Biooptics Newsletter 08/2020

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 Department of Pharmacology at Peter-Mayr-Strasse 1a and a 2-photon microscope in cooperation with the Department of Physiology, Schöpfstrasse 41. You can find more information on the official website.

New microscope trainings

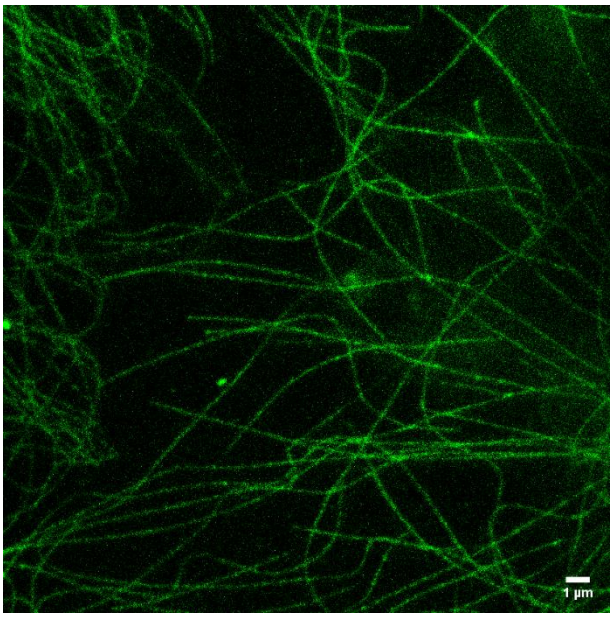
During the onset of the corona crisis, we have started to implement no/low contact trainings on all microscopes. These trainings consist of a two-step process; as a first step the trainee is asked to watch a video that was recorded in order to demonstrate the most important hardware operations on the microscopes (how to switch it on and off). Upon request the training may be recorded on video transferred to the trainee for reference purposes.

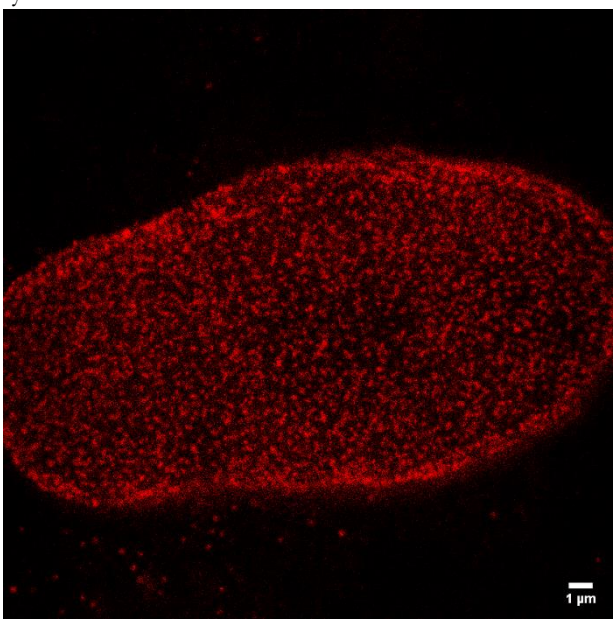
New microscope LSM980 AiryScan2

After several delays the new confocal microscope from Zeiss was delivered in 2020 and a general online training 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 This microscope is intended as a general replacement of the outdated SP5.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 detector spreads the fluorescent light onto an array of 32 individual detectors that are geometrically arranged in a honeycomb-like shape. This enables captureing additional image information which is used to improve signal to noise ratio and/or (!) resolution and/or (!) acquisition speed in a very flexible manner.

2D STED with 775 nm

If you are mostly interested in 2D information about your sample, 2D STED will be the best choice. In this case you can also expect the best resolution in the xy direction. All the energy of the 775 STED laser donut will be applied in xy only. There won't be any STED laser applied along the z-axis. Expected resolution will be 35 nm in xy, resolution in z: similar to confocal around 800 to 1000 nm. The required pixelsize **in xy will be <20 nm** in order to recover the available resolution. This approach will work well for intrinsically flat samples, since out of focus light along the z-axis will have a negative impact on the resolution in xy. Even if you are interested in 2D information it still can be useful to take a few (!) z-sections (4 to 5) at a **z-distance of 150 nm.** These additional z-planes can be used in the Huygenssoftware in order to improve the resolution in the xy direction even more (2D STED deconvolution). Image 1: 2D 775 nm STED of microtubules of a Cos7 cell, Alexa 594. Image 2: 2D 775 nm STED of a Cos7 cell, Nuclear Pore Complex, stained with Star635P.

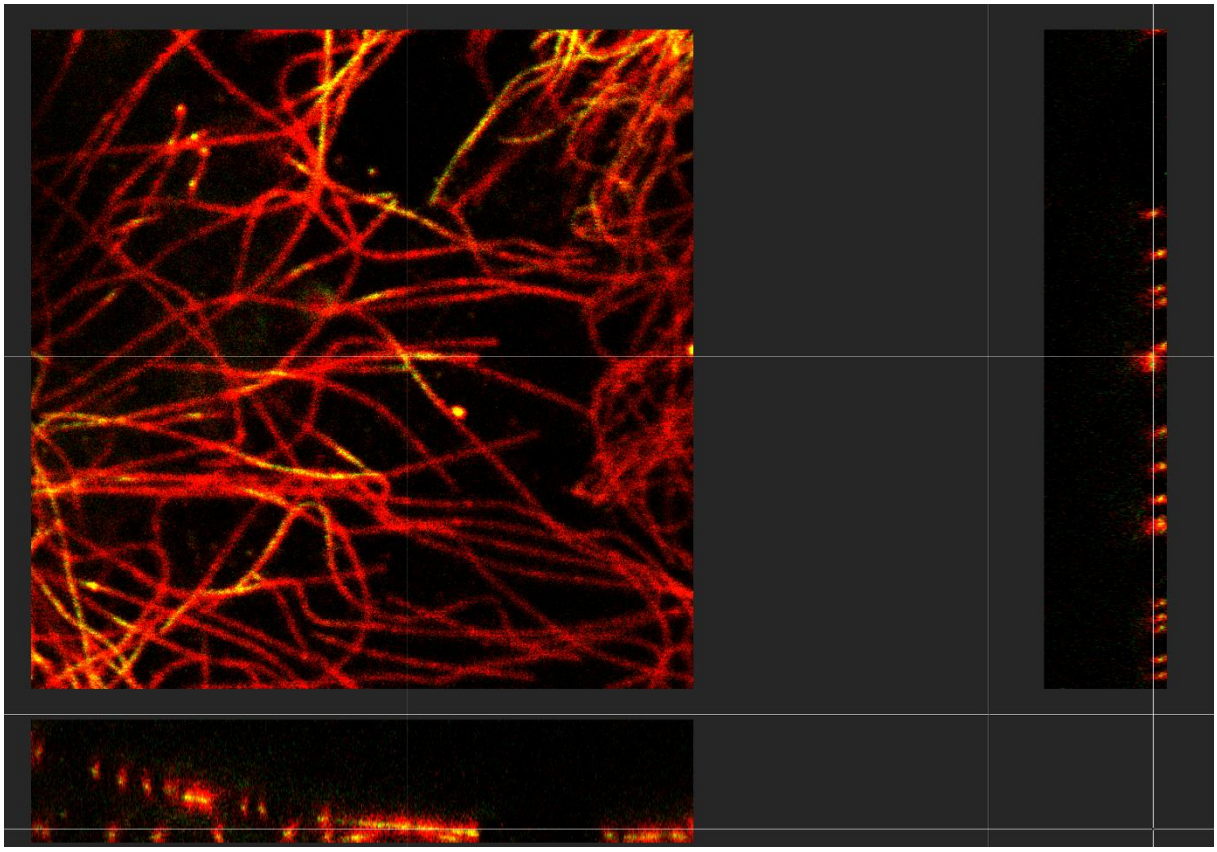




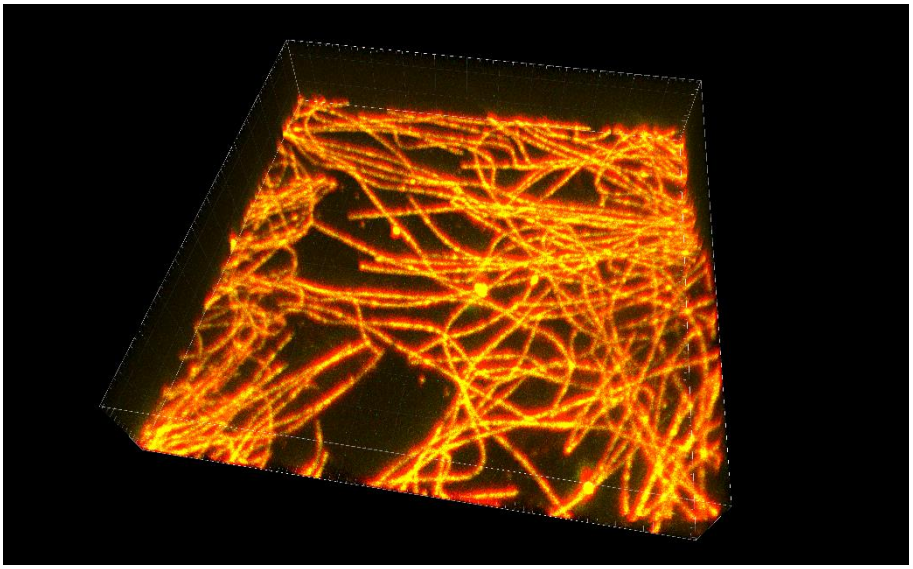
3D STED with 775 nm

If your sample is clearly 3D and/or you are interested in 3D information, you should go for 3D STED. In 3D STED the 775 nm STED laser is applied onto the sample as 2 donuts, one in xy and one in z-direction. Hence, the energy of the STED laser is split over the optical axes, resulting in some loss of resolution in xy and an improvement in z. This 3D STED option is very powerful and it provides you with an excellent tool against the (intrinsically) bad z-resolution in confocal microscopy. In our system it is also possible to tune the amount of z- vs xy STED light to be applied from 0% z (= 2D STED) to 100% z- STED light (= 3D STED). You can also decide for e.g. 2.5D STED. The final resolution will also depend on this z-donut setting; for full 3D STED it is expected to be around 60 nm for xy and slightly lower for z.

In order to visualize this different 3D performance of 3D STED vs Confocal I did a confocal scan in parallel to a 3D STED (100%) using again Alexa594 stained microtubules**. Red=confocal, low z-resolution**;

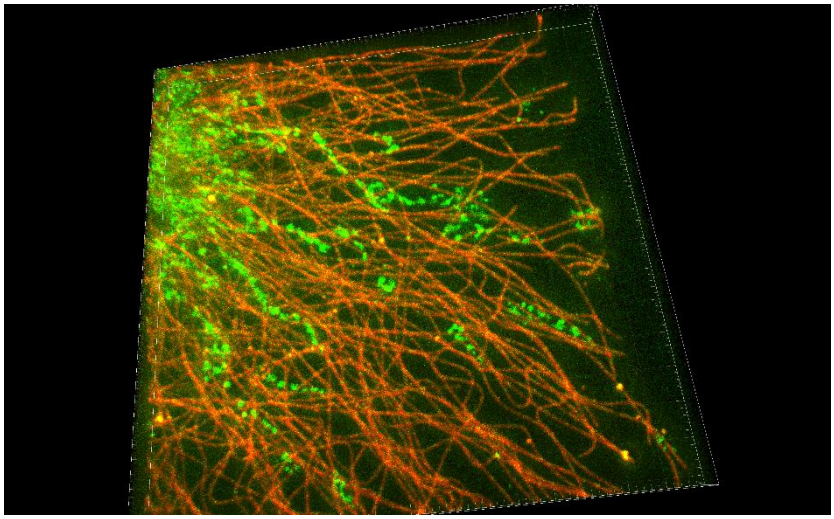


green=3D STED, high z-resolution: Only the part of the MTs which is really "in focus" is seen in green in this ortho-section view. Also note the elongated structures in red in the two smaller images (xz and yz views). Image 2: Same image in 3D view. In general, green structures (3D STED) are thinner (=have higher resolution) than the red ones (Confocal).



2 color STED using the 775 STED laser with two dyes

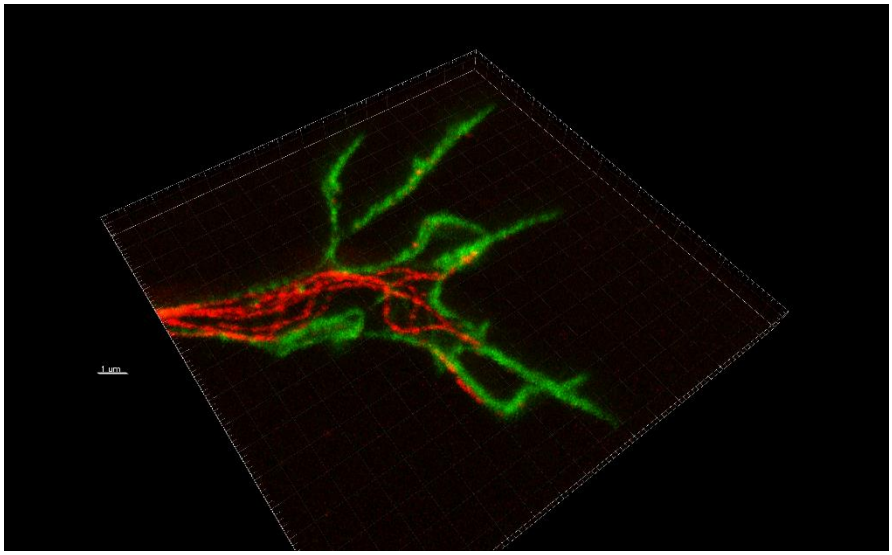
There are now 2 possible strategies for doing multi-color STED experiments. There more commonly used and easier one is to use two different dyes that can both be used with one STED laser, such as the 775 STED laser. It is easier to setup and to perform than a two-STED laser experiment. For this (as discussed above) both dyes need to be suitable for the 775 laser. The two dyes – such as Alexa 594 and STAR 635P - can be imaged in a sequential scan setup between lines or frames (even stacks). Linear unmixing ("dye separation") may be required for a good separation- it is the preferred approach nevertheless. In addition, a simultaneous scan using two Detectors (HyDs) is also possible. Again the user has full freedom to either decide for 2D or 3D STED.



Example: Cos7 cell with MTs (A594) and the mitochondrial Marker Tom20 (STAR 635P), sequential scan between lines, 3D STED for both dyes.

Advanced: 2 color STED using the 592 AND the 775 STED Laser

In this approach the sequence of the overall imaging procedure is very critical. It is mandatory to first image with the 775 Laser and then with the 592 STED Laser- not vice versa! Any (even short) exposure of the far-red 775 fluorophores to the 592 STED laser will immediately bleach all far-red fluorescence. For z-stacks the switch between the dyes needs to be done "between stacks". In case of failure, a new cell/structure needs to be found on the slide, re-imaging is not possible. Suitable dyes are green STED dyes such as Oregon Green 488 plus far-red dyes such as STAR RED. Example (in cooperation with Rüdiger Schweigreiter, Neurobiochemistry): Growth cone of a sensory neuron (DRG): Actin was stained with Phalloidin-OregonGreen 488, MTs were stained with AB and STAR Red. Microtubules were imaged first (complete zStack, 3D STED); thereafter the ActinOG488 was imaged (complete z-stack, 3D STED).



Final remarks

During the setup of the upgrade also the **old 592 STED laser has been realigned extensively**. The performance of 592 STED is clearly better than before (less bleaching, than it used to be; but certainly more bleaching than with the 775 Laser).

The 93 x glyerol objective together with the 775 laser enables imaging of **thick samples such as thick tissue sections.** I was not able to test it since I had no sample around, but it is described to work very well. All scientists interested in such thick samples should consider to try it on the new setup - including those who failed in the past with the 592 Laser + oil objective – it will certainly be a lot better.

In the Huygens software, we are currently able to process 2D STED images only; I have requested the 3D STED option and hope to get it soon. All images in this newsletter have not been processed with Huygens (this would result in a further improvement).

Please contact me for any further questions regarding this newsletter or for any introductions to the new upgrade on the microscope.

Contact and further information

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