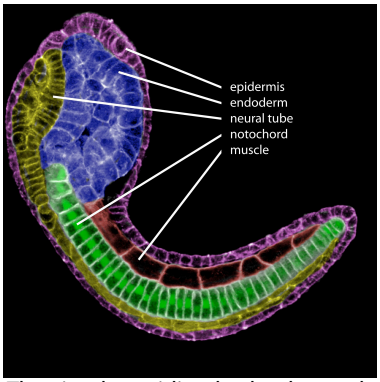
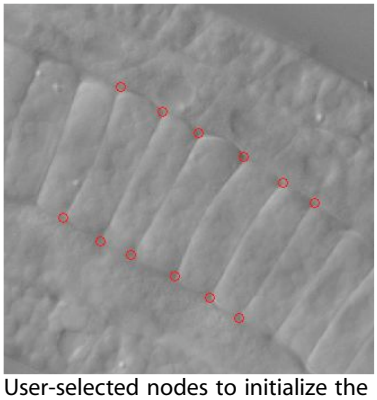
Morphomic Analysis of a Simple Chordate

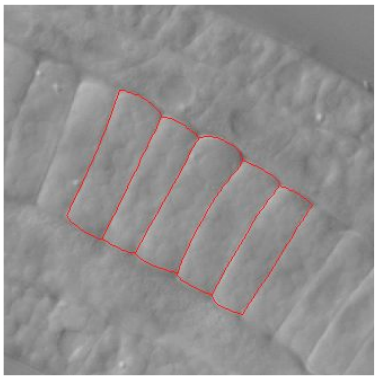
Michael Veeman*, Boguslaw Obara*, Jae Hyeok Choi*, William Smith and B.S. Manjunath



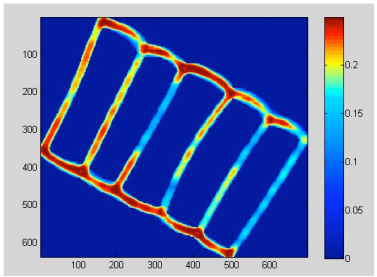
The simple ascidian body plan and tissue architecture



User-selected nodes to initialize the network of cell boundaries



Final segmentation after snake evolution



Local Motility Map showing the standard deviation of distance from each cell's centroid to the periphery calculated over 5 successive frames

A quantitative systems-level understanding of chordate morphogenesis will require the acquisition and analysis of extremely large multi-dimensional (3D plus time sequence) image sets with the challenging requirements of having sufficient resolution to capture the behavior of single cells while still encompassing entire developing tissues or organisms. The small size, small cell number, simple tissue architecture and high optical clarity of the ascidian *Ascidiella aspersa* provide a unique opportunity to capture key aspects of chordate morphogenesis with both high spatiotemporal resolution and an embryo-wide field of view. Here we present several Differential Interference Contrast (DIC) 2D timelapse datasets of ascidian notochord morphogenesis with sufficient temporal resolution (1 frame every 15 seconds) to smoothly capture the rapidly moving cell protrusions thought to drive many morphogenetic processes.

Although DIC imaging is widely used for its excellent optical sectioning abilities on unlabelled tissues and extremely fast exposure times, it is not straightforward from an image processing perspective as it does not provide a direct mapping of cell edges. A reasonable, albeit gappy, edge map can be provided, however, by convolution with directional derivatives of the Gaussian, and individual notochord cells segmented using "network snakes" (Butenuth, 2006), a modified parametric active contour that can be initialized as a network of given topology. Although originally developed to segment checkerboard patterns of roads and fields in aerial imagery, the network snake concept is also extremely effective at segmenting cells in a tightly packed tissue as the manual initialization of network topology guarantees that the image will be partitioned into the expected number of regions. As long as the network of cell boundaries does not change topology, timelapse segmentation requires only the iterative use of the final segmentation of one frame as the network initialization for the subsequent frame.

In addition to the many standard parameters that can be derived from the segmented data, a mapping of strictly local cell motility can be generated by measuring the standard deviation of the distance of each point on a cell's periphery with respect to that cell's centroid. For the post-intercalation notochord, this local motility map provides a quantitative and compelling visualization of an unexpected degree of cell motility at the notochord boundary. We are currently testing how this unexpected lateral motility relates to the ongoing narrowing and lengthening of the tail.

This work was supported by NSF awards ITR-033169 and EY-00888 to BSM, NIH awards HD038701 and GM075049 to WS and a Santa Barbara Foundation/Tri-Counties Blood Bank fellowship to MV.

BO, JHC and BSM are with the Center for BioImage Informatics, Department of Electrical and Computer Engineering, University of California Santa Barbara, USA. Email: {obara,jaehyok, manj}@ece.ucsb.edu. MV and WS are with the Department of Molecular, Cell and Developmental Biology, University of California Santa Barbara, USA. email:{veeman,w_smith}@lifesci.ucsb.edu

* MV, BO and JHC contributed equally to this work