# A SMALL ANIMAL TIME-RESOLVED OPTICAL TOMOGRAPHY PLATFORM USING WIDE-FIELD EXCITATION

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## ABSTRACT

Small animal imaging plays a critical role in present day biomedical research by filling an important gap in the translation of research from the bench to the bedside. Optical techniques constitute an emerging imaging modality which have tremendous potential in preclinical applications. Optical imaging methods are capable of non-invasive assessment of the functional and molecular characteristics of biological tissue. The three-dimensional optical imaging technique, referred to as diffuse optical tomography, provides an approach for the whole-body imaging of small animal models and can provide volumetric maps of tissue functional parameters (e.g. blood volume, oxygen saturation etc.) and/or provide 3D localization and quantification of fluorescence-based molecular markers *in vivo*. However, the complex mathematical reconstruction problem associated with optical tomography and the cumbersome instrumental designs limits its adoption as a high-throughput quantitative whole-body imaging modality in current biomedical research. The development of new optical imaging paradigms is thus necessary for a wide-acceptance of this new technology.

In this thesis, the design, development, characterization and optimization of a small animal optical tomography system is discussed. Specifically, the platform combines a highly sensitive time-resolved imaging paradigm with multi-spectral excitation capability and CCD-based detection to provide a system capable of generating spatially, spectrally and temporally dense measurement datasets. The acquisition of such data sets however can take long and translate to often unrealistic acquisition times when using the classical point source based excitation scheme. The novel approach in the design of this platform is the adoption of a wide-field excitation scheme which employs extended excitation sources and in the process allows an estimated ten-fold reduction in the acquisition time.

The work described herein details the design of the imaging platform employing DLP-based excitation and time-gated intensified CCD detection and the optimal system operation parameters are determined. The feasibility this imaging approach and accuracy of the system in reconstructing functional parameters and fluorescence

markers based on lifetime contrast is established through phantom studies. As a part of the system characterization, the effect of noise in time-resolved optical tomography is investigated and propagation of system noise in optical reconstructions is established. Furthermore, data processing and measurement calibration techniques aimed at reducing the effect of noise in reconstructions are defined. The optimization of excitation pattern selection is established through a novel measurement-guided iterative pattern correction scheme. This technique referred to as Adaptive Full-Field Optical Tomography was shown to improve reconstruction performances in murine models by reducing the dynamic range in photon flux measurements on the surface.

Lastly, the application of the unique attributes of this platform to a biologically relevant imaging application, referred to as Förster Resonance Energy Transfer is described. The tomographic imaging of FRET interaction *in vivo* on a whole-body scale is achieved using the wide-field imaging approach based on lifetime contrast. This technique represents the first demonstration of tomographic FRET imaging in small animals and has significant potential in the development of optical imaging techniques in varied applications ranging from drug discovery to *in vivo* study of protein-protein interaction.