Biooptics Newsletter 04/2021

Biooptics Core Facility-in general

The Biooptics Core Facility of the MUI exists since early 2009 and is located in the CCB, Innrain 80-82, first floor, rooms M01.370 and M01.381. We are currently hosting five microscopes in-house, a neuron-tracing instrument in cooperation with the Institute of Pharmacology at Peter-Mayr-Strasse 1a and a 2-photon microscope in cooperation with the Institute of Physiology, Schöpfstrasse 41. You can find more information on the official website.

Microscope trainings

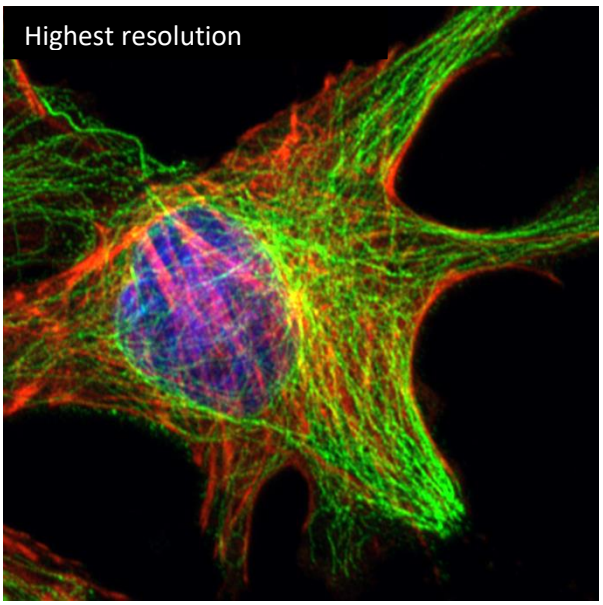
During the onset of the corona crisis, we have started to implement no/low contact trainings on all microscopes. These trainings consist of a two-step process; as a first step the trainee is asked to watch a video that was recorded in order to demonstrate the most important hardware operations on the microscopes (how to switch it on and off), followed by an interactive training. Upon request the training may be recorded on video and transferred to the trainee for reference purposes. Presently the trainings are mostly held in a hybrid online/presence format. The long-term (post-Covid) goal will be to unify the "best of both worlds" (documentation via video and personal assistance).

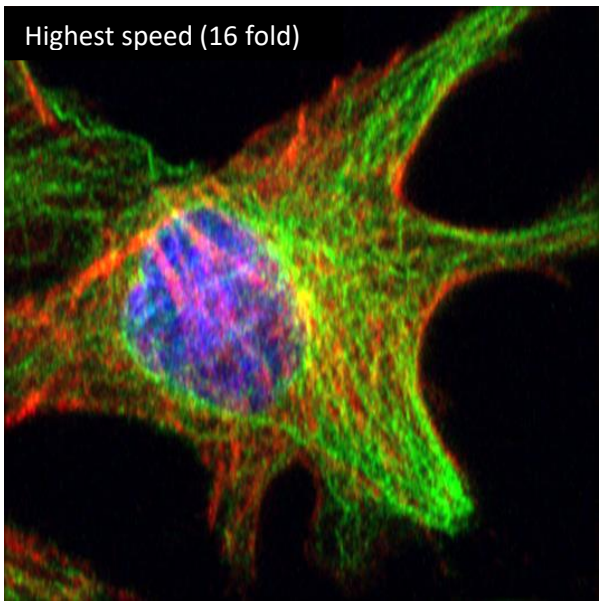
New computer and screen on the SP8

The old PC (from 2013) and the corresponding computer screen were replaced by new ones. The operating system has been changed (to Win 10) as well in early 2021.

New microscope LSM980 AiryScan2

After several delays the new confocal microscope from Zeiss was delivered in spring 2020 and a general online training from Zeiss was done in two separate sessions (beginner and advanced). This microscope is intended as a general replacement of the outdated SP5. Apart from general confocal imaging this microscope is equipped with a special AiryScan detector. This imaging mode spreads the fluorescent light onto an array of 32 individual detectors that are geometrically arranged in a honeycomb-like shape. Thus additional image





information is recorded, which is used to improve signal-to-noise-ratio and/or (!) resolution and/or (!) acquisition speed in a very flexible manner. Depending on the needs of the researcher there are 5 different airyscan-imaging modes available from very high precision (resolution) to very high speed at reduced precision. Example shows a cell stained for actin, tubulin and DNA. In addition, a general confocal spectral detector is also available, albeit most users are imaging with the AiryScan detector

The microscope is equipped with 5 objectives (air, glycerol and water immersion), 6 Lasers, an incubation box, temperature and CO2 control. Larger objects (tissue sections) can be scanned using the sample navigator. All standard experiments such as z-stacks, time series, bleaching (FRAP) or a combination thereof are easily possible. More complex experiments can be planned using the experiment designer.

This microscope offers fluorescence correlation spectroscopy (FCS) a novel technique for Innsbruck in its most basic version as well. A special training was done (and recorded) in 2020. Briefly, this method can be considered as supplementary to FRAP to measure molecular speeds and binding events (like FRAP). FCS is very useful in low expression situations (avoiding any overexpression problems) and will yield fully quantitative data on concentration of molecules (in mol/l) and their movement speeds. Feel free to contact me for more information.

2-Photon microscope: UPDATE

In 2018 the Biooptics Core Facility of the MUI purchased a Leica SP8 MP multiphoton microscope, which is hosted and supervised by the Institute of Physiology (Director: Univ.-Prof. Michaela Kress; Supervision: Kai Kummer, PhD).

2-photon microscopy makes use of long-wavelength light produced by infrared (IR) excitation lasers. At the level of physics **two photons, with half the energy i.e. the double wavelength** are used for the excitation of the fluorophore (in standard confocal, STED, AiryScan there is always one photon used). 2-Photon microscopy allows imaging deep into tissues with highest sensitivity thus depicting finest details of cellular and subcellular processes (for more information visit https://www.leica-microsystems.com/products/confocal-microscopes/p/leica-tcs-sp8-mp). 2-photon microscopy is generally most useful in all situations requiring to image deeply within thick samples (100s of microns are typically achievable).

© Institute of Physiology, MUI, SP8 2Photon

The setup is currently equipped with a Mai Tai DeepSee IR laser (690-1040nm; Spectra-Physics) together with a standard blue laser (488 nm) and filter sets for GFP/mCherry and CFP/YFP imaging, as well as a temperature control system (Scientifica) for perfusion of acute brain slices. The microscope is controlled by the Leica LAS X SP8 control software.

Currently, only a few research groups make use of this microscope setup, so if you are interested please get into contact with us to discuss further arrangements or request an introduction using PPMS.

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LSM980 or SP8: which confocal microscope to use?

Having now two modern (and one outdated) confocal microscopes the SP8 (including STED) and the LSM980 (including airyscan) many existing and potential new users might wonder, which is the better choice. There is not always a straightforward answer to that and there clearly is a partial (!) functional overlap between the two instruments. Nevertheless, it is possible to point out several unique and specific features for both microscopes considering the three major aspects resolution, speed and sensitivity.

resolution: Both microscopes can yield **"superresolution"** images, but at very different levels and using different staining and imaging procedures. The **LSM980** can reach down **to 120 nm resolution**, whereas the **SP8gSTED** will achieve around **35 nm xy resolution in 2D STED** mode. This clearly indicates that the SP8 is superior in achieving very high resolutions. The downside is the slightly more complicated sample preparation (essentially an indirect IF using special dyes). Moreover, the achieved resolution using the LSM980 will be asymmetric by default (xy= 120 nm; z=600 nm), whereas 3D STED on the SP8 will result in (almost) isotropic resolution in all 3 dimension (xyz= 80 nm). If highest resolution is the main goal, the STED mode of the SP8 is clearly preferable to the superresolution mode of the airy scan detector in the LSM980.

speed: At its **highest speed (CO-8Y)** the **AiryDetector** of the LSM980 will be **faster** than the resonant scanner (RS) of the SP8 for a **single dye**. Additionally, the signal-to-noise ratio of the airy scan detector will be superior to the one of the RS in the SP8. The overall speed comparison will also depend on the amount of dyes to be imaged. On the LSM980 there is just one AiryScan detector that needs to image all dyes (the confocal detector of the LSM980, will be a lot slower than the RS mode of SP8). If there are multiple dyes to be imaged the speed of the SP8 might still be better due to the availability of more than one detector for high speed imaging. For high speed applications the choice of the microscope will thus depend also on the number of dyes to be analyzed. Note that high speed is not only limited to live samples - a thick and large fixed tissue section may also require "high speed" in order to avoid very long imaging times.

sensitivity: In general, the **AiryScan** detector will have advantages over the HyDs of the SP8. The fluorescence signal is detected in 32 channels, therefore all **noise** will be **averaged out** even at very low light levels. The overall **sensitivity of the AiryScan detector** is therefore one of the main advantages of the LSM980, although the HyDs of the SP8 are also very sensitive detectors.

moreover: Additional instrument specific aspects include the pulsed WLL of the SP8 and the confocal spectral detector of the LSM980, which both represent unique features on the respective instrument that may be usefully applied to specific imaging problems. Starting with the WLL it offers a lot of flexibility considering the choice of excitation wavelengths. Whereas in typical setups (such as the LSM980) you have just one laser line such as 488 nm to excite any green chromophore, in the SP8 you can freely choose between 1 and 8 lines from 470 to 670 nm. This enables exciting exactly at the excitation maxima of chromophores, which is very useful for separating many spectrally overlapping chromophores

(note that e.g. the excitation maximum of Alexa 488 is not at 488 nm but at 498 nm). It is also possible to remove unwanted reflections in the sample using the WLL in combination with gating. The Sp8 is also equipped with a very convenient autofocus called AFC that is able to prevent any focus drift of the microscope for hours to days, which makes it very useful for prolonged live cell imaging. For the LSM980 the overview scan is very useful for any researcher working on large tissue sections since it enables generating maps of the sections very easily. Spectral detection is very well implemented on the emission side using the confocal spectral detector (≠airyscan). For newbies the software of the LSM980 is slightly easier to operate than the one on the SP8. Leica users, who have worked on a different Leica microscope in the past, will find it very easy to operate the SP8 software.

Contact and further information

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