EVOS® fl

USER GUIDE

Digital Inverted Microscope

for **Fluorescence** *and*
Transmitted Light Applications

mercury-free
energy-efficient

AMG
ADVANCED MICROSCOPY GROUP

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CONTENTS

WARNINGS, PRECAUTIONS & NOTICES

Throughout this manual, the following types of notifications require your close attention:

⚠ ***WARNING!*** *This type of notification tells how to avoid serious personal injury.*

ⓘ ***IMPORTANT!*** *This type of notification tells how to avoid damaging the microscope and/or voiding your warranty. (Contact your local distributor for more warranty information.)*

C *This symbol indicates information specific to the color camera version.*

MONOCHROME CAMERA VS. COLOR CAMERA

The EVOS fl microscope is factory-configured with either a monochrome camera or a color camera. Monochrome cameras are commonly used for high-performance fluorescence applications, and provide the best sensitivity for detection of faint fluorescence signals. Color cameras have lower fluorescence sensitivity but have the advantage of being able to differentiate structures by color in transmitted light, e.g., imaging stained tissue samples. Throughout this *User Guide*, any operational differences between the two versions of the microscope have been noted.

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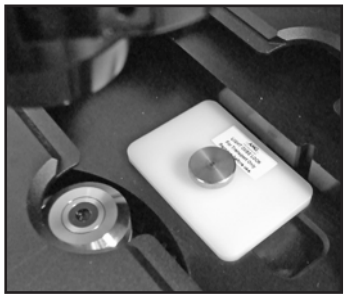
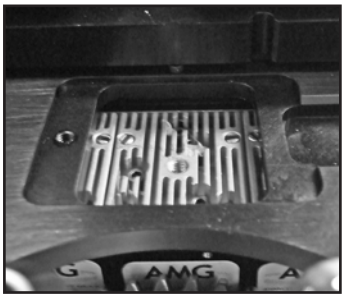
SETUP



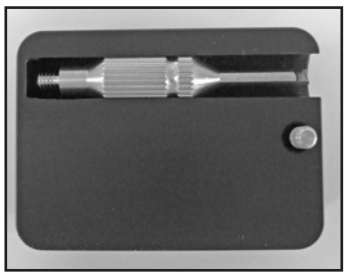
LED light cube lock
(in place under stage)



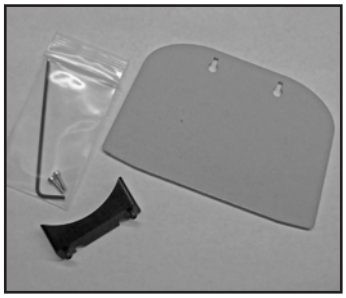
LED light cubes (in place under LED light cube lock)

Condenser shield, removable

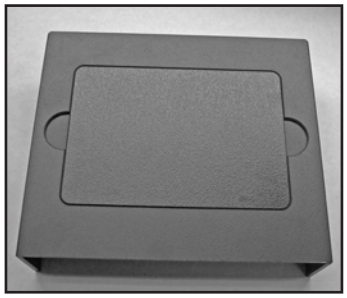
Light cube access cover (top)



Light cube access cover (bottom, with tool)



UV shield assembly kit



Light shield box

STANDARD ITEMS INCLUDED

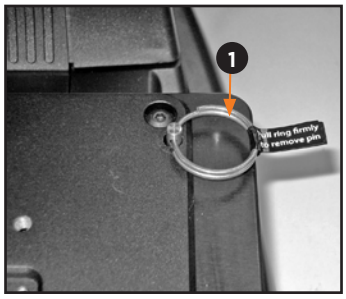
Before setting up your new EVOS, unpack the unit and accessories and verify all parts are present. Contact your distributor if anything is missing.

Note: *If you do not have your distributor information, you can look it up at the AMG website or contact AMG Customer Service (see p. 34).*

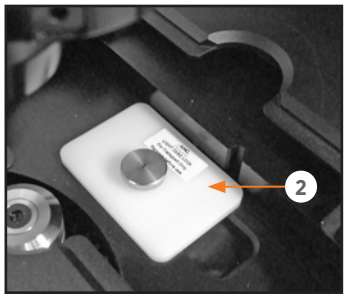
- **EVOS$_{fl}$ microscope, per order**
- **Condenser shield, removable**
- **Power adaptor**
- **Dust cover**
- **Mouse pad**
- ***Quick Start Guide***
- **USB flash drive (includes *User Guide*)**
- **Accessories boxes**

  Smaller box
  - Light shield box
  - USB mouse
  - Power cord

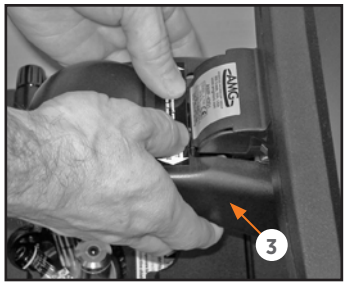
  Larger box (for storage)
  - UV shield assembly (mount, shield, screws & L-shaped hex key)
  - Light cube access cover (includes LED light cube installation tool); install light cube access cover before using EVOS
  - Condenser sliders: Pinhole, Diffuser, Meniscus A, Meniscus B, and Block



Stage lock pin ❶ engaged



Light cube lock ❷ engaged



Grasp under support arm ❸ with both hands to lift EVOS

Light shield box ❹ on stage

MOVING/TRANSPORTING EVOS

- ALWAYS lock stage with the stage lock pin ❶ before moving microscope.
- When transporting or shipping EVOS$_{fl}$, secure the LED light cubes in place with the light cube lock ❷.
- Lift the microscope by grasping it firmly with both hands under the support arm ❸, balancing the weight as shown at left.
- To transport EVOS to a different facility, use the original packaging materials if possible. Always be sure the microscope is properly cushioned and braced to prevent damage.

ⓘ ***IMPORTANT!*** *Never allow EVOS to be subjected to sudden impact or excessive vibration. Handle the microscope with care to prevent damage.*

OPERATING ENVIRONMENT

- Place the microscope 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 microscope to allow for proper ventilation and prevent overheating of electronic components.
- Set up EVOS away from direct light sources, such as windows. Ambient room lighting can enter the imaging path and affect the image.

  Note: *Place the light shield box ❹ on the stage over the sample to reduce the effects of ambient light and improve image quality.*
- Operating temperature range: 4°–32°C (40°–90°F).
- Relative humidity range: 30–90%.

ⓘ ***IMPORTANT!*** *Never subject EVOS to UV sterilization. UV degrades many materials, including plastic. Damage from UV exposure is not covered under the manufacturer's warranty.*

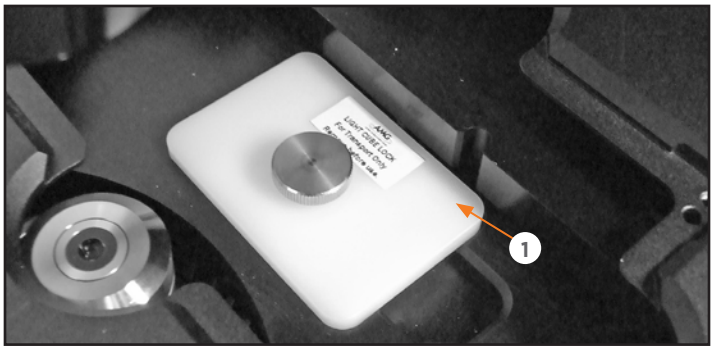
MECHANICAL STAGE

STAGE LOCK PIN

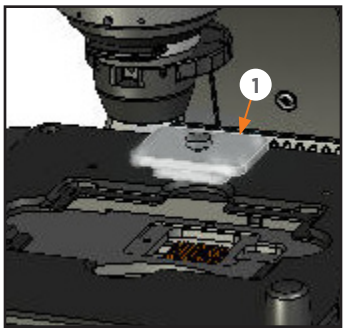
Before moving the mechanical stage for the first time, remove the stage lock pin ❶ from the back right-hand corner of the stage plate. Pull firmly to remove this pin.

You may store the stage lock pin in your accessories box for future use.

Note: *Always secure the mechanical stage with the stage lock pin before moving the microscope.*



LED light cube lock ❶ engaged



Remove light cube lock ❶

Secure light cube access cover ❷ over opening

LED LIGHT CUBES

ⓘ ***IMPORTANT!*** *Before changing light channels, ALWAYS be sure the light cube lock has been removed. Applying force to the light cube selection lever while the lock is in place may seriously damage the mechanism. This type of damage is not covered by the manufacturer's warranty.*

1. Move the stage back to allow access to the light cube lock ❶, which is centered under the back of the stage.
2. Loosen the thumbscrew to remove the light cube lock. You may store the light cube lock in your accessories box for future use during transport or shipping.
3. Place the light cube access cover ❷ into the opening and tighten thumbscrew.

Note: *For information about adding optional LED light cubes, refer to* ***Changing LED Light Cubes (p. 23)****.*

Power switch

Power adaptor plugged in

POWER SUPPLY

1. Turn the power switch to the "○" (OFF) position before connecting the power adaptor.

ⓘ ***IMPORTANT!*** *Always use the correct power supply. The power adaptor specifications appear on the serial number label (front of LCD hinge) and in the* ***SPECIFICATIONS (p. 36)****. Damage due to an incompatible power adaptor is not covered by warranty.*

2. Connect the power adaptor to the power jack on the right side of the microscope base, attach the cord to the adaptor, and plug the cord into an outlet.

Mouse and USB flash drive plugged in

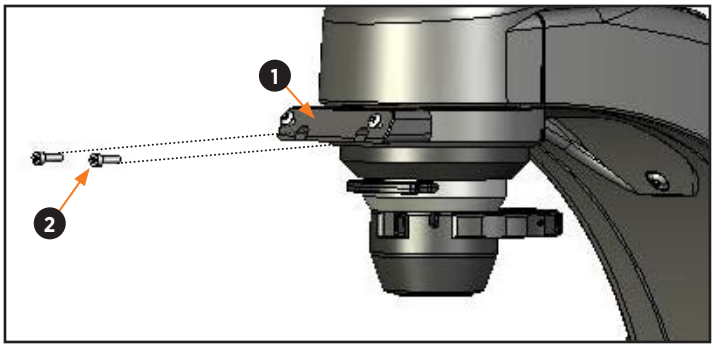
DVI cable (not included) plugged in

USB PORTS

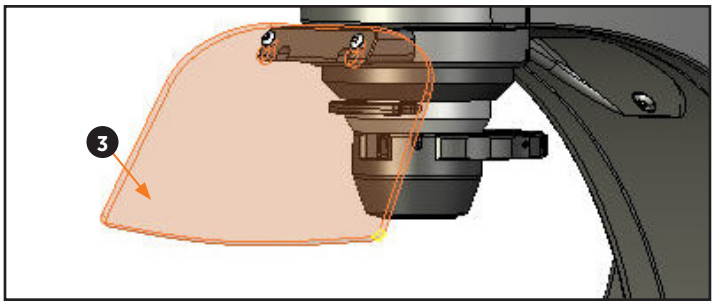
Plug the mouse and the USB flash drive into the USB ports located on the bottom right of the support arm. You may also plug in a USB keyboard (not included) for text input.

DVI OUTPUT PORT

A DVI port is available for output to a projector or other display. (DVI cable not included.) This port produces digital output only; EVOS is compatible with either a DVI-D or DVI-I display.



Attach UV shield mount ❶



UV shield ❸ in place

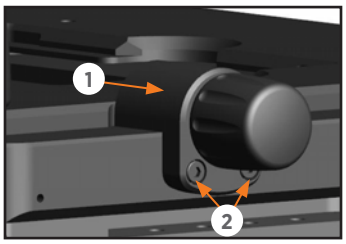
UV SHIELD

⚠ ***WARNING! UV LIGHT HAZARD!*** *This microscope uses a Class 3B ultraviolet LED for the DAPI channel. Avoid exposure to the UV beam and use protective shields. NEVER look directly at light.*

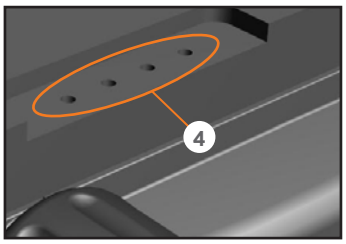
For your protection, follow this procedure to install the UV safety shield before using the DAPI fluorescence channel.

1. Attach the UV shield mount ❶ to the front of the condenser with the two screws ❷ provided.
2. Remove the protective coverings from both surfaces of the UV shield ❸.
3. Place the holes in the UV shield over the screws on the mount and push the slots down on the screws to secure the shield in place, as shown.

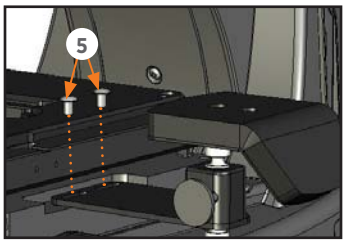
   Note: *The UV shield is removable for access to the condenser sliders used in transmitted light mode. Simply unhook it from the screws on the UV mount.*



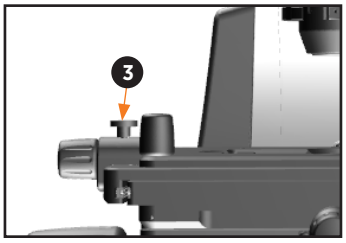
Remove Y-axis stage knob ❶



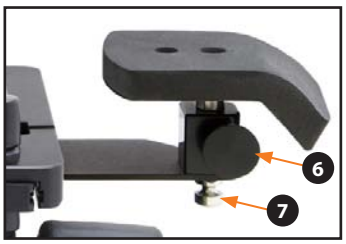
Slide stage to expose holes ❹



Attach arm rest to microscope



Stage brake ❸ on left Y-axis knob



Adjustment pin ❻ and arm rest post ❼

ARM REST (OPTIONAL)

An optional arm rest kit is available. (See ***PARTS & ACCESSORIES (p. 35)*** for ordering information.) The arm rest fits on either side of the stage. To attach the arm rest, it is necessary to remove a Y-axis stage knob ❶ (right knob shown). Store this knob in your accessories box or other safe place.

1. Use the smaller L-shaped hex key supplied in the arm rest kit to loosen the 2 stage knob screws ❷.

Note: *The Y-axis stage brake ❸ is on the left knob. If you are placing the arm rest on the left side, remove the right knob and replace it with the knob that has the stage brake. The stage brake is useful for time lapse captures.*

2. Slide the stage all the way to the side opposite the intended arm rest position. This will expose the holes ❹ for the arm rest screws.
3. Align the arm rest base slots over the holes as desired and use the larger hex key to attach the base to the stage with the arm rest screws ❺.
4. Adjust arm rest height, if desired:
   a. Pull the adjustment pin ❻ almost all the way out to allow the arm rest post ❼ to move up or down, and set the arm rest to the preferred height.
   b. Push the adjustment pin in. It may be necessary to move the arm rest slightly so the pin can fit through the groove on the post.

EVOS installed in a cell culture hood

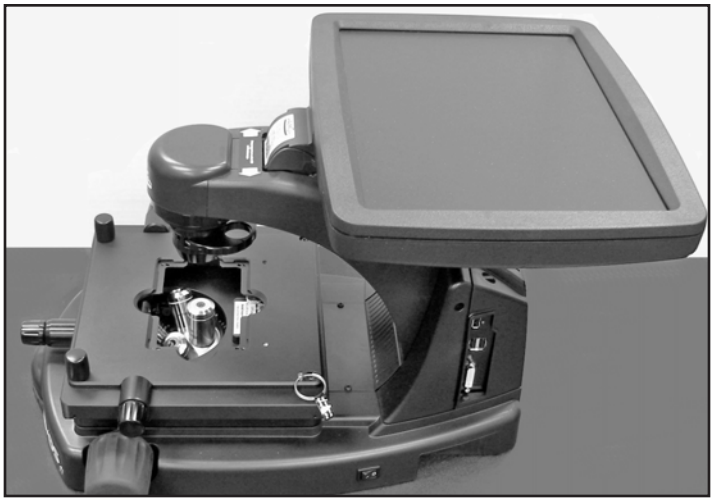
INSTALLING EVOS IN A CELL CULTURE HOOD

EVOS' small footprint, simple power connection, and easily-viewed display make it quick to install and convenient to use in a cell culture hood.

DIMENSIONS

EVOS will fit in cell culture hoods that are at least 20 ½ inches (520 mm) deep. If your cell culture hood is smaller, it may be possible to turn the EVOS at a slight angle to fit.

	ENGLISH	METRIC
DEPTH	18.5 in	47.0 cm
WIDTH	14.0 in	35.5 cm
HEIGHT, TRANSPORT	12.75 in	32.4 cm
HEIGHT, DISPLAY	22.75 in	57.8 cm
WEIGHT	33.7 lbs	15.3 kg



LCD tilted back into transport position

INSTALLATION

Note: *Refer also to the illustrations on p. 4 for more details about moving EVOS.*

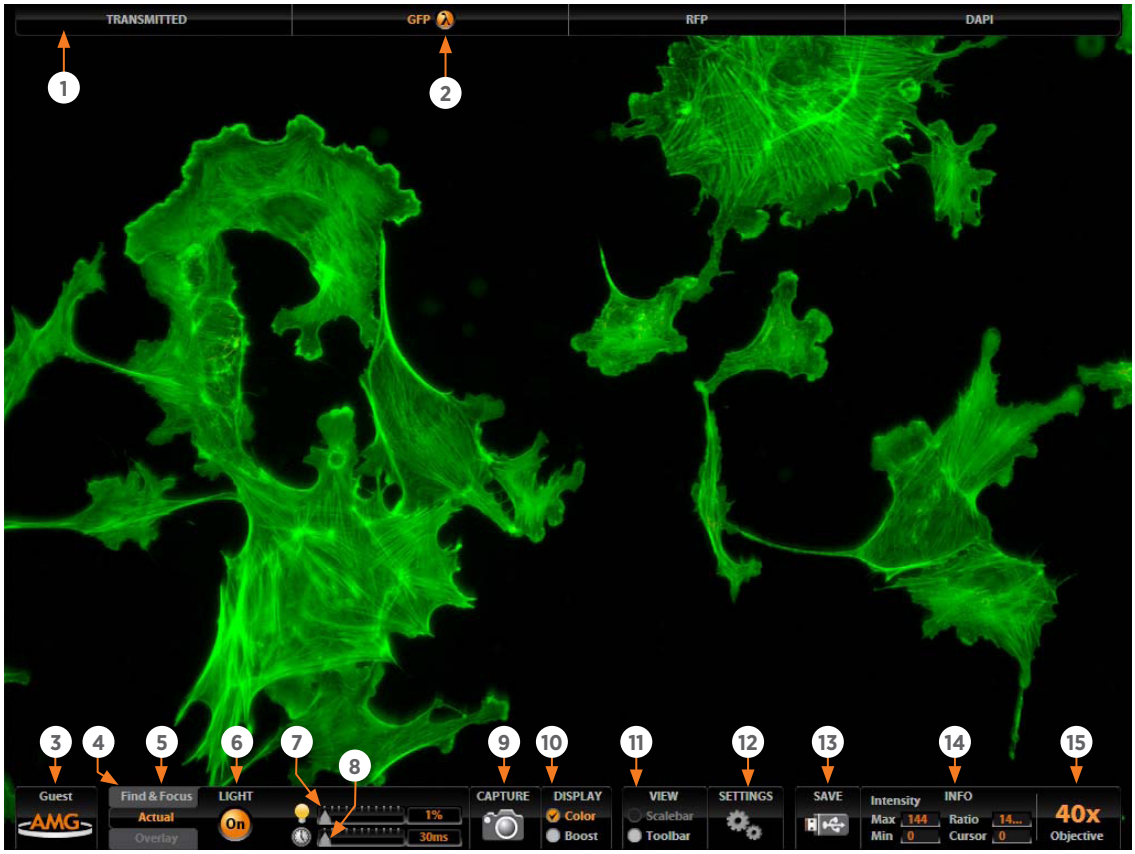
1. Secure the stage with the stage lock pin, switch EVOS off, and disconnect the power cord, mouse and, if connected, keyboard.
2. Tilt the LCD screen back until it is parallel with the tabletop.
3. Lift the microscope by grasping it firmly with both hands under the support arm just behind the condenser.
4. Gently place the microscope on a lab cart and transport it to the cell culture hood.

   Note: *Verify that the hood sash is raised enough for the microscope to slide underneath (approximately 14" or higher).*
5. Lift the microscope as before and move it into the hood.
6. Tilt the LCD monitor upright.
7. Remove the stage lock pin, connect the power cord, mouse and, if desired, keyboard, and switch EVOS on.

REMOVAL

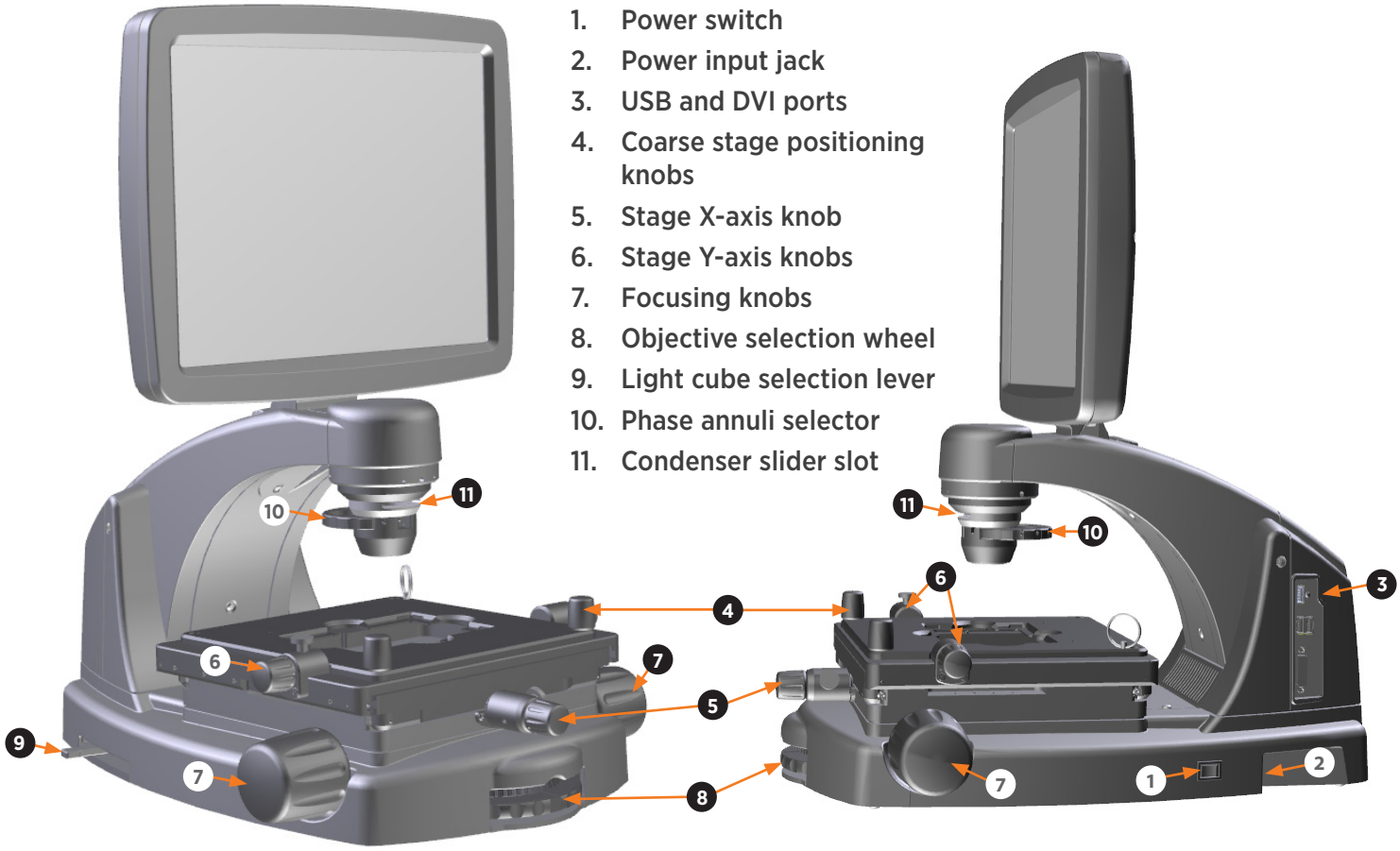
Reverse the sequence above to remove EVOS from the cell culture hood.

1. Channel indicator bar
2. Active channel (highlighted)
3. Login button
4. Control bar
5. Control bar tabs:
   - Find & Focus
   - Actual
   - Overlay
6. LIGHT ON/OFF button
7. Illumination slider
8. Exposure time slider
9. Image capture button
10. Color/boost options
11. Scalebar/toolbar options
12. Settings control button
13. Save image button
14. Info display bar*
15. Selected objective



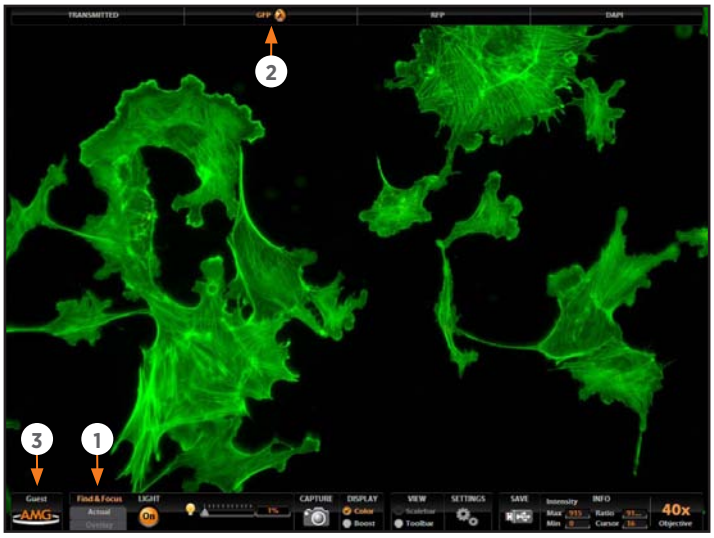
C **The color camera version shows the QuickSave option instead of the info display bar.*

1. Power switch
2. Power input jack
3. USB and DVI ports
4. Coarse stage positioning knobs
5. Stage X-axis knob
6. Stage Y-axis knobs
7. Focusing knobs
8. Objective selection wheel
9. Light cube selection lever
10. Phase annuli selector
11. Condenser slider slot

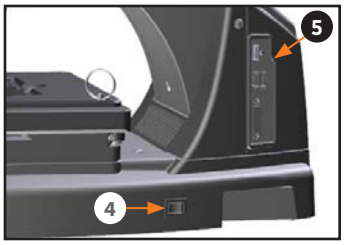


***Note:** Refer to the **CONTROLS GLOSSARY (p. 25)** for more details about onscreen and mechanical controls.*

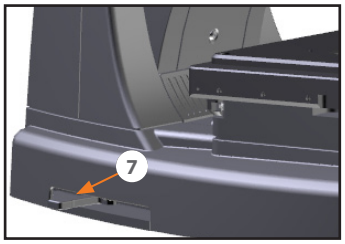
BASIC OPERATION



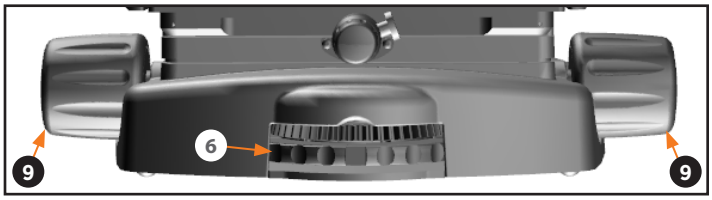
Software control bar ❶, channel bar ❷ and login button ❸



Power switch ❹ and data ports ❺



Light cube selection lever ❼



Objective selection wheel ❻ and focusing knobs ❾

LIGHT ON button ❽ in the control bar

Light shield box ❿ in place on stage

The EVOS$_{fl}$ microscope uses both mechanical and software controls for operation. Mechanical controls include the stage X-Y axis knobs, focusing knobs, objective selection wheel, and the light cube selection lever. Software controls are located in the control bar ❶ at the bottom of the screen. The channel indicator bar ❷ at the top of the screen shows the selected filter cube or transmitted light position. The login button ❸ displays the current user ID.

Refer to the ***QUICK-REFERENCE DIAGRAMS (p. 8)*** as needed. See also the ***CONTROLS GLOSSARY (p. 25)*** for more details.

FLUORESCENCE OPERATION

1. Turn on the microscope using the power switch ❹ on the right side of the microscope base.
2. Plug a USB flash drive into one of the USB ports ❺ on the right side of the microscope arm.
3. Place the sample on the stage, using a vessel holder if needed.

   Note: *Place slides with coverslips face up.*
4. Set the magnification using the objective selection wheel ❻ on the front of the microscope.
5. Move the light cube selection lever ❼ on the left side of the microscope all the way toward you. (The channel bar will highlight the "Transmitted" position.)
6. Turn on illumination using the LIGHT ON button ❽, located on the left side of the control bar.
7. Focus the sample using the focusing knobs ❾.
8. Place the light shield box ❿ on the stage, over the sample. This is important for optimal image quality.

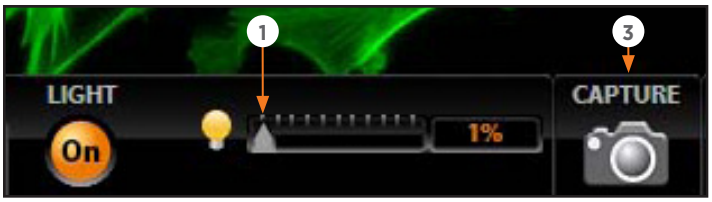
   Note: *If your application requires access to the sample, work in a dark room and use the Block slider to block light reflected from the condenser.*
9. Move the light cube selection lever to the desired fluorescence channel. (The channel indicator bar will highlight the selected light cube.)
10. Click the Find & Focus tab.
11. Click the LIGHT ON button to turn on fluorescence illumination.

⚠ ***WARNING! UV LIGHT HAZARD!*** *When using the DAPI channel, avoid exposure to beam and use protective shields. NEVER look directly at light.*

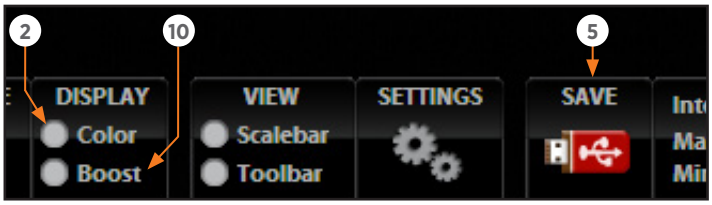
12. Adjust the focus as needed.
13. Adjust the illumination intensity if necessary, using the Illumination slider ❶ on the control bar or the mouse scroll wheel.

continued on next page

Fluorescence Operation, continued

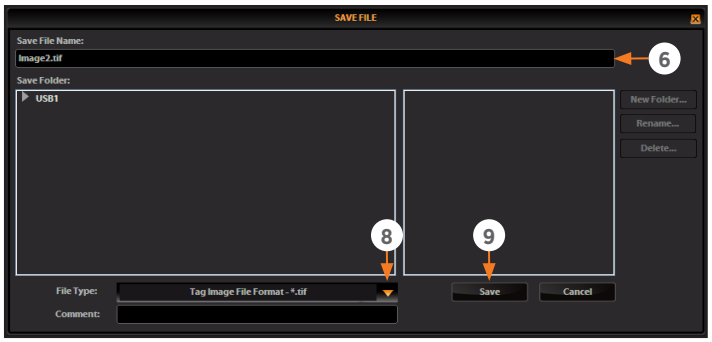


Illumination slider ❶ and Capture button ❸ in the control bar



Color ❷ and Boost ❿ options; Save button ❺

Overlay tab ❹



Save File dialog box

Virtual keyboard

Actual tab

Note: *When the Color option ❷ is off, overexposed pixels will appear red. Dim the illumination until the red highlights disappear to get the maximum level of brightness without any overexposed areas. See* ***p. 27*** *for instructions on changing the overexposed pixel display.*

C *For the color camera version, the overexposed pixels are always highlighted in red unless this feature is disabled in the settings.*

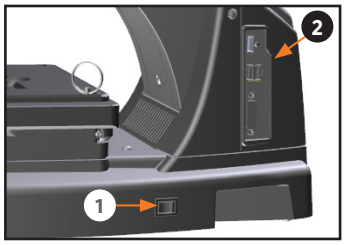
14. Click the Capture button ❸ to acquire the image.
15. Move the light cube selection lever to the next position and repeat steps 10-14 to acquire each fluorescence channel as desired.
16. Click the Overlay tab ❹ to show all channels in color overlay.
17. Adjust brightness and contrast for each channel to bring them into balance with each other.
18. Click the Save button ❺ to save the color image. The Save File dialog box will pop up.
19. Click in the Save File Name text field ❻ to enter the file name. A virtual keyboard will pop up. After entering the file name, click the Accept button ❼ at the lower right of the keyboard.
20. Choose the file type ❽ and click the Save button ❾.

Note: *See* ***Saving Images & Working with Files (p. 14)*** *for more information.*

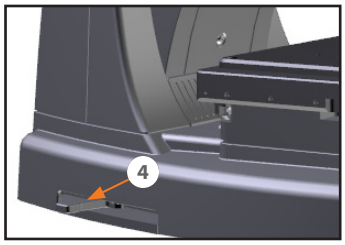
HELPFUL TIPS

- The Boost option ❿ amplifies faint signals near the background. This only changes the display; it does not alter the data.
- Turn off the Color option ❷ to display the image in grayscale. This often shows more detail than a color image.
- Find & Focus uses a shorter exposure time (100 ms) and lower illumination (appx. 60%) compared to image capture settings. This minimizes photobleaching and phototoxicity effects. When you capture an image, illumination and exposure time automatically adjust for best image quality and then reset to lower levels after the capture.
- The Actual tab provides full-powered illumination and actual exposure times for live viewing of the sample.

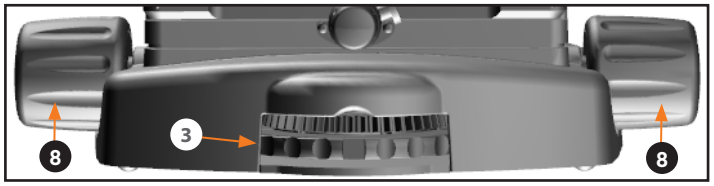
Note: *With longer exposure times (more than 200 ms) there will be a lag between focusing the image and seeing the focus change onscreen.*



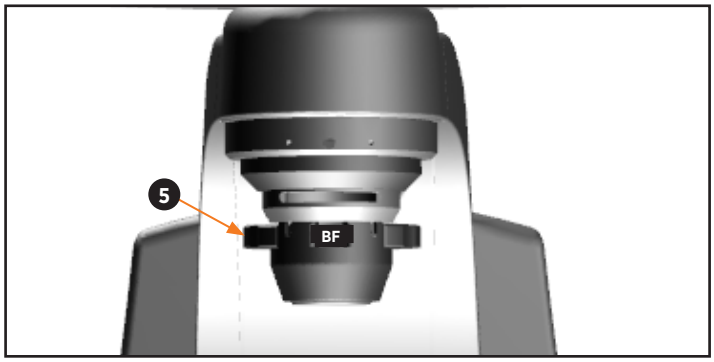
Power switch ❶ and data ports ❷



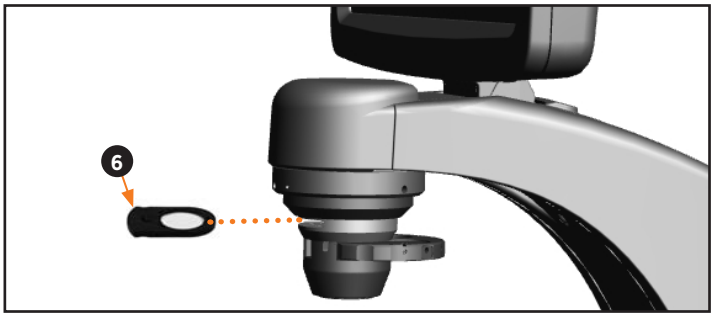
Light cube selection lever ❹



Objective selection wheel ❸ and focusing knobs ❽



Phase annuli selector ❺



Insert slider ❻ into condenser

LIGHT ON button ❼ in the control bar

BRIGHTFIELD OR PHASE CONTRAST OPERATION

1. Turn on the microscope using the power switch ❶ on the right side of the microscope base.
2. Plug a USB flash drive into one of the USB ports ❷ on the right side of the microscope arm.
3. Place the sample on the stage, using a vessel holder if needed.

   Note: *Place slides with coverslips face up.*
4. Set the magnification using the objective selection wheel ❸ on the front of the microscope.
5. Move the light cube selection lever ❹ on the left side of the microscope all the way toward you. (The channel indicator bar will highlight the "Transmitted" position.)
6. Turn the phase annuli selector ❺ to the position that corresponds to the selected objective and contrast method.
7. Insert the appropriate condenser slider ❻ into the slot on the condenser assembly for optimal image quality.

   Five condenser sliders are included with EVOS *fl* microscopes. For brightfield applications you may use the **Diffuser** or **Pinhole** slider, or a **Meniscus** slider.

   Due to variances in sample size, color and thickness, actual slider use may differ from the suggested slider use described below.

   Diffuser slider: Brightfield, 2x or 4x (for flat field illumination)

   Pinhole slider: Brightfield, all magnifications (to enhance contrast)

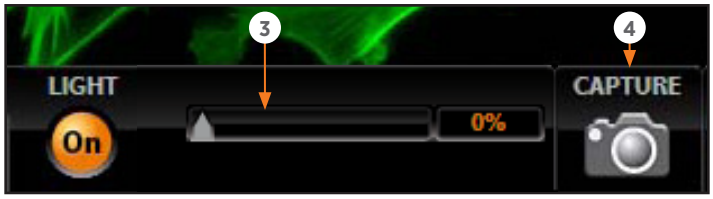
   Meniscus A slider: Brightfield, 2x (for low-volume fluid in a multi-well dish)

   Meniscus B slider: Brightfield, 2x (for high-volume fluid in a multi-well dish)
8. Turn on illumination using the LIGHT ON button ❼, located on the left side of the control bar.
9. Focus the sample using the focusing knobs ❽.

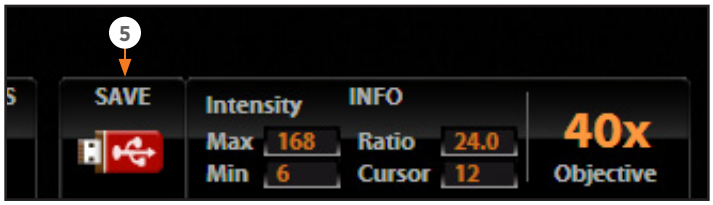
continued on next page

Brightfield or Phase Contrast Operation, continued

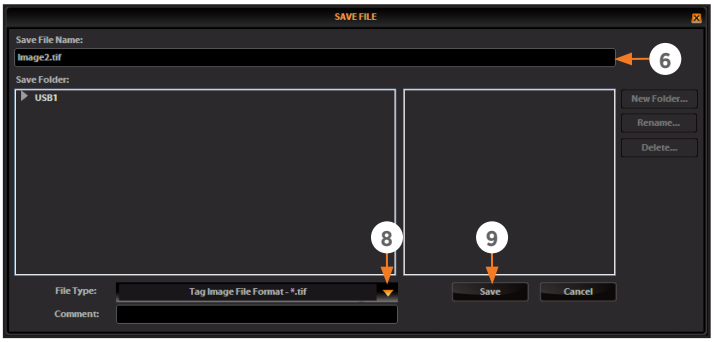
Light shield box ❶ in place on stage (cover ❷ shown in place)



Illumination slider ❸ and Capture button ❹ in the control bar



Save button ❹



Save File dialog box

Virtual keyboard

10. When switching from fluorescence to transmitted light with the light shield box ❶ on the stage, remove the light shield box cover ❷ so the light from the condenser can pass through.

11. Adjust the illumination intensity if necessary, using the Illumination slider ❸ on the control bar or the mouse scroll wheel.

    Note: *Overexposed pixels will appear red. Dim the illumination until the red highlights disappear to get the maximum level of brightness without any overexposed areas. See* ***p. 27*** *for instructions on changing the overexposed pixel display.*

12. Click the Capture button ❹ to acquire the image.

13. Click the Save button ❺ to save the image. The Save File dialog box will pop up.

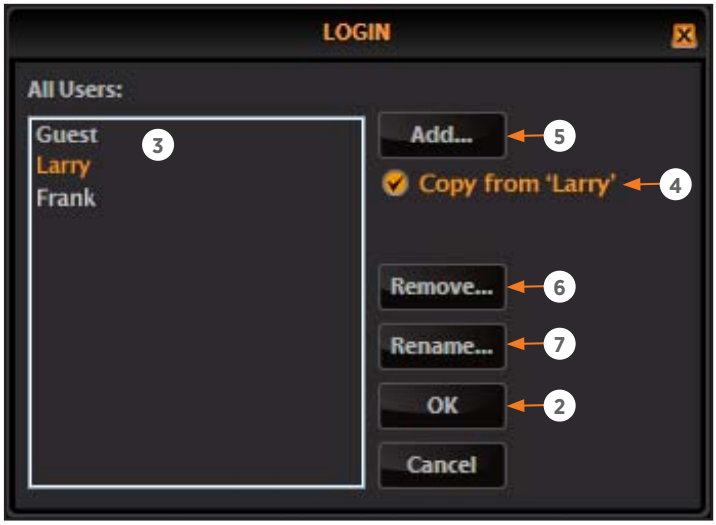
14. Click in the Save File Name text field ❻ to enter the file name. A virtual keyboard will pop up. After entering the file name, click the Accept button ❼ at the lower right of the keyboard.

15. Choose the file type ❽ and click the Save button ❾.

    Note: *See* ***Saving Images & Working with Files (p. 14)*** *for more information.*

ADVANCED OPERATION

Login button ❶ (set to Guest profile)



Login dialog box

Virtual keyboard

Settings: Basic Tab (to change default login)

LOGGING IN/CREATING NEW USER LOGINS

EVOS keeps settings in memory for each user ID, so multiple users can work with the same EVOS microscope without having to reset their preferences.

To use this feature, set up a user profile for each regular user. You may also assign user IDs for experiments in progress.

Note: *User profiles are not password protected. All users should verify they are logged in correctly to avoid changing others' settings.*

LOG IN WITH AN EXISTING PROFILE

1. Click the login button ❶ at the bottom left of the screen. (This is the AMG logo with the current user profile indicated above.)
2. Select the desired user profile and click OK ❷.

   Note: *No password is necessary to log in.*

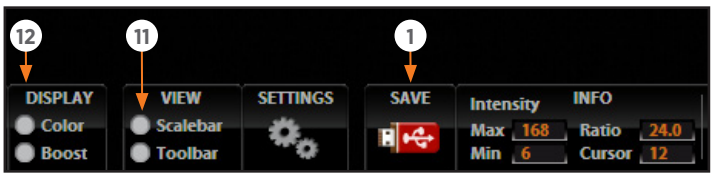
ADD OR REMOVE A USER PROFILE

1. Click the login button ❶.
2. To copy an existing profile, highlight the profile in the user list ❸, select the "Copy from 'name'" option ❹, and click the Add button ❺. The virtual keyboard will pop up so you can name the new profile.
3. To create a new user profile without copying any settings, deselect the "Copy from" option ❹, click the Add button ❺, and enter a user name.
4. After adding a new user profile, click OK ❷ to log in under that name and adjust settings as desired. When you switch off, EVOS will save your settings to memory.
5. To remove a user profile, highlight it and click the Remove button ❻. A confirmation dialog box will pop up. Deleting the user profile will remove all its associated settings from memory.
6. To rename a user profile, click the Rename button ❼ and enter the new name.

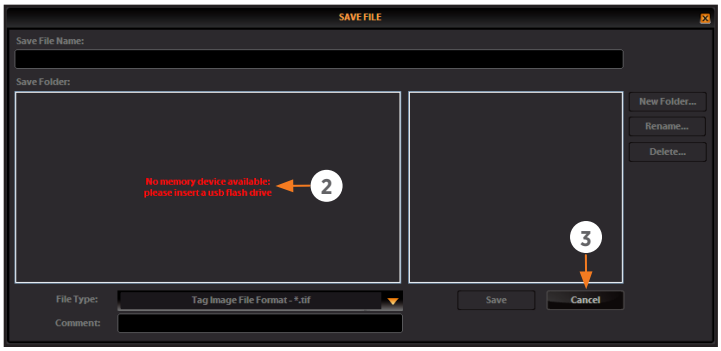
CHANGE THE DEFAULT LOGIN

The default user login is Guest; to set the default login as the last active user, go to the Basic tab of the Settings dialog box and uncheck the "Default to Guest on startup" ❽ option.

Note: *For multiple users, we recommend leaving the "Default to Guest on startup" option checked.*



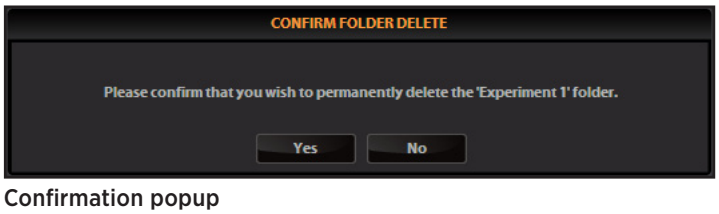
Save button ❶, Scalebar option ⓫, and Display options ⓬



Save File information message

Save File dialog box

Virtual keyboard



Confirmation popup

SAVING IMAGES & WORKING WITH FILES

When you click the Save button ❶, the Save File dialog box appears. If there is no USB flash drive or network connection in place, an information message ❷ will appear. Click the Cancel button ❸ to clear this message.

In the Save Folder list ❹ and the saved files list ❺, selected items will appear orange. If a USB keyboard is installed, the virtual keyboard will not appear. Note that pressing the Enter key on a physical keyboard is like pressing the Save button in the Save file dialog box.

1. Click in the Save File Name text field ❻. Enter a file name and click the Accept button ❼.

   To overwrite a file, simply select the name of the file from the saved files list ❺ instead of clicking on the Save File Name text field. A Save As confirmation dialog box will pop up. It is not possible to recover an overwritten file.

2. Click on the name of a folder in the Save Folder list ❹ to select the destination for the new image.

3. To create a new folder, first click the name of the parent folder, and then click the New Folder button ❽ to enter a folder name and, if desired, date.

   Note: *Clicking the Date button ❾ anywhere within a text field will automatically insert the current date (MM-DD-YYYY) wherever the cursor is in that field.*

4. Select a file format (.tif, .png, .jpg or .bmp) from the File Type drop-down menu ❿.

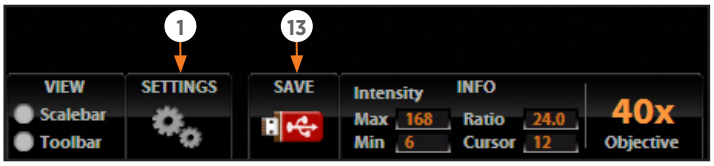
   Note: *To save a 16-bit image, select .tif or .png and ensure the Scalebar ⓫ and Color/Boost ⓬ options are off. File types .jpg and .bmp (as well as images of all types with the Scalebar, Color, or Boost options engaged) only save at 8-bit depth.*

5. Click in the Comment text field ⓭ to enter a comment and date (optional).

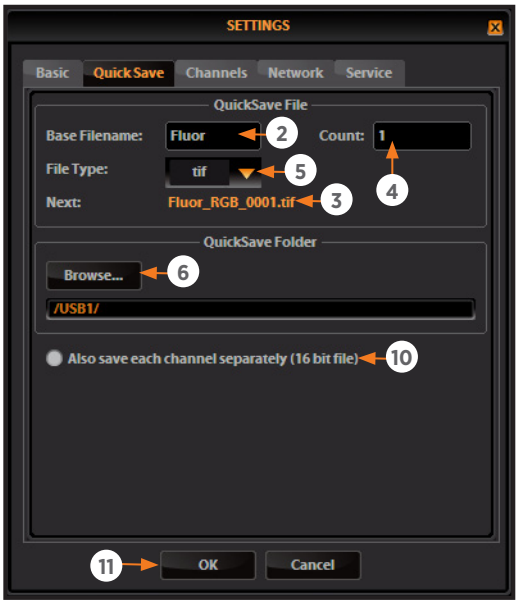
6. Click the Save button ⓮ to save the file.

7. To delete a file or folder, highlight the item on the list and click the Delete button ⓯. A confirmation dialog box will pop up. It is not possible to recover a deleted file.

8. To rename a file or folder, highlight the item on the list and click the Rename button ⓰. The virtual keyboard will pop up; you can use the Clear button ⓱ to reset to a blank field.



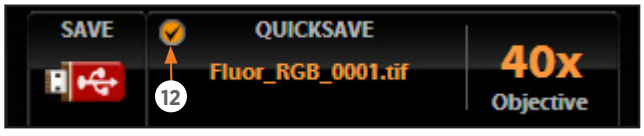
Settings button ❶ and Save button ⓭



Settings: QuickSave Tab

Virtual keyboard

Browse popup (to select a destination folder)



QuickSave option radio button ⓬

USING THE QUICKSAVE OPTION

QuickSave allows you to save multiple images under a single base file name. Simply specify the settings and select the QuickSave option (in the Overlay tab), and EVOS will save each image with a single click of the Save button.

1. Click the Settings button ❶ to open the Settings dialog box, and then select the QuickSave tab.
2. Click in the Base Filename text field ❷ and enter a name that describes the imaging session. The orange "Next" file name ❸ will reflect the information entered.
3. Click in the Count text field ❹ to enter the starting number, if you do not want to start at 1. The orange "Next" file name will reflect the information entered.
4. Select a file format (.tif or .png) from the File Type drop-down menu ❺.
5. Click the Browse button ❻ to select a destination folder for the QuickSave files. In the Browse popup, highlight the desired folder and click OK ❼.
6. To create a new folder, first click the name of the desired parent folder, and then click the New Folder button ❽. Enter a folder name and date, if desired. After creating the new folder, click OK ❼ to close the Browse popup.

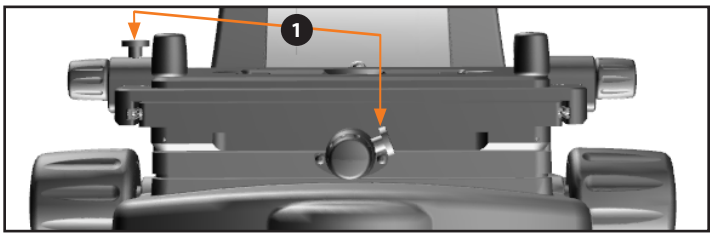
   Note: *Clicking the Date button ❾ anywhere within a text field will automatically insert the current date (MM-DD-YYYY) wherever the cursor is in that field.*
7. Select "Also save each channel separately" ❿ to save multiple channels for each image. This will create up to five files per captured image, named according to the following conventions:

   - BaseName_RGB_0001.tif (Overlay image)
   - BaseName_channel_0001.tif (where "channel" is the selected channel, such as GFP, RFP, DAPI, or TRANS)

   When optional LED light cubes are installed, the files will automatically save channels with their names. See ***Changing LED Light Cubes (p. 23)*** for more information.
8. Click OK ⓫ to accept QuickSave settings.
9. Select the Overlay tab and click the radio button for the QuickSave option ⓬.

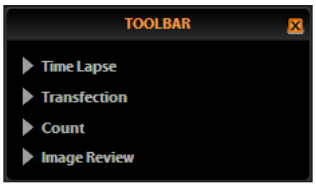
C ***Note:*** *The color camera version displays the QuickSave option in all tabs.*

10. After acquiring an image with the Capture button, click Save ⓭. The image will be saved as specified in the QuickSave settings.

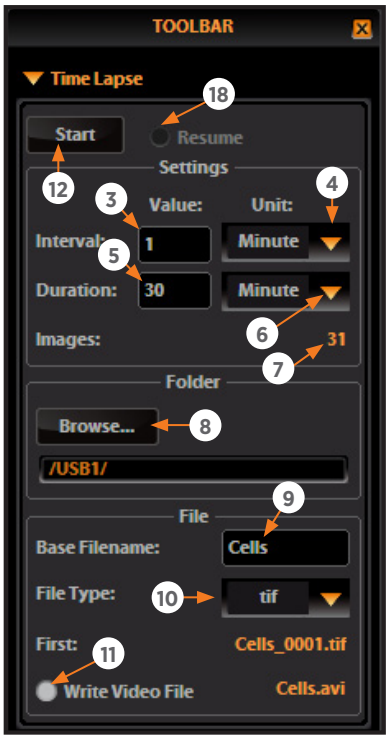


X-axis and Y-axis stage brakes ❶

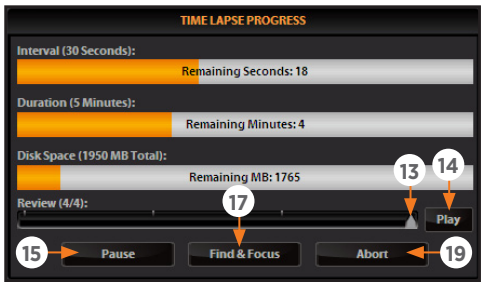
Toolbar option ❷ in the control bar



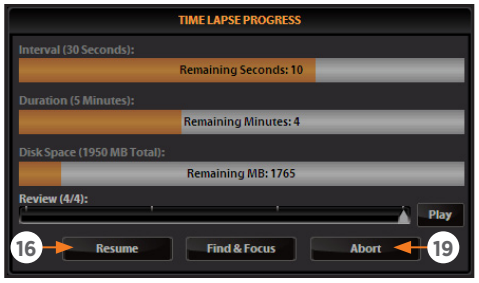
Toolbar



Time Lapse tool



Time Lapse Progress popup



Time Lapse Progress paused

RECORDING TIME LAPSE IMAGES

With EVOS, you can set up your cells and program the microscope to record time lapse images. To use this feature, open the Time Lapse tool in the toolbar, specify the settings, and click Start. You may pause or cancel sessions in progress.

START A TIME LAPSE SESSION

1. Once the specimen is focused and ready, tighten the stage brakes ❶ to prevent the stage from drifting during the session.
2. Open the Toolbar ❷ and expand the Time Lapse tool.
3. Click the Interval text field ❸ and enter a value.
4. Choose a unit of measure for the capture interval from the interval Unit drop-down menu ❹.
5. Click the Duration text field ❺ and enter a value.
6. Choose a unit of measure for the duration from the duration Unit drop-down menu ❻.

   Note: *The Images field ❼ shows the total number of images for the session.*
7. To save the session in a folder other than the default location, click the Browse button ❽ to select the destination.
8. In the Browse popup (shown on ***p. 15***), highlight the desired folder and click OK.
9. Under File, click the Base Filename text field ❾ to enter a name, and then choose a file type (.png or .tif) from the File Type drop-down menu ❿.
10. To create a video (.avi) file, select Write Video File ⓫.
11. Click the Start button ⓬ to begin the time lapse session. EVOS will display the Time Lapse Progress popup as long as the session is active.

    Note: *The Review slider ⓭ lets you review the images already captured during the current session. The Play button ⓮ shows all the images in sequence.*

PAUSE AND RESTART A TIME LAPSE SESSION

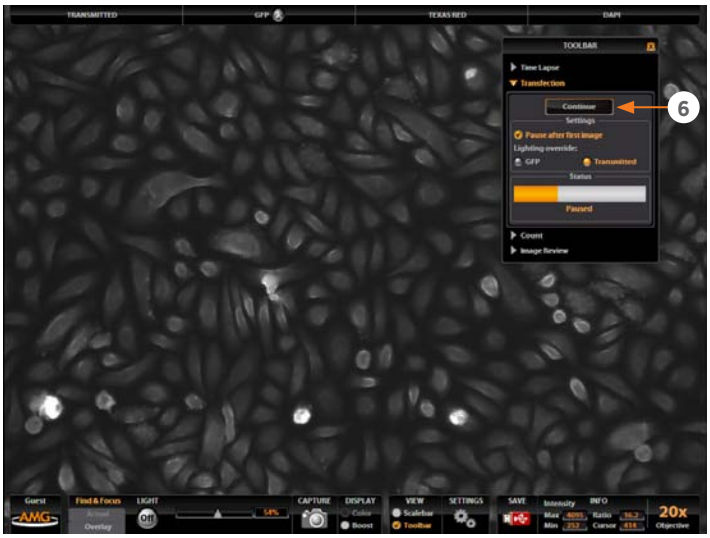
In the progress popup, click the Pause button ⓯ to suspend the time lapse captures. The progress popup will dim, and a Resume button ⓰ will replace the Pause button.

Alternatively, to pause and adjust the settings, click the Find & Focus button ⓱. Click the Start button ⓬ to resume the time lapse capture sequence, or uncheck the Resume radio button ⓲ and start a new time lapse session.

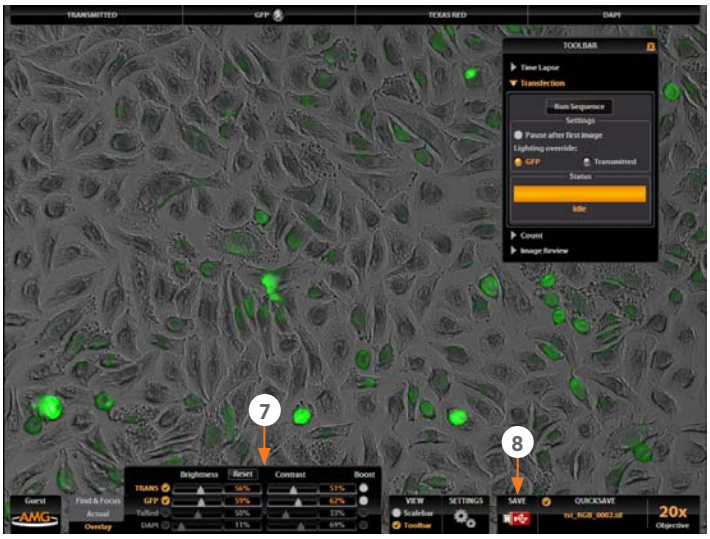
ABORT A TIME LAPSE SESSION

In the progress popup, click the Abort button ⓳. A dialog box will pop up, giving you the option to delete or keep the files already saved. Clicking Cancel will resume the session.

Sample ready for transfection analysis



Sequence paused between images



Transfection overlay image

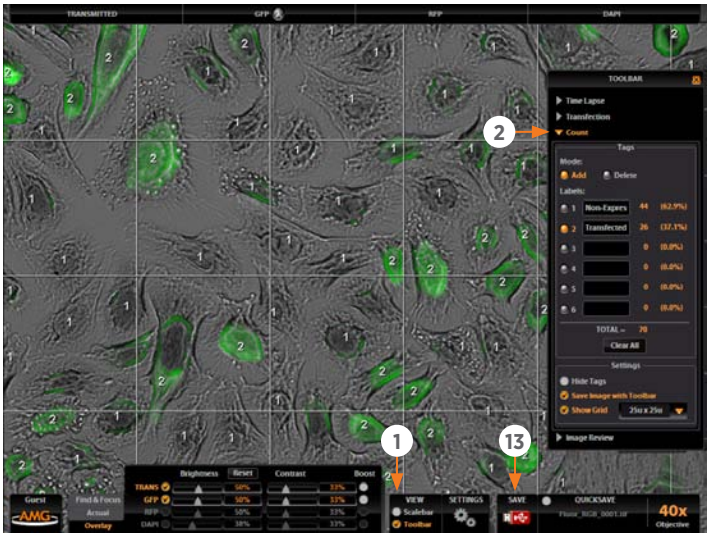
USING THE TRANSFECTION TOOL

EVOS' Transfection tool expedites the capture and overlay of images for transfection analysis.

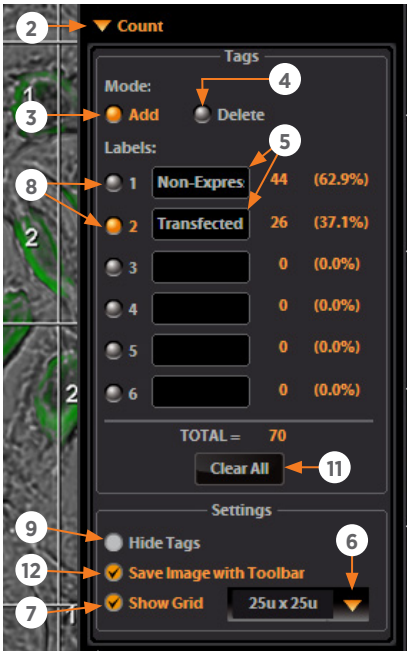
1. Choose a light cube, focus on the sample, and adjust the lighting. See steps 1-13 of ***Fluorescence Operation (p. 9)*** for detailed instructions.
2. Open the Toolbar ❶ and expand the Transfection tool ❷.

   Note: *The "Pause after first image" option ❸ allows you to adjust focus, if necessary, before capturing the transmitted light channel.*
3. Click the Run Sequence button ❹. The sequence always starts with the fluorescence channel and finishes with the transmitted light channel.

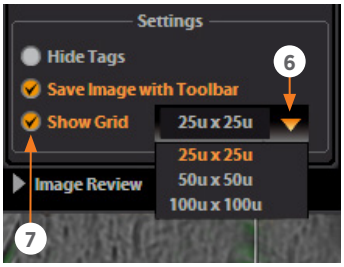
   Note: *The Lighting Override feature ❺ allows you to select a channel to activate with the LIGHT ON/OFF button.*
4. If the Pause option ❸ is selected, adjust focus and click the Continue button ❻. If Pause is deselected, go to step 5.
5. The dual-channel image will display with the Overlay tab selected. Adjust the brightness and contrast settings ❼ as desired, and save the image ❽. See ***Saving Images & Working with Files (p. 14)*** for detailed instructions.



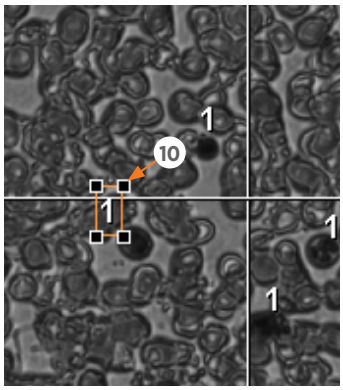
Toolbar option ❶ and Count tool ❷



Count tool



Detail of grid size menu ❻ and Show Grid option ❼



Selected tag ❿; drag to move

COUNTING CELLS

The Count tool streamlines cell counting by marking items with up to 6 labels onscreen. As you tag items, EVOS will keep a running tally of counts with percentages for each label assigned. Document your results simply by saving the tagged image, with the Count tool displaying the totals.

1. Acquire an image. See ***BASIC OPERATION (p. 9)*** for detailed instructions.
2. Open the Toolbar ❶ and expand the Count tool ❷.

   Note: *Use the Add mode ❸, which is active by default. The Delete mode ❹ is for removing tags that have already been added.*
3. Click in a black Label text field ❺ to name a label. You may use up to 6 labels.
4. Under Settings, you may choose a grid size in the drop-down menu ❻ or leave the Show Grid option ❼ inactive.
5. Select a Label button ❽ and left-click at each point onscreen to tag the items for that category. Switch labels as desired; EVOS will tag for the selected label.

   Note: *To use Digital Zoom while counting cells, first suspend the Count tool. Either select the Hide Tags setting ❾, click the triangle ❷ to minimize the Count tool, or click the Toolbar option ❶ to minimize the whole Toolbar. When the Count tool is suspended, the left mouse button will behave according to the rules described in* ***Using Digital Zoom (p. 20)****. After zooming, reactivate the Count tool by deselecting Hide Tags or reopening the tool.*
6. To move a tag, select ❿ and drag it. Left-click anywhere else onscreen to deselect.
7. To delete a tag, right-click it, or choose the Delete mode ❹ and left-click it. You may also use the Clear All button ⓫ to delete all tags for all labels.
8. To save an image showing the labels, counts and percentages as shown in the Count tool, select the Save Image with Toolbar option ⓬ and click the Save button ⓭. Deselecting this option will produce an image saved with the tags only.

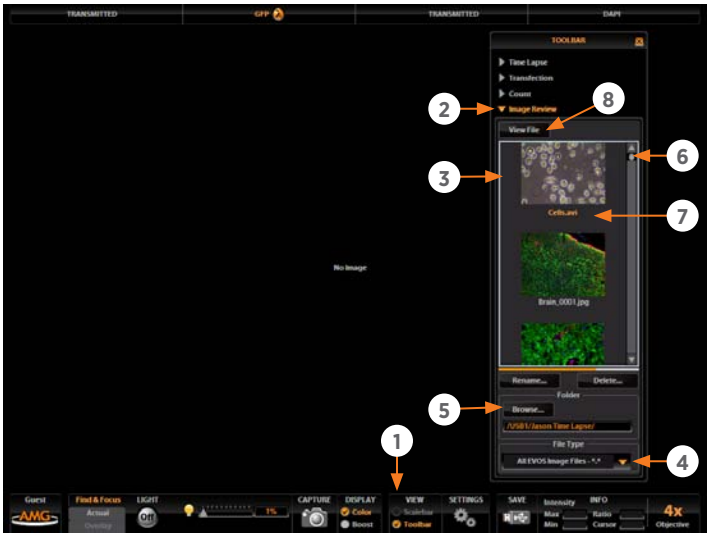


Image Review Tool: Find and select a file

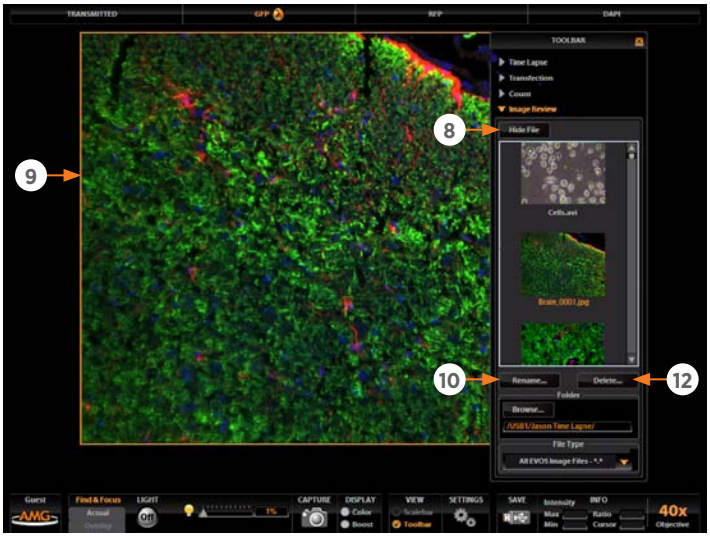
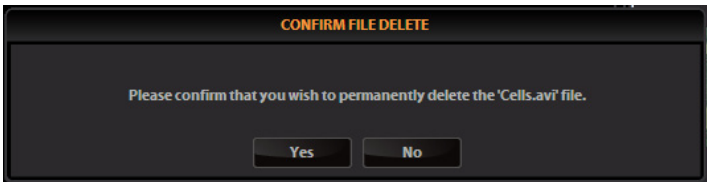


Image Review Tool: View, rename or delete a file

Virtual keyboard



Confirmation popup

REVIEWING IMAGES

The Image Review tool allows you to review still images or play video files from the USB drive or network connection. You may also use this tool to rename or delete saved files.

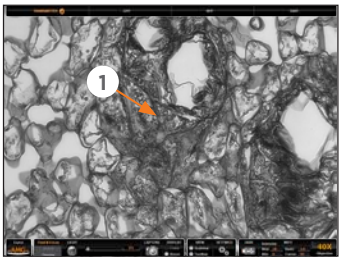
1. Open the Toolbar ❶ and expand the Image Review tool ❷.
2. The preview list ❸ displays thumbnail images for all viewable files in the selected directory. (The top-level USB directory is selected by default.) If there are no viewable files in the directory, the preview area will be empty.

   Note: *The File Type drop-down menu ❹ filters files by type. By default, it is set to display all files with .png, .tif, .jpg, .bmp, or .avi extensions.*
3. If the image or video file you wish to review is not in the directory displayed, click the Browse button ❺ to find and open the desired directory.
4. Use the scroll bar ❻ as needed to search the preview list for the desired file. Click the image to select it. The selected file name appears orange ❼.
5. Click the View File button ❽ to display the image in the image review port ❾. This button toggles between View File and Hide File. Hiding the file closes the image review port.

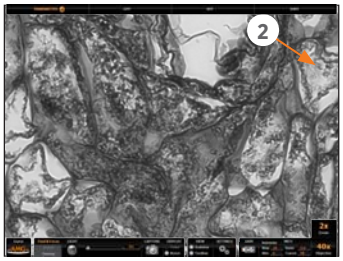
   Note: *Double-clicking the thumbnail image will also toggle between displaying and hiding the file.*
6. To zoom the image in the review port, double-click the area of interest; right-click to restore normal magnification. Refer to ***Using Digital Zoom (p. 20)*** for more detailed instructions.
7. To rename a file, select it and click the Rename button ❿. The Virtual Keyboard will pop up. Enter the new file name and click the Accept button ⓫.
8. To delete a file, select it and click the Delete button ⓬. A confirmation dialog box will pop up. It is not possible to recover a deleted file.

USING DIGITAL ZOOM

EVOS can zoom the image onscreen, quickly allowing a closer look. Simply double-click live or captured images to zoom them. In the images below, the numbered arrows indicate click points. Also note that the zoom factor display appears over the selected objective display.



Live image at 40x magnification



Live 40x image zoomed 2x

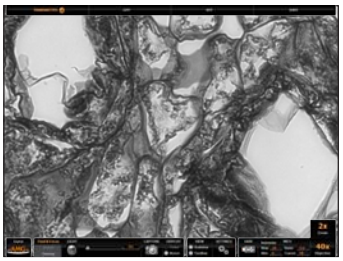
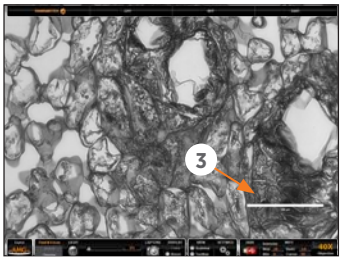


Image recentered

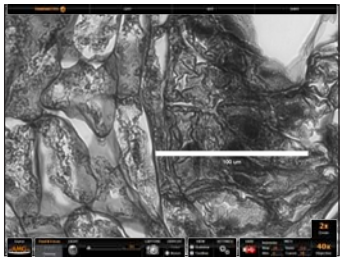
ZOOM AND RECENTER LIVE IMAGES

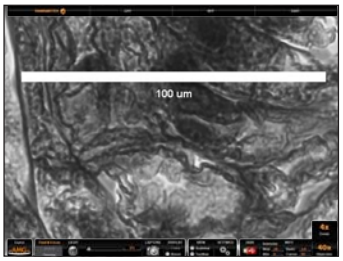
Note: *Live images can only zoom to 2x. To zoom an image at higher levels, you must first capture it.*

1. Double-click the area of interest ❶ in the image onscreen. EVOS will display a view zoomed 2x, centered on the point clicked.
2. In the enlarged image, double-click on any point in the middle area of the screen to recenter the image ❷. (EVOS will place points from the outer edges of the screen as close to the center as possible.) You may recenter repeatedly.
3. Right-click anywhere on the image to restore the view to unzoomed magnification.

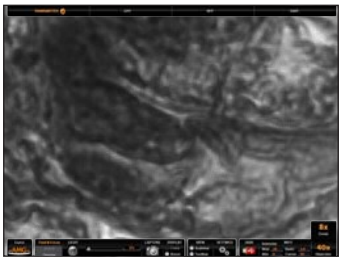


Captured image at 40x magnification, with scalebar


Captured 40x image zoomed 2x



Captured 40x image zoomed 4x


Captured 40x image zoomed 8x

ZOOM CAPTURED IMAGES

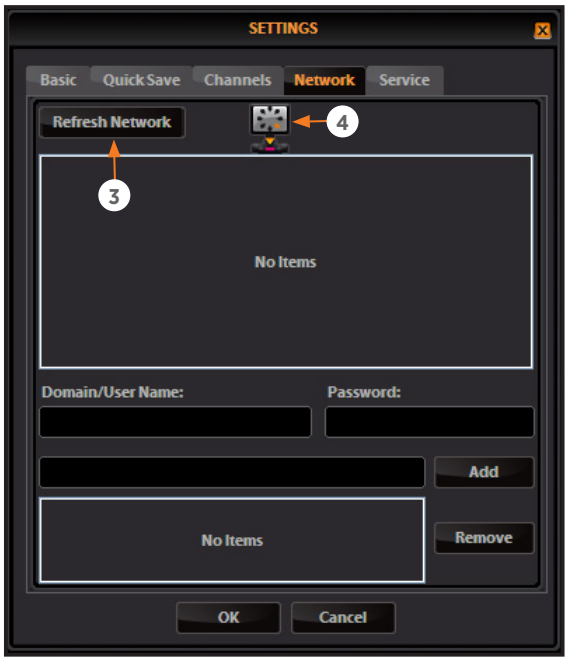
1. Double-click the area of interest ❸ in the image onscreen. A view zoomed 2x, centered on the point clicked, will appear.
2. In the enlarged image, double-click again on any point ❸ to double the digital zoom level. The enlarged image will center around the point clicked.
3. Continue double-clicking to double the digital zoom value as desired. It is possible to zoom in to the pixel level of the digital image.
4. Right-click to restore the view to unzoomed magnification.

Note: *Capturing and saving a zoomed image will result in a file showing the actual magnification, not the zoomed magnification. If the scalebar is active, it will appear in the file.*

Recommended USB-to-Ethernet adapters

Login button ❶ (set to Guest profile)

Settings button ❷



Refreshing the network connection

CONNECTING EVOS TO A NETWORK

You can log EVOS onto a Windows/SMB network via an Ethernet cable connection and save captured images directly to shared folders on the network.

Note: *If your network is on a Linux server, it will have to use Samba in order for EVOS to find it. Contact your network administrator for help if a physically connected EVOS cannot find the network.*

ITEMS NEEDED FOR ETHERNET CONNECTION

To set up the network connection, you need the following items (not included):

- Ethernet cable
- USB-to-Ethernet adaptor

  Note: *For best results, use one of these compatible USB-to-Ethernet adapters:*

  - Belkin model F5D5050
  - Cisco Linksys model USB300M
  - D-Link model DUB-E100
  - TRENDnet model TU-ET100C
  - TRENDnet model TU2-ET100

LOGGING ONTO THE NETWORK

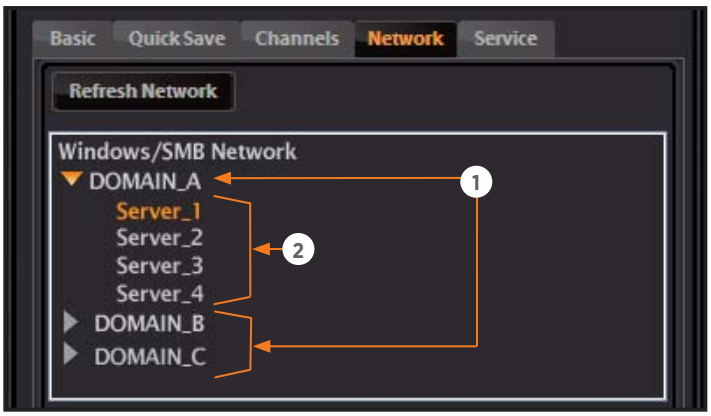
1. Verify the microscope is powered on and the network cable is plugged into the correct jack and connected via the adapter to the USB port.
2. Be sure you are logged in under your own EVOS user ID. The current user ID ❶ is displayed above the AMG logo in the bottom left corner of the screen. See ***Logging In/ Creating New User Logins (p. 13)*** for more information.
3. Click the Settings button ❷ to open the Settings dialog box, and then select the Network tab.

   Note: *EVOS will try to connect for about 30 seconds. If there is a problem with the connection, the Network page will display "No Items."*

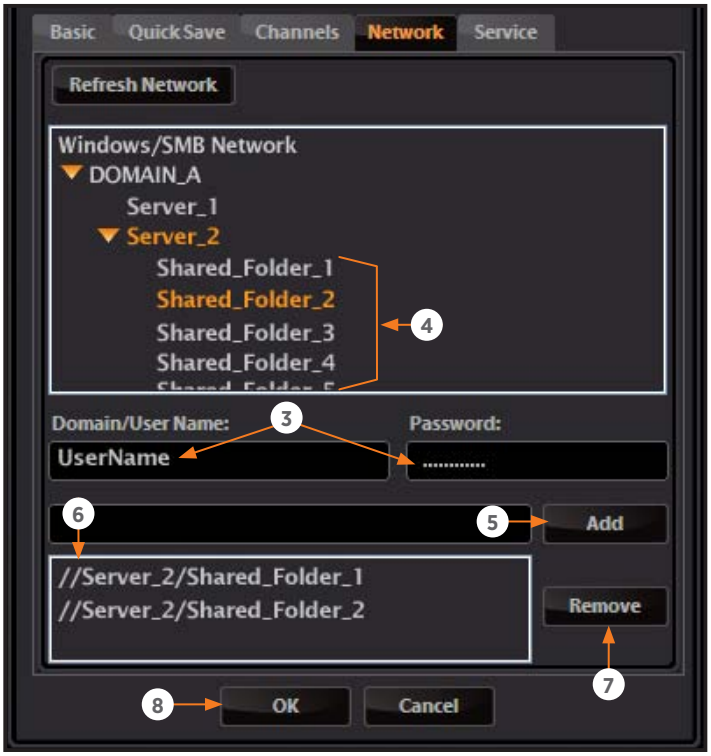
   Double-check the physical connections and click the Refresh Network button ❸. During the refresh, a progress icon ❹ will appear.

   Unless there is an issue with the network, or you are using an incompatible adapter, refreshing the connection should resolve the problem within a few moments. Contact your network administrator for help if the problem persists.

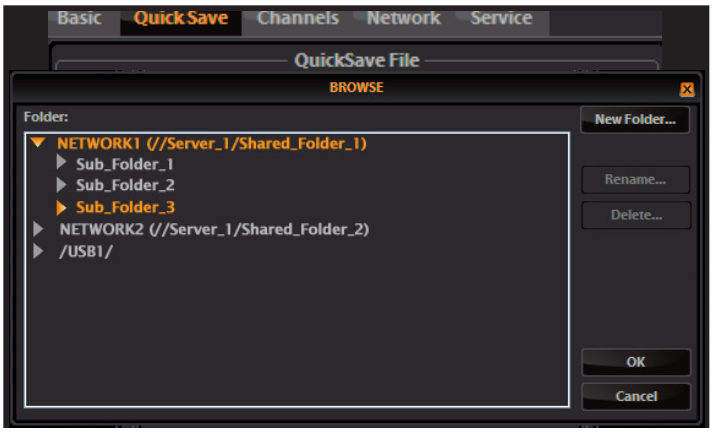
continued on next page



Windows/SMB Network, with available domains ❶ and servers ❷



Adding shared folders; ***The Network page only displays the top-level folders. View subfolders in the Browse popup when saving a file.***



Network destinations in the QuickSave Browse popup

4. The upper list box of the Network page will display the top level (available domains) of the Windows/SMB network file tree. Click the triangle icon, or double-click the domain name, to expand a domain ❶ and display the available servers ❷.

   Note: *If a domain, server, or shared file appears on the file tree without a triangle icon, and you are not able to expand or open it, your permission to access that item is restricted.*

5. Enter your network user name and password in the login fields ❸ and select a server to view the top level of shared folders ❹ on that server. You may not navigate below the top-level shared folders on the Network page.

   Note: *If you are using a new user name or password, verify your login on a PC before using it to log EVOS onto the network.*

6. After the network accepts your login, it will display the list of available shared folders ❹ on the selected server. Select a shared folder and click the Add button ❺ to include it in the list of possible file destinations. The folder should appear on the list box ❻ below the Add button. If it does not, contact your network administrator for help.

   Note: *You may add multiple shared folders to the list, but you can only use a single login on any one server or domain. We recommend that each EVOS user establish network connections under his or her own EVOS user ID. See* ***Logging In/ Creating New User Logins (p. 13)*** *for more details on EVOS user IDs.*

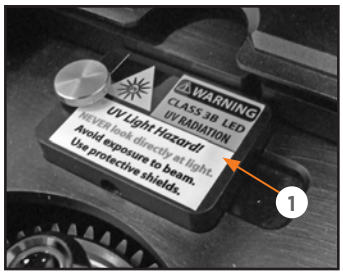
   Your EVOS user ID will remember your network login. (It is encrypted in the microscope's non-volatile memory.) All your connections and login information will be hidden from other EVOS user profiles.

7. If you need to remove a shared folder from the destinations list, select the folder name and click the Remove button ❼.

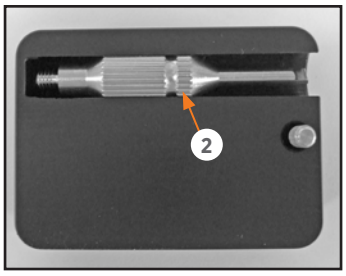
8. Click OK ❽ to save your network settings.

9. To verify your list of network destination folders, go to the QuickSave tab and click the Browse button to display the QuickSave Browse popup.

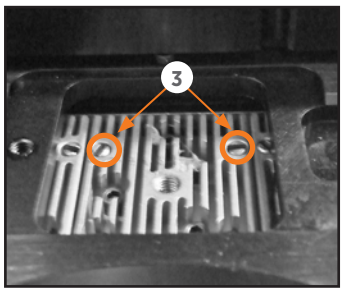
   All your selected network destinations, as well as any USB flash drives currently plugged in, will appear in the Browse popup. These locations will also be available in the Save dialog box and through the Browse button in the Time Lapse and Image Review tools.



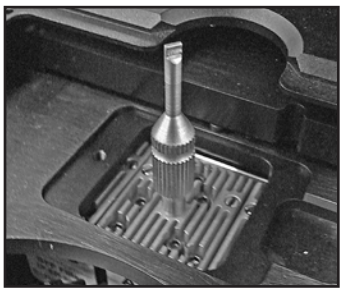
Remove LED light cube access cover ❶ (under stage)



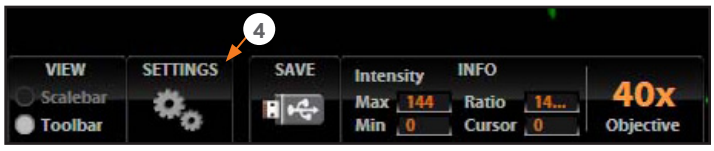
Light cube tool ❷ (under access cover)



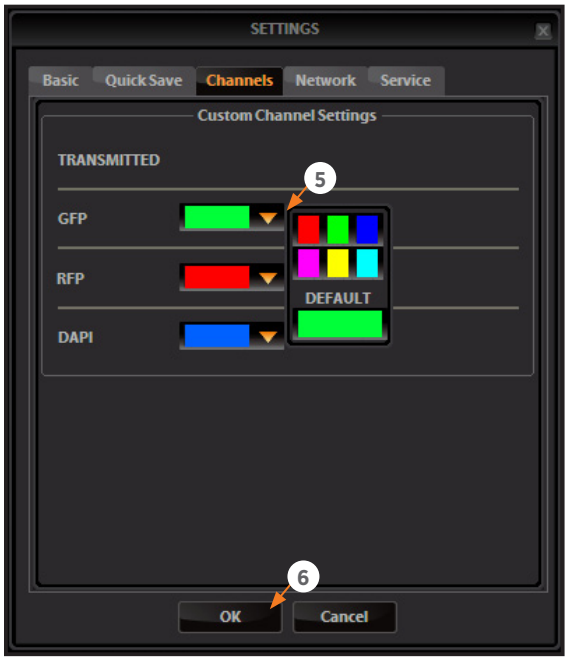
Loosen slotted screws ❸ with tool



Light cube tool attached to light cube



Settings button ❹



Assigning custom channel display colors
(not applicable for the color camera version)

CHANGING LED LIGHT CUBES

INSTALL LIGHT CUBE

Optional LED light cubes are available; see ***PARTS & ACCESSORIES (p. 35)*** for details. Each LED light cube is coded so EVOS will automatically recognize it in any position.

⚠ ***WARNING! UV LIGHT HAZARD!*** *This microscope uses a Class 3B ultraviolet LED for the DAPI channel.* ***Before changing LED light cubes, power off the microscope.***

1. Move the stage back to allow access to the light cube access cover ❶, centered under the back of the stage.
2. Loosen the thumbscrew to remove the light cube access cover and remove the light cube tool ❷ under it.
3. Move the light cube selection lever to the position you want to use for the new cube.
4. Use the light cube tool to loosen the 2 slotted screws ❸ as shown in photo at left.
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 then 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 2 slotted screws so that the screw heads sit flush with the ridges on the light cube.
9. Slide the tool into its storage slot before replacing the light cube access cover.

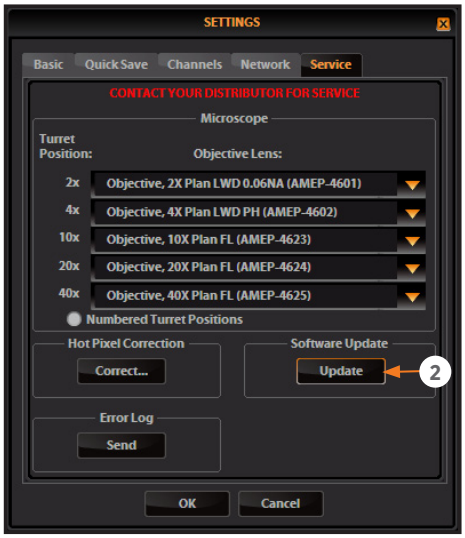
ASSIGN CUSTOM CHANNEL DISPLAY COLORS

You may assign custom display colors for each light cube in the Channels tab of the Settings dialog box.

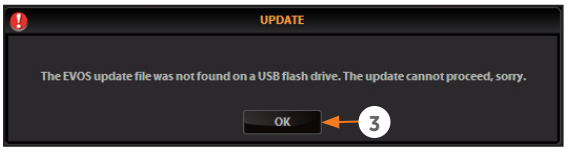
C ***Note:*** *The color camera version does not include this feature.*

1. Click the Settings button ❹ to open the Settings dialog box, and then select the Channels tab.
2. Select the appropriate color from the drop-down menu ❺ for each light cube you wish to customize.
3. Click OK ❻ to accept the custom color assignments. The display change will take effect with the next image acquired in the customized channel.

Settings button ❶



Settings: Service Tab



Missing update notification

Verification progress bar

Update confirmation popup

Installation progress bar

UPDATING SOFTWARE

Periodically, AMG adds functionality and other improvements to the EVOS user interface. We recommend keeping your EVOS$_{fl}$ microscope up to date with the latest software. If you have any questions about software updates, contact your local distributor. If you do not have your distributor information, you can look it up at the AMG website or contact AMG Customer Service (see p. 34).

DOWNLOAD SOFTWARE UPDATE

Software updates are available under the Support menu at the AMG website.

1. Download the update directly to the top level of a USB flash drive with at least 30MB available. Do not open or rename the file on your computer; EVOS will verify and install it during the update process.

2. Download the current user guide for EVOS$_{fl}$ from the AMG website. The updated user guide covers the new software functionality when features are added.

Alternatively, you can get the latest software and documentation updates from your local EVOS distributor.

INSTALL SOFTWARE UPDATE

1. Plug the USB stick into the data port on the lower right side of the EVOS support arm.

2. Click the Settings button ❶ to open the Settings dialog box, and then select the Service tab.

   Note: *Changing settings in the Service tab will affect the microscope's performance. If service beyond the software update is needed, please contact your EVOS distributor.*

3. Click the Update button ❷ in the Service tab. A verification progress bar should appear. If a missing update notification pops up, be sure the USB with the zipped update file is fully plugged in. Click OK ❸ and then click the Update button again.

4. After file verification, an update confirmation dialog will pop up. Check the revision details and click Yes ❹ to start the update.

5. The screen will display update progress. When the update is complete, the main EVOS$_{fl}$ screen will reappear.

   ⓘ ***IMPORTANT!*** *Do not power off, unplug the USB stick, or add/remove any devices during the update.*

CONTROLS GLOSSARY

This section describes all the onscreen and mechanical controls in detail. To see the most commonly used controls in context, refer to the ***QUICK-REFERENCE DIAGRAMS (p. 8)***.

ONSCREEN CONTROLS

This glossary is not alphabetized. Onscreen items are listed from top to bottom first, and then from left to right.

Channel indicator bar with GFP channel highlighted

CHANNEL INDICATOR BAR

The ***channel indicator bar*** (top) highlights whichever one of the following light channels is currently selected:

- Lever position 1 (Transmitted in example; closest to front of microscope)
- Lever position 2 (GFP in example; second from front)
- Lever position 3 (RFP in example; second from back)
- Lever position 4 (DAPI in example; closest to back)

See also ***Light Cube Selection Lever (p. 30).***

Login button (set to Guest profile)

LOGIN BUTTON

The ***Login button*** (AMG logo, bottom left) allows for logging in and creating or changing user profiles. This button also displays the current user profile.

Control bar variations: Find & Focus, Actual & Overlay

CONTROL BAR

The ***control bar*** (bottom left) varies depending on which tab is selected. Choose a tab by clicking on it in the left end of the control bar:

- ***Find & Focus***: to avoid photobleaching while setting up a fluorescence specimen
- ***Actual:*** to view image using actual acquisition parameters (LED brightness and exposure time)
- ***Overlay:*** to view multiple fluorescence channels; also, the QuickSave option is available on this tab.

Note: *In all tabs, the color camera version displays the QuickSave option instead of the Info bar.*

Find & Focus tab

FIND & FOCUS TAB

Use the ***Find & Focus tab*** to view the sample with either transmitted light (to minimize photobleaching) or fluorescence, and to acquire images. This feature displays 10 frames/second for focusing and captures images at longer-exposure, high-quality settings. The following controls are available in the Find & Focus tab:

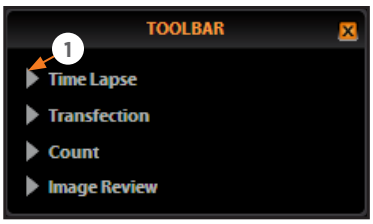
- LIGHT ON/OFF button
- Illumination slider
- Image capture button
- Color option
- Boost option

Actual tab

Overlay tab

Scalebar

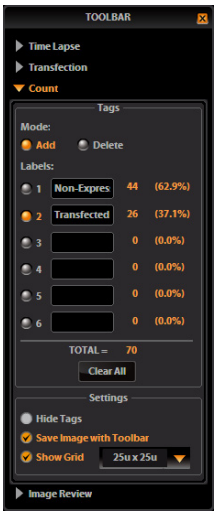
View: Scalebar and Toolbar options



Toolbar

Time Lapse tool

Transfection tool



Count tool

ACTUAL TAB

Use the ***Actual tab*** with fluorescence channels to view the image at the actual exposure time used for high-quality image capture. With Actual tab selected, EVOS responds more slowly to stage position and focus changes, depending on the user-selected exposure time for the camera. The following controls are available in the Actual tab:

- LIGHT ON/OFF button
- Illumination slider
- Exposure time slider
- Image capture button
- Color option
- Boost option

OVERLAY TAB

Use the ***Overlay tab*** to select and overlay multiple fluorescence channels as a single multicolor image. The following controls are available in the Overlay tab:

- Brightness and Contrast sliders for each channel
- Boost option for each channel
- QuickSave option (to the right, not shown)

VIEW: SCALEBAR OPTION

The ***Scalebar option*** (bottom center) is a toggle button that displays or hides the Scalebar tool. This option is only available after an image is captured. To move the Scalebar, simply click and drag it.

VIEW: TOOLBAR OPTION

The ***Toolbar option*** (bottom center) includes the Time Lapse, Transfection, Count, and Image Review tools. Click the small gray triangle ❶ to open each tool.

TIME LAPSE TOOL

The ***Time Lapse tool*** allows you to set up the interval/duration and the file name/destination for time lapse sessions. See ***Recording Time Lapse Images (p. 16)***.

TRANSFECTION TOOL

The ***Transfection tool*** automatically captures and overlays images for transfection analysis. See ***Using the Transfection Tool (p. 17)***.

COUNT TOOL

The ***Count tool*** allows you to tag cells and other features using up to 6 labels, and it calculates the totals and percentages for each label. See ***Counting Cells (p. 18)***.

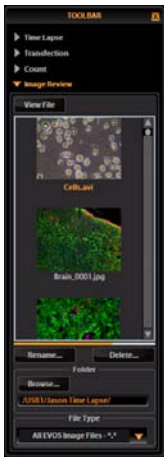


Image Review Tool

IMAGE REVIEW TOOL

The ***Image Review tool*** allows you to review still images or play video files from the USB drive or network connection. You may also use this tool to rename or delete saved files. See ***Reviewing Images (p. 19)*** for more details.

Settings button

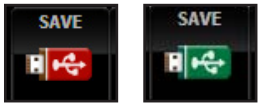
SETTINGS BUTTON

The ***Settings button*** (bottom center) opens the Settings dialog box. Within the Settings dialog box, the following options are available:

- ***Basic Tab***
  - *"This EVOS"* section displays the serial number and software version.
  - *"Login"* section controls default Guest login.
  - *"Display"* section controls the following options:
    - **"Highlight saturated pixels in red"** indicates overexposed areas onscreen;
    - **"Reset Scalebar Location"** moves the Scalebar back to its default position in the lower right corner of the screen;
    - **"Enable Mouse Wheel"** allows the mouse scroll wheel to control illumination levels;
    - **"LCD Backlight"** controls the LCD lighting.
  - *"File Save"* section allows you to specify 8-bit as the default format for TIFF files.
- ***QuickSave Tab*** allows you to set up a file name, count, file type, and folder to save captured images automatically, as well as the option to save each channel in a separate file for each captured image.
- ***Channels Tab*** (not shown) allows you to assign a custom display color to each fluorescence channel (monochrome camera version only). See ***Assign Custom Channel Display Colors (p. 23).***
- ***Network Tab*** (not shown) allows you to set up connections to shared folders via Ethernet. See ***Connecting EVOS to a Network (p. 21).***
- ***Service Tab*** (not shown) allows for maintenance functions; contact your distributor if service is needed.

Settings: Basic Tab
(monochrome version shown)

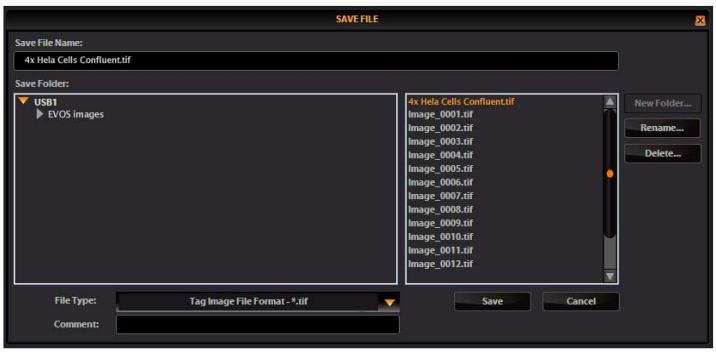
Settings: QuickSave Tab
(color version shown)



Save button

SAVE BUTTON

The ***Save button*** (bottom right) saves the current image to the USB flash drive or network folder. A red USB icon indicates that the current image has not been saved yet. After a file is saved, the USB icon turns green.



Save File dialog box

SAVE FILE DIALOG BOX

The ***Save File dialog box*** (popup) allows options for naming and filing captured images. The following options are available in the ***Save File dialog box:***

- *Save File Name text field:* Click to enter a file name.
- *New Folder... button:* Click to enter a folder name and date (optional).
- *Save Folder list:* The captured image will save to the selected (orange) folder.
- *File list:* (to the right of the *Save Folder list*) Displays saved files already in the selected folder.
- *File type menu:* Drop-down menu allows .tif, .png, .jpg, or .bmp formats.
- *Comment text field:* Click to enter a comment and date (optional).

Note: *For more information, refer to* ***Saving Images & Working with Files (p. 14).***

Virtual keyboard

VIRTUAL KEYBOARD

The ***Virtual keyboard*** popup allows text entry anytime a text field or text-related button is selected. Where applicable, a Date button is available to include the current date in the text field. Click the Accept button to enter the text in the field.

Note: *You may plug in a USB keyboard for text input. When a keyboard is connected, the virtual keyboard does not pop up except when you add a new user ID or rename a file with the Image Review tool.*

Info display variations

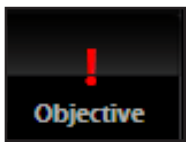
Note: *In all tabs, the color camera version displays the QuickSave option instead of the Info bar.*

INFO DISPLAY *(monochrome camera version only)*

The ***Info display*** (bottom right) shows the following context-sensitive data in the Find & Focus and Actual tabs:

- Intensity (Max and Min)
- Ratio (Max to Min)
- Cursor position
- Current objective selected

In Overlay, the info display shows the QuickSave option with the QuickSave file name (see ***QuickSave tab*** under ***Settings button, p. 27***) and the current objective selected.

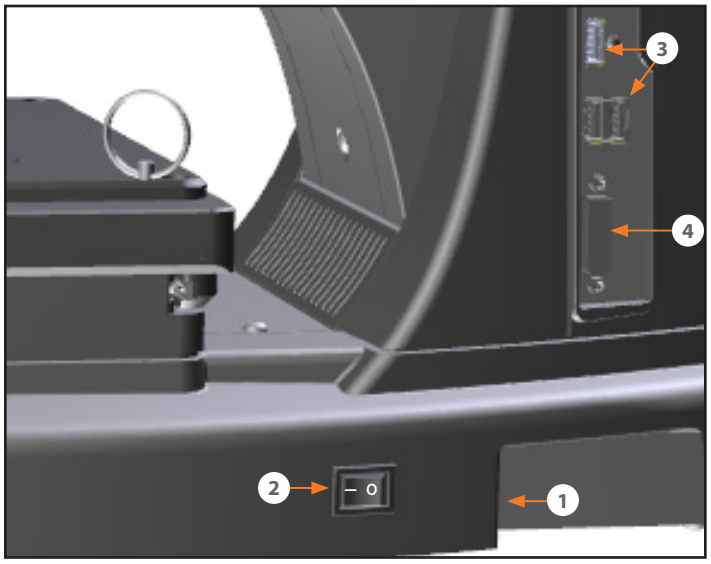


Objective turret between positions

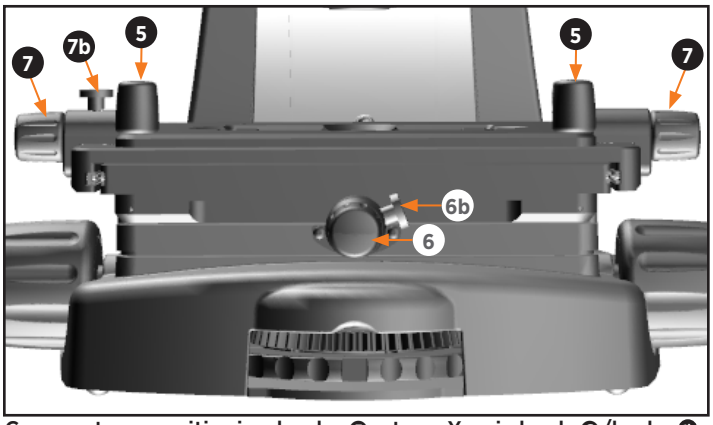
Digital Zoom Value display

DIGITAL ZOOM VALUE DISPLAY

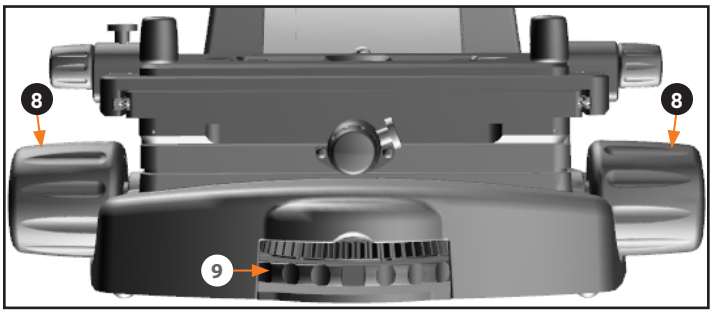
The ***Digital Zoom Value display*** (bottom right) appears above the selected objective display to show the zoom value when you zoom an image.



Power input jack ❶, power switch ❷ USB ports ❸ and DVI port ❹



Coarse stage positioning knobs ❺, stage X-axis knob ❻/brake ❻b and stage Y-axis knobs ❼/brake ❼b



Focusing knobs ❽ and objective selection wheel ❾

MECHANICAL CONTROLS

This controls glossary is not alphabetized. Mechanical controls are listed in the order they are normally used.

POWER INPUT JACK

Plug the power adaptor into the ***power input jack*** ❶.

POWER SWITCH

Set the ***power switch*** ❷ to "—" to turn the microscope on or "O" to turn it off.

USB AND DVI PORTS

Plug the mouse and flash drive into the ***USB ports*** ❸. Use the ***DVI port*** ❹ for digital output to a projector or other display.

COARSE STAGE POSITIONING KNOBS

Use the ***coarse stage positioning knobs*** ❺ to position the specimen within the field of view, particularly at low magnifications.

STAGE X-AXIS KNOB

Use the ***stage X-axis knob*** ❻ for fine left-right movements to position the specimen within the field of view, particularly at high magnifications. To secure the stage in place for time-lapse image captures, tighten the ***X-axis stage brake*** ❻b.

STAGE Y-AXIS KNOBS

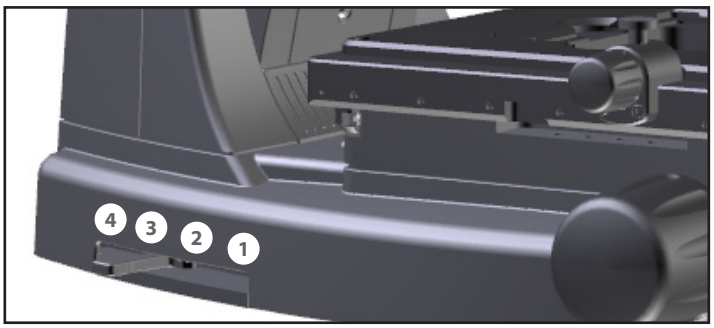
Use the ***stage Y-axis knobs*** ❼ for fine front-back movements to position the specimen within the field of view, particularly at high magnifications. To secure the stage in place for time-lapse image captures, tighten the ***Y-axis stage brake*** ❼b.

FOCUSING KNOBS

Use the ***focusing knobs*** ❽ to bring the specimen into focus.

OBJECTIVE SELECTION WHEEL

Turn the ***objective selection wheel*** ❾ to change magnifications. The objective turret will click into place at each position.



Light cube selection lever in position ❸

LIGHT CUBE SELECTION LEVER

Move the ***light cube selection lever*** to change light channels. The lever will click into place for each of the following positions:

- Position ❶ (closest to front)
- Position ❷ (second from front)
- Position ❸ (second position from back)
- Position ❹ (furthest position to the back)

Note: *The channel indicator bar across the top of the screen displays the active channels for each lever position (see* ***p. 25****).*

Phase annuli selector ❺ (side and front views) in BF position

PHASE ANNULI SELECTOR

Set the ***phase annuli selector*** ❺ to the position that corresponds with your selected objective for transmitted light observations. The selector will click into place for each of the following positions:

- BF (use for brightfield observations)
- 4/10 PH (use for phase observations at 4x or 10x)
- 20/40 PH (use for phase observations at 20x or 40x)

Condenser slider slot ❻

CONDENSER SLIDER SLOT

Transmitted Light Applications
Enhance image quality by inserting a Diffuser or Meniscus condenser slider all the way into the slot ❻. (See slider usage guidelines, ***p. 11***.)

The Pinhole slider is useful to enhance contrast.

Fluorescence Applications
The Block slider is useful in a dark environment to block fluorescent light from being reflected by the condenser and improve image quality. It allows more access to the sample than the light shield box.

MOUSE SCROLL WHEEL

When the "Enable Mouse Wheel" option is selected in the Basic tab of the Settings dialog box (see ***p. 27***), the mouse scroll wheel will adjust the illumination intensity. Roll the scroll wheel away from you for brighter illumination, or roll it toward you for dimmer illumination.

CARE & 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 microscope, turn off the power immediately and wipe dry.
- Do not exchange objectives between microscopes unless you know that the components have been approved and recommended by AMG.
- After using, cover the microscope with the supplied dust cover.

ⓘ ***IMPORTANT!*** *Never disassemble or service the microscope yourself. Unauthorized repairs may damage the microscope or alter its functionality, which may void your warranty. Contact your local EVOS distributor to arrange for service.*

OBJECTIVE LENS CARE

Clean each objective periodically or when necessary with a lens paper and lens cleaning solution. To avoid scratching soft lens coatings, use only optical-grade cleaning materials.

Note: *To protect all optical components of the microscope, use the dust cover when the microscope is not in use.*

STAGE CARE

- Clean the stage as needed with paper towels or Kimwipes dampened with 70% ethanol.
- When moving EVOS, be sure to lock the stage with the stage lock pin as shown on ***p. 4*** to prevent the stage from sliding.

STERILIZATION PROCEDURES

To sterilize the EVOS, please follow these procedures:

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 or optics.*
3. Lightly wipe EVOS working surfaces (stage top, focusing knobs, objective selection wheel, housing) with paper towels or Kimwipes dampened with 70% ethanol or 4,000 ppm hydrogen peroxide (H_2O_2).

   ⓘ ***IMPORTANT!*** *Do not allow sterilization solution to get into lubricated areas, such as the stage roller bearings, or any points of rotation such as axles for the stage knobs, condenser wheel, etc. Do not soak any surface in sterilization solution. NEVER spray liquid anywhere on the EVOS. Always wipe surfaces with dampened paper towels instead.*
4. If it is necessary to sterilize the condenser, do not apply solution directly to the condenser assembly. Instead, select the desired phase ring, and then cover the condenser with clear plastic wrap and wipe the wrap with sterilization solution.

   ⓘ ***IMPORTANT!*** *Never subject EVOS to UV sterilization. UV degrades many materials, including plastic. Damage from UV exposure is not covered under the manufacturer's warranty.*

TROUBLESHOOTING

IMAGE QUALITY ISSUES

PROBLEM	POSSIBLE SOLUTIONS
Misaligned overlay image	▶ Re-capture images in each channel.
Transmitted light image is too dim (at higher magnifications)	▶ Set the phase annuli selector to the BF position. ▶ Remove condenser slider, if one is in place. ▶ Remove light shield box, if it is in place.
Specks, dots, or blurs on image	▶ Follow instructions under ***Objective Lens Care (p. 31)*** to clean objectives.
Uneven focus across screen	▶ Position specimen flat on the stage; be sure the specimen's thickness is even.
Trouble 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.)
LCD screen is black	▶ Click the LIGHT ON button (onscreen). ▶ Move objective selection wheel so that light shines through objective. ▶ Verify that the phase annuli selector on condenser is not stuck between settings. ▶ Center specimen over objective. ▶ Verify power supply is connected and power switch is on.
LCD screen is red, or red patches cover parts of the screen	▶ Dim the illumination until the red highlights disappear to get the maximum level of brightness without any overexposed areas. ▶ Disable the "Highlight saturated pixels in red" option in the Settings (see ***p. 27)***.

SOFTWARE INTERFACE ISSUES

PROBLEM	POSSIBLE SOLUTIONS
Image does not respond to changes in focus or stage position	▶ Click the LIGHT ON button. (Note that a red USB icon on the Save button indicates there is an unsaved image, which will be lost unless it is saved before clicking LIGHT ON.)
LIGHT ON/OFF button is inactive	▶ Verify that the light cube selection lever is clicked into a position. ▶ Verify that the objective selection wheel is clicked into a position.
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. The USB icon on the Save button is red when there is a captured image to save.
DVI output does not work on an external LCD monitor or projector	▶ Power EVOS off, check the cable connections, and power on the microscope. ▶ Verify that the external display accepts DVI-D input (i.e. digital input). A monitor with analog input only (DVI-A) will not display the EVOS' digital output.
Unable to connect to network	▶ Verify physical cable connections; confirm the Ethernet jack is active. ▶ Use a compatible network adaptor (see ***p. 21*** for a list of supported adapters). ▶ If the physical connections are good, and the problem persists, contact your network administrator to resolve any network issues. Note that a Linux network must use Samba for EVOS to be able to find it.

MECHANICAL ISSUES

PROBLEM	POSSIBLE SOLUTIONS
LED light cube selection lever does not move	▶ NEVER force lever! ▶ Remove LED light cube lock and replace with light cube access cover (see ***p. 5***).
Mechanical stage does not move	▶ Remove stage lock pin. ▶ Check stage brakes (on the stage knobs) and loosen as needed.
Vessel does not sit securely on moving stage	▶ Use the correct vessel holder for the application (refer to the *EVOS Vessel Holders* spec sheet, included on the USB flash drive).
Mechanical stage drifts during time lapse sessions	▶ Tighten the stage brakes (on the stage knobs) securely before starting a time lapse session (see ***p. 16)***.
Mechanical stage tension is loose	▶ Tighten the stage brakes (on the stage knobs) as desired to increase tension.

Note: *For additional technical support, contact your local EVOS distributor. If you do not have your distributor information, you can look it up at the AMG website or contact AMG Customer Service (see p. 34).*

CUSTOMER & TECHNICAL SERVICE

AMG CONTACT INFORMATION

- Toll-free (US & Canada) **866-614-4022**
- Local **425-368-0444**
- International **01-425-368-0444**
- Fax **425-368-0555**
- E-mail **customerservice@amgmicro.com**
- Web site **www.amgmicro.com**

Advanced Microscopy Group Customer Service business hours are 7:00 a.m. – 4:00 p.m. Pacific Standard Time. After hours, you may leave a telephone message. We will return your call the following business day.

ADVANCED MICROSCOPY GROUP
22025 20th Ave SE
Suite 100
Bothell, WA 98021

Thanks for being our valued customer! Advanced Microscopy Group is a Seattle-area (Bothell, WA) optical equipment design and manufacturing firm with a domestic and international client base. Advanced Microscopy Group designs, develops, and manufactures optical systems and software for scientific, biotechnology, industrial and educational fields.

DISCLAIMER

EVOS DISTRIBUTOR INFORMATION

The AMG website lists all authorized EVOS distributors by region, country and state. If you need help finding a distributor in your area, please contact AMG Customer Service directly.

SERVICE AND WARRANTY INFORMATION

ⓘ ***IMPORTANT! For microscopes purchased outside the United States or Canada,*** *contact your authorized EVOS distributor for their warranty and service policy. For microscopes purchased within the United States and Canada, contact AMG Customer Service directly to arrange for service or repairs.*

RETURNED GOODS POLICY FOR REPAIR OR REPLACEMENT PARTS

AMG offers a 60-day return policy on unused non-custom items in original packaging. Products returned for reasons other than quality issues are subject to a restocking fee of $20.00 or 10% of the price, whichever is greater. After 60 days, no product will be accepted for return unless it is being returned under warranty. The customer is responsible for the return shipping charges. Once received, the returned product will be inspected and credit will be issued, except in cases where the item is damaged, incomplete, or not in original packaging. Special or custom products are not eligible for return. ***AMG will not accept an unauthorized return; contact your distributor first if you did not purchase the microscope directly from AMG.***

Please call 866-614-4022 to obtain a Return Material Authorization (RMA) number before shipping the product back to AMG. The RMA number must be indicated on all paperwork and labels for return. ***No returns will be accepted without a RMA number.***

To return goods for repair or replacement, please contact Advanced Microscopy Group Customer Service by one of the numbers above. Please be prepared to supply the following information:

- Your name, return shipping address and telephone number
- Catalog/Model number of the item(s) you are returning
- Serial Number(s), if applicable
- Description of the product's problem or reason for the return
- Date the item was purchased
- Distributor information, if applicable

Serial number label on LCD hinge (top view)

An AMG Representative will issue you a Return Materials Authorization (RMA) number. Please label the outside of your shipping container with this number. For any additional information, please call Customer Service: 866-614-4022 or 1-425-368-0444.

PARTS & ACCESSORIES

VESSEL HOLDERS & STAGE PLATES

Item	Part Number
Holds two 26 mm x 76 mm standard slides	AMEP-VH001
Holds four 35 mm Petri dishes	AMEP-VH002
Holds two 60 mm Petri dishes	AMEP-VH003
Holds one 100 mm Petri dish	AMEP-VH004
Holds two 25 cm^2 T-25 flasks, rectangular or triangular	AMEP-VH005
Holds one 75 cm^2 Nunc T-75 flask	AMEP-VH006
Holds one 76 mm x 33 mm hemocytometer	AMEP-VH007
Holds one 75 cm^2 Greiner T-75 flask	AMEP-VH008
Holds all vessel types (plain-stage functionality)	AMEP-VH009
Holds one 25 cm^2 BD or Greiner T-25 flask	AMEP-VH010
Holds one NUNC/SPL IVF 4-well dish (66 mm square)	AMEP-VH011
Holds one SPL T-75 flask	AMEP-VH012
Holds four Ibidi 35 mm Petri dishes	AMEP-VH013
Holds two Ibidi 50 mm Petri dishes	AMEP-VH014
Stage Plate for heating tray, Tokai Hit Cat. No. MATS-UAXKW-D	AMEP-4684
Stage Plate for heating stage, BioFlux by Fluxion	AMEP-4685
Stage Plate for multi-well vessels; also holds one Corning T-75 flask	AMEP-4686
Stage Plate for multi-well vessels; allows max. objective clearance	AMEP-4687
Stage Plate with 160x110 mm opening	AMEP-4691
Adaptor, 160x110 mm opening to standard-sized opening	AMEP-4693

LED LIGHT CUBES

Item	Part Number
DAPI	AMEP-4650
GFP	AMEP-4651
RFP	AMEP-4652
CFP	AMEP-4653
YFP	AMEP-4654
TX RED	AMEP-4655
CY5	AMEP-4656

Item	Part Number
Q-Dot 525	AMEP-4657
Q-Dot 545	AMEP-4658
Q-Dot 565	AMEP-4659
Q-Dot 585	AMEP-4660
Q-Dot 605	AMEP-4661
Q-Dot 625	AMEP-4662
Q-Dot 655	AMEP-4663
Q-Dot 705	AMEP-4664
Q-Dot 800	AMEP-4665
Q-Dot 525-800	AMEP-4666

OBJECTIVES

Item	Part Number
Objective, 2x Plan	AMEP-4601
Objective, 4x Plan	AMEP-4602
Objective, 4x Plan Fluor	AMEP-4622
Objective, 10x Plan Fluor	AMEP-4623
Objective, 20x Plan Fluor	AMEP-4624
Objective, 40x Plan Fluor	AMEP-4625
Objective, 60X Plan Fluor	AMEP-4626

See ***SPECIFICATIONS (p. 36)*** for more details on objectives listed.
For a full list of currently supported objectives, please call Customer Service: 866-614-4022 or 1-425-368-0444.

REPLACEMENT PARTS

Item	Part Number
Arm Rest Kit	AMEP-4618
UV Shield Kit	AMEP-4643
Light Shield Box	AMEP-4639
Dust Cover	AMEP-4642
Light Cube Access Cover	AMEP-4630
Light Cube Lock	AMEP-4638
Power Adapter	AMEP-4641
USB Mouse	AMEP-4617
Pinhole Slider	AMEP-AS15
Diffuser Slider	AMEP-DFS1
Meniscus A Slider	AMEP-MN3
Meniscus B Slider	AMEP-MN4
Block Slider	AMEP-4688

Note: *To place an order, contact your local EVOS distributor. If you do not have your distributor information, please look it up at the AMG website or contact AMG Customer Service (see p. 34).*

SPECIFICATIONS

<table>
<tr><td>Optics</td><td colspan="2">Infinity-corrected optical system; RMS-threaded objectives with 45 mm parfocal distance</td></tr>
<tr><td>Objectives</td><td colspan="2">LWD; actual objectives included vary per order.
<table>
<tr><th>Part Number</th><th>Mag.</th><th>Description</th><th>N.A.</th><th>W.D. (mm)</th></tr>
<tr><td>AMEP-4601</td><td>2x</td><td>Plan N INF/- FN22</td><td>0.06</td><td>5</td></tr>
<tr><td>AMEP-4602</td><td>4x</td><td>UPlan N Ph INF/-/FN22</td><td>0.13</td><td>17</td></tr>
<tr><td>AMEP-4622</td><td>4x</td><td>PlanFluor INF/1.2</td><td>0.13</td><td>19.7</td></tr>
<tr><td>AMEP-4623</td><td>10x</td><td>PlanFluor INF/1.2</td><td>0.30</td><td>8.3</td></tr>
<tr><td>AMEP-4624</td><td>20x</td><td>PlanFluor INF/1.2</td><td>0.45</td><td>7.1</td></tr>
<tr><td>AMEP-4625</td><td>40x</td><td>PlanFluor INF/1.2</td><td>0.65</td><td>2.8</td></tr>
<tr><td>AMEP-4626</td><td>60x</td><td>PlanFluor INF/1.2</td><td>0.75</td><td>2.2</td></tr>
</table></td></tr>
<tr><td>Objective Turret</td><td colspan="2">5-position; front-mounted control</td></tr>
<tr><td>Light Cubes
U.S. Patent No. 7,502,164</td><td colspan="2">DAPI: 360 nm excitation, 447 nm emission
GFP: 470 nm excitation, 525 nm emission
RFP: 530 nm excitation, 593 nm emission</td></tr>
<tr><td>Illumination</td><td colspan="2">LED (50,000-hour life); adjustable intensity</td></tr>
<tr><td>Contrast Methods</td><td colspan="2">Fluorescence and transmitted light (brightfield & phase contrast)</td></tr>
<tr><td>Condenser</td><td colspan="2">3-position turret for brightfield & phase contrast</td></tr>
<tr><td>Condenser Sliders</td><td colspan="2">Pinhole, Diffuser, Meniscus, and Block filters</td></tr>
<tr><td>Condenser Working Distance</td><td colspan="2">53 mm</td></tr>
<tr><td>Mechanical “Glide” Stage</td><td colspan="2">▸ X-Y axis fine-positioning controls, 69 mm (2.7”) per rotation; 110 mm x 110 mm (4.3” x 4.3”) range of motion
▸ Z-axis focusing controls, 480 µm/rotation
▸ Interchangeable vessel holders available for most common shapes & sizes</td></tr>
<tr><td>LCD Display</td><td colspan="2">15” color, 1024 x 768 pixels; adjustable tilt</td></tr>
<tr><td>Image Acquisition</td><td colspan="2">Onboard microprocessor; built-in software for image acquisition via mouse control</td></tr>
<tr><td>Camera</td><td>Monochrome:
Sony ICX285AL monochrome CCD,
2/3” 1360 x 1024, 1.4 Megapixels</td><td>Color:
Sony ICX285AQ color CCD,
2/3” 1360 x 1024, 1.4 Megapixels</td></tr>
<tr><td>Captured Images</td><td>Monochrome camera:
16-bit monochrome TIFF or PNG (12-bit dynamic range); 24-bit color TIFF or PNG;
jpeg, bmp (1360 x 1024 pixels)</td><td>Color camera:
16-bit color TIFF or PNG (12-bit dynamic range);
24-bit color TIFF or PNG;
jpeg, bmp (1360 x 1024 pixels)</td></tr>
<tr><td>Output Ports</td><td colspan="2">3 USB and 1 DVI (digital output)</td></tr>
<tr><td>Power Supply</td><td colspan="2">AC Adapter; Input 100-240V, 50-60Hz;
Output 5 VDC/4.15A ⊖-●-⊕</td></tr>
<tr><td>Dimensions</td><td colspan="2">Operating height: 57.8 cm (22.75”)
Storage/transport height: 32.4 cm (12.75”)
Depth: 47.0 cm (18.5”); Width: 35.5 cm (14.0”)</td></tr>
<tr><td>Weight</td><td colspan="2">15.3 kg (33.7 lbs)</td></tr>
</table>

***Note:** Specifications are subject to change without notice. Go to the EVOS fl product page online to download the latest product information.*