

Nikon Instruments Eclipse Ti-E Inverted Microscope coupled with Nipkow spinning disk confocal imager CARV II

Location

Room D / basement / 048 (l. 2472)

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Contacts

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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 after the manager's approval. 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).

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 side, 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. 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 side into its default state.

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.

Czech-BioImaging project:

- „Supported by MEYS (LM2015062 Czech-BioImaging)” or
- *We acknowledge the BioImaging Facility, Institute of Physiology, supported by the Czech-BioImaging large RI project (LM2015062 funded by MEYS CR) for their support with obtaining scientific data presented in this paper*

OPPK Microscopic systems project:

- „Supported by project OPVK Mikroskopický systém CZ.2.16/3.1.00/28034“.

Usage fees: see the document - link below:

Internal users:

http://intranet.fgu.cas.cz/pristroje_sluzby/PublishingImages/Stranky/Kontakty_a_obecne_informace/Poplatky%20za%20využití%20zobrazovacích%20technik%20FGÚ%20zapojených%20do%20projektu%20Czech-BioImaging.pdf

External users:

http://www.fgu.cas.cz/upload/files/Fees_Imaging_Core_Facilities_IPHYS_Czech_Bioimaging.pdf

Specification of the confocal microscope Nikon Ti-E / Crest CARV II

1. **Microscope.** The inverted fluorescent microscope Nikon Ti-E with motorized stage Prior Proscan III enables one to conduct multiple ROI, Tile Scan and Mark&Find experiments. The microscope is coupled with Nipkow spinning disc imager CARV II that contains a unit for confocal and FRAP imaging. The whole system is placed on an active pneumatic anti-vibration table STable© Supertech.

2. Available objectives.

Nikon CFI Plan Fluor 10X, DIC, 10x/0.3 NA, WD = 16 mm;

Nikon CFI ADL 10X, Ph, 10x/0.25, WD = 6.2 mm;

Nikon CFI Plan Fluor 20X, DIC, 20x/0.5 NA, WD = 2.1 mm;

Nikon CFI S Fluor 40X Oil, DIC, 40x/1.3 NA, WD = 0.22 mm;

Nikon CFI Plan Apo Lambda 60X Oil, DIC, 60x/1.4 NA, WD = 0.13 mm;

Nikon CFI Plan Apo VC 100X Oil, DIC, 100x/1.4 NA, WD = 0.13 mm;

For more information, please visit:

https://www.nikoninstruments.com/en_EU/Product-Selectors/Objective-Selector

3. Illumination path. The sample can be illuminated by a halogen lamp in transmission mode or by the mercury lamp X-Cite® 120PC Q in epi-fluorescent mode. The provided spectrum ranges from 300 nm to 700 nm and the illumination path is optimized for common dyes (DAPI, GFP, Cy3, Texas Red, Cy5).

4. Detection path. The emission path is equipped with band pass filters that are optimized for the above mentioned dyes. The image is acquired by a cooled monochromatic 16bit CCD camera Hamamatsu Orca-R² with resolution 1344 (H) x 1024 (W), pixel size 6,45 µm x 6,45 µm, acquisition speed 16,2 fps and quantum efficiency over 70 %.

5. Box incubator. The system Okolab UNO-COMBINED-CONTROLLER controls the environmental parameters such as temperature (ambient temperature to 50° C), CO₂ (0 to 15 %) and relative humidity (up to 75 % for 37° C). The size of the box is 85,5 x 127,7 x 25,0 mm.

6. Software. The whole system is controlled through NIS-Elements AR that supports a tool for running complex tasks (module Jobs).

Useful websites

- Reservation system at the Institute of Physiology, Czech Academy of Sciences:
<https://www.biomed.cas.cz/rezervace/>

- Specification of optical microscopes at the Institute of Physiology, Czech Academy of Sciences:

<http://fgu.cas.cz/research/167-shared-equipment>

- Detailed description of the microscope:

Internal users:

http://intranet.fgu.cas.cz/pristroje_sluzby/Stranky/Kontakty_a_obecne_informace.aspx

External users:

<http://www.fgu.cas.cz/en/articles/567-carv-ii-system>

- Information on the equipment that has been included into the Czech-BioImaging project:

<http://www.fgu.cas.cz/articles/529-czech-bioimaging-2016-2019>

- Training protocol – internal users:

(http://intranet.fgu.cas.cz/pristroje_sluzby/Stranky/Kontakty_a_obecne_informace.aspx)

including the rules for entering the facilities with laser scanning confocal microscopes at the Institute of Physiology, Czech Academy of Sciences, template

(http://intranet.fgu.cas.cz/pristroje_sluzby/PublishingImages/Stranky/Kontakty_a_obecne_informace/Z%c3%a1znam%20o%20za%c5%a1kolen%c3%ad%20formul%c3%a1%c5%99%20Czech-BioImaging%20VZOR.pdf)

- Training protocol – external users: <http://www.fgu.cas.cz/articles/529-czech-bioimaging-2016-2019>

Image processing in free software NIS-Elements Viewer
https://www.nikoninstruments.com/en_EU/Products/Software/NIS-Elements-Advanced-Research/NIS-Elements-Viewer

or

image processing and analysis with open source program Fiji (Fiji is Just ImageJ) that includes a number of useful plug-ins. For example:

Bioformats (former LOCI Tools): <http://imagej.nih.gov/ij/> ;

SLIM Curve: http://fiji.sc/SLIM_Curve, <https://slim-curve.github.io/> ;

ImageJ world mailing list: <http://imagej.nih.gov/ij/list.html>.

Interactive day spectra viewer:

ThermoFisher (Life Technologies) interactive Spectra Viewer.

<http://www.thermofisher.com/cz/en/home/life-science/cell-analysis/labeling-chemistry/fluorescence-spectraviewer.html.html>

Tables of fluorescent dyes spectra:

<http://www.fluorophores.tugraz.at/substance/>