Super-Resolution Microscopy in Germany

5th Anniversary of NIC Heidelberg

On October 29 the Nikon Imaging Center (NIC) at the University of Heidelberg celebrated its 5th Anniversary. Initiated by Prof. Dr. Thomas Holstein, Head of the Department of Molecular Evolution & Genomics, University of Heidelberg and Dr. Jörg Kukulies, General Manager of the Microscopy Division at Nikon Germany, the NIC Heidelberg is the second of today eight core facilities located in North America, Europe, and Asia.

Since the first opening at Harvard University, USA, in 2001, each facility is connected to a well-reputed university and offers high-end instrumentation for advanced light microscopy research (www.nikonimagingcenters.com). As Ulrike Engel, Director of the NIC Heidelberg, pointed out in her speech at the anniversary symposium, the mission of the institution is to provide all researchers of life sciences at the University with access and training to state-of-the-art light microscopy.

After the welcome notes contributed by board members of the University and Nikon representatives, selected users gave some insights in their research results in the field of light microscopy. Dr. Dimitris Liakopoulos from the Institute of Biochemistry opened the session with presenting his work on controlling astral microtubule function during spindle positioning in yeast. As the second speaker Annette Schmidt from the Institute of Zoology presented data of imaging and manipulation of the zebrafish retina. The morning session was finished by Prof. Dr. Robert Grosse, University of Marburg. He reported on cytoskeletal dynamics through formins, a protein family, which is involved in the polymerization of actin and associate with the fast-growing end of actin filaments.

As a preview to the afternoon session on super-resolution microscopy the on-site structured illumination microscope (SIM) from Nikon was proudly presented to the birthday guests during lunch time (Fig. 1). It was the first system which has been installed in Europe and the second one already runs at the Nikon Imaging Center at the Curie Institute in Paris.

To bypass the limit of optical resolution defined by the Abbe theory, today three different technologies are commercially available to provide images in super-
In addition to the SIM system Nikon also offers an instrument for stochastic optical reconstruction microscopy (STORM). Invited speakers for the super-resolution microscopy session were Prof. Dr. Heinrich Leonhardt from the University of Munich and Dr. Mike Heilemann from the University of Bielefeld. In his talk "visualization and manipulation of the invisible" Dr. Leonhardt introduced the technology and applications of SIM. In this context he described nanobodies, a small-type antibody as an appropriate labeling tool to minimize the distance between chromophore and region of interest. Statements to tailored illumination, nonlinear fluorophore responses, or the precise localization are summarized in the review "A guide to super-resolution fluorescence microscopy" he recently published as a coauthor in the Journal of Cell Biology (2010, 190(2), 165-75).

After that Dr. Heilemann gave a lecture on STORM including working principle, applications in biology, photo-switchable fluorescent probes, and practical considerations. As an outlook for three-dimensional super-resolution he presented three concepts for 3D STORM: astigmatic imaging, Bi-plane imaging and Double-helical PSF. Astigmatic imaging features a cylindrical lens introduced into the imaging path which effects image resolution of 20 to 30 nanometers in the lateral dimensions and 50 to 60 nanometers in the axial dimension (Huang et al., Science 2008). For the Bi-plane method collected fluorescence is divided by a beam splitter, creating two separate image planes on the same CCD camera and can be combined to a 3D raw data stack (Juette et al., Nat. Meth. 2008). The key feature for the double-helical PSF imaging system is a spatial light modulator which will be placed in the Fourier plane. Two molecules as close as 14 nm (x), 26 nm (y) and 21 nm (z) can be resolved by this technique (Pavani et al., PNAS 2009). Further perspectives and challenges for STORM is single particle tracking, the use of EGFP as a redox switch, and live cell imaging.
The session was followed by a birthday party where all guests were welcomed to taste the specially designed birthday cake (Fig. 2).

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