

MICROSCOPIC IMAGE THRESHOLDING

Report submitted for the fulfillment of the requirements for the degree of Bachelor of Technology in **Information Technology**

Submitted by

Abhrak Chatterjee (11700214003)

Abhirup Biswas (11700214001)

Poushali Choudhury (11700214048)

Under the Guidance of Mr. Hiranmoy Roy

[Assistant Professor, Department of IT]



RCC Institute of Information Technology
Canal South Road, Beliaghata, Kolkata – 700 015
[Affiliated to West Bengal University of Technology]

ACKNOWLEDGEMENT

We would like to express our sincere gratitude to Mr. Hiranmoy Roy of the department of Information Technology, whose role as project guide was invaluable for the project. We are extremely thankful for the keen interest he took in advising us, for the books and reference materials provided for the moral support extended to us.

Last but not the least we convey our gratitude to all the teachers for providing us the technical skill that will always remain as our asset and to all non-teaching staff for the gracious hospitality they offered us.

Place: RCCIIT, Kolkata

Date: 18.05.2018

.....
Abhrak Chatterjee (11700214003)

.....
Abhirup Biswas (11700214001)

.....
Poushali Choudhury(11700214048)

Department of Information Technology
RCCIIT, Beliaghata,
Kolkata – 700 015,
West Bengal, India

APPROVAL

This is to certify that the project report entitled “Microscopic Image Thresholding” prepared under my supervision by Abhrak Chatterjee (IT/2014/001), Abhirup Biswas (IT/2014/002), Poushali Choudhury (IT/2014/029), be accepted in fulfillment for the degree of Bachelor of Technology in Information Technology.

It is to be understood that by this approval, the undersigned does not necessarily endorse or approve any statement made, opinion expressed or conclusion drawn thereof, but approves the report only for the purpose for which it has been submitted.

.....
Dr. Abhijit Das
(Associate Professor & Head Dept. of IT)

.....
Mr. Hiranmoy Roy
(Assistant Professor Dept. of IT)

INDEX

<u>Contents</u>	<u>Page Numbers</u>
<i>1. Abstract</i>	<i>5</i>
<i>2. Introduction</i>	<i>6</i>
<i>3. Problem Definition</i>	<i>7</i>
<i>4. Literature Survey</i>	<i>8</i>
<i>5. SRS(System Requirement Specification)</i>	<i>11</i>
<i>6. Planning</i>	<i>12</i>
<i>7. Design</i>	<i>13</i>
<i>8. Results and Discussion</i>	<i>18</i>
<i>9. Conclusion and Future Scope</i>	<i>24</i>
<i>10. References / Bibliography</i>	<i>25</i>
<i>11. Paper Acceptance</i>	<i>26</i>
<i>12. List of Tables</i>	<i>28</i>
<i>13. List of Figures</i>	<i>29</i>

ABSTRACT

Ubiquitous use of microscope in the field of medical diagnosis influences the development of automated systems. The inbuilt noise, illumination and contrast variations make microscopic image processing an emerging field of computer vision applications. This project presents a novel and fast thresholding technique for microscopic data. To represent the inherent image vagueness, we use a fast processing fuzzy membership value generation technique using restricted equivalence function (REF). Then, a fuzzy entropy value is used to measure the total fuzziness present in the object and the background of the image. Finally, to search the optimal threshold value we use the well popular Bat algorithm. We have also implemented a multilevel thresholding technique for processing some complex fluorescence microscopy images. Experimental results on microscopic data and also on normal images show the superiority of the proposed thresholding technique. Proposed method is superior than other state-of-the-art methods not only in processing time but also in quantitative results.

INTRODUCTION

An image is a visual representation of something. In information technology, image processing is a processing of images using mathematical operations by using any form of signal processing for which the input is an image, a series of images, or a video, such as a photograph or video frame; the output of image processing may be either an image or a set of characteristics or parameters related to the image.

In the field of science and technology, image segmentation is the branch of image processing dealing with the partitioning of an image into various parts (technically, segments) with each part having some common properties. Various methods exist for image segmentation. The most common technique is thresholding method. Thresholding is a general and important topic in the area of image processing segmentation. This method is used to fetch an object from its background. Similarly, multilevel thresholding is the process of extracting multiple objects from an image. Different types of image thresholding technique have been proposed. Still the thresholding field of image processing is one of the most important and challenging one.

Microscopes use the simple visible light refracting lenses, Electrons, x-rays and infrared rays. They are to detect the smaller and smaller structures. Scanning electron microscopes are able to resolve the viruses which are far smaller than any cell. Microscopes are used to view the cellular structures of organs, germs and bacteria. They play a very important role in laboratory for the tissues and organisms which are too small to be seen clearly with the naked eye. To detect any kind of disease, doctors use microscopic results. Microscopic data has an important role in the field of medical science.

The aim of our project is to present a fast thresholding technique for microscopic images that will help to detect diseases in a faster way. We will process an image and calculate fuzzy membership value using restricted equivalence function (REF). Then we will calculate fuzzy entropy value to measure total fuzziness in the object and the background of the image. At last, we will apply bat algorithm (BA) to minimize the searching time of threshold value.

PROBLEM DEFINITION

We have worked on image processing domain and used MATLAB (R2017a) platform to build our program in a 4 GB RAM machine. Our main objective is to fetch object(s) from a given image using multi level thresholding and minimize the searching time of threshold values in order to find an optimized result. In this project we are mainly focused on microscopic images that will help to detect any abnormality in the image in very less time. But our algorithm works on the other images as well.

In the medical field, imaging has become an integral step for detecting and diagnosing diseases. This allows the doctors to get a clear view of the conditions inside the body without cutting. The oldest technique for such imaging is X-ray which has been in use for a very long time. Since diseases are caused by malfunction of organs (or cells), a malfunctioning cell, tissue or organ will look different from a perfect one and this difference helps doctors to identify and detect for diagnosing. Therefore, images captured by the microscope and modern fluorescence microscope are ubiquitous in the medical diagnosis. But these microscopic images need various pre and post processing for the image to be easily understandable by doctors. Here comes the integral role of image segmentation. By this process only the region of interest (ROI) of the image is differentiated from the other non-important parts of the image. For instance, in the field of pathology, a blood sample is to be tested for finding out the constituents of that random sample. So after capturing a magnified image of the sample, the various constituents need to be differentiated in the image. So Image Segmentation will play the major role here to extract out each of these similar types of objects from the image for further study. Various methods of segmentation techniques and algorithms have been devised and formulated, but this remains a great problem because of the huge diversity in the cases of images, noises and the presence of various other objects in the adjoining areas of the ROI. Most of the well known thresholding techniques are used in the medical diagnosis image processing field, but very few works have been done purely dedicated towards the microscopic image thresholding. In this project, we devise the segmentation mechanism using thresholding technique. Since this technique involves the extensive searching of the optimum threshold values for extracting out almost all portions of the object(s), reducing searching time is one of the greatest concerns for this method. So, in order to reduce the searching time, a nature inspired algorithm will be the best one to employ. Here we employ Bat Algorithm. Since the microscopic images are used by doctors for diagnosis, fast processing is very much essential. Again, these images having very low contrast, and noise, which creates lots of ambiguity in object boundary detection. Therefore, a fast fuzzy domain representation with fast searching technique is essential. To fulfill all these requirements, we use restricted equivalence function (REF) based fuzzy domain representation along with Bat algorithm.

LITERATURE SURVEY

The table below lists the research papers along with their authors which have been referred so far till now:-

Table 1: *Literature Survey*

Paper Name	Authors(s)
A Survey of Thresholding Techniques	P. K. Sahoo, S. Soltani, and A. K. C. Wong
A Threshold Selection Method from Gray-Level Histograms	N. Otsu
Image enhancement and thresholding by optimization of fuzzy compactness	S. K. Pal, and A. Rosenfield
Image thresholding by minimizing the measure of fuzziness	L. K. Huang, and M. J. Wang
Image segmentation using fuzzy homogeneity criterion	H. D. Cheng, C. H. Chen, and H. H. Chiu
A Novel Color Image Multilevel thresholding based Segmentation using Nature Inspired Optimization Algorithms	A. K. Bhandari, A. Kumar, S. Chaudhary, and G. K. Singh
Multilevel thresholding algorithm based on particle swarm optimization for image segmentation	C. Wei, and F. Kangling
Image segmentation by three-level thresholding based on maximum fuzzy entropy and genetic algorithm	W. Tao, J. Tian, and J. Liu
A Fuzzy Entropy Based Multi-Level Image Thresholding Using Differential Evolution	S. Sarkar, S. Pal, R. Burman, S. Das, and S. S. Chaudhuri
Image thresholding using LBP and GA-based optimal parameter selection for 2D Tsallis-Havrda-Charvat entropy	R. Tewari, S. Dhar, and H. Roy
A novel method for image thresholding using interval type-2 fuzzy set and Bat algorithm	S. Dhar, and M. K. Kundu
Image thresholding using restricted equivalence functions and maximizing the measures of similarity	H. Bustince, E. Barrenechea, and M. Pagola
Fuzzy Sets	L. A. Zadeh
Bat Algorithm: A Novel Approach for Global Engineering Optimization	X. Yang, and A. H. Gandomi

One of the most popular thresholding techniques is global histogram based thresholding. Otsu's method is the most popular one in histogram based thresholding. At first we used Otsu's method for image thresholding but, the problem with this method is: it gives better results only in the case of high contrast images. But our project is mainly concerned about microscopic images and every image might not have high intensity difference between object and background. So, we switched to Fuzzy algorithms. Some well known fuzzy set theoretic based image thresholding techniques work well in the low contrast and noisy images. They have applied the fuzzy concept in normal gray level image. S. K. Pal, and A. Rosenfield proposed a fuzzy compactness measure for thresholding in "*Image enhancement and thresholding by optimization of fuzzy compactness*". On the other hand, L. K. Huang and M. J. Wang applied fuzzy entropy-based objective function in "*Image thresholding by minimizing the measure of fuzziness*". Crisp logic deals with information that can be either entirely true or entirely false but nothing in between. However, this bi-valued logic fails to contemplate complex structures, like edge pixels, boundary pixels, and threshold values. Hence, a system with multi-valued logic and which allow smooth transitions between 'true' and 'false' is essential. Zadeh introduced fuzzy sets to deal such imprecise information problems. To threshold the object and background from a microscopic image we have implemented fuzzy set theory, rather than conventional histogram based methods, to boost the overall accuracy. After analyzing all these methods, we decided to use a fast processing fuzzy membership value generation technique using restricted equivalence function (REF). Then, a fuzzy entropy value is used to measure the total fuzziness present in the object and the background of the image.

In a two class problem, we need to select one threshold value from 'L' different gray levels to segment the object and background. The problem of threshold selection becomes more difficult for multi-class thresholding. Now the threshold selection problem becomes an optimization problem, where we have to search the threshold value for which the total fuzzy entropy of the image becomes minimum. Again, the searching time increases drastically from linear to polynomial in the case of multi-class thresholding. But in medical diagnosis we need a fast processing thresholding technique. Different nature inspired segmentation methods are also there to minimize the searching. C. Wei, and F. Kangling proposed a multi-level thresholding technique using particle swarm optimization (PSO) in "*Multilevel thresholding algorithm based on particle swarm optimization for image segmentation*". W. Tao, J. Tian, and J. Liu proposed a multi-level segmentation using fuzzy entropy and genetic algorithm(GA) in "*Image segmentation by three-level thresholding based on maximum fuzzy entropy and genetic algorithm*". S. Sarkar, S. Pal, R. Burman, S. Das, and S. S. Chaudhuri proposed a thresholding technique using differential evolution (DE) in "*A Fuzzy Entropy Based Multi-Level Image Thresholding Using Differential Evolution*". R. Tewari, S. Dhar, and H. Roy applied the fuzzy set concept in local binary domain (LBP) domain and improved the result in the case of low contrast images in "*Image thresholding using LBP and GA-based optimal parameter selection for 2D Tsallis-Havrda-Charvat entropy*". S. Dhar, and M. K. Kundu proposed a type-II fuzzy technique with Bat algorithm (BA) in "*A novel method for image thresholding using interval*

type-2 fuzzy set and Bat algorithm". Among them, Bat algorithm converges very fast to the optimal value rather than the other methods. We have showed quantitatively in result section that Bat algorithm is faster than other optimization techniques in terms of convergence to the optimal value. Therefore, we have used Bat algorithm to search the threshold value. X. Yang, and A. H. Gandomi proposed bat algorithm by simulation the behavior of bat when it finds the food. It takes into account the major advantages of particle swarm optimization and simulated annealing.

SRS

(SOFTWARE REQUIREMENT SPECIFICATION)

Table 2: *Software Requirement Specification*

64-Bit MATLAB, Simulink, and Polyspace Product Families				
Operating Systems	Processors	Disk Space	RAM	Graphics
Windows 10 Windows 8.1 Windows 7 Service Pack 1 Windows Server 2016 Windows Server 2012 R2 Windows Server 2012 Windows Server 2008 R2 Service Pack 1	Any Intel or AMD x86-64 processor AVX2 instruction set support is recommended With Polyspace, 4 cores is recommended	2 GB for MATLAB only, 4–6 GB for a typical installation	2 GB With Simulink, 4 GB is required With Polyspace, 4 GB per core is recommended	No specific graphics card is required. Hardware accelerated graphics card supporting OpenGL 3.3 with 1GB GPU memory is recommended.

PLANNING

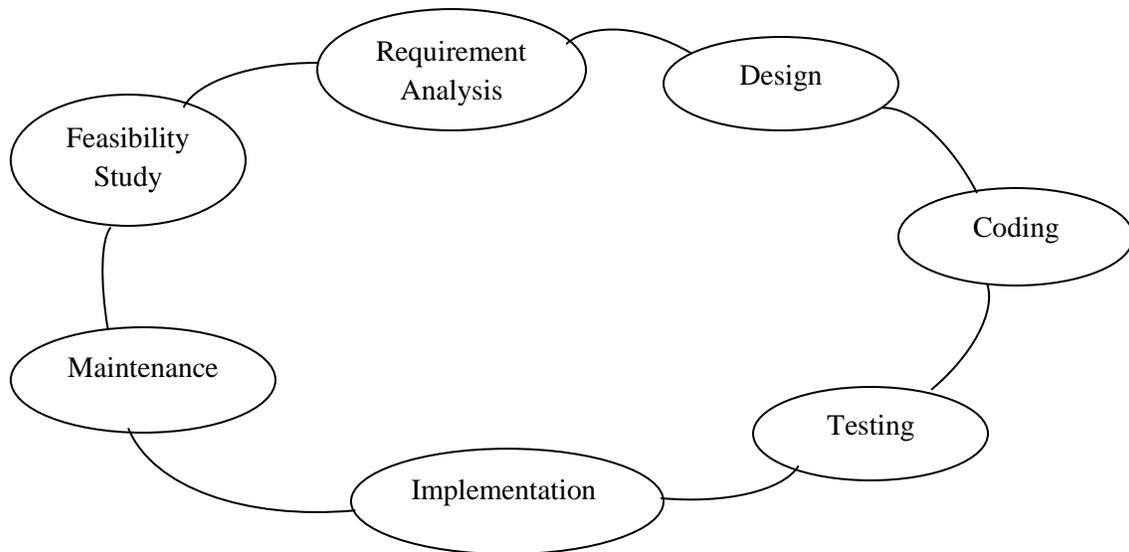


Fig. 1: *Software Development Life Cycle Model*

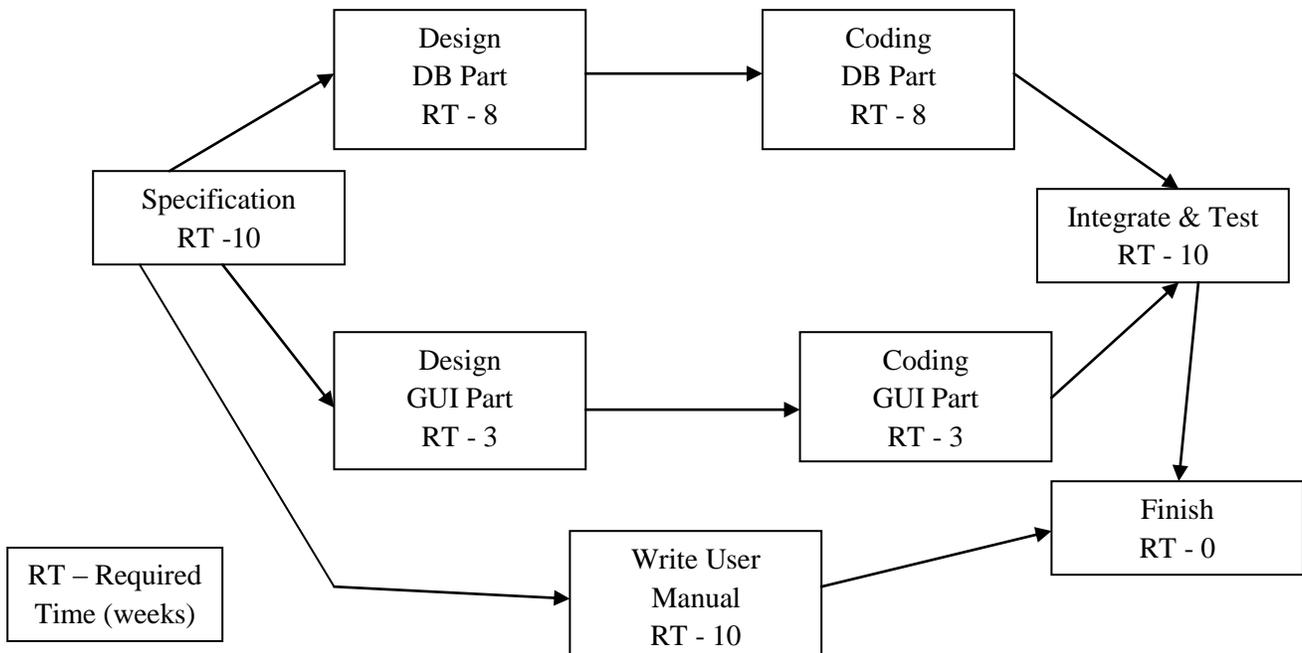


Fig. 2: *Activity Networks*

DESIGN

PROPOSED METHOD:

Our proposed algorithm has four parts and successful completion of each part has led us closer to our main objective of the project.

- A. Restricted equivalence function
- B. Construction of fuzzy set
- C. Fuzziness measure using fuzzy entropy
- D. Optimized threshold value selection using Bat algorithm

A. RESTRICTED EQUIVALENCE FUNCTION

Restricted equivalence function is the derived idea of the equivalence function with some conditions. Due to its fast processing, it has been used as a fuzzy membership function in many thresholding algorithms.

Definition 1: A function $REF : [0, 1]^2 \rightarrow [0, 1]$ is called restricted equivalence function, if it satisfies the following conditions:

- 1) $REF(x, y) = REF(y, x)$ for all $x, y \in [0, 1]$;
- 2) $REF(x, y) = 1$ if and only if $x = y$;
- 3) $REF(x, y) = 0$ if and only if $x = 1$ and $y = 0$ or $x = 0$ and $y = 1$;
- 4) $REF(x, y) = REF(c(x), c(y))$ for all $x, y \in [0, 1]$, c being a strong negation;
- 5) For all $x, y, z \in [0, 1]$, if $x \leq y \leq z$, then $REF(x, y) \geq REF(x, z)$ and $REF(y, z) \geq REF(x, z)$.

Many different restricted equivalence functions exist, such as:

- 1) $REF(x, y) = 1 - |x - y|$;
- 2) $REF(x, y) = 1 - |x - y|^2$;
- 3) $REF(x, y) = 1 - |x - y|^{0.5}$;

For our fuzzy membership generation, we have chosen the 1st function with simple linear form, and we call it REF_1 .

B. CONSTRUCTION OF FUZZY SET

In an image, each and every image pixel is a fuzzy set of 'n' numbers of elements, where 'n' depends on number of segmented regions, e.g. for single level thresholding the value of $n = 2$ i.e. object and background. The membership value of each pixel in the corresponding fuzzy set is expressing the relation between the image pixel with the object and with the background. Therefore, each pixel has 'n' membership values associated with 'n' number of classes: C_1, C_2, \dots, C_n . In two class thresholding, C_1 belongs to object and C_2 belongs to background. The REF_1 require two arguments, one is the pixel intensity value and another is the average intensity value of any one class for which the

fuzziness is measured. The higher value of the restricted equivalence function means higher value of the membership function.

For every pixel 'I', let the intensity value of that pixel is 'P' and the min and max intensity values of the image are 0 and (L - 1). In normal gray image the value of L = 256 i.e. gray value distribution is from '0' to '255'. If there are 'n' number of classes present in the image then for thresholding we need (n-1) threshold values. Let, the threshold values are : t_1, t_2, \dots, t_{n-1} . Then, the membership values of pixel I with intensity P can be represented using the REF as follows:

$$\mu(I) = \begin{cases} REF\left(\frac{P}{L-1}, \frac{C_1(t_1)}{L-1}\right), & P \leq t_1 \\ REF\left(\frac{P}{L-1}, \frac{C_2(t_1 \rightarrow t_2)}{L-1}\right), & P > t_1 \ \& \ P \leq t_2 \\ \vdots \\ REF\left(\frac{P}{L-1}, \frac{C_n(t_{n-2} \rightarrow t_{n-1})}{L-1}\right), & P > t_{n-2} \ \& \ P \leq t_{n-1} \end{cases} \quad (1)$$

Where C_1, C_2, \dots, C_n are the different classes average intensity value. The average intensity values of different classes are measured as follows

$$\begin{aligned} C_1 &= \frac{\sum_0^{t_1} P}{\text{no. of pixels in that class}} \\ C_2 &= \frac{\sum_{t_1+1}^{t_2} P}{\text{no. of pixels in that class}} \\ &\vdots \\ &\vdots \\ C_n &= \frac{\sum_{t_{n-2}+1}^{t_{n-1}} P}{\text{no. of pixels in that class}} \end{aligned} \quad (2)$$

For two class problem, where we need one single threshold (t), one class is object and another is background. The membership generation will be represented as follows:

$$\mu(I) = \begin{cases} REF\left(\frac{P}{L-1}, \frac{C_o(t)}{L-1}\right), & P \leq t \\ REF\left(\frac{P}{L-1}, \frac{C_b(t)}{L-1}\right), & P > t \end{cases}$$

$$C_0 = \frac{\sum_0^t P}{\text{no. of pixels in the object}}$$

$$C_b = \frac{\sum_{t+1}^{L-1} P}{\text{no. of pixels in the background}} \quad (3)$$

C. FUZZINESS MEASURE USING FUZZY ENTROPY

The next step is to measure the degree of fuzziness present in the image pixels, which are already converted to fuzzy domain using REF in the previous subsection. There are different approaches available to measure the fuzziness. One of the most popular fuzziness measure is the measure of entropy, which is established from Shannon's function of entropy measure in information theory. For a fuzzy set 'F' the entropy measure for the set is given using the following equations:

$$E(F) = \frac{1}{m \times \ln 2} \sum_m S(\mu_A(P_i)) \quad (4)$$

$$S(\mu_A(P_i)) = -\mu_A(P_i) \ln[\mu_A(P_i)] - [1 - \mu_A(P_i)] \ln[1 - \mu_A(P_i)] \quad (5)$$

where, P_i represent the fuzzy value of pixel P , which to be in class 'i'. 'i' represents number of different classes present in the image i.e. $i \in 1, 2, \dots, n$. 'm' represents total number of image pixels in the image.

For 'n' number of classes, we have to measure the fuzzy entropy of each class. Then, the sum of all these entropy values will give us the total fuzzy entropy measure of the image. Let the total fuzzy entropy of the image with 'n' classes is E_{Tn} , then it will be represented using the following equation:

$$E_{Tn} = E_1 + E_2 + \dots + E_n \quad (6)$$

where, E_1, E_2, \dots, E_n are the fuzzy entropy values of 'n' different classes. In the case of 2 class thresholding Eq.6 becomes:

$$E_{T2} = E_o + E_b \quad (7)$$

where, E_o is the fuzzy entropy of the object and E_b is the fuzzy entropy of the background, respectively. For the selection of best or optimal threshold value this total entropy value should be minimum.

D. OPTIMIZED THRESHOLD VALUE SELECTION USING BAT ALGORITHM

Three main rules are used for executing the bat algorithm:

1) Echolocation is used by all bats to measure distance. The bats are able to differentiate between food/prey and background barriers. In the echolocation system, high frequency is emitted by a bat and it is followed by listening to returning echoes. The distance and

location of the prey are decided by the differences between the emitted frequencies and the echoed frequencies.

2) Bats move randomly with velocity at a position. It has a fixed frequency and varying wavelength. It also has a loudness to search for prey. They can adaptively alter the wavelength of their emitted pulses and also alter the rate of pulse emission based on the closeness of the target.

3) Though the loudness varies in many ways, here the basic assumption is that the loudness changes from a large (positive) to a minimum constant value.

Main steps of the algorithm are given below:

1) Initialization;

Repeat

2) Generating the new solutions;

3) Local searching for minimizing the effect of local optima;

4) Generating new solution with the random fly;

5) Getting the current best solution until termination criteria are met;

The proposed algorithm is shown in the form of a flow control diagram:

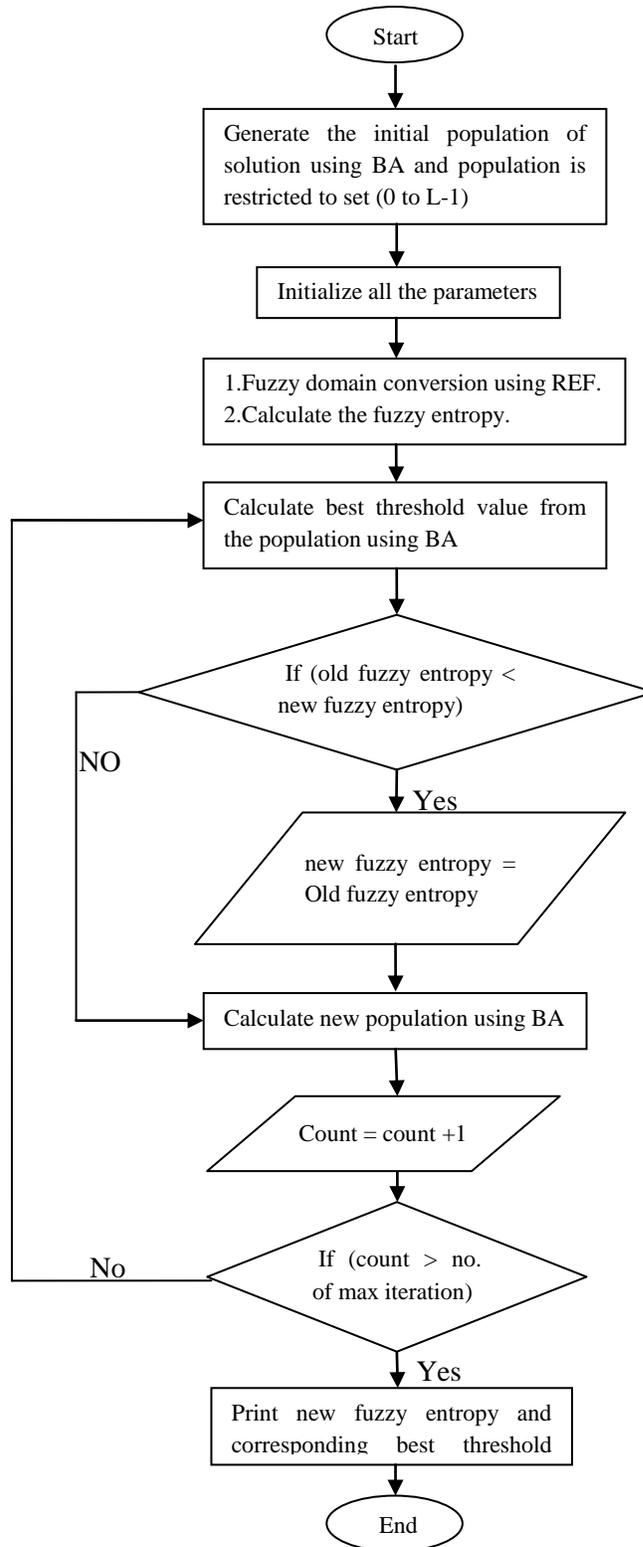


Fig. 3: *Flow control diagram of our proposed method*

RESULTS AND DISCUSSION

Simulation of the proposed algorithm has been carried out over a large number of microscopic images available from the Broad Institute microscopic blood sample database, and also over some natural well known images. We compared the performance of our proposed algorithm with popular state-of-the-art methods, like Otsu's method, PSO based method, GA based method, and DE based method. Different parameters of the Bat algorithm and their respective values used in the proposed method are as follows:

- 1) Population size: 40
- 2) Number of generations: 20
- 3) Loudness: 0.5
- 4) Pulse rate: 0.5
- 5) Minimum frequency: 0
- 6) Maximum frequency: 2

Some results of two level thresholding are shown below:

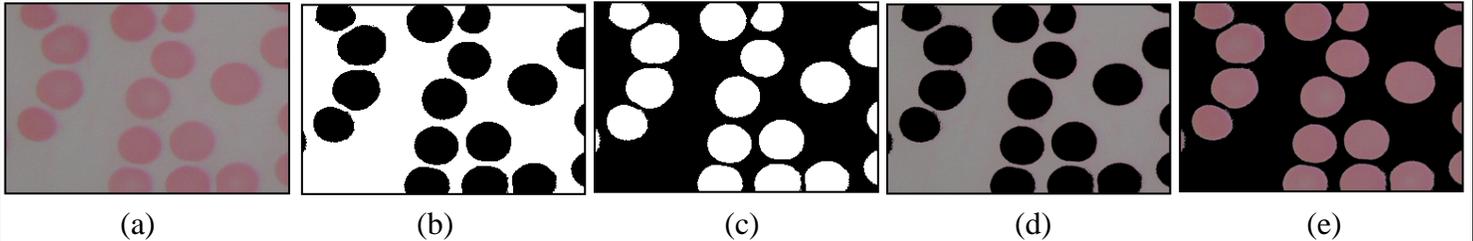


Fig. 4: *Microscopic-1.*(a) *Original image*, (b) *Background threshold image*, (c)*Foreground threshold image*, (d) *Background segmented image*, (e) *Object segmented image*

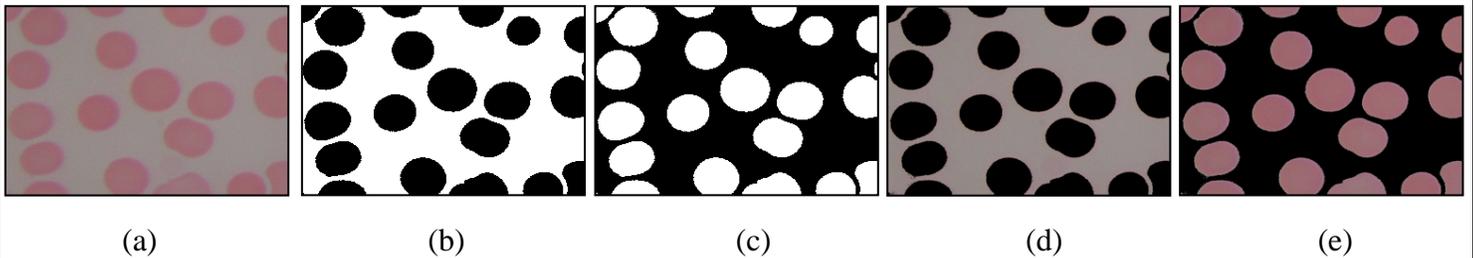


Fig. 5: *Microscopic-2.*(a) *Original image*, (b) *Background threshold image*, (c)*Foreground threshold image*, (d) *Background segmented image*, (e) *Object segmented image*

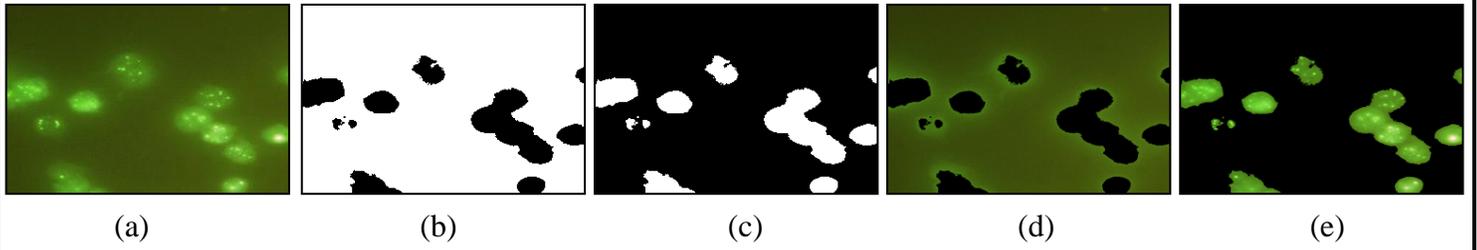


Fig. 6: *Fluorescence Microscopic-1.*(a) *Original image*, (b) *Background threshold image*, (c)*Foreground threshold image*, (d) *Background segmented image*, (e) *Object segmented image*

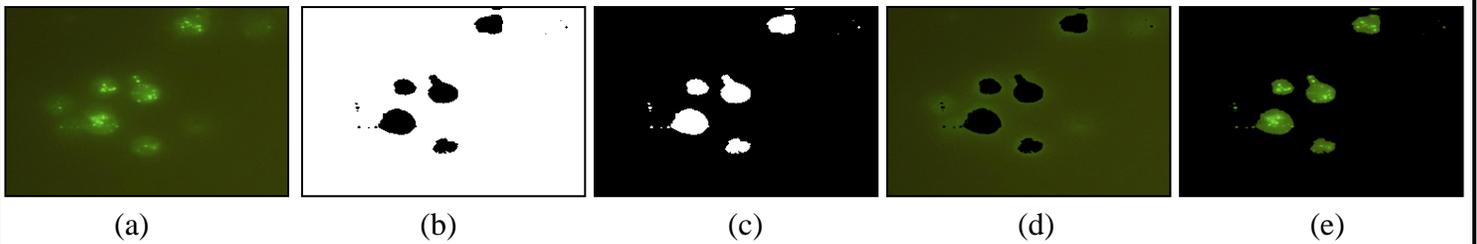


Fig. 7: *Fluorescence Microscopic-2.*(a) *Original image*, (b) *Background threshold image*, (c)*Foreground threshold image*, (d) *Background segmented image*, (e) *Object segmented image*

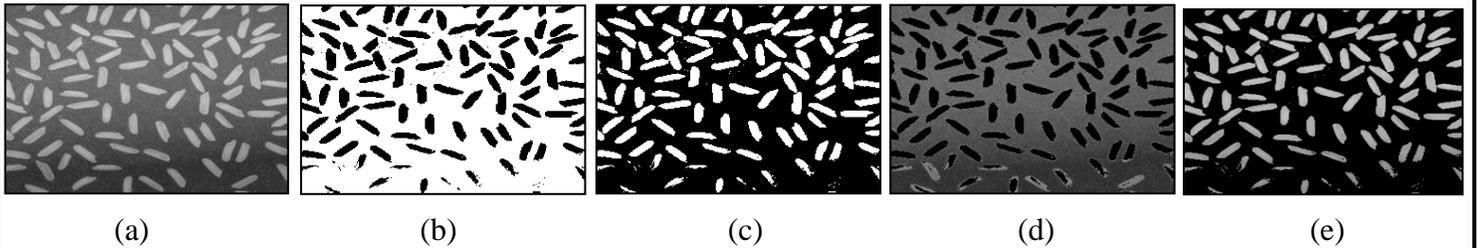


Fig. 8: *Rice.*(a) *Original image*, (b) *Background threshold image*, (c)*Foreground threshold image*, (d) *Background segmented image*, (e) *Object segmented image*

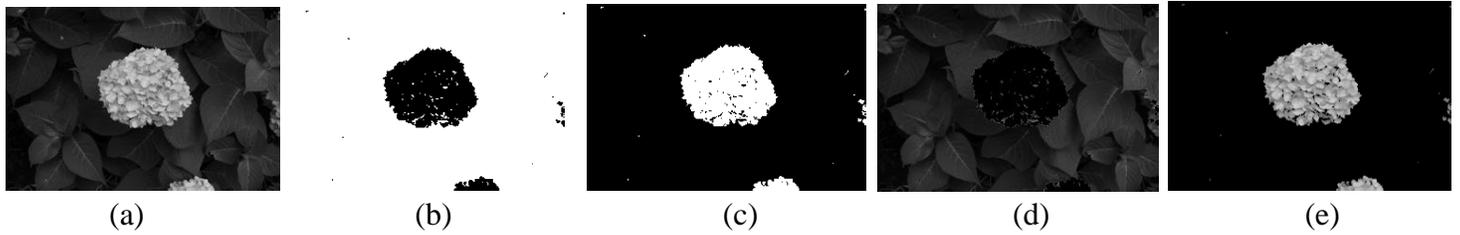


Fig. 9: *Flower.* (a) *Original image*, (b) *Background threshold image*, (c) *Foreground threshold image*, (d) *Background segmented image*, (e) *Object segmented image*

Table 3: *Performance Measurement (M.E.) On Different Images By The Proposed Method And Other State-Of-The-Art Methods Using Two Level Thresholding*

Test Images	Otsu	Wei et al.(PSO)	Tao et al.(GA)	Sarkar et al.(DE)	Proposed (BA)
Microscopic-1	0.51	0.50	0.48	0.49	0.49
Microscopic-2	0.50	0.48	0.48	0.46	0.43
Fluorescence Microscopic-1	0.61	0.58	0.59	0.60	0.51
Fluorescence Microscopic-2	0.65	0.61	0.59	0.62	0.59
Rice	0.41	0.39	0.38	0.40	0.35
Flower	0.40	0.37	0.35	0.34	0.31

Some results of three level thresholding are shown below:

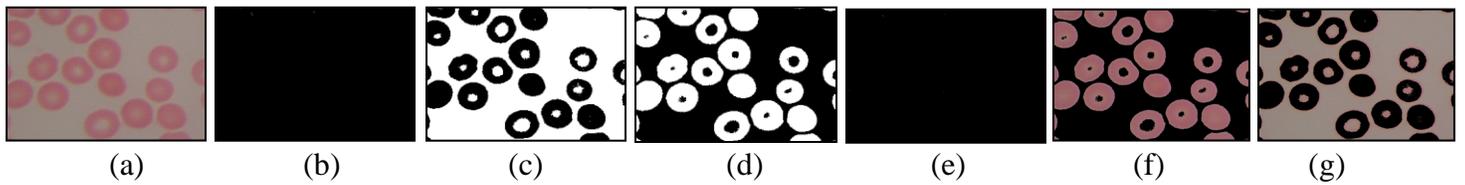


Fig. 10: *Microscopic-3. (a) Original image, (b) Background threshold image, (c) Object-1 threshold image, (d) Object-2 threshold image, (e) Background segmented image, (f) Object-1 segmented image, (g) Object-2 segmented image.*

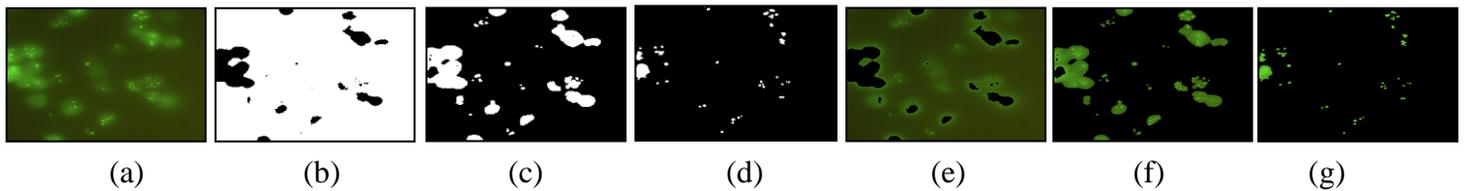


Fig. 11: *Fluorescence Microscopic-3. (a) Original image, (b) Background threshold image, (c) Object-1 threshold image, (d) Object-2 threshold image, (e) Background segmented image, (f) Object-1 segmented image, (g) Object-2 segmented image.*

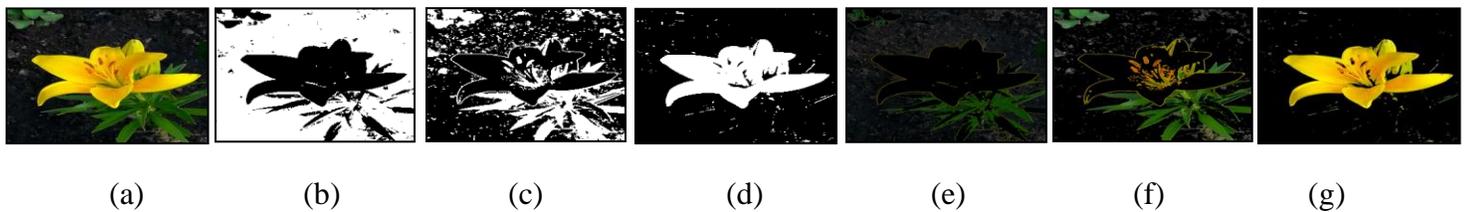


Fig. 12: *Lily Flower-2. (a) Original image, (b) Background threshold image, (c) Object-1 threshold image, (d) Object-2 threshold image, (e) Background segmented image, (f) Object-1 segmented image, (g) Object-2 segmented image.*

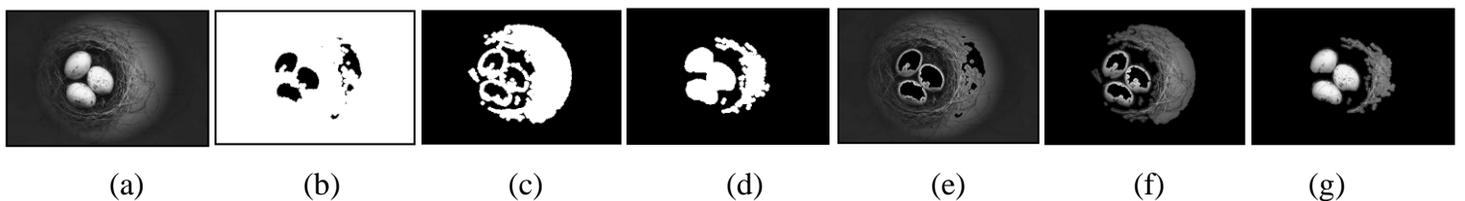


Fig. 13: *Nest-Egg. (a) Original image, (b) Background threshold image, (c) Object-1 threshold image, (d) Object-2 threshold image, (e) Background segmented image, (f) Object-1 segmented image, (g) Object-2 segmented image.*

Table 4: *Performance Measurement (M.E.) On Different Images By The Proposed Method And Other State-Of-The-Art Methods Using Three Level Thresholding*

Test Images	Otsu	Wei et al.(PSO)	Tao et al.(GA)	Sarkar et al.(DE)	Proposed (BA)
Microscopic-3	0.61	0.60	0.62	0.63	0.42
Fluorescence Microscopic-3	0.67	0.66	0.63	0.66	0.53
Lily Flower-2	0.61	0.58	0.55	0.57	0.51
Nest-Egg	0.67	0.62	0.63	0.65	0.59

Upper bound and lower bound of threshold values will vary from image to image. Therefore, it is set by determining the max and min intensity value present in the image.

The proposed method is compared with other four state-of-the-art methods Otsu, Wei et al., Tao et al., and Sarkar et al.. Where Wei's method is based on PSO, Tao's method is based on GA, and Sarkar's method is based on DE. All the above mentioned methods are implemented and well tuned according to their respective papers. We use the metric; misclassification error (M.E.) to compare the performance of the proposed method against other methods. M.E. is defined using the following equation:

$$M.E. = 1 - \frac{|B_o \cap B_t| + |F_o \cap F_t|}{|B_o| + |B_t|} \quad (8)$$

where, B_t and F_t are the background and foreground of the test images and B_o and F_o are the original images, respectively. Table-3 and Table-4 show the performance measurement (M.E.) of the proposed method compared with other state-of-the-art methods on different microscopic and normal images. Our proposed method gives lesser M.E. than other compared methods. Table-3 shows the M.E. values of different methods when only two level thresholding is performed. Fig.4 to Fig.9 shows the sample output results of the proposed method using two level thresholding. Table-4 shows the M.E. values of different methods when three level thresholding is performed. Fig.10 to Fig.13 shows the sample output results of the proposed method using three level thresholding. Although a three class segmentation is performed on microscopic image, interestingly the output result shows only two classes. It proves the superiority of the proposed method. Two level thresholding results of the proposed method on microscopic, fluorescence microscopic and natural images. (a) Original image, (b) Background threshold image, (c) Foreground threshold image, (d) Background segmented image, (e) Object segmented image. Finally, Table 5 compares our proposed method with other state-of-the-art methods in terms of required approximate processing time represented in terms of second. Since we are talking about fast thresholding is essential in the

case of medical diagnosis, the required time is really better than other state-of-the-art methods. Fig.14 shows the User Interface (UI) of our project.

Table 5: *Comparison Of The Proposed Method Against State-Of-The-Art Methods In Terms Of Processing Time*

Methods	Approx. Processing time (in seconds)
Wei et al.	6.18
Tao et al.	8.22
Sarkar et al.	6.01
Proposed	2.04

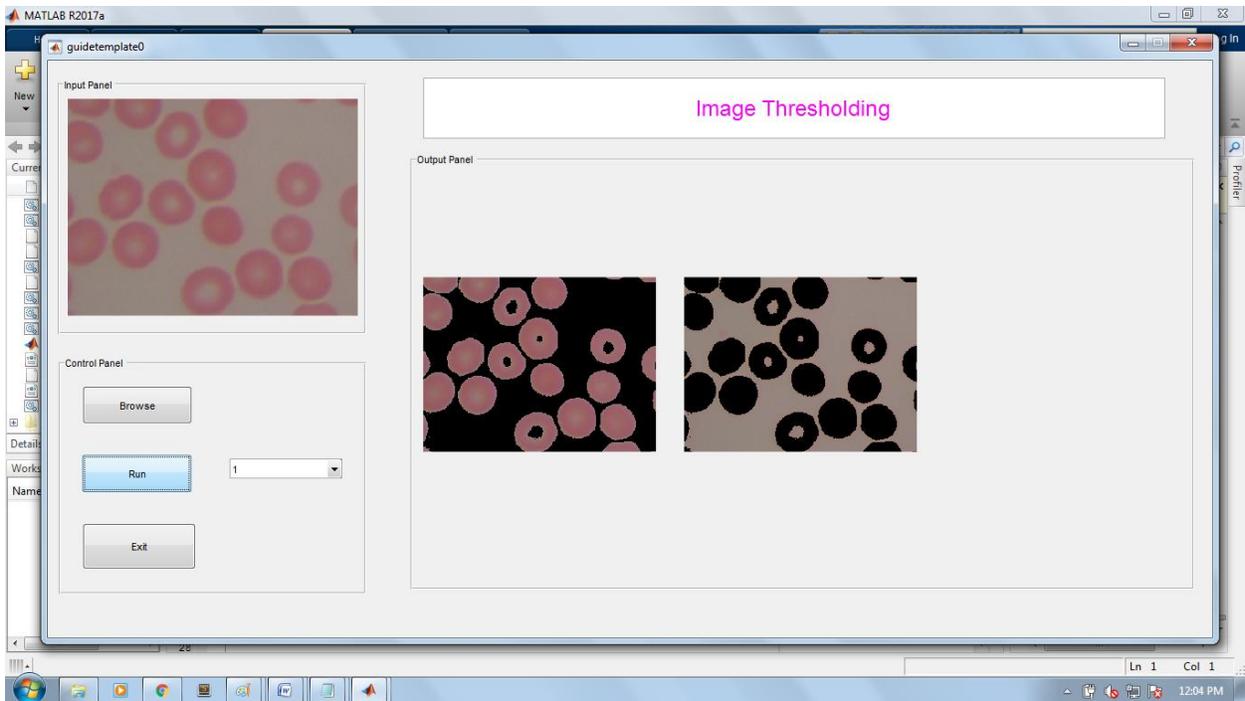


Fig. 14: *UI Design*

CONCLUSION AND FUTURE SCOPE

Image thresholding is one of the most important task in the field of computer vision to be able to select the essential ROI. In this project a novel algorithm is proposed which is time efficient and competent in both microscopic and natural image segmentation. In medical diagnosis segmentation of microscopic image is very essential. The proposed method use fast processing REF to represent the fuzzy information, nature inspired Bat algorithm to select the optimal thresholding in quick time. The performance of the proposed scheme is also verified with some state-of-the-art optimization techniques. Simulation results show that the output image is well segmented. In near future we aim to make the algorithm more faster for effective real-time application.

REFERENCES

- [1] P. K. Sahoo, S. Soltani, and A. K. C. Wong, "A Survey of Thresholding Techniques", *J. Computer Vision, Graphics, and Image Processing*, vol. 41, pp. 233–260, 1988.
- [2] N. Otsu, "A Threshold Selection Method from Gray-Level Histograms", *IEEE Trans. On Systems, Man, and Cybernetics*, vol. 9, no. 1, pp. 62–66, 1979.
- [3] S. K. Pal, and A. Rosenfield, "Image enhancement and thresholding by optimization of fuzzy compactness", *J. Pattern Recogn. Lett.*, vol. 7, pp. 77–86, 1988.
- [4] L. K. Huang, and M. J. Wang, "Image thresholding by minimizing the measure of fuzziness", *J. Pattern recogn.*, vol. 28, no. 1, pp. 41–51, 1995.
- [5] H. D. Cheng, C. H. Chen, and H. H. Chiu, "Image segmentation using fuzzy homogeneity criterion", *Inform. Sci.*, vol. 98, pp. 237–262, 1997.
- [6] A. K. Bhandari, A. Kumar, S. Chaudhary, and G. K. Singh, "A Novel Color Image Multilevel thresholding based Segmentation using Nature Inspired Optimization Algorithms", *J. Expert Systems With Applications*, vol. 63, pp. 112–133, 2016.
- [7] C. Wei, and F. Kangling, "Multilevel thresholding algorithm based on particle swarm optimization for image segmentation", In *Proc. International conference on Control (ICC)*, 2008.
- [8] W. Tao, J. Tian, and J. Liu, "Image segmentation by three-level thresholding based on maximum fuzzy entropy and genetic algorithm", *J. Pattern Recognition Letters*, vol. 24, pp. 3069–3078, 2003.
- [9] S. Sarkar, S. Pal, R. Burman, S. Das, and S. S. Chaudhuri, "A Fuzzy Entropy Based Multi-Level Image Thresholding Using Differential Evolution", Chapter in *Sentiment Detection in Online Content: A WordNet Based Approach*, pp. 386–395, 2015.
- [10] R. Tewari, S. Dhar, and H. Roy, "Image thresholding using LBP and GA-based optimal parameter selection for 2D Tsallis-Havrda-Charvat entropy", In *Proc. International conference on Research in Computational Intelligence and Communication Networks (ICRCICN)*, pp. 89–94, 2016.
- [11] S. Dhar, and M. K. Kundu, "A novel method for image thresholding using interval type-2 fuzzy set and Bat algorithm", *Applied Soft Computing*, vol. 63, pp. 154–166, 2018.
- [12] H. Bustince, E. Barrenechea, and M. Pagola, "Image thresholding using restricted equivalence functions and maximizing the measures of similarity", *J. Fuzzy sets and systems*, vol. 158, pp. 496–516, 2007.
- [13] L. A. Zadeh, "Fuzzy Sets", *Information and Control*, vol. 8, no. 3, pp. 338–353, 1965.
- [14] X. Yang, and A. H. Gandomi, "Bat Algorithm: A Novel Approach for Global Engineering Optimization", *Engineering Computations*, vol. 29, no. 5, pp. 464–483, 2012.
- [15] <http://data.broadinstitute.org/bbbc/BBBC005/>

PAPER ACCEPTANCE

Our paper, entitled with “*Microscopic Image Thresholding using Restricted Equivalence Function based Fuzzy Entropy Minimization and Bat Algorithm*” has been accepted in the 2nd International conference on Electronics, Materials Engineering and Nano Technology 2018 (IEMENTech 2018). The authors of the paper are Hiranmoy Roy, Soumyadip Dhar, Poushali Choudhury, Abhirup Biswas, Abhrak Chatterjee, Department of Information Technology, RCC Institute of Information Technology Kolkata, India. Fig.15 shows the presentation certificate, received by Abhrak Chatterjee and fig.16 shows the Participation certificate, received by Abhirup Biswas and Poushali Choudhury in the conference, occurred in IEM, Kolkata, India.

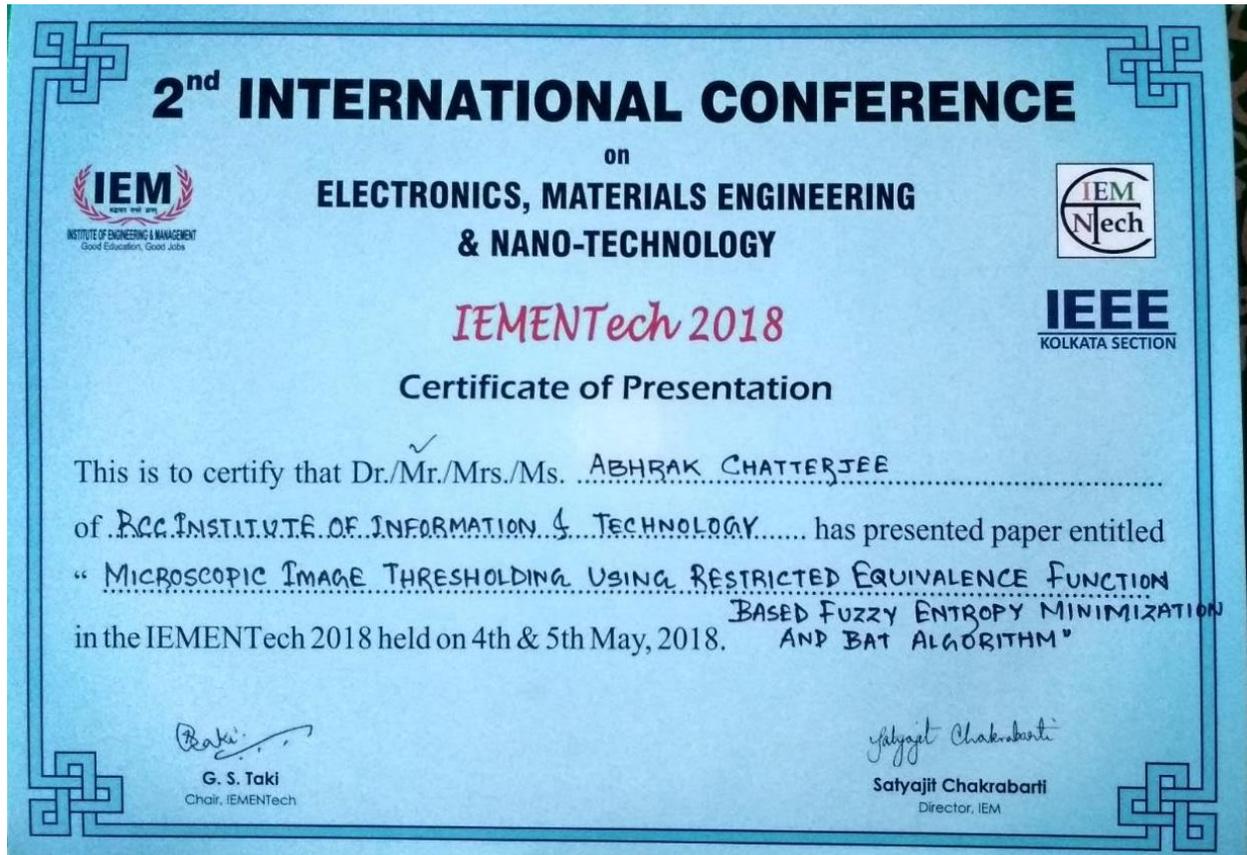


Fig. 15: *Presentation Certificate*



Fig. 16 (a): *Participation Certificate*



Fig. 16 (b): *Participation Certificate*

LIST OF TABLES

Table No.	Title	Page No.
1	Literature Survey	7
2	Software Requirement Specification	11
3	Performance Measurement (M.E.) On Different Images By The Proposed Method And Other State-Of-The-Art Methods Using Two Level Thresholding	19
4	Performance Measurement (M.E.) On Different Images By The Proposed Method And Other State-Of-The-Art Methods Using Three Level Thresholding	20
5	Comparison Of The Proposed Method Against State-Of-The-Art Methods In Terms Of Processing Time	21

LIST OF FIGURES

Figure No.	Title	Page No.
1	Software Development Life Cycle Model	11
2	Activity Networks	11
3	Flow control diagram of our proposed method	16
4	Microscopic-1	17
5	Microscopic-2	17
6	Fluorescence Microscopic-1	18
7	Fluorescence Microscopic-2	18
8	Rice	18
9	Flower	19
10	Microscopic-3	20
11	Fluorescence Microscopic-3	20
12	Lily Flower-2	20
13	Nest-Egg	20

Figure No.	Title	Page No.
14	UI Design	22
15	Presentation Certificate	25
16	Participation Certificate	26