EVOS™ FL Auto 2

Imaging System for Fluorescence and Transmitted Light Applications

Catalog Number AMAFD2000

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Revision history MAN0014072

Revision	Date	Description
B.0	14 April 2017	Removed references to discontinued products.
A.0	17 February 2017	New user guide

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Contents

About this guide

Audience

This user guide is for laboratory staff operating, maintaining, and analyzing data using the Invitrogen™ EVOS™ FL Auto 2 Imaging System.

User attention words

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

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

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

Safety alert words

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

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

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

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

1. Product information

Product description

EVOS™ FL Auto 2 Imaging System

The Invitrogen™ EVOS™ FL Auto 2 Imaging System (Cat. No. AMAFD2000) is a fully automated, digital, inverted multi-channel fluorescence and transmitted light imaging system. The system is designed for a broad range of applications including, but not limited to, multi-channel fluorescence imaging, multiple-position vessel scanning, area scanning with montage or tile stitching, and time-lapse imaging.

EVOS™ FL Auto 2 Software

The EVOS™ FL Auto 2 Imaging System is controlled by the integrated Invitrogen™ EVOS™ FL Auto 2 Software accessed by the touch-screen monitor or the computer mouse and keyboard. The software is pre-installed to the computer workstation supplied with the instrument, and the software icon (i.e., shortcut) is placed on the computer desktop and under **Start ▶ All Programs ▶ Auto 2.**

Key features of the EVOS™ FL Auto 2 Software include:

- **Capture:** Allows control over every aspect of the system for image capture through a simple user interface. All images acquired can be saved in TIF, PNG, C01, DIB, and JPEG formats, or compiled into a video sequence in AVI or WMV formats.

- **Automate:** Allows the creation, saving, and running of user-defined routines to automate image collection.

- **Autofocus:** Can be setup in three different modes to optimize speed and accuracy.

- **Image stitching and montage:** Allows the scanning of an area to acquire multiple images to build a tiled and stitched image. The Review tool allows zooming in/out and panning of the composite image. The entire scan or only regions of interest can be exported.

- **Z-stacking:** Captures a series of images along the z-axis that can be saved individually or combined into a Z-stack projection with a greater depth of field than any of the individual source images.

- **Time lapse:** Creates and runs time lapse movies based on user specifications.

- **Review:** Allows you to review captured images.

Product use

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

Standard items included

Before the EVOS™ FL Auto 2 Imaging System is installed (page 9), unpack the unit and accessories and verify all parts are present. Examine the instrument carefully for damage incurred during transit. Contact your distributor if anything is missing. Damage claims must be filed with the carrier; the warranty does not cover in-transit damage.

Note: If you do not have your distributor information, contact Technical Support (see page 175).

- EVOS™ FL Auto 2 Imaging System
- Computer
- Touch-screen monitor
- Keyboard
- Mouse
- Accessories box (located in the instrument box), containing:
  - Power cable*

    *For use with North American outlets; instruments intended for use outside North America must order a separate power cord.
  - Power supply
  - Display Port to Display Port cable
  - USB 2.0 type A to B cable (black)
  - USB 3.0 type A to B cable (2×) (blue)
  - Dust cover
- Light cubes, as ordered
- Light cube tool
- Objectives, as ordered
- Vessel holder(s) for Well Plates (Cat. No. AMEP-VH022) and Double Slides (Cat. No. AMEP-VH021) and as ordered (located in the accessories box)
- Light box with cover (located in the accessories box)
- Sliders: Block slider, Diffuser slider
- USB flash drive (includes *User Guide and Quick Start Guide*)

IMPORTANT! Wiping the computer supplied with the EVOS™ FL Auto 2 Imaging System (i.e., erasing the hard drive to remove all programs, files, and the operating system) voids the product warranty. Do not install third party software or update the Windows™ operating system.

Instrument exterior components

Top view
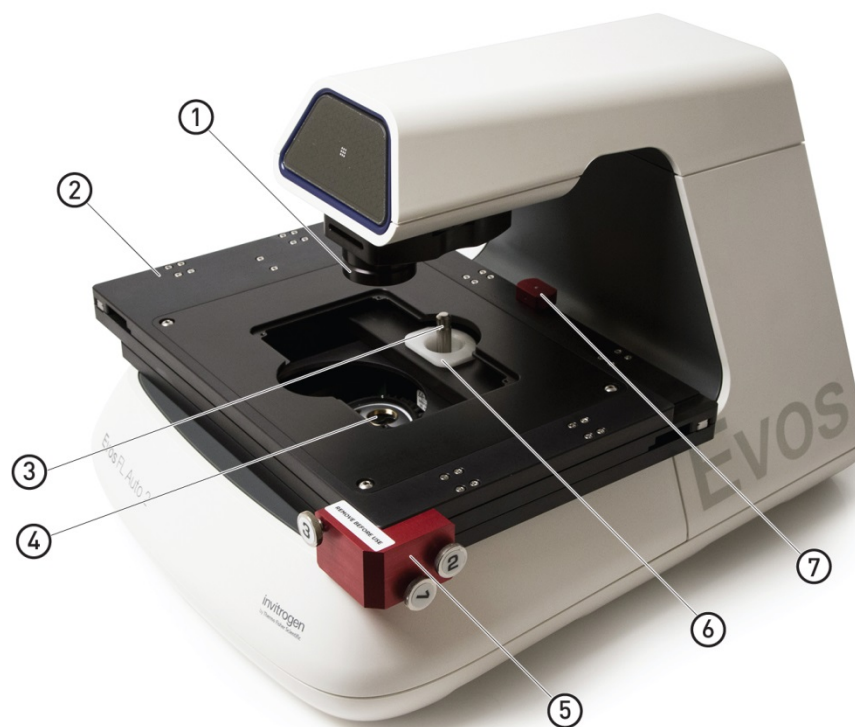
1. Condenser
2. Automatic X-Y axis stage
3. Light cube tool
4. Objective turret (accomodates up to 5 objectives)
5. X-Y stage shipping restraint
6. Light cube shipping restraint
7. Camera shipping restraint

Side view

① Condenser
② Automatic X-Y axis stage
③ Handholds

Rear view

① Power switch
② 4-pin power input port (24 VDC, 5 A)
③ USB 2.0 port, black
④ USB 3.0 ports, blue (2×)

Graphical user interface (GUI)

The functions of the EVOS™ FL Auto 2 Imaging System is controlled by the integrated Invitrogen™ EVOS™ FL Auto 2 Software through a graphical user interface (GUI), which is accessed by the touch-screen monitor or the computer mouse and keyboard.

GUI layout

The GUI of the system consists of the Viewing area on the left and a series of tabs representing the main functions of the software (Capture, Automate, Review, and Settings) on the right. Each tab contains the controls necessary to execute the selected function. The Locations button, Area/Field View toggle, and the Zoom slider are located above the Viewing area.
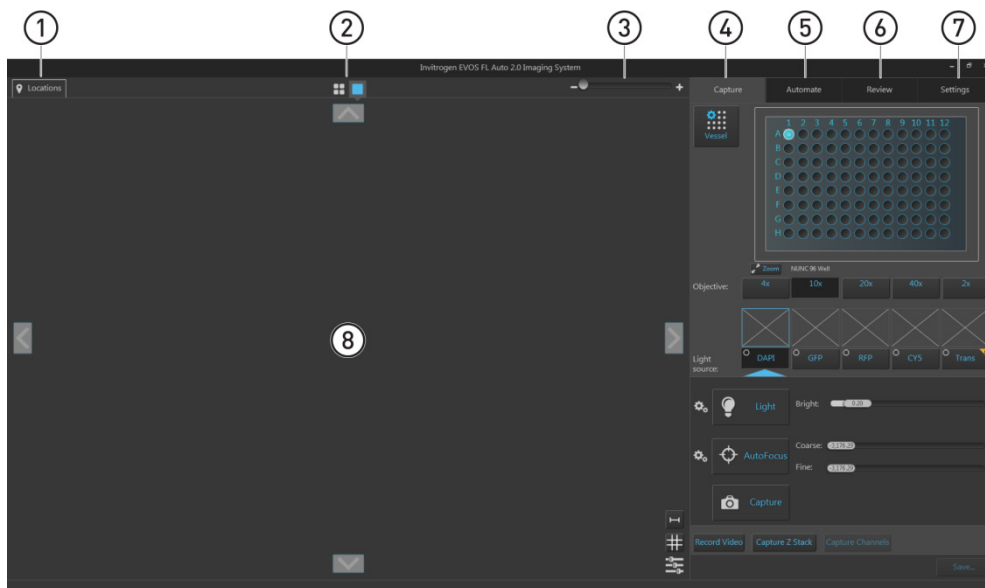
①   **Locations:** Opens the Locations controls, which allows you to select location for automated scan protocols.

②   **Area View/Field View toggle:** Switches between Area and Field Views.

③   **Zoom slider:** Zooms in and out of the Viewing area.

④   **Capture tab:** Contains the controls for the manual capture of images.

⑤   **Automate tab:** Allows you to create and run automated scan protocols and time lapse experiments.

⑥   **Review tab:** Allows you to review and annotate captured images.

⑦   **Settings:** Contains controls to select and adjust basic and advanced system options and instrument functions.

⑧   **Viewing area:** Displays the sample in Area View or Field View modes.

Note: Click on the **View Help Content** icon to open the Help window, which provides EVOS™ FL Auto 2 Imaging System Help.

Hover your pointer over the **Tooltip** icon without clicking it to view a small hover box with additional information.

Note: For more information and detailed descriptions of software controls, see "Appendix C: Graphical user interface (GUI)", page 84.

2. Installation

IMPORTANT! Do not perform the initial installation of the EVOS™ FL Auto 2 Imaging System yourself. A Thermo Fisher Scientific representative will contact you to schedule the installation.

When the installation is scheduled:

1. Receive and inspect the system.
2. Move the crated instrument to the installation site.

Operating environment and site requirements

- The EVOS™ FL Auto 2 Imaging System has a footprint of approximately 92 cm × 92 cm (36 in × 36 in); entire system includes the instrument, computer, and 23-inch touch-screen, high-resolution LCD monitor.

- If the system includes the optional EVOS™ Onstage Incubator (Cat. No. AMC1000), then add 40 cm (15.8 in) to the width of the bench, for a total bench width of ~132 cm (51.8 in).

- The EVOS™ FL Auto 2 Imaging System should be placed on a level surface away from vibrations from other pieces of equipment. Tabletop centrifuges, vortex mixers and other laboratory equipment can vibrate the instrument during a run that can cause a decrease of instrument performance.

- Allow at least 5 cm (2 in) free space at the back of the instrument to allow for proper ventilation and prevent overheating of electronic components.

- The EVOS™ FL Auto 2 Imaging System should be installed away from direct light sources, such as windows. Ambient room lighting can enter the imaging path and affect the image quality.

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

- Relative humidity range: 30–90%.

- Operating power: 100–240 VAC, 1.8 A

- Frequency: 50–60 Hz

- Electrical input: 24 VDC, 5 A

IMPORTANT! Do not position the instrument so that it is difficult to turn off the main power switch located on the back of the instrument base (see page 7). In case of an instrument malfunction, turn the main power switch to the OFF position and disconnect the instrument from the wall outlet.

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.

3. Unpack the instrument and accessories and verify all parts are present.

   See "Set up", page 11, for detailed instructions on how to unpack the instrument and accessories.

   See page 5 for the list of standard items included in the shipment.

4. Examine the instrument carefully for damage incurred during transit. Contact your distributor if anything is missing. Damage claims must be filed with the carrier; the warranty does not cover in-transit damage.

IMPORTANT! Do not lift the EVOS™ FL Auto 2 Imaging System by stage or condenser arm. Lift the instrument by using the handholds in the base.

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.** Do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons

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IMPORTANT! Do not subject the EVOS™ FL Auto 2 Imaging System to sudden impact or excessive vibration. Handle the instrument with care to prevent damage.

Set up

IMPORTANT! The following set-up instructions are provided for informational purposes only. Do **not** attempt the initial set-up of the instrument on your own. The initial set-up of the EVOS™ FL Auto 2 Imaging System will be performed by your Thermo Fisher Scientific service representative, who will also provide some basic operator training.

Unpack and connect the monitor

1. Open the case and remove the monitor and accessories.
2. If a VGA cable is attached to the monitor, take it off.
3. Remove protective covering from monitor.
4. Plug power cord into monitor.
5. Plug the USB touch screen cable into monitor.

Unpack and connect the computer

1. Open the box and remove the keyboard.
2. Unpack the keyboard from its box.
3. Unpack the computer.
4. Unpack the mouse and power cord from the accessory holder.
5. Plug in mouse, keyboard, and power cord.
6. Plug the USB cord already connected to the monitor into the computer.

IMPORTANT! Wiping the computer supplied with the EVOS™ FL Auto 2 Imaging System (i.e., erasing the hard drive to remove all programs, files, and the operating system) voids the product warranty.

Unpack and connect the instrument

1. Open the box and remove the accessory box.
2. Carefully lift the instrument out of the box, holding it by two of the four handholds in the base (see page 7).

   IMPORTANT! Do not lift the EVOS™ FL Auto 2 Imaging System by stage or condenser arm. Lift the instrument by using the handholds in the base.

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. Remove the following from the accessory box (located in the instrument box):
   - Power cable (for North America and as ordered)
   - Power supply
   - Display Port to Display Port cable
   - USB 2.0 type A to B cable (black)
   - USB 3.0 type A to B cable (2×) (blue)
   - White cardboard box (contains the light box and vessel holders)
   - Dust cover
5. Confirm that the power switch is OFF (located on the back; see page 7).
6. Plug the power cable into the power supply and check for the light on the power supply.
7. Plug the power supply connector into the instrument.

8. Use the Display Port-to-Display Port cable to connect the Display Port output on the computer to the DVI input on the monitor.

9. Use the blue USB 3.0 type A to B cables to connect the blue USB 3.0 ports on the back of the instrument and to the blue ports on the back of the computer. These cables are custom to the cameras and connect them to the computer.

10. Use the black USB 2.0 type A to B cables to connect the black USB 2.0 port on the back of the instrument and to the black port on the back of the computer. This cable connects the instrument to the computer.
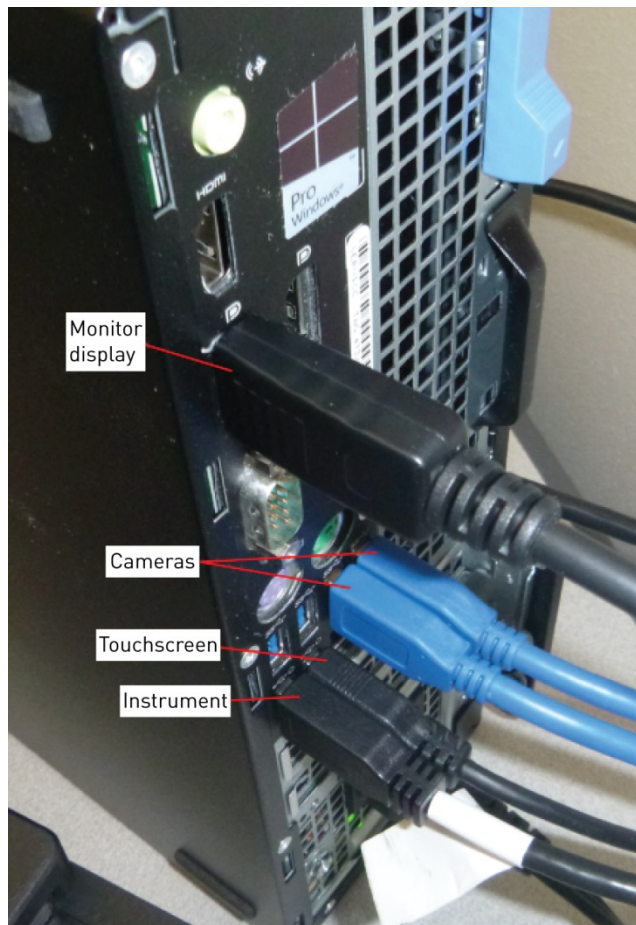
Note: At this point, everything should be plugged in and OFF. Save the packaging for future shipping/storage of the instrument.
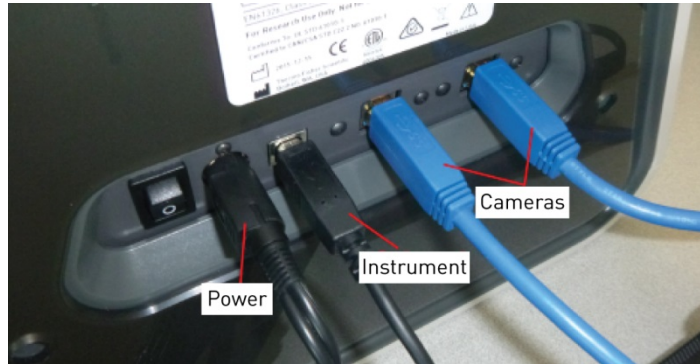
Rear view of instrument showing the cable connections after the setup is completed.

Rear view of computer showing the cable connections after the setup is completed.

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After installation is complete

**Training**

When the installation of the EVOS™ FL Auto 2 Imaging System is complete, the Thermo Fisher Scientific service representative will perform installation tests.

During and/or after installation, the Thermo Fisher Scientific service representative will provides some basic operator training. For additional training and reference information, see the user documents provided with the instrument

Remove shipping restraints

The EVOS™ FL Auto 2 Imaging System is equipped with three shipping restraints (X-Y stage, light cube, and camera shipping restraints), which prevent damage to the instrument from shock and vibration during transport. The shipping restraints must be removed before the EVOS™ FL Auto 2 Imaging System is powered on.
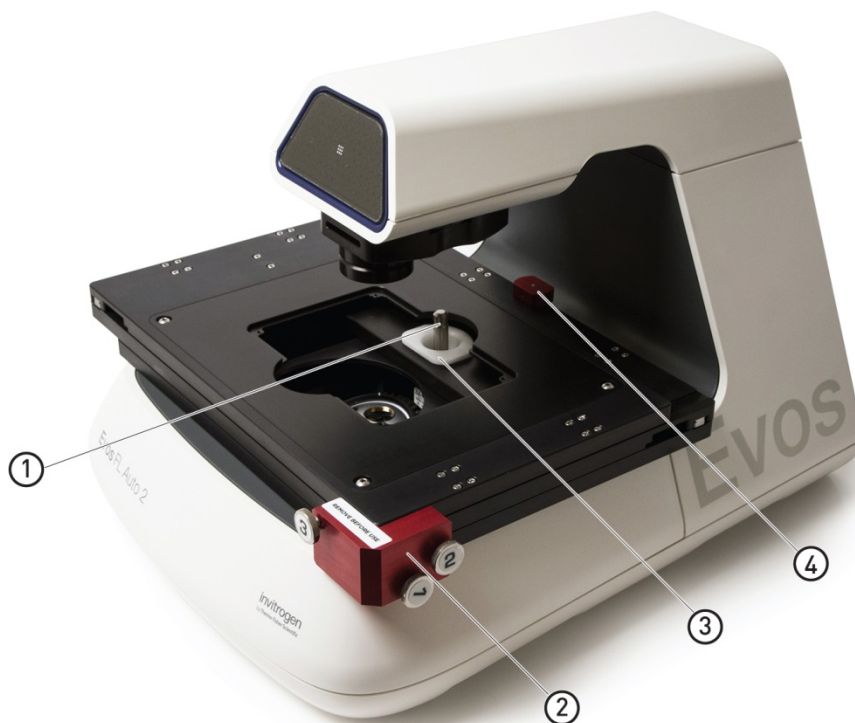
| ① Light cube tool | ③ Light cube shipping restraint |
| ② X-Y stage shipping restraint | ④ Camera shipping restraint |

1. Unscrew the three thumb screws on the X-Y stage shipping restraint until they spin freely. You do not need to remove them from the restraint altogether.
2. Gently pull the X-Y stage shipping restraint forward, away from the unit.
3. Unscrew the light cube tool (the cylinder) and remove the light cube shipping restraint (the block) through the opening on the X-Y stage.
4. Unscrew the camera shipping restraint and pull it up to remove it from the X-Y stage.

Note: Store the shipping restraints and the light cube tool in the accessories box for future use. To reinstall the shipping restraints, see page 73.

IMPORTANT! Do not subject the EVOS™ FL Auto 2 Imaging System to sudden impact or excessive vibration. Handle the instrument with care to prevent damage.

Turn ON the EVOS™ FL Auto 2 Imaging System

1. Turn the instrument power switch located in back of the instrument base to the ON position.

2. Turn the computer and monitor ON.

3. When the computer shows the Windows™ desktop and the X-Y stage of the instrument has stopped moving, click the Auto II icon on the desktop to start the EVOS™ FL Auto 2 software.

4. Once the **Capture** tab is displayed, the EVOS™ FL Auto 2 Imaging System is ready to use.
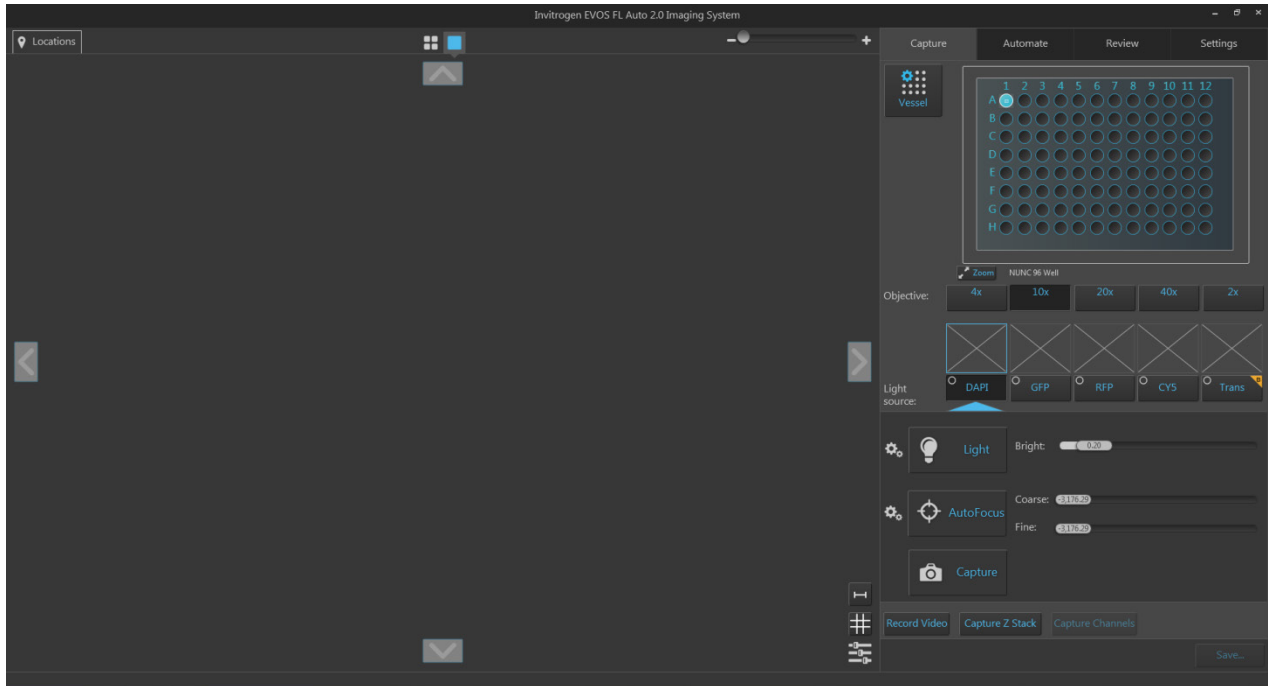
IMPORTANT! All shipping restraints must be removed before turning on the EVOS™ FL Auto 2 Imaging System to prevent damage (see page 13).
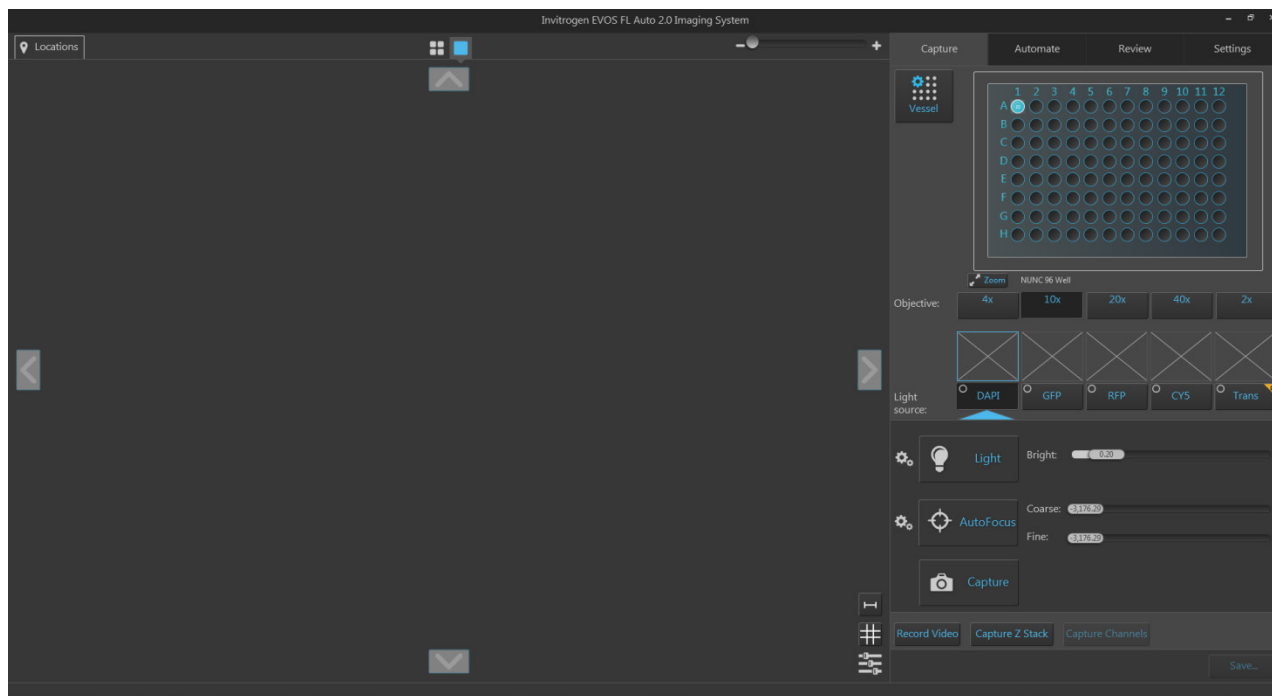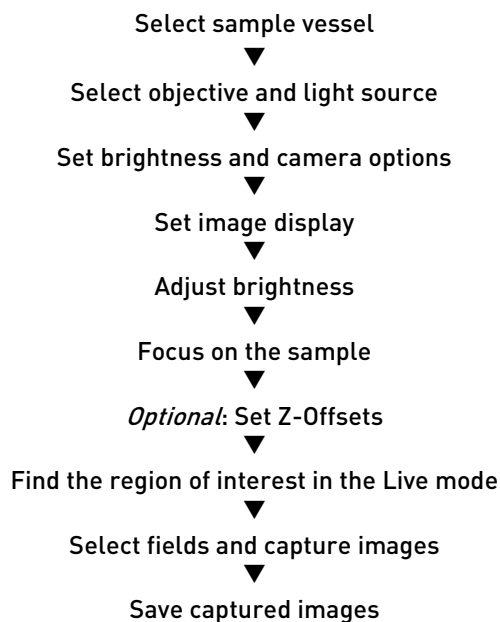
3. Capture and save images

Overview

Capture tab

The basic functions of the EVOS™ FL Auto 2 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.

Note: For a detailed description of the Capture tab controls, see "Capture tab" in "Appendix C: Graphical user interface (GUI)", page 97.

Workflow

Select sample vessel
▼
Select objective and light source
▼
Set brightness and camera options
▼
Set image display
▼
Adjust brightness
▼
Focus on the sample
▼
Optional: Set Z-Offsets
▼
Find the region of interest in the Live mode
▼
Select fields and capture images
▼
Save captured images

Capture images

Select sample vessel

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

   Note: For the types of vessel holders available for the EVOS™ FL Auto 2 Imaging System, visit **thermofisher.com/evos** or contact Technical Support (page 175).

2. On the **Capture tab**, click the **Vessel** button to open the Vessel Selection Wizard.

3. Select the **Vessel category** that corresponds to your sample vessel. Available options are:

   – **Well Plates**

   – **Flasks**

   – **Dishes**

   – **Slides**

4. Select the appropriate **Holder** and **Vessel type** from the available dropdown menus.

   Note: The Vessel Selection wizard is contextual; the dropdown menus display only the options available for the selected Vessel category.
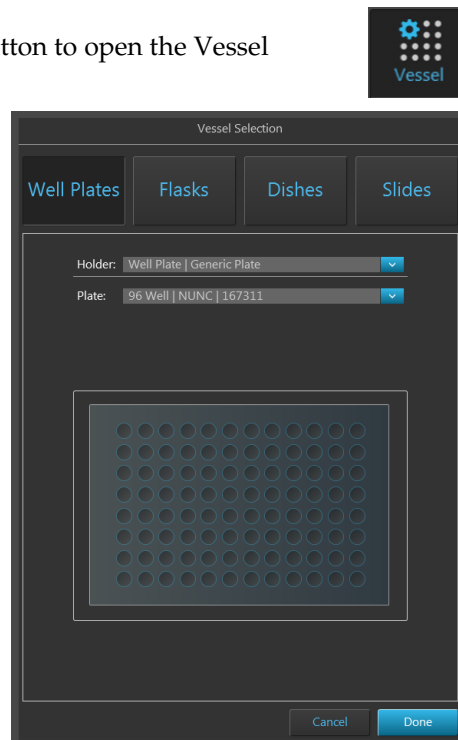
5. Click **Done** to complete your selection and close the wizard. The **Vessel map** on the Capture tab displays your selected vessel.

   By default, the first well on the sample vessel is selected and indicated in blue on the Vessel map. The Viewing area displays the selected well.
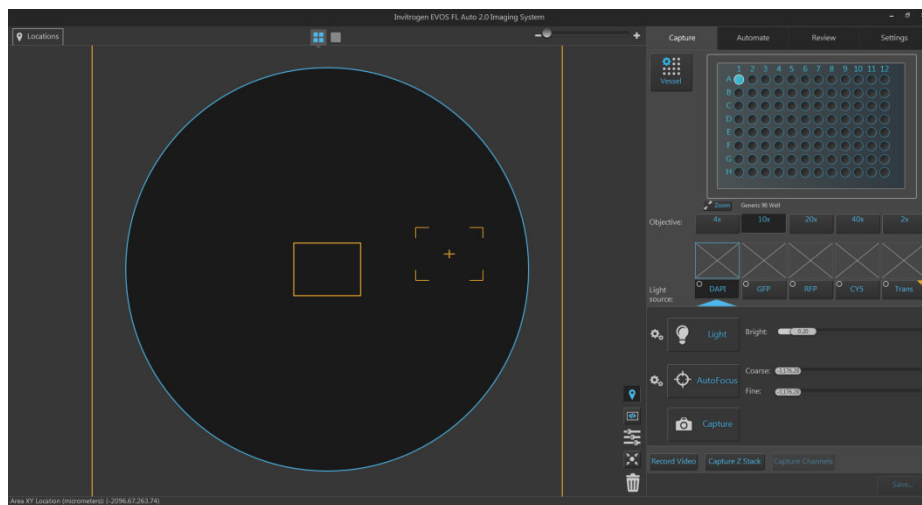
6. To select another well, click on the desired well on the **Vessel map**.

Select objective and light source

1. Click the desired **Objective** button to select the corresponding magnification. You can select only one objective at a time. In the example below, 10X objective has been selected.

Objective:	4x	10x	20x	40x	2x

2. Select the desired **Light source** from the available options for which you wish to adjust brightness and set focus. In the example below, the DAPI channel has been selected.

Light source:	○ DAPI	○ GFP	○ RFP	○ CY5	○ Trans

> **Note:** You can select only one light source at a time to adjust brightness and set focus. However, you can display and capture multiple channels simultaneously (see "Capture multiple channels simultaneously", page 26).

Set brightness and camera options

1. Click the **Brightness and camera settings** button to expand the controls for setting mode, camera, and phase options.

Mode:	Simple	Actual
Camera:	Mono	Color
Phase:	Not available for the selected channel	
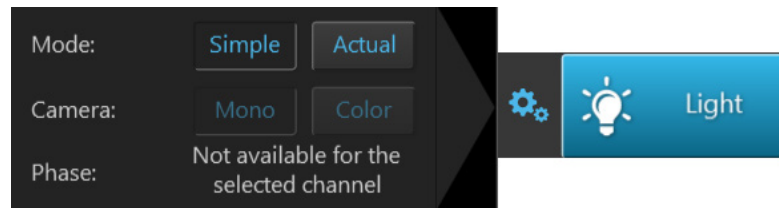
🔆 Light

2. For **Mode**, select **Simple** or **Actual**.
   – **Simple** mode allows you to control **Brightness** as a single parameter.
   – **Actual** mode allows you to adjust **Light** (i.e., LED intensity), **Exposure**, and **Gain** parameters individually.

3. For **Camera**, select **Mono** (monochrome) or **Color**.
   – **Mono** is used for image capture in fluorescence and transmitted light (brightfield) channels
   – **Color** is used for image capture in the brightfield channel only

4. *Optional*: Choose **Phase** options. Available options are:
   – **Small Ring:** Used for objectives with low magnification (i.e., Olympus™ 4× PH)
   – **Medium Ring:** Used for objectives with medium magnification (i.e., EVOS™ 4×/10× PH)
   – **Large Ring:** Used for objectives with high magnification (i.e., EVOS™ 20×/40× PH)
   – **Brightfield** (phase contrast off)

> **Note:** Phase options are available only for the transmitted light (brightfield) channel; they are not available for fluorescence channels. The phase contrast option does not require phase contrast objectives. However, to obtain a phase image, you must first install a phase contrast objective.

5. Click the **Brightness and camera settings** button again to collapse the controls.

Adjust brightness

1. Click the **Light** button to turn on the excitation light for the currently selected light source and enter the instrument in the **Live mode.**
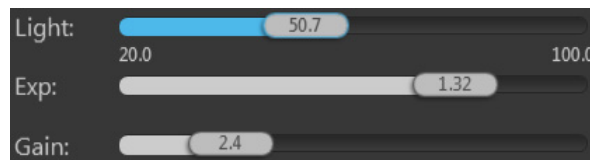

   In the Live mode, the Viewing Area shows the sample illuminated with the selected light source and the image is displayed in pseudo-color in the default emission color.

2. Adjust brightness using the **Brightness** controls:

   – In the **Simple mode**, adjust the brightness by moving the **Brightness** slider in the desired direction.

     Alternatively, double-click the slider handle to activate the brightness text box and directly enter the desired brightness value (0%–100%).

   – In the **Actual mode**, adjust the brightness parameters individually by moving the **Light** (LED intensity), **Exposure**, and **Gain sliders** in the desired direction.

     Alternatively, double-click the slider handle to activate the corresponding text box and directly enter the desired value for the selected parameter (0%–100% for Light, 0–4.00 seconds for Exposure, or 1.0–8.0 for Gain).

Note: For best results, optimize the brightness parameters as follows:

- **When searching for sample:** Increase **Gain** for a brighter signal and decrease **Exposure** for faster frame rate during navigation around the vessel.

- **When capturing image:** Decrease **Gain** to reduce background noise and increase **Exposure** to regain signal intensity, as needed.

- **For brighter signal:** Increase **Light** intensity for brighter illumination. If needed, follow by increasing **Gain**.

- **For time lapse imaging:** Increase **Gain** and **Exposure**, and decrease **Light** intensity to reduce photobleaching and phototoxicity.

  For example, for overnight time lapse experiments, capture one image every 30 minutes or less, limit the use of autofocus, and use a channel other than DAPI for autofocus.
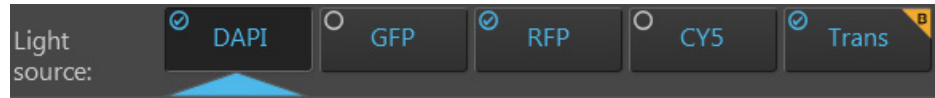
Set image display options

1. Select the **channels** you wish display in the Viewing Area by clicking on the small circular checkboxes on the upper left of the corresponding **Light source** buttons.

   You can select multiple channels to display in the Viewing Area. In the example below, DAPI, RFP, and transmitted light (brightfield) channels have been selected.

2. Click **Image Display settings** button to expand the controls for adjusting the image display parameters (brightness, contrast, gamma) for the selected channels.
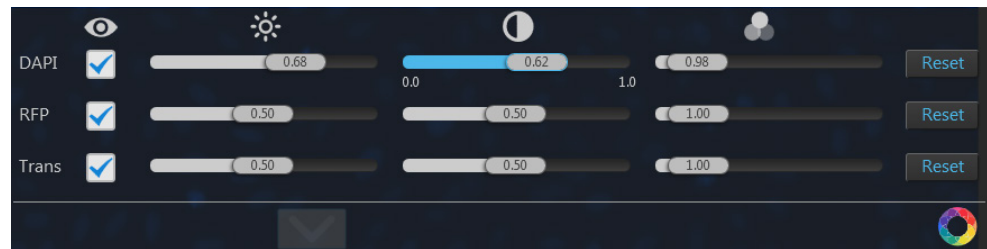
   Note: The controls for image display settings are contextual; only the controls for the selected channels will be available. In the example above, only the controls for the selected DAPI, RFP, and transmitted light (brightfield) channels are displayed.

3. *Optional*: To remove a channel from displaying in the Viewing Area, unselect the corresponding **Display** checkbox. To display an available channel not shown in the Viewing Area, re-select the checkbox.

4. Adjust the **Brightness**, **Contrast**, and **Gamma** settings for each of the selected channels using the corresponding sliders.

5. Click the **Image color display** button to display the sample pseudo-colored in the default emission color of the selected channels. By default, color display option is on.

6. Click the **Image Display settings** button again to collapse the controls.

   Note: Adjustments made to Image display settings only affect how the image is displayed in the Viewing Area; they do not change how the image is captured.

**Focus on the sample**

1. Click **Autofocus** to run the autofocus procedure to find the best focal plane for the current channel and sample.

   Alternatively, use the **Coarse** and **Fine** focus sliders to manually find the best focal position for the current channel and sample.
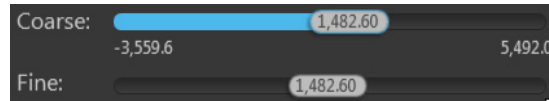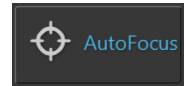
> **Note:** If you run the autofocus procedure with the light off, the autofocused image will be captured and stored in the image cache for that channel.
>
> If you run the autofocus procedure with the light on (i.e., with the **Light** button pressed), the instrument will simply find the optimal focus, but will not capture the autofocused image.

2. To choose a different autofocus method, click **Advanced focus settings** button located to the left of the Autofocus button, then select from the **AutoFocus Method** dropdown.
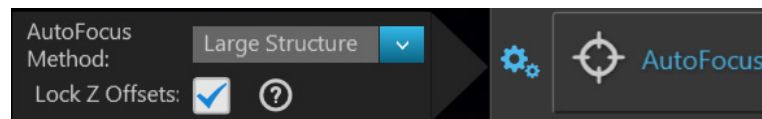

   Available options are:

   – **Small Structure:** Choose this option for samples with many fine, hair-like structures (e.g., filaments or structural stains).

   – **Large Structure:** Choose this option when your sample contains large structures (e.g., whole cell stains).

   – **Small Bright Objects:** Choose this option when capturing samples with localized staining (e.g., nuclei).

   For more information on advanced focus settings, see page 104.

Optional: Set Z-Offsets

Z-Offsets allow you to specify the optimal focus position in each channel relative to the focus position in other channels.
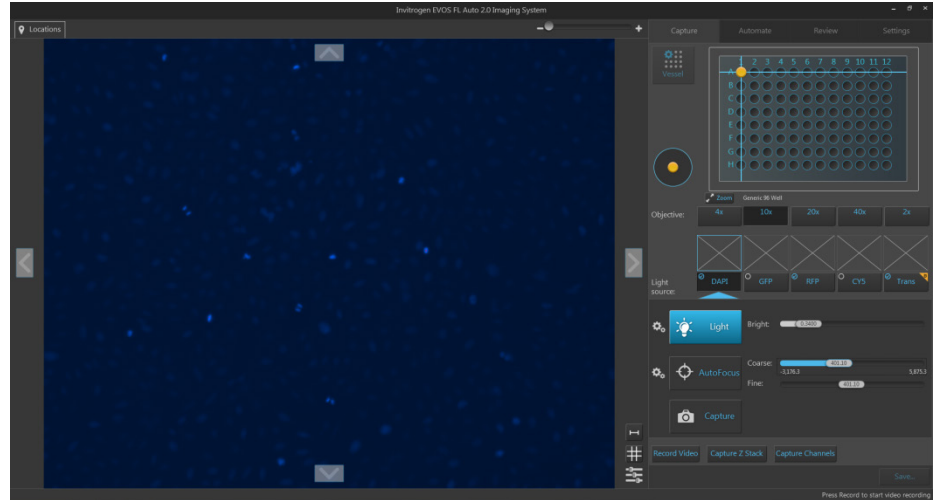
1. Verify that the **Lock Z-Offsets** option is checked in the Advanced focus settings window, then click the **Advanced focus settings** button again to close the window.
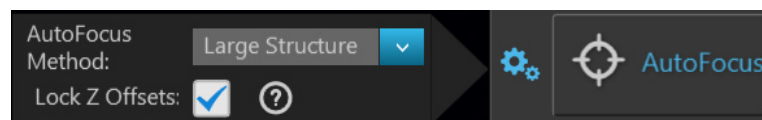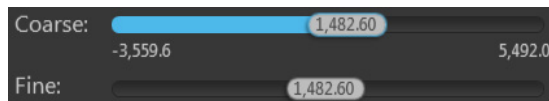

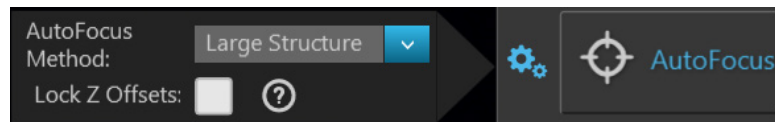2. Select the **Objective** and **Light source** (i.e., channel) you wish to capture.
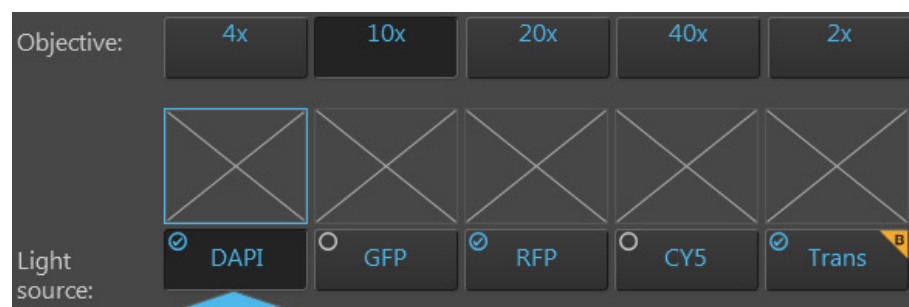
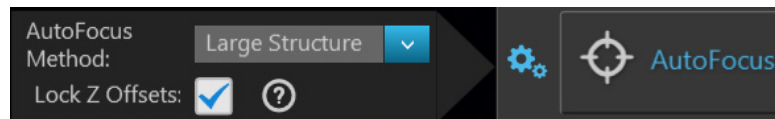3. Click the **Light** button to turn the light on and enter the Live mode.

4. Focus on the sample manually using the **Coarse focus** and **Fine focus** sliders or automatically by clicking the **AutoFocus** button.

    You can also double-click on the handle of the focus slider to activate the text box and enter the desired value for the focus position.

5. After you have found the optimal focus position in the first channel, click the **Advanced focus settings** button, then uncheck the **Lock Z-Offsets** option.


6. Without changing the objective, select the next **Light source** (i.e., channel) you wish to capture.

7. If the light is off, click the **Light** button to turn it on, then focus on the sample manually using the **Coarse focus** and **Fine focus** sliders or automatically by clicking **AutoFocus**.

8. Repeat this procedure for every channel you wish to capture.

9. When you have found the optimal focus position in all the additional channels you wish to capture, click the **Advanced focus settings** button, then check the **Lock Z-Offsets** option.


    When you have completed this procedure, the focus position in each selected channel will be offset relative to each other.

Find the region of interest in the Live mode

1. While in Field View, click the **Light** button and enter the instrument in the **Live mode.**

2. To go to a specific location on the sample vessel, click and drag the **crosshair** to the corresponding location on the virtual vessel.
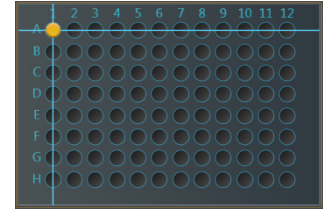
3. If needed, click on the **Zoom button** to open a larger view of the Vessel map for easier navigation to the desired location.
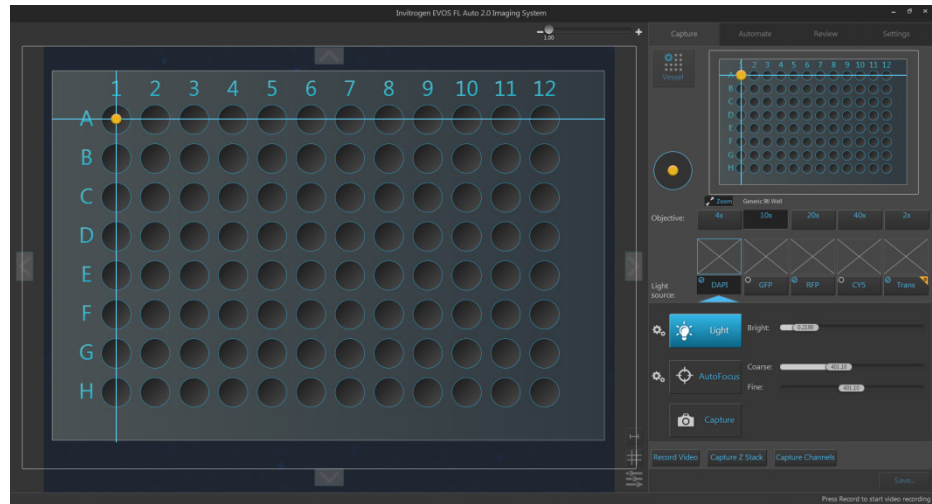

Click the **Zoom button** again to close the zoom window.

4. While in the **Live mode**, use the **Jog Control** to move the stage at an intermediate pace to the desired location as you view the sample until you find the field of view you wish to capture.


Alternatively, click on the **navigation arrows** (**up**, **down**, **left**, **right**) on the Viewing area to move in the corresponding direction exactly one field of view. You can also click and drag the field of view itself to move around the sample vessel.
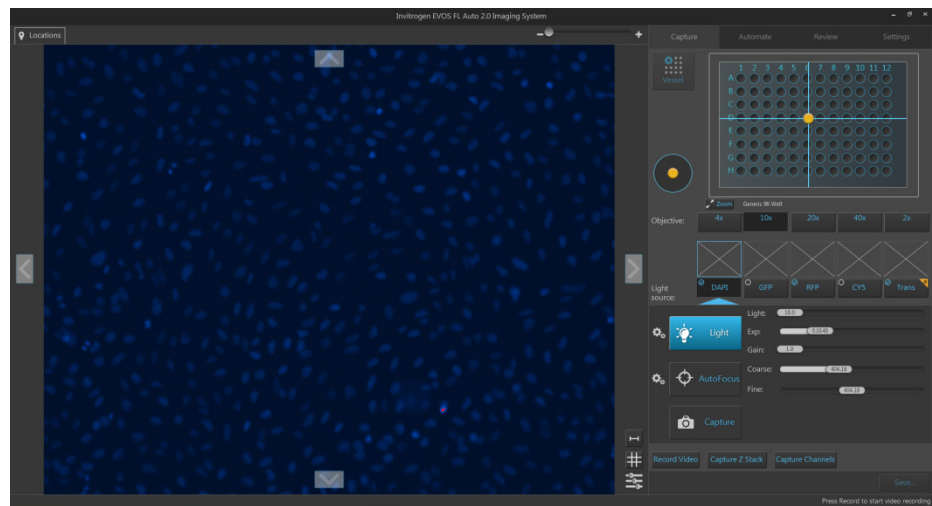
5. Click the **Light** button again to exit the Live mode in Field View.
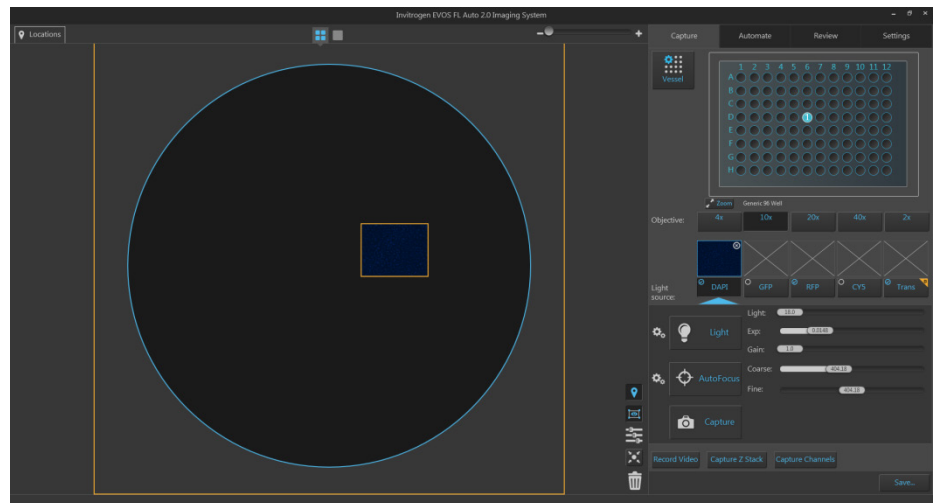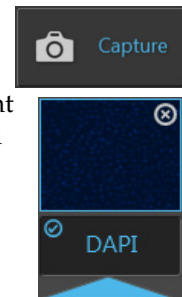
Select fields and capture images

1. In **Area View**, position the **capture crosshair** over the region of interest and click to select the field you wish to capture.

   Alternatively, find the field you wish to capture in the **Live mode**.
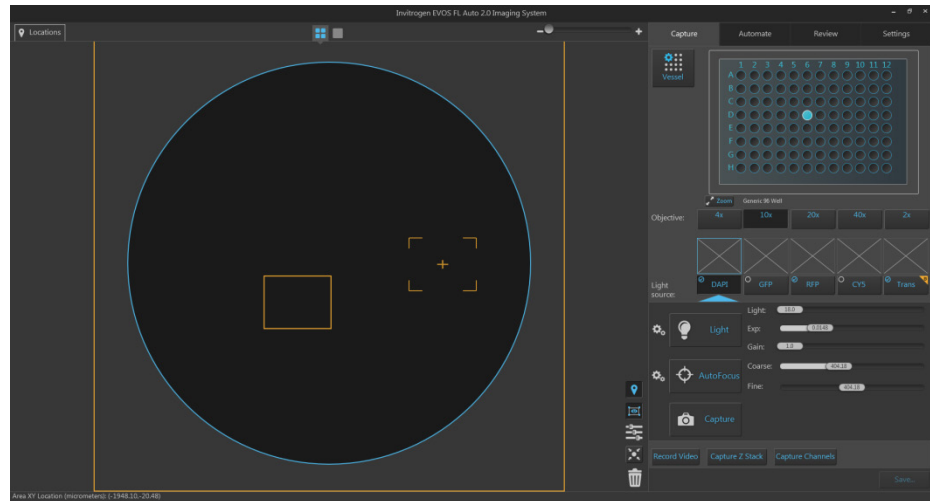
2. Click **Capture** to acquire an image of the selected field using the current capture settings.

   A thumbnail of the captured image is displayed above the Light source button for the channel in which the image was captured (in this example, DAPI).

   The Viewing area displays the captured image at the location of its capture when the zoom level is greater than one field of view.


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

3. To capture the same field in another channel, select the desired channel using the corresponding **Light source** button.


4. If needed, readjust **brightness** and **focus**, then click **Capture**.

A thumbnail of the captured image is displayed above the Light source button for the new channel in which the image was captured (in this example, RFP).
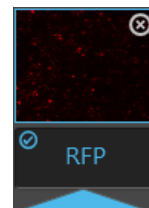
The Viewing area displays a multicolor overlay of the images captured in multiple channels at the location of their capture.

In this example, the second image was captured in the RFP channel, and the Viewing area displays a multicolor overlay of the images in the DAPI and RFP channels.
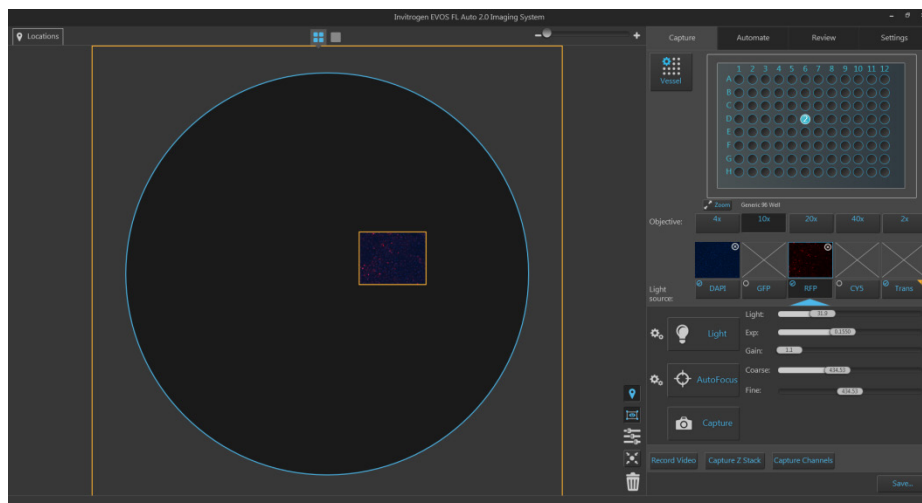
5. To zoom in on the Viewing area, use the **Zoom slider** located above the Viewing area.
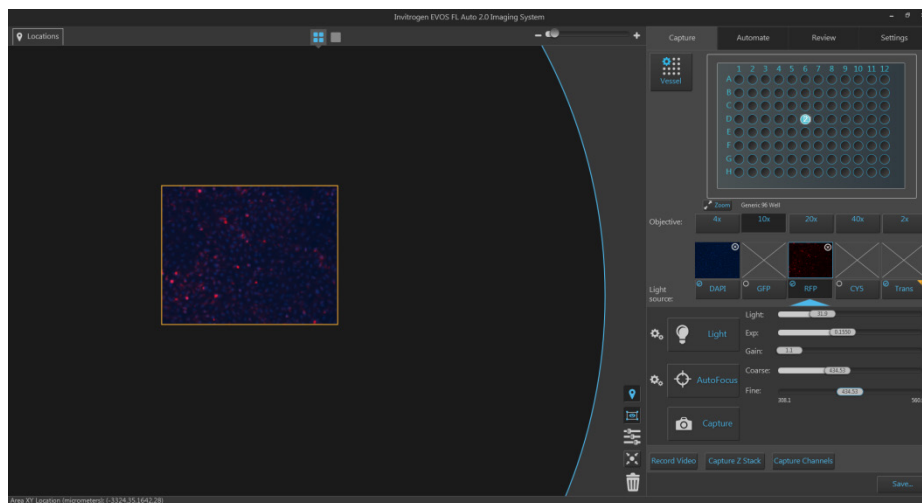
6. To capture another field, position the **capture crosshair** over the new region of interest, and click to select the new field.

   Alternatively, find the new field you wish to capture in the Live mode.

7. Click the **Light source** button to select the corresponding channel, readjust **brightness** and **focus** (if needed), then click **Capture**.

   In the example below, a second field was captured in the DAPI channel to the left of the first field, which was captured in DAPI and RFP channels.
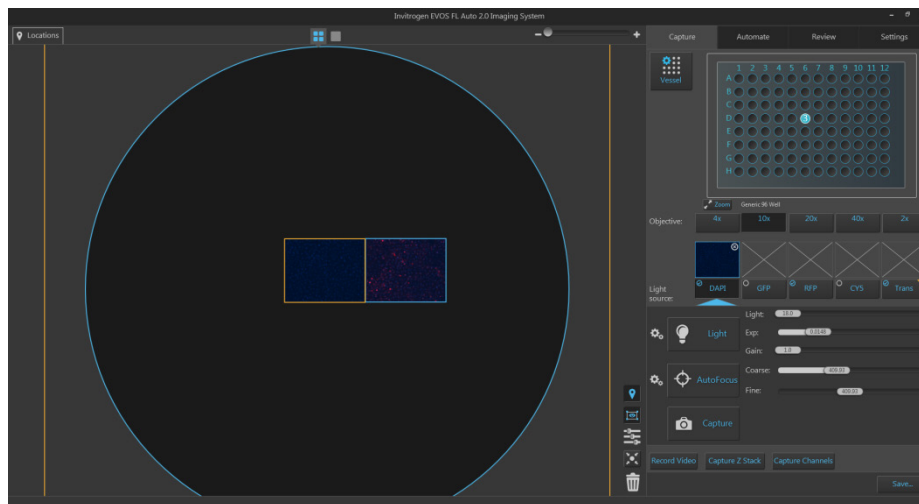

8. To capture a field using a different magnification, select the desired **Objective**.

9. Click the **Light source** button to select the corresponding channel. If needed, readjust **brightness** and **focus**.

10. Position the **capture crosshair** over the new field of interest, click to select the new field, then click **Capture**.

    In the example below, a third field located was captured in the DAPI channel using the 4X objective.
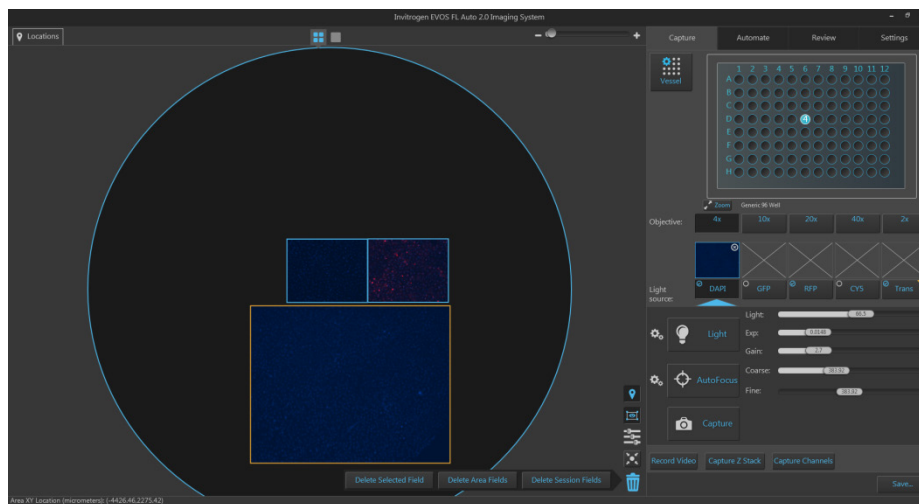
11. To save your captured images, click **Save…**. For more information, see "Save" (page 35).

Capture multiple channels simultaneously

1. To capture a selected field in multiple channels simultaneously, check the **Toggle channel display** option for the channels you wish to capture.

   The Toggle channel display checkboxes are located on the top left corner of the corresponding light source buttons. In the example below, DAPI and RFP channels have been selected.
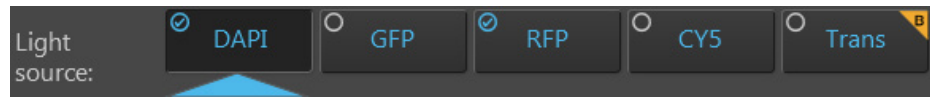
2. Click the desired **Objective** button to select the corresponding magnification. You can select only one objective at a time. In the example below, 4X objective has been selected.


3. Adjust **brightness** and **focus** for each of the selected channels as described before.

4. Position the **capture crosshair** over the new region of interest, and click to select the new field. Alternatively, navigate to the region of interest you wish to capture in the **Live mode**.

5. Click **Capture Channels**. The instrument captures an image of the selected field in each of the selected channels using the current capture settings.

   A thumbnail of the captured image is displayed above the Light source button for each channel in which it was captured.

   The Viewing area displays a multicolor overlay of the images captured in the selected channels at the location of their capture.

   In the example below, the selected field was captured in the DAPI and RFP channels using the 4X objective, and the Viewing area displays the merged image captured in these channels.
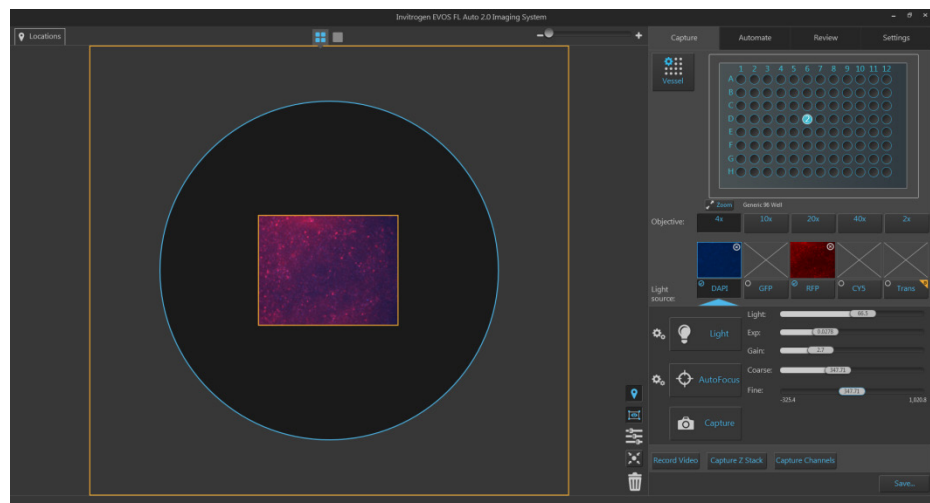
6. To save your captured images, click **Save…**. For more information, see "Save" (page 35).

Capture Z-Stack

Capture Z-Stack acquires multiple images of a selected field at different focal planes along the z-axis and combines them to generate a final image with a greater depth of field than any of the individual source images.

1. Select the **Objective** and **Light source** (i.e., channel) you wish to capture.


   Note: You can capture z-stack images in only one channel at a time; however, you can save the z-stacks images captured individually in separate channels into a single merged video (see "Save Z-Stack as a video", page 33).

   ---

2. Position the **capture crosshair** over the new region of interest, and click to select the new field. Alternatively, navigate to the region of interest you wish to capture in the **Live mode**.

3. Adjust **brightness** and **focus** using the corresponding sliders on the Capture tab. The focus position you set in this step is the Default Focus position.
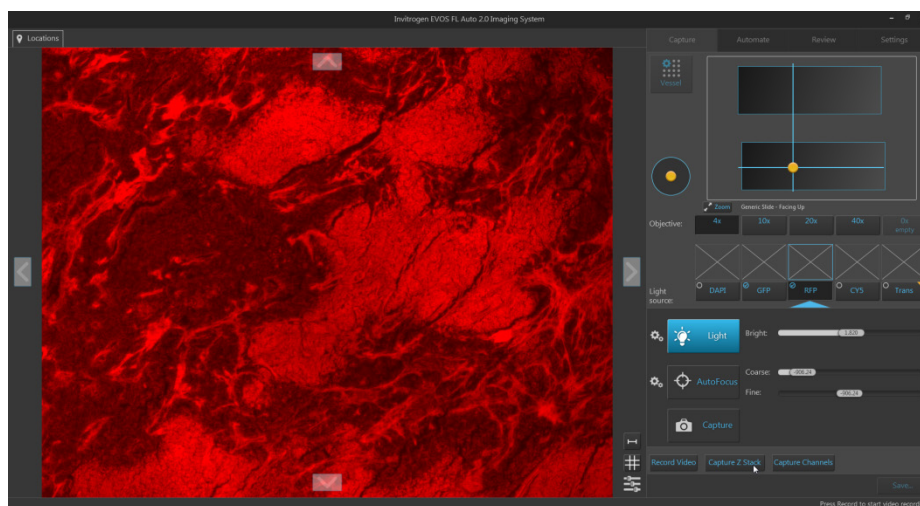

4. Click **Capture Z-Stack** to open the Z-Stack Settings window. You can move the Z-Stack Settings window anywhere on the screen.
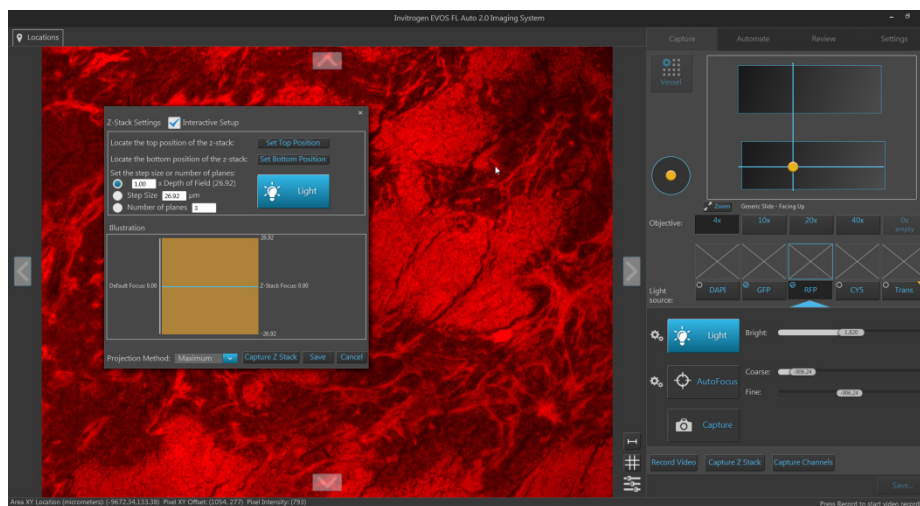

- To enter the z-stack parameters manually, go to Step 5 (page 28).
- To set the z-stack parameters interactively, go to Step 6 (page 29).

5. To set the z-stack parameters manually, enter the desired values for the following parameters, then proceed to Step 10 (page 29).
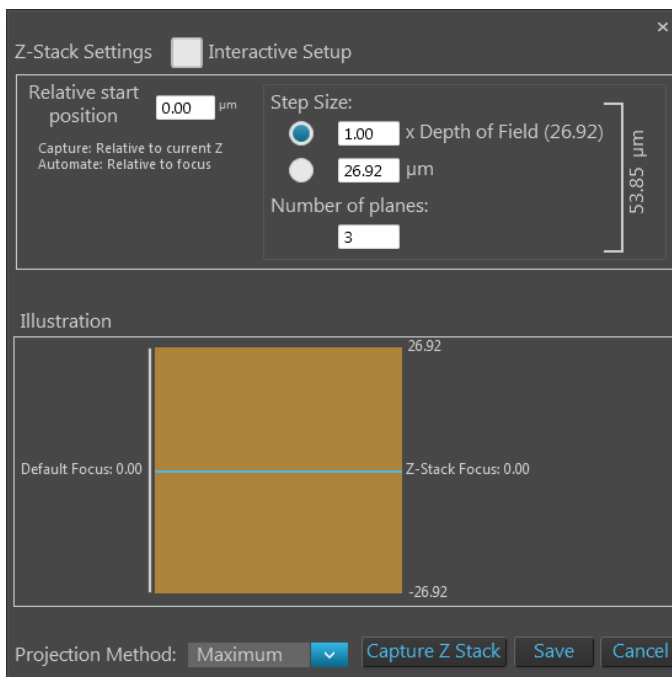
- **Start position relative to focus position:** Specifies the z-stack focus position (i.e., the middle point between the top and the bottom planes of the z-stack) relative to the default focus position.

- **Step Size:** Specifies the z-distance in μm between focal planes.

  You can designate the step size as a **multiple of Depth of Field** or directly enter the **Step Size** in μm in the corresponding text box.

- **Number of planes:** Specifies the number of focal planes that make up the z-stack.

Note: As you enter the z-stack parameters, **Illustration** is automatically updated to show the positions of the z-stack focus position, top and bottom planes relative to the default focus position, and the z-stack depth.
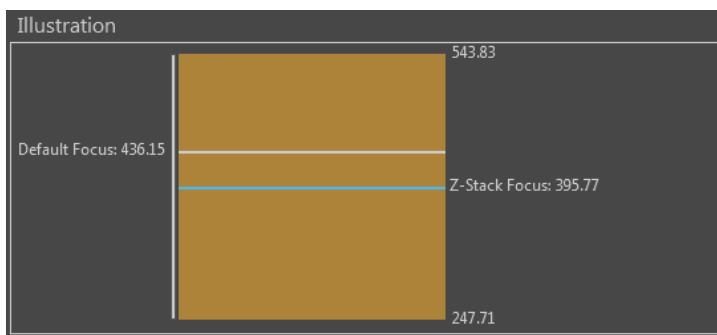
6. To set the z-stack parameters interactively, click the **Interactive** checkbox. The Z-Stack Settings window displays the interactive controls.
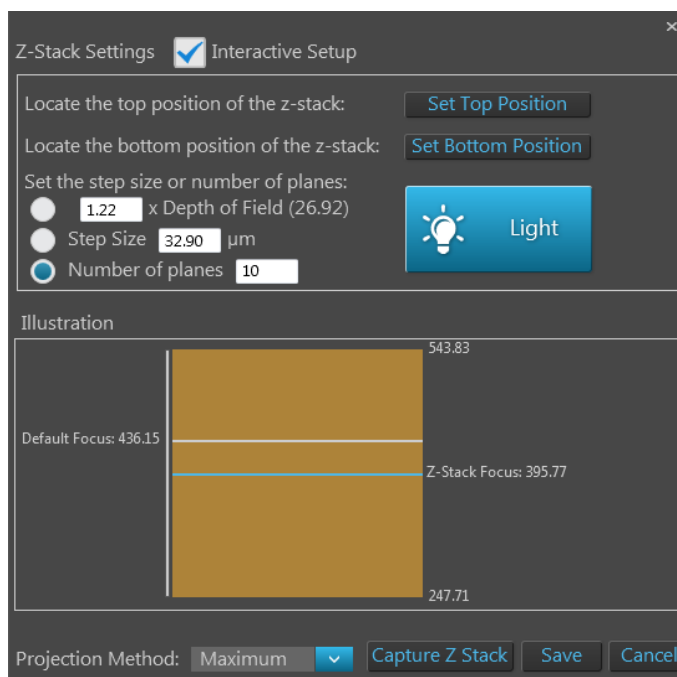
7. If not already on, click the **Light** button to illuminate the sample as you locate the top and bottom boundaries of the z-stack.

8. Using the **focus** sliders on the Capture tab, locate the top position of the z-stack, then click **Set Top Position**.

   Repeat the procedure for the bottom position, then click **Set Bottom Position**.

   This sets the top and bottom boundaries of the z-stack image set.

9. Enter the **Step Size** (in μm) or the **Number of planes** in the corresponding text box, or define the z-stack as a multiple of **Depth of Field**.

   These parameters determine the number of "optical sections" captured in the z-stack.

10. Select the **Projection Method** from the dropdown menu.

    This is the mathematical algorithm used to extract the most in-focus pixels from the images captured at different focal planes when generating the z-stack projection. Available options are:

    – **Maximum:** For fluorescent images that are bright on dark background.

    – **Standard deviation** (StDev)**:** For unstained transmitted images where the objects are identified by the contrast instead of the intensity.

    – **Average:** For fluorescent or histochemically-stained transmitted images when assessing concentration of the stain/marker.

11. To save the z-stack parameters you have set up, click **Save**. This allows you to capture z-stack images in other channels using the same specifications.

    Note: Clicking **Save** closes the Z-Stack Settings window. To capture z-stack images, click **Capture Z-Stack** to re-open the Z-Stack Settings window.

12. To capture the z-stack, click **Capture Z-Stack** in the Z-Stack Settings window. The instrument captures multiple images of the sample along the z-axis based on your specifications.

A thumbnail of the z-stack projection is displayed above the Light source button for the channel in which the z-stack images were captured.

The z-stack icon on the top left corner of the thumbnail image identifies it as a z-stack projection.
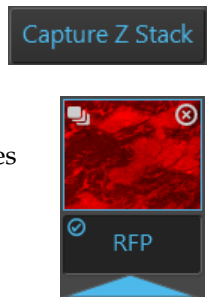
13. The Area View displays the z-stack projection at the location of its capture.

The z-stack icon on the top left corner of the captured field identifies it as a z-stack projection.
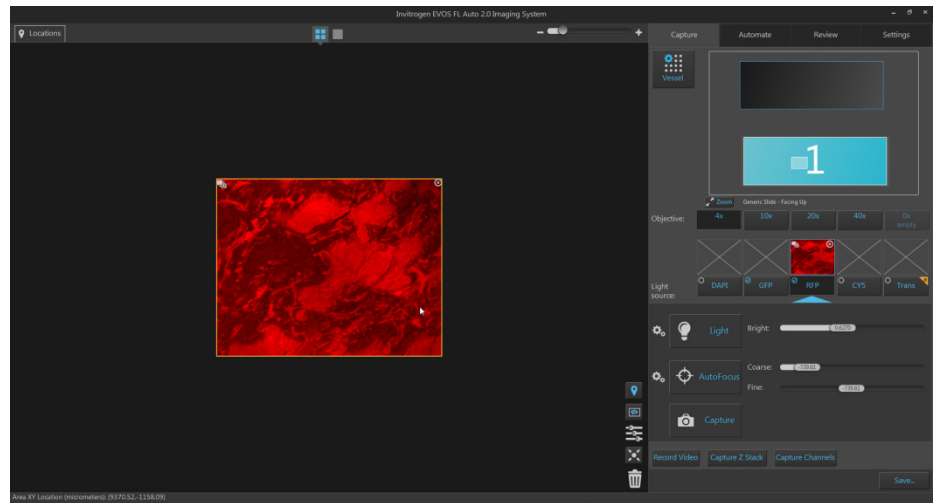
14. To view the individual planes that make up the z-stack image set, double-click the field that shows the z-stack projection in Area View or click the Field View icon.

The Field View displays the z-planes and the z-stack controls appear to the left of the Viewing area.
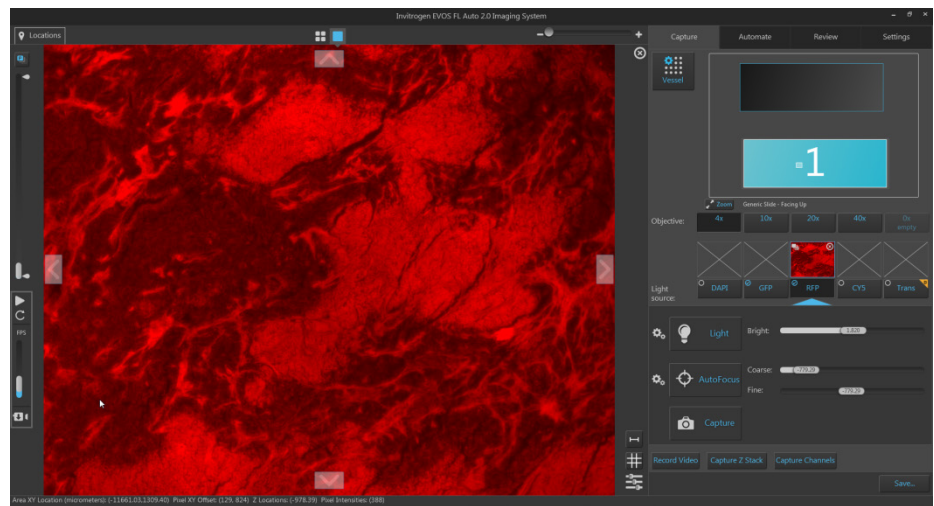
15. Move the **z-stack slider** up or down to view the z-planes (i.e., the "optical sections") in the sequence they were captured.

    Move the slider up to display the z-planes towards the top position of the z-stack.

    Move the slider down to display the z-planes closer to the bottom position.

    The z-slice number that is displayed above the slider handle as you move it up or down indicates the order of the plane within the z-stack.

16. To view the the z-stack projection, click the **Toggle Projection Image** button.

    The Viewing area displays the z-stack projection, which is generated by combining the most in-focus pixels from each z-plane. By default, the projection image is on.

17. To view the z-planes in sequence as a movie, click the **Start z-stack playback** button located below the z-stack slider.

    The Viewing area displays the z-planes in sequence, starting at the bottom slice (Z-plane: 1) and stopping at the top.

18. To play the z-stack movie in a loop, click the **Repeat playback** button.

    The Viewing area will display the z-planes in a continuous loop, starting at the bottom slice and looping back to beginning after displaying the top slide.

    To change the playback speed, move the **Playback speed** slider up (faster) or down (slower). The playback speed is expressed in frames per second (FPS).
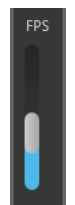
   To stop the z-stack movie, click **Stop z-stack playback**.

Note: During playback, individual z-planes that make up the z-stack are shown sequentially in the channel in which they were captured. The projection image is not displayed (i.e., the Toggle Projection Image is unselected).

19. To limit the z-stack movie to specific z-planes, drag the **z-stack calipers** to the desired z-planes to set upper and lower boundaries.

When replaying the z-stack movie or saving it as a separate video file, only the z-planes that fall within the z-stack calipers are included in the video file.

20. To capture z-stack images in another channel, select the next **Light source** (i.e., channel) you wish to capture, then repeat the procedure described above.
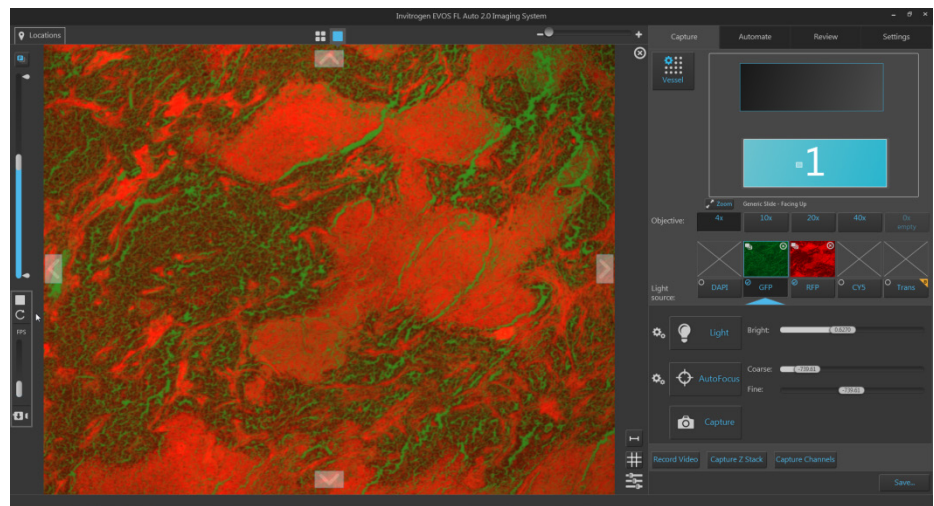
Note: To view the z-stack images captured in multiple channels as an overlay, check the **Toggle channel display** option for the channels you wish to include in the overlay.

The Toggle channel display checkbox is located on the top left corner of the corresponding light source button. In the example below, GFP and RFP channels have been selected.
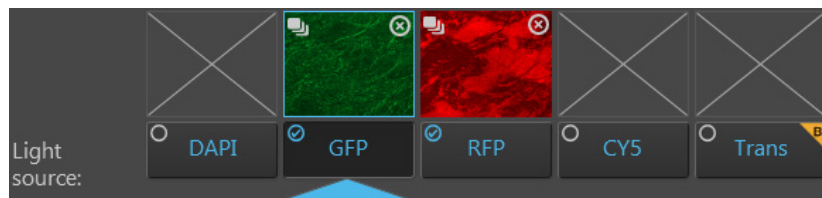
21. To save the z-stack movie as a separate file, see "Save Z-Stack as a video" (page 33).

22. To save the z-stack projection and individual z-planes for each field and each channel as separate images, click **Save…** to open the Save dialog. For more information, see "Save", page 35.

Save Z-Stack as a video

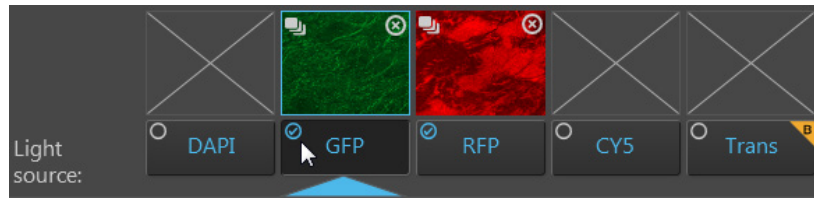1. Check the **Toggle channel display** option for the channels you wish to include in your z-stack video. In the example below, GFP and RFP channels contain z-stacks and both have been selected for the z-stack video.

   Note: Selected channels must contain captured z-stack images to be included in the z-stack video. If multiple channels with z-stacks are selected, the z-stack video displays an overlay of the z-stack images captured in different channels.

2. Click **Save as Video** icon to save the z-stack images as a video sequence. The Save Z-Stack Videos dialog opens.
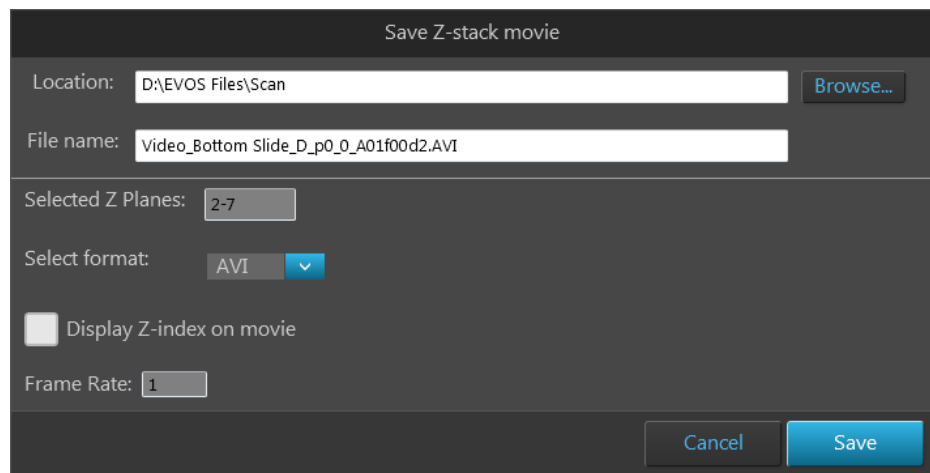
3. Click **Browse**, navigate to the folder in which you wish to save your z-stack video, then click **Select**.
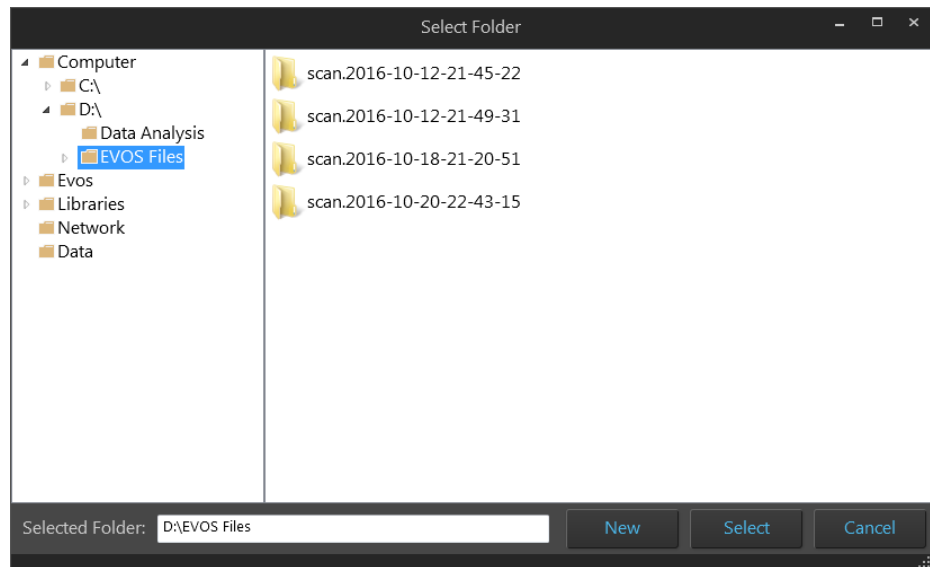
   Note: We recommend that you save your captured images to an external hard drive.

Alternatively, click **New** to create a new folder at the desired location, type in the name of the newly created folder, then click **Select**.

4. If desired, enter a new name for the z-stack video file in the **File name** textbox.

5. Confirm that the **Selected Z Planes** text box displays the correct z-indices for the planes that you wish to include in your z-stack video.

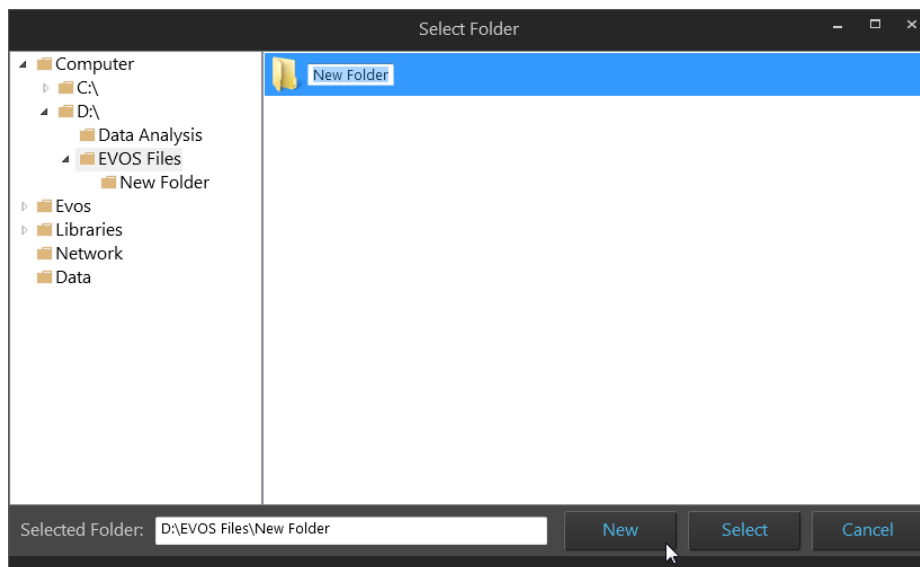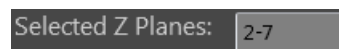> **Note:** When saving the z-stack movie as a separate video file, only the z-planes that fall within the z-stack calipers are included in the movie.
>
> To change the z-planes you wish to include in the z-stack movie, click **Cancel** and readjust the **z-stack calipers** as described on page 32.

6. Select the **file format** for the z-stack video from the dropdown menu. Available options are **AVI** and **WMV**.

7. To display the z-index of each plane (i.e., the order within the z-stack) in the video, check **Display Z-Index on movie**, then select the desired **Location**, **Font Size**, and **Font Color** from the drop-down menus.

By default, this option is not checked.
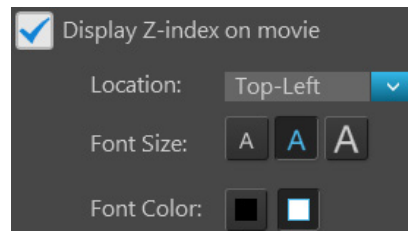
8. Confirm that the **Frame rate** text box displays the correct **Playback speed** for your z-stack video.

> **Note:** The **Frame rate** of your z-stack video sequence is set using the **Playback speed** slider. To change the Frame rate for your z-stack movie, click **Cancel** and readjust the Playback speed slider as described on page 32.
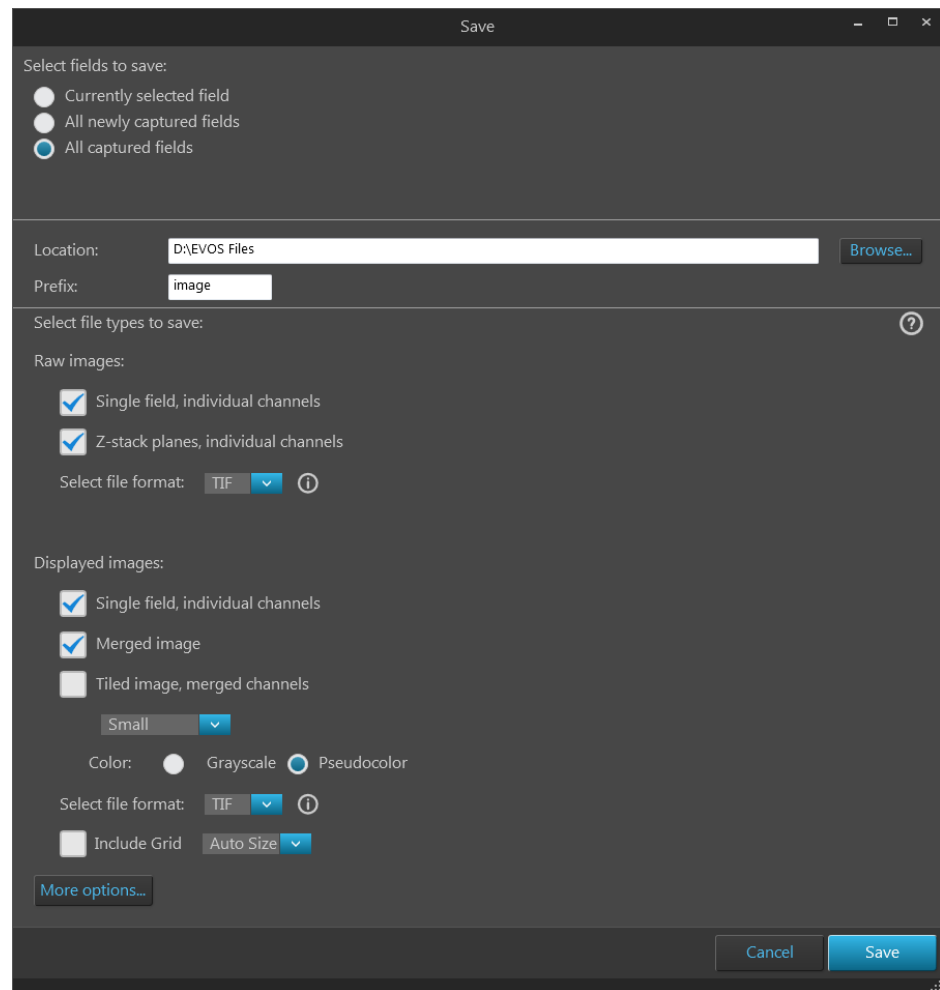
9. Click **Save** to save the z-stack images as a video sequence based on your specifications.

Save

Save captured images

1. When finished capturing images, click **Save…** to open the Save window.

2. Select the captured fields you wish save. Available options are:

    - **Currently selected field:** Saves images only from the currently selected field.

    - **All newly captured fields:** Saves images that have been captured and stored in the image cache, but not yet saved. This option is available only if you have previously saved images from the same session.

    - **All captured fields:** Saves images from all captured fields that are held in the image cache. This is typically all of the images that you captured during an imaging session.

    Note: If the software remains open while users access the instrument back to back, there may be several sets of images stored in the image cache. To clear these images from the cache, click the trash can icon (see "Delete image options", page 88).

    Saving the same images in the cache again does **not** overwrite the existing files in the destination directory; newly saved files simply have a differentiator added to the name.

3. Click **Browse**, navigate to the folder in which you wish to save your captured images, then click **Select**.
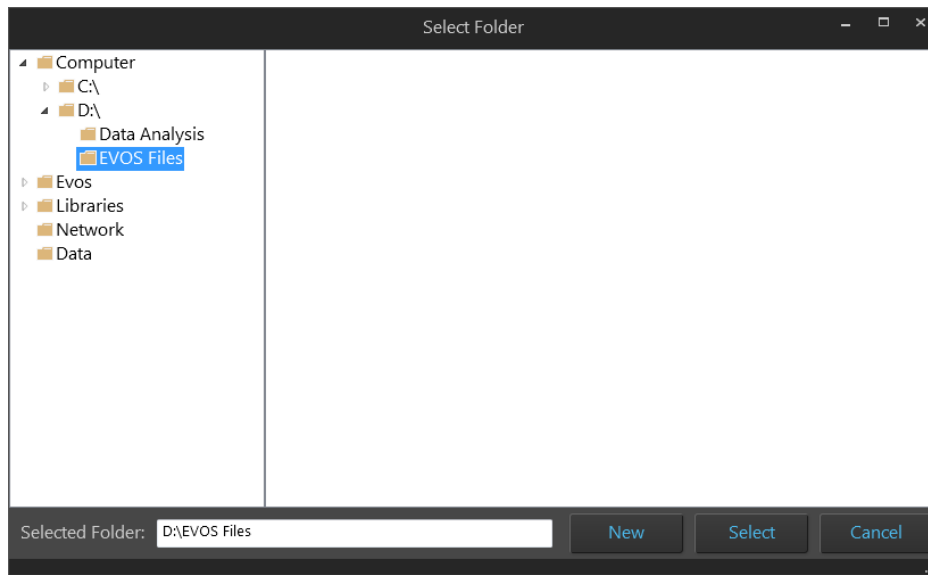
To create a new folder in which to save your captured images, navigate to the desired location, click **New**, type in the name of the newly created folder, then click **Select**.
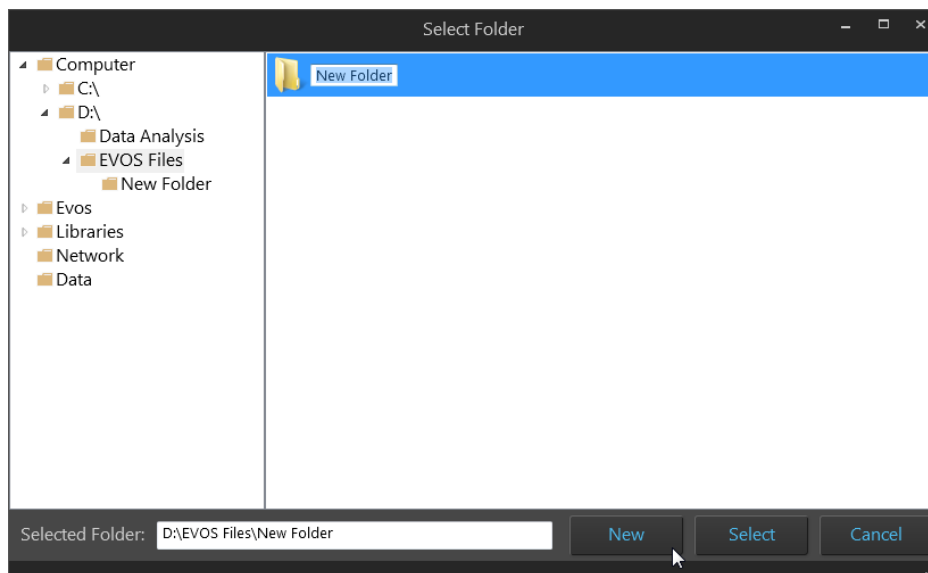
Note: We recommend that you save your captured images to an external hard drive.

4. If desired, type the prefix you wish to use for your save images in the **File name prefix** textbox.

Note: For an overview of the file naming convention for saved images, see "File naming convention", page 148.

5. Select **File types to save** by checking the corresponding checkboxes. You can choose multiple file types for your captured images.

    Available options for file type are **Raw images** and **Displayed images**.

    - **Raw images:** Saves images captured in different channels individually as 16-bit raw images. This is the recommended format for image analysis.

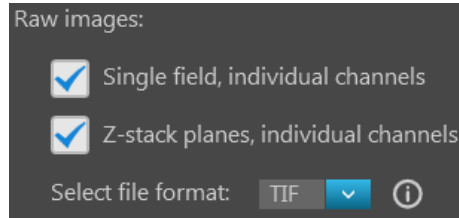   Available **File format** for raw images are **TIF**, **PNG**, **C01**, and **DIB**.

    - **Displayed images:** Saves images in a format that can be viewed in most image display applications.
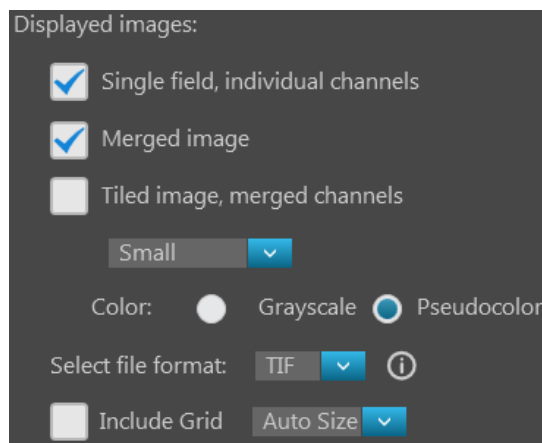
        Available **Color** options for displayed images are **Grayscale** (16-bit) and **Pseudocolor** (24-bit RGB; 8-bit per RGB channel).

        Available **File format** for displayed images are: **TIF**, **PNG**, and **JPEG**.

        If desired, check the **Include Grid** option and select the grid size.

    Note: While pseudocolors help differentiate the channels used in multi-channel overlays, grayscale images usually show more detail. 24-bit images (8-bit per RGB channel) are NOT recommended for image analysis as not all channels will display in many image analysis applications.

6. To see more file type options in a table format, click **More options…**

Full menu of save options are displayed in a convenient table format, allowing you to save your captured images in a number of formats simultaneously.

7. Select the desired file types and save options by checking the corresponding checkboxes. You can select multiple file types and options.

Available file type options are **Raw images** and **Displayed images**.

- **Raw images**:Saves each channel individually.
  - **Single field**, **Z-Stack planes**, and **Tiled images** can be saved as raw images.
  - Available **File format** for raw images are **TIF**, **PNG**, **C01**, and **DIB**.
- **Displayed images:** Saves each channel individually or as a merged image in a format that can be viewed in most image display applications.
  - **Single field**, **Z-Stack planes**, and **Tiled images** from individual channels can be saved as displayed images.

    However, **Z-planes** can only be saved from individual channels.

    Single fields and **Tiled images** can be saved from individual channels or as merged view.
  - Available **Color** options for displayed images are **Grayscale** (16-bit) or **Pseudocolor** (24-bit RGB; 8-bit per RGB channel).
  - Available **File format** for displayed images are **TIF**, **PNG**, and **JPEG**.

8. After you selected save options for your captured images, click **Save**.

Note: For more information on each save option available for raw and displayed images, see the detailed descriptions in "Appendix C: Graphical user interface" under **Capture tab ▶ Save** (page 111).

4. Create and run automated scan

Overview

Automate tab

The EVOS™ FL Auto 2 Imaging System allows you to create and recall scan protocols, which are a series of automatically executed steps to capture multiple images over an area and/or time period based on your specifications. For repeat experiments, automated scan protocols can be saved, recalled, even edited.

The scan protocols are created using the **Automate tab**, which is organized into a series of panels that contain the necessary controls that organized by functionality (i.e., Load, Hardware, Scan Area, AutoFocus and Z Stacks, Time Lapse and Incubator, Image Save Settings, Save, and Run).

You can access the **Capture**, **Review**, and **Settings** at any time by selecting the corresponding tab.

Note: For a detailed description of each of the Automate tab controls, see "Automate tab" in "Appendix C: Graphical user interface (GUI)", page 114.

Workflow

Set hardware options
▼
Select scan areas
▼
Set Z-Offsets in Live mode
▼
Configure AutoFocus settings
▼
Set Time Lapse options
▼
Set incubator options
▼
Configure image save settings
▼
Save automated scan protocol
▼
Run automated scan protocol
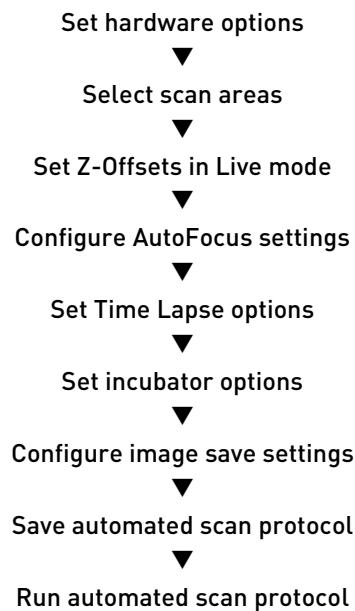
Create a scan protocol

Set hardware options

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

   For the types of vessel holders available for the EVOS™ FL Auto 2 Imaging System, visit **thermofisher.com/evos** or contact Technical Support (page 175).

2. On the **Capture tab**, click the **Vessel** button to open the Vessel Selection wizard, then select the sample vessel as described on page 16.

   Note: If you are using the EVOS™ Onstage Incubator, set it up for operation as described in "Appendix D: EVOS™ Onstage Incubator", page 156.
   ---

3. In the **Automate tab**, click **Hardware ▶ Edit** to open the Hardware panel.

   The controls in this panel allow you to select the objective and the channels you wish to use for the automated scan protocol, and to adjust channel settings.
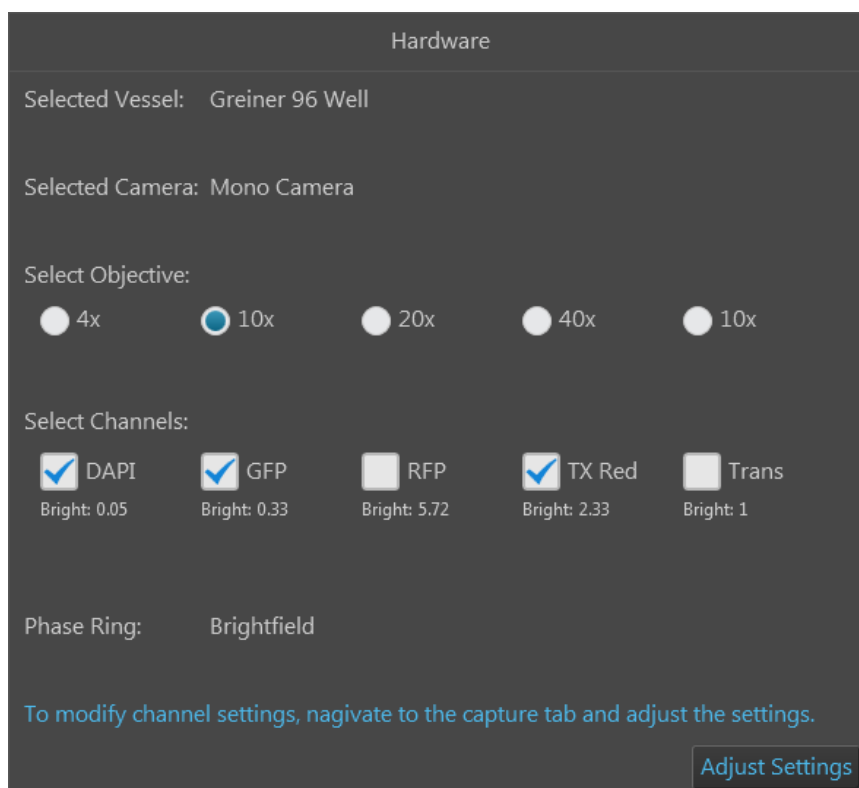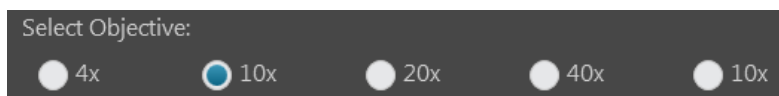

   Note: For a detailed description of the controls available in the Hardware panel, see "Hardware" in "Automate tab", page 115.
   ---

4. Select the **Objective** you wish to use for the scan protocol. You can select only one objective at a time. In the example below, 10X objective has been selected.

5. Select the **Channels** you wish to use for the scan protocol. You can select multiple channels. In the example below, DAPI, RFP, and TX Red channels have been selected for capture.
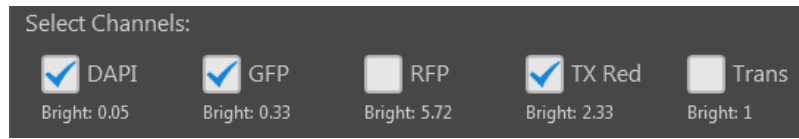

6. To modify channel settings, click **Adjust Settings** to navigate to the Capture tab.

7. Click **Brightness and camera settings** button to configure the **Mode**, **Camera**, and **Phase** options as descibed in "Set brightness and camera options", page 17.
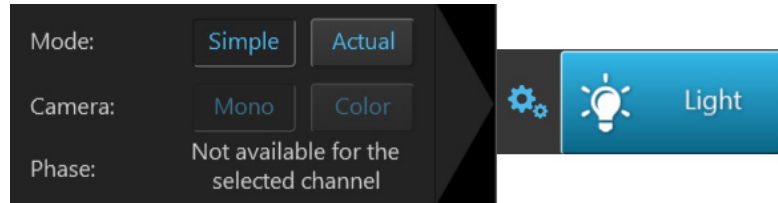
8. Click the **Light** button to turn on the excitation light for the currently selected channel (i.e., Light source) and enter the Live mode.

9. While in the Live mode, adjust the brightness settings using the **Brightness** controls as described in "Adjust brightness", page 18.

10. If you have selected the **Simple mode**, adjust the brightness by moving the **Brightness** slider in the desired direction.
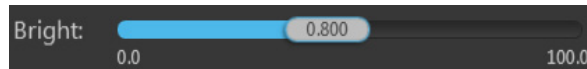
If you have selected the **Actual mode**, adjust the brightness parameters individually by moving the **Light** (LED intensity), **Exposure**, and **Gain sliders** in the desired direction.
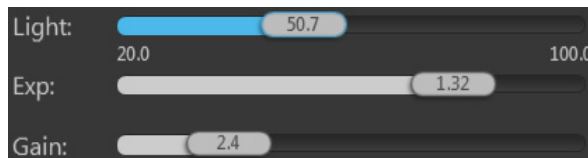
11. While still in the Live mode, select each of the remaining channels that will be used for the automated scan and adjust the brightness settings.

12. When finished with the channel settings, click the **Automate** tab to return to the Hardware panel.

13. Click **Done** to save hardware options, then proceed to "Select scan areas", page 42.

Select scan areas

1. In the **Automate tab ▶ Scan Area**, click the **Edit** button to open the Scan Area panel. The controls in this panel allow you to define the areas and fields you wish to scan and specify the order in which they are captured.
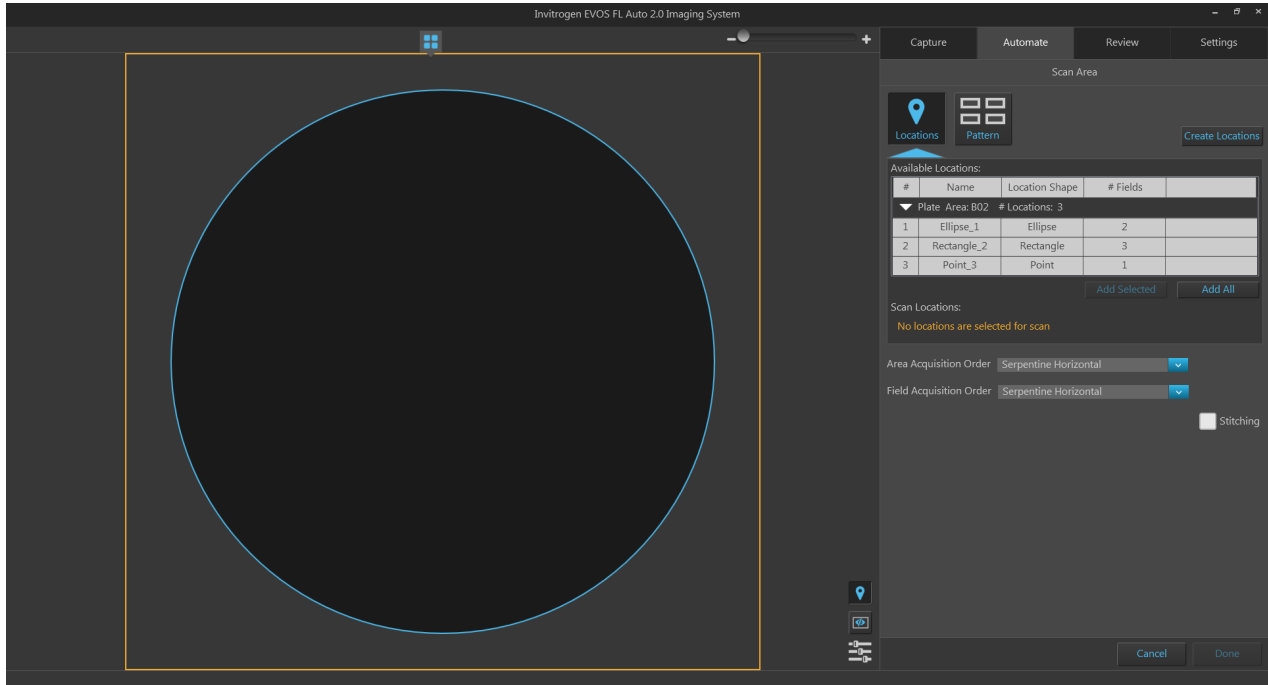
Note: For a detailed description of the controls available in the Scan Area panel, see "Scan Area" in "Automate tab", page 116.

To define the areas and fields you wish to scan, you have two options:

- **Scan Locations** tool (page 118) assigns scan regions based on the selections made with the **Locations** tool (page 92).

  To use the Scan Locations tool to select regions for automated scan, go to Step 2 (page 43).

- **Scan Pattern** tool (page 123) allows you to define the capture regions for the scan protocol as a pattern based on defined parameters.

  To use the Scan Pattern tool to select regions for automated scan, go to Step 8 (page 45).
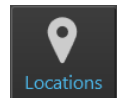
2. Click the **Select Scan Locations** button to open the **Scan Locations** tool.

- If you have already defined locations using the **Locations** tool, they will be listed in the Available Locations table.
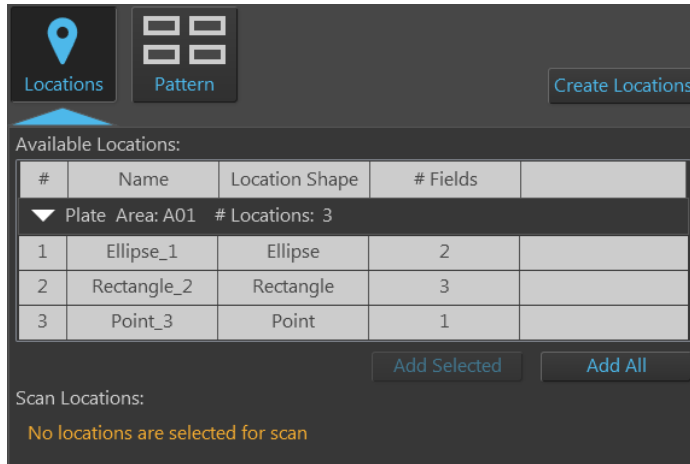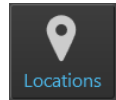
- If there are no previously defined locations, the Available Locations table displays the message "No locations are available for selection".
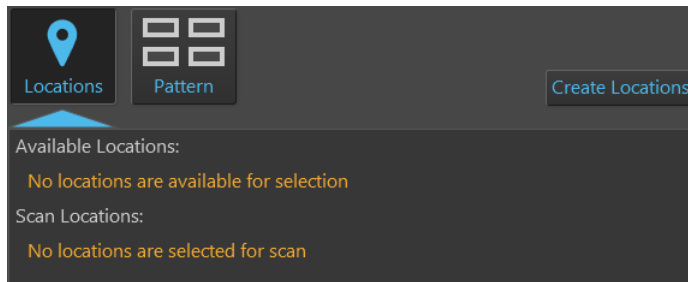
3. To create new locations for the scan protocol, click **Create Locations** to open the **Locations** tool (page 92).

| Locations – Area View | Locations – Field View |

4. Using the Locations tool, define the locations as described (page 93), then navigate back to the **Automate tab ▶ Scan Locations**.

Newly created locations will be listed in the Available Locations table.

#	Name	Location Shape	# Fields	
▼ Plate Area: A01 # Locations: 4				
1	Ellipse_1	Ellipse	1	
2	Rectangle_2	Rectangle	0	
3	Point_3	Point	1	
4	Freeform_4	Polygon	2	

Available Locations:

In the following example, the first location in the list (an ellipse) is selected. The Viewing are displays the selected location and the field group that best fits the shape of the location.

5. To add a location to the scan protocol, click the desired location in the Available Locations table to select, then click **Add Selected**.

To add multiple locations to the scan protocol simultaneously, Shift-click the desired locations in the Available Locations table to select them, then click **Add Selected**.

To add all of the locations listed in the Available Locations table, click **Add All**.

The locations added to the scan protocol are displayed in the **Scan Locations** table and removed from the Available Locations table.

Available Locations:

#	Name	Location Shape	# Fields	
▼ Plate Area: A01 # Locations: 1				
1	Ellipse_1	Ellipse	2	

Add Selected   Add All

Scan Locations:

#	Name	Location Shape	# Fields	
▼ Plate Area: A01 # Locations: 2				
2	Rectangle_2	Rectangle	3	
3	Point_3	Point	1	

Assign scan area   Remove Selected   Remove All

6. To assign a scan area for the selected scan locations, click **Assign scan area** to open the **Vessel map**, then select the desired areas from the Vessel map to define the scan area as described (page 122).
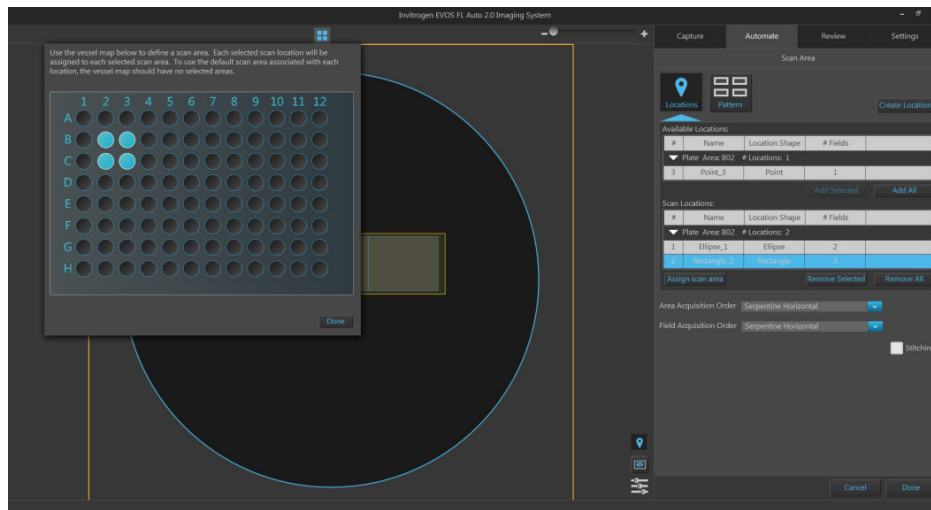
7. Click **Done** to close the Vessel map and exit the area selection mode.

8. If you wish to to define the capture regions for the scan protocol as a pattern based on defined parameters, click the **Select Scan Pattern** button to open the **Scan Pattern** tool.
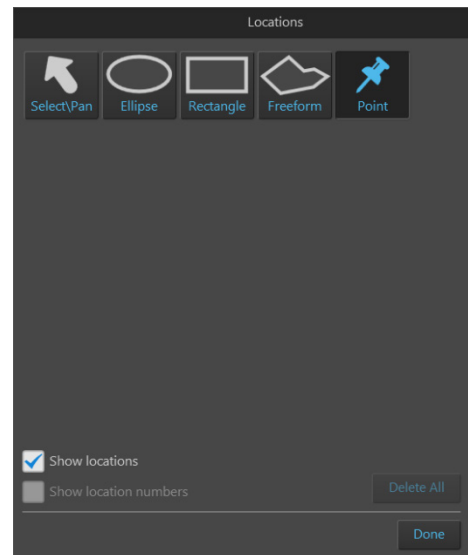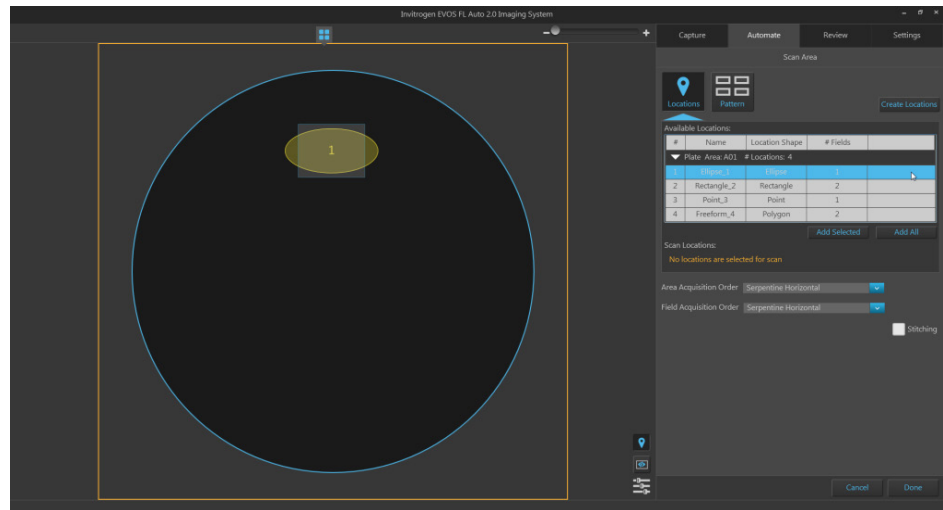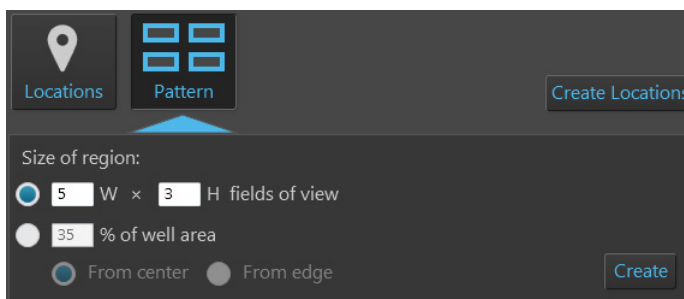

- To define the capture region as a matrix, select **W × H fields of view**, then enter the desired dimensions into the corresponding text boxes. You can only enter integers (page 123).

- To define the capture region as a percentage of area covered by scan fields, select **% of well area**, enter the desired value (an integer between 1 and 100) into the text box, then select **From center** or **From edge** (page 124).

9. When you are finished defining the parameters for the capture pattern, click **Create**.

Note: The region you define with the Field selection tools in Area View will be replaced by a set of capture fields that best fit the drawn shape or, in the case of the Pattern tool, satisfy the criteria set for the region (see examples on pages 123–124).

In a multi-well plate, multi-chamber slide, or multi-vessel holder, the fields defined in a selected well, chamber, or vessel holder will also apply to the other selected wells, chambers, or vessel holders.

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10. If needed, click the **Delete field group** button that is displayed when you hover over the field group to delete that field group.

   To delete all field groups from Area View, click the **Clear field selections** button located at the bottom right corner of Area View.

11. To assign a scan area for the selected scan locations, click **Assign scan area** to open the **Vessel map**, then select the desired areas from the Vessel map to define the scan area as described (page 122).
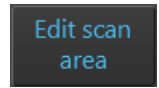
   If you wish to view a larger version of the Vessel map, click the **Zoom button**. Click the zoom button again closes the zoom window.
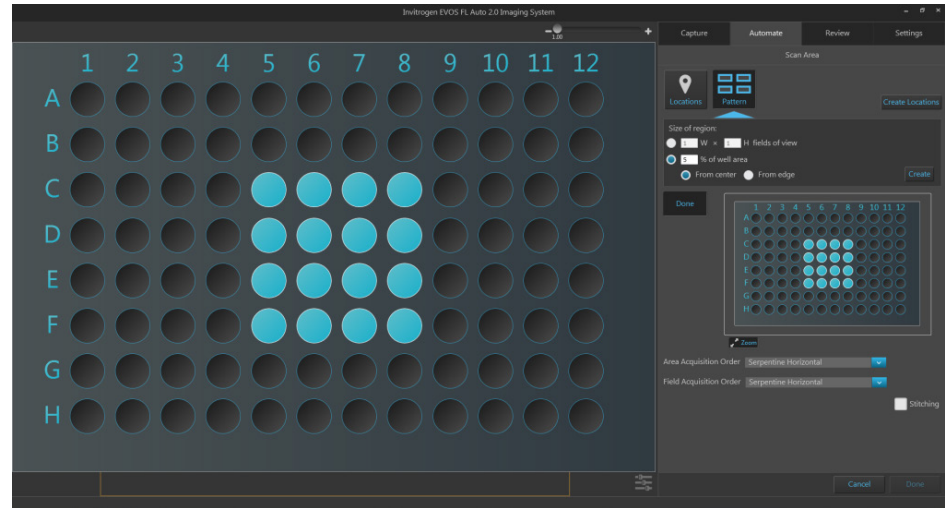
12. When finished defining the scan areas, click **Done** to exit the selection mode.

13. Click **Area Acquisition Order** to you specify the order in which selected areas are captured in the automated scan protocol.

   Available options are:

   – **Serpentine horizontal**

   – **Serpentine vertical**

   – **Random selection**
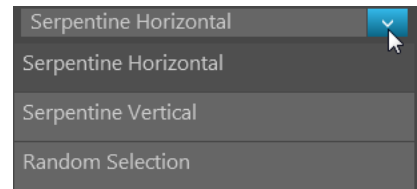
14. Click **Field Acquisition Order** to you specify the order in which the fields are captured in each selected area.

   Available options are:

   – **Spiral Outward Counterclockwise**

   – **Spiral inward Clockwise**

   – **Serpentine Horizontal**

   – **Serpentine Vertical**

   – **Random Selection**
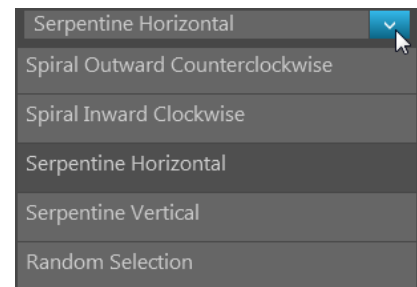
15. To use stitching in your scan area, check the **Stitching** box.

    Stitching function applies an overlap when assembling captured images into a mosaic image of the scan area.
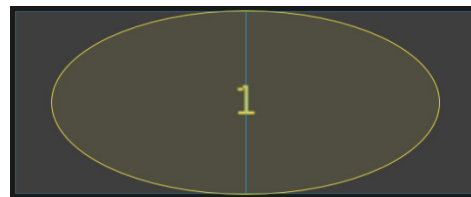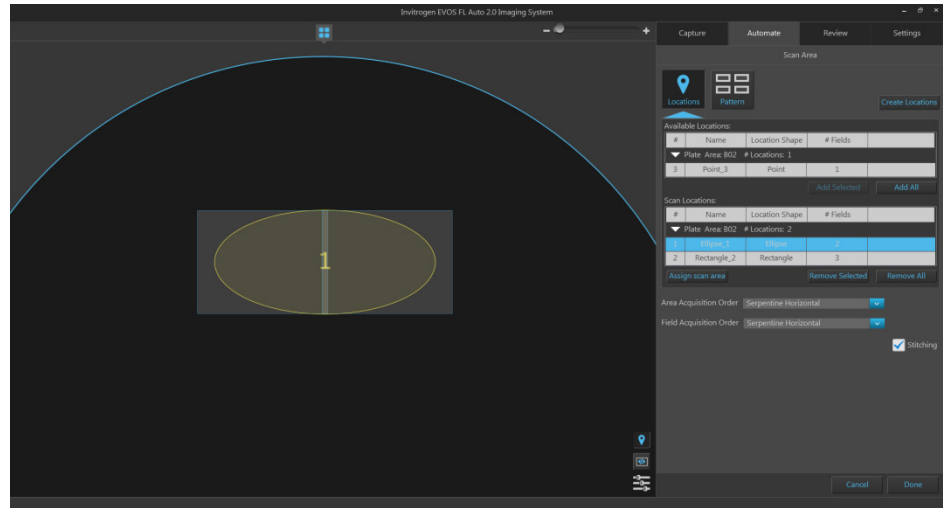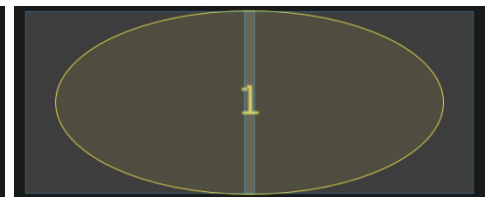
| Stitching off | Stitching on |

16. When finished with area selection, click **Done** to save your selections and return to the Automate tab.

Set Z-Offsets in Live mode

Z-Offsets allow you to specify the optimal focus position in each channel relative to the focus position in other channels. Setting the correct Z-Offsets is especially important when the fluorescent markers in different channels are in different focal planes. For best results, optimize the Z-Offsets in the Capture tab, and then carry the offsets over to the Automate tab.

1. In the **Automate tab ▶ AutoFocus and Z Stacks** panel, ensure that the **Capture Z-Stacks** option is unchecked, then click the **Capture tab**.

> **Note:** The **Z-Offsets** option is not available as one of the Focus Position controls when the **Capture Z-Stacks** option has been selected. For more information, see "Z-Stack Settings", page 130.

2. In the **Capture tab**, click the **Advanced focus settings** button and verify that the **Lock Z-Offsets** option is selected. By default, Lock Z-Offsets option is checked.
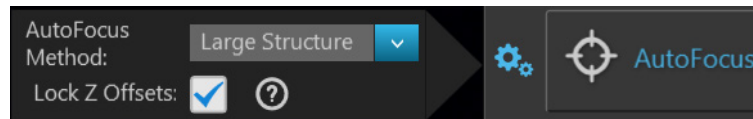
3. Select the **Objective** and **Light source** (i.e., channel) you wish to capture.
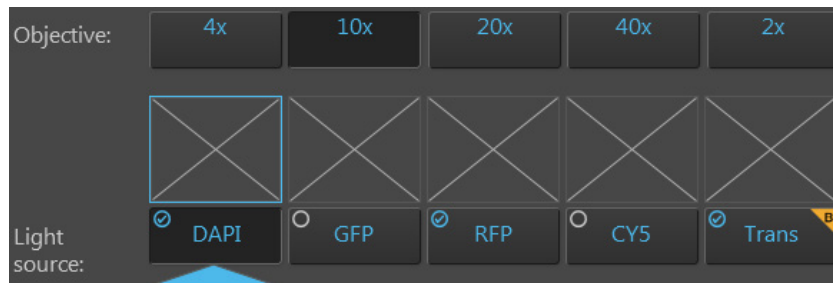
4. Click the **Light** button to enter the Live mode (see page 101).
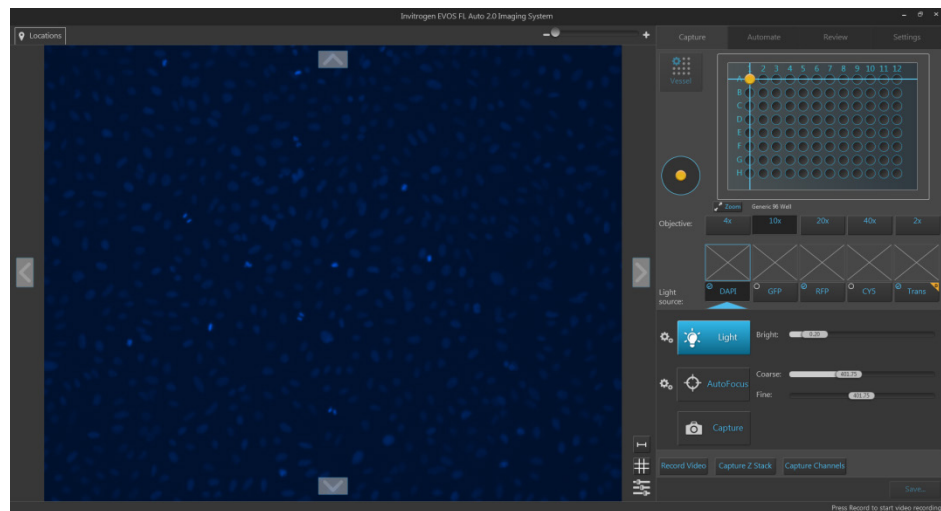
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5. Focus on the sample manually using the **Coarse focus** and **Fine focus** sliders or automatically by clicking the **AutoFocus** button.

   You can also double-click on the handle of the focus slider to activate the text box and enter the desired value for the focus position.
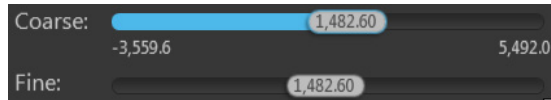
6. After you have found the optimal focus position in the first channel, click the **Advanced focus settings** button, then uncheck the **Lock Z-Offsets** option.

7. Without changing the objective, select the next **Light source** (i.e., channel) you wish to capture.

8. If the light is off, click the **Light** button to enter the Live mode, then focus on the sample manually using the **Coarse focus** and **Fine focus** sliders or automatically by clicking **AutoFocus**.
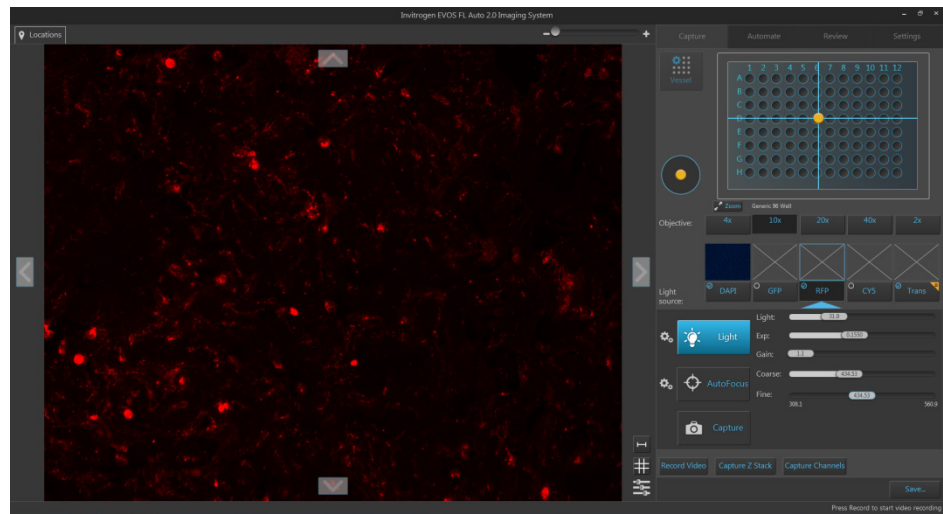
9. Repeat this procedure for every channel you wish to capture.

10. When you have found optimal focus in all of the channels you wish to capture, click the **Advanced focus settings** button, then check the **Lock Z-Offsets** option.
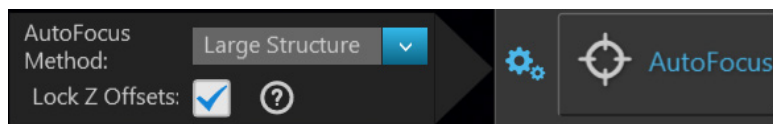
11. If the light is on, click the **Light** button to turn off the Light and exit the Live mode.

12. In the **Automate tab ▶ AutoFocus and Z Stacks**, click the **Review** button to open the AutoFocus and Z Stacks panel.

You will see the new settings carried over to Z-Offsets under the Focus Position and the focus positions will be offset relative to each other.

In the example below, DAPI channel is focused to 265.499 µm.

The Z-Offsets for the RFP and transmitted light channels relative to the DAPI channel are set to 20.19 µm and 15.30 µm, respectively.

Therefore, the instrument will focus to 285.689 µm (265.499 µm + 20.19 µm) in the RFP channel and to 280.799 µm (265.499 µm + 15.30 µm) in the transmitted light channel.
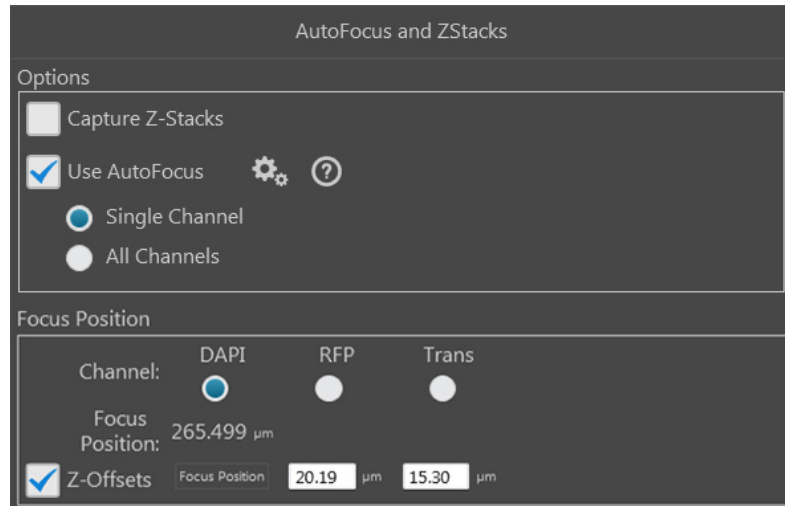
Because the Z-Offsets are preserved, selecting the RFP channel for the default focus position at 265.499 µm refocuses the instrument to 245.309 µm in the DAPI channel (265.499 µm – 20.19 µm) and to 260.609 µm in the transmitted light channel (265.499 µm – 4.89 µm).
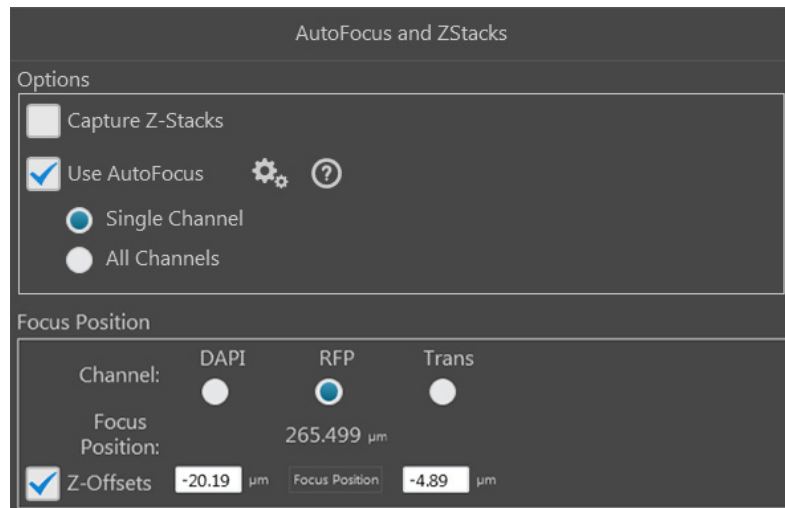
Note: If you need to adjust the Z-Offsets while in the Automate tab, make sure to close out the Autofocus and Z Stacks panel by clicking the **Done** button before navigating back to the Capture tab to make the adjustments.

<table>
<tr><td>Configure
AutoFocus settings</td><td>1.</td><td>In the **Automate tab ▶ AutoFocus and Z Stacks**, click the **Edit** button to open the AutoFocus and Z Stacks panel. Note that if you have previously adjusted any settings in the AutoFocus and Z Stacks panel, the Edit button will have changed to Review button.</td></tr>
</table>

> **Note:** The options available on the AutoFocus and Z Stacks panel are contextual; the controls that are displayed depend on the selections made in this panel or elsewhere in the Automate tab. For more information, see "AutoFocus and Z Stacks" in "Automate tab", page 129.

2. Check the **Use AutoFocus** box. The panel reveals the controls to configure the autofocus settings.
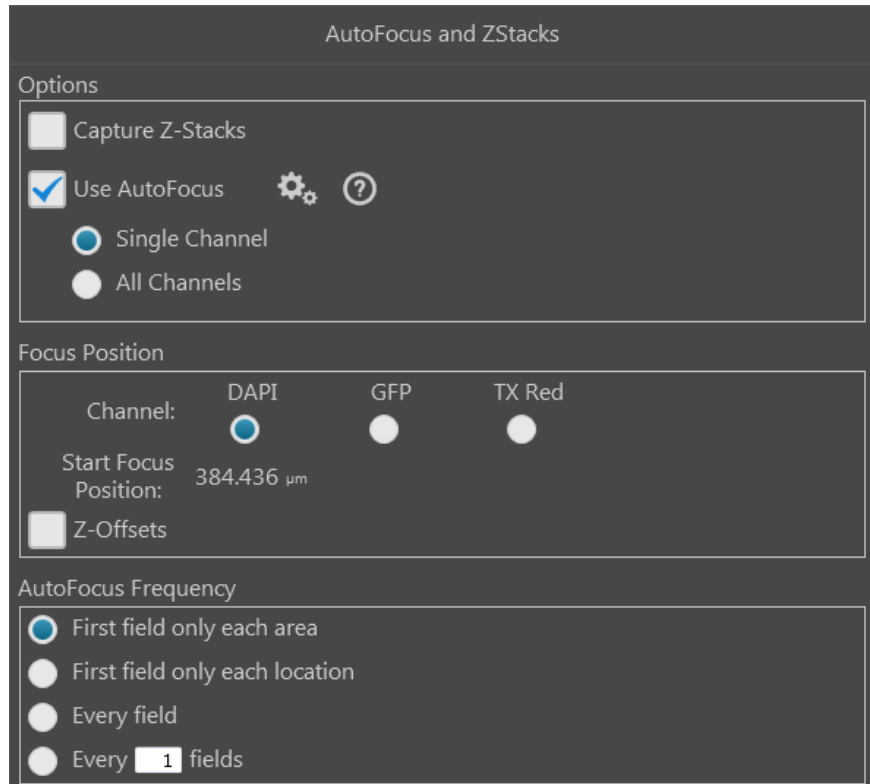
3. Select **Single Channel** or **All Channels** to autofocus during the scan protocol.
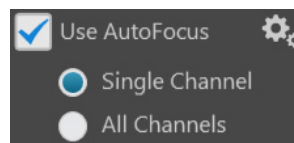
- – **Single Channel:** Autofocus procedure is run in a single channel and applied to all other selected channels. This option preserves the Z-Offsets between the channels (if they were set previously).
- – **All Channels:** Autofocus procedure is run separately in all selected channels. This option does not preserve the Z-Offsets between channels.

4. If you have selected **Single Channel**, choose the **Channel** for which to use autofocus. The focal plane identified in this channel will be used for all other channels. Note that only the channels selected in the Hardware panel (page 41) will be available to choose.
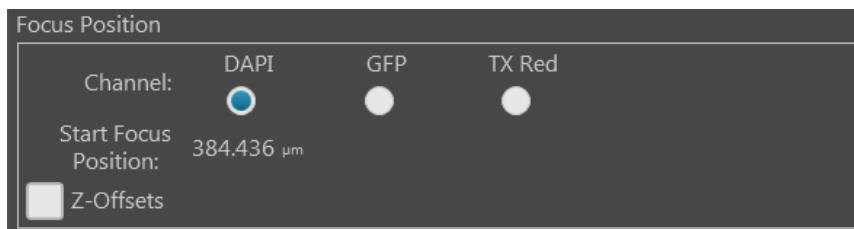
5. Click the **Advanced AutoFocus Configuration** button to reveal the options for autofocus methods.

6. Select the desired **AutoFocus Method** for each channel from the corresponding dropdown menu, then click **Close**.
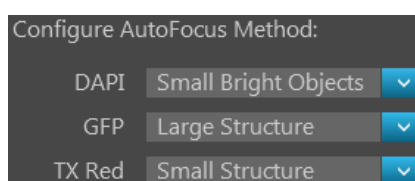
Available options are:

–   **Small Structure:** Choose for samples with many fine, hair-like structures (e.g., filaments or structural stains). This method is able to measure the presence or absence of small size image features.

–   **Large Structure:** Choose for samples with large structures (e.g., whole cell stains). This method looks for large changes in image content as parts of the image go in and out of focus.

–   **Small Bright Objects:** Choose for samples with localized staining (e.g., nuclei). This method looks for brightness changes in the center of the cell and optimizes the focus on cells that have a bright center with a dark surround.

Note: The effectiveness of the autofocus method depends on the plate type, sample type, biomarker intensity, magnification, and channel. It may be necessary to use different autofocus methods for different channels.

7. *Optional*: To carry over the Z-Offsets set up in the Capture tab, check the **Z-Offsets** box.

If you have not yet set the Z-Offsets, but would like to use them in your scan protocol, navigate back to the Capture tab to set them as described previously (see page 48), then navigate back to the Automate tab.

8. Set the **AutoFocus Frequency**.
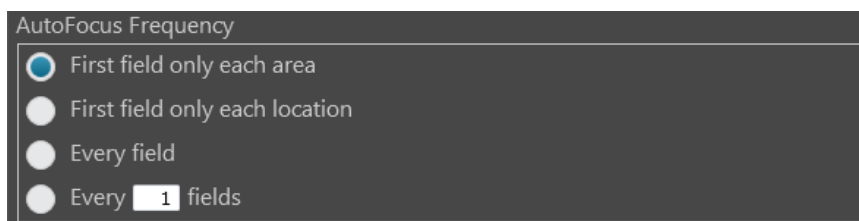
Available options are:

- **First field only each area:** Performs the autofocus operation only on the first field of each scan area. No further autofocus is performed on subsequent fields in the scan area. The focus position obtained on the first field is used for the remaining fields in the scan area.

- **First field only each location:** Performs the autofocus operation only on the first field of each location in every scan area. No further autofocus is performed on subsequent fields..

- **Every field:** Performs the autofocus operation on every field of the scan area.

- **Every <X> fields:** Allows you to specify the distance between fields in each scan area before the autofocus is triggered.

  The distance between fields is expressed as "unit fields" (equivalent to a field of view as determined by the objective used); it is not determined by the order in which the fields are collected.

  For example, if you wish to autofocus every 3 fields, autofocus will only occur when the stage is three fields away from another autofocused field. If the stage is less than three fields away, autofocus will not occur.

Note: For a single culture dish or flask, the scan area corresponds to the entire dish or flask. For a multi-well plate or a multi-chamber slide, each scan area corresponds to a single well or chamber.

Specifying fewer fields per area to autofocus reduces the overall scan times. Frequent autofocusing may not be necessary for fields that are close together or for samples with consistent z-locations on very flat vessel surfaces.

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9. When finished with your selections, click **Done** to return to the Automate tab and proceed to "Set Time Lapse options", page 54.

Set Time Lapse options

A time lapse routine automatically captures individual images of the fields that were defined in the Scan Area at given intervals over a time period based on your specifications. The images can then be stitched together into a video.

Note: The capture parameters defined in the Automate tab (z-stacks, exposures, channels, etc.) are applied to every time point of a time lapse routine.

1. In the **Automate tab ▶ Time Lapse and Incubator**, click the **Edit** button to open the Time Lapse and Incubator panel, then select **Use Time Lapse**.

The time lapse controls in the panel become active.
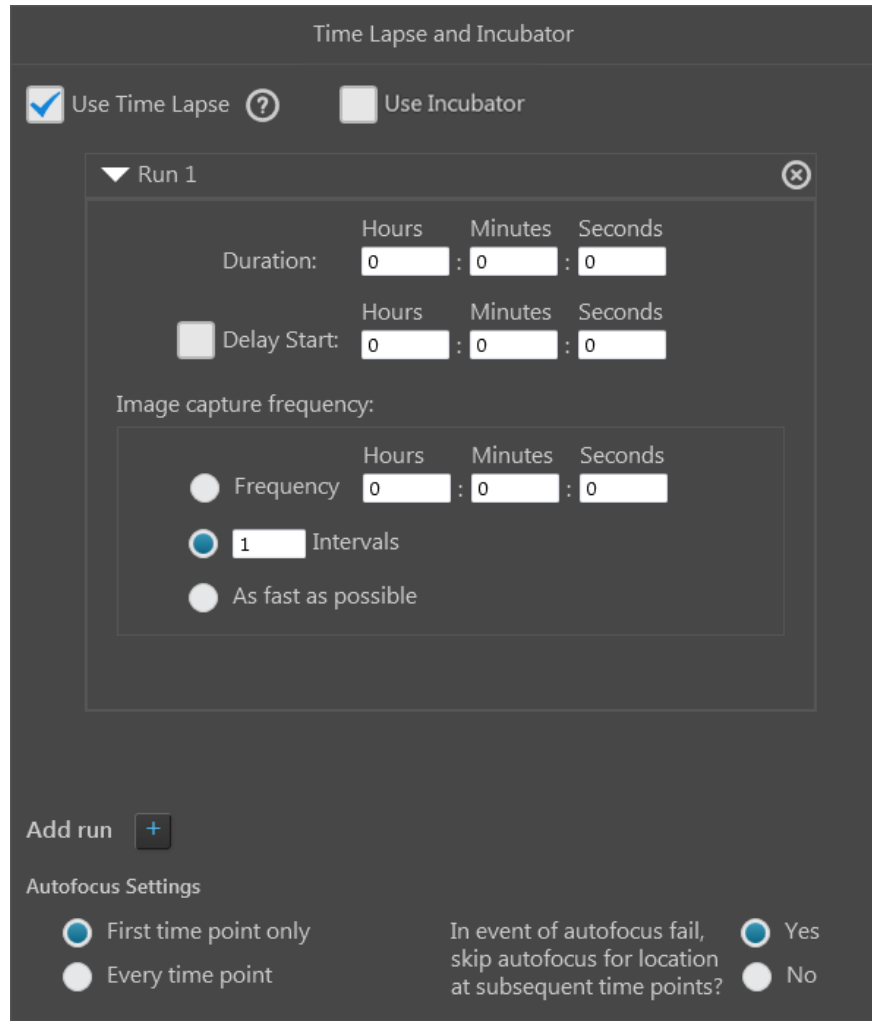
2. Enter the **Duration** of the run into the corresponding **Hours**, **Minutes**, and **Seconds** fields.
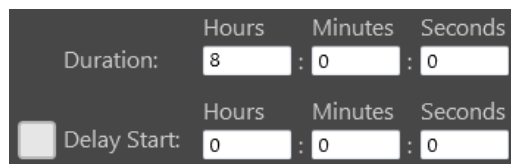
3. *Optional*: Under Run 1, select **Delay Start**, then enter the desired time interval into the corresponding **Hours**, **Minutes**, and **Seconds** fields.

    The delay start determines the time interval after the start of the scan protocol in which no images are captured. The duration of the run is automatically adjusted to reflect the time interval entered into the delay start fields.

4. Set the Image capture frequency.

    Available options are:

    *   **Frequency**: Enter the time period 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.

        Note that the images are collected at the end of an interval. For example, in an 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 capture set in the order specified without any delay between images sets.

        Note that the speed with which the images are captured depends on your specifications for the scan protocol such as the autofocus frequency and exposure settings.

5. *Optional*: Click **Add run** to add another run with its own parameters (delay start, duration, image capture frequency, incubator settings) to the time lapse routine.
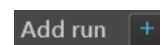
    Note: The runs are performed in chronological order, beginning with Run 1 and ending with Run N. You can remove a run from the time lapse routine by clicking the corresponding **Delete Run** button (page 141).

6. Set the **Autofocus Settings** for the time lapse routine.
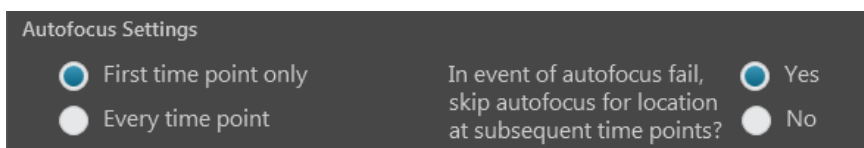
Available options are:

- **First time point only**: Select this option if you wish to autofocus at the selected locations only at the first time point of the time lapse routine.

- **Every time point**: Select this option if you wish to autofocus at the selected locations at every time point of the time lapse routine.

- **Autofocus fail options**: Click **Yes** to skip an autofocus location at subsequent time points in the event of an autofocus failure at that location.

  Click **No** to attempt autofocus at each selected autofocus location regardless of previous success or failure.

7. If you wish to use the EVOS™ Onstage Incubator during your time lapse routine, see "Set incubator options", page 57.

Otherwise, click **Done** to return to the Automate tab and proceed to "Configure image save settings", page 58.

Set incubator options	**IMPORTANT!** Before using the EVOS™ Onstage Incubator in your time lapse experiments, make sure that:

- The EVOS™ Onstage Incubator has been set up for use (see "Set up the EVOS™ Onstage Incubator", page 156).
- The gas inputs have been configured (see "Configure gas inputs", page 162).
- The oxygen sensor has been calibrated (see "Calibrate oxygen sensor", page 163).

1. In the **Automate ▶ Time Lapse** panel, select **Use Incubator**.

The Incubator controls in the panel become active.
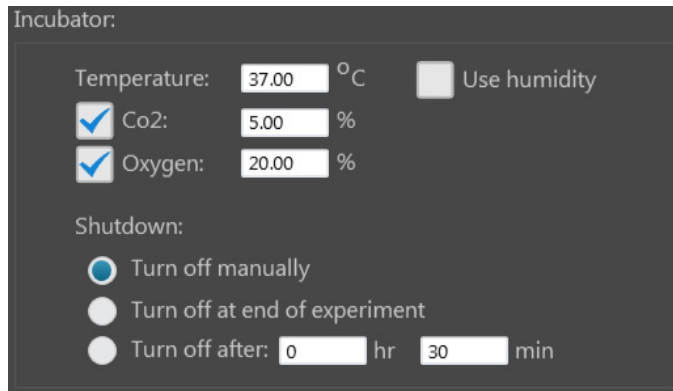
> **Note:** **Use Time Lapse** option must be previously checked for the **Use Incubator** option to be available.

2. Enter the target values for **Temperature**, **CO₂**, and **Oxygen**.
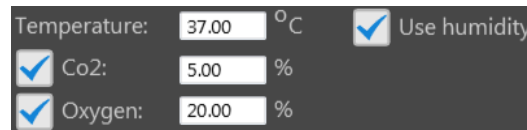
3. If desired, select **Use humidity**.

4. Select the desired **Shutdown** option for the incubator.
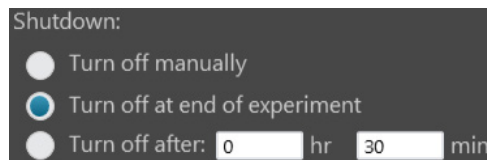
Available options are:

- **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.

5. When finished with your selections, click **Done** to return to the Automate tab and proceed to "Configure image save settings", page 58.

Configure image save settings

1. In the **Automate tab ▶ Image Save Settings**, click the **Edit** button to open the Image Save Settings panel.
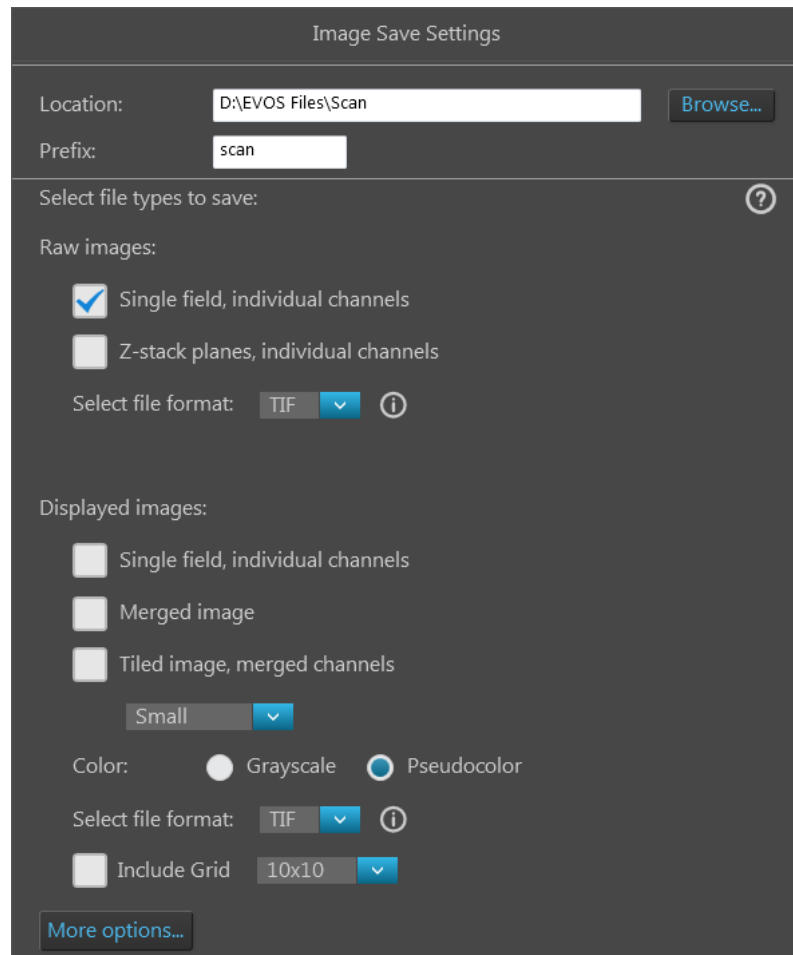
2. Click **Browse**, navigate to the folder in which you wish to save your images captured during the automated scan protocol, then click **Select**.

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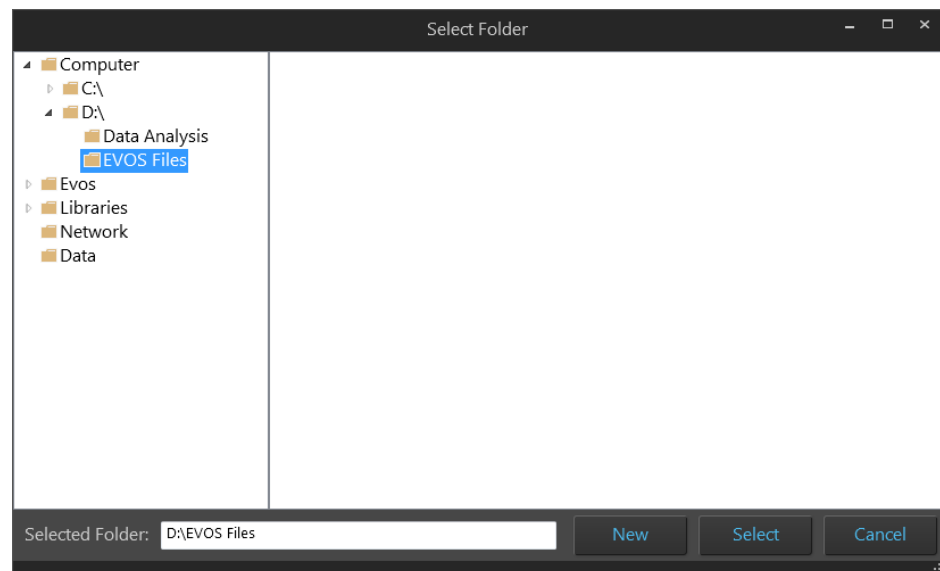
> **Note:** We recommend that you save your captured images to an external hard drive.

3. To create a new folder for the scan protocol, navigate to the desired location, click **New**, type in the name of the newly created folder, then click **Select**.
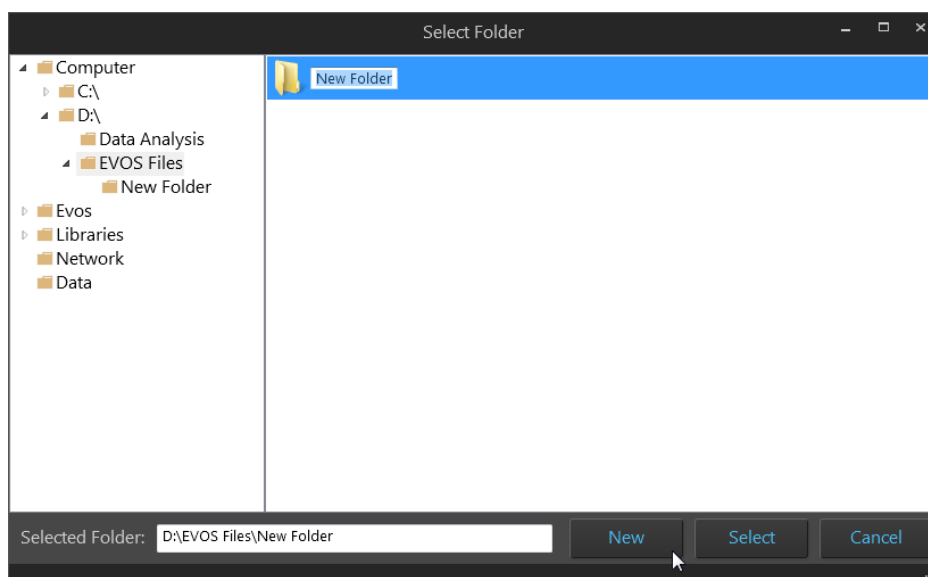
4. If desired, type the prefix you wish to use for your saved images in the **Prefix** textbox.

> **Note:** For file naming conventions for saved images, see "File naming convention", page 148.

5. Select **File types to save** by checking the corresponding checkboxes. You can choose multiple file types for your images captured during the automated scan protocol.

Available file type options are **Raw images** and **Displayed images**.

- **Raw images:** Saves images captured in different channels individually as 16-bit raw images. This is the recommended format for image analysis.
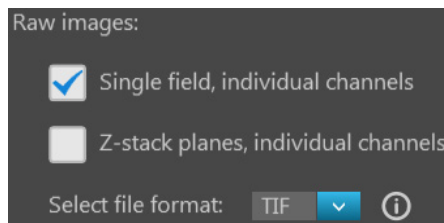
  – Available **raw image** options are:

    Single field, individual channels: Saves images captured in each field and each channel individually.

    Z-stack planes, individual channels: Saves the z-stack projection and each z-plane for each field and each channel as a separate image.

  – Available **File format** for raw images are **TIF**, **PNG**, **C01**, and **DIB**.

- **Displayed images:** Saves images in a format that can be viewed in most image display applications.
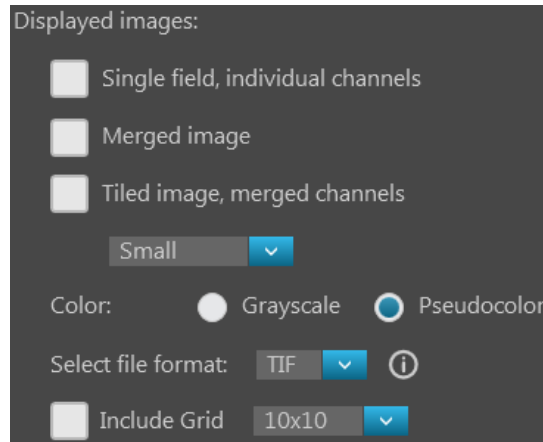
– Available **displayed image** options are:

Single field, individual channels: Saves images captured in each field and each channel individually.

Merged image: Saves the images of a field captured in different channels as a multi-channel overlay.

Tiled image, merged channels: Merges the images captured in each channel, then aligns them close together into a tiled format.

You can create tiled images with one of the following dimensions: **Small** (2000 × 2000 pixels), **Medium** (4000 × 4000 pixels), **Large** (10,000 × 10,000 pixels), or **Maximum** (26,000 × 26,000 pixels).

– Available **Color** options for displayed images are:

Grayscale: 16-bit.

Pseudocolor: 24-bit RGB (8-bit per RGB channel).

– Available **File format** for displayed images are: **TIF**, **PNG**, and **JPEG**.

Note: While pseudocolors help differentiate the channels used in multi-channel overlays, grayscale images usually show more detail.

Note: 24-bit images (8-bit per RGB channel) are NOT recommended for image analysis as not all channels will display in many image analysis applications.

6. Check the **Include Grid** option to superimpose a grid on the displayed images.

You have the following options for the grid size (in pixels):

Auto, **10 × 10**, **50 × 50**, **100 × 100**, **200 × 200**, and **500 × 500**.

7. To see more file type options in a table format, click **More options…**

Full menu of save options is displayed in a convenient table format, allowing you to save your captured images in a number of formats simultaneously.

8. Select the desired file types and options by checking the corresponding checkboxes. You can select multiple file types and options.

Available file type options are **Raw images** and **Displayed images**.

- **Raw images**: Saves each channel individually.
  - **Single fields**, **Z-planes**, and **Tiled images** can be saved as raw images.
  - Available **File format** for raw images are **TIF**, **PNG**, **C01**, and **DIB**.

- **Displayed images:** Saves each channel individually or as a merged image in a format that can be viewed in most image display applications.
  - **Single fields**, **Z-planes**, and **Tiled images** from individual channels can be saved as displayed images.

    However, you can only save **Single fields** and **Tiled images** as a displayed image in merged view; **Z-planes** can only be saved from individual channels.
  - Available **Color** options for displayed images are **Grayscale** (16-bit) or **Pseudocolor** (24-bit RGB; 8-bit per RGB channel).
  - Available **Brightness** options for displayed images are **High** and **Full**.
  - Available **File format** for displayed images are **TIF**, **PNG**, and **JPEG**.

9. After you have configured Image Save Settings for your automated scan protocol, click **Done** to return to the Automate tab.

Save automated scan protocol

When you have finished creating your automated scan protocol, the total number of images that will be captured in the scan protocol and the total file size for the captured images are displayed next to the Save button in the Automate tab.

Total number of images: 26040   Save

Total file size: 1820MB

At this point, we recommend saving the automated scan protocol for future recall in repeat experiments. For convenience, you can also edit recalled scan protocols to create new routines rather than creating them from the very beginning.

1.   In the **Automate tab**, click **Save** to open the Save As dialog, then navigate to the location in which you wish to save your scan protocol.
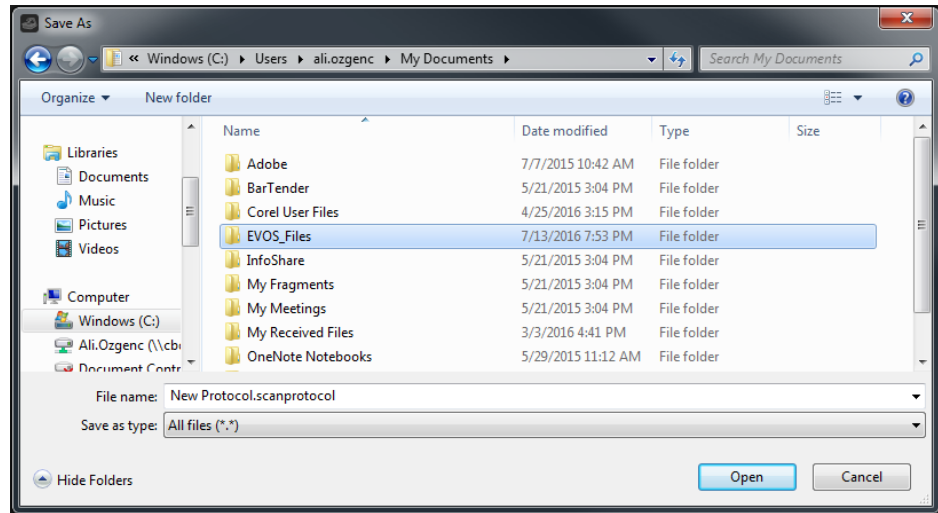
2.   In the **File name** text box, type in the name of your new routine.

The default name for new scan protocols is "New Protocol.scanprotocol".

3.   Click **Save** to save your scan protocol and close the Save As dialog.

4.   To run your automated scan protocol, proceed to "Run newly created scan protocol", page 63.

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Run automated scan protocol

Run newly created scan protocol

1. In the **Automate tab**, click **Run** to run the automated scan protocol you have just created.

    The EVOS™ FL Auto 2 Imaging System starts executing the steps specified in your automated scan protocol to capture multiple images over an area and/or time period.

    The Viewing area displays the fields as they are being captured during the scan, while the Vessel map indicates the area being captured.
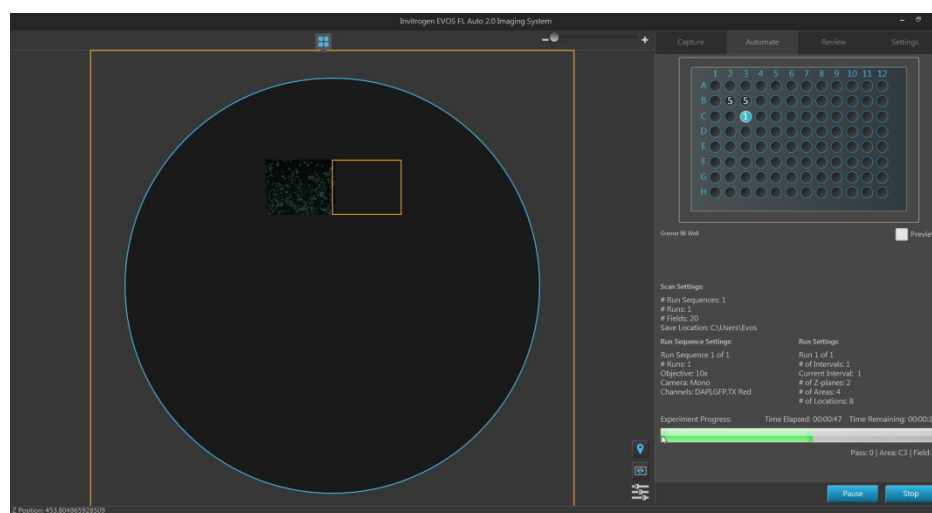
    The Scan Settings, Run Sequence Settings, and Run Settings specified in your scan protocol are displayed below the Vessel map, and the Experiment Progress bar tracks the progress of the run.
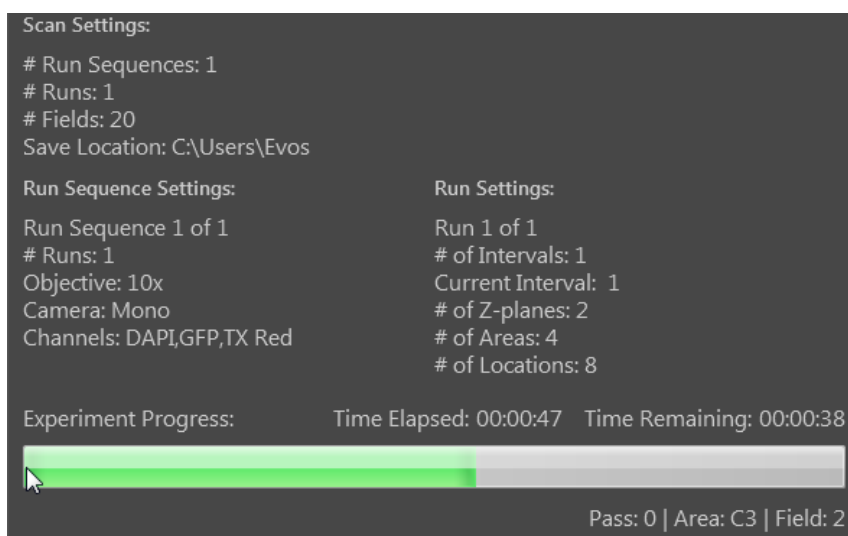
2. Click **Pause** to pause the automated scan protocol anytime during a run.

    Click **Resume** to restart the scan protocol from the step where it was paused.

3. If you wish to abort the scan protocol before it is completed, click **Stop**.

    Click **Save** to save the images acquired during the aborted scan protocol in the location specified in Image Save Settings, or click **Discard** to delete them.

4. When the scan protocol is completed, click **Done**.
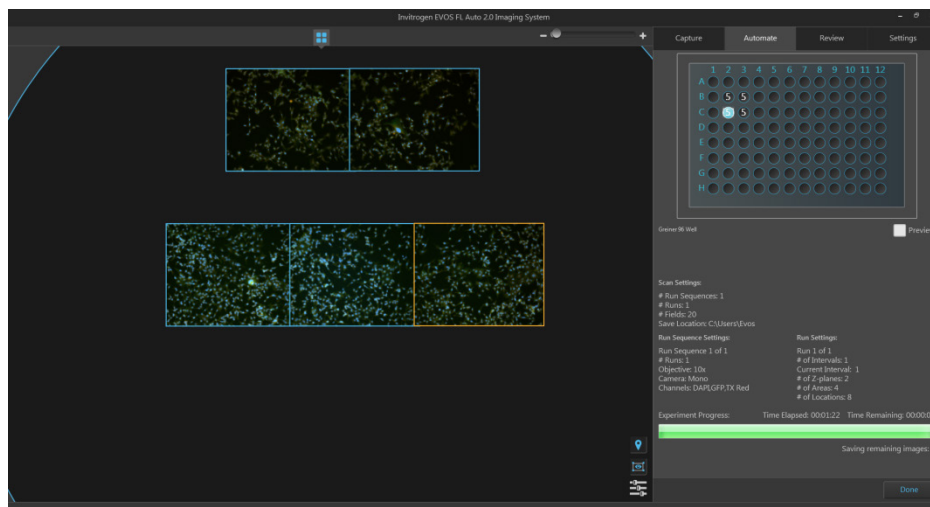
Load and run a saved scan protocol

For repeat experiments, you can recall and run a saved scan protocol. If desired, you can also edit the settings of the recalled scan protocol.

1. On the Automate tab, click **Load**, then navigate to the folder containing the saved scan protocol you wish to recall.
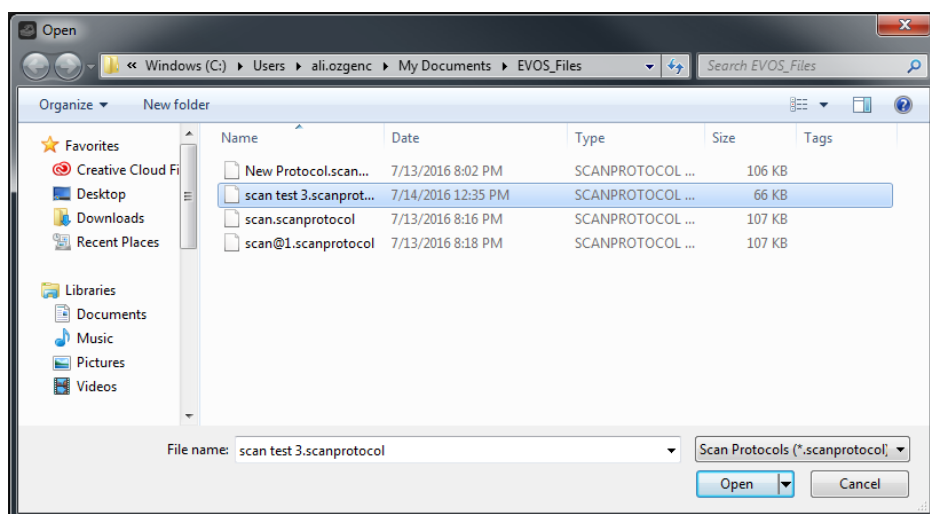
2. Click the saved scan protocol of interest to select it, then click **Open**.

3. If you wish to edit the recalled scan protocol, click **Review** to open the desired Automate tab panel, adjust the settings you wish to change, then click **Done** to return to the Automate tab.

4. Click **Run** to run the recalled routine.

5. When the scan protocol is completed, click **Done**.

Note: Saved scan protocols retain the information about the scan areas as well as the camera and lighting options set when creating the protocol. To change these settings for a new experiment, click **Review** after loading the saved scan protocol and make the desired changes.

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5. 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 (front of LCD hinge) and in the Specifications. Damage due to an incompatible power adaptor is not covered by warranty.

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

IMPORTANT! If you have any doubt about the compatibility of decontamination or cleaning agents with parts of the equipment or with material contained in it, contact Technical Support (page 175) 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™ FL Auto 2 Imaging System, be sure to lock the X-Y stage using the Shipping Restraint as shown on page 71 to prevent the stage from sliding.

Decontamination procedures

> **IMPORTANT!** The EVOS™ FL Auto 2 Imaging System should not be subjected to UV decontamination. UV degrades many materials, including plastic. Damage from UV exposure is not covered under the manufacturer's warranty.

In case hazardous material is spilt onto or into the components of the EVOS™ FL Auto 2 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™ FL Auto 2 Imaging System (stage top, objective turret, housing, etc.) with paper towels or Kimwipes™ tissues dampened with 70% ethanol or 4,000 ppm hydrogen peroxide (H_2O_2).

   > **IMPORTANT!** Do not allow decontamination solution to get into lubricated areas, such as the stage roller bearings, or any points of rotation such as stage motors, condenser wheel, etc.
   >
   > Do not soak any surface in decontamination solution.
   >
   > NEVER spray liquid anywhere on the EVOS™ FL Auto 2 Imaging System.
   >
   > Always wipe surfaces with dampened paper towels instead.

Calibrate the stage

Stage Calibration function allows the EVOS™ FL Auto 2 software to establish internal coordinates to properly execute its functions. The calibration procedure requires the EVOS™ FL Auto 2 calibration slide supplied with the instrument (also available separately; Cat. No. AMEP4720).

Vessel alignment procedure

1. Go to **Settings** tab, then click **Stage Calibration** to open the Stage Calibration tool.
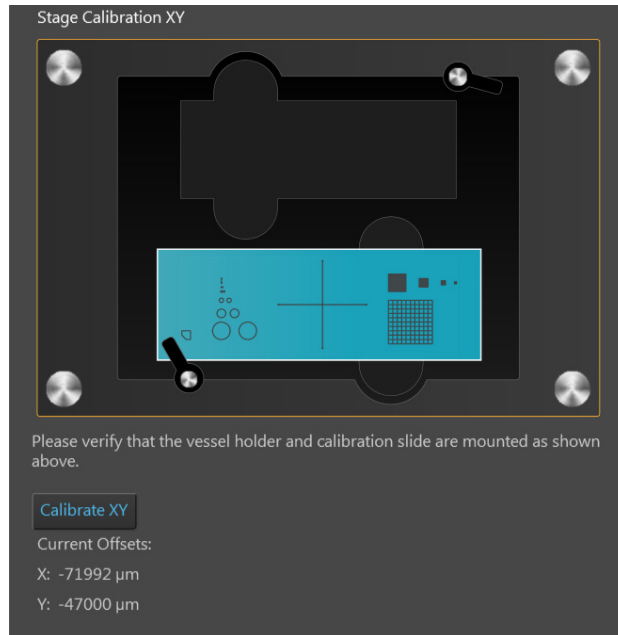
2. Mount the vessel holder and the calibration slide as shown on the screen, then click **Calibrate XY**. The instrument proceeds with the automated calibration procedure, during which the stage moves around to various and a progress bar displays on the screen.
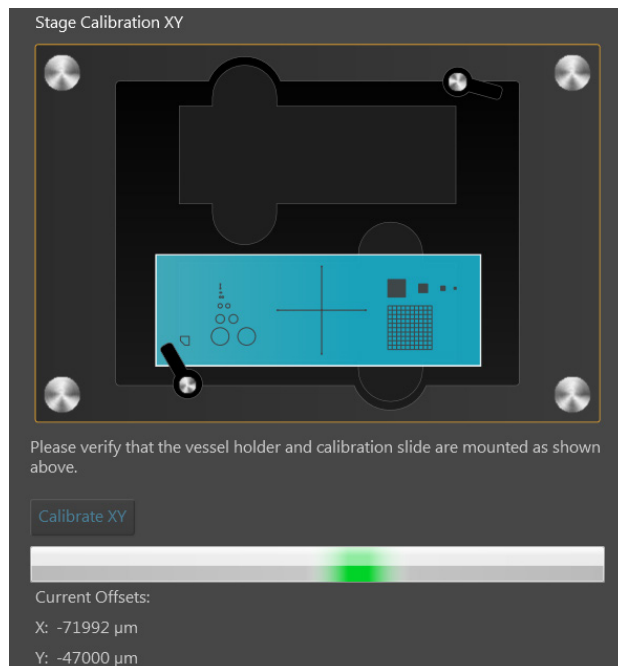
3. When the calibration is complete, click **OK** in the completion dialog box, then click **Next** to move to Z Stage Calibration.

Stage Calibration XY

Please verify that the vessel holder and calibration slide are mounted as shown above.

Calibrate XY   Status: Stage Calibration Complete

Current Offsets:

X: -72662.2541016811 µm

Y: -46464.6795950361 µm

The XY Stage Calibration is complete. Please click **Next** to proceed to Z Stage Calibration.
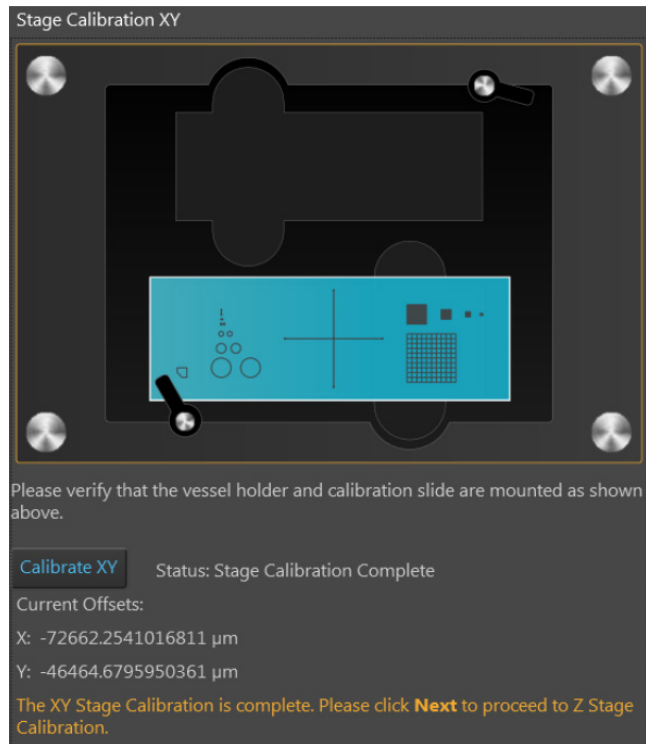
4. Use the **Brightness** and **Focus** sliders to bring the image of the crosshairs into focus (see page 69), then click **Done**.

Stage Calibration Z
Please adjust the controls to bring the cross hair into focus and then press Done.

Adjust lighting

Mode: Simple   Actual

Brightness: 0.013510

Adjust focus

Autofocus

Coarse: -2.81

Fine: -2.81
-294.5   288.9

Current Z Offset: 3574.10666666667 µm

New Z Offset: 3571.30000000001 µm
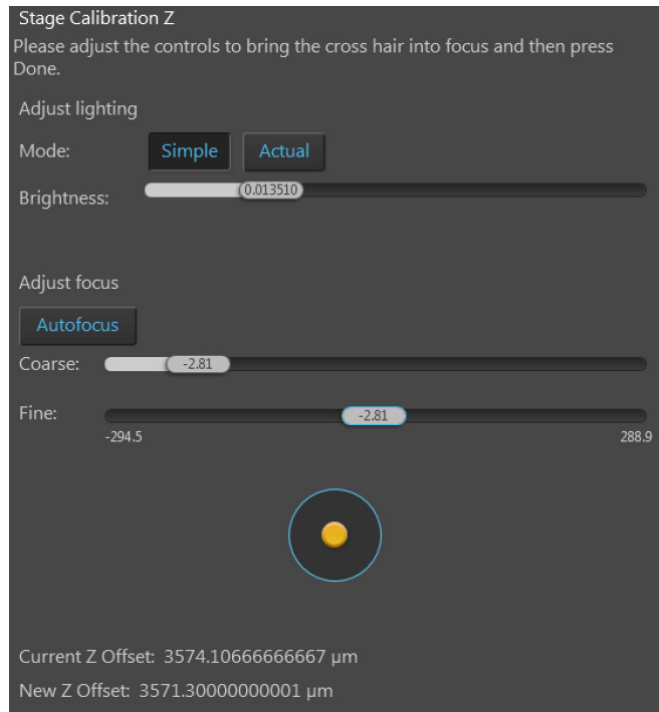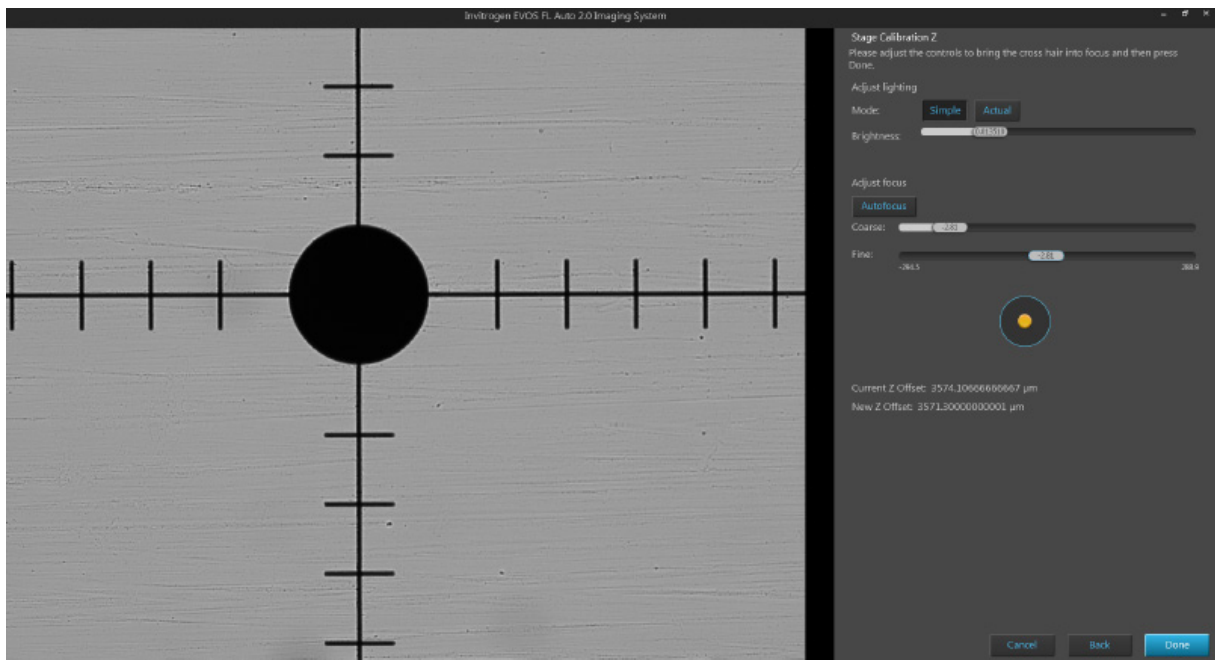
Change EVOS™ LED light cubes

To customize your EVOS™ FL Auto 2 Imaging System, you can add and remove LED light cubes to fit the instruments functionality to your own specific research needs. For a complete list of available light cubes and to inquire about custom light cubes, go to **thermofisher.com/evos** or contact Technical Support (page 175).

Procedure for LED light cube change

1. On the **Settings ▶ Filter Cubes** tab, select the position of the **Light cube** you wish to change, then click **Move to filter cube change position**.

2. Use the light cube tool to loosen the two slotted screws (white arrows) that are flush with the ridges on the light cube.

3. Screw the threaded end of the light cube tool into the hole in the center of the light cube as shown.

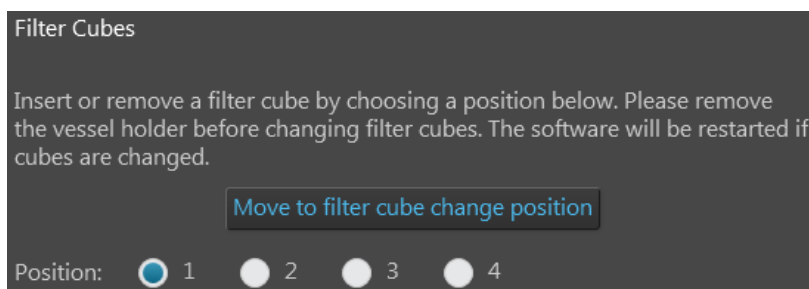4. Use the tool to tilt the light cube slightly toward you and lift out gently, and then remove tool from cube.

5. 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.

6. Use the light cube tool to gently tighten the two slotted screws so that the screw heads sit flush with the ridges on the light cube.

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

Change the objectives

To customize your EVOS™ FL Auto 2 Imaging System, you can add and remove objectives to fit the instruments functionality to your own specific research needs.

Procedure for objective change

1. Remove the objective you wish 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 the following example, a new objective is installed into the turret position 5.

3. Go to the **Settings ▶ Objectives** tab, and find objective in the **Profiles** list on the left that matches the newly installed objective.

4. Click and drag the **objective profile** for the new objective to the appropriate **turret position** on the right. In the following example, EVOS™ 4X objective with 19.7-mm working distance is selected and dragged to the turret position 2.
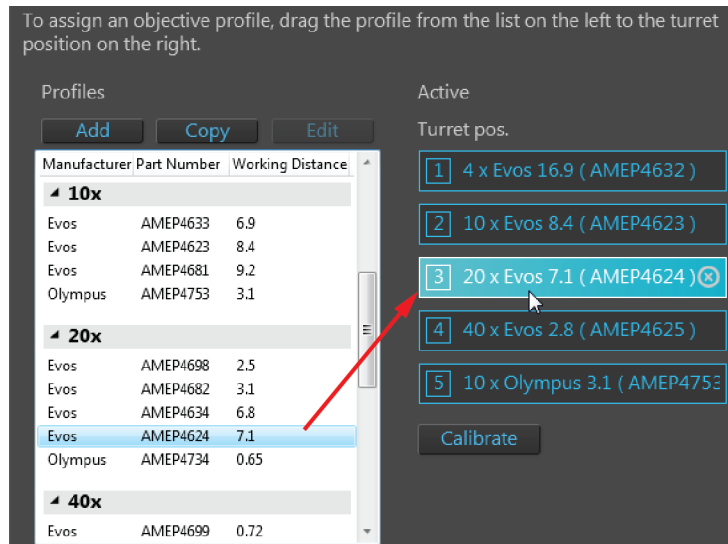
5. *Optional*: If desired, type in a label in the **Display Name** text box. This creates a label that is displayed on the Objective button in the Capture tab.

In the following example, "NoPH" is entered as the Display Name.
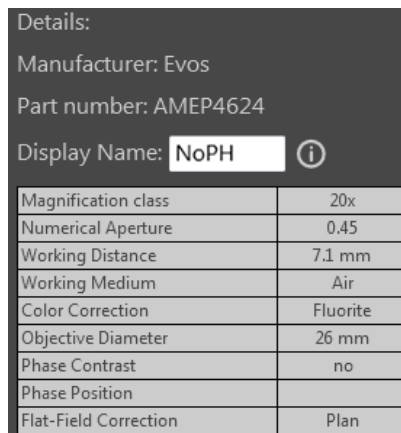
Therefore, the Objective button in the Capture tab will display the label "NoPH" below the objective magnification.

6. When finished, click **Done** to return to the Settings tab.

Note: For best results, calibrate the newly installed objectives before using it in your experiments (page 73).

Calibrate the objectives

Calibrate Objective function allows the calibration of the field of view, parfocality, and parcentration parameters of the selected objective.

Calibrating the parfocality ensures that the sample stays in focus when the objective is changed, and calibrating the parcentration ensures that an object in the center of the field of view will stay in the center of the field no matter which objective is being used.

Note: The pre-installed objectives supplied with the EVOS™ FL Auto 2 Imaging System have been pre-calibrated. You do not need to calibrate them again unless they are reinstalled after removal from the instrument.

Calibration procedure

Calibration procedure involves two distinct steps and requires the use of the EVOS™ FL Auto 2 calibration slide supplied with the EVOS™ FL Auto 2 Imaging System (also available separately; Cat. No. AMEP4720).

First, the calibration crosshair on the screen is matched to the crosshair on the calibration slide; then, the diameters of the reference circles on the calibration slide are measured.
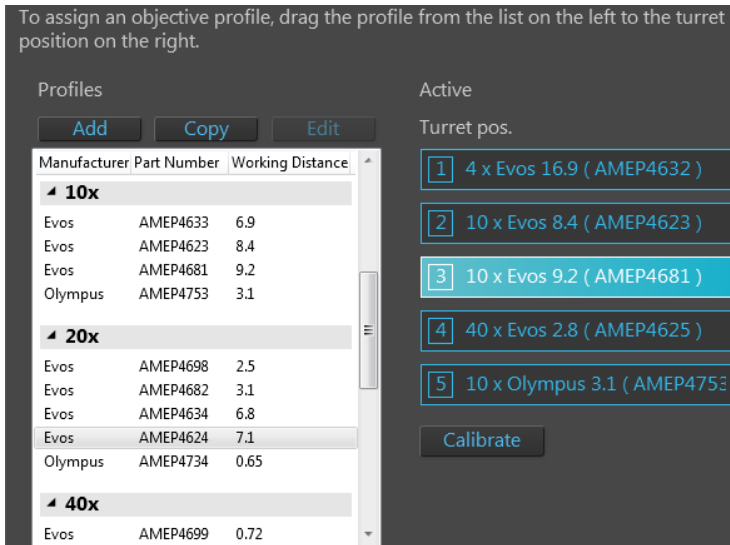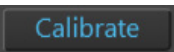
Each step is performed using both the monochrome and the color camera. Total time required objective calibration is about 5 minutes.

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

1. Go to the **Settings ▶ Objectives** tab, then click **Calibrate** to launch the Objective Calibration tool.

2. Mount the EVOS™ Calibration Slide in the vessel holder as shown on the screen (see Note below), select the objective you wish to calibrate, then click **Calibrate Objective**.

The stage moves to the calibration target and the instrument automatically finds the calibration crosshairs on the calibration slide (see page 75).

Note: Depending on the type of objective being calibrated (e.g. Long Working Distance vs. Coverslip Corrected objective), the calibration slide needs to be mounted face up or face down. When mounting the slide, make sure to match the orientation of the slide to the graphic shown on the calibration screen.

3. Adjust the lighting and focus using the **Brightness** and **Focus** controls and bring the image of the crosshairs into focus.
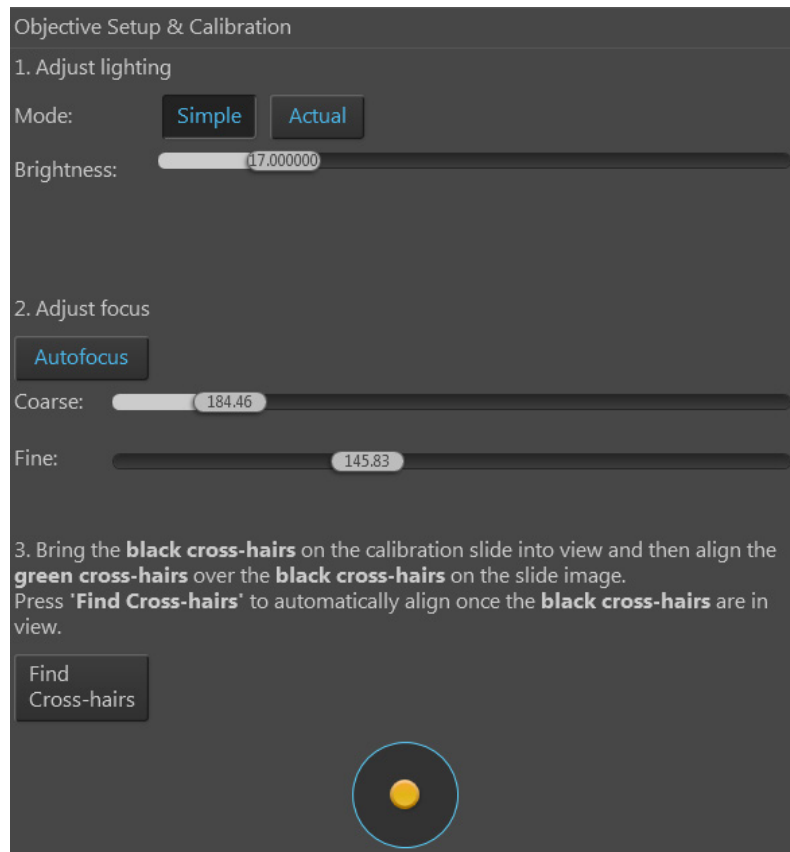
4. Use the **Jog Control** button to bring the black crosshairs on the slide image into view, then click **Find Cross-hairs** to automatically align the black and green crosshairs.
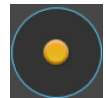
5. Click **Next**, then repeat the alignment procedure for the color camera.

6. Click **Next** to proceed to the second part of the calibration procedure, measuring the diameter of the reference circle.

   The stage automatically moves to the calibration circle and the imaging system automatically selects the appropriate reference circle on the slide, which depends on the specific objective you are calibrating.
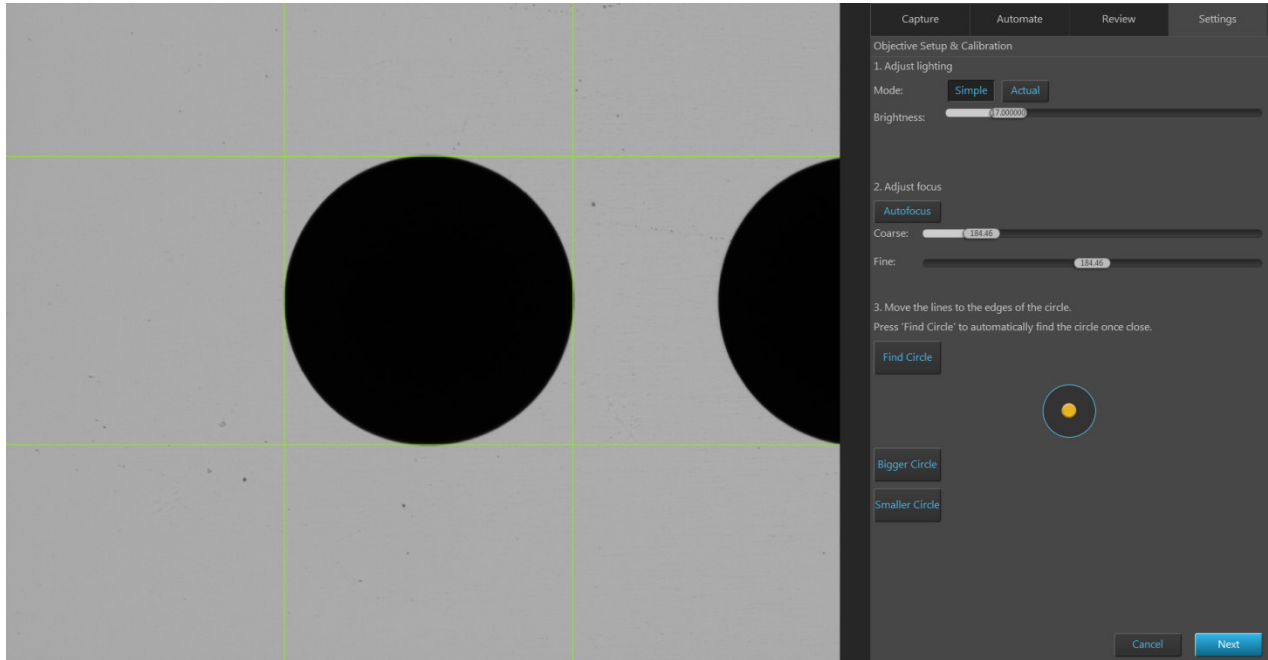
Note: If the circle on the screen is too large or too small, click **Smaller Circle** or **Bigger Circle** buttons as appropriate to move to a more appropriately sized circle. For best results, use the largest circle possible.

7. Adjust the lighting and focus using the **Brightness** and **Focus** controls, then click **Find Circle** to move the green calibration lines to the edges of the reference circle. You can also manually move the lines by clicking and dragging them into position.

8. Click **Next**, then repeat the procedure for measuring the diameter of reference circles for the color camera.

9. When finished, click **Done** to complete the calibration. Repeat the calibration process for each additional objective to be calibrated.

Install the shipping restraints

Use the Shipping Restraints whenever you package the unit for shipment. If the unit is being hand-carried and is not at risk of drops or excessive vibration there is no need to install the restraint. If you need to remove light cubes or objectives, remember to do so before installation of the Shipping Restraint.

Install procedure

1. Start with the instrument turned on and the software running. If a vessel holder is installed, remove it.

2. Move the stage to the center of travel until the front right corner of all three plates are aligned.

3. From **Brightness and camera settings**, select **Monochrome Camera**.
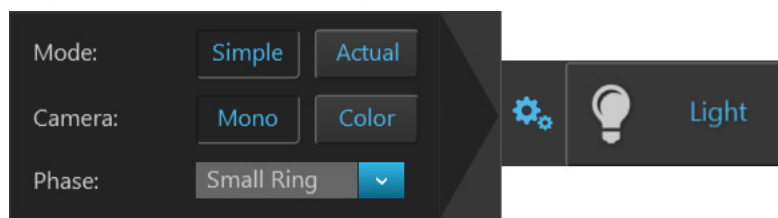
4. From the **Settings ▶ Objectives** tab, select the first populated **Light cube** position.
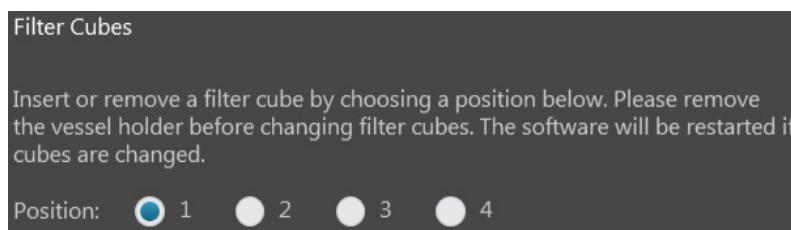
Alternatively, click the **Light source** button to select the corresponding Light cube.

5. Move the z-position to the middle of the focus range using the focus sliders.
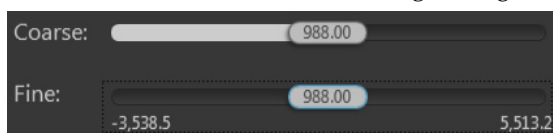
6. Go to **Settings ▶ Service** tab, then click **Move to Shipping Position**.

7. Turn off the power to the instrument and shut down the software.

8. To install the **X-Y Stage Shipping Restraint**, attach to threaded holes in the front right corner of stage, then tighten the screws in order from bottom to top, 1-2-3.

   If necessary, you can gently push on the side or front of the stage to move the plates into alignment.

   IMPORTANT! Do not over-tighten the screws. Once the lock washer compresses, stop tightening.

9. To install the **Light Cube Shipping Restraint**, place the white Light Cube Shipping restraint into the Light Cube access hole, then insert the Light Cube Tool into the hole in the shipping restraint. Secure the restraint by screwing the Light Cube Tool into the top of the Light Cube.

   IMPORTANT! Do not over-tighten; the Light Cube Tool is secure when it moves with the Light Cube. The Light Cube should still be able to move slightly side to side with respect to the X-Y Stage.

10. To install the **Camera Shipping Restraint**, insert the Camera Shipping Restraint into the hole at the back of the X-Y Stage, then screw it into the camera carriage until the red knob touches the top of the XY Stage. Do not over-tighten.
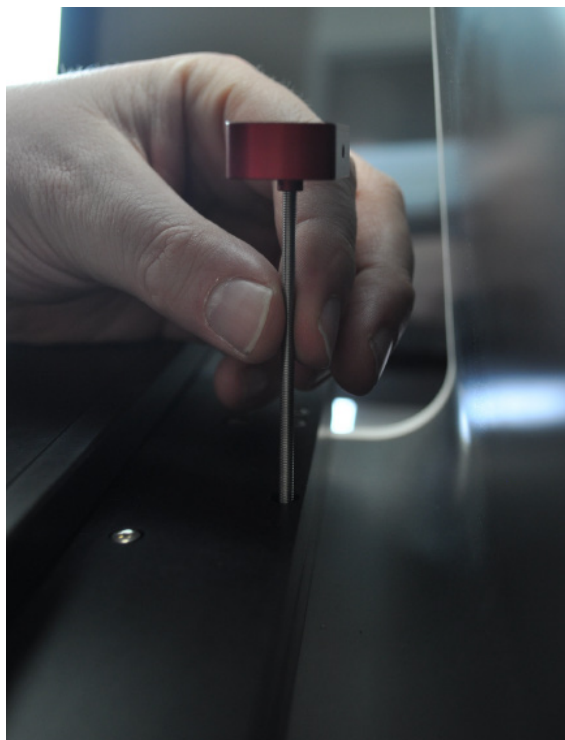
IMPORTANT! Do not power the instrument back on until the Shipping Restraints have been removed.

Appendix A: Troubleshooting

Note: For additional technical support, contact your local EVOS™ distributor. If you do not have your distributor information, visit **thermofisher.com/evos** or contact Technical Support (page 175).

Image quality issues

Problem	Possible solutions
Image is too dim (at higher magnifications)	Remove condenser slider, if one is in place.
Specks, dots, or blurs on image	Follow instructions under "Objective lens care" (page 65) to clean objectives.
Uneven focus across screen	• Position sample, so that it lies flat on the stage; ensure that the sample's thickness is even. • Be sure vessel holder is mounted flat with respect to stage.
Difficulty focusing on coverslipped sample on standard slide	Place the slide so the coverslip is facing up (long working-distance objectives require a thick optical substrate, and image best through 1.0–1.5 mm of glass or plastic).
Image display is black	• Click the Power button (onscreen). • Center sample over objective. • Verify power supply is connected and power switch is on.
Image display is red, or red patches cover parts of the screen	• Dim the illumination until the red highlights disappear to get the maximum level of brightness without any overexposed areas. • Disable the "Show saturated pixels" option in the **Settings ▶ General** tab.

Software interface issues

Note: We recommend keeping the EVOS™ FL Auto 2 Imaging System up to date with the latest software.

Problem	Possible solutions
Image does not respond to changes in focus or stage position	Click the **Light** button to return to live observation.
Some of the software controls are not available	The controls available on the EVOS™ FL Auto 2 Imaging System are contextual; only the controls relevant for the chosen task will be available.
Save button does not respond when clicked	Click capture first; it is only possible to save an image that is captured.
Unable to connect to network	• Verify physical cable connections; confirm the Ethernet jack is active. • Contact your network administrator to resolve any network issues.

Mechanical issues

Problem	Possible solutions
Automatic stage does not move	Remove shipping restraint.
Filter Cube Axis does not move	Remove shipping restraint.
Camera Axis does not move	Remove shipping restraint.
Vessel does not sit securely on moving stage	Use the correct vessel holder for the application (visit **thermofisher.com/evos**).

Appendix B: System overview

Technical specifications

Note: Technical specifications of the EVOS™ FL Auto 2 Imaging System are subject to change without notice. For the latest product information, see the product page (**thermofisher.com/evos**).

Physical characteristics	**Dimensions (H × D × W):** 32 × 48 × 37 cm (12.5 × 19.0 × 14.5 inches)
	Weight: 16 kg (35 lb)
	Footprint: Approximately 92 cm × 92 cm (36 in × 36 in); entire system includes the instrument, computer, and 23-inch touch-screen, high-resolution LCD monitor.
	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: 24 VDC, 5 A
Hardware	**Optics:** Infinity-corrected optical system; RMS-threaded objectives with 45 mm parfocal distance.
	Illumination: Adjustable intensity LED (>50,000-hour life per light cube).
	Light cubes (not included): 5 position chamber for 4 fluorescence cubes plus brightfield. Broad selection standard and specialty light cubes (see page 83).
	Contrast methods: Fluorescence and transmitted light (brightfield & phase contrast).
	Objective turret: 5-position; front-mounted control.
	Objectives (not included): Wide selection of high-quality LWD and coverslip-corrected objectives.
	Condenser: 60 mm long working distance condenser, 4 position turret with a clear aperture and 3 phase annuli.
	Stage: Motorized X-Y scanning stage. Travel range of 120 mm × 80 mm with sub-micron resolution, drop-in inserts to receive vessel holders and lock down holders to fix sample in place during long scans.
	Focus mechanism: Automated focus mechanism with sub-micron resolution.
	LCD display: 23-inch high-resolution, touch-screen color monitor (also fully controllable via mouse); 1920 × 1080 pixel resolution.
	Cameras: High-sensitivity 1.3 MP CMOS monochrome camera with 1328 × 1048 pixels; high-sensitivity 3.2 MP CMOS color camera with 2080 × 1552 pixels.
	Computer: External PC with 16 GB RAM running Windows™ 7 Pro designed to operate with touchscreen monitor and instrument.
	Captured images: 16-bit monochrome (12-bit dynamic range) TIF, PNG, C01, or DIB; 8-bit per RGB channel TIF, PNG, or JPG,; time-lapse AVI or WMV.
	Output ports: Microscope: 2 USB 3.0, 1 USB 2.0, Power Computer: multiple USB 3 ports, Display Port, HDMI, Ethernet
	Networking capability: Connection through Windows/SMB network via an Ethernet cable connection.
	Power supply: AC adapter with country-specific power cords.

Operation principles and technical overview

LED illumination

The EVOS™ FL Auto 2 Imaging System utilizes an adjustable intensity LED light source provided by the proprietary, user-interchangeable LED light cube (see below). Because the LED light source is as close as possible to the objective turret, the number of optical elements in the channel is minimized. High-intensity illumination over a short channel increases the efficiency of fluorophore excitation, providing better detection of weak fluorescent signals.

In contrast to traditional fluorescence microscopy light sources that use mercury, a toxic carcinogen requiring special handling and disposal, the LED light source of the EVOS™ FL Auto 2 Imaging System is more environmentally friendly, energy efficient, and has a significantly longer life span (>50,000 hours versus 300 hours for a typical mercury bulb and 1,500 hours for a metal halide bulb).

LED light cubes

Each user-interchangable, auto-configured light cube contains an LED, collimating optics, and filters. In addition to the channel dedicated to the transmitted light from the condenser for brightfield contrast applications, the EVOS™ FL Auto 2 Imaging System can accommodate up to five fluorescent or specialty light cubes for multiple-fluorescence research applications.

The table below lists some of the common fluorescent and specialty light cubes available from Thermo Fisher Scientific. For a complete list of available light cubes and to inquire about custom light cubes, go to **thermofisher.com/evos** or contact Technical Support (see page 175).

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

Appendix C: Graphical user interface (GUI)

The EVOS™ FL Auto 2 Imaging System is controlled by the integrated Invitrogen™ EVOS™ FL Auto 2 Software through a graphical user interface (GUI), which is accessed by the touch-screen monitor or the computer mouse and keyboard.

GUI layout

The GUI of the EVOS™ FL Auto 2 Imaging System consists of the Viewing area on the left and a series of tabs representing the main functions of the software (Capture, Automate, Review, and Settings) on the right. Each tab contains the controls necessary to execute the selected function. The Locations button, Area View/Field View toggle, and the Zoom slider are located above the Viewing area, which can contain additional controls depending on the view mode and the tab selected.
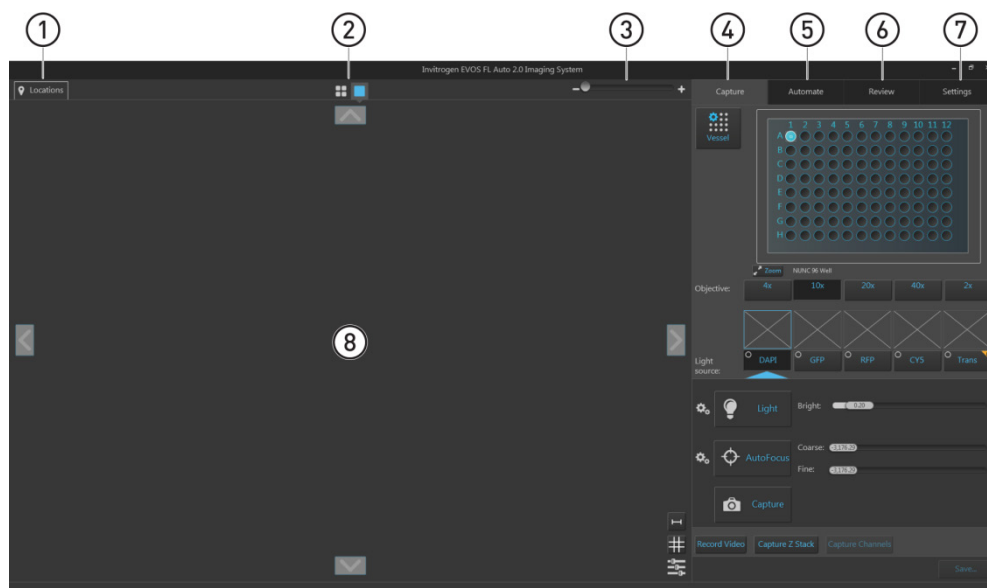
① **Locations:** Opens the Location tools, which allow you to select locations or designate capture fields for automated scan protocols (page 92).

② **Area View/Field View toggle:** Switches between Area View and Field View.

③ **Zoom slider:** Zooms in and out of the Viewing area.

④ **Capture tab:** Contains the controls for the manual capture of images (page 95).

⑤ **Automate tab:** Used to create and run automated scan protocols (page 114).

⑥ **Review tab:** Allows you to review captured images.

⑦ **Settings tab:** Contains controls to select and adjust basic and advanced system options and instrument functions (149).

⑧ **Viewing area:** Displays the sample in Area View or Field View mode (page 85).

Note: Click on the **View Help Content** icon to open the Help window, which provides EVOS™ FL Auto 2 Imaging System Help.

Hover your pointer over the **Tooltip** icon without clicking it to view a small hover box with additional information.

Viewing area

Overview

Viewing area displays the sample in **Area View** or **Field View**. The Area View/ Field View toggle and the Zoom slider are located above the Viewing area.

Area View

In Area View, the Viewing area displays a graphical representation (i.e., map) of the selected target area in its entirety. Depending on the vessel in use, the target area may represent a single well of a multiwell plate, a chamber of chamber slide, or an entire culture dish.

Area View allows you to select individual fields of view for manual image capture.
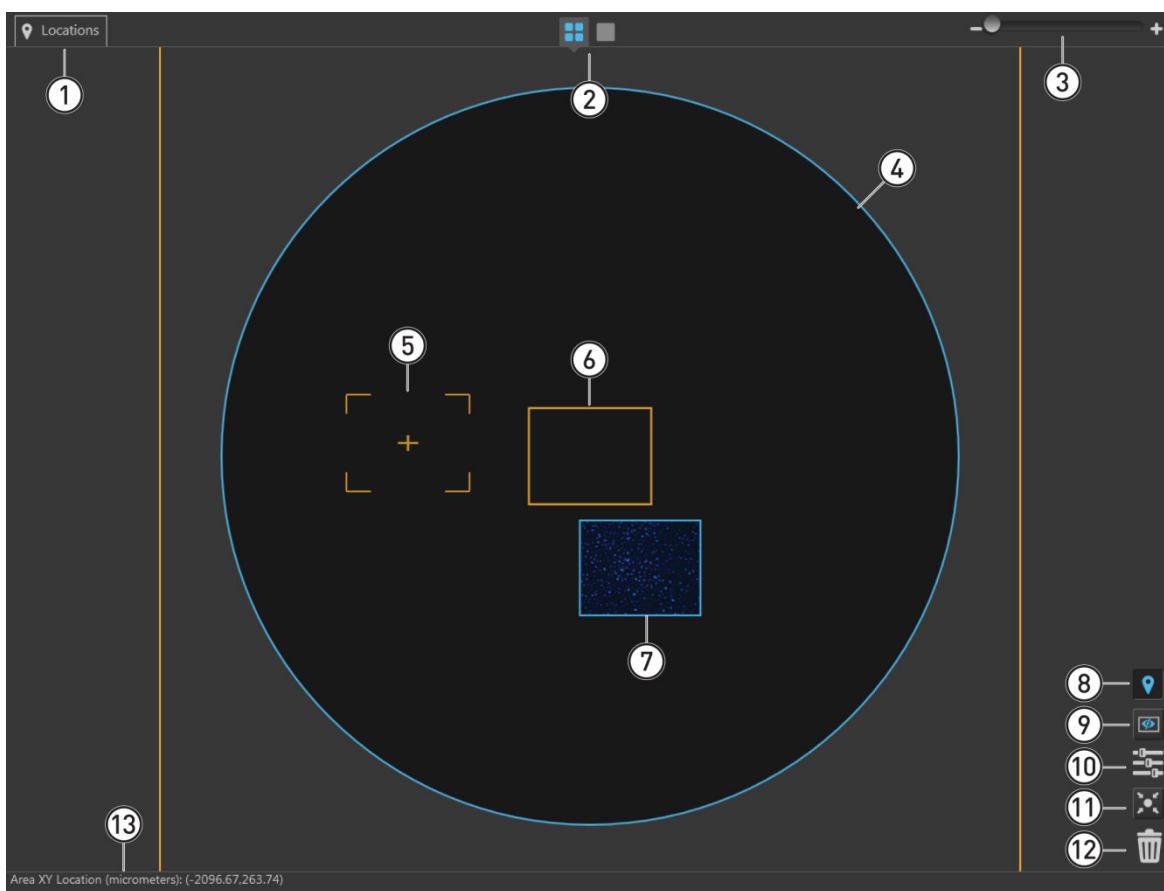
① **Locations** (page 92)
② **Area View/Field View toggle** (page 86)
③ **Zoom slider** (page 86)
④ **Area map** (page 86)
⑤ **Field selection tool** (page 86)
⑥ **Selected field** (page 87)
⑦ **Captured field** (page 87)
⑧ **Toggle locations display** (page 87)
⑨ **Toggle field borders** (page 87)
⑩ **Image display settings** (page 87)
⑪ **Center on selected field** (page 88)
⑫ **Delete image options** (page 88)
⑬ **Area and pixel information** (page 88)

Area View controls

① **Locations button:** Opens the Locations tools, which allow you to mark ("pin") specific points or draw fields of interest in the Viewing area, which can be revisited for further examination or captured during automated scan protocols.
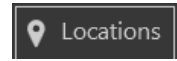
Note that the Location tools accessed from the Area View differ from those that are accessed through the Field View.

For detailed description of the Locations function, see page 92.

② **Area View/Field View toggle:** Switches between Area View and Field View.

③ **Zoom slider:** Zooms in and out of the Viewing area.

The minimum and maximum zoom for Area View are 1X and 2X, respectively.

④ **Area map:** Represents the current sample area selected for view or capture (i.e., target area). The target area is selected using the **Vessel map** (page 99).
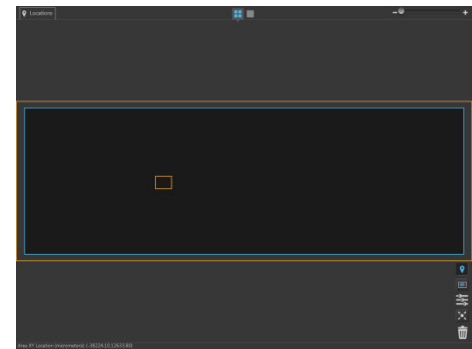

Note: If using a single culture dish, flask, or slide, the scan area corresponds to the entire dish, flask, or slide. As such, the scan area displayed in Area View represents the entire dish or flask selected.

In a multi-well plate, multi-chamber slide, or multiple dishes on multi-dish holders, each scan area corresponds to a single well, chamber, or dish.

The following examples show the area map of a single well of a 96-well plate (left) and a sample slide on a double-slide holder (right). The orange square in each examle correspond to a single field view at 4X magnification and illustrate the relative size of a single well in a 96-well plate and a sample slide.
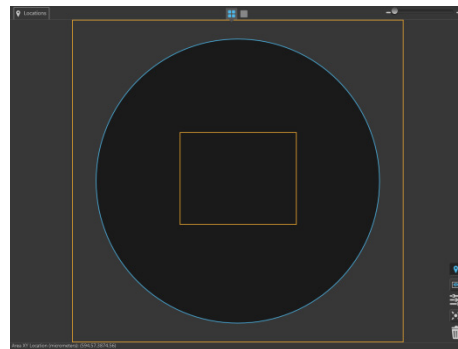
Area map – Single well of a 96-well plate   Area map – Sample slide

⑤ **Field selection tool:** Selects the target field you wish to capture.

- The selection tool consists of an orange crosshair within a broken orange rectangle. The crosshair indicates the position of the objective lens over the target and the rectangle represents the actual imaging area.

- The relative size of the field selection tool depends on the zoom level and the selected objective.

⑥ **Selected field:** Field that has been selected. Selected field corresponds to the field of view displayed in the **Field View**.

- Selected field, whether captured or not, is indicated by orange borders around it, which cannot be turned off.

- Clicking the **Capture** button (page 105) captures an image of the selected field and stores it in the memory buffer.

⑦ **Captured field:** Field that has been captured. A thumbnail of the captured image is displayed within the field borders.

If multiple images are captured for the same field in different channels, the captured images are displayed as an overlay.
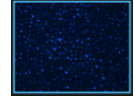
⑧ **Toggle locations display:** Switches the display of locations (defined with the Location tools) in Area View on and off.

Locations display is on   Locations display is off

⑨ **Toggle field borders:** Switches the display of the borders around captured fields in Area View on and off. This option is not available in Field View.

Field borders are on   Field borders are off

- When field borders are turned on, all captured fields in Area View are displayed with blue borders around them.

- Currently selected field, whether captured or not, is displayed with an orange border, which cannot be hidden.

⑩ **Image display settings:** Opens the Image display settings window, which allows you to adjust image display parameters (brightness, constrast, gamma) for the Viewing Area. Clicking the button again closes the window.

	👁	☀	◑	🎨	
GFP	✔	0.50	0.50	1.00	Reset
DAPI	✔	0.50	0.50	1.00	Reset
RFP	✔	0.50	0.50	1.00	Reset
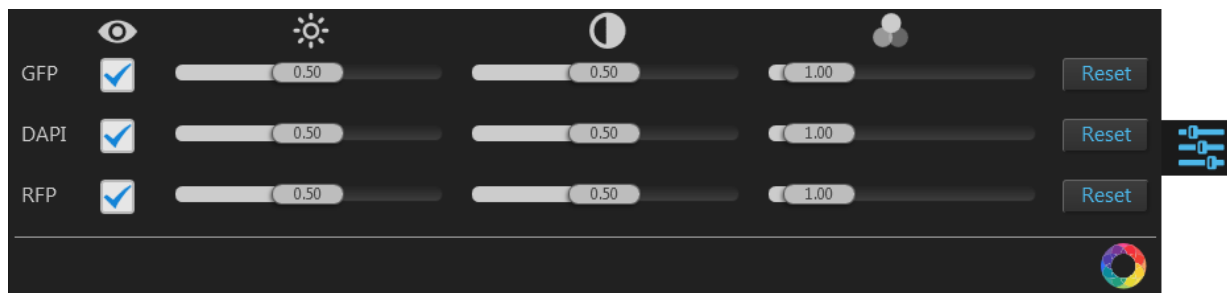
Note: Adjustments made to Image display settings only affect how the image is displayed in the Viewing Area; they do not affect how the image is captured.

- **Visible Channels:** Visible channels 👁 are the channels selected for display in the Viewing area. They are selected using the **Toggle channel display** checkbox located on the top left corner of the corresponding **Light source** button (see page 101).

Note: The controls for image display settings are contextual; only the controls for the visible channels will be available. In the example above, only the controls for the GFP DAPI, and RFP channels are displayed.

- To remove a channel from display, uncheck the corresponding **Visible Channel** 👁 checkbox. To display it again, recheck the checkbox.

- **Brightness** ☀, **Contrast** ◐, and **Gamma** 🌓 parameters for each of the selected channels are adjusted using the corresponding sliders.

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

  When on, images are displayed in default pseudocolor corresponding to the channel in which they were captured. You can change the default pseudocolor using in the **Settings ▸ Filter Cubes** tab (page 149).

  When off, images are displayed in grayscale.

| Color display is on | Color display is off |

(11) **Center on selected field:** Centers the Viewing area on the selected field.

(12) **Delete image options:** Opens the Delete image options window, which contains the controls listed below. Clicking the button again closes the window.

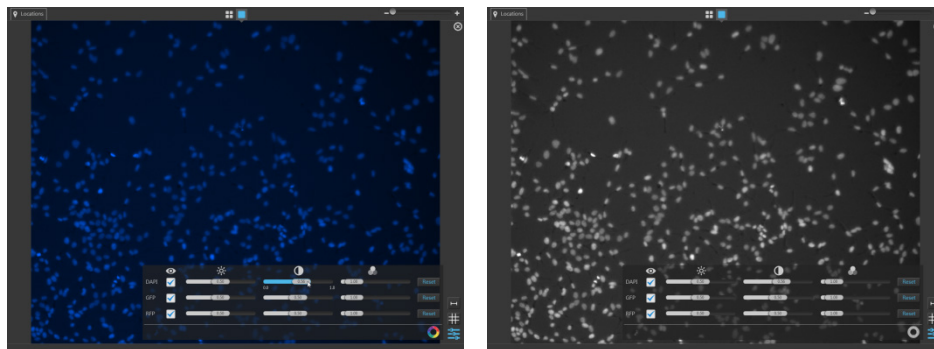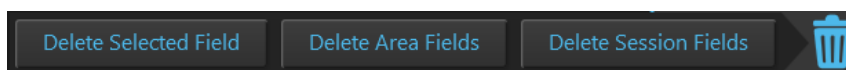- **Delete Selected Field:** Deletes only the selected field, if the selected field contains a captured image. If the selected field is empty, this option is not available.

- **Delete Area Fields:** Deletes only the fields that were captured in the current scan area. Images captured in other areas are not deleted.

- **Delete Session Fields:** Deletes all fields in all areas that were captured in the current session.

(13) **Area and pixel information:** Displays the coordinates of the pointer and pixel intensity at the pointer location.

Area XY Location (Microns): (-389.57,475.19) Pixel XY Offset: (80, 80) Z Locations: (N/A, -3538.33, N/A, N/A, N/A) Pixel Intensities: (N/A, 341, N/A, N/A, N/A)

- **Area XY Location (Microns):** Location of your pointer in relation to the center of the selected well along the x-y axis, expressed in μm.

- **Pixel XY Offset:** Location of your pointer in relation to the top left corner of the captured field, expressed in pixels along the x-y axis.

- **Z Locations:** Location of the captured plane along the z-axis, in μm.

- **Pixel Intensities:** Pixel intensity at the pointer location as reported by the camera. It represents the number of photons detected by the camera sensor.

- Area XY Location at the pointer location is displayed for the entire scan area. Pixel XY Offsets, Z Locations, and Pixel Intensities are displayed only for captured fields.

Field View

Field View displays the currently selected field in the Viewing area. It allows you to preview the sample and to adjust imaging parameters (e.g., brightness, focus) in the Live mode before capture.
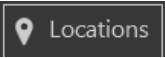
- If the selected field contains a captured image and the display is turned on for the channel in which the image was captured, Field View displays the captured image, allowing you to preview it before saving.

- If the field was captured in multiple channels, Field View displays an overlay of the captured images.
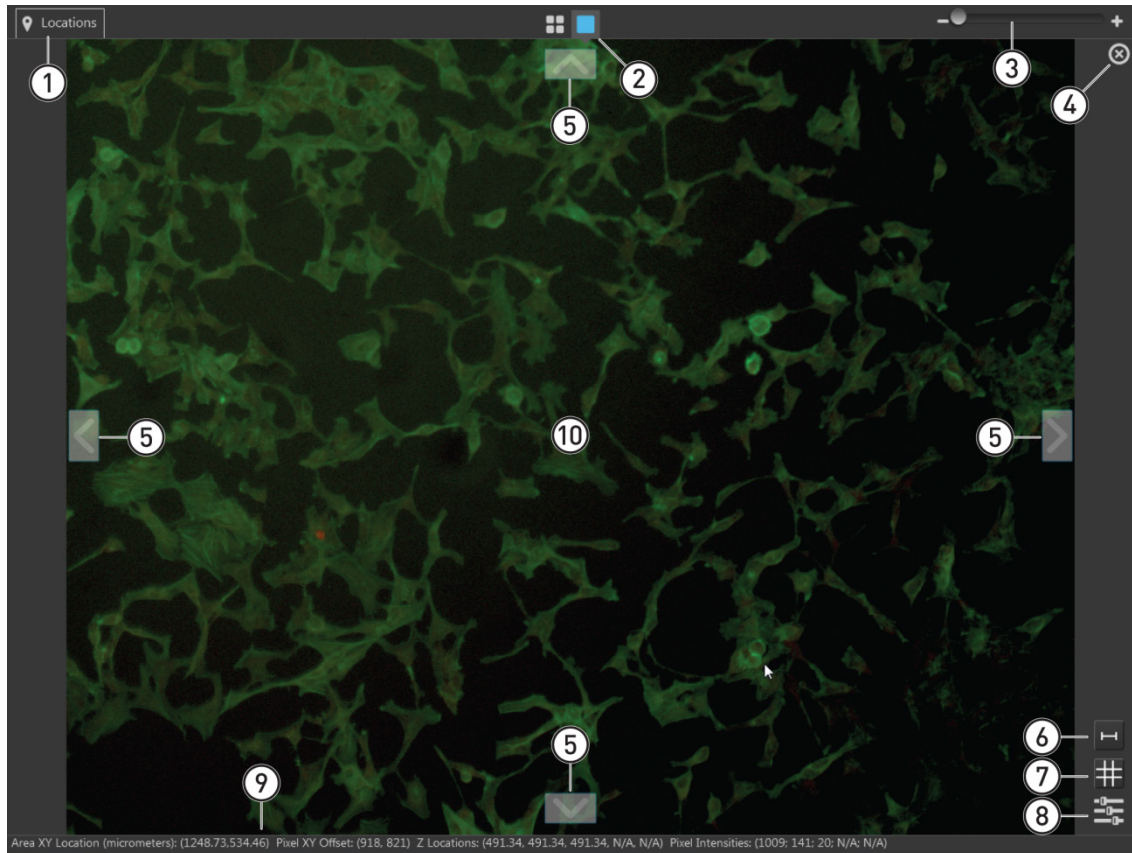
①	**Locations** (page 92)	⑥	**Toggle scale bar** (page 90)
②	**Area View/Field View toggle** (page 90)	⑦	**Toggle sample grid** (page 90)
③	**Zoom slider** (page 90)	⑧	**Image display settings** (page 90)
④	**Delete field** (page 90)	⑨	**Area and pixel information** (page 91)
⑤	**Navigation arrows** (page 90)	⑩	**Selected field of view** (page 91)

Field View controls

① **Locations button:** Opens the Locations tool, which allows you to mark ("pin") specific points in Field View.

The pinned points can be revisited later or used in a automated scan protocol.

Note that the Location tools accessed from the Field View differ from those that are available through the Area View.

For detailed description of the Locations function, see page 92.

② **Area View/Field View toggle:** Switches between Area View and Field View.

③ **Zoom slider:** Zooms in and out of the Viewing area.

The minimum and maximum zoom for Field View are 1X and 4X, respectively.

④ **Delete field:** Deletes the image captured in the current field on view. This button is available only if there is an image captured in the current field on view.

⑤ **Navigation arrows:** Moves the stage exactly one field of view in the corresponding direction (**up**, **down**, **left**, **right**).

⑥ **Toggle scale bar:** Switches the display of the scale bar in the Viewing area on and off.

⊢ Scale bar display is on ⊢ Scale bar display is off

⑦ **Toggle sample grid:** Switches the display of the sample grid in the Viewing area on and off.

Sample grid display is on Sample grid display is off

⑧ **Image display settings:** Opens the Image display settings window, which allows you to adjust image display parameters (brightness, constrast, gamma) for the Viewing Area. Clicking the button again closes the window.

For more information on Image display settings window, see page 87.

	👁	☀	◑	♣	
GFP	✓	0.50	0.50	1.00	Reset
DAPI	✓	0.50	0.50	1.00	Reset
RFP	✓	0.50	0.50	1.00	Reset

⑨ **Area and pixel information:** Displays the coordinates of the pointer and pixel intensity at the pointer location.

Area XY Location (Microns): (96.34,1215.38) Pixel XY Offset: (638, 930) Pixel Intensity: (424)

This information is displayed only if the Light is on for the selected channel, or if the current field of view contains a captured image and image display is on for the channel in which the image was captured.

- **Area XY Location (Microns):** Location of your pointer in relation to the center of the selected well along the x-y axis, expressed in µm.

- **Pixel XY Offset:** Location of your pointer in relation to the top left corner of the captured field, expressed in pixels along the x-y axis.

- **Z Locations:** Location of the captured plane along the z-axis, in µm.

- **Pixel Intensities:** Pixel intensity at the pointer location as reported by the camera. This represents the number of photons detected by the camera sensor.

⑩ **Selected field of view:** Displays the entire selected field of view.
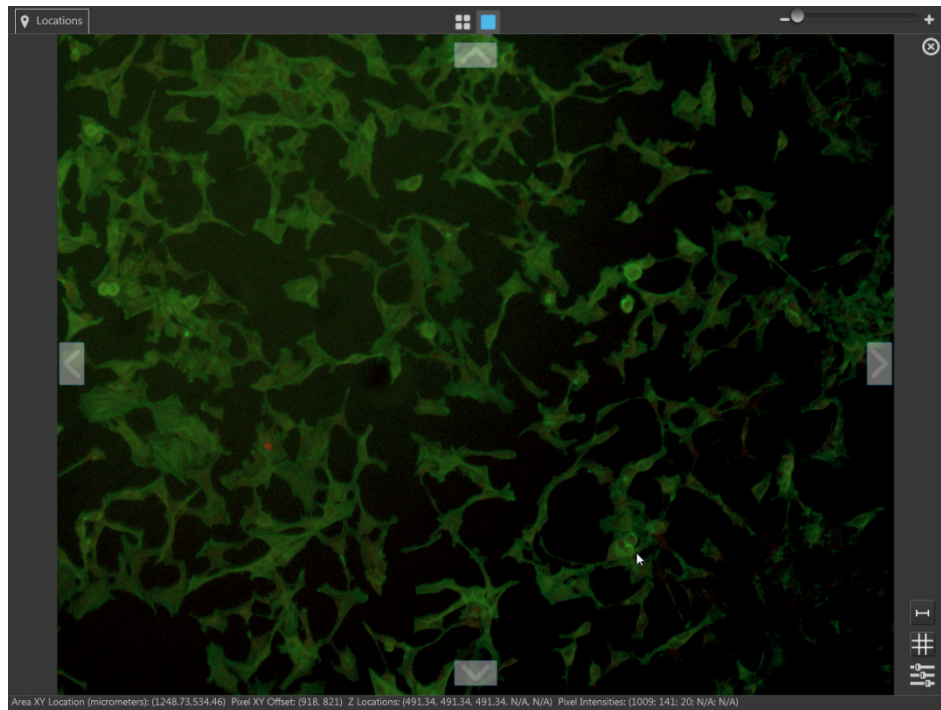
- If a previously captured field is selected in Area View, Field View displays the image captured in the selected field.

- If the selected field is captured in multiple channels, Field View displays an overlay of the captured images.

- Switching the Field View to **Live mode** (i.e., **Light** button on) displays the live image of the selected field in the current channel, whether the field contains a captured image or not.

- Switching the **Live mode** off (i.e., **Light** button off) returns the Field View to its previous state (blank or displaying the previously captured image).

Locations

Locations function allows you to mark ("pin") specific points or draw fields of interest in the Viewing area. These locations of interest can then be revisited for further examination or captured during automated scan protocols.

Note that the Location tools are contextual; the tools available depend on whether the instrument is in Field View or Area View.
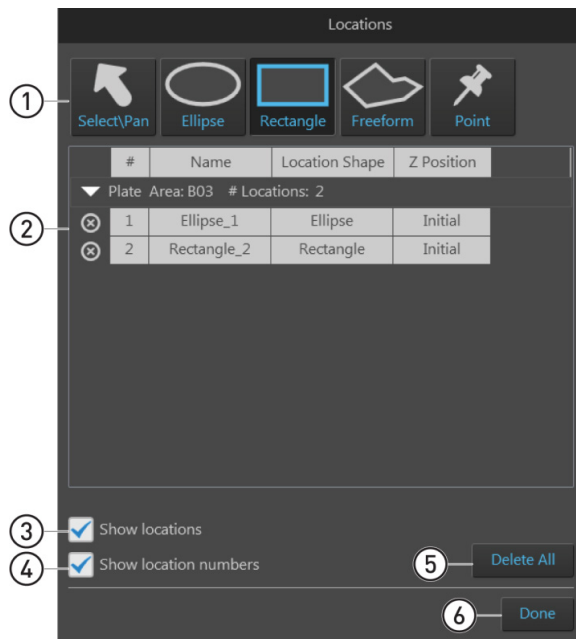
Locations – Area View
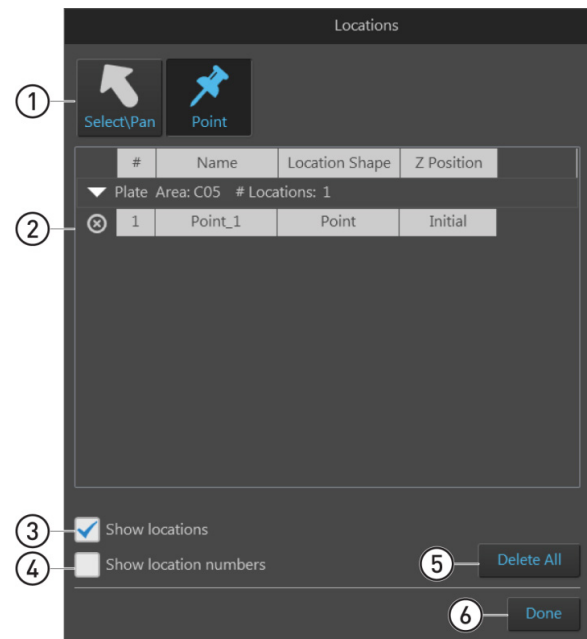
Locations – Field View

① **Location tools** (page 93)	④ **Show location numbers** (page 96)
② **Locations directory** (page 95)	⑤ **Delete all** (page 96)
③ **Show locations** (page 96)	⑥ **Done** (page 96)

Locations controls   ① **Location tools:** Used to define points and fields of interest.

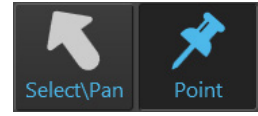Location tools – Area View   Location tools – Field View

- **Select\Pan:** Allows you to explore different regions of the sample vessel in the Viewing area when the Locations controls are active.
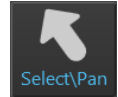
  To navigate to a different region in the Viewing area, select **Select\Pan**, then click-drag the Viewing area in the desired direction.

- **Ellipse:** Allows you to define a field group that best fits an ellipse drawn in Area View.
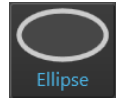
  To define the capture region, select **Ellipse**, then click-drag an ellipse shape in Area View.
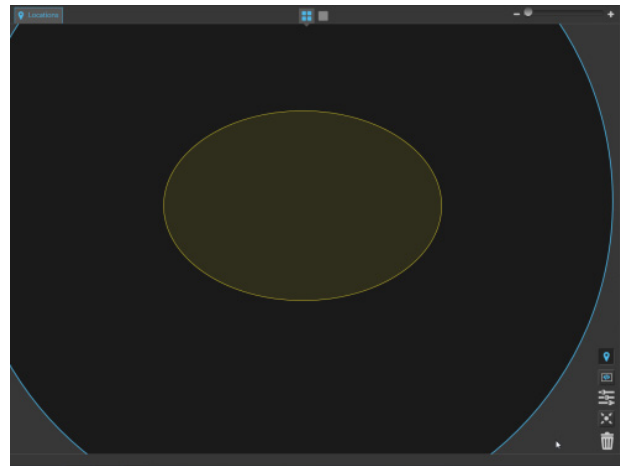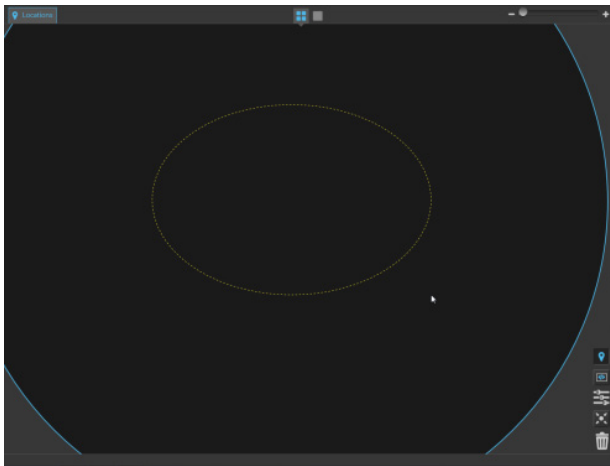
- **Rectangle:** Allows you to define a field group that best fits a rectangle drawn in Area View.

  To define the capture region,select **Rectangle**, then click-drag a rectangle shape in Area View.
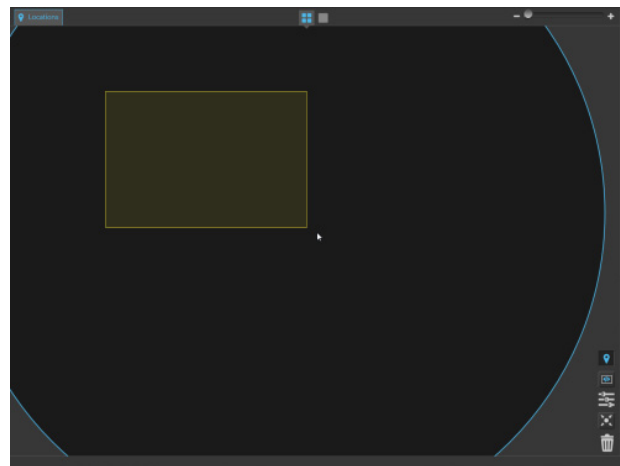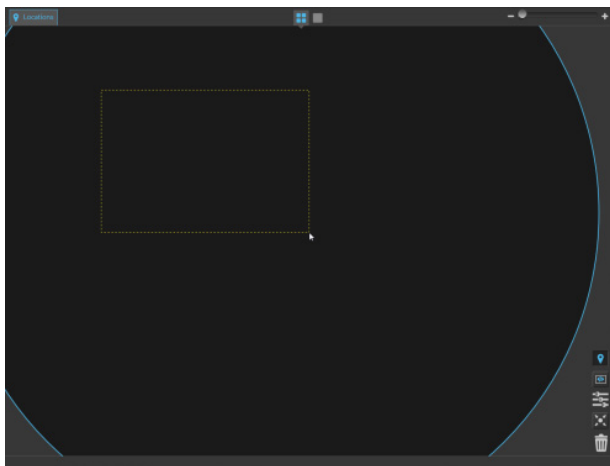
- **Freeform:** Allows you to draw a freeform shape based on straight-sided polygonal shapes in Area View that will encompass the capture fields.
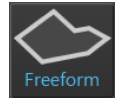
  To define the freeform region, select **Freeform**, then click on the desired location in Area View to add your first anchor point.

  At the next location, click again to add your second anchor point. When you release the mouse button, both points will be joined by a straight line.

  Continue moving around the Area View, clicking to add new anchor points and fastening the end of the line to each new point as you go.

  To close your freeform shape, double-click on your initial anchor point. Alternatively, double-click anywhere on Area View to close your freeform shape with a straight line from this point to your initial starting point.
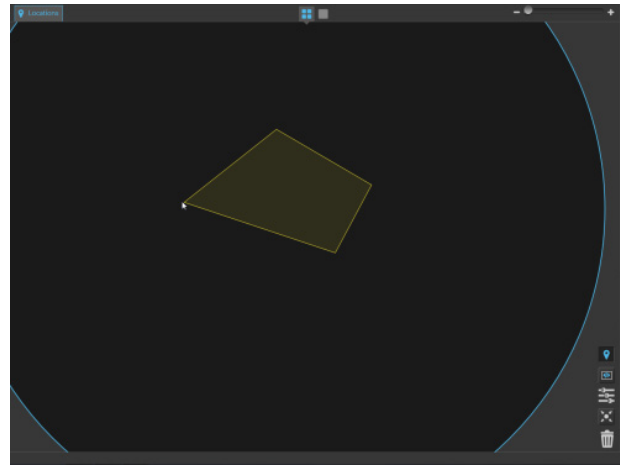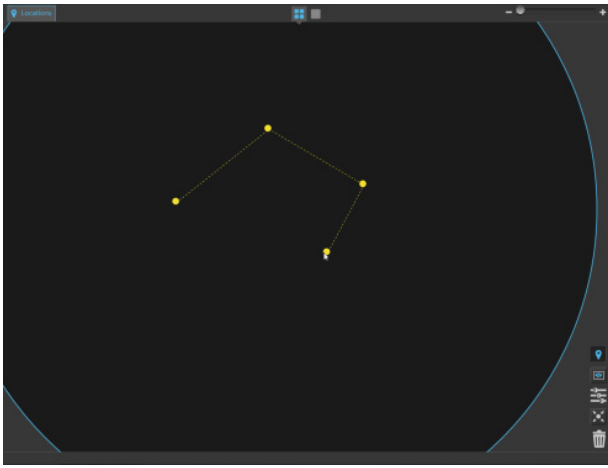
- **Point:** Allows you to mark ("pin") specific points in Area and Field Views as locations of interest.
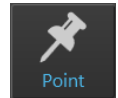
  To mark a point in Area or Field View, select **Point**, then click on the desired location.
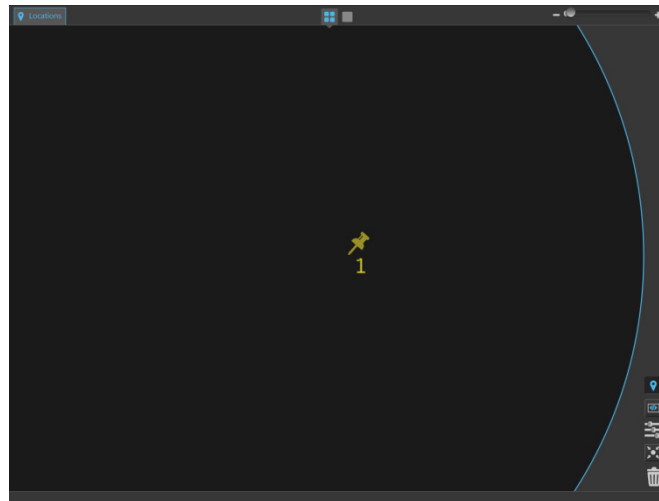
② **Locations directory:** Lists the locations (points or shapes) created with the Location tools in a table format. You can select a location from the directory to view the sample and/or set the z-focus at that location.

In the following example, the directory lists three locations created in the first well (Area: A01) of a 96-well plate: an ellipse, a rectangle, and a point.

#	Name	Location Shape	Z Position
▼ Plate Area: A01 # Locations: 3			
1	Ellipse_1	Ellipse	Initial
2	Rectangle_2	Rectangle	Initial
3	Point_3	Point	Initial

- To select a location, click the desired location from the list. The X-Y axis stage moves to align the objective with the selected ("current") location.

In the example below, location number 3 ("Point_3") is selected in the Locations directory and the objective is centered on that location (as indicated by the orange rectangle showing the field of view).

#	Name	Location Shape	Z Position	
▼ Plate Area: A01 # Locations: 3				
1	Ellipse_1	Ellipse	Initial	
2	Rectangle_2	Rectangle	Initial	
3	Point_3	Point	Initial	Set Z
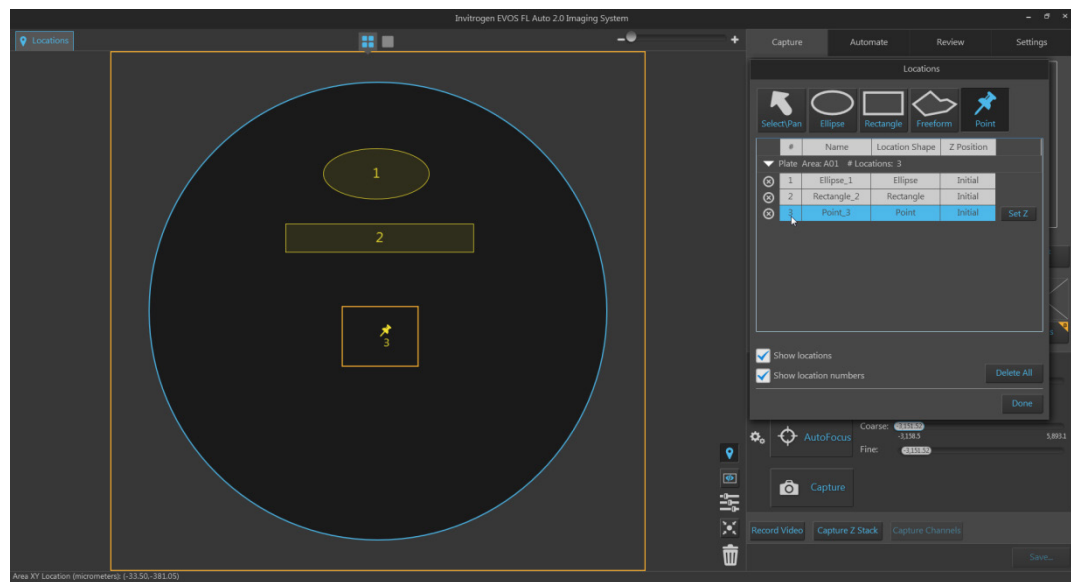
- To set the z-focus for a location, select the location from the list, then click **Set Z**. The z-focus will be set at the current z-position for that location.

- To change the z-position at a selected location, exit the Locations tool and adjust the focus using the **focus sliders** in the Capture tab, then **Set Z** for that location as described.

- To remove a location from the list, select the location, then click the **Delete Location** button.

③ **Show locations:** Toggles the display of existing locations in the Viewing area.
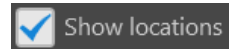
④ **Show location numberss:** Toggles the display of location numbers for the existing locations in the Viewing area.
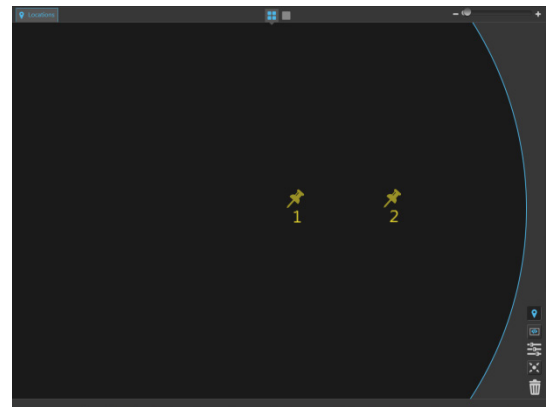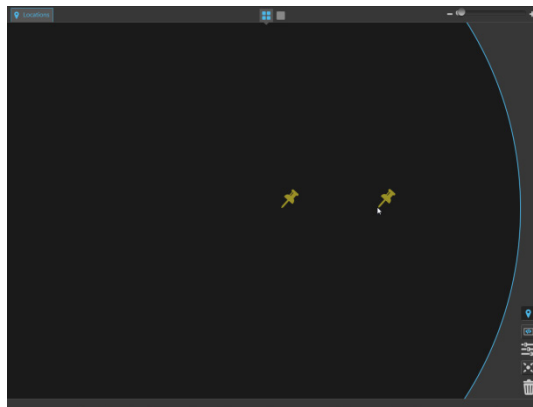
⑤ **Delete all:** Deletes all locations in the Locations directory and removes them from the Viewing area..

⑥ **Done:** Finalizes the location creation procedure and returns to the Capture tab.

Capture tab

Overview

Capture tab contains the controls for the manual capture of images. You can access the **Automate**, **Review**, and **Settings** at any time by selecting the corresponding tab. All current selections and settings within the Capture tab are preserved if you navigate away from the Capture tab.
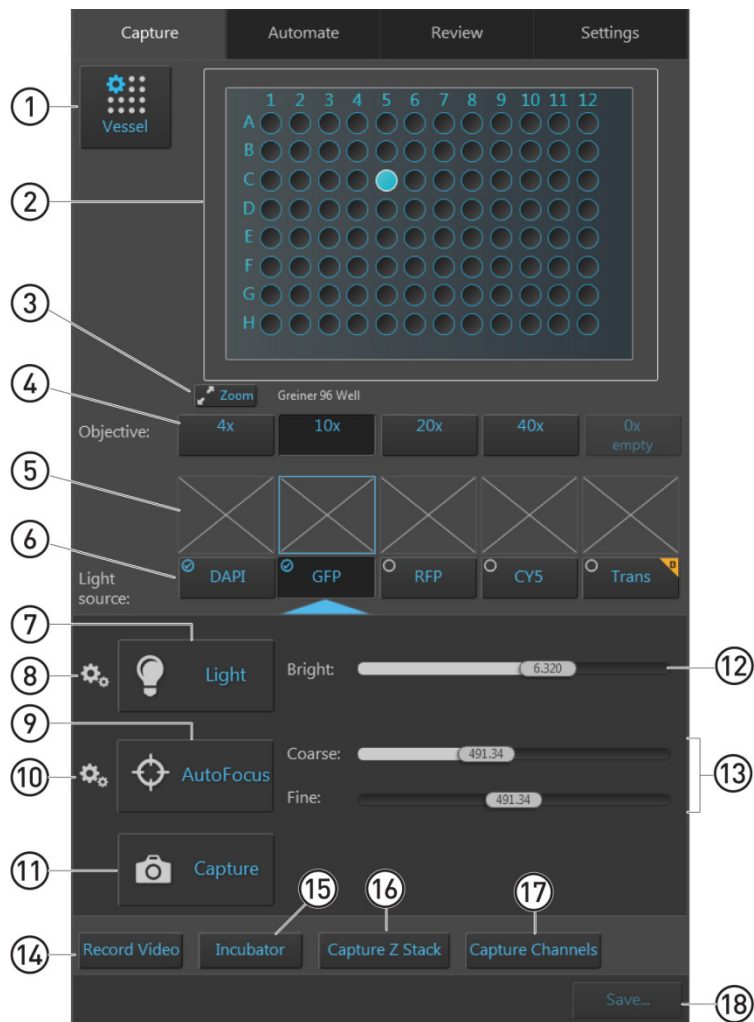
① **Vessel** (page 98)
② **Vessel map** (page 99)
③ **Zoom** (page 100)
④ **Objective** (page 100)
⑤ **Thumbnail image**s (page 100)
⑥ **Light source (Channel)** (page 101)
⑦ **Light (Live mode)** (page 101)
⑧ **Brightness and camera settings** (page 102)
⑨ **AutoFocus** (page 103)

⑩ **Advanced focus settings** (page 104)
⑪ **Capture** (page 105)
⑫ **Brightness controls** (page 106)
⑬ **Focus sliders** (page 107)
⑭ **Record Video** (page 107)
⑮ **Incubator** (page 108)
⑯ **Capture Z Stack** (page 109)
⑰ **Capture Channels** (page 111)
⑱ **Save** (page 111)

Capture tab controls

① **Vessel:** Opens the Vessel Selection wizard, used for specifying the vessel and vessel holder in use. You MUST select a vessel prior to imaging.
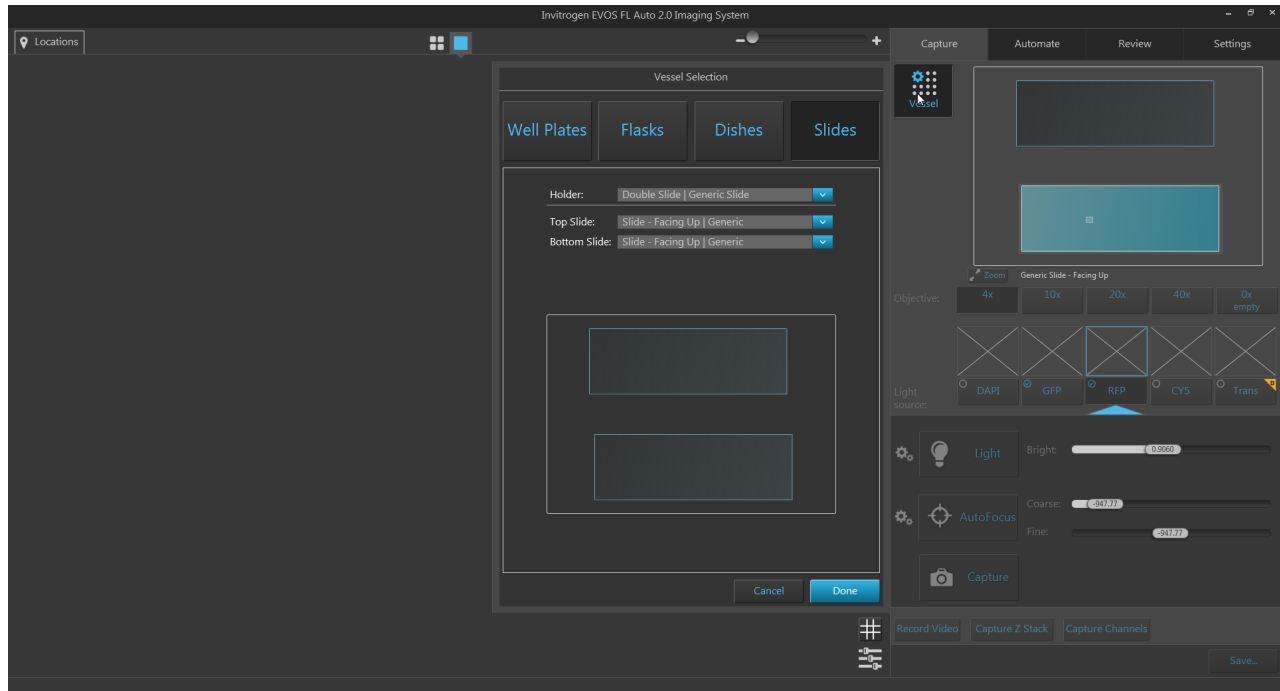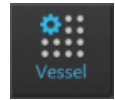
- Select the **Vessel category** that corresponds to your sample vessel to display the holder and vessel type selections available for that category.

  Available vessel categories are **Well Plates**, **Flasks**, **Dishes**, and **Slides**.
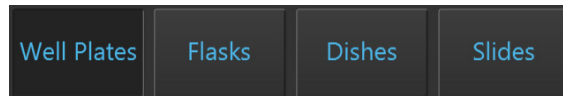
  The following images are example screens from the Vessel Selection wizard for each of the vessel categories.
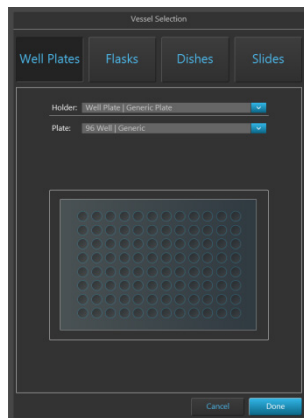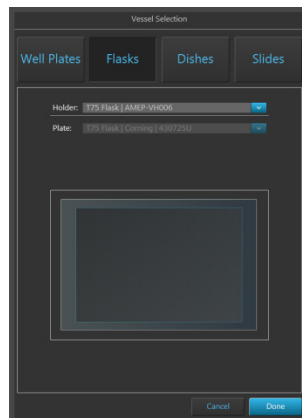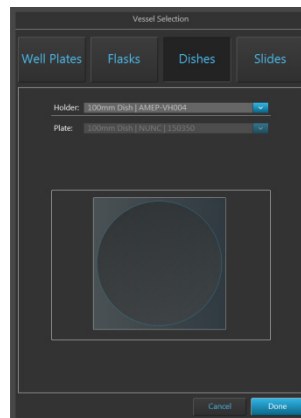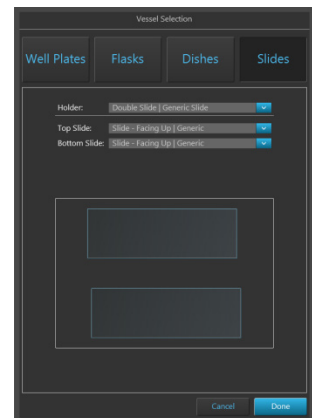
| Well Plates | Flasks | Dishes | Slides |

Note: The controls available in the Vessel Selection wizard are contextual; the dropdown menus display only the options available for the selected vessel category.

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- Select the appropriate **Holder** and **Vessel type** from the dropdown menus available for your vessel category.

  The following images are example screens from the Vessel Selection wizard for vessel holder and vessel type selection for each of the vessel categories.

| Holder: | Well Plate \| AMEP-VH061 | ∨ |
| Plate: | 96 Well \| Generic | ∨ |

Well Plates

| Holder: | 100mm Dish \| AMEPVH004 | ∨ |
| Plate: | 100mm Dish \| NUNC \| 150350 | ∨ |

Dishes

Holder:	T25 Double Flask | AMEP-VH-005	∨
Left Flask:	T25 Flask | Falcon | 353108	∨
Right Flask:	T25 Flask | Falcon | 353108	∨

Flasks

Holder:	Double Slide | AMEP-VH001	∨
Top Slide:	Slide - Facing Up | Generic	∨
Bottom Slide:	Slide - Facing Up | Generic	∨

Slides

② **Vessel map:** Represents the vessel container (vessel + vessel holder) in use, and it is determined by the selection made in the Vessel selection dropdown. The well selected for imaging is indicated in blue on the Vessel map.

- To select a well to display in the Viewing area, click on the desired well, flask, dish, or slide.

  In the following examples, the first well of a 96-well plate and the first dish on a 35-mm quad dish holder have been selected.
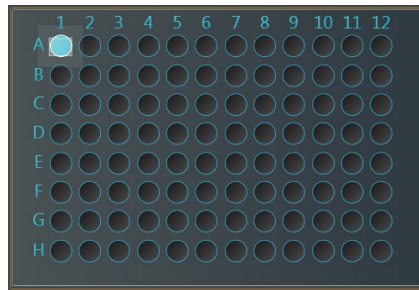
96-well plate
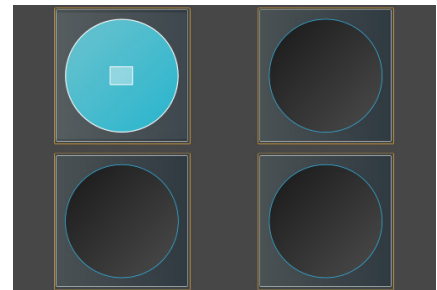
35-mm dishes on a quad dish holder

- When in the **Live mode** (page 101), the Vessel map displays crosshair that aid in navigation around the vessel or well, as shown below.
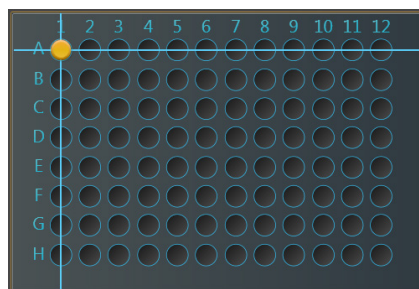
96-well plate
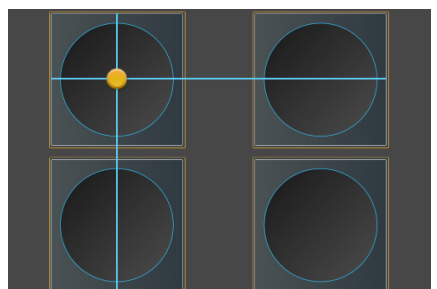
35-mm dishes on a quad dish holder

- To move the stage to another location on the sample vessel to display a different field, click and drag the **crosshair** to the corresponding location on the virtual vessel.

- When the Light is off (i.e, not in the Live mode), click on a different well on the Vessel map to move the **crosshair** to the same relative position as the previous well.

③ **Zoom:** Opens a larger view of the Vessel map.
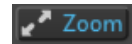
- This view shows the same content as the smaller Vessel map and behaves the same way.

- Clicking the button again closes the zoom window.
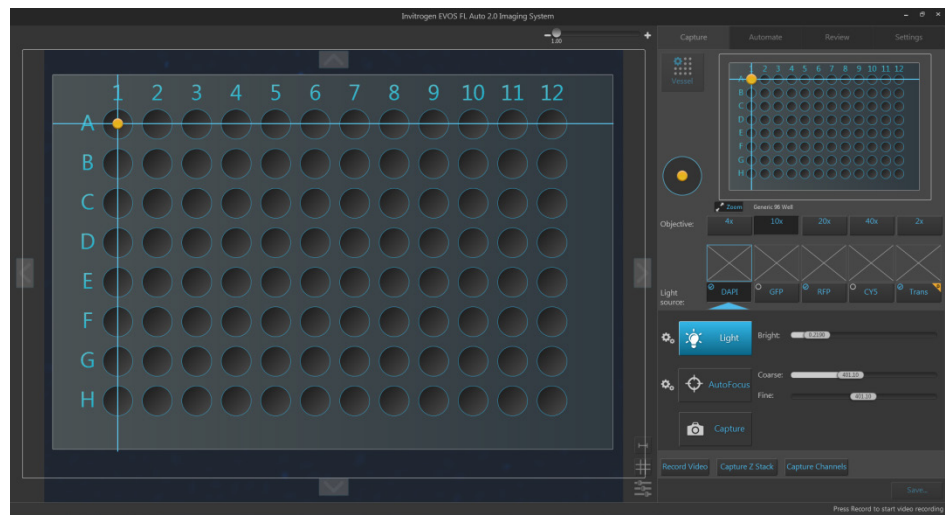
④ **Objective:** Used to select from the currently installed objectives.

- The magnification displayed on the objective button reflects the profile specified in Objective setup on the Settings tab.

- You can select only one objective at a time. In the example below, the 10X objective is selected.

⑤ **Thumbnail images:** Display the most recently captured image stored in the memory buffer for the specific channel.

- If unsaved, newly captured images will overwrite the previously captured image in the channel.

- If no image has been captured for the channel, the corresponding thumbnail will be blank.

- If z-stack was captured in the channel, the **z-stack** icon on the top left corner of the thumbnail image for that channel identifies the image as a z-stack projection.

- You can delete an image captured in a channel by clicking the **Delete channel image** icon on the top right corner of the thumbnail image for that channel.

In the example below, GFP channel displays the thumbnail for a z-stack projection while the RFP channel displays the thumbnail for a field captured at single focal plane. The rest of the channels do not have any images captured.

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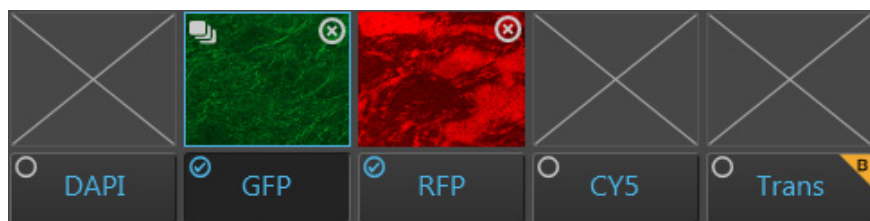
⑥ **Light source:** Selects the desired light source from the installed LED light cubes (fluorescent channels) or transmitted light from the condenser (brightfield).

- Click a **Light source** button to select the corresponding channel for capture. The selected channel is indicated by the blue arrow underneath the corresponding light source button. You can select only one light source at a time for capture.

- Check the **Toggle channel display** option located on the top left corner of the Light source button to select the corresponding channel for display in the Viewing area. You can select multiple channels for display.

  In the example below, the GFP channel is selected for capture and both GFP and RFP channels are selected for display.
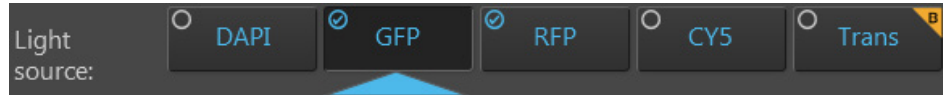
⑦ **Light (Live mode):** Turns on the excitation light for the currently selected light cube and enters the instrument in the **Live mode**. The Live mode allows rapid visualization of a large sample area illuminated with the selected light source before image capture.
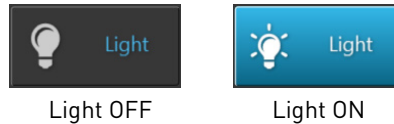
Light OFF   Light ON

- Clicking the **Light button** a second time or capturing an image turns the light off and exists the instrument from the Live mode.

- In the Live mode, the Viewing area shows the sample illuminated under the selected light source in pseudo-color with the default emission color.

  In the example below, the sample is illuminated in the GFP channel.
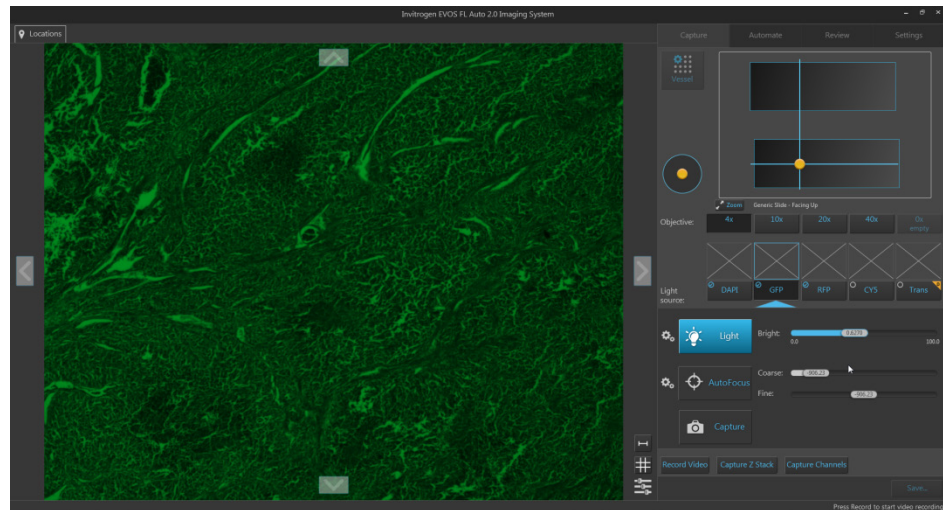
- The Vessel map displays the navigation crosshair, and the **Jog Control** button appears next to the Vessel map.

- **Jog Control** moves the stage at an intermediate pace, allowing the quick scanning of the sample in different parts of the sample vessel.

⑧ **Brightness and camera settings:** Opens the Brightness and camera settings window, which allows you to set Mode, Camera, and Phase options for the selected light source. Clicking the button again closes the window.
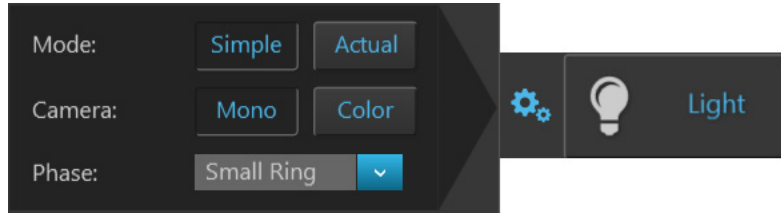
- **Mode:** Toggles between **Simple** and **Actual modes** for brightness controls.
    - **Simple mode** allows you to control light intensity as a single **Brightness** parameter (page 106).
    - **Actual mode** allows you to adjust **Light** (i.e., LED intensity), **Exposure**, and **Gain** parameters individually.
- **Camera:** Toggles between the **Mono** (monochrome)and **Color** cameras.
    - **Mono** is used for image capture in fluorescence and transmitted light (brightfield) channels. It uses a high-sensitivity 1.3 MP CMOS monochrome camera with 1328 × 1048 pixel resolution.
    - **Color** is used for image capture in the brightfield channel only. It uses a high-sensitivity 3.2 MP CMOS color camera with 2080 × 1552 pixel resolution.
- **Phase:** Selects between **Phase** options and **Brightfield** in the transmitted light channel only. Phase contrast options change the phase annuli being used to match the objective with a specific magnification. Available options are:
    - **Small Ring:** Used for objectives with low magnification (i.e., Olympus™ 4× PH)
    - **Medium Ring:** Used for objectives with medium magnification (i.e., EVOS™ 4×/10× PH)
    - **Large Ring:** Used for objectives with high magnification (i.e., EVOS™ 20×/40× PH)
    - **Brightfield** (phase contrast off)

Note: Phase options are available only for the transmitted light (brightfield) channel; they are not available for fluorescence channels. The phase contrast option does not require phase contrast objectives. However, to obtain a phase image, you must first install a phase contrast objective.

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⑨ **Autofocus:** Runs the autofocus algorithm in the currently selected channel to find the best focus position for the sample. This button is available on both Capture and Automate tabs.
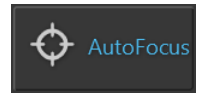
- The Autofocus window displays the image from the selected channel in real time as the instrument searches for the optimal focus.
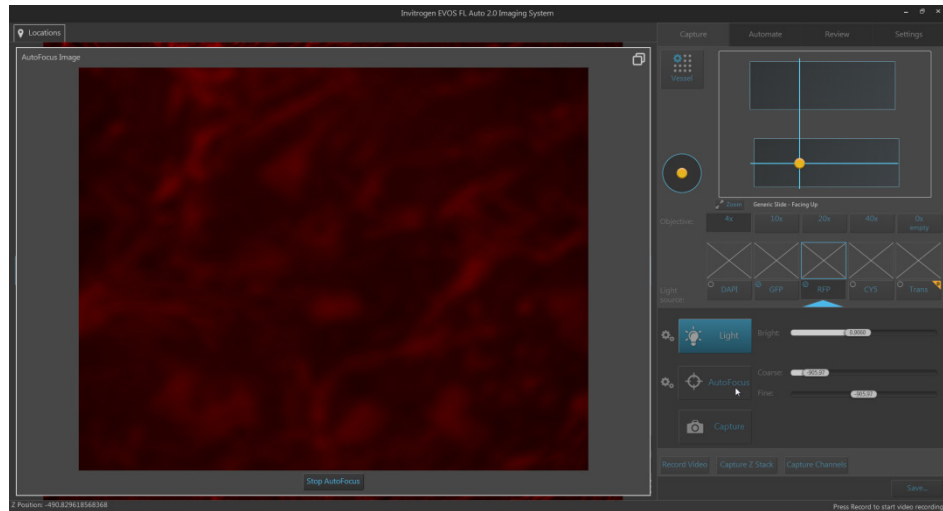
- To minimize the Autofocus window, click the **minimize** button.
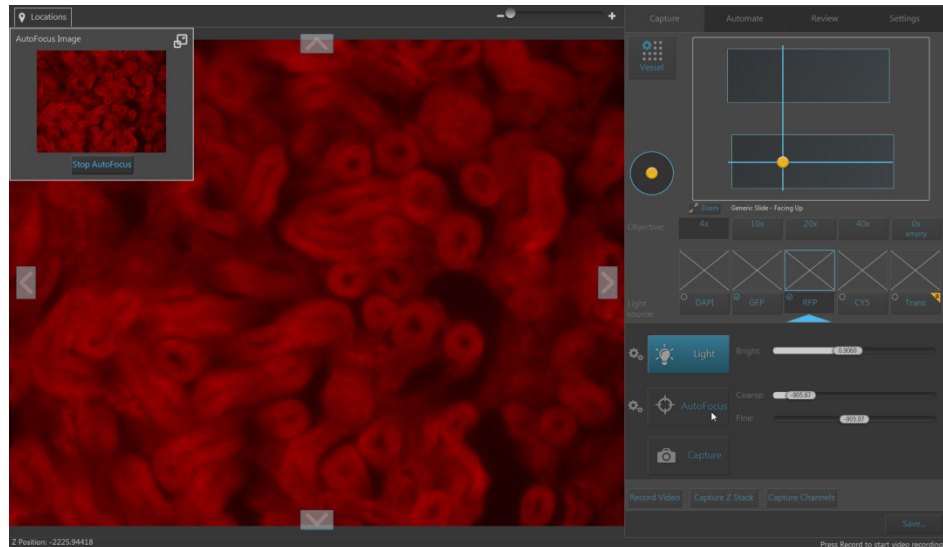
Once minimized, the Autofocus window can be moved around the Viewing area.

To expand the Autofocus window, click the **expand** button.

- Click **Stop AutoFocus** in the Autofocus window to abort the autofocus procedure.

⑩ **Advanced focus settings:** Opens the Advanced focus settings window, which contains the controls listed below. Clicking the button again closes the window.
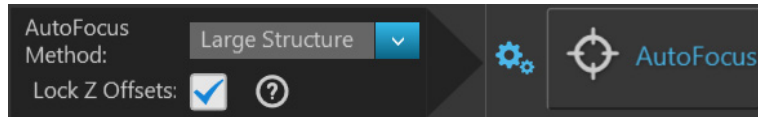
- **Autofocus method:** Allows you to select the autofocus method best suited for your experimental needs. Available options are:

  – **Small Structure:** This method computes the energy according to the size of image features and is able to measure the presence/absence of small image features.

    Choose this method when capturing images of samples that have many fine, hair-like structures (e.g., filaments or structural stains).

  – **Large Structure:** This method is statistics-based and looks for large changes in image content as parts of the image go in and out of focus.

    Choose this method when your sample contains large structures (e.g., whole cell stains).

  – **Small Bright Objects:** This method looks for changes in brightness at the center of the cell and optimizes focus on cells that have a bright center with a dark surround area.

    Choose this method when capturing samples with localized staining (e.g., nuclei).

Note: The effectiveness of the autofocus method depends on the plate type, sample type, biomarker intensity, magnification, and channel. It may be necessary to use different algorithms for different channels. This ensures the best autofocus is used for your sample.

- **Lock Z-Offsets:** Allows you to lock the Z-Offsets, which specify the optimal focus position in each channel relative to the focus position in other channels.

  – When locked, adjusting the Z-position in one channel changes the Z-position in all channels, preserving the relative Z-positions of the channels.

  – When unlocked, adjusting the Z-position in one channel does not affect the Z-position set for the other channels.

  For an example, see "Note", below. For more information on configuring the autofocus settings for an automated scan protocol, see page 133.

Note: Consider a scenario where the initial Z-positions of the DAPI and RFP channels are 50 μm and 80 μm, respectively. The autofocus is run from the DAPI channel and finds the best focal plane at a Z-position of 60 μm.

If the autofocus is selected to run from a single channel, then the Z-Offsets will be locked, and the RFP channel will be focused to a Z-position of 90 μm.

If the autofocus is run from all channels, then the Z-Offsets will not be locked, and the autofocus algorithm will find the best focal plane for the RFP channel independently of the DAPI channel.

⑪ **Capture:** Captures an image of the selected field using the current capture settings and stores it in the image cache of the channel in which it was captured.

- Field View displays the captured image. If multiple images are captured in the same field of view using different channels, the captured images are displayed as an overlay.

- The captured image is also be displayed above the Light source button for the specific channel in which the image is captured.

- A thumbnail of the captured image is displayed in Area View at the location of its capture when the zoom level is greater than one field of view.

The examples below show the Field View (top) and the Area View (bottom) after the same field has been captured in DAPI and GFP channels.
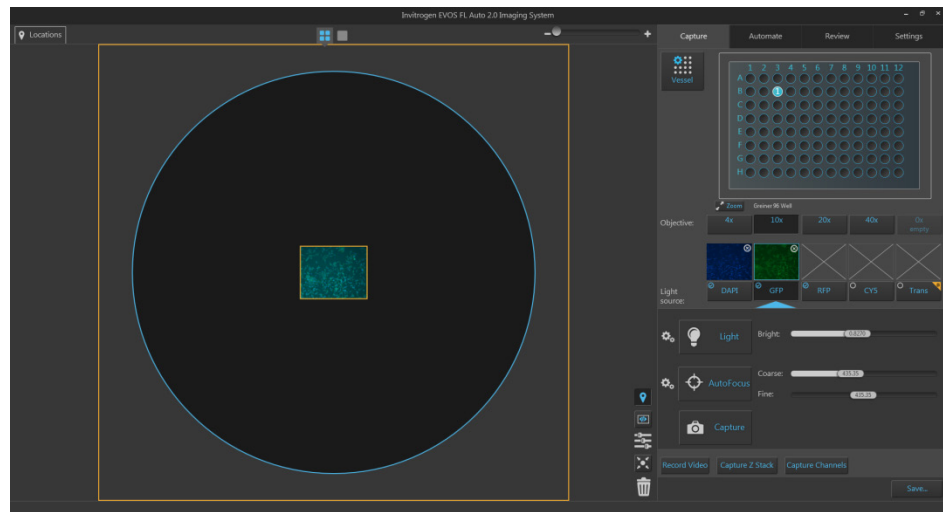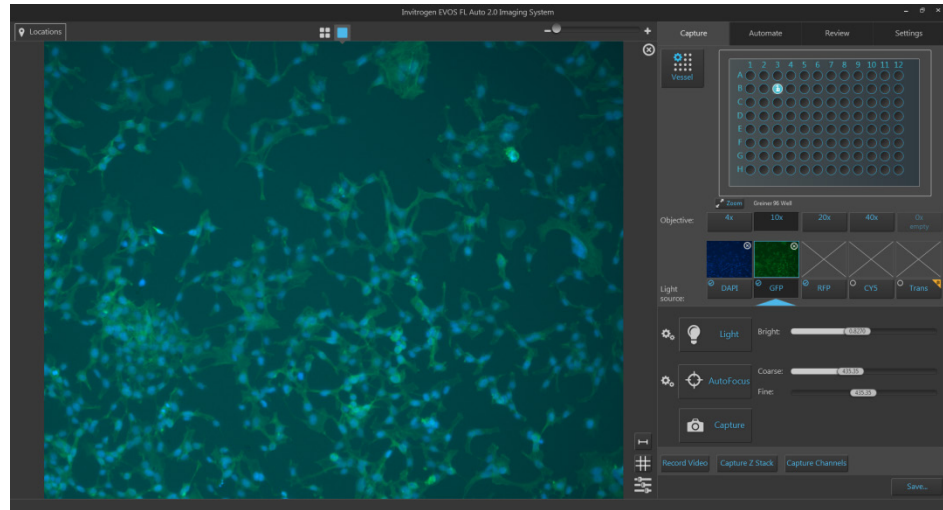
Note: Captured images are stored in the image cache for the selected channel. If unsaved, newly captured images of the same field will overwrite the previously captured image in that channel.

⑫ **Brightness controls:** Control the brightness settings for the selected channel. You can adjust the brightness settings for each channel independently without affecting the settings for other channels.

To adjust the settings for a given parameter, move the corresponding slider in the desired direction or enter the desired value in the **text box**.

- In the **Simple mode**, light intensity is controlled as a single **Brightness** parameter and expressed as a percentage between 0% and 100%.
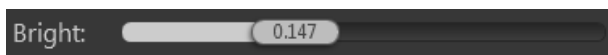
Brightness slider (Simple mode)

- In the **Actual mode**, brightness is controlled as individual **Light** (LED intensity), **Exposure**, and **Gain** parameters.
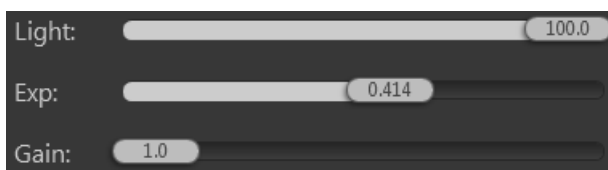
Light Exposure, and Gain sliders (Actual mode)

Brightness parameters in the Actual mode have the following ranges:

Light: 0–100%
Exposure: 0–4 seconds
Gain: 1–8

Note: For best results, optimize the brightness parameters as follows:

- **When searching for sample:** Increase **Gain** for a brighter signal and decrease **Exposure** for faster frame rate during navigation around the vessel.

- **When capturing image:** Decrease **Gain** to reduce background noise and increase **Exposure** to regain signal intensity, as needed.

- **For brighter signal:** Increase **Light** intensity for brighter illumination. If needed, follow by increasing **Gain**.

- **For time lapse imaging:** Increase **Gain** and **Exposure**, and decrease **Light** intensity to reduce photobleaching and phototoxicity.
  For example, for overnight time lapse experiments, capture one image every 30 minutes or less, limit the use of autofocus, and use a channel other than DAPI for autofocus.

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⑬ **Focus sliders:** Used for the manual adjustment of focus for the current channel. The focus position is expressed in μm along the z-axis.
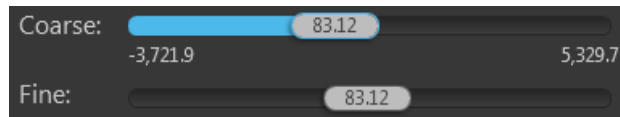
- The coarse focus slider bar represents the full focal range of the instrument while the fine focus slider bar represents only a small fraction of the full focal range.
- **Coarse focus slider handle** can be placed at any point within the focal range of the instrument.
- **Fine focus slider handle** can be dragged as far as either end of the slider bar, but it will snap back to the center position upon release.
- You can adjust the focus by moving the **focus sliders** in the desired direction.

  Alternatively, you can double-click on the handle of the focus slider to activate the text box and enter the desired value for the focus position.
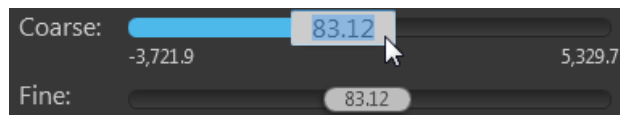
- If the instrument is in the **Live mode**, the live image changes in effective real-time to reflect the changes made to the focus.

⑭ **Record Video:** Opens the Record Video controls, which allow you to record capture a series of live images and compile them into a video.
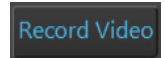
- Record Video controls are available only in the **Live mode**.
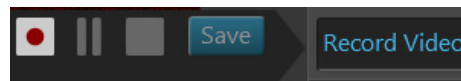
- Click **Record** to start recording live images.

  Duration and frames recorded are displayed under the Record Video controls.
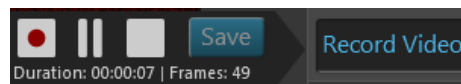
- Click **Pause** to pause recording live images.
- Click **Stop** to finish recording.
- Click **Save** to open the Save Video window.

  The Save Video window follows the standard Windows file navigation system and allows you to save the captured video in the desired location.

  You can choose to record your video in **AVI** or **WMV** formats.

⑮ **Incubator:** Opens Incubator tab, which allows you to control the EVOS™ Onstage Incubator and monitor its status during your experiments.

- **Use Incubator**: Enables the use of the EVOS™ Onstage Incubator.
- **Temperature:** Sets the incubator temperature (ambient to 40°C).
- **CO₂:** Sets the CO_2 level (0% to 20%).
- **Oxygen:** Sets the oxygen level (0% to 20%).
- **Use humidity:** Enables the use of humidified atmosphere in the incubation chamber.
- **Shutdown:** Allows you select between manual shutdown or automatic shutdown after a specified time period.
- **Incubator status:** Displays the current temperature, humidity, CO_2, and oxygen values against the target values.

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

⑯ **Capture Z Stack:** Opens Z-Stack Settings window, which allows you to capture multiple images along the z-axis based on your specifications.
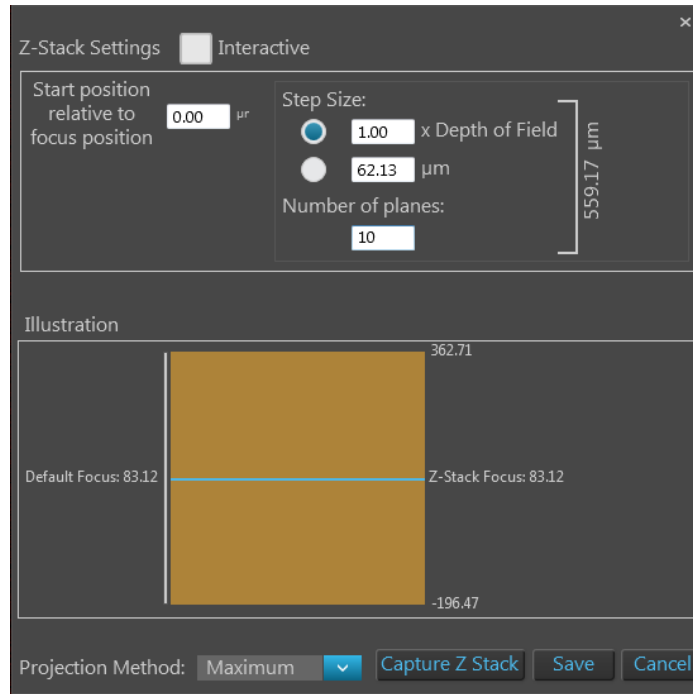
- **Start position relative to focus position:** Specifies the Z-Stack Focus position relative to the current focus position.

  The Z-Stack Focus represents the middle position between the top and the bottom planes of the z-stack; half of the images captured for the z-stack will be above this position and half will be below it.

- **Step Size:** Specifies the z-distance in μm between the successive focal planes that are captured for the z-stack.

  You can specify the step size as a **multiple of Depth of Field** or directly enter the **Step Size** in μm in the corresponding text box.

- **Number of planes:** Specifies the number of focal planes that are captured to generate the z-stack. Each captured image along z-axis represents a single "optical section" of the sample.

- **Illustration:** Graphically demonstrates the z-stack parameters (see image above). As you enter the z-stack parameters, **Illustration** is automatically updated to reflect the new settings.

  – The Z-Stack Focus is represented by a blue line. It is the sum of current focus position and the start position relative to the focus position.

  – The orange rectangle represents the z-stack depth. It is the product of step size and number of planes.

  – The positions of the bottom and the top focus positions and the position of the Z-Stack Focus are indicated in μm next to the orange rectangle representing the z-stack depth.

- **Interactive:** When checked, displays the interactive z-stack controls, which allow you to set the top and bottom positions of the z-stack manually.
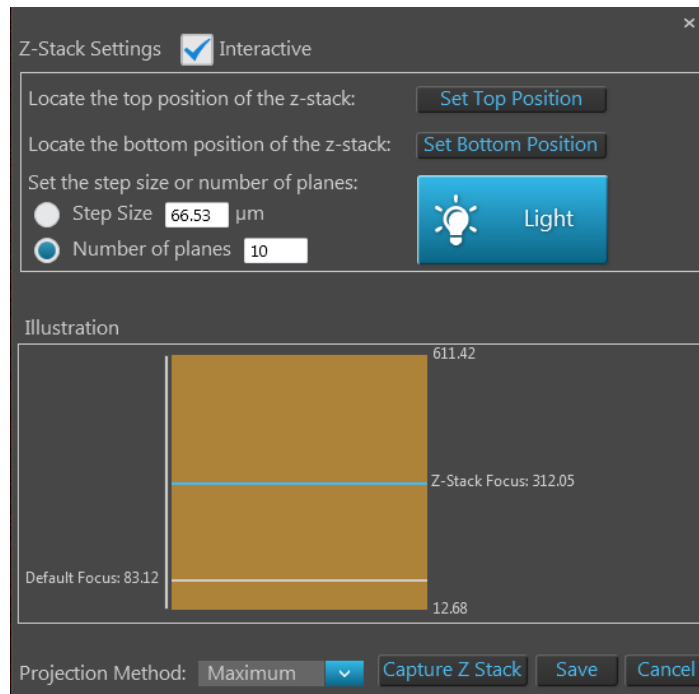
- **Light:** Turns on the light source in the selected channel to illuminate the sample as you locate the top and bottom boundaries of the z-stack using the focus sliders. Clicking the button again turns off the light.

- **Set Top Position:** Sets the current focal plane reached using the focus sliders as the top position of the z-stack.

- **Set Bottom Position:** Sets the current focal plane reached using the focus sliders as the bottom position of the z-stack.

- **Projection Method:** Determines the mathematical algorithm that will be used to extract the most in-focus pixels from the images captured at different focal planes to generate the z-stack projection.

- **Maximum:** For fluorescent images that are bright on dark background.

- **Standard deviation** (StDev)**:** For unstained transmitted images where the objects are identified by the contrast instead of the intensity.

- **Average:** For fluorescent or histochemically-stained transmitted images when assessing concentration of the stain/marker.

- **Capture Z Stack:** Captures the z-stack images based on your specifications.

- **Save:** Saves the z-stack settings for future use in the current or another selected channel.

  Clicking **Save** closes the Z-Stack Settings window. To capture z-stack images, click **Capture Z-Stack** to re-open the Z-Stack Settings window.

- **Cancel:** Closes the Z-Stack Settings window without capturing z-stack images or saving z-stack settings.

Note: You can capture z-stack images in only one channel at a time; however, you can save z-stack images of a field captured in different channels as a single merged video.

⑰ **Capture Channels:** Captures a selected field in multiple channels simultaneously.

Capture Channels

Before capture, each channel to be acquired must be selected by clicking on the small circular checkbox located on the upper left corner of the corresponding light source button.

In the example below, DAPI, RFP, and transmitted light (brightfield) channels have been selected for simultaneous capture.

| Light source: | ○ DAPI | ⊘ GFP | ⊘ RFP | ○ CY5 | ○ Trans |

⑱ **Save:** Opens the Save options window, which allows you to select a save location and to set save options.

Save...

Save

Select fields to save:
- ○ Currently selected field
- ○ All newly captured fields
- ○ All captured fields

Location: D:\EVOS Files\Scan   Browse...
Prefix: image

Select file types to save: ⓘ

Raw images:
- ☑ Single field, individual channels
- ☑ Z-stack planes, individual channels
- Select file format: TIF ▾ ⓘ

Displayed images:
- ☑ Single field, individual channels
- ☑ Merged image
- ☐ Tiled image, merged channels
  - Small ▾
  - Color: ○ Grayscale ● Pseudocolor
  - Select file format: TIF ▾ ⓘ
  - ☐ Include Grid Auto Size ▾

More options...

Cancel   Save

- **Select fields to save:** Allows you to select the captured fields you wish to save. You can choose from the following options:
  - **Currently selected field:** Saves images only from the current field.
  - **All newly captured fields:** Saves images that have been captured and stored in the image cache, but not yet saved. This option is available only if you have previously saved images from the same session.
  - **All captured fields:** Saves images from all captured fields that are held in the image cache. This is typically all of the images that you have captured during an imaging session.

- **Location:** Describes the location of the current folder or file.

Location: C:\Users\ali.ozgenc\Documents\EVOS_Files

- **File name prefix:** Allows you assign a prefix to your saved images.

File name prefix: image

The default prefix for images captured in the Capture tab is "image".

- **Browse:** Opens the Select Folder window, which allows you to assign a destination folder for your saved images.

Browse...
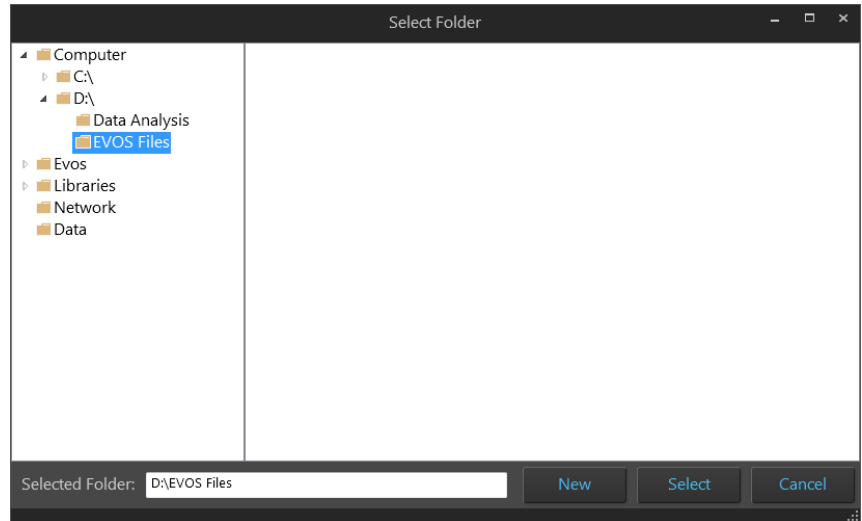
- **Select file types to save:** Allows you to set Image save options. You can select one or more of the following options:

- **Raw images:** Saves images from individual channels as 16-bit raw images. This is the recommended format for performing image analysis. Available save options for raw images are:
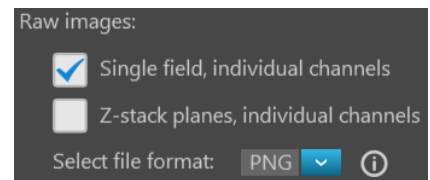
  – **Single field, individual channels:** Saves images captured in each field and each channel individually.

  – **Z-stack planes, individual channels:** Saves individual z-stack images as well as the z-stack projection for each field and channel.

  Available file formats for raw images are:

  – **TIF:** No or low image compression with no loss in image quality.

  – **PNG:** High image compression with no loss in image quality.

  – **C01:** Thermo Fisher™ proprietary format. Only images captured by monochrome cameras may be saved in this format.

  – **DIB:** Color corrects across devices for true viewing. Only images captured by monochrome cameras may be saved in this format.

Note: When viewing 16-bit images on most standard image viewers, they will appear very dark or almost totally black, especially if a low exposure setting was used. These images either need to be converted for display or opened in a software application that allows viewing of 16-bit images such as the Thermo Scientific™ Celleste™ Image Analysis Software (Cat. No. AMEP4816).

- **Displayed images:** Allows you to save images in a format that can be viewed in most image display applications.

  Displayed images from individual channels can be saved separately or as a merged or tiled image, using either 24-bit pseudocolor (8-bit per RGB channel) or grayscale (16-bit).
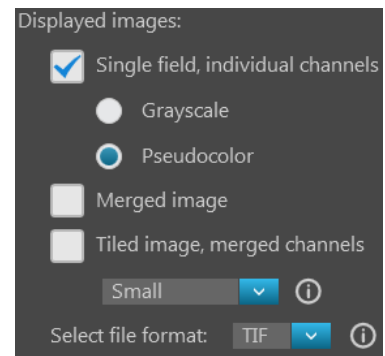
Note: While pseudocolors help differentiate the channels used in multi-channel overlays, grayscale images usually show more detail.

Note: 24-bit images (8-bit per RGB channel) are NOT recommended for image analysis as not all channels will display in many image analysis applications.

Available save options for raw images are:

- **Single field, individual channels:** Saves images captured in each field and each channel individually. You can select **Grayscale** (16-bit) or **Pseudocolor** (24-bit RGB; 8-bit per RGB channel).

- **Merged image:** Combines the images captured in different channels into a multicolor overlay and saves the merged image in 24-bit Pseudocolor (8-bit per RGB channel).

- **Tiled image:** Merges the images captured in each channel and places them very close together into a tiled format without applying an algorithm. The resulting image is not seamless, but the process is quicker than stitching and is often sufficient for most applications.

  You can create tiled images with the following dimensions:
  - **Small** (2000 × 2000 pixels)
  - **Medium** (4000 × 4000 pixels)
  - **Large** (10,000 × 10,000 pixels)
  - **Maximum** (26,000 × 26,000 pixels)

Note: If you are analyzing images that cross fields such as images of neurites, and the images are slightly misaligned, you might want to consider **stitching** the images. Stitching applies an algorithm to apply a slight overlap, and then removes the seam so you can visualize objects that cross fields.

Available file formats for raw images are:

- **TIF:** No or low image compression with no loss in image quality.

- **PNG:** High image compression with no loss in image quality.

- **JPEG:** High image compression with little loss in image quality; good for sharing.

- **BMP:** No image compression with no loss in image quality.

Note: If you plan to perform image analysis, you should save the image as a 16-bit TIF raw image (preferred), a C01, or a DIB. There is information in the headers of these files and OME information that might be useful.

Automate tab

Automate tab consists of a series of panels, which are organized by functionality and contain the controls necessary to create and recall automated scan protocols.
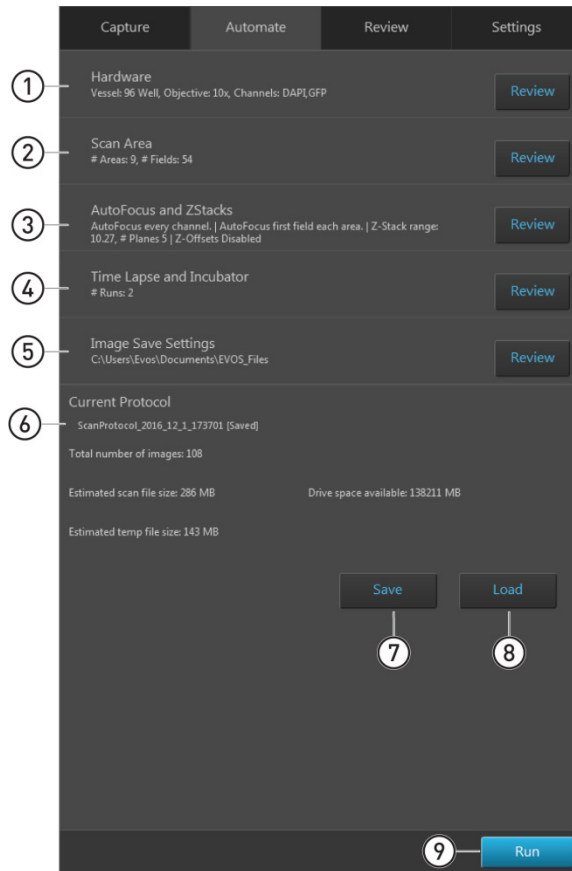
1. **Hardware:** Allows you to configure hardware options (such as sample vessel, objective, light source etc.) for the scan protocol (page 115).

2. **Scan Area:** Allows you to specify the scan areas and fields to capture for the scan protocol (page 116).

3. **AutoFocus and Z Stacks:** Allows you to configure AutoFocus options and Z-Stack settings for the scan protocol (page 129).

4. **Time Lapse and Incubator:** Allows you to specify time lapse options (duration, capture frequency etc.) and incubator settings (temperature, oxygen etc.) for the scan protocol (page 137).

5. **Image Save Settings:** Allows you to select a save location for captured images and to set image save options (page 143).

6. **Current Protocol:** Displays the name of the currently selected scan protocol and provides additional information (total number of images, estimated scan file size, estimated temporary file size, and drive space available).

7. **Save:** Saves the automated scan protocol for future experiments.

8. **Load:** Opens the Load dialog, which allows you to recall a previously saved scan protocol to run with new samples.

9. **Run:** Runs the automated scan protocol (newly created or recalled).

Hardware

Hardware panel allows you to select and edit hardware options (such as sample vessel, objective, light source etc.) and adjust settings for the current scan protocol.

① Selected Vessel
② Selected Camera
③ Select Objective
④ Select Channels
⑤ Phase Ring
⑥ Adjust Settings
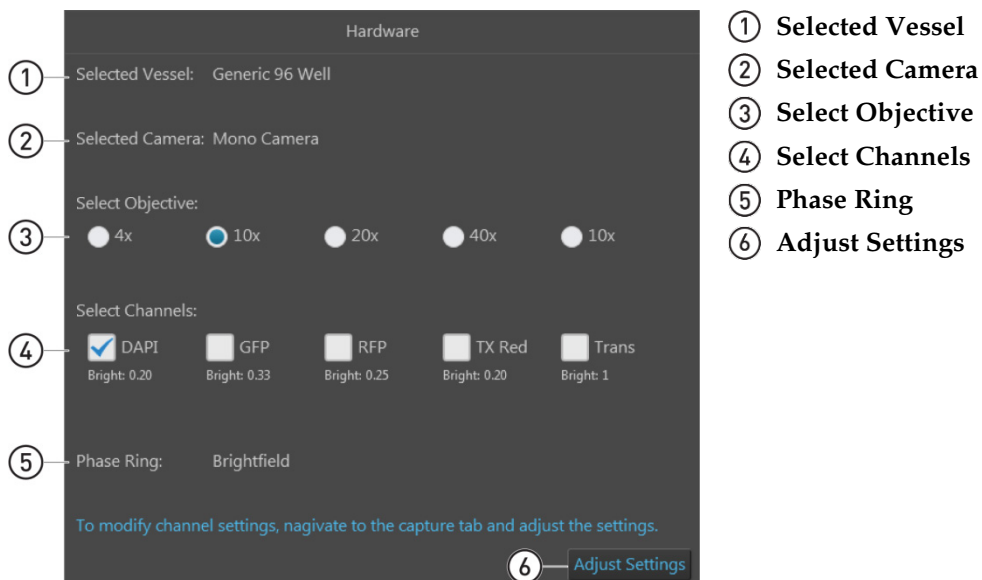
Hardware controls

① **Selected Vessel:** Displays the selected vessel type. For more information, see "Vessel" in "Capture tab controls", page 98.

② **Selected Camera:** Displays the selected camera. For more information, see "Brightness and camera settings" in "Capture tab controls", page 102.

③ **Select Objective:** Allows you select the objective for the scan protocol from the currently installed objectives.

You can select only one objective at a time. In the example below, the 10X objective is selected.
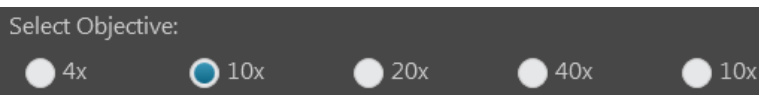
④ **Select Channels:** Allows you to select the channels you wish to capture in the scan protocol. The brightness value set in the Capture tab for each channel is displayed below the corresponding checkbox.

You can select multiple channels by checking the corresponding channel boxes. In the example below, the DAPI and GFP channels have been selected.
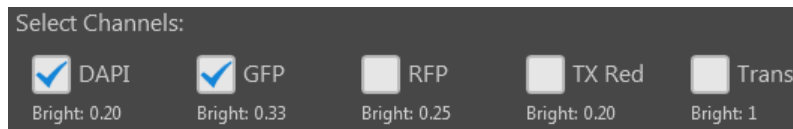
⑤ **Phase Ring:** Displays the phase option selected for the transmitted light (brightfield) channel. For more information, see "Phase" in "Capture tab controls", page 102.

⑥ **Adjust settings:** Navigates to the Capture tab (page 92), which allows you to modify channel settings for the scan protocol.

In the capture tab, you can change **Brightness and camera settings** (page 102), adjust **Brightness** parameters (page 106), and select a different **Vessel** (page 98) and **Objective** (page 100).

Scan Area

Scan Area panel allows you to define the areas and fields you wish to scan in the automated scan protocol and specify the order in which they are captured.
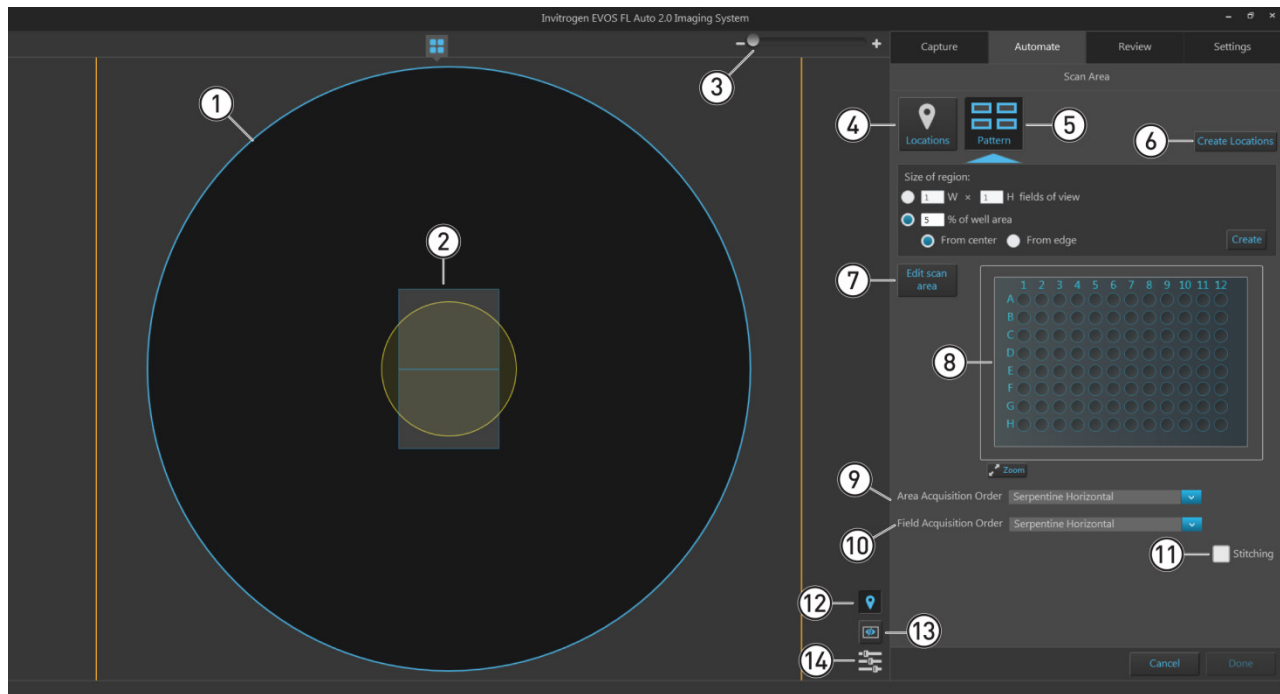
①	**Area View** (page 117)	⑧	**Vessel map** (page 126)
②	**Capture region** (page 118)	⑨	**Area acquisition order** (page 126)
③	**Zoom slider** (page 118)	⑩	**Field acquisition order** (page 126)
④	**Select Scan Locations** (page 118)	⑪	**Stitching** (page 127)
⑤	**Select Scan Pattern** (page 123)	⑫	**Toggle locations display** (page 127)
⑥	**Create Locations** (page 124)	⑬	**Toggle field borders** (page 127)
⑦	**Edit scan area** (page 125)	⑭	**Image display settings** (page 128)

Note: If using a single culture dish, flask, or slide, the scan area corresponds to the entire dish, flask, or slide. As such, the scan area displayed in Area View represents the entire dish or flask selected.

In a multi-well plate, multi-chamber slide, or multiple dishes on multi-dish holders, each scan area corresponds to a single well, chamber, or dish.

You can specify multiple areas to scan, but the Area View displays only the current (i.e., active) scan area.

Scan Area controls ① **Area View:** Represents the target scan area from which specific fields are selected for the scan protocol.

- In a single culture dish or flask, the scan area corresponds to the entire dish or flask. As such, the scan area displayed in Area View represents the entire dish or flask selected.

  In the example below, the sample vessel is a single 60-mm culture plate, and the Area View corresponds to the entire plate.
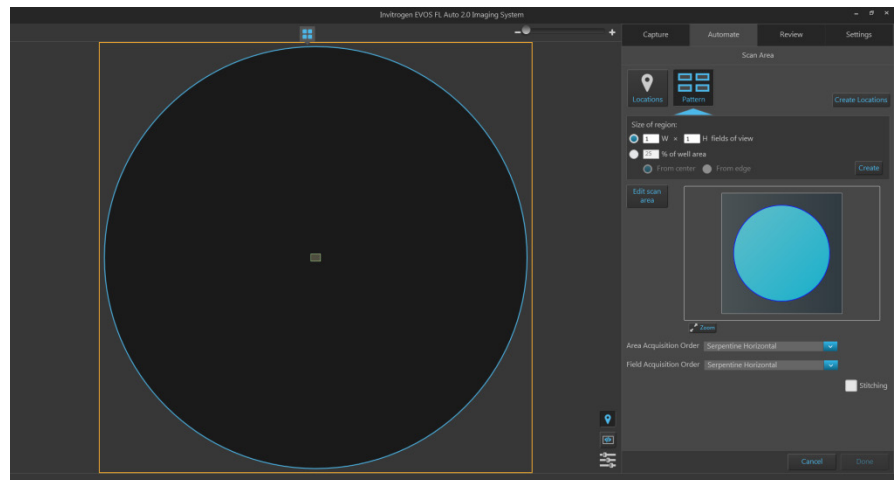
- In a multi-well plate or a multi-chamber slide, each scan area corresponds to a single well or chamber. You can specify multiple areas to scan, but the Area View displays only the current (i.e., active) scan area.

  In the example below, the sample vessel is a 6-well plate. Although the first three wells have been selected for capture, the Area View displays only the active first well.
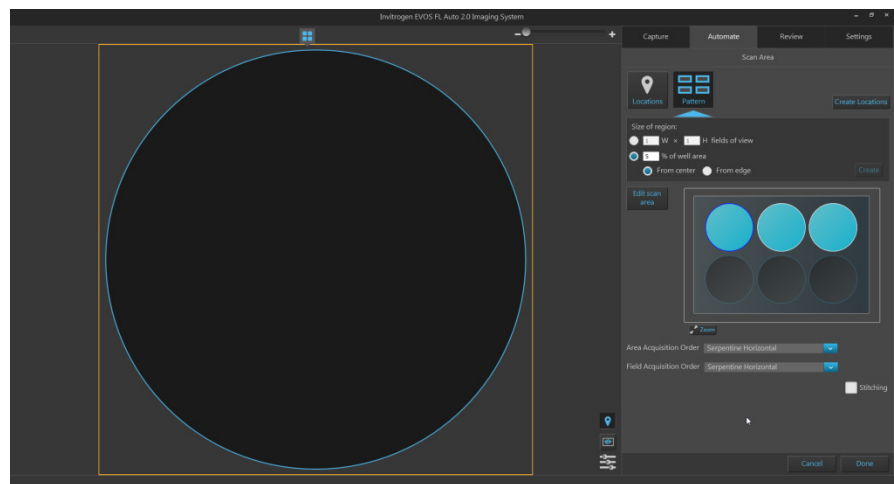
- When using the **Select Scan Locations** tool (page 118), the scan areas are selected using the **Assign scan area** button (page 121).

  When using the **Select Scan Pattern** tool (page 123), the scan areas are selected from the **Vessel map** using the **Edit scan area** button (page 125).

- When multiple scan areas have been defined, you can specify the order in which they are captured using the **Area Acquisition Order** (page 126).

- You can specify the order in which the selected fields are captured using the **Field Acquisition Order** (page 126).

② **Capture region:** Represent the regions to be captured in a scan protocol.

The regions to be captured consist of fields of view that are defined using the **Select Scan Locations** or **Select Scan Pattern** tools. You can select multiple regions for automated capture.

In the following example, the capture region was defined as 15 fields of view (5 W × 3 H) using the Select Scan Pattern tool.
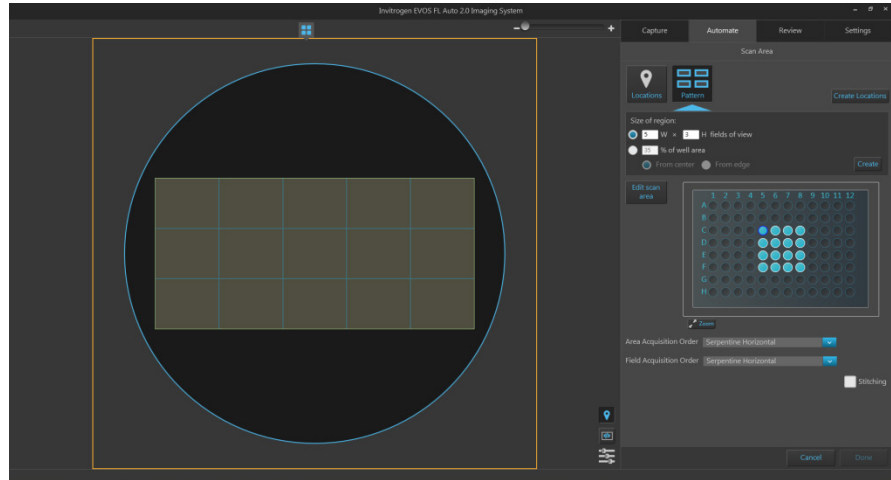
③ **Zoom slider:** Zooms in and out of the Viewing area.

④ **Select Scan Locations:** Opens the **Scan Locations** tool, which allows you to define the capture regions and assign scan areas for the scan protocol.
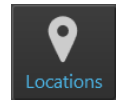
- Locations are defined using the **Locations** tool (page 92).

- Locations that have already been defined with the Locations tool are listed in the **Available Locations** table.

  The Available Locations table also provides information about the name and shape of each location, and the number of fields it contains.

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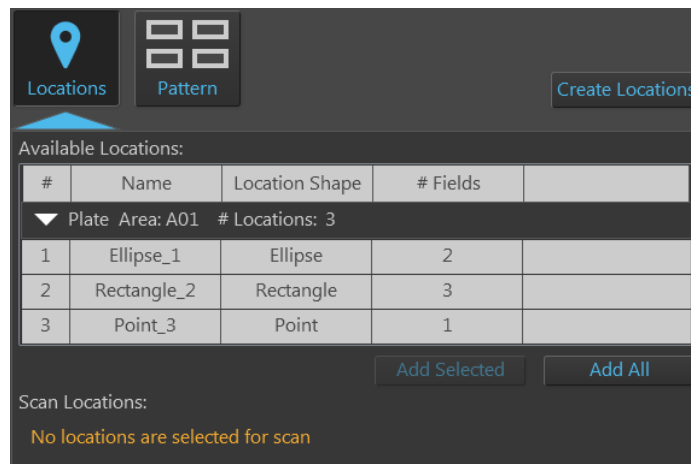
- If there are no previously defined locations, the **Available Locations** table displays the message "No locations are available for selection".
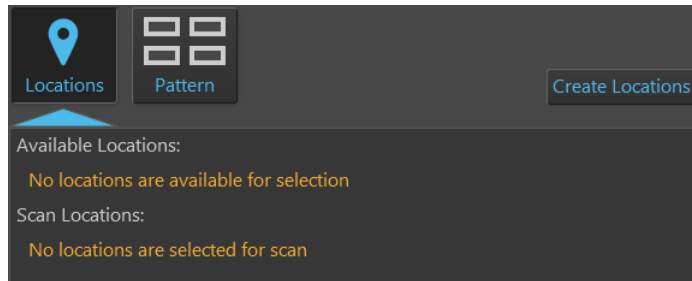
- To create new locations for the scan protocol, click **Create Locations** to open the **Locations** tool (page 92).

  Using the Locations tool, define the locations as described (page 93), then navigate back to the **Automate tab ▶ Scan Locations**.

  Newly created locations will be listed in the Available Locations table.

- To select a location listed in the Available Locations table, click the desired location. The selected location will be highlighted in blue and the Viewing area will display the selected location and shape (if the **Toggle locations display** is on).

  In the following example, the first location in the list (an ellipse) is selected. The Viewing are displays the selected location and the field group that best fits the shape of the location.

- To add a location to the scan protocol, click the desired location in the Available Locations table to select, then click **Add Selected**.

- To add multiple locations to the scan protocol simultaneously, Shift-click the desired locations in the Available Locations table to select them, then click **Add Selected**.

- To add all of the locations listed in the Available Locations table, click **Add All**.

- The locations added to the scan protocol are displayed in the **Scan Locations** table and removed from the Available Locations table.

  The Scan Locations table also provides information about the name and shape of each location, and the number of fields it contains.

  In the example below, two locations have been added to the Scan locations list, and one location remains in the Available Locations table.

- To remove a location from the Scan Locations table, click the desired location in the table to select, then click **Remove Selected**.

- To remove multiple locations from the Scan Locations table simultaneously, Shift-click the desired locations in the table to select them, then click **Remove Selected**.

- To remove all of the locations listed in the Scan Locations table, click **Remove All**.

- The locations removed from the Scan Locations table appear in the Available Locations table.

- To assign a scan area for the selected scan locations, click **Assign scan area** to open the **Vessel map**, then select the desired areas from the Vessel map to define the scan area (see page 122).

  Depending on the vessel in use, scan areas can consist of wells in multi-well plates, chambers in multi-chamber slide, or entire culture flasks or dishes.
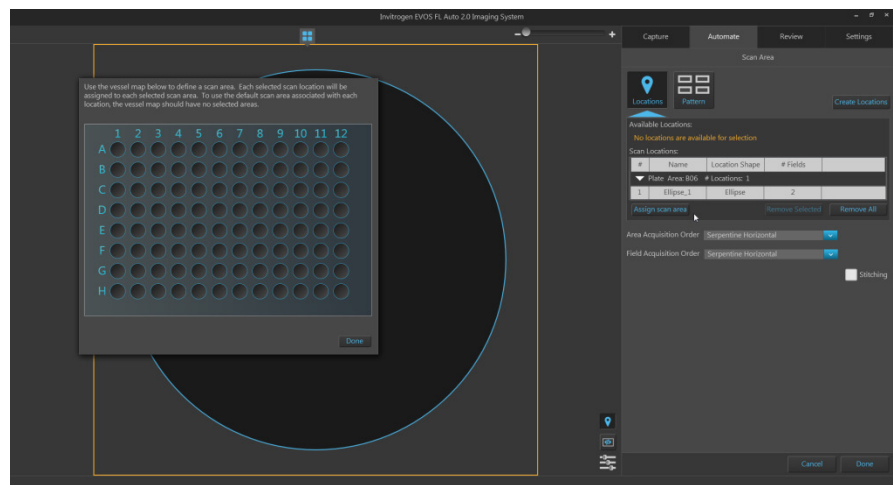
- When assigning scan areas, each selected scan location (i.e., highlighted in Scan Locations table) is assigned to each selected scan area.

  If no location is selected in the Scan Locations table, all scan locations listed in the table are assigned to each selected scan area.

  If the Vessel map contains no selected scan areas, the scan protocol uses the default scan area associated with each scan location.

- To define scan areas in the Vessel map:
  - **Click** on a single area (well, chamber, or dish) to select it. Selected area will be displayed in blue.
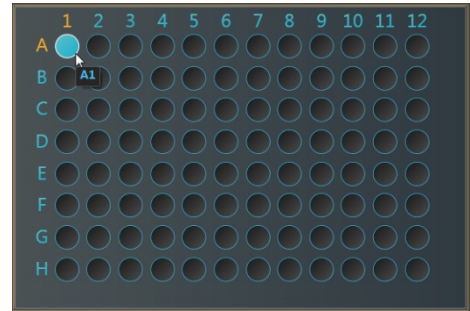
  - **Ctrl-click** to select multiple areas individually. Selected areas will be displayed in blue.
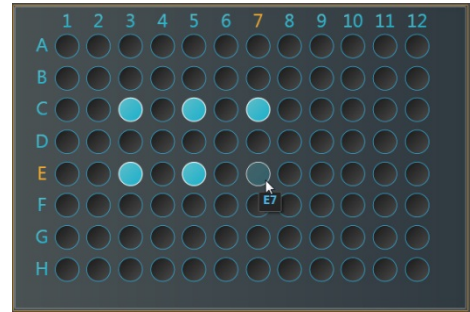
  - **Click-drag** to select multiple areas as a single block on the Vessel map.
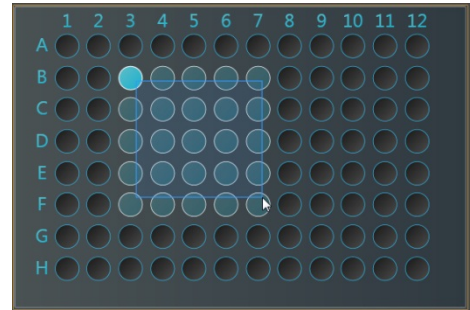
  - **Ctrl-click-drag** to select multiple blocks on the Vessel map.
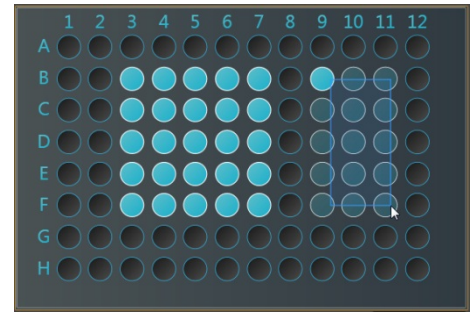
- To unselect scan areas:
  - To unselect a single area, Ctrl-click the area you wish to unselect.
  - To unselect all areas, click on any region on the Vessel map that is not selectable (i.e., not a well, chamber, or dish).
- Click **Done** to close the Vessel map and exit the area selection mode.

⑤ **Select Scan Pattern:** Opens the **Scan Pattern** tool, which allows you to define the capture regions for the scan protocol as a pattern based on defined parameters.

- You can define the scan pattern as a matrix (X fields wide × Y fields high) or as a percentage of area covered by scan fields, counted either from the center or from the edges.

  In the following example, the capture region has been defined as a matrix of 5 fields wide × 3 fields high.
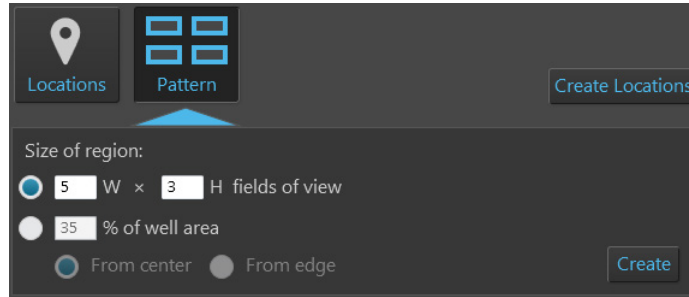
- As you define the parameters, the Area View displays a preview of the selected fields.
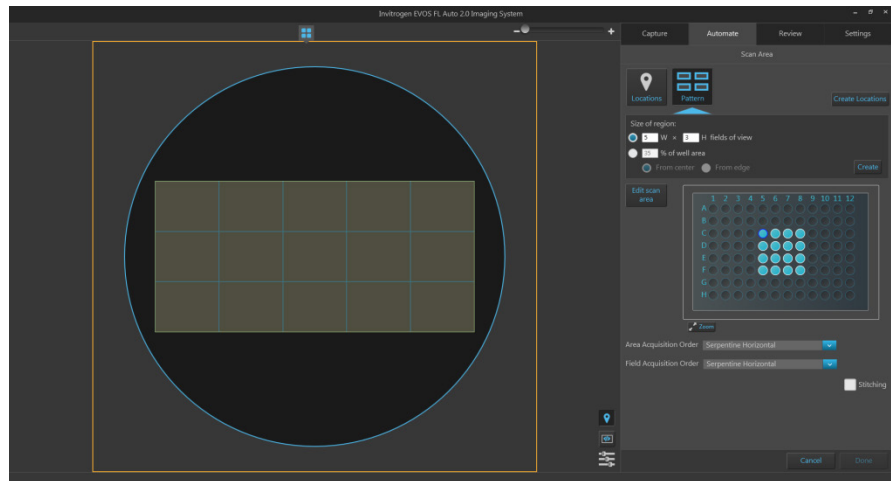
- To define the capture region as a matrix, select **W × H fields of view**, then enter the desired dimensions into the corresponding text boxes. You can only enter integers.

  In this example, the capture region is defined as a pattern of 5 fields wide × 3 fields high.
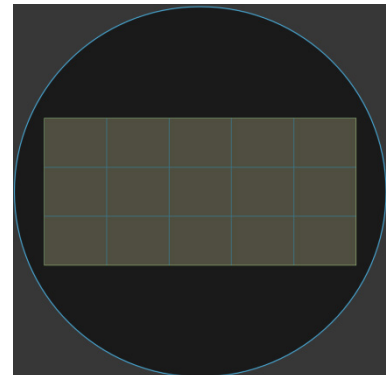
- To define the capture region as a percentage of area covered by scan fields, select **% of well area**, enter the desired value (an integer between 1 and 100) into the text box, then select **From center** or **From edge**.

In this example, the capture region is defined as 50% of well area covered by the scan fields, counted from the edge.
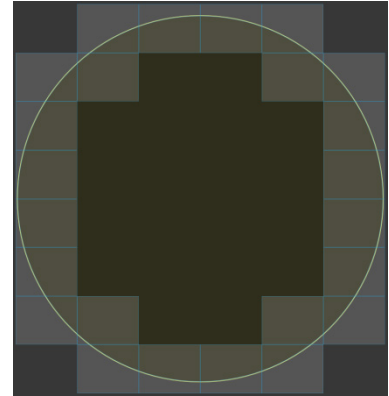
In this example, the capture region is defined as 50% of well area, counted from the center.
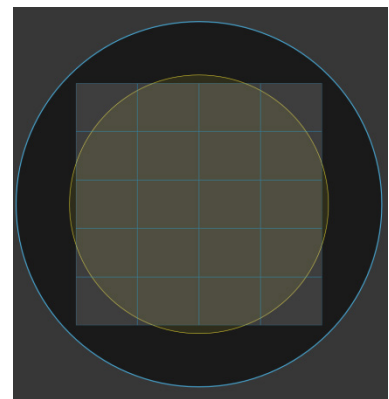
- When you are finished defining the parameters for the capture pattern, click **Create**.

⑥ **Create Locations:** Open the **Locations** tool (page 92), which allows you to create new locations for the scan protocol.

Using the Locations tool, define the locations as described (page 93), then navigate back to the **Automate tab ▶ Scan Locations**.

Newly created locations will be listed in the Available Locations table.

⑦ **Edit scan area:** When using the Select Scan Pattern tool, it allows you to specify the areas you wish to capture by selecting the corresponding areas on the **Vessel map.**

Edit scan area

- Click **Edit scan area** to enable area selection on the Vessel map. Edit scan area button changes to **Done** button and the Vessel map becomes active.
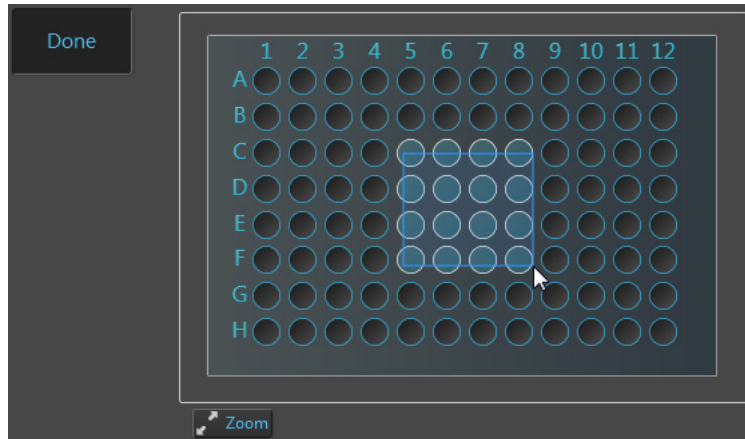
- To define scan areas in the Vessel map, follow the same procedure as described for the Assign scan area (page 122).

- If you wish to view a larger version of the Vessel map, click the **Zoom button**. The larger map displays the same content as the smaller vessel map and behaves the same way.

Zoom
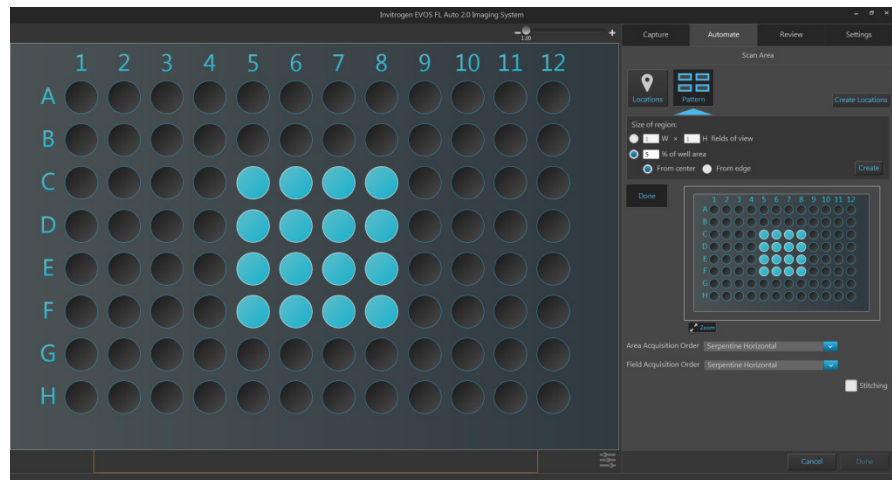
- When in the **edit mode** (i.e., **Edit scan area** button is clicked), clicking on an area in the larger map selects the corresponding area in the smaller map and marks it for the scan protocol.

- Clicking the zoom button again closes the zoom window.

- When finished defining the scan areas, click **Done** to exit the selection mode.

⑧ **Vessel map:** Represents the vessel container (vessel + vessel holder) in use. In the **Automate tab ▶ Scan Area** panel, the Vessel map is used to select specific areas for the automated scan protocol.

- To select an area (well, chamber, or dish) for the scan protocol, click **Edit scan area**, then click on the corresponding area on the Vessel map (page 125). A blue fill indicates that the area has been selected for the scan protocol.

- The scan area shown in the Viewing area is indicated with a dark blue circle on the Vessel map. Areas that are not active have white circles.

- In the example below, A1, A2, B1, and B2 wells have been selected for the scan protocol. A1 is the active scan area (with dark blue flame), while the inactive wells (A2, B1, B2) display white frames. The Area View shows the active well A1.
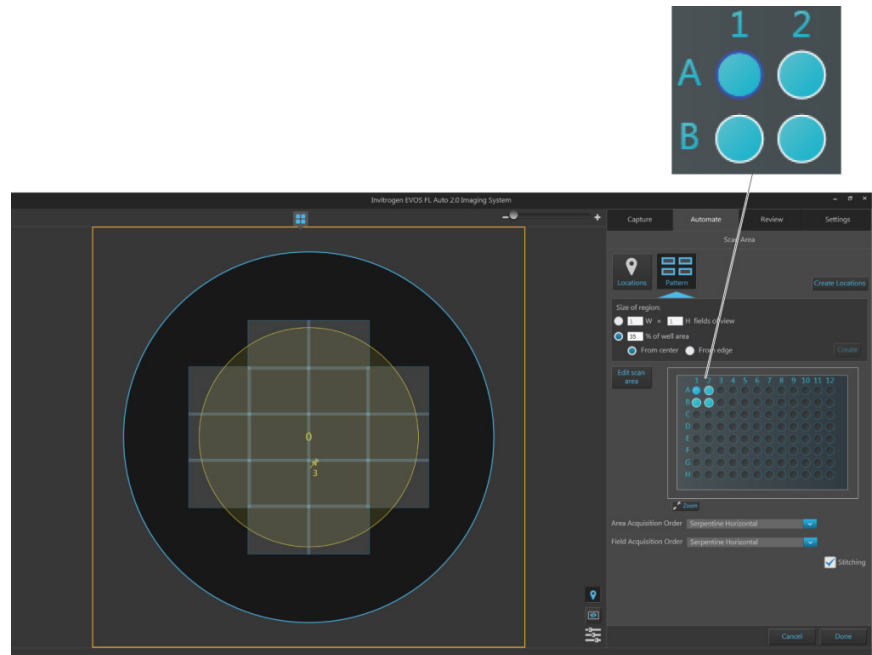
⑨ **Area Acquisition Order:** Allows you specify the order in which the selected areas are captured in the scan protocol. Available options are:

- **Serpentine Horizontal**
- **Serpentine Vertical**
- **Random Selection**
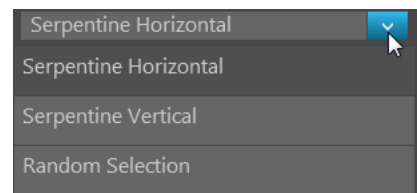
⑩ **Field Acquisition Order:** Allows you specify the order in which the fields are captured in each area selected for the scan protocol. Available options are:

- **Spiral Outward Counterclockwise**
- **Spiral inward Clockwise**
- **Serpentine Horizontal**
- **Serpentine Vertical**
- **Random Selection**
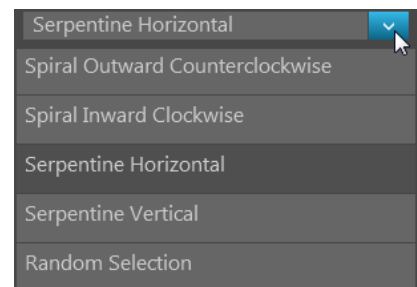
⑪ **Stitching:** Applies an overlap when assembling captured images into a mosaic image of the scan area, which allows you to visualize objects in the sample that cross fields.
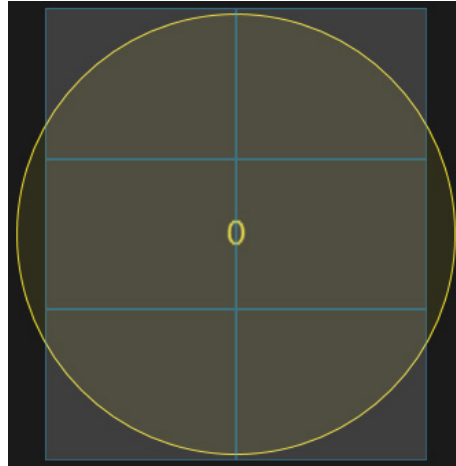
 Stitching
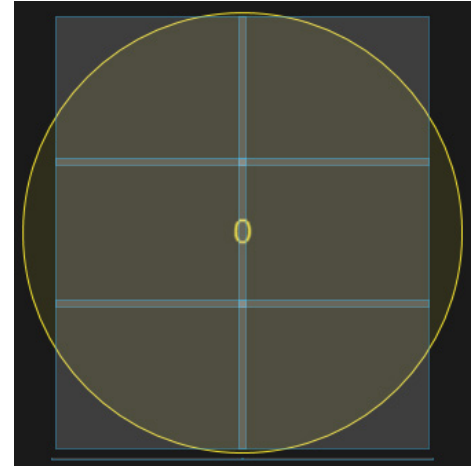
Stitching off   Stitching on

⑫ **Toggle locations display:** Switches the display of locations (defined with the Location tool) in Area View on and off.

 Field borders are on   Field borders are off

⑬ **Toggle field borders:** Switches the borders around captured fields in Area View on and off. This option is not available in Field View.

 Field borders are on   Field borders are off

- When field borders are turned on, all captured fields in Area View are displayed with blue borders around them.
- Currently selected field, whether captured or not, is displayed with an orange border, which cannot be hidden.

(14) **Image display settings:** Opens the Image display settings window, which allows you to adjust image display parameters (brightness, constrast, gamma) for the Viewing Area. Clicking the button again closes the window.

	👁	☀	◐	⚫	
GFP	☑	0.50	0.50	1.00	Reset
DAPI	☑	0.50	0.50	1.00	Reset
RFP	☑	0.50	0.50	1.00	Reset

Note: Adjustments made to Image display settings only affect how the image is displayed in the Viewing Area; they do not change how the image is captured.

- **Visible Channels** 👁 : Visible channels are the channels selected for display in the Viewing area. They are selected using the **Toggle channel display** checkbox located on the top left corner of the corresponding **Light source** button (see page 101).

Note: The controls for image display settings are contextual; only the controls for the visible channels will be available. In the example above, only the controls for the GFP, DAPI, and RFP channels are displayed.

- **Brightness** ☀, **Contrast** ◐, and **Gamma** ⚫ parameters for each of the selected channels are adjusted using the corresponding sliders.

- **Reset** button resets the **Brightness**, **Contrast**, and **Gamma** parameters to their default values.

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

  When on, images are displayed in default pseudocolor corresponding to the channel in which they were captured. You can change the default pseudocolor in the Settings tab (page 149).

  When off, images are displayed in grayscale.
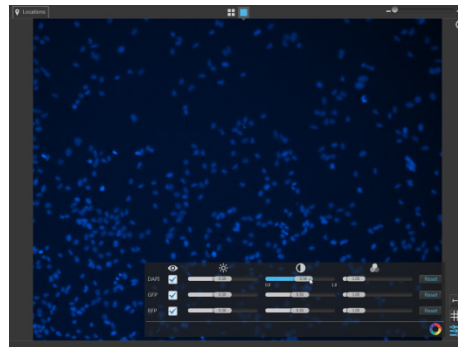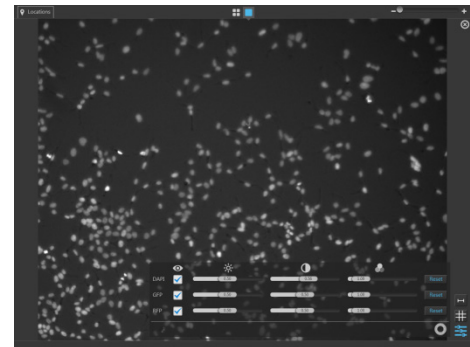
Color display is on   Color display is off

AutoFocus and Z Stacks

AutoFocus and Z Stacks panel allows you to configure the autofocus options and determine the Z-Stack settings for the scan protocol.

① **Capture Z-Stacks**
② **Use AutoFocus**
③ **Channel**
④ **Focus Position**
⑤ **Z-Offsets**
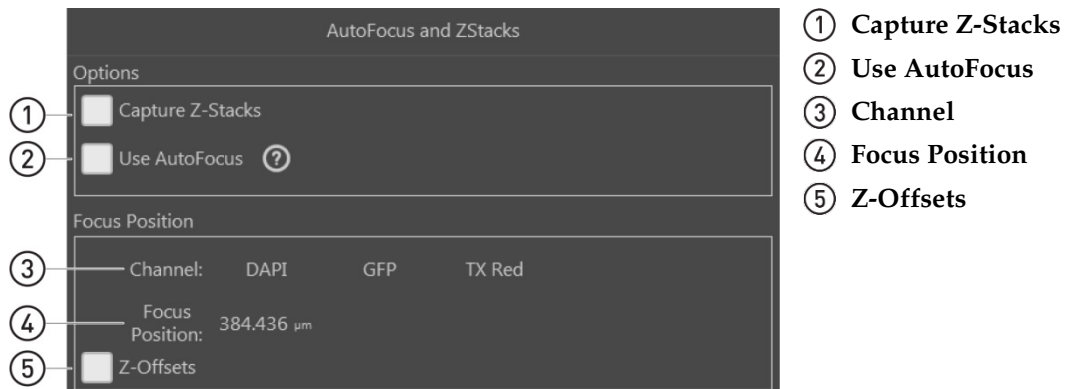
Note: The options available on this panel are contextual; the controls that are displayed depend on the selections made in this panel or elsewhere in the Automate tab. Objects and controls that are not available as a function in a particular context are not shown in the panel.

Note: Click on the **View Help Content** icon to open the Help window, which provides EVOS™ FL Auto 2 Imaging System Help.

AutoFocus and Z Stacks controls

① **Capture Z-Stacks:** Allows you to capture a series of images at various focal planes along the z-axis within the sample.

Checking this option reveals the controls for **Z-Stack Settings** (page 130).

② **Use AutoFocus:** Allows you configure the autofocus method and set the autofocus frequency to use during automated scan.

Checking this option reveals the controls for **AutoFocus Settings** (page 133).

③ **Channel:** Indicates the channels selected for capture in scan protocol, and allows you set the default focus position.

Depending on the context, **Channel** displays channel selection buttons for the Default Focus Position and the Z-Offsets.

④ **Focus Position:** Indicates the active z-stage position (i.e., the position of the focal plane along the z-axis) for the selected channel (page 135).

⑤ **Z-Offsets:** Allows you to specify the focus position in each channel relative to the focus position in other channels when the fluorescent markers in different channels are in different focal planes (page 135).

This option is not available when the scan protocol is set to capture images in a single channel.

Z-Stack Settings

Z-Stacking allows you to capture multiple images along the z-axis based on your specifications during the automated scan protocol.

Images captured for the z-stack can be used to create a Z-Stack Projection, which is a digital image processing technique that combines multiple images taken at different focal planes to generate an image with a greater depth of field than any of the individual source images.

Note that the settings for the Z-Stack procedure are configured in the Z-Stack Settings window in the Capture tab (**Capture tab ▶ Capture Z-Stack**, page 109).
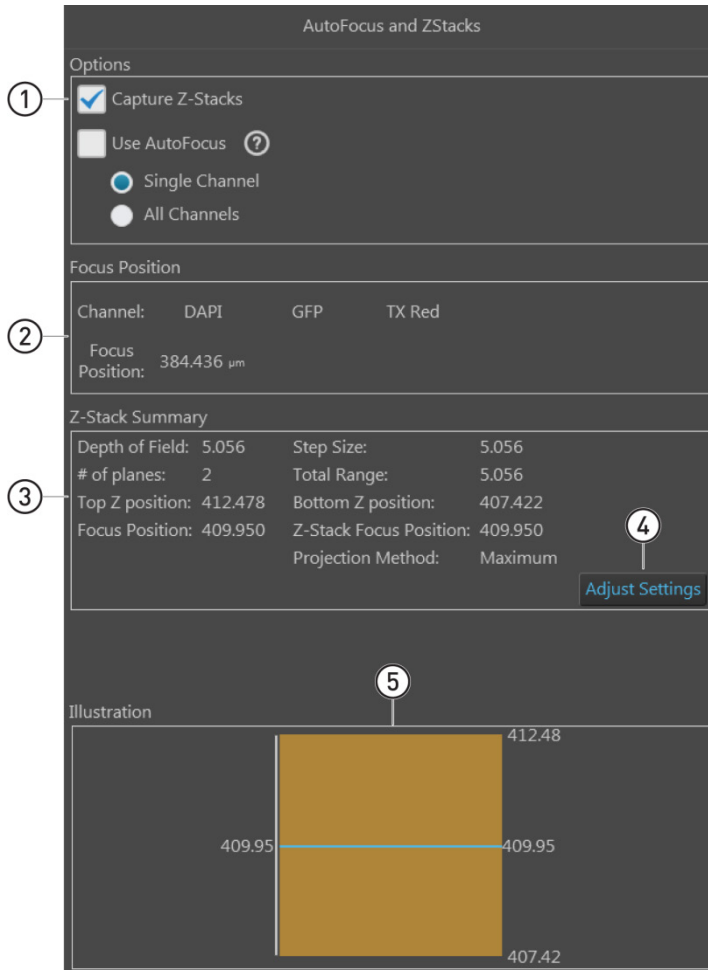
① **Capture Z-Stacks** (page 131)   ④ **Adjust Settings** (page 131)
② **Focus Position** (page 131)   ⑤ **Illustration** (page 132)
③ **Z-Stack Summary** (page 131)

Note: When the **Capture Z-Stacks** option is checked, the Z-Offsets option is not available as one of the Focus Position controls and the Z-Offsets that have been previously set are not preserved.

Note: Click on the **View Help Content** icon to open the Help window, which provides EVOS™ FL Auto 2 Imaging System Help.

Z-Stack Settings controls

① **Capture Z-Stacks:** Allows you to capture z-stack images based on your specifications during your automated scan protocol.
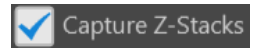
Capture Z-Stacks

When the **Capture Z-Stacks** option is checked, the AutoFocus and Z Stacks panel displays the Z-Stack Summary, the Adjust Settings button, and Illustration.

② **Focus Position:** Indicates the position of the current focal plane along the z-axis for the selected channel, which is used as a reference point when defining the different focal planes captured for the Z-Stacks.

Focus Position

Channel:	DAPI	GFP	TX Red

Focus Position: 384.436 µm
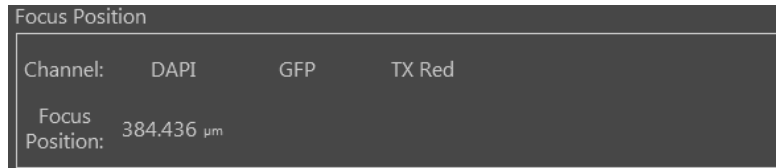
③ **Z-Stack Summary:** Provides a summary of the Z-Stack Settings that were set up in the Capture tab (**Capture tab ▶ Capture Z-Stack**, page 109).

The summary includes information on depth of field, step size, number of planes, total range of the z-stack, top and bottom z-positions, default focus position of the selected channel, and the z-stack focus position.

Z-Stack Summary

Depth of Field:	5.056	Step Size:	5.056
# of planes:	2	Total Range:	5.056
Top Z position:	412.478	Bottom Z position:	407.422
Focus Position:	409.950	Z-Stack Focus Position:	409.950
		Projection Method:	Maximum
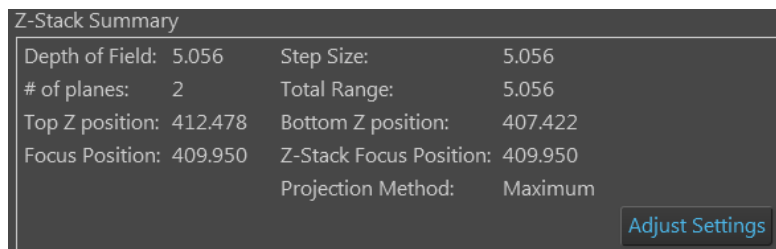
Adjust Settings

④ **Adjust Settings:** Opens the Z-Stack Settings window in the Capture tab, which allows you to modify the z-stack settings for the scan protocol (see "Capture Z-Stack", page 109).

Adjust Settings

Z-Stack Settings ☑ Interactive Setup

Locate the top position of the z-stack: Set Top Position
Locate the bottom position of the z-stack: Set Bottom Position
Set the step size or number of planes:
   ● 1.00 x Depth of Field (26.92)
   ○ Step Size 26.92 µm
   ○ Number of planes 3

💡 Light

Illustration

26.92

Default Focus: 0.00   Z-Stack Focus: 0.00

-26.92

Projection Method: Maximum ⌄   Capture Z Stack   Save   Cancel

⑤ **Illustration:** Graphically demonstrates the Z-Stack Settings. Illustration is available only if Capture Z-Stacks option is selected (page 129).

- The Z-Stack Focus is represented by a blue line. It is the sum of Default Focus and Start position relative to focus position.

- The orange rectangle represents the Z-Stack depth. It is the product of step size and the number of planes.

- The positions of the lowest and the highest focal depths and the position of the Z-Stack Focus are indicated in μm next to the orange rectangle representing the Z-Stack depth.

Illustration

412.48

409.95 409.95

407.42
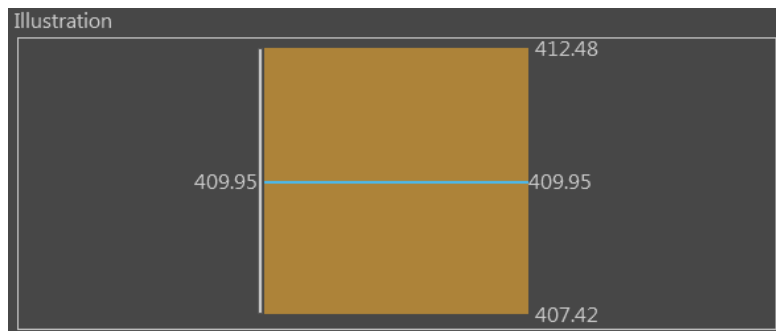
AutoFocus Settings

Autofocus performance depends on the z-information contained within a vessel and vessel holder combination. When the system is properly calibrated for the selected vessel, autofocus is able to use the dimensions used during the vessel creation to find the optimal focal plane.

During automated scan, if the autofocus is unable to find the focal plane using a small range, it extends the range within which it searches for focal plane.
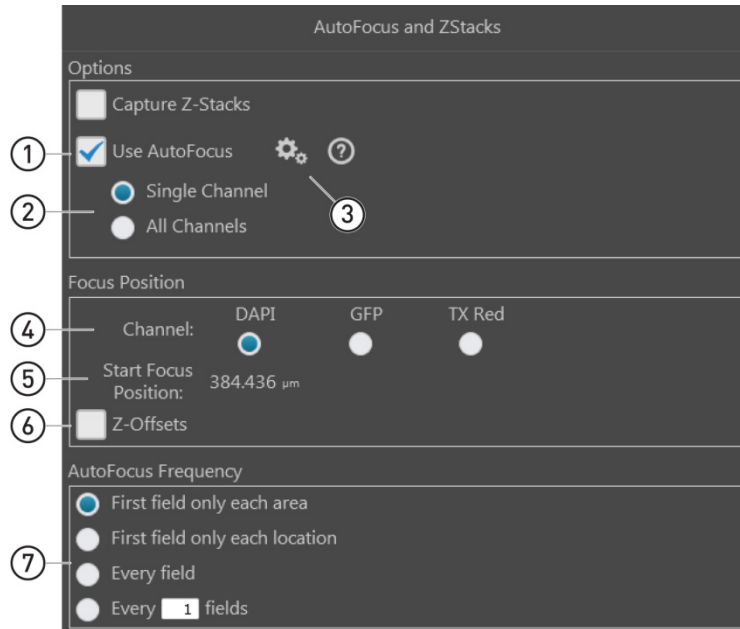
① **Use AutoFocus** (page 134)   ⑤ **Start Focus Position** (page 135)
② **Single Channel/All Channels** (page 134)   ⑥ **Z-Offsets** (page 135)
③ **Configure AutoFocus Method** (page 134)   ⑦ **AutoFocus Frequency** (page 136)
④ **Channel** (page 135)

Note: Click on the **View Help Content** icon to open the Help window, which provides EVOS™ FL Auto 2 Imaging System Help.

**AutoFocus Settings controls**

① **Use AutoFocus:** Reveals the controls for setting autofocus options for the scan protocol.
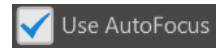
☑ Use AutoFocus

② **Single Channel/All Channels:** Option to run the autofocus in a single channel or in all channels selected for the scan.

⦿ Single Channel
◯ All Channels
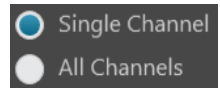
- When **Single Channel** is selected, autofocus is run in a single channel. This option preserves the Z-Offsets between the channels.

- By default, the last channel used for imaging or viewing a live image is selected for single channel autofocus. You can change the autofocus channel using the **Channel** radio buttons (page 135).

- When **All Channels** is selected, autofocus is run separately in all channels that were selected in the Hardware panel (page 115). This option does not preserve the Z-Offsets between channels.

③ **Configure AutoFocus Method:** Reveals the controls to configure the autofocus method for each channel selected for the scan protocol.
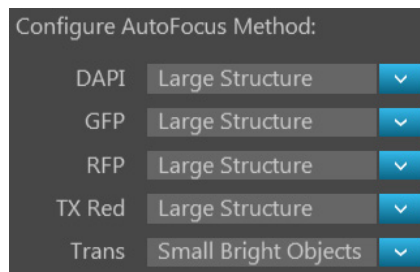
Configure AutoFocus Method:

DAPI	Large Structure ⌄
GFP	Large Structure ⌄
RFP	Large Structure ⌄
TX Red	Large Structure ⌄
Trans	Small Bright Objects ⌄

Available options are:

- **Small Structure:** This method computes the energy according to the size of image features and is able to measure the presence/absence of small image features. Choose this option when capturing samples that have many fine, hair-like structures (e.g., filaments or structural stains).

- **Large Structure:** This method is statistics-based and looks for large changes in image content as parts of the image go in and out of focus. Choose this option when your sample contains large structures (e.g., whole cell stains).

- **Small Bright Objects:** This method looks for changes in brightness at the center of the cell and optimizes focus on cells that have a bright center with a dark surround area. Choose this option to capture samples with localized staining (e.g., nuclei).

After selecting the autofocus method, click **Close** to return to the AutoFocus and Z Stacks panel.

Note: The effectiveness of the autofocus method depends on the plate type, sample type, biomarker intensity, magnification, and channel. It may be necessary to use different autofocus methods for different channels. This ensures that the best autofocus method is used for the relevant biology.

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④ **Channel:** Allows you to select the channel in which to run the autofocus during the automated scan when the **Single Channel** option is selected.

- Only the channels that have been selected for the automated scan protocol in the Hardware panel will be available for selection.

- In the example below, DAPI channel has been selected for the autofocus procedure during the scan protocol.

⑤ **Start Focus Position:** Indicates the currently active z-stage position (i.e., position of the current focal plane along the z-axis) for the selected channel.

- The Start Focus Position is used as starting point around which the autofocus algorithm searches for optimal focus.

- You can only select a single channel for the Start Focus Position.
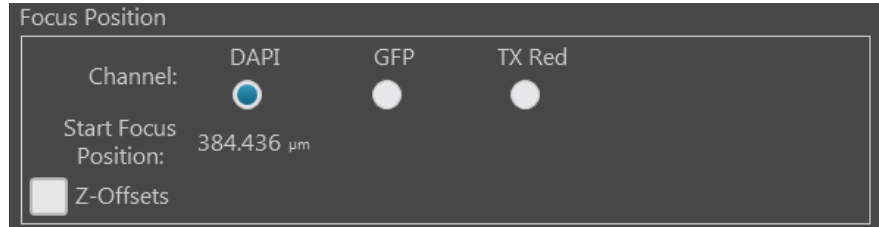
- When Z-Offsets option is checked, Start Focus Position is used as reference point against which the focus position in other channels is set.

⑥ **Z-Offsets:** Allows you to specify the focus position in each channel relative to the focus position in other channels. Setting the correct Z-Offsets is especially important when the fluorescent markers in different channels are in different focal planes.
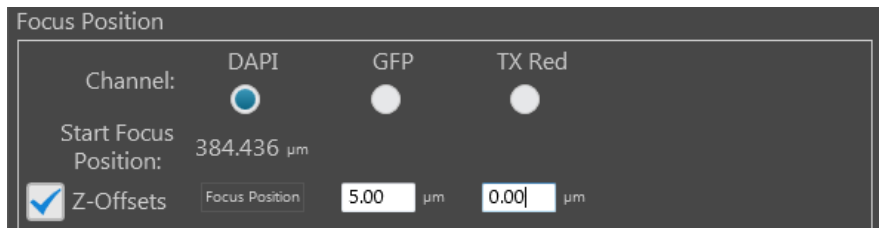
- The Z-distance difference between channels is expressed as an offset from the Start Focus Position in the reference channel.

- You can enter both positive and negative values in μm for the Z-Offsets.

  In the example above, the reference start focus position is set to 384.44 μm in the DAPI channel, while the Z-Offset for the GFP channel is 5.00 μm. Therefore, the autofocus algorithm will use 389.44 μm as the default focus position for the GFP channel.

- If the autofocus algorithm is configured to run on a single channel (page 134), the current Z-distance differences between channels will be preserved as if the Z-Offsets have been locked (page 104).

- If the autofocus algorithm is configured to run on all channels (page 134), then the autofocus run in one channel will not affect the Z-distances of the other channels as if the Z-Offsets have been unlocked (page 104).

- The **Z-Offsets** option is not available when the **Capture Z-Stacks** option has been selected (page 130).

Note: Consider a scenario where the initial Z-positions of the DAPI and GFP channels are 50 µm and 80 µm, respectively. The autofocus is run from the DAPI channel and finds the best focal plane at a Z-position of 60 µm.

If the autofocus is selected to run from a single channel, then the Z-Offsets will be locked, and the GFP channel will be focused to a Z-position of 90 µm.

If the autofocus is run from all channels, then the Z-Offsets will not be locked, and the autofocus algorithm will find the best focal plane for the GFP channel independently of the DAPI channel.

⑦ **AutoFocus Frequency:** Allows you to specify which fields in a scan area are used for autofocus during the automated scan protocol. Specifying fewer fields per scan area for autofocus reduces the overall scan times.

AutoFocus Frequency
● First field only each area
○ First field only each location
○ Every field
○ Every ⬚1 fields
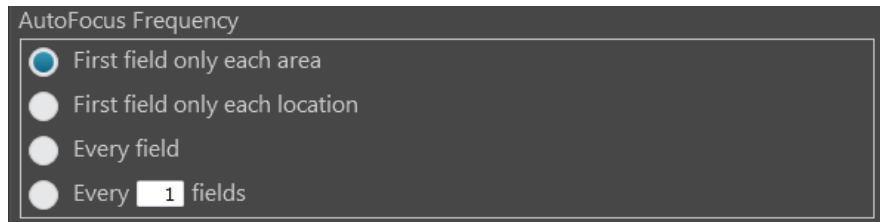
Available options for AutoFocus Frequency are:

- **First field only each area:** Performs the autofocus operation only on the first field of each scan area. No further autofocus is performed on subsequent fields in the scan area. The focus position obtained on the first field is used for the remaining fields in the scan area.

- **First field only each location:** Performs the autofocus operation only on the first field of each location in every scan area. No further autofocus is performed on subsequent fields..

- **Every field:** Performs the autofocus operation on every field of the scan area.

- **Every <X> fields:** Allows you to specify the distance between fields in each scan area before the autofocus is triggered.

  The distance between fields is expressed as "unit fields" (equivalent to a field of view as determined by the objective used); it is not determined by the order in which the fields are collected.

  For example, if you wish to autofocus every 3 fields, autofocus will only occur when the stage is three fields away from another autofocused field. If the stage is less than three fields away, autofocus will not occur.

Time Lapse and Incubator

Time Lapse and Incubator panel gives you the option of running your automated scan protocol over a time period, with or without the use of the EVOS™ Onstage Incubator, where the selected fields are captured at given intervals based on your specifications. The images captured in a time lapse scan protocol can then be compiled into a video sequence.
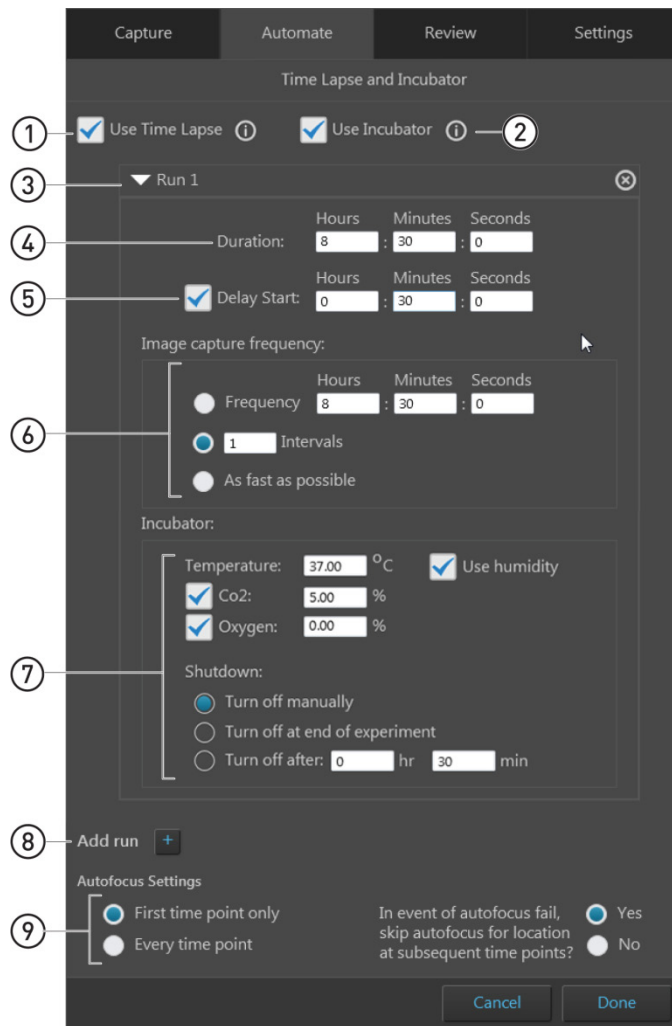
① **Use Time Lapse** (page 138)
② **Use Incubator** (page 138)
③ **Run** (page 139)
④ **Duration** (page 139)
⑤ **Delay Start** (page 139)

⑥ **Image capture frequency** (page 140)
⑦ **Incubator** (page 141)
⑧ **Add run** (page 141)
⑨ **Autofocus Settings** (page 142)

Note: The options on this panel are contextual. Objects and controls that are not available as a function in a particular context are not shown in the panel.

Note: Click on the **View Help Content** icon to open the Help window, which provides EVOS™ FL Auto 2 Imaging System Help.

Time Lapse and Incubator controls

① **Use Time Lapse:** Allows you to create time lapse routines to capture individual images at given intervals over a time period based on your specifications.

> ☑ Use Time Lapse

- Checking the **Use Time Lapse** box enables all of the other controls in this panel, allowing you to set the specific options for your time lapse routine (e.g., imaging frequency, use of the incubator etc.).
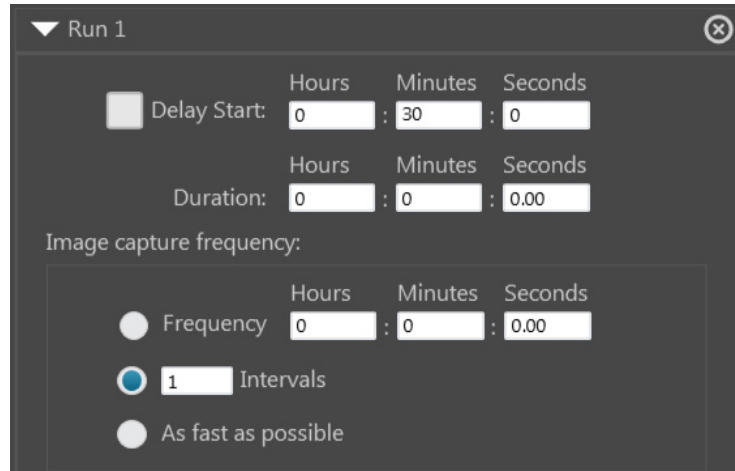
- A time lapse routine can be divided into individual "runs", which allow for different imaging frequency, duration, and incubator settings (see "Run", below).

- To add additional runs to your time lapse routine, click the **Add run** button (page 141).

- The fields and locations to be captured during a time lapse experiment are selected in the **Scan Area** panel (page 116).

- The autofocus options for the time lapse routine are set in the **AutoFocus and Z Stacks** panel (page 129).

- The captured images are stored with time lapse information in their headers and can be stitched together into a video.

② **Use Incubator:** Enables the use the EVOS™ Onstage Incubator your time lapse experiments. This option is available only when the EVOS™ Onstage Incubator is connected to the EVOS™ FL Auto 2 Imaging System.

When checked, **Use Incubator** option reveals the controls for configuring the incubator settings (see "Incubator", page 141).
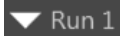
Note: The EVOS™ Onstage Incubator (Cat. No. AMC1000) is an optional accessory that enables the incubation of cells on the automatic X-Y stage, allowing the capture of images from the same sample over long periods of time and the recording of time lapse movies.

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③ **Run:** Contains the controls for the corresponding run.
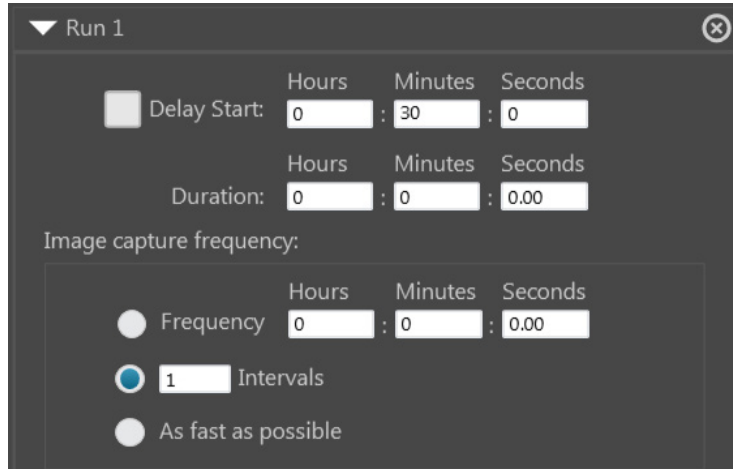
- The options available in this area are contextual and depend on the selections made for **Use Time Lapse** and **Use Incubator**.

- Click the **collapse/expand** button ▼ Run 1 to hide the controls for the run. Click the button again to expand the controls.

- To add additional runs to your time lapse routine, click the **Add run** button (page 141).

- If there is more than one run in the time lapse routine, each run has its own set of controls that are similarly collapsible/expandable.

- Click the **Delete Run** button ⊗ to delete a run and remove it from the panel.

④ **Duration:** Allows you to specify the duration of the corresponding run in the time lapse routine.
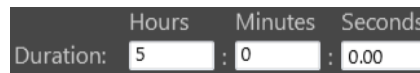
- Enter the desired values for the duration of the run into the corresponding **Hours**, **Minutes**, and **Seconds** fields.

⑤ **Delay Start:** Allows you to postpone image capture for the corresponding run of the time lapse routine and specify the duration of the delay.
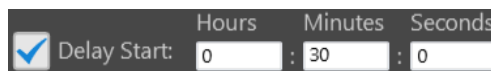
- Enter the desired values for the delay into the corresponding **Hours**, **Minutes**, and **Seconds** fields.

- When the Delay Start option is not selected, image capture starts immediately after the time lapse experiment is initiated.

- By default the Delay Start option is deselected, with 0 hours entered for the duration. After this feature has been used once, the most recent delay period entered is used as the default value.
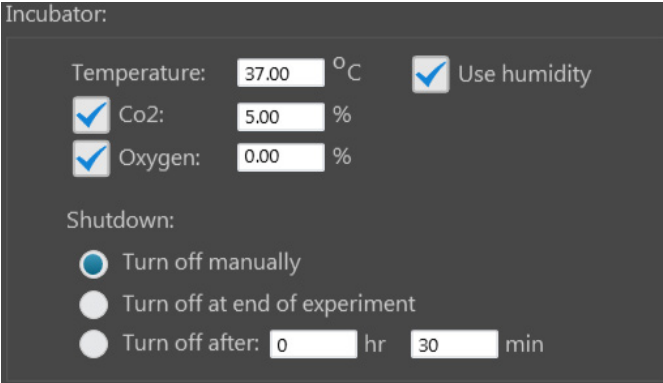
⑥ **Image capture frequency:** Sets the frequency with which the image sets are captured for the corresponding run in the time lapse routine.

The fields and scan areas are captured in the order specified in the **Scan Area** panel (page 126).
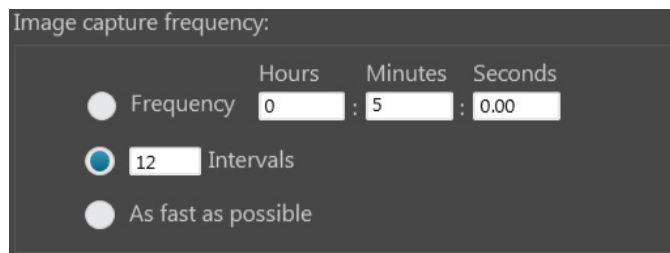
Available options for image capture frequency are:

- **Frequency:** Determines the time period that must elapse before a new set of images are captured. Note that the initial set of images is captured at time point 0.

  For example, if your run duration is 1 hour and you have entered 12 minutes for image capture frequency, the instrument captures one set of images every 12 minutes starting at time point 0 for a total of 6 image sets (60 minutes/12 minutes per image set plus the initial image set at time point 0).

- **Intervals:** Determines the total number of time intervals between captured image sets for a given run duration. Note that the initial set of images is captured at time point 0.

  For example, if your run duration is 1 hour and you have specified 5 Intervals, the instrument captures a total of 6 image sets 12 minutes apart (60 minutes/5 intervals plus the initial image set at time point 0).

- **As fast as possible:** Captures a new set of images immediately after completing the capture of the previous set without any delay between the image sets.

  Note that the speed with which the images are captured depends on your specifications for the scan protocol such as the autofocus frequency and exposure settings.

  When this option is selected, the instrument captures all selected fields in each scan area in the order specified, and then repeats the image capture process without any delay, starting with the first field in the first area for the duration of the run.

⑦ **Incubator:** Determines the incubation parameters (i.e., culture conditions) and the shutdown method for the EVOS™ Onstage Incubator.

- The incubation parameters are set separately for each run in the time lapse routine.

- **Temperature:** Sets the incubator temperature (ambient to 40°C).

- **CO₂:** Sets the CO_2 level (0% to 20%).

- **Oxygen:** Sets the oxygen level (0% to 20%).

- **Use humidity:** Enables the use of humidified atmosphere in the incubation chamber.

- **Shutdown:** Allows you to select the shutdown method for the EVOS™ Onstage Incubator at the end of your time lapse routine.

    – **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.

⑧ **Add run:** Adds a new run to the time lapse routine.

- Clicking the **Add run** button adds a new set of run controls to the panel, which are identical to the existing run controls.

    This allows you set the parameters for each run (delay start, duration, capture frequency, and incubator settings) independently for each run.

- The runs are performed in chronological order, beginning with Run 1 and ending with Run N.

- You can remove a run from the time lapse routine by clicking the corresponding **Delete Run** button .

⑨ **Autofocus Settings:** Allows you to set the autofocus frequency and autofocus fail options for the time lapse routine only.
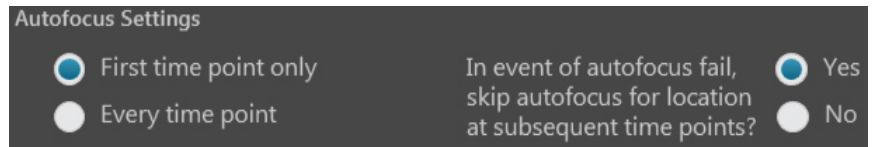
- **First time point only:** Performs the autofocus procedure only once at the first time point of the run before proceeding with image capture.

- **Every time point:** Performs the autofocus procedure at every time point of the run, ensuring that the sample is in focus for each image set captured.

- **Autofocus fail options:** Selecting **Yes** causes the instrument to skip the autofocus procedure at locations where no optimal focal plane was found at subsequent time points.

  Selecting **No** ensures that the autofocus is run at each selected autofocus location every time regardless of previous autofocus failure.

Note: The Autofocus Settings options available in this panel apply only to the time lapse routine. Autofocus locations and method are determined separately in the AutoFocus and Z Stacks panel (page 133).

Image Save Settings

Image Save Settings panel allows you to assign a destination directory for your saved images and to specify the file types to save.
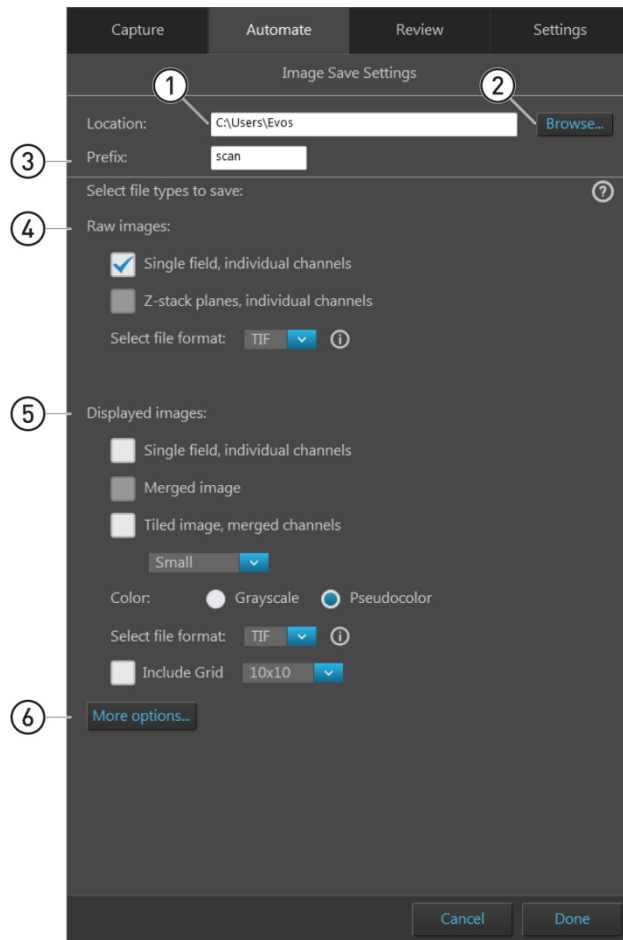
① **Location** (page 144)   ④ **Raw images** (page 145)

② **Browse** (page 144)   ⑤ **Displayed images** (page 146)

③ **File name prefix** (page 144)   ⑥ **More options** (page 147)

Note: Click on the **View Help Content** icon to open the Help window, which provides EVOS™ FL Auto 2 Imaging System Help.

Hover your pointer over the **Tooltip** icon without clicking it to view a small hover box with additional information.

Image Save Settings controls

① **Location:** Describes the location of the current folder or file.

Location:	C:\Users\Evos

② **Browse:** Opens the Select Folder window to assign a destination directory for your saved images.

Browse...

The Select Folder window follows the standard Windows file navigation system.
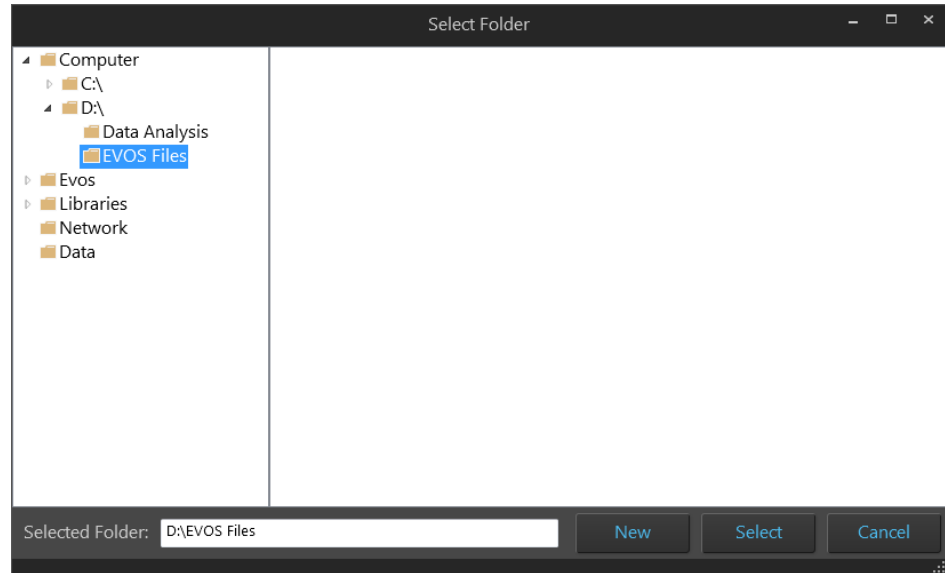
- **Selected folder:** Displays the location of the current folder.
- **New:** Creates a new folder in the current location.
- **Select:** Selects the current folder as the destination directory for your saved files. If the destination directory contains other files that could get overwritten, a warning message is displayed in the Select Folder window.

Note: We recommend that you save your captured images to an external hard drive.

③ **File name prefix:** Allows you to assign a prefix to the automatically generated file names when saving captured images.

Prefix:	scan

The default prefix for images captured in an automated scan is "scan".

Note: For more information on the file naming convention when saving, see "File naming convention", page 148.

④ **Raw images:** Allows you to save images and image sets captured in individual channels as 16-bit raw images. This is the recommended format for performing image analysis.
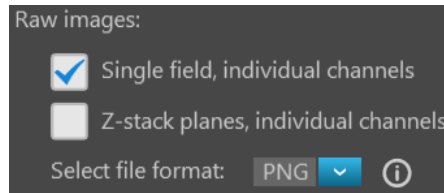
Available save options for raw images are:

- **Single field, individual channels:** Saves images captured in each field and each channel individually.

- **Z-planes, individual channels:** Saves individual images captured along the z-axis as well as the z-stack projection for each field and channel.

Available file formats for raw images are:

- **TIF:** No or low image compression with no loss in image quality.

- **PNG:** High image compression with no loss in image quality.

- **C01:** Thermo Fisher™ proprietary format. Only images captured by monochrome cameras may be saved in this format.

- **DIB:** Color corrects across devices for true viewing. Only images captured by monochrome cameras may be saved in this format.

Note: If you plan to analyze your image, save it as a 16-bit TIF raw image (preferred), a C01, or a DIB. There is information in the headers of these files and OME information that might be useful.

Note: When viewing 16-bit images on most standard image viewers, they will appear very dark or almost totally black, especially if a low exposure setting was used. These images will either need to be converted for display or opened in a software application that allows viewing of 16-bit images.

---

⑤ **Displayed images:** Allows you to save images in a format that can be viewed in most image display applications.

You can save displayed images in 24-bit pseudocolor (8-bit per RGB channel) or 16-bit grayscale, either individually from each channel or as a merged image.

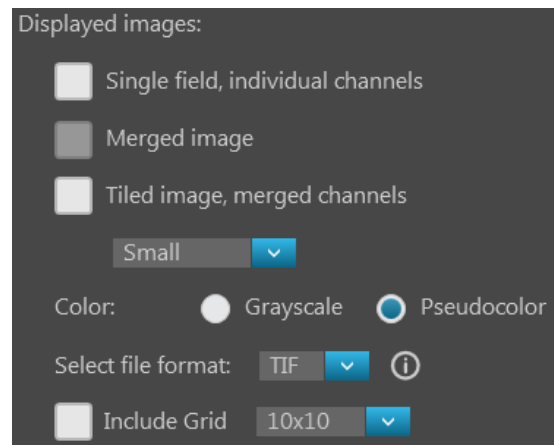**Note:** While pseudocolors help differentiate the channels used in multi-channel overlays, grayscale images usually show more detail.

24-bit images (8-bit per RGB channel) are NOT recommended for image analysis as not all channels will display in many image analysis applications.

Available save options for displayed images are:

- **Single field, individual channels:** Saves images captured in each field and each channel individually in **Grayscale** (16-bit) or **Pseudocolor** (24-bit RGB; 8-bit per RGB channel).

- **Merged image:** Combines images of a field captured in different channels into a multicolor overlay and saves the image in 24-bit Pseudocolor (8-bit per RGB channel).

- **Tiled image, merged channels:** Merges the images captured in each channel and aligns them close together into a tiled format without applying a stitching algorithm. The resulting tiled image is not seamless, but this method is quicker than stitching and is often sufficient for most applications. You can create tiled images with the following dimensions:
    - **Small** (2000 × 2000 pixels)
    - **Medium** (4000 × 4000 pixels)
    - **Large** (10,000 × 10,000 pixels)
    - **Maximum** (26,000 × 26,000 pixels).

Note: If you are analyzing images that cross fields such as images of neurites, and the images are slightly misaligned, you might want to consider **stitching** the images (see page 127). Stitching applies an algorithm to apply an overlap, and then removes the seam so you can visualize objects that cross fields.

Available file formats for raw images are:

- **TIF:** No or low image compression with no loss in image quality.

- **PNG:** High image compression with no loss in image quality.

- **JPEG:** High image compression with little loss in image quality; good for sharing.

Include Grid option superimposes a grid on the displayed images.

You have the following options for the grid size (in pixels): **Auto**, **10 × 10**, **50 × 50**, **100 × 100**, **200 × 200**, and **500 × 500**.

⑥ **More options:** Displays the full menu of save options in a convenient table format, allowing you to save your images in various formats simultaneously.

More options...

Select file types to save:

	Raw images (saves each channel individually)	Displayed image- individual channel	Displayed image- merged view
Single field	☑	☑	☐
Z-Stack planes	☐	☐	☐
Tiled images	☐	☐ Small ⌄	☑ Small ⌄

TIF ⌄ ⓘ

Color:
○ Grayscale ⦿ Pseudocolor

TIF ⌄ ⓘ

☑ Include Grid 10x10 ⌄

Fewer options...

File naming convention
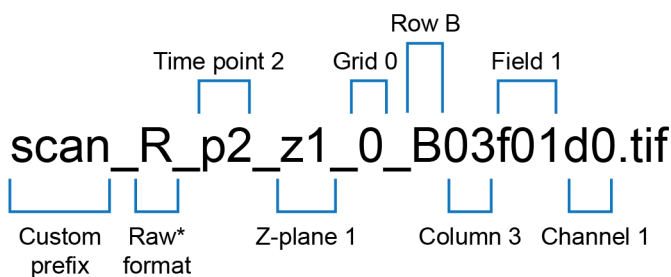
Files are saved using the following file naming convention:

<Prefix>_Time point_Z-plane_Grid_Row_Column_Field_Channel_File type

where:

- **<Prefix>:** User defined prefix that is assigned to the automatically generated default file name.

  By default, the prefix is "image" for images captured manually in the Capture tab, and "scan" for images captured automatically in the Automate tab.

- **Time point:** Describes the time point at which the image was captured and has the format p0, p1, p2... etc.

  For manually captured images, the time point is set to "p0".

- **Z-plane:** Describes the plane in the z-axis where the image was captured and has the format z0, z1, z2…etc.

- **Grid:** Refers to vessels containing multiple grid patterns on them, such as slides with groupings of microarrays. It has the format 0, 1, 2… etc.

- **Row:** Refers to the row number in the selected culture vessel.

- **Column:** Refers to the column number in the selected culture vessel.

- **Field:** Refers to the field of view that was captured and has the form f01, f02, f03… etc. Fields are numbered based on the order in which they were captured.

- **Channel:** Refers to the channel in which the image was captured and has the form d0, d1, d2… etc. A single-channel assay will be labeled with d0 in the file name.

For example, the image with the following name was captured at time point 2 through channel 1 in a time lapse z-stack scan. It shows the field 1 in the z-plane 1 in grid 0, row B, and column 3.

Row B
Time point 2   Grid 0   Field 1

scan_R_p2_z1_0_B03f01d0.tif

Custom   Raw*   Z-plane 1   Column 3   Channel 1
prefix   format

* R = Raw
  D = Display
  M = Merged

Settings

Settings tab overview

Settings tab is used for selecting basic and advanced system options and for performing calibration and maintenance procedures.

To access the Settings tab, click the Settings tab anytime from the Capture, Automate, and Review tabs.

To close the Settings tab and return to the previous tab, click **Done**.

General	>
Objectives	>
Stage Calibration	>
Incubator	>
Service	>
Camera Rotation	>
Filter Cubes	>
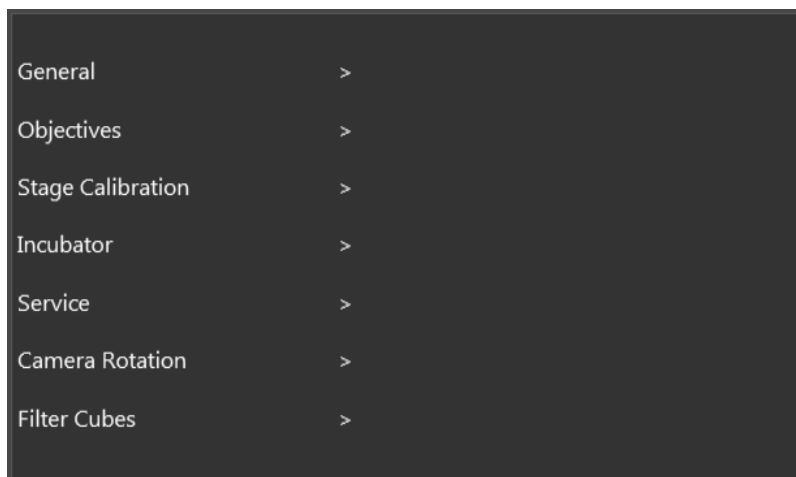
① **General:** Allows you to set the Saturated Pixel, Jog Control, Scale Bar, and Grid Settings options (page 150).

② **Objectives:** Allows you to set up and calibrate objectives and assign objective profiles. For more information, see "Change the objectives" (page 71) and "Calibrate the objectives" (page 73).

③ **Stage Calibration:** Allows you to calibrate the automatic X-Y axis stage for accurate instrument function. For more information, see "Calibrate the stage" (page 67).

④ **Incubator:** Allows you to configure the gas inputs, calibrate the oxygen sensor, and set the temperature offsets for the EVOS™ Onstage Incubator.

⑤ **Service:** Provides version information about instrument software and firmware.

⑥ **Camera Rotation:** Allows you to calibrate the camera rotation for accurate instrument function.

⑦ **Filter Cubes:** Allows you to add or remove EVOS™ LED light cubes. For more information, see "Change EVOS™ LED light cubes" (page 70).

General

General panel in the Settings tab allows you to set the Saturated Pixel, Jog Control, Scale Bar, and Grid Settings options.

To expand the controls for a setting, click the corresponding **expand/collapse** button. To close it, click the button again.

Capture	Automate	Review	Settings

General
1. ▶ Saturated Pixels
2. ▶ Jog Control
3. ▶ Scale Bar
4. ▶ Grid Settings
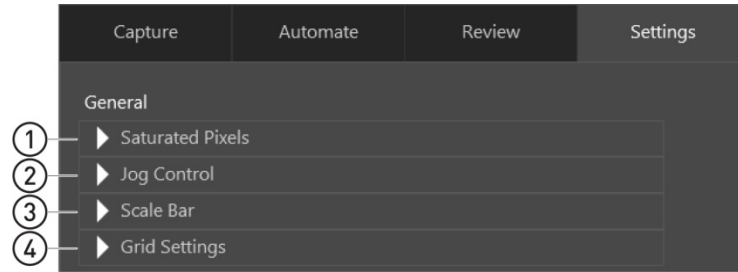
① **Saturated Pixels** (below) ③ **Scale Bar** (below)
② **Jog Control** (below) ④ **Grid Settings** (page 151)

General settings controls

① **Saturated Pixels:** Displays the pixels in saturated areas on an image in the selected color, which provides a visual aid for optimal illumination when adjusting the brightness settings.

Available colors for saturated pixels are **Blue**, **Red**, and **Green**.
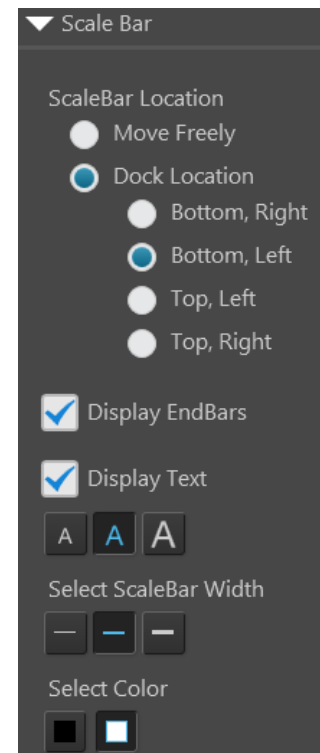
▼ Saturated Pixels

☑ Show saturated pixels:

Blue ▾
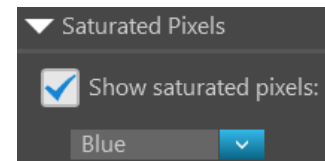
② **Jog Control:** Allows you to invert the direction of the stage movement as input by the Jog Control button (page 101).

Available options are **Invert Horizontally** and **Invert Vertically**.

▼ Jog Control

Jog Control Options
☐ Invert Horizontally
☐ Invert Vertically
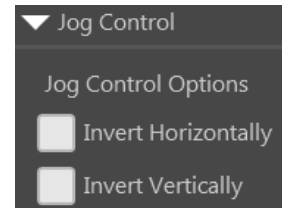
③ **Scale Bar:** Allows you to set the display options for the scale bar.

- Select **Move Freely** to be able to drag the scale bar anywhere in the Viewing area or select **Dock Location** to dock it in the **bottom** or **top left** or **right** corners of the Viewing area.

  When set to move freely, click **Reset to Default** to place the scale bar in the bottom left corner.

- Other scale bar display options include:
  - **Display End Bars:** Adds end bars to the scale bar.
  - **Display Text:** Displays the size of the scale bar. You can select to display the scale information using **small**, **medium**, or **large** sized fonts.
  - **Select Scale Bar Width:** Allows you select the width of the scale bar (**narrow**, **medium**, **wide**).
  - **Select Color:** Allows you to select the color of the scale bar (**black** or **white**).

▼ Scale Bar

ScaleBar Location
○ Move Freely
● Dock Location
  ○ Bottom, Right
  ● Bottom, Left
  ○ Top, Left
  ○ Top, Right

☑ Display EndBars

☑ Display Text
A A A

Select ScaleBar Width
— — —

Select Color
■ □

④ **Grid Settings:** Allows you to set the display options for the sample grid that is displayed in the Viewing area.


- **Select Color:** Sets the color of the grid. Available options are **white**, **light grey**, **dark grey**, and **black**.

- **Grid Alignment:** Determines how the grid is aligned to the Viewing area. Available options are:
  - **Cross-hair at center**
  - **Grid Square at the center**

- **Grid Size:** Allow you select the grid size (i.e., the unit size of the squares that form the grid), which is expressed in µm.

  You have the following options for the grid size (in pixels):

  Auto, **10 × 10**, **50 × 50**, **100 × 100**, **200 × 200**, and **500 × 500**.

  When the **Auto Size** option is selected, the software automatically adjusts the grid spacing if the chosen value results in more than 50 grid lines per image.

- **Show grid size label:** Displays the unit size of the squares that form the grid.
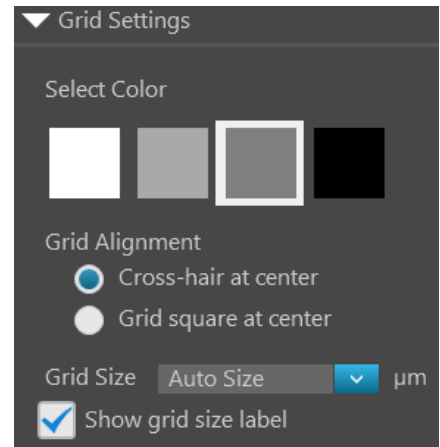
Appendix D: EVOS™ Onstage Incubator

EVOS™ Onstage Incubator

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

The EVOS™ Onstage Incubator consists of a Stagetop Environmental Chamber that is placed on the automatic X-Y stage of the imaging system and a separate Control Unit that supplies the power and gas (air or air-CO_2 premix, CO_2-only, and nitrogen-only). The onstage incubator is controlled by the same software and user interface that controls the EVOS™ FL Auto 2 Imaging System.

Standard items included

- Stagetop Environmental Chamber
- Control unit
- Master Stage Plate (also available separately as EVOS™ Onstage Master Plate, Cat. No. MEPVH035)
- Vessel holder for multi-well plates (also available separately as EVOS™ Onstage Vessel Holder, Multiwell Plates, Cat. No. AMEPVH028)
- Cable with 6-pin connector
- Cable, USB A-to-B, 180 cm/6 ft
- Heated hose with temperature control, 180 cm/6 ft (also available separately as EVOS™ Onstage Incubator Hose, Cat. No. AMEP4728)
- Gas line, 1/8" ID, 1/4" OD (also available separately as EVOS™ Onstage Incubator Gas Line, Cat. No. AMEP4732)
- Push-to-connect gas line adaptor (3 each)
- Standard-head open-end wrench
- Hex screw driver
- Power Cord, Type A (North America)

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

Technical specifications

Physical characteristics

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

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

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

CO_2 range: 0% to 20%

O_2 range: 0% to ambient

Operating power: 100–240 VAC, 1.8 A

Frequency: 50–60 Hz

Electrical input: 24 VDC, 5 A

Hardware

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

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

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

EVOS™ Onstage Incubator components

Control unit and environmental chamber

EVOS™ Onstage Incubator consists of an environmental chamber and a separate control unit that supplies the power and gas (air or air-CO_2 premix, CO_2, and N_2 for O_2 displacement in hypoxia experiments), and controls the humidity and temperature.

① Control unit

② Hose heater connector

③ Heated hose

④ Sensor data cable with 6-pin connector

⑤ Environmental chamber

Control unit rear view

① Sensor data cable jack

② USB control cable jack

③ Power input jack

④ Port 1: Air

⑤ Port 2: N_2

⑥ Port 3: CO_2

⑦ Power switch

Environmental chamber

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

The environmental chamber sits on the onstage incubator master plate attached to the X-Y stage of the EVOS™ FL Auto 2 Imaging System (see "Assemble the environmental chamber", page 157).
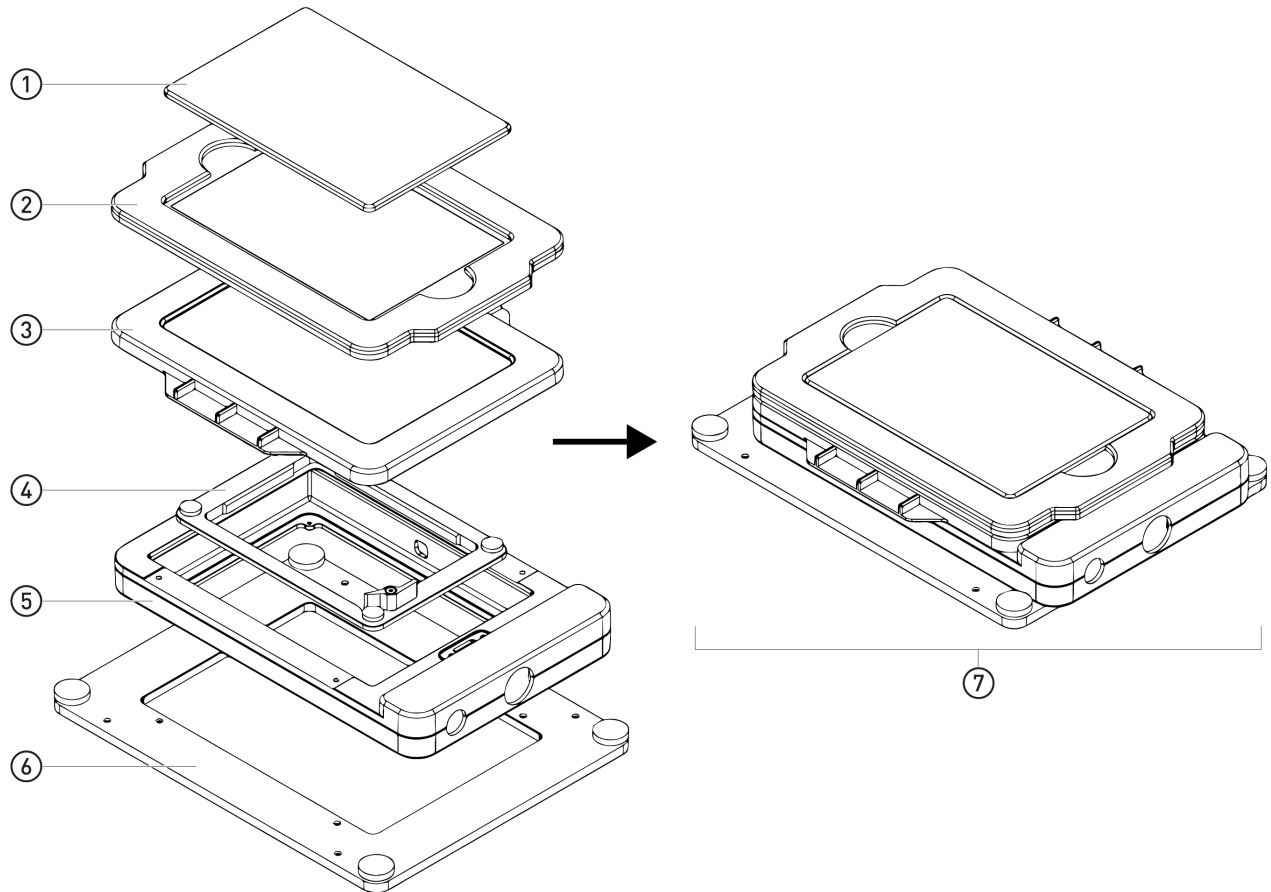
1. Light shield cover
2. Light shield
3. Heated glass lid
4. Vessel holder/adaptor
5. Incubator chamber
6. Master plate
7. Assembled environmental chamber

Set up the EVOS™ Onstage Incubator

Install the Onstage Incubator Master Plate

1. Remove the X-Y stage base plate ① from the X-Y stage by unscrewing the four 3.0-mm screws (indicated by red arrows) on the base plate ② If necessary, unscrew and remove the vessel holder/adaptor ③ before removing the base plate.
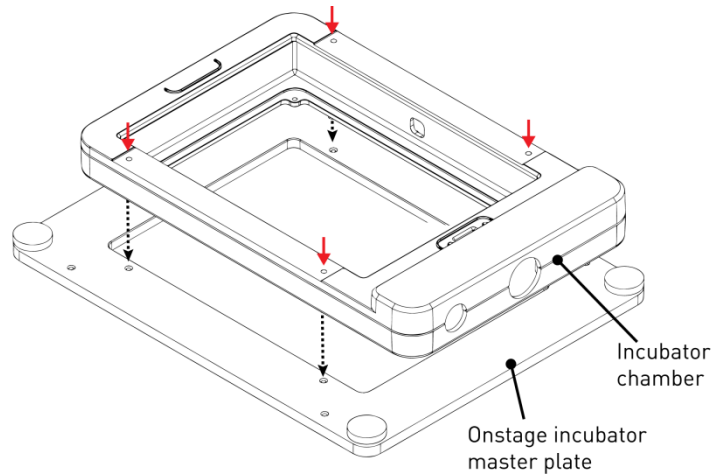

2. Secure the onstage incubator master plate ④ to the X-Y stage using the four thumb screws (indicated by red arrows).
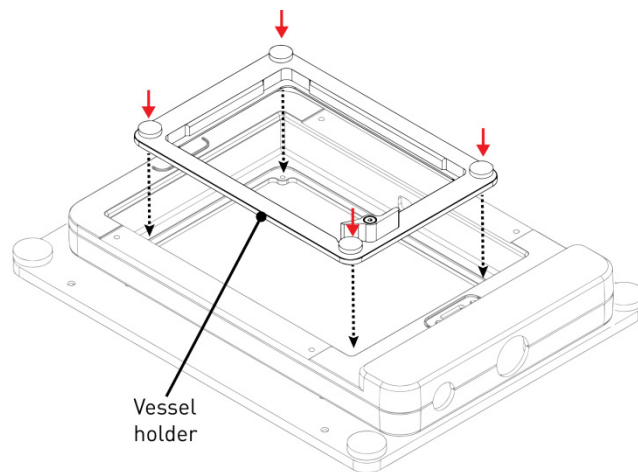
Assemble the environmental chamber

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

Incubator chamber

Onstage incubator master plate

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

Vessel holder

Note: Place an empty "dummy" culture plate into the vessel holder/adaptor for the initial warm up and equilibration to prevent build-up of condensation on the optical components and the inside of the EVOS™ FL Auto 2 Imaging System.

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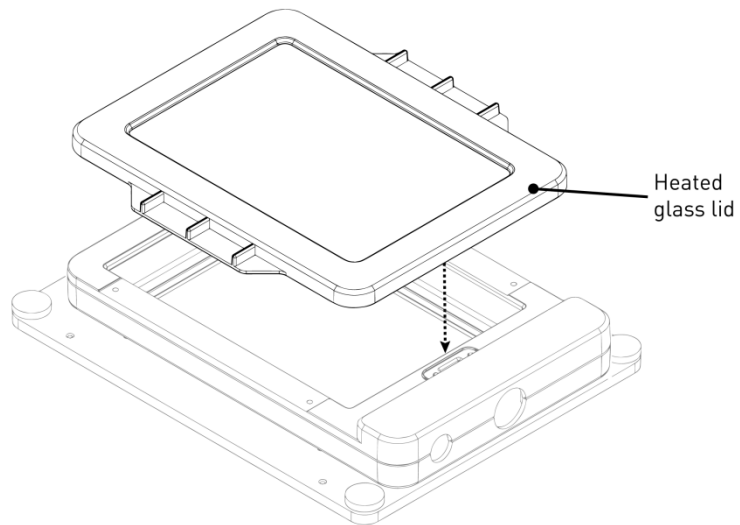
Heated glass lid

4. Place the light shield with tinted plastic window on top of the heated glass lid. **Use of the light shield is required for fluorescence imaging applications.**

5. If desired, place the light shield cover on the light shield for fluorescence imaging applications. The light shield cover completely blocks any ambient light from entering the environment chamber and improves image quality in fluorescence imaging applications.


Light shield cover

Light shield
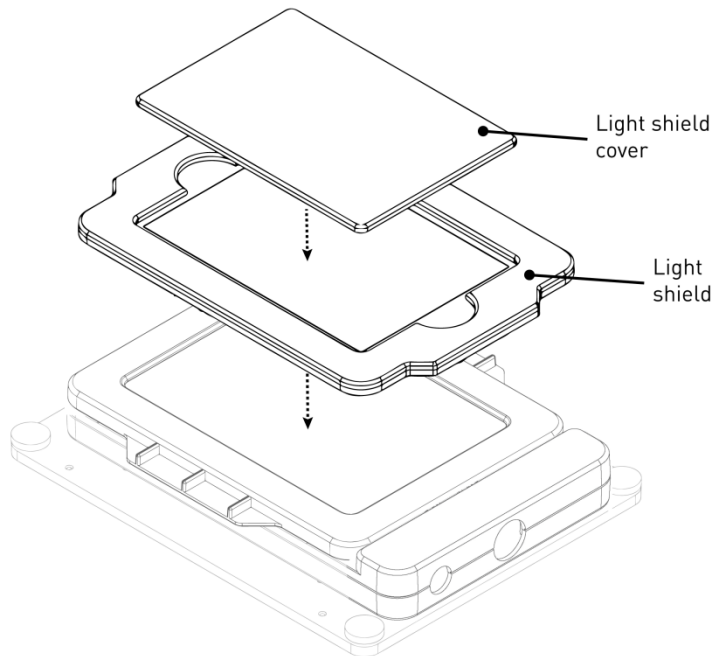
Set up for operation   Follow the procedure below to set up the EVOS™ Onstage Incubator for operation. For the locations of the various input jacks and gas ports, refer to "Control unit rear view", page 154.

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

1. Plug power cord into the power input jack on the control unit and the wall outlet.

2. Plug USB cable into the USB control cable jack on the control unit and the USB port on the computer.

3. Connect each gas line to the appropriate gas tank via the PTC (push-to-click) connectors threaded into the regulator. To do this, push the tubing into the PTC connector until it clicks into place. Pull on tubing slightly to ensure a tight connection; the tubing should not come out.
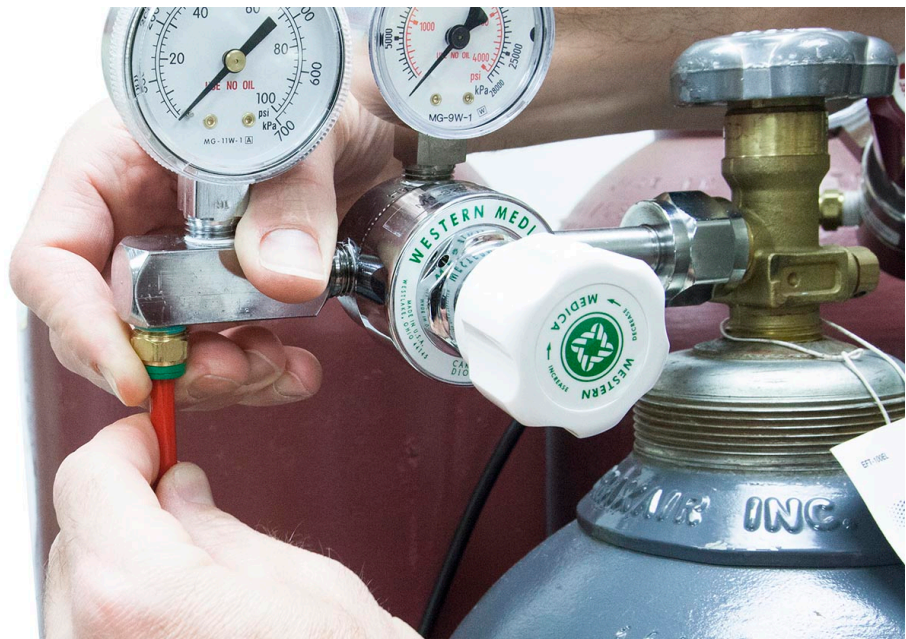

4. Attach the gas lines to the control unit via the PTC connectors for the appropriate gas intake port.

   - If using pre-mixed air, attach to **Port 1: Air In**

   - If using compressed air and CO_2, attach to **Port 1: Air In** and **Port 3: CO₂ In**

   - For oxygen displacement, attach to **Port 1: Air In** and **Port 2: Nitrogen In**

5. Plug the 6-pin sensor data cable to the environmental chamber and the appropriate input jack on the control unit.

6. Assemble the water reservoir and add warm water (approximately 50°C) to the max fill line through the fill hole (see image below). **Do not overfill** the water reservoir.
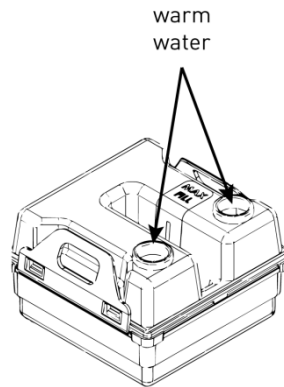
7. Place the water reservoir into the control unit **with the fill holes to the front** and close the lid.
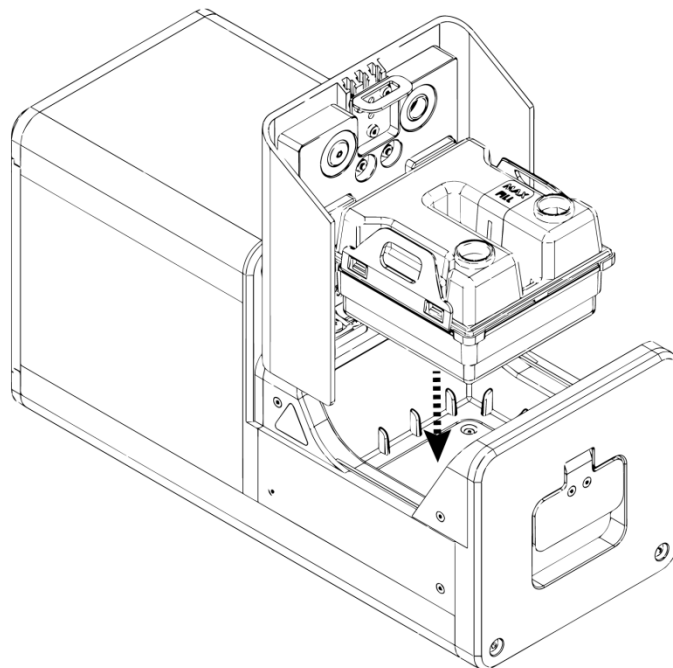
8. Attach the heated hose between the environmental chamber and the control unit

9. Plug the hose heater cable to the connector on the heated hose.

Use the EVOS™ Onstage Incubator

Turn ON the EVOS™ Onstage Incubator

1. Turn ON the EVOS™ FL Auto 2 Imaging System as described on page 14.

2. Turn the computer and monitor ON.

3. Turn ON the power switch to the control unit (see page 154 for location).

4. Restart the Auto 2software.

5. On the **Capture** tab, click the **Incubator** button to display the Incubator control panel.

6. Select **Use Incubator**, then enter the target values for **Temperature**, **CO₂**, and **Oxygen**. If desired, select **Use humidity**.

7. Select the desired **Shutdown** option:

   - **Turn off manually:** The incubator will remain on until the **Use Incubator** option is manually deselected and the **Apply** button is clicked.

   - **Turn off after:** Enter the time period in **hours** and **minutes** that must elapse before the incubator is shut down automatically.

8. Click **Apply**. Incubator status will change to "Running" and the control panel will display the Current, Target, and Actual values for Temperature, Humidity, CO₂, and Oxygen.

9. Click **Done** to return to the Capture tab.

Note: Place an empty "dummy" culture plate into the vessel holder/adaptor for the initial warm up and equilibration to prevent build-up of condensation on the optical components and the inside of the EVOS™ FL Auto 2 Imaging System.

Configure gas inputs

1. In the **Settings** tab (page 149), click **Incubator** to open the incubator configuration panel.
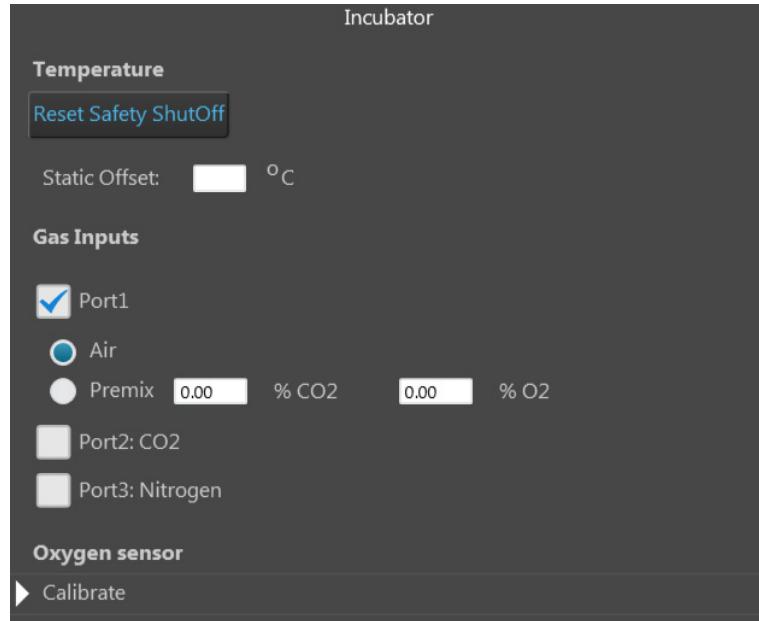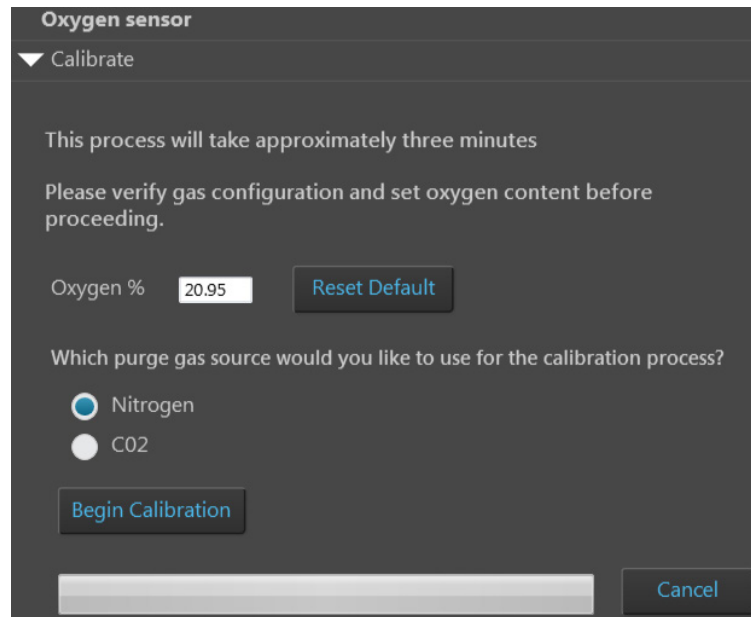

2. Select the appropriate options for the **Gas Inputs** that reflects your set-up for the EVOS™ Onstage Incubator.

    • For **Port 1**, you may select **Air** or **Premix**.

      If you select **Premix**, manually enter the percentage of the CO_2 and O_2 to reflect the specifics of your set-up.

    • **Port 2** is reserved for Nitrogen only.

    • **Port 3** is reserved for CO_2 only.

3. Click **Done** once you have configured the gas connections for each port.

4. Turn on the regulators on the gas tanks. The meters on the regulators show the tank fill on the right and gas flow on the left.

5. Set the flow on the regulators as follows. Do not exceed 50 psi of pressure.

    • **Air:** 40–50 psi

    • **CO_2:** 40–50 psi

    • **Nitrogen:** 40–50 psi

Calibrate oxygen sensor

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

1. In the **Settings** tab (page 149), click **Incubator** to open the incubator configuration panel, then expand the **Calibrate** panel under **Oxygen Sensor**.

2. Verify that your gas connections have been correctly configured.

3. Verify that the **Oxygen %** has been set correctly. If not, enter the correct value for oxygen content.

4. Select the **purge gas source** for your calibration (**Nitrogen** or **CO$_2$**).

5. Click **Begin Calibration**. The EVOS™ FL Auto 2 Imaging System automatically calibrates the oxygen sensor for the proper functioning of the EVOS™ Onstage Incubator. The entire calibration process takes approximately three minutes.

Appendix E: Safety

Safety conventions used in this document

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

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

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

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

Symbols on instruments

Electrical symbols on instruments

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

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

Safety symbols

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

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

Environmental symbols on instruments

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

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

Safety labels on instruments

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

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

General instrument safety

⚠ **WARNING! PHYSICAL INJURY HAZARD.** Use this product only as specified in this document. Using this instrument in a manner not specified by Thermo Fisher Scientific may result in personal injury or damage to the instrument.

Moving and lifting the instrument

⚠ **CAUTION! PHYSICAL INJURY HAZARD**. The instrument is to be moved and positioned only by the personnel or vendor specified in the applicable site preparation guide. If you decide to lift or move the instrument after it has been installed, do not attempt to lift or move the instrument without the assistance of others, the use of appropriate moving equipment, and proper lifting techniques. Improper lifting can cause painful and permanent back injury. Depending on the weight, moving or lifting an instrument may require two or more persons.

Moving and lifting stand-alone computers and monitors

⚠ **WARNING!** Do not attempt to lift or move the computer or the monitor without the assistance of others. Depending on the weight of the computer and/or the monitor, moving them may require two or more people.

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

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

Operating the instrument

Ensure that anyone who operates the instrument has:

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

Cleaning or decontaminating the instrument

⚠ **CAUTION!** Using cleaning or decontamination methods other than those recommended by the manufacturer may compromise the safety or quality of the instrument.

Removing covers or parts of the instrument

⚠ **CAUTION! PHYSICAL INJURY HAZARD.** The instrument is to be serviced only by trained personnel or vendor specified in the user guide. Do not remove any covers or parts that require the use of a tool to obtain access to moving parts. Operators must be trained before being allowed to perform the hazardous operation.

Safety requirements for EVOS™ Onstage Incubator

⚠ **WARNING!** Thermo Fisher Scientific recommends the use of nitrogen, oxygen, and carbon dioxide gas with the Onstage Incubator. The use of alternative gasses is currently not supported and may adversely affect system performance

Gas cylinders

You must supply the required nitrogen, oxygen, and carbon dioxide gas cylinders and accessories for the installation. This instrument requires pressurized house lines, or one size 1-A gas cylinder that holds approximately 7.2 m^3 (257 ft^3) of gas when full for each gas. Use only pre-purified gasses of 99.9% or greater purity.

⚠ **CAUTION!** Damage to the instrument and its products can result from using impure gas, gases other than specified, or an inadequate amount of gas.

⚠ **WARNING! EXPLOSION HAZARD.** Pressurized gas cylinders are potentially explosive. Always cap the gas cylinder when it is not in use, and attach it firmly to the wall or gas cylinder cart with approved brackets or chains.

⚠ **WARNING!** Gas cylinders are heavy and may topple over, potentially causing personal injury and tank damage. Cylinders should be firmly secured to a wall or work surface. Please contact your environmental health and safety coordinator for guidance on the proper installation of a gas cylinder.

Pressure regulator

You must supply a two-gauge regulator with a Compressed Gas Association (CGA) 580-cylinder adaptor on the inlet side and a Swagelok™-type end-fitting that accepts 6.35-mm (0.25-in.) o.d. tubing. The primary gauge (0 to 3000 psi; 0 to 25,000 kPa recommended) measures tank pressure, and the secondary gauge (0 to 200 psi; 0 to 2000 kPa recommended) measures regulated pressure. The secondary gauge must allow regulation to 50 psi. Compressed Gas Association (CGA) 580-cylinder adaptor with a needle-type shutoff valve on the exit side. The needle valves should have Swagelok™-type end-fittings ready for connection to 6.35-mm (0.25-in.) o.d. tubing.

Attaching the cylinder

Attach the pressurized gas cylinder firmly to a wall or gas cylinder cart by means of approved straps or chains.

Ventilation requirements

⚠ **WARNING!** The Onstage Incubator should be installed and operated in a well-ventilated environment as defined as having a minimum airflow of 6–10 air changes per hour. Please contact your environmental health and safety coordinator to confirm that the Ion instruments will be installed and operated in an environment with sufficient ventilation.

Ventilation requirements

Allow at least 50 cm (20 in) of clearance around the Instrument for ventilation.

Chemical safety

Chemical hazard warning

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

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

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

General safety guidelines

To minimize the hazards of chemicals:

- Read and understand the Safety Data Sheets (SDSs) provided by the chemical manufacturer before you store, handle, or work with any chemicals or hazardous materials. (See "Safety Data Sheets (SDS)")

- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.

- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.

- Check regularly for chemical leaks or spills. If a leak or spill occurs, follow the manufacturer's cleanup procedures as recommended in the SDS.

- Comply with all local, state/provincial, or national laws and regulations related to chemical storage, handling, and disposal.

Chemical waste safety

Chemical waste hazard

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

Chemical waste safety guidelines

To minimize the hazards of chemical waste:

- Read and understand the Safety Data Sheets (SDSs) provided by the manufacturers of the chemicals in the waste container before you store, handle, or dispose of chemical waste.

- Provide primary and secondary waste containers. (A primary waste container holds the immediate waste. A secondary container contains spills or leaks from the primary container. Both containers must be compatible with the waste material and meet federal, state, and local requirements for container storage.)

- Minimize contact with chemicals. Wear appropriate personal protective equipment when handling chemicals (for example, safety glasses, gloves, or protective clothing). For additional safety guidelines, consult the SDS.

- Minimize the inhalation of chemicals. Do not leave chemical containers open. Use only with adequate ventilation (for example, fume hood). For additional safety guidelines, consult the SDS.

- Handle chemical wastes in a fume hood.

- After emptying the waste container, seal it with the cap provided.

- Dispose of the contents of the waste tray and waste bottle in accordance with good laboratory practices and local, state/provincial, or national environmental and health regulations.

Waste disposal

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

- Characterize (by analysis, if necessary) the waste generated by the particular applications, reagents, and substrates used in your laboratory.

- Ensure the health and safety of all personnel in your laboratory.

- Ensure that the instrument waste is stored, transferred, transported, and disposed of according to all local, state/provincial, and/or national regulations.

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

Electrical safety

⚠ **DANGER! ELECTRICAL SHOCK HAZARD.** Severe electrical shock can result from operating the EVOS™ FL Auto 2 Imaging System without its instrument panels in place. Do not remove instrument panels. High-voltage contacts are exposed when instrument panels are removed from the instrument.

Fuses

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

Power

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

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

⚠ **DANGER! ELECTRICAL HAZARD.** Plug the system into a properly grounded receptacle with adequate current capacity.

Overvoltage rating

The EVOS™ FL Auto 2 Imaging System has an installation (overvoltage) category of II, and is classified as portable equipment.

Physical hazard safety

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

Biological hazard safety

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

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

In the U.S.:

*   U.S. Department of Health and Human Services guidelines published in *Biosafety in Microbiological and Biomedical Laboratories*

    (stock no. 017-040-00547-4;
    www.cdc.gov/OD/ohs/biosfty/bmbl4/bmbl4toc.htm)

*   Occupational Safety and Health Standards, Bloodborne Pathogens

    (29 CFR§1910.1030;
    www.access.gpo.gov/nara/cfr/waisidx_01/29cfr1910a_01.html)

*   Your company's/institution's Biosafety Program protocols for working with/handling potentially infectious materials.

*   Additional information about biohazard guidelines is available at:

    www.cdc.gov

In the EU:

*   Check your local guidelines and legislation on biohazard and biosafety precaution, and the best practices published in the World Health Organisation (WHO) Laboratory Biosafety Manual, third edition

    www.who.int/csr/resources/publications/biosafety/WHO_CDS_CSR_LYO_2004_11/en/

Safety and electromagnetic compatibility (EMC) standards

This section provides information on:

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

U.S. and Canadian safety standards

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

European safety and EMC standards

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

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

Australian EMC standards

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

Documentation and support

Obtaining support

Technical support For the latest services and support information for all locations, visit **www.thermofisher.com**.

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities

- Search through frequently asked questions (FAQs)

- Submit a question directly to Technical Support (**thermofisher.com/support)**

- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents

- Obtain information about customer training

- Download software updates and patches

Safety Data Sheets (SDS) Safety Data Sheets (SDSs) are available at **thermofisher.com/support**.

> (!) **IMPORTANT!** For the SDSs of chemicals not distributed by Thermo Fisher Scientific contact the chemical manufacturer.

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

> (!) **IMPORTANT!** Wiping the computer supplied with the EVOS™ FL Auto 2 Imaging System (i.e., erasing the hard drive to remove all programs, files, and the operating system) voids the product warranty.