

# EVOS™ FL Auto 2 Imaging System

For fluorescence and transmitted light applications

Catalog Number AMAFD2000

Pub. No. MAN0016154

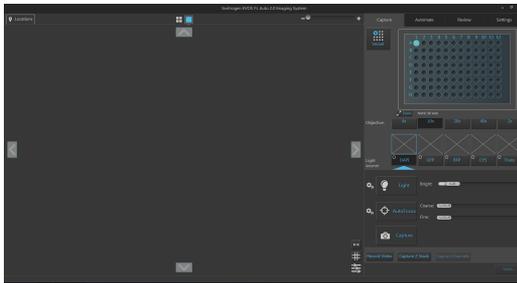
Doc. Part No. ZP-PKGA-0687 Rev. B.0

**Note:** For safety and biohazard guidelines, see the “Safety” appendix in the *EVOS™ FL Auto 2 User Guide* (Pub. No. MAN0014072). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

This document is intended as a benchtop reference for the users of the EVOS™ FL Auto 2 Imaging System (Cat. No. AMAFD2000). See the *EVOS™ FL Auto 2 User Guide* for detailed instructions and troubleshooting. The user guide is provided on the USB flash drive and is also available for download at [thermofisher.com](http://thermofisher.com).

## Capture images

- 1 Turn on the EVOS™ FL Auto 2 Imaging System
  - a. Turn the instrument power switch located in back of the instrument base to the ON position.
  - b. Turn the computer and monitor ON.
  - c. When the computer shows the Windows™ desktop and the X-Y stage of the instrument has stopped moving, click the **Auto 2** icon to start the EVOS™ FL Auto 2 software.
  - d. Once the Capture tab is displayed, the EVOS™ FL Auto 2 Imaging System is ready to use.



- 2 Select sample vessel
  - a. Place the vessel containing your sample on the stage using the appropriate vessel holder.
  - b. On the **Capture** tab, click the **Vessel** button to open the **Vessel Selection Wizard**.
  - c. Select the **Vessel category** that corresponds to your sample vessel, then select the appropriate **Holder** and **Vessel type** from the available dropdown menus.
  - d. Click **Done** to complete your selection and close the wizard. The Vessel map on the Capture tab displays your selected vessel.



- 3 Select objective and light source
  - a. Click the desired **Objective** button to select the corresponding magnification.



- b. Select the desired **Light source** for which you wish to adjust brightness and set focus.



## 4 Set brightness and camera options

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



- b. For **Mode**, select **Simple** or **Actual**.

- **Simple** mode allows you to control **Brightness** as a single parameter.
- **Actual** mode allows you to adjust **Light** (i.e., LED intensity), **Exposure**, and **Gain** individually.

- c. For **Camera**, select **Mono** (monochrome) or **Color**.

- **Mono** is used for image capture in fluorescence and transmitted light (brightfield) channels
- **Color** is used for image capture in the brightfield channel only

- d. *Optional:* Choose **Phase** options.

**Note:** Phase options are available only for the transmitted light (brightfield) channel; they are not available for fluorescence channels.

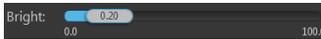
- e. Click the **Brightness and camera settings** button again to collapse the controls.

## 5 Adjust brightness

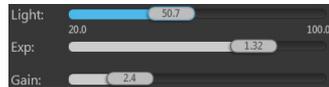
- a. Click the **Light** button to turn on the excitation light for the selected light source and enter the instrument in the Live mode.



- b. Adjust brightness using the **Brightness** controls.



Brightness controls in Simple mode



Brightness controls in Actual mode

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

- **When searching for sample:** Increase **Gain** for a brighter signal and decrease **Exposure** for faster frame rate during navigation around the vessel.
- **When capturing image:** Decrease **Gain** to reduce background noise and increase **Exposure** to regain signal intensity, as needed.
- **For brighter signal:** Increase **Light** intensity for brighter illumination. If needed, follow by increasing **Gain**.
- **For time lapse imaging:** Increase **Gain** and **Exposure**, and decrease **Light** intensity to reduce photobleaching and phototoxicity.

## 6 Set image display options

- a. Choose the **channels** you wish display in the Viewing Area by selecting the appropriate **channel checkbox** on the corresponding Light source button.

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



- b. Click the **Image Display settings** button to expand the image display controls for the selected channels.



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

- d. *Optional:* To remove a channel from displaying in the Viewing Area, unselect the corresponding **Display** checkbox.

To display an available channel not shown in the Viewing Area, re-select the checkbox.

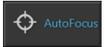
- e. Click the **Image color display** button to display the sample pseudo-colored in the default emission color of the selected channels. By default, color display option is on.

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

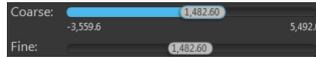
**Note:** Adjustments made to Image display settings only affect how the image is displayed in the Viewing Area; they do not change how the image is captured.

## 7 Focus on the sample

- a. Click **AutoFocus** to run the autofocus procedure to find the best focal plane for the current channel and sample.



Alternatively, use the **Coarse** and **Fine focus** sliders to manually find the best focal position for the current channel and sample.



- b. To choose a different autofocus method, click **Advanced focus settings** button, then select from the **AutoFocus Method** dropdown.



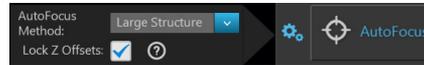
Available options for AutoFocus Method are:

- **Small Structure:** Choose this option for samples with many fine, hair-like structures (e.g., filaments or structural stains).
- **Large Structure:** Choose this option when your sample contains large structures (e.g., whole cell stains).
- **Small Bright Objects:** Choose this option when capturing samples with localized staining (e.g., nuclei).

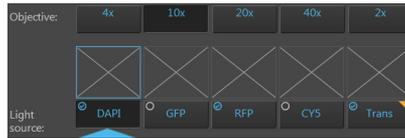
## 8 Optional: Set Z-Offsets

Z-Offsets allow you to specify the optimal focus position in each channel relative to the focus position in other channels.

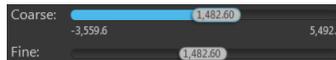
- a. Verify that the **Lock Z-Offsets** option is checked in the Advanced focus settings window, then click the **Advanced focus settings** button again to close the window.



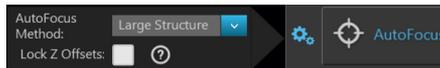
- b. Select the **Objective** and the **Light source** (i.e., channel) you wish to capture.



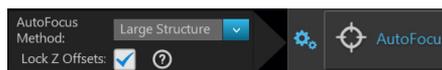
- c. Click the **Light** button to enter the Live mode, and focus on the sample manually using the **Coarse** and **Fine focus** sliders or automatically by clicking the **AutoFocus** button.



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



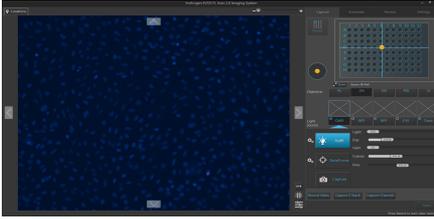
- e. Without changing the objective, select the next **Light source** (i.e., channel) you wish to capture.
- f. If the light is off, click the **Light** button to turn it on, then focus on the sample manually using the **Coarse** and **Fine focus** sliders or automatically by clicking **AutoFocus**.
- g. Repeat this procedure for every channel you wish to capture.
- h. When you have found the optimal focus position in all the additional channels you wish to capture, click the **Advanced focus settings** button, then check the **Lock Z-Offsets** option.



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

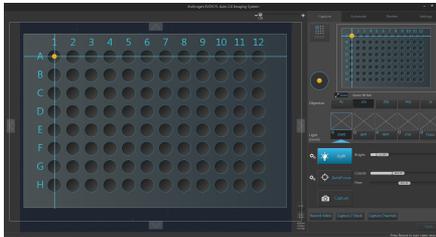
**9 Find the region of interest in the Live mode**

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



- b. To go to a specific location on the sample vessel, click and drag the **crosshair** to the corresponding location on the virtual vessel.

If needed, click on the **Zoom** button  to open a larger view of the Vessel map for easier navigation to the desired location. Click the **Zoom** button again to close the zoom window.



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

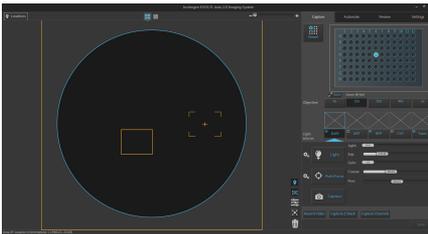
Alternatively, click on the **navigation arrows (up, down, left, right)** on the Viewing area to move in the corresponding direction exactly one field of view. You can also click and drag the field of view itself to move around the sample vessel.

- d. Click the **Light** button again to exit the Live mode in Field View.



**10 Select field and capture images**

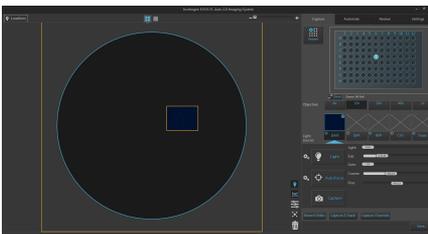
- a. In **Area View**, position the **capture crosshair** over the region of interest and click to select the field you wish to capture. Alternatively, find the field you wish to capture in the Live mode.



- b. Click **Capture** to acquire an image of the selected field using the current capture settings.

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

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



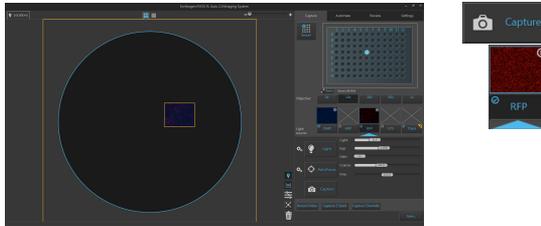
- c. To capture the same field in another channel, select the desired **channel** using the corresponding **Light source** button.



- d. Readjust the **brightness** and **focus** (if needed), then click **Capture**.

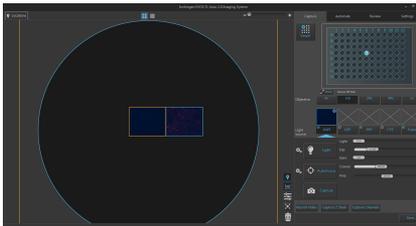
A thumbnail of the captured image is displayed above the Light source button for the new channel in which the image was captured.

The Viewing area displays a multicolor overlay of the images at the location of their capture.

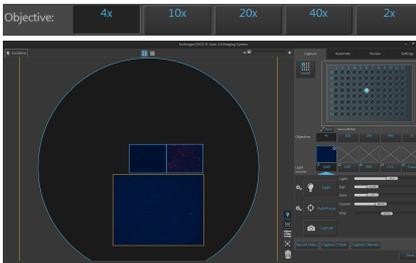


- e. To capture another field, position the capture **crosshair** over the new region of interest, then click to select the new field.

- f. Readjust the **brightness** and **focus** (if needed), then click **Capture**.



- g. To capture a field using a different magnification, select the desired **Objective**, then repeat the capture procedure with the new magnification as described.



- h. To capture a selected field in multiple channels simultaneously, select the **channels** you wish to capture by checking the small circular checkboxes on the corresponding light source buttons.

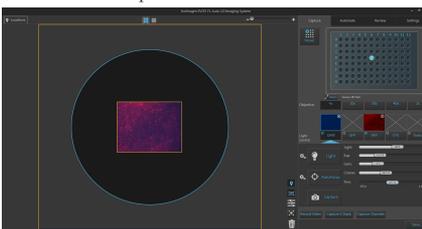
In the following example, **DAPI**, **RFP**, and **transmitted light** (brightfield) channels have been selected.



- i. Readjust the **brightness** and **focus** (if needed), then click **Capture Channels**.

**Capture Channels**

The instrument captures an image of the selected field in each of the selected channels using the current capture settings. After capture, the Viewing area displays a multicolor overlay of the captured images at the location of their capture.

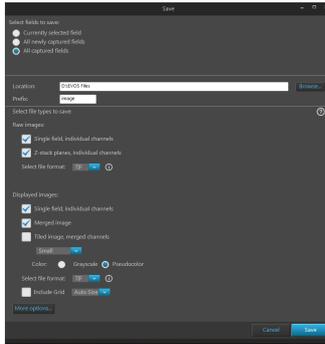


## Save captured images

### 1 Select captured field to save

- a. When finished capturing images, click **Save...** to open the Save window.

Save...



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

- **Currently selected field:** Saves images only from the currently selected field.
- **All newly captured fields:** Saves images that have been captured and stored in the image cache, but not yet saved. This option is available only if you have previously saved images from the same session.
- **All captured fields:** Saves images from all captured fields that are held in the image cache. This is typically all of the images that you captured during an imaging session.

### 2 Select save location

- a. Click **Browse**, navigate to the desired folder, then click **Select**.

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

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

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



### 3 Select file types to save

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

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

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

Available **raw image options** are:

- **Single field, individual channels:** Saves images captured in each field and each channel individually.
- **Z stack planes, individual channels:** Saves the z-stack projection and each z-stack plane for each field and each channel as a separate image.

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

- **Displayed images:** Saves images in a format that can be viewed in most image display applications.

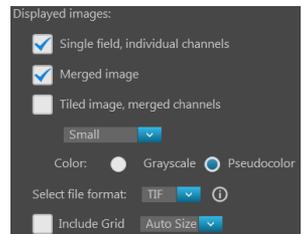
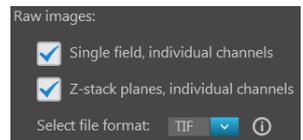
Available **displayed image options** are:

- **Single field, individual channels:** Saves images captured in each field and each channel individually.
- **Merged image:** Saves the images of a field captured in different channels as a multi-channel overlay.
- **Tiled image, merged channels:** Merges the images captured in each channel, then aligns them close together into a tiled format.

Available sizes for tiled images are **Small** (2000 × 2000 pixels), **Medium** (4000 × 4000 pixels), **Large** (10,000 × 10,000 pixels), or **Maximum** (26,000 × 26,000 pixels)

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

- **Grayscale:** 16-bit
- **Pseudocolor:** 24-bit RGB (8-bit per RGB channel).



Available **Brightness options** for displayed images are:

- **High:** Limits pixel depth and reduces exposure time and dynamic range.
- **Full:** Provides more detail for quantitative imaging.

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

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

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

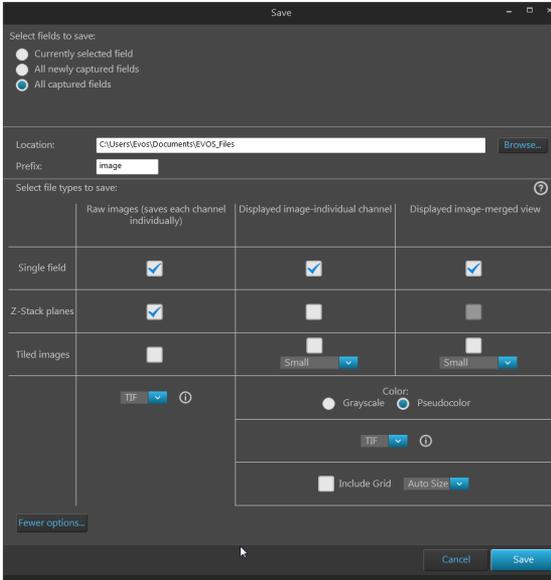
- b. If desired, check the **Include Grid** option and select the grid size. 

When checked, this option superimposes a grid on the displayed images.

You have the following options for the grid size (in pixels): **Auto, 10 x 10, 50 x 50, 100 x 100, 200 x 200, and 500 x 500.**

- c. To see more file type options in a table format, click **More options...**

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



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



The instrument will save the images in the designated folder based on your specifications.

# Documentation and support

## Technical support

For the latest services and support information for all locations, go to [thermofisher.com/support](http://thermofisher.com/support).

At the website, you can:

- Access worldwide telephone and fax numbers to contact Technical Support and Sales facilities
- Search through frequently asked questions (FAQs)
- Submit a question directly to Technical Support ([thermofisher.com/support](http://thermofisher.com/support))
- Search for user documents, SDSs, vector maps and sequences, application notes, formulations, handbooks, certificates of analysis, citations, and other product support documents
- Obtain information about customer training
- Download software updates and patches

## SDS

Safety Data Sheets (SDSs) are available at [thermofisher.com/support](http://thermofisher.com/support).

## Limited Product Warranty

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

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

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Revision	Date	Description
B.0	22 January 2018	Reformatted
A.0	01 February 2017	New document

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