# Automated Classification System for HEp-2 Cell Patterns

by

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Dissertation submitted in partial fulfilment of
the requirements for the
Bachelor of Engineering (Hons)
(Electrical and Electronics)

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CERTIFICATION OF APPROVAL

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(ELECTRICAL AND ELECTRONICS)

Approved by,

_____
(Ms Zazilah May)

UNIVERSITI TEKNOLOGI PETRONAS
TRONOH, PERAK
September 2015

# 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 acknowledgements, and that the original work contained herein have not been undertaken or done by unspecified sources or persons.

_____

NUR ASHIQIN BT NOR SHAHARIM

# ABSTRACT

Human Epithelial Type-2 (HEp-2) cells are essential in diagnosing autoimmune diseases. Indirect immunofluorescence (IIF) imaging is a fundamental technique for detecting antinuclear antibodies in HEp-2 cells. The four main patterns of HEp-2 cells that are being identified are nucleolar, homogeneous, speckled and centromere. The most commonly used method to classify the patterns is manual evaluation. This method is prone to human error. This paper will propose an automated method of classifying HEp-2 cells patterns. The first stage is image enhancement using Histogram equalization contrast adjustment and Wiener Filter. The second stage uses Sobel Filter and Mean Filter for segmentation. The third stage feature extraction based on shape properties data extraction. The last stage uses classification based on different properties data abstracted. The results obtained are more than 90% for nucleolar and centromere and about 70% for homogenous and speckled. For future work, another feature extraction method need to be introduced to increase the accuracy of the classification result. The method suggested is to analyze and obtain the data based on the texture of the image.

# ACKNOWLEDGEMENTS

First and foremost, with the completion of my Final Year Project FYP, I would like to express my outmost gratitude to my university, Universiti Teknologi PETRONAS for accommodating and providing me all the needed things for my project throughout the 2 semesters.

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Last but not least, special thanks to my fellow classmates from January 2011 batch that are willing to share their knowledge and also not to forget my family who are very supportive and motivates me throughout the FYP.

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# ABBREVIATIONS AND NOMENCLATURES

FYP: Final Year Project

UTP: Universiti Teknologi PETRONAS

CREST: Calcinosis, Raynaud syndrome, Esophogeal dysmotility, Sclerodactyly, Telangiectasia

HEp-2: Human Epithelial Type 2

ANA: Antinuclear Antibodies

IIF: Immunofluorescence

SLE: Systemic Lupus Erythematosus

MATLAB: Matrix Laboratory

MIVIA: Macchine Intelligent per il riconoscimento di Video, Immagini e Audion

HE: Histogram Equalization

AHE: Adaptive Histogram Equalization

UM: Unsharp Masking

GF: Gabor Filter

SLE: Active Systemic Lupus Erythematosus

# CHAPTER 1

# INTRODUCTION

## 1.1 Background

Antibodies are produced by a person's immune system as a defense mechanism against foreign protein (antigens). In a normal condition, the antibodies will only attack antigens. However, there are an exception condition where the antibodies destroy the human proteins (autoantigens) instead. This condition is consider as an abnormality. When this condition occurs, the immune system produces a group of antibodies called Antinuclear Antibodies (ANA). The ANA attacks autoantigens instead of antigens (Wiliem, Hobson et al. 2014).

ANA test is conducted by collecting sample of blood serum from an individual. Human Epithelial type 2 (HEp-2) cells will be present inside the collected blood serum. HEp-2 cells can be seen and captures the image by using Indirect Immunofluorescent (IIF) protocol (Doshi and Schaefer 2013, Schaefer, Doshi et al. 2013). The IIF image is then analyze to determine whether the individual HEp-2 cells is normal or otherwise. The HEp-2 cells have distinct pattern according to the diseases associated with the antibodies abnormality. The following Figure 1 shows the HEp-2 cells pattern and its associated diseases:

| Nucleolar | Homogenous | Speckled | Centromere |
|---|---|---|---|
| • Sclerodema<br>• Polymyositis | • Mixed connective tissue disease.<br>• Active Systemic Lupus Erythematosus( SLE)<br>• Drug-induced lupus. | • Mixed connective tissue disease.<br>• SLE<br>• Sjogren Syndrome<br>• Sclerodema<br>• Polymyositis<br>• Rheumatoid arthritis | • Sclerodema<br>• Calcinosis, Raynaud syndrome, Esophogeal dysmotility, Sclerodactyly, Telangiectasia (CREST). |

FIGURE 1: HEp-2 cells patterns and its associated diseases.

### 1.2 Problem Statement

Currently, ANA test are analyze manually by an expert physician at the hospitals. This method have several limitation that will affect the accuracy of the test diagnosis. Manual diagnosis have the following limitations as listed below:

i) Subjectivity of diagnosis.

The quality of the diagnosis are subjective due to the dependency on the experience and expertise of the physician which varies from one physician to another.

ii) Accuracy of diagnosis.

Accuracy of diagnosis can be affected by the physician condition (Fatigue and stress can affect the accuracy).

iii) Standardization of result.

Manual diagnosis by different physician will be lacked of standardization which may leads to incomparability and difficult interpretation of IIF result.

iv) Lack of automation solutions.
Medical field nowadays are advancing to more automation solution which are more reliable and time saving.

## 1.3 Objectives and Scope of Study

### 1.3.1 The objectives of this project are listed as follows:

i) To enhance the images of the cell patterns.
ii) To perform segmentation process on the images.
iii) To perform feature extraction on the images.
iv) To automatically classify the image sample according to the cell pattern.

### 1.3.2 The scope of this project are listed as follows:

i) Learning on HEp-2 cells (general information, type of pattern, disease associated).
ii) Learning on digital image processing (image enhancement, segmentation, feature extraction, pattern classification).
iii) Revise and improve on knowledge in using MATLAB software.

# CHAPTER 2

# LITERATURE REVIEW

This project requires the author to understand about each steps which are equally important in order to have an accurate classification of the HEp-2 cells pattern. Therefore the authors have gone through a few research papers that have explain about the topics related to the author's project. The accuracy of the classification are dependent on the separate algorithms used in image enhancement, segmentation, feature extraction, and classification process (Agarwal, Tiwari et al. 2014).

The first step is image enhancement. Image enhancement are crucial because the IIF image could be of low quality. This process will enhance the image quality therefore preventing problems to arise due to a not clear image of HEp-2 cells. Throughout the years, many algorithm especially for image enhancement are develop and improved. One of the methods are Histogram Equalization (HE). HE increase global contrast of the image which result in improvised intensity distribution over the histogram. However, this method disadvantages are it may cause over-enhancement of image contrast and poorly-suited for retaining local detail (Hossain, Alsharif et al. 2010). Adaptive Histogram Equalization (AHE) method is then implemented to improve HE method by computing several histogram and redistributed the lightness value which result in ability to retain local detail and reduce over-enhancement of image. Although there are improvements, AHE method enhance the noise together with the image (Aggarwal and Garg 2014).

Another method to be discussed is the Unsharp Masking (UM). UM method uses the blurred image of the original image to produce a mask which is then combined together to reduce the blurriness of the original image. The downside of this technique is that it is sensitive to noise due to the absence of noise suppression model (Hossain, Alsharif et al. 2010). A new model named Adaptive Unsharp Mask Algorithm (AUM) is develop by emphasizing the medium-contrast details (Kanwal, Girdhar et al. 2011). Nevertheless, the AUM technique also has its on weakness which is it is unable to detect low contrast edge (Agarwal, Tiwari et al. 2014).

Other than these method, there are many methods that are also able to enhance an image with their own advantages and disadvantages. Combination of the techniques are more preferable nowadays because the techniques are able to complement each other weaknesses and strength (Chaira 2012). There are no perfect technique because the enhancement of the image quality depends on the original image condition.

Next process after image enhancement is the segmentation process. This process involves the partitioning the image into several section in order to locate the HEp-2 cells. One of the method that have been develop for this process is the Gabor Filter (GF). GF advantage is that the invariant properties of the extracted feature (Tian 2011). As for the weakness, it is found out that the inside image of the cell will not be clear. Another method is Unsupervised Grow-Cut (UGC) method which applies the Grow-Cut algorithm but improved to be able to be unsupervised (Ghosh, Antani et al. 2011).

The third process is the feature extraction (FE) process. FE process considered the

heart of this project because the accuracy of the classification of HEp-2 cells depends on how clear and distinct the feature extracted from the cells. Each of the cell classification have a unique pattern of the cells (Mohan and Thirugnanam 2013). The following Figure 2 shows the categories of the cell pattern of HEp-2 cells that will be compared and classified as.

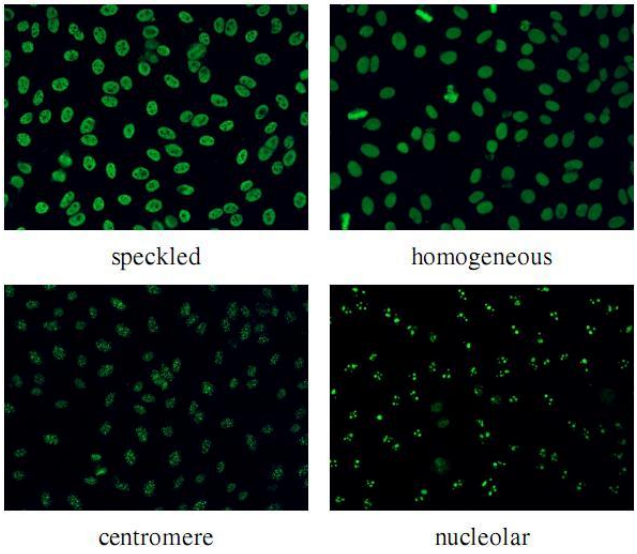



FIGURE 2: HEp-2 cells pattern.

One of the method available is Multi-dimensional Local Binary Pattern (MD-LBP) where the texture features are extracted to characterize the cell area (Schaefer, Doshi et al. 2014). Another available method is Gray-Level Co-Occurrence Matrix (GLCM) which is done by extracting texture information about an image from the spatial relationship between intensity values at specified offsets. However, this method has lower performance in discriminating irregular staining pattern especially fine-speckled cell pattern.

Last but not least, the last process is the classification process. This process mainly compared the standard cell pattern of HEp-2 cells of each category with the HEp-2 cells pattern that was extracted the feature previously (Setty, Srinath et al. 2013).

One of the method available is by using Codebook Based Descriptors (CDR). This method splits a cell image into small patches, which are then grouped into sets representing the inner and edge regions of the cell. Each region is the described as a histogram of visual words. The downside of this method is, it is intuitive and lacks a theoretical explanation therefore reducing it performance. Margin distribution based bagging pruning (MAD-Bagging) takes the input from the feature extracted image for classifier ensemble (Schaefer, Doshi et al. 2014). Discrete Meyer wavelet is the most discriminating for classification task compare to other wavelet and spatial domain algorithm (Katyal, Kuse et al. 2014).

# CHAPTER 3

# METHODOLOGY

## 3.1 Research Methodology.

The following Figure 3 shows the general flow outline for FYP 1. A more detailed explanation of the steps are entail in the Project Activities section (Figure 5).

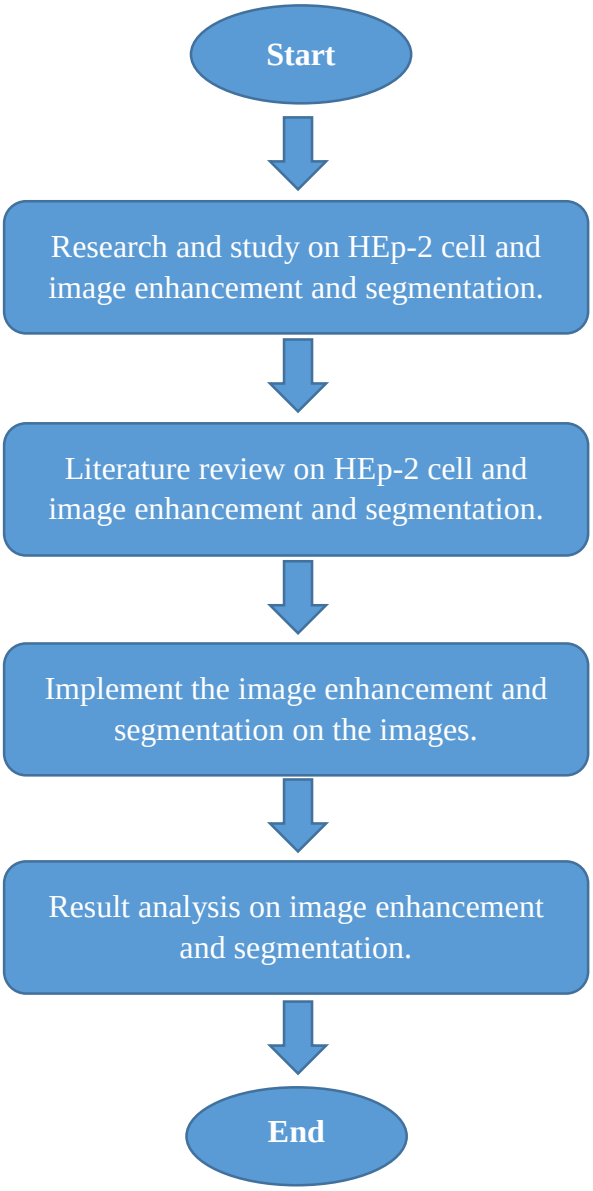



FIGURE 3: General flow outline for FYP 1

The following Figure 4 shows the general flow outline for FYP 2. A more detailed explanation of the steps are entail in the Project Activities section (Figure 6).

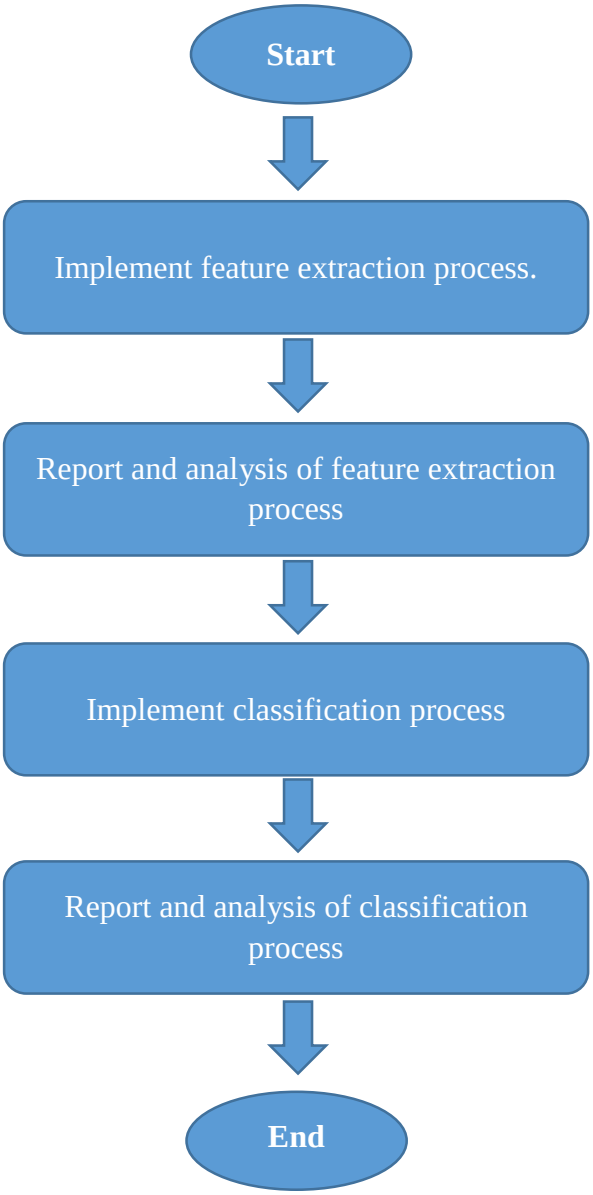



FIGURE 4: General flow outline for FYP 2

### 3.2 Project Activities

The following Figure 5 shows the research activities details for FYP 1.

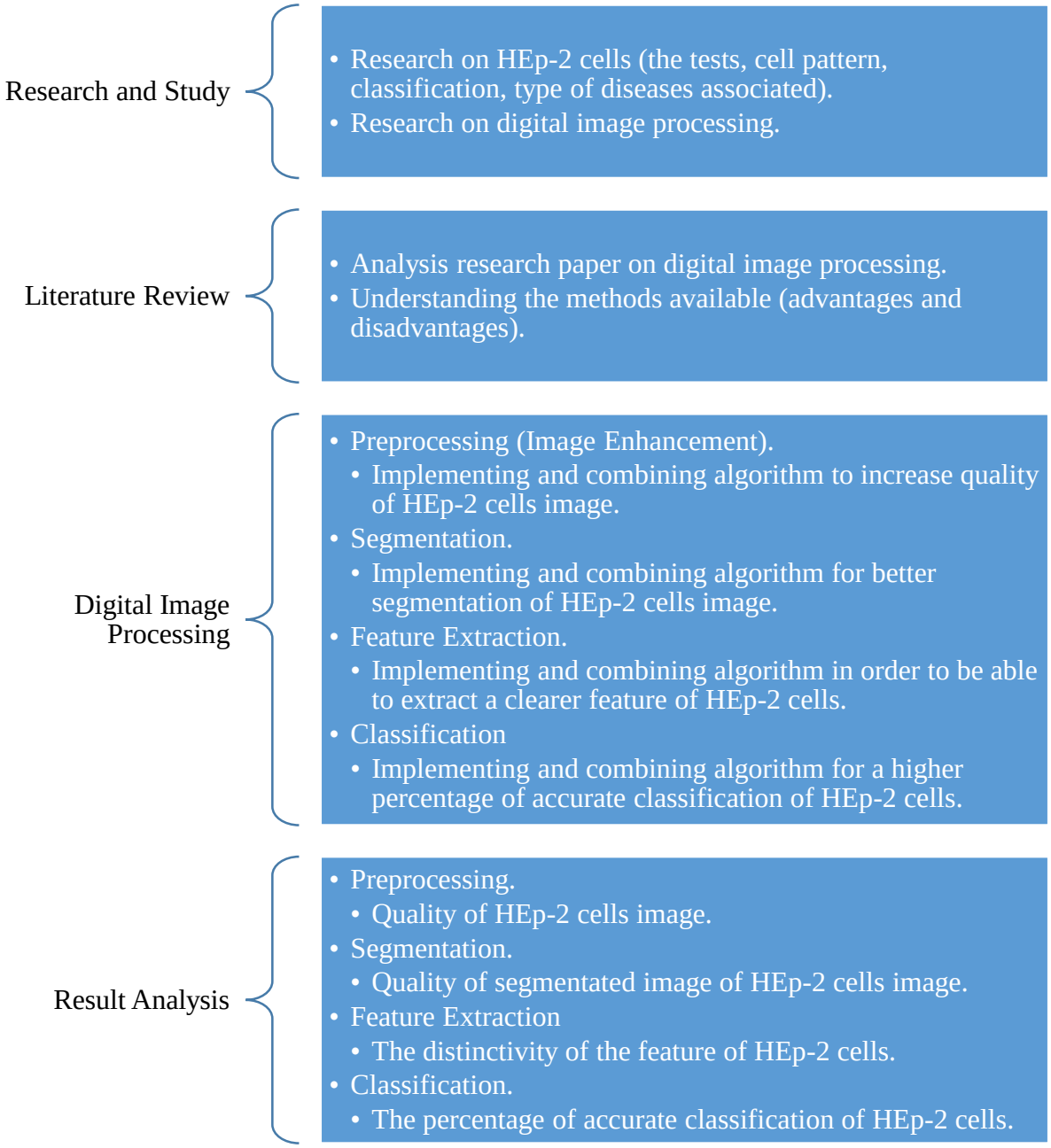



FIGURE 5: Research activities details for FYP 1

The following Figure 6 shows the research activities details for FYP 2.

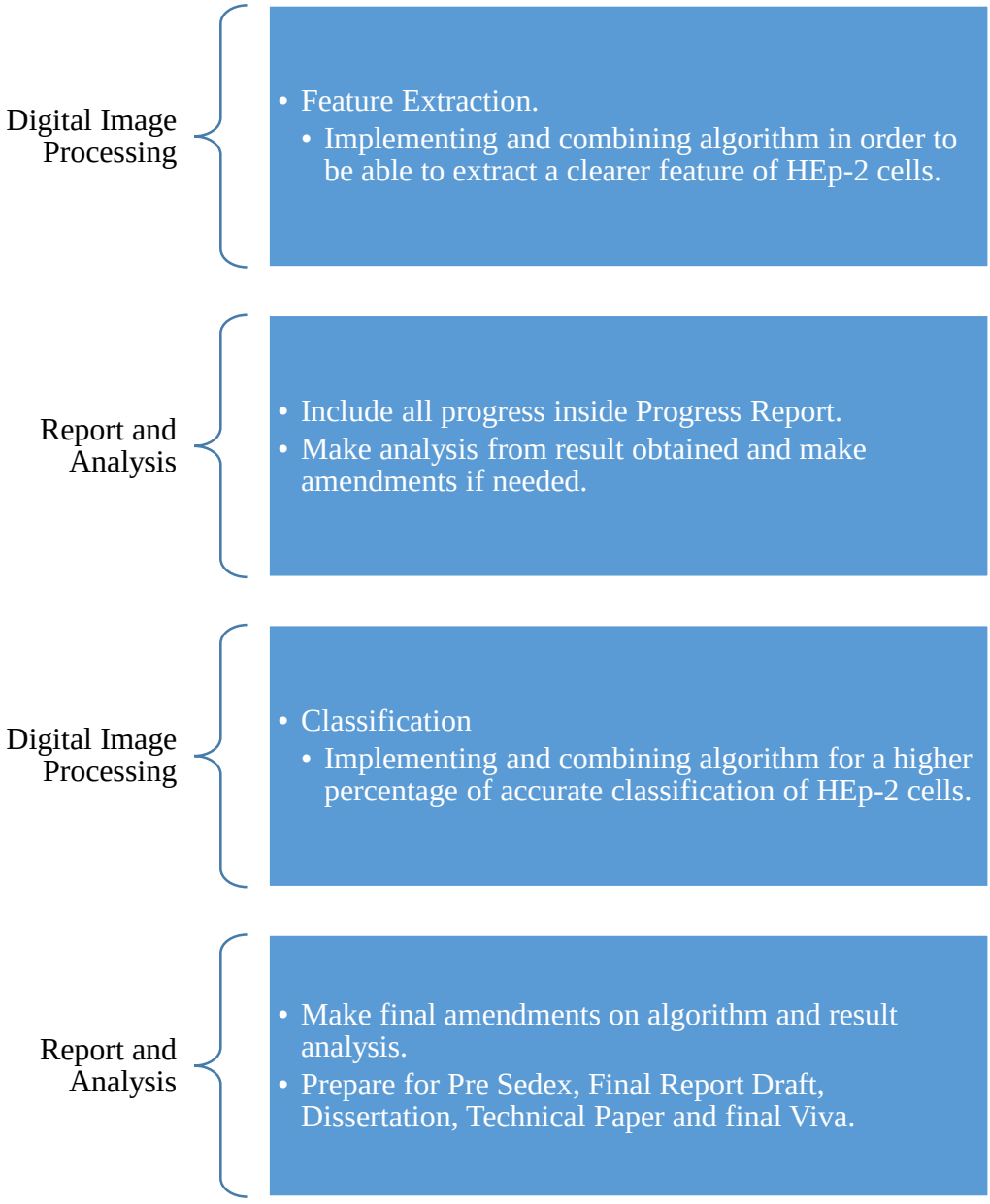



FIGURE 6: Research activities details for FYP 2

### 3.3 Key Milestone.

The following Figure 7 is the overall key milestone for FYP 1.

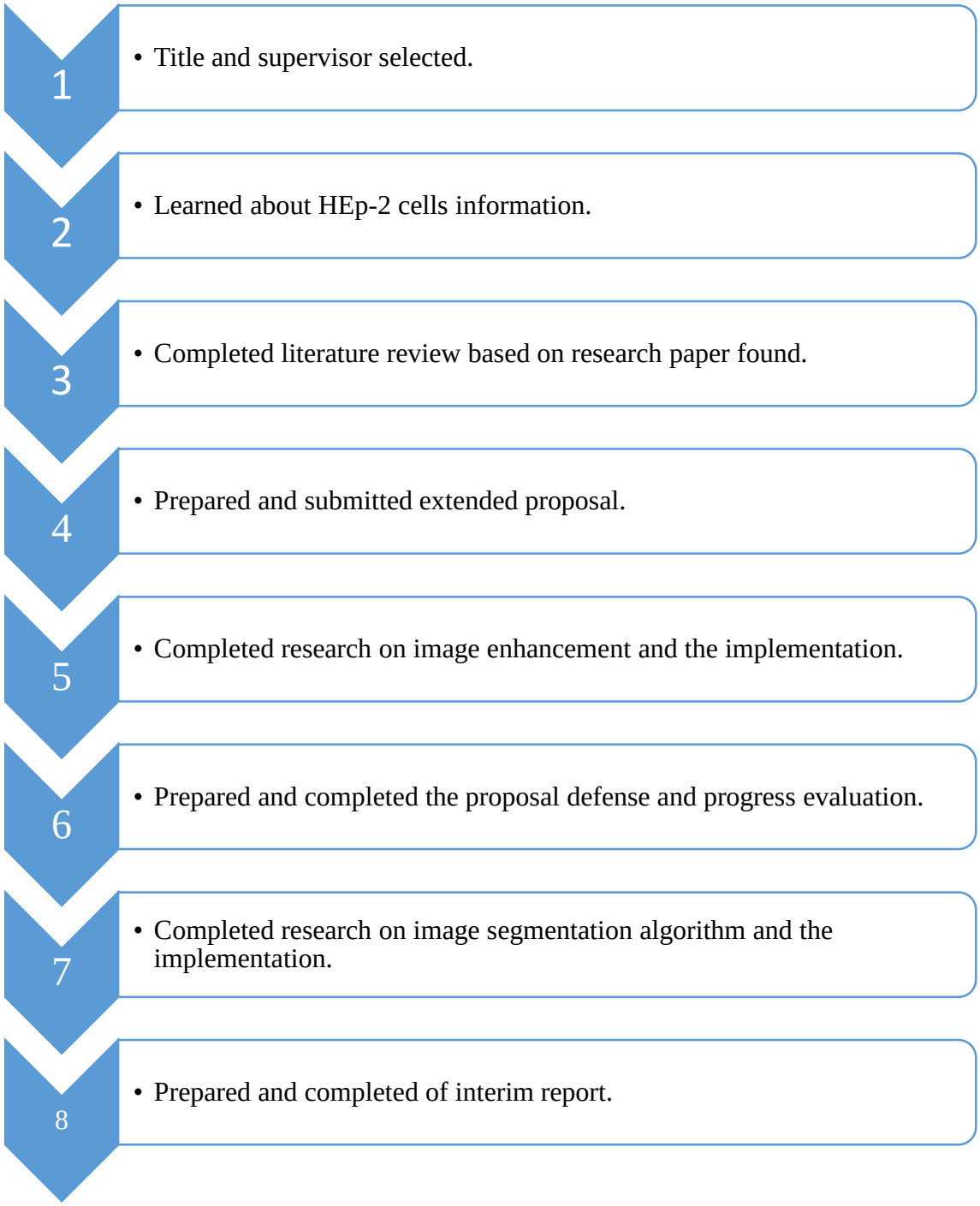



FIGURE 7: Key Milestone for FYP 1

The following Figure 8 is the overall key milestone for FYP 2.

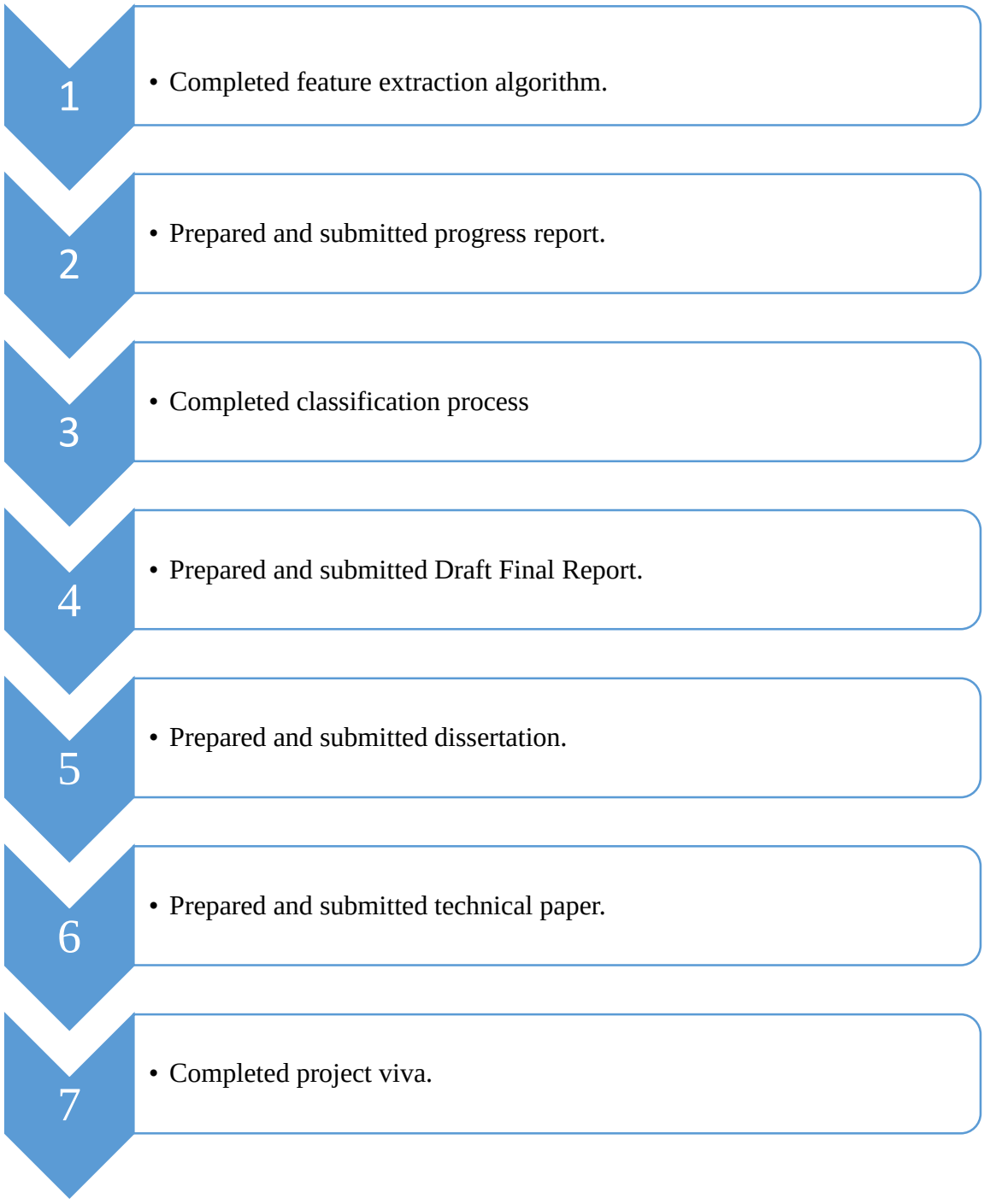



FIGURE 8: Key Milestone for FYP 2

### 3.4 Gantt Chart.

The following Figure 9 is the Gantt chart for FYP 1.

| Details | Week | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | 13 |
| Tittle and supervisor selection | | | | | | | | | | | | | |
| Research on HEp-2 cells information. | | | | | | | | | | | | | |
| Make literature review based on research paper found | | | | | | | | | | | | | |
| Preparation and submission of Extended Proposal | | | | | | | | | | | | | |
| Research on image enhancement algorithm and implement | | | | | | | | | | | | | |
| Preparation for Proposal Defense and Progress Evaluation | | | | | | | | | | | | | |
| Research on image segmentation algorithm and implement | | | | | | | | | | | | | |
| Preparation of Interim Report | | | | | | | | | | | | | |
| Submission of Interim Report | | | | | | | | | | | | | |

TABLE 1: Gantt Chart for FYP 1

The following Figure 10 is the Gantt chart for FYP 2.

| Details | Week | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 01 | 02 | 03 | 04 | 05 | 06 | 07 | 08 | 09 | 10 | 11 | 12 | 13 |
| Feature extraction if HEp-2 cells algorithm | | | | | | | | | | | | | |
| Preparation & submission of Progress Report | | | | | | | | | | | | | |
| Classification of HEp-2 cells algorithm | | | | | | | | | | | | | |
| Pre-SEDEX | | | | | | | | | | | | | |
| Preparation & submission of Draft Final Report | | | | | | | | | | | | | |
| Preparation & submission of Dissertation | | | | | | | | | | | | | |
| Preparation & submission of Technical Paper | | | | | | | | | | | | | |
| Project Viva | | | | | | | | | | | | | |

TABLE 2: Gantt Chart for FYP 2

### 3.5 Tools Required.

Software:

i) MATLAB.

ii) Microsoft Office.

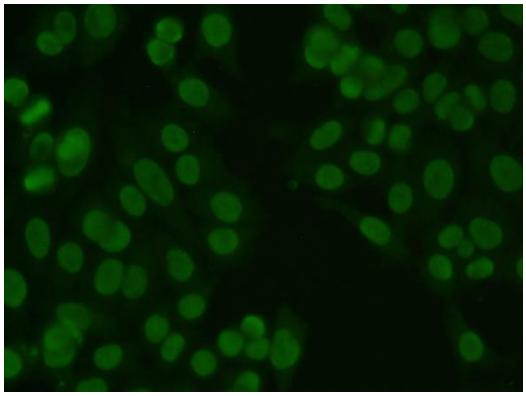
iii) Image processing toolbox.

# Chapter 4

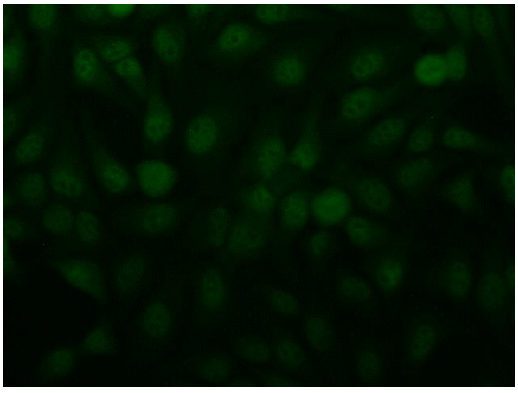
# Results and Discussions

1. Original IIF image.

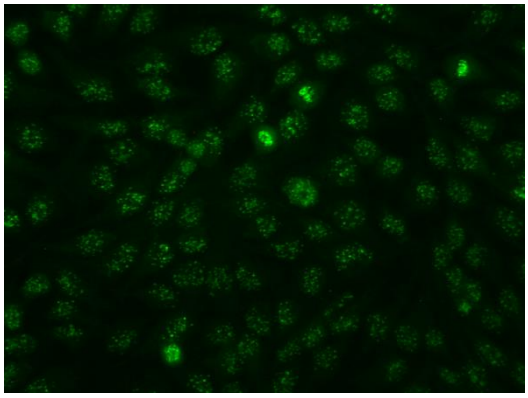
   The image sample dataset is obtained from Macchine Intelligent per il riconoscimento di Video, Immagini e Audio (MIVIA) dataset (http://mivia.unisa.it/datasets/biomedical-image-datasets/hep2-image-dataset/). There 28 main image dataset combination of the 4 patterns of HEp-2 cells.



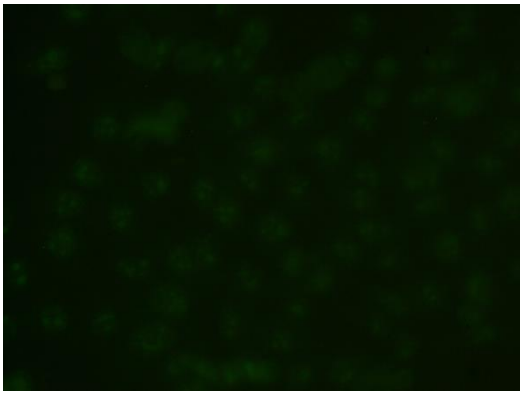
a) Homogeneous



b) Speckled



c) Centromere

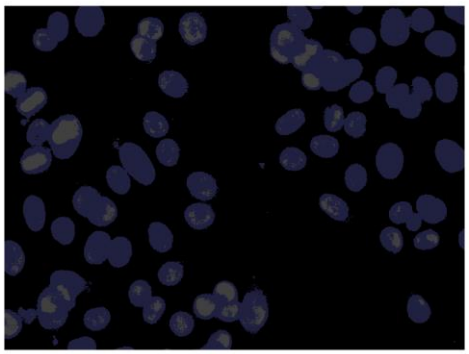


d) Nucleolar

FIGURE 9: Original IIF Images of HEp-2 cells.

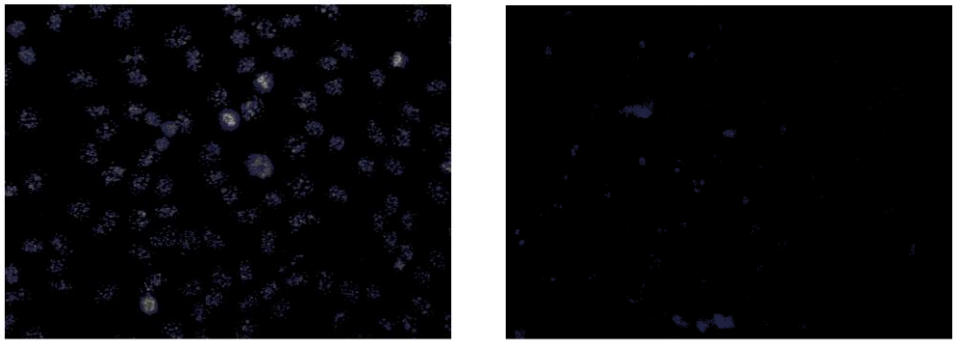
2. Convert to grayscale.

   The image sample is then converted to grayscale by using built-in function inside MATLAB. Grayscale is chosen instead of Red Green Blue (RGB), Hue Saturation Value (HSV), Hue Saturation Lightness (HSL) and Hue Saturation Intensity (HSI). This is because analyzing in grayscale is simpler and less complex. It is also easier to visualize and distinguish the depth of the image.



a) Homogeneous

b) Speckled

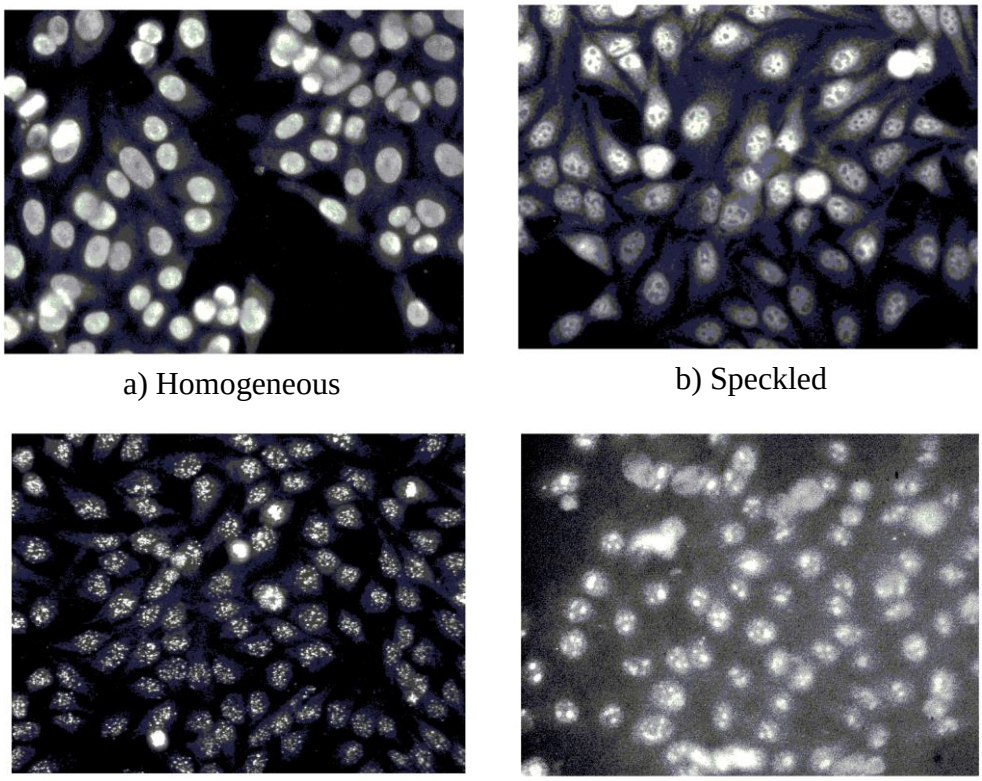


c) Centromere d) Nucleolar

FIGURE 10: Grayscaled Images of HEp-2 cells.

3. After image enhancement.

   The image samples are enhanced by applying modified histogram based contrast adjustment. This will increase the visibility of the cells inside the image sample.



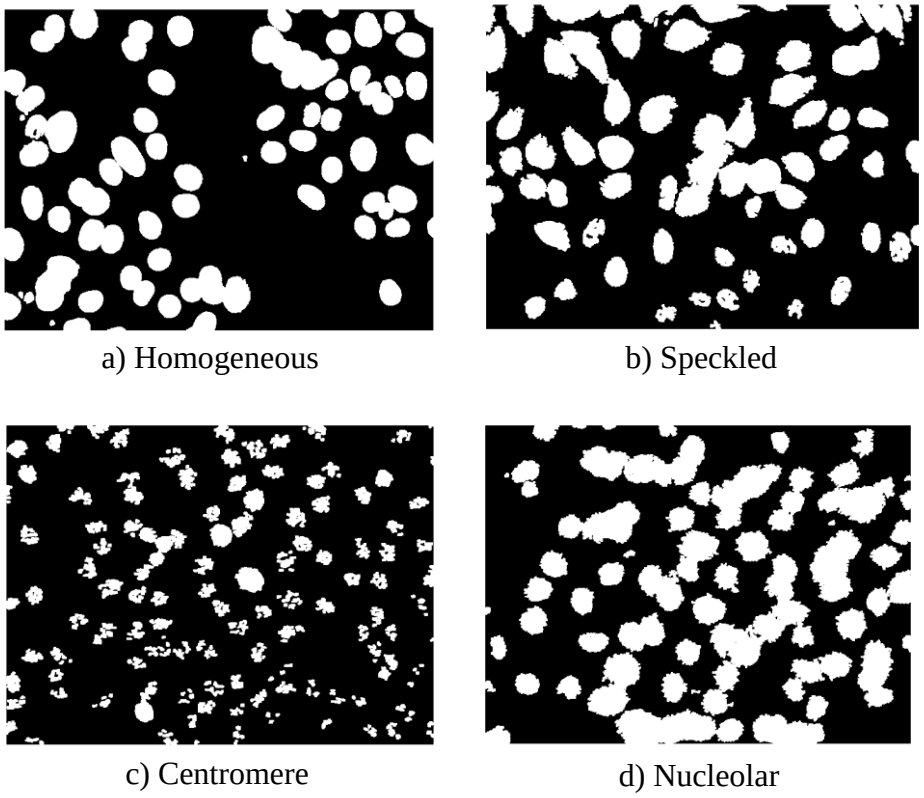
a) Homogeneous b) Speckled

c) Centromere d) Nucleolar

FIGURE 11: Enhanced Images of HEp-2 cells.

4. After applying Wiener's Filter.

   Wiener's filter is used to filter the noise so that the image of the cells are more distinct and clearer.



a) Homogeneous b) Speckled

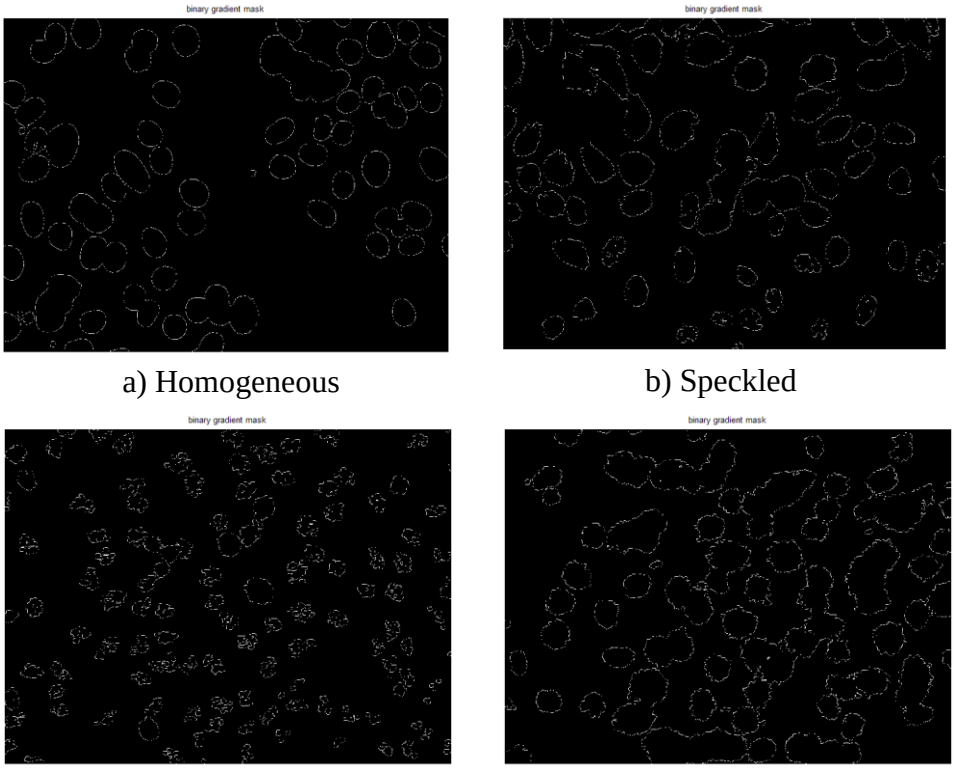
c) Centromere d) Nucleolar

FIGURE 12: Images of HEp-2 cells after applying Wiener's Filter.

5. Applying Sobel's Filter for edge detection.

a) Binary gradient mask.

Edge and the Sobel operator are used to calculate the threshold value. Next, the threshold value is tuned and edge is used again to obtain a binary mask that contains the segmented cell.



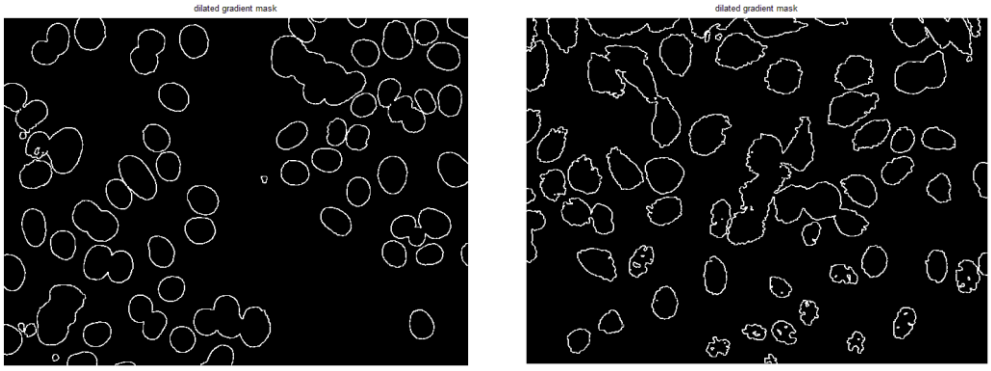

a) Homogeneous b) Speckled

c) Centromere d) Nucleolar

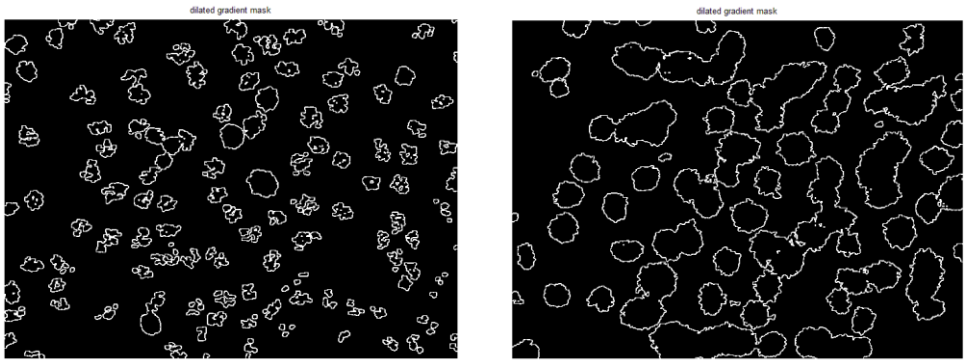
FIGURE 13: Binary gradient mask of images of HEp-2 cells.

b) Dilated gradient mask.

The lines from binary gradient mask do not quite delineate the outline of the object of interest. In order to solve this, the Sobel image is dilated using linear structuring elements.




a) Homogeneous b) Speckled

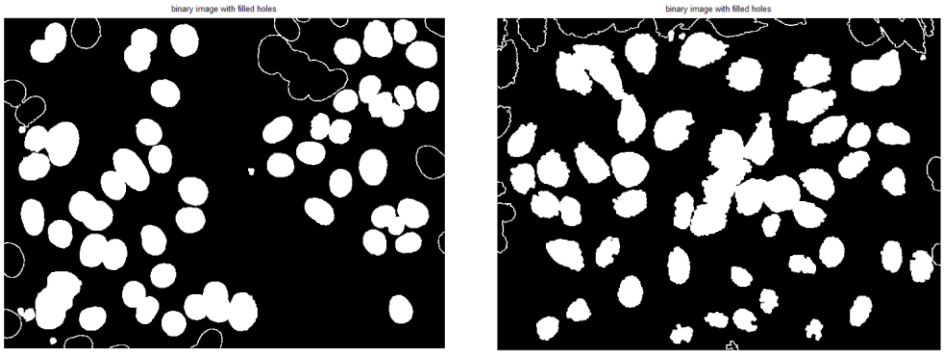



c) Centromere d) Nucleolar

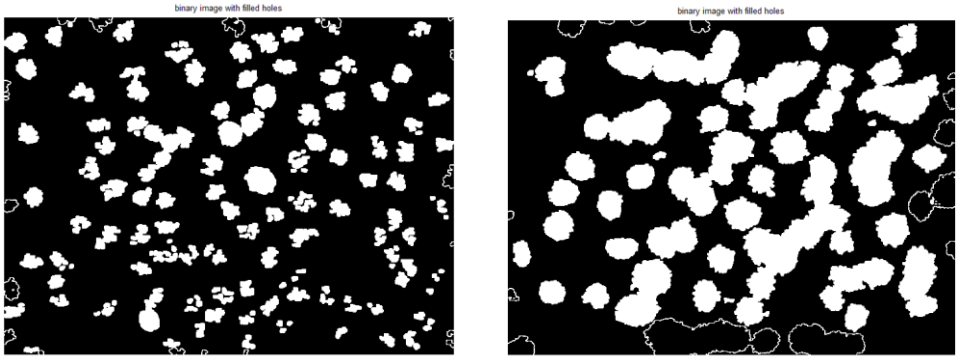
FIGURE 14: Dilated gradient mask of images of HEp-2 cells.

6. Filling holes into binary image.

   The holes in the interior of the cell after dilation is filled.




a) Homogeneous b) Speckled

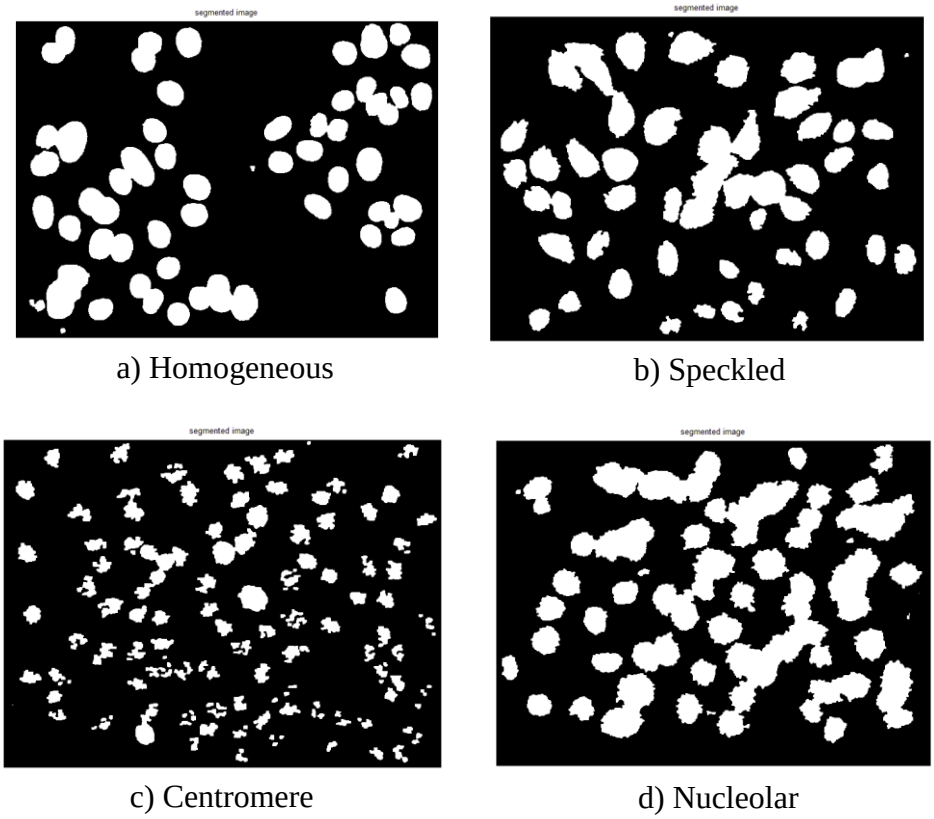



c) Centromere d) Nucleolar

FIGURE 15: Binary images of HEp-2 cells filled with holes.

7. Cleared border and segmented image.

   The border image is cleared and only the image of complete cells (the one which is filled) are kept for further processing.



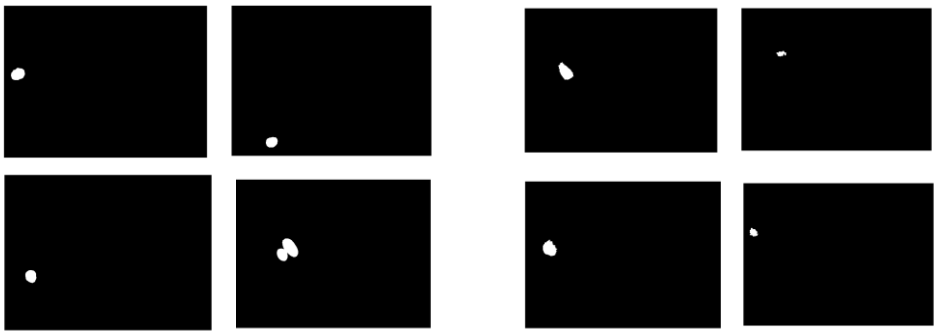

a) Homogeneous b) Speckled

c) Centromere d) Nucleolar

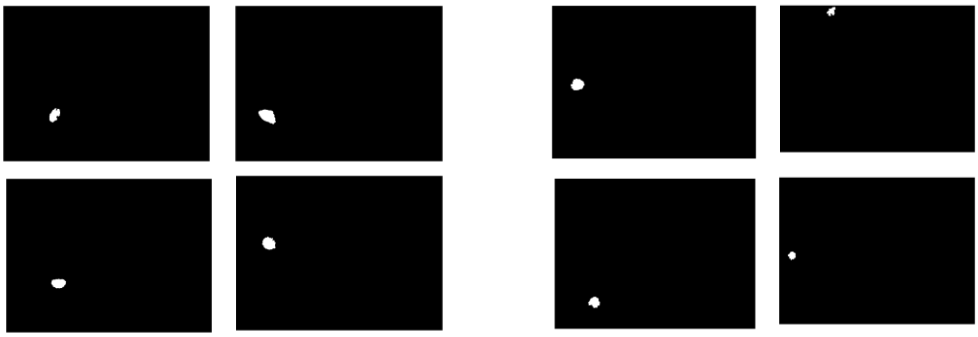
FIGURE 15: Fully completed segmented image of HEp-2 cells.

8. Identify and examine each cells inside the sample image.

   All detected cells are to be analyze (only shows 4 sample for report purpose).



a) Homogeneous b) Speckled



c) Centromere d) Nucleolar

FIGURE 16: Sample of each of the HEp-2 cells.

9. Median of shape properties of every cell detected.

   The properties can be extracted are area, perimeter, major axis, minor axis. Each of the cell patterns exhibit different value for each of the properties.

| Cell Pattern / Properties | Homogenous | Nucleolar | Speckled | Centromere |
|---|---|---|---|---|
| Area | 7073.4651 | 11056.0526 | 4539.4444 | 1404.6385 |
| Perimeter | 333.1484 | 514.9970 | 241.4421 | 169.3999 |
| Major Axis | 109.9729 | 150.1935 | 77.6189 | 51.0907 |
| Minor Axis | 78.2701 | 86.3228 | 42.2334 | 33.25098 |

TABLE 3: Shape properties of each of the cell patterns

10. The cell pattern is classified according to the properties extracted from previous stage. Homogeneous and Speckled have less accuracy based on the results. This is due to there are 2 type of speckled which are fine speckled and coarse speckled. This contributed to the less accuracy because fine speckled have almost similar properties with homogeneous.

| Cell pattern / Identify as | Homogenous (%) | Nucleolar (%) | Speckled (%) | Centromere (%) |
|---|---|---|---|---|
| Homogenous | 78.6 | 3.0 | 16.2 | 2.1 |
| Nucleolar | 3.2 | 92.5 | 4.4 | 1.3 |
| Speckled | 17.1 | 2.5 | 74.3 | 3.4 |
| Centromere | 1.1 | 2.0 | 5.1 | 93.2 |

Table 4: Classification process accuracy result

# Chapter 5

# Conclusion and Recommendation

A vast amount of Digital Image Processing techniques have been developed. The right combination of method for each part: image enhancement, segmentation and feature selection are very important because it will ease the process of classification. This will eventually lead to higher percentage of accurate analysis of the cell type classification. Combining some already available algorithm are able to produce the desired result and improvement can be made possible.

This final year project will be entailing the methods and algorithm used for each steps in order to produce a working and high accuracy of classification of HeP-2 cells based on its pattern. All image processing will be done in MATLAB software.

The combination of Modified Histogram based Contrast Adjustment and Wiener Filter for image enhancement, Sobel Filter and Median Filter for segmentation, Shape Properties Extraction for feature extraction and classification according to the data from feature extraction manage to produce a satisfactory result. The percentage of correct identification is acceptable except for homogeneous and speckled pattern. This is due to the shape of fine speckled is almost similar to homogenous therefore identifying them as homogenous. Course speckled cell pattern has no problem to be identify with this method.

Future works that can be done to improve this project is by adding another method to the feature extraction process. The suggested method is the textural analysis extraction. Each of the cell patterns will have different textural data which can be obtain by using histogram analysis. By analyzing and interpreting the data, we can use it to improve the accuracy of classification of the cell pattern.

## References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## Appendices

Coding sample:

```
clc;
close all;
clear all;

%read image
I = imread('11.bmp');

%change image to grayscale
I2 = rgb2gray(I);
figure, imshow(I2)

%increase contrast
I3 = imadjust(I2);
figure, imshow(I3);

%threshold the image
level = graythresh(I3);
d = im2bw(I3,level);
d = bwareaopen(d, 50);
figure, imshow(d)

%filter the noise using wiener filter
K = wiener2(d,[5 5]);
figure, imshow(K)

[~, threshold] = edge(K, 'sobel');
fudgeFactor = .5;
BWs = edge(K,'sobel', threshold * fudgeFactor);
figure, imshow(BWs), title('binary gradient mask');

se90 = strel('line', 3, 90);
se0 = strel('line', 3, 0);
BWsdil = imdilate(BWs, [se90 se0]);
figure, imshow(BWsdil), title('dilated gradient mask');

BWdfill = imfill(BWsdil, 'holes');
figure, imshow(BWdfill);
title('binary image with filled holes');

BWnobord = imclearborder(BWdfill, 4);
figure, imshow(BWnobord), title('cleared border image');

seD = strel('diamond',1);
BWfinal = imerode(BWnobord,seD);
BWfinal = imerode(BWfinal,seD);
figure, imshow(BWfinal), title('segmented image');

Z = medfilt2(BWfinal);
figure, imshow(Z);
```

```
m = medfilt2(Z);
figure, imshow(m);

BWoutline = bwperim(m);
Segout = I;
Segout(BWoutline) = 255;
figure, imshow(Segout), title('outlined original image');

cc=bwconncomp(m,8);
n=cc.NumObjects;

Area = zeros(n,1);
Perimeter = zeros(n,1);
MajorAxis = zeros(n,1);
MinorAxis = zeros(n,1);

F = regionprops (cc, 'Area', 'Perimeter', 'MajorAxisLength',
'MinorAxisLength');

for i = 1:n
    Area(i) = F(i).Area;
    Perimeter(i) = F(i).Perimeter;
    MajorAxis(i) = F(i).MajorAxisLength;
    MinorAxis(i) = F(i).MinorAxisLength;
end

graindata(1,1) = mean(Area);
graindata(2,1) = mean(Perimeter);
graindata(3,1) = mean(MajorAxis);
graindata(4,1) = mean(MinorAxis);

if ( (graindata(1,1) >= 9000) && (graindata(2,1) >= 450) )
    display('Nucleolar')
elseif ( (graindata(1,1) <= 3000) && (graindata(3,1) <= 60) )
    display('Centromere')
elseif ( (graindata(1,1) <= 6000) && (graindata(4,1) <= 60) )
    display('Speckled')
else
    display('Homogeneous')
end
```