ADDIS ABABA UNIVERSITY

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SCHOOL OF GRADUATE STUDIES

CENTER OF BIOMEDICAL ENGINEERING

Mobile Phone Microscope Imaging for e-health Applications at Low Resource Setting; Image Processing for Automatic Counting of Blood Cells

Master's Thesis

Submitted in Partial Fulfillment of the Requirement for the Degree of
Master of Science in Biomedical Engineering (Instrumentation and Imaging)

By

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I, the undersigned, declare that this thesis is my original work. It has never been presented for a degree in any other institution and that all sources of materials used in it have been duly acknowledged.

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Certificate of Examination

This is to certify that the thesis prepared by Mulugeta Mideksa Amene entitled "***Mobile Phone Microscope Imaging for e-health Applications at Low Resource Setting; Image Processing for Automatic Counting of Blood Cells"*** and submitted in Partial Fulfillment of the Requirement for the Degree of Master of Science (Biomedical Engineering) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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_____

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Head of CBME or Graduate Program Coordinator

Next to God to my Family

(My beloved wife Tigina and son Jossy)

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Mulugeta Mideksa Amene

Addis Ababa, Ethiopia
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Preface

Most of the chapters presented in this thesis are extensions of a course project work, the National technology innovation award of EFDRE Ministry of Science & Technology (MOST) and posters presented at forums.

Publications

1. **Mulugeta Mideksa** (2013). "Mobile Phone Microscope" Project sponsored by MOST**,** won the best clinical project Award2013 of the MOST.
2. **Amene, Mulugeta Mideksa,** WHO Medical device 3rd Global forum, May 10-12/2017, Geneva, Switzerland…(Poster # R43 page 22) (https://www.who.int/medical_devices/global_forum/3rd_gfmd/May8-3GFMDfinal_programme.pdf?ua=1).

ABSTRACT

"Mobile Phone Microscope Imaging for e-health Applications at Low Resource Setting; Image Processing for Automatic Counting of Blood Cells"

Mulugeta Mideksa Amene, Addis Ababa University, 2021

BACKGROUND: currently, there is limited information on study to design web page that incorporates a clinical laboratory atlas and to couple, portable mobile phone- microscope imaging (PMPMI) device for e-health application in low resource settings (specially in Ethiopia) to enable the health extension workers (HEW)/community health workers (CHWs) promote early detection and protection of diseases by means of automatic complete blood cell (CBC) count.

MATERIAL and METHODS: collect HP-model mobile phone, Microscope Eye piece, objective lens, 300 clinical laboratory slides; develop a comprehensive technique that includes designing and assembling a mobile attached microscope, a web page that incorporates a clinical laboratory atlas, image sharing apps and rigorous mathematical algorithm for automatic CBC count to test the quality and functionality of the new device.

RESULTS: the proposed coupled mobile phone-microscope imaging device is able to share sample images with next level lab technologists/pathologists via image sharing open applications and the developed image processing scheme allows automated CBC count on images acquired through the new coupled system. The counting algorithm offered an overall accuracy of 90% in RBC count and 99.9% in WBC count. Additionally, JossyBME.com web site is developed to upload lab-atlas images for further reference.

CONCLUSIONS: The new coupled mobile phone-microscope device functions in white light settings. The work promotes early detection and protection of diseases and presented here as a cost-effective option. The device has been designed in such a way that it could be used not only by HEWs but also by the higher-level hospital laboratory personals. The effectiveness of the developed cell counting algorithm could show the great promises of the proposed imaging system and the new device.

Keywords: Mobile Phone Microscope, Health Extension Worker (HEW), Tel-laboratory, e-health, Website, Atlas, Complete Cell Count (CBC), Mathematical Algorithm.

LIST OF Figures

List of Tables

Abbreviations

ANC	Anti-natal Care
BME	Biomedical Engineering
CBC	Complete Blood Cell Count
CCD	Charge Coupled Device
CHW	Community Health Worker
DIN	Deutsches Institutfür Normung
DOF	Depth of focus
EDTA	Ethylenediamine Tetraacetic Acid
ERS	Electronic Recording System
FMOH	Federal Ministry of Health
Hb or Hgb	Hhemoglobin
Hct	Hhematocrit
HC	Health Center
HEW	Health Extension Worker
HIV/AIDs	Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome
HP	Health Post
HSDP	Health Sector Development Plan
HSV	Hue-Saturation-Value
JIS	Japanese Industrial Standards
GUI	Graphic User Interface
LIS	Laboratory Information System
LIC	Low Income Country
LRS	Low Resource Setting
MAPA	Microanatomy and Pathology Atlas
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
MCV	Mean corpuscular volume
NA	Numerical Aperture
PD	Parfocal Distance
PF	Plasmodium Falciparum
PIP	Product Innovation Process

PMPMI	Portable Mobile Phone Microscope Imaging
RBC	Red Blood Cell
RGB	Red Green Blue
RMS	Royal Microscopical Society
SDG	Sustainable Development Goals (Global goal)
SPP	Species Pluralis, (Latin for multiple species).
TB	Tuberculosis
UHC	Universal Health Coverage
UNICEF	United Nations International Children's Emergency Fund
UN-SDG	United Nation-Sustainable Development Goal
WBC	White Blood Cell
WHO	World Health Organization
YCbCr	Green (Y), Blue (Cb), Red (Cr) or (Luminance; Chroma: Blue; Chroma: Red).

Table of Contents

CHAPTER ONE

INTRODUCTION

1.1 Background

There are several trails to reach unreached population; those who are living in remote areas of low resource setting and children left behind. These communities' potential problems need to be addressed with big strategic involvement of special healthcare programs. In the Ethiopian context, there exists the Health Extension Program (HEP) which is a community-based strategy designed to deliver health promotion, disease prevention and selected curative health services at the community level [1]. It is a mechanism to provide health service in an equitable manner to all segments of the population in the country. The services are provided free of charge. It needs enablers for the system with e-health technology to accomplish the four major components of the sixteen health package categories [1, 2]. The four major areas are (our focus is on 2nd and 3rd components) promotion of hygiene and environmental sanitation, prevention and control of major communicable diseases, promotion and provision of family health services and health education and communication.

Aiming for a more equitable and diversified coverage as well as quality healthcare services for its dwellers, Ethiopia has set successive Health Sector Development Plans (HSDP) since 2000 each spanning five years. The priority areas of the HEP for HSDP IV were scaling up of urban and pastoralist HEPs, maintaining and improving the quality of rural health extension programs and organization of health development army.

1.2 Health System and the HEP

The national health policy of Ethiopia is aimed to standardize packages of basic services in all primary health care facilities through providing and regulating a comprehensive package of promotive, preventive, curative and rehabilitative health services. The types of health facilities, ranging from community outreach provided by community health workers (CHWs) at Health Posts (serving 3,000-5,000 people), through outpatient care at Health Centers (serving 15,000-25,000 people) and to inpatient services at primary hospitals (serving 60,000-100,000 people), general hospitals (serving 1-1.5 million people) and specialized hospital (serving 3.5 -5 million people). Figure 1 presents Ethiopia's three tier public health care system.

The HEP is an innovative community-based program started in 2003 by deploying over 38,000 Health Extension Workers (HEWs) to over 16,000 health posts (with a catchment area population of 5,000 on average) [26]. The HEWs are bringing basic health services to the doorstep of Ethiopia's large, rural population, facilitating closer contact between health workers and communities and importantly mobilizing communities to change behaviors.

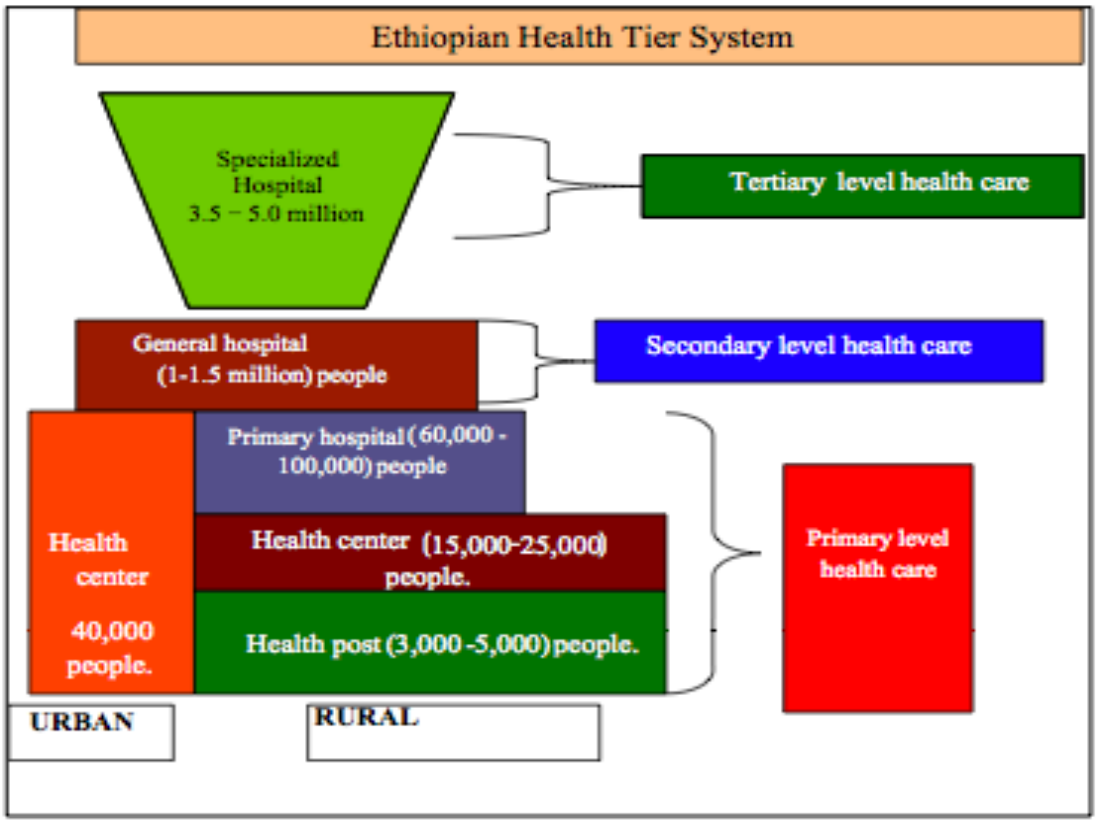


Figure 1: Ethiopia's three tier public health system

(Source: Ethiopian Health Sector Development Plan 2010-2015)

The HEP provides health post-based basic services, including improved access and utilization of latrines, increased contraceptive acceptance rate, antenatal care (ANC), assisted delivery, improved health seeking behavior, expanded vaccination services, malaria control and prevention and reduction of new HIV infections [1, 2]. Moreover, HEWs have also started treating common childhood diseases, including pneumonia and severe acute mal-nutrition [1, 3]. Case referral to health centers is also provided when more complicated care is needed. The HEP contributes to the country's move toward universal health coverage (UHC).

To satisfy the increasing demand of the community, HEW's knowledge and skills need to be improved. The Federal Ministry of Health (FMoH) is working to improve the skills and competency of HEWs through integrated refresher in-service training and upgrading of HEWs to the next level. The HEWs provide numerous homecare clinical services while availability of skilled laboratory technologists is highly scarce. There are several diseases putting tremendous societal burdens that could be significantly lessened by enhancing the knowledge and skills of HEWs and other health professionals so that they deliver effective healthcare services. Malaria, tuberculosis (TB) and other blood parasites are among those societal issues being major killing diseases particularly in the low resource settings. Making accessible simpler and affordable technological solutions should be one way towards addressing such and related issues.

Microscope is used routinely in many clinics for diagnosing various diseases while interpreting microscopic readings is not necessarily straightforward. In places where laboratory technologists are too scarce, the problem gets even more pronounced. Table 1 Percentage of medical problems reported by microscope operators. The issues raised include:

1. The Ergonometric effect as headaches, shoulder pain and back pain demonstrated on almost 70% of the clinical lab technologists, who are working on analog microscope examination procedures.
2. More than 30% of the clinical lab technologists, who are working on analog microscope examination procedures, also suffer from pains on their legs, feet, wrists, hands and fingers.

Table 1: Percentage of medical problems reported by microscope operators

Anatomical Location	Employee Percentage
Neck	50-60
Shoulders	65-70
Back (Total)	70-80
Lower Back	65-70
Lower Arms	65-70
Wrists	40-60
Hands and Fingers	40-50
Legs and Feet	20-35
Eyestrain	20-50
Headaches	60-80

1.3 High Burden Diseases

Seven million children are estimated to die every year due to lack of basic health resources (UNICEF). CHWs are frequently the last-mile of hope for many young patients dealing with malaria, pneumonia and diarrhea. Malaria and TB are the two major killer tropical diseases in sub-saharan African countries. In Ethiopia, the prevalence and rate of morbidity/mortality due to these diseases is higher than HIV/AIDs. According to reports by the Ethiopian FMOH, almost 75% of the nation is malarious with about 68% of the total inhabitants living in areas at risk of malaria [4]. The problem is compounded by increasing frequency and magnitude of malaria epidemics [5]. There is a similar trend in the case of TB too. Common methods for diagnosis are blood tests for malaria and sputum tests in the case of TB.

1.4 Problem Statement

Medical coverage in Ethiopian context remains still in the low side irrespective of the current nationwide move to put such a discrepancy to its minimum. The required medical device is either unavailable or insufficiently portable, and operators may not possess adequate training to make full use of the images obtained. This is particularly more pronounced in remote areas where medical devices and medical professionals are very scares, which is often considered as one of those hindering factors for HEWs carry out their duties effectively. In this regard, development of easy to use, low cost devices can play a vital role. Particularly, this is true with regards to communicable diseases including malaria, TB, and HIV. The first intent of this thesis was to design a low cost, portable, microscopic device coupled with a smart phone system and that works in both white light and fluorescence settings. However, for fluoroscopic setting, we require additional filters, dark rooms and high skills and that is not meant for low resource settings. So, for simplicity, this thesis project focuses on the bright field (white light) setting.

The complete blood cell (CBC) count helps for the performance testing and validation of the design technology. CBC count provides valuable information about the blood and to some extent the bone marrow, which is the blood-forming tissue. In a clinical setting, the CBC count comprises a group of tests used for the determination of the number, size, shape, and proportion of each blood cell types (red blood cells (RBC), white blood cells (WBC), and platelets) in a given sample of blood. The CBC also provides information about the amount of hemoglobin in RBCs, determines the percentage and absolute number of the five white blood cell types, and used in early detection of abnormal blood cells.

In general, not all medicine is delivered in hospitals as well as in doctors' offices due to geo-barriers and scares skilled clinicians. In fact, clinicians are now-a-days tele-reporting from homes. The practice of telemedicine the remote transmission of clinical data using the internet, wireless, satellite, and telephone media is at an all-time high, and it is improving access, equity and quality to care for patients across the globe. *Telemedicine also is cutting down on healthcare costs and play a significant role to meet SDG goals[69].* It is the intent of the current thesis project to design and develop a simple and portable microscopic device for use in automatic CBC procedures which could be incorporated easily as part of a telemedicine app.

The data clearly show that there is a huge gap yet to be filled and that requires a carful intervention mechanism. This calls for the development of technological solutions that could be used to reverse the situation targeting paradigm shift.

There are two major issues in this regard:

- One is developing a simple and portable microscopic technology that could be used by most users as point of care, human error free, cost effective and life saving device.
- The second one is reduction of ergonometric outcomes and digitalizing the manual system to retrieve or share data for easy access to the next referral level and electronic recording system (ERS)/laboratory information system (LIS).

In this thesis, a coupled mobile phone-light microscope-imaging device has been designed and developed and an image processing algorithm has been developed for automated CBC application including a web based clinical decision support system. Captured images are manually compared with clinical laboratory specimens' atlas.

1.5 Organization of the Thesis

The rest of the thesis has been organized into four chapters. Chapter 2 discusses theoretical background and related works on RBCs, CBC, Mobile Microscopes, Telemedicine, IP, Classification algorithms, web development and CBC count in general. Chapter 3 presents materials required and methods followed in the current study. It includes the design, development and testing of the mobile phone microscope, webpage development, data collection and the digital image processing scheme utilized for the CBC count. Chapter 4 compiles the overall results of the thesis, discussions on the results, and some of the limitations and challenges with the current study. Finally, Chapter 5 concludes the thesis and forwards some recommendations.

1.6 Research Objectives

The General objective is

- To develop a mobile phone microscope imaging device for e-health applications coupled with an image-processing algorithm for automatic counting of blood cells.

The specific objectives are:

- Design and develop a coupled mobile phone-microscope device and check its efficacy.
- Assess the image quality captured by the proposed device and check its accuracy.
- Develop an algorithm that could be used to do CBC count automatically from the generated microscopic images based on digital image processing concepts.
- Quantify the performance of the CBC count scheme based on known quantitative matrices.

1.7 Significance of the Study

The thesis project will have important implications in the efforts to alleviate the efficiency of HEWs and the health care referral system (primary-secondary-tertiary) thereby supporting the national e-health strategy that is crucial to achieving universal health coverage (to meet SDG-3). The study promotes the safe, equitable and quality of clinical service for low resource settings in Ethiopia. The study also demonstrates Tele-laboratory system. The automated scheme for CBC count is particularly developed for the following purposes:

- Remote follow-up of cancer patients taking treatments (chemo/radio-therapy);
- Detection of infections and anemia diagnosis;
- Identification of acute and chronic illnesses, bleeding tendencies, and white blood cell disorders such as leukemia;
- Promoting early detection and protection of malaria and TB epidemics, and
- Treatment monitoring for anemia and other blood diseases.

CHAPTER TWO

MOBILE MICROSCOPY, CBC COUNT and LITRATURE REVIEW

2.1. Mobile Microscope

Theory of Microscopes

A microscope is an optical device used to image an object onto the human eye or a video device. The earliest microscopes, consisting of two elements, simply produced a larger image of an object under inspection than what the human eye could observe. The design has evolved over the microscope's history to now incorporate multiple lenses, filters, polarizers, beam splitters, sensors, illumination sources, and a host of other components. To understand this important optical device, we need to consider a microscope's components, key concepts and specifications, and applications.

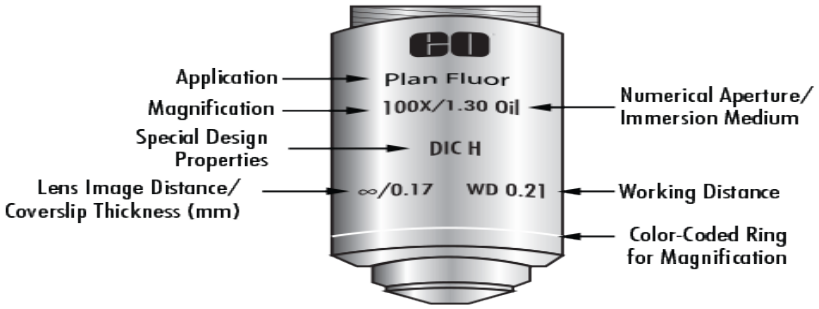
Basic Components of a Microscope

There are different types of microscopes including simple, compound, stereo, confocal, scanning electron, and transmission electron microscope. Our focus in the current thesis project is compound microscope. A compound microscope is one that contains multiple lens elements (as compared to a simple microscope). It works like a simple magnifier which utilizes a single lens to magnify a small object in order for the human eye to discern its details. With a simple magnifier, the object is placed within the focal length of the single lens. This produces a magnified, virtual image. With a microscope, a relay lens system replaces the single lens; an objective and an eyepiece work in tandem to project the image of the object onto the eye, or a sensor – depending upon the application. There are two parts to a microscope that increase the overall system magnification: the objective and the eyepiece. The objective, located closest to the object, relays a real image of the object to the eyepiece. This part of the microscope is needed to produce the base magnification. The eyepiece, located closest to the eye or sensor, projects and magnifies this real image and yields a virtual image of the object. Eyepieces typically produce an additional 10X magnification, but this can vary from 1X – 30X.

Key Concepts and Specifications

Most microscope objective specifications are listed on the body of the objective itself: the objective design/standard, magnification, numerical aperture, working distance, lens to image

distance, and cover slip thickness correction. Figure 2 shows how to read microscope objective specifications. Since the specifications are located directly on the body of the objective, it is easy to know exactly what one has, a very important fact when incorporating multiple objectives into an application. Any remaining specifications, such as focal length, field of view, and design wavelength, can easily be calculated or found in the specifications provided by the vendor or manufacturer.



Magnification	1X	2X	3X	4X	10X	20X	40X	60X	100X
Color Code	Black	Gray	Red	Yellow	Green	Light Blue	Light Blue	Dark Blue	White

Figure 2: Typical transmissivity microscope objective (100X, White used in this thesis).

The Objective Standard (DIN and JIS)

There are two main microscope international standards: DIN (Deutsches Institutfür Normung) and JIS (Japanese Industrial Standards). Most compound microscopes employ DIN standard. The image distances are different for DIN and JIS, there is no difference in optical performance; they are equal in quality. Similarly, each standard utilizes the same Royal Microscopical Society (RMS) mounting thread of 0.7965" x 36TPI. Some microscope manufacturers prefer to list the tube lens length by the optical properties instead of the mechanical. Lastly, there is a dimension typically listed for objectives to allow the user to consistently know what length it is: the parfocal distance (PD). The parfocal distance is the distance from the flange of the objective to the object under inspection.

Table 2 compares the two microscope standards while Figure 3 presents microscope objective international standards.

Table 2: Microscope international standards: DIN and JIS

No	Parameters	DIN	JIS	Remark
1	Tube length (Eyepiece - objective)	160mm	170mm	Mechanical Tube L.
2	Eyepiece diameter	23mm	23-30mm	
3	Object mean focal distance	45mm	36mm	Parfocal Focal Distance
4	Distance to the eyepiece field	150 mm	145 mm	Optical Tube Length
5	Internal real image from tube	10mm	10mm	
6	Eyepieces diameter	195mm	195mm	
7	Objectives thread diameter	0.7965"	0.7965"	RMS mounting thread
8	Whitworth threading	36 TPI, 55	36 TPI, 55	RMS mounting thread

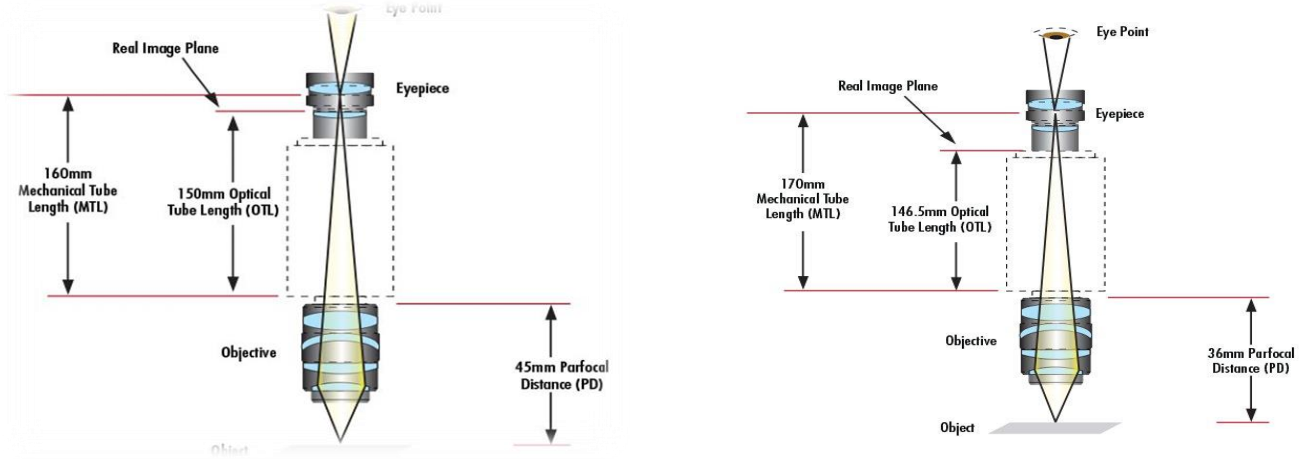


Figure 3: Microscope objective international standards: DIN (left) and JIS (right).

Magnification

Eyepieces and objectives both have magnification that each contribute to the overall system magnification. Magnification is usually denoted by an X next to a numeric value. Most objectives contain a colored band around the entire circumference of the body that indicates their magnification. For example, a yellow band denotes a 10X magnification.

Numerical Aperture

The Numerical Aperture (NA) of an objective is a function of the focal length and the entrance pupil diameter. Large NA objectives sometimes require the use of immersion oils between the object under inspection and the front of the objective. This is because the highest NA that can be achieved within air is an NA of 1 (corresponding to 90° angle of light). To get a larger angle and increase the amount of light entering the objective (Equation 1), it is

necessary to use immersion oil (index of refraction typically = 1.5) to change the refractive index between the object and the objective. High NA objectives in conjunction with immersion oil are a simple alternative to changing objectives, a move that may be costly.

$$NA = n \sin \theta \textbf{(2)} \qquad \text{EQ (1)}$$

Field of View

Field of view is the area of the object that is imaged by a microscope system. The size of the field of view is determined by the objective magnification. When using an eyepiece-objective system, the field of view from the objective is magnified by the eyepiece for viewing. In a camera-objective system, that field of view is relayed onto a camera sensor. The sensor on a camera is rectangular and therefore can only image a portion of the full circular field of view from the objective. In contrast, the retina in our eye can image a circular area and captures the full field of view. Therefore, the field of view produced by a camera-microscope system is typically slightly smaller than that of an eyepiece-microscope system. Equations 2 and 3 can be used to calculate the field of view in the systems.

$$Field\ of\ View_{Camera-Objective} = \frac{Camera\ Sensor\ Size}{Magnification_{Objective}} \qquad \text{EQ (2)}$$

$$Field\ of\ View_{Eyepiece-Objective} = \frac{Field\ Stop_{Eyepiece}}{Magnification_{Objective}} \qquad \text{EQ (3)}$$

Depth of Focus

When one focuses a microscope on an object, there is a finite range above and below this object in which other objects appear in sharp focus. This range is called the depth of focus of the microscope. It varies markedly with objective NA. In other word, the focal depth refers to the depth of the specimen layer which is in sharp focus at the same time, even if the distance between the objective lens and the specimen plane is changed when observing and shooting the specimen plane by the microscope. As human eyes are individually different in the ability of their focus adjustment, each person's perception of the focal depth varies. At present, the Berek formula is generally used, because it gives a focal depth value that often coincides with that obtained through experiments (see Equation 4) [6]. In the case of a video smart phone camera, the focal depth varies according to number of pixels of the smart phone or CCD, optical magnification, and numerical aperture. Depth of focus can be calculated using formulae presented on Equation 4 [6].

Focal Depth Formula: Visual Observation (Berek Formula) EQ (4)

$$D.O.F. = \frac{\omega \times 250{,}000}{NA \times M} + \frac{\lambda}{2(NA)^2} \quad (\mu m)$$

D.O.F. : Depth Of Focus

ω: Resolving power of eyes 0.0014
(when optical angle is 0.5 degrees)
M: Total magnification
(objective lens magnification x eyepiece magnification)
λ: Wevelength
(550nm)

$$\rightarrow \quad D.O.F. = \frac{350}{NA \times M} + \frac{0.275}{(NA)^2}$$

This indicates that the focal depth becomes smaller as the numerical aperture becomes larger.

Example With MPLFLN100×(NA=0.90), WHN10×:

$$D.O.F. = \frac{350}{0.90 \times 1000} + \frac{0.275}{0.81} = 0.39+0.34 = 0.73\mu m$$

Or

$$\frac{d = \lambda\sqrt{N2 - (NA)^2}}{(NA)^2}$$

Where d is the depth of focus for photomicrography, λ (lamda) is wave length (550nm) and N is *index of refraction (1.25).* For visual use, one must add further depth, since the eye is capable of a certain amount of accommodation. In this case, the depth d' becomes: ***d' = d + (250 /M2)***

where M is the magnification of the microscope. The assumption made here is that the eye can accommodate an image 250mm away. Putting actual numbers in the above expression, we find that for the three most common objectives, we have the following depths of focus:

Objective	Eyepiece	Photomicro	Visual Depth of Focus
10x, 0.25 N.A.	10x	0.0080mm	0.0335mm
40x, 0.65 N.A.	10x	0.0010mm	0.0026mm
100x, 1.25 N.A.	10x	0.0003mm	0.0005mm

The visual depth of focus of the 100X, 1.25 N.A. oil-immersion objective is here shown to be only 0.0005 mm, or about a wavelength of light. This very tiny value indicates how closely one must focus when using such a high-power objective and indicates why a microscope needs a very finely controlled focusing motion.

Cover Slip Thickness

When viewing fluid materials such as bacteria, cell cultures, blood and the like, it is necessary to use a cover slip to protect the object under inspection and microscope components from contamination. A cover slip, or glass microscope slide, changes the way light refracts from the object into the objective. As a result, the objective needs to make proper optical corrections to produce the best quality image. Therefore, objectives denote a range of cover slip thicknesses for which they are optimized. Typically, this is listed after the infinity symbol (which denotes that an objective is an infinite conjugate, or infinity corrected design) and ranges from zero (no cover slip correction) to 0.17mm.

2.2. Complete Blood Count (CBC)

The complete blood count (CBC) is one of the most commonly ordered blood tests in most clinics and hospitals. The CBE test measures the quantity of all the different types of cells in the blood which are mainly white blood cells (WBC), red blood cells (RBC) and platelets. The CBE also provides some valuable information on other parameters related to the WBC and RBC but those are not considered in the current study. The RBC count is commonly performed on an automated hematology analyzer using well mixed whole blood that is added to a chemical called EDTA to prevent clotting. The complete tests are usually carried out in less than one minute. When the performance limit of the automated hematology analyzer is exceeded, however, sample dilution/sample pre-treatment, manual smear review, or manual cell counts may be required. Each laboratory has established rules for determining the need for manual smear review based upon specific RBC parameters. For example, a manual differential is always performed when nucleated immature RBCs are found on an electronic cell count.

RBC count: determines the total number of red cells (erythrocytes) in a sample of blood. Most anemias are associated with a low RBC count and hemoglobin.

WBC count: most CBC counts include both a WBC count and an automated differential. A differential determines the percentage of each of the five types of mature WBCs. An elevated WBC count occurs in infection, allergy, systemic illness, inflammation, tissue injury, and leukemia. A low WBC count may occur in some viral infections, immunodeficiency states, and bone marrow failure. The WBC count provides clues about certain illnesses, and helps physicians monitor a patient's recovery among other things. The differential will reveal which WBC types are affected most.

Platelet Count: platelets are disk-shaped structures formed by the detachment of cytoplasm from megakaryocytes. They aid in the coagulation process by attaching or adhering to the walls of injured blood vessels, where they stick together to form the initial platelet plug. A low platelet count may occur in patients with AIDS, for example.

In general, the human RBC values vary by age and sex. The normal values for men and non-pregnant women can be found in the literature [7]. The manual count of blood parameters has some obvious shortcomings: it is often time-consuming task, very subjective in nature and hardly repetitive. Automated procedures should alleviate some of these shortcomings. In this

regard, the current project intends to make the counting processes automated based on the concept of image analysis performed on color images of known data samples/smears acquired through the mobile phone microscope imaging device developed in this thesis study. This is usually considered the first step to identifying whether a patient has infection or not, to monitor treatment responses diagnosed with various forms of cancers, and the like.

Blood specimens are stored at room temperature for more than 1 day (up to 3 days or possibly longer) are acceptable with some limitations for CBC but not for the differential (identifying different types of WBCs). In general, when it is done inside clinics, the entire CBC procedure offers the following information [8]:

- RBC count – tells us the total number of RBCs. It gives number of RBCs in all SPP (species pluralis, the Latin for multiple species) in millions per micro liter of blood.
    - Low RBC count = Anemia
    - High RBC count = Polycythemia - draw blood to fix.
- WBC count – tells the total number of WBCs. The normal range of WBCs in all SPP equals 1,000's to 10,000's per microliter of blood.
    - High WBCs = Leukemia
    - Low WBCs = Immuno compromised, steroids, overwhelming infection.
- Hhemoglobin (Hb or Hgb) level – indicates the total amount of hemoglobin in the blood.
- Hhematocrit (Hct) – is the fraction of the blood made up of RBCs.
- Mean corpuscular volume (MCV) – the average RBC size.
- Mean corpuscular hemoglobin (MCH) – the average amount of hemoglobin per RBC.
- Mean corpuscular hemoglobin concentration (MCHC) – the average concentration of hemoglobin per RBC.
- Platelet count – the number of platelets.
- WBC differential count – the different types of WBCs expressed as a percentage.

2.3. Literature Review

2.3.1. CBC

The manual (Hemocytometer and microscope) counting of total blood cells is often boring, labor intensive, time consuming, and leads to the inaccurate results due to human errors. Currently, there are several systems available for automatic counting of complete blood cells (CBC) in the market. These systems allow counting of RBCc, WBCc and platelets within the blood smear slides but these counting machines are too expensive. Some of the techniques used in CBC count are:

- Clinical Approach (automation techniques - electronic blood cell counting): Electronic blood cell counting is based upon the principle of impedance (resistance to current flow). Some hematology analyzers combine both impedance counting with light scattering to measure platelets. A small sample of well mixed ethylenediamine tetraacetic acid (EDTA) blood is aspirated into a chamber (the WBC counting bath) and diluted with a balanced isotonic saline solution that is free of particles. The diluted blood sample is split into two parts, one for counting RBCs and platelets and the other for counting WBCs. The RBC portion is transferred to the RBC/platelet counting bath where it is diluted further. The other portion remains in the WBC bath and a detergent (lysing agent) is added to destroy (hemolyze) the RBCs. A small portion of the diluted fluid in each bath can flow past a small aperture. An electrical current is produced in each aperture by two electrodes, one on the inside and the other on the outside of the aperture. The saline solution is responsible for conducting current between the electrodes. The cells move through the aperture one at a time, when a cell enters the aperture, it displaces a volume of electrolyte equal to its size. The cell acts as an electrical resistor and impedes the flow of current. This produces a voltage pulse, the magnitude of which is proportional to the size of the cell. Instrument electronics are adjusted to discriminate voltage pulses produced by different cells, these adjustments are called thresholds. For example, the threshold for counting a RBC is equivalent to a cell volume of 36 femtoliters or higher. The optical density of the cyanmethemoglobin is proportional to hemoglobin concentration. The voltage pulses produced by the WBCs depend upon the size of the cell and its nuclear density. *One known issue with this technique of Electronics CBC is its expensiveness with associated maintenance and running costs.*

- *Thresholding Based Method*: The WBCs and RBCs are counted by using the gray thresholding algorithm proposed by Pooja R. Patil et al. [9]. The first step of their method involves RGB to gray conversion of the microscopic images of the samples. After that, median filtering is applied to remove noise in the background. Otsu's method is used for binarization of image. For proper segmentation of blood cells, holes presented in the binary image are filled using morphological procedures. The cells near the border contain less information and are removed to reduce the complexity. Labeling algorithm is applied to count the connected objects. Finally, form factor calculation is done to get the total RBC count.

- *Watershed Transform Based Method*: Hemant Tulsani et al. presented a method for counting of blood cells based on analysis of microscopic images [10]. The image processing techniques used for counting are spatial filtering, morphological operations and segmentation using watershed transformation. In the preprocessing step, smoothening of the images is done using an average filter. In the next step, the RGB images are converted to YCbCr color space and the Cb component is extracted to get nucleus and platelets from the images. Blood smear image is binarized and morphological opening is done to get the mask of WBCs and platelets. Individual images for WBCs and platelets can be obtained with another opening and image subtraction. Next, grayscale image of the blood smear is applied to opening by reconstruction and closing by reconstruction. Finally, the binary base image containing all the cells is obtained and mask is subtracted from it to get RBC binary image. The binary images are segmented using watershed transform.

- *Hough Transform Based Methods*: Venkatalakshmi B. et al. presented a method for automatic RBC counting using the Hough transform. The algorithm for estimating the RBCs consists of five major steps: input image acquisition, pre-processing, segmentation, feature extraction and counting. During the pre-processing stage, original blood smear is converted into Hue-Saturation-Value (HSV) color space. As the saturation component (saturation image) often shows the bright components clearly, it is further used for analysis. First step of segmentation is to find out lower and upper threshold from histogram information. The saturation image was then divided into two binary images based on this information. Morphological area closing

was applied to lower pixel value image and morphological dilation and area closing are applied to higher pixel value image. Morphological XOR operation was applied to two binary images and circular Hough transform is applied to extract RBCs.

- In another study, Vinutha H. R. introduced an automatic RBC and WBC counting using *computer vision* [11]. Similar to the study by Venkatalakshmi B. et al., the estimation of RBCs involves several steps: input image acquisition, preprocessing of acquired image, segmentation, feature extraction and finally counting. The pre-processing step consists of conversion of the original blood smear image into saturation image. Histogram thresholding and morphological operations were used for segmentation. Feature extraction is done with the help of morphological operations to differentiate between RBCs, WBCs, Platelets and background. Last step is to measure number of RBCs by using Hough Transform from the blood smear image.

2.3.2. Mobil Microscope

There are a number of efforts made to design and develop a mobile microscope by different scholars. Breslauer D. N. et al. published a paper and tried to demonstrate the use of a mobile microscope for use in capturing high quality bright field images of malaria parasites and sickle blood cells as well as fluorescence images of cells infected with the bacterium that causes TB [12]. The device could potentially become an important tool for medical diagnostics in the LRS/LIC, where resources are limited and laboratory facilities are scarce, but where mobile phone networks are universal. In another study Admasu Abate developed a mobile-based tele-pathology system for a low resource setting in Ethiopia [13]. There are several more mobile microscopy techniques proposed in the literature and readers could consult materials published online to learn more about the techniques. *Unlike our proposal, most of them not included either the full design of Microscope or tested with the common laboratory diagnostics CBC count or the web page for further reference for interpretation*

2.3.3. Web Development

Webs are developed to support diagnosis and other clinical laboratory practices. For example, the internet pathology laboratory for medical education hosted by the University of Utah Eccles health science library consists of general and systemic pathology, virtual patient, examination, anatomy – histology, pathology and other multi subject tutorials. Another site called WebPath educational resource contains over 2700 images with text that illustrate gross

and microscopic pathologic findings along with radiologic imaging associated with human disease conditions. For self-assessment and self-directed study, it incorporates over 1300 examination items. It also includes more than 20 tutorials in specific subject areas. Such computer-aided instructional materials support educational programs in the health sciences [14].

Another is the online Histology and Pathology Atlas for medical students, which is an instructional aid to self-directed learning. The primary aim of this web was to develop and pilot an on-line and accessible atlas of histology and histopathology, the Microanatomy and Pathology Atlas (MAPA), for use by medical students as an adjunct to learning histology and pathology in a medical school curriculum [15]. MAPA and similar web-based learning tools can be used to encourage and support self-directed medical students learning in histology and pathology and suggests that similar instructional tools may be useful in other disciplines to promote more active student learning [30]. The detailed is available in the following site: https://www.researchgate.net/publication/321639135_Design_of_an_Online_Histology_&_Pathology_Atlas_for_Medical_Students_an_Instructional_Aid_to_Self-Directed_Learning.

CHAPTERE THREE

PROPOSED MOBILE MICROSCOPE DEVICE AND CBC TECHNIQUE

The first stage in the development of the proposed CBC technique was design and assembly of mobile attachment microscope using simple smart phone, one objective and eyepiece lenses and other locally available materials so as to capture microscopic images of blood samples. The second stage involved developing a web page and uploads the clinical laboratory atlas for further interpretation of the laboratory image results. The last stage comprised of developing rigorous mathematical algorithm for CBC to count RBCs and WBCs from given blood samples.

3.1. Design and Assembling a Mobile Microscope

3.1.1. Coupled Microscope Mobile Design and Image Acquisition

The image acquisition device used for imaging purpose was made of a microscope coupled with a smart mobile. The first procedure is comprised of collecting the materials needed for the design of the proposed instrument: HTC-one and Samsung Galaxy III or IV Mobile phone, HP or Toshiba Intel Core i5 laptop, Microscope Eye pieces (10x, …), Microscope Objectives, Filters, Power supply (such as LED, 2x1.5A Battery, wires, connectors), Sample Holder (open and adjustable in 3D plane), Attachment Microscope Holder (closed and adjustable in 3D plane), Microscope holder cover and others. The next phase includes assembling the different parts of the microscope and couple to the mobile smart phone device using the designed holder. Though automation is possible, in this work the distance between the lenses and the samples is to be adjusted manually but based on scientific theory following the JIS standard. The whole coupled device is fixed in a simple holder made up of a plastic.

The mobile is attached to the eye piece of the microscope for easily sending the result to a lab information system (LIS) like in tel-radiography or in radiology information system (RIS). Below, we will visit the basic components of the microscope and their properties.

- ***Condenser:*** is designed to collect, control and concentrate light from the lamp onto the specimen as shown in Figure 4. The iris controls the amount of light.

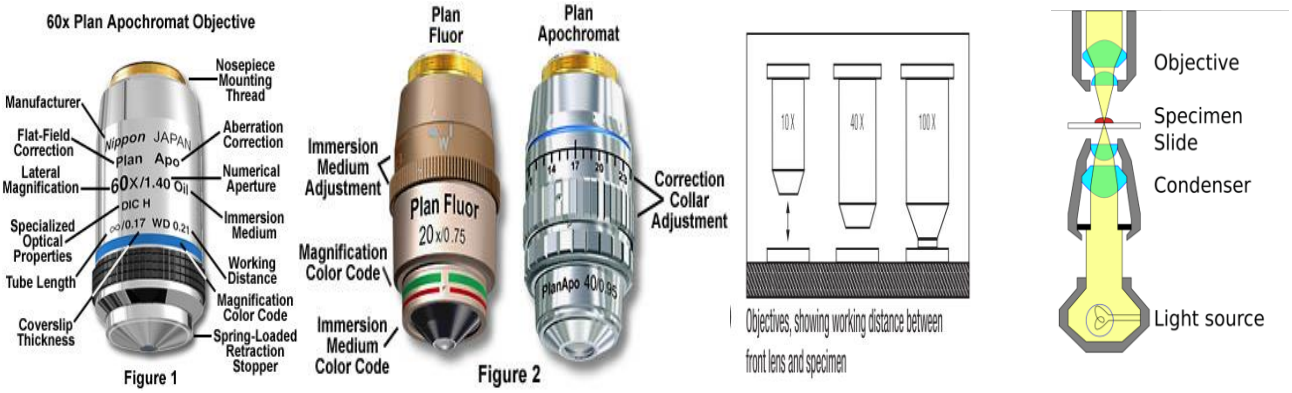


Figure 4: Microscope objective and condenser specification.

- ***Magnification:*** determines how big the image looks. As magnification increases, area seen decreases and magnification does not always mean better resolution. High magnification without resolution is not useful; this is known as 'empty magnification'.
- ***Numerical Aperture (NA):*** dictates how much light is collected from a specimen from the objective. This is a critical value that indicates the light acceptance angle, which in turn determines the light gathering power, the resolving power, and depth of field of the objective. NA determines resolution. Theoretically, the highest angular NA obtainable with a standard microscope objective would be 180 degrees, resulting in a value of 90 degrees for the half-angle utilized in the NA equation. The sine of 90 degrees is one, which indicates that NA is limited not only by the angular aperture, but also by the imaging medium refractive index, i.e. NA = n. sin(ø) = 1.52 sin (90º) = 1.52 x 1 = 1.52.
- ***Immersion Medium:*** most objectives are designed to image specimens with air as the medium between the objective and the cover glass but oil immersion has been used in the current work. Immersion in media with higher refractive index than air (such as oil) increase the amount of light collected, hence creates higher NA and greater resolution. At high magnification, more light can be captured if immersion medium such as oil is used.
- ***Working Distance:*** this tells how close the objective must be for optimal function.
- ***Depth of Focus/field:*** this is about the range of distance of the objective from the specimen within which the image will stay in focus. Depth of focus decreases with higher numerical aperture. The image will go out of focus with small movements of the objective when depth of focus is small.

- Eyepiece: is the viewing unit of a microscope. It can be mono ocular (one eye) or binocular when both eyes are used. Eyepieces add magnification (10x – 20x) to the image produced by the objective.
- Determining the Focal Length of the Lens: focal length of converging lens has radii of 10 cm and index of refraction 1.52. This results: 1/f = (n-1) (1/R1 – 1/R2) = (1.52 – 1) (1/10cm – 1/ (-10) cm) = 9.6cm.
- Cover Glass Thickness: Most transmitted light objectives are designed to image specimens that are covered by a cover glass (or cover slip). The thickness of these small glass plates is now standardized at 0.17 mm for most applications, although there is often some variation in thickness within a batch of coverslips. For this reason, some of the more advanced objectives have an adjustment of the internal lens elements to compensate for this variation. Abbreviations for the correction collar adjustment include Corr, w/Corr, and CR, although the presence of a movable, knurled collar and graduated scale is also an indicator of this feature.
- Parfocal Distance: This is another specification that can often vary by manufacturers. Most companies produce objectives that have a 45 millimeter parfocal distance, which is designed to minimize refocusing when magnifications are changed (see the illustration in Figure 5).

Figure 5: Parfocal length of the objective (45mm is standard).

- **Working Distance:** This is the distance between the objective front lens and the top of the cover glass when the specimen is in focus. In most instances, the working distance of an objective decreases as magnification increases. Working distance values are not included on all objectives and their presence varies depending upon the

manufacturer. Common abbreviations are L, LL, LD, and LWD (long working distance), ELWD (extra-long working distance), SLWD (super-long working distance), and ULWD (ultra-long working distance). Newer objectives often contain the size of working distance (in millimeters) inscribed on the barrel. The objective illustrated in Figure 5 above has a very short working distance of 0.21mm.

- **Focal Length of Eye Piece**: $F_e = L/M_e = 250/10 = 25mm$ assuming $M_e = 10x$ for the eye piece.
- **Focal Length of Objective**: $F_o = L/M_o = 250/100 = 2.5mm$ assuming $M_o = 100x$ for the objective.
- **Mechanical Tube Length**: This is the length of the microscope body tube between the nosepiece opening, where the objective is mounted, and the top edge of the observation tubes where the oculars (eyepieces) are inserted. Tube length is usually inscribed on the objective as the size in number of millimeters (160, 170, 210, etc.) for fixed lengths, or the infinity symbol (∞) for infinity-corrected tube lengths. The objective illustrated in Figure 4 is corrected for a tube length of infinity, although many older objectives will be corrected for tube lengths of either 160 (Nikon, Olympus, Zeiss) or 170 (Leica) millimeters.
- **Total Magnification:** M = Me x Mo = 10 x 100 = 1000x.
- **Resolution:** The quality of the image was not influenced by the brand or model of the mobile-phone used, but only by its digital resolution, with any resolution above 0.8 megapixels resulting in images enough for diagnosis [16]. In this thesis study, a 5MP HTC mobile camera was used.

Following the above listed specifications of the coupled microscope-camera device, in order to test its efficacy, tests were carried out through imaging well-established blood samples available on smears found at Tikur Anbessa Specialized Hospital (TASH) in terms of its capabilities in malaria and TB detection. Best images were used for further image analysis to develop the proposed robust CBC tool. The basic parts of the mobile microscope proposed in this thesis, the assembled version as well as the detailed components are shown in Figure 6.

Figure 6: Basic parts of the proposed mobile microscope (left), another view of the basic parts of the mobile microscope (middle) and assembled version of the mobile microscope (right).

3.4. Mobile Microscope Specification and Design Parameters

In order to design the proposed mobile microscope device, the DIN optical design and the finite scheme has been used in this thesis (see also

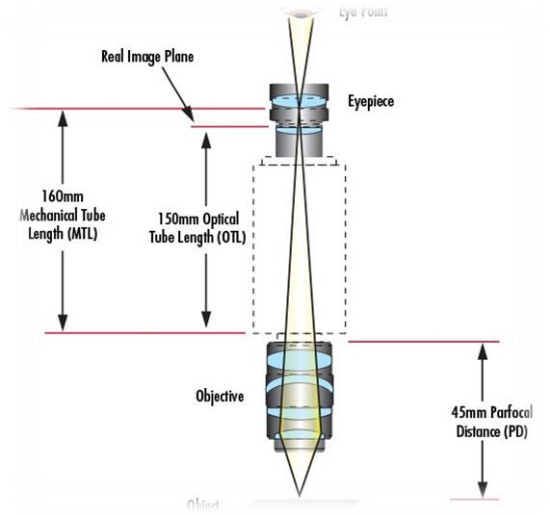


Figure 7. This design nearly followed this DIN Standard due to the height of the tube length

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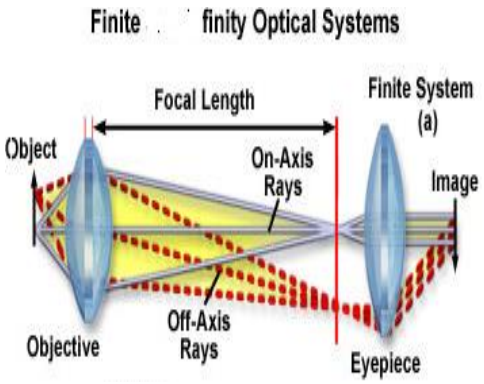


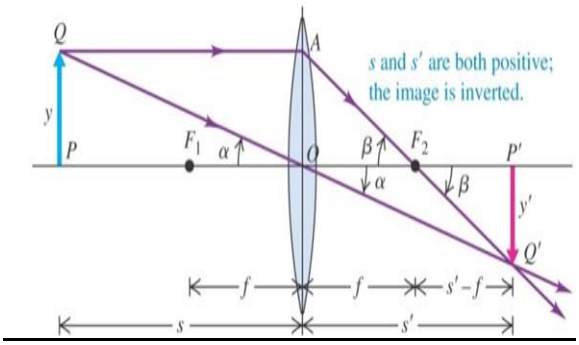


Figure 8a: The Finite optical system Figure. Figure 8b: Optical image formation by converging lens.

***Mobil** Phone **and Eyepiece Holder:** Made of Aluminum and tube length is 130mm as shown in Figure 9.*

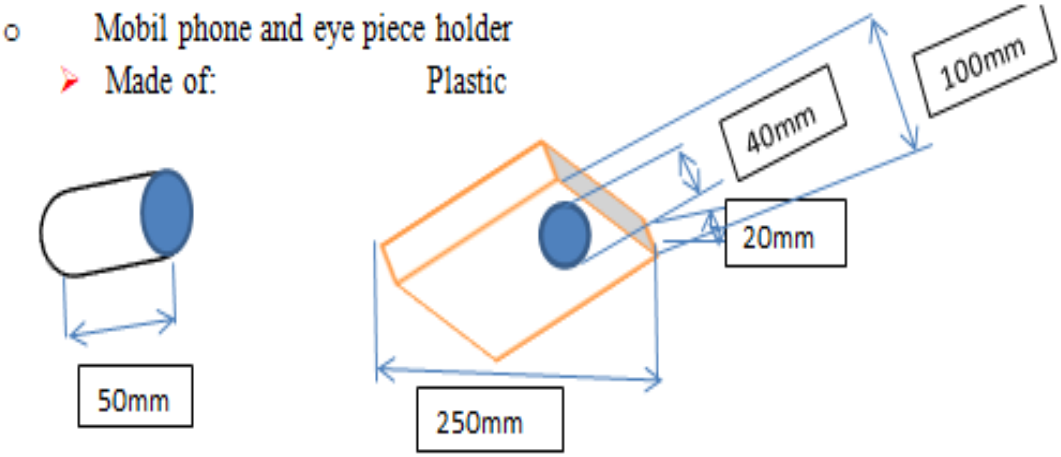


Figure 9a: Eye piece Holder & Mobile camera Attachment/Holder

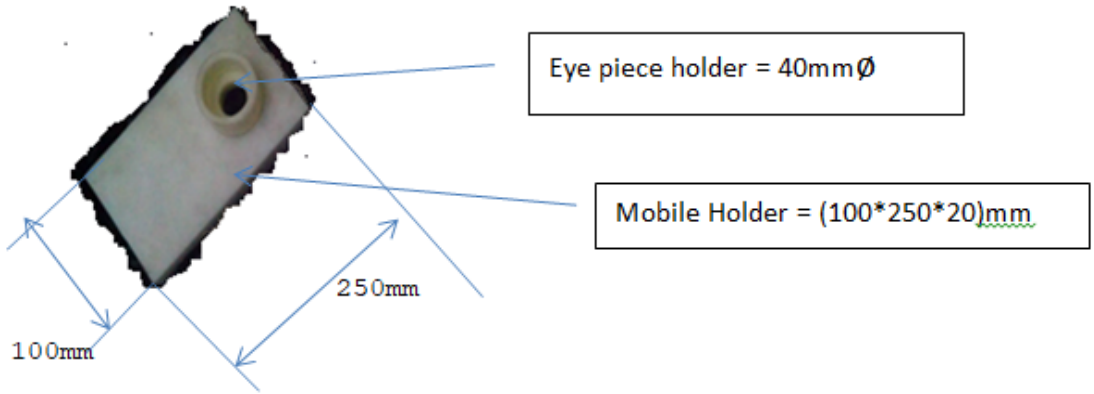


Figure 7 b: Mobile and eye piece attachment specification.

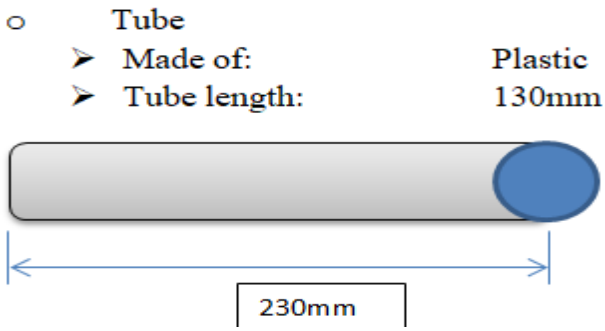


Figure 8a. Mobile Microscope Tube length.

In its simplest form, the microscope consists of two positive lenses, which are mounted at either end of a tube. The first lens has a very short focal length, and the object is placed in front of this lens just beyond the focal distance. Since the lens is a positive lens, it forms a real, inverted image at a distance L behind the lens. Since the distance from the first lens to the image is much greater than from the object to the first lens, this image is magnified by the ratio of these two distances. The size of the length L is set by the geometry of the device, and is often about 16 cm.

The objective holder used in the current thesis was made of metallic material in the 1st design but changed to plastic during the 2nd re-designing. The eye piece specifications include:

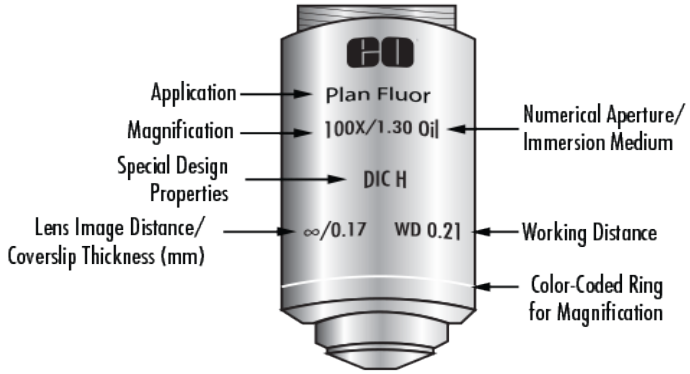
- View field diameter (SWF 10x Eyepiece): 2.12mm
- Amplification: 10X
- Model: GWH
- XXX: 1 R
- Company of origin: Japan
- Manufacturer Name: Olympus

3.5 Objective Specification (100X Plan Achromat)

Microscope objectives are usually designed to be used with a specific group of oculars and/or tube lenses strategically placed to assist in the removal of residual optical errors. As an example, older Nikon and Olympus compensating eyepieces were used with high numerical aperture fluorite and apochromatic objectives to eliminate lateral chromatic aberration and improve flatness of field. Newer microscopes (from Nikon and Olympus) have objectives that are fully corrected and do not require additional corrections from the eyepieces or tube lenses. Figure 10 presents objective lens basics.

The objective specifications adopted in the current thesis work are listed below.

- Manufacturer: LIESS
- Country of origin: Japan
- Thread type: Nosepiece mounted Thread
- Plan Field Correction: Plan
- Aberration Correction: Achromat
- Magnification: 100X
- Magnification color code: White
- Immersion medium: 1.25 Oil
- Working distance (WD): 0.21
- Coverslip thickness: 0.17
- Tube Length: Infinitive (∞) but in our design TL is finite ~ 13cm
- Stopper type: Spring loaded retraction stopper
- Sp. Optical property: Achro



Magnification	1X	2X	3X	4X	10X	20X	40X	60X	100X
Color Code	Black	Gray	Red	Yellow	Green	Light Blue	Light Blue	Dark Blue	White

Figure 9: Objective lens basics [1].

The f-number is the focal length divided by the aperture size, also called the f/D ratio. For example, a 50 mm lens with an aperture size D = 25 mm has a f-number =f/D = 50 mm / 25 mm = 2. So, it would be said to have a f-stop of f/2. Since exposure time depends on the area of the aperture, f-stops changing by square-root of 2 change the exposure time by a factor of 2. Typical f-stops are f/2, f/2.8, f/4, f/5.6, f/8, f/11 and f/16.

Example: A common telephoto lens of a 35 mm camera has a focal length of 200 mm; its f-stops range from f/2.8 to f/22. Then the range of apertures is from D=f/f-number=200mm/2.8=71mm to D= 200mm/22=9.1mm, i.e. between 9.1mm-71mm. Intensity is proportional to square of D. Hence, the corresponding intensity ratio is (71mm/9.1mm)2= (22/ 2.8)2 ~= 62. That means, for an exposure of 1/1000s at f/2.8, we would have to expose

for 62/1000 s ~= 1/16 s at f/22. Note that the larger the f-number, the smaller is the aperture opening.

Figure 11 presents a comparison between general setup of a CCD camera, the scientific camera and that of a mobile phone camera. Figure 12 compares the mobile attachment distances used in the current study against the JIS standard.

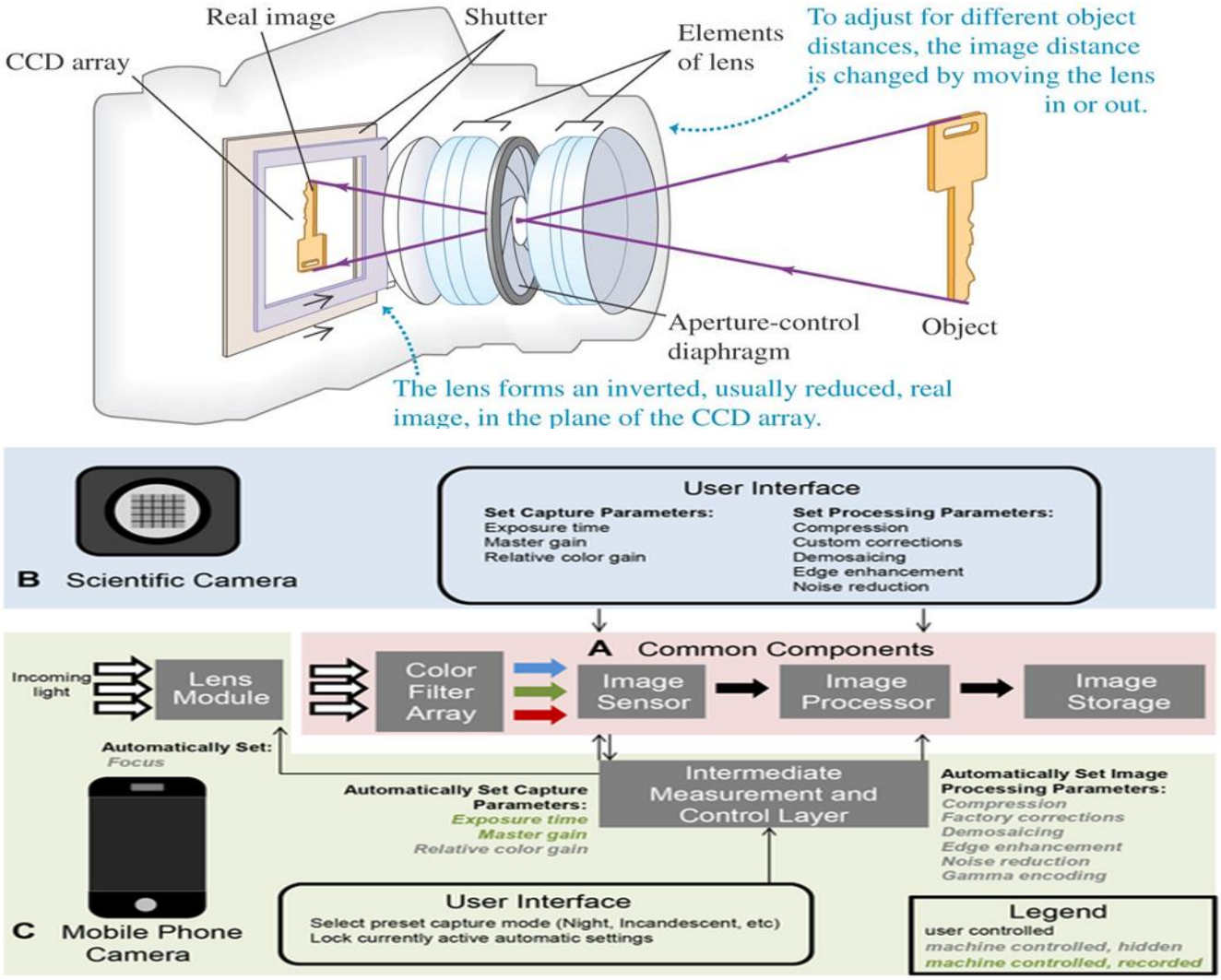


Figure 10: Camera design and parts: CCD camera (top), scientific camera (middle), and Mobile phone camera (bottom).

HTC Mobile Phone Specifications: listed below are the detailed specifications assumed for the HTC mobile used in this thesis work.

- Manufacturer: AT&T, Dolby Mobile
- Model: IMEI/IMEISV
- Hard Ware

- Memory (camera): 768MB
- Main Camera: 8M
- Support: Wi-Fi, Bluetooth
- Display: 4.3’ = 4.3*204cm = 10cm
- Memory (SD): 1.83GB
- Memory Internal: 0.97GB
- Software
    - Operating System: Android Version 2.3.3
    - Sensor: Sensor Version 2.1
    - Based Band Version: 12.56.60.25V/ 26.10.04.03-M
    - Kernel version: 2.6.35.10-GD 2564FB, HTC/Kernel@&18-2#1
    - CST: Jun 2011
    - Build #: 2.47.502.7 CL93396
    - Software#: 2.47.502.7
    - Browser version: Web kit/533.1
    - Legal info
    - HTC: Legal
    - Google: Legal
    - Open source: Legal
    - Mobile Networking for tele-medicine and tele-laboratory
    - Acquired image – stored in HTC mobile – shared with Viber – to ref. Lab.
    - Received image – download – image process – CBC result – back to HEW.
    - Image processing (Hough transform) for CBC.

Dimensions and Specifications: Summary

- Mobile Microscope: HTC
- Software: Android
- Hardware: Tell HTC
- Objective: 45mm
- Eye piece: 28mm
- Condenser: 60mm condenser with eyepiece diaphragm
- Light: 65mm LED/battery powered
- Sample holder, base and stand

- Space b/n condenser & stage: 5mm
- Sample holder: 75x25x1mm
- Sample stage: 100x78x2mm
- Base: 184x175x2mm
- Stand: 60x60x192mm
- Stand Expander: 70mm
- Fine & Coarse adjustment: Standard
- Tube: 130mm
- Mobile tele-medicine and tele-laboratory Networking
- Hardware: Tablet/Desktop/Laptop (min. dual core)
- Software: Viber, Math lab, Hough transform
- Mobil Microscope

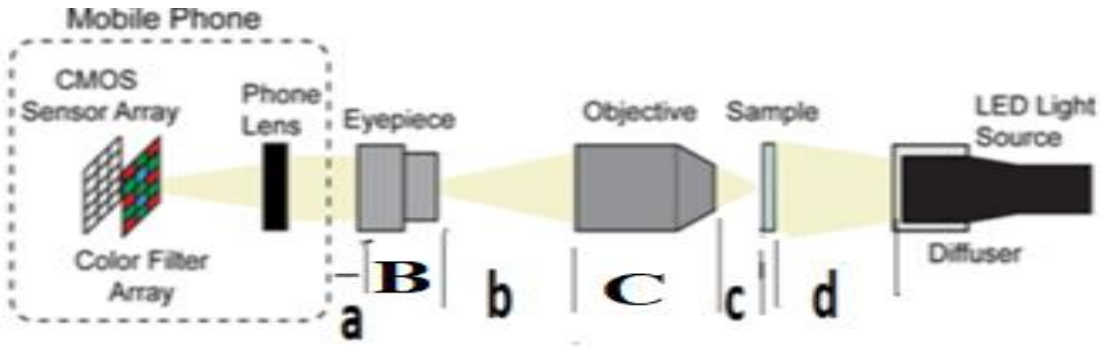
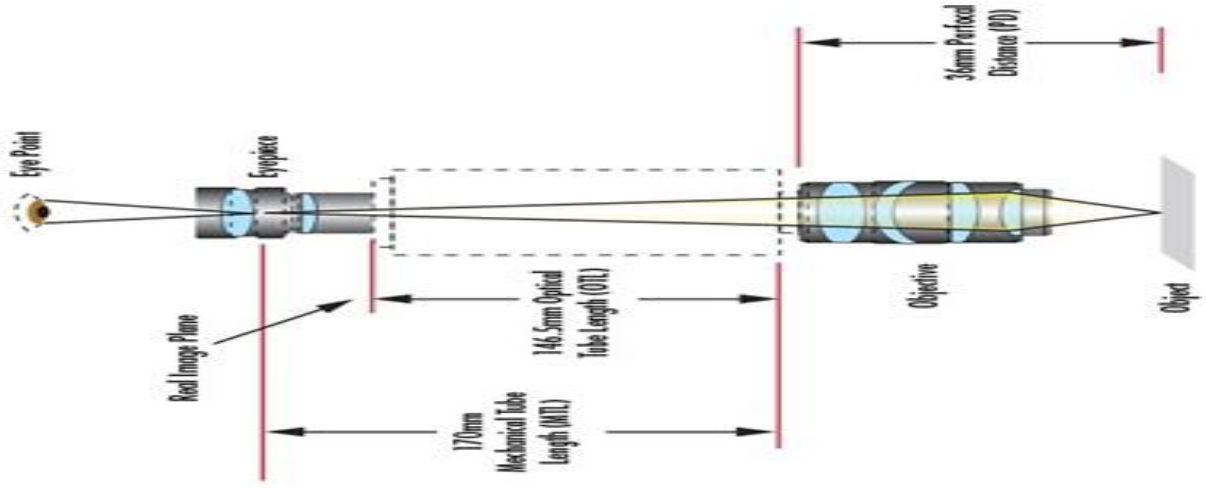


Figure 11: (Top) As per the JIS standard, the following mobile attachment distances have been adopted: OTL=b=146mm, MTL=B+b=170mm, PO=C+c=36mm, a=1mm, and d=12mm (adjustable); (Bottom) the JIS standard.

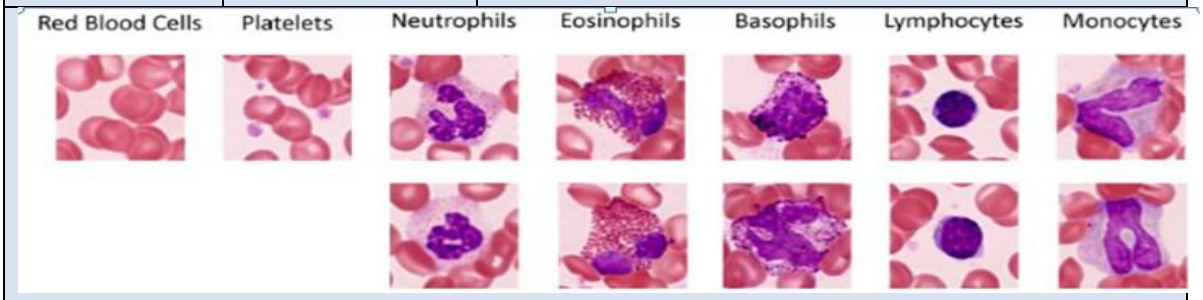


3.6 Data Collection

The website developed in this study is named "jossyBME.com". Few snap shots of the website are included in the annex section of this thesis document (annex D). Uploaded Atlas of malaria parasite images from WHO was imported into the jossyBME site. The web site is available for free use by lab technologists and biomedical professionals. Images were captured using the proposed mobile microscope device using samples collected from TASH. Some selected sample images are displayed in the result section as well as in Annex B. The abnormal samples contained malaria cases confirmed by experts. Note that some of the results in this thesis study have been presented at WHO 2nd and 3rd global medical device conference in Geneva, Switzerland to get feedback from experts who attended the conferences.

Note: CBC test parameters including RBC, WBC and Platelets

RBC	Platelets	WBC



3.7 Mathematical Algorithm for CBC

As said in previous chapters, CBC count is needed for identifying many diseases. Traditionally, RBCs and WBCs are counted manually by experts from blood smears with haemocytometer. The manual procedure is very time consuming and prone to inaccuracies depending on the skills of the lab technology expert dealing with the subject. CBC is done to provide information about a person's general health, assess how well the blood-forming organs (for example, bone marrow and spleen) are functioning, check for anemia (a reduction in the number of healthy RBCs), check for *infection,* provide a baseline to compare with future CBCs during and after treatment, and *monitor the effects of therapy*, especially therapies that can cause bone marrow suppression (a condition in which the bone marrow does not produce normal number of RBCs, WBCs and platelets).

CBC test is done in laboratories based on some known steps. Blood is usually taken from a vein in the arm. A tourniquet or elastic band is wrapped around the upper arm to apply

pressure to the area and make the vein swell. Then the skin is cleaned and disinfected. A needle is inserted into the vein and a small amount of blood is removed. After the sample collection, smearing and staining follow. Then images are captured through a microscopic examination. Figure 13 summarizes the microscopic examination procedures, preparation of thin and thick blood films on a sample slide together with a normal and a leukemic microscopic sample images.

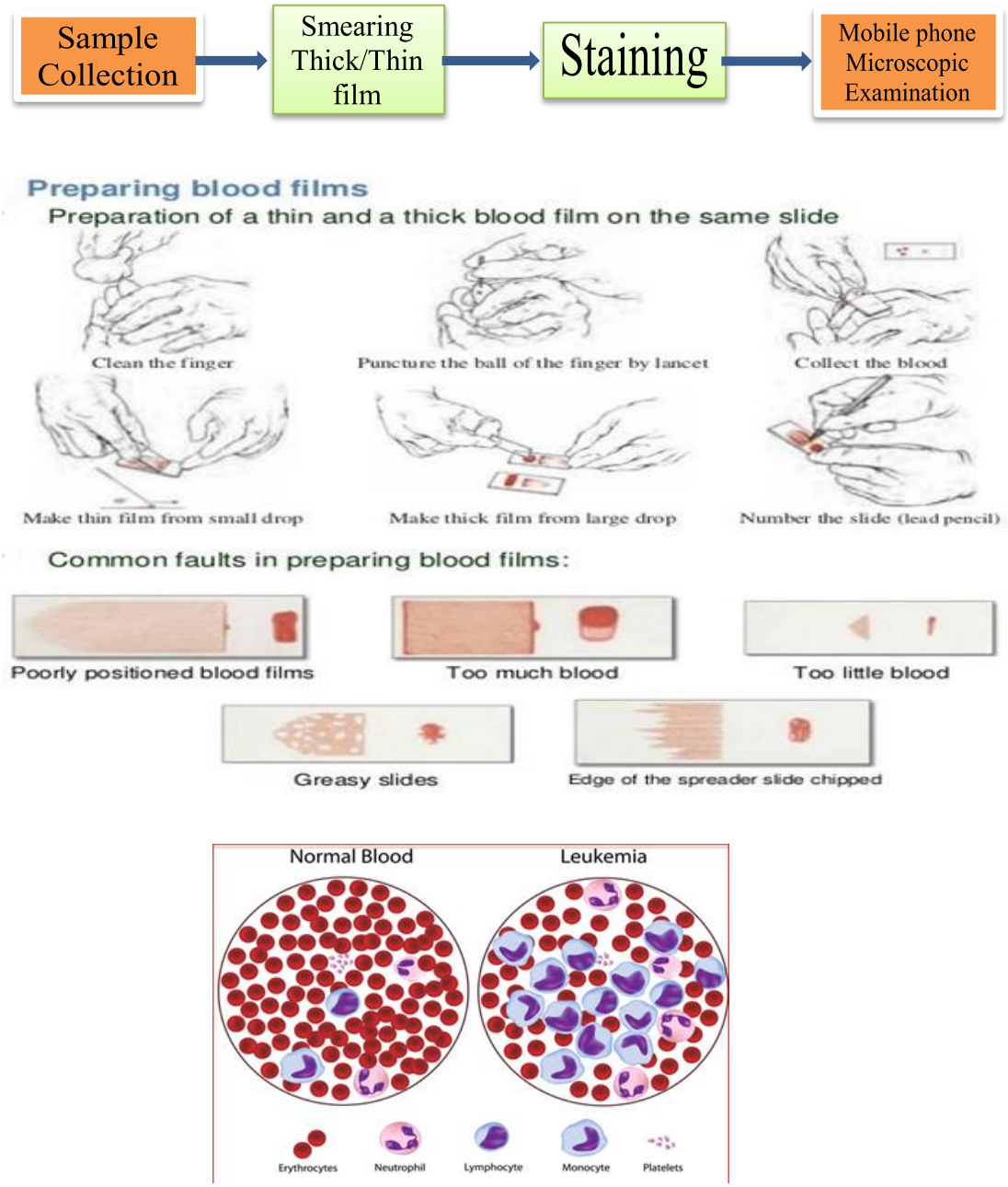


Figure 13: Summary of the microscopic examination procedures (top), preparation of thin and thick blood films on sample slide (middle), normal and leukemic microscopic images (bottom). (Source: www.MedGurus.org).

Once the images are captured, they are stored in a JPG format which is the default format set in the mobile microscope in the current study. The CBC count involves three main steps. First one is pre-processing which incorporates RGB to grayscale conversion. The second step is the application of Hough transform used to segment and separate the different blood cells inside the given sample. The last stage is the counting of the different blood cells and display of the results. The general steps are summarized in Figure 14. All steps are implemented in a Matlab environment. The counting is essential to classify the samples into normal and abnormal (Leukemia, Anemic, etc...).

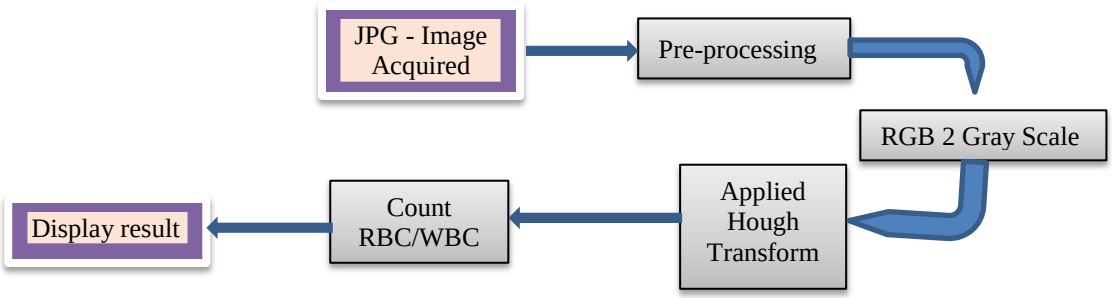


Figure 12: The general steps involved in the proposed CBC count scheme.

The performance of the proposed scheme is compared against traditional blood cell counting methods that rely on geometric features extracted from raw images (such as simple diameter and area of the RBCc, WBCs and the platelets). See also Appendix C. Final validation is made against available gold standard which is the physician/pathologist manual assessment together with output from Hematology Analyzer. Accordingly, quantitative performance evaluation of the proposed CBC counting scheme is carried out in terms of its efficiency, which in this thesis is quantified in terms of relative errors.

3.8 The Web and its e-health Applications

The images acquired through the proposed coupled smart phone-microscope device are primarily saved on the device for different purposes: To compare against the existing standard Atlas (see also Appendix A) images from "jossyBME.com"; To be sent through email or viber to either a pathologist, a lab technologist or other expert for disease diagnosis (malaria, TB detection) and feedbacks could be sent back to the smart phone; or To be sent to a PC or laptop for further image analysis work for CBC.

Data from beneficiaries (community) is collected by CHWs/HEWs using a mobile data collection tool that wirelessly sends pictures to the cloud. Then it can be accessed by a referral healthcare laboratory technologist or pathologist on a laptop platform. Figure 15 depicts the information flow diagram. The web, jossyBME.com, was built on a "JOOMLA" platform. It contains the lab Atlas and microscopic training materials, and a link to national/international resources related to HTM and BME. Particularly in places where a referral Lab Information System (LIS) is available, this system can have tremendous importance.

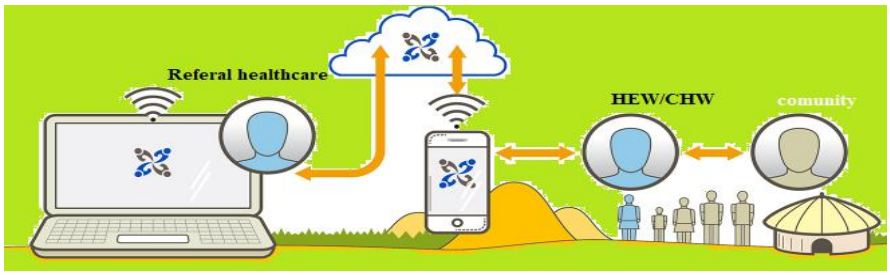


Figure 13: This is the most basic version of an information flow diagram.

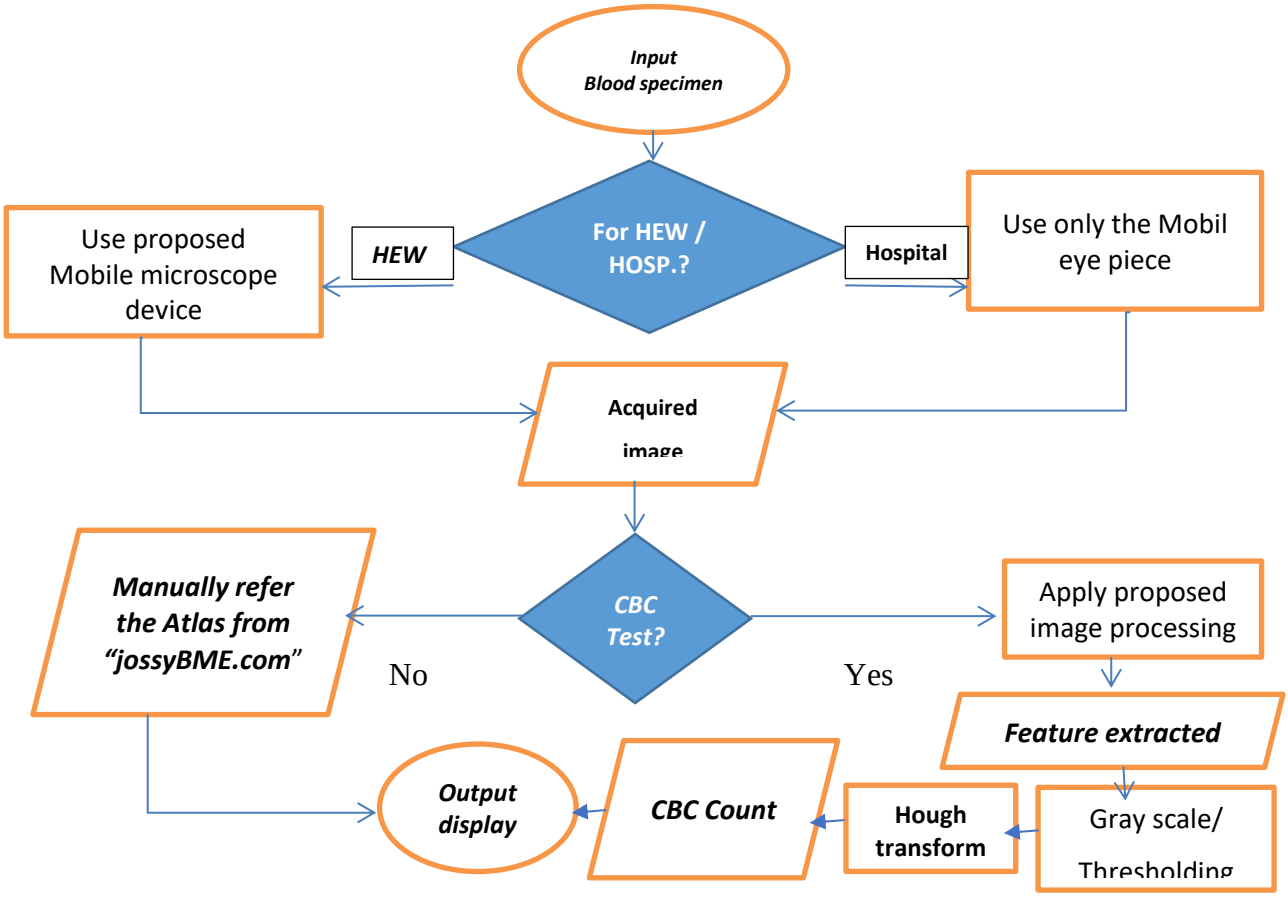


Figure 14: General flow diagram of the proposed scheme.

3.9 Project Feasibility Study

3.9.1 Technical Feasibility

The project has been assessed for its technical, financial, legal and operational feasibility. The financial assessment typically involved a cost/benefits analysis of the project. The proposed device has been clinically approved by the IRB (Institute of Review Board) and is under process of patent registration by the Ethiopian patent right agency. The IRB is the decision-making body established to govern all PIPs (product innovation processes). Figure 17 presents the product innovation processes from gate 0 to gate 3. The project fulfilled the design-dependent parameters including reliability, maintainability, supportability, usability, reducibility, disposability, sustainability, affordability, accessibility and others. It promotes the e-health policy of the government of Ethiopia.

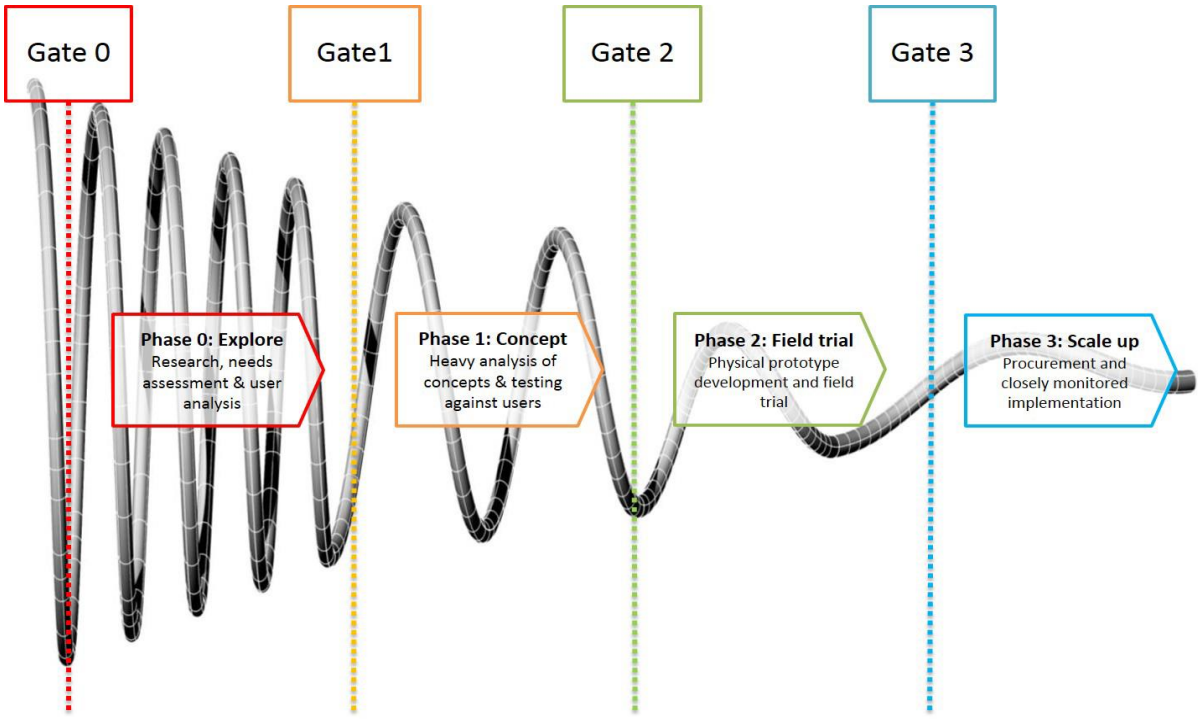


Figure 15: The product innovation processes (PIPs).

The process for developing PIPs consists of four phases and corresponding Gates with criteria that projects must meet to pass through a Gate (UNICEF). The purpose of the process is to ensure an iterative learning-based approach. The coils in the diagram represent ever decreasing iterations through the project's life-cycle. Iterative based learning innovation projects, by their nature, contain uncertainties, more so during their initial phases than near their completion. Planning details, both long and short term, will depend on what is

discovered whilst addressing these uncertainties. Rather than focusing on sticking to predefined project plans, the project should focus on the end-deliverable (s) and allow the planning to change according to the learnings generated. A project's initial phase will contain substantial and frequent iterations (as illustrated by the larger coils above). Towards the project's completion, iterations are anticipated to be fewer and lighter because of less uncertainty meaning that more detailed planning can be made covering a longer period. During all phases several uncertainties specific to that phase should be addressed. In addition, many generic uncertainties should be addressed. Note that the device proposed in this thesis is at stage of phase 2 and it needs to be tested in different environment conditions before it reaches phase 3 which is the scales up phase.

3.9.2 Ethical Considerations

The use of patient samples was approved jointly by the AAU and the health institutions (Health Science College (HSC) and TASH) IRBs. Written consent was obtained for all patient samples.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 Results

Mobile microscope designed

The new mobile-microscope assembled with its different parts of the prototype microscope and coupled with the mobile smart phone device using the designed holder. HTC-one and Sony-Xperia Mobile phone, HP Intel Core i5 laptop, Microscope Eye pieces (10x), Microscope Objectives(40x, 100x), Power supply with condenser and Attachment Microscope Holder. Though automation is possible, in this work the distance between the lenses and the samples is to be adjusted manually but based on scientific theory following the JIS standard. The whole coupled device is fixed in a simple holder made up of aluminum.

The mobile was not only attached to the eye piece to the new designed device to capture the clinical laboratory sample image but also on the standard microscope for hospitals to reduce ergonometric effect and for further consultation and evaluation, a role important enough that a medical communication standard (DICOM) has been widely adopted for the handling of digital images or easily sending the result to a lab information system (LIS) like in tel-radiography or in radiology information system (RIS). Below, we made the V-1 and V-2 designed mobile microscope which encourage the local production of Medical device in Ethiopia.

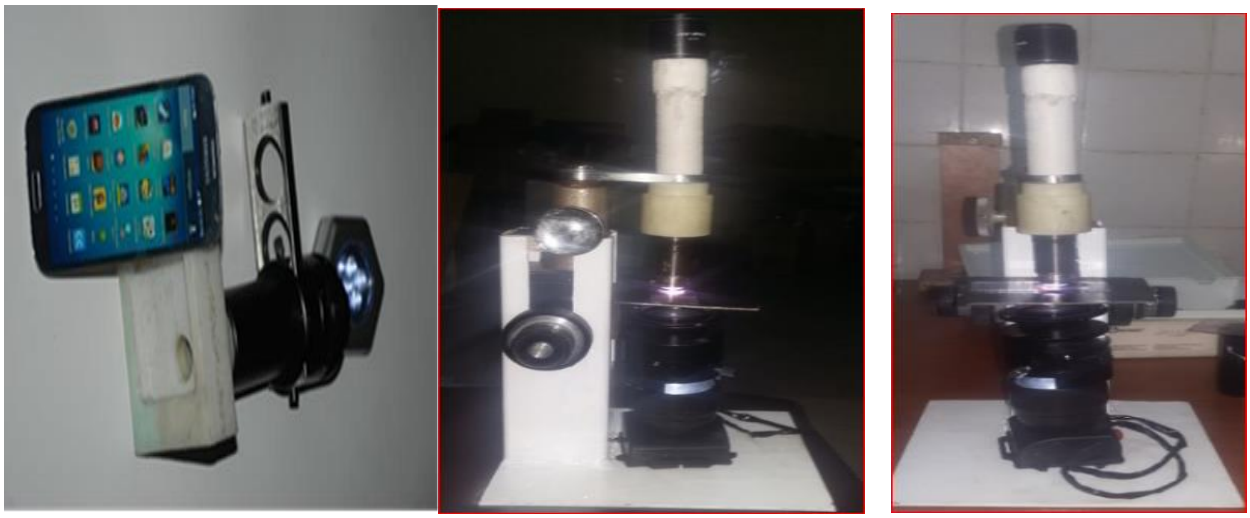


Figure 166: The first mobile phone microscope assembly result (left) and two views of the re-designed version of the mobile microscope (middle-right).

Web designed: http://www.jossyBME.com

Figure 177: A snap shot of the jossyBME.com website.

Table 3 presents the different types of sample images considered in the current thesis work. The interpretation of the samples was done by a senior lab technologist. It was on these types of images that the proposed CBC system was applied. A total of 100 specimens were used to test the efficacy of the proposed system. The result is compared against available gold standards (hematology analyzer and manual CBC). The result is summarized in Table 4 below and plotted in Figure 18. Accordingly, the proposed algorithm was able to detect WBCs with perfect accuracy (100%) assuming the manual method as a gold standard. The accuracy, which was measured in terms of relative error, of the method in detecting RBCs was between 86.7% and 100% or 93,5% in average after all samples were considered.

Validation of the designed mobile microscope with real clinical lab blood sample in TASH

Table 3: Different types of sample images considered in the current thesis work. Interpretation was done by a senior lab technologist.

No	Sample type	Lab unit	Tested sample type	Result	Remark
01	Hematology cell	Teaching lab	Blood	RBC, WBC	
02	Hematology cell	Teaching lab	Blood	RBC, WBC	
03	Bacteriology tissue	Teaching lab	Blood	Not clear	Poor slide
04	Bacteriology tissue 2	Teaching lab	Blood	Not clear	Poor slide
05	Symptoms	Teaching lab	Blood	Acid fast bacilli	
06	Symptoms 2	Teaching lab	Blood	Acid fast bacilli	
07	Hematology parasite	Teaching lab	Blood	Malarial parasites	
08	Hematology parasite 2	Teaching lab	Blood	Malarial parasites	
09	Hematology cell	Teaching lab	Blood	Blast cell immature	
10	Hematology cell 2	Teaching lab	Blood	Blast cell immature	
11	Whole blood cell	Teaching lab	Blood	MM	
12	Whole blood cell	Teaching lab	Blood cancer	AML	
13	Whole blood cell	Teaching lab	Blood cancer	CMML	
14	Whole blood cell	Teaching lab	Blood	Malaria Parasite	
15	Whole blood cell	Teaching lab	Blood	Neutrophil	
16	Whole blood cell	Teaching lab	Blood	RBC blood cell	
17	Whole blood cell	Teaching lab	Blood	Hair cell	

Table 4: Result validation through comparison with existing gold standards manual (CBC).

Specimen #	Gold Standard (Manual)		Proposed Algorithm		Efficiency (%)		Remark
	#RBC	# WBC	#RBC	#WBC	% RBC	% WBC	
Specimen 1	35	3	31	3	88.6	100	
Specimen 2	22	0	25	0	88.0	100	
Specimen 3	37	2	35	2	94.6	100	
Specimen 4	30	2	30	2	100	100	
Specimen 5	45	1	39	1	86.7	100	
Specimen 6	29	3	31	3	***93.5***	100	
Specimen	…	…	…	…	…	…	…
Specimen 100	30	1	29	1	96.7	100	

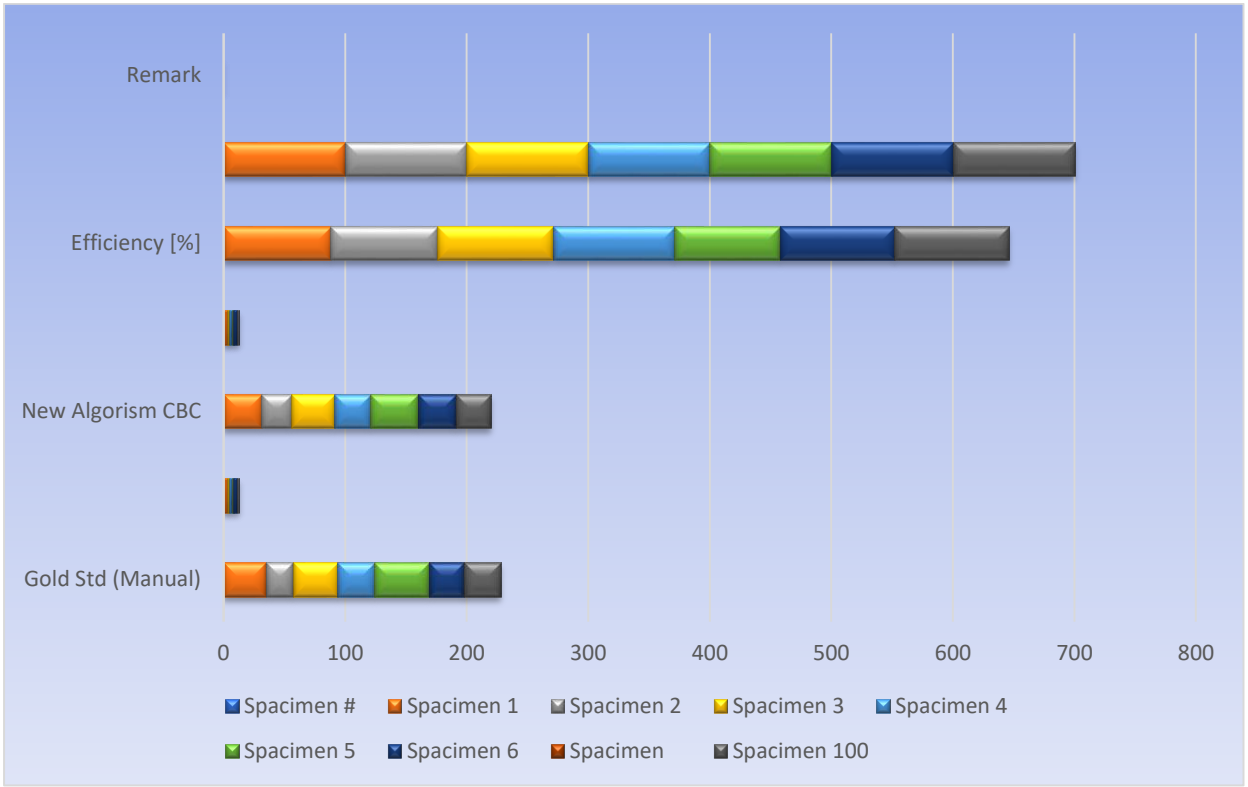


Figure 18: Validation of the results through comparison with manual CBC count.

4.2 Discussion

The result clearly show the great promises the proposed device and CBC scheme in this thesis showing its potentials to be the candidate of the current merged technology for LRS/LIC.

The new designed Microscope does not need Healthcare infrastructure such as clinical laboratory setup and electrical power. It is mobile/handheld that able to serve the Pastorals society and reach the un reachable community. Promote the task shifting to Tel-laboratory.

It is Validated through CBC counting using Hough Transform algorism and resolved the following problem which exhibited in the existing manual and Automatic CBC counting system

The manual (Hemocytometer and microscope) counting of total blood cells is often

1. Boring,
2. Labor intensive,
3. Time consuming, and
4. Inaccurate results due to human errors.

The automatic Electronics CBC count is expensive with associated

1. Maintenance and
2. Running costs.

The new device encourages the implementation of the following in Tele laboratory

1. E-health(tele diagnosis, e-learning),
2. M-health strategy of the MOH.to meet the universal health coverage (UHC)

Moreover, it supported in reduction of the ergonometric effect on the clinical laboratory technologist.

This CBC count served as a proof of principle that clinical imaging of hematologic and infectious diseases is possible with smart mobile phone camera technology combined with our new mobile microscopy attachment.

The web page is serving to support the clinical lab technologist to refer there tested sample with the global atlas clinical laboratory images.

4.3 Limitations

- The sample collection is varied based on skill and commitment of the expert.
- Preservation and despatch of some of the samples was poor.
- The Atlas automated mobile application software is not yet developed.
- The proposed solution works for piloting but not as full-fledged as Medico legal on clinical trial practices.
- Additional fund is needed for improvement and scale up for real world applications.

4.4 Challenges

- The species and each stages of malaria parasite exhibit versatile shape and size. That limited the analysis only on Plasmodium Falciparum Trophozoite stage, that accounted for more than 90% epidemic than the other type of Malaria species in Ethiopia.
- Blood specimens stored at room temperature for more than 1 day (up to 3 days or possibly longer) were found to be acceptable with some limitations for CBC (but this is not true if a differential has to be done which is not considered in the current thesis work).
- The project took longer time to be feasible or to reach at this level. Some of the reasons include: fresh malaria samples were unavailable in Addis Ababa hospitals and the other regions as the case is seasonal. So the samples used in the current study were taken from two different sources: one set is collected from preserved samples while the other was collected from regions outside Addis and during malaria epidemic.
- Poor blood sample collection and preparation in both (thin and thick) film.

CHAPTER FIVE

CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

The proposed coupled smart phone-microscope device is a simple technology that can be used to acquire microscopic images of a given blood sample. It is composed of both software as well as hardware components. Once the device is installed, it can easily identify malaria and other blood parasites which are invisible with the naked eye and difficult to detect from microscopes by less skilled lab technicians. The proposed solution should alleviate the problems with the subjective and time consuming manual procedures carried out inside clinics. Particularly, in low resource settings, its use is assumed to be tremendous. For referral and University hospitals, the device can be used for teaching purposes. For general hospitals, the device can replace use of CCD cameras for disease diagnosis. It can be integrated into a lab information system (LIS) as part of HIMS. For primary hospitals and health centers, it can be used to get further skills to the clinical professional and to get support from the next referral and university hospitals through its networking capability. For HEWs, the device highly supports and strengthens homecare services. That increases accessibility, availability, equity and quality of healthcare services. It can save more lives as a portable, point of care device and that makes the HEWs to be capable of implementing effective home to home services. Moreover, it reduces human errors and percentage of medical problems reported by microscope operators in high level hospital laboratories.

The new device not only alleviated the problem mentioned in this paper such as percentage of ergonometric effect on clinical lab technologist, inadequate access to clinical microscopy in LIC, but it would provide remote access to digital patent result record keeping, automated sample analysis (CBC counting), involve high skilled expert diagnosticians via tele-laboratory system , and epidemiological monitoring – the latter enhanced by the ease of location-tagging patient data by GPS location data. Combining the mobile phone microscopy system with automated sample preparation systems could address challenges associated with use by minimally-trained health extension workers.

The new device has been clinically tested using more than 100 blood samples and it was found that it yields more that 90% correlation to a lab technologist’s diagnosis. The device is

flexible and comes with a simple design to couple a mobile phone with a microscope. Furthermore, it should be a cost-effective alternative to existing devices.

5.2 Recommendations and Future Works

The proposed device is leading as a benchmark in projects related to m-health local production and technology. It can simply be converted into a blood chemistry machine in the clinical laboratory. With the increasing acceptance of mobile phones as sensing and health tools, however, existence of the camera toolkit for biomedical applications would have a significant impact. Introduction and scaling up of such technologies through innovative ways helps low resource setting countries to meet certain United Nations Sustainable Development Goals (UN-SDG).

Acknowledgment: This project has received funding from the Ethiopian Ministry of Science and Technology.

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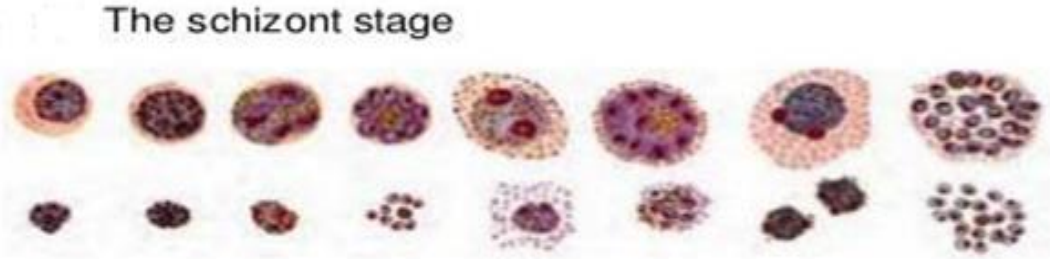
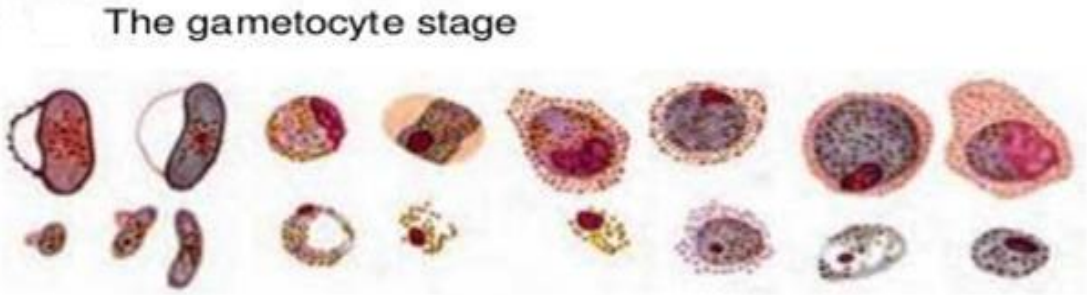
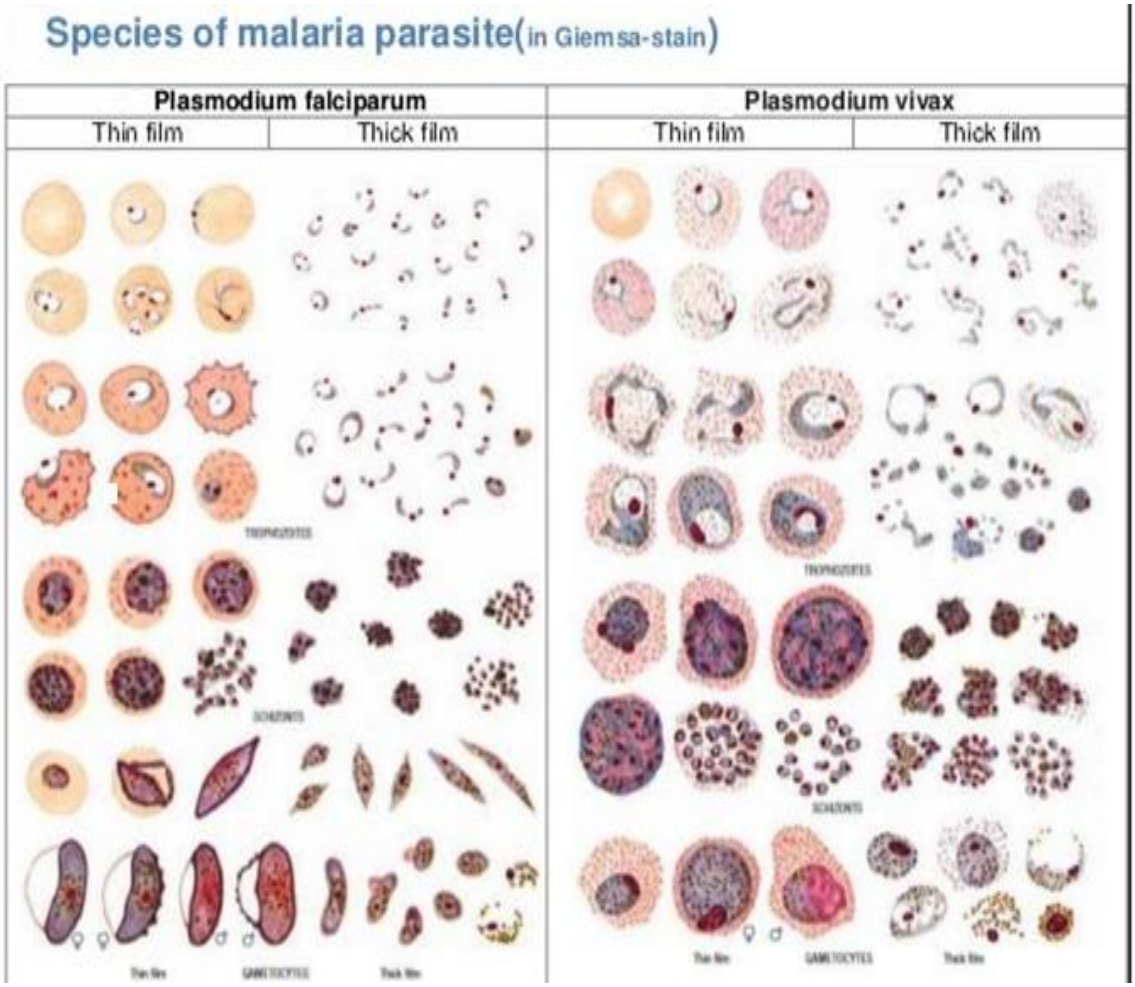
APPENDICS

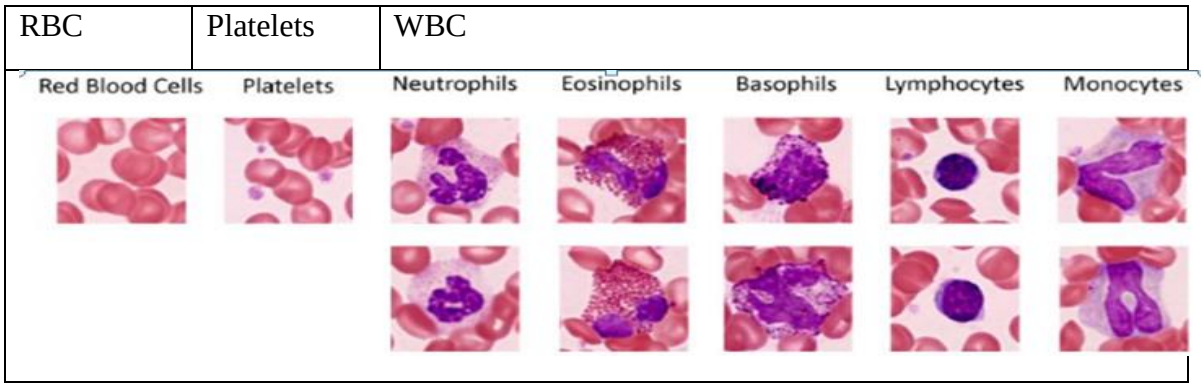
Annex A. The clinical laboratory atlas for blood sample







Annex B. Different laboratory sample images taken by the proposed mobile microscope.



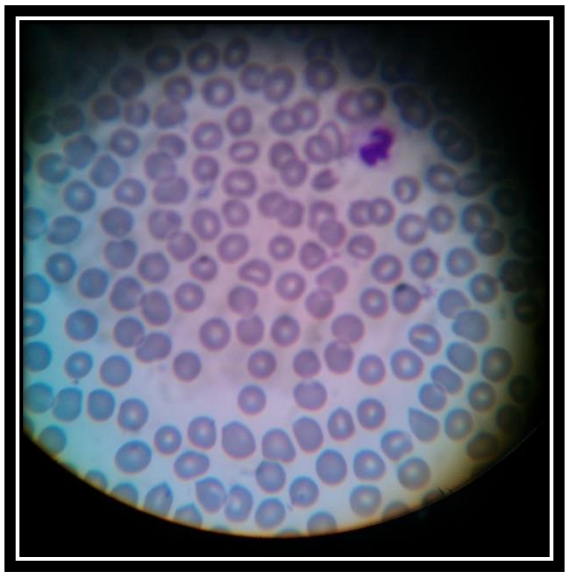


Image 31

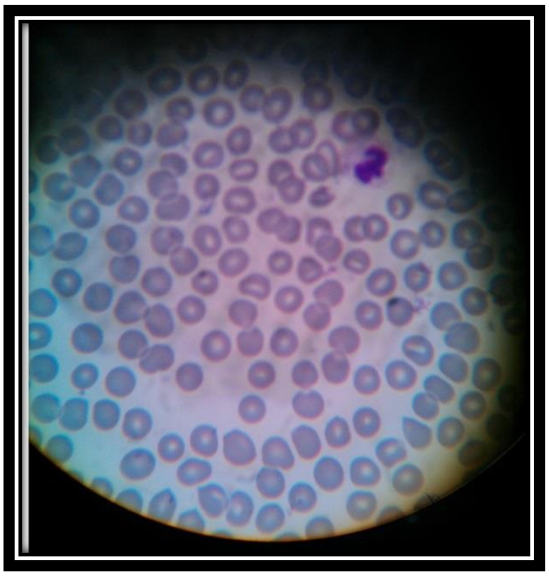


Image 32

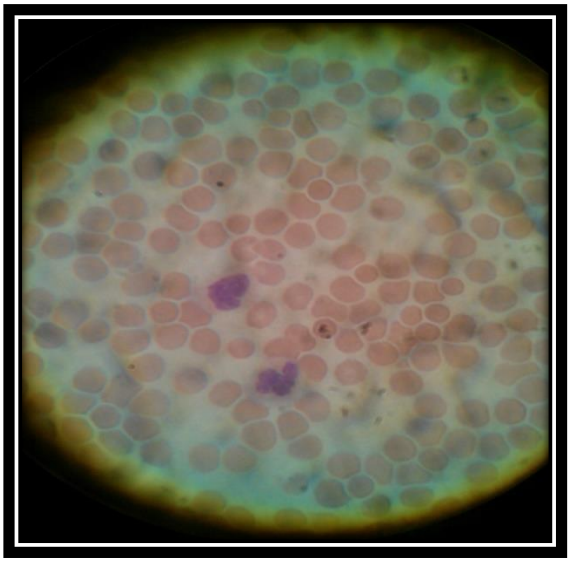


Image 33

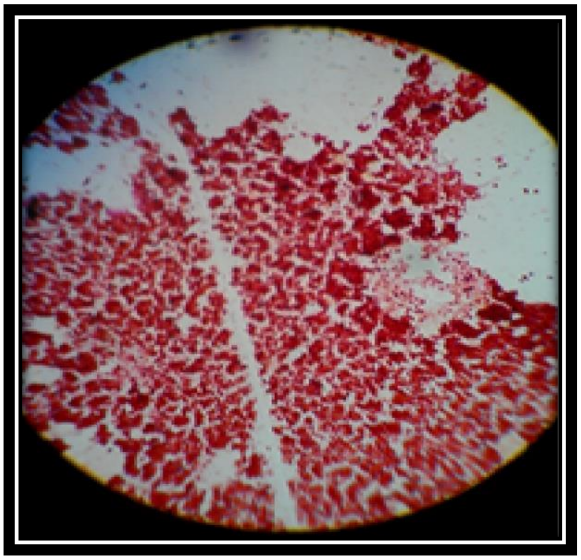


Image 34

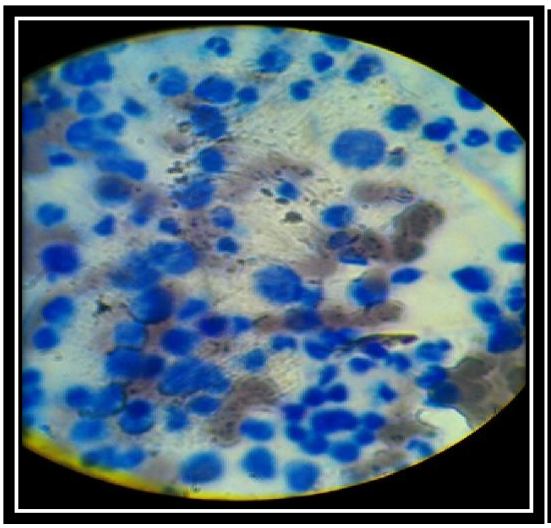


Image 35

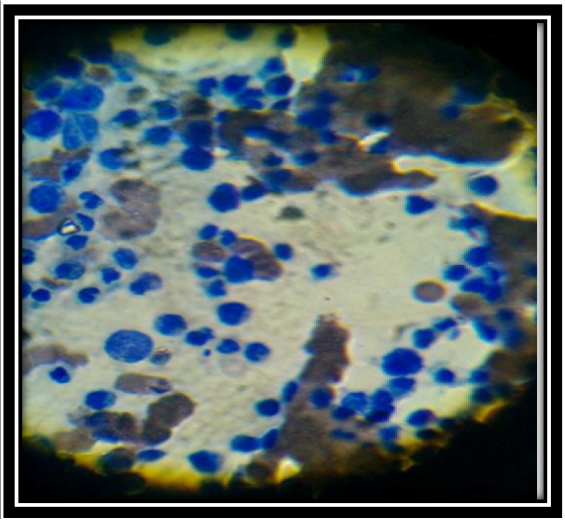
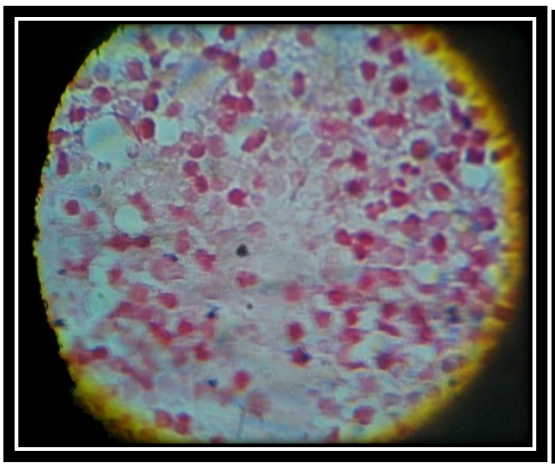


Image 36



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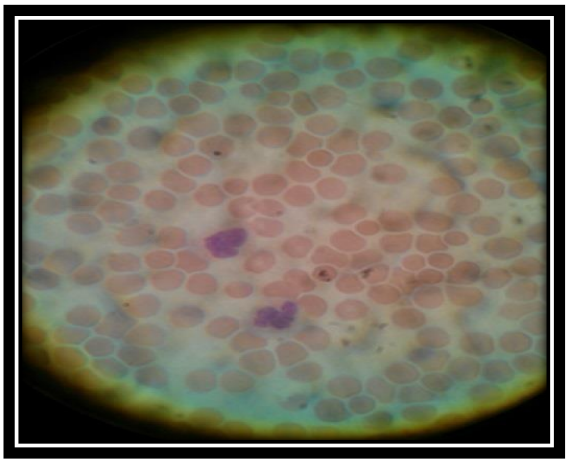


Image 38

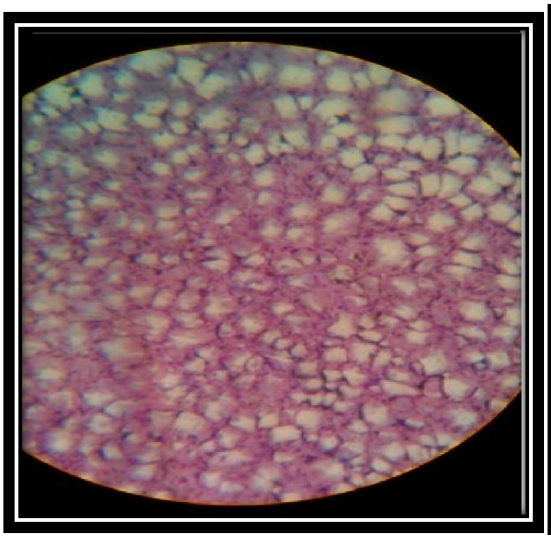


Image 39

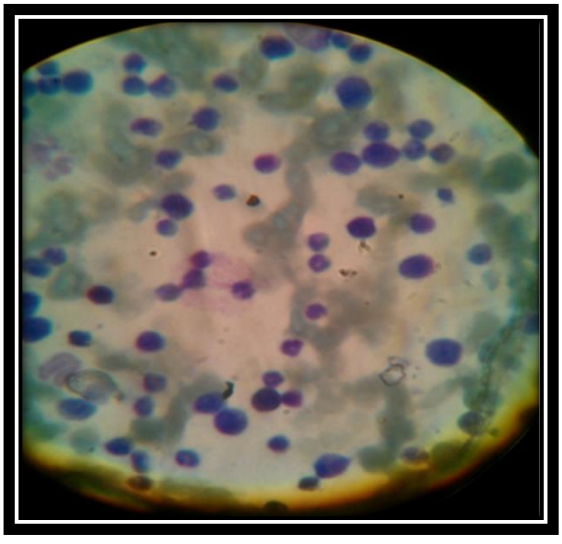


Image 40

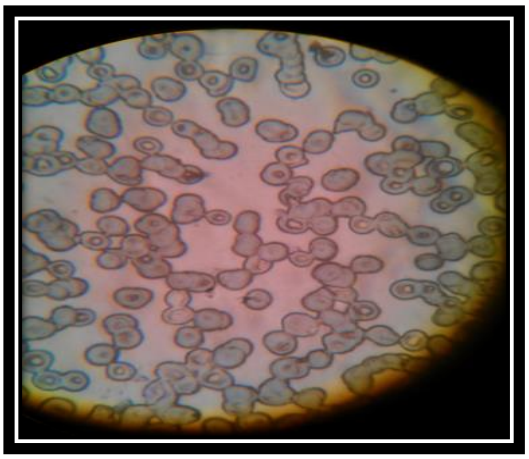


Image 41

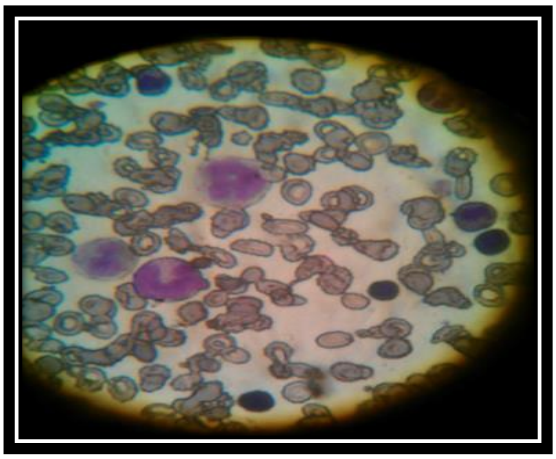


Image 42

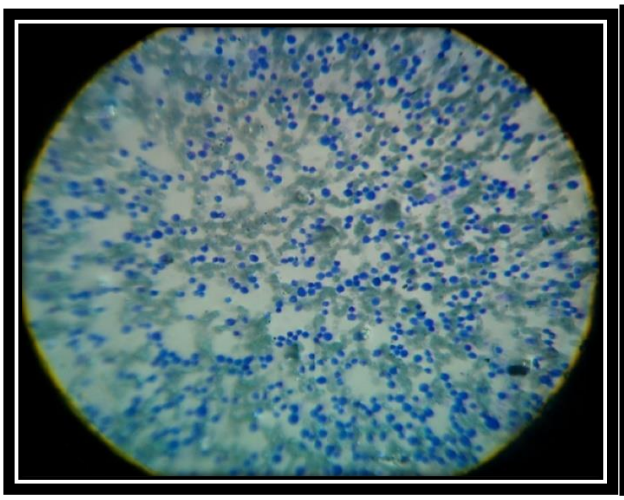


Image 43

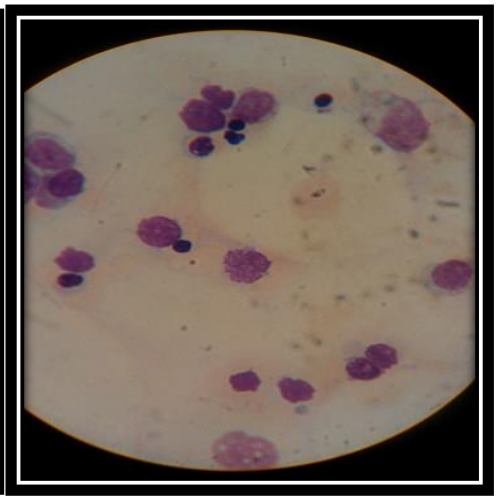


Image 44

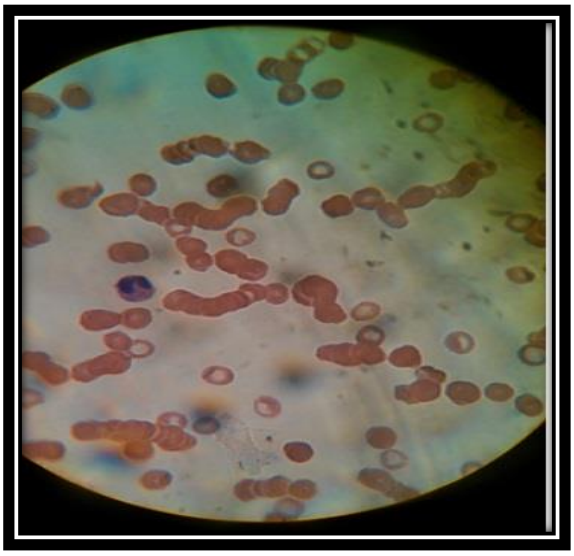


Image 45

Annex C. Counting blood cell by hematocytometer and its calculation detailed.

Preparing Hemocytometer

- If using a glass hemocytometer and coverslip, clean with alcohol before use. Moisten the coverslip with water and affix to the hemocytometer. The presence of Newton's refraction rings under the coverslip indicates proper adhesion.
- If using a disposable hemocytometer (for example, INCYTO DHC-N01), simply remove from the packet before use.

Preparing Cell Suspension

- Gently swirl the flask to ensure the cells are evenly distributed.
- Before the cells have a chance to settle, take out 0.5 mL of cell suspension using a 5 mL sterile pipette and place in an Eppendorf tube.
- Take 100 µL of cells into a new Eppendorf tube and add 400 µL 0.4% Trypan Blue (final concentration 0.32%). Mix gently.

Counting

- Using a pipette, take 100 µL of Trypan Blue-treated cell suspension and apply to the hemocytometer. If using a glass hemocytometer, very gently fill both chambers underneath the coverslip, allowing the cell suspension to be drawn out by capillary action. If using a disposable hemocytometer, pipette the cell suspension into the well of the counting chamber, allowing capillary action to draw it inside.
- Using a microscope, focus on the grid lines of the hemocytometer with a 10X objective.
- Using a hand tally counter, count the live, unstained cells (live cells do not take up Trypan Blue) in one set of 16 squares (Figure 1). When counting, employ a system whereby cells are only counted when they are set within a square or on the right-hand or bottom boundary line. Following the same guidelines, dead cells stained with Trypan Blue can also be counted for a viability estimate if required.
- Move the hemocytometer to the next set of 16 corner squares and carry on counting until all 4 sets of 16 corners are counted.

Viability

To calculate the number of viable cells/mL:

- Take the average cell count from each of the sets of 16 corner squares.
- Multiply by 10,000 (104).
- Multiply by 5 to correct for the 1:5 dilution from the Trypan Blue addition.

The final value is the number of viable cells/mL in the original cell suspension.
Example:

- If the cell counts for each of the 16 squares were 50, 40, 45, 52, the average cell count would be:
- (50 + 40 + 45 +52) ÷ 4 = 46.75
- 46.75 x 10,000 (104) = 467,500
- 467,500 x 5 = 2,337,500 live cells/mL in original cell suspension

To calculate viability:
If both live and dead cell counts have been recorded for each set of 16 corner squares, an estimate viability can be calculated.

- Add together the live and dead cell count to obtain a total cell count.
- Divide the live cell count by the total cell count to calculate the percentage viability.

Example:

- Live cell count: 2,337,500 cells/mL
- Dead cell count: 50,000 cells/mL
- 2,337,500 + 50,000 = 2,387,500 cells
- 2,337,500 ÷2,387,500 = 97.9% viability

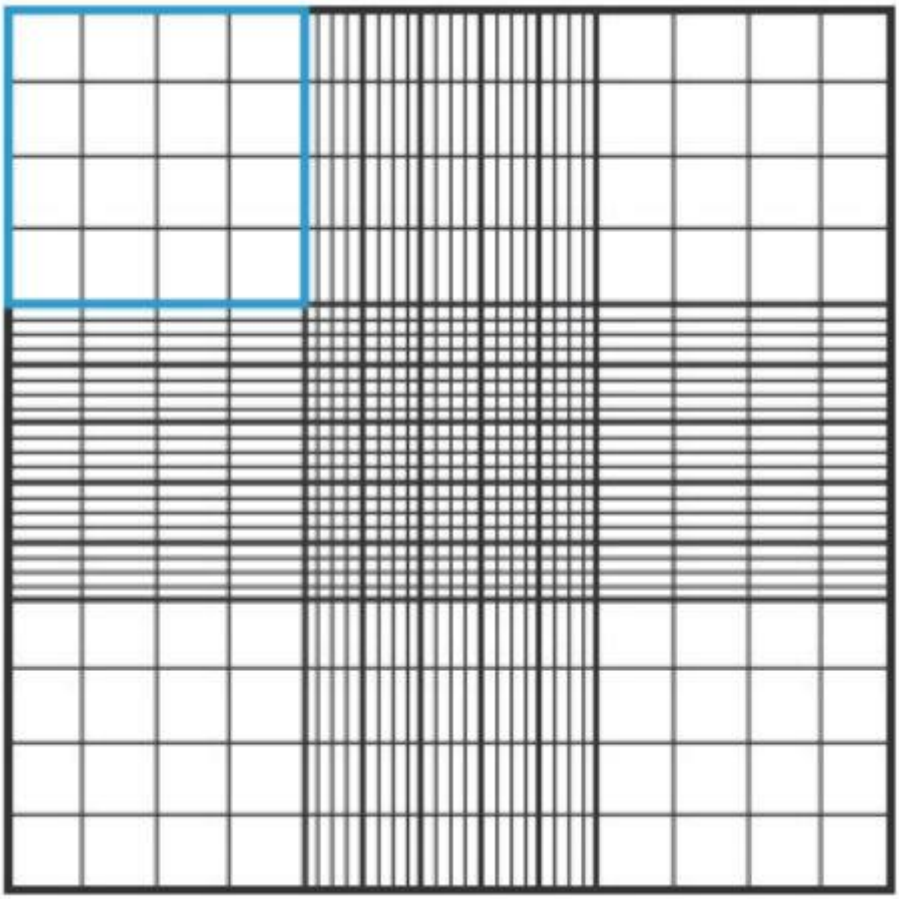
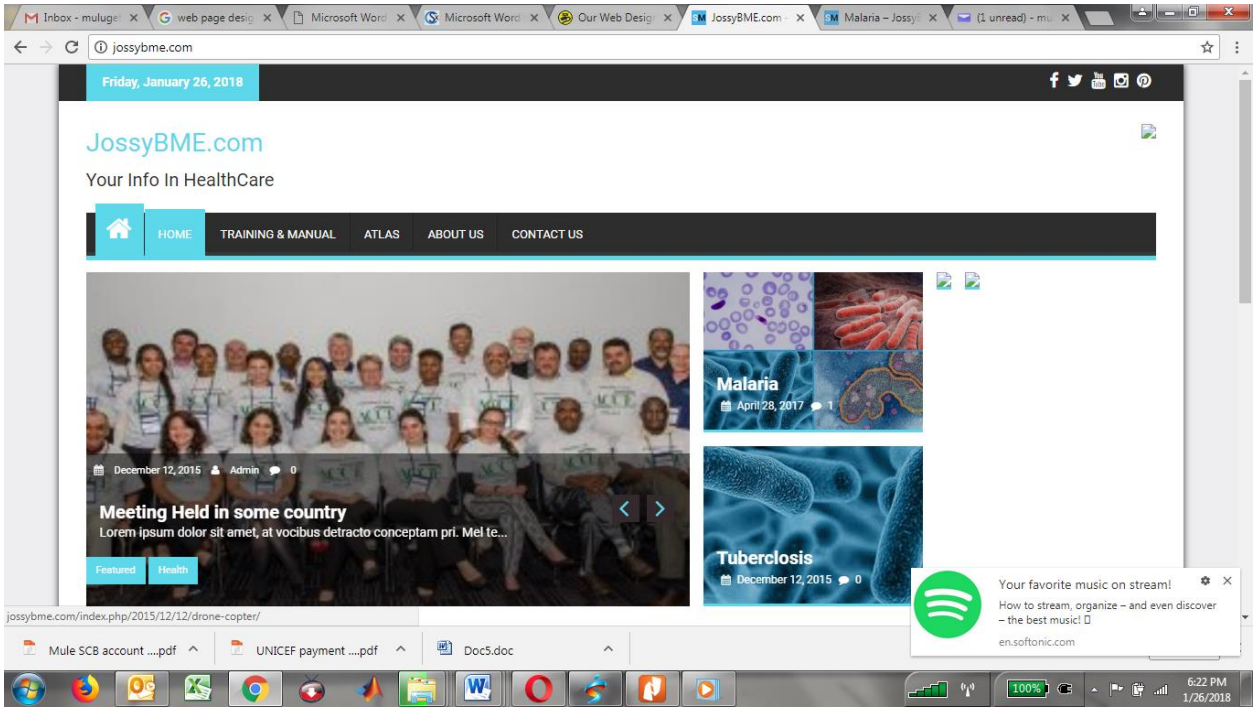
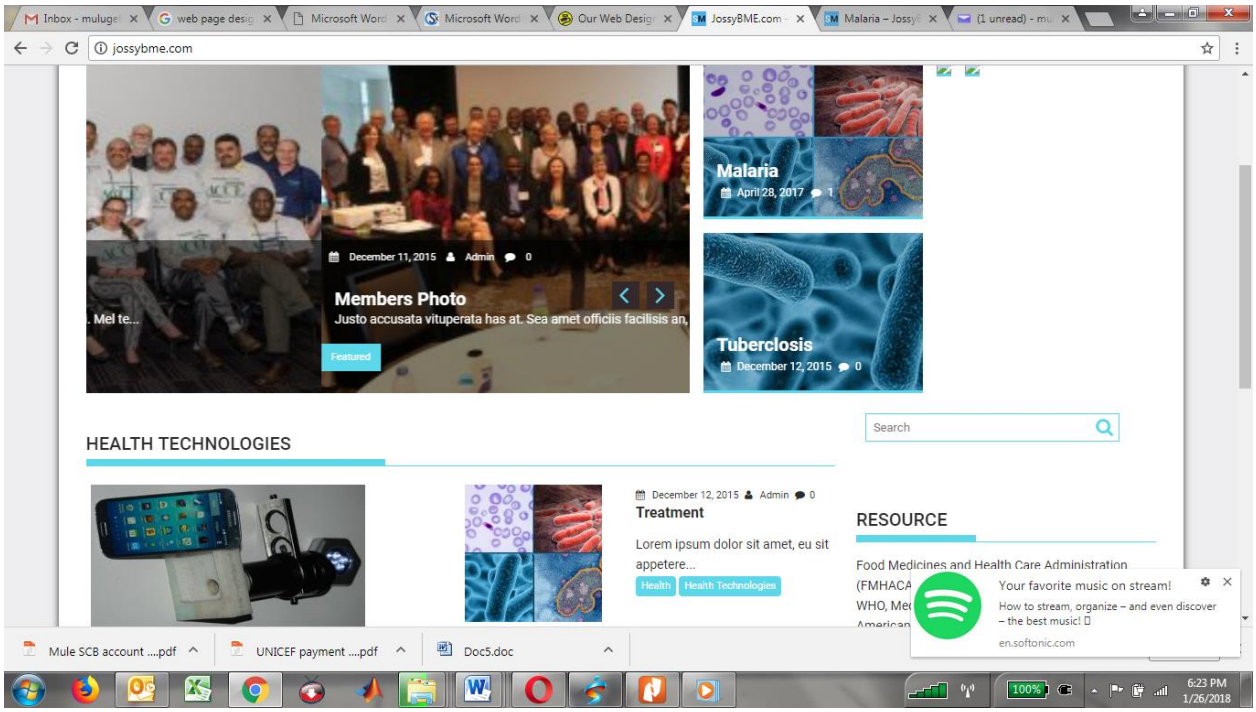
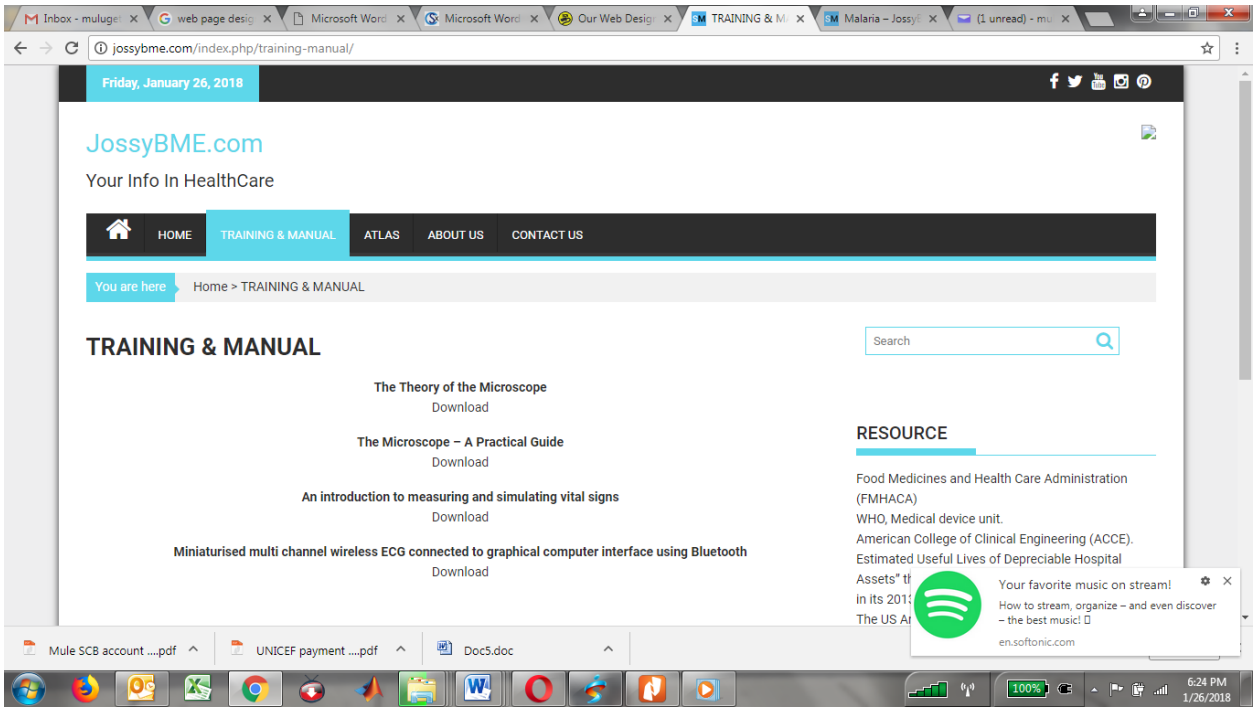


Figure 1. Hemocytometer gridlines.
Hemocytometer diagram indicating one of the sets of 16 squares that should be used for counting.

Annex D. Snapshots of the “JossyBME” web site.



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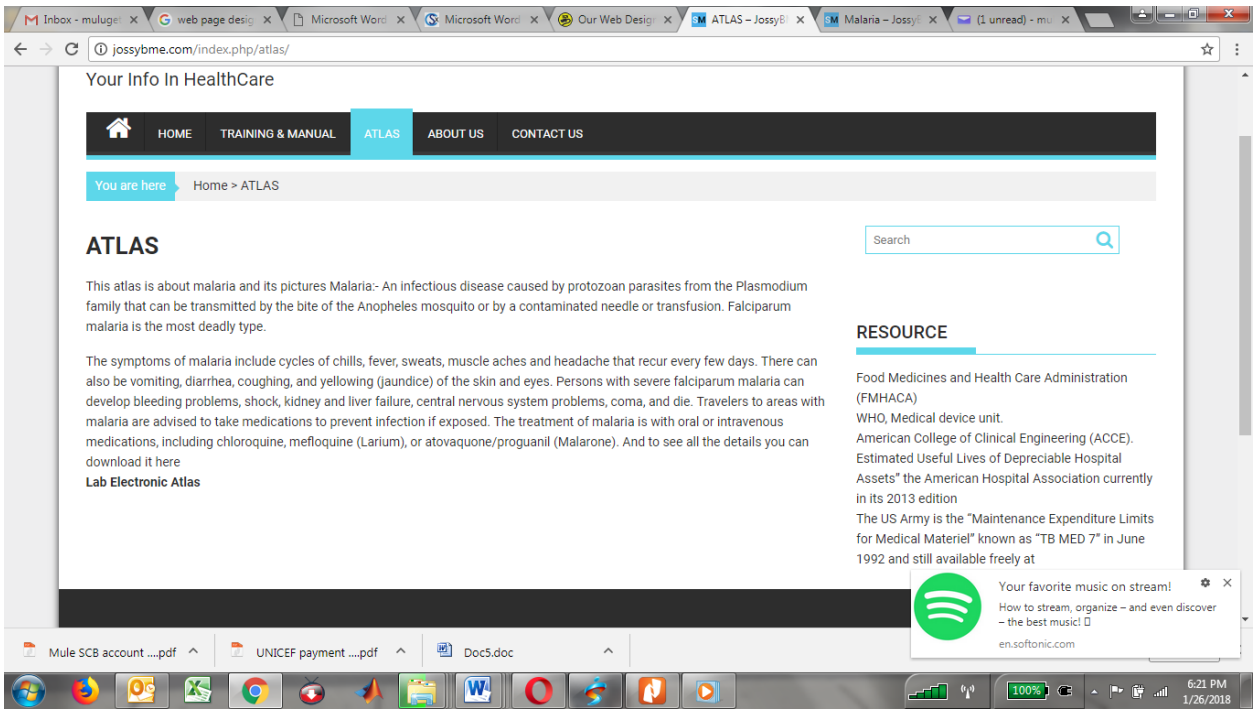
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This atlas is about malaria and its pictures Malaria:- An infectious disease caused by protozoan parasites from the Plasmodium family that can be transmitted by the bite of the Anopheles mosquito or by a contaminated needle or transfusion. Falciparum malaria is the most deadly type.
The symptoms of malaria include cycles of chills, fever, sweats, muscle aches and headache that recur every few days. There can also be vomiting, diarrhea, coughing, and yellowing (jaundice) of the skin and eyes. Persons with severe falciparum malaria can develop bleeding problems, shock, kidney and liver failure, central nervous system problems, coma, and die. Travelers to areas with malaria are advised to take medications to prevent infection if exposed. The treatment of malaria is with oral or intravenous medications, including chloroquine, mefloquine (Larium), or atovaquone/proguanil (Malarone). And to see all the details you can download it here
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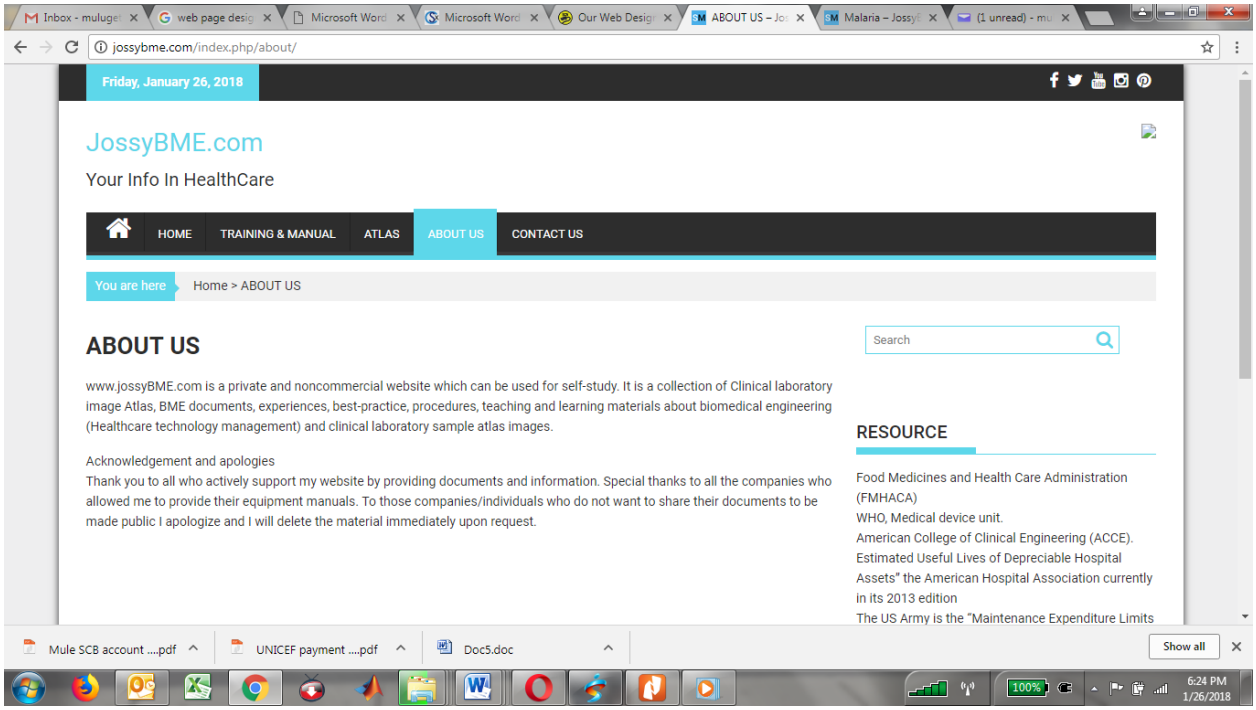
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Acknowledgement and apologies
Thank you to all who actively support my website by providing documents and information. Special thanks to all the companies who allowed me to provide their equipment manuals. To those companies/individuals who do not want to share their documents to be made public I apologize and I will delete the material immediately upon request.
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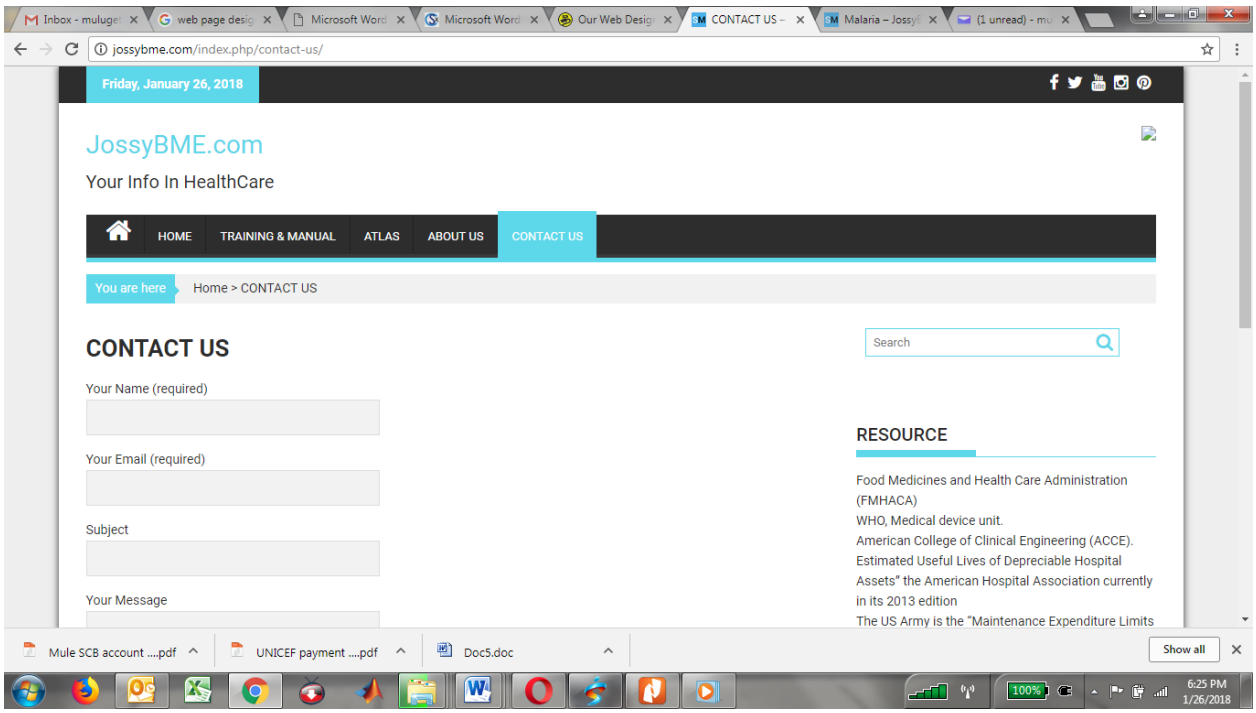
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Annex E Mathlab code for CBC counting by Hough Transform Algorithm

```
%Test classificaion - with trinions...
clear all
close all
clc

% s_num = 27;

worg=imread('C:\Desktop\AAIT\image_lab_sample\Figure_1');


w = double(worg);

T_R = w(:,:,1);
T_G = w(:,:,2);
T_B = w(:,:,3);

w(:,:,1) = T_R;
%w(:,:,1) = (w(:,:,1) - mean(mean(w(:,:,1)))./std(std(w(:,:,1)));
w(:,:,2) = T_G;
%w(:,:,2) = (w(:,:,2) - mean(mean(w(:,:,2)))./std(std(w(:,:,2)));
w(:,:,3) = T_B;
%w(:,:,3) = (w(:,:,3) - mean(mean(w(:,:,3)))./std(std(w(:,:,3)));

%++++++++++Normalization++++++++++
w_old(:,:,1) = double(w(:,:,1)./max(max(abs(w(:,:,1)))));
w_old(:,:,2) = double(w(:,:,2)./max(max(abs(w(:,:,2)))));
w_old(:,:,3) = double(w(:,:,3)./max(max(abs(w(:,:,3)))));


figure, imshow(double(w_old))

w=w_old;
%% HSI
HHH = w(:,:,1);
SSS = w(:,:,2);
LLL = w(:,:,3);
FL = (HHH+SSS+LLL)/sqrt(3.0);
V_1 = (2.0*LLL-HHH-SSS)/sqrt(6.0);
V_2 = (HHH-SSS)/sqrt(2.0);
si = size(V_1);
FS = sqrt(V_1.^2+V_2.^2);

FH = (atan2(V_2,V_1));%(atan(V_2./V_1));%unwrap(angle(V_1 + V_2*i));
FA = sqrt(HHH.^2+SSS.^2+LLL.^2);
FPHI = acos(FL./FA);

for qa_1 = 1:si(1)
    for qa_2 = 1:si(2)
        if (V_2(qa_1,qa_2) >= 0)
            if (V_1(qa_1,qa_2) >= 0)
                FH(qa_1,qa_2) = ((FH(qa_1,qa_2))./(pi)).^3;
            else
                FH(qa_1,qa_2) = ((FH(qa_1,qa_2))./(pi)).^3;
            end;
        else
            if (V_1(qa_1,qa_2) >= 0)
```

```
                FH(qa_1,qa_2) = (abs(FH(qa_1,qa_2))./(pi)).^1;
            else
                FH(qa_1,qa_2) = (abs(FH(qa_1,qa_2))./(pi)).^1;
            end;
        end;
    end;
end;

w(:,:,1) = FH;
w(:,:,2) = FS;

w(:,:,3) = FL;

sz = size(w(:,:,1));
las_x = sz(1) - 2;
las_y = sz(2) - 2;

st_pt_x = 1;
st_pt_y = 1;

%% trinion

for r_s_x = 1:las_x
    for r_s_y = 1:las_y

trt = 1;
[L_1,L_2,L_3] = PH_trinion_typeI_non_localized(w(st_pt_x+r_s_x-
trt:st_pt_x+r_s_x+trt,...
   st_pt_y+r_s_y-trt:st_pt_y+r_s_y+trt,1),w(st_pt_x+r_s_x-
trt:st_pt_x+r_s_x+trt,...
   st_pt_y+r_s_y-trt:st_pt_y+r_s_y+trt,2),w(st_pt_x+r_s_x-
trt:st_pt_x+r_s_x+trt,...
   st_pt_y+r_s_y-trt:st_pt_y+r_s_y+trt,3));
% [L_1,L_2,L_3] = PH_trinion_typeII_non_localized(w(st_pt_x+r_s_x-
trt:st_pt_x+r_s_x+trt,...
%     st_pt_y+r_s_y-trt:st_pt_y+r_s_y+trt,1),w(st_pt_x+r_s_x-
trt:st_pt_x+r_s_x+trt,...
%     st_pt_y+r_s_y-trt:st_pt_y+r_s_y+trt,2),w(st_pt_x+r_s_x-
trt:st_pt_x+r_s_x+trt,...
%     st_pt_y+r_s_y-trt:st_pt_y+r_s_y+trt,3));

%% PCA Extraction
% [r_1,r_2,r_3] =
Vectorial_PCA_Transform_with_trinions(abs(L_1),abs(L_2),abs(L_3));
[r_1,r_2,r_3] = Vectorial_PCA_Transform_with_trinions(L_1,L_2,L_3);



mi = min(min(min(r_1(:)),min(r_2(:))),min(r_3(:)));
if (min(r_1(:)) < 0)
        r_1 = r_1 + abs(mi);%abs(min(r_1(:)));%
end;
if (min(r_2(:)) < 0)
        r_2 = r_2 + abs(mi);%abs(min(r_2(:)));%
end;
if (min(r_3(:)) < 0)
        r_3 = r_3 + abs(mi);%abs(min(r_3(:)));%
end;
```

```
ma = max(max(max(abs(r_1(:))),max(abs(r_2(:)))),max(abs(r_3(:))));
mi = min(min(min(r_1(:)),min(r_2(:))),min(r_3(:)));
if(max(abs(r_1(:))) > 1)
         r_1 = r_1./ma;%r_1./max(abs(r_1(:)));%(r_1 - mi)./(ma - mi);%
end;
if(max(abs(r_2(:))) > 1)
         r_2 = r_2./ma;%r_2./max(abs(r_2(:)));%(r_2 - mi)./(ma - mi);%
end;
if(max(abs(r_3(:))) > 1)
         r_3 = r_3./ma;%r_3./max(abs(r_3(:)));%(r_3 - mi)./(ma - mi);%
end;

% r_0 = r_1+r_2+r_3;
% r_1 = r_1./r_0;
% r_2 = r_2./r_0;
% r_3 = r_3./r_0;

% r_1 = abs(L_1);
% r_2 = abs(L_2);
% r_3 = abs(L_3);

mx_1 = 0.0; mx_2 = 0.0; mx_3 = 0.0; my_1 = 0.0; my_2 = 0.0; my_3 = 0.0;
for zo_1 = 1:3
    for zo_2 = 1:3
        mx_1 = double(mx_1) + double(zo_1)*r_1(zo_1,zo_2);
        my_1 = double(my_1) + double(zo_2)*r_1(zo_1,zo_2);
        mx_2 = double(mx_2) + double(zo_1)*r_2(zo_1,zo_2);
        my_2 = double(my_2) + double(zo_2)*r_2(zo_1,zo_2);
        mx_3 = double(mx_3) + double(zo_1)*r_3(zo_1,zo_2);
        my_3 = double(my_3) + double(zo_2)*r_3(zo_1,zo_2);
    end;
end;
% feature extraction
for z_1 = 1:3
    for z_2 = 1:3
        %Variance
         SM_1(z_1,z_2) = 0.5*(double(z_1) -
mean(r_1(:)))^2*r_1(z_1,z_2) + 0.5*(double(z_2) -
mean(r_1(:)))^2*r_1(z_1,z_2);
         SM_2(z_1,z_2) = 0.5*(double(z_1) -
mean(r_2(:)))^2*r_2(z_1,z_2) + 0.5*(double(z_2) -
mean(r_2(:)))^2*r_2(z_1,z_2);
         SM_3(z_1,z_2) = 0.5*(double(z_1) -
mean(r_3(:)))^2*r_3(z_1,z_2) + 0.5*(double(z_2) -
mean(r_3(:)))^2*r_3(z_1,z_2);

%                     Contrast
%          SM_1(z_1,z_2)=0.5*double(z_1 + z_2).*r_1(z_1,z_2).^2;
%          SM_2(z_1,z_2)=0.5*double(z_1 + z_2).*r_2(z_1,z_2).^2;
%          SM_3(z_1,z_2)=0.5*double(z_1 + z_2).*r_3(z_1,z_2).^2;

          %cluster Shed
%          SM_1(z_1,z_2)=abs(double(z_1)+double(z_2)-mx_1-
my_1)^3*r_1(z_1,z_2);
%          SM_2(z_1,z_2)=abs(double(z_1)+double(z_2)-mx_2-
my_2)^3*r_2(z_1,z_2);
```

```
%          SM_3(z_1,z_2)=abs(double(z_1)+double(z_2)-mx_3-
my_3)^3*r_3(z_1,z_2);

         % Maximum Propablity
%          SM_1(z_1,z_2)=max(r_1(:));
%          SM_2(z_1,z_2)=max(r_2(:));
%          SM_3(z_1,z_2)=max(r_3(:));

         %
%          SM_1(z_1,z_2)=r_1(z_1,z_2)*abs(double(z_1) - double(z_2));
%          SM_2(z_1,z_2)=r_2(z_1,z_2)*abs(double(z_1) - double(z_2));
%          SM_3(z_1,z_2)=r_3(z_1,z_2)*abs(double(z_1) - double(z_2));

          %Homogeneity
%          SM_1(z_1,z_2)=r_1(z_1,z_2)/(1+abs(double(z_1) -
double(z_2)));
%          SM_2(z_1,z_2)=r_2(z_1,z_2)/(1+abs(double(z_1) -
double(z_2)));
%          SM_3(z_1,z_2)=r_3(z_1,z_2)/(1+abs(double(z_1) -
double(z_2)));

         %Energy
%          SM_1(z_1,z_2)= r_1(z_1,z_2).^2;
%          SM_2(z_1,z_2)=r_2(z_1,z_2).^2;
%          SM_3(z_1,z_2)=r_3(z_1,z_2).^2;

          %
%          SM_1(z_1,z_2)=r_1(z_1,z_2)/(1+abs(double(z_1) -
double(z_2)).^2);
%          SM_2(z_1,z_2)=r_2(z_1,z_2)/(1+abs(double(z_1) -
double(z_2)).^2);
%          SM_3(z_1,z_2)=r_3(z_1,z_2)/(1+abs(double(z_1) -
double(z_2)).^2);
%

          %Clastur Prominace
%        SM_1(z_1,z_2)=0.5*(double(z_1+z_2-mx_1-my_1).^4)*r_1(z_1,z_2);
%        SM_2(z_1,z_2)=0.5*(double(z_1+z_2-mx_2-my_2).^4)*r_2(z_1,z_2);
%        SM_3(z_1,z_2)=0.5*(double(z_1+z_2-mx_3-my_3).^4)*r_3(z_1,z_2);

%             %entropy
%             SM_1(z_1,z_2)=r_1(z_1,z_2)*log(r_1(z_1,z_2)+1);
%             SM_2(z_1,z_2)=r_2(z_1,z_2)*log(r_2(z_1,z_2)+1);
%             SM_3(z_1,z_2)=r_3(z_1,z_2)*log(r_3(z_1,z_2)+1);
    end;
end;

Summean_1 = sum(SM_1(:));
Summean_2 = sum(SM_2(:));
Summean_3 = sum(SM_3(:));

PCA_Q_Eax(r_s_x,r_s_y,1) = (Summean_1);%abs((Summean_3) -
(Summean_1));%
PCA_Q_Eax(r_s_x,r_s_y,2) = (Summean_2);%abs((Summean_3) -
(Summean_2));%
PCA_Q_Eax(r_s_x,r_s_y,3) = (Summean_3);%abs((Summean_2) -
(Summean_1));%
```

```
clear L_1 L_2 L_3 r_1 r_2 r_3 mx_1 mx_2 mx_3 my_1 my_2 my_3
clear Te_1 Te_2 Te_3
    end;
end;

toc



% figure
% imshow(PCA_Q_Eax./max(abs(PCA_Q_Eax(:))),[])

if (max(max(abs(PCA_Q_Eax(:,:,1)))) ~= 0.0)
    %PCA_Q_Eax(:,:,1) =
PCA_Q_Eax(:,:,1)./max(ones(size(PCA_Q_Eax(:,:,1))),(PCA_Q_Eax(:,:,1)+PC
A_Q_Eax(:,:,2)+PCA_Q_Eax(:,:,3)));
    PCA_Q_Eax(:,:,1) =
PCA_Q_Eax(:,:,1)./max(max(abs(PCA_Q_Eax(:,:,1))));
end;
if (max(max(abs(PCA_Q_Eax(:,:,2)))) ~= 0.0)
    %PCA_Q_Eax(:,:,2) =
PCA_Q_Eax(:,:,2)./max(ones(size(PCA_Q_Eax(:,:,1))),(PCA_Q_Eax(:,:,1)+PC
A_Q_Eax(:,:,2)+PCA_Q_Eax(:,:,3)));
    PCA_Q_Eax(:,:,2) =
PCA_Q_Eax(:,:,2)./max(max(abs(PCA_Q_Eax(:,:,2))));
end;
if (max(max(abs(PCA_Q_Eax(:,:,3)))) ~= 0.0)
    %PCA_Q_Eax(:,:,3) =
PCA_Q_Eax(:,:,3)./max(ones(size(PCA_Q_Eax(:,:,1))),(PCA_Q_Eax(:,:,1)+PC
A_Q_Eax(:,:,2)+PCA_Q_Eax(:,:,3)));
    PCA_Q_Eax(:,:,3) =
PCA_Q_Eax(:,:,3)./max(max(abs(PCA_Q_Eax(:,:,3))));
end;

figure
imshow(sqrt(PCA_Q_Eax(:,:,1).^2+PCA_Q_Eax(:,:,2).^2+PCA_Q_Eax(:,:,3).^2
),[])
title('Amplitude of the entropies')
figure,subplot(1,2,1),imshow(PCA_Q_Eax);
%
subplot(1,2,2),imshow(w(1:las_x,1:las_y,:)-PCA_Q_Eax);
% d=w(1:las_x,1:las_y,:)-PCA_Q_Eax;


 %% Cell Counter

% PCA_Q_Eax1=w(1:las_x,1:las_y,:)-PCA_Q_Eax;
cform = makecform('srgb2lab');
Im_lab = applycform(PCA_Q_Eax,cform);
figure, subplot(2,2,1),imshow(w);
subplot(2,2,2),imshow(Im_lab),title('Color Transform')
%// Apply threshold

Im_lab = im2bw(Im_lab(:,:,3),.7);
subplot(2,2,3),imshow(Im_lab),title('binary image')
%// Detect ~circles
```

```
[centers, radii] = imfindcircles(Im_lab,[13
27],'Sensitivity',0.94,'Edge',0.46,'ObjectPolarity','dark');

% subplot(2,2,4),
figure,
imshow(worg(2:sz(1)-1,2:sz(2)-1,:)),title('Hough Transform');
hold on
viscircles(centers, radii,'EdgeColor','k','LineWidth',4);

NumCircles = numel(radii);

%// Display message box
Msg = sprintf('You have just found %i RBCs!!!\n',NumCircles);

msgbox(Msg)
```