

Olympus BX-51 Microscope --- User's Guide

Version 1.0

Edited by Dr. Hartmut G. Hedderich

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The following guide describes the use of the Olympus BX-51 Microscope. The guide is intended to assist instrument users after the initial training session.

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The Olympus BX-51 Microscope can perform the following measurements:

- Bright Field Microscopy in transmission and epi modes
- Dark Field Microscopy in epi mode
- Fluorescence Microscopy in epi mode
- Polarization Microscopy in epi and transmission modes



1. Hardware

The microscope has a total of 3 light sources: two tungsten lamps for transmission and epi microscopy and a mercury lamp for fluorescence microscopy. **WARNING: never use the mercury lamp with the dark field cube or dark field objectives!** The intense light will destroy the cube! The intensity of the tungsten lamps can be set with the illumination adjustment knob at the front right edge of the microscope.

The specimen plate has a holder for microscope slides. When using slides full **x-y-z** control is available. The slide holder can be removed for large size substrates (i.e. well plates). When using substrates that do not fit the slide holder the **x** control is lost! Always re-install slide holder!

In the standard setup the Olympus is equipped with the following objectives: x2, x10, x40 and x100 for bright field (BF) and x10 and x100 for dark field (DF) microscopy. The DF objectives are easily recognized by their size – they are much bigger than the BF objectives. Only BF objectives are used for fluorescence microscopy!

The filter cube changer has a total of 6 positions. 5 of those are permanently occupied by the following filter cubes (*see data sheets in addendum*):

- position 1 – BF
- position 2 – U/B (ultraviolet excitation/blue emission) – model U-MWU2
- position 3 – B/G (blue excitation/green emission) – model U-MWB2
- position 4 – G//R (green excitation/red emission) – model U-MWG2
- position 5 – DF
- position 6 – open, can be used for user specific filters (user needs to provide excitation, emission and dichroic filters)

There is a shutter at the bottom right corner of the filter cube changer. It needs to be in the open position in order to get light to the eye piece or the camera.

The Olympus has an extra module to connect an external detector or spectrograph. Those need to be provided by the user.

The analyzer for epi polarization microscopy is permanently installed below the eye piece.

A high-end DP-71 camera is used to take pictures, time-lapse pictures or movies as well as standard movies.

All levers have pictograms pointing out how a component performs under different lever positions. **Hint:** the extra module *camera* position (lever in) points the mirror to the back! Pull the lever all the way out to have the light go to the DP-71 camera!

There is a neutral density (ND) filter at the arm of the microscope. Use the filter for BF microscopy. Remove the ND filter once a fluorescence cube is chosen or you perform DF or polarization microscopy!

2. Startup

Covid-19 Regulations: please watch the following YouTube movie (<https://youtu.be/-VqncPchM1l>) to see how the microscope is cleaned prior to use and after finishing your experiments!

Turn on tungsten (white light) source. The on/off switch is at the right side of the microscope. There is also a switch to change between top (epi) and bottom (transmission) illumination. Set the intensity with the illumination adjustment knob.

The mercury lamp does have its own power supply. Please follow the instructions on top of the power supply. Specifically, leave the lamp on for a minimum of 15 minutes before switching it off. Leave the lamp off for at least 15 minutes, so it has time to cool off! The life time of the mercury lamp is limited to 200-300 hours. Not following those rules will dramatically diminish the lifetime of the lamp!

Place your sample on the specimen table. You may focus your sample through the eye piece.

3. Software

Start the software by clicking the **DP Controller** icon on the desktop. This will start the camera software. Push the *Start* button on the left side of the **Capture tab** to turn the camera on. The monitor will show a preview screen with a live image.

There are a total of 7 tabs beneath the image screen. Those are from left to right: **Capture**, **Color**, **Level**, **Scale**, **Timelapse/Movie**, **Microscope** and **User Setting**.

The **Capture tab** contains the most important information and will help you to get good images. The following parameters can be set here:

- *Image Size* – it will start up with 4080x3072 resolution. Please change it to 1360x1024 for images or 680x512 for movies (frame rate limited).
- *Exposure Mode* – it starts up in Auto mode. I strongly recommend to switch to **Manual mode**. This will give you an image exactly how you want it.
- *Exposure Time* – move the slider to set the exposure time while watching the live image on the screen (range: 1/44,000s to 60s)
- *ISO Sensitivity* – for DSLR cameras use the lowest possible ISO setting to avoid the emergence of white noise.
- *Objective* – if you want a scale on your image you need to have the correct objective being displayed here. Since two 10x and 100x objectives (one each for BF and DF) are installed it helps to go to the **Microscope tab** to select the correct objective. Each objective was calibrated individually!
- *Accumulation* – leave at Average mode

The **Color tab** contains two options: white and black balance. They cannot be used together! White balance provides the possibility to adjust the RGB (red-green-blue) components of the image. This can be helpful to make certain particles stand out more. Black balance darkens the image and sharpens the edges of blurry particles.

The **Level tab** gives the possibility to move the spectral range of a BF image.

The **Scale tab** gives the user many options to put a scale on the image. There are options for color changes of line and numbers, length of scale line, positioning, etc. Choose what will work best for you.

Timelapse/Movie tab becomes interesting if you have a reaction going on or you have something moving on your substrate. The options are timelapse which will take images in a user-defined time interval and total time. This will produce a stack of picture. It is also possible to combine those pictures to a movie by using timelapse movie. The last option is a straight up movie. Please make sure to set the image size to 680x512 pixels!

Microscope tab is used to tell the program which objectives are installed and which one is active. It is important to choose the correct objective in order to have a correct scale imprinted. Each objective was calibrated individually.

UserSetting tab gives the possibility to save a certain setup. Since the microscope is setup for each new slide, it usually is not an option that is needed.

Use the **x-y-z** stage to get your specimen in focus and to the correct position. Use the exposure time setting to get optimum light characteristics. Hit the camera button to take an image. The image will show up in a second window. There are several options to manipulate your image post capture. The most important one is image composition which allows to combine several images to a new picture. This is an important option to see for example which parts of your specimen fluoresce and which ones do not.

4. Known Problems/Maintenance

Sometimes it seems impossible to get the sample in focus. This is most often seen with the x40 and x100 objectives. The reason is that the objective lens got dirty. Usually, this happens when a user runs the objective into the specimen. Please inform the person-in-charge of the microscope right away. He will clean the objectives with solvent and special tissue paper. **Please do not attempt to clean microscope objectives yourself!**

When the mercury lamp gets close to the end of its lifetime, it will be harder to get the burner started or it won't start at all. Please inform us about this observation. We will change the mercury lamp and re-align the microscope correctly.

5. Turn Off/Shut Down Microscope

Save your images and copy them to a flash drive if necessary. Close the program. Logoff the computer.

Turn off ALL light sources. This is especially important for the mercury lamp which has a limited lifetime. Remove all your samples. Please do not leave any samples with the microscope. Samples and substrates that are left will be discarded!

Please return the microscope to the following positions:

- Move the specimen table to the bottom
- Switch to the x2 objective
- Close shutter
- Return filter changer to position 1 (BF)
- Put ND filter back in its position
- Push lamp lever to the inward position (tungsten lamp)

Finally, clean the microscope as demonstrated in the cleaning movie!

6. Addendum

- Position 2: Data Sheet for Filter Cube U-MWU2:

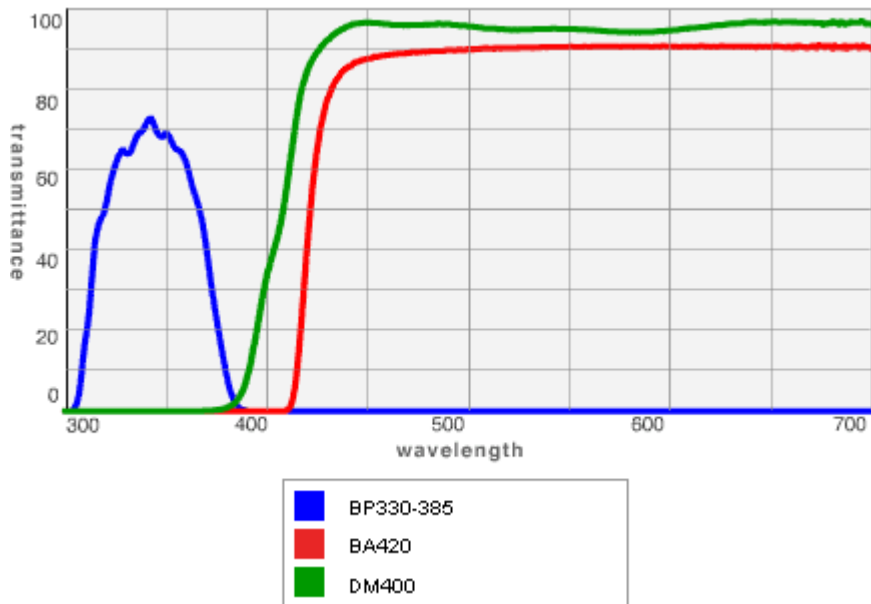
UIS2 series : Fluorescence Mirror Unit
U-MWU2



Excitation filter	Emission filter	Dichromatic filter
330 - 385	420	400

Transmittance/Wavelength

[.CSV download](#)



The values of transmittance were measured using Lambda900 (PerkinElmer Life & Analytical Sciences).

- **Position 3: Data Sheet for Filter Cube U-MWB2:**

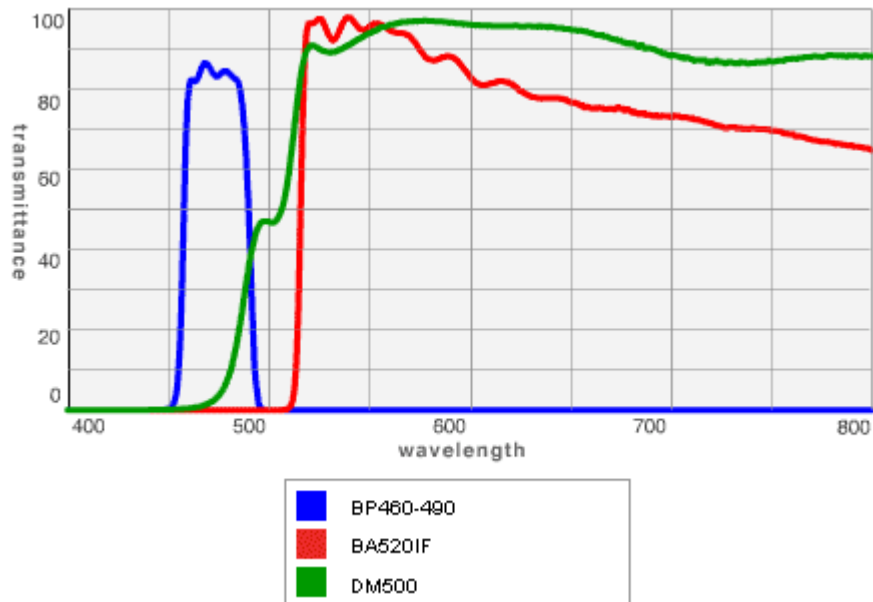
UIS2 series : Fluorescence Mirror Unit
U-MWB2



Excitation filter	Emission filter	Dichromatic filter
460-490	520IF	500

Transmittance/Wavelength

[.CSV download](#)



The values of transmittance were measured using Lambda900 (PerkinElmer Life & Analytical Sciences).

- Position 4: Data Sheet for Filter Cube U-MWG2:

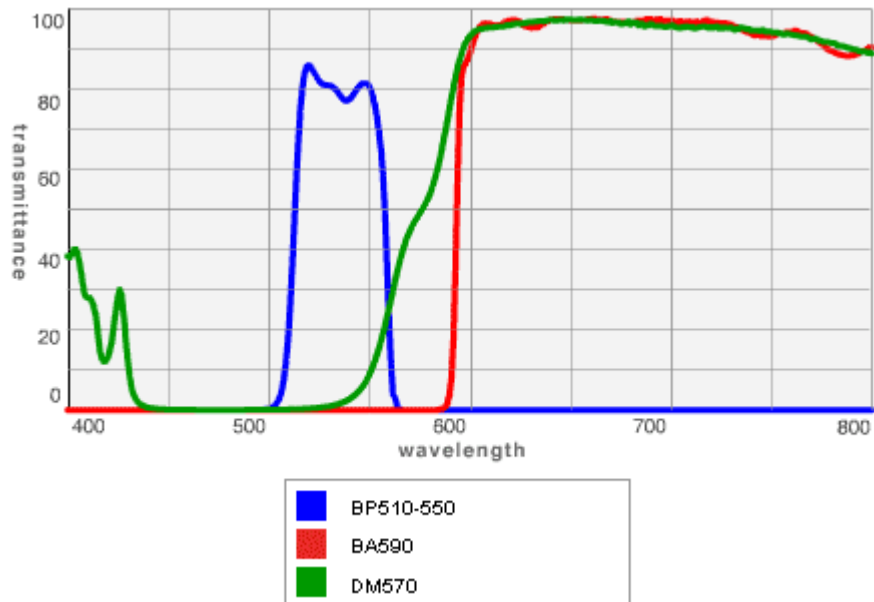
UIS2 series : Fluorescence Mirror Unit
U-MWG2



Excitation filter	Emission filter	Dichromatic filter
510-550	590	570

Transmittance/Wavelength

[.CSV download](#)



The values of transmittance were measured using Lambda900 (PerkinElmer Life & Analytical Sciences).