Pathogen Imaging Applications

The ability to image live pathogens *in vitro* and *in vivo* is integral to the study of infectious diseases, to garner information on pathogen virulence strategies, invasion processes, disease dissemination and host responses and different imaging modalities can offer novel perspectives on each of these. Imaging approaches can also then be translated into treatment stratagems through improving throughput for compound and vaccine screening using both *in vitro* and *in vivo* model systems and can reduce the costs involved in treatment development. This special issue on the imaging of live pathogens brings together a series of articles examining different aspects of imaging from *in vitro* analyses to imaging of the activity of immune response genes in whole animals.

High content image analysis in conjunction with fluorescently-tagged bacteria has been instrumental in allowing the screening of large compound libraries for novel antimicrobials. Tanya Parish and colleagues describe such a system for the identification of compounds targeting *Mycobacterium tuberculosis* in infected macrophages [1]. This technology is widely applicable to different microbes including protists and helminths as well as bacteria.

Fluorescence-based imaging has been instrumental in recent studies of intracellular pathogen invasion of host cells. Here Polly Roy and Bjorn Mohl demonstrate a technique for the labelling of non-enveloped RNA viruses with a limited capacity for insertion of markers. This involves insertion of a small peptide sequence which then binds a fluorophore-conjugated arsenical molecule, thus obviating the inability to incorporate fluorescent protein genes into these viruses [2]. Jost Enninga and co-workers meanwhile provide a comprehensive review of the different imaging approaches that can be used to look at the role of macropinocytosis in pathogen invasion *in vitro* using *Shigella* as an example [3]. The relative merits of different systems are discussed along with the problems that may be encountered while using these probes. The approaches they describe can also be applied to other methods of pathogen invasion.

Pathogen invasion also causes alterations in many aspects of host cell biology and one of these is lipid metabolism. Ella Sklan and colleagues describe methodology for examining the kinetics of lipid droplet (LD) formation and their dynamics in hepatitis

C virus-infected cells by a live cell imaging approach which allows real-time investigations to be performed [4]. This method overcomes several of the disadvantages observed in previous systems which are largely dependent on end-point labelling and therefore do not yield information on the events of LD biogenesis during infectious processes.

One of the major successes of modern imaging technology has been the leap from *in vitro* methodologies, such as those described above, to the ability to visualise infections within a living animal (usually a mouse but other models are also utilised such as zebrafish). This has been made possible both by advances in reporter gene and probe development and by enhanced photo-detection modalities. Natalie Suff and Simon Waddington review the different methodologies used for *in vivo* imaging, in particular the method of using somatic transgenesis with lentiviral reporter constructs to monitor the dynamics of host responses to infection [5]. Peter Taylor and colleagues describe a three dimensional approach to *in vivo* imaging, diffuse light imaging tomography with integrated micro-computed tomography which allows loci of infection to be pinpointed more accurately within the animal than does conventional bioluminescence imaging, using the example of *Escherichia coli* K1 [6]. Rogerio Amino *et al*, demonstrate the use of bioluminescence imaging over time to look at tissue tropisms of different parasitic organisms including *Plasmodium* and trypanosomes [7]. Bioluminescence imaging can reveal many previously unrecognised facets of infectious processes, however it is limited when information is required at the cellular level. The use of intra-vital microscopy allows researchers to circumvent this limitation in combination with fluorescent reporters for pathogen or host. Nathan Peters and co-workers show how this technology can be used to study infections of the skin, with the parasitic protist *Leishmania* as their model. They combine green fluorescent reporter mice with red fluorescent pathogens to examine the interactions of parasite and host cells at different time points within a naturally transmitted infection [8]. Jim Brewer and his colleagues use intra-vital microscopy to examine the interactions of host and parasite within the central nervous system (CNS). Here they present a detailed description of the technique as applied to CNS infections within the cortical meninges [9].

Imaging techniques also provide non-invasive methods of examining the effects a pathological infectious process can have upon the host. Jean Rodgers, Barbara

Bradley and Peter Kennedy illustrate this by the use of contrast enhanced magnetic resonance imaging to follow the breakdown of the blood-brain barrier in an experimental model of African sleeping sickness [10].

One of the issues highlighted in many of the articles is the importance of ensuring virulence of the pathogen once labelled or under the imaging conditions used. For example, engineering a label to be genetically encoded in an organism might impact its ability to infect cells [2], or the organism may express the label at low levels or the label may have low wavelength emission that make it difficult to detect [6]. A futher consideration is that many of the pathogens mentioned in these articles are cause of significant disease to humans and/or animals and it is important to note that pathogen containment approaches were appropriately invoked by the authors but that these necessary precautions differ significantly between countries and between institutes and are not covered in detail.

In summary the articles presented here cover a range of imaging applications as used in the field of infectious diseases ranging from *in vitro* methods for drug discovery and basic cell biology to whole animal imaging for tracking the dynamics of infection, including host-directed methods for analysing immune responses and pathology.

The guest editors would like to thank all of the authors and expert reviewers for their time and expertise.

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3. Kühn, S., et al., *Imaging macropinosomes during Shigella infections.* Methods, 2017.
4. Nevo-Yassaf, I., et al., *Live cell imaging and analysis of lipid droplets biogenesis in HCV infected cells.* Methods, 2017.
5. Suff, N. and S.N. Waddington, *The power of bioluminescence imaging in understanding host-pathogen interactions.* Methods, 2017.
6. Witcomb, L.A., et al., *Non-invasive three-dimensional imaging of Escherichia coli K1 infection using diffuse light imaging tomography combined with micro-computed tomography.* Methods, 2017.

7. Tavares, J., et al., *In vivo imaging of pathogen homing to the host tissues.* Methods, 2017.
8. Carneiro, M.B., et al., *Use of two-photon microscopy to study Leishmania major infection of the skin.* Methods, 2017.
9. Coles, J.A., et al., *The mouse cortical meninges are the site of immune responses to many different pathogens, and are accessible to intravital imaging.* Methods, 2017.
10. Rodgers, J., B. Bradley, and P.G.E. Kennedy, *Delineating neuroinflammation, parasite CNS invasion, and blood-brain barrier dysfunction in an experimental murine model of human African trypanosomiasis.* Methods, 2017.