Chapter 1

Introduction

Light-sheet microscopy is at the cutting edge of live-organism imaging. It uses decoupled and orthogonal optical illumination and detection to image volumes of biological samples. The technique is fast and minimally invasive enabling the imaging of live model organisms and earning it the Nature Method of the Year in 2014. In the coming years it will help move biology out of the petri dish and back into the animal.

The aim of this work was to develop a light-sheet microscope imaging system which permitted the three dimensional dynamic tracking of particles in live biological samples. This system was based on the seminal work of Ernst Stelzer *et. al*, who pioneered digital light-sheet technology [1].

1.1 Motivation

The technology developed during this work was applied in two biological investigations which required the tracking of particles in live biological systems: the egress of viral particles in live cells, and the mechano-biology of live and developing model organisms.

1.1.1 The live tracking of viral egress

Viruses are carriers of infectious disease in humans, by hijacking the internal working of the cell the virus replicates using the machinery of the cell. 80 % of adults in the UK are thought to be infected with Herpes Simplex Virus (HSV)-1 (cold sores) which is currently medically incurable [2]; only the symptoms can be suppressed. Understanding virus pathology is a requirement for assisting in therapeutic intervention.

Currently, the virus structure is well understood through high resolution techniques such as Atomic Force Microscopy (AFM) and Electron Microscopy (EM). Contemporary biological models of viral infectivity dynamics are based on *in vitro* studies. Virus particles are smaller than the optical diffraction limit (20 nm-200 nm) and have been observed using optical super-resolution techniques [3]. The structure of HSV-1, for instance, has also been eluclidated *in vitro* using super-resolution techniques [4]. However, virus particles move tens of nanometres on the time scale of milliseconds [5] meaning these slow super-resoltuon do not provide the temporal resolution needed to accurately track virus particles in three dimensions, nor *in vivo*.

Wide-field particle localisation techniques are compatible with fast light-sheet microscopy and can be used to accurately localise particles to sub-diffraction limited positions in two dimensions. Full three dimensional sub-diffraction limited tracking is viable using a light-sheet microscope when adding an axially encoding optical element in the detection optical path [6]; with a greatly extended three dimensional field of view. This work intended to implement such a technique to enable the *in vivo* study of virus trafficking through a host cell with unparalleled temporal resolution.

1.1.2 Mechano-biology of developing organisms

Cell mechanics plays a vital role in the development of organisms; internal stresses within tissues induce cellular migrations that can govern the organism's resultant

anatomy [7]. Despite genetics screens providing the essential structural, patterning and signalling pathways the process of morphogenesis remains a mystery [8, 9]. Live imaging of organism development can reveal patterns in cell behaviour, but it has not yet provided a quantitive description of force generation. Only with an understanding of how forces propagate within tissues can morphogenesis be fully elucidated. In this work a technique was developed to mechanically probe deep tissue using the presented light-sheet microscope. A non-invasive magnetic field was used to to move a magnetic bead embedded in a live embryo. The light-sheet microscope was used to image the cells and the bead in real-time and three dimensions.

1.1.3 Structure

Initially, the fundamental physics of light microscopy will be presented, leading into a discussion on techniques which can provide full volumetric fluorescence imaging. From there, contemporary light-sheet microscope technology from the literature will be presented and discussed. Using this, a design for a custom and purpose-built light-sheet microscope (which will be used in this work) will be presented. Following on, the methods for the generation of waveforms, for digital light-sheet microscopes, will be critically consideration. The projective mathematics from the generation of these waveforms will then be applied (in more detail) to the projective tomographic reconstructions in volumetric light microscopy. In preparation for the chapters requiring the mounting of biological samples, a custom, open-hardware, live imaging, sample chamber will be presented. This will enable the mounting of live cells with the intent for single particle tracking of virus particles, in cells, as discussed in the motivation section. Single particle tracking will then be characterised in the system presented here, alongside an analysis of the localisation errors associated with dynamic particle tracking. The light-sheet microscope, built for this work, will then be used for the fast imaging of a model organism the Zebrafish. Embedded magnetic beads will then be shown to mechanically perturb live Zebrafish in order to extract viscoelastic properties of the organism as it develops. In the final two chapters, the results of the preceding chapters will be summarised and reviewed; followed by a discussion on how the contributions from this body of work should be developed in the future.

References

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