1. Publishable Summary

Summary description

Disease caused by microorganisms accounts for a significant financial and economic burden in the modern world. Taken together, viral, fungal and bacterial infections cost billions of pounds a year not only in human disease, leading to lost work hours, but also within an agricultural context where microorganisms can have a significant impact on domesticated animals such as beef and dairy cattle.

The initial event in the vast majority of this disease is the interaction of the pathogen with the host animal. Animals have many barrier systems in place including the skin, mucosal systems and sloughing epithelial cells lining outward-facing tissues. It is with these epithelial cells that pathogens must first interact in order to colonise the host. These initial interactions are complex and highly dependent upon specific facets of both the pathogen and the host, providing tropism for particular microorganisms. Influenza virus, for example, interacts with the respiratory epithelial cells, whereas *Salmonella* bacteria principally interact with the intestinal lining. Even after an initial contact, these host-pathogen interactions play a critical role in disease progression. Many fungal pathogens for example, upon entering the blood stream perform a second round of host interaction, with the cells of the blood-brain barrier.

Broadly, the main objectives of the project were:

(a) To develop a model system with which to study host-pathogen interactions

(b) To develop and use high temporal and spatial resolution 4-dimensional microscopy to study the mechanistic basis of host-pathogen interactions.

Work performed and main results

Since the start of the project in March 2012, the majority of the project time has been spent studying the interactions of different pathogens with host-cell systems. After spending several months optimising acquisition parameters at the Birmingham Advanced Light Microscope (BALM) facility, we were able to acquire a suitable spatial resolution (equivalent to a volume of ~120µm x 120 µm x 30 µm) in quick enough succession (up to 50 frames per second) to image fluorescent microbes, both free-swimming in the media and on the surface of cells (see Figure 1 for schematic).

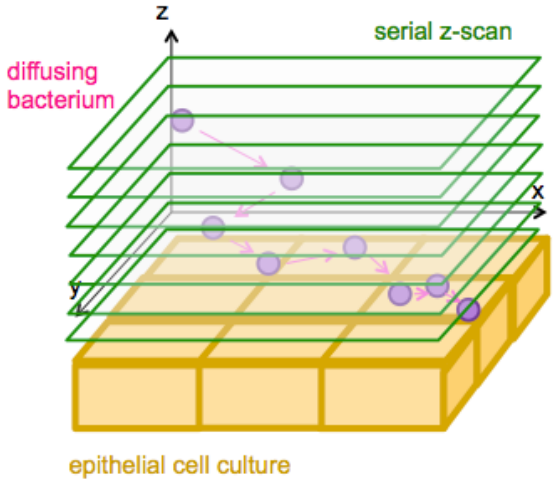

Figure 1: Schematic of analysis technique

Several different microbes were genetically transformed to produce fluorescent proteins (a requirement of this acquisition technique). Whilst the original target for the project, *Streptococcus pneumoniae*, was at the time, found to be unsuitable for imaging, several strains of *Salmonella spp*. *Escherichia spp*. as well as the fungal pathogen, Cryptococcus neoformans, were found to be ideal for imaging using these techniques (see Figure 2 for reconstructed 3D volume).

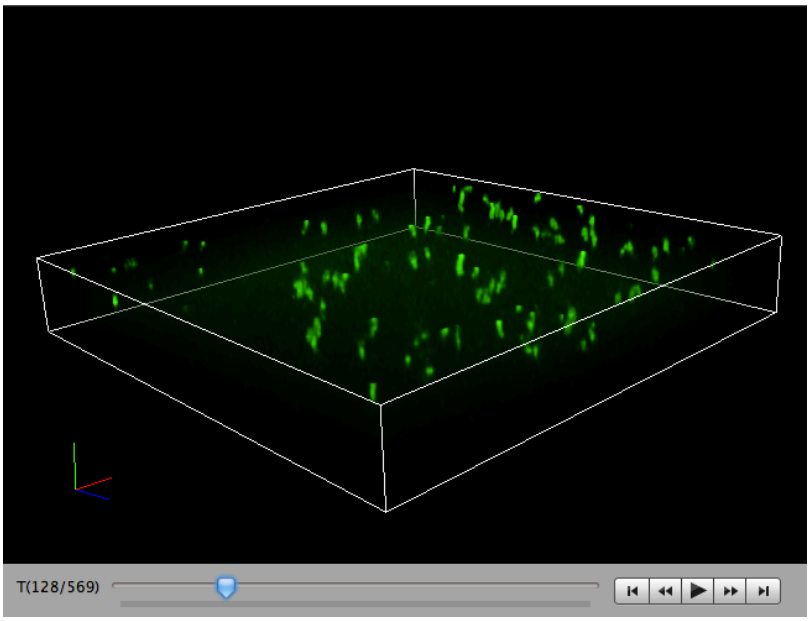

Figure 2: An typical frame from a 4D experiment

A major objective of the project was to develop a post-acquisition analysis technique in order to analyse these images such that quantitative data from experiments could be used to draw conclusions about the mechanistic basis for host-pathogen interactions. In collaboration with bio-informatitions at the University of Birmingham, suitable object identification and tracking algorithms were used in concert with bespoke code to extract what was deemed key information about the host-pathogen interactions. Where possible, this work was validated by comparing our results with other published studies. In some cases, however, this could not be done as the data were fully novel.

Final results and impact

After acquisition and quantitation of a large amount of data, we found that many parameters could be gleaned from high resolution 4D imaging experiments. The choice of parameters depended largely on the model system. For example, when looking at *Salmonella* interactions with host epithelial cells, the most useful (and scientifically interesting) parameters extracted from our analysis were the dwell times and the number of dwell events. By comparing wild-type *Salmonella* with identical bacteria lacking known adhesins (for example, those encoded by the *Salmonella* Pathogenicity Island 1), we saw a reduction in the average dwell time of *Salmonella*, but not the number of interactions. This supports published data in the field but adds a potential mechanistic understanding; that SPI1 affects the strength of adhesion for bacteria already bound to cells, but does not affect the initial interactions, which are likely mediated by another class of adhesins.

These and similar findings are important because they are hard or impossible to see using conventional techniques. Furthermore, through collaboration with other researchers at Birmingham, the technique has been applied to other systems including those of Cryptococcus neoformans in brain endothelia. Finally, although it does not require the same resolution, the principal of the technique has also been used to track and analyse the migration of fibroblasts in two spatial dimensions over time, with the intention of moving the system to true 4D in the future to better understand the migration of cells within a 3D matrix.

In summary, the techniques developed in this project have far reaching applicability and have strengthened collaboration between individuals and groups, both at the University of Birmingham and further afield.