3680-Pos Board B541
Super Resolution Drift Correction with Intermittently Observable Markers
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Microscope drift correction necessary for super-resolution microscopy over extended time periods generally requires either significant modification of the microscope setup or the introduction of large fiduciary markers into the sample, which may perturb the cell. Further, existing drift correction methods expect the fiduciary markers to be continually observable, ruling out most quantum dots due to blinking. Similarly, single fluorophores have insufficient signal to make ideal markers, and as such, large particles or fluorescent beads are common fiduciary markers. Here, a drift correction method is developed allowing for intermittent observations, allowing quantum dots, which are less likely to perturb cells, to serve as fiduciary markers. A statistical comparison of intuitive methods of drift correction with the method developed here reveals that naively applying typical methods to intermittent particles introduces significant amounts of additional uncertainty, sufficient to significantly reduce the effective localization accuracy over the course of super-resolution experiments. The method developed here works equally well whether particle observations are intermittent or continual, as long as a comparable number of total particle observations are available.

3681-Pos Board B542
Super-Resolution Imaging using Randomly Diffusing Probes
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Recent advances in super-resolution microscopy allow imaging of biological tissues labeled with fluorescent dyes with unprecedented resolution making it possible to study cellular structures on a new length-scales. These techniques, such as photo-activated localization microscopy and stochastic optical reconstruction microscopy, rely on the fact that single emitters can be localized with nm accuracy. When multiple emitters reside within a diffraction-limited spot one has to ensure that they emit one at a time, which is typically done by serial photo-switching. This localization approach however has not been applied yet to other imaging modalities, for example imaging local electromagnetic field enhancement, mainly because serial photoswitching would be infeasible for investigating a local field. Here we present a super-resolution imaging technique which circumvents the requirement for serial photoswitching by using the random motion of single dye molecules to scan the surface in a stochastic manner. The scanning combined with point-spread function localization allows us to image electromagnetic field enhancement of a single hot spot with 1.2 nmn accuracy. Hotspots, utilized for example in surface-enhanced Raman spectroscopy (SERS), appear when light illuminating rough metallic surface is concentrated to the nanometer scale, producing an intense electromagnetic field. The mechanism generating the enhancement is still not well understood and probing the electromagnetic field of the hotspots can offer key insight. We mapped the field of hot spots on the surfaces of aluminium thin films and silver nanoparticle clusters and discovered strong fluorescence enhancements, decaying exponentially from the center, which correlates inversely with the size of the hotspot.

3682-Pos Board B543
Imaging Subresolution Membrane Curvature in Living Cells by Back Focal Plane Positioning Polarized Total Internal Reflection Microscopy (TIRFM)
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Induction of membrane curvature is an essential step in the formation of endocytic vesicles and viral particles. Axelrod and co-workers have shown that polarized total internal reflection fluorescence microscopy (pol-TIRFM) can be used to visualize membrane curvature in cells labeled with the lipophilic fluorophore DiI. We describe an approach for creating s-pol and p-pol TIRF fields using a commercial microscope system equipped with a 2-D scan head to position polarized laser beams at distinct azimuthal positions in the back focal plane (BFP) of a high numerical aperture TIRF lens. This configuration allows for reduction of interference fringing of the coherent light by alternating illumination at azimuthal positions separated by 180 degrees. Additional reduction of fringing could be obtained by sweeping the laser through small arcs in the BFP of about 8 degrees with minimal impact on the polarization of the TIRF fields. We investigated the performance of this pol-TIRFM approach for visualization of endocytosis and viral budding in cells labeled with DiI and either the endocytic marker clathrin-YFP or viral budding marker HIV Gag-YFP.

3683-Pos Board B544
Optimizing Single-Wavelength Control of Superresolution Localization Microscopy
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Superresolution localization microscopy (e.g. PALM, STORM) requires the acquisition of many frames containing non-overlapping single-fluorophore images. In any given frame, only a small fraction of the fluorophores in the sample are actually emitting light, and the user typically controls the fraction of fluorophores activated in each frame. Two competing considerations determine the fraction of fluorophores that the user should activate: Activating a large fraction of the fluorophores increases the rate at which information is acquired, but also increases the probability of nearby fluorophores activating simultaneously, producing overlapping images. Further complicating the analysis is bleaching, which decreases the number of fluorophores present over time and thus reduces the probability of overlaps.
We have performed a mathematical analysis, using variational calculus, that takes into account the effects of bleaching and overlaps, to determine the optimal time-dependent activation probability per molecule that maximizes the number of single-molecule images acquired in a given time. When the experiment is controlled by two wavelengths (one for activating molecules, the other for exciting fluorescence) the optimal scheme is one with a constant error rate, and is insensitive to the detailed kinetics of the bleaching process. However, when the experiment is controlled by a single wavelength, activation and excitation are coupled variables, and the optimal scheme depends quite sensitively on the details of the bleaching process, particularly whether the ground state of the fluorophore is dark or activated. Depending on the detailed kinetics of the activation and bleaching processes, acquisition with a low error rate might maximize or minimize the number of single-molecule images. Schemes that deactivate molecules by sending some of them to a long-lived triplet state are particularly problematic. We also analyze robustness, to determine how error rates are affected by deviations from the optimal scheme.

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Using Label-Free Screening to Investigate Stem-Cells from their Microanatomical Niche
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An understanding of both embryonic organogenesis and adult tissue regeneration relies on isolating and characterizing stem cells. In general, these cells are difficult to study because they constitute minute populations in organ niches and express multiple cell-surface markers, only some of which are identified. Furthermore, their properties change quickly in vitro and possibly even during isolation procedures. We have recently developed a label-free cell-analysis platform to characterize key cell-surface markers in single, functional organ stem cells directly isolated from their microanatomical niche, i.e. muscle (satellite) stem cells from single myofibers. Our platform is based on measuring the transit time of single cells as they transit a microchannel functionalized with a specific antibody. Cells that express the complementary surface antigen have a longer transit time as compared to those that do not. With this method, we have discovered that individual myofibers significantly differ from each other with respect to expression of the markers Sca-1, CXCR4, β1-integrin, and M-cadherin on their associated satellite cells. Furthermore, we have found that this heterogeneity extends to other important markers such as Notch1. Our data are the first to show the phenomenon of microniche-dependent variation in adult stem cells of the same tissue, emphasizing the complexity of the muscle stem-cell population and prompting new studies aimed at understanding the purpose for such heterogeneity. Ultimately, our platform introduces a new method for stem-cell analysis, as FACS and MACS do not allow for niche-specific characterization. Microscopy, while capable of imaging stem cells in their respective niches, is neither precise nor quantitative. Overall, our method can be broadly applied to the quantitative analysis of single stem cells isolated from their microniches in other adult and developing organs, leading to new discoveries on stem-cell properties and regenerative potential.