cells on the molecular level is poorly understood. The vitrification model posits that the role of sugars are merely to inhibit ice formation altogether. The water replacement model predicts sugars preferentially bind to the surfaces of protein forming a protective layer against the denaturing ice formation. Under this paradigm, one would expect the hydrodynamic radius of diffusing protein to increase with increasing sugar concentration. In order to test this hypothesis, we have developed both one-photon and two-photon fluorescence correlation spectroscopy (FCS) to measure the hydrodynamic radius of fluorescent particles in glucose solutions. The two-photon technique was developed to investigate tryptophan-containing proteins. However, to date, we have only succeeded in measuring the diffusion of reasonably large, Avidin-coated polystyrene spheres that possess sufficient fluorescence to be reliably detected. Work is continuing on using this technique to study the diffusion of the protein hemocyanin. Given these difficulties with the limited brightness of tryptophan, the one-photon FCS setup is being used to investigate green fluorescent protein (GFP), a much smaller, but considerably brighter, fluorophore.
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1778-Pos Board B670
Isotropic Resolution in Localization-Based Super-Resolution Microscopy by Single Objective Emission Interference
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The three-dimensional localization of single molecules is a key feature for localization based super-resolution microscopy. Simple methods for molecule localization along the optical axis typically provide a precision that is about 2.5 times worse than lateral. Dual objective microscopes with 4Pi emission detection can improve the axial localization precision to match the lateral. However, 4Pi detection requires complicated instrumentation, intricate maintenance and expensive parts. Therefore, dual objective 4Pi detection is not suitable for everyday biological use. We demonstrate that 4Pi detection for localization based super-resolution microscopy can be realized by using only one objective and a mirror, which eliminates the drawbacks of dual objective 4Pi detection, but still provides isotropic localization precision.

1779-Pos Board B671
FLIM Phasor Analysis for Time-Domain and Frequency-Domain Data
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The phasor analysis of FLIM images provides a fit free global view of molecular species and their interaction in cells and tissues. Different techniques are used to collect the original data either in the time domain or in the frequency domain. The ''phasor transformation'' which is based on the calculation of Fourier components should in principle make the phasor plot independent of the domain of data collection. However, technical differences between the modalities of data acquisition in various instruments result in slightly different phasor calculations. In this poster we discuss the origin of the variations between the different methods of data acquisition. In particular we compare data obtained with the classical analog frequency domain instrument, data obtained with the FLIMbox principle that is based on a digital equivalent of the frequency domain instrument and data obtained with the popular time-correlated single photon counting instrument. We discuss how to minimize these differences which could results in phasors plots that can be directly compared form data obtained with different instruments. We also discuss and compare methods of data filtering which can decrease the noise in the phasor plot without affecting the resolution of FLIM images. Finally we compare phasor plots obtained for different harmonics of the laser repetition frequency. We show that the phasor plot at high harmonics from autofluorescence tissue samples can distinguish between various extracellular components such as the weak fluorescence from collagen and elastin. Work supported in part by NIH-P41 P41-RRO3155, 8P41GM103540 and P50-GM076516

1780-Pos Board B672
Measurements of Fluorescence Decay Times by the Digital Parallel Frequency-Domain Method (FLIMbox)
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The digital parallel frequency domain design is a powerful approach that offers the possibility to implement a variety of different applications in fluorescence spectroscopy and microscopy. It allows lifetime measurements in a cuvette, fluorescence lifetime imaging and FCS as well as multi frequency and multi wavelength tissue imaging in small portable medical devices. It dramatically reduces the acquisition time from the several minutes scale to the seconds scale, and performs every signal process in a digital fashion, avoiding any RF emission and it is extremely inexpensive. This development is the result of a systematic study carried on the previous design known as the FLIMbox. The extensive work done in maximizing the performance of the original FLIMbox led us to develop a new hardware solution with exciting and promising results and potentials that were not possible in the previous hardware realizations. The new design permits acquisition of the full harmonic content of the sample response when it is excited with a pulsed light source in one single measurement using the digital mixing principle that was developed in the original FLIMbox. Furthermore, it is very stable, has very low power requirements and higher precision, and allows the multi exponential analysis. The FLIMbox can be synchronized with lasers that are intrinsically modulated or can generate a frequency to amplitude modulate a laser diode or LED. It provides up to four input channels, it has a saturation feedback control to avoid any time information loss, and it is only limited by the number of photons collected rather than by the sampling window implementation scheme. Work supported by NIGMS, 8P41GM103540.

1781-Pos Board B673
Dual Channel Detection of Ultra Low Concentration of Bacteria in Real Time and via Scanning Fcs
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The rapid quantification and identification of infectious disease agents is of primary importance for medical diagnosis, public health, food safety and environment monitoring. Here we describe an alternative, simple and rapid method to detect very low concentrations of bacteria in water. Our device consists of a small confocal microscope with a horizontal geometry with large pinhole and a holder for cylindrical cuvettes. Two motors provide a rotational and slower vertical inversion motion of the cuvette, so as to scan a total volume of 1ml/min. The device looks like a simplified flow cytometer without flow. Bacteria are stained by two nucleic acid dyes that fluoresce green and red and excited with two lasers. When a bacterium passes through the observation volume emits both red and green fluorescence. The light emitted from the sample is directed by a system of lens toward a dichroic beam splitter and then separated into two light paths for red and green fluorescence detection respectively. Data are analyzed with a correlation filter program based on particle passage pattern recognition. The passage of a particle trough the illumination volume is mimicked with a Gaussian pattern in both channels. The width of the Gaussian correlates with the time of passage of the particle. When the program finds a match with a Gaussian in both channels one particle is counted. The concentration of particles in the sample is deduced from the total number of coincident hits and the total volume scanned. This portable setup provides higher sensitivity (up to few bacteria per ml), rapid results, low cost and a wide use ranging from clinical applications to pollution monitors and water and air quality control.
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1782-Pos Board B674
Measurement of Membrane GPI-GFP Confinement and Dynamics by Image Correlation Spectroscopy
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Current models of the cell membrane assume a heterogenous environment containing domains. Sphinomyelin and cholesterol enriched nano- and micro- domains form a subcategory of membrane heterogeneities, which are thought to functionally sequester proteins. Membrane proteins may also be sequestered by the meshwork membrane proximal actin cytoskeleton. Here we show that image correlation spectroscopy (ICS) based techniques, applied to standard TIRF fluorescence microscopy image series, can be used to characterize these domains. To validate ICS measurements for such systems, we simulated a confined tracer particle diffusion in meshwork and microdomains where we varied the domain size, domain density, confinement probability and diffusion coefficients over ranges reported for cells. We show how one can extract from the correlation function data the characteristic parameters of the system such as apparent domain sizes and characteristic diffusion coefficients. We establish the limits due to the spatio-temporal sampling and noise. Using the simulations, we established the minimum number of domains that need to be sampled by tracer particles for the emergence of confined dynamics features in the correlation function. Finally, we applied this analysis to the study of dynamics of raft associated GPI-GFP in COS-7 cell membranes and verified that our tools can detect changes in the confinement parameters following the application of drugs that disrupt rafts. The recovered large spatial scale diffusion coefficient increased from 0.06 to 0.1 um^2/s when cells were exposed to cholesterol