

# General Usage Rules for Confocal Microscopes (Leica SP8, Leica SPE, Leica SP2, CARV II, Bruker ULTIMA)

## Usage rules

1. **Reservation.** The microscope can be reserved at <https://biomed.cas.cz/rezervace/>. The access is granted only for registered users and the permission is issued by the IT Department [helpdesk@fgu.cas.cz](mailto:helpdesk@fgu.cas.cz) after an approval of microscope's manager ("Manager") (<http://www.fgu.cas.cz/articles/663-kontakty-czbi>). The user's login credentials will be sent back to the user's e-mail address. For non-registered users the microscope can be reserved only through the Manager. To display the reservations, click on "Plánování a vytvoření rezervací" and then on the blue triangle next to the heading "Přehled rezervací". Then select "Bioimaging Facilities" and reserve the time slot at the microscope. The right to use the microscope is exclusively reserved for the person stated in the reservation system. The users are obliged to conduct an experiment at the reserved time slot, otherwise, the reservation has to be edited or cancelled. Any problems with the reservation system should be reported to IT Helpdesk, IPHYS CAS ([helpdesk@fgu.cas.cz](mailto:helpdesk@fgu.cas.cz)).

Reservation through the website can be created maximum one month in advance. The reservation has to be edited/cancelled as soon as the need of change is known – in any case the edit/cancellation cannot be made later than 24 hours before the beginning of the session. To edit or cancel the reservation within 24 hours before the start, contact the Manager. The reservations which are not cancelled will be charged the full price. If the reserved time slot is not used repeatedly, the reservation time at the microscope will be restricted. The facility safety rules and conditions for acquiring the login credentials are stated on page 2 of Training protocol see the relevant document – link below.

2. **Users training.** After registration into the reservation system, the user gains a status of the untrained user. To acquire a status of the trained user (who can work on the microscope independently), it is necessary to complete a mandatory training. This training includes working safety rules, microscope control and operation and application tips. The Training protocol is issued and signed after the successful training. For training please contact the Manager.

3. **Guidelines for handling the microscope.** The user is obliged to fill in all the items in the Microscope attendance book (name, start time, end time, affiliation, financial source for payment, experiment duration, purpose and notes), which is located at the microscope location, and sign at the end of the experiment.

All the microscope configuration changes (hardware, for example filters) must be consulted with the Manager in advance. The configuration can be changed by the user if and only if the user is trained and explicitly allowed by the Manager to change the

specific microscope configuration. However, the microscope configuration changes have to be returned to the default state at the end of the experiment unless stated otherwise by the Manager.

The mercury lamp should not be switched on earlier than 5 minutes in advance. Do not turn off the mercury lamp when it is planned to use the mercury lamp again within 20 minutes. Do not turn on the mercury lamp when it is still hot due to potential exposure to mercury vapour.

Do not directly touch any optical parts by hand (objective lenses, filters, etc.). Do not use excessive force to change the microscope setting (objectives, incubator box, etc.) and rather contact the Manager.

At the end, the user is obliged to clean the working area, i.e. place used glass into the dedicated container and remove the immersion media from the objectives by using lens cleaning tissues. The user also immediately reports any unusual behaviour of the system to the Manager and states the problem into the Microscope attendance book. If not stated otherwise by the Manager, the user always returns the microscope site into its default state.

At the end, the user will fill in specifications of his/her usage into the Microscope attendance book (name, start time, end time, affiliation, financial source for payment, experiment duration, purpose and notes), if any of the information was not filled at the start. Sign the information filled.

Any further questions (e.g. experimental design) and requirements (e.g. different set of filters) should be requested via the Manager.

When the results are used for a publication, the users have to indicate that in Acknowledgments. The precise form is stated below and any questions will be answered by the Manager.

## Safety hazards and prevention measures

1. **Microscope.** Microscopes are delicate devices, which require careful caretaking. Just as you would be driving a car, you need to be aware of what you are doing and that you are responsible for the damages to the equipment and harm to the people caused by your mishandling of the microscope usage.

2. **Supervision.** All microscope users must be supervised during the use of microscope, unless previously trained by responsible facility staff. Even when trained user status is acquired, the user is responsible to contact the Manager prior any activity with the microscope that is new to the user. Untrained personal is not allowed without prior permission into the microscopy rooms as they are unaware of the workplace hazards. The facility staff can remove any user who breaks the usage rules.

3. **Samples.** Every sample brought to a microscope must follow the guidelines of Institute of Physiology. The user is responsible for correct disposal of the samples after

the microscopy session has ended, as well as for removing the contamination and residues of samples.

4. **Laser safety.** When entering the room, you are required to turn on the warning lights to indicate you are using lasers. Always use laser specific protective eyewear when using multiphoton laser.

DO NOT USE the multiphoton laser without the laser protective eyewear!

DO NOT stare into the laser beams.

Lasers can cause eye and skin injury. When using a laser, you must be aware of standard behaviour of the machine and where the lasers are after opening shutters. If you do not have the information, ask the Manager, otherwise you are not allowed to use the lasers without supervision.

DO NOT search for the multiphoton laser beam, it is infrared, therefore invisible to the human eye.

5. **General caretaking.** While working with microscopes, you will be in contact with some of its parts more often than with others. Take greater care around the following parts:

### I. Objective.

**A.** Objective is a key component to your imaging. Each type of objective requires specific immersion media, except for dry objectives.

**Dry objectives:** **DO NOT apply** any immersion media!

**DO NOT CONTAMINATE** non-immersion objectives.

**Water objectives:** ALWAYS use **ONLY DEIONIZED or DESTIL WATER!**

**Oil objectives:** ALWAYS use **system specific Immersion OIL**  
**NEVER** place **oil on a water** objective **or water on an oil** objective!  
ALWAYS CLEAN an oil, water or glycerol immersion from both the **sample glass** and from **the objective** before switching to another objective or ending the work.

**B. Cleaning procedure:** Fold a lens cleaning tissue and gently drag it over an objective lens in a **straight unidirectional motion** approximately 5 times using a different part of the lens cleaning tissue each time. Fold a lens cleaning tissue and apply a drop of Diethyl-ether. In a straight unidirectional motion, drag the tissue over an objective lens once.

**DO NOT WIPE** in a **CIRCULAR** motion!

**ONLY USE** dedicated **lens cleaning tissues!**

**C. Application of immersion:** Apply **only one drop** of oil (oil objectives) or water (water objectives) to an objective's lens or your sample. A drop should have a nice **circular dome shape**. Too much oil or water will

result in contaminating other parts of the microscope, potentially causing damage!

Slowly approach with the objective to the sample until the immersion drop connects with the sample's coverglass. **DO NOT CRASH the objective** lens against the coverglass. Watch the approach to the sample from the side without the presence of any laser light.

**D. Tilescan, Mark&Find, and other stage movement:** **ALWAYS** check that **no obstacles** are present that could potentially harm the objective or condenser.

**Be highly aware of the stage movement** especially with custom-made solutions of microscope inserts. Familiarize yourself with the software controls and indication of movement.

## II. Detectors.

**DO NOT EXPOSE** hybrid detectors or GaAsP detectors **to ambient light** or any other light source aside from the fluorescence from your sample. Light overexposure could cause not only damage to the detectors, but also your imaging data might be lost. Some detectors have an overload protection, but you should still **start your imaging with very low intensities of the laser.**

## 6. Microscope-specific Usage rules.

**Leica SP8:** Lasers in general must be turned off in LAS X software. Special attention must be pointed to Argon laser usage, which requires special system shut down procedure. DO NOT turn off the hardware laser power button, the system must stay turned on for about 15 minutes for Argon laser to cool down. Not following this rule will result in decreased service life of Argon laser or to its complete malfunction.

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**Usage fees:** see the document - link below:

[Internal users](#)

[External users](#)