A novel approach to understanding parasite nutrient uptake and metabolism at a subcellular scale using Nanoscale Secondary Ion Mass Spectrometry

Macaulay Turner*#, Kathryn Else#, Katie L. Moore*

*Department of Materials, Photon Science Institute, Faculty of Science and Engineering

#Lydia Becker Institute of Immunology & Inflammation, Faculty of Biology, Medicine and Health, The University of Manchester, Oxford Road, Manchester, M13 9PL

Trichuris trichiura is a major public health concern infecting around half a billion people and causing the loss of around 640,000 disability adjusted life years (DALYs). The parasite inhabits the caecum and proximal colon of infected individuals with its anterior end embedded within a unique intracellular epithelial cell niche. Currently we do not know what the parasite feeds on, or the main route of nutrient uptake. Although the parasite has a mouth it lacks a muscular pharynx, arguably making feeding through the mouth unlikely. Trichuris spp have a structure termed the bacillary band, which occupies one third of the circumference of its anterior end. The function of the bacillary band is enigmatic, with it ascribed both secretory and absorptive in function. Previous work has shown that fluorescently labelled glucose is taken up by the worm at the bacillary band pores and localises in the central stichocyte cells. Whilst the use of fluorescent tags is an incredibly powerful tool, the addition of a fluorophore can drastically alter a molecule's kinetics and may change uptake mechanisms as well as how it is metabolised within the parasite. Thus, alternative methodologies to localise compounds at a subcellular level are required.

NanoSIMS is a high-resolution secondary ion mass spectrometry instrument (beam size can be focused to 50 nm) that can be used to image and measure elemental and isotopic distributions in samples at subcellular scale. It has extremely high sensitivity which makes it possible to detect elements at parts per million concentrations depending on the element. NanoSIMS can be used in tandem with stable isotope probing, with this method an organism is exposed to a compound labelled with a stable isotope The NanoSIMS instrument can then detect and localise isotopic enrichment in the sample at the subcellular scale to infer mechanism of uptake, and utilisation of the compound. The use of stable isotope labelling has the advantage that it does not alter the size or structure of the compound, therefore will mirror the uptake mechanisms, and will be metabolised the same as an unlabelled compound.

We have used stable isotope probing with the NanoSIMS to reveal how *T. muris* utilises glucose after uptake. Worms were exposed to ^{13}C labelled glucose for different times and the anterior end of the worms were imaged with NanoSIMS at a high lateral resolution (~90 nm). We have shown that glucose, or glucose metabolites, localise to the stichocyte granules, stichocyte membrane, muscle and in small (150 nm) circular structures within the bacillary band cells. Additionally, we have shown the amino acid alanine localises to the stichocyte granules.

Our data thus (a) reveals a possible novel function of the stichocyte granules as a nutrient store and (b) showcases the potential of NanoSIMS in understanding the biology of large multicellular parasites. NanoSIMS and stable isotope probing therefore offer a new approach to understanding nutrient uptake and metabolism in the field of parasitology at the subcellular scale.