Segmentation on *Drosophila* wing - Internship report

Ana Domingues, Corinna Blasse[1]

[1]Max Planck Institute for Cell Biology and Genetics, Dresden 01307 Germany

Abstract

During the development stage (embryogenesis), drosophila wing present different aspect until is fully devolved, for example cells suffer elongation in some cases [1–3]. It is also during this stage that different regions of the wing became visible, such as blade or several veins [4].

In this project we use a full develop drosophila wing. Through the study of the different regions - cells with different aspects – we try, with success, to improve the automatic cell segmentation. Trainable Weka Segmentation, a Fiji plugin was the tool used.

Training of the human eye

The first step of this project, and one of the most imports, was the training of the human eye. Due to the size and different color intensity or lack of it, is really difficult to distinguish between the cell membrane from the rest (background). To overcome this difficulty we used the tool Brightness/Contrast available in Fiji (Image > Adjust > Brightness/Contrast). This tool enhances the different between the bright and the dark pixels dues helping us to see the membranes.

Trainable Weka Segmentation – Fiji Plugin

The plugin Trainable Weka Segmentation combined the image processing tools of Fiji with Weka machine learning power [5]. Through the input of the user this plugin is enabled to train and learn, thenceforward performing the some task to in to the unknown. In this particular case we trained two different labels: "Membrane" and

"Background". With the intention of improving previously outcomes a study of the different features available in this plugin was made.

Identification of the best regions to label

Several tests, all with the same group of features and conditions (see Figure 1), were done to infer the best regions to label. Due to the different regions in the wing and also to the different conditions that the images were captured, this is an important step on wing segmentation study.

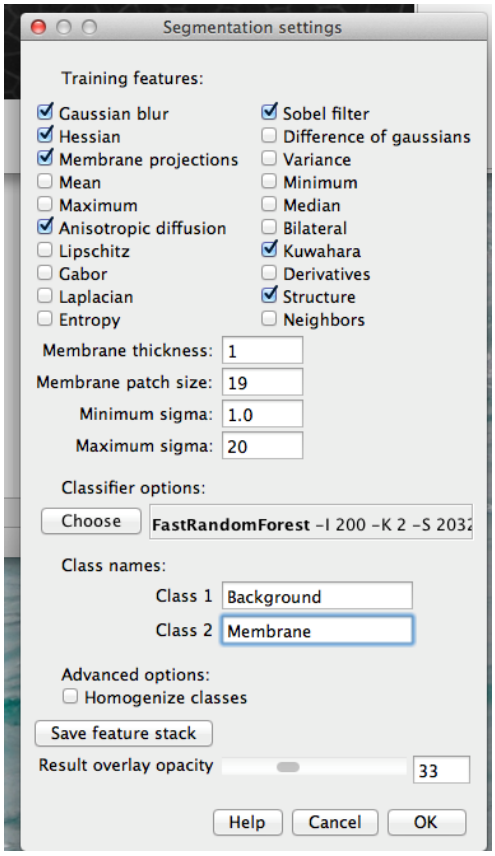

Figure 1: Printscreen of the constants conditions used to test the best areas to label

First of all is necessary to define the label procedure adopted. In every different spot that must be labeled, is important to have 2 kinds of label, one label as a membrane and another one a near to the membrane (if possible parallel) as a background (Figure 2A). The second kind of label is inside the cell, label a big amount of pixels inside a cell as a background, although is not necessary to label the full inside cell (Figure 2B). Due to the amount of pixel labeled as background in the previously, is necessary to label more membranes – is important to have the same number of pixels of each label, membrane and background.

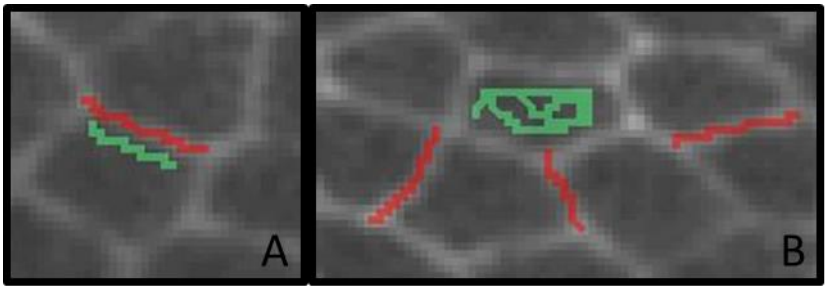

Figure 2: The type of label chosen. **A.** selected the membrane and a near background. **B.** Selected a full cell as background, and several as membranes to had the similar number of pixels as for each label.

After several tests we conclude that are 4 important spot that need to be labeled for a good segmentation:

- Bright spots
- Dark spots
- Elongated cells
- Small cells

In the Figure 3 is possible to observe diverse kinds of cell membrane. In certain parts of the wing is really difficult to see the membrane due the lack contrast of pixel intensity between the membrane and the background, for example, the wing border or in the spots where the cells appear elongated. In fact, we noticed that this two areas of the wing are the ones here the automatic segmentation perform the worst results. Normally is necessary to labels more in this areas to improve the results.

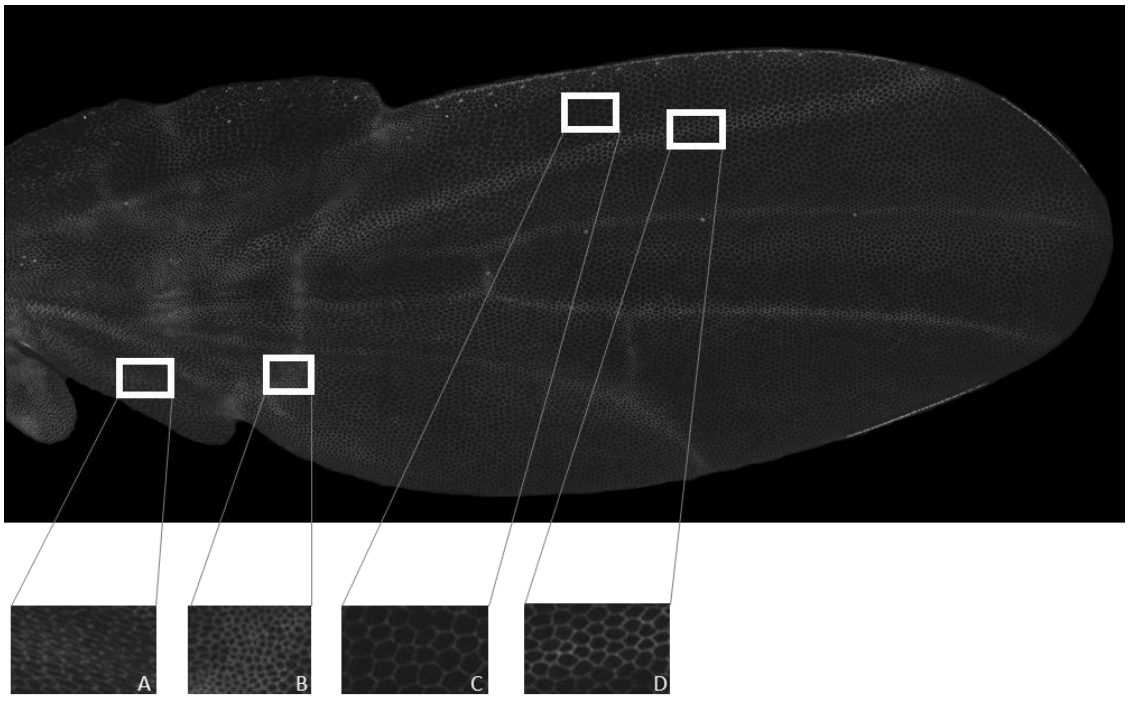

Figure 3: In this drosophila wing is emphasized as an example, the 4 spots to label: **A.** elongated cells, **B.** small cell, **C.** dark spot, **D.** bright spots.

Besides the problem (common also on dark spots, and small cells) mention above, area of the wing with elongated cells is problematic due to the type to background, sometimes is not more than a line.

Another area that present some trouble is the small cells. In this case, beside the contrast or the lack of contrast between the membrane and the background, the problem is the number of pixels in each label. In the small cells compared to the other cells the number of pixels in a membrane or in the background is much smaller. Due to this fact is also necessary to label in this areas, so the training set know that there are membrane or backgrounds only with a few number of pixels.

The bright spot, normally the wing veins, only present a problem because the rank of the pixel intensity, in this area the rank of the pixel intensity are superior.

The last, and one of the trickiest is the dark spot. In this case, something the membrane and the background appear almost with the some pixel intensity and is really difficult for the human eye to distinguish. In this case is crucial to use the contrast tool to help with this task.

Not that is also important to not over label the wing, since that can compromise the final result and also improve the time and resources spend to segment the wing.

Training features

After the definition of the best areas to label was mandatory to establish the best set training features for our images.

After consulting the guide provided by Fiji, we create a resume table (Table 1) with some notes [5]. While this study we also test the behavior of a specific feature in our image are only simple notes that will helps in the next step of this internship.

Table 1: Resume information of the training features available in Trainable Weka Segmentation.

Training feature	Information provided by Fiji	Notes
Gaussian blur	Reduce image noise; Reduce detail;	Not god to find the membrane
Sobel filter	Edge detection;	The membrane was good, but add some background in the middle.
Hessian	God for corner detection;	Very good for the membrane, but add “noise” to the background
Difference	Compute 2 Gaussian blur images from the	Better result of finding the

Training feature	Information provided by Fiji	Notes
of Gaussians	original image and subtract one from the other, i.e., compute two gaussians with two different sigma values, resulting in a bigger differences between pixel intensity. Improving in the end the edge detection.	membrane than the Gaussian Blur.
Membrane projections		Bad results, the membrane isn't defined and it is mixed with the background
Anisotropic diffusion	2^n smoothing per iterations; Edge threshold set to the membrane size.	Because of the smoothing the membrane are not that defined
Bilateral	Similar to the Mean filter but better preserves edges while averaging/blurring other parts of the image. The 'closeness' of other neighborhood pixels to the current pixels is determined by the specified threshold.	Good for reducing small variation in the pixel intensity.
Lipschitz	Cover of an image that is equivalent to a grayscale opening by a cone. The Lipschitz cover can be applied for the elimination of a slowly varying image background by subtraction of the lower Lipschitz cover (a top-hat procedure).	The membrane need smoothness, probable is good combined with other that smooth the image.
Kuwahara	Another noise-reduction filter that preserves edges	Add too must noise.
Gabor	Linear filter used for edge detection	Good result in general
Derivatives	Calculates high order derivatives of the input image	Poor result; Bright emphasis.
Laplacian	Computes the Laplacian of the input image. It uses smoothing.	Add noise in the background.
Struture	Calculates for all elements in the input image, the eigenvalues (smallest and largest) of the so-called structure tensor. During this computation two image are created, one with the brigth enhance and the other more blurry.	Brigth pixels in strange places.
Entropy		Need more labels to improve the results
Neighbors		Good result

Results

Once we understood the different features we did several test with different subsets of features. The main idea was to choose a set that was a good compromise

between the features that blur and smooth the image and the ones that preserve edge and reduce noise.

The probability map was saved and we only used the membrane image of the probability map to induce the result of the tests.

The follow table present the several test and the mean and standard deviation compute with a different program created *in the house* to infer the best set of training features for the drosophila wing segmentation.

Table 2: Results of the tests perform to find the best training features set in order to improve the mean probability of a correct membrane classification**.**

	T1	***T2***	***T3***	***T4***	***T5***	***T6***	***T7***	***T8***	***T9***
Gaussian blur	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*
Sobel filter	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*
Hessian	*X*	*X*	*X*	*X*		*X*	*X*	*X*	*X*
Difference of Gaussians	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*
Membrane projections		*X*	*X*						
Anisotropic diffusion	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*
Bilateral								*X*	*X*
Lipschitz	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*	*X*
Kuwahara		*X*		*X*	*X*	*X*	*X*	*X*	*X*
Gabor	*X*								
Derivatives						*X*			
Laplacian								*X*	
Struture							*X*		
Entropy									
Neighbors									
Mean	*0.9327*	*0.9346*	*0.9366*	*0.9367*	*0.9361*	*0.9308*	*0.927*	*0.9374*	*0.9385*
SD	*0.0839*	*0.0864*	*0.0851*	*0.0867*	*0.0905*	*0.0848*	*0.0861*	*0.0879*	*0.0859*

The basic set of training features was: Gaussian blur, Sobel filter, Difference of Gaussians and Anisotropic diffusion. This basic set was establish in previous tests with different labels and was not include in Table 2. All the tests in Table 2 have the some labels therefore, we not going to present the pre-tests that help us to establish the basic that of training features. The next step was testing different set features to improve the mean value, at the time ~0.86. As we can observe the test T1 was an improvement of the existing values. Some of the tests the mean provability of the membrane to be corrected classified is not significative but we can see that when Hessian (T5) was excluded the result decreased, so we can claim that this in an important feature. Also when Structure feature is include the result gets worse thus, is not a good feature for our type of images.

The Entropy and Neighbors features were excluded before testing. Entropy is the degree of system disorder, so for this type of images it does not make sense to tested, in the case of the Neighbors features, this take in consideration the *n* pixels

close by, in the case of a wing, with the several different regions, is not a good feature.

Also the Gabor feature was excluded because the time and the resource consumed.

Despite the some small increase between tests the test T9 present the best mean score.

Conclusion

This internship was composed for diverse steps. Since the biological field was new, it was necessary and additional effort to a success conclusion. Despite the study and research done in this field this does only superficial to a better understanding the problem. Also the image segmentation field was a new field, so was necessary a more extensive (for the time available).This study include the math and algorithms behind the convolution techniques for example, useful to a better understanding of the training features.

Drosophila wing was several different areas, thus we conclude that is necessary to label in 4 spots: Bright spots, Dark spots, Elongated cells and Small cells. There is also some rules to a good labeling: label a membrane and a near background and also an "all" cell as a background and complement this with several membrane labels so that the number of pixel label as a membrane and as background are a similar number (really important).

Due to the tests perform, we established that, the best set to training features is: Gaussian blur, Sobel filter, Hessian, Difference of Gaussians, Anisotropic diffusion, Bilateral, Lipschitz and Kuwahara.

After the tests performed we improve the mean probability of a correct membrane classification for ~86% to almost 94%. To this good result is important the rules for labeling and also the set of training features established.

References

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