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doi:10.1017/S1431927618007730
Microsc. Microanal. 24 (Suppl 1), 2018


# 3D Modeling of the Stink Bug Alimentary System from Serial Resin Sections

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Stink bugs are rapidly emerging as a new pest of crop plants. In contrast to beetle larvae and caterpillars, which have a "tube-within-a-tube" alimentary system [1], stink bugs are more closely related to piercing and sucking plant pests, such as aphids, and so have a highly 3-dimensional, multi-chambered digestive system. In order to understand and characterize their digestive system, we chose to use resin-based embedding and sectioning due to the fidelity of the preservation, the high resolution of the section detail, and the wide range of length scales available. However, it is challenging to obtain 3D information from high resolution resin sections of relatively large specimens. In this study, we combined 2D image stitching, segmentation, and stacking to create a 3D model of the entire alimentary system of the brown stink bug (BSB) in a process generally known as Array Tomography [2].

BSB nymphs (~3x5mm) were fixed in 4% formaldehyde, dehydrated in graded ethanol, infiltrated with graded LR white, and heat polymerized. Half-micron serial sections were cut with a diamond knife and one section was collected onto a slide every 10 sections (5 microns); in total, we collected 171 sections through the central region of a BSB nymph. Sections were stained with toluidine blue, mounted, and imaged by RGB laser absorbance/transmittance with a Leica SP5 confocal microscope [3]. A motorized stage and automated image stitching routines in LAS-AF software were used to capture tiled images of entire sections at higher magnification. Features in these tiled images were used to align all the images into a Z-stack with TrakEM2. The chambers of the gut were then manually segmented, and the segmented image stack was rendered and exported into a video file for viewing and analysis.

The generation of a 3D model from the serial sections allowed us to understand the order and connectivity of the different chambers of the stink bug alimentary system. The stink bug digestive system is comprised of six distinct chambers (M1, M2, M3, M4b, M4, and hindgut; Figure 1A, 2B). Similar to serial section SEM (ssSEM), it is possible to re-examine the original slides and sections at higher magnification if study of the 3D model generated from the segmented 2D images yields features of interest and/or new hypotheses because the imaging process is non-destructive (unlike, for example, focused ion beam milling or serial block face SEM techniques). Study of the 3D model and the corresponding resin sections of stink bug allowed us to determine that there is a high degree of similarity between the alimentary system of stink bugs and the bean bug, *Riptortus pedestrus* [4]. In addition to the creation of the 3D model, the use of LR white sections allowed the study and analysis of fine features, such as the microvilli lining the cells of the M1 chamber (Figure 1B), the bacterial symbionts that reside in M4 gut chamber (Figure 1C), and the constriction zones between different gut chambers (Figure 1D). In summary, while TrakEM2 was written and is mainly used for the 3D analysis of serial sections by electron microscopy, we were able to use it successfully to stack, segment, and render 3D volumes from toluidine-stained semi-thin sections at the light level.

References:

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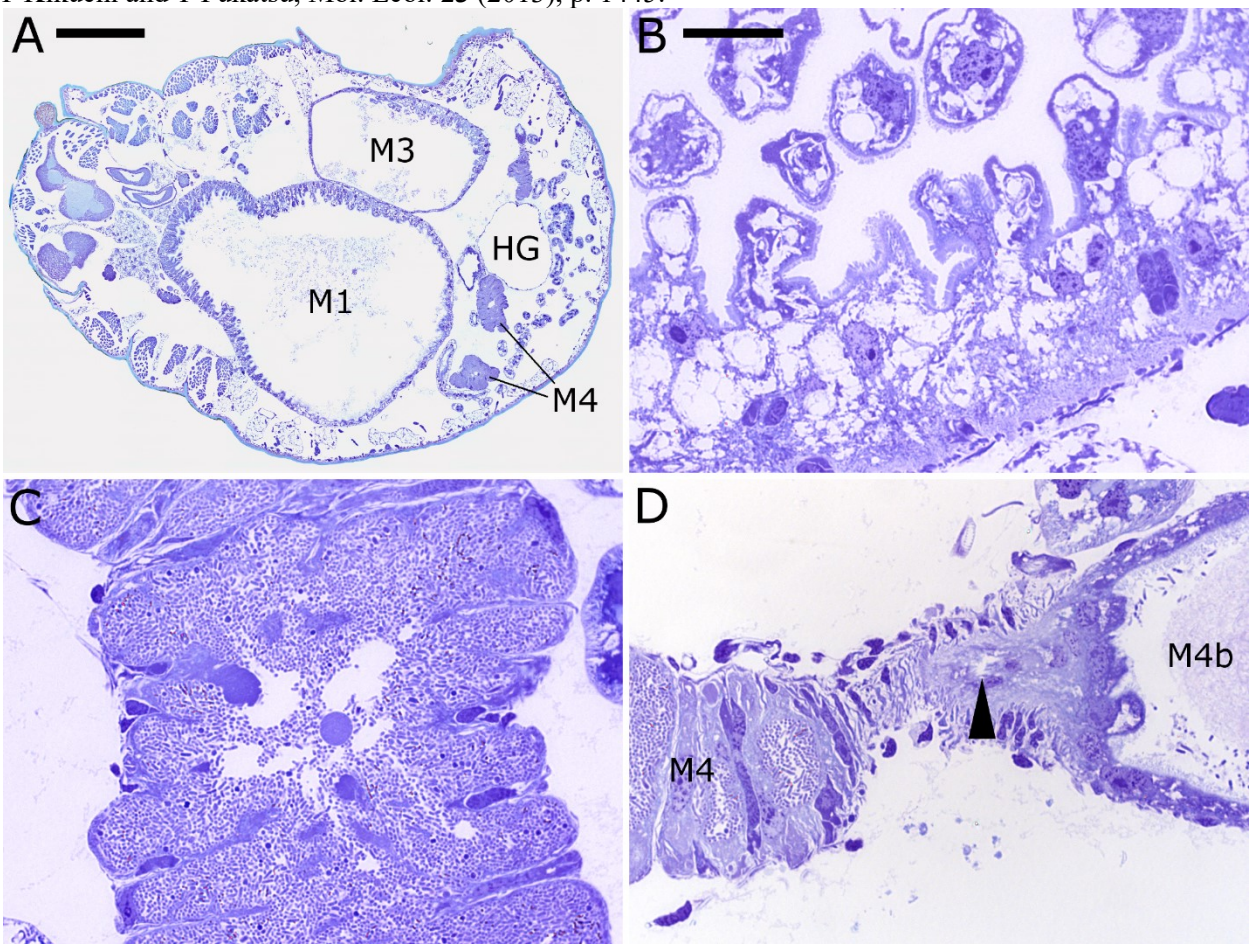


**Figure 1:** 2D images of BSB. A.) A stitched image of an entire section of a BSB nymph, showing several chambers of the alimentary tract; however, no direct evidence of connectivity is visible in this image. B.) The constriction zone (arrowhead) between the M3 and M4B gut chambers. C.) The M4 chamber is full of symbiotic bacteria. D.) The cells lining the M1 chamber have many fine microvilli on their apical/lumenal surfaces. HG = hindgut. Scale bars = A - 250 µm, B, C, D - 20 µm.

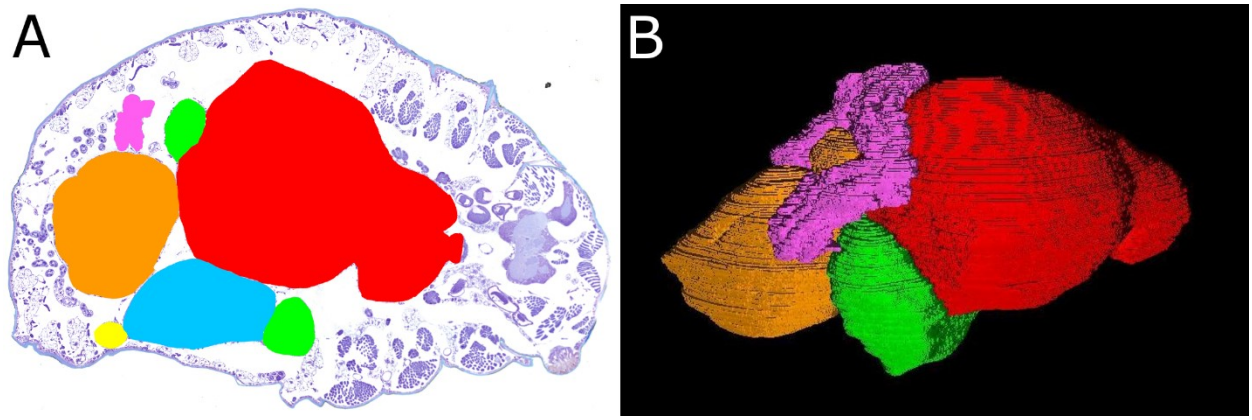


**Figure 2:** 3D image creation process. A.) A stitched image of an entire BSB section with the individual chambers of the gut manually segmented. B.) A 3D rendering of an entire BSB alimentary system from manually segmented images of serial sections.

https://doi.org/10.1017/S1431927618007730 Published online by Cambridge University Press