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Computer-Aided Framework for the Dielectrophoretic Studies of Human Erythrocytes

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ABSTRACT: The present study is on the Computer-aided Framework for the Dielectrophoretic studies of Human Erythrocytes. The presented software oriented automated framework helps in studying various Bio-physical properties of human erythrocytes. The framework can be used for the end-to-end study of the properties of the human Red Blood cells. This automated approach saves a lot of time and achieves a great percentage of accuracy as compared with the classical manual approaches for the human Red Blood cell studies. The properties like Excess Dielectric Constant, Excess Permittivity and various other Bio-physical properties of the human erythrocytes can be efficiently studied using this automated framework. The blood sample is placed at the Electrode chamber and Non-Uniform Electric Field is applied. The video and pictorial recordings of the Red Blood Cells are captured through the High-end microscope which is connected to the CCTV camera. Required parameters are measured and analysed using the software and finally the objective of the study is achieved with a greater efficiency and accuracy.

KEYWORDS: Computer-aided Framework, Dielectrophoresis, Human Erythrocytes, Computer aided Instrumentation, Dielectrophoretic studies, Dielectrophoresis automation, Bio-physical properties.

I. INTRODUCTION

The human blood consists of different elements, of which Red Blood Cells (Erythrocytes) are the important ones. They transport the life giving oxygen from lungs to the body cells and carry away the carbon dioxide. Various Bio-Chemical properties pertaining to these were studied upon. Advancements were also made in investigating its Bio-physical properties as well. The Dielectrophoretic principle is applied to study the bio-physical properties. Various experimental techniques and approaches were used to study its properties. Scientific investigations were done on the Bio-physical properties of human erythrocytes and its characteristics were studied. The studies involved the general common classical manual approach and technique which was time consuming and at the same time the accuracy was not achieved to the extent required. The present study is an attempt to automate the process with a computer aided setup which eases the job of the researcher in studying various properties of human Blood Cells. The proposed framework is software driven one. It caters all the investigation needs by covering the study, end-to-end. The framework does not involve any complex architecture or integrations. It is very simple and can be applied for the Dielectrophoretic studies of the human erythrocytes with a greater ease. The results obtained from this study were of greater accuracy as compared with the ones obtained by using the general manual techniques.

II. RELATED WORK

Razali T et al. [1] have proposed a computer aided automated framework for the detection and identification of Red Blood Cells from the blood smear image. The proposed method uses Artificial Neural Network classifier through which the Red Blood Cells are classified as Normal or Abnormal from the cluster. Initially, the global threshold method applied on the green channel colour image is used for extracting RBCs region from the background.

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Later, the morphological filter and connected component labelling are utilized for abolishing the noise and holes in the Red Blood Cells. The framework operates through the following – Image acquisition, Colour space reduction, Red Blood Cell segmentation and post processing, Feature extraction and RBC classification. The Image acquisition is done through a light microscope, which captures the image. In Colour space reduction, the individual component or RGB (Red, Green and Blue) channel is investigated to find out the optimal colour channel to distinguish between the background and the RBC. The segmentation process is next implemented for partitioning an image into a region of homogeneous representation corresponding to the object of interest in the acquired image. In the post processing, to remove the noise and unwanted foreground cell from the segmented image, the methods like- Connected component labelling, morphological operation and bounding box filter are used. In the feature extraction module, the seven HU moment features are used to represent the Red Blood Cell shape. And the RBC Classification module uses the ANN (Artificial Neural Network) classifier.

E.A. Mohammed et al. [2] have presented on and reviewed on the approaches for the blood smear image segmentation and analysis. The main aim of image segmentation in this study is the isolation of the regions of interest in the image. This isolation of regions is done by either extracting one or more specific objects from the image or by partitioning the image into few connected semantic regions. This segmentation process is the key factor and main reason for the success of the overall detection process of White blood Cells from the microscopic images of the peripheral blood smear sample. The Peripheral Blood Smear image examination approach with the suggested approach helps in many ways and overcomes the Manual method which is tedious and time consuming and with a lot of variations. The proposed automated approach facilitated a lot and saves a lot of time and achieves a lot of accuracy. Also, the problem of blood smear image segmentation has been addressed in this study. The study also discusses on the supervised learning algorithm called SVM (Support Vector Machine) which is used in recognizing patterns and analysing data. It also discusses about ANN (Artificial Neural Network) technique which uses Machine learning algorithm. These are used for supervised and unsupervised learning methods. The study suggests few software tools like MATLAB, Octave and others that help in development of analysis techniques. Blood image analysis systems like HemaCAM, CellaVision and others were compared in this study. These are computer based automated systems which eases the job of image analysis.

A Hamouda et al. [3] have introduced an efficient method for Red Blood Cell counting. This method is better than the traditional method which is more costly and a time consuming one. The study involved the automatic Red Blood Cell counting and recognition. It uses the Image processing and the decision tree for RBC's classification. The RBC's classified yielded a very good classification high sensitivity and with a very good accuracy percentage. In the First phase of RBC counting called Histogram equalization, intensity value adjustments are done using Image Intensity Transformation. In the second phase of RBC counting (Segmentation), the blood cells are detected. It is done by differentiating the blood cells from the background in terms of contrast.In the second phase of RBC counting (Segmentation), the blood cells are detected. It is done by differentiating the blood cells from the background in terms of contrast. By using the image processing operators which calculate the gradient of image, e changes in contrast is detected. The Threshold is then applied for the creation of Binary Mask containing the Segmented Cell. The study also gives an overview of the Red Blood Cell classification and clustering algorithms including the K-means and the ID3 algorithms. Finally, the performances of the techniques discussed were investigated using the accuracy and the sensitivity parameters. It yielded the best sensitivity with accuracy of 97%.

N.D. Jambhekar [4] has presented his study on the Red Blood Cells classification using an automated diagnosis method. The automated method is used to classify the Blood cells using the Image Processing technique. The Blood cells image is captured through the microscope or recorded from the scanning electron microscope. The captured image is considered as a matrix of light intensity levels which can be manipulated using computer algorithms using MATLAB software. Image processing operations such as Edge smoothing, Edge detection, Feature extraction, Image procession and Image registration. Histogram method is used for analysing the image. It provides a very good representation of the colour spread of the captured image. The Histogram is also used for equalizing the image and for obtaining a large number of statistics. The study also discusses about the classification of blood cells using the advanced Artificial Neural Network technique. The output is obtained by applying this Neural Network technique on the captured blood cell image which contains the RBC's, WBC's and the sickled cells. The case study shows an accuracy of 81% using this multilevel perceptron model.

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M Veluchamy et al. [5] have presented an approach for the classification of blood cells using Artificial Neural Network. The approach follows a classification of Normal and abnormal blood cells in the microscopic images using the statistical and moment invariant analysis. The automated setup contained the process-(a) Image Data collection, (b) Blood Cell segmentation and (c) Feature extraction. At the image data collection unit, the blood specimens are obtained. The image of each blood cell contains number of normal and abnormal cells. At the blood cell segmentation unit, the image segmentation process is used to detect the entire blood cells. To detect the blood cells, the edge detection and basic morphology tools are used. The morphological operations used are erosion, dilation, opening and closing. And, using the Feature extraction method, 27 features were extracted from each cell image captured. The extracted feature included, 16 Statistical features, 4 geometrical features and 7 moment invariant features. The blood cells classification provided through this method is really appreciable. It can help the technician to objectively decide on the nature of the blood. The proposed system can be used as an aiding system for the pathologists.

Ateeba Shazi et al. [6] have presented a study on the Excess Permittivity of erythrocytes of patients suffering from Iron Deficiency (Anaemia) through a Dielectrodynamic technique. The Dielectrodynamic collection rate (DCR) of the human erythrocytes suffering from Anaemia is measured and the Excess Permittivity parameter is calculated for the study. Blood samples of normal person and those suffering from Anaemia are collected and it is stored by adding anti-coagulant (EDTA). The experimental work is then completed within one hour of the sample collection time. Erythrocytes of normal person and anaemic person are isolated from plasma by centrifuging. These are then subjected to Non-Uniform Electric Field and the Dielectrodynamic collection Rate (DCR) is measured. The parameter Excess Permittivity of erythrocytes is thus calculates using the relation with Micro polar parameter and with the Yield or DCR. The Dielectrodynamic Technique thus helped in studying the nature of the blood of patients suffering from Iron deficiency (Anaemia).

M Birgatti et al. [7] have demonstrated the separation of White Blood Cells from the erythrocytes using a Dielectrophoresis based Lab-on-a-chip device. This device involves miniaturisation of various complex analytical procedures. This device is expected to enable the laboratory testing to move from the laboratory employing the complex equipment's into a non-laboratory setting. The set up involves both Hardware and Software for this demonstration. The system developed is very much useful for movement, levitation and separation of large number of blood cells exhibiting identical dielectrophoretic properties. This dielectrophoretic based PCB device has parallel electrodes which are used to separate erythrocytes from the White Blood cells. It was also demonstrated that the White Blood Cells isolated through the dielectrophoresis based Printed Circuit Board (PCB) are suitable for the PCR-based molecular diagnosis of Thalassemia and Cystic Fibrosis mutations. The study further suggested that the dielectrophoresis based Lab-on-a-chip; PCB device can be designed to automatically isolate the blood cells which is suitable for robotized molecular diagnosis. It further suggests the possibility of developing fully automated procedures for the Blood Cells isolation, PCR and genetic testing.

D Mehta et al. [8] have proposed an automated system for the counting of Red Blood Cells using Image processing techniques. In this system, the image processing algorithm is used which works on the image captured by the high-end microscope with a greater accuracy. After capturing the image, it is pre-processed to extract the information. Techniques like Median filtering, low pass filtering are applied for reducing the noise and redundancy from the captured image. Applying these techniques makes the image suitable for further processing. The proposed automated system uses Python IDLE and other open source software's and libraries for the image processing task. The system uses the following – (a) Image reading, (b) Converting the image to Grey scale image, (c) High Pass filtering to filter the background elements, (d) Canny Edge detection to detect the edges of the cell, (e) Closing Morphological operations and (f) Contouring the Image. This system thus addresses the problem of image processing in the Red Blood Cells by utilizing the morphological approaches for the segmentation, extraction and estimation of the blood cells. The efficient algorithm enables the accurate detection, segmentation and estimation of the Red Blood Cells. This proposed method thus, minimizes the cost of the equipment and promotes the mobility of the device for relocation to remote parts for different pathological tests.

N Dhaygude et al. [9] have presented a system which provides user friendly software which allows a good user interaction with a simple tool for counting Red Blood Cells and White Blood Cells from the captured image. The counting results in turn are used to determine the deficiency or capability of the body system. The study uses Pulse Coupled Neural Network(PCNN) algorithm which is used for separating overlapping cells very easily. The proposed

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system architecture includes the following- (a) Sample Image, which is taken from the laboratory, (b) Scale Image Conversion (RGB to Grey scale) which also uses High Pass Filters and Canny Edge Detection (c) Grey scale image conversion into Binary image, (d) Detection and Counting of Red Blood Cells and White Blood Cells and (e) Disease detection from the sample Image. The study uses the following method and techniques - (a) Image segmentation, (b) Noise Removal, where PCNN parameters are set to remove small disturbing objects or elements successfully and (c) Cell Counting. The study uses the basic model of PCNN which has 3 main parts to form a neuron-Dendritic tree, Linking modulation and the Pulse generator.

Madhur G B and D S Patil [10] have presented a Preliminary study on the automatic Blood Cell analysis method by using Digital image processing. This process includes the automatic blood cell counting. In order to create clearer and cleaner blood cell images, few pre-processing and post-processing techniques have been implemented. The digital image process encompasses a broad range of software and hardware. The basic steps to analyse the blood cell of this system include- (a) Image acquisition, (b) Image Pre-processing, (c) Image Enhancement, (d) Image Segmentation, (e) Feature Extraction, (f) Morphological operations and (g) Abnormality detection. Various segmentation techniques used with the help of classifier for differentiating between the different types of Red blood cells and the White blood cells are, KNN, NN, W-KNN, Bayes, SVM, NNet. The different types of Artificial Neural Networks (ANN) used in this study are, local linear map, feed-forward back-propagation and fuzzy cellular Neural Network. The system uses MATLAB software for the image analysis. The analysis of Blood Cell images was seen more accurate with the help of this digital image processing. Also, the system seems to be more efficient in terms of time and cost as compared with other techniques of blood cell analysis. This method can be very much applied for the medical diagnosis and blood cell counting.

Ateeba Shazi et al. [11] have presented a study on the excess permittivity of Diabetic Human erythrocytes using Computer-aided Dielectrodynamic technique.This technique can serve as a tool for the detection, diagnose, analysis and monitoring of Diabetes mellitus. In the study, the Non uniform Electric Field is generated using a pin-pin electrode configuration. This field is applied on the sample. The cells in motion are collected at the electrodes in the form of chains which is observed under microscope employing CCTV. The CCTV camera is interfaced with the computer. The length of the chain formed at electrodes for a fixed time, called Dielectrodynamic collection rate (DCR) is measured this computer interface with CCTV. The excess permittivity is thereby calculated using the relation for DCR or yield. The Excess permittivity of diabetic human erythrocytes is thus computed knowing the yield and micro-polar parameter.Based on the experimental study, it suggests that the human diabetic erythrocytes are less dielectric as compared with the normal human erythrocytes. The technique thus serves as a potential tool for diagnosing and detecting and thereby monitoring the human diabetic erythrocytes.

M Abdul Malik and M K M Zafar [12] have presented a lone cell Dielectrophoresis technique for finding the Excess Dielectric constant of human erythrocytes. The technique involves the measurement of velocity of a selected single cell. The erythrocytes are suspended in the glycine-glucose medium and subjected to the Non-Uniform Electric Field (NUEF). A wire-wire electrode configuration is used in this setup. The experimental set up consists of a Compound microscope, a signal generator of frequency ranging from 1 KHz to 20 MHz, digital storage oscilloscope and the electrode chamber. After the application of Non-Uniform electric field, the observations are made through the eyepiece micro meter marked into divisions. Velocity of the RBC is then measured and the excess dielectric constant of human erythrocytes is then calculated using the relation with micro polar parameter. The velocity of the human erythrocytes were determined at constant frequency of 1 MHz for different voltage ranges from 10 to 26 Volts rms and a graph is plotted between square of voltage and the velocity of the human erythrocytes for different blood groups. The study finally concluded that the Excess dielectric constant of Human Erythrocytes is more or less one and the same irrespective of the blood groups. This novel technique thus helps in understanding the behaviour of the erythrocytes.

III. PROPOSED FRAMEWORK

Fig.1. shows the proposed computer aided framework used for the Dielectrophoretic studies of human erythrocytes. The framework consists of the following modules which cover the investigation, end-to-end, right from sample collection, providing inputs, obtaining the outputs and finally the report generation based on the results analysis.

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(1) *Sample Collector& Electrode Chamber:*
The human Red Blood Cells for investigation are collected with EDTA added as anti-coagulant. The sample is suspended in the glycine-glucose medium at the electrode chamber. The simple wire-wire electrode is used in the current study to evaluate the framework setup. Other electrode types can be used according to the output requirement and input data. This sample electrode chamber is mounted on the microscope connected to the CCTV.

(2) *Input Signal Generator:*
The electrodes are connected to the software driven signal generator which produces signals with frequencies ranging from 1 KHz to 20MHz. The software is installed on windows platform and managed by the standalone computer system. The frequencies are customizable and can be calibrated for various ranges. There is an option of even connecting this one to the image analysis software (MATLAB).

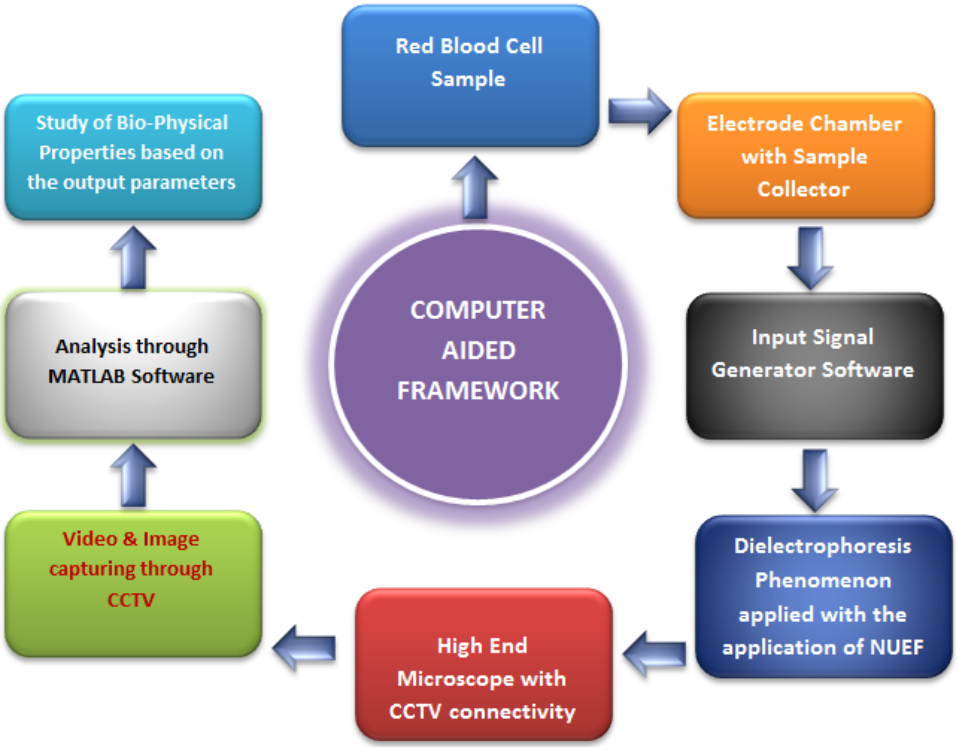

Fig.1. Proposed Computer-aided Framework for the Dielectrophoretic studies of human erythrocytes.

(3) *Red Blood Cell Image Capturing module:*
The Dielectrophoresis phenomenon is exhibited with the application of Non-Uniform Electric field. The video and images of the human RBC's under investigation is captured through the CCTV camera connected to the microscope. The output of the Video and images is sent to the computer which captures the required investigation activity in the form of video files and image files for the selected or configured timestamp.

(4) *Video and Image Analysis module:*
The captured video and image files are analysed through the MATLAB software. At this module, the statistical and data analysis is done using this software which uses add-on application specific solutions (Tool Boxes). These tool boxes are used for Image and video visualization and helps in performing Image processing, image analysis, image visualization and algorithm development. Various other properties and parameters pertaining to

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images like Edge detection, Boundary tracing and image statistics can also be included in the study based on the requirement and scope. The MATLAB software also has a built-in analysis via GUI (Graphical User Interface) mode, which facilitates the end user in analysing the input data.

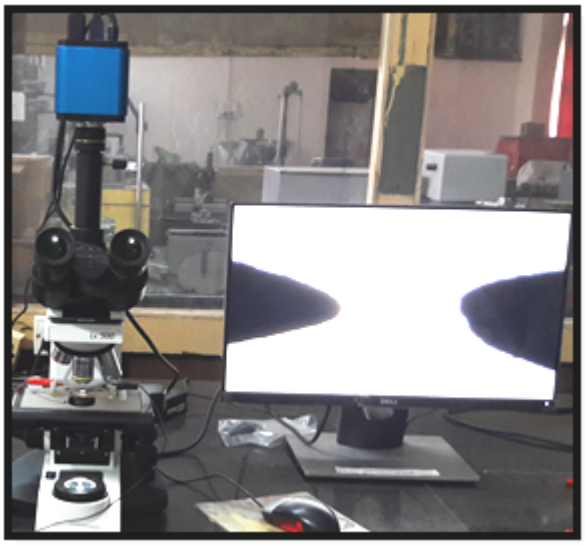

Fig.2. High-end microscope with CCTC connected to software.

Fig.2. shows the Microscopic setup at test lab. This high-end microscope has the electrode chamber mounted on it and also connected to the CCTV which is again connected to the computer. The video and image capturing mode is set on at the computer before the application of NUEF. The live view of the activity helps in identifying any distortions or impurity in the sample and can be paused and set again with fresh sample and reconfigurations of the setup.

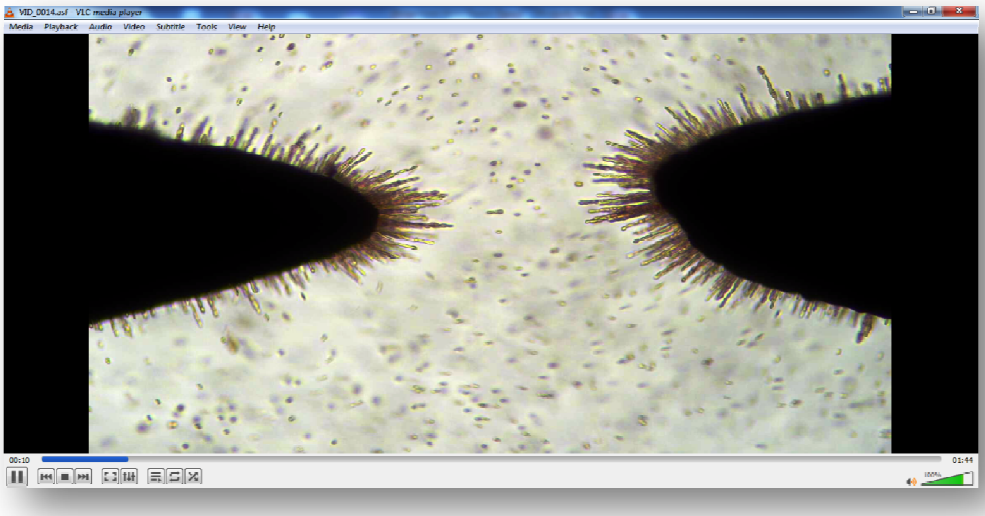

Fig.3.Video file of DEP phenomenon of human RBC captured through CCTV.

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Fig.3. shows the snapshot of the video file captured during the investigation. As mentioned above, the microscope is connected to the CCTV which in turn is connected to the computer. The output can be captured in the form of either video file or image files of different formats. These files can be used for the drill down and analysis using MATLAB software.

IV. SCOPE AND LIMITATIONS

The framework is developed on windows operating system and all the software's used are compatible on windows platform. The software's are compatible for both 32 and 64 bit operating systems. All the software's are user interactive with GUI support. The current proposed framework is limited to windows platform. However, it can be customized and upgraded to overcome its limitations to support for other platforms as well based on the necessity and requirement. The scope of the framework can also be broadened with few customizations and software integrations with other 3rd party software's, add-ons and plug-ins. Based on the requirements of the scientific investigations, other software's for the same purpose can also be used to cater the need, but only after a thorough evaluation of tools and hardware's.

V. CONCLUSION

The replacement of the manual driven activity with software driven automated set up for the scientific investigations of Human Red Blood Cells is the need of the hour. A lot of studies were done on the Bio-chemical and Bio-physical properties of Human Red Blood Cells using the Dielectrophoretic technique. Most of the scientific investigations done so far on Human erythrocytes involved the classical Manual techniques which involved a lot of manual activity andthe output obtained lacked accuracy. The framework presented is an automated one and replaces all the manual activity and gives a very good output with a greater percentage of accuracy. The parameters under investigations can be measured and studied at length with a greater drill-down. The framework uses a very good automated analysis tool. Few Bio-physical properties like Excess Dielectric constant and Excess permittivity of human erythrocytes were studied using this framework. Similarly, scientific investigations can also be done for various other properties pertaining to Red Blood Cells. The framework can also be customized with different other software's and tools based on the input and output requirement. Apart from vendor based tools or software's, freeware's (Open Source Tools) can also be used or integrated within this framework accordingly to cater the investigation needs. The current framework is designed to work on windows platform. It can be further customized and upgraded to work on other platforms as well based on the hardware and software compatibility of the experimental setup.

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