mobility in a frozen solvent prevents many photoactivatable small molecule fluorophores and fluorescent proteins from undergoing the necessary isomerizations or other rearrangements required for photoactivation or blinking. In this work, we have identified a red fluorescent protein which exhibits photoactivation at cryogenic temperatures. Using a custom fluorescence microscope capable of maintaining a sample at cryogenic temperatures during imaging, we have characterized the photoswitching behaviour and obtained superresolution images of previously studied proteins in the model organism *Caulobacter crescentus*.

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Fluorescence Microspectroscopy with Nanometer Peak Position Resolution: Novel Applications of Environment-Sensitive Probes

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Fluorescence microspectroscopy (FMS) with environment-sensitive probes provides information about local molecular surroundings at microscopic spatial resolution. Until recently, only probes exhibiting large spectral shifts due to local changes have been used. Herein, we show that appropriate measuring procedure and data analysis enable nanometer spectral peak position resolution, even for photosensitive fluorophores [1].

The reach of our approach is demonstrated in several examples. The first application shows how we can distinguish lipid vesicles in different lipid phases with two commonly used polarity-sensitive probes. A synthesized NBD-based fatty acid red-shifted its emission maximum by 1.5-2 nm going from gel to liquid-disordered phase in DPPC. Between these two phases Laurdan exhibits a large 50 nm red-shift. We therefore chose a more challenging combination - gel and liquid ordered phase, realized by DPPC and DPPC/Chol (40 mol%), respectively, where we were able to detect a 3 nm blue-shift with Laurdan [1]. The second example shows application of a synthesized rhodamine-based pH-activatable probe that is sensitive to aggregation. We studied a receptor-mediated internalization in dendritic cells and measured a 3 nm aggregation-induced emission spectral shift due to probe accumulation in endosomes and lysosomes [2,3].

The results show that peak position resolution, characteristic for spectrofluorimetric measurements on bulk samples, could readily be achieved at micrometer spatial scale.

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Boosting the Localization Precision in Super-Resolution Microscopy: booSTORM

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Over the last decade, single molecule switching and localization have become the key for super-resolved visualization of molecular architecture and dynamics. Here, we show that biocompatible plasmonic coatings on standard microscope cover glasses significantly improve the resolution of a direct stochastic optical reconstruction microscopy (dSTORM) experiment. The enhanced signal-to-noise ratio sharpens the location precision by a factor of 1.8 as demonstrated in super-resolution images of the nuclear pore complex (Heil et al., bioRxiv doi: 10.1101/136739). Together with recent advances in image processing and analysis (Franke et al., 2016, Nat. Methods, 14, 41-6) we demonstrate the reliability of our optoplasmonic approach (booSTORM) and proof that the effect leads to an increased total photon yield per fluorophore. The strength of booSTORM is that the enhancement is solely induced by the metal-dielectric coatings on the cover glass and does not require any additional modification of the microscope setup. This makes booSTORM very versatile, and applicable as a booster for many other fluorescence approaches including for example live cell Fluorescence Correlation Spectroscopy and Fluorescence Resonance Energy Transfer studies.

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Force Spectroscopy of Phagocytosis with High Frame Rate 3D Light Sheet Imaging

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A critical part to the immune system is the search and elimination of foreign pathogens by macrophages, neutrophils and other cells. These cells eliminate large particles (>0.5μm) by consuming them through a process called phagocytosis. Understanding the mechanics of how a macrophage engulfs a target requires careful monitoring of forces and high quality florescence imaging of the membrane and cytoskeleton. Using an atomic force microscope (AFM) with a versatile optics system, we can monitor piconewton scale forces while imaging the phagocytosis engulfment from the side using Pathway Rotated Imaging for Sideways Microscopy with vertical light sheet (PRISM-LS). In addition, the versatile optics system can be used to record high frame rate three dimensional images of macrophage engulfment. In this study, we look at the effects of different applied forces on phagocytotic uptake and mechanical response of macrophage cells. Preliminary results show the macrophage produces a dynamic response to the application of a several hundred piconewton force with an IgG covered AFM tip during its engulfment. Light sheet imaging with controlled force data will inform mechanical models of phagocytosis which will improve understanding of this important immunological process and inform mammalian disease progression.

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Bacterial Proteins Associated with Cell Shape Homeostasis Localize to Specific 3D Geometries

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The bacterial kingdom exhibits a wide variety of cell shapes and sizes. The shapes of these cells are crucial for their lifestyle. Over the past few years we have developed an image-processing framework allowing us to extract precise 3D shapes of bacterial cells from fluorescence microscopy data. From these xyz coordinates, we calculate geometric parameters such as local curvatures, surface areas, and the relative enrichment of fluorescent signals. We use this to measure the geometric localization of bacterial proteins responsible for establishing and maintaining the characteristic shape of various Gram-negative bacteria such as the straight rod *Escherichia coli*, the curved rod *Vibrio cholerae* and the helical rod *Helicobacter pylori*. In *Escherichia coli,* we focused on the bacterial actin MreB which localizes away from positive Gaussian curvature and toward zero and low curvature. MreB's many cell shape roles are modulated by interactions with the transmembrane protein RodZ. In the absence of *rodZ*, MreB loses its curvature localization and cells are not able to maintain their uniform rod-like morphology. Additionaly, we have used antibiotic treatment and genetic perturbations to confirm that our method and normalization strategies perform well in a variety of geometries. In contrast to MreB's enrichment at low Gaussian curvature, the cholera protein CrvA localizes to regions of negative Gaussian curvature. We have also developed a cell wall pulse-chase labeling strategy (Quantitative Analysis of Sacculus Architectural Remodeling, QuASAR), to determine that CrvA reduces the effective exponential growth constant for the inner curve relative to the outer curve. This results in cells which curve up over time. Recently, we have begun to examine the localization of the multiple cell shape determinants in the bacterial carcinogen *H. pylori*, not all of which share the same curvature localization preferences.

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Uncovering Hidden Dynamics in Live-Cell Single Molecule Data with Bayesian Statistics

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Single-molecule imaging techniques localize and track individual molecules inside living cells with nanometer precision and millisecond timing; this capability has allowed researchers to investigate many open questions across biology. However, single-molecule image analysis is fundamentally limited by *a priori* model selection, parameter unidentifiability, and other supervisory biases. To address these issues, we have developed an analysis framework for Single-Particle Tracking data based on nonparametric Bayesian inference. By encoding any information we have about the system into the ''prior'', iteratively determining the maximal parameter values and data selection by the