

EVOS™ M5000 Imaging System

USER GUIDE

For Fluorescence and Transmitted Light Applications

Catalog Number AMF5000 and AMF5000SV

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| 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 | 8 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 | 7 August 2018 | New user guide for the EVOS™ M5000 Imaging System. |

The information in this guide is subject to change without notice.

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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.



Product description

EVOS™ M5000 Imaging System

The Invitrogen™ EVOS™ M5000 Imaging System (Cat. No. [AMF5000SV](#)) is a fully integrated, digital, inverted cell 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 x 1080 pixel resolution), a highly sensitive monochrome CMOS camera (2048 x 1536 pixel resolution, 3.2 Megapixels) to acquire images seamlessly through an intuitive user interface using a mouse for easy control, and a position-sensing stage that enables sample locations to be mapped and stored for future relocation.

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 and starts automatically when the instrument is powered on.

Key features of the EVOS™ M5000 Software include:

- **Stage View:** Integrates a precision manual stage with sensors for accurately mapping and marking points of interest in various vessel types, including plates, slides, and petri dishes.

Note: *Stage View* requires an updated stage manufactured in late-2024 for all new EVOS™ M5000 systems. Prior EVOS™ M5000 systems having a stage with separate X- and Y-axis control knobs are not compatible with **Stage View** but can be updated to have all of the other software features described in this user guide.

- **Capture:** Enables control over every aspect of image capture through a simple user interface. All images acquired can be saved in JPG, PNG, and TIFF formats, or compiled into a video sequence in AVI or MP4 formats.
- **Autofocus:** Enables autofocus in fluorescence or 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 the individual source images.
- **Time lapse:** Enables creating a time-lapse movie using captured images.
- **Review:** Enables reviewing, measuring, and annotating captured images.
- **Cell count:** Enables automatic or manual counting of cells post-acquisition.
- **Confluence:** Enables calculating the percentage confluence of the culture based on selected reference objects and background.

- **Transfection Efficiency:** Enables measuring percent transfection in a population of cells, as determined after measuring confluence.
- **Network and Connect connectivity:** Allows Wi-Fi and Ethernet connectivity to the network and to your Connect account as part of the Connect-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 with position-sensing technology
- 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
- 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, preloaded with user documentation)
- EVOS™ Vessel Holder, Multiwell Plate (Cat. No. [AMEPVH022](#))
- EVOS™ Vessel Holder, Two 25 × 75 mm slides (Cat. No. [AMEPVH001](#))
- EVOS™ Vessel Holder, Large Universal, 1 inch (Cat. No. [AMEPVH113](#))
- EVOS™ Calibration Slide (Cat. No. [AMEP4720](#))
- EVOS™ Light Shield
- 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 key, 2 mm
- Mouse pad

EVOS™ M5000 Imaging System user documentation

- EVOS™ M5000 Imaging System 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 Imaging System User Guide (Pub. No. MAN0017763)
 - EVOS™ M5000 Imaging System Quick Start Guide (Pub. No. MAN0017765)
 - EVOS™ M5000 Imaging System Installation Guide (Pub. No. MAN0017783)

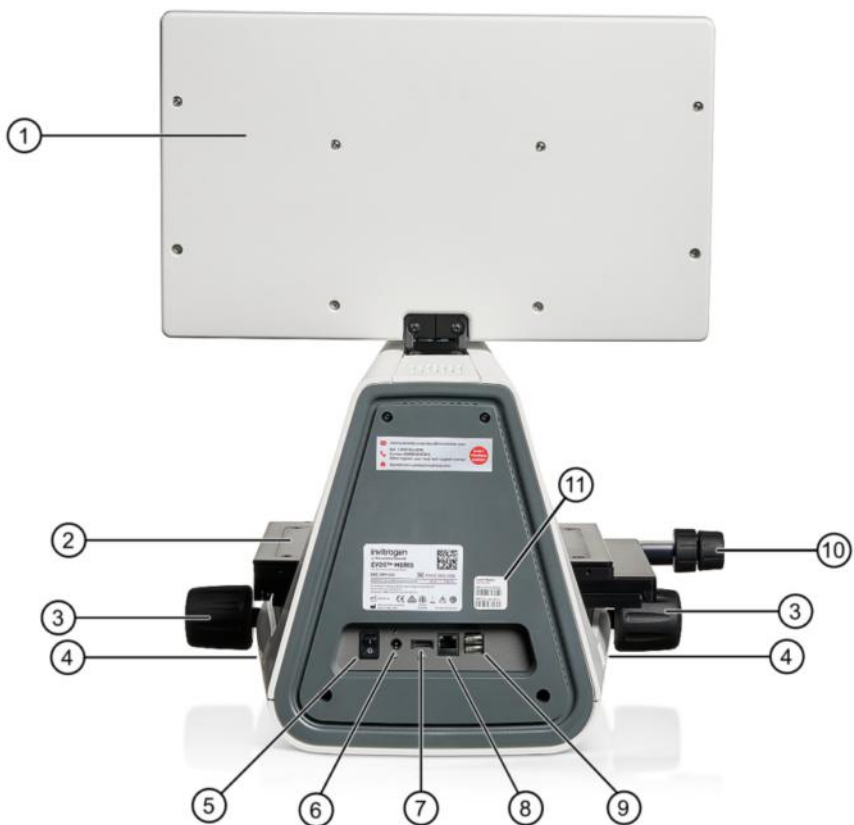
Instrument exterior components and mechanical controls

Front view



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

Rear view

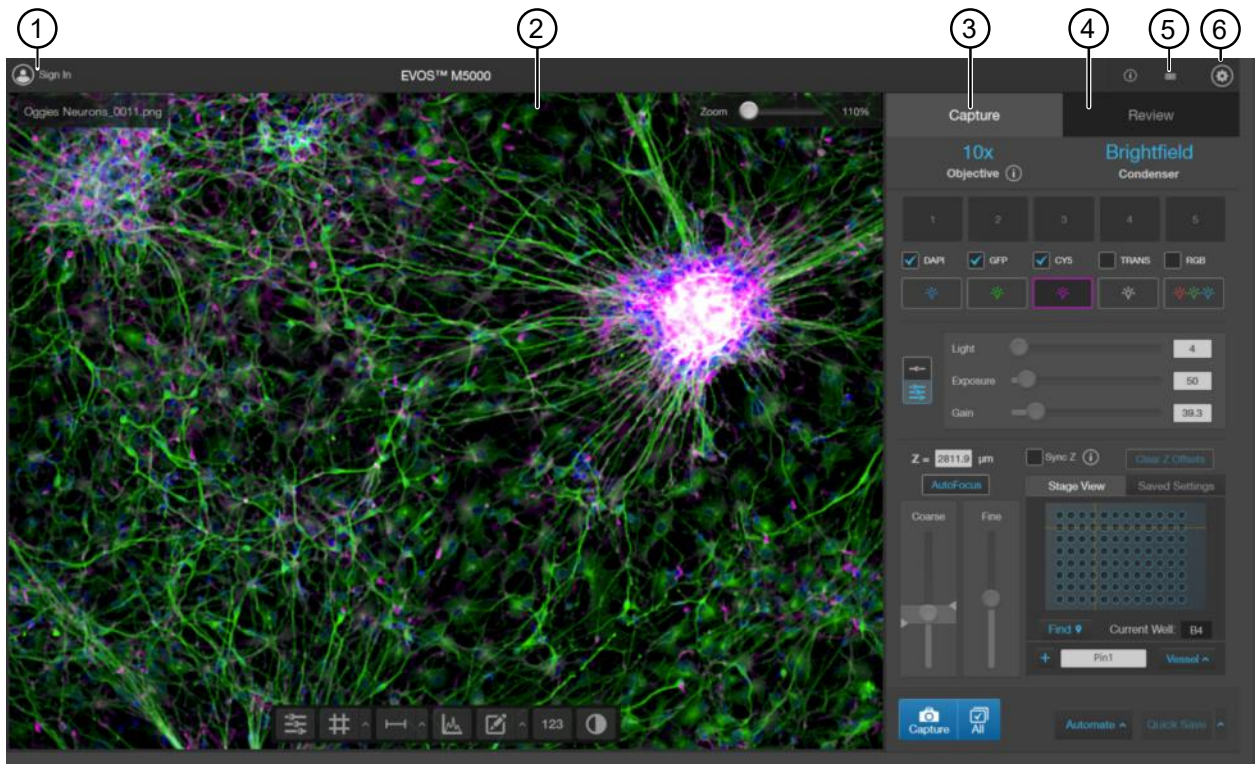


- | | |
|---|---|
| ① LCD monitor | ⑦ DisplayPort (video output) |
| ② Mechanical X-Y stage | ⑧ Network port |
| ③ Focusing knobs | ⑨ USB-A 2.0 ports (2x) |
| ④ USB-A 3.0 port (2x) | ⑩ Stage X- and Y-axis positioning knobs |
| ⑤ Power switch | ⑪ Instrument passwords sticker |
| ⑥ Single-pin power input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

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, Connect-based platform, for image storage and analysis with the EVOS™ Image Analysis application.
- ② **Viewing area:** Displays live or captured images of 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)”.

Note: For more information on the EVOS™ Image Analysis application, see Appendix D, “EVOS™ image analysis”.

Operating environment and site requirements

- The dimensions of the EVOS™ M5000 Imaging System are 18 × 23 × 18 in (46 × 59 × 46 cm) (W × H × D). The system requires a benchtop of approximately 36 × 36 in (92 × 92 cm).
- If the system includes the optional EVOS™ Onstage Incubator (Cat. No. [AMC2000](#)), then add 16 in (40 cm) to the width of the bench.
- Allow at least 2 in (5 cm) of 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 operation and interfere with instrument performance.
- If possible, install the EVOS™ M5000 Imaging System 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 “Rear view” on page 14). In case of an instrument malfunction, turn the main power switch to the OFF position and disconnect the instrument from the wall outlet.

Cell culture 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 “Standard items included” on page 11 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. 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.



Figure 1 Before removing the stage lock pin.

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

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

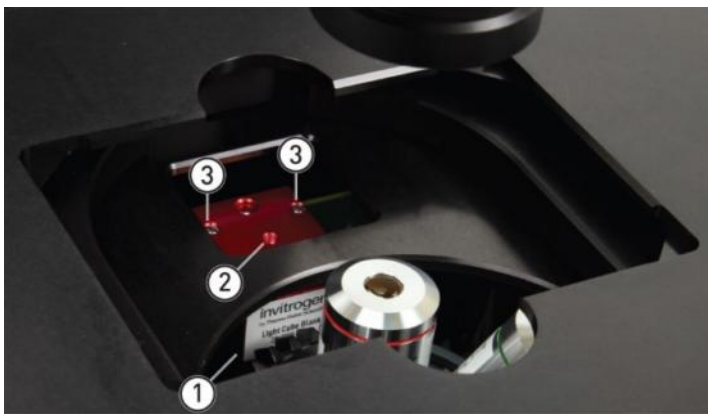
Note: The light cube shipping restraint is secured with the light cube tool to the blank light cube installed in the light cube turret. Used together, they immobilize the light cube turret to protect it during transport.



Figure 2 After removing the stage lock pin and moving the stage to access the shipping restraint.

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

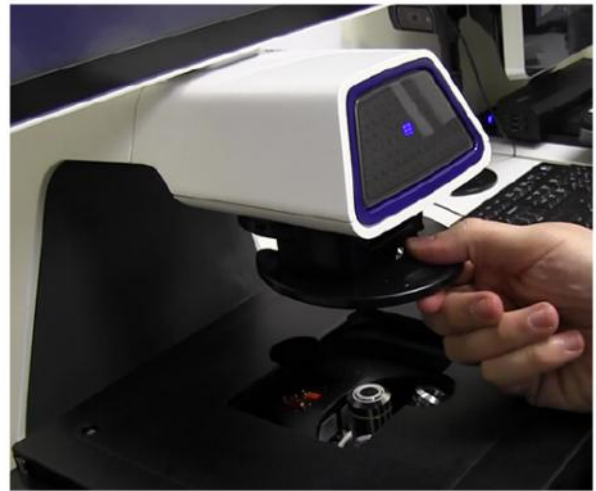
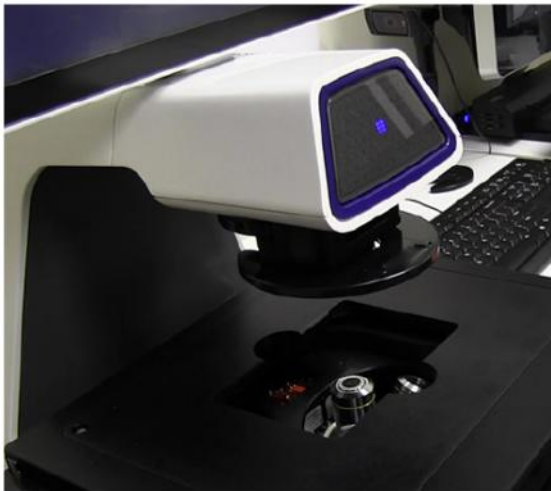
5. Using the light cube tool, loosen the two screws that secure the blank light cube to the instrument. You do not need to remove the screws.
6. Screw the light cube tool in to the threaded hole in the blank light cube, then lift the blank light cube up and out of the microscope. Store the blank light cube and the light cube tool in the accessories box.

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

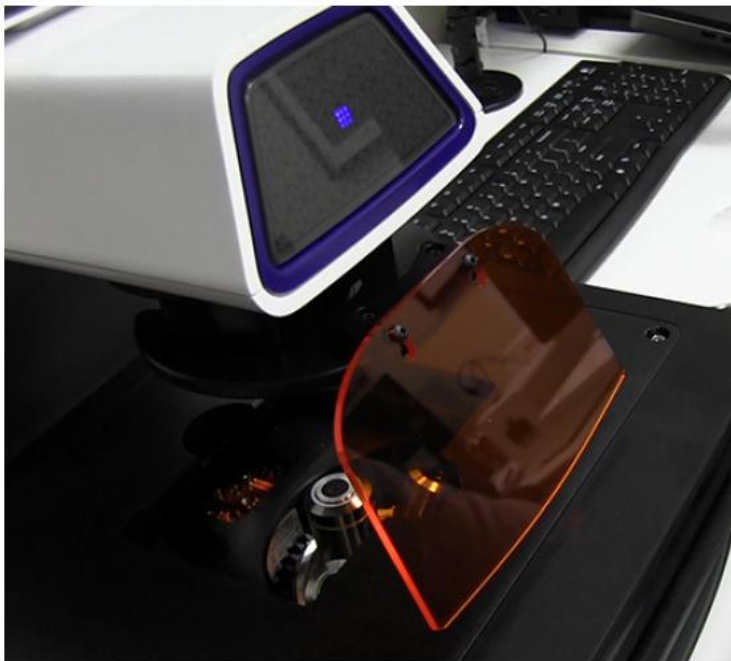
1. Verify that the EVOS™ Condenser Light Shield™ is installed on the condenser assembly. The condenser shield is pre-installed and helps reduce the potential effects of overhead lighting on your image.
2. Pull the condenser light shield out from the condenser head.



3. Secure the UV light shield mount to the top of the condenser light shield using the two screws and 2 mm hex key 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 “Rear view” on page 14).
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 (“Rear view” on page 14).
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 (“Rear view” on page 14).
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 “Rear view” on page 14) to the **ON** position.
2. When the **Capture** tab is displayed, the EVOS™ M5000 Imaging System is ready to use.

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, Connect-based platform, to store your image files and analyze them with the EVOS™ Image Analysis application (“EVOS™ image analysis” on page 138).

For instructions on how to connect to a Wi-Fi network and how to map a network drive for saving your images, see “Configure network settings” on page 102.

Set date and time

For instructions on how to configure the date and time to the local time, see “Set date and time” on page 105.

Connect to the Thermo Fisher™ Connect Platform


About the Thermo Fisher™ Connect Platform

The Thermo Fisher™ Connect 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.

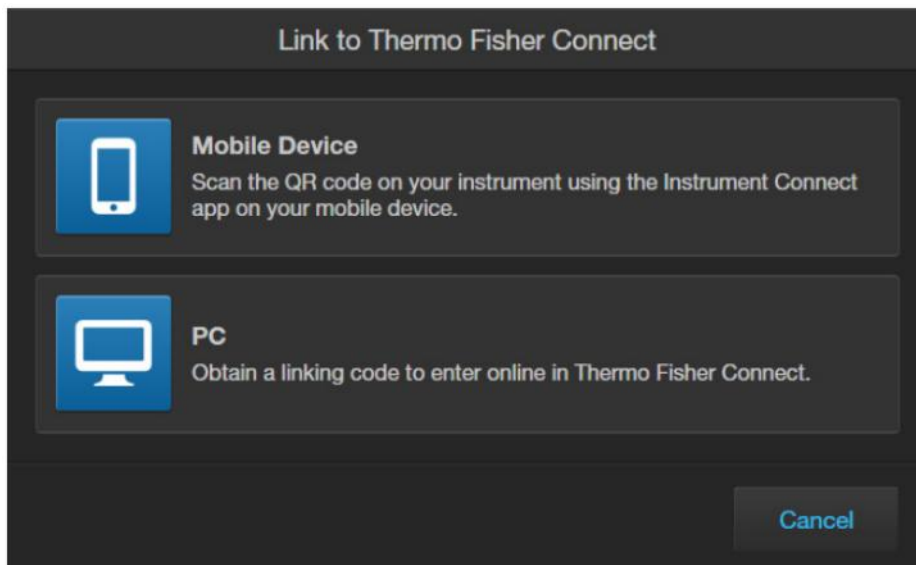
Create a PIN number

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 (“Add instrument to your Connect account with QR code (Mobile Device)” on page 25).
2. **PC (linking code):** Obtain a linking code to enter online in Thermo Fisher™ Connect Platform (“Add instrument to your Connect account with QR code (Mobile Device)” on page 25).

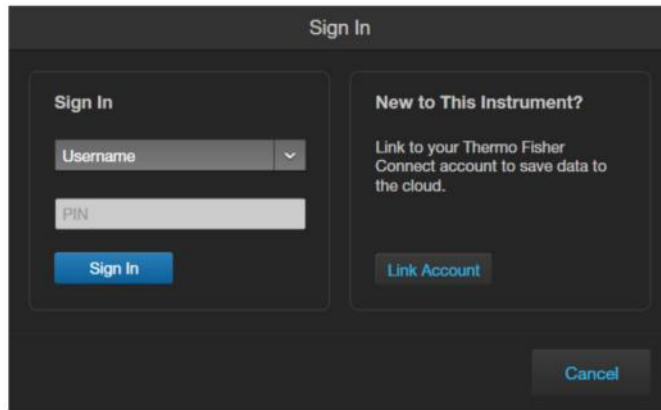


When the instrument is linked, you can save captured images in your unique user account in addition to local storage.

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 26.




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

1. Click  (**Sign In**) 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 Platform with linking code (PC)

1. Click  (**Sign In**) 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  (**Sign In**) 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 (“Create a PIN number” on page 23).

4. Click **Sign In**.

When you connect to your Connect account, the **Sign In** button changes to display your user name (for example, ).

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 online EVOS™ Image Analysis application.

For more information on the online EVOS™ Image Analysis application, see Appendix D, “EVOS™ image analysis”.

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.



Capture images

Overview

Workflow

| Capture images | |
|----------------|---|
| | Select the objective and light source page 28 |
| | Focus on the sample page 30 |
| | Find the region of interest page 31 |
| | Capture an image in a single channel page 32 |
| | Save page 62 |
| | Analyze and annotate the captured images page 36 |

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.



Capture images in a single channel

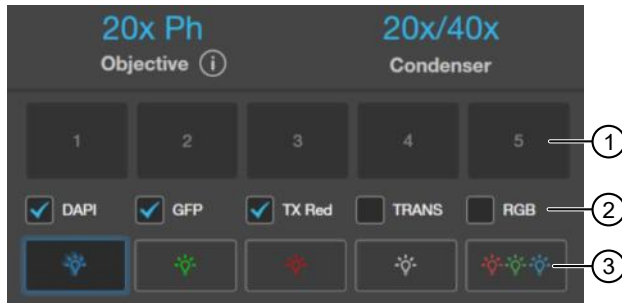
Select objective and light source

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 (“Front view” on page 13).
3. Select the desired **Phase** option by turning the phase ring selector (“Front view” on page 13). Available options are:
 - **Oly 4x:** Used for Olympus™ 4x phase contrast objectives (Olympus™ 4x PH)
 - **4x/10x:** Used for EVOS™ 4x or 10x phase contrast objectives (EVOS™ 4x/10x PH)
 - **20x/40x:** Used for EVOS™ 20x or 40x phase contrast objectives (EVOS™ 20x/40x PH)
 - **Brightfield** (phase contrast off)

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



- ① Memory buffer thumbnail images
- ② Auto-capture channels (via the **All** button)
- ③ Channel selection/light activation 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 (Condenser) is displayed in orange.



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

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

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

Adjust brightness

Brightness is controlled with the use of **Light**, **Exposure**, and **Gain** slider bars in the **Capture** control panel. Selecting one of the two **Brightness** slider panels enables control of the brightness settings.

- Select the single slide panel to adjust the **Light** setting and allow the system to automatically determine the camera **Exposure** and **Gain** settings to minimize photobleaching.



- Select the triple slider panel to individually adjust the **Light**, **Exposure**, and **Gain** settings.



Note: Optimize the brightness parameters as follows:

- **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 longer, limit the use of autofocus, and use a channel other than DAPI for autofocus.
 - In the **Settings** ▶ **Display** 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.



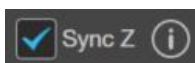
Alternatively, click **AutoFocus** to autofocus on the sample.


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 (“Front view” on page 13).

Note: Do NOT use autofocus with oil-immersion objectives, as this can create air bubbles in the oil or cause the objective to collide with the sample.

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



Click  next to the **Sync Z** selection as a reminder of how **Sync Z** affects the focusing.


- When synced, focus adjusts for all channels.
- When unsynced, focus only adjusts the selected channel.

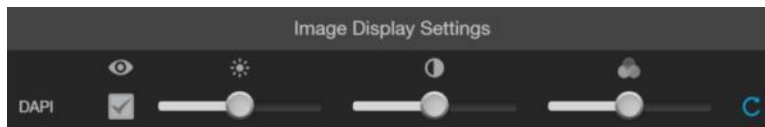
Note: Z-Offset specifies the focus position in the selected channel relative to the focus position in other channels. Setting the correct Z-Offset is especially important when the fluorescent markers in different channels are in different focal planes.






Optional: Adjust display settings

1. If not already visible, hover the pointer over the Viewing area to reveal the buttons for **Display Settings** and **Analysis Tools**.



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



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

Note: The **Gamma** slider allows non-linear contrast adjustments; moving it to the left suppresses low intensities whereas moving it to the right boosts low intensities. For images with nonspecific background fluorescence (often called "autofluorescence") moving the **Gamma** slider to the left may help reduce undesired background fluorescence. For images with very faint fluorescence signals, moving the **Gamma** slider to the right may help increase faint signals and bring them out of the background, making them easier to see or detect at the lower limits of brightness.

Find a region of interest

1. Use the **X-axis** and **Y-axis** positioning knobs ("Front view" on page 13) 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 100–1000%.

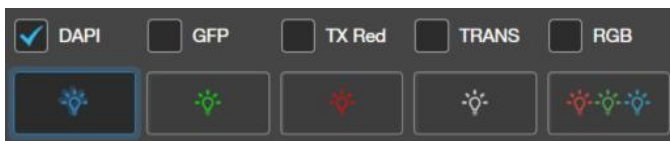


You can also double-click on the image to center and zoom by 200% on the clicked location, to a maximum of 1000%. Right-clicking on a zoomed image resets the zoom to 100%.

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

Capture images for each channel

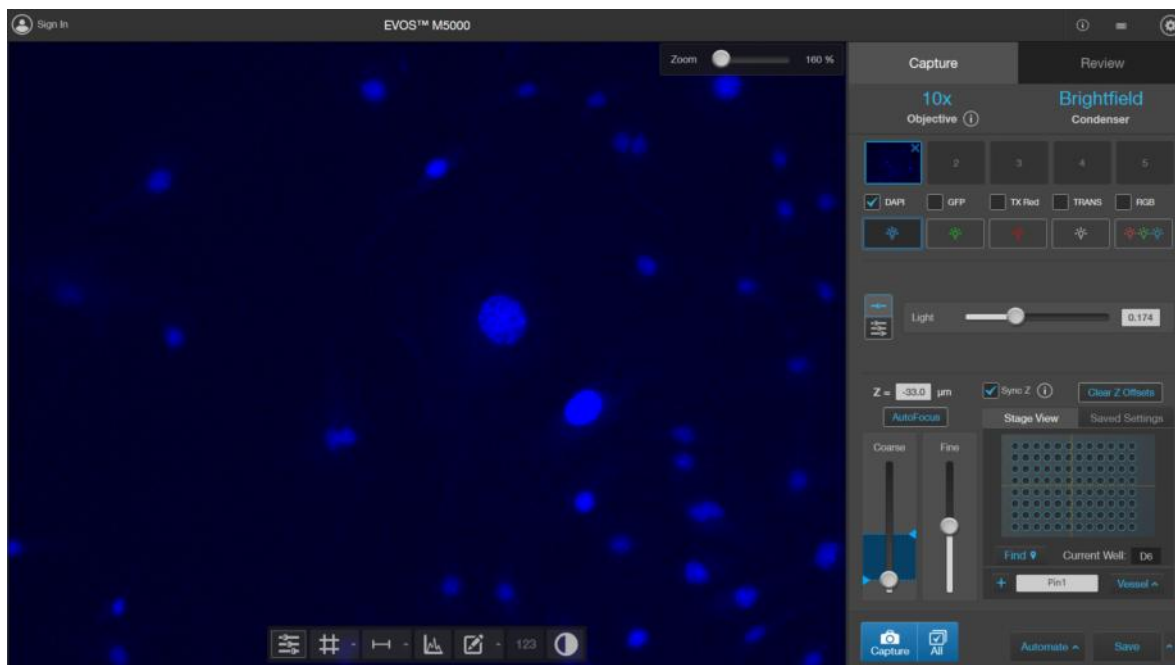
1. Ensure that the **Channel** you want to capture is selected.



When you select a **Channel**, the corresponding Capture channel checkbox above the button is automatically checked.

Note: If you exit the **Live** mode, the current channel remains selected, as indicated by the highlighted color surrounding the channel button.

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



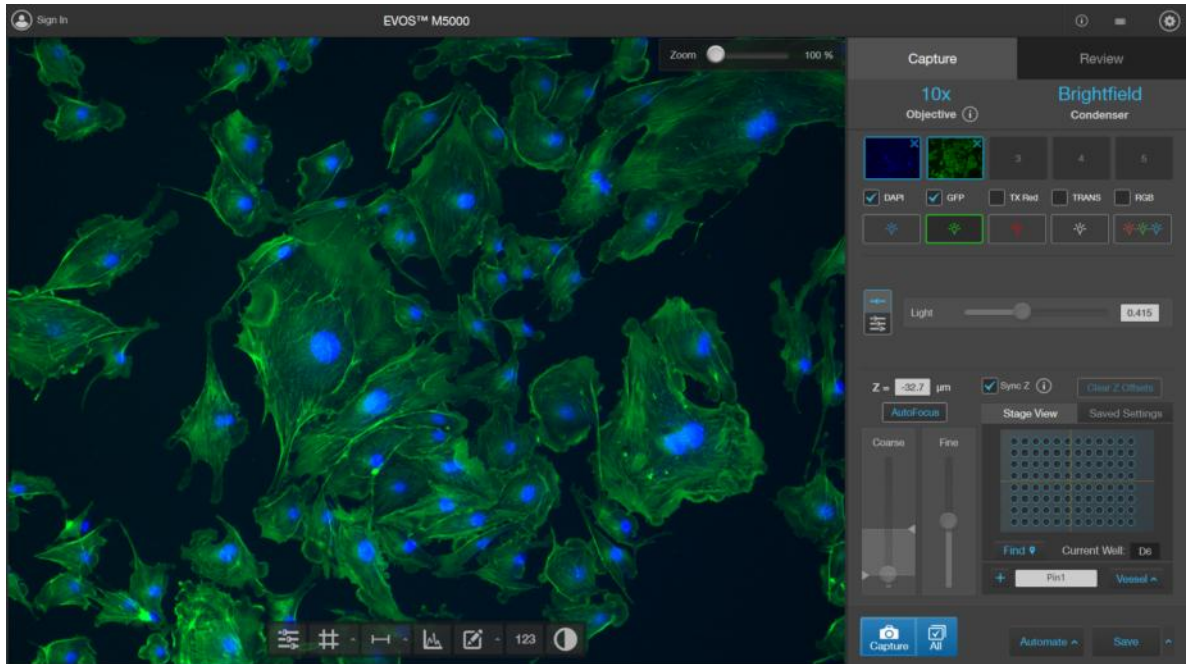
A thumbnail of the captured image is displayed and highlighted for the selected channel (in this example, DAPI) beneath the **Objective** display.

IMPORTANT! Captured images are stored in individual memory buffers with one for each channel. Memory buffers are for display and are not automatically saved. If unsaved, newly captured images overwrite the previously captured image in the selected channel. Images captured in other fields and channels are not affected.

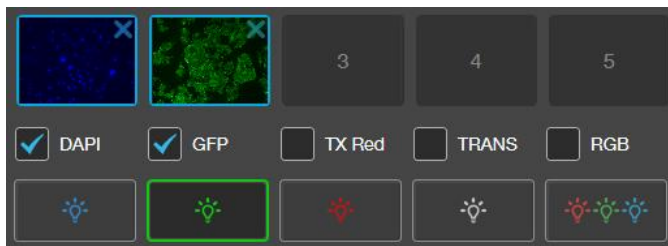
3. To capture the same field of view in another channel, select the desired **Channel** (for example, GFP, TX RED, etc.).

- If needed, readjust 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.

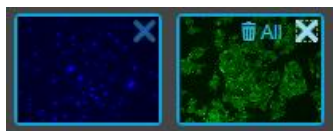


A thumbnail of the image captured in the new channel is displayed along with the thumbnail of the image from the previously captured channel.



Repeat the above steps to capture each channel as needed.

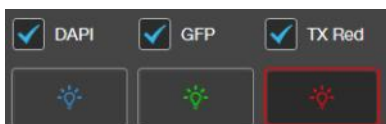
- To remove a captured image from the memory buffer, click the **X** on the desired image, then click the **X** again to remove only the selected image. To remove all captured images from the memory buffer, click the **X** on any of the captured images, then click **All (Clear All)**.



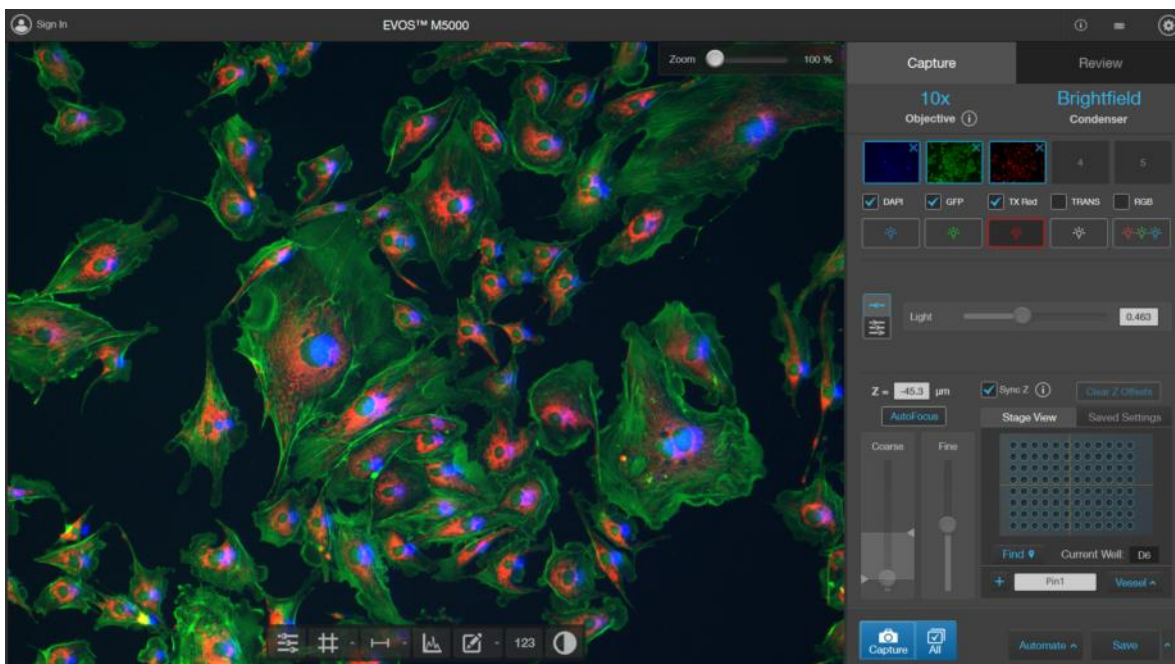
Capture images in multiple channels

Capture multiple channels automatically

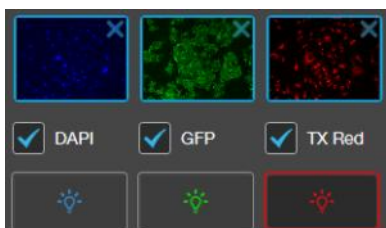
1. To capture multiple channels automatically, select the desired channels by checking the corresponding boxes.




2. If needed, adjust brightness and focus for each of the selected channels as described.
3. Click **All** to automatically acquire an image in each of the selected channels.
The Viewing area shows a multicolor overlay of the images captured for 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 individual memory buffers with one for each channel. Memory buffers are for display and are not automatically saved. If unsaved, newly captured images overwrite the previously captured image in the selected channel. Images captured in other fields and channels are not affected.

4. To remove a captured image from the memory buffer, click the **X** on the desired image, then click the **X** again to remove only the selected image. To remove all captured images from the memory buffer, click the **X** on any of the captured images, then click  **All (Clear All)**.

5

Measure, annotate, and analyze 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. If not already showing, hover the pointer over the Viewing area to reveal the buttons for **Display Settings and Analysis Tools**.



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

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 (“Configure display settings” on page 88)
 - Adjust image display settings (“Adjust image display settings” on page 37)
 - Display a grid (“Display grid” on page 38)
 - Display a scale bar (“Display scale bar” on page 39)
 - Align channels (“Align channels” on page 40)

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 (“Show Align Channels tool” on page 102).




-
- View pixel intensity histogram (“View pixel intensity histogram” on page 41)
 - Add and show measurements and annotations (“Add measurements and annotations” on page 42)

- Analyze cell culture (“Analyze cell culture” on page 44):
 - Perform Auto Count (“Perform auto count” on page 45)
 - Perform Manual Count (“Perform manual count” on page 51)
 - Measure confluence (“Measure confluence” on page 54)
 - Calculate transfection efficiency (“Calculate transfection efficiency” on page 58)
- Save analysis results (“Save analysis results” on page 92)

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, are part of any saved display image. In contrast, saved analysis images (that is, 16-bit individual channel files) consist of the raw data and do not preserve any edited changes.





To save raw image data for image analysis, select the **Save individual channels for analysis** option when saving captured images (“Save” on page 62).

Adjust image display settings

1. Click  (**Image Display Settings**) 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 illumination light turned on). In the example above, only the controls for DAPI, GFP, and TX Red channels are displayed.

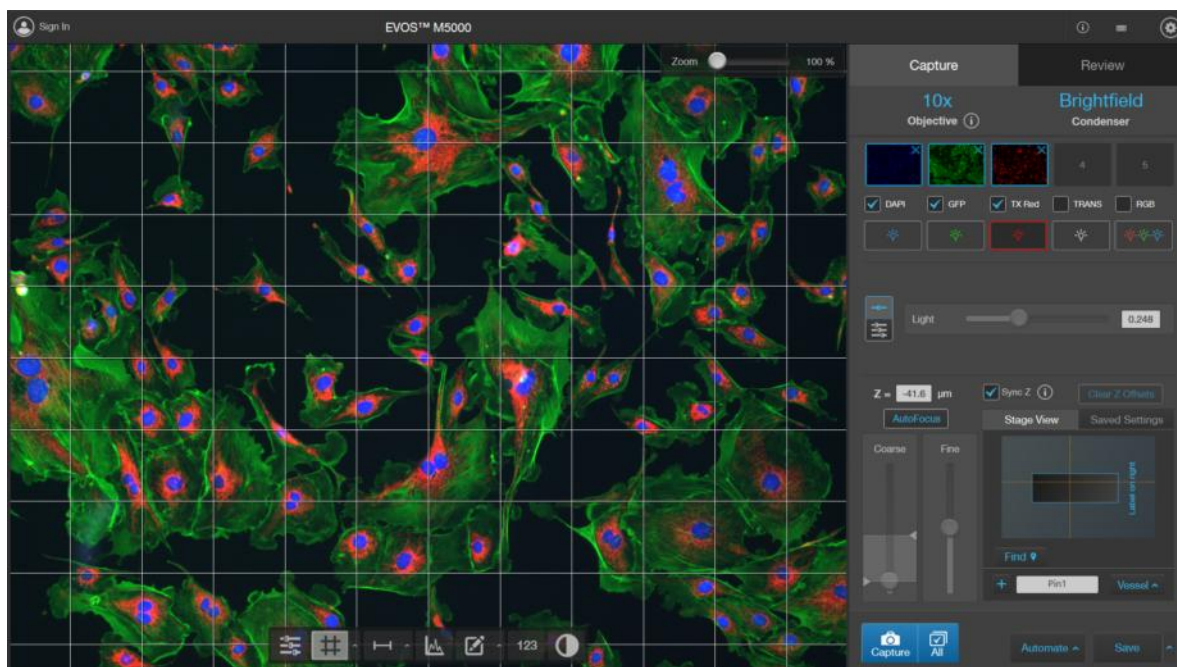
2. (Optional): To remove a channel from displaying in the Viewing Area, unselect the corresponding checkbox.
To display a channel with a captured image that is not shown in the Viewing Area, reselect the checkbox.
3. Adjust the  (**Brightness**),  (**Contrast**), and  (**Gamma**) settings for each of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


Note: The **Gamma** slider allows non-linear contrast adjustments. Moving the slider to the left suppresses low intensities. Moving the slider to the right boosts low intensities. For images with nonspecific background fluorescence (also known as autofluorescence), moving the **Gamma** slider to

the left may help reduce undesired background fluorescence. For images with very faint fluorescence signals, moving the **Gamma** slider to the right may help the intensity of faint signals to stand out from the background. This can help to see or detect signals at the lower limits of brightness..

Display grid

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




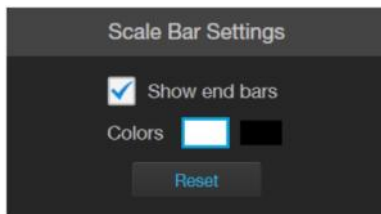
2. To change the grid size, click  (**Grid Settings**) (arrow on the **Grid** split button) to open the **Grid Settings** tool.
3. Select the **Size** for the grid. Available grid sizes depend on the magnification of the selected objective. Also provided is the option to display the center point to identify the center of the field of view.
4. Click the **Grid Settings** button again to save your settings and close the tool.

Display scale bar

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



2. To change **Scale Bar Settings**, click  (**Scale Bar Settings**) (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**) again to save your settings and close the tool.

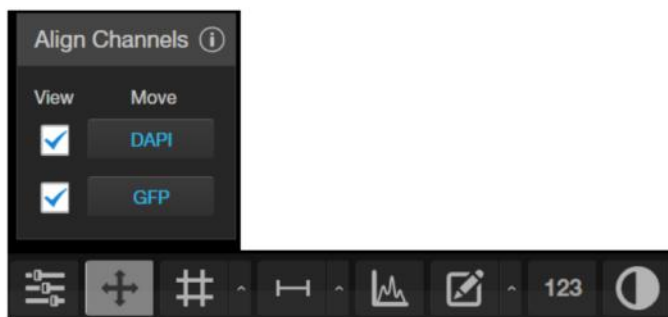
Align channels

Align channels

The **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 (“Show Align Channels tool” on page 102).

1. Click **+** (**Align Channels**) 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.



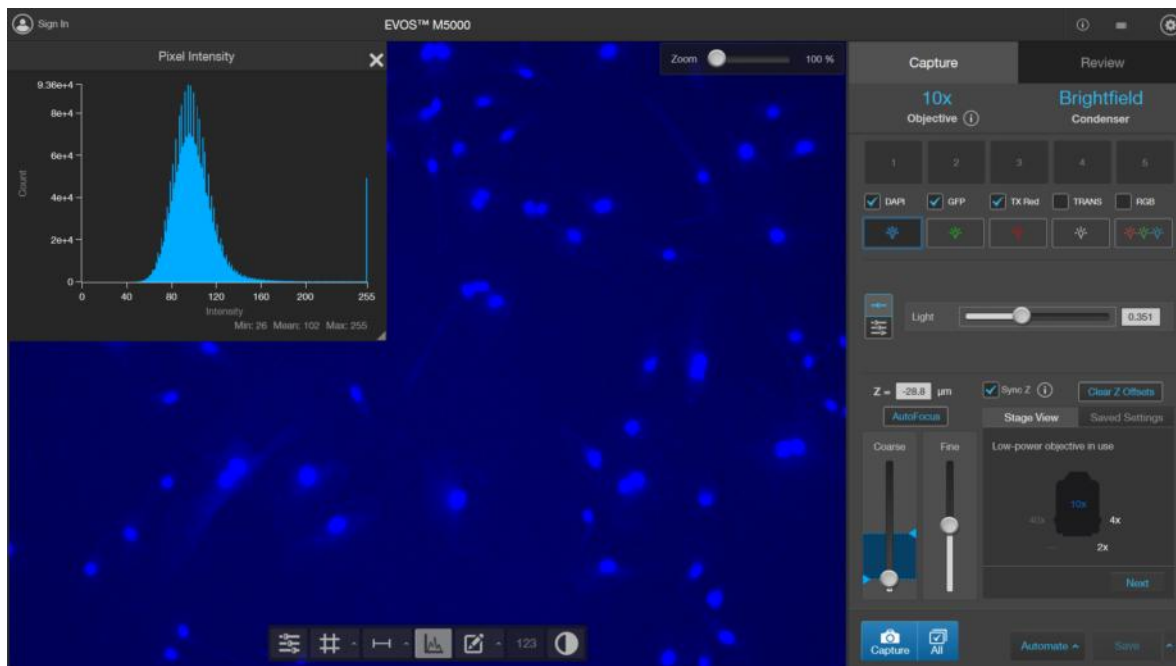
3. *Optional:* To remove a channel from displaying in the Viewing Area, unselect the corresponding **View** checkbox. To display a channel with a captured image that is not shown in the Viewing Area, re-select the corresponding **View** checkbox.
4. Use the keyboard arrow keys to move the image in the 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.

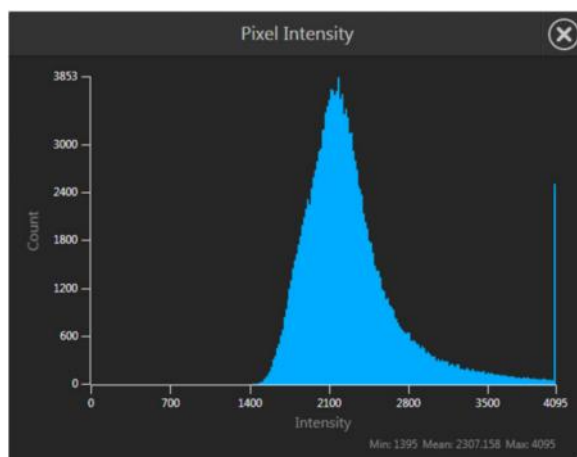
View pixel intensity histogram

Display histogram

1. Click  (**Histogram**) to open the Intensity Histogram plot.




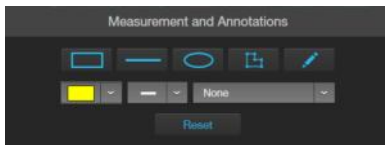
2. The Pixel Intensity histogram shows the Pixel count vs. Intensity data of the image displayed in the Viewing area as well as the minimum, mean, and maximum pixel intensities.



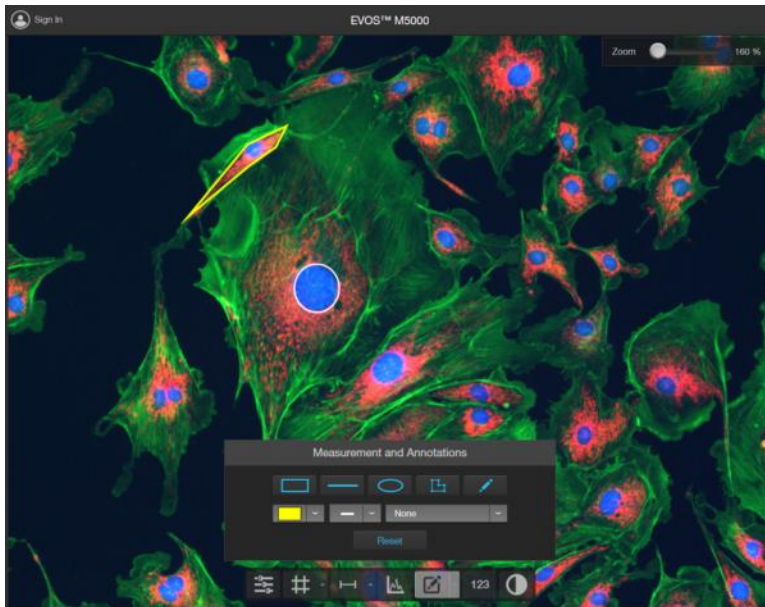
3. To move the histogram, click within the plot heading area and drag the plot to the desired location.
4. To resize the histogram, click the grey triangle at the lower right corner of the plot, then drag the plot to the desired size.
5. Click the **Histogram** button again to close the Pixel Intensity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

Add measurements and annotations

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

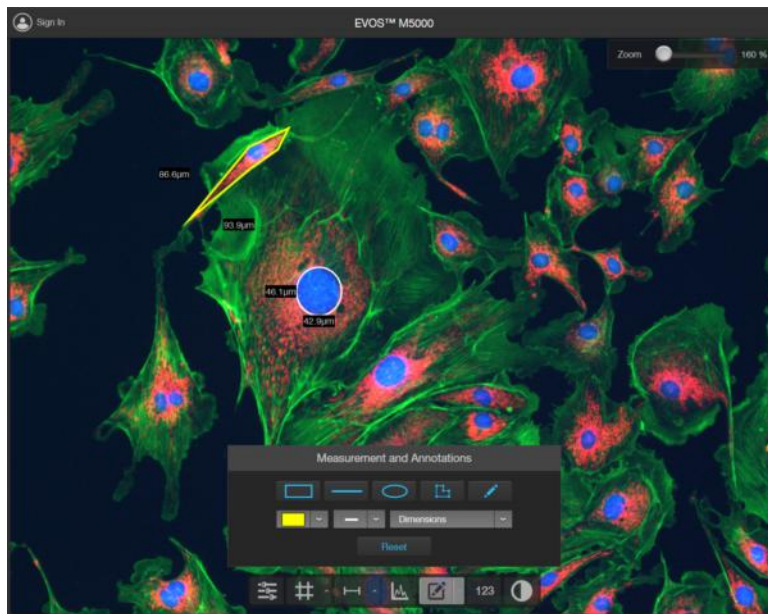


2. Using the **Annotations** tools, draw a **rectangle**, **line**, **ellipse**, **polygon**, or a **free-form** shape over the region of interest on the Viewing area. You can draw multiple shapes of different types.



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


4. If desired, choose 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** (main part of the split button) to turn the display on and off.
 - Click **Measurement and Annotations Controls** (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

If not already showing, hover the pointer over the Viewing area to reveal the **Display Settings and Analysis Tools** toolbar, then click  (**Show Cell Count**) to display **Auto Count**, **Manual Count**, and **Cell Culture** options in the tabs area.



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

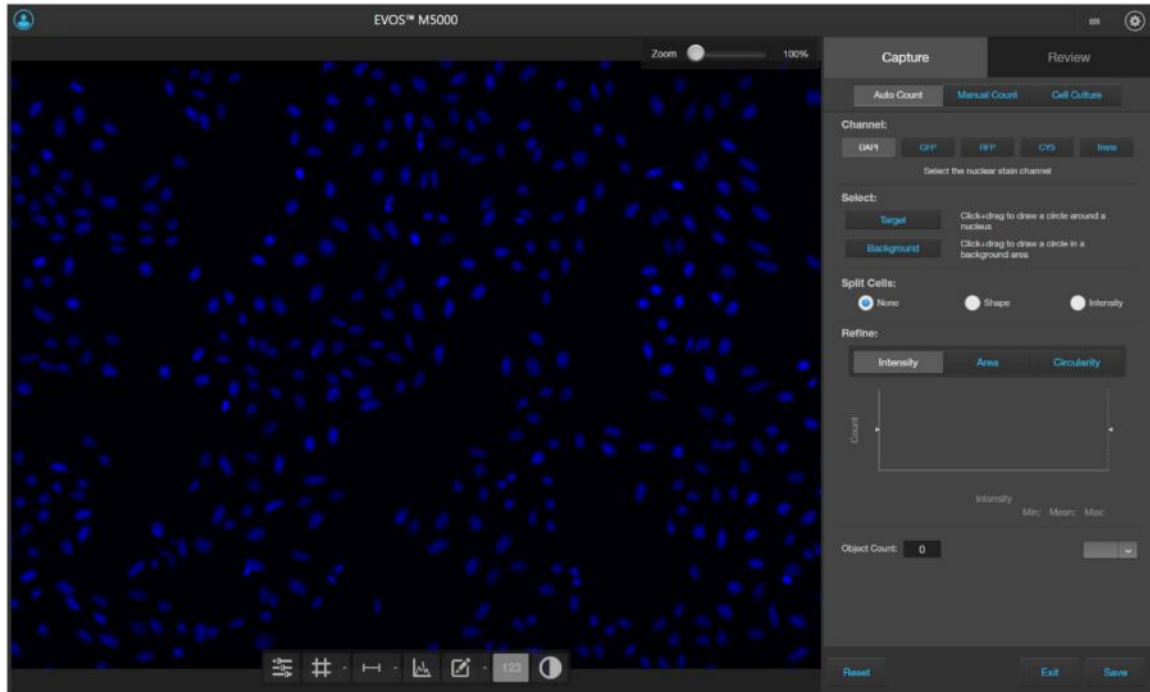
- **Auto Count:** Automatically counts the objects displayed in the Viewing area based on your specifications (“Count cells – auto count” on page 45). With **Auto Count**, you can count objects only in a single fluorescence channel (nuclear stain channel).
- **Manual Count:** Allows you to tag objects 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 (“Count cells – manual count” on page 51). 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 (for example, cells) and one background reference in your image, then automatically calculates the percentage of confluence of your culture (“Count cells – manual count” on page 51).
 - **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 (“Calculate transfection efficiency” on page 58).
- **Batch Analysis:** Allows you to save and apply the analysis parameters set in the **Auto Count**, **Confluence**, and **Transfection Efficiency** tools to other images that you have collected and saved an image folder (“Batch analysis” on page 94). **Batch Analysis** is not available for **Manual Count**.

IMPORTANT! For analysis, only use 16-bit image files (TIFF or PNG). The 16-bit images of individual channels contain the full dynamic range and metadata needed for quantitative analysis, whereas display image files do not.

Count cells – auto count

Perform auto count

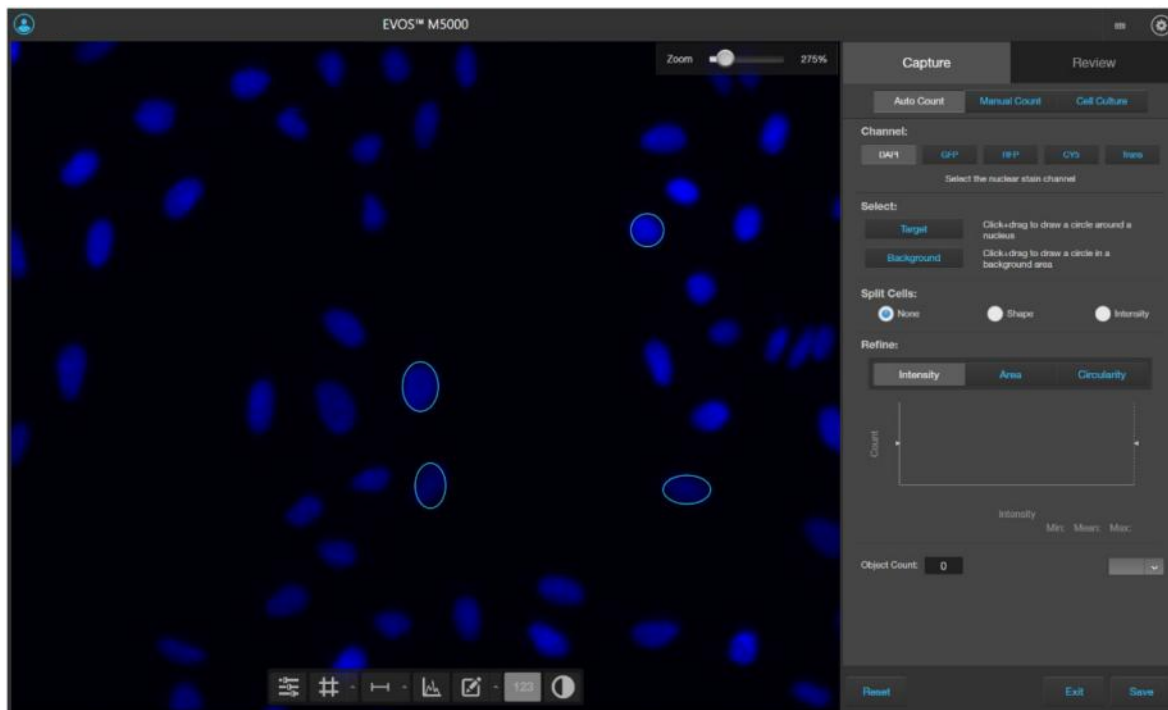
1. Click **123** (Show Cell Count), then select **Auto Count**.



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.

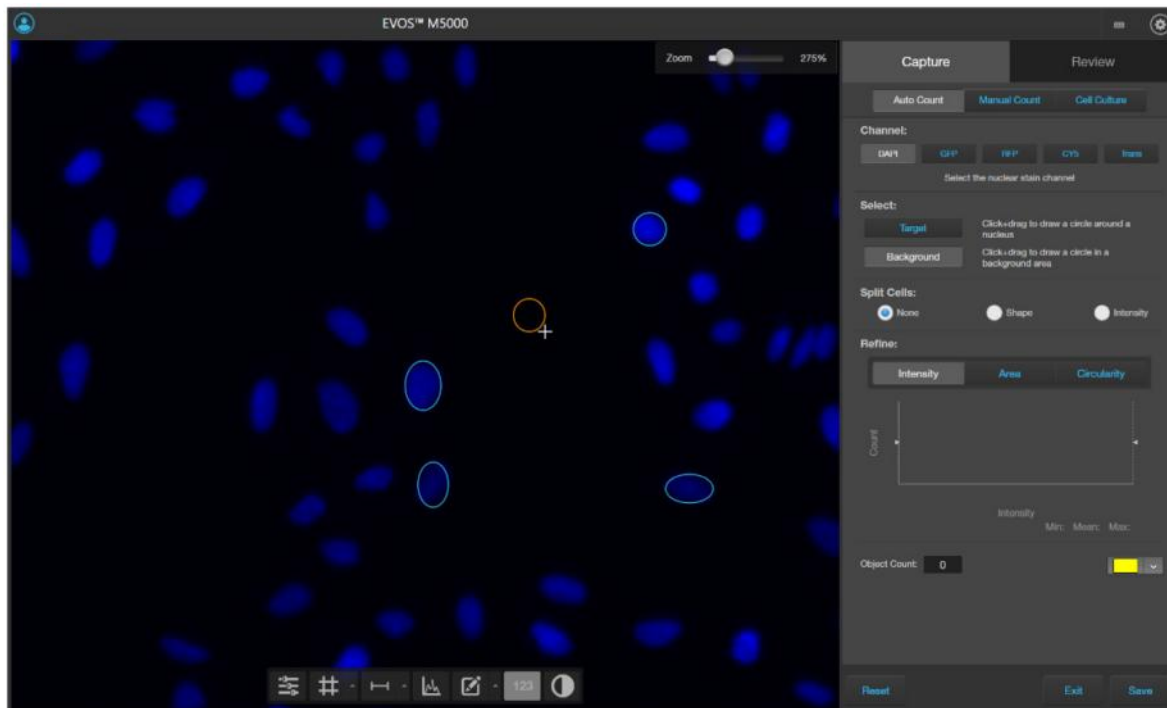


4. If needed, click **Target** again to identify other targets (for example, nuclei that might appear different) to improve the accuracy of your count.

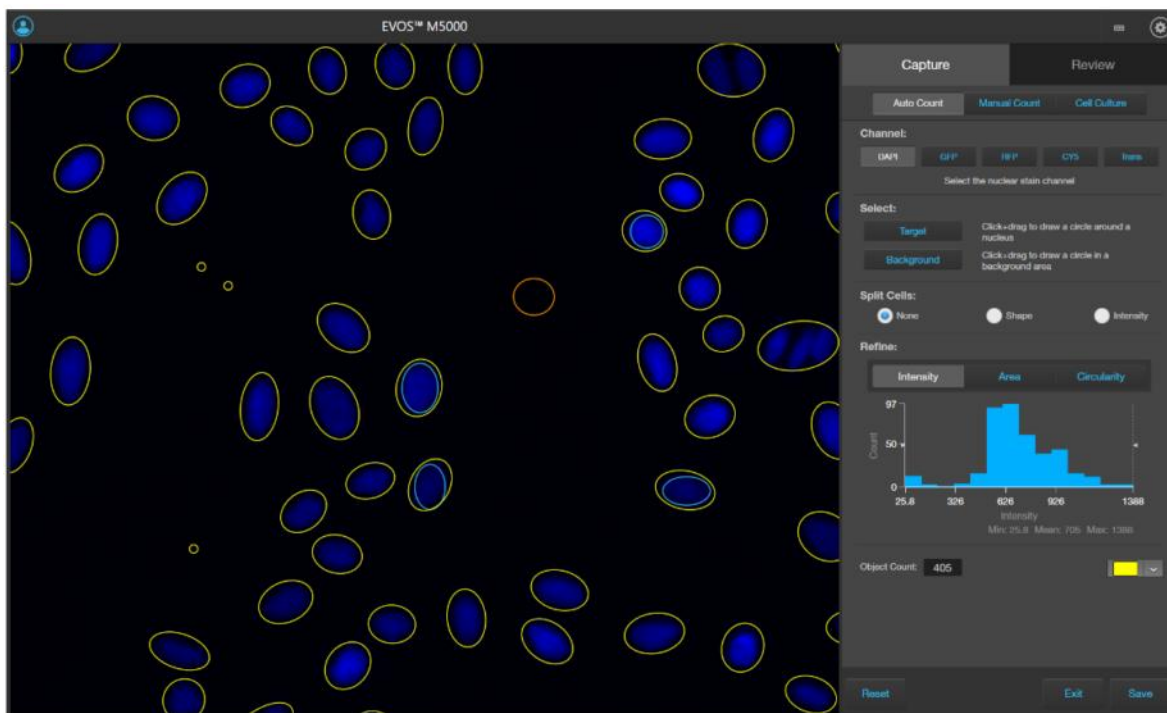
Note: For best results, follow these guidelines when identifying target objects:

- 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.
-

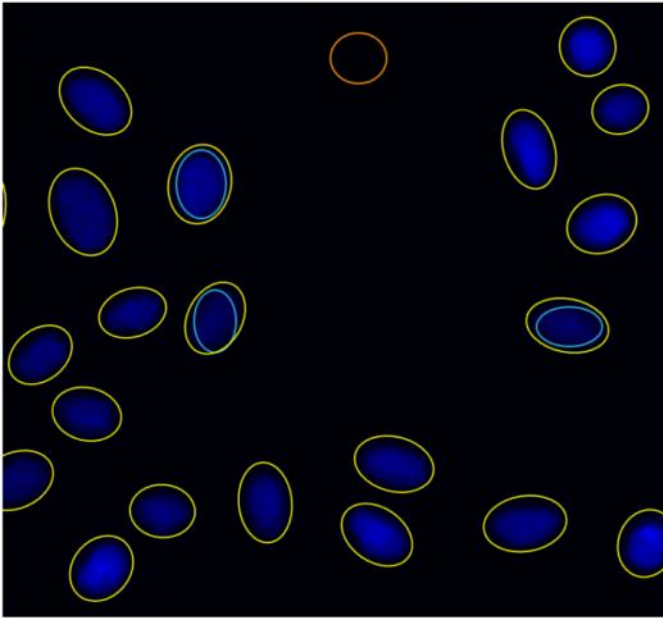
- To distinguish the target from background, click **Background**, then click and drag to draw a circle (orange) in a background area.



- 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.

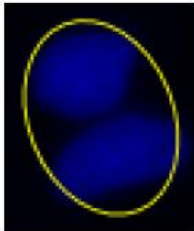


Figure 3 Undercount – 2 cells counted as 1

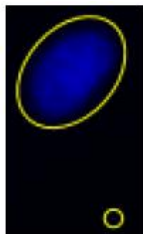


Figure 4 Overcount – 1 cell counted as 2

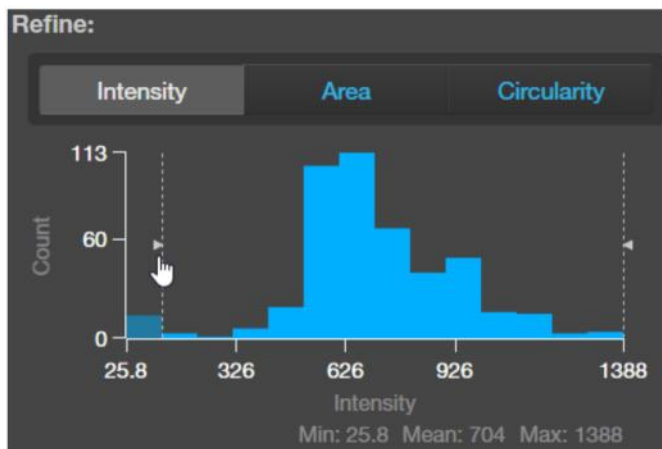
To obtain a more accurate count:

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

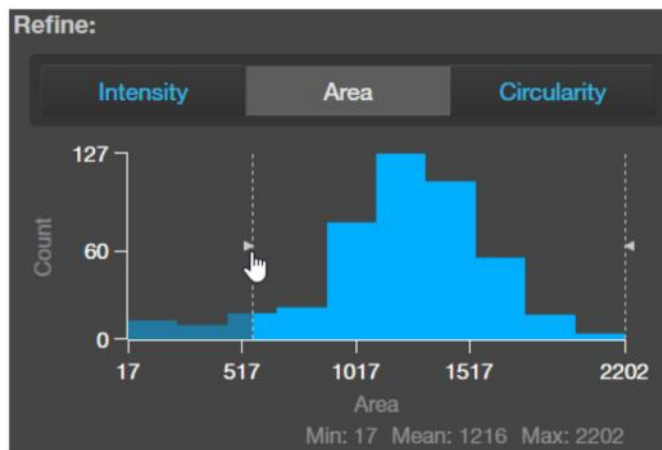
7. To count closely grouped cells that are touching or overlapping as distinct objects, select from the **Split Cells** options:



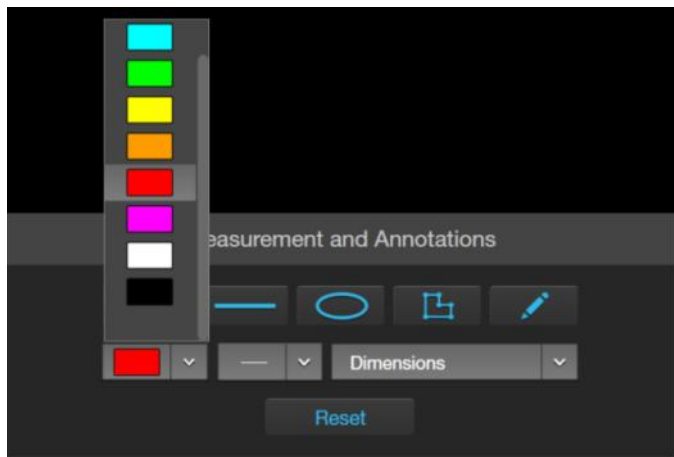
- **None:** Touching or overlapping objects are not counted separately.
 - **Shape:** Distinct objects are identified and counted based on shape.
 - **Intensity:** Distinct objects are identified and counted based on pixel intensity.
8. The Refine section displays a histogram plot showing **Count** versus **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.



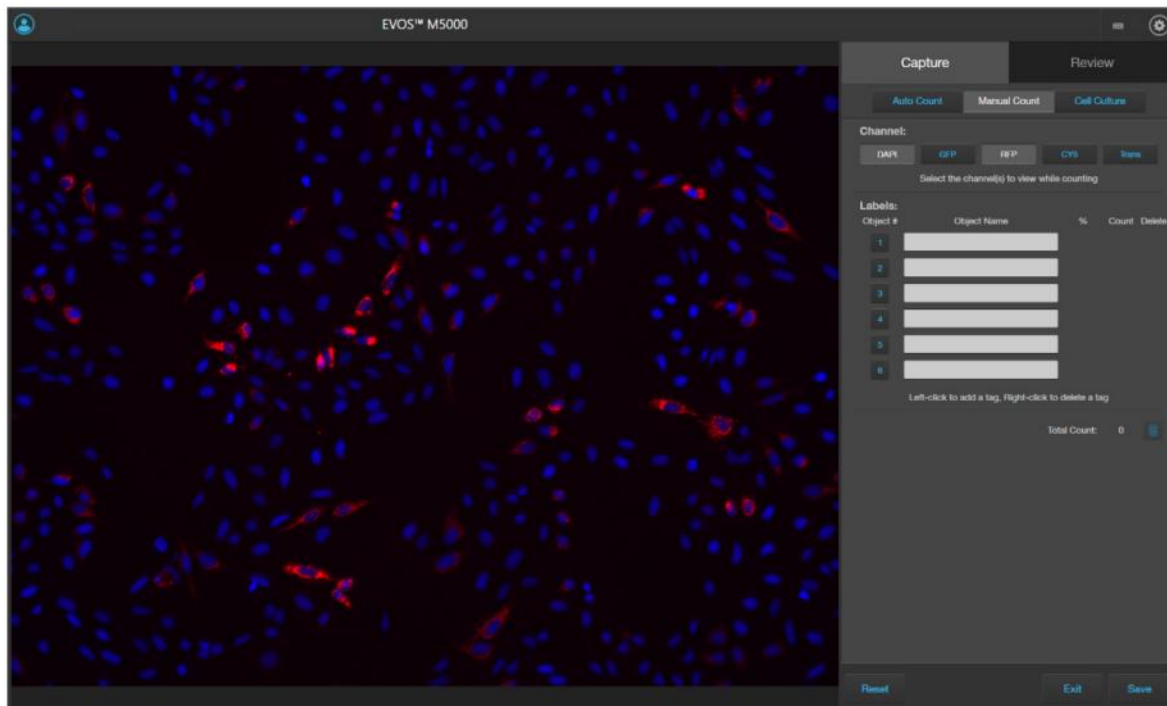
11. When finished with the count, save your count results (see “Save analysis results” on page 92).

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

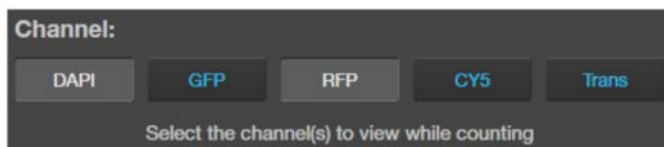
Perform manual count

1. Click **123** (**Show Cell Count**), 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.



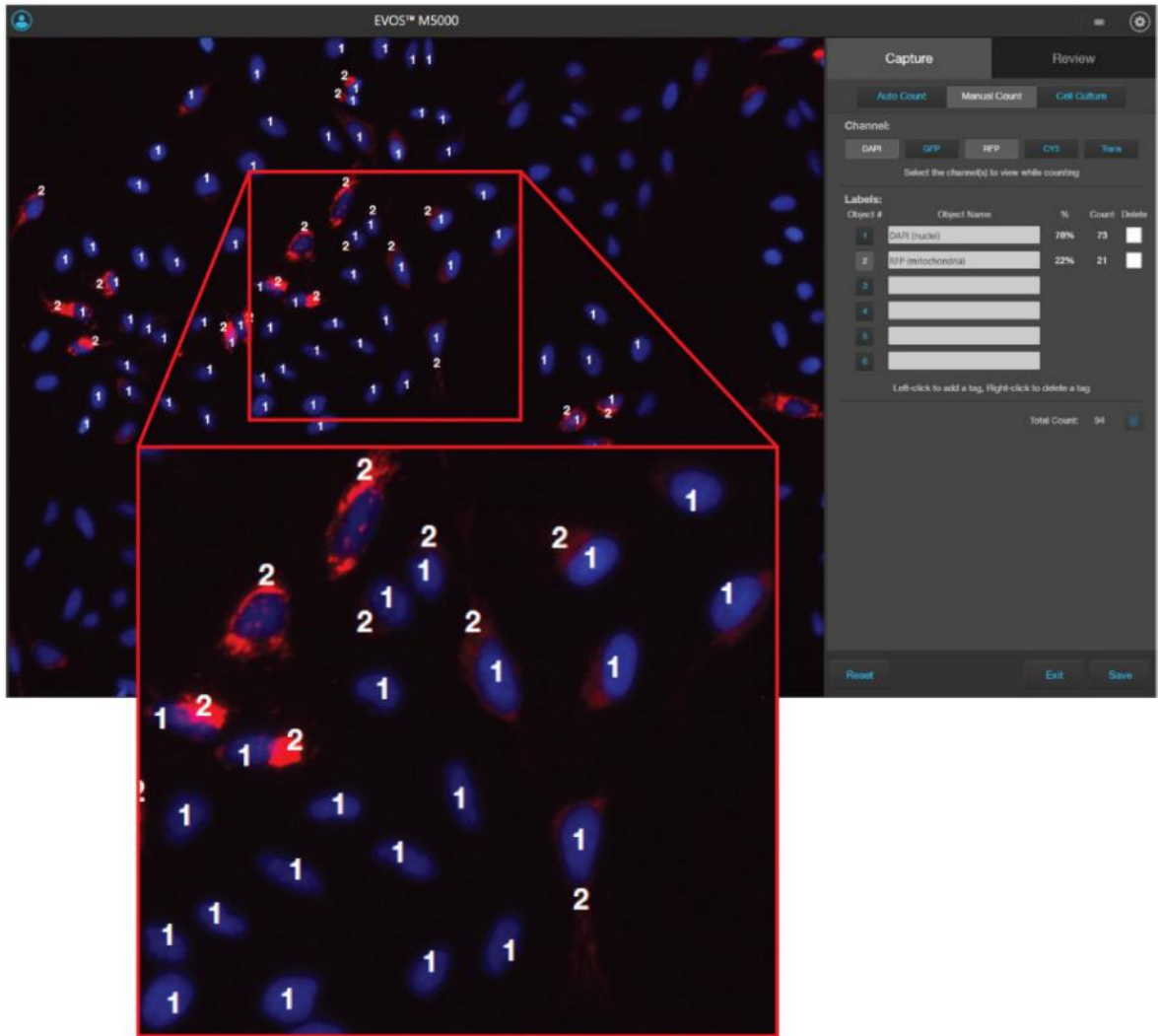
3. Click in an **Object Name** field to enter a name for that label. You can use up to six labels to tag objects for the manual count.

Labels:

| Object # | Object Name | % | Count | Delete |
|----------|--------------------|---|-------|--------|
| 1 | DAPI (nuclei) | | | |
| 2 | RFP (mitochondria) | | | |
| 3 | | | | |
| 4 | | | | |
| 5 | | | | |
| 6 | | | | |

Left-click to add a tag, Right-click to delete a tag

- 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” on page 92).

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** or **10x** magnification. Set the phase ring to **Oly 4x** (for Olympus™ 4x phase objective) or **4x/10x** (for EVOS™ phase objectives) using the phase ring selector (“Front view” on page 13).
- 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 image files (TIFF or PNG), 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

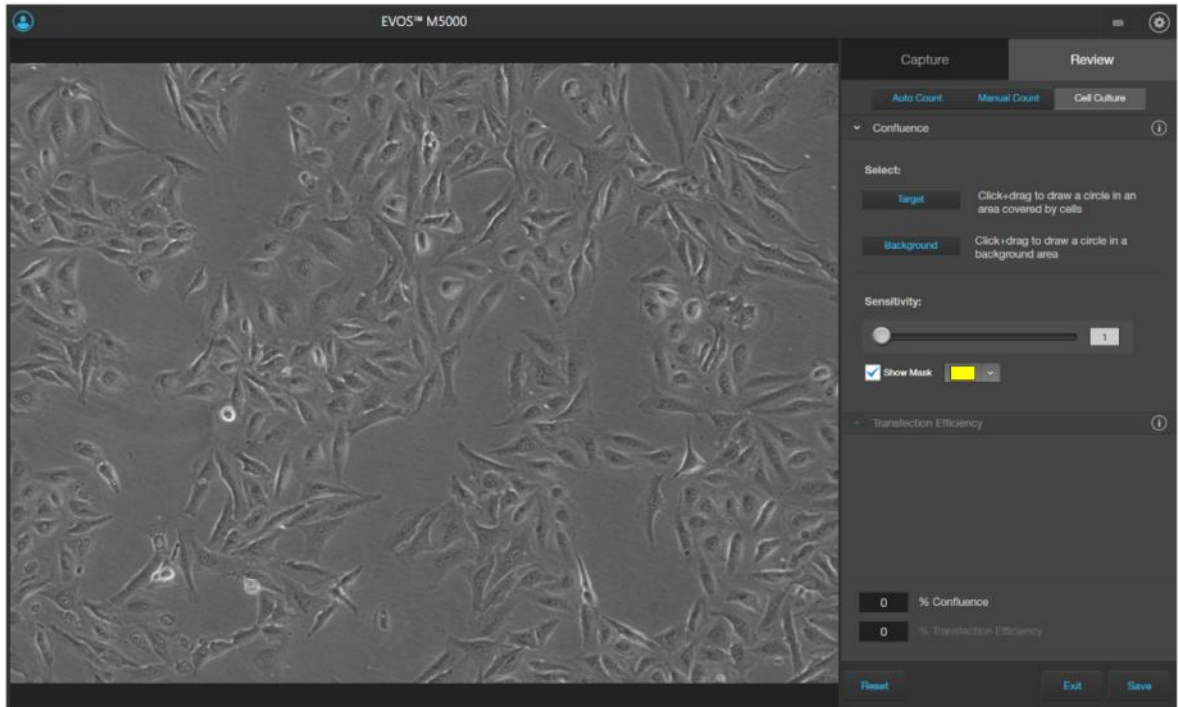
1. In the transmitted light channel, adjust lighting and focus on the sample, then click **Capture** to acquire an image of your culture.

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. If not visible, hover the pointer over the Viewing area to reveal **Display Settings and Analysis Tools**, then click the **Show Cell Count (123)** button.

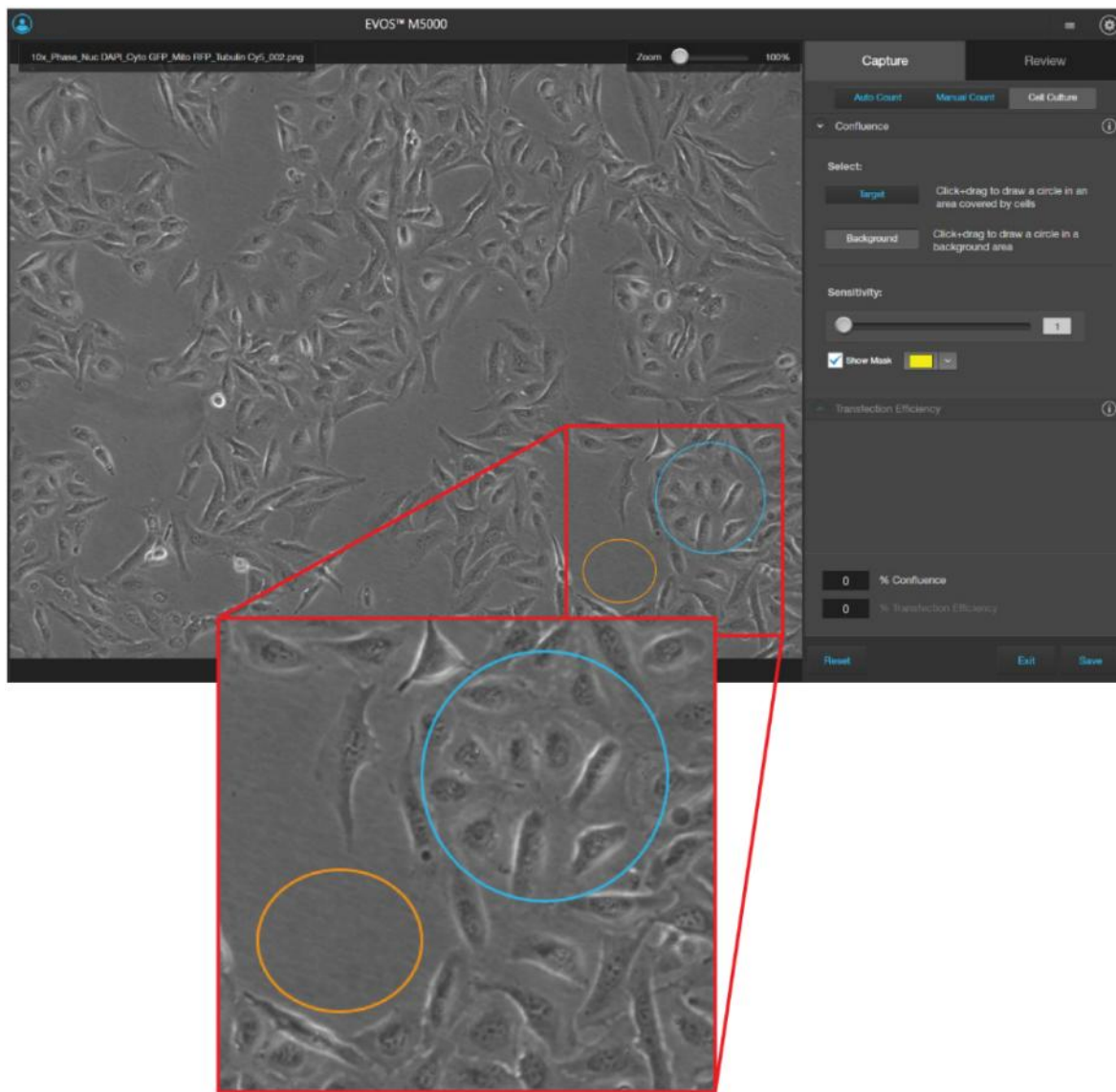


3. In the tabs area, select **Cell Culture** to display the **Confluence** tool.



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

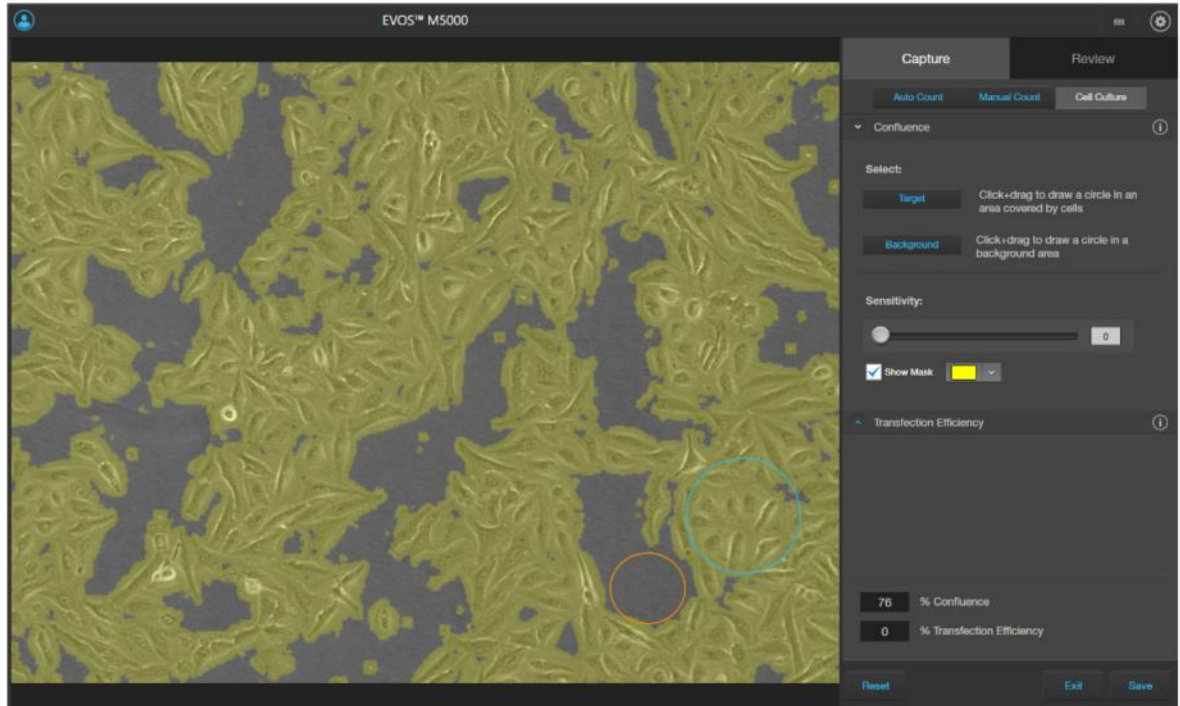
5. Click **Background**, then click and drag on the image to draw a circle around a background area that does not contain any cells (orange circle).



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.

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



- 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.

- When you complete the confluence measurement, the **Transfection Efficiency** tool becomes available. To calculate transfection efficiency, go to “Calculate transfection efficiency” on page 58.
- To save your **Confluence** results without calculating transfection efficiency, click **Save** (see “Save analysis results” on page 92).

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.

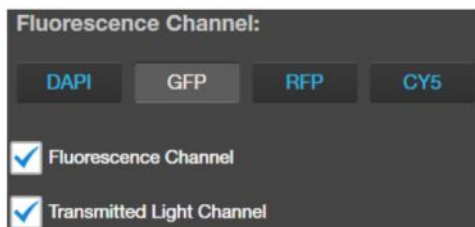
The **Transfection Efficiency** tool calculates the fluorescence area (transfected cells expressing the fluorescence marker) divided by the entire cell area in the image.

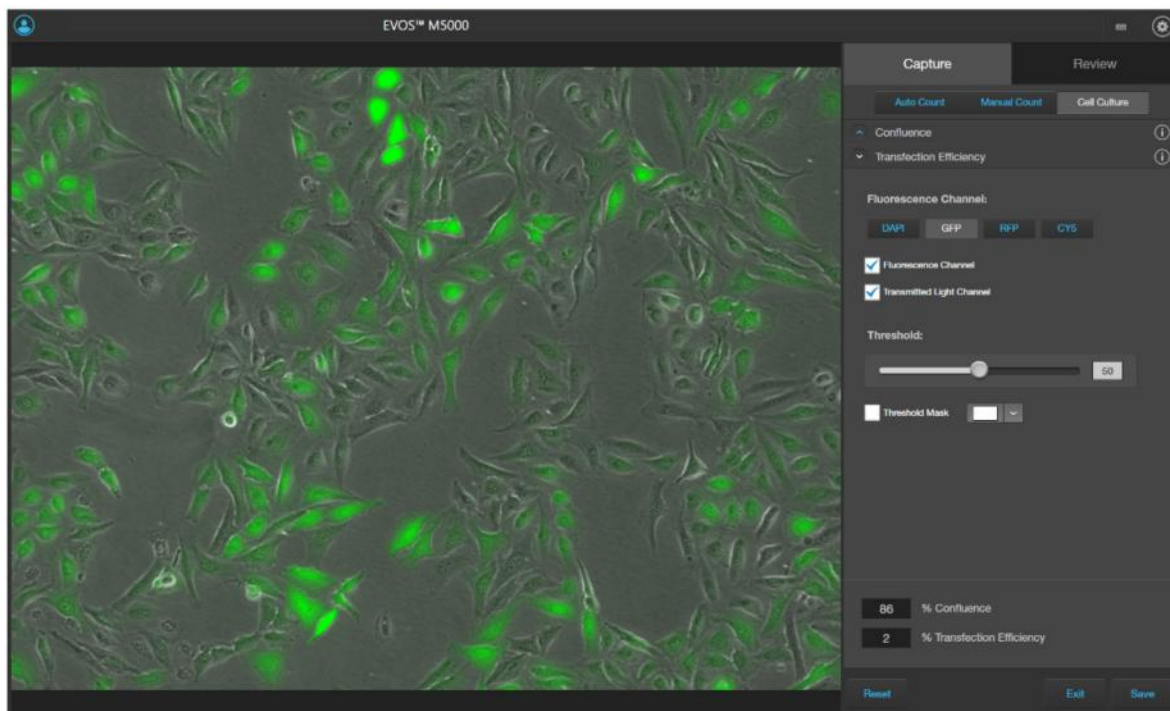
IMPORTANT! For analysis, only use 16-bit image files (TIFF or PNG), which contain the full dynamic range and metadata needed for quantitative analysis.

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.

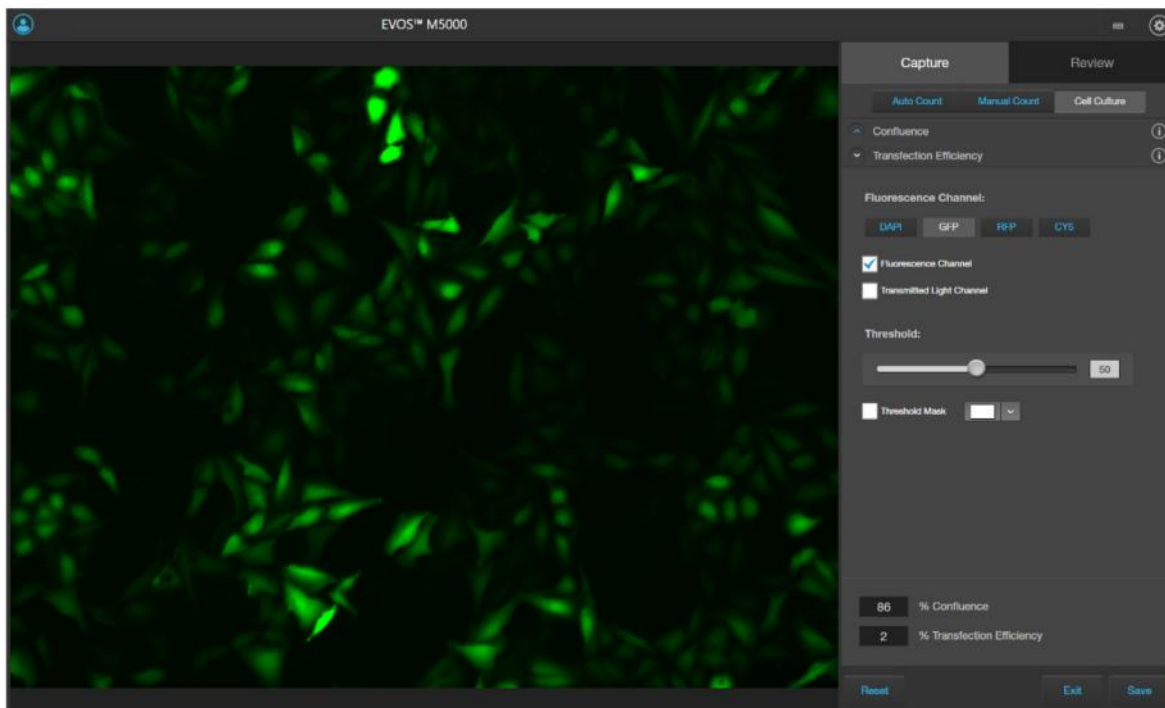




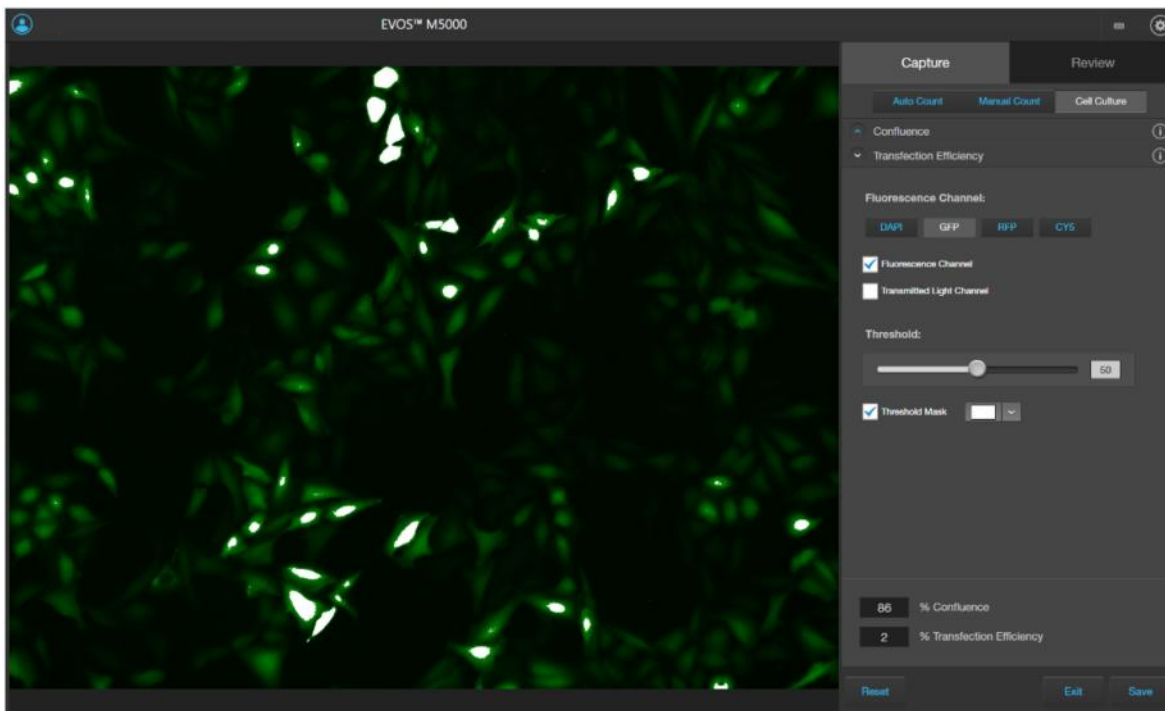
The software automatically calculates the **Transfection Efficiency** and displays the results as **% Confluence** and **% Transfection Efficiency**.

Note: The **Transfection Efficiency** calculation is based on the final measured confluence (Step 8 on page 57) 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 step 5 on page 61).

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



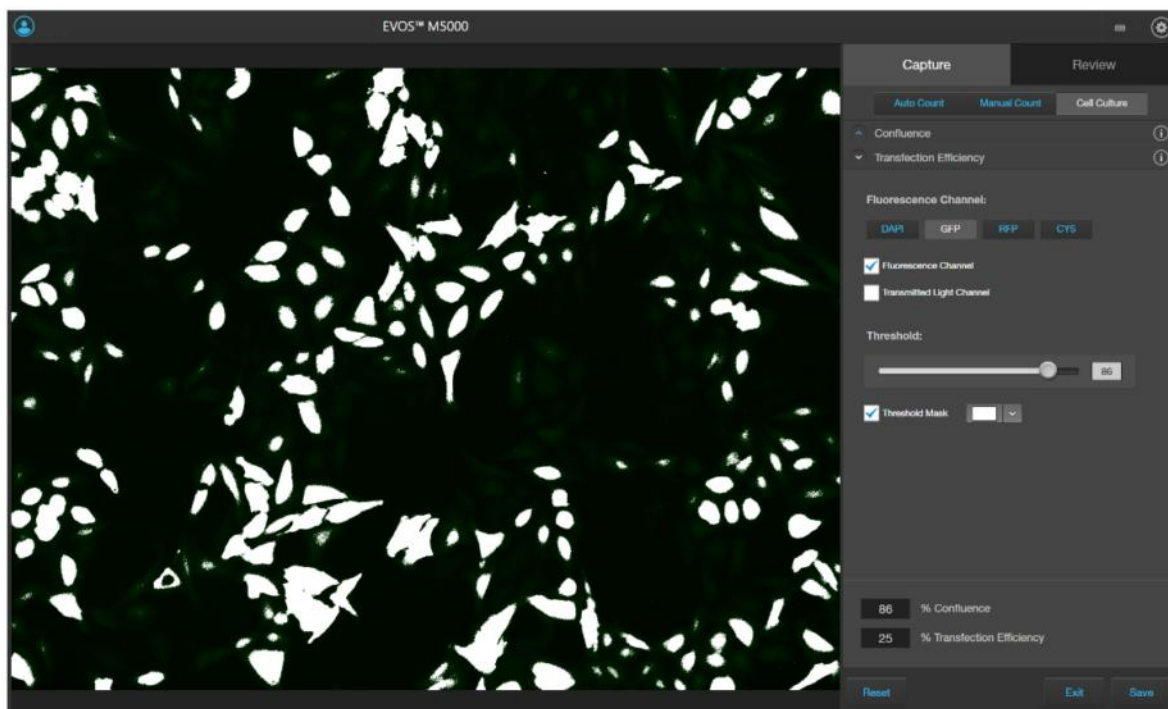
4. Select **Threshold Mask** to highlight the fluorescence areas included in the Transfection Efficiency calculation.



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” on page 92.

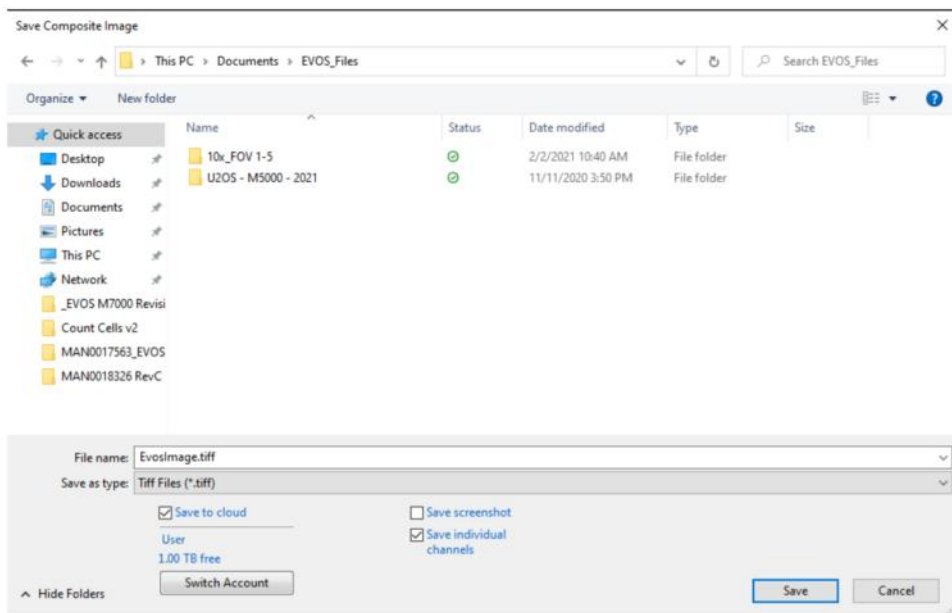
6

Save captured images

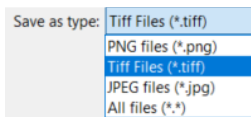
Save

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**, and **JPEG**.

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. Saving as type TIFF or PNG are the recommended formats for image analysis.

Leave the **Save individual channels** option unselected to save only the captured image displayed in the viewing area. The image is saved in display format, which is not useful for analysis.

IMPORTANT! Save individual channels as type TIFF or PNG as these are the recommended formats for image analysis, because they allow you to save raw image data from each selected channel.

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

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

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

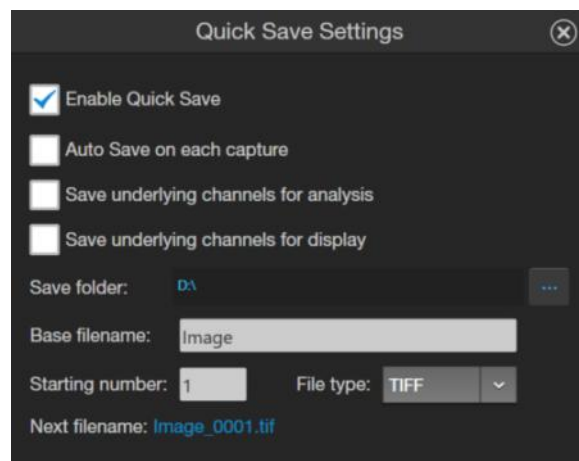
6. Click **Save** to save the image.

Quick Save images

(Optional) Enable Quick Save

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

1. Next to the **Save** button, click **^ (Up)** to open the **Quick Save Settings** dialog.





2. Click **Enable Quick Save**. The **Save** button on the **Capture** panel will change to **Quick Save**.
3. Set the save to location drive by clicking **... (Browse)** to open the **Select Folder** dialog, navigate to the folder for storing images, then click **Select Folder**.

4. Enter a **Base Filename**. The **Starting number** automatically changes to 1 when the **Base Filename** is changed.

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**, and **JPEG**.
6. To save the separate channels for the image displayed in the viewing area, select **Save underlying channels for analysis** for later analysis, **Save underlying channels for display** to show separate pseudocolored images for paper or slide presentation, or you can also enable both selections.
7. To automatically save captured images without having to click **Save** after each capture, select **Auto Save on each capture**.
8. Click  to close the dialog.
The button now displays  (**Quick Save**).

7

Capture time lapse images

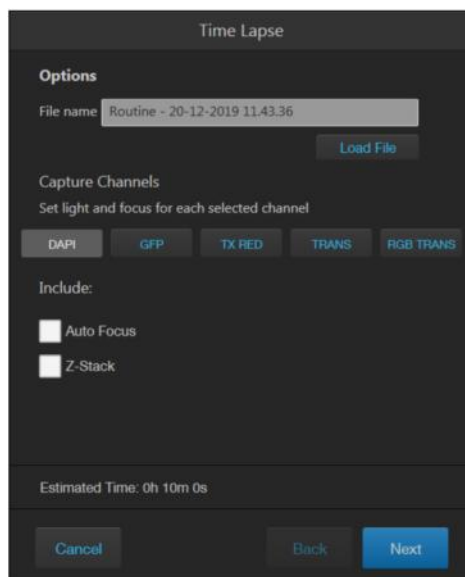
Time lapse tool

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. Click on the desired capture channels (for example, **DAPI**, **GFP**, etc.). Multiple channels can be selected for capturing time-lapse images.
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 running 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. (Optional) If the EVOS™ Onstage Incubator is installed and used for a time-lapse routine, click **Use Incubator**.

Note: 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.
 - The gas inputs have been configured.
 - The oxygen sensor has been calibrated.
-

6. Adjust brightness and focus for each selected channel as described in “Capture images in a single channel” on page 28, then click **Next**.
7. If you have opted to run **Auto Focus**, select the **Auto Focus** channel (for example, **DAPI**, **GFP**, and so on) to use.

Note: In a time lapse routine, you can run the autofocus procedure only in a single channel. 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” on page 68.

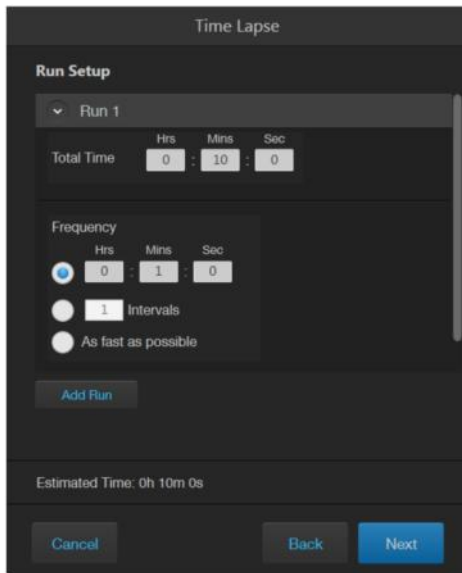
9. If you have opted to capture **Z-Stack** images, define the **Top**, **In Focus**, and **Bottom** positions for the Z-Stack image set.
- a. If not already on, click the **Light** button to illuminate the sample as you locate the boundaries and the **In Focus** position of the Z-Stack.
 - b. To accept 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 **Z number** 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. Click **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**, **CO₂**, 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.
17. When finished with adding Runs, click **Next** to set the Save options.
18. Click ... (**Choose Save Folder**), navigate to the desired destination folder for the **Time Lapse** images, then click **Select Folder**.
19. Select the **File Type: PNG** or **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**.

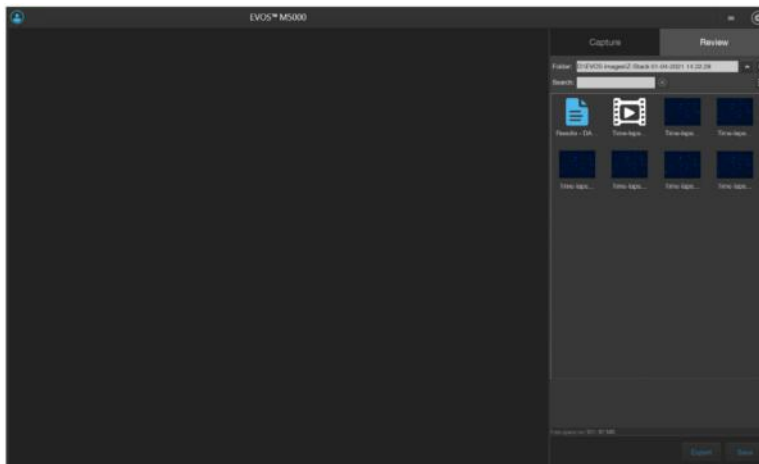
22. Select the **Frame Rate** for image capture: **1**, **3**, or **5** images/second.

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 (step 3 on page 72).

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.

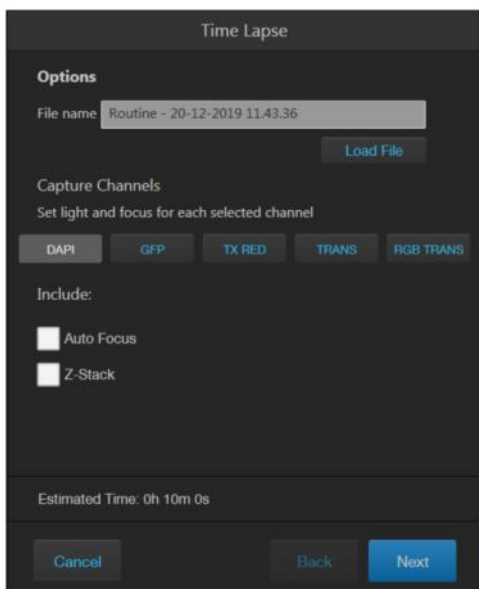
24. When the **Time Lapse** routine is completed, the **Review** tab opens to the files captured in the routine.



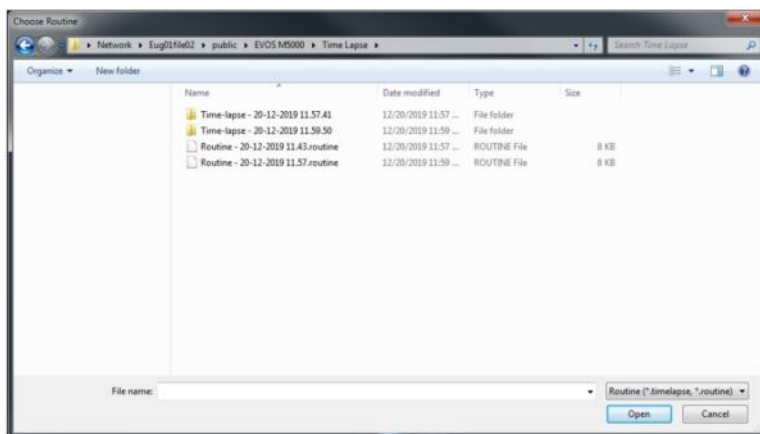
Run a saved time lapse routine

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.

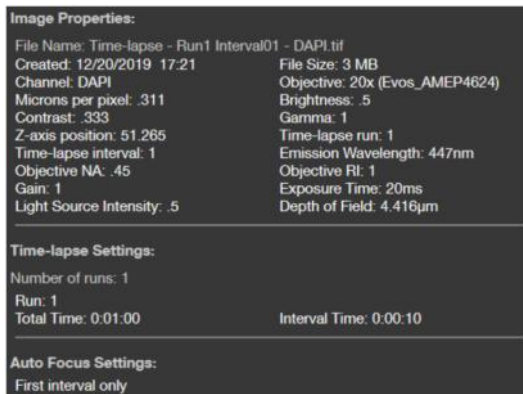
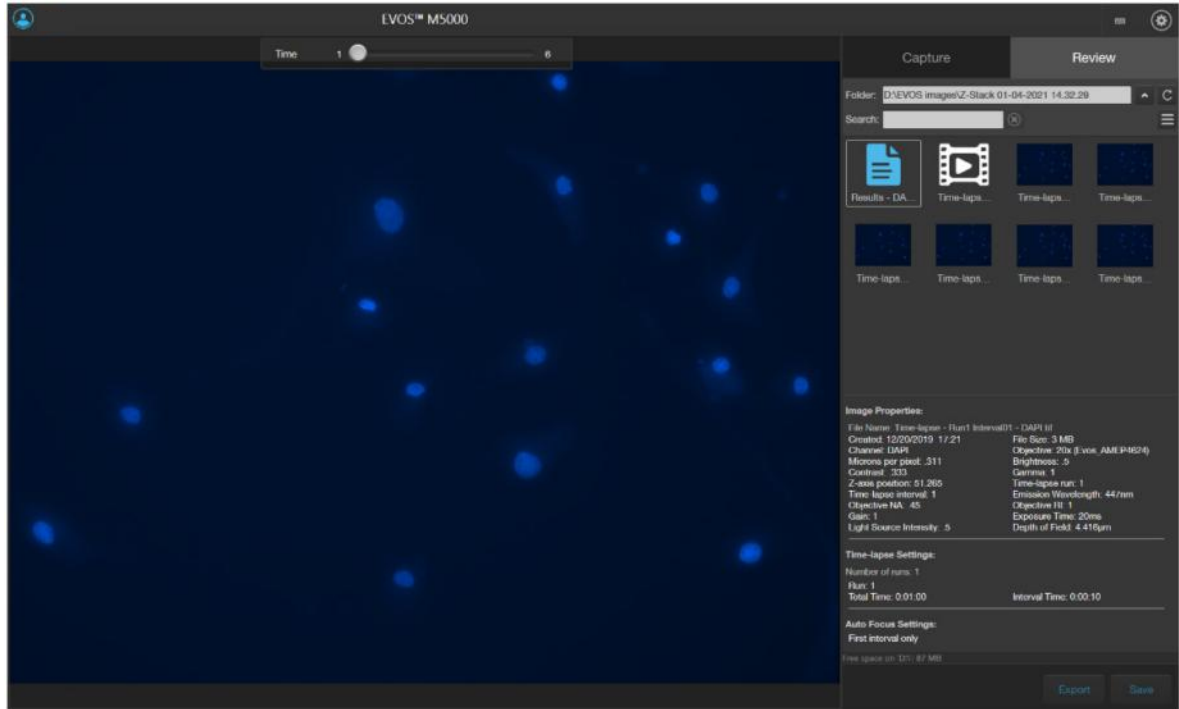


3. Select the routine you want to run, then click **Open**.
4. If needed, make changes to the routine as described for new routines.
5. Click **Start** to run the **Time Lapse** routine.

Review images and video captured in a time lapse routine

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

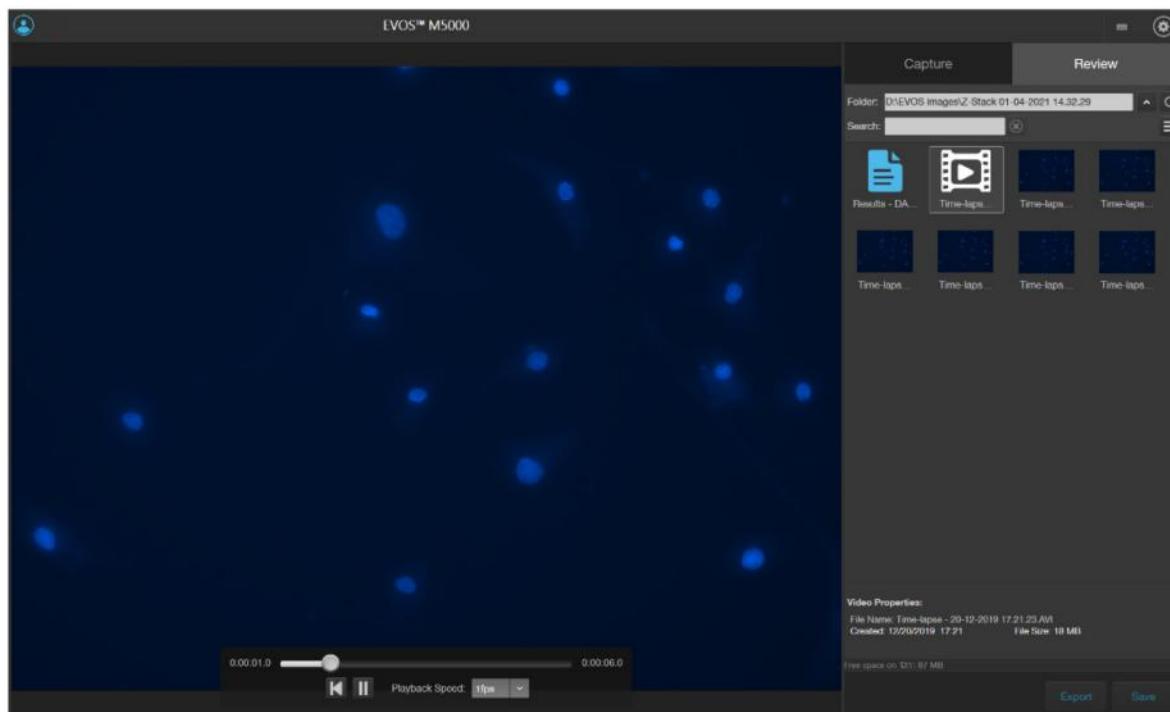
The viewing area displays the first image captured in the time lapse routine and the **Review** tab shows the **Image Properties**, and **Time Lapse** and **Auto Focus Settings**.



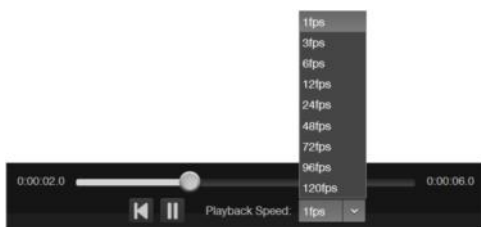
2. To view the subsequent images captured in the time-lapse routine, use the **Time Slider** at the top of the viewing area.



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 (step 19 on page 68). You can view the saved time lapse recording at different playback speeds ranging from 1 fps (frames per second) to 120 fps (step 3 on page 72).

- 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**.

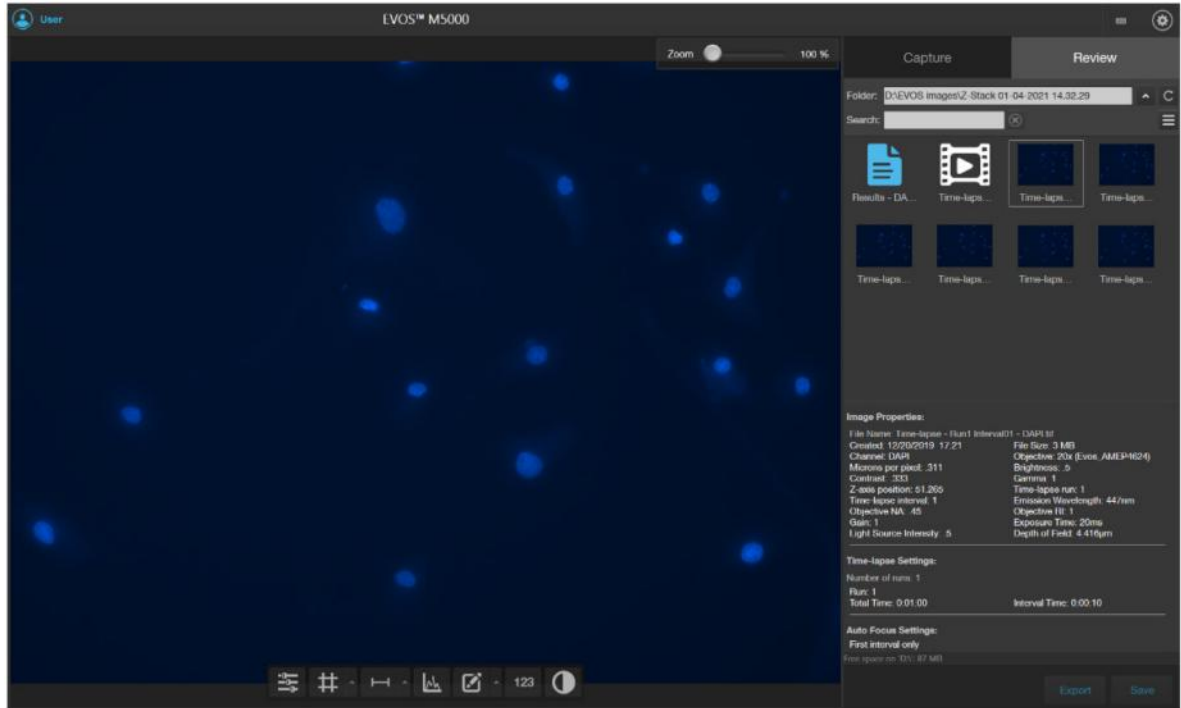


Image Properties:

| | |
|--|--------------------------------|
| File Name: Time-lapse - Run1 Interval01 - DAPI.tif | File Size: 3 MB |
| Created: 12/20/2019 17:21 | Objective: 20x (Evos_AMEP4624) |
| Channel: DAPI | Brightness: .5 |
| Microns per pixel: .311 | Gamma: 1 |
| Contrast: .333 | Time-lapse run: 1 |
| Z-axis position: 51.265 | Emission Wavelength: 447nm |
| Time-lapse interval: 1 | Objective RI: 1 |
| Objective NA: .45 | Exposure Time: 20ms |
| Gain: 1 | Depth of Field: 4.416µm |
| Light Source Intensity: .5 | |

Time-lapse Settings:

| | |
|---------------------|------------------------|
| Number of runs: 1 | |
| Run: 1 | |
| Total Time: 0:01:00 | Interval Time: 0:00:10 |

Auto Focus Settings:

First interval only

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



- To annotate captured images and perform cell count and confluence measurements, use the **Display Settings and Analysis Tools** as described in Chapter 5, "Measure, annotate, and analyze captured images".



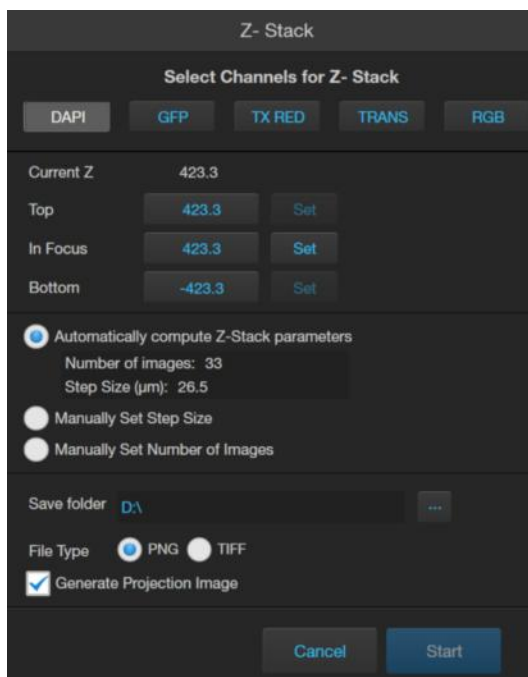
Capture Z-Stack

Z-Stack tool

The **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, the **DAPI** channel is selected.
3. If not already on, click **Light** 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**.
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 **Browse** to open the **Choose Save Folder** dialog.
9. Go to the destination folder for the Z Stack images, then click **Select Folder**.
10. Select the **File Type: PNG** or **TIFF**.
11. Select **Generate Projection Image** to generate a projection image.

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

When unselected, the system only saves the individual Z-Stack images.
12. Click **Start** to capture the Z-Stack image sets based on your specifications.

The system provides information about the progress of the Z Stack protocol.
13. When the Z-Stack routine is completed, the **Review** tab opens to the files captured in the routine.



Stage View

Overview






Note: **Stage View** requires an updated stage manufactured in late 2024 for all new EVOS™ M5000 systems. Prior EVOS™ M5000 systems using a stage with separate X- and Y-axis control knobs do not have access to the **Stage View** feature but can be updated to have all of the other software features described in the this user guide.

Stage View provides a method for marking points of interest in your sample in multiple vessel types. **Stage View** also shows a map of specific vessels to display the actual location in the well or area you are viewing. Vessel maps have been created for 6 precision-mount spring-retention EVOS™ vessel holders.

- Well plates in 6-, 12-, 24-, 48-, 96-, and 384-well formats (Non-OSI compatible) (Cat. No. [AMEP-VH022](#) and [AMEP-VH061](#))
- Well plates in 6-, 12-, 24-, 48-, 96-, and 384-well formats (OSI compatible) (Cat. No. [AMEP-VH028](#) and [AMEP-VH040](#))
- Standard glass slides (Non-OSI compatible) (Cat. No. [AMEP-VH044](#))
- 35 mm Petri dishes (OSI compatible) (Cat. No. [AMEP-VH110](#))

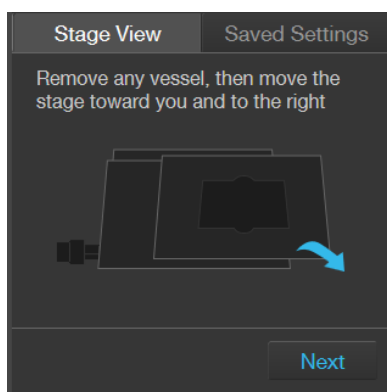
For optimally finding and marking pins, we recommend using an appropriate precision-mount spring-retention EVOS vessel holder. However, any non-precision EVOS™ vessel holder can work provided the holder is consistently positioned with each use.

Workflow

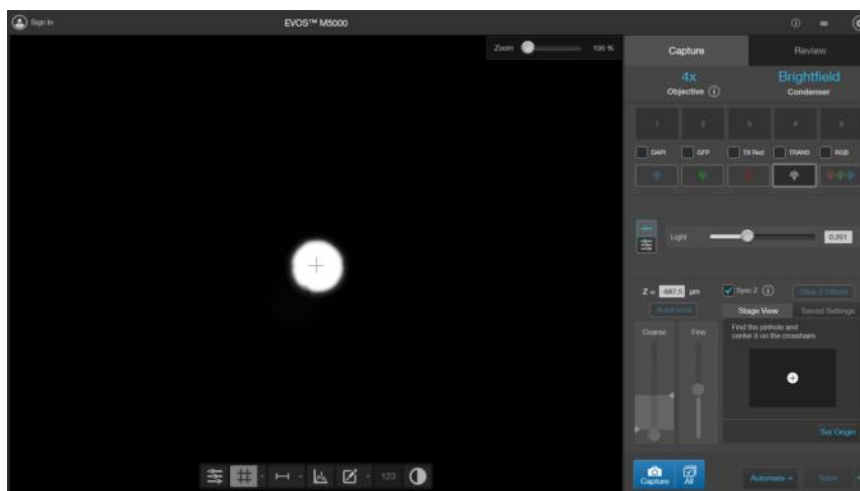
| Stage View | |
|---|--|
|  | Set the Stage Origin page 78 |
|  | Mark the pin locations page 81 |
|  | Find the pins using the Find Target screen page 81 |
|  | Save the pin maps page 81 |
|  | Load the pin map and find the pins using the Find Target screen page 84 |

Set Stage Origin

1. Select the **Stage View** tab.
2. Switch to a low-power objective (2x, 4x, or 10x). This allows for easier locating of the calibration pinhole and ensures enough space for the objective beneath the stage for movement.
3. Click **Next**. This turns on the transmitted light and moves the **Coarse** focus slider to the bottom.
4. Remove any vessel on the stage and move the stage toward you and to the right using the stage knobs.

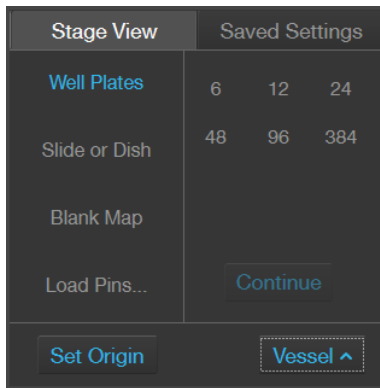


5. Click **Next**. The center point (+) appears.
6. Find the pinhole (white circle in image view) and position the center point within the hole. Below shows the screen view as the pinhole is lined up with the center point.

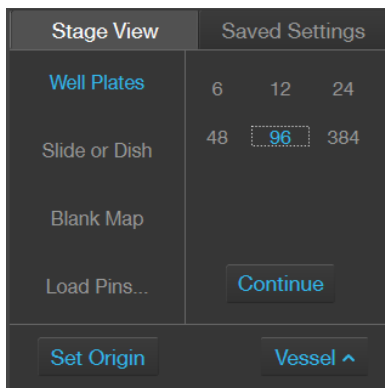


Note: When the objective is in position under the far left corner of the vessel holder only a small rotation of the X and Y control knobs is needed to find and center the pinhole. From this corner position the X-axis (outer knob) is rotated approximately 5 mm toward you and the Y-axis (inner knob) is rotated approximately 9 mm away from you to see the pinhole.

7. Click **Set Origin**. The stage is now initialized for tracking its position.
8. Click **Vessel** to select a vessel type:
 - **Well Plates**
 - **Slide or Dish**
 - **Blank Map**
 - **Load Pins**



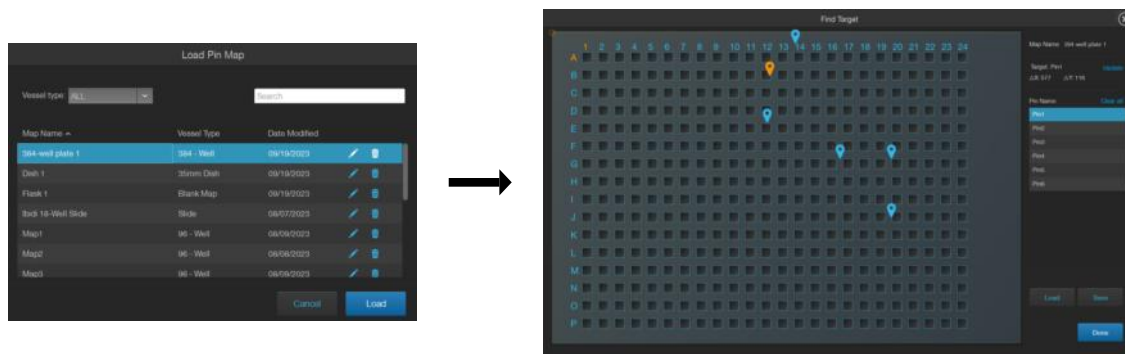
9. Click the plate size or vessel size/type or load a pin map from the **Load Pin Map** window:



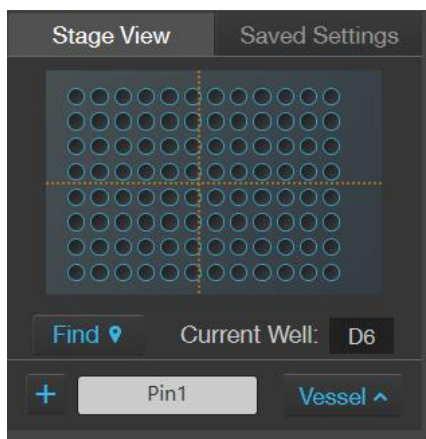
- **Well Plates:** 6, 12, 24, 48, 96, or 384 wells
- **Slide or Dish:** 25 x 75 mm slide or 35 mm Petri dish
- **Blank Map:** For all other vessels, such as a T-flask, large Petri dish, and so on

- **Load Pins:** Loads a previous map with pins and the appropriate vessel. Click **Load Pins....** Click the desired **Map Name** row. Click **Load**. The **Find Target** window will open. Click **Done** to return to the main screen.

Note: When selecting a well plate, any captured images will automatically add the well ID to the saved image filename.



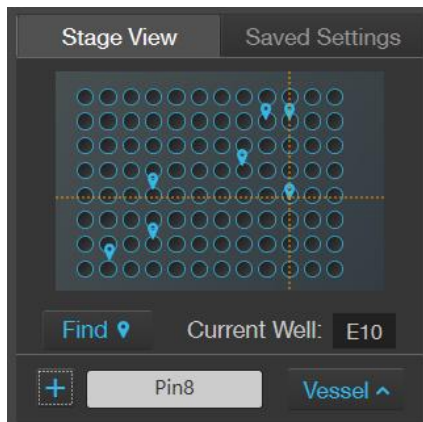
10. Click **Continue** to load the vessel map. A representation of the vessel layout will appear in the **Stage View** tab. Also visible is an amber moveable crosshairs that tracks the stage position as the stage is moved.



11. Insert the vessel into the holder. **Carefully** seat the vessel tightly into the corner opposite the spring clip and ensure the spring clip correctly secures the vessel. For plates, the **Current Well** will display A1 and update the well ID as the stage is moved to different wells (other vessel types will not have the **Current Well** display). You can now move the amber crosshairs around the small vessel map by moving the stage.

Mark pin locations

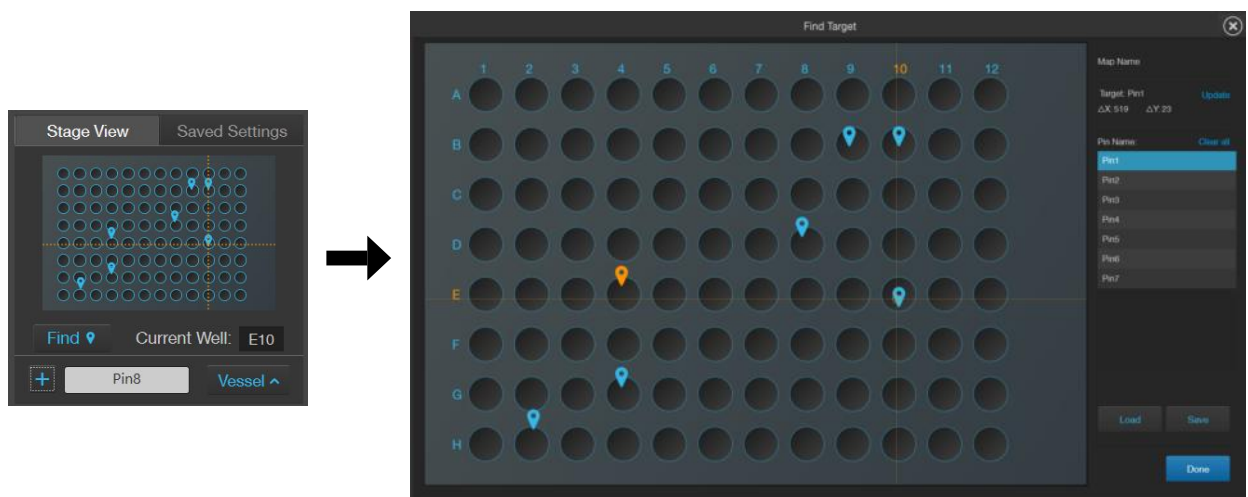
1. Find a point of interest by moving the stage using the knobs.
2. Once on a desired spot, click **+** (**Add pin**). This will be **Pin 1** or can be custom named by clicking in the field next to the **+** icon. Subsequent pins will be Pin 2, Pin 3, and so on, unless custom named previously.



3. Mark any other points of interest in the vessel by moving the stage, then clicking **+** to add each pin.
4. Once all of the desired pins are created, click **Find**. The large **Find Target** map will appear showing a more detailed view of marked pin locations in the vessel.

Find pins using the Find Target screen

The **Find Target** screen is an expanded view of the small vessel map.



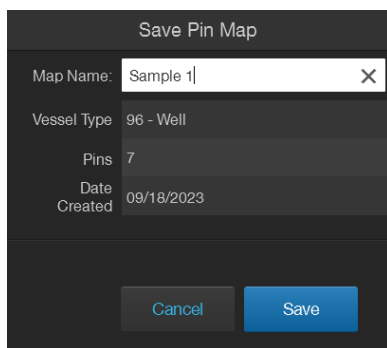
1. Select a target by clicking on the desired pin or the pin name in the list. The pin will change color from blue to amber and **Target** will update with the name of the selected pin.
2. To find the exact location of the current target (amber), move the stage using the knobs to move the crosshairs. As the crosshairs move towards the target pin, the ΔX and ΔY values will change. When the stage is close to the target pin, the crosshairs turn green. The pin is exactly located when the values of ΔX and ΔY equal zero.






3. Click **Done** to close the **Find Target** screen, then turn on the illumination and check to verify that the desired target is visible.
4. (Optional) Before closing **Find Target**, click **Save** to go to the **Save Pin Map** window. If needed, enter a **Map Name**. Click **Save**.

The **Save** window also displays the following information:

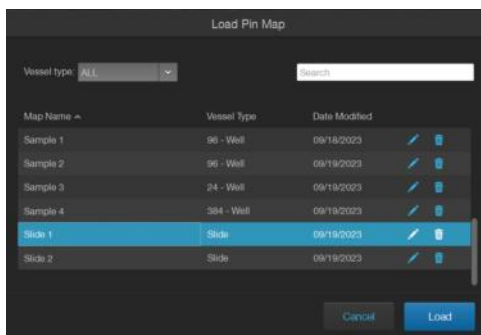
- Vessel Type
- Number of Pins
- Date Created



5. (Optional) Each pin name in the **Find Target** window can be modified or deleted by hovering the mouse over the pin name, then clicking  (**Edit**) or  (**Delete**). Click  to complete the edit. All pins can be deleted by clicking **Clear all** above the pin list.



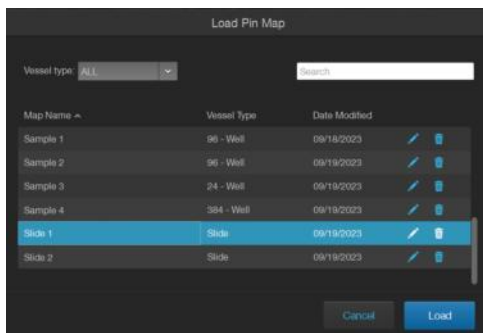
6. To use a previous map, click **Load**. This opens the **Load Pin Map** window (see “Load Pin Map” on page 84).



This window allows for searching and editing of saved maps. Saved maps can be selected from the list, using **Search**, or sorting by vessel type, map name, or date modified.

Load Pin Map

- To use a previous map, select a row, then click **Load**.



- This window also allows for searching and editing saved maps. Saved maps can be selected from the current list by using the **Search** function or sorting by vessel type.
- Map names can be changed by clicking (**Edit**) or (**Delete**). Click to complete the edit.

Update the target pin location

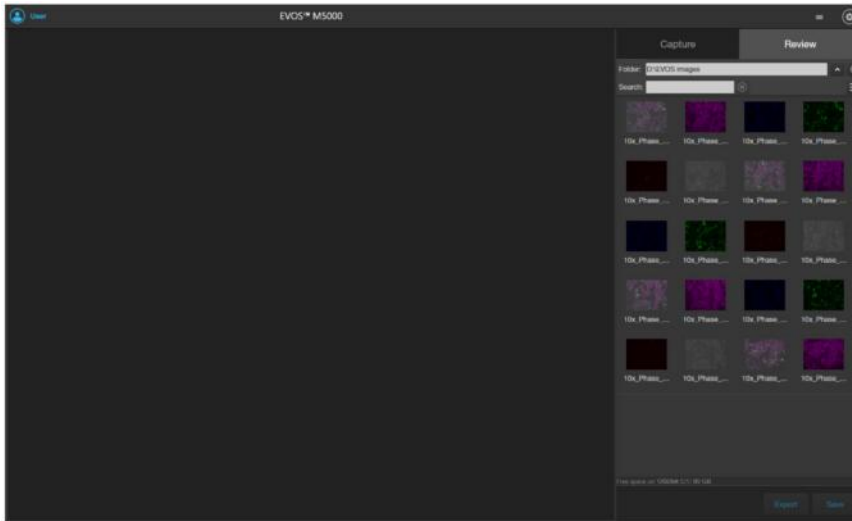
If the target has shifted in the field of view relative to the pin (for example, your cell of interest has migrated over time), you can update the pin's position to correct it. After clicking **Done** to dismiss the **Find Target** window and verifying that the target has shifted relative to the pin, do the following:

1. Re-center the target using the stage knobs in live observation mode (that is, with the light on).
2. Open **Find Target**.
3. Select the pin to correct. By default, the first pin is selected when opening the window, so you will likely need to re-select the pin to be adjusted.
4. Click **Update** within the Target $\Delta X/\Delta Y$ area to move the pin to the current stage location. A window will display asking for confirmation you are moving the correct pin. Click **Confirm**.

10

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 (“Review images” on page 86)
- Configure display settings (“Configure display settings” on page 88)
 - Adjust image display settings (“Adjust display settings for saved images” on page 88)
 - Display grid (“Display grid” on page 89)
 - Display scale bar (“Display scale bar” on page 89)
- View pixel intensity histogram (“View pixel intensity histogram” on page 89)
- Add measurements and annotations to saved images (“Add measurements and annotations to saved images” on page 90)
- Analyze cell culture using saved images (“Analyze cell culture using saved images” on page 90):
 - Perform Auto Count (Auto Count on page 91)
 - Perform Manual Count (Manual Count on page 91)
 - Measure confluence (Confluence on page 91)
 - Calculate transfection efficiency (Transfection Efficiency on page 91)
- Save analysis results (“Save analysis results” on page 92)
- Perform batch analysis of saved images (“Save analysis results” on page 92)

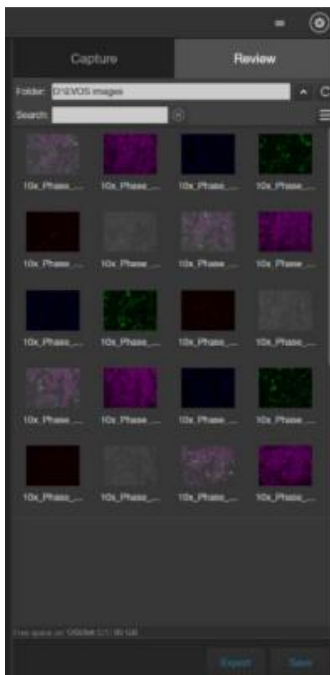
Note: For a detailed description of the **Review** tab controls, see Appendix B, “Graphical user interface (GUI)” (“Review tab” on page 126).

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, “System overview”.

Review images

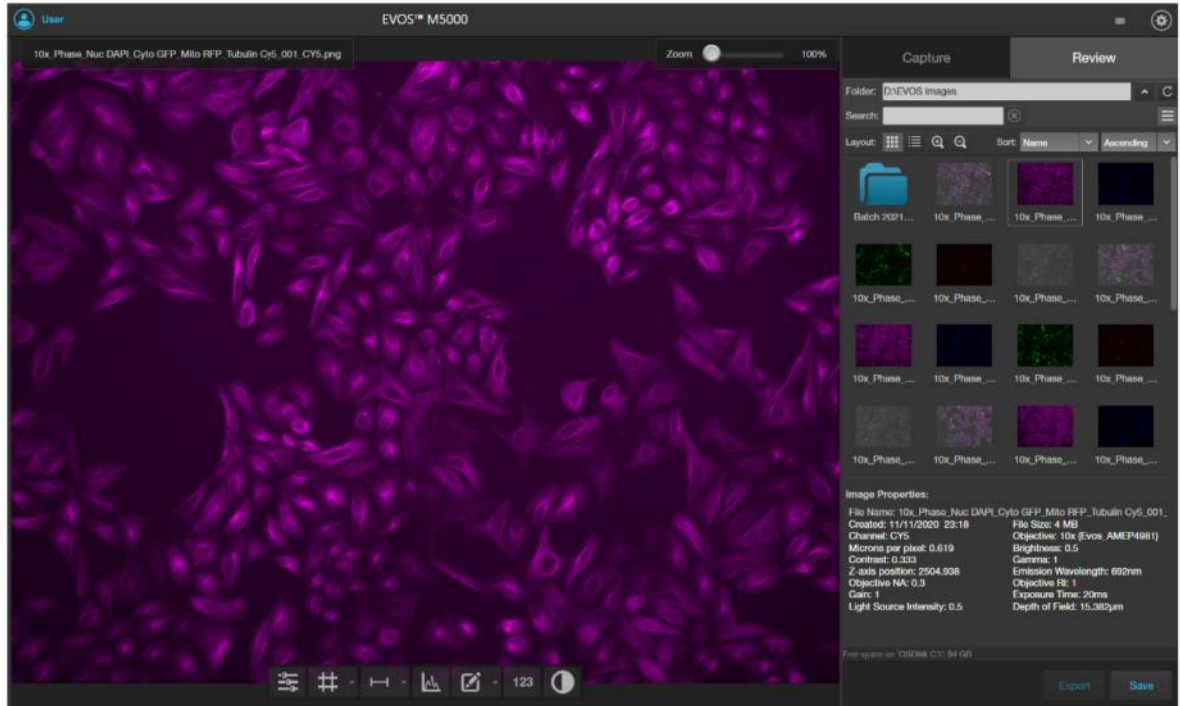
Review images

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 ☰ (**Display Settings**) to expand the **Display Settings** controls.
4. To display the folder/image preview in list format, click ≡ (**List view**).
5. To display the folder/image preview in grid format, click ☰ (**Grid view**).

6. Click the image to select. 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


Adjust 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  (**Image Display Settings**) to expand the controls for image display parameters (brightness, contrast, gamma). For detailed instructions, see “Adjust image display settings” on page 37.


Note: The controls for image display settings are available only for captured channels. In the example below, the controls are available only for the DAPI, GFP, and Tx Red channels.



Display grid

- Click  (**Grid**) to superimpose a grid over the Viewing area. For instructions on how to change the **Grid** settings, see “Display grid” on page 38.

Display scale bar

- Click  (**Scale Bar**) to superimpose a scale bar over the Viewing area. For instructions on how to change scale bar settings, see “Display scale bar” on page 39.

View pixel intensity histogram

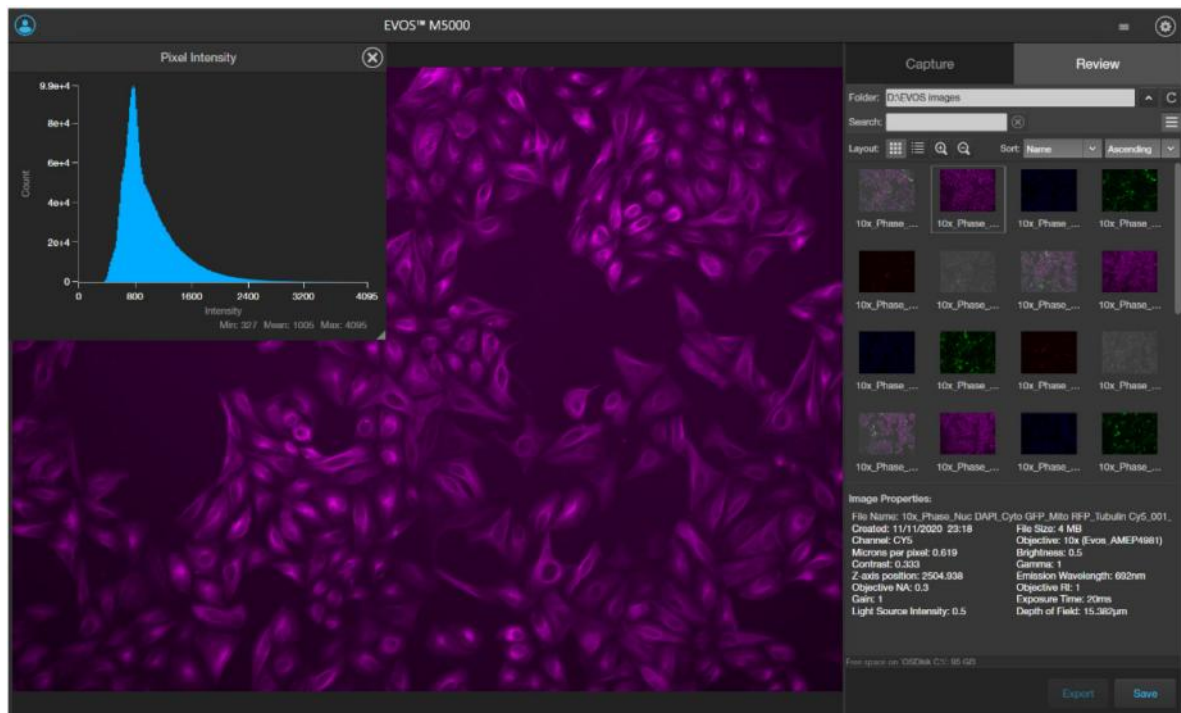
Display histogram

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




2. Click  (**Histogram**) to open the **Intensity Histogram** plot.

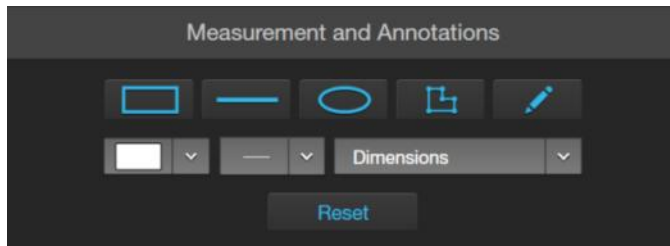
The Pixel Intensity histogram shows the Pixel count vs. Intensity data of the image displayed in the Viewing area as well as the minimum, mean, and maximum pixel intensities. See “Align channels” on page 40 for more information on the Histogram tool.



Note: The **Histogram** tool is available in both the **Capture** and the **Review** tabs and functions 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** at the bottom of the screen.
2. Click  (**Measurement and Annotations**) to open the measurement and annotations tools.



For instructions on how to add and show measurements and annotations to your images, see “Add measurements and annotations” on page 42.

Note: **Measurement and Annotations** tools are available in both the **Capture** and **Review** tabs and functions 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 **123 (Show Cell Count)** to display **Auto Count**, **Manual Count**, and **Cell Culture** options below the **Capture** tab.



IMPORTANT! For analysis, only use 16-bit image files (TIFF or PNG). The 16-bit images of individual channels contain the full dynamic range and metadata needed for quantitative analysis, whereas **Display** 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 only count objects in a single fluorescence channel (typically a nuclear stain channel).
For instructions on how to perform **Auto Count**, see “Count cells – auto count” on page 45.
- **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 “Count cells – manual count” on page 51.
- **Cell Culture:** Allows you to measure the confluence of your culture and calculate the transfection efficiency.
 - **Confluence:** Allows you to select up to 5 reference objects for the target (i.e., cells) and one background reference in your image, then automatically calculates the percentage confluence of your culture.
For instructions on how to measure the confluence of your culture, see “Count cells – manual count” on page 51.
 - **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 “Calculate transfection efficiency” on page 58.

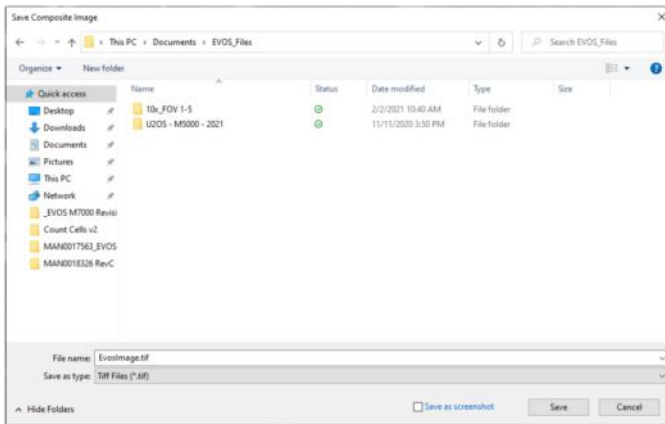
3. If desired, perform **Batch Analysis** to the remainder of the images in the image folder (“Save analysis results” on page 92).

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 the **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 lists the various options available for saving 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 | Not applicable. |
| 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 | Not applicable. |
| 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, % transfection efficiency, threshold, and other options as displayed in the Transfection Efficiency tool | Not applicable. |

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.

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.

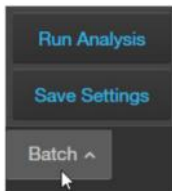
The **Batch Analysis** function is available as a **Save Settings-Run Analysis** split-button on the **Auto Count** and **Cell Culture** panels on the **Review** tab. It is not available for **Manual Count**.

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 image files (TIFF or PNG) that contain the full dynamic range and metadata needed for quantitative analysis.

Save current analysis settings

1. When finished with **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

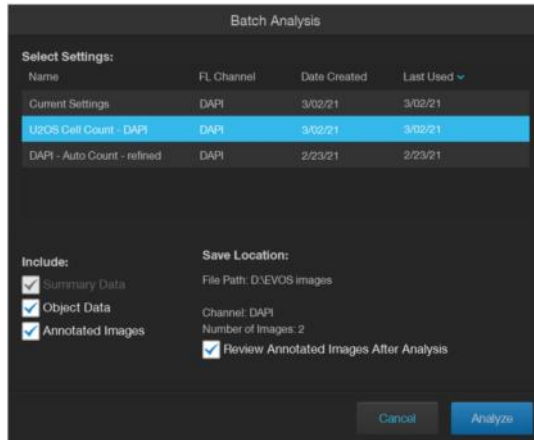
1. Navigate to the folder that contains the images you wish to analyze using the **Batch Analysis** tool, then click on a representative image to open it.
2. Perform **Auto Count**, **Confluence** measurement, or **Transfection Efficiency** calculation on the open image as described previously.
3. To 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 on page 95).

4. Select the **Settings** to use for **Batch Analysis**.

- To use the current analysis settings (that is, analysis parameters that you have used in Step 2 on page 94), 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_.






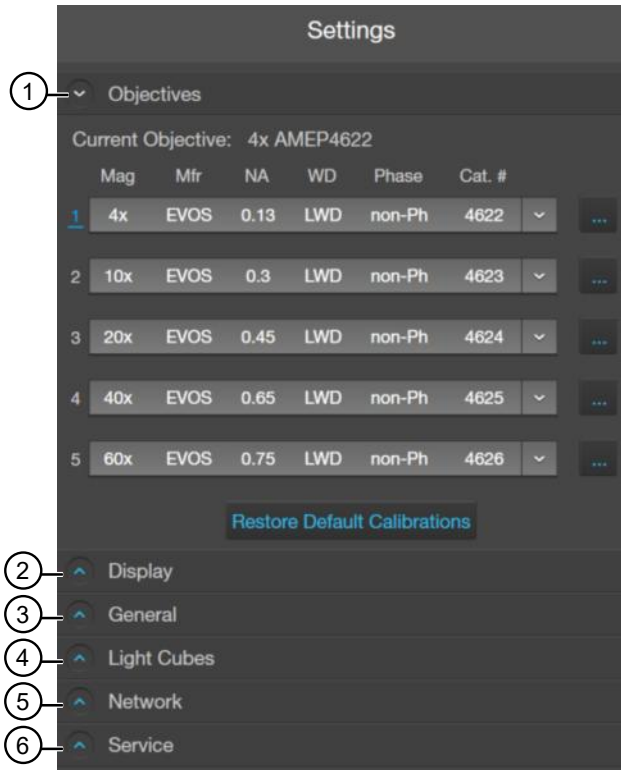
Configure instrument settings

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  (**Settings**).
- To expand or collapse any of the controls, click the corresponding arrow ( or ).



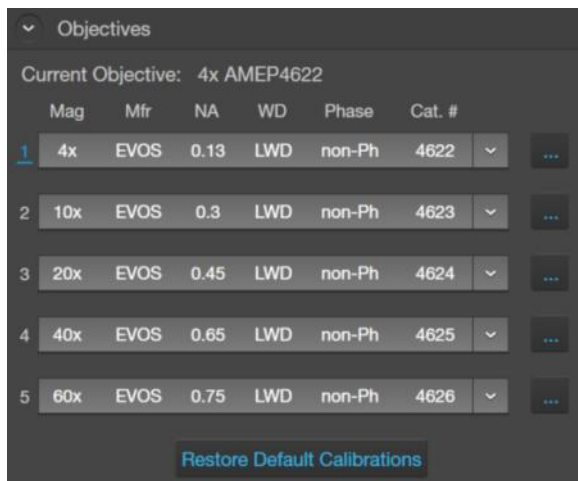
- ① **Objectives:** Set up and calibrate objectives and assign objective profiles (“Adjust objective settings” on page 98).
- ② **Display:** Use White Balance Calibration and Hot Pixel Correction to adjust the white balance for the color camera (“Calibrate white balance” on page 100) and set saturated pixel options (“Set saturated pixel options” on page 101).
- ③ **General:** Set general instrument options for focus wheel action, Capture All Channels, and Align Channels options (“General settings” on page 101).
- ④ **Light Cubes:** Add or remove EVOS™ LED light cubes and assign pseudocolors for installed light cubes (“Change EVOS™ LED light cubes” on page 109).
- ⑤ **Network:** Configure network settings (“Configure network settings” on page 102).
- ⑥ **Service™:** Copy error logs, set date and time, and update software and firmware from the cloud or the USB flash drive (“Service” on page 105).

Adjust objective settings

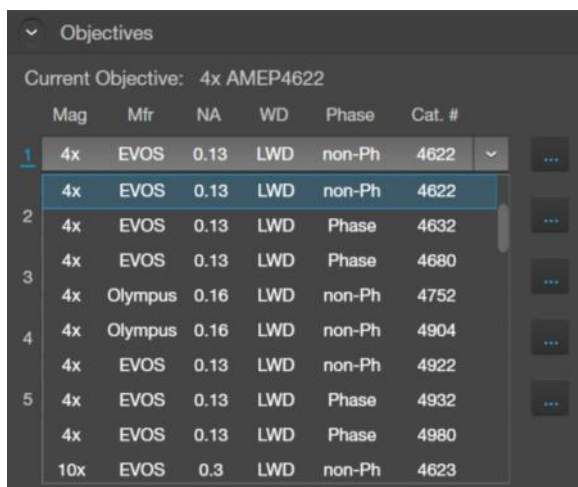
Assign objectives

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” on page 110.

1. Go to the **Settings** ▶ **Objectives** tab.



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

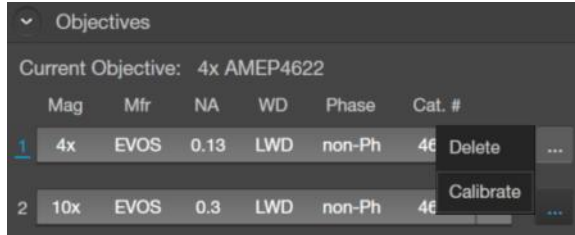


3. Click **Done**.

IMPORTANT! For best results, calibrate the newly installed objective before using it in your experiments (see “Calibrate objective magnification” on page 112).

Calibrate objective magnification

1. Go to the **Settings ▶ Objectives**, click **⋮ (Actions)** next to the newly installed objective, then select **Calibrate** to launch the Objective Calibration tool.

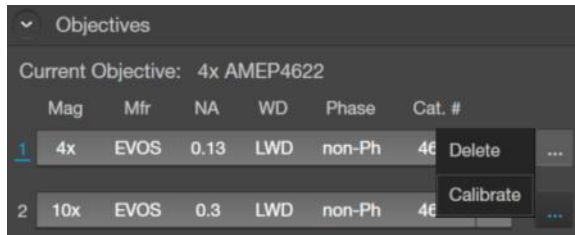


2. Mount the EVOS™ Calibration Slide in the 2-slide vessel holder (Cat. No. [AMEP-VH001](#)) and select the objective you want to calibrate by rotating the objective turret to the active position.
3. Click **⋮ (Actions)** next to the active objective to select **Calibrate**.
4. Follow the onscreen instructions to calibrate the objective magnification for the selected objective. For more information, see “Calibrate the objectives” on page 112.

Delete objectives

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

- Go to **Settings ▶ Objectives**, click **⋮ (Actions)** next to the turret position from which you have removed the objective, then select **Delete**.

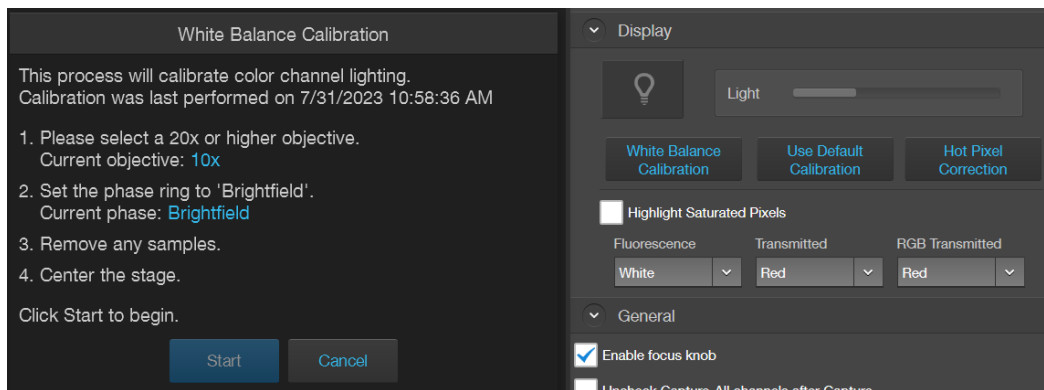


Calibrate white balance

Calibrate white balance

Calibrate White Balance calibrates color channel lighting.

1. Go to **Settings** ▶ **Display** tab, then click **White Balance Calibration**.



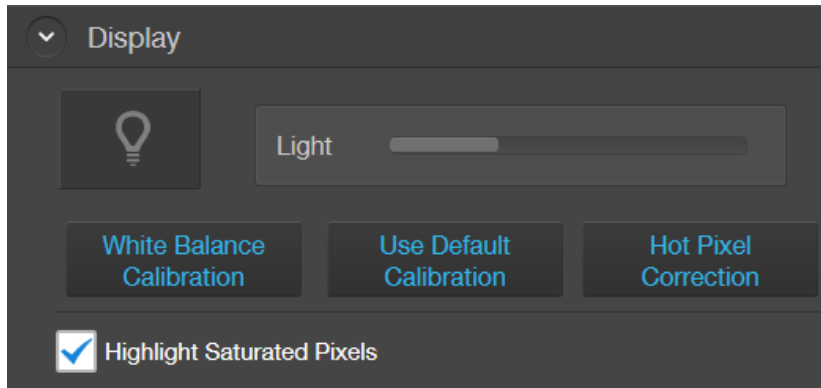
2. Follow the screen instructions. Select a 20X or higher objective, then set the phase ring to **Brightfield** using the Phase ring selector (“Rear view” on page 14).
3. Remove any samples from the X-Y stage, then center the stage using the stage positioning knobs (“Rear view” on page 14).
4. Click **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.

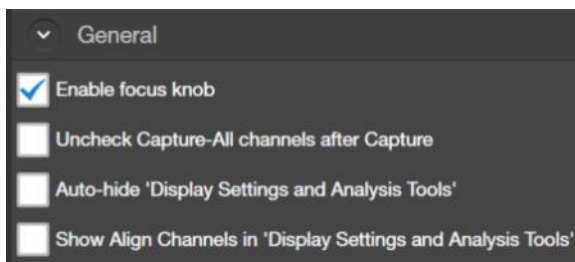
1. Go to **Settings** ▶ **Display**, then click the **Highlight Saturated Pixels** checkbox.



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**.

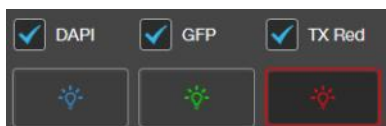
General settings

General settings options



Define 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” on page 34).



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 Tools** 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 Tools** 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 Tools** 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 Tools** 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.

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, Connect-based platform, to store your image files and analyze them with the EVOS™ Image Analysis application.

Connect to a Wi-Fi network

1. Click **Settings**.
2. Click **Network**.



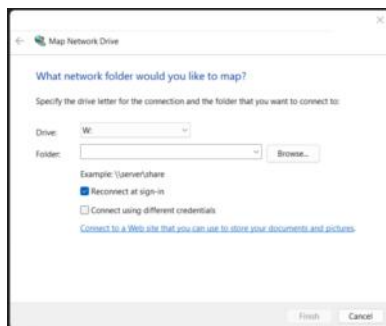
3. Click **Show Wi-Fi Networks**, then select the network you want to join.



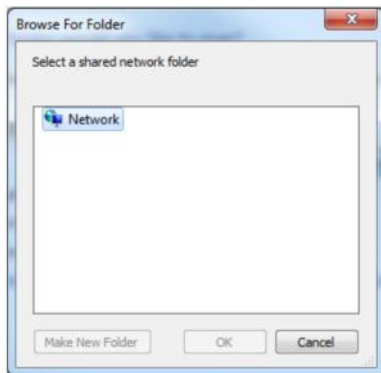
4. Click **Close**.

Map network drive

1. Go to **Settings > Network**, then click **Map Network Drive**.



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 to reconnect to the selected drive and folder when you turn on the instrument, click **Reconnect at logon**.
6. If you want to reconnect as a different user, click **Connect using different credentials**.
7. To connect to a website for storing your documents and pictures, click the hyperlink, then navigate to the desired website.
8. After you have mapped the desired network drive and folder or connected to the website, click **Finish**.

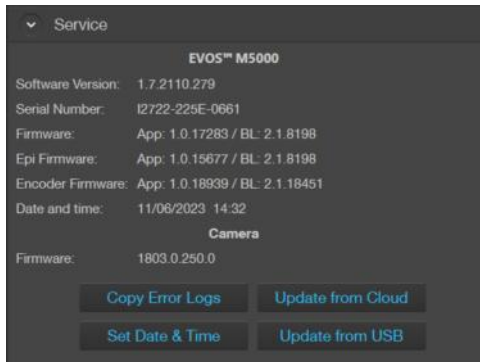
Note: When **Reconnect at logon** is checked and a successful connection is established, the system will attempt a reconnection to any previously connected networks or mapped drives every time the instrument is turned on until you manually disconnect from the connected networks or mapped drives. If no connection can be successfully established to the previously mapped drive during start up, the instrument displays the **Reconnect to Mapped Network Drive** dialog.



Service

The **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.

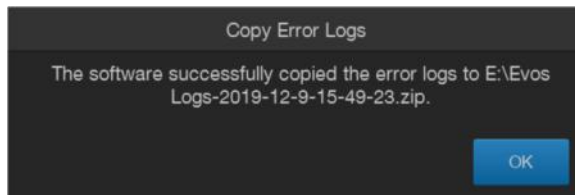
To view the **Service** tab, click **⚙️ (Settings)**, then select **Service**.



Copy error logs

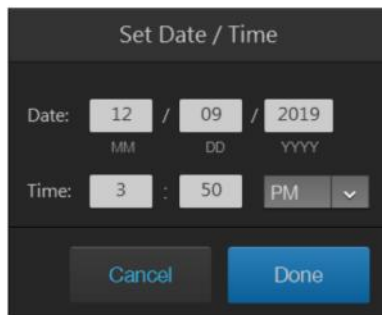
- On the **Service** tab, click **Copy Error Logs**.

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 **24HR** format or **AM** or **PM** for a 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 (“Connect to the Thermo Fisher™ Connect Platform” on page 23).
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 the 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 of 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**.
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 the 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.

12

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 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 in “Remove shipping restraints” on page 18 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 (H₂O₂).

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.

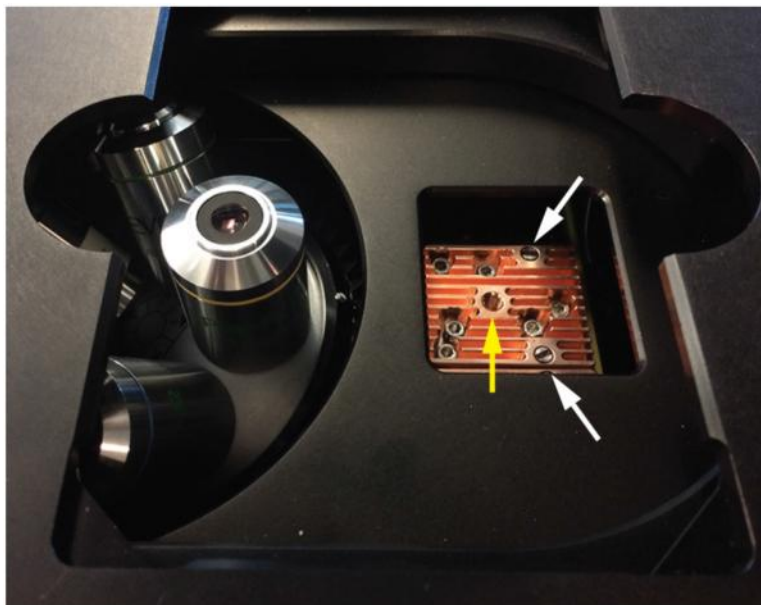
For a complete list of available light cubes and to inquire about custom light cubes, go to thermofisher.com/evos or contact Technical Support.



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 **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 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** ▶ **Objectives** tab, then find the objective in the **Objectives** list that matches the newly installed objective.

Objectives

Current Objective: 4x AMEP4622

| | Mag | Mfr | NA | WD | Phase | Cat. # | |
|---|------|---------|------|-----|--------|--------|-----|
| 1 | 4x | EVOS | 0.13 | LWD | non-Ph | 4622 | ... |
| 2 | 10x | EVOS | 0.3 | LWD | non-Ph | 4623 | ... |
| 3 | 20x | EVOS | 0.45 | LWD | non-Ph | 4624 | ... |
| 4 | 40x | EVOS | 0.65 | LWD | non-Ph | 4625 | ... |
| 5 | 60x | Olympus | 0.9 | CC | non-Ph | 4845 | ... |
| | 60x | Olympus | 1.42 | CC | non-Ph | 4694 | |
| | 60x | Olympus | 0.9 | CC | non-Ph | 4845 | |
| | 60x | Olympus | 0.16 | CC | non-Ph | 4910 | |
| | 60x | EVOS | 0.75 | LWD | non-Ph | 4926 | |
| | 100x | EVOS | 1.28 | CC | non-Ph | 4696 | |
| | 100x | Olympus | 1.4 | CC | non-Ph | 4733 | |
| | 100x | Olympus | 1.45 | CC | non-Ph | 4913 | |
| | 100x | EVOS | 1.25 | CC | non-Ph | OP100 | |

Note: Calibrate the newly installed objective before use (“Calibrate the objectives” on page 112).

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.

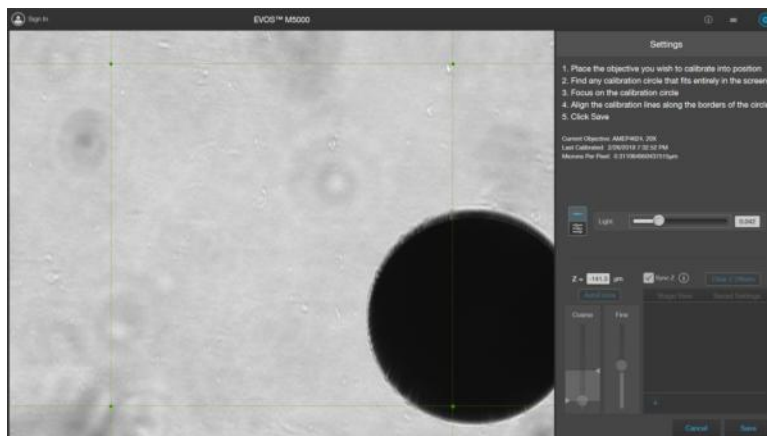
Note: The pre-installed objectives supplied with the EVOS™ M5000 Imaging System have been pre-calibrated. You do not need to calibrate them again unless they are reinstalled after removal from the instrument.

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 approximately 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 slide vessel holder (you can use either of the 2 slots).
2. Select the objective to calibrate using the Objective selection wheel (“Rear view” on page 14).
3. Go to **Settings** ▶ **Objectives**, click **⋮ (Action)** for the newly installed objective, then select **Calibrate** to launch the Objective Calibration tool.

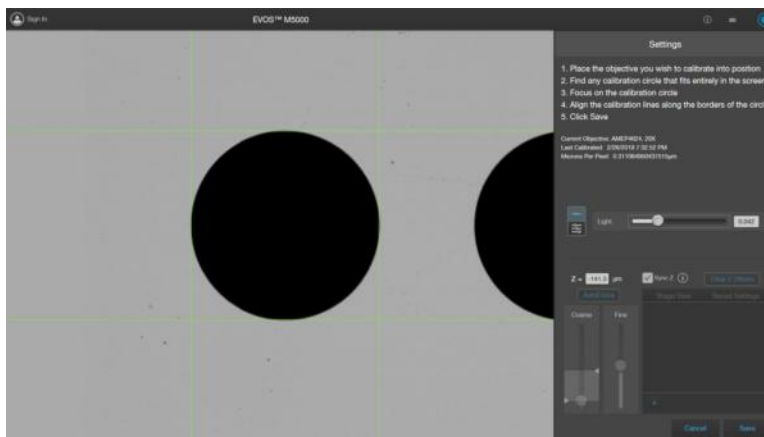


4. Find a calibration circle that fits entirely in the field of view.

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**.



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.

Image quality issues

| Observation | Recommended action |
|--|---|
| Transmitted light 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” (“Objective lens care” on page 108) to clean the objectives. |
| Uneven focus across screen | <ul style="list-style-type: none">• 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 | <ul style="list-style-type: none">• Click a light 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 | <ul style="list-style-type: none">• 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. |

Stage view issues

| Observation | Recommended actions |
|--|--|
| Cannot locate pinhole for aligning the stage | Use a low-power objective (10x or less). |
| Cannot find original pin locations on the vessel | Note the orientation of your slide or vessel in the vessel holder when creating pins. Use the same orientation when re-locating pins. |
| | Use a low-power objective (10x or less). |
| | For microtiter plates, confirm plate is oriented with well A1 in the upper left corner of the vessel holder. |
| | Use Stage View-compliant vessel holders. These vessel holders have screws in the corners and a spring clip to precisely align the vessel holder on the stage and make re-locating pins easier. Additionally, the vessel holders have index marks that align with the stagetop marks to confirm the correct vessel holder orientation in case of removal and re-installation. |
| Pin map has substantially shifted | Check the vessel position in the vessel holder to ensure it is placed tightly in the reference corner. |
| | Vessel was placed incorrectly before imaging and marking pins. Confirm vessel and vessel holder were installed correctly before initial imaging and pin placement. |
| | Confirm the vessel holder thumbscrews are secured finger-tight to ensure accurate alignment. |

Software interface issues

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

| Observations | Recommended actions |
|--|--|
| Image does not respond to changes in focus or stage position | Click Light 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. |

(continued)

| Observations | Recommended actions |
|------------------------------|--|
| Unable to connect to network | <ul style="list-style-type: none"> • 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. |

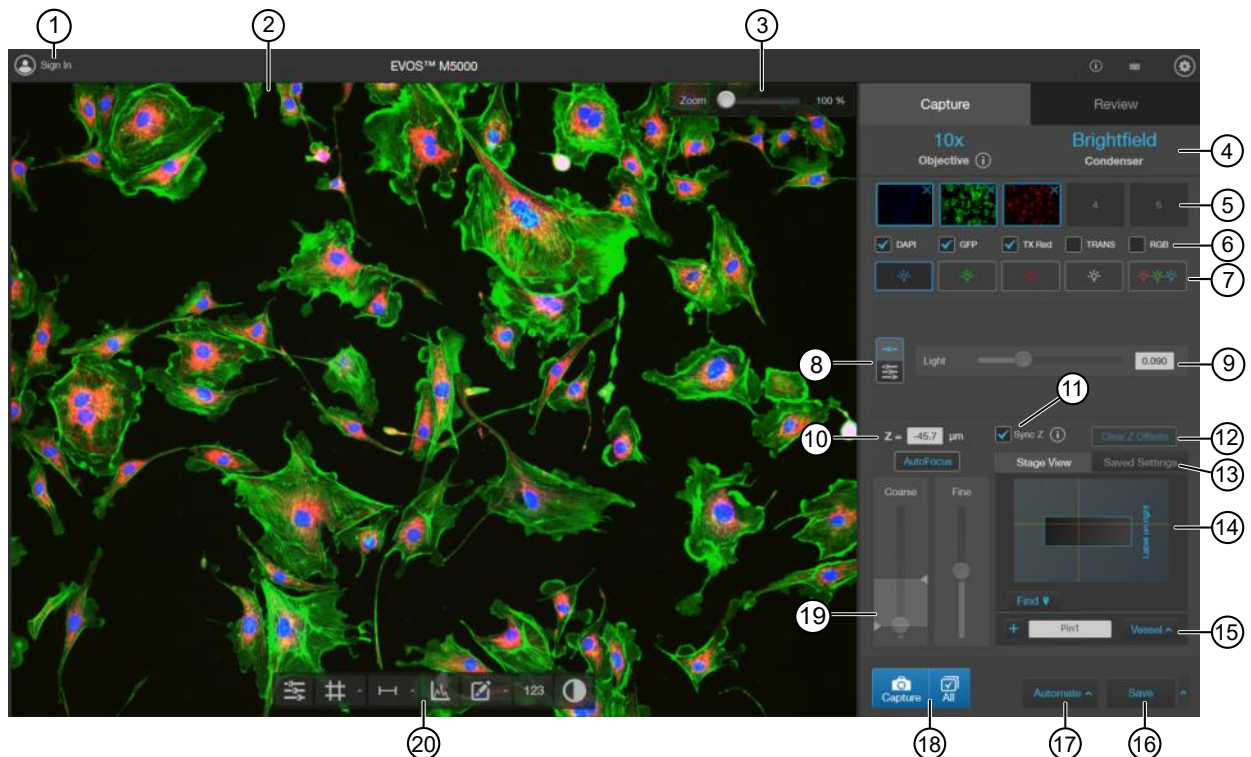
Mechanical issues


| Observations | Recommended actions |
|--|--|
| 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). |



Graphical user interface (GUI)

Capture tab



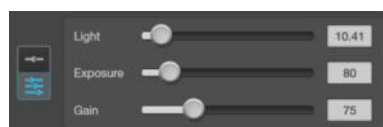
1. **Sign In/User:** Allows you to sign in to and sign out of your Connect account.
2. **Viewing area:** Displays live or captured images of the sample.
3. **Zoom slider:** Zooms in and out of the Viewing area. The zoom range is 100% to 1000%.
4. **Active objective and phase ring:** Displays the active objective and phase ring information.
 - If the selected objective and phase ring match, the selected phase ring is depicted in blue font. If the objective and phase ring do not match, the phase ring is depicted in orange font.
 - To learn more about the current objective, click .
5. **Thumbnails:** Display the most recently captured image stored in the memory buffer for the channel.
6. **Capture channels:** Checkmarked channels to be captured using the **All** button.

7. **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 view in the **Live** mode. In **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 highlighted color around the channel button.
8. **Brightness mode:** Toggles between Simple and Advanced modes for brightness controls.
 - Simple mode allows you to control light intensity as a single **Light** parameter. The system will automatically determine exposure and gain in order to minimize photobleaching.
 - Advanced mode allows you to adjust **Light** (that is, LED intensity), **Exposure**, and **Gain** parameters individually.
9. **Brightness controls:** Adjust the brightness for the selected channel using the simple or advanced slider controls. The **Exposure** and **Gain** advanced sliders can help to optimize image details.

Simple slider control for **Light** adjustment.



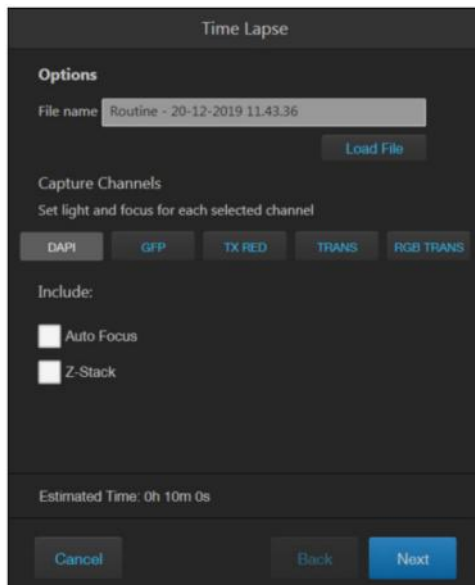
Advanced slider controls for **Light**, **Exposure**, and **Gain** adjustments.



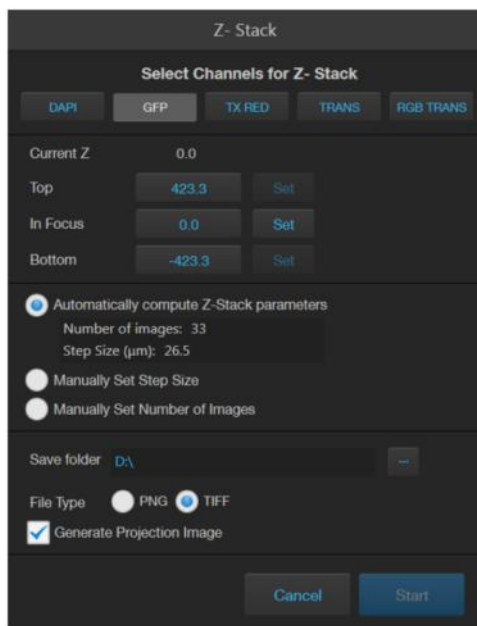
10. **Z = (μm):** Displays the current focus position in μm along the Z-axis as controlled by the focus knobs or focus sliders in the user interface. You can also change the Z-axis focus position by entering the desired value into the **Z =** text box.
11. **Sync Z:** Allows you to sync the Z-axis offsets, which specify the optimal focus position for each channel relative to the focus position in other channels.
 - When synced, movement of the Z-position in one channel changes the Z-position in all channels, preserving their relative Z-positions.
 - When unsynced, adjusting the Z-position only affects the currently selected channel.
12. **Clear Z Offsets:** Clicking **Clear Z Offsets** sets all of the channels to the same focal plane value as the selected active channel. For example, if GFP is selected as the active channel and its focal plane is -712, then clicking **Clear Z Offsets** will set the other 3 channels with focal planes of -745, -694, and -715 to be -712.
13. **Saved Settings:** Allows you to save Z-axis focus positions and brightness settings for future use. Click **+** to save the current settings.
14. **Stage View:** Visual tool that allows precise marking of points of interest on various vessel types, as well as the relocation of these points at a later time.
15. **Vessel type for Stage View:** Allows selection of the vessel type being viewed. Vessel types include, slides, petri dishes, and plates from 6 to 384 wells.
16. **Save (Quick Save, Auto Save):** Saves the currently displayed image (all of the currently visible channels) to the hard drive, USB drive, or network drive. After saving an image, the filename is displayed in the upper-left corner of the screen.
17. **Automate:** Allows you to display the controls for the **Time Lapse** and **Z-Stack** tools, and the optional 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.

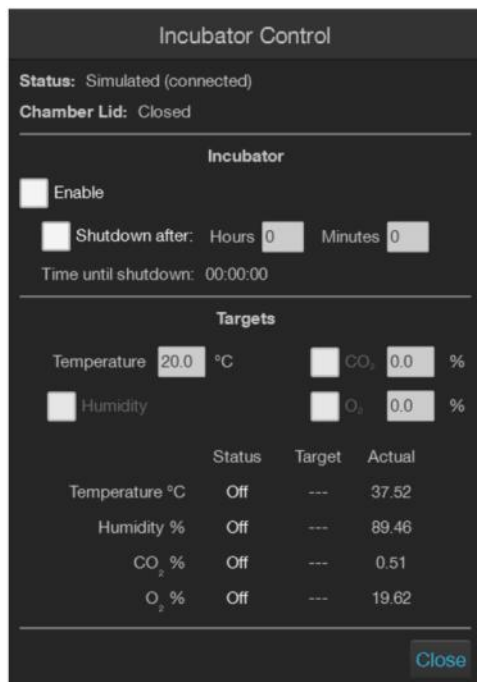


Z-Stack: Opens the Z-Stack tool, which allows you to capture multiple images along the Z-axis based on your specifications. You can use the images captured with the Z-Stack tool to generate a Z-Stack Projection.



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

Note: The Incubator Control window only appears if an EVOS™ Onstage Incubator is connected to the EVOS™ M5000 instrument.



18. **Capture** and **All:** Captures one channel (**Capture**) or multiple channels (**All**) using the current capture settings and stores the image in the image cache of the channel in which it was captured.

19. **Focus controls:**

- **Autofocus:** Runs autofocus in the selected channel.

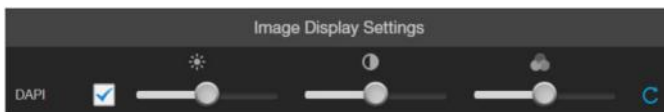
Note: Do NOT use autofocus with oil-immersion objectives, as this can create air bubbles in the oil or cause the objective to collide with the sample.


- **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 (when active) triangles on the **Coarse** focus slider), you can limit autofocus to the selected region along the Z-axis.

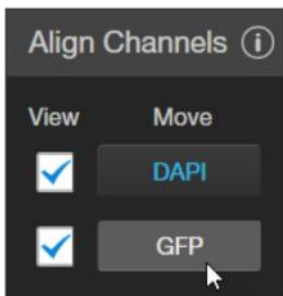
20. **Display Settings and Analysis Tools:** Allow you to change image display settings in the Viewing area, and analyze and annotate captured images.




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

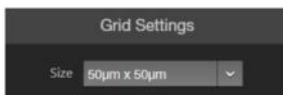



 **(Align Channels):** Allows you to correct pixel shifts that can occasionally appear when performing multichannel fluorescence imaging at higher magnifications. The **Align Channels** tool only displays the controls for the channels in which the selected multichannel fluorescent image has been captured.




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.

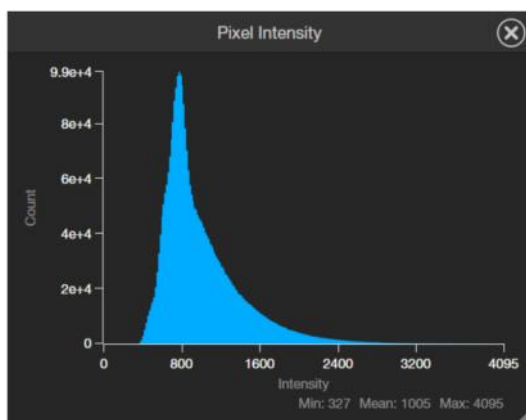
 **(Display Grid/Grid Settings):** **Display Grid** button switches the grid display in the Viewing area on and off. **Grid Settings** allows you to set the grid size.




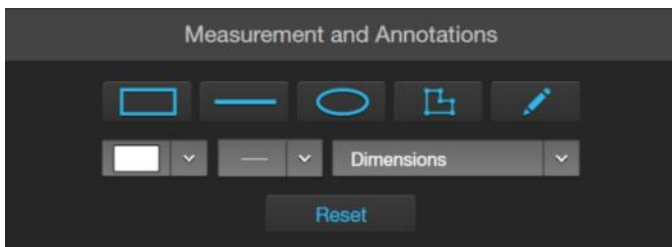
 **(Display Scale Bar/Scale Bar Settings):** **Display Scale Bar** button switches the display of the scale bar in the Viewing area on and off. **Scale Bar Settings** allows you to select scale bar color and to display or hide end bars.



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

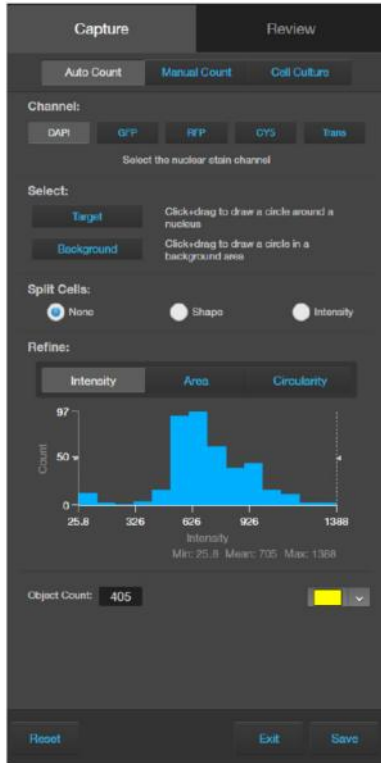


 **Measurements and Annotations:** Allows you to draw regions of interest (rectangle, ellipse, polygon, line, or free-form) on the captured image and measure dimensions, area, or perimeter of the drawn region.



123 (Show Cell Count): 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.

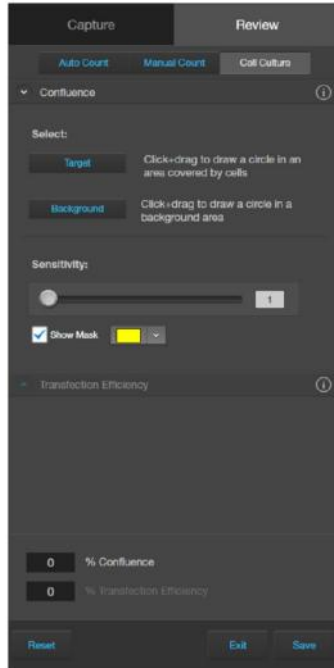
- **Auto Count:** Allows you to define auto count parameters by selecting representative target objects, then count the objects by intensity, area, and circularity. See “Auto count controls” on page 128 for **Auto Count** controls.



- **Manual Count:** Allows you to manually mark items onscreen using up to 6 separate labels and keep a running tally of the counts with percentages for each label. See “Manual count controls” on page 129 for **Manual Count** controls.

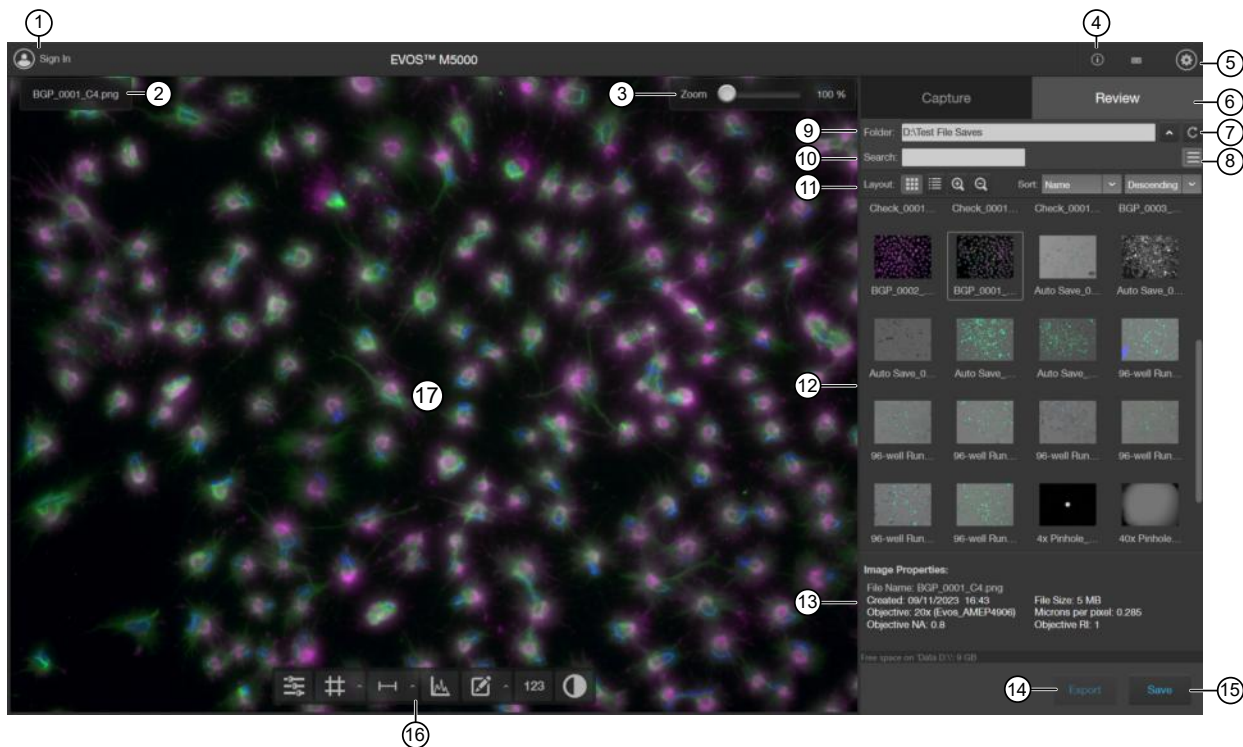


- **Cell Culture:** Allows you to measure the confluence of your culture and calculate the transfection efficiency. See “Cell culture – confluence controls” on page 130 for **Confluence** controls and “Cell culture – transfection efficiency controls” on page 131 for **Transfection Efficiency** controls.



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

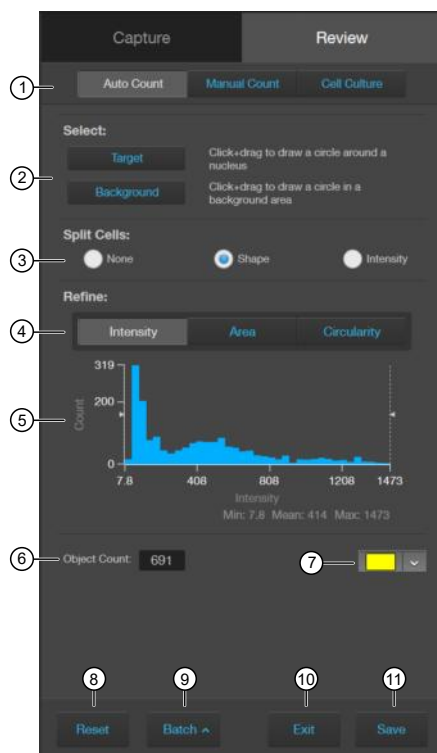
Review tab



1. **Sign In/User:** Allows you to sign in to and sign out of your Connect account.
2. **Image file name:** File name of the image displayed in the Viewing area.
3. **Zoom slider:** Zooms in and out of the image. The zoom range is 100% to 1000%.
4. **Keyboard Shortcuts:** Instrument actions triggered by keyboard combinations instead of mouse actions.
5. **Settings:** Opens the **Settings** tab. See “Settings” on page 132 for more information.
6. **Capture and Review tabs:** Allows you to toggle between the **Capture** and **Review** tabs.
7. **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.
8. **Display Settings:** Displays or hides the **Layout** controls.
9. **Folder:** Displays the location of the current folder or image.
10. **Search:** Allows you to search images by file name in the selected folder.
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. **Export:** Allows you to export the currently selected folder to a storage device.
15. **Save:** Saves the currently opened image.
16. **Display settings and analysis tools:** Allow you to change image display settings in the Viewing area, and analyze and annotate captured images. See “Display settings and analysis tools” on page 36 for more information.
17. **Viewing area:** Displays the selected 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. **Select Target and Background:** Allows you to select representative target objects and background areas for **Auto Count**.
3. **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.
4. **Refine:** Selects intensity, area, or circularity by which to refine **Auto Count** results using the **Count Histogram**.
5. **Count Histogram:** Allows you to set pixel intensity, area, or circularity thresholds to refine the **Auto Count** results.
6. **Object Count:** Displays the object count based on the **Auto Count** parameters.
7. **Object color:** Selects the color by which the counted objects are identified.
8. **Reset:** Resets the count to 0 and clears the selected targets and background areas.
9. **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 “Batch analysis” on page 94 for more information).

10. **Exit:** Exits the **Auto Count** tool and displays the **Review** tab.
11. **Save:** Saves the analysis results as an image in the selected file format (see “Save analysis results” on page 92 for more information).

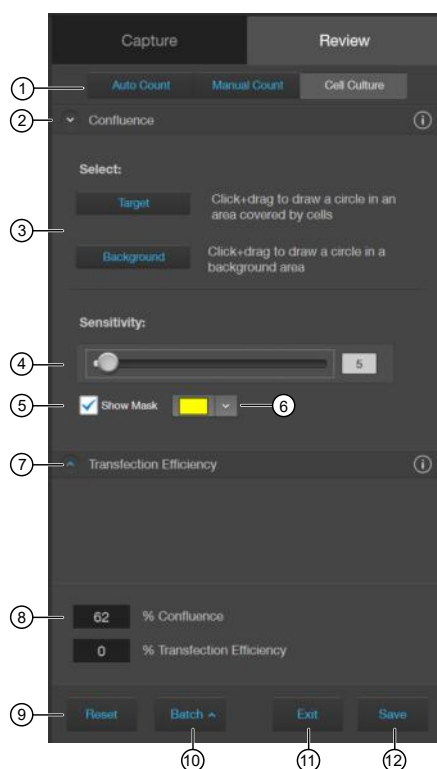
Manual count controls



1. **Analysis tools:** Allows you to toggle between **Auto Count**, **Manual Count**, or **Cell Culture** (Confluence and Transfection Efficiency) tools for image analysis.
2. **Object #** and **Object Name:** 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 “Count cells – manual count” on page 51 for more information. **Object Name** allows you to assign a name for the **Object #**.
3. **% and Count:** Displays the label count and its percentage of the total object count (total count of all labels).
4. **Delete:** Selects a label for deletion.
5. **Trash:** Deletes the tags for the label and resets the label count to 0.
6. **Total Count:** Displays the total count of objects tagged with all labels.
7. **Reset:** Resets all label counts and the total count to 0 and clears the Viewing area of all tags.

8. **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 “Batch analysis” on page 94 for more information).
9. **Exit:** Exits the **Manual Count** tool and displays the **Review** tab.
10. **Save:** Saves the analysis results as an image in the selected file format (see “Save analysis results” on page 92 for more information).

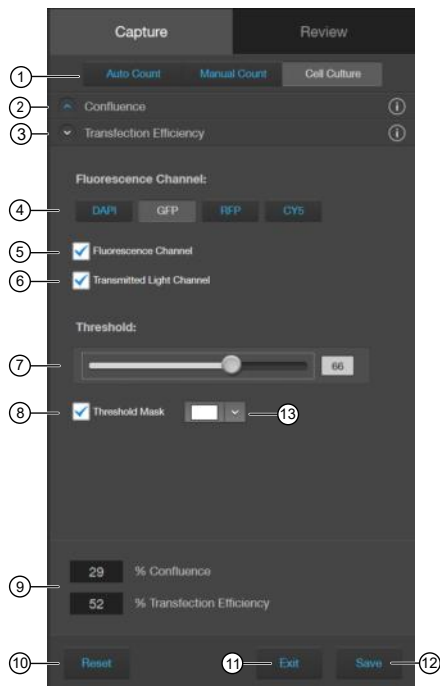
Cell culture – confluence controls



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 “Batch analysis” on page 94).
11. **Exit:** Exits the **Cell Culture** tool and displays the **Review** tab.
12. **Save:** Saves the analysis results as an image in the selected file format (see “Save analysis results” on page 92).

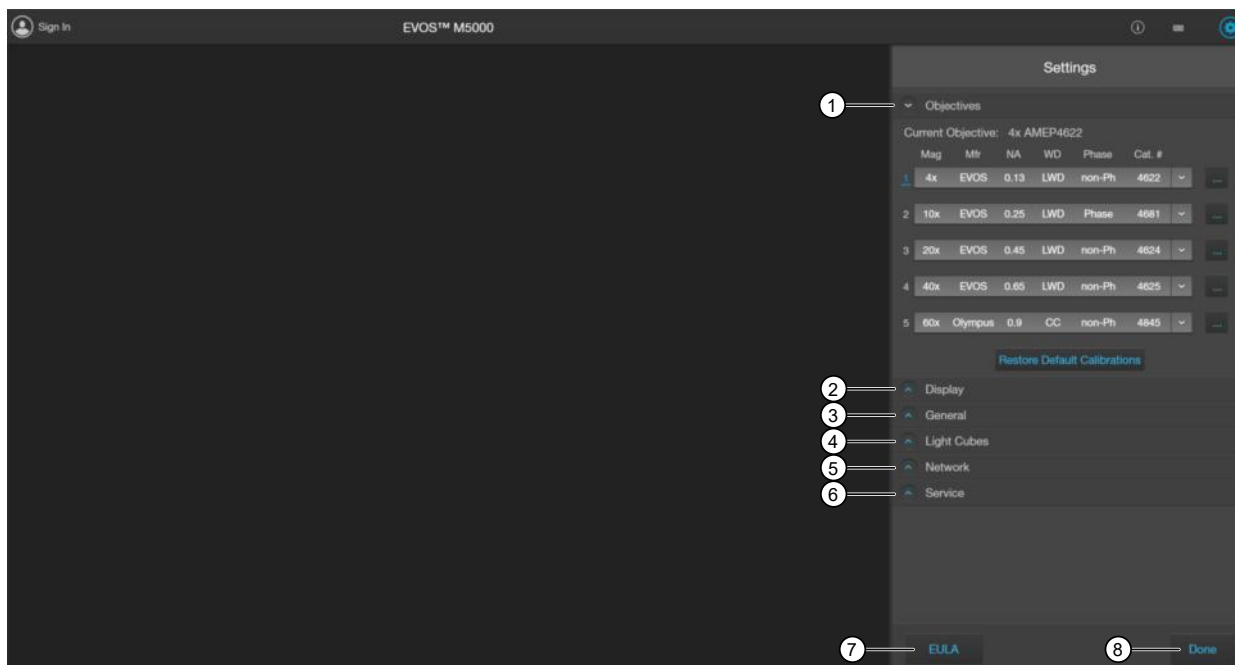
Cell culture – transfection efficiency controls



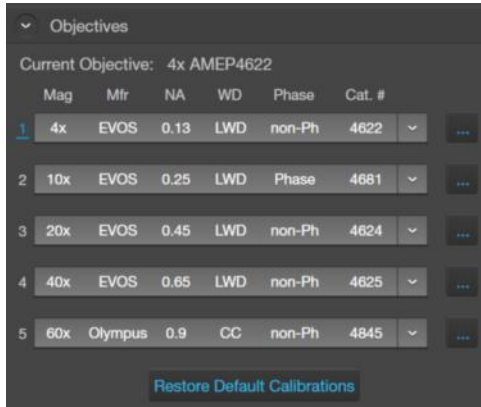
1. **Analysis tools:** Allows you to toggle between **Auto Count**, **Manual Count**, or **Cell Culture** (Confluence and Transfection Efficiency) tools for image analysis.
2. **Confluence:** Expands or hides the controls for the **Confluence** tool.
3. **Transfection Efficiency:** Expands or hides the controls for the **Transfection Efficiency** tool.
4. **Fluorescence Channel selection:** Selects the fluorescence channel for transfection efficiency calculation.

5. **Fluorescence Channel:** Toggles the display of the fluorescence channel.
6. **Transmitted Light Channel:** Toggles the display of the transmitted light.
7. **Threshold:** Adjusts the fluorescence threshold value. Only the cells that express above the set threshold are used in the tranfection efficiency calculation.
8. **Threshold Mask:** Indicates the areas above the set threshold value and are included in the transfection efficiency calculation.
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. **Exit:** Exits the **Cell Culture** tool and displays the **Review** tab.
12. **Save:** Saves the analysis results as an image in the selected file format (see “Save analysis results” on page 92).
13. **Mask Color:** Selects the threshold mask color.

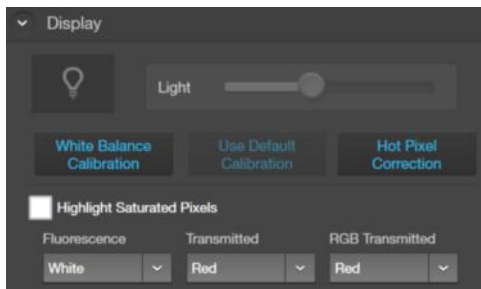
Settings



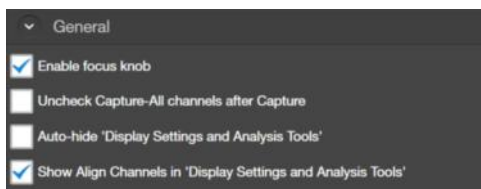
1. **Objectives:** Allows you to assign and unassign objectives on the objective turret, and to calibrate objective magnification.



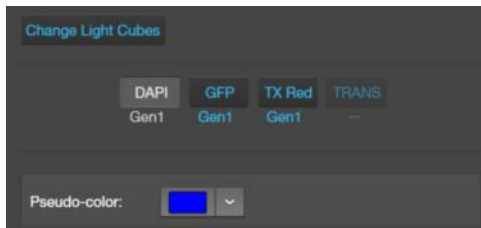
2. **Display:** Allows you to calibrate color channel lighting (**White Balance Calibration**) and to assign saturated pixel colors in the Fluorescence, Transmitted, and RGB Transmitted channels.



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.



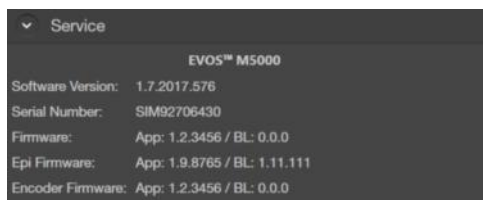
4. **Light Cubes:** Allows you to add or remove EVOS™ LED light cubes from the instrument and to assign pseudocolors to specific channels.



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, Connect-based platform, or using a USB flash drive, and to copy the error logs.



7. **Done:** Closes the **Settings** tabs and returns to the **Capture** tab.





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): 4-position chamber for 4 fluorescence cubes plus brightfield. Wide selection of standard and specialty light cubes (see “LED light cubes” on page 136).

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 one clear aperture and 3 phase rings.

Stage: Manual X-Y position-sensing stage. Travel range of 120 mm × 80 mm, drop-in inserts to receive vessel holders and lock down holders to fix the sample in place.

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 pixels, 3.2 Megapixels) with 3.45 μm pixels.



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

Output ports: 4-pin power input port (12 VDC, 5 A), 2 USB 3.0, 2 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 uses an adjustable-intensity LED light source provided by the proprietary, user-interchangeable LED light cube (see below). The LED light source is as close as possible to the objective and the number of optical elements is minimized. High-intensity illumination over a short light path increases the efficiency of fluorophore excitation, providing better detection of weak fluorescent signals.

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

LED light cubes

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

The table below lists some of the common fluorescence 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.

| 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 |



(continued)

| Light cube | Dye |
|-----------------------|--|
| Cy5™ | Cy5™, Alexa Fluor™ 647, Alexa Fluor™ 660, DRAQ5™ |
| Cy™5.5 | Cy™5.5, Alexa Fluor™ 660, Alexa Fluor™ 680, Alexa Fluor™ 700 |
| Cy7™ | Cy7™, IRDye™ 800CW |
| Specialty light cubes | Dye |
| CFP-YFP em | CFP/YFP (for FRET applications) |
| AO | Acridine orange + RNA, simultaneous green/red with FL color |
| White | Reflected light applications |



EVOS™ image analysis

EVOS™ image analysis

EVOS™ Image Analysis is an easy to use online 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.

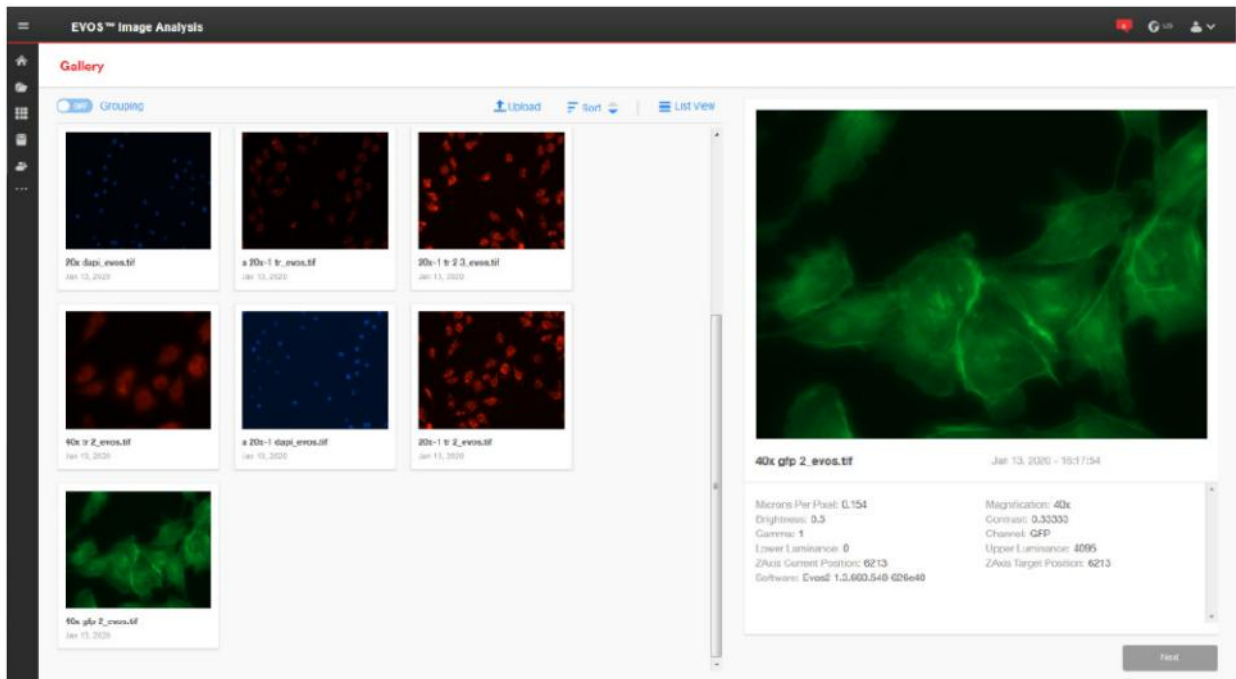
The EVOS™ Image Analysis application is available for free on the Connect platform. However, you need a current Connect account to sign in. To create your Connect account online, go to thermofisher.com/connect.

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 (that is, perform **Auto Count** and **Manual Count**), select the desired image in the **Gallery**, then click **Next** to open the **Edit Image** screen (“Edit image” on page 139).

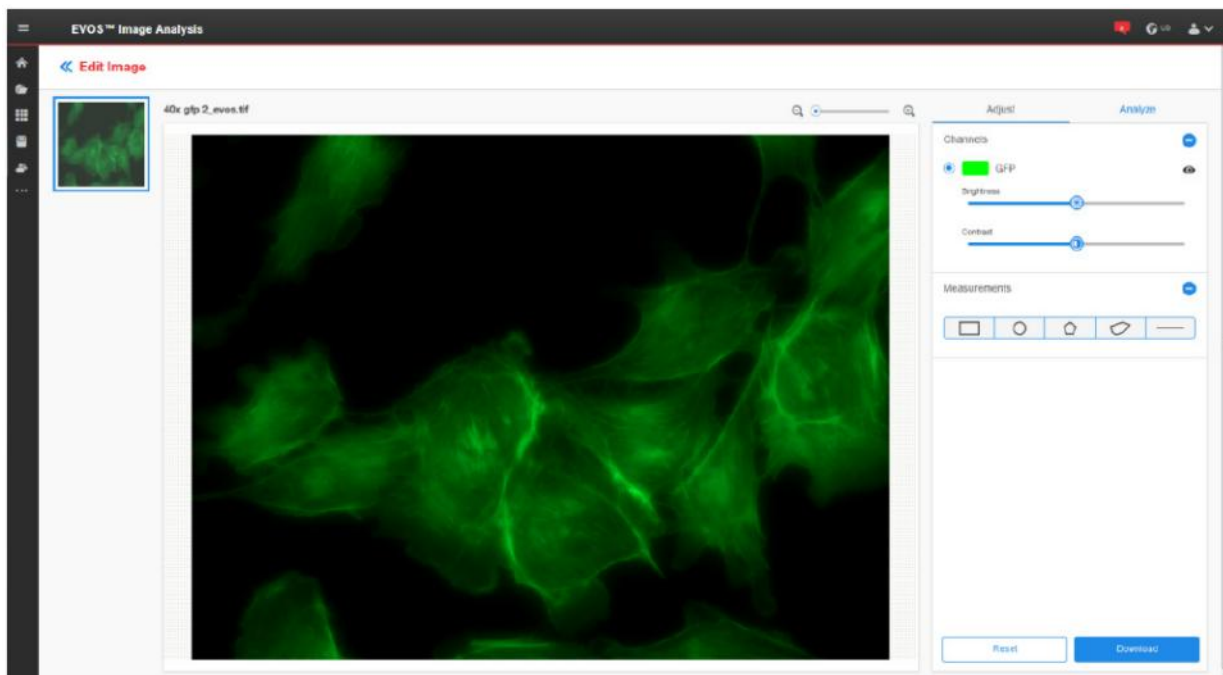


Edit image

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

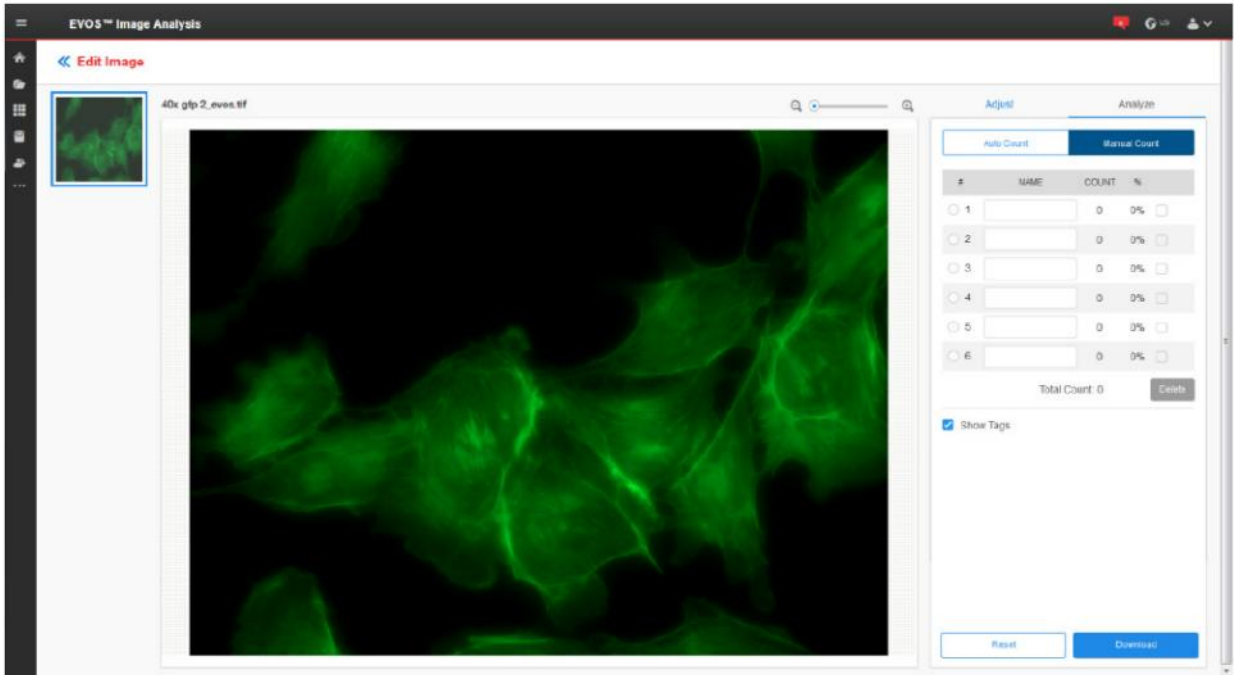
Adjust tab

The **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 (Chapter 5, “Measure, annotate, and analyze captured images”).



Analyze tab

The **Analyze** tab allows you to use **Auto Count** and **Manual Count**. The **Auto Count** and **Manual Count** functions of the EVOS™ Image Analysis application are identical to the count tools of the EVOS™ M5000 Imaging System (“Analyze cell culture” on page 44).





Software controls and functions

- **Autofocus:** The **Autofocus** button triggers the microscope to move through a Z-axis range (defined by the triangle sliders in the **Coarse** focus control) and search for the optimal focal plane. The display shows a live view as the objective sweeps through the defined Z-axis range. Autofocus uses an image-based focusing algorithm to achieve the desired focus.

Note: Do NOT use autofocus with oil-immersion objectives, as this can create air bubbles in the oil or cause the objective to collide with the sample.

- **Brightness, Contrast, and Gamma:** The **Image Display Settings** panel provides slider controls for adjusting the brightness, contrast, and gamma of the current image. These controls can be used to darken or lighten the image and increase or decrease contrast. The **Gamma** slider allows for non-linear contrast adjustments. Moving this slider to the left can help reduce undesired background fluorescence, particularly in images with nonspecific background fluorescence. Conversely, moving this slider to the right can enhance fluorescence signals and make them more visible, especially in images with very faint signals, and can be particularly useful for detecting signals at the lower limits of brightness.
- **Keyboard shortcuts:** Keyboard shortcuts are helpful for efficiently performing instrument functions without relying on mouse actions. These shortcuts typically involve holding down the **Control** key and pressing a designated key to trigger a specific action. Keyboard shortcuts for the EVOS™ M5000 instrument can be used for a range of tasks, including selecting the illumination channel, toggling the current channel on and off, running autofocus, capturing the current channel, capturing all selected channels, and saving the displayed image using the current **Save** settings. By familiarizing yourself with these shortcuts you can streamline your workflow and enhance productivity while operating the instrument.
- **Simple and Advanced illumination controls:** **Simple** illumination operates with one slider using an intelligent algorithm to adjust the LED brightness, exposure time, and camera gain at the same time. **Advanced** illumination allows control of the same parameters using 3 separate sliders. Clicking the icon with 3 sliders from the default **Simple** illumination control (1 slider) switches to the **Advanced** option.

Note: The **Simple** illumination single slider uses an intelligent algorithm designed to maximize the signal-to-noise ratio and minimize photobleaching. For most imaging work we recommend using the single slider to adjust the illumination. For more advanced users it may be useful to control these settings individually, but please note it is also possible to photobleach the sample or degrade images if you use the wrong combination of settings.

- **Sync Z:** The **Sync Z** feature is designed to enable the user to focus on a single channel while the other channels are adjusted automatically. This is accomplished by first adjusting the focus of each channel individually with the **Sync Z** feature unchecked. This will establish unique offsets for each channel. Once this initial adjustment is complete, the **Sync Z** feature can be enabled and thereafter focusing on one channel will automatically adjust the focus of the others using the same offsets that were established during the individual setup. If at any point the offsets become misaligned, the **Clear Z Offsets** button can be used to quickly reset and start again.

Image files

- **Channel:** A channel refers to a specific illumination range used to capture images. Fluorescence channels are defined by their excitation and emission wavelengths, which enable the detection of different cell populations or subcellular structures. Transmitted light is full-spectrum and can be used either alone or in conjunction with phase-contrast optics to provide images based on the light that passes through the sample. The interaction of the light with the sample as it passes through cells or tissue provides contrast for transmitted light images.
- **Channel overlay:** When multiple channels are captured from a single location, they can be overlaid in an image to visualize the relationships between different cells or structures identified by each channel. The overlaid channels are pseudocolored, meaning that distinct colors are assigned to each channel for better differentiation. It is important to note that an image with overlaid channels is intended for display purposes only and should not be used for quantitative analysis.
- **Analysis image:** An analysis image refers to a single-channel image that is saved in a lossless format using the full dynamic range of the camera (12 bits). These images are saved in 16-bit format, which may require specialized viewing software for proper display. When viewed on other software, analysis images are displayed in grayscale monochrome, representing the pure data that was captured. To enhance visualization, the EVOS™ software automatically displays analysis images using the appropriate pseudocolor. It's important to note that the EVOS™ software does not modify analysis images in any way. This means that features like scalebars or annotations cannot be added, and changing the contrast onscreen does not alter the underlying data in the image file.
- **Display image:** A display image refers to a modified version of an original image that can be used outside of the EVOS™ M5000 software. These images are usually pseudocolored, either representing a single channel or multiple overlaid channels, and are intended for purposes such as PowerPoint™ presentations or sharing with colleagues. It's important to note that display images are saved in an 8-bits per channel file format, which may compromise the intensity information and result in lower quality images for quantitative analysis. While display images can be useful for manual tasks like cell counting or size/distance measurements, it is not recommended to use them with analysis tools like **Auto Count**, **Confluence**, or **Transfection Efficiency**, as these measurements rely on the complete intensity information that might be lost in display images. Display images can be customized with features like scale bars or annotations, and adjusted brightness and contrast values for visual presentation; as such they are not the original data and should not be used for analysis purposes.

- **Pseudocolor image:** A pseudocolor image is a modified version of a grayscale image that uses a single color (for a fluorescence channel) or multiple distinct colors (for a fluorescence overlay image) to represent the image as it would be seen through a microscope's oculars. The chosen color(s) typically correspond to the emission wavelength of the fluorescence channel used to capture the image. With the EVOS™ M5000 instrument, which uses a highly sensitive monochrome camera, all fluorescence images are initially captured as monochrome. These images can then be saved as either monochrome 16-bit images for analysis or pseudocolored 8-bit images for display purposes. It's important to note that any saved pseudocolor image is always considered to be a display image.
- **TIFF image file:** TIFF is an acronym for "tagged image file format", a common digital image file encoding standard. "Tags" consist of information (also known as metadata) stored in the image file along with the image. TIFF images for microscopy can contain tags about the objective, illumination info, camera, exposure time, and other details of image capture. TIFF files are not compressed and can be saved in analysis or display format. EVOS™ TIFF files have the following naming conventions:
 - Basefilename_quicksavenumber_**channel**_well.tiff = 8-bit pseudocolor channel for display
 - Basefilename_quicksavenumber_**channel**_well.tif = 16-bit monochrome channel for analysis
 - Basefilename_quicksavenumber_well.tif = 8-bit, RGB multi-channel pseudocolor overlay for display

Note: Bolded text in the file names indicates variables that change between the types.

- **PNG image file:** PNG is an acronym for "portable network graphics", a common digital image file encoding standard. Like TIFF files, PNG files contain information (known as "chunks") stored along with the image. PNG images for microscopy can contain chunks (that is, metadata) about the objective, illumination information, camera, exposure time, and other details of image capture. PNG files use lossless compression and can be saved in analysis or display format. EVOS™ PNG files have the following naming conventions:
 - Basefilename_quicksavenumber_**channel**_well_**D**.PNG = 8-bit pseudocolor channel for display
 - Basefilename_quicksavenumber_**channel**_well.png = 16-bit monochrome channel for analysis
 - Basefilename_quicksavenumber_well.png = 8-bit RGB multi-channel pseudocolor overlay for display

Note: Bolded text in the file names indicates variables that change between the types.

- **JPEG image file:** JPEG is an acronym for "joint photographic experts group", a common digital image file encoding standard. As opposed to TIFF or PNG files, JPEG image files are often highly compressed, which results in data loss. In addition to lossy compression, standard JPEG images do not contain metadata and have limited dynamic range (only 8-bit images). These images are useful for display, sharing, and viewing and can easily be shared and viewed, however, they are not optimal for analysis. EVOS™ JPEG files have the following naming conventions:
 - Basefilename_quicksavenumber_**channel**_well.jpg = 8-bit pseudocolor channel for display
 - Basefilename_quicksavenumber_well.jpg = 8-bit RGB multi-channel pseudocolor overlay for display

Note: Bolded text in the file names indicates variables that change between the types.

Microscope controls and optics

- **Light cube:** A light cube is a proprietary optical module that can be easily installed by the user in an EVOS™ fluorescence microscope or fluorescence-capable Countess™ cell counter. Each light cube contains a high-power LED as well as the electronics and optical elements needed to illuminate and capture a single fluorescence channel. Upon powering on the instrument, light cubes are automatically detected and assigned in the control software, requiring no additional configuration from the user. This design allows for easy integration and use, providing a simple and efficient way to enhance fluorescence imaging capabilities.
- **Optical substrate:** The optical substrate refers to the optical material (typically made of glass or plastic) located between the objective and the sample in a microscope. The optical properties and thickness of this material play a crucial role in determining image quality. Microscope objectives are specifically designed to be used with a particular thickness of optical substrate. Using an incorrect thickness or material can lead to issues such as difficulties in focusing, excessive background autofluorescence, or even the inability to focus on the cells. It is important to note that oil-immersion objectives require specialized oil as part of the optical substrate, which must have the correct refractive index for optimal imaging results.
- **Stage X axis:** Movement of the stage in the horizontal direction using the X-axis stage control knob.
- **Stage Y axis:** Movement of the stage in the vertical direction (that is, away or toward the user) using the Y-axis stage control knob.
- **Turret:** A mechanical rotary control in the microscope that allows the user to move optical elements into or out of the light path. An objective turret allows the selection of different magnifying objectives, whereas a condenser turret allows use of various phase-contrast rings or an open aperture for transmitted light illumination.
- **Vessel:** A holder for a biological specimen made of glass or plastic, with optical properties suitable for use with a microscope.
- **Vessel Holder:** An accessory that mounts on the stage to hold a particular type of sample vessel (for example, an opening for a microtiter plate, glass slide, or 35 mm Petri dish)
- **Z plane:** The focal plane of the microscope. The **Z plane** position is controlled by the focus knobs, the focus sliders in the user interface, or by running the **Autofocus** function.

Stage View feature

- **Crosshairs:** The crosshairs are a visual aid that provides real-time information about the position of the objective lens relative to the stage. The crosshairs can be seen on both the small vessel map in the **Stage View** tab and the larger vessel map in the **Find Target** window. When the crosshairs are within 4 units of the target in both the X and Y directions in the **Find Target** window, they change color from amber to green. This change indicates that the crosshairs are close enough to the target to typically have it within the field of view.
- **Pin:** A precise stage location created by the user which can be re-located in either the same imaging session or a different imaging session.
- **Pinhole:** A precise location on a **Stage View** vessel holder which is used to set the stage origin after powering on the instrument.
- **Pin map:** A collection of pins corresponding to multiple areas of interest on a biological sample (for example, in different wells of a microtiter plate, various locations on a histology tissue slide, in a 35 mm Petri dish, etc.).



WARNING! GENERAL SAFETY. Using this product in a manner not specified in the user documentation may result in personal injury or damage to the instrument or device. Ensure that anyone using this product has received instructions in general safety practices for laboratories and the safety information provided in this document.

- Before using an instrument or device, read and understand the safety information provided in the user documentation provided by the manufacturer of the instrument or device.
- Before handling chemicals, read and understand all applicable Safety Data Sheets (SDSs) and use appropriate personal protective equipment (gloves, gowns, eye protection, and so on). To obtain SDSs, visit thermofisher.com/support.

Symbols on this instrument



Symbols may be found on the instrument to warn against potential hazards or convey important safety information. In this document, the hazard symbol is used along with one of the following user attention words.






- **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.

Standard safety symbols

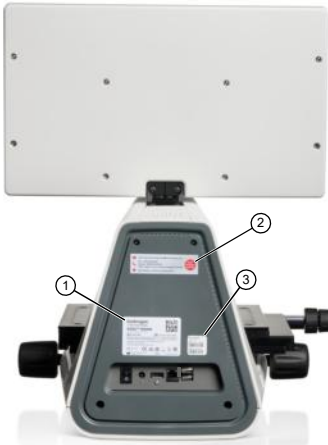
| Symbol and description | |
|------------------------|--|
| | CAUTION! Risk of danger. Consult the manual for further safety information. |
| | CAUTION! Risk of electrical shock. |
| | CAUTION! Hot surface. |

(continued)

| Symbol and description | |
|---|--------------------------------------|
|  | CAUTION! Potential biohazard. |
|  | CAUTION! Ultraviolet light. |

| Symbole et description | |
|---|--|
|  | MISE EN GARDE ! Risque de danger. Consulter le manuel pour d'autres renseignements de sécurité. |
|  | MISE EN GARDE ! Risque de choc électrique. |
|  | MISE EN GARDE ! Surface chaude. |
|  | MISE EN GARDE ! Danger biologique potentiel. |
|  | MISE EN GARDE ! Rayonnement ultraviolet. |


Location of labels

| Labels and location |
|---|
|  |
| <p>Figure 5 Instrument information labels (back panel)</p> <ul style="list-style-type: none"> ① Instrument information label ② Technical support information ③ Instrument passwords label |





Control and connection symbols

| Symbols and descriptions | |
|--------------------------|---|
| | On (Power) |
| ○ | Off (Power) |
| ⏏ | Protective conductor terminal (main ground) |
| ~ | Alternating current |

Conformity symbols

| Conformity mark | Description |
|---|---|
|  | Indicates conformity with safety requirements for Canada and U.S.A. |
|  | Indicates conformity with China RoHS requirements. |

(continued)

| Conformity mark | Description |
|---|---|
|  | Indicates conformity with European Union requirements. |
|  | Indicates conformity with Australian standards for electromagnetic compatibility. |
|  | INDICATES CONFORMITY WITH THE WEEE DIRECTIVE 2012/19/EU.  CAUTION! To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options. |

Safety information for instruments not manufactured by Thermo Fisher Scientific

Some of the accessories provided as part of the instrument system are not designed or built by Thermo Fisher Scientific. Consult the manufacturer's documentation for the information needed for the safe use of these products.

Instrument safety

General



CAUTION! Do not remove instrument protective covers. If you remove the protective instrument panels or disable interlock devices, you may be exposed to serious hazards including, but not limited to, severe electrical shock, laser exposure, crushing, or chemical exposure.

If covers are removed, do not use the instrument. Contact Technical Support.



CAUTION! Solvents and Pressurized fluids. Wear eye protection when working with any pressurized fluids. Use caution when working with any polymeric tubing that is under pressure:

- Extinguish any nearby flames if you use flammable solvents.
- Do not use polymeric tubing that has been severely stressed or kinked.
- Do not use polymeric tubing with tetrahydrofuran or nitric and sulfuric acids.
- Be aware that methylene chloride and dimethyl sulfoxide cause polymeric tubing to swell and greatly reduce the rupture pressure of the tubing.
- Be aware that high solvent flow rates (~40mL/min) may cause a static charge to build up on the surface of the tubing and electrical sparks may result.

Physical injury



CAUTION! Moving and Lifting Injury. Improper lifting can cause painful and permanent back injury.

Things to consider before lifting or moving the instrument or accessories:

- Depending on the weight, moving or lifting may require two or more persons.
- If you decide to lift or move the instrument after it has been installed, do not attempt to do so without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques.
- Ensure you have a secure, comfortable grip on the instrument or accessory.
- 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.
- For smaller packages, rather than lifting the object from the packing box, carefully tilt the box on its side and hold it stationary while someone else slides the contents out of the box.



CAUTION! Moving Parts. Moving parts can crush, pinch and cut. Keep hands clear of moving parts while operating the instrument. Disconnect power before servicing.

Electrical safety



WARNING! Ensure appropriate electrical supply. For safe operation of the instrument:

- Plug the system into a properly grounded receptacle with adequate current capacity.
- Ensure the electrical supply is of suitable voltage.
- Never operate the instrument with the ground disconnected. Grounding continuity is required for safe operation of the instrument.



AVERTISSEMENT ! Veiller à utiliser une alimentation électrique appropriée. Pour garantir le fonctionnement de l'instrument en toute sécurité :

- Brancher le système sur une prise électrique correctement mise à la terre et de puissance adéquate.
- S'assurer que la tension électrique est convenable.
- Ne jamais utiliser l'instrument alors que le dispositif de mise à la terre est déconnecté. La continuité de la mise à la terre est impérative pour le fonctionnement de l'instrument en toute sécurité.



WARNING! Power Supply Line Cords. Use properly configured and approved line cords for the power supply in your facility. If the line cord is damaged, contact Technical Support.



AVERTISSEMENT ! Cordons d'alimentation électrique. Utiliser des cordons d'alimentation adaptés et approuvés pour raccorder l'instrument au circuit électrique du site.



WARNING! Disconnecting Power. To fully disconnect power either detach or unplug the power cord, positioning the instrument such that the power cord is accessible.



AVERTISSEMENT ! Déconnecter l'alimentation. Pour déconnecter entièrement l'alimentation, détacher ou débrancher le cordon d'alimentation. Placer l'instrument de manière à ce que le cordon d'alimentation soit accessible.

Overvoltage rating

The EVOS™ M3000 Imaging System has an installation (overvoltage) category of II, and is classified as portable equipment.

Cleaning and decontamination



CAUTION! Cleaning and Decontamination. Use only the cleaning and decontamination methods that are specified in the manufacturer user documentation. It is the responsibility of the operator (or other responsible person) to ensure that the following requirements are met:

- No decontamination or cleaning agents are used that can react with parts of the equipment or with material that is contained in the equipment. Use of such agents could cause a HAZARD condition.
- The instrument is properly decontaminated a) if hazardous material is spilled onto or into the equipment, and/or b) before the instrument is serviced at your facility or is sent for repair, maintenance, trade-in, disposal, or termination of a loan. Request decontamination forms from customer service.
- Before using any cleaning or decontamination methods (except methods that are recommended by the manufacturer), confirm with the manufacturer that the proposed method will not damage the equipment.



MISE EN GARDE ! Nettoyage et décontamination. Utiliser uniquement les méthodes de nettoyage et de décontamination indiquées dans la documentation du fabricant destinée aux utilisateurs. L'opérateur (ou toute autre personne responsable) est tenu d'assurer le respect des exigences suivantes:

- Ne pas utiliser d'agents de nettoyage ou de décontamination susceptibles de réagir avec certaines parties de l'appareil ou avec les matières qu'il contient et de constituer, de ce fait, un DANGER.
- L'instrument doit être correctement décontaminé a) si des substances dangereuses sont renversées sur ou à l'intérieur de l'équipement, et/ou b) avant de le faire réviser sur site ou de l'envoyer à des fins de réparation, de maintenance, de revente, d'élimination ou à l'expiration d'une période de prêt (des informations sur les formes de décontamination peuvent être demandées auprès du Service clientèle).
- Avant d'utiliser une méthode de nettoyage ou de décontamination (autre que celles recommandées par le fabricant), les utilisateurs doivent vérifier auprès de celui-ci qu'elle ne risque pas d'endommager l'appareil.

Instrument component and accessory disposal

To minimize negative environmental impact from disposal of electronic waste, do not dispose of electronic waste in unsorted municipal waste. Follow local municipal waste ordinances for proper disposal provision and contact customer service for information about responsible disposal options.

Safety and electromagnetic compatibility (EMC) standards

The instrument design and manufacture complies with the following standards and requirements for safety and electromagnetic compatibility.



Safety standards

| Reference | Description |
|---|---|
| EU Directive 2014/35/EU | European Union “Low Voltage Directive” |
| EN 61010-1 UL 61010-1 CAN/CSA C22.2 No. 61010-1 | <i>Safety requirements for electrical equipment for measurement, control, and laboratory use – Part 1: General requirements</i> |
| EN 61010-2-081 | <i>Safety requirements for electrical equipment for measurement, control and laboratory use – Part 2-081: Particular requirements for automatic and semi-automatic laboratory equipment for analysis and other purposes</i> |

EMC standards

| Reference | Description |
|--------------------------------|--|
| EU Directive 2014/30/EU | European Union “EMC Directive” |
| EN 61326-1 | <i>Electrical Equipment for Measurement, Control and Laboratory Use – EMC Requirements – Part 1: General Requirements</i> |
| AS/NZS CISPR 11 | <i>Limits and Methods of Measurement of Electromagnetic Disturbance Characteristics of Industrial, Scientific, and Medical (ISM) Radiofrequency Equipment</i> |
| ICES-003, Issue 7 | <i>Industrial, Scientific and Medical (ISM) Radio Frequency Generators</i> |
| FCC Part 15 Subpart B (47 CFR) | <p><i>U.S. Standard Radio Frequency Devices</i></p> <p>This equipment has been tested and found to comply with the limits for a Class A digital device, pursuant to part 15 of the FCC Rules. These limits are designed to provide reasonable protection against harmful interference when the equipment is operated in a commercial environment. This equipment generates, uses, and can radiate radio frequency energy and, if not installed and used in accordance with the instruction manual, may cause harmful interference to radio communications. Operation of this equipment in a residential area is likely to cause harmful interference in which case the user will be required to correct the interference at his own expense.</p> <p>This equipment has been designed and tested to CISPR 11 Class A. In a domestic environment it may cause radio interference, in which case, you may need to take measures to mitigate the interference.</p> <p>Do not use this device in close proximity to sources of strong electromagnetic radiation (e.g. unshielded intentional RF sources), as these can interfere with the proper operation.</p> |

Environmental design standards

| Reference | Description |
|--|--|
| Directive 2012/19/EU | European Union “WEEE Directive” –Waste electrical and electronic equipment |
| Directive 2011/65/EU and Commission Delegated Directive 2015/863 | European Union “RoHS Directive” – Restriction of hazardous substances in electrical and electronic equipment |
| SJ/T 11364-2014 | “China RoHS” Standard – Marking for the Restricted Use of Hazardous Substances in Electronic and Electrical Products For instrument specific certificates, visit our customer resource page at www.thermofisher.com/us/en/home/technical-resources/rohs-certificates.html . |
| Regulation (EC) 1907/2006 | Registration, Evaluation, Authorisation, and Restriction of Chemicals (REACH) |

Chemical safety



WARNING! GENERAL CHEMICAL HANDLING. To minimize hazards, ensure laboratory personnel read and practice the general safety guidelines for chemical usage, storage, and waste provided below. Consult the relevant SDS for specific precautions and instructions:

- 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. To obtain SDSs, see the "Documentation and Support" section in this document.
- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing).
- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with sufficient ventilation (for example, fume hood).
- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer cleanup procedures as recommended in the SDS.
- Handle chemical wastes in a fume hood.
- Ensure use of 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.)
- After emptying a waste container, seal it with the cap provided.
- Characterize (by analysis if needed) the waste generated by the particular applications, reagents, and substrates used in your laboratory.
- Ensure that the 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.



AVERTISSEMENT ! PRÉCAUTIONS GÉNÉRALES EN CAS DE MANIPULATION DE PRODUITS CHIMIQUES. Pour minimiser les risques, veiller à ce que le personnel du laboratoire lise attentivement et mette en œuvre les consignes de sécurité générales relatives à l'utilisation et au stockage des produits chimiques et à la gestion des déchets qui en découlent, décrites ci-dessous. Consulter également la FDS appropriée pour connaître les précautions et instructions particulières à respecter :

- Lire et comprendre les fiches de données de sécurité (FDS) fournies par le fabricant avant de stocker, de manipuler ou d'utiliser les matériaux dangereux ou les produits chimiques. Pour obtenir les FDS, se reporter à la section « Documentation et support » du présent document.
- Limiter les contacts avec les produits chimiques. Porter des équipements de protection appropriés lors de la manipulation des produits chimiques (par exemple : lunettes de sûreté, gants ou vêtements de protection).
- Limiter l'inhalation des produits chimiques. Ne pas laisser les récipients de produits chimiques ouverts. Ils ne doivent être utilisés qu'avec une ventilation adéquate (par exemple, sorbonne).
- Vérifier régulièrement l'absence de fuite ou d'écoulement des produits chimiques. En cas de fuite ou d'écoulement d'un produit, respecter les directives de nettoyage du fabricant recommandées dans la FDS.
- Manipuler les déchets chimiques dans une sorbonne.

- Veiller à utiliser des récipients à déchets primaire et secondaire. (Le récipient primaire contient les déchets immédiats, le récipient secondaire contient les fuites et les écoulements du récipient primaire. Les deux récipients doivent être compatibles avec les matériaux mis au rebut et conformes aux exigences locales, nationales et communautaires en matière de confinement des récipients.)
- Une fois le récipient à déchets vidé, il doit être refermé hermétiquement avec le couvercle fourni.
- Caractériser (par une analyse si nécessaire) les déchets générés par les applications, les réactifs et les substrats particuliers utilisés dans le laboratoire.
- Vérifier que les déchets sont convenablement stockés, transférés, transportés et éliminés en respectant toutes les réglementations locales, nationales et/ou communautaires en vigueur.
- **IMPORTANT !** Les matériaux représentant un danger biologique ou radioactif exigent parfois une manipulation spéciale, et des limitations peuvent s'appliquer à leur élimination.

Biological hazard safety



WARNING! Potential Biohazard. Depending on the samples used on this instrument, the surface may be considered a biohazard. Use appropriate decontamination methods when working with biohazards.



WARNING! BIOHAZARD. Biological samples such as tissues, body fluids, infectious agents, and blood of humans and other animals have the potential to transmit infectious diseases. Conduct all work in properly equipped facilities with the appropriate safety equipment (for example, physical containment devices). Safety equipment can also include items for personal protection, such as gloves, coats, gowns, shoe covers, boots, respirators, face shields, safety glasses, or goggles. Individuals should be trained according to applicable regulatory and company/ institution requirements before working with potentially biohazardous materials. Follow all applicable local, state/provincial, and/or national regulations. The following references provide general guidelines when handling biological samples in laboratory environment.

- U.S. Department of Health and Human Services, *Biosafety in Microbiological and Biomedical Laboratories (BMBL)*, 6th Edition, HHS Publication No. (CDC) 300859, Revised June 2020
www.cdc.gov/labs/pdf/CDC-BiosafetymicrobiologicalBiomedicalLaboratories-2020-P.pdf
- Laboratory biosafety manual, fourth edition. Geneva: World Health Organization; 2020 (Laboratory biosafety manual, fourth edition and associated monographs)
www.who.int/publications/i/item/9789240011311



Documentation and support

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 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and its affiliates warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have questions, contact Life Technologies at www.thermofisher.com/support.

