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
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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
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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
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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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[4] P.P.Mondal et al., *Journal of Applied Physics* **102**, 44701, (2007).

3292-Pos Board B339
FRET Imaging Through A White Light Laser (wll) Source
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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.
(1) Chen Y. et al.2008. Journal of Microscopy 228: 139-152.
(2) Caorsi, V. al. 2007 Microsc Res Tech 70(5): 452 - 458.
(3) McConnell, G. 2005 Applied Physics B: Lasers and Optics 81(6): 783-786.

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
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