TOPIC: New Developments in Optical Probes and Instrumentation
Title: High Resolution Imaging of Optical Molecular Markers in Mesoscopic Diffusion Regimes

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Introduction: Progress in the biological sciences has often been associated with the evolution of optical imaging and the corresponding capacity to identify specific anatomical and molecular biomarkers. The underlying physical barrier for extending high-resolution (diffraction limited) optical imaging beyond current mean free path-length (MFPL) limits of several hundred microns is the significant light diffusion in living tissues. In techniques like Optical Projection Tomography (OPT), complications from scattering are resolved by imaging very early development stages or naturally transparent organisms or otherwise by chemically treating the specimen of interest post-mortem, in order to make it transparent[2]. Conversely, it is also possible to perform optical tomography through entire mice in full diffusion regime, albeit with low resolution[1] (~1mm or worse). Mesoscopic scale therefore applies to organisms and tissues whose dimensions are usually between 1mm-1cm, for which neither ballistic nor diffuse photon propagation regimes apply. Herein, we research on methods for imaging of optical contrast and molecular markers in living tissues with high spatial resolution and penetration range of many millimeters to centimeters of tissue, not limited by light diffusion. *In-vivo* imaging beyond one MFPL could offer an important visualization tool for many areas of biology that involve the study of insects, fish, worms and other small-sized living organisms or organs.
Methods: We have investigated several methods for high resolution imaging of fluorescent markers in-vivo in the presence of scattering. In strongly forward-scattering objects, we report on the use of a mesoscopic fluorescence tomography (MFT) method that utilizes Fermi simplification to the Fokker-Planck solution of photon transport theory[3]. Using this theoretical model and a modified microscopic experimental setup we constructed a tomographic scheme for fluorescence tomography of D. melanogaster in prepupal and early pupal stages, not accessible by any of the existing optical microscopy techniques. We also investigate the use of multi-spectral optoacoustic tomography (MSOT) to extend high-resolution imaging of optical molecular contrast deep into highly scattering organisms and tissues[4]. The technique is based on the optoacoustic phenomenon that retains both high optical contrast and ultrasonic scattering-free resolution. We investigate the utility of MSOT for whole-body imaging of several mesoscopic size model organisms, i.e. Drosophila pupa, Earthworm, Zebrafish and mouse extremities, and compare it to OPT and MFT.
Results: Using MFT we demonstrate whole-body three-dimensional visualization of the morphogenesis of GFP-expressing salivary glands and wing imaginal discs in living Drosophila melanogaster pupae in vivo and over time. Furthermore, MSOT demonstrated robust spatial resolution on the order of 37μm in visualizing fluorescent molecular markers deep in objects of different size, from Drosophila pupa with characteristic diameters of about 800μm to mouse leg with cross sections of up to 10mm.
Conclusions: In order to move beyond the one MFPL limit and allow *in-vivo* visualization of organisms and structures in the mesoscopic range, for instance worms, insects or small animal extremities, we investigated herein the utility of MFT and MSOT for whole-body imaging of mesoscopic scale living organisms, never optically visualized in the past. Both approaches were found as highly capable mesoscopic imaging modalities that can close the imaging gap existing between current optical microscopy imaging approaches and state-of-the-art optical tomography methods that work in full-diffusion (macroscopic) regimes.

References:

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