TB in 3D: A novel approach to quantifying tuberculosis infection in three dimensions using Mesoscopy and optical clearing techniques

Francis, R.[1], McCann, L.[2], Khatri, B.[3], Keeble, J.[3], Dagg, B.[3], Amos, B.[2], Ho, M.M.[3], MacLellan-Gibson, K.[1] and McConnell, G.[2]

[1] Biological Imaging Group, National Institute of Biological Standards and Control (NIBSC), MHRA, United Kingdom, [2] Centre for Biophotonics, SIPBS, University of Strathclyde, United Kingdom, [3] TB and Malaria Group, NIBSC, United Kingdom

Tuberculosis (TB) is the world's deadliest infectious disease, claiming ~1.7million lives / year. TB vaccine testing relies on *in vivo* models including the mouse aerosol challenge model. Previous methods of determining 3D changes in the tissue and quantification of the disease within the tissue have relied on serial sectioning of histopathological sections, with stitching required to obtain cellular resolution for precise quantification. The histopathological approach has several disadvantages including the distortion of data in 3D and data mismatch. To overcome this, we used an optimised optical clearing technique with whole TB infected lung lobes or 0.5mm slices, in combination with CLSM and Mesoscopy (which has a 4x magnification with 0.47 numerical aperture to image a 6 x 6 mm area at cellular resolution), and isosurface rendering, to resolve infected lung to a cellular resolution over a large field of view in 3D. Using these techniques, we have shown that it is possible to quantify the volume of TB infection within optically clear lobes, to resolve individual TB colonies by Mesoscopy, and to compare different TB strains. We now hope to use these techniques to compare between vaccinated and non-vaccinated mice, and to incorporate other fluorescent stains for immune cell types, hopefully aiding in the understanding of the host response to TB.