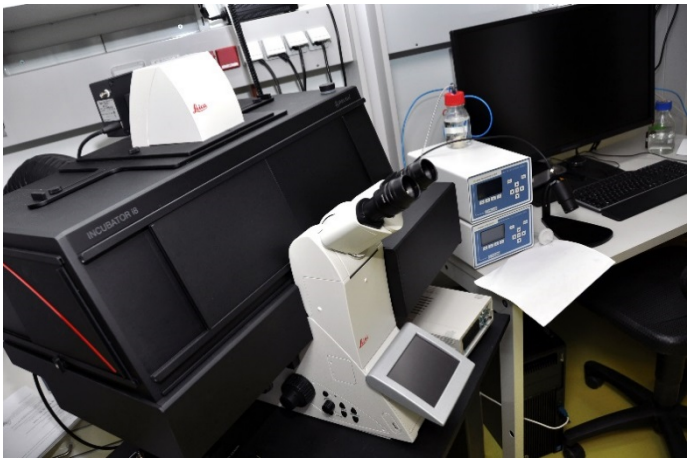
Biooptics Newsletter 06/2017

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 four microscopes in house and an additional one in cooperation with the Department of Pharmacology at Peter-Mayr-Strasse 1a. You can find more detailed general information on the official website. This newsletter is a new initiative and I will sent it out at regular intervals. **Any suggestions for contributions for the next issue are very welcome!**

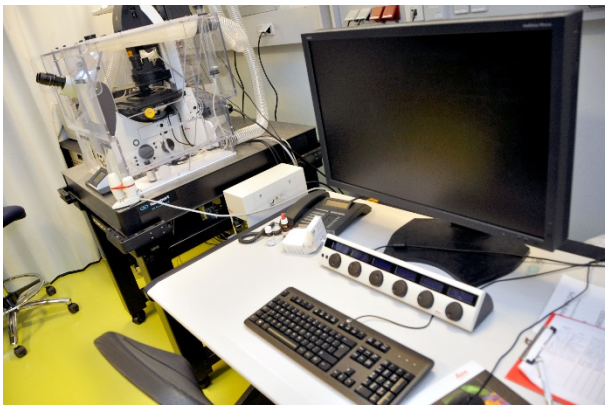


Upgrade of gSTED

Our gSTED microscope - purchased in 2013 in a cooperative effort with LFUI- has been used by several groups at the MUI and LFUI and has resulted in several publications. However, several factors have turned out to be disadvantageous:

1) Due to the very intense and continuous illumination at 592 nm (current STED wavelength), samples tend to bleach rather rapidly under STED illumination limiting the maximal amount of STED images per sample;

2) At present, we cannot do STED deep inside a sample, say a brain section, the STED range is limited to 10-20 µm in z.



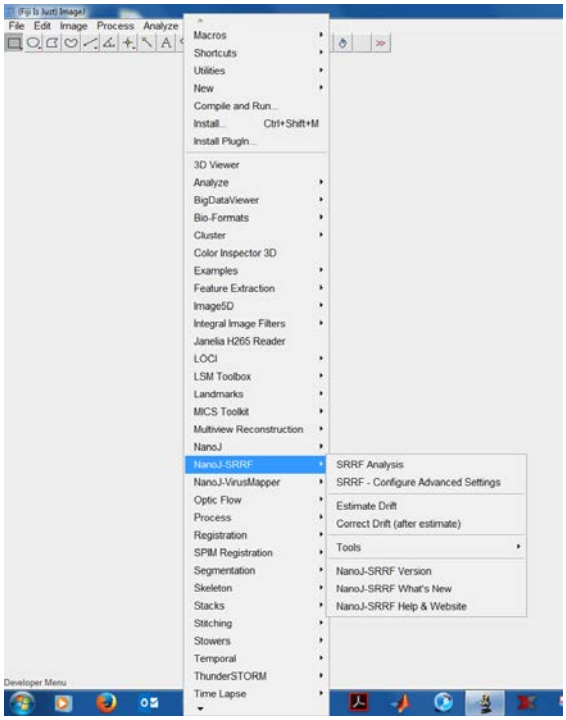
3) Tissue samples, in general, are often showing autofluorescence in the yellow and green range, not allowing the usage of yellow-green fluorophores, which are, however, mandatory on our current system.

4) Our system is capable of STED superresolution only in the xy direction, whereas the z resolution is just as good/bad as the usual confocal z-resolution (~ 800-1000 nm).

Technical solutions of all these problems have in the meantime become available. The application of **far-red STED depletion lasers** is nowadays state-of the art in STED imaging, since it results in higher resolution in combination with less autofluorescence and bleaching. A pulsed, far-red STED Laser at 775 nm is available as an upgrade for the SP8 microscope. In addition, we can extend our 2D STED to a 3D STED by using advanced optics. Finally, deep STED (within thick samples) imaging has become available using a motorized corrected 93x Glycerolobjective (since early 2017).

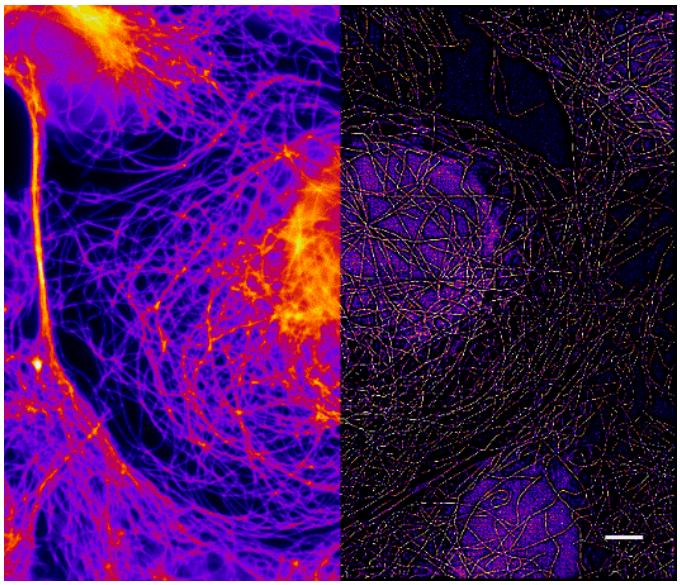
We are currently **collecting interested scientific groups in order to write an investment proposal.** In case of interest, or if you just need more detailed information you are welcome to contact me directly. We need to present sound scientific arguments (projects) in order to justify the costs of these upgrades.

SRRF analysis - how to do superresolution on any microscope

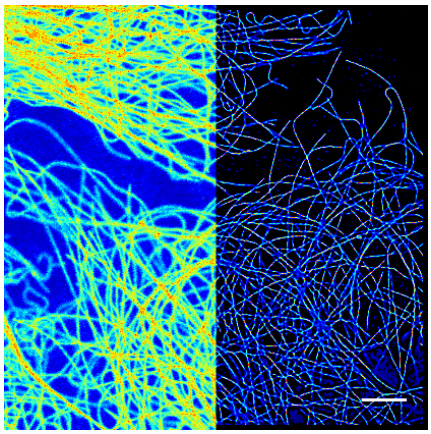


In Innsbruck, we are running a gSTED superresolution microscope, which is delivering superresolved images. There are, however, situations when it cannot be used due to a number of constraints. Gustafsson and colleagues (Gustafsson et al., 2016) recently published a set of mathematical methods in order to do superresolution on virtually any fluorescence microscope. Their method, termed SRRF, is based on the analysis of fluorescence fluctuations using fitting for radial structures; it is thus comparable to STORM and SOFI. One of the big advantages is the ease of use and implementation. It is actually available as a Fiji plugin-called NanoJ-SRRF.

There are very few experimental requirements; you need to have a fluorescently labeled sample, you need to have access to any fluorescence microscope (I have tried Widefield, Confocal-LSM, Confocal-Spinning Disk and TIRF). Preferentially, 100 or more images should be acquire at high speed, high illumination intensity and short exposure times (per frame). These conditions will favor the presence of fluctuations that are the principle of the analysis. The method is applicable for both live and fixed cells, the usefulness for live cells depends on the speed of movement that is expected (...you need to take 100 images per timepoint…). Some show images for **wide-field (>)**, confocal-LSM and

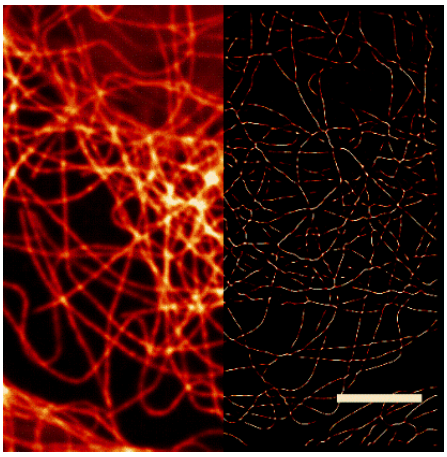


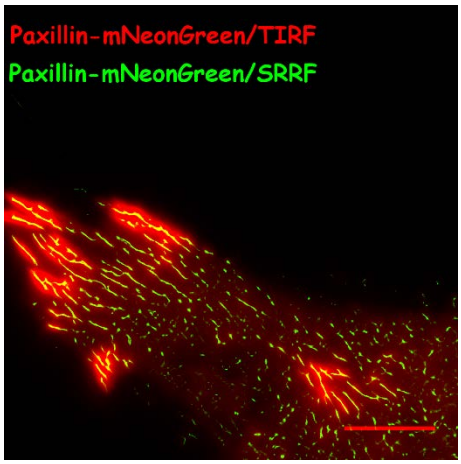
confocal-Spinning Disk are presented. HeLa cells with microtubules stained with OregonGreen488 are shown, left part, unprocessed. Bar=5 um.



(<) Same for **Confocal LSM**; the resonance scanner was used (remember: you need to be fast….).

You can also use a **spinning disk (>) confocal (iMIC)** in combination with an EM-CCD camera. The best data that I have obtained in pilot experiments have been acquired using TIRF imaging on the iMIC.





Albeit all tested methods have resulted in a clear increase in resolution, **(<) TIRF** has clearly resulted in the **best improvement** as seen using the focal adhesion marker Paxillin-mNeonGreen (mNG is very effective on the gSTED either, by the way…).

The down-side: Essentially the method is **useful for 2D imaging, only;** for 3D the results are really bad. For 3D images, Huygens Deconvolution (https://www.i-med.ac.at/itservices/systeme/huygens) is much more powerful in improving the resolution.

News in Brief

Did you know that we can do STORM imaging as well? Briefly, you need an Alexa647 labelled sample, STORM imaging buffer of your choice and the iMIC microscope! Use the TIRF imaging mode and full laser power @642 nm, blinking of Alexa647 is induced efficiently…

A new Biooptics Advisory Board has been formed: This internal board consists of several scientists from the different research focus areas of the MUI (also including an LFUI representative) and is supposed to develop perspectives on new investments that are needed by the users of the biooptics facility for up-to-date research.

As of April 2017 the Huygens software has received an upgrade: A new GPU (“graphics card”) now enables much faster data processing than previously. Moreover, old data (> 1 month) will automatically be deleted in order to keep the hard disk clean and available for image processing (not storage) tasks.

A new superresolution method is "ante portas": The MINFLUX technique described by the Hell lab (Balzarotti et al., 2017) enables much higher resolution (approx. 1 nm) at lower light exposure than STED, STORM or SIM. In addition, it is very fast. Commercial instruments are not (yet?) available.

Contact and further information

Martin Offterdinger, PhD
Medical University Innsbruck, CCB
Division of Neurobiochemistry /Biooptics
Innrain 80-82, room 01.370
A-6020 Innsbruck
Austria
phone : +43-512-9003-70287
mail: martin.offterdinger@i-med.ac.at
http://www.i-med.ac.at/neurobiochemistry/neurobiochemistry/Biooptics/Main.html