How can Nanoscale Secondary Ion Mass Spectrometry (NanoSIMS) be used to localise anthelminthics at a sub-cellular level in *Trichuris spp*

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1. Whipworm Background and Aims

- *Trichuris spp* are soil-transmitted helminths that infect mammals with the human infective species *T. trichiura* infecting **464.6 million people**[1].
- Current intervention strategies include mass drug administration programmes.
- However, we **lack efficacious drugs.**
- *T. muris* is the mouse infective whipworm that can be used as a model for *T. trichiura* infection.
- **Levamisole** is an anthelminthic that is extremely efficacious against *T. muris,* but **lacks efficacy against *T. trichiura in vivo***[2][3].
- **Levamisole binds nicotinic acetylcholine receptors** in parasites causing continued receptor stimulation which leads to parasite muscle paralysis[4] .
- We aim to identify **the uptake mechanism** and **subcellular localisation** of deuterium labelled (2H) **Levamisole** and other anthelminthics through the use of NanoSIMS.

2. An Introduction to NanoSIMS

- NanoSIMS is **a high-resolution secondary ion mass spectrometry** instrument that can be used to image and measure elemental and isotopic distributions in samples at **subcellular scale.**
- A **primary ion beam** is scanned over a sample surface which **generates secondary ions** that are detected and analysed by a **mass spectrometer.**
- NanoSIMS has extremely high sensitivity which makes it possible to detect elements at parts per million concentrations.
- Stable isotope probing (**NanoSIP**) involves the exposure of a sample to a compound labelled with a **stable isotope**, then investigating the isotopic **enrichment** in the sample to infer mechanism of uptake and incorporation.

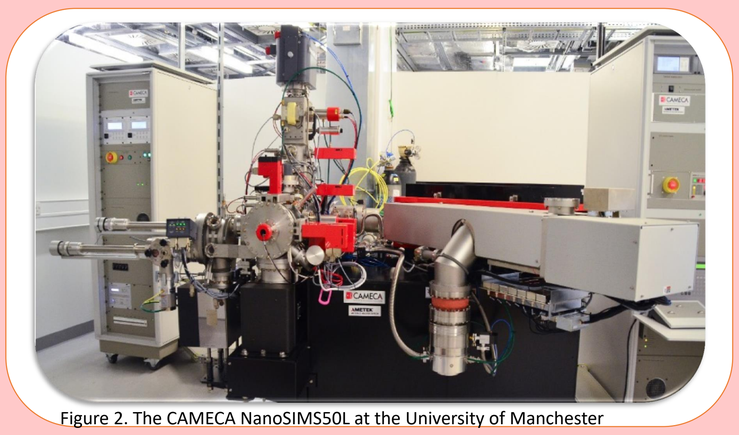

Figure 2. The CAMECA NanoSIMS50L at the University of Manchester

3. Experimental Design

To investigate the subcellular localisation of Levamisole in *T. muris*:

1. Mice were infected with **150 *T. muris* eggs.**
2. Adult *T. muris* worms were isolated at day **35 post infection** from the mice.
3. Worms were soaked in **20 µg/ml concentration of deuterated Levamisole** for 24 hours.
4. Worms were fixed in 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1M Hepes buffer.
5. *T. muris* worms were then dehydrated in an ethanol series then embedded in LR White resin.
6. Embedded worms were sectioned to **1 µm and placed on a Si wafer**.
7. Sections were then analysed using **NanoSIMS.**

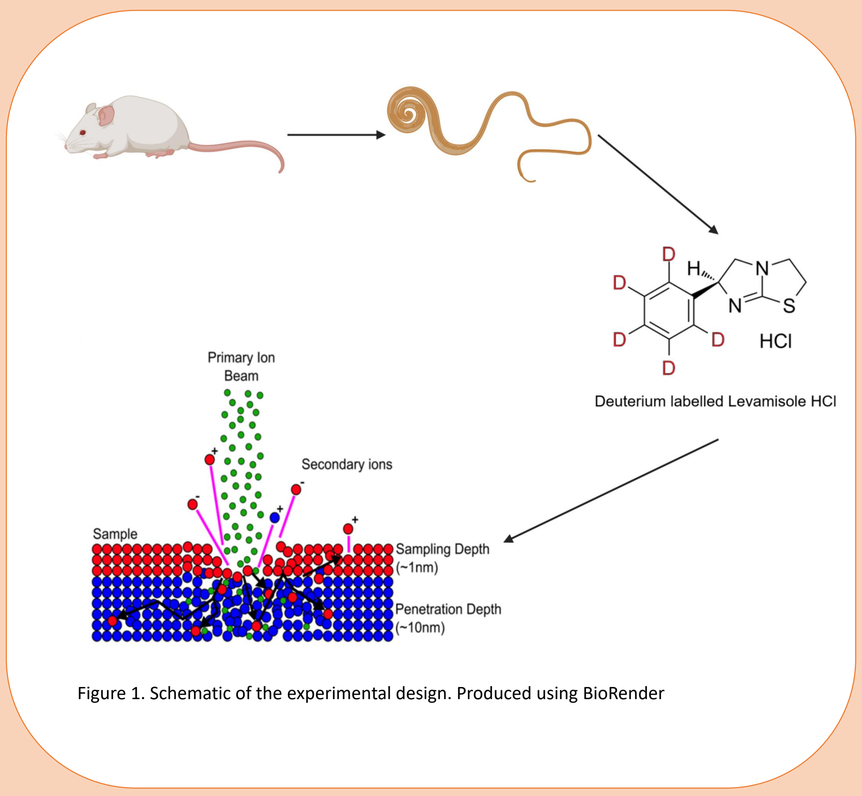

Figure 1. Schematic of the experimental design. Produced using BioRender

4. Results

3a.

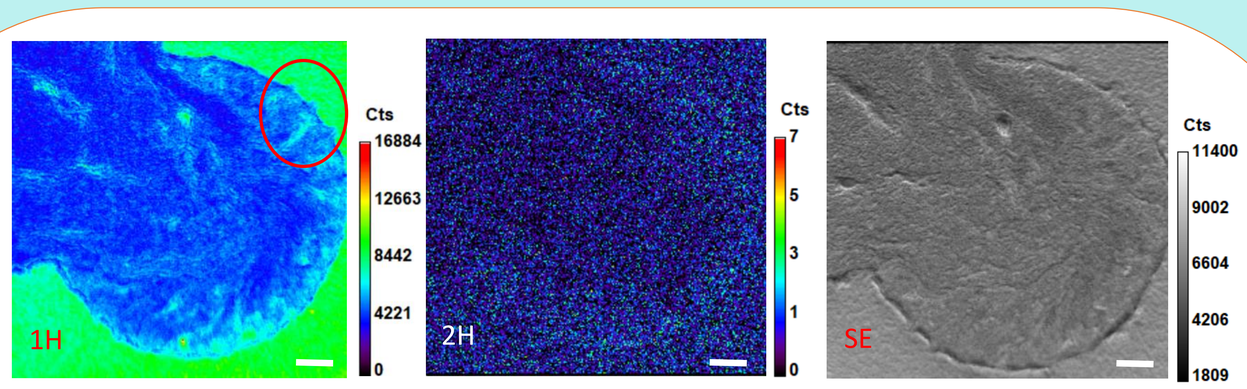

Figure 3a. High resolution NanoSIMS image of the anterior section of *T. muris* worms treated with deuterium labelled Levamisole. Circled are what appears to be the bacillary band pores. Detectors were aligned to $1H^-$,$2H^-$, the colour of each pixel indicates the number of secondary ions of $1H^-$ or $2H^-$ when the primary ion beam was scanned over than area of the sample. Secondary electron (SE) also shown. All scale bars are 5 µm.

3b.

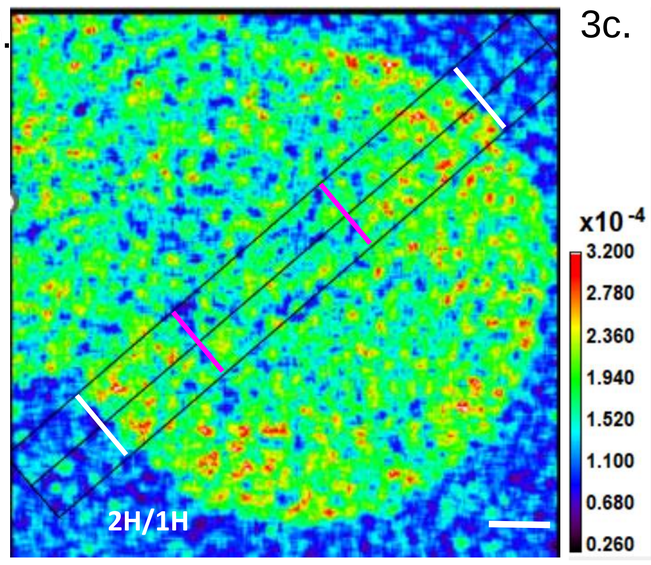

Figure 3b. A ratio of 2H counts and 1H counts was taken for each pixel in Figure 3a. Localisation of deuterium labelled Levamisole is indicated by an enrichment of 2H in comparison to 1H, i.e. an increase in the 2H/1H ratio. Blue area shows the resin which has no enrichment above the natural ratio, so no Levamisole. Inside the worm there is an increase in the ratio indicating Levamisole localisation. Scale bar is 5 µm.

3c.

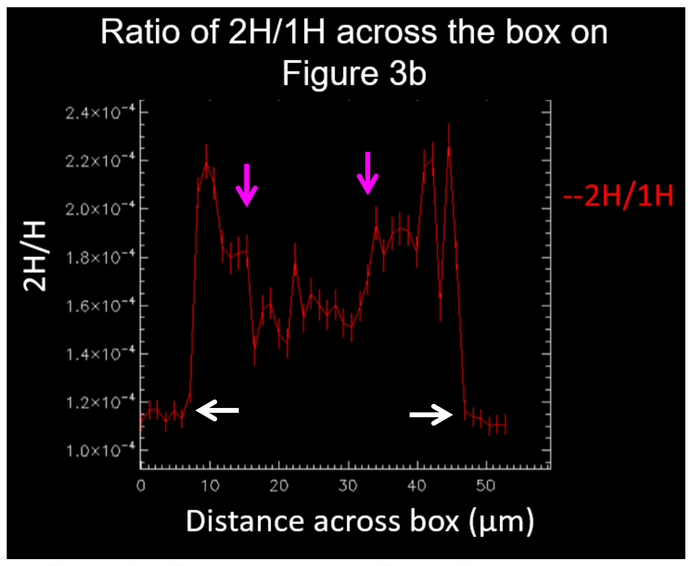

Figure 3c. The black box on Figure 3b was drawn, and the ratio of 2H/1H was recorded at each micron across the box. The ratio of the resin (white arrow) was the ratio of 2H found naturally (~0.015%). There is then an increase in the 2H/1H ratio at the hypodermal layer of the worm (between white and purple). This indicates that deuterated Levamisole is localised to this portion of the worm. There was also some enrichment in the centre of the worm (between purple) but lower enrichment than the hypodermal layer.

5. Conclusions

An enrichment of deuterium was found inside the anterior end of the worm when compared with the resin it was embedded in (Figure 3b/3c). This indicates Levamisole is present in the anterior end of the worm localising in the hypodermal layer. The protrusions circled in Figure 3a appear to be the bacillary band openings, as inferred by their size, shape and location. We conclude that:

1. We have successfully shown that detection of anthelminthics in *T. muris* is possible through NanoSIMS.
2. Levamisole accumulates in the anterior end of the hypodermal layer below the bacillary band.
3. Accumulation below the bacillary band could indicate this is a site of uptake.
4. Accumulation in the hypodermal layer suggests nicotinic acetylcholine receptors are present.

6. Future Work

- Further characterise the accumulation of Levamisole at different timepoints.
- Investigate any potential differences in localisation of the drug when deuterated Levamisole is applied *in vivo* to infected mice.
- Characterise the localisation of other anthelminthics, such as Mebendazole and Ivermectin to help inform on their lack of *in vitro* efficacy.
- Use NanoSIP with stable isotope labelled nutrients to elucidate the whipworm's feeding mechanism.

References

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Acknowledgments

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