR form accompanies the returned parts.

- If shipping the knives, it is recommended that they are labelled and handled as a potential biohazard. Please be sure to follow local safety rules when shipping the used knives and treat them as "used sharps".
  Of all the components of the Teneo VS the knife carries the highest risk. It is used for every slice on multiple samples, as such if there is a pocket of viable/toxic material the knife will be sure to pass through it, potentially picking up material.

Caution! (for Instrument Supervisors, FEI Engineers and Third Party Service Individuals)
When the stage is removed, it is highly likely there will be some trapped material, which the general user cannot clean whilst the instrument is installed. When removing covers and disassembling the units please take care to do so slowly on a designated work area, cleaning the deposits as they are discovered. It is a good idea not to invert the unit until it is over the designated work area to prevent the uncontrolled release of trapped material.