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688-Pos

High resolution fluorescence lifetime maps from minimal photon counts

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Fluorescence lifetime imaging microscopy (FLIM) can be used to probe concentration profiles of key marco-molecules within sub-cellular environments. However, current analyses for FLIM require a large number of photons per pixel to provide such a quantitative picture of life. In order to acquire such large number of photons, we must either increase data acquisition time, which limits temporal resolution, or increase laser intensity, which causes greater photo damage to the sample, or both. Here, we propose to analyze FLIM data by leveraging tools from the Bayesian paradigm. Our framework also takes into account details of the experiment such as point spread function (PSF) and instrument response function (IRF) of arbitrary shapes. We show that our method is robust with respect to species lifetimes from below the IRF to exceeding inter-pulse times. Moreover, we achieve direct blind unmixing of lifetimes with sub-nanosecond resolution and sub-pixel spatial resolution using limited photon budgets. We benchmark our method using a variety of synthetic and experimental data. If time allows, we will discuss our recently developed method toward learning wavefront distortions introduced by optically inhomogeneous sub-cellular environments.

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Fluorescence fluctuation spectroscopy with a cooled SPAD array detector to unravel molecular processes in living cells

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The molecular dynamics and protein interactions in living cells remain poorly understood despite their importance to several cellular processes. RNA molecules, for example, play a major role in many processes, from encoding and regulating gene expression to catalyzing biological reactions. Alterations of the metabolism of RNAs and RNA-binding proteins are involved in neurodegenerative diseases, such as ALS in neuronal cells. In particular, the connection between biomolecular dynamic processes and cellular function/organization is still unclear.Here, we propose a new class of Fluorescence Fluctuation Spectroscopy (FFS) techniques performed with a novel single-photon-avalanche-diode (SPAD) array detector to better decipher molecular dynamics, interactions and structural changes directly in living cells with microsecond time resolution. FFS is a family of techniques for quantifying dynamics of (bio)-molecular processes. FFS methods are based on the measurement of the spatial/temporal fluctuations generated by a population of fluorescent molecules passing through a small microscope focal volume (in our case a confocal detection volume). Recently, we showed how to further enhance the classical confocal FFS methods by introducing a SPAD array detector with an active cooling system. By spatially and temporally tag single-photons (the temporal tags allow leveraging the fluorescence lifetime), we can quantify not only molecular dynamics but also changes in the cell organization at a single-molecule level. Moreover, by cooling the detector to $-15°C$ we showed an improvement in the signal-to-noise ratio and dynamic range due to a 10-fold decrease in the dark-count rate. As a proof-of-principle, we will demonstrate how combining single-molecule labeling and FFS with a SPAD array detector can be employed to investigate different RNA-based molecular processes in living cells.

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Quantitative DNA-paint imaging of AMPA receptors in live neurons

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DNA-PAINT can image biological specimens with potentially nanometer resolution and obtain absolute stoichiometry. The technique, however, has not been used in living system due to high salt concentration in the buffer required for specific binding of DNA-imager to DNA-docker probes. Here, we used multiple binding motifs from 1-16x, of the docker to accelerate the binding speed of the imager under physiological buffer condition without compromising spatial resolution. We imaged endogenous AMPARs—critical proteins involved in nerve communication in live neurons. We found ~50% of synaptic AMPARs are immobile in nanodomains, with the other half are mo-

bile. We also found that DNA-PAINT provides tracking results of AMPARs with more than 5-fold higher throughput than traditional single particle tracking due to its high labeling density and photobleaching-resistance, enabling us to detect the difference in diffusion of AMPARs between individual neurons.

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Quantitative analysis of hypericin interaction with SARS-CoV 2 and with a model membrane

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Hypericin (Hyp), a pigment extracted from *Hypericum perforatum*, is a naturally occurring photosensitizer (PS), i.e. a compound that sensitizes the production of singlet oxygen ($^1O_2$) upon visible photo-excitation. $^1O_2$ is an oxidizing molecule capable of damaging biomolecules such as lipids, nucleic acids and amino acids. The combination of a PS, visible light and molecular oxygen is at the basis of photodynamic therapy (PDT), a clinically approved treatment against cancer cells, but also effective against pathogenic bacteria, fungi and viruses.

Hyp represents a very interesting PS, because it shows high singlet-oxygen quantum yield ($\varphi\Delta=0.33$) and high fluorescence quantum yield ($\varphi F=0.35$) in DMSO. Due to the hydrophobic character of the molecule, these properties are lost in aqueous solution, where Hyp tends to form photo-inactive aggregates. The photo-physical and photo-dynamic properties are recovered when Hyp is bound to apolar systems, such as phospholipidic membranes or protein hydrophobic pockets. These features are useful for localization of the PS by fluorescence microscopy. In particular, Hyp was found to effectively inactivate viruses with a phospholipidic envelope, like SARS-CoV-2 upon light exposure, and sometimes also in dark conditions.We used fluorescence spectroscopy and microscopy to explore the interaction between SARS-CoV-2 and Hyp, and correlative AFM-fluorescence microscopy on a bilayer model membrane, to study the morphological changes induced by the presence of the PS. The aim of this research project is to assess the binding and photosensitizing properties of Hyp with SARS-CoV-2 and a model membrane, in order to provide more data for a future therapeutic implementation of PDT with this antiviral agent against SARS-CoV-2 and, more generally, all enveloped viruses.

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Super resolved 3D tracking of cargo transport through nuclear pore complexes

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Nuclear pore complexes (NPCs) embedded within the nuclear envelope mediate rapid, selective, and bidirectional traffic between the cytoplasm and the nucleoplasm. The human NPC scaffold consists of a pair of ~100 nm diameter rings separated by ~50 nm. Hundreds of largely intrinsically disordered polypeptides attached to this scaffold generate the permeability barrier and provide binding sites for cargo complexes undergoing transport. Deciphering the mechanism and dynamics of this process is challenged by the need for high spatial and temporal resolution. We report here a multi-color imaging approach that enables direct 3D visualization of cargo transport trajectories relative to a super-resolved double-ring structure of the NPC scaffold. An adaptive optics system generates chromatic correction and the astigmatic spot shape necessary for 3D information, and provides a z-dependent tunable precision in x, y, and z as demanded by the needs of the experiment. The success of this approach is enabled by the high positional stability of NPCs within permeabilized cells, as verified by a combined experimental and simulation analysis. Using the HMSiR blinking dye tethered to Nup96, the octagonal symmetry of both rings of the NPC scaffold are well-resolved by applying a rotational algorithm to individual pores that is effective even for a low number of fluorophore localizations. Hourglass-shaped translocation conduits for two cargo complexes representing different nuclear transport receptor (NTR) pathways indicates rapid migration through the permeability barrier on or near the NPC scaffold.