Detection of *Plasmodium falciparum*-infected red blood cells by optical stretching

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1 Introduction

The most severe form of human malaria is caused by the parasite *Plasmodium falciparum* (*Pf*). The infection is accompanied by profound changes in the mechanical properties of the infected host red blood cells (RBCs), contributing to reduced blood flow in the microcirculation and vasoconstriction. A precise assessment of the mechanical properties of infected red blood cells (IRBCs) is thus essential for an improved understanding of the disease, for the assessment of potential treatments, and for improved diagnostic purposes. At the single-cell level, micropipette aspiration, optical tweezers, and magnetic traps offer powerful tools for precise measurements of cell compliance. These methods have either low throughput efficiencies or are limited in the information they yield. They are also difficult to incorporate into online diagnostic tools. Bulk methods such as ektacytometry have fast acquisition, but deliver only ensemble measurements liable to deliver false negative results when samples contain small subpopulations of IRBCs among many uninfected cells. We report here the development and use of a novel hematological tool for the detection of IRBCs, a microfluidic optical stretcher, tested in *Pf* cultures. We show that the technique is ideally suited for the quantification of alterations in the mechanical properties of RBCs during early stages of infection. The method combines the sensitivity of traditional single-cell measurement techniques with the potential for high throughput analysis.

The optical stretcher can determine IRBC elasticity in a noncontact mode by trapping cells directly between two divergent counterpropagating laser beams. Figure 1 illustrates the schematics of the setup used. The capillary and optical fibers are supported and aligned by a cross-shaped photolithographic pattern on a glass slide. The trapped cell is stretched along the axis of the laser beams by simply modulating the light intensity. The stretching forces are generated by the momentum transfer that occurs at the interface between the sample cell and the surrounding medium due to a change in refractive index. These surface forces pull the cell apart, analogous to a tug-of-war situation. The resulting stretching force can be 1 to 2 orders of magnitude larger than the net trapping forces, the latter arising from the asymmetric force field for cells positioned slightly off the center in the trap. Spheroidal cell shapes greatly facilitate theoretical modeling, and consequently the only quantitative measurements of erythrocyte mechanical properties with the optical stretcher have been performed in the past on osmotically swollen erythrocytes. To measure erythrocyte deformability without altering its original shape, the analysis of surface forces becomes more involved, but is tractable in principle using finite element method (FEM) calculations. An alternative approach, taken here, is to employ a simplified phenomenological model to quantify cell compliance. Here we use the simple Kelvin-Voigt model, assuming only a global viscosity \( \eta \) and elasticity constant \( E \) for the whole cell. According to this model, the strain \( \varepsilon(t) \) following an instantaneous constant stress \( \sigma \) can be described as:

\[
\varepsilon(t) = a \cdot \left[ 1 - \exp(-\lambda t) \right],
\]

where \( \lambda = E \cdot \eta^{-1} \) is the characteristic deformation rate and \( a = \sigma \cdot E^{-1} \) is the relative amplitude of the stretch.

2 Materials and Methods

2.1 Cultures and Preparation of Cells

Red cells infected with *Pf* A4-BC6 clone (kindly provided by B. Elford at the Institute of Molecular Medicine, Oxford, United Kingdom), derived from clone A4, were cultured under a low-oxygen atmosphere by standard methods. Parasite development and replication were assessed in cultures by microscopic inspection of Giemsa-stained thin blood smears and parasite count, as reported before. IRBCs with trophozoite-stage parasites were concentrated from culture samples by...
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Fig. 1 (a) Schematic of the optical stretcher illustrating the photolitho-
graphical SU/8 pattern used for aligning the fibers and capillary. Two
lights delivering opposing fibers are automatically aligned when
placed into the grooves, which are narrower than the fiber diameter.
The superposition of the light creates a stable trap. Cells can be
trapped and stretched, depending on the light intensity. (b) Sequence
of operation during experiments. After a cell is positioned in the trap,
the capillary flow is halted. The image acquisition is started and after
one second the stretcher light intensity is instantaneously stepped up
for approximately one second. The cells stretch and relax according
to the light intensity. (c) Example of an uninfected RBC in the trap at low
light power. The image analysis routine automatically locates the cell
boundaries and displays corresponding cell edge pixels. (d) The same
cell as in (c), elongated due to increased power in the optical
stretcher. (e) Deformation behavior of uninfected RBCs compared to
IRBCs. Cohorts (squares, n=32) and controls (triangles, n=46) be-
have similarly and exhibit small standard errors, whereas the IRBCs
(circles, n=24) show a greatly reduced compliance and larger stan-
dard errors.

gelatine flotation immediately prior to experimentation. Con-
trol samples of normal, uninfected RBCs, obtained from
healthy volunteers by venipuncture into a syringe with hep-
arin, after informed written consent, were tested in parallel.

2.2 Optical Stretcher

The microfluidic optical stretcher was custom built [see Fig.
1(a)] for the IRBC experiments, modifying an integrated mi-
crofluidic design. The laser beams were arranged to enter and
cross the capillary 15 μm above its bottom wall. This off-
center arrangement, which is lower than in previous designs,
became necessary due to the rapid sedimentation of the unin-
fected RBCs to the capillary bottom, where they lay flat
(1.5 μm height), and would otherwise not have been picked up
by the optical trap. The flow capillary (number 8510, Vit-
room Limited, Mountain Lakes, New Jersey) was connected
with tubing and fittings (Upchurch Scientific, Oak Harbor,
Washington) to two Eppendorf reservoirs. The photostretching
light source was a 5-W cw linear-polarized single-mode Yb fiber
laser (YLR-LP-5, IPG Photonics, Oxford, Massachusetts),
emitting at a wavelength of 1070 nm. The laser light in the
optical fibers (PM980-XP, Nufern Limited, East Granby, Con-
ecticut) was split 50/50 in a polarization-maintaining Y-fiber
beam splitter (Gould Fiber optics, Millersville, Maryland). The
photolithographic pattern aligning the capillary and laser
fibers was made from SU-8 2025 photoresist (MicroChem
Corporation, Newton, Massachusetts) as described in Ref. 5.
The beam waist radius at the center of the trap was calculated
to be \( \omega \approx 14 \mu m \).

2.3 Stretching Procedure

The cell compliances measurements were performed as previ-
ously described.6 When cells were trapped (with the major
axis of the uninfected, biconcave-disk-shaped cells aligned
parallel to the laser fibers), the flow was stopped for the
time of the measurement. The laser emission was then
switched on and set to a low trapping power density
(8.4·10^5 W/cm^2 at the trap center/40 mW power in each
fiber). The power density was instantly increased (\( t_{rise} 
\approx 50 \mu s \)) to 53.4·10^5 W/cm^2 (laser power of 2×250 mW)
for one second, and then switched back to the previous lower
trapping power, as sketched in Fig. 1(b). In the malaria-
infected sample, cohort cells were visually distinguishable
from trophozoite-containing IRBCs, as the latter contained
dark hemozoin crystals and had irregular shape.

2.4 Imaging and Image Analysis

The microfluidic optical stretcher was mounted on a Leica
DM IRBE inverted microscope with a 63× long working
distance objective (HCX-PL-FLL 63×, Leica Microsystems,
Wetzlar, Germany) in phase-contrast mode. The stretching se-
quences were recorded with a microscope camera PL-A662
(Pixelink, Ottawa, Canada) at \( \approx 12 \) frames per sec. Image
analysis and edge detection were performed with custom soft-
ware written in Labview Vision (National Instruments, Austin,
Texas). The cell elongation was measured by performing an
eclipse fit of the digitally recognized cell edge pixels, report-
ing the axis length parallel to the optical fibers. Figures 1(e)
and 1(d) are two phase-contrast images of a stretching se-
cence. They show a typical sample of cell edges in trapped
Fig. 1(c) and stretched Fig. 1(d) states.

3 Results/Discussion

Optical stretcher measurements of cell compliance were done
in three different types of cells: 1. trophozoite-containing IR-
BCs, 2. uninfected RBCs of the IRBC culture (cohorts), and
3. controls. A reduced compliance was observed for IRBCs,
confirming previous results obtained with optical tweezers.4
Both cohorts and controls retained their normal biconcave
disk-like shape. Figure 1(e) shows a comparison between the
stretch responses of controls (n=46), cohorts (n=32), and
IRBCs (n=24). Both uninfected samples display homoge-
eous behavior in the optical stretcher, with a similar compli-
cance and a small standard error [Fig. 1(e)]. In contrast, the
IRBCs are less compliant, with a relative stretch amplitude \( a \)
reduced by almost half. The stretch response of IRBCs was
much more variable than that of uninfected cohorts and con-
trols, reflected in the much larger error bars.

The fitted curves in Fig. 1(e) represent the exponential strain-time relation from the Kelvin-Voigt model [Eq. (1)].
The parameters for these fits and their standard errors of the mean (SEM) are shown in Table 1.

Optical stretching of infected and uninfected red blood cells
confirms results obtained with other single-cell mechanics-measurement techniques.\[ Measurements with optical
tweezers had found IRBCs to stiffen significantly during
parasite maturation, with shear moduli increasing up to tenfold in the late schizont stage. Here we have shown that even at an early trophozoite stage, when the increase in apparent stiffness (the relative stretching amplitude $a$) by a factor of $\sim 2$ is relatively modest, IRBCs can be easily detected with the optical stretcher. Also, the values found for the elasticity-viscosity ratio (the deformation rate $\lambda$) for uninfected RBCs are in good agreement with earlier published values in the range of 5 to 10 s$^{-1}$. This ratio decreased by a factor of $\sim 3$ for the infected cells in our experiments.

Our study shows that uninfected cohort cells from $ Pf $ cultures and control RBCs do not have significantly different mechanical properties in vitro. This is in contrast to one clinical study performed with ectakymetry. Dondorp et al. attributed the reduced deformability of red blood cells (infected and uninfected) from patients with severe malaria also to alterations in the uninfected RBC (not to be confused with “uncomplicated malaria” in Ref. [3]). The ectakymeter, however, cannot distinguish between infected and uninfected RBCs. The extension to uninfected RBCs is made in this study because of the low fraction of parasitized cells present (< 20%), and the finding that the measured deformability did not correlate with different parasitaemia levels. It remains to be elucidated whether reduced elasticity can be observed with the optical stretcher in ex vivo RBC samples from patients with severe malaria.

Quantifying the absolute elasticity modulus remains an open task. It is solvable in principle and has been demonstrated for spherical shapes. But the biconcave disk-like shape of uninfected RBCs, and the irregular shape of IRBCs require theoretical modeling and SEM simulations to extract absolute numbers for forces and elasticity modulus. However, the simplified phenomenological model used for analysis here proved sufficient for detecting the mechanical differences in diseased cells. The characteristic deformation rate $\lambda$ is independent of the actual magnitude of stress on the cell. Both parameters $a$ and $\lambda$ change significantly during infection (with the probability of the null hypothesis that the means are not different $p < 0.001$), and establish the use of this optofluiddic device in applications that involve automatic cell sorting, early stage diagnostics, or screening of drugs that reduce cell stiffening.

In summary, optical stretching is a promising novel technique to measure red blood cell mechanical properties and their changes during disease. Its main advantage is that it operates entirely in a noncontact manner and can thus render results that are free from artifacts induced by adhesion to surfaces or physical contact required in other single-cell mechanical-measurement techniques. In addition, by incorporation into a suitable microfluidic environment and automation, throughput rates approaching one cell per second can in principle be achieved, which compares favorably with other single-cell mechanical-measurement techniques.

Acknowledgments

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References


**Table 1**

<table>
<thead>
<tr>
<th>Cell type</th>
<th>$n$</th>
<th>$a$</th>
<th>$\lambda$ (s$^{-1}$)</th>
<th>$\chi^2$</th>
<th>$D$ (µm)</th>
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<tr>
<td>Infected</td>
<td>24</td>
<td>(36±3)·10$^{-3}$</td>
<td>2.5±0.7</td>
<td>8·10$^{-6}$</td>
<td>4.2</td>
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<tr>
<td>Controls</td>
<td>46</td>
<td>(64±1)·10$^{-3}$</td>
<td>9.6±0.4</td>
<td>2·10$^{-6}$</td>
<td>4.6</td>
</tr>
<tr>
<td>Cohort</td>
<td>32</td>
<td>(66±1)·10$^{-3}$</td>
<td>8.6±0.7</td>
<td>8·10$^{-6}$</td>
<td>4.6</td>
</tr>
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