

# **EVOS™ M5000 Imaging System**

For Fluorescence and Transmitted Light Applications

Catalog Number AMF5000

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Revision C.0

Information in this document is subject to change without notice.

#### Revision history MAN0017563

Revision	Date	Description
C.0	14 January 2020	Add information about adjustable scale bar, Clear All button for images in the memory buffer, Hot Pixel Correction, Z-Stack infographics, additional objective data, additional metadata for PNG and TIFF images, Time/Date setup, automatic reconnect to WiFi networks and mapped network drives, new authentication code requirement for first-time Connect™ account connection for added login security, and update the screens throughout the document.
B.0	08 January 2019	Add Connect™ account sign in and save, Confluence tool, Time Lapse video save, review, and playback, EVOS™ Onstage Incubator, and EVOS™ Image Analysis sections, update GUI section and the screens throughout the document.
A.0	07 August 2018	New user guide for the EVOS™ M5000 Imaging System

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# About this guide

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<b>Audience</b>	This user guide is for laboratory staff operating, maintaining, and analyzing data using the Invitrogen™ EVOS™ M5000 Imaging System.
<b>User attention words</b>	<p>Two user attention words appear in this document. Each word implies a particular level of observation or action as described below.</p> <hr/> <p><b>Note:</b> Provides information that may be of interest or help but is not critical to the use of the product.</p> <hr/> <p><b>IMPORTANT!</b> Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.</p> <hr/>
<b>Safety alert words</b>	<p>Three safety alert words appear in this document at points where you need to be aware of relevant hazards. Each alert word—<b>CAUTION, WARNING, DANGER</b>—implies a particular level of observation or action, as defined below:</p> <hr/> <p> <b>CAUTION!</b> – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.</p> <hr/> <p> <b>WARNING!</b> – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.</p> <hr/> <p> <b>DANGER!</b> – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.</p> <hr/>

# 1. Product information

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## Product description

### EVOS™ M5000 Imaging System

The Invitrogen™ EVOS™ M5000 Imaging System (Cat. No. AMF5000) is a fully integrated, digital, inverted imaging system for four-color fluorescence and transmitted-light applications.

It combines precision optics, a five-objective turret, an 18.5 inch high-resolution LCD display (1920 × 1080 pixel resolution), and a highly sensitive Sony IMX265 monochrome CMOS camera (2048 × 1536 pixel resolution, 3.2 Megapixels) to acquire images seamlessly through the intuitive user interface using a mouse for easy control.

### EVOS™ M5000 Software

The EVOS™ M5000 Imaging System is controlled by the integrated Invitrogen™ EVOS™ M5000 Software through a graphical user interface (GUI), which is accessed by the computer mouse and keyboard. The software is pre-installed to the instrument and starts automatically when the instrument is powered on.

Key features of the EVOS™ M5000 Software include:

- **Capture:** Allows control over every aspect of the system for image capture through a simple user interface. All images acquired can be saved in BMP, JPG, PNG, and TIFF formats, or compiled into a video sequence in AVI or WMV formats.
- **Autofocus:** Allows autofocus in fluorescence and brightfield modes.
- **Z-stacking:** Captures a series of images along the z-axis that can be saved individually or combined into a Z-stack projection with a greater depth of field than any of the individual source images.
- **Time lapse:** Allows you to create a time lapse movie using captured images.
- **Review:** Allows you to review, measure, and annotate captured images.
- **Cell count:** Allows you to automatically or manually count cells in fluorescence mode post-acquisition.
- **Confluence:** Allows you calculate the percentage confluence of your culture based on selected reference objects and background.
- **Network and Connect™ connectivity:** Allows Wi-Fi and Ethernet connectivity to the network and to your Connect™ account, Thermo Fisher's cloud-based platform, to store and access your data files.

### Product use

For Research Use Only. Not for use in diagnostic procedures.

## Standard items included

### **EVOS™ M5000 Imaging System**

- EVOS™ M5000 Imaging System, includes the following components and pre-installed accessories:
  - 18.5 in articulated LCD monitor (1920 × 1080 pixel resolution)
  - Embedded PC (4 GB RAM)
  - Light cube shipping restraint (remove before use)
  - Stage lock pin (remove before use)
  - EVOS™ Condenser Light Shield, 110 mm
  - Light cube tool (remove before use)
  - Blank light cube (remove before use)
  - LED light cubes, as ordered
  - Objectives, as ordered

### **EVOS™ M5000 Accessories Kit**

- EVOS™ M5000 Accessories Kit (located in the instrument box), contains:
  - Wireless mouse and keyboard
  - USB receiver (for wireless mouse and keyboard connection)
  - USB Wi-Fi adaptor (for wireless network connection to Connect™ applications and mapped network drives)
  - USB 3.0 flash drive, 16 GB (for image storage, and preloaded with user documentation)
  - EVOS™ Vessel Holder, Two 25 × 75 mm slides (Cat. No. AMEPVH001)
  - EVOS™ Vessel Holder, Universal (Cat. No. AMEPVH009)
  - EVOS™ Calibration Slide (Cat. No. AMEP4720)
  - EVOS™ Light Shield Box
  - UV shield assembly
  - Condenser Slider, Block (Cat. No. AMEP4688)
  - Condenser Slider, 4X Pupil (Cat. No. AMEP4738)
  - Condenser Slider, Diffusion for Brightfield Applications (Cat. No. AMEPDFS1)
  - Universal power supply (12 V, 5 A) and power cord (type B, North America)
  - EVOS™ Dust Cover
  - Accessories box with adjustable compartments
  - Hex driver, 2 mm
  - Mouse pad

### **EVOS™ M5000 user documentation**

- EVOS™ M5000 Quick Start Guide, printed (Pub. No. MAN0017765)
- EVOS™ M5000 Imaging System Installation Guide, printed (Pub. No. MAN0017783)
- Pre-loaded to USB 3.0 flash drive (located in the accessories box):
  - EVOS™ M5000 User Guide (Pub. No. MAN0017763)
  - EVOS™ M5000 Quick Start Guide (Pub. No. MAN0017765)
  - EVOS™ M5000 Imaging System Installation Guide (Pub. No. MAN0017783)

# Instrument exterior components and mechanical controls

Front view



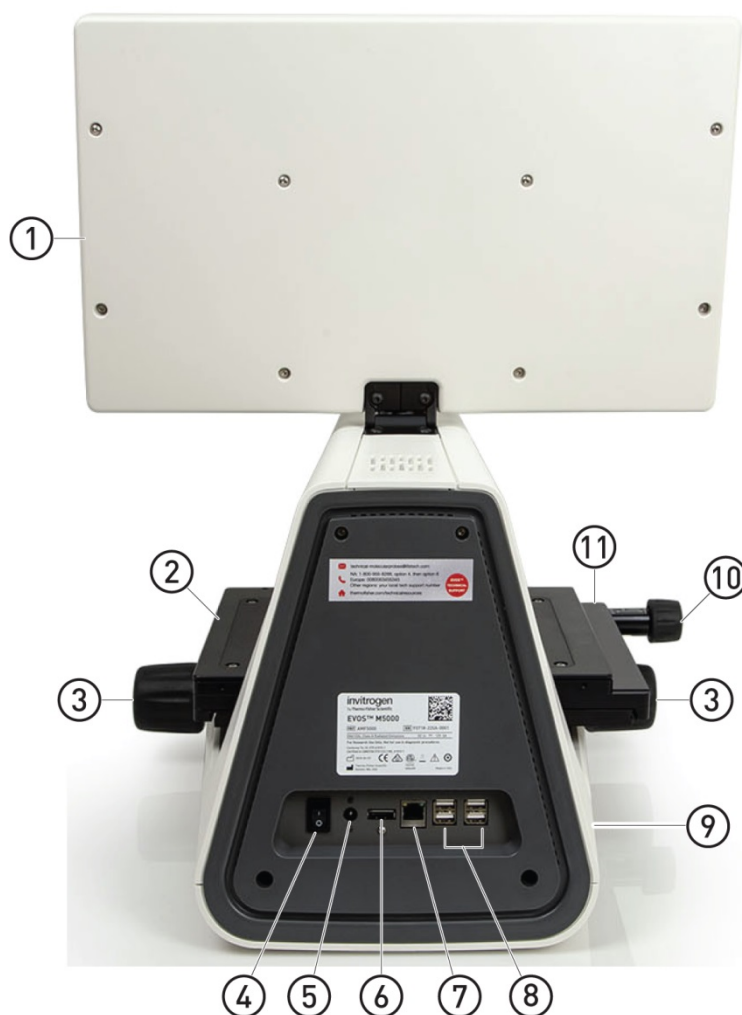
- |                                 |                             |
|---------------------------------|-----------------------------|
| ① LCD monitor                   | ⑦ Focusing knobs            |
| ② Condenser slider slot         | ⑧ Objective selection wheel |
| ③ Condenser head                | ⑨ Vessel holder             |
| ④ Mechanical X-Y stage          | ⑩ Objective                 |
| ⑤ Stage X-axis positioning knob | ⑪ Phase annuli selector     |
| ⑥ Stage Y-axis positioning knob |                             |

## Stage positioning knobs and brakes



- |                                 |
|---------------------------------|
| ① Mechanical X-Y stage          |
| ② Stage X-axis positioning knob |
| ③ Stage brake for X-axis        |
| ④ Stage Y-axis positioning knob |
| ⑤ Stage brake for Y-axis        |

## Rear view



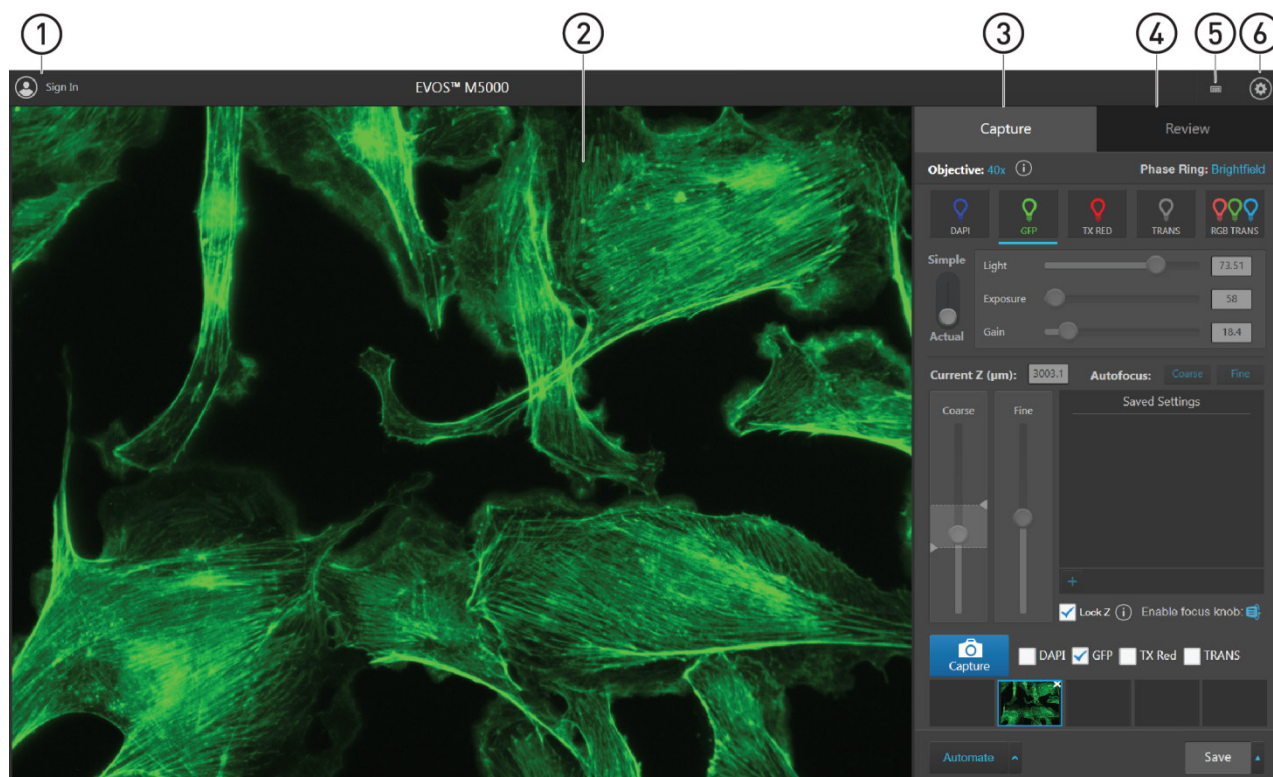
- |  |                                  |
|--|----------------------------------|
| ① LCD monitor                          | ⑦ Network port                   |
| ② Mechanical X-Y stage                 | ⑧ USB-A 2.0 ports (4x)           |
| ③ Focusing knobs                       | ⑨ USB 3.0 port (1x)              |
| ④ Power switch                         | ⑩ Stage X-axis positioning knob  |
| ⑤ 4-pin power input port (12 VDC, 5 A) | ⑪ Stage Y-axis positioning knob* |
| ⑥ Display port (video output)          |                                  |

\* Not visible in this perspective.

# Graphical user interface (GUI)

## GUI layout

The GUI of the system consists of the Viewing area on the left and Capture and Review tabs and the Settings and Virtual keyboard buttons on the right. Each tab and button opens the controls necessary to execute the selected function.



- ① **Sign In/User:** Allows you to sign in to your Connect™ account, Thermo Fisher's cloud-based platform, for image storage and analysis with the EVOS™ Image Analysis application.
- ② **Viewing area:** Displays the sample.
- ③ **Capture tab:** Contains the controls for image capture.
- ④ **Review tab:** Allows you to review and annotate captured images.
- ⑤ **Keyboard button:** Opens the virtual keyboard.
- ⑥ **Settings button:** Opens the Settings tabs, which allow you to select and adjust basic and advanced system settings.

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**Note:** For more information and detailed descriptions of GUI controls, see "Appendix B: Graphical user interface (GUI)", page 80.

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**Note:** For more information on the EVOS™ Image Analysis application, see "Appendix E: EVOS™ Image Analysis", page 104.

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## 2. Installation

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### Operating environment and site requirements

- The dimensions of the EVOS™ M5000 Imaging System are 18 × 23 × 18 in (W×H×D) (46 × 59 × 46 cm). The system requires a benchtop of approximately 36 × 36 in (92 × 92 cm).
- If the system includes the optional EVOS™ Onstage Incubator (Cat. No. AMC1000), then add 16 in (40 cm) to the width of the bench.
- Allow at least 5 cm (2 in) free space at the back of the instrument to allow for proper ventilation and prevent overheating of electronic components.
- Place the EVOS™ M5000 Imaging System on a level surface away from vibrations from other pieces of equipment. Tabletop centrifuges, vortex mixers, and other laboratory equipment can vibrate the instrument during a run and cause a decrease in instrument performance.
- The EVOS™ M5000 Imaging System should be installed away from direct light sources such as windows. Ambient light can enter the imaging path and affect the image quality.
- Operating temperature range: 4°–32°C (40°–90°F).
- Relative humidity range: 0–90%.
- Operating power: 100–240 VAC, 1.8 A
- Frequency: 50–60 Hz
- Electrical input: 12 VDC, 5 A

---

**IMPORTANT!** Do not position the instrument so that it is difficult to turn off the main power switch located on the back of the instrument base (see page 8). In case of an instrument malfunction, turn the main power switch to the OFF position and disconnect the instrument from the wall outlet.

---

#### Hood setup

The EVOS™ M5000 Imaging System fits in cell culture hoods that are at least 24 in (61 cm) deep and 36 in (92 cm) high with a 30 in (76 cm) opening. If your cell culture hood is smaller, it may be possible to fit the instrument by turning it at a slight angle.



## Prepare for installation

- |   |   |
|---|---|
| <b>Receive and inspect the shipment</b>             | <ol style="list-style-type: none"><li>1. Verify that the items shown on the shipping list are the same items that you ordered at the time of purchase.</li><li>2. Carefully inspect the shipping containers and report any damage to the Thermo Fisher Scientific service representative. Record any damage or mishandling on the shipping documents.</li></ol> |
| <b>Move the instrument to the installation site</b> | <ol style="list-style-type: none"><li>1. Clear the installation site of all unnecessary materials.</li><li>2. If possible, move the crated instrument and other shipping containers to the installation site.</li></ol>   |



**CAUTION! PHYSICAL INJURY HAZARD.** Lift or move the instrument using proper lifting techniques. We recommend that you lift or move the crated instrument with the assistance of others and the use of appropriate moving equipment. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

---

## Install the instrument

- |                              |  |
|------------------------------|--|
| <b>Unpack the instrument</b> | <ol style="list-style-type: none"><li>1. Open the shipping box and remove the accessory box.</li><li>2. Carefully lift the instrument out of the box by grasping it firmly with both hands under the support arm.</li><li>3. Place the instrument on a flat, level surface that will be free from vibration and leave enough room around it for the stage to move freely.</li><li>4. Tilt the LCD monitor upright.</li><li>5. Examine the instrument carefully for damage incurred during transit.</li><li>6. Unpack the accessories box and verify all parts are present. See page 6 for the list of standard items included in the shipment.</li></ol> |
|------------------------------|--|

---

**IMPORTANT!** Do not subject the EVOS™ M5000 Imaging System to sudden impact or excessive vibration. Handle the instrument with care to prevent damage.

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**Note:** Contact your distributor if anything is missing. If you do not have your distributor information, contact Technical Support (page 116). Damage claims must be filed with the carrier; the warranty does not cover in-transit damage.

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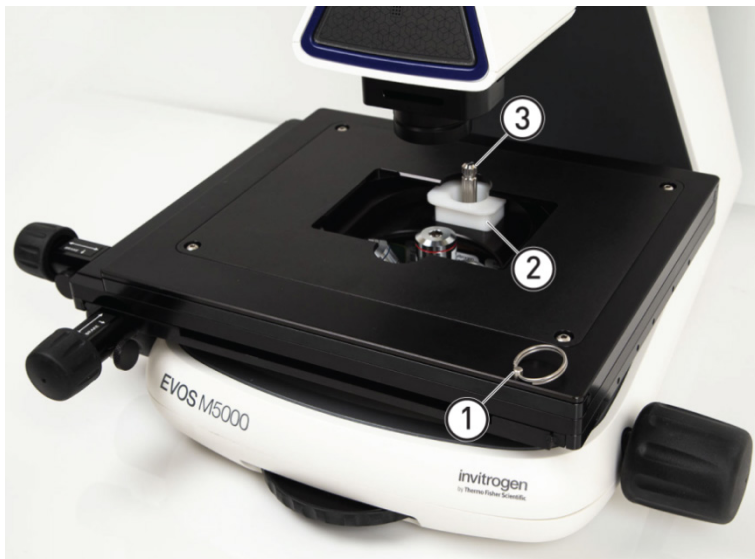
---

**Note:** Make sure to set aside packaging and foam for future transport and storage. Re-install the stage lock pin and the light cube shipping restraint before moving or transporting the instrument. Always ensure that the instrument is properly cushioned and braced to prevent damage.

---

## Remove shipping restraints

The EVOS™ M5000 Imaging System is equipped with two shipping restraints (stage lock pin and light cube shipping restraint) to protect the instrument from shock and vibration during transport. You must remove the shipping restraints before you power on the EVOS™ M5000 Imaging System.



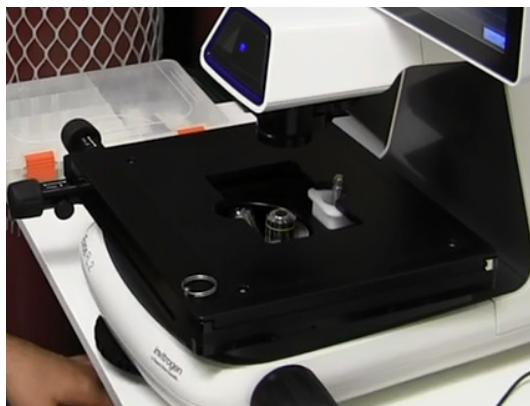
- ① Stage lock pin
- ② Light cube shipping restraint
- ③ Light cube tool

1. Pull firmly to remove the stage lock pin and release the X- and Y-axis brakes.
2. Using the X-axis and Y-axis stage positioning knobs, move the stage back to obtain access to the light cube shipping restraint, which is centered under the back of the stage.

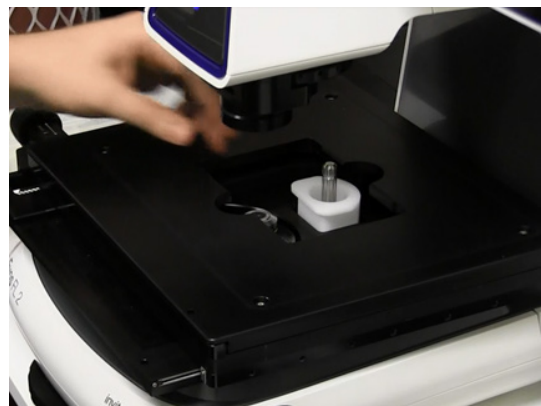
---

**Note:** The light cube shipping restraint is secured with the light cube tool to the blank light cube installed in the light cube turret. Used together, they immobilize the light cube turret to protect it during transport.

---



Before stage reposition



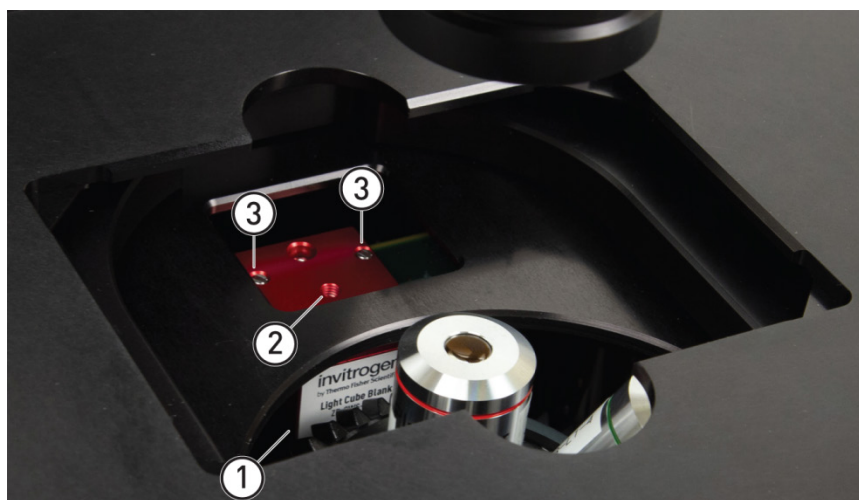
After stage reposition

3. Unscrew and remove the light cube tool, which secures the shipping restraint block to the blank light cube.
4. Remove the shipping restraint block and store it in the accessories box. Removal of the restraint block provides access to the blank light cube in the light cube turret.

---

**Note:** The blank light cube is red and does not have the grooved copper top of the LED light cubes.

---



- ① Blank light cube
- ② Thread hold
- ③ Screws

5. Using the light cube tool, loosen the two screws that secure the blank light cube to the instrument. You do not need to remove the screws.
6. Screw the light cube tool to the thread hold on the light cube, then lift the blank light cube up and out of the light cube turret. Store the blank light cube and the light cube tool in the accessories box.
7. Place the desired EVOS™ Vessel Holder on the stage.

---

**Note:** Store the shipping restraints and the light cube tool for future use in the accessories box provided with your system. Always re-secure the X-Y stage with the stage lock pin and re-install the light cube restraint before moving the instrument.

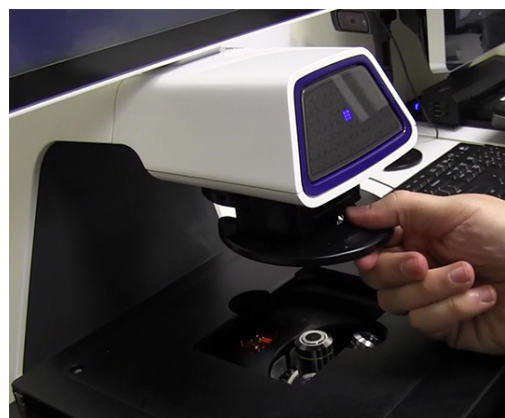
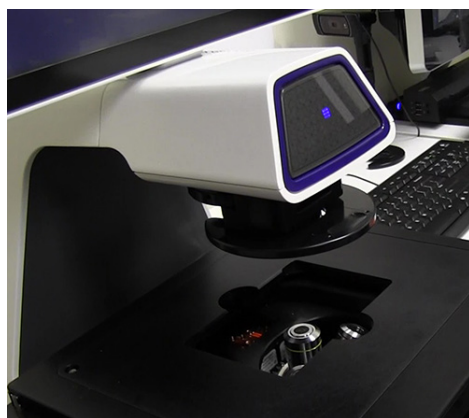
---

**IMPORTANT!** Before changing light channels, ALWAYS verify the light cube restraint has been removed. Attempting to change the light channels while the restraint is in place can seriously damage the mechanism. This type of damage is not covered by manufacturer's warranty.

---

## Install the UV light shield

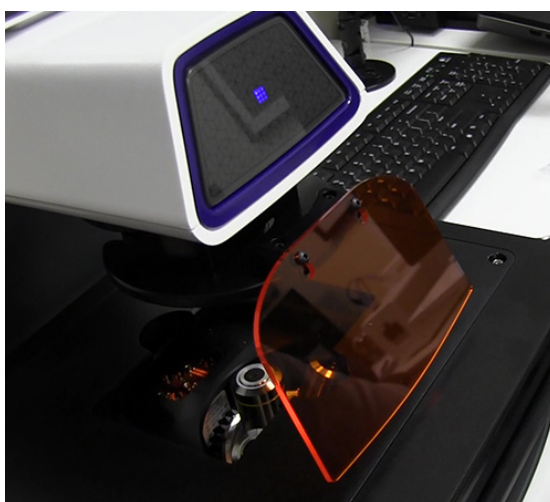
1. Verify that the EVOS™ Condenser Light Shield is installed on the condenser assembly. The condenser shield is pre-installed and helps reduce the potential effects of overhead lighting on your image.
2. Pull the condenser light shield out from the condenser head.



3. Secure the UV light shield mount to the top of the condenser light shield using the two screws supplied with the UV shield assembly (not the protruding screws on the mount).



4. Clip the condenser light shield with the attached UV light shield mount back onto the condenser head.
5. Peel the protective paper from the UV light shield, then slide the orange UV light shield over the two protruding screws on light shield mount to attach it to the instrument.



---

**Note:** The UV light shield is provided as a safety feature and should be installed whenever the unit is in operation. The UV light shield is removable for access to the condenser sliders used in transmitted light mode. Simply unhook it from the screws on the UV light shield mount.

---

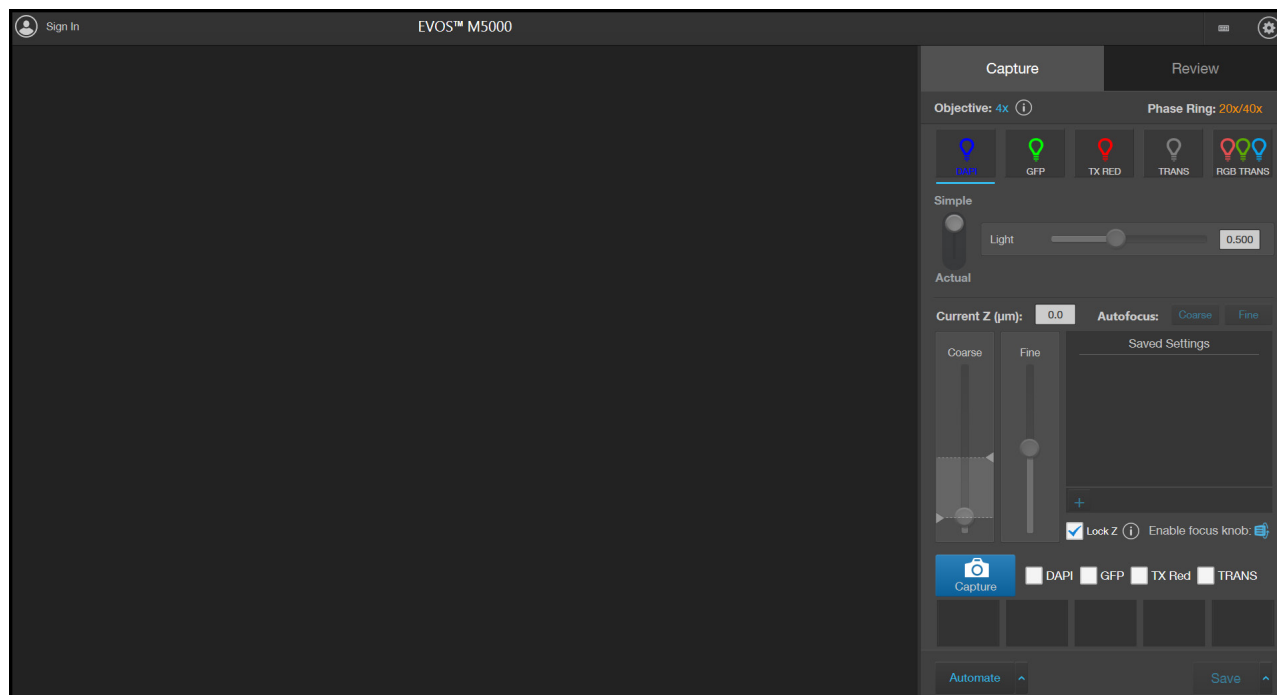
## Connect the instrument

1. Confirm that the power switch is OFF (located on the back; see page 8).
2. Connect the USB receiver for the wireless mouse and keyboard to an available USB-A 2.0 port (page 8).
3. Connect the power adaptor and power cord. Ensure a tight connection
4. Plug the power adaptor into the power input port on the instrument (page 8).
5. Plug the power cord into a power outlet and check for the light on the power supply.



## Turn on the EVOS™ M5000 Imaging System

1. Turn the instrument power switch (located on the back; see page 8) to the ON position.
2. When the **Capture** tab is displayed, the EVOS™ M5000 Imaging System is ready to use.



---

**IMPORTANT!** All shipping restraints must be removed before turning on the EVOS™ M5000 Imaging System to prevent damage (page 12).

---

### Connect to a Wi-Fi network

You can connect the EVOS™ M5000 Imaging System to a network via an Ethernet cable or Wi-Fi adaptor and save captured images directly to shared folders on the network. You can also connect to your Connect™ account, Thermo Fisher's cloud-based platform, to store your image files and analyze them with the EVOS™ Image Analysis application (page 16).

For instructions on how to connect to a Wi-Fi network and how to map a network drive to save your images, see “Configure network settings”, page 67.

### Set date and time

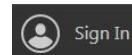
For instructions on how to configure the date and time to the local time, see “Set date and time”, page 70.

## Sign in to your Connect™ account

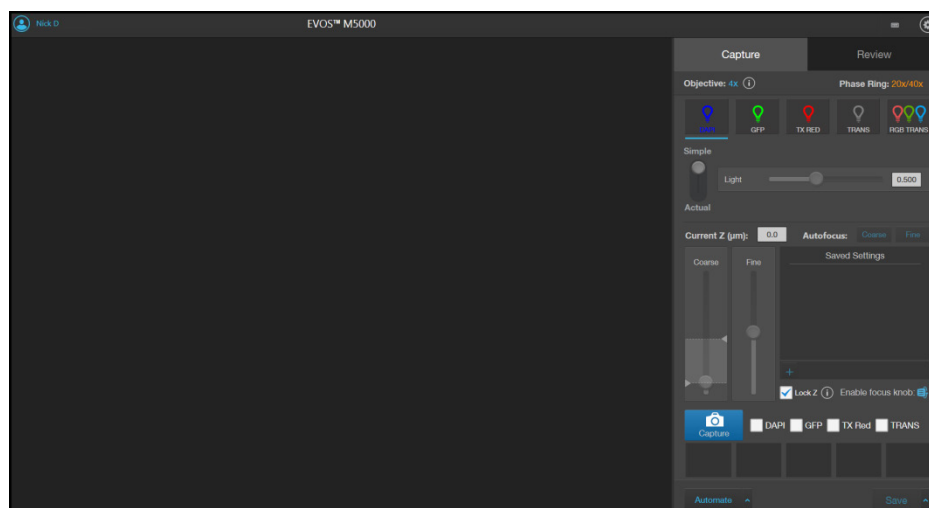
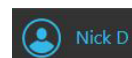
Connecting to your Connect™ account, Thermo Fisher's cloud-based platform, allows you to save captured images in your unique user account in addition to local storage.

**Note:** You must have a Connect™ account or create one to sign in. To create your Connect™ account online, go to [thermofisher.com/connect](https://thermofisher.com/connect).

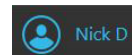
1. Click the **Sign In** button on the top left corner of the screen to open the Connect to the Cloud dialog.



2. Enter your Connect™ user name and password, then click **Sign In**.  
When you connect to your Connect™ account, the Sign In button changes to display your user name.



3. To sign out of your Connect™ account, click your **User** button to open the Sign Out dialog, then click **Sign Out**.



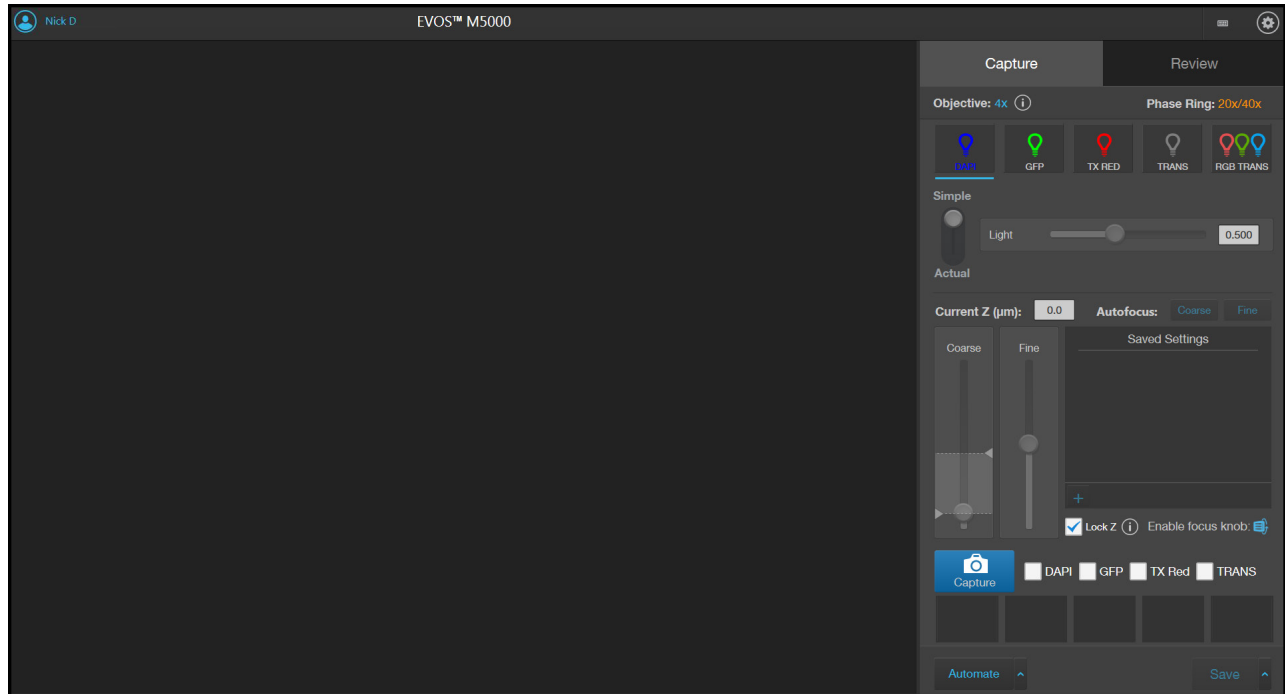
**Note:** You can choose to save captured images to your Connect™ account in addition to local storage. Images saved to your account in the Connect™ platform can then be viewed and analyzed with the EVOS™ Image Analysis application. For more information on the EVOS™ Image Analysis application, see “Appendix E: EVOS™ Image Analysis”, page 104.

## 3. Capture images

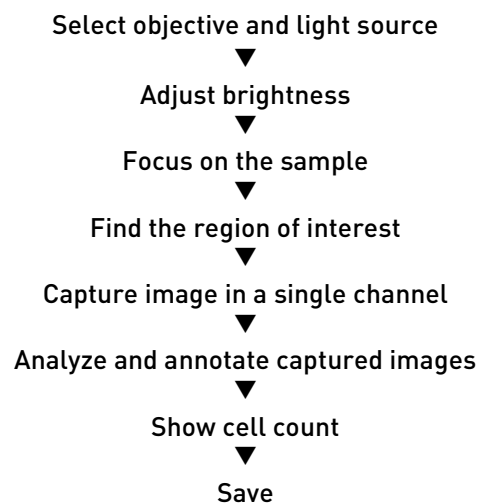
### Overview

#### Capture tab

The basic functions of the EVOS™ M5000 Imaging System, such as viewing the sample, setting optimal focus, and capturing and saving images are performed in the **Capture tab**, which is the first screen after start-up.



#### Workflow





## Capture images in a single channel

### Select objective and light source

1. Place the vessel containing your sample on the stage using the appropriate vessel holder.

---

**Note:** When capturing images in fluorescence channels, place the light shield box on the stage, over the sample. This is important for optimal image quality.

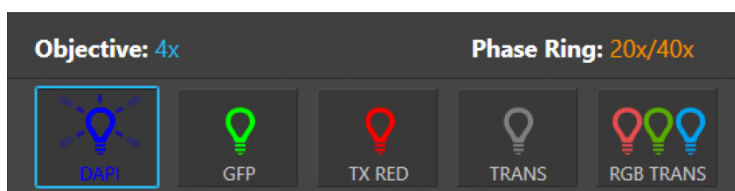
---

2. Set the magnification using the objective selection wheel (page 7).
3. Select the desired **Phase** option by turning the phase annuli selector (page 7). Available options are:

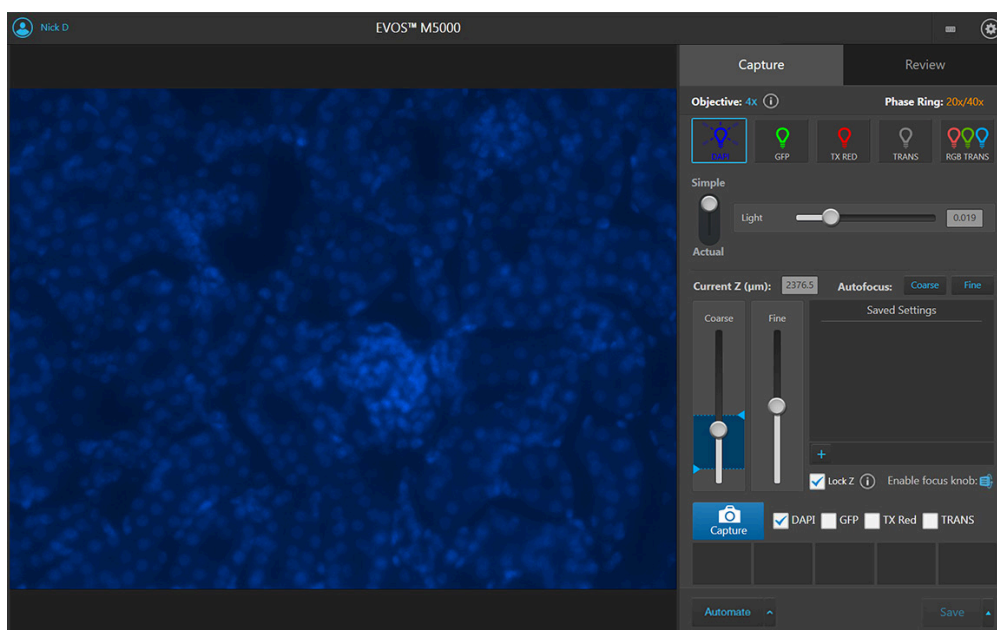
- **Oly 4x:** Used for low magnification objectives (Olympus™ 4× PH)
- **4x/10x:** Used for medium magnification objectives (EVOS™ 4×/10× PH)
- **20x/40x:** Used for high magnification objectives (EVOS™ 20×/40× PH)
- **Brightfield** (phase contrast off)

The active objective and phase ring information is displayed above the Channel buttons.

4. Select a **Channel** to turn on the excitation light and enter the instrument in the **Live mode**. In this example, the DAPI channel has been selected.



In the Live mode, you can adjust the brightness and configure display settings for the selected channel, and set the focus on the sample.



---

**Note:** You can select only one light source at a time for Live mode to adjust brightness and set focus. However, you can display and capture multiple channels simultaneously.

---

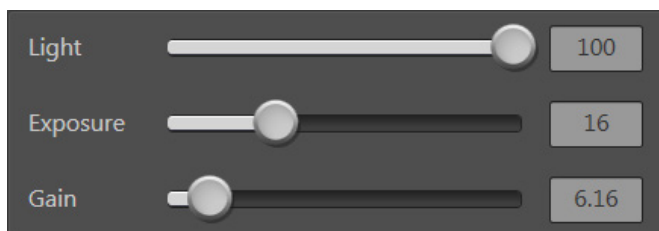


## Adjust brightness

1. For **Brightness mode**, select **Simple** or **Actual**.
  - **Simple** mode allows you to control **Brightness** as a single parameter.
  - **Actual** mode allows you to adjust **Light** (i.e., LED intensity), **Exposure**, and **Gain** parameters individually.
2. Adjust brightness using the **Brightness** controls:
  - In the **Simple** mode, move the **Light** slider to adjust brightness. Alternatively, enter the desired brightness value (0%–100%) directly in the brightness field.



- In the **Actual mode**, move the **Light** (LED intensity), **Exposure**, and **Gain** sliders to adjust the brightness parameters individually. Alternatively, enter the desired value for each parameter (0%–100% for Light, 0–4.00 seconds for Exposure, or 1.0–8.0 for Gain) directly in the corresponding field.



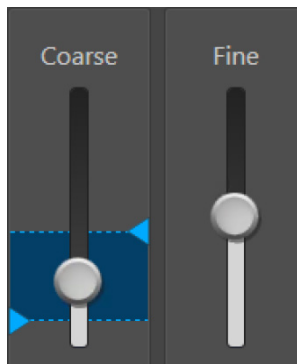
---

**Note:** Optimize the brightness parameters as follows:

- **When searching for sample:** Increase **Gain** for a brighter signal and decrease **Exposure** for faster frame rate during navigation around the vessel.
  - **When capturing image:** Decrease **Gain** to reduce background noise and increase **Exposure** to regain signal intensity, as needed.
  - **For brighter signal:** Increase **Light** intensity for brighter illumination. If needed, follow by increasing **Gain**.
  - **For time lapse imaging:** Increase **Gain** and **Exposure**, and decrease **Light** intensity to reduce photobleaching and phototoxicity.  
For example, for overnight time lapse experiments, capture one image every 30 minutes or less, limit the use of autofocus, and use a channel other than DAPI for autofocus.
  - In the **Settings ▶ Visuals** tab, select **Highlight Saturated Pixel** to display the overexposed pixels in the color of your choice. To obtain the maximum level of brightness without any overexposed areas, dim the illumination until the highlights disappear.
-

## Focus on the sample

1. Use the **Coarse** and **Fine** focus sliders to focus on the sample.



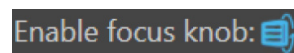
Alternatively, click **Coarse** and **Fine autofocus** to autofocus on the sample.



2. To limit the autofocus algorithm to a specific region along the Z-axis, use the **Autofocus Maximum** and **Autofocus Minimum** controls (blue triangles on the Coarse focus slider) to set the upper and lower bounds.

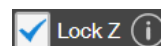
**Note:** You can also focus on the sample manually using the **focusing knobs** on the instrument (page 7).

Click **Enable focus knob**, then turn the focusing knob until the region of interest is in focus.




3. Repeat the focus procedure for each channel you want to capture.

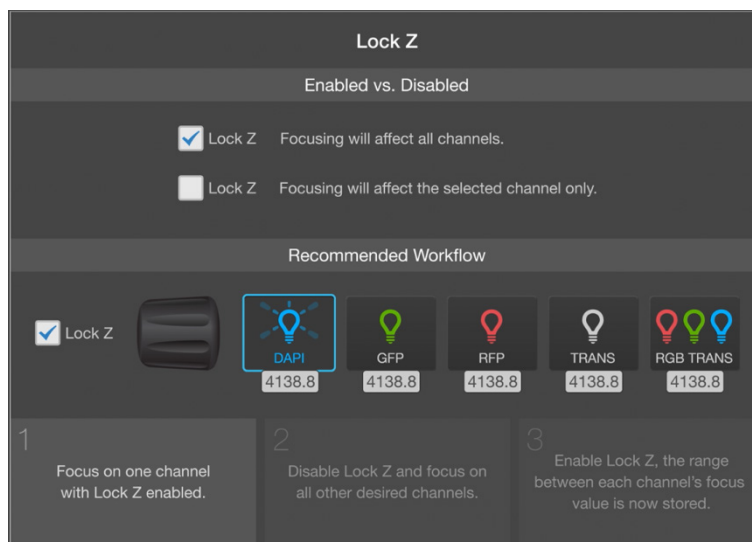
4. If you want to preserve the Z-Offsets between the channels, select **Lock Z**.



- When locked, focusing in one channel affects all channels.
- When unlocked, focusing affects only the selected channel.

**Note:** Z-Offset specifies the focus position in the selected channel relative to the focus position in other channels. Setting the correct Z-Offset is especially important when the fluorescent markers in different channels are in different focal planes.

Click  next to Lock Z selection to view the recommended workflow for Z-Offset set-up.

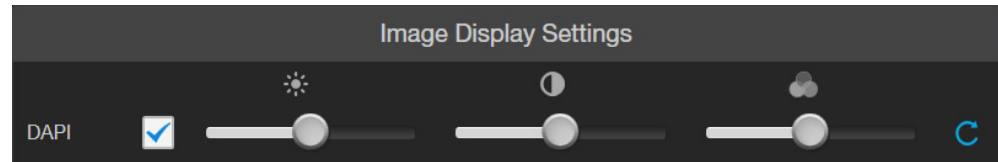






### Optional: Configure display settings

1. Hover the pointer over the Viewing area to reveal the buttons for Display Settings and Analysis Tools.



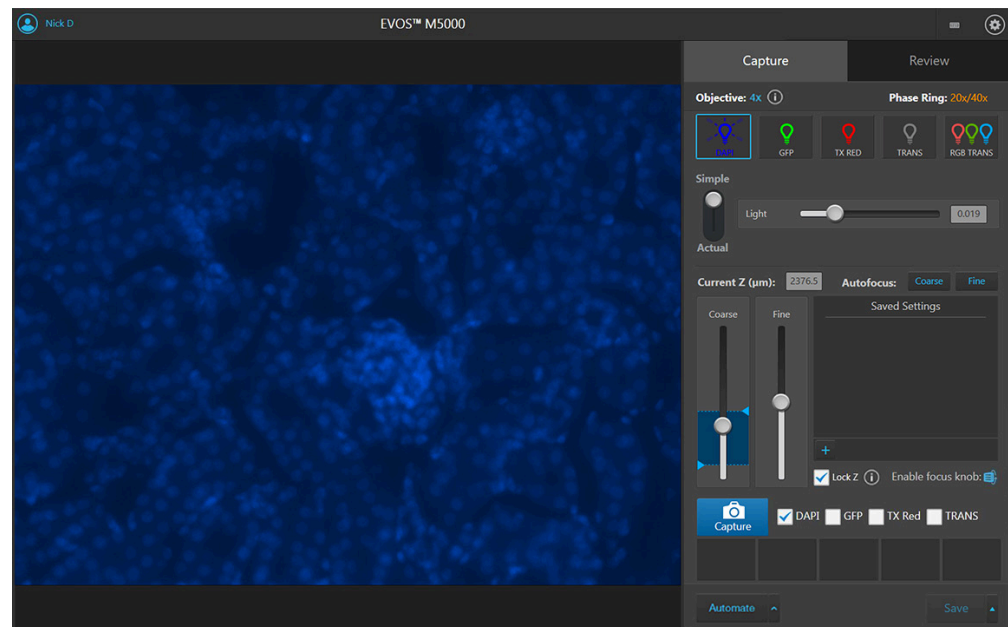
2. Click **Image Display Settings** button to expand the controls for image display settings for the selected channel.



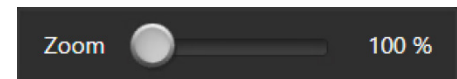
3. Adjust **Brightness** , **Contrast** , and **Gamma**  using the corresponding sliders.
4. Click the **Reset** button  to return the display settings to their default values.
5. Click the **Image Display Settings** button again to collapse the controls.

### Find the region of interest

1. Use the **X-axis** and **Y-axis positioning knobs** (page 7) to move the sample stage and bring the region of interest into the field of view.



2. To zoom in and out of the Viewing area, use the **Zoom** slider. The zoom range is 100% to 1000%.

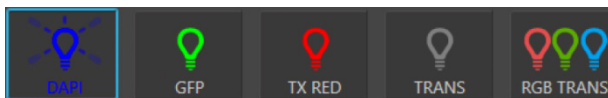


3. If needed, readjust the brightness and focus, then proceed to capture the image.

**Note:** If desired, enable **Quick Save** (page 41) before proceeding to image capture. Quick Save function allows you to set the save options in advance and save your captured images with a single click directly from the Capture tab or automatically after each capture.

## Capture image in a single channel

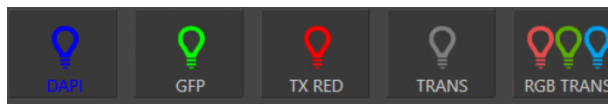
1. Ensure that the **Channel** you want to capture is selected.



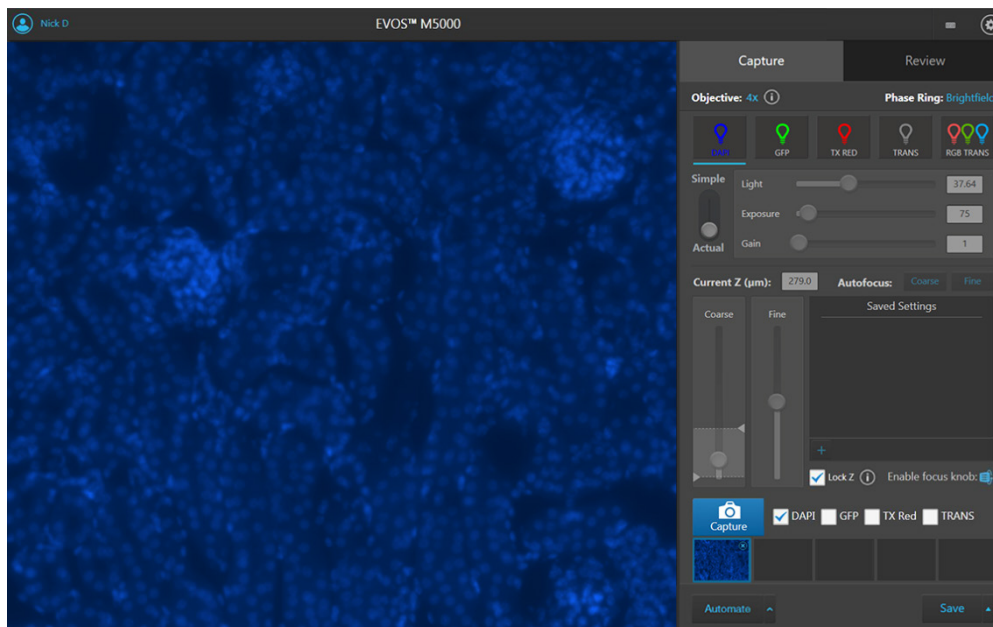
When you select a **Channel**, the corresponding **Capture channel** box is automatically checked.



**Note:** If you exit the Live mode, the current channel remains selected, as indicated by the blue line underneath the channel button.



2. Click **Capture** to acquire the image.  
The viewing area shows the newly captured image.



A thumbnail of the captured image is displayed for the selected channel (in this example, DAPI).

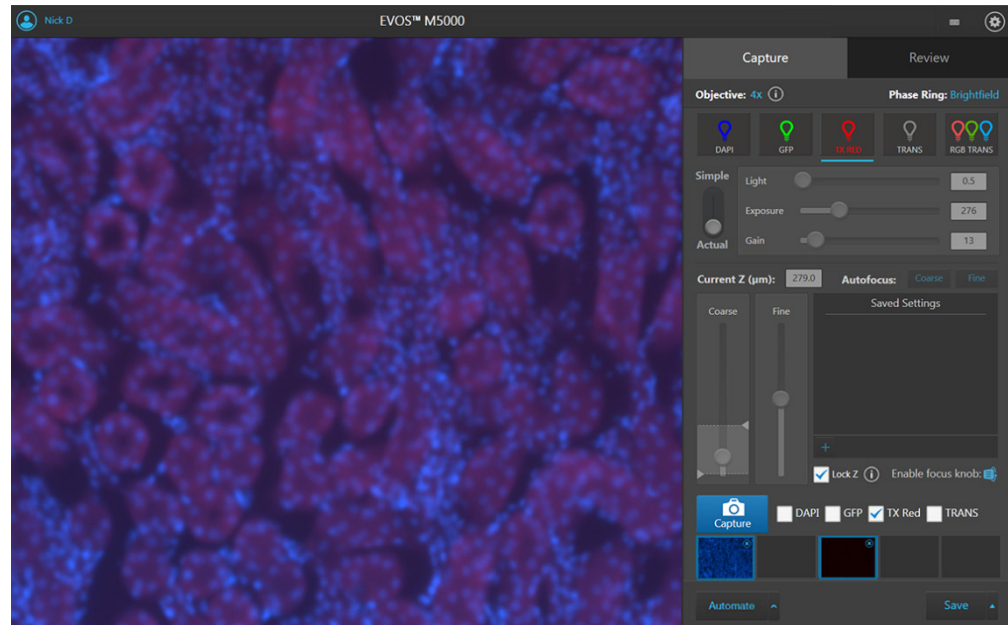


**IMPORTANT!** Captured images are stored in the memory buffer. If unsaved, newly captured images overwrite the previously captured image in the selected channel. Images captured in other fields and channels are not affected.

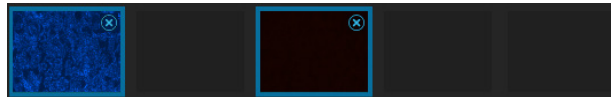
- To capture the same field of view in another channel, select the desired **Channel**.



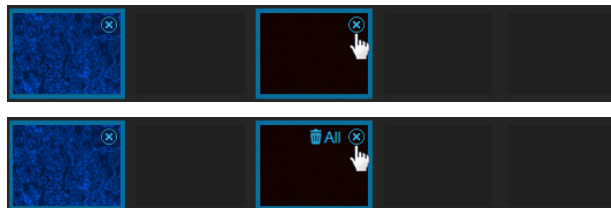
- If needed, re-adjust the brightness and focus, then click **Capture**.  
The viewing area shows the image captured in the new channel superimposed on the image captured in the previous channel.



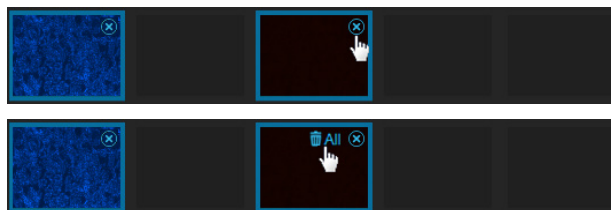
A thumbnail of the image captured in the new channel is displayed along with the thumbnail of the image from the previously captured channel.



- To remove a captured image from the memory buffer, click the **X** on the desired image, then click the **X** again to remove only the selected image.



- To remove all captured images from the memory buffer, click the **X** on any of the captured images, then click **Clear All**.





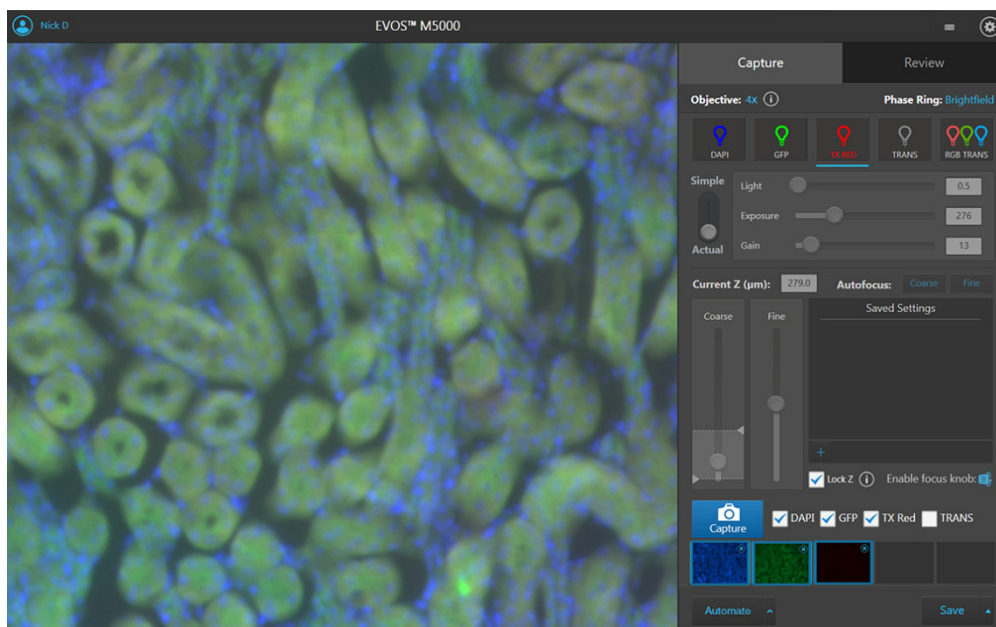
## Capture images in multiple channels

### Capture multiple channels simultaneously

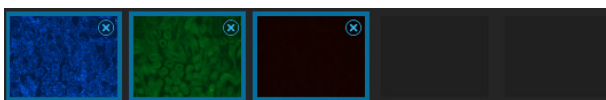
1. To capture multiple channels simultaneously, select the desired channels by checking corresponding boxes.



2. If needed, adjust **brightness** and **focus** for each of the selected channels as described.
3. Click **Capture** to acquire an image in each of the selected channels.  
The Viewing area shows a multicolor overlay of the images captured in each selected channel.



A thumbnail of the captured image is displayed for each captured channel (in this example, DAPI, GFP, and Texas Red).



---

**IMPORTANT!** Captured images are stored in the memory buffer. If unsaved, newly captured images overwrite the previously captured image in the selected channel. Images captured in other fields and channels are not affected.

---

7. To remove a captured image from the memory buffer, click the **X** on the desired image, then click the **X** again to remove only the selected image.



8. To remove all captured images from the memory buffer, click the **X** on any of the captured images, then click **Clear All**.



## 4. Analyze and annotate captured images

### Display settings and analysis tools

Display settings and analysis tools allow you to change image display settings for live and captured images in the Viewing area, annotate captured images, and perform cell count and confluence measurements.

1. Hover the pointer over the Viewing area to reveal the buttons for Display Settings and Analysis Tools.



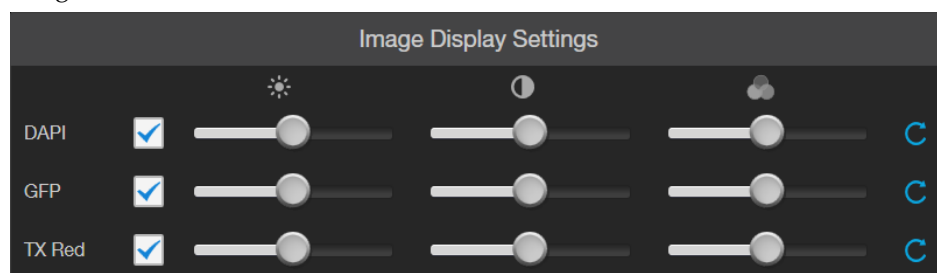
2. Click a button to open the corresponding tool; click the button again to close it.

**IMPORTANT!** Changes made to images in the Viewing area with the display settings and analysis tools, including changes made to image parameters, display of the grid and the scale bar, as well as any annotations and measurements, persist when the images are saved. If you want to save raw image data that you can use for analysis, make sure to select **Save individual channels** option when saving captured images (see page 39).





### Configure display settings

#### Configure image display settings

1. Click the **Image Display Settings** button to expand the controls for image display parameters (brightness, contrast, gamma) for captured images.

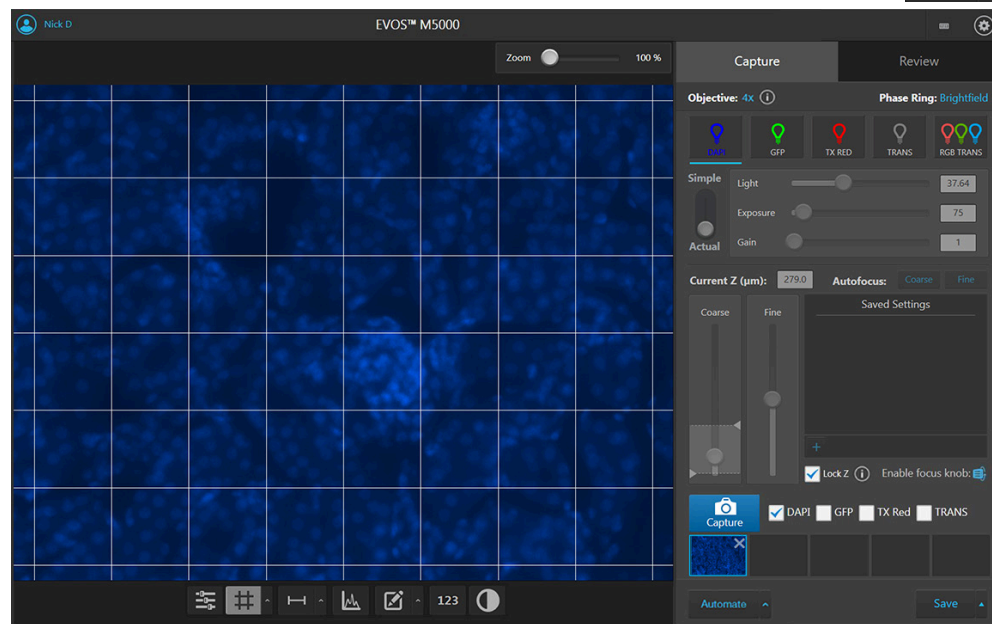


**Note:** The controls for image display settings are contextual; they are available only for channels with captured images or for a single channel in the Live mode (with the excitation light turned on). In the example above, only the controls for DAPI, GFP, and TX Red channels are displayed.

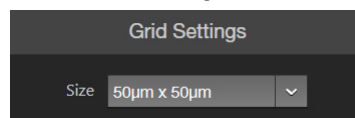
2. *Optional:* To remove a channel from displaying in the Viewing Area, unselect the corresponding checkbox.  
To display a channel with a captured image that is not shown in the Viewing Area, re-select the checkbox.
3. Adjust the **Brightness** , **Contrast** , and **Gamma**  settings for each of the selected channels using the corresponding sliders.
4. Click the **Reset** button  to return the image display settings to their default values.
5. Click the **Image Display settings** button again to collapse the controls.

## Display grid

1. Click the **Grid** button to superimpose a grid over the Viewing area.



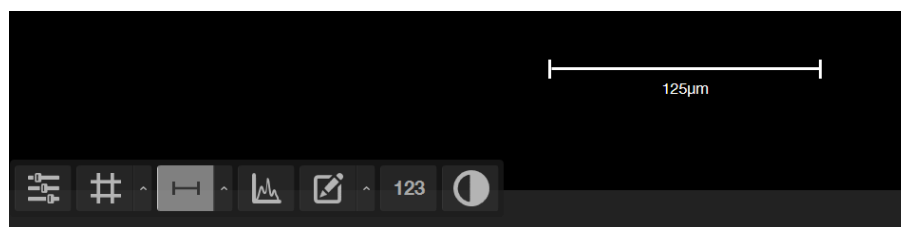
2. To change the grid size, click the **Grid Settings** button (arrow on the Grid split button) to open the Grid Settings tool.



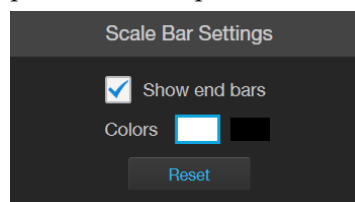
3. Select the **Size** for the grid. Available grid sizes depend on the magnification of the selected objective.
4. Click the **Grid Settings** button again to save your settings and close the tool.

## Display scale bar

1. Click the **Scale Bar** button to superimpose a scale bar over the Viewing area.



2. To change scale bar settings, click the **Scale Bar Settings** button (arrow on the Scale Bar split button) to open the Grid Settings tool.



3. Select **Show End Bars** to display the scale bar with the end bars.
4. Select the **Color** for the scale bar.



- To move the scale bar, hover your pointer over the scale bar until a bounding box appears, then click within the box and drag the scale bar to the desired location within the Viewing area.



- To adjust the length of the scale bar, hover your pointer over the scale bar until a bounding box appears, then click left or right side of the box and drag the box to the desired length.

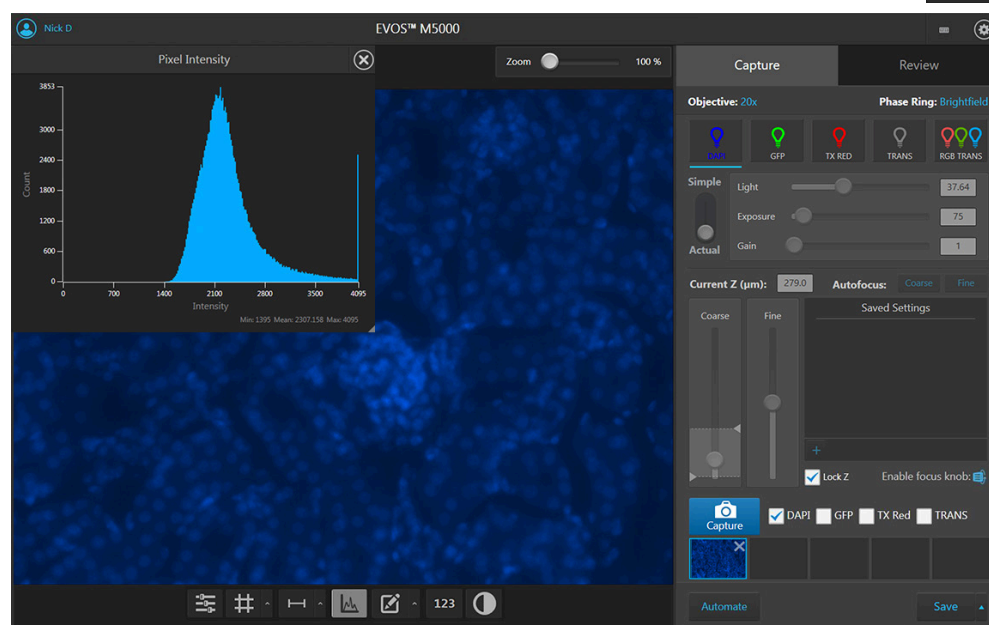
You can adjust the length by pre-fixed increments based on the objective magnification.



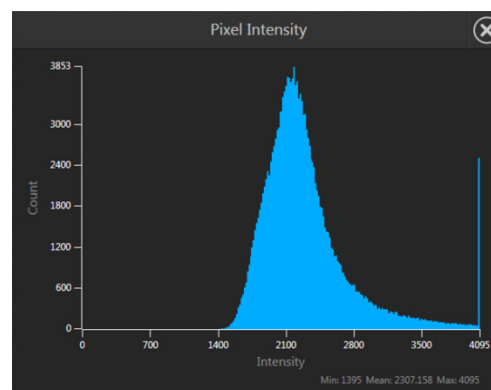
- Click **Scale Bar Settings** button again to save your settings and close the tool.

## Display histogram

- Click the **Histogram** button to open the Intensity Histogram plot.

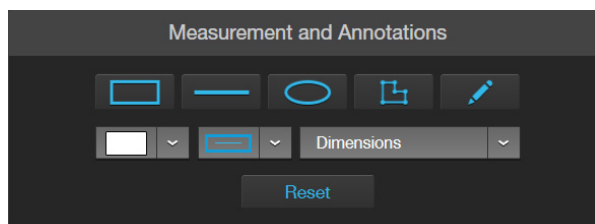


- Intensity Histogram plot shows the Pixel count vs. Intensity data of the image displayed in the Viewing area.
  - Click the **Histogram** button again to close the Intensity Histogram plot.
- You can also close to plot by clicking the X on the plot.

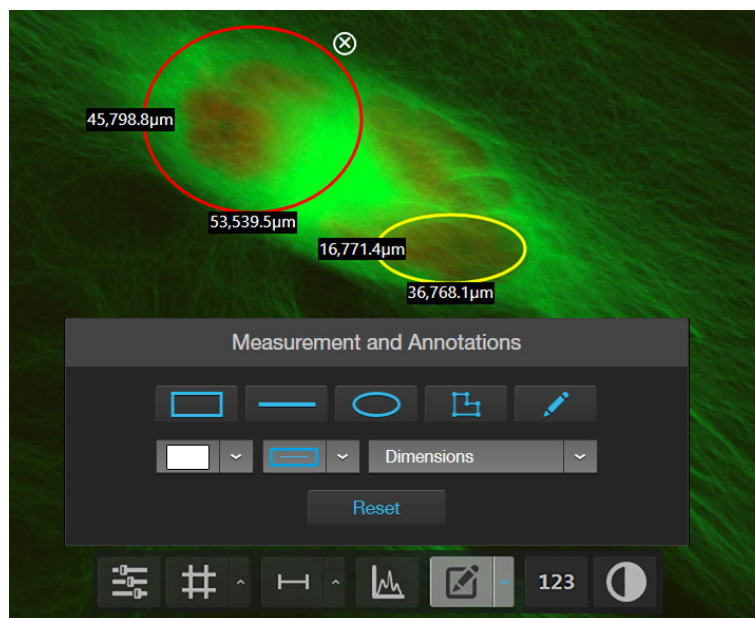


## Add and show measurements and annotations

1. Click the **Measurement and Annotations Tools** button (the arrow on the Show Measurements and Annotations split button) to open the measurement and annotations tools.

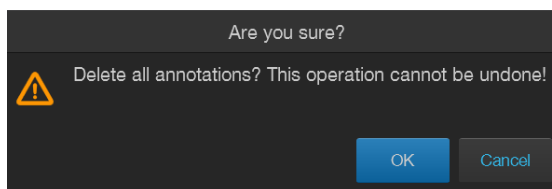


2. Using the **Draw** tools, draw a **rectangle**, **line**, **ellipse**, **polygon**, or a **free-form** shape over the region of interest on the Viewing area. You can draw multiple shapes of different type.



3. If needed, change the **Color** and **Thickness** of the annotation to make it more visible over the image.
4. If desired, select to display the **Dimensions**, **Area**, or **Perimeter** information for the annotation from the dropdown menu.
5. To delete a selected annotation, click the **X** on the shape that appears when you hover your pointer over it.

To delete all annotations, click **Reset**, then click **OK** in the dialog that opens.

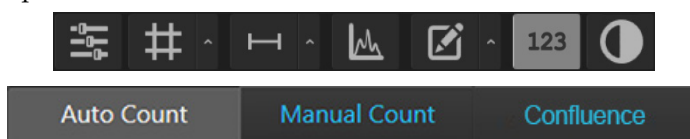


6. Click the **Measurement and Annotations Tools** button (arrow on the split button) again to close the tools.
7. Click the **Show Measurements and Annotations** button (main part of the split button) to turn the display on and off.

## Show cell count

### Cell count tool

Hover the pointer over the Viewing area to reveal **Display Settings and Analysis Tools**, then click the **Analysis** button to display **Auto Count**, **Manual Count**, and **Confluence** options in the tabs area.



- **Auto Count:** Automatically counts the objects displayed in the Viewing area based on your specifications (page 30). With Auto Count, you can count objects only in a single channel.
- **Manual Count:** Allows you to tag objects in the Viewing area with up to six labels. As you tag objects, the system keeps a running tally of the counts with percentages for each label assigned (page 34). With Manual Count, you can count objects in multiple channels simultaneously, provided that they contain images.
- **Confluence:** Allows you to select up to 5 reference objects for the target (i.e., cells) and one background reference in your image, then automatically calculates the percentage confluence of your culture (page 36).

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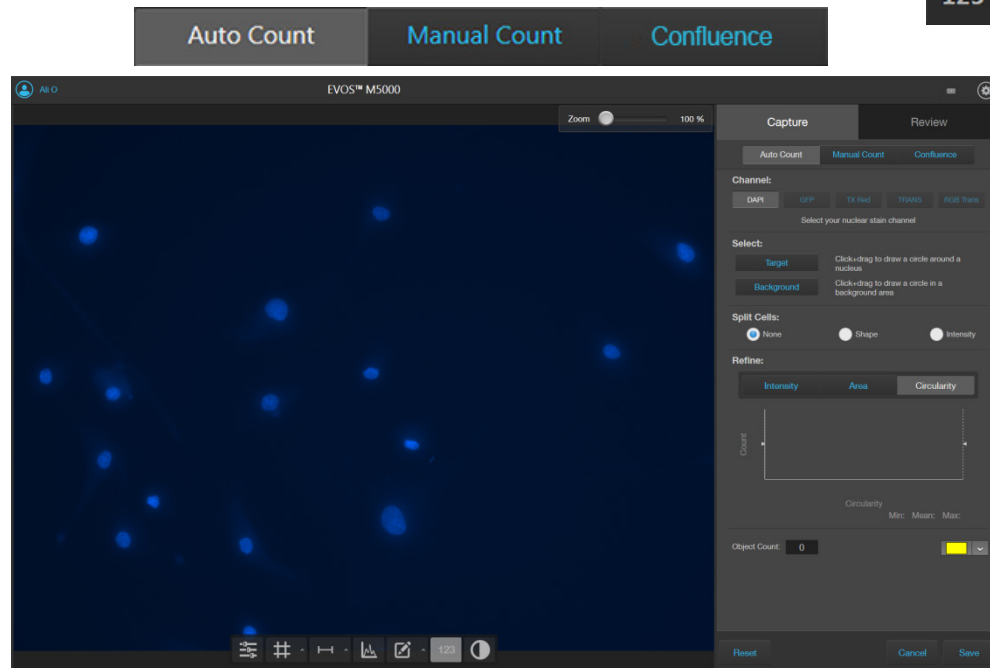
**Note:** Display Settings and Analysis Tools are available in both the Capture and the Review tabs.

---

## Perform Auto Count

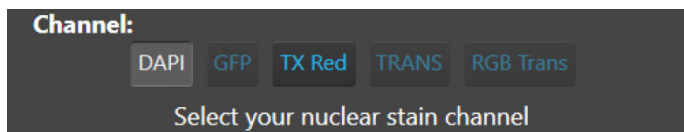
1. Click the **Analysis** button, then select **Auto Count**.

123

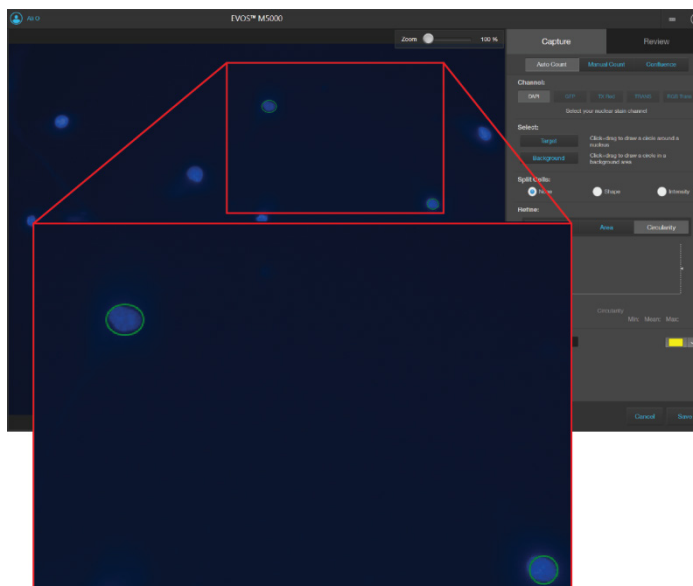


2. If counting from the Capture tab, select the **Channel** in which to count objects. This option is available only when you are counting from the Capture tab.

Available options depend on the LED light cubes installed, and you can only select channels that contain captured images. In this example, only DAPI and TX Red channels contain captured images, and DAPI is selected for auto count.



3. To identify the target objects to include in your count, click **Target**, then click and drag to draw a circle (green) around a representative target.



4. If needed, click **Target** again to identify further objects to include in your count (for example, targets that might appear different).

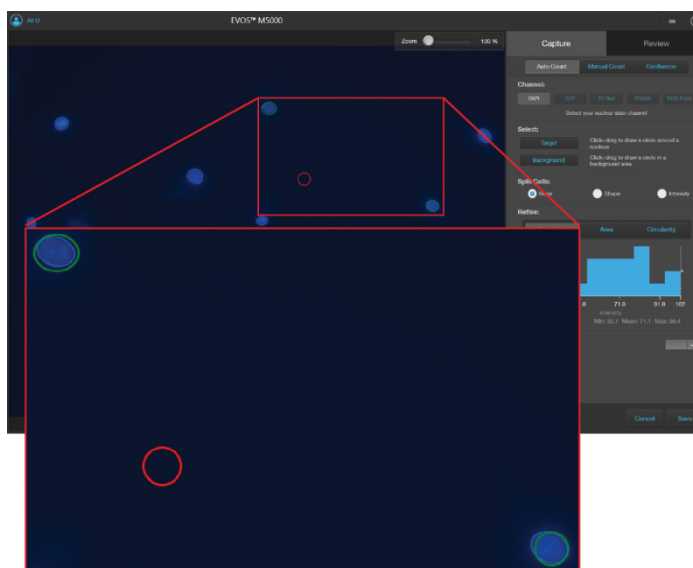
Target

**Note:** For best results, follow these guidelines when identifying target objects:

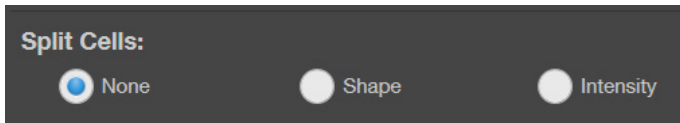
- When selecting objects, circle the entire object and include a slight border around it.
- To include objects of lower intensity in your count, select dimmer objects during identification.
- Circle only one object at a time to help define object size for segmentation.

5. To distinguish the target from background, click **Background**, then click and drag to draw a circle (red) in a background area.

Background

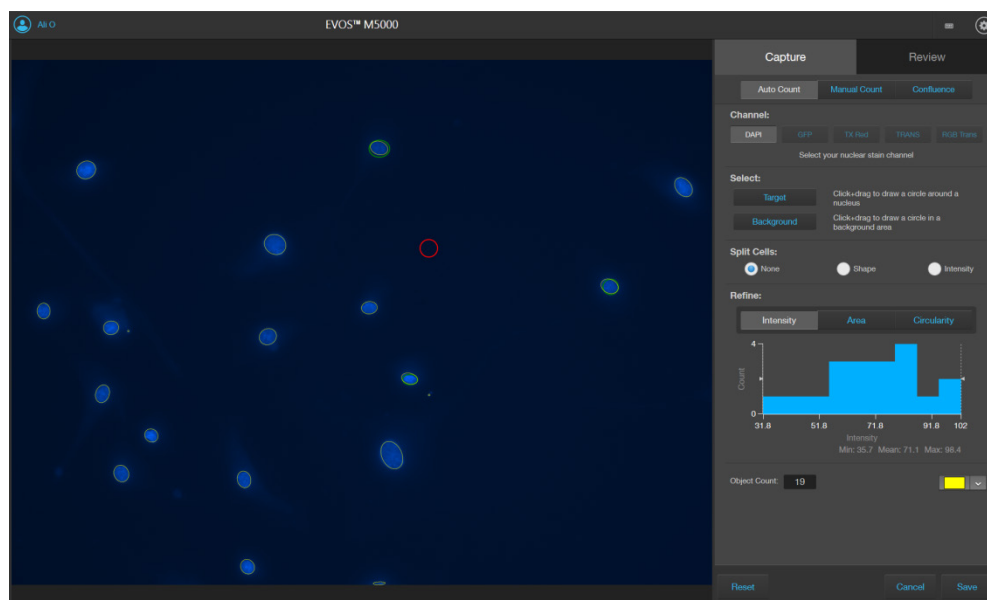


6. To count closely grouped cells that are touching or overlapping as distinct objects, select from the **Split Cells** options:



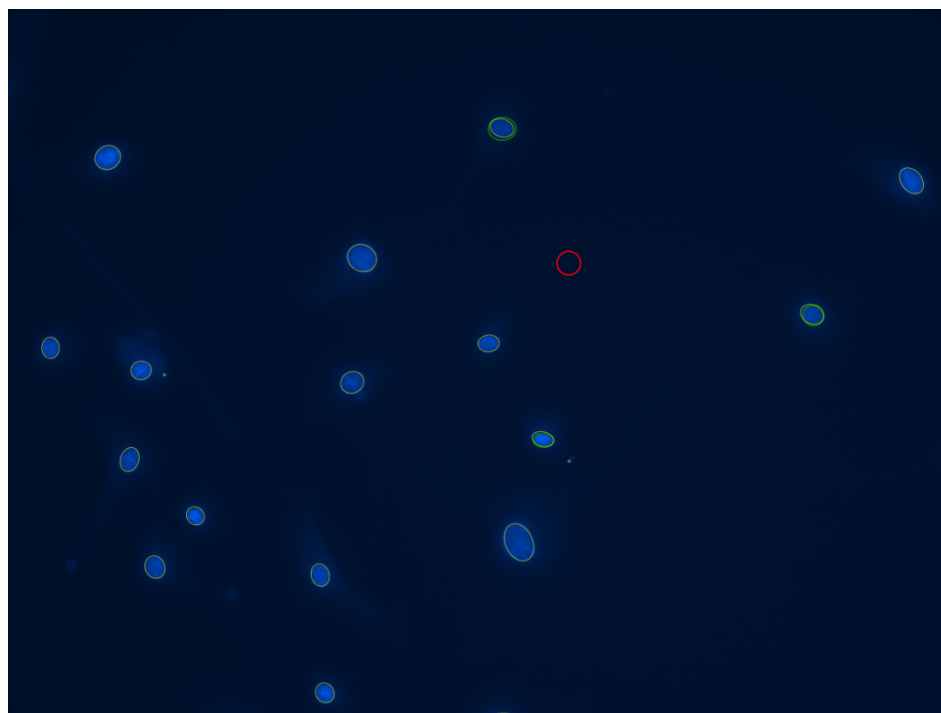
- **None:** Touching or overlapping objects are not counted separately.
- **Shape:** Distinct objects are identified and counted based on shape.
- **Intensity:** Distinct objects are identified and counted based on pixel intensity.

7. When you are finished defining the target and background areas, the software automatically counts the objects based on your criteria.



Viewing area identifies the objects that were counted with colored circles and the Object Count field displays the number of objects included in the count.

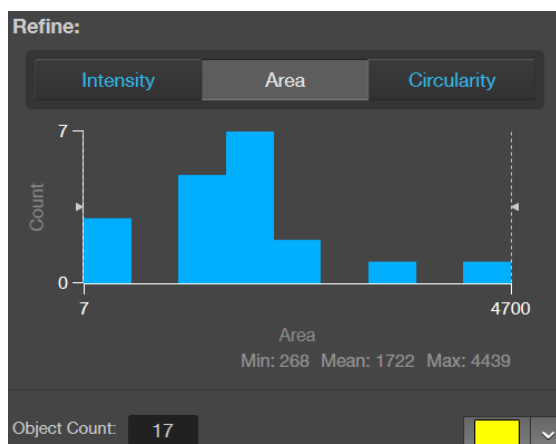
Object Count: 19



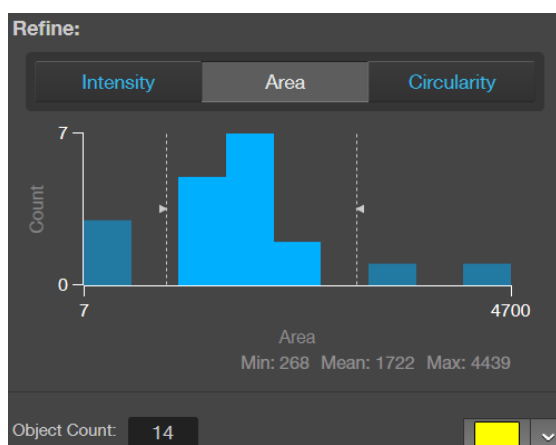
8. To change the color of the circles that identify the objects included in the count, select the desired color from the **Count Color** dropdown.



9. The Refine section displays a histogram plot showing **Count** versus **Intensity**, **Area**, or **Circularity**. In this example, **Area** is selected.



10. To refine your count, select **Intensity**, **Area**, or **Circularity**, move the gate handles to set the upper or the lower boundary for the selected parameter. You can refine the count by a single parameter or by multiple parameters. The software applies the selected boundaries and recalculates the count.



11. When finished with the count, save your count results (see “Save analysis results”, page 38).

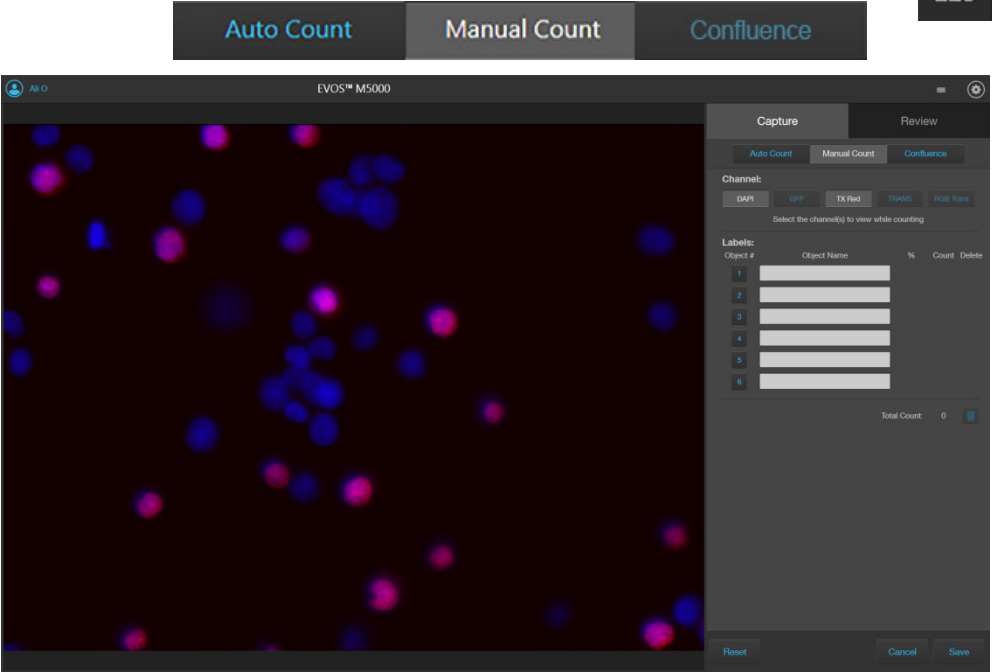
---

**IMPORTANT!** If you navigate away from the Auto Count screen, your count will be lost. To preserve the count results, make sure to save it before you navigate away from the count screen.

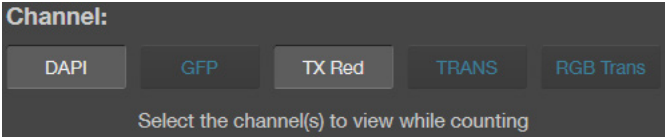
---

Perform Manual Count

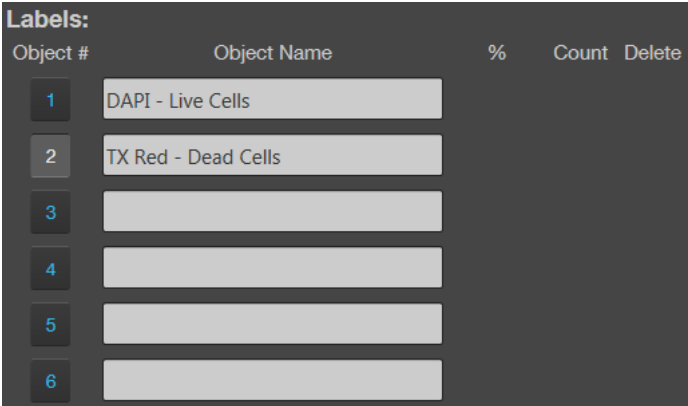
1. Click the **Analysis** button, then select **Manual Count**.



2. Select the **Channels** to display in the Viewing area for manual count. You can select multiple channels, provided that they contain captured images.
- In this example, DAPI and TX Red channels contain captured images, and both are selected for the manual count.

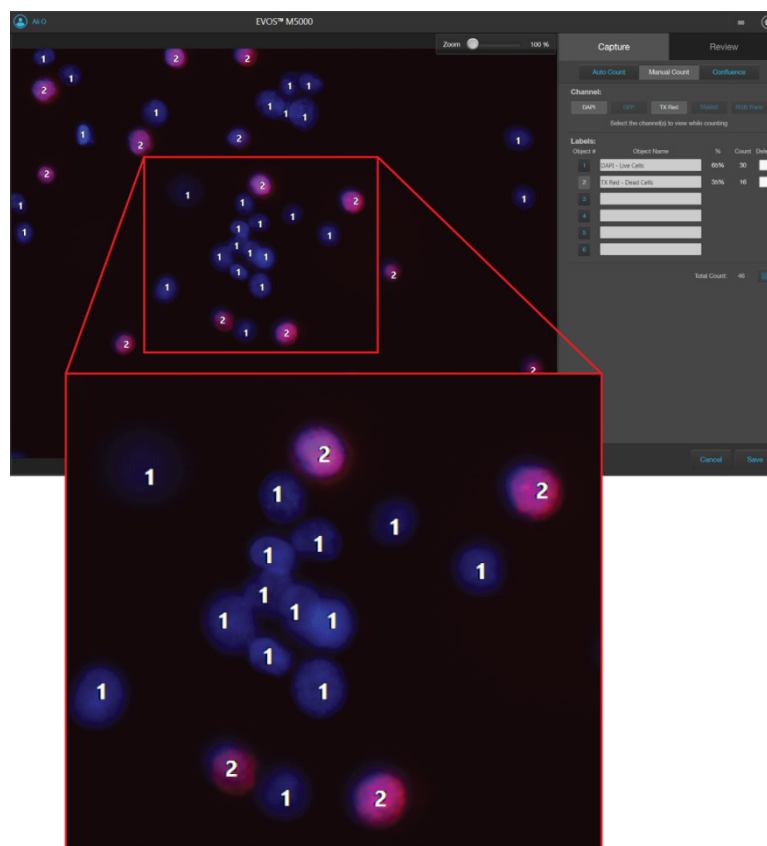


3. Click in an **Object Name** field to enter a name for that label. You can use up to six labels to tag objects for the manual count.





- Click on the **Label number** to select a label, then left-click at each point on the Viewing area to tag the items in that label category. You can switch labels as desired.



As you tag the objects onscreen with the selected label, the system keeps a running tally of the counts with percentages for each label assigned.

Labels:				
Object #	Object Name	%	Count	Delete
1	DAPI - Live Cells	65%	30	<input type="checkbox"/>
2	TX Red - Dead Cells	35%	16	<input type="checkbox"/>
3				
4				
5				
6				
Total Count:			46	

- To delete all tags for a label, check the **Delete** box for the label, then click the **Trash** button.
- To delete all tags for all labels, check the **Delete** boxes for each label, then click the **Trash** button.
- When finished with the count, save your count results (see “Save analysis results”, page 38).



# Measure confluence

## Confluence tool

Confluence is a measure of how tightly cells are packed in a culture. When all available growth area is utilized in a culture vessel and the cells make close contact with one another, the culture is at 100% confluence.

## Guidelines for confluence measurements

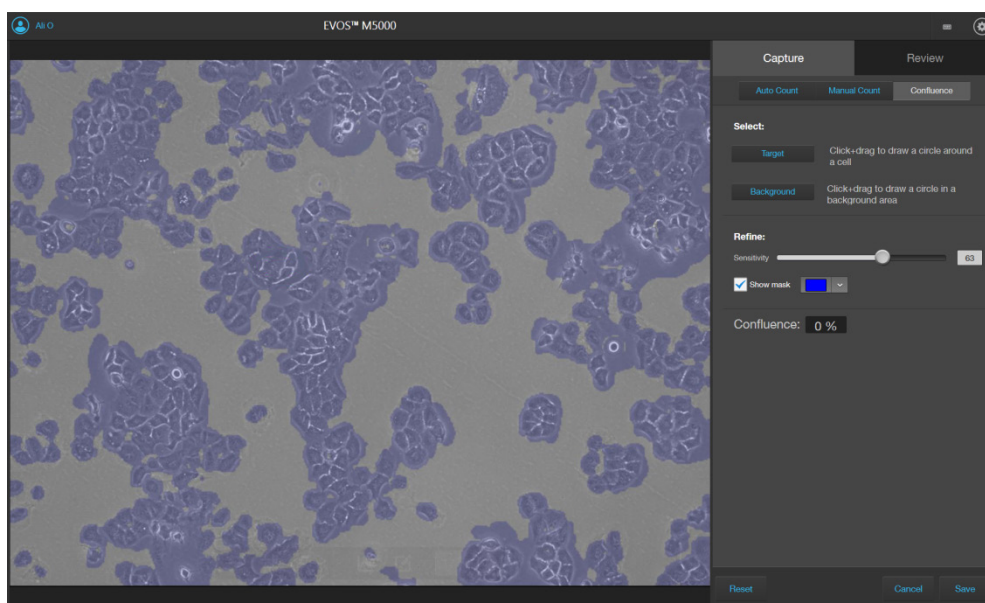
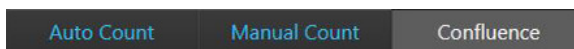
- We recommend that you visualize your cells using transmitted light and a phase objective with **4X** to **10X** magnification. Set the phase ring to **Oly 4x** (for Olympus™ 4X phase objective) or **4x/10x** (for EVOS™ phase objectives) using the phase annuli selector (page 7).
- Increasing the number of targets and background areas improves accuracy. You can select up to 5 target areas and 5 background areas.
- The Confluence tool uses a texture and intensity based algorithm. The sensitivity slider adjusts the algorithm sensitivity to pixel intensity (higher intensity = more pixels included). Decreasing the sensitivity reduces the confluence value.
- Different cell types have different confluence “patterns”, and variability in morphology and contrast can influence the absolute confluence measurement between different cell types. However, with a given cell type, the reproducibility from measurement to measurement can be optimized. Reproducibility in confluence measurements is more important than absolute percentages.

## Measure confluence

1. In the transmitted light channel, adjust lighting and focus on the sample, then click **Capture** to acquire an image of your culture.
2. Hover the pointer over the Viewing area to reveal **Display Settings and Analysis Tools**, then click the **Analysis** button.



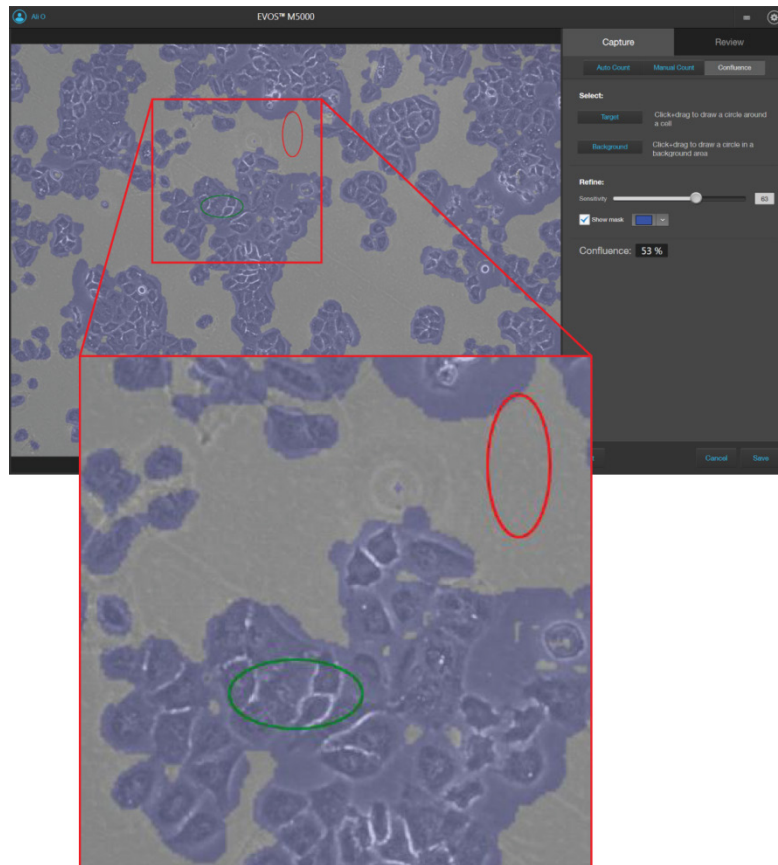
3. In the tabs area, select **Confluence** to display the Confluence tool.



4. Click **Target**, then click and drag on the image to draw a circle around an area that contains cells (green circle).
5. Click **Background**, then click and drag on the image to draw a circle around a background area that does not contain any cells (red circle).

Target

Background




---

**Note:** You can select up to 5 targets and 5 background areas. Increasing the number of targets and background areas improves the accuracy and reproducibility of the confluence measurements.

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6. Refine the sensitivity of the Confluence measurement using the **Sensitivity** slider. Decreasing the sensitivity reduces the confluence value.



7. The software automatically calculates the confluence of your culture and displays the results as a percentage of confluence.

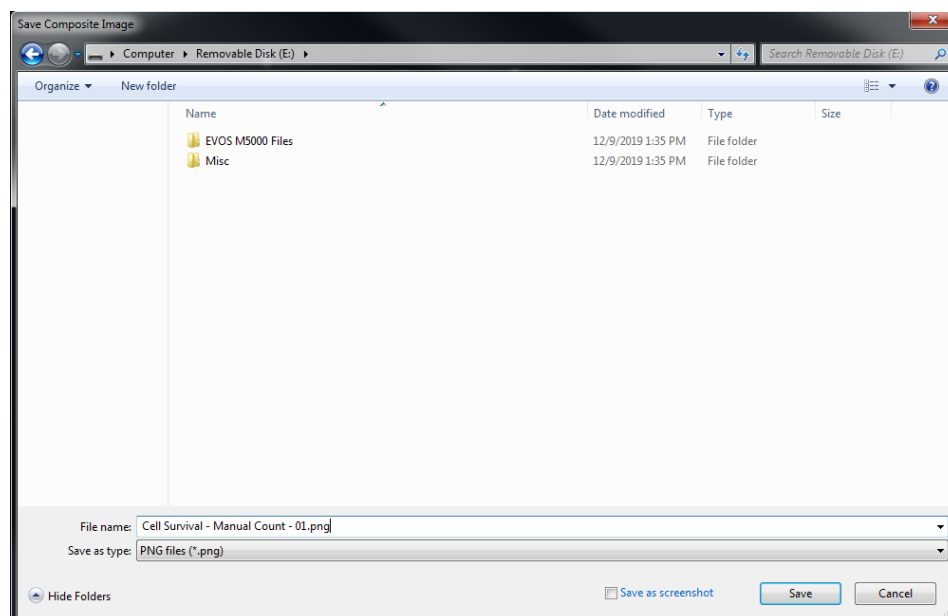
Confluence: 53 %

8. When finished with the confluence measurement, save your results (see “Save analysis results”, page 38).

# Save analysis results

## Save

1. When finished with the Auto count, Manual count, or Confluence measurement, click **Save** to open the Save Composite Image dialog, then navigate to the destination folder to save your count results.



2. Enter **File name** and select **File type**.
3. Select **Save screenshot** to preserve a detailed account of your count results as an image. ☒ **Save screenshot**
  - For Auto Count, the screenshot shows the count histogram and the selections made in the Auto Count tool.
  - For Manual Count, the screenshot shows the labels, counts, and percentages as shown in the Manual Count tool.
  - For Confluence, the screenshot shows an image of the culture, selected target and background areas, sensitivity, and percentage of confluence as shown in the Confluence tool.
  - Deselecting the Save screenshot option produces an image saved with the tags and the total count or confluence information only.
4. If you have performed an Auto count, select **Save data** to save a CSV file of your count results with individual object brightness, area, and circularity data. ☒ **Save data**

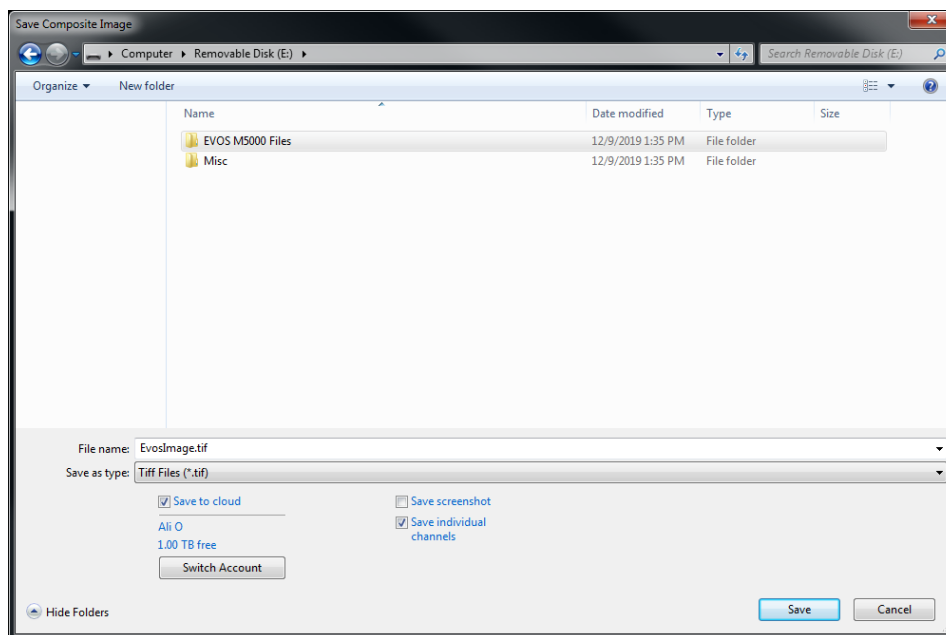
This option is not available with Manual count or Confluence measurement.

## 5. Save captured images

### Save

#### Save images manually

1. After capturing an image in a single channel or a set of images in multiple channels, click **Save** to open the Save dialog, then navigate to the destination folder to save your captured images.



2. Enter a new file name for your saved images or use the default file name, then select the file format from the **Save as type** dropdown.

Available file formats are **BMP, JPG, PNG, and TIFF**.

3. Select **Save to cloud** to save a copy of your captured image to your Connect™ account.

To save the copy of your image to another account in the Connect™ platform, click **Switch Account**, then sign in to the appropriate account.



4. Select **Save individual channels** to save images captured in different channels individually. This is the recommended format for image analysis.



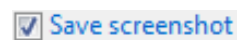
Leave the **Save individual channels** option unselected to save only the captured image displayed in viewing area.

---

**IMPORTANT! Save individual channels** is the recommended format for image analysis, because it allows you to save raw image data from each selected channel.

---

5. Select **Save screenshot** to save an image of the entire instrument screen in addition to the captured image.



Leave the **Save screenshot** option unselected to save only the captured image.

---

**Note:** Save screenshot function allows you retain an image of the annotations and image display selections as seen in the viewing area along with the instrument controls.

---

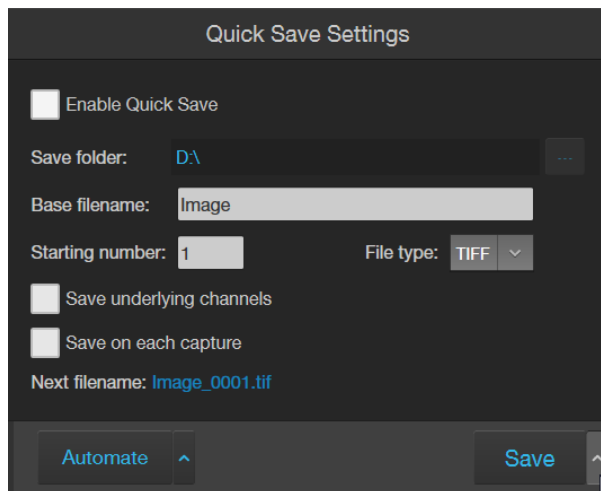
6. Click **Save** to save the image.


## Quick Save images

### *Optional:* Enable Quick Save

Quick Save function allows you to set the save options in advance and save your captured images with a single click directly from the Capture tab or automatically without having to click Save after each capture.

1. Click the **Quick Save Settings** button (the arrow on the Save split button) to open the Quick Save Settings dialog.



2. Select **Enable Quick Save**. ☒ Enable Quick Save
3. Click the **Browse** button  to open the Select folder dialog, navigate to the folder where you want to save captured images, then click **Select Folder**.



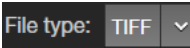



4. Enter a **Base Filename** and **Starting number** for saved images.



**Note:** The default base filename is Image. Base filename is appended by a numerical suffix and channel name.

Starting number determines the starting numerical suffix, which is increased by one for each subsequently saved image. For example, Image\_0001, Image\_0002, and so on.

**Next filename: Image\_0001.tif**

5. Select the File Type from the dropdown menu. Available options for file type are **PNG, TIFF, JPEG, and BMP**. 
6. To save the image displayed in the viewing area and each underlying channel individually, select **Save underlying channels**. 
7. To save captured images automatically without having to click Save after each capture, select **Save on each capture**. 
8. Click the **Quick Save Settings** button again to save your settings and close the dialog. 

## 6. Capture time lapse images

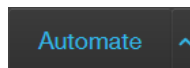
### Time Lapse tool

With the Time Lapse tool, you can set up your cells and program the EVOS™ M5000 Imaging System to capture images (including Z-Stack images) at given intervals over a time period based on your specifications. For repeat experiments, time lapse routines can be saved, recalled, and edited.

### Run a time lapse routine

#### Run a new time lapse routine

1. On the Capture tab, click **Automate**, then select **Time Lapse** to open the Time Lapse tool.



2. Select the **Capture Channels**. You can select multiple channels in which to capture time lapse images. In the following example, **DAPI** and **TX Red** channels have been selected.

3. Select **Auto Focus** to run the autofocus algorithm during the time lapse routine.



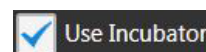
Unselect Auto Focus to capture time lapse images using the initial focus positions set before you run the routine.



4. Select **Z-Stack** to capture multiple images along the Z-axis based on your specifications.

Unselect Z-Stack to capture images only at the focus position set for each channel (either Auto Focus as part of the routine or initial focus positions).

5. If you have the optional EVOS™ Onstage Incubator installed and want to use it during your time lapse routine, select **Use Incubator**.



This option is only available if you have installed and connected the EVOS™ Onstage Incubator to your EVOS™ M5000 instrument.

---

**IMPORTANT!** Before using the EVOS™ Onstage Incubator in your time lapse experiments, ensure that:

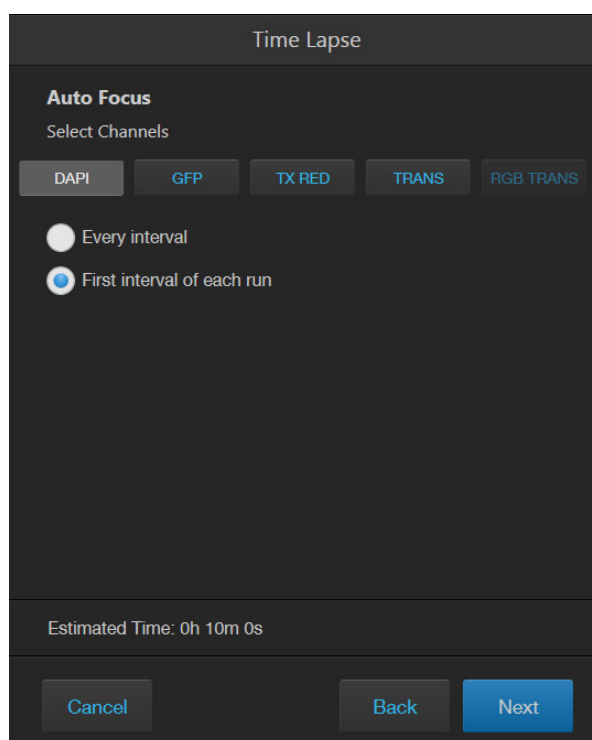
- The EVOS™ Onstage Incubator has been set up for use (page 95).
- The gas inputs have been configured (page 102).
- The oxygen sensor has been calibrated (page 103).

6. Adjust brightness and focus for each selected channel as described in “Capture images” (page 18), then click **Next**.

---

**Note:** When the sample is focused and ready, tighten the stage brakes (page 7) to prevent the stage from drifting during the time lapse routine.

7. If you have opted to run **Auto Focus**, select the **Auto Focus Channel** to use.

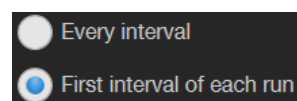


---

**Note:** In a time lapse routine, you can run the autofocus procedure only in a single channel (in this example, DAPI). The focal plane identified in this channel is then used for all other channels.

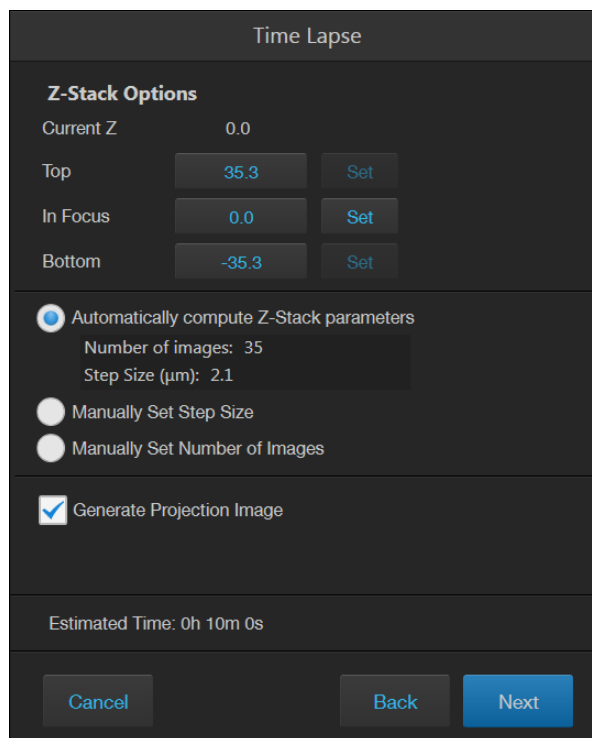
---

8. Select to autofocus at **Every interval** or at **First interval of each run**, then click **Next**.

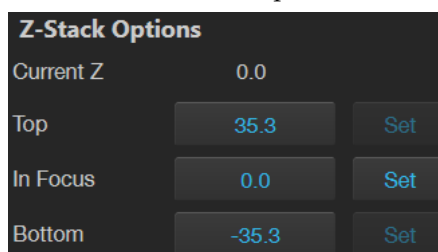


**Note:** In a time lapse routine, images are captured at the end of each interval of a run. For more information, see “Intervals”, page 46.

9. If you have opted to capture **Z-Stack** images, define the **Top**, **In Focus**, and **Bottom** positions for the Z-Stack image set.



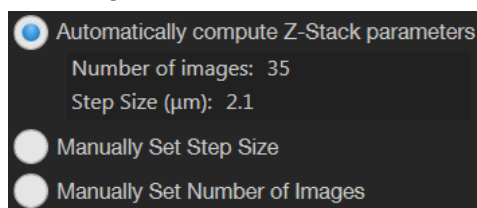
- a. If not already on, click the **Light** button to illuminate the sample as you locate the boundaries and the In Focus position of the Z-Stack.

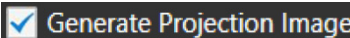


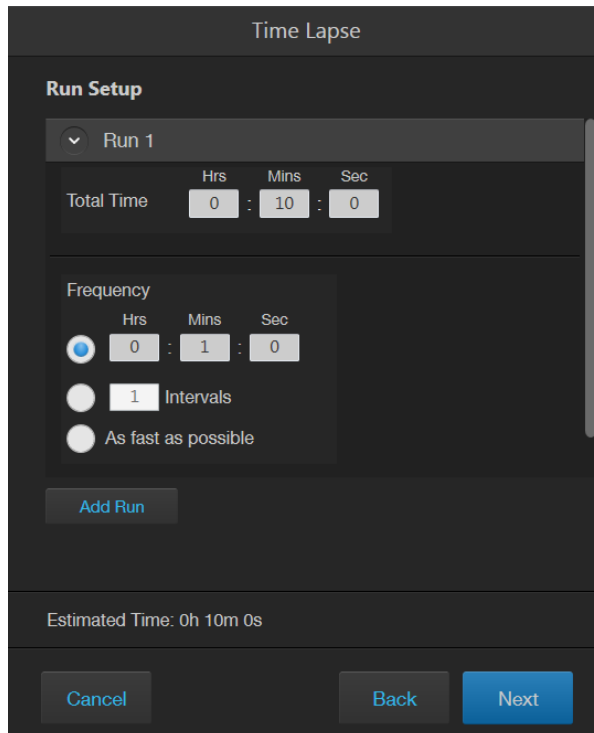
- b. To accept the **Current Z** as the **In Focus** position, click **Set**.  
To change the **In Focus** position, move the **Coarse** and **Fine focus** sliders on the Capture tab to focus on the sample, then click **Set**.
- c. Move the focus sliders up from the Current Z position to the desired **Top** position (upper boundary of the Z-Stack), then click **Set** for Top.
- d. Move the focus sliders down from the Current Z position to the desired **Bottom** position (lower boundary of the Z-Stack), then click **Set** for Bottom.

**Note:** Click the **Z number** button for the Top, In Focus, and Bottom positions to jump to that position along the Z-axis.

10. Define the **Z-Stack parameters** to determine the number of “optical sections” captured in the Z-Stack image set:

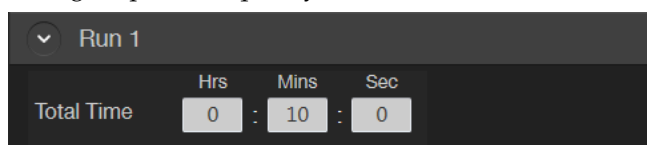


- **Automatically compute Z-Stack Parameters:** Select to calculate the number of images and their Z-positions automatically based on the Z-Stack boundaries and the default Step Size.
  - **Manually Set Step Size:** Select to enter the **Step Size** (Z-distance in  $\mu\text{m}$  between focal planes) in the corresponding text box. The system calculates the number and Z-positions of the images based on your entry.
  - **Manually Set Number of Images:** Select to enter the **Number of Images** (Z-distance in  $\mu\text{m}$  between focal planes) in the text box. The system calculates the Step Size and the Z-positions of the images based on the number of images.
11. Select **Generate Projection Image** to generate a projection image. 
- When selected, the system uses the most in-focus pixels from images captured at different focal planes to generate a composite in-focus image. Each image in the Z-Stack is also separately saved.
- When unselected, the system only saves the individual Z-Stack images.
12. Click **Next** to set up a Run.



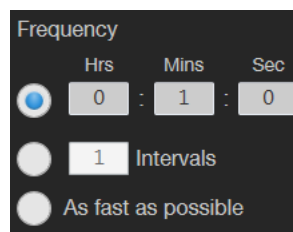
13. Enter the **Total Time** for Run 1 in **Hours**, **Minutes**, and **Seconds**.

You can create multiple Runs for a Time Lapse Routine, each with its own duration and image capture frequency.



The screenshot shows a dark-themed window titled 'Run 1'. Below the title bar, there are three input fields for 'Total Time': 'Hrs' (0), 'Mins' (10), and 'Sec' (0). The fields are separated by colons.

14. Set the **Image capture options** for Run 1. Available options are **Frequency**, **Intervals**, and **As fast as possible**.



The screenshot shows a dark-themed window titled 'Frequency'. It has three radio buttons: 'Frequency' (selected), 'Intervals', and 'As fast as possible'. The 'Frequency' option has associated input fields for 'Hrs' (0), 'Mins' (1), and 'Sec' (0).

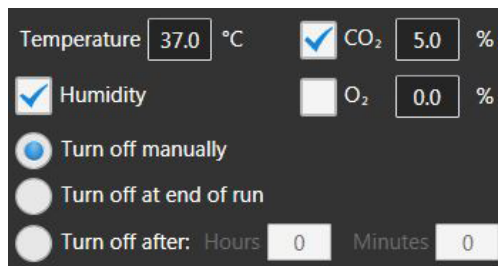
- **Frequency:** Enter the time in **Hours**, **Minutes**, and **Seconds** that must elapse before a new set of images are captured.  
For example, in an experiment with an image capture frequency of 2 minutes and 30 seconds, the images will be captured every 2 minutes and 30 seconds after the initial set of images are acquired at time point 0.
- **Intervals:** Enter the total number of **time intervals** between the captured image sets for a given run duration.  
Images are collected at the end of an interval. For example, in a Run experiment with a duration of 5 minutes and 2 intervals, the images will be captured every 2 minutes and 30 seconds after the initial set of images are acquired at time point 0.
- **As fast as possible:** Select this option to capture a new set of images immediately after completing the previous set without any delay between the sets.  
The speed with which the images are captured depends on the specific choices made for the routine such as the autofocus frequency and exposure settings.

---

**IMPORTANT!** To reduce phototoxicity, minimize the capture frequency and light intensity, and maximize gain.

---

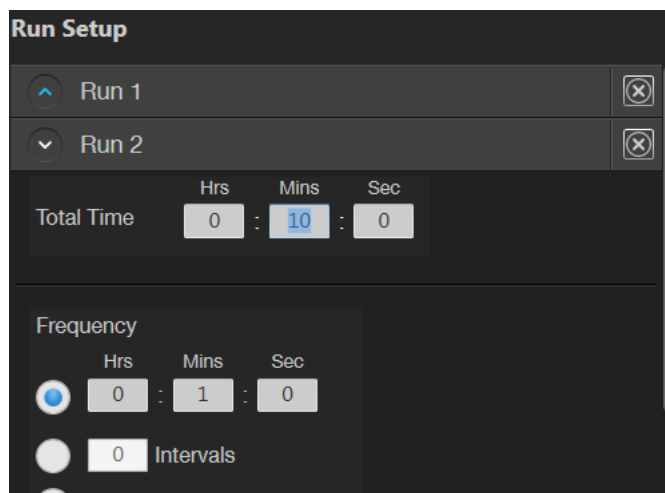
15. If you have opted to use the EVOS™ Onstage Incubator in your time lapse routine, set the **Incubator options** for Run 1.



Temperature  °C ☒ CO<sub>2</sub>  %  
☒ Humidity ☐ O<sub>2</sub>  %  
☒ Turn off manually  
☐ Turn off at end of run  
☐ Turn off after: Hours  Minutes

- Enter the target values for **Temperature**, **CO<sub>2</sub>**, and **Oxygen**.
  - If desired, select **Humidity** to use a humidified atmosphere in the incubator.
  - Select the **Shutdown** option for the incubator.
    - Turn off manually:** The incubator remains on until the **Use Incubator** option is manually deselected.
    - Turn off at the end of experiment:** Heat, humidity, and the flow of gas are automatically turned off at the end of the experiment.
    - Turn off after:** Enter the time period in **hours** and **minutes** that must elapse before the heat, humidity, and the flow of gas are automatically turned off.
16. *Optional:* Click **Add Run** to add another Run to the routine, then define the Run options as described for Run 1.

**Add Run**



**Run Setup**

Run 1

Run 2

Total Time Hrs Mins Sec  
 :  :

Frequency Hrs Mins Sec  
☒  :  :   
☐  Intervals

To remove a Run, click the **Delete** button  for the Run you wish to remove from the routine.

17. When finished with adding Runs, click **Next** to set the Save options.

The screenshot shows a dark-themed dialog box titled "Time Lapse". Under the "Save Options" section, the "Save folder" is set to "\\Eug01file02\\public\\Ali O\\EVOS M5000\\Time L..." with a browse button "...". The "File type" section has two radio buttons: "PNG" (unselected) and "TIFF" (selected). Below this, there is a checkbox for "Save Time Lapse Movie" which is currently unchecked. Underneath the checkbox, "Video Type" is set to "AVI" and "Frame Rate" is set to "1" images per second. At the bottom, the "Estimated Time" is "0h 10m 0s". There are three buttons at the very bottom: "Cancel", "Back", and "Start".

18. Click the **Browse** ... button, navigate to the desired destination folder for the Time Lapse images, then click **Select Folder**.

This screenshot shows a close-up of the "Save folder:" field in the dialog box. The text "\\Eug01file02\\public\\Ali O\\EVOS M5000" is entered, followed by a browse button "...".

19. Select the **File Type: PNG** or **TIFF**.

This screenshot shows the "File type" section of the dialog box. It features two radio buttons: "PNG" and "TIFF". The "TIFF" radio button is selected, indicated by a blue dot.

20. If desired, select **Save Time Lapse Movie** to create a video from the images captured in the time lapse routine.

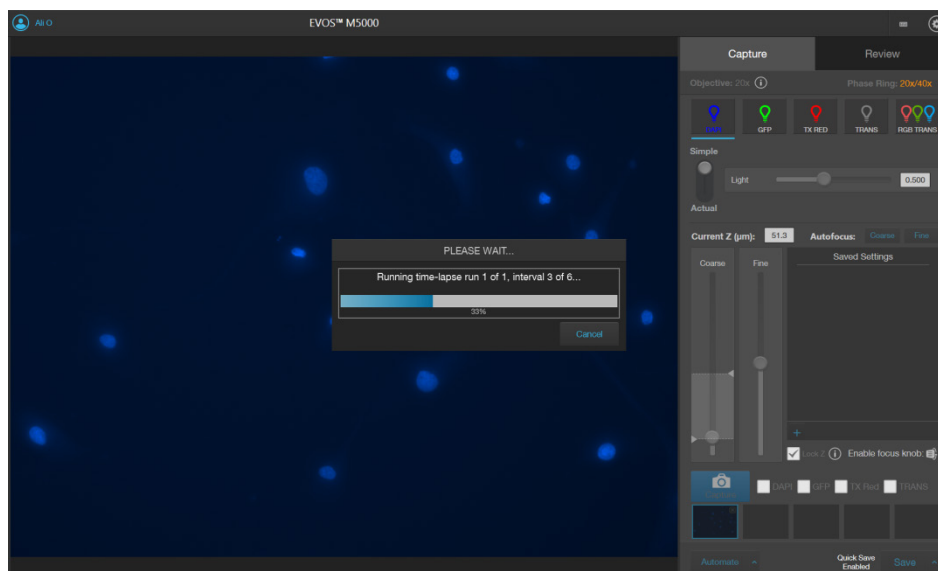
This screenshot shows the "Save Time Lapse Movie" section. The checkbox "Save Time Lapse Movie" is checked. Below it, "Video Type" is set to "AVI" and "Frame Rate" is set to "1" images per second.

21. Select the **Video Type: MP4** or **AVI**.  
22. Select the **Frame Rate: 1, 3, or 5 images/second**.

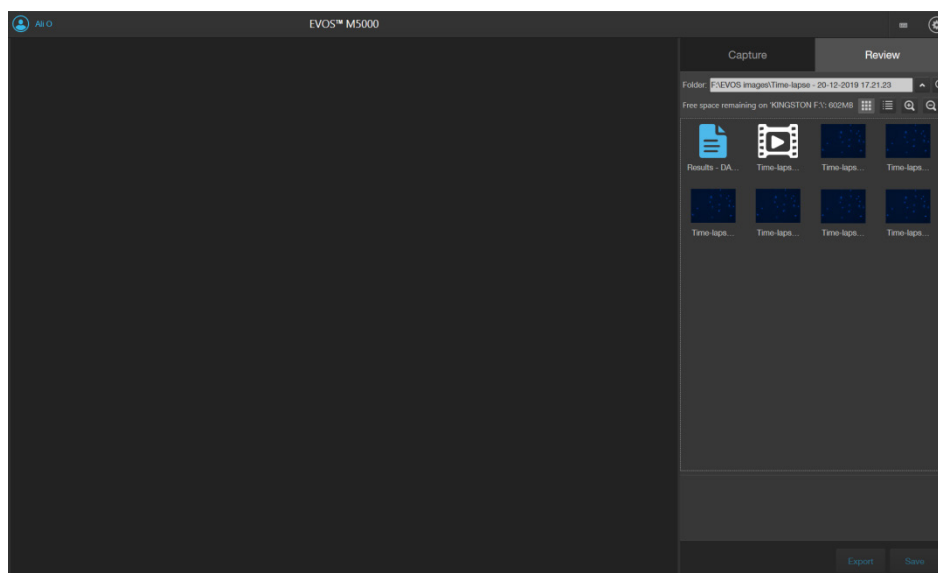
23. Click **Start** to run the Time Lapse routine.

Start

The system provides information about the progress of the Time Lapse routine as it captures the images based on the routine specifications.



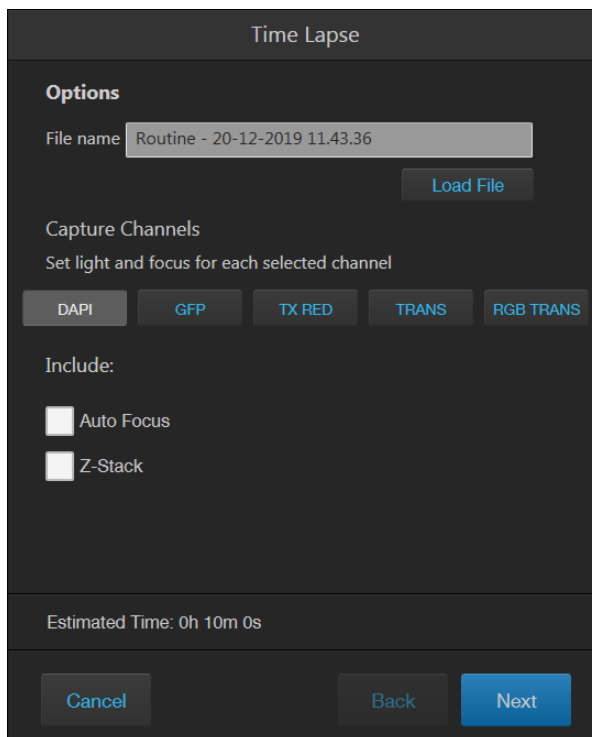
24. When the Time Lapse routine is completed, the Review tab opens to the files captured in the routine.



## Run a saved time lapse routine

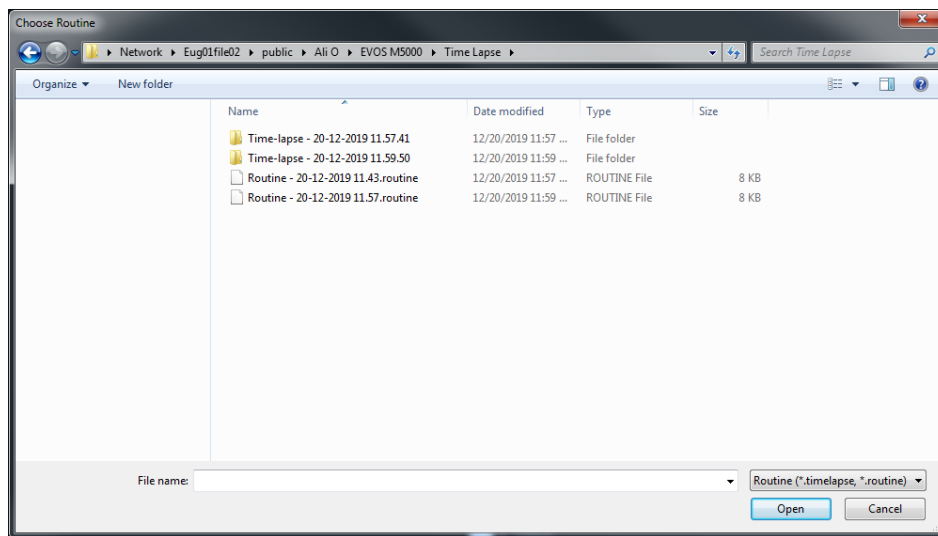
Each time you run a Time Lapse routine, the system saves the specifics of the routine, which you can then recall and run with a new sample.

1. On the Capture tab, click **Automate**, then select **Time Lapse** to open the Time Lapse tool.



The Time Lapse configuration window has a dark background. At the top, it says "Time Lapse". Below that is an "Options" section with a "File name" field containing "Routine - 20-12-2019 11.43.36" and a "Load File" button. The "Capture Channels" section says "Set light and focus for each selected channel" and has five buttons: "DAPI", "GFP", "TX RED", "TRANS", and "RGB TRANS". Below that is an "Include:" section with two checkboxes: "Auto Focus" and "Z-Stack". At the bottom, it shows "Estimated Time: 0h 10m 0s" and three buttons: "Cancel", "Back", and "Next".

2. Click **Load File** to open the Choose Routine dialog.

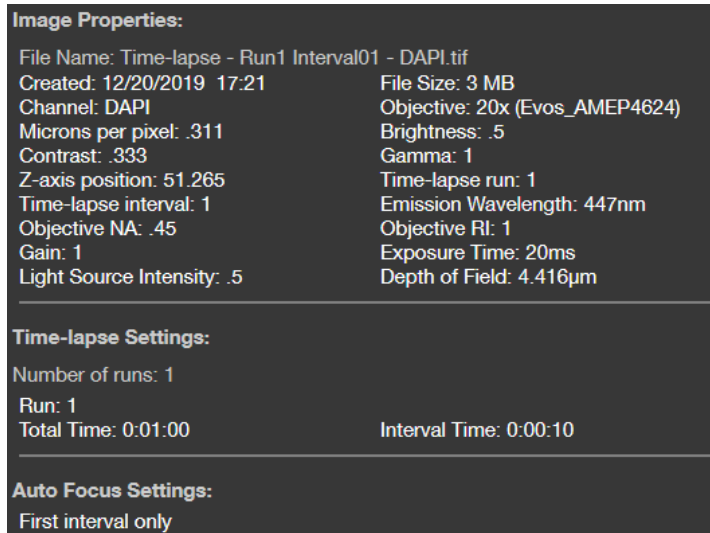
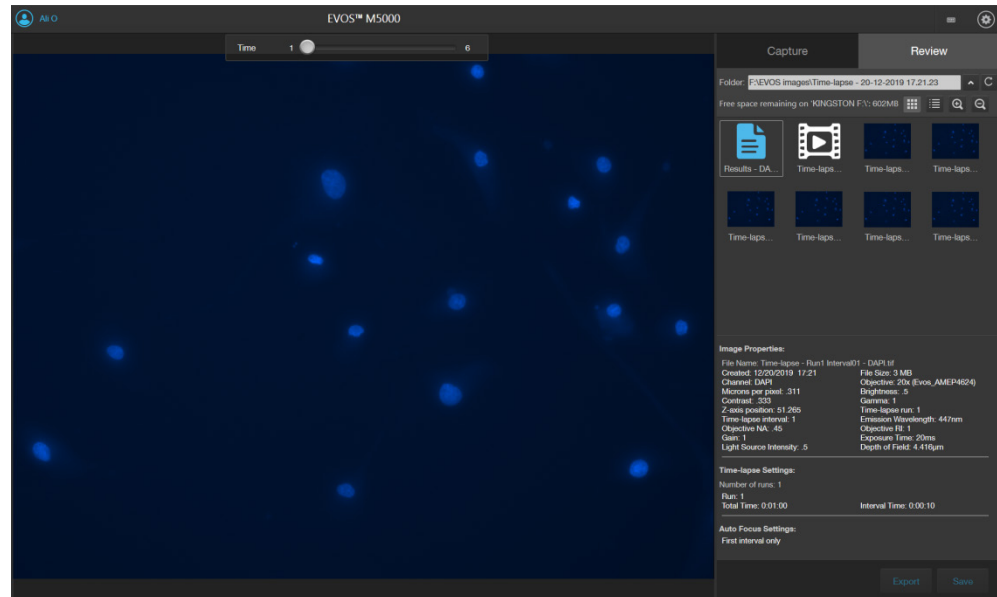


3. Select the routine you want to run, then click **Open**.
4. If needed, make changes to the routine as described for new routines.
5. Click **Start** to run the Time Lapse routine.




Review images and video captured in a time lapse routine

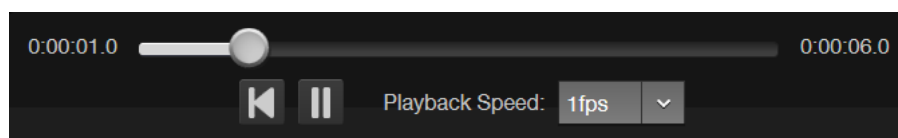
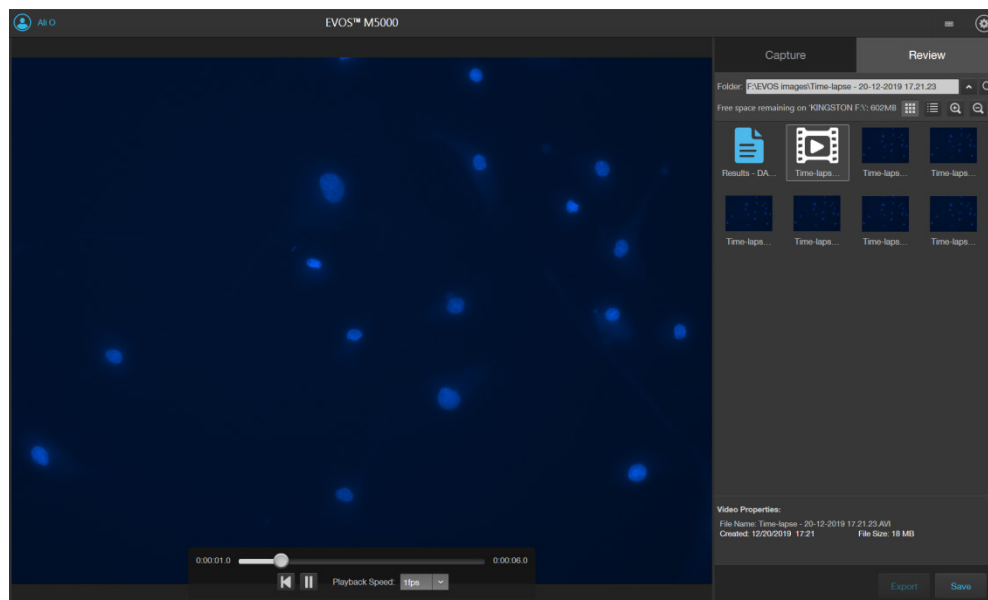
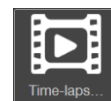
1. To review the results from your completed time lapse routine, click **Results**. The viewing area displays the first image captured in the time lapse routine and the Review tab shows the Image Properties, and Time Lapse and Auto Focus Settings.



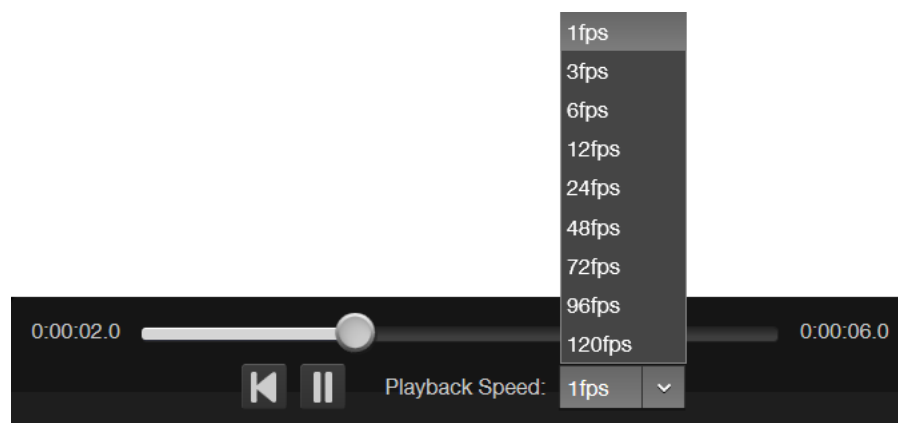
2. To view the subsequent images captured in the time lapse routine, use the Time Slider at the top of the viewing area.



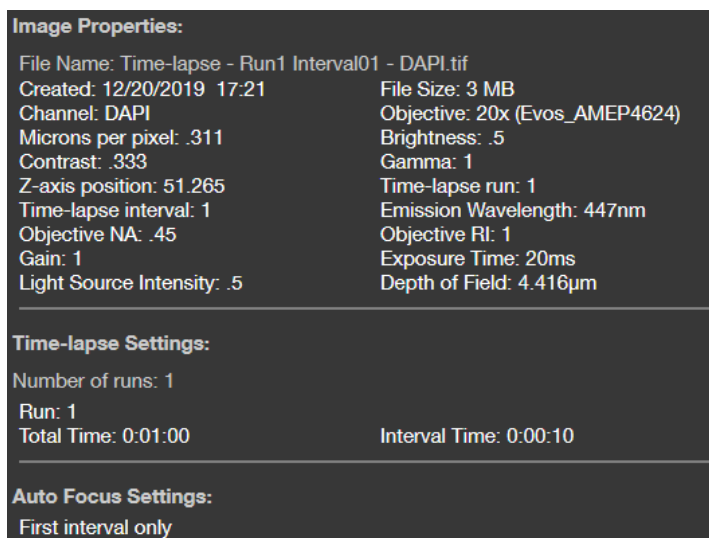
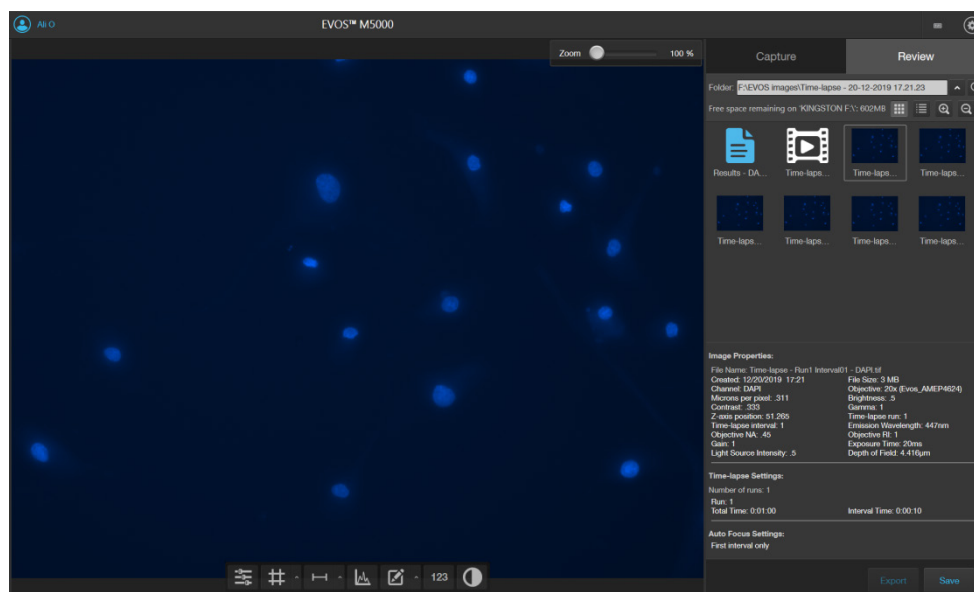
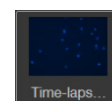
3. To play the time lapse movie, select the **Time-lapse Video** file, then click **Play**  on the **Time Lapse Video Player**.



4. To change the playback speed, select the desired speed from the **Playback Speed** menu.



- To review individual images captured during the time lapse routine, select the desired image from the Review tab. The viewing area displays the selected image and the Review tab shows the Image Properties, and Time Lapse and Auto Focus Settings.



- To zoom in on the image, use the **Zoom** slider.



- To annotate captured images and perform cell count and confluence measurements, use the **Display Settings and Analysis Tools** as described on page 25.



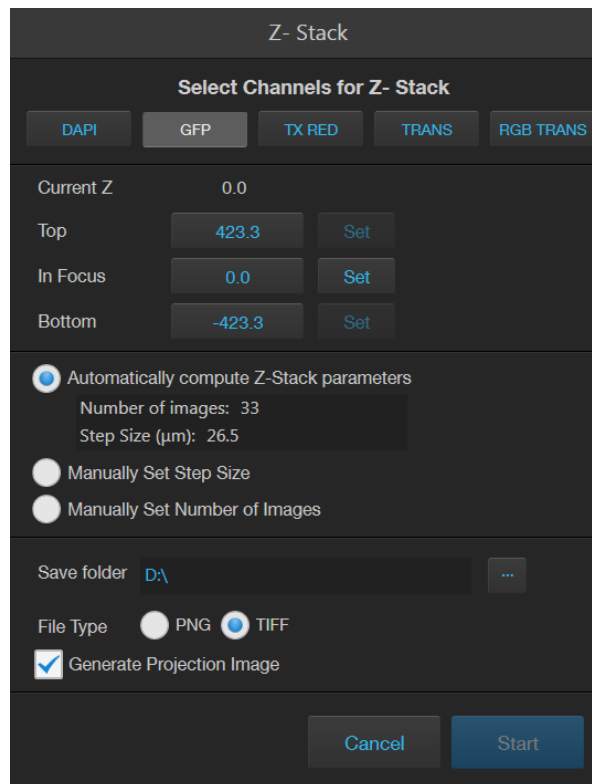
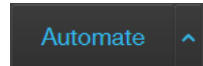
## 7. Capture Z-Stack

### Z-Stack tool

Z-Stack tool allows you to capture multiple images of a selected field at different focal planes along the Z-axis and combine them to generate a final image with a greater depth of field than any of the individual source images.

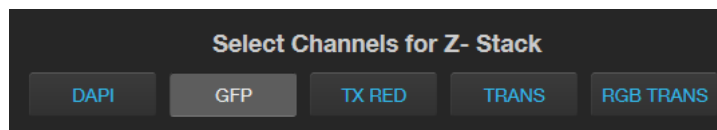
### Capture Z-stack images

1. On the Capture tab, click **Automate**, then select **Z Stack** to open the Z-Stack tool.



The Z-Stack tool dialog box is titled "Z- Stack". It features a section "Select Channels for Z- Stack" with five buttons: DAPI, GFP, TX RED, TRANS, and RGB TRANS. Below this, the "Current Z" is set to 0.0. There are three rows for Z-axis parameters: "Top" with a value of 423.3 and a "Set" button; "In Focus" with a value of 0.0 and a "Set" button; and "Bottom" with a value of -423.3 and a "Set" button. Three radio buttons are present: "Automatically compute Z-Stack parameters" (selected), "Manually Set Step Size", and "Manually Set Number of Images". The "Automatically compute Z-Stack parameters" option shows "Number of images: 33" and "Step Size (μm): 26.5". Below these is a "Save folder" field with the path "D:\\" and a browse button "...". The "File Type" section has two radio buttons: "PNG" and "TIFF" (selected). A checkbox "Generate Projection Image" is checked. At the bottom are "Cancel" and "Start" buttons.

2. Select the **Channels for Z Stack** that you want to capture. You can select multiple channels. In this example, **GFP** channels is selected.



This dialog box shows the "Select Channels for Z- Stack" section with five buttons: DAPI, GFP, TX RED, TRANS, and RGB TRANS. The GFP button is highlighted with a blue border, indicating it is selected.

3. If not already on, click the **Light** button to illuminate the sample, then adjust **brightness** and **focus** using the corresponding sliders on the Capture tab.  
The focus position you set in this step is the initial Current Z position.
4. To accept the **Current Z** as the **In Focus** position, click **Set** for In Focus.

Current Z	73.9	
Top	73.9	Set
In Focus	73.9	Set
Bottom	-35.3	Set

To change the **In Focus** position, move the **Coarse** and **Fine focus** sliders on the Capture tab to focus on the sample, then click **Set**.

5. Move the focus sliders up from the Current Z position to the desired **Top** position (upper boundary of the Z-Stack), then click **Set** for Top.
6. Move the focus sliders down from the Current Z position to the desired **Bottom** position (lower boundary of the Z-Stack), then click **Set** for Bottom.

Current Z	-50.2	
Top	100.9	Set
In Focus	73.9	Set
Bottom	-35.3	Set

---

**Note:** You can click the **Z number** button for the Top, In Focus, and Bottom positions to jump to those positions along the Z-axis.

---

7. Define the **Z-Stack parameters** to determine the number of “optical sections” captured in the Z-Stack image set:

- **Automatically compute Z-Stack Parameters:** Select to calculate the number of images and their Z-positions automatically based on the Z-Stack boundaries and the default Step Size.

<input checked="" type="radio"/>	Automatically compute Z-Stack parameters
	Number of images: 6
	Step Size (μm): 27.3

- **Manually Set Step Size:** Select to enter the **Step Size** (Z-distance in μm between focal planes) in the text box. The system calculates the number and Z-positions of the images based on your entry.

<input checked="" type="radio"/>	Manually Set Step Size
	Step Size (μm): 34.1
	Number of images: 5

- **Manually Set Number of Images:** Select to enter the **Number of Images** (Z-distance in μm between focal planes) in the text box. The system calculates Step Size and the Z positions of the images.

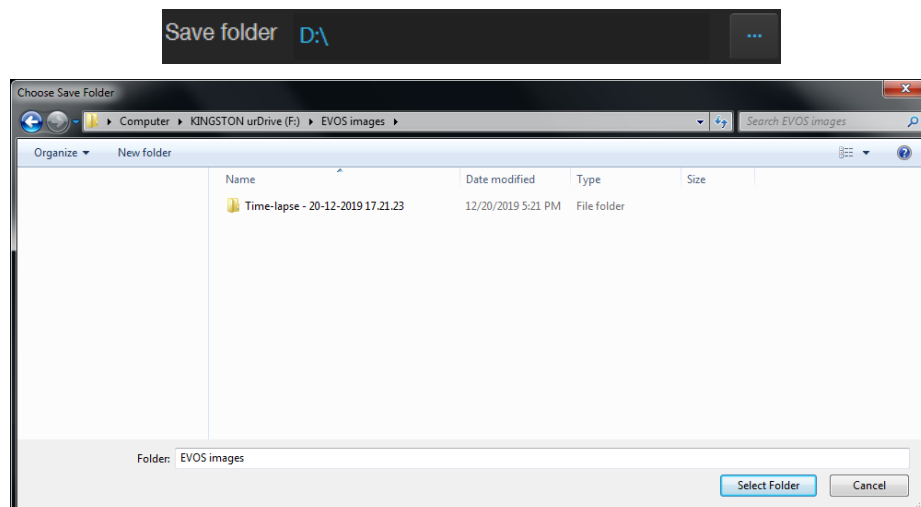
<input checked="" type="radio"/>	Manually Set Number of Images
	Number of images: 4
	Step Size (μm): 45.4

---

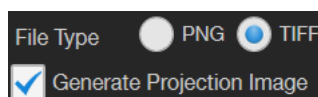
**Note:** You can take up to 100 images (“optical sections”) for a Z-Stack.

---

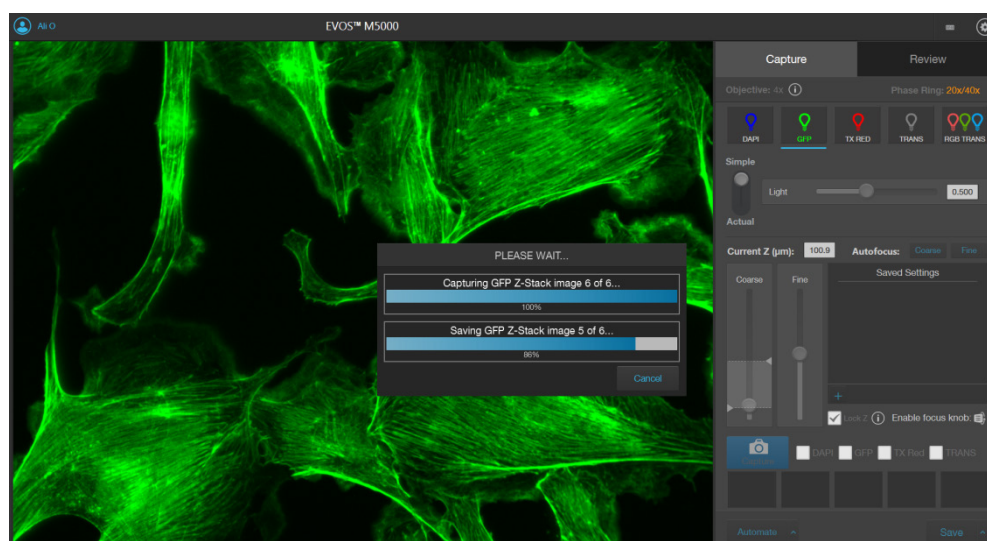
8. Click the **Browse** button to open Choose Save Folder dialog.



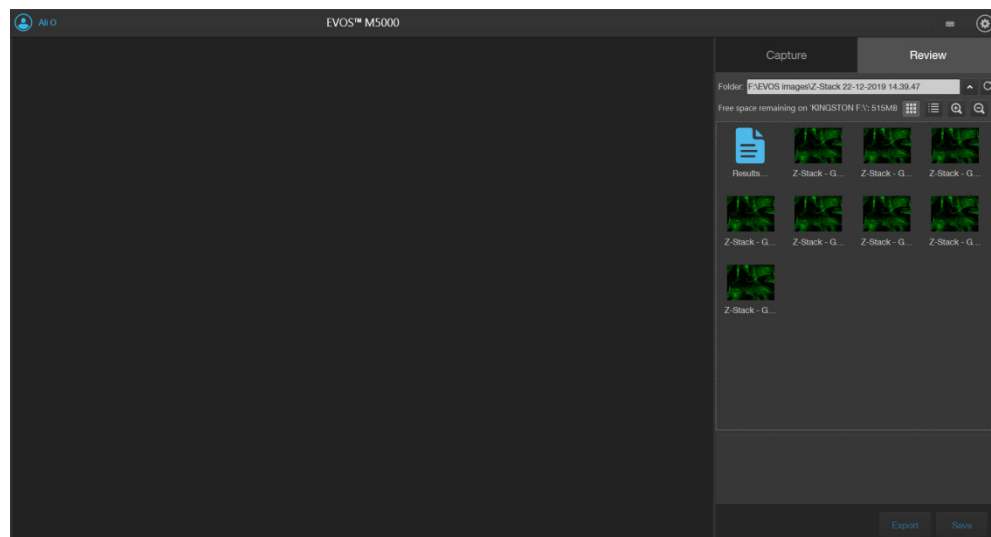
9. Go to the destination folder for the Z Stack images, then click **Select Folder**.
10. Select the **File Type**: PNG or TIFF.



11. Select **Generate Projection Image** to generate a projection image.  
When selected, the system uses the most in-focus pixels from images captured at different focal planes to generate a composite in-focus image. Each image in the Z-Stack is also separately saved.  
When unselected, the system only saves the individual Z-Stack images.
12. Click **Start** to capture the Z-Stack image sets based on your specifications.  
The system provides information about the progress of the Z Stack protocol.



13. When the Z-Stack routine is completed, the Review tab opens to the files captured in the routine.



## 8. Review saved images

The **Review tab** allows you to review still images, including those captured in Time Lapse routines and Z-Stacks. You can also use this tool to annotate the saved images, and to re-save or delete saved files. For the descriptions of the controls available in the Review tab, see page 86.

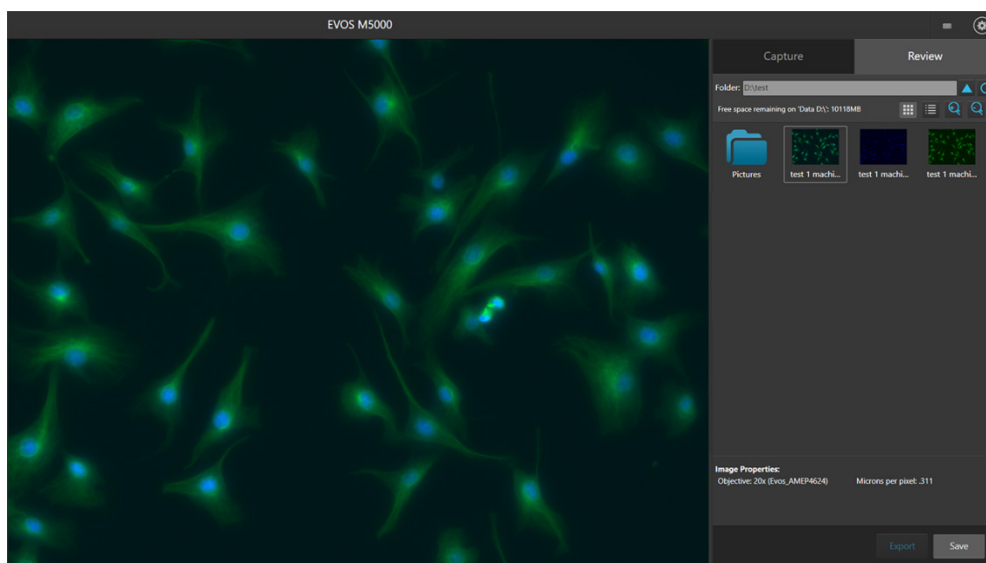
**Note:** You can view and analyze captured images that are saved to your Connect™ account with the EVOS™ Image Analysis application.

For more information on the EVOS™ Image Analysis application, see “Appendix C: EVOS™ Image Analysis”, page 104.

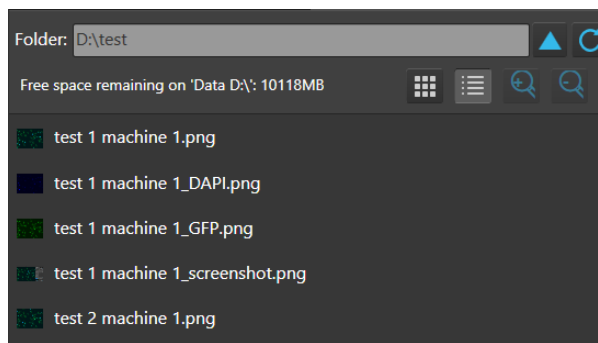
### Review images

#### Review images

1. Click the **Review** tab.
2. The **folder/image preview** area displays thumbnail images for all viewable files in the selected directory (the top-level USB directory is selected by default). If there are no viewable files in the directory, the preview area will be empty.

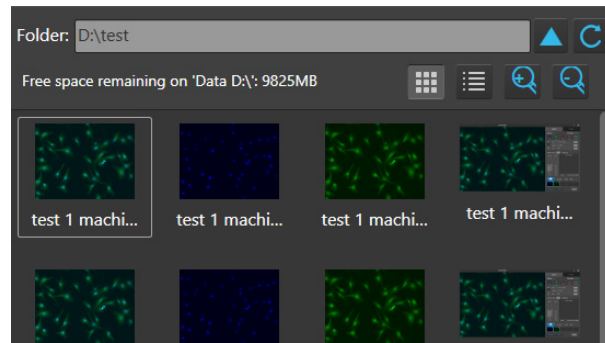


3. To display the folder/image preview in list format, click the **List view** button.

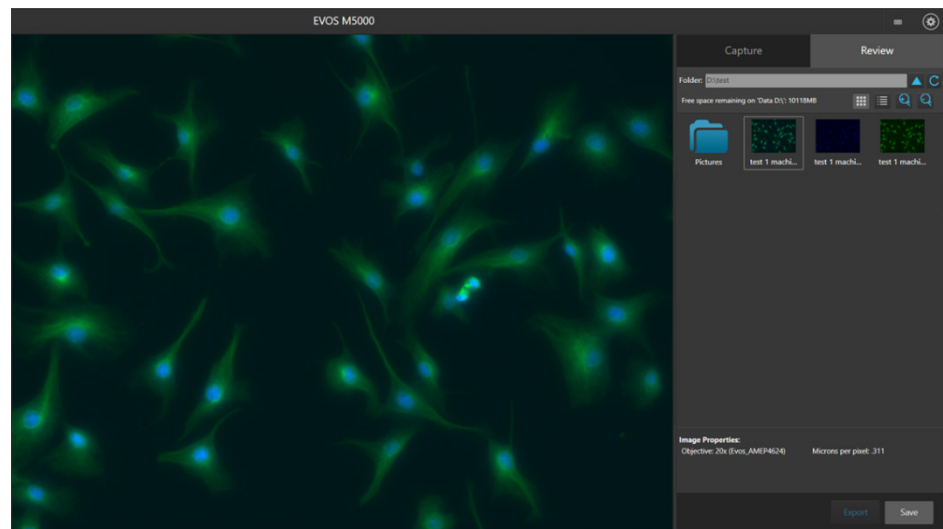




- To display the folder/image preview in grid format, click the **Grid view** button.



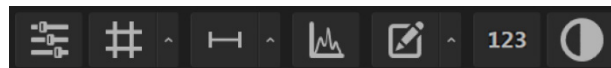
- If needed, use the **scroll bar** to search the preview list for the desired image file.
- Click the **image** to select it. The selected image is indicated with a box around it and the viewing area displays the selected image.



## Change display settings for saved images

Display settings and analysis tools allow you to change image display settings, and to analyze and annotate captured images.

Hover the pointer over the Viewing area to reveal the buttons for Display Settings and Analysis Tools.



The display tools function in the same way in the Capture and Review tabs:

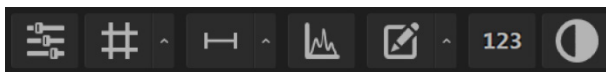
- Click the **Image Display Settings** button to expand the controls for image display parameters (brightness, contrast, gamma). For detailed instructions, see page 25.
- Click the **Grid** button to superimpose a grid over the Viewing area. For instructions on how to change the Grid settings, see page 26.
- Click the **Scale Bar** button to superimpose a scale bar over the Viewing area. For instruction on how to change scale bar settings, see page 26.






## Analyze saved images

### Analyze and annotate saved images

Hover the pointer over the Viewing area to reveal the buttons for Display Settings and Analysis Tools.



The analysis and annotations tools function in the same way in the Capture and Review tabs:



- Click the **Histogram** button to open the Intensity Histogram plot. The Intensity Histogram plot shows the Pixel count vs. Intensity data of the image displayed in the Viewing area (see page 27). 
- Click the **Measurement and Annotations Tools** button (the arrow on the Show Measurements and Annotations split button) to open the measurement and annotations tools. For instructions on how to add and show measurements and annotations to your images, see page 28. 
- Click the **Analysis** button to display the **Auto Count**, **Manual Count**, and **Confluence** options in the Review tab.
  - For instructions on how to perform an Auto Count, see page 30.
  - For instructions on how to perform a Manual Count, see page 34.
  - For instructions on how to measure the confluence of your cell culture sample, see page 36.

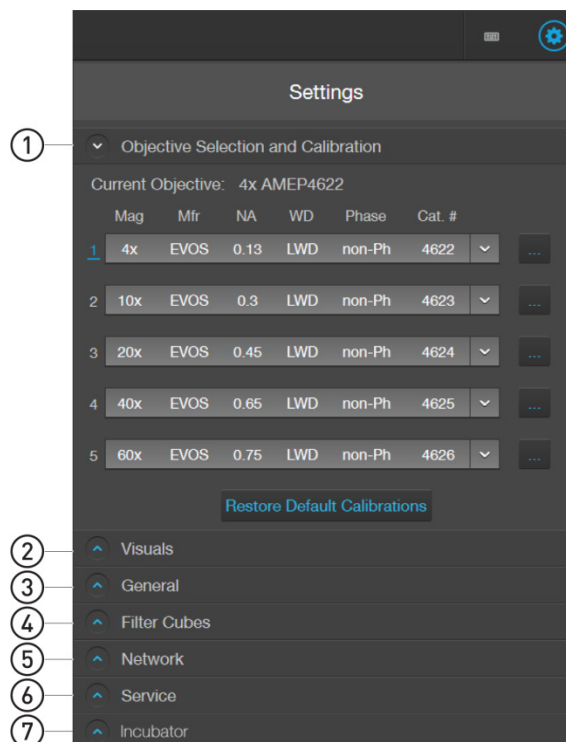
## 9. Adjust instrument settings

### Overview

#### Settings tab

The **Settings** tab allows you to assign objectives to the objective turret, to calibrate objective magnification, to set image and general instrument options, add and remove light cubes, and to connect to a Wi-Fi network and to map network drives.

- To open the settings tab, click the **Settings** button. 
- To expand or collapse any of the controls, click the corresponding arrow. 



- ① **Objective Selection and Calibration:** Set up and calibrate objectives and assign objective profiles (page 62).
- ② **Visuals:** Adjust White Balance for the color camera (page 64) and set saturated pixel options (page 65)
- ③ **General:** Set general instrument options, such as savings options for TIFF files, focus wheel action, Saved Settings options, and Capture All Channels options (page 66)
- ④ **Filter Cubes:** Add or remove EVOS™ LED light cubes and to assign pseudocolors for installed light cubes (page 74).
- ⑤ **Network:** Configure network settings (page 67)
- ⑥ **Service:** Copy error logs, set date and time, and update software and firmware from the cloud or the USB flash drive (page 70).
- ⑦ **Incubator:** Configure the gas inputs, calibrate the oxygen sensor, and set the temperature offsets for the EVOS™ Onstage Incubator (page 91).

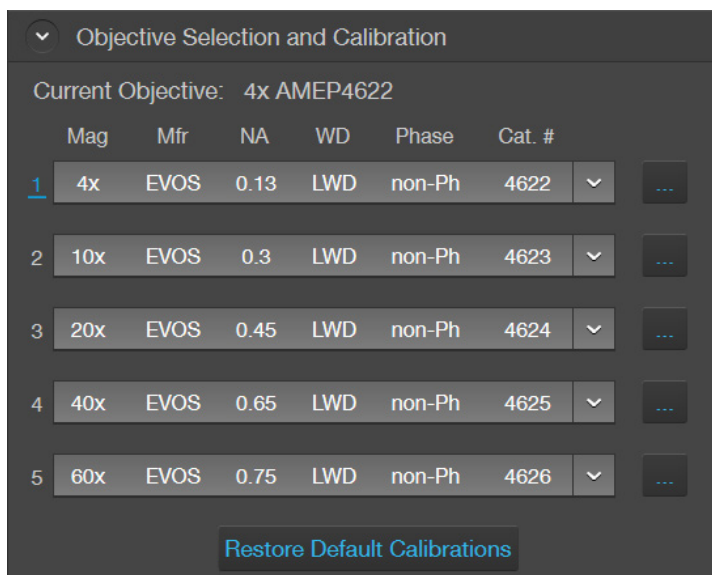
The Incubator panel is only visible when an EVOS™ Onstage Incubator is connected to your EVOS™ M5000 Imaging System.

## Adjust objective settings

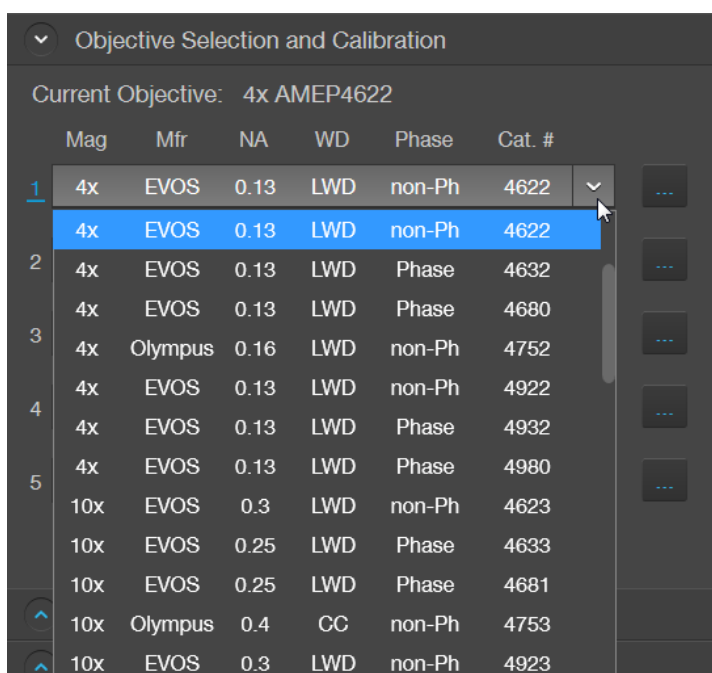
### Assign objectives

After adding a new objective to the objective turret or replacing an older objective, assign the new objective to the appropriate turret position. For instructions on how to remove an objective, see “Change the objectives”, page 75.

1. Go to the **Settings ▶ Objective Selection and Calibration** tab.




2. Find the newly installed objective in the **Objectives** list for the corresponding turret position.

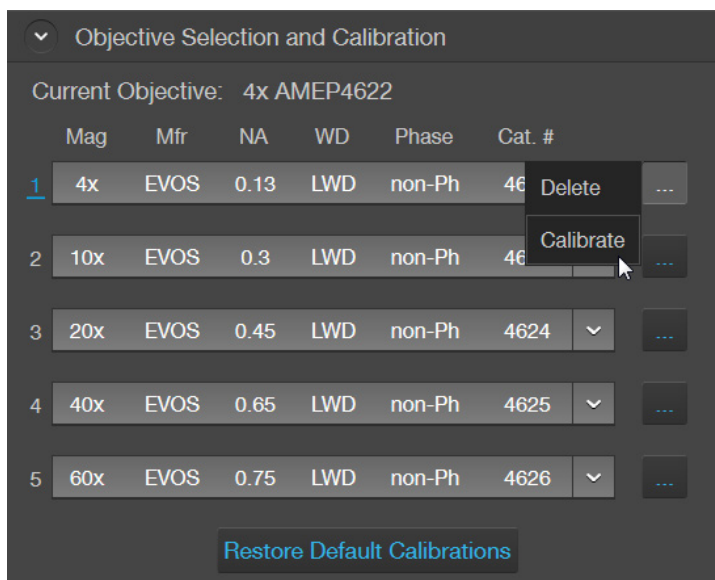


3. Click **Done**.

**IMPORTANT!** For best results, calibrate the newly installed objective before using it in your experiments (see “Calibrate objective magnification”, page 63).

## Calibrate objective magnification

1. Go to the **Settings ► Objective Selection and Calibration**, click the **Action** button  next to the newly installed objective, then select **Calibrate** to launch the Objective Calibration tool.

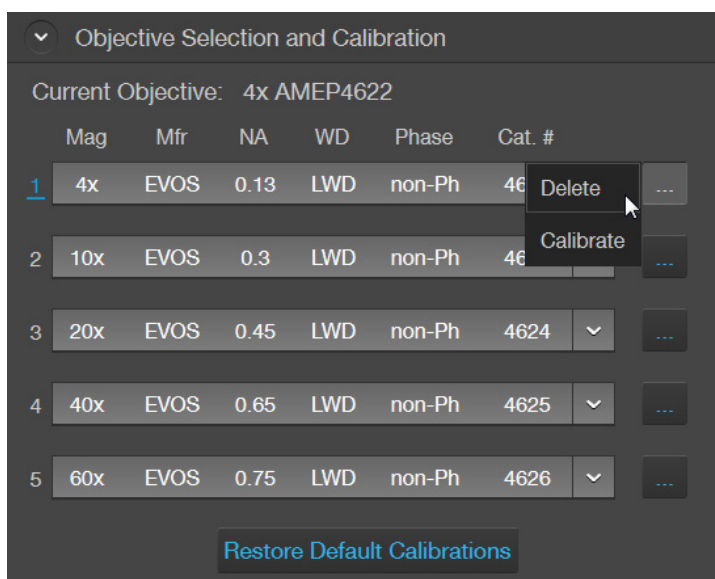


2. Mount the EVOS™ Calibration Slide in the vessel holder, select the objective you want to calibrate, then click **Calibrate Objective**.
3. Follow the onscreen instructions to calibrate the objective magnification for the selected objective. For more information, see “Calibrate the objectives”, page 76.

## Delete objectives

After removing an objective from the objective turret, unassign the objective from the corresponding turret position.

1. Go to the **Settings ► Objective Selection and Calibration**, click the **Action** button  next to the turret position from which you have removed the objective, then select **Delete**.

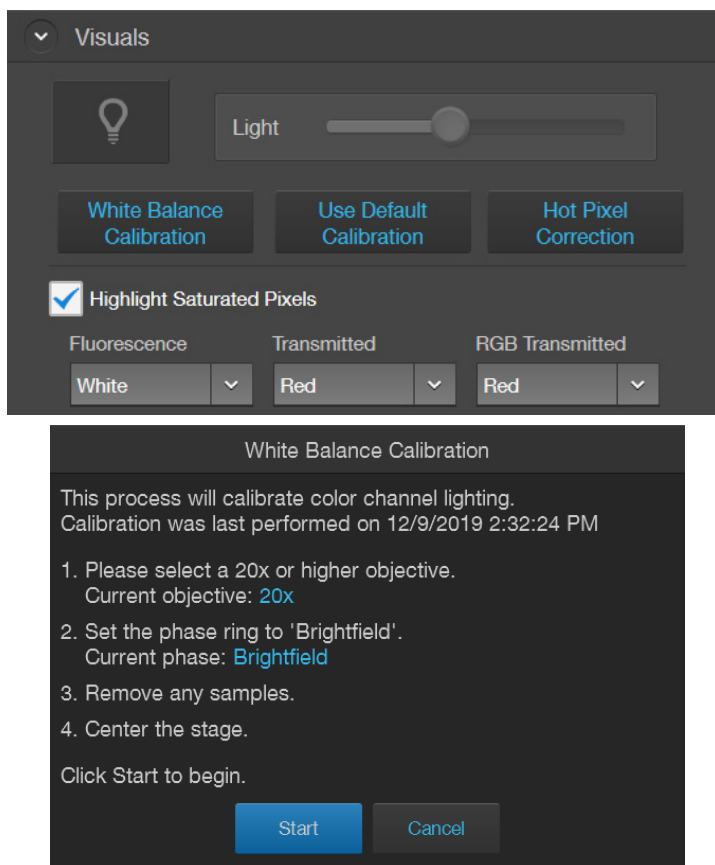


# Calibrate white balance

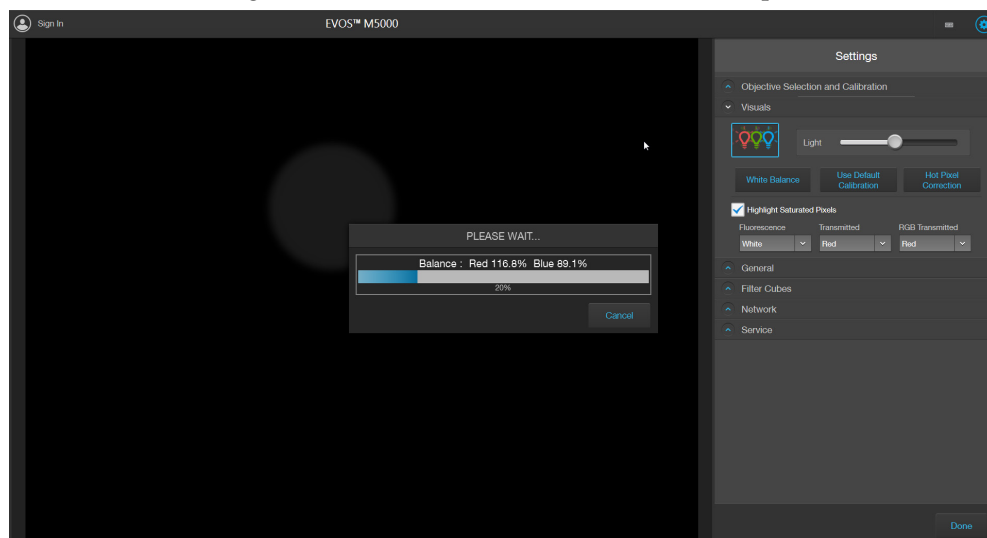
## Calibrate white balance

Calibrate White Balance calibrates color channel lighting.

1. Go to the **Settings ► Visuals** tab, then click **White Balance Calibration** to open the White Balance Calibration tool.



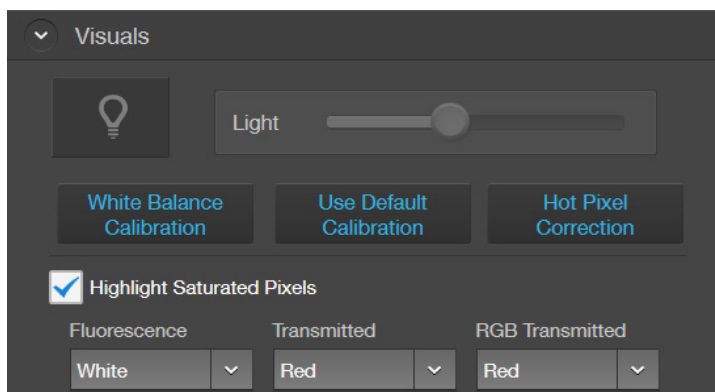
2. Select a 20X or higher objective, then set the phase ring to **Brightfield** using the Phase annuli selector (page 7).
3. Remove any samples from the X-Y stage, then center the stage using the stage positioning knobs (page 7).
4. Click **Start** to begin the automatic white balance calibration procedure.



## Set saturated pixel options

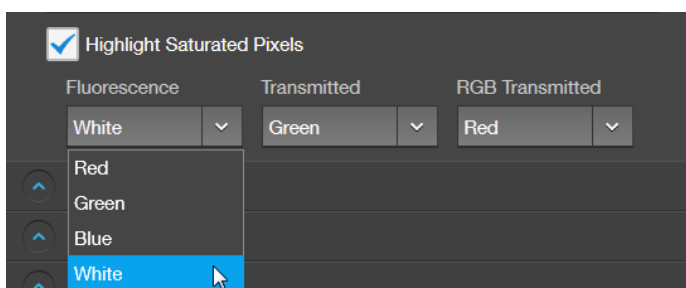
**Highlight saturated pixels** Highlight saturated pixels function displays overexposed pixels on an image with the user-defined color, which provides a visual aid for optimal illumination when adjusting the brightness settings.

1. Go to the **Settings ► Visuals** tab, then click the **Highlight Saturated Pixels** checkbox.



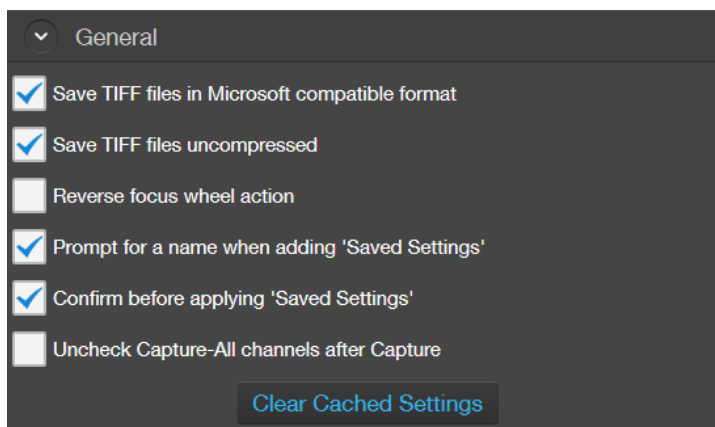
2. To assign a specific color to the saturated pixels in a channel, select the desired color from the corresponding channel dropdown (**Fluorescence**, **Transmitted**, or **RGB Transmitted**).

Available colors for saturated pixels are **Red**, **Green**, **Blue**, and **White**.



# General settings

## General settings options



### Define saving options for TIFF files

1. To save **TIFF files in a Microsoft compatible format**, select the corresponding option.
2. To save **TIFF files uncompressed**, select the corresponding option.
3. When finished with your selections, click **Done**.

### Reverse focus wheel action

1. Select **Reverse focus wheel action**.
2. Click **Done**.

### Define savings settings for “Saved Settings”

1. To set saving options for “Saved Settings” in the Capture tab, go to the **Settings ▶ General** tab.
2. If you want to assign specific names to each saved setting, select **Prompt for a name when adding ‘Saved Settings’**.
3. If you want to see a confirmation dialog before applying saved Settings, select **Confirm before applying ‘Saved Settings’**.
4. When finished with your selections, click **Done**.

### Adjust “Capture All” checkbox behavior

By default, channel checkboxes stay checked after image capture when you select multiple channels for simultaneous capture (see “Capture images in multiple channels”, page 24). You can reverse this behavior in the General settings.



1. To automatically unselect channel checkboxes, go to the **Settings ▶ General** tab.
2. Click **Uncheck Capture-All channels after Capture**.
3. Click **Done**.

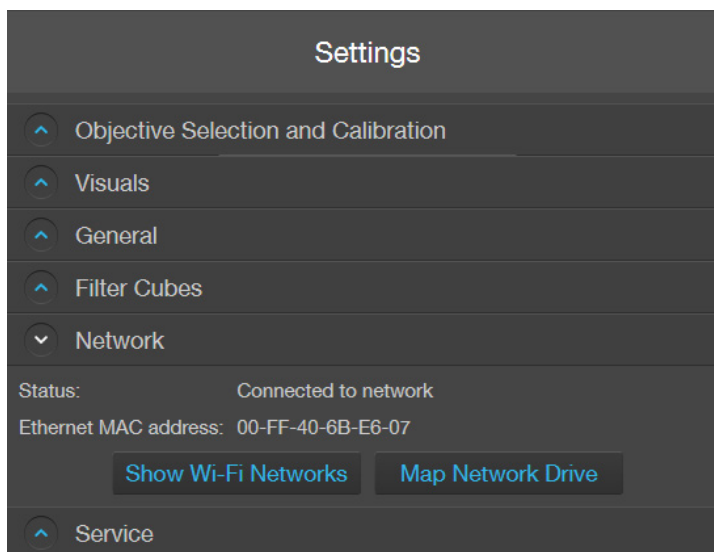


## Configure network settings

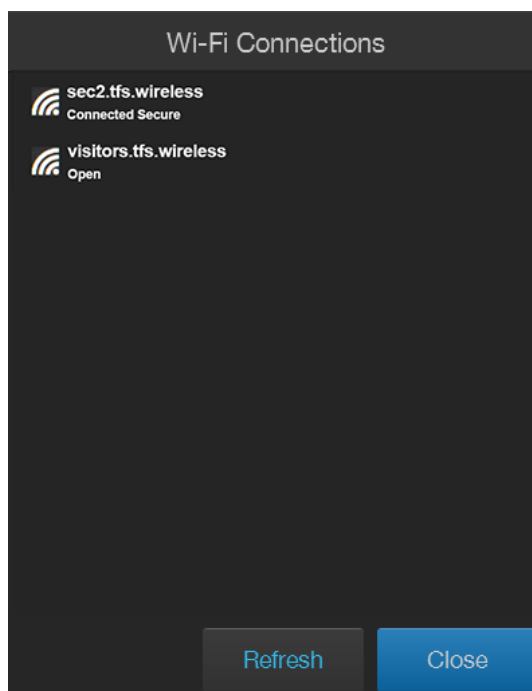
You can connect the EVOS™ M5000 Imaging System to a network via an Ethernet cable or Wi-Fi adaptor and save captured images directly to shared folders on the network. You can also connect to your Connect™ account, Thermo Fisher's cloud-based platform, to store your image files and analyze them with the EVOS™ Image Analysis application.

### Connect to a Wi-Fi network

1. Click the **Settings** button to open the Settings tab.
2. Select **Network** to show the Network options.

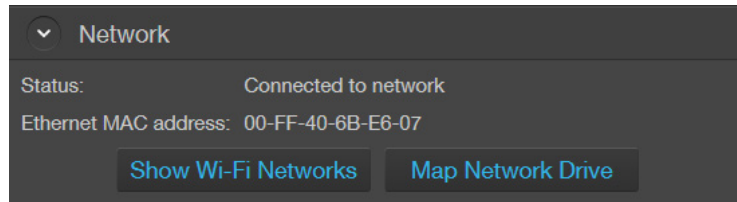


3. Click **Show Wi-Fi Networks**, then select the network you want to join.

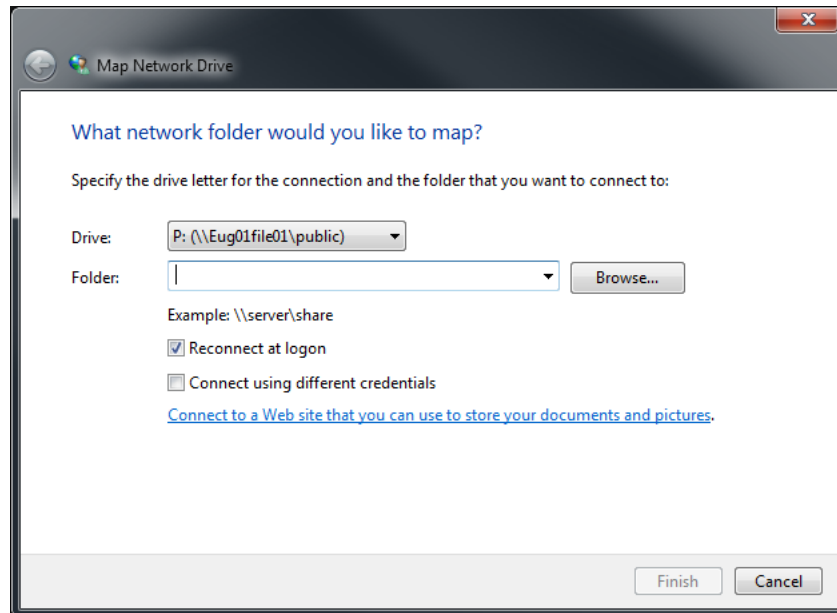


4. Click **Close**.

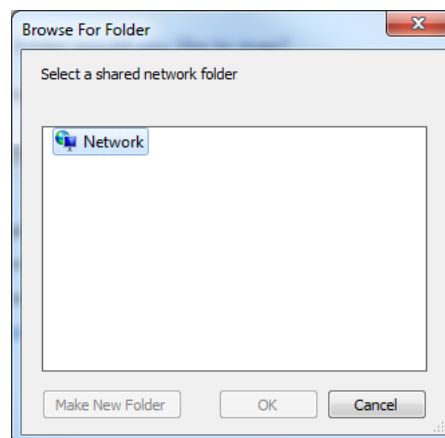
- Map network drive**
1. Go to the **Settings ▶ Network** tab, then click **Map Network Drive**.



Map Network Drive dialog opens.



2. Select the drive you want to map from the **Drive** menu.
3. Click **Browse** to open the Browse for Folder dialog.



4. Navigate to the folder you want to map, then **OK**.
5. If you want the reconnect to the selected drive and folder when you turn on the instrument, check **Reconnect at logon**.

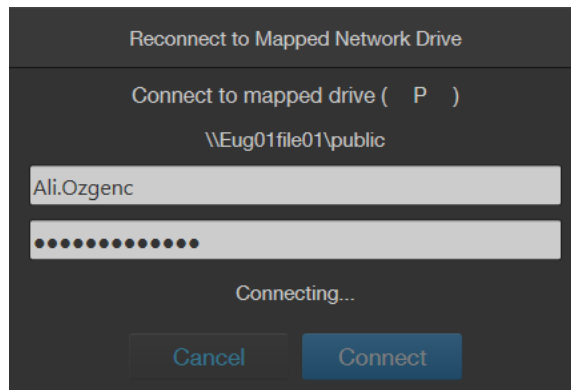
☒ **Reconnect at logon**

6. If you want to reconnect as a different user, check **Connect using different credentials**. ☐ Connect using different credentials
7. To connect to a Web site that you can use to store your documents and pictures, click the hyperlink, then navigate to the desired Web site.  
[Connect to a Web site that you can use to store your documents and pictures.](#)
8. After you have mapped the desired network drive and folder or connected to the website, click **Finish**.

---

**Note:** When **Reconnect at logon** is checked and a successful connection is established, the system will attempt a reconnection to any previously connected networks or mapped drives everytime the instrument is turned on until you manually disconnect from the connected networks or mapped drives.

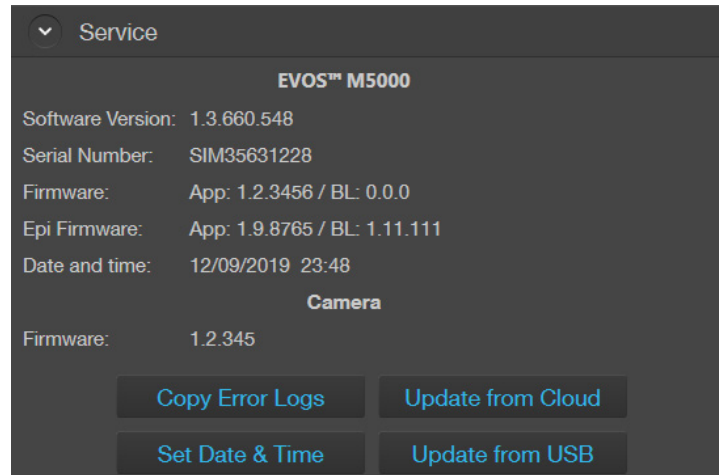
If no connection can be successfully established to the previously mapped drive during start up, the instrument displays the "Reconnect to Mapped Network Drive" dialog.



# Service

Service tab displays the current software and firmware versions of the system and allows you to copy error logs, set date and time, and update software and firmware from the cloud or the USB flash drive.

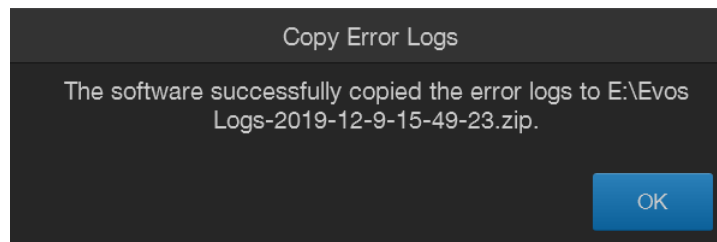
To view the Service tab, click the **Settings** button, then select **Service**.



## Copy error logs

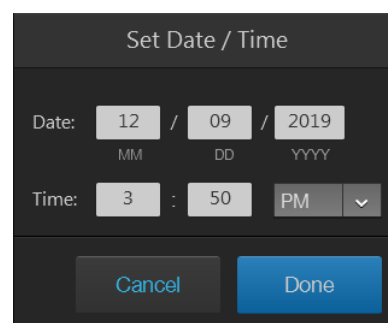
1. On the **Service** tab, click **Copy Error Logs**.

The instrument automatically saves a copy of the error logs in the previously mapped drive.



## Set date and time

1. On the **Service** tab, click **Set Date & Time**.



2. Enter the **Date** and **Time**. For Time, select **24 hour** format or **AM** or **PM** for 12 hour format.
3. Click **Done** when finished.

---

**Note:** You cannot set the time more than 24 hours in the past.

---

## Update from Cloud

1. Restart your EVOS™ M5000 instrument before an update to optimize the update process.
2. To update the instrument software and firmware from the Cloud, sign in to your Connect™ account (page 16).
3. On the **Service** tab, click **Update from Cloud**.  
The instrument automatically searches your Connect™ account, for the update.
4. If a new update is available and the appropriate files are detected, the software pauses for a few moments and then restarts.
5. When a Windows™ OS Setup dialog box opens, click **Next** to proceed with software update.
6. The screen displays the update progress. When the update is complete, the system restarts and the M5000 software automatically boots up again.

## Update from USB

1. Download the latest software from [thermofisher.com/evos](http://thermofisher.com/evos) to a fresh USB flash drive with at least 200 MB available space.
2. Restart your EVOS™ M5000 instrument before an update to optimize the update process.
3. Plug the USB flash drive containing the newest software update into the EVOS™ M5000 instrument.
4. On the **Service** tab, click **Update from USB**. A windows dialog opens.
5. Locate the update file from your USB Drive, then click **Open**.
6. When a Windows™ OS Setup dialog box opens, click **Next** to proceed with software update.
7. The screen displays the update progress. When the update is complete, the system restarts and the M5000 software automatically boots up again.

## 10. Instrument care and maintenance

---

### General care

- When cleaning optical elements, use only optical-grade materials to avoid scratching soft lens coatings.
- Use the appropriate cleaning solutions for each component, as indicated in the Decontamination Procedures below.
- If liquid spills on the instrument, turn off the power immediately and wipe dry.
- Do not exchange objectives between instruments unless you know that the components have been approved and recommended by Thermo Fisher Scientific.
- After using, cover the instrument with the supplied dust cover.

---

**Note:** Always use the correct power supply. The power adaptor specifications appear on the serial number label (above ports and plugs on the rear of the instrument) and in the Specifications. Damage due to an incompatible power adaptor is not covered by warranty.

---



**CAUTION!** Never disassemble or service the instrument yourself. Do not remove any covers or parts that require the use of a tool to obtain access to moving parts. Operators must be trained before being allowed to perform the hazardous operation. Unauthorized repairs may damage the instrument or alter its functionality, which may void your warranty. Contact your local EVOS™ distributor to arrange for service.

---

**IMPORTANT!** If you have any doubt about the compatibility of decontamination or cleaning agents with parts of the equipment or with material contained in it, contact Technical Support (page 116) or your local EVOS™ distributor for information.

---

### Objective lens care

Clean each objective periodically or when necessary with an optical-grade swab and a pre-moistened lens wipe (or lens paper moistened with lens cleaning solution). To avoid scratching the soft lens coatings, use only optical-grade cleaning materials and do not rub the lens.

---

**Note:** To protect all optical components of the instrument, use the dust cover when the instrument is not in use.

---

## Stage care

- Clean the X-Y stage as needed with paper towels or Kimwipes™ tissues dampened with 70% ethanol.
- When moving the EVOS™ M5000 Imaging System, be sure to lock the X-Y stage using the Shipping Restraint as shown on page 75 to prevent the stage from sliding.

## Decontamination procedures

In case hazardous material is spilt onto or into the components of the EVOS™ M5000 Imaging System, follow the decontamination procedure as described below.

1. Turn power OFF.
2. Clean the LCD display.
  - a. Use a soft, dry, lint-free cloth to wipe off any dust from the screen.
  - b. Clean the LCD display with a non-alcohol based cleaner made for flat-panel displays.

---

**IMPORTANT!** Do not spray cleaning fluid directly onto the screen, as it may drip into the display.

---

3. Lightly wipe working surfaces of the EVOS™ M5000 Imaging System (stage top, objective turret, housing, etc.) with paper towels or Kimwipes™ tissues dampened with 70% ethanol or 4,000 ppm hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>).

---

**IMPORTANT!** Do not allow decontamination solution to get into lubricated areas, such as the stage roller bearings, or any points of rotation such as stage motors, condenser wheel, etc.

Do not soak any surface in decontamination solution.

NEVER spray liquid anywhere on the EVOS™ M5000 Imaging System.

Always wipe surfaces with dampened paper towels instead.

---

## Change EVOS™ LED light cubes

To customize your EVOS™ M5000 Imaging System, you can add and remove LED light cubes to fit the instrument's functionality to your own specific research needs. Each LED light cube is coded to allow the EVOS™ FL Imaging System to automatically recognize it in any position.

For a complete list of available light cubes and to inquire about custom light cubes, go to [thermofisher.com/evos](http://thermofisher.com/evos) or contact Technical Support (page 116).

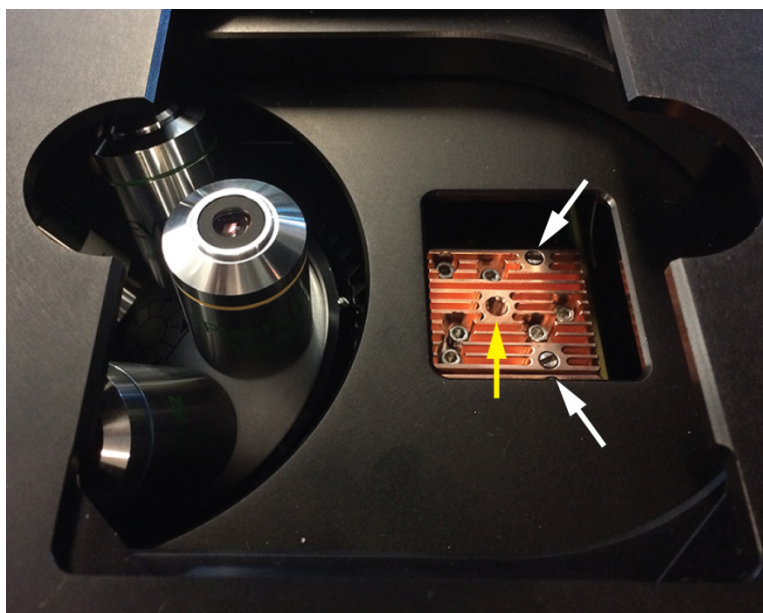


**WARNING! UV LIGHT HAZARD!** The EVOS™ M5000 Imaging System uses a Class 3B ultraviolet LED for the DAPI channel. **Before changing the LED light cubes, ensure that the excitation light is turned OFF** (the instrument is **not** in the Live mode).

---

### Change LED light cube

1. Select the position of the **Light cube** you want to change.
2. Move the stage back to allow access to the light cube, which is centered under the back of the stage.



3. Use the light cube tool to loosen the two slotted screws (white arrows) that are flush with the ridges on the light cube.
4. Screw the threaded end of the light cube tool into the hole in the center of the light cube (yellow arrow).
5. Use the tool to tilt the light cube slightly toward you and lift out gently, and then remove tool from cube.
6. Attach the tool to the new light cube and lower the cube into position so that the electronic connection aligns properly (facing the back of the microscope) and the cube sits squarely in place with the label facing toward the front.
7. Unscrew the light cube tool from the cube, then use it to gently tighten the two slotted screws flush with the ridges on the light cube.

---

**IMPORTANT!** If the screws are not flush with the top of the light cube they may catch on the stage while moving and damage the system.

---

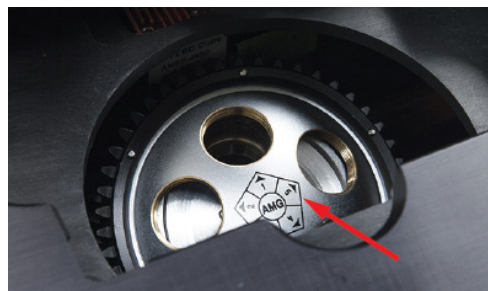


## Change the objectives

To customize your EVOS™ M5000 Imaging System, you can add and remove objectives to fit the instrument's functionality to your own specific research needs.

### Procedure for objective change

1. Remove the objective you want to replace from the objective turret. You may need to move the stage so that the objectives are accessible. Note the indicated position (1–5) of the removed objective on the turret (red arrow).



2. Screw the new objective into the open position in the objective turret. Note the part number of the objective and the turret position. In this example, the new objective is installed into the turret position 5.



3. Go to the **Settings ▶ Objective Selection and Calibration** tab, then find the objective in the **Objectives** list that matches the newly installed objective.

Objective Selection and Calibration							
Current Objective: 60x AMEP4626							
	Mag	Mfr	NA	WD	Phase	Cat. #	
1	4x	EVOS	0.13	LWD	non-Ph	4622	...
2	10x	EVOS	0.3	LWD	non-Ph	4623	...
3	20x	EVOS	0.45	LWD	non-Ph	4624	...
4	40x	EVOS	0.65	LWD	non-Ph	4625	...
5	60x	EVOS	0.75	LWD	non-Ph	4626	...
	40x	Olympus	0.95	CC	non-Ph	4754	
	40x	EVOS	0.65	LWD	non-Ph	4925	
	40x	EVOS	0.65	LWD	Phase	4935	
	40x	EVOS	0.65	LWD	Phase	4983	
	50x	EVOS	0.95	CC	non-Ph	OP050	
	60x	EVOS	0.75	LWD	non-Ph	4626	
	60x	Olympus	1.42	CC	non-Ph	4694	
	60x	EVOS	0.75	LWD	non-Ph	4926	
	100x	EVOS	1.28	CC	non-Ph	4696	
	100x	Olympus	1.4	CC	non-Ph	4733	
	100x	EVOS	1.25	CC	non-Ph	OP100	

**Note:** Calibrate the newly installed objective before using it (page 76).

## Calibrate the objectives

**Calibrate objective magnification** allows the calibration of the field of view, parfocality, and parcentration parameters of the selected objective. When calibrated, parfocality ensures that the sample stays in focus when the objective is changed, and parcentration ensures that an object in the center of the field of view stay in the center of the field no matter which objective is being used.

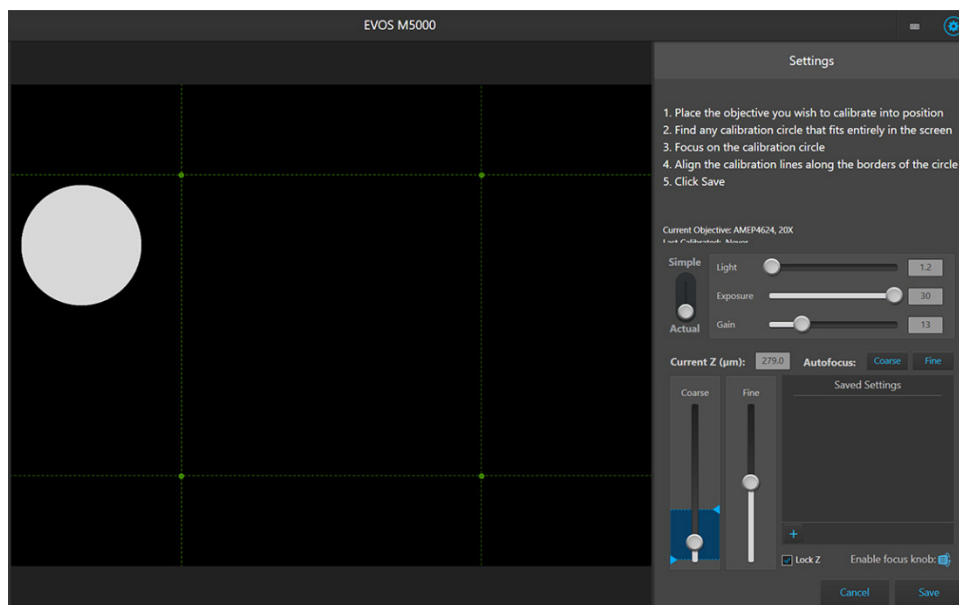
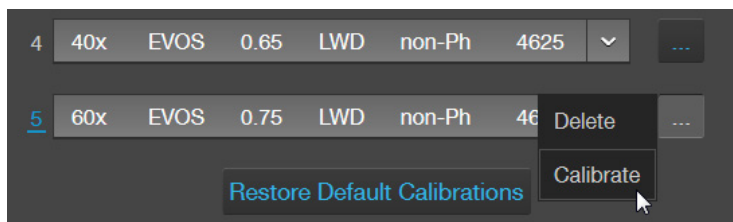
**Note:** The pre-installed objectives supplied with the EVOS™ M5000 Imaging System have been pre-calibrated. You do not need to calibrate them again unless they are reinstalled after removal from the instrument.

### Calibrate objective magnification

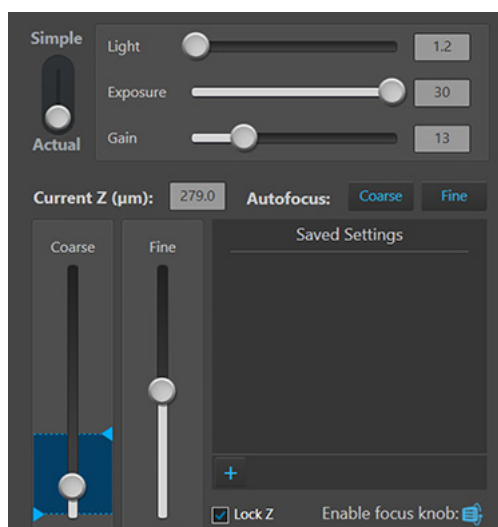
Calibration procedure requires the use of the EVOS™ M5000 calibration slide supplied with the EVOS™ M5000 Imaging System (Cat. No. AMEP4720). Total time required objective calibration is about 5 minutes.

**Note:** For best parfocality and parcentration, calibrate the installed objectives one after the other without removing the calibration slide.

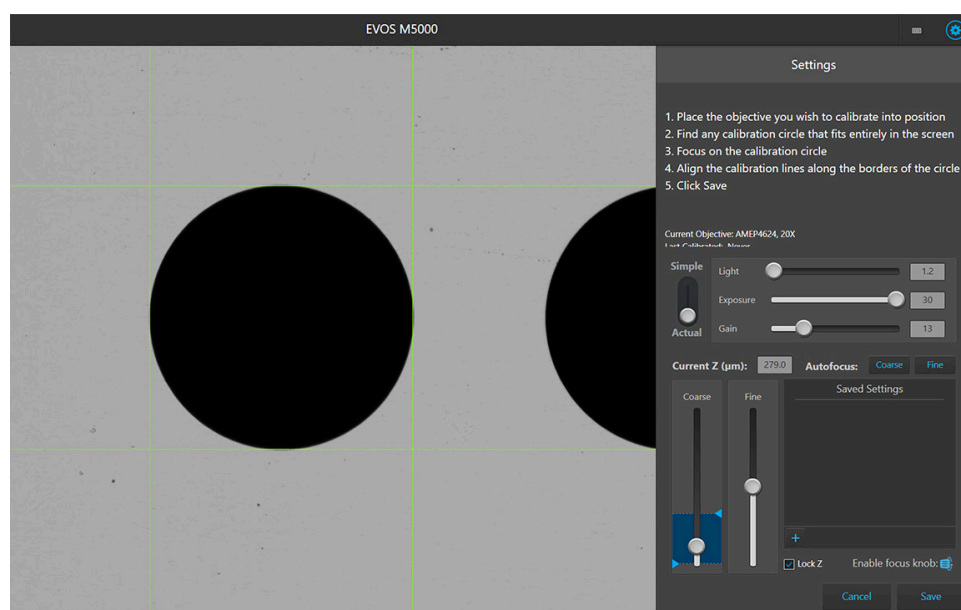
1. Mount the EVOS™ Calibration Slide **face down** in the vessel holder for slides (you can use either of the two slots).
2. Select the objective to calibrate using the Objective selection wheel (page 7).
3. Go to the **Settings ► Objective Selection and Calibration**, click the **Action** button for the newly installed objective, then select **Calibrate** to launch the Objective Calibration tool.



4. Find a calibration circle that fits entirely in the field of view.
5. Focus on the calibration circle using the focus controls.



6. Click and drag the green calibration lines to align them along the outer borders of the calibration circle.



7. When finished, click **Save** to complete the calibration. Repeat the calibration process for each additional objective to be calibrated.
8. When finished calibrating all of the installed objectives, click **Done**.

# Appendix A: Troubleshooting

**Note:** For additional technical support, contact your local EVOS™ distributor. If you do not have your distributor information, visit [thermofisher.com/evos](http://thermofisher.com/evos) or contact Technical Support (page 116).

## Image quality issues

Problem	Possible solutions
Image is too dim (at higher magnifications)	Remove condenser slider, if one is in place.
Specks, dots, or blurs on image	Follow instructions under “Objective lens care” (page 72) to clean objectives.
Uneven focus across screen	<ul style="list-style-type: none"><li>• Position the sample so that it lies flat on the stage; ensure that the sample’s thickness is even.</li><li>• Be sure vessel holder is mounted flat with respect to stage.</li></ul>
Difficulty focusing on coverslipped sample on standard slide	Place the slide so the coverslip is facing up (long working-distance objectives require a thick optical substrate, and image best through 1.0–1.5 mm of glass or plastic).
Image display is black	<ul style="list-style-type: none"><li>• Click the Power button (onscreen).</li><li>• Center sample over objective.</li><li>• Verify power supply is connected and power switch is on.</li></ul>
Image display is red, or red patches cover parts of the screen	<ul style="list-style-type: none"><li>• Dim the illumination until the red highlights disappear to get the maximum level of brightness without any overexposed areas.</li><li>• Disable the “Highlight Saturated Pixels” option in the <b>Settings</b> tab.</li></ul>

## Software interface issues

**Note:** We recommend keeping the EVOS™ M5000 Imaging System up to date with the latest software.

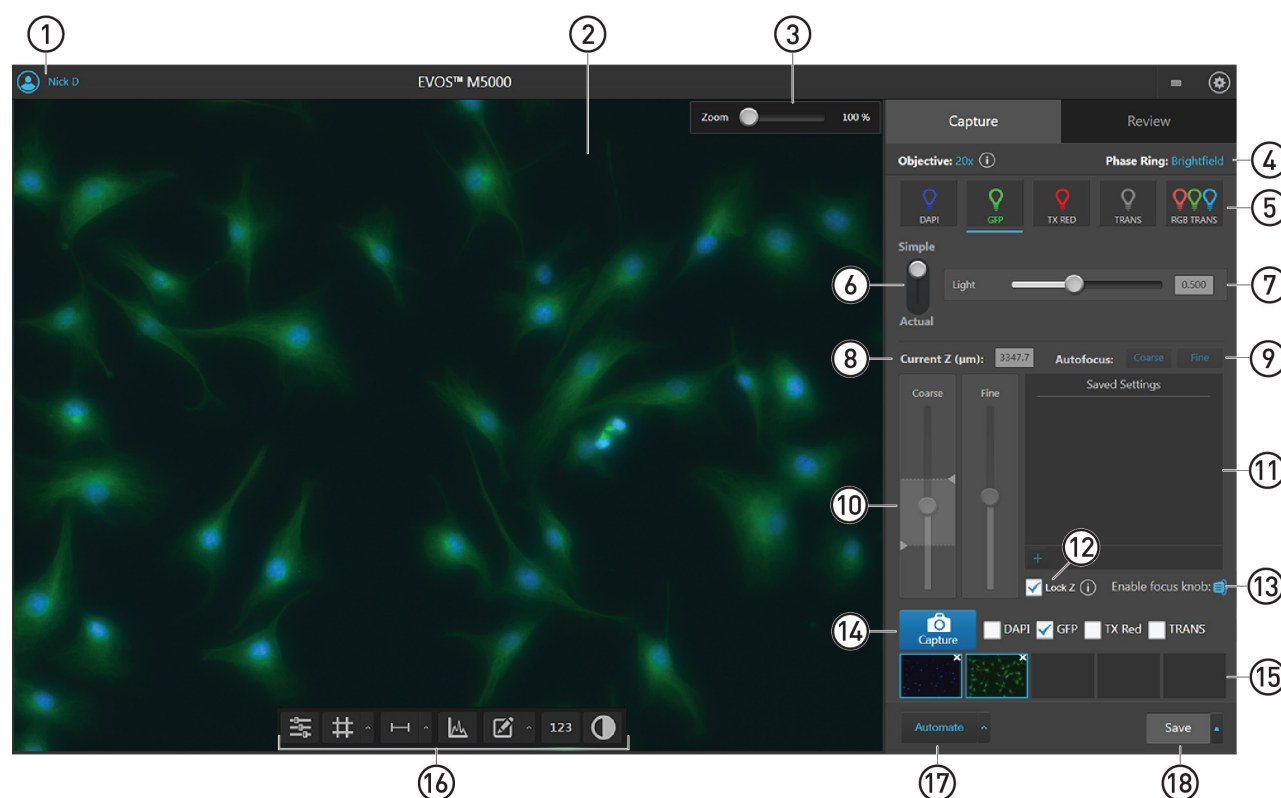
Problem	Possible solutions
Image does not respond to changes in focus or stage position	Click the <b>Light</b> button to return to live observation.
Some of the software controls are not available	The controls available on the EVOS™ M5000 Imaging System are contextual; only the controls relevant for the chosen task will be available.
Save button does not respond when clicked	Click capture first; it is only possible to save an image that is captured.
Unable to connect to network	<ul style="list-style-type: none"><li>• Verify physical cable connections; confirm the Ethernet jack is active (for Ethernet connection).</li><li>• Verify that the USB Wi-Fi adaptor for wireless network connection is installed into one of the USB ports in the back (for wireless connection).</li><li>• Contact your network administrator to resolve any network issues.</li></ul>

## Mechanical issues

Problem	Possible solutions
X-Y stage does not move	Remove shipping restraint and loosen the X- and Y-axis brakes.
Filter Cube Axis does not move	Remove shipping restraint.
Vessel does not sit securely on moving stage	Use the correct vessel holder for the application (visit <a href="https://www.thermofisher.com/evos">thermofisher.com/evos</a> ).

# Appendix B: Graphical user interface (GUI)


## Capture tab



① **Sign In/User:** Allows you to sign in to and sign out of your Thermo Fisher Connect™ account.

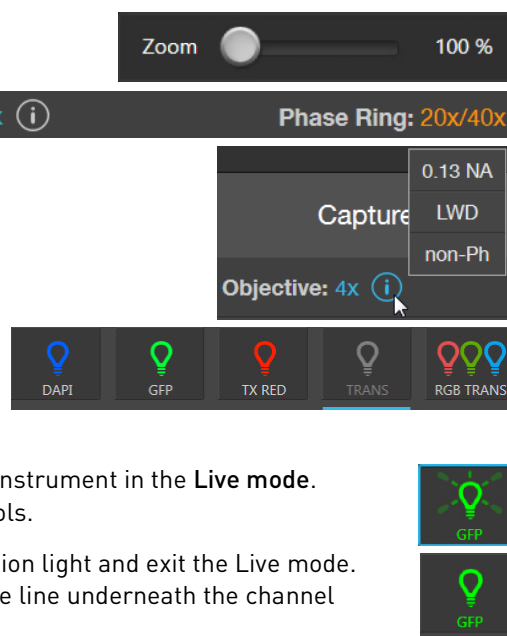
② **Viewing area:** Displays the sample.

③ **Zoom slider:** Zooms in and out of the Viewing area. The zoom range is 100% to 1000%.

④ **Active objective and phase ring:** Displays the active objective and phase ring information. To learn more about the current objective, click .

⑤ **Channel:** Selects the light source from the installed LED light cubes (fluorescent channels) or from the condenser (transmitted light). RGB Trans option outputs a color image from interlaced RGB images in the Live mode.

- Select a channel to turn on the excitation light and enter the instrument in the **Live mode**. In the Live mode, you can use the brightness and focus controls.
- Click the selected Channel button again to turn off the excitation light and exit the Live mode. The current channel remains selected as indicated by the blue line underneath the channel button.



- ⑥ **Brightness mode:** Toggles between Simple and Actual modes for brightness controls.
- **Simple mode** allows you to control light intensity as a single **Light** parameter.
  - **Actual mode** allows you to adjust **Brightness** (i.e., LED intensity), **Exposure**, and **Gain** parameters individually.



- ⑦ **Brightness controls:** Adjust the brightness for the selected channel.

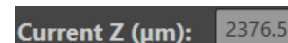


Simple mode



Actual mode

- ⑧ **Current Z (μm):** Displays the current focus position in μm along the Z-axis. To change the Z-axis focus position, enter the desired value into the text box.

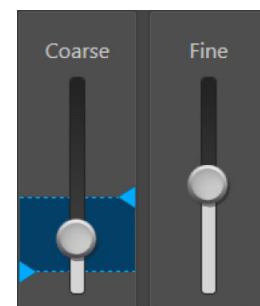


- ⑨ **Autofocus (Coarse and Fine):** Runs the coarse and fine autofocus algorithms in the selected channel.




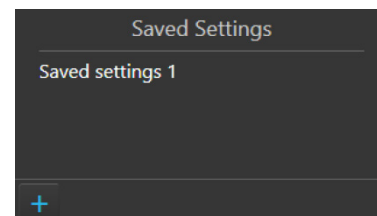
- ⑩ **Coarse and Fine focus sliders:** Allow you to adjust the focus in the selected channel when in the Live mode.


Using the Autofocus Maximum and Autofocus Minimum controls (blue triangles on the Coarse focus slider), you can limit the autofocus algorithm to the selected region along the Z-axis.

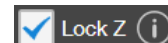


- ⑪ **Saved Settings:** Allows you to save and edit Z-axis focus positions and brightness settings for future use.

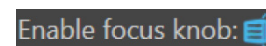
Press the  button to save the current settings.



- ⑫ **Lock Z:** Allows you to lock the Z-axis offsets, which specify the optimal focus position in each channel relative to the focus position in other channels.
- When locked, movement of the Z-position in one channel changes the Z-position in all channels, preserving their relative Z-positions.
  - When unlocked, adjusting the Z-position only affects the currently selected channel.
  - Click  next to Lock Z selection to view the recommended workflow for Z Offset set-up (page 20).



- ⑬ **Enable focus knob:** Enables the manual focus knobs.



- ⑭ **Capture:** Captures an image using the current capture settings and stores it in the image cache of the channel in which it was captured.



You can capture multiple channels by selecting the corresponding channel checkboxes.






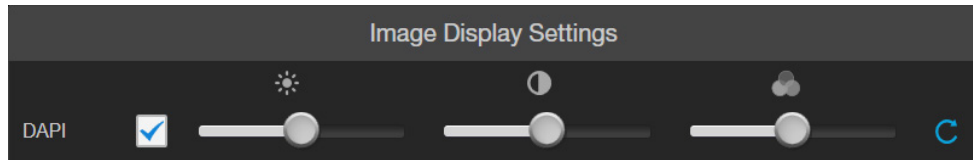
- ⑮ **Thumbnails:** Display the most recently captured image stored in the memory buffer for the channel.



- ①6 **Display settings and analysis tools:** Allow you to change image display settings in the Viewing area, and analyze and annotate captured images.

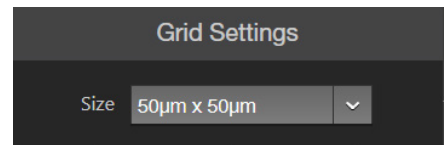


**Image Display Settings:** Opens the Image display settings tool, which allows you to adjust image display parameters (Brightness , Contrast , Gamma Correction ) for the selected channels.



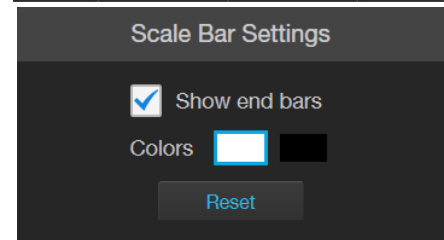
**Display Grid/Grid Settings:** Display Grid button switches the grid display in the Viewing area on and off.

Grid Settings allows you to set the grid size.

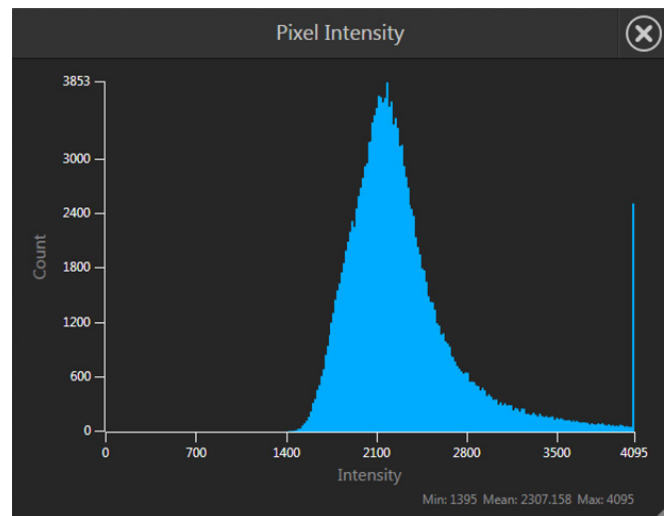


**Display Scale Bar/Scale Bar Settings:** Display Scale Bar button switches the display of the scale bar in the Viewing area on and off.

Scale Bar Settings allows you to select scale bar color and to display or hide end bars.

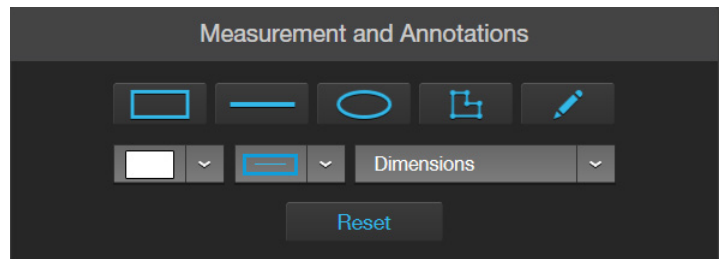


**Pixel Intensity:** Opens the Pixel Intensity window, which displays the Pixel count vs. Intensity histogram, where Intensity is the number of photons detected by the camera sensor.



**Measurements and Annotations:**

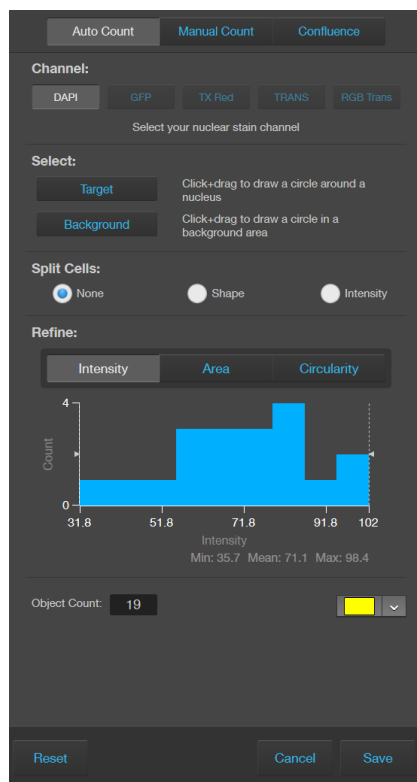
Allows you to draw regions of interest (rectangle, ellipse, polygon, line, or free-form) on the captured image and measure dimensions, area, or perimeter of the drawn region.



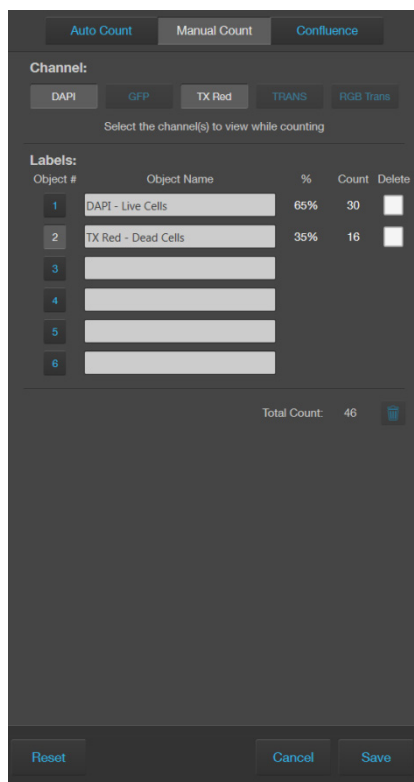


**Show Cell Count:** Allows you to perform cell counts using the Auto Count or Manual Count tools.

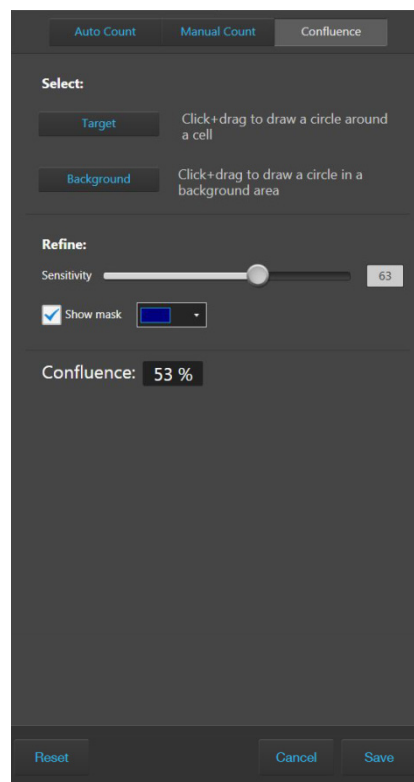
- **Auto Count:** Allows you define auto count parameters by selecting representative target objects, then count the objects by intensity, area, and circularity (page 30).
- **Manual Count:** Allows you to manually mark items onscreen using up to six separate labels and keep a running tally of the counts with percentages for each label (page 34).
- **Confluence:** Allows you to select up to 5 reference objects each for target (i.e., cells) and background in your image to automatically calculate the percentage confluence of your culture (page 36).



Auto Count



Manual Count

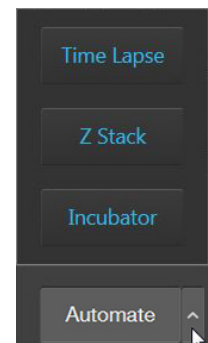


Confluence



**Toggle pseudocolor:** Allows you to display images in pseudocolor or in grayscale in the Viewing area. By default, color display is on.

- ⑪ **Automate:** Allows you to display the controls for the Time Lapse and Z-Stack tools, and the EVOS™ Onstage Incubator.



**Time Lapse:** Opens the Time Lapse tool, which allows you to create and run time lapse routines to capture images at given intervals over a time period based on your specifications.

**Z-Stack:** Opens the Z-Stack tool, which allows you to capture multiple images along the Z-axis based on your specifications. You can use the images captured with the Z-Stack tool to generate a Z-Stack Projection.

**Incubator:** Opens the Incubator Controls, which allow you to control the EVOS™ Onstage Incubator and monitor its status during your experiments.

Incubator Control

Status: Simulated (connected)  
Chamber Lid: Closed

Incubator

☐ Enable

Shutdown after:

Hours

0

Minutes

0

Time until shutdown: 00:00:00

Targets

Temperature

20.0

°C

☐ CO<sub>2</sub>

0.0

%

☐ Humidity

☐ O<sub>2</sub>

0.0

%

	Status	Target	Actual
Temperature °C	Off	---	37.52
Humidity %	Off	---	89.46
CO <sub>2</sub> %	Off	---	0.51
O <sub>2</sub> %	Off	---	19.62

Close

- ⑱ **Save:** Opens the Save dialog, which allows you to select a save location, set save options, and enable the Quick Save option.

Quick Save Settings

☐ Enable Quick Save

Save folder:

D:\

...

Base filename:

Image

Starting number:

1

File type:

TIFF

▼

☐ Save underlying channels

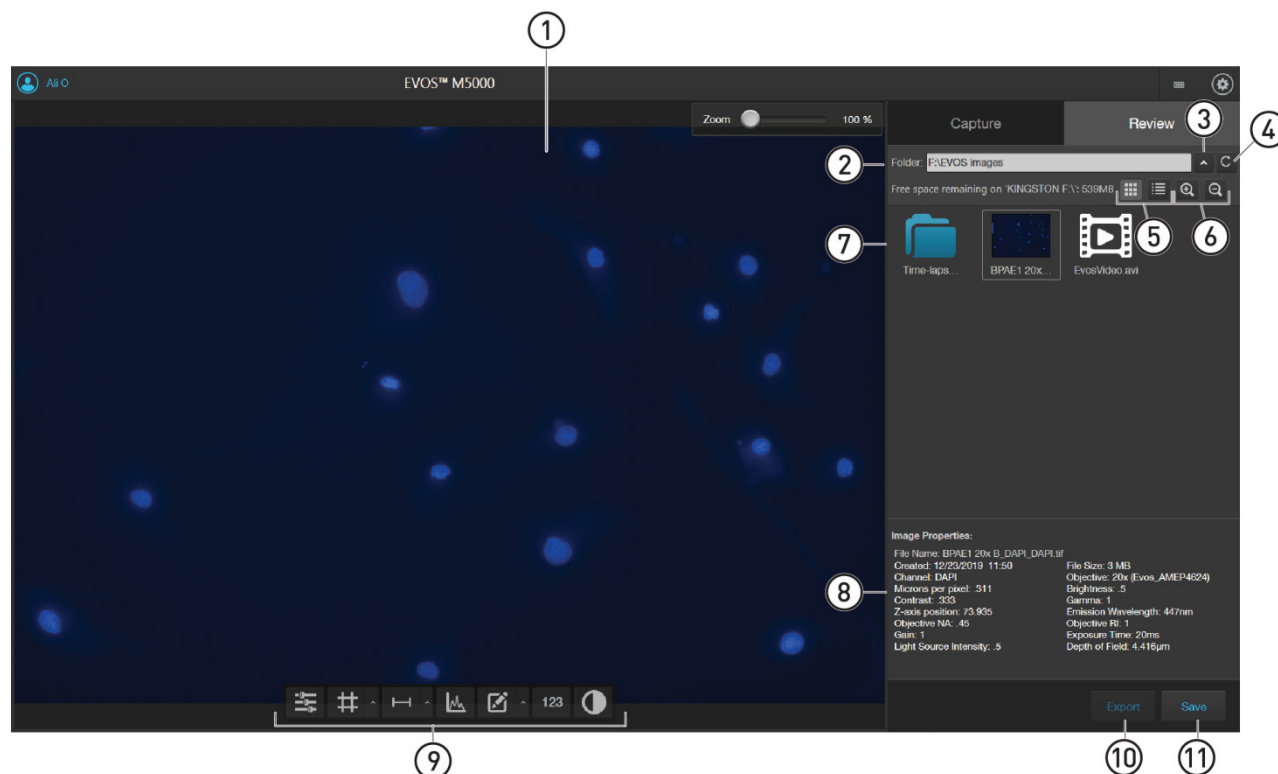
☐ Save on each capture





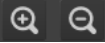
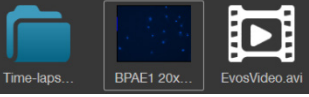
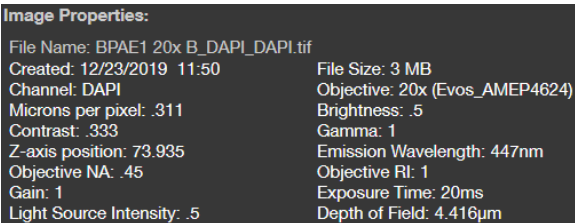
Next filename: Image\_0001.tif

Automate

Save

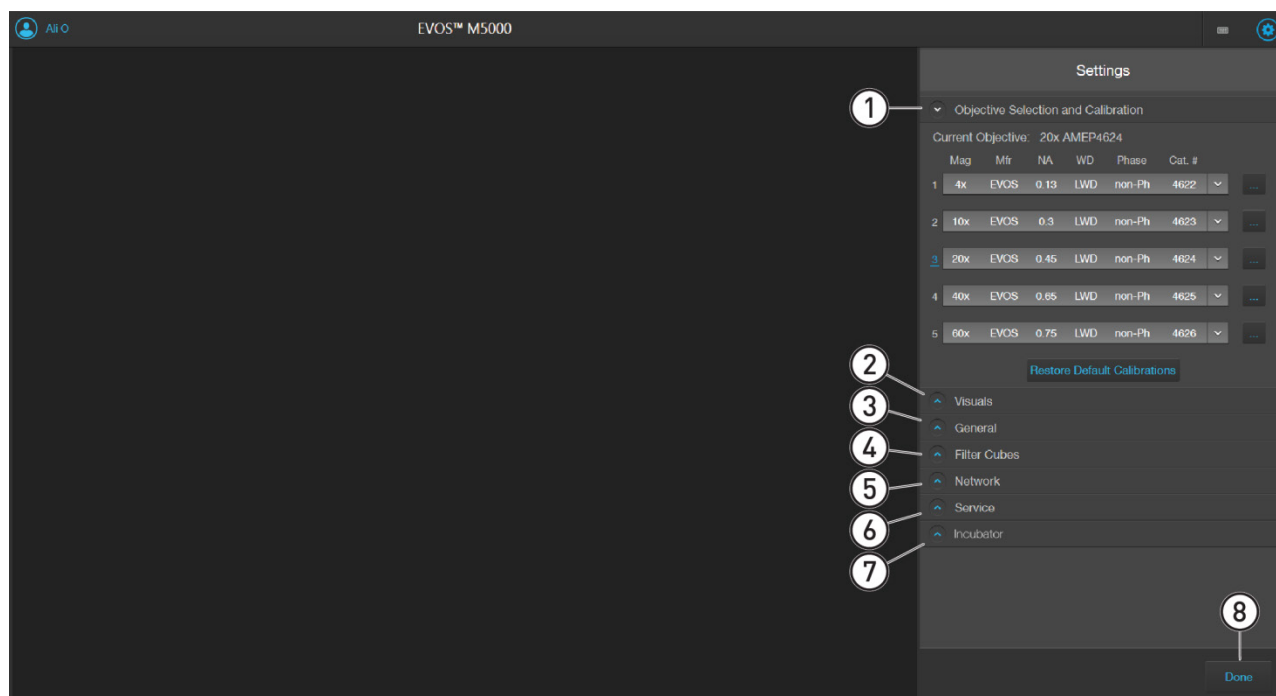
## Review tab



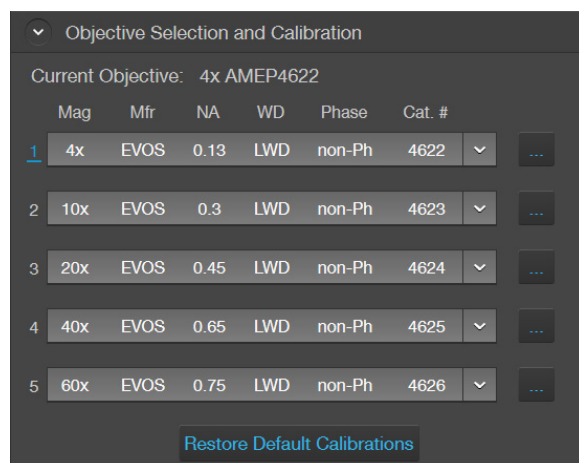
- ① **Viewing area:** Displays the sample.
- ② **Folder:** Displays the location of the current folder or image.  

- ③ **Up:** Goes up one folder from the current folder.  

- ④ **Refresh:** Refreshes the list or grid of images in the current folder.  

- ⑤ **Grid view and List view buttons:** Display the folders or saved images in grid or list view.  

- ⑥ **Folder/Image display size:** Increase and decrease the display size of the folders or image files.  

- ⑦ **Browse:** Allows you to navigate to the folder containing your saved images and to select the image to display in the viewing area. Currently selected folder or image is indicated with a box around it.  

- ⑧ **Image properties:** Displays the metadata for the selected image file.  


**Image Properties:**  
 File Name: BP AE1 20x B\_DAPI\_DAPI.tif  
 Created: 12/23/2019 11:50  
 Channel: DAPI  
 Microns per pixel: .311  
 Contrast: .333  
 Z-axis position: 73.935  
 Objective NA: .45  
 Gain: 1  
 Light Source Intensity: .5  
 File Size: 3 MB  
 Objective: 20x (Evos\_AMEP4624)  
 Brightness: .5  
 Gamma: 1  
 Emission Wavelength: 447nm  
 Objective RI: 1  
 Exposure Time: 20ms  
 Depth of Field: 4.416µm
- ⑨ **Display settings and analysis tools:** Allow you to change image display settings in the Viewing area, and analyze and annotate captured images. See page 82 for more information.
- ⑩ **Export:** Allows you to export the currently selected folder or image to a storage device.
- ⑪ **Save:** Saves the currently opened image.

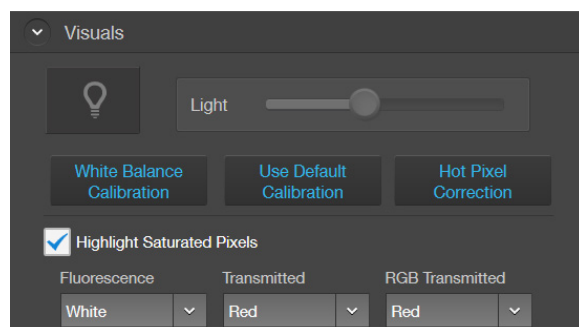
# Settings



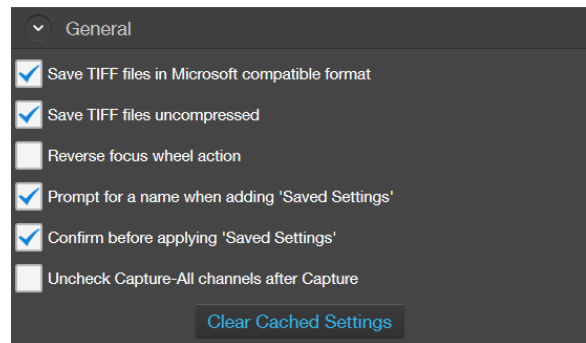
- ① **Objective Selection and Calibration:** Allows you to assign and unassign objectives on the objective turret, and to calibrate objective magnification.



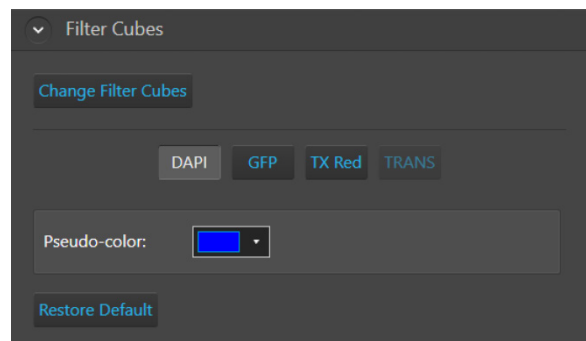
- ② **Visuals:** Allows you to calibrate color channel lighting (White Balance Calibration) and to assign saturated pixel colors in the Fluorescence, Transmitted, and RGB Transmitted channels.



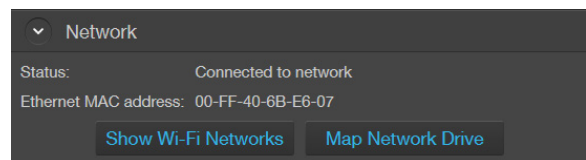
- ③ **General:** Allows you to define saving options for TIFF files and Saved Settings, and to reverse the focus wheel action.



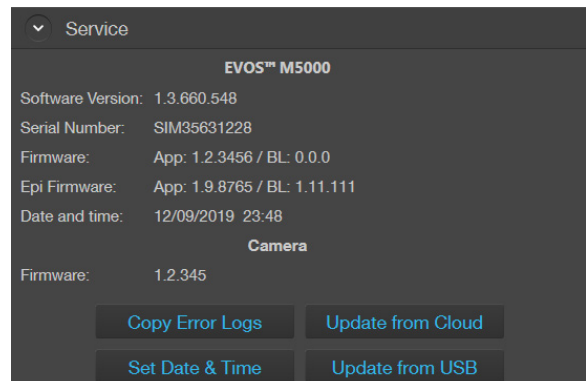
- ④ **Filter Cubes:** Allows you to add or remove EVOS™ LED light cubes from the instrument and to assign pseudocolors to specific channels.



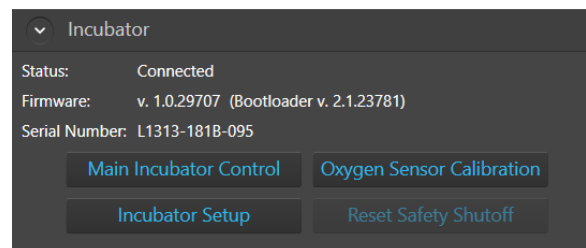
- ⑤ **Network:** Allows you to connect to a Wi-Fi network and to map network drives.



- ⑥ **Service:** Displays the EVOS™ M5000 hardware and software information, allows you to update the EVOS™ M5000 firmware and software from Connect™, Thermo Fisher's cloud-based platform, or using a USB flash drive, and to copy the error logs.



- ⑦ **Incubator:** Allows you to set up and configure the EVOS™ Onstage Incubator. The Incubator tab is visible only if the the EVOS™ Onstage Incubator is connected to the EVOS™ M5000 instrument.



**Done:** Closes the Settings tabs and returns to the previously opened tab.

Done

## Appendix C: System overview

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### Technical specifications

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**Note:** Technical specifications of the EVOS™ M5000 Imaging System are subject to change without notice. For the latest product information, see the product page ([thermofisher.com/evos](https://thermofisher.com/evos)).

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Physical characteristics	<p><b>Dimensions (W × H × D):</b> 46 × 59 × 46 cm (18 × 23 × 18 in)</p> <p><b>Weight:</b> 16 kg (35 lbs.)</p> <p><b>Footprint:</b> Approximately 92 cm × 92 cm (36 in × 36 in)</p> <p><b>Operating temperature:</b> 4°–32°C (40°–90°F)</p> <p><b>Operating humidity:</b> &lt;90%, non-condensing</p> <p><b>Operating power:</b> 100–240 VAC, 1.8 A</p> <p><b>Frequency:</b> 50–60 Hz</p> <p><b>Electrical input:</b> 12 VDC, 5 A</p>
Hardware	<p><b>Optics:</b> Infinity-corrected optical system; RMS-threaded objectives with 45 mm parfocal distance.</p> <p><b>Illumination:</b> Adjustable intensity LED (&gt;50,000-hour life per light cube).</p> <p><b>Light cubes</b> (not included): 5 position chamber for 4 fluorescence cubes plus brightfield. Broad selection standard and specialty light cubes (see page 90).</p> <p><b>Contrast methods:</b> Fluorescence and transmitted light (brightfield &amp; phase contrast).</p> <p><b>Objective turret:</b> 5-position; front-mounted control.</p> <p><b>Objectives</b> (not included): Wide selection of high-quality LWD and coverslip-corrected objectives.</p> <p><b>Condenser:</b> 60-mm long working distance condenser, 4 position turret with a clear aperture and 3 phase annuli.</p> <p><b>Stage:</b> Manual X-Y scanning stage. Travel range of 120 mm × 80 mm with sub-micron resolution, drop-in inserts to receive vessel holders and lock down holders to fix sample in place during long scans.</p> <p><b>Focus mechanism:</b> Automated focus mechanism with sub-micron resolution.</p> <p><b>LCD display:</b> 18.5-inch high-resolution LCD display (1920 × 1080 pixel resolution)</p> <p><b>Camera:</b> High-sensitivity monochrome CMOS camera (2048 × 1536 pixel resolution, 3.2 Megapixels)</p> <p><b>Captured images:</b> 16-bit monochrome (12-bit dynamic range) TIFF or PNG; 8-bit per RGB channel TIF, PNG, BMP, or JPG.</p> <p><b>Output ports:</b> 4-pin power input port (12 VDC, 5 A), 1 USB 3.0, 4 USB 2.0</p> <p><b>Networking capability:</b> Connection through Windows/SMB network via an Ethernet cable connection or wirelessly using the supplied Wi-Fi adaptor.</p> <p><b>Power supply:</b> AC adaptor with country-specific power cords.</p>

# Operation principles and technical overview

## LED illumination

The EVOS™ M5000 Imaging System utilizes an adjustable intensity LED light source provided by the proprietary, user-interchangeable LED light cube (see below). Because the LED light source is as close as possible to the objective turret, the number of optical elements in the channel is minimized. High-intensity illumination over a short channel increases the efficiency of fluorophore excitation, providing better detection of weak fluorescent signals.

In contrast to traditional fluorescence microscopy light sources that use mercury, a toxic carcinogen requiring special handling and disposal, the LED light source of the EVOS™ M5000 Imaging System is more environmentally friendly, energy efficient, and has a significantly longer life span (>50,000 hours versus 300 hours for a typical mercury bulb and 1,500 hours for a metal halide bulb).

## LED light cubes

Each user-interchangeable, auto-configured light cube contains an LED, collimating optics, and filters. In addition to the channel dedicated to the transmitted light from the condenser for brightfield contrast applications, the EVOS™ M5000 Imaging System can accommodate up to four fluorescent or specialty light cubes for multiple-fluorescence research applications.

The table below lists some of the common fluorescent and specialty light cubes available from Thermo Fisher Scientific. For a complete list of available light cubes and to inquire about custom light cubes, go to [thermofisher.com/evos](https://thermofisher.com/evos) or contact Technical Support (see page 116).

Light cube	Dye
DAPI	DAPI, Hoechst™, BFP
TagBFP	TagBFP
CFP	ECFP, Lucifer Yellow, Evans Blue
GFP	GFP, Alexa Fluor™ 488, SYBR™ Green, FITC
YFP	EYFP, acridine orange + DNA
RFP	RFP, Alexa Fluor™ 546, Alexa Fluor™ 555, Alexa Fluor™ 568, Cy™3, MitoTracker™ Orange, Rhodamine Red, DsRed
Texas Red	Texas Red™, Alexa Fluor™ 568, Alexa Fluor™ 594, MitoTracker™ Red, mCherry, Cy™3.5
Cy5	Cy™5, Alexa Fluor™ 647, Alexa Fluor™ 660, DRAQ5™
Cy5.5	Cy™5.5, Alexa Fluor™ 660, Alexa Fluor™ 680, Alexa Fluor™ 700
Cy7	Cy™7, IRDye 800CW
Specialty light cubes	Dye
CFP-YFP em	CFP/YFP (for FRET applications)
AO	Acridine orange + RNA, simultaneous green/red with FL color
AORED	Acridine orange + RNA, CTC formazan, Fura Red™ (high Ca <sup>2+</sup> )
White	Refracted light applications



## Appendix D: EVOS™ Onstage Incubator

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### EVOS™ Onstage Incubator

The EVOS™ Onstage Incubator (Cat. No. AMC1000) is an optional accessory for the EVOS™ FL Auto Imaging System that enables the incubation of cells on the automatic X-Y stage, allowing the capture of images from the same sample over long periods of time and recording of time lapse movies.

The EVOS™ Onstage Incubator consists of a Stagetop Environmental Chamber that is placed on the automatic X-Y stage of the imaging system and a separate Control Unit that supplies the power and gas (air or air-CO<sub>2</sub> premix, CO<sub>2</sub>-only, and nitrogen-only). The onstage incubator is controlled by the same software and user interface that controls the EVOS™ M5000 Imaging System.

### Standard items included

- Stagetop Environmental Chamber
- Control unit
- Master Stage Plate (also available separately as EVOS™ Onstage Master Plate, Cat. No. AMEPVH035)
- Vessel holder for multi-well plates (also available separately as EVOS™ Onstage Vessel Holder, Multiwell Plates, Cat. No. AMEPVH028)
- Cable with 6-pin connector
- Cable, USB A-to-B, 180 cm/6 ft
- Heated hose with temperature control, 180 cm/6 ft (also available separately as EVOS™ Onstage Incubator Hose, Cat. No. AMEP4728)
- Gas line, 1/8 in ID, 1/4 in OD (also available separately as EVOS™ Onstage Incubator Gas Line, Cat. No. AMEP4732)
- Push-to-connect gas line adaptor (3 each)
- Standard-head open-end wrench
- Hex screw driver
- Power Cord, Type A (North America)

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**Note:** A country-specific power cord must be ordered separately in regions not using the Type A power plug.

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## Technical specifications

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**Note:** Specifications of the Onstage Incubator are subject to change without notice. Refer to the EVOS™ product page at [www.thermofisher.com/evos](http://www.thermofisher.com/evos) for the latest product information.

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### Physical characteristics

	Stagetop Environmental Chamber	Control Unit
<b>Height:</b>	25 cm (9.7 in)	37 cm (15 in)
<b>Depth:</b>	19 cm (7.6 in)	16 cm (6.3 in)
<b>Width:</b>	3.7 cm (1.5 in)	20 cm (7.9 in)
<b>Weight:</b>	1.5 kg (3.3 lb)	10 kg (22 lb)
<b>Temperature range:</b> Ambient to 40°C (± 0.1°C)		
<b>Humidity:</b> >80% relative humidity (RH) at 37–40°C		
<b>CO<sub>2</sub> range:</b> 0% to 20%		
<b>O<sub>2</sub> range:</b> 0% to ambient		
<b>Operating power:</b> 100–240 VAC, 1.8 A		
<b>Frequency:</b> 50–60 Hz		
<b>Electrical input:</b> 24 VDC, 5 A		

### Hardware

**Compatible vessels:** Multi-well plates, 35-mm Petri dishes, T-25 flasks

**Gas input ports:** Air or air-CO<sub>2</sub> premix, CO<sub>2</sub>-only, and N<sub>2</sub>-only (max. 50 psi input)

**Stagetop environmental chamber accessories:** Master Stage Plate, Vessel holder for multi-well plates

## EVOS™ Onstage Incubator components

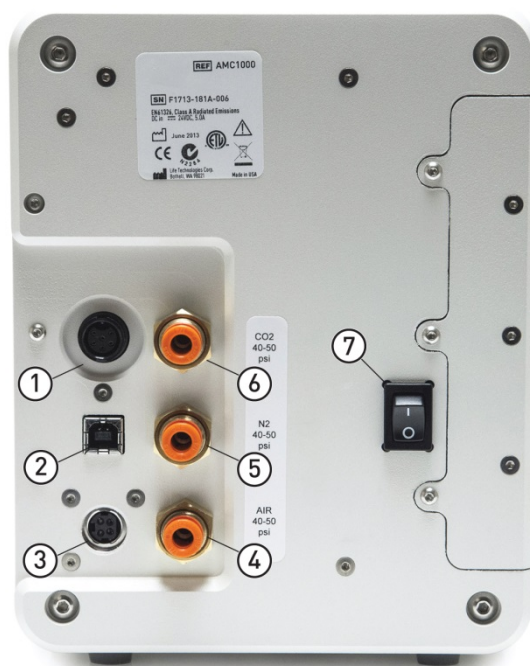
### Control unit and environmental chamber

EVOS™ Onstage Incubator consists of an environmental chamber and a separate control unit that supplies the power and gas (air or air-CO<sub>2</sub> premix, CO<sub>2</sub>, and N<sub>2</sub> for O<sub>2</sub> displacement in hypoxia experiments), and controls the humidity and temperature.



- ① Control unit
- ② Hose heater connector
- ③ Heated hose
- ④ Sensor data cable with 6-pin connector
- ⑤ Environmental chamber

### Control unit rear view

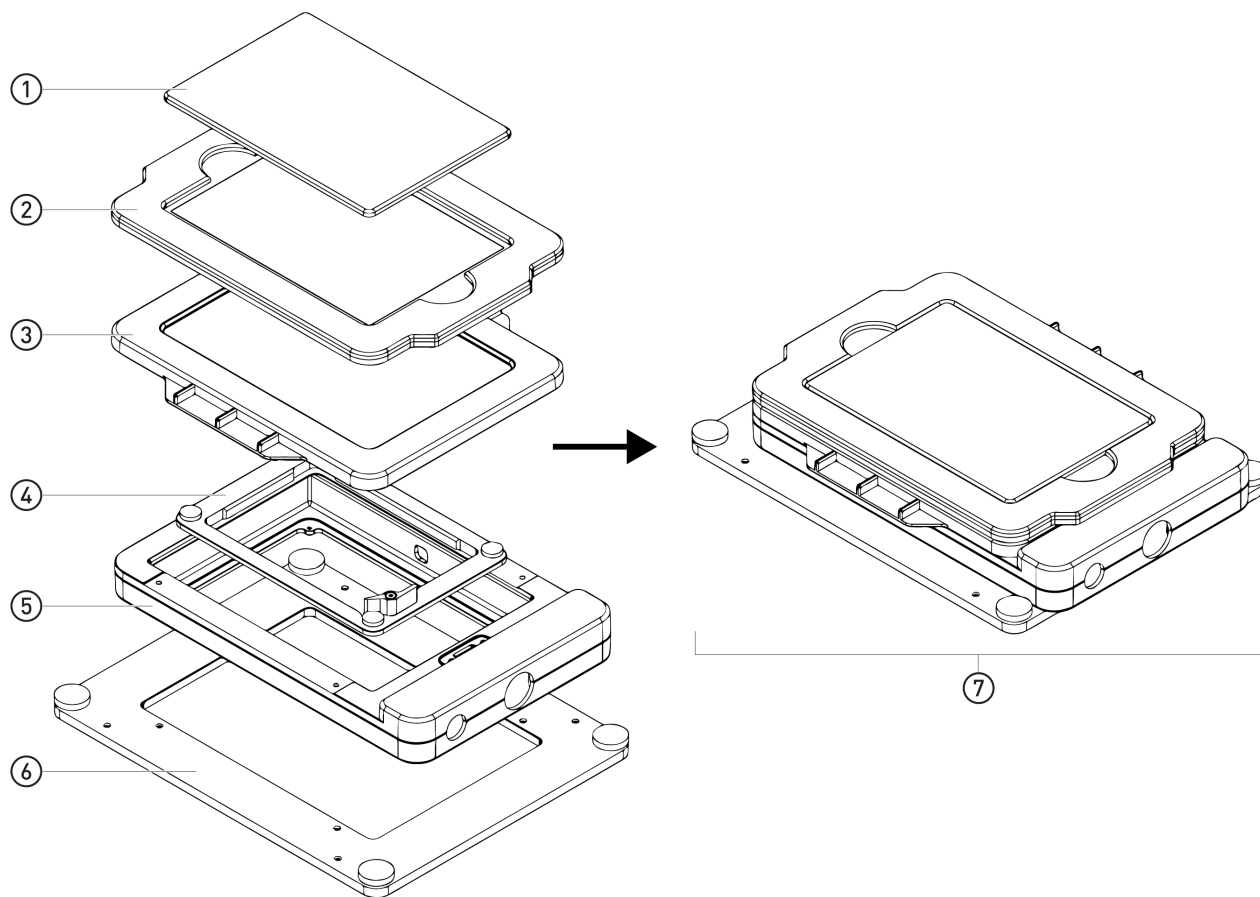


- ① Sensor data cable jack
- ② USB control cable jack
- ③ Power input jack
- ④ Port 1: Air
- ⑤ Port 2: N<sub>2</sub>
- ⑥ Port 3: CO<sub>2</sub>
- ⑦ Power switch

## Environmental chamber

The environmental chamber of the EVOS™ Onstage Incubator consists of the incubator chamber, the vessel holder/adaptor, the heated glass lid, the light shield, and the light shield cover.

The environmental chamber sits on the onstage incubator master plate attached to the X-Y stage of the EVOS™ M5000 Imaging System (see “Assemble the environmental chamber”, page 96).



- ① Light shield cover
- ② Light shield
- ③ Heated glass lid
- ④ Vessel holder/adaptor
- ⑤ Incubator chamber
- ⑥ Master plate
- ⑦ Assembled environmental chamber

## Set up the EVOS™ Onstage Incubator

### Install the Onstage Incubator Master Plate

1. Remove the X-Y stage base plate from the X-Y stage by unscrewing the four 3.0-mm screws (indicated by red arrows) on the base plate. If necessary, unscrew and remove the vessel holder/adaptor before removing the base plate.

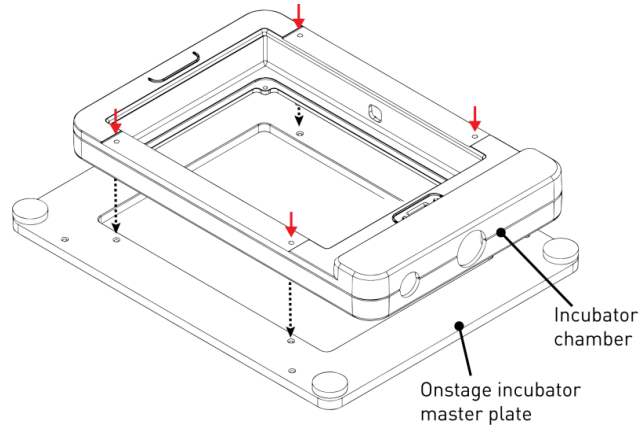


2. Secure the onstage incubator master plate to the X-Y stage using the four thumb screws.

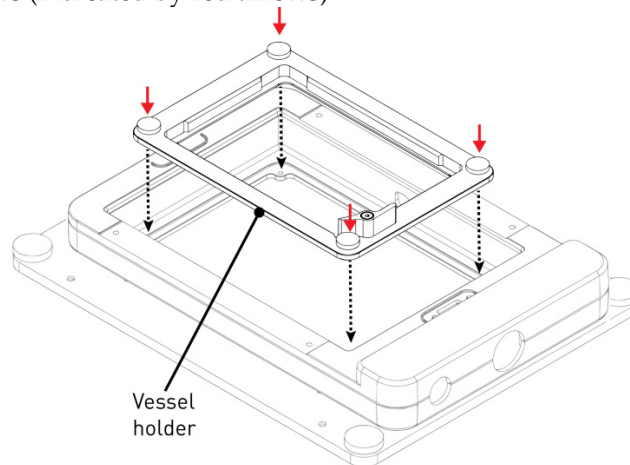


## Assemble the environmental chamber

1. Place the incubator chamber on the onstage incubator master plate and secure it in place using the four 2.0-mm hex screws (indicated by red arrows).



2. Attach the vessel holder/adaptor to the incubator chamber using the four thumb screws (indicated by red arrows).

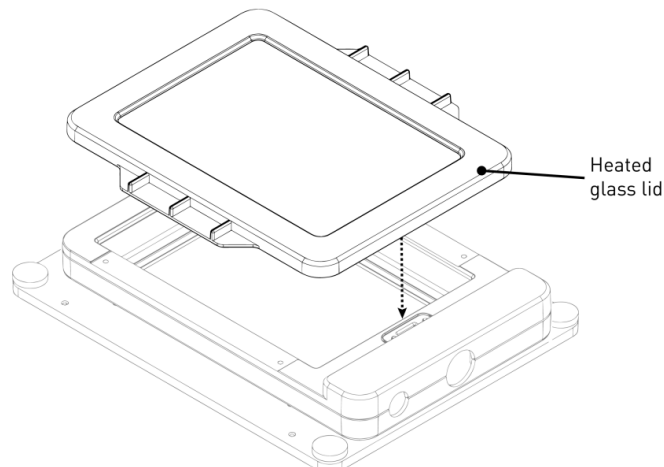


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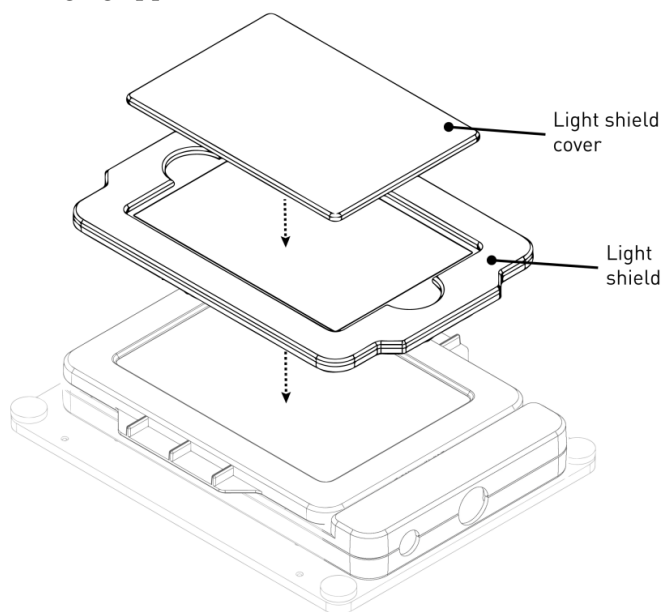
**Note:** Place an empty “dummy” culture plate into the vessel holder/adaptor for the initial warm up and equilibration to prevent build-up of condensation on the optical components and the inside of the EVOS™ M5000 Imaging System.

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3. Place the heated glass lid with the no-fog glass window on the incubator chamber. The heated glass lid is guided and held secure in its place by the two magnets on its rim.



4. Place the light shield with tinted plastic window on top of the heated glass lid. **Use of the light shield is required for fluorescence imaging applications.**
5. If desired, place the light shield cover on the light shield for fluorescence imaging applications. The light shield cover completely blocks any ambient light from entering the environment chamber and improves image quality in fluorescence imaging applications.





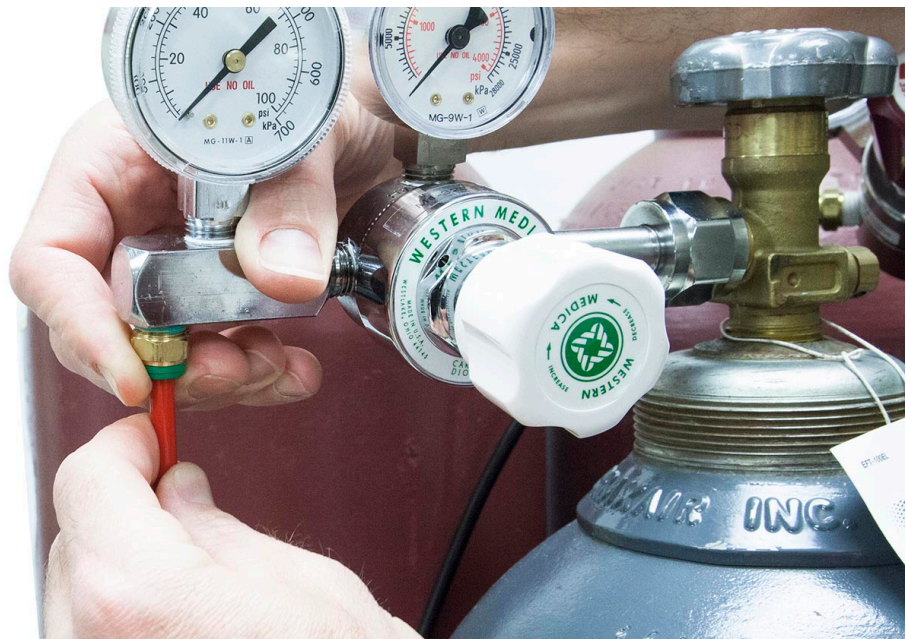
**Set up for operation** Follow the procedure below to set up the EVOS™ Onstage Incubator for operation. For the locations of the various input jacks and gas ports, refer to “Control unit rear view”, page 93.

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**IMPORTANT!** Do not position the control unit so that it is difficult to turn off the main power switch. In case of an instrument malfunction, turn the main power switch to the OFF position and disconnect the power cord from the wall outlet.

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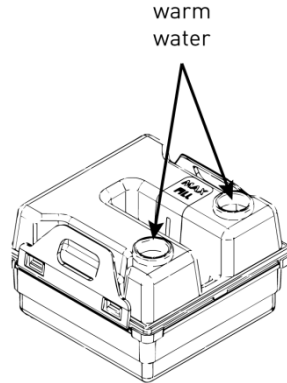
1. Plug power cord into the power input jack on the control unit and the wall outlet.
2. Plug USB cable into the USB control cable jack on the control unit and the USB port on the computer.
3. Connect each gas line to the appropriate gas tank via the PTC (push-to-click) connectors threaded into the regulator. To do this, push the tubing into the PTC connector until it clicks into place. Pull on tubing slightly to ensure a tight connection; the tubing should not come out.



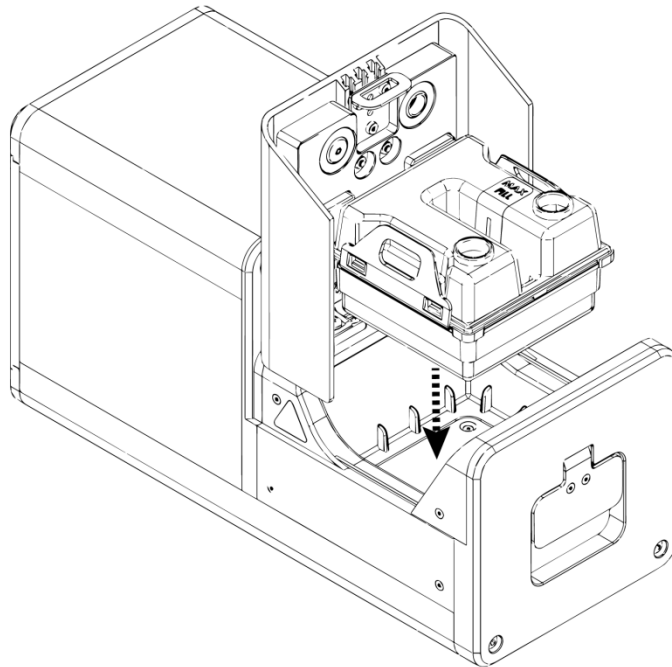
4. Attach the gas lines to the control unit via the PTC connectors for the appropriate gas intake port.
  - If using pre-mixed air, attach to **Port 1: Air In**
  - If using compressed air and CO<sub>2</sub>, attach to **Port 1: Air In** and **Port 3: CO<sub>2</sub> In**
  - For oxygen displacement, attach to **Port 1: Air In** and **Port 2: Nitrogen In**
5. Plug the 6-pin sensor data cable to the environmental chamber and the appropriate input jack on the control unit.



6. Assemble the water reservoir and add warm water (approximately 50°C) to the max fill line through the fill hole (see image below). **Do not overfill** the water reservoir.



7. Place the water reservoir into the control unit **with the fill holes to the front** and close the lid.



8. Attach the heated hose between the environmental chamber and the control unit
9. Plug the hose heater cable to the connector on the heated hose.

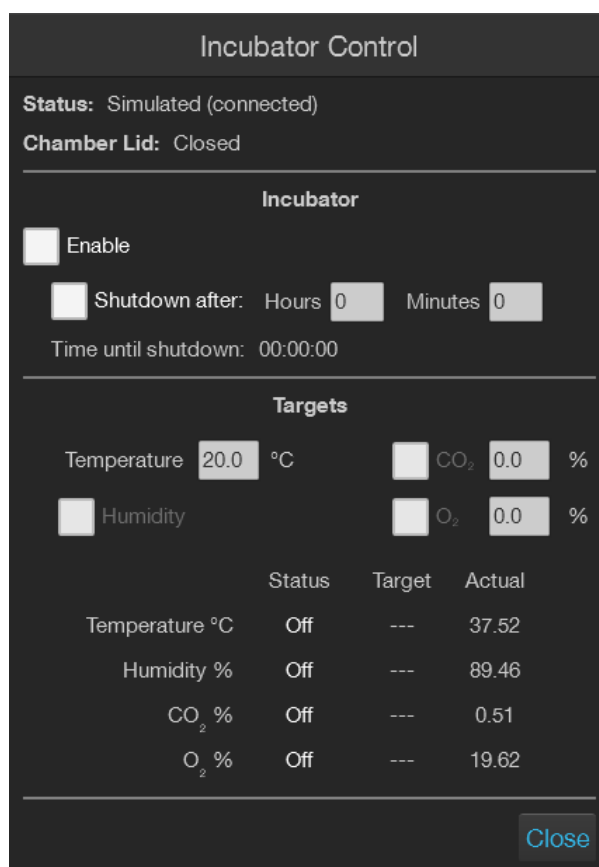
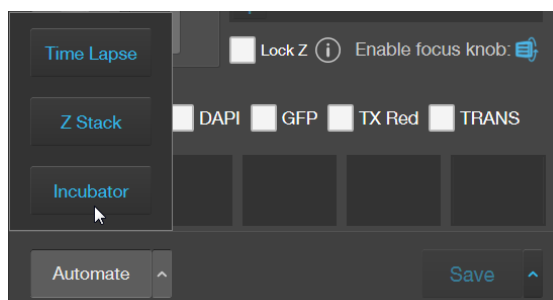
## Use the EVOS™ Onstage Incubator

**IMPORTANT!** Before using the EVOS™ Onstage Incubator in your experiments, make sure that:

- The gas inputs have been configured (see “Configure gas inputs”, page 102).
- The oxygen sensor has been calibrated (see “Calibrate oxygen sensor”, page 103).

### Turn ON the EVOS™ Onstage Incubator

1. Turn ON the EVOS™ M5000 Imaging System as described on page 15.
2. Turn ON the power switch to the EVOS™ Onstage Incubator control unit (page 93).
3. Start the M5000 software. The M5000 software automatically recognizes the EVOS™ Onstage Incubator upon connection.
4. On the **Capture** tab, click **Automate**, then select **Incubator** to open the Incubator Control window.



**Note:** Alternatively, go to the **Settings** tab, click **Incubator** to display the Incubator panel, then click **Main Incubator Control** to open the Incubator Control window.

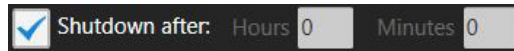
### Main Incubator Control

5. Select **Enable** to turn on the incubator.



6. Select the desired Shutdown option:

- **Manually shutdown:** The incubator will remain on until the **Enable** option is manually deselected and the **Close** button is clicked.
- **Automatic shutdown:** Select **Shutdown after**, then enter the time in **Hours** and **Minutes** that must elapse before the incubator is shut down automatically.



7. Enter the target values for **Temperature**, **CO<sub>2</sub>**, and **Oxygen**. If desired, select **Humidity** to use a humidified atmosphere in the incubator chamber.

Targets			
Temperature	20.0 °C	<input checked="" type="checkbox"/> CO <sub>2</sub>	5 %
<input checked="" type="checkbox"/> Humidity		<input checked="" type="checkbox"/> O <sub>2</sub>	18 %

Incubator status will change to “Running” and the control panel will display the Target and Actual values for Temperature, Humidity, CO<sub>2</sub>, and Oxygen.

	Status	Target	Actual
Temperature °C	Adjusting	20.00	37.51
Humidity %	Good	80.00	89.53
CO <sub>2</sub> %	Adjusting	5.00	0.00
O <sub>2</sub> %	Adjusting	18.00	19.55

8. Click **Close** to return to the Capture tab.

**Note:** Place an empty culture plate into the vessel holder/adaptor for the initial warm up and equilibration to prevent build-up of condensation on the optical components and the inside of the EVOS™ M5000 Imaging System.

### Optional: Set Temperature Offset

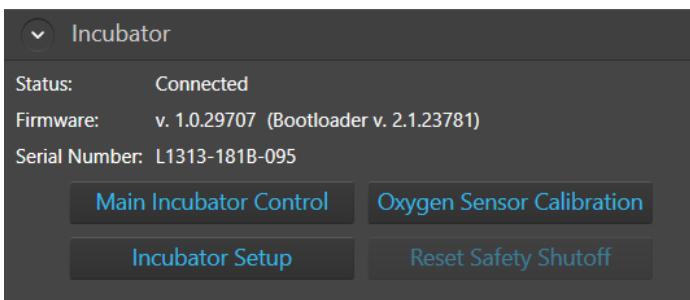
Ambient Offset allows you to adjust temperature readings based on the ambient room temperature. This should remain at 0 by default.

1. Go to the **Settings** tab, then click **Incubator** to display the Incubator panel.
2. Click **Incubator Setup** to open the Incubator Setup window.
3. Enter the appropriate temperature for the **Ambient Offset** in the Incubator Setup window, then click **Apply**.

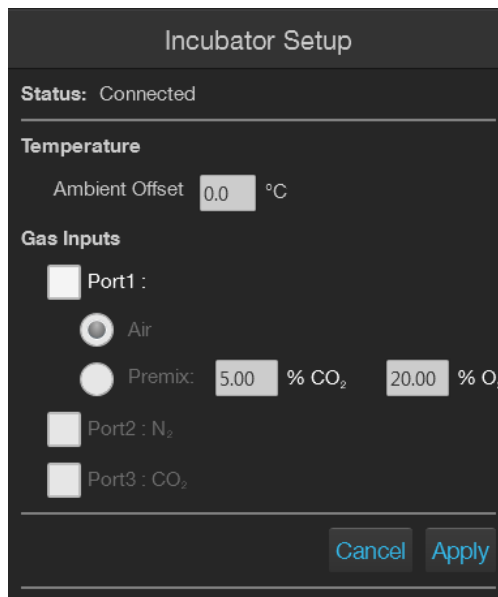
Incubator Setup	
Status:	Connected
Temperature	
Ambient Offset	0.0 °C

## Configure gas inputs

1. Go to the **Settings** tab, then click **Incubator** to display the Incubator panel.



2. Click **Incubator Setup** to open the Incubator Setup window.

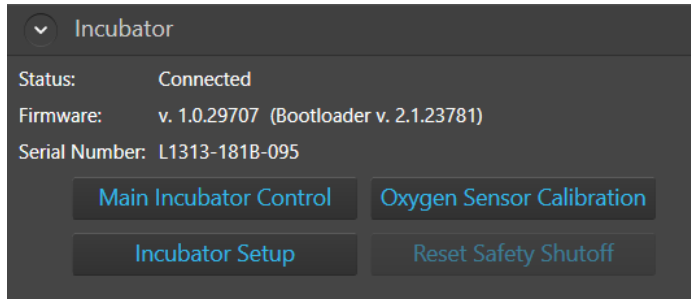


3. Select the appropriate options for the **Gas Inputs** that reflects your set-up for the EVOS™ Onstage Incubator.
  - For **Port 1**, you may select **Air** or **Premix**.  
If you select **Premix**, manually enter the percentage of the **CO<sub>2</sub>** and **O<sub>2</sub>** to reflect the specifics of your set-up.
  - **Port 2** is reserved for Nitrogen only.
  - **Port 3** is reserved for CO<sub>2</sub> only.
9. Click **Apply** after you have configured the gas connections for each port.
10. Turn on the regulators on the gas tanks. The meters on the regulators show the tank fill on the right and gas flow on the left.
11. Set the flow on the regulators as follows. Do not exceed 50 psi of pressure.
  - **Air:** 40–50 psi
  - **CO<sub>2</sub>:** 40–50 psi
  - **Nitrogen:** 40–50 psi

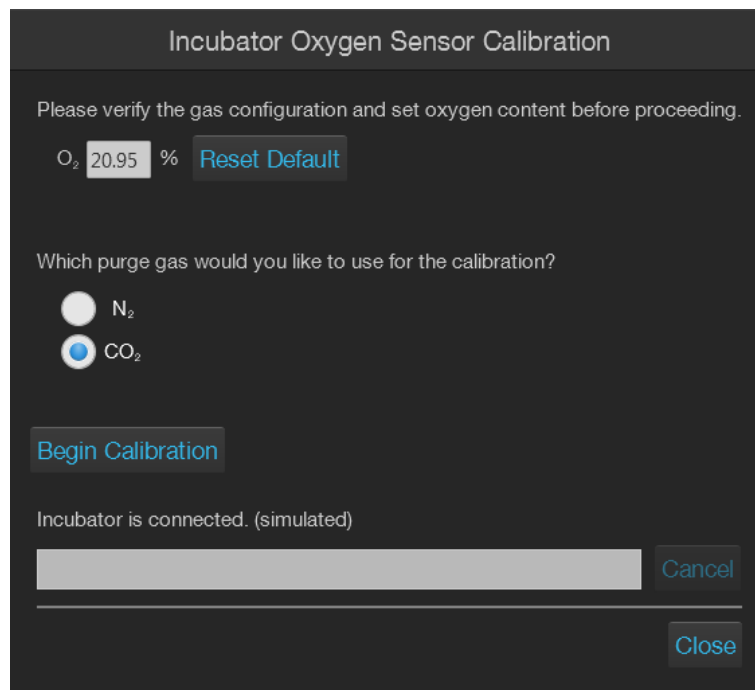
## Calibrate oxygen sensor

Calibrating the oxygen sensor ensures that the atmosphere in the environmental chamber is replenished with the appropriate gasses in the correct proportion.

1. Go to the **Settings** tab, then click **Incubator** to display the Incubator panel.



2. Click **Oxygen Sensor Calibration** to open the Incubator Setup window.



3. Verify that your gas connections have been correctly configured.
4. Verify that the **Oxygen** % has been set correctly. If not, enter the correct value for oxygen content.
5. Select the **purge gas source** for your calibration (**Nitrogen** or **CO<sub>2</sub>**).
6. Click **Begin Calibration**. The EVOS™ M5000 Imaging System automatically calibrates the oxygen sensor for the proper functioning of the EVOS™ Onstage Incubator. The entire calibration process takes approximately three minutes.

# Appendix E: EVOS™ Image Analysis

## EVOS™ Image Analysis

The EVOS™ Image Analysis is an easy to use application for storing, viewing, and analyzing images from your EVOS™ M5000 Imaging System.



After you have signed in to your Connect™ account from the EVOS™ M5000 Imaging System, you can choose to save captured images directly to the application in addition to local storage.

You can then use the application for editing images (e.g., to adjust brightness and contrast, annotations, pseudo coloring, etc.) as well as for identification and quantification of cells.

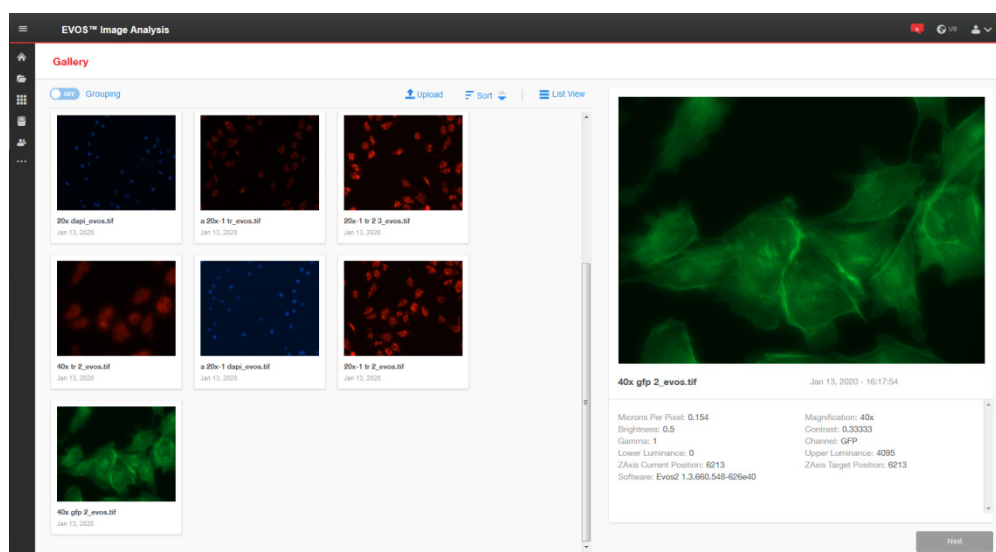
The EVOS™ Image Analysis application is available for free on the Connect™ platform. However, you need a current Connect™ account to sign in. To create your Connect™ account online, go to [thermofisher.com/connect](https://thermofisher.com/connect).

**Note:** This sections provides an overview of the EVOS™ Image Analysis application. For detailed instructions on how to use the EVOS™ Image Analysis application, refer to the *EVOS™ Image Analysis Help System*. A PDF version of the *EVOS™ Image Analysis Help System* (Pub. No. MAN0018314) is also available for download at [thermofisher.com](https://thermofisher.com).

## Gallery

The EVOS™ Image Analysis Gallery contains the images saved from your EVOS™ M5000 Imaging System to your Connect™ account. You can view the images and image metadata in this tab, and select individual images for further image adjustment and analysis.

To adjust an image or to analyze it (i.e., perform Auto or Manual count), select the desired image in the **Gallery**, then click **Next** to open the Edit Image screen (page 105).

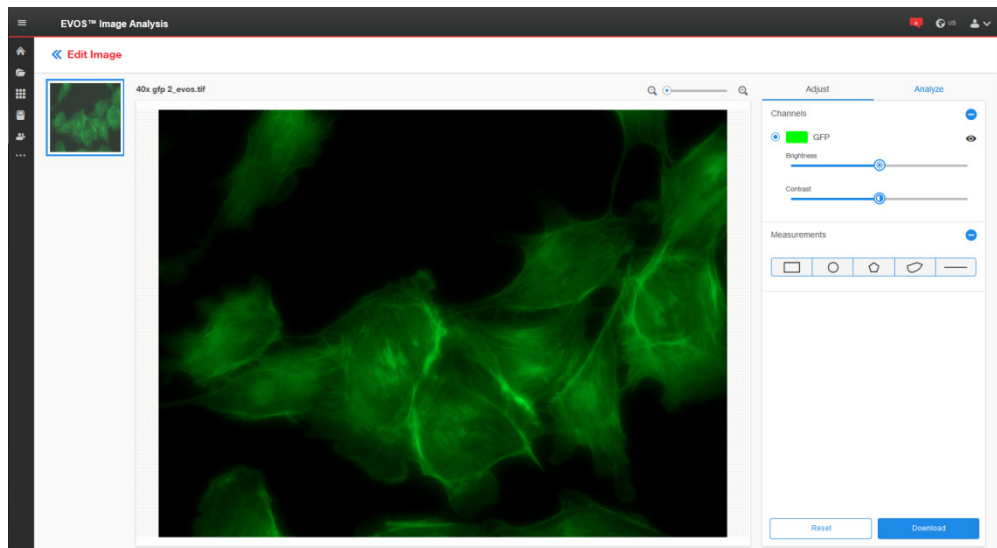


## Edit Image

Edit Image screen contains the **Adjust** and **Analyze** tabs.

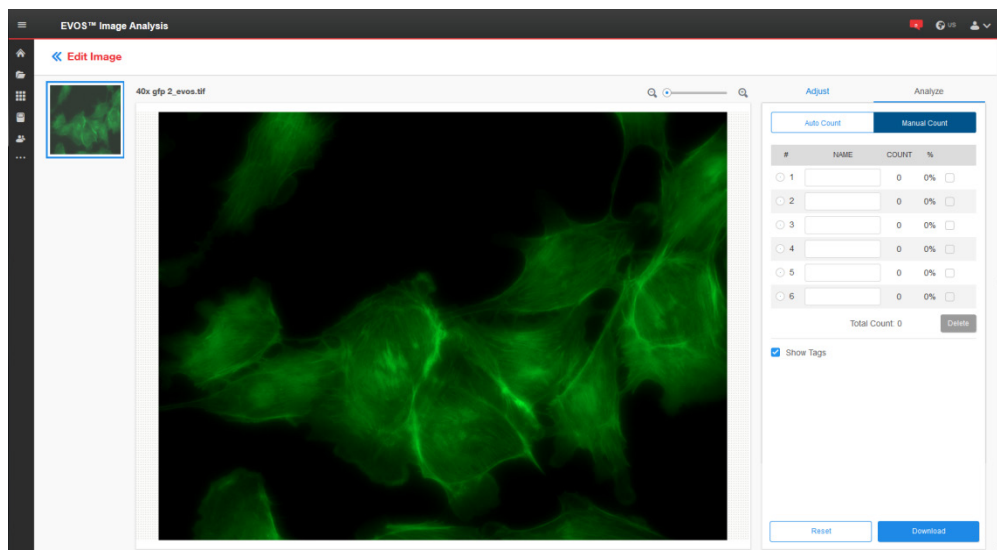
### Adjust tab

Adjust tab allows you to zoom in to your selected image, adjust brightness and contrast values, and add line measurements and annotations. The measurement and annotation functions of the EVOS™ Image Analysis application are identical to the Analysis tools of the EVOS™ M5000 Imaging System (page 25).



### Analyze tab

Analyze tab allows you to perform Auto and Manual counts. The Auto and Manual Count functions of the EVOS™ Image Analysis application are identical to the count tools of the EVOS™ M5000 Imaging System (page 29).



## Appendix F: Safety

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### Safety conventions used in this document

**Safety alert words**      Three safety alert words appear in this document at points where you need to be aware of relevant hazards. Each alert word—**CAUTION**, **WARNING**, **DANGER**—implies a particular level of observation or action, as defined below:



**CAUTION!** – Indicates a potentially hazardous situation that, if not avoided, may result in minor or moderate injury. It may also be used to alert against unsafe practices.

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**WARNING!** – Indicates a potentially hazardous situation that, if not avoided, could result in death or serious injury.

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**DANGER!** – Indicates an imminently hazardous situation that, if not avoided, will result in death or serious injury. This signal word is to be limited to the most extreme situations.









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## Symbols on instruments








### Electrical symbols on instruments

The following table describes the electrical symbols that may be displayed on Thermo Fisher Scientific instruments.

Symbol	Description
	Indicates the <b>On</b> position of the main power switch.
	Indicates the <b>Off</b> position of the main power switch.
	Indicates a standby switch by which the instrument is switched on to the <b>Standby</b> condition. Hazardous voltage may be present if this switch is on standby.
	Indicates the <b>On/Off</b> position of a push-push main power switch.
	Indicates a terminal that may be connected to the signal ground reference of another instrument. This is not a protected ground terminal.
	Indicates a protective grounding terminal that must be connected to earth ground before any other electrical connections are made to the instrument.
	Indicates a terminal that can receive or supply alternating current or voltage.
	Indicates a terminal that can receive or supply alternating or direct current or voltage.


## Safety symbols

The following table describes the safety symbols that may be displayed on Thermo Fisher Scientific instruments. Each symbol may appear by itself or in combination with text that explains the relevant hazard (see “Safety labels on instruments”). These safety symbols may also appear next to **DANGERS**, **WARNINGS**, and **CAUTIONS** that occur in the text of this and other product-support documents.

Symbol	Description
	Indicates that you should consult the manual for further information and to proceed with appropriate caution.
	Indicates the presence of an electrical shock hazard and to proceed with appropriate caution.
	Indicates the presence of a hot surface or other high-temperature hazard and to proceed with appropriate caution.
	Indicates the presence of a laser inside the instrument and to proceed with appropriate caution.
	Indicates the presence of moving parts and to proceed with appropriate caution.
	Indicates the presence of a biological hazard and to proceed with appropriate caution.
	Indicates the presence of an ultraviolet light and to proceed with appropriate caution.





## Environmental symbols on instruments

The following symbol applies to all Thermo Fisher Scientific electrical and electronic products placed on the European market after August 13, 2005.






Symbol	Description
	<p><b>Do not dispose of this product as unsorted municipal waste.</b> Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE).</p> <p><b>European Union customers:</b> Call your Customer Service representative for equipment pick-up and recycling. See <a href="http://www.thermofisher.com">www.thermofisher.com</a> for a list of customer service offices in the European Union.</p>

## Safety labels on instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on Thermo Fisher Scientific instruments in combination with the safety symbols described in the preceding section.

Hazard Symbol	English	Français
	<b>CAUTION!</b> Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	<b>ATTENTION!</b> Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant toute manipulation de produits.
	<b>CAUTION! HAZARDOUS WASTE.</b> Refer to SDS(s) and local regulations for handling and disposal.	<b>ATTENTION!</b> Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la réglementation locale associées à la manipulation et l'élimination des déchets.
	<b>DANGER!</b> High voltage.	<b>DANGER!</b> Haute tension.
	<b>WARNING!</b> To reduce the chance of electrical shock, do not remove covers that require tool access. No user-serviceable parts are inside. Refer servicing to Thermo Fisher Scientific qualified service personnel.	<b>AVERTISSEMENT!</b> Pour éviter les risques d'électrocution, ne pas retirer les capots dont l'ouverture nécessite l'utilisation d'outils. L'instrument ne contient aucune pièce réparable par l'utilisateur. Toute intervention doit être effectuée par le personnel de service qualifié venant de chez Thermo Fisher Scientific.
	<b>DANGER!</b> Class 3B visible and/or invisible laser radiation present when open. Avoid exposure to beam.	<b>DANGER!</b> Rayonnement visible ou invisible d'un faisceau laser de Classe 3B en cas d'ouverture. Evitez toute exposition au faisceau.
	<b>CAUTION!</b> Moving parts. Crush/pinch hazard.	<b>ATTENTION!</b> Pièces en mouvement, risque de pincement et/ou d'écrasement.

## General instrument safety

	 <b>WARNING! PHYSICAL INJURY HAZARD.</b> Use this product only as specified in this document. Using this instrument in a manner not specified by Thermo Fisher Scientific may result in personal injury or damage to the instrument.
Moving and lifting the instrument	 <b>CAUTION! PHYSICAL INJURY HAZARD.</b> The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.
Moving and lifting stand-alone computers and monitors	 <b>WARNING!</b> Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people. <b>Things to consider before lifting the computer and/or the monitor:</b> <ul style="list-style-type: none"><li>• Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.</li><li>• Make sure that the path from where the object is to where it is being moved is clear of obstructions.</li><li>• Do not lift an object and twist your torso at the same time.</li><li>• Keep your spine in a good neutral position while lifting with your legs.</li><li>• Participants should coordinate lift and move intentions with each other before lifting and carrying.</li><li>• Instead of lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone slides the contents out of the box.</li></ul>
Operating the instrument	Ensure that anyone who operates the instrument has: <ul style="list-style-type: none"><li>• Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.</li><li>• Read and understood all applicable Safety Data Sheets (SDSs). See “Safety Data Sheets (SDS)”.</li></ul>
Cleaning or decontaminating the instrument	 <b>CAUTION!</b> Using cleaning or decontamination methods other than those recommended by the manufacturer may compromise the safety or quality of the instrument.
Removing covers or parts of the instrument	 <b>CAUTION! PHYSICAL INJURY HAZARD.</b> The instrument is to be serviced only by trained personnel or vendor specified in the user guide. Do not remove any covers or parts that require the use of a tool to obtain access to moving parts. Operators must be trained before being allowed to perform the hazardous operation.

# Chemical safety

## Chemical hazard warning



**WARNING! CHEMICAL HAZARD.** Before handling any chemicals, refer to the Safety Data Sheet (SDS) provided by the manufacturer, and observe all relevant precautions.



**WARNING! CHEMICAL HAZARD.** All chemicals in the instrument, including liquid in the lines, are potentially hazardous. Always determine what chemicals have been used in the instrument before changing reagents or instrument components. Wear appropriate eyewear, protective clothing, and gloves when working on the instrument.



**WARNING! CHEMICAL STORAGE HAZARD.** Never collect or store waste in a glass container because of the risk of breaking or shattering. Reagent and waste bottles can crack and leak. Each waste bottle should be secured in a low-density polyethylene safety container with the cover fastened and the handles locked in the upright position. Wear appropriate eyewear, clothing, and gloves when handling reagent and waste bottles.

## General safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See “Safety Data Sheets (SDS)”)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer’s cleanup procedures as recommended in the SDS.
- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

# Chemical waste safety

## Chemical waste hazard



**CAUTION! HAZARDOUS WASTE.** Refer to Safety Data Sheets (SDSs) and local regulations for handling and disposal.

## Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.
- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.
- Handle chemical wastes in a fume hood.
- After emptying the waste container, seal it with the cap provided.
- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

## Waste disposal






If potentially hazardous waste is generated when you operate the instrument, you must:

- Characterize (by analysis, if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure the health and safety of all personnel in your laboratory.
- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.



**IMPORTANT!** Radioactive or biohazardous materials may require special handling, and disposal limitations may apply.

## Electrical safety

	 <b>DANGER! ELECTRICAL SHOCK HAZARD.</b> Severe electrical shock can result from operating the EVOS™ M5000 Imaging System without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.
Fuses	 <b>WARNING! FIRE HAZARD.</b> For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.
Power	 <b>DANGER! ELECTRICAL HAZARD.</b> Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.
	 <b>DANGER! ELECTRICAL HAZARD.</b> Use properly configured and approved line cords for the voltage supply in your facility.
	 <b>DANGER! ELECTRICAL HAZARD.</b> Plug the system into a properly grounded receptacle with adequate current capacity.
Overvoltage rating	The EVOS™ M5000 Imaging System has an installation (overvoltage) category of II, and is classified as portable equipment.

## Physical hazard safety

### Moving parts



**WARNING! PHYSICAL INJURY HAZARD.** Moving parts can crush and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing the instrument.

## Biological hazard safety



**WARNING! BIOHAZARD.** Biological samples such as tissues, body fluids, and blood of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local, state/provincial, and/or national regulations. Wear appropriate protective eyewear, clothing, and gloves. Read and follow the guidelines in these publications.

**ATTENTION! BIOHAZARD.** Les échantillons biologiques tels que les tissus, les fluides corporels et le sang des humains et d'autres animaux ont la possibilité de transmettre des maladies infectieuses. Suivre tous les règlements municipaux, provinciaux/provincial et / ou nationales en vigueur. Porter des lunettes de protection approprié, des vêtements et des gants.

### In the U.S.:

- U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories* (stock no. 017-040-00547-4; [www.cdc.gov/OD/ohs/biosfty/bmbl4/bmbl4toc.htm](http://www.cdc.gov/OD/ohs/biosfty/bmbl4/bmbl4toc.htm))
- Occupational Safety and Health Standards, Bloodborne Pathogens (29 CFR§1910.1030; [www.access.gpo.gov/nara/cfr/waisidx\\_01/29cfr1910a\\_01.html](http://www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html))
- Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.
- Additional information about biohazard guidelines is available at: [www.cdc.gov](http://www.cdc.gov)

### In the EU:

- Check your local guidelines and legislation on biohazard and biosafety precaution, and the best practices published in the World Health Organisation (WHO) Laboratory Biosafety Manual, third edition [www.who.int/csr/resources/publications/biosafety/WHO\\_CDS\\_CSR\\_LYO\\_2004\\_11/en/](http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/)



## Safety and electromagnetic compatibility (EMC) standards

This section provides information on:

- U.S. and Canadian safety standards
- European safety and EMC standards
- Australian EMC standards

### U.S. and Canadian safety standards



The **CSA C/US Mark** signifies that the product meets applicable U.S. and Canadian standards, including those from CSA, CSA America, ANSI, ASME, ASSE, ASTM, NSF and UL.

### European safety and EMC standards



The **CE Mark** symbolizes that the product conforms to all applicable European Community provisions for which this marking is required. Operation of the instrument is subject to the conditions described in this manual.

The protection provided by the instrument may be impaired if the instrument is used in a manner not specified by Thermo Fisher Scientific.

### Australian EMC standards



The **C-Tick Mark** indicates conformity with Australian and New Zealand standards for electromagnetic compatibility.

# Documentation and support

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## Related documentation

The publication numbers in this section are for the latest product versions available at the time of publication. For documentation supporting newer product versions, go to [thermofisher.com/support](http://thermofisher.com/support).

Document	Pub. No.
EVOS™ M5000 Imaging System Quick Reference Guide	MAN0017565
EVOS™ M5000 Imaging System Installation Guide	MAN0017783

## Customer and technical support

Visit [thermofisher.com/support](http://thermofisher.com/support) for the latest in services and support, including:

- Worldwide contact telephone numbers
- Product support, including:
  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including user guides, manuals, and protocols

## Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at [www.thermofisher.com/us/en/home/global/terms-and-conditions.html](http://www.thermofisher.com/us/en/home/global/terms-and-conditions.html). If you have any questions, please contact Life Technologies at [www.thermofisher.com/support](http://www.thermofisher.com/support).

