Making sense of mitochondria: Novel techniques for measuring dynamics, (dys)function and interaction.

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There is an emerging recognition that disordered mitochondrial dynamics contribute to the pathogenesis of complex diseases that are not classically considered to involve mitochondria. While microscopic methods have become increasingly important as fundamental tools to assess mitochondrial (dys)function in living cells and/or organisms, in all instances, the ability to obtain temporal information at high resolution is critical to the success of these studies. In this paper, we describe the combined use of novel genetically targeted probes and high resolution optical imaging technologies to explore mitochondrial function/dysfunction at the level of the single cell, as well as in livng zebrafish (Danio rerio).

Super resolution methods circumvent the diffraction limit (~200 nm in xy) of conventional fluorescence imaging to provide spatial resolutions in the range of 10 - 100 nm. STED (STimulated Emission Depletion) microscopy, developed by Stefan Hell [2] is essentially a point scanning approach based around confocal technology, however, emission from the Gaussian spot used to excite the fluorophore in confocal microscopy is restricted to a smaller focal volume by a longer wavelength depletion source. This depletion source is shaped into a "donut" which quenches the fluorescence at the periphery of the excitation spot by stimulated emission. The advantages of using STED microscopy to study mitochondrial morphology/dynamics include the ability maintain accurate z-resolution at depth leading to accurate reproducible measurements mitochondrial size, fragmentation and volume.

The dye technology consists of single chain antibodies (Fluorogen Activating Peptides - FAPs) that bind to and activate fluorescence of nonfluorescent molecules [3]. These single-chain antibodies can be expressed as fusion constructs with cellular proteins, and the expressed proteins are labeled by addition of the complementary dye molecules with specific properties. This FAP-fluorogen technology, using triarylmethane (MG-based) fluorogens has been previously shown to be suitable for super-resolution microscopy [4,5]. We extended these findings to demonstrate the feasibility of using time-lapse STED imaging of mitochondrially targeted FAP in live cells with sufficient temporal and spatial resolution to study mitochondrial dynamics, as well as mitochondrial-endoplasmic reticulum interactions without appreciable photo-toxicity. We have also been successful in using these approaches to look at mitochondrial function in the context of the zebrafish (Danio rerio) embryo.

References:

[1] da Silva, A.F., et al., *Mitochondria dynamism: of shape, transport and cell migration.* Cell Mol Life Sci, 2014. **71**(12): p. 2313-24.

[2] Klar, T.A., E. Engel, and S.W. Hell, Breaking Abbe's diffraction resolution limit in fluorescence microscopy with stimulated emission depletion beams of various shapes. Phys Rev E Stat Nonlin Soft Matter Phys, 2001. 64(6 Pt 2): p. 066613.

[3] Szent-Gyorgyi C, *et al*. Fluorogen-activating single-chain antibodies for imaging cell surface proteins. Nat Biotechnol. 2008;26(2):235-40. doi: 10.1038/nbt1368. PubMed PMID: 18157118.

[4] Fitzpatrick JA, *et al*. STED nanoscopy in living cells using Fluorogen Activating Proteins. Bioconjug Chem. 2009;20(10):1843-7. PubMed PMID: 20976031; PMCID: 2957894.

[5] Yan Q, *et al.* Localization microscopy using noncovalent fluorogen activation by genetically encoded fluorogen-activating proteins. Chemphyschem. 2014;15(4):687-95. PubMed PMID: 24194371; PMCID: 3967550.