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MULTISPECIES SINGLE MOLECULE IMAGING WITH QUANTUM DOTS

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ABSTRACT

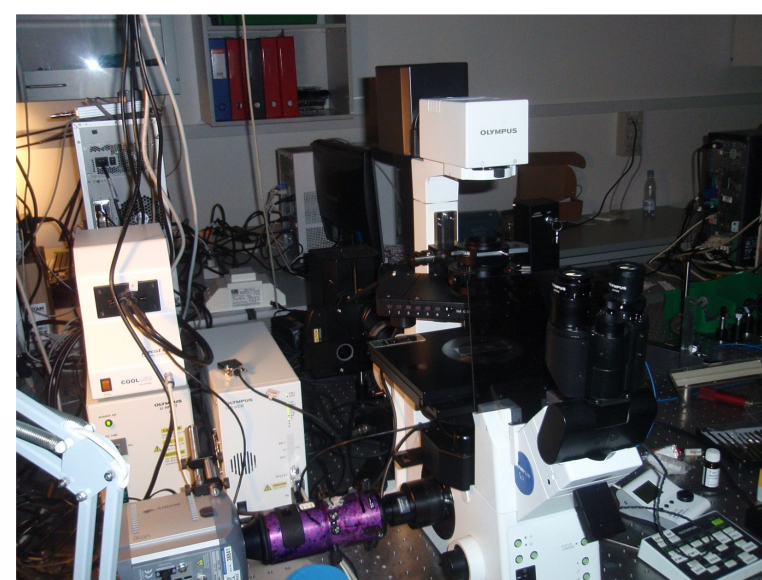
Quantum dots (Qdots) are fluorescent nanoparticles that have far superior signal intensity and signal stability compared to more conventional fluorescent molecules. We find that imaging with Qdots can easily be extended to the simultaneous visualization of up to four different molecular species at single molecule sensitivity and millisecond time integration. We find that this technique can easily be adapted towards studying the spatial and temporal organization of various combinations of lipids and proteins in the cellular plasma membrane.

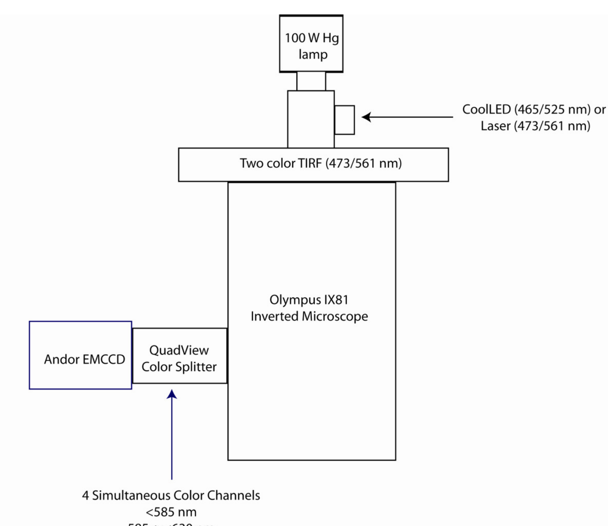
SUMMARY

Our main interest is in using Qdots in single molecule imaging microscopy for investigating the spatial and temporal nano organization of the cellular plasma membrane. As part of this work, we are interested in the single molecule behavior of Qdots (i.e. brightnesss, photostability, and spectral separation), and in investigating various methods for targeting Qdots with high specificity and avidity to a variety of lipids and membrane proteins.

MICROSCOPY SET UP

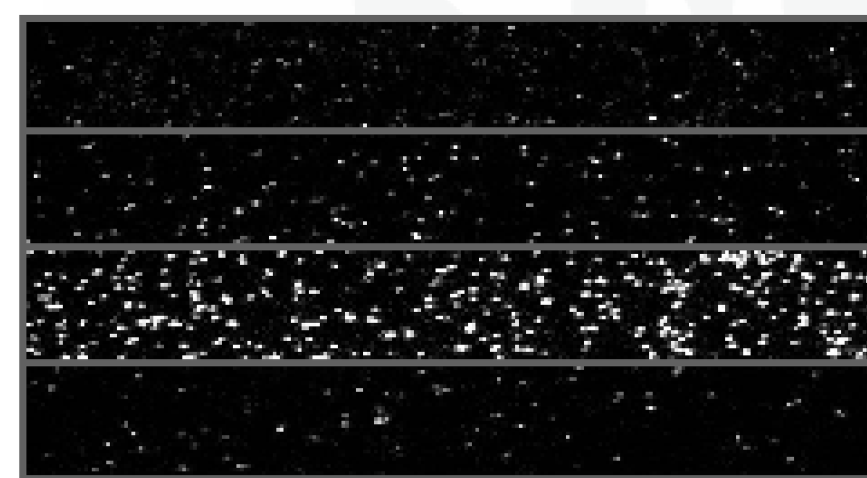
Our microscope is an inverted Olympus IX- 81 fluorescence microscope equipped with a very sensitive Andor EMCCD. On this microscope, we can illuminate specimen with either a conventional Hg arc lamp, a CoolLED (465 and 525 nm), or laser illumination (473 nm) in epi or TIRF mode. On the detection side, we also have a QuadView image splitter for simultaneous image acquisiton in up to 4 separate color channels.

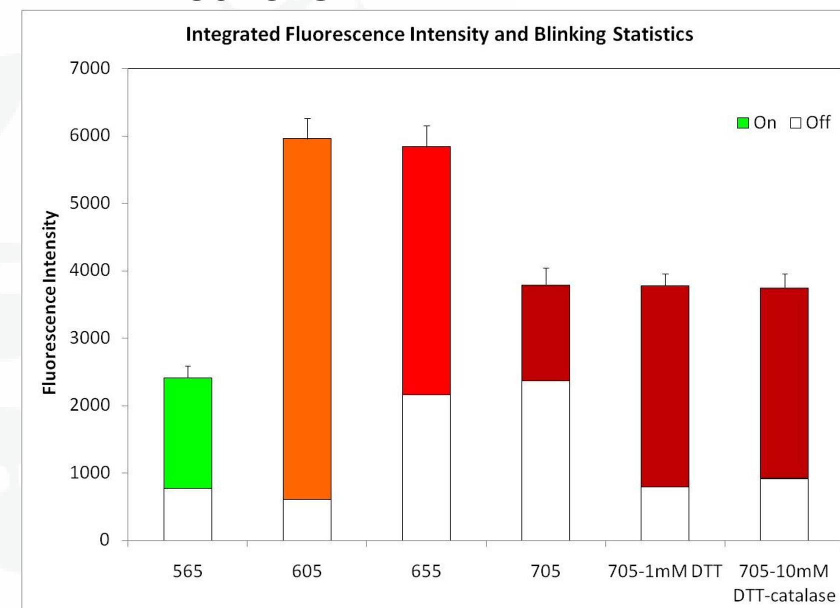




EXAMPLES OF CURRENT PROJECTS

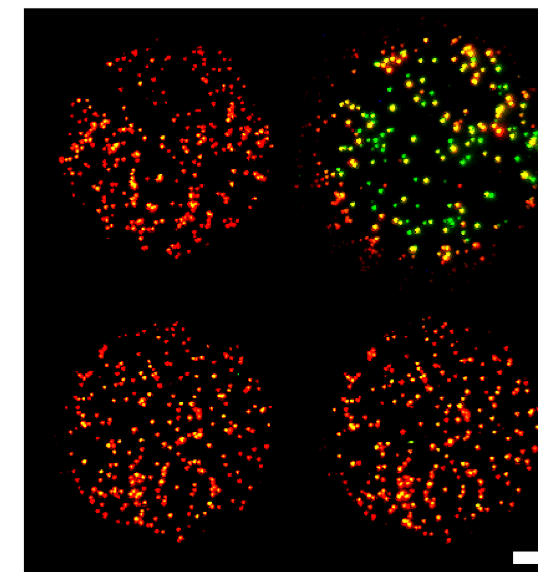
QDOT INTENSITY AND BLINKING STATISTICS



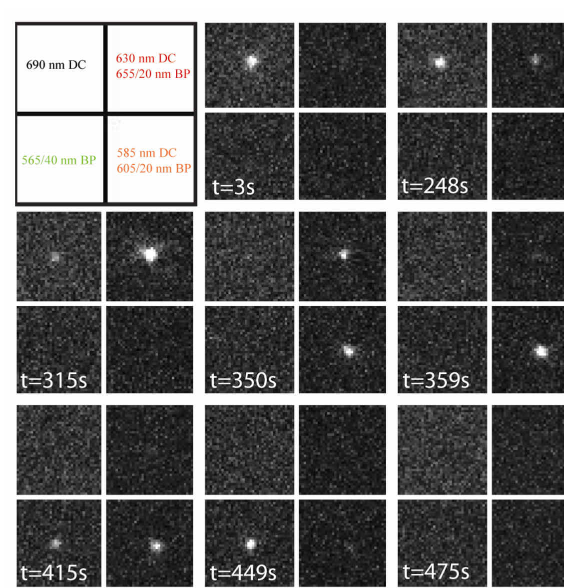


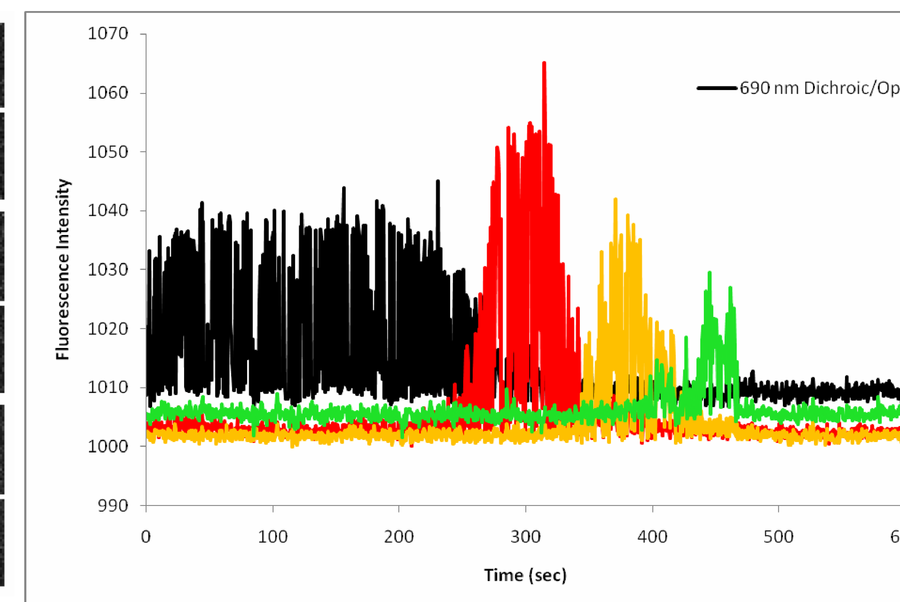
Qdot intensity comparison of from top to bottom, 565, 605, 655 and 705 emitting Qdots adsorbed on glass. Time lapse images in each instance were acquired with 10 ms integration using a Hg arc lamp. In these image sequences, we observe frequent Qdot intensity fluctuations between an on and an off state for all Qdots studied to date. We find that the observed Qdot brightness and intermittency is color dependent, where 605 nm Qdots exhibits the best combination of Qdot brightness (~6000 counts above background with 10 msec integration) and on frequency. (~90/10 on/off frequency). In contrast, 705 nm Qdots while moderately bright (~4000 counts above background) are primarily in an off state (40/60 on/off frequency). Consistent with previous results, we do also find that small reducing agents (β - mercaptoethanol, DTT) in the 1-10 mM range improves the on/off frequency with no effect on the Qdot brightness.

QDOT COLOR STABILITY



We also find that Qdots switch color in a continuous, power dependent manner. This color switching is almost completely inhibited with 25 μM β- mercaptoethanol. Fluorescence color switching imaged on a CoolSNAP Pro$_{cf}$ CCD camera with 10 sec integration time. Left and right images were acquired about 7 minutes apart after continuous illumination. Top images show color switching while bottom images show the inhibition of the color switching in the presence of β – mercaptoethanol.

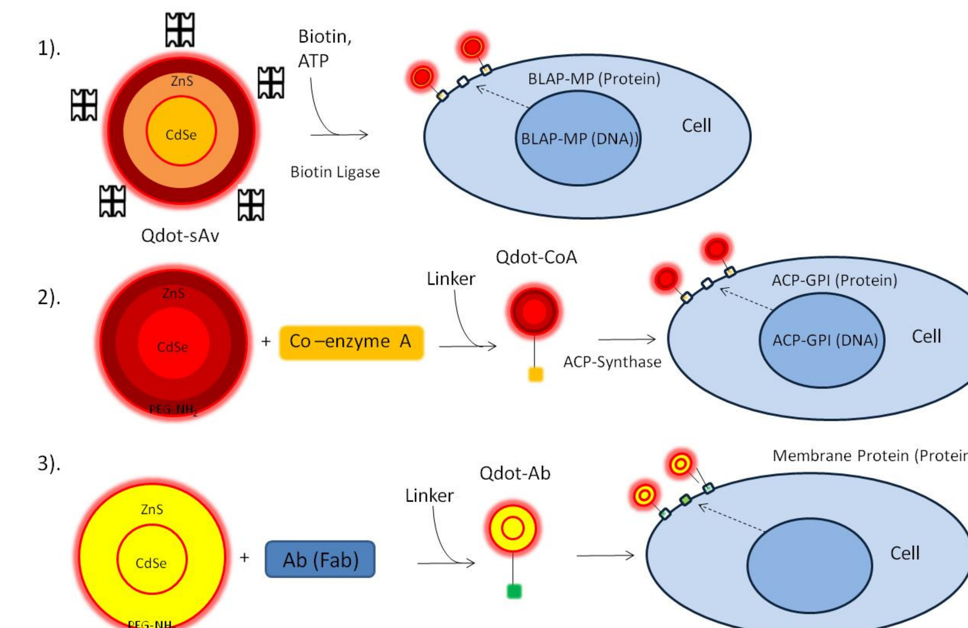


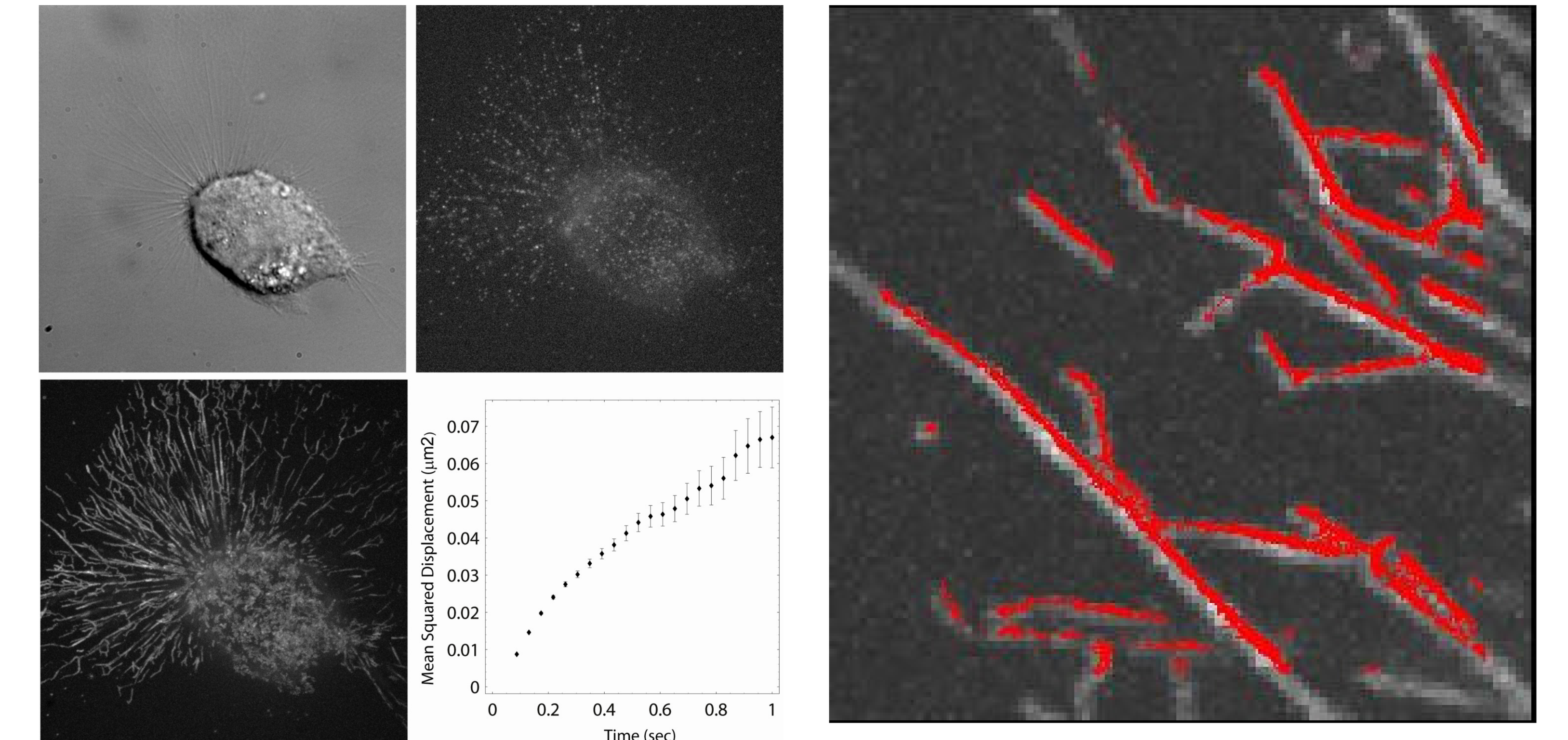


(Right) Raw image data examples of single 705 nm Qdot showing the gradual color switching behavior. Fluorescence emission was split into four separate image channels by a QuadView emission splitter onto a single Andor EMCCD. (Left) Integrated fluorescence intensity of a single 705 nm Qdot undergoing a gradual color switch. In the example given the fluorescence signal of the single Qdot remains in the upper channel (>690 nm) for about the first 250 sec (black curve) when it undergoes a gradual switch into the 655/20 nm channel remaining there for about 100 sec (red curve) followed by a switch into the 605/20 nm channel (75 sec), into 565/40 nm channel (75 sec) and finally into a dark state for the last 125 sec

TARGETING OF QDOTS TO SPECIFIC MEMBRANE PROTEINS AND LIPIDS

We are applying Qdots for tracking the spatial and temporal dynamics of lipids and membrane proteins (MPs). In the case of membrane proteins, we are currently exploring three targeting schemes: 1) Targeting of recombinant and native membrane proteins by genetic insertion of a 15 amino acid biotin ligase acceptor peptide (BLAP) substrate to bacterial biotin ligase in combination with streptavidin (sAv) conjugated Qdots, 2) Targeting of recombinant membrane proteins by genetic insertion of a 77 amino acid acyl carrier protein (ACP) substrate to ACP Synthase in combination with custom CoA conjugated Qdots, and 3) Custom antibody Qdot conjugates. In the case of lipids, we are exploring the use of cholera toxin conjugates for tracking the sphingolipid G_{M1} and sAv conjugated Qdots for tracking biotinylated lipids.





Example of single Qdot imaging of unknown genetically modified native membrane protein in mouse embryo fibroblasts. (top left) DIC image of genetically modified cell. (top middle) Single fluorescence image of genetically modified cell labeled with sAv conjugated Qdots. Image is from a 600 frame time lapse which was acquired with a 100X, 1.25 NA objective, and illumination with a 100W Hg arc lamp (10 ms integration). (bottom left) Maximum intensity projection of showing diffraction- limited map of the cellular space that was visited during over a 600 image frame movie lasting 23 sec. (bottom middle) Average mean squared displacement of all single Qdot molecules over first 120 frames corresponding to greater than 26,000 particle positions. (right) Close up of sub diffraction limited map obtained from linking the centroids of each single Qdot position as determined by least square curve fitting to a spatial 2D Gaussian.

MULTICOLOR SINGLE MOLECULE IMAGING OF LIPIDS AND PROTEINS

Our main goal is now to implement and apply our results from the characterization of single Qdots in order to investigate the nano organization of the cellular plasma membrane by high speed, multicolor, simultaneous single molecule imaging of a combination of lipids and membrane proteins. A preliminary example of such a combination is shown below where we have combined cholera toxin conjugated Qdots (705 nm) for tracking G_{M1} (bottom right), sAv conjugated Qdots (605 nm) for tracking epidermal growth factor receptor (EGFR) BLAP (bottom left), and yellow fluorescent protein (YFP) plasma membrane marker for visualizing the plasma membrane.