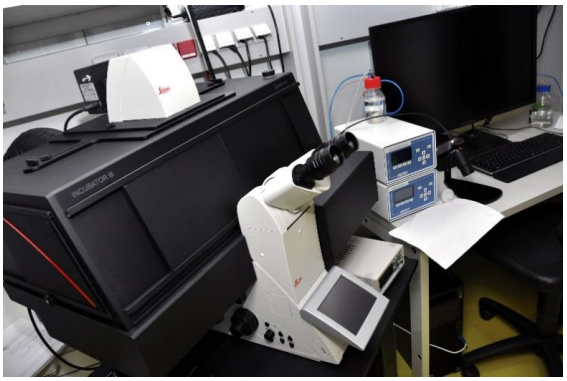
Biooptics Newsletter 06/2018

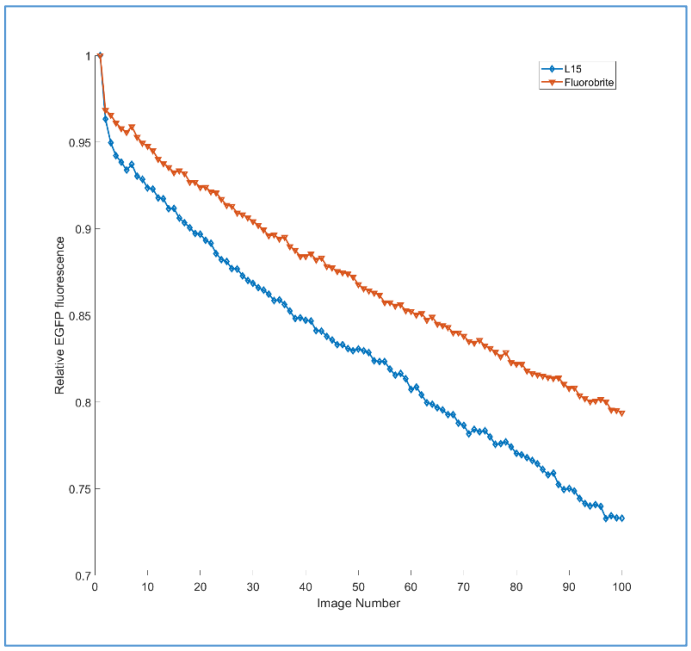
Biooptics Core Facility-in general



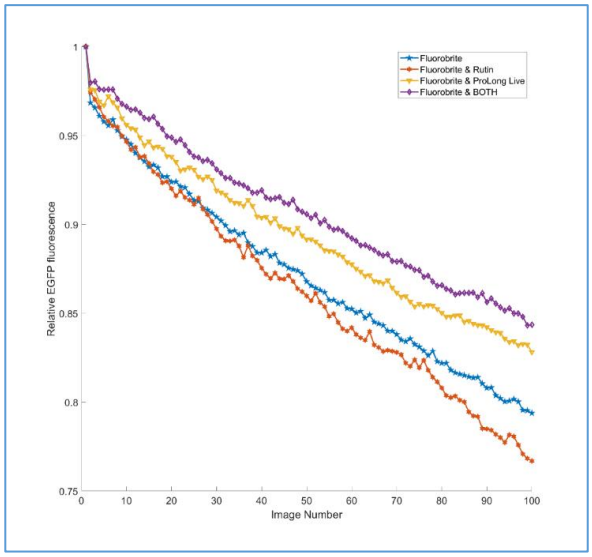
The Biooptics Core Facility of the MUI exists since early 2009 and is located in the CCB, Innrain 80-82, first floor, rooms M01.370 and M01.381. We are currently hosting four microscopes in house and an additional one in cooperation with the Department of Pharmacology at Peter-Mayr-Strasse 1a. You can find more information on the official website.

Live cell imaging: Signal-to noise and bleaching

Working with living samples on the microscope poses additional challenges to sample preparation and imaging conditions. Frequently, there is a need to acquire a number of images at low bleaching and low photoxicity. This may be addressed at several levels, at the level of the microscope itself, but also at the level of sample preparation. At the CFB, many people are working with phenol-red free Leibovitz's L15 medium, which has a rather low autofluorescence combined with the ability of pH-stabilization in CO2-free cultivation conditions. There are also special optimized imaging media (Fluorobrite, ThermoFisher) and anti-bleach compounds available, which I have evaluated at the CFB recently, using the same illumination conditions for all samples. To improve bleaching the previously described compound Rutin and a special formulation (Thermo-Fisher, "ProLong Live") with both Standard L15 (w/o Phenol Red) or Fluorobrite Medium (Thermo-Fisher, supplemented with 20 mM HEPES) were tested.

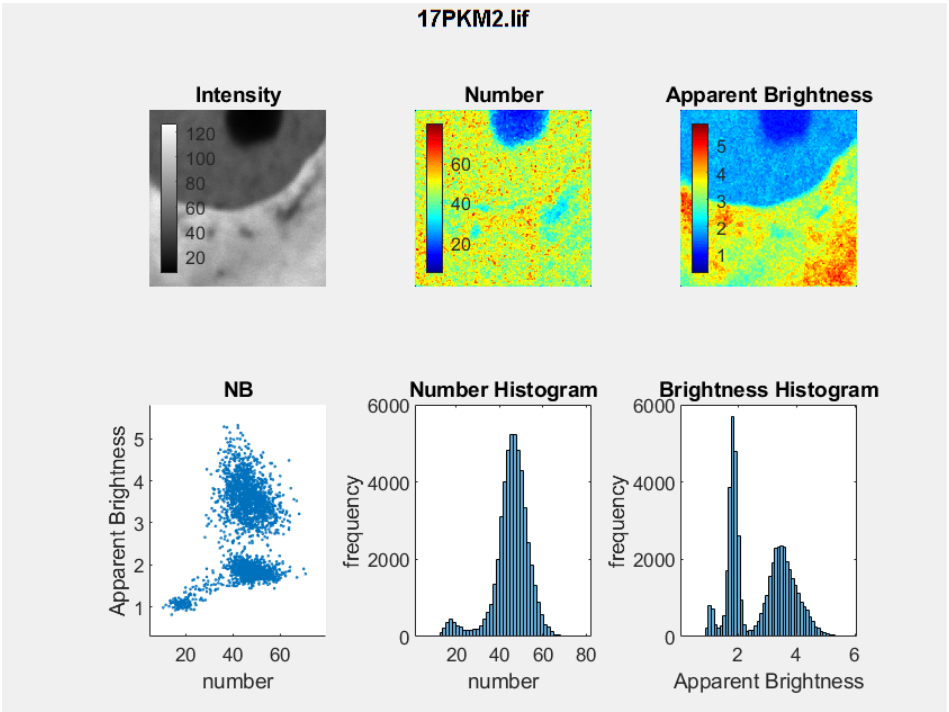


The performance of **Fluorobrite** in absence of any additive was better than for L15. Second, both additives were tested with both media. As an example, the results for Fluorobrite are shown. These data indicate that Fluorobrite is well suited as a general live cell imaging medium AND can be further improved with the additive ProLong Live in order to achieve the overall best live cell imaging performance. This does not exclude that L15 can still be a good choice. In general, I recommend against Standard DMEM with Phenol Red, which suffers from an overall low signal to noise ratio due to high autofluorescence.



Live cell imaging: number and brightness

Live cell imaging is mostly associated with imaging of dynamic processes such as intracellular transport or mitosis. In addition, the F-Methods such as FRAP and FRET are known to the scientific community. The less popular “Number and Brightness” (N&B) analysis (see Digman et al., 2008; Trullo et al., 2013) uses statistical analysis to answer questions on protein dimerization, or more general protein complex stoichiometry within specific localizations in live cells. Briefly, fluorescence fluctuations are analyzed in a time-course on a pixel-by-pixel basis to get an information on the number of particles (N) within the confocal volume of the laser scanning microscope. In addition, the brightness (B) of the particle is obtained; this allows drawing conclusions on the association between EGFP-tagged fusion proteins. The basic idea is rather simple: at any given pixel the fluctuations of fluorescence are proportional to the number of molecules (N) and the fluorescence emission (B) per molecule. If there are many particles with low emission per particle (eg EGFP-monomers), there will be a low standard deviation per pixel over time. If there are fewer, but brighter particles, there will be a higher standard deviation per pixel. A simple way of N&B can even be implemented in Fiji/ImageJ. I have set up a more complete analysis in MATLAB, which is available upon request.



Example: **Pyruvate kinase M2-EGFP:** this classical enzyme of the glycolysis pathway can form dimers or tetramers; tetramers have high enzymatic activity and are mostly found in the cytosol; dimers in contrast play a certain role as transcriptional modulators in the nucleus of cancer cells. A time series (100 frames) of PKM2-EGFP expressing HeLa cells was done using a pixelsize of 50 nm and an autofocus (using AFC) at the SP8 microscope (more details upon request!). The MATLAB-based analysis shows a brightness of approx. 2 in the nucleus and a brightness of approx. 4 in the cytosol (as expected!). In case of interest please contact me, I will be happy to supply further assistance!

Light Sheet Microscope Demo: 17.9./18.9.2018, CCB

We will be testing a light sheet microscope (Zeiss). There are currently two major applications for this technology. First, it is used to image larger living samples such as organoids, zebra fish embryos, but also plants. The main advantages are high frame rates and low phototoxicity. Second, it is used for large fixed samples, such as whole mouse brains, which were prepared using tissue clearing (CLARITY, iDISCO, CUBIC, …). Here, the main advantage compared to a standard confocal setup, is the increased axial resolution and improved axial light yield due to the geometry of the light sheet illumination. The Zeiss system will be able to image both living organisms and cleared samples.

News in brief

New IMARIS computer

Since May 2018 we have a new IMARIS computer, which has huge amount of RAM and an excellent graphics board. It enables the efficient analysis of even larger and more complex datasets.

New 2-Photon microscope

A 2-photon microscope will be delivered and installed at the end of July. It will be hosted by the Department of Physiology. Typical applications of 2P microscopy include imaging of very thick - 100s of micron to sub-mm range - specimens at confocal resolution, such as living tissues (e.g. brain). More detailed information will be provided in the next newsletter in autumn.

Rules on SP5 microscope

Due to the overbooking of the SP5 microscope we will reinforce (the already existing!) rule: A maximum booking of 4 h/week and user, twice per week. Additional bookings only evening, night and weekends. Due to increasing unavailability of spare parts, the SP5 may be discontinued in 2020. The SP8 will show equal or better performance for all experiments and can be used as well.

Contact and further information

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