HIGH THROUGHPUT TOMO
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Summary and Description of the project objectives

One of the reasons why optical tomography techniques have not become mainstream is the lack of high-throughput. Optical Projection Tomography (OPT), Selective Plane Illumination Microscopy (SPIM) and similar microscopy techniques rely on a single measurement session per specimen, and in the case of OPT a single reconstruction procedure for each measurement session. This means that the process is time-consuming and thus one not useful for imaging large numbers of specimens, or, more importantly, for time-lapse imaging. The amount of time required for a complete time-lapse imaging session on enough *D. melanogaster* or zebrafish subjects to obtain statistically significant results, for example, is impractical. It is for this reason that these experiments at the forefront of current biological research are pursued otherwise, typically using planar microscopy techniques. A similar problem is present in Fluorescence Molecular Tomography: each imaging session is typically in the order of 10 minutes per wavelength measured and must be done one subject at a time. If to this we add the time necessary to prepare and place the mouse, and the amount of time required to solve the inverse problem, we may easily reach time near the 30 minute range possibly one hour if multispectral measurements are acquired. This means that if 3D imaging is required for a drug development study, for example, this is done on a limited number of animals, resorting to planar imaging approaches (epi-fluorescence, for example) for the high throughput. Unfortunately these planar approaches are incapable of giving 3D information and are not quantitative in nature.

To address the problem of high throughput, two main imaging approaches will be addressed, namely, High-Throughput OPT/SPIM and High-Throughput FMT:

- **High-Throughput OPT/SPIM**. The developments expected for the OPT and SPIM setup include:
    - *Helical Optical Projection Tomography (hOPT)*: This technique is based on rotating the sample as in current OPT setups while at the same time displacing the sample vertically giving it a helical motion. This increases the throughput while significantly reducing the exposure and thus photo-toxic effects, being able to stack several samples or specimens for imaging. Novel advances both in terms of software (implemented in CUDA to optimize speed) and hardware are unique and innovative.
    - *Combined hOPT/SPIM*: It is straightforward to implement a SPIM arm in our current OPT or in the future hOPT setup. In this manner we will be able to obtain high-throughput low resolution images using hOPT and then focus a specific

areas using SPIM thus optimizing the throughput of SPIM with the aid of hOPT information.
    - *GPU developed inverse Radon Transform*: In order to optimize throughput all codes will be developed in CUDA, a parallel processing language based on the use of the Graphics Processing Units (GPUs). The language I am most familiar with is CUDA, however other approaches will also be considered.
    - *Accounting for scattering in OPT*: one of the tasks will be to account for scattering in OPT measurements. There is currently only one approach which attempts to account for scattering in the reconstruction based on the Fokker-Plank approximation. This approach however, is time-consuming. We shall develop ultra-fast reconstruction algorithms which account for scattering even if in a more approximate manner, in order to optimize the resolution/throughput ratio of the technique. Ideally, we would like to be able to improve resolution at the cost of longer computing times.
- **High-Throughput FMT**. The developments expected for the FMT setup include:
    - Multi-subject FMT: Up to date there are no setups which can perform optical tomographic measurements in more than one small animal. The approach that will be employed here is novel and will consist of adding beamsplitters and mirrors in order to control the excitation and collection of fluorescence from at least 3 subjects simultaneously. If the methodology is successful, this will be extended to 5 subjects, which is currently the number used for high-throughput planar imaging.
    - GPU-based inverse problem for Diffuse light: Additionally to the current approaches which have already significantly boosted speed for a detailed description of the methodology used in the inverse problem to achieve reconstructions in under one minute), we will rewrite the currently existing code so that it may make use of the computing speed of GPUs. Additionally, the existing code would need to be restructured in order to account for more than one subject simultaneously.

Description of the work performed since the beginning of the project and main results
The main issues addressed since the beginning of the project have been with respect to improving the throughput of SPIM/OPT setups, working both on hardware and software development. As main important results from this first two years of project we should emphasize:

- Implementation and experimental setup of Helical OPT for high throughput imaging
- Development of CUDA GPU software for Helical OPT
- Successful implementation of Helical OPT for high throughput imaging of up to 13 specimens of *D. melanogaster*.
- Implementation of ultra-fast measurements on the beating zebrafish heart

The high impact of this work may be understood by the publication of a peer-reviewed paper in a Nature journal, Scientific Reports. This new approach will enable imaging of several samples in development simultaneously (see Fig. 1).

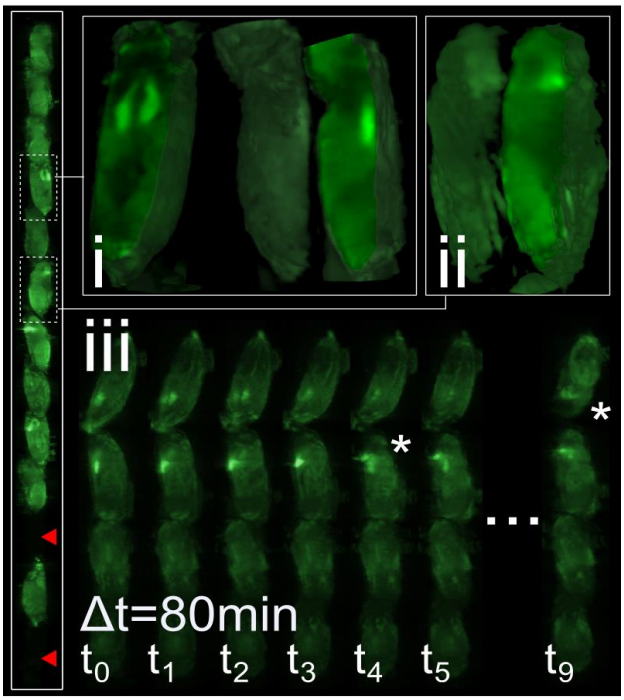

FIG. 1. Volume renders of 13 *D. melanogaster* pupae which express GFP ubiquitously, imaged simultaneously using hOPT. Volume render details of an early stage pupa (two views shown) (i) and at a later developmental stage (ii). Red arrowheads indicate non-expressing GFP individuals. (iii) Time-lapse imaging for over 12 hours (hOPT images taken every 15 minutes, results shown for 80 minute time intervals) of a set of 7 *D. melanogaster* pupae of which 4 are shown here. Asterisks indicate when the process of head eversion takes place in different individuals at different time points.

Current work within this project point towards the use of this technique for other species such as the developing zebrafish, for which a very important development which took place during the second part of the project. In order to image fast processes such as the heart beat in real time, within this project we develoed of ultrafast imaging approaches, which are key for high throughput imaging.

- *Ultra-fast measurements*: making use of fast cmos cameras and tunable lenses, we are now capable of generating ten 3D volumes per second in light sheet microscopy

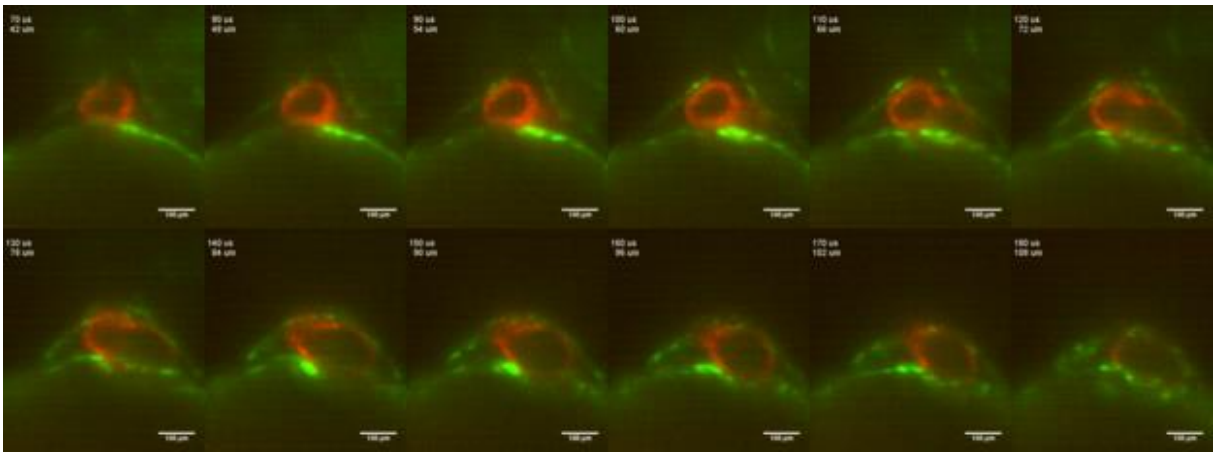

Figure 2, Selected Frames of a single beat of a zebrafish heart, taken at 200fps. The frequency of the heart beat is approximately 2Hz, we see here 12 z-planes each measurement taking 10ms. Manuscript in preparation.

More information on the development of the project can be found at:
http://highthroughputtomo.weebly.com/