Chapter 1

Introduction

Light-sheet microscopy is at the cutting edge of live-organism imaging. 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 tracking of particles through biological samples. This system was based on the work of Ernst Stelzer who pioneered digital light sheet technology[**Huisken2004**]. A light sheet microscope uses orthogonal illumination and detection to optically section biological samples. A previous system was built in order to study developmental biology. This work intended to improve upon this design so as to facilitate fast 3D particle tracking.

1.1 Motivation

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 1 (cold sores) which is currently medically incurable[**Herpes**]; only the symptoms can be suppressed. Understanding virus pathology is a requirement for assisting in therapeutic intervention. The virus structure is well understood through high resolution techniques such as Atomic Force Microscopy and Electron Microscopy. In this group we have used super resolution techniques to study the Herpes Simplex Virus 1 structure *in vitro*[**Laine2015**]. Contemporary biological models of viral infectivity dynamics are based on *in vitro* studies Studying these dynamics *in vivo* and following a virus through its entire process in a living organism could provide new, useful insights and understanding which could be used to suppress or reverse viral infection in humans. Virus particles are smaller than the diffraction limit (20 nm-200 nm); optical super resolution techniques can image sub-diffraction limit and have observed Human Immunodeficiency Virus 1[**Pereira2012**]. Virus particles move tens of nanometres on the time scale of milliseconds[**Brandenburg2007**], these techniques currently do not produce the temporal resolution required to accurately track virus particles[**Brandenburg2007**] in three dimensions and are limited to *in vitro* studies.

Particle localisation techniques are compatible with light sheet microscopy and can be used to accurately localise particles to sub-pixel, sub-diffraction limited positions in two dimensions. In conjunction with a novel third dimensional tracking technique, exclusive to light sheet, full sub-diffraction limited tracking is viable[**Spille2015a**]. This will then enable the *in vivo* study of virus trafficking through a host cell and protein propagation in neurons with unparalleled temporal resolution.

1.1.1 Structure