

**FLUORESCENCE INTENSITY POSITIVITY CLASSIFICATION
OF HEP-2 CELLS IMAGES USING FUZZY LOGIC**

by

DAYANG FARZANA BT ABANG SAZALI

FINAL PROJECT REPORT

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

Universiti Teknologi PETRONAS
Bandar Seri Iskandar
31750 Tronoh
Perak Darul Ridzuan

©Copyright 2013
by
Dayang Farzana Bt Abang Sazali, 2013

CERTIFICATION OF APPROVAL

**FLUORESCENCE INTENSITY POSITIVITY CLASSIFICATION
OF HEP-2 CELLS IMAGES USING FUZZY LOGIC**

By

Dayang Farzana Bt Abang Sazali

13050

A project dissertation submitted to the
Department of Electrical & Electronic Engineering
Universiti Teknologi PETRONAS
in Partial Fulfillment of the Requirements
for the Degree
Bachelor of Engineering (Hons)
(Electrical & Electronic Engineering)

Approved:

Dr. Josefina Barnachea Janier

Project Supervisor

UNIVERSITI TEKNOLOGI PETRONAS
TRONOH, PERAK

CERTIFICATION OF ORIGINALITY

This 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.

DAYANG FARZANA BT ABANG SAZALI

ABSTRACT

Indirect Immunofluorescence (IIF) is a gold standard used for antinuclear autoantibody (ANA) test using Hep-2 cells to determine specific diseases. Automated interpretation is crucial to assure high accuracy to determine the autoantibody type of diseases. There are different classifier algorithm methods that have been proposed in previous works to classify the fluorescence intensity, however, there is still no valid algorithms to set as a standard. The purpose of this study is to classify the fluorescence intensity by using fuzzy logic algorithm to determine the positivity of the Hep2-cell serum samples. The scope of study of this project involves converting the RGB colour space of images to LAB colour space and the mean value of the lightness channel and chromaticity layer (a) channel is extracted and classified by using fuzzy logic algorithm based on the standard score ranges of ANA fluorescence intensity which are 4+, 3+, 2+, 1+ and 0. Based on the results, the accuracy of intermediate and positive class is 85% and 87% respectively.

ACKNOWLEDGEMENT

First and foremost, the author would like to take this opportunity to express her deepest gratitude and thankful to God for His kind blessing by giving a chance, strength and determination to complete her Final Year Project in a good condition, health and well-being. Efforts have been taken in this project, however, it would not have been possible without the kind support and help of many individuals and organizations. The author is highly indebted to Dr. Josefina Barnachea Janier for her guidance and constant supervision as well as for providing necessary information regarding the project and also her support in completing the project.

Special thanks as well to the co-supervisor, Ms. Zazilah Bt. May for her patient guidance in the technical and non-technical knowledge. Not to forget, a special gratitude and thanks to Dr Ibrahim Faye for his support and cooperation in completing the project. The author also would also like to thank Dr. Nazreen Bt Badruddin, the FYP coordinator for conducting several seminars and briefings to assist the FYP students as well as helping to arrange the schedule for ELECTREX and Viva for students by inviting external examiners for evaluation. Besides that, the author thanks and appreciation also goes to her colleagues and beloved families for their endless support and encouragement throughout the duration of the project.

TABLE OF CONTENTS

ABSTRACT.....	v
ACKNOWLEDGEMENT	vi
LIST OF TABLES	ix
LIST OF EQUATIONS	ix
LIST OF FIGURES	ix
LIST OF ABBREVIATIONS	x
CHAPTER 1 INTRODUCTION.....	1
1.1 Background Study	1
1.2 Problem statement	2
1.3 Objectives	3
1.4 Scope of Study	3
1.5 Relevancy of the Project.....	4
1.6 Feasibility of Project	4
CHAPTER 2 LITERATURE REVIEW.....	5
2.1 Fluorescence Intensity.....	5
2.2 CIELAB 5	
2.3 Other Classifier for Fluorescence Intensity.....	6
2.4 Fuzzy Logic	8
2.4.1 Fuzzy Logic in Health Industry Applications	9
2.4.2 Colour Classification.....	10
2.4.3 Life Estimation of Phase Angle Controlled Induction Motors.....	10
CHAPTER 3 METHODOLOGY	12
3.1 Research Methodology	12
3.2 Flow Chart	14
3.3 Project Tasks	16
3.3 Gantt Chart and Milestones.....	17
3.4 Tools	17
CHAPTER 4 RESULTS AND DISCUSSIONS	18
4.1 Feature Extractions.....	18
4.2 Fuzzy Logic Classification.....	19

CHAPTER 5 CONCLUSION & RECOMMENDATION.....	25
REFERENCES.....	26
APPENDICES	28
APPENDIX A FYP1 & FYP2 GANTT CHART AND MILESTONES	29
APPENDIX B RESULTS OF LAB FEATURES FOR TRAINING IMAGES	30
APPENDIX C POSITIVE CLASS MEAN VALUE GRAPH.....	47
APPENDIX D INTERMEDIATE CLASS MEAN VALUE GRAPH	48
APPENDIX E RESULTS OF FUZZY LOGIC FLUORESCENCE INTENSITY CLASSIFICATION FOR INTERMEDIATE CLASS	49
APPENDIX F RESULTS OF FUZZY LOGIC FLUORESCENCE INTENSITY CLASSIFICATION FOR POSITIVE CLASS	51
APPENDIX G MATLAB CODING FOR FEATURE EXTRACTION	53

LIST OF TABLES

Table 1: Fluorescence Intensity Classification Criteria	5
Table 2: Existing Fluorescence Intensity Classifier	8
Table 3: Existing Fuzzy Logic Classifier	9
Table 4: LAB Features Extractions	18
Table 5: Ranges of LAB Mean Value for Positive and Intermediate Class	19
Table 6: Class of Fluorescence Intensity Based on Fuzzy Logic Output	21

LIST OF EQUATIONS

Equation 1: Triangular function	9
Equation 2: Trapezoidal function	9

LIST OF FIGURES

Figure 1: CIE Chromacity Diagram	6
Figure 2: Example of classification rule used to vary the error tolerance of the classification system	7
Figure 3: Partitioning H dimension with triangular membership function [9]	10
Figure 4: Research Flow	12
Figure 5: Scheme of Image Processing and Classification	13
Figure 6: Flow Chart	14
Figure 7: Flow Process of Fluorescence Intensity Classification	15
Figure 8: Project Tasks	16
Figure 9: Fluorescence Intensity Fuzzy Logic Model	19
Figure 10: Membership Function for 'L' Mean Value	20
Figure 11: Membership Function for 'a' Mean Value	20
Figure 12: Fuzzy Rule	21
Figure 13: Fluorescence Intensity Output	21
Figure 14: Graphical Application of Fuzzy Rules for Positive Results	22
Figure 15: Graphical Application of Fuzzy Rules for Intermediate Results	23
Figure 16: Graphical User Interface (GUI)	23

LIST OF ABBREVIATIONS

AAB	Autoantibody
ANA	Antinuclear Autoantibody
ARD	Autoimmune Rheumatic Diseases
CAD	Computer Aided System
CMYK	Cyan-Magenta-Yellow-Black
DAPI	6-diamidino-2-phenylindol
ELISA	Enzyme-linked Immunosorbent Assay
Hep-2	Human Epithelial Type 2
HSL	Hue, Saturation, Luminosity
IIF	Indirect Immunofluorescence
K-NN	K-Nearest Neighbour
LAB	Luminosity layer (L), Chromaticity layer 'a' and 'b' (A) and (B)
MES	Multiple Expert Systems
MLPs	Multi Layer Perceptrons
RBF	Radial Basis Network
RGB	Red-Green-Blue
RI	Reactivity Index

CHAPTER 1

INTRODUCTION

1.1 Background Study

Autoantibody (AAB) is type of proteins or antibodies that directed against the individual's protein. AAB is produced when the immune systems cannot recognise self and non-self protein. Therefore, the antinuclear antibodies (ANA) test is performed to determine type of autoimmune disease if ANA is positive. There are various developed technologies development to diagnose the disease-specific of AABs such as enzyme-linked immunosorbent assay (ELISA) and multiplexing technologies, however, indirect immunofluorescence (IIF) assay is set as a standard method to perform on human epithelial (Hep-2) cells today.

The immunofluorescence image will be evaluated to determine positive and negative screening results and staining pattern recognition. Previously, the samples classification and pattern recognition is visually evaluated by medical doctor or the experts. As it resulting as time consuming and lack of expertise to diagnose ANA screening, rate of misclassifications errors will be increased. Thus, an automated evaluation is developed in order to decrease the rate of errors [1],[2]. In medical context, computer aided system (CAD) has been successfully employed which serves as second reader capable to examine the image more accurately by reducing errors and works as a training tool for specialized medical personnel.

An ANA Hep-2 test results is considered positive when a clear ANA pattern is observed. There are varieties of patterns for specific disease. According to [3], the study made an analysis on ANA test on healthy individuals and patients with autoimmune rheumatic diseases (ARD). The results obtained from ANA patterns for ARD patient mostly are homogenous, nuclear coarse speckled, and centromeric. Whereas the nuclear fine speckled was observed only in healthy person. In addition, to obtain the specific pattern, dilution of 1:80 is usually used. More description of pattern can be found in [4].

In most research, the classification technique to determine the fluorescence intensity of ANA test still lacks of accuracy. Determining occurrences of auto immune disease is crucial where these AAB are detected by specific fluorescence intensity. Hence, this project intends to presents a system to classify the fluorescence intensity to determine either the serum samples are positive or negative using fuzzy logic algorithm before proceeding with pattern classification.

1.2 Problem statement

Some research groups in previous works have proposed different algorithms for the analysis of IIF images. The validation of proposed methods had been carried out on small and private dataset. In this proposal, the author will discuss on the dominant fluorescence intensity of ANA test. The significance on determining the fluorescence intensity of the serum samples is to identify the ANA pattern for a specific autoimmune disease. The proposed method will divide the fluorescence intensity into five classes, which are positive (4+, 3+, 2+), intermediate (1+) and negative (0). The crucial issue that needs to be highlighted here are, the datasets are manually interpreted by the medical doctor which resulting in time consuming, no valid standard has been set for the classification due to lack of the accuracy in most research and most of the classification errors are made on the intermediate images since its intensity samples usually exhibit low contrast where even the experts found the classification task difficult.

1.3 Objectives

The objectives of this work are:

1. To study and identify different features in images that contributes to positivity and negativity of the samples.
2. To develop classification algorithm using fuzzy logic that is able to accurately divide the images into five classes: positive (4+, 3+, 2+), intermediate (1+), and negative (0).
3. To assess the performance of classification algorithm.
4. To validate the accuracy of classification results.

1.4 Scope of Study

Fluorescence intensity of hep-2 cell images are extracts into colour saturation and lightness to determine the classes of the intensity. The image is pre-process and converts the original image to LAB colour space. LAB color space is used to identify different colors in an image by analyzing Luminosity layer (L), Chromaticity layer 'a' where the color spectrum is from green color to red color (A), and Chromaticity layer 'b' where the color spectrum is from blue to yellow color (B) [5]. After converting the color space, the mean value of L and 'a' are chosen as main features for extraction.

Then, fuzzy logic classifier will be used to classify the fluorescence intensity based on the features which are mean value of L, and (a). It uses a fuzzy rule (linguistic IF-THEN statement) and combines with fuzzifying input according to membership function or features to get an output distribution [6].

1.5 Relevancy of the Project

This project mainly involves on understanding of image processing and using the knowledge to design algorithm that can be used in medical application. As a whole, this work encompasses digital signal processing knowledge on how each image is represented or interpreted and helps to optimize the exploitation of the information that permits the processing faster.

1.6 Feasibility of Project

The time frame to develop the classification algorithm using fuzzy logic depends on how fast the basic designs can be understood. Eight months of time frame to complete Final Year Project (FYP) is feasible for completing the project.

CHAPTER 2

LITERATURE REVIEW

2.1 Fluorescence Intensity

According to the Centre for Disease Control and Prevention in Atlanta, Georgia, the scores range for fluorescence intensity is divided into two classes which are positive and negative; four subgroups in the positive class are shown in TABLE 1 [7].

Table 1: Fluorescence Intensity Classification Criteria

Subgroups	Description
4+	Maximal fluorescence (Brilliant green)
3+	Less brilliant green fluorescence
2+	Defined pattern but diminished fluorescence
1+	Very subdued fluorescence
0	Negative

However, the medical doctors in [8] referred to the same guideline (TABLE 1) suggested to classify into three classes, namely positive, intermediate, and negative since the technical problems can affect the test sensitivity and specificity. Hence, the scores range for positive class is 2+ and above, intermediate class is 1+ and 0 indicates negative.

2.2 CIELAB

Colour space systems are created by Commission Internationale de l'Eclairage or International Commission on Illumination (CIE) in 1931 which defined the CIEXYZ (X-Red, Y-Green, Z- Blue) colour space that represented the entire possible colour by human eyes. As for CIELAB, it contains a large colour gamut that can represent the entire colour visible by the human eyes. Its L component closely matches human perception of lightness. However, as it consists of larger gamut, the

bitmap image represented as LAB requires more data per pixel for computer displays or even human vision. As for RGB colour model, its model has a limited gamut of colour, while CIELAB attempts to model all colours [9]. Besides, according to paper [10] and [11] which evaluates the appearance and colour of food and leukocyte detection for medical image processing, respectively, used l^*a^*b colour space as it has uniform distribution of colours, and as well as very close to human perception. Other colour spaces such as RGB and CMYK, in practice, widely used for display and printing, while HSB and HSL are used for colour selection. However, all mentioned colour spaces are subsets of the human colour gamut as measured and defined by CIE which is shows in Figure 1.

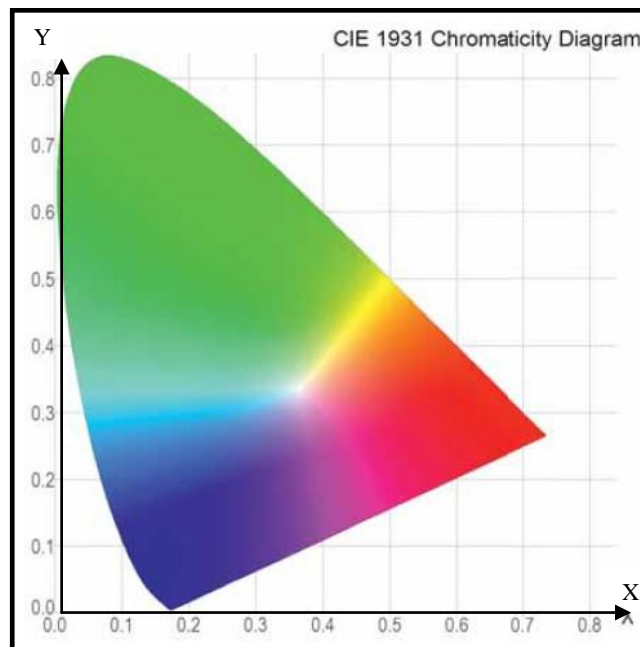


Figure 1: CIE Chromacity Diagram

2.3 Other Classifier for Fluorescence Intensity

There are automated system with different algorithm implementation have been created. One of it is ALKIDES interpretation systems, it is a helpful tool which is able to analyze different cells and bead-based cells fluorescence assays [12]. It consists of modules for device and autofocus control, image analysis and pattern recognition algorithm. All algorithms of ALKIDES are implemented using C++ programming language and OpenMP for parallelization task. As for autofocus control it is using Haralick's characterization to analyse grey scale transition that

used 6-diamidino-2-phenylindol (DAPI) as fluorescent dye for object recognition and focusing. According to [1], the result obtained using ALKIDES is 90.1% agreement compared to visual interpretation as for the assessment of data, reactivity index (RI) is calculated by combining the image intensity, contrast, and number of grey-scale levels of the overall image [1].

Other recent approach regarding on the system for fluorescence intensity classifier is by using a Multiple Expert System (MES). There are two types of classifier which work as measurement level: Multi-Layer Perceptrons (MLPs) and Radial Basis Network (RBF). The combination of these classification rules using two neuron outputs that belongs to positive and negative classes and the samples are presented in vector by considering in xy-plane where (-1,1) is negative, (1,-1) is positive and otherwise is dubious (refer to Figure 2) [13]. The point O_i represents the i th output vector. This classification method has an encouraging results and still needs to be improved.

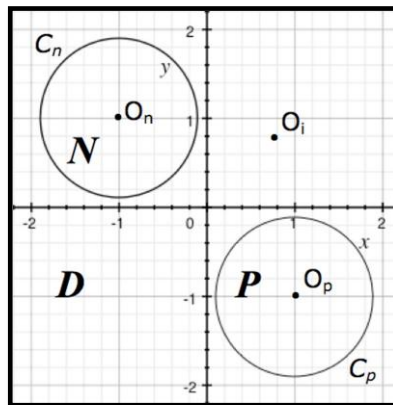


Figure 2: Example of classification rule used to vary the error tolerance of the classification system

The other technology for IIF ANA screening is EUROPattern system. It is a closed system which is fast and comprehensive IIF pattern recognition system providing objective test results. The classification of this software is implemented using k-nearest neighbour (k-NN) algorithm. As for classification of overall samples, a very high agreement is achieved between both interpretations. The system have achieved a good result as stated in [2] “ its sensitivity and specificity amounted to 100% and 97.5%, respectively, while the positive and negative predictive value were

99.3% and 100%” after a comparison was made with visual interpretation. As mentioned in [12] again, k-NN classifier is simpler and performs good classification. It works by memorizing the whole training data and performs classification if the attribute test object matches one of the training examples exactly. Table 2 below summarizes the techniques have been used to classify the fluorescence intensity.

Table 2: Existing Fluorescence Intensity Classifier

AUTHOR	TECHNIQUE	APPLICATIONS	ADVANTAGES	LIMITATIONS	RESULTS
A. Willitzki et al. (2012)	<ul style="list-style-type: none"> Segmentation: Histogram-based mixture model threshold algorithm followed by watershed transformation Reactivity Index 	<u>AKLIDES platform technology</u> <ul style="list-style-type: none"> Model background Intensity Calculates and evaluates image intensity 	Multipurpose bioanalytical tool that able to analyze different kind of cells and bead-based fluorescence assays	Positive findings provided need to be always confirmed by an expert.	Reported data showed high agreement between manual and AKLIDES interpretation. Egerer et al. reported positive samples revealed an agreement 90.1% and 92.7% for university and private laboratory respectively.
J. Voigt et al. (2012)	<ul style="list-style-type: none"> K-NN 	<u>Development of EuroPattern Suite</u> Classification of fluorescence intensity and pattern.	<ul style="list-style-type: none"> High sensitivity and specificity High agreement with manual evaluations. Can recognize most of the important ANA patterns. 	Difficulty in recognition of mixed pattern.	Comparison with manual interpretation: Positive: 100% Negative: 97.5% Pattern: 94%
P. Soda, G. Iannello, and M. Vento (2009)	<ul style="list-style-type: none"> Multi Layer Perceptrons Radial Basis Network 	<u>Multi-expert Systems</u> Fluorescence Intensity Classifications	Combined both classifier can recognize high percentage of dubious class and low error rate.	Results are encouraging but still need to be improved as still requesting human expresses the final classifications.	Error rate for FP and FN is 0.9% and 98.5% for dubious class recognition.

2.4 Fuzzy Logic

This classifier aims at modeling the human thinking and reasoning and applying the model to problems according to needs. Various types of field have used this classifier in their industry pertaining to requirements.

2.4.1 Fuzzy Logic in Health Industry Applications

Events and mechanisms are analyzed in a fuzzy system and can be modeled by considering the related verbal and substantially uncertain information as complementary to the events in preference to equations that are accepted only in implications of certain rules and assumptions [14]. In this paper, new method of fuzzy logic has been proposed besides Sugeno and Mamdani method. The modified Mamdani method is defined by equation (1) and (2) which combining fuzzification formulation of trapezoidal and triangular functions. The 3 methods are performed for cancer risk analysis and the results are compared as shown in the Table 3.

$$\begin{aligned}
 &\text{if } a > x \parallel c < x \Rightarrow 0 \\
 &\text{if } a \leq x \leq b \Rightarrow \frac{\max' \times [\sin(x) - \sin(a)]}{[\sin(b) - \sin(a)]} \\
 &\text{if } b \leq x \leq c \Rightarrow \frac{\max' \times [\sin(c) - \sin(x)]}{[\sin(c) - \sin(b)]}
 \end{aligned} \tag{1}$$

$$\begin{aligned}
 &\text{if } a > x \parallel d < x \Rightarrow 0 \\
 &\text{if } a \leq x \leq b \Rightarrow \frac{\max' \times [\sin(x) - \sin(a)]}{[\sin(b) - \sin(a)]} \\
 &\text{if } b \leq x \leq c \Rightarrow 1 \\
 &\text{if } c \leq x \leq d \Rightarrow \frac{\max' \times [\sin(d) - \sin(x)]}{[\sin(d) - \sin(c)]}
 \end{aligned} \tag{2}$$

Table 3: Existing Fuzzy Logic Classifier

AUTHOR	TECHNIQUE	APPLICATIONS	ADVANTAGES	LIMITATIONS	RESULTS
A. Yilmaz and K. Ayan (2012)	Modified Mamdani fuzzy logic	Cancer risk analysis	Higher accuracy compared to Sugeno and Mamdani method fuzzy logic.	100% accuracy cannot be detected since the risk status of a person having a low risk rate may change in future with different living conditions and factors.	Average Accuracy: Modified Mamdani :79.5% Mamdani :76.7% Sugeno: 66.9%

Therefore, in this paper, the modified Mamdani method will be used in this study as it results in higher accuracy agreement in analyzing cancer risk.

2.4.2 Colour Classification

Fuzzy logic has been used on determining colour based on human perception. In [15], colour classification usually used for colour image segmentation. HSL (hue, saturation, lightness) is used for representing three dimensions. A colour can be coded by giving the membership function with different values of hues range. The other two dimensions (S, L) are usually divided into three parts: weak, medium and strong. Then, each dimension of S and L will be presented in 1D trapezoidal membership function and then by multiplying those functions, a set of 2D membership function is generated. Further explanation on colour classification can be referred to [15]. FIGURE 3 shown below illustrate a triangular membership function which assumes the value interval [0, 1]. The membership function is described by the fuzzy set which is colours. Therefore, in this paper, the colour classification can be determined by using fuzzy logic.

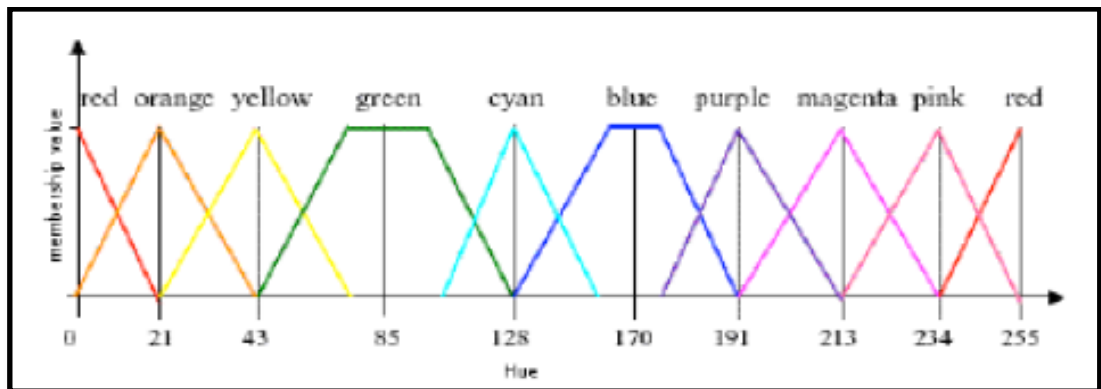


Figure 3: Partitioning H dimension with triangular membership function [9]

2.4.3 Life Estimation of Phase Angle Controlled Induction Motors

In this application, fuzzy logic is used to estimate the life estimation of an induction motor. There are three feature extractions used that cause the insulation stress: voltage peak, rate of rise of voltage and thermal loss. In addition, the stress input parameters has set to four fuzzy set namely low, medium, high and very high. Then, the triangular membership function is presented with each of features with respect to reference value of fuzzy set [16]. Therefore, from these three features, the estimation life can be estimated by using fuzzy expert system.

Briefly, besides the two applications of fuzzy logic stated above, there are a lot of other application such as image segmentation [17], texture features classification [18] and others. Therefore, in this paper, the aim of this project is to design a fuzzy logic algorithm for the fluorescence intensity of IIF image diagnosis. It is vital as the intensity tends to determine the positivity of the serum sample based on several image's features such as saturation and lightness to determine the autoimmune disease.

CHAPTER 3

METHODOLOGY

3.1 Research Methodology

This final year project is to be carried out with the following phases (see Figure 4) and the description of each step.

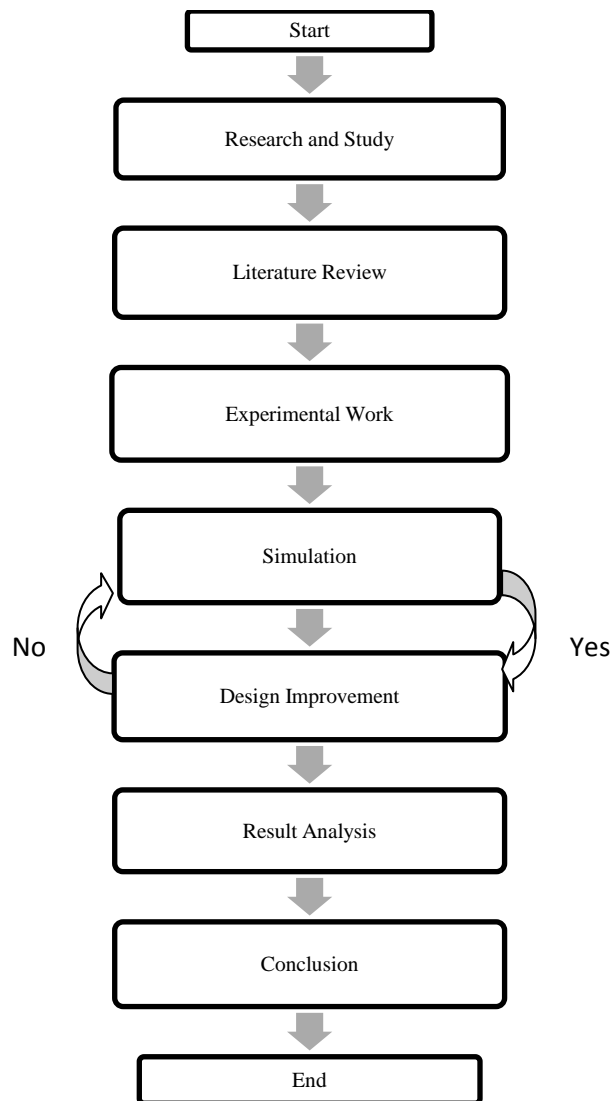


Figure 4: Research Flow

In research stage, general approaches of autoimmune disease detection, identification and classification of AAB Hep-2 cell are studied. Next, the segmentation and pre-processing techniques such as Gaussian filter; median filter, thresholding and etc are studied. The types of colour space for a conversion from RGB are also studied. Besides that, classification techniques such as KNN, MLPs, and RBF are studied. Based on reading and research that have been made, type of colour space, features extraction and classification are chosen.

After finalizing on the techniques, the experimental work is started by executing and initiating the project with MATLAB software prior to implementation. The implementation of the coding will be based on the sequence as shown in the Figure 5 starting from image pre-processing, conversion of colour space, mean calculation and classification. After implementation, the coding is tested and troubleshoots for improvement at the end of the project.

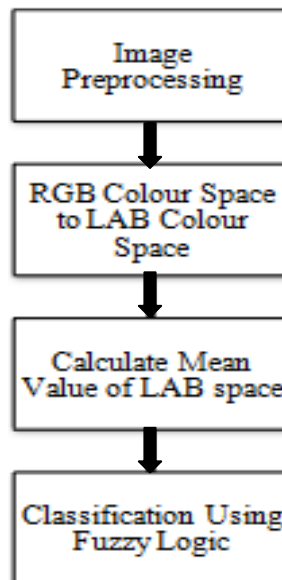


Figure 5: Scheme of Image Processing and Classification

Figure 5 displays the process in order to obtain the mean value of lightness, chromaticity colour a , and chromaticity colour b , and then classify the features obtained from the training datasets using fuzzy logic. The Hep-2 cell images are acquired from a fluorescence microscope (40-fold magnification) coupled with 50W mercury lamp with a digital camera (SLIM system by Das slr). The camera has a CCD with square pixel of 6.45 micro-meters. The images have a resolution of 1388x1038 pixels, a colour depth of 24 bits and stored in BMP format. The

biomedical engineer manually segmented the image by using a tablet PC and subsequently, each image was verified and annotated a medical doctor specialized in immunology.

The acquire image is converted to LAB colour space and the mean value of each luminosity layer ' L ', and chromaticity layer ' a ' where colour falls along the red-green axis from the training dataset is calculated and extracted. After obtaining the mean values based on intermediate and positive classes for training purposes (except negative), the rule in the fuzzy logic is given based on the image that contains three input variables of spectrum range as recorded in Results sections.

3.2 Flow Chart

Figure 6 displays the flow of approached planned to be carried out in order to realize the idea of the proposed project.

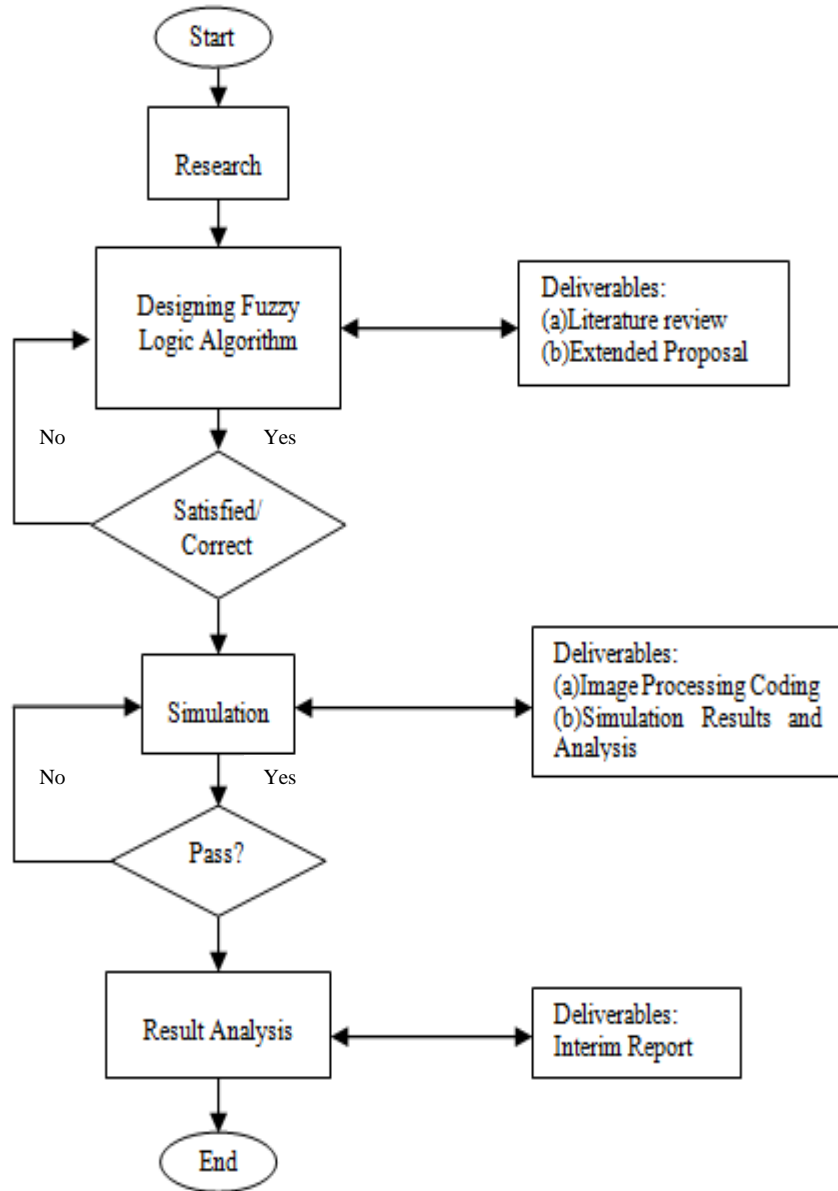


Figure 6: Flow Chart

Figure 7 below shows the flow of process in order to determine the fluorescence intensity classification.

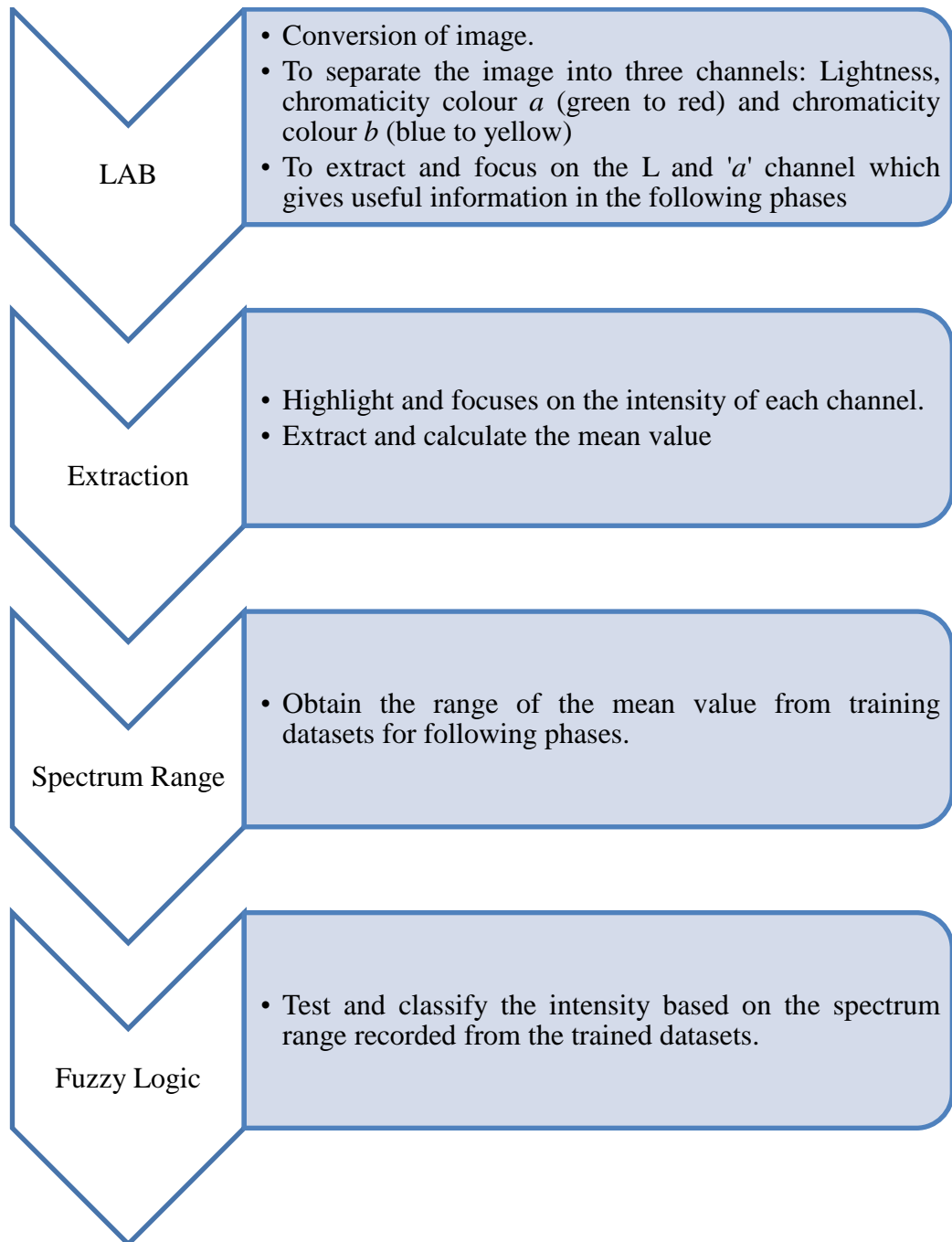


Figure 7: Flow Process of Fluorescence Intensity Classification

3.3 Project Tasks

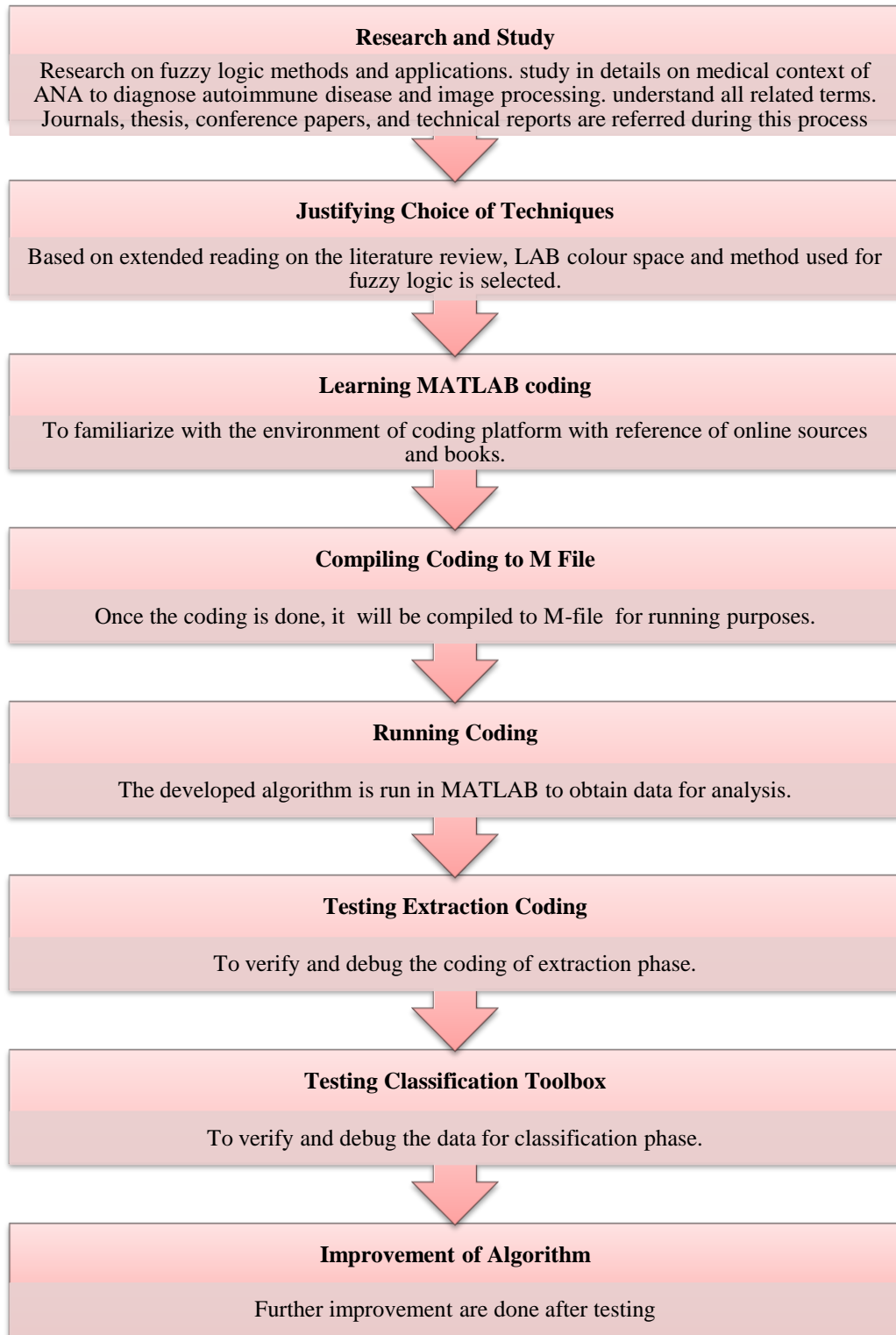


Figure 8: Project Tasks

3.3 Gantt Chart and Milestones

Please refer to the attached document in APPENDIX A.

3.4 Tools

This research requires the following software:

MATLAB R2012a :

- i. Image Processing Toolbox
- ii. Fuzzy Logic Toolbox

CHAPTER 4

RESULTS AND DISCUSSIONS

More than 700 datasets were given by MIVIA research lab of the University of Salerno that active in pattern recognition and computer vision. However, the datasets are only consisting of positive and intermediate images. This section will presents and discusses on the findings of this study which classify the classification of fluorescence intensity: 4+, 3+, 2+, 1+ and 0. The given images have been segmented and annotated by the experts, hence, the simulations involved are extraction of L and 'a' mean value and fuzzy logic classifications.

4.1 Feature Extractions

Table 4 shows three extraction values after converting the original image into LAB colour space of two types fluorescence intensity; positive and intermediate. The mean value of each layer of the converted image which is lightness, chromaticity (a) and chromaticity (b) are calculated and extracted as shown. The value of positive and intermediate intensity shows a distinct difference. 721 train databases have evaluated and the mean value of lightness, chromaticity (a) and (b) are shown in the APPENDIX B. After obtaining all mean values of the train datasets, the graph of positive and intermediate intensity is plotted in APPENDIX C and APPENDIX D from the lowest to highest of each mean values. Both graph shows that there is a few datasets that are overlapping and shows in a distinct ranges.

Table 4: LAB Features Extractions

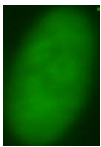


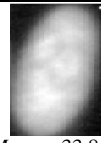

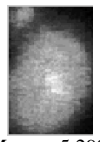
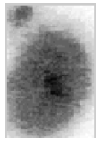
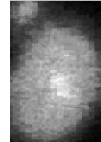
No.	Original Image	Lightness (L) Image	Chromaticity (a) Image	Chromaticity (b) Image	Fluorescence Intensity
1		 Mean = 29.261	 Mean = -33.469	 Mean = 33.813	POSITIVE
2		 Mean = 5.299	 Mean = -10.108	 Mean = 7.636	INTERMEDIATE

Table 5: Ranges of LAB Mean Value for Positive, Intermediate and Negative Class

CHANNEL	RANGES (MEAN)		
	POSITIVE	INTERMEDIATE	NEGATIVE
L	5.987 to 54.782	3.13 to 14.8	< 3.13
A	-9.178 to (-53.286)	-4.439 to (-22.294)	< -22.294
B	8.386 to 55.078	4.225 to 20.434	< 4.225

After obtaining the mean values based on intermediate and positive classes for training purposes, channel L and channel ‘a’ are selected as both of it satisfied and highlighted the criteria of fluorescence intensity. Meanwhile, negative class value is chosen lower than the intermediate minimum value as the class of negative is not provided in the datasets. The rule in the fuzzy logic is given based on the image that contains two input variables (Channel L and Channel A) as recorded in spectrum range as shown in Table 5.

4.2 Fuzzy Logic Classification

Figure 9 below shows the fluorescence intensity fuzzy logic model with two parameters; mean value *L* and mean value *a* are taken as inputs and one output with 5 membership functions which are classes of fluorescence intensity: 4,3, 2, 1 and 0.

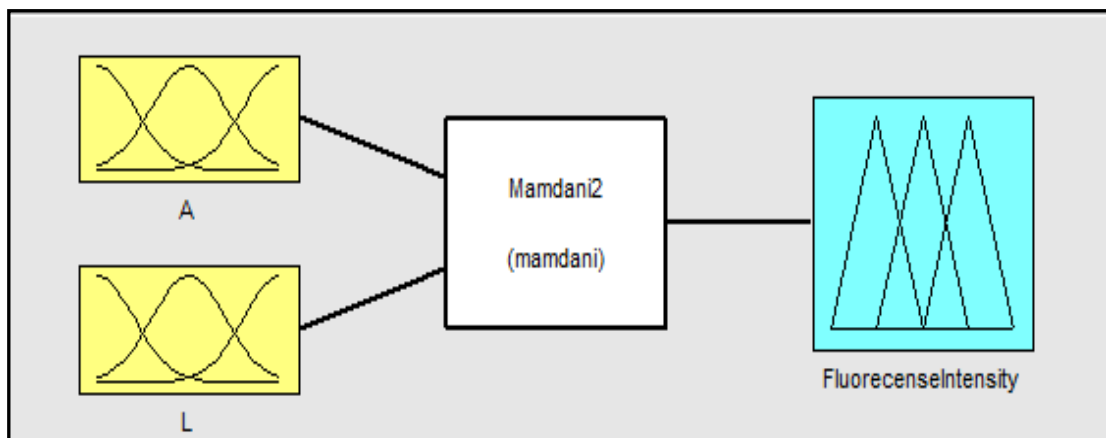


Figure 9: Fluorescence Intensity Fuzzy Logic Model

Based on the range recorded from the experimental results, the *Lightness* input parameter is classified as dull and bright according to the magnitudes while ‘a’

is classified as very bright green, bright green, medium green, low green and red. The membership functions for lightness and chromaticity colour a are shown in Figure 10 and Figure 11 shows the trapezoidal and triangular membership functions are selected for the input as well as the output.

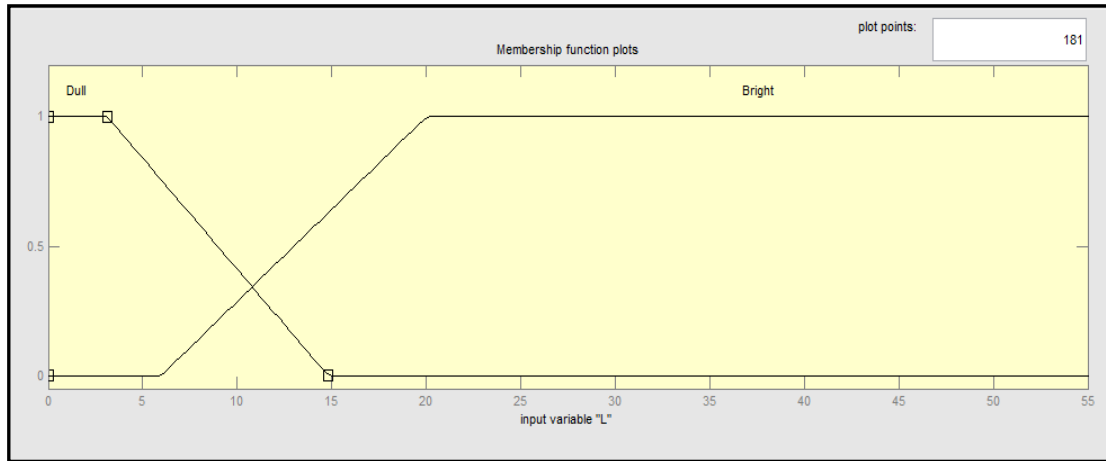


Figure 10: Membership Function for ' L ' Mean Value

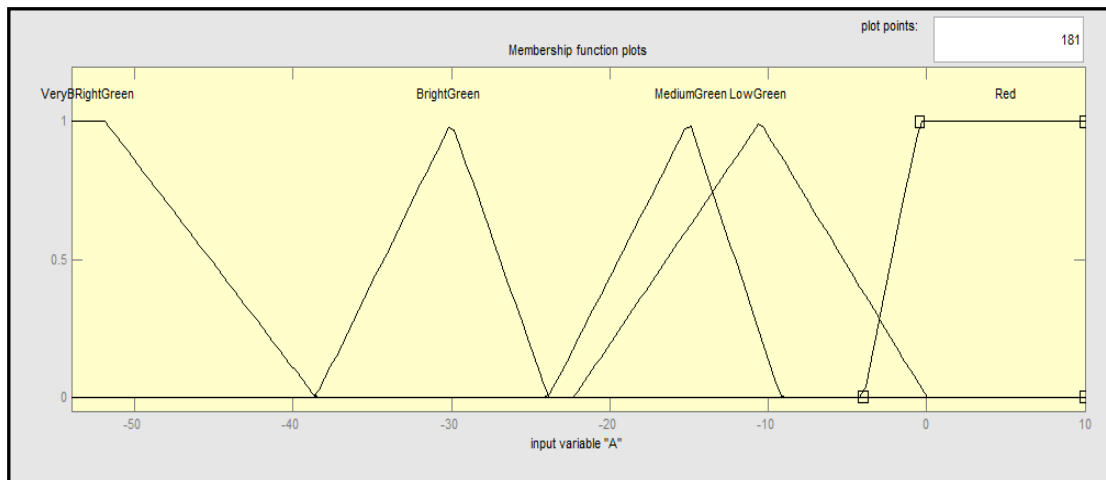


Figure 11: Membership Function for ' a ' Mean Value

The membership functions and the rules are framed by considering all the possible combinations of the inputs computed from the experimental results. A total of 6 rules are framed (see Figure 12). The test data is computed in per unit value from 0 to 1. The output of the fuzzy expert systems is presented in Table 6.

1. If (A is Red) and (L is Dull) then (Fluorescence_Intensity is 0) (1)
2. If (A is Red) and (L is Bright) then (Fluorescence_Intensity is 0) (1)
3. If (A is LowGreen) and (L is Dull) then (Fluorescence_Intensity is +1) (1)
4. If (A is MediumGreen) and (L is Bright) then (Fluorescence_Intensity is +2) (1)
5. If (A is BrightGreen) and (L is Bright) then (Fluorescence_Intensity is +3) (1)
6. If (A is VeryBrightGreen) and (L is Bright) then (Fluorescence_Intensity is +4) (1)

Figure 12: Fuzzy Rule

Table 6: Class of Fluorescence Intensity Based on Fuzzy Logic Output

Class	Output
0	0.00 – 0.20
+1	0.21 – 0.40
+2	0.41 – 0.60
+3	0.61- 0.80
+4	0.81 – 1.00

Figure 12 shows the ‘if-then’ linguistic statement or rules based on the input-output membership functions. There are 6 rules shown. The results will show 0 (negative) when there is red colour existing regarding any lightness. Four classes of positive intensity is decided according to range of input variables.

After all the rules in the fuzzy logic are framed, 200 datasets are tested. The output variables are classified into 5 classes which are 0, 1, 2, 3 and 4. Negative and intermediate class is indicated by 0 and 1 respectively, while from 2 to 4 is positive that divided to 3 classes corresponds to its intensity as shown in Figure 13.

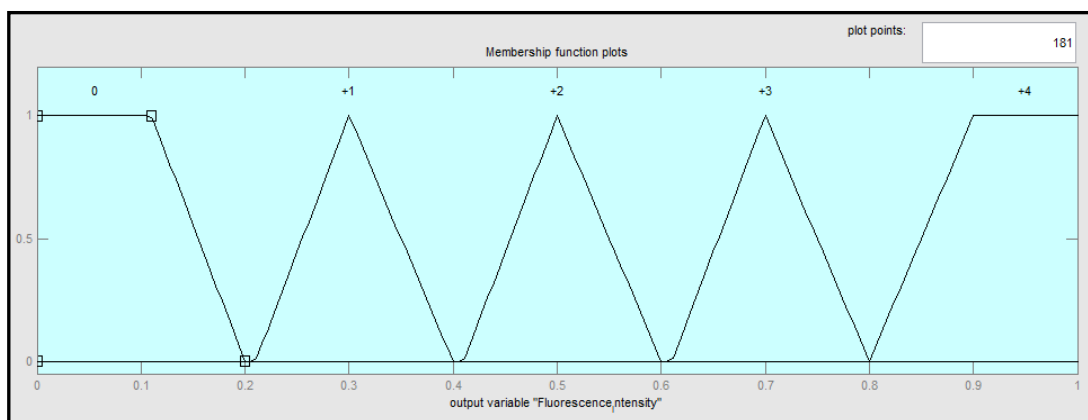


Figure 13: Fluorescence Intensity Output

The graphical application of the fuzzy rules shows a positive Hep-2 cell image when L mean value is 29.3 and ' a ' mean value is -33.4. as shown in Figure 14. The features of input parameters corresponding to L and ' a ' mean values are shown at the top of the respective blocks. The defuzzification of the resultant membership function is performed using largest output maximum (lom) algorithm that shows on the last block of the resultant output – Results.

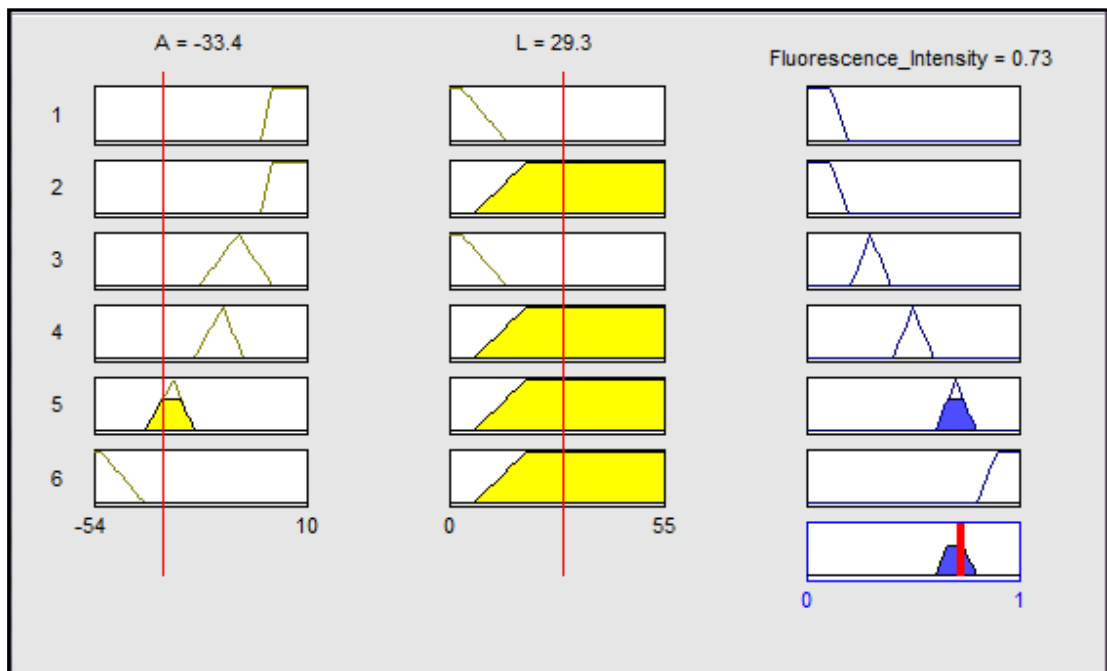


Figure 14: Graphical Application of Fuzzy Rules for Positive Results

The observation can be made based on the figure for positive class is, the output membership function consists of five triangles start from 0, 1, 2, 3, and 4, and the shaded blue colour is mostly on the third triangle and results in class of 2-positive with an output of 0.73.

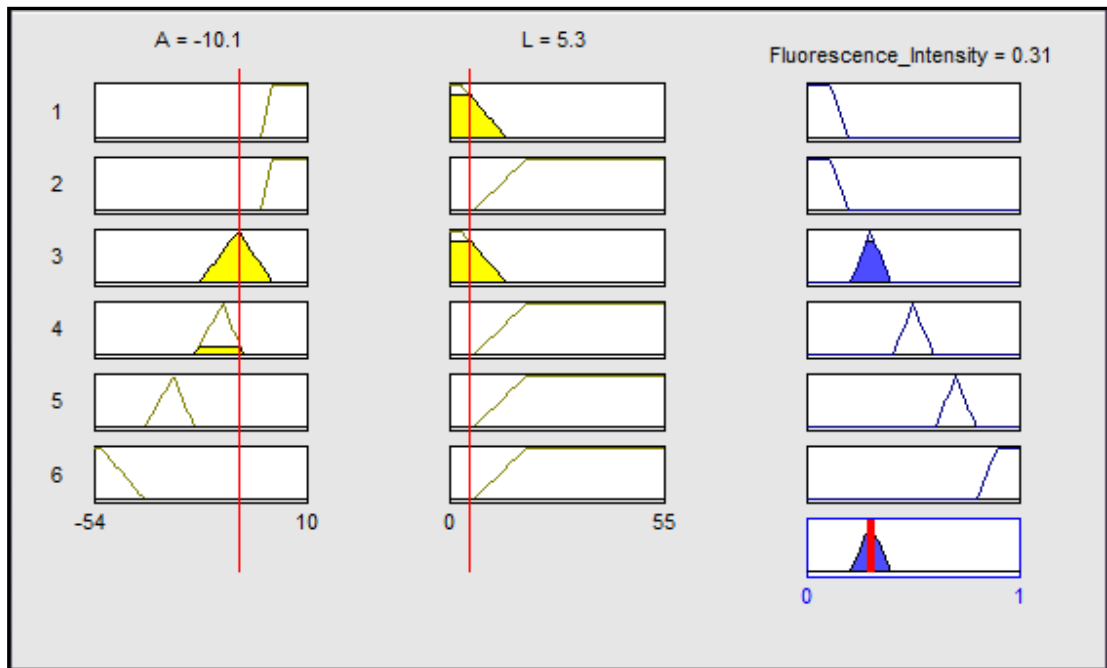


Figure 15: Graphical Application of Fuzzy Rules for Intermediate Results

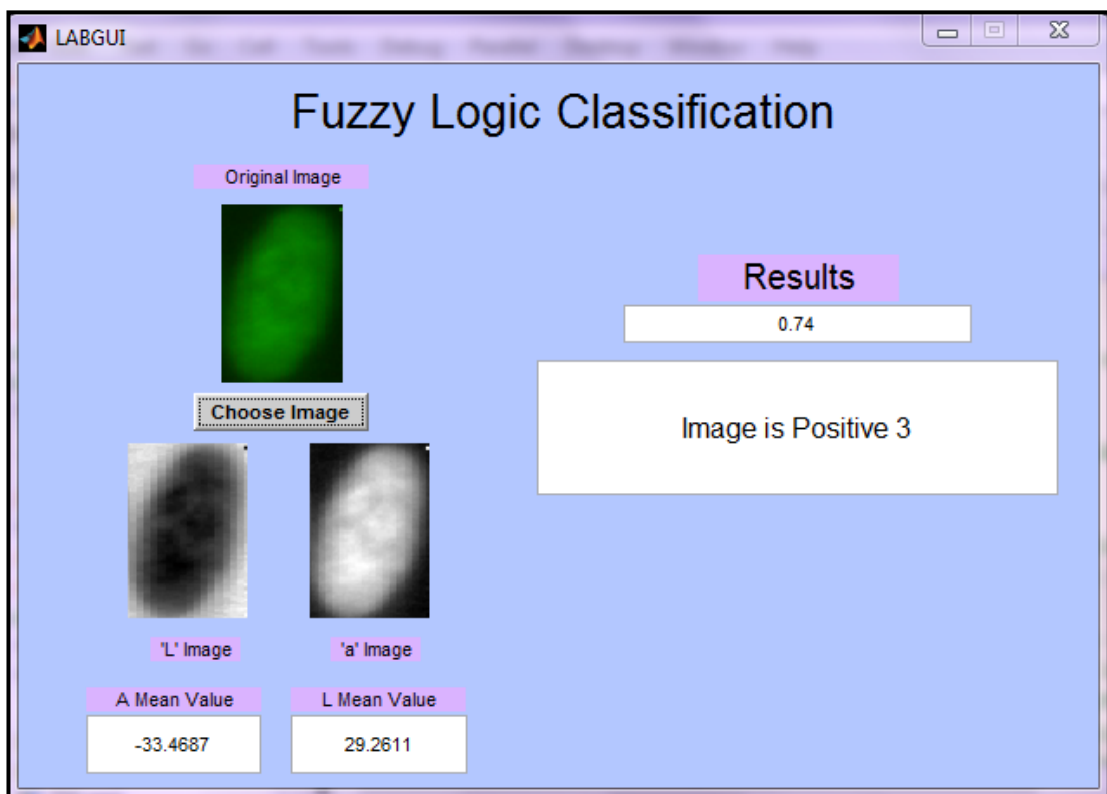


Figure 16: Graphical User Interface (GUI)

On the other hand, the intermediate class (Figure 15) is on the second triangle function indicates intermediate class with an output of 0.31. Figure 16 shows the GUI that has been created for the ease of use and as the primary interface for human-machine interaction.

Referring to APPENDIX E and APPENDIX F, 200 datasets of positive and intermediate fluorescence intensity are used for testing the performance measurements of fuzzy logic. It is seen that, 15 out of 100 intermediate data and 13 out of 100 positive data held in fuzzy logic classifier are misclassified, then, the accurate results are obtained and performance measurement is ensured at a rate of 85% for intermediate class and 87% for positive class. However, larger database is needed to observe the accuracy of the proposed method.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

In conclusion, this project aims to classify the fluorescence intensity of Hep-2 cells fluorescence image to determine specific diseases through ANA test. In most research, there is still no validation of proposed methods from other research groups. Therefore, fuzzy logic classifier is proposed in this study.

Based on research that has been done, LAB colour space is selected as the entire colour is visible and close to human perception. It consists of three features that can be measured which are lightness, chromaticity (a) and chromaticity (b). After the RGB or original image is converted to LAB colour space, the mean value for two main features: L and 'a', are calculated and the range is obtained from the experimental on training datasets. The ranges for two features then are used in the input variable for fuzzy logic classifier. Type of fuzzy logic used in this study is the modified Mamdani method as it uses triangle and trapezoid membership functions. As the output result of data received from these factors, the classes of fluorescence intensity can be determined.

From the results that have been obtained, it is seen that the proposed algorithm provides full information for the classifications and shows that the fuzzy expert systems is reliable with high agreement of accuracy measurement which are 85% for intermediate class and 88% positive. However, 100% accuracy in the system could not be detected as there are few datasets determine by the expert (medical doctor) may be misclassified and needs to be further analyzed.

As the results are encouraging, in the future, a large database will be engaged to further test and improve the developed tools.

REFERENCES

- [1] K. Egerer, D. Roggenbuck, R. Hiemann, M.-G. Weyer, T. Buttner, B. Radau, *et al.*, "Automated evaluation of autoantibodies on human epithelial-2 cells as an approach to standardize cell-based immunofluorescence tests," *Arthritis Research & Therapy*, vol. 12, p. R40, 2010. {, 2012 #69}
- [2] J. Voigt, C. Krause, E. Rohwader, *et al.*, "Automated Indirect Immunofluorescence Evaluation of Antinuclear Autoantibodies on HEp-2 Cells," *Clinical and Developmental Immunology*, vol. 2012, p. 7, 2012.
- [3] H. A. Mariz, E. I. Sato, S. H. Barbosa, S. H. Rodrigues, A. Dellavance, and L. E. Andrade, "Pattern on the antinuclear antibody-HEp-2 test is a critical parameter for discriminating antinuclear antibody-positive healthy individuals and patients with autoimmune rheumatic diseases," *Arthritis Rheum*, vol. 63, pp. 191-200, Jan 2011.
- [4] A. Dellavance and e. al, "Third Bbrazilian Consensus for Autoantibodies Screening in HEp-2 Cells. Recommendations for Standardization of Autoantibodies Screening Trial in HEp-2 cells, Quality Control and Clinical Association," 2009 2009.
- [5] Stafson, "The Known Colors Palette Tool - Revised," 2011. Available: <http://www.codeproject.com/Articles/243610/The-Known-Colors-Palette-Tool-Revised>
- [6] E.Sivasankar and R.S.Rajesh, "Knowledge Discovery in Medical Datasets Using a Fuzzy Logic rule based Classifier," 2010.
- [7] P. Foggia, G. Percannella, P. Soda, and M. Vento, "Benchmarking HEp-2 Cells Classification Methods," *Medical Imaging, IEEE Transactions on*, vol. PP, pp. 1-1, 2013.
- [8] A. Rigon, P. Soda, D. Zennaro, G. Iannello, and A. Afeltra, "Indirect Immunofluorescence in Autoimmune Diseases: Assessment of Digital Images for Diagnosis Purpose," vol. 72, pp. 427-477, 2007.
- [9] M. Abeln. (2012). *Color Spaces, Part 4: Lab*. Available: <http://therefractedlight.blogspot.com/2012/02/color-spaces-part-4-lab.html>
- [10] K. Leon, D. Mery, and F. Pedreschi, "Color Measurement in L*a*b* units from RGB digital images," 2005.
- [11] D. M. Ushizima, R. T. Calado, and E. G. Rizzatti, "Leukocyte Detection Using Nucleus Contour Propagation," *Medical Imaging and Augmented Reality*, 2006.

- [12] A. Willitzki, R. Hiemann, V. Peters, U. Sack, P. Schierack, *et al.*, "New Platform Technology for Comprehensive Serological Diagnostics of Autoimmune Diseases," *Clinical and Developmental Immunology*, vol. 2012, p. 8, 2012.
- [13] P. Soda, G. Iannello, and M. Vento, "A multiple expert system for classifying fluorescent intensity in antinuclear autoantibodies analysis," *Pattern Analysis and Applications*, vol. 12, pp. 215-226, 2009/09/01 2009.
- [14] A. Yilmaz and K. Ayan, "Cancer Risk Analysis by Fuzzy Logic Approach and Performance Status of The Model," *Turkish Journal of Electrical Engineering and Computer Science*, 2011.
- [15] P. Puranik, P. Bajaj, A. Abraham, P. Palsodkar, and A. Deshmukh, "Human Perception-Based Color Image Segmentation Using Comprehensive Learning Particle Swarm Optimization," in *Emerging Trends in Engineering and Technology (ICETET), 2009 2nd International Conference on*, 2009, pp. 630-635.
- [16] T. G. Arora, M. V. Aware, and D. R. Tutakne, "Fuzzy logic application to life estimation of phase angle controlled induction motors," in *Diagnostics for Electric Machines, Power Electronics and Drives, 2009. SDEMPED 2009. IEEE International Symposium on*, 2009, pp. 1-5.
- [17] M. R. Khokher, A. Ghafoor, and A. M. Siddiqui, "Image segmentation using fuzzy rule based system and graph cuts," in *Control Automation Robotics & Vision (ICARCV), 2012 12th International Conference on*, 2012, pp. 1148-1153.
- [18] R. V. Rajesh, J. Veerappan, S. K. Sujitha, and E. A. Kumar, "Classification and retrieval of images using texture features," in *Computing Communication & Networking Technologies (ICCCNT), 2012 Third International Conference on*, 2012, pp. 1-5.

APPENDICES

APPENDIX A

FYP1 & FYP2 GANTT CHART AND MILESTONES

Details	Week													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
Title selection														
Research on autoimmune disease														
Research on existing classifier for fluorescence Intensity														
Preparation and submission of extended proposal														
Research on fuzzy logic applications														
Research on colour spaces														
Preparation for proposal defence														
Proposal defence and progress evaluation														
Extended researches and submission draft of interim report														
Improvement of interim report														
Interim Report Submission														

Details	Week														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Image pre-processing and segmentation of colour using k-mean clustering															
Extraction of mean value (L*a*b)															
Create loop to read images															
Results gathering and data analysis															
Image classification using fuzzy logic															
Progress reports submission															
Create Graphical User Interface (GUI)															
ELECTREX presentation															
Final Report and Technical report (soft bound)															
Oral presentation															
Dissertation (hard bound)															



Process



Suggested Key Milestone

APPENDIX B

RESULTS OF LAB FEATURES FOR TRAINING IMAGES

No	L	A	B	INTENSITY
1	29.261	-33.469	33.813	Positive
2	19.394	-24.025	24.930	Positive
3	15.298	-21.078	20.748	Positive
4	8.412	-11.706	11.078	Positive
5	9.780	-13.867	12.783	Positive
6	7.518	-12.910	10.592	Positive
7	3.909	-5.408	5.269	Intermediate
8	4.095	-7.811	5.901	Intermediate
9	17.544	-23.045	23.251	Positive
10	6.963	-12.359	9.901	Positive
11	8.041	-12.757	11.209	Intermediate
12	7.401	-13.949	10.645	Intermediate
13	27.137	-32.037	33.087	Positive
14	17.058	-24.399	22.213	Positive
15	22.721	-27.515	28.446	Positive
16	20.702	-25.804	26.515	Positive
17	3.508	-6.693	5.055	Intermediate
18	54.782	-53.286	55.078	Positive
19	7.700	-12.716	10.623	Positive
20	3.603	-5.153	4.818	Intermediate
21	5.299	-10.108	7.636	Intermediate
22	5.533	-10.554	7.973	Intermediate
23	9.130	-14.358	12.702	Intermediate
24	10.164	-16.200	13.791	Positive
25	8.189	-12.874	11.396	Intermediate
26	33.016	-36.497	37.206	Positive
27	21.029	-26.090	26.868	Positive
28	8.557	-14.143	11.832	Positive
29	17.586	-23.507	22.168	Positive
30	29.950	-33.084	34.267	Positive
31	3.843	-7.334	5.538	Intermediate
32	18.088	-25.108	22.934	Positive
33	3.836	-7.300	5.529	Intermediate
34	5.201	-9.925	7.495	Intermediate
35	14.196	-20.979	18.874	Positive
36	11.468	-18.095	16.351	Intermediate
37	9.287	-15.051	12.686	Positive

38	14.854	-20.733	20.114	Positive
39	5.133	-9.742	7.394	Intermediate
40	6.841	-11.507	9.490	Positive
41	20.543	-25.396	25.862	Positive
42	27.375	-30.817	31.786	Positive
43	7.249	-10.286	9.748	Positive
44	12.452	-19.317	16.882	Positive
45	11.883	-16.731	16.284	Positive
46	21.510	-26.387	27.099	Positive
47	34.073	-37.486	38.489	Positive
48	6.052	-11.534	8.720	Intermediate
49	5.103	-7.326	7.000	Intermediate
50	17.919	-23.791	23.840	Positive
51	6.039	-11.457	8.695	Intermediate
52	8.896	-15.938	12.693	Intermediate
53	5.086	-9.684	7.327	Intermediate
54	19.038	-24.018	24.315	Positive
55	3.896	-7.434	5.614	Intermediate
56	8.522	-11.587	11.079	Positive
57	5.801	-11.063	8.359	Intermediate
58	10.471	-14.849	13.835	Positive
59	6.245	-11.821	8.989	Intermediate
60	3.943	-5.450	5.320	Intermediate
61	7.577	-13.123	10.672	Positive
62	8.231	-11.533	10.954	Positive
63	13.825	-22.127	19.284	Intermediate
64	7.833	-13.288	10.969	Positive
65	18.819	-23.827	24.592	Positive
66	10.058	-15.786	13.569	Positive
67	4.210	-6.202	5.699	Intermediate
68	21.098	-27.820	26.456	Positive
69	13.055	-19.749	17.270	Positive
70	4.415	-6.439	5.994	Intermediate
71	8.772	-15.646	12.510	Intermediate
72	22.265	-26.581	27.515	Positive
73	23.762	-28.443	29.804	Positive
74	8.840	-13.932	12.322	Intermediate
75	11.622	-17.719	15.532	Positive
76	9.569	-15.461	13.129	Positive
77	8.155	-11.556	10.913	Positive
78	3.870	-7.385	5.578	Intermediate
79	3.269	-6.238	4.711	Intermediate
80	3.316	-6.328	4.779	Intermediate

81	8.809	-12.346	11.584	Positive
82	9.623	-16.142	13.443	Positive
83	27.907	-31.371	32.774	Positive
84	4.774	-9.093	6.878	Intermediate
85	37.881	-40.310	41.342	Positive
86	21.471	-26.544	27.243	Positive
87	10.596	-17.222	14.670	Positive
88	11.739	-19.874	16.578	Intermediate
89	4.194	-7.992	6.045	Intermediate
90	18.049	-22.785	23.194	Positive
91	4.955	-9.431	7.138	Intermediate
92	9.327	-15.507	12.963	Positive
93	17.946	-24.498	23.108	Positive
94	9.736	-15.776	13.346	Positive
95	22.921	-27.024	28.124	Positive
96	22.847	-27.431	28.436	Positive
97	5.245	-9.994	7.557	Intermediate
98	20.823	-25.476	26.451	Positive
99	4.013	-5.570	5.421	Intermediate
100	6.674	-12.572	9.599	Intermediate
101	24.807	-29.016	30.326	Positive
102	9.857	-12.893	12.321	Positive
103	23.379	-29.719	28.785	Positive
104	10.856	-17.073	14.696	Positive
105	6.955	-13.201	10.015	Intermediate
106	5.517	-10.517	7.949	Intermediate
107	8.805	-13.807	12.241	Intermediate
108	4.406	-8.340	6.346	Intermediate
109	8.693	-12.162	11.475	Positive
110	18.522	-23.634	23.768	Positive
111	3.695	-5.066	4.961	Intermediate
112	5.379	-10.265	7.752	Intermediate
113	32.082	-35.780	36.454	Positive
114	8.525	-13.688	11.537	Positive
115	18.838	-24.683	24.894	Positive
116	22.119	-26.691	27.290	Positive
117	6.425	-12.153	9.246	Intermediate
118	19.034	-24.353	24.855	Positive
119	6.753	-12.803	9.723	Intermediate
120	15.015	-21.549	19.476	Positive
121	26.967	-30.545	31.882	Positive
122	3.577	-6.826	5.155	Intermediate
123	9.485	-15.278	12.983	Positive

124	20.135	-24.994	25.482	Positive
125	18.794	-24.278	24.610	Positive
126	3.656	-5.039	4.904	Intermediate
127	26.505	-30.150	31.232	Positive
128	4.925	-9.285	7.082	Intermediate
129	5.648	-10.654	8.106	Intermediate
130	20.702	-25.767	26.271	Positive
131	4.225	-8.059	6.088	Intermediate
132	24.158	-30.118	28.945	Positive
133	3.636	-5.258	4.862	Intermediate
134	8.261	-13.502	11.314	Positive
135	3.975	-5.552	5.365	Intermediate
136	9.548	-15.023	12.994	Positive
137	20.476	-25.369	25.884	Positive
138	10.374	-16.221	14.001	Positive
139	16.921	-23.699	21.648	Positive
140	5.100	-9.643	7.343	Intermediate
141	36.660	-39.334	40.251	Positive
142	3.777	-7.196	5.443	Intermediate
143	8.823	-13.944	12.236	Intermediate
144	6.340	-11.914	9.117	Intermediate
145	26.292	-30.020	31.364	Positive
146	5.159	-9.826	7.433	Intermediate
147	9.330	-12.865	12.181	Positive
148	9.417	-14.646	13.098	Intermediate
149	21.624	-26.782	27.547	Positive
150	4.821	-9.200	6.947	Intermediate
151	21.878	-28.618	27.269	Positive
152	10.747	-17.165	14.691	Positive
153	7.485	-12.871	10.519	Positive
154	7.478	-11.775	10.397	Intermediate
155	23.605	-27.917	28.889	Positive
156	52.063	-51.211	52.710	Positive
157	3.382	-4.763	4.502	Intermediate
158	8.048	-12.818	11.210	Intermediate
159	20.772	-25.426	26.072	Positive
160	20.034	-25.417	25.962	Positive
161	9.333	-15.499	12.992	Positive
162	28.313	-31.778	33.257	Positive
163	9.377	-13.770	12.534	Positive
164	6.161	-10.819	8.678	Positive
165	5.823	-10.950	8.376	Intermediate
166	7.883	-14.675	11.317	Intermediate

167	3.741	-7.139	5.391	Intermediate
168	4.924	-9.347	7.094	Intermediate
169	22.675	-27.059	28.254	Positive
170	8.018	-12.665	11.153	Intermediate
171	8.556	-14.101	11.834	Positive
172	22.149	-26.574	27.420	Positive
173	9.240	-12.971	12.239	Positive
174	20.496	-25.465	26.071	Positive
175	4.394	-8.352	6.332	Intermediate
176	28.686	-32.706	32.894	Positive
177	8.272	-14.787	11.798	Intermediate
178	7.047	-11.554	9.764	Intermediate
179	3.406	-4.439	4.546	Intermediate
180	18.383	-23.967	24.416	Positive
181	3.765	-5.257	5.061	Intermediate
182	6.561	-12.391	9.429	Intermediate
183	7.060	-9.776	9.085	Positive
184	4.465	-6.246	6.078	Intermediate
185	9.096	-13.057	12.160	Positive
186	7.625	-12.128	10.612	Intermediate
187	18.731	-24.240	24.480	Positive
188	5.261	-10.038	7.582	Intermediate
189	19.699	-24.168	25.138	Positive
190	10.524	-15.638	14.259	Positive
191	23.784	-27.970	28.780	Positive
192	9.711	-15.295	13.066	Positive
193	18.387	-23.416	23.625	Positive
194	5.248	-9.928	7.556	Intermediate
195	13.958	-22.019	19.420	Intermediate
196	4.533	-8.649	6.533	Intermediate
197	6.796	-12.118	9.661	Positive
198	34.877	-38.043	38.983	Positive
199	3.923	-5.741	5.284	Intermediate
200	15.295	-22.280	20.300	Positive
201	8.223	-11.269	10.760	Positive
202	9.385	-14.812	12.750	Positive
203	15.390	-22.528	20.061	Positive
204	9.172	-14.350	12.769	Intermediate
205	8.262	-14.823	11.794	Intermediate
206	3.742	-5.102	5.024	Intermediate
207	3.192	-4.564	4.225	Intermediate
208	19.146	-24.032	24.398	Positive
209	5.273	-10.061	7.598	Intermediate

210	4.428	-8.421	6.381	Intermediate
211	26.408	-30.049	31.269	Positive
212	37.131	-39.579	40.310	Positive
213	9.050	-12.632	11.865	Positive
214	22.742	-26.694	27.843	Positive
215	35.564	-38.417	39.096	Positive
216	19.316	-26.189	24.430	Positive
217	13.395	-18.388	18.188	Positive
218	17.563	-23.059	23.200	Positive
219	7.084	-12.370	10.040	Positive
220	11.122	-17.825	15.277	Positive
221	11.469	-17.924	15.598	Positive
222	50.702	-50.179	51.550	Positive
223	3.827	-5.224	5.153	Intermediate
224	20.351	-25.450	26.214	Positive
225	3.365	-4.509	4.484	Intermediate
226	22.692	-26.714	27.769	Positive
227	32.445	-35.649	35.980	Positive
228	25.841	-29.404	30.500	Positive
229	23.080	-27.525	28.594	Positive
230	23.501	-28.303	29.732	Positive
231	11.704	-18.200	15.828	Positive
232	8.098	-11.123	10.658	Positive
233	6.693	-12.225	9.575	Intermediate
234	11.372	-18.064	15.550	Positive
235	39.366	-41.452	42.478	Positive
236	30.574	-34.454	34.742	Positive
237	12.054	-18.954	16.299	Positive
238	10.757	-15.051	14.175	Positive
239	3.990	-7.591	5.751	Intermediate
240	8.040	-11.648	10.847	Positive
241	15.492	-20.515	20.735	Positive
242	5.214	-9.880	7.509	Intermediate
243	5.628	-10.507	8.085	Intermediate
244	8.182	-12.899	11.395	Intermediate
245	9.810	-15.481	13.241	Positive
246	18.744	-24.481	24.880	Positive
247	18.144	-23.655	23.588	Positive
248	23.316	-27.885	28.759	Positive
249	7.762	-14.003	11.087	Intermediate
250	4.031	-5.613	5.447	Intermediate
251	6.516	-12.185	9.360	Intermediate
252	5.514	-10.504	7.944	Intermediate

253	3.872	-7.389	5.581	Intermediate
254	13.100	-19.266	18.342	Intermediate
255	3.818	-5.165	5.138	Intermediate
256	22.652	-27.576	28.429	Positive
257	17.282	-22.527	22.527	Positive
258	3.621	-6.909	5.218	Intermediate
259	4.576	-8.732	6.595	Intermediate
260	12.265	-19.019	16.571	Positive
261	27.457	-30.957	32.237	Positive
262	8.139	-15.275	11.699	Intermediate
263	9.428	-13.799	12.667	Positive
264	10.268	-15.835	14.544	Intermediate
265	26.996	-30.580	31.804	Positive
266	12.102	-16.657	15.819	Positive
267	4.071	-7.748	5.867	Intermediate
268	4.244	-8.061	6.116	Intermediate
269	4.556	-6.406	6.210	Intermediate
270	30.804	-34.852	35.499	Positive
271	3.188	-6.081	4.590	Intermediate
272	20.457	-25.513	26.324	Positive
273	17.083	-22.319	22.387	Positive
274	12.955	-19.325	16.940	Positive
275	34.106	-37.359	38.048	Positive
276	10.576	-16.892	15.110	Intermediate
277	21.399	-26.504	27.238	Positive
278	5.931	-11.278	8.543	Intermediate
279	23.839	-28.385	29.198	Positive
280	7.507	-13.966	10.776	Intermediate
281	20.575	-27.184	25.844	Positive
282	5.984	-11.419	8.623	Intermediate
283	4.089	-7.803	5.892	Intermediate
284	21.130	-26.369	26.874	Positive
285	8.976	-16.361	12.844	Intermediate
286	25.340	-29.401	30.708	Positive
287	8.047	-14.921	11.545	Intermediate
288	23.750	-28.605	29.833	Positive
289	7.252	-13.639	10.428	Intermediate
290	7.349	-11.636	10.210	Intermediate
291	5.016	-9.544	7.225	Intermediate
292	8.070	-14.970	11.580	Intermediate
293	21.202	-25.627	26.464	Positive
294	23.300	-28.074	28.967	Positive
295	7.824	-11.171	10.168	Positive

296	9.929	-16.463	13.836	Positive
297	8.265	-13.145	11.502	Intermediate
298	7.651	-13.145	10.776	Positive
299	15.141	-20.065	20.272	Positive
300	5.637	-10.753	8.123	Intermediate
301	4.725	-9.004	6.809	Intermediate
302	24.253	-28.330	29.611	Positive
303	5.436	-10.371	7.834	Intermediate
304	16.543	-21.730	21.811	Positive
305	4.308	-8.221	6.209	Intermediate
306	11.506	-19.430	16.238	Intermediate
307	3.461	-6.604	4.988	Intermediate
308	8.936	-16.208	12.774	Intermediate
309	8.590	-14.059	11.846	Positive
310	5.137	-9.779	7.401	Intermediate
311	21.485	-28.266	27.202	Positive
312	21.440	-27.726	26.046	Positive
313	32.444	-36.046	36.706	Positive
314	7.494	-12.012	10.405	Intermediate
315	3.637	-5.043	4.874	Intermediate
316	4.802	-9.154	6.920	Intermediate
317	37.785	-40.015	40.695	Positive
318	26.462	-30.264	31.758	Positive
319	9.428	-15.727	13.035	Positive
320	4.522	-6.680	6.152	Intermediate
321	5.138	-9.762	7.389	Intermediate
322	24.261	-28.821	30.311	Positive
323	11.229	-17.289	15.892	Intermediate
324	10.129	-16.175	13.839	Positive
325	10.577	-17.130	15.161	Intermediate
326	17.331	-22.547	22.737	Positive
327	8.503	-12.019	11.132	Positive
328	16.594	-24.128	21.957	Positive
329	8.391	-11.819	10.989	Positive
330	3.729	-7.115	5.375	Intermediate
331	17.377	-23.103	23.107	Positive
332	20.958	-26.296	27.127	Positive
333	19.098	-24.427	25.186	Positive
334	8.913	-15.751	12.678	Intermediate
335	5.865	-11.192	8.452	Intermediate
336	8.853	-14.870	12.390	Positive
337	3.875	-5.385	5.220	Intermediate
338	17.725	-25.124	23.129	Positive

339	25.828	-30.345	30.361	Positive
340	10.427	-16.825	14.247	Positive
341	5.340	-10.150	7.692	Intermediate
342	7.941	-13.436	11.109	Positive
343	7.853	-10.939	10.237	Positive
344	10.045	-13.929	13.363	Positive
345	28.544	-32.732	33.074	Positive
346	12.203	-18.709	16.289	Positive
347	4.553	-8.646	6.561	Intermediate
348	13.887	-18.968	18.809	Positive
349	30.745	-34.634	35.141	Positive
350	8.863	-15.217	12.541	Intermediate
351	13.641	-20.239	17.951	Positive
352	3.778	-7.208	5.444	Intermediate
353	11.848	-18.355	15.941	Positive
354	7.166	-13.432	10.299	Intermediate
355	7.682	-12.098	10.683	Intermediate
356	11.008	-18.106	15.461	Intermediate
357	6.237	-11.624	8.957	Intermediate
358	18.003	-23.772	23.479	Positive
359	6.364	-12.048	9.163	Intermediate
360	4.405	-8.380	6.348	Intermediate
361	26.453	-30.204	31.549	Positive
362	4.621	-8.782	6.659	Intermediate
363	34.952	-38.094	38.995	Positive
364	10.030	-13.843	13.032	Positive
365	5.378	-10.258	7.750	Intermediate
366	17.727	-22.752	23.303	Positive
367	16.072	-21.035	21.330	Positive
368	6.550	-9.178	8.768	Positive
369	36.359	-39.059	40.110	Positive
370	7.955	-13.049	11.041	Intermediate
371	29.438	-33.312	33.501	Positive
372	6.717	-12.684	9.664	Intermediate
373	4.335	-6.119	5.887	Intermediate
374	25.682	-29.482	30.756	Positive
375	6.858	-12.943	9.867	Intermediate
376	5.085	-9.679	7.326	Intermediate
377	13.721	-19.800	19.053	Intermediate
378	35.597	-38.510	39.350	Positive
379	3.588	-5.013	4.803	Intermediate
380	7.398	-13.851	10.630	Intermediate
381	7.777	-12.315	10.828	Intermediate

382	3.545	-6.766	5.109	Intermediate
383	4.234	-8.059	6.101	Intermediate
384	3.133	-5.976	4.515	Intermediate
385	25.552	-29.908	31.096	Positive
386	5.828	-11.104	8.397	Intermediate
387	20.013	-24.858	25.342	Positive
388	5.923	-11.110	8.516	Intermediate
389	5.666	-10.722	8.157	Intermediate
390	12.845	-21.181	18.036	Intermediate
391	19.867	-25.275	25.814	Positive
392	3.958	-7.550	5.703	Intermediate
393	9.246	-12.995	12.255	Positive
394	7.960	-12.955	11.059	Intermediate
395	9.595	-15.152	12.819	Positive
396	13.028	-21.379	18.281	Intermediate
397	32.190	-35.297	37.090	Positive
398	8.847	-12.408	11.634	Positive
399	6.051	-11.489	8.714	Intermediate
400	4.769	-6.735	6.518	Intermediate
401	9.002	-12.632	11.847	Positive
402	13.524	-21.645	18.866	Intermediate
403	7.259	-13.695	10.443	Intermediate
404	5.959	-11.359	8.586	Intermediate
405	12.026	-18.322	16.071	Positive
406	6.683	-12.600	9.611	Intermediate
407	3.746	-5.094	5.037	Intermediate
408	9.564	-14.368	13.299	Positive
409	8.333	-13.071	11.609	Intermediate
410	7.343	-11.593	10.215	Intermediate
411	7.694	-11.279	10.439	Positive
412	10.640	-16.829	14.345	Positive
413	20.968	-26.118	26.863	Positive
414	9.956	-15.636	14.217	Intermediate
415	8.799	-12.187	11.564	Positive
416	6.252	-11.817	9.000	Intermediate
417	14.510	-19.181	19.398	Positive
418	3.564	-4.982	4.768	Intermediate
419	16.071	-23.385	21.284	Positive
420	3.493	-6.645	5.035	Intermediate
421	8.965	-12.495	11.662	Positive
422	3.130	-5.973	4.511	Intermediate
423	35.139	-37.975	38.646	Positive
424	3.783	-7.218	5.452	Intermediate

425	8.947	-15.899	12.748	Intermediate
426	12.178	-20.065	17.086	Intermediate
427	3.943	-7.482	5.682	Intermediate
428	4.575	-8.718	6.593	Intermediate
429	11.326	-17.522	15.313	Positive
430	3.770	-7.175	5.432	Intermediate
431	3.917	-7.444	5.644	Intermediate
432	11.938	-18.751	16.204	Positive
433	30.428	-34.312	34.589	Positive
434	8.148	-11.395	10.787	Positive
435	7.530	-11.988	10.481	Intermediate
436	7.009	-13.197	10.080	Intermediate
437	19.515	-24.369	24.770	Positive
438	3.487	-4.720	4.659	Intermediate
439	12.614	-20.398	17.618	Intermediate
440	18.500	-23.941	24.371	Positive
441	14.184	-22.294	19.684	Intermediate
442	9.761	-16.088	13.470	Positive
443	5.443	-10.369	7.841	Intermediate
444	9.368	-12.971	12.047	Positive
445	32.966	-36.346	36.889	Positive
446	13.626	-18.760	18.502	Positive
447	4.160	-7.901	5.995	Intermediate
448	11.564	-17.379	15.320	Positive
449	11.706	-17.814	16.536	Intermediate
450	20.681	-25.876	26.635	Positive
451	7.565	-12.833	10.570	Positive
452	20.448	-26.946	25.407	Positive
453	5.524	-10.389	7.946	Intermediate
454	22.190	-26.761	27.708	Positive
455	8.807	-13.941	12.250	Intermediate
456	5.742	-10.950	8.274	Intermediate
457	21.159	-26.142	26.822	Positive
458	8.852	-14.837	12.334	Positive
459	6.592	-12.497	9.491	Intermediate
460	14.788	-21.230	20.434	Intermediate
461	21.559	-26.794	27.530	Positive
462	21.024	-25.697	26.723	Positive
463	9.662	-17.352	13.791	Intermediate
464	20.766	-25.989	26.671	Positive
465	11.853	-20.049	16.734	Intermediate
466	27.564	-31.198	32.302	Positive
467	9.936	-17.770	14.174	Intermediate

468	8.716	-11.986	11.132	Positive
469	3.975	-7.585	5.729	Intermediate
470	18.180	-23.195	23.499	Positive
471	3.876	-5.288	5.143	Intermediate
472	7.784	-13.312	10.869	Positive
473	22.108	-28.625	27.347	Positive
474	8.908	-14.569	12.256	Positive
475	20.533	-24.970	25.808	Positive
476	3.560	-4.816	4.764	Intermediate
477	6.891	-13.102	9.926	Intermediate
478	5.932	-11.120	8.527	Intermediate
479	11.830	-18.492	16.794	Intermediate
480	20.355	-24.867	25.719	Positive
481	9.331	-13.302	12.371	Positive
482	4.496	-8.580	6.479	Intermediate
483	20.602	-25.764	26.359	Positive
484	8.414	-13.657	11.545	Positive
485	24.940	-28.942	30.150	Positive
486	13.876	-20.349	19.416	Intermediate
487	7.199	-12.076	9.996	Positive
488	4.812	-9.177	6.935	Intermediate
489	9.447	-13.136	12.349	Positive
490	14.224	-21.122	18.880	Positive
491	39.445	-41.536	42.719	Positive
492	12.245	-18.708	17.262	Intermediate
493	5.790	-10.790	8.315	Intermediate
494	9.122	-12.985	12.104	Positive
495	5.365	-10.185	7.725	Intermediate
496	10.046	-15.871	14.361	Intermediate
497	4.350	-6.037	5.913	Intermediate
498	5.400	-7.905	7.427	Intermediate
499	19.436	-24.515	24.907	Positive
500	4.287	-8.181	6.178	Intermediate
501	16.371	-22.085	22.017	Positive
502	9.161	-14.341	12.755	Intermediate
503	3.744	-5.231	5.030	Intermediate
504	5.145	-9.818	7.414	Intermediate
505	5.706	-10.660	8.212	Intermediate
506	14.061	-21.954	19.475	Intermediate
507	12.054	-18.524	17.094	Intermediate
508	8.109	-11.553	10.726	Positive
509	3.850	-5.273	5.186	Intermediate
510	10.336	-15.726	14.649	Intermediate

511	18.437	-24.008	24.350	Positive
512	7.622	-10.749	10.065	Positive
513	14.617	-19.553	19.667	Positive
514	9.420	-14.802	12.672	Positive
515	3.683	-4.982	4.946	Intermediate
516	11.948	-16.356	15.458	Positive
517	7.708	-11.045	10.288	Positive
518	3.401	-6.489	4.901	Intermediate
519	6.992	-13.202	10.060	Intermediate
520	3.827	-7.301	5.516	Intermediate
521	21.865	-26.661	27.400	Positive
522	19.575	-26.576	25.068	Positive
523	13.790	-21.083	18.561	Positive
524	20.143	-24.976	25.390	Positive
525	8.272	-12.982	11.511	Intermediate
526	5.081	-9.641	7.318	Intermediate
527	9.911	-15.628	14.150	Intermediate
528	3.200	-6.107	4.612	Intermediate
529	8.282	-13.164	11.549	Intermediate
530	4.847	-9.181	6.980	Intermediate
531	11.501	-17.221	16.222	Intermediate
532	10.849	-17.428	14.878	Positive
533	14.758	-19.898	19.959	Positive
534	8.592	-13.996	12.328	Intermediate
535	20.841	-25.544	26.119	Positive
536	7.796	-13.484	11.001	Positive
537	26.835	-30.401	31.553	Positive
538	8.676	-13.660	12.027	Intermediate
539	11.188	-17.882	15.989	Intermediate
540	4.222	-8.022	6.084	Intermediate
541	21.262	-26.674	27.316	Positive
542	13.396	-22.074	18.840	Intermediate
543	3.646	-5.056	4.887	Intermediate
544	21.557	-26.639	27.434	Positive
545	9.382	-14.613	13.043	Intermediate
546	11.783	-19.441	16.558	Intermediate
547	24.855	-29.149	30.302	Positive
548	23.231	-27.635	28.511	Positive
549	8.142	-14.792	11.644	Intermediate
550	8.767	-12.392	11.685	Positive
551	22.404	-27.016	27.814	Positive
552	21.192	-26.463	27.106	Positive
553	20.299	-24.863	25.744	Positive

554	18.543	-24.112	24.610	Positive
555	22.561	-27.062	27.816	Positive
556	10.085	-16.385	13.883	Positive
557	22.994	-29.417	28.519	Positive
558	20.471	-25.918	26.585	Positive
559	26.912	-30.656	31.723	Positive
560	3.382	-6.453	4.874	Intermediate
561	24.378	-28.911	30.200	Positive
562	6.872	-13.054	9.897	Intermediate
563	39.646	-41.683	42.640	Positive
564	26.027	-30.674	30.753	Positive
565	4.501	-8.562	6.486	Intermediate
566	8.582	-13.660	11.949	Intermediate
567	6.828	-12.877	9.822	Intermediate
568	8.851	-14.035	12.321	Intermediate
569	22.335	-27.298	28.282	Positive
570	11.956	-20.111	16.866	Intermediate
571	24.243	-30.188	29.068	Positive
572	22.507	-27.051	27.642	Positive
573	29.740	-33.131	33.285	Positive
574	15.570	-22.565	20.452	Positive
575	3.742	-5.144	5.029	Intermediate
576	8.092	-14.726	11.569	Intermediate
577	6.892	-13.069	9.923	Intermediate
578	4.052	-5.758	5.471	Intermediate
579	16.794	-24.265	21.992	Positive
580	17.111	-23.240	22.756	Positive
581	25.792	-30.212	31.551	Positive
582	20.868	-27.766	26.130	Positive
583	5.536	-10.508	7.972	Intermediate
584	20.135	-25.191	25.787	Positive
585	12.070	-18.417	16.182	Positive
586	3.736	-5.127	5.020	Intermediate
587	5.466	-10.430	7.877	Intermediate
588	12.759	-19.476	17.032	Positive
589	16.919	-21.830	22.328	Positive
590	4.790	-6.718	6.550	Intermediate
591	8.433	-12.027	11.199	Positive
592	7.063	-13.363	10.165	Intermediate
593	4.247	-8.102	6.121	Intermediate
594	3.478	-4.734	4.630	Intermediate
595	3.344	-4.607	4.450	Intermediate
596	4.693	-8.941	6.762	Intermediate

597	6.021	-11.387	8.664	Intermediate
598	21.793	-26.030	27.068	Positive
599	7.312	-10.352	9.547	Positive
600	28.371	-31.584	32.852	Positive
601	9.330	-14.756	13.002	Intermediate
602	9.318	-14.968	12.733	Positive
603	5.698	-10.870	8.211	Intermediate
604	19.105	-24.049	24.519	Positive
605	4.134	-7.888	5.958	Intermediate
606	5.051	-9.640	7.280	Intermediate
607	5.582	-10.615	8.041	Intermediate
608	5.501	-10.499	7.928	Intermediate
609	8.915	-14.162	12.403	Intermediate
610	8.417	-11.663	11.005	Positive
611	20.765	-26.047	26.510	Positive
612	20.620	-25.877	26.659	Positive
613	6.137	-11.693	8.842	Intermediate
614	34.763	-37.849	38.566	Positive
615	3.759	-7.163	5.418	Intermediate
616	5.347	-10.081	7.695	Intermediate
617	25.289	-29.034	30.141	Positive
618	4.350	-8.282	6.269	Intermediate
619	3.794	-5.279	5.101	Intermediate
620	33.758	-37.044	37.753	Positive
621	3.732	-5.117	5.011	Intermediate
622	9.190	-13.059	12.224	Positive
623	5.881	-11.098	8.461	Intermediate
624	16.303	-21.320	21.128	Positive
625	16.880	-22.584	22.302	Positive
626	5.987	-10.297	8.386	Positive
627	10.369	-14.192	13.463	Positive
628	21.456	-26.604	27.269	Positive
629	5.740	-10.939	8.271	Intermediate
630	7.492	-12.843	10.532	Positive
631	18.736	-23.713	24.322	Positive
632	13.741	-20.418	18.228	Positive
633	24.992	-30.298	30.617	Positive
634	19.378	-26.346	24.508	Positive
635	3.538	-4.787	4.732	Intermediate
636	5.734	-10.934	8.262	Intermediate
637	8.281	-15.099	11.850	Intermediate
638	3.579	-4.836	4.795	Intermediate
639	4.984	-9.512	7.183	Intermediate

640	7.966	-13.033	10.958	Positive
641	11.311	-17.918	16.154	Intermediate
642	11.033	-15.967	15.245	Positive
643	5.650	-10.775	8.141	Intermediate
644	17.941	-25.567	23.801	Positive
645	29.421	-33.670	34.895	Positive
646	8.421	-13.482	11.713	Intermediate
647	7.371	-12.761	10.378	Positive
648	18.377	-22.819	24.014	Positive
649	3.278	-6.254	4.724	Intermediate
650	4.842	-6.859	6.620	Intermediate
651	18.276	-22.939	23.698	Positive
652	17.179	-22.697	22.828	Positive
653	10.058	-15.595	14.330	Intermediate
654	4.011	-5.682	5.411	Intermediate
655	20.691	-25.914	26.570	Positive
656	35.884	-38.765	40.031	Positive
657	10.554	-16.544	14.210	Positive
658	11.036	-17.028	14.836	Positive
659	3.468	-4.920	4.628	Intermediate
660	26.910	-30.489	31.868	Positive
661	19.012	-24.678	24.912	Positive
662	19.612	-24.518	25.366	Positive
663	21.179	-26.026	26.782	Positive
664	4.310	-8.221	6.211	Intermediate
665	21.169	-25.780	26.505	Positive
666	8.417	-11.694	11.173	Positive
667	7.729	-12.204	10.757	Intermediate
668	15.808	-23.257	21.079	Positive
669	7.097	-11.230	9.854	Intermediate
670	5.116	-9.764	7.373	Intermediate
671	4.659	-8.745	6.702	Intermediate
672	21.416	-28.222	26.972	Positive
673	10.473	-17.014	14.306	Positive
674	8.520	-14.243	11.834	Positive
675	22.015	-26.553	27.173	Positive
676	23.257	-27.465	28.316	Positive
677	7.889	-11.262	10.336	Positive
678	3.386	-6.460	4.879	Intermediate
679	18.314	-25.241	23.323	Positive
680	20.363	-25.825	26.354	Positive
681	11.934	-16.843	16.351	Positive
682	7.152	-13.296	10.266	Intermediate

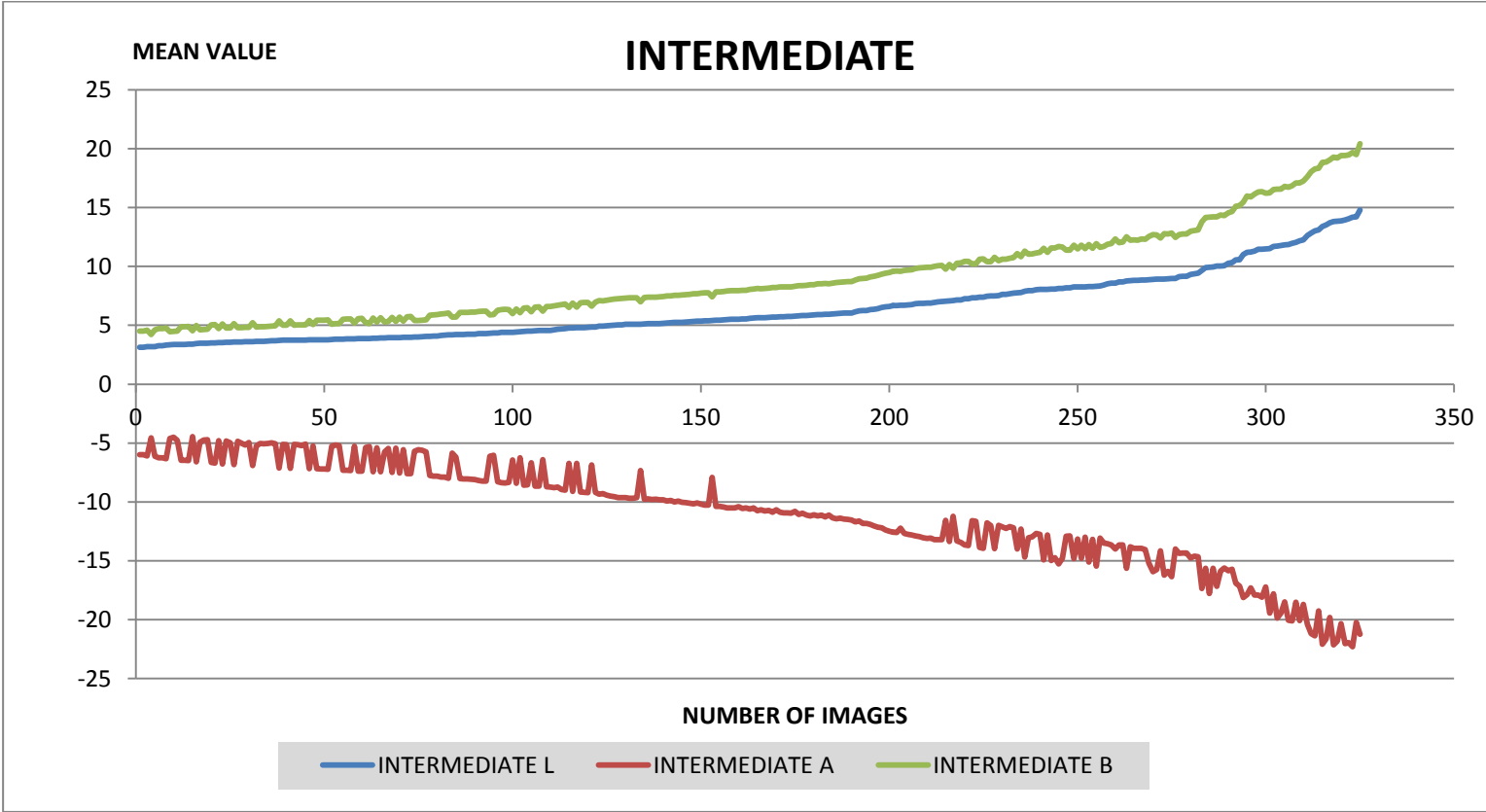
683	8.311	-15.443	11.927	Intermediate
684	9.267	-12.942	12.111	Positive
685	18.526	-24.260	24.641	Positive
686	19.831	-26.660	25.079	Positive
687	5.729	-10.878	8.251	Intermediate
688	36.650	-39.221	39.968	Positive
689	5.917	-11.203	8.517	Intermediate
690	15.250	-20.239	20.467	Positive
691	11.452	-17.895	16.326	Intermediate
692	10.670	-16.874	14.402	Positive
693	12.262	-18.446	16.196	Positive
694	9.123	-12.880	11.756	Positive
695	26.258	-29.900	30.957	Positive
696	38.400	-40.661	41.745	Positive
697	17.029	-21.949	22.016	Positive
698	3.944	-5.440	5.322	Intermediate
699	17.451	-22.943	22.802	Positive
700	13.855	-21.830	19.199	Intermediate
701	8.983	-13.993	12.460	Intermediate
702	22.676	-27.099	28.166	Positive
703	17.308	-24.707	22.658	Positive
704	14.211	-20.242	19.512	Intermediate
705	17.147	-22.966	22.947	Positive
706	17.195	-24.596	22.511	Positive
707	4.204	-5.842	5.698	Intermediate
708	13.243	-20.619	17.910	Positive
709	8.688	-13.660	12.083	Intermediate
710	24.132	-28.227	29.467	Positive
711	7.641	-12.253	10.622	Intermediate
712	5.042	-9.622	7.266	Intermediate
713	21.037	-25.521	26.590	Positive
714	6.742	-12.759	9.704	Intermediate
715	8.530	-13.536	11.891	Intermediate
716	11.393	-17.358	15.136	Positive
717	10.035	-17.177	14.215	Intermediate
718	3.606	-4.941	4.831	Intermediate
719	31.749	-34.533	35.904	Positive
720	21.864	-26.898	27.721	Positive
721	31.968	-34.600	36.314	Positive

APPENDIX C

POSITIVE CLASS MEAN VALUE GRAPH



INTERMEDIATE CLASS MEAN VALUE GRAPH



APPENDIX E

RESULTS OF FUZZY LOGIC FLUORESCENCE INTENSITY CLASSIFICATION FOR INTERMEDIATE CLASS

No	A	L	INTENSITY	AGREEMENT	RESULTS	CLASS
1	-9.703	6.678	INTERMEDIATE	Y	0.330	1
2	-6.740	3.533	INTERMEDIATE	Y	0.330	1
3	-8.865	4.741	INTERMEDIATE	Y	0.310	1
4	-9.850	6.873	INTERMEDIATE	Y	0.320	1
5	-9.143	6.378	INTERMEDIATE	Y	0.320	1
6	-21.998	15.400	INTERMEDIATE	N	0.570	2
7	-8.352	4.464	INTERMEDIATE	Y	0.320	1
8	-9.875	6.824	INTERMEDIATE	Y	0.330	1
9	-10.543	5.912	INTERMEDIATE	Y	0.320	1
10	-6.640	3.525	INTERMEDIATE	Y	0.330	1
11	-11.228	7.521	INTERMEDIATE	Y	0.330	1
12	-8.280	4.396	INTERMEDIATE	Y	0.320	1
13	-6.021	3.155	INTERMEDIATE	Y	0.340	1
14	-10.625	5.571	INTERMEDIATE	Y	0.320	1
15	-19.196	12.586	INTERMEDIATE	N	0.550	2
16	-18.059	11.541	INTERMEDIATE	N	0.560	2
17	-9.870	5.173	INTERMEDIATE	Y	0.310	1
18	-10.177	6.800	INTERMEDIATE	Y	0.330	1
19	-8.244	4.320	INTERMEDIATE	Y	0.320	1
20	-20.904	14.183	INTERMEDIATE	N	0.560	2
21	-9.440	6.580	INTERMEDIATE	Y	0.320	1
22	-7.806	4.181	INTERMEDIATE	Y	0.320	1
23	-10.874	6.113	INTERMEDIATE	Y	0.320	1
24	-8.374	4.522	INTERMEDIATE	Y	0.320	1
25	-15.595	9.406	INTERMEDIATE	Y	0.350	1
26	-10.454	7.100	INTERMEDIATE	Y	0.330	1
27	-8.592	4.625	INTERMEDIATE	Y	0.310	1
28	-10.411	5.476	INTERMEDIATE	Y	0.320	1
29	-10.220	6.926	INTERMEDIATE	Y	0.330	1
30	-11.184	7.582	INTERMEDIATE	Y	0.330	1
31	-19.136	12.664	INTERMEDIATE	N	0.550	2
32	-8.514	4.600	INTERMEDIATE	Y	0.310	1
33	-15.793	8.419	INTERMEDIATE	Y	0.340	1
34	-13.308	7.186	INTERMEDIATE	Y	0.330	1
35	-11.100	7.422	INTERMEDIATE	Y	0.330	1
36	-6.651	3.490	INTERMEDIATE	Y	0.330	1
37	-10.774	7.312	INTERMEDIATE	Y	0.330	1
38	-6.728	3.550	INTERMEDIATE	Y	0.330	1
39	-8.164	4.351	INTERMEDIATE	Y	0.320	1
40	-9.326	4.895	INTERMEDIATE	Y	0.310	1
41	-10.990	5.791	INTERMEDIATE	Y	0.320	1
42	-8.527	4.675	INTERMEDIATE	Y	0.310	1
43	-7.867	4.122	INTERMEDIATE	Y	0.320	1
44	-8.835	4.630	INTERMEDIATE	Y	0.310	1
45	-10.447	6.731	INTERMEDIATE	Y	0.330	1
46	-8.391	4.510	INTERMEDIATE	Y	0.320	1
47	-7.090	3.717	INTERMEDIATE	Y	0.330	1

48	-11.460	6.006	INTERMEDIATE	Y	0.320	1
49	-14.302	8.007	INTERMEDIATE	Y	0.340	1
50	-9.840	6.810	INTERMEDIATE	Y	0.330	1
51	-9.014	4.831	INTERMEDIATE	Y	0.310	1
52	-9.167	4.813	INTERMEDIATE	Y	0.310	1
53	-18.945	12.308	INTERMEDIATE	N	0.550	2
54	-11.253	7.627	INTERMEDIATE	Y	0.330	1
55	-8.258	4.382	INTERMEDIATE	Y	0.320	1
56	-21.983	15.412	INTERMEDIATE	N	0.570	2
57	-12.218	6.543	INTERMEDIATE	Y	0.320	1
58	-14.822	7.934	INTERMEDIATE	Y	0.340	1
59	-10.840	7.364	INTERMEDIATE	Y	0.330	1
60	-13.241	8.014	INTERMEDIATE	Y	0.340	1
61	-21.893	15.362	INTERMEDIATE	N	0.570	2
62	-10.083	5.331	INTERMEDIATE	Y	0.310	1
63	-12.470	6.543	INTERMEDIATE	Y	0.320	1
64	-10.076	6.936	INTERMEDIATE	Y	0.330	1
65	-13.072	6.992	INTERMEDIATE	Y	0.330	1
66	-12.635	6.651	INTERMEDIATE	Y	0.330	1
67	-9.471	5.168	INTERMEDIATE	Y	0.310	1
68	-16.616	10.129	INTERMEDIATE	Y	0.350	1
69	-10.502	7.091	INTERMEDIATE	Y	0.330	1
70	-7.885	4.135	INTERMEDIATE	Y	0.320	1
71	-8.232	4.386	INTERMEDIATE	Y	0.320	1
72	-19.034	12.440	INTERMEDIATE	N	0.550	2
73	-9.256	5.100	INTERMEDIATE	Y	0.310	1
74	-18.661	12.003	INTERMEDIATE	N	0.550	2
75	-13.367	7.498	INTERMEDIATE	Y	0.330	1
76	-11.458	6.016	INTERMEDIATE	Y	0.320	1
77	-11.200	7.605	INTERMEDIATE	Y	0.330	1
78	-13.894	7.726	INTERMEDIATE	Y	0.330	1
79	-10.644	6.986	INTERMEDIATE	Y	0.330	1
80	-8.285	4.345	INTERMEDIATE	Y	0.320	1
81	-6.489	3.449	INTERMEDIATE	Y	0.330	1
82	-9.683	6.684	INTERMEDIATE	Y	0.330	1
83	-21.192	14.487	INTERMEDIATE	N	0.560	2
84	-11.126	7.580	INTERMEDIATE	Y	0.330	1
85	-7.710	4.042	INTERMEDIATE	Y	0.320	1
86	-10.737	5.627	INTERMEDIATE	Y	0.320	1
87	-7.511	3.938	INTERMEDIATE	Y	0.320	1
88	-10.824	7.022	INTERMEDIATE	Y	0.330	1
89	-16.768	10.666	INTERMEDIATE	Y	0.360	1
90	-11.885	6.233	INTERMEDIATE	Y	0.320	1
91	-15.452	9.021	INTERMEDIATE	Y	0.350	1
92	-12.573	6.605	INTERMEDIATE	Y	0.320	1
93	-18.717	12.311	INTERMEDIATE	N	0.550	2
94	-18.967	12.329	INTERMEDIATE	N	0.550	2
95	-20.512	13.862	INTERMEDIATE	N	0.560	2
96	-6.636	3.506	INTERMEDIATE	Y	0.330	1
97	-8.242	4.322	INTERMEDIATE	Y	0.320	1
98	-9.205	4.826	INTERMEDIATE	Y	0.310	1
99	-7.509	4.060	INTERMEDIATE	Y	0.320	1
100	-19.197	12.470	INTERMEDIATE	N	0.550	2

APPENDIX F

RESULTS OF FUZZY LOGIC FLUORESCENCE INTENSITY CLASSIFICATION FOR POSITIVE CLASS

No	A	L	INTENSITY	AGREEMENT	RESULTS	CLASS
1	-20.317	13.575	POSITIVE	Y	0.550	2
2	-18.090	11.123	POSITIVE	Y	0.560	2
3	-16.513	11.129	POSITIVE	Y	0.560	2
4	-38.728	34.875	POSITIVE	Y	1.000	4
5	-20.711	15.327	POSITIVE	Y	0.560	2
6	-30.147	23.645	POSITIVE	Y	0.700	3
7	-22.513	15.515	POSITIVE	Y	0.580	2
8	-27.434	21.559	POSITIVE	Y	0.740	3
9	-23.728	19.054	POSITIVE	Y	0.590	2
10	-7.033	3.750	POSITIVE	N	0.330	1
11	-4.503	2.365	POSITIVE	N	0.350	1
12	-25.885	19.775	POSITIVE	Y	0.760	3
13	-28.511	22.843	POSITIVE	Y	0.720	3
14	-19.590	14.478	POSITIVE	Y	0.550	2
15	-33.138	27.670	POSITIVE	Y	0.730	3
16	-26.886	22.891	POSITIVE	Y	0.750	3
17	-17.302	10.598	POSITIVE	N	0.360	1
18	-7.224	4.013	POSITIVE	N	0.330	1
19	-22.718	16.180	POSITIVE	Y	0.580	2
20	-19.509	12.726	POSITIVE	Y	0.550	2
21	-26.623	20.405	POSITIVE	Y	0.750	3
22	-23.492	16.902	POSITIVE	Y	0.590	2
23	-26.064	20.173	POSITIVE	Y	0.760	3
24	-21.027	16.112	POSITIVE	Y	0.560	2
25	-4.906	2.590	POSITIVE	Y	0.350	1
26	-25.308	19.321	POSITIVE	Y	0.770	3
27	-25.565	19.315	POSITIVE	Y	0.770	3
28	-30.055	26.507	POSITIVE	Y	0.700	3
29	-23.044	16.650	POSITIVE	Y	0.590	2
30	-35.378	32.791	POSITIVE	Y	0.760	3
31	-21.998	15.035	POSITIVE	Y	0.570	2
32	-24.260	19.593	POSITIVE	Y	0.790	3
33	-23.404	19.017	POSITIVE	Y	0.590	2
34	-21.068	14.267	POSITIVE	Y	0.560	2
35	-21.604	14.705	POSITIVE	Y	0.570	2
36	-23.031	16.383	POSITIVE	Y	0.590	2
37	-19.671	12.965	POSITIVE	Y	0.550	2
38	-36.419	31.844	POSITIVE	Y	0.770	3
39	-18.830	13.576	POSITIVE	Y	0.540	2
40	-25.140	20.771	POSITIVE	Y	0.770	3
41	-21.238	16.062	POSITIVE	Y	0.570	2
42	-30.030	24.952	POSITIVE	Y	0.700	3
43	-25.994	19.905	POSITIVE	Y	0.760	3
44	-22.435	15.183	POSITIVE	Y	0.580	2
45	-27.154	21.164	POSITIVE	Y	0.740	3
46	-25.107	19.572	POSITIVE	Y	0.770	3
47	-17.297	10.677	POSITIVE	N	0.360	1
48	-22.443	14.324	POSITIVE	Y	0.580	2
49	-17.447	10.962	POSITIVE	Y	0.560	2

50	-23.809	19.107	POSITIVE	Y	0.590	2
51	-23.154	16.528	POSITIVE	Y	0.590	2
52	-23.602	17.072	POSITIVE	Y	0.590	2
53	-19.968	13.106	POSITIVE	Y	0.550	2
54	-19.789	12.695	POSITIVE	Y	0.550	2
55	-10.050	6.020	POSITIVE	N	0.320	1
56	-20.218	13.657	POSITIVE	Y	0.550	2
57	-22.124	15.185	POSITIVE	Y	0.580	2
58	-28.177	22.309	POSITIVE	Y	0.720	3
59	-18.294	12.975	POSITIVE	Y	0.550	2
60	-19.477	12.633	POSITIVE	Y	0.550	2
61	-20.952	16.130	POSITIVE	Y	0.560	2
62	-42.995	40.435	POSITIVE	Y	1.000	4
63	-19.947	13.691	POSITIVE	Y	0.550	2
64	-31.815	28.477	POSITIVE	Y	0.720	3
65	-25.813	20.837	POSITIVE	Y	0.760	3
66	-41.463	38.509	POSITIVE	Y	1.000	4
67	-24.493	18.039	POSITIVE	Y	0.780	3
68	-23.650	16.699	POSITIVE	Y	0.590	2
69	-23.167	15.557	POSITIVE	Y	0.590	2
70	-6.889	3.679	POSITIVE	N	0.330	1
71	-16.608	10.287	POSITIVE	Y	0.360	1
72	-26.512	21.355	POSITIVE	Y	0.750	3
73	-24.591	18.269	POSITIVE	Y	0.780	3
74	-23.887	17.496	POSITIVE	Y	0.790	3
75	-25.357	21.034	POSITIVE	Y	0.770	3
76	-8.300	4.814	POSITIVE	N	0.320	1
77	-27.731	21.766	POSITIVE	Y	0.730	3
78	-34.464	29.347	POSITIVE	Y	0.750	3
79	-19.405	12.604	POSITIVE	Y	0.550	2
80	-17.248	12.059	POSITIVE	Y	0.550	2
81	-31.377	28.062	POSITIVE	Y	0.710	3
82	-17.776	11.163	POSITIVE	Y	0.560	2
83	-39.673	36.164	POSITIVE	Y	1.000	4
84	-23.171	16.594	POSITIVE	Y	0.590	2
85	-25.281	19.199	POSITIVE	Y	0.770	3
86	-23.993	17.236	POSITIVE	Y	0.790	3
87	-18.312	11.550	POSITIVE	Y	0.560	2
88	-6.207	3.315	POSITIVE	N	0.340	1
89	-19.770	14.219	POSITIVE	Y	0.550	2
90	-16.551	10.009	POSITIVE	N	0.350	1
91	-26.836	19.649	POSITIVE	Y	0.750	3
92	-20.216	15.150	POSITIVE	Y	0.550	2
93	-23.783	19.369	POSITIVE	Y	0.590	2
94	-22.871	17.935	POSITIVE	Y	0.580	2
95	-18.980	12.070	POSITIVE	Y	0.550	2
96	-19.185	12.588	POSITIVE	Y	0.550	2
97	-19.491	12.796	POSITIVE	Y	0.550	2
98	-17.868	11.123	POSITIVE	Y	0.560	2
99	-18.098	12.883	POSITIVE	Y	0.550	2
100	-15.998	9.325	POSITIVE	N	0.350	1

APPENDIX G

MATLAB CODING FOR FEATURE EXTRACTION

```
%chose the number of images to give input
N = ___ ;

%change the desired input image name here only
prefix_image='';

%change the desired input image format here only
fileformat='.png';

for j=1:N

    image= imread(strcat(prefix_image,num2str(j),fileformat));
    B=im2double(image);

    cform = makecform('srgb2lab');
    C = applycform(B,cform);

    LChannel = C(:, :, 1);
    aChannel = C(:, :, 2);
    bChannel = C(:, :, 3);

    subplot(3, 4, 2);
    imshow(LChannel, []);

    subplot(3, 4, 3);
    imshow(aChannel, []);

    subplot(3, 4, 4);
    imshow(bChannel, []);

    meanl=mean(LChannel(:));
    meana=mean(aChannel(:));
    meanb=mean(bChannel(:));

    LAB_Value = {'N','meanl', 'meana', 'meanb';j,meanl,meana,meanb};
    fprintf('%d\t %0.3f\t %0.3f\t %0.3f\n',j,meanl,meana,meanb);

end
```