microscopy.core@mssm.edu; 212-241-0400 June 2022



The Olympus MVX10 Stereoscope is a highly versatile imaging platform that bridges the gap between observation by eye and high-magnification exploration by compound microscope. The system is equipped for axial transmitted illumination; oblique transmitted illumination; oblique reflected light illumination; and multi-color fluorescence.

Three front lenses are available for the system: 0.63x, 1.0x, and 2.0x. These lenses provide the main source of magnification. The zoom body further magnifies the image from a factor of 0.63x to 6.3x. There is an additional Magnification Changer that can be set to 1.0x or 2.0x.

Final magnification ranges from 0.4x (0.63x front lens, 0.63x zoom body setting, 1.0x Mag Changer) to 25.4x (2.0x front lens, 6.3x zoom body, 2.0x Mag Changer).

DAPI, FITC and TRITC single-bandpass filter sets are available standard on the system. A Cy5 filter set can also be installed for four-color imaging.

The DP80 dual-CCD camera is set up for both brightfield imaging as well as fluorescence, and it is capable of overlaying images from both techniques so that fluorescence signals can be placed in context. Quantitative fluorescence measurements down to cellular levels are possible when the camera is set to the monochrome mode.



System Overview

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Focus Wheel in Detail

Fluorescence Turret in Detail



Transmitted Light Base in Detail



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Startup

- 1. Turn on the required illumination:
 - a. For fluorescence, turn on the fluorescence lamp located under the air table. Rotate the Fluorescence Turret to the first filter set and open the Shutter (slide selector forward).
 - b. For transmitted light, turn on the Transmitted Light Source. Start with 50% power.
 - c. For reflected oblique light (0.63x and 1.0x front lens only), turn on the Reflected Oblique Light Source. Start with 50% power. *Ask CoRE personnel for assistance attaching the lens accessory.*
- 2. Flip the Focus Control to ON.
- 3. Turn on the computer and log on. Use the credentials you established during the training session.
- 4. Start Olympus CellSens Dimension from the desktop.

Initial Setup

- 1. Place sample directly on the center of the glass plate. *For reflected light samples, consider placing the matte black background underneath the specimen.*
- 2. Push the Binocular/Camera Selector in (for eyepieces).
- 3. Rotate the Front Lens turret to the desired lens. It should "click" into place toward the front of the stereoscope.
- 4. Set the Zoom Body to 0.63x. The current setting is lined up with the indent on the Zoom Body.
- 5. Look through the eyepieces. Rotate the Focus Wheel under focus is achieved. Use the XY stage controls to center the specimen in the eyepieces.
 - a. When using reflected oblique illumination, rotate the polarizer located at the bottom edge of the lens accessory to reduce speculars (bright spots) and eliminate reflections.
- 6. Adjust the Zoom Body to the desired setting. Refocusing may be necessary.
- 7. Pull the Binocular/Camera Selector to send light to the camera.

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Software Setup – Transmitted Light/Reflected Oblique

- 1. Click to change the camera to the **RGB Mode**. (Red/Green/Blue stripes)
- Set Exposure to "Automatic," the Region to "Spot 0.1%," and the Sensitivity to "ISO 200."







4. Move the **Exposure box** on the Live image to the brightest area of the image (usually a background or empty space).

Move your sample around to find the brightest area.





- Switch Exposure from Automatic to Manual to hold this exposure setting.
- 6. Double-check sample focus for the camera image.



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 Click on the White Balance tool. Move the cursor to the Live image and click on a pixel that you know should be white (usually background).

If you are using the matte black background, temporarily add a white sheet of paper to the Live image. You may need to temporarily re-adjust the exposure - <u>do not adjust the lamp setting</u>!!



8. Update the **Zoom settings** in the software before taking a picture. *This step is crucial to ensuring your scaling data/measurements are correct.*



Current Front Lens (lens is closest to you)

9. Click **Snapshot** to capture a final image.

Mag Changer (image shows 1X setting)



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Software Setup – Fluorescence

1. Under **Camera Control**, switch to the monochrome camera mode.



 Switch Exposure to "Automatic," Region to "Spot 0.1%," and Gain to 1.0x. Exposure: 2.4 s



- 3. Click Live.
- Move the Fluorescence Turret to the first desired filter set (Positions 1, 2 or 3). Open the Shutter.
- 5. Move the **Exposure box** to the brightest area in the image field (look for the brightest signal in your sample).



TRITC

TBF



SHUTTER

0

6. Switch **Exposure** from Automatic to Manual to hold this exposure setting.

NOTE: If you will be using these images for quantitative analysis, record and reuse the exposure times for a given fluorescence channel!

Exposure: 35	5.71 ms
Manual Automatic	5
35.71 ms	-

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7. Update the Zoom settings in the software before taking a picture. *This step is crucial to ensuring your scaling data/measurements are correct.*





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- 11. To create multi-color TIF overlays, go to **Image > Combine Channels**.
- 12. Input the three images under the **Images** column. *The pseudocolor will be automatically applied.*

Click **OK** to generate the overlay.

If "Convert to RGB" is left unchecked, the resulting image can be saved as a multi-page TIF. Each channel will be saved in a RAW format and will be independently available for analysis.

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Saving Images / Shutting Down

- 1. Save all images to the HIVE.
 - a. <u>Remember the data policy</u>: All images must be saved to the HIVE! Any data stored locally on the PC is subject to immediate deletion without warning!
- 2. Close the CellSens software. Log off your account, or shut down the PC.
- 3. Turn off the Focus Control switch.
- 4. Turn off any light sources that were used: Transmitted Light Source, Reflected Oblique Light Source, or the Fluorescence Lamp.