proteins consistent with single-particle tracking data. For system (2), we perform drug-trial experiments on living COS-7 cells to show that kICS can quantify the degree to which the drugs will affect kinetic binding/unbinding rates. By labeling the glycolipid membrane receptor GM1 with a fluorescent cholera toxin B-subunit, we show that cytoskeleton perturbations with the drug cytochalasin D result in significant changes ($k_u = 0.013 \pm 0.004\ s^{-1}$ to $0.08 \pm 0.02\ s^{-1}$) to the undocking rate of GM1 to the actin cytoskeleton. With these two proof-of-concept experiments, we pave the way to using kICS as an efficient and reliable method to elucidate kinetic binding rates and transport dynamics parameters for studies in biomembranes.

3054-Pos Board B746

A New Efficient Implementation of 2PE-STED Microscopy

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Time-gated detection substantially reduces the requested intensity for reaching effective sub-diffract resolution in stimulated emission depletion (STED) microscopy. When the time-gated detection is combined with STED beam operating in continuous-wave (CW), a simple, cheap, but also efficient, STED microscopy implementation is obtained, the so called gated CW-STED (gCW-STED) microscopy. The virtues of two-photons excitation (2PE) microscopy have been recently combines with those of STED microscopy. Two mainly implementations are explored. Chronologically, the first implementation relied on STED beams running in CW. Nevertheless, this implementation is relative simple, its resolution performance is strongly limited. The second implementation relied on pulsed STED beams. In this case the resolution performance improves but also the cost and the technical skills necessary for its routine use.

In this scenario, a natural solution is the combination of 2PE microscopy with gCW-STED microscopy. Although this implementation is rather straightforward, the small 2PE cross-section, which results in weak signals, conflicts with the major disadvantages of gCW-STED microscopy. Namely, time-gated detection surely reduces the signal, hence, in a situation of weak signal the images degrade in terms of signal-to-noise/background ratio (SNR and SBR).

Here, we propose a combination of different hardware- and software-based approaches for recovering both SNR and SBR. In particular, we explored the use of synchronous detection methods to remove all the background potentially induced by the STED beam. As well as, we improved the signal by accelerating the pulse repetition rate of the 2PE laser beam. As a side effect, this approach speeds up the acquisition time, reducing potential photodamage effects. The collection efficiency of the microscope is also optimized by using dedicated time gate electronics. Finally, raw images are processed by ad-hoc deconvolution algorithms to further enhance the SNR and SBR.

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Dual Color STED Microscopy with Ultrafast Photon Counting

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To more precisely quantify association between cardiac proteins, we built a dual-channel super-resolution stimulation emission depletion (STED) microscope with an ultrafast photon-counting acquisition system. STED microscopy is unique among super-resolution technologies due to its capability of optical sectioning and fast imaging speed. A major limitation of STED is the aggravated photobleahching, to which fast scanning speed is an excellent solution. In order to keep up with fast scanning speed and to reduce counting nonlinearity, we invented a photon counting acquisition system based on ultra-fast analog-to-digital conversion (ADC), pushing the readout rate to the gigahertz range. Incorporated into a resonant scanning confocal microscope, this system can acquire images at 16,000 lines per second and 58,000 pixels per line. By adding a continuous wave (CW) depletion laser and photon arrival time control elements, the confocal microscope was converted to a time-gated STED microscope. A second STED channel was built with picosecond depletion pulses. The dual-channel STED microscope is able to achieve a resolution of ~40 nm in biological samples which allows a more stringent analysis of colocalization in native systems.

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Efficient ROI Selection for Multi-Emitter Fitting Approaches in Single-Molecule Super-Resolution Microscopy

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Single molecule super-resolution techniques such as PALM and STORM circumvent the diffraction limit by using fluorophore switching to isolate single emitters in space and time. The requirement that individual fluorophore signals are well separated, however, limits the speed of these methods. Recent advances have relaxed this requirement through the use of model functions which allow multiple overlapping emitters within one image region. Building on the methods used for sparse localisation, multi-emitter fitting methods typically extract fixed size rectangular regions of interest (ROIs) around candidate molecules and perform fitting within these regions. These ROIs will often intersect and it is common for molecules to be present in multiple ROIs and/or to only be partially enclosed within a ROI. This requires a number of empirical solutions to collate molecules and ensure that they are not over-counted, and to ensure convergence when attempting to fit partial molecules. Fitting molecules repeatedly within multiple ROIs also incurs significant computational overhead.

Rather than using fixed size rectangular ROIs at candidate molecule positions, an alternative approach can be constructed based on the selection of contiguous regions of pixels which have a non-zero information content. In short, the algorithm determines which pixels in the image contain useful information by performing a statistical test to determine if pixels (or groups of neighbouring pixels) are significantly higher than the background, dilates this mask to introduce a 'safety factor', and uses a labelling algorithm to identify contiguous groups of pixels within this mask. These contiguous, non-rectangular, regions become the new ROIs. The approach has the dual advantages of fitting each emitter only once, and incorporating the full information about every emitter within each ROI so as to avoid any convergence problems.

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Highly Efficient HIV-1 Entry Mediated by Nonspecific Virion-Cell Interactions Quantified by Real-Time Single Particle Imaging

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The apparent low infectivity of HIV-1 is purportedly explained by virion-cell interactions that take place prior to viral entry in which the virus must first diffuse to the cell surface and bind to specialized receptors; these interactions are speculated, from in vitro studies, to be the rate-limiting step involved in the infection process yet the underlying mechanisms are not completely understood. Furthermore, receptor binding, in addition to nonspecific interactions limiting infectivity, may dictate the subsequent steps, i.e. entry pathways, of infection. Using single-virus tracking methods with spatial and temporal resolutions of ~20 nm and 40 ms, respectively, we have quantified the dynamics of mCherry labeled HIV-1 virions with varied envelope (Env) glycoprotein incorporation interacting with TZM-bl cells, a HeLa-derived cell line expressing CD4 and co-receptors at high surface densities. The number of viral-cell touching events and corresponding contact lifetimes of most trajectories suggest few and transient interactions with the cell surface before permanent dissociation. The fraction of virions that become immobilized is heavily influenced by nonspecific interactions in both the presence and, to a lesser extent, absence of DEAE-dextran, contributing to receptor-independent endocytic entry as revealed by time-lapse and confocal imaging studies. Measured internalization efficiencies as high as 80% appear to be independent of Env content despite proportionally higher infectivities; thus, nonspecific binding and subsequent endocytosis may actually lower the apparent HIV-1 infectivity. Moreover, we directly observed a prevalence of these internalized virions to undergo recycling and/or transcytosis implicating this pathway as being involved, albeit indirectly, with infection. (Supported by NIH 1DP2OD008693).

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Development of Stable Small Quantum Dots for AMPA Receptor Tracking at Neuronal Synapses

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AMPA receptors are important excitatory neurotransmitter receptors in the brain. Trafficking of AMPA receptors in and out of synapses affects synaptic plasticity. Quantum dots have been used as fluorescent probes for tracking diffusion of AMPA receptors. However, the size of commercially available quantum dots is ~20 nm, likely limiting their ability of accessing the synaptic cleft which is ~30 nm. Therefore, photo-stable fluorescent probes with smaller sizes are needed. In this study, we developed a new method for coating quantum dot cores to obtain stable quantum dots with diameters about 9 nm.