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# 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 ( $\sim 10 \mu\text{s}/\text{frame}$ ), 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.

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## References

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