Novel insights into cell nucleus

An introduction to Structured Illlumination Microscopy

Structured illumination microscopy has proved to be an excellent alternative to several existing microscopy techniques. Extending the capabilities of classical multi-colour fluorescence wide-field or confocal microscopic systems, it can provide sufficient resolution needed for analyses of fine intracellular structures like nuclear pore complexes or NPCs. These complex protein complexes are situated in the envelope surrounding cell nucleus and provide exchange pathway for number of molecules. NPCs mediate passage of RNA, ribosomes and signal molecules through the nucleus–cytoplasm boundary. This process assures continuous flow of information required for synthesis of proteins inside the living cell. Direct observation of NPC complexes with structured illumination microscopy allowed for the first time to perform series of analyses of subcellular structures beyond the diffraction limit and opened new routes to unravel new features of cell nucleus superstructure.

A new structured illumination microscopy approach that delivers ‘sub-diffraction’ resolution at high speed has been developed by a team from the University of California, San Francisco, led by Prof. John Sedat, Assist. Prof. Mats Gustafsson, and Dr. David Agard, and is based on the employment of diffracted laser light to create a pattern of multiple interfering illumination beams. Foundations to this study have been laid by fundamental paper of Mats Gustaffson, in which he described the principle of using Moiré interference to overcome Abbe resolution limit. This work led to formalisation of saturated structured-illumination microscopic (SSIM) approach which has been tested and proved to be an excellent experimental tool. Capture of images over several orientations of the beams incident on the sample, followed by intensive computational analysis, produces final images with spatial resolution that is at least twice as fine as the 200 – 300 nm best case resolution of classical fluorescence microscopy.

The UCSF research team has recently published their study of nuclear pore complexes in C2C12 cells imaged with a derivative of SSMI named three-dimensional structured illumination microscopy or 3D-SIM. After initial tests of images and resolution achieved with their system it became clear that 3D-SIM could deliver images surpassing the quality offered by wide-field and confocal setups. Despite somewhat lower resolution than typically found in other super-resolution techniques on similar specimens, the Sedat group was able to dissect individual nuclear pore complexes, discern and quantify chromatin within the nucleus and the lamina from NPCs. Interestingly, the UCSF researchers reported presence of membrane invaginations previously observed only with transmission electron microscope.

The detection employed by this instrument came in the shape of a parallel array of Andors iXon3 897 EMCCD cameras, facilitating enhanced sensitivity and speed of the approach over multiple probe wavelengths. This performance boost was critical to realise the implementation of this ‘multi-shot’ protocol for the eventual high resolution imaging of challenging live specimens within a reasonable measurement period.

By doubling the resolution the Lothar Schermelleh and his colleagues have shown 3D-SIM to be the first super-resolution technique capable of multi-colour three-dimensional images of whole cells and their organelles. This microscopic approach is very promising alternative to complex and expensive systems that often require dedicated and unique fluorescent dyes as well as technically challenging optical set-ups.