1013-Pos Board B799
The Effects of Zero-Length and Non-Zero Length Cross-Linking Reagents on the Optical Spectral Properties and Structures of Collagen Hydrogels
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We compared the effects of zero-length cross-linkers 1-Ethyl-3 (3dimethylaminopropyl) carbodiimide (EDC) and non zero-length cross-linkers glycolaldehyde and glyceraldehyde on the optical and structural properties of three dimensional (3D) collagen hydrogels. We evaluated these effects by multiphoton microscopy (MPM) that combined two-photon fluorescence (TPF) and second harmonic generation (SHG) contrasts and transmission electron microscopy (TEM). The collagen hydrogels were incubated separately with the above reagents present at the concentration of 0.1 M. The incubation with glycolaldehyde and glyceraldehyde induced strong auto-fluorescence within the gels. We followed the formation of fluorescence with TFP signals in situ and in real time as well as characterized the micro- and nanostructures within cross-linked hydrogels by examining SHG and TEM images respectively. As detected in the SHG images, glycolaldehyde and glyceraldehyde modified 5 - 10 µm 'fiber-like' collagen structures to longer, 20 µm and more, aggregated strands while EDC had minimal effect on the microstructure. TEM revealed that glycolaldehyde and glyceraldehyde either completely eliminated collagen's characteristic native fibrillar striations or generated uncharacteristic fibrils with extensions. EDC preserved the native striation patterns, decreased the fibril diameters and effectively homogenized the fibrils within hydrogels assembled at 1.8 g/l to 4.68 g/l collagen concentrations and 37 °C. Our findings provide a clear understanding on how different cross-linking reagents have very different effects on the collagen hydrogels. This understanding is critical for advancing tissue engineering and wound healing applications.

1014-Pos Board B800
Phasor Analysis with a New Widefield Photon-Counting Flim Detector
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Fluorescence lifetime imaging microscopy (FLIM) is a powerful technique for distinguishing molecular species, studying molecular interactions or assembly, and observing Förster resonance energy transfer (FRET). We present a new widefield photon-counting detector which uses the principles of phasor analysis to achieve FLIM with access to dynamics across a broad range of timescales. In previous widefield photon-counting FLIM detectors, low quantum efficiency has limited sensitivity, and global count rate limitations have constrained frame rates. Our detector, called the H33D Gen II, achieves a quantum efficiency of 15% across the visible spectrum, and achieves global count rates of several MHz. Photons are collected across the entire field of view in a widefield manner, and assigned a position in the image using a cross-strip anode. Each photon is also timed with sub-nanosecond precision using analog-to-digital converters (ADCs) and a field-programmable gate array (FPGA), which removes the dead time present in Gen I from the time-to-digital converter (TDC). This stream of photon counts is then binned into frames by software to achieve very fast frame rates, limited only by the number of photons collected and the desired statistics. We use phasor analysis with custom software to process the lifetime information, resulting in very fast generation of FLIM movies. We demonstrate the range of capabilities of this new detector and the applicability of this approach to FLIM for single particle and cellular imaging. This system provides high-precision access to nanosecond scale lifetimes, while also being capable of observing a broad timescale of dynamics (millisecond scale and higher) simultaneously across an image.

1015-Pos Board B801
Measurements of Cell Mass Distribution during Cell Division with Quantitative Phase Microscopy
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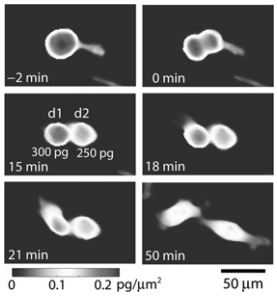
Despite much attention to the regulation of genetic material partitioning during cell division, relatively little is known about the partitioning of cell mass, an essential outcome of successful cytokinesis. Recent work suggests that mispartitioning of cellular contents during division may constitute a form of epigenetic memory, however, conventional techniques cannot accurately quantify daughter cell masses. Quantitative phase microscopy, in which the phase shift of light as it passes through and interacts with matter inside a cell is measured, in combination with computer vision techniques, provides a new approach for directly measuring the masses of hundreds of paired daughter cells and tracking the resultant cell fates. We will show that, across several cell types, approximately one in ten cell divisions results in a highly asymmetric partitioning of mass. Additionally, we have found that specific disruption of actomyosin activity using small molecule inhibitors leads to a marked increase in the percentage of highly-asymmetric cell divisions. This suggests that sub-cytotoxic concentrations of chemotherapeutics may lead to dramatic variation in cancer cell population mass distributions and potential epigenetic effects on cell function and disease progression.



1016-Pos Board B802
Rapid, Accurate Single Particle Tracking Based on Radial Symmetry Center Determination
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Accurately tracking particles in images is a crucial task in areas as diverse as super-resolution microscopy, membrane biophysics, and soft-matter microrheology. Tracking errors can easily propagate into flawed conclusions about mechanisms underlying particle dynamics. The commonly used method of locating the center of a particle by direct fitting of a two-dimensional Gaussian function to a measured intensity profile is very accurate, but is computationally intensive and not generalizable to non-point-like particles. Its slowness is a necessary consequence of iteratively, numerically searching through a large fitting parameter space. I introduce a new approach to sub-pixel particle tracking based on exploiting the radial symmetry of particle images, valid for any radially symmetric intensity profile. I provide an algorithm that employs an analytic, non-iterative calculation of the best-fit symmetry center to determine the particle location. Over a wide range of signal-to-noise ratios, this approach yields tracking accuracies nearly identical to those of Gaussian fitting with execution times over two orders of magnitude faster and with greater robustness in the presence of nearby particles. The performance of this algorithm is tested on simulated images as well as experimental data from several fields.

1017-Pos Board B803
Novel Genetically Encoded Probes for Observing Molecular Processes in Living Cells by Two-Photon Polarization Microscopy
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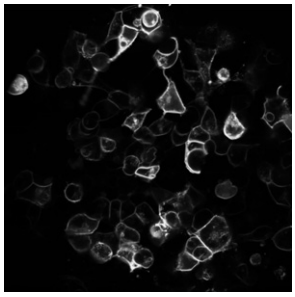
Two-photon polarization microscopy (2PPM) can be used for imaging a wide range of dynamic molecular events in living cells and tissues. The technique allows sensitive monitoring of protein-protein interactions and of conformational changes, in a variety of proteins. 2PPM is sensitive enough to allow imaging of even submillisecond events. Due to 2PPM's requirement for only a single fluorescent protein tag, many existing fluorescently tagged constructs can act as probes of protein function. Here we present our results in using 2PPM for monitoring G-protein activation and calcium imaging, and a first generation of genetically encoded probes developed specifically for 2PPM.



Micro & Nanotechnology: Nanopores I

1018-Pos Board B804
Voltage Gating of Abiotic Nanopores with Electromechanical DNA Gates
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The ability to control liquid flux across porous membranes is important in separation, purification, and sensing. Here we describe a way to open and close a nanoscale pore by a voltage and pH. We produce track-etched pores in polyethylene terephthalate (PET) and chemically etch them into a conical shape. This is followed by a pore modification where we attach 30 mer single-stranded DNA to the pore walls. This process is applied to pores that have a narrow opening diameter between 3nm and 20nm. The DNA modification is restricted to the region next to the small opening of a pore. We find that the