SBEMimage

Getting started

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This is a brief guide for getting started with *SBEMimage*. A more detailed manual is in preparation. Questions? Contact benjamin.titze@fmi.ch

Requirements

These are the requirements to use version 2.0 of *SBEMimage*. Future releases may support SEMs and microtomes from other manufacturers.

- ZEISS SEM with *SmartSEM* software (version 5.4 or above – older versions may work, but have not been tested.)
- Gatan 3View system with *DigitalMicrograph* software (GMS 2 or 3; GMS 2 is fully tested, GMS 3 is experimental)
- Remote control capability for *SmartSEM*: `CZEMApi.ocx` must be registered on support PC. Ask ZEISS for further information.
- Adapter to feed BSE detector signal to the ZEISS microscope. The adapter can be built with the following parts: FCT Electronic FM-11W1P-K120, FCT Electronic FMX-008P102, and standard BNC cable, see image on the right.

- Find the amplified output signal of the Gatan BSE detector (or any other detector – does not matter as long as you can get the signal onto a BNC cable), then connect that output signal BNC cable to the BNC end of the adapter shown above. Then find the input boards at the back of the microscope where the signals from the different detectors are fed in. Choose one that you don't regularly use. We always use the EsB detector input. Connect the other end of the adapter to that circuit board. The image you will now obtain in the *SmartSEM* software when the EsB detector is selected is the signal from your BSE detector.

Installation

1) Install Python on the support PC (the PC on which *DigitalMicrograph* is running).

We recommend the Anaconda Python distribution:
https://www.anaconda.com/download/#windows

Important: Download Python version **3.6** (not 2.7)!

2) PyQt 5 is already included in the Anaconda distribution. Several additional Python packages are required. Install them by typing the following commands in the console window after the Anaconda Python distribution has been installed:

- `pip install win32com`
- `pip install pythoncom`
- `pip install validators`
- `pip install validate_email`

If you don't use Anaconda, there may be other packages that you need to install. If you receive an error message that package XYZ is missing, you can usually install it the same way as shown above: `pip install XYZ`

3) Copy all files from the GitHub repository **including the folder structure** (subdirectories 'src', 'img', 'gui'…) into the folder `C:\pytools\SBEMimage`

You can choose a folder other than `C:\pytools` but please ensure the program has full read/write access. In the DM communication script, you have to update the variable `install_path` if you use a directory other than `c:\pytools`.

The path to SBEMimage.py should be: `\SBEMimage\src\SBEMimage.py`

Starting the software

Run the script `SBEMimage_DMcom_GMS2.s` (for GMS 2) or `SBEMimage_DMcom_GMS3.s` (for GMS 3) in *DigitalMicrograph*. It can be found in `C:\pytools\SBEMimage\dm`.

Open this file in DM and click on "Execute". Further information is provided in the script file.

Now run the main application by starting the batch file `SBEMimage.bat` or typing `python SBEMimage.py` in the console (current directory must be `C:\pytools\SBEMimage\src`).

Load `default.ini` to get started. This configuration will be in simulation mode. Simulation mode should always work because the APIs for the SEM and 3View are disabled. This mode can be used to set up acquisitions, calculate estimates, or look at existing data. If you can run *SBEMimage* in simulation mode, your Python environment works and the installation of *SBEMimage* was successful.

If you wish to switch to the normal application mode, do the following:

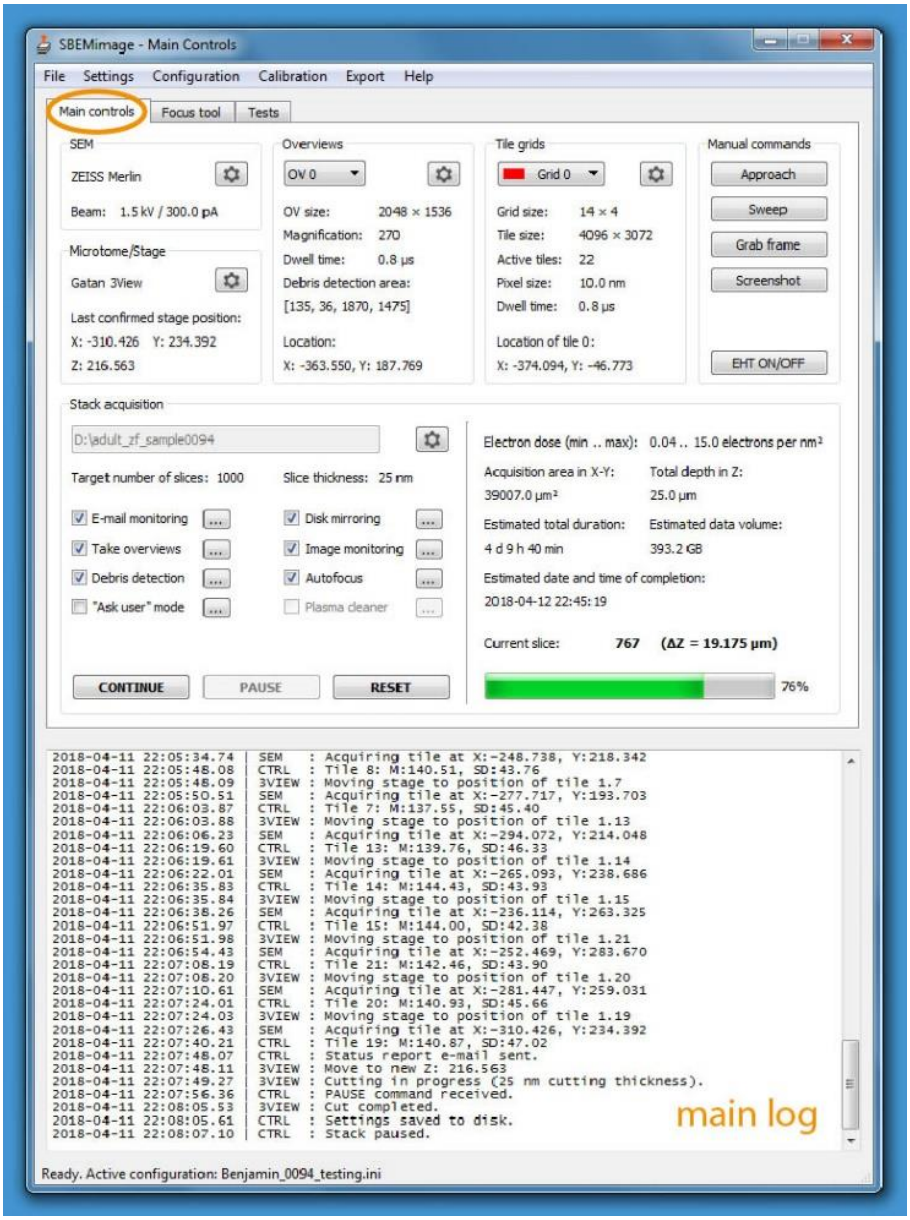
Open `default.ini` and under the section `[sys]` change `simulation_mode = True` to `simulation_mode = False`

If the communication script is running in *DigitalMicrograph* and the *SmartSEM* remote API is active and set up correctly, the software should now be fully operational. If the APIs cannot be initialized, you will receive an error message when starting up the application.

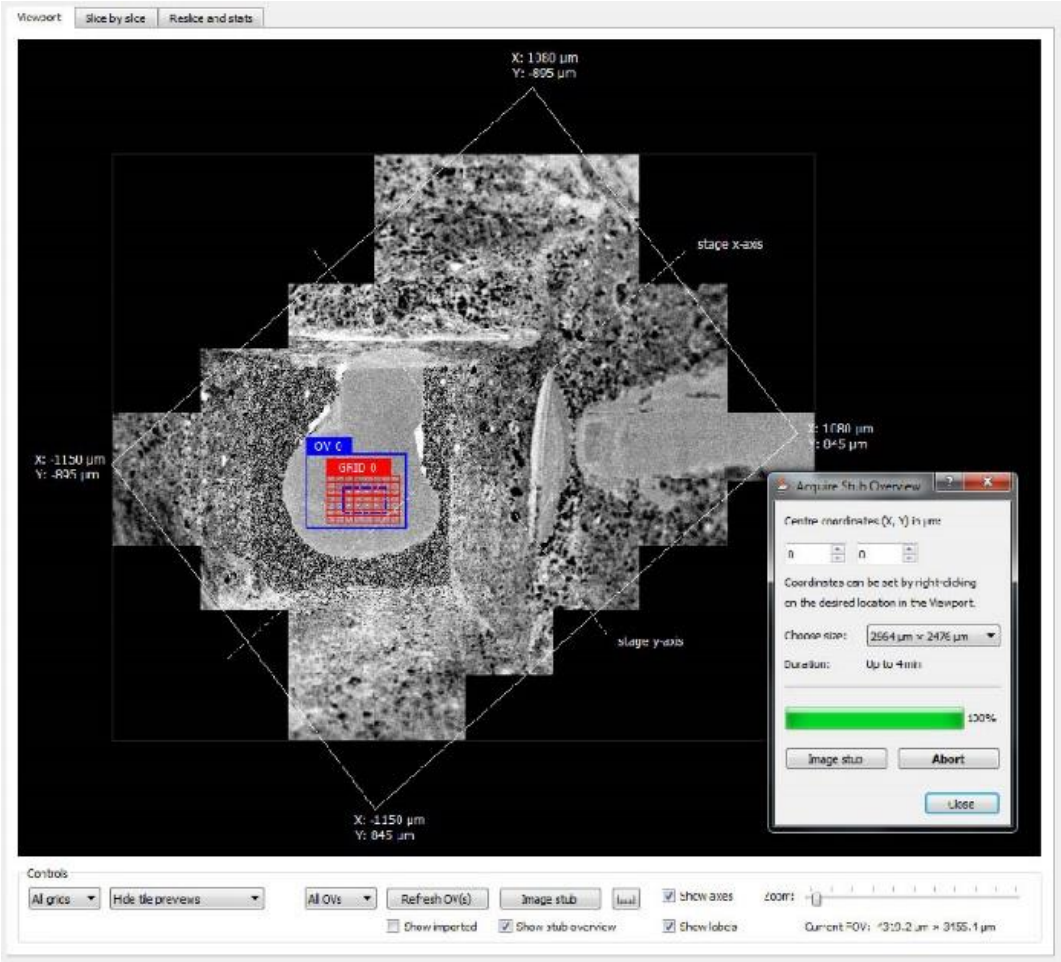
User interface: Main Controls and Viewport

The graphical user interface consists of two windows that fit next to each other on a wide screen (1920 × 1080 is recommended). It was designed with remote desktop software such as *TeamViewer* and *VNC* in mind: All functions are accessible on a single screen.

The window **'Main Controls'** on the right displays at a glance all relevant settings, the acquisition status, the current electron dose, and real-time estimates for the duration of the acquisition and the total data size. Here you can set up all acquisition parameters. Click on the buttons with a cogwheel icon to open dialog windows for changing settings (available for the panels "SEM", "Microtome/Stage", "Overviews", "Tile grids" and "Stack acquisition". Click on the tool option buttons ("…") to change the settings for the different features (for example, debris detection) that can be activated during stack acquisitions. Two additional tabs contain a focus tool and various functions for testing and debugging.



The other, larger window (positioned on the left by default) is the **Viewport**. The workspace shown in the Viewport's main tab covers the entire accessible range of the stage motors. When sufficiently zoomed out, the stage boundaries are shown as solid white lines, and the x and y stage axes as dashed white lines. To obtain an overview of the entire surface of the sample holder ('stub') mounted on the 3View stage, click on the button "Image stub". A large low-resolution (372 nm pixel size) mosaic will be acquired and placed in the workspace as a background image (see screenshot below).

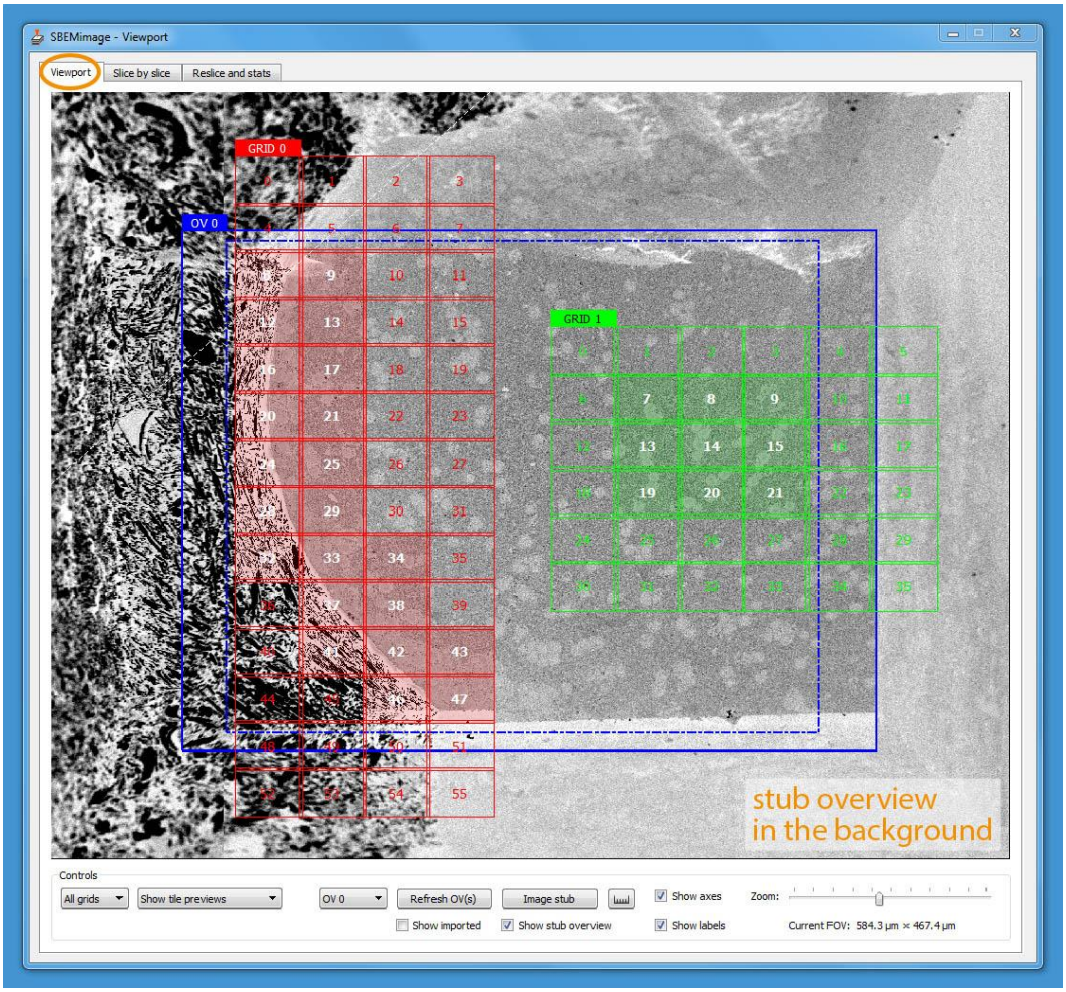


You can then use the stub overview image to locate the region of interest. In the region of interest, you can acquire a smaller overview image at higher resolution (typically 100-200 nm pixel size). Press the CTRL key and click on the blue rectangle "OV 0" and drag it to the position where you wish to acquire the overview image.

To acquire image tiles at the target resolution for analysis (typically 5-20 nm pixel size), you can set up a tile grid in the region of interest. Grid size, tile size, overlaps/gaps between tiles, and acquisition parameters (frame size, pixel size, and dwell time) are specified for each grid. The default tile grid is "Grid 0". By pressing the ALT key and clicking on a grid, you can drag it to a new position.

Tiles can be individually selected or deselected for imaging (press SHIFT and click on a tile). For complex acquisition tasks, multiple overviews can be set up to cover the region(s) of interest, and multiple grids can be created with different imaging parameters. You can choose for each overview

image and for each grid whether it should be acquired on every slice, or in intervals. This permits, for example, to image an area with alternating pixel sizes, or to acquire an overview stack at low resolution with a high-resolution mosaic on every tenth slice. The screenshot below shows an overview image (“OV 0”) and two tile grids (“GRID 0” and “GRID 1”). The highlighted tiles have been selected for imaging. A low-resolution stub overview mosaic is displayed in the background.

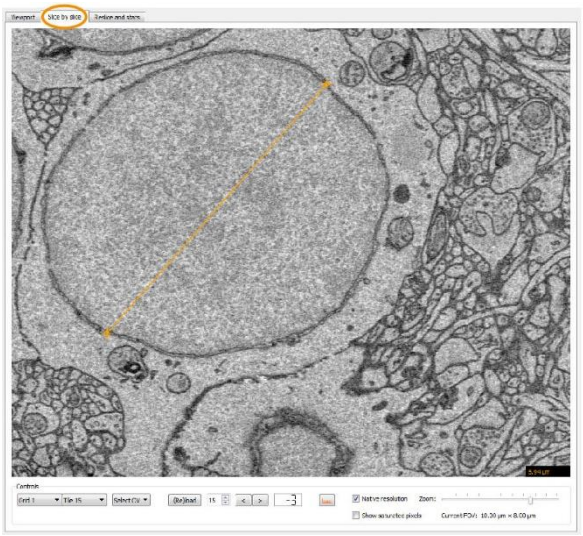


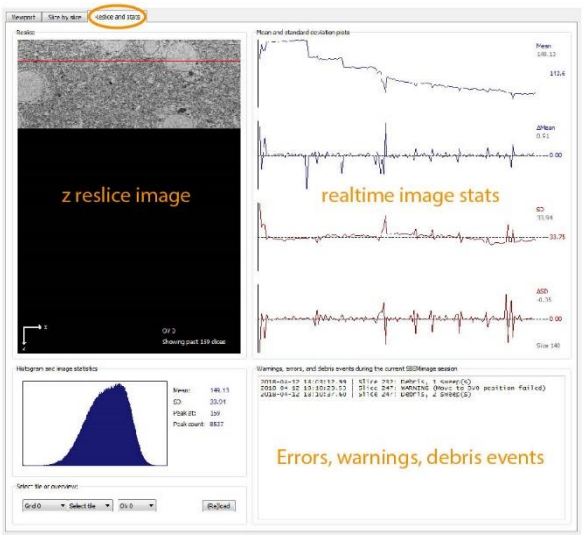
The basic elements described above are displayed in different layers inside the viewport. The background layer consists of the stub overview image, which provides the main reference frame for an acquisition. The next layer contains the overview images that cover the regions of interest. They are primarily used for debris detection and to position the tile grids. The tile grids are usually located above the overview images, but they can also be placed on any other part of the workspace within the accessible motor range. Finally, additional imported images are shown in the foreground. You can choose whether to show or hide elements by using the controls at the bottom of the window.

The visual scene can be panned by left-click dragging, and zoomed in and out with the mouse wheel or the zoom slider in the bottom-right corner. The viewport is fully functional even while an acquisition is running.

Select the second and third tab to use the **slice-by-slice viewer** and to show **reslices and statistics** (see screenshots below). In each tab, use the grid/tile selector on the bottom to choose the data source, then click on “(Re)load”. In the slice-by-slice viewer, click on the ruler icon to measure distances. When the button is activated (orange colour), mark the starting point for the measurement by clicking with the right mouse button. Mark the end point with a second right click. The distance is displayed in the bottom right corner. To deactivate the measurement function, click on the ruler icon again (colour changes back to black). The measurement tool works the same way in the viewport.

In the “reslice and stats” tab, you can select a slice by left-clicking on the area where the plots are shown. The selected slice is marked with a vertical line in the plot area and a red line in the reslice. The histogram and the mean/SD values are shown for the selected slice.





Mouse and key commands

Left click:	Drag to pan field of view
Double click:	Zoom in
Right click:	Context menu (Tile selection, image import…)
Left click + Shift:	Select or deselect tiles
Left click + Alt:	Drag to shift grid
Left click + Ctrl:	Drag to shift overview image
Left click + Alt + Ctrl:	Drag to shift imported image
Mouse wheel:	Zoom in and out (in mosaic viewer); Forward and backward through image series (in slice viewer)
Measuring tool:	Activate by clicking on measure button (ruler icon), then right-click on two different points between which you wish to measure the distance.