

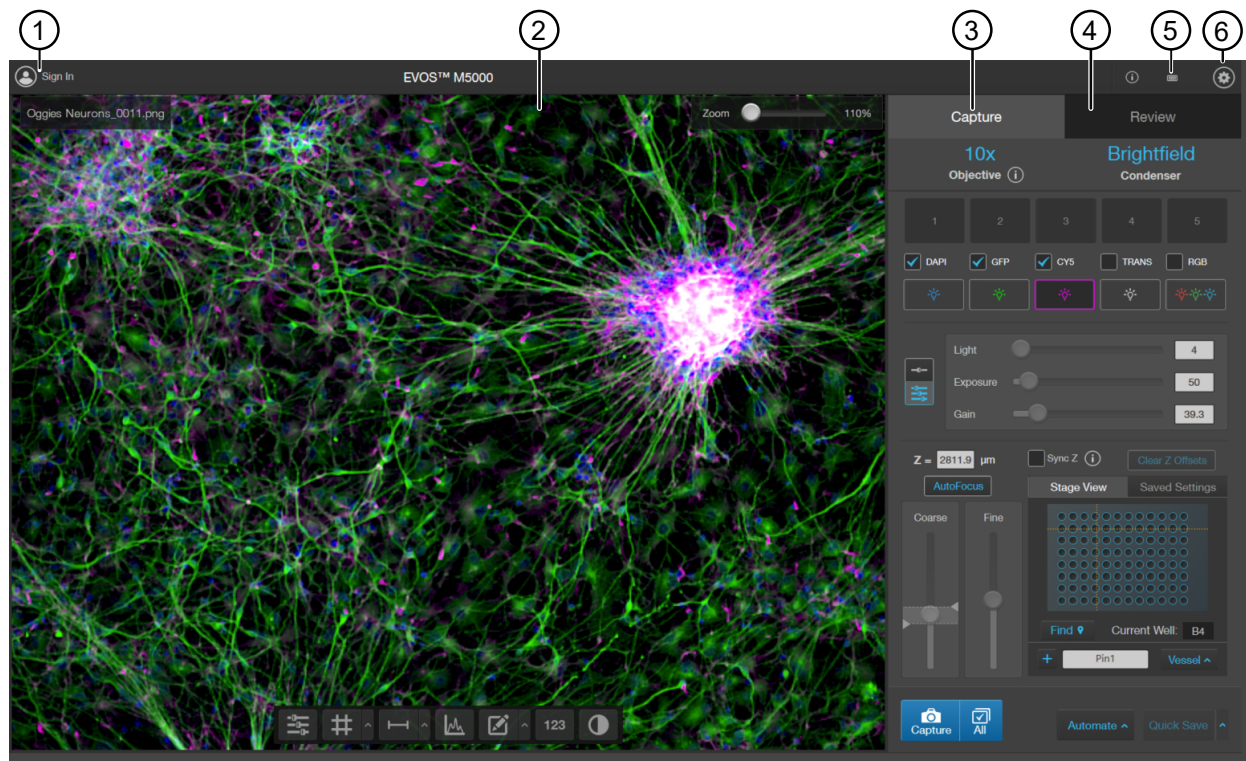
EVOS™ M5000 Imaging System

Doc. Part No. 710209 Pub. No. MAN0017765 Rev. C.0

Note: For safety and biohazard guidelines, see the “Safety” appendix in the following product documentation: EVOS™ M5000 Imaging System User Guide (Pub. No. MAN0017563). Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Graphical user interface (GUI)

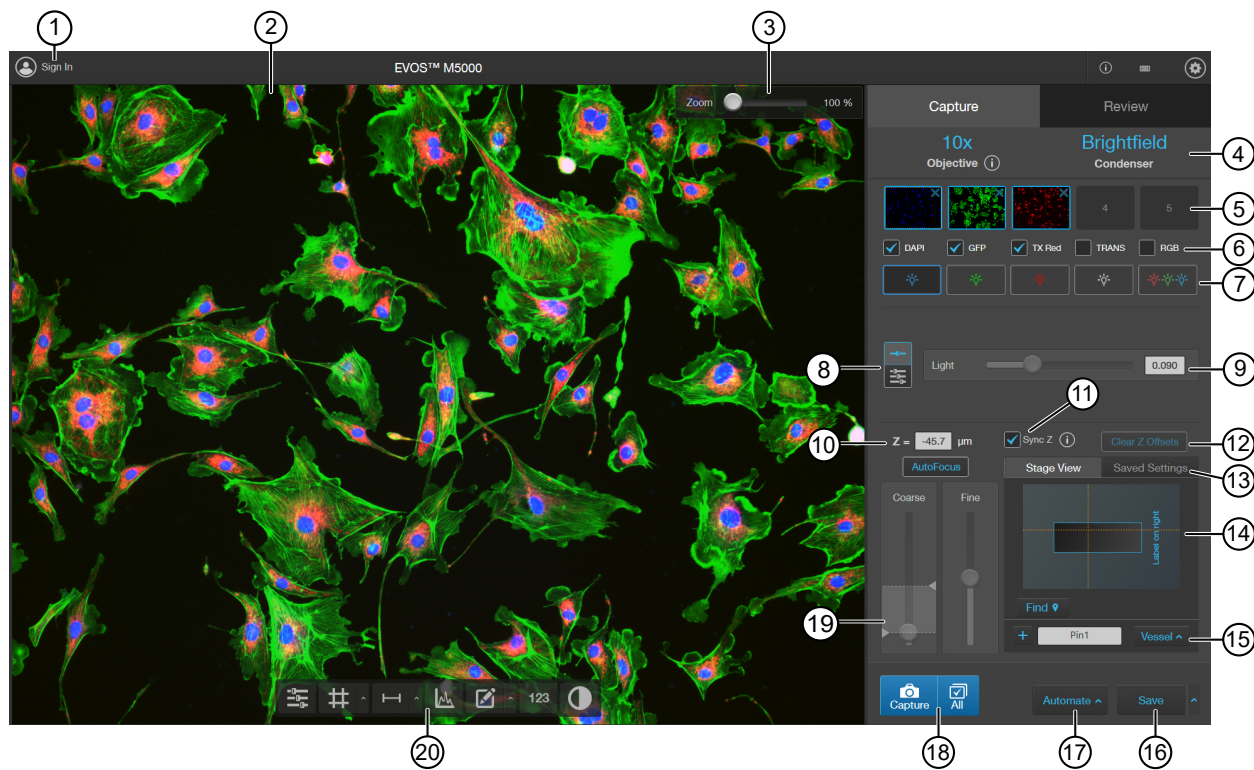
The graphical user interface (GUI) of the system consists of the Viewing area on the left and the **Capture** and **Review** tabs and **Settings** and **Virtual** keyboard buttons on the right. Each tab and button opens the controls necessary to execute the selected function.




1. **Sign In/User:** Allows you to sign in to your Connect account, Connect-based platform, for image storage and analysis with the EVOS™ Image Analysis application.
2. **Viewing area:** Displays images of the sample.
3. **Capture tab:** Contains the controls for image capture.
4. **Review tab:** Allows you to review and annotate captured images.
5. **Keyboard button:** Opens the virtual keyboard.
6. **Settings button:** Opens the **Settings** panel, which allows you to select and adjust basic and advanced system settings.

Capture tab

The basic functions of the EVOS™ M5000 Imaging System, such as viewing the sample, setting optimal focus, and capturing and saving images are performed in the **Capture** tab, which is the first screen after start-up.

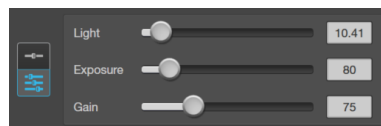


- 1. Sign In/User:** Allows you to sign in to and sign out of your Connect account.
- 2. Viewing area:** Displays the live or captured images of the sample.
- 3. Zoom slider:** Zooms in and out of the Viewing area. The zoom range is 100% to 1000%.
- 4. Active objective and condenser:** Displays the active objective and condenser information.
 - If the selected objective and phase ring match, the selected phase ring is depicted in blue font. If the objective and phase ring do not match, the phase ring is depicted in orange font.
 - To learn more about the current objective, click .
- 5. Thumbnails:** Click to show/hide the most recently captured image stored in the memory buffer for the channel.
- 6. Capture channels:** Checkmarked channels are captured when the **All** button is selected.
- 7. Channel:** Selects the light source from the installed LED light cubes (fluorescent channels) or from the condenser (transmitted light). RGB Trans option outputs a color image from interlaced RGB images in the Live mode.
 - Select a channel to turn on the excitation light and put the instrument into **Live** mode. In **Live** mode, you can use the brightness and focus controls.
 - Click the selected **Channel** button again to turn off the excitation light and exit **Live** mode. The current channel remains selected as indicated by the highlighted border of the channel button.
- 8. Brightness mode:** Toggles between Simple and Advanced modes for brightness controls.
 - Simple mode allows you to control image brightness as a single **Light** parameter.
 - Advanced mode allows you to individually adjust **Light** (that is, LED intensity), **Exposure**, and **Gain** parameters.
- 9. Brightness controls:** Adjust the brightness for the selected channel using the simple or advanced slider controls. The **Exposure** and **Gain** advanced sliders help to optimize image details.

Simple slider control for total brightness adjustment.

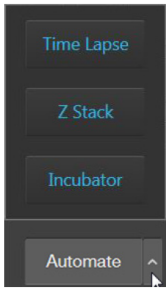


Advanced slider controls for **Light**, **Exposure**, and **Gain** adjustments.

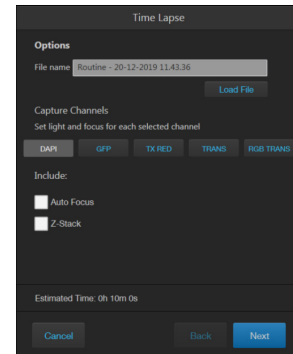


- 10. Z = (μm):** Displays the current focus position in μm along the Z-axis as controlled by the focus knobs or focus sliders in the user interface. You can also change the Z-axis focus position by entering the desired value into the **Z =** text box.
- 11. Sync Z:** Allows you to synch the Z-axis offsets, which specify the optimal focus position in each channel relative to the focus position in other channels.
 - When synched, movement of the Z-position in one channel changes the Z-position in all channels, preserving their relative Z-positions.
 - When unsynched, adjusting the Z-position only affects the currently selected channel.
- 12. Clear Z Offsets:** Clicking **Clear Z Offsets** sets all of the channels to the same focal plane value as the selected active channel. For example, if GFP is selected as the active channel and its focal plane is -712, then clicking **Clear Z Offsets** will set the other 3 channels with focal planes of -745, -694, and -715 to be -712.

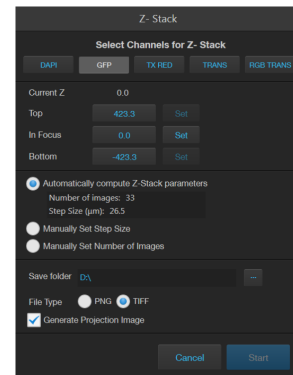
13. **Saved Settings:** Allows you to save Z-axis focus positions and brightness settings for future use.
Click **+** to save the current settings.
14. **Stage View:** Visual tool that allows precise marking of points of interest on various vessel types, as well as the relocation of these points at a later time.
15. **Vessel type for Stage View:** Allows selection of the vessel type being viewed. Vessel types include, slides, petri dishes, and plates from 6 to 384 wells.
16. **Save (Quick Save, Auto Save):** Saves the currently displayed image (all of the currently visible channels) to the hard drive, USB drive, or network drive. After saving an image, the filename is displayed in the upper-left corner of the screen.
17. **Automate:** Allows you to display the controls for the **Time Lapse** and **Z-Stack** tools, and the optional EVOS™ Onstage Incubator.



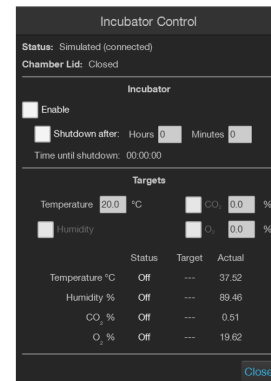
Time Lapse: Opens the **Time Lapse** tool, which allows you to create and run time lapse routines to capture images at given intervals over a time period based on your specifications.



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



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



18. **Capture and All:** Captures one channel (**Capture**) or multiple channels (**All**) using the current capture settings and stores images in the image cache of the channel in which it was captured.
19. **Focus controls:**
 - **Autofocus:** Runs autofocus in the selected channel.

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

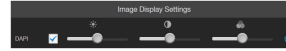
- **Coarse and Fine focus sliders:** Allow you to adjust the focus in the selected channel when in the Live mode.

Using the **Autofocus Maximum** and **Autofocus Minimum** controls (blue (when active) triangles on the **Coarse** focus slider), you can limit autofocus to the selected region along the Z-axis.

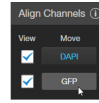
20. **Display Settings and Analysis Tools:** Allow you to change image display settings in the Viewing area, and analyze and annotate captured images.



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

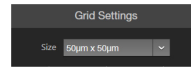


(Align Channels): Allows you to correct pixel shifts that can occasionally appear when performing multichannel fluorescence imaging at higher magnifications. The **Align Channels** tool only displays the controls for the channels in which the selected multichannel fluorescent image has been captured.



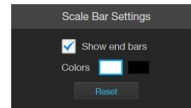
Note: The button to display the **Align Channels** tool is visible only if the **Show Align Channels in Display Settings and Analysis Tools** option is selected in the **Settings > General** tab.

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



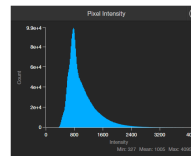
Grid Settings allows you to set the grid size.

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

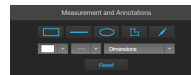


Scale Bar Settings allows you to select scale bar color and to display or hide end bars.

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



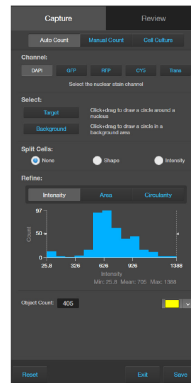
Measurements and Annotations: Allows you to draw regions of interest (rectangle, ellipse, polygon, line, or free-form) on the captured image and measure dimensions, area, or perimeter of the drawn region.



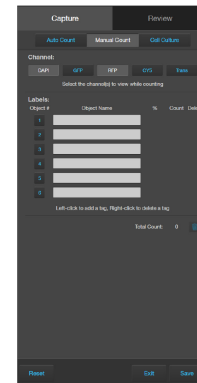
(Show Cell Count): Allows you to perform cell counts using the **Auto Count** or **Manual Count** tools and to measure the confluence and transfection efficiency of your culture from the captured image using the **Cell Culture** tools.

- **Auto Count:** Allows you to define auto count parameters by selecting representative target objects, then count the objects by intensity, area, and circularity.
- **Manual Count:** Allows you to manually mark items onscreen using up to 6 separate labels and keep a running tally of the counts with percentages for each label.
- **Cell Culture:** Allows you to measure the confluence of your culture and calculate the transfection efficiency.

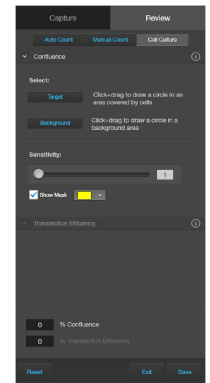
Auto Count



Manual Count

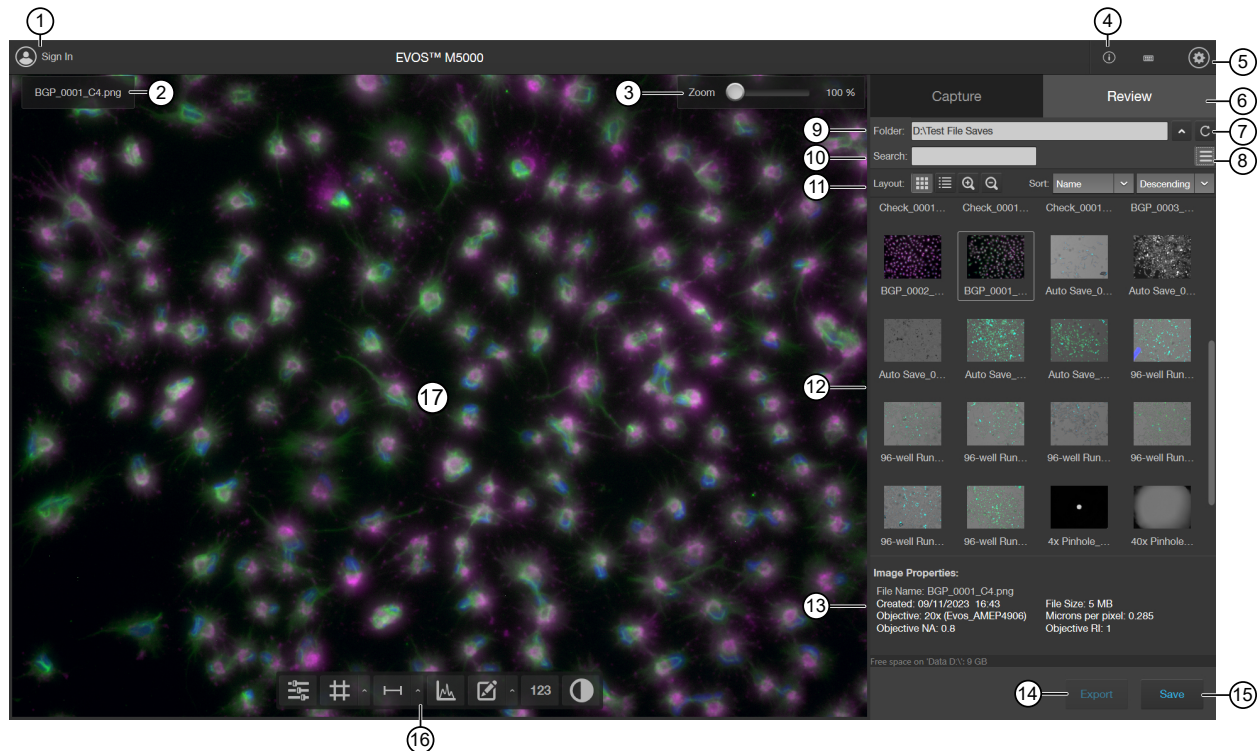


Cell Culture



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

Review tab

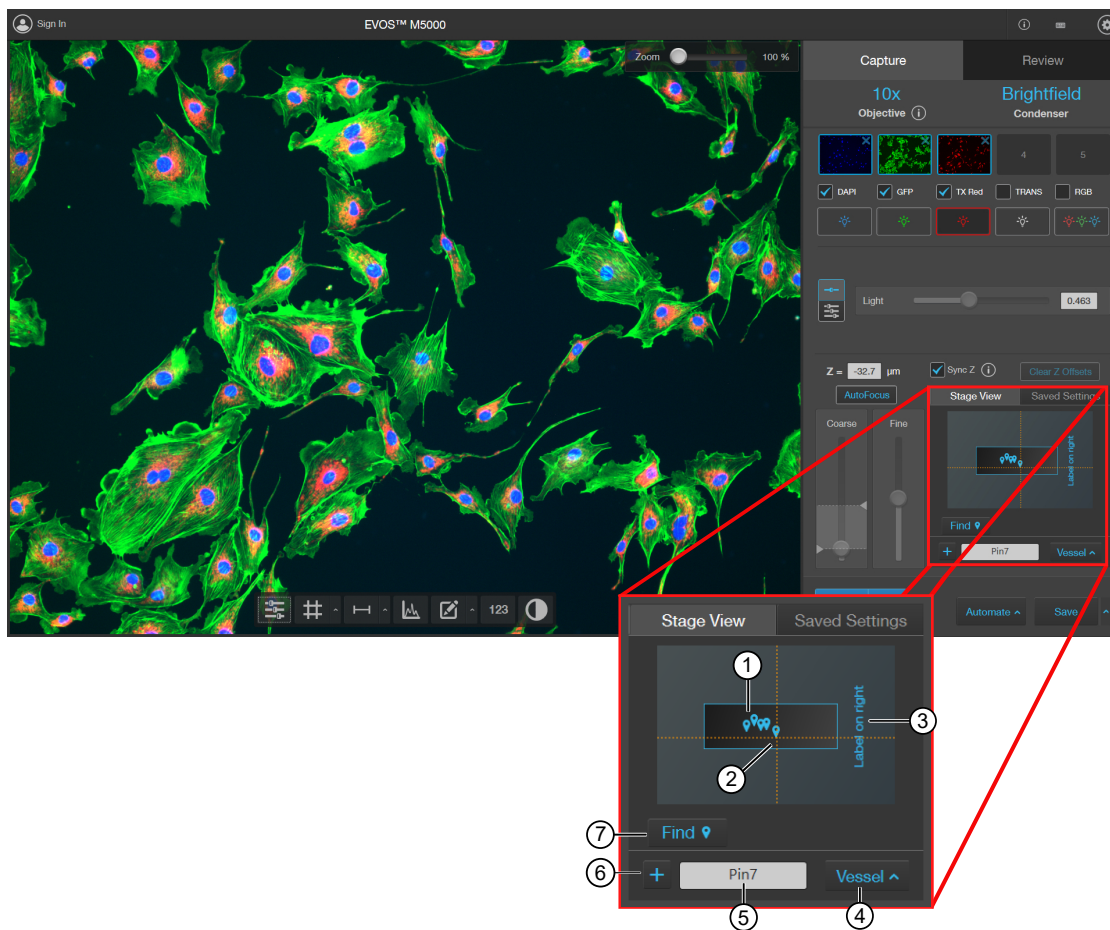


1. **Sign In/User:** Allows you to sign in to and sign out of your Connect Platform account.
2. **Image file name:** File name of the image displayed in the Viewing area.
3. **Zoom slider:** Zooms in and out of the image. The zoom range is 100% to 1000%.
4. **i (Keyboard Shortcuts):** Instrument actions triggered by keyboard combinations instead of mouse actions.
5. **⚙️ (Settings):** Opens the **Settings** tab.
6. **Capture and Review tabs:** Allows you to toggle between the **Capture** and **Review** tabs.
7. **^ (Up)** and **↻ (Refresh):** **Up** allows moving up one folder from the current folder. **Refresh** refreshes the list or grid of images in the current folder.
8. **Display Settings:** Displays or hides the Layout controls.
9. **Folder:** Displays the location of the current folder or image.
10. **Search:** Allows you to search images by file name in the selected folder.
11. **Layout:** Allows you to toggle between grid or list view, increase or decrease the display size of the folders or image files, and sort files by name, file type, or date created in ascending or descending order.
12. **Image preview/Image list:** Displays the preview or list of the image files and subfolders in the current folder.
13. **Image Properties:** Displays the metadata for the selected image file.
14. **Export:** Allows you to export the currently selected folder to a storage device.
15. **Save:** Saves the currently opened image.
16. **Display settings and analysis tools:** Allow you to change image display settings in the Viewing area and analyze and annotate captured images.
17. **Viewing area:** Displays the selected image.

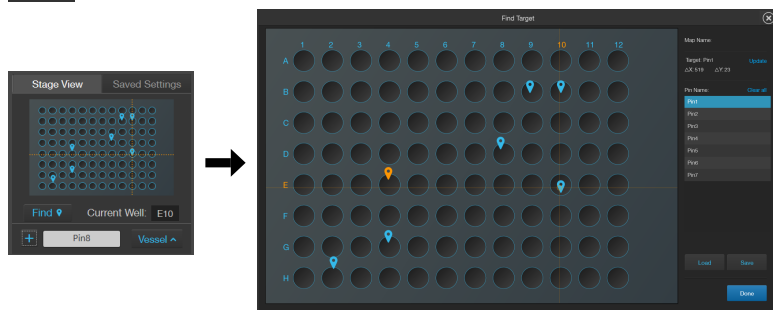
Stage View

Stage View provides a precise method for marking points-of-interest in your sample. With the selection of various vessel types, including plates, slides, and petri dishes, Stage View also displays a map of each specific vessel to provide a way to locate the well or area you are viewing.

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



1. **Vessel map:** A small representation of the currently-selected vessel and its associated wells, created pins, and so on.
2. **Crosshairs:** Displays real-time information about the position of the objective relative to the stage. The crosshairs appear on the small vessel map in the **Stage View** tab as well as the large vessel map in the **Find Target** window.
3. **Slide orientation:** To ensure correct repeated slide placement, **Label on right** is displayed as a reminder.
4. **Vessel:** Allows for selecting a specific vessel type.
5. **Pin identification:** Displays the next automatically assigned or custom name for a new pin.
6. **+ (Add pin):** Creates a new pin in the current stage location.
7. **Find** enlarges the small view to a larger window that provides details for each pin and its associated location in the vessel.



Settings

The **Settings** tab allows you to assign objectives to the objective turret, calibrate objective magnification, set image and general instrument options, add and remove light cubes, connect to a Wi-Fi network, or map network drives.

- To open the settings tab, click **Settings**.
- To expand or collapse any of the controls, click the corresponding arrow (▲ or ▼).

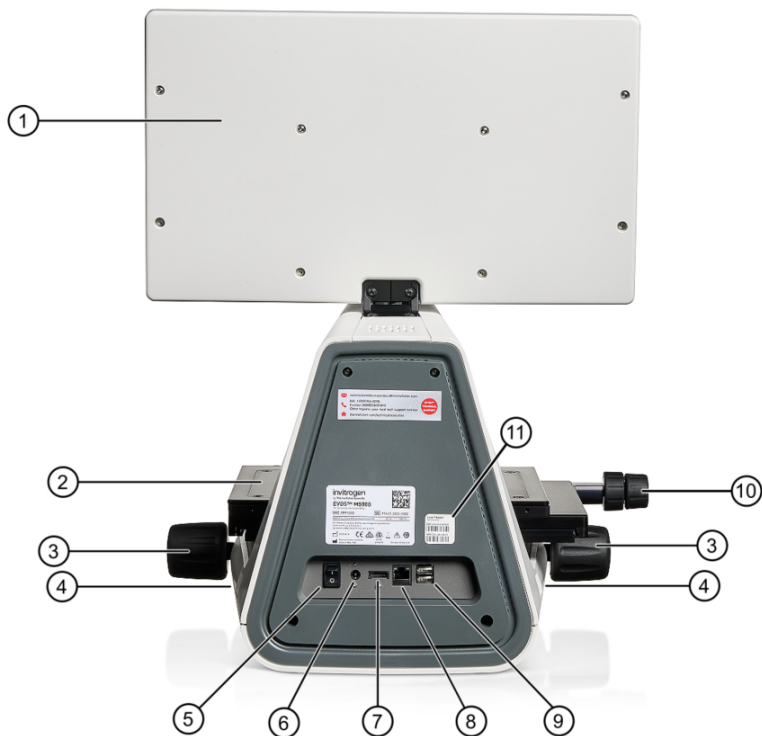
Instrument exterior components and mechanical controls

Front view



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

Rear view



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

Limited product warranty

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Revision history: Pub. No. MAN0017765 C.0

Revision	Date	Description
C.0	14 November 2023	Add Stage View tool and update screens and content.
B.0	25 January 2019	Add Thermo Fisher Cloud sign in and Confluence tool, and update the screenshots.
A.0	27 August 2018	New document for EVOS™ M5000 Imaging System.

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

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