

An X-ray Micro-CT study in Bombus terrestris: how sublimated lodine enhances external and internal anatomical definition.



Woπderful Scieπtific

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Introduction

X-ray micro-CT is an established method for studying insects¹. Often, internal soft tissues have similar Xray attenuation values, causing investigators to resort to using contrast enhancing agents (CEAs) such as iodine in the form of either Lugol's iodine or lodine dissolved in 100% ethanol to enhance tissue definition². The results obtained can be excellent³ but there are often downsides.

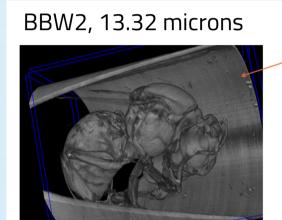
Iodine sublimination has previously been reported to enhance micro-CT image clarity in mammalian tissue⁴. Here, we show that exposing bumble bees (*Bombus terrestris*) to sublimated lodine (from 5 - 100 days) can enhance image tissue definition in whole-body insects.

Methodology

We collected 36 bees from commercial colonies (14 workers, 10 gynes, 10 males, and 2 queens), and 4 bees from the wild (2 spring foraging queens and 2 workers), stored them all at -80 °C. We then thawed and scanned the bees using a benchtop Skyscan 1072 X-ray scanner at isotropic interpixellar distances varying from 9 – 20 µm. 6 individuals were also scanned at 2-7 µm using a Neoscan80. Following the first scan each bee was placed in airtight plastic screw top bottles at room temperature either with (N=35) or without (N=3) their containing lodine crystals. The bee were rescanned after varying periods between 5 days and 106 days. Scans were reconstructed using NRECON and viewed using Disect and Tomomask

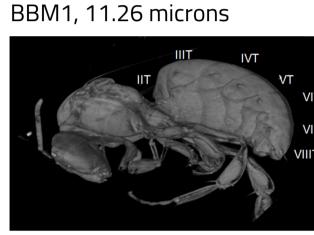
Results – Scans using Skyscan 1072

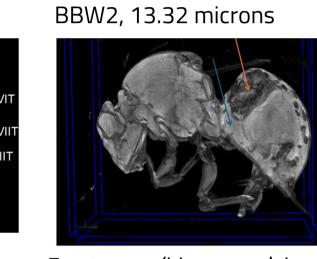
Before exposure to iodine vapour (Fig 1) most external bee features (where there was good X-ray contrast between the surrounding low attenuation air and the chitinous exoskeleton) were well seen except for the pile making the bees appear bald. Internal anatomical detail was limited, and fat stores were only seen in large gynes.

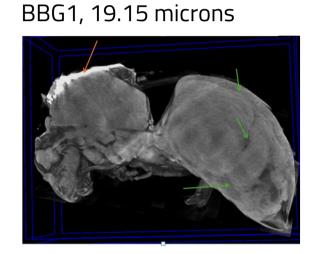


anatomical detail









Male features, spiracles, tergite /sternite segments well seen

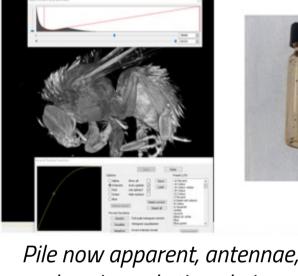
Empty crop (blue arrow), large Full crop, abdominal air sacks, abdominal air sacks (red arrow) parietal/visceral fat observed, tippex marker (red arrow)

Fig. 1 Unstained (i.e., no lodine) Bombus terrestris worker (BBW), male (BBM), and gyne (BBG) scanned with Skyscan 1072.

In bees rescanned after 1 week in iodine vapour (Fig. 2) pile became apparent, and where there was an air/chitin interface, lodine staining worked well (e.g., detail seen on antennae ,proboscis, sting recess (in females), and phallus (in males)). In contrast, internal staining of some structures were variable after 1 week (e.g., flight muscles, crop, proventricular valve, midgut, hind gut, ovarioles and Ventral Nerve Cord (VNC)). The trachea and air sacs were seen equally well before and after 1-3 weeks in lodine.

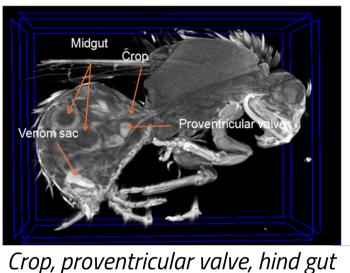
BBW2lo7, 12.2 microns – all images

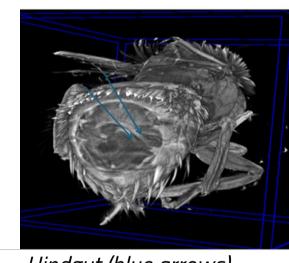
Pile not evident – but good external

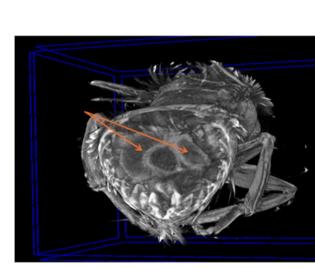








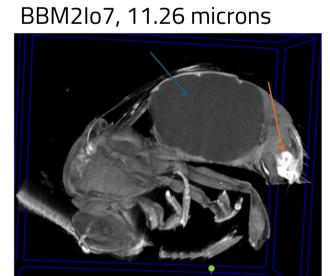




Hindgut (blue arrows) Midgut (red arrows)

proboscis, and sting obvious visible, VNC not seen Fig. 2 Bombus terrestris worker (BBW) scanned after 1 week in Iodine vapour (Io7) with Skyscan 1072 at 12.2 microns.

In the larger males, gynes and queen bees (Fig. 3) little evidence of internal staining was obtained at 7 days and most required periods of several weeks for optimal staining. Little extra staining was obtained after 50 days, and several bees scanned after this showed evidence of desiccation. The 3 bees left in the air-tight bottles but not exposed to iodine rapidly showed signs of internal decomposition and became covered by fungal hyphae (images not shown). Specimens exposed to iodine showed no such features.



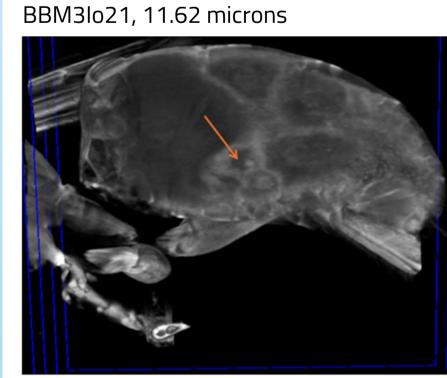
Male: fluid filled crop readily distinguished from other abdominal tissue (blue arrow). Stained endophallus (red arrow). Unlike workers, no staining of mid/hind gut

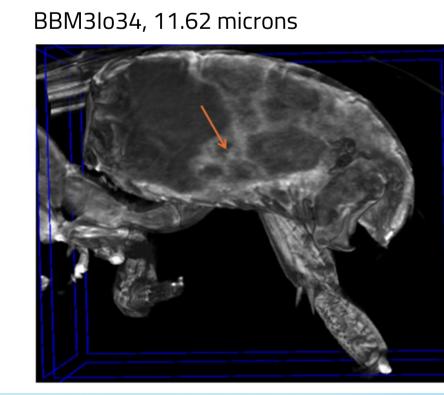
BBG1lo7, 13.32 microns

Gyne: large crop with low attenuation contents (red arrow), Proventricular gland (blue arrow) seen, mid/hind gut poorly seen

BBQ1lo7, 13.95 microns

Queen: poor internal staining at 1 week





Large crop. Proventricular valve (red arrows) in males different shape to queens, gynes, and workers

Fig. 3 Larger Bombus terrestris individuals including males (BBM), gynes (BBG), and queens (BBQ) scanned after 1 week in lodine vapour using with Skyscan 1072. BBM3 was scanned at 21 days, and 34 days.



Results continued – Scans using Neoscan 80

The best images were seen on the on the higher resolution NeoScan80 (Fig. 4 – 5). On this machine, and after 40 days in lodine vapour, features of the head, glands, and brain were seen in good detail (Fig. 4). Likewise, features of the thorax (including individual flight muscle fibres) and abdomen (including entire digestive and reproductive systems) were seen well (Fig. 5).

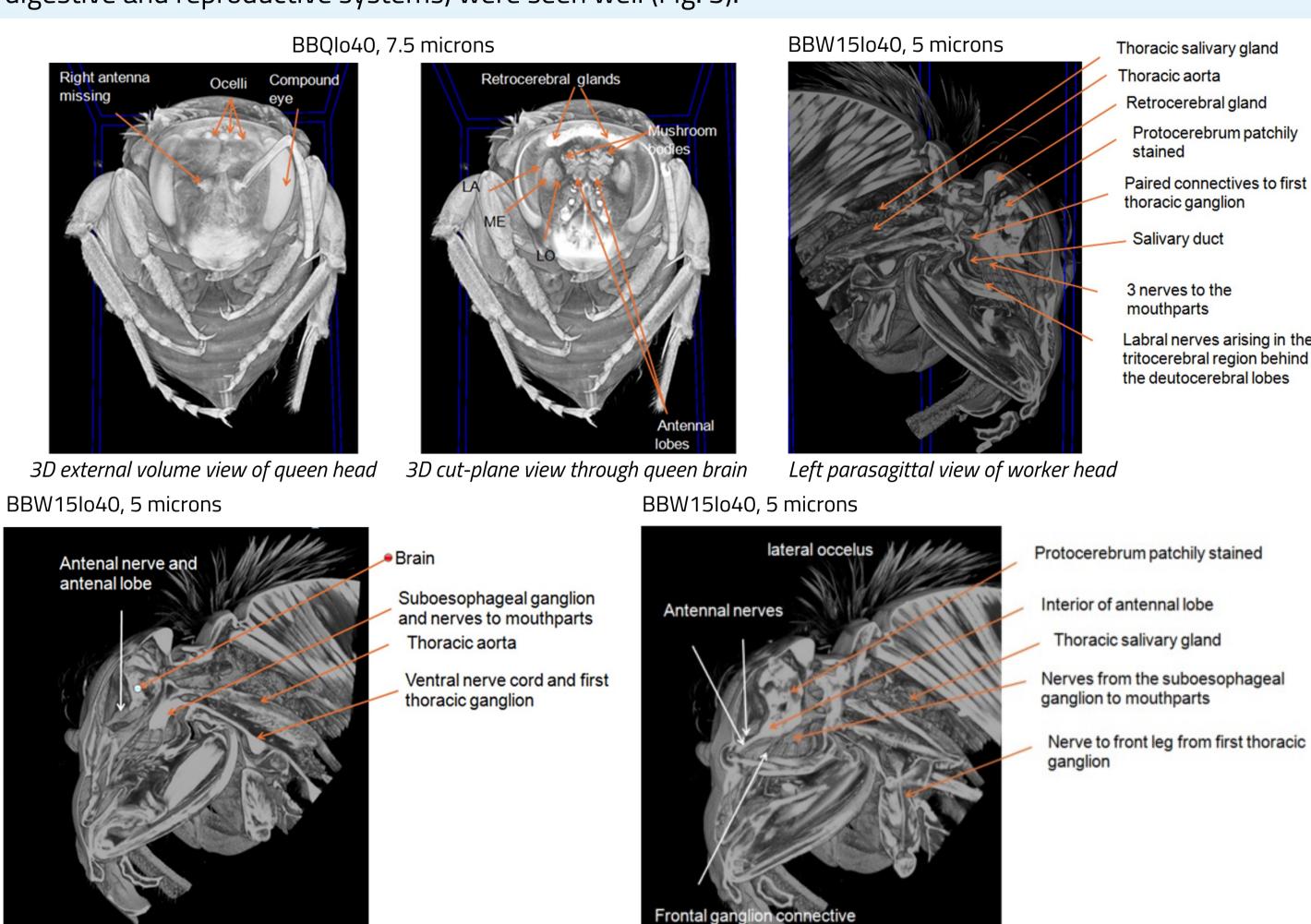
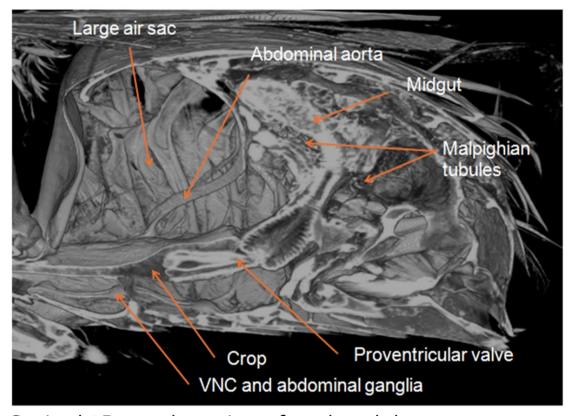


Fig. 4 Head scans of *Bombus terrestris* queens (BBQ) and workers (BBW) scanned after 40 days in Iodine vapour using Neoscan 80.

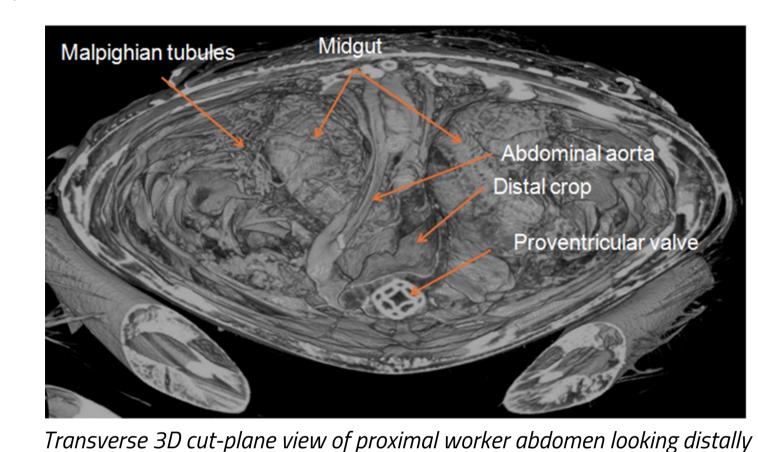
Right parasagittal view of worker head





Sagittal 3D cut-plane view of worker abdomen

Midline sagittal 3D cut-plane view of worker head



Brain BBQ2lo40, 7.5 microns Flight muscles Ovariolar ligaments BBQ2lo40, 7.5 microns Ovary with 4 Ovaries with ovarioles Proventricular VNC and abdominal Sting in sting recess

Sagittal 3D cut-plane view of queen whole body

3D dorsal cut-plane view of queen whole body

Fig. 5 Whole-body, and abdomen scans of *Bombus terrestris* including males, gynes, and queens scanned after 40 days in Iodine vapour using Neoscan 80.

Conclusion

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We have shown that sublimated iodine penetrates and stains biological tissues and is a useful adjunct to performing micro-CT in insects. We conjecture that sublimated iodine penetrates the internal tissues by direct passage through the exoskeleton and by diffusion through the spiracles, tracheal vessels and air sacs. Optimal staining is achieved more quickly in smaller individuals and would presumably be even quicker in much smaller insects such as Drosophila.

The method, while slow, has the advantage of preserving tracheal structure. The proventricular valve (the thickest and most muscular structure in the GI tract) proved a valuable marker between the crop and mid gut. We were able to confirm the previously reported⁶ marked differences between the shape of the proventricular valve of the male and female bees. The size and lengths of the midgut and hindgut was very similar to that described almost a 100 years ago in a related American *Bombus* species⁷.

Iodine exposed specimens showed remarkably little sign of desiccation or bacterial decomposition over the study period. Theoretically, quicker internal staining could be achieved with live anaesthetised bees because the iodine vapour would enter the tracheal system by active ventilation as well as passive diffusion.

References

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