''line scan'' scanning scheme increases the contrast (signal to noise ratio) of the image by sqrt(N). It also improves the image resolution because it eliminates the mechanical backlash of the vertical scanning mirror when each frame is scanned multiple times in regular raster scanning schemes. Supported by NIH grant HL088640.

931-Pos

High Throughput High Sensitivity Depth Resolved Wide Field Microscopy

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3D optical microscopies including confocal microscopy, two-photon excitation microscopy, and coherent anti-Stokes Raman scattering microscopy have optical sectioning capability, but their image acquisition is relatively slow due to the sequential nature of raster scanning. Recently, scanningless nonlinear microscopy based on temporal focusing was introduced as an alternative to using the diffraction-limited spot. However, comparable optical sectioning has not been proved without optimizing the optical design and high-throughput capability has not been achieved due to the optical power limitation. In this presentation, high-throughput high-sensitivity depth-resolved wide-field two-photon microscopy is proposed. To quantify depth discrimination capability, a comprehensive mathematical model for depth-resolved wide-field illumination is derived and experimentally validated. By optimizing optical design parameters through numerical simulation, the best 3D resolution is shown to be close to diffraction limit. In addition, single particle detection sensitivity and high-throughput imaging capability are demonstrated by incorporating quantum dots, which are known to have high two-photon cross section, as a contrast agent into the proposed system. Finally, depth-resolved single particle tracking is evaluated to study the transport process in the cells with the developed microscopy, which confirms that this microscopy holds the potential in the fields of biology and medicine where both sensitivity and throughput are required.

932-Pos

Optimizing Multi-Photon Fluorescence Microscopy Light Collection from Living Tissue by Non-Contact Total Emission Detection (TEDII)

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A benefit of multiphoton fluorescence microscopy is the inherent optical sectioning that occurs during excitation at the diffraction-limited spot. The scanned collection of fluorescence emission is incoherent; i.e., no real image needs to be formed on the detector plane. The isotropic emission of fluorescence excited at the focal spot allows for new detection schemes that efficiently funnel all attainable photons to detector(s). We previously showed (JOM v.228, p.330-7, 2007) that parabolic mirrors and condensers could be combined to collect the totality of solid angle around the spot for tissue blocks, leading to ~8-fold signal gain. We now apply a version of this Total Emission Detection instrument modified to make non-contact images inside tissue *in vivo*. The device is mounted on a periscope (LSM Tech) to avoid touching tissue and is simpler, and in some cases more effective, than hybrid objective and fiber optic ring based systems for emission collection enhancement. Images of live brain and kidney show that the gain using this optical scheme varies as a function of imaging depth and the characteristics of the sample being imaged. Brain imaging (through a tiny region of thinned skull) of GFP labeled microglia showed up to a 1.8 fold increase in emission collection, while the gain in whole *ex vivo* brain samples showed up to a 2.5 fold increase (vs. light collected by a 20X water 0.95NA lens alone). Rat kidney imaging of blood vessels labeled with anneps (Invitrogen) *in vivo* showed up to a 2 fold enhancement in emission collection. These results show that multi-photon imaging using the TEDII device will permit scanning at twice the rate with the same SNR in these tissues or allow reduction of laser power by 60% to reduce photo-damage.

933-Pos

Sub-Diffraction Limited Wide Field Imaging and Microfabrication Based on Surface Plasmons

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Standing-wave surface plasmon resonance fluorescence (SW-SPRF) is a microscopy technique combining standing-wave total internal reflection fluorescence (SW-TIRF) microscopy and surface plasmon resonance (SPR) or surface plasmon-coupled emission (SPCE). Previous studies have shown that SW-TIRF technique can enhance lateral image resolution by more than twice utilizing standing evanescence waves. Further improvement may be generated using surface plasmons by reflecting light on the gold surface through the cover glass at a specific angle inducing collective excitation of electrons in the metal. In this study, we developed imaging and lithography method with less than 100 nm resolution by applying SW-SPRF microscopy with corrugated gold surface. We used corrugated gold surface to induce surface plasmon waves with larger wave number compared to uncorrugated one. This matching process requires proper optimization of parameters including grating constant, perturbation depth, incidence angle of the beam, and excitation wavelength. The fabrication of the corrugated gold surface was done by e-beam etching. For imaging, sub-diffraction size fluorescent particles were used to measure point spread function. For lithography, nano-patterns were produced by the exposure of interfering evanescent waves on azo dye (Congo-Red) thin films produced by spin-coating. The resultant patterns were measured with AFM. We gratefully acknowledge funding from the Singapore-MIT Alliance (SMA-2), the Singapore-MIT Alliance for Research and Technology (SMART), and the Samsung Scholarship.

934-Pos

High Resolution Wide Field Stimulated Raman Scattering Microscopy

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Fluorescent imaging modalities, such as STED, PALM and STORM, has demonstrated the feasibility of super-resolution imaging. However, no comparable super-resolution imaging has been achieved based on non-fluorescent contrast mechanisms. We present a novel super-resolution approach based on incorporating stimulated Raman scattering (SRS) contrast into a standing-wave (SW) total internal reflection microscope. SW-SRS microscopy has the potential to improve the lateral resolution of current SRS microscopy in total internal reflection geometry. There is a critical difficulty to implement SW-SRS microscopy. Stimulated Raman gain, SRG, is a weak modulation of the intensity of the Stokes beam. The ratio of the SRG to Stokes beam intensity is a function of pump beam instantaneous intensity. The need for wide field imaging further reduces pump beam flux resulting in very unfavorable SRG to Stokes beam intensity ratio. As an example, using a standard Ti-Sapphire laser exciting a 100×100 micron square region, SRG to Strokes beam intensity can be as low as 10^-9. This low signal to noise ratio is particularly challenging for wide field imaging that requires area detectors, such as CCD cameras, with limited dynamic range. To overcome these difficulties, we show that SRG to Stokes beam ratio can be improved to 10^-4 by utilizing mJ pulses using a regenerative amplifier, optimization of pulse durations and bandwidths, and destructive interference of the Strokes beam background. We gratefully acknowledge funding from the Singapore-MIT Alliance (SMA-2), the Singapore-MIT Alliance for Research and Technology (SMART), and the Samsung Scholarship.

935-Pos

Single Point FCS on a Commercial Confocal Laser Scanning Microscope with Analog Detectors

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Fluorescence Correlation Spectroscopy is a technique invented in the early 1970s to measure diffusion coefficient, chemical reaction rates and photo physical processes. It is a common belief that in order to obtain single point FCS data, one needs either a sophisticated FCS instrument with photon counting detectors or avalanche photon detectors or an instrument custom made for this type of experiments. Here we show that we can obtain single point FCS data on a commercial confocal laser scanning microscope without any modifications (Nikon C1). We successfully measured the diffusion coefficient and the concentration of Rhodamine B in solution for concentrations ranging from 5 nM to 280 nM. We also determined the diffusion coefficient of two different labeled lipid analogs (1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate and BODIPY TMR phosphatidylinositol (4,5) bisphosphate) incorporated in the membrane of giant unilamellar vesicles. The results obtained for these lipid analogs are in good agreement with previously published data. Finally, we highlighted the fact that the actual proportion of labeled lipid analogs incorporated in the membrane of the giant unilamellar vesicle (formed by the electroformation method) is significantly different than the proportion of these lipids in the organic solvent stock solution.

936-Pos

Computational and Statistical Limits to Palm, Storm, and Related Sub-Diffraction Fluorescence Microscopy Techniques

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Techniques such as PALM and STORM enable fluorescence microscopy with subwavelength resolution, using molecules that can be activated from a dark