Theoretical models for near forward light scattering by a *Plasmodium falciparum* infected red blood cell

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A number of experimental elastic light scattering studies have been performed in the past few years with the aim of developing automated *in vivo* tools for differentiating a healthy red blood cell from a *Plasmodium falciparum* infected cell. This paper examines some theoretical aspects of the problem. An attempt has been made to simulate the scattering patterns of healthy as well as infected individual red blood cells. Two models, namely, a homogeneous sphere model and a coated sphere model have been considered. The scattering patterns predicted by these models are examined. A possible method for discriminating infected red blood cells from healthy ones has been suggested.

**Keywords:** medical and biomedical optics; malaria; *Plasmodium falciparum*; red blood cell characterization

1. Introduction

Malaria is a serious disease which affects more than 240 million people worldwide resulting in about 781,000 deaths every year [1]. The majority of these deaths are caused by a parasite known as *Plasmodium falciparum*. The conventional diagnosis of malaria relies on a microscopic examination of blood smears. The method requires invasive acquisition of blood samples, significant training for health workers and it also involves time delay in the diagnosis. It is, therefore, desirable to develop faster, noninvasive and innovative automated methods which are independent of operator bias and skill. With these requirements in mind, there is considerable interest currently in examining the potential of the elastic light scattering technique as an alternative to conventional methods for quick and reliable diagnosis of malaria [2–12].

Methods relying on elastic light scattering have been extensively used for retrieval of size, shape and refractive index information of scatterers in many disciplines of science and engineering including biomedical diagnostics [13–21]. Scattering by a single scatterer as well as scattering and propagation of light in a collection of scatterers has been employed for this purpose. Whereas flow cytometric type methods could be used for developing systems based on scattering by one particle at a time (for example, for RBCs), biomedical tissues have been modeled often as a collection of scatterers. Measurements including angular scattering, back scattering, extinction spectra, variation of diffuse reflectance as a function of source detector separation, change in linear polarization and circular polarization, etc. have been analyzed for diagnostic purposes.

Subsequent to the invasion of RBCs by *P. falciparum*, intraerythrocytic development begins with what is known as the ring stage, followed by the trophozoite stage as the parasite grows and culminating with the schizont stage. Considerable changes in the physical structure of the RBC occur during this development. As a result, appreciable changes in the light scattering features are also expected. This suggests that it should be possible to discriminate the malaria-parasite invaded cells from the normal ones from their light scattering pattern.

Park et al. [2] have recently investigated structural, biochemical and mechanical modifications in healthy RBCs at various post infection stages using tomographic phase microscopy and diffraction phase microscopy. In another study, static and dynamic light scattering of healthy and malaria-parasite invaded RBCs was examined experimentally by Park et al. [3]. For a single infected RBC, it was noted that not only did the scattering pattern change, the scattered intensity at forward scattering also decreases. It was concluded from the static scattering maps of individual RBCs that the scattering pattern can distinguish the specific disease state. Lee and Lu [4,5] have studied experimentally the light scattering patterns of healthy *P. falciparum* parasitized red blood cells. By measuring the wavelength dependent scattering of blood samples at discrete angles of both forward and

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backward directions, it was concluded that signal can distinguish between healthy and ring stage infected red blood cells. The absorption and scattering properties of the parasite in the three developmental stages have been studied theoretically as well as experimentally by Serribrennikova et al. [6]. For this purpose parasites were extracted from host erythrocyte cultures. The experimentally measured spectra were interpreted theoretically using a core-shell Mie theory for the structure of *P. falciparum*. Hemoglobin content and sizes of infected erythrocytes have been studied by Hanssen et al. [7] using soft X-rays. The scattering characteristics of malaria byproduct hemozoin, including its scattering distribution and depolarization, have also been examined [9,10]. Brief reviews [11,12] on the biophotonic techniques for the study of malaria-infected red blood cells have been published recently.

It is well known that a normal human RBC has a biconcave discoid shape. However, in the absence of availability of exact solutions, early investigations have used a homogeneous sphere as the model for the RBC shape [22–26]. Steinke and Shepherd [22] have performed scattering experiments showing fair agreement between measured scattering cross-sections and those calculated. In a highly diluted suspension Hammer et al. [23] measured angle-resolved intensity of scattered light and compared with predictions of Mie theory. The measurements were in reasonable agreement with the predictions of Mie theory in the near forward direction. The absorption and scattering cross-sections obtained from Mie theory were used by Steinke and Shepher [24] to describe light transport in the blood. More recently, rigorous simulations of light scattering by realistic RBCs have become possible employing a variety of numerical methods [27–33]. The results generally conclude that the approximation of RBC shape by a volume-equivalent sphere is not sufficiently accurate. Nevertheless, the spherical shape is still a useful model because of the following reasons. (i) It is possible to sphere RBCs through a chemical treatment. Kim and Orenstein [25] developed a procedure for isovolumetrically sphering RBCs. The advantage of sphering is obvious. The spherical shape can be handled conveniently and accurately using the Mie theory [34]. The use of the Mie theory then is not an approximation. Tycko et al. [26] found good agreement in flow cytometric measurements of spherical RBCs with the Mie theory. More recently Kinnunen et al. [35] have compared the scattering patterns from actual RBCs as well as from a spherical RBC with Mie results. Good correlation was found in comparisons of Mie theory and sphered RBCs. Tarasov et al. [30] have noted that sphering close to ideal can be achieved in the presence of sodium dodecyl sulfate. Physiologically, the sphering could result in a small change in thickness of the RBC membrane. However, this change is so insignificant that it does not affect the scattering from the RBC [25]. In fact, it is known that even the entire membrane contributes negligibly to the scattering properties of a RBC [26,30]. (ii) It has been observed that RBC volume and refractive index are the main factors in determining changes in scattering cross-sections and not the shape [35,36]. Thus, for blood in physiological concentrations, the Mie theory offers a good description of the absorption coefficient, even without sphering, in the spectral range 600 to 1100 nm where absorption is low [36]. (iii) Although the homogeneous sphere model is not very accurate, it is still a useful approximate model for near forward scattering to the extent of extracting qualitative conclusions.

The present work attempts to study the changes in the angular light scattering pattern of a RBC at various stages of *P. falciparum* infection, modeling the RBC as (a) a homogeneous sphere and (b) a core shell structure. A flowcytometric arrangement is envisaged in which one RBC at a time passes through the scattering region. That is, the scattering pattern of each RBC is analyzed separately. The infection stage is thus detected on a cell by cell basis. In malaria infection, after invading the red blood cells, *P. falciparum* digests much of erythrocyte's hemoglobin and the digested heme is converted into a substance called hemozoin, which is sequestered in the digestive vacuole of the parasite. Consequently, it would appear that the core–shell structure is a better model for a RBC. We examine both the models: (i) healthy as well as infected RBCs are modeled as equivalent volume homogeneous spheres by defining and computing an effective refractive index for the whole RBC for each infection stage. (ii) The homogeneous sphere model is then modified to a core–shell structure with the parasite vacuole as the core and the host RBC as the shell. Concentric as well as non-concentric (off-centered core) structures are examined. An attempt has been made to identify signatures in the scattering pattern which could be used to discriminate diseased RBCs from the healthy ones. The paper is organized as follows. Section 2 examines the validity of the homogeneous sphere model for a single RBC. By carefully analyzing the simulated scattering patterns, a method for simultaneous determination of refractive index and size of the RBC is suggested. This retrieval of size and refractive index completely characterizes the RBC. Section 3 considers the core–shell model for an infected RBC and examines its validity in simulating the scattering pattern. Finally, Section 4 concludes by summarizing the main results of this study.
2. The homogeneous sphere model

To generate angular light scattering pattern of a scatterer, data on its size shape and refractive index is needed. The same applies to RBCs. Thus, the first task in the process of simulating scattering patterns for a RBC is to build a morphological model. We do this on the basis of some recent experimental results. It has been noted by Park et al. [2] that healthy RBCs have an homogeneous distribution of refractive index. Infected RBCs, on the other hand, are optically inhomogeneous. In another study [7], the X-ray absorption profiles indicate that the ring stage parasite is cup shaped with a large invagination of host cytoplasm. The early trophozoite maintains an invagination feature but by the mid-trophozoite stage the parasite expands to a more spherical shape. X-ray dense features are evident within the parasite, suggesting these are aggregates of hemozoin crystals. It is a difficult, if not impossible, task to model such a complex object realistically. However, given that the RBCs have been successfully modeled as homogeneous spheres in the past [22–26] and also in recent times [9,35], the simplest model that could be contemplated for healthy RBCs is a homogeneous sphere. This is particularly acceptable because it is possible to sphere the RBCs. Infected RBCs, although optically inhomogeneous, may also be approximated as homogeneous spheres as a first step. Intracellular inhomogeneities of a RBC, including that due to parasite, may be thought of as contributing to an effective refractive index for the RBC. A number of effective medium theories [37,38] have been developed over the years for approximating inhomogeneous media by an homogeneous one. The choice of mixing rule to be used depends on the type of inhomogeneities involved.

2.1. Basic data

The mean refractive index of cytoplasmic volume obtained by Park et al. [2] is 1.399 ± 0.006, 1.395 ± 0.005, 1.383 ± 0.005 and 1.373 ± 0.006 for healthy, ring, trophozoite and schizont stage, respectively. These measurements were performed at 14–20 h (ring stage), 20–36 h (trophozoite stage) and 36–48 h (schizont stage) post invasion. The tomographic phase microscopy was used for these measurements which provides three-dimensional structural information. The volumetric information gives healthy RBC volume as 93.1 ± 7.9 fl, which for a spherical volume, works out to a radius range 2.730 to 2.889 μm. The value 93.1 fl corresponds to a radius of 2.8116 μm.

For infected RBCs, the mean values of the cytoplasmic volume, as given by Park et al. [2] are 88.5 ± 11.8, 57.5 ± 13.8, and 34.2 ± 15.1 fl for ring, trophozoite and schizont stage RBCs. It is well known (see, for example [7]) that whereas the parasite volume inside the RBC expands during various stages, the whole RBC volume stays relatively constant. Thus, the RBC volume is taken to be same in all infection stages. For simulations in the present work, we take cytoplasmic volumes to be 93.1, 88.5, 57.5, 34.2 fl for the four progressive RBC stages. The parasite vacuole size, which is obtained by subtracting the parasite volume from the whole RBC volume, works out to be 4.6, 35.6 and 58.9 fl for ring, trophozoite and schizont stages, respectively.

The effective refractive index for the homogeneous sphere model may be computed using one of the many mixing rules [37,38]. Here, we use the Gladstone–Dale law of mixtures which has been often used to compute the effective refractive index in the context of bioparticles. Using this law, the effective refractive index for the RBC, may be expressed as

\[
\eta_{RBC} = \frac{V_{shell}\eta_{shell} + V_{core}\eta_{core}}{V_{shell} + V_{core}},
\]

where \(\eta\) and \(V\) represent the refractive index and the volume, respectively. The subscripts ‘core’ and ‘shell’ refer to parasite vacuole and cytoplasmic shell respectively. From refractive index maps of RBCs given in Park et al. [2], we take the refractive index values of the parasite vacuole to be 1.36 for the ring stage and 1.38 for trophozoite and schizont stages by eye estimation. The effective refractive index of the homogeneous spherical RBC then works out to be 1.399, 1.393, 1.382 and 1.377, respectively, for healthy, ring, trophozoite and schizont stages.

2.2. Scattered intensity

Figure 1 shows a plot of simulated scattered intensities against scattering angle \(\theta\) on a semi-log scale for four stages of a RBC modeled as an homogeneous sphere. The radius of the sphere, denoted by \(a\), is 2.8116 μm in each case. The refractive index is taken to be 1.399, 1.393, 1.382 and 1.377 for healthy, ring, trophozoite and schizont RBC stages respectively. The wavelength of the incident light, denoted by \(\lambda\), is 0.6328 μm and the refractive index of the surrounding medium is taken to be 1.35. The scattered intensities depicted in Figure 1 correspond to the element \(S_{11}\) of the scattering matrix and have been obtained by using the well-known computer code, BHMIE, of Bohren and Huffman [27]. In this paper it is denoted by \(I(\theta) \equiv S_{11}\). Forward scattered intensities in Figure 1 can be seen to decrease as the RBC stage alters from normal to ring to trophozoite to schizont. This is in agreement with experimental observation of Park et al. [3], who found
that forward scattered intensity decreased by 16.6 % (ring stage), 30.7 % (trophozoite stage); and 65.7% (Schizont stage) as compared to forward scattered intensity of healthy RBCs. Corresponding results from the homogeneous sphere model are 14.9% (ring stage), 45.5 (trophozoite stage) and 59.3% (schizont stage). The qualitative agreement is apparent. We have noticed that the qualitative agreement continues in the near forward scattering region. The decreasing intensity trend can be readily understood in terms of smaller refractive index mismatch between the RBC and the surrounding medium as a higher infection stage is reached.

A part of the difference between experimental and model results could perhaps be attributed to use of parasite vacuole refractive index by eye estimation. However, the major reason for the disagreement is perhaps the assumption of modeling a real RBC by a homogeneous sphere. The model results are expected to agree with experiments only for the sphered RBCs. To the best of our knowledge, the angular scattering results for sphered infected RBCs are not available at this point in time.

The forward scattering amplitude, \( S(0) \), is related to the extinction efficiency by the following relation [34],

\[
Q_{\text{ext}} = \frac{4}{x^2} \text{Re} S(0),
\]

where \( S(0) \) is related to \( I(0) \) by the relation \( I(0) = |S(0)|^2 \) and \( x = 2\pi a/\lambda \) is the size parameter, which is a measure of the size of the RBC in units of wavelength of light. Table 1 gives extinction efficiency values for the four stages of RBCs. Note that extinction efficiencies show considerable change as a RBC goes from the healthy stage through the ring, trophozoite and schizont stages. The extinction efficiency of a normal RBC decreases to about 40% of its original value when it reaches the schizont stage.

The study of the variation of RBC extinction efficiency with infection stage is aimed only at getting a fuller picture of the scattering by an infected sphered RBC. It has not been used in arriving at the algorithm for characterizing the RBC. The characterization algorithm is entirely based on the near forward angular scattering.

### 2.3. Characterization of a RBC modeled as homogeneous sphere

Figure 2 provides a closer look at the forward lobe scattering patterns of Figure 1. The y-axis scale has been changed from log in Figure 1 to linear in Figure 2 for clear display of slopes in scattering patterns. The impression of minimum intensity nearing zero in Figure 2 is a consequence of this change. It is evident from this figure that the slopes of scattering patterns in all four stages are different. This implies that it should be possible to characterize a RBC from a measure of its near forward scattering slope. To quantify the slope, the ratio of the scattered intensities at a pair of suitable scattering angles,

\[
R(\theta_1, \theta_2) = I(\theta_1)/I(\theta_2),
\]

may be defined as a measure of the slope. Variation of \( R(\theta_1, \theta_2) \) for two angle pairs, \( R(1^\circ, 4^\circ) \) and \( R(1^\circ, 5^\circ) \), is shown in Figure 3. The refractive index has been varied in the range 1.37 to 1.40. The refractive index values of the RBCs are expected to lie within this range. Note that the graphs are single valued. Hence, \( n \) can be inferred unambiguously from a measured value of \( R(\theta_1, \theta_2) \). The knowledge of \( n \), in turn, characterizes the state of the RBC. The intensity ratio is single valued for the entire range \( 2.70 \leq a \leq 2.9 \) as shown in Figure 4 for the angle pair \( \theta_1 = 1^\circ \) and \( \theta_2 = 4^\circ \). Table 2 shows the intensity ratios for various stages of the RBC predicted by the model. The angle pairs are the same as those shown in Figure 3. The table shows that for deciphering the refractive index from the measured intensity ratios, a larger angle difference pair is preferable.
because the change in the value of $R(\theta_1, \theta_2)$ for this pair is larger for the same change in refractive index. The sensitivity of the technique is expected to be greater for such an angle pair.

Table 2. The ratio, $R(\theta_1, \theta_2)$, of the scattered intensities at two angle pairs within the forward scattering lobe computed for a homogeneous sphere model. Here $a=2.8116$ and the refractive indices are 1.399, 1.393, 1.382, 1.377, respectively, for healthy, ring, trophozoite and schizont stages of the RBC.

<table>
<thead>
<tr>
<th>$I(\theta_1)/I(\theta_2)$</th>
<th>Healthy</th>
<th>R-stage</th>
<th>T-stage</th>
<th>S-stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>$I(1^\circ)/I(4^\circ)$</td>
<td>4.91</td>
<td>4.79</td>
<td>4.63</td>
<td>4.60</td>
</tr>
<tr>
<td>$I(1^\circ)/I(5^\circ)$</td>
<td>15.90</td>
<td>15.45</td>
<td>14.75</td>
<td>14.58</td>
</tr>
</tbody>
</table>

The intensity ratio technique has been used in the past in various contexts [15,39]. Hodgkinson [39] had first noted that the measurement of scattering intensities at a pair of convenient angles within the forward scattering lobe could yield useful estimates of the size of the scatterer. More recently, Sharma [14] has demonstrated the potential of this method for characterizing a soft biomedical tissue modeled as a collection of scatterers with fractal size distribution. It was shown that size distribution as well as refractive index can be determined simultaneously and unambiguously from the scattering pattern using this method. The technique has the additional advantage that some of the measurement errors are annulled because of the fact that the ratios of the intensities and not the intensities themselves are involved.

The above description of the intensity ratio technique assumes that the size of the RBC is known 

because the change in the value of $R(\theta_1, \theta_2)$ for this pair is larger for the same change in refractive index. The sensitivity of the technique is expected to be greater for such an angle pair.
using Mie theory yields $R(1^\circ, 4^\circ) = 4.60$ and $R(1^\circ, 5^\circ) = 14.58$. These values of $R$ are considered as measured experimental values for the purpose of this illustration. Figure 5 shows graphs for a set of $(a, n)$ values which yield $R(1^\circ, 4^\circ) = 4.60$ (solid line) and $R(1^\circ, 5^\circ) = 14.58$ (dashed line). These curves correspond to all possible $(a, n)$ values that can produce $R(1^\circ, 4^\circ) = 4.60$ and $R(1^\circ, 5^\circ) = 14.58$ in the $a$ and $n$ range shown in the figure. The curves have been obtained using Mie computations. We first fix $n$ and vary $a$ to find desired $a$ values which generate $R(1^\circ, 4^\circ) = 4.60$ and $R(1^\circ, 5^\circ) = 14.58$. We repeat this for a number of $n$ values in the range $1.37 \leq n \leq 1.4$. The two curves can be seen to intersect at the point $(2.81, 1.38)$ which is the correct size and refractive index of the RBC.

### 3. Core–shell model for the infected RBC

In their experiments, Park et al. [3] noted that the scattering pattern of a healthy RBC shows a distinct regular periodic feature. The oscillatory features change for infected red blood cells, especially at higher angles ($\theta \geq 10^\circ$). Simulations in Figure 1, however, show that the scattering patterns in the homogeneous sphere model are very regular and periodic even for infected RBCs. Clearly, the homogeneous sphere model predictions do not agree with experimental observations even qualitatively at $\theta \geq 10^\circ$. The homogeneous sphere model, therefore, needs modification if it is to more resemble actual morphology and possibly be able to predict larger angle scattering correctly. An obvious choice that presents itself is a core–shell morphology with the parasite vacuole as the core embedded in the host RBC.

#### 3.1. Basic data

As already discussed in Section 2.2, we have taken 1.399, 1.395, 1.383, 1.373 as the shell refractive index for the four stages of the RBCs. We again assume the core refractive index to be 1.36 for the ring stage and 1.38 for the trophozoite and schizont stages. The radius of the RBC is 2.8116 $\mu$m and the core radii are 1.0317, 2.0407 and 2.4137 $\mu$m, respectively, for the ring, trophozoite and schizont stages.

#### 3.2. Scattered intensity

Figure 6 is a semi-log plot of the scattered intensities generated by a healthy RBC and three infection stages of the RBC modeled as a core–shell structure. The host and the inclusion have been taken to be concentric spheres while generating these graphs. The decrease in forward scattered intensity from this model is 12.23%, 45.56% and 58.92% relative to healthy RBCs for ring, trophozoite and schizont stages, respectively. These values are very similar to those predicted by the homogeneous sphere model. In fact, a comparison of graphs in Figures 6 and 1 shows that the scattering patterns obtained in the two models do not differ significantly over the entire forward lobe. Significant changes appear in scattering pattern only outside the forward lobe. The scattering patterns of infected RBCs are less smooth and less regular. This can be seen more clearly in Figure 7 where ring stage RBC scattering patterns from Figures 1 and 6 are plotted on the same axes. This is indeed the trend that has been seen in the experiments. This suggests that the core–shell model is perhaps a more realistic model for an infected RBC in comparison to the homogeneous sphere model.
The computations of the scattered intensity for the core–shell model have been carried out using the computer program developed for a non-concentric coated sphere by Ngo et al. [40]. The computer code is freely available on the Internet.

We have also computed the extinction efficiencies for this model. In Table 1 these are compared with those obtained from a homogeneous sphere model. Predictions of both the models are similar. A maximum difference of about 6% occurs for the schizont stage.

### 3.3. Off-centered core

Figure 8 shows a comparison of the scattering pattern of a concentric sphere model for the ring stage RBC with scattering patterns obtained when the core is non-concentric. The separation between core and shell centers is 0.2, 0.6 and 1.0 μm along the direction of incidence of the light. The size and refractive index of the core are 1.0317 μm and 1.393, respectively. The shell size is 2.8116 μm and the refractive index is 1.393. Plots in the figure clearly show that the scattering patterns for all four stages of the RBC are nearly identical up to about θ = 20° scattering angle. The differences appear at larger scattering angles. Clearly, the position of the core inside the host does not affect the scattering pattern appreciably up to about θ = 20° but becomes appreciable beyond 20°. We have verified that this is true for other infection stages too. A similar outcome was arrived at by Quirantes and Bernard [41] in the context of algae-like particles. They observed that there was little dependence on centered or off-centered coated spheres on scattering, absorption and extinction efficiencies.

### 3.4. Intensity ratio technique

Figure 9 shows a close up of the forward scattering lobe from Figure 6. It is clear that the slope of the scattered intensities in the forward lobe is different in the four stages of the RBC. Consequently it should be possible to choose a suitable angle pair for which \( R(\theta_1, \theta_2) \) would be single valued. However, the characterization of RBC using the intensity ratio technique is more complex in the core–shell model. The complexity arises because there are four variables in the coated sphere model (two refractive indices and two sizes). In contrast, in the homogeneous sphere model, there are only two variables. Thus, characterizing RBCs is much simpler in the homogeneous sphere model. The refractive index and size of the RBC can be determined simultaneously using the intensity ratio technique without any a priori knowledge about its size and refractive index.

### 4. Conclusions

This paper attempts to simulate the scattering pattern of a *P. falciparum* infected red blood cell at various stages of infection and suggests a method for characterizing the RBC from its near forward angular scattering pattern. Two models for RBCs have been investigated: (i) homogeneous sphere model and (ii) core–shell sphere model. Predictions of both the models show good qualitative agreement with experimentally observed intensity patterns in the forward scattering lobe. However, the experimental data available in the public domain, which is for non-sphered RBCs, is not adequate for detailed quantitative
assessments of the models. At angles beyond the first scattering lobe, the core–shell sphere model seems to be a more appropriate model. As expected, the scattering patterns are less regular and less periodic in this model. Scattering patterns from the concentric core model have been contrasted with predictions of the non-concentric core–shell model. The difference in the two scattering patterns becomes significant only for $\theta \geq 20^\circ$. Clearly, the core–shell model appears to be a better morphological model for an infected RBC. However, it is not clear how to use these features of scattering pattern for characterizing a RBC uniquely.

The extinction efficiencies have also been examined. Predicted extinction efficiencies in the two models are in close proximity. This is consistent with the observation that the scattered intensity decrease in the forward direction at various infection stages is nearly the same in the two models. This observation is also consistent with earlier observations [35] that the scattering cross-section does not significantly depend on the shape of the RBC. This observation is of significance from the point of view of light transport problems in blood.

It has been demonstrated that the scattered intensity ratios at two angle pairs within the forward scattering lobe can be used to completely characterize a spherical RBC in terms of its size and refractive index. It should be possible to infer the infection stage from this information. Quantitative verification, however, can be performed only when the experimental data on single spheres infected RBCs becomes available. We recognize that the intensity ratio technique is also an invasive technique but it at least enables one to get rid of operator bias. It may be concluded from this study that a simple homogeneous sphere model for RBCs is a potentially useful model for characterizing RBCs after sphering. For non-sphered RBCs only qualitative conclusions may be obtained.

The relationship between refractive index of a RBC and its infection stage was eye estimated in this paper from refractive index maps given by Park et al. [2]. But, it should be clearly understood that the error so introduced does not affect the accuracy of retrieval of the refractive index and size of the RBC in any way. The eye estimated values were employed essentially to demonstrate the single valuedness of the intensity ratio and to demonstrate the capability of the intensity ratio technique for characterizing the RBCs in terms of refractive index and size. A more accurate relationship between the infection stage and the refractive index of the RBC may be obtained by using values from the actual measurements in place of an eye estimation.

References


