to study gene regulation of processes in living cells. The approach can be readily applied to other viruses as well.

1005-Plat
Quantitative TCSPC FRET-FLIM Applied to Donors Exhibiting Multi-Exponential Decays: Spatio-Temporal Interaction Between Calmodulin and the Kv7.2 Potassium Channel in Living Cells
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The spatio-temporal study of the dynamics of molecular interactions using FRET-FLIM is generally compromised by the large number of photons required to fit the multiple-lifetime decay of the donor population in each pixel of an image. Long acquisitions prevent interacting dynamics to be detected in an image, while the use of high excitation intensities results in artifactual measurements due to bleaching. The computation of the minimal fraction of donor molecules (mf_D) undergoing FRET allows quantitative imaging of molecular interactions with either single or multi-lifetime donors such as CFP, where the complexity associated to fitting a fluorescent decay with more than two components hampers quantification with traditional least-squares fitting strategies. This novel non-fitting analysis has been recently applied to wide-field time gated FLIM systems and we now extend it to more widely available TCSPC systems.
The use of mf_D analysis has allowed us to study the spatio-temporal dynamics of the interaction between CFP-tagged Kv7.2 channels (donor) and YFP-labeled calmodulin (acceptor) in HEK293 living cells on a TCSPC system. We show the existence of discrete interacting domains that can be followed as a function of time (every 12-15s), where the fraction of interacting KCNQ2 with calmodulin oscillates at least between 15% and 25%.
In summary, we have shown that the mf_D analysis allows quantitative study of the spatio-temporal dynamics of molecular interactions on TCSPC systems. Using this non-fitting strategy we have quantitatively imaged the interacting dynamics of CFP-tagged Kv7.2 channels and YFP-labeled calmodulin in living cells.

1006-Plat
N-Way FRET Microscopy for Imaging Multiple Protein Interactions Within a Single Living Cell
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Fluorescence Resonance Energy Transfer (FRET) microscopy has emerged as a powerful tool for probing nanoscale protein interactions while capturing the microscale organization of these interactions. However, current FRET microscopy approaches are limited to the analysis two interacting fluorescently labeled proteins at a time. This limitation precludes the use of FRET microscopy for simultaneous measurement of multiple biochemical activities. Here we present a new FRET microscopy method that generalizes quantitative FRET microscopy to any number of fluorophores interacting in any combination. This approach makes use of Parallel Factor Analysis (PARAFAC) to define excitation/emission spectral fingerprints for FRET between any number of interacting fluorophores from observations of reference samples on any instrument. The resultant spectral fingerprints are then used in a simple linear unmixing model recover the distributions of free and interacting fluorophores as well as their apparent FRET efficiencies. Input data can consist of either complete spectral data or filter-based methods. The approach was validated using control constructs consisting of fluorescent protein fusions inside living cells. This method will enable intracellular analysis of sequential biochemical interactions that could not previously be observed.

1007-Plat
Single Cell Sensing and Manipulation by Scanning Nanopore Microscopy
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Signal Transduction by Ion Nano-Gating (STING) technology is a label-free biosensor based on quartz nanopipettes capable of detecting DNA and proteins. Using conical quartz nanopipettes a Scanning Ion Conductance Microscope (SICM) was developed and used for single cell imaging, sensing and manipulation. The height of the nanopore above a surface can be accurately controlled by measuring the ionic current through the pore as it approaches a surface. By maintaining a predefined distance from a surface and raster scanning the pore, a topographical image can be acquired. Additionally, material can be ejected through nanopipette using electrophoretic and electroosmotic properties. The cost of fabrication and ease of use of nanopipettes provides an ideal system for developing cellular systems for both cell detection and control. We demonstrate the capability of this sensor to image living cells, deposit material for controlled single cell growth on a substrate, and a platform for single cell injection and detection using an integrated microfluidic chip. The combination of STING technology with traditional SICM technology can be leveraged to acquire both topographical data and functional data, such as local ion concentration. This form of complementary microscopy is henceforth termed functional Scanning Ion Conductance Microscopy (fSICM). Preliminary fSICM results will be presented. This work illustrates the applicability of the STING sensor to a variety of applications, from single cell detection to definition of cellular circuits.

1008-Plat
In Vivo Identification of Changes in Metabolic State as Stem Cells Differentiate, by Phasor Analysis of Fluorescence Lifetime Imaging
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In stem cell research there is a high demand of techniques to investigate self-renewal and differentiation mechanisms and to develop stem-cell-based therapies for regenerative medicine. Here we develop a label-free method to identify and classify stem cells and differentiating cells according to their metabolic state. We use the phasor approach to fluorescence lifetime imaging and intrinsic biochemical fluorescence biomarkers such as NADH, flavins, retinoids and porphyrin. The organ studied is the C. elegans germ line, expressing a histone-GFP fusion protein that allows us to identify the differentiation state of the germ cells. We calculate the average phasor value of the intrinsic fluorescence of germ cells and we plot the cell phasor fingerprints in a scatter diagram. Cell phasor fingerprints cluster according to their differentiation state. Different metabolic fingerprint of cells reflect changes in binding sites of NADH with different coenzymes during differentiation. The phasor approach to lifetime imaging provides a label-free, fit-free and sensitive method to identify different metabolic state of cells during differentiation, to sense small changes in the redox state of cells and may identify symmetric and asymmetric divisions and predict cell fate. This method is also a promising non-invasive optical tool for monitoring metabolic pathways during differentiation or disease progression, and for cell sorting.
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1009-Plat
Multiplexed Time Lapse Fluorescence Lifetime Readouts in an Optically Sectioning Time-Gated Imaging Microscope
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The aim of our study is to follow the temporal and spatial interactions of proteins within different signalling networks in live cells. To map protein-protein interactions, we apply Förster resonant energy transfer (FRET) readout using fluorescence lifetime imaging (FLIM) and we present an optically sectioned FLIM microscope capable of time-lapse readouts of multiplexed FRET pairs. We note that FLIM FRET provides robust measurements, permits the use of dark acceptors and helps minimise the impact of spectral cross-talk.
Wide-field FLIM is implemented using a gated optical intensifier synchronized with the excitation pulses from a fibre-laser pumped supercontinuum source or a frequency doubled Ti:Sapphire laser and enables FLIM FRET in live cells with acquisition times below ~10 seconds. Optical sectioning is implemented using a Nipkow disk unit and improves quantitation compared to wide-field imaging, e.g. permitting separation of signals from the plasma membrane and the cytosol.
To demonstrate the capabilities of this instrument, we have mapped the intracellular changes of calcium levels using interleaved time lapse FLIM acquisitions of live cells labelled with two calcium probes following stimulation with ionomycin and calcium solutions. HEK293 cells were labelled with the genetically expressed biosensor, Troponin TN-XL, for which changes in calcium levels are read out by FRET between the CFP and YFP fluorophores, and with a calcium sensing dye, GFP-Certified™ FluoForte™. The emission profile of the latter is spectrally separate from CFP and YFP, making it useful for multiplexing with the many available CFP/YFP FRET biosensors.
We aim to apply this instrument to follow the spatial and temporal changes of simultaneous activation of signalling pathways during fibroblast migration in a concentration gradient of a chemo-attractant (PDGF), particularly using specifically designed FRET biosensors for small GTPases (Rac), phosphoinositide pathway (IP3) and calcium.