ONE BY ONE – LOCALIZATION MICROSCOPY IN NANOPHYSIOLOGY

Christian Sieben

Nanoscale Infection Biology, Helmholtz Centre for Infection Research
Inhoffenstr. 7, 38124 Braunschweig, Germany

E-Mail : christian.sieben@helmholtz-hzi.de

KEY WORDS: super-resolution microscopy, live-cell imaging, data analysis, clustering, tracking, co-localization

How do proteins organize in cells to fulfill their designated function? Proteins have a limited operating range and, consequently, their activity occurs at the nanometer scale, which requires advanced microscopy techniques to study associated processes. Focusing on localization microscopy, I will discuss imaging and analysis techniques to investigate protein organization and dynamics at the nanoscale.

Single-molecule localization microscopy (SMLM) relies on the temporally separated emission of individual molecules. While the acquired image is still diffraction limited, due to their sparsity, each molecule can be localized at nanometer precision and their coordinates accumulated to form a super-resolved image. But the power of SMLM goes far beyond generating images. Computational analysis tools allow extracting the spatial organization, colocalization, clustering and dynamics of individual molecules, which has transformed the study of nanoscale biology over the past decade.

In this tutorial, I will first briefly introduce experimental methods established in localization microscopy while discussing practical considerations towards their advantages, limits and common artifacts. I will then spend the remaining time discussing several biological studies and examples where SMLM techniques have helped to understand subcellular structures, their organization and dynamics.