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Research Article

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Malaria parasite detection-Employability of foldscope in malarial diagnosis.

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Abstract

Malarial diagnosis at the initial stage of the infection still remains a global challenge. Since malaria is mostly restricted to the under developed countries, precision in diagnosis is not affordable by the lower socio-economic group. Foldscope can be a boon to many of these problems like easy handling, low maintenance cost, least sourcing and employability of a technician with minimal knowledge. Also, the non-requirement of battery and electricity to operate is a major breakthrough, hence can be utilized as an alternative to detect malarial parasite at an early stage in the most difficult geographical terrain. Our study focused on employ ability of the foldscope in identifying the different stages of *Plasmodium falciparum*. An optimized method has been established which allows for maintaining a healthy, highly synchronous culture for longer period of time. The study so far was successful in identifying and synchronizing the stages of *P. falciparum* and validating the same through foldscope images**.**

Keywords: Malaria, *Plasmodium falciparum*, culture, CO_2 incubator, Synchronization.

I. Introduction

Parasitic diseases cause millions of morbidities and mortalities per annum and impose serious health and socioeconomic consequences, mainly in developing countries. By combining principles of optical design with origami. Foldscope is the ultra-affordable, paper microscope, designed to be extremely portable, durable, and to give optical quality similar to conventional research microscopes (magnification of 140X and 2 micron resolution), foldscope brings hands-on microscopy to new places. Smartphone-assisted foldscopes can be applied for the diagnosis of parasite diseases, one being malaria and can be efficiently and as a portable diagnostic tool. Foldscope when assembled produced 20 X magnifications onto a Smartphone camera sensor for the identification of *P. falciparum* in blood smears.

Manual microscopy of malaria is performed by visual inspection after Giemsa staining. (Chemical for highlighting the parasites). Stained blood smears of two types, that is thick and thin blood smears. The thick blood smears are prepared with thick layer of

blood on the slide. The thick blood smears are mainly used for the detection of malaria parasites (results is +ve or –ve of malaria). The problems associated with thick blood smears are lack of clear visibility and unable to differentiate the RBC's from other components of blood like WBC's and Platelets. The thin blood smears are prepared with a thin layer of blood. The thin blood smears are used for detailed examination. But the lack of expertise and feasibility to afford microscope can hamper the early diagnosis in rural and endemic regions.

The current project tested the efficiency of foldscope in malarial parasite identification. The focus was mainly to estimate the parasitemia enabling diagnosis at early stage. Since foldscope is portable, durable and cost effective. Thus we have user friendly technique can prove as breakthrough in malarial diagnosis.

II. Objectives

In view of the above concerns the project aimed at employing foldscope in malarial diagnosis, with the objectives:

1. To identify the malaria parasite *P.falciparum* under foldscope.
2. To clearly identify the different stages of the parasite development.
3. To estimate the parasitemia levels.
4. To compare the relative clarity of the images, taken in foldscope and light microscope.

III. Materials and Methods

Reagents

RPMI media, uninfected erythrocytes (blood group O-positive), sodium chloride, freshly prepared Giemsa stain, sterile pipettes, Culture flasks.

Equipment

Cryogenic equipment at 4°C, Cryogenic equipment at –20°C,Cryogenic equipment at –196°C, Laminar flow hood, Incubator with a reliable source of CO_2, Microscope with a 100x oil immersion objective, Centrifuge, Water-bath or heater block, Shaker.

The milestone of the current project was establishment of malarial parasite culture laboratory. The lab is successfully maintained for the culturing of Plasmodium.spp.

Sample collection

Plasmodium falciparum culture was obtained from the institute of International centre for Genetic Engineering & Biotechnology (ICGEB).

Culturing

In-vitro cultivation of *Plasmodium falciparum* (*Fairlamb et al*., 1985): In brief, suspensions (50%) of infected cells with complete media (RPMI with 15% serum) were prepared. Appropriate amount of uninfected cells were added to get an initial parasitemia of 0.5 to 1.0 % and diluted with complete media to get 5% cell suspension (5 % Haematocrit). Culture was kept in CO_2 incubator at 37°C.Thin-blood smears of *Plasmodium falciparum* (ring stage), were prepared from the culture samples. *P. falciparum* was identified in Giemsa and Leishman stained blood films using foldscope attached to a Smartphone. The method achieved as high diagnostic tool, specificity, suggesting that this could be a valuable for malaria screening programs.

Synchronization of Parasite Culture

Synchronization was done by the method of (Lambros and Vanderberg, 1979), based on the differential permeability of parasitized RBC membrane. Pre-warm an aliquot of 5% D-sorbitol, complete medium, and RPMI at 37°C. Centrifuge the culture at 1800 rpm for 5 minutes at room temperature, discard the supernatant, and resuspend in 5 volumes of pellet to pre-warmed sorbitol solution. Incubate the sample for 5 minutes. Centrifuge at 1800 rpm. Remove the supernatant, and wash twice with 25ml of complete medium. After the last wash, remove the supernatant and adjust the pellet to a Haematocrit of 50% and use it to inoculate the culture in a culture flask, adding fresh RBCs to maintain Haematocrit at 5% for synchronous cultures.

After synchronization, parasitemia was estimated by thin film technique and parasitemia was calculated as following:

Formulae:

- Standardized RBC-count was taken for 1:400 dilutions through,

Total RBC/mm^3 = (No. of RBC counted* Dilution factor* Depth factor)/ (No. of chambers counted)

- Percentage of parasitemia or percent of infected RBC's,

Percent of infected RBC's = (No. of infected RBC/Total no. of RBC-counted)*100

- Parasites/µl is given by,

Parasites/µl = (No. of Parasitized RBC cells *5*10^6) / (No. of RBC-cells counted)

IV. Results

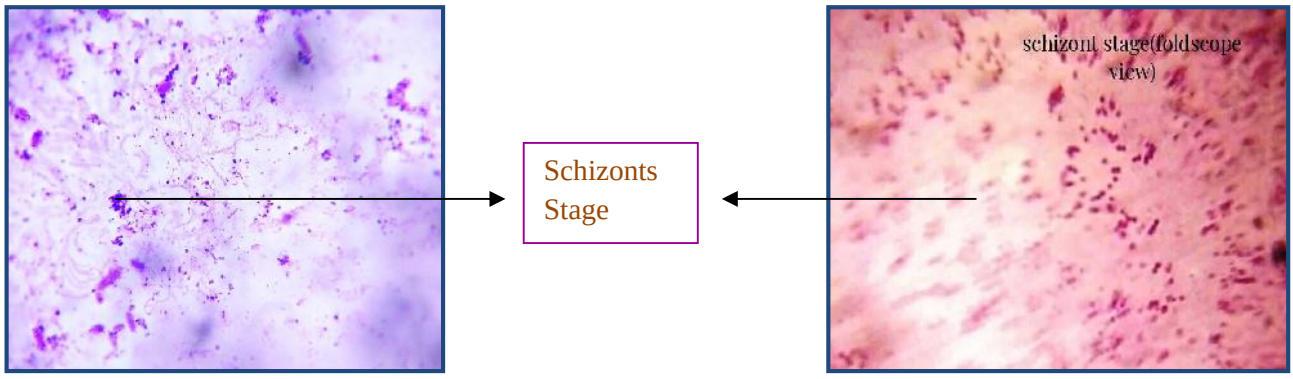

Fig 1a: Microscope view Fig 1b: Foldscope view

Fig 1: Estimation of Parasitemia at 5% Haematocrit at 48 hrs under Microscope (100X) and Foldscope.

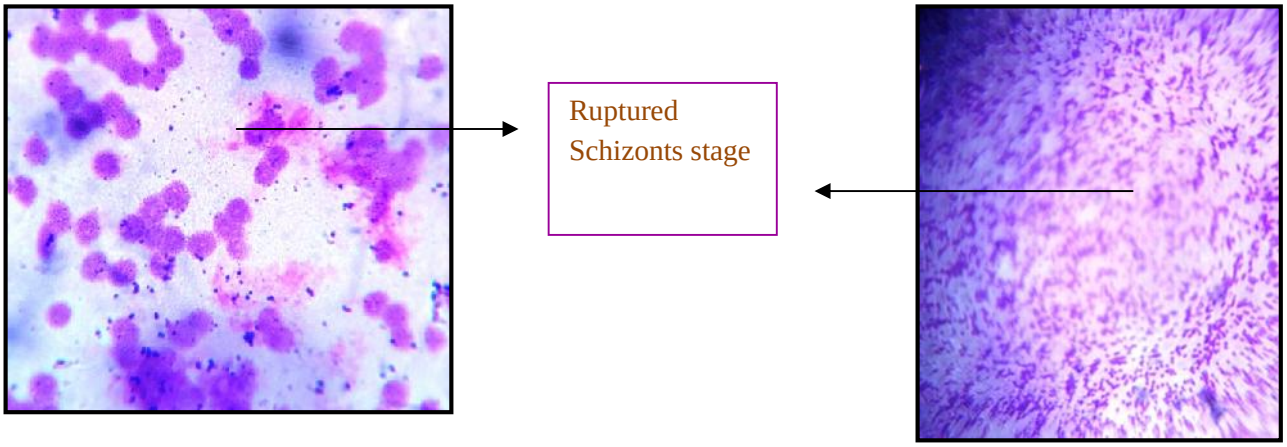

Fig 2a: microscopic view Fig 2b: Foldscope view

Fig 2: Estimation of Parasitemia at 5% Haematocrit at 24hrs under Microscope (100X) and Foldscope.

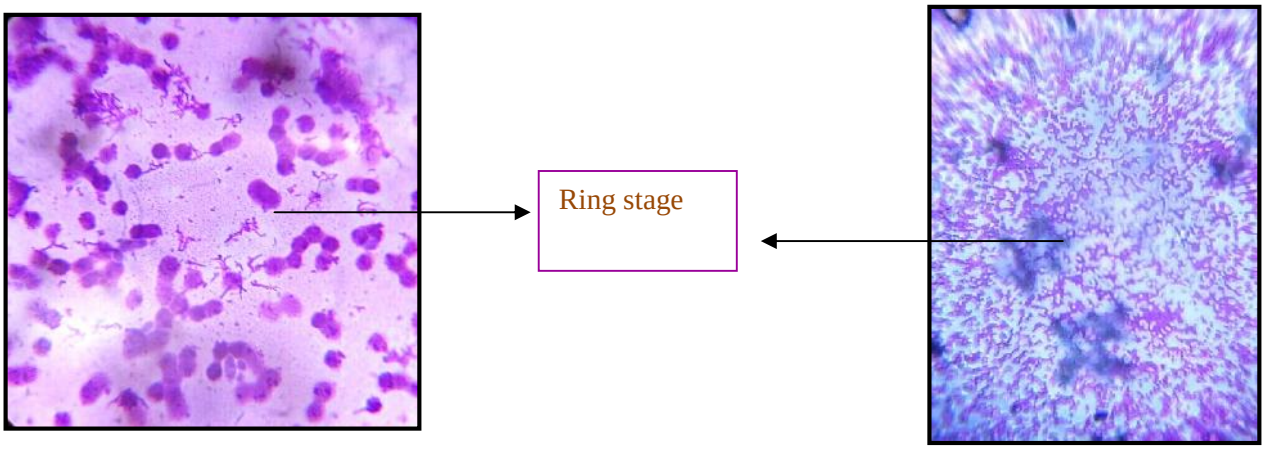

Fig 3a: Microscopic view Fig 3b: Foldscope view

Fig 3: Estimation of Parasitemia at 5% Haematocrit at 72hrs under Microscope (100X) and Foldscope.

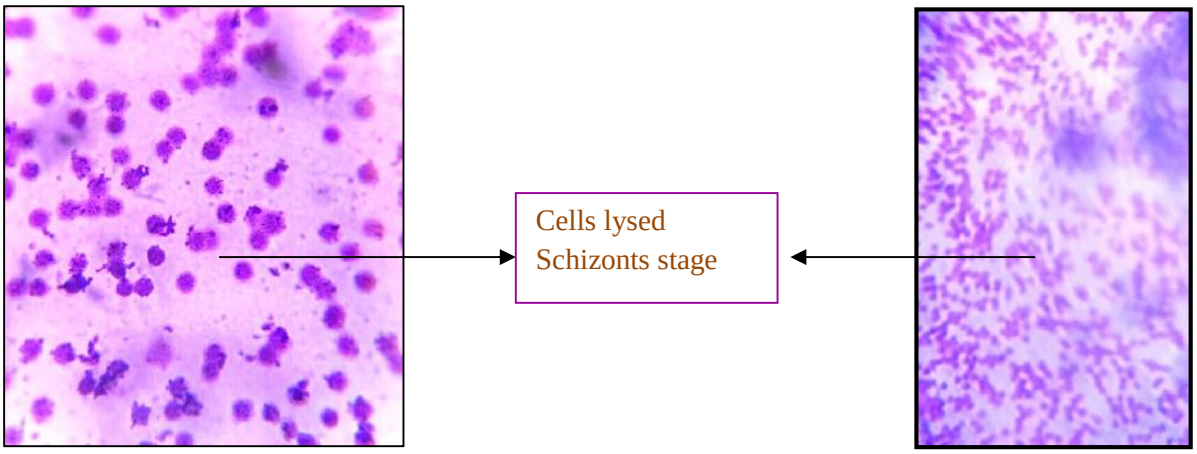

Fig 4a: Microscope view Fig 4b: Foldscope view

Fig 4: Estimation of Parasitemia at 15% Haematocrit at 72 hrs under Microscope (100X) and Foldscope.

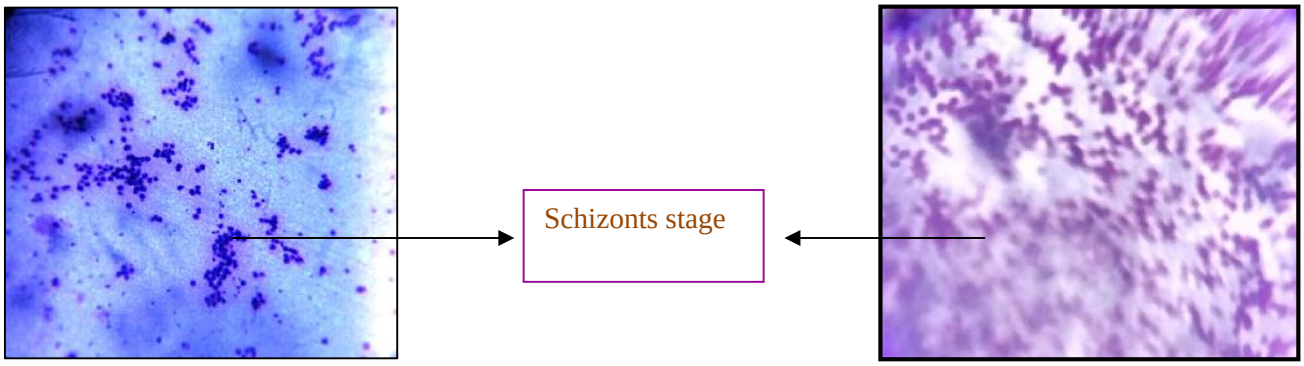

Fig 5a: Microscope view Fig 5b: Foldscope view

Fig 5: Estimation of Parasitemia at 15% Haematocrit at 24 hrs under Microscope (100X) and Foldscope.

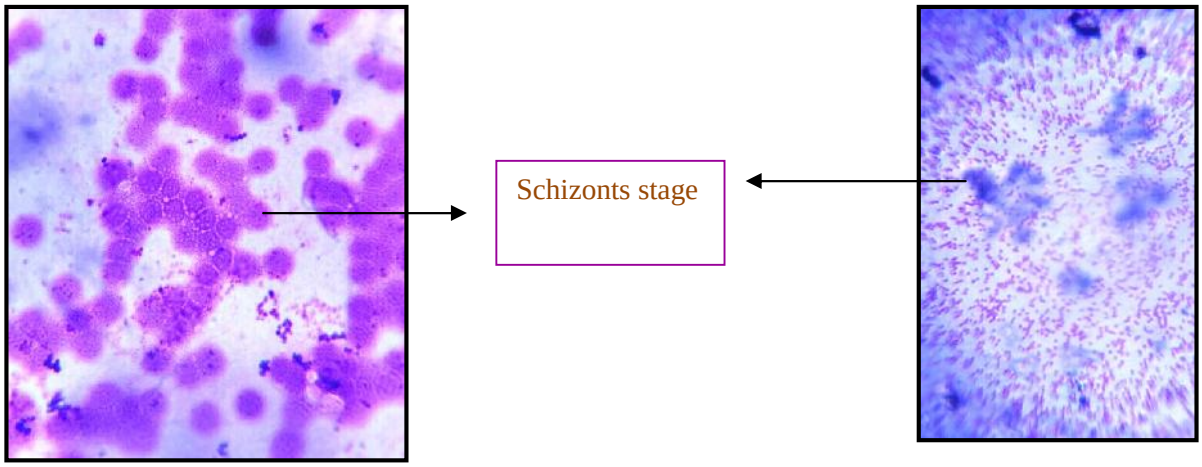

Fig 6a: Microscope view Fig 6b: Foldscope view

Fig 6: Estimation of Parasitemia at 15% Haematocrit at 48 hrs under Microscope (100X) and Foldscope.

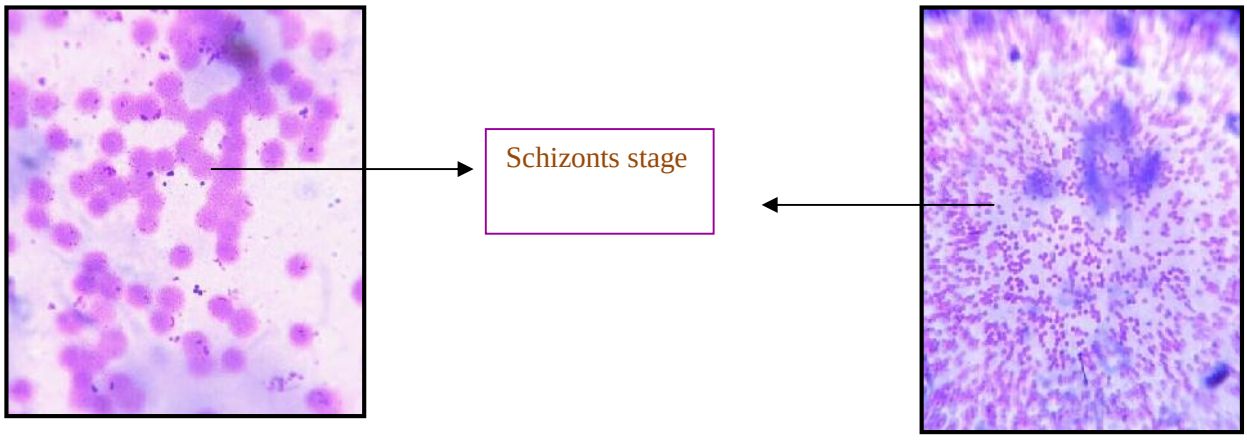

Fig 7a: Microscope view

Fig 7b: Foldscope view

Fig 7: Estimation of Parasitemia at 25% Haematocrit at 24 hrs under Microscope (100X) and Foldscope.

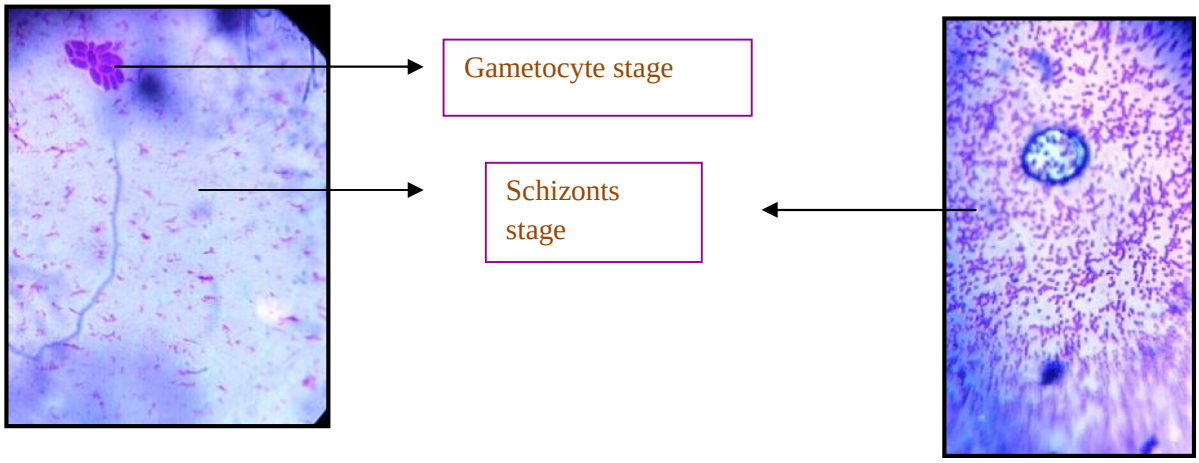

Fig 8a: Microscope view

Fig 8b: Foldscope view

Fig 8: Estimation of Parasitemia at 25% Haematocrit at 72 hrs under Microscope (100X) and Foldscope

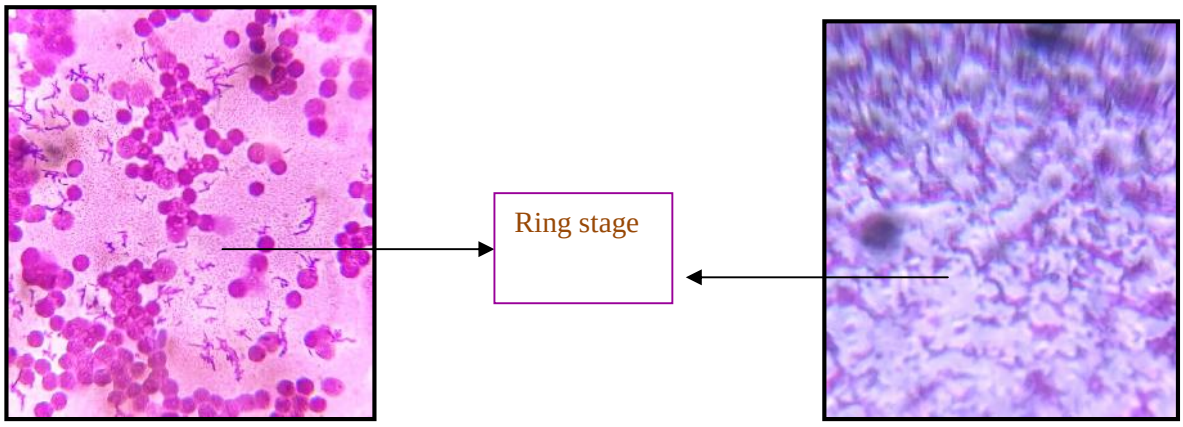

Fig 9a: Microscope view

Fig 9b: Foldscope view

Fig 9: Estimation of Parasitemia at 25% Haematocrit at 48 hrs under Microscope (100X) and Foldscope

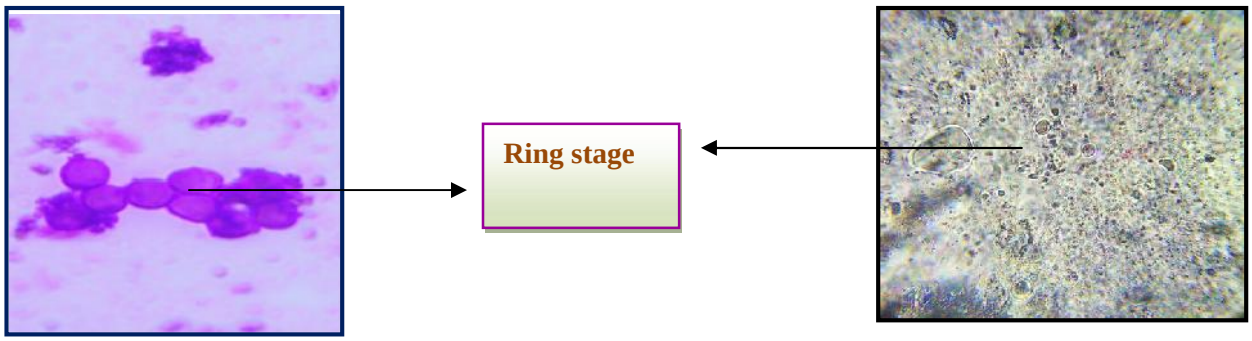

Fig 10a: Microscope view

Fig 10b: Foldscope view

Fig 10: Synchronized Ring stage viewed under Microscope (100X) and Foldscope

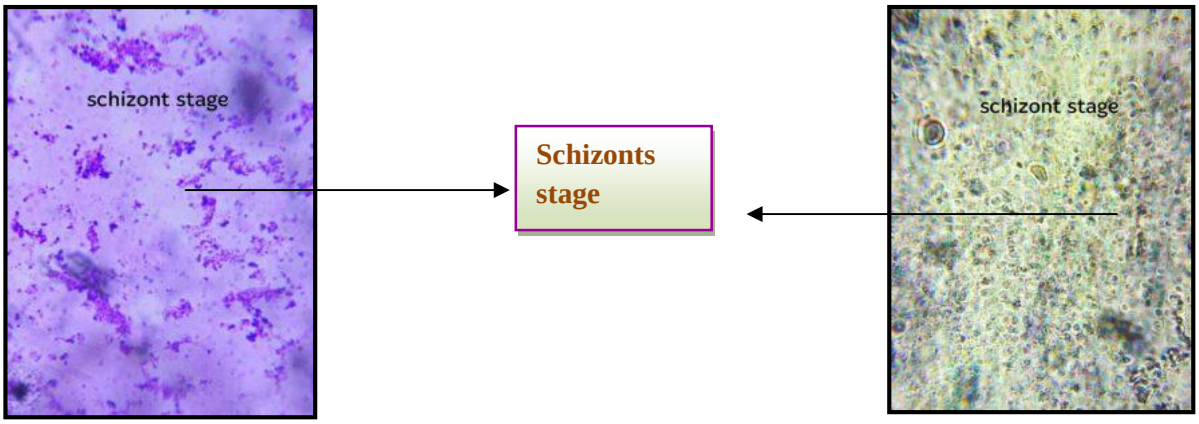

Fig 11a: Microscope view

Fig 11b: Foldscope view

Fig 11: Synchronized Schizonts stage viewed under Microscope (100X) and Foldscope

Table 1a: Percentage of parasitemia estimated at 1: 400 dilutions as observed under Microscope.

SL.No	Percent of Haematocrit	Percentage of Parasitemia 1:400 dilutions
1.	5% 24hrs	44.6
	48 hrs	30.3
	72 hrs	36.8
2.	15% 24hrs	35.9
	48hrs	43.2
	72 hrs	83.7
3.	25% 24 hrs	46.6
	48 hrs	58.04
	72 hrs	30.59

Table 1b: Percentage of parasitemia estimated at 1: 400 dilutions as observed under Foldscope

SL.No	Percent of Haematocrit	Percentage of Parasitemia 1:400 dilutions
1.	5% 24hrs	30.5
	48 hrs	22.6
	72 hrs	29.7
2.	15% 24hrs	56.6
	48hrs	59.1
	72 hrs	63.59
3.	25% 24 hrs	36.7
	48 hrs	32.0
	72 hrs	37.7

V. Discussion

The culture, *Plasmodium falciparum* was established and different stages of the parasite were studied and each stage image was uploaded to foldscope (Website details: microcosmos.foldscope.com). Cultures were maintained and medium renewal was carried out without contamination. Parasites were developed and parasitemia estimation was done with 100% of parasitemia achievement.

- Maximum ring stages were achieved at Haematocrit (72 hrs-36.16%) and 25% Haematocrit (48 hrs-58.04%).
- 100% of parasitemia (Table 1a) is obtained at 15% H [72 hrs-83.7%] and 50% of parasitemia is obtained at 25%H [48 hrs- 58.04%]

Hence the study resulted in successful identification of stages of malarial parasites through foldscope. The parasitemia estimation was carried out with different percentage of haematocrit. The clarity and magnification of the images as observed under foldscope was satisfactory. Therefore foldscope promises as an alternative to microscope. Thus making malarial diagnosis affordable and easy.

Acknowledgments

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