# Emerging Single Molecule Techniques II

**3036-Pos**
**Extending the Nano-Positioning System (NPS)**
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Single-Pair Fluorescence Resonance Energy Transfer (FRET) experiments reveal structural and dynamic information about macro-molecules by monitoring the change in FRET efficiency between fluorescent dyes attached to a macromolecule. The Nano-Positioning System (NPS) developed recently [1] uses data from several of such experiments to infer the position of a dye attached to protein sites unresolved by x-ray crystallography. Briefly, we perform probabilistic data analysis that allows us to calculate the distribution of possible dye positions in a simple and objective way without relying on ad-hoc procedures. Up to now NPS was limited to the triangulation of just one fluorescently labelled position based on FRET measurements to several other positions known from crystal structure [1,2]. Here, we discuss ways to extend the present model beyond this basic triangulation principle. In particular, we show how to gain three dimensional distance information by analysing triangulation networks where FRET is measured between arbitrary labelling sites in absence of other structural information.
[1] A. Muschielok, J. Andrecka, A. Jawhari, F. Brückner, P. Cramer & J. Michaelis, Nat. Meth. 5, 965-971 (2008)
[2] J. Andrecka, B. Treutlein, M.A. Izquierdo Arcusa, A. Muschielok, R. Lewis; A.C.M. Cheung, P. Cramer, and J. Michaelis, NAR doi:10.1093/nar/gkp601 (2009)

**3037-Pos**
**Single Molecule Tracking Inside Individual Living Bacterial Cells**
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We have developed a single-molecule fluorescence assay to directly observe the stringent response in individual living E. coli cells. For this purpose, we have created chromosomal fusions of both RelA and three ribosomal proteins with a photo-activatable fluorescent protein.
The stringent factor RelA binds to a small fraction of ribosomes, where it synthesizes the global transcriptional regulator ppGpp in response to amino acids deprivation. Our objective is to study the binding kinetics of individual RelA molecules to the ribosome in living cells and to observe how its kinetics changes during a nutritional down-shift.
While E. coli contains on average about 100 RelA molecules and 20000 ribosomes, using a photo-activatable fluorescent probe we can activate only a few fluorescent molecules per cell at any given time. We induce stringent response by rapid addition of amino acid hydroxamates. Since our fluorescent tag is photoconvertible, we can repeat tracking experiments many times in the same E. coli cell.
We record trajectories of individual RelA molecules diffusing in living E. coli cells with a laser exposure of 1 millisecond, a frame time of 5 milliseconds, and a spatial precision of 50 nanometers. The high resolution of the experiments makes it possible to characterize RelA binding kinetics under varying growth conditions. When the cell grows exponentially, RelA trajectories closely resemble trajectories of fluorescently tagged ribosomal proteins. After nutritional downshift, RelA binding kinetics changes rapidly. Results suggest that under amino-acid starvation, RelA is only transiently bound to the ribosome. The data is consistent with an order of magnitude drop in affinity to the ribosome.

**3038-Pos**
**Development of a Plasmonic Nanoparticle-Based Assay to Observe Nanoscale Biological Dynamic Interactions**
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To measure nanoscale distances relevant to quantifying bio-molecular dynamics, a widely used technique is fluorescence resonance energy transfer (FRET). While FRET is sensitive to distances between donor and acceptor fluorophores below 10nm, the technique suffers from a low signal-to-noise ratio, limited range of distance sensitivity, and rapid photobleaching of the fluorescent dyes. These unavoidable experimental shortcomings greatly hinder the robustness of the technique in measurements characterizing dynamics at the single-molecule level. A new approach that exploits the plasmon coupling of gold nanoparticles has been introduced, which has a high signal-to-noise ratio, distance ranges from sub-nanometer to hundreds of nanometers (depending on particle size), and superior photostability. Previous work has demonstrated the viability of plasmon coupling to report the distance between two gold nanoparticles conjugated to the ends of DNA. We have extended the technique such that the distance between the gold nanoparticles and the plasmon coupling spectral response are independently determined through the use of image analysis and spectrophotometry. In this manner, this technique can be extended to observe the dynamics of any protein interaction where gold nanoparticles may be conjugated. We have designed and implemented a custom spectrometer with high spectral resolution (0.015nm) and low integration times (millisecond), surpassing commercially available instruments, by using a sensitive CCD array and off-axis parabolic mirrors. By simultaneously analyzing the collected spectra of single gold nanoparticle pairs and images of their diffraction-limited spots using a 2-D Gaussian fitting algorithm, the spectra are correlated to the measured distance between the gold nanoparticles. As a proof-of-concept, we are now creating calibrations between the plasmon coupling spectra and the distance between dually-labeled DNA molecules. This experimental technique has the potential to study DNA-protein and protein-protein interactions with high spatial (sub-nanometer) and temporal (millisecond) resolution.

**3039-Pos**
**Advanced Multidimensional Optics to Investigate Biological Complexity at the Single Molecule Level in Living, Functional Cells**
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Fluorescence microscopy offers a minimally-perturbative, non-invasive means to probe functional living cells. This project aims to develop super-resolution, multi-colour, multi-polarization fluorescence microscopy capable of simultaneous imaging of multiple bio-molecules, such as genomically encoded fluorescent protein fusion constructs. It will enable dynamically-modulated high-contrast imaging to a bandwidth of ~1kHz, facilitate ~millisecond tracking of single protein molecules in a single cell to ~10nm spatial precision, and permit nanoscopic length scale conformational change to be measured. It will be constructed to permit future addition of laser-tweezers and will be used to perform investigations on single living cells. Under investigation will be the cellular processes that bring about assembly of individual proteins into functional biological machines. The microscope will allow precise determination of how many components such machines have, dynamics of assembly and disassembly processes, mechanistic interactions, molecular stability and how machines of different biological processes may co-operate to produce compounded effects across the whole cell.

**3040-Pos**
**Detection of Rare Interaction Events Via Combined Photobleaching and Single Molecule Microscopy**
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Gathering information on the interplay between different membrane components and the characteristics of protein interactions in live cells requires the design of sensitive methods that allow for the simultaneous study of different probe molecules. Especially the aim to single out rare interaction events under a vast excess of non-interacting molecules remains challenging.
The two-color single molecule imaging technique presented here is the advancement of a recently presented approach to virtually dilute fluorescently labeled membrane constituents by photobleaching [1]. Using this technique, single molecule microscopy can be performed at almost arbitrarily high surface densities of fluorescent probe molecules. The method yields information on the fraction of colocalized particles and their position within 40nm accuracy. Supplemental data on the mobility and stoichiometry of the labeled molecule species can also be deduced. The sensitivity of our two-color single molecule imaging technique is significantly increased by tracking colocalized spots over consecutive images. We present a detailed statistical description of false positives and false negatives and quantify the sensitivity of our method.
Proof of principle experiments were performed by measuring the interaction between Alexa647-labeled Cholera Toxin B (CTX-B-Alexa647) and Bodipy-labeled GM1 (Bodipy-GM1) diffusing in a fluid supported lipid bilayer. We directly observed single Cholera Toxin molecules bound to Bodipy-GM1 and quantified their occupancy via brightness analysis. We demonstrate that extremely low interaction probabilities of only 2.5% can be unambiguously identified by tracking colocalized spots.
We also present preliminary results on the application to live cells investigating the interaction between Bodipy-GM1 and Ab-Alexa647 labeled CD59.
[1] Moertelmair et al., Appl. Phys Lett. 87, 263903, 2005.

**3041-Pos**
**Single Molecule Chemical Reactions within Femtoliter Volume Containers**
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We create and observe controlled single molecule chemical reactions within femtoliter containers called hydrosomes. Hydrosomes are stable aqueous