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.
[1] S. Santer, Macromolecules, 39, 3056-3061, 2006
[2] L. Novotny, S. Stranick, Annu.Rev.Phys,Chem, 57, 303-331, 2006.
[3] B. Dragnea, Encyclopedia of Nanoscience and Nanotechnology, Dekker, 2703-2712, 2004.
[4] B. Knoll, F. Keilmann, Nature, 399 (6732), 134-137, 1999.
[5] I. Kopf et. al. J.Phys.Chem. C, 111, 8166-8171, 2007.

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