Superresolution in Light Microscopy-annotation

In light microscopy, there are three general superresolution (SR) techniques widely used nowadays. SR methods based on widefield microscopy are Structured illumination microscopy (SIM) and Single-Molecule Localization Microscopy (SMLM). In SMLM there are two approaches based on the same principle - Stochastic optical reconstruction microscopy (STORM) and Photoactivation localization microscopy (PALM). The third SR method is based on confocal scanning microscopy - Stimulated emission depletion (STED) microscopy.

Generally, all these techniques work using a simple principle – to decrease the number of emitting fluorophores in particular place in the microscope field of acquisition, which simplifies the localization of their position.

In the SIM, the ON-OFF state is created via patterned light when the grid pattern is generated and superimposed directly onto the specimen in the objective focal plane. The final image is calculated by a mathematical algorithm based on Fourier transform. The resolution of a final reconstructed image is approximately two times better than the Abbe limit for the particular wavelength and could be around 100 nm.

The SMLM is based on the stochastic blinking of individual fluorophores (which introduces the ON-OFF state system again) and then the position (localization) of each blink is precisely localized by the image post-processing. This technique could give the resolution around 25 nm.

The Stimulated emission depletion (STED) microscopy is a superresolution confocal microscopy technique using two laser lines for the scanning – one for an excitation and the second for an emission depletion. The lasers are co-aligned to form a donut shape when the inner spot (ON state) is the area with the only emission used for the acquisition and the emission from the outer ring (OFF state) is pushed by the depletion laser to longer wavelengths and is not acquired. The resolution of STED microscopy is theoretically unlimited, while practically could achieve 35 nm. The image is created by the same step-wise manner principle like in any confocal scanning microscope.

The course gives you an opportunity to work hands-on with DeltaVision OMX™ for SIM microscopy, the Leica TCS SP8 STED 3X with 660 cw depletion laser will be used for the STED technique and Nikon N-STORM microscope for STORM localization microscopy.