Quantitative characterization of transcription factor nuclear dynamics and interactions in live cells by functional Fluorescence Microscopy Imaging (fFMI)

Vladana Vukojević
Department of Clinical Neuroscience,
Karolinska Institutet, Stockholm, Sweden

Lack of quantitative information on the detailed kinetics of molecular interactions in living cells is one of the main obstacles hampering systematic progress in many areas of biomedical research. The aim of this talk is to introduce functional Fluorescence Microscopy Imaging (fFMI) and show that critical progress has been made in methodology and instrumentation development to enable quantitative measurements in live cells with a rigor that is approaching the precision of in-solution measurements.

The audience will learn about fFMI, a high-resolution experimental modality that builds on quantitative fluorescence microscopy techniques with single-molecule sensitivity, fluorescence imaging by fast signal acquisition using Avalanche Photodiode Detectors (APD), so-called APD imaging [1], and Fluorescence Correlation Spectroscopy (FCS) [2]. fFMI enables us to visualize fluorescent molecules in live cells expressing the molecules of interest at physiologically relevant levels, and quantitative characterize the determinants of their biological function (local concentration, diffusion constants, kinetic rate constants and the equilibrium binding constants) with ultimate sensitivity, high temporal resolution (μs) and great localization precision (200 nm). Application of fFMI for quantitative characterization of transcription factor nuclear dynamics and interactions with chromatin will be discussed [3].

References

1. Vukojević V, Heidkamp M, Ming Y, Johansson B, Terenius L, Rigler R. Quantitative single-molecule imaging by Confocal Laser Scanning Microscopy. Proc Natl Acad Sci USA, 2008, 105, 18176-18181.
2. Vukojević V, Pramanik A, Yakovleva T, Rigler R, Terenius L, Bakalkin G. Study of Molecular Events in Cells by Fluorescence Correlation Spectroscopy. Cell. Mol. Life. Sci. 2005, **62:**535-550.
3. Vukojević V, Papadopoulos DK, Terenius L, Gehring W, Rigler R. Quantitative study of synthetic Hox transcription factor–DNA interactions in live cells. Proc Natl Acad Sci USA 2010, **107:**4087-4092.