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3D Virtual Histology of Polychaetes Using Micro-CT

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Traditionally the only techniques available to investigate the internal anatomy of soft-bodied invertebrates were dissection and histological sectioning. These techniques are destructive and do not provide an accurate *in situ* representation of the internal anatomy. Micro-computed tomography (Micro-CT) has recently been shown to be an effective, non-destructive method to image the three-dimensional (3D) internal anatomy of an unstained polychaete, *Nephtys* [1]. The aim of this study was to determine if other families of polychaetes can be imaged without staining; and to determine if stains can be used to provide detailed images of the pharyngeal musculature.

Polychaetes from six families within the clade Aciculata were scanned in this study. The specimens were prepared for scanning and three were stained with phosphotungstic acid (PTA) or iodine. They were micro-CT scanned at the Natural History Museum, London (NHM), using the X-Tek HMX ST 225 scanner (Nikon Metrology, Tring), or at the Hellenic Centre for Marine Research (HCMR), using the Skyscan 1172 scanner (Bruker, Belgium). The specimens were scanned with either a molybdenum, silver or tungsten target, between 55-130kV and 160-200µA. Three-dimensional models were created, from the tomographic datasets, and manipulated using the Drishti software suite [2].

In the unstained specimens density gradients were observed on the greyscale level as a consequence of differences in tissue density and type. For example, the jaws of all unstained specimens were easily elucidated whereas the clarity of internal muscles is dependent upon their intrinsic density being higher than the alcohol preservative [3]. PTA is a protein stain and so preferentially stains muscles [4]. Therefore, specimens stained with PTA produced scans where the musculature was very clearly defined, (figure 1). Iodine appears to stain the tissue indiscriminately; therefore in iodine stained specimens all the internal structures appeared fully stained but it was difficult to differentiate between some tissue types (figure 2).

This technique has significant advantages over the traditional techniques of dissection and serial sectioning. It allows a 3D visualisation of the specimen that can be virtually sectioned in any angle or plane. The data can also be segmented to isolate individual structures, allowing them to be viewed separately from the surrounding tissue but still maintaining their *in situ* configuration. Whilst all the specimens could be imaged without staining, the level of detail was significantly lower than the scans of stained specimens. In the stained specimens, the entire pharyngeal muscle system could be imaged, although the definition between features was higher with PTA staining. The development of a wider variety of tissue-specific stains would increase the amount of information that can be extracted from micro-CT scans. Future technological advances in micro-CT, 3D rendering software, and computer processing will create higher quality data sets. Subsequently this will enable researchers to access and study soft bodied specimens more easily and quickly. It also has a major advantage over traditional methods that it is non-destructive; this allows us to scan rare specimens in collections, such as at the

NHM. This will allow scans of holotypes and rare specimens to be distributed electronically to researchers, around the world, without the risk of damage or loss of the specimen; allowing them to perform their own virtual histology on the same specimen [5]. Such an approach could come to play a major role in species identification, taxonomy and studies of functional anatomy.

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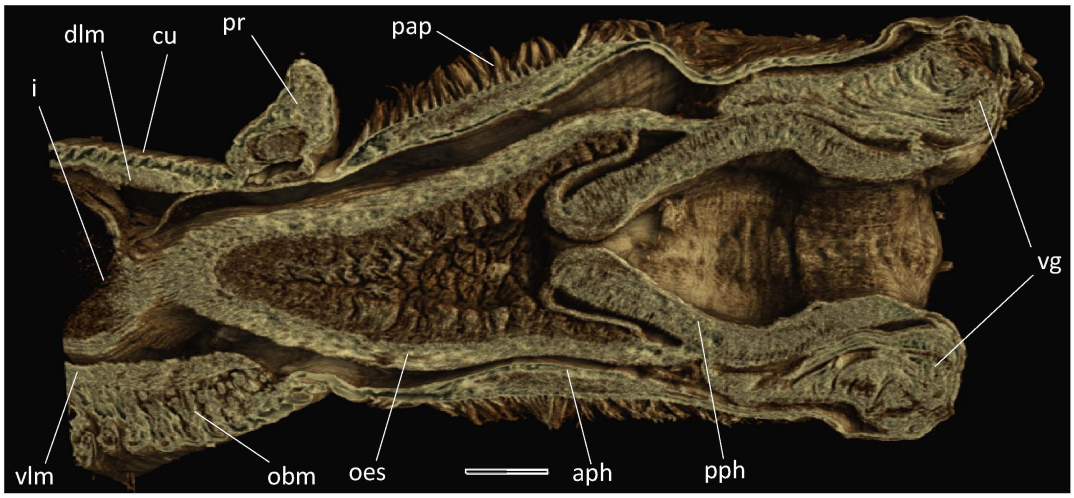

Figure 1. *Glycera tesselata*, with everted pharynx and stained with PTA, longitudinal section. (aph – anterior pharynx; cu – cuticle; dlm – dorsal longitudinal muscle; i – intestine; obm – oblique muscle; oes – oesophagus; pap – papillae; pph – posterior pharynx; pr – prostomium; t – jaw; vg – venom gland; vlm – ventral longitudinal muscles). Scale bar = 200µm.

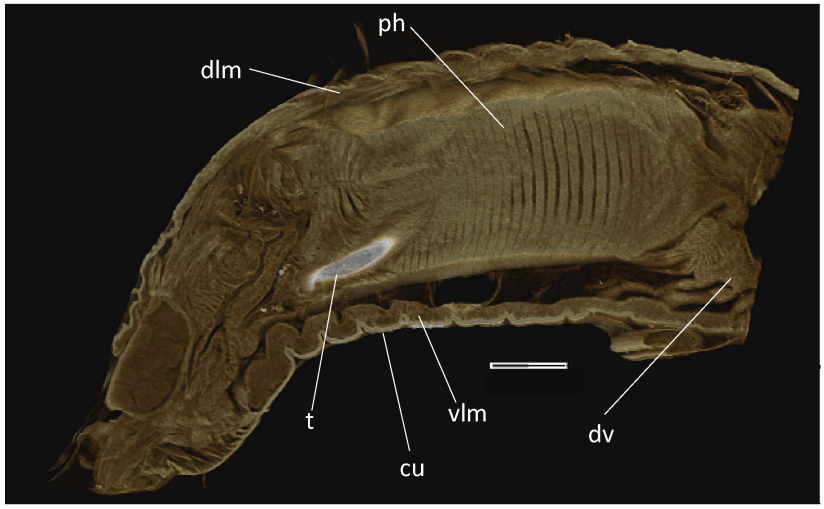

Figure 2. *Lepidonotus clava*, stained with iodine, longitudinal section. (cu – cuticle; dlm – dorsal longitudinal muscle; dv – diverticula (intestine); ph – pharynx; t – jaw; vlm – ventral longitudinal muscle). Scale bar = 100µm.

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