objective glass is significantly reduced, and that the technique is useful for imaging biological samples. Supported by NIH NIAMS R01AR049277.

919-Pos

Microscope Objective Based Surface Plasmon Resonance Imaging of Cell-Substrate Contacts

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Assembly of a high numerical objective platform for surface plasmon resonance imaging (SPRI) was used to investigate a high resolution diffraction limited refractive index image of cell-substrate contacts. SPRI is a highly sensitive biochemical surface sensor measurement technique that has only recently been applied to the field of cell-biology. The nature of the refractive index measurement made at the cell-surface interface is investigated. We present SPRI measurements of several different cell types as well as multimode image comparisons with fluorescence and transmission mode microscopies to scrutinize the signal composition of the SPRI refractive index signal. While the primary correlation of the SPRI signal appears to be the cellular membrane distance to substrate, depending on the cell type, cell focal contacts, and cellular organelles can also contribute to subcellular refractive index changes at the cell-substrate interface.

920-Pos

A Comparison of Objective Lenses for Multiphoton Microscopy: Improved Epifluorescence Collection from Turbid Samples

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Over the past two decades, multiphoton microscopy (MPM) has launched a revolution in high-resolution *in vivo* imaging with its combination of molecular contrast, optical sectioning and depth penetration. However, as with other optical microscopies, the imaging depth of MPM is ultimately limited by the scattering of both excitation light and emitted fluorescence. Since multiphoton excitation is largely confined to a diffraction-limited focal volume, MPM can theoretically utilize any fluorescence as signal and most collection schemes seek to collect as many photons as possible. The collected fraction depends on the choice of objective lens and the design of the post-objective light collection path, as well as the optical properties of the specimen. In particular, this fraction decreases significantly in turbid samples. Recently, there has been a concerted effort by lens manufacturers to develop objective lenses that are specially tailored for MPM and aim to collect as much scattered light as possible. In order to assess the advancements made by this ''second generation'' of MP objectives, we directly measure the collected fraction from a sample consisting of a fluorescein layer below a solution of scattering microspheres. Trans- and epi-fluorescence are collected in separate channels to give a relative measure of the collected fraction and the dependence on sample scattering is compared for five different lenses. We also observe that the angular divergence of the light cone exiting the objective back increases with sample scattering- a practical detail that has implications on design of collection pathways.

921-Pos

Pixel Multiplexing for Simultaneous High Resolution High Speed Image Capture

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We introduce a imaging modality that works by transiently masking image-subregions during a single exposure of a CCD frame. By offsetting subregion exposure time, temporal information is embedded within each stored frame, allowing simultaneous acquisition of a full high spatial resolution image and a high-speed image sequence without increasing bandwidth. The technique is demonstrated by imaging calcium transients in heart cells at 250 Hz with a 10 Hz megapixel camera.

922-Pos

A Multifocal Two-Photon Microscopy Setup for Parallel 3D Tracking of Gold Nanorods

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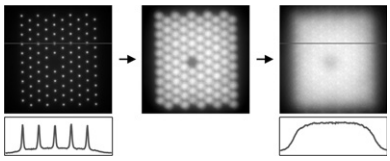
We have constructed a laser scanning two-photon fluorescence microscopy setup that combines advantages of multiphoton microscopy (confocal 3D resolution, low phototoxicity to cells and tissue, low background autofluorescence) with the speed and sensitivity of widefield (epi) detection. A hexagonal pattern of 100 confocal scanning beams is created using a diffraction optical element (DOE). We scan this array in a spiral fashion, such that each spot forms a 2D Gaussian in the focal plane that partly overlaps with neighboring spots. In this way, homogeneous illumination is achieved with minimal load on the scanning mirror (see figure).

By detecting fluorescence with an EMCCD camera, we can create images with milliseconds exposure times. This allows us to obtain 3D fluorescence images of cells within 2 seconds.



We use this setup to track the motion of multiple individual fluorescent nanoparticles. We compare the suitability of gold nanorods and quantum dots for two-photon 3D intracellular tracking. We show that the bright two-photon luminescence of gold nanorods allows video-rate tracking of single nanorods with ten nanometer accuracy. Furthermore, we explore possibilities of distinguishing closely spaced nanorods based on polarization-sensitive luminescence.

923-Pos

3D Confocal Microscope Image Enhancement by Richardson-Lucy Deconvolution Algorithm with Total Variation Regularization: Parameters Estimation

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Deconvolution is an efficient tool for enhancing both fluorescence and confocal microscopy images. Although in confocal microscopy the point spread function is rather small and images are much sharper compared to fluorescence microscopy, deconvolution can considerably improve image contrast and reduce noise.

Several deconvolution methods have been proposed for 3D microscopy. In this work, we used Richardson-Lucy (RL) iterative algorithm assuming Poisson noise. In the presence of noise, the RL algorithm does not always give a optimal solution. To reduce the effects of noise, the RL algorithm is combined with total variation regularization. As it has been shown before, total variation (TV) with a carefully chosen regularization parameter reduces intensity oscillations in homogeneous areas and helps enhance contrast on edges.

The aim of this work was to find good estimates to deconvolution algorithm parameters from the input to obtain optimal results. The test images generated from experimental data by smoothing out the noise but keeping typical structures. The analysis showed that optimal algorithm parameter Lambda is strongly correlating with the noise level. On the other hand finding the optimal parameter value is a very tedious process and so we derived formula estimating the value from the input image. The estimated Lambda turned out to give a more robust stopping criterion then the popular criterion using the threshold level for the relative change between two successive iteration steps.

We applied the deconvolution algorithm to study mitochondrial organization in rat cardiomyocytes. An open source software for deconvolving 3D images is available in http://sysbio.ioc.ee/software/.

924-Pos

A Generation-3 Programmable Array Microscope with Digital Micro-Mirror Device

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We present a new and improved implementation of a programmable array microscope (PAM) based on a digital micro-mirror device (DMD) spatial light modulator. The PAM provides optical sectioning in a wide-field microscope with greatly enhanced speed and sensitivity compared to conventional point-scanning confocal microscopes.[1-3] Furthermore, one obtains the ability to structure the spatial distribution and dose of illumination in the field of view according to the brightness levels observed in the sample. Such optimized light exposure (OLÉ) prolongs the available duration of imaging in time lapse experiments because of reduced photobleaching.[4]

Recent improvements in DMD reflectivity (up to 68%), format size (1080p), and speed (25 KHz binary frame rate) have enabled the design and development of a more light-efficient dual path PAM. The performance of this instrument will be described and compared to that of conventional point-scanning and spinning disk confocal microscopes.

[1] Q.S. Hanley et al., J. Microsc. **196**, 317-331 (1999); [2] R. Heintzmann et al., J. Microsc. **204**, 119-137 (2001) ; [3] Hagen et al., Micros. Res. Techniq., **72**, 431-440 (2009). [4] W. Caarls et al., manuscript in prepration