# thermoscientific

# Microscope Companion<sup>™</sup> 1.16

# User Guide

PN 1552687

Revision A • January 2025 Limited Rights



# **Copyright and Trademarks**

# **Technical Publications**

Technical Publications Team - Hillsboro

Copyright © 2025 by FEI Company, a part of Thermo Fisher Scientific. The information and materials contained herein are proprietary to Thermo Fisher and are provided for your organization's internal use on a need-to-know basis. They cannot be duplicated, published, or disseminated to any third party without the express written consent of Thermo Fisher.

# Trademark Acknowledgments

All trademarks are the property of Thermo Fisher Scientific and its subsidiaries unless otherwise specified. FEI and the FEI logo are registered trademarks of FEI Company (part of Thermo Fisher Scientific and its affiliates). All other trademarks belong to their respective owners.

Excel and Microsoft are registered trademarks of Microsoft Corporation.

# Limited Rights

The following notice applies to the U.S. Government and other purchases with federal funds:

Contractor Name: Thermo Fisher Scientific

Contractor Address: 5350 NE Dawson Creek Drive, Hillsboro OR 97124

The Government's rights to use, modify, reproduce, release, perform, display, or disclose these technical data are restricted to those rights specified in DFARS 252.227-7015(b)(2), FAR 52.227-14(g)(2)(Alternate II) and FAR 12.211. Any reproduction of technical data or portions thereof marked with this legend must also reproduce the markings. Any person, other than the Government, who has been provided access to such data, must promptly notify the above named Contractor.

# **Document History**

Rev A, January 2025

# **Release Notes**

# Purpose

This section describes the Thermo Fisher Scientific Microscope Companion<sup>™</sup> (MiCo) software releases. (referred to as MiCo in the remainder of this document). For details about the use and features of the MiCo software, see the Microscope Companion<sup>™</sup> User Manual.

# Hardware Requirements

The MiCo software can be used on the Microscope PC of Thermo Fisher Scientific and FEI TEM systems with a compatible TEM Server software version and compatible cameras and/or detectors.

# System, Software and Configuration Compatibility

MiCo is a hardware-oriented application with live-viewing of camera's and detectors for diagnostic purposes only. The application is not intended to be used for data acquisition. Data acquisition can be performed in other dedicated software applications like Thermo Fisher Scientific Velox, EPU, etc. In some situations it may be necessary to use the extended (camera/detector) functionalities in other applications in combination with MiCo.

Below sections shows the preferred version of MiCo software in combinations with the microscope software and the system configuration compatibility for the latest version.

## Preferred MiCo Version per Microscope Software Version

The best user experience and access to the latest features is achieved in combination with the latest versions of the microscope software. We intend to make MiCo backward compatible with a limited range of Microscope Software versions, i.e., six versions in particular. We make this choice in order to deliver improvements on a more frequent basis. Therefore, you may or may not see some features described in these Release Notes based on your system and software versions. We recommend upgrading to the latest MiCo version that supports the current Microscope Software as detailed in the table below. Software version combinations outside of the range specified in the table are not supported.

The following table lists the preferred version of MiCo software in combination with the microscope software.

MiCo / Apollo	Titan	Talos	
MiCo 1.16	3.23 - 3.17	2.23 - 2.17	

### **Compatible Microscopes and Supported Functionalities**

Thermo Fisher Scientific Microscope Companion<sup>™</sup> software contains functionalities for a limited range of Thermo Fisher Scientific transmission electron microscopes and is installed only on those by default.

The following microscopes are supported and their main functionalities are described.

Microscope Name	Functionality
Tundra	Automated alignments, SmartCam, Recovery options (Vacuum, Source/HT)
Glacios (G1 and G2), Arctica, L120C, F200	Automated alignments, SmartCam
Krios, Glacios, Arctica	Autoloader temperature and cryo cycle (requires Autoloader SW upgrade).
Spectra Ultra	EDS detector calibration
Metrios 6	EDS detector calibration, SmartStage insert/retract

MiCo offers live-viewing most camera's and (STEM) detectors on any microscope (on Windows 10) for diagnostic purposes only. Only some Falcon/Ceta reference image are supported (not all and not SmartCam).

 Note: It is recommended to close FlucamViewer before using SmartCam in MiCo, see known issues.

## Microscope Companion<sup>™</sup> (MiCo) 1.16

This release includes:

- Changed the implementation of Source and HT recovery. This feature is compatible with all FEGs, but not with Thermionic sources.
- All the hardware pages now feature the column valves button.

## Known issues

The following are known issues that are being released with this version of the software.

ID	Description	Workaround
1	The startup time of MiCo may be longer than usual for some systems during the first time after TEM server start, taking from about 10 to 60 seconds.	None available for now.
2	Grey progress images are shown for few alignments using a Falcon (3) camera	None available for now.
3	Dose measurement for Falcon camera's is too sensitive and may prevent live-viewing of the camera.	Use other applications, like EPU.
4	Running SmartCam in the application blocks other software from using the same (like automated alignments) and it may cause alignments to fail quickly.	Close SmartCam manually. Note that MiCo does not need to remain open when working in other applications.
5	Gun Shift alignment requires an inserted holder for X- Ray safety on systems without Autoloader. However, it requires vacuum/no sample in beam.	Holder can be navigated to hole or holder can be slightly retracted from microscope.
6	Stage recovery error is thrown with recovery of stage.	Ignore the error, the stage will be enabled
7	Missing reference image manager links for some camera's	Use Microscope Software Launcher to access the reference image managers.
8	Removed the links to reference image managers for Camera's	These are available from the Microscope Software Launcher

# Introduction

Thermo Fisher Scientific Microscope Companion<sup>™</sup> software is an application to prepare Thermo Fisher Scientific transmission electron microscopes for data acquisition in other dedicated software applications like Thermo Fisher Scientific Velox, EPU, etc. In the rest of this document, and wherever practical, we use MiCo as an abbreviation of Microscope Companion<sup>™</sup>.

MiCo software is aimed at supervisors (thus not at all users) of Thermo Scientific Transmission Electron Microscopes. It is intended as a helpful assistant in identifying and correcting issues. Additionally, MiCo allows you to adjust basic microscope settings, do some sample navigation and perform hardware management activities like calibrations. The supervisor should be able to successfully perform the following:

- Evaluate the quality of acquired data.
- Identify the cause of accuracy and quality issues with the acquired data.
- Perform the necessary corrective actions to resolve the identified issues, provided that these actions are available to the user. For higher level corrective actions, the assistance of a Thermo Fisher Scientific engineer may be required.

The available functionality of the Microscope Companion software depends on the microscope type, configuration, and software versions. Therefore, some functionality described in this user manual may not be available, and the appearance of the User Interface (UI) may differ from those shown.

# Installation and Start-Up

The MiCo software is included in the TEM Server software installation and should be installed by a qualified Thermo Fisher Scientific service engineer during a microscope software upgrade. Additionally, the software can be installed or updated using an installer provided by a Thermo Fisher Scientific service engineer. To ensure compatibility with your microscope software versions, it is highly recommended that you upgrade to the latest version of MiCo. For compatibility specifics, please consult the Release Notes for your particular MiCo software version.

Before initiating the MiCo software, ensure that the TEM server is operational. To start MiCo, you can either use the Windows Start menu (navigate to All Programs > Thermo Scientific MiCo > MiCo) or the microscope software launcher. Upon starting the software, the main window will appear, as shown in the image below.

**Note:** The images in this document depend on the microscope and may look slightly different.



đ

# User Interface Layout and Features

The Microscope Companion user interface is divided into different parts as shown in the following image. These parts are:

- 1. Menu and Acquisition toolbar.
- 2. Hardware Management panel.

3. Image Viewing Area is the location where the live-view windows of cameras and detectors appear in the UI.

4. Side panels.



## Menu and Acquisition Toolbar

The main menu area contains the following items.

Menu item	Description
File	Close the application.
View	Layout of the image windows.
Optics	Optical settings like magnification, optical mode (STEM/TEM), projection mode (Imaging/Diffraction) and probe mode (microprobe/nanoprobe).
Filter	Set the system to EFTEM mode and set up or modify settings of the energy filter on the system.
Detectors	Available cameras on the system.
Help	User manual and release notes.

The acquisition toolbar primarily focuses on sample navigation using live-view windows from cameras and detectors, rather than data acquisition, as seen in other applications. The toolbar consists of the items outlined in the table below.

Toolbar	Description / Main purpose
SmartCam	Start SmartCam live-view and beam blank button.
Optics	Change optical mode (TEM/STEM).
Annotations	Measure the dimension of a feature on a sample in the image window.
Detectors	Select a Camera or detector for live-view.
Camera	Set up properties of live-view of various cameras.
Scan Rotation	Change scanning direction of STEM live-view.
STEM Imaging	Set up properties of live-view of various detectors.
Energy Filter	Set up properties of the energy filter.
Dose rate	See and measure dose on the sample.

## Side Panels

The right side of the UI grants access to the following panels:

- 1. Histogram.
- 2. Fast Fourier Transform (FFT).
- 3. Object Properties, which displays parameters for a selected live-view or annotation, such as image size and conditions under which a live-view is being recorded.
- 4. Detector Layout.
- 5. Detector Settings, which offers parameters and functions to fine-tune the STEM detectors.

The Histogram, FFT, and Detector Layout panels are discussed in the subsequent sections. The Detector Settings panel is addressed in conjunction with live-views of STEM detectors.

## Histogram

The Histogram is shown for a live-view window as the first tab of the display settings panel and as shown in the figure below. It should be noted that it is possible to have multiple live-view windows open and there is only one histogram at a time. The Histogram is displayed for the selected image window where the title of the image window is high-lighted.

The purpose of the Histogram is to adjust the live-viewing of cameras without changing the controls of the camera or detector itself.



Microscope Companion<sup>™</sup> 1.16

#### Options of a Live-View Window

The appearance of the live-view is determined by a number of parameters:

- Black-level is the histogram intensity value represented by black. Any intensity value below the black-level will be black. In the figure above, the black-level of 443.4 is applied.
- White-level is the histogram intensity value represented by white. Any intensity value above the white-level will be white. In the figure above, the white-level of 1496.2 is applied.
- Gamma value is the gradient applied to the histogram intensity value. In the figure above, a linear gradient is applied with a gamma value of 1.00. The linear gradient is represented by

the diagonal line in the graph. Select the **Gamma** button (**U**) to reset the Gamma value to 0.50 for diffraction pattern images, or to 1.00 for all other image types.

• Look-up-Table (LUT) describes the relation between the histogram intensity value and screen color for the range of black-level to white-level. There are a number of predefined LUTs available. In the figure above, the Grayscale LUT is used. It is also possible to invert

the LUT 🛄

For a reference of Cubehelix LUT please see: Green, D. A., 2011, `A colour scheme for the display of astronomical intensity images', Bulletin of the Astronomical Society of India, 39, 289.

#### Adjust a Live-View Window

There are several ways in which to adjust the live-viewing window:

- Automatically adjust Black and White level ( ) or White level ( ) only. The automatic adjustment of Black and White level is the default option for all images, except diffraction pattern images.
- Manually interact with the graph by
  - Moving the vertical lines using the mouse to adjust the minimum intensity value (blacklevel) and maximum intensity value (white-level).
  - Changing the shape of the curve by moving the line using the mouse.
- Manually use the controls underneath the graph.

Optimize a Live-View Window

The Detector Saturation Indicator shows the saturation of the detector as shown below. The Detector Saturation Indicator can be used to optimize the imaging conditions, so the detector captures the full range of intensities without overexposure.

## Fast Fourier Transform

The Fast Fourier Transform (FFT) can be found in the second tab of the Display settings side panel, as illustrated in the figure below. It displays a high-speed live FFT of the selected image window. The interactive window allows for zooming in or zooming out using the mouse wheel when the cursor hovers over the FFT.



You can draw a circle at a specified spatial frequency by selecting the **Show spatial frequency** check box and entering the value and unit (pm, nm, mm). Alternatively, double-clicking within the FFT sets the spatial frequency at the cursor's location.

A right-click in the FFT reveals a menu with several additional options, including:

- Resetting the zoom.
- Zooming to one FFT pixel per display pixel (zoom 1:1).
- Utilizing interpolation (anti-aliasing) to enhance the smoothness of the displayed image.
- Applying a Hamming window (windowed FFT) to reduce horizontal and vertical lines caused by image borders.

## **Detector Layout**



Detector Layout panel with an HAADF and a Super-X G2 detector. For the Super-X G2 detector, segments 1, 3 and 4 are enabled.



Detector Layout panel with a Dual-X detector.

Depending on the system configuration and Optics mode, the Detector Layout panel shows:

Detector status or Detector Segment status

The inserted/retracted or enabled/disabled status for each detector or detector segment. Select the detector-specific button to toggle its status.

Depending on the type of detector, additional properties can be displayed.

- Super-X and Dual-X sensors: the count rate and dead time percentage.
- STEM detectors: the collection angle.
- Main Screen

Insert or retract the FluScreen.

Beam status

No Beam: Column valves closed, FEG Standby, High Tension off

If no beam is present, the reason is shown here.

Magnification

Magnification – + 5.5 kx 🕶

The available magnification values are nominal. Select [+] or [-] or select a value from the drop-down list.

Camera length



Select [+] or [-] or select a value from the drop-down list.

Field of View



The available magnification values are calibrated.

Select [+] or [-] or select a value from the drop-down list, or enter the desired value in the list box.

• Proj. Mag.



Select [+] or [-] or select a value from the drop-down list.

# Hardware Management Panel

The hardware management panel is divided into four distinct parts: the Toolbar, the Header, the management pages, and the log window. The following sections describe each part.

## Hardware Toolbar

The toolbar grants access to pages as outlined in the table below. The selected page will be highlighted on the toolbar, with the button appearing in a purple color. Please note that pages marked with an asterisk (\*) are only available on supported systems.

Page	lcon	Brief description of main functionalities
Home		Status overview of main system functionality, Column valve status and open/close, Column pressure, Stage type and status
Actions	\$	Cryo-cycle, Evacuate Column, Recover Source and High- Tension, Recover Stage, Recover Optics boards
Electron Beam	<b>Y</b>	Source and High-Tension On/Off status
Alignments*	+	Overview of automated alignments Executing automated alignments with progress indication, information and diagnostic images
Detectors	<b>4</b>	Camera status EDS status and controls
Optics	X	Change beam diameter in TEM imaging*

## Status Indications

The hardware management toolbar and panels provide feedback on the status of various subsystems, modules and functionality of the microscope. Status symbols and coloring are used to indicate status and it should be obvious where issues occur. The symbols and coloring are shown in the following table.

Symbol	Status	Remarks
Ø	Good, Ready, On	Good, Ready, On
()	Warning	Indicates something may require attention. For example, it indicates that the column valves are closed on the vacuum page. These valves are opened automatically when required by some other actions on the microscope, like viewing an live-view.
0	Expired	Indicates something is expired. Typically, the status of something like an alignment may expire after not being executed for a specific time period.
?	Unknown	Not sure what the status is at this moment. Status will be overwritten after execution. However, it should be noted that some status may not be persistent and/or stored and the indication may return after restarting the application.
0	Bad, Not Ready, Off	Something is not in an operational state and requires attention and/or recovery actions. For example, alignments may fail when they were executed under non-intended conditions.

## Resizing the Panel

The hardware management panel is fully open by default and the home page is shown when starting the application. The button for the currently displayed page becomes purple.

It is possible to collapse the panel completely by clicking on the icon of the selected page in the toolbar.

Additionally, the toolbar provides a way to re-size the Hardware Management Panel at the bottom as indicated in the figure below. The panel can be open (left), half-way open (middle) or closed (right).



## Hardware Management Header

The header of the hardware management panel, located above the pages, displays the system status as depicted in the figure below. The status elements in the header include:

- The optical mode, which may comprise TEM, EFTEM, or STEM.
- Spotsize (1-11).
- Probe mode (microprobe, nanoprobe).
- The Magnification range, which can be either High-Magnification (HM) or Low-Magnification (LM). Furthermore, you can toggle between the HM and LM by clicking the button.
- The Liquid Nitrogen level in the Column and Autoloader or Cryo Loading Station (CLS). The liquid nitrogen level will be shown on Autoloader systems only when in Cryo temperature mode. In all other cases, the highest temperature will be displayed to indicate the system is (not yet) in Cryo temperature mode. It is recommended to look at the home page for all temperatures available for column or Autoloader.

MiCo	TEM	Spotsize 3	Microprobe	LM	40 x	Column 85%	Autoloader 85%	
Home								

# Hardware Management Pages

The toolbar provides access to various pages, which are described in the sections below.

## Home Page

The Home Page offers a status overview of the main system functionality, along with a generalized microscope image. It does not display the status of every component within the microscope. This page also contains links to other pages.

The page contains control over the column valves and shows its status.

It shows stage type and status. Optionally, holder status and insert/retract with progress information is available on supported systems.

### Autoloader Functionality

Additional functionality will be available on the Home page on microscopes equipped with an Autoloader. The microscope will have a Column and Autoloader compartment with several temperature sensors. The temperatures of all the sensors available on the microscope will be displayed.

The page also has controls to change the temperature mode or run a cryo cycle. The image below shows the buttons and the page for running a cryo-cycle. The temperature mode after the cryo-cycle should be selected before starting the cryo-cycle. Additionally, the cryo-cycles for column and autoloader should be started separately. Otherwise formulated, it is currently not possible to start both at the same time using a single button. It should be noted, it is not possible to delay the transition of temperature mode at this moment and the temperature mode will be set immediately after the cryo-cycle is finished. A second note, the temperature mode will only be set as long as MiCo is not closed during the cryo-cycle as it will not remember the temperature state after the cryo-cycle.

Running a cryo-cycle or changing the temperature mode will show a progress page, but no time information is currently available. The change in temperature mode will show a blinking Autoloader or Column temperature in the header toolbar.

In case there are problems with the filling of liquid nitrogen, an error dialog will be presented on the home page with recommended action.



### SmartStage Functionality

Additional functionality will be available on the Home page on microscopes equipped with a SmartStage (Metrios only). The microscope will have an extended Stage section with status information as shown below.



The page provides access to the automatic insert and retracts of the holder with progress reporting.

Additionally, the SmartStage supports a manual drift compensation that can be applied directly after a holder is inserted using a live-view of a camera or STEM detector to observe the drift. The user is left to determine the drift compensation vector. The drift compensation allows to capture of an image under quasi-static conditions. However, the drift will change over time as the holder will (thermally) stabilize inside the microscope. Therefore, the manual compensation should or could be reduced to zero after some point in time. It should be reduced to zero (reset) when the (automated and) active drift compensation takes over. The compensate drift button provides access to the drift compensation graph. Here, a three-dimensional drift compensation vector can be set. The two-dimensional graph corresponds roughly to the imaging plane. The vertical direction can be compensated if the image is moving out of focus. The actual compensation vector is shown below the graph.



• Note: The compensation is not automatically reset and is left up to the user. It is recommended to reset after holder stabilization within the microscope or after holder retraction.

Note: The scan rotation in STEM mode or TEM image rotation is not taken into account.

## Actions Page

The actions page contains:

Evacuate Column (previously known as "evacuate all")

Recover Source and High-Tension, the following should be taken into account:

- The Recover Source and High-Tension button enables to start source from an off or poweron state to emitting.
- Set the High-Tension to a default value, which may not match the intended (maximum) value for the microscope. Adjust the High-Tension value using the TEM UI.
- The system reports the progress of the source, which may take more than 10 minutes to reach an emitting state.
- It is compatible with all FEGs, but not with Thermionic sources.

Recover Stage, previously known as Enable stage.

Recovery of Optics Board degradation.

Recover Autoloader, Autoloader temperature system, and column temperature system with recommendations if available. The temperature system recovery will only be enabled when needed, but the Recover Autoloader will remain enabled. It is recommended to try this functionality in case of problems experienced with the Autoloader. If it does not resolve your issues, then it is recommended to contact your local service engineer.

Start Cryo-cycle for supported systems.

Start Heating Cycle for supported system, documentation for this feature will be provided elsewhere.

## **Electron Beam Page**

The pages displays the Source and High-Tension status. The set and measured HT value are available.

Note: The Source status is incorrectly shown for Thermionic sources and is not yet supported, as mentioned in the Release Notes (Known Issues).

## **Alignments Page**

The Alignments Page is only available on systems with automated alignments, such as Tundra. The page displays various sequences with automated alignment modules. These modules are tailored to specific systems and workflows for other applications, like EPU.

Consider the following points when interacting with the Alignments Page:

- Automated alignment modules offer an alternative to manual alignments using the TEM user interface. However, it should be assessed (by the supervisor) whether the alignments should be performed on the microscope.
- These automated processes are designed to operate under the default microscope settings defined in the sequences. Deviations from these defaults may result in module failure.
- Most (but not all) alignment modules have default expiration times. These should be used as guidelines rather than strict timelines.
- Most (but not all) alignment modules store the status. Some precondition and fast or necessary modules do not store the result and may show unknown status after restart.
- The execution of any automated alignment module can be initiated on-demand from MiCo, regardless of its expiration time.
- Adjusting microscope settings while automated alignments are running is not advised, even though it may be technically possible.
- Note: The SmartCam live-view is not automatically paused and its continuation may hinder successful execution of alignments (Known Issues).

This page includes:

- Alignment status, error messages, and expiration times.
- Alignment sequence execution with progress messages and diagnostic images.
- Start/Stop: Begins the automated alignment procedure for the selected Alignments set.
- Other features: Individual alignment selection, progress indication per running alignment, and messages indicating the progress and results of the automated alignments procedure.



It should be noted that the microscope state is automatically saved upon successful completion of each complete alignment sequence. In case of a failure for one or more modules, it is possible to manually perform these alignments using TEM User Interface. In such case, it is recommended to save the state (wrench icon) of the microscope manually after finishing.

In the case of Tundra microscopes, it's possible to manually save/load the state to perform alignments using the TEM user interface as well. This procedure typically involves saving the state, opening the TEM UI, loading the state, executing alignments, saving the state again, closing the TEM UI, and finally, loading the state.

#### Alignments Sequence

User Alignments Sequence

This sequence performs the basic alignment of the microscope. It is meant to run without a sample, and aligns appropriate alignments in LM, HM  $\mu$ P, and HM nP modes.

It consists of a Preparation part, and three sub-sequences. The sub-sequences and their purpose are as follows:

#### • Hole/Eucentric (HM μP)

Roughly aligns the system in HM  $\mu P$  mode at a magnification defined in HM Precondition block (about 45 kx).

• Data Acquisition (HM nP)

Aligns the system in HM nP mode at a magnification defined in HM Precondition block (above 100 kx) to prepare it for the Data Acquisition.

• Atlas (LM)

Aligns the system in LM mode at its highest magnification (around 2 kx) to prepare it for the Atlas screening.

**Optimize Optics Sequence** 

This sequence performs fine tuning of the microscope. It is meant to run on a sample and aligns appropriate alignments in HM nP mode.

It is executed on the current optical settings. In particular, the following parameters are used:

- Gun lens (does not apply for Tundra)
- Spot size
- C2 Aperture
- Magnification
- Objective Aperture

#### Alignments and Blocks Description

This blocks can be divided into two groups system actions, and Alignments.

#### System Actions

#### Load Aligned State

- Makes sure that the initial state of the microscope is the last aligned state.
- It loads the \*APM\*.alg file located in C:\Tecnai\Alg folder (as it comes aligned from factory and service).
- It also loads the last aligned state of all apertures (currently, this file is not available for user access).

#### HM µP Preconditions

- Sets the default optical settings for the HM  $\mu$ P sequence. These settings are:
  - Sets Eucentric focus
  - Probe mode: μP
  - Magnification:
    - Tundra, F200C: 43 kx
    - Glacios, Arctica: TEM 45kx, EFTEM 540 kx
  - Spot size: 4
    - Glacios with C-FEG: variable (based on Gun lens value)
    - C2 aperture: 150 um

#### HM nP Preconditions

- Sets the default optical settings for the HM nP sequence. These settings are:
  - Sets Eucentric focus
  - Probe mode: nP
  - Magnification:
    - Tundra, F200C: 110 kx
    - Glacios non-FFI non-EFTEM, Arctica: 120 kx
    - Glacios FFI non-EFTEM: 45 kx
    - Glacios EFTEM (both non-FFI and FFI): 540kx
  - Spot size: 4
    - Glacios with C-FEG: variable (based on Gun lens value)
  - C2 aperture:
    - Tundra: 70 μm
    - Glacios and Glacios FFI: 50  $\mu m$

#### **LM Preconditions**

- Sets the default optical settings for the LM sequence. These settings are:
  - Sets Eucentric focus
  - Probe mode: μP
  - Magnification: Highest LM magnification
  - Spot size: 4
    - Glacios with C-FEG: variable (based on Gun lens value)
  - C2 aperture: 150 μm

#### **Optimize Optics Preconditions**

- Sets the default optical settings for the optimize optics sequence. These settings are:
  - Sets Eucentric focus
  - Magnification: Glacios FFI non-EFTEM: 17500 x
  - C2 aperture: Glacios FFI: 50 um

#### Move Stage To Vacuum

- Moves stage to a vacuum position defined within the EPU.
- It is not possible to define the position in MiCo (this block is skipped when run from MiCo).

#### Move Stage To Site

- Moves stage to a sample position defined within the EPU.
- It is not possible to define the position in MiCo (this block is skipped when run from MiCo).

#### Restore C2 Aperture

• Inserts the C2 aperture defined by the user.

#### Set Magnification For C2

- Decreases the magnification to see the whole beam in the FluCam Viewer.
- The target magnification depends on the size of the C2 aperture. The larger the C2 aperture, the lower the magnification.

#### **Restore Magnification**

• Restores the magnification to the one defined by the user.

#### Alignments

#### Find Beam

- Assures the electron beam is visible on the FluScreen.
- It maximizes the screen current value by changing the beam position and its size.
- Find beam may also change the magnification to find the beam faster.
- Other alignments can internally call the Find Beam procedure as well, if they need help with finding the electron beam.

#### C2 Aperture

- Aligns the important C2 apertures.
- It minimizes the beam movement while changing the beam size.

#### Gun Tilt

- Centers the image of the extractor aperture onto the C2 aperture.
- It maximizes the screen current value by changing the position of the extractor's image with respect to the C2 aperture.

#### Gun Shift

- Minimizes the beam movement while changing the Spot size value.
- It minimizes the beam movement while changing the Spot size values between 3 and 9.
- To do that, it centers the beam using a beam shift parameter on Spot size 9, while on Spot size 3, it centers the beam using a gun shift parameter.

#### Beam Center

- Moves the electron beam to the center of the FluCam.
- It minimizes the distance from the center by changing the beam position.

#### **Beam Astigmatism**

- Makes the electron beam circular.
- It minimizes the eccentricity of the electron beam's shape using the condenser stigmators.

#### **Objective Aperture**

- Centers the objective apertures with respect to the electron beam in diffraction.
- First, it finds the target position by focusing the beam to a spot in diffraction.
- Then, it spreads the beam to see the objective aperture edge and minimizes the objective aperture distance from the target position using the objective aperture movement.

#### Parallel Illumination

- Sets the parallel illumination on the sample.
- It switches to diffraction mode and minimizes the beam size.

#### Image Astigmatism

- Minimizes the astigmatism within an image.
- It acquires the image of an amorphous area, performs a FFT of the image, fits the Thon rings, calculates the image astigmatism, and minimizes it using the objective stigmators.

#### Image Coma

- Minimizes the coma aberration within an image.
- It acquires more images for different electron beam angles, calculates the coma value, and minimizes it using the beam tilt parameter.

## **Detectors Page**

The Detectors Page contains tiles related to the detectors available on the system, such as the SuperXG2, Ceta 16M speed, Falcon 3EC, and SmartCam in the following image.

Microscope Com	spanion			
File View Optics	s Detectors Help			
* 🛶 🚆	CRG CRG			
SmartCam	Optics Annotations Detectors			
MiCo			TEM LM 700 x 🛱 Liquid 6	••• ED
) Home				문
Q <sup>(0</sup> Vacuum	النر ال			
Electron Beam	SuperXG2	Ceta 16M speed	Falcon 3EC	
*				
Stage	Status:	Acquire	Acquire	
		Reference Images	Reference Images	
Detectors				
ş				
Optics				
	Defrost			
	Canterace			
	SmartCam			

#### EDS detector section

Depending on the type of EDS detector, the following functions may be offered:

- Shutter / Position Depending on the type of detector, either:
  - Shutter status: Open or Closed
  - Detector position: Inserted or Retracted

The microscope software has automatic safety functions. Depending on the status of the microscope and its subsystems, these safety functions can deny the execution of actions that could damage the EDS detector.

- Temperature The cooling status of the EDS detector.
- Status toggle Depending on the type of detector, either:
  - Shutter status: Open or Close
  - Detector position: Insert or Retract
- Defrost

Warm up the EDS detector.

While warming up, a timer is displayed below the Defrost button. The defrost procedure can be aborted at any time.

Calibrate

Opens an external calibration tool.

Status

Displays status and progress messages for the EDS detector.

• External Tools Opens external tools such as EDX Calibration.

#### **Energy Filter section**

The Energy Filter section shows the name of the filter and information on its status.

#### Camera section

The Camera's on the system are displayed.

### **Optics Page**

This page includes control of the beam diameter, only available for TEM imaging on supported systems.

#### Changing beam diameter

It is recommended to use a SmartCam or camera live-view and half-way open hardware management panel, as shown in the figure below. The beam diameter control consists of a purple-boxed area with two options for manipulating the beam diameter:

- Use the mouse-wheel when the cursor is over the boxed area.
- Use mouse-clicks on the two halves of the purple-boxed area, where the left and right halves represent the mouse buttons.

Currently, no keyboard shortcuts are available for adjusting the beam diameter.

# Live-View of Cameras and Detectors

The live-views of the cameras and detectors can be initiated from the Acquisition Toolbar. The toolbar also includes a beam blank button for manually blanking/unblanking the beam. Typically, the required camera settings include image size and exposure time. The live-view of the cameras and detectors will be displayed in the Image Viewing area of the User Interface.

The following topics are discussed in separate sections:

- SmartCam (previously known as FluCam).
- STEM detectors.

## SmartCam

The SmartCam is a low-resolution and low-sensitivity camera that can be used to set up the microscope or for sample navigation, for example. The camera looks at the retractable Main Screen (a fluorescent plate, previously known as FluScreen) inside the microscope as indicated in the detector layout on the right side.

The live-view is started from the Acquisition Toolbar at the top left of the application. The small SmartCam toolbar contains a button for blanking and unblanking the beam. Also, it has a button to insert the beam stop, which will alternate between retracted, half-inserted, and fully inserted.



## Automatic Start of SmartCam Live-View

The SmartCam toolbar initiates the automatic start-up of a SmartCam live view. The start-up consists of the following steps:

- A Message Box is presented to ask whether the Column valves should be open to see a beam on the camera.
- The Main Screen is inserted. Additionally, the Main Screen can be manually inserted and retracted from the detector layout.

- The beam is unblanked and is visible in the detector layout. The beam blank button is available next to the SmartCam start button in the Acquisition Toolbar.
- A SmartCam live-view is shown in the Image and Data Viewing area in the middle of the application.

### Manual Actions for SmartCam Live-View

There are several possible manual actions:

- It is possible to run SmartCam with a blanked beam or with column valves closed, although the image will be back. The beam can be blanked by clicking the button next to the SmartCam start button. The column valves can be closed via the Home page.
- It is not possible to run SmartCam with a retracted Main Screen.
- The appearance of the live-view can be adjusted using the histogram (Black-level, White-level, Gamma, Color Lookup Table).
- Right-clicking with the computer mouse on the SmartCam display provides access to additional features such as the data bar and screen markings.

#### Modes



The SmartCam display can be set in different modes to optimize the imaging conditions.

- **Natural**: Natural mode displays the image as it would appear on the flu-screen. The camera sensitivity is adapted to the highest intensity in the image. This mode is most suitable for general use.
- Linear: In Linear mode gamma is set to 1, this mode gives more contrast image compared to *Natural*.
- **High Contrast**: Features with very low contrast can be difficult to observe in *Natural* mode. *High Contrast* adapts the gamma level to produce more contrast compared to Linear and Natural.

- **High Dynamic Range**: Some images (mostly diffraction patterns) have an extreme intrascene dynamic range, e.g. there are both bright and dark areas with information. Adapting the exposure time is not enough, the camera lacks dynamic range to image both the bright spots and the patterns in the background. To enhance the dynamic range the exposure time is alternated between a short and long exposure and an image is reconstructed from these images. To fit the dynamic range in the display a gamma curve is chosen that enhances the dark areas and suppresses contrast in the highlights. Several exposure time pairs can be chosen in the camera control panel or by using the mouse wheel in the image.
- Manual (using the slider): Automatic adjustment is paused, the camera parameters can be adjusted by hand by using the slider on the SmartCam display.

## Notes on SmartCam

The reference images for SmartCam (dark and gain) are applied automatically and should be recorded elsewhere (FlucamViewer or the new Reference Image Manager from the Microscope Software Launcher).

## **STEM Detectors**

## Use the STEM Live-View to Prepare and Acquire a Single STEM Image

The basic steps to acquire a STEM image with MiCo are as follows:

1. Select the **Optics > STEM** mode .



- 2. Insert the Detector Layout > Main Screen and open the column valves.
- 3. Select the **SmartCam** to start a live view of the Main Screen.



A Ronchigram should be visible in the SmartCam live view.

4. Use the handpanels to optimize the Ronchigram.

- 5. Depending on the configuration, insert one or more detectors. Either:
  - In the Detectors toolbar, activate one or more detectors or detector segments.



Detectors ribbon bar for a system with HAADF and a BF/DF detector.



Detectors ribbon bar for a system with HAADF and a Panther STEM BF-S/DF-S detector. The DF-S option combines the DF-I (inner)and DF-O (outer) signals.

• In the Detector Layout panel, activate one or more detectors or detector segments.

∧ Detector Layout				
	Field of view	- +	111.0 kx 💌	
	Camera length	- +	160 mm 👻	
	HAADI	F: 37 - 200	mrad	
	Main Screen			
	DF4: 14 - 35 mrad			
	DF2: - mrad			
	BF: 12 mrad			
Bases active				
Beam active				

6. Select **STEM Imaging > Start Scanning** to start a STEM live view.



For each selected detector, MiCo opens an Image Display tab and starts scanning in View mode. While scanning, the current scan line position is indicated by an orange line that runs along the side of the image(s).

If a detector is retracted or deactivated, then the related image is removed again.

- 7. If necessary, use the handpanels to optimize the STEM live view.
- (Optional) Maximize the dynamic range for the selected detector image. For the HAADF detector and for the Panther STEM BF-S and DF-S detectors it is also possible to use the Auto Gain function for an automatically optimized dynamic range. For instructions, see How to use Auto Gain.
  - a. Select the STEM image of the detector for which Offset and/or Gain need to be adjusted.
  - b. Select the **Detector Settings** > **Scope tool**.

▲ Detector Settings			
Detector	HAADF		
Gain		41.205 %	💿 Auto
Offset		42.700 %	
Scope tool		Center pattern	

A counts plot appears in the image of each selected detector.



c. Select STEM Imaging > Beam Blank.



- d. Adjust **Detector Settings** > **Offset**, so that the entire *counts* plot stays just above the lower blue line (not the dotted blue line).
- e. Select STEM Imaging > Beam Blank again to unblank the beam.
- f. Adjust **Detector Settings** > **Gain**, so that the entire *counts* plot stays just below the upper red line.

- g. Verify that the Display Settings > Histogram > Detector Saturation Indicator is entirely in the green, but close to the blue and red areas. This means that the Offset and Gain are set properly.
- 9. Select or specify the desired **Field of view** or **Magnification**. Either:
  - Use the Handpanels > Magnification knob.
  - Select or specify the desired Detector Layout side panel > Field of view.
    - Select [+] or [-]
    - Select a value from the drop list.
    - Specify a custom value



• Select the desired **Optics** menu > **Magnification**.

File	Edit	<u>V</u> iew	Optics DPC Help		
			Magnification (111.0 kx)	Þ.	5.0 kx
	E		Camera length (14 mm)	Þ	7.0 kx
Lavout	-	Appotat	TEM		9.9 kx
STEM			14.0 kx		
			Imaging		19.8 kx
✓ Diffraction		✓ Diffraction		28.0 kx	
			Microprobe		39.6 kx
V Nanoprobe			56.0 kx		
					70.01

In STEM Imaging mode, the Optics menu does not offer the *Field of View* parameter. Instead it offers a set of *Magnification* values, where Magnification = 0.1/Field of view with the assumption that the width of the field of view is 10 cm. 10. Select the STEM Imaging > Scan Size.



The preset Scan Size values include square and non-square areas of various aspect ratios and dimensions. Non-square scan sizes are not supported for DPC and iDPC acquisitions.

- 11. Select the STEM Imaging > Dwell Time or specify a custom value.
  - For most acquisitions, a dwell time between 5 μs and 40 μs results in good quality images.
     A very short dwell time may cause noisy images.
  - The overhead time is an estimation. For very short dwell times, the overhead time may be significantly larger than estimated.
  - The frame time is calculated from the dwell time, the scan size, and the overhead time.
- 12. Select **STEM Imaging > Start Scanning** again to stop the STEM live view.
- 13. (Optional) Select **STEM Imaging > Start Acquiring** to acquire a single image.



It is also possible to start a single image acquisition without first stopping the STEM live view.

### How to Use Auto Gain

The Auto Gain function is available for the HAADF detector and for the Panther STEM BF-S and DF-S detectors. The Auto Gain function automatically adjusts the Gain value, so that the optimal dynamic range of the STEM detector is used.

- In live viewing mode, the Auto Gain function checks the Gain value after each frame and adjusts it if necessary.
- The Gain value is not updated during the acquisition of an image or series.

The Auto Gain function also sets the Offset value to a pre-defined default value, so that the count is (nearly) zero in areas where no current is detected.

1. In the **Image Display**, select the image of the detector for which the Gain and Offset value need to be adjusted.



2. Activate the **Detector Settings > Auto** function.

<ul> <li>Detector Settings</li> </ul>			
Detector	HAADF		
Gain		41.205 %	💿 Auto
Offset		42.700 %	
Scope tool		Center pattern	

To deactivate the Auto Gain function, either:

- Select Detector Settings > Auto again,
- Manually adjust the Gain or Offset value.

### The Live Scan Rotation Functionality

The Scan Rotation functionality adjusts the orientation of the scanning pattern.



Rotate tool



Specify the Image Rotation angle by drawing an artificial horizon in the Image Display.

#### Rotation angle



Either: Select [+] and [-] to fine tune the current orientation. Select the input field and enter the desired rotation angle. Select the input field and drag the mouse to the left or to the right. The *Rotation angle* value must be in the [-180, 180] degrees range. If the entered value is outside the valid range, then MiCo corrects the angle to the [-180, 180] degrees range.

- Rotate left / right 90 degrees
- Reset rotation to 0 degrees

Navigation with the joystick takes the Rotation Angle into account, so that the image on screen moves in the same direction as the joystick.

If the specimen is moved via the *TEM User Interface* > *Stage* control panel or another software application, then the Rotation Angle is *not* accounted for.

## Sample Navigation

Any camera or detector window (live-view or paused) can be used for Sample Navigation by using the Click-Center functionality. This feature makes it easier and faster to center a feature or region of interest in the Field of View by double-clicking on it.

Depending on the preceding stage moves, the accuracy of the first Click-Center move may vary. The Click-Center functionality does not apply backlash correction to absorb any mechanical tolerances of the stage. If the selected feature is not accurately centered the first time, then center it once more.

Alternatively, it is possible to use the **Arrow** keys on the keyboard to move the stage, which will move the stage approximately 80% of the Field of View in the selected direction. Using the **Shift+Arrow** keys reduces the shift to approximately 40% of the Field of View.