ThermoFisher SCIENTIFIC

Sherpa

User Manual

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thermo scientific

Contents

1 Introduction

The Sherpa software offers automation and calibration applications for Thermo Scientific and FEI Transmission Electron Microscopes. The Sherpa software aims to:

- Improve the ease of use of the microscope for regular and advanced users.
- Optimize microscope performance and reliability.

1.1 Audience

This manual is aimed at users of Thermo Scientific Transmission Electron Microscopes. In particular, you should be able to successfully identify issues with the microscope. Morover, you should be able perform the necessary corrective actions to resolve the identified issues, provided that these actions are available. For higher level corrective actions, the assistance of a Thermo Fisher Scientific service engineer may be required.

1.2 Availability of Sherpa Applications

The availability of Sherpa applications depends on the system configuration and software version. This document and the table below only apply to functionalities that are available to users and supervisors. For functionalities that are restricted to Thermo Fisher Scientific engineers, please see the service documentation.

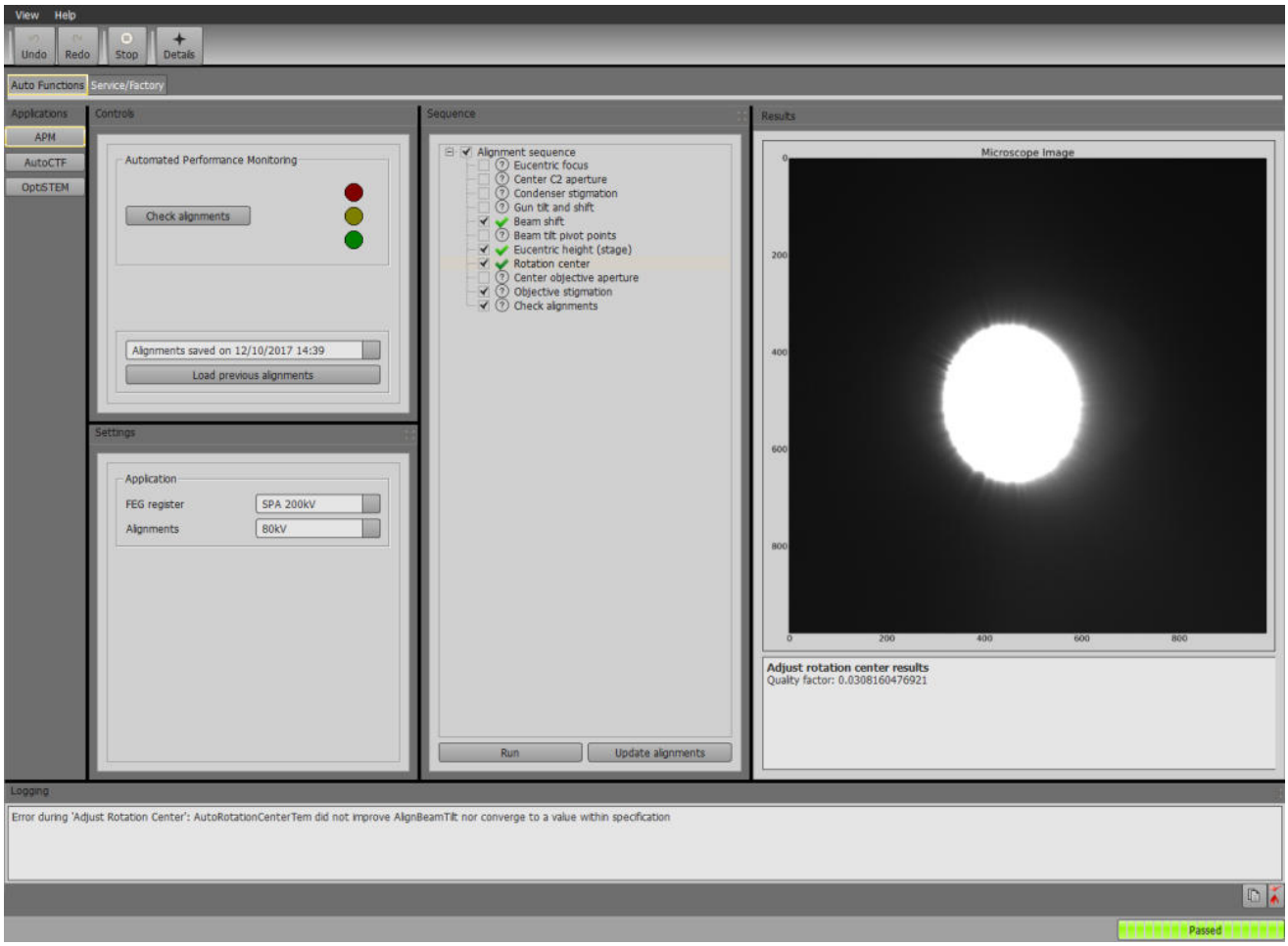
Application	System Types	Introduction	Remarks
OptiSTEM	Titan / Themis	Titan 2.6	Only C1 / A1 correction.
	ETEM		
	Metrios		
	Spectra / Krios G4	Titan 3.1	
OptiSTEM+	See OptiSTEM	See OptiSTEM	Adds A2 / B2 correction to OptiSTEM. Not available on Krios G4.
AutoSTEM	Talos F200X/C/S/i	Talos 1.9	Only C1 / A1 correction. Requires automated aperture mechanisms.
	Talos Arctica G2		
	Glacios		
OptiMono	All Titan	Titan 2.6	High Base with Monochromator and EELS filter.
	Spectra	Titan 3.1	
OptiMono+	Spectra	Titan 3.3	Adds Ultimono conditions to OptiMono.

Application	System Types	Introduction	Remarks
TEM Distortion Correction	Talos F200E		Only compatible with Ceta cameras.
Energy Filter	Krios	Titan 3.6	Systems with a Selectris or Selectris X Filter.
	Glacios	Talos 2.6	
AutoCTF	Titan Krios, Krios G3i	Titan 2.11	
	Titan Halo		
	Krios G4	Titan 3.1	
	Talos F200C	Talos 1.12	
	Talos Arctica (G2)		
	Glacios		
	Talos L120	Talos 2.1	
APM	Krios G3i	Titan 2.13	
	Krios G4	Titan 3.1	
	Talos Arctica	Talos 1.14	
	Glacios		
HT Conditioning	Talos L120	Talos 2.6	Systems with a Thermionic source.
Spot Saturation	Talos L120	Talos 2.3	
Align Genie	Talos F200 X/C/S/i	Talos 2.3	Not described in this User Manual. See *Sherpa* > *Align Genie* > *Help*

2 The User Interface

2.1 User Interface Layout and Common Functions

The Sherpa user interface provides multiple applications. Depending on the system configuration and software version, these applications are made available to the user via the Sherpa User Interface. The Sherpa User Interface is accessible from the **Microscope Software Launcher** > **Tools** menu.



Menu

- View: select which frames are displayed in the Auto Functions tab.
- Help: online help.

Toolbar

When a command or action is being executed, the button becomes yellow.

- Undo: restore settings to the values before a task or sequence was executed.
- Redo: restore settings to the values before the preceding *Undo* action.
- Stop: abort execution of an application and all changes made by the application so far.

- Details: display/hide detailed logging and intermediate results.

Auto Functions tab

The Auto Functions tab contains a selection of panels to select and perform manual and automated tasks.

- Applications: selection of the Sherpa applications that are available for the system configuration and software version.
- Controls: selections and buttons to execute tasks.
- Settings: selections and values used for task execution.
- Sequence: ordered list of checks, actions, or other items to orchestrate task execution.
- Results: images, charts and other (graphical) data that are acquired or generated during task execution.
- Logging: detailed information about the task execution.

The size and position of the panels can be customized.

- To hide a frame, select the cross in the top right corner.
- To show it again, select it in the *View* menu.

Service/Factory tab

Applications that are only available for Thermo Fisher Scientific engineers, e.g. calibrations. The applications in the Service/Factory tab are not described in this manual.

Status bar

Task execution status, progress or result.

When a function fails, a button appears with the number of errors. Select this button to view the reported errors.

2.2 Undo and Redo

Select **Undo** to restore the microscope settings to the values they had prior to starting a procedure.

Select **Redo** to restore the microscope settings to the values they had just before the preceding Undo action.

The buttons become yellow when a procedure is active. When the values are successfully restored, the result is shown in the Logging area. In case a procedure fails, an error message is shown in the Logging area.

The Undo/Redo mechanism works stack-based.

- For each successfully completed procedure, the corresponding settings are added to a stack.
- When the cursor moves over the Undo button, a tooltip indicates for which procedure the settings are restored.
- The stack is limited to 100 actions. When this limit is reached, the oldest settings are removed.

- The entire stack is cleared when Sherpa is restarted.

The Undo/Redo function does not behave the same for all Auto Functions.

Application	**Undo/Redo supported**	**Remarks**

OptiSTEM (+)	Yes	• Settings are cleared from the undo stack when the High Tension setting changes.
		• The Undo and Redo functions only work when the microscope settings are identical to the settings that were active at the moment that the application procedure is started:
		  • The optical mode must be STEM probe nP
		  • Magnification must be in the SA range.
		  • C2 lens and, if present C3 lenses must be on.

AutoSTEM	Yes	
OptiMono and OptiMono+	Yes	No remarks
Energy Filter	Yes	No remarks
all other applications	No	

3 OptiSTEM and OptiSTEM+

3.1 Introduction

OptiSTEM and OptiSTEM+ are Sherpa apllications that reduce the time-to-image for high resolution STEM images.

The *OptiSTEM* application automatically tunes the defocus (C1) and two-fold condenser astigmatism (A1) aberrations. In comparison to manual tuning, this saves a considerable amount of time.

For ultimate STEM performance, the optional *OptiSTEM+* application also optimizes coma (B2) and three-fold condenser astigmatism (A2). Traditionally, these second-order aberrations are tuned with the probe corrector software on a cross grating specimen. By the time the experiment can be started on the specimen of interest, the aberrations may have drifted just far enough to loose optimal performance. OptiSTEM+ optimizes coma and three-fold astigmatism in only a few minutes and on the specimen of interest.

Note **OptiSTEM is optimized for use with a DCOR probe corrector.**
Although tested on a wide range of materials at all available High Tension values, performance may vary with the specimen type, and is not guaranteed on non-probe-corrected microscopes.

3.2 Preconditions

The following conditions must be fulfilled before OptiSTEM and/or OptiSTEM+ can run successfully:

- Drift and contamination levels are within limits.
- Optics is aligned for HR-STEM imaging.
- For best performance, the Probe corrector must be tuned so that third-order aberrations and higher are not limiting resolution. From experience, these aberrations are stable for more than a week in case the system runs stable at constant high tension.
- System is set to STEM nP (SA mode) in three condenser mode.
- Specimen is at eucentric height.
  If desired, the specimen orientation is set to the zone axis.
- The microscope is not too far from:
  - Focus
  - Two-fold astigmatism.
  - Three-fold astigmatism.
  - Coma.

3.3 Procedure

Before starting the *C1 / A1* or *A2 / B2* procedure, make sure that:

- High Tension is on.
- The FEG is operational.
- The column valves are open.

1. In the **TEM User Interface**, set the system to **STEM nP** in **Three condenser** mode
2. Select an **SA magnification**.
3. Move the specimen to the **Region of Interest**
4. Bring the specimen to **Eucentric Height**.
5. If desired, adjust **Alpha** and/or **Beta** tilt to match the specimen zone axis.
6. Adjust the **Stigmators** and adjust **Focus** until there is atomic resolution.
7. In the **Controls** panel, depending on the coma and three-fold astigmatism status:
   - Select **A2 / B2** to optimize defocus, two-fold astigmatism, coma and three-fold astigmatism. This action will typically take a few minutes.
   - Select **C1 / A1** if coma and three-fold astigmatism are already within acceptable limits. This action will typically take less than 1 minute.

   When a procedure is started, a plot appears that shows the measured aberrations after each optimization cycle. In a successful run, the aberrations decrease towards zero. If a procedure fails or when a procedure is aborted all adjusted parameters are reverted to the values they had before the procedure was started.
8. In the **TEM User Interface** > **STEM Imaging** control panel:
   a. If **Blank** is yellow, select **Blank** to unblank the beam.
   b. Select **Acquire**.

3.4 Controls

- **C1 / A1**
  The first order aberration correction procedure is executed.
  This procedure optimizes defocus and two-fold astigmatism.
- **A2 / B2**
  The first order aberration correction procedure (C1 / A1) is executed, automatically followed by the second order aberration correction procedure (A2 / B2). The second order procedure optimizes coma and three-fold astigmatism.
  The A2 / B2 function is only available for OptiSTEM+, not for regular OptiSTEM.

3.5 Tips & Tricks

- The C1 / A1 procedure usually takes less than 1 minute. When the initial defocus and twofold astigmatism are already close to the optimal values, the procedure will be completed even faster.

  The 1st order aberrations have to be tuned every few minutes for optimal results, for example after changing the region of interest.
- The A2 / B2 procedure optimizes all 1st and 2nd order aberrations. This procedure will take much longer than the 1st order aberration correction procedure.
  The 2nd order aberrations not as volatile as the 1st order aberrations. Execute the A2 / B2 procedure once or twice per few hours while using the microscope.
  To minimize the execution of time-consuming corrector alignments in the CEOS Corrector User Interface and swapping samples, run the A2 / B2 procedure at least once per day.
- The 3rd and higher order aberrations cannot be tuned with OptiSTEM or OptiSTEM+.

- For a probe-corrected system, typically only three reflection pairs need to be visible in the FFT for a crystalline sample (e.g. silicon 110).

  For non-probe corrected systems, the coarse tuning of the aberrations must be closer to the optimal value than for probe corrected systems due to spherical aberrations.
- For some amorphous specimens like amorphous carbon, OptiSTEM will also work well at lower magnifications. This feature can sometimes be exploited for HR-STEM imaging as well. For instance, for a cross grating sample it is possible to optimize aberrations in a lower magnification range (up to 200k - 300k), and then zoom in to higher magnification for HR-STEM imaging.

3.6 Troubleshooting

OptiSTEM uses STEM images to correct aberrations. It is therefore important create a contrast-rich STEM image before starting OptiSTEM(+). For crystalline specimens, typically three reflection pairs in the FFT of the STEM image are enough for OptiSTEM to work.

The resolution of the final STEM image depends on various characteristics and settings.

- Optical alignment: degree of 1^{st} and 2^{nd} order aberrations, probe corrector alignments (state of higher order aberrations), optical settings (convergence angle, magnification).
- Stage settings: eucentric Z-height of sample, zone-axis orientation (depends on the specimen).
- Specimen: elemental composition, thickness, crystallinity, lattice parameters, contamination levels.
- Optical, thermal and mechanical drift.

OptiSTEM and OptiSTEM+ optimize the 1^{st} and 2^{nd} order aberrations, but have no influence on the other settings or sample characteristics. In case OptiSTEM fails, often the reason is that preconditions are not met. The Sherpa logging may provide guidance to find the cause and correct it.

3.6.1 Suggestions to Improve the Preconditions for OptiSTEM(+)

- Try a different part of the specimen.

  OptiSTEM works better with specimens that are thin. For crystalline samples typically three reflection pairs in the FFT are enough for OptiSTEM+ to work.
- Make sure that the detector used by OptiSTEM is aligned with respect to the optical axis.

  Read the Tips & Tricks section on what detector is used by OptiSTEM. In case another detector is preferred, insert the preferred detector and retract all others. If not all detectors can be retracted, at least retract the ones higher up in the following top-down hierarchy: HAADF, DF4, DF2, BF.
- Try to change the magnification level to improve the contrast in the image. Especially for specimens that have a low contrast (like graphene), it can help to tune on a feature in the sample. In this case, OptiSTEM can be used at a lower magnification to tune the lower order aberrations, but one can zoom in to higher magnification for the high resolution image.
- Tune the probe corrector with the CEOS Corrector User Interface.

  Select **TEM User Interface** > **STEM Autotuning** control panel > **Settings** tab > **Reset** before tuning the corrector.
- Make sure there is no excessive contamination on the specimen. Try plasma cleaning the sample or another means to limit the contamination during STEM.
- Make sure that drift is within limits.
- Check the HR-STEM optical alignments.

In case all the suggestions above fail, try to perform the OptiSTEM(+) procedure with a reference sample, for example silicon 110. If no HR-STEM image can be made with a reference sample, then there is probably something wrong with the system alignments.

Note **Non-Probe Corrected systems:**
Do not renew the focus calibration unless it is absolutely required.

The focus calibration influences the calibration of OptiSTEM. When the focus calibration is redone, also the OptiSTEM calibration must be performed again. This calibration is only available to service and factory engineers, not to customers.

4 AutoSTEM

4.1 Introduction

The AutoSTEM application corrects focus and astigmatism in STEM mode. The application works best at STEM magnifications up to 1 million. AutoSTEM provides a shorter time to improved imaging conditions.

Without AutoSTEM, the tuning of the defocus (C1) and twofold condenser astigmatism (A1) must be performed by the user. This can be a time-consuming process and in addition can deteriorate the sample quality. AutoSTEM automates this process which leads to better results in a reduced time frame.

AutoSTEM requires Automated Aperture Mechanisms (AAM).

4.2 Preconditions

The following conditions must be fulfilled before AutoSTEM can run successfully:

- Drift and contamination levels are within limits.
- Optics is aligned for HR-STEM imaging.
- System is set to STEM nP (SA mode).
- Specimen is at eucentric height.
  If desired, the specimen orientation is set to the zone axis.
- The microscope is not too far from:
  - Focus
  - Two-fold astigmatism.
  - Three-fold astigmatism.
  - Coma.

4.3 Controls

- **C1 / A1**
  Select C1 / A1 to start the first order aberration correction procedure for defocus and two-fold astigmatism. During the C1/A1 procedure, a plot appears which shows the evolution of the aberrations with respect to the algorithm iteration. A successful run will have the C1 and A1 iterating towards zero.

4.4 Procedure

1. In the **TEM User Interface**, set the system to **STEM nP** mode.
2. Select an **SA magnification**.
3. Move the specimen to the region of interest.
4. Bring the specimen to **Eucentric Height**.
5. If desired, adjust **Alpha** and/or **Beta** tilt to match the specimen zone axis.

6. Adjust the **Stigmators** and adjust **Focus** until there is atomic resolution.
7. Select **Controls** > **C1 / A1** to optimize defocus and two-fold astigmatism.
   This action will typically take up to 1 minute.
8. In the **TEM User Interface** > **STEM Imaging** control panel:
   a. If **Blank** is yellow, select **Blank** to unblank the beam.
   b. Select **Acquire**.

4.5 Tips & Tricks

- The first order aberration correction procedure (C1/A1 button) is a fast procedure. It takes usually less than 1 minute. In case the initial conditions were already close to the optimal settings for defocus and two-fold astigmatism, the procedure will be faster than when the conditions are further away from the optimum.
- The C1 / A1 button can be used after changing the region of interest. The first order aberrations have to be tuned every few minutes for optimal results.
- For some amorphous samples like amorphous carbon, AutoSTEM will work also well at lower magnification. This feature can sometimes be exploited for HR-STEM imaging as well. For instance, for a cross grating sample it is possible to optimize aberrations at a lower magnifications (100kX - 300kX) and then zoom in to higher magnification for HR-STEM imaging.
- Optimization of the aberrations will be done on only one inserted detector. In case multiple detectors are available and inserted, the optimization will be done on the detector that is first in the following sequence:
  HAADF > DF4 > DF2 > BF.
  In case no detector is inserted, the detector that is highest in the hierarchy will be inserted automatically.

4.6 Troubleshooting

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5 OptiMono

5.1 Introduction

The OptiMono application improves the ease of use of the monochromator. With a few mouse-clicks, OptiMono achieves optimal energy resolution and optimizes mono-stigmation and focus for a given mono excitation.

5.2 Preconditions

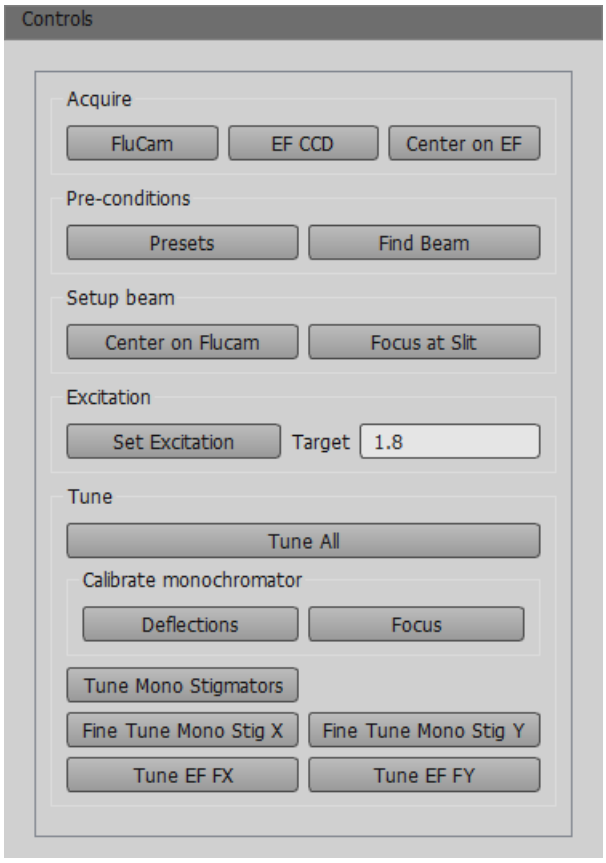
The following conditions must be fulfilled before OptiMono can run successfully:

- The Monochromator and mono slits are aligned.
- The energy filter is:
  - in EELS mode,
  - aligned,
  - set to the desired dispersion.

  Although OptiMono includes an automated fine focus for the filter, the energy offset, coarse focus, stigmation and AC compensation must be manually adjusted before the OptiMono functions are used.

5.3 Procedure

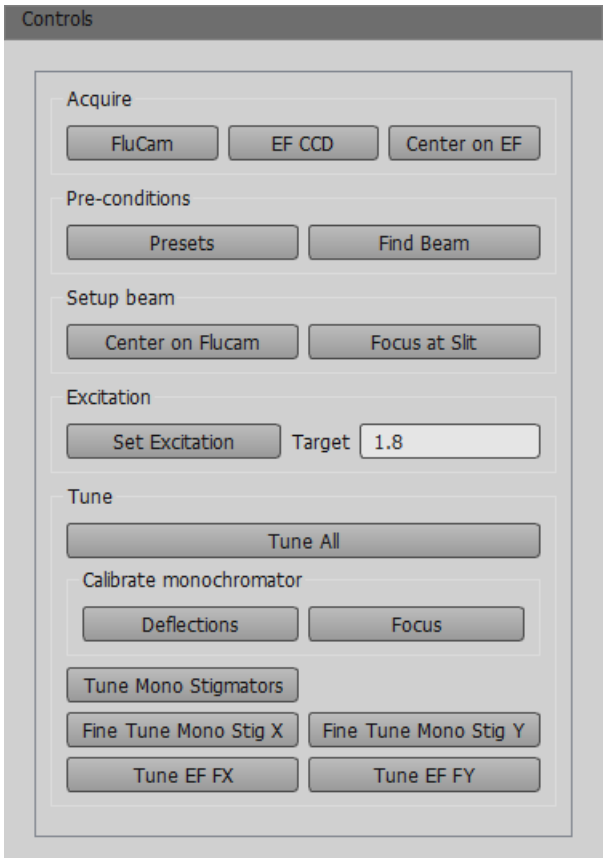
The typical workflow to get an optimized monochromated beam is performed in the Controls panel.



1. In **Pre-conditions**:
   a. Select **Presets** to select the correct optical setting.
   b. If the beam is not visible on the FluScreen, select **Find beam**.
2. In **Setup beam**:
   a. Select **Center on FluCam** to optimize beam alignment with the filter.
   b. Select **Focus at Slit** to correct the image plane.
3. In **Excitation**:
   a. Specify the desired **Target** excitation.
   b. Select **Set Excitation** to ramp to the specified Target excitation.
   Wait until the Target excitation is reached.
4. In **Tune**:
   a. Select **Tune All** to optimize the mono shifts, focus and stigmators, and fine tune the EF focus.
   b. If desired, select **Fine Tune Mono Stig X** and **Fine Tune Mono Stig Y** to fine-tune the Mono Stigmation.

5.4 Controls

The OptiMono functions are grouped according to their purpose and chronological usage.



Acquire

The Acquire functions are used to look at the beam either on the FluCam or the EF CCD. They do not specify or adjust any values.

- **FluCam**
  Displays the FluCam image, including the EF entrance position. Additionally, the fitted beam center position is reported in the detailed logging.
- **EF CCD**
  Displays the image from the post-filter camera. Additionally, the energy spread of the beam is fitted to the image.
- **Center on EF**
  Center the beam on the EF entrance aperture.

Preconditions

The precondition functions ensure that the microscope has the correct optical mode to optimize the monochromated beam and allows you to find the beam if no beam is visible.

- **Presets**
  This function sets the microscope to the correct optical settings:
  - The Monochromator is set to filtered mode.
  - System is set to two-condenser imaging mode.
  - Magnification is set to the lowest SA magnification.
  - Aperture mechanisms are retracted, or are set to the largest aperture.

- **Find Beam**

  This function works the same as the **TEM User Interface** > **Monochromator** control panel > **Find Beam** function. If necessary or desired, select optional pre- and post-conditions in the **Settings** panel.

Setup Beam

The setup beam functions will fine tune the optical settings so that the monochromator can be excited and properly focused and stigmated.

- **Center on FluCam**

  This function centers the beam and optimizes the optics and gun lens for optimal visibility.

- **Focus at Slit**

  This function automatically inserts a monochromator slit and adjusts the intensity such that the slit plane is imaged (analogous to the manual slit focusing using the slit wobbler).

Excitation

- **Set Excitation**

  This function will automatically ramp the excitation to the specified *Target value*. While ramping, the function will focus the beam and adjust the mono shifts to keep the beam on the FluScreen.

Tune

The Tune functions will optimize the monochromator focus, shift and stigmation as well as the EF focus to get to a properly focused and stigmated monochromated beam that is centered on the EF entrance as well as measure the energy spread of the beam.

- **Tune All**

  This function will perform all the steps necessary in a predefined order to get to the final result:

  - Calibrations
  - Coarse Tuning
  - Fine Tuning
  - EF Focus Tuning

- **Calibrate Deflections**

  This function will calibrate the deflections caused by the different monochromator elements. These calibrations are then used to compensate for these shifts and keep the beam in the correct position.

- **Calibrate Focus**

  This function is used to determine the optimal focus for different values of the mono stigmator X. These results are used to keep the beam focused while optimizing the mono stigmator X.

- **Tune Mono Stigmators**

  Tuning of the monochromator focus and stigmators based on the FluCam image. This function provides a basic setting that can later be fine-tuned on the energy filter.

- **Fine Tune Mono Stig X**

  Perform the fine tuning of the monochromator stigmator X based on minimization of the energy spread as measured on the energy filter.

- **Fine Tune Mono Stig Y**

  Fine tune the monochromator stigmator Y by minimizing the width of the dispersion as measured on the FluCam.

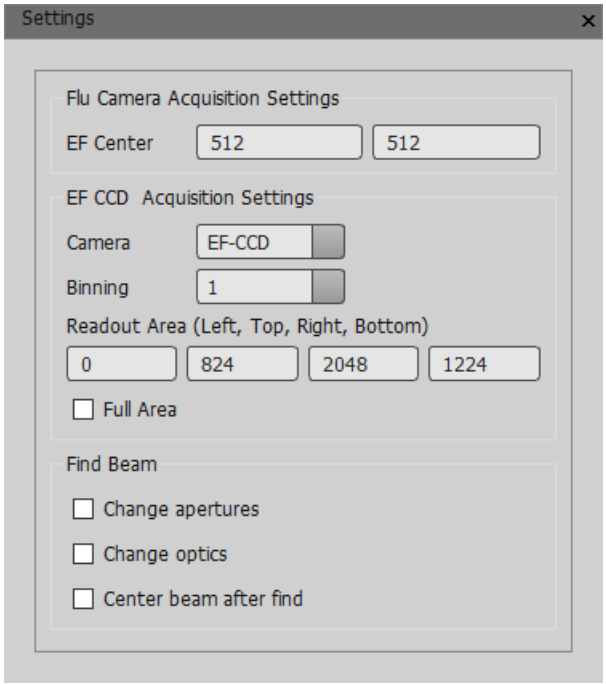
- **Tune EF FX**

  Optimize the EF focus X by minimizing the width of the dispersion.

- **Tune EF FY**
  Optimize the EF focus Y by minimizing the angle of the dispersion.

5.5 Settings

The OptiMono Settings can be used to adjust some of the inputs for the automated functions.



Flu Camera Acquisition Settings

- **EF Center**
  Specify the X and Y coordinate of the beam center on the FluScreen.
  See Align the Energy Filter Entrance Position with the Energy Filter Entrance Aperture on page 21.

EF CCD Acquisition Settings

- **Camera**
  If the Energy Filter has more than one post-filter camera, then select the camera for image acquisition.
- **Binning**
  Set the binning used to acquire an EF CCD image. Note that higher binning value will give less accurate results.
- **Readout Area (Left Top, Right, Bottom)**
  Define the area of the post-filter camera sensor that is displayed and used for the various functions.
- **Full Area**
  Set the Readout Area values to the entire post-filter camera sensor.

Find Beam

- **Change apertures**
  Before starting the Find Beam algorithm, the largest C1 / C2 / C3 apertures are inserted and the Objective and SA apertures are retracted. This maximizes the beam diameter at the FluScreen and increases the speed at which the beam can be found.
- **Change optics**
  Before starting the Find Beam algorithm, the lowest SA magnification is set and Spot size 1 is selected. This maximizes the beam diameter at the FluScreen and increases the speed at which the beam can be found.
- **Center beam after find**
  Automatically centers the beam after the beam has been found.

5.6 Tips & Tricks

Intentionally left blank.

5.7 Troubleshooting

General

- Verify that the preconditions are met.
  See Preconditions on page 16.
- In the **Controls** section:
  - Select **Preconditions**: **Presets** to make sure that the microscope is in the correct optical mode.
  - Select **Acquire**: **FluCam** and **Acquire**: **EF CDD** to verify that both cameras are working properly.

Find Beam fails

- Verify that there is no specimen or holder in the column that blocks the beam.
  If necessary, unload the specimen or retract the holder.
- Check the **error logging**.

No beam or misaligned beam on the Energy Filter

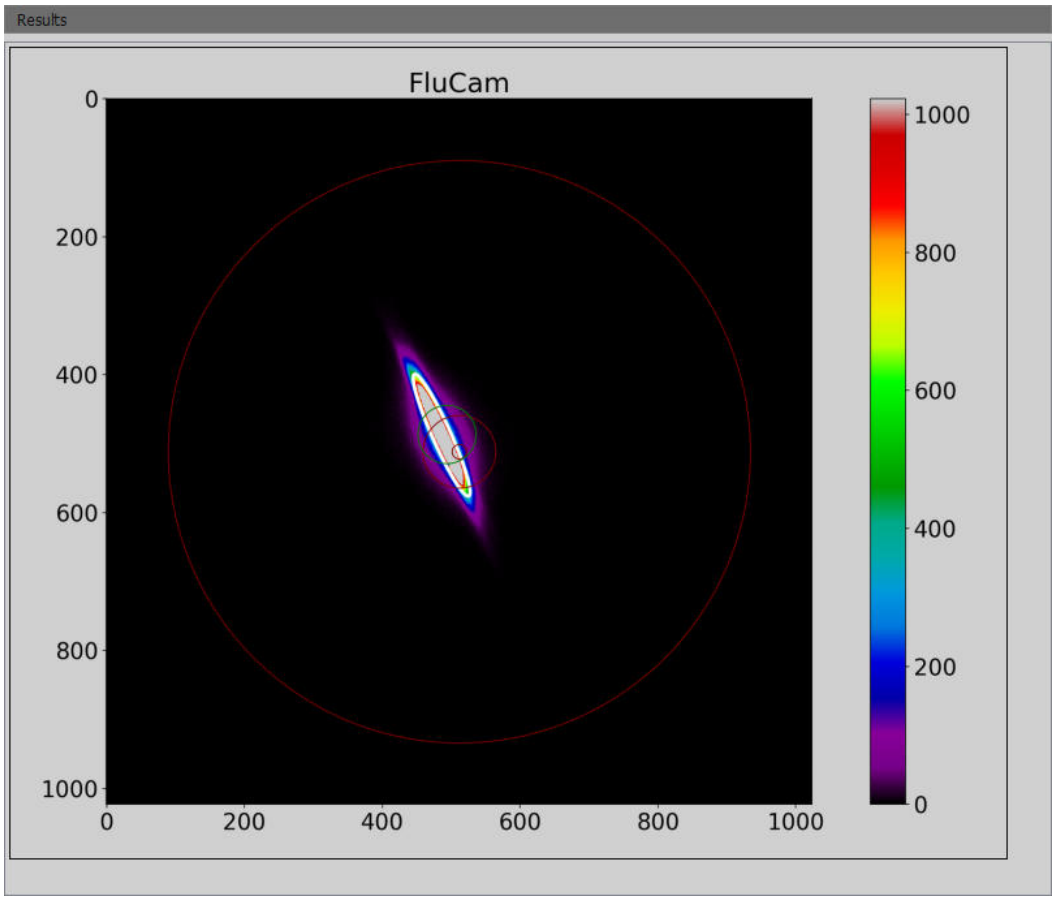
- Verify that the preconditions are met.
  See Preconditions on page 16.
- Verify that the center of the beam is aligned with the entrance of the energy filter.
  See Align the Energy Filter Entrance Position with the Energy Filter Entrance Aperture on page 21.

5.7.1 Align the Energy Filter Entrance Position with the Energy Filter Entrance Aperture

The *Energy Filter (EF) entrance position* is the position of the beam center on the FluScreen when acquiring a spectrum image. If this position is not the same as the mechanical position of the EF entrance aperture, then the filter might not be able to accurately determine the energy spread of the beam.

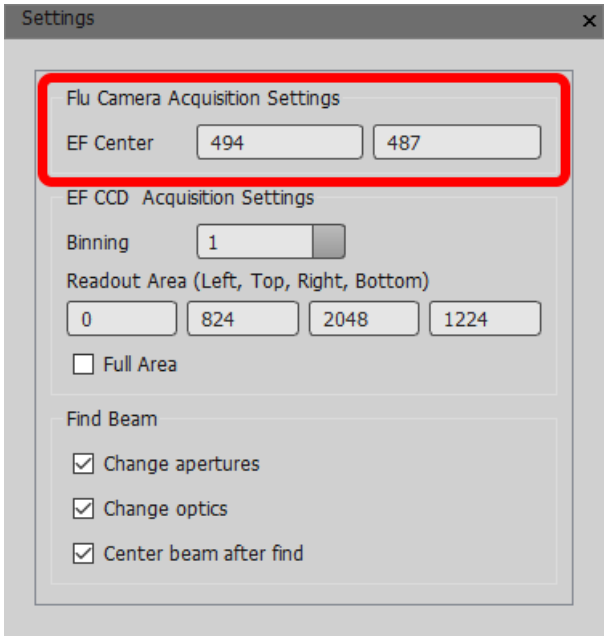
To verify and align the *EF entrance position* with the *EF entrance aperture*, follow the steps below.

1. Verify that the *EF entrance position* is aligned with the *EF entrance aperture*:
   a. Retract the **FluScreen**
   b. Adjust **Beam Shift** to maximize the signal on the post-filter camera.
   The *center of the beam* is now aligned with the *EF entrance aperture*.
   c. Insert the **FluScreen**
   d. Select **Controls** > **Acquire**: **FluCam**
   e. Verify that the *EF entrance position* (green circle) is located at the *center of the beam*.
   Note that the center of the beam does not have to be at the center of the FluScreen.



2. If the *EF entrance position* is not at the *center of the beam*,
   then adjust the **EF Center** position coordinates.
   a. In the toolbar, select **Details**
   b. In the **Logging** panel, look for the **X and Y coordinates** of the beam center position.

c. In **Settings** > **Flu Camera Acquisition Settings**: **EF Center**, enter the **X and Y coordinates** of the beam center position.



d. Select **Controls** > **Acquire**: **FluCam** to acquire a new FluCam image.

e. Verify that the *EF entrance position* is aligned with the *center of the beam*.

6 OptiMono+

6.1 Introduction

OptiMono+ is an extension of the OptiMono application for systems with the Ultimono option. With OptiMono+, the monochromator can be tuned automatically under Ultimono conditions.

6.2 Preconditions

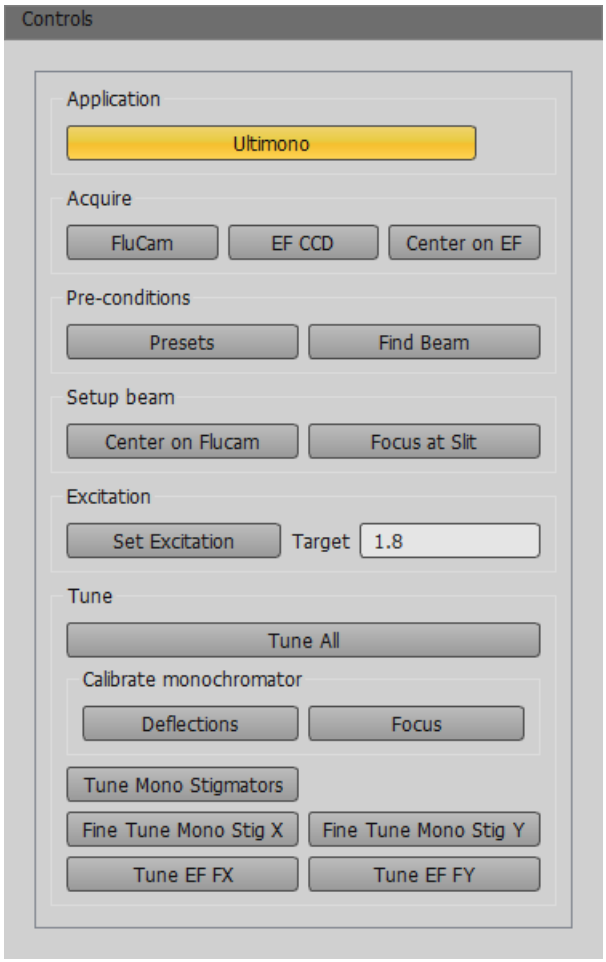
The Preconditions for OptiMono+ are the same as for the regular OptiMono auto-function.

- The Monochromator and mono slits are aligned.
- The energy filter is:
  - in EELS mode,
  - aligned,
  - set to the desired dispersion.

  Although OptiMono includes an automated fine focus for the filter, the energy offset, coarse focus, stigmation and AC compensation must be manually adjusted before the OptiMono functions are used.

6.3 Procedure

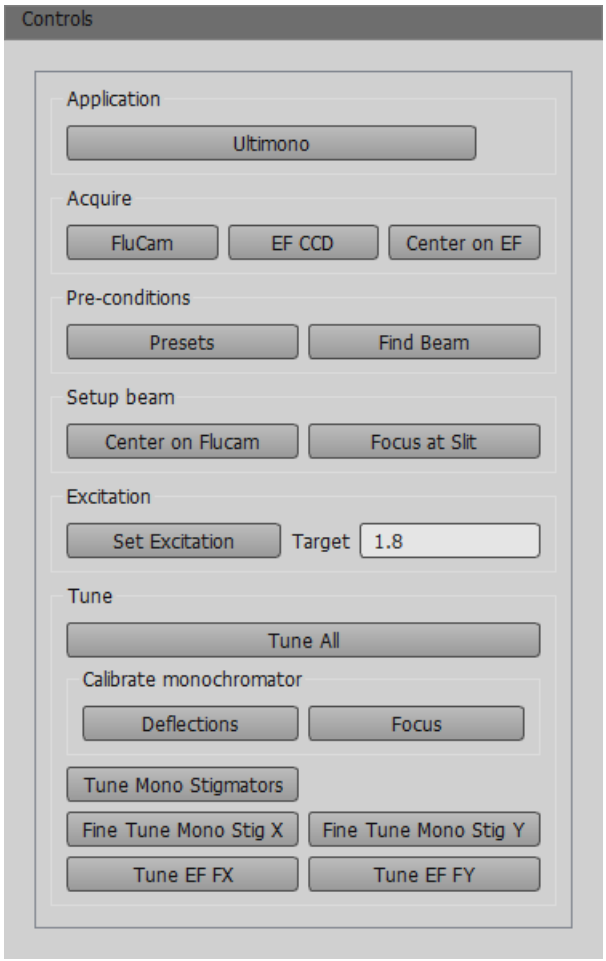
The typical workflow for getting an optimized monochromated beam under Ultimono conditions is performed in the Controls panel:



1. Select **Ultimono** to enable ultimono mode.
2. In **Pre-conditions**:
   a. Select **Presets** to select the correct optical setting.
   b. If the beam is not visible on the FluScreen, select **Find beam**.
3. In **Setup beam**:
   a. Select **Center on FluCam** to optimize beam alignment with the filter.
   b. Select **Focus at Slit** to correct the image plane.
4. In **Excitation**:
   a. Specify the desired **Target** excitation.
   b. Select **Set Excitation** to ramp to the specified Target excitation.
   Wait until the Target excitation is reached.
5. In **Tune**:
   a. Select **Tune All** to optimize the mono shifts, focus and stigmators, and fine tune the EF focus.
   b. If desired, select **Fine Tune Mono Stig X** and **Fine Tune Mono Stig Y** to fine-tune the Mono Stigmation.

6.4 Controls

The OptiMono+ controls are grouped according to their functionalities and chronological sequence.

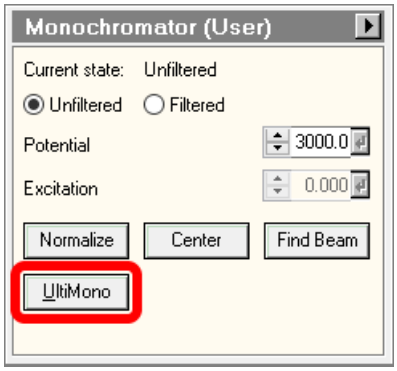


Application

- **Ultimono**

  Enable or disable Ultimono mode.

  The Ultimono status in Sherpa is synchronized with the Ultimono status in the **TEM User Interface** > **Monochromator** control panel.



Pre-conditions

- **Presets**

  If Ultimono is enabled, then Presets sets the microscope to Ultimono tuning conditions:

  - The Monochromator is set to filtered mode.

- The Monochromator potential is set to 800 V.
- System is set to two-condenser imaging mode.
- Magnification is set to the lowest SA magnification.
- Aperture mechanisms are retracted, or are set to the largest aperture.
- *Find beam* is automatically started to re-center the beam.

- **Find Beam**
  This function works the same as the **TEM User Interface** > **Monochromator** control panel > **Find Beam** function. If necessary or desired, select optional pre- and post-conditions in the **Settings** panel.

Setup Beam

The setup beam functions will fine tune the optical settings so that the monochromator can be excited and properly focused and stigmated.

- **Center on FluCam**
  This function centers the beam and optimizes the optics and gun lens for optimal visibility.
- **Focus at Slit**
  This function automatically inserts a monochromator slit and adjusts the intensity such that the slit plane is imaged (analogous to the manual slit focusing using the slit wobbler).

Excitation

- **Set Excitation**
  This function will automatically ramp the excitation to the specified *Target value*. While ramping, the function will focus the beam and adjust the mono shifts to keep the beam on the FluScreen.

Tune

The Tune functions will optimize the monochromator focus, shift and stigmation as well as the EF focus to get to a properly focused and stigmated monochromated beam that is centered on the EF entrance as well as measure the energy spread of the beam.

- **Tune All**
  This function will perform all the steps necessary in a predefined order to get to the final result:
  - Calibrations
  - Coarse Tuning
  - Fine Tuning
  - EF Focus Tuning
- **Calibrate Deflections**
  This function will calibrate the deflections caused by the different monochromator elements. These calibrations are then used to compensate for these shifts and keep the beam in the correct position.
- **Calibrate Focus**
  This function is used to determine the optimal focus for different values of the mono stigmator X. These results are used to keep the beam focused while optimizing the mono stigmator X.
- **Tune Mono Stigmators**
  Tuning of the monochromator focus and stigmators based on the FluCam image. This function provides a basic setting that can later be fine-tuned on the energy filter.

- **Fine Tune Mono Stig X**
  Perform the fine tuning of the monochromator stigmator X based on minimization of the energy spread as measured on the energy filter.
- **Fine Tune Mono Stig Y**
  Fine tune the monochromator stigmator Y by minimizing the width of the dispersion as measured on the FluCam.
- **Tune EF FX**
  Optimize the EF focus X by minimizing the width of the dispersion.
- **Tune EF FY**
  Optimize the EF focus Y by minimizing the angle of the dispersion.

6.5 Settings

The Settings for OptiMono+ are identical to the Settings for the regular OptiMono auto-function.
See Controls on page 17.

6.6 Tips & Tricks

Intentionally left blank.

6.7 Troubleshooting

Troubleshooting for OptiMono+ is the same as for the regular OptiMono auto-function.
See Troubleshooting on page 21.

7 TEM Distortion Correction

Linear Distortion in TEM (TEM LD) causes anisotropic magnification of the acquired image in two perpendicular directions. The TEM Distortion Correction plugin is intended for automated correction of TEM LD over the whole SA magnification range (from 4.3kx to 630kx) below the specified threshold of 1%. It is designed to work only on crystalline silicon sample with {110} orientation.

The automated process can be divided into three phases:

1. Optical alignments

   An optional phase selectable via a checkbox, it ensures the correct conditions for LD data acquisition are set. This phase is designed to move the compustage and perform the algorithms on three pre-aligned specimen area types: a hole/no sample, an amorphous area, and a crystalline area. If selected, each area must be specified by moving the compustage to the given area and pressing the *"Set"* button before the procedure can be started. Users can verify the stage position using the *"Go to"* button.

2. Measurement of LD

   This phase is intended only for the inspection of LD values. The SA magnification range is divided into two intervals – Low SA and High SA. For Low SA, it is needed to set crystalline and no sample area. For High SA, only a crystalline sample area is necessary. The appropriate position(s) must be specified by moving the compustage to the given area and pressing the *"Set"* button before the procedure can be started. Users can verify the stage position using the *"Go to"* button.

3. Correction of LD

   The goal is to end up with LD below the specified threshold. This iterative phase will aim to minimize the linear distortion regardless of whether it is already below the specified threshold or not. The SA magnification range is divided into two intervals – Low SA and High SA. For Low SA, it is needed to set the crystalline and no sample areas. For High SA, only a crystalline sample area is necessary. The appropriate position(s) must be specified by moving the compustage to the given area and pressing the *"Set"* button before the procedure can be started. Users can verify the stage position using the *"Go to"* button.

   The LD is calculated as:

$$Linear\ Distortion\ [\%] = \left(1 - \frac{d_{min}}{d_{max}}\right) \cdot 100\%\ ,$$

   where d_{max} and d_{min} are the dimensions of the measured feature in the direction of largest and lowest anisotropic magnification, respectively.

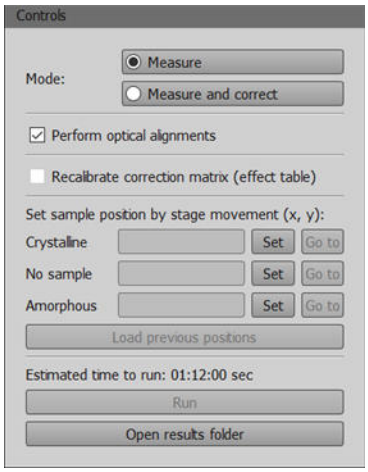
Note **TEM Linear Distortion Correction is only compatible with Thermo Scientific Ceta cameras.**

7.1 Preconditions

- Optics aligned for HR-TEM imaging (µP TEM).
- Suitable crystalline silicon sample for LD measurement and correction in the SA magnification range. In order to perform also the optical alignments, crystalline, no sample, and amorphous areas are necessary.
- Sample drift within limits (<1 nm/min).
- System cooled down for at least 2 hours.
- Sample at correct z-height and zone-axis orientation (double-tilt holder recommended).
- Magnification calibration must be performed.
- Bias/Gain corrections must be set correctly.

7.2 Controls

The controls tab features the following settings and functions:



Mode

Selection between LD measurement, or LD correction followed by measurement.

Perform optical alignments

Checkbox for the automated execution of optical alignments before the LD measurement/correction.

Recalibrate correction matrix (effect table)

The LD correction is dependent on magnitude and orientation, which may vary. The correction matrix maps the behavior and ensures a quick convergence in subsequent iterations.

Set positions by compustage movement

The optical alignments and the measurement/correction process require an indication of one or more areas within the sample. The user is required to locate the required sample positions via compustage movement and store their coordinates by hitting the *"Set"* button. Users can verify the stage position by using the *"Go to"* button.

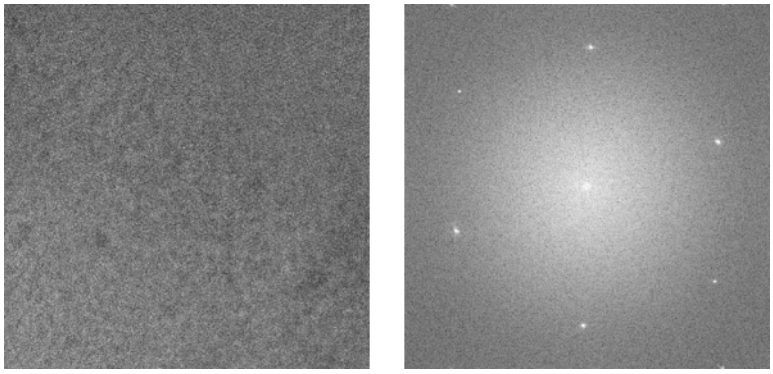
If the checkbox *"Perform optical alignments"* is not checked and the user selects LD measurement/correction at magnifications from the High SA interval, only the "Crystalline" sample position is required. If some magnifications from the Low SA range are selected, the "Crystalline" and "No sample" sample areas are required.

- No sample

  Some of the optical alignments are optimized to run without interaction with the sample. This sample position is also necessary for LD measurement/correction at magnifications from the Low SA interval. Choose and store an area without the specimen in the whole Field of View (FOV) of the FluCam Viewer at 4.3kx magnification.

- Crystalline

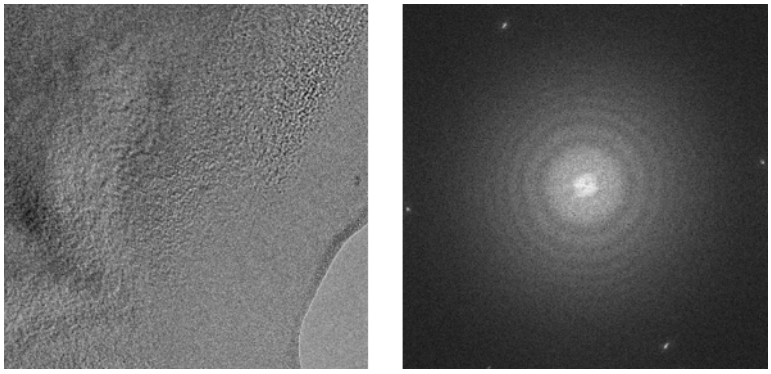
  The crystalline sample area is required for the LD measurement/correction at any magnification. Find a flat and thin part of the silicon sample which is visible over the whole field of view of FluCam Viewer at 94kx magnification. This sample position requires the zone-axis orientation of the sample (see Tips & Tricks section for a short zone-axis orientation guide).



An example of a crystalline area of the silicon sample image together with its FFT.

- Amorphous

  Some of the optical alignments require an amorphous specimen area. This area is usually located near the borders of the specimen (applicable for standard HR-STEM silicon specimen). Ensure that the amorphous area is visible in the image and check that the Thon rings are easily visible in the FFT (with some defocus applied).



An example of an amorphous area of the silicon sample together with its FFT.

Load previous positions

Loads the compustage positions that were used during the last run of the algorithm. Keep in mind, that the positions might be changed due to specimen drift.

Time Estimation

When the sequence is not running, the UI displays a time estimate of the currently selected options. While the sequence is running, the UI displays a countdown.

Run

Executes the selected algorithms at chosen magnifications.

Open results folder

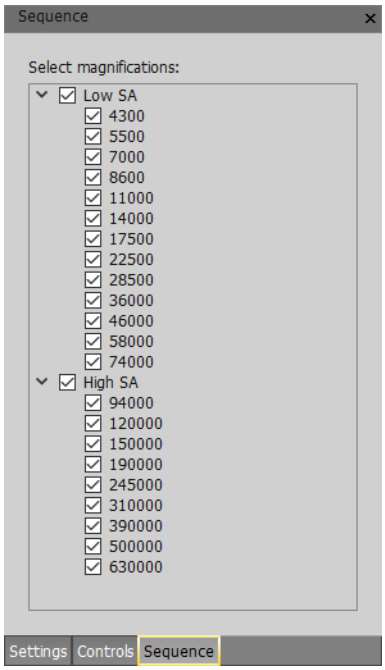
Opens a folder where all the LD measurement reports are stored.

7.3 Settings

Intentionally left blank.

7.4 Sequence

It is possible to (de)select several magnifications for LD measurement/correction in the sequence tab. The LD measurement/correction at the selected magnifications from the High SA interval is performed at the *"Crystalline"* stage position. The LD measurement/correction at the selected magnification from the Low SA interval is performed at the *"Crystalline"* and *"No sample"* stage positions.



7.5 Procedure

1. Verify that all the **preconditions** are fulfilled.
2. Select the **desired mode**.
3. Ensure the correct **optical conditions**
   (see description in Tips & Tricks on page 33)
   or select *“Perform optical alignments”.*
4. Set the required **sample positions** by moving the CompuStage and acquiring the positions.
   a. At 94kx magnification
      - Crystalline
      - Amourphous
   b. At 4.3kx magnification
      - No sample
5. Select **Run**

7.6 Tips & Tricks

Correction matrix recalibration

The LD correction is dependent on the correction matrix. If the correction fails multiple times, recalibrate the correction matrix.

Optical alignments

For a successful LD measurement, the optical alignments must be set correctly. To fully automate the LD measurement process, a set of automatic optical alignment procedures was developed. The process consists of the following alignment blocks:

- find beam,
- focus beam,
- center beam,
- center C2 aperture,
- align beam tilt pivot points,
- stigmate beam (condenser),
- rotation center,
- stigmate image (AutoCTF),
- coma correction (AutoCTF),
- focus image.

It may be turned on/off by checking/unchecking the *“Perform optical alignments”* checkbox on the *“Controls”* tab. It may happen that automation cannot overcome some of situations. In that case it is recommended to verify:

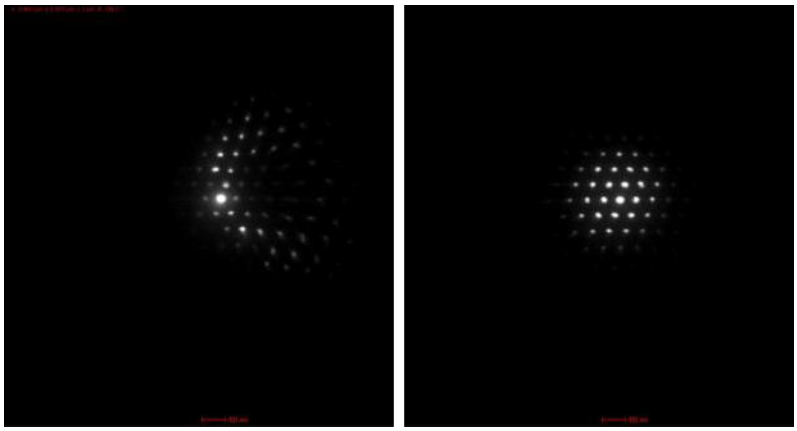
- Starting conditions,
- correct setting of sample positions – most importantly the amorphous part of the specimen,
- check the log window in Sherpa to identify the probable source of algorithm failure. If none of the actions referred above helps, it is recommended to perform the following alignments manually:

- center C2 apertures – check 50 and 150 µm on spot size 5 and 10,
- beam shift – check spot size 5 and 10,
- beam tilt pivot points,
- rotation center,
- condenser astigmatism – check spot size 5 and 10,
- objective astigmatism,
- coma-free alignment,
- focus image.

Sample positions

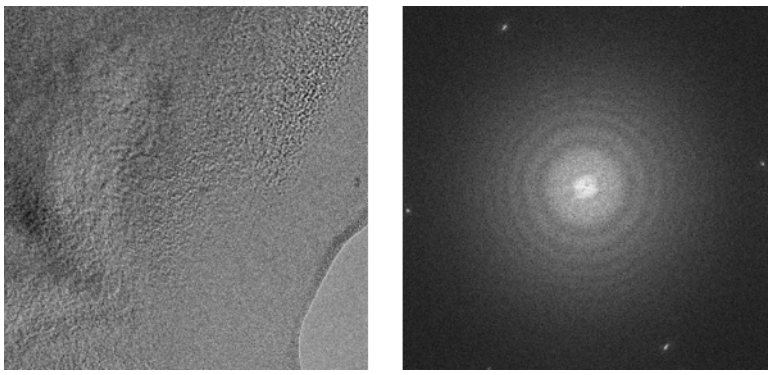
It is important to set the sample positions accurately.

- For the crystalline sample area, zone-axis orientation is mandatory. This process is performed in the TEM diffraction mode, where it is necessary to find a symmetrical diffraction pattern in the region of interest by adjusting the alpha and beta tilt. The process is summarized in the images below:



An example of a zone-axis orientation alignment with unadjusted (left) and adjusted (right) alfa and beta tilt.

- The amorphous area is required for several automated optical alignments. It is recommended to verify this area using the bottom-mounted camera acquisition, where multiple Thon-rings are easily visible (see below):



An example of an amorphous area of the silicon sample together with its FFT.

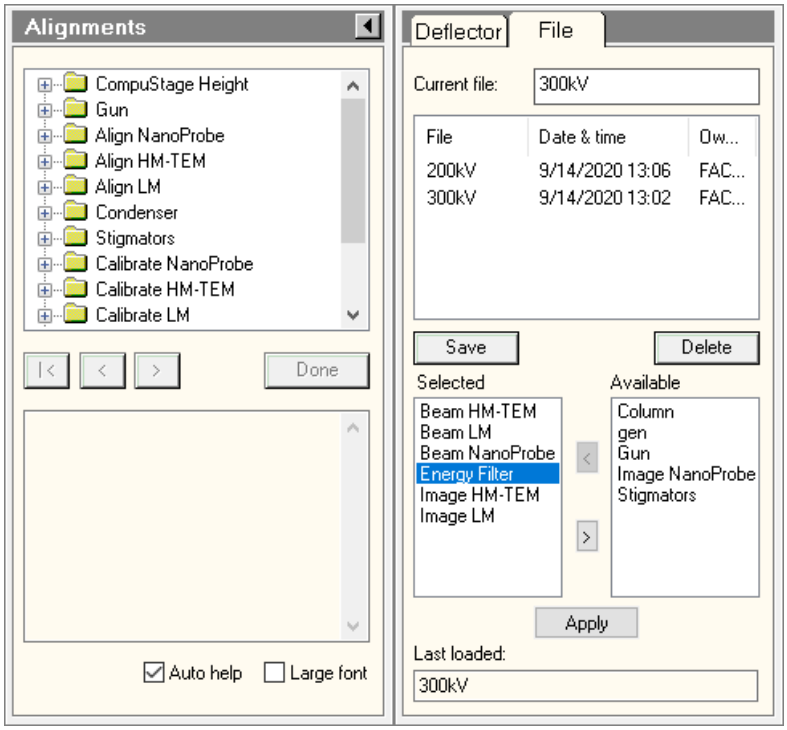
8 Energy Filter

8.1 Introduction

The Energy Filter application provides automated procedures for tuning the energy filter. These procedures include centering of the beam with respect to the energy selecting slit, tuning isochromaticity and tuning image distortions. It also provides procedures for measuring isochromaticity and image distortions.

8.2 Preconditions

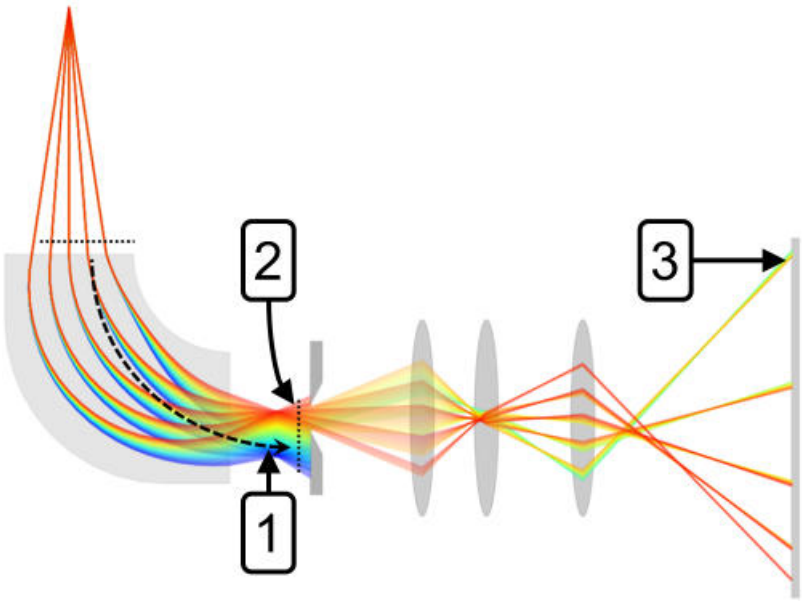
- The system has a Selectris or Selectris X Energy Filter.
- The specimen is either (in order of preference):
  - Unloaded.
  - Positioned at a hole, so that the beam passes through.
  - Positioned at a very thin area (100 nm or less).
- In the **TEM User Interface** > **Alignments** control panel> **File** tab:



  - An appropriate **Alignment file** is loaded, see *Last loaded:*
  - The **Selected** list contains the **Energy Filter** alignment.
  - The selected alignments are applied.
- The intensity of the beam at the post-filter camera is approximately 50% of the saturation intensity. The tuning procedures will work with lower intensities, but the results may be less accurate.

8.3 Procedure

Without optimization of the Energy Filter alignment, the beam may travel through the Energy Filter in a misaligned and aberrated way, as shown in the image below.



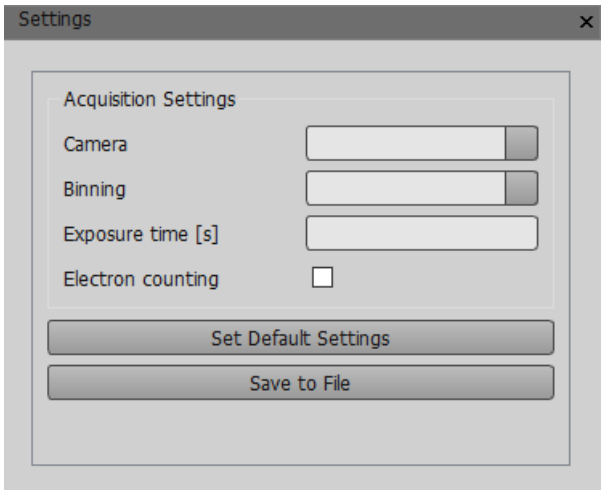
The procedure to align and tune the Energy Filter aims to achieve the following goals:

Phase	Controls - Function Group	Goal
1	Zero Loss	Center the Zero Loss Peak at the Energy Selection Slit.
2	Isochromaticity	Correct the aberrations before the Energy Selection Slit.
3	Geometric and Chromatic Distortions	Correct the distortions after the Energy Selection Slit.

How to prepare the camera settings and how to achieve these goals is described in the instructions below.

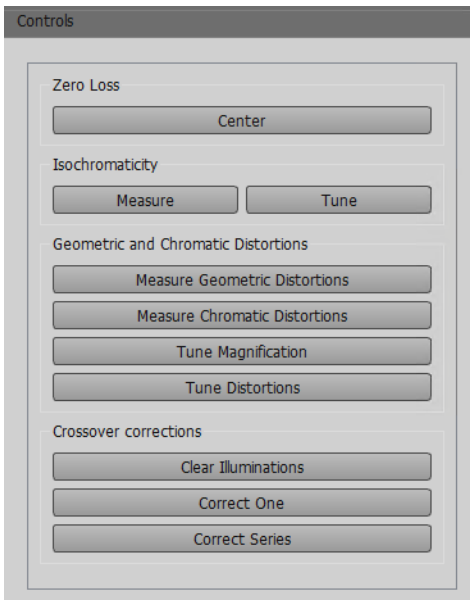
1. Prepare for the Energy Filter measurements and optimizations:
   a. Unload the **specimen**,
      or move the **specimen** so that the beam passes through a hole,
      or through an area of very thin carbon foil.
   b. In the **FlucamViewer**, verify that the beam illuminates the entire Filter Entrance Area.
   c. If the Filter Entrance Area is not fully illuminated, then adjust **Intensity** and/or **Spot Size**

2. In **Sherpa** > **Energy Filter** > **Settings**:



   a. Select **Camera**: **EF-Falcon**
   b. Select **Binning**: **4**
   c. Specify **Exposure time**: **0.2**
   d. Clear **Electron counting**
   e. If desired, select **Save to File** to store the current values for the next Energy Filter session.

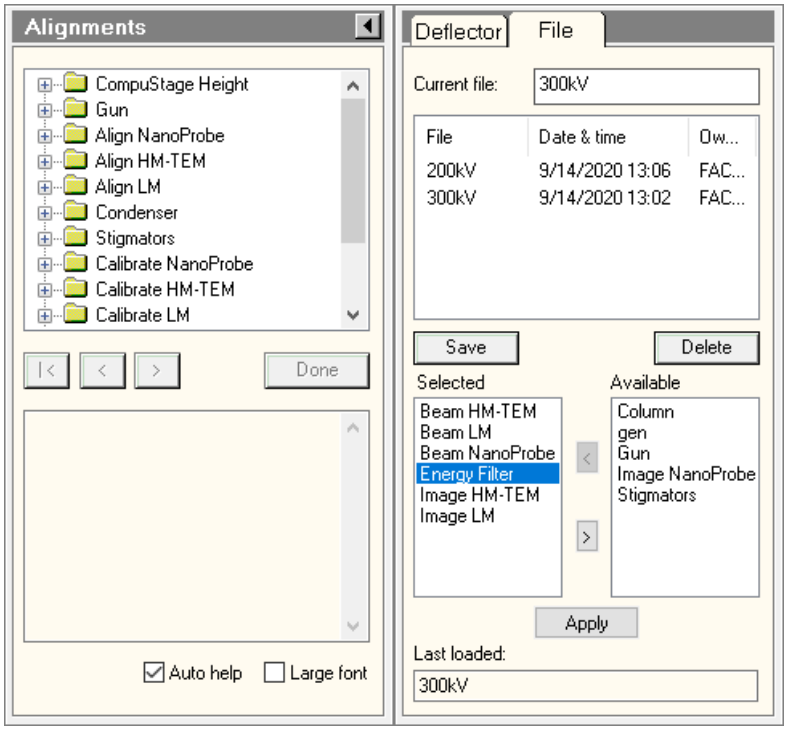
The following actions are performed in the **Sherpa** > **Energy Filter** > **Controls** panel:



3. In **Zero Loss**, select **Center** and wait until the procedure is completed.
4. In **Isochromaticity**:
   a. Select **Measure** and wait until the measurement procedure is completed.
   b. If the **Max Non-Isochromaticity** value does not meet the specification, then select **Tune**
5. In the **Settings** control panel, either:
   - Select **Binning**: **1** for ultimate accuracy of the *Geometric and Isochromatic Distortions* functions.
   - Select **Binning**: **2** for high accuracy of the *Geometric and Isochromatic Distortions* functions at a reasonable response time.

- Select **Binning**: **4** for a fast response time of *Geometric and Isochromatic Distortions* functions with reasonable accuracy.

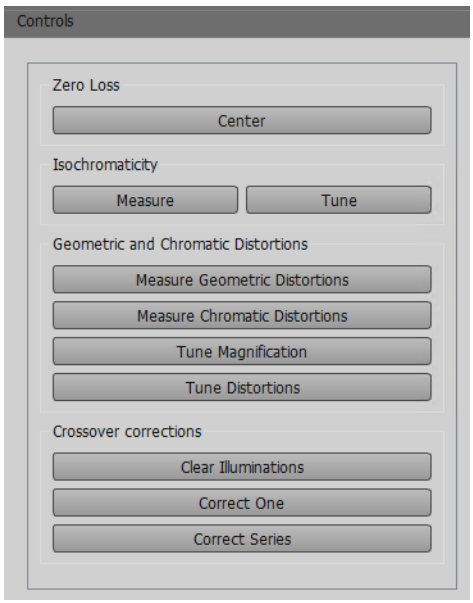
6. In **Geometric and Chromatic Distortions**:
   a. Either:
      - Select **Measure Chromatic Distortions** to measure all correctable orders of distortions.
      - Select **Measure Geometric Distortions** to measure only the geometric distortions, but at a faster response time than the *Measure Chromatic Distortions* function.
   b. If the measured **Distortion** or **Chromatic Distortion** is far out of specification (> 2%), then select **Tune Magnification** to correct the zero and first order distortions.
   c. If the measured **Distortion** or **Chromatic Distortion** is almost within specification (< 2%), then select **Tune Distortions** to correct all remaining geometric and chromatic distortions. It may be necessary to select *Tune Distortions* more than once to bring the measured distortions within specification.
7. If desired, save the optimized *Energy Filter* alignment.
   In the **TEM User Interface** > **Alignments** control panel > **File** tab:



   a. Select or specify the appropriate Alignment file:
      - Select an existing **File** from the table.
        The name of the selected *File* appears in *Current file*
      - Specify a new file name in **Current file**
   b. Select **Save** to update or create the *Current file*.
      All alignments in the *Selected* list and the *Available* list are saved to the *Current file*.

The progress and results of all functions in the *Controls* panel are displayed in the *Results* panel and are available as a PDF report in `C:\ProgramData\FEI\EnergyFilter\reports\`

8.4 Controls



The various measurement and optimization functions are grouped by their purpose, and are presented from top to bottom in the advised order of execution.

The *Crossover correction* functions are only available to *Supervisor* users.

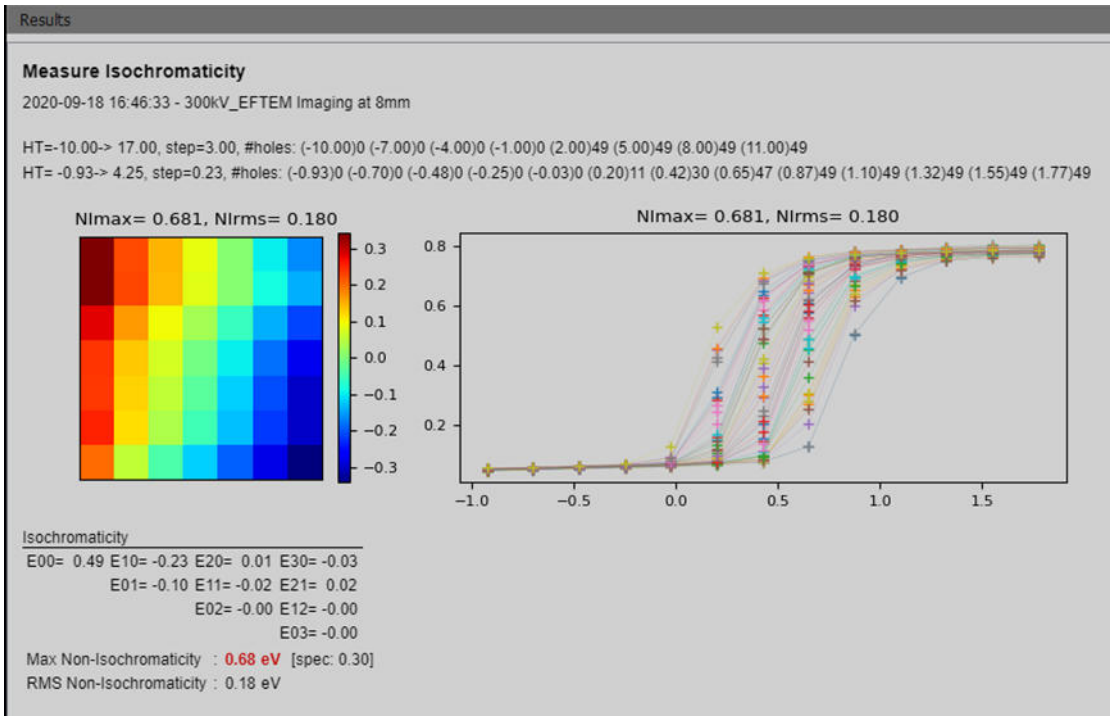
- **Zero Loss**: **Center**

  Finds the center of the Zero Loss Peak and aligns it with the Energy Selection Slit.

  The *Center* function corrects for possible hysteresis in the magnetic prism. Hysteresis may occur after switching to a different High Tension voltage, or may be the result of drift due to room temperature variations.

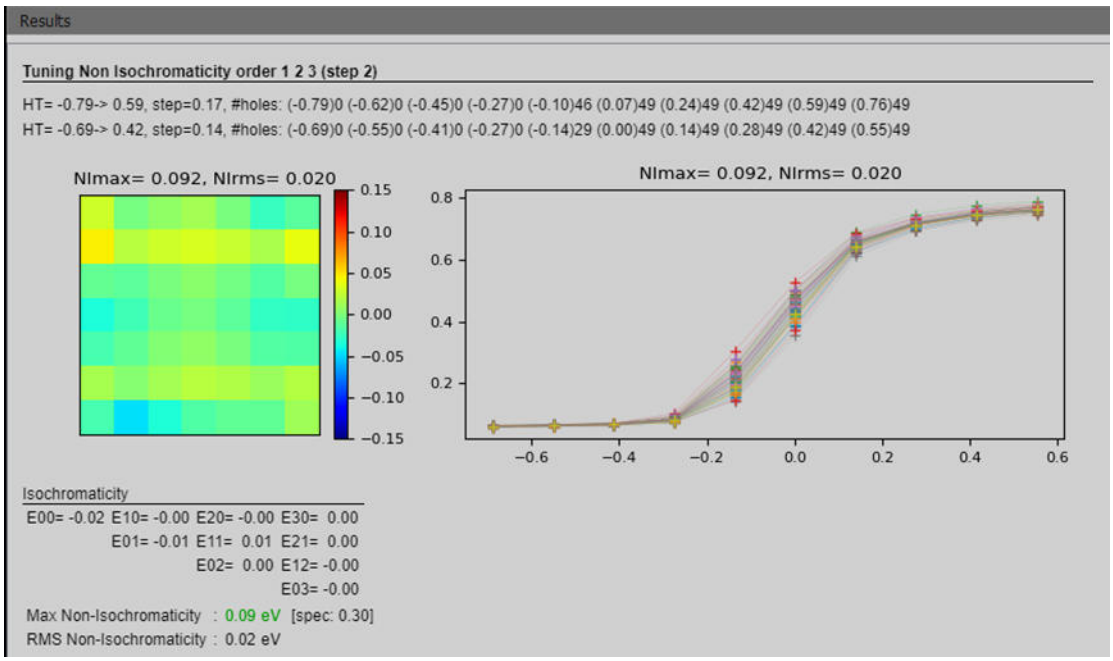
- **Isochromaticity**:
  - **Measure**:

    Measures the isochromaticity of the Energy Filter and determines if the result meets the specification.



Example of an isochromaticity measurement result that is far out of specification.

- **Tune**:
  Corrects for aberrations at the Energy Selection Slit, so that the isochromaticity value is improved.



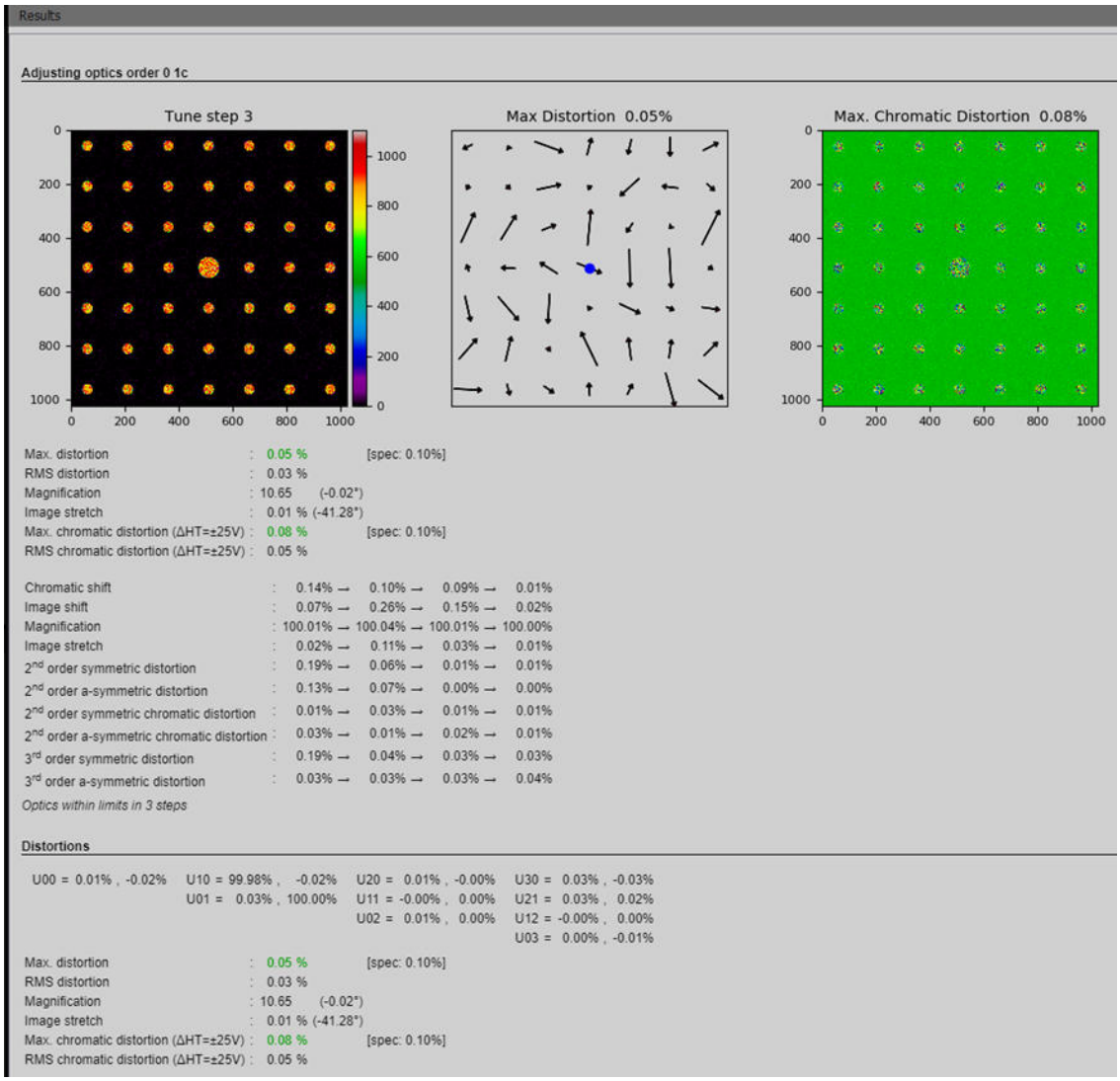
Example of an isochromaticity tuning result that is within specification.

The *Tune* function iterates fine-tuning the pre-slit multipoles and measuring the isochromaticity until either:

- The measured isochromaticity is within specification.
- A maximum number of iterations has been reached.

When the maximum number of iterations has been reached and the isochromaticity is not within specification, select *Tune* again to start a new series of iterations.

- **Geometric and Chromatic Distortions**:
  - **Measure Geometric Distortion**:
    Measures the Geometric Distortion and determines if the result meets the specification.
  - **Measure Chromatic Distortion**:
    Measures the Chromatic Distortion and determines if the result meets the specification.
  - **Tune Magnification**:
    Corrects zero and first order optics (mask position and size).
    The *Tune Magnification* function is especially useful as a coarse pre-tuning when the measured distortions are far out of specification (> 2%).
  - **Tune Distortions**:
    Corrects all geometric and chromatic distortions.



Example of a distortions tuning result that is within specification.

The *Tune Distortions* function iterates tuning the post-slit multipoles and measuring the distortions until either:

- no further improvement of the individual distortion coefficients is achieved.
- all individual distortion coefficients are each below their limit.
- a maximum number of iterations has been reached.

As a consequence, the routine may continue to iterate even though the total distortion is already within specification, or the routine may stop iterating even though the total distortion is not yet within specification. In the latter case, select *Tune Distortions* again to start a new series of iterations.

- **Crossover corrections**

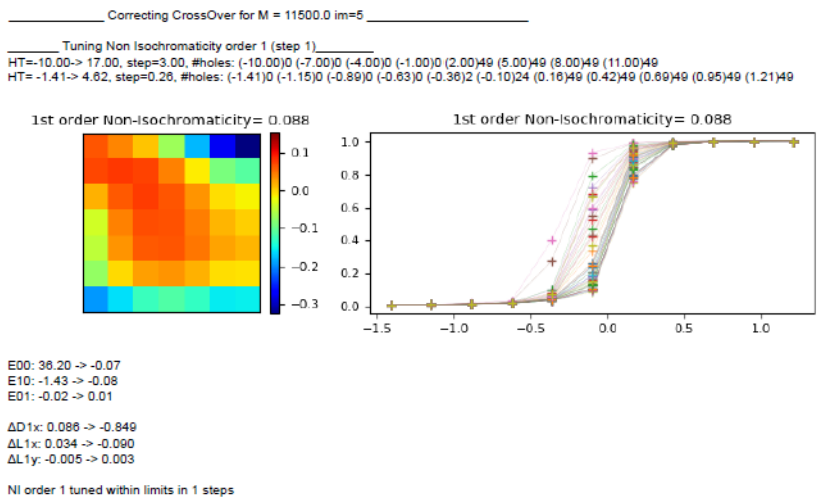
Only available for Supervisor users and for Thermo Fisher Scientific engineers.

Note

The Crossover corrections must be performed *only once* after:
• the system is installed.
• the energy filter is installed.
• a service action in which the column is split and re-assembled.

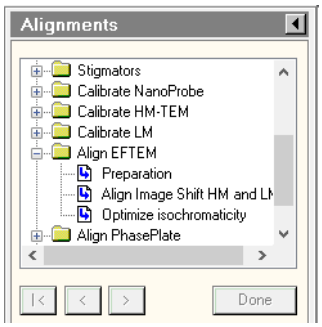
When a different magnification is selected, the final crossover point at the end of the column can shift a little bit. This shift is caused by minute mechanical tolerances in how the lenses are assembled in the column. Because the beam enters the filter slightly different at various magnifications, there is also a small change of focus at the energy selection slit when a different magnification is selected.

The *Crossover corrections* functions compensate for the magnification-dependent shifts of the beam at the energy filter entrance.



Example of a Crossover correction result that is within specification.

The Crossover correction functions can be used together with the *TEM User Interface* > *Alignments* > *Align EFTEM* procedure.



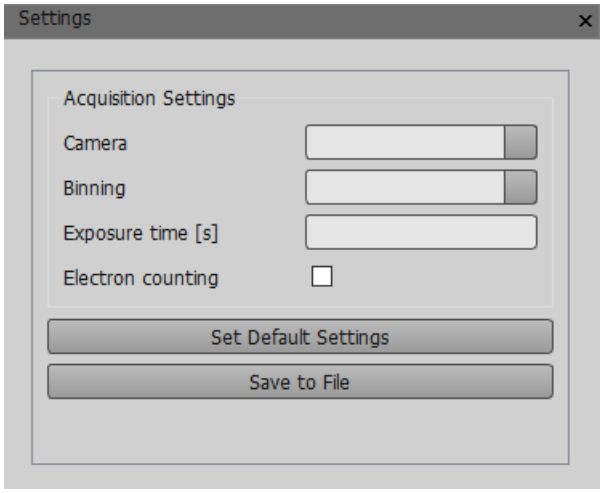
- **Clear Illuminations**

  Clears the stored beam settings for all magnifications used to center the beam on energy filter entrance. Use this function to clear any cached illumination data that may have been stored by the *Correct Series* function. This is required when the beam settings are no longer valid.
- **Correct One**

  Corrects the crossover for the current magnification, and determines if the result meets the specification. Requires manual centering of the beam on the energy filter entrance.
- **Correct Series**

  Corrects the crossover for the current magnification and all lower magnifications in the selected magnification range (LM, SA, Mh), and determines if the results meet the specifications. For each magnification this function automatically centers the beam on the filter entrance position and stores the associated beam settings throughout the runtime of the application.

8.5 Settings



- **Camera**:
  If the Energy Filter has more than one camera, then select the camera for image acquisition.
- **Binning**:
  Set the binning for the selected camera.
  - For the *Zero Loss*: *Center* function and the *Isochromaticity*: *Measure* and *Tune* functions, a higher Binning value increases the speed of the procedure without sacrificing accuracy.
    Binning 4 will give good results at a fast response time.
  - For the *Geometric and Chromatic Distortions* functions, a high Binning value may give less accurate results.
    Binning 1 or 2 is preferred for ultimate accuracy.
- **Exposure time [s]**:
  The integration time for a single image acquisition.
- **Electron counting**:
  - **Ticked**: the camera acquires images in *electron counting* mode.
  - **Cleared**: the camera acquires images in *linear* mode.
- **Set Default Settings**:
  Restores the built-in default acquisition settings.
- **Save to File**:
  Stores the current settings.
  Changes that are not stored are lost when Sherpa is closed. The Energy Filter application restores the most recently stored settings at start-up.

8.6 Tips & Tricks

8.6.1 Binning and Illumination

The response time and accuracy of the measurement and tuning functions depend on the binning value for the camera and the intensity of the beam at the post-filter camera sensor.

Function Group	Advised Binning	Result		
		Response Time	Accuracy at ±10% saturation	Accuracy at ±50% saturation
Zero Loss	4	Fast	High	High
Isochromaticity	4	Fast	Good	High
Geometric and Chromatic Distortions	1	Slow	Good	High
	2	Moderate	Moderate	Good
	4	Fast	Moderate	Moderate

- For all function groups, a higher binning value results in a faster response time.
- For the *Geometric and Chromatic Distortions* function group, a higher binning value results in a lower accuracy and slower convergence.
  For the *Zero Loss* and *Isochromaticity* functions the binning value has no impact on the accuracy.
- The illumination intensity has an impact on the accuracy of the results in all function groups.

Note **Avoid illumination intensities at which the camera becomes saturated.**

8.6.2 Measurement and Tuning when a Specimen is present in the Beam

The measurement and tuning routines perform best when no specimen or a thin specimen (< 100 nm) is inserted.

If the specimen is so thick that the intensity in the plasmon peaks becomes comparable or higher than the intensity in the Zero Loss Peak, then the *Zero Loss*: *Center* function and the *Chromatic Distortions* functions become unreliable.

The measurement and tuning routines can handle images in which part of the camera is blocked, for example by a grid bar or an aperture. In that case, the *Measure* and *Tune* functions only use the part of the camera sensor that is sufficiently illuminated.

8.7 Troubleshooting

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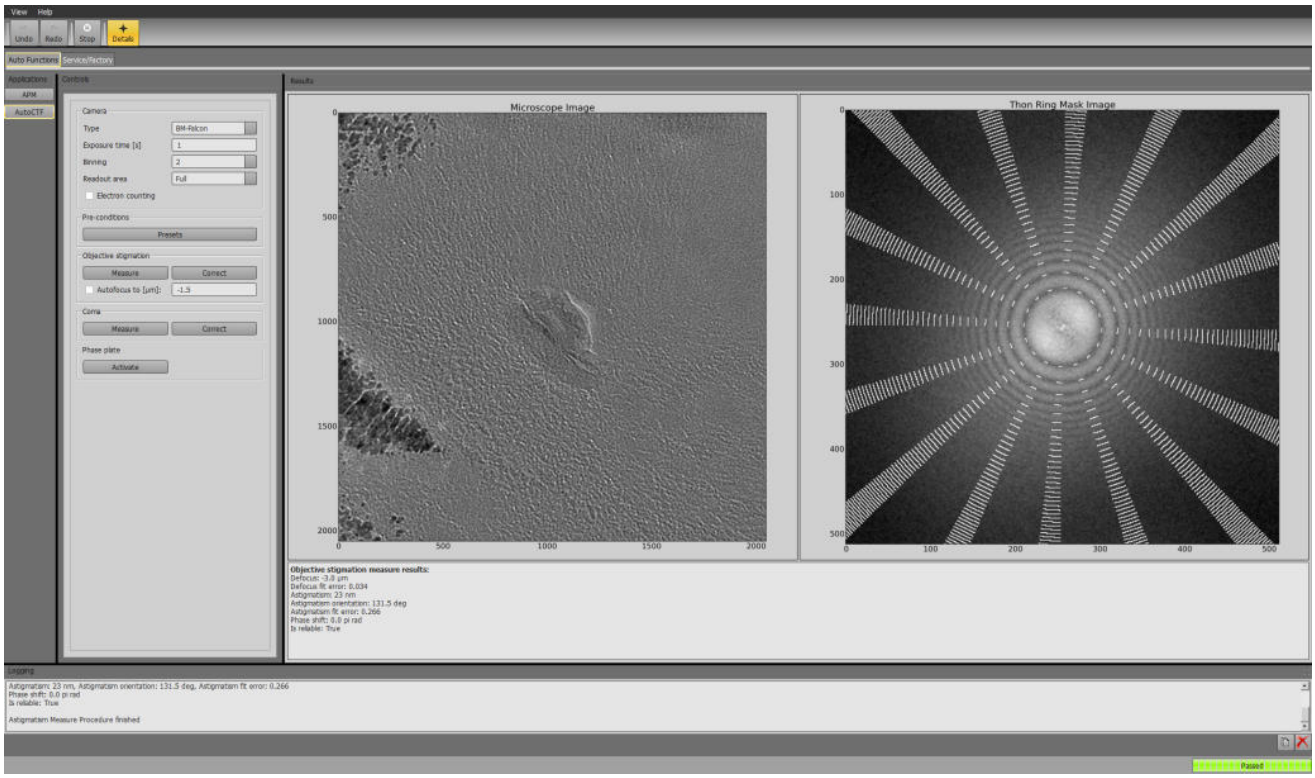
9 AutoCTF

9.1 Introduction

The AutoCTF application includes the following functionalities:

- Measure defocus and astigmatism by fitting Thon rings in image power spectra.
- Measure coma by fitting Thon rings of a beam tilt tableau.
- Measure the phase shift induced by the Volta phase plate.
- Automatically correct for astigmatism and optionally set the defocus to a specified value.
- Automatically correct for coma.
- Activate the phase plate to a specified phase shift value.

The Results panel shows:



- Left image: the acquired TEM image.
- Right image: the corresponding power spectrum with superimposed dashed lines representing the fitted Thon rings.
- Below the images: the measurement results.

9.2 Preconditions

The following conditions must be fulfilled before AutoCTF can run successfully:

- The **TEM User Interface** and **TIA** are both running.
- The magnification calibrations have been performed.

- The **EPU** > **Auto Functions** tab > **Autofocus** calibration has been performed.
- A specimen is loaded which enables the display of Thon rings in the power spectrum of the TEM image.

  This is typically a thin amorphous carbon film, such as Quantifoil, Lacey Carbon, or a Cross Grating sample.
- The microscope is properly aligned.

  Before using AutoCTF, check the Direct Alignments (especially rotation center) and roughly correct objective astigmatism.

9.3 Procedure

1. Use the handpanels to set suitable optics values for good quality Thon rings.

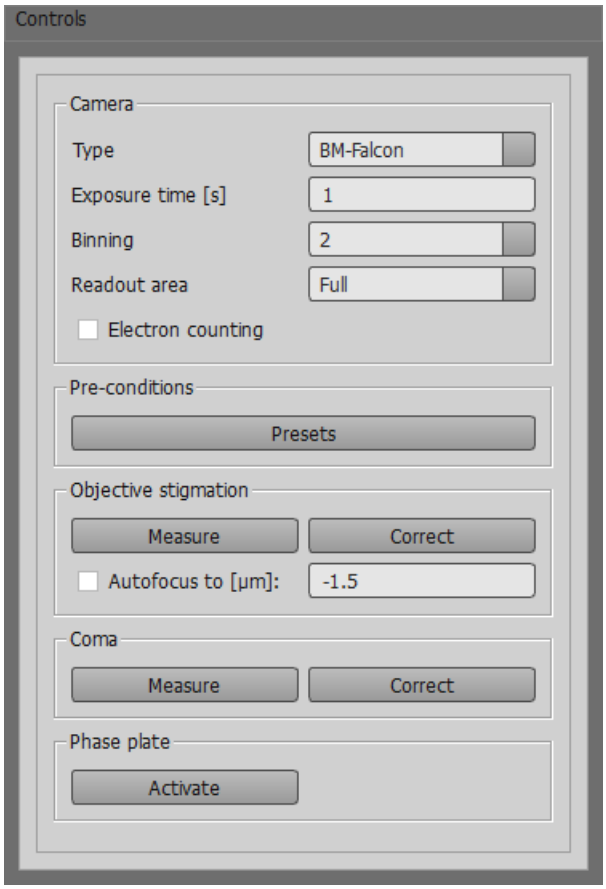
   Typical settings and recommendations:
   - **Magnification**: approximately 150kX, or use the magnification that will be used for the planned Automated data acquisition run.
   - **Spot Size**:
     - When using the high dose camera: select Spot Size 3 or higher.
     - When using the high sensitivity camera: check the dose rate to select a suitable Spot Size.

       Do *not* use Spot Size 1 or 2.
   - **Illuminated Area** / **Intensity**: the beam diameter can be narrowed a bit to increase the signal strength. When the beam is too narrow, less Thon Rings will be visible.
   - **Defocus**: typically between -1 μm and -3 μm.
2. Move the **specimen** to an area with **thin, uninterrupted, amorphous carbon foil** that is *not* close to a grid bar.
3. Accurately set the **specimen** to **eucentric height**, using the CompuStage Alpha tilt wobbler.
4. In the **Settings** panel:
   a. Specify the **Convergence settings** values.
   b. If desired, specify a custom **Coma** > **Beam tilt** value.
   c. Specify the **Phase plate activation** > **Target phase shift** value.
5. In the **Controls** panel:
   a. Specify the **Camera** settings.
   b. Specify the **Objective stigmation** > **Autofocus** value to the same value as used in step 1.
   c. Select **Objective stigmation** > **Measure** and wait for the result.
   d. If the current astigmation is not OK, then:
      - Select **Correct**
      - Wait until the procedure is completed.
   e. Select **Coma** > **Measure** and wait for the result.
   f. If the current coma correction is not OK, then:
      - Select **Correct**
      - Wait until the procedure is completed.
6. Select **Phase plate** > **Activate** to generate a new phase plate.
7. Wait until the activation procedure has reached the specified target phase shift.

Note **AutoCTF does not support the Undo/Redo functions.**

9.4 Controls

The Controls tab features the following settings and functions.



Camera

- **Type**
  Same as *CCD/TV Camera* control panel > *Camera*
- **Exposure time [s]**
  Same as *CCD/TV Camera* control panel > *Integration time*
- **Binning**
  Same as *CCD/TV Camera* control panel > *Binning*
- **Readout area**
  Same as *CCD/TV Camera* control panel > *Readout area*
- **Electron counting**
  Same as *CCD/TV Camera* control panel > *Settings* tab > *Electron Counting*
  If the selected camera supports electron counting and it is enabled, then AutoCTF will use the selected camera in electron counting mode. If the box is not ticked, images are acquired in linear acquisition mode.

Pre-conditions

- **Presets**
  Apply the optics settings that are used by APM for Coma Correction and Objective Stigmation.

Objective stigmation

- **Measure**
  A single TEM image is acquired, after which defocus and astigmatism are measured. In case a phase plate is inserted also phase shift is measured.
  The *Results* panel displays the acquired image and the power spectrum with dashed lines showing fitted Thon rings. If the confidence score is not high enough, the measurement is considered failed. The power spectrum is crossed out and the *Is reliable* status is set to *False*.
- **Correct**
  An iterative procedure is started to remove astigmatism.
  - Autostigmate with Autofocus might perform extra iterations with 1 µm extra defocus to determine if the microscope is in under-focus or in over-focus range.
  - If Thon ring detection fails, the correction algorithm assumes it was too close to focus, and adds 1 µm extra under-focus for the next iteration.
  - In case the astigmatism cannot be corrected within a set number of iterations, the optical settings will revert to what they were prior to starting the correction procedure.
  - When *Stop* is selected during the correction procedure, the settings are restored to what they were prior to starting the correction procedure.
- **Autofocus to [um]**
  - Ticked:
    The specified defocus is applied after the Autofocus function is executed. Thon rings are evaluated with the specified defocus.
  - Cleared:
    No defocus is applied after the Autofocus function is executed. Thon rings are evaluated in focus.

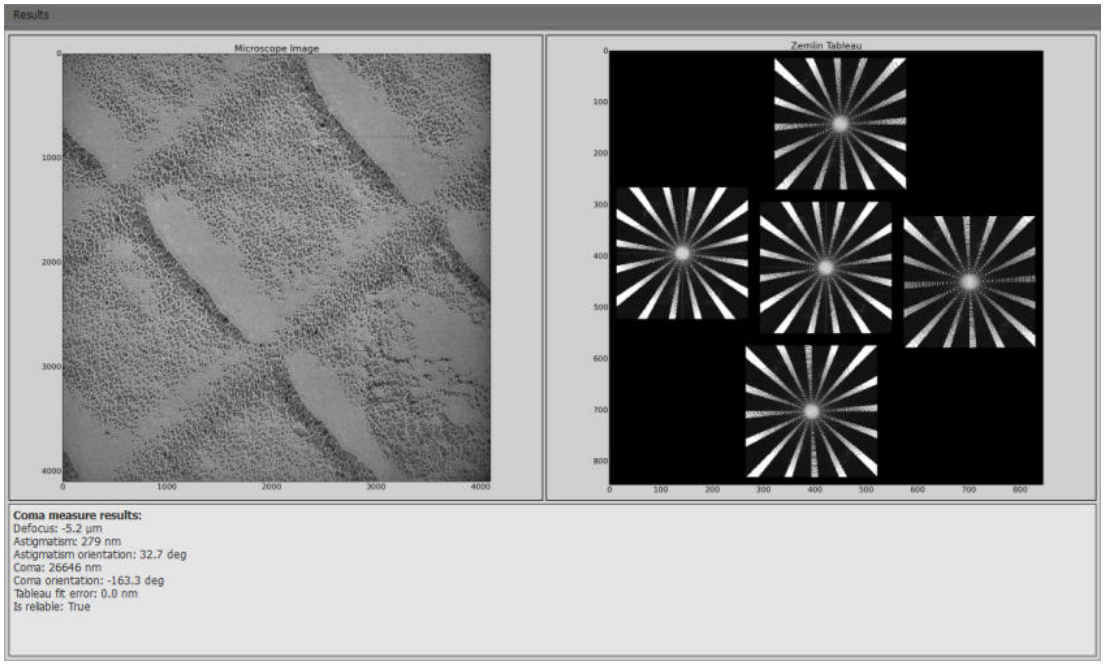
Coma

Note **Verify that the Objective aperture mechanism is retracted before starting a coma measurement and/or correction.**

- **Measure**

  A beam tilt tableau is created with beam tilts in the positive and negative X and Y directions. Specify the beam tilt *Settings > Coma > Beam tilt*.

  Based on the tableau, the current coma is measured. The result will be displayed in the *Results* panel:



  - Left image: the last acquired TEM image.
  - Right image: the power spectra, also known as Zemlin tableau.
  - Below the images: measurement results.

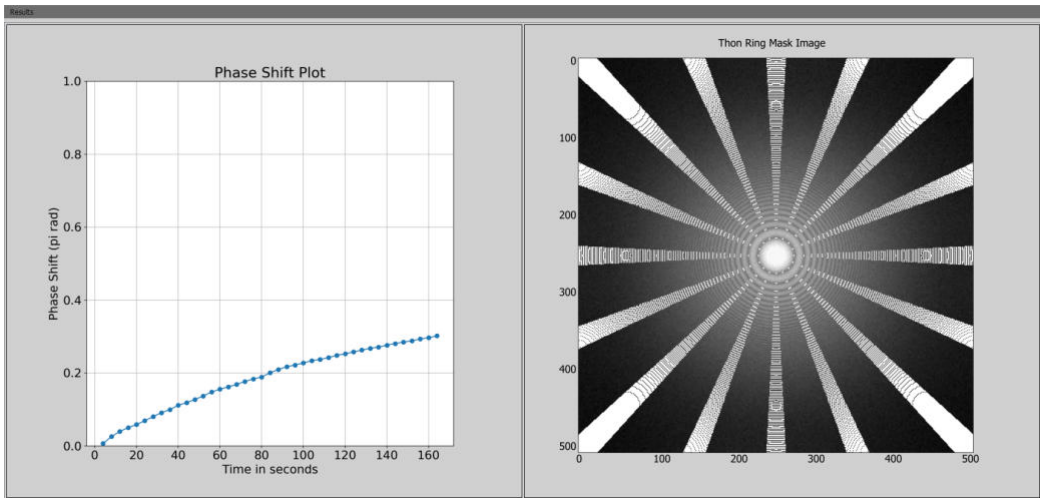
- **Correct**

  An iterative procedure is started to correct for coma. In case the astigmatism cannot be corrected for within the number of set iterations, the optical settings will revert to what they were before the start of the auto function.

  When *Stop* is selected during the correction procedure, the settings will be restored to what they were prior to starting the correction procedure.

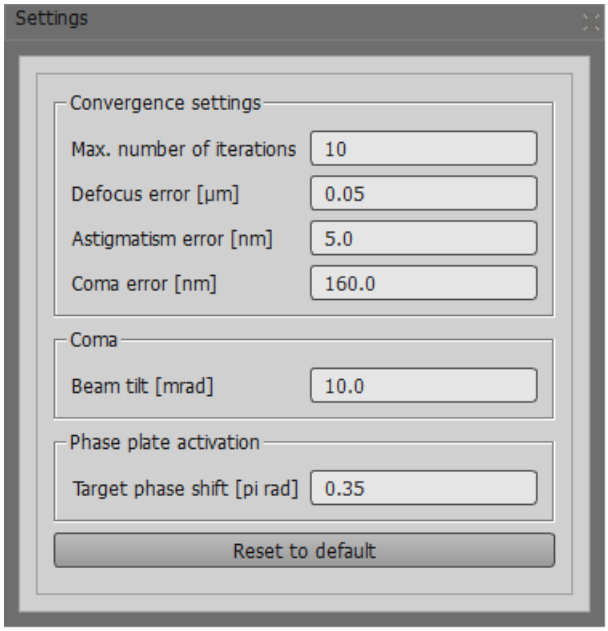
Phase plate

- **Activate**
  Phase shift is measured in successive exposures until the specified *Target phase shift* is reached. As part of the phase plate activation algorithm, *Auto stigmate* is performed to correct for phase plate-induced objective astigmatism.



9.5 Settings

The following settings are available:



Convergence settings

- **Max number of iterations**
  Maximum number of iterations that are performed during a correct action (auto-stigmate or auto-coma free alignment). In case the maximum number of iterations are done and the convergence criteria as defined by the error settings are not reached, the optical settings from before the correct action are restored.
  The default number of iterations is 10.

- **Defocus error**, **Astigmatism error** and **Coma error**
  Maximum remaining errors for defocus, astigmatism and coma that are acceptable to complete the auto-function task.
  The default values (defocus = 50 nm, astigmatism = 5 nm and coma = 160 nm) are well suitable to optimize the microscope alignments, but can be adapted if more accurate results are desired (lower value) or a faster convergence is desired while compromising on accuracy (higher value).
  Note that setting the error too restrictive can cause the AutoCTF function to fail.

Coma

- **Beam tilt**
  Defines the beam tilt for coma measurement and correction. Default value is 10 mrad.

Phase plate activation

- **Target phase shift**
  The activate phase plate will continue to acquire images until the measured phase shift is larger than the Target phase shift.

- **Reset to default**
  Restores all Settings parameters to their default values.
  - Changes that are made by a supervisor are stored automatically. The next time any user executes the AutoCTF function, the stored values are used.
  - Changes that are made by a regular user are volatile. When the user logs out, the Settings parameters are automatically reverted to the most recent stored values. If no changed values are stored by the supervisor, then the default settings are used.

9.5.1 Background Information: Coma Value in Sherpa vs the AutoCTF Tool

There are two definitions of coma:

- Sherpa uses the same coma definition as the Cs image corrector software. Coma values measured and displayed in Sherpa can be directly related to the Cs image corrector software.
- The prototype AutoCTF tool uses a different definition, which gives values that are exactly a factor 3 larger.

As an example: when the prototype AutoCTF tool measures 1200 nm coma, the Sherpa AutoCTF function measures 400 nm coma. The default value of the coma convergence setting in Sherpa must therefore also be set a factor 3 lower than in the prototype stand-alone AutoCTF tool.

9.6 Tips & Tricks

General notes

- Thon ring fitting requires good contrast in the power spectrum. If Thon ring fitting fails especially at low defocus values, either increase the exposure time or increase the dose rate.
- For fast and good results of the AutoCTF functions, the microscope must be properly aligned. Before using AutoCTF, check the Direct Alignment (especially rotation center) and roughly correct objective astigmatism. If these alignments are too far off, a high number of iterations might be necessary to achieve a decent result.

- If AutoCTF fails to converge to the set target threshold after the maximum number of iterations is reached, the initial optics parameters will be restored. Starting AutoCTF again with the same settings will result in the same failure. Increase the **Max. number of iterations** or correct astigmatism and coma more accurately before starting the AutoCTF function again.

Auto-stigmate

- To increase the accuracy of the objective stigmation in case the **Autofocus to** option is selected:
  - Repeat **Measure** and **Correct** until the astigmation at the specified defocus is achieved.
  - Clear the **Autofocus to** checkbox.
  - Select **Measure** and **Correct** one more time at the current defocus value. The defocus value will not be reset.

Auto coma-free alignment

- For auto coma-free alignment, the Objective aperture mechanism has to be retracted! During the alignment a beam tilt is applied. If the Objective aperture is not retracted it may block the tilted beam.
- Depending on the microscope optics, reliable tableau formation for coma-free alignment either requires high defocus (-3 µm or lower) or a reduction in beam tilt angles for tableau formation (e.g. default Tableau beam tilt of 10 mrad might be reduced to 5 mrad in Settings tab).
- In case of a Cs image corrector, the correct coma functionality is not available within Sherpa AutoCTF. It is possible to *measure* the coma, for instance to check if it is within limits, but the coma *correction* must be done with the Cs image corrector software. Sherpa AutoCTF uses the beam tilt to correct coma for non-Cs image corrected systems, but this is not desired for Cs image corrected systems.

Phase shift

- Phase shift is a value between 0 and 1 [pi radians]. Without the phase plate, the phase shift must be 0. With phase plate, a value of 0.5 pi radians gives an optimal contrast.

  Note that phase is in fact a periodic quantity with period 2 pi radians, but Thon ring fitting only allows resolving pi radians.

9.7 Troubleshooting

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10 APM

10.1 Introduction

The Automated Performance Monitoring (APM) application minimizes the preparation time for reliable high quality data acquisition. The application evaluates if the microscope alignments are well suited for high quality data acquisition, and offers to optimize the alignments if the evaluation result indicates improvements are desirable or even necessary.

When APM is used on a regular basis, the reliability, performance and ease of use of the microscope are maintained on a continuously high level.

The full APM functionality is only available for the Supervisor user-level. Regular users can check the current alignment status, but cannot use the optimization functions.

10.1.1 Functions Overview

1. **Check alignments**
   Loads the current Alignment set and evaluates if the alignments are fit for data acquisition.
   See The APM Alignment Set on page 64 for more information.
2. **Sequence**
   Execute manual and automated actions to improve the alignment status.
3. **Results**
   Live images and processing results from the running action.
4. **Logging**
   Detailed information about executed actions and their results.
5. **Load previous alignments**
   Select and load an Alignment set that is saved earlier.

Note **Only use *Load previous alignment* as a last resort, when the current Alignment set cannot be improved.**

10.2 Preconditions

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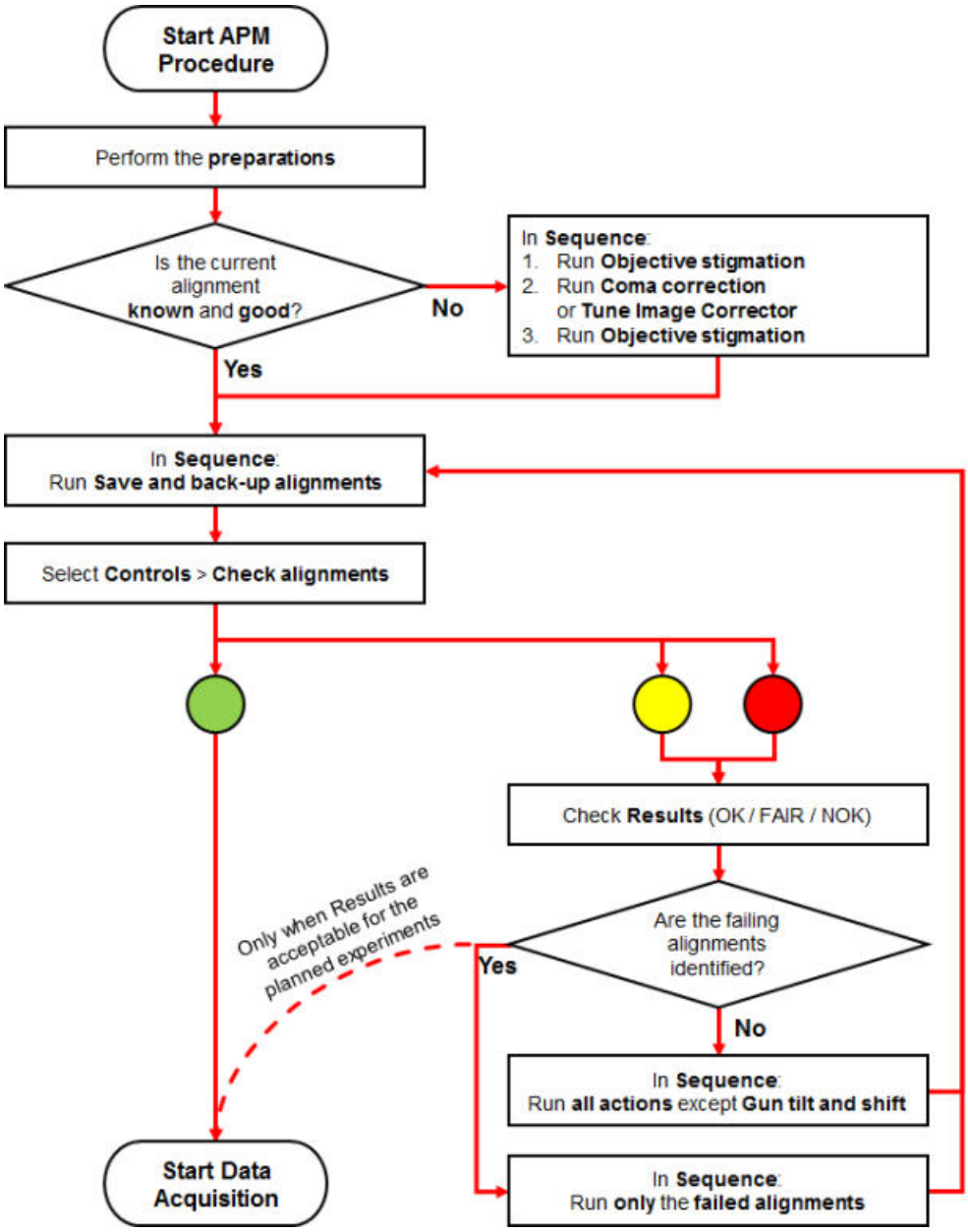
10.3 Procedure

10.3.1 Quick Reference Flowchart

Make sure to start the APM procedure with performing the Preparations on page 56.



Note **Only execute the *Gun tilt and shift* alignment when absolutely necessary.**
After the *Gun tilt and shift* alignment is executed, the *Dose calibration* and *Dose protector* for the Falcon camera may need to be updated also.

10.3.2 Preparations

1. In the **TEM User Interface**, verify that the **FEG Register file** and **Alignment file** for APM are loaded.

System types	**FEG Register file**	**Aligment file**
High End (Titan software)	`APM_[serial].feg` This FEG Register file contains the following FEG Register(s): ● `APMTEM_[HT###]` for TEM mode. ● `APMEFTEM_[HT###]` for EFTEM mode. (*only on systems with an Energy Filter*)	`Factory[serial][HT###].alg`

Mid Range
(Talos software)
	`usr_factory.feg`
	This FEG Register file contains the following FEG Register:
	`Factory_APMTEM_[serial][HT###].feg`
		`Factory[serial][HT###].alg`

In the file names above:
- `[serial]` is either:
  - High End systems: `D####`
  - Mid Range systems: `SN#######`
- `[HT###]` is the High Tension voltage.

Note **If the required FEG Register and/or Alignment file is not available, then contact your Thermo Fisher Scientific service representative to create the missing files.**

2. Select **Handpanels** > **Eucentric Focus**
3. Move the **specimen** to an area of **amorphous carbon foil** that is *not* close to a grid bar.
4. Right-click on **Sequence** > **Eucentric height (stage)** and select **Run selected**
5. Accurately **focus** the **specimen** with the following settings:
   - Camera: BM-Ceta
   - Magnification: 96kX
   - Spot size: 4
   - Gun lens: 4
   - Illuminated area: 2 µm
6. Select **Handpanels** > **Reset Defocus**
7. Retract the **Objective aperture mechanism** and **Selected Area aperture mechanism**
8. Right-click on **Sequence** > **Save and back-up alignments** and select **Run selected**

10.3.3 Check the Current Alignment Status

1. In the **Control** panel, check the date and time of the last saved Alignment set.
   If manual adjustments have been made since the most recent Alignment set was saved, right-click on **Sequence** > **Save and back-up alignments**, and select **Run selected** . This will create an Alignment set that contains the manual adjustments.
2. Select **Handpanels** > **Eucentric Focus**
   This sets the Objective lens to the eucentric focus preset and normalizes all lenses.
3. Move the **specimen** to an area of **amorphous carbon foil** that is *not* close to a grid bar.
4. Set the specimen to eucentric height. Either:
   - Right-click on **Sequence** > **Eucentric height (stage)** and select **Run selected**
   - Use the **Stage Wobbler** method
5. Select the following **optics values:**
   - Magnification: 96kX
   - Spot size: 4
   - Gun lens: 4
   - Illuminated area: 2 µm

   These values are similar to the optics settings for the **Check alignments** function.
6. Accurately **focus** the **specimen** manually via the FFT from the Ceta camera, or by running an autofunction.
7. Select **Handpanels** > **Reset Defocus**
   and verify the TEM User Interface that Defocus is 0.
8. Retract the **Objective aperture mechanism** and **Selected Area aperture mechanism**
9. If the current alignment status is not known, or not optimal:
   a. Right-click on **Sequence** > **Objective stigmation**, and select **Run selected**
   b. Right-click on **Sequence** > **Coma correction** or **Tune image corrector**, and select **Run selected**
   c. Right-click on **Sequence** > **Objective stigmation** again, and select **Run selected**
10. Right-click on **Sequence** > **Save and back-up alignments** and select **Run selected**

Note **The Check Alignments action in the next step loads the current APM Alignment set. This means that any manual alignments will be overwritten.**
To avoid loosing the manual alignments, right-click on *Sequence* > *Save and back up alignments* and select *Run Selected*.

11. Select **Controls** > **Check Alignments**
    The *Check Alignments* function performs the following actions:
    - Loads the last saved Alignment set.
    - Performs a set of astigmatism and coma measurements at the optical axis and at 2000 nm offsets from the optical axis.
    - Evaluates the measurement results to classify the alignment status.
      - The acquired images and evaluation results are displayed in the *Results* panel.
      - The alignment status is displayed by the *traffic light*.
12. Wait for the **traffic light** to the alignment status.
    - **Green**:

The alignment status is fit for high quality data acquisition. No improvement is needed, the system is ready for data acquisition.

- **Yellow**:
  The alignment status is fair. Whether or not improvement is needed depends on the required resolution of the planned experiment. See Coma, Phase Shift and Resolution on page 65 for guidelines.
  Continue at Improve the Alignments with APM on page 58 by selecting the *Objective stigmation* actions and the *Coma correction* or *Tune image corrector* actions in the sequence.
- **Red**:
  The alignment status is not fit for data acquisition, improvement is necessary.
  In the sequence, select all actions, except the *Gun tilt and shift* action. Then continue at Improve the Alignments with APM on page 58.

Note **After execution of the *Gun tilt and shift* action, the *Dose calibration* and *Dose protector* for the Falcon camera must be updated also.**

If the result is not green, then the Logging panel may provide suggestions how to continue. For details about how to handle sub-optimal results, see chapters Tips & Tricks on page 64 and Troubleshooting on page 66.

10.3.4 Improve the Alignments with APM

The Sequence panel presents the typical workflow to achieve and maintain an optimal alignment status for high quality data acquisition.

Before performing an APM check or sequence, make sure that the system is stable. For example, after an LN2 refill there may be thermal drift.

1. In the **Sequence** panel, make a selection of the available actions:
   a. Tick the **Load alignments** action to apply the last saved APM Alignment set.

Note **If the alignments have been improved outside Sherpa APM, do *not* tick Load Alignments.**

For instructions how to manually adjust the alignments outside Sherpa APM,
see How to Adjust the Alignments in the TEM User Interface on a System with Sherpa APM on page 65.

   b. Tick the first **Save and back-up alignments** action to store the current alignment settings in an Alignment set.
   This makes it possible to revert to the initial Alignment set in case executing the Sequence does not improve the alignment status.
   c. Tick the **alignment actions** that you wish to run.
      - If the *Results* and/or *Logging* panels clearly identify which alignments are not within limits,
        then tick **only the identified alignment actions**
      - If it is not clear what causes the yellow or red traffic light,
        then tick **all alignment actions** in the sequence, except the *Gun tilt and shift* alignment.

Note

Only execute the *Gun tilt and shift* alignment when absolutely necessary.
After execution of the *Gun tilt and shift* alignment, the *Dose calibration* and *Dose protector* for the Falcon camera must be updated also.

d. Tick **Check alignments** to automatically evaluate if the selected alignment actions are effective.

In contrast to the *Control* > *Check alignments* function, the *Sequence* > *Check alignments* action does not start with loading the last saved APM Alignment set. This way, the *Check alignments* action only evaluates the result of the ticked alignment actions.

e. Tick the last **Save and back-up alignments** action to automatically store the results.

The selected set of actions is stored. At startup, Sherpa automatically loads the most recent selection.

2. Select **Run** to execute the selected actions.

Some alignments require manual actions by the user. For these alignments, the *Results* panel displays the instructions.

After completion of a manual alignment, select **Resume** to continue with the the next selected action in the sequence.

3. In case an action fails (red cross), execution of the sequence is aborted, not paused.

Perform the following steps to correct a failed alignment:

a. Perform the failed action *manually*, for example in th *TEM User Interface* > *Direct Alignments* control panel.

b. Right-click on the **failed action** and select **Run Selected** to repeat the alignment in APM.

For the following actions a follow-up action is recommended for the best result:

- **Rotation center**

  The *Rotation center* alignment affects coma. Run the *Coma correction* or *Tune image corrector* action after the *Rotation Center* alignment is performed.

- **Coma correction** and **[m]Tune image corrector**

  Coma correction affects Objective lens astigmatism. Run the *Objective stigmation* alignment after the *Coma correction* or the *[m]Tune image corrector* action.

c. If the repeated alignment passes (green checkmark), then execute the succeeding selected actions *one-by-one* (right-click > Run selected).

Do *not* select *Run*. The Run function always executes all selected actions.

4. Check the **Result** panel for the evaluation result:

- All **OK**:

  The alignment status is fit for high quality data acquisition. No further improvement is needed.

  End of procedure.

- Some are **FAIR**, the rest is **OK**:

  The alignment status is fair. Whether or not improvement is needed depends on the required resolution of the planned experiments. See Coma, Phase Shift and Resolution on page 65 for guidelines.

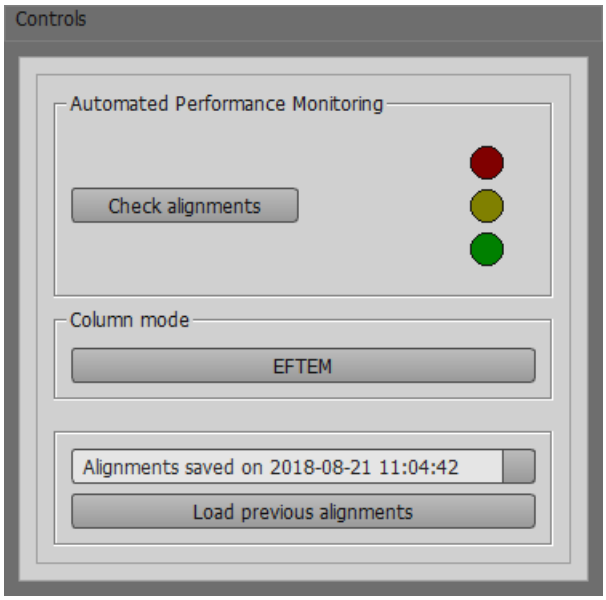
  A FAIR result on a perfect sample still allows high resolution data acquisition. If an improvement in the coma values is desired, this can be achieved using autofunctions or by repeating the full or partial alignment sequence in APM.

- **NOK** (Not OK):

The alignment status is not fit for data acquisition. To improve the alignment status:

- First, repeat this improvement procedure from step 1 onwards.
- If repeating the APM procedure does not lead to the desired result, use the **TEM User Interface** > **Direct Alignments** to troubleshoot the failing alignments.
  After all manual corrections are completed, run the **Sequence** > **Save and back-up alignments** action, and repeat the APM procedure from the start with selecting the **Check alignments** button.
- If all the above actions still results in *NOK*, then select **Control** > **Load previous alignment**, select a known good Alignment set. Then repeat this improvement procedure from step 1 onwards.

10.4 Controls



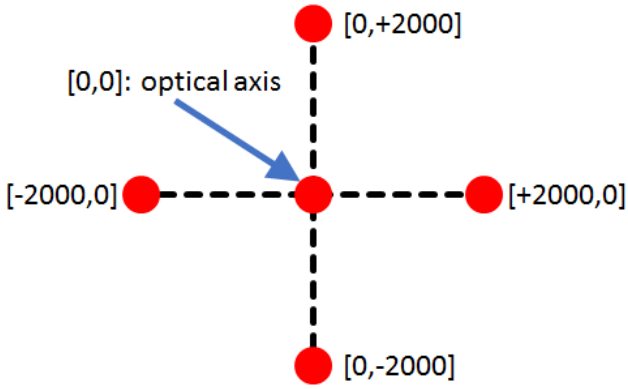
- **Check Alignments**
  Loads the last saved Alignment set for the current optical mode (TEM or EFTEM) and assesses whether or not the alignment status is fit for high quality data acquisition.

Note **If the alignments have been improved manually, any manual adjustments are overwritten by the last saved Alignment set and will be lost.**

For instructions how to manually adjust the alignments outside Sherpa APM, see How to Adjust the Alignments in the TEM User Interface on a System with Sherpa APM on page 65.

To assess the alignment status, Sherpa performs a set of astigmatism and coma measurements at the optical axis and at 2000 nm offsets from the optical axis. For the measurements at the offset positions, Sherpa applies a beam shift and a matching stage shift, so that the same area is illuminated at each beam shift.

In TEM mode, the optical axis is the center of the FluScreen. In EFTEM mode, the optical axis is shifted to the Energy Filter entrance position.



For each measurement, Sherpa APM assesses the amount of coma and astigmatism. If a measurement fails or if the result is not satisfactory, then Sherpa APM skips the remaining measurement positions. The alignments status is indicated by the *Traffic Light*.

Depending on the system's optical mode, the bottom-mounted or post-filter High Sensitivity camera is used. If there is no High Sensitivity camera available, Sherpa will use the High Dose camera. If necessary, Sherpa APM lifts the FluScreen, inserts the camera, and applies the appropriate acquisition settings. The acquired images and evaluation results will be displayed in the *Results* frame.

- **Traffic Light**

  Indicates whether or not the alignment status meets the criteria for high quality data acquisition.
  - Green: alignment status is OK, high quality data acquisition feasible.
  - Yellow: alignment status is fair. High quality data acquisition feasible, but depending on the required resolution for the planned experiments, improvement is recommended.
  - Red: alignment status is not suitable, or the *Check alignments* function failed. Improvement is required.

  When the result is not *green*, the *Results* and *Logging* panels may provide suggestions how to continue.

  For instructions how to improve the alignment status, see Procedure on page 55.
- **EFTEM**

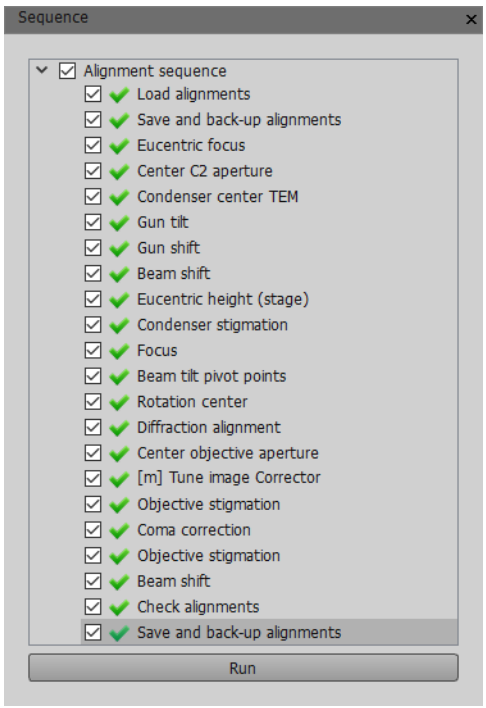
  Toggles the system's optical mode between *TEM* and *EFTEM* and loads the settings for the active mode from the active Alignment set.

  If the system's optical mode is switched via the *TEM User Interface* > *EFTEM* control panel, then Sherpa APM follows automatically.
- **Load previous alignments**

  Applies the selected Alignment set. This functionality is typically used to load a preceding Alignment set when the current Alignment set cannot be improved to a desired alignment status.

  An Alignment set contains the most recent FEG Register settings for TEM mode and for EFTEM mode at the time the backup is saved. It is therefore not necessary to select an Alignment set that was saved when the system was in the same optical mode as the current optical mode.

10.5 Sequence



The availability and order of the actions depends on the system configuration and the software version.

The top-down order of the actions corresponds to the preferred execution sequence for a reliable result. Although it is possible to execute the actions individually and in any chosen order, this may lead to unpredictable results. The selected set of actions is stored. At startup, Sherpa automatically loads the most recent selection.

- **Alignment sequence**
  Presents the available actions and their status. Which actions are available depends on the system configuration.
  - Question mark: action has not been executed.
  - Running man: action is being executed.
  - Green checkmark: action has succeeded.
  - Red cross: action has failed.
- **Run / Resume**
  Starts or continues the sequence of ticked actions.
  When a guided manual action is ongoing, the Run button changes to a Resume button.
- **Context menu**
  The Alignment sequence offers a context menu with the following options:
  - **Run Selected**: executes only the selected action.
  - **Clear History**: reverts the status of the selected action to 'not executed'.

10.5.1 Overview of the APM Sequence actions

Actions with an `[m]` prefix are guided manual tasks. All other actions are fully automated routines.

Seq.	Action	Description / Instructions / Remarks
1st	Load alignments	Loads the active Alignment set.
2nd	Save and back-up alignments	Saves a timestamped backup of the active Alignment set.
The availability and order of the actions depends on the system configuration and software version.		
	Eucentric focus	Performs the following functions: Normalize all > Eucentric Focus > Reset Defocus
	Center C2 aperture	Performs the *Center C2 Aperture* direct alignment
	Condenser center TEM	Performs the *Condenser Center TEM* direct alignment.
	Gun tilt	Performs the *Gun tilt* direct alignment. *Only for systems with Fringe Free Imaging that do not have a Falcon 3EC camera*.
	Gun shift	Performs the *Gun shift* direct alignment. *Only for systems with Fringe Free Imaging that do not have a Falcon 3EC camera*.
	[m] Gun tilt and shift	By default, this alignment is not selected. Only select and perform this alignment when absolutely necessary. If the Gun Tilt and/or Gun Shift alignment is changed, then the dose calibration and dose protector may require updates as well. ● Perform **Direct Alignments** > **Gun Tilt** ● Perform **Direct Alignments** > **Gun Shift** ● Select **Sherpa** > **APM** > **Sequence** > **Resume** *For systems without Fringe Free Imaging and/or with a Falcon 3EC camera*.
	Beam shift	Aligns the Beam Shift using the FluCam.
	Eucentric height (stage)	Performs the *Auto-eucentric by stage* function.
	[m] Condenser stigmation	Follow the instructions to perform Condenser stigmation, preferably on the high dose camera, but if necessary on the high sensitivity camera.
	Focus	Adjusts the Objective lens to focus the specimen by fitting the Contrast Transfer Function (CTF) to the FFT, then resets the *Defocus* value.
	Beam tilt pivot points	Aligns the Beam Tilt Pivot Points.
	Rotation center	Aligns the Rotation Center using the FluCam.

Seq.	Action	Description / Instructions / Remarks
	Diffraction alignment	Aligns the Diffraction Pattern: ● TEM mode: with the center of the FluScreen. ● EFTEM mode: with the entrance position of the Energy Filter.
	Center objective aperture	Inserts and aligns the 100 micron objective aperture.
	[m] Tune Image Corrector	● Tune the Image Corrector in the **Corrector User Interface**. ● Select **Sherpa** > **APM** > **Sequence** > **Resume** This action is only available on systems with an Image Corrector.
	Objective stigmation	Performs Objective stigmation, preferably on the high dose camera, but if necessary on the high sensitivity camera.
	Coma correction	Performs Coma Correction on the high-sensitivity camera. This action is only available on systems without an Image Corrector.
Check, save and backup the optimized Alignment set.		
Before last	Check alignments	Evaluates the current alignment status.
Last	Save and back-up alignments	Updates the active Alignment set, and saves a timestamped back-up.

10.6 Tips & Tricks

10.6.1 The APM Alignment Set

Sherpa APM uses Alignment sets. An Alignment set consists of an Alignment file and a FEG Register file.

- FEG Register file: `APM_Dxxxx.feg`
  where `Dxxxx` is the serial number of the system.
  This FEG Register file contains the following FEG Register(s):
  - `APMTEM_HT###` register for TEM mode.
  - On a system with an Energy Filter:
    `APMEFTEM_HT###` register for EFTEM mode.
- Alignment file: `FactoryDxxxxHT###.alg`
  where
  - `Dxxxx` is the serial number of the system.
  - `HT###` is the High Tension voltage.

Note **If the required FEG Register and/or Alignment file is not available, then contact your Thermo Fisher Scientific service representative to create the missing files.**

Before working with APM for the very first time, the Alignment set must be prepared manually. This is typically done by a Thermo Fisher Scientific engineer.

Once Sherpa APM is used to maintain the alignments, the FEG Register and the Alignments file are no longer loaded and maintained via the TEM User Interface. Instead, the Alignment set must be loaded and maintained in the Sherpa user interface.

10.6.2 How to Adjust the Alignments in the TEM User Interface on a System with Sherpa APM

On a microscope with Sherpa APM, the TEM User Interface control panels are still available to manually adjust the microscope alignments and settings. Changes that are made outside Sherpa APM are not automatically merged into the active APM Alignment set.

Follow the steps below to save a manually adjusted alignment as the active APM Alignment set:

1. Right-click on **Sequence** > **Save and back-up alignments** and select **Run selected** to save a timestamped backup of the *current* alignments and settings.
2. Use the **TEM User Interface** > **FEG Register** and/or **Alignment** control panel to manually adjust the microscope settings and alignments.
3. Right-click on **Sequence** > **Save and back-up alignments** and select **Run selected** to save a timestamped backup of the *adjusted* alignments and settings.

If the above steps are not executed, then the manually adjusted values will be overwritten by the active Alignment set as soon as APM is used.

10.6.3 How to Maintain the Microscope Alignment with Sherpa APM and Permission Management

Sherpa APM maintains a dedicated alignment set, consisting of a FEG Register and an Alignments file. To safeguard the consistency of the alignments and settings that are maintained by APM, the *Permission Management* option restricts some of the TEM User Interface functions for regular users and supervisors.

For a specification of the restricted TEM User Interface functions per system type and per user level, please see the *TEM User Interface - Permission Management User Manual*. This document is available on the Microscope PC.

10.6.4 Coma, Phase Shift and Resolution

Coma introduces a frequency dependent Phase Shift [rad] of:

$$\text{Phase Shift} = 2\pi \times B \times \lambda^2 \times q^3$$

in which:

- B is coma [m].
- λ is the relativistic wavelength [m].
  - for 300 kV this is 1.97 picometer.
  - for 200 kV this is 2.51 picometer.
- q is the frequency component [1/m].
  Frequency component value q = 1/(3 Å) corresponds to a resolution of 3 Å.

A commonly used criterion is that the phase shift introduced by the aberrations shall not exceed 45 degrees, or $\pi/4$ [Uhlemann&Haider, ultramicroscopy 1998]. The maximum allowed coma B to meet this phase shift criterion is:

Required resolution	HT = 300 kV (λ = 1.97 pm)	HT = 200 kV (λ = 2.51 pm)
2 Å	B < 258 nm	B < 159 nm
3 Å	B < 870 nm	B < 536 nm

In practice, the coma criteria are not as strict as the above calculation suggests. When multiple particles are averaged over different orientations as is done in a Single Particle Analysis experiment, the frequency components are still conserved in some directions, so a better resolution is still possible.

10.7 Troubleshooting

Several APM autofunctions depend on accurate bias and gain reference images for the FluCam, the bottom-mounted High Sensitivity camera and/or the post-filter High Sensitivity camera. If an APM autofunction fails repeatedly, check and if necessary renew the bias and gain reference images.

Center objective aperture

This autofunction first calibrates the shift of the aperture in X and Y direction, and then centers the objective aperture over the bright central diffraction spot.

Symptom	Possible cause	Possible solution
Objective aperture centering keeps failing	Diffraction calibration is not OK.	Redo diffraction calibration via microscope UI/ calibrations. In case this does not resolve the issue, please contact your local service engineer. Mitigation: Select the **TEM User Interface** > **Apertures** control panel to align the Objective aperture.
	Sample position not suitable for objective aperture centering.	Make sure the beam is centered on a suitable piece of carbon film. The objective aperture centering routine relies on the presence of diffraction rings.
	Objective aperture centering too far off. The objective aperture is blocking the diffraction rings completely.	• Switch to **Diffraction** mode. • Select the **TEM User Interface** > **Apertures** control panel to adjust the Objective aperture mechanism.

11 HT Conditioning for Systems with a Thermionic Source

11.1 Introduction

The HT Conditioning application provides a fully automatic and safe ramp-up from zero to the maximum High Tension voltage. It stabilizes the emission current and automatically handles any sparks and flashovers that may occur during the ramp-up procedure. After the procedure is completed, the accelerator is conditioned and ready for regular use.

The HT Conditioning procedure must be performed after the accelerator has been exposed to atmosphere, for example after replacing the emitter or cleaning the accelerator.

11.2 Preconditions

Before starting the HT Conditioning procedure, verify that:

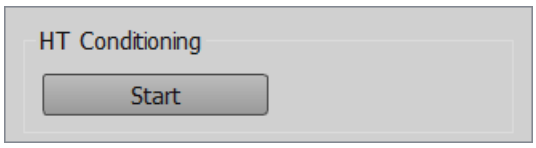
- The vacuum system is in *All vacuum (Closed)* state.
- The Column Valves are *closed*.

11.3 Procedure

Follow the steps below to perform the HT Conditioning procedure:

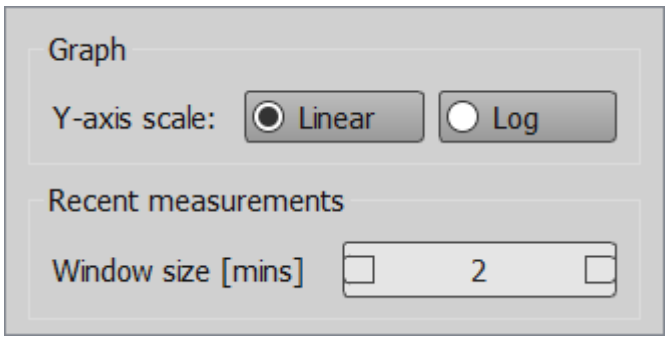
1. Make sure that **all preconditions** are met.
2. Select **Controls** > **Start**
3. If desired, select **Details** in the toolbar.
   When *Details* is active, a more verbose logging is displayed in the *Logging* panel.
4. Wait until the HT Conditioning procedure is completed.
   - If the procedure is completed successfully, then the Logging panel displays the message `HT Conditioning finished`
   - If the procedure fails, then the Logging panel displays the appropriate error message.
5. If desired, inspect the **HT Conditioning report** (PDF) in
   `C:\ProgramData\ThermoScientific\HT Conditioning\Reports\`

11.4 Controls



Start:
Checks if the preconditions are fulfilled and then starts the HT Conditioning procedure. If the preconditions are not fulfilled, an appropriate error message appears in the *Logging* panel.

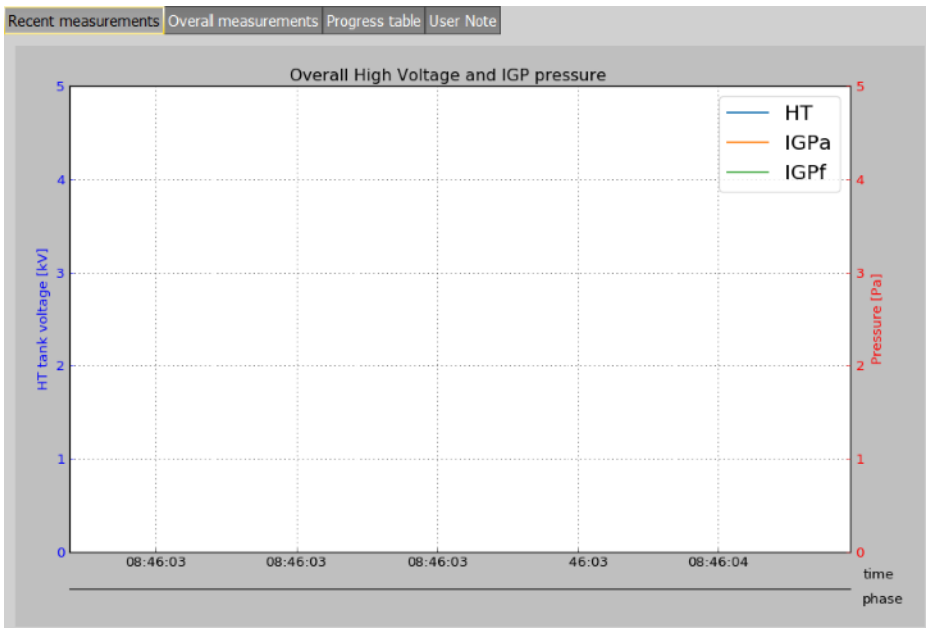
11.5 Settings



- **Graph – Y-axis scale**:
  Sets the scale of the vertical axis in the *Results* panel > *Recent measurements* graph and *Overall measurements* graph
- **Recent measurements - Window size [mins]**:
  Sets the amount of time that is captured in the *Results* panel > *Recent measurements* graph.

11.6 Results

The **Recent measurements** and **Overall measurements** show the IGPa and IGPf pressure, and HT Tank voltage. The axis scales and range are specified in the *Settings* panel.

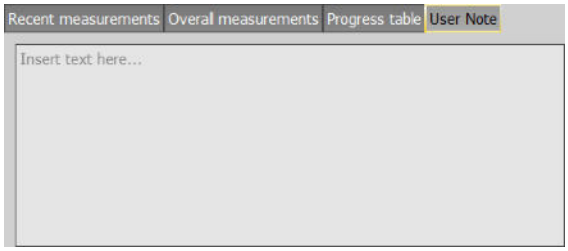


The **Progress Table** shows a progress and results summary for the HT Conditioning phases.

Recent measurements | Overal measurements | Progress table | User Note

		Description	IGPa pressure [Pa]	IGPa Spark count	IGPf pressure [Pa]	IGPf Spark count	Arc count	Flashover count	Retry count	Actual Time	Min Time
1	☐	Initial pressure wait									0:04:59
2	☐	Prologue									0:31:00
3	☐	Level 1									0:48:00
4	☐	Level 2									0:36:00
5	☐	Level 3									0:12:00
6	☐	Level 4									0:24:00
7	☐	Level 5									2:00:00
8	☐	Level 6									2:00:00
9	☐	Level 7									2:00:00
10	☐	Level 8									6:06:00
11	☐	Eplogue									0:00:30
12		**Total**		**0**		**0**	**0**	**0**	**0**	**0:00:00**	**14:42:29**

The **User Note** is a free text entry field. The User Note is included in the HT Conditioning report.



11.7 Tips & Tricks

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11.8 Troubleshooting

Symptom	Possible cause	Possible solution
Error message: "High dark current for HT ramp-up"	The accelerator is not clean.	Clean the accelerator, then start the HT Conditioning procedure again.

12 Spot Saturation

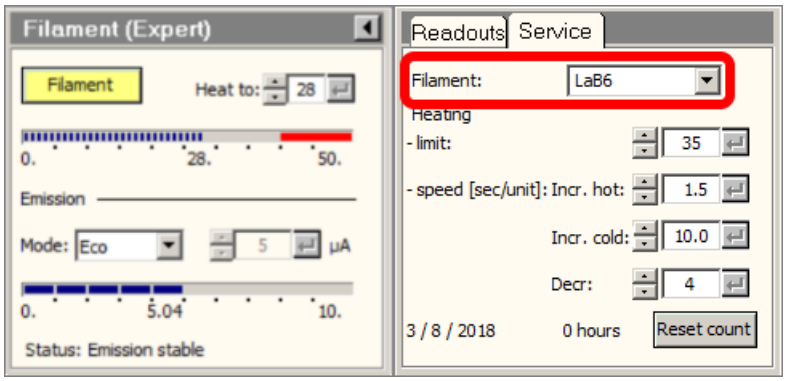
12.1 Introduction

The Spot Saturation application maximizes the beam current, as measured on the FluScreen, and optimizes the shape of the spot. The automated Spot Saturation procedure does this without overheating the filament. In comparison to traditional methods that are based on increasing the filament heating, the automated Spot Saturation procedure results in a longer filament lifetime.

12.2 Preconditions

Verify that:

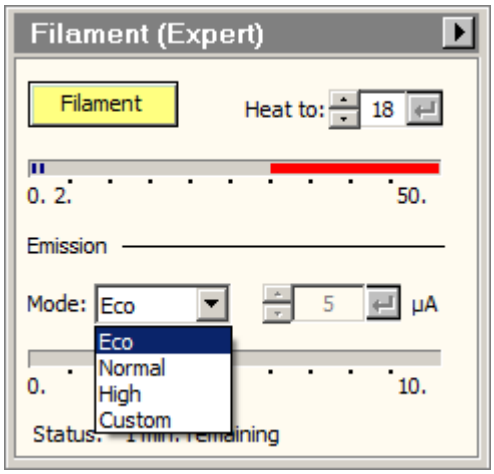
- **TEM User Interface** > **Filament** control panel > **Service** > **Filament** is **LaB6** (not *Tungsten*)



- The **vacuum** system is in *All Vacuum* state.
- No **holder** is inserted.

12.3 Procedure

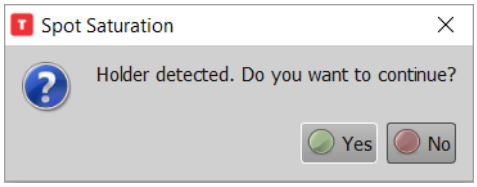
1. Verify that the preconditions are fulfilled.
2. In the **TEM User Interface** > **Filament** control panel, select the desired emission current.



The recommended values are:

- **Mode**: **Normal**
- **Current**: **10** µA

3. In **Sherpa** > **Spot Saturation**, select **Controls** > **Start**
4. If the *Start* function detects a holder, then a warning appears:

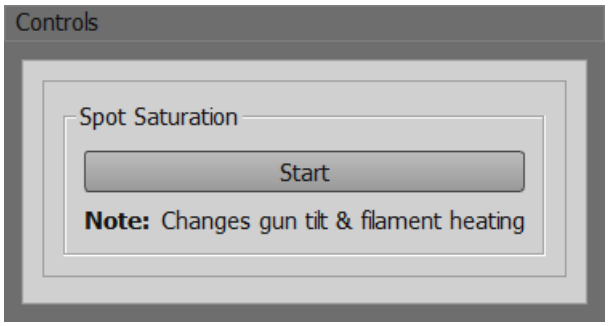


It is strongly recommended to:

a. Select **No**
b. Remove the **holder**
c. Start this procedure from step 1 onwards.

5. If desired, select **Details** to display the FluScreen image and a visualization of the Spot Saturation algorithm.
6. Wait until the procedure is completed.

12.4 Controls

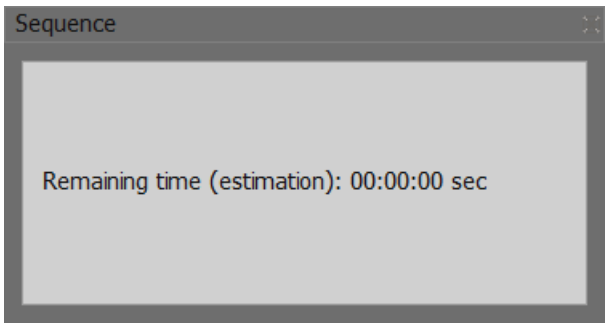


Start

The Start function checks if the preconditions are fulfilled and then starts the Spot Saturation procedure.

- If the preconditions are not fulfilled, then a warning appears with the choice to continue or not.
- If no violations are detected, then the Spot Saturation procedure starts.

12.5 Sequence



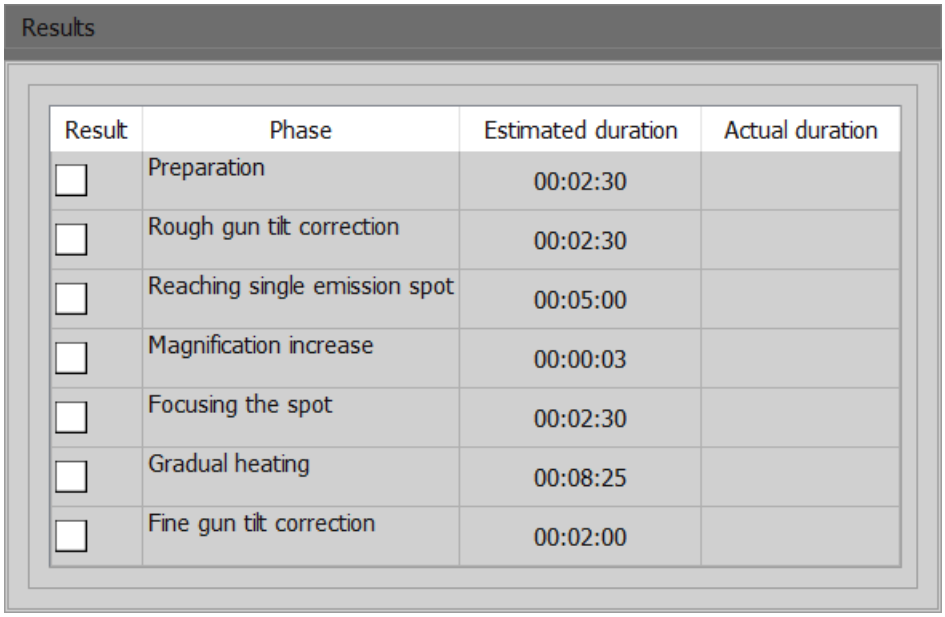
The Sequence panel displays the estimated remaining time until the Spot Saturation procedure is completed. If more time is needed to complete the procedure, then an appropriate warning appears.

The Spot Saturation procedure goes through the following phases:

1. Preparation
2. Rough gun tilt correction
3. Reaching single emission point
4. Magnification increase
5. Focusing the spot
6. Gradual heating
7. Fine gun tilt correction

12.6 Results

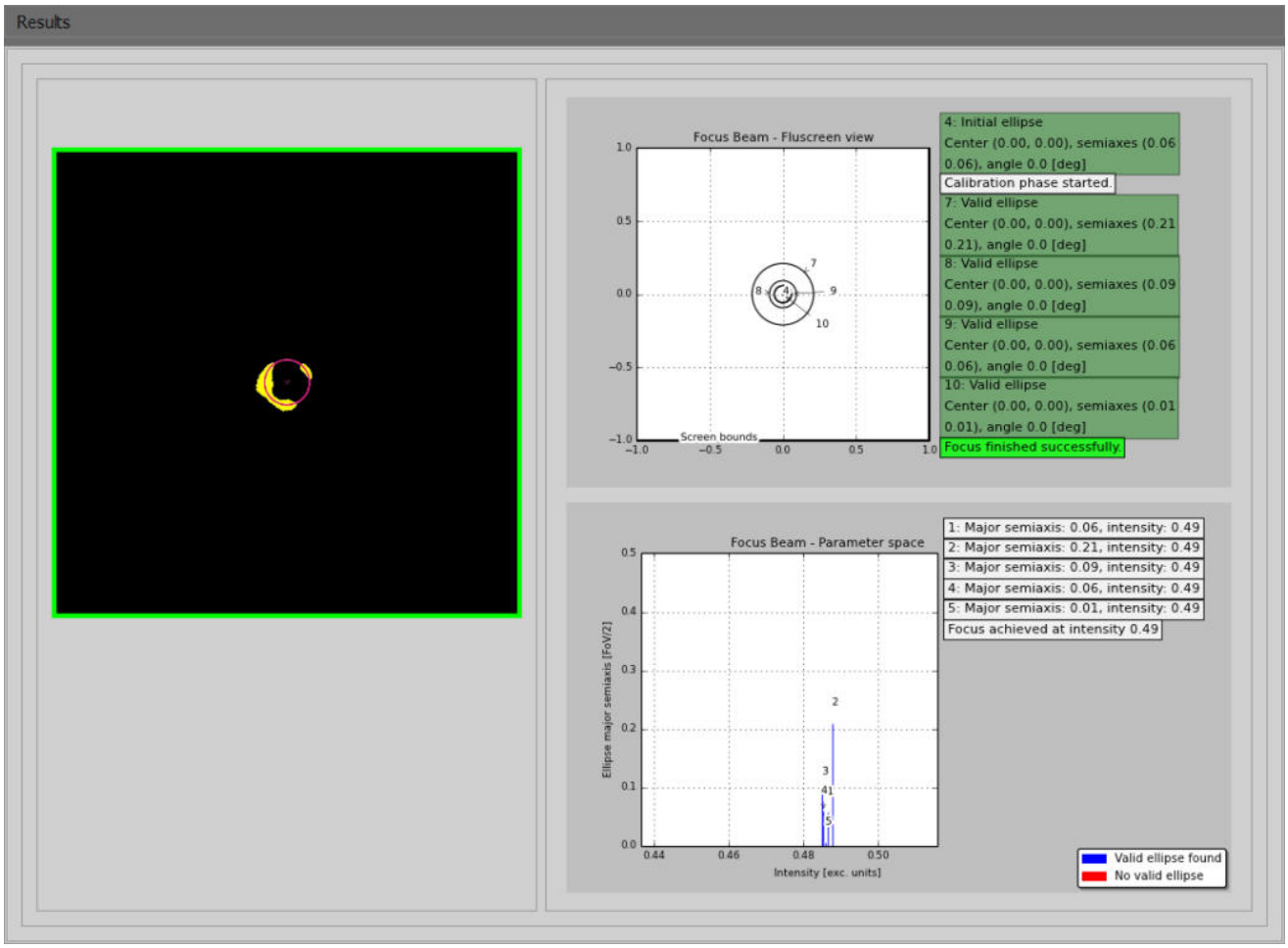
If *Details* is not selected, then the Results panel displays a summary of the progress and results of the phases of the Spot Saturation procedure.



The results table shows the current phase, its result, and the estimated and actual duration. The result is represented as follows:

- White – Phase not started.
- Blue – Phase started.
- Gray – Phase interrupted by the user.
- Green – Phase finished successfully.
- Yellow – Phase finished with warning.
- Red – Phase failed.

If *Details* is selected, then the Results panel displays the following information:



- The FluScreen image
- The intermediate results of the processing steps.

12.7 Tips & Tricks

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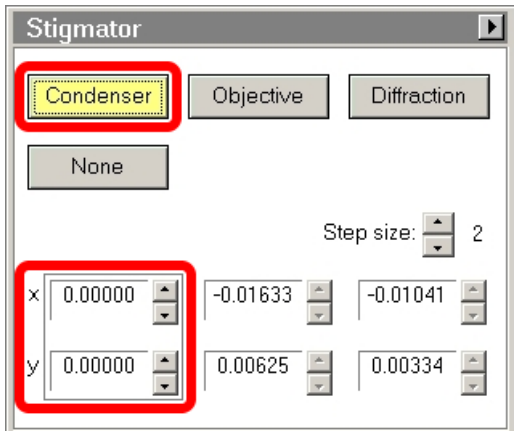
12.8 Troubleshooting

Symptom	Possible cause	Possible solution
The Spot Saturation algorithm fails to find the initial spot.	The raw screen current is less than 15 pA	In the **TEM User Interface** > **Filament** control panel, select a higher **emission current**
	A large condenser astigmatism is present	• Select **TEM User Interface** > **Stigmator** control panel > **Condenser** • For **x** and **y**, enter **0.0** 

13 Copyright, Limited Rights and Revision History

Copyright

Limited Rights

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Revision Table

Revision	Date	Description of Changes
A	14-SEP-2017	Initial version
B	12-MAR-2018	• Add the AutoCTF application • Update for the TEM Server 6.11 release
C	01-JUN-2018	• Add the APM application for launching customers • Update for the TEM Server 6.12 release
D	12-SEP-2018	• Make APM also available for regular customers • Add the HT Ramp-up application • Update for the TEM Server 6.13 release
E	28-NOV-2018	• Add the Glacios system for APM • Update for the TEM Server 6.14 release
F	15-MAR-2019	• Add Preconditions > Presets to AutoCTF > Controls • Update for the TEM Server 6.15.1 and 6.15.2 releases
G	14-MAY-2019	• Re-arrange the APM chapter and add instructions for manual adjustments. • Update for the TEM Server 7.1 release
H	17-SEP-2019	• Update the APM chapter • Update for the TEM Server 7.2 release
I	10-OCT-2019	Update for the TEM Server 6.15.3 release, based on Revision F.

Revision	Date	Description of Changes
J	29-JAN-2020	● Add the Spot Saturation application ● Add the OptiMono+ application ● Update for the TEM Server 7.3 release, based on Revisions H and I.
K	06-MAR-2020	Update for the TEM Server 7.4 release
L	30-JUN-2020	Update for the TEM Server 7.5 release
7.6	09-OCT-2020	● Add the HT Conditioning application ● Add the Energy Filter application ● Update for the TEM Server 7.6 release
7.7	17-DEC-2020	● Add the TEM Distortion Correction application ● Update for the TEM Server 7.7 release
7.8	07-APR-2021	● Remove HT Ramp Up (succeeded by HT Conditioning) ● Update for the TEM Server 7.8 release ● Update for TEM Distortion correction
7.9	01-JUL-2021	Update for TEM Server 7.9 release
7.9.A	14-JUL-2021	APM: add FEG Register and Alignment file names for Mid Range systems.
7.11	14-DEC-2021	Update for TEM Server 7.11 release
7.13	16-AUG-2022	TEM Distortion Correction: restrict camera applicability to Ceta only.
7.13.1	07-SEP-2022	APM > Condenser Stigmation is reverted from automated to manual action.

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