

USER GUIDE

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EVOS[®] FL Auto

EVOS[®] Onstage Incubator

Imaging System for **Fluorescence**
and **Transmitted Light** Applications

Catalog Numbers AMAFD1000, AMC1000

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Revision C.0

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About This Guide

Audience This user guide is for laboratory staff operating, maintaining, and analyzing data using the EVOS[®] FL Auto Imaging System.

Revision history

| Revision | Date | Description |
|----------|---------------|---|
| C.0 | July 2014 | <ul style="list-style-type: none">• Updated objective calibration procedure• Updated user interface screenshots• Added Movies section |
| B.0 | December 2013 | <ul style="list-style-type: none">• Added French translation of Biological Hazard Safety• Added product warranty disclaimer |
| A.0 | November 2013 | <ul style="list-style-type: none">• Added EVOS[®] Onstage Incubator section• Updated user interface screenshots |
| 1.0 | April 2013 | Establish new user guide |

User attention words

Two user attention words appear in Life Technologies user documentation. 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

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



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



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. Installation

Standard Items Included

Before setting up the EVOS® FL Auto Imaging System, unpack the unit and accessories and verify all parts are present. Contact your distributor if anything is missing.



Note: If you do not have your distributor information, contact Technical Support (see page 83).

- EVOS® FL Auto Imaging System
- Computer
- Touch-screen monitor
- Keyboard
- Mouse
- Light cubes, as ordered
- Objectives, as ordered
- Vessel holder(s), as ordered
- Light box with cover
- Light cube tool
- Sliders: Block slider, Diffuser slider
- Dustcover
- USB flash drive (includes User Guide and Quick Start Guide)

Operating Environment

- Place the EVOS® FL Auto Imaging System on a level surface away from vibrations from other pieces of equipment.
- 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.
- Set up the EVOS® FL Auto Imaging System 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%.



IMPORTANT! Do not position the instrument so that it is difficult to turn off the main power switch located on the right side of the instrument base (see page 58). In case of an instrument malfunction, turn the main power switch to the OFF position and disconnect the instrument from the wall outlet.

Set Up

- Unpack 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 USB touch screen cable into monitor.

- Unpack 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 Imaging System (i.e., erasing the hard drive to remove all programs, files, and the operating system) voids the product warranty.

- Unpack the instrument**
1. Open the box and remove the accessory box.
 2. Carefully lift the instrument out of the box, holding it by one of the four handholds in the base (see page 58).



IMPORTANT! Do not lift the EVOS® FL Auto 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
 - Power supply
 - Display Port to DVI cable
 - USB type A to B cable
 - White cardboard box (contains the light box and vessel holders)
 - Dust cover
5. Confirm that the power switch is OFF (located on the right side of the instrument base; see page 58).
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-DVI cable to connect the Display Port output on the computer to the DVI input on the monitor.
9. Use the USB cable to connect 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.

Remove the shipping restraint

The shipping restraint prevents the X-Y stage from moving during shipping. If the stage is not secured during shipping, shock and vibration can damage the motors that move the stage. The shipping restraint must be removed before the EVOS® FL Auto Imaging System is powered on.



1. Unscrew the three thumb screws until they spin freely. You do not need to remove them from the shipping restraint altogether.
2. Gently pull the shipping restraint forward, away from the unit.



Note: Store the shipping restraint in your accessories box for future use. To reinstall the shipping restraint, see page 53.



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

Turn ON the EVOS® FL Auto Imaging System

1. Turn the instrument power switch located on the right side of the instrument base (see page 58) to the ON position.
The automatic X-Y stage will begin to move within a few seconds of the instrument being powered on.
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 EVOS® logo next to the start button on the desktop to start the EVOS® FL Auto software.
4. The EVOS® loading screen will be displayed while the software is starting up. This may take a minute.
5. Once the **Image ▶ Capture** tab (i.e., the Capture tab under Image, see page 7) is displayed, the EVOS® FL Auto Imaging System is ready to use.

2. Basic Operation

The EVOS® FL Auto Imaging System is a fully automated imaging system controlled by the integrated EVOS® FL Auto software. The software, accessed by the touch-screen monitor or the mouse, controls the automated X-Y axis stage and the objective turret, and features imaging and analysis tools.

Image Capture

Select the **Image ▶ Capture** tab (i.e., the Capture tab under Image) on any screen to access "Capture Controls". Note that the **Image ▶ Capture** tab is the default screen after start-up.

Capture tab controls

The screenshot shows the 'CAPTURE' tab of the EVOS FL Auto Imaging System software. The interface is organized into several functional areas:

- Vessel Expert:** Located at the top left, it includes a 'VESSEL EXPERT' button (1) and a 'VIRTUAL VESSEL' display (2).
- Objective Turret:** Features a 'MEDIUM JOG' button (3) and five objective buttons (4) labeled 20x, 10x, 20x, 2x, and 40x.
- Light and Camera:** Contains a 'POWER' toggle (6) with 'ON' and 'OFF' states, and a 'LIGHT' button (7). Below these are five light channel buttons (8) labeled 'TxRed', 'GFP', 'CY5', 'TRANS', and 'PHASE', each with a 'No Image' status.
- Focus and Capture:** Includes a 'SNAP TO LAST' button (9), a 'FIND SAMPLE' button (11), an 'AUTO FINE' button (12), and a 'CAPTURE' button (15). Below these are focus sliders for 'QUICK' (10), 'COARSE' (13), and 'FINE' (14). A 'Z-STACK' button (16) and a 'CAPTURE FOCUS NOMINAL' button (17) are also present.
- Save:** A 'SAVE' button (18) is located at the bottom right.

Red numbered callouts (1-18) point to these specific controls.



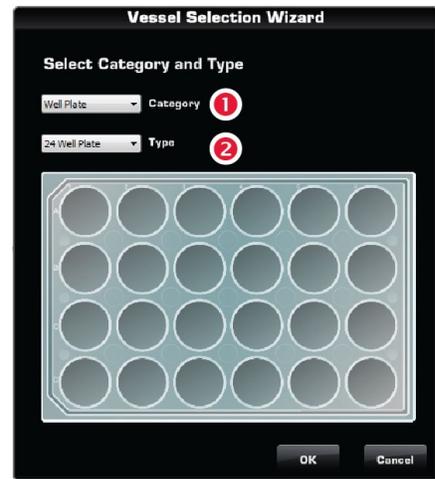
Note: 15, 16, 17, and 18 are "Hot buttons". Hot buttons are user-specified, programmable buttons that customize the imaging system for your specific needs. For more information on customizing Hot buttons, see page 21.

Vessel Expert button

Vessel Expert button opens the Vessel Selection Wizard, which is used for selecting the sample vessel category (such as well plate, Petri dish, culture flask, chamber slide, etc.) and vessel type (such as 24-well plate, 35-mm Petri dish, T75 flask, etc.) from the **Category 1** and **Type 2** drop-down menus.

With appropriate vessel holders, the EVOS[®] FL Auto Imaging System can accommodate a wide variety of common culture vessels types in standard sizes.

Note that a vessel **MUST** be selected prior to imaging.

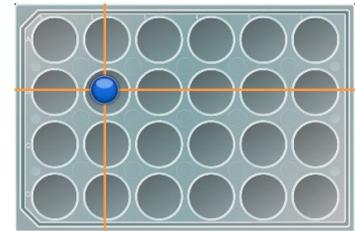


Note: For a list of the vessel holders and stage plates available for the EVOS[®] FL Auto Imaging System, refer to www.lifetechnologies.com/evosflauto or contact Technical Support (page 83).

Virtual vessel

Virtual vessel is a graphical representation of the sample vessel as selected with the Vessel Expert.

- To choose the location on the sample vessel you wish to view and image, click and drag the **crosshairs** to the corresponding location on the virtual vessel.
- Click on a well to move the **crosshairs** to the center of the well.



Medium Jog button

Medium Jog button moves the stage at an intermediate pace while viewing sample, allowing the quick scanning of the sample in different parts of the sample vessel.

Objective buttons

Objective buttons control the active objective turret position. The magnifications displayed on the Image Capture tab reflect the objective profiles chosen on the System Service tab.

Power toggle

Power toggle turns the LED light source in the selected channel ON and OFF.

Light channel buttons

Light channel buttons are used for selecting the desired channel from the number of installed LED light cubes or the transmitted light from the condenser. The selected channel is indicated by the blue color of the corresponding Light channel button.



Note: If there are images in the memory buffers, they will be displayed as thumbnails below the corresponding light cube indicators.

Light and Camera button

Light and Camera button opens the Light and Camera selection tool for the **Monochrome camera** ③ (for fluorescence and brightfield) or the **Color camera** ④ (for brightfield only).

Apply Pseudo Color ⑤ allows the display of the images in user-defined pseudo color (see page 21) when using the Monochrome camera in fluorescence channels.



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

Actual Mode ⑥ allows you to individually adjust the LED intensity, camera gain, and exposure (rather than controlling them as a single parameter with the **Light Intensity slider**).

Clear All Channels ⑦ clears out all memory buffers, which are temporary holding areas for captured images.

Snap to Last button

Snap to Last button reverts to the focus position of the last captured image.

Quick slider

Quick slider moves the focus through a small subset of the total focal range as determined by the current vessel. This focal range is centered around the “Focus Nominal” of the selected vessel (see Hot buttons, below). Utilizing the Quick slider reduces the need to use the Find Sample button and the Coarse Focus slider.

Find Sample and Auto Fine buttons

Find Sample and **Auto Fine** Buttons are used for automatic coarse and fine focusing on the sample.

Coarse and Fine Focus sliders

Coarse and **Fine Focus sliders** are used to manually adjust coarse and fine focus.

Hot buttons

“Hot buttons” are user-specified, programmable buttons that customize the imaging system. Up to four Hot buttons can be displayed on the **Image ▶ Capture** tab. For detailed information on customizing Hot buttons to your specific needs, see page 21.

Capture images using the Capture tab

1. Place the vessel containing your sample on the stage using the appropriate vessel holder. For the types of vessel holders available for the EVOS® FL Auto Imaging System, refer to www.lifetechnologies.com/evosflauto or contact Technical Support (page 83).
2. Use the **Capture tab controls** to select the desired imaging options (such as vessel type, area to capture, lighting, magnification, etc.) and adjust the illumination and the focus.
3. Click the desired **Capture** button to acquire your image.
4. Click **Save Image** to save the image to the desired location (see page 10).

Save

Clicking the **Save Image** button on any screen opens the **Image ▶ Save** tab, which allows you to name and save an image file or a scan in a desired location in a USB drive or the network, and to choose the desired Save Options.



Save tab controls

Folder address bar 1 : Describes the location of the current folder or the file.

Up folder button 2 : Navigates up to a higher-level folder.

New folder button 3 : Creates a new folder in the current location.

Current folder 4 : Contains lower level folders and/or saved files. Click on the lower level folders to navigate there.

File Name text field 5 : Allows you to enter a file name for your image.

Save Options 6 : Allows you to save your image using the Default, Quantitative Monochrome, or Custom options.

Bit Depth 7 : Allows you to set your color depth to 8-bit (256 colors) or 16-bit (thousands of colors; high-color).

File Type drop-down menu 8 : Allows you choose the format of your saved image file. Available options are JPEG, BMP, TIFF, and PNG.

Save As 9 : Allows you to choose between Color or Monochrome.

Include Image Annotations 10 : Allows you to include image annotations in your saved image.

Save Underlying Channels 11 : Allows you to include the underlying channels in your saved image.

Save Image 12 : Saves your image using the selected options.



Note: The **Default option** saves images in 8-bit color in TIFF format with the image annotations included.

To save a 16-bit image, select TIFF, and ensure that the Color option is deselected.

File types JPEG and PNG (as well as images of all types with the Color options engaged) only save at 8-bit depth.

3. Edit and Analyze Images

Overlay

Overlay tab controls

The **Image ▶ Edit ▶ Overlay** tab allows further adjustments to the brightness and contrast in each channel before the captured image is saved. Each channel can be turned ON and OFF independently and the brightness and contrast for each channel can be adjusted separately using controls described below.

ON/OFF buttons 1 : Toggle the display of corresponding channel in the overlay.

Light channel buttons 2 : Select the light channel for adjusting the brightness and contrast. The channel being adjusted is indicated by the blue color of the corresponding button. In the example on the right, the RFP channel is selected for adjustment.

Brightness and Contrast sliders 3 : Adjust the brightness and contrast of the image in the selected channel.

Reset All Channels 4 : Returns the brightness and contrasts settings to their previous value.

Clear Channel 5 : Clears the memory buffer for the selected channel.

Save Image 6 : Saves the image in the desired location. For more information, see page 10.



Note: Captured images can be saved in JPEG, BMP, TIFF, and PNG formats. For more information and additional save options, see page 10.

Measure

Measure tab controls

The **Image ▶ Edit ▶ Measure** tab is used for adding annotations, creating histograms for visualizing data, and drawing up to 12 regions of interest (ROIs) in the captured image prior to saving.

Picture

Current Image 1: Selects an image from memory buffer to annotate.

Annotations

Add 2: Activates the Add mode to add an annotation using the Annotation tools (see below).

To add an annotation, click **Add**, select an **Annotation tool 5**, and then click the area of the image where you want to add the annotation.

Select 3: Activates the Select mode to edit the properties of an existing annotation or the contents of a text box.

Zoom 4: Activates the digital zoom function onscreen, allowing a closer look at live or captured images.

To zoom on an image, click **Zoom**, and then double-click the area on the screen you wish to enlarge. To restore the view to unzoomed magnification, right-click anywhere on the image.

Annotation Tools 5: Include the text box, arrow, histogram, rectangle, ellipse, and line tools.

If there is no keyboard connected to the system, a virtual keyboard will appear when adding or editing a text box.

Annotation properties

Name 6: Allows you to label the annotation that you have added.

Color Edit 7: Allows you to select the color of an annotation.

Measure 8: Selects what is measured in a region of interest (ROI).

Lock Aspect Ratio 9: Locks the aspect ratio of the selected annotation tool.

Width 10: Changes the line width of the selected annotation.

Delete Selected 11: Deletes selected annotations.

Edit Text 12: Allows you to edit existing text in a selected text box.

Plot 13: When an intensity plot is displayed for a line, allows you to shades the area under each line.

Smooth 14: Smooths the histogram plot.



Misc.

Show Annotations ¹⁵: Option to display or hide the annotations on screen.

Save Image as Screenshot ¹⁶: Option for saving the image shown onscreen as its own file.

Show Annotation after Add ¹⁷: Option to edit annotations as they are added.

Units ¹⁸: Selects the units of measurement used for the annotations.

Show Histogram/Intensity ¹⁹: Option to display or hide the histogram for a selected ROI. If the line tool is selected, an intensity plot is displayed instead of a histogram. Histogram/intensity data include information from underlying channels if the ROI is selected using images from Image Capture, but not from Image Review.

Delete All Annotations ²⁰: Deletes all existing annotations.

Export All ²¹: Exports histogram/intensity data, including underlying channel data when coming from Image Capture, as a .csv file. When coming from Image Review, underlying channel data are not included.

Save Image ²²: Saves the image and the annotation(s) as a single file. For more information, see page 10.



Note: All annotations will remain whether or not a new image is captured. To start over, click **Delete All Annotations** ²⁰.

Count

Count tool

The Count tool, located in the **Image ▶ Edit ▶ Count** tab, allows you to manually mark items onscreen using up to six separate labels. As you tag each item to assign one of the six labels (thus grouping similar items together), the system keeps a running tally of the counts with percentages for each label assigned.



Note: You can use the Count tool on both newly captured and saved images, and document the results of your count by saving the tagged image, with the Count tool displaying the totals.

Count tool controls

Tags

Add 1: Activates the Add mode to tag items onscreen using the selected Label (see below).

Select 2: Activates the Select mode to select existing tags onscreen.

Zoom 3: Activates the digital zoom function for a closer look at live or captured images.

Labels 4: Select the label used for tagging on-screen items. Each label row features a text field for entering a **label name 5** and displays a running tally of the **count with percentage 6**. Clicking in the trash bin icons deletes the tags on the screen for that label set.

Total 7: Displays the total count of all labels assigned.

Misc.

Delete All Tags 8: Deletes all existing tags.

Show Tags 9: Displays or hides the tags onscreen.

Show Grid 10: Displays or hides the onscreen counting grid.

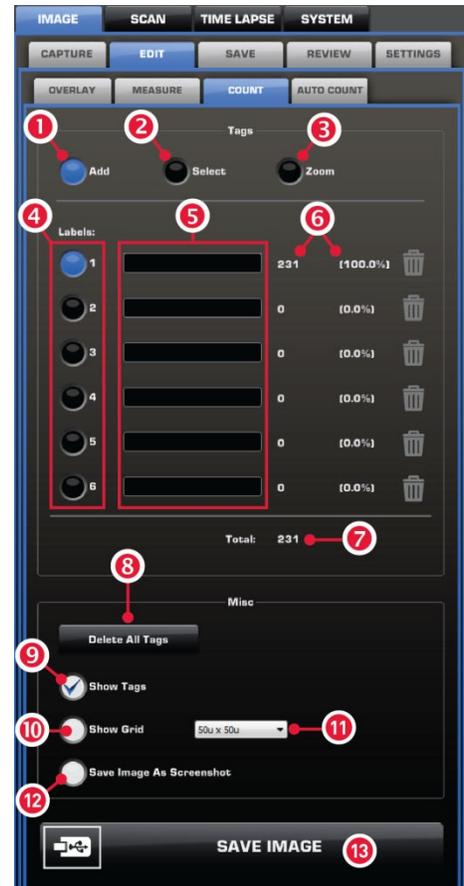
Grid size 11: Selects the grid size from a drop-down menu.

Save Image as Screenshot 12: Option to save the image as displayed in the Count tool, showing the labels, counts, and percentages. Deselecting this option produces an image saved with the tags only.

Save Image 13: Saves the image in the desired location.

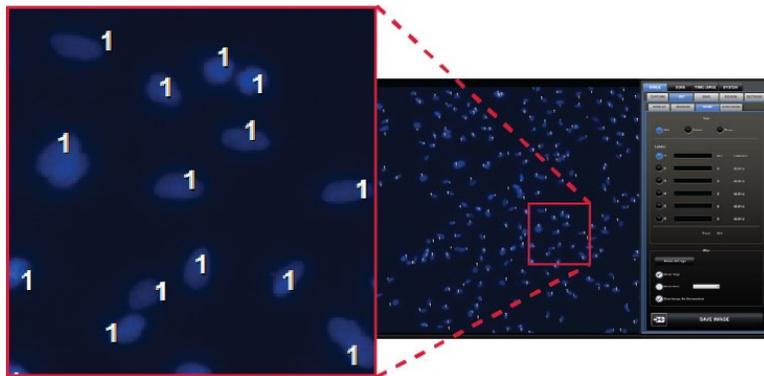


Note: You can document the results of your count by saving the tagged image with the **Save Image as Screenshot 12** option selected; this will save the image showing the labels, counts, and percentages as displayed in the Count tool.



Count procedure

1. Begin by acquiring an image in the **Capture** tab (see page 9) or by opening a saved image using the **Review** tab (see page 18).
2. Click **Show Grid** and choose **Grid size** from the drop-down menu. You may also choose to leave this option inactive.
3. Click the **text field** next to a label to enter a name for that label category. Repeat for the remaining labels, as necessary. You can use up to six labels per count.
4. To begin counting, click **Add** to activate the Add mode.
5. Select a **Label** button, and then left-click at each point onscreen to tag the items for that category. Each tagged item will show the label used, and a running tally of the count with the percentage for the label used and the total count will be displayed in the Count tool.



Note: You may switch labels as desired during a count; the EVOS[®] FL Auto software will tag each item for the label selected.

6. To use digital zoom while counting, click **Zoom** to select the Zoom mode, and then double-click the area onscreen you wish to enlarge. To resume counting, click **Add** to reselect the Add mode.
7. To move a tag, click **Select** to activate the Select mode, and then click and drag the tag to the desired location. Left-click anywhere on the screen to deselect it.
8. To delete a tag, right-click it. You may also click **Delete All Tags** to delete all tags for all labels.
9. To save the image with the tags, showing the labels, counts, and percentages as displayed in the Count tool, select **Save Image as Screenshot**, and then click **Save Image** to save the image in the desired location.



Note: Deselecting the **Save Image as Screenshot** option will produce an image saved with the tags only.

Auto Count

Auto Count tool

The Auto Count tool, located in the **Image ▶ Edit ▶ Auto Count** tab, is used for automatic counting of regions of interest (ROI) on an image.

Based on a histogram of the captured image and gated by signal intensity, the tool uses an algorithm to mark and count the regions of interest (ROI) on the screen (see the example below).

Auto Count controls

Count ①: Initiates the Auto Count procedure for the captured or live image on the screen.

Count From File ②: Opens a previously saved image to perform Auto Count.

Separate touching cells ③: Option to separately count the cells that are touching, which increases the accuracy of the count.

Aggressive boundaries ④: Option to more aggressively tighten the boundaries around regions, resulting in more ROI.

Count field ⑤: Displays the results of the count (i.e., the number of ROI on the screen above the signal threshold).

Brightness ⑥: Controls the sensitivity of the Auto Count tool by changing the threshold for brightness.

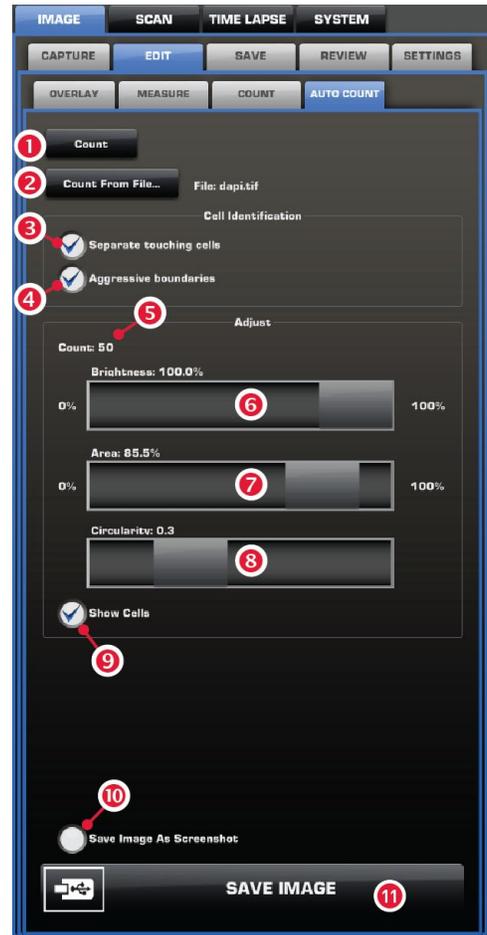
Area ⑦: Controls the sensitivity of the Auto Count tool by changing the threshold for area. Increasing the area threshold causes only larger cells to be counted, while decreasing it allows a wider range for the cell size.

Circularity ⑧: Controls the sensitivity of the Auto Count tool by changing the threshold for circularity.

Show cells ⑨: Displays the counted objects outlined in green to help you optimize the parameters used for object segmentation and counting.

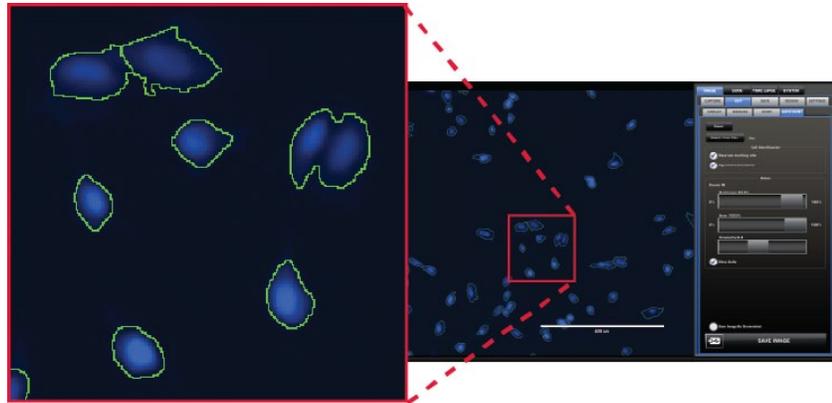
Save Image as Screenshot ⑩: Option to save the image as displayed in the Auto Count tool, showing the labels, counts, and percentages. Deselecting this option produces an image saved with the tags only.

Save Image ⑪: Saves the image in the desired location.

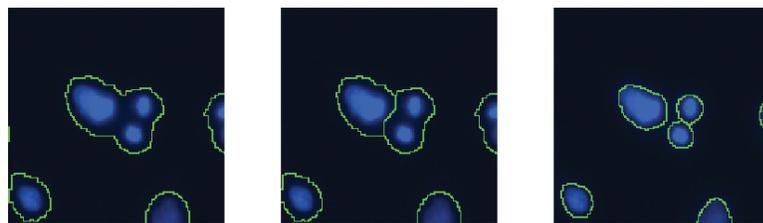


Auto Count procedure

1. Begin by acquiring an image using the **Capture** tab (see page 9).
2. Go to the **Auto Count** tab, and then click **Count** to initiate the Auto Count procedure.
Alternatively, click **Count From File** to perform the Auto Count procedure on a previously saved image.
3. Based on a histogram of the captured image, the EVOS[®] FL Auto software will use an algorithm to mark and count the regions of interest (ROI) on the screen (see the example below). The result of the count (i.e., the number of ROI on the screen above the signal threshold) is displayed in the **Count field**.
4. Select **Show cells** to visualize the boundaries used by the software to mark and count the ROI during the count.



5. Review the ROI marked on the screen and select **Separate touching cells** and **Aggressive boundaries** options, if desired. Separately counting the cells that are touching increases the accuracy of the count and aggressively tightening the boundaries around regions results in more ROI to be counted.



Normal boundaries

Separate touching cells

Separate touching cells
Aggressive boundaries

6. Adjust the sensitivity of the count by moving the **Brightness** and **Area** sliders in the **100%** or **0%** direction. This changes the thresholds for brightness and area, respectively, to be designated as ROI and included in the count.



Note: Moving the slider in the **100%** direction increases the count sensitivity by lowering the threshold above which the ROI are counted and results in a greater number of ROI and a higher count; moving it in the **0%** direction increases the threshold value, thereby reducing sensitivity and lowering the count.

7. Adjust the threshold for circularity by moving the **Circularity** slider in the high or low direction.
8. To save the image with the tags, select **Save Image As Screenshot**, and then click **Save Image**.

Image Review

The **Image ▶ Review tab** utilizes a folder system for easily locating saved files and allows you to review still images or play video files from the USB drive or the network connection. You may also use this tab to rename or delete saved files.

Review tab controls

Folder address bar 1 : Describes the location of the current folder or the file.

Up folder 2 : Navigates up to a higher-level folder.

Create folder 3 : Creates a new folder in the current location.

Current folder 4 : Contains lower level folders and/or saved files.

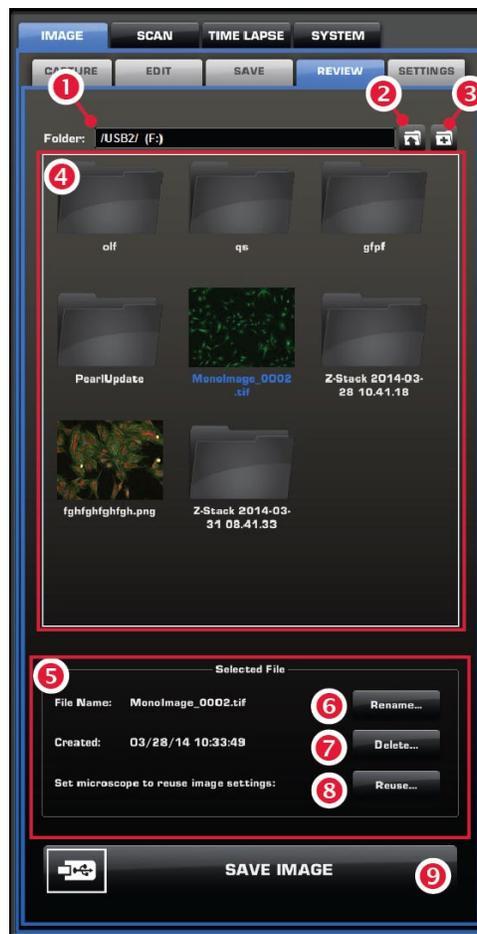
Selected file 5 : Displays the name and date of creation for the selected file.

Rename 6 : Allows you to rename the selected file.

Delete 7 : Deletes the selected file.

Reuse 8 : Recalls settings from image file and applies them to the microscope, including vessel selection, XY position, focus position, objective, light channel, and light slider settings.

Save Image 9 : Saves the image in the desired location. For more information, see page 10.



4. Advanced Operations

Image Settings

The **Image ▶ Settings** tab allows you to customize the user interface of the EVOS® FL Auto Imaging System for your specific needs. Select **Image ▶ Settings ▶ Mono** tab to customize the settings for the Monochrome camera or select **Image ▶ Settings ▶ Color** for the Color camera.

Image settings for monochrome camera

Image Capture Reprogrammable Buttons 1 : Assign up to four user-programmable image capture buttons (i.e., “Hot buttons”) to be displayed on the **Image ▶ Capture ▶ Mono** tab (see page 21).

Custom Channel Settings 2 : Applies a pseudo color to each channel to help differentiate the images in multi-channel overlays when using the Monochrome camera.

Highlight Saturated Pixels in Red 3 : Option to display pixels in saturated areas on a live image in red so that the LED intensity, gain, and exposure time can be adjusted individually in the **Actual mode** (page 9) or together by using the **Light intensity slider** (page 7).

Block Condenser in Fluorescence 4 : Moves the condenser to blocking position when fluorescence channel is selected. This can improve fluorescence captures when light box is not used over the sample, but it can significantly increase the time it takes to change channels and objectives. Not recommended for most users.

Underlying Channel Save 5 : Allows you to save the underlying channel in 8-bit color (256 colors) or 16-bit monochrome.

Capture All selection 6 : Assigns the channels captured with the Capture All button.

Quick Save settings 7 : Determines the Quick Save settings, such as base name, file type, save path, and the options for saving the underlying images and color.

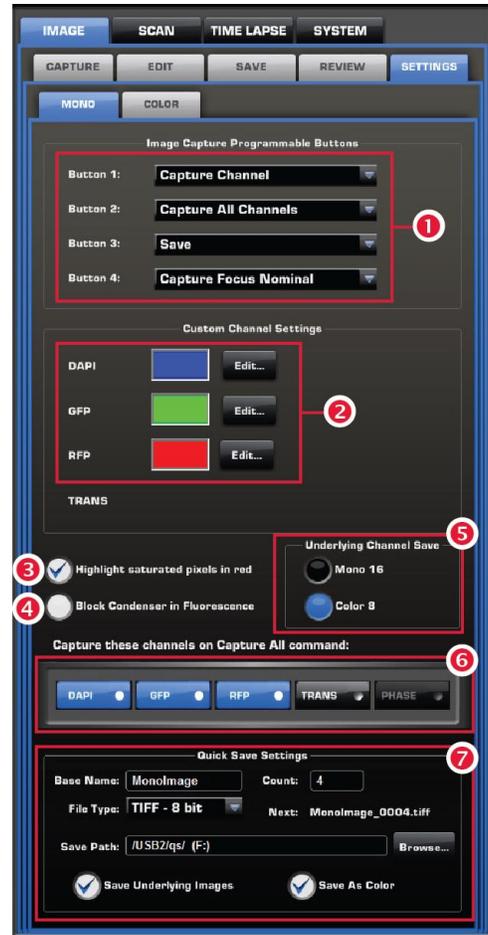


Image settings for color camera

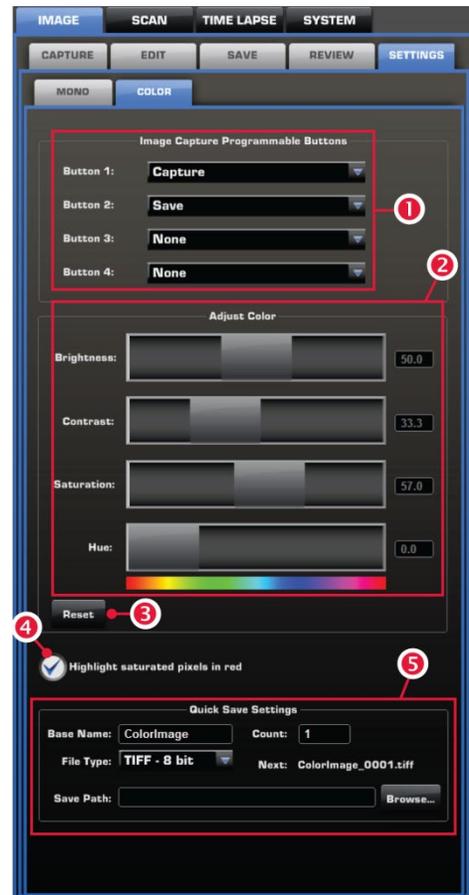
Image Capture Reprogrammable Buttons 1 : Assigns up to four user-programmable image capture buttons (i.e., “Hot buttons”) to be displayed on the **Image ▶ Capture ▶ Color** tab (see page 21).

Adjust color 2 : Contains slider controls that adjust color settings (Brightness, Contrast, Saturation, Hue) for all future captures using the Color camera.

Reset 3 : Resets the color settings to default values.

Highlight Saturated Pixels in Red 4 : Option to display pixels in saturated areas on a live image in red so that the LED intensity, gain, and exposure time can be adjusted individually in the **Actual mode** (page 9) or together by using the **Light intensity slider** (page 7).

Quick Save settings 5 : Determines the Quick Save settings, such as base name, file type, and save path.

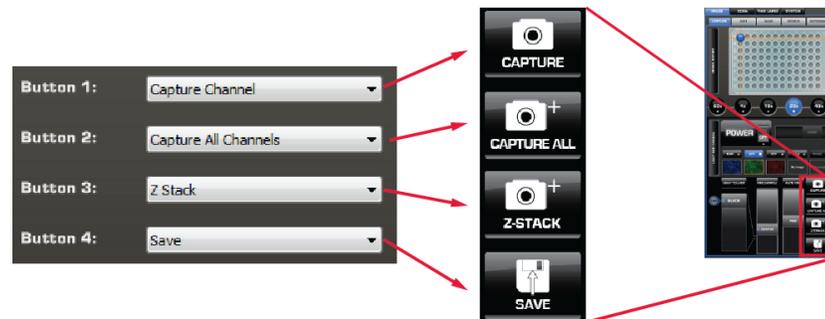


Assign Hot buttons

1. To assign up to four user-programmable image capture buttons (i.e., Hot buttons) to be displayed on the **Image ▶ Capture** tab, click the **Mono** or **Color** tab under **Image ▶ Settings ▶ Color** to select the camera for which you wish to apply the Hot buttons.
2. Select up to four Hot buttons from the drop-down menus under **Image Capture Reprogrammable Buttons** in the order you want them displayed (Buttons 1–4).

You can choose from the following options:

- **Capture:** Captures the current image on the screen.
- **Capture All:** Captures an image using each of the installed light cubes.
- **Transfection:** Creates a two-image overlay using one fluorescent image and one transmitted light image.
- **Save:** Saves the captured images.
- **Z-Stack:** Captures a series of images of the sample along the z-axis.
- **Capture Focus Nominal:** Sets the focus nominal for the current vessel. Focus nominal is the Z-point, around which the focal range of the Find Sample autofocus and the Quick slider is centered.
- **Quick Save:** Allows single-click saving of captured images. There are separate monochrome and color settings for Quick Save, which can be accessed via the Image Settings tab.



Select pseudo colors

When using the Monochrome camera in multi-channel overlays, applying a pseudo color to each channel helps differentiate the images. To assign a custom pseudo color to a channel, follow the procedure below.

1. In **Image ▶ Settings**, select the **Mono** tab for the Monochrome camera.
2. Under **Custom Channel Settings**, click **Edit** for the desired channel, then select the color you wish assign.
3. Repeat for the remaining channels.



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

Scan

Routines tab controls

The **Scan** tab gives you the option to create and recall “scan routines”, a series of steps to acquire a tiled and stitched image. For repeat experiments, Scan routines can be saved, recalled, even edited.

The **Scan ▶ Routines** tab is used for creating and running new scan routines, and running, editing, and deleting existing routines.

Create New Routine 1 : Initiates the Scan Wizard to guide you through a series of steps to create, save, and run a new scan routine based on your specifications.

Folder 2 : Describes the location of the current folder or routine.

Up folder 3 : Navigates up to a higher-level folder.

Create folder 4 : Creates a new folder in the current location.

Stored list 5 : Contains lower lever folders and/or saved routines.

Run 6 : Runs the selected routine.

Edit 7 : Allows you to change the parameters of an existing routine.

Delete 8 : Deletes the selected routine.



Create a scan routine

1. Go to **Scan ▶ Routines** tab, and then click **Create New Routine** to open the Scan Wizard.

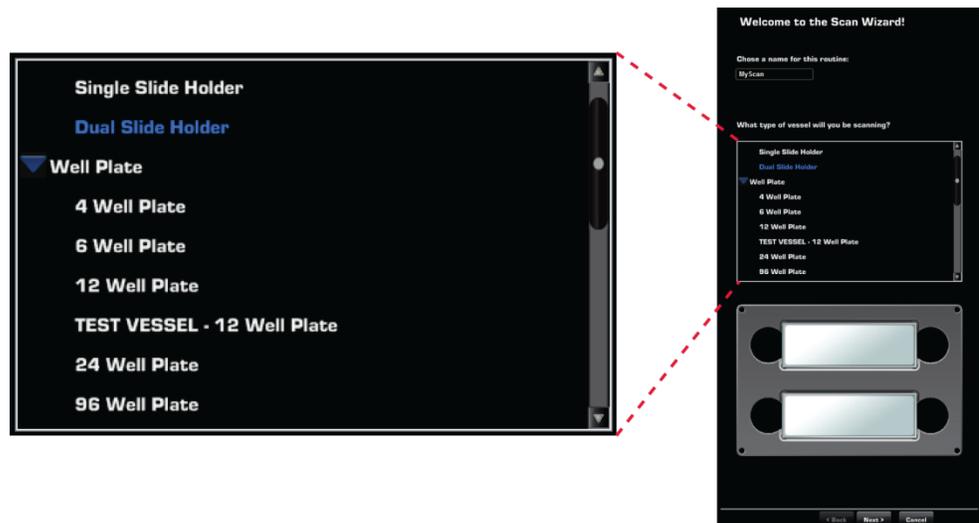
The Scan Wizard will guide you through the process of creating your own scan routine as you make a series of onscreen selections.

After making your selection at each step, click **Next** for the next prompt or click **Back** to revisit the previous step. Click **Cancel** to abort the process.

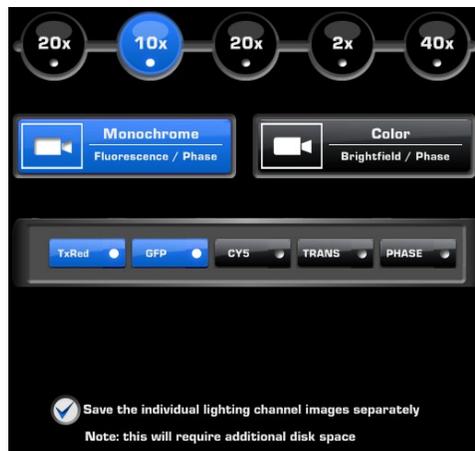


Note: To have the option of changing a step of a saved scan routine any time you recall and run the scan, select the **Show this page again** option at the bottom of the page you wish to revisit.

- When prompted, enter the routine name and select a **Vessel** from the list. Selected vessel type will be highlighted in blue and a virtual vessel will appear under the list (in the example below, a dual slide holder is selected).



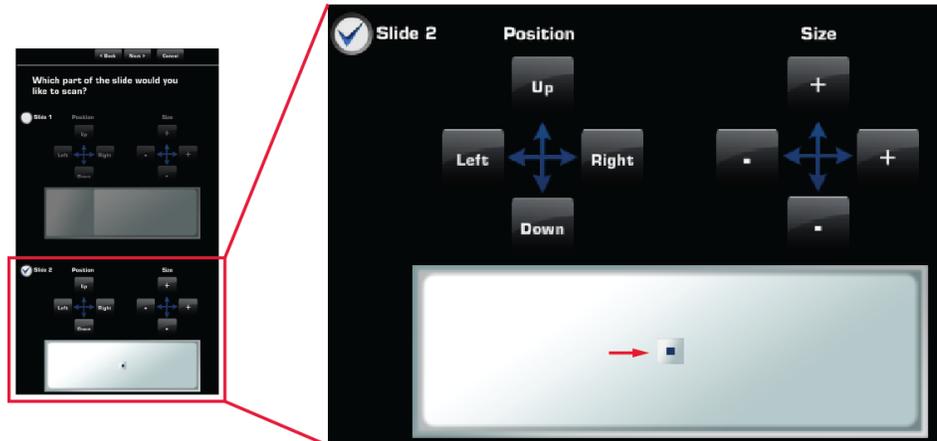
- Select the **objective**, **camera**, and the desired **light channels** to use in your scan routine.



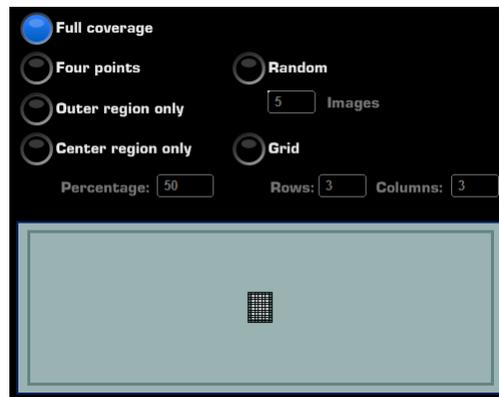
- If desired, select **Save the individual lighting channel images separately**. Note that saving images separately requires more disk space.
- When prompted, choose how to select the areas you would like to cover in your scan. You have two options:
 - Select area rectangle:** Move and size a “region of interest rectangle” on your virtual vessel. If using this method, continue with “Scan Routine Options: Select Area Rectangle”, page 24.
 - Place beacons on the live microscope image:** Mark the opposite corners of your area of interest on the live image to designate your area(s) of interest. If using this method, continue with “Scan Routine Options: Place Beacons on Live Image”, page 25.

Scan routine options: select area rectangle

1. When prompted, choose **Select area rectangle**.
2. Using the virtual vessel (vessel image representing the sample vessel), select the **wells** or **slides** to cover in your scan (in the example below, Slide 2 in a dual slide holder is selected).
3. Select the **area** of the vessel you wish to scan by moving and sizing the **area rectangle** (indicated by red arrow in the example) using the **Position** and **Size** controls.



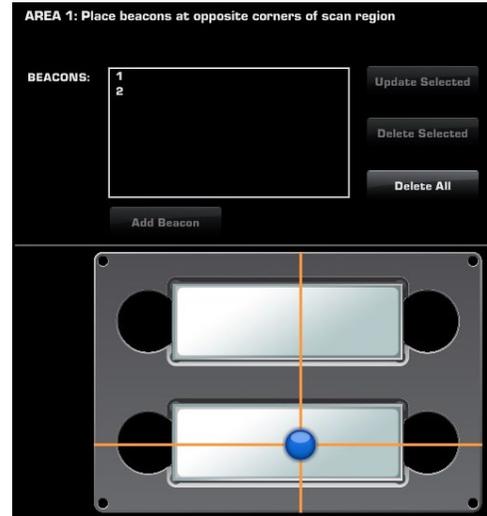
4. Determine how often you would like the system to refocus during a scan by selecting **Find Sample** and **Auto Fine** options for the scan.
 - **Find Sample:** searches over a wide range to provide coarse focus.
 - **Auto Fine:** searches over a narrow range to provide fine focus.
 - You may choose to run either, both, or neither of the two focus functions during a scan routine.
5. Select the amount of coverage. You may choose from the available coverage options (full coverage, four points, etc.), select to enter a number of random images, enter a percentage of the vessel to scan, or create a custom coverage grid. A larger area requires longer scan time and results in a bigger file size.



6. When prompted, adjust focus and lighting for each of the channels that you have selected.
7. Proceed to "Finish Creating a Scan Routine", page 26.

Scan routine options: place beacons on live image

1. When prompted, choose **Place beacon on the live microscope image** and enter the **number of regions** you would like to define.
2. Click **Add Beacon**, and using the crosshairs on the virtual vessel, pan to your area of interest (in example on the right, center of Slide 2).
3. Using the crosshairs on the **virtual vessel**, pan to your ROI and then click on the **live image** to select the location to add your beacon. The beacon will be placed at the location as determined by the crosshairs on the live image.
 - **Beacon 1** determines the top left starting position of the scan.
 - **Beacon 2** determines the bottom right of the scan.
4. To update an existing beacon, select it from the Beacons list, make your changes, and then click **Update Selected**.



To delete an existing beacon, select it, and then click **Delete Selected**.

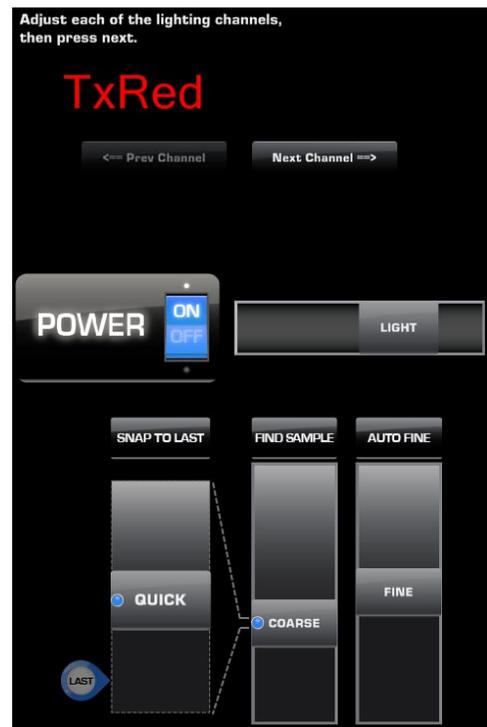
To delete all existing beacons, click **Delete All**.

Adjust focus and lighting

1. After placing your beacons, adjust the **focus** and **lighting** for each of the channels that you have selected.
2. Determine how you would like the system to refocus during a scan by selecting **Find Sample** and **Auto Fine** options for the scan.

You may choose to run either, both, or neither of the two focus functions during a scan routine.

- **Find Sample:** searches over a wide range to provide coarse focus.
 - **Auto Fine:** searches over a narrow range to provide fine focus.
3. Click **Next Channel** to proceed to the next selected light channel. Click **Prev Channel** to return to the previous light channel.
 4. After you have adjusted the **focus** and **lighting** for each of the channels, click **Next** to proceed to “Finish creating a scan routine”, page 26.

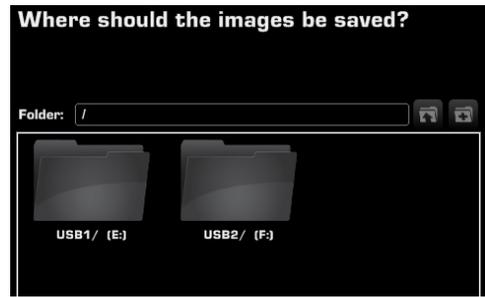


Finish creating a scan routine

1. When prompted, navigate to location in the network or the USB drive where you wish to save the images captured during the scan and select the desired folder.

To create a new save folder, click **Create folder** button.

2. If you wish to change the location where the images are saved each time you run the scan, select **Show this page again when I run the scan**.
3. Click **Next** to proceed to the next step.
4. Click **Finish** to save your routine in the selected location without running it. Alternatively, click **Start** to run your scan routine immediately.



Recall a saved scan routine

The **Stored** list in the **Scan ▶ Routines** tab allows you to recall a saved scan routine and run it with new samples.

1. On the **Scan ▶ Routines** tab (page 22), navigate to the folder containing the saved scan routine you wish to recall.
2. Click the routine of interest in **Stored** list to select it. Note that the icon for a saved scan routine is different from the folder icon depicting saved scans, which contain the images captured during a scan routine.
3. Click **Run** to run the selected routine.



Click **Edit** to make changes to the routine parameters.

Click **Delete** to delete the routine.



Note: Saved scan routines retain the information about the placement of the beacons as well as the camera and lighting options you have selected when creating the routine. You can change these routine parameters by clicking **Edit** after selecting the scan routine of interest from the **Stored** list.

Edit a scan

Use the **Scan ▶ Edit** tabs (**Overlay**, **Measure**, and **Count**) to edit your scans.

The controls for editing scans are identical to the controls used for editing single captured images in **Image ▶ Edit** tabs.

Refer to “Edit and Analyze Images” on page 11 for detailed information on using these tools.

Export a scan

Scans are saved as “.sti” files, which are very large. To make scans more user-friendly, you have the option of exporting the scan using a different image quality. You may also use the digital zoom to select a specific area of the image and then export that particular part of the screen.

1. Go to **Scan ▶ Edit** tab.
2. In Save Settings, select desired **Quality (Low, Medium, or High)**. The export tool will display the resolution and approximate size of the selected option.
3. Click **Export Scan** to save it in the quality selected.



| Quality: | Resolution: | Approximate Size: |
|---------------------------------------|-------------|-------------------|
| <input type="radio"/> Low | 364x273 | 0.30 MB |
| <input type="radio"/> Medium | 910x682 | 1.86 MB |
| <input checked="" type="radio"/> High | 3640x2730 | 29.81 MB |

Review a scan

The **Scan ▶ Review** tab utilizes the same folder system as the **Image ▶ Edit** tab (page 18) for easily locating saved files. It also allows you to review still images or play video files from the USB drive or the network connection. You may also use this tab to rename or delete saved files.

Time Lapse

The **Time Lapse** tab gives you the option of creating and running a time lapse routine. A time lapse routine automatically captures individual images of a region of interest (ROI) at given intervals over a time period based on your specifications. The images can then be stitched together into a video.

You will first specify the conditions for the entire “movie” (e.g., image capture options, ROI), and then you will build “scenes” that determine the overall length of the routine and environmental conditions along the way.

Routines tab controls

The **Time Lapse ▶ Routines** tab is used for creating and running new time lapse routines, and running, editing, and deleting existing routines.

Create New Routine 1 : Initiates the Scan Wizard to guide you through a series of steps to create, save, and run a new scan routine based on your specifications.

Folder 2 : Describes the location of the current folder or routine.

Up folder 3 : Navigates up to a higher-level folder.

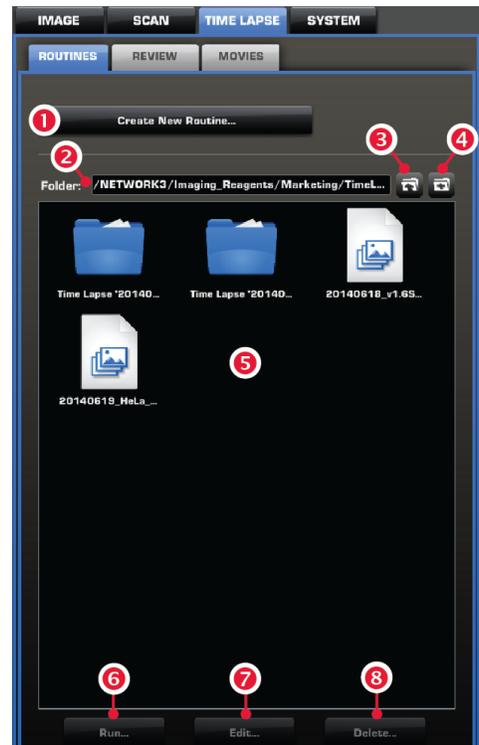
Create folder 4 : Creates a new folder in the current location.

Stored list 5 : Contains lower lever folders and/or saved routines.

Run 6 : Runs the selected routine.

Edit 7 : Allows you to change the parameters of an existing routine.

Delete 8 : Deletes the selected routine.

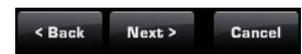


Create a time lapse routine

1. Go to **Time Lapse ▶ Routines** tab, and then click **Create New Routine** to open the Scan Wizard.

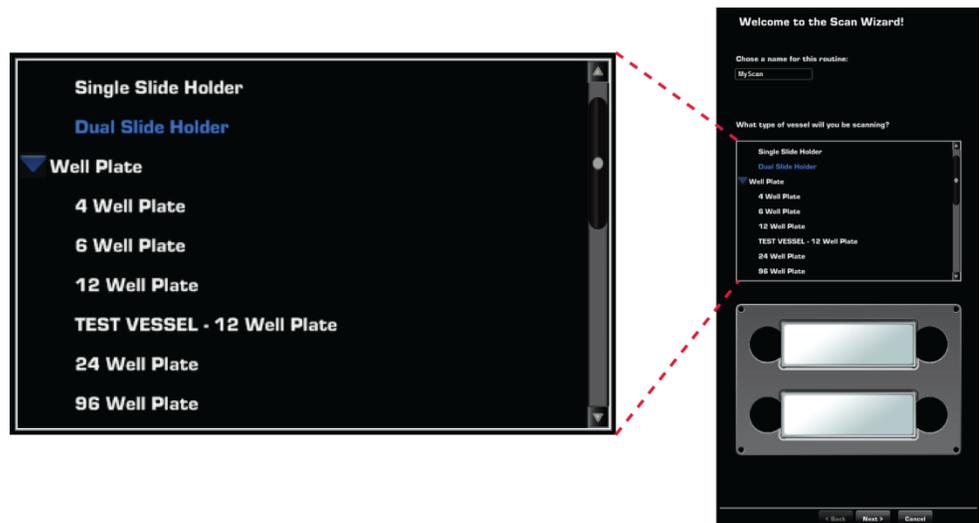
The Scan Wizard will guide you through the process of creating your own scan routine as you make a series of onscreen selections.

After making your selection at each step, click **Next** for the next prompt or click **Back** to revisit the previous step. Click **Cancel** to abort the process.



Note: To have the option of changing a step of a saved scan routine any time you recall and run the scan, select the **Show this page again** option at the bottom of the page you wish to revisit.

- When prompted, enter the routine name and select a Vessel from the list. Selected vessel type will be highlighted in blue and a virtual vessel will appear under the list (in the example below, a dual slide holder is selected).



- When prompted, enter the name of the routine and the number of scenes.
- Select the **objective, camera** (monochrome or color), and the **light channels** to use in your time lapse routine.
- If desired, select to use the **Auto find sample, Auto fine focus, Z-Stack, and Auto lighting** options. The Auto find sample and Auto fine focus options also allow you select the channels on which to run these options.
 - Auto find sample** searches over a wide range to provide coarse focus to your sample during the time lapse routine.
 - Auto fine focus** option provides fine focus over a narrow range.
 - Z-Stack** option captures a series of images along the z-axis.
- Click **Next** to proceed to setting beacons (page 30).



Set beacons

1. Click **Add Beacon** to manually place the beacons to determine your area of interest.

Unlike a scan routine, where you can add only two beacons, you can add up to 384 beacons in a time lapse routine.

2. Using the crosshairs on the **virtual vessel**, pan to your ROI and then click on the **live image** to select the location to add your beacon. The beacon will be placed at the location as determined by the crosshairs on the live image.
3. Alternatively, click **Populate Wells** to automatically place a beacon at the center of each well of the selected culture vessel.
4. To update an existing beacon, select it from the **Beacons** list, make your changes, and then click **Update Selected**.

To delete an existing beacon, select it, and then click **Delete Selected**.

To delete all existing beacons, click **Delete All**.

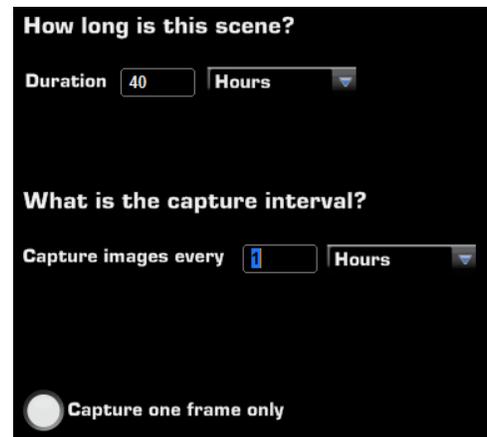
5. After placing your beacons, adjust the **focus** and **lighting** for each of the channels that you have selected.
6. Enter the **time lapse options** (e.g., option to create video for each beacon, whether to include watermarks, and whether to save individual channels).



Create scenes

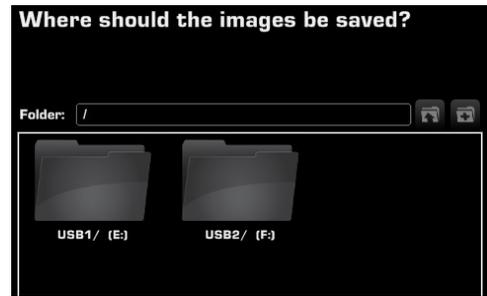
1. When prompted, enter **length of scene** and **capture interval**.
2. Select **Capture one frame only** to capture images on each beacon only once and then end the routine. This option is available only if you have specified a single scene.
3. Next, enter the **culture conditions** in the environmental chamber conditions (e.g., temperature, humidity, atmosphere).
4. If desired, add additional scenes and enter the desired conditions for each scene.

5. After setting up all the scenes, click **Next** to proceed to “Finish creating a time lapse routine”, page 31.



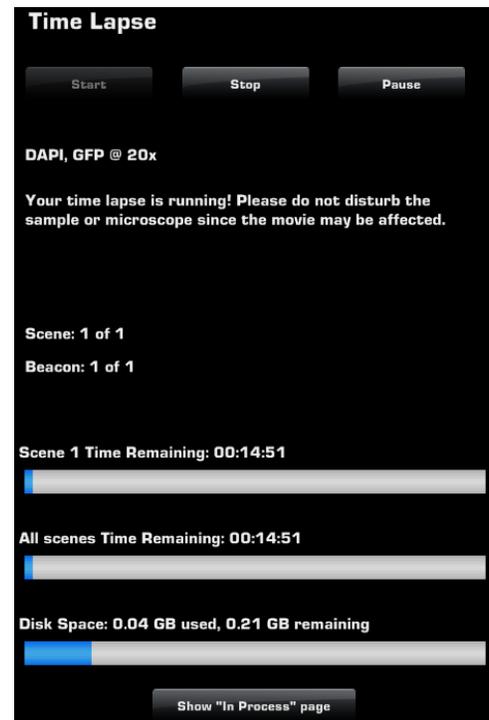
Finish creating the time lapse routine

1. When prompted, navigate to location in the network or the USB drive where you wish to save the images captured during the scan and select the desired folder.
To create a new save folder, click **Create folder** button.
2. If you wish to change the location where the images are saved each time you run the scan, select **Show this page again when I run the scan**.
3. Click **Next** to proceed to the next step.
4. Click **Finish** to save your routine in the selected location without running it. Alternatively, click **Start** to run your time lapse routine immediately.



Run the time lapse routine

1. To run the time lapse routine, click **Start**.
2. To adjust the parameters for the time lapse routine anytime during a run, click **Pause** and adjust the desired parameters (see page 32).
Click **OK** to resume the routine.
3. Click **Show "In Progress" page** to view the captured images and the progress of the time lapse routine.
4. Click **Stop** to abort the time lapse routine.



Recall a saved time lapse routine

The **Stored** list in the Routines tab allows you to recall a saved scan routine and run it with new samples.

1. On the **Time Lapse ▶ Routines** tab (page 28), navigate to the folder containing the saved scan routine you wish to recall.
2. Click the routine of interest in the **Stored** list to select it. Note that the icon for a saved time lapse routine is different from that of the time lapse folder, which contains the images captured during a time lapse routine.
3. Click **Run** to run the selected routine.
Click **Edit** to make changes to the routine parameters.
Click **Delete** to delete the routine.



Note: Saved time lapse routines retain the information about the placement of the beacons as well as the camera and lighting options you have selected when creating the routine. You can change these routine parameters by clicking **Edit** after selecting the scan routine of interest from the **Stored** list.

Edit a saved time lapse routine

1. To edit a saved time lapse routine, select the routine of interest from the **Stored** list and click **Edit**.
2. Edit the desired conditions for the routine, using the same procedures as described for creating a routine (page 28), setting beacons (page 30), and creating scenes (page 30).
You can change the image capture options, add or delete beacons, change scene options (e.g., length of a scene, the capture interval etc.), as well as modifying the culture conditions (e.g., temperature, humidity, atmosphere).
3. Click **Finish** to save your routine in the selected location without running it.
Alternatively, click **Start** to run the edited time lapse routine immediately.

Adjust routine parameters

You can adjust the parameters for a time lapse routine anytime during a run. This allows you to quickly fine-tune your time lapse routine in response to changing experimental or culture conditions. In contrast to editing a routine, which saves the changes made, adjustments made to the routine parameters during a time lapse run do not persist after the run is completed.

1. To adjust the parameters for a time lapse routine anytime during a run, click **Pause**.
2. Adjust the desired conditions for the routine, using the same procedures as described for setting beacons and creating scenes (page 30).
3. Click **OK** to resume the routine.

Review a time lapse

The **Time Lapse ▶ Review** tab utilizes the same folder system as the **Image ▶ Review** tab (page 18) for easily locating saved files. It also allows you to review still images or play video files from the USB drive or the network connection. You may also use this tab to rename or delete saved files.

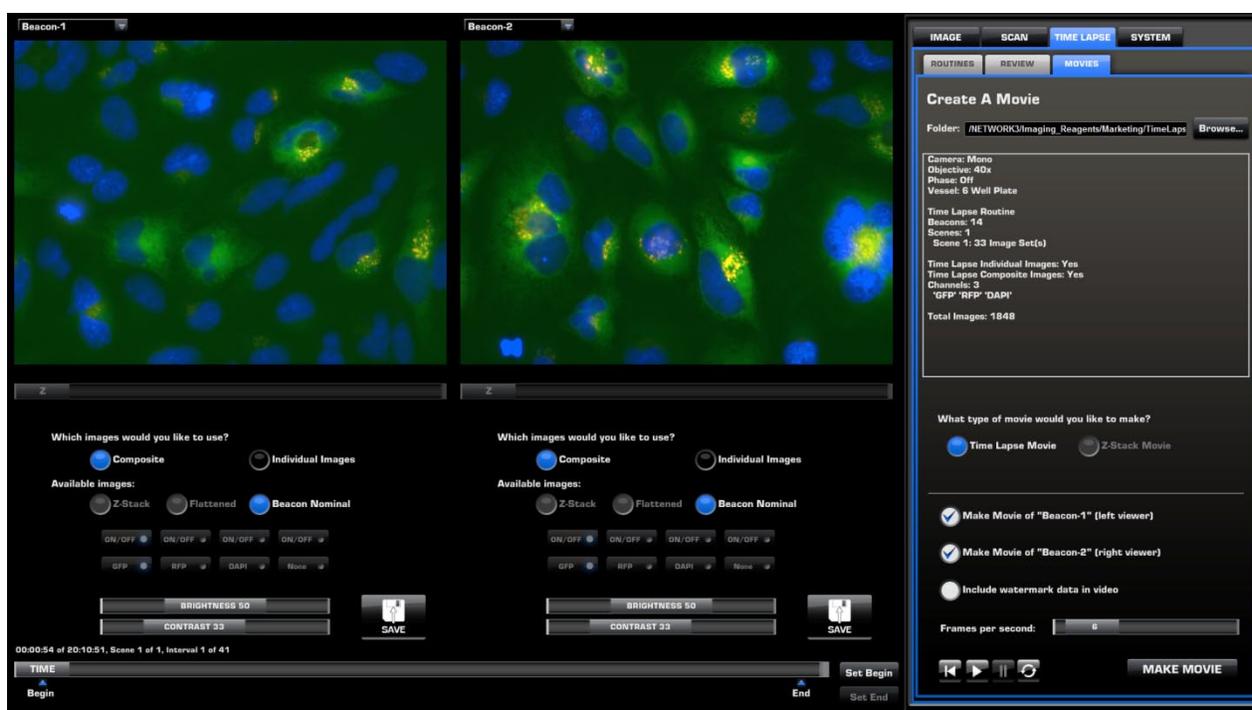
Movies

The **Time Lapse ▶ Movies** tab allows you to create a movie by stitching together previously captured images. There are two types of movies::

- **Time Lapse movie:** Uses images of region(s) of interest (ROI) captured over a time period. Using the images from the same source folder, you can create two separate movies following the same timeline but each displaying a different ROI (i.e., displaying the region around two different beacons).
- **Z-Stack movie:** Uses images of the sample captured along the Z-axis. This option allows you to create two separate movies showing the same ROI along the Z-axis captured in a different channel (e.g., GFP and Texas Red)

Overview of Movies tab

The controls available on the Movies tab depend on whether you choose to create a Time Lapse or a Z-Stack movie. Controls that do not apply to the selected movie type are grayed out. The example below shows the controls for creating a Time Lapse movie.



- The left side of the screen contains the controls for setting the beginning and end of the time line or the Z-Stack (for Time Line and Z-Stack movies, respectively), adjusting the lighting, and selecting the image type. The two separate viewing panes are used for viewing the source images for the movie(s) as well as the playback of the final assembled movie(s).
- The right side of the screen contains the tools for selecting the source image folder, the movie type, the frames per second, and movie playback. Options for creating a movie from the left viewing pane, right viewing pane, or from both viewers, and for adding watermark data to the movie are also located here.

Controls for Time Lapse Movies – right panel

Folder address bar 1: Describes the location of the current folder or the file.

Browse 2: Allows you to navigate to the source folder containing images captured in a time lapse or scan routine.

Movie data 3: Displays source image data for the selected folder (camera, objective, light channels, and vessel type), time lapse routine (number of beacons, scenes, and image sets), and movie conditions set by the user.

Movie type 4: Option to select Time Lapse or Z-Stack movies.

Depending on the selection made here, appropriate controls in the Movies tab become active or grayed out.

Viewer selection 5: Option to make a movie from images in the left viewing pane, the right viewing pane, or both.

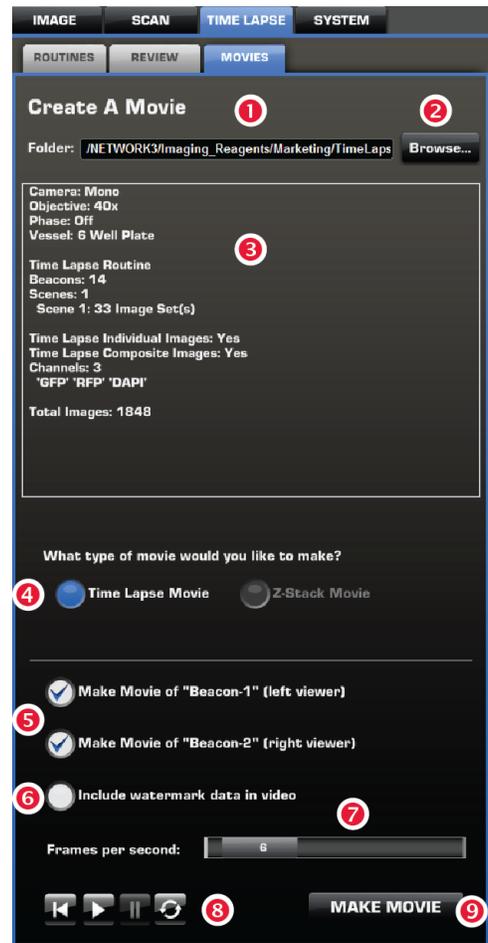
For the **Time Lapse Movie** option, each viewing pane covers the region of interest (ROI) as determined by the beacon selected from the appropriate Beacon dropdown.

Watermark 6: Option to include watermark data in the final movie.

Frames per second 7: Controls how many images are shown in one second of video. Smaller numbers result in longer movies.

Playback controls 8: Controls (from left to right) to **Rewind**, **Pause**, **Play**, and play in a **Loop** the assembled movie.

Make Movie 9: Assembles the selected images into the final movie.



Controls for Time Lapse Movies – left panel

Beacon dropdown menus **1** : Allows you to select the region of interest (ROI) to include in your assembled Time Lapse movie. ROIs are determined by the specific beacons set during the selected time lapse routine (see page 30). Beacon selection controls are not available for Z-Stack movies.

Viewing panes **2** : Display the captured images that you can assemble into the final movie.

Image selection **3** : Options to display **Composite** or **Individual Images**. Composite images display images from all light channels superimposed.

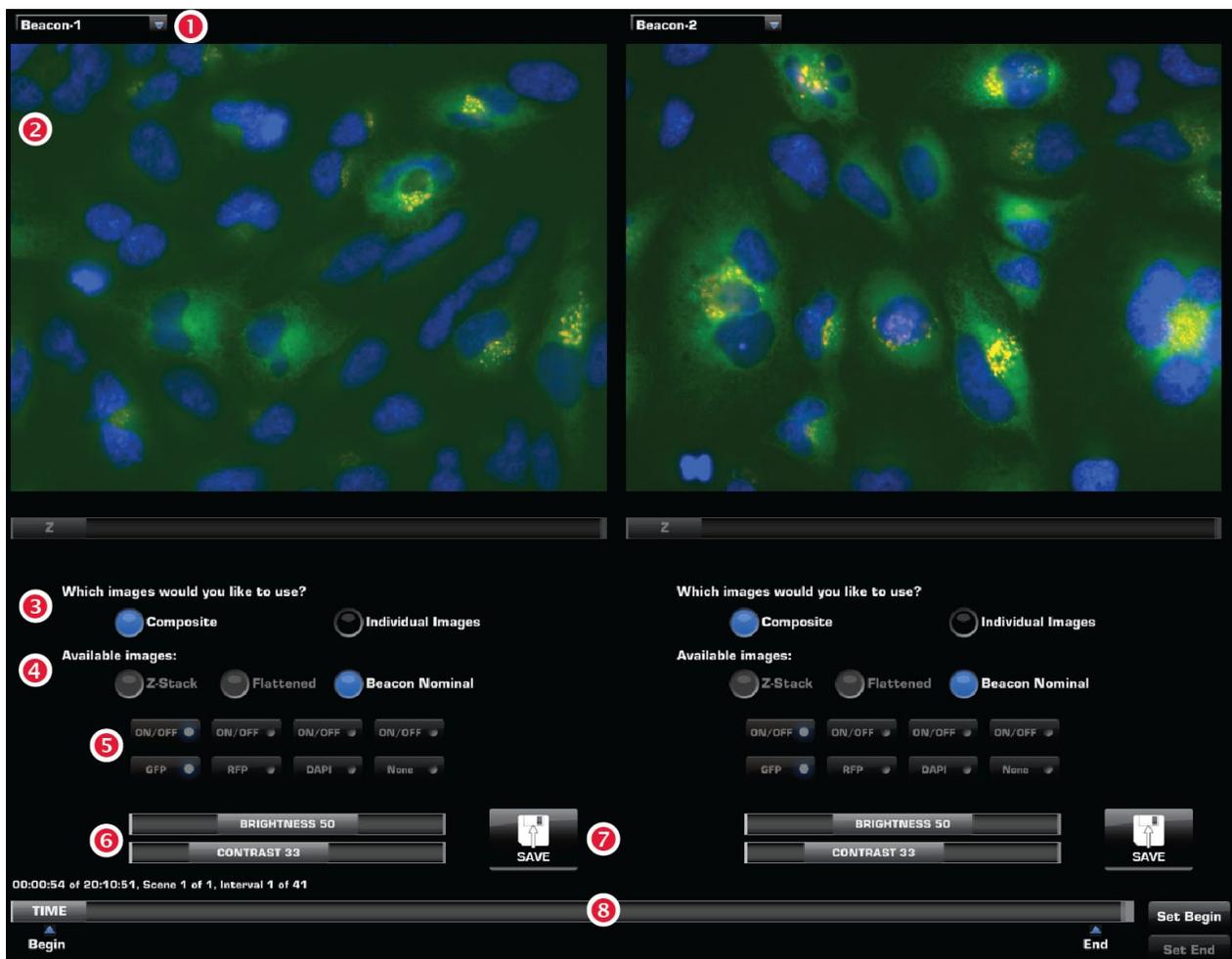
Available images **4** : Options to select the image type available for the movie. Only the appropriate options for your movie will be selectable; all other options will be grayed out.

Light channel buttons **5** : Allow you to select individual light channels for display. The selected channel is indicated by the blue color of the corresponding button. These buttons are not available when **Composite** image option is selected.

Brightness and Contrast sliders **6** : Adjust the brightness and contrast of the image in the selected channel.

Save **7** : Saves the image displayed in the viewer.

Timeline controls **8** : Allow you to scan through the captured images in the selected source folder and define the beginning and the end of your movie.



Create a Time Lapse Movie

1. Go to **Time Lapse ▶ Movies** tab and click **Browse**.
2. Navigate to the desired **Time Lapse folder** and click to select it. Do not double-click to open the folder.
3. For the movie type, select **Time Lapse Movie**.
4. Using the **Beacon** dropdown menus, select the **beacon** that represent the region of interest (ROI) you wish to include in the movie.
For each viewing pane, you can choose one beacon that was set for the selected time lapse routine.
5. Select **Composite** or **Individual Images**.
Composite option displays images from all light channels superimposed, while the Individual Images option allows you select the light channels you wish to display.
6. Select the desired image type from **Available images**. Only the appropriate options for your movie will be selectable; all other options will be grayed out.
7. If available, select the desired **light channels**. The available options depend on the selected time lapse routine and whether you have selected to display **Individual Images**.
8. Adjust the **Brightness** and **Contrast**.
9. Using the **timeline slider**, review the captured images in the selected folder.
10. Drag the little triangle labeled **Begin** beneath the timeline to the desired start point for your movie and click **Set Begin**.
11. Drag the little triangle labeled **End** to the desired end point for your movie and click **Set End**.
12. Select the left, right, or both **viewing panes** to include in your movie.
13. If desired, select **Include watermark data in video**.
14. Using the **Frames per second** slider, set the speed of the final movie. Choosing fewer frames per second will display each image for longer, resulting in a longer overall movie.
15. Click **Make Movie**. The EVOS® FL Auto software automatically assembles the selected images into the final movie using the conditions you have set.
16. After the movie is created, you can review it using the **playback controls**.
You can also save the assembled movie and play it using a third-party media player.

Controls for Z-Stack Movies – right panel

Folder address bar 1: Describes the location of the current folder or the file.

Browse 2: Allows you to navigate to the source folder containing images captured in a time lapse or scan routine.

Movie data 3: Displays source image data for the selected folder (camera, objective, light channels, and vessel type), Z-Stack routine, and movie conditions set by the user.

Movie type 4: Option to select Time Lapse or Z-Stack movies. Depending on the selection made here, appropriate controls in the Movies tab become active or grayed out.

Viewer selection 5: Option to make a movie from images in the left viewing pane, the right viewing pane, or both.

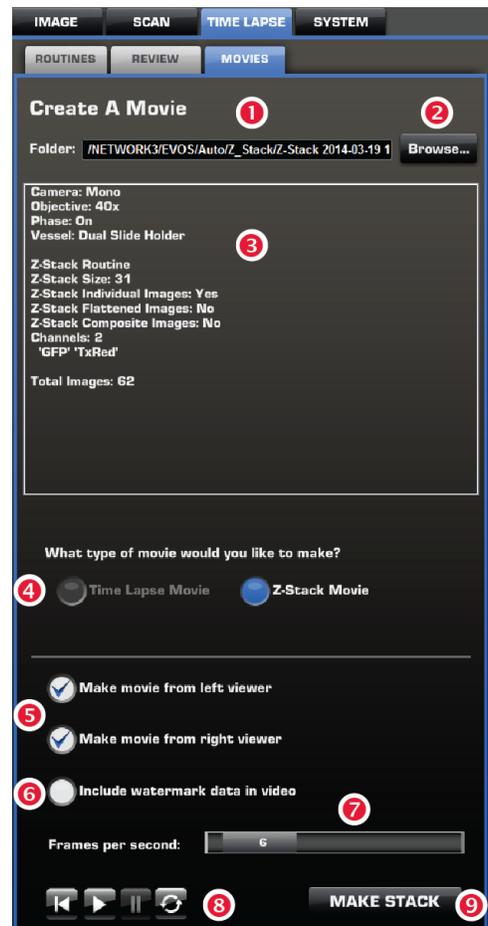
For the **Z-Stack Movie** option, each viewing pane covers the same region of interest (ROI), but imaged using a different light channel.

Watermark 6: Option to include watermark data in the final movie.

Frames per second 7: Controls how many images are shown in one second of video. Smaller numbers result in longer movies.

Playback controls 8: Controls (from left to right) to **Rewind**, **Pause**, **Play**, and **Loop** the assembled movie.

Make Stack 9: Assembles the selected images into the final movie.



Controls for Z-Stack Movies – left panel

Beacon dropdown menus ① : Allows you to select the region of interest (ROI) to include in your assembled Time Lapse movie. ROIs are determined by the specific beacons set during the selected time lapse routine (see page 30). Beacon selection controls are not available for Z-Stack movies.

Viewing panes ② : Display the captured images that you can assemble into the final movie.

Z-Slice controls ③ : Allow you to scan through the captured images in the selected source folder and define the beginning and the end of your movie.

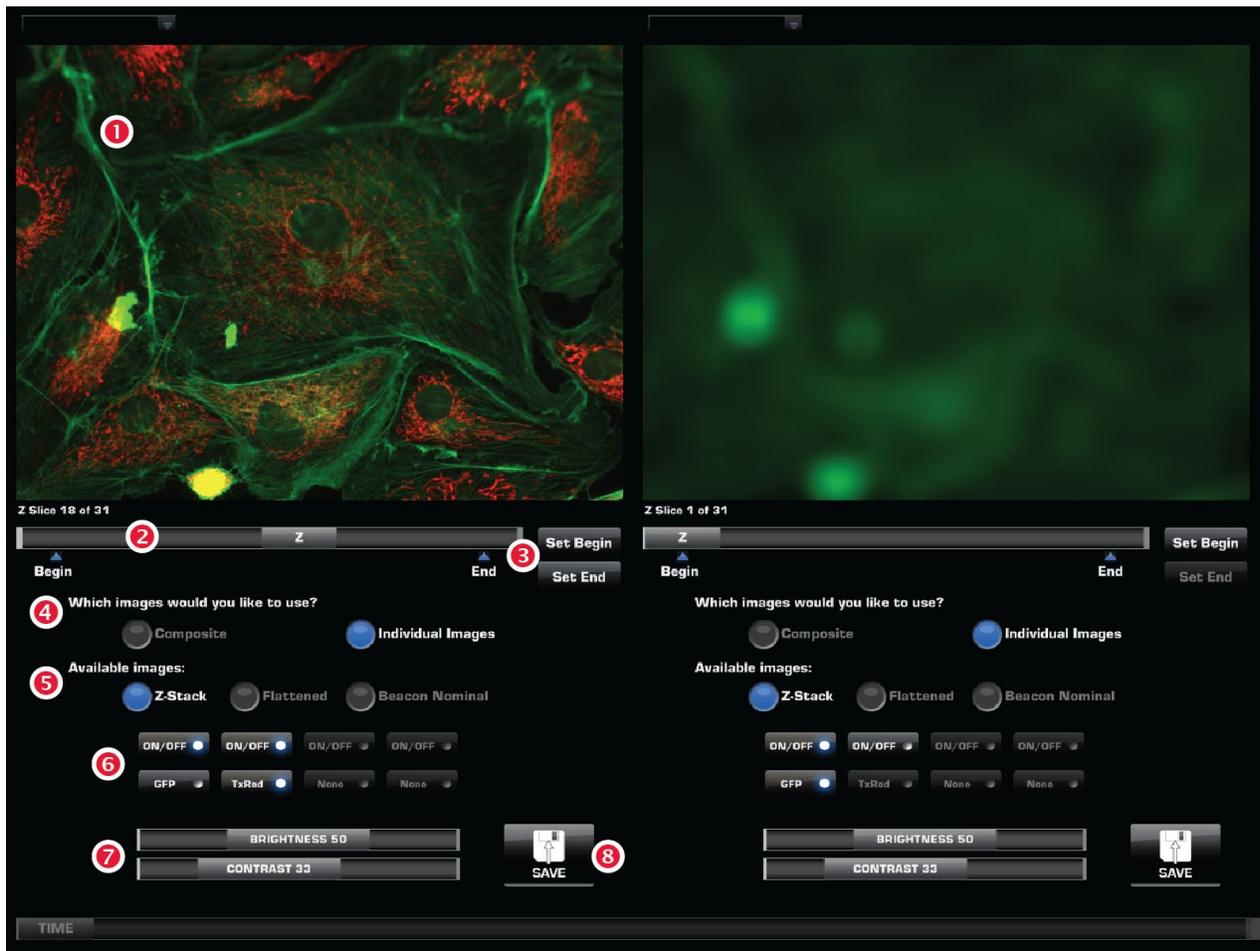
Image selection ④ : Option to display **Composite** or **Individual Images**. Composite images display images from all light channels superimposed.

Available images ⑤ : Option to select **Flattened** or **Beacon Nominal** images for display.

Light channel buttons ⑥ : Allow you to select individual light channels for display. The selected channel is indicated by the blue color of the corresponding button. These buttons are not available when **Composite** image option is selected.

Brightness and Contrast sliders ⑦ : Adjust the brightness and contrast of the image in the selected channel.

Save ⑧ : Saves the image displayed in the viewer.



Create a Z-Stack Movie

1. Go to **Time Lapse ▶ Movies** tab and click **Browse**.
2. Navigate to the desired **Z-Stack folder** and click to select it. Do not double-click to open the folder.
3. For the movie type, select **Z-Stack Movie**.
4. **Individual Images** and **Z-Stack** options will be automatically selected.
5. Select the desired **light channels** for each viewing pane. The available options depend on the light channels used when the selected Z-stack routine was run.
6. Adjust the **Brightness** and **Contrast** for each of the selected light channels.
7. Using the appropriate **Z-Slice slider**, review the captured images in each viewing pane.
8. Drag the little triangle labeled **Begin** beneath each Z-Slice slider to the desired start point for your movie and click **Set Begin**.
9. Drag the little triangle labeled **End** to the desired end point for your movie and click **Set End**.
10. Select the left, right, or both **viewing panes** to include in your movie.
11. If desired, select **Include watermark data in video**.
12. Using the **Frames per second** slider, set the speed of the final movie. Choosing fewer frames per second will display each image for longer, resulting in a longer overall movie.
13. Click **Make Stack**. The EVOS[®] FL Auto software automatically assembles the selected images into the final movie using the conditions you have set.
14. After the movie is created, you can review it using the **playback controls**.
You can also save the assembled movie and play it using a third-party media player.

5. System

Basic Tab

Basic tab controls

The **System** ▶ **Basic** tab allows you to select basic system functions.

Live Expand/Pinch Gesture ① can be selected based on user preference.

Show Scale bar ② option displays the scale bar on captured images.

Show Getting Started Button ③ displays the shortcut button to the Home screen on the user interface.

Time Lapse: retract objective ④ retracts the objective between image captures.

Reset Scale Bar ⑤ returns the scale bar to its original location on the screen.

Capture Focus Nominal ⑥ is used to calibrate the Z-range (i.e., depth) of vessels and to fine tune the focal plane used by system (see page 41).

Calibrate Vessel Alignment ⑦ opens the Vessel Alignment Tool used for adjusting the alignment positions for the current vessel (see page 50).

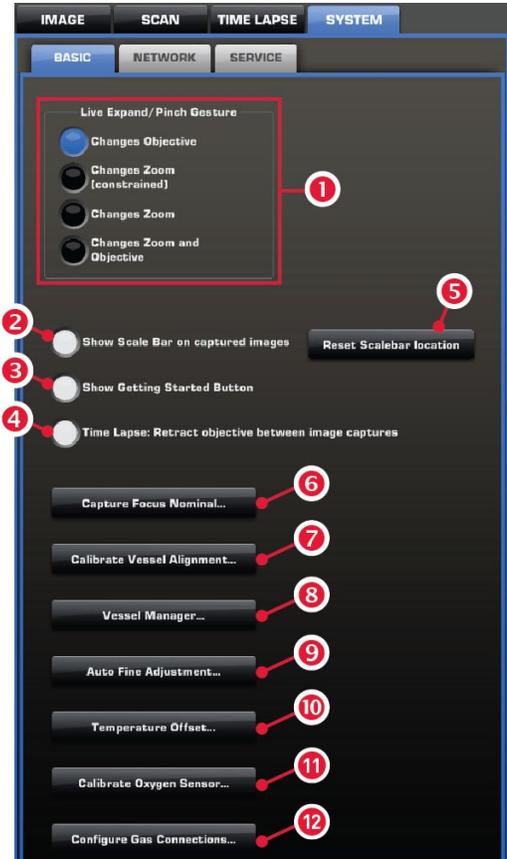
Vessel Manager ⑧ allows you to add new or custom vessels to the Vessel Expert tool (see page 41).

Auto Fine Adjustment ⑨ allows you to change the range of the Auto Fine focus function (see page 41).

Temperature Offset ⑩ allows users to set temperature offsets for the EVOS® Onstage Incubator.

Calibrate Oxygen Sensor ⑪ is used for calibrating the oxygen sensor for the proper operation of the EVOS® Onstage Incubator (see page 70).

Configure Gas Connections ⑫ is used for configuring the gas connection ports on the control unit of the optional EVOS® Onstage Incubator (see page 69).



Note: The Calibrate Oxygen Sensor and Configure Gas Connections buttons are only available when the optional EVOS® Onstage Incubator is installed with the EVOS® FL Auto Imaging System. See page 60 for more information on the optional EVOS® Onstage Incubator.

Capture Focus Nominal

Capture focus nominal is used to calibrate the Z-range (i.e., depth) of vessels that are not yet pre-configured within the auto-user interface, or to fine tune the focal plane used by system for Find Sample and Auto Fine focus functions. To use this feature, follow the steps below:

1. Navigate to **Image Capture** tab, adjust the focusing sliders to get the sample in focus, and capture the image.
2. Go to the **System ▶ Basic** tab and click **Capture Focus Nominal**.

Vessel Manager

Vessel Manager wizard allows you to add new or custom vessels to the Vessel Expert tool (see page 41). To add a new vessel, follow the steps below:

1. Navigate to the **System ▶ Basic** tab and click **Vessel Manager** to open the Vessel Manager wizard.
2. Select **Save the current vessel as** checkbox.
3. In the **New Item** field, enter a unique item name that specifies the vendor, style, or part number of the vessel. For example, Costar T-3406.
4. In the **New Display Name** field, enter the name with which you want to identify the new vessel in the Vessel Expert list (page 8). For example, 96-well – Costar T-3406.
5. If desired, add any pertinent notes about the vessel in the **Notes** field.
6. Click **Finish** to add the vessel. The new vessel will be available in the Vessel Expert list and added to the **User Created Vessel Files** list in the Vessel Manager.
7. To remove a vessel from the list, select it in the **User Created Vessel Files** list and click **Remove**.

The screenshot shows the 'Vessel Manager' dialog box. It has a title bar 'Vessel Manager'. Below the title bar, there are fields for 'Current Vessel' with sub-fields: 'Category: Other', 'Type: Viewer', 'Item: Generic', and 'Display Name: Viewer'. A checked checkbox 'Save the current vessel as:' is followed by a 'New Item:' text box containing 'Custom'. Below this is a descriptive text: 'The item name specifies the vendor, style or part number of the vessel. It must be unique within the category and type. I.e., "Costar T-3406"'. Another 'New Display Name:' text box contains 'Viewer - Custom', with a descriptive text: 'The display name is how the vessel will show up in the Vessel Expert list. It should uniquely identify the vessel. I.e., "96 Well - Costar T-3406"'. There is a 'Notes:' text box with the instruction: 'Any pertinent notes about this vessel; who added it, why, etc.'. At the bottom, there is a 'User Created Vessel Files:' section with a large empty box and a 'Delete' button. At the very bottom are '< Back', 'Finish', and 'Cancel' buttons.

Auto Fine Adjustment

Auto Fine function searches over a narrow focal range to provide fine focus to the region of interest. To change the range of the Auto Fine operation, follow the steps below:

1. On the **Image ▶ Capture** tab, select the camera and the objective for which you wish to change the range of the Auto Fine operation.
2. Navigate to the **System ▶ Basic** tab and click **Auto Fine Adjustment**.
3. On the Auto Fine Adjustment dialog, change the focal range of the operation using the **Auto Fine range slider**, and click **OK**.

The screenshot shows the 'Auto Fine Adjustment' dialog box. It has a title bar 'Auto Fine Adjustment' with a close button 'X'. The main text says 'Change the size of the range for the Auto Fine operation'. Below this is a slider control with a value of '100% of default'. Underneath the slider, it displays 'Auto Fine range: 26.84 microns', 'Camera: Monochrome', and 'Objective: 10x'. At the bottom right are 'OK' and 'Cancel' buttons.

Network tab

Using the **System ▶ Network** tab, you can log the EVOS® FL Auto Imaging System onto a Windows/SMB network via an Ethernet cable connection and save captured images directly to shared folders on the network.



Note: SMB is the only supported protocol. No other protocols (such as HTTPS, FTP, or WebDAV) are currently supported. If you are connecting to a Linux server, it will have to use Samba for EVOS® FL Auto Imaging System to find it. Contact your network administrator for help if a physically connected instrument cannot find the SMB network.

Connect to network

The EVOS® FL Auto Imaging System recognizes the network automatically when it is connected to it via an Ethernet cable, the **DHCP** **1** option is selected, and **Connect** **3** is clicked.

If the network is not recognized automatically, do the following:

1. Enter your network domain, user name, and password in **Network Credentials** fields **4** and select a server to view the top level of shared folders on that server.

You may now navigate below the top level shared folders on the Network page.

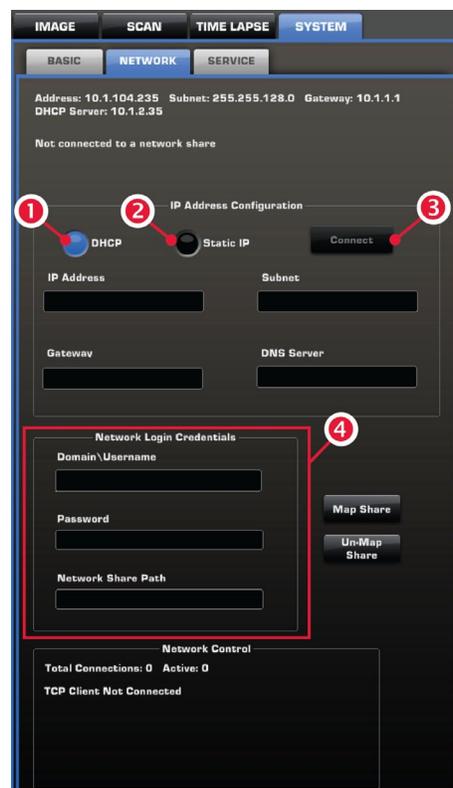
2. After the server accepts your login, the EVOS® system will display the list of available shared folders on the selected server.

Select a shared folder and click **Add** to include it in the list of possible file destinations. You may also type in the file path and click **Add**.

3. The folder should appear on the list box below the Add button. If it does not, contact your network administrator for help. If you need to remove a shared folder from the destinations list, select the folder name and click **Remove**.

Helpful hints

- The EVOS® FL Auto Imaging System will try to connect for about 30 seconds. If there is a problem with the connection, the Network page will display “Not connected to a network share.”
- Double-check the physical connections and click the Refresh Network button. During the refresh, a progress icon will appear.
- Unless there is an issue with the network, or you are using an incompatible adaptor, refreshing the connection should resolve the problem within a few moments. Contact your network administrator for help if the problem persists.
- If your configuration requires using a static IP address, select **Static IP Address** **2**, enter your IP Address, Subnet Mask, Gateway Address, and DNS Server Address, and then click **Connect** **3**.



Service Tab

Service tab contains information about the EVOS® FL Auto Imaging System as well as several user preferences and settings. Using the Service tab, you may view and/or change the following:

Service tab controls

This EVOS Auto 1 : Displays the Serial Number specific to each instrument and Software Revision, which is the current version of the software running on the system.

Update Software 2 : Use the button to upload new software versions from a USB flash drive (see page 46).

Import Vessels 3 : Allows you to add additional vessel types to the Vessel Selection Wizard.

Error Log 4 : Used for capturing error logs when prompted by the software. Plug the USB flash drive into the computer and select this button. Error log will be automatically downloaded to be sent to Life Technologies.

Microscope 5 : Use the drop down menus to select the correct objective for each objective turret position.

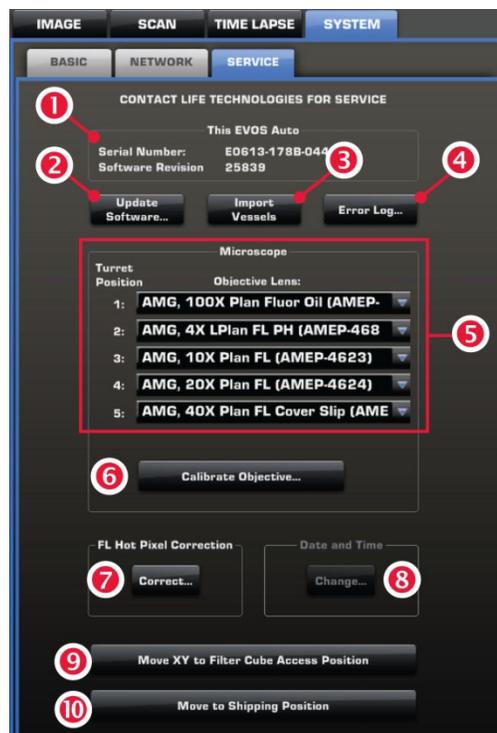
Calibrate Objective 6 : Allows the calibration of the Field of View, Parfocality, and Parcentration parameters of the selected objective (see Note below).

FL Hot Pixel Correction 7 : Allows you to correct hot pixels on the camera. This operation requires you to prevent stray light from entering the optical path by covering the stage containing the light box.

Date and Time 8 : Click to set the date and time.

Move XY to Filter Cube Access Position 9 : Clicking this button will move the stage to a position that will allow for easy installation of light cubes (see page 51).

Move to Shipping Position 10 : Follow prompts to properly install the shipping restrain for safe shipment of system (see page 52).



Note: 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. For more information and instructions on calibrating the EVOS® FL Auto Imaging System, see page 47.

6. 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 Sterilization 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 Life Technologies.
- 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 83) 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 Imaging System, be sure to lock the X-Y stage using the Shipping Restraint as shown on page 52 to prevent the stage from sliding.

Sterilization Procedures



IMPORTANT! The EVOS® FL Auto Imaging System should not be subjected to UV sterilization. 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 Imaging System, follow the sterilization 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 Imaging System (stage top, objective turret, housing, etc.) with paper towels or Kimwipes® tissues dampened with 70% ethanol or 4,000 ppm hydrogen peroxide (H₂O₂).



IMPORTANT! Do not allow sterilization 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 sterilization solution. NEVER spray liquid anywhere on the EVOS® FL Auto Imaging System. Always wipe surfaces with dampened paper towels instead.

Software Updates

Periodically, Life Technologies adds functionality and other improvements to the EVOS® user interface. We recommend keeping your EVOS® FL Auto Imaging System up to date with the latest EVOS® FL Auto software. If you have any questions about software updates, contact your local EVOS® distributor. If you do not have your distributor information, contact Technical Support (page 83).

Download software update

Software updates are available from the EVOS® FL Auto Imaging System product page at www.lifetechnologies.com/evosflauto.

1. Download the update directly to the top level of a USB flash drive with at least 100 MB available. Do not open or rename the file on your computer; EVOS® FL Auto Imaging System will verify and install it during the update process.
2. Download the current user guide for the EVOS® FL Auto Imaging System from our website. The updated user guide covers the new software functionality when features are added.
3. Alternatively, you can get the latest software and documentation updates from your local EVOS® distributor or by contacting Technical Support (page 83).

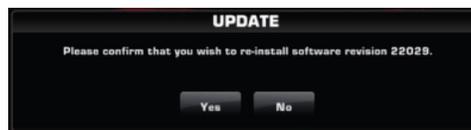
Install software update

1. Plug USB flash drive into computer.
2. Click **System tab** in EVOS® FL Auto software, and then select the **Service tab**.
3. Click **Update Software** **1** in the service tab. A verification progress bar should appear.



Note: If a missing update notification pops up, be sure the USB flash drive with the update file is fully plugged in. Click **OK** and then click the update button again.

4. After file verification, an update permission dialog will pop up. Check the revision details and click **Yes** to start the update.
5. A Windows® Setup dialog box will pop up. Click **Next** to proceed with the software update.
6. The screen will display the update progress. When the update is complete, the Windows® desktop will appear. Click the EVOS® FL Auto icon in Windows® to complete the update process and start the software.



IMPORTANT! Do not power off, unplug the USB flash drive, or add or remove any devices during the update.

Calibrate 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 Imaging System have been pre-calibrated. You do not need to calibrate them again unless they are reinstalled after removal from the instrument.

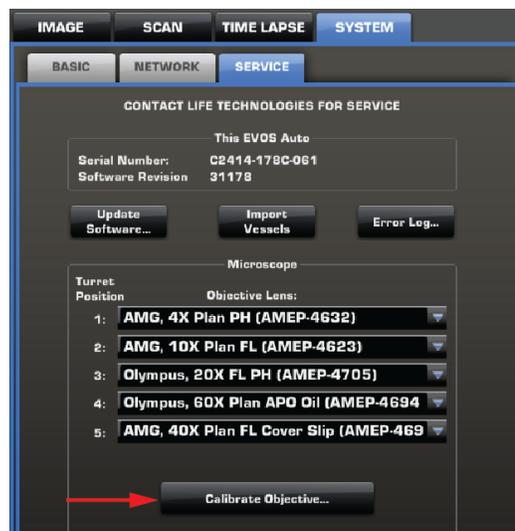
Calibration procedure

Calibration procedure involves two distinct steps and requires the EVOS[®] FL Auto calibration slide (supplied with the EVOS[®] FL Auto Imaging System) (also available separately; Cat. no. AMEP4720). First, the calibration crosshair on the screen is matched to the crosshairs 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, the objectives should be calibrated one after the other without removing the calibration slide.

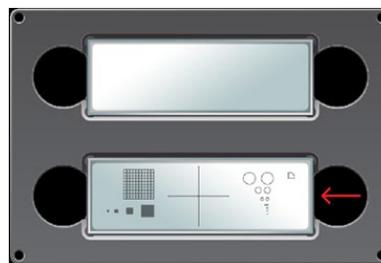
1. Go to **System ▶ Service** tab (page 43), and click **Calibrate Objective** to launch the Calibration Wizard.
2. Mount the EVOS[®] Calibration Slide in the vessel holder as shown on the screen (see Note below).



Note: When calibrating Long Working Distance (LWD) objectives, mount the calibration slide face up. When calibrating Coverslip Corrected (CS) objectives, mount the calibration slide face down.



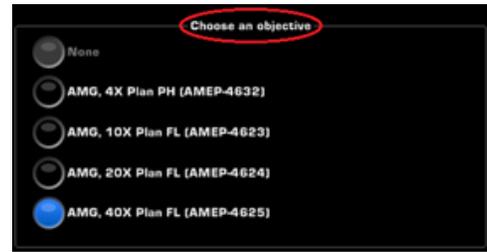
Slide mounted for LWD objective



Slide mounted for CS objective

3. Select an objective from the options available and click **Next**.

The stage automatically moves to the calibration target and the imaging system automatically finds the numbered crosshairs on the calibration slide.



Note: If calibrating a Coverslip Corrected (CS) objective, select the **Calibrate a cover slip corrected objective** checkbox before clicking **Next**.



4. Adjust the lighting using the **Light intensity slider**.

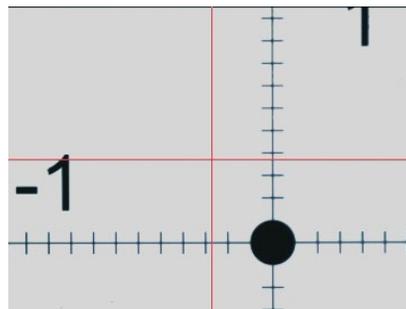
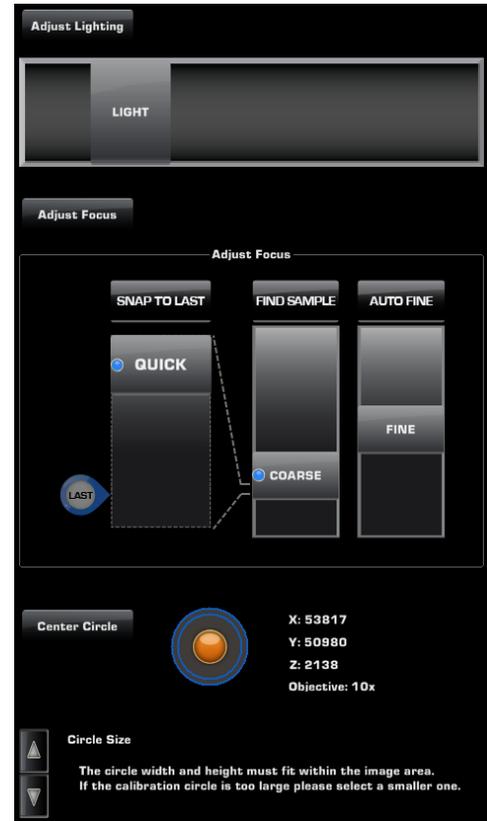
First, increase the intensity of transmitted until saturated (red) pixels appear in the view, and then reduce the illumination until the image is bright without saturated pixels.

Alternatively, click **Adjust Lighting** to perform this step automatically.

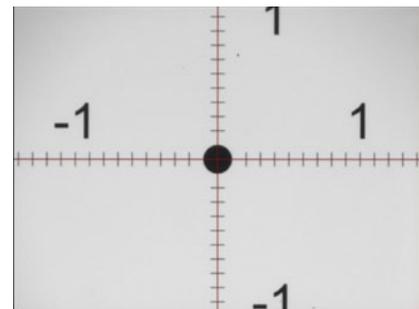
5. Adjust the focus using the **Focus controls** until optimum focal plane is achieved by eye.

Alternatively, click **Adjust Focus** to perform this step automatically.

6. Manually align the red crosshairs on the screen to the calibration target using the **Medium Jog** button.
7. When the red crosshairs are close to the calibration target, click **Center Crosshair** to center them on the calibration target.
8. If the crosshairs do not appear fully centered on the target, click **Center Crosshair** again to re-center (see image below).



Coarse alignment with Manual Jog



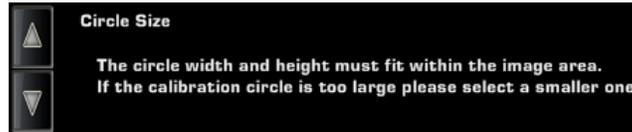
Fine alignment with Center Crosshair

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

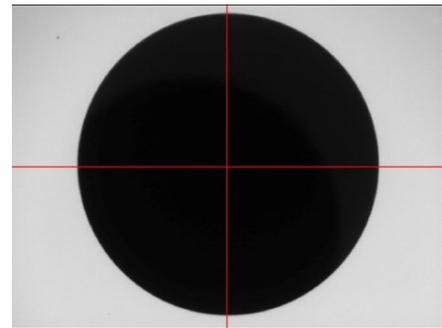
The stage automatically moves to the calibration circle and the imaging system automatically selects the appropriate reference circle on the slide, which depends on the specific objective you are calibrating.



Note: If the calibration circle does not fit within the field of view, use the selection arrows to move to a smaller circle. For best results, use the largest circle possible.



- Adjust the lighting and focus as described in steps 4 and 5 (page 48).
- Manually align the red crosshairs on the screen to the calibration circle on the slide using the **Medium Jog** button.
- When the red crosshairs are close to the calibration circle, click **Center Circle** to center them on the calibration circle.

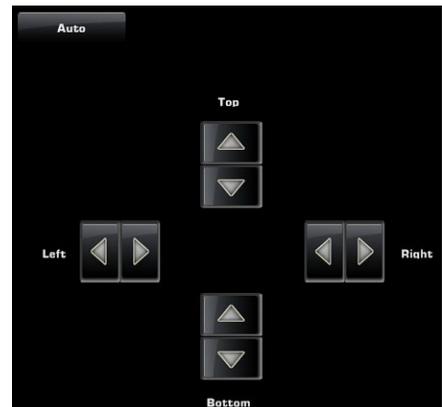


If the crosshairs do not appear fully centered on the target, click **Center Crosshair** again to re-center.

- Click **Next** to proceed to measuring the diameter of the reference circle.

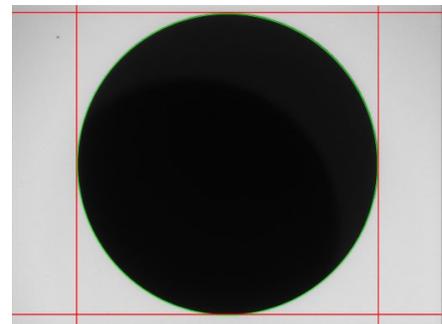
The calibration screen displays the calibration gridlines in red and the calibration circle outlined in green.

- Click **Auto** to align grid lines to the edge of the circle.
- Use the **arrow** buttons to move the lines until they are just touching the outside edges of the circle. Moving one grid line will not affect the position of others.



The line becomes yellow where the red gridlines and the green circle around the calibration target touch.

- Click **Next** to repeat the entire procedure (aligning crosshairs and measuring the diameter of reference circles) for the Color camera.



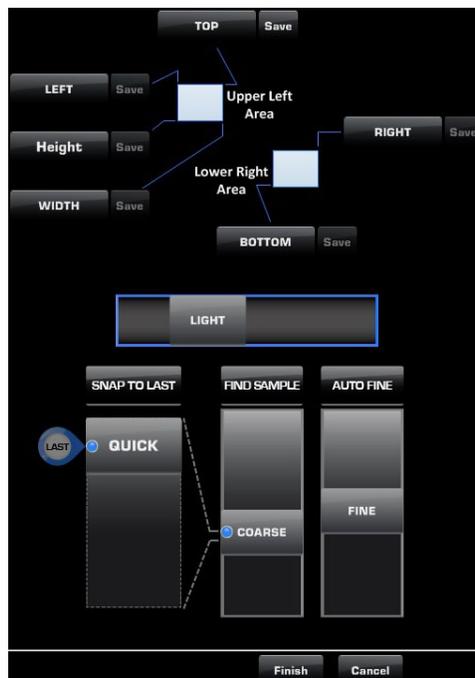
- After completing the calibration procedure for both cameras, click **Finish**.
The imaging system displays the "Calibration Complete" message.
- Click **OK** to repeat steps 3–16 for each additional objective.

Calibrate Vessel Alignment

Calibrate Vessel Alignment function allows the EVOS[®] FL Auto software to establish internal coordinates for the instrument to properly execute high-resolution mosaic tiling, multi-position well scanning, image stitching, and measurement functions.

Vessel alignment procedure

1. Go to **System ▶ Basic** tab (page 40), and then click **Calibrate Vessel Alignment** to open the Vessel Alignment Tool.
The Vessel Alignment Tool provides step-by-step instructions for adjusting the alignment positions of the current vessel.
 2. Adjust the lighting and focus on the vessel using the **Light slider** and **Focus controls**.
 3. Press the **TOP** button to move to the current top position.
 4. Adjust the view so that the top edge of the vessel exactly touches the horizontal crosshair on the screen.
 5. Click **Save** to lock in the new top position.
 6. Repeat the procedure for each remaining parameter (**LEFT**, **RIGHT**, and **BOTTOM**).
 7. Once you have calibrated the vessel alignment for each parameter, click **Finish**.



Note: Verify that you have the correct vessel selected before starting the vessel calibration process.

Change the EVOS® LED Light Cube

To customize your EVOS® FL Auto Imaging System, you can add and remove LED light cubes to fit the instrument's functionality to your own specific research needs. For instructions on how to change the Light Cubes, see below. For a complete list of available light cubes and to inquire about custom light cubes, go to www.lifetechnologies.com/evos or contact Technical Support (see page 83).

Procedure for LED light cube change

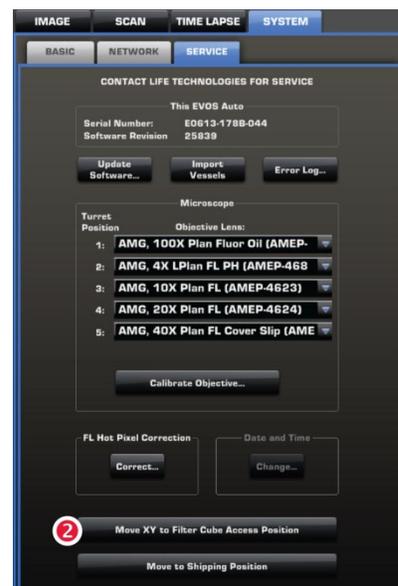
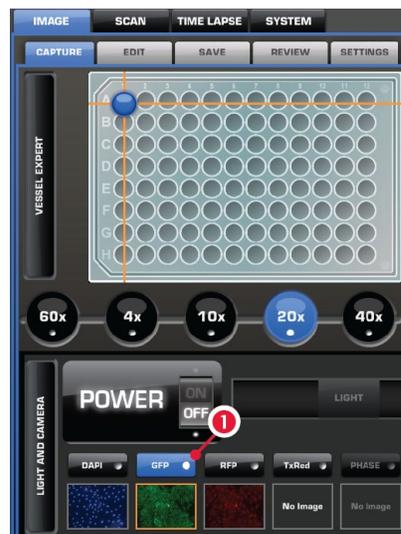
1. On the **Image Capture** tab, select the **channel 1** of the filter cube you want to remove/replace.
2. On the **System Service** tab, click **Move XY to Filter Cube Access Position 2**.
3. If no cube is installed, proceed to Step 7.
4. Use the light cube tool to loosen the two slotted screws as shown below.



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



6. Use the tool to tilt the light cube slightly toward you and lift out gently, and then remove tool from cube.
7. 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.
8. Use the light cube tool to gently tighten the two slotted screws so that the screw heads sit flush with the top of 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 Imaging System, you can add and remove objectives to fit the instruments functionality to your own specific research needs. Instructions on how to change the objectives are provided below.

Procedure for objective change

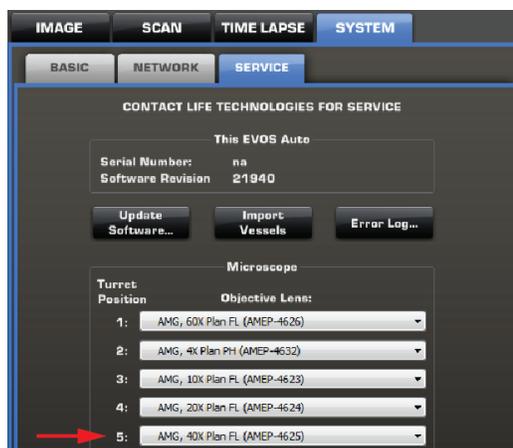
1. Remove the objective you wish to replace from the objective turret. You may need to move the stage so that the objectives are accessible. Note the indicated position (1–5) of the removed objective on the turret (see red arrow).



2. Screw the new objective into the open position in the objective turret. Note the part number of the objective and the turret position.



3. Navigate to the **System** ► **Service** tab, and find the turret position that matches the newly installed objective in the **Objective lens** section.
4. From the dropdown menu for that turret position, find and select the appropriate objective.



Install the Shipping Restraint

Use the Shipping Restraint 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 microscope turned on and the software running. Navigate to the **System ▶ Service** tab, and click **Move to Shipping Position**.
2. A dialog box will appear detailing Step 1. Follow the instructions and click **Move**.
3. Wait while the microscope moves into position (the mouse cursor will be busy during this operation). Once the stage comes to a halt, instructions for Step 2 will appear.
4. Align the pins with the holes on the mid plate of the stage and secure thumbscrew #1. Press **Move** to bring the top plate into position. Step 3 will appear.
5. Secure thumbscrew #2 and press **Move** to bring the bottom plate into position. Step 4 will appear.
6. Secure thumbscrew #3 and press **Finish** to advance to Step 5.
7. Verify that all thumbscrews are secure. Click **Done** and power down the instrument.



IMPORTANT! Do not power the instrument back on until the Shipping Restraint has been removed.

7. Troubleshooting



Note: For additional technical support, contact your local EVOS® distributor. If you do not have your distributor information, refer to www.lifetechnologies.com/evosfauto or contact Technical Support (page 83).

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 44) to clean objectives. |
| Uneven focus across screen | <ul style="list-style-type: none">• Position specimen, so that it lies flat on the stage; ensure that the specimen’s thickness is even.• Be sure vessel holder is mounted flat with respect to stage. |
| Difficulty focusing on coverslipped specimen on standard slide | Place the slide so the coverslip is facing up (long working-distance objectives require a thick optical substrate, and image best through 1.0–1.5 mm of glass or plastic). |
| Image display is black | <ul style="list-style-type: none">• Click the Power button (onscreen).• Center specimen over objective.• Verify power supply is connected and power switch is on. |
| Image display is red, or red patches cover parts of the screen | <ul style="list-style-type: none">• Dim the illumination until the red highlights disappear to get the maximum level of brightness without any overexposed areas.• Disable the “Highlight saturated pixels in red” option in the Settings ▶ Basic tab (page 40). |

Software Interface Issues

We recommend keeping the EVOS® FL Auto Imaging System up to date with the latest software. For more information, see “Software Updates” (page 46).

| Problem | Possible Solutions |
|--|---|
| Image does not respond to changes in focus or stage position | Click the Power button to return to live observation. |
| Scalebar does not appear when clicked | Capture image first; scalebar is only available after capturing an image. |
| Save button does not respond when clicked | Click capture first; It is only possible to save an image that is captured. |
| Unable to connect to network | <ul style="list-style-type: none">• 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. |
| Vessel does not sit securely on moving stage | Use the correct vessel holder for the application (refer to www.lifetechnologies.com/evosflauto). |

Appendix A: Description of EVOS[®] FL Auto Imaging System

Technical Specifications



Note: Specifications of the EVOS[®] FL Auto Imaging System are subject to change without notice. For the latest product information, see the EVOS[®] FL Auto Imaging System product page (www.lifetechnologies.com/evosflauto).

Physical characteristics

Height: 32.2 cm (12.7 in)

Depth: 47.2 cm (18.6 in)

Width: 34.3 cm (13.5 in)

Weight: 20.0 kg (44.1 lb)

Footprint: Approximately 92 cm × 92 cm (36 in × 36 in); entire system includes the instrument, computer, and 22" 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

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

Contrast methods: Epifluorescence and transmitted light (brightfield and phase contrast)

Objective turret: 5-position, automated control

Fluorescence Channels: Simultaneously accommodates up to 4 fluorescent light cubes

Condenser working distance: 60 mm

Stage: Automated X-Y scanning stage (travel range of 115 mm × 78 mm with sub-micron resolution); interchangeable vessel holders available

LCD display: 22" inch high-resolution, touch-screen color monitor

Camera: Dual (monochrome and color camera)

Monochrome: high-sensitivity interline CCD

Color: high-sensitivity CMOS

Output ports: Multiple USB ports, 1 display output with DVI adaptor (supports direct output to USB and networked storage)

EVOS® FL Auto Software

The integrated EVOS® FL Auto software, accessed by a touch-screen monitor or the mouse, features standard functions such as a scale bar and image review tool as well as a variety of advanced imaging and analysis tools. All images acquired can be saved in JPEG, BMP, TIFF, and PNG formats.

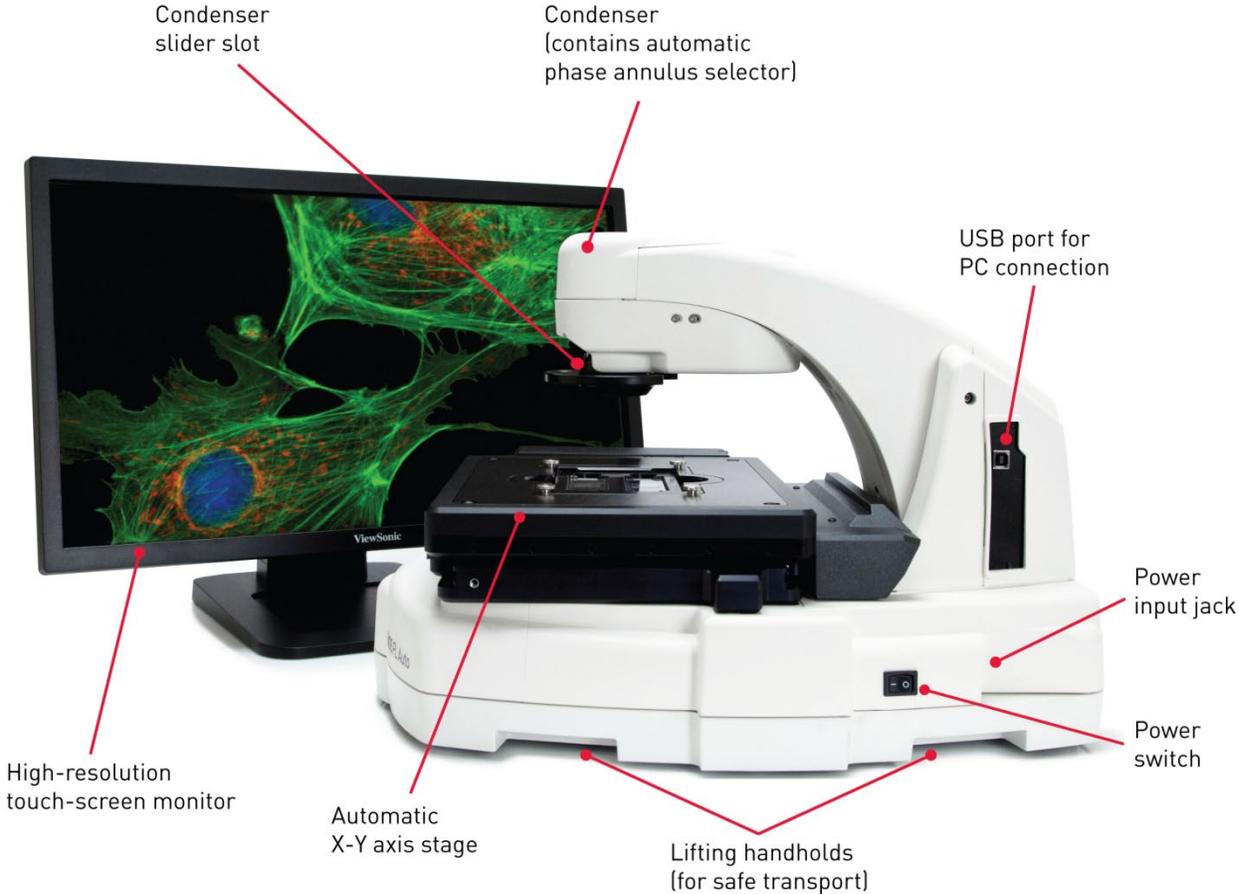
Key features of the EVOS® FL Auto software include:

- **Quick Image Wizard:** Provides step-by-step instructions for capturing perfect images; ideal for even the first-time users.
- **Manual Exploration:** Allows control over every aspect of the system through a simple user interface using either touch-screen monitor or mouse.
- **Routines:** Allows the creation, saving, and running of user-defined routines to automate image collection.
- **Autofocus:** Can be setup in several different modes to optimize speed and accuracy.
- **Image Stitching:** Allows the scanning of an area to acquire multiple images to build a tiled and stitched image. The Scan 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. The **Z-Stack Flat Focus** feature collects a series of images, extracts the most "in focus" pixels from each image, and then returns a single, focused image even if the sample is very thick.
- **Time Lapse:** Creates and runs time lapse movies based on user specifications.
- **Image Editing:** Provides built-in image analysis tools in the user interface, including the Manual Counting Tool, Measure and Annotate, and Image Review.

Applications

The EVOS® FL Auto system is designed to be used for a broad range of applications including, but not limited to, multi-channel fluorescence imaging, cell density assays, multiple-position vessel scanning, and time-lapse imaging.

Instrument Exterior Components



Note: The EVOS® FL Auto Imaging System is a fully automated imaging system; no manual adjustment is required for the objective, focusing controls, light cube and camera selection, etc.

Operation Principles and Technical Overview

LED illumination The EVOS[®] FL Auto 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 Imaging System is more environmentally friendly, energy efficient, and has a significantly longer life span (>50,000 hours versus 300 hours for a typical mercury bulb and 1,500 hours for a metal halide bulb).

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

The table below lists some of the common fluorescent and specialty light cubes available from Light Technologies. For a complete list of available light cubes and to inquire about custom light cubes, go to www.lifetechnologies.com/evosflauto or contact Technical Support (see page 83).

| 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 B: 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-CO₂ premix, CO₂-only, and nitrogen-only). The onstage incubator is controlled by the same software and user interface that controls the EVOS[®] FL Auto 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: Note that a country-specific power cord must be ordered separately in regions not using the Type A power plug.

Technical Specifications



Note: Specifications of the Onstage Incubator are subject to change without notice. Refer to the EVOS® FL Auto Imaging System product page at www.lifetechnologies.com/evosflauto for the latest product information.

Physical characteristics

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

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

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

CO₂ range: 0% to 20%

O₂ range: 0% to ambient

Operating power: 100–240 VAC, 1.8 A

Frequency: 50–60 Hz

Electrical input: 24 VDC, 5 A

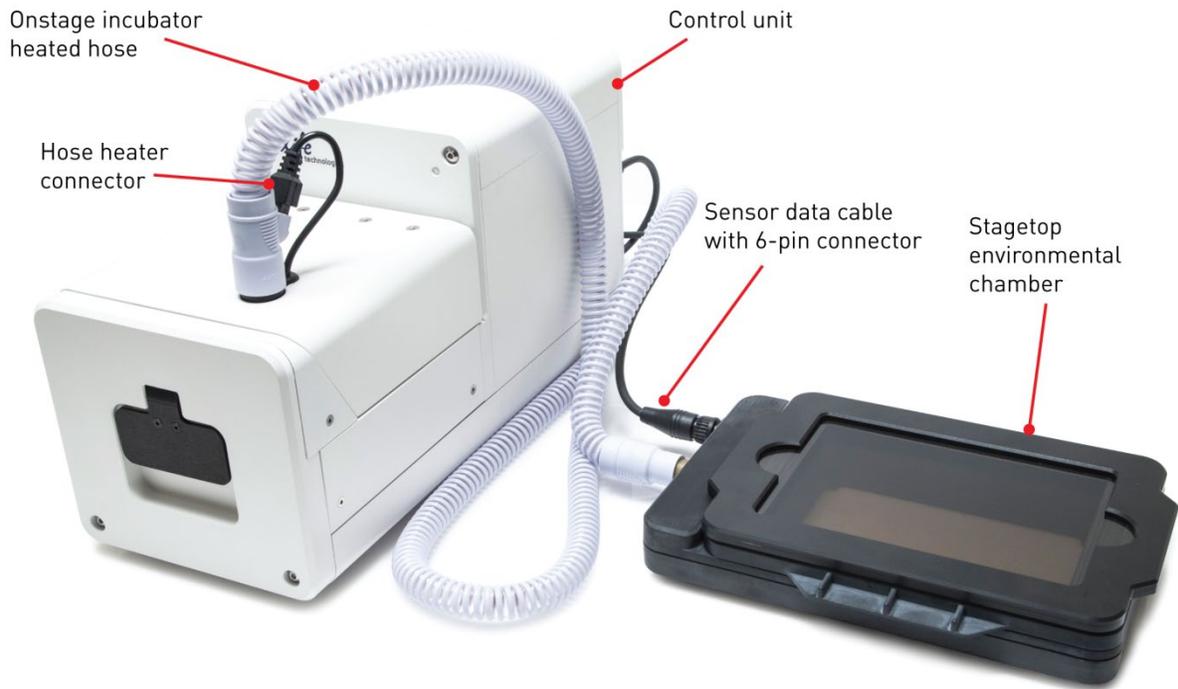
Hardware

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

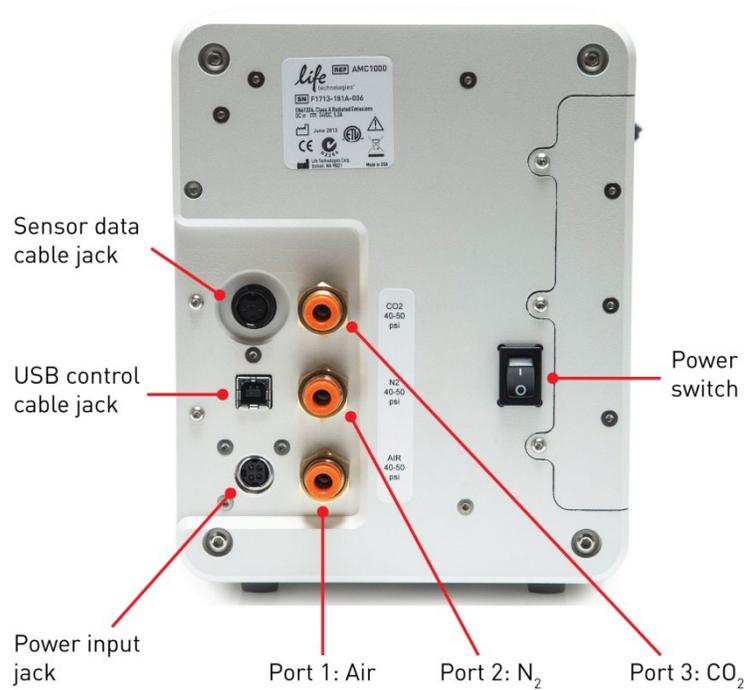
Gas input ports: Air or air-CO₂ premix, CO₂-only, and N₂-only (max. 50 psi input)

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

EVOS[®] Onstage Incubator Exterior Components



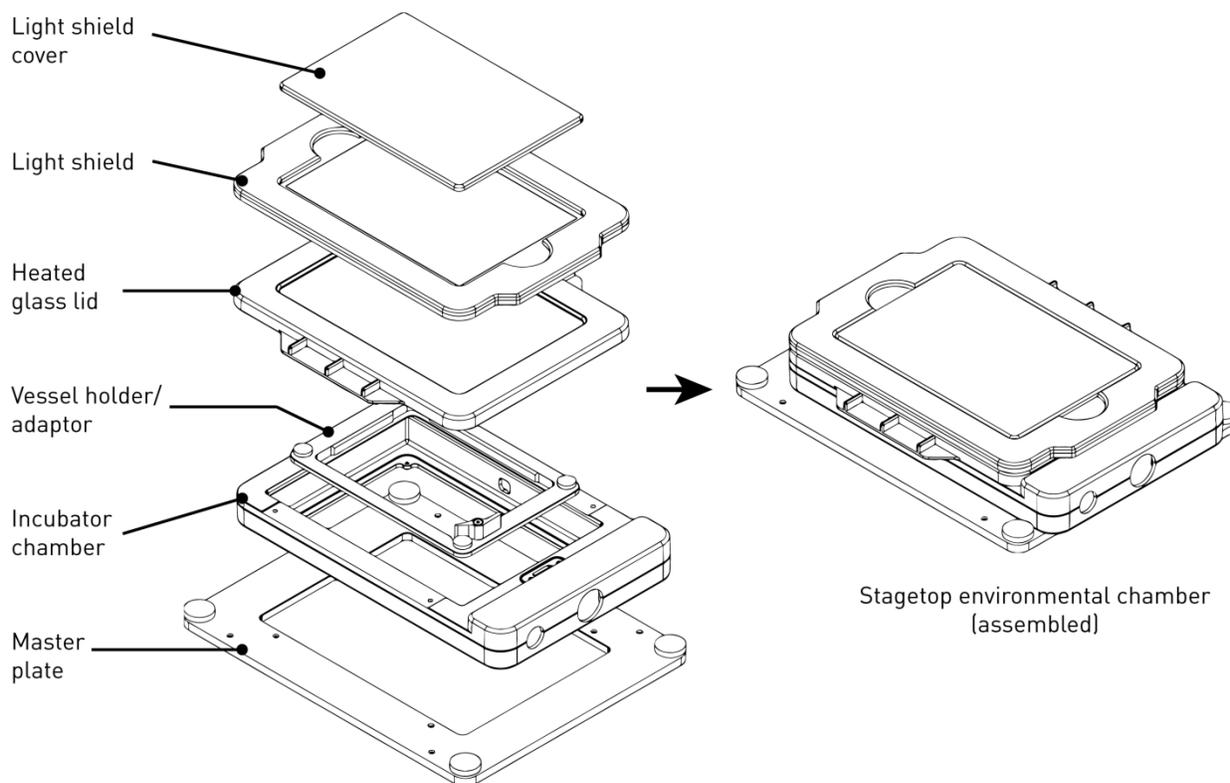
EVOS[®] Onstage Incubator Control Unit Instrument Panel



EVOS® Onstage Incubator Stagetop Environmental Chamber

The Stagetop 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 Stagetop Environmental Chamber sits on the onstage incubator master plate attached to the X-Y stage of the EVOS® FL Auto Imaging System (see “Assemble the Stagetop Environmental Chamber”, page 65).

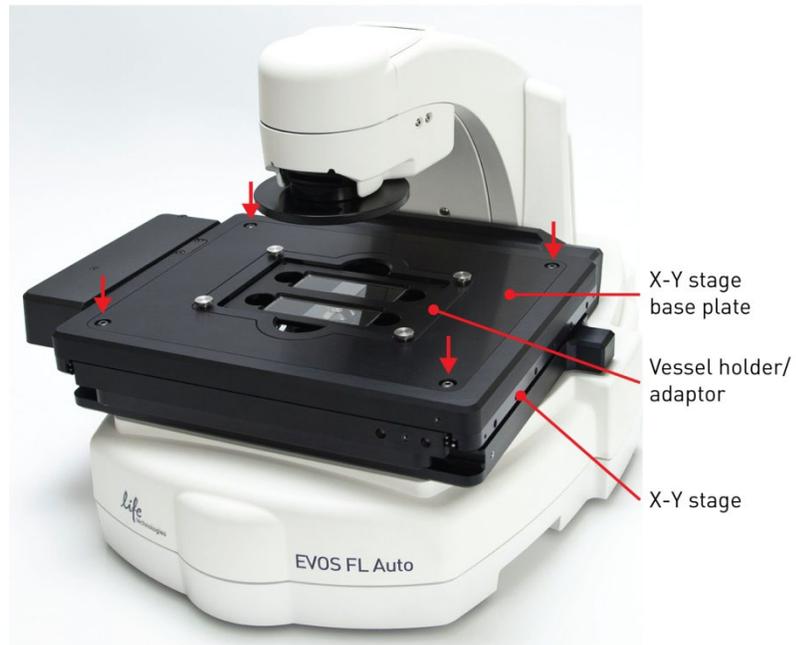


Installing the EVOS[®] Onstage Incubator

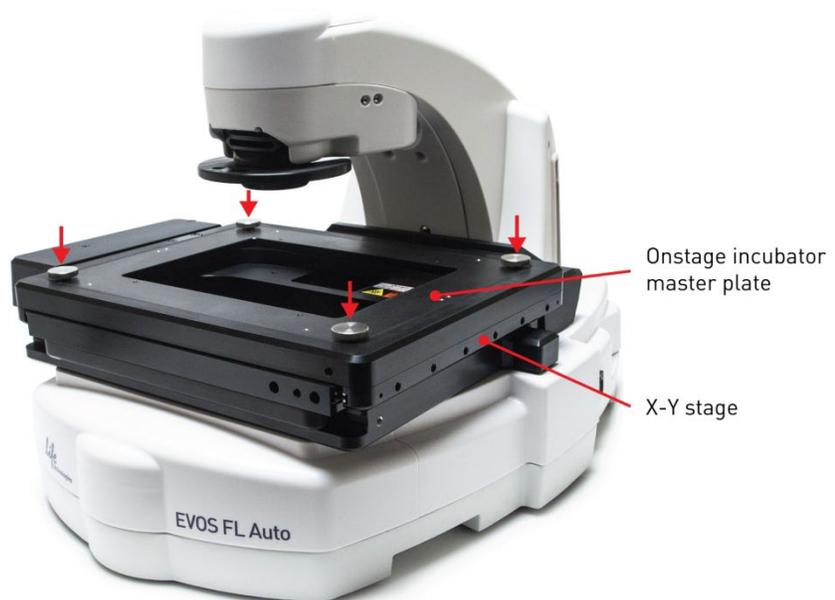
Before setting up the EVOS[®] Onstage Incubator, unpack the unit and accessories and verify all parts are present. Refer to page 60 for a list of the standard items included with the EVOS[®] Onstage Incubator. Contact your local distributor or Technical Support (see page 83) if anything is missing.

Install the Onstage Incubator Master Plate

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

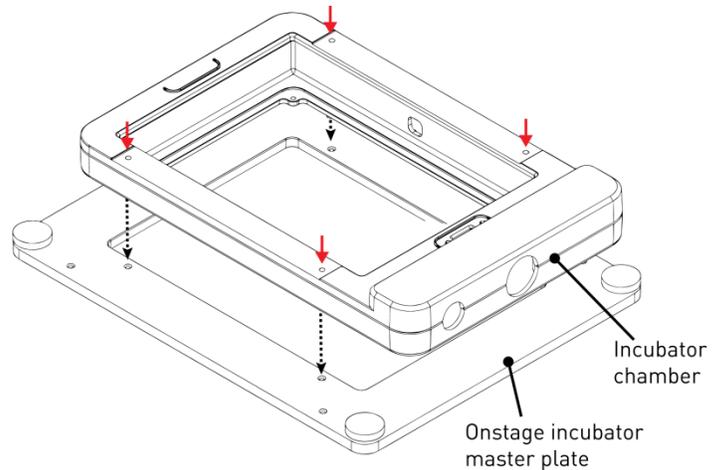


2. Secure the onstage incubator master plate to the X-Y stage using the four thumb screws (indicated by red arrows).

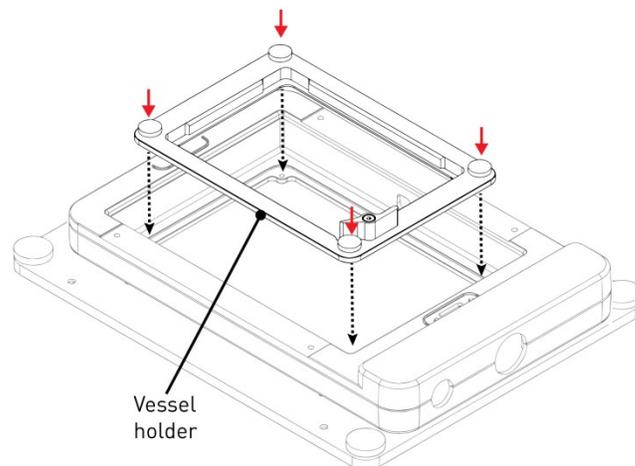


Assemble the Stagetop Environmental Chamber

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

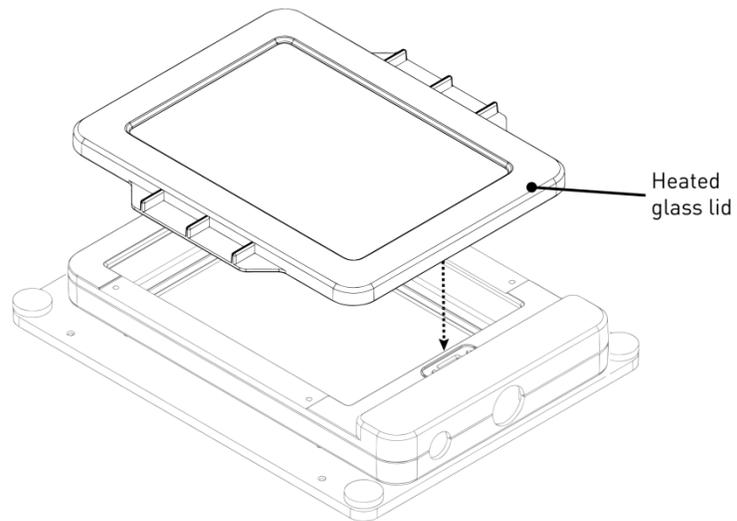


2. Attach the vessel holder/adaptor to the incubator chamber using the four thumb screws (indicated by red arrows).

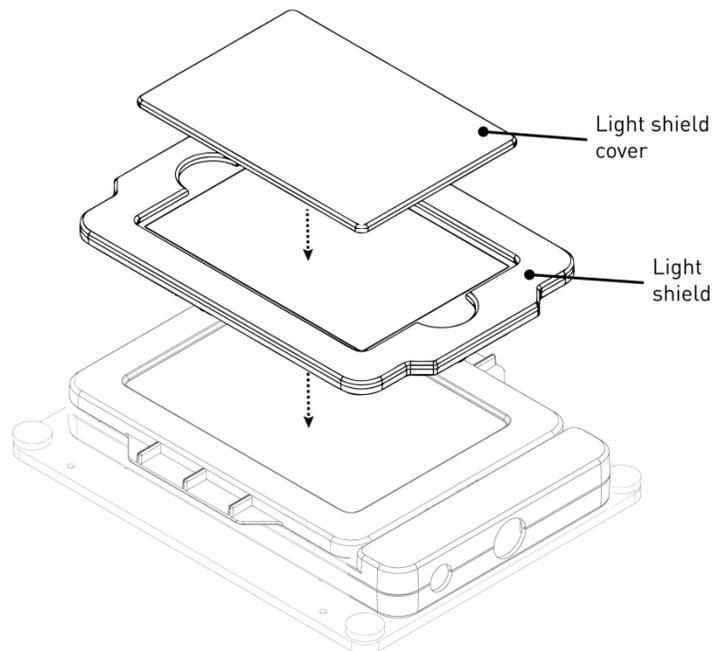


Note: Place an empty “dummy” culture plate into the vessel holder/adaptor for the initial warm up and equilibration to prevent build up of condensation on the optical components and the inside of the EVOS® FL Auto Imaging System.

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



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



Set up for operation Follow the procedure below to set up the EVOS® Onstage Incubator for operation. For the locations of the various input jacks and gas ports, refer to “EVOS® Onstage Incubator Control Unit Instrument Panel”, page 62.

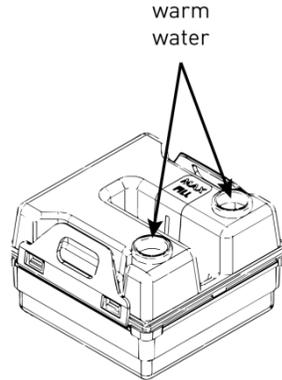
! **IMPORTANT!** Do not position the Control Unit so that it is difficult to turn off the main power switch (see page 62 for the location of the power switch). In case of an instrument malfunction, turn the main power switch to the OFF position and disconnect the power cord from the wall outlet.

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 EVOS® FL Auto 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.

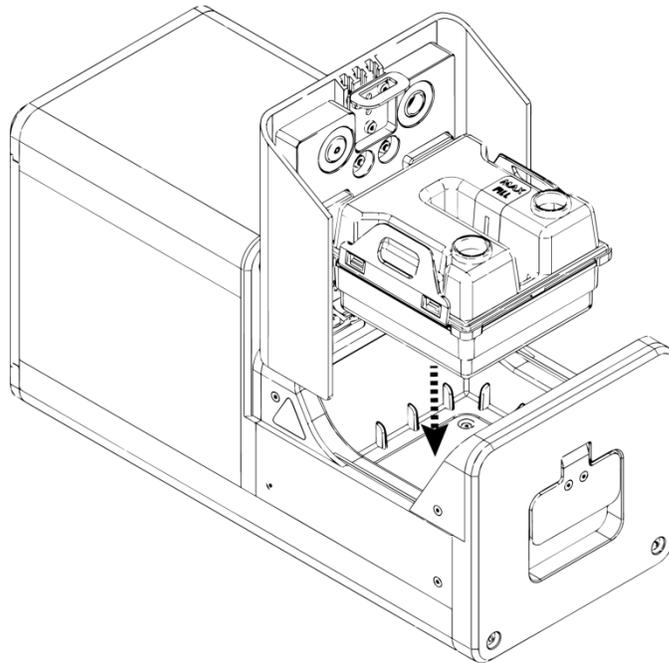


4. Attach the gas lines to the Control Unit via the PTC connectors for the appropriate gas intake port.
 - If using pre-mixed air, attach to **Port 1: Air In**
 - If using compressed air and CO₂, attach to **Port 1: Air In** and **Port 3: CO₂ In**
 - For oxygen displacement, attach to **Port 1: Air In** and **Port 2: Nitrogen In**
5. Plug the 6-pin sensor data cable to the Stagetop 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.

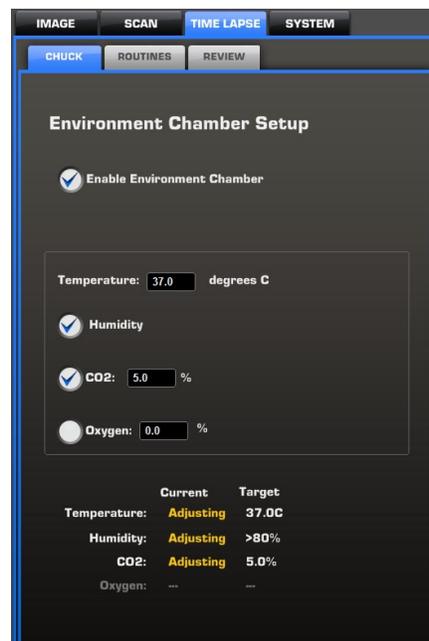


8. Attach the heated hose between the Stagetop Environmental Chamber and the Control Unit.
9. Plug the hose heater cable to the connector on the heated hose.

Using the EVOS[®] Onstage Incubator

Turn ON the EVOS[®] Onstage Incubator

1. Turn ON the EVOS[®] FL Auto Imaging System as described on page 6.
2. Turn the computer and monitor ON.
3. Turn ON the power switch to the Control Unit.
4. Restart the EVOS[®] FL Auto software.
5. Go to the **Time Lapse** tab. The Time Lapse tab will now display the **Incubator** tab.
3. Click **Enable Onstage Incubator** under **Time Lapse ▶ Incubator** 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 Imaging System.

Configure gas connections

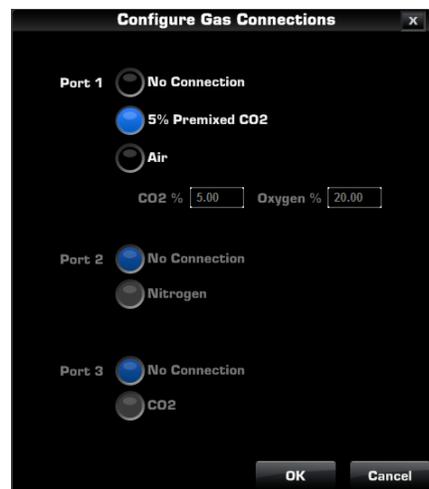
1. Go to **System ▶ Basic** tab (page 40), and then click **Configure Gas Connections**.
2. Select the appropriate option for each **Port** that reflects your set-up for the EVOS[®] Onstage Incubator.

For **Port 1**, you may choose 5% pre-mixed CO₂ or air, or manually enter the percentage of the CO₂ and oxygen to reflect the specifics of your set-up.

Port 2 is reserved for Nitrogen, and **Port 3** is reserved for CO₂ only.

Choose **No Connection** if you are not using that particular port in your set-up.

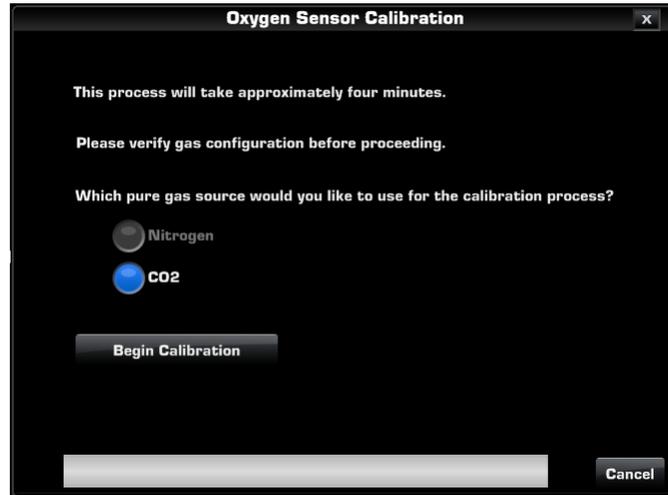
3. Click **OK** once you have configured the gas connections for each port.
4. Turn on the regulators on the gas tanks. The meters on the regulators show the tank fill on the right and gas flow on the left.
5. Set the flow on the regulators as follows. Do not exceed 50 psi of pressure.
 - **CO₂**: 40–50 psi
 - **Air**: 40–50 psi
 - **Nitrogen**: 40–50 psi



Calibrate oxygen sensor

Calibrating the oxygen sensor ensures that the atmosphere in the Stagetop Environmental Chamber is replenished with the appropriate gasses in the correct proportion.

1. Go to **System** ▶ **Basic** tab (page 40), and then click **Calibrate Oxygen Sensor**.



2. Verify that your gas connections have been correctly configured.
3. Select the **Pure Gas Source** for your calibration (Nitrogen or CO₂).
4. Click **Begin Calibration**. The EVOS® FL Auto Imaging System automatically calibrates the oxygen sensor for the proper functioning of the EVOS® Onstage Incubator. The entire calibration process takes approximately four minutes.

General Care of the EVOS[®] Onstage Incubator

- When cleaning the EVOS[®] Onstage Incubator, use only optical-grade materials to avoid scratching soft lens coatings.
- Use the appropriate cleaning solutions for each component, as indicated in the Sterilization Procedures below.
- If liquid spills on the instrument, turn off the power immediately and wipe dry.
- Clean the EVOS[®] Onstage Incubator as needed with paper towels or Kimwipes[®] tissues dampened with 70% ethanol.



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 83) or your local EVOS[®] distributor for information.

Sterilization Procedures



IMPORTANT! The EVOS[®] Onstage Incubator should not be subjected to UV sterilization. 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[®] Onstage Incubator, follow the sterilization procedure as described below.

1. Turn power OFF.
2. Lightly wipe working surfaces of the EVOS[®] Onstage Incubator with paper towels or Kimwipes[®] tissues dampened with 70% ethanol or 4,000 ppm hydrogen peroxide (H₂O₂).



IMPORTANT! Do not soak any surface in sterilization solution. NEVER spray liquid anywhere on the EVOS[®] Onstage Incubator. Always wipe surfaces with dampened paper towels instead.

Appendix C: Safety

Safety Conventions Used in this Document

Safety alert words Four safety alert words appear in Life Technologies user documentation at points in the document where you need to be aware of relevant hazards. Each alert word—**IMPORTANT**, **CAUTION**, **WARNING**, **DANGER**—implies a particular level of observation or action:

Definitions



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



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.

Except for **IMPORTANT!** safety alerts, each safety alert word in Life Technologies document appears with an open triangle figure that contains a hazard symbol. These hazard symbols are identical to the hazard icons that are affixed to Life Technologies instruments (see “Safety symbols”).

Symbols on Instruments

Electrical symbols on instruments

The following table describes the electrical symbols that may be displayed on Life Technologies 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 Life Technologies 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 Life Technologies electrical and electronic products placed on the European market after August 13, 2005.

| Symbol | Description |
|---|--|
|  | <p>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).</p> <p>European Union customers: Call your Customer Service representative for equipment pick-up and recycling. See www.lifetechnologies.com for a list of customer service offices in the European Union.</p> |

Safety Labels on Instruments

The following CAUTION, WARNING, and DANGER statements may be displayed on Life Technologies 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 réglementation 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 Life Technologies 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 Life Technologies. |
|  | 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. Évitez 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 Life Technologies 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! Life Technologies 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 m³ (257 ft³) 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 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 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/bmb14/bmb14toc.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 Life Technologies.

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, go to www.lifetechnologies.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 (techsupport@lifetech.com)
- 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 www.lifetechnologies.com/sds.



IMPORTANT! For the SDSs of chemicals not distributed by Life Technologies 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.lifetechnologies.com/termsandconditions. If you have any questions, please contact Life Technologies at www.lifetechnologies.com/support.



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

For support visit lifetechnologies.com/support or email techsupport@lifetech.com

lifetechnologies.com

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