

Zeiss Stereo Lumar V12

User guide



The Zeiss Stereo Lumar is a fluorescence capable and fully automated stereoscope with an increased focusing range, which allows imaging of whole small animals (eg. zebrafish, flies, *C. elegans*), as well as cell cultures in Petri dishes. Magnifications range is equivalent to ~1-12x.

Contrast methods: Fluorescence, brightfield, darkfield, oblique illumination

Objective: ApoLumar S 1.2x

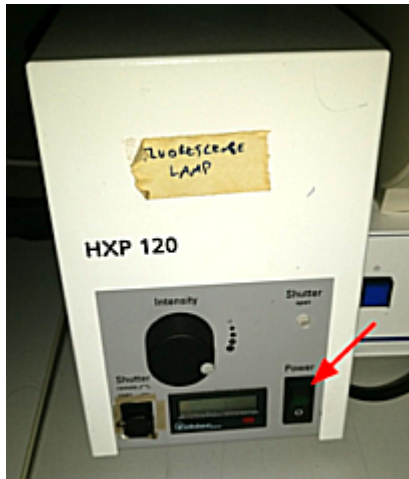
Eyepiece: 10/23x

Camera : Hamamatsu Orca-ER monochrome (1.3MPx)

Operating conditions : room temperature

Turn on procedure

1. Turn on the computer and log in using your Agendo username and password.
2. Turn on the mercury lamp for fluorescence if needed. If the lamp was used less than 30 minutes before, check if it has cooled down and only then turn it on.



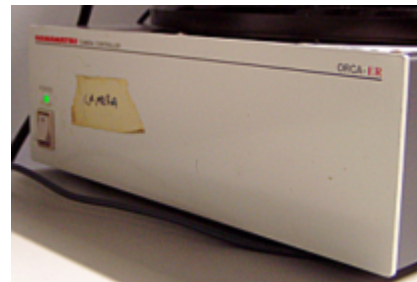
3. Turn on the transmitted light source if needed.



4. Turn on the stereoscope control box.

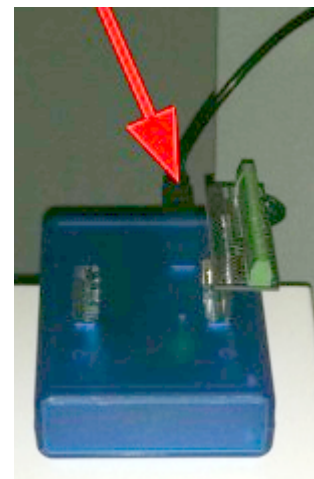


5. Turn on the camera controller (below the monitor).



6. Start the Micro-Manager software.

In case everything is on but you get an error at Micro-Manager boot, unplug and plug back in the cable in the blue box on top of the mercury lamp.



Turn off procedure

1. Close all programs and log off from Windows.
2. Turn off the camera controller.
3. Turn off the stereoscope control box.
4. If you used the mercury light source and there is no one scheduled to use the stereoscope for the next 30 minutes, turn it off.
5. Clean the area.

Control panel

Joystick - zoom in and out (right, left) and focus (up, down)

Buttons:

TL↑ and TL↓ - change the intensity of the transmitted light lamp

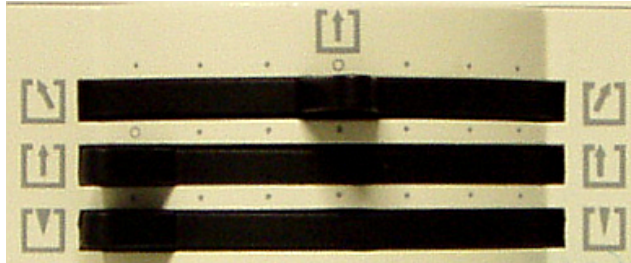
TL/FL - switch between transmitted light and fluorescence








FL shutter - open/close the shutter for fluorescence



Controls for transmitted light illumination

(right side of the microscope base)



 The bundle of light emerges vertically (reflector inclined by 45°)	
 Inclination of the bundle of light towards the operator (risk of being dazzled!)	 Inclination of the bundle of light off the operator
 Bundle of light emerges centrally	 Translation of the bundle of light towards the operator
 Diffuse light quality through white reflector surface	 Directed light quality through specular reflector surface

Switch between the camera and eyepiece ports

(top right of the microscope)



Iris

(right side of the microscope)

- regulates the depth of field for transmitted light
- should be fully open for fluorescence



Filter wheels for fluorescence

Available filters:

Filter	Excitation	Emission
CFP	436/20	480/40
YFP	500/20	535/30
GFP	470/40	525/50
TxRed	560/40	630/75

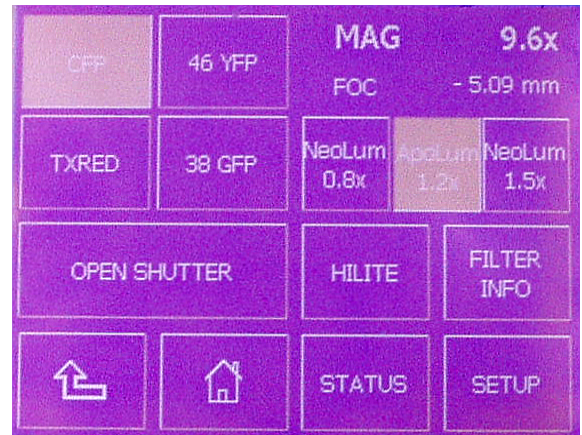


switch

selected filter

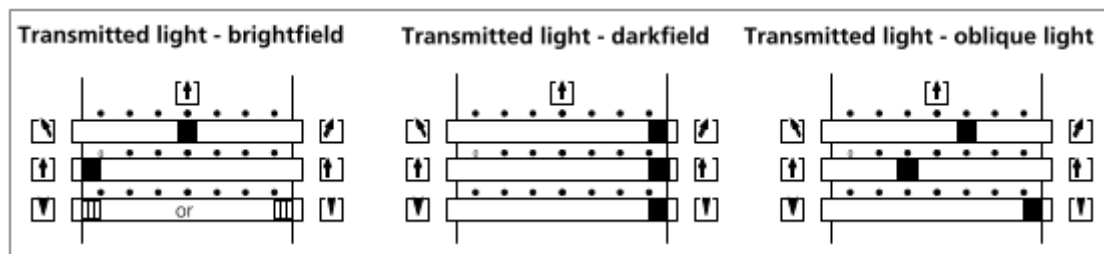
Setup for fluorescence

1. Fully open the iris with the wheel on the right side of the microscope.
2. Choose the fluorescence filter with the button on the right side of the microscope or from the Menu on the touchscreen – go to *Function* → *Fluorescence*. There are four fluorescence filters, for blue (CFP), green (38 GFP), yellow (46 YFP), and red (TxRED) fluorescence.
3. Micro-Manager controls the fluorescence lamp shutter, so it is necessary to go Live in the software.
4. Open and close the microscope shutter with the control panel button.
5. Make sure that the orange protective screen is in place when using UV illumination.



Setup for transmitted light

Select the desired technique via the sliders in the base of the microscope. For oblique light, use intermediate positions to optimize the contrast. The sliders control the position and surface quality of the reflector that is directing the light at the sample. The top slider controls the inclination of the reflector, the middle slider moves the reflector front to back, and the bottom slider changes the surface quality of the reflector between a mirror and a white surface.



Recording images

Activate *Live* to start imaging. Adjust the exposure time and light intensity. To save the image, stop the live recording and use *File* → *SaveAs ... tiff* from the ImageJ menu or the saving buttons in the *Live* window. It is only possible to record one color at a time.

Pixel size

Micro-Manager does not have the information about the zoom factor of the microscope and therefore the images are not calibrated. Write down the magnification shown on the display of the control panel and use the calculator on the website to calculate the pixel size. Make sure that the correct objective and eyepiece is selected on the control panel (objective ApoLum 1.2x, eyepiece 10/23x), otherwise the shown magnification is incorrect. To select objective/eyepiece, go to *Setup* → *Objective/Eyepiece*.

Function Menu – useful functions

Zoom clickstop – buttons with predefined zoom levels

Focus speed – change the focus speed

Zoom speed – change the zoom speed

Recording color images

The camera is monochrome, but it is possible to obtain color images in brightfield mode, using the same filters that are used for fluorescence. Set the light source to 2700 K, about 15% intensity.

Deactivate the light manager in the control panel as it changes the light intensity depending on zoom (*Function → Light Manager*). Acquire 3 images using the TxRed, GFP and CFP filters with brightfield illumination. Choose the exposure times so that the three images have a similar brightness (look at the histogram in Micro-Manager). Load the three images as a stack in ImageJ, assign the red color to the TxRed image, Green color to the GFP image, blue color to the CFP image. Convert the image to RGB Color and you now have a color image of your sample. Adjust the white balance if necessary: check if the white areas of the sample are really white, if not, adjust the brightness of the individual images so that the mean values in the white areas are similar.