Making Data Interactive: 3D EM of Dividing Cells in a Regenerating Planarian

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We understand the world around us by using as many of our senses as we can to tackle a problem, why not apply that curiosity and approach to our work as microscopists?

From materials to biology, the images we take of data are two dimensional representations of the 3-dimensional physical specimen we are imaging. For quite some time we’ve been able to combine this xy planar data with z sections taken via a confocal microscope, or more recently with higher resolution using a serial blockface SEM (SBFSEM) to get 3-D data related to the question at hand. When it comes to visualizing this 3D data there are many options, yet all these methods are arguably rendered in two dimensions and lean heavily on engaging only one of the senses, sight.

Here we explore the morphologies of dividing stem cells in the context of a regenerative flatworm, \textit{Schmidtea mediterranea} by incorporating sight and touch, displaying 3D biological SBFSEM data as 3D prints. To enrich for dividing cells in our dataset, we imaged both intact developing embryos and regenerating adults. The regenerating adults were amputated post-pharyngeally across the whole animal to incite a regeneration response and fixed 48 hours after amputation where there is an increase in cell division particularly in the anterior fragment near the wound site [1].

Regenerating planarian samples were processed for 3View by staining with reduced osmium, thioarbohydrazide, and aqueous osmium tetroxide, further staining with uranyl acetate and lead citrate, dehydration through a graded series of acetone, and infiltration and curing in a hard formulation of Spurr’s resin [Electron Microscopy Sciences]. Images were acquired on a Zeiss Merlin with Gatan’s 3View 2XP system using a Diatome knife at 15 nm pixels and 60nm slices using Zeiss’ Focal Charge Compensator at 45% nitrogen to reduce charging. Stacks were aligned and mitotic cells were modeled using IMOD [2] and further visualized in Blender or Rhino. The 3D models output by IMOD are sliced in Cura to create g-code for the printers. These models were printed using FDM (fused deposition modeling) with PLA (polylactic acid) on Lulzbot’s Taz6.

Moving forward we using this method of displaying information to interrogate the nuances of cell division in the context of the microenvironment within the planarians to better understand their ability to regenerate.

References:


Figure 1. Blender render showing asymmetrical dividing cell that is 25um across long axis.

Figure 2. Same asymmetrical dividing cell 3D printed shown from a top down view, mounted to a muscle cell using magnets to show context within the animal. This was printed using fused deposition modeling (FDM) with Polysmooth PLA (polylactic acid) by Polymaker, a plastic that allows the surface layer to be smoothed over with a vapor of isopropyl alcohol.