invitrogen

EVOS™ M5000 Imaging System

For Fluorescence and Transmitted Light Applications

Catalog Number AMF5000

Publication Number MAN0017563
Revision E.0

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ThermoFisher SCIENTIFIC

Information in this document is subject to change without notice.

Revision history MAN0017563

Revision	Date	Description
E.0	14 December 2022	Update Connect account sign in, add information about Align Channels tool, update relevant screens.
D.0	01 April 2021	Add information about Transfection Efficiency calculation and Batch Analysis, update Appendix B – Review tab, update the screens throughout the document.
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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Manufacturer: Life Technologies Corporation | 22025 20TH Ave SE | Bothell, WA 98021.

Trademarks

Contents

About this guide

Audience

This user guide is for laboratory staff operating, maintaining, and analyzing data using the Invitrogen™ EVOS™ M5000 Imaging System.

User attention words

Two user attention words appear in this document. Each word implies a specific level of observation or action as described below.

Note: Provides information that may be of interest or help but is not critical to the use of the product.

IMPORTANT! Provides information that is necessary for proper instrument operation, accurate installation, or safe use of a chemical.

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.

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

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.

1. Product information

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 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, TIFF, and RAW TIFF formats, or compiled into a video sequence in AVI or MP4 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:** Allow 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)
- Manual X-Y stage
- 5-position objective turret
- Condenser with 4-position turret
- 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

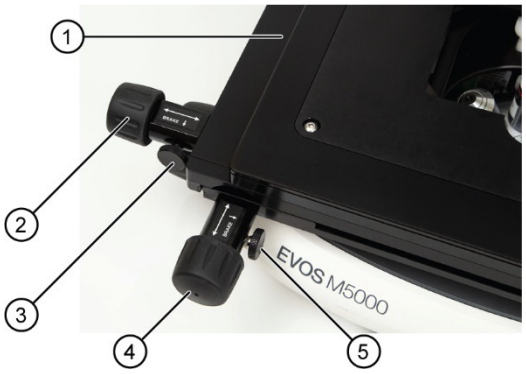
- EVOS™ M5000 Quick Start Guide, printed (Pub. No. MAN0017765)
- EVOS™ M5000 Imaging System Installation Guide, printed (Pub. No. MAN0017783)
- Pre-loaded to USB 3.0 flash drive (located in the accessories box):
  - EVOS™ M5000 User Guide (Pub. No. MAN0017763)
  - EVOS™ M5000 Quick Start Guide (Pub.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

Instrument exterior components and mechanical controls

Front view

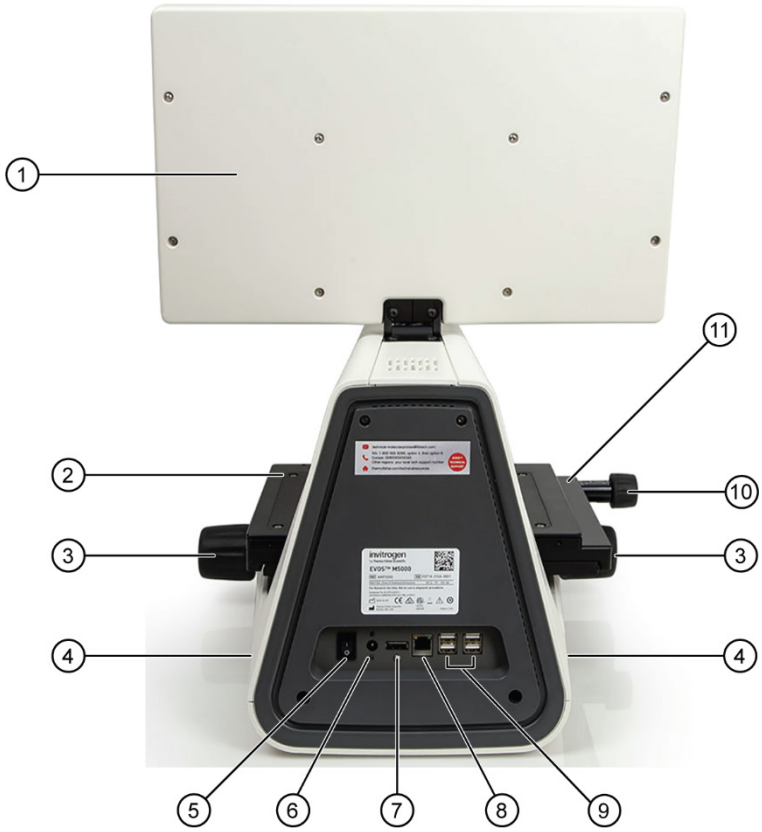
1. LCD monitor
2. Condenser
3. Condenser light shield
4. Mechanical X-Y stage
5. Stage X-axis positioning knob
6. Stage Y-axis positioning knob
7. Focusing knobs
8. Objective selection wheel
9. Vessel holder
10. USB-A 3.0 port
11. Objective
12. Phase annuli selector

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



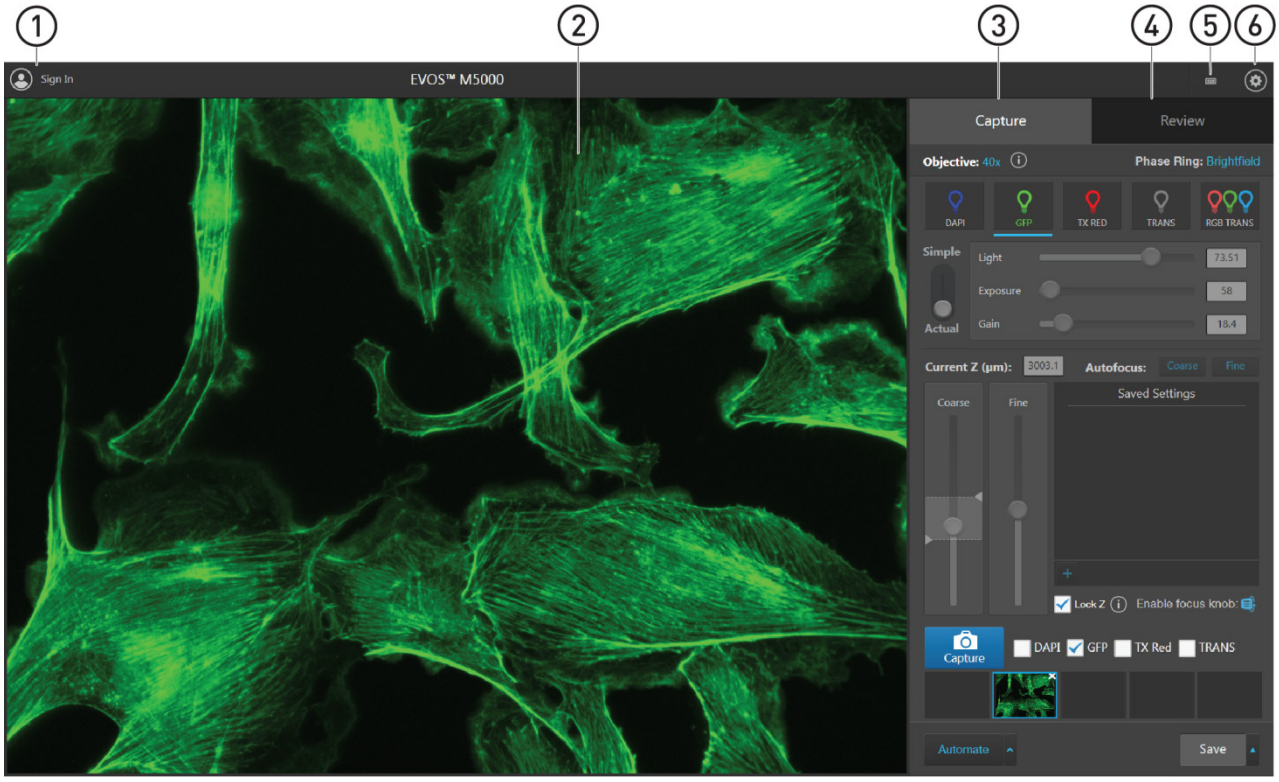
1. LCD monitor
2. Mechanical X-Y stage
3. Focusing knobs
4. USB-A 3.0 port (2×)
5. Power switch
6. 4-pin power input port (12 VDC, 5 A)
7. Display port (video output)
8. Network port
9. USB-A 2.0 ports (4×)
10. Stage X-axis positioning knob
11. Stage Y-axis positioning knob*

* 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.

Note: For more information and detailed descriptions of GUI controls, see "Appendix B: Graphical user interface (GUI)", page 101.

Note: For more information on the EVOS™ Image Analysis application, see "Appendix E: EVOS™ Image Analysis", page 129.

2. Installation

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 9). 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

Receive and inspect the shipment

1. Verify that the items shown on the shipping list are the same items that you ordered at the time of purchase.
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.

Move the instrument to the installation site

1. Clear the installation site of all unnecessary materials.
2. If possible, move the crated instrument and other shipping containers to the installation site.

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

Unpack the instrument

1. Open the shipping box and remove the accessory box.
2. Carefully lift the instrument out of the box by grasping it firmly with both hands under the support arm.
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.
4. Tilt the LCD monitor upright.
5. Examine the instrument carefully for damage incurred during transit.
6. Unpack the accessories box and verify all parts are present. See page 6 for the list of standard items included in the shipment.

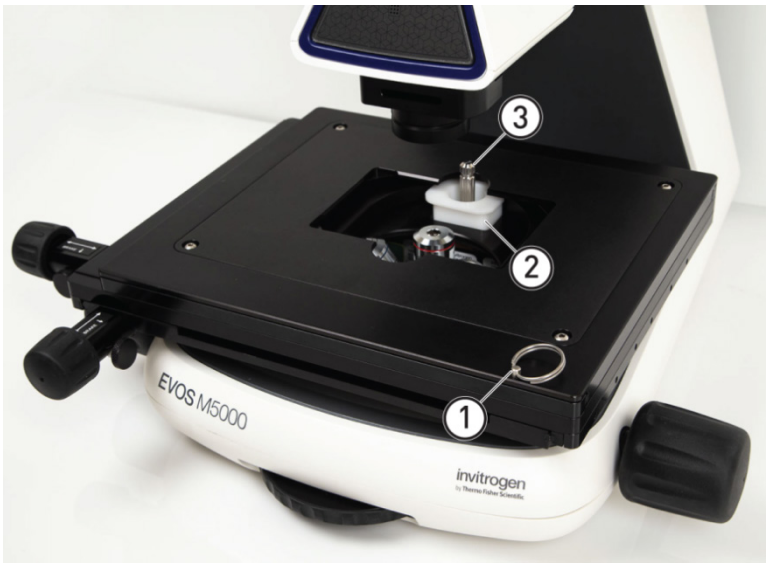
IMPORTANT! Do not subject the EVOS™ M5000 Imaging System to sudden impact or excessive vibration. Handle the instrument with care to prevent damage.

Note: Contact your distributor if anything is missing. If you do not have your distributor information, contact Technical Support (page 141). Damage claims must be filed with the carrier; the warranty does not cover in-transit damage.

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.



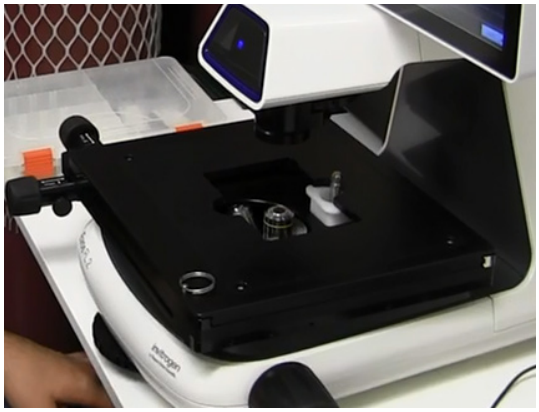
① Stage lock pin

② Light cube shipping restraint

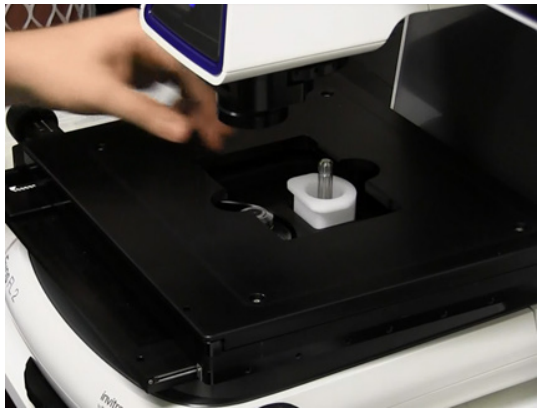
③ Light cube tool

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.



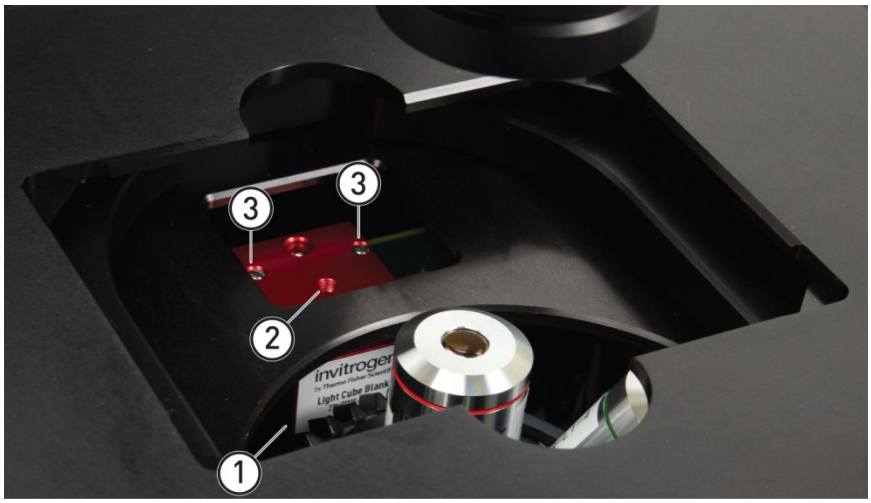
Before stage reposition



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

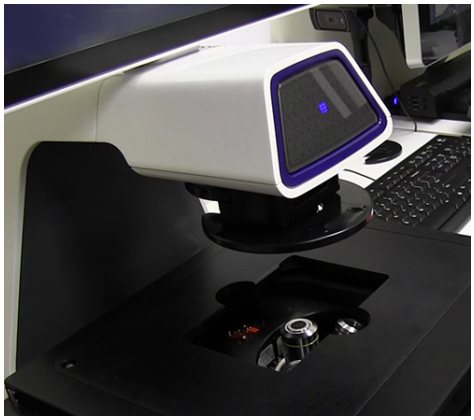
5. Using the light cube tool, loosen the two screws that secure the blank light cube to the instrument. You do not need to remove the screws.
6. Screw the light cube tool to the thread hold on the light cube, then lift the blank light cube up and out of the light cube turret. Store the blank light cube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

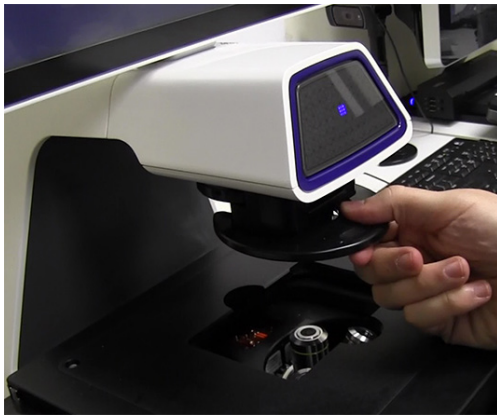
Note: Store the shipping restraints and the light cube tool for future use in the accessories box provided with your system. Always re-secure the X-Y stage with the stage lock pin and re-install the light cube restraint before moving the instrument.

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 the manufacturer's warranty.

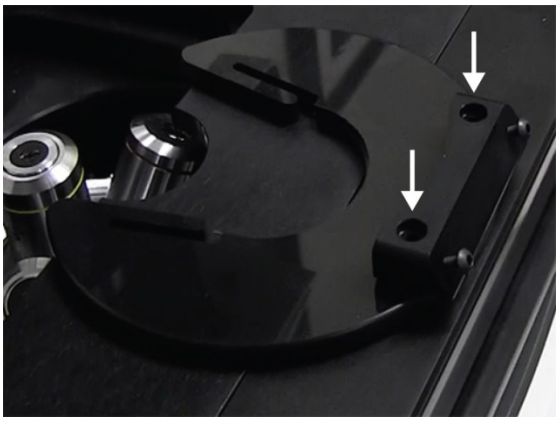
Install the UV light shield

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.

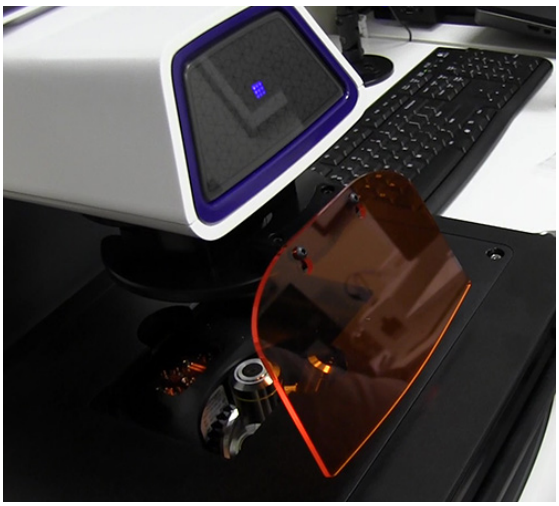




3. Secure the UV light shield mount to the top of the condenser light shield using the two screws supplied with the UV shield assembly (not the protruding screws on the mount).



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.

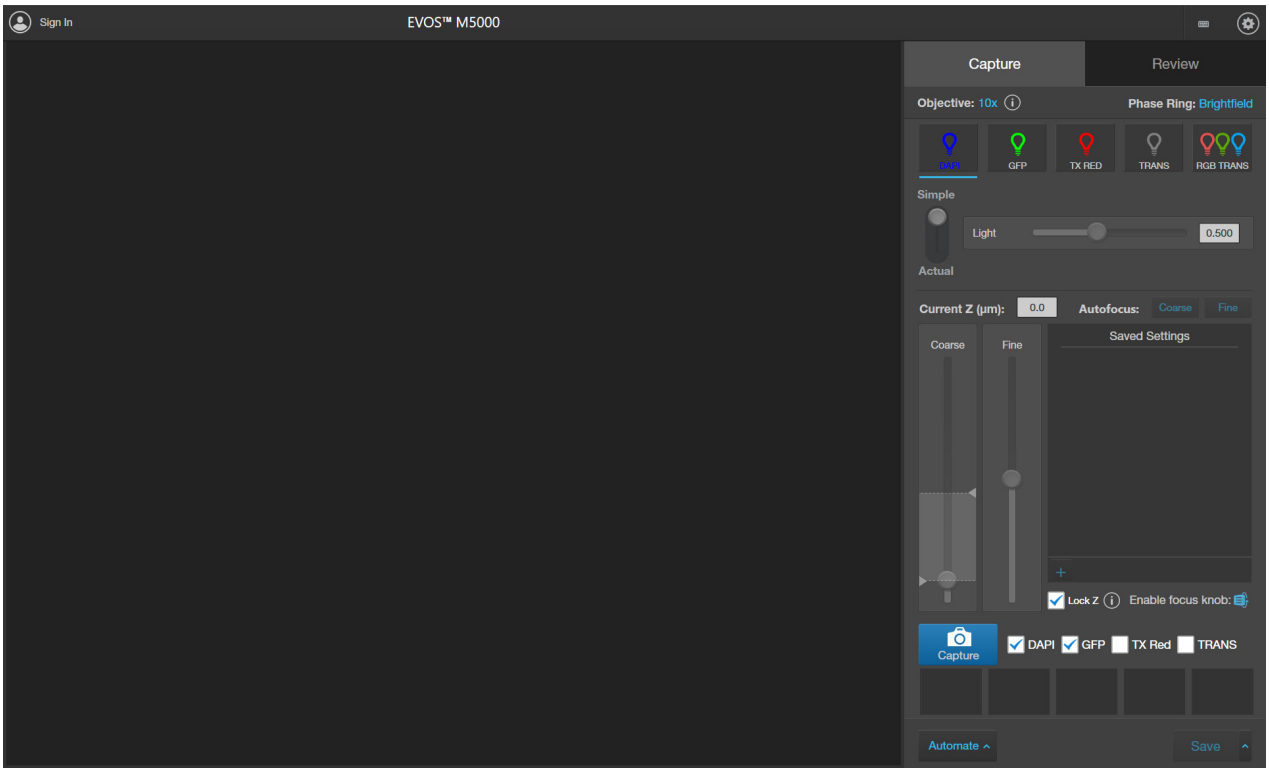
Connect the instrument

1. Confirm that the power switch is OFF (located on the back; see page 9).
2. Connect the USB receiver for the wireless mouse (located inside the flap of the mouse box) and keyboard to an available USB-A 2.0 port (page 9).

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 9).
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 9) 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 13).

Connect the instrument to the internet

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 **Error! Bookmark not defined.**).

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 88.

Set date and time

For instructions on how to configure the date and time to the local time, see "Set date and time", page 91.

Connect to the Thermo Fisher Connect Platform

About the Thermo Fisher Connect Platform

The Thermo Fisher Connect Platform, Thermo Fisher's cloud-based platform, enables access to your EVOS™ M5000 instrument through Instrument Connect by way of a web browser or mobile device. Connecting to your Connect account allows you to save captured images in your unique user account in addition to local storage.

Create a Connect account

1. Go to **thermofisher.com/connect** from your web browser.
2. Click **Sign up now** and follow the prompts to create an account.

   Your e-mail address is used as your username.
3. When signed in, click **Update PIN number**.
4. Enter a PIN number in the **new** and **confirm** fields.

   The PIN number is necessary to sign in to Connect from the instrument.

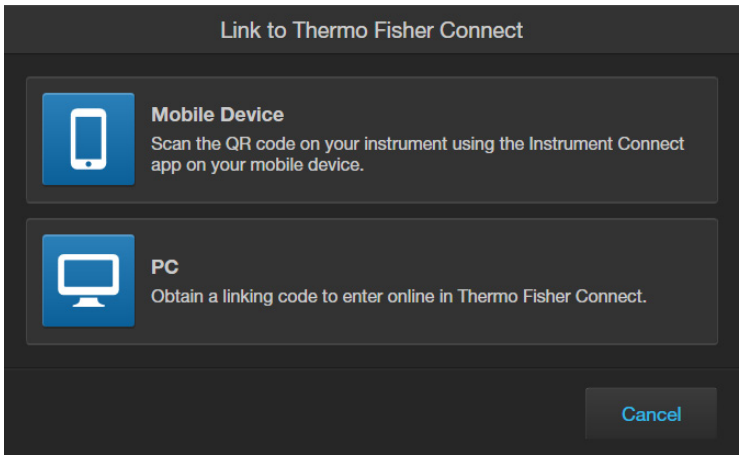
Create a PIN number

1. Log in to your Connect account using a web browser.
2. Navigate to **(Instrument Connect)**.
3. Select **Update PIN number**.
4. Confirm the PIN number.

Link instrument to your Connect account

You can link the EVOS™ M5000 instrument to your Connect account using one of the following options:

1. **Mobile Device (QR code):** Scan the QR code on your instrument using the Instrument Connect application on your mobile device (page 18).
2. **PC (linking code):** Obtain a linking code to enter online in Thermo Fisher Connect (page 18).

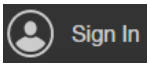


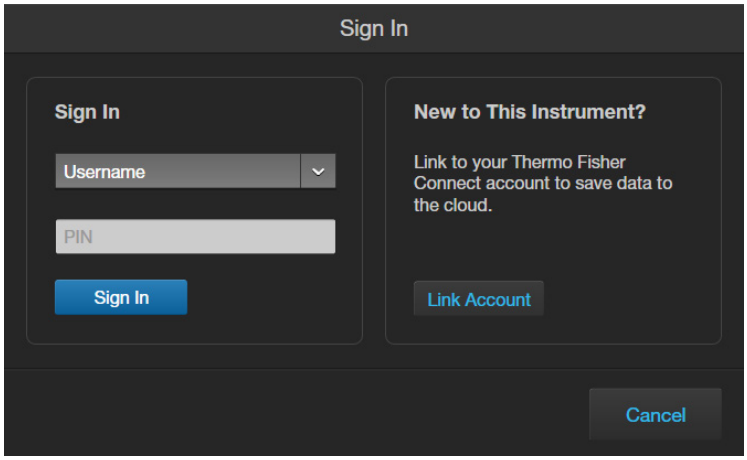
When the instrument is linked, you can save captured images in your unique user account in addition to local storage.

IMPORTANT! The first Connect account that links to the instrument becomes Administrator by default. If the first user needs to be unlinked from the instrument, a new user must be assigned the Administrator role beforehand. Failure to do so will result in the loss of instrument connectivity for all other linked users. For instructions on how to setup a new Administrator see "Set up a new Administrator" on page 19.

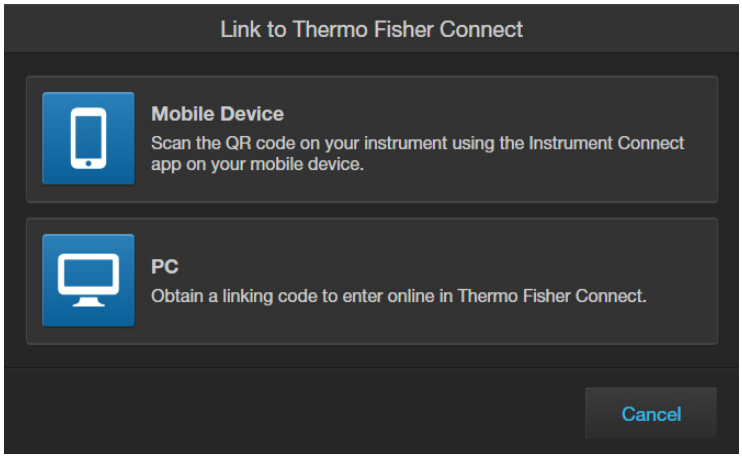
Add instrument to your Connect account with QR code (Mobile Device)

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





2. Click **Link Account**, then select **Mobile Device**.



3. Scan the QR code on your instrument using the Instrument Connect application on your mobile device.

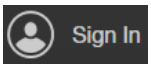
Add instrument to Thermo Fisher Connect with linking code (PC)

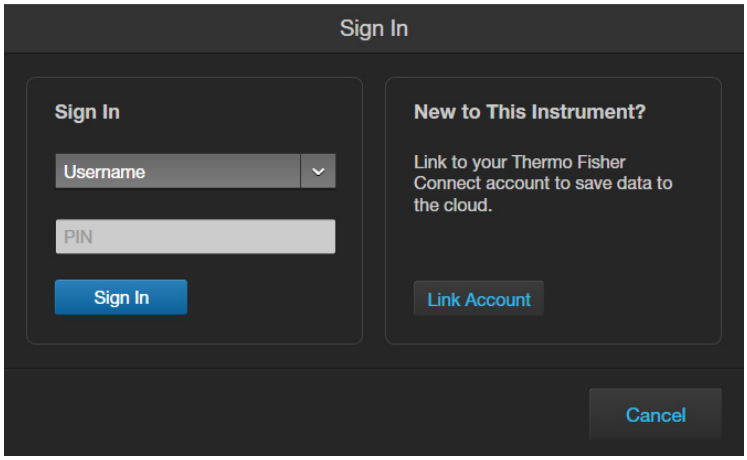
1. Click the **Sign In** button on the top left corner of the screen to open the Sign In dialog.
2. Click **Link Account**, then select **PC**. Note the linking code provided by the instrument.
3. Log in to your Connect account using a web browser.
4. Select **(InstrumentConnect)** from the left navigation strip.
5. Select **(Add an Instrument)** from the top navigation strip.
6. Select **EVOS™ M5000** from the **Instrument type** dropdown menu, then click **Next**.
7. Enter the linking code generated by the instrument in the text box, then click **Send**.

   Upon successful authentication, the instrument is linked to Connect.

Sign in to your Connect account from the EVOS™ M5000 instrument

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



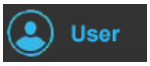


Note: If another user account is displayed, select the username to sign out and connect a different user account.

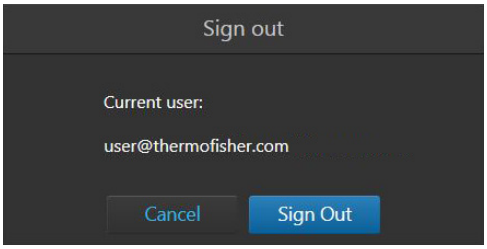
2. Select your user name from the list of linked accounts in the **Username** dropdown.
3. Enter your Connect **PIN** number.

   If you do not have a PIN number, set the PIN number in the Instrument Connect application (page 17).
4. Click **Sign In**.

   When you connect to your Connect account, the Sign In button changes to display your user name.



5. 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 Connect account 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 129.

Set up a new Administrator

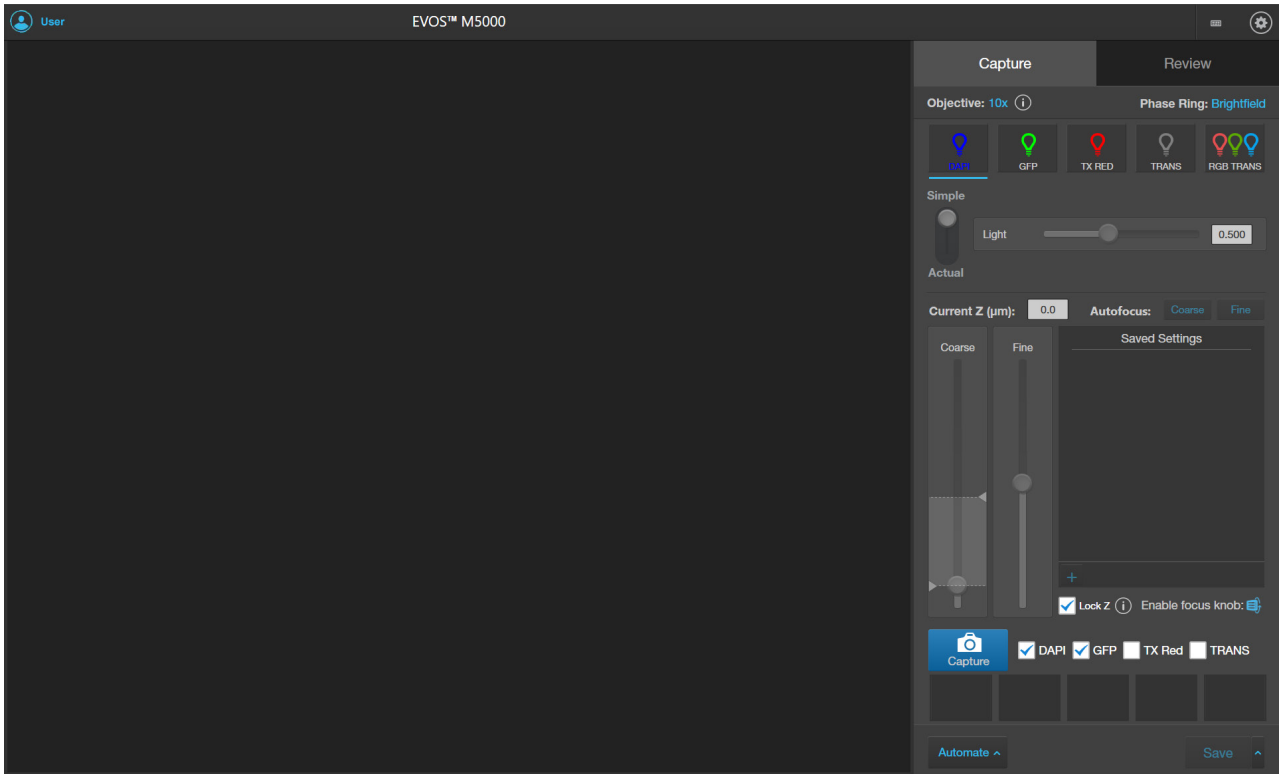
1. Log the current Administrator into their Connect account.
2. Select **Instruments**.
3. Select the EVOS™ M5000 instrument the user is linked to.
4. Select **Manage users** from the top navigation strip.
5. Set Administrator privileges to another user linked to the same instrument.

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

Select objective and light source

▼

Adjust brightness

▼

Focus on the sample

▼

Find the region of interest

▼

Capture image in a single channel

▼

Analyze and annotate captured images

▼

Save

Capture images in a single channel

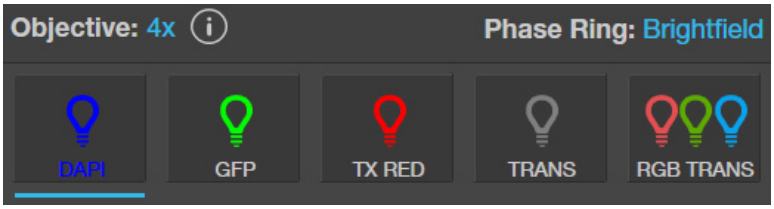
Select objective and light source

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 8).
3. Select the desired **Phase** option by turning the phase annuli selector (page 8). Available options are:
   - **Oly 4×:** Used for low magnification objectives (Olympus™ 4× PH)
   - **4×/10×:** Used for medium magnification objectives (EVOS™ 4×/10× PH)
   - **20×/40×:** 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.

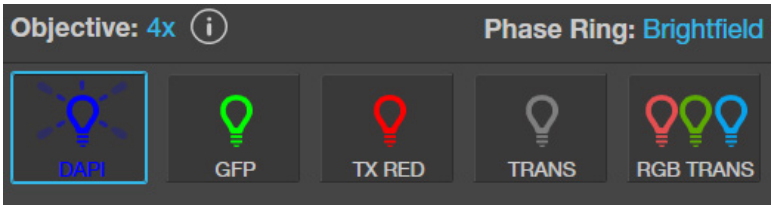


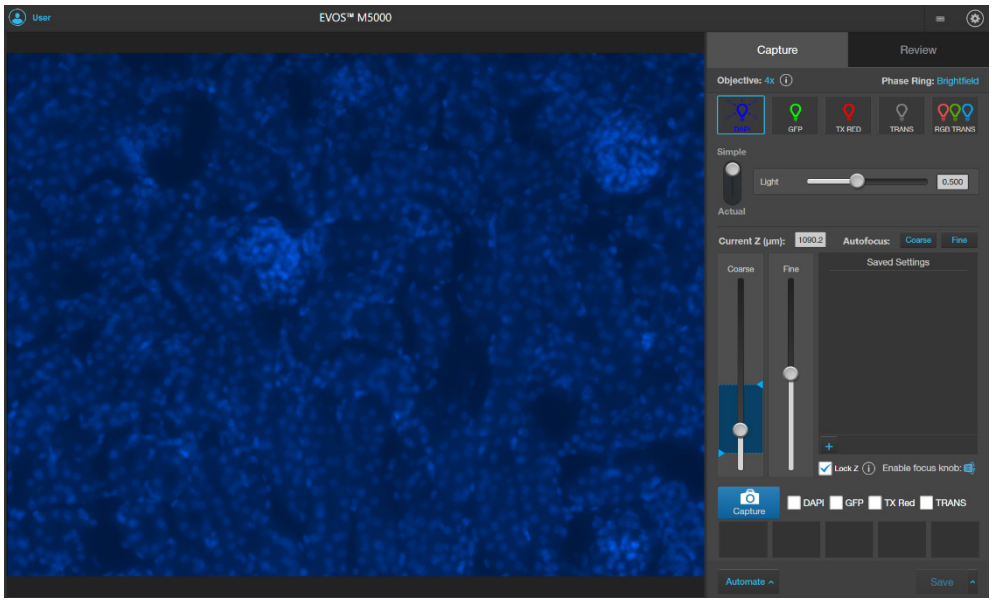
   Note: If the phase ring correctly matches the objective, the selected phase ring is depicted in blue. If the objective and the phase ring are mismatched, the phase ring is depicted in orange.

4. Select a **Channel** to turn on the excitation light and enter the instrument in the **Live mode**.

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

   In this example, the DAPI channel has been selected.



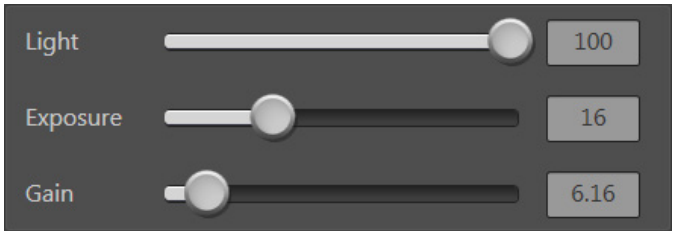


Note: You can only select a single light source for Live mode when adjusting brightness and focus. However, you can display and capture multiple channels simultaneously.

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 directly in the corresponding field. Note that the allowed values for Light, Exposure, and Gain depend on the selected objective and channel.



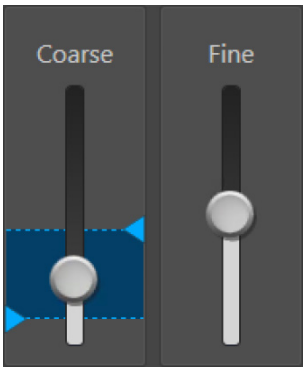
Note: Optimize the brightness parameters as follows:

- **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**, 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 Pixels** 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.

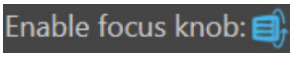


Alternatively, click **Coarse** and **Fine autofocus** to autofocus on the sample.

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.

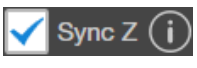
Note: You can also focus on the sample manually using the **focusing knobs** on the instrument (page 8).

Enable focus knob toggles the manual focus knob function on and off.



If the function is turned off, click **Enable focus knob,** then turn the focusing knob until the region of interest is in focus.

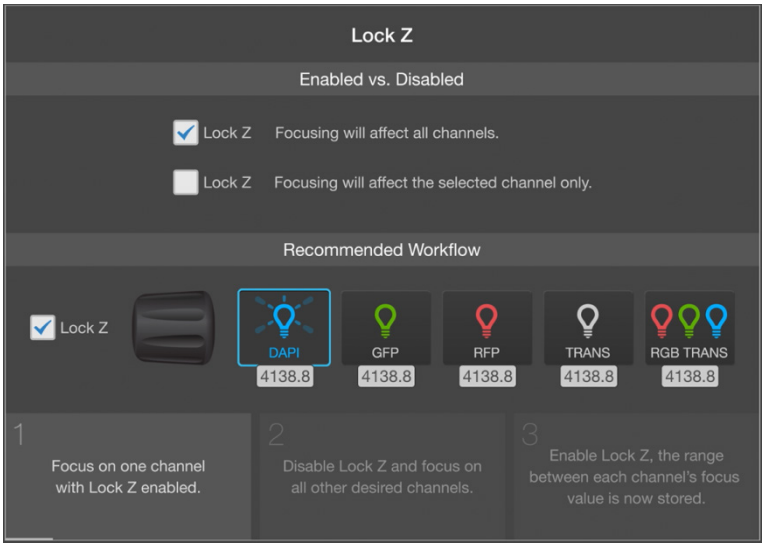
3. Repeat the focus procedure for each channel you want to capture.
4. If you want to preserve the Z-Offsets between the channels, select **Synch Z**.



- When synched, focusing in one channel affects all channels.
- When unsynched, 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.

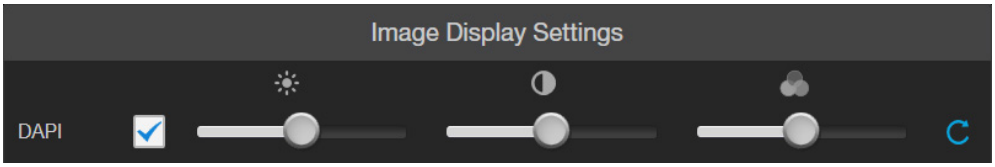
Click  next to Synch Z selection to review the recommended workflow for Z-Offset set-up.



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. 

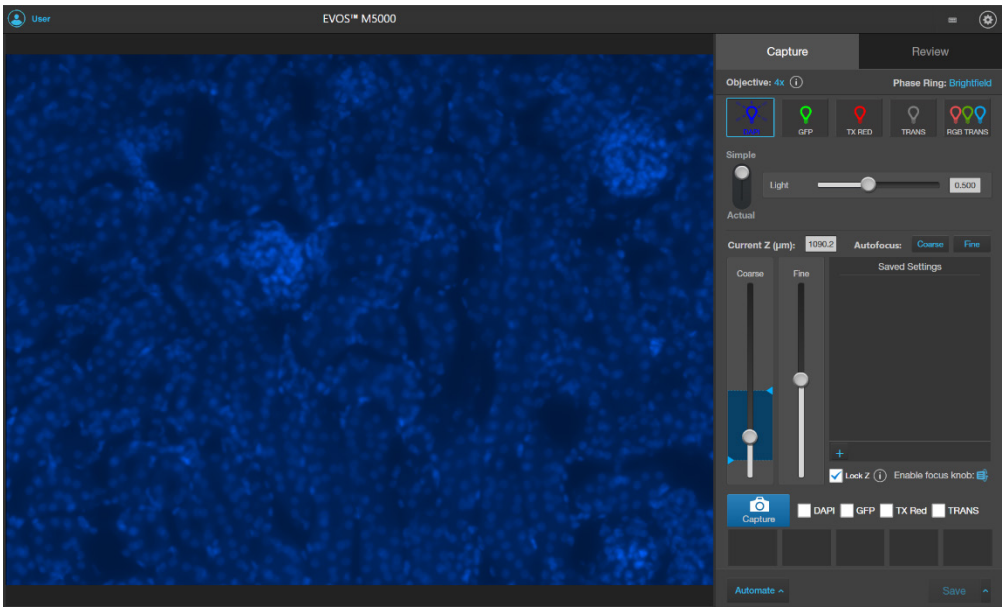


3. Adjust **Brightness**  , **Contrast**  , and **Gamma**  using the corresponding sliders.
4. Click the **Reset** button  to return the display settings to their default values.
5. Click the **Image Display Settings** button again to collapse the controls.

Note: In color overlay images, varying the amount of gamma correction changes not only the brightness, but also the ratios of red to green to blue. This is to account for the fact that you can change gamma for individual monochrome-displayed channels in the EVOS™ M5000 software as well (e.g., the transmitted light channel).

Find the region of interest

1. Use the **X-axis** and **Y-axis positioning knobs** (page 8) 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.

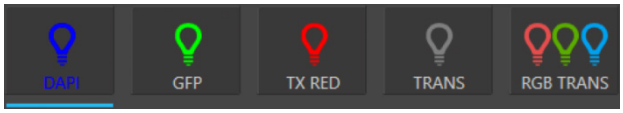
Note: If desired, enable **Quick Save** (page 52) before proceeding to image capture. Quick Save function allows you to set the save options in advance and save your captured images with a single click directly from the Capture tab or automatically after each capture.

Capture image in a single channel

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

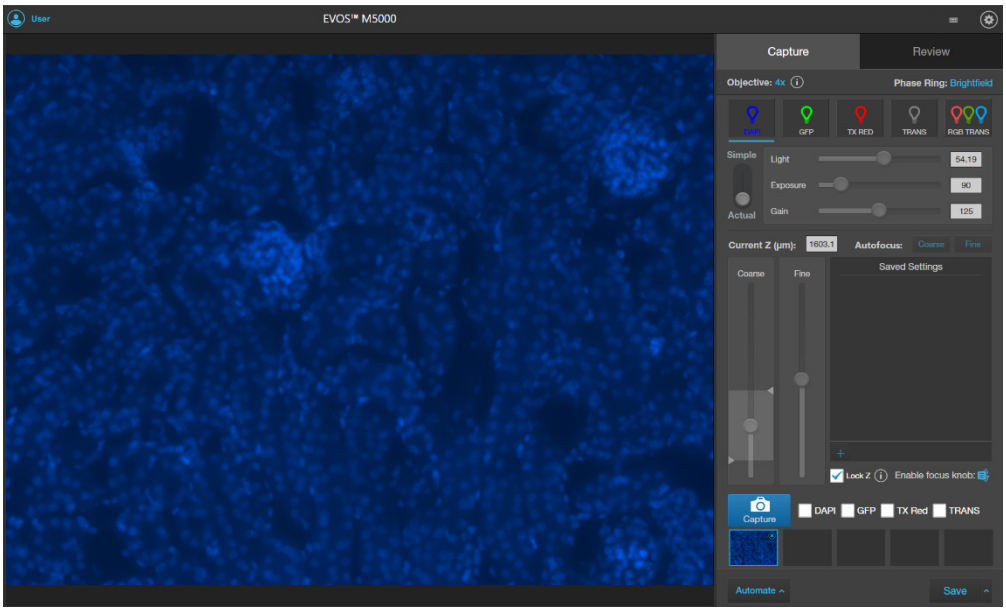
When you select a **Channel**, the corresponding **Capture channel** box is automatically checked.

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.



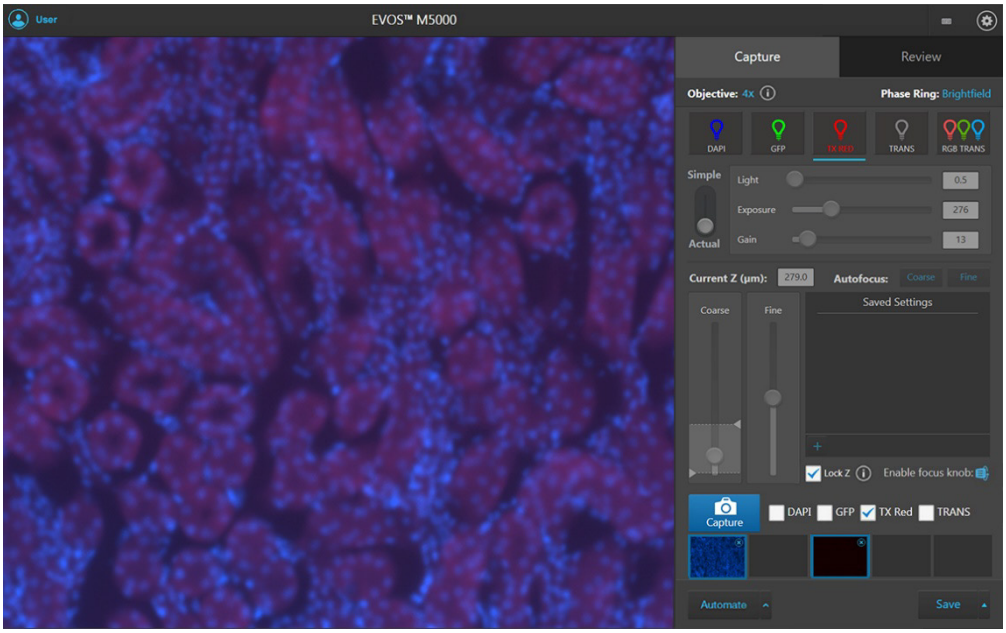
A thumbnail of the captured image is displayed for the selected channel (in this example, DAPI).

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.

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

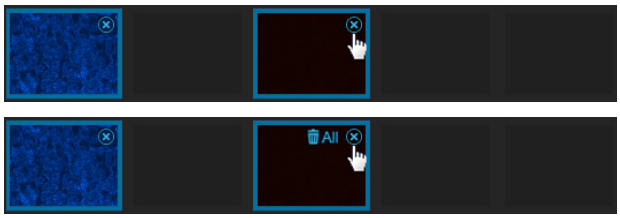
4. If needed, re-adjust the brightness and focus, then click **Capture**.

   The viewing area shows the image captured in the new channel superimposed on the image captured in the previous channel.

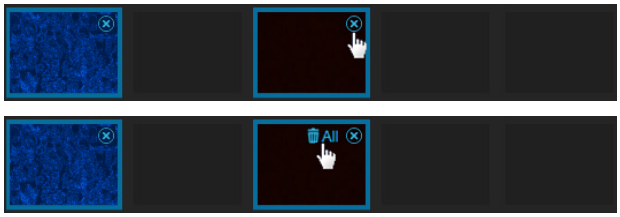


   A thumbnail of the image captured in the new channel is displayed along with the thumbnail of the image from the previously captured channel.

5. 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.



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



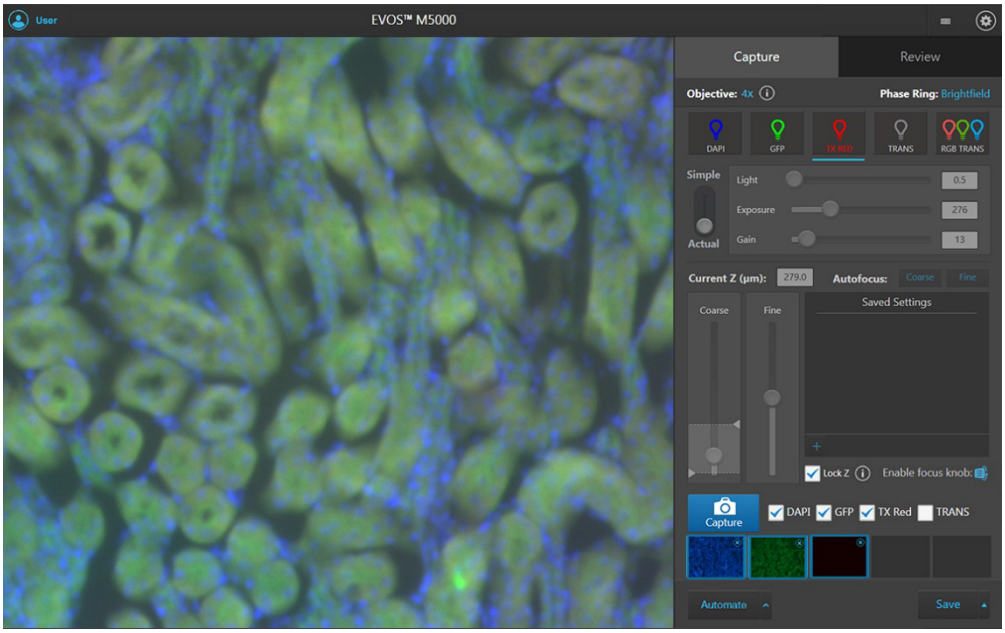
Capture images in multiple channels

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, correct pixel shifts that can occur at higher magnifications in multichannel fluorescent images, annotate captured images, and perform cell count, measure confluence, and calculate transfection efficiency.

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. Using the Display settings and analysis tools, you can:
   - Configure display settings (page 30)
     - Adjust image display settings (page 30)
     - Display grid (page 31)
     - Display scale bar (page 32)
   - Align channels (page 33)

Note: The button to display the Align Channels tool (indicated by the red arrow) is visible only if the **Show Align Channels in Display Settings and Analysis Tools** option is selected in the **Settings ▸ General** tab (page 87).

   - View pixel intensity histogram (page 34)
   - Add and show measurements and annotations (page 35)
   - Analyze cell culture (page 37):
     - Perform Auto Count (page 38)
     - Perform Manual Count (page 42)
     - Measure confluence (page 44)
     - Calculate transfection efficiency (page 47)
   - Save analysis results (page 76)

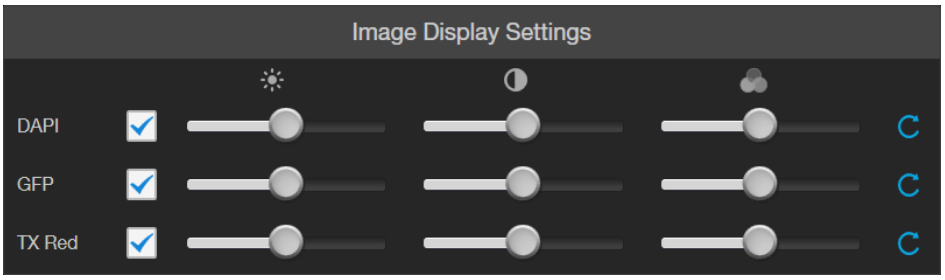
IMPORTANT! Changes made to images in the Viewing area with the display settings and analysis tools, including changes made to image parameters, display of the grid and the scale bar, as well as any annotations and measurements, persist when the images are saved.

To save raw image data for image analysis, select the **Save individual channels** option when saving captured images (page 50).

Configure display settings

Adjust 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.

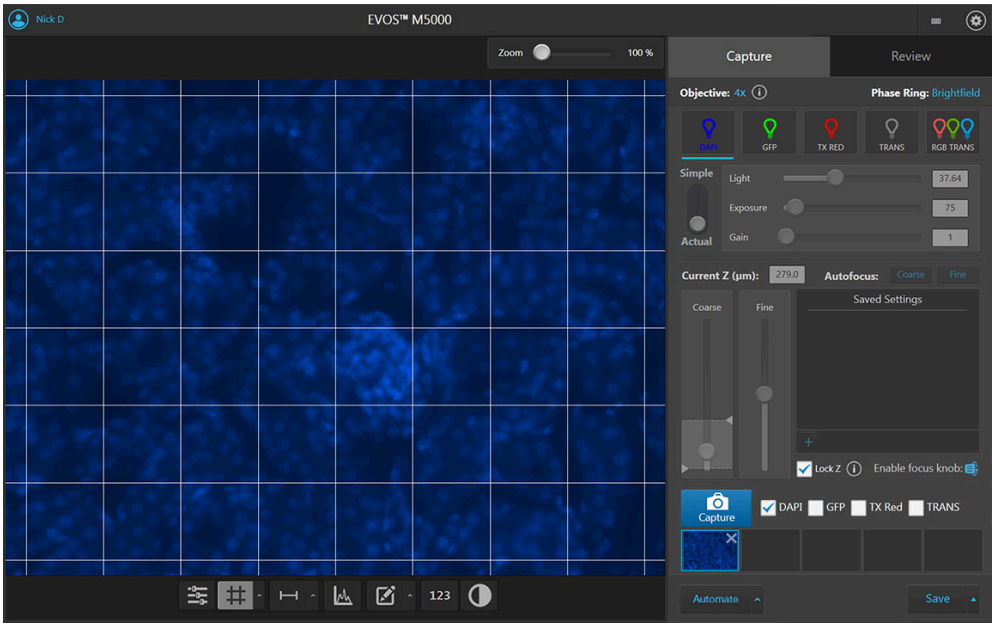
   To display a channel with a captured image that is not shown in the Viewing Area, re-select the checkbox.

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.

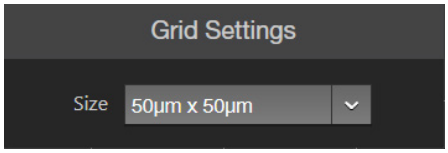
> **Note:** In color overlay images, varying the amount of gamma correction changes not only the brightness, but also the ratios of red to green to blue. This is to account for the fact that you can change gamma for individual monochrome-displayed channels in the EVOS™ M5000 software as well (e.g., the transmitted light channel).

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.

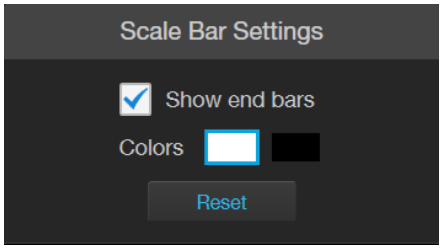


3. Select the **Size** for the grid. Available grid sizes depend on the magnification of the selected objective.
4. Click the **Grid Settings** button again to save your settings and close the tool.

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.
5. 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.

6. To adjust the length of the scale bar, hover your pointer over the scale bar until a bounding box appears, then the 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.

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

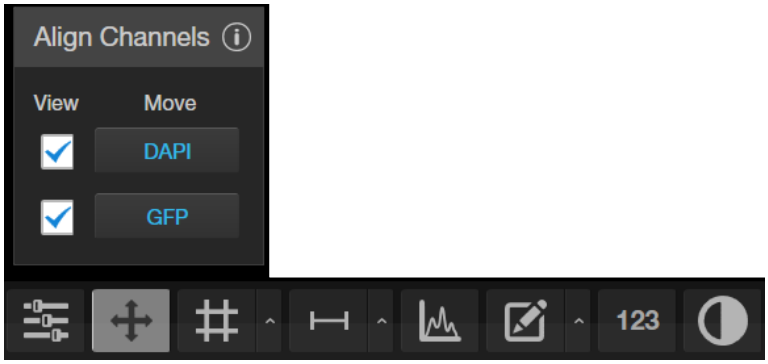
Align channels

Align channels

Align Channels tool allows you to correct pixel shifts that can occasionally appear when performing multichannel fluorescence imaging at higher magnifications. You can select individual channels in a multichannel image, move them to the correct position relative to other channels, and then save the corrected image.

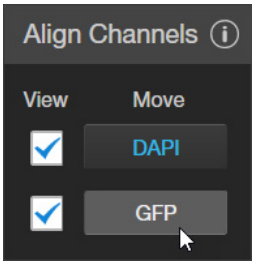
Note: The button to display the Align Channels tool is visible only if the **Show Align Channels in Display Settings and Analysis Tools** option is selected in the **Settings ▸ General** tab (page 87).

1. Click the **Align Channels** button to expand the controls for the Align Channels tool.



Note: The controls for the Align Channels tool are contextual. The tool only displays the controls for the channels in which the selected multichannel fluorescent image has been captured. In this example procedure, the multichannel image was captured in the DAPI and GFP channels.

2. Select the channel for which you wish to correct pixel shifts. In the following example, the GFP channel is selected.



3. *Optional*: To remove a channel from displaying in the Viewing Area, unselect the corresponding **View checkbox**.

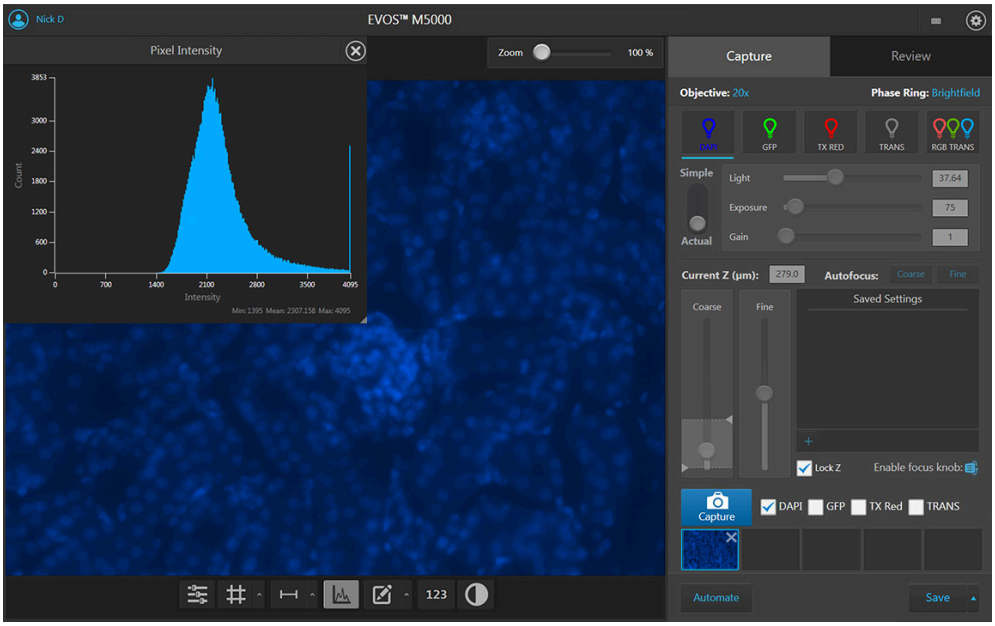
   To display a channel with a captured image that is not shown in the Viewing Area, re-select the corresponding View checkbox.

4. Use the keyboard arrow keys to move the image in the selected channel to the desired position to correct for the pixel shift.
5. If the multichannel image was captured in more than two channels, repeat the process for the other channels until all the channels are correctly aligned.
6. Click **Save** to save the corrected image, then click the **Align Channels** button to hide the channel alignment controls.

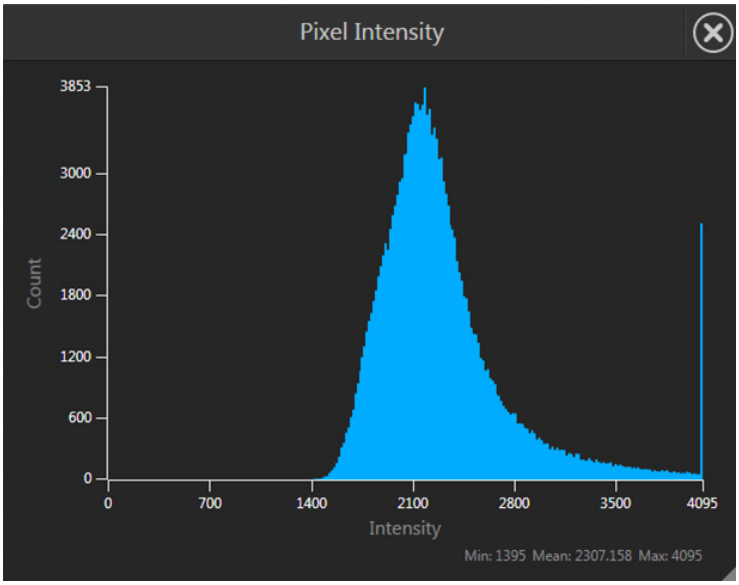
View pixel intensity histogram

Display histogram

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



2. The Pixel Intensity histogram shows the Pixel count vs. Intensity data of the image displayed in the Viewing area as well as the minimum, mean, and maximum pixel intensities.

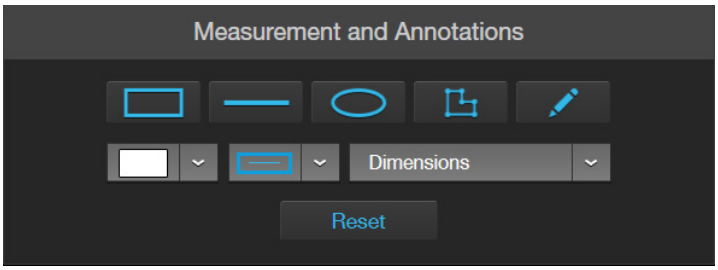


3. To move the histogram, click within the plot heading area and drag the plot to the desired location.
4. To resize the histogram, click the grey triange at the lower right corner of the plot, then drag the plot to the desired size.
5. Click the **Histogram** button again to close the Pixel Intensity histogram.

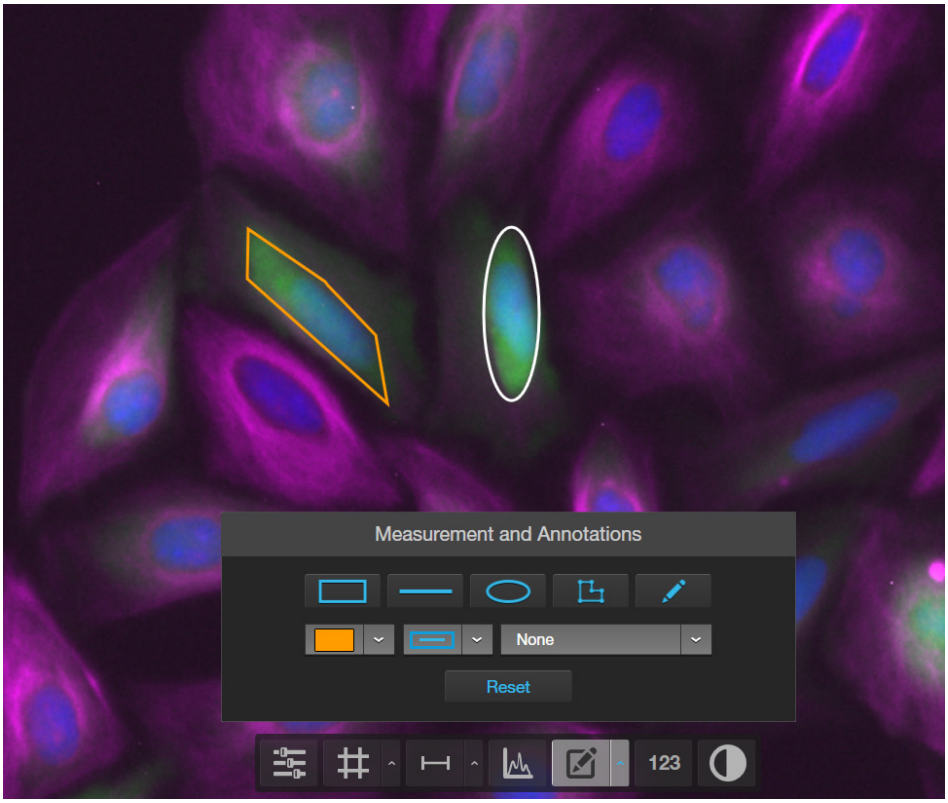
   Alternatively, click the **X** on the plot to close the histogram.

Add measurements and annotations

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

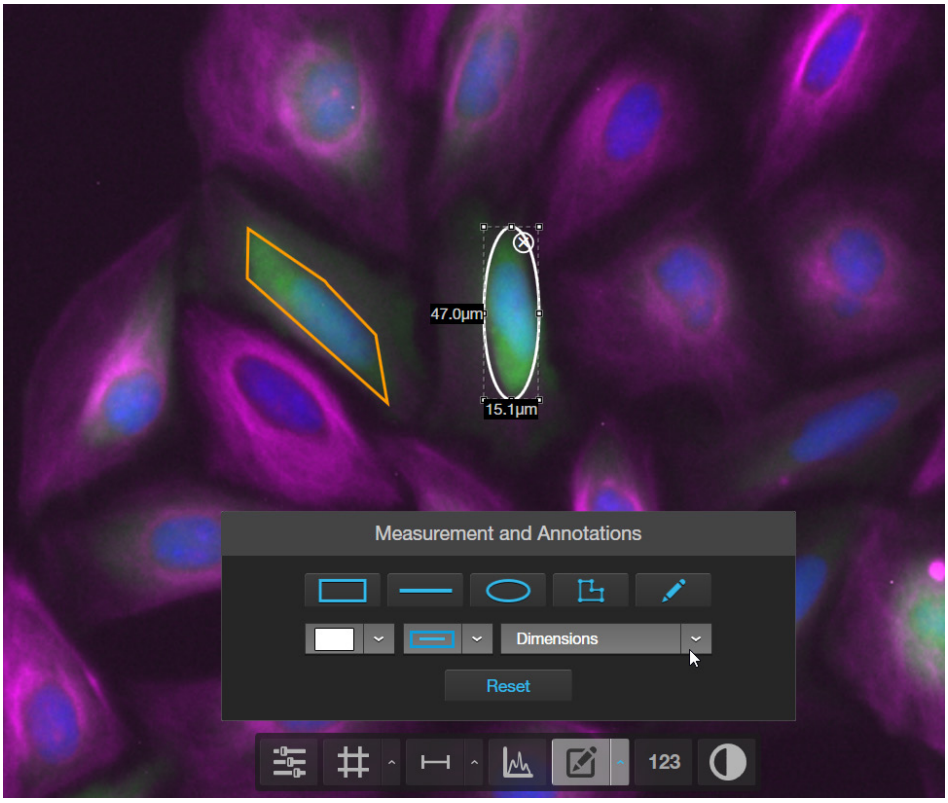


2. Using the **Annotations** tools, draw a **rectangle**, **line**, **ellipse**, **polygon**, or a **free-form** shape over the region of interest on the Viewing area. You can draw multiple shapes of different type.



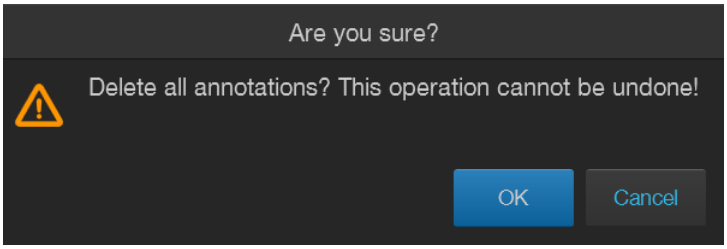
3. If needed, change the **Color** and **Thickness** of the annotation to make it more visible over the image.

4. If desired, select to display the **Dimensions**, **Area**, or **Perimeter** information for the selected 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.

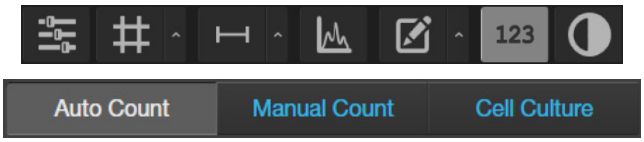


6. Click the **Measurement and Annotations Tools** button (arrow on the split button) again to close the tools.
7. After you have added measurements and annotations to your image:
   - Click **Show Measurements and Annotations** button (main part of the split button) to turn the display on and off.
   - Click **Measurement and Annotations Controls** button (the arrow on split button) to display the controls to add new measurement and annotations or to delete existing ones.

Analyze cell culture

Analysis tools

Hover the pointer over the Viewing area to reveal **Display Settings and Analysis Tools**, then click the **Show Cell Count (123)** button to display **Auto Count**, **Manual Count**, and **Cell Culture** options in the tabs area.



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

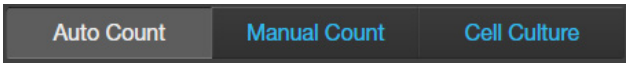
- **Auto Count:** Automatically counts the objects displayed in the Viewing area based on your specifications (page 38). With Auto Count, you can count objects only in a single fluorescence channel (nuclear stain channel).
- **Manual Count:** Allows you to tag objects in the Viewing area with up to six labels. As you tag objects, the system keeps a running tally of the counts with percentages for each label assigned (page 42). With Manual Count, you can count objects in multiple channels simultaneously.
- **Cell Culture:** Allows you to measure the confluence of your culture and calculate the transfection efficiency.
  - **Confluence:** Allows you to select up to five 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 42).
  - **Transfection Efficiency:** Allows you to estimate the transfection efficiency of your culture by calculating the ratio of fluorescence area (i.e., cells expressing the fluorescence marker) to the total cell area in your culture (page 47).
- **Batch Analysis:** Allows you to save and apply the analysis parameters set in Auto Count, Confluence, and Transfection Efficiency tools to other images that you have collected and saved an image folder (page 78). Batch Analysis is not available for Manual Count.

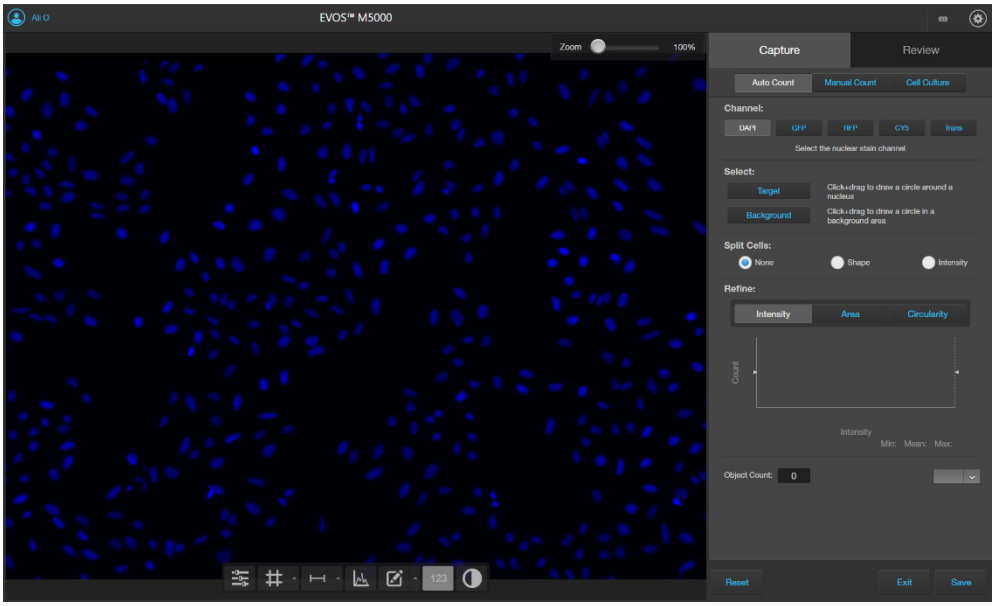
IMPORTANT! For analysis, only use 16-bit Raw image files (TIFF RAW). Raw image files contain the full dynamic range and metadata needed for quantitative analysis, whereas Display and Merged image files do not.

Count cells – Auto Count

Perform Auto Count

1. Click the **Show Cell Count (123)** button, then select **Auto Count**.

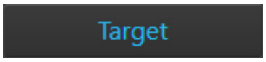


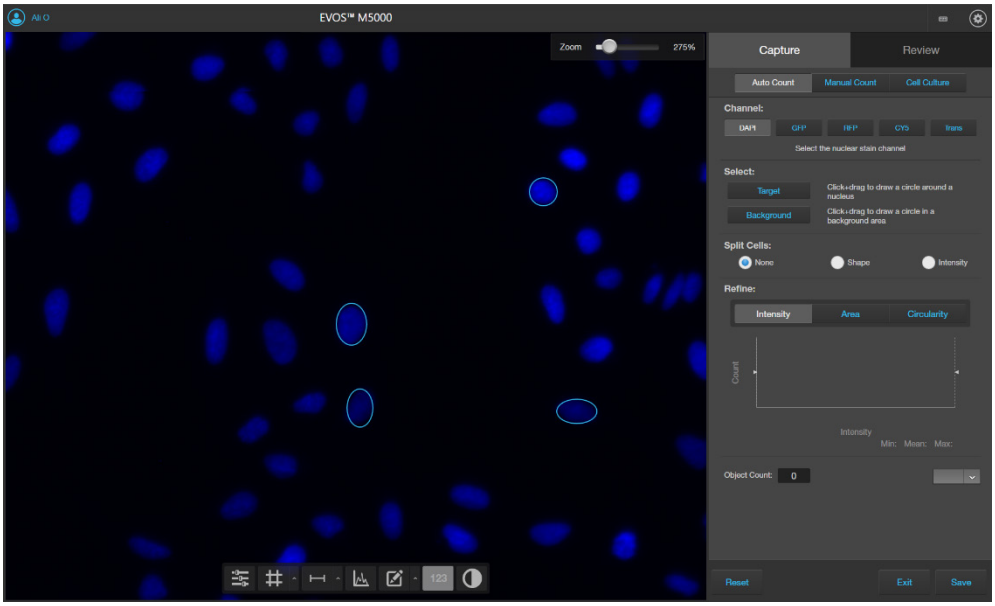


2. If counting from the Capture tab, select the **Channel** in which to count objects.

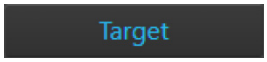
   Available options depend on the channels used when the image was captured. In this example, all channels contain captured images, and DAPI is selected for auto count.

3. To identify targets, click **Target**, then click and drag to draw a circle (blue) around a representative target.





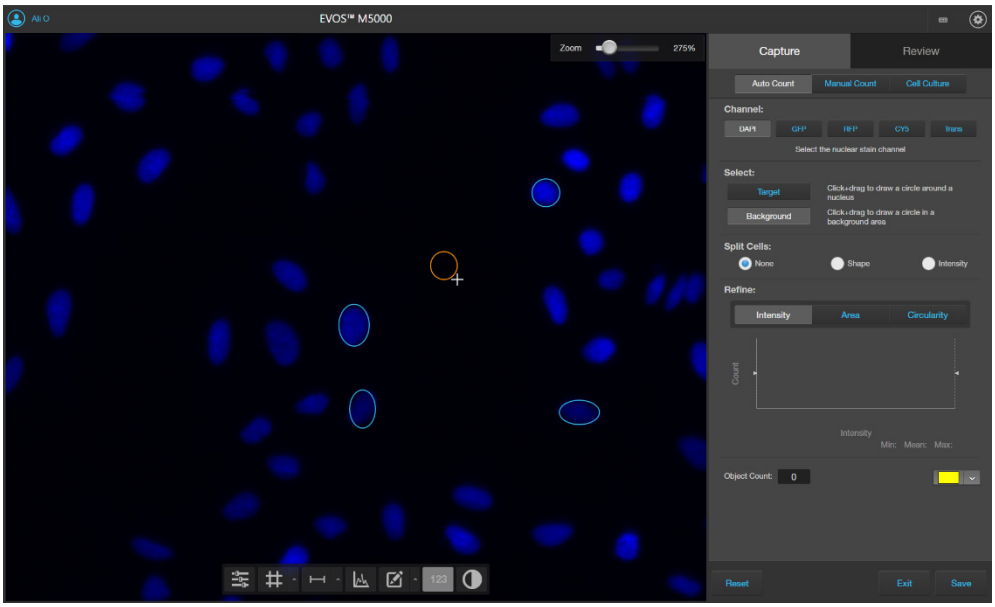
4. If needed, click **Target** again to identify other targets (for example, nuclei that might appear different) to improve the accuracy of your count.



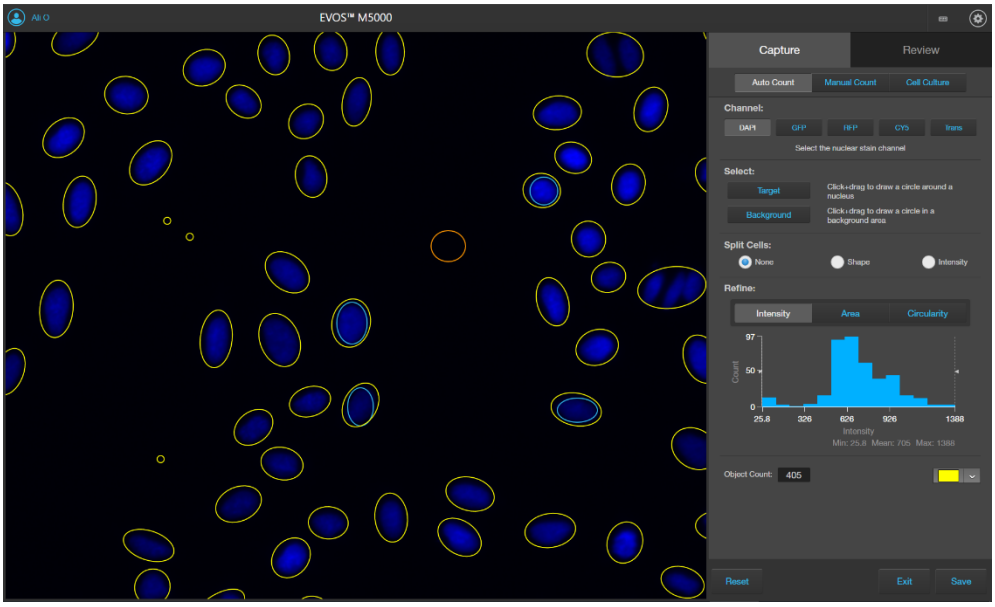
Note: For best results, follow these guidelines when identifying target objects:

- 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.

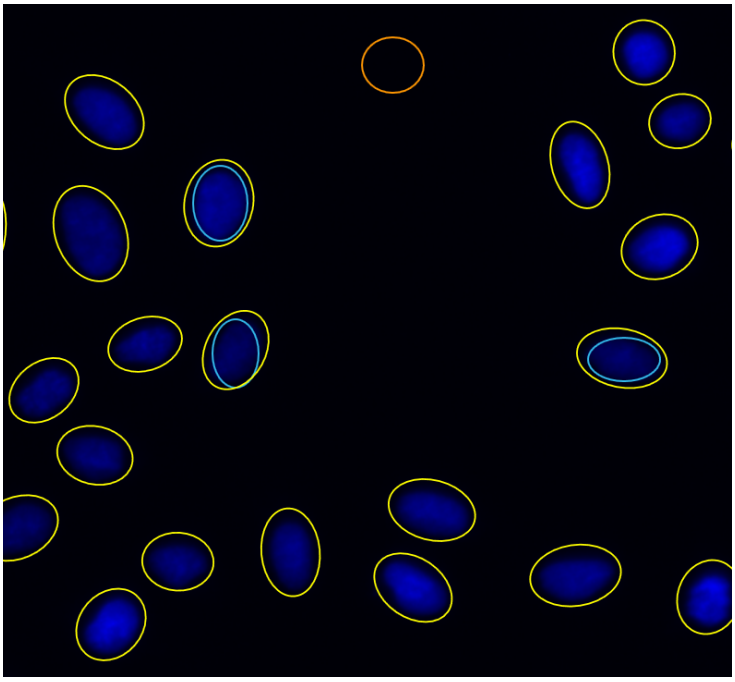
5. To distinguish the target from background, click **Backround**, then click and drag to draw a circle (orange) in a background area.

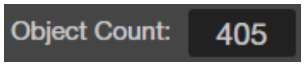


6. After you define the target and background areas, the software automatically counts the objects based on your criteria.

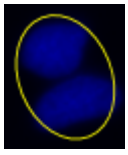


The target objects that were counted are identified with colored circles (in this example, yellow) and the Object Count field displays the number of objects included in the count.





Note: Depending on the quality of the image and representative targets and background that you have selected, the auto count algorithm can overcount or undercount the cells in the image.



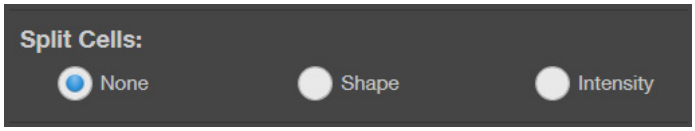
Undercount – 2 cells counted as 1

Overcount – 1 cell counted as 2

To obtain a more accurate count:

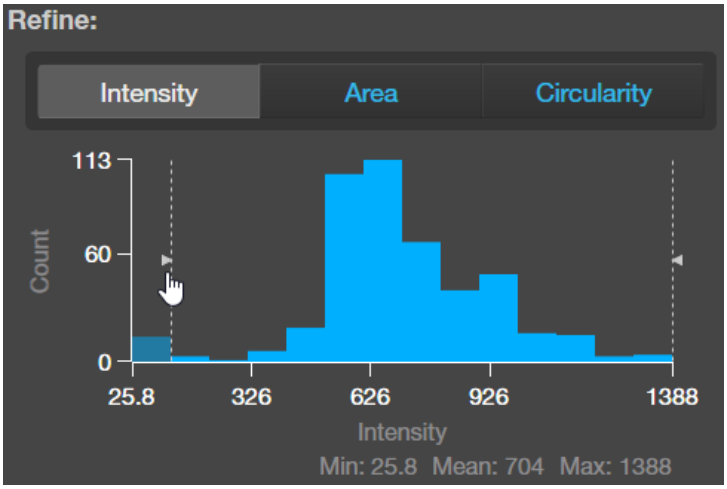
- Split cells by shape or intensity.
- Refine your count by intensity, area, or circularity.

7. 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.

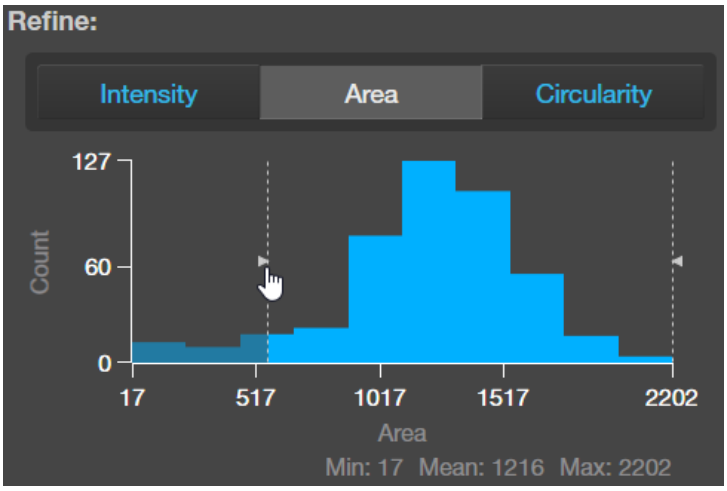
8. The Refine section displays a histogram plot showing **Count** versus **Intensity**, **Area**, or **Circularity**. In this example, **Intensity** is selected.



9. 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.



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

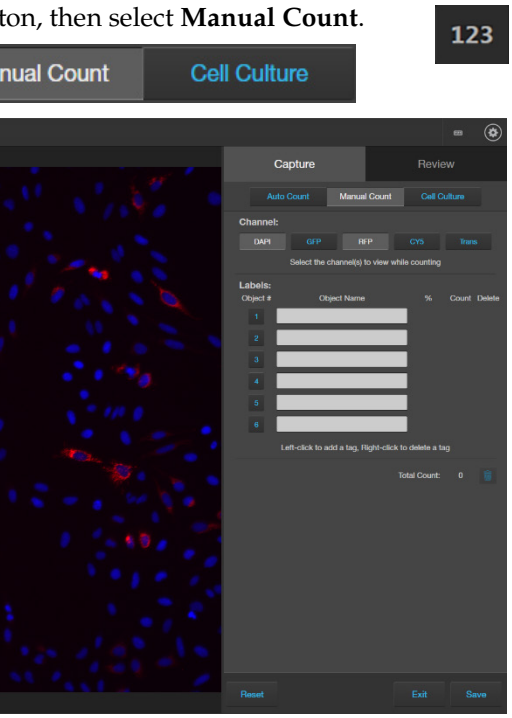
11. When finished with the count, save your count results (see "Save analysis results", page 76).

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.

Count cells – Manual Count

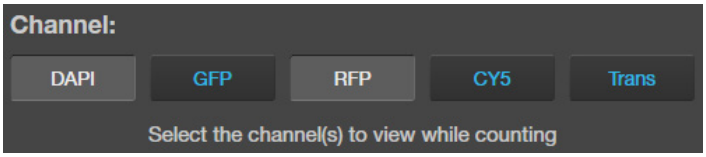
Perform Manual Count

1. Click the **Show Cell Count (123)** button, then select **Manual Count**.

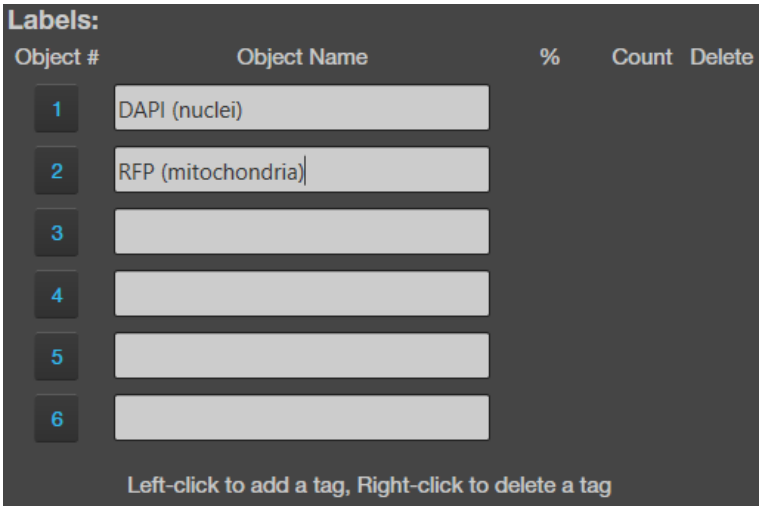


2. Select the **Channels** to display in the Viewing area for manual count. You can select multiple channels that contain captured images.

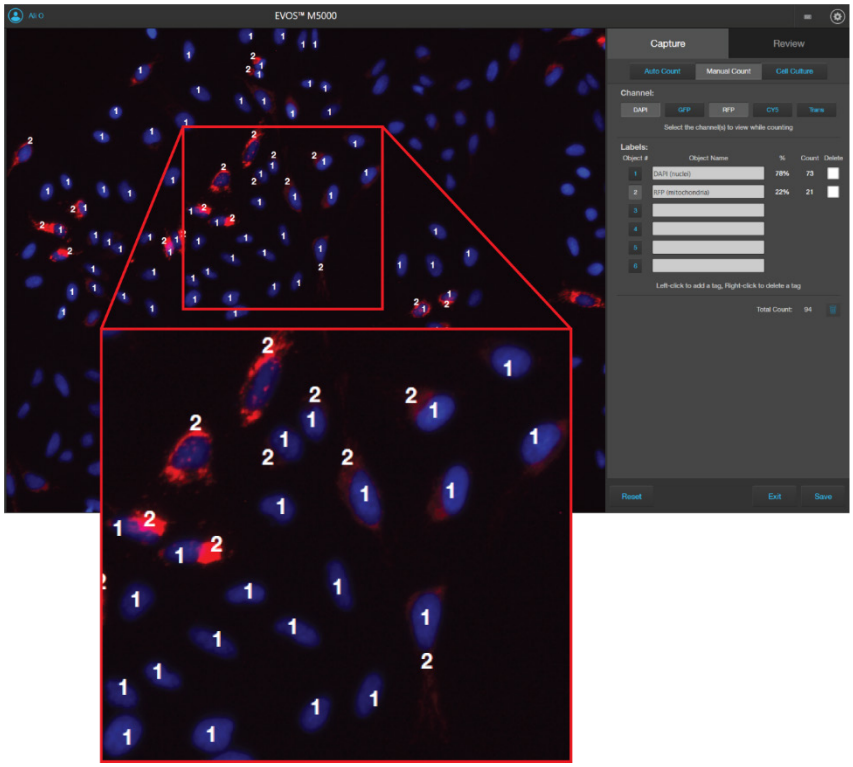
   In this example, DAPI and RFP channels contain captured images, and both are selected for the manual count.



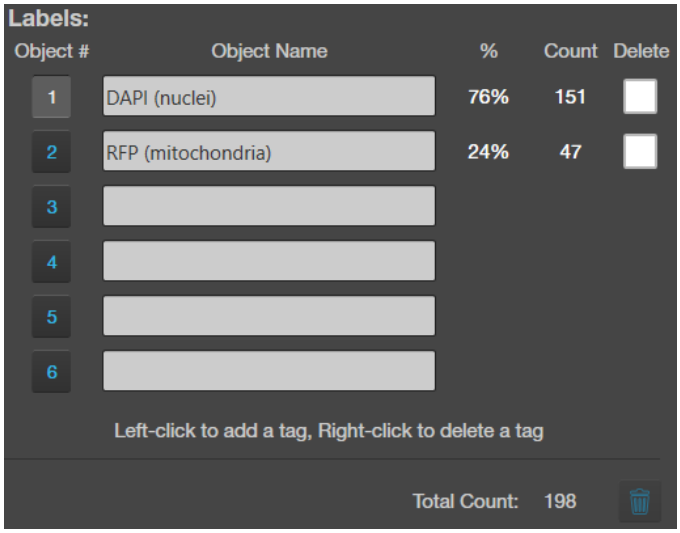
3. Click in an **Object Name** field to enter a name for that label. You can use up to six labels to tag objects for the manual count.



4. 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.



5. To delete a tag, right-click on the tag you wish to delete
6. To delete all tags for a label, check the **Delete** box for the label, then click the **Trash** button.
7. To delete all tags for all labels, check the **Delete** boxes for each label, then click the **Trash** button.
8. When finished with the count, save your count results (see "Save analysis results", page 76).

Measure confluence

Confluence tool

Confluence is a measure of how densely cells are distributed in 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.

The Confluence tool measures the percent area covered by cells in the image, which is required to calculate transfection efficiency.

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 4×** (for Olympus™ 4X phase objective) or **4×/10×** (for EVOS™ phase objectives) using the phase annuli selector (page 8).
- If you plan to calculate the transfection efficiency after measuring the confluence, capture the image in both the transmitted light channel and the fluorescence channel for the marker that your cells are expressing.
- For analysis, only use 16-bit Raw image files (TIFF RAW), which contain the full dynamic range and metadata needed for quantitative analysis.
- 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, within a given cell type, you can optimize the reproducibility of your measurements. Reproducibility in confluence measurements is more important than absolute percentages.

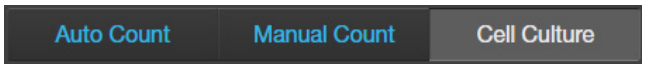
Measure confluence

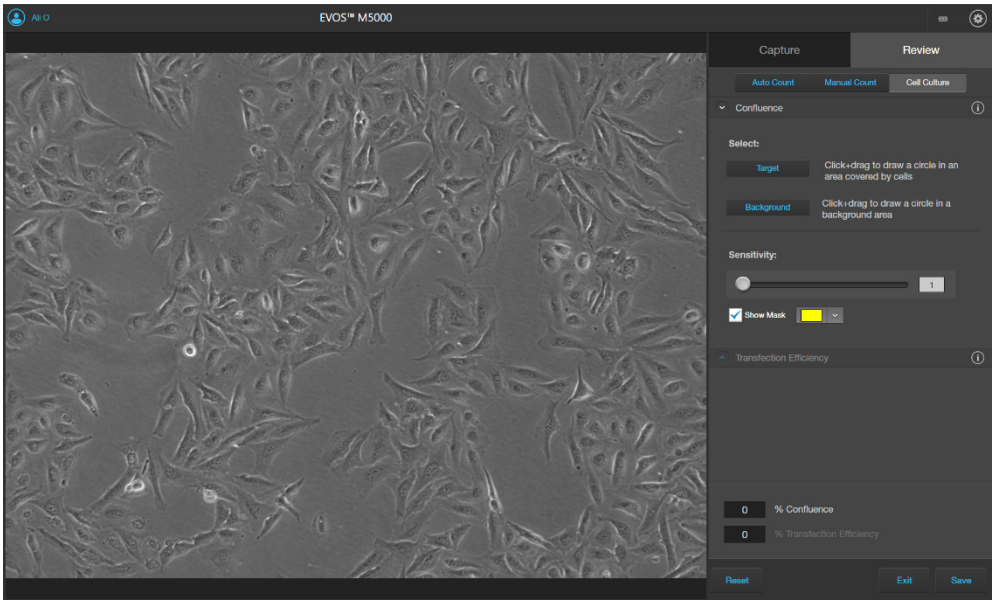
1. In the transmitted light channel, adjust lighting and focus on the sample, then click **Capture** to acquire an image of your culture.

   Note: If you plan to calculate the transfection efficiency after measuring the confluence, capture the image in both the transmitted light channel and the fluorescence channel for the marker that your cells are expressing.

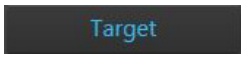
2. Hover the pointer over the Viewing area to reveal **Display Settings and Analysis Tools**, then click the **Show Cell Count (123)** button.

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

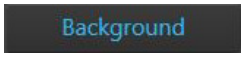


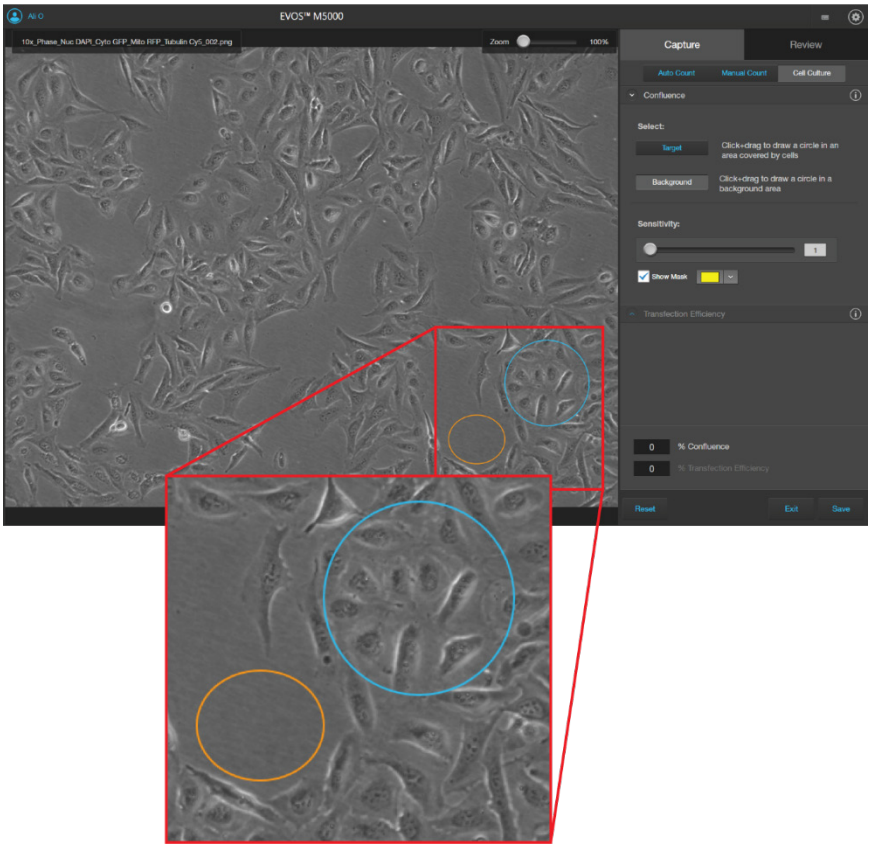


4. Click **Target**, then click and drag on the image to draw a circle around an area that contains cells (blue circle).



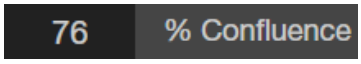
5. Click **Background**, then click and drag on the image to draw a circle around a background area that does not contain any cells (orange circle).



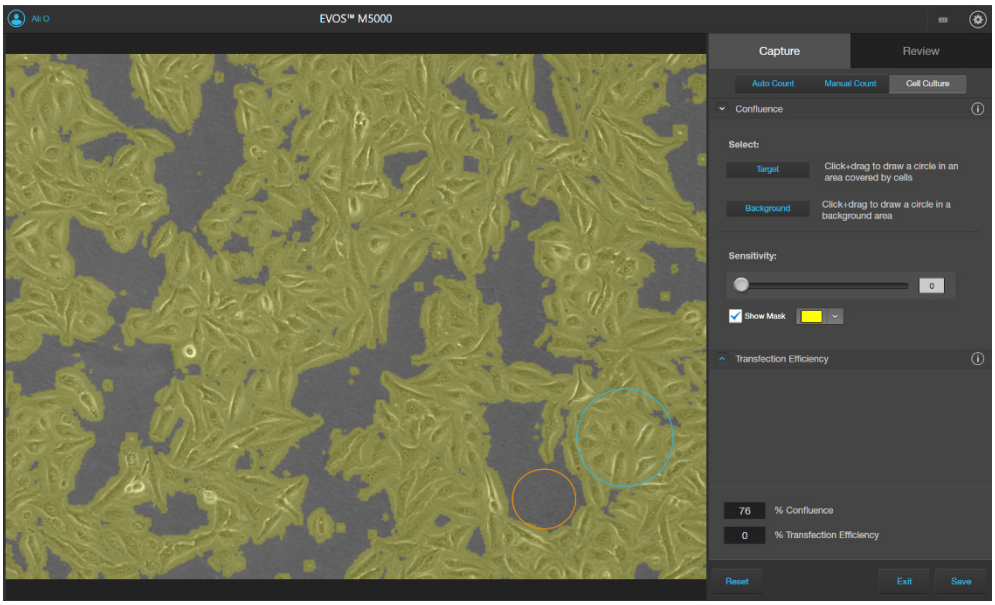


Note: You can select up to 5 targets and 5 background areas. Increasing the number of targets and background areas improves the accuracy and reproducibility of the confluence measurements.

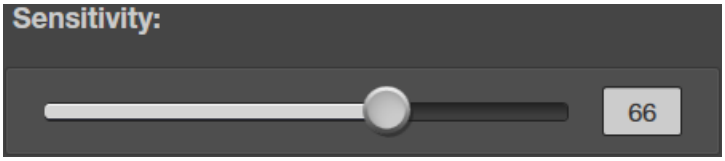
6. The software automatically calculates the confluence of your culture and displays the results as a percentage.



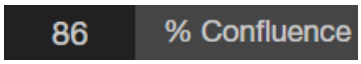
7. To view the areas of the image counted as Target, select **Show Mask**. The areas counted as Target are highlighted in the selected color.



8. Refine the sensitivity of the Confluence measurement using the **Sensitivity** slider. Increased the sensitivity results in higher confluence value.



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



Note: As you adjust sensitivity, observe the image with the **Show Mask** option on. Ensure that the target areas are selected with minimal coverage of the background areas.

9. When you complete the confluence measurement, Transfection Efficiency tool becomes available. To calculate transfection efficiency, go to "Calculate transfection efficiency", page 47.
10. To save your Confluence results without calculating transfection efficiency, click **Save** (see "Save analysis results", page 76).

Calculate transfection efficiency

Transfection Efficiency tool

Transfection of a cell population typically results in a varying number of cells expressing the desired genes of interest. Transfection efficiency is the percentage of cells that are transfected compared to the entire population.

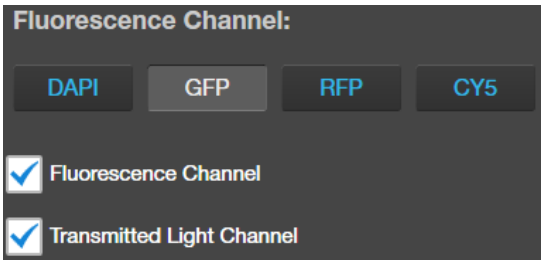
The Transfection Efficiency tool calculates the fluorescence area (transfected cells expressing the fluorescence marker) divided by the entire cell area in the image.

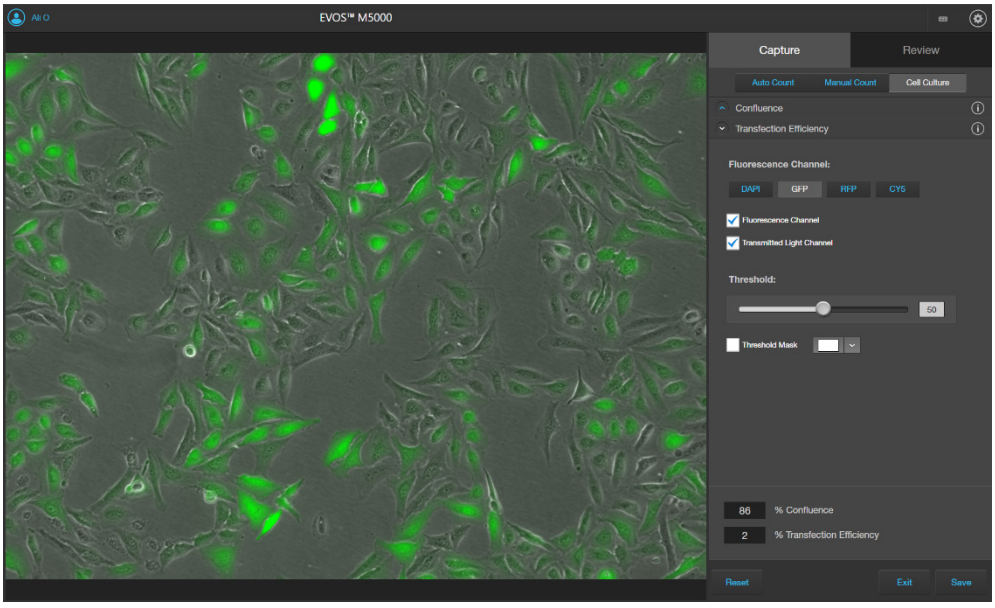
IMPORTANT! For analysis, only use 16-bit Raw image files (TIFF RAW), which contain the full dynamic range and metadata needed for quantitative analysis.

Calculate transfection efficiency

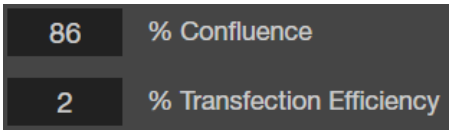
1. After you have completed the confluence measurement, click **Transfection Efficiency** to expand the controls for the Transfection Efficiency tool.
2. Select the **Fluorescence Channel** for which you wish to calculate the transfection efficiency.

   In this example, we want to calculate the percentage of cells that express GFP. Therefore, the **GFP** channel is selected. For display options, **Fluorescence Channel** and the **Transmitted Light Channel** options are checked, so that both channels are displayed in the viewing area.



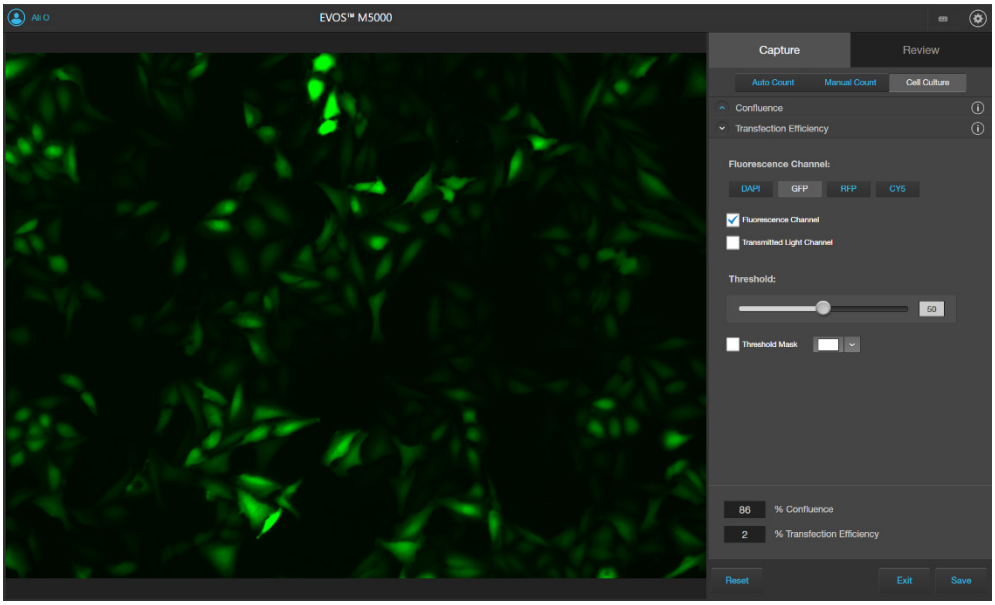


The software automatically calculates the Transfection Efficiency and displays the results as % Confluence and % Transfection Efficiency.

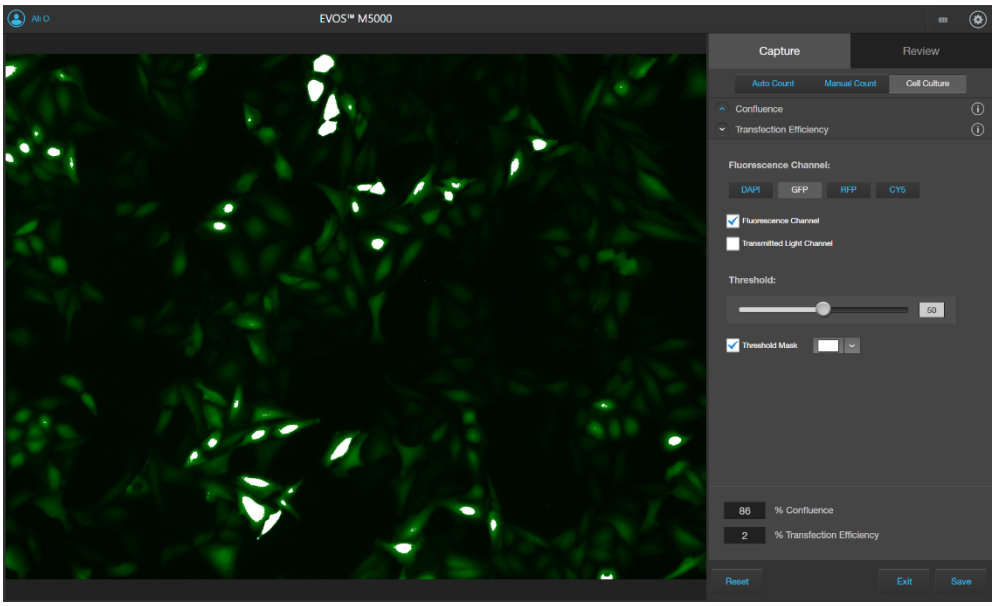


Note: Transfection Efficiency calculation is based on the final measured confluence (Step 8, page 46) and the fluorescence area that is above the set fluorescence threshold value. To refine the Transfection Efficiency calculation, adjust the Threshold such that only the cells that express at the desired level are included in the calculation (see page 49).

3. To view the pure fluorescence signal and to observe the various levels of fluorescence marker (GFP) expression, uncheck the **Transmitted Light Channel**.

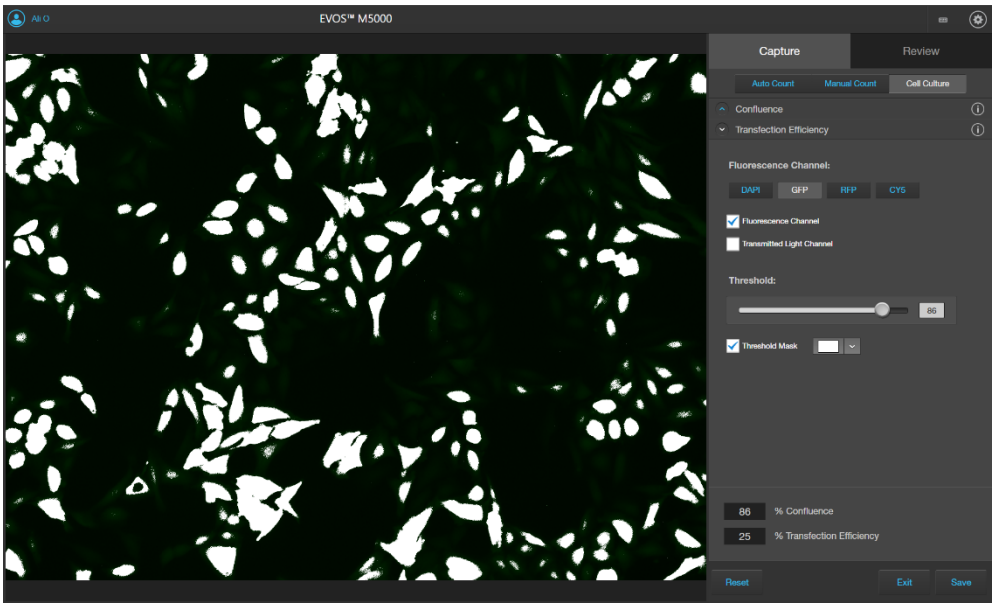


4. Select **Threshold Mask** to highlight the fluorescence areas included in the Transfection Efficiency calculation.

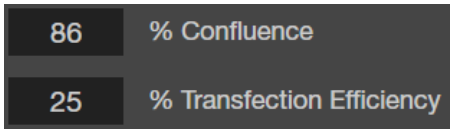


5. To refine the Transfection Efficiency calculation, adjust the **Threshold** slider until all the cells that express at the desired level are included in the calculation.

Note: Viewing the image only in the **Fluorescence Channel** and toggling the **Threshold Mask** on and off will help you determine the best **Threshold** value for your experiment.



As you adjust the **Threshold**, the software updates the calculation and displays the new Transfection Efficiency value.



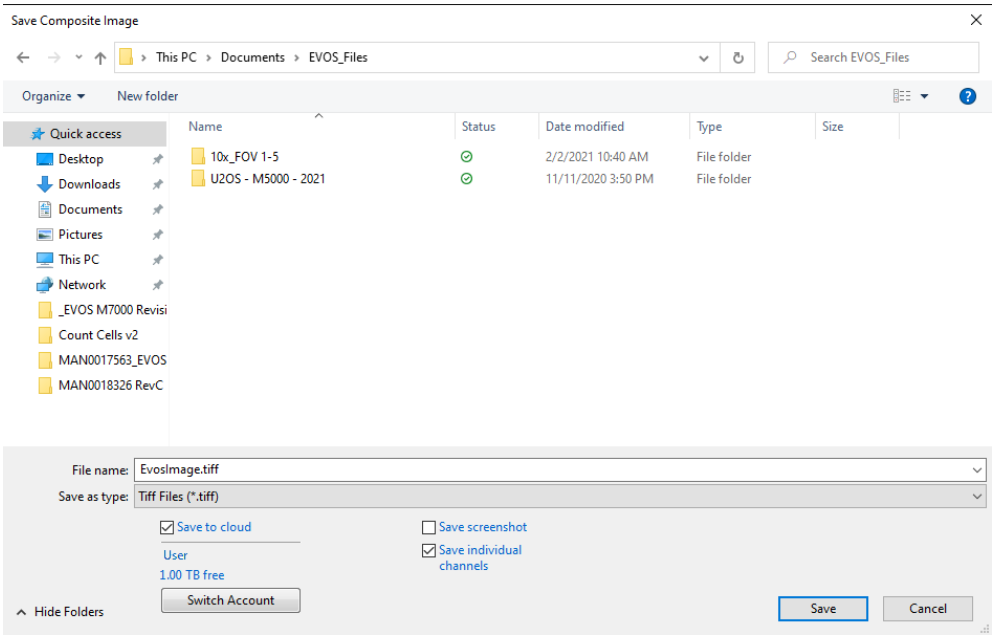
6. To save your Confluence and Transfection Efficiency results, go to “Save analysis results”, page 76.

5. Save captured images

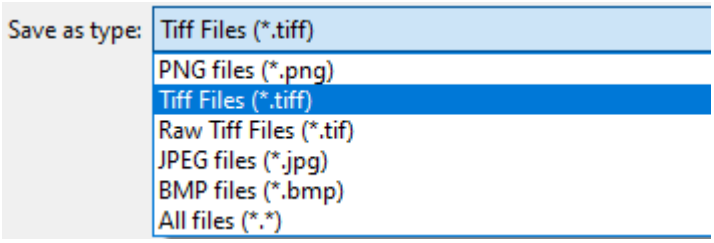
Save

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.

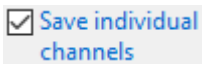


Available file formats are **PNG**, **TIFF**, **Raw TIFF** (16-bit uncompressed), **JPEG**, and **BMP**.

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.

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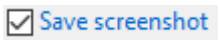
5. Select **Save screenshot** to save an image of the entire instrument screen in addition to the captured image.



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.

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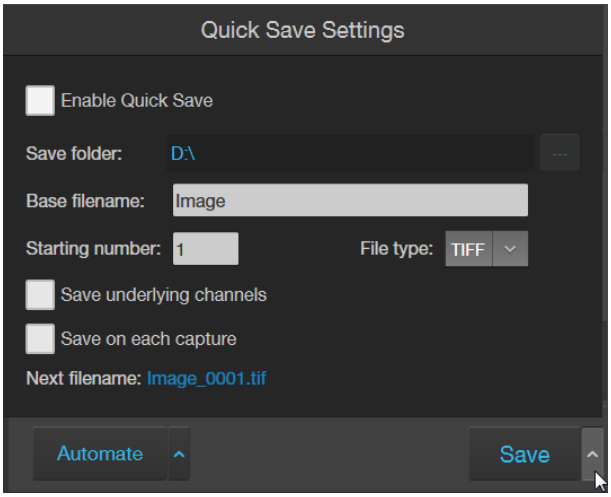
6. Click **Save** to save the image.

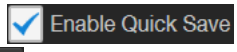
Quick Save images

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.



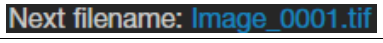
2. Select **Enable Quick Save**. 

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.

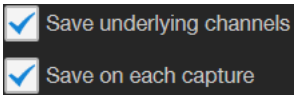
Starting number determines the starting numerical suffix, which is increased by one for each subsequently saved image. For example, Image_0001, Image_0002, and so on.



---

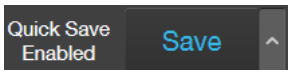
5. Select the File Type from the dropdown menu. Available options for file type are **PNG**, **TIFF**, **TIFF (RAW)**, **JPEG**, and **BMP**.

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

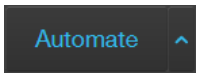
Time Lapse tool

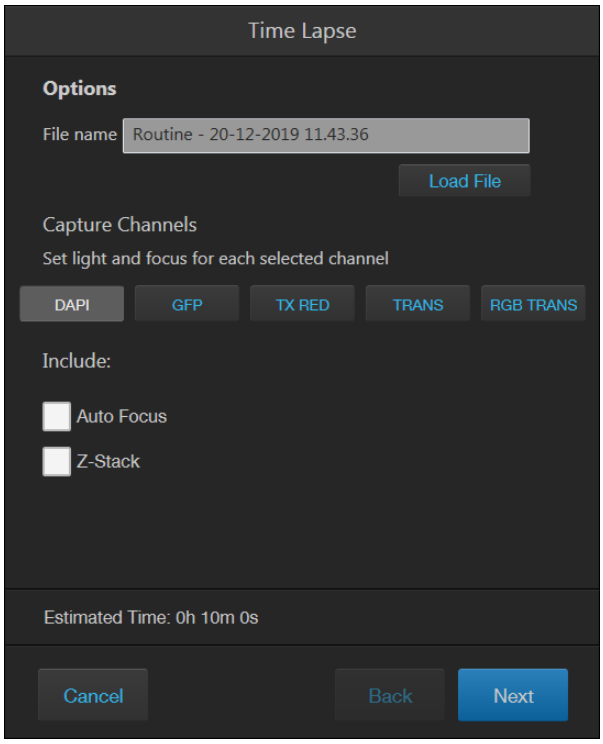
With the Time Lapse tool, you can set up your cells and program the EVOS™ M5000 Imaging System to capture images (including Z-Stack images) at given intervals over a time period based on your specifications. For repeat experiments, time lapse routines can be saved, recalled, and edited.

Run a time lapse routine

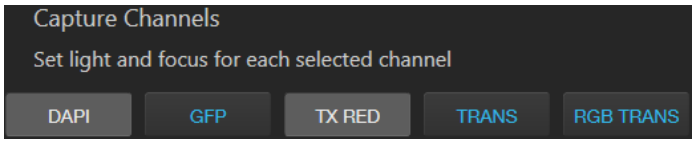
Run a new time lapse routine

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

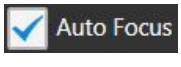




2. Select the **Capture Channels**. You can select multiple channels in which to capture time lapse images. In the following example, **DAPI** and **TX Red** channels have been selected.



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.

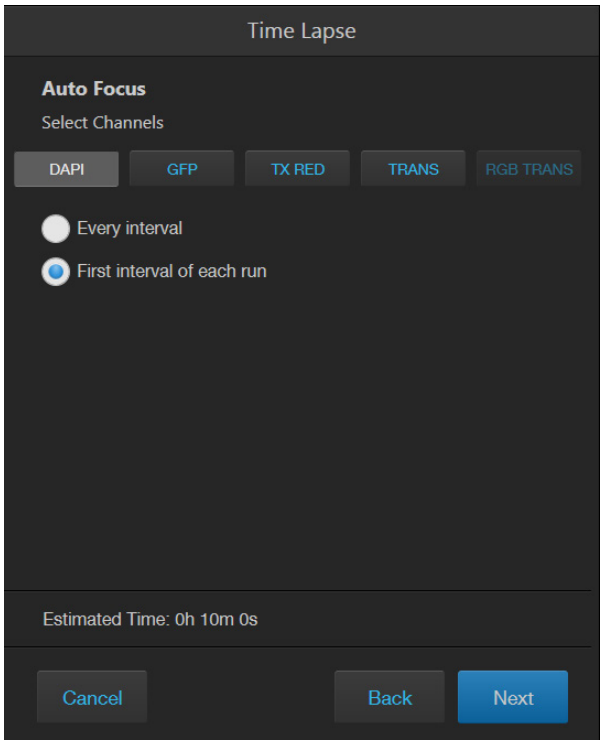
IMPORTANT! Before using the EVOS™ Onstage Incubator in your time lapse experiments, ensure that:

- The EVOS™ Onstage Incubator has been set up for use (page 120).
- The gas inputs have been configured (page 127).
- The oxygen sensor has been calibrated (page 128).

6. Adjust brightness and focus for each selected channel as described in "Capture images" (page 21), then click **Next**.

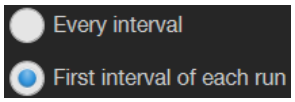
Note: When the sample is focused and ready, tighten the stage brakes (page 8) to prevent the stage from drifting during the time lapse routine.

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



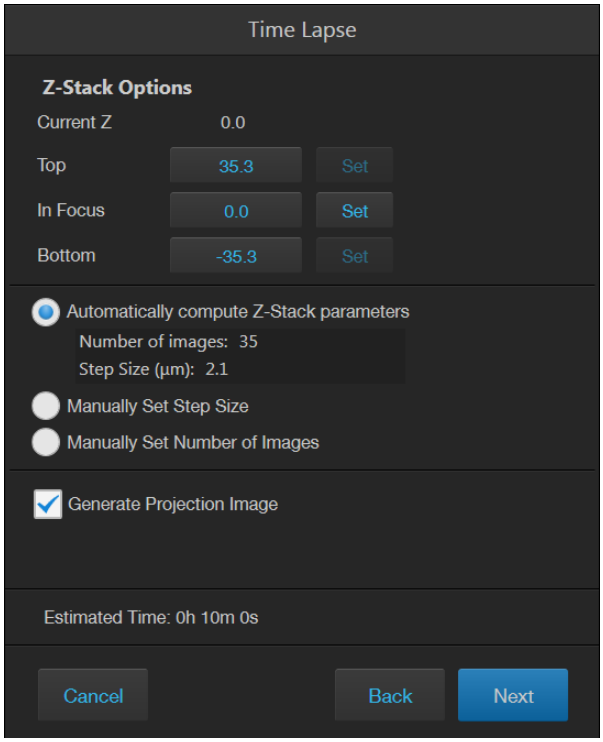
Note: In a time lapse routine, you can run the autofocus procedure only in a single channel (in this example, DAPI). The focal plane identified in this channel is then used for all other channels.

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

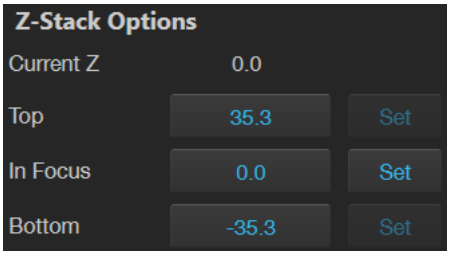


Note: In a time lapse routine, images are captured at the end of each interval of a run. For more information, see "Intervals", page 57.

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**.

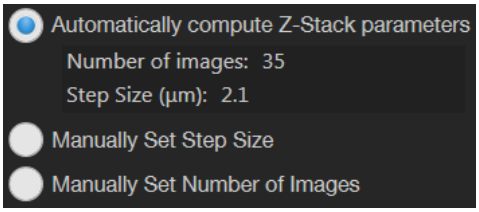
   To change the **In Focus** position, move the **Coarse** and **Fine focus** sliders on the Capture tab to focus on the sample, then click **Set**.

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:



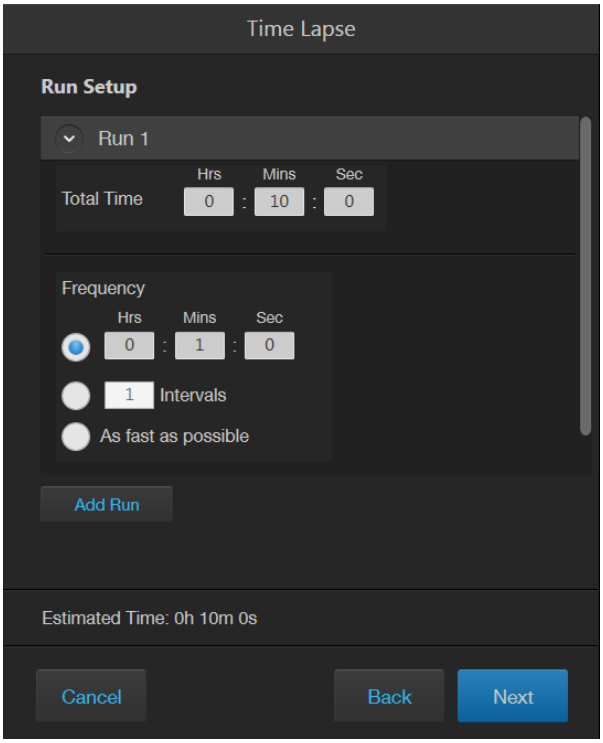
- **Automatically compute Z-Stack Parameters:** Select to calculate the number of images and their Z-positions automatically based on the Z-Stack boundaries and the default Step Size.
- **Manually Set Step Size:** Select to enter the **Step Size** (Z-distance in µm between focal planes) in the corresponding 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 µm 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.

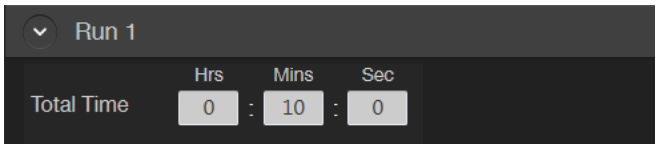
When unselected, the system only saves the individual Z-Stack images.

12. Click **Next** to set up a Run.

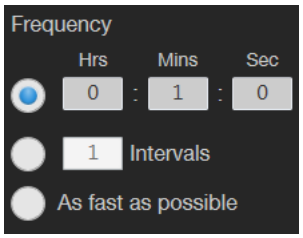


13. Enter the **Total Time** for Run 1 in **Hours**, **Minutes**, and **Seconds**.

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



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



- **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.

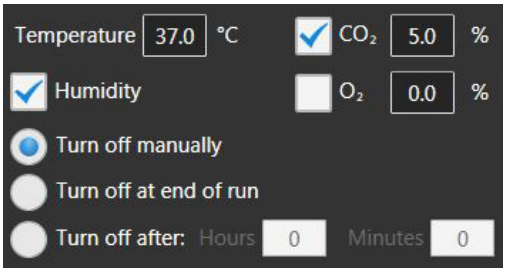
  Images are collected at the end of an interval. For example, in a Run experiment with a duration of 5 minutes and 2 intervals, the images will be captured every 2 minutes and 30 seconds after the initial set of images are acquired at time point 0.

- **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.



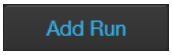
a. Enter the target values for **Temperature**, **CO_2**, and **Oxygen**.

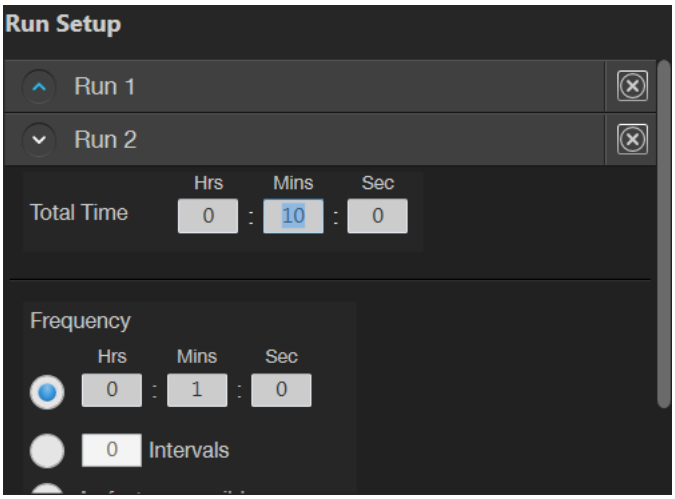
b. If desired, select **Humidity** to use a humidified atmosphere in the incubator.

c. 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.

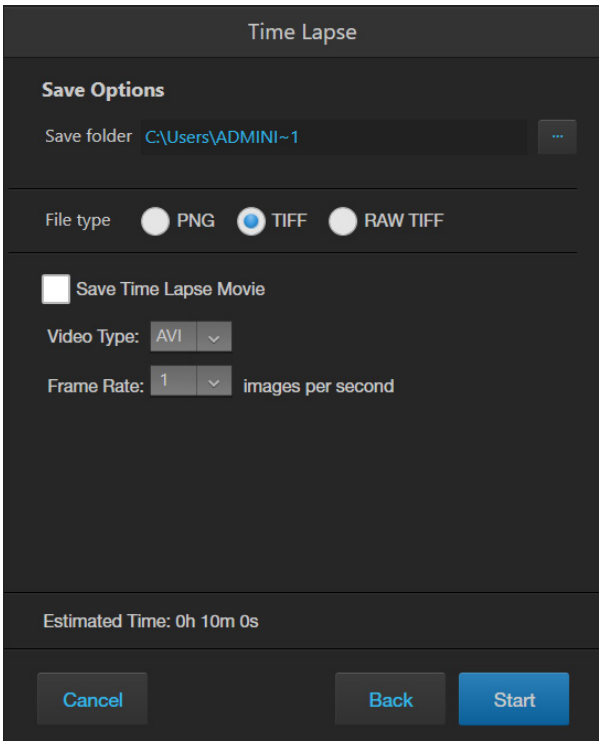
16. *Optional*: Click **Add Run** to add another Run to the routine, then define the Run options as described for Run 1.





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

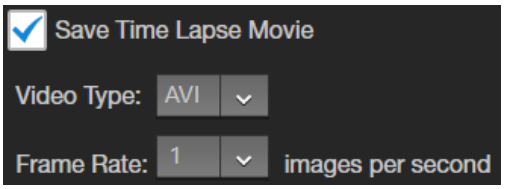
17. When finished with adding Runs, click **Next** to set the Save options.



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

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

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



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

Note: Frame Rate specifies how many images are captured per second during the time lapse recording. You can view the saved time lapse recording at different playback speeds ranging from 1 fps (frames per second) to 120 fps (page 63).

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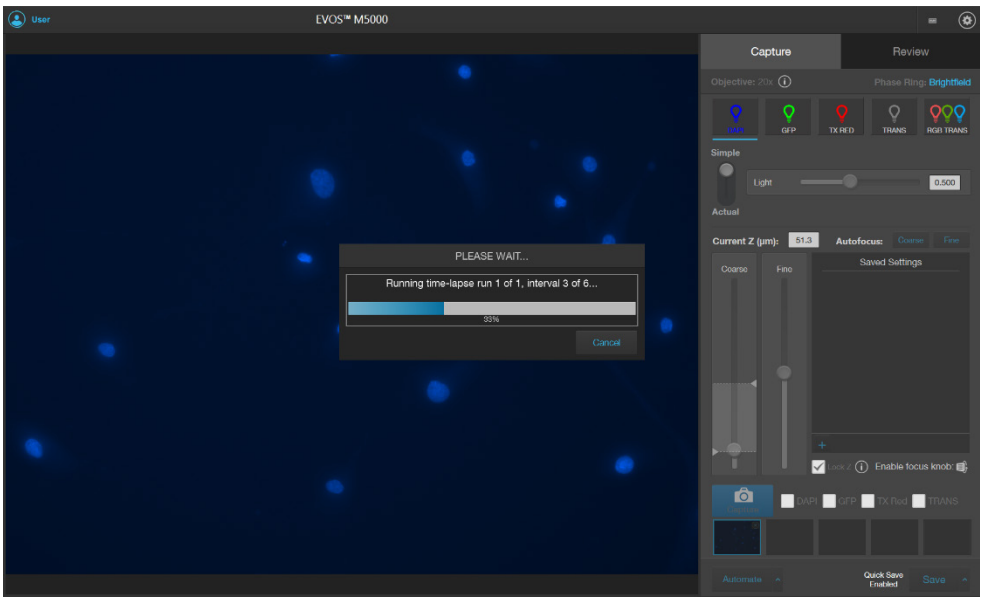
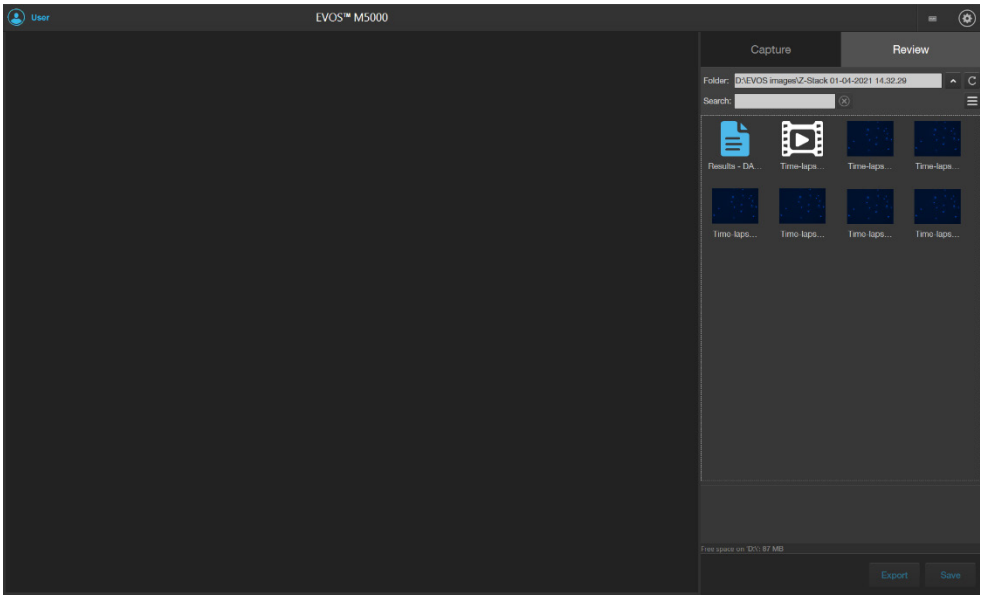
23. Click **Start** to run the Time Lapse routine.

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



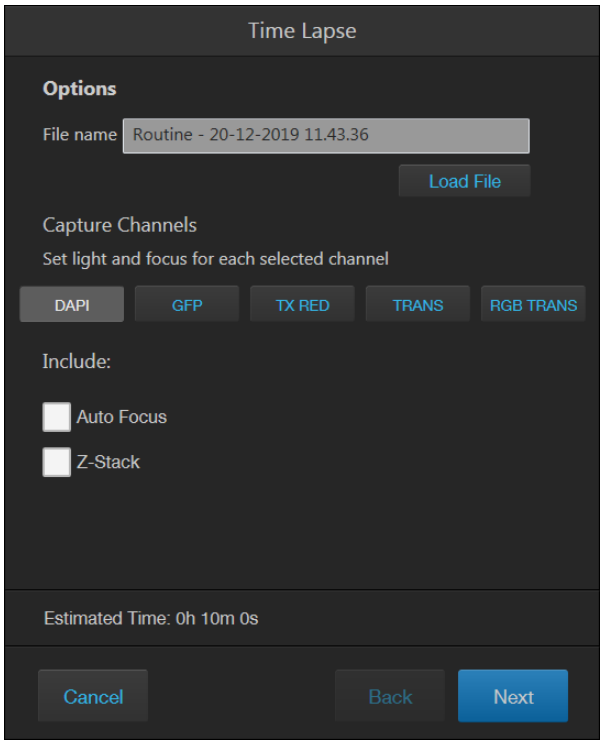
24. When the Time Lapse routine is completed, the Review tab opens to the files captured in the routine.



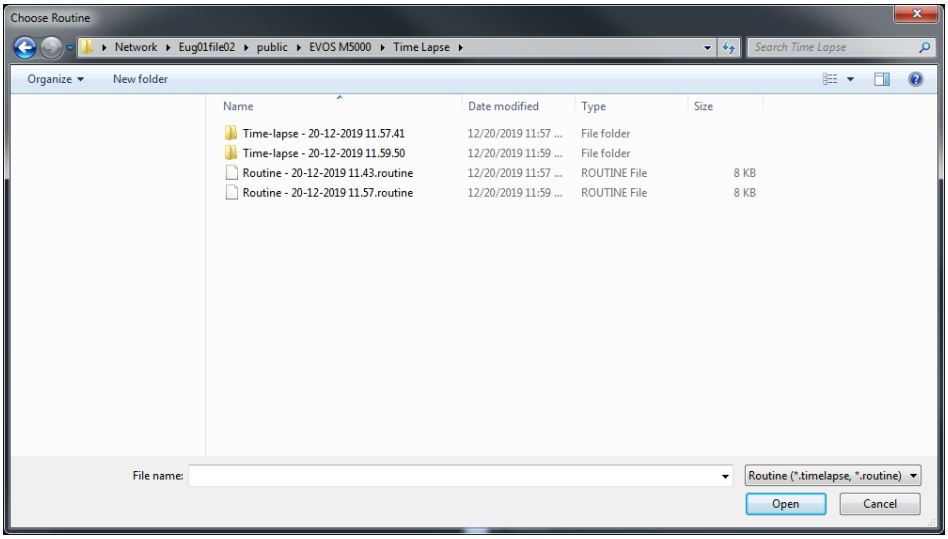
Run a saved time lapse routine

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.



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

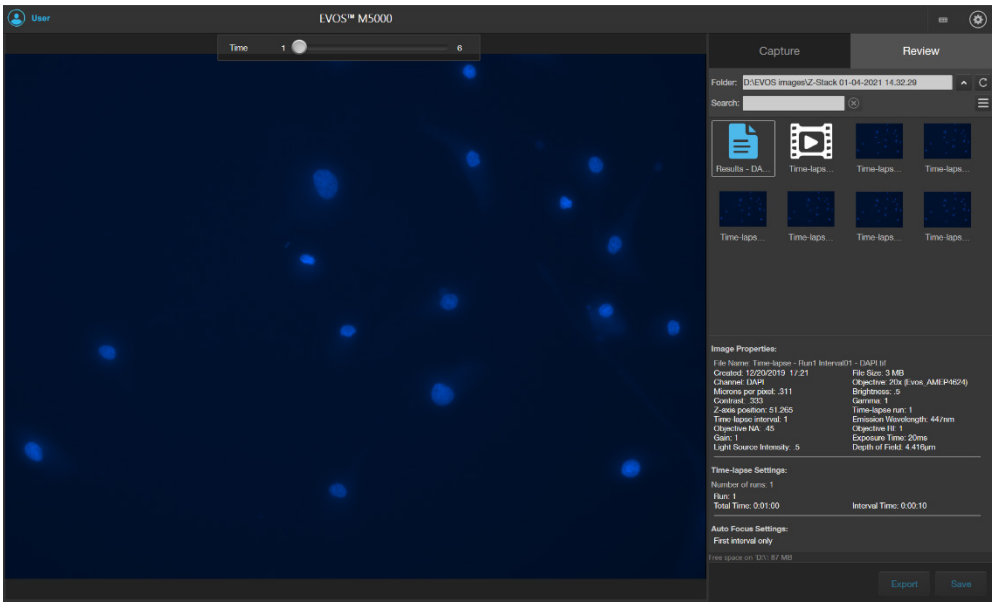


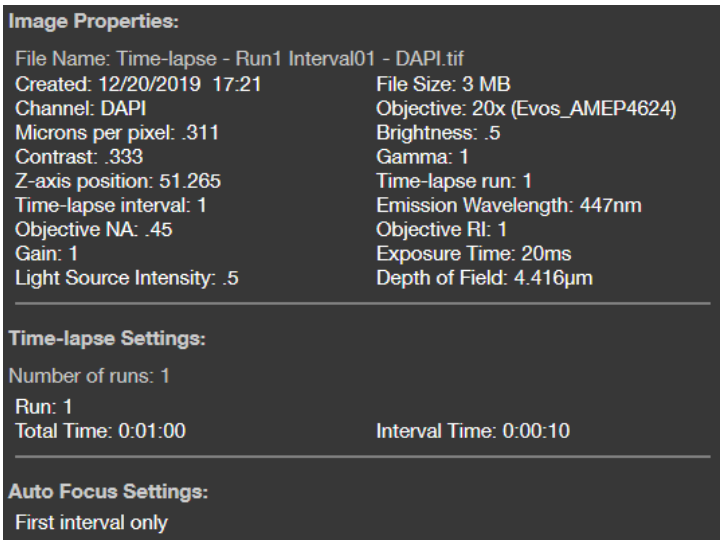
3. Select the routine you want to run, then click **Open**.
4. If needed, make changes to the routine as described for new routines.
5. Click **Start** to run the Time Lapse routine.

Review images and video captured in a time lapse routine

1. To review the results from your completed time lapse routine, click **Results**.

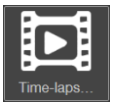
   The viewing area displays the first image captured in the time lapse routine and the Review tab shows the Image Properties, and Time Lapse and Auto Focus Settings.

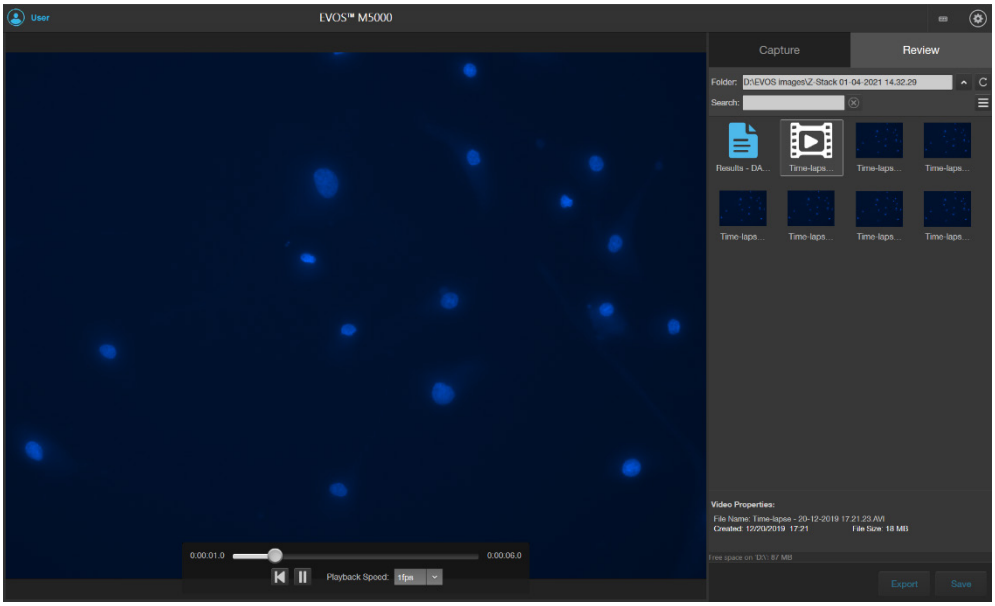


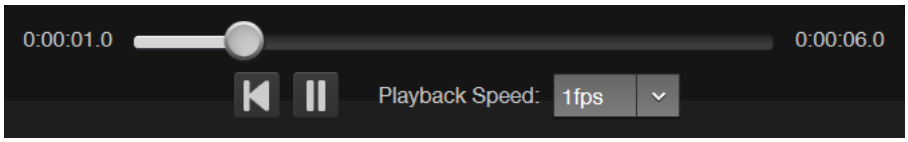


2. To view the subsequent images captured in the time lapse routine, use the Time Slider at the top of the viewing area.

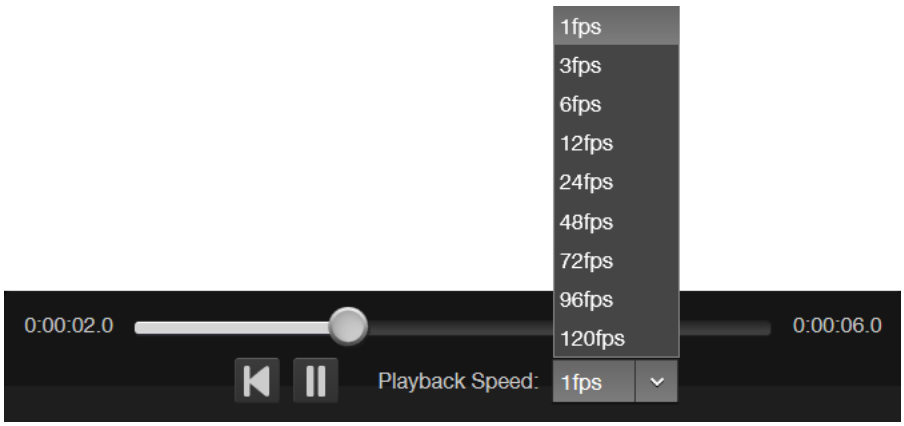
3. To play the time lapse movie, select the **Time-lapse Video** file, then click **Play** on the **Time Lapse Video Player**.





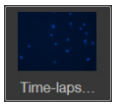


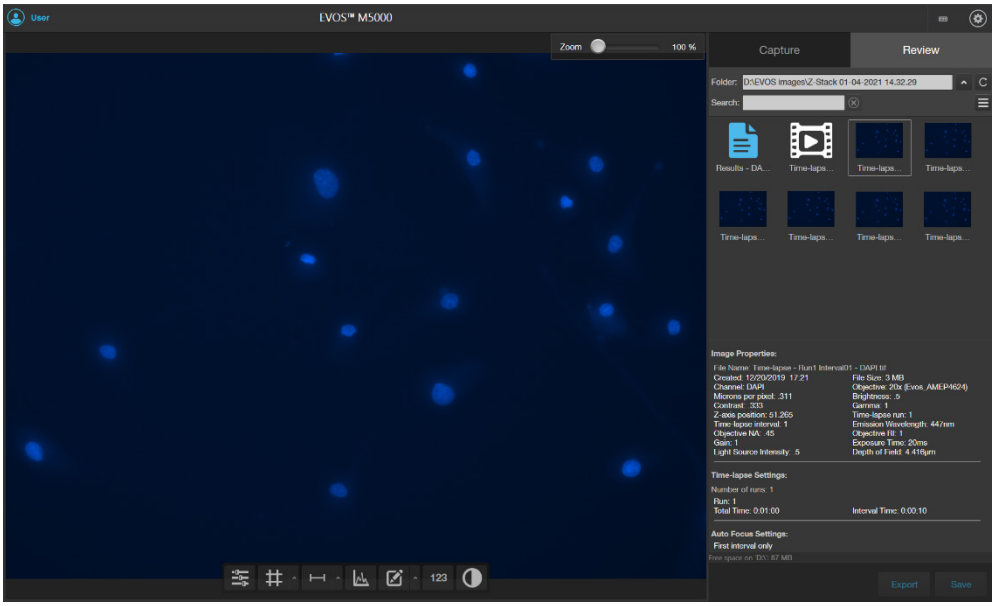
4. To change the playback speed, select the desired speed from the **Playback Speed** menu.

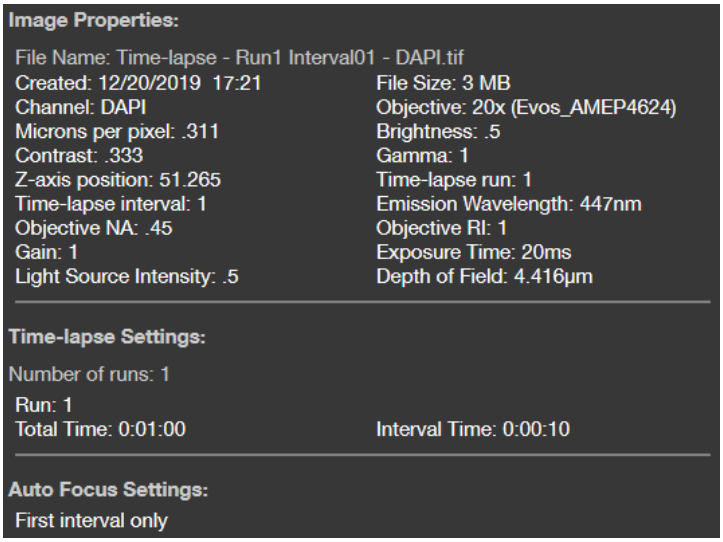


Note: Playback Speed is independent of the Frame Rate, which specifies how many images are captured per second during the time lapse recording (page 59). You can view the saved time lapse recording at different playback speeds ranging from 1 fps (frames per second) to 120 fps (page 63).

5. 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.







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

7. To annotate captured images and perform cell count and confluence measurements, use the **Display Settings and Analysis Tools** as described on page 29.

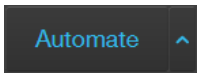
7. Capture Z-Stack

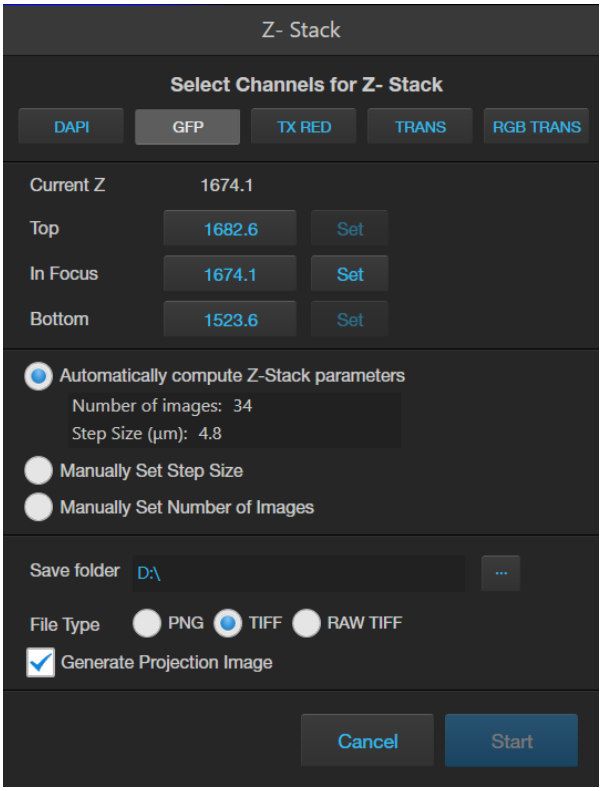
Z-Stack tool

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.

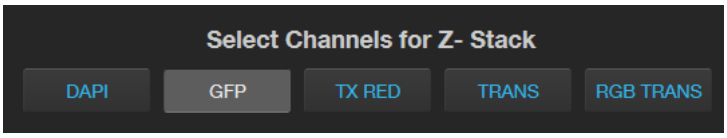
Capture Z-stack images

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





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

   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**.

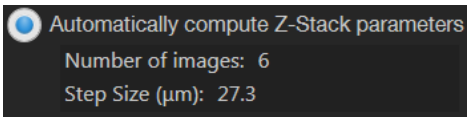
5. Move the focus sliders up from the Current Z position to the desired **Top** position (upper boundary of the Z-Stack), then click **Set** for Top.

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.

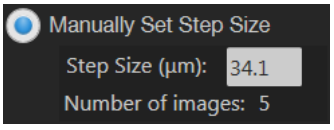
Note: You can click the **Z number** button for the Top, In Focus, and Bottom positions to jump to those positions along the Z-axis.

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

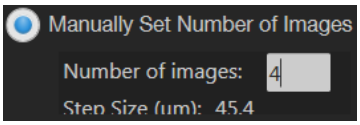
   - **Automatically compute Z-Stack Parameters:** Select to calculate the number of images and their Z-positions automatically based on the Z-Stack boundaries and the default Step Size.



   - **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.

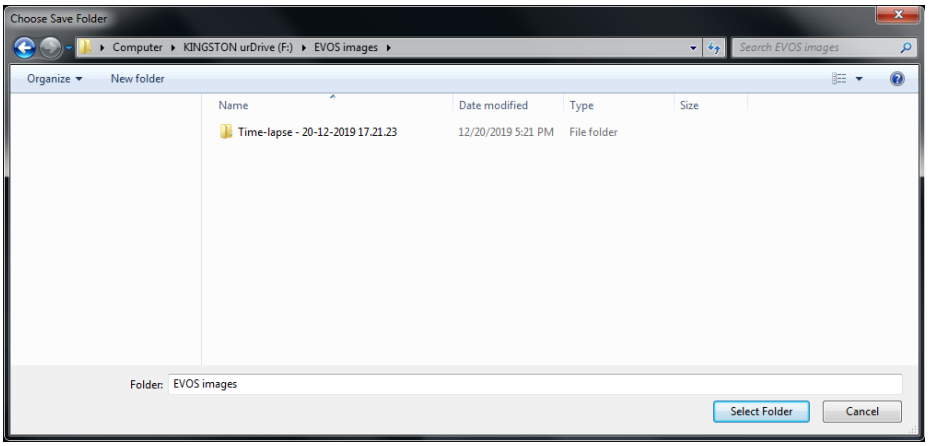


   - **Manually Set Number of Images:** Select to enter the **Number of Images** (Z-distance in µm between focal planes) in the text box. The system calculates Step Size and the Z positions of the images.

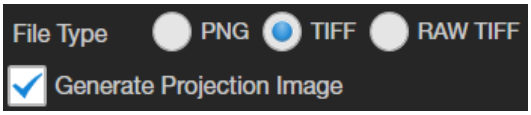


Note: You can take up to 100 images ("optical sections") for a Z-Stack.

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**, **TIFF**, or **RAW TIFF**.

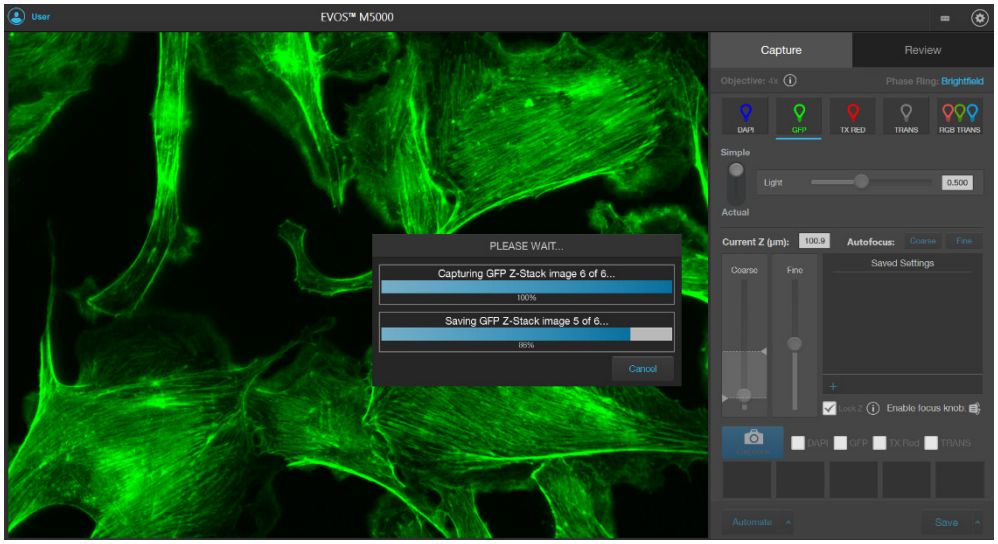


11. Select **Generate Projection Image** to generate a projection image.

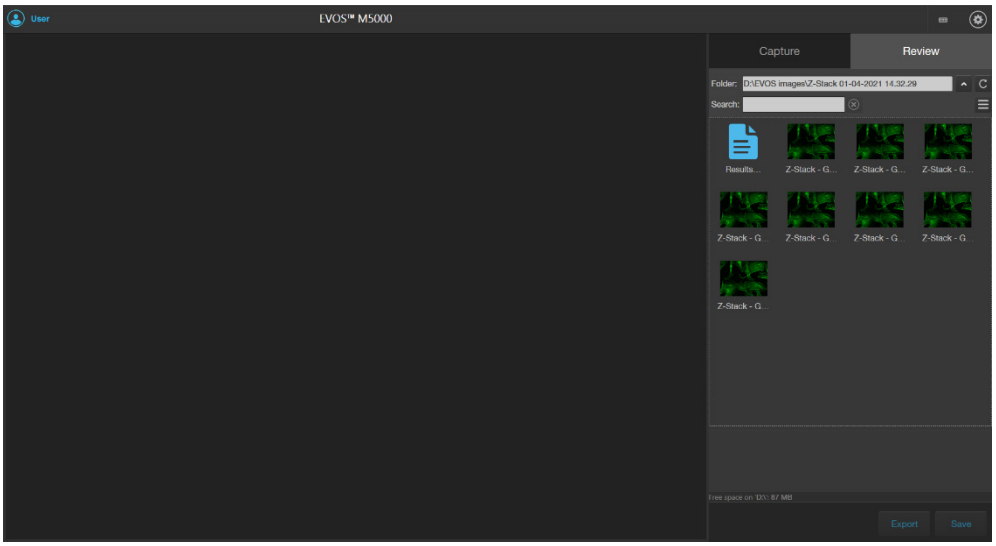
    When selected, the system uses the most in-focus pixels from images captured at different focal planes to generate a composite in-focus image. Each image in the Z-Stack is also separately saved.

    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.

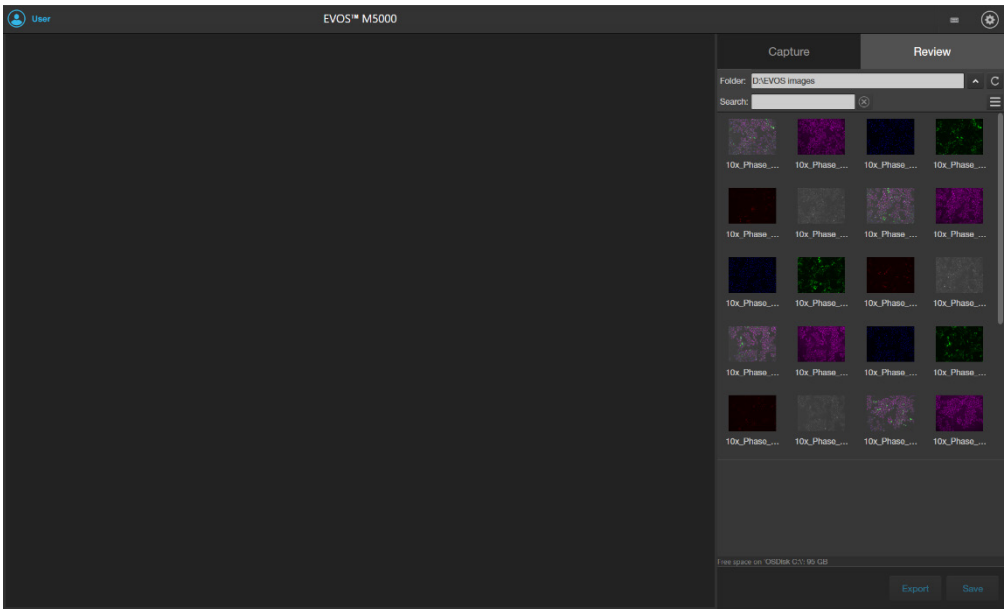


13. When the Z-Stack routine is completed, the Review tab opens to the files captured in the routine.



8. Review and analyze saved images

The **Review tab** allows you to review, annotate, and analyze still images, including those captured in Time Lapse routines and Z-Stacks. You can also re-save or delete saved files.



- Review saved images (page 70)
- Configure display settings (page 72)
  - Adjust image display settings (page 72)
  - Display grid (page 72)
  - Display scale bar (page 72)
- View pixel intensity histogram (page 73)
- Add measurements and annotations to saved images (page 74)
- Analyze cell culture using saved images (page 75):
  - Perform Auto Count (page 75)
  - Perform Manual Count (page 75)
  - Measure confluence (page 75)
  - Calculate transfection efficiency (page 75)
- Save analysis results (page 76)
- Perform batch analysis of saved images (page 76)

Note: For a detailed description of the Review tab controls, see "Review tab" in "Appendix B: Graphical user interface (GUI)" (page 107).

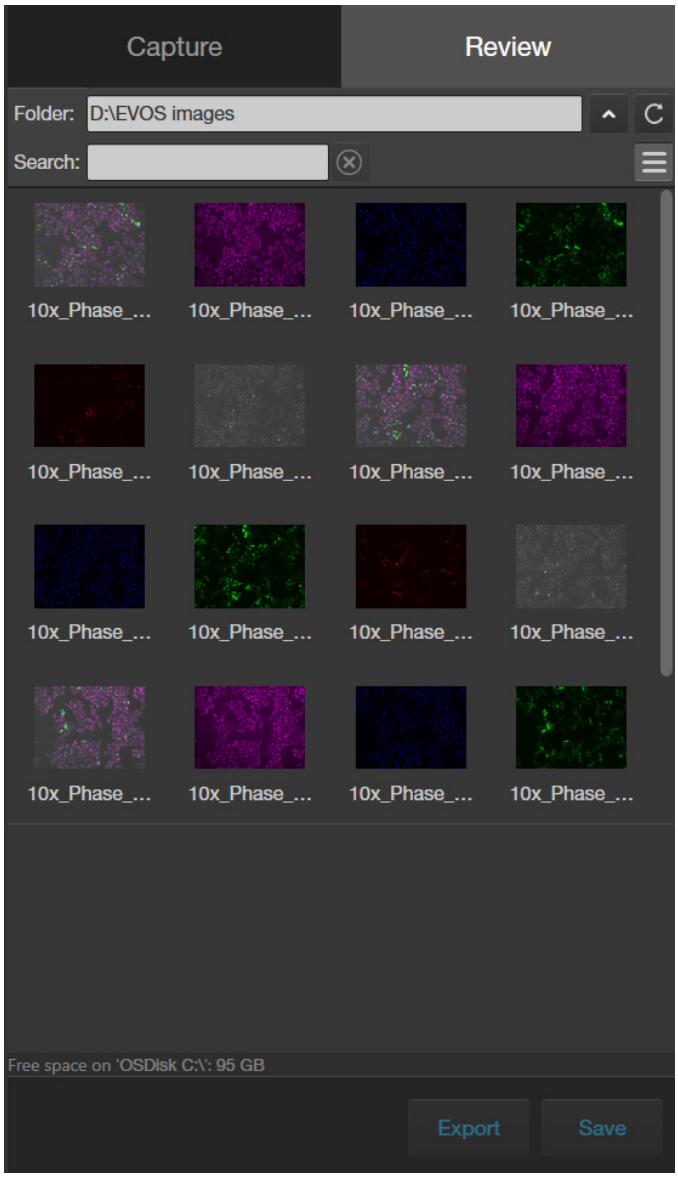
Note: You can also view and analyze captured images that are saved to your Connect account with the EVOS™ Image Analysis application.

For more information on the EVOS™ Image Analysis application, see "Appendix C: EVOS™ Image Analysis", page 129.

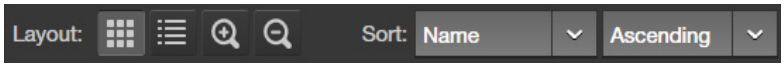
Review images

Review images

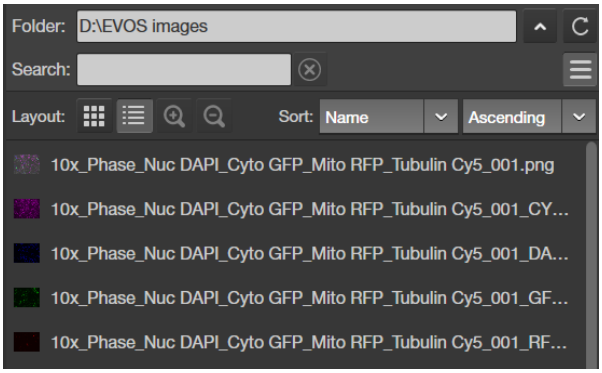
1. Click the **Review** tab.
2. In the **Folders** panel, navigate to the folder containing your saved images, then click to select it. The **folder/image preview** area displays thumbnail images for all viewable files in the selected directory (the top-level USB directory is selected by default). If there are no viewable files in the directory, the preview area will be empty.



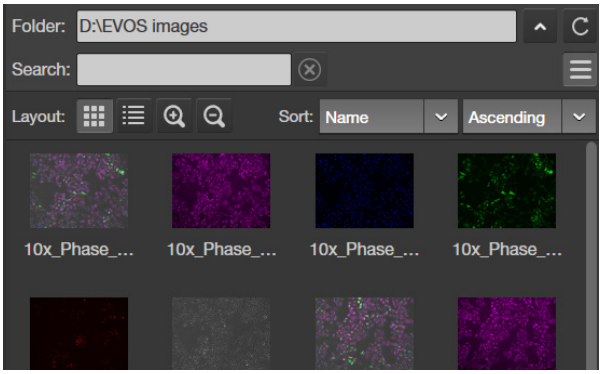
3. To change the layout of the saved images or to sort the saved images by name, file type, or date created, click the **Display Settings** button to expand the Display Settings controls.



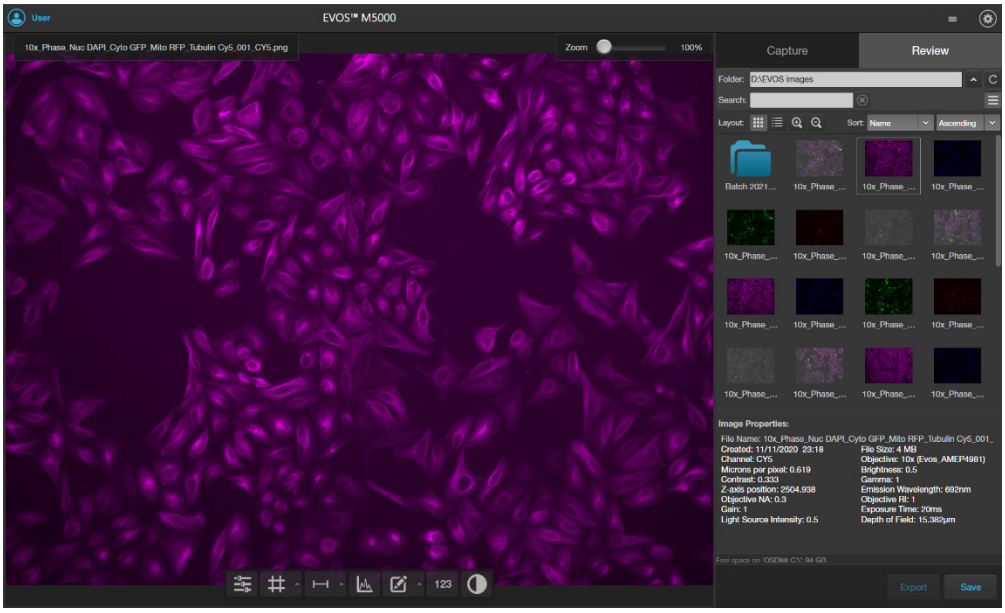
4. To display the folder/image preview in list format, click the **List view** button.



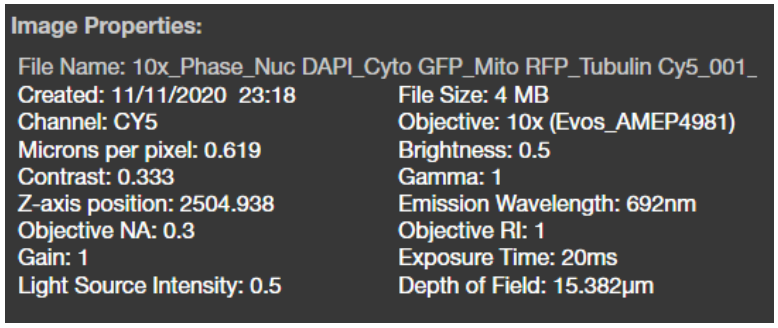
5. To display the folder/image preview in grid format, click the **Grid view** button.



6. Click the **image** to select it. The selected image is indicated with a box around it and the viewing area displays the selected image.



The Image Properties panel shows the metadata associated with the selected image.



Configure display settings

Change display settings for saved images

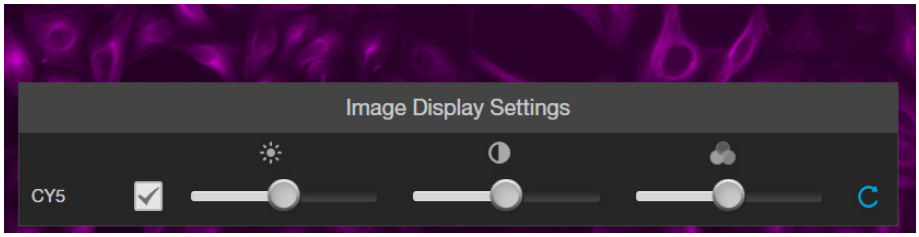
Display settings and analysis tools allow you to change image display settings, and to analyze and annotate captured images.

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

Note: Display Settings are available in both the Capture and the Review tabs and function the same way.

- Click the **Image Display Settings** button to expand the controls for image display parameters (brightness, contrast, gamma).
  For detailed instructions, see page 30.

Note: The controls for image display settings are available only for captured channels. In the example below, the controls are available only for the CY5 channel, because the image that is displayed is captured in the CY5 channel.



Display Grid

- Click the **Grid** button to superimpose a grid over the Viewing area.
  For instructions on how to change the Grid settings, see page 31.

Display Scale bar

- 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 32.

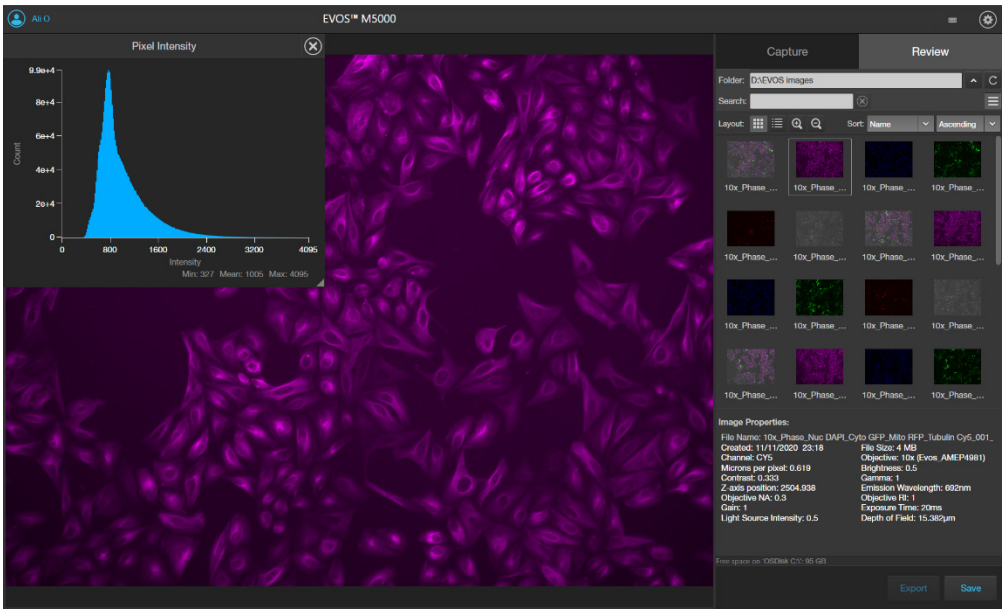
View pixel intensity histogram

Display histogram

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

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

The Pixel Intensity histogram shows the Pixel count vs. Intensity data of the image displayed in the Viewing area as well as the minimum, mean, and maximum pixel intensities. See page 33 for more information on the Histogram tool.

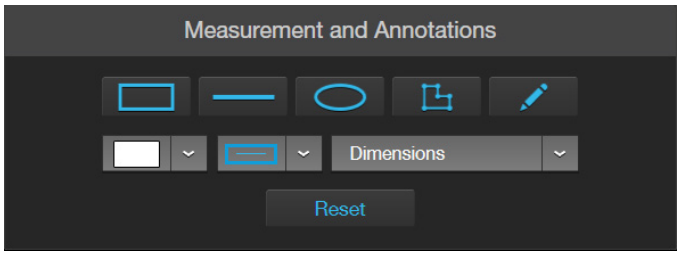


Note: Histogram tool is available in both the Capture and the Review tabs and function the same way.

Add measurements and annotations to saved images

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

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

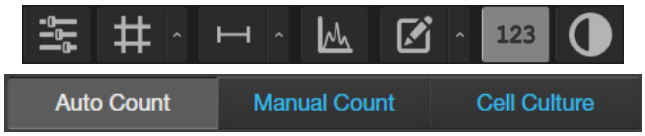


For instructions on how to add and show measurements and annotations to your images, see page 35.

Note: Measurements and Annotations tools are available in both the Capture and the Review tabs and function the same way.

Analyze cell culture using saved images

1. Hover the pointer over the Viewing area to reveal **Display Settings and Analysis Tools**, then click the **Show Cell Count (123)** button to display **Auto Count**, **Manual Count**, and **Cell Culture** options in the tabs area.



IMPORTANT! For analysis, only use 16-bit Raw image files (TIFF RAW). Raw image files contain the full dynamic range and metadata needed for quantitative analysis, whereas Display and Merged image files do not.

2. Click to select from the following analysis options:
   - **Auto Count:** Automatically counts the objects displayed in the Viewing area based on your specifications. With Auto Count, you can count objects only in a single fluorescence channel (nuclear stain channel).

     For instructions on how to perform Auto Count, see page 38.
   - **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. With Manual Count, you can count objects in multiple channels simultaneously.

     For instructions on how to perform Manual Count, see page 42.
   - **Cell Culture:** Allows you to measure the confluence of your culture and calculate the transfection efficiency.
     - **Confluence:** Allows you to select up to five reference objects for the target (i.e., cells) and one background reference in your image, then automatically calculates the percentage confluence of your culture.

       For instructions on how to measure the confluence of your culture, see page 42.
     - **Transfection Efficiency:** Allows you to estimate the transfection efficiency of your culture by calculating the ratio of fluorescence area (i.e., cells expressing the fluorescence marker) to the total cell area in your culture.

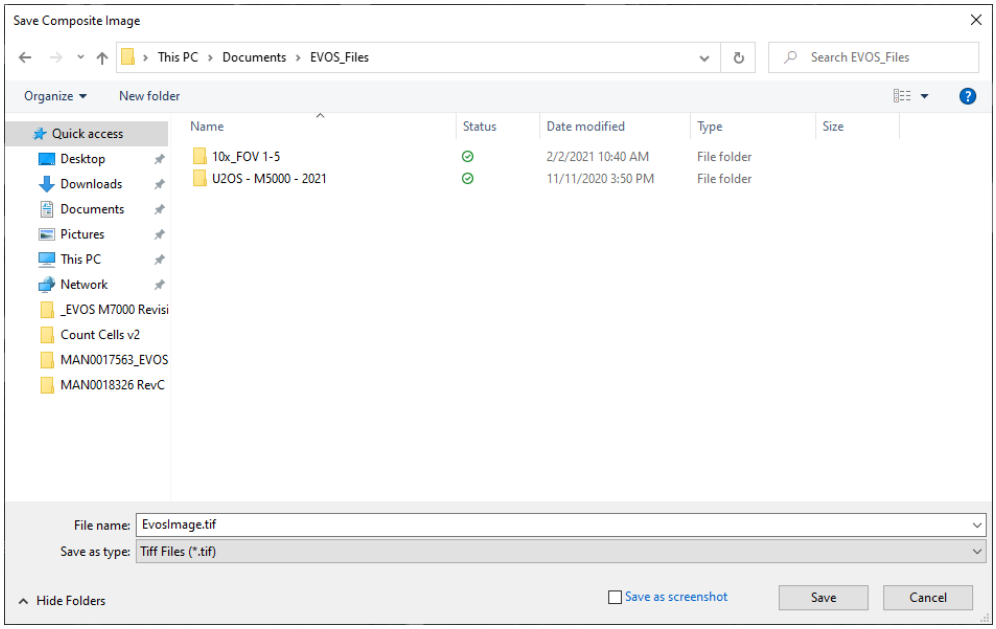
       For instructions on how to calculate the transfection efficiency, see page 47.
3. If desired, perform **Batch Analysis** to the remainder of the images in the image folder (page 76).

Note: Batch Analysis allows you to save and apply the analysis parameters set in Auto Count, Confluence, and Transfection Efficiency tools to other images that you have saved in an image folder. Batch Analysis is not available for Manual Count.

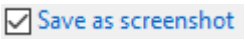
Save analysis results

Save

1. When finished with the Auto count, Manual count, or Cell Culture analysis (Confluence and Transfection Efficiency), 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 as screenshot** to preserve a detailed account of your count results as an image.



   When Save as screenshot is not selected, the software only saves the image as displayed in the Viewing area.

   Table 1 (page 77) lists the various options available for saving analysis results
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.

   This option is not available with Manual count, Confluence measurement, or Transfection Efficiency calculation.
5. Click **Save** to save your analysis results.

Table 1. Save options for analysis tools.

	Save	Save as screenshot	Save data
Auto Count	Image and object count; counted objects and user selected target and background areas identified	Image (same as Save) and Auto Count tab with object count, histogram, and count options as displayed in the Auto Count tool	Separate image (same as Save) and CSV files; CSV file contains brightness, area, and circularity data
Manual Count	Image and total object count; object labels as tagged by user during count	Image (same as Save) and Manual Count tab with labels, object counts, percentages, and total count as displayed in the Manual Count tool	N/A
Confluence	Image in transmitted light channel (with or without mask) and % confluence; user selected target and background areas identified	Image (same as Save) and Cell Culture Confluence tab with % confluence and sensitivity as displayed in the Confluence tool	N/A
Transfection Efficiency	Image in selected channels (with or without mask), % confluence, and % transfection efficiency in the fluorescence channel	Image (same as Save) and Cell Culture Transfection Efficiency tab with % confluence, % transfe ction efficiency, threshold, and other options as displayed in the Transfection Efficiency tool	N/A

N/A: Not applicable.

Batch Analysis

Batch Analysis function

Batch analysis allows you to save and apply the analysis parameters set in Auto Count, Confluence, and Transfection Efficiency tools to other images that you have collected and saved in an image folder.

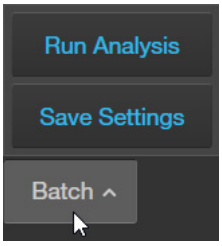
Batch Analysis function is available as a Save Settings-Run Analysis split-button on the Auto Count and Cell Culture panels on the Review tab. It is not available for Manual Count.

IMPORTANT! Images to be batch analyzed should all be of the same cell type and have the same magnification and illumination settings. For consistent measurements, do not mix different cell types, magnifications, or illumination settings in the same folder when performing batch analysis.

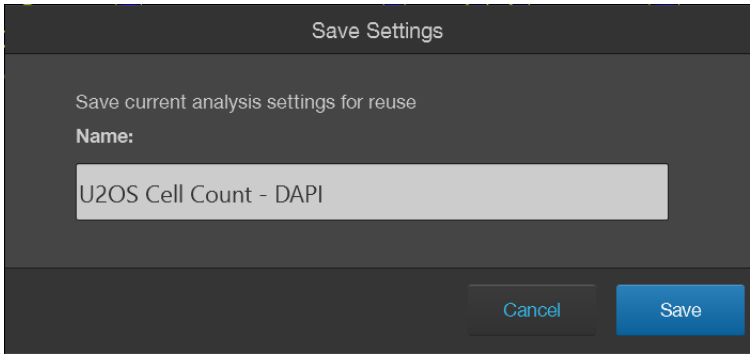
IMPORTANT! For batch analysis, only use 16-bit Raw image files (TIFF or PNG)., which contain the full dynamic range and metadata needed for quantitative analysis.

Save current analysis settings

1. When finished with the Auto count, Confluence measurement, or Transfection Efficiency calculation, click **Batch**, then select **Save Settings**.

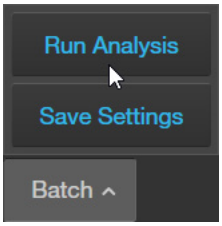


2. Enter the name for the settings, then click **Save**. The current analysis settings are saved for reuse, which you can apply to other images that you have collected and saved an image folder.



Run Batch Analysis

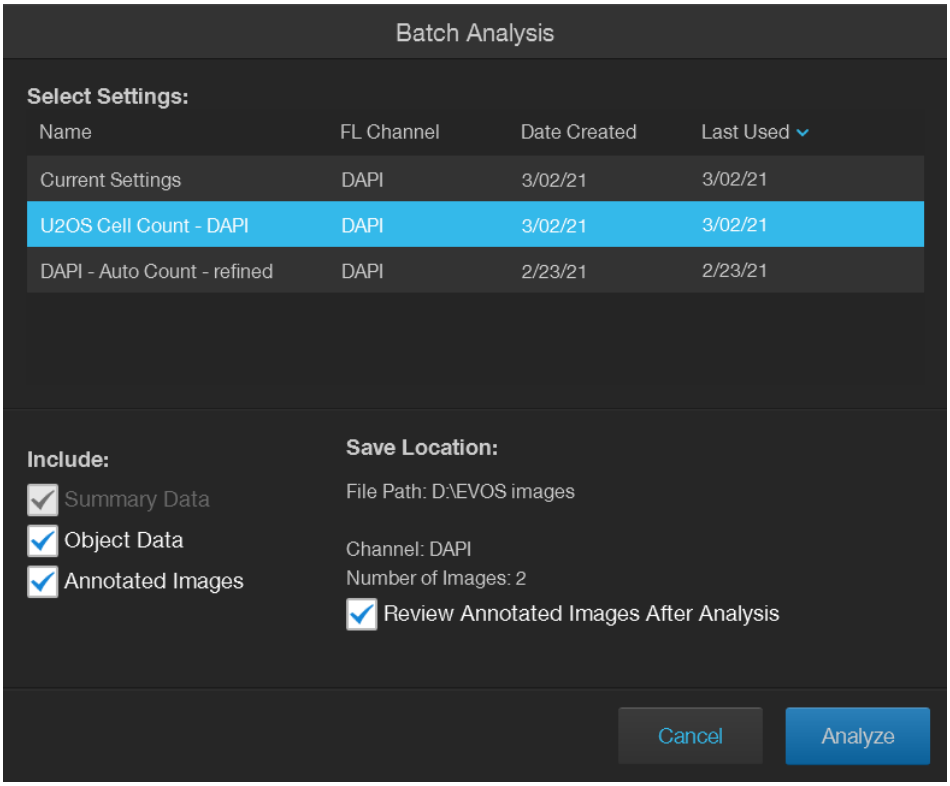
1. Navigate to the folder that contains the images you wish to analyze using the Batch Analysis tool, then click on a representative image to open it.
2. Perform Auto count, Confluence measurement, or Transfection Efficiency calculation on the open image as described previously.
3. To analyze the remaining images in the same image folder, click **Batch**, then select **Run Analysis**.



Note: You can also directly run Batch Analysis for images in an image folder using previously saved Batch Analysis settings without first performing Auto count, Confluence measurement, or Transfection Efficiency calculation (see Step 4).

4. Select the **Settings** to use for Batch analysis.
   - To use the current analysis settings (i.e., analysis parameters that you have used in Step 2), select **Current Settings**.
   - To use previously saved analysis settings, select the desired option from the **Select Settings** list. You can sort the list by fluorescence channel, date created, or last date used.

     In this example, the previously saved **U2OS Cell Count - DAPI** setting is selected.



5. To save the **Intensity**, **Area**, and **Circularity** data of objects counted in Auto Count, select **Object Data**. When Object Data is selected, the data is saved as separate CSV files for each analyzed image in the analysis folder. The option to save Object Data is available only for Auto Count.
6. To embed the measurements from the batch analysis in the images so that you can compare them after the analysis, select **Annotated Images**.

Note: Summary Data is always selected. After the analysis, summary data is included in the analysis folder as a separate CSV file.

7. To review the annotated images immediately after the analysis is complete, select **Review Annotated Images After Analysis**.
8. Click **Analyze** to run batch analysis using the selected settings.

   The software applies the analysis parameters used for the representative image to all the images in the image folder.

   When batch analysis is completed, the software saves the analysis results in a separate folder in the same location as the analyzed images.

   If **Review Annotated Images After Analysis** is selected, the software switches to the Review mode and displays the list of analyzed images in the Review panel.

Note: The analysis folder is named using the following format:

Batch <AnalysisDate><Unique Analysis ID>

For example, Batch 2021-03-02T112538

The images in the analysis folder retain their original name, but they are given the prefix AN_.

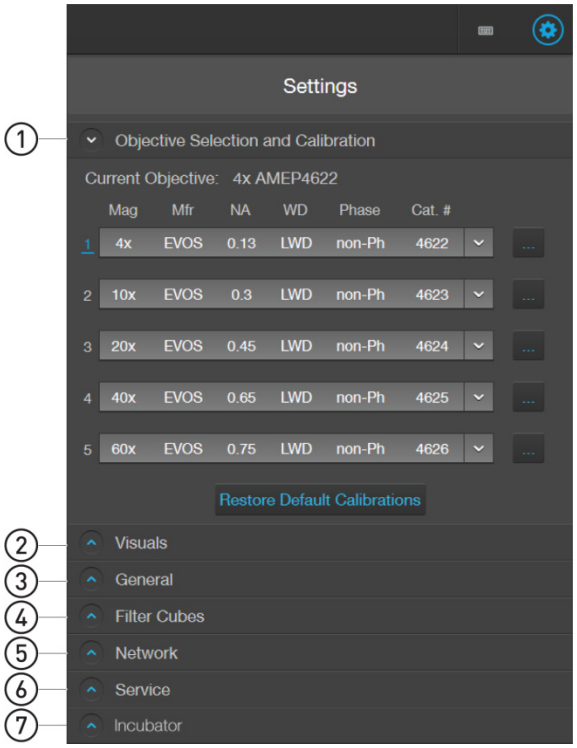
9. Adjust instrument settings

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 the any of the controls, click the corresponding arrow.



① **Objective Selection and Calibration:** Set up and calibrate objectives and assign objective profiles (page 82).

② **Visuals:** Adjust White Balance for the color camera (page 84) and set saturated pixel options (page 85)

③ **General:** Set general instrument options such as save options for TIFF files, focus wheel action, Saved Settings, Capture All Channels, and Align Channels options (page 86)

④ **Filter Cubes:** Add or remove EVOS™ LED light cubes and to assign pseudocolors for installed light cubes (page 95).

⑤ **Network:** Configure network settings (page 88)

⑥ **Service:** Copy error logs, set date and time, and update software and firmware from the cloud or the USB flash drive (page 91).

⑦ **Incubator**: Configure the gas inputs, calibrate the oxygen sensor, and set the temperature offsets for the EVOS™ Onstage Incubator (page 116).

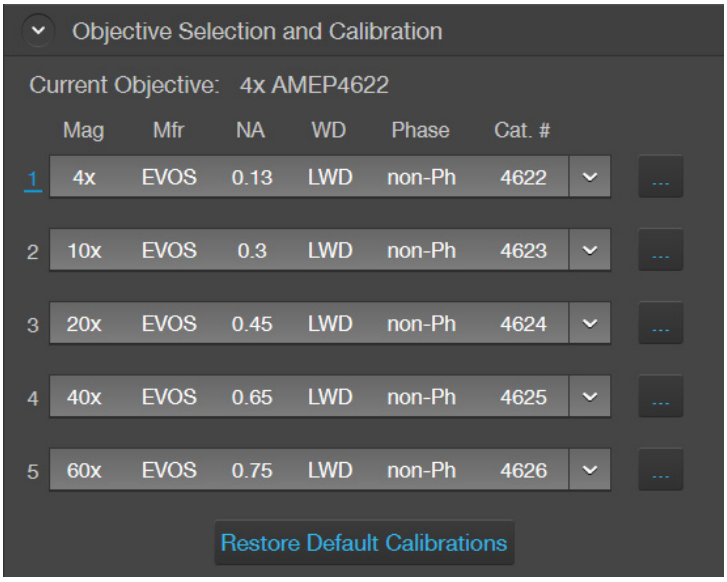
The Incubator panel is only visible when an EVOS™ Onstage Incubator is connected to your EVOS™ M5000 Imaging System.

Adjust objective settings

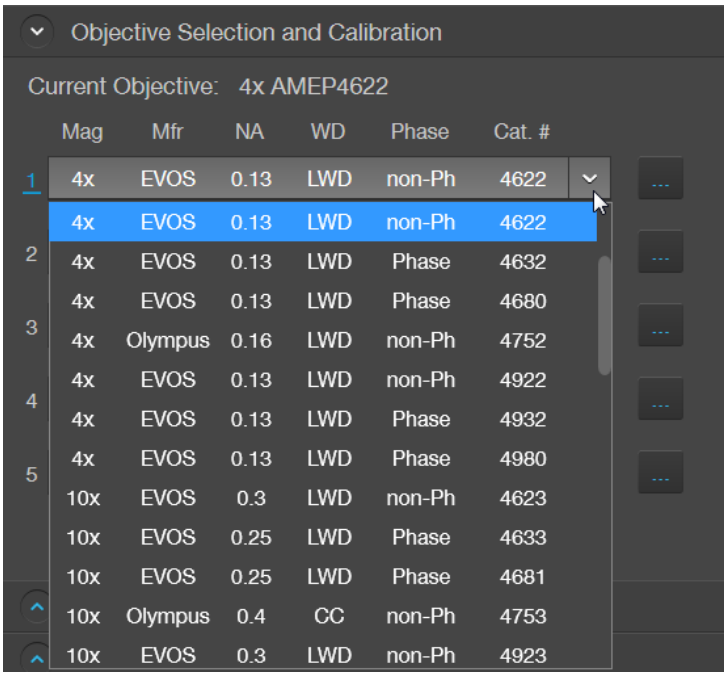
Assign objectives

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 96.

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.

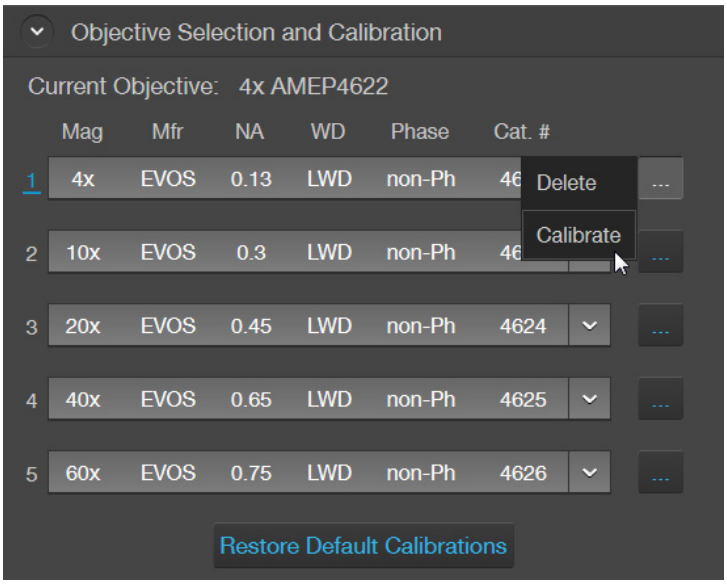


3. Click **Done**.

IMPORTANT! For best results, calibrate the newly installed objective before using it in your experiments (see “Calibrate objective magnification”, page 83.

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.

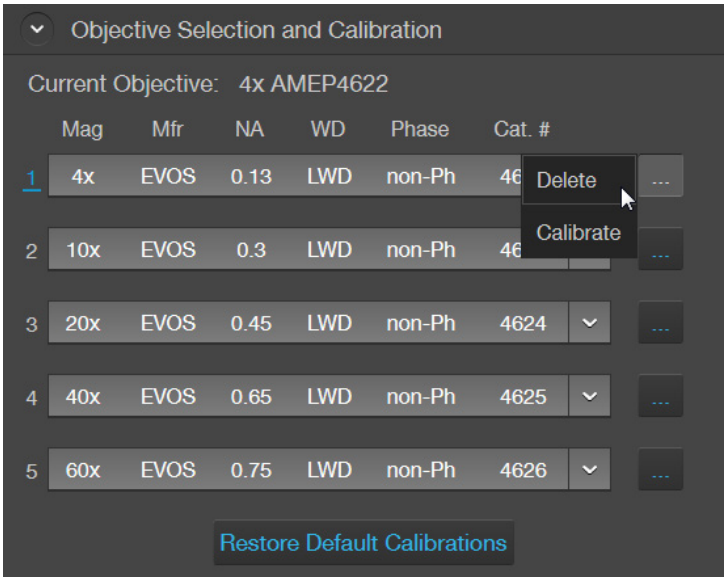


2. Mount the EVOS™ Calibration Slide in the vessel holder, select the objective you want to calibrate, then click **Calibrate Objective**.
3. Follow the onscreen instructions to calibrate the objective magnification for the selected objective. For more information, see "Calibrate the objectives", page 97.

Delete objectives

After removing an objective from the objective turret, unassign the objective from the corrsponding 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**.

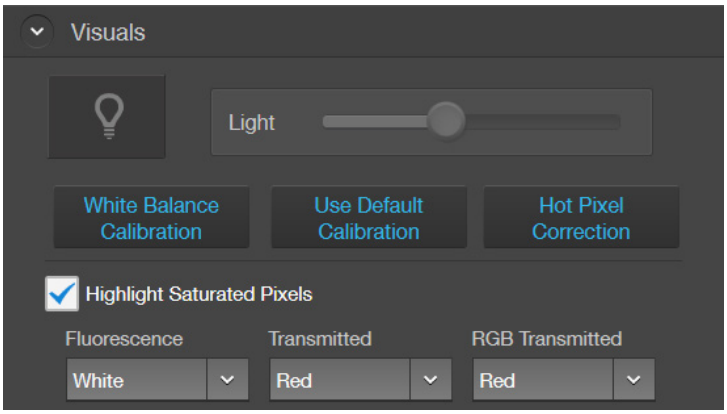


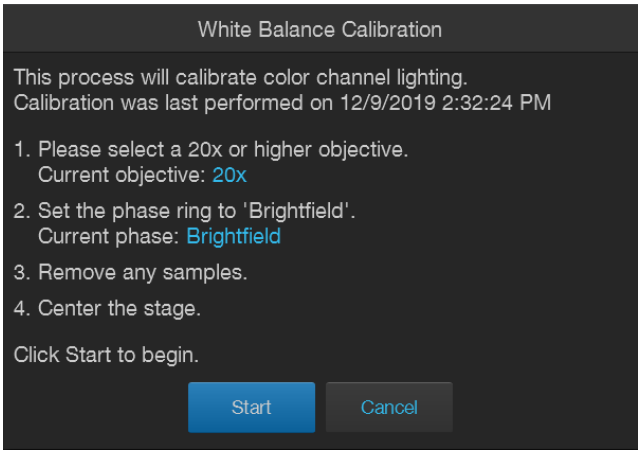
Calibrate white balance

Calibrate white balance

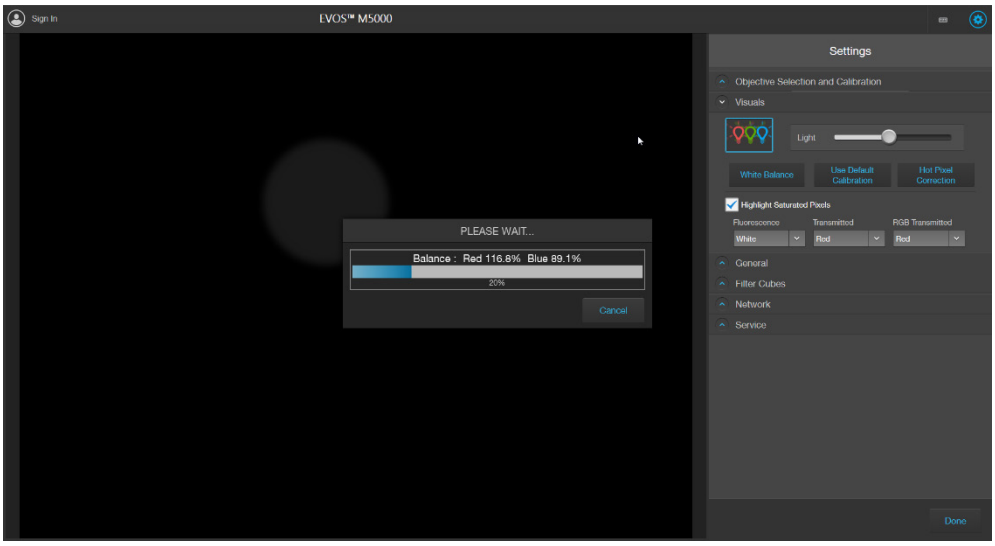
Calibrate White Balance calibrates color channel lighting.

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 8).
3. Remove any samples from the X-Y stage, then center the stage using the stage positioning knobs (page 8).
4. Click **Start** to begin the automatic white balance calibration procedure.

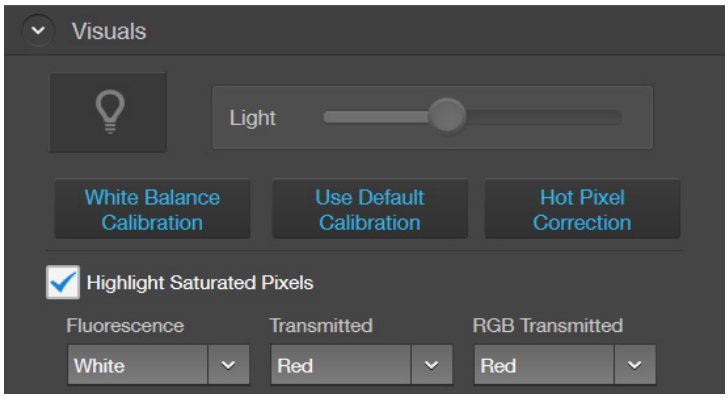


Set saturated pixel options

Highlight saturated pixels

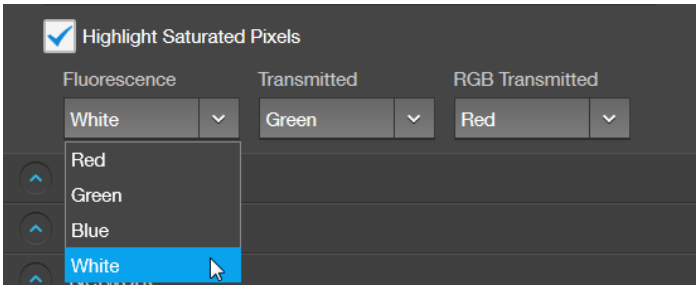
Highlight saturated pixels function displays overexposed pixels on an image with the user-defined color, which provides a visual aid for optimal illumination when adjusting the brightness settings.

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



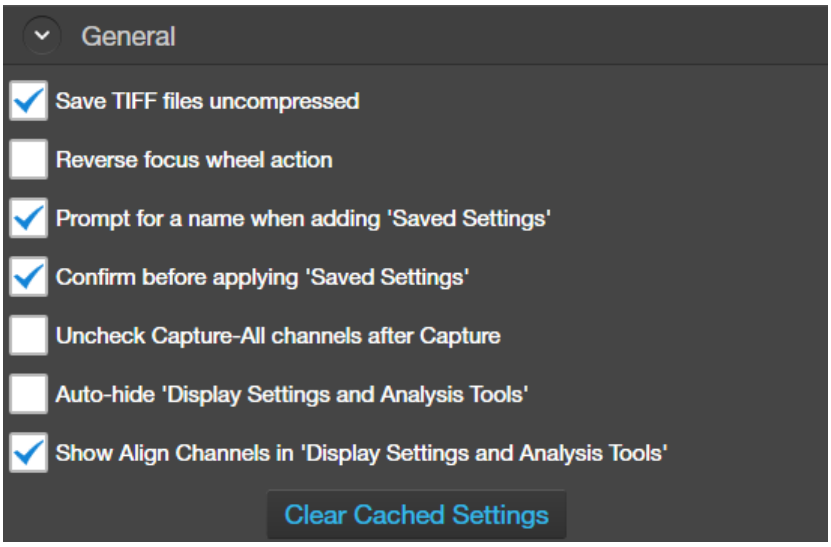
2. To assign a specific color to the saturated pixels in a channel, select the desired color from the corresponding channel dropdown (**Fluorescence**, **Transmitted**, or **RGB Transmitted**).

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



General settings

General settings options



Define save options for TIFF files

1. To save **TIFF files uncompressed**, select the corresponding option.
2. When finished with your selections, click **Done**.

Reverse focus wheel action

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

Define "Saved Settings" options

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**.

Define "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 28).

1. To automatically unselect channel checkboxes after multichannel image capture, click **Uncheck Capture-All channels after Capture** in the **Settings ▸ General** tab.
2. Click **Done**.

Define "Display Settings" toolbar behavior

By default, the Display Settings and Analysis toolbar is displayed automatically after image capture and remains visible. You can change this behavior in the General settings.

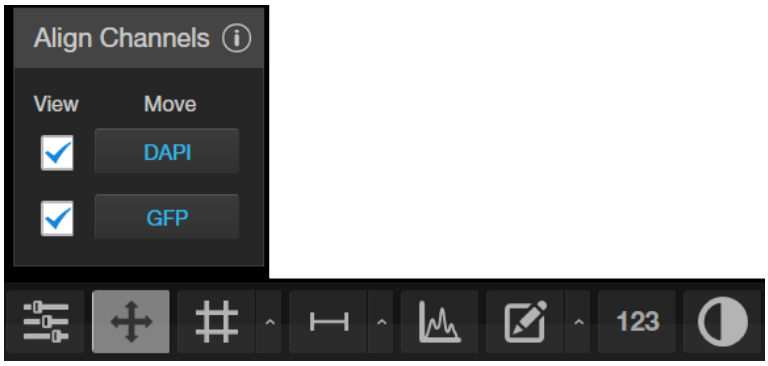
1. To automatically hide the Display Settings and Analysis toolbar, click **Auto-hide 'Display Settings and Analysis Tools'**.
2. Click **Done**. The toolbar will be displayed when you hover the cursor over the Viewing area after image capture. The toolbar will automatically hide if no action is taken.

Show "Align Channels" tool

By default, the button to display the Align Channels tool (indicated by the red arrow) is not shown in the Display Settings and Analysis toolbar. You can add the Align Channels tool to the toolbar in the General settings.

1. Select **Show Align Channels in 'Display Settings and Analysis Tools'** in the **Settings ▸ General** tab.
2. Click **Done**. The Display Settings and Analysis toolbar will display the Align Channels button.

Note: The controls for the Align Channels tool are contextual. The tool only displays the controls for the channels in which the selected multichannel fluorescent image has been captured. In the following example, the tool only shows the controls for DAPI and GFP channels, because the multichannel image was captured in those channels.



Clear Cached Settings

1. Click **Clear Cached Settings** to clear all saved lighting and Z-settings when moving between channels and objectives.
2. Click **Done**.

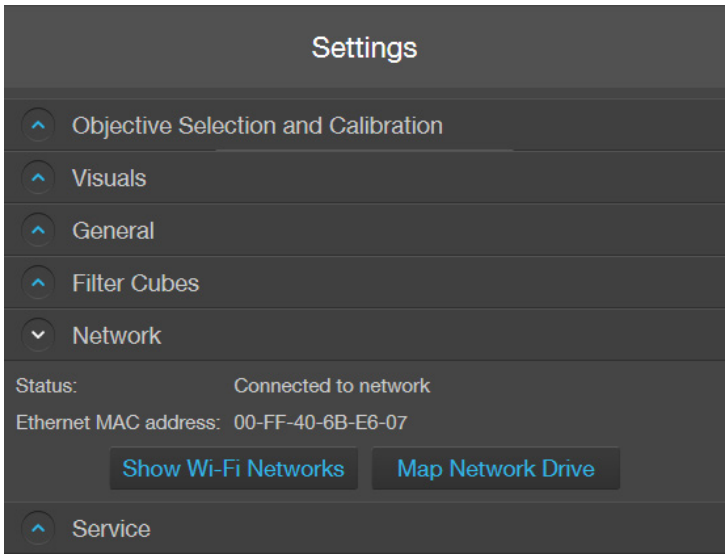
Configure network settings

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.

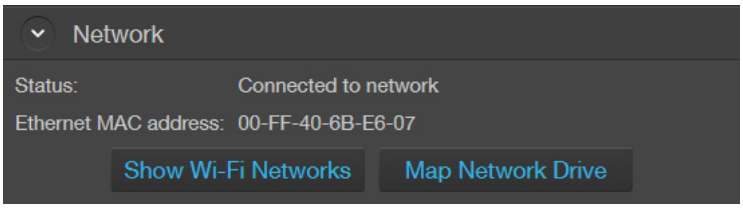


3. Click **Show Wi-Fi Networks**, then select the network you want to join.

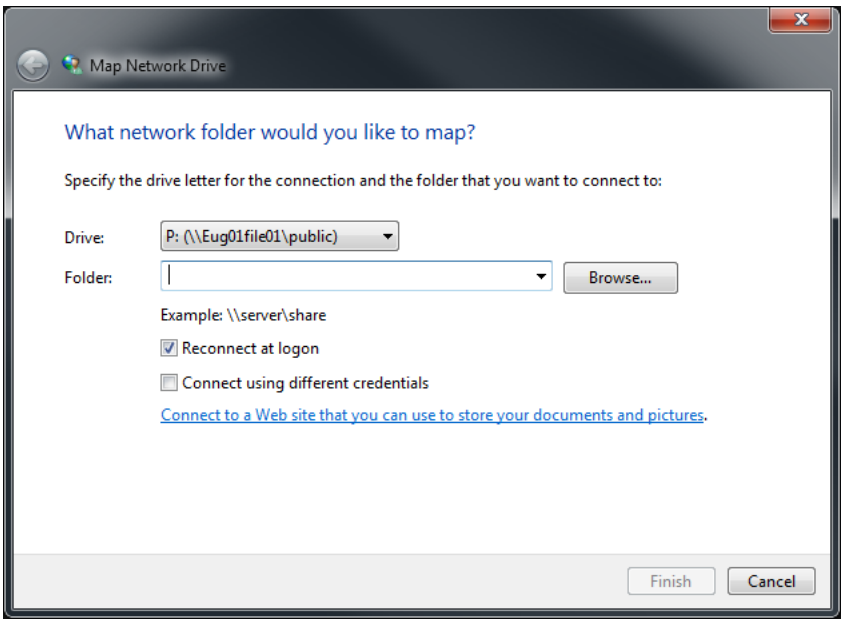
4. Click **Close**.

Map network drive

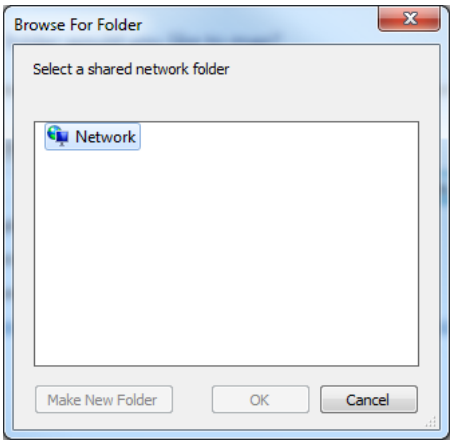
1. Go to the **Settings** ▸ **Network** tab, then click **Map Network Drive**.



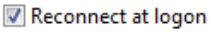
Map Network Drive dialog opens.



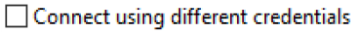
2. Select the drive you want to map from the **Drive** menu.
3. Click **Browse** to open the Browse for Folder dialog.



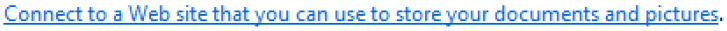
4. Navigate to the folder you want to map, then **OK**.
5. If you want the reconnect to the selected drive and folder when you turn on the instrument, check **Reconnect at logon**.



6. If you want to reconnect as a different user, check **Connect using different credentials**.



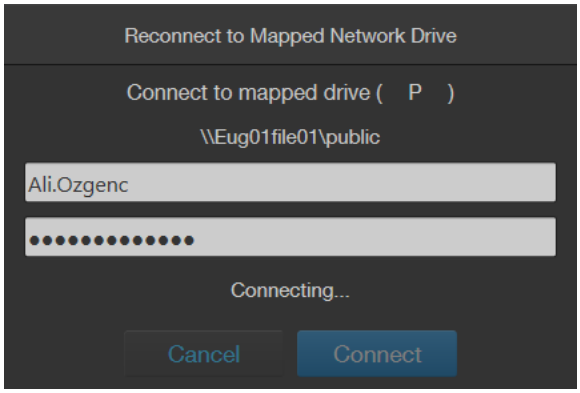
7. To connect to a Web site that you can use to store your documents and pictures, click the hyperlink, then navigate to the desired Web site.



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.

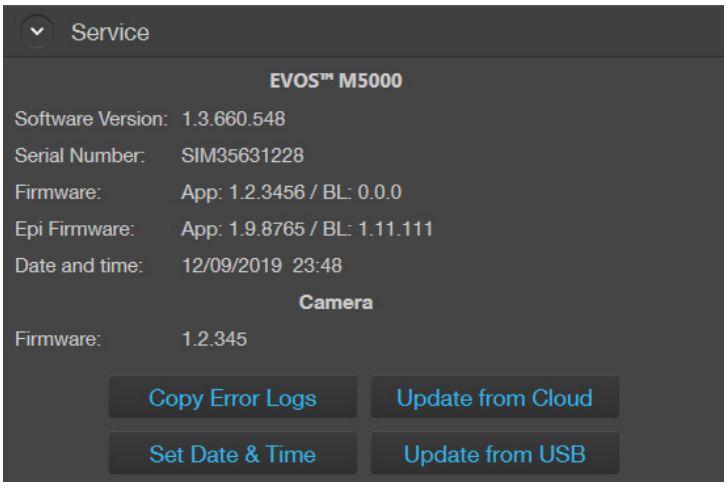
If no connection can be succesfully established to the previously mapped drive during start up, the instrument displays the "Reconnect to Mapped Network Drive" dialog.



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.

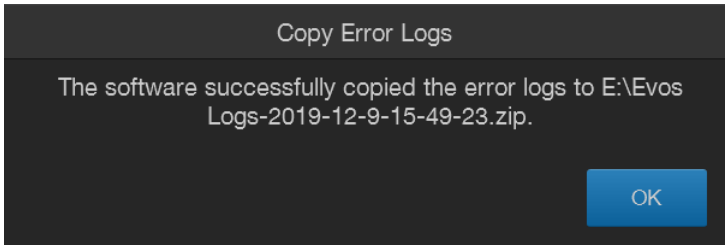
To view the Service tab, click the **Settings** button, then select **Service**.



Copy error logs

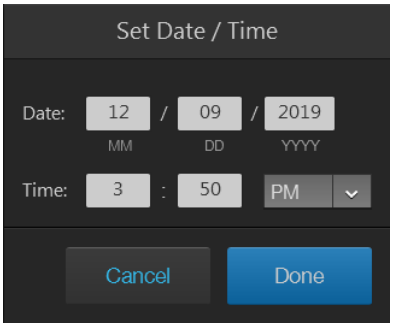
1. On the **Service** tab, click **Copy Error Logs**.

   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 **Error! Bookmark not defined.**).
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** 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 141) 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 96 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_2O_2).

   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

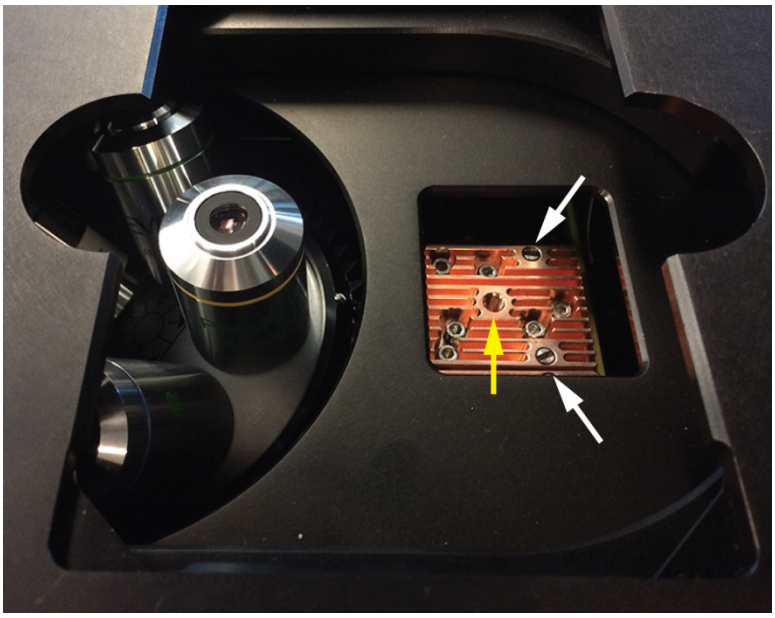
To customize your EVOS™ M5000 Imaging System, you can add and remove LED light cubes to fit the instrument's functionality to your own specific research needs. Each LED light cube is coded to allow the imaging system to automatically recognize it in any position.

For a complete list of available light cubes and to inquire about custom light cubes, go to **thermofisher.com/evos** or contact Technical Support (page 141).

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.

Change the objectives

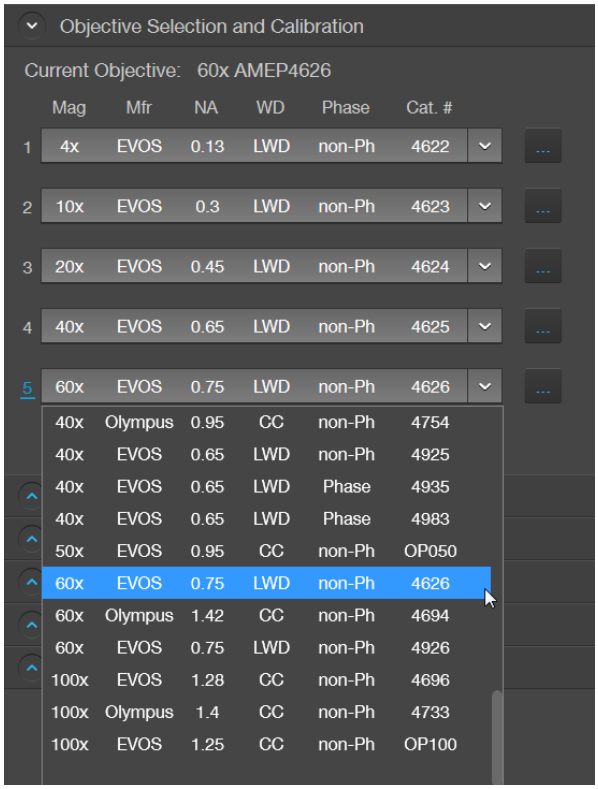
To customize your EVOS™ M5000 Imaging System, you can add and remove objectives to fit the instrument's functionality to your own specific research needs.

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.



Note: Calibrate the newly installed objective before using it (page 97).

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.

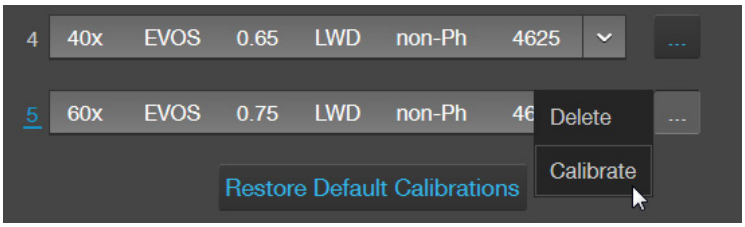
Note: The pre-installed objectives supplied with the EVOS™ M5000 Imaging System have been pre-calibrated. You do not need to calibrate them again unless they are reinstalled after removal from the instrument.

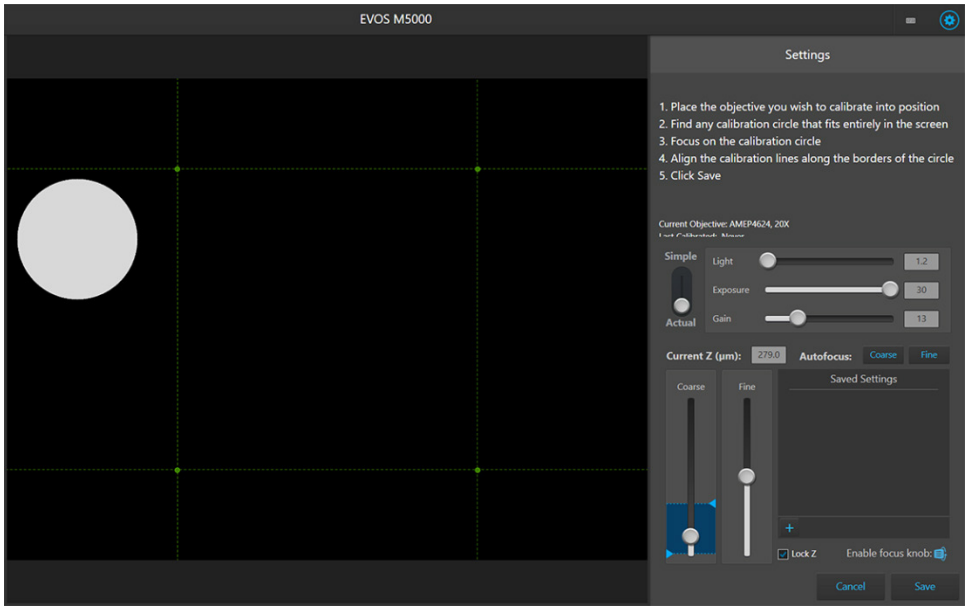
Calibrate objective magnification

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

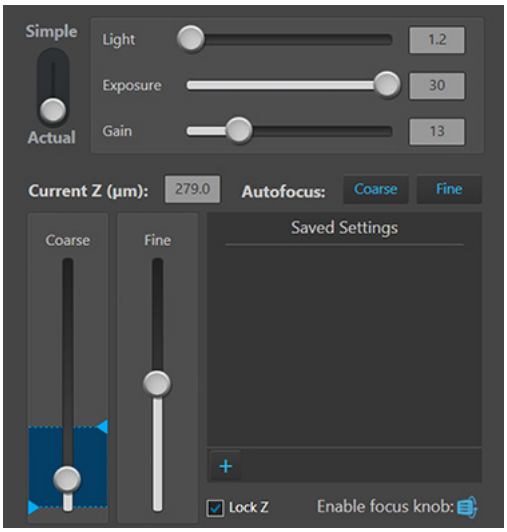
Note: For best parfocality and parcentration, calibrate the installed objectives one after the other without removing the calibration slide.

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 8).
3. Go to **Settings** ▸ **Objective Selection and Calibration**, click **Action** button ... for the newly installed objective, then select **Calibrate** to launch the Objective Calibration tool.

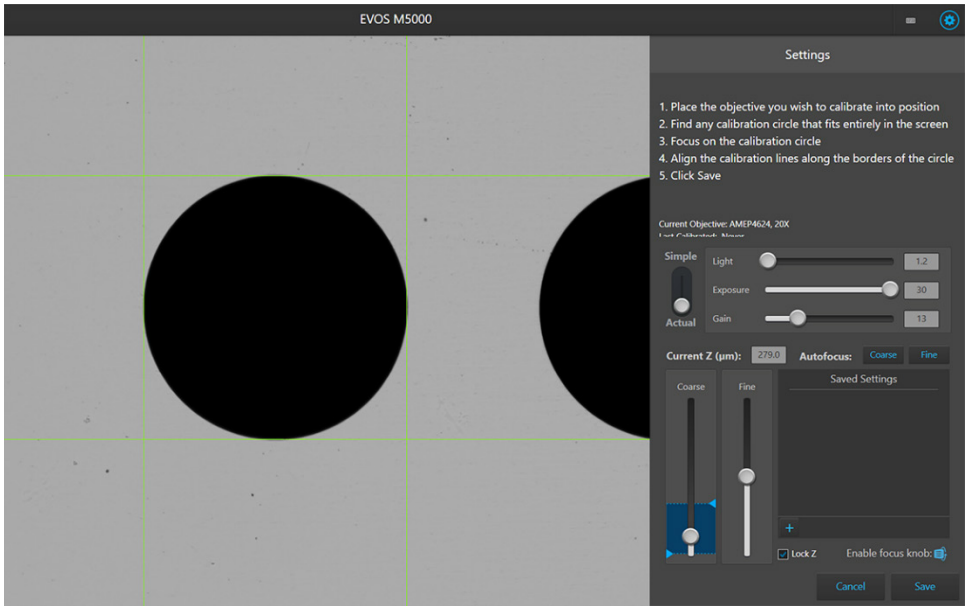




4. Find a calibration circle that fits entirely in the field of view.
5. Focus on the calibration circle using the focus controls.



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 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** or contact Technical Support (page 141).

Image quality issues

Problem	Possible solutions
Image is too dim (at higher magnifications)	Remove the condenser slider, if one is in place.
Specks, dots, or blurs on image	Follow the instructions under "Objective lens care" (page 93) to clean the objectives.
Uneven focus across screen	• Position the sample so that it lies flat on the stage; ensure that the sample's thickness is even. • Ensure that the vessel holder is mounted flat with respect to the stage.
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	• Click the **Power** button (onscreen). • Center the sample over the objective. • Verify that the power supply is connected and the power switch is on.
Image display is red, or red patches cover parts of the screen	• Dim the illumination until the red highlights disappear to get the maximum level of brightness without any overexposed areas. • Disable the "Highlight Saturated Pixels" option in the **Settings** tab.

Software interface issues

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

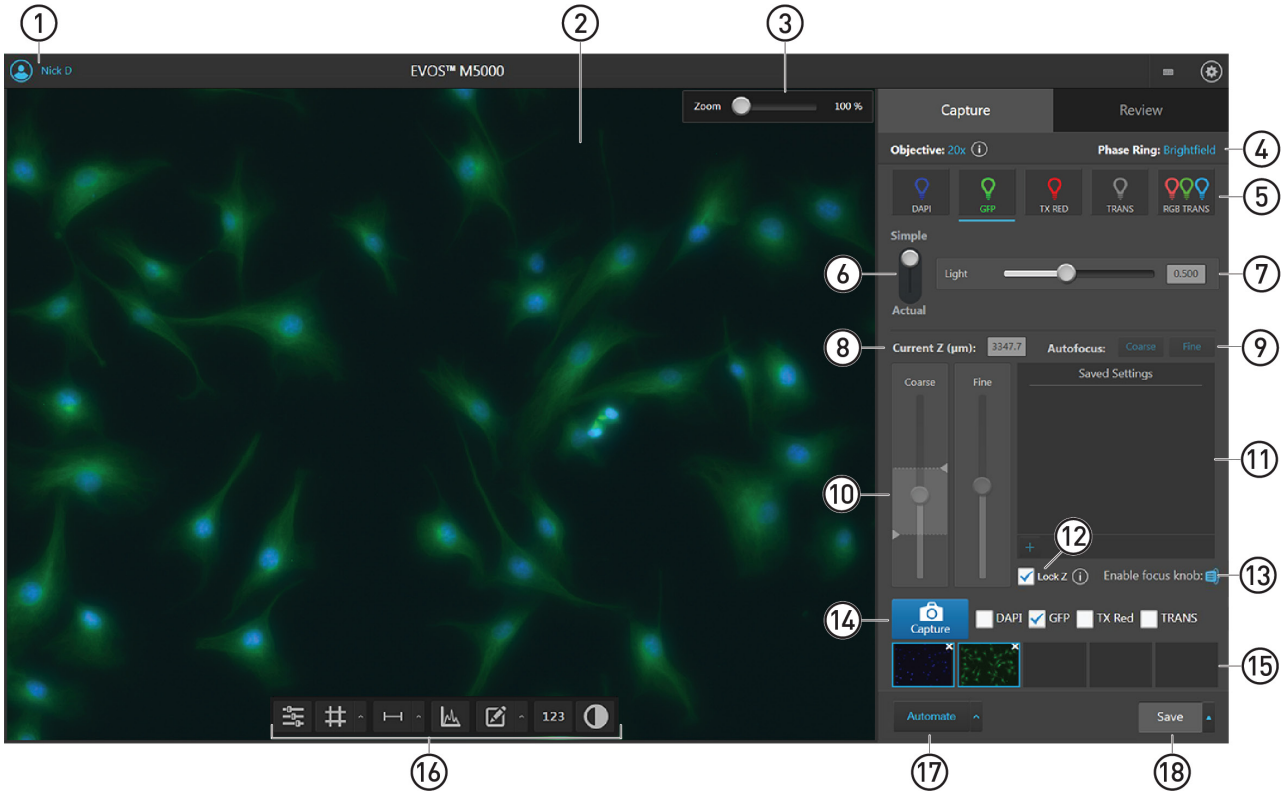
Problem	Possible solutions
Image does not respond to changes in focus or stage position	Click the **Light** button to return to live observation.
Some of the software controls are not available	The controls available on the EVOS™ M5000 Imaging System are contextual; only the controls relevant for the 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	• Verify physical cable connections; confirm that the Ethernet jack is active (for Ethernet connection). • 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). • Contact your network administrator to resolve any network issues.

Mechanical issues

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

Appendix B: Graphical user interface (GUI)

Capture tab

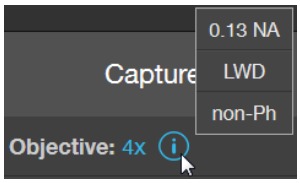


1. **Sign In/User:** Allows you to sign in to and sign out of your Thermo Fisher Connect account.
2. **Viewing area:** Displays the sample.
3. **Zoom slider:** Zooms in and out of the Viewing area. The zoom range is 100% to 1000%.

4. **Active objective and phase ring:** Displays the active objective and phase ring information.

If the selected objective and the phase ring match, the selected phase ring is depicted in blue font. If the objective and the phase ring do not match, the phase ring is depicted in orange font.

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.

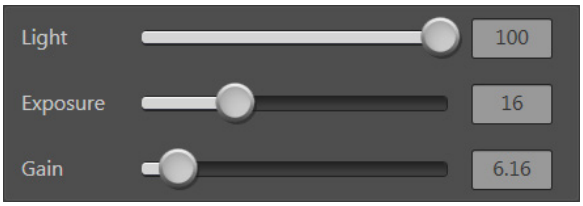
- Select a channel to turn on the excitation light and enter the instrument in the **Live mode**. In the Live mode, you can use the brightness and focus controls.
- Click the selected Channel button again to turn off the excitation light and exit the Live mode. The current channel remains selected as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



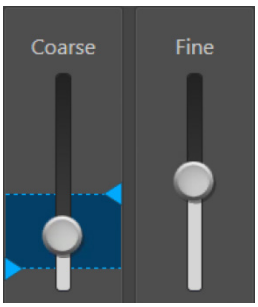
Actual mode

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.

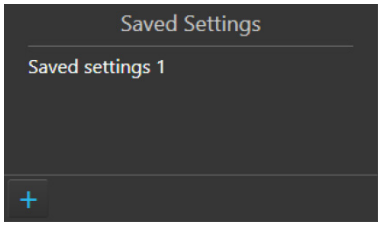
⑩ **Coarse and Fine focus sliders:** Allow you to adjust the focus in the selected channel when in the Live mode.

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.

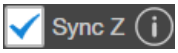


⑪ **Saved Settings:** Allows you to save and edit Z-axis focus positions and brightness settings for future use.

Press the + button to save the current settings.

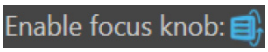


⑫ **Synch Z:** Allows you to synch the Z-axis offsets, which specify the optimal focus position in each channel relative to the focus position in other channels.



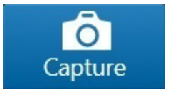
- When synched, movement of the Z-position in one channel changes the Z-position in all channels, preserving their relative Z-positions.
- When unsynched, adjusting the Z-position only affects the currently selected channel.
- Click ⓘ next to Synch Z selection to view the recommended workflow for Z Offset set-up (page 24).

⑬ **Enable focus knob:** Enables and disables 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.

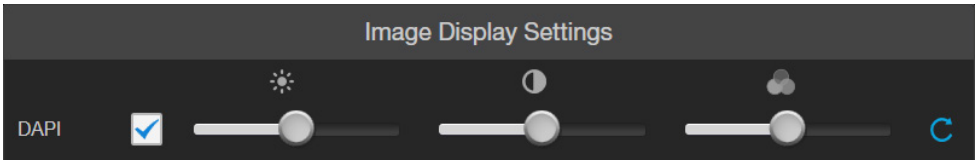
You can capture multiple channels by selecting the corresponding channel checkboxes.



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

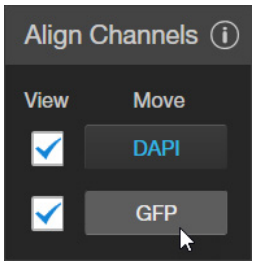
⑯ **Display settings and analysis tools:** Allow you to change image display settings in the Viewing area, and analyze and annotate captured images.

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.



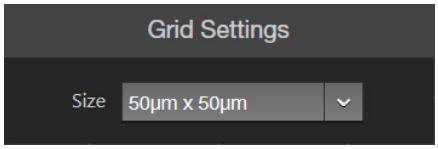
Align Channels: Allows you to correct pixel shifts that can occasionally appear when performing multichannel fluorescence imaging at higher magnifications. The Align Channels tool only displays the controls for the channels in which the selected multichannel fluorescent image has been captured.

The button to display the Align Channels tool is visible only if the **Show Align Channels in Display Settings and Analysis Tools** option is selected in the **Settings ▸ General** tab.



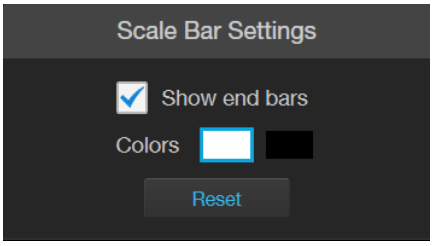
Display Grid/Grid Settings: Display Grid button switches the grid display in the Viewing area on and off.

Grid Settings allows you to set the grid size.

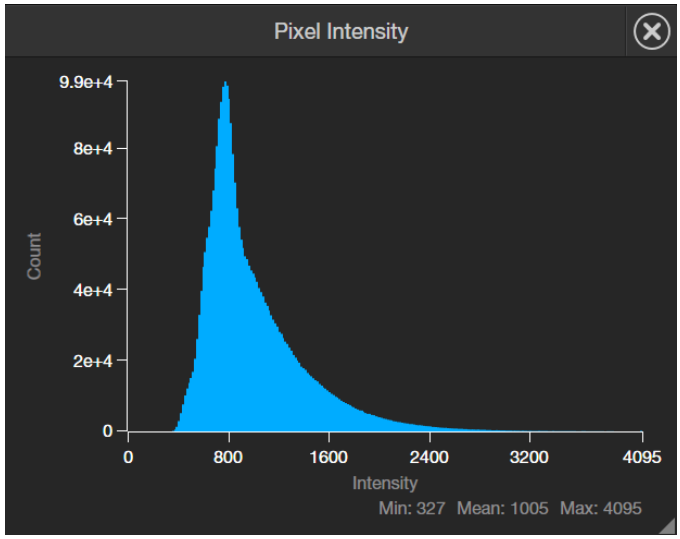


Display Scale Bar/Scale Bar Settings: Display Scale Bar button switches the display of the scale bar in the Viewing area on and off.

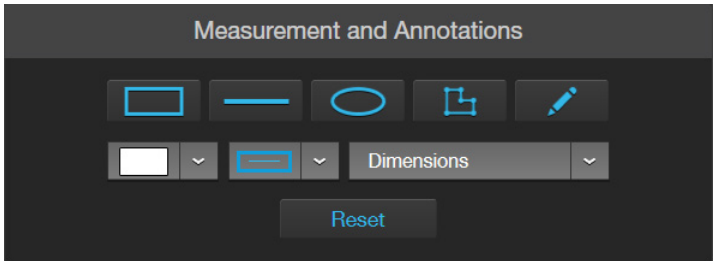
Scale Bar Settings allows you to select scale bar color and to display or hide end bars.



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.

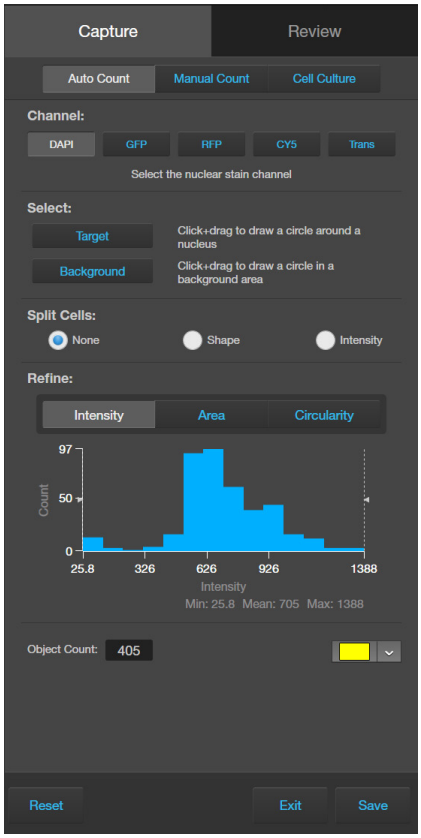


Measurements and Annotations: Allows you to draw regions of interest (rectangle, ellipse, polygon, line, or free-form) on the captured image and measure dimensions, area, or perimeter of the drawn region.

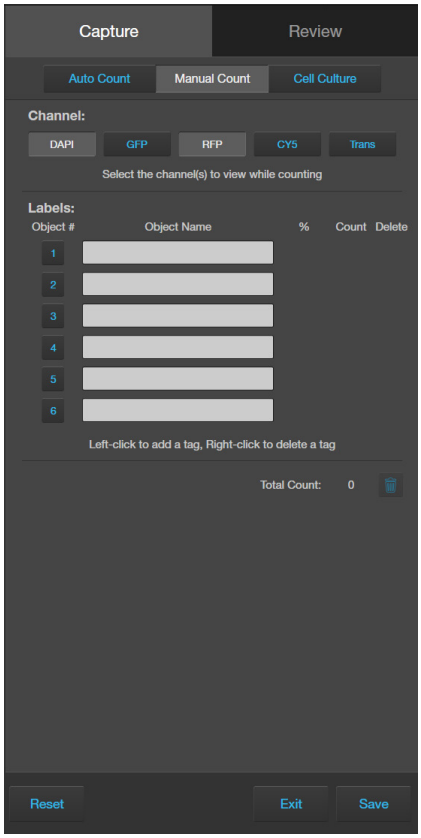


Show Cell Count (123): Allows you to perform cell counts using the Auto Count or Manual Count tools and to measure the confluence and transfection efficiency of your culture from the captured image using the Cell Culture tools.

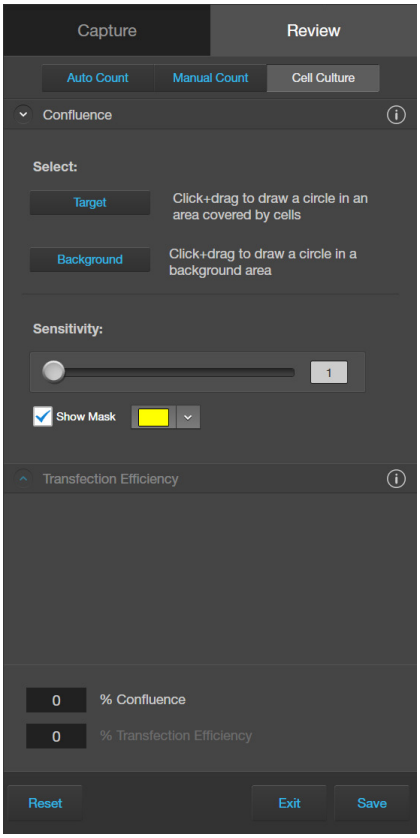
- **Auto Count:** Allows you define auto count parameters by selecting representative target objects, then count the objects by intensity, area, and circularity. See page 108 for Auto Count controls.
- **Manual Count:** Allows you to manually mark items onscreen using up to six separate labels and keep a running tally of the counts with percentages for each label. See page 109 for Manaul Count controls.
- **Cell Culture:** Allows you to measure the confluence of your culture and calculate the transfection efficiency. See page 110 for Confluence controls and page 111 for Transfection Efficiency controls.



Auto Count



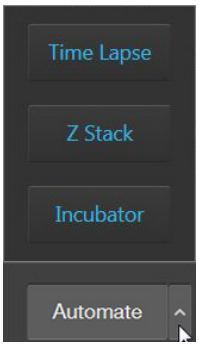
Manual Count



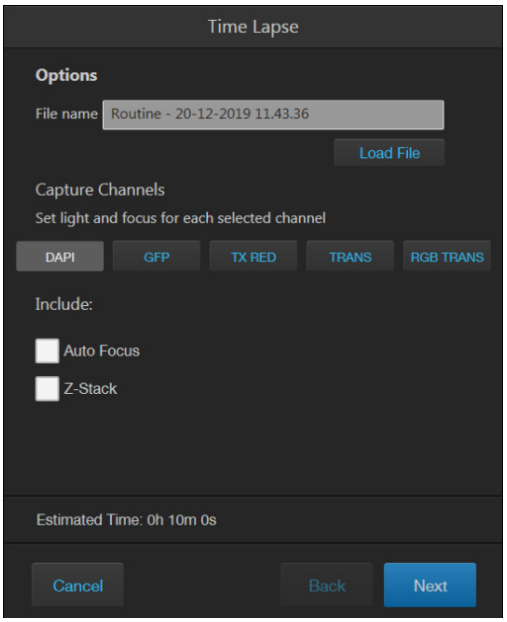
Cell Culture

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

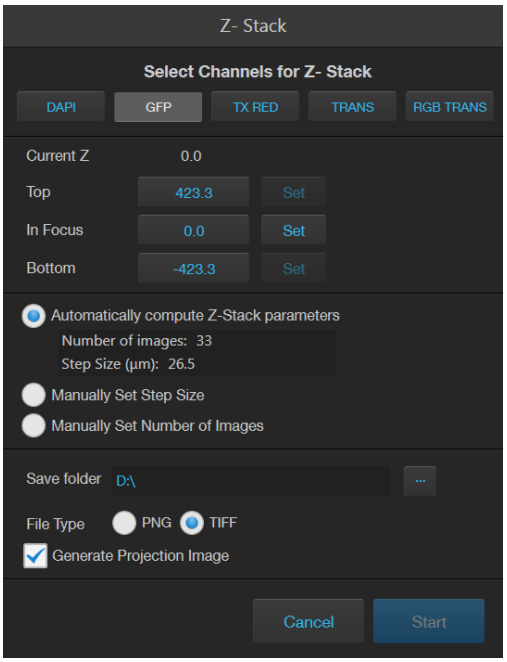
⑰ **Automate:** Allows you to display the controls for the Time Lapse and Z-Stack tools, and the EVOS™ Onstage Incubator.



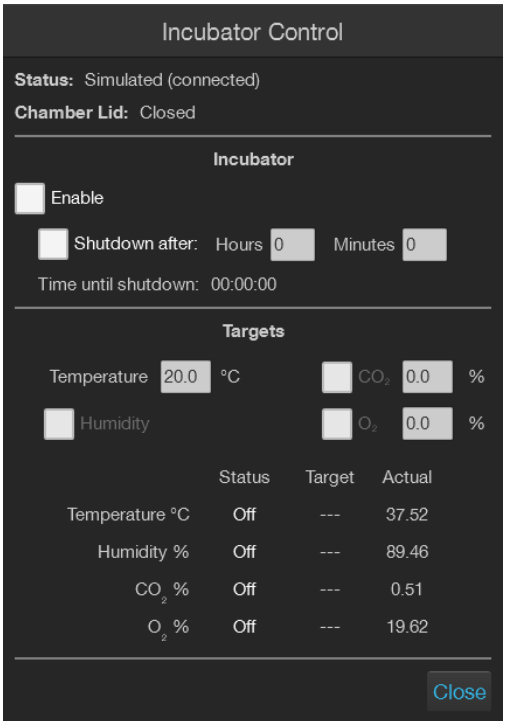
Time Lapse: Opens the Time Lapse tool, which allows you to create and run time lapse routines to capture images at given intervals over a time period based on your specifications.



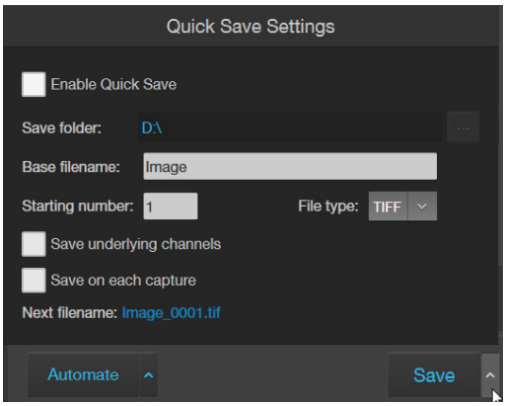
Z-Stack: Opens the Z-Stack tool, which allows you to capture multiple images along the Z-axis based on your specifications. You can use the images captured with the Z-Stack tool to generate a Z-Stack Projection.



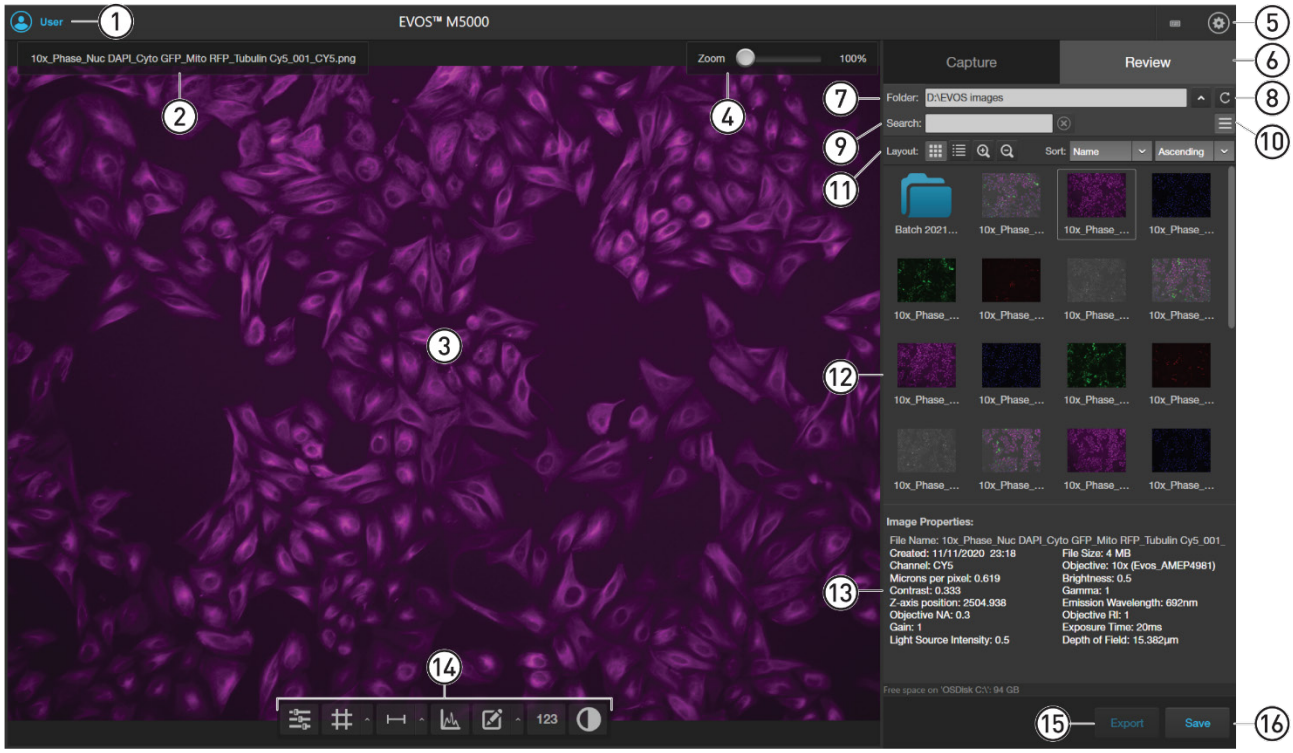
Incubator: Opens the Incubator Controls, which allow you to control the EVOS™ Onstage Incubator and monitor its status during your experiments.



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



Review tab



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

② **Image file name:** File name of the image displayed in the Viewing area.

③ **Viewing area:** Displays the selected image.

④ **Zoom slider:** Zooms in and out of the image. The zoom range is 100% to 1000%.

⑤ **Settings:** Opens the Settings tab. See page 112 for more information.

⑥ **Capture and Review tabs:** Allows you to toggle between the Capture and Review tabs.

⑦ **Folder:** Displays the location of the current folder or image.

⑧ **Up and Refresh:** Up button allows you go up one folder from the current folder. Refresh button refreshes the list or grid of images in the current folder.

⑨ **Search:** Allows you to search images by file name in the selected folder.

⑩ **Display Settings:** Displays or hides the Layout controls.

⑪ **Layout:** Allows you to toggle between grid or list view, increase or decrease the display size of the folders or image files, and sort files by name, file type, or date created in ascending or descending order.

⑫ **Image preview/Image list:** Displays the preview or list of the image files and subfolders in the current folder.

⑬ **Image Properties:** Displays the metadata for the selected image file.

⑭ **Display settings and analysis tools:** Allow you to change image display settings in the Viewing area, and analyze and annotate captured images. See page 103 for more information.

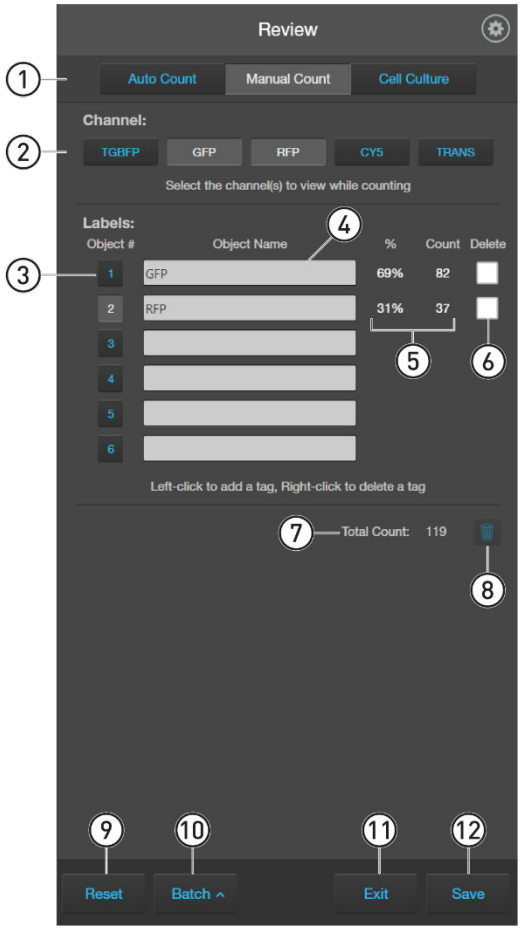
⑮ **Export:** Allows you to export the currently selected folder or image to a storage device.

⑯ **Save:** Saves the currently opened image.

Auto Count controls

1. **Analysis tools:** Allows you to toggle between Auto Count, Manual Count, or Cell Culture (Confluence and Transfection Efficiency) tools for image analysis.
2. **Channel:** Selects the nuclear stain channel for Auto Count. You can select only a single channel.
3. **Select Target and Background:** Allows you to select representative target objects and background areas for Auto Count.
4. **Split Cells:** Allows you to split multiple objects that have been counted as one into individual objects based on shape or pixel intensity to increase the count accuracy.
5. **Refine:** Selects intensity, area, or circularity by which to refine Auto Count results using the Count Histogram.
6. **Count Histogram:** Allows you to set pixel intensity, area, or circularity thresholds to refine the Auto Count results.
7. **Object Count:** Displays the object count based on the Auto Count parameters.
8. **Object color:** Selects the color by which the counted objects are identified.
9. **Reset:** Resets the count to 0 and clears the selected targets and background areas.
10. **Batch Analysis:** Allows you to save and apply the analysis parameters to other images that you have collected and saved in an image folder (see page 78 for more information).
11. **Exit:** Exists the Auto Count tool and displays the Review tab.
12. **Save:** Saves the analysis results as an image in the selected file format (see page 76 for more information).

Manual Count controls



① **Analysis tools:** Allows you to toggle between Auto Count, Manual Count, or Cell Culture (Confluence and Transfection Efficiency) tools for image analysis.

② **Channel:** Selects the channels for Manual Count. You can select multiple channels.

③ **Object #:** Allows you to select the label (Object #) with which to tag objects in the Viewing area. Left-click on the objects in the Viewing area to tag them with the selected label; right-click to delete a tag. In this example, Object #2 is selected. You can switch labels as desired. See page 42 for more information.

④ **Object Name:** Allows you assign a name for the Object #.

⑤ **% and Count:** Displays the label count and its percentage of the total object count (total count of all labels).

⑥ **Delete:** Selects a label for deletion.

⑦ **Total Count:** Displays the total count of objects tagged with all labels.

⑧ **Trash:** Deletes the tags for the label and resets the label count to 0.

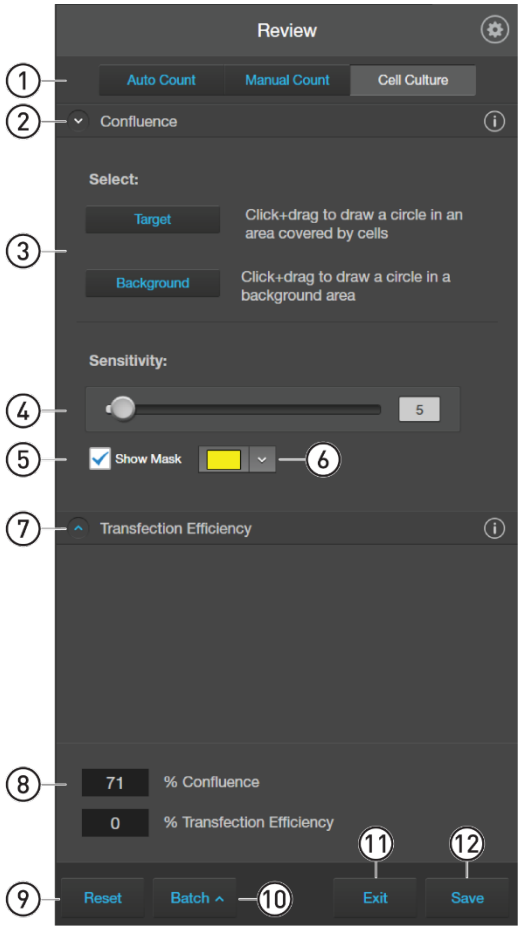
⑨ **Reset:** Resets all label counts and the total count to 0 and clears the Viewing area of all tags.

⑩ **Batch Analysis:** Allows you to save and apply the analysis parameters to other images that you have collected and saved in an image folder (see page 78 for more information).

⑪ **Exit:** Exists the Manual Count tool and displays the Review tab.

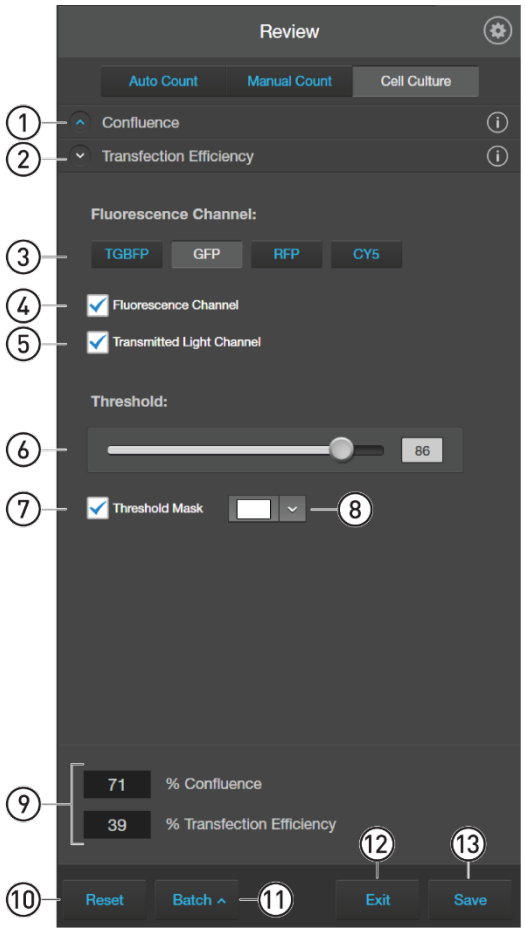
⑫ **Save:** Saves the analysis results as an image in the selected file format (see page 76 for more information).

Cell Culture – Confluence controls



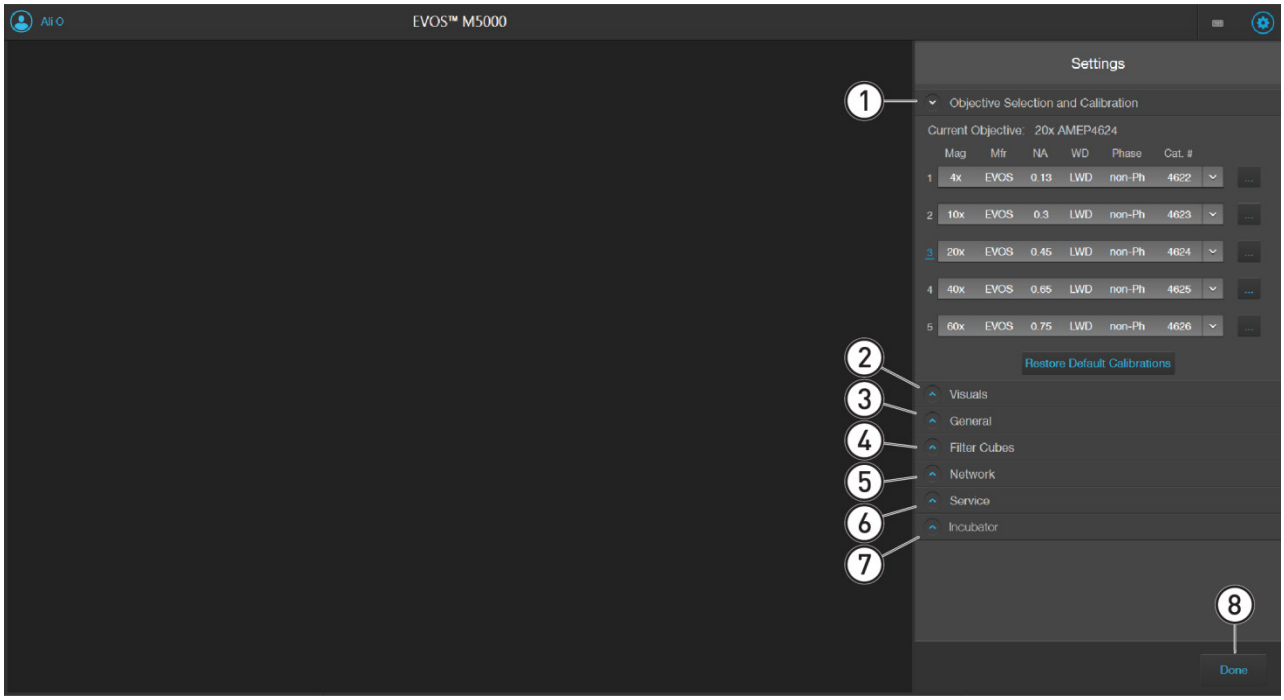
1. **Analysis tools:** Allows you to toggle between Auto Count, Manual Count, or Cell Culture (Confluence and Transfection Efficiency) tools for image analysis.
2. **Confluence:** Expands or hides the controls for the Confluence tool.
3. **Select Target and Background:** Allows you to select representative cell and background areas for the confluence measurement.
4. **Sensitivity:** Adjusts the algorithm sensitivity to pixel intensity (higher intensity = more pixels included). Decreasing the sensitivity reduces the confluence value.
5. **Show Mask:** Indicates the areas included in the confluence measurement.
6. **Mask Color:** Selects the mask color.
7. **Transfection Efficiency:** Expands or hides the controls for the Transfection Efficiency tool. The Transfection Efficiency tool is inactive until the confluence measurement is completed.
8. **% Confluence:** Displays the percentage of the area covered by cells in the image, based on the selected target and background areas and sensitivity.
9. **Reset:** Resets the Confluence and Transfection Efficiency measurements to 0 and clears the selected targets and background areas.
10. **Batch Analysis:** Allows you to save and apply the cell culture analysis parameters to other images that you have collected and saved in an image folder (see page 78).
11. **Exit:** Exists the Cell Culture tool and displays the Review tab.
12. **Save:** Saves the analysis results as an image in the selected file format (see page 76).

Cell Culture – Transfection Efficiency controls

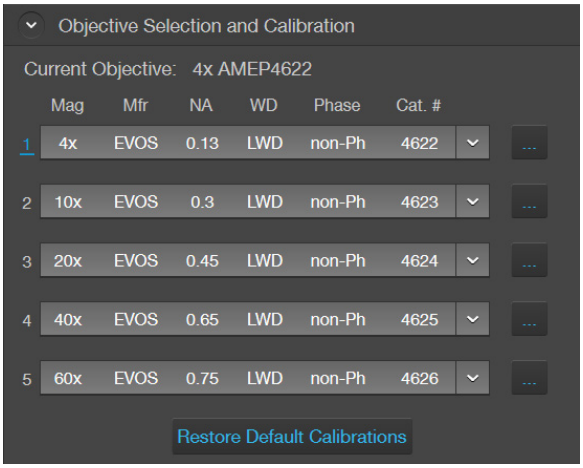


1. **Confluence:** Expands or hides the controls for the Confluence tool.
2. **Transfection Efficiency:** Expands or hides the controls for the Transfection Efficiency tool.
3. **Fluorescence Channel selection:** Selects the fluorescence channel for transfection efficiency calculation.
4. **Fluorescence Channel:** Toggles the display of the fluorescence channel.
5. **Transmitted Light Channel:** Toggles the display of the transmitted light.
6. **Threshold:** Adjusts the fluorescence threshold value. Only the cells that express above the set threshold are used in the tranfection efficiency calculation.
7. **Threshold Mask:** Indicates the areas above the set threshold value and are included in the transfection efficiency calculation.
8. **Mask Color:** Selects the threshold mask color.
9. **% Confluence and %Transfection Efficiency:** Displays the calculated confluence and transfection efficiency values.
10. **Reset:** Resets the Confluence and Transfection Efficiency measurements to 0 and clears the selected targets and background areas.
11. **Batch Analysis:** Allows you to save and apply the cell culture analysis parameters to other images that you have collected and saved in an image folder (see page 78).
12. **Exit:** Exists the Cell Culture tool and displays the Review tab.
13. **Save:** Saves the analysis results as an image in the selected file format (see page 76).

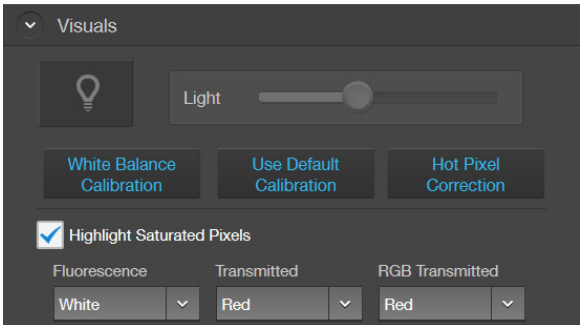
Settings



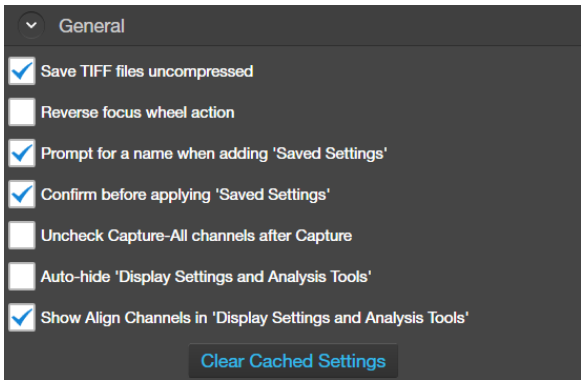
① **Objective Selection and Calibration:** Allows you to assign and unassign objectives on the objective turret, and to calibrate objective magnification.



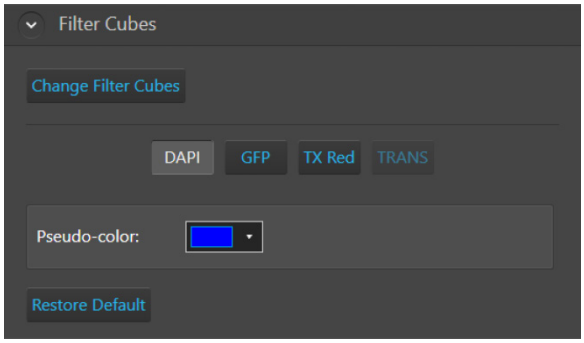
② **Visuals:** Allows you to calibrate color channel lighting (White Balance Calibration) and to assign saturated pixel colors in the Fluorescence, Transmitted, and RGB Transmitted channels.



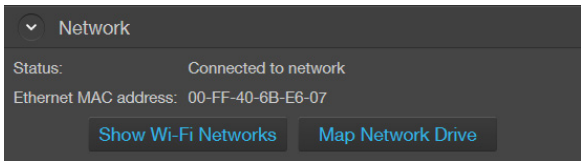
③ **General:** Allows you to define general instrument options such as save options for TIFF files, focus wheel action, Saved Settings, Capture All Channels, and Align Channels options, and to clear cached settings.



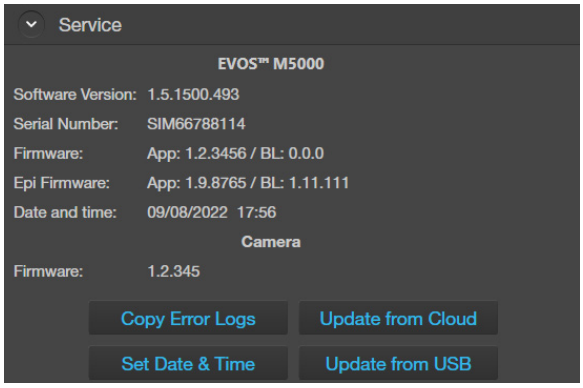
④ **Filter Cubes:** Allows you to add or remove EVOS™ LED light cubes from the instrument and to assign pseudocolors to specific channels.



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

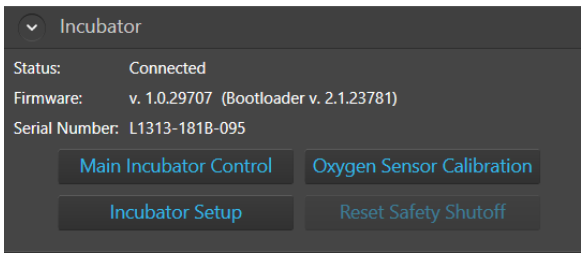


⑥ **Service:** Displays the EVOS™ M5000 hardware and software information, allows you to update the EVOS™ M5000 firmware and software from Connect, Thermo Fisher's cloud-based platform, or using a USB flash drive, and to copy the error logs.

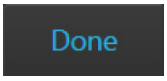


⑦ **Incubator:** Allows you to set up and configure the EVOS™ Onstage Incubator or the EVOS™ Onstage Incubator (OSI-2).

The Incubator tab is visible only if an Onstage Incubator is connected to the EVOS™ M5000 instrument.



⑧ **Done:** Closes the Settings tabs and returns to the previously opened tab.



Appendix C: System overview

Technical specifications

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**).

Physical characteristics

Dimensions (W × H × D): 46 × 59 × 46 cm (18 × 23 × 18 in)

Weight: 16 kg (35 lbs.)

Footprint: Approximately 92 cm × 92 cm (36 in × 36 in)

Operating temperature: 4°–32°C (40°–90°F)

Operating humidity: <90%, non-condensing

Operating power: 100–240 VAC, 1.8 A

Frequency: 50–60 Hz

Electrical input: 12 VDC, 5 A

Hardware

Optics: Infinity-corrected optical system; RMS-threaded objectives with 45 mm parfocal distance.

Illumination: Adjustable intensity LED (>50,000-hour life per light cube).

Light cubes (not included): 5 position chamber for 4 fluorescence cubes plus brightfield. Broad selection standard and specialty light cubes (see page 115).

Contrast methods: Fluorescence and transmitted light (brightfield & phase contrast).

Objective turret: 5-position; front-mounted control.

Objectives (not included): Wide selection of high-quality LWD and coverslip-corrected objectives.

Condenser: 60-mm long working distance condenser, 4 position turret with a clear aperture and 3 phase annuli.

Stage: 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.

Focus mechanism: Automated focus mechanism with sub-micron resolution.

LCD display: 18.5-inch high-resolution LCD display (1920 × 1080 pixel resolution)

Camera: High-sensitivity monochrome CMOS camera (2048 × 1536 pixel resolution, 3.2 Megapixels) with 3.45-µm pixel resolution

Captured images: 16-bit monochrome (12-bit dynamic range) TIFF; 8-bit per RGB channel TIFF, PNG, BMP, or JPG.

Output ports: 4-pin power input port (12 VDC, 5 A), 1 USB 3.0, 4 USB 2.0

Networking capability: Connection through Windows/SMB network via an Ethernet cable connection or wirelessly using the supplied Wi-Fi adaptor.

Power supply: AC adaptor with country-specific power cords.

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-interchangable, 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** or contact Technical Support (see page 141).

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^{2+})
White	Refracted light applications

Appendix D: EVOS™ Onstage Incubator

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_2 premix, CO_2-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)

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

Technical specifications

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

Physical characteristics

	Stagetop Environmental Chamber	Control Unit
Height:	25 cm (9.7 in)	37 cm (15 in)
Depth:	19 cm (7.6 in)	16 cm (6.3 in)
Width:	3.7 cm (1.5 in)	20 cm (7.9 in)
Weight:	1.5 kg (3.3 lb)	10 kg (22 lb)

Temperature range: Ambient to 40°C (± 0.1°C)

Humidity: >80% relative humidity (RH) at 37–40°C

CO_2 range: 0% to 20%

O_2 range: 0% to ambient

Operating power: 100–240 VAC, 1.8 A

Frequency: 50–60 Hz

Electrical input: 24 VDC, 5 A

Hardware

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

Gas input ports: Air or air-CO_2 premix, CO_2-only, and N_2-only (max. 50 psi input)

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

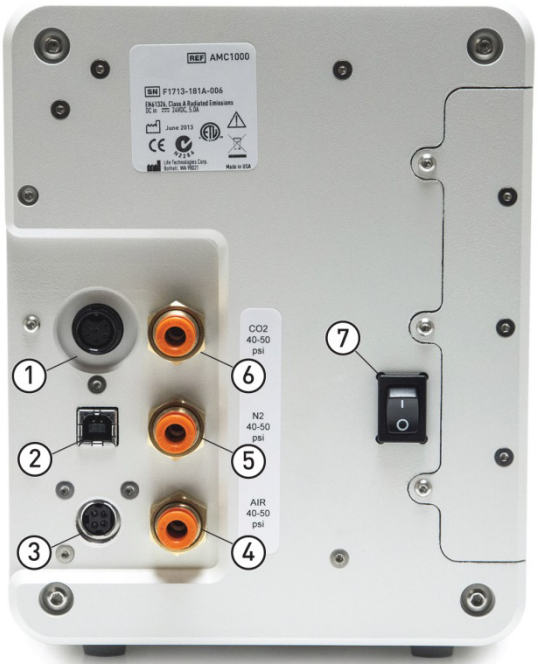
EVOS™ Onstage Incubator components

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_2 premix, CO_2, and N_2 for O_2 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

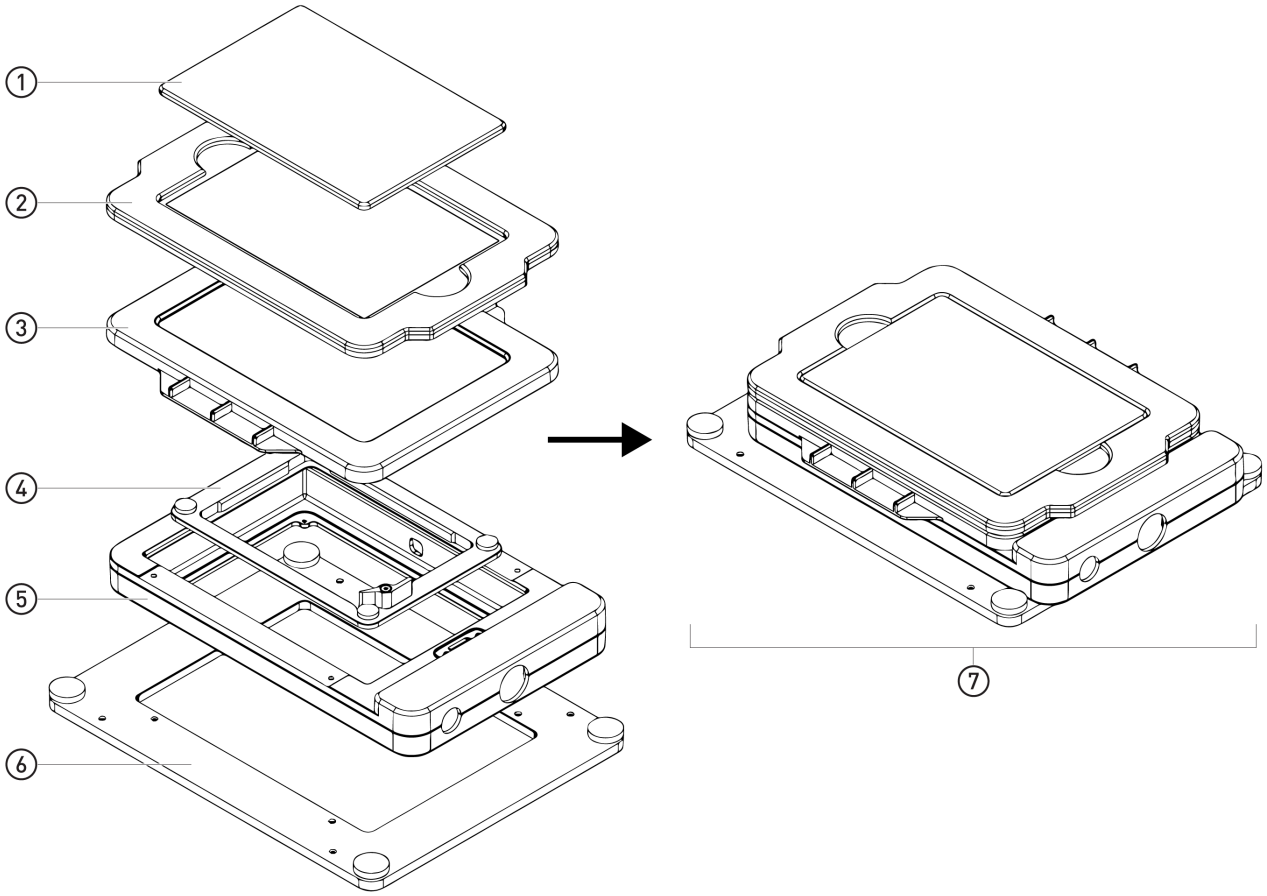


1. Sensor data cable jack
2. USB control cable jack
3. Power input jack
4. Port 1: Air
5. Port 2: N_2
6. Port 3: CO_2
7. Power switch

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 121).



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

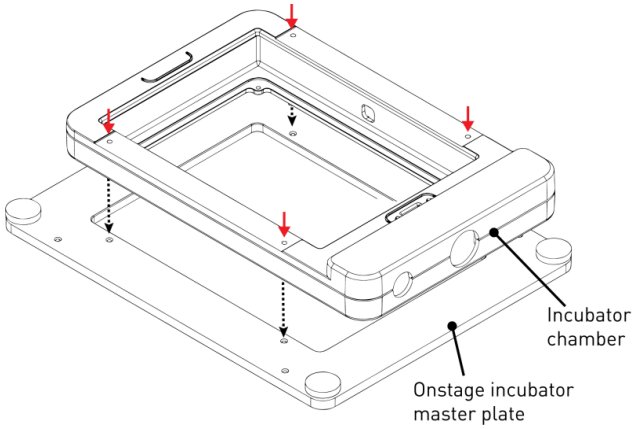
Install the Onstage Incubator Master Plate

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.

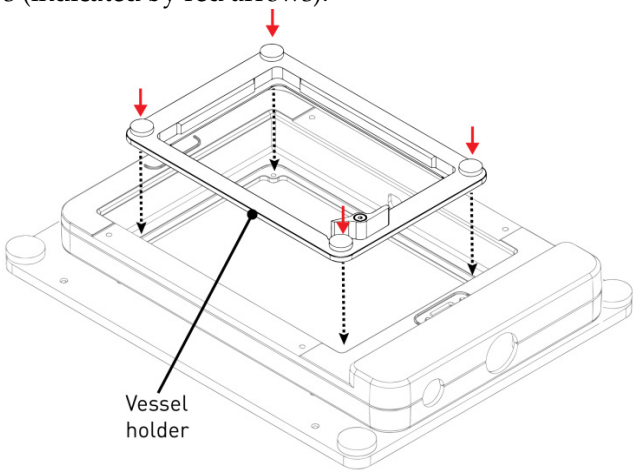
2. Secure the onstage incubator master plate to the X-Y stage using the four thumb screws.

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).

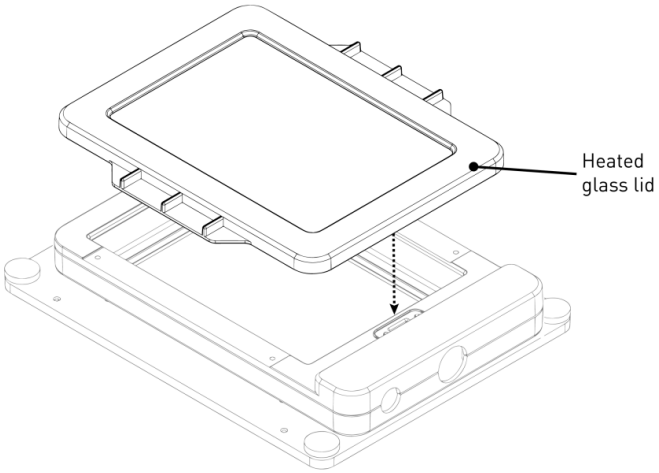


2. Attach the vessel holder/adaptor to the incubator chamber using the four thumb screws (indicated by red arrows).

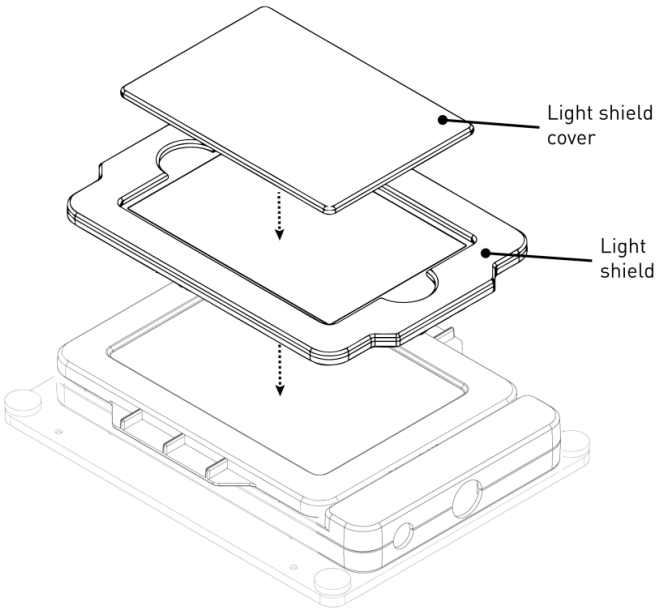


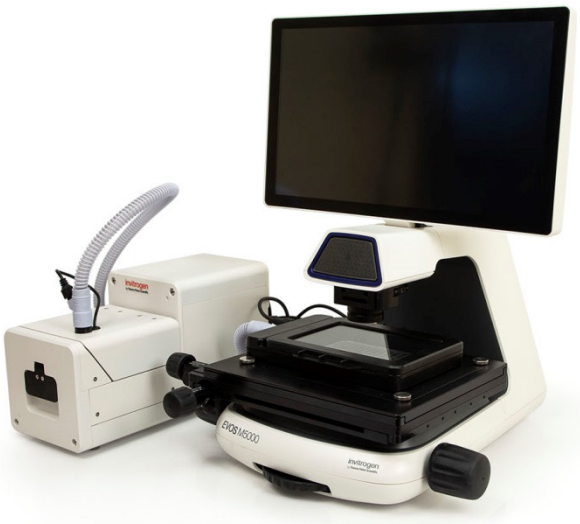
Note: Place an empty "dummy" culture plate into the vessel holder/adaptor for the initial warm up and equilibration to prevent build-up of condensation on the optical components and the inside of the EVOS™ M5000 Imaging System.

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.



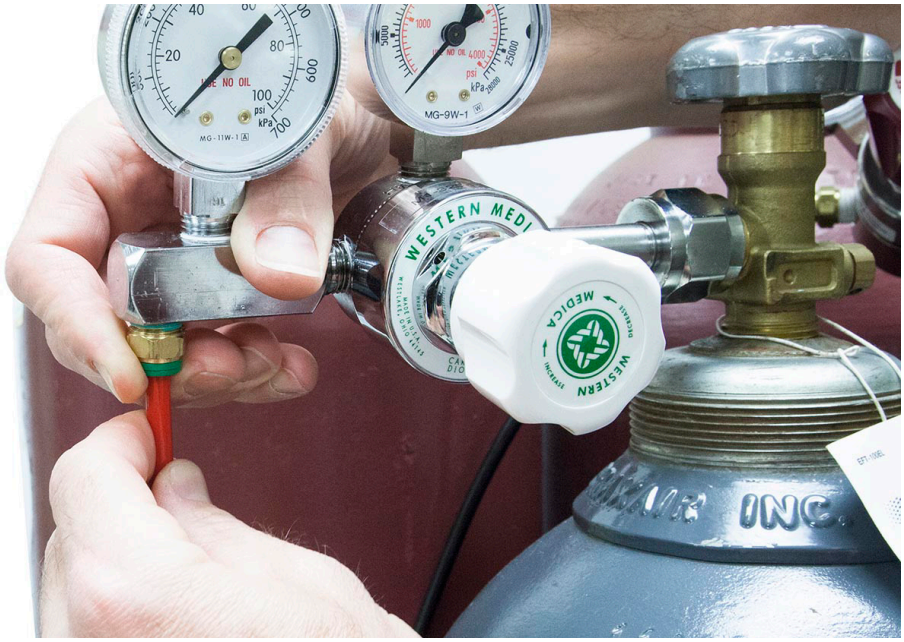


Set up for operation

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 118.

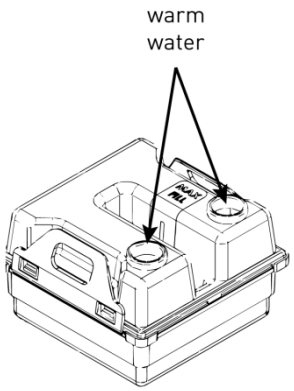
IMPORTANT! Do not position the control unit so that it is difficult to turn off the main power switch. In case of an instrument malfunction, turn the main power switch to the OFF position and disconnect the power cord from the wall outlet.

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.

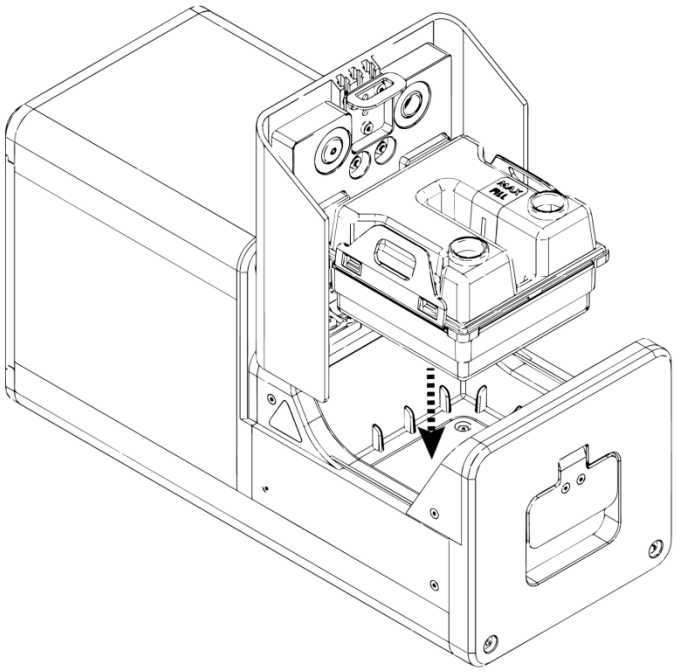


4. Attach the gas lines to the control unit via the PTC connectors for the appropriate gas intake port.
   - If using pre-mixed air, attach to **Port 1: Air In**
   - If using compressed air and CO_2, attach to **Port 1: Air In** and **Port 3: CO_2 In**
   - For oxygen displacement, attach to **Port 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.

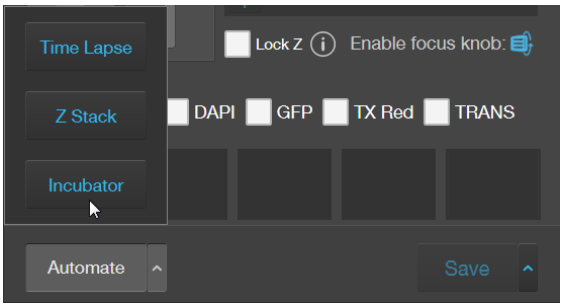
Use the EVOS™ Onstage Incubator

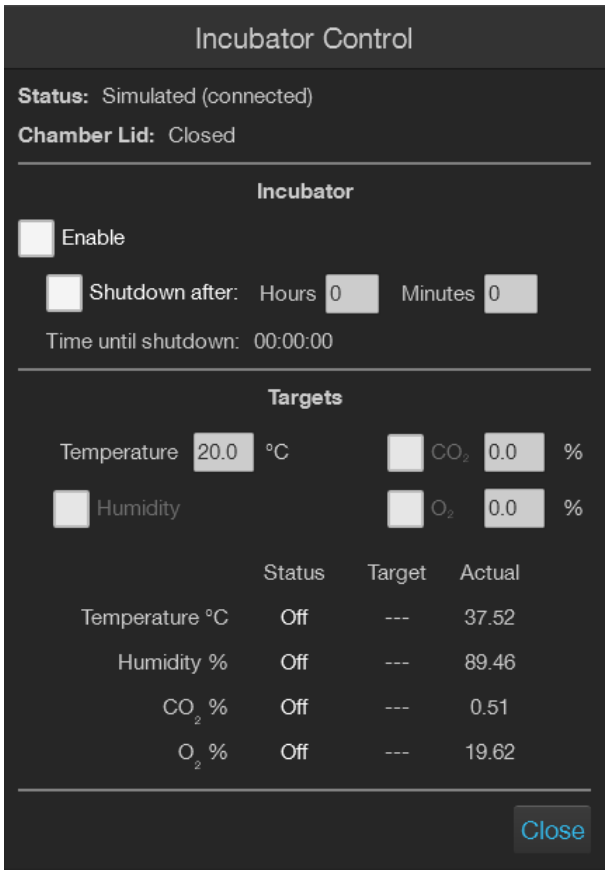
IMPORTANT! Before using the EVOS™ Onstage Incubator in your experiments, make sure that:

- The gas inputs have been configured (see "Configure gas inputs", page 127).
- The oxygen sensor has been calibrated (see "Calibrate oxygen sensor", page 128).

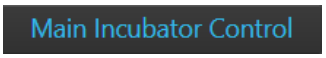
Turn ON the EVOS™ Onstage Incubator

1. Turn ON the EVOS™ M5000 Imaging System as described on page 16.
2. Turn ON the power switch to the EVOS™ Onstage Incubator control unit (page 118).
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.



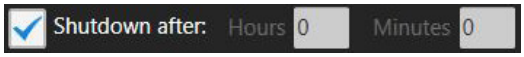


Note: Alternatively, go to the **Settings** tab, click **Incubator** to display the Incubator panel, then click **Main Incubator Control** to open the Incubator Control window.

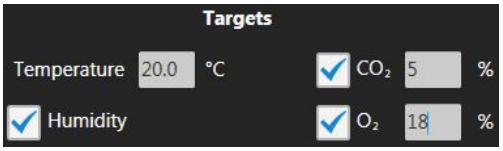


5. Select **Enable** to turn on the incubator.

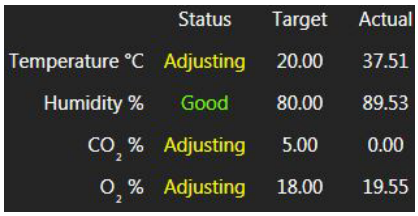
6. Select the desired Shutdown option:
   - **Manually shutdown:** The incubator will remain on until the **Enable** option is manually deselected and the **Close** button is clicked.
   - **Automatic shutdown:** Select **Shutdown after**, then enter the time in **Hours** and **Minutes** that must elapse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



7. Enter the target values for **Temperature**, **CO_2**, and **Oxygen**. If desired, select **Humidity** to use a humidified atmosphere in the incubator chamber.



   Incubator status will change to "Running" and the control panel will display the Target and Actual values for Temperature, Humidity, CO_2, and Oxygen.



	Status	Target	Actual
Temperature °C	Adjusting	20.00	37.51
Humidity %	Good	80.00	89.53
CO_2 %	Adjusting	5.00	0.00
O_2 %	Adjusting	18.00	19.55

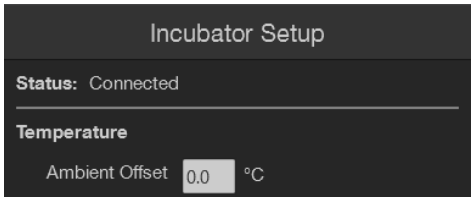
8. Click **Close** to return to the Capture tab.

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

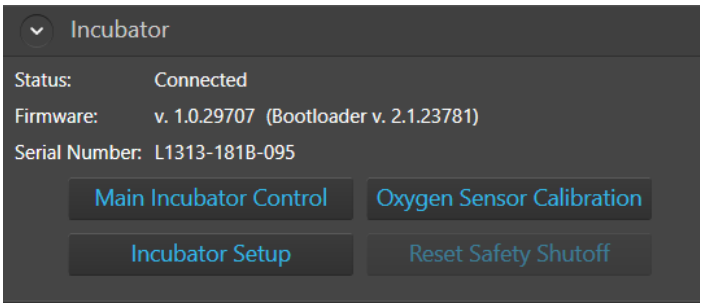
Ambient Offset allows you to adjust temperature readings based on the ambient room temperature. This should remain at 0 by default.

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**.

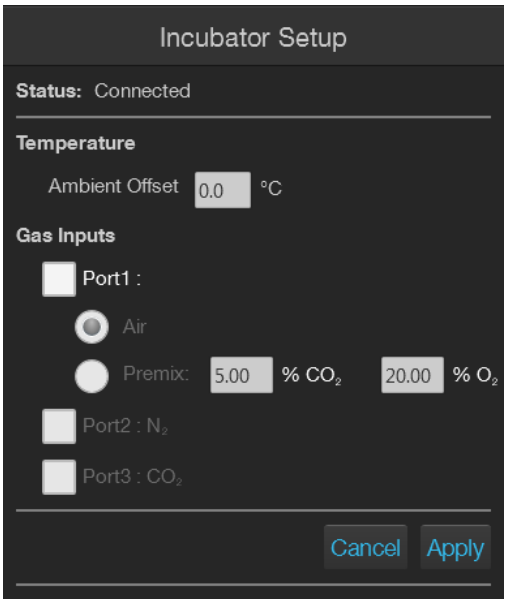


Configure gas inputs

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**.

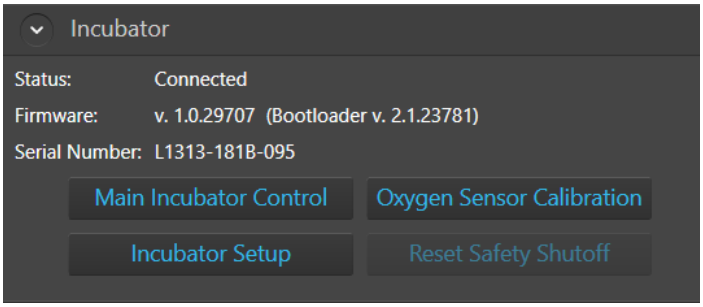
     If you select **Premix**, manually enter the percentage of the **CO_2** and **O_2** to reflect the specifics of your set-up.
   - **Port 2** is reserved for Nitrogen only.
   - **Port 3** is reserved for CO_2 only.

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_2:** 40–50 psi
    - **Nitrogen:** 40–50 psi

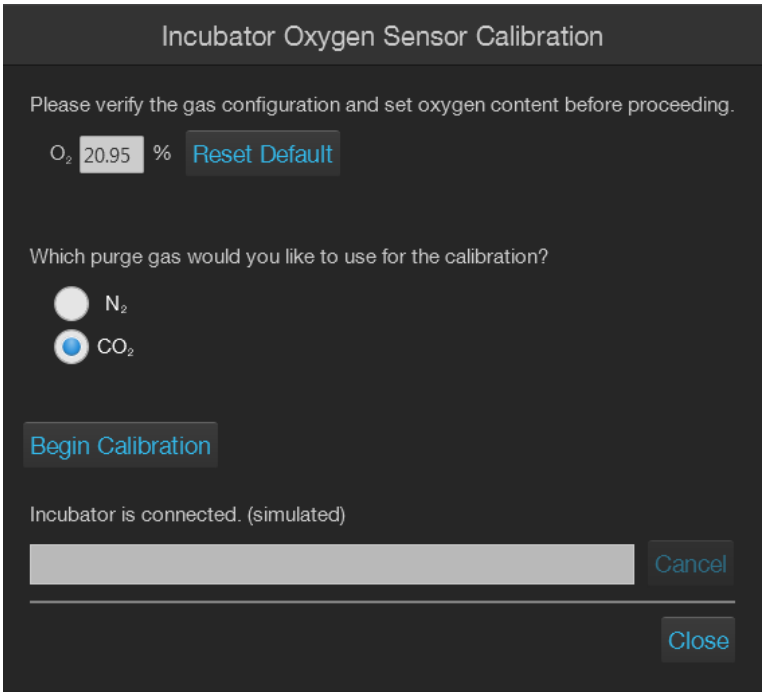
Calibrate oxygen sensor

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_2**).
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.

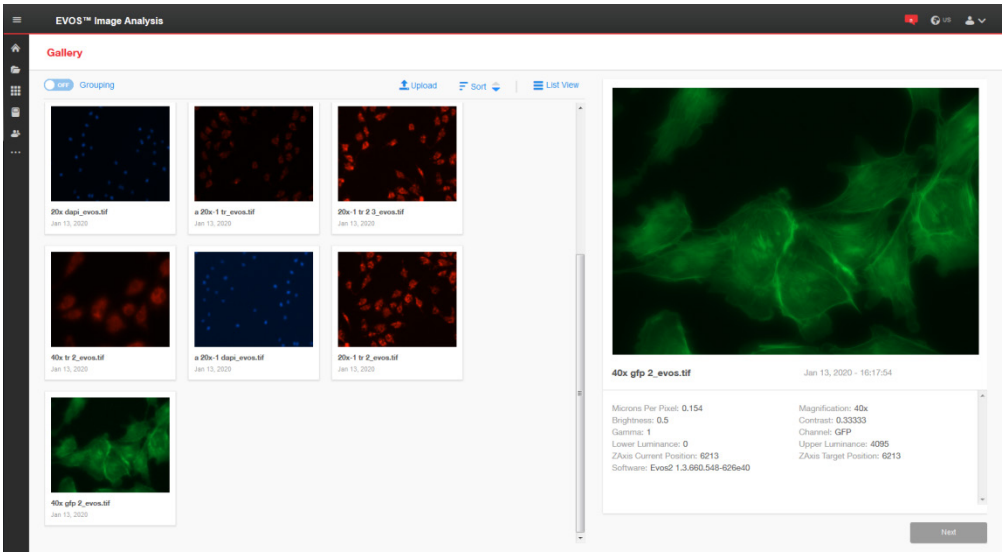
The EVOS™ Image Analysis application is available for free on the Connect platform. However, you need a current Connect account to sign in. To create your Connect account online, go to **thermofisher.com/connect**.

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**.

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 130).

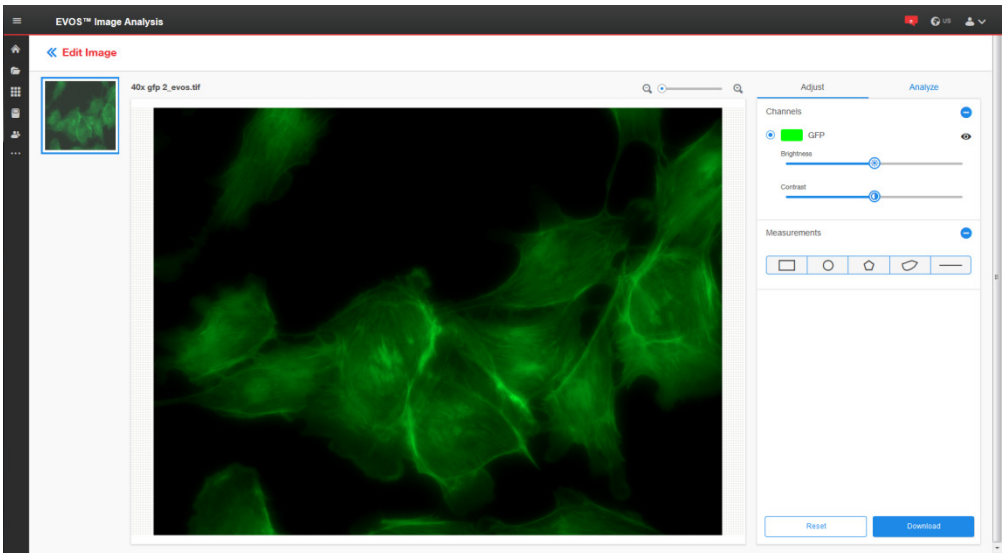


Edit Image

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

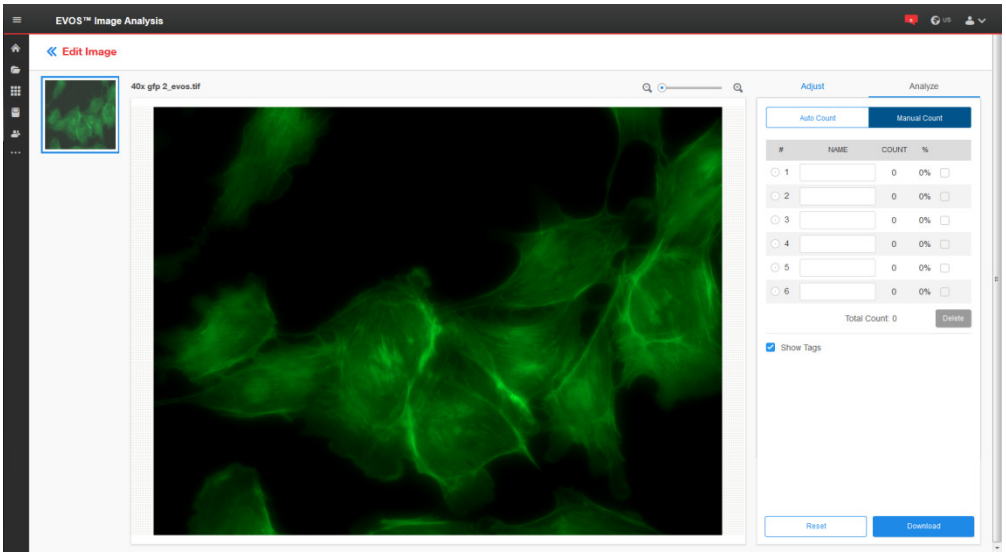
Adjust tab

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 29).



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 37).



Appendix F: Safety

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.

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

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.

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 **On** position of the main power switch.
	Indicates the **Off** position of the main power switch.
	Indicates a standby switch by which the instrument is switched on to the **Standby** condition. Hazardous voltage may be present if this switch is on standby.
	Indicates the **On/Off** 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
	**Do not dispose of this product as unsorted municipal waste.** Follow local municipal waste ordinances for proper disposal provisions to reduce the environmental impact of waste electrical and electronic equipment (WEEE). **European Union customers:** Call your Customer Service representative for equipment pick-up and recycling. See **www.thermofisher.com** for a list of customer service offices in the European Union.

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
	**CAUTION!** Hazardous chemicals. Read the Safety Data Sheets (SDSs) before handling.	**ATTENTION!** Produits chimiques dangereux. Lire les fiches techniques de sûreté de matériels avant toute manipulation de produits.
	CAUTION! HAZARDOUS WASTE. Refer to SDS(s) and local regulations for handling and disposal.	**ATTENTION!** Déchets dangereux. Lire les fiches techniques de sûreté de matériels et la régulation locale associées à la manipulation et l'élimination des déchets.
	**DANGER!** High voltage.	**DANGER!** Haute tension.
	WARNING! 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.	**AVERTISSEMENT!** 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.
	**DANGER!** Class 3B visible and/or invisible laser radiation present when open. Avoid exposure to beam.	**DANGER!** Rayonnement visible ou invisible d'un faisceau laser de Classe 3B en cas d'ouverture. Evitez toute exposition au faisceau.
	**CAUTION!** Moving parts. Crush/pinch hazard.	**ATTENTION!** Pièces en mouvement, risque de pincement et/ou d'écrasement.

General instrument safety

WARNING! PHYSICAL INJURY HAZARD. 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

CAUTION! PHYSICAL INJURY HAZARD. 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

WARNING! 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.

Things to consider before lifting the computer and/or the monitor:

- Make sure that you have a secure, comfortable grip on the computer or the monitor when lifting.
- Make sure that the path from where the object is to where it is being moved is clear of obstructions.
- Do not lift an object and twist your torso at the same time.
- Keep your spine in a good neutral position while lifting with your legs.
- Participants should coordinate lift and move intentions with each other before lifting and carrying.
- 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.

Operating the instrument

Ensure that anyone who operates the instrument has:

- Received instructions in both general safety practices for laboratories and specific safety practices for the instrument.
- Read and understood all applicable Safety Data Sheets (SDSs). See “Safety Data Sheets (SDS)”.

Cleaning or decontaminating the instrument

CAUTION! 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

CAUTION! PHYSICAL INJURY HAZARD. 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

DANGER! ELECTRICAL SHOCK HAZARD. 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

WARNING! FIRE HAZARD. For continued protection against the risk of fire, replace fuses only with fuses of the type and rating specified for the instrument.

Power

DANGER! ELECTRICAL HAZARD. Grounding circuit continuity is vital for the safe operation of equipment. Never operate equipment with the grounding conductor disconnected.

DANGER! ELECTRICAL HAZARD. Use properly configured and approved line cords for the voltage supply in your facility.

DANGER! ELECTRICAL HAZARD. 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)
- Occupational Safety and Health Standards, Bloodborne Pathogens

  (29 CFR§1910.1030;
  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

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/

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

Indicates conformity with safety requirements for Canada and U.S.A..

European safety and EMC standards

Indicates conformity with European Union requirements.

CE

Australian EMC standards

Indicates conformity with Australian and New Zealand standards for electromagnetic compatibility.

Documentation and support

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**.

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** 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**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.