

**SEGMENTATION OF ANTI-NUCLEAR ANTIBODY IMAGES BASED ON
WATERSHEDS AND FAST REGION MERGING**

By

NOOR LIYANA BINTI MEHFAR

FINAL PROJECT REPORT

Submitted to the Department of Electrical & Electronic Engineering
in Partial Fulfilment of the Requirements
for the Degree
Bachelor of Engineering (Hons)
(Electrical & Electronic Engineering)

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By

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CERTIFICATION OF APPROVAL

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15170

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Approved:

AP Dr. Ibrahima Faye

Project Supervisor

UNIVERSITI TEKNOLOGI PETRONAS

TRONOH PERAK

December 2013

CERTIFICATION OF ORIGINALITY

This is to certify that I am responsible for the work submitted in this project, that the original work is my own except as specified in the references and acknowledgments, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

Noor Liyana Mehfar

ABSTRACT

In this paper, segmentation based on watersheds and fast region merging is proposed to be the main segmentation technique in this project. Segmentation of Anti-Nuclear Antibody (ANA) images is the data or input of this project by concentrating in four different patterns of ANA images. Watershed segmentation algorithms are combining edge and region based techniques. Furthermore, watershed segmentation algorithm has several drawbacks which are the image to be over segmented and separation of homogenous region. Therefore, fast region merging techniques are proposed and implement to overcome the problem. In addition, this technique is used to merge homogenous region based on several criteria which is intensity and some other homogenous parts. Moreover, in this paper, basic Gaussian filter is been implement to enhance the watershed segmentation by reducing some of the unwanted noise inside the images and highlighting the edge of cells. Gaussian filter is the fundamental technique in segmentation process of an image. This paper describes the watershed and fast region merging segmentation techniques to be implement in ANA images and the result will be the behaviour of each ANA images based on the selected pattern to be use in this paper.

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Chapter 1

PROJECT BACKGROUND

1.1 Background of study

Medical image processing provides a valuable contribution to lab technologist in medical image analysis. Medical image segmentation is an essential method used in clinical diagnosis and assessment. The result enhance the efficiency of diagnostic and beneficial for organs and tissue recognition[1]. In this paper, edge based detection method is proposed for anti-nuclear antibody (ANA) images segmentation.

In our body, normally we have antibodies to avoid any invaders into our body such as virus but for anti-nuclear antibody (ANA), it gives a signal to body to against itself because of antibodies identify a self-protein as foreign. Besides that, ANA can be produced with infections, lung diseases, cancer and autoimmune diseases. ANA have a capability to combine with several blood nucleuses inside the blood serum.

In order to detect the present of ANA inside the blood serum, a test called Anti-Nuclear Antibody test need to be done using either two techniques. The two techniques are Indirect Immunofluorescence (IIF) and Enzyme-Linked Immunosorbent Assay (ELISA). In this project, IIF test is the primary method to be used instead ELISA. In fact, IIF is the preferred method for ANA detection.

Furthermore, for the purpose of ANA detection, the main screening method has been introduced which is Indirect Immunofluorescence (IIF) with HEp-2 cells. [2]. Hep-2 cells are a catalyst to detect ANA pattern. In this paper, only four main patterns will be taken into consideration.

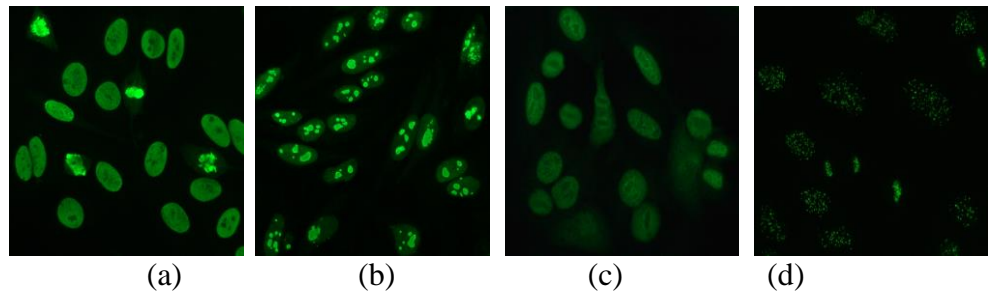


Figure 1: Anti-Nuclear Antibody Images (a) Homogenous pattern, (b) Nucleolar pattern, (c) Speckled pattern and (d) Centromere pattern

1.2 Problem statement

Indirect Immunofluorescence (IIF) is the main method for detection of auto-antibodies using Anti-Nuclear Antibody(ANA)[3]. Current detection of ANA is based on manual human evaluation are time consuming, suffer from high variability, which impacts on the reliability of results. Due to these disadvantages of manual testing, automation and standardization are necessary. A Computer Aid Diagnostic System of ANA by IIF is necessary but the background and the nucleus cell of ANA need to be accurately segmented/distinguished in order to come up with a fast and accurate diagnostic system.

1.3 Objective

- To develop segmentation algorithm using watershed and fast region merging to enhance Anti-Nuclear Antibody (ANA) images in order to be able to accurately segment the nucleus of ANA cell and according to the edge and region of the cells.
- To evaluate the performance of proposed segmentation method on a dataset of ANA images.

1.4 Scope of project

- i. This study is focusing on four patterns of ANA cells which are nucleolar, homogenous, centromere and speckled.
- ii. Watersheds and fast region merging are the main technique that will be used in this study.
- iii. To design and implement an algorithm using Matlab and evaluate the performance of the algorithm on ANA images.

1.5 Feasibility of the project

Feasibility of this project is considered as possible to be done since all the equipment and software are needed are available at place of study and all the information needed can be found through online website or books available at place of study.

Chapter 2

LITERATURE REVIEW

2.1 Watershed segmentation

In general, watershed segmentation algorithm is the combination of edge and region based techniques[4]. Moreover, the watershed segmentation is been developed quite a long year back with many advance and improvement method and still going on until now. Hence, from the improvement that has been developed year by year, several drawback has been decreased due to the research done so far. Furthermore, current watershed segmentation is not longer to standalone in order to segment the images but it is combining with some other segmentation techniques. Moreover, several researchers used watershed as proposed techniques and providing a positive result and some drawback also exist [5-7]. Among the most crucial are as follows[6]:

(i) Over segmentation

When the watershed segmentation is perform, the result is usually will be over segmentation and hardly useful. Therefore, the common solution for this problem is to use marker image[8].

(ii) Sensitivity to noise

Watershed effect is worst when it use of high pass filters to measure the gradient which will amplify the noise. As a solution, an isotropic filters need to be use in order to reduce the noise.

(iii) Poor detection in low contrast contour

The watershed transform is unable to detect the low contrast contour due to signal to noise ratio is not sufficient enough.

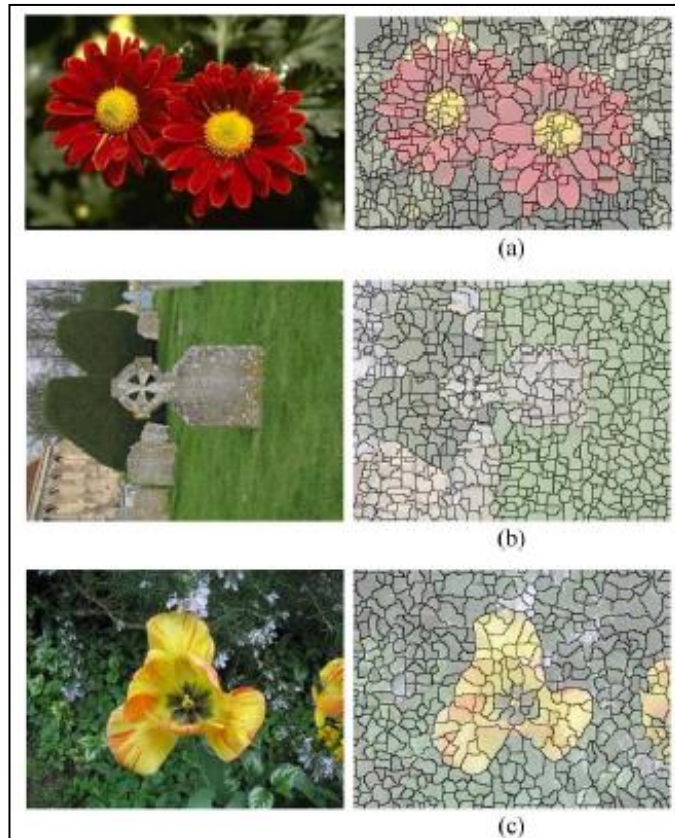


Figure 2 : Over segmented images[9]

Currently for edge detection, another method is selected to be the technique to detect the edge since watershed segmentation is tending to over segmentation. Moreover, for region, it is practical to used watershed since it separated the region but by applying another method, this problem can be solved. In this paper [2], watershed segmentation is proposed with otsu thresholding as the preliminary technique in order to get accurate result. After applying otsu method, watershed segmentation is implement and divided in three techniques which are foreground, background and marker controlled watershed segmentation. Watershed segmentation can be a powerful segmentation method when it combine with any morphological tools[10].

2.1.1 Watershed transformation

Edge based detection is basically a segmentation method to detect the contour or boundary of edge of an image by using intensity gradient and grouping the images to detect the continuities of the contour[11]. The images are labelled according to edge and non-edge pixels. However, there is some error on the detection of contour due to noise and another one is due to the contour being broken into small edge groups. Watershed transformation is used to overcome the disconnected contour using gradient magnitude image. Moreover, region detection technique can be used to produce a close contour image.

2.1.2 Two staged watershed segmentation

In this paper [12], it proposed two staged watershed segmentation as a pre-processing method to obtain the accurate outline of the cells. On the first stage of segmentation, this particular stage is to merge the homogenous or connected region. Moreover, threshold is set to eliminate the region that has unreasonable size which is below 100 pixels. As the result, the selected region is counted; RN_1 is used to check whether the region is under predefined threshold. If the selected region is under specification, the first stage segmentation is positive and produces the outline of the cells.

As in second stage of the segmentation, the concept of markers is used to draw the outline of the over-segmented image. Region that have pixel over the threshold is going through pre-processing again. There are two types of cell images which is nucleolar and discrete speckled patterns. Furthermore, cyan component is used because the dissimilarity of background and region is high. Therefore, the outline can be detected due to the dissimilarity. After going through the pre-processing procedure, otsu's thresholding was implement to generate the marker for watershed segmentation.

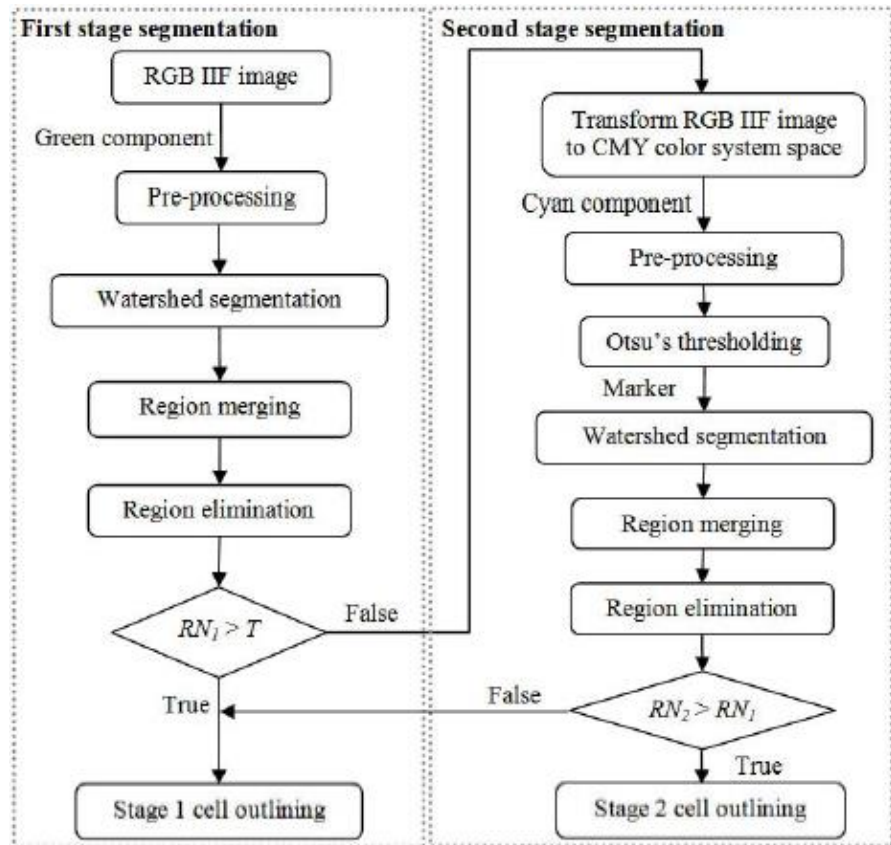


Figure 3: Flowchart of two staged watershed segmentation

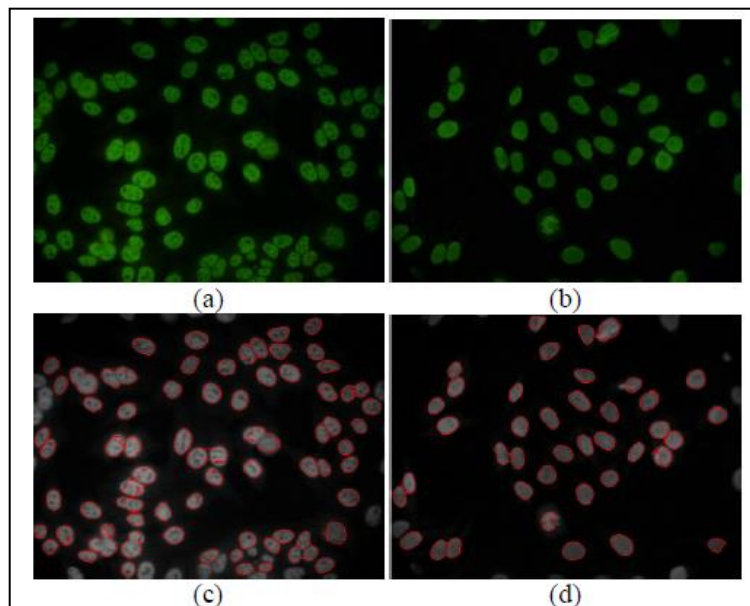


Figure 4: (a)(b)Nucleolar and speckled pattern (c)(d) Segmented cells

1.1 Fast Region Merging[13]

Watershed segmentation method is currently having many drawbacks due to many factors that cannot be solved by using watershed algorithm itself. Therefore, fast region merging is proposed to be second method or solving method of over segmented images after the pre-processing process. In an image, there are some criteria that allow the image to be merged together. Furthermore, region can be define as a set of homogenous properties that can be classified as the same pixels that grown by merging smaller regions [13] .

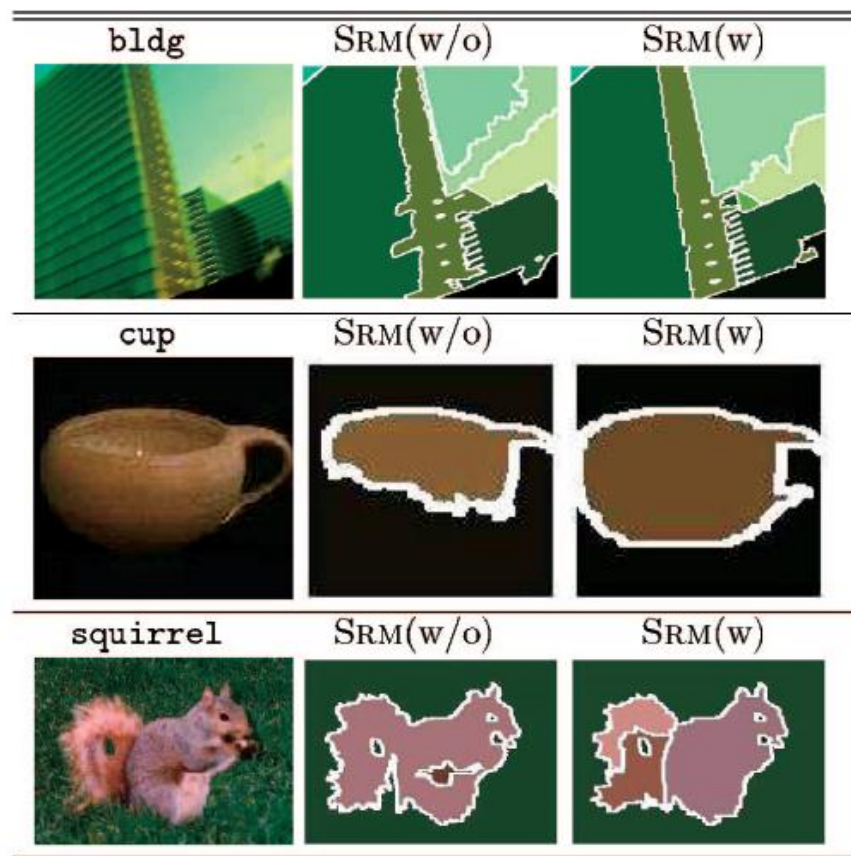


Figure 5: Example of Statistical Region Merging Technique [13]

2.3 Analysis of literature

Watershed segmentation is quite a classic segmentation method compare to other advance method. Furthermore, watershed is still reliable method to be used in segmentation method. The fundamental of watershed itself give a valuable contribution to image processing process to get the best and positive result. Based on research done so far, successful rate on watershed segmentation is quite high. The result is not 100% perfect but still can be useful in image processing. In this paper, the author focusing on watershed segmentation method with additional fast region merging to overcome the drawback from watershed which is over segmented image.

Chapter 3

METHODOLOGY

3.1 Research methodology

In this part, this will explain the research methodology by phases accordingly

(a) Literature research

In literature research, medical image processing is the first thing to know about because this project is related in that particular scope. Therefore, to have some basic knowledge on image processing is important. To deal with image segmentation, there are many techniques in image processing. Some research on main techniques of image segmentation need to be done. To evaluate each of the techniques, it is suggested to read research paper done so far for that particular topic

(b) Background study

Segmentation techniques are used in many image processing applications. For this paper, image segmentation process for medical imaging will be studied. It is beneficial to know others techniques beside proposed techniques.

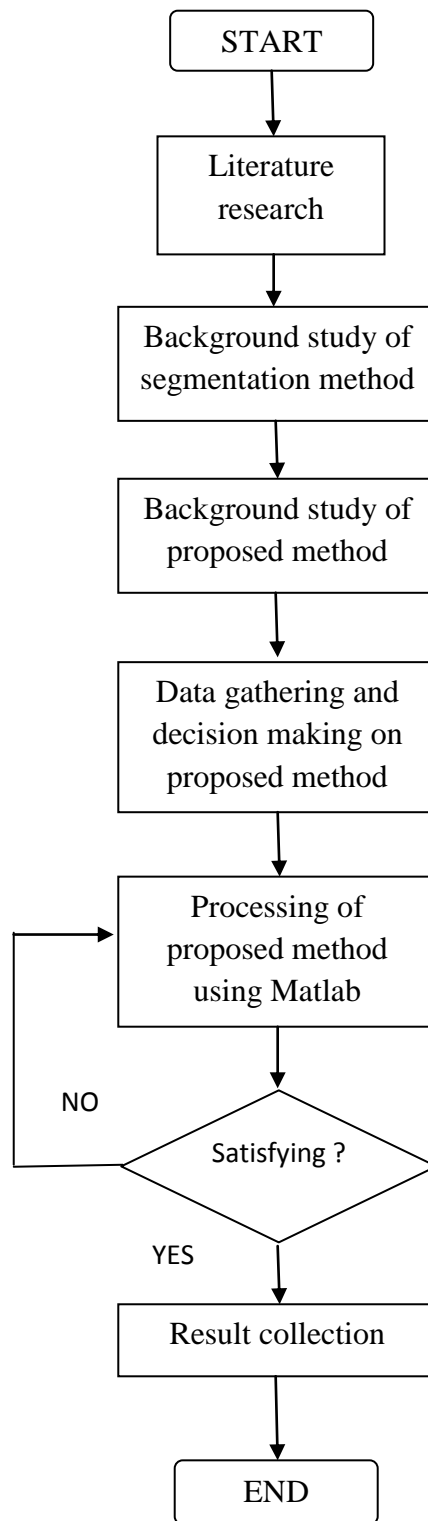
(c) Image dataset gathering

The proposed techniques need to process four types of pattern. Therefore, image dataset need to be compile according to the type. For the image dataset, author obtains from MIVIA.

(d) Image Processing

For final result, pre-processing need to be done to obtain a good output. Furthermore, watershed segmentation method needs to undergo several pre-processing before watershed can be applied. Hence, in order to achieve a good result, image pre-processing is a vital process in image processing.

3.2 Flow chart



3.3 Coding development

3.3.1 Data Gathering and Analysis

In this project, after having discussion with respective supervisor, from overall cell in one image, only one cell need to undergo segmentation process since the performance of cell can be evaluate by using only one cell. Furthermore, the objective of this project is to segment the nucleus inside the cell. Therefore, by using only one cell and segment the nucleus, the cell can proceed to classification process.

By using Matlab pre-processing toolbox, this can project can be done which is to segment Anti-Nuclear Antibody Images in order to achieve the objectives. Matlab capable to perform image processing that have a set of reference standard algorithm, visualization, functions etc. Image processing toolbox supports every range of image with different resolution and also contrast.

(i) Image pre-processing

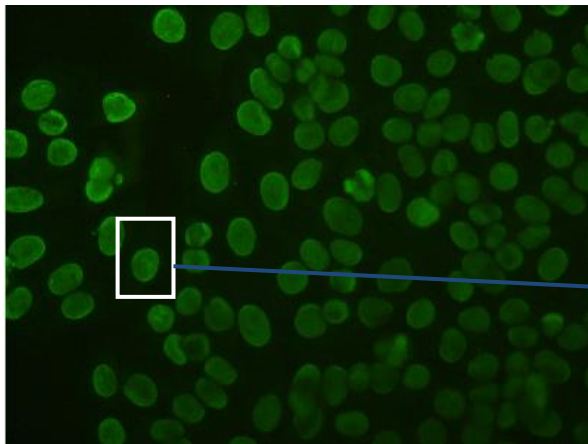


Figure 6: Original image

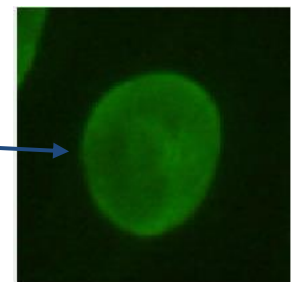


Figure 7: Selected cell

```
I = imread('22.bmp');  
crp=imcrop(I);  
figure(2), imshow(crp);  
conv=rgb2gray(crp);  
I_eq=conv;  
bw = im2bw(I_eq, graythresh(I_eq))  
figure, imshow(bw)
```

Firstly, by using imread function, the selected cell image name needs to be called out. Previously, the selected image are using imadjust function to increase the intensity but there are drawback in the result which is it ruin the original structure of the image. Therefore, imadjust function cannot be used in this project due to the drawback occur. Furthermore, after cropping the selected cell, the image is converted into gray scale which using rgb2gray function. The result is in figure 8 below.

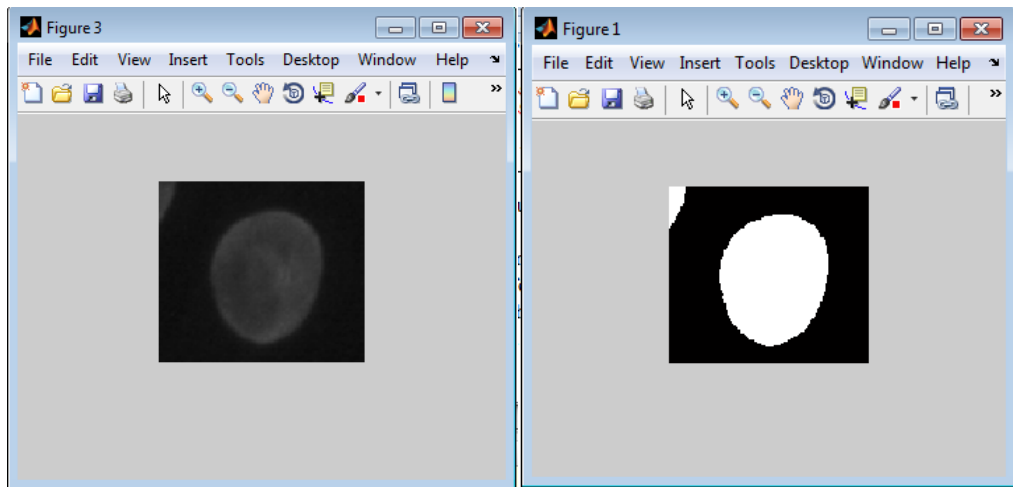


Figure 8: rgb2gray result

Figure 9: Global threshold result

Furthermore, the rgb2gray function converts the image into grey scale. It is easy for the process to be executed because by using grey colour space it easy to indicate the intensity differences. In figure 9, global threshold is used to convert the image into black and white or binary image to preserve the edge and region of the image.

(ii) Watershed segmentation

For watershed segmentation process, image need to undergo several processes such as compute morphological process because without this process, image will tend to over segmented. Furthermore, the process is divided into two sub-process which is segmentation the outer cell and inner cell.

```
bw3 = imopen(bw, ones(1,1));  
figure, imshow(bw3);  
bw4 = bwareaopen(bw3, 40);  
figure, imshow(bw4);  
bw4_perim = bwperim(bw4);  
figure, imshow(bw4_perim);  
overlay1 = imoverlay(I_eq, bw4_perim, [.3 1 .3]);  
figure, imshow(overlay1)
```

For *imopen* function, it used to perform morphological opening only on grayscale or binary image. It include the erosion followed by a dilation using the same particular image components. The erosion is used to shrink the image according to the structuring element and eliminate objects that smaller than the image shape. Next is the dilation, it dilates the remaining objects by the same particular image shape.

For *bwareaopen* function, 40 is P value which is indicating the pixel of the image. The function will delete the white areas that below than P value. The areas that exceed P value is preserve and the result of this particular function is mostly similar with *imopen* function. Since the image is in binary which is two dimension images, the default connected component is 8.

To find the perimeter of the cell in binary image, *bwperim* function is used to observe the perimeter pixels of input image. A non-zero pixel is indicate as part of the perimeter or else at least it is connected to one zero valued pixel. The default connectivity for two dimension image is 4.

Hence, as the result of the morphological operation for outer cell, *imoverlay* function is introduced to superimpose the result of the morphological operation with grayscale image which is the original image. Basically it overlay one image on top of another to get better result. As in the coding, *I_eq* indicate the binary image and it overlay with *bw4_perim* which is the result from *bwperim* function.

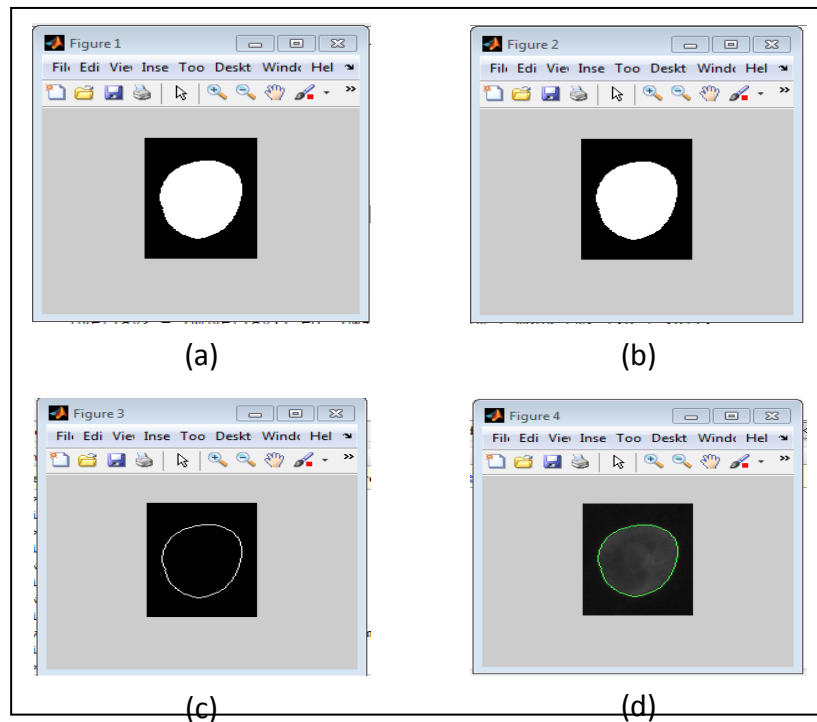


Figure 10: (a) *imopen* result (b) *bwareaopen* result (c) *bwperim* result
(d) *imoverlay* result

```

mask_em = imextendedmax(I_eq, 15);
figure(1), imshow(mask_em);
mask_em = imclose(mask_em, ones(5,5));
figure(2), imshow(mask_em);
mask_em = bwareaopen(mask_em, 40);
figure(3), imshow(mask_em);
overlay2 = imoverlay(I_eq, bw4_perim | mask_em, [.3 1
.3]);
figure, imshow(overlay2)

```

This process is to segment the inner cell to develop the nucleus of the cell. By using *imextendedmax* function, it can identify all regional minima or maxima that follow the specified threshold. The input of this function is grayscale image and the output is in binary image. In this function, it computes the maximum region which is the high intensities area.

For *imclose* function, it is inverted with *imopen* function whereby the process is starting with dilation followed by an erosion of the image. The next function is *bwareaopen*, it is same with previous function but applied in different image.

As the result for morphological for inner cell, *imoverlay* function is used to compute the result of the inner cell.

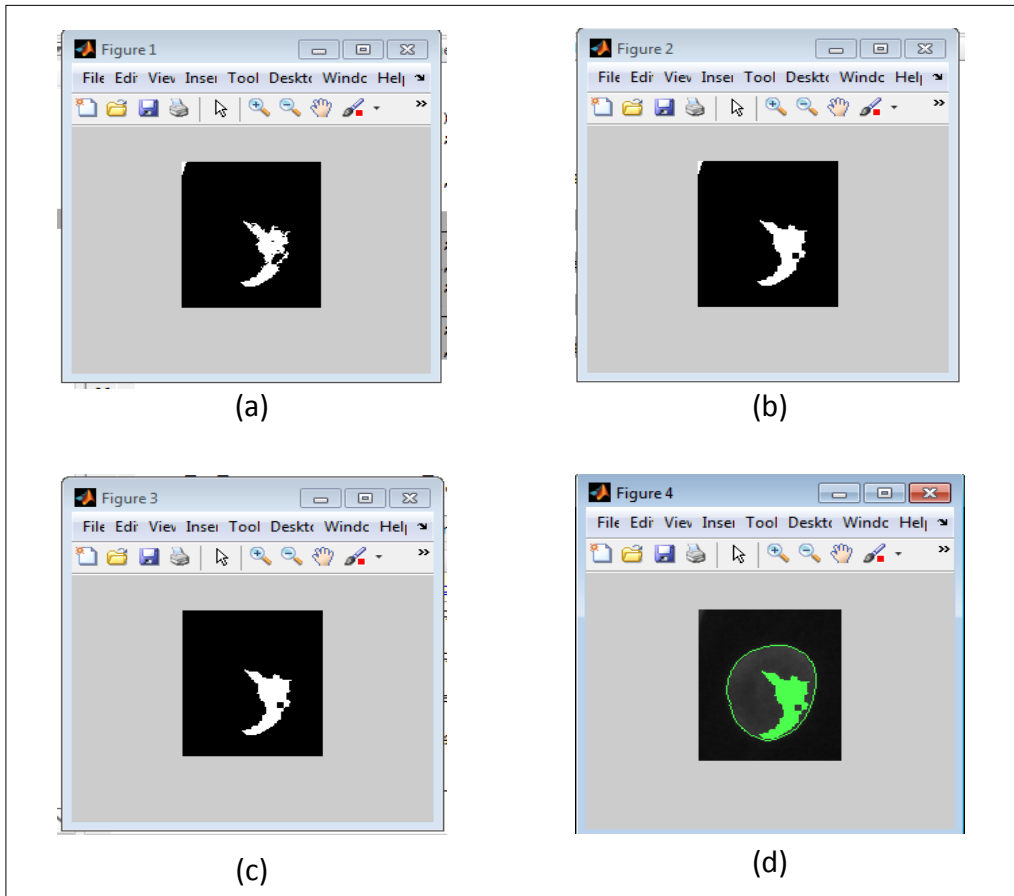


Figure 11: (a) *imextendedmax* result (b) *imclose* result (c) *bwareaopen* result (d) *imoverlay*

Furthermore, to compute the watershed segmentation process, there is several processes need to completed. For *imcomplement* function, this function focusing on inverted the intensity such as black will become white and white will became black. The low intensity will become darker and the high intensity will become lighter. From this process, it appears the nucleus as black and the rest is white. By using *imimposemin* function, it modified the intensity of minima regional. Furthermore, to compute the distance between

pixels, *bwdist* function is used by assigning a number that is distance between pixel and nearest non-zero pixel.

Lastly to compute the watershed segmentation process, *watershed* function is used. By referring to figure (), watershed image is superimposed with *imoverlay2* result. Therefore, the nucleus inside the cell is visible and the objective of this project is achieved.

```
I_eq_c = imcomplement(I_eq);
I_mod = imimposemin(I_eq_c, ~bw4 | mask_em);
D = bwdist(~I_mod);
figure(4), imshow(D, [], 'InitialMagnification', 'fit')
title('Distance transform of ~bw')

D = -D;
D(~I_mod) = -Inf;

L = watershed(D);
imshow(L);
rgb = label2rgb(L, 'jet', [.7 .8 .9]);
figure(4), imshow(rgb, 'InitialMagnification', 'fit')
title('Watershed transform of D')

figure, imshow(overlay2), hold on
himage = imshow(label2rgb(L));
set(himage, 'AlphaData', 0.2);
```

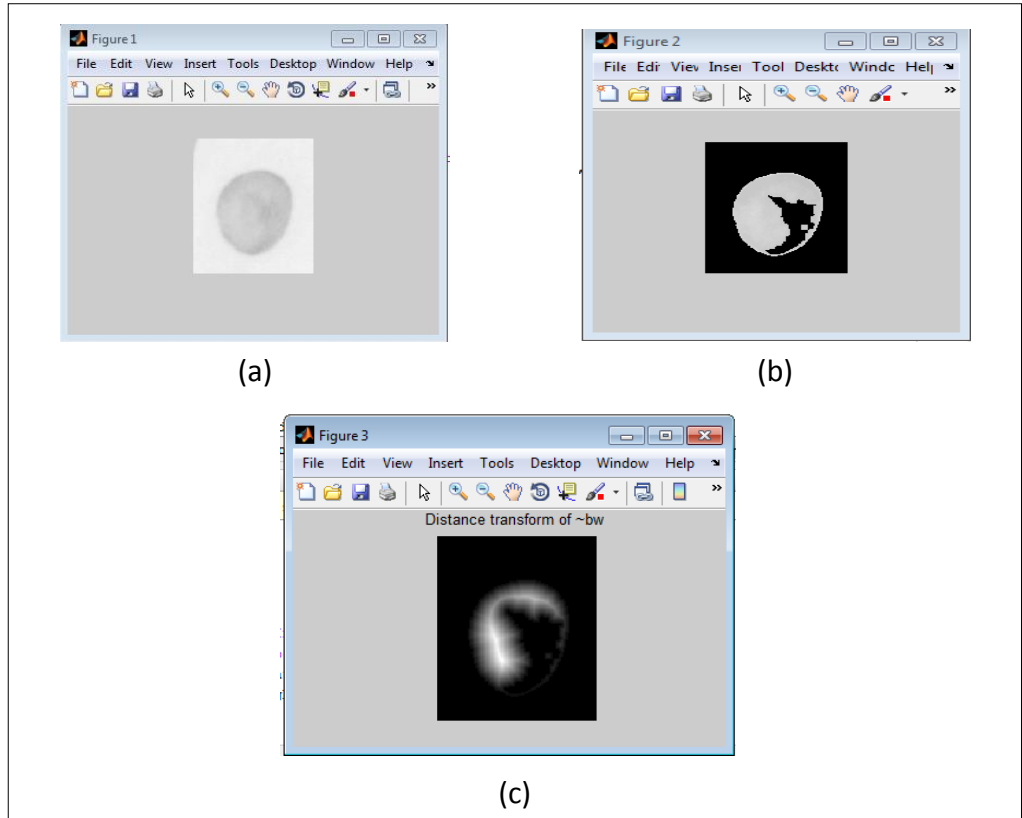


Figure 12: (a) *imcomplement* result (b) *imimposemin* result (c) *bwdist* result

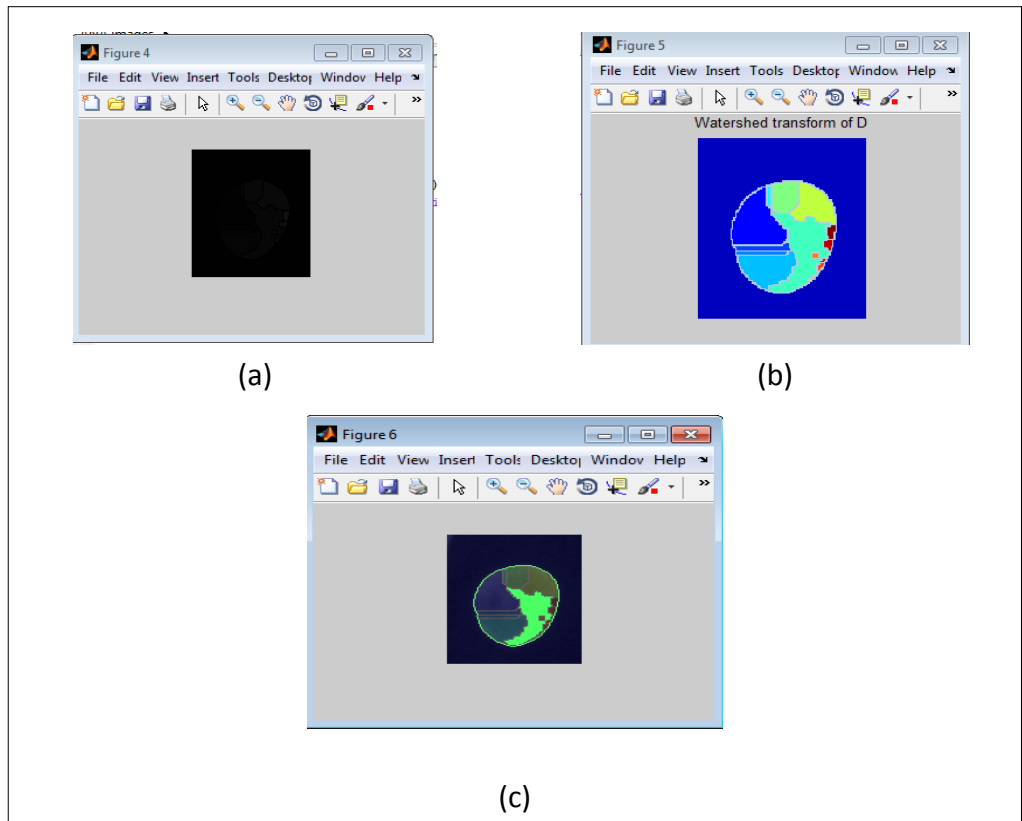


Figure 13: (a) *watershed* result (b) *label2rgb* result (c) *imoverlay* result

(iii) Fast Region Merging

For the second method which is fast region merging, the result is not good enough and the nucleus cannot be detected since this method is to merge the same region. Meanwhile for this particular image, the intensity for every image is not the same. Hence if the image is process using watershed, it creates many regions. Therefore, this method is not suitable for this particular image. Furthermore, the process itself make the image is no longer in the actual structure.

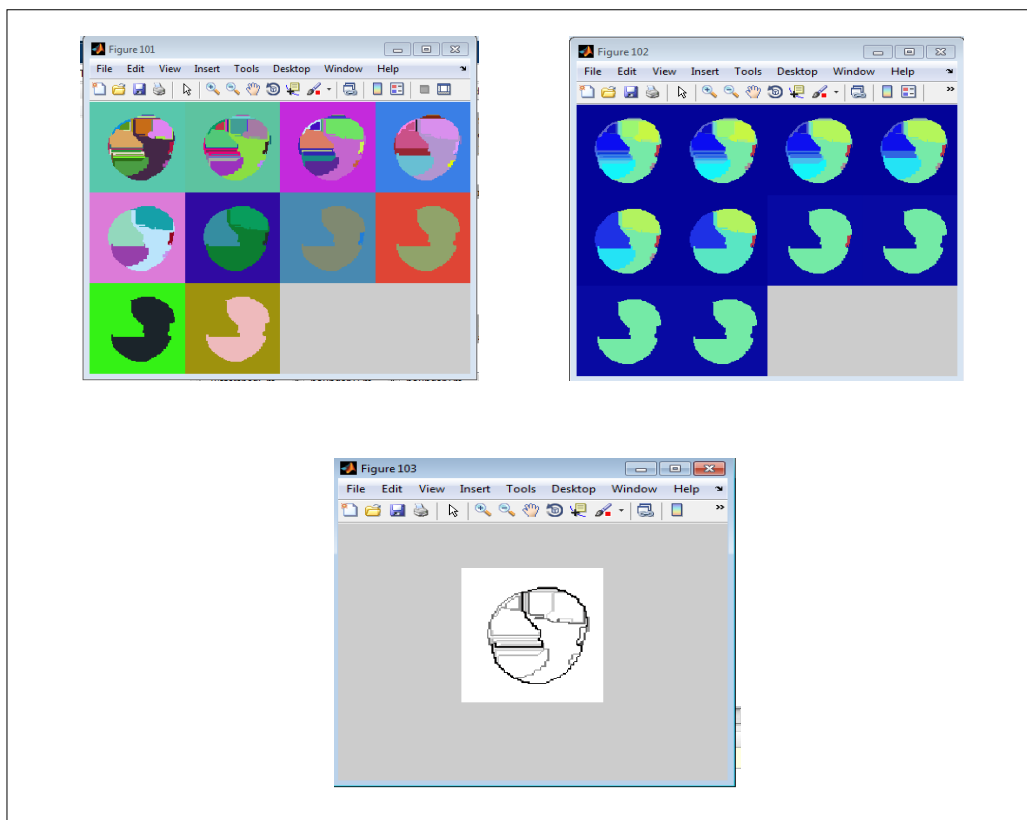


Figure 14: Statistical Region Merging

3.4 Project activities

(a) Attending image processing short course

Image processing short course is conducted inside place of study. This course is using image processing toolbox in Matlab. This course covered generally about image processing, image enhancement and image segmentation. Moreover, some practical on coding using Matlab has been

performed during the course together with instructor. Figure 4 is the coding that has been completed during the course while in figure 5, is the result of every gradient operators.

```
%% Edge detection
% Declare a figure to display images
figure(1);
% Read and image named Blue with JPEG extension and put it
in a variable
% named 'Image'
Image = imread('MyHouse.jpg');
subplot(3,3,1);
imshow(Image), title('Original image');
% convert the image to grayscale
Image = rgb2gray(Image);
% Apply edge detection using sobel operator
EdgeImageRoberts = edge(Image, 'roberts');
subplot(3,3,2);
imshow(EdgeImageRoberts), title('Edge image using Robert
operator');
% Apply edge detection using sobel operator
EdgeImageSobel = edge(Image, 'sobel');
subplot(3,3,3);
imshow(EdgeImageSobel), title('Edge image using Sobel
operator');
% Apply edge detection using Canny operator
EdgeImageCanny = edge(Image, 'canny');
subplot(3,3,4);
imshow(EdgeImageCanny), title('Edge image using Canny
operator');
%Apply edge detection using Roberts operator
EdgeImageRoberts = edge(Image, 'roberts');
subplot(3,3,5);
imshow(EdgeImageRoberts), title('Edge image using Roberts
operator');
%Apply edge detection using zerocross operator
EdgeImageZerocross = edge(Image, 'Zerocross');
subplot(3,3,6);
imshow(EdgeImageZerocross), title('Edge image using
Zerocross operator');
```

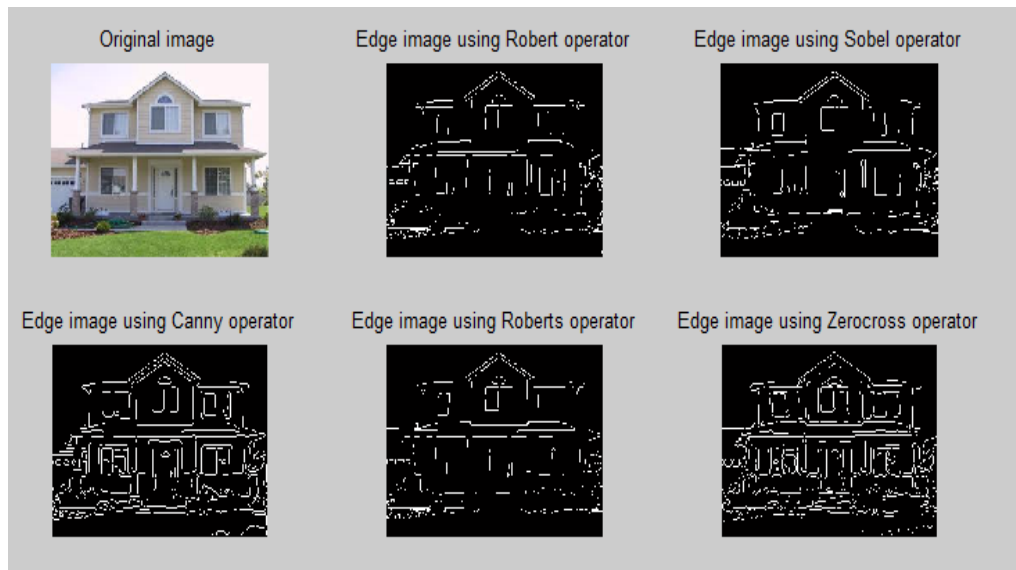


Figure 15: Output of each operator

(b) Automatic cropping process

For the starting of image pre-processing, cell is manually crop by user. Therefore, an automatic cropping function needs to be applied. By using function of *bwconncomp*, the connected component in binary image can be found. Therefore, by combining with *cellfun* function, the idea is to crop the highest intensity which is the highest connected component in an image. There is a limitation on using this function because ANA images have a constant intensity and the cropping function would not able to classify the highest intensity unless a range of intensity level is set.

```

I = imread('polo.jpg');
BW = im2bw(rgb2gray(I), 0.25);figure;imshow(BW);
BW1 = BW;
CC = bwconncomp(BW);
numPixels = cellfun(@numel,CC.PixelIdxList);
[biggest,idx] = max(numPixels);

BW(CC.PixelIdxList{idx}) = 0;
figure, imshow(BW);
figure, imshow(BW1);
Ir = imsubtract(BW1,BW);
figure;imshow(Ir)

```

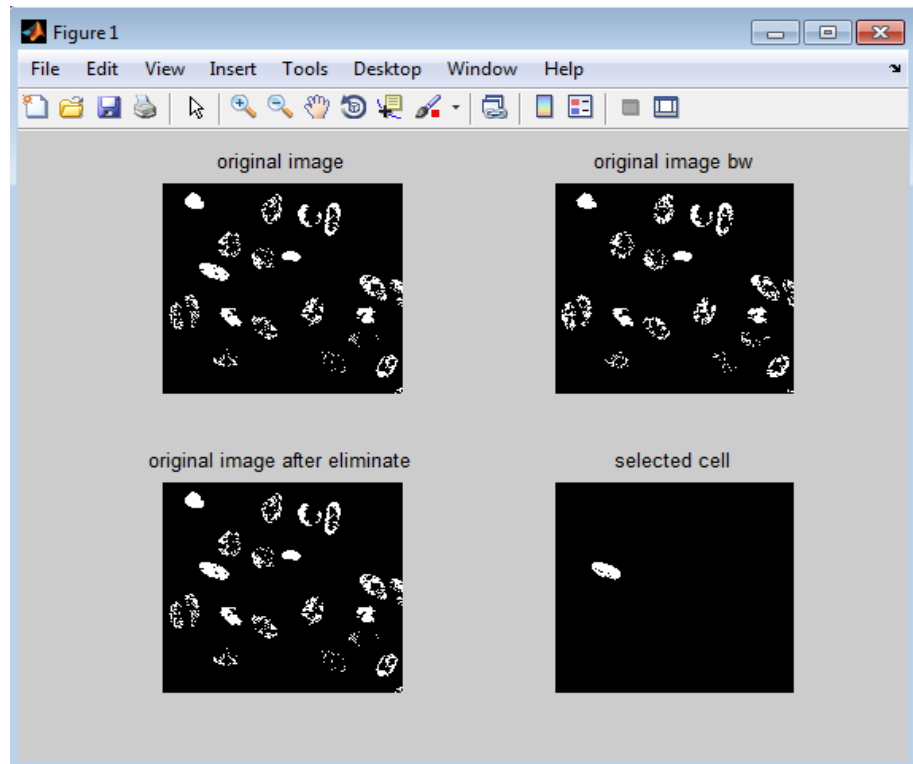


Figure 14: Automatically crop image

(c) Meeting with supervisor

Weekly meeting with project supervisor discussing on some problem or improvement that need to be done.

3.5 Equipment and tools

For equipment and tools, this project using Matlab software. Image processing toolbox is used to segment the image.

3.6 Gantt chart

In table 2, all tasks or works are included in the Gantt chart based on weeks. It is divided in two sections which FYP1 and FYP2. Total weeks for FYP1 and FYP2 are 14weeks respectively. Furthermore, key milestone in each submission of documentation such as report and proposal are also stated in Gantt chart based on date line. Refer to Appendix A and B.

Chapter 4

RESULT AND DISCUSSION

4.1 Segmentation result

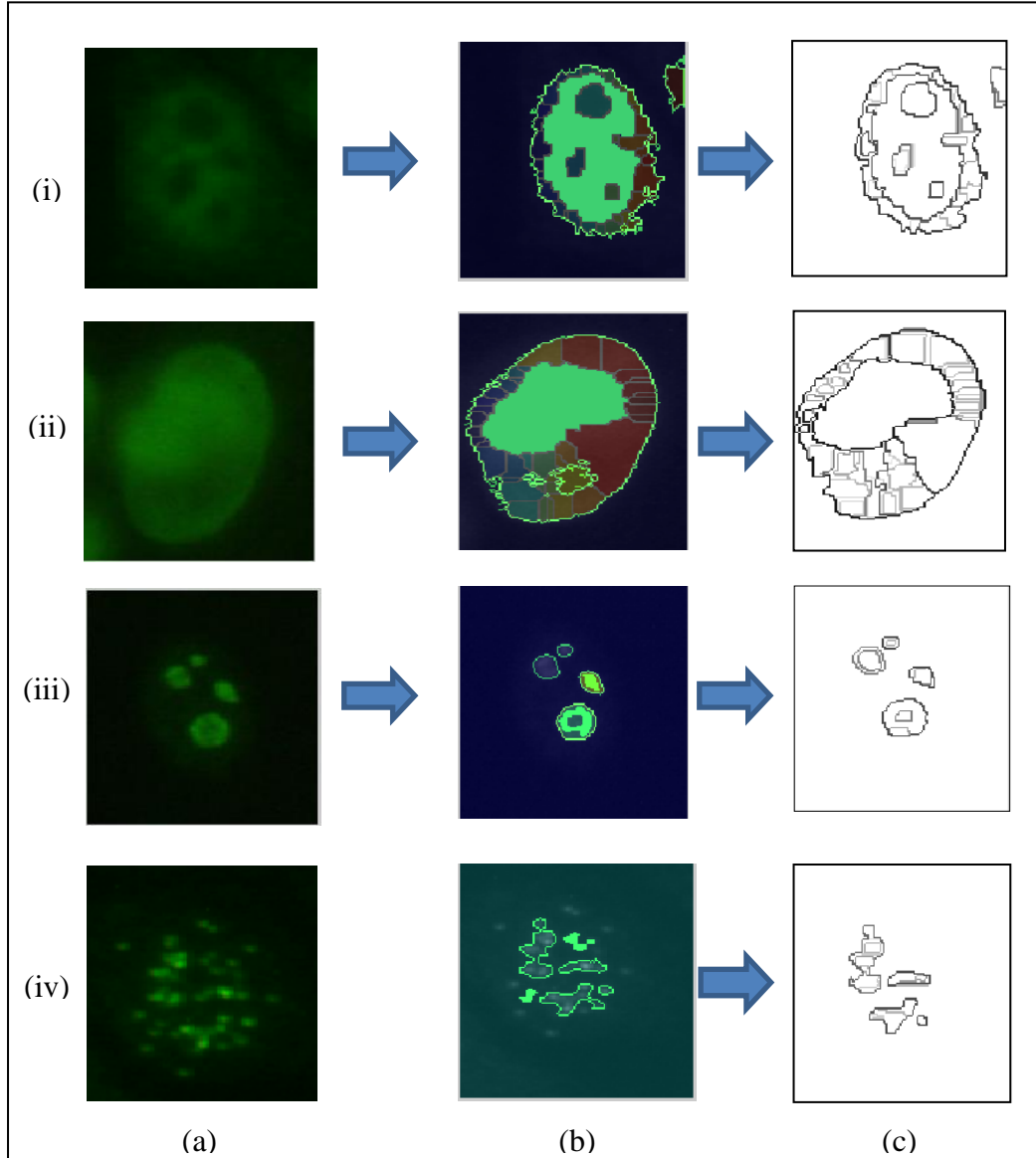


Figure 15:(a) Original image (b) Watershed result (c) Fast Region Merging
(i) Speckled pattern (ii) Homogenous pattern (iii) Nucleolar pattern
(iv) Centromere pattern

In figure 15(a) column, is the cropped image by selection of cell desired by user. Furthermore, in figure 15(b), is the result for first method which is watershed segmentation and for figure 15(c) is the result for fast region merging method. By author observation, the result by using the first method is the best

compared to second method which is fast region merging. Furthermore, fast region merging method is used to overcome the drawback from watershed segmentation method but the result for this particular method is not as author expected. The original structure of cell is not preserved because the method itself not able to identified the homogenous region since in original image, the region of the cell is depends on the intensities of image. For this particular input, the intensities in an image are varying. Therefore, this process has difficulties to merge the cell according to same region. Meanwhile for first method, the result is better than the first method and the nucleus inside the cell is visible and the structure of original image is preserved.

The output of each pattern has been evaluated by using pass and fails method. This pass and fails method is using naked eyes to evaluate the image output since author did not have any ground truth or any reference to be compared. Furthermore, most of research paper is not concentrate on segmented the nucleus of the cell but it concern on the edge of the cell. Therefore, it is hard for author to obtain any info or result related to this project. By using naked eyes, author invites several colleagues to participate in a test to observe the nucleus inside the original image and compared it with segmented image using naked eyes. If the participant give a positive result is categorize into pass and vice versa. Author run 30 images for each pattern which is homogenous, speckled, centromere and nucleolar. Each participant evaluates five (5) random images and the accuracy for each pattern is calculated depends on pass test. The result is in table 1.

	Pass	Fail	Accuracy
Homogenous	24	6	80%
Nucleolar	25	5	83%
Speckled	14	16	47%
Centromere	10	20	33%

Table 1 : Segmentation accuracy result

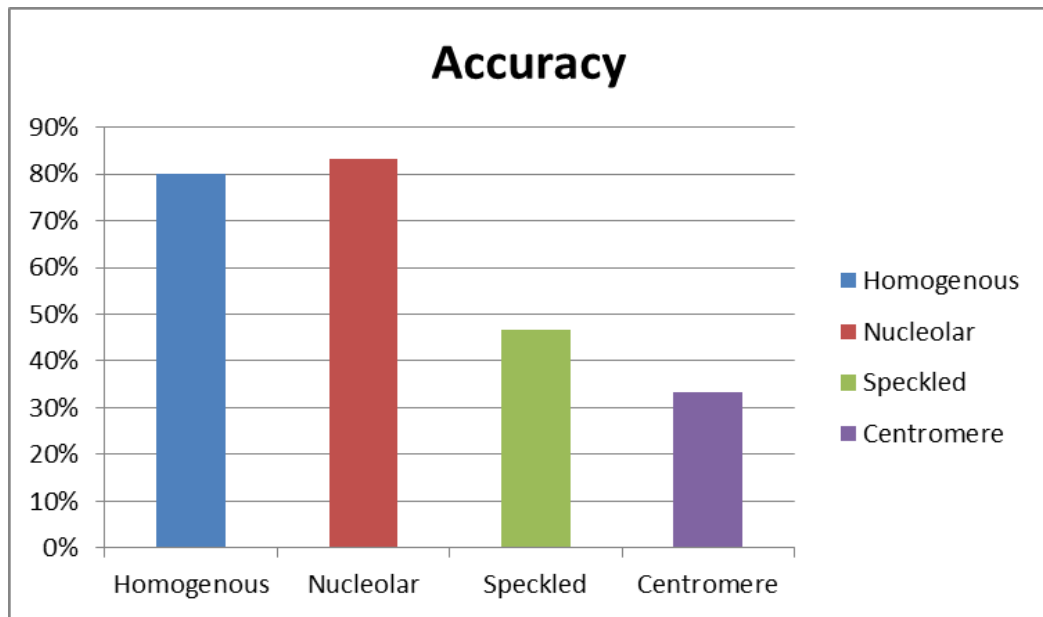


Figure 16 : Segmentation accuracy result

For figure 16, the most successful pattern is nucleolar followed by homogenous. For nucleolar pattern, the nucleus inside the cell is easily to identify since the edge of the cell is less visible. Refer to figure 15(iii). Therefore, the accuracy for this process to segment the nucleus is high. For second highest accuracy is homogenous pattern. Refer to figure 15(ii). Since the nucleus for homogenous is in single object, therefore the nucleus for this pattern is easily to process. Centromere pattern is the lowest accuracy since the cell contains too many nucleuses. Therefore, this process cannot accurately segment the nucleus and tend to over-segment the nucleus. Refer figure 15(iv).

4.2 Pattern Classification

For Anti-Nuclear Antibody cells, the pattern can be classified using several types of properties such as number, size and shape. Hence, segment the nucleus of the cell, the pattern of the cell can be classified using object number method. Every each of the cells has different object number which means the nucleus inside the cell. Therefore, in this project, the output of segmented image can be used to classify the cell according to the pattern.

Pattern	Properties
Homogenous and speckled	Single object inside cell
Centromere	Corresponding to human cell which is around 46 nucleus inside one cell
Nucleolar	Nucleolar corresponding to nucleoli inside human cell. The nucleus is around one to ten in one cell.

Table 2: Patterns properties [14]

4.3 Segmentation system

For the result of the overall segmentation process, author had create a platform which is automated segmentation system for make it easier to observe the final result of this segmentation process. Hence, GUI is used to create a system can be easily manage in order to obtain the final result but the final result for this system is watershed segmentation method. For fast region merging method, it will automatic obtain the output after this system is completely finish. It is because fast region merging is not from matlab developer. Therefore, author faces some difficulty to create the output of fast region merging inside this system. In figure 17, it show the step on how to use this system.

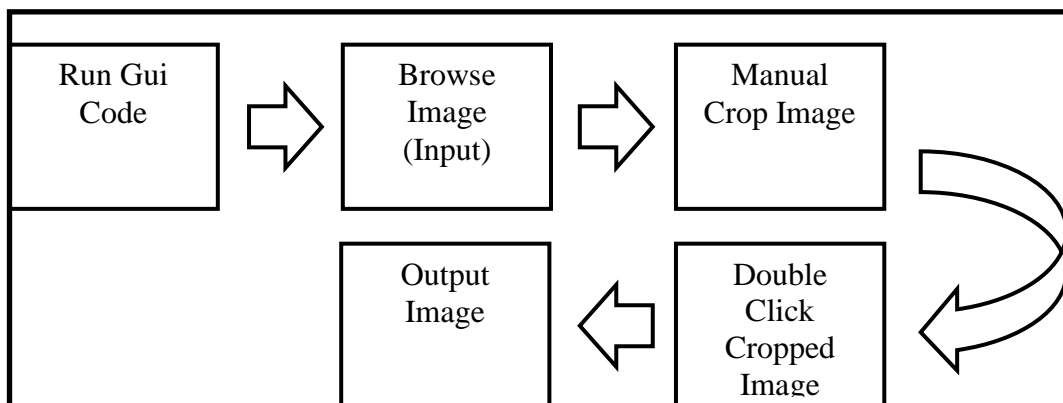


Figure 17: Segmentation System Guideline

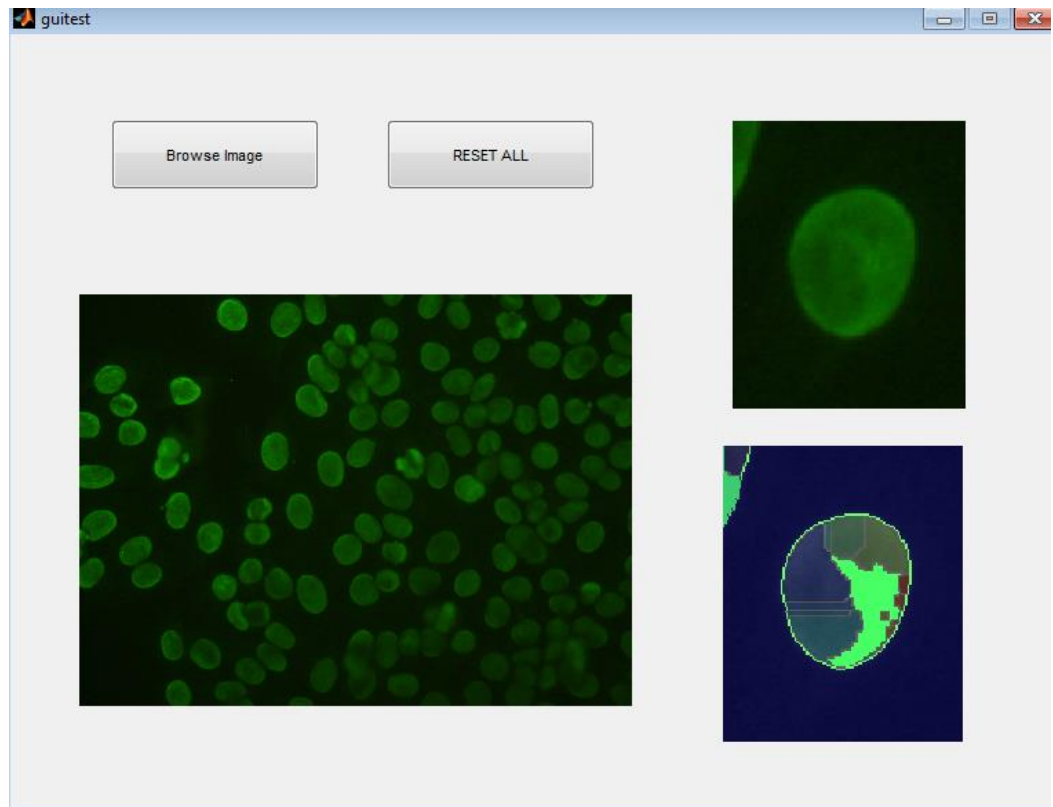


Figure 18: Segmentation system

Chapter 5

CONCLUSION

Author can conclude that image pre-processing is a vital process since the image must be in less noise condition and clear. Furthermore, it will affect the next process which is the segmentation process. Therefore, author take some time on image pre-processing since watershed need to undergo several morphological process before computing watershed function. The result for each of the cell is different. It is because each pattern has its own properties and structure, in order to preserve the structure of the cell, author need to make sure that watershed method is given the best output. In the future, automatically image segmentation system will become an important system. Furthermore, it can save time to process the image and it is beneficial for scientist or medical expertise for having this automatic system. It also can contribute to reduce human error and increase the accuracy of the output. Even though it cannot give a perfect image but less or more it can contribute to medical image processing something valuable that can give many positive impacts to them.

REFERENCES

- [1] C.-Y. Chang and P.-C. Chung, "Medical image segmentation using a contextual-constraint-based Hopfield neural cube," *Image and Vision Computing*, vol. 19, pp. 669-678, 8/1/ 2001.
- [2] C. Chung-Chuan, T. Jin-Shiuh, H. Tsu-Yi, and T. Chin-Wang, "Segmentation of anti-nuclear antibody images based on the watershed approach," in *Industrial Electronics and Applications (ICIEA), 2010 the 5th IEEE Conference on*, 2010, pp. 1695-1700.
- [3] C. W. Liu, T. Y. Hsieh, W. C. Pai, and C. H. Lin, "Segmentation of diffused type for ANA image based on edge model," in *Networked Computing and Advanced Information Management (NCM), 2011 7th International Conference on*, 2011, pp. 26-29.
- [4] M. G. Liu, J. Jiang, and C. H. Hou, "Hybrid image segmentation using watersheds and adaptive region growing," in *Visual Information Engineering, 2003. VIE 2003. International Conference on*, 2003, pp. 282-285.
- [5] J. M. Gauch, "Image segmentation and analysis via multiscale gradient watershed hierarchies," *Image Processing, IEEE Transactions on*, vol. 8, pp. 69-79, 1999.
- [6] A. N. Rao, V. V. Kumar, and C. Nagaraju, "A New Segmentation Method using Watersheds on Grey Level Images," in *Advanced Computing and Communications, 2006. ADCOM 2006. International Conference on*, 2006, pp. 275-278.
- [7] A. N. Moga and M. Gabbouj, "Parallel image component labelling with watershed transformation," *Pattern Analysis and Machine Intelligence, IEEE Transactions on*, vol. 19, pp. 441-450, 1997.
- [8] L. Vincent, "Morphological grayscale reconstruction in image analysis: applications and efficient algorithms," *Image Processing, IEEE Transactions on*, vol. 2, pp. 176-201, 1993.
- [9] P. Bo, D. Zhang, and D. Zhang, "Automatic Image Segmentation by Dynamic Region Merging," *Image Processing, IEEE Transactions on*, vol. 20, pp. 3592-3605, 2011.
- [10] L. Vincent and P. Soille, "Watersheds in digital spaces: an efficient algorithm based on immersion simulations," *Pattern Analysis and Machine Intelligence, IEEE Transactions on*, vol. 13, pp. 583-598, 1991.
- [11] K. Haris, S. N. Efstratiadis, N. Maglaveras, and A. K. Katsaggelos, "Hybrid image segmentation using watersheds and fast region merging," *Image Processing, IEEE Transactions on*, vol. 7, pp. 1684-1699, 1998.
- [12] H. Yu-Len, J. Yu-Lang, H. Tsu-Yi, and C. Chia-Wei, "Adaptive Automatic Segmentation of HEp-2 Cells in Indirect Immunofluorescence Images," in *Sensor Networks, Ubiquitous and Trustworthy Computing, 2008. SUTC '08. IEEE International Conference on*, 2008, pp. 418-422.
- [13] R. Nock and F. Nielsen, "Statistical region merging," *Pattern Analysis and Machine Intelligence, IEEE Transactions on*, vol. 26, pp. 1452-1458, 2004.
- [14] G. V. Ponomarev, V. L. Arlazarov, M. S. Gelfand, and M. D. Kazanov, "ANA HEp-2 cells image classification using number, size, shape and localization of targeted cell regions," *Pattern Recognition*.

APPENDIX A

```
function varargout = guitest(varargin)
% GUITEST MATLAB code for guitest.fig
%   GUITEST, by itself, creates a new GUITEST or raises the
existing
%   singleton*.
%
%   H = GUITEST returns the handle to a new GUITEST or the handle
to
%   the existing singleton*.
%
%   GUITEST('CALLBACK',hObject,eventData,handles,...) calls the
local
%   function named CALLBACK in GUITEST.M with the given input
arguments.
%
%   GUITEST('Property','Value',...) creates a new GUITEST or
raises the
%   existing singleton*. Starting from the left, property value
pairs are
%   applied to the GUI before guitest_OpeningFcn gets called. An
%   unrecognized property name or invalid value makes property
application
%   stop. All inputs are passed to guitest_OpeningFcn via
varargin.
%
%   *See GUI Options on GUIDE's Tools menu. Choose "GUI allows
only one
%   instance to run (singleton)".
%
% See also: GUIDE, GUIDATA, GUIHANDLES

% Edit the above text to modify the response to help guitest

% Last Modified by GUIDE v2.5 04-Dec-2013 02:33:59

% Begin initialization code - DO NOT EDIT
gui_Singleton = 1;
gui_State = struct('gui_Name',       mfilename, ...
                  'gui_Singleton',  gui_Singleton, ...
                  'gui_OpeningFcn', @guitest_OpeningFcn, ...
                  'gui_OutputFcn',  @guitest_OutputFcn, ...
                  'gui_LayoutFcn',  [] , ...
                  'gui_Callback',   []);
if nargin && ischar(varargin{1})
    gui_State.gui_Callback = str2func(varargin{1});
end

if nargout
    [varargout{1:nargout}] = gui_mainfcn(gui_State, varargin{:});
else
    gui_mainfcn(gui_State, varargin{:});
end
% End initialization code - DO NOT EDIT
```



```

% --- Executes just before guitest is made visible.
function guitest_OpeningFcn(hObject, eventdata, handles, varargin)
% This function has no output args, see OutputFcn.
% hObject    handle to figure
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
% varargin   command line arguments to guitest (see VARARGIN)

% Choose default command line output for guitest
handles.output = hObject;

% Update handles structure
guidata(hObject, handles);

% UIWAIT makes guitest wait for user response (see UIRESUME)
% uiwait(handles.figure1);

% --- Outputs from this function are returned to the command line.
function varargout = guitest_OutputFcn(hObject, eventdata, handles)
% varargout  cell array for returning output args (see VARARGOUT);
% hObject    handle to figure
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)

% Get default command line output from handles structure
varargout{1} = handles.output;

% --- Executes on button press in pushbutton1.
function pushbutton1_Callback(hObject, eventdata, handles)
% hObject    handle to pushbutton1 (see GCBO)
% eventdata  reserved - to be defined in a future version of MATLAB
% handles    structure with handles and user data (see GUIDATA)
[fn pn]=uigetfile('*.bmp','select bmp file');
complete = strcat(pn,fn);
Image1=imread(complete);
axes(handles.axes1);
imshow(Image1);
Image2 = imcrop(Image1);
axes(handles.axes2);
imshow (Image2);
%-----%
-----%

```

```

conv=rgb2gray(Image2);

I_eq=conv;
bw = im2bw(I_eq, graythresh(I_eq));
bw3 = imopen(bw, ones(1,1));
bw4 = bwareaopen(bw3, 40);
bw4_perim = bwperim(bw4);
overlay1 = imoverlay(I_eq, bw4_perim, [.3 1 .3]);

mask_em = imextendedmax(I_eq, 15); %extended maxima transform
mask_em = imclose(mask_em, ones(5,5));
mask_em = bwareaopen(mask_em, 40);
overlay2 = imoverlay(I_eq, bw4_perim | mask_em, [.3 1 .3]);

I_eq_c = imcomplement(I_eq);
I_mod = imimposemin(I_eq_c, ~bw4 | mask_em);
D = bwdist(~I_mod);

D = -D;
D(~I_mod) = -Inf;

L = watershed(D);
rgb = label2rgb(L, 'jet', [.7 .8 .9]);

axes(handles.axes3), imshow(overlay2), hold on
himage = imshow(label2rgb(L));
set(himage, 'AlphaData', 0.2);

%-----%
imwrite(rgb, 'L.jpg');

image=double(imread('L.jpg'));
Qlevels=2.^(-1:0);
[maps, images]=srm(image, Qlevels);
srm_plot_segmentation(images, maps);
imshow(images, maps);

% --- Executes on button press in pushbutton2.
function pushbutton2_Callback(hObject, eventdata, handles)
% hObject      handle to pushbutton2 (see GCBO)
% eventdata    reserved - to be defined in a future version of MATLAB
% handles      structure with handles and user data (see GUIDATA)
Axeshandles = [handles.axes1, handles.axes2, handles.axes3];
for h = Axeshandles
    cla(h);
end

```

APPENDIX B: GANTT CHART FOR FYP I

Activities/Weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Project Selection	■	■												
Literature research			■	■	■									
Submission extended proposal						★								
Study of proposed method							■	■						
Proposal defence presentation								★						
Pre-processing proposed method									■	■	■			
Draft interim report											■	■	■	
Submission interim report														★
Algorithm test														■

Work in progress: ■

Key milestone: ★

APPENDIX C: GANTT CHART FOR FYP II

Activities/Weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Algorithm test														
Techniques selection														
Preferred technique test														
Submission of progress report								★						
Segmentation process														
Draft final report														
Submission final report														★

Work in progress:

Key milestone:

