### Fluorescence Microscopy: OI-1 Modification

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The OI-1 device is a simple vertical illuminator, created for metallographic observations was manufactured between 1940 -1950 by the firm "Progress" (today is knowing as LOMO). Sometimes an OI-1 is sold on the Internet (eBay) occasionally.

If incorporating this device to the microscope it results in elongation of the length of the tube to more than 160 mm. Biological microscopes, such as the MBU-4A, have a threaded extension that can be removed and replaced by the OI-1, but it will remain a tube length of 174 mm (it would be desirable for an extensible tube to correct the tube length to 160 mm).

The OI-1 has a semi-transparent mirror that can be replaced by a dichroic mirror of a multimedia projector, in this case, before cutting and sizing. To ensure that it is correct and adapts to the needs of the fluorescence microscopy, it was determined that this mirror reflects the light of the blue-violet coloration, letting past the colors after the blue (Transmittance > 450 nm).

A light source was prepared with UV band LED's (365 nm, 385 nm, 400 nm) that operate with DC batteries (3.5 or 3.6 V). The LED's must be mounted on threaded bushings of small incandescent bulbs of 2.5 V or 3.6 V, because the base of the OI-1 is specific made for these dimensions.

Apart from the light source, you must take a care about getting filters for eliminating residual red-light emitted from LED's, for to protect the eyes when they are observing. In this case, we used LOMO's Crystal filters whose list is presented below (transliteration of the Cyrillic characters of the filters):

#### **Excitation filters in correct wavelength**

- UFS-6:	325 nm	ultraviolet (LED 365 nm by 325 nm	n)
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- FS-1: 385 nm blue-violet (LED 385 nm)
- SS-15: 400 nm blue (LED 400 nm)

#### Length removal filters red and infrared wavelength

- SZS7: 320/750 nm

- SZS21: 325/650 nm

- SZS24: 270/950 nm

# Barrier filters, to remove the UV, the blue when observing the sample.

- SHC3: 400/3000 nm Weak yellow
- SHC18: 450/3000 nm Strong yellow

The big task is just starting, you must cut the excitation filters and the red/IR filter until they have a diameter of 15 or 16 mm, which must go inside the lighting tube. One will serve to select the correct wavelength and the other to limit the passage of the red and infrared light from the light source.

Barrier filters are available on sale on eBay as SHC3 or SHC18, or sometimes as GG9, GG13 and Orange (OG1). You can also use the sliding filters from Carl Zeiss, Leitz, Reichert, etc., although they are much more expensive. Some filters are prepared to fit the eyepiece, others must be reconditioned using: glass cutter, tweezers, adhesive tape, Emery 080 paper and patience, yes, a lot of patience. At the end of the document some photos show the result of the patient work.



*Photo 1. Parts of the illuminator transformed. The mirror dichroic is shown, approximately rectangular form. The filters SS-15 and SZS-21 (cut to 16 mm in diameter). The metal frame in which the mirror is mounted.* 



*Photo* 2. Showing the reflection detail of the dichroic mirror reflecting the wavelengths from ultraviolet to blue.



*Photo* 3.Light source, consisting of a 16 mm heatsink mounted on a 3.5 V bulb socket and secured with silicone heat glue.



Photo 4.Installation sequence of the filters and the light source in the OI-1, first the SS-15 (or the FS-1), in second place the SZS-21 then, finally, the light source at its base.



Photo 5, OI-1 Vertical illuminator with 10x luminescent lens and light source on.



Photo 6. MBU-4a microscope with OI-1 illuminator and 10x luminescent lens.



Photo 7.A picture is displayed without dealing with the edge of a cross section of a fern leaf. A 10x luminescent lens, a 7x eyepiece, a 1 mm-thick shc18 barrier filter and an MFN-12 device were used; equipped with a Lumix camera with a 15-second exposure. The sample was mounted on a modification of the formula PVA-G Walter Dioni (PVA-G.-medium polyvinyl alcohol-glycerol).



Photo 8.Chloroplasts in a fern leaf, taken with a 40x target of water immersion, 7x eyepiece, 1mm thick shc18 barrier filter. The exposure time is 15 seconds. Untreated image. The fern leaf specimen is mounted on a modification of the PVA-G formula by Walter Dioni (PVA-G.-Polyvinyl Alcohol-Glycerol Medium).

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