fraction for such *adapted cells* varies from phi = 0.16 at 0.10 Osm to phi = 0.36 at 1.45 Osm. For cells grown at 0.28 Osm, a similar range of is obtained by *plasmolysis* (sudden osmotic upshift) using NaCl as the external osmolyte, after which the cellular response is passive loss of cytoplasmic water. Using fluorescence recovery after photobleaching (FRAP), we measure the effective axial diffusion coefficient $D_{GFP}$ of green fluorescent protein in the cytoplasm of live *E. coli* cells as a function of for both plasmolyzed and adapted cells. For *adapted* cells the median diffusion coefficient $D_{GFP}{}^{m}$ decreases by only a factor of 2.1 as phi increases from 0.16 to 0.36. In sharp contrast, for *plasmolyzed* cells $D_{GFP}{}^{m}$ decreases by a factor of 70 as phi increases from 0.16 to 0.33. Clearly GFP diffusion is not determined by phi alone. By comparison with quantitative models, we show that the plasmolysis data cannot be explained by simple crowding theory in a homogeneous medium. We will also report on measurements of time-resolved fluorescence anisotropy of GFP in the cytoplasm, diffusion of RNA polymerase in the cytoplasm, and diffusion of GFP in the periplasm of *E. coli.* Time lapse measurements monitor the recovery of cell volume and GFP diffusion after plasmolysis, which may be a key determinant of the time scale of the recovery of growth.

**156-Pos Board B35**

**Measuring the Number of LuxR Proteins in a Single Cell of *V. harveyi***

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We have determined the number *N* of LuxR proteins in a single cell of the bacterium *V. harveyi* by measuring the distribution functions of cell volumes and protein-fluorescence intensities during cell division. In quorum sensing, the LuxR protein population, which regulates many (~70) genes, is sensitive to the concentration of auto-inducer molecules (AIs). We utilized a strain that is incapable of producing AIs. The LuxR proteins are tagged by a red fluorescent protein (mCherry). In the absence of AIs, the cells maintain a baseline residual concentration of LuxR that is remarkably constant over 8-10 cycles of cell division. We recorded the growth of a single cell into a large colony by imaging both phase contrast and mCherry intensity every 2 minutes in a 6-hour movie. The phase-contrast image was used to measure the volume of each cell, while the mCherry intensity monitored the LuxR population. At each cell-division event, we determined the fractional partitioning of the cell volume and the LuxR population. From the large number of cell-division events (~300), we obtained the normalized distributions of both the volumes and the mCherry intensities. Our procedure allows an accurate measurement of the width of the volume distribution ($\sigma = 0.031 \pm 0.003$). Significantly, the width of the LuxR distribution was observed to be much broader, presumably because of small-number fluctuations. By deconvoluting the Gaussian distributions, we find that the average LuxR copy number *N* equals $140 \pm 10$ just before cell division. Repeating the experiment at successively higher levels of applied AI concentration, we confirmed that when *N* is 10 times larger, the 2 distributions converge to the same width σ. This technique may be applied quite generally to other systems.

**157-Pos Board B36**

**Influence Of P-selectin Structure On Its Mobility In The Weibel-Palade Body And Plasma Membranes**

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The leukocyte adhesion molecule P-selectin is stored in Weibel-Palade bodies (WPBs), a secretory organelle of endothelial cells. The extracellular domain of P-Selectin comprises 9 consensus repeats (CRs), an EGF domain (E) and a Lectin domain (L) at the N-terminus, forming a rod-like structure approximately 48nm in length. Although truncation of extracellular CRs of P-Selectin impairs leukocyte capture under flow conditions, how such modifications affect the mobility of P-Selectin in the WPB membrane and in the plasma membrane (PM) after exocytosis is not known. Using single WPB FRAP or TIRFM with single fluorophore (SF) detection and tracking the diffusion of P-Selectin-EGFP and N- and C-terminal truncations of P-Selectin-EGFP was investigated in WPB or PM during ionomycin (1 μM) -evoked WPB exocytosis at 37°C.

P-Selectin-EGFP was immobile in the WPB membrane, but its N-terminal truncations rendered it mobile. On exocytosis SFs of P-Selectin-EGFP and its mutations were found to diffuse approximately freely in the PM in the vicinity of WPB fusion sites. The diffusion coefficient ***D*** for P-Selectin-EGFP was 0.14 $\mu m^2/s$, (n=2890 SF). Deletion of 8 of the 9 CRs increased ***D*** to 0.18 $\mu m^2/s$, (n=3907 SF). Removal of the L domain alone increased ***D*** to 0.24 $\mu m^2/s$, (n=1716 SF). Deleting both L and 8CRs increased ***D*** to 0.29 $\mu m^2/s$ (n=1818 SF). Removing E had no effect. C-terminal truncation also altered ***D***. The structure of P-Selectin influences its mobility in the WPB and PM.

**158-Pos Board B37**

**Quantitative Analysis of Spatial Protein-protein Proximity in Fluorescence Confocal Microscopy**

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Colocalization between fluorescently-labeled proteins has turned to be a measure of protein-protein interactions and a tool in cell biology. However, its evaluation has inherent caveats. The popular overlay method is qualitative and greatly depends on user setting for threshold values. Quantitative methods are also available, but the results can be unreliable because of the questionable assumption that proteins are uniformly distributed, and of the failure to minimize the influence of nonspecific labeling and random fluorescence noise. In order to quantify colocalization in a more absolute manner, we extended the use of image cross-correlation spectroscopy (ICCS) 1 to minimize the effect of protein distribution, non-specific labeling and random noise. The numerical procedure to separate the fluorescent components is based on the fact that the crosscorrelation and autocorrelation image values as function of x,y pixel shift have a peak at zero pixel shift decaying with sharp and shallow components as a function of x and y pixel shift. The sharp component corresponds to the co-localized proteins while the shallower one corresponds to non-specific labeling. By fitting the sharp and shallow landscapes of the crosscorrelation and autocorrelation functions to the sum of two Gaussian distributions, one can extract the peak amplitude of the specific sharp components to calculate the protein proximity index (PPI) from the ratio between the crosscorrelation and autocorrelation values at x,y=0 pixel shift. In summary, our method extracts the colocalization value from background generating consistent results from both computer simulated images and biological confocal images. Thus, it is a powerful microscopy tool to determine the nature of macromolecular complexes and their dynamic changes in biological processes.

1. Comeau JW, Costantino S, Wiseman PW. A guide to accurate fluorescence microscopy colocalization measurements. Biophys J. 2006;91:4611-22.

**159-Pos Board B38**

**The White Confocal - Controlling Spectral Fluorescence**

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Fluorescence has evolved to the most important tool in modern biological research. Specific histological stainings, antibody-based protein-markers and DNA-hybridization were the classical targets. Fluorescent proteins and other advanced stainings allow tracing molecules and structures in living samples - both by classical imaging as well as by modern analytical approaches; e.g. fluorescence correlation and its derivatives.

A very beneficial phenomenon in fluorescence is the fact that it comes in an infinite number of colors - which is at the same moment the most challenging feature. Multiple colors are available simultaneously - but at the price of a very elaborate illumination scheme, tricky beam splitting and efficient but selective detection of the various colors which are used to stain different structural elements in the sample.

All three modules, that are required for incident light fluorescence measurement instruments, are now spectrally tunable: white laser light sources, programmable acousto optical beam splitters and tunable multi-band emission detectors. These tunable elements allow for any spectral combination both on the excitation and the emission side. Reduction of crosstalk, more specificity and new measurements like excitation-emission correlation are some of the benefits of these developments. And as a side-effect: the acousto-optical devices transmit much better as compared to commonly used filters and dichroics.

Here, new approaches to use spectral information of both emission and excitation are presented and examples are given.

**160-Pos Board B39**

**Identifying Components Of Astroglial Autofluorescence Using The Spectral Separability Index, Xijk**

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In in multi-color fluorescence, endogenous fluorophores have been considered more of a nuisance than a signal. Many of them co-exist (e.g., mitochondrial NADH and flavins) or, as ceroids and lipofuscins, have intrinsically broad fluorescence excitation and emission spectra. Thus, the presence of autofluorescence, along with cross-excitation and fluorescence bleed-through of one color channel into the neighboring one, bring up the question to which extent different color channels contain truly independent fluorophore information.