

SerialFIB Tutorial

Preface

A tutorial for step by step explanations of setting up a SerialFIB session. All functionalities are going to be displayed, including their designer modules. If you are running into problems, please contact klumpe@biochem.mpg.de

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Starting up SerialFIB and setting up a session

Cool down system, load sample, potentially already identify positions of interest and set coincident point for those position.

Then: Open SerialFIB, either by opening the shortcut that has been created for you on the desktop (see image below) or by moving to the SerialFIB folder and typing:

`python SerialFIB.py`



The graphical user interface is going to open up, looking somewhat like Figure 1:

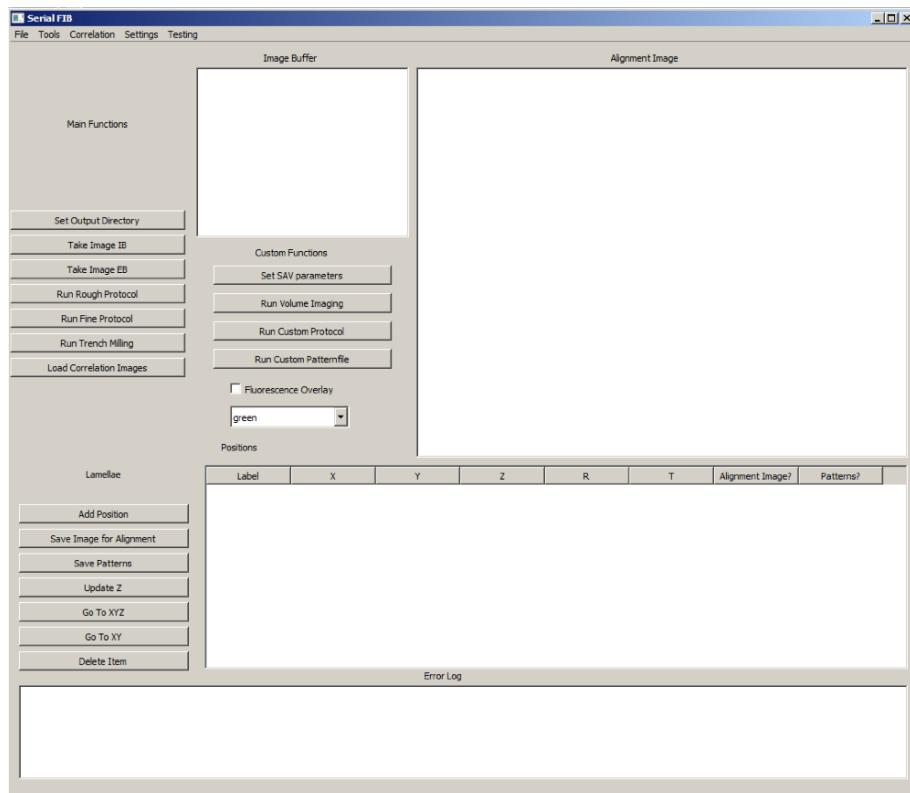
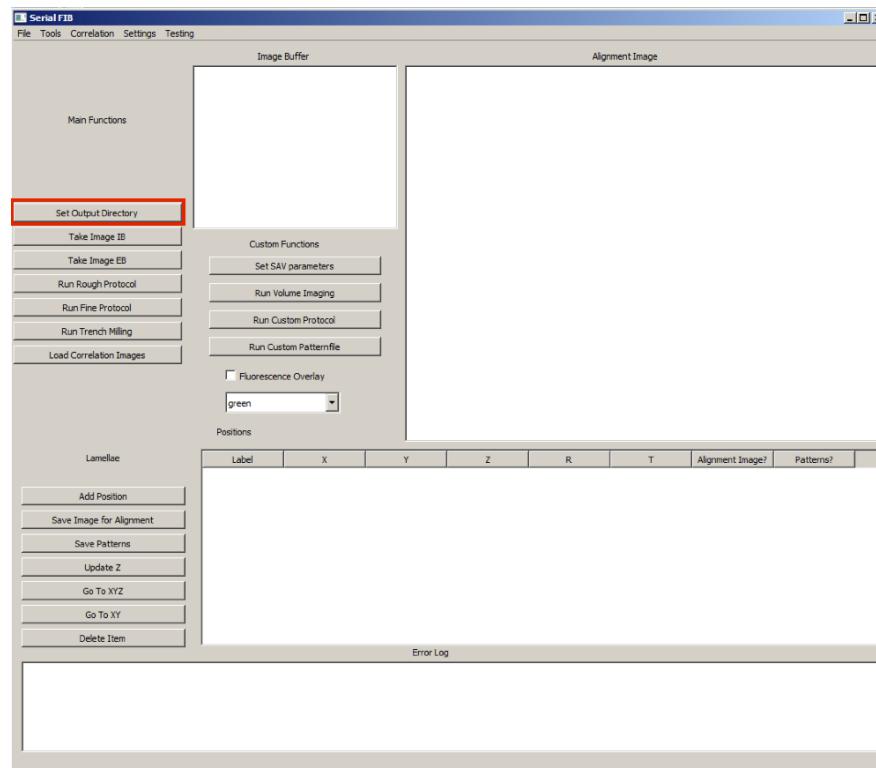


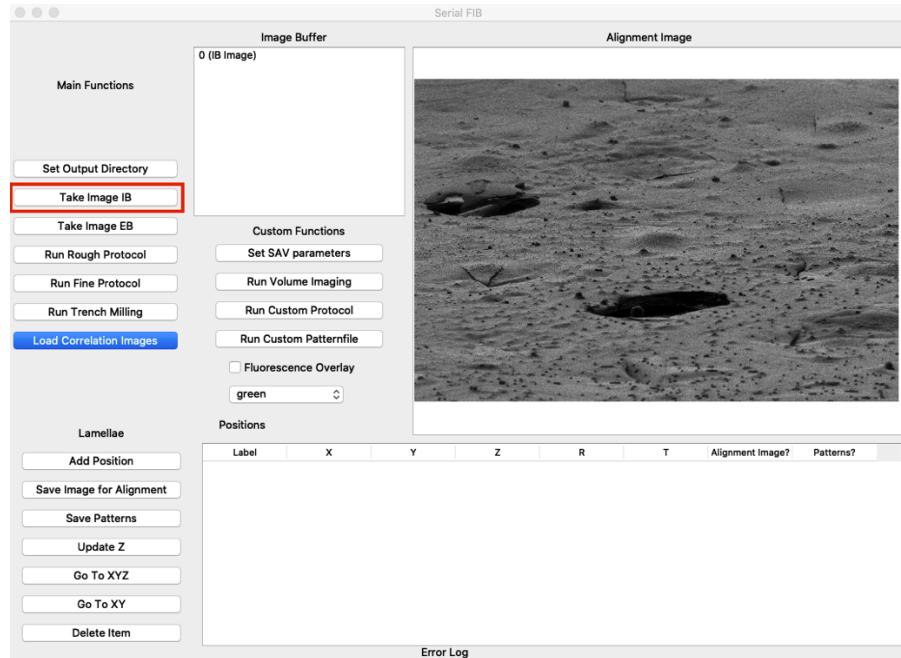
Figure 1: Graphical user interface

First thing you do is to set the output directory (see Step 1) . This will be the place where everything that SerialFIB writes out will be stored such as output images from Volume Imaging, patterns used for setting up positions as well as patterns that are used for milling, and the alignment images.



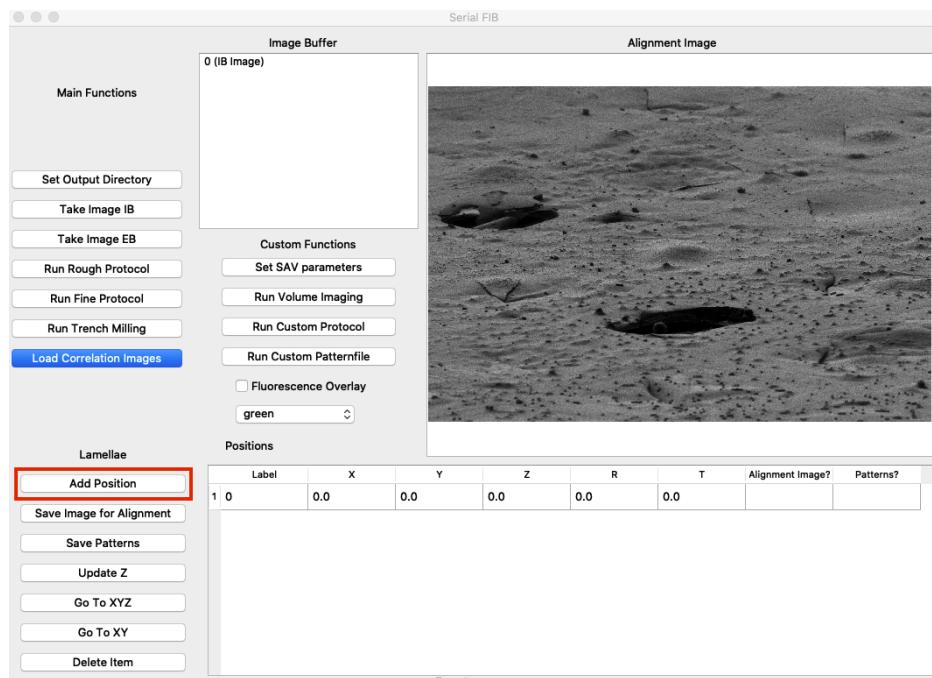
Step 1: Setting the output directory for the SerialFIB run

Now you can maneuver to the desired position using the user interface of the microscope. Once you are at your lamella site, pressing "Take Image IB" (Step 2) will take an ion beam image that we can use for realignment during the milling process.

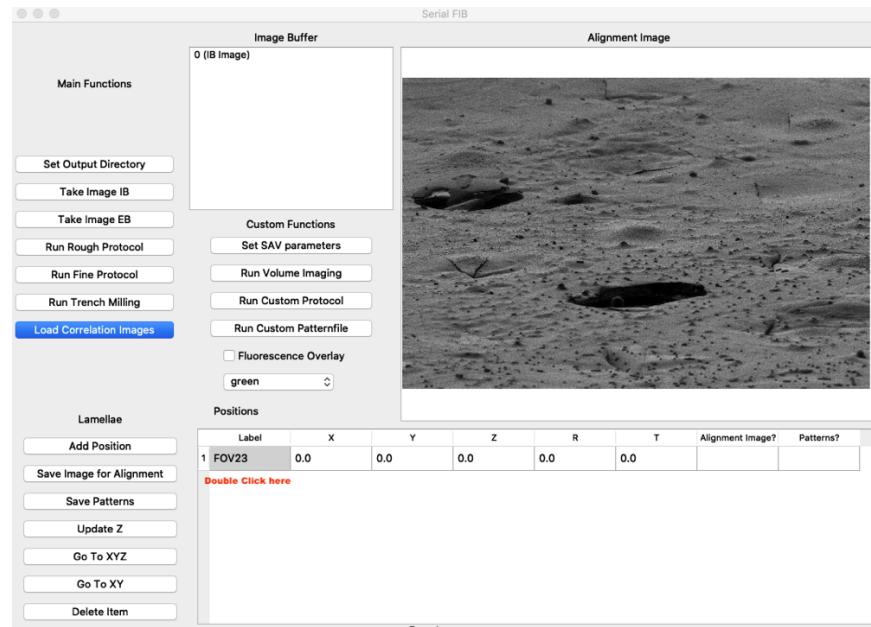


Step 2: Taking an image for realignment during automated milling procedure

While still being at the same stage position, add a lamella position to the table at the bottom of the GUI by pressing "Add Position" (Step 3). Note that the numbers shown here are dummies, so they should differ when working at the microscope. By double clicking into the "Label" panel in the table, you can give your site a name if desired.



Step 3: Adding position to the lamellae list



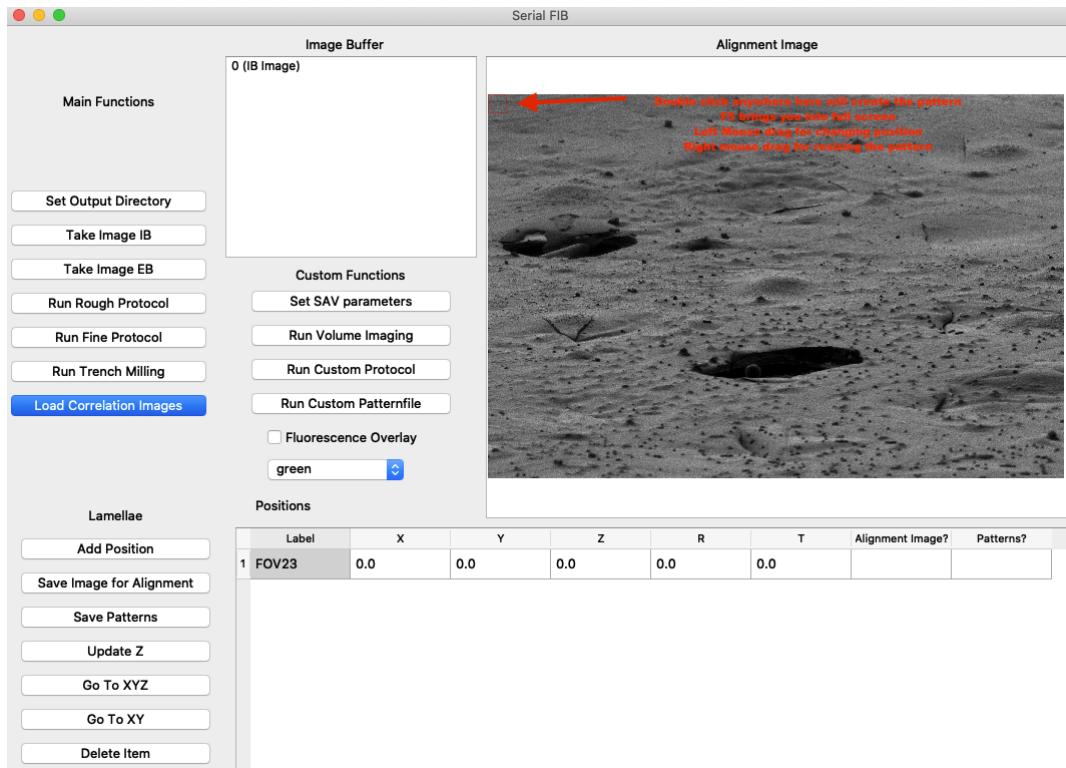
Step 4: Add a Label to the position, if desired.

Now we can define our lamella position. For that, create patterns within the Alignment Image by double clicking into the window. A pattern is going to appear that you can drag around using left mouse drag and resize using the right mouse drag. If you want to go into fullscreen mode for that, press "F5" (Step 5 and 6).

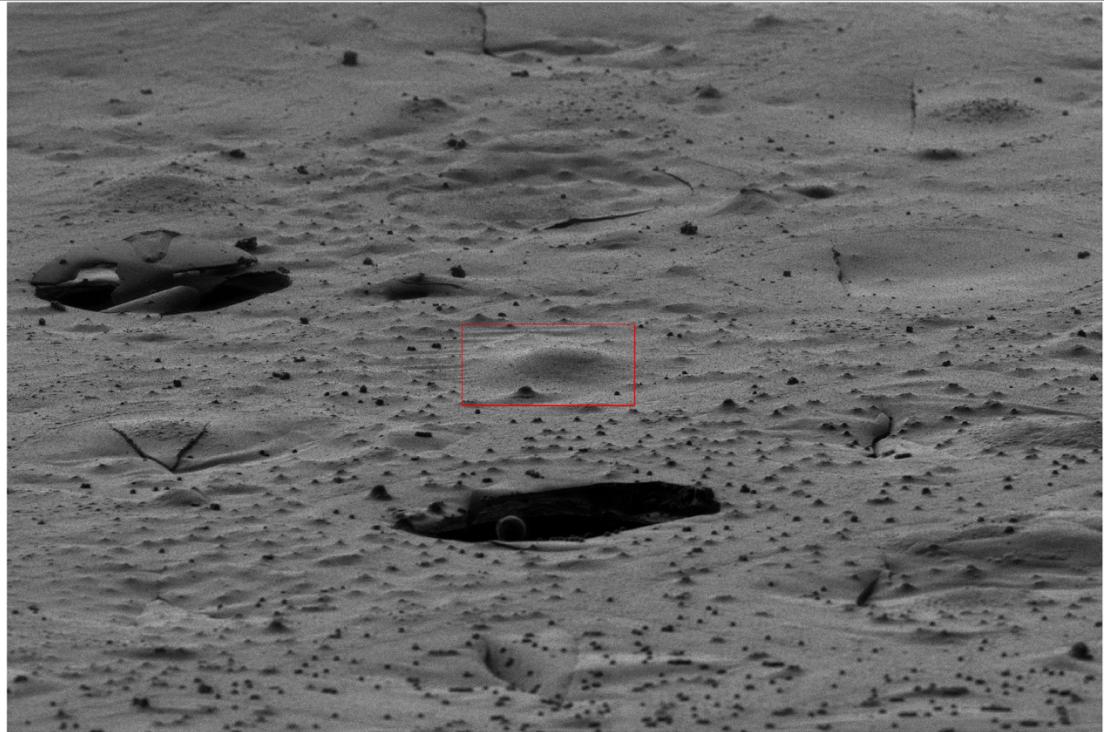
!!!! BUG ALERT !!!!

Select the image that you define the patterns for from the ImageBuffer by double clicking on the list item. Please also do that for the first position, it is currently still a bug that needs to be fixed.

!!!! BUG ALERT !!!!

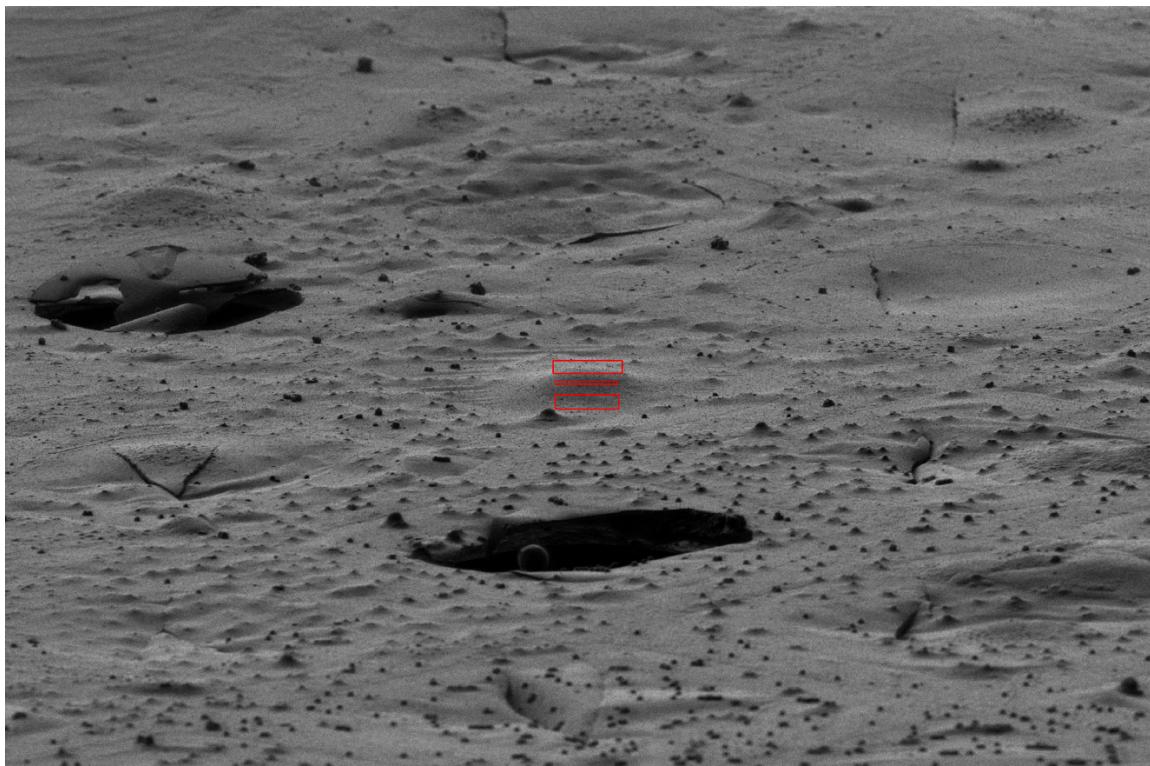


Step 5: Add and resize patterns to define lamella position



Step 6: You can do this in full screen mode by pressing F5. To get out of full screen mode, just press F5 again

Define three patterns in a way that corresponds to . If you created too many, you can just select it by left mouse click and delete them by pressing “Backspace” or “Del” on the keyboard. You can also select multiple patterns to drag around by simply holding the “Ctrl” key while selecting pattern. At the end, your site should look somewhat like Step 7.



Step 7: Your patterns should in the end look something like the above

And the patterns define positions as explained in Figure 2.

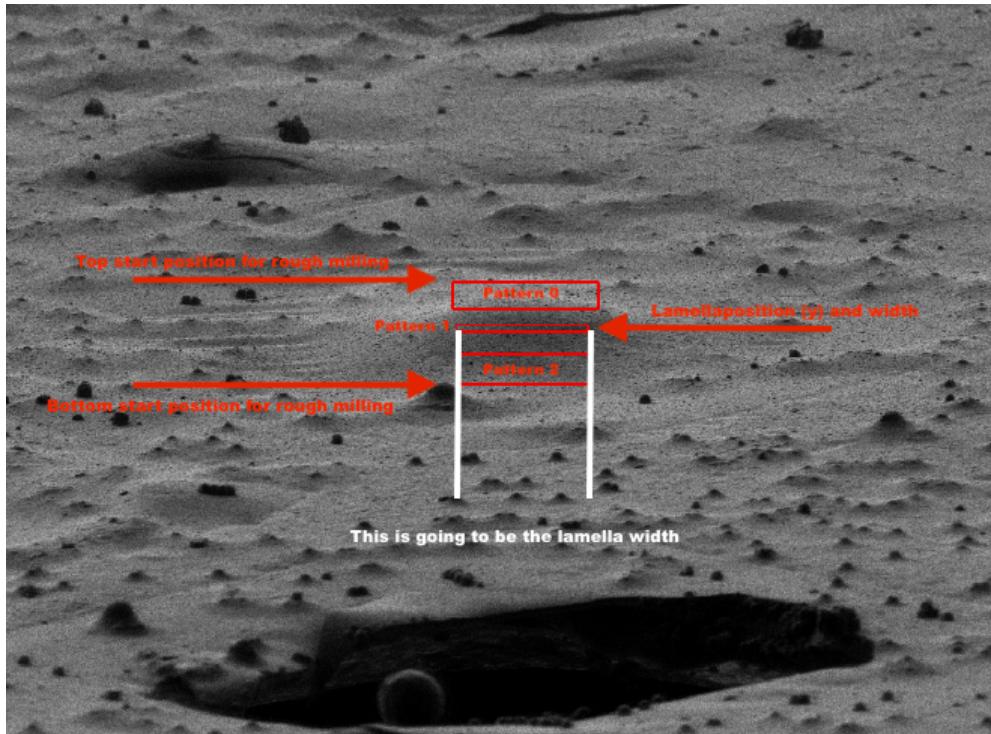
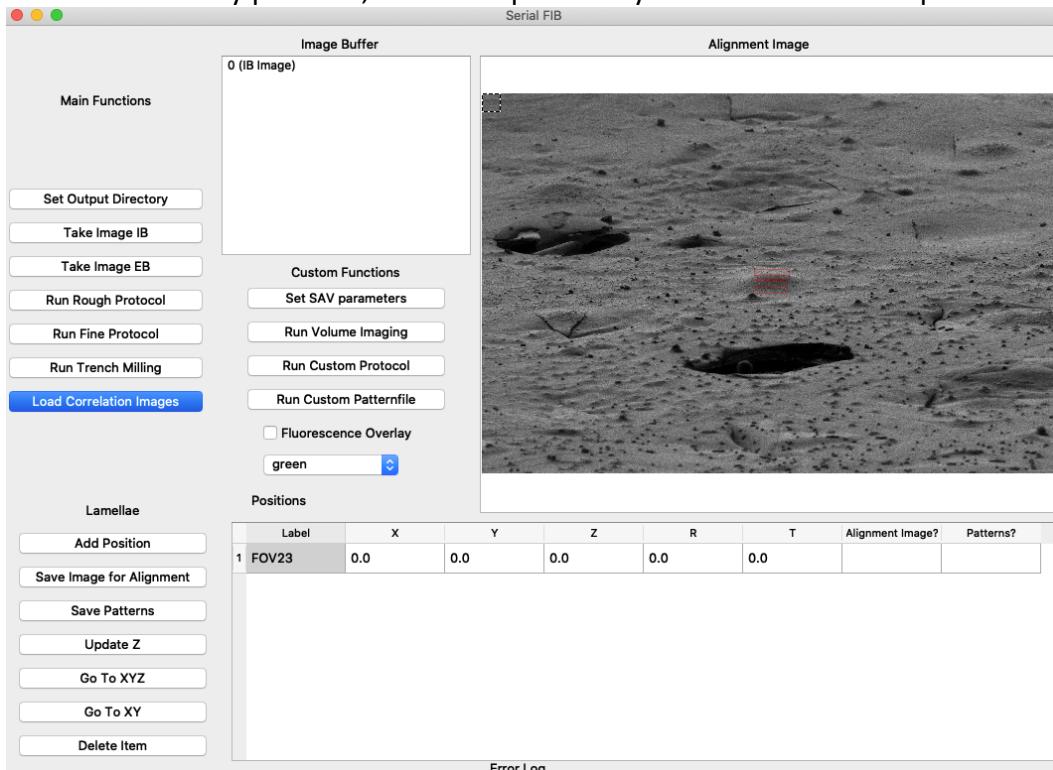


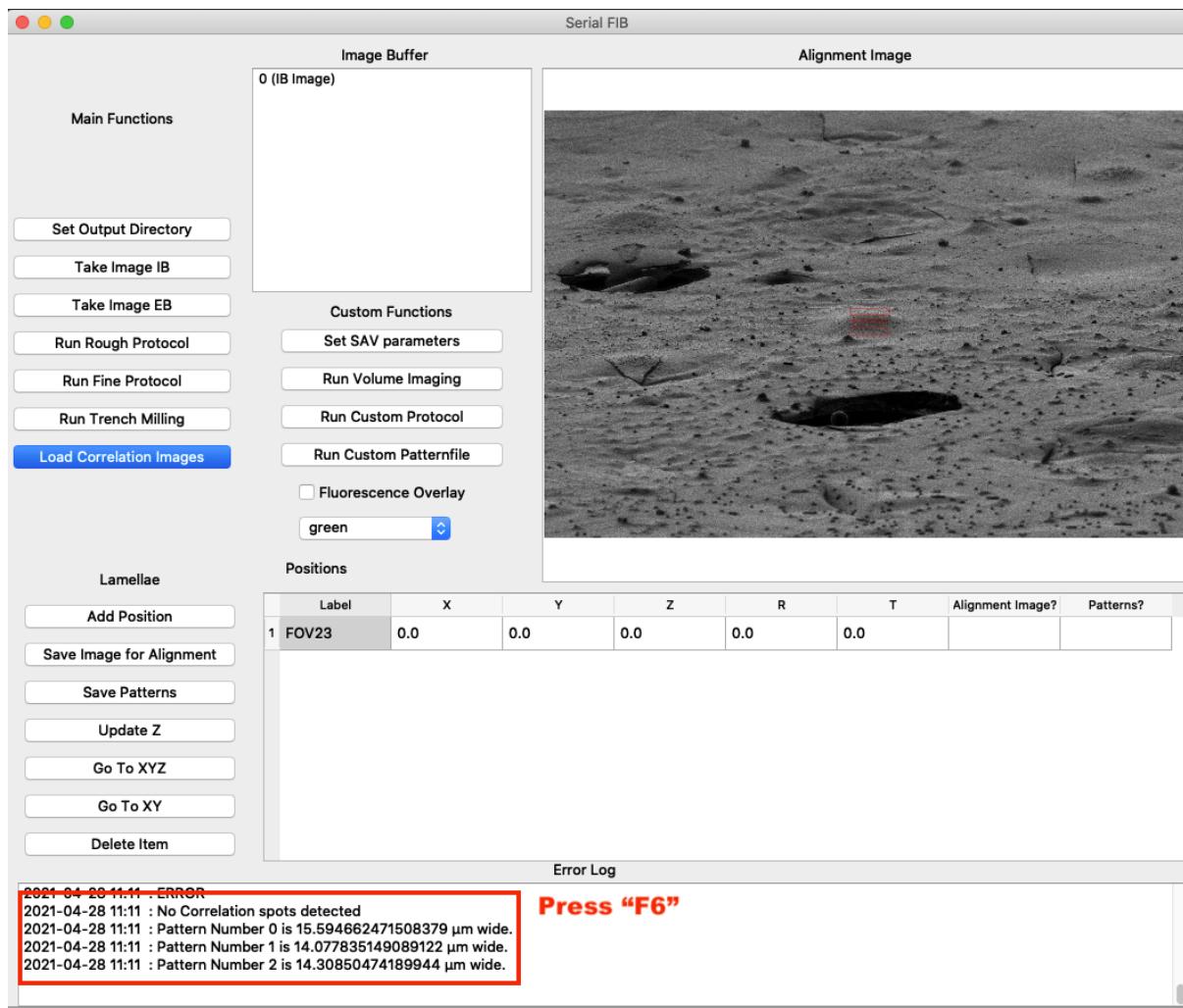
Figure 2: Explanation of the patterns and their relevant positions

If you created too many patterns, select the pattern by left mouse click and press delete.



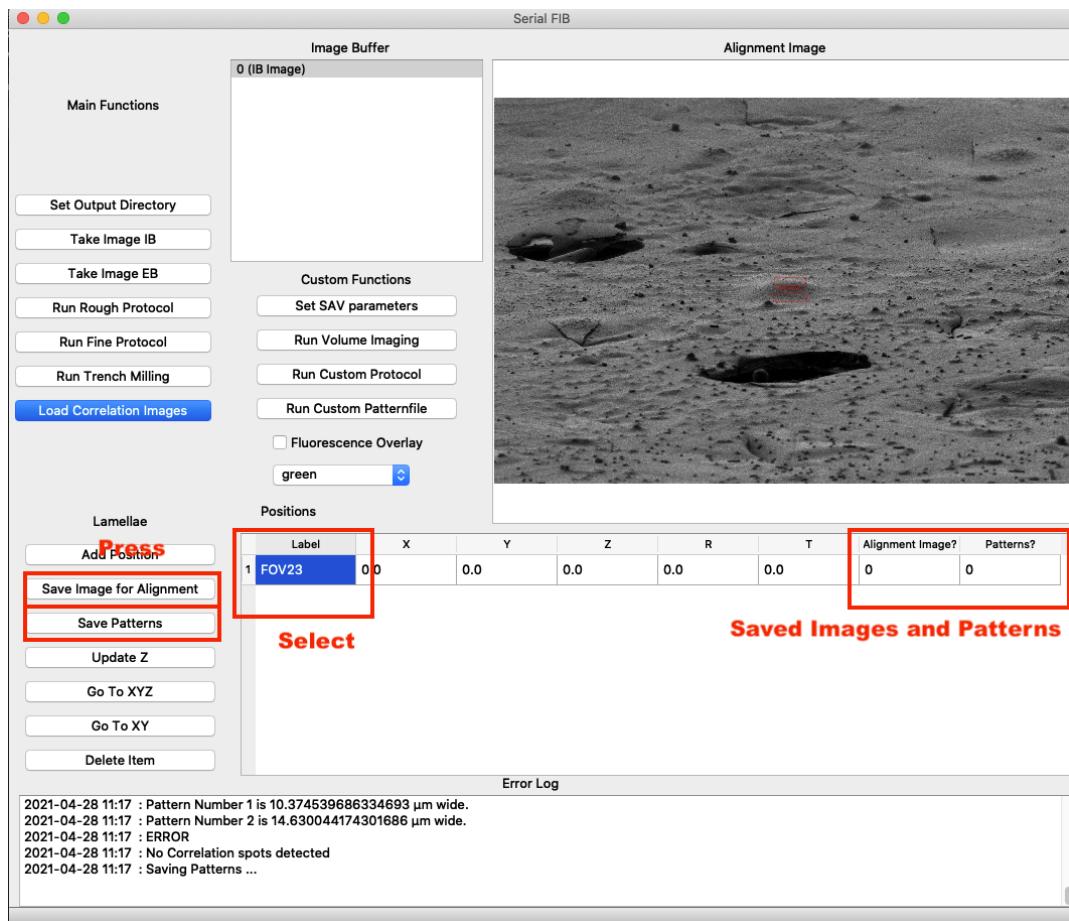
Step 8: Selecting and Deleting patterns by left mouse click and pressing "Backspace" or "Del"

If you want to know how big your patterns are, press F6. In the Log, the pattern widths from top to bottom are going to appear.



Step 9 Press F6 to find out the width of your patterns. They are numbered 0,1,2 from top to bottom, so the lamella width is always the "Pattern Number 1" value.

In order to assign the Pattern and Image to the stage position, simply select the label of the desired stage position in the table and then press Save Image for Alignment and Save Patterns.



Now you need to define the protocols that you want to run. We will come back later to creating those protocol files. You simply define them by going to Settings and selecting the files corresponding to your use case (e.g. Rough Mill Protocol, Fine Mill Protocol, SAV parameters). You can also simply save and load a settingsfile. Once you either defined the files or loaded a settingsfile, you can save the session by going to “File -> Save Session”. Loading a session is also going to reload your settings. If you haven’t saved or loaded one yet, it will write your settings into the output folder so that you know what parameters you used for your run. Loading the session is going to reload output directory, settings, images, stage positions and patterns. You can load the session once you saved it out by going to “File → Load Session”.

Once you set up all your positions as described above, you can hit any of the protocol buttons and milling is going to start based on the files you provided in your settings. Setting up those files will be discussed in the specific sections below. A window is going to pop up where you can cancel the operation at the next safe spot (which is after alignment of images and before pattern starts). Don’t worry, your sample is not going to be exposed by milling.

If, for some reason, you don’t want to use 10 pA as your alignment current for the ion beam, you can change the ion beam current for alignment by typing in a value (in pA) into the box that appears when going to “Testing→Change Alignment Current”. Just type in e.g. 1.5 into the text box and hit okay if you want your alignments to be performed at 1.5 pA.

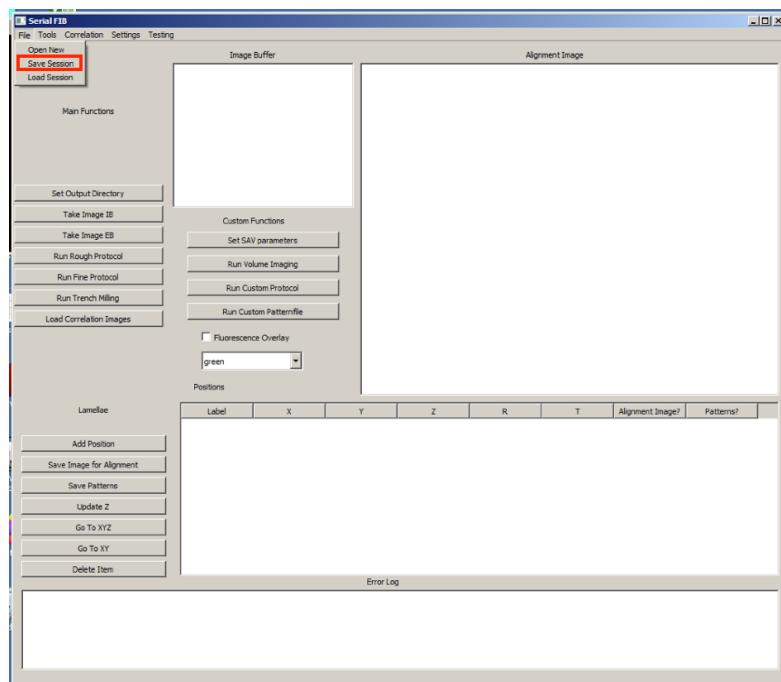


Figure 3 File Dropdown menu

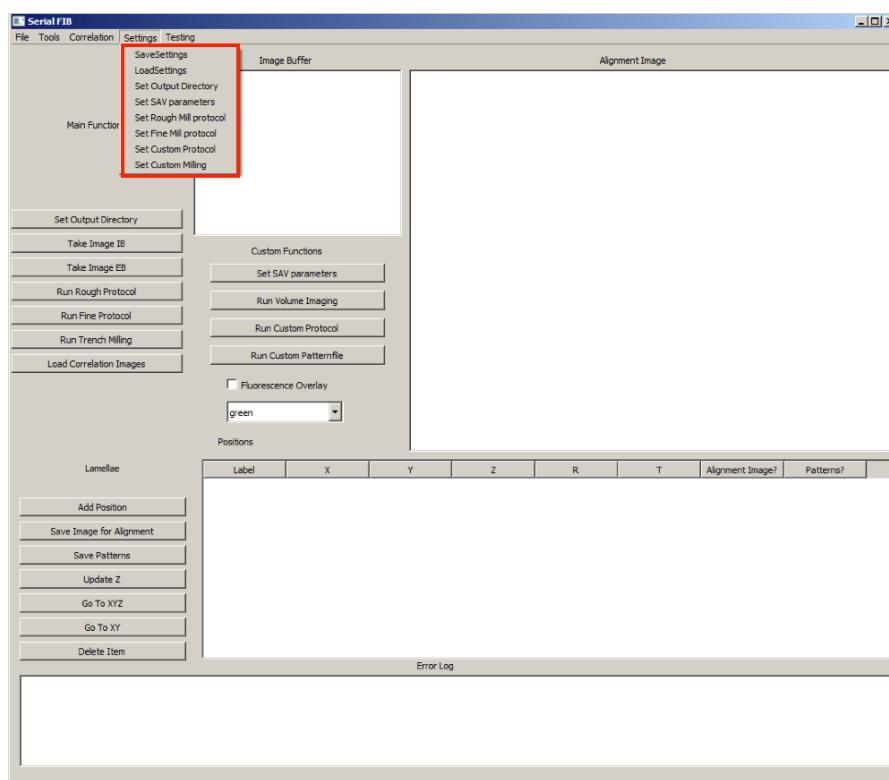
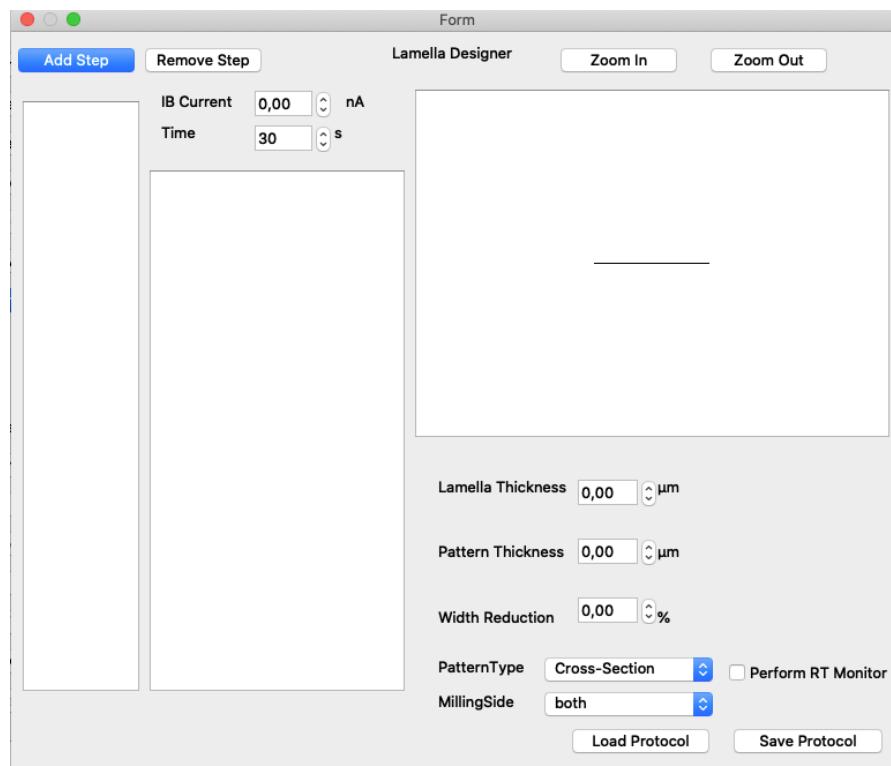


Figure 4: Settings Dropdown menu

Working with Protocols – Rough and Fine milling with the LamellaDesigner

In order to set up protocol files for rough and fine milling for lamella preparation, you can go to the LamellaDesigner, which you find by going to “Tools → LamellaDesigner”. It will open up a window as shown below.



You can add and Remove Steps by clicking the respective button in the top left and you can select the steps by clicking once within the list on the left. The list on the right is a dummy that will show you top and bottom pattern, but they are both going to be displayed at the same time in the schematic representation on the right. For each step, you can assign the IB current, the milling time, the lamella thickness you would like to achieve in that step and the thickness the milling patterns should have. Note that typical, the ion beam mills further than one would expect based on their separation. Thus, a separation of 350-400 nm of the final milling steps usually gives you lamellae thinner than 250 nm. Note also that typically, prolonged milling times lead to thinner lamellae than specified by the target thickness.

Note also that when starting with this, especially in fine milling, you need to play a little with the parameters since every sample is different. Generally, I like to use a site I can spare where I test parameters and subsequently set several positions up with those. If you are not so optimistic regarding fine milling, what also usually helps is going back to the positions and re-defining the lamella position manually before starting fine milling. While we do have results where this wasn't necessary and still produced nice lamellae, success rate tends to be higher and it usually gives a better feeling to the users as well. ☺

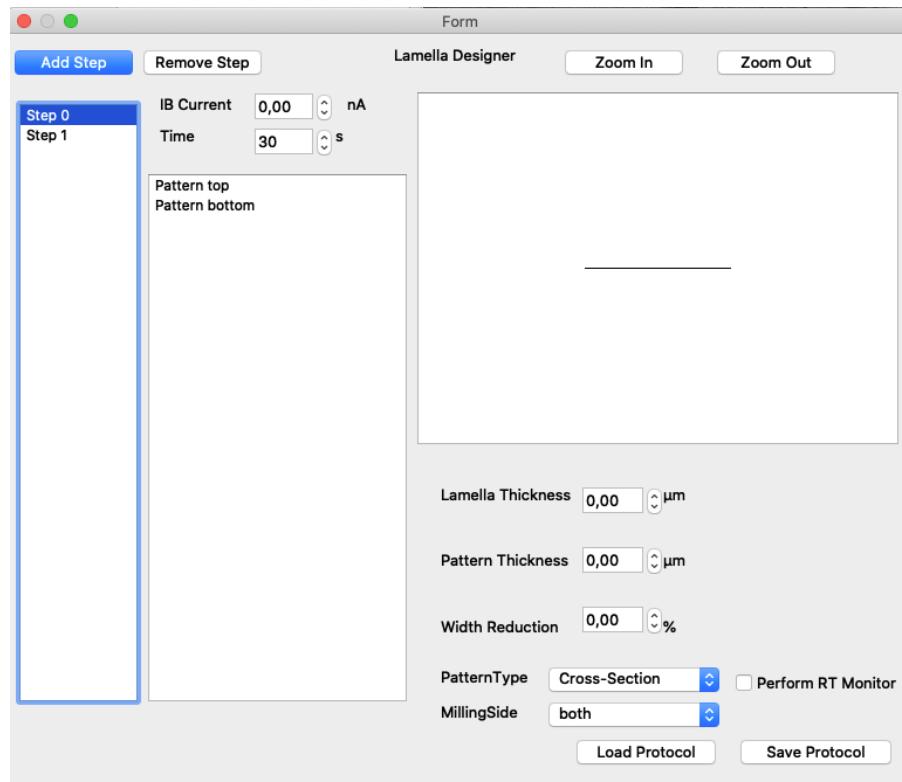


Figure 5: LamellaDesigner with Steps added

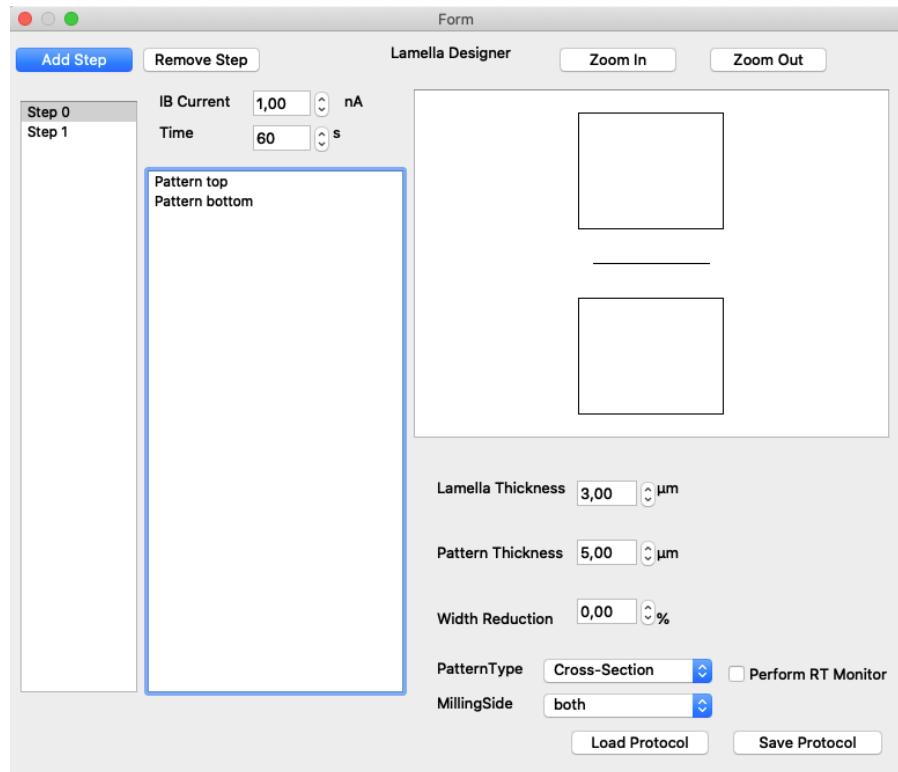


Figure 6: LamellaDesigner with values added to the parameters

If desired, you can also rename the steps by double clicking in the list on the left. When you are ready with your protocol, you can save it by pressing “Save Protocol” and write it somewhere into your output folder or wherever you like. Please remember to set the protocol before you run it within the GUI by going to “Settings→Set Rough Mill Protocol” and

“Settings→ Set Fine Mill Protocol”, respectively. Note that if you were to do both rough and fine milling in one go, you can simply increase the steps in your Rough Mill Protocol. The parameters and protocol files are described below.

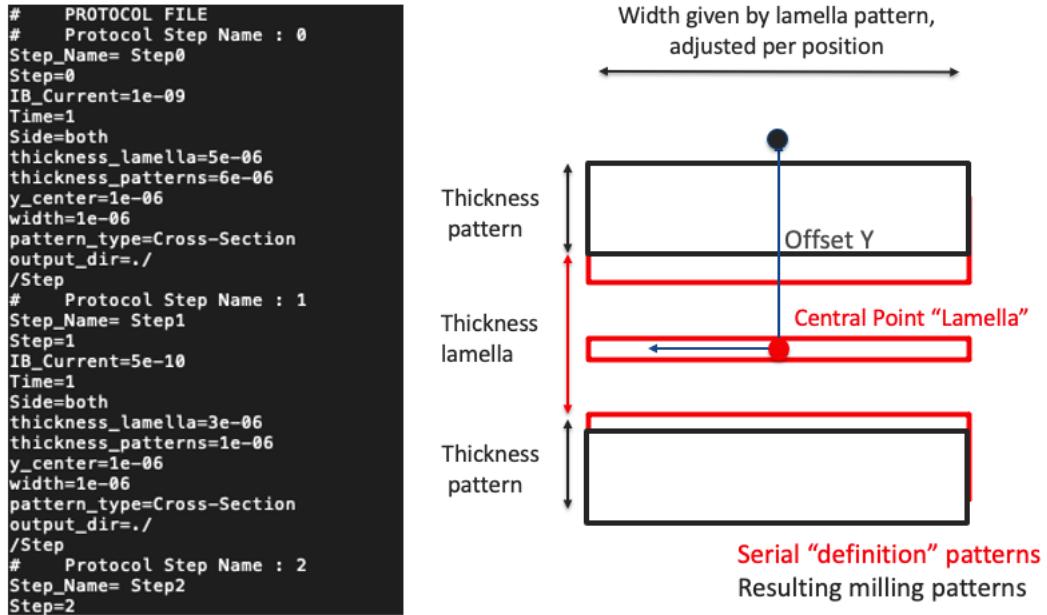


Figure 7: Illustration of a protocol file (left) and the logic behind protocol files (right)

So, why do we have separate steps for the two protocols? First, that allows you to split the procedure into two steps, circumventing any redeposition on your lamellae while they are still in the FIB/SEM. Also, the difference between the buttons “Run Rough Protocol” and “Run Fine Protocol” is, that the extreme point of where to start rough milling ablation are taking into account only for the “Rough Protocol”.

Note that width reduction isn’t implemented yet in the current version of SerialFIB since it was a feature we were thinking about but proved to not be 100% necessary. Remember that the width of each lamella and their position are going to be defined by the setup as described in the prior section, so these are just dummy values in the protocol file.

Working with Protocols – FIB-SEM Volume Imaging with the VolumeDesigner

To perform volume imaging using SerialFIB, you can design your parameters in the VolumeDesigner, which you can find by going to “Tools→ VolumeDesigner”. You can fill the parameters there and reload them as well if you want to make adjustments. Save them to a location of your choice and please don’t forget to define them in the GUI by going to “Settings→ Set SAV params”.

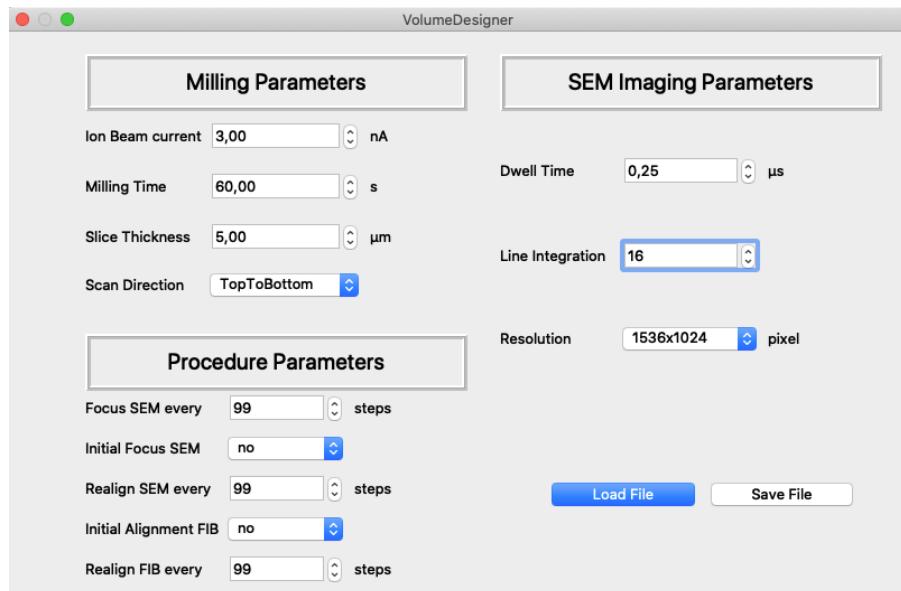


Figure 8: Volume Designer module with sample parameters

Generally, the depth of focus in the SEM is very large. So if you are looking only at a single site, focusing manually once should be good enough for approximately 5 μm of volume. This being said, the autofocus function generally doesn't perform too great on the surface at cryogenic temperatures as there is too little features. While it is possible to autofocus, optimizing that function is still ongoing work and so far, I haven't seen a system where it actually works reliably. If you do have ideas, please let me know at klumpe@biochem.mpg.de. That would be great!

Realigning the SEM also is a little tricky if your coincident point is not set 100% correct since I restrict it to using the Beam shift rather than moving the stage as moving the stage is also going to change the ion beam image. So, if you want to use it, please make sure your coincident point is as accurate as you can get it. Otherwise, set your field-of-view a little larger if you can afford it. Same goes for realigning the FIB. Generally, the stages are okay-ishly low in drift these days, so you don't need to do it every couple of nanometers but rather after quite some time of data collection.

The definition of the volume is done in the same way as for the lamellae in the main GUI. Start and end position are adjusted so that you can potentially use the same position definitions for both volume imaging and lamella milling at the same position.

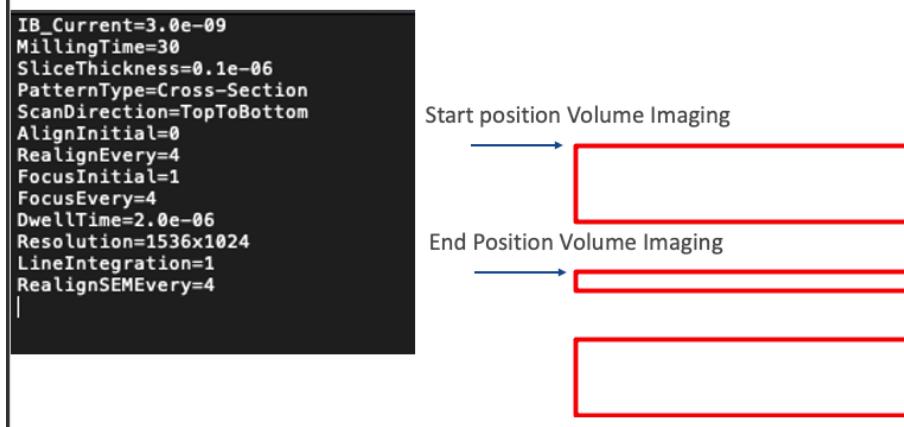


Figure 9: SAV params file (left) and logic (right) behind volume imaging position definition using the three patterns used for lamella site preparation

Working with Patternfiles – Creating your own workflows for development

If you want to do something outside the norm of the use cases provided (lamellae milling, correlative site preparation, volume imaging), such as lift-out milling or, potentially with some scripting, the waffle method, you can do whatever you like with the PatternDesigner. It is similarly structured as the LamellaDesigner (note that the LamellaDesigner is actually based on the PatternDesigner).

Operation of the PatternDesigner is very similar to the LamellaDesigner. The only difference is that you can add and remove as many Patterns as you want. They are defined by a central point which is given from the central point of the middle pattern in the site-specific setup.

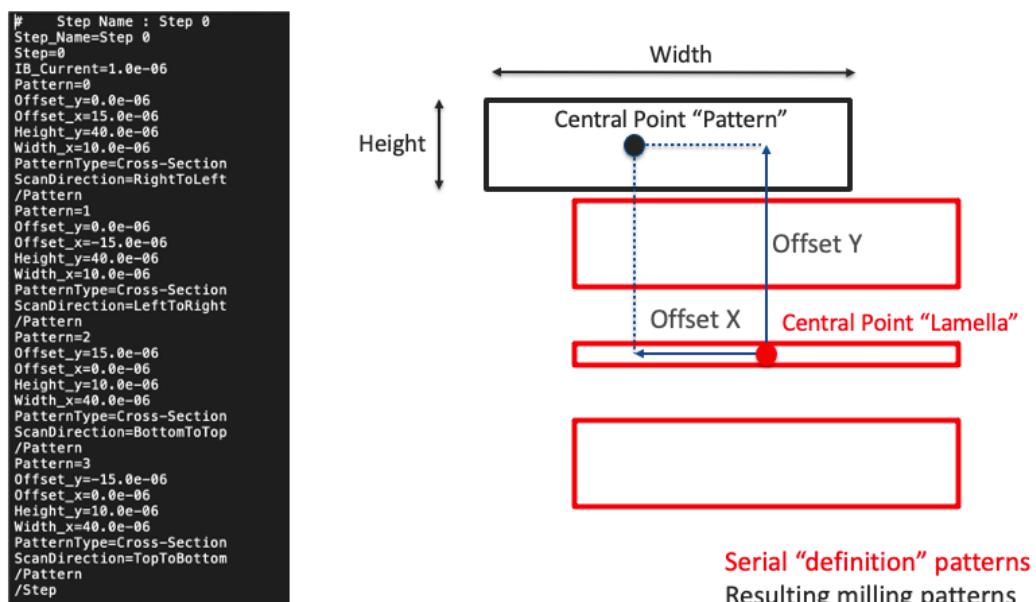


Figure 10: Pattern sequence file (left) and logic behind a pattern sequence file (right).

Working with correlative data – Loading FIB images + correlation spots from 3DCT

If you are working with correlative approaches and use the 3D correlation toolbox for site-specific lamella preparation, you can load the image and correlation spots into SerialFIB as alignment image and in order to guide the setup of your lamellae. For that, simply press “Load Correlation Images” either in the main GUI or under “Correlation → Load Correlation Images”, then select the FIB image prior to correlation as well as the output file created by 3DCT. The loaded image is going to look somewhat like Figure ... and the correlation spots (though already visible in the image but very tiny) become a little clearer when going into full screen mode as green dots in the image (cf. Figure 11). Currently, please save your FIB images as 8- or 16-bit images without databar if you want to load correlation images. While this will be improved in the future to make it less error-prone, you might experience problems otherwise.

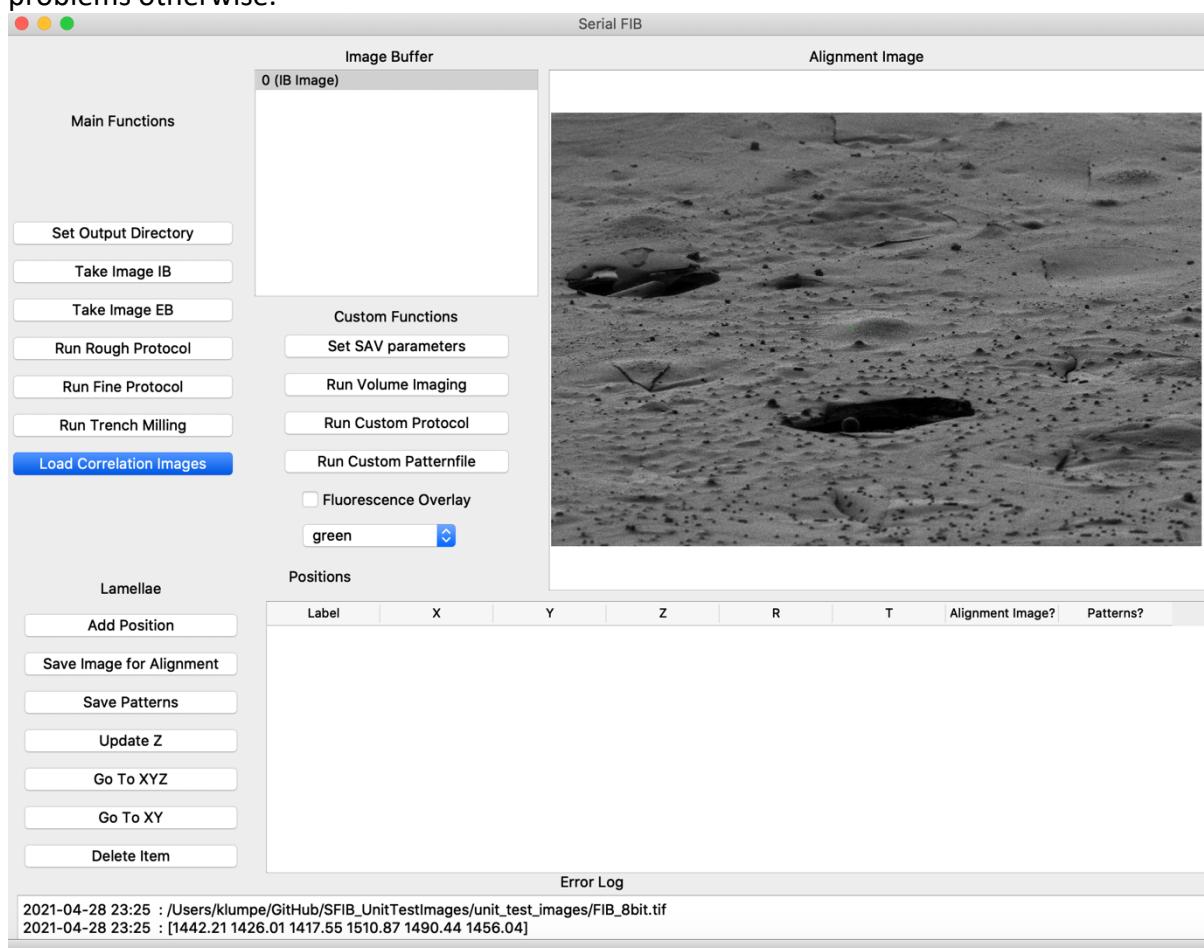


Figure 11: GUI after loading an image from the 3DCT output.

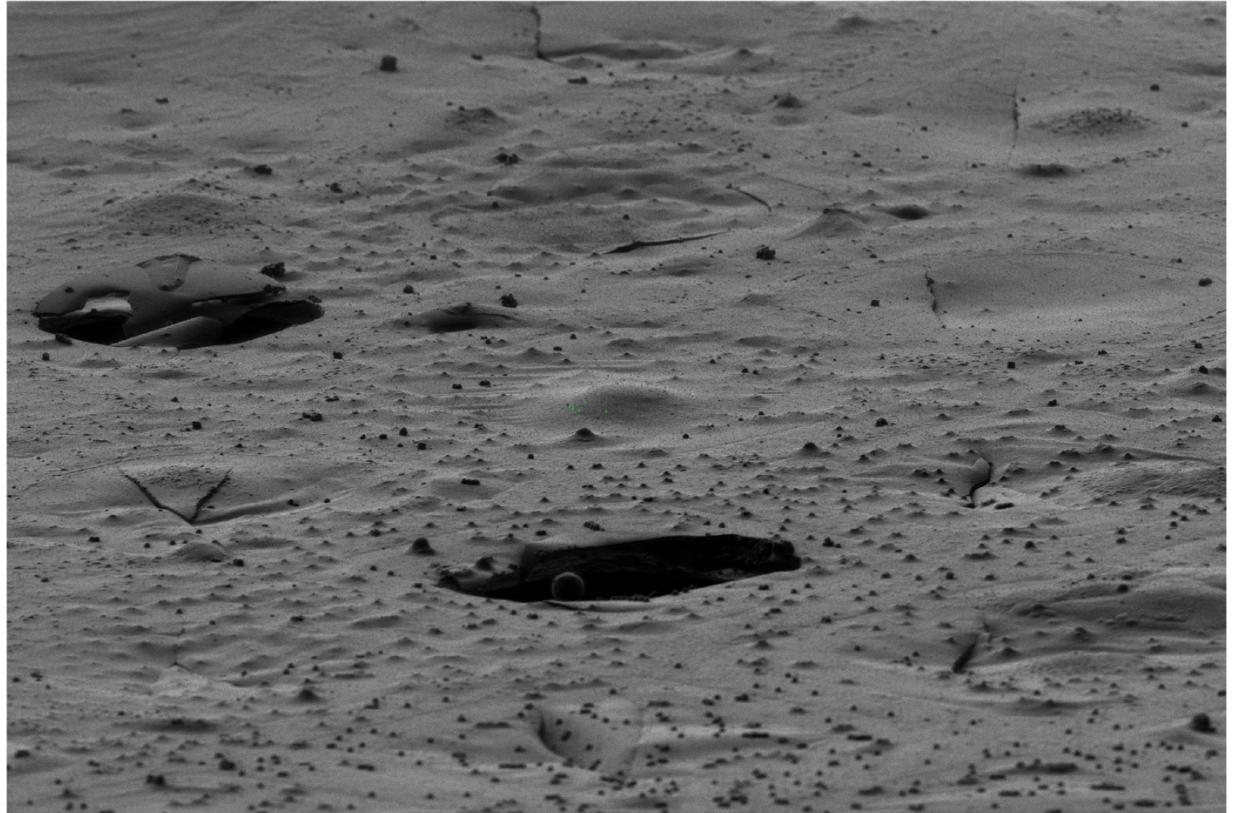


Figure 12: 3DCT correlation images loaded into the GUI show the correlation spots as green dots.

The approach works both with 3D- and 2D-correlation outputs from 3DCT. You can also let the program draw little patterns at the correlation spots by pressing “Testing→Draw Corr Patterns”. Their right-most edge will correspond to the proper positions after aligning to the prior image by choosing it from the ImageBuffer (double clicking on the list item in the image buffer) and pressing “Testing→ Align to Image Buffer”.

Working with the ScriptEditor – Creating your own scripts

Every function we use for the use cases as well as setup of positions can be called in the ScriptEditor. The microscope itself is a class called “fibsem” and to do a stagemovement you would e.g. write “fibsem.moveStageAbsolute(stageposition)”.

Stagepositions are given as a python list called “stagepositions” (they correspond to a dictionary with keys x,y,z,r,t), images are obtainable from a list called “images” (given as AdornedImages from AutoScript, so to see the data just type images[0].data). The patterns are given as a list “patterns” and can be obtained in the same way.

Documentation of all commands is under construction.

With the buttons “Open Script” you can open a saved script which will be loaded only into the opened “Choose script” value (this is where you can zap through the library). With Open library, you open all scripts within the loaded library with the associated “Choose script numbering”. Save Scripts and Library do the same.

Form

Open Script Open Library Script Editor Save Script Save Library
Choose script 0

```
### Testing cryo-FIB-SEM volume imaging ###

### User Input ###
output_dir=r'D:/User Data/Sven/20210201_SerialFIB_cleaning/folder9'
img_index=0
stagepos_index=0
pattern_index=0

#####
### Definition of variables ###
fibsem.output_dir=output_dir '/'
label=stagepositions[stagepos_index]['label']
alignment_image=images[img_index]
pattern_dir=output_dir+'/'+str(label)+'/'

### Writing pattern directories ###
fibsem.write_patterns(label,patterns[pattern_index],alignment_image,output_dir)

### Creating SAV patternfile ###
fibsem.create_SAV_patterns(pattern_dir,str(label)+'_lamella.ptf',str(label)+'_tp.ptf',str(label)
+'_bp.ptf',pattern_dir+'/SAV_pattern_file.ptf')

### Running SAV ###

```

Run Script