Currently, we have successfully made small quantum dots with different emission wavelengths (527 nm, 615 nm, 620 nm and 655 nm), and functionalized them with streptavidin. Furthermore, we tested the small quantum dots on cultured neurons and found that all of them bind to AMPA receptors with high specificity. We found that the diffusion behavior of AMPA receptors labeled with our small quantum dots is different from that with commercial quantum dots, indicating that bigger quantum dots may have hindered the AMPA receptors' accessibility to the synaptic cleft. In addition, we identified micro-domains of approximately 100 nm x 100 nm where the AMPA receptors tend to bind and diffuse within these domains.

3059-Pos Board B751
Thermodynamically Driven Blinking for Super-Resolution Microscopy
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Super-resolution microscopy enables imaging of structures smaller than the diffraction limit, defined by Abbe's law. Over the last years, a continuously increasing toolset of physical methods, fluorescent probes and labeling strategies has emerged, making this imaging approach accessible to a broad range of applications. Single-molecule based stochastic methods, as STORM, PALM and their derivates require the least complex instrumentation, which gained these methods fast growing popularity among scientist of very diverse backgrounds. However, the relative simplicity of the instrumentation comes with a trade-off in probe requirements. These methods rely on the partial suppression of the detectable on-state, which can be achieved by photo-activation of an initially dark state or reversible switching between a bright (on) and a dark (off) state. Most synthetic dyes allow for the latter activation scheme, but require special treatment in order to do so. Chemical additives, as reducing reagents and oxygen scavenger, complex illumination schemes, molecular scaffolds, high irradiation intensities or a combination of the above is usually required to optimize the ratio between on- and off-state that enables a maximal densely labeling of the structure of interest. Here, we report a rhodamine derivate, which converts between a bright and a dark state in response to pH changes. At pH7, an average of $>1\%$ is fluorescent and tumbles between the two states on the molecular level. The resulting blinking occurs on timescales of up to several seconds and can therefore be exploited for e.g. STORM without further sample treatment or complex illumination schemes. Its ease-of-use and its outstanding photo-stability and brightness render this dye an excellent tool for super-resolution imaging techniques based on stochastic read-out.

3060-Pos Board B752
Multiplexed Imaging of Osteocytes in Bone
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Osteocytes are embedded in bone and account for 90% of cells within mature bone. Although previously viewed as quiescent cells, recent research has revealed the importance of osteocytes in regulation of bone remodeling, both as a mechanosensor and a source of signaling molecules for regulating osteoclasts and osteoblasts. Many osteocyte imaging techniques rely on imaging the lacunocanalicular space around the cell rather than directly imaging osteocytes themselves. To better characterize differences in osteocyte morphology and structure in aging and disease, we have developed a multiplexed imaging technique with the goal of examining osteocytes in 3D within their bone environment. 50-100 micron-thick decalcified bone sections were prepared and a variety of imaging dyes were used in combination with confocal microscopy to image bone matrix, lacunocanalicular space, osteocyte cell membrane, nuclei, and cytoskeleton in various combinations. We have simultaneously imaged collagen matrix (using a transgenic mouse line expressing GFP-tagged-collagen), lacunocanalicular space (using a fixable Texas Red-labeled dextran), osteocyte cytoskeleton (using alexa-647-phalloidin) and nuclei (using DAPI). We have also employed DiO labeling of the cell membrane in addition to dextran labeling of the lacunocanalicular system and DAPI imaging of cell nuclei. DiI labeling of the membrane in combination phalloidin labeling of the cytoskeleton was problematic in bone slices with either incomplete penetration of DiI in the sample or loss of phalloidin specificity. Mounting of bone slices in 2-2'-thiodiethanol increased imaging depth and resolution but resulted in loss of GFP and phalloidin signal over time, therefore requiring imaging immediately after mounting. From multiplexed confocal image stacks it is possible to simultaneously study several aspects of osteocyte structure in demineralized bone matrix with sufficient resolution to render detailed 3-D reconstructions of imaged volumes. These methods can be applied to studying osteocyte structure/morphology in normal and diseased bone tissues.

3061-Pos Board B753
Structural Studies by Correlative Stochastic Optical Reconstruction Microscopy and Electron Microscopy
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In cell biology, visual techniques such as light and electron microscopy are essential tools for analysis of cellular structures and function. The development of a super-resolution fluorescence microscopy method, stochastic optical reconstruction microscopy (STORM), has allowed multicolor and three-dimensional imaging with nanometer-scale resolution and enabled the study of cellular processes at the molecular scale. Electron microscopy (EM) can reveal not labeled structures, like organelles, membranes, macromolecules in higher resolution. By combining STORM with EM, we were able to understand cellular complexity and localization of molecules of interest in relation to other structures in high resolution. Here, we demonstrate that this method can be used to elucidate the ultrastructural details of cellular events by bridging the gap between light and electron microscopy in cell biology applications.

3062-Pos Board B754
4D Multiplexed Functional Imaging in Deep Tissue
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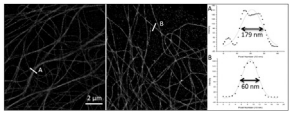
We present a deep tissue multiplexed functional imaging method that probes multiple cellular conditions in live model organisms. The method uses FRET-based biosensors to sense cellular conditions such as calcium and cAMP concentration levels, and uses fluorescence lifetime quantification to interpret the cellular conditions reported by the FRET biosensors. The method is based on Fourier lifetime excitation-emission matrix (FLEEM) spectroscopy [1] that simultaneously measures fluorescence lifetimes at multiple excitation and emission wavelengths within 23 microseconds, allowing quantifications on multiple FRET biosensors simultaneously in live model organisms. Samples are imaged in 3D by combining FLEEM spectroscopy with scanning laser optical projection tomography (SLOT) [2]. We demonstrate the method in zebrafish embryos transiently expressing cAMP FRET biosensor, which showed an increase in cAMP concentration upon physiological stimulus with forskolin and IMBX. Calcium and cAMP concentration levels during zebrafish embryonic development were monitored with time lapse 3D functional imaging of the embryo from 12 hours to 22 hours post fertilization. The method opens the door to multiplexed functional imaging of cellular biochemistries in whole live organisms.



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3063-Pos Board B755
Design and Implementation of 3D Focus Stabilization for Fluorescence Microscopy
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Focus stabilization is critical for many imaging modalities like TIRF, PALM and STORM. The focus stabilization device presented here, named pgFocus, is an open source and open hardware solution that can be integrated into microscopes with an existing objective positioner. pgFocus is a programmable and inexpensive circuit board consisting of a micro-controller, linear sensor array, DAC and an ADC. While pgFocus can stabilize on a single focal plane within ± 3nM at 30Hz, it can also follow and correct 3D focus changes when imaging multiple Z positions. pgFocus works by monitoring the reflection of an 808nm laser beam that is internally reflected at a glass/water interface. The translation of the reflected laser beam is converted into Δ distance change between the objective and the glass/water interface. The relationship between movement of the objective and the translation of the return laser beam is determined through an calibration procedure. This Δ distance change measurement is used to modify and adjust a pass-through voltage signal that is directed to a piezo objective positioner. The pass-through voltage is continually adjusted to move the reflected laser beam back to the original focus position.