enhancement. The change in fluorescence intensity of the LDL particle is observed with fluorescence microscopy and analyzed using single particle tracking. Dequenching events are observed as a single-step increase in intensity. Wortmannin, a drug that inhibits late endosome fusion, is used to block the transport of LDL to the lysosome. Individual dequenching events are not observed in wortmannin-treated cells suggesting that degradation of the LDL particle requires late endosome-lysosome interactions. This LDL labeling scheme will be extended to two-color single particle tracking experiments to determine the fraction of late endosome-lysosome interactions that lead to enzymatic degradation of LDL particles.

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Activation Pathway Of paGFP In Living Cells

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Particle tracking inside the cell largely benefits of the ability to spatially and temporally mark specific structures to follow their ''signalling'' over a ''dark'' background as made possible since the advent of the photo-activatable markers. In terms of spatial confinement of the photo-activation process, the use of multiphoton excitation provides several favourable aspects compared to single photon confocal microscopy in photomarking biological structures to be tracked: the confined excitation volumes, of the order of magnitude of subfemtoliter, due to the non-linear requirements provide a unique control of the excitation and consequently photoactivation in the 3D space. In this context photoactivation experiments can be used to assess quantitative information about the binding kinetics of a macromolecule expressed in different cellular compartments. In this work we extended to photoactivation procedures and models originally developed for the quantitative analysis of FRAP experiments and we evaluated, for different proteins of medical interest (Rac-paGFP), the diffusive behaviour in the cytoplasm and the binding kinetics at the large endosomes. The results are compared with standard photobleaching experiments, in order to evidence the gained sensitivity obtained with photo-activatable proteins.

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Organelle Specific Associations of HIV-1 Nef with HLA-I A2 and CD4 using Fluorescence Cross Correlation Spectroscopy

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Among HIV encoded proteins, ***Nef*** is critically required for virus replication and AIDS pathogenesis and is a major effector of immune-evasion mechanisms, in part by modulating cell surface expression of CD4 and HLA-I receptors. Genetic and biochemical studies have suggested two different mechanisms for *Nef* induced downregulation of CD4 and HLA-I. This might reflect distinct and differential interactions between *Nef* and the receptors in the various subcellular organelles. To evaluate this possibility, we measured the interactions of *Nef* -cerulean with HLA-I A2-eYFP protein in the TGN, ER and plasma membrane in transiently transfected HeLa cells using single point Fluorescence Cross Correlation Spectroscopy (FCCS). We found that between 27 to 37% of the total concentration of *Nef* was found to be associated with HLA-I A2 in these organelles.

We also studied the interactions of a CD4-eYFP on the plasma membrane with *Nef*-cerulean. We found that most of the *Nef* present in the plasma membrane binds CD4. Two diffusion components for CD4 were observed, with most of the binding between CD4 and *Nef* occurring in the more mobile fraction. Most of the *Nef* at the plasma membrane was also found to bind CD4 LL/AA mutant, which is not downregulated by *Nef* efficiently and thus was thought to bind *Nef* less avidly. We also demonstrate that removal of cholesterol by β-methyl cyclodextrin abolished the larger complexes.

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Raster Image Correlation Spectroscopy (RICS) with One Photon Excitation and Analog Detection: Some Practical Considerations for GUVs and Cell Membranes

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Raster Image Correlation Spectroscopy (RICS) allows for mapping the local translational diffusion coefficient(s). The applicability of the technique has recently been extended by implementation on confocal laser scanning microscopes (CLSM) having one-photon laser excitation and analog detection [1-4]. To better understand the reproducibility and accuracy of RICS analysis of cellular membranes and the top membranes of Giant Unilamellar Vesicles (GUVs) [4], the influence and the constraints imposed by instrumentation characteristics and by sample properties on the retrieved diffusion values have been simulated. Similarly to Brown et al [1], particle numbers in the observation volume and analysis brick size were varied. In addition the workable scan speed range was explored as well as the amount of allowed detection noise. We present evidence for a drop in D values when mapping towards the GUV perimeter and corroborate results when both the total number of particles and the mapping brick size get small. Experimental results show that the magnitude of correlated detection noise in our Zeiss LSM 510 META confocal system (build 2002) along the fast x-scan axis ($\psi = 0$) is considerably larger than reported by others [1-3]. The effect of omitting the $\psi = 0$ line in the analysis was investigated. Both the LFD, UCI RICS package as well as our Matlab software based on routines supplied by D. Kolin (McGill University) gave similar simulation and translational diffusion mapping results.

[1] Brown 2008, J. Microsc. *229*, 78-91.
[2] Dalal 2008, Microscopy Research and Technique *71*, 69-81.
[3] Sanabria 2008, Biophys J doi:10.1529/biophysj.108.138974.
[4] Gielen 2008, J. Fluoresc. *18*, 813-819.

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Effects of Oversampling and Scanning Artifacts on the Accuracy of Spatial Fluorescence Intensity Fluctuation Analysis Methods

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We present a systematic simulation and experimental study of potential measurement artifacts that arise due to raster scan acquisition for the image fluctuation analysis methods image correlation spectroscopy (ICS) and spatial intensity distribution analysis (SpIDA). With computer simulations, we show that photobleaching occurring during the sampling of sequential pixels affects the density and quantal brightness measurements obtained from single laser scanning microscopy images. This effect is even more pronounced when the images are oversampled with by using a small pixel size. The magnitude of this artifact was a function of the bleaching coefficients. The simulation results were compared to actual experimental data collected using a confocal laser-scanning microscope for a variety of dyes and sampling conditions. We analyzed images of monomeric fluorescent dyes covalently bound to coverslips, and monomeric green fluorescent protein (EGFP) transfected in mammalian cells. Moreover, we investigated the effects of non-constant emission of fluorescent probes, the presence of background noise and shot noise on the accuracy of the image fluctuation methods. We found that as the laser power increases (below saturation), some asymmetry in the spatial autocorrelation function appears which leads to systematic errors in the ICS measured number densities. SpIDA is also affected in the same manner by this artifact. Finally we looked at the accuracy of both techniques as a function of the quantal brightness of the particles. We generated computer simulated images of point emitters with different quantal brightness and then added shot noise to the images to determine accuracy and precision ranges for the measurements.

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Biocompatible Quantum Dots for Intravital Kidney Imaging

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Quantum dots (QDs) are emerging alternatives to traditional dyes for fluorescence imaging applications. Quantum dots are attractive due to their photostability, broad excitation, narrow emission bands, high quantum yields and relatively long fluorescence lifetimes for contrast enhancement. We are interested in applying quantum dots (CdSe/ZnS) with biocompatible coatings for functional imaging of the kidney in vivo. Towards that end, we carried out experiments to characterize the bio- and photo-physical properties of CdSe/ZnS quantum dots with lipopolymer and aminoethoxy ethanol (AEE) coatings, and in vivo imaging of the kidney using surface coated quantum dots. We found that the fluorescence of the CdSe/ZnS quantum dots with surface coatings is enhanced in the presence of blood serum and it is serum concentration dependent. In addition, these surface coated quantum dots exhibit photo-induced fluorescence enhancement (PFE) and the PFE varies with specific surface coatings. Previously, PFE phenomenon is observed in CdSe quantum dots without surface coatings and has been studied under a number of different experimental conditions. To our knowledge, PFE of quantum dots with a surface coating has not been reported in the literature. For imaging application, we have