oxidase or spingomyelinase. In addition, the effective domain area explored by tracer particles increased post drug treatments, from 0.25 to 0.31 um². These observations suggest that the domain boundaries are affected by drugs leading to more permeable domains.

1783-Pos Board B675
Experimental Determination of the Forster Critical Distance
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Forster resonance energy transfer (FRET) can be used as a spectroscopic ruler to measure nanometer-scale distances. The recovery of inter-dye distance depends on a calibration factor known as the Forster critical distance (R₀). This distance is currently estimated based on measurements of the quantum yield of the donor dye, the overlap integral between the donor and acceptor dyes, and assumptions about the index of refraction and the relative orientation of the donor and acceptor dye molecules.

Here, we report a method to experimentally measure R₀ using B-DNA as a structural reference. Fifteen donor (Cy3)-labeled oligonucleotides were generated, by placing donor-labeled Thymidines at positions 11, 14, ..., 39. A single complementary strand was synthesized with acceptor (AlexaFluor647) at position 10. The strands were annealed, producing dsDNA consisting of a 30 base pair (bp) ruler with a 10 bp cap on each end. For each freely diffusing construct, the mean transfer efficiency (TE) was measured by single-pair FRET (sp-FRET) and ensemble (en-FRET). The TE’s as a function of bp were fit to a reduced representation model of B-DNA that provided the absolute inter-dye distances. The reduced model was formulated based on an atomistic model of dye-labeled B-DNA, R₀ was recovered from the fit. We repeated our approach using three different donor/acceptor pairs, each with a different R₀.

1784-Pos Board B676
How can the Enhanced Sensitivity and Favourable Noise Characteristics Conferrer by Electron Multiplication Improve Fluorescence-Guided Surgery?
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Mammalian cells express two classes of photoporphyrin IX (PPIX), designated Types II and III, which phosphorylate photoporphyrin IX to generate P4P. A number of studies indicate that these enzymes are important for Golti trafficking and early as well as late stages of endocytosis. In this study, we focus on PI4KIIβ, a protein that is evenly distributed between immobile and soluble fractions and is believed to participate in stimulus-dependent phosphoinositide signaling. Using molecular brightness analysis, we found that EGFP-tagged PI4KIIβ exists as two distinct species in the cytoplasm, a soluble monomer and a high order complex enriched with multiple copies of PI4KIIβ. This observation is confirmed by autocorrelation analysis which identifies two species with distinct mobilities. We further demonstrate that the high order complex enriched with PI4KIIβ is sensitive to inhibition of palmitoylation, indicating that it is associated with membranes, very likely vesicles. Indeed, we show that the high order PI4KIIβ complex is sensitive to expression of dynam 2-K44A, a dominant-negative inhibitor of endocytos.

We investigate whether or not electron multiplication devices (EMCCD) can confer sensitivity and acquisition time advantages in fluorescence-guided neurosurgery when compared to scientific-grade charge-coupled devices (CCD). We present a demonstration of the sensitivity of PPIX detection that can be obtained with a new EMCCD camera that was developed by NituV Cameras. This device significantly reduces the noise generated during the read-out process (at least 10x) and presents a previously unreachable sensitivity in photon counting mode. Such major reduction in the noise threshold represents an opportunity to detect very faint levels of PPIX with smaller integration times than was previously achieved with CCDs. The goal of this study was to open the way to a less disruptive in vivo fluorescence detection technique to allow surgeon to perform more accurate resections on a more varied range of intracranial tumors.

1785-Pos Board B677
Diffusion-Enhanced Luminescence Resonance Energy Transfer in the Cytoplasm of Live Bacterial Cells
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We previously found that a 17-amino acid lanthanide-binding tag (LBT) expressed as a fusion with a cytoplasmic protein in E. coli takes up Tb³⁺ in live cells (Biochemistry 50, 6789, 2011). The protein is called DAL after its designation with a new EMCCD camera that was developed by Nu¨ vu¨ Cameras. This camera is a powerful tool to characterize cellular cytosolic vesicles that are otherwise difficult to characterize by other techniques. This work is supported by the National Institutes of Health (R01 GM64589) and the National Science Foundation (PHY-0346782).