

Short instructions. Olympus FV1000

Version 140416

This short instruction will guide you through the turn on and off sequence of the microscope system. For a detailed instruction in system operation refer to the Olympus user manual.

This instruction also contains important information on laser safety and general guidelines.

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1. Laser safety

Safety notes for operating the laser scanning microscope:

During scanning, the laser radiation is freely accessible after exiting the objective in the specimen area of the laser scanning microscope. This circumstance demands special attention and caution. If the laser radiation comes in contact with the eyes, it may cause serious eye injuries. For this reason, prudent handling is absolutely necessary as soon as one or several laser emission warning indicators are lit. If used as prescribed and observing the safety notes during the operation of a laser scanning microscope, there are no dangers to the user.

1. According to IEC/EN 60825-1, this laser scanning microscope is a laser device of Class 3B.
2. Never look directly into a laser beam or a reflection of the laser beam. Avoid all contact with the laser beam. Otherwise, your eyesight may be permanently damaged. A reflected laser beam is just as dangerous as a direct beam.
3. Never deactivate the laser protection devices. Read the “Safety Guide” in the Olympus User’s Manual to familiarize yourself with the safety devices of the laser scanning microscope. If you find any of the laser protection devices inactivated, please contact the facility staff immediately.
4. Note that objects (such as micromanipulators) in the specimen area may cause laser light to exit the safe beam path during the scanning in an uncontrolled manner by means of reflection or scattering and endanger the environment.
5. Do not change a specimen during scanning.
6. Do not change any objectives during scanning.
7. All unused positions of the objective turret should be closed with a cap.
8. Do not change any filter cubes or beam splitters during scanning. Never disconnect an optical waveguide.
9. Never remove the scan head from the microscope stand during operation. Before removing the scan head, the system must be completely switched off.
10. Do not remove the microscope condenser.

2. General guidelines

1. Lab rules:

This room is a S2 laboratory.

At times when S2 experiments are performed in this room, you are not allowed to enter the room. S2 experiments will be indicated by a sign at the outside of the door and in the online booking system.

When no S2 experiments are performed everyone who is authorized by the facility staff can enter the room.

You always have to wear a lab coat. Lab coats can be left in the cupboard, first door left from the entrance.

2. Booking and keys:

Before you can use a microscope you have to make a reservation in the online booking system.

Always pick up a key from Wolfgang Posselt in room 1052. Office hours: Monday to Friday. If you are planning to use a microscope outside these hours, pick up a key in advance.

When you are leaving lock the microscope room and after 17:00 also lock the main door of the facility. Return the key to Wolfgang Posselt or to the mail box of the Laser Microscopy Facility, level S, in the hallway left from the stair case, second row, in the center.

3. Climatisation control:

Please note in the online reservation the desired temperature.

Wolfgang Posselt will switch on the temperature control early in the morning to ensure that the incubation chamber has reached the temperature when you start your experiment.

But you are responsible for setting CO₂ and humidity as well as for switching off the climatisation control. Read chapter 4. "Using the climatic chamber".

4. Immersion oil:

Always remove old immersion oil, before you add new ones.

Only use the lens tissues provided by the Laser Microscopy Facility staff.

In case there is oil on no-oil objectives, don't try to clean them but leave a note in the log book.

5. Changing objectives:

Never change the objectives manually!

To change objectives use the microscope control menu within the Olympus software or the panel box left of the microscope stand.

6. Changing the specimen:

Before you change the specimen, drive down the objective nosepiece.

Don't forget to remove the old oil from the objective before you add new ones.

7. Lasers:

Lasers for the lines 491, 532, and 559 nm have to be switched on manually. Read chapter 3 for details.

8. Data transfer:

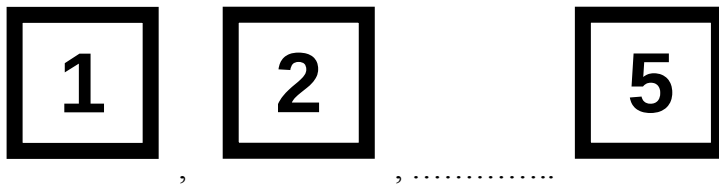
Never store any data on the microscope computer.

There are three ways to transfer your data from the microscope computer:

- a. Transfer through the MHH network. Save your data on hard disk P: of your department.
- b. USB-sticks or movable hard disks connected via USB. The USB sockets are located at the back of the computer.'
- c. Writable CDs. This computer supports only CD-R, -RW. Other formats will not be recognized. Open the Nero Start Smart software and follow the instructions.

3. Switching on the microscope

1. Follow the procedure by the numbers:



2. Switch on the **HBO lamp (no. 1)**.
3. Switch on **all the labelled buttons of the main switches no. 2 and 3**. Microscope stand, laser and scan controllers, and the computer start automatically.
4. Start the Olympus software: double click on icon “**FluoViewStart**”.
5. Select your **User ID**, type in your password, and press OK.
6. **Do not perform the next step until the software start up is completed.**
7. This microscope provides the following **laser lines**:
 - 405 nm
 - 440 nm
 - 491 nm
 - 532 nm
 - 559 nm
 - 635 nm

The lasers for 405, 440, and 635 nm are controlled entirely through the Olympus software. These lasers are switched on by selecting an acquisition protocol from the dye list in the “Image Acquisition” window or by directly clicking on the laser box in the “Acquisition Setting” window.

The lasers for 491, 532, and 559 nm have to be switched on manually.

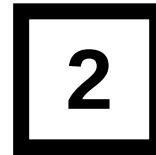
Note, the software will not recognize, if they are not switched on.

Switch on only the lasers you need.
8. To switch on 491 and 532 nm, after the green diode stopped blinking, turn the ignition key (no. 4) to the vertical position. A red diode flashes for about 60 sec. Then the green and 2 red diodes should be on.
9. To switch on 559 nm (no. 5), wait until the diode 'TEMP' stops blinking. Then turn the ignition key (no. 5) to the ON position. The red diodes “Laser” will blink for a few min. Then they should stay red.

4. Using the climatic chamber

1. Check the water bottle at the control unit behind the microscope table. Refill with distilled water only. Distilled water is in Wolfgang Posselt's office.

Eventually open the valve underneath the water bottle.



2. Turn on the power switch at the right side of the control panel no. .
3. Switch on the microscope following the procedure in chapter 3. The climatisation control software opens automatically.
4. In the software set the desired temperature between 30° and 42° C. Cooling is not possible. **Do not activate the humidity before the temperature is stable to avoid condensation which will damage the microscope equipment.**
5. If you are using the CO₂ open the gas bottle at the wall behind the microscope table. Turn the main valve counter clockwise, not more than a quarter turn.
6. When the temperature is stable set the desired values for CO₂ concentration (between 0 and 10%) and relative humidity.
Don't set the relative humidity higher than 60%. Higher values will cause condensation which will damage the microscope equipment. The software will open a warning. Confirm by pressing no.
7. For shutting down, select first the dry mode in the software. The values for CO₂ and humidity will be set to minimum and the temperature to 37°C. **Let the dry mode run until the humidity is less than 25%.**
8. Close the gas bottle, if used..



9. Turn off the power switch at the control panel no. .

5. Switching off the microscope

1. Exit the control software.
2. Shut down Windows.
3. Turn the key switch of the 491/532 nm laser to off (no. 4).
4. Turn the key switch of the 559 nm laser to off (no. 5).
5. Switch off all buttons of switch boards no. 3 and 2.
6. Switch off the HBO lamp (no. 1).