One such method makes use of the stochastic fluorescence emission of individual molecules. Massive oversampling of the fluorescence emission of these particles allows the determination of their positions with high accuracy and, thus, the construction the image of a fluorescently labeled, biological sample with a resolution below the diffraction limit. This and related techniques, however, are limited to the imaging of fixed samples and require many minutes or hours to construct a single image. Another approach is making use of certain photophysical properties of fluorophores and a combination of illumination lasers to decrease the size of the excitation focus in confocal microscopy. Disadvantages of this method include the need for sophisticated laser equipment, very specific requirements for the fluorescent labels, and long times to obtain images. In general, live cell imaging at the timescales required to study the dynamics of intracellular processes is impractical with these newly developed super-resolution techniques.
Here, we present a drastically different approach to sub-diffraction-limited imaging that utilizes a propagating, nanoscopic beam of visible light with a diameter of a few 10s of nm. This phenomenon relies on the resonance of surface plasmons with the photons at the dielectric/metal interface. The width of the transmitted photon beam is independent of wavelength remains constant over length scales of 100s of nm.

3289-Pos Board B336
UV Ratiometric Imaging Of Isolated Ventricular Cardiomyocytes Using An LED Based Illuminator
Martyn Reynolds[1], Niall MacQuaide[2], Martin Thomas[1], Godfrey Smith[2].
[1]Cairn Research Ltd, Faversham, United Kingdom, [2]University of Glasgow, Glasgow, United Kingdom.
Ratiometric fluorescence microscopy methods allow researchers to obtain calibrated images of dynamic changes in the physical properties of cells and tissues independent of dye concentration.The wavelength changes required for ratiometric imaging are routinely achieved using a short-arc source in combination either with a diffraction grating or interference filters mounted in a filter wheel. Filter wheels typically switch positions in around 50ms, scanning monochromators can achieve wavelength changes within a ms. These approaches generally limit ratio imaging rates to a little over 10Hz, and have the inherent drawbacks of short lamp life and high thermal emissions. An LED based system has much higher stability than a short-arc source, and with sub-microsecond wavelength switching times allows the very highest speeds to be obtained. Here we made use of the popular Ca2+ probe Fura-2 to record images of intracellular [Ca2+] in isolated ventricular cardiomyocytes at frame rates in excess of 100Hz using a simple and inexpensive LED based system.
Transient spatial gradients of Ca2+ can exist in single ventricular cardiomyocytes during spontaneous release of Ca2+ from the sarcoplasmic reticulum. This can result intracellular Ca2+ waves traveling at 100-200microns/s along the length of the cell. Rapid imaging is required to resolve the time course and pattern of intracellular Ca2+ release.
Conventionally Fura-2 ratios are calculated by monitoring the fluorescence signal elicited from excitation at 340 and 380nm, however short wavelength LEDs (340nm) are not available currently. Alternatively, reliable ratiometric measurements can be made by exciting Fura-2 at its isobestic point (360nm) and 380nm. We have followed fast spatial changes in Ca2+ by switching wavelength in the microsecond time domain using commercial LEDs emitting at peak wavelengths of 365nm and 385nm.

3290-Pos Board B337
Theoretical Analysis of Nano-scale Imaging by Ion Conductance Microscopy
Samantha J. L. Lee[1], Eero J. Willman[1], David Klenerman[2], F. Anibal Fernández[1], Guy W. J. Moss[1].
[1]University College London, London, United Kingdom, [2]Cambridge University, Cambridge, United Kingdom.
Ion conductance microscopy (ICM) is a powerful new technique that allows non-contact, nano-scale imaging of the topography of living cells in physiological solutions (Hansma et al., *Science* 243:641-643, 1989; Korchev et al., *Biophys. J.* 73:653-658, 1997). ICM works by measuring the reduction of current that occurs when a nano-pipette probe, in a conducting solution, approaches a non-conducting surface, such as the cell membrane. In ICM a point is chosen in the x-y plane and the probe is lowered towards the surface. The z-position at which the probe current is reduced by a specified amount (determined by the current set-point) indicates the relative height of the sample at that location. Measuring such heights at each position as the sample is scanned in the x-y plane allows the cell topography to be determined. We have developed a finite element-based computational model that can simulate nano-scale ICM imaging for small (~100 nm) probes. This model allows us to make a systematic study of how objects of arbitrary size and geometry appear in images obtained using a variety of current set-points and/or probes of different shapes. The implications of these simulations for producing an optimized scanning routine and the feasibility of using data from simulations to correct the recorded image so that it represents a true topography are also examined.

3291-Pos Board B338
Enhancing Signal to Noise Ratio in linear and non-linear excitation microscopy
Emiliano Ronzitti[1,2], Valentina Caorsi[1], Francesca Cella[1], Alberto Diaspro[1,2].
[1]LAMBS-MicroSCoBiO, Department of Physics, University of Genoa,, Genoa, Italy, [2]IFOM-SEMM, Foundation FIRC Institute for Molecular Oncology, Milan, Italy.
The resolution capability of an optical system can be completely characterized by the vectorial diffraction theory[1], which defines the intensity distribution of a point like source imaged by a lens assuming ideal imaging conditions. Unfortunately, these conditions can not be completely reached as noise affects a recorded microscope image. A detailed characterization of the imaging process in linear and non-linear fluorescence microscopy allows to evaluate the noise deterioration effect on the resolution capability.
In this work we propose optical set-up schemes towards an image quality improvement in terms of Signal to Noise Ratio. In order to reach this aim, we insert a proper amplitude ring filter on the illumination arm of the microscope[2]. The effect induced by the filter results in a redistribution of the spatial frequencies of the OTF, in particular with high frequencies information collected at improved SNR[3].
The optical system response of the proposed scheme has been characterized in the spatial and in the frequency domain by using a computational simulation mainly based on a vectorial approach.
Analysis reveals that, the practical imaging quality in presence of noise can be significantly improved in the ring filtering scheme. Further improvements can be reached by the usage of the proposed annular filters in combination with image restoration[4].
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3292-Pos Board B339
FRET Imaging Through A White Light Laser (wll) Source
Valentina Caorsi[1], Paolo Bianchini[1,2], Emiliano Ronzitti[1,2], Giuliano Colombetti[3], Alberto Diaspro[1,2].
[1]LAMBS-MicroSCoBiO, Department of Physics, University of Genoa, Genoa, Italy, [2]IFOM-SEMM, Foundation FIRC Institute for Molecular Oncology, Milan, Italy, [3]Institute of Biophysics, CNR, Pisa, Italy.
FRET (Forster Resonance Energy Transfer) imaging has been largely used as a useful tool to investigate intermolecular interactions, allowing to measure distances in 1-10nm range. A large variety of methods, based on both steady state and lifetime measurements, has been developed to carry out quantitative results in the evaluation of the FRET efficiency1. However a quantitative data interpretation can be difficult due to donor-acceptor spectral overlap which leads to contaminations of the FRET signal2. These contaminations are worsen by the limited choice of excitation wavelengths available on conventional microscopes. Recently, a new generation of laser sources has been proposed, mainly based on supercontinuum laser technology3 . We recently tested a Koheras SuperK compact WLL combined with a Leica TCS SP5 AOBS system, to analyse FRET imaging. Moreover, lifetime measurements have been performed coupling the system with a time-correlated single-photon counting (TCSPC) electronics (Becker and Hickl), exploiting the 90 MHz repetition frequency in the visible excitation range. In all cases, we benefited of the flexibility of the WLL excitation wavelength choice as well as the capability of performing excitation spectra directly on the sample under investigation: on one hand it has been possible to fully characterize the FRET couple; on the other it has been possible to optimize the excitation wavelengths thus limiting the spectral contaminations.
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(2) Caorsi, V. al. 2007 Microsc Res Tech 70(5): 452 - 458.
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3293-Pos Board B340
Cell Image Quality and Point Spread Function Depends on Photomultiplier Sensitivity in a Fast Frame-Rate Multi-color Laser-Scanning Confocal Fluorescence Microscope
Yi-Kuang Liu, Mansoureh Eghbali, Enrico Stefani.
UCLA School of Medicine, Los Angeles, CA, USA.

A typical parameter used for describing the resolving capability of a fluorescence microscope is the full width at half maximum (FWHM) obtained by fitting a Gaussian curve to the point spread function (PSF) generated by a fluorescent bead.
In this work we used a custom-built video-rate confocal microscope at the diffraction limit to measure variations of PSF FWHM as a function of PSF intensities at different photomultiplier (PMT) sensitivities. Images were obtained by summation (128-256) of pixels with 62.5 ns dwell time. We used fluorescence beads and fluorescence-conjugated IgGs excited at 488 and 561 nm with emission recorded centered at 536 nm and 607 nm, respectively. In general, FWHM as a function of PSF peak intensity remains invariant using maximum photomultiplier (PMT) sensitivity. On the other hand, when using lower PMT sensitivities, FWHM values were correlated with PSF intensities, being at the diffraction limit.
In agreement with this finding, we found that at a lower PMT sensitivity the image quality of cells (e.g. isolated heart myocytes labeled with anti-L-type Ca2+ channel and anti-ryanodine receptors) is greatly improved. These proteins accumulate along the tubular transversal structure. It was remarkable that when using high pixel sensitivity (25-16 nm/pixel) we could discriminate discrete molecular clusters near the diffraction limit (~250 nm). In summary, proper usage of PMT sensitivity helps revealing more subcellular structures using a multi-color laser-scanning fluorescence microscope.

3294-Pos Board B341
Infrared-Nanoscopy of Surface Patterns in Mixed Polymer Brushes
Marlena Filimon.
Ruhr University Bochum, Bochum, Germany.
Polymer system in their diversity may offer a range of alternative, especially in the form of suitably designed thin films. Thin films made of so-called polymer brush show unique properties that allow changing their topography between different morphologies. Mixed polymer brushed consisting of two homopolymers (polymethyl methacrylate and polystyrene) being covalently with one end to a solid substrate have attached abiding interest because of their ability to switch properties such as the surface energy and/or surface topography in response to challenge of their environment.[1] For this reason, a conventional microscopic technique (AFM or STM) cannot provide information on specificity of biomolecular interaction.
A near field microscope incorporating vibrational spectroscopy as a contrast mechanism would allow chemical mapping in the so called ''fingerprint region'', with the high spatial resolution of SNOM [2-4]. Using a scattering scanning near-field microscope (s-SNIM) allows us to simultaneously record topography and frequency-dependent near-field signal of organic and biological samples with sub-diffraction limited resolution of up to 90 nm [4]. For chemical imaging of surface patterns in mixed polymer brushes, we used two tunable lasers, a CO laser (4.8μm - 6.3μm) and a high power continuous wave infrared optical parametric oscillator (OPO) (3.2μm - 4.1μm) as radiation source. We performed measurements around 1740 cm-1 (C=O stretching mode of methyl methacrylate) and 2930cm-1 (C-H stretching mode of styrene). An advanced image processing of the topography and the near-field image provided the evaluation of frequency dependent contrast showing spectroscopic signature.
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3295-Pos Board B342
A Scheme for Increasing the Collection Efficiency of Multiphoton Microscopy
Jesse D. McMullen, Warren Zipfel.
Cornell University, Ithaca, NY, USA.
Conventional widefield microscopy is hindered in thick specimens by the scattering of the light from the focal plane. By confining the source of fluorescence to a known volume, multiphoton microscopy turns all emitted light into potential signal. Most microscope objective lenses currently in use for two-photon microscopy are designed along conventional widefield imaging paradigms where rejection of scattered rays is critical. For this reason, collection of highly scattered light by typical objective lenses can be inefficient. To help increase collection in highly scattering samples we demonstrate a collar of light pipes that can be added to existing physiological objectives to dramatically increase the net fluorescence collected. Unlike other schemes, such as the use of parabolic reflectors, this setup is usable for *in vivo* imaging of animals and thick tissue explants.
Figure - Images of a hypocampal YFP slice imaged via epi-fluorescence through a 20x Olympus objective and through our collection collar.

3296-Pos Board B343
Monitoring Voltage-dependent Protein Dynamics Using Fringe-field Electric Impedance Tomography
Sameera Dharia, Gregory Dittami, Jeffery Wyrick, Richard Rabbitt.
University of Utah, Salt Lake City, UT, USA.
Fringe-field Electric Impedance Tomography (ff-EIT) is a non-invasive electrophysiological technique that monitors the local dielectric properties around resting and excited cell membranes. In our implementation of ff-EIT, a *Xenopus Oocyte* is positioned between a circumferentially distributed array of gold electrodes and is simultaneously excited using whole-cell voltage clamp. Radio frequencies (10 kHz-10 MHz) are passed between pairs of electrodes around the *Oocyte*. Data collected using the ff-EIT system can be used to temporally resolve the electrical response of the membrane during cellular excitation. *Xenopus Oocytes* were selected as a model cell to be used in the ff-EIT system due to their large size, visually polarized hemispheres, and ability to express exogenous membrane-bound proteins on their own membranes. Measurements made during cellular excitation show a significant difference in impedance change between either hemisphere of a native *Oocyte,* thereby demonstrating the ability of the ff-EIT system to record with subcellular resolution. Furthermore, results collected from *Oocytes* injected with potassium (Shaker) ion-channels indicate that the ff-EIT system can be used to sense 1) the voltage dependence of membrane-impedance change associated with ion-channel activation and 2) kinetic information associated with ion-channel inactivation. Preliminary data also suggests the potential use of ff-EIT in detecting voltage-sensor movement and in monitoring dielectric changes due to the various conformational states of voltage-sensitive proteins.

3297-Pos Board B344
Theoretical Limits To Errors, Acquisition Rates, And Resolution In Microscopy Of Switchable Fluorophores: Replacing The Diffraction Limit With The Algorithm Limit
Alexander R. Small, Forrest Hippensteel.
California State Polytechnic University, Pomona, Pomona, CA, USA.
We calculate error rates, and their effects on imaging speed and resolution, in techniques that overcome the diffraction limit by using switchable fluorescent molecules. Recent experimental work has beaten the diffraction limit in fluorescence microcopy by activating and localizing subsets of the fluorescent molecules in the specimen, and repeating this process until all of the molecules have been imaged. Examples include PhotoActivation Localization Microscopy (PALM), STOchastic Reconstruction Microscopy (STORM), and microscopy of blinking quantum dots. In all these techniques there is a tradeoff between speed (activating more molecules per imaging cycle) and error rates (activating nearby molecules and producing overlapping images that hide information on molecular positions), and so intelligent image-processing approaches are needed to identify and reject bright spots containing multiple molecules. We show that there is a maximum acquisition rate determined by this trade-off, and that how closely one can approach this acquisition rate depends on the capabilities of the algorithm used to distinguish single-molecule spots from multi-molecule spots. In particular, we calculate the error rates of commonly-used algorithms that use the shape of the bright spot rather than the overall intensity. This technique is used in STORM because fluorescent dyes have fluorescence efficiencies that can be strongly affected by the local environment. We show that the capabilities of these algorithms, in combination with the target contrast between fluorophores and background, determine whether the resolution is limited by the capabilities of the algorithm or the number of photons collected, leading to photon-limited and algorithm-limited resolution regimes. Finally, we consider algorithms that can infer molecular positions from images of overlapping blurs, and derive the dependence of the minimimum acquisition time on algorithm performance for this class of algorithms.

3298-Pos Board B345
High-speed Super-resolution Imaging through Interpolated Deconvolution of Live-cell TIRF Images
Shaohui Huang, Lawrence Lifshitz, Karl Bellve, Clive Standley,
Kevin Fogarty, Michael Czech.
University of Massachusetts Medical School, Worcester, MA, USA.
Recent progresses in overcoming the diffraction-limited optical resolution have mostly relied on spatial modulation of fluorophore's distribution between its bright and dark photophysical states. Such feat is often accomplished through complex and expensive experimental setups, and almost all the related super-resolution imaging techniques are hostile towards live-cell studies. In contrast, super-resolution imaging through interpolated deconvolution (Carrington et al., 1995, Science, 268:1483) is a post-acquisition image-processing technique that is independent on microscope platform. Its efficient collection and utilization of fluorescence photons also make the technology potentially a preferred method