Radiocontrast micro-CT imaging optimised for differential tissue segmentation in *Calliphora vicina* (blowfly)

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Keywords: Iodine, x-ray micro tomography, segmentation

The analysis of internal and external structures in whole animals is at the heart of a wide range of morphology based investigations. Detailed and reliable knowledge of morphological structures, their differences and relationships are crucial in fields ranging from animal development, comparative and behavioural morphology to systematics, biodiversity and evolution. Histological techniques employing conventional microscopy provide morphological data in high resolution, but suffer from drawbacks such as long preparation times, intricate staining and preparation procedures, which can introduce shrinkage and distortion. Additionally these techniques are destructive and limited to only one viewing plane per specimen [1]. Micro computed-tomography (CT) has been shown to be a powerful tool for anatomical and morphological investigations. It requires less time for preparation and can be used with the complete specimen, therefore allowing the study of morphological features in situ and in 3D. But its contrast and resolution are limited in soft tissue, such as muscle or neural tissue. Iodine in an aqueous solution has been shown to be a powerful contrasting agent for soft tissue in both vertebrates and invertebrates [1, 2, 3, 4]. Here we analyse for the first time the relationship between iodine concentration and soft tissue contrast in micro-CT for a number of important soft tissue types including muscle and neural tissue and provide an analysis of the relationship between iodine staining procedures and tissue contrast.

Adult *Calliphora vicina* were fixed in ethanol and then transferred into iodine solutions of different concentrations. Specimens were kept in the solutions for 2 days and then washed directly before scanning with 100% ethanol. The CT scans were performed using a Nikon HMX ST 225 system. The cans lasted 26 min each. To quantitatively assess the contrast between tissue and background as well as between different tissue types we determined the contrast-to-noise ratio (CNR), calculated as:

CNR = [mean(tissue) – mean(bg)] / sigma_squared(bg)]

Our study shows clearly that Iodine as a contrasting agent will enhance the contrast of the specimen for all Iodine concentration used independent of procedural detail when compared to unstained specimen (Fig. 1). This corroborates earlier findings [1, 2] by showing the efficiency of Iodine as a contrast agent for muscle tissue in micro-CT scans of both vertebrate and invertebrate preparations. Our study demonstrates that iodine in low to medium concentrations can enhance contrast between different tissue types. Both results are evident in the CNRs (Fig. 1) calculated for different tissues using different Iodine concentrations as well as in the scans themselves (Fig. 2, 3). Figure 1 clearly reveals that the CNR values for all tissue types increase with increasing iodine concentration throughout the range of iodine concentrations used. The largest contrasts between different tissue types are achieved with Iodine concentrations of 0.3 to 1.0%. Figure 3a shows a virtual section through the head capsule of a fly, the different regions of the brain can clearly be discriminated from each other. This tissue specific contrast allows the segmentation of different tissue types as shown in Figure 3b, in which we segmented the gut of the fly as well as the optic neuropiles and rendered them as 3D volumes in the outline of the scanned fly head. These advances make contrast enhanced microCT a powerful technique, complementing conventional morphological methods.

References

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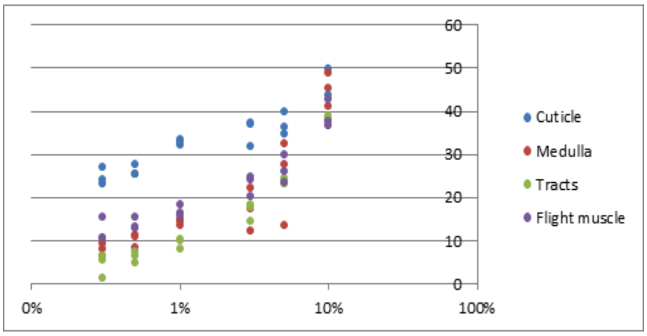


Figure 1) *CNR values of all tissue types increase with increasing Iodine concentration. Morphologically important tissue types, neuronal tissue (Medulla and neuronal tracts) muscle and cuticula are measured individually in three flies. The clusters of individual tissue types show the best separation for low to moderate concentrations.*

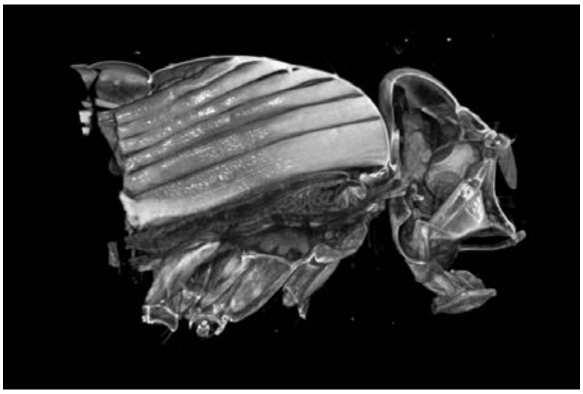
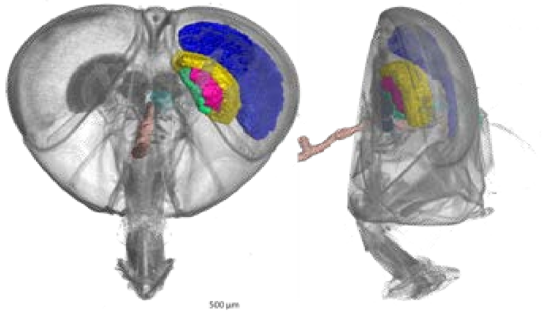


Figure 2*) A rendering of a scan of a male blowfly. The visualisation shows a longitudinal section through the thorax and head of the fly, the large flight muscles are prominent in the thorax. Rendering was performed using Drishti 2.0 (program developed by Ajay Limaye from Australian National University).*

a)



b)

Figure 3 *a) This example of a reconstructed slice through the head capsule of a fly shows high contrast between air, cuticle and soft tissue, scale bar 0.8 mm. b) Within the brain, intensity differences are sufficient for the manual segmentation of major brain regions, as well as nerves and other soft tissues, such as the oesophagus running through the brain. Sensory organs and cuticular tissues are also visible, and have been rendered to create an in-situ model of the fly brain. Colour code: Dark blue: lamina; Yellow: medulla; magenta: lobula plate; green: lobula; beige: gut.*