Visualising the parasitic whipworm *Trichuris muris* using X-ray micro-computed tomography

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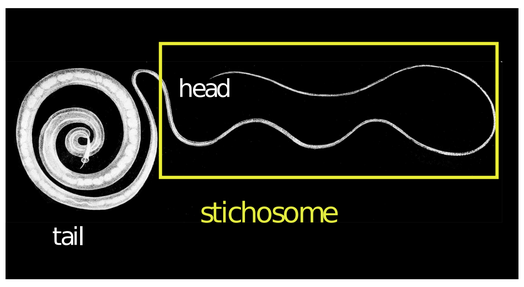
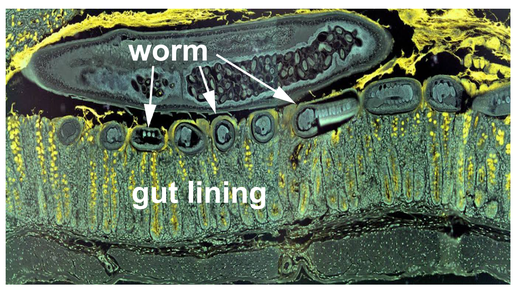
Background





• Whipworm parasites (left) infect the large intestine

• Human whipworm (*T. trichiura*) infects ~500 million worldwide

• The anterior of the worm contains the stichosome which is a hub for metabolic activity

• Eggs hatch in the gut and worms burrow through cells lining the gut creating a "mucosal tunnel"

• Above: pseudocolored cross-section of the mucosal tunnel of the mouse whipworm *T. muris*

• Little is known about *Trichuris* feeding and development

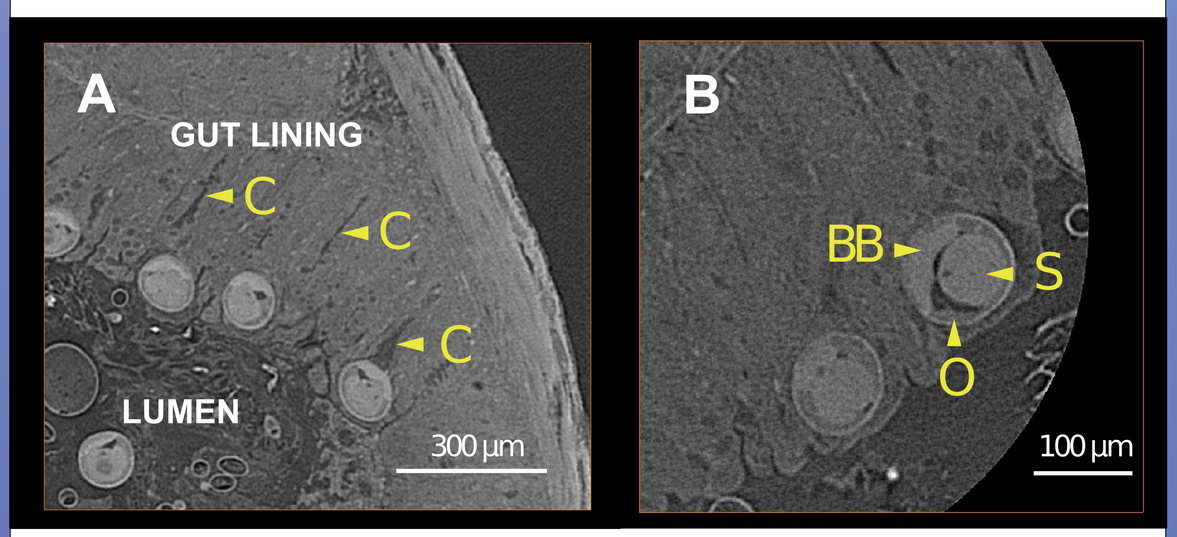
• Current drugs have poor efficacy; increasing our understanding of *Trichuris* lifestyle may inform new control strategies

• 2D imaging is damaging to the mucosal tunnel and thus provides limited insight, but non-destructive X-ray µCT can address the 3D relationship between the gut epithelium and the worm

Research Questions

1. Does an OsO_4 stain provide good contrast to view morphology?
2. How is the worm positioned in relation to the gut wall?
3. How much of the stichosome is covered by the mucosal tunnel?

1 Osmium tetroxide stains worms and gut effectively

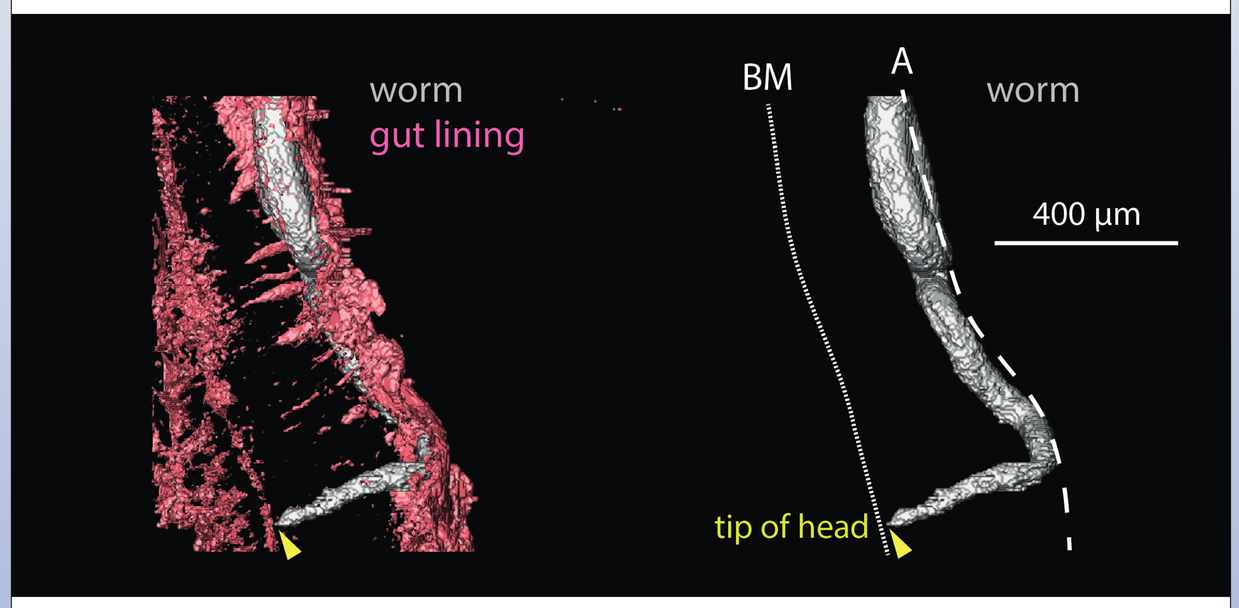


Orthoslices of two regions of a 0.5cm-long section of mouse gut infected with *T. muris*, showing host gut and parasite morphology. Dissected gut tissue was stained with 1% Osmium tetroxide and imaged with a Zeiss Xradia VersaXRM-520 tomograph (50kV, 4W).

Slice A: Crypts of Lieberkühn (C), which are secretory pits present in the gut wall. Worm larvae invade the gut at the base of these.

Slice B: higher magnification scan showing the stichosome (S), Bacillary Band (BB, involved in feeding) and Oesophagus (O).

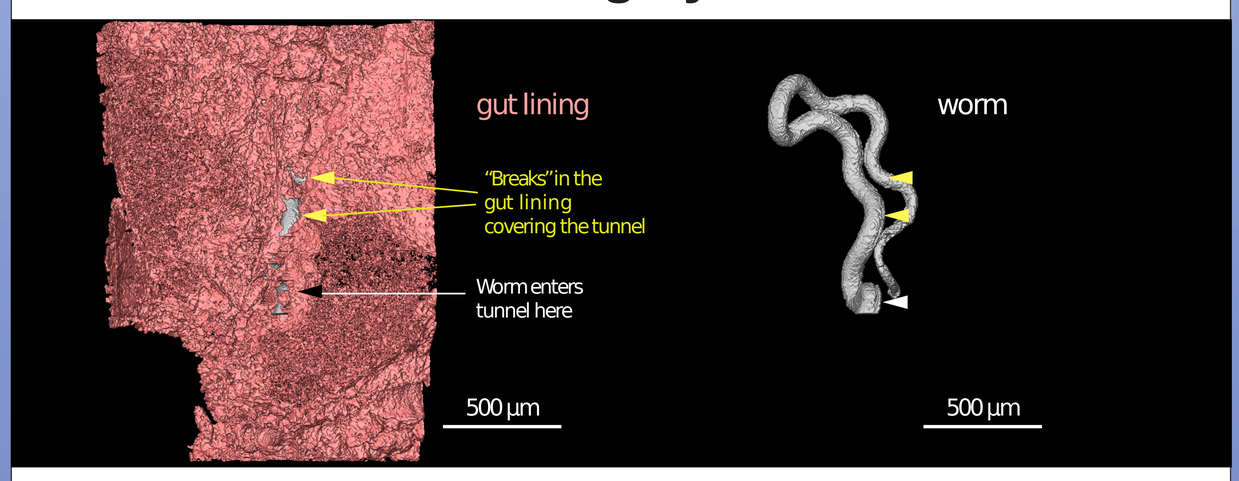
2 Worms 'burrow' towards the basement membrane



Images showing isosurfaces of a segmented worm (grey) with and without the host gut (pink). Images were segmented manually using AVIZO. White dotted lines indicate the approximate planes of the basement membrane (BM) and the absorptive surface of the gut (A).

4 out of 6 worms in the gut sample had heads turned down the crypts of Lieberkühn towards the basement membrane.

3 Worm stichosome coverage by the tunnel is highly variable



total tunnelled length including epithelial breaks (µm)	cumulative length of exposed stichosome (µm)	cumulative length of unexposed stichosome (µm)	proportion of stichosome exposed (%)
3141.42	182.85	2958.57	5.82
7757.46	0.00	7757.46	0.00
3994.84	696.88	3297.96	17.44

Images showing isosurfaces of a segmented worm (grey) with and without the host gut (epithelium, pink). Lengths of embedded portions of stichosome were estimated using AVIZO's native centerline tree algorithm and are displayed in the table above.

Conclusions

• X-ray tomography lends itself to visualising the 3D relationship between worm and gut. OsO_4 was an effective stain, but alternatives such as inorganic iodine and uranyl acetate may also be worth investigating

• The tendency of worms to "burrow" toward the basement membrane could be a behavioural adaptation to avoid expulsion by the host. Alternatively, worms may grow backwards from a fixed point

• High variability in epithelial coverage of worm may have implications for drug development - perhaps effective drugs will need to be able to pene-trate the mucosal tunnel so that the whole stichosome may be targeted

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