



## **Eyer: Automated counting of ommatidia using image processing techniques.**

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### **Introduction**

The *UAS/GAL4* ectopic expression system (reviewed in Brand *et al.*, 1994) is a widely used tool for the overexpression of transgenes in *Drosophila melanogaster*. This bipartite expression system allows for the expression of transgenes in specific tissues, including the *Drosophila* eye using the *glass multiple reporter (GMR)-GAL4* driver line (Freeman, 1996). During this expression, it is of interest to measure differences in morphological characteristics such as ommatidia number, ommatidial area, and overall area of the eye. This measurement is both time consuming and labor intensive given the number of *Drosophila* required for analysis and the limitations of currently available programs. Here, we describe a novel program designed for simple, morphological analysis such as counting and area measurements. Although initially designed for analysis of the *Drosophila* eye, this program could be used for analysis of various tissues, and could be used for other applications apart from expression studies.

### **Design of Program**

#### *Software*

The Eyer software was developed as the user interface for a segmentation algorithm optimized for the *Drosophila* eye. It was written for the Windows platform using Visual Studio C# and WPF. The image processing routines used by Eyer are performed by the AForge.NET open-source imaging library and the Vincent-Soille immersion watershed algorithm. The program was developed using images at a resolution of 1.33 pixels per micrometer. This equates to ommatidia that are approximately 20 pixels in diameter at the center of the eye in adult *Drosophila*.

#### *User interface*

The Eyer software is a single purpose application, which allows for a completely tailored interface to make the eye analysis task as quick and easy as possible. Instead of opening individual image files, a folder is selected for editing, and all images are shown as a list of thumbnails. The user can switch between active images by clicking on its thumbnail. An image can be easily panned and zoomed via mouse or keyboard. All measurements carried out on an image are automatically recorded in an associated text file and stored with the image in the original folder.

#### *Algorithm*

There are several challenges presented to standard segmentation algorithms by the images of *Drosophila* eyes. The convex nature of the eye results in ommatidia size and shape variations across the eye leading to partially obscured ommatidia around the edge. The lighting used when taking

SEM images can vary over the surface of the eye and also from image to image. Light spots and shadows cause the intensity of an ommatidium to be unpredictable. The bristles that grow between *Drosophila* ommatidia reflect intense light, creating further challenges for a segmentation algorithm. These bristles, from an image processing perspective, appear to split many of the ommatidia while following along the edge of others. For these reasons, the goal set for the development of the segmentation algorithm was not to segment the eye completely, but to segment the largest area possible with the highest accuracy. The portion of the eye not segmented by the software is then completed manually.

The solution to the segmentation problem described above required a set of processes that remove color variation and highlight the ommatidia edges uniformly. Once that had been accomplished, an immersion watershed algorithm was used to separate and mark each ommatidium. The complete algorithm can be described in five steps. (1) *Contrast Enhancement*: This is achieved with a combination of histogram equalization and contrast stretching. (2) *Edge Detection*: Both horizontal and vertical edges are detected with a standard edge detection routine. The edge detection results are added to the contrast-improved image to further distinguish the lines around the ommatidia from the centers. (3) *Local Thresholding*: Removal of all color intensity to create a black and white image. The typical conversion method uses a threshold value, where intensities above that value are white, and below are black. Since the intensity of the ommatidia varies over the surface of the eye, an adaptive local threshold has to be used, where the value of the threshold changes for each pixel, dependent on the surrounding area. After this step most edges are visible; however, many ommatidia still appear joined. (4) *Watershed*: The watershed algorithm is able to separate connected blobs. This step processes the image as if it is a topographic relief, identifying locations where water would pool if poured onto the image. (5) *Blob Analysis*: The resulting watershed lines are then used to segment the eye. The list of segmented objects is filtered by size and shape by a blob analysis package to determine which objects are valid ommatidia.

## Sample Analysis and Discussion

### *Drosophila*

Flies of various genotypes were aged three days past eclosion and frozen at  $-80^{\circ}\text{C}$ . Flies were mounted, desiccated overnight, and coated in gold before photography at 170 times magnification with a Hitachi S-570 scanning electron microscope as per standard methods.

### *Area*

To measure area, or to segment an image for counting purposes, the region of interest (ROI) must be defined by the user. The ROI tool creates a point based polygon where points can be easily added and removed while defining the area. The polygon can be made with as many points as needed, resulting in a clearly defined area without the labor intensive process of using a freehand tool. The total area of the eye is calculated in real time and recorded in the associated text file as mentioned.

### *Counting*

The resolution of the image affects the performance and speed of the segmentation required for counting. Higher resolutions will result in a higher segmentation percentage, with a reduction in speed.

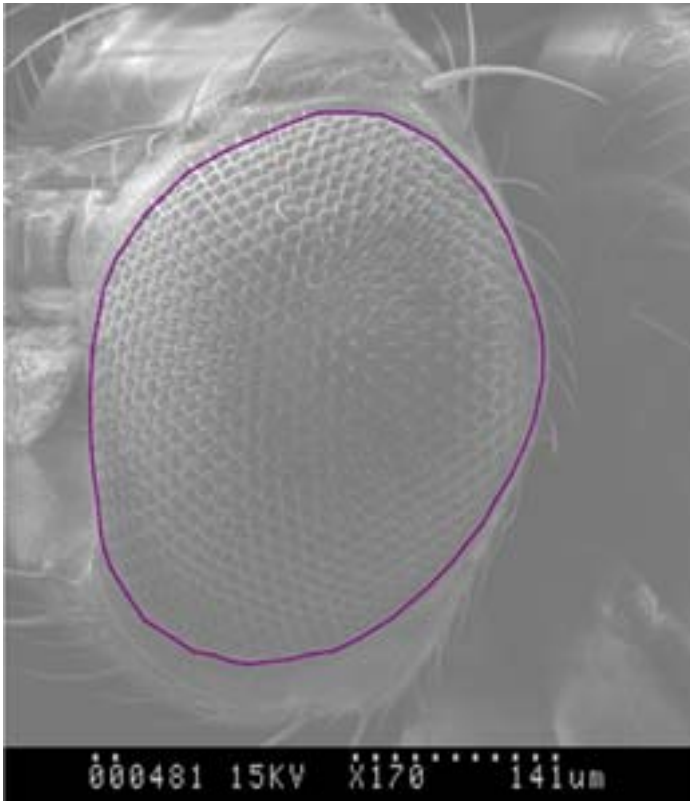


Figure 1. Original image with user-defined ROI.

The contrast enhancement performed by Eyer can be applied to the entire image separately to improve the visibility of detail on the subject. This was found to be useful when examining features such as the overall area of the eye.

After the segmentation process, identified ommatidia are marked with red dots. In this example, segmentation took 4.8 seconds to complete, and it completed with 561 of 688 ommatidia correctly marked. Four dots were placed incorrectly. The four false positives and remaining unmarked ommatidia can be completed manually before the results are saved.

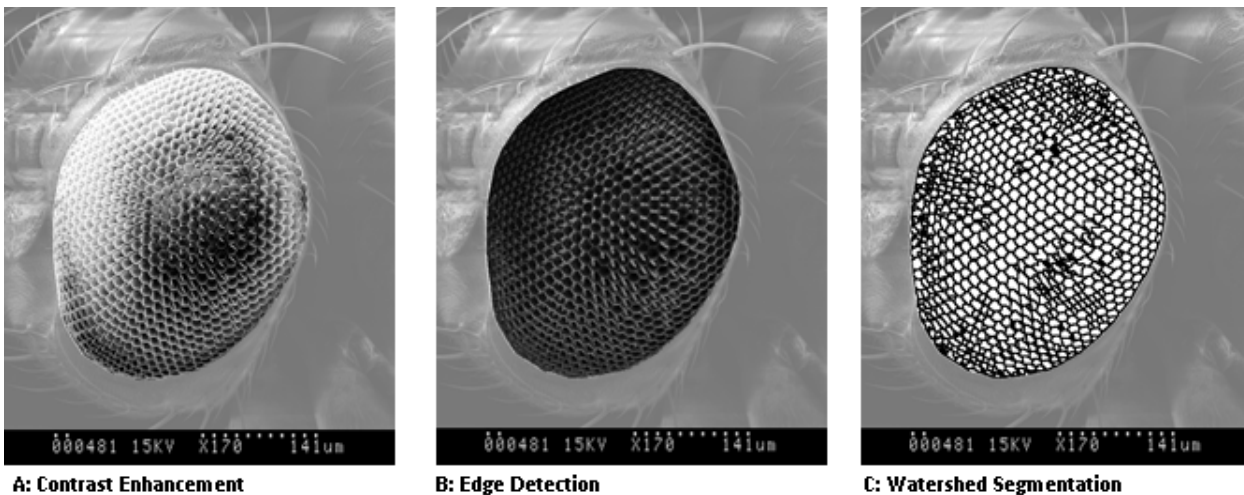


Figure 2. Image processing stages.

## Conclusions

Morphological analysis in *Drosophila* is widely used during expression studies to investigate gene and protein function. In the *Drosophila* eye, measurement of characteristics such as ommatidia number, ommatidial area, and area of the eye are often time consuming and labor intensive. Our novel program, Eyer, has been shown to be a valuable tool for simple morphological analysis of the

*Drosophila* eye. This program could reduce the time needed for morphological analysis of various *Drosophila* tissues, as well as tissues in other organisms.

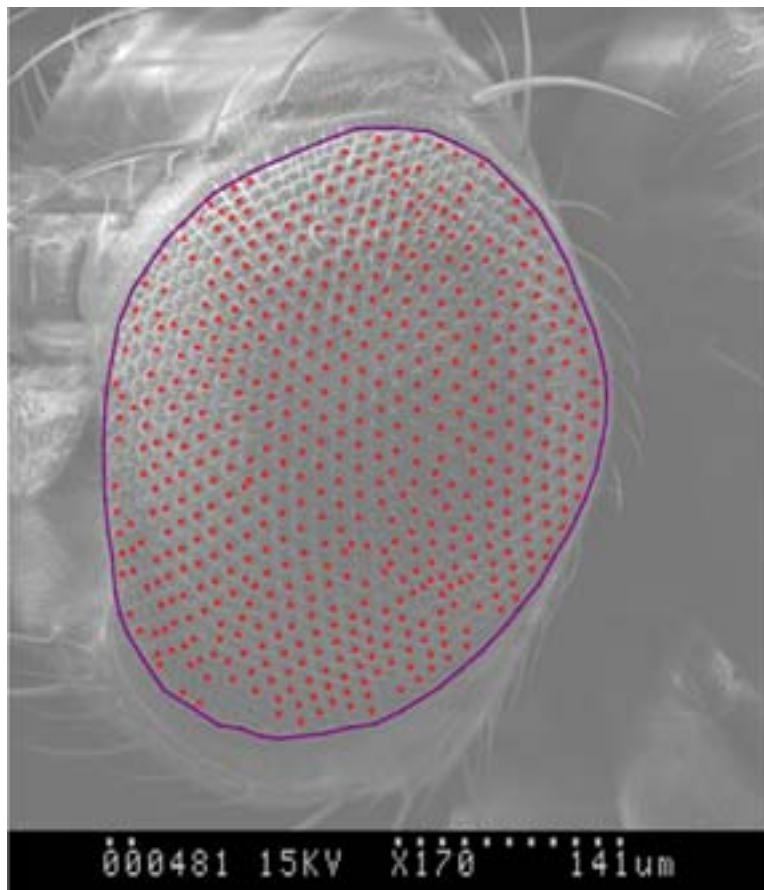


Figure 3. Identified ommatidia marked by Eyer.

References: Kirillov, A., 2011, Aforge.NET: Framework. Retrieved from <http://www.aforogenet.com/framework>; Brand, A.H., A.S. Manoukian, and N. Perrimon 1994, *Methods Cell Biol.* 44: 635-654; Freeman, M., 1996, *Cell* 87: 651-660; Prajdić, M., 2008, *Watershed Image Segmentation in C#*. Retrieved from <http://weblogs.sqlteam.com/mladenp/archive/2008/02/11/Watershed-Image-Segmentation-in-C.aspx>; Vincent, L., and P. Soille 1991, *IEEE Transactions on Pattern Analysis and Machine Intelligence* 13(6): 583-598.

### Call for Papers

Submissions to *Drosophila* Information Service are welcome at any time. The annual issue now contains articles submitted during the calendar year of issue. Typically, we would like to have submissions by mid-December to insure their inclusion in the regular annual issue. but articles can be accepted for this volume until 31 December. Details are given in the Guide to Authors or on the DIS web site: [www.ou.edu/journals/dis](http://www.ou.edu/journals/dis).