sampling surveys of the single-molecule trajectories. With this system, we measured the duration difference of restriction-site-searching by these enzymes. The simplicity and versatility of the method suggest the possibility of more practical process analyses in living organisms.

Imaging & Optical Microscopy II

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Dronpa-1, Dronpa-2 And Dronpa-3 Separation Using Phase Resolved Optical Lock-in Detection (pholid) Microscopy

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In living cells, protein complexes are dynamic structures that control many distinct aspects of cell behavior. Addressing the complexity of protein function requires a comprehensive understanding of subcellular localization and protein stoichiometry. Fluorescence microscopy provides a high contrast method to determine specific localizations of individual proteins contained within living cells. Determining localizations of protein pairs is made difficult by inherent photobleaching, cross-talk, and autofluorescence contained within the sample being imaged. Traditionally, to separate fluorescent proteins (FPs) with overlapping spectra, techniques such as linear unmixing and fluorescence lifetime imaging (FLIM) have been used. Such techniques rely on complex microscope configurations and often require a compromise in signal to noise ratios. In these studies we use phase information derived from optical lock in detection (OLID) (Mao et al. Biophys J. 2008 Jun;94(11):4515-2) to separate the photoswitchable FPs Dronpa-1, Dronpa-2, and Dronpa-3 from GFP and autofluorescence with no effect in signal to noise. Additionally, relative stochiometric ratios between each of the Dronpa variants contained within microinjected zebrafish embryos were obtained with a standard confocal microscope.

1490-Pos Board B334

Near Infrared Emitting Dye Di-4-ANBDQBS for Recording Action Potentials in Isolated Cardiomyocytes

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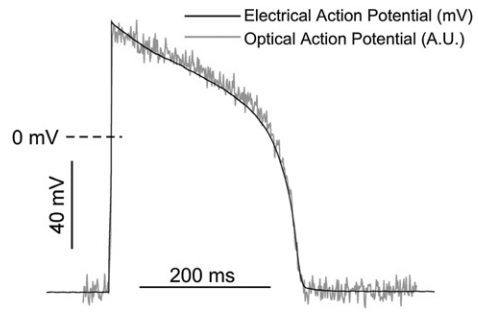
The use of voltage-sensitive dyes (VSDs) for noninvasive measurement of action potentials (AP) in isolated cells is limited by photodynamic damage. Here we tested a new red-shifted VSD Di-4-ANBDQBS as an optical AP (OAP) reporter. **Methods**: Guinea-pig ventricular myocytes were loaded with Di-4-ANBDQBS (18.4-73.6 μM), paced (CL=1-2s) and imaged (excitation, 660 nm laser, emission, >750 nm) in an inverted microscope (40x objective) using an EMCCD camera at 860 frames/s. The VSD signal was integrated over the cell image. **Results**: Limited exposure to laser (700 ms every 5 min) yielded OAPs with fast upstrokes (2-3 ms), stable duration (APD) and signal-to-noise (SNR) exceeding 20 for at least 30 min. The OAP faithfully followed the electrical AP obtained via patch pipette (Figure) yielding a high correlation between the respective APD measurements (R^2=0.985). Longer laser exposures (10 s on/50 s off for 5 minutes) caused APD prolongation which could be alleviated by reducing laser power and/or dye concentration at the expense of reduced SNR. **Conclusion**: With careful consideration of laser exposure, Di-4-ANBDQBS shows significant promise for noninvasive AP recordings in cardiomyocytes with an acceptable SNR.



1491-Pos Board B335

Optical Switchable Spironaphthoxazine (NISO)-derived Probes for Optical Lock-in Detection (OLID) Imaging Microscopy and OLID-FRET

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An optical switch probe based on spironaphthoxazine (NISO) harboring a O^6-benzylguanine (BG) functionality was synthesized and characterized for applications in optical lock-in detection (OLID) Image microscopy. NISO undergoes rapid and reversible, high-fidelity transitions between a colorless spiro (SP) state and a blue colored MC-state that serves as an acceptor probe in Förster resonance energy transfer (FRET) with GFP, YFP and other green or red emitting donor probes. The transition from SP to MC is brought about with high quantum yield by exciting SP with 365 nm or 720 nm (2-photon) while the MC to SP transition is rapidly affected upon excitation of MC with 543~632 nm light. Thus a defined waveform of optical or opto-thermal manipulations of the NISO switch provides a simple means to modulate the intensity of a donor probe via FRET. NISO was linked to BG via a polyethylene glycol (PEG) linker and this substrate was shown to efficiently label O^6-benzylguanine-DNA alkyltransferase (AGT) and AGT-fusion proteins. The optical switching properties BG-PEG-NISO coupled to AGT in fusion proteins with GFP (GFP-AGT/PEG-NISO) and mCherry-(mCherry-AGT/PEG-NISO) were studied. *In vitro* OLID-FRET imaging studies showed the suitability of NISO as an acceptor probe for GFP and mcherry. The R_0 for FRET between GFP and MC-NISO is 4.7 nm and GFP-AGT fusion protein covalently labeled with BG-PEG-NISO exhibited a 55% decrease in GFP fluorescence intensity upon conversion of SP to MC. Other examples of *in vitro* and *in vivo* OLID-FRET using GFP-AGT and mcherry-AGT labeled with NISO-BG will be presented.

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rsCherryRev and NISO Red-shifted Optical Switch Probes for Optical Lock-in Detection (OLID) Imaging and 2-colour OLID-FRET

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Optical lock-in detection (OLID) microscopy using synthetic or genetically encoded optical switches is was developed by our group to generate high-contrast images of the distributions and interactions of proteins in the presence of high and time-varying background signals such as those found in living cells in culture and in live tissue. OLID requires an optical switch probe whose fluorescence intensity can be modulated through deterministic optical control of its fluorescent and non-fluorescent states. Our initial studies focused on Dronpa, while in this study we show how the genetically-encoded optical switch rsCherryRev can be used to extend the wavelength region of OLID to the red (>550 nm) and for 2-colour OLID imaging in combination with Dronpa.

Optical lock in detection (OLID) of Foerster resonance energy transfer, OLID-(FRET) using optical switches as acceptor probes can overcome several limitations of FRET imaging of protein interactions in living cells, including detecting low levels of protein complexes that result from endogenous unlabeled proteins, and non-stoichiometric formation of protein complexes between donor and acceptor probes. We have previously used NitroBIPS as an optically switchable acceptor probe for GFP, and in this study we show how the synthetic, red-shifted optical switch spironaphthoxazine (NISO) serves as a switchable acceptor for both GFP and mCherry in OLID-FRET. These new probes are used for 2-colour OLID-FRET of protein interactions in living cells.