Contribution ID: **189** Contribution code: **PT-11** Type: **Plenary talk (virtual)**

Quantitative Scanning-Free Confocal Microscopy with Single-Molecule Sensitivity and Fluorescence Lifetime Imaging for the Study of Fast Dynamic Processes in Live Cells

Wednesday, 31 August 2022 09:00 (45 minutes)

Timing is everything in biology – biological systems exchange information by controlling the spatio-temporal behavior of biological molecules, using dynamics to encode and decode information. Dynamic changes in the concentration of biological molecules are therefore an integral part of biological networks' function and inadequate spatial distribution and temporal dynamics are characteristic of disease states.

Living cells control the concentration, spatial distribution, and temporal dynamics of biological molecules through molecular interactions and transporting processes, most notably diffusion. Through reaction-diffusion processes biomolecules are integrated in specific dynamical networks and perform specialized biological functions in the cell, such as gene expression. These networks are complex – they are made up of many constituents (different interacting molecules); they are tightly intertwined – products of one reaction are reactants in another one; and they are dynamically controlled – the rates at which biochemical transformations occur are autocatalytically regulated by molecules produced in the same biological network. Consequently, these complex networks may acquire a new quality – the capacity to self-adjust their essential variables to control their biological functions.

To understand how these dynamical networks are controlled and self-regulated, the concentration and mobility of interacting molecules – which are in addition to chemical reactivity the determinants of chemical kinetics, need to be quantitatively characterized in live cells.

To this aim, we have developed quantitative scanning-free confocal microscopy with single-molecule sensitivity, high temporal resolution (~10 μs/frame), and fluorescence lifetime imaging by integrating massively parallel fluorescence correlation spectroscopy [1] with fluorescence lifetime imaging microscopy (mpFCS/FLIM)[2].

The capacity of this method to characterize in live cells compartmentalization of molecular processes by measuring local excited-state decay *via* FLIM, and their dynamic integration by measuring diffusion/active transport using mpFCS will be discussed.

Acknowledgments: This work was supported by grants from the Swedish Research Council (2018-05337); Olle Engkvists Foundation (199-0480); Magnus Bergvall Foundation (2019- 03381, 2020-04043); Karolinska Institutet's Research Foundation Grants (2020-02325); Swedish Foundation for Strategic Research (SBE13-0115); NIH/NIAAA (R01AA028549); Nakatani Foundation for Advancement of Measuring Technologies in Biomedical Engineering; Strategic Research Program in Neuroscience (StratNeuro); Yoshida Foundation for Science and Technology; ERASMUS+: European Union Programme for Education, Training, Youth, and Sport; Qatar National Research Foundation (PPM 04-0131-200019); and Science Fund of the Republic of Serbia (call PROMIS, Grant no. 6066079).

References

1. A.J. Krmpot *et al.*, Anal. Chem. **91(17)**, 11129 (2019).
2. S. Oasa, A.J. Krmpot, S.N. Nikolić *et al.* Anal. Chem. **93(35)**, 12011 (2021).

Primary authors: NIKOLIC, Stanko N. (Institute of Physics Belgrade, University of Belgrade, 11080 Belgrade, Serbia); KRMPOT, Aleksandar J. (Institute of Physics Belgrade, University of Belgrade, 11080 Belgrade, Serbia); OASA, Sho (Karolinska Institute, Department of Clinical Neuroscience (CNS), Center for Molecular Medicine (CMM)); CLAYTON, Andrew H. A. (Swinburne University of Technology, School of Science, Department of Physics and Astronomy); TERENIUS, Lars (Karolinska Institute, Department of Clinical Neuroscience (CNS), Center for Molecular Medicine (CMM)); BELIĆ, Milivoj R. (Texas A&M University at Qatar); RIGLER, Rudolf (Karolinska Institute, Department of Medical Biochemistry and Biophysics (MBB)); VUKOJEVIC, Vladana (Karolinska Institute, Department of Clinical Neuroscience (CNS), Center for Molecular Medicine (CMM))

Presenter: VUKOJEVIC, Vladana (Karolinska Institute, Department of Clinical Neuroscience (CNS), Center for Molecular Medicine (CMM))

Session Classification: Plenary Talks

Track Classification: Scientific Sections: S13 Biophysics and Medical Physics