of protein oligomers in living cells (Raicu et al., Nature Photonics, **3**, 2009). In an ongoing research project in our lab, we are are probing the movement of protomers in an oligomer by appropriately tagging and systematically altering a fluorophore position within each protomer. In the work described here, we show the application of this method in yeast cells (*S. cerevisiae*) that express the sterile 2 alpha factor protein (Ste2p, a G protein-coupled receptor) tagged with two different variants of the green fluorescent protein (GFP). Previously, we showed by tagging GFP2/YPF (donor/acceptor fluorophores) variants at the C-terminus of Ste2p that Ste2p is self-assembled into a rhombus shaped tetramer. The measured FRET efficiencies of the Ste2p oligomers, which were tagged with fluorophores at various locations in the Ste2p amino acid sequence, were calibrated against FRET reference standards in order to extract information regarding the relative orientations of the fluorescent tags. The effect of an agonist, the yeast mating pheromone alpha factor, on the measured FRET efficiencies was quantified and compared for the various fluorescent tag locations to reveal information regarding the relative movement of the Ste2p protomer segments upon binding of the agonist.

761-Pos Board B561
Two-Photon Imaging in Turbid Media to a Few MM Depths
Viera Crosignani, Alexander Dvornikov, Enrico Gratton.
We present a new detection method for two-photon fluorescence imaging of turbid media that extends imaging depth to few mm. Compared to conventional two-photon microscopy, the imaging depth enhancement is achieved by use of a more efficient detection of fluorescence, propagating in multiple-scattering media. The imaging depth in turbid media depends on two major factors: the ability of the imaging system to deliver necessary excitation light power to a certain depth to induce two-photon fluorescence and the ability of the detection system to collect and detect fluorescence photons scattered by the media. In two-photon imaging, usually, fluorescence is collected by the same microscope objective that is used for excitation. This optical scheme has its principal limitation in efficiency of fluorescence detection because of the narrow angle and area from which fluorescence photons can be collected. As a result, the maximum depth of tissue imaging that was reached so far by two-photon fluorescence microscopy is about 1mm. Our system utilizes for excitation the conventional two-photon microscopy scheme, however, for fluorescence detection we used 1'' cathode area PMT directly placed on the turbid sample from the opposite side of excitation. The PMT was equipped with a mechanical shutter and optical filters to reject excitation light and transmit only fluorescence. All optical components of the detector were coupled with index matching compounds to assure refractive index continuity from the sample to the PMT photocathode to minimize light losses due to reflection at boundaries. This detection scheme, while simple in construction and practically not requiring any optics, was proved to be very efficient in the collection of weak fluorescence from a wide area of a turbid sample and allowed to obtain high resolution images at depths up to 3mm.

762-Pos Board B562
High Resolution Microscopy in Live-Cell Imaging
Gerd U. Nienhaus, Per N. Hedde, René Dörlich, Jochen Fuchs, Susan Gayda, Franz Oswald, Joerg Wiedenmann.
We have used PALM (photoactivation localization microscopy) imaging to study the formation and disassembly of focal adhesions of live HeLa cells in a high resolution pulse chase experiment using a monomeric variant of the photoactivatable protein IrisFP [1]. mIrisFP is a photoactivatable fluorescent protein that combines irreversible photoconversion from a green- to a red-emitting form with reversible photoswitching between a fluorescent and a nonfluorescent state in both forms [2]. A subpopulation of mIrisFP molecules is photoconverted to the red form by irradiating a specific region of the cell with a pulse of violet light. Migration of the tagged proteins out of the conversion region can be studied by subsequently localizing the proteins in other regions of the cell by PALM imaging, exploiting the photoswitching capability of the red species. Online image analysis was performed by using our recently software [3].
[1] Adam et al., Proc. Natl. Acad. Sci. USA. *105* (2008) 18343.
[2] Fuchs et al., Nat. Methods *7* (2010) 627.
[3] Hedde et al., Nat. Methods *6* (2009) 689.

763-Pos Board B563
Quantification of Fluorescence Signals in Purified Mitochondria
Yong Wu, Harpreet Singh, Rong Lu, Jean Chrisostome Bopassa, Ligia Toro, Enrico Stefani.
Studies of localization and transient association of proteins with mitochondria are limited by the lack of appropriate purification, high resolution imaging and quantification techniques. Here, we investigated the localization of mitochondrial proteins and quantified them in isolated mitochondria from murine heart. Mitochondria were rapidly isolated and purified using a Percoll gradient. The degree of purity of three fractions (M1-M3) was verified by Western blots using markers of different cellular components along with mitochondrial markers. M3 fraction was the purest with negligible amounts of contaminating proteins from the plasma membrane (0.2%), nucleus (6.5%), Golgi complex (1.8%), and endoplasmic reticulum (2.2%) as compared to the whole cell lysate fractions, but was enriched with mitochondrial markers VDAC1 (126%) and COX4 (139%). Thus, M3 fraction was used for sequential labeling with Mitotracker (100 μM) and specific antibodies for mitochondrial proteins. Images were obtained using confocal microscopy. To extract signal information of distinct mitochondria, we utilized the Statistical Region Merging (SRM) and Robust Automatic Threshold Selection (RATS) algorithms to minimize the human bias introduced by the commonly used empiric thresholding. The criteria to positively identify isolated mitochondria were: Mitotracker labeling, average intensity and size. Once mitochondria were selected, the degree of protein co-labeling was quantified. As a proof of concept, we show that VDAC1 and COX4 highly colocalize with Mitotracker labeled mitochondria but not alpha 1C Ca channel. These techniques pave the way to further study mitochondrial proteins and their temporal association with non-mitochondria proteins. Supported by NIH.

764-Pos Board B564
Analysis of Spot Detection and Localization Algorithms for PALM and STORM
Avtar Singh, Paul Lee, Sarah A. Shelby, Rebecca M. Williams, Warren R. Zipfel.
Until recently, one of the major pitfalls of using optical microscopy to study cell biology has been the gap between the instrument resolution, constrained by diffraction to λ/NA (~250 nm), and the size of biological macromolecules, which are roughly two orders of magnitude smaller. With the advent of super-resolution microscopy, optical techniques can now be used to probe structural content that was previously only accessible to electron microscopy with the added benefit of being able to image live specimen. In particular, localization-based methods such as PALM and STORM have been readily adopted by many labs due to their ease of implementation, requiring not much more than a widefield or TIRF system and relatively simple image analysis software. Here, we compare a number of algorithms for image filtering, spot detection and PSF localization, investigating their Type I and II error rates, speed, precision and bias. Computational routines are written in C/C++ and tested on synthetic images and real data recorded from an EMCCD. In addition, we explore the viability of using a solid-state ''light engine'' (Lumencor, Inc.) for both activation and readout of photo-activatable probes; this novel illumination source allows for computer-controlled millisecond switching and attenuation of up to seven high-power (> 100 mW) spectral bands.

765-Pos Board B565
Single Cell Refractive Index Measurements for Optical Biomechanics
Robert P. Thomen, Semere M. Woldemariam, Michael G. Nichols.
Accurate measurements of the optical properties of living cells are essential for both therapeutic and diagnostic applications of biomedical optics. Changes in the index of refraction of red blood cells, for example, have been used to chart the progress of the malaria parasite (1). The cellular index of refraction is also of critical importance in cellular biomechanics measurements. In the optical stretcher, a dual-beam optical trap, small variations in the index of refraction can lead to substantial errors in the assessment of single-cell elasticity. To determine the cell-to-cell variation of the index of refraction, we have integrated a Mach-Zehnder interferometer with a conventional transmitted light microscope to obtain high-resolution interferograms of individual cells. Quantitative phase images are obtained by performing a Hilbert transform and using Goldstein's algorithm to unwrap the wrapped phase image. The decoupling technique of Rappaz et al. was used to obtain cell height and index of refraction maps (2). By employing several laser sources, the cellular index of refraction can be measured as function of wavelength. Measurements of multiple cell lines at several wavelengths indicate that the refractive index can, in fact, vary significantly from cell-to-cell. Along with these results we will discuss the implications for accurate single-cell biomechanical measurements made with the optical stretcher.
1. Park et al. Proc. Natl. Acad. Sci. USA *105*, 1730 (2008)
2. Rappaz et al. Opt. Express *13*, 9361 (2005)
Supported by NIH P20 RR16469 from the National Center for Research Resources (NCRR) and NIH R15 GM085776