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A Technology Assessment of Light-Emitting Diode (LED) applied to the Optical Microscope for the Diagnosis of Malaria

C. Hildenberg L. De Oliveira[1], M. A. Dalla Costa[2], G. Holsbach Costa[3]

*Department of Electrical Engineering, Federal University of Rondônia, Brazil
Email: berg@unir.br
** GEDRE, Federal University of Santa Maria, Brazil
*** Center of Exact Sciences and Technology, University of Caxias do Sul, Brazil

ABSTRACT: *Optical methods, such as light microscopy, are a standard method for the diagnosis of malaria. Some critical determining factors for the accurate diagnosis are adequate lighting and a good visual acuity of the technician. In the age of high-quality light-emitting diode (LED) illumination and portable solar battery chargers, microscopy has become more feasible in remote areas. However, very few theoretical studies have been reported so far. The purpose of this paper is to examine and compare the lighting source models that are available in clinical microscopy for the diagnosis of malaria. Two lighting sources, halogen lamp and white LED, are compared. The variables that are investigated in this study are the color rendering index (CRI), temperature, entropy and detection of the first parasite.*

I. INTRODUCTION

Malaria is a serious disease that affects mainly the tropical regions of the world [1]. Because of the overlap of symptoms with other tropical diseases, misdiagnosis is not uncommon and often can cause a delay in initiating appropriate treatment [2]. The abusive and incorrect use of antimalarial drugs simply with the appearance of fever without a specific diagnosis can lead to resistance development, toxicity and economic losses [3][4].

The Giemsa stain, based on a thick drop of blood stained, is considered the most effective method for the control of malaria due to its low cost, as it can differentiate species of malaria and quantify the parasitic [5][6].

Under adequate lighting condition, this procedure is reliable [6]. Nevertheless, the optimization of the test would be a great advantage, instead of rerunning it when the lighting or sample does not result in reliable reading of the strip. Fast diagnoses are an alternative, however, it is highly expensive, and they tend to provide qualitative results rather than quantitative [5].

The accuracy of clinical results varies according to the level of precision in the sample reading, which impacts in the identification of errors of the species and estimation errors of parasite density. An important variable in defining the accuracy and speed of clinical research in optical microscopy is the lighting [5][7].

The halogen lamps have been employed as a light source in optical microscopy for many years. Most of the energy emitted by these lamps is scattered as heat in the infrared wavelengths. Additionally, these lamps have a lower correlated colour temperature (CCT), these CCT values should highlight the reddish colours, which could distort other colours in some cases.

Light Emitting Diodes (LED) are efficacious because of their low power consumption, durability, and low heat. LED are well suited for microscopy lighting because they emit neither UV nor IR radiation, which could damage biological sample on microscope slides. However, there have been no studies investigating the effectiveness of white LED for microscope lighting.

The aim of this study was to evaluate the effectiveness of white LED for microscope lighting, the characteristics of the image formation and colour reproduction when exposed to LED was investigated. In the search, two microscope slides were read by one experienced microscopist and the temperature in the microscope was measured, which have the different source of illumination. Also, the efficiency of LED in the detection time of the first parasite was compared with the halogen lamp.

II. MALARIA

Malaria remains as the single biggest cause of death by parasitic diseases in the world. It is considered the most significant threats to the health nowadays, causing from 1.5 to 2.7 million of deaths annually [1][4].

These deaths are mainly of children under five years old. It is estimated that it kills 483,000 children a year, and about 1,300 lose their lives every day [1]. Although not all adult cases of malaria are fatal, the disease forces workers to stop for several weeks. Overall, malaria costs to Africa about $ 12 billion a year in lost of productivity [1], [4].

This disease is caused by a parasite called Plasmodium, the one which most impacts the world, threatening the health of 40% of the global population over 100 countries [1]. There are four species of Plasmodium that can cause human infection: falciparum, vivax, ovale and malariae [5].

The disease diagnosis is made through blood collection, by digital puncture. It is spread on the test strip, stained and read [5]. This technique shows the presence (or absence) of malaria parasite. If a parasite is found, the next step is to determine the species and its stage of life. After this, another evaluation is performed to find out the degree of infection to seek a better treatment to the patient.

Recent studies suggest that diagnostic delays lead to an escalation of cases of malaria [8]. The test, when taken during its early stage, is the key for reducing mortality rates. It ensures proper treatment to prevent the spread of malaria once the natural period of transmission of malaria is linked to the existence of host (humans reservoirs) and vectors (mosquito) [5],

2.1. The Diagnosis

The lack of specific symptoms and signs to identify malaria leads to different diagnostics. The diagnostic needs to have high accuracy; otherwise, it may lead to wrong clinic decisions, causing serious health issues and even death [1]. The blood microscopy in the diagnosis of malaria seeks to identify the morphology of the parasite [5]. These parasites vary in size, shape and appearance; they can also be mistaken with strange contaminant elements present in the blood sample such as fungi and bacteria [5]. Temperature is an important factor which contributes for preserving the morphological characteristics of the analyzed specimen. This test is very complex, which makes the diagnosis very difficult to be performed.

III. LIGHT SOURCE LAMP CHARACTERISTICS

The light microscopy, so-called because it uses visible light to detect small structures, requires pigments in the background to identify mixed structures. The microscope is an indispensable accessory in the clinical diagnosis of malaria, because it allows to analyze the morphology of biological samples, ensuring the differentiation of normal cells and infected cells [5]. The high microscope resolution is necessary for analysis of Plasmodium, which is the degree of clarity and sharpness microscope's field of view. The quality of the light source impacts directly in the visualization of the tiny bodies to be highlighted. These small bodies range from 0,5 to 20 microns in diameter, coloured red (core) and blue (cytoplasm) [5]. The most common light source used in these microscopes is halogens bulb and, more recently, LED.

3.1. Halogen Lamp

As the tungsten filament lamp works like a thermal radiator, it means that the light is generated by the heating of a solid object (the filament) at a very high temperature. Tungsten light presents spectral emission which resembles that of a black body. The spectral output profile of tungsten- halogen bulbs is qualitatively similar to tungsten and carbon filament bulbs.

The halogen lamp is the primary source of lighting used in modern microscopy, except to those used to investigate in fluorescent microscopy. These microscopes present low-voltage supply, usually between 6V and 12V. The heat radiated or conducted through by the halogen lamp, when used for extended periods of time, can affect the analyzed sample if the temperature continues to rise in the slice, consequence of protein denaturation with the partial or complete loss of a protein's three-dimensional structure [9].

3.2. LED

LED is the most promising technology for lighting in a large range of fields [10]. Its recent advances have provided several advantages such as reduced costs, large useful life, robustness, and high efficacy. It is generally fed by low-cost power supplies, such as low-voltage batteries or switch-mode power supplies [11].

In the optical microscope, high power diodes generate sufficient intensity to provide a useful source of illumination for a broad range of clinical applications [12]. However, few studies have examined the characteristics of this light source and compared to the conventional halogen lamp.

IV. EXPERIMENTAL SETUP

Experiments have been set up in order to evaluate and compare the light sources. The wavelength range is measured with a spectro-colorimeter from Inventfine model CMS-5000 and an integrating sphere, the schematic as shown in Fig. 1.

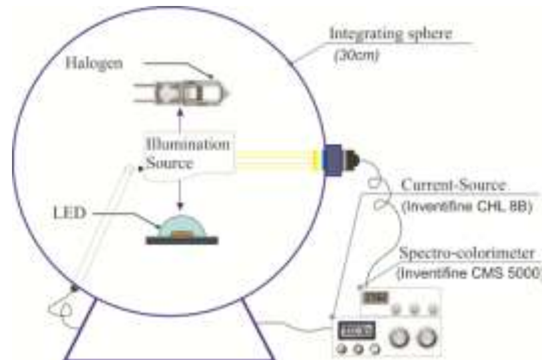

Fig. 1. Setup for the measurement of static luminous flux.

20W Philips halogen bulb and Philips® Lumileds® Luxeon Rebel LED LXML-PWC1-0100 are used in tests. The LED brightness control is regulated via Pulse Width Modulation (PWM). An infrared camera (Fluke Ti25) was used for monitoring the temperature. A microscope with a 100x oil objective and 1.4 numerical aperture was used to obtain the images. Besides, a Micron MT9N001 CMOS 10MP Colour Digital Camera system was attached to the microscope. The pixel resolution was 720 x 480. The images were stored in TIF file format, with 24 bits per pixel, in colour. Giemsa microscopic slides were prepared by the Research Center for Tropical Medicine of Rondônia Brazil (CEPEM/SESAU).

In order to make a more precise analysis of the image results, the entropy characteristics were evaluated and assessed in each image for each colour components, denoted by R, G and B [13].

The image entropy or image uncertainty describes how much randomness (or uncertainty) there is in an image (how much information is provided by image). In other words, the value of clutter that relates inversely to the second angular momentum is given by the equation (1).

$$e(\mathrm{I}) = \sum_{w=0}^{H-1} P_I(w)\log_2\left(P_I(w)\right) \qquad (1)$$

where H is the number of different intensity levels and $P_I(w)$ is the joint probability distribution of the pixels associated with the image I, determined as:

$$P_I(i) = \frac{\eta_i}{\eta} \qquad (1)$$

where η_i is the number of occurrences of the intensity.

V. EXPERIMENTAL RESULTS

At this point, the experiments have been set up to evaluate and to compare the influence of two different luminous sources on this microscope's application possibilities.

5.1. Led Characteristic

The Forward Current vs. Forward Voltage curve of the LED were calculated from datasheet values, as shown in Fig 2.

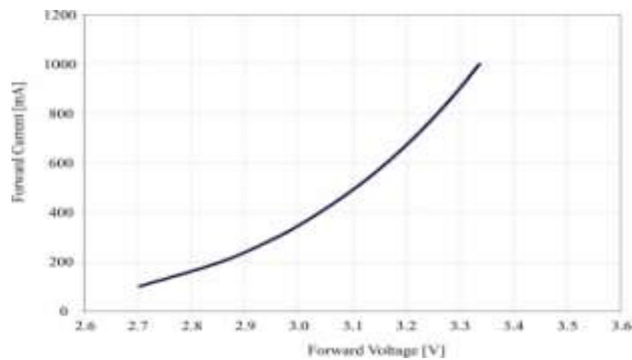

Fig. 2. Forward current versus forward voltage.

5.2. Spectral Analysis

The CIE trichromatic system was used, which represents the colours according to their chromaticity and luminance. It ensured that colour can be defined regardless of its peripheral variables such as subjective perception of colour definition. Colour variation measure (CVM) of light sources is calculated in CIE colour space, as shown in Fig. 3.

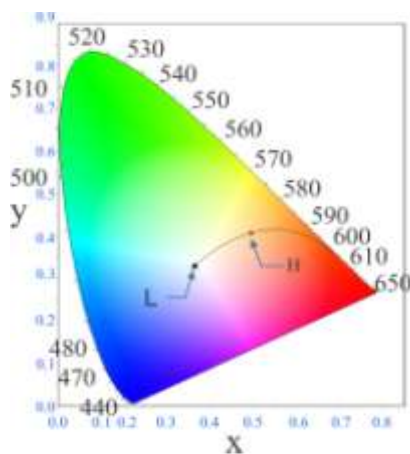

Fig. 3. The chromaticity space and position of sources of light obtained from measurements in an integrating sphere. H - the halogen lamp; L - LED.

The Fig. 3 shows that the halogen lamp presents characteristics of highlighting colours ranging from yellow to red. These lamps have a lower correlated colour temperature (CCT), these CCT values should highlight the reddish colours, which could distort colours in some cases.

5.3. Temperature

The halogen lamp has radiation in the infrared region and presents thermal energy conversion, transferring heat by convection. The core of the light source region reached 95 [°C], and the heat radiated to the stage, where the microscope slide is found with the material analysis, reached 35.4 [°C].

The halogen was replaced by the LED, working with 0.6[W] power, this produced a drastic change in its thermal characteristics. The core region of the light source now goes to 33 [°C] and no heat radiated to the stage. The blade with the material for analysis reached 26.9 [°C].

5.4. Biological Samples

To check the effects of lighting on the formation of the image to be observed, the prepared slide described in the previous section was analyzed with a 100X immersion objective outlined in section 4, as seen in the Fig. 4.

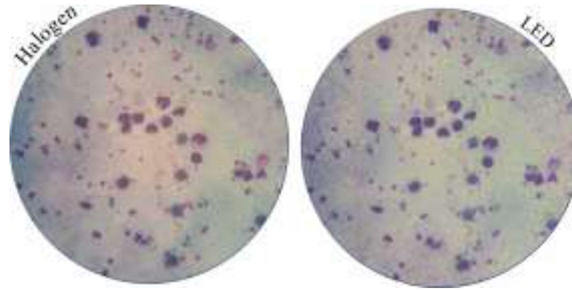

Fig. 4. Two images captured from the same field viewed by different light source. Halogen and LED.

According to the information theory, as higher the entropy of a signal, higher will be the amount of information contained in this signal. Considering this, in the light microscopy applications shown in Fig. 4, an image with higher entropy is supposed to give more information to the diagnosis. Considering the entropy in each colour components (R, G and B), the results of the measurement are shown in Fig. 5.

Fig. 5. Entropy comparison results of per colour channel.

5.5. Detection Of The Fist Parasite

The determination of the first parasite is crucial to go on to the next stage of research [5][2], which is the identification of the species and life-cycle stage producing the infection and calculation of the degree of disease, by calculating the ratio of parasites. A total of 20 slides were used in order to compare the detection time using Kaplan-Meier plot and log rank test (p= 0.62) between the methods (Fig.6). This result shows the gain of the proposed lighting method, decreasing the time to identify the first parasite (decrease by around 33%).

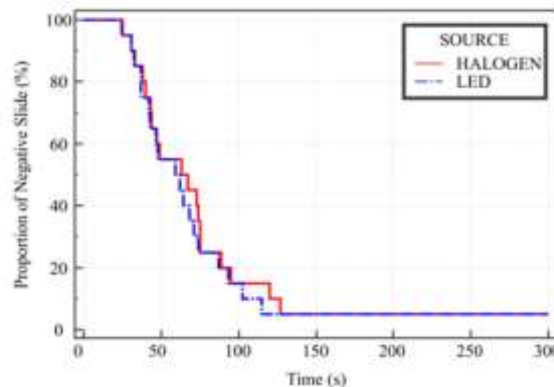

Fig. 6. Parasite detection time. Kaplan-Meier plot with log rank test (p = 0:62) to show time until detection of the first parasite (truncated at 5 min).

VI. Conclusion

In order to obtain the comparison between a halogen lamp and a white LED applied to an optical microscope in the diagnosis of malaria, this work proposed to investigate the colour characteristics and visual performance that differ between two light sources in the formation of the image, from the same sample. As a result, the proposed approach made it possible to extract the differences in colour reproduction in a biological

sample illuminated by the two lighting methods. The Kaplan-Meier method was used in order to measure the efficacy of time detection of the first parasite, it was shown that the long half-life, of the LED, was lower. The image produced by LED visualization presents an image with more entropy, consequently, more apparent details on the scene, indicating that this may be a useful economic system for rapid diagnosis of malaria.

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