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Original Article

Diagnosis of malarial infection using change in properties of optically trapped red blood cells

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ABSTRACT

Background: In previous work studying the properties of red blood cells (RBCs) held in an optical tweezers trap, we observed an increase in the spectrum of Brownian fluctuations for RBCs from a *Plasmodium falciparum* culture—due to increased rigidity of the cells—compared to normal RBCs. We wanted to extend the study to patient samples, since the earlier work was done with cultures grown in the lab.

Methods: Individual RBCs were held in an optical-tweezers trap. Its position fluctuations were measured and the power spectrum determined. The corner frequency (f_c) of the spectrum gave a quantitative measurement of the spectrum.

Results: The value of f_c was 25 Hz for normal cells, which increased to 29 Hz for infected cells—both for *P. falciparum* and *Plasmodium vivax* infections.

Conclusion: The technique of measuring f_c can be used as a screening tool for malaria in patients with fever, since RBCs not carrying the parasite will also show the change due to the bystander effect, irrespective of whether it is caused by *P. falciparum* or *P. vivax*.

Early and accurate diagnosis of malaria is essential to manage this deadly disease because it continues to remain a global public health problem [1,2]. In earlier work [3], we had studied the properties of red blood cells (RBCs) trapped in an optical-tweezers trap, and found an increase in the spectrum of Brownian fluctuations from nRBCs (normal cells) to iRBCs (infected cells). The change was primarily due to increased rigidity of iRBCs, which, though slightly controversial, is fairly well established [4–6], and what prevents diseased cells from behaving normally. Interestingly, we found a **bystander effect** [7], in which hosting and non-hosting RBCs showed the same change in their properties. Further experiments with various inhibitors have confirmed that the substance responsible for the bystander effect is mediated by ATP or cAMP [8,9]. The bystander effect is consistent with several recent reports which discuss the role of extracellular vesicles in influencing cell-to-cell communication among RBCs [10–12].

However, the use of the tweezers method for disease diagnosis remained questionable, partly because the earlier studies were done with cultures grown in the lab. The cultures only studied *Plasmodium falciparum* infection, but could not be applied to *Plasmodium vivax* infection. This was because *P. vivax* predominantly infects reticulocytes, and it is quite difficult to maintain long-term cultures of such cells in the lab.

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At a glance commentary

Scientific background on the subject

Optically trapped red blood cells (RBCs) could be potentially used for diagnosis of malaria, particularly because it takes advantage of the bystander effect. However, its use in actual patients suffering from malaria remained questionable because earlier studies were done with cultures grown in the lab.

What this study adds to the field?

This study extended our previous work by extending it to patients suffering from malaria and admitted to nearby hospitals. It also helped us to study *P. vivax* infection, which are difficult to culture in the lab (because they predominantly infect reticulocytes) even though they form the majority of patient infections.

In this work, we extend the use of this method to blood samples drawn from patients suffering from malaria, obtained from hospitals in and around Bangalore. This has now allowed us to study *P. vivax* infection, since they form the majority of samples. Consistent with our earlier work using cultures, the corner frequency—which is a measure of the Brownian spectrum—increased from nRBCs to iRBCs for both kinds of infections. The results give a direct confirmation of the bystander effect because:

(a) For *P. falciparum* samples, no attempt was made to see if the cell actually hosted the parasite or not. Given the low parasitemia count in patients, it is likely that most of the RBCs being studied are non-hosting, and therefore made rigid by the substance released into the blood stream.

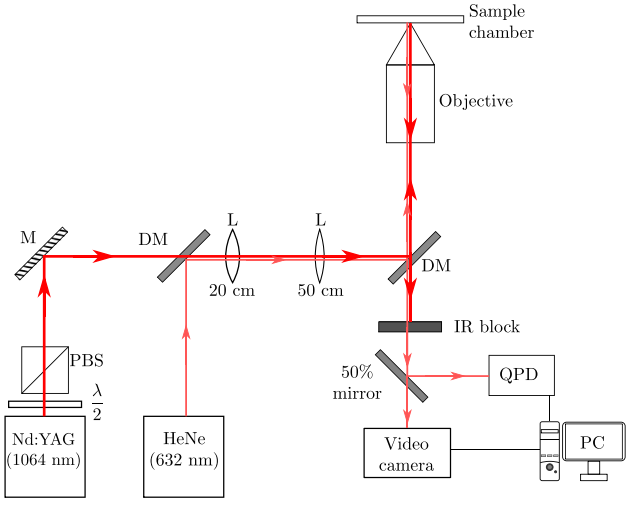

Fig. 1 Experimental schematic of the optical tweezers set up. Abbreviations used: M: mirror; DM: dichroic mirror; L: lens; QPD: quadrant photo detector; IR: infrared.

(b) For *P. vivax* samples, which predominantly infects reticulocytes and since our study only looks at mature RBCs, there is negligible chance that the parasite is inside the RBC. Therefore, the change in rigidity can only be caused by some substance other than the parasite.

The bystander effect has been studied in detail in our previous work with cultures—e.g. by looking exclusively at non-hosting RBCs, or by incubating nRBCs in a spent medium.

The above results show that the tweezers technique can be used as a general screening test for all kinds of malarial infection. It has the following advantages over other existing methods.

(i) It is easily automated.
(ii) It is statistical, and hence does not require trained personnel.
(iii) It is independent of the stage of development of the parasite, and hence does not require the blood sample to be drawn at a particular time—during a febrile episode, for example.
(iv) It can be used during the earliest stage of the disease when the parasitemia count is extremely low and the only symptom is high fever, because it takes advantage of the bystander effect and hence does not require the RBC to host the parasite.

Methods

Optical tweezers

The set up for the optical-tweezers trap has been described in detail in our earlier work [3], and is reviewed here for completeness. As shown in Fig. 1, it consisted of a 100× oil-immersion objective coupled to a Zeiss inverted microscope. The trapping laser was formed from an infrared laser operating at 1064 nm. The output of the laser was imaged on to the 5 mm back plane of the objective using a pair of lenses. The incident power at the back plane of the lens was 300 mW, which got reduced to 10% of this value at the sample plane due to transmission of the objective at the trapping wavelength of 1064 nm.

A small amount of red laser beam (from a HeNe laser operating at 633 nm) was mixed with the trapping laser. The back-scattered light from this beam was used to monitor the position of the trapped particle—its position was measured using a quadrant photo-detector (QPD). The corner frequency f_c of Brownian fluctuations for each trapped RBC was measured as follows:

(i) Its position along the x direction was measured using the QPD.
(ii) The position was measured as a discrete time series at a sampling rate of 16 kHz for a total sample length of 100,000 points.
(iii) The data were fast Fourier transformed (FFT) to yield the spectrum of Brownian fluctuations.
(iv) The spectrum was fitted to a Lorentzian function to get f_c.

The trapped RBC was imaged with a video camera.