Version 2.1
April 2021

Selectris Applications Note

Table of contents

1. EFTEM and zero-loss filtering

Selectris is a post-column image filter that enables Energy Filtered Transmission Electron Microscopy (EFTEM).

When an electron beam interacts with the sample some of the electrons lose part of their energy (inelastically scattered electrons) and for some electrons their initial energy remains unchanged (elastically scattered electrons). In conventional TEM, all electrons (both elastically and inelastically scattered) contribute to the image formation (Fig.1a). In short, elastically scattered electrons are contributing to the image formation and inelastically scattered electrons play a role in adding noise to the image. As the sample becomes thicker, it produces more inelastically scattered electrons and therefore the signal to noise ratio in the image decreases.

In case of cryoEM where signal in the image is weak, the sample thickness becomes an important factor that increases the noise level and significantly reduces the quality of the image.

A prism inside the energy filter can sort the electrons that went through the sample according to their energy (produce an electron energy loss spectrum) and a slit mechanism makes it possible to choose the electrons of a particular energy to contribute to the image formation (Fig.1b).

The electrons with the highest energy are those which were elastically scattered during interaction with the sample and have not lost their initial energy. Those are zero-loss electrons. Zero-loss filtering is a procedure of filtering out all inelastically scattered electrons which leads to only zero-loss electrons contributing to the image formation. Zero-loss filtering is widely used in cryoEM, and especially in cryo electron tomography data acquisition where the sample “becomes thicker” with increasing tilt angles. But now this technique is also becoming popular for SPA.

In order to have an effective filtering the slit width should be adjusted in such a way that it lets all elastically scattered electrons go through and block all inelastically scattered electrons. For the majority of cryo EM samples this optimal slit width is 10eV.

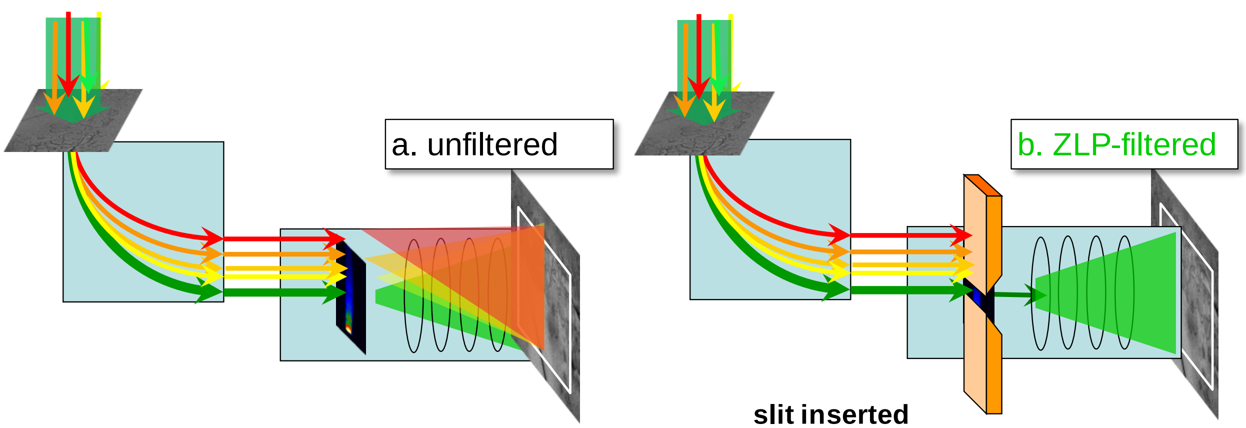


Fig. 1. Zero-loss filtering. (a) Unfiltered image: electrons with all energies contribute to the image formation. (b) ZLP-filtered image: slit mechanism allows the selection of zero-loss electrons for contributing to the image formation.

The energy loss spectrum of the electrons that contribute to the image formation without filtering is shown on Fig. 2. There are two major peaks: zero-loss peak (red arrow) and plasmon peak (blue arrow). The plasmon peak represents the electrons that lost part of their energy. It starts around 6-7eV and has its maximum at ~20eV on the scale of energy loss. That means that in order to remove those electrons effectively we need to put one edge of the slit at the position of ~5eV. The other edge of the slit will be at the position ~-5eV to have the slit centered around ZLP. That is why a slit width of 10eV is considered optimal for filtering out all the inelastically scattered electrons and including all the zero-loss electrons.

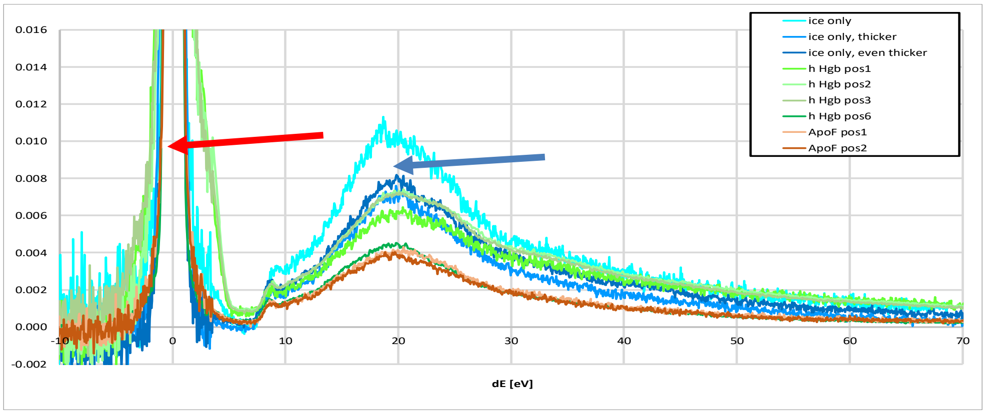


Fig. 2. Electron energy loss spectrum of various cryo EM specimen. Red arrow – zero loss peak, blue arrow – plasmon peak.

2. Selectris tuning in Sherpa

When the Selectris has not been tuned for a long time or is severely misaligned it is advised to start with loading the latest alignment in the Alignment tab in the user interface (UI) (Fig.3).

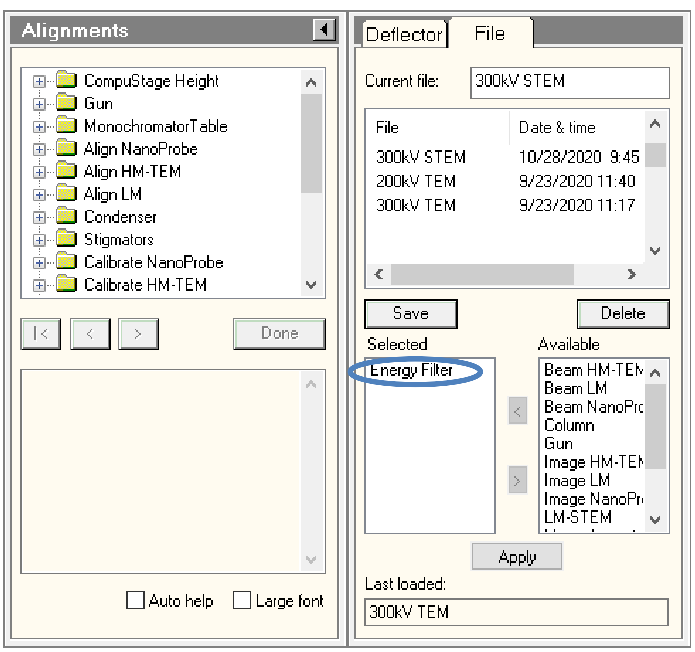


Fig. 3. Loading of Selectris alignment file in the user interface.

Tuning of the Selectris is performed in Sherpa (Fig.4). In brief, there is a 4-step sequence for this procedure:

1) ZLP centering;
2) Isochromaticity tuning;
3) Tune Magnification;
4) Tune Distortions.

A more detailed description of the steps follows further below.

The changes introduced during tuning in Sherpa are not saved automatically. In order to store the filter tuning for later one should save an alignment file in the user interface.

Although the tuning routines work well on thin and modestly thick specimens, the tuning is most accurate when the specimen is removed from the field of view (for example, by moving the stage to an empty area). You can use the same optical settings (magnification, gun lens, condensor aperture, beam diameter) as those which will be used for data acquisition. Check that the camera is illuminated sufficiently (3-10 e/px/sec in counting mode, more than 20 e/px/sec in linear mode for Falcon4). If the camera is illuminated with too low intensity, lower the spot number temporarily during filter tuning. In the "settings" box (Fig.4) check that a proper camera mode is selected for filter tuning: choose Falcon in linear or counting mode depending on the mode that will be used for data acquisition. Default Acquisition settings of binning 4 and exposure time 0.5 sec work well in majority of the cases.

When the filter is tuned (ZLP centering, isochromaticity, distortions) at one magnification, the tuning is also good at all other EFTEM magnifications[1]. This is ensured by the so-called "cross-over corrections". These correct for the small shifts in the position of the final cross-over in the microscope that can occur when the magnification is changed. Such shifts are caused by imperfect mechanical alignment of the lenses. When you change magnification, you can check that the filter is still properly tuned using the "Measure" buttons. These cross-over corrections can be tuned in the Service/Factory tab of the Energy Filter in Sherpa.

[1] However, the isochromaticity can increase to a few eV at the very lowest magnifications in SA an at the very lowest magnifications in LM

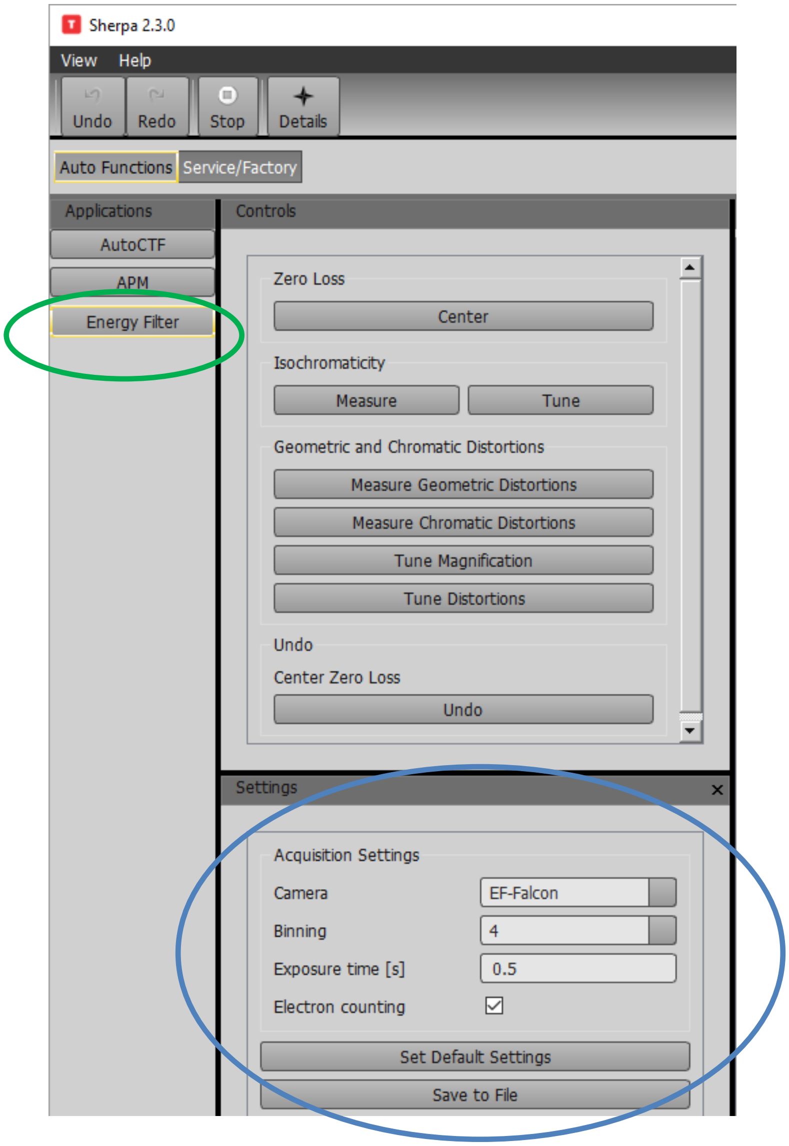


Fig. 4. Selectris tuning in Sherpa. The green circle indicates where to start the tuning plug-in. The blue circle indicates the settings box to specify exposure parameters.

2.1 Zero-loss peak centering

The first step of the tuning is zero-loss peak (ZLP) centering (Fig. 5). This automatic procedure makes sure that the slit is centered around the ZLP. The only output that is provided after the procedure is finished is the shift in eV which had been applied to bring the slit around the ZLP (Fig. 5).

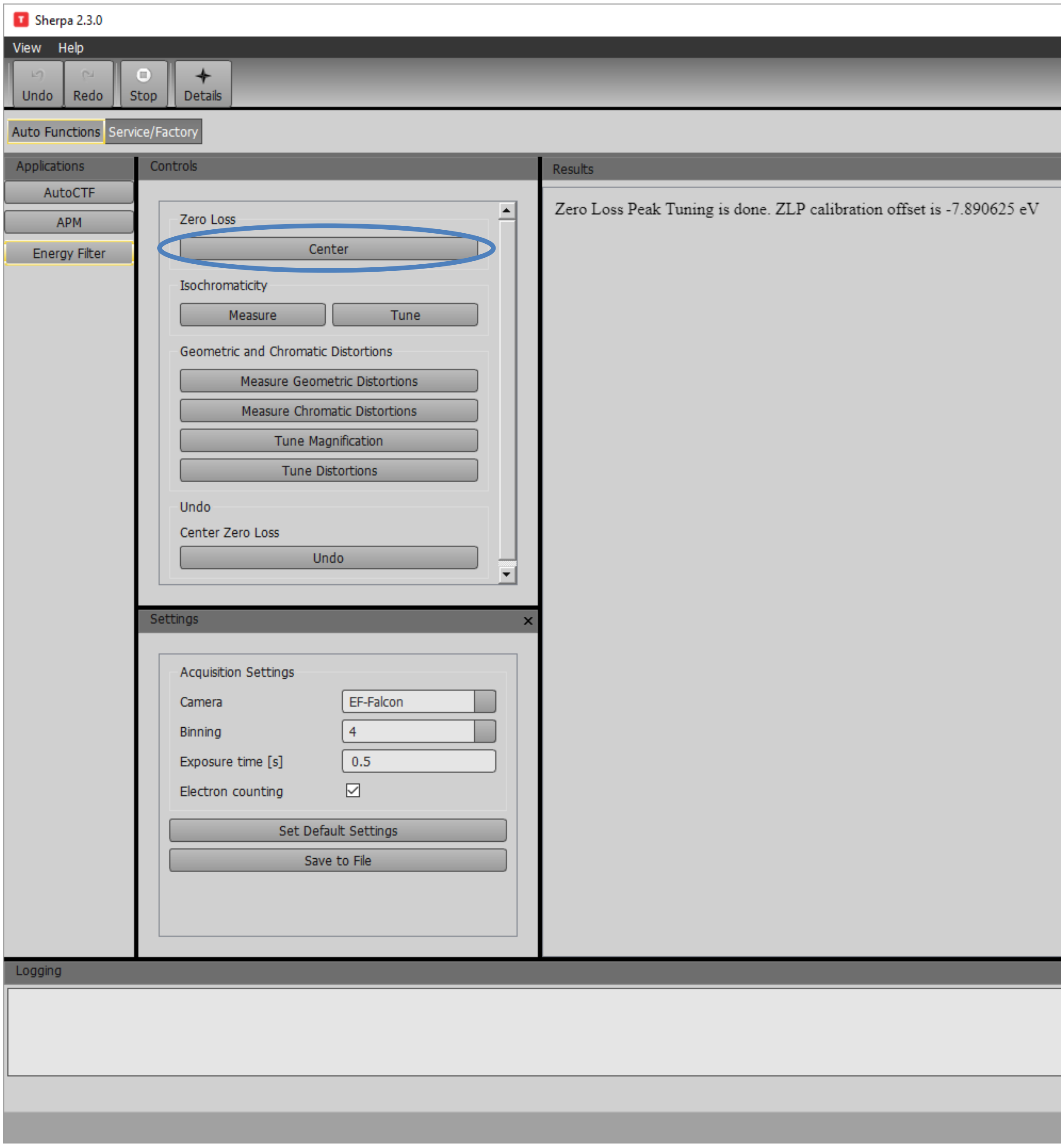
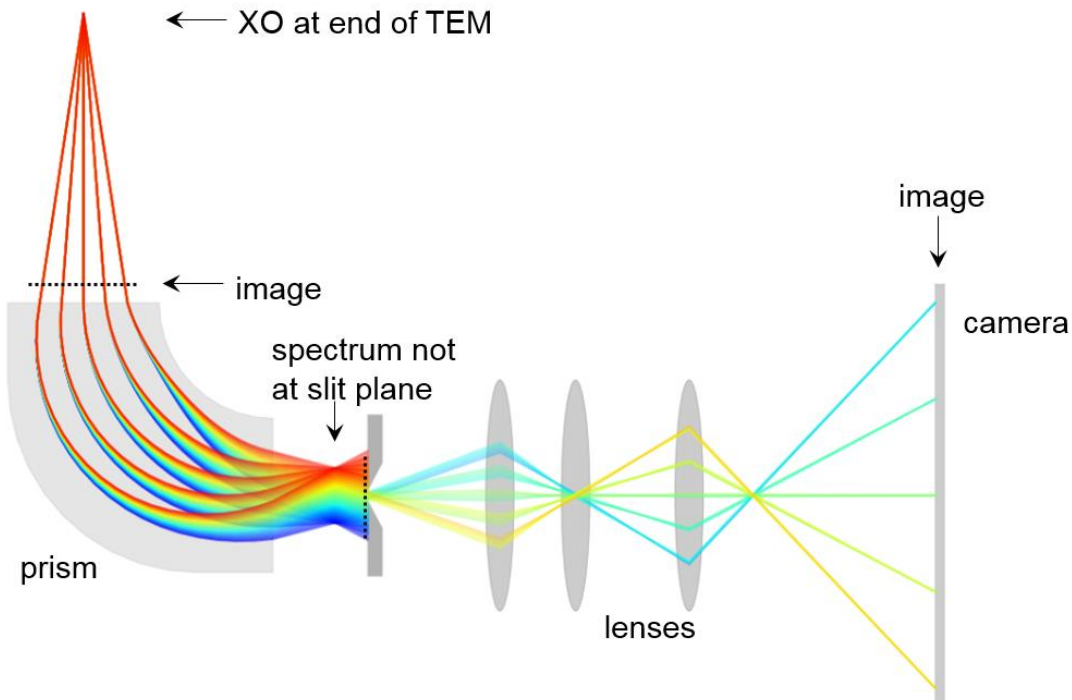


Fig. 5. Zero loss centering procedure in Sherpa.

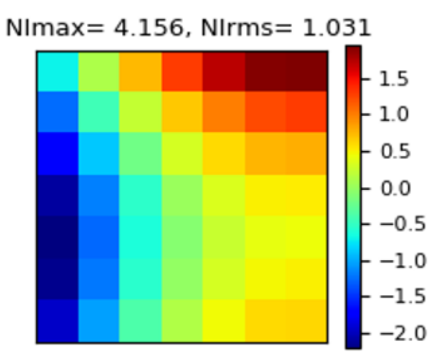
2.2 Isochromaticity tuning

When the Selectris is not optimally tuned, it can occur that the spectrum plane is not properly focused at the plane of the slit. If that is the case, the slit does not select a specific energy. Instead, different energies are selected for different positions in the image (Fig.6).

The effect of the selected energy not being the same for all positions in the image is called non-isochromatism (non=not, iso=same, chroma=color, tism=happening).



a



b

Fig. 6. (a) Non-isochromaticity ray diagram. (b) An image in Sherpa isochromaticity tuning procedure showing a large amount of non-isochromaticity (different colors in the image correspond to different energies of electrons).

Tuning of isochromaticity starts by clicking the “Tune” button in the Isochromaticity box in Sherpa. During the tuning the software first measures the isochromaticity and then improves it until the measured value reaches specification (Fig. 7).

The displayed measurement comprises of the amount of residual non-isochromaticity after the tuning and the specification for that value. If the measurement doesn’t reach specification it is colored in red. Once it reaches the specification, it turns green.

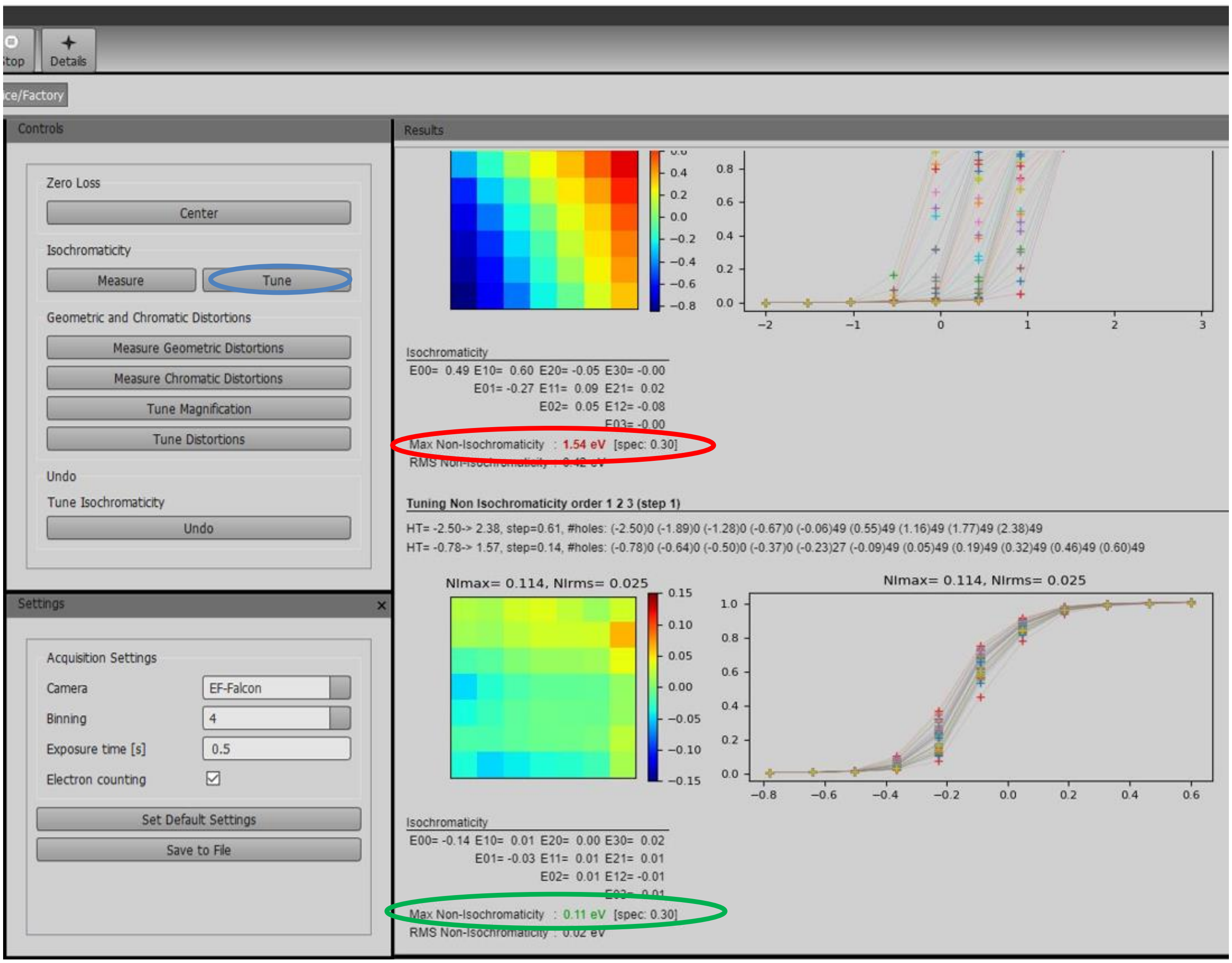


Fig. 7. Non-isochromaticity tuning procedure in Sherpa.

There is no need to measure Isochromaticity or Distortions before tuning them. When tuning is started, both values will be measured automatically and then improved. The measure function can be used in order to check current Isochromaticity or Distortion values without changing them.

2.3 Chromatic and Geometrical distortions tuning

The third and fourth steps of filter tuning are Chromatic and Geometrical distortion tuning.

1) Geometrical distortion

The lenses should properly project the entrance plane of the filter to the camera, without distortion and with uniform magnification. When lenses, stigmators or other multipoles are mistuned, the image may become (geometrically) distorted (Fig. 8).

These distortions are measured by inserting a mask with a hole pattern at the filter's entrance and analyzing the uniformity of the recorded mask image. The distortions and required lens corrections can be calculated from the relative positions of the holes in the camera image (Fig. 9).

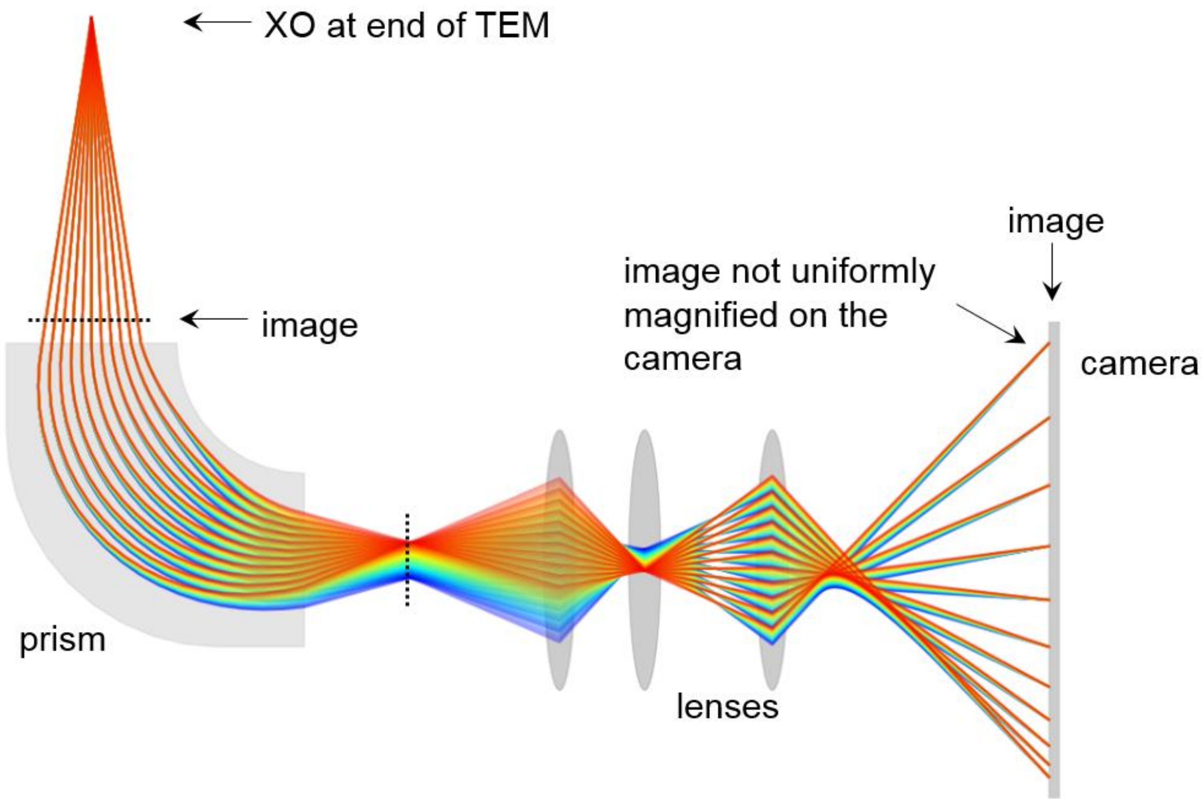


Fig. 8. Geometrical distortion ray diagram.

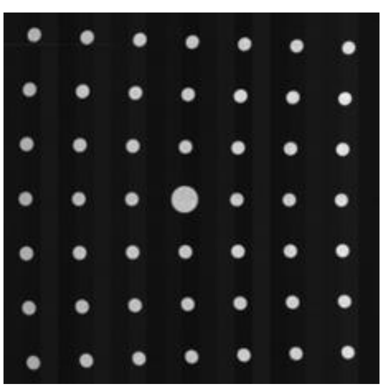


Fig. 9. Image with geometric distortion before tuning.

Rough tuning of Geometrical distortion is done by clicking the “Tune magnification” button (Fig. 10). During this procedure the value of tuned distortion does not typically reach the specification. That is a normal behavior and the residual Geometrical distortion will be fixed during the next step of filter tuning.

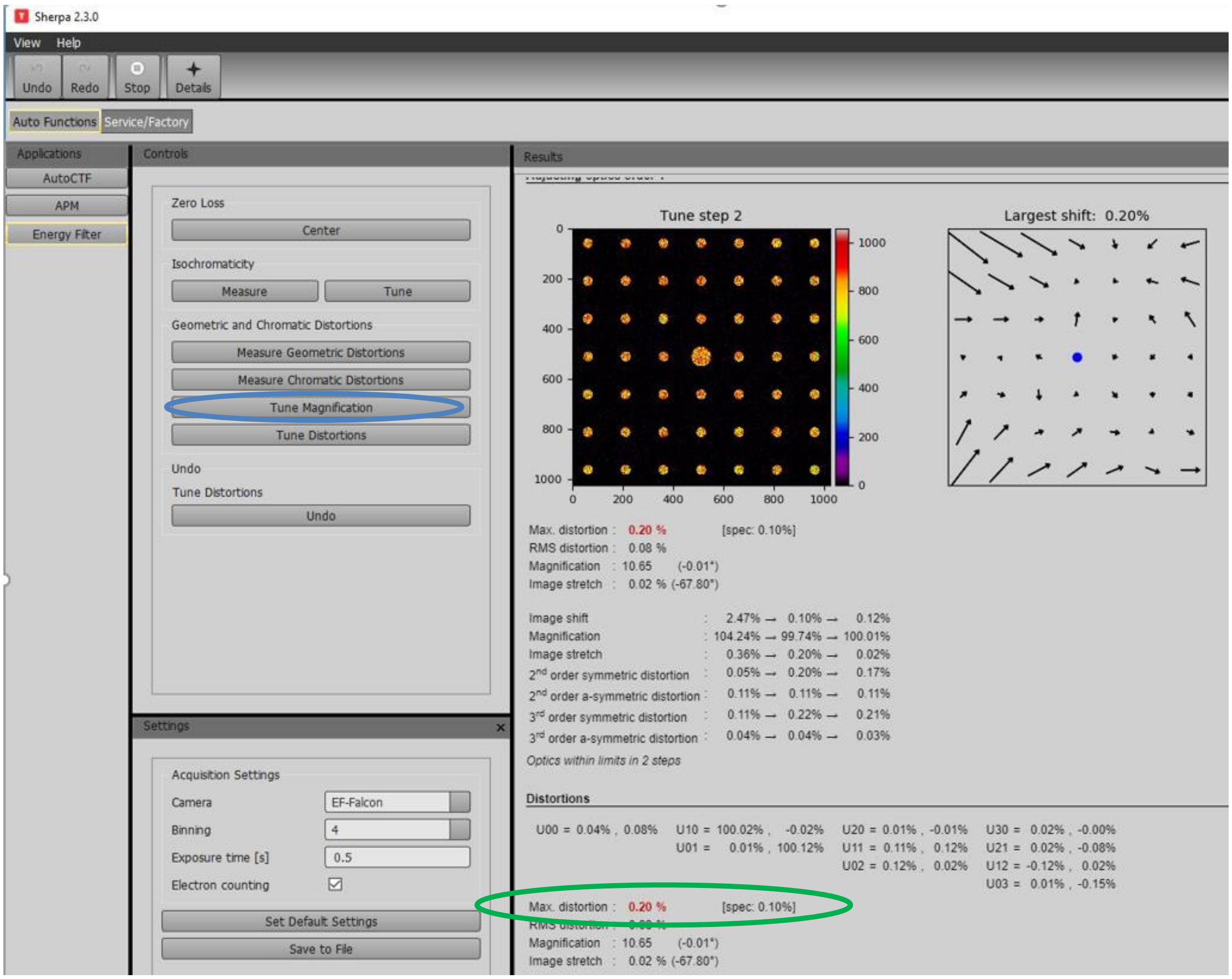
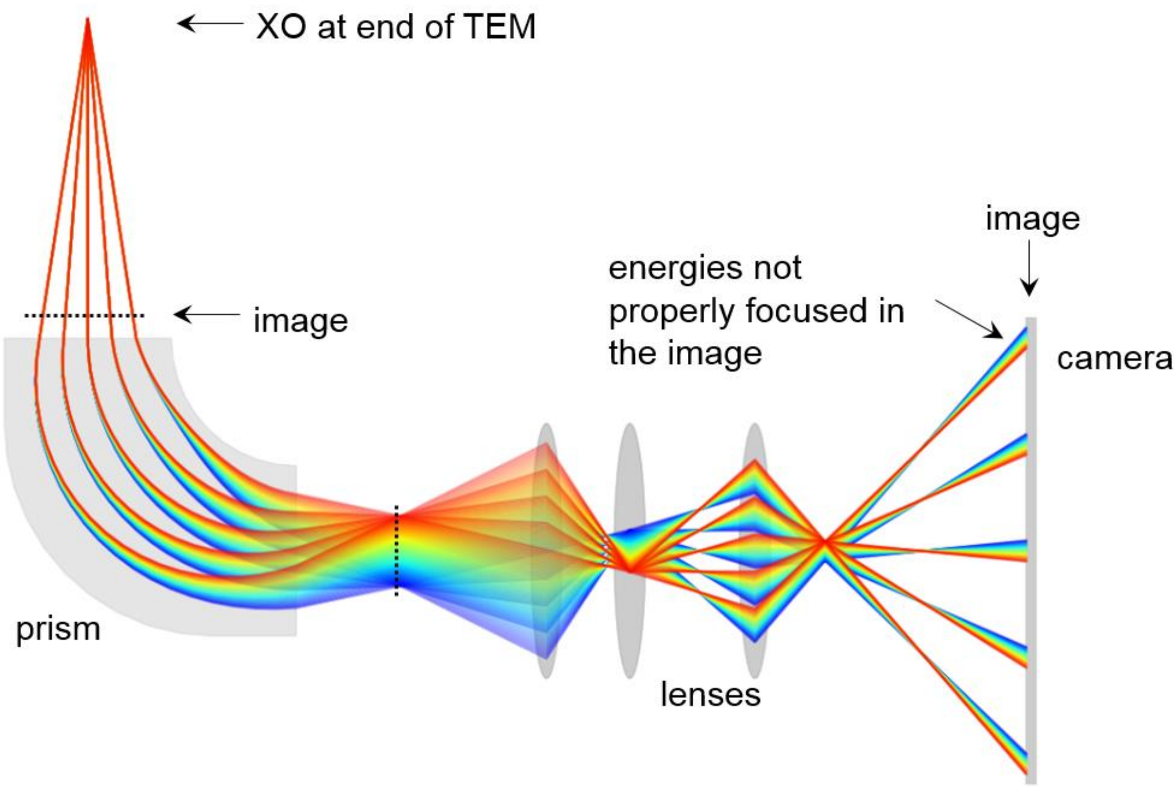


Fig. 10. Coarse tuning procedure of geometrical distortion in Sherpa.

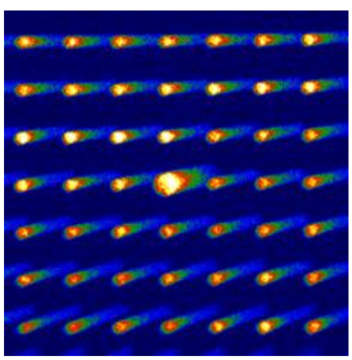
2) Chromatic distortion

All electrons coming from a specific position in the sample plane should be focused at the same position on the image plane at the camera, irrespective of their energies.

When this focus is not well tuned, electrons of different energies may be imaged at slightly different positions on the camera (Fig. 11).



a



b

Fig. 11 (a) Chromatic distortion ray diagram. (b) Image with large chromatic distortion before tuning: every mask hole is smeared like a small EELS spectrum.

Chromatic distortion is tuned together with fine Geometrical distortion tuning by clicking the “Tune Distortions” button. When both measured distortions reach specifications, they are highlighted in green in the output window (Fig. 12)

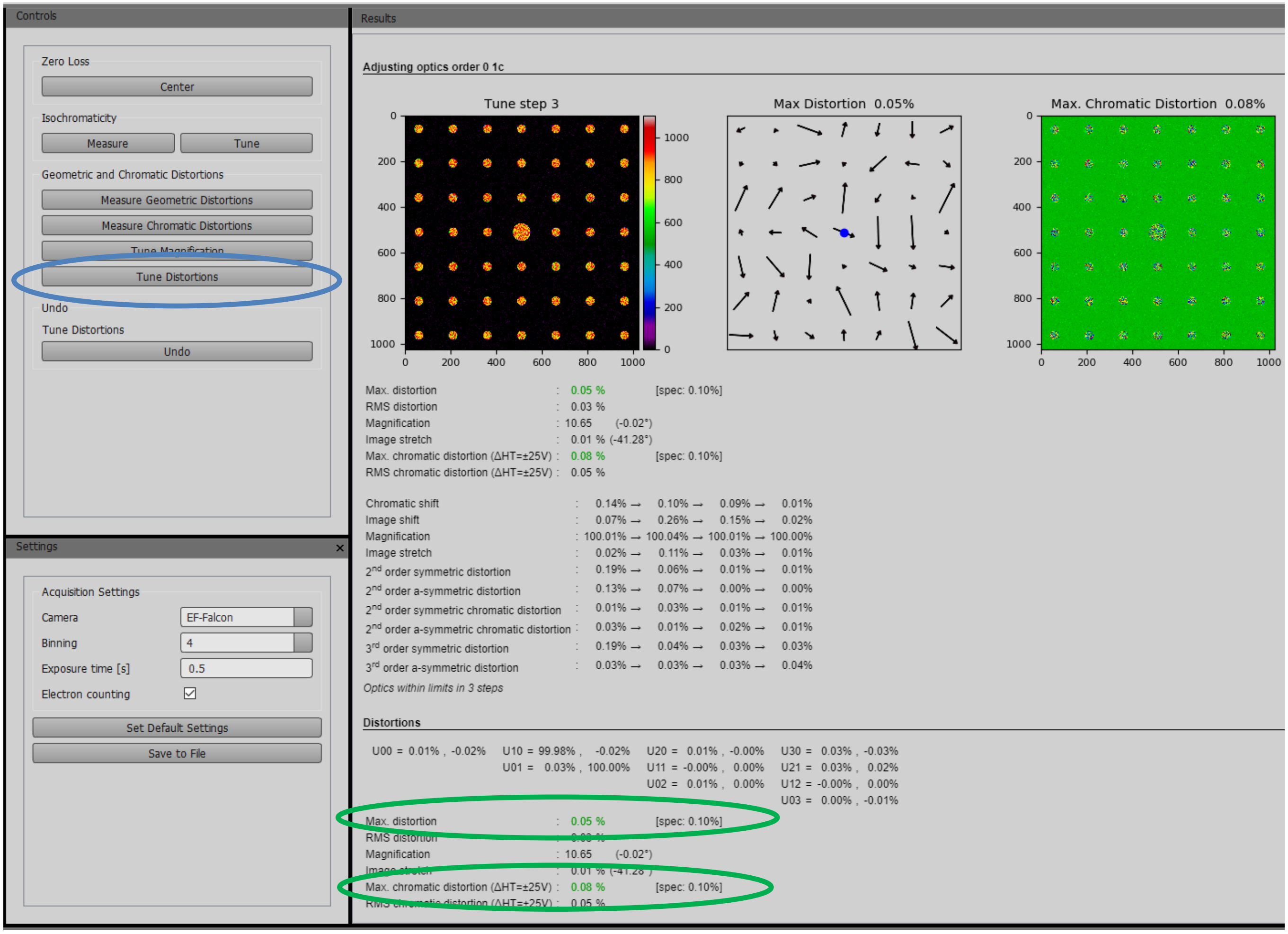


Fig. 12

After completing these steps, the filter is fully tuned.

3. How to use Selectris with EPU

Setting up data collection in EPU software using Selectris includes a few additional filter-related steps compared to data collection on a bottom mounted camera.

First, a user should decide on presets which will be used during the run. At this point the option “Insert slit” should be turned to “no” for all the presets.

There is an additional preset “Zero Loss” which is used to perform ZLP-centering in Autofunctions and during the data collection (Fig. 13). Optical conditions for this preset should be identical to the ones set in “Data Acquisition”. If for data acquisition the user chooses a dose rate on the camera that is in a lower range (below 3e/px/sec in counting mode) then the spot size for “Zero Loss” should be decreased appropriately. Exposure time for this preset should be ~0.5-1 sec.

Once optical conditions for all presets are defined, the filter tuning can be performed. In order to do that move the stage over a hole (best accuracy is obtained with no specimen in the field of view), set the data acquisition preset, check centering of the beam around the filter entrance aperture on the FluCam Viewer. Check that the gain reference is valid for these illumination conditions. If not, collect a new gain reference.

Then perform the filter tuning as described above.

When the filter has been tuned for one magnification, the “cross-over corrections” ensure that the tuning is also good for the other EFTEM magnifications. When you change the magnification, you can check that the filter is still properly tuned using the “Measure” buttons.

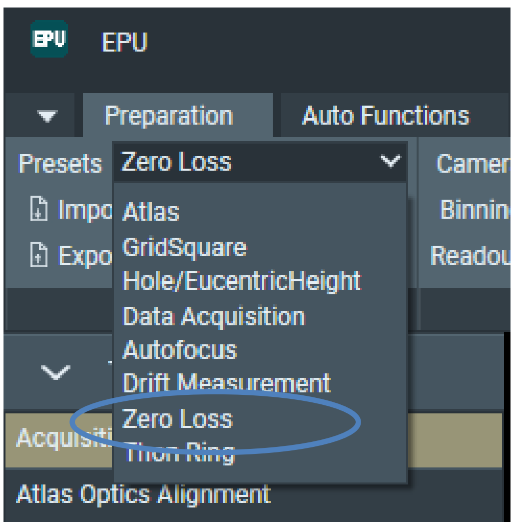


Fig. 13. Zero loss preset in EPU.

Once the tuning is finished, select “Insert Slit” to “YES” for all high magnification presets (Data Acquisition, Focusing, Zero Loss, etc.) and for Hole/Eucentric Height (Fig. 14). For the majority of cases, 10 eV is the best slit width. If the 10 eV slit comes in the field of view in Hole/Eucentric Height preset images, that indicates that the cross-over correction is not perfectly aligned. In that case, the slit width maybe increased to 20-30 eV for this preset and it is advised to re-align the cross over correction for the future (N.B.: this cross-over correction is not the same that is performed in the Direct alignments box of the UI, this cross-over correction is available in the Service tab in Sherpa).

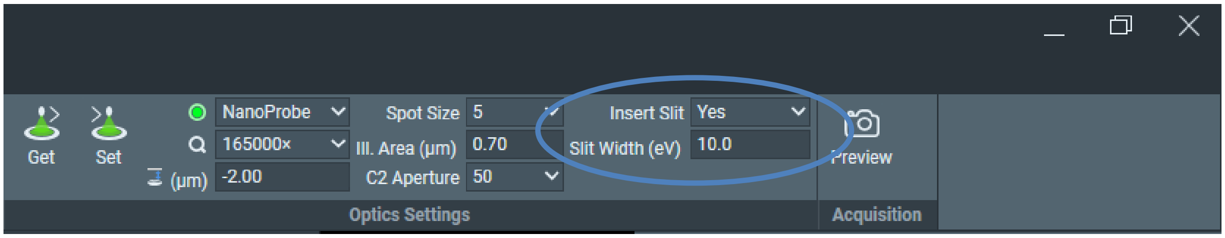


Fig. 14. Slit insertion box in EPU preset.

If during an EPU run ZLP readjustment is needed it will be performed at the optical and imaging conditions specified in Zero Loss peak preset. In order to check how this preset will work for automated ZLP alignment during data collection, go to Auto Functions tab, choose “Zero Loss” in the preset menu and run the Auto Zero Loss procedure (Fig 15).

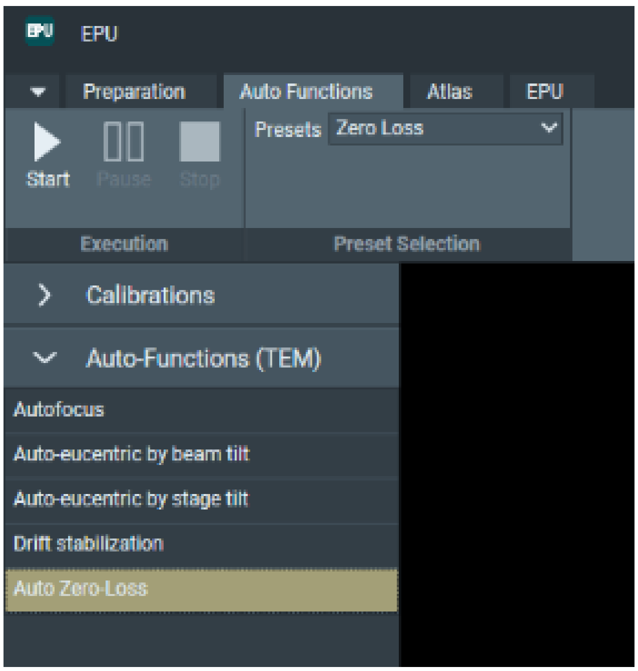


Fig. 15. Zero loss peak centering autofunction.

Then the EPU run can be set up as normal. Once it is done, the periodicity of the zero less peak centering can be adjusted in the EPU tab in “Automated acquisition” (Fig. 16). Set Auto Zero Loss to “yes” and specify after how many hours the centering procedure will be repeated. Centering after each 8 hours is a good starting point for a 10eV slit. More often centering may be needed if smaller slit width is used during data acquisition.

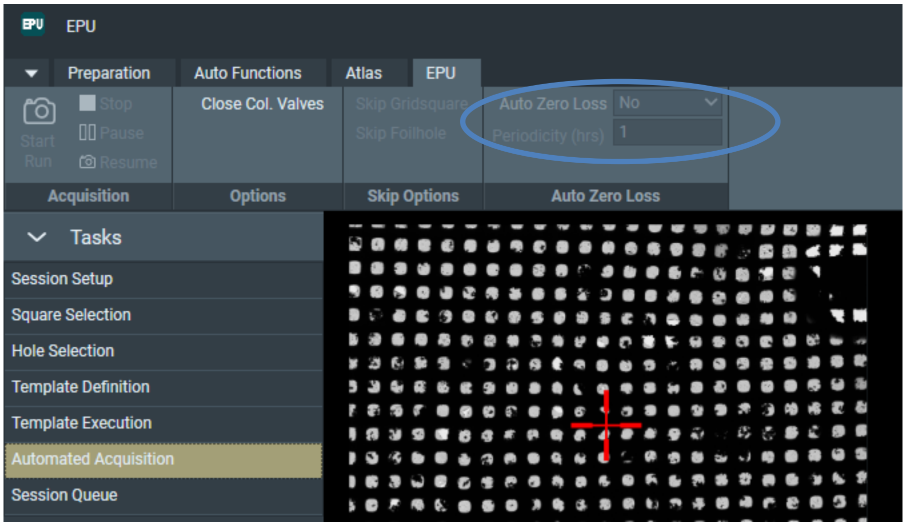


Fig. 16. Setting of periodicity of ZLP centering in EPU.

3. How to use Selectris with Tomography

Setting up data collection in Tomography software using Selectris includes a few additional filter-related steps compared to data collection on a bottom mounted camera.

First, a user should decide on presets that will be used during the run. At this point the option “Insert slit” should be turned to “no” for all the presets.

There is an additional preset “Zero Loss” to perform ZLP-centering in autofunctions and during the data collection (Fig. 17). Optical conditions for this preset should be identical to “Exposure” preset optics settings. If for data acquisition user chooses a dose rate on the camera that is in a lower saturation range (below 3e/px/sec in counting mode) then the spot size for “Zero Loss” should be decreased by 1 step. Exposure time for this preset should be ~0.5-1 sec.

Once the optical conditions for all presets are defined the filter tuning can be performed. In order to do that, move the stage over a hole (best accuracy is obtained with no specimen in the field of view), set the exposure preset to the microscope, check centering of the beam around the filter entrance aperture on the FluCam Viewer. Check that the gain reference is valid for these illumination conditions. If not, collect a new gain reference.

Then perform the filter tuning as described above.

When the filter has been tuned for one magnification, the “cross-over corrections” ensure that the tuning is also good for the other EFTEM magnifications. When you change the magnification, you can check that the filter is still properly tuned using the “Measure” buttons.

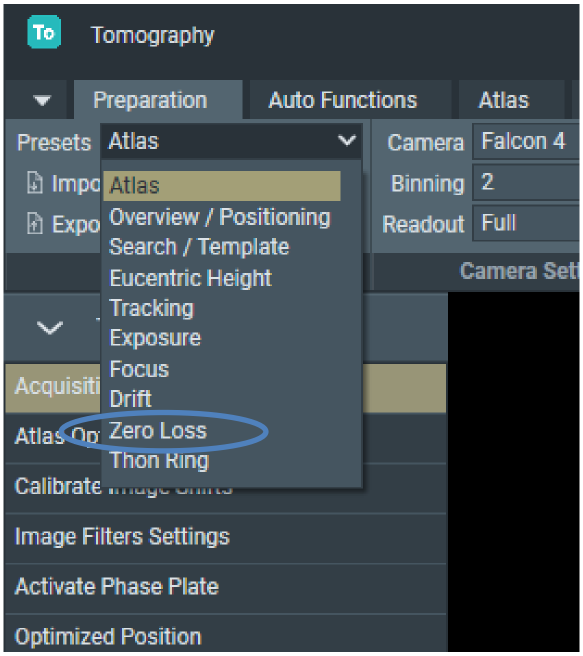


Fig. 17. Zero loss preset in Tomo.

Once the tuning is finished , select "Insert Slit" to "Yes" for all high magnification presets (Tracking, Exposure, Focus, Zero Loss, etc.) and for Search/Template (Fig. 18). For the majority of cases, 10 eV is the best slit width. If the 10 eV slit comes in the field of view in Search/Template images, that means that the cross-over correction is not perfectly aligned. In that case, the slit width maybe increased to 20-30 eV for this preset and it is advised to re-align the cross over correction for the future (N.B.: this crossover correction is not the same that is performed in the Direct alignments box of UI, this cross-over correction is available in the Service tab in Sherpa).

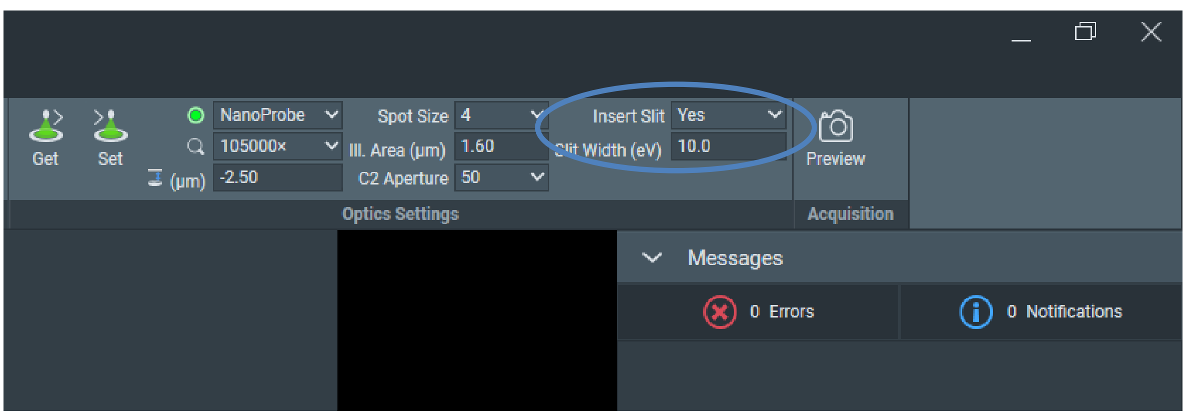


Fig. 18. Slit insertion box in Tomo preset

In order to check how the Zero Loss preset performs during data collection, go to Auto Functions tab, choose "Zero Loss" in the preset menu and run the Auto Zero Loss procedure (Fig. 19).

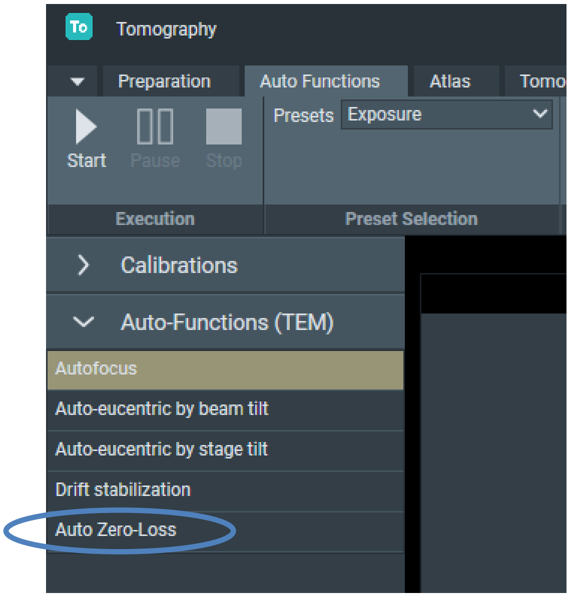


Fig. 19. Zero loss peak centering autofunction.

Then the Tomography run can be set up as normal. Once it is done the periodicity of the zero less peak centering can be adjusted in the Tomography tab in "Data acquisition" (Fig. 20). Tick the box Adjust ZLP to "yes" and specify after how many images the centering procedure will be repeated in the flap out menu. In general, for short Tomo runs (less than 8 hours) no ZLP centering is needed. For longer runs set the periodicity equal to number of images in the tilt series. So the centering is triggered in front of each next tilt series.

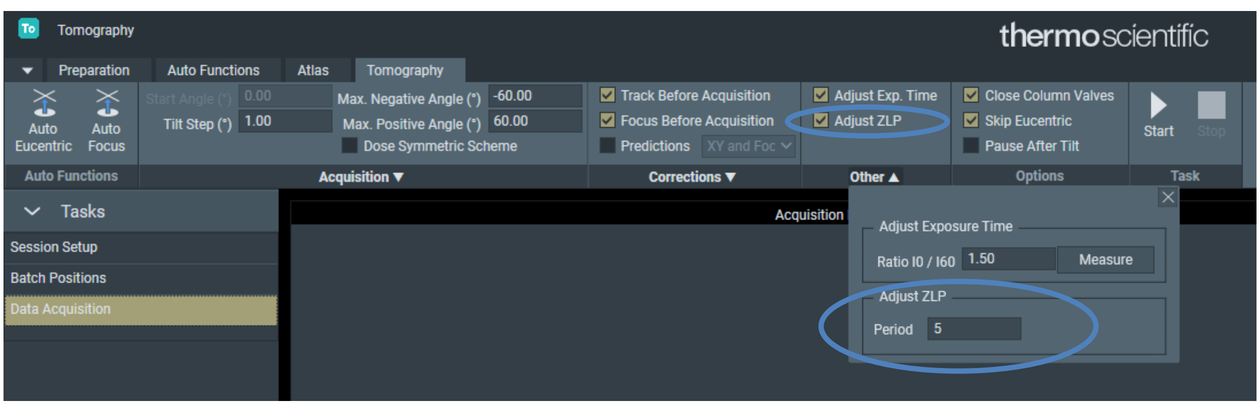


Fig. 20. Setting of periodicity of ZLP centering in Tomo.