Advances in Fluorescence Correlation Spectroscopy: Quantitation of Biomolecular Interactions and Imaging of Membrane Dynamics

The overall aim of our laboratory is to develop techniques to investigate molecular actions and functions under physiological relevant conditions to gain insights into important biological questions. In the last years we have developed two different Fluorescence Correlation Spectroscopy (FCS) modalities that allow for the first time a) the quantitative measurement of molecular interactions in organisms, and b) the imaging of a whole cell membrane in a spectroscopic mode. The first method is single wavelength excitation fluorescence cross-correlation spectroscopy (SW-FCCS) which allows the determination of biomolecular interactions in vivo. We demonstrate how SW-FCCS can be used to measure the dissociation constants of Cdc42, a small Rho-GTPase, with different interacting molecules (N-WASP, IRSp53, IQGAP1) in live cells and zebrafish embryos. Secondly, to multiplex FCS measurements and acquire spatial maps of concentrations and diffusion coefficients, we developed a total internal reflection (TIR) based FCS approach using EMCCDs as detectors. Imaging Total Internal Reflection FCS (ITIR-FCS) can take more than 3000 measurements simultaneously and thus can yield spatial maps of concentrations and diffusion coefficients of lipid bilayers and cell membranes. As an example, we monitored cell membrane changes by using raft and non-raft associated molecules upon the removal of cholesterol. Our results indicate that the cell membrane organization on the nano- and micron-scale follow different patterns on cells. Both tools, SW-FCCS and ITIR-FCS, are new tools which allow quantitative measurements in cells which can yield new insights into biological phenomena.

About the Speaker

Prof. Thorsten Wohland studied Physics at the Technical University of Darmstadt and the University of Heidelberg in Germany from 1989-1995. He completed his diploma thesis in physics at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany, where he worked on the influence of light polarization on the forces in optical tweezers under the supervision of Ernst H.K. Stelzer. In 1996 he completed his national service at the National Research Institute for Environment and Health (GSF) in Munich, Germany, where he worked on single photon emission computer tomography (SPECT) with Wolfgang Kreyling. In 1997 he joined the research group of Prof. Horst Vogel at the Swiss Federal Institute of Technology in Lausanne (ETHL/EPFL), Switzerland. In the year 2000, he obtained his doctoral degree in the field of biophysics for the study of theoretical and practical aspects of fluorescence correlation spectroscopy (FCS) and its application to integral membrane proteins. Following another two years in the group of Richard N. Zare at Stanford in the USA working on single molecule detection and protein immobilization he finally arrived at the National University of Singapore (NUS) in June 2002 where he joined as Assistant Professor. He was promoted to Associate Professor in July 2008. His current interests are in application and development of fluorescence spectroscopy techniques and the investigation of protein function especially in cells and small organisms. For that purpose he was on sabbatical from July to December 2009 at EMBL Heidelberg under an Alexander von Humboldt Foundation scholarship. Since January 2010 he returned to Singapore.