Biological materials present unique challenges for microscopic imaging techniques. In Scanning Electron Microscopy (SEM), uncoated biological materials may collect charge as they are scanned by the electron beam. This can reduce the overall scan quality and introduce artefacts into the dataset. This problem is largely overcome by employing variable pressure SEM. VPSEM offers nm resolution of external features, but unfortunately cannot visualise internal information. Confocal microscopy and micro-CT scanning offer avenues to visualise internal morphology, but are not without their own drawbacks. Confocal microscopy offers high resolution but can be limited with regards to sample size and penetration, and specimens may require specialist stains if they are not autofluorescent. However, when the sample is appropriate, the results are stunning! Micro-CT, whilst offering very good tissue penetration capabilities, presents problems for biological tissues, since their X-ray absorption coefficient is rather poor. As a result, the contrast displayed in the images tends to be low and thus complicates the reconstruction and subsequent interpretation of the 3D datasets that are produced. Lab based micro-CT scanning also falls well short of confocal and SEM imaging with regards to resolutions achieved.

Research ventures that combine these techniques facilitate discoveries in morphology and systematics, yet no single preparation method is currently applicable to all. Non-destructive and reversible preparations and techniques have the advantage of preserving type materials for future generations and future analyses, thus opening up the vast collections housed within regional and national museums across the world.

At the Natural History Museum (NHM) we are exploring methodologies which will allow us to employ all three techniques to investigate biological samples. Here I present the findings of a comparative study using various fixing, staining and drying techniques using simple crustacean models Artemia salina and Daphnia sp. In micro-CT, heavy metal stains like phosphotungstic acid (PTA), iodine and osmium tetroxide have been shown to differentially stain tissues and improve contrast by increasing the X-ray absorption of target tissues. But do these stains survive the drying processes necessary for SEM based work (e.g. nano-CT)? How do they compare to confocal datasets? Can the protocols be reversed to allow specimens to be returned to the Museum’s collections?
Fig. 1: Confocal micrograph of unstained female Artemia salina specimen. This is a composite image of 100 tiles, in a 10X10 grid, each scanned with a step size in z of 7.2µm for a total of 64 steps. The image was taken on the NHM’s Nikon A1SI Confocal Microscope system using a 10X objective lens to give a final resolution of 2.49µm per pixel.

Fig. 2: Rendered micro-CT data set of male Artemia salina specimen stained with iodine and PTA. Colours represent different tissue groups i.e. red-dense gut muscles; blue-finer musculature, lumen and nervous tissues. The specimen was scanned on the NHM’s Nikon HMXST 225 micro-CT system at 105kV, 90µA and 500ms exposure. Final resolution was 5µm per pixel.

Fig. 3: SEM image of the Artemia salina ciliated thoracopods. This image was acquired from an uncoated specimen using the NHM’s FEI Quata 650 ESEM FEG at 7kV and a chamber pressure of 70Pa. Final resolution is 3.3µm per pixel.