CZECH BIOIMAGING

Imaging principles of life

NEWSLETTER AUTUMN 2022

FOCUS ON TECHNOLOGIES

BRINGING SIM MICROSCOPY TO A NEW LEVEL

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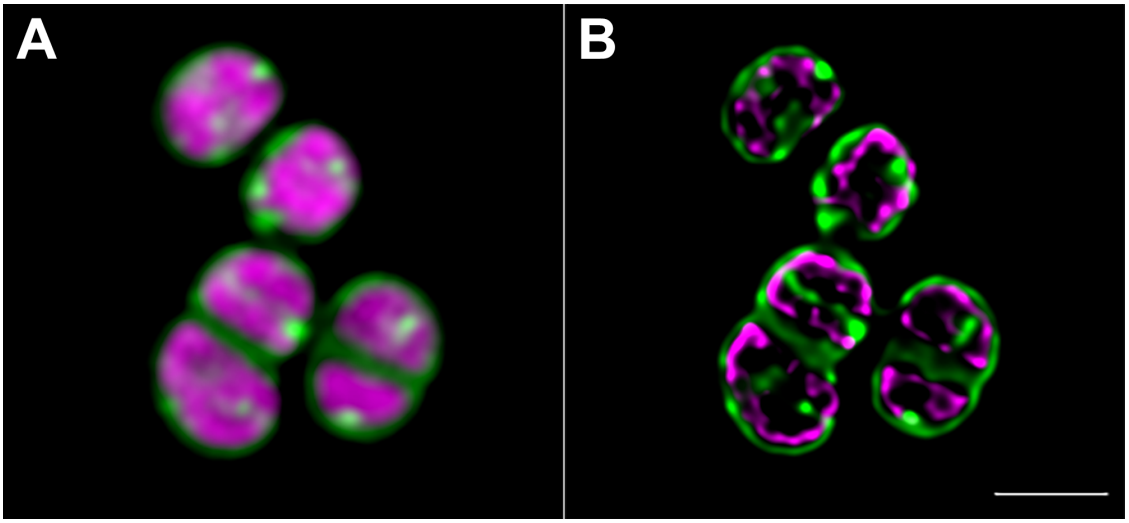
Structured **I**llumination **M**icroscopy (SIM) is a super-resolution fluorescent microscopy technique that allows to achieve resolution higher than the diffraction limit of light. Unlike other super-resolution methods such as **S**ingle **M**olecule **L**ocalization **M**icroscopy (SMLM) or **S**timulated **E**mission **D**epletion **M**icroscopy (STED), SIM does not require special sample preparation, and it can be used with almost any sample type, live or fixed.

The main limitation of conventional SIM is slow acquisition speed due to rotation of the mechanical grid. Rotation is necessary due to the fact, that multiple images with different grid orientation must be captured, to calculate final super-resolution image. At the **Cell**ular **Im**aging (CELLIM) Core Facility of Ceitec Masaryk University, we have the Elyra7 lattice SIM microscope from Carl Zeiss. Compared to conventional SIM, it has the advantage of using a lattice pattern instead of grid lines, which removes the need for grid rotation and increases acquisition speed. Therefore, faster biological processes can be observed and captured compared to conventional SIM. The lattice SIM offers similar resolution to a conventional SIM microscope (i.e., 100 nm on the lateral axis, a twofold improvement compared to a wide field system).

Recently, we implemented two upgrades for this instrument. First, the number of images (phases) needed to obtain a final image was decreased from 15 to 9. This improve acquisition speed, is less phototoxic to the sample and minimizes photobleaching. Therefore, a live specimen can be imaged for a longer period of time without detrimental phototoxic effects. Second, an improvement in the SIM reconstruction algorithm improved overall resolution.

Image reconstruction using new SIM2 is divided into two steps. First, denoising and frequency suppression is performed, and a digital SIM point spread function (PSF) is obtained. Then, the PSF is used for iterative image deconvolution. The result is a major improvement in resolution down to 60 nm—without any changes to the staining protocol or sample preparation.

For more information visit us at **https://cellim.ceitec.cz/** or contact us directly at **cellim@ceitec.muni.cz**.



Membrane and DNA of bacterial cells (Staphylococcus aureus) are labelled with DiOC6 dye (green) and POPO1 dye (purple). Raw images were acquired using microscope Carl Zeiss Elyra 7 with lattice SIM. Image A) was processed using standard SIM algorithm; image B) was processed using new SIM2 algorithm. You can see clear improvement in image resolution using new SIM2 algorithm. Scale bar represents 1 µm.
Image courtesy: Michaela Prochazkova, Pavel Plevka, Ceitec MU.

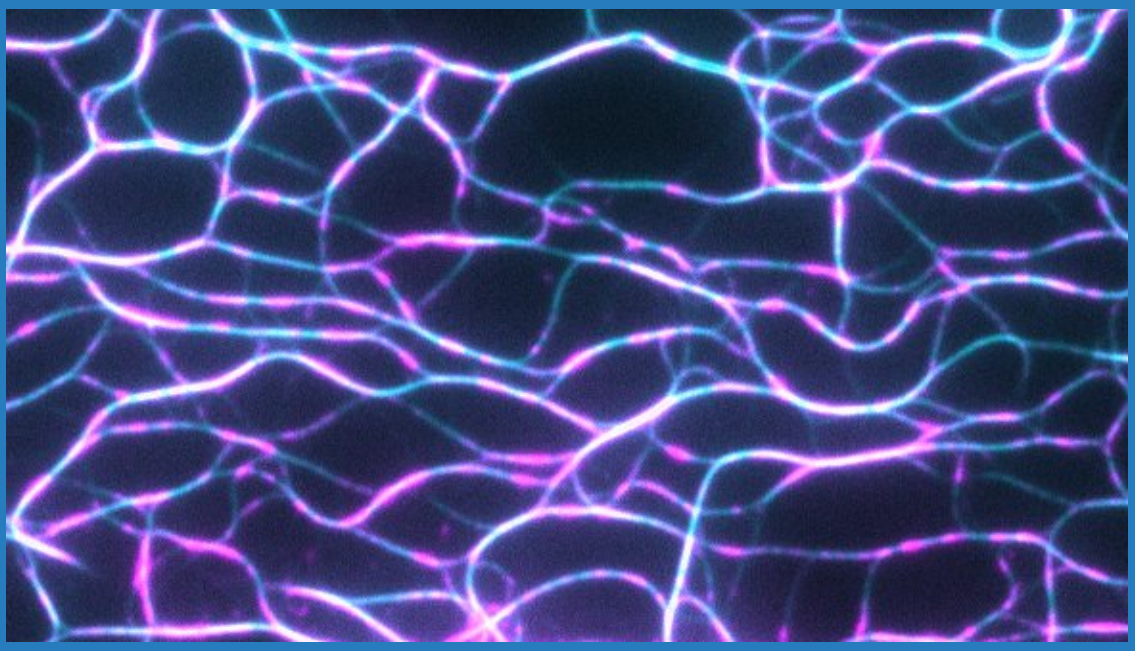
HIGHLIGHTS OF USER RESULTS

ALZHEIMER'S DISEASE-ASSOCIATED PROTEIN TAU SQUEEZES MICROTUBULES

Author: Z. Lánský

Tau is a microtubule-associated protein, which is plays a role in the Alzheimer's disease. The physiological functions of tau however remain unclear. New work from the Institute of Biotechnology of the Czech Academy of Sciences shows that tau recognizes the structural states of microtubules and suggests that this can lead to regulation of microtubule-related cellular processes.

Tubulin in the microtubule lattice can exist in either an expanded or compacted state, dependent on its nucleotide state. Tau molecules can form a protective envelope on the surface of microtubules, which regulates the access of other microtubule-associated proteins to the microtubule lattice. We have used single molecule microscopy to visualize the formation of tau envelopes and optical tweezers to probe the effect of envelopes on the microtubule structure. We found that tau envelopes favor the compacted state of the microtubule lattice and their formation can convert an expanded lattice to the compacted conformation. Our results reveal a tight interplay between the microtubule structure and binding of tau and suggest that that reversible self-association of tau into envelopes coating the microtubule lattice is a physiological mechanism for tau function in cells.



UPCOMING EDUCATIONAL ACTIVITIES (AUTUMN - WINTER)

VIRTUAL REALITY TOOLS IN BIOLOGY DATA ANALYSIS

Course | November 3, 2022 | IPHYS, Prague

FLIM NOT ONLY FOR BIOLOGISTS

Practical course | November 14 - 16, 2022 | UK, BIOCEV, Vestec

TRANSMISSION ELECTRON MICROSCOPY IN LIFE SCIENCES

Specialised course | November 21 - 25, 2022 | IMG, Prague

NEUROIMAGING: MAPPING THE FUNCTION AND STRUCTURE OF BRAIN

Course | November 28 - 30, 2022 | CEITEC MU, Brno

IMAGE OF LIFE 2022

Course | December 2022 | IMG, Prague

USEFUL LINKS

Czech-bioimaging Scientific Conference 2022

Czech-bioimaging – pro veřejnost

Czech-bioimaging – technologies

Euro-BioImaging website

Velké výzkumné infrastruktury

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www.czech-bioimaging.cz