

1.1 Motivation

The technology developed during this work was ^{developed to support} 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, ^{which replicate 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 elucidated *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-resolution 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, ^{BY} when adding an axially-encoding optical element in the detection optical path [6]; with a greatly extended three dimensional field of view. This work ^{is} 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

Careful: HSV-1 rarely needs treatment.

Although most cases require no treatment, it can be needed by immunocompromised patients and the

2 Reasons to study - MODEL VIRUS - RARE, SERIOUS CASES

which canoes cold sores, as well as serious conditions in some patients, and which is an important model virus.

but they can be tracked using fluorescence microscopy (and imaged with super-resolution fluorescence microscopy), so their pathing movement through infected cells can be observed.

the development of an...

CHANGES IN SHAPE OR STRUCTURE OF THE VIRUS MOVES THROUGH THE CELL (Particle tracking is possible, already Time-resolved imaging of structure is the challenge.)

BY TIME-RESOLVED IMAGING.

Say what type of organism is of interest as a model, here.

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 quantitative 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 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 of this thesis

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 considered. 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.

(Zebrafish)

As part of this work,

A separate application of the

specimens

information from

about

single

(Zebrafish)

minimally —

SPECIALIST BACKGROUND

PRESENTED FOR

to accurately register illumination to the imaging plane will be presented.

(GIVE CHAPTER NUMBERS!)

should have mentioned in 1.1.2