3D Reconstruction and Visualization of the Developing *Drosophila* Wing Imaginal Disc at Cellular Resolution

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I. INTRODUCTION

Biological tissues display a large diversity in shape and size. The size and shape of tissues depend on the number, size, shape, and arrangement of the constituting cells. To better understand the mechanisms that guide tissues into their final shape, it is important to investigate the cellular arrangement within tissues. To this end we are studying the epithelial morphogenesis of the developing wing of the fruit fly *Drosophila melanogaster* at the late larval stage. The *Drosophila* wing develops from the central region of the wing imaginal disc, a simple single cell-layered epithelium. The goal of our research is to create a detailed 3D model of the individual cells in the epithelium. To date, 3D models of the wing imaginal disc have been created, and the apicolateral cell boundaries have been identified, allowing for the calculation of cell parameters, e.g. apical cross-sectional area of cells.

The 3D models are being generated from data derived from confocal image stacks of the central region of larval wing imaginal discs stained for E-cadherin, a marker of adherens junctions, and phalloidin, a marker of filamentous (F-) actin. These image stacks visualize both the adherens junctions present at the apicolateral side of cells, as well as the entire cell outlines as identified by the F-actin meshwork underlying the cell plasma membrane. The images have a resolution of 0.155 μm/pixel, with an inter-image distance of 1 μm. Since our initial work focuses on generating 3D models of the cell boundaries on the apical surface, we process the blue channel (E-cadherin stain) of the images, which highlights the disc's apical surface. See Figure 1.

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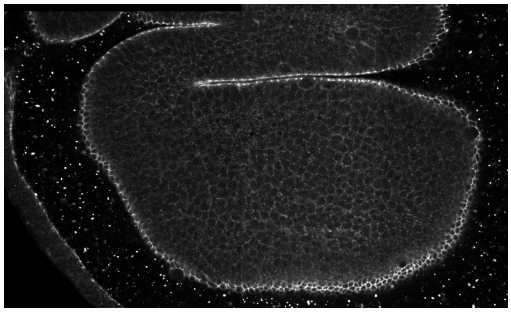

Fig. 1. A single blue-channel image from the confocal image stack of the wing imaginal disc stained for E-cadherin.

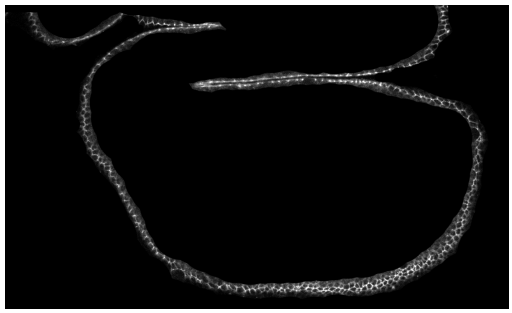

Fig. 2. The image has been manually edited in order to isolate the apical surface highlighted by the E-cadherin stain.

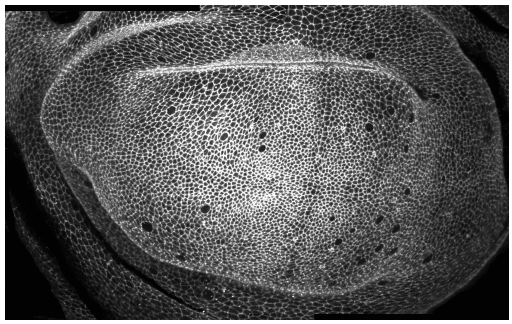

Fig. 3. The highlighted outlines of the apical surface present in the edited image stack are merged together into a single image.

II. METHODS

3D models and visualizations of the wing imaginal disc are produced via the following steps.

- Individual blue-channel images are manually edited to isolate the stained and highlighted adherens junctions on the apicolateral surface. See Figure 2.
- The stack of edited images are merged into a single image using a maximum intensity projection. This results in an image that displays the two-dimensional cell mesh consisting of the network of adherens junctions on the tissue's apical surface. See Figure 3.
- A 2D geometric model of the cell mesh is extracted from the image using the program "packing analyzer v2.0" [1], producing the 2D coordinates of the vertices of the mesh. See Figure 4.

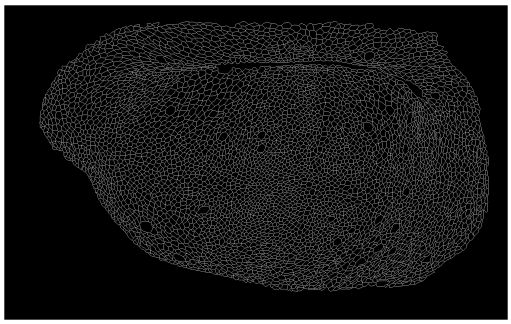

Fig. 4. A 2D geometric description of the cell boundaries is derived from the merged image.

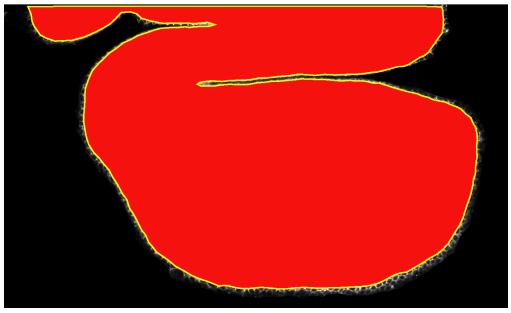

Fig. 5. The edited images (e.g. Figure 2) are manually contoured and segmented to identify the interior of the imaginal disc.

- The edited images (e.g. Figure 2) are manually contoured, then filled, to produce a stack of images that define the surface and interior of the wing imaginal disc. See Figure 5.
- The filled image stack is the input to a 3D surface reconstruction method that utilizes MPU implicit functions [2] to create a 3D model which approximately fits to the contours embedded in the stack. See Figure 6.
- The 2D cell mesh (Figure 4) is projected onto the upper (apical) surface of the reconstructed imaginal disc model to produce a 3D model of the apical cell boundaries.
- The areas of the indivdual 3D cell apical faces are calculated and mapped to the Hue channel of the HSV color space with the formula

  $$((max\_area - area)/(max\_area - min\_area))^2 * 240.$$

  The resulting color distribution ranges from dark blue for the smallest cells to bright red for the largest. The quotient is squared in order to produce a greater color spread in the blue to green range, which provides better differentiation of the cells in the center of the disc. See Figure 7.

III. CONCLUSIONS AND FUTURE WORK

The color-shaded 3D cell mesh produced by our method demonstrates that cells at the center of the wing imaginal disc, in close proximity to the signaling sources of the morphogens *Dpp* and *Wingless*, are apically more constricted than cells further away from the sources. Together with functional experiments [3], [4], this shows the direct influence

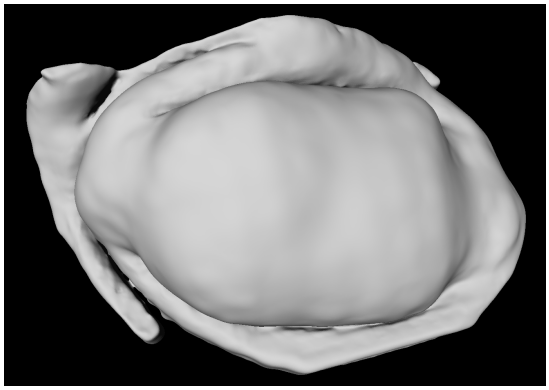

Fig. 6. 3D surface model of the wing imaginal disc produced from a stack of contoured and segmented confocal microscopy images.

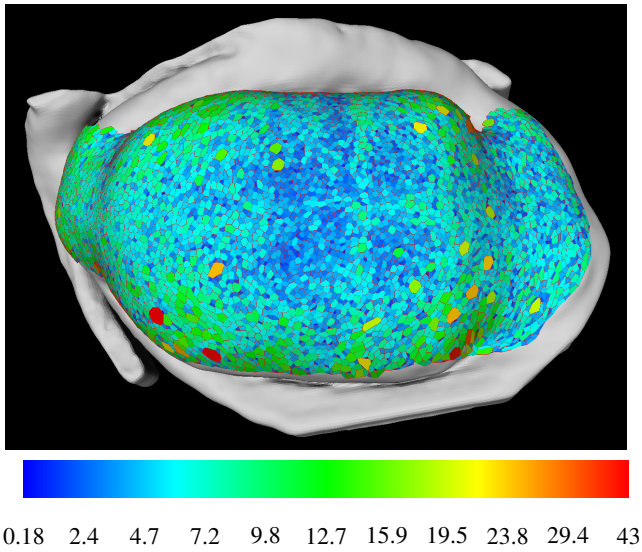

Fig. 7. Projected cell mesh displayed with the reconstructed surface. The cells' apical faces have been color-shaded as a function of their area. The area units are microns squared.

of the morphogen signals on the shape of the cells. Thus, morphogens may control not only the identity, but also the shape of their target cells.

Future work will involve projecting and deforming the cell mesh from the apical to the basal surface in order to produce 3D models of the disc's individual epithelial cells.

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