

HCIimage

Leica DMI8 Microscope Guide



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INSTALLATION

Registered users can download the latest version of HCImage from the HCImage website using the following link - <https://hcimage.com/download/login/> (login required). For access to HCImage downloads, complete the software registration form (<https://hcimage.com/register/>), including a valid dongle number and email address, and an email will be sent with the HCImage download details.

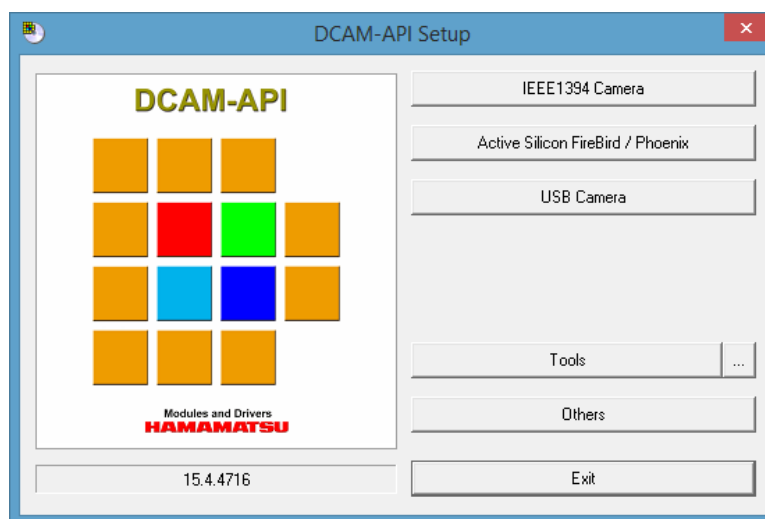
HCImage

1. Insert the HCImage installation DVD into the DVD-ROM drive. If autoplay is enabled, the HCImage setup will run automatically. If autoplay fails to start, locate your DVD-ROM drive and double-click on **setup.exe**.
 2. Click **Yes**, if prompted by the User Account Controls.
 3. To begin the installation wizard, click **Next**.
 4. Follow the instructions on each installation page.
- 
5. Securely connect the dongle () to a USB port after the software installation has finished.
 6. Install the appropriate DCAM-API drivers, please see "**DCAM-API Drivers**" below.
 7. Turn the camera power on prior to launching HCImage.
 8. Click the **HCImage** icon on your Desktop to launch HCImage.
 9. Register the software to receive technical support, please go to www.hcimage.com and click **Register**.

DCAM-API Drivers

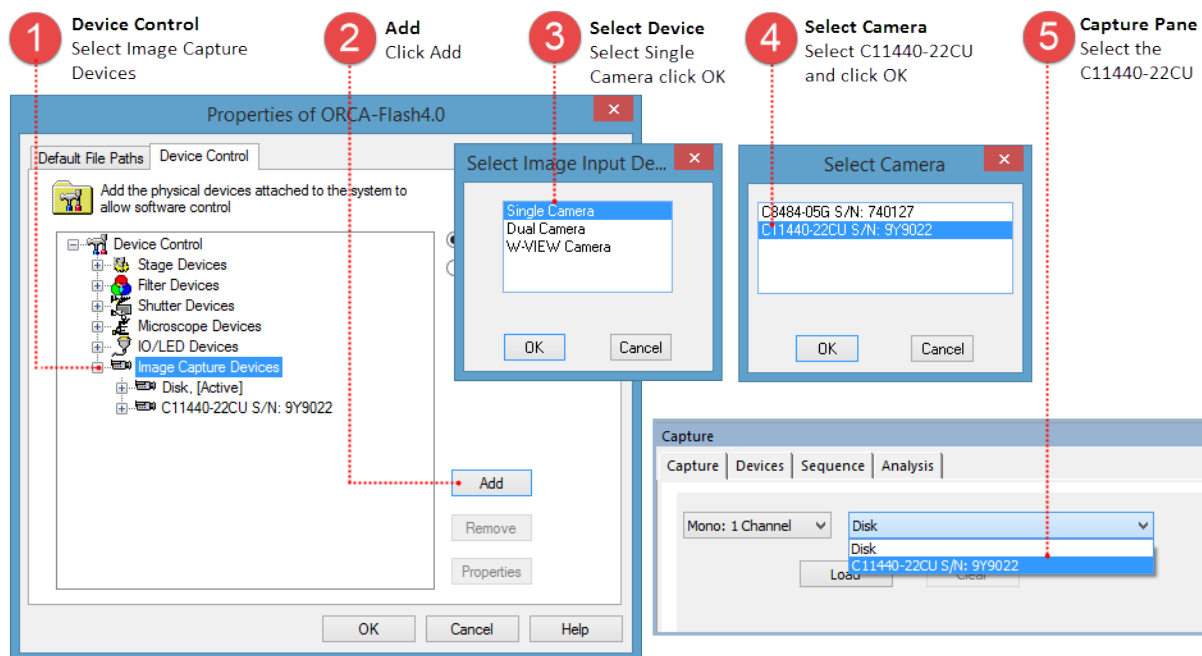
The current version of DCAM-API is available for download at <http://dcam-api.com/>. Before installing the camera driver, make sure that the camera is turned off.

1. After installing HCImage Live from the DVD, you will be prompted to install DCAM-API, click **Yes**. If you downloaded HCImage Live, please go to <http://www.dcam-api.com/> and download the DCAM-API drivers for Windows.
2. Click **Yes**, if prompted by the User Account Controls.
3. **[Camera Link]** Select the **Active Silicon FireBird / Phoenix** module. **[USB 3.0]** Select the **USB Camera** module. **[Firewire]** Select the **IEEE 1394 Camera** module.
4. Click **Next** to begin the installation.
5. Follow the instructions on each installation page.
6. Click **Finish** when the installation is complete.



Add the camera

Launch HCImage, go to File, select Current Profile and then follow the steps below to add a camera to the profile.



Leica Driver

The latest version of the Leica SDK Hardware Configurator is available for download at http://www.leica-microsystems.com/fileadmin/downloads/Leica%20LAS%20X/Software/Leica_SDK_Hardware_Configurator_Setup_website.zip.

1. Download and unzip the installer.
2. Double-click the **Leica SDK Hardware Configurator Setup.exe** and follow the installation instructions.
3. Click **Yes**, if prompted by the User Account Controls.

Files necessary to run Leica microscopes in 64-bit:

When running the 64-bit version of HCImage, please copy the files below found on the HCImage DVD in the Leica x64 folder under microscope drivers (Drivers\Microscopes\Leica\x64\)) and paste them into the HCImage directory (i.e., C:\Program Files\HCImage).

ahmconfig.xml	cmserial2.dll
ahmcore.dll	cmusb.dll
ahmpersist.dll	logger.dll
cmemulator.dll	valentine.dll

CONFIGURE THE MICROSCOPE

The microscope drivers have been installed, time to configure it and then add it as a device in HCImage.

Leica Hardware Configurator

Make sure that the microscope is connected to the computer and turned on, then proceed with the steps below.

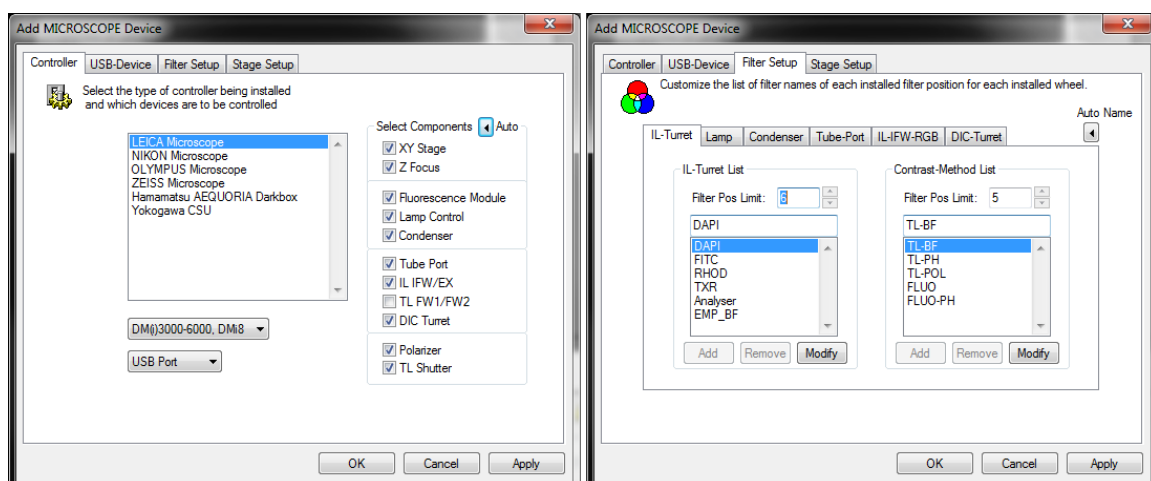
1. Launch the **Leica SDK Hardware Configurator** from the desktop.
2. Go to **Hardware Setup** and select **Leica DMI8** from the microscope list.
3. Select **USB** (or COM #) for the **Connection** and click **OK**.
4. Go to **Configure** and setup the individual components of the microscope.
5. When the setup is complete, exit the Hardware Configurator, the microscope settings will be saved automatically as **ahmconfig.xml**.
6. Copy **ahmconfig.xml** (located in C:\ProgramData\Leica Microsystems\Leica Hardware Configurator) to the local HCImage folder.

Note: If the ahmconfig.xml file is not copied into the HCImage directory, the microscope can be added to a profile and will function normally, the problem occurs the next time HCImage is launched. HCImage will not be able to communicate with the microscope and will display and error message.

Add Microscope to a Profile

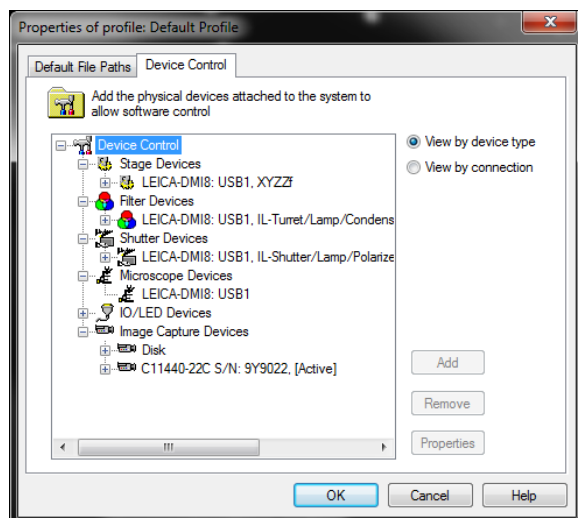
Once the microscope has been setup, the next step is to add the microscope to a profile and configure it in HCImage.

1. Launch HCImage, go to **File** and select **Current Profile**. In the **Device Control** tab, select **Microscope Devices** and click **Add**.
2. Click on **Leica Microscope** and select **DM(i)3000-6000, DMI8** from the drop-menu. Go to the **USB-Device** tab and select the **LEICA 1** device that is listed.



3. Go to the **Controller** tab, click **Auto** and HCImage will update components list.
4. Verify that the components list is accurate, add or remove components if necessary. Go to the **Filter Setup** tab to see the settings for the individual components.
5. Click **OK** to accept the microscope settings and return to the Current Profile. The Leica DMI8 is now listed as a Microscope Device and the components are listed as Stage, Filter and

Shutter Devices. Click **OK** to save the device settings to the profile and close the window.



- Go to the **Devices** pane and expand the **Microscope Setup** panel to access the individual component controls for the fluorescence module, objectives, condenser, etc.

Microscope Setup Panel

The Microscope Setup panel is located in the Devices pane and provides access to the microscope's controls as shown below. The focus controls are available in the Z Setup panel also located in the Devices pane.

Fluorescence Shutter
Open and close the shutter

Contrast Methods
Select from available contrast methods

Objectives
Select magnification to change objective, the properties are displayed below

Condenser
Select the optical element for applicable objective and observation method

Fluorescence Lamp
Turn On/Off and attenuate the illumination level

Transmitted Light
Turn On/Off and adjust the light intensity level (lamp voltage)

Filter Cubes
Select from available filters, details displayed below. Right-click on filter to add color

Test All Contrast Methods
Test all components of the microscope

Link to Calibration
Enable objective link to calibration

Objective Type
Switch between Dry and Immersion objectives

Top Lens
Move lens In or Out of the light path

Light Path
Displays the current light path

Field & Aperture Diaphragm
Adjust the size of diaphragm opening

Shutter Controls
Polarizer - In or Out of the light path
TL Shutter - Open or Close

DIC Turret
Select DIC object prism disk

Filter Setup Examples

Follow the steps below to configure the microscope for brightfield and fluorescence observation.

Part 1 - Filter Setup for Default Idle Positions

The screenshot shows the 'Filter Setup' dialog box. It includes sections for 'Advanced Settings', 'Filter Group', and 'Shutter Controls'. Red dotted lines with numbered circles (1, 2, 3) point to specific settings.

1 Advanced Settings
Enable Return to Idle On Exit, Return to Idle After Capture and Return to Idle During Delay

2 Filter Group
Select Default Idle Positions

3 Shutter Controls
Set the shutter status to Closed for the IL and the TL Shutters

Filter Setup

☒ Enable Automated Filter/Shutter Control

☐ IO/LED Devices ☐ XYZ Stage Offset

Advanced Settings ☒ Return to Idle On Exit

☒ Return to Idle After Capture ☐ Dazzle Protection

☒ Return to Idle During Delay ☐ Exposure Protection

☐ Default Idle Positions ☐ Test

Time Delay

☒ None ☐ Manual ☐ Automatic: 0.1 Sec.

Delay Position: Pre-Exposure

Filter-Shutter

Filters

DMI8B Contrast-Method USB1	Don't care
DMI8B IL-Turret USB1	Don't care
DMI8B Lamp USB1	Don't care
DMI8B IL-FIM USB1	Don't care
DMI8B Condenser USB1	Don't care
DMI8B Tube-Port USB1	Don't care
DMI8B DIC-Turret USB1	Don't care

Shutters

DMI8B IL-Shutter USB1	Closed
DMI8B Lamp USB1	Closed
DMI8B Polarizer USB1	Open
DMI8B TL-Shutter USB1	Don't care

Part 2 - Filter Setup for Brightfield

Filter Setup

☒ Enable Automated Filter/Shutter Control

☐ IO/LED Devices ☐ XYZ Stage Offset

Advanced Settings ☒ Return to Idle On Exit

☒ Return to Idle After Capture ☐ Dazzle Protection

☒ Return to Idle During Delay ☐ Exposure Protection

☐ Default Idle Positions

☒ BF

Add

Copy

Remove

☐ Test

Time Delay

☒ None ☐ Manual ☐ Automatic: 0.1 Sec.

Delay Position: Pre-Exposure

Filter-Shutter

☒ Filters

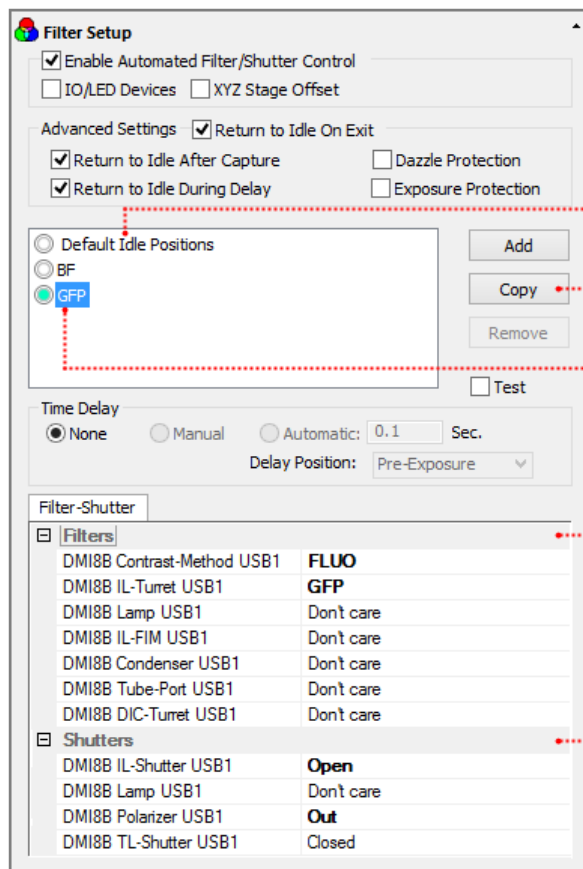
DMI8B Contrast-Method USB1	BF-BF
DMI8B IL-Turret USB1	BF
DMI8B Lamp USB1	Don't care
DMI8B IL-FIM USB1	Don't care
DMI8B Condenser USB1	BF
DMI8B Tube-Port USB1	Don't care
DMI8B DIC-Turret USB1	Don't care

☒ Shutters

DMI8B IL-Shutter USB1	Closed
DMI8B Lamp USB1	Open
DMI8B Polarizer USB1	Out
DMI8B TL-Shutter USB1	Open

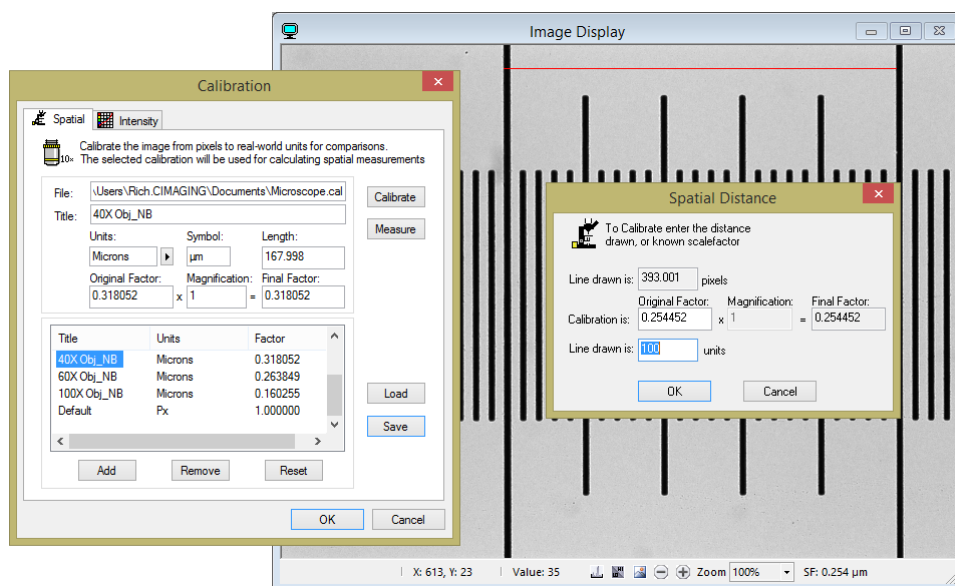
- 1 Filter Group**
Select Default Idle Positions
- 2 Copy Filter Group**
Click Copy, enter BF and click OK
- 3 Filter Group**
Select BF
- 4 Filter Controls**
Set the Contrast Method to BF-BF, IL-Turret to BF and the Condenser to BF
- 5 Shutter Controls**
Set the shutter status to Open for the Lamp and the TL shutters and the Polarizer to Out

Part 3 - Filter Setup for Fluorescence



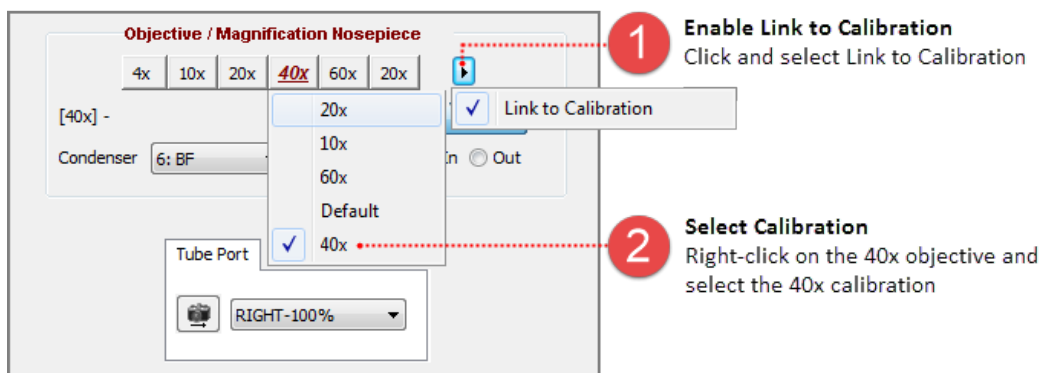
CALIBRATE OBJECTIVES

1. Starting with the lowest power objective, click **Live**, bring the micrometer into focus and center it.
2. Click **Abort** and then click on the **Calibration Properties** icon ( Calibration) in the Analysis toolbar.
3. Click **Add**, in the Title box and enter the objective magnification, contrast method and immersion type if applicable (e.g., 40x, 40x Oil, 40x DIC, etc.).
4. Select **Microns** from the **Units** drop-menu and then click **Calibrate**. Move the cursor to a starting position on the scalebar; click and drag a line to span the distance to measure.
5. Enter the known distance of the line and click **OK**. The Calibration Factor in the Spatial Calibration Menu will be updated.
6. Click **OK**, to close the **Calibration Properties** dialog and repeat the process for the remaining objectives.
7. **[Save Calibration]** Click **Save**, select file path, enter the file name and click **Save**. The Spatial Calibration is displayed in the lower right-hand corner of the image file.



Link Calibration to Objective

To link the calibration to an objective, go to the Microscope Setup panel in the Devices pane and follow the steps below.



ADAPTIVE FOCUS CONTROL

The Leica Adaptive Focus Control (AFC) actively maintains the desired plane of observation precisely in focus, avoiding focus drift due to temperature changes. Please contact your Leica representative or review the documentation provided with the system for information about the supported objectives and the recommended slides and dishes for use with the Leica AFC.

Focus Modes and Properties

The AFC provides two modes of operation that can be enabled through HCImage.

Continuous Mode

In the Continuous mode, the AFC actively maintains the desired plane of observation precisely in focus, avoiding focus drift due to temperature changes. The setup is easy, turn on the AFC, bring the sample into focus and enable the Continuous mode by setting Hold Focus Position. The sample will be held in focus for the during image acquisition until it is turned off.

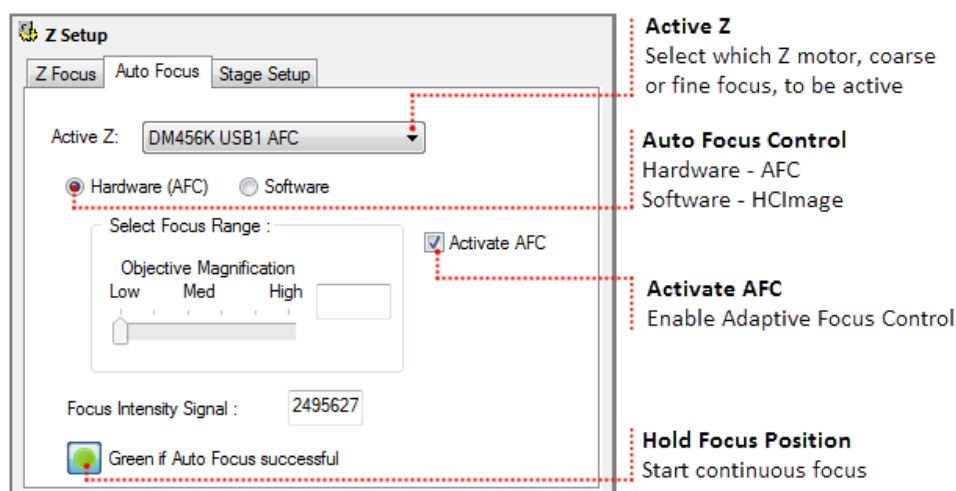
Note: When continuous focus is enabled, the Z focus position and objectives cannot be adjusted or changed.

On Demand Mode

The On Demand mode provides focus stabilization at defined intervals based on the needs of the experiment. In this mode, the AFC is only active at specified points during the time lapse. When active, the AFC calculates and adjusts to the optimal focal position.

Auto Focus Properties

The AFC will automatically be included when the DMI8 is added to the Profile. The Z Setup panel, located in the Devices pane, provides access to the microscope's focus controls in the Z Focus, Auto Focus and Stage Setup tabs. The controls for the AFC are available in the Auto Focus tab shown below. The auto focus controls are also available in the Sequence pane, as shown in the Auto Focus Examples in the following section.



Auto Focus Examples

Continuous Mode

Get a live image, configure the capture settings and focus on the sample, then go to the Sequence pane and follow the steps below.

1 Scan Type
Select Time Lapse

2 Auto Save
Click the ellipses icon, select CXD and enter the file location and naming convention

3 Field Delay
Select 0 Delay

4 Duration
Select Continuous

5 DISK
Select to DISK

6 Refresh Z Focus
Disable Refresh Z Focus

7 Auto Focus Options
Select Auto-Focus Properties

8 Auto Focus Type
Select Hardware (AFC)

9 Activate AFC
Enable AFC

10 Hold Focus Position
Click button to enable continuous focus

11 Save Settings
Click OK

12 Start Acquisition
Click Start

On Demand Mode

Get a live image, configure the capture settings and focus on the sample, then go to the Sequence pane and follow the steps below.

1 Scan Type
Select Time Lapse

2 Auto Save
Click the ellipses icon, select CXD and enter the file location and naming convention

3 Field Delay
Enter 30 s

4 End Time
Enter 3 h

5 DISK
Select to DISK

6 Refresh Z Focus
Enable Refresh Z Focus Every 1 Field

7 Auto Focus Options
Select Use Fine Focus to Autofocus

8 Auto Focus Properties
Select Auto-Focus Properties

9 Auto Focus Type
Select Hardware (AFC)

10 Activate AFC
Enable AFC

12 Save Settings
Click OK

13 Start Acquisition
Click Start