Contour Extraction of Drosophila Embryos Using Active Contours in Scale Space

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CONTOUR EXTRACTION OF DROSOPHILA EMBRYOS USING ACTIVE CONTOURS IN SCALE SPACE

A Thesis
Presented To
The Faculty of the Department of Computer Science
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By
Soujanya Siddavaram Ananta

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CONTOUR EXTRACTION OF DROSOPHILA EMBRYOS USING
ACTIVE CONTOURS IN SCALE SPACE

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Soujanya Siddavaram Ananta
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Contour extraction of Drosophila embryos is an important step to build a computational system for pattern matching of embryonic images which aids in the discovery of genes. Automatic contour extraction of embryos is challenging due to several image variations such as size, shape, orientation and neighboring embryos such as touching and non-touching embryos. In this thesis, we introduce a framework for contour extraction based on the connected components in the Gaussian scale space of an embryonic image. The active contour model is applied on the images to refine embryo contours. Data cleaning methods are applied to smooth the jaggy contours caused by blurred embryo boundaries. The scale space theory is applied to improve the performance of the result. The active contour adjusts better to the object for finer scales. The proposed framework contains three components. In the first component, we find the connected components of the image. The second component is to find the largest component of the image. Finally, we analyze the largest component across scales by selecting the optimal scale corresponding to the largest component having largest area. The optimal scale at which maximum area is attained is assumed to give information about the feature being extracted. We tested the proposed framework on BDGP images, and the results achieved promising accuracy in extracting the targeting embryo.
INTRODUCTION

Genetics provide the most powerful approach available to understand the function of the human genes. Model organisms, such as Drosophila melanogaster, share many genes with humans. The Drosophila embryo is one of the most well known model organism that is widely used in biological research particularly in genetics and developmental biology [32]. Drosophila is also used in life history evolution. A model organism is a species that is studied to understand a biological phenomena, with the expectation that the experiments made on the model organism will provide awareness in the research of other organisms. The Drosophila embryo is a useful model organism for studying many aspects of development because it is small and cheap to culture in the lab. It also has a short life span.

Images of Drosophila embryos consist of significant gene expression patterns [21]. Information of the gene expression is captured for understanding the development of embryos at various stages. Analysis of similar gene expression patterns is important in understanding the interaction of genes that generate the body plans of Drosophilas, humans and other metazoans [17]. The recent technique used to study gene expression patterns is in situ hybridization [25]. In situ hybridization(ISH) is a protocol used to determine patterns of gene expressions during embryogenesis of Drosophila [30].

Comparison of expression patterns is most biologically meaningful when images from a similar time point (developmental stage range) are compared [11]. A set of embryonic images contain information on the spatial and temporal patterns that are extremely useful for the study of gene-gene interaction which is a biological problem. Spatiotemporal gene expression is the activation of genes of a particular location at a particular time during development. Dark regions in an embryonic image, as shown in Figure 1.1, indicate a significant gene expression pattern. Given two standardized images
of embryos at same development stage, the interaction strength of two genes can be quantified by computing the similarity of expression patterns. Traditionally, Drosophila development is divided manually into stages by qualitative visual inspection. The manual labeling of stages has become a bottleneck with the dramatically increasing data, e.g., the high resolution embryonic images contributed by The Berkley Drosophila Genome Project (BDGP) [30, 1].

![Embryo images containing variations in size, shape, orientation and appearance in addition to its neighboring context](image)

(a) embryo with variation in the orientation  
(b) image with partial embryo  
(c) embryo image with proper alignment

Figure 1.1: Embryo images containing variations in size, shape, orientation and appearance in addition to its neighboring context

Biologists spend significant time in manually annotating images from large scale experiments like BGDP [1]. Annotation includes data such as view, orientation, and stages of development of the embryos [14]. The advanced microscopes have led to the rapidly growing digital data in biology. In order to deal with these data sets, it is necessary to develop automated methods for extracting and analyzing images. Embryo contour extraction is an important step to build pixel-to-pixel correspondence between embryos of interest so that the comparisons between embryonic images in a computation system are biologically meaningful [21]. A comparative analysis of the images contributes to the study of regulatory networks governing embryonic development [13, 10].
Several challenges are faced due to image variations such as size, shape, orientation and context of the neighboring embryos. Size and shape variations are due to different development stages. Another image variation is due to the context of neighboring embryos i.e. the embryo of interest may or may not intersect neighboring embryos. The problem of contour extraction from a blurred digital image is some sort of pattern recognition problem.

In this thesis, we design an automated framework for contour extraction using active contours with scale space. The scale space theory used in this thesis is contributed by Lindeberg [23]. The scale-space theory focuses on the basic fact that the image structures, like objects in the world, exist as meaningful entities over certain ranges of scale [22]. In general, one cannot expect to know what scales are appropriate in describing those image structures. The active contour adjusts better to the object for finer scales [29].

The proposed framework has three main stages. The first stage is applying the active contour segmentation and finding the connected components of the image after segmentation. The second step is to extract the largest connected component of the image. The final step is to apply a criterion to decide the optimal scale to locate a targeting embryo. The corresponding scale with the largest area is selected as the optimal scale for a particular image. The scale $\sigma$ which is a gaussian kernel for suppression of noise in our experiment ranges from 0.5-30 in sampled space. Active contour model is described in detail in Chapter 2.

This thesis is organized as follows: Chapter 2 gives a review of the two main existing methods for contour extraction: edge detection and active contour, and the advantages of the active contour over edge detection. Chapter 3 presents the proposed framework used for contour extraction. Chapter 4 shows results of the proposed framework and Chapter 5 provides the summary, conclusion, and the future scope of this research.
EXISTING METHODS FOR CONTOUR EXTRACTION

This chapter mainly focuses on the existing methods of contour extraction. Edge detection and the active contour models are the most popular methods used for contour extraction for wide range of applications, such as image segmentation and motion tracking. This chapter is organized as follows: Section 1 describes the various edge detection methods, comparison between them and the limitations. Section 2 introduces contour extraction, its significance and the active contour model which is the main technique used for extraction of the contour in this thesis.

2.1 Edge detection

Edge detection is a basic rule in the field of image processing and computer vision in the areas of feature detection and extraction. It refers to the process of determining sharp discontinuities in an image. The image given as input is stored in a matrix form which contains the pixels of the image. The gradient of the matrix is calculated and if the gradient is large then the pixel is determined as an edge pixel. Edge detection techniques may be grouped into two different categories:

1. Gradient based edge detection
   - Sobel
   - Prewitt
   - Roberts

2. Laplacian based edge detection
   - Laplacian
   - Zero-cross
The key of edge detection is the choice of threshold. The choice of threshold directly determines the results of edge detection. The lower the threshold, the more the edges will be detected, and the result will be increasingly susceptible to noise and detect edges of irrelevant features in the image. Conversely, a high threshold may miss subtle edges, or result in fragmented edges. If the edge threshold is applied just to the gradient magnitude image, the resulting edges in general, will be thick and some type of edge thinning post-processing is necessary.

Edge thinning is a technique used to remove the undesired false points on the edge of an image. This technique is applied after the image has been filtered for noise (using median, gaussian filter, etc.). The edge operator has been applied to detect the edges after the edges have been smoothed using an appropriate threshold value. This removes all the undesired points and if applied carefully, results in one pixel thick edge elements.

2.1.1 Edge detection methods

Sobel Filter

The Sobel filter finds edges using the Sobel approximation to the derivative. It returns edges at those points where the gradient of image is maximum. The operator consists of a pair of convolution kernels.

Prewitt Filter

The Prewitt filter finds edges using the Prewitt approximation to the derivative. It returns edges at those points where the gradient of image is maximum.

Roberts Filter

The Roberts filter finds edges using the Roberts approximation to the derivative. It returns edges at those points where the gradient of image is maximum.

Laplacian Of Gaussian Filter

The Laplacian of Gaussian filter finds edges by looking for zero crossings after filtering image with a Laplacian of Gaussian filter.
**Zero Cross Filter**

The Zero-cross filter finds edges by looking for zero crossings after filtering image with a filter you specify.

**Canny Method**

The Canny method finds edges by looking for local maxima of the gradient of image. The gradient is calculated using the derivative of a gaussian filter. The method uses two thresholds to detect strong and weak edges, and includes the weak edges in the output, only if they are connected to strong edges. This method is therefore more likely to detect true weak edges.

### 2.1.2 Comparison between edge detection methods

Gradient based algorithms such as Sobel and Prewitt are sensitive to noise. The Sobel operator consists of a pair of 3*3 convolution kernels. One kernel is the other rotated by 90°. Using the kernel the absolute magnitude of the gradient is found. The Roberts operator consists of a pair of 2*2 convolution kernels. The absolute value of the gradient is computed. Prewitt operator is similar to the Sobel operator as illustrated in Figure 2.1. They are good at detecting vertical and horizontal edges in images.

The Laplacian is a 2-D measure of the 2\(^{nd}\) spatial derivative of an image. The Laplacian is often applied to an image that has first been smoothed with something approximating a gaussian smoothing filter in order to reduce its sensitivity to noise. Because these kernels are approximating a second derivative measurement on the image, they are very sensitive to noise. To counter this, the image is often gaussian smoothed before applying the Laplacian filter. This pre-processing step reduces the high frequency noise components prior to the differentiation step.
The Canny edge detector is the optimal edge detection method. Canny followed a list of criteria to improve the previous edge detection methods [7].

1. Low error rate - It is important not to miss the edges that occur in the image and there should be no responses to non-edges.

2. Edge points should be well localized - The difference between the edge pixels and the actual edge should be minimum.

3. Only one response to a single edge - The previous methods does not eliminate the possibility of multiple responses to an edge.

The Canny edge detector first smoothes the image to eliminate the noise and then finds the image gradient. The pixels that are less than the maximum gradient are suppressed and the gradient is further reduced by hysteresis. Hysteresis is used to track along the remaining pixels that have not been suppressed. Hysteresis uses two thresholds which allows more flexibility than a single threshold approach. If the magnitude is below the first threshold, it is made as non edge. If the magnitude is above the high threshold, it is made as an edge. Canny edge detection algorithm has a number of adjustable parameters, which have an impact on the effectiveness of the algorithm and the computation time.

1. Size of the gaussian filter

2. Threshold values

Figure 2.1 shows results of the various edge detection methods.

2.1.3 Summary and limitations of edge detection

Gradient based algorithms such as Sobel, Prewitt and Roberts are sensitive to noise and the size of kernel filter and coefficients are fixed and cannot be adapted to a
An adaptive edge detection algorithm is necessary to provide a robust solution that is adaptable to varying noise levels. The performance of the Canny algorithm depends heavily on the adjustable parameters, sigma, the standard deviation for the gaussian filter, and two threshold values ('T_1' and 'T_2'). The parameters can be adapted to different environments. This implies more blurring, necessary for noisy images, as well as detecting larger edges. The user can tailor the algorithm by adjusting these parameters to adapt to different environments.

Based on comparison shown in Figure 2.1, we observe that Canny algorithm gives the optimal result as the parameters can be adaptive to different environments. The maximum number of edges are detected using Canny edge detector. Most of the edges are obtained by varying the parameters using Canny edge detector whereas in Sobel, Prewitt and Roberts not all edges are detected because of fixed parameters. These algorithms are not able to detect the edges while removing the noise whereas Canny does.

Canny’s edge detection algorithm is computationally more expensive compared to Sobel, Prewitt and Roberts operator. However, the Canny’s edge detection algorithm performs better than all these operators under almost all scenarios.
Limitations of edge detection

1. Edges of a neighboring embryo are also detected. Note that an embryonic image may contain a neighboring embryo, in addition to a targeting embryo.

2. All image points that have the maximum gradient are treated as edges. The gradient can be maximum inside the embryo and that point is also treated as an edge where as in reality it is not.

2.2 Contour extraction

In contrast to edge detection, contour extraction is a higher-level formulation that aims to extract the boundary shape of an object of interest in an image. The image processing stage is necessary in order to provide a standardized image database for gene expression pattern comparison [27]. A lot of research is being done in these areas and many approaches were reported so far. Contour extraction can be generally classified into two categories:

1. Region-based [6, 4]

2. Edge-based [6, 24]

Edge-based techniques rely on discontinuities in image values between distinct regions and the goal is to accurately distinguish the boundary separating these regions. Edge-based models consist of evolving a contour in homogeneous areas, and locally stopping it when it reaches high image gradients [28]. They have several advantages like local coherence and robustness to region inhomogeneities, but they also have important drawbacks that make them inefficient on noisy images or when the contour initialization is not completely inside or outside the region to segment. Region-based searches for equality inside a sub-region, based on a desired property, e.g. intensity, color, and texture. Region-based techniques depend on common patterns in intensity values within a cluster
of neighboring pixels [6]. These models take account of the whole region and are thus more robust to noise and to initialization than edge-based models. Due to minimization of global energy, region-based active contours do not have any limitation placing the initial contour. Region-based active contour can detect interior boundaries regardless of the placement of the initial contour.

Contour extraction is an important step for feature detection. Once the contour is extracted, different characteristics of the contour will be examined and used as features which will later be used in pattern matching of the images. The region of interest is extracted and used to compare gene expression patterns of the image at different stages of development. An embryo image taken during embryogenesis usually contains multiple embryos with region of interest at the center of the image. The neighboring embryo may be touching or non touching.

The two main approaches of active contour based on mathematical implementation are: Snakes and level-sets. Snakes explicitly shift predefined contour points based on an energy minimization technique, while level-set approaches move contours completely as a particular level of a function.

Snakes or the active contours, are used extensively in computer vision and image processing applications, particularly to locate object boundaries. The active contour is an important model for defining an object outline from a noisy image using techniques of curve revolution [5]. Snakes are curves that can deform within the image plane and capture a desired feature. Methods to evolve these contours were introduced to computer vision by Kass, Witkin and Terzopoulos [15]. This model is an energy minimizing spline guided by external forces and influenced by image forces that pull it towards features such as edges [16]. The active contour model is more consistent than edge detection.

The active contours can be classified into two broad categories: parametric active contours [15, 34] and geometric active contours [19]. The parametric active contours represent contours explicitly as parameterized curves where as the geometric
contours represent contours implicitly as level-sets of two dimensional functions. Geometric active contours are classified into two categories: models based on boundary functionals and models based on area functionals. It is a flexible curve which will dynamically adapt to the required edges in the image. The active contour consists of a set of controlled points or contour points as shown in Figure 2.2.

![Figure 2.2: Basic form of the active contour](image)

The initial contour must be specified. The contour will then be attracted to features in the image extracted by internal energy creating an attractor image. The energy function is a weighted combination of internal and external forces. Snake is defined by:

1. a set of contour points
2. an external energy term
3. an internal energy term

An initialized contour is considered. The active contour is used to refine the initialized contour of an embryo of interest. It dynamically moves towards the region of interest by minimizing its energy iteratively. Minimization of energy from high to low is an aspect of optimization. We need to shrink or expand the active contour depending on the image which is known as external energy. There are several aspects of defining energy...
such as smoothness, elasticity, etc. The fundamental idea of the active contour is to minimize an initial snake according to its energy. Representing the position of a snake perimetrically by $v(s) = (x(s), y(s))$ [15], we can write its energy functional as

$$E_{\text{snake}} = \int_0^1 E_{\text{snake}}(v(s)) \, ds$$

$$E_{\text{snake}} = \int_0^1 E_{\text{int}}(v(s)) + E_{\text{ext}}(v(s)) \, ds$$

where $E_{\text{int}}$ represent the internal energy of the spline, and $E_{\text{ext}}$ gives rise to the external constraint forces.

The internal energy can be written as,

$$E_{\text{int}} = (\alpha(s) \| v' \|_2^2 + \beta(s) \| v'' \|_2^2 )/2$$

The first order term is controlled by $\alpha(s)$ and the second order term is controlled by $\beta(s)$. $\alpha(s)$ controls the deformability of the shape as it controls elasticity and $\beta(s)$ controls the rigidity. Internal energy enforces a shape on the contour and maintains a constant distance between the control points by shrinking or expanding the deformation curve. Internal energy $E_{\text{int}}$ describes elasticity and stiffness of the contour. Elasticity parameters control the smoothness of the contour and makes its shrinking tendency stronger. The contour roughly follows the model shape, which allows greater fluctuations in shape if the elasticity is low. If high, the contour holds tight to the model shape.

$E_{\text{ext}}$ is called external energy function, and is derived from image features such as image gradient field, or from other sources like knowledge base, users input, etc. External energy $E_{\text{ext}}$ is smoothed with a gaussian filter with variance. At the location of the desired boundary, $E_{\text{ext}}$ should be its minimum [3]. The external energy is written as,

$$E_{\text{ext}}(x,y) = -\| \nabla I(x,y) \|^2$$

$$E_{\text{ext}}(x,y) = -\| \nabla G_\sigma(x,y) * I(x,y) \|^2$$
where \( I(x,y) \) is the gray scale image, \( G_\sigma(x,y) \) is a two dimensional gaussian kernel that convolute with the image \( I(x,y) \). \( \nabla \) is the gradient operator. The effect of using gaussian kernel convolute with image \( I(x,y) \) is to smooth the image, and to blur the image boundaries. The external forces are responsible for putting the snake near the desired local minimum. The active contour has the total energy \( E \).

During the process of contour deformation, the curvature based internal force maintains the contour smoothness, while the gradient-based external force attracts the contour to the desired boundaries in the image. The deformation finally stops when the snake reaches a local energy minimum. The active contours have become an invaluable base tool for segmentation and object recognition.

### 2.2.1 Advantages of the active contour over edge detection

1. Active contours are self-adaptive in search of minimal energy state.
2. The external and internal energy forces can be used to manipulate the active contour.
3. They can be made sensitive to image scale by incorporating gaussian smoothing in the image energy function. Gaussian smoothing is used to suppress the noise in the digital images.

The only drawback of active contour model is the existence of local minima in the active contour energy. The main difficulty with parametric active contour algorithm is that the initial contour must be close to the true boundary or else it will likely converge with the wrong result [34]. The active contour segmentation may also result in multiple components and also the contour results are sensitive to scales. The largest of the components is expected to be the target contour.
This chapter focuses on the proposed framework for contour extraction. Section 1 describes the outline of the framework. Section 2 addresses the active contour segmentation and the active contour models used for the experiments and the comparison between them. Section 3 illustrates the Distance-Normalized technique. Section 4 provides the details of the optimal scale selection criterion.

Information of the gene expression is captured for understanding development of the embryo at various stages. In order to discover the interaction of genes automatically, we build contour point-to-point correspondence between embryos of interest. The first step is to extract the contour of the target embryo automatically. Embryo contour extraction is an important step to build pixel-to-pixel correspondence between embryos of interest so that the comparisons between embryonic images in a computation system are biologically meaningful. Contour extraction is very challenging due to image variations. Variations include size, shape, noise, orientation, partial embryos. Figure 3.1 shows the variations in an embryonic image.

(a) image with partial touching embryo  (b) image with partial non-touching embryo

(c) embryo with variation in the orientation

Figure 3.1: Variations of an embryonic image

Edge detection results in lot of noise after segmentation. All the points having
the gradient maximum are treated as edges. The gradient can be maximum inside as well as outside the embryo. Therefore more advanced technique called the active contour is used to eliminate the limitations of edge detection technique. The active contour model provides an efficient way for image segmentation in which the boundaries of the object are detected. Due to serious image variations, the existing techniques failed to obtain desirable results. The main criteria of the research work is largest of the largest, i.e., largest area of the resultant largest component of the image obtained after segmentation. The criterion is to determine an automatic procedure to determine the optimal scale for a specific image.

3.1 Outline of the framework

In this section, we proposed an approach to remove contour pixels of neighboring embryos. We assumed that the embryo of interest dominates the neighboring context in an image. Furthermore, we categorized the neighboring context into overlapping and non-overlapping context. It is expected that the first case is easy to handle than the second case due to the overlapping region.

The data set is passed through the active contour model. The main scale is considered to be $\sigma$ which represents the gaussian kernel. $\sigma$ was used to suppress the noise in the digital image. $\sigma$ gives the standard deviation of the gaussian low pass filter. Convolution of the image with the gaussian filter is performed and then the convoluted image is passed through the active contour model. The resulting images might have jaggy contours as well as multiple connected components. Jaggy contours are eliminated by applying the smoothness constraint. We applied the proposed criterion to extract the largest component which is expected to be the target embryo. The proposed framework is divided into three components. The first component is to find the number of connected components of the resulting images of the active contour model. A connected component is defined as a connected region whose pixels belong to the same class in a segmented image. The second component is to find the largest component among all the connected
components. The largest component is assumed to be the target embryo. The third component is to apply the proposed criterion and find the optimal scale corresponding to the largest component with the largest area. The corresponding $\sigma$ value is considered to be the optimal scale. Figure 3.2 shows the outline of the proposed framework.

![Figure 3.2: Proposed framework for contour extraction in scale space](image-url)
3.2 Active contour segmentation

As described in the last chapter, the active contour model is a methodology based on the use of deformable contours, which adapt its border to the diverse shapes of the objects in the images [31]. In some methods based on this methodology, the results are very conditioned by the selection of the initial position of the contour. The active contour methods provide an effective way for segmentation, in which boundaries of the objects are detected by evolving curves.

This technique is more advanced compared to edge detection. This method is applied to refine the contour of target embryo in an image based on an initialized contour. The active contours writhe and move under the influence of external and internal forces, toward a feature of interest in an image, usually an edge. The active contours are most often used with images to find and describe regions of interest [18].

We consider an initialized contour and input image. We deform the set of points to match with the initialized contour called the active contour so that it converges with the real contour. It is mainly based on optimization of energy. The minimization of the energy associated with the current contour is a sum of an internal and external energy. The energy functional which is minimized is a weighted combination of internal and external forces. Low energy implies that the contour is more stable.

\[ E = \alpha_1 E_{int} + \alpha_2 E_{ext} \]

Level-set and Chan-Vese are the two active contour models used for our experiments. The main difficulty in the active contour model is the choice of the initial contour and value of the parameters. The only drawback of the active contour model is the existence of local minima in the active contour energy. The existence of local minima in its functional energy makes the initial contour critical to extract meaningful objects lying in images.
3.2.1 Level-set

Level-set theory, a formulation to apply the active contours, was proposed by Osher and Sethian [26]. Level-set methods have been widely used in image processing and computer vision. The basic idea is to represent a contour as the zero level-set of a higher dimensional function, called the level-set function (LSF). Given an initial contour and high dimensional continuous function, we deform at each pixel where zero level-set corresponds to the actual position of the curve. Slide and get the optimal solution so that the energy is minimum. Instead of tracking a curve through time, the level-set method evolves a curve by updating the level-set function at fixed coordinates through time [33]. The idea behind deformable models, for image segmentation is quite simple. The user specifies an initial guess for the contour, which is then moved by image driven forces to the boundaries of the desired images.

Level-set method consists of initializing the active contour in a distance function and re-initializing it periodically during the evolution. In conventional level-set method, the level-set function develops irregularities during its evolution, which may cause numerical errors and eventually destroy the stability of the evolution [20]. Re-initialization is a remedy but it raises problem and also affects numerical accuracy. Re-initialization also makes the method computationally expensive. The distance regularization effect in our model eliminates the need for re-initialization and thereby avoids its induced numerical errors.

Distance regularization is defined with a potential function such that derived level-set evolution has a unique forward and backward diffusion effect which results in maintaining desired shape. Potential function is aimed to maintain signed distance property, i.e., how close a given pixel point is to the boundary. \( \sigma \) is the scale parameter in the gaussian kernel and \( \alpha \) denotes the weight of the weighted area term. Figure 3.3 shows the resulting images after applying level-set model.
Figure 3.3: Contour results obtained by different scales by level-set. The result is not satisfying because of (i) jaggy contour (ii) not smooth
3.2.2 Chan-Vese

Chan-Vese model implements level-set as well as the active contour. Chan and Vese proposed multi-phase active contour model [9, 8], which increases the amount of subsets that the active contours can locate simultaneously. In this segmentation technique, we pass external energy factor $\alpha$ as a parameter to the function. The energy in this technique is defined in terms of segmentation. This is a nice way to segment images whose foregrounds and backgrounds are statistically different and homogeneous [9].

There are some objects whose boundaries are not well defined through the gradient. The main idea of Chan and Vese model is to consider the information inside the regions not only at their boundaries. Chan and Vese introduce a new active contour model, called ”without edges”. Region based active contour models, e.g Chan and Vese [9] are equivalent to boundary based active contour models. Boundary based methods handle changes in topology and provide robust stopping terms to detect the goal contours. Most Region-based models consists of two parts: the regularity, which determines the smooth form of the contours, and the energy minimization part, which determines for equality of a preferred feature within a subset. Region-based active contours divides an image into several sub-regions. These regions exist either inside or outside the contour. The active contour model proposed by Chan and Vese is very robust to initialization and gives good results when there is a difference between the foreground and background means.

$\sigma$ and $\alpha$ are the two main parameters used in the Chan-Vese model to minimize the energy function. $\alpha$ value represents the weight of the external energy factor. The higher the value of $\alpha$, the smoother is the image, which is one of the aspect of defining energy. $\sigma$ is the scale parameter in the gaussian kernel. Smoothing of the image is done using gaussian convolution. A gaussian filter smooths an image by calculating weighted averages in a filter box.
Figure 3.4: Results are satisfying but the performance depends on scales
The Figures 3.3 and 3.4 demonstrate that the contour results are sensitive to scales. The contour results are different for different parameters. These figures demonstrates the motivation of this research. We now compare the two methods, level-set and Chan-Vese to see which method yields better results and then apply the proposed criteria to that method.

3.2.3 Comparison between level-set and Chan-Vese segmentation

We compare the resultant output images of the level-set and Chan-Vese segmentation. There are two main comparisons to be done:

1. Comparison of output images by considering different scale values in each of the models.

2. Comparison between the level-set and Chan-Vese methods.

The contour is influenced by internal and external forces varying its shape adaptively. Chan-Vese yields the better results than level-set. Chan-Vese segmentation results in more smoothness which is one of the aspect of defining external energy. The level-set model is the edge-based active contour model where as Chan-Vese is region based active contour model. Edge based active contour models evolve the contour towards one way, inside or outside. Therefore, an initial contour must be placed completely inside or outside the region of interest. Edge-based active contours may remove the blurry boundaries, and they are sensitive to noise as edge-based segmentation does. Figure 3.5 shows the comparison between the resulting images of level-set and Chan-Vese segmentation.
Figure 3.5: Comparison between level-set and Chan-Vese
The performance of the Chan-Vese model is good but depends on scale. This is the main motivation of this research. We need to find the scale which yields the best result. All the current segmentation techniques iterate till the energy is minimized. The main concern is that there is no guarantee that these techniques take global minimal energy under consideration. The sub-optimal energy might be considered as the minimal energy which is not the optimal solution.

The next step is to do some kind of pre-processing before segmentation. The initial pre-processing technique that has been proposed is to calculate the weight (normalized distance) of each pixel and multiply it with the original image pixel value and then perform the segmentation.
3.3 Distance-Normalized technique

A pre-processing technique for contour extraction which considers an additional energy factor is proposed. The idea is to consider the normalized distance of a pixel to the center of the embryo. This pre-processing technique is applied to the active contour model to make the resultant image more smooth. The additional energy factor that we consider is the normalized distance at the position of the pixel under consideration.

Algorithm Distance-Normalized

1. For each image in the data set
2. Compute the longest distance from the pixel at the center to the pixel at the corner of the image
3. Compute the distance of the pixel under consideration to the corner pixel
4. Compute ratio of the distance of the current pixel to the largest distance
5. Multiply the weight with the RGB value of the current pixel
6. The RGB values are combined to get the true color image

This pre-processing technique was applied to obtain the results after contour extraction with more accuracy. This technique is expected to remove the neighboring embryos at least to some extent.
3.4 Algorithm for optimal scale selection

The proposed criteria extracts the largest component of the image after segmentation. The main aim is to design automatic procedure by selecting the largest area of the largest component of all scales and determine the optimal scale for a specified image. The contour extracted by Chan-Vese method highly depends on scale. From the Figure 3.4, we observe that the results obtained from the Chan-Vese segmentation are satisfying but the performance depends on scale. Different kinds of image structures give rise to contours at different scales.

The connected components of the images obtained from the active contour segmentation are found. As the scale increases, the number of connected components decrease. The image is processed repeatedly by modifying the $\sigma$ value. $\sigma$ is the gaussian kernel used in this framework. Gaussian smoothing is low-pass filter. Filter blurs everything that is smaller than the filter. It suppresses high-frequency detail (noise, but also edges), while preserving the low-frequency parts of the image (i.e. those that don’t vary so much). We focus on analyzing largest connected components across scales. Our criterion is to select the optimal scale by taking into account the resultant contour which has maximum area i.e. total number of pixels compared to the resultant contours of other scales, and its corresponding scale.
**Algorithm**  Selection of parameter based on largest of the largest criteria

1. For each image in the data set
2. For each of the n scales
3. Perform Active contour segmentation
4. For every 15 pixels
5. Compute average
6. Average value is replaced for 15 pixels // for smoothing of the image
7. Find the largest component of the image
8. Compute the area for the largest component
EXPERIMENT

This chapter mainly focuses on the experiments done on the proposed framework. Section 1 describes the main setup required i.e. about the data sets, the tool used in implementing the framework and the parameters considered. Section 2 shows the results of initial pre-processing technique proposed. Section 3 describes the case of the rejected contours and the criterion applied. It also addresses the smoothness constraint applied in the framework. Section 4 displays results of the framework obtained by comparing different scales.

We will test the proposed framework on BDGP (Berkley Drosophila Genome Project) images [1]. These images were captured using advanced microscopes for the determination of the gene expression patterns of Drosophila embryos in different development stages. Each image is a high-resolution spatial representation of an embryo that might be neighbored by other embryos. The algorithm makes use of the active contour model which is also called as snake. The active contour is a framework which defines an image outline from a noisy image. The active contours do not solve the entire problem in finding target contours in images. They depend on information in image data or higher level image understanding process. When snake is placed near the object contour, it moves dynamically towards object contour by minimizing its energy iteratively.
4.1 Setup

In this section, we describe the setup of proposed framework. Firstly, we focus on the data set images obtained from BDGP. We then address the tool used for the development environment. Finally, we talk about the parameters used in the framework.

4.1.1 Data sets

The BDGP gene disruption project consists of a large collection of Drosophila strains where each strain contains a single genetically engineered P transposable element inserted in a defined genomic region. The resulting images of this project are in very large number. Sophisticated tools are needed to annotate this amount of data. BDGP aims to provide common data sets to be shared among various research groups as a stable basis for the evaluation and comparison of different methods for the analysis of human DNA sequences [1]. These data sets are used by algorithms which are aimed towards gene finding and the identification of regulatory sequence. We use high throughput images to determine patterns of gene expression during embryogenesis.

4.1.2 MATLAB

We use MATLAB to implement the proposed framework. MATLAB is a high level language and user friendly interactive environment for numerical computation, visualization and programming. The language tools and built-in functions in MATLAB enable the user to explore multiple approaches and reach a solution at a faster pace. MATLAB can be used for wide range of applications including image processing and computational biology. Image processing toolbox provides set of standard algorithms and graphical tools for image processing, analysis, visualization and algorithm development. Feature detection, noise reduction, image segmentation can be performed using MATLAB [2]. Graphic tools allow us to explore diverse set of images, create contours, extraction of
a contour and manipulate region of interest. Edge-detection algorithms identify object boundaries in an image. Image segmentation algorithms determine region boundaries in an image.

4.1.3 Parameters

The parameters we focus are $\alpha$ which is the external energy factor and $\sigma$ which defines the gaussian kernel. $\alpha$ is the weight of the smoothing term where as gaussian kernel is used for suppression of noise. A gaussian filter is created and then convoluted with the image before passing it to the active contour model. The gaussian smoothing operator is a 2D convolution operator that is used to blur images and suppress the noise in the image [12]. Gaussian filters are effective low pass filters from the perspective of both the spatial and frequency domains with standard deviation $\sigma$. An edge is a local feature in an image, and a smoothing operation that gives more significance to pixels farther away will distort its features. Properties that make the gaussian smoothing effective are:

1. Gaussian low pass filter is rotationally symmetric, i.e., the amount of smoothing performed by the filter in all directions will be same.

2. The gaussian function has a single lobe, i.e., each image pixel is replaced with a weighted average of neighboring pixels resulting in smoothing. The weight given to a neighbor decreases monotonically with distance from the central pixel, giving most weight to central pixels.

3. The relationship between $\sigma$ and degree of smoothing is a larger sigma implies wider gaussian filter and great smoothing.

4. Implementation of large gaussian filter can be done efficiently.
4.2 Distance-Normalized technique

This technique is applied before performing the segmentation. The resultant image after performing distance normalization is passed to the Chan-Vese method. This technique is expected to smoothen the image and remove the neighboring context to some extent. The output obtained was not as expected. The extracted contour is not the same as the region of interest. Figure 4.1 shows the resulting images after applying the Distance-Normalized technique and performing contour extraction.

Figure 4.1: Unsuccessful case of extracted contours using Distance-Normalized algorithm
4.3 Rejected case of contour extraction

Some of the results obtained from the Chan-Vese active contour model may have the partial embryo along with the region of interest. There is also a chance that the image might have multiple components. This case is caused due to the complex image structure which includes gene expression patterns or overlapping regions. To reject such kind of cases, we apply the next criterion.

Our criterion is to reject the image if the angle between any three points on the extracted contour is less than $30^\circ$. For each 3 points on the image we create a vector between 2 points. The angle between the vectors is calculated. If the angle is less than $30^\circ$, the image is rejected.

Therefore, if the segmentation results in multiple components, we extract the largest component which is expected to be the actual target contour. The resultant images might have jaggy contours. Jaggy contours are eliminated by applying the smoothness constraint. We apply data cleaning method to smooth the image. Figure 4.2 shows the rejected contours after applying the criterion specified above.

Figure 4.2: Rejected contours with angle less than 30
4.3.1 Applying smoothness constraint

The output images obtained after passing through Chan-Vese segmentation may contain jaggy contour. We smooth the image using bin means. This is one of the methods of data cleaning which is a data pre-processing technique. We divide the pixel points into bins of size 15 and the mean value is assigned to all the pixel values of that particular bin. Figure 4.3 shows a comparison of the embryonic image with and without smoothness constraint. With smoothness constraint, the largest connected component extracted is consistent with the real boundary.

(a) without smoothness constraint          (b) with smoothness constraint

Figure 4.3: Largest connected components: a) without, b) with smoothness constraint
4.4 Results obtained from the framework

We apply the proposed framework in the gaussian scale space with \( \sigma \) ranging from 0.5 to 30. We give an experimental analysis of the proposed framework on the BGDP embryonic images. We then present the successful results achieved by the criterion. We present the images along with its optimal scale at which the image has the largest area. We will also present an analysis of a failure case to illustrate the limitation of the proposed framework.

4.4.1 Successful case of extracted contours

The characteristic scale of a local image pattern is the scale parameter at which the gaussian kernel provides a local maximum. At finer scales, greater detail is represented. For a specified image, the scale at which the area is largest for the largest component is determined as the optimal scale. We present a set of successful results achieved by the criteria used in the framework. The optimal scale for each image is displayed. The scale in our experiment ranges from 0.5 to 30. Figure 4.4 shows that the largest component of the embryo extracted based on the gaussian scale space.
Figure 4.4: Successfully extracted contours with optimal scale in gaussian scale space
4.4.2 Failure case of extracted contours

Figure 4.5 presents failure case of the proposed method which is caused by the occurrence of various gene expression patterns and overlapping neighboring context of the embryo. This case illustrates the main limitation of the proposed framework. The future goal of this research may be to extract the actual contour even in the occurrence of the complex image structure caused by a number of gene expression regions. With the increment of the scale, i.e., the gaussian kernel $\sigma$, neighboring objects tend to diffuse or merge into the region of interest. Thus, the largest component may contain not only the region of interest but also non-region of interest objects.

![Unsuccessful case of extracted contours with optimal scale](image)

Figure 4.5: Unsuccessful case of extracted contours with optimal scale

$\sigma = 2$  $\sigma = 9$  $\sigma = 2$

$\sigma = 7$  $\sigma = 1$  $\sigma = 2$
4.4.3 Successful case of extracted contours with energy parameter alpha

Chan-Vese method has two main parameters $\alpha$ and $\sigma$. $\alpha$ is the weight of the smoothing term i.e. the external energy factor. Higher $\alpha$ implies more smoothing. $\sigma$ is the gaussian kernel which is used for suppression of the noise. The external energy factor $\alpha$ does not have any significance in terms of the scale space in contour extraction. The scale $\alpha$ ranges from 0.5 to 1.3. Figure 4.6 shows the resulting images after extraction of largest component by modifying the external energy factor.

Figure 4.6: Successfully extracted contours with optimal scale with energy parameter alpha
CONCLUSION

5.1 Outcome

The main outcome of this thesis includes developing a framework for contour extraction of drosophila embryonic images. All our experiments are tested on BDGP (Berkeley Drosophila Genome Project) [1] images. BDGP has large amounts of data sets. In order to deal with these data sets, we developed an automated model for extracting and analyzing images. Contour extraction is an important step in comparing the images at various development stages.

We first applied the edge detection methods on the BDGP data set. The edge detection methods are one of the existing methods for segmentation of images. Edge detection works well only in the images with sharp intensity transitions and relatively low noise. Canny edge detector gives the best results among all the edge detection methods. It consists of complex computations and is time consuming. Canny edge detection is computationally more expensive.

We then applied the active contour methods which are more advanced than edge detection. Snakes have proven to be useful in many applications such as image processing, etc. The active contour models integrated with the external and internal forces pulling the contours to the exact boundaries have shown their powerful abilities in object segmentation. Snakes and level-set are two important models of the active contours. We applied level-set method and Chan-Vese method on the data set. Comparison of the two methods was done and we concluded that the Chan-Vese method provides decent accuracy.

Parameters of these methods play a major role in the segmentation of the image by minimizing the energy. We observed that performance of the active contour model depends on the scales. The scale in our experiments refers to the gaussian kernel.
Convolution of image is done using the kernel and the Chan-Vese model is applied on the convoluted image. To improve the performance, the first proposed pre-processing technique is distance normalization. Applying this technique before segmentation did not prove to be useful. The resulting images after segmentation were not accurate. Hence, this method was discarded.

Few results of the Chan-Vese segmentation are obtained with the partial embryo along with the region of interest. There are also few cases where the resulting image contains multiple components. These cases can be eliminated by implementing the heuristic, or the angle between the vectors that should be greater than 30°. Any image with an angle between the vector that is less than 30° is eliminated. Our proposed framework extracts the largest component among all the connected components, which is expected to be the actual target contour. The jaggy contours may be eliminated by applying the smoothness constraint. We smoothed the image by applying bin means to handle jaggy contour. Bin means is a data cleaning technique.

We proposed a contour extraction framework for Drosophila embryonic images. Given a high resolution image, we applied the Chan-Vese active contour model. The active contour model is applied to refine the initialized contour. The proposed framework calculates the connected components of the image after passing through the active contour model. The largest component with the largest area is extracted. This process is repeated by modifying the scale values. The scale at which the maximum area is attained is assumed to give information about the feature being extracted. Each image might have a different scale at which the area is maximum for the largest component. The $\sigma$ value corresponding to the largest component is considered to be optimal scale. The active contour adjusts better to the finer scales. We present a set of successful results achieved by the best criterion. The scale $\sigma$ in our experiment ranged 0.5-30 in sampled space, which is, $[0.5, 1, 3, 5, 7, 10, 11, 15, 20, 27]$. Some of the images with inner and outer contour had different band width concentration. Confusion of contour points was caused during
convergence. In the proposed method, the failure case was caused by the occurrence of various gene-expression patterns and overlapping neighboring context of the embryo.

5.2 Future scope

Experimental results show that the proposed criterion can achieve results with promising accuracy. One of the limitations of the framework is the processing time. The processing time is quite long as we are working with the original size of the image to preserve the details of the image. If the image is resized, there is a possibility of losing a few details of the image. The accuracy of the result is governed by the convergence criteria, used in energy minimization. To make the results more accurate, tighter convergence criteria is required. Hence, this results in longer computation time. The second limitation is the larger scale space. Each image might have a different optimal scale. If we plot a graph taking image as x axis and scale as y axis, we obtain a scattered graph. Figure 5.1 gives a picture of the graph.

Figure 5.1: Graph showing optimal scale of the images
To address this problem, a classification model may be developed based on the scale space. The scale space can be divided into a range of values and each range of values can be classified as a particular class. This classification might give us some information about range of scales of the images having single embryo, partially touching embryo and non touching partial embryos. In this way, we might come to the conclusion that if the image is having single embryo, then it might belong to a particular class, and we get the information of the range of optimal scales. Similarly, we might come to a conclusion with touching and non touching embryos.
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