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Anisotropic light scattering of individual sickle red blood cells

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Abstract. We present the anisotropic light scattering of individual red blood cells (RBCs) from a patient with sickle cell disease (SCD). To measure light scattering spectra along two independent axes of elongated-shaped sickle RBCs with arbitrary orientation, we introduce the anisotropic Fourier transform light scattering (aFTLS) technique and measured both the static and dynamic anisotropic light scattering. We observed strong anisotropy in light scattering patterns of elongated-shaped sickle RBCs along its major axes using static aFTLS. Dynamic aFTLS analysis reveals the significantly altered biophysical properties in individual sickle RBCs. These results provide evidence that effective viscosity and elasticity of sickle RBCs are significantly different from those of the healthy RBCs. © 2012 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.JBO.17.4.040501]

Keywords: red blood cell; sickle cell disease; light scattering; quantitative phase microscopy.

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Sickle cell disease (SCD) is an inherited blood disorder where a point mutation in the β-globin gene results into production of sickle hemoglobin (HbS) instead of hemoglobin (HbA).1 Under deoxygenated condition, HbS self-assembles inside the red blood cell (RBC) and dramatically damages the RBC membrane structure, often resulting into a sickle-shaped RBC. This sickle RBC has a considerably reduced deformability, causing abnormal rheological properties of sickle blood and eventually vaso-occlusion and organ damage.

Characterizing sickle cell properties, especially at the single-cell level, plays crucially important roles in understanding the pathophysiology of SCD.2 However, the characterization of individual sickle RBCs is complex and not fully addressed. It is presumably because of the limitations of the measurement techniques.2 Here, we report both static and dynamic light scattering results from individual sickle RBCs that are enabled by introducing anisotropic Fourier transform light scattering (aFTLS).

Light scattering techniques have been extensively used for characterizing biological molecules, cells, and tissues.3 Static light scattering can also measure the volume and cytoplasmic hemoglobin concentration of the RBCs.4 Temporal fluctuation of scattering signals with respect to a specific scattering angle provides information about motion—a diffusion coefficient of the scattering object.

Angular light scattering has traditionally been measured with goniometer-based instruments and several measurement techniques have been used to study light scattering of objects.56 Recently, a significant breakthrough in light scattering detection sensitivity has been achieved by the development of Fourier transform light scattering (FTLS) technique.7 In FTLS, light scattering patterns from a sample can be obtained from the electric field (E-field) of the sample via quantitative phase imaging technique. The E-field E(r, t) is then numerically propagated to far-field by 2-D Fourier transform as I(φ, t) = |∫ E(r, t) exp[−jq·r]2|/2π, where q is the spatial frequency vector. The growing scientific interest and increased number of studies using FTLS is indicative of its effectiveness in the study of various phenomena in biophysics and cell biology.8-11

To measure light scattering from sickle RBCs, we prepared blood samples extracted from a patient with SCD as well as from a healthy individual under a research protocol approved by the institutional review board (IRB). The SCD patient was under treatment with hydroxyurea. The blood was collected in ethylenediaminetetraacetic acid (EDTA) anticoagulant and stored at 4°C. For measurement, the blood sample was first diluted with a phosphate-buffered saline. To quantitatively measure the E-field maps of sickle RBCs, we employed diffraction phase microscopy.12,13 For each sickle RBC, we measured the E-field E(r, t) = A(r, t) exp[iΔφ(r, t)], where A(r, t) and Δφ(r, t) are the amplitude and phase delay, respectively. All the measurements were performed at ambient oxygen concentration (21%) and room temperature (23°C). The time-averaged cell height maps can be retrieved from the measured phase maps as h(r) = (Δφ(r, t)) / (2π · Δn), where λ is the wavelength of the laser and Δn is the difference in refractive index between RBC cytoplasm and surrounding medium. The sickle RBCs are classified in accordance with the morphology: echinocyte (type II), discocyte (type III), and crescent-shaped irreversibly sickled cell (type IV; ISC) (Fig. 1). This classification corresponds with sickle RBC fraction II–IV by density separation.14 The measured topographies of the sickle cells are consistent with a recent study using quantitative phase imaging.15

To retrieve anisotropic light scattering, we introduce aFTLS analysis (Fig. 2). We first processed the E-field of individual sickle RBCs such that the horizontal axis of the E-fields is parallel to the long axis of the cell. Then the angular light-intensity scattering pattern of the sickle RBC is retrieved by applying 2D Fourier transformations to the E-field. The spatial frequency vector and the scattering angle can be related as |q| = 2πn sin θ/λ, where n is the refractive index of medium. The light scattering patterns along the long and short axes are retrieved by selecting the pattern of polar angle width of 30 deg along the horizontal and vertical axes, respectively. Then, the light scattering signals along the long and short axes are retrieved as a function of scattering angle after...
is not statistically different. The \( \omega \) can be directly and \( \Gamma \) and \( \beta \) was added \( \Gamma \) values of sickle values. 0

The static light-intensity scattering patterns associated with indi-

1. Phase image of a typical sickle RBC. (b) The long axis is

However, the human RBC membrane has a complex

The dynamic light scattering from membrane fluctuations

To measure the dynamic light scattering of sickle RBCs, we

To our knowledge, there has been no prior study of light

1. it is difficult to classify individual sickle RBCs in
different morphological groups without imaging the

2. the scattered power from an individual sickle RBC is

3. even if successful in both classification and detection,

For sickle RBCs of types II-IV, the intensity autocorrelation

We then retrieved the scattering of sickle RBCs in different

dissymetric scattering signals along the long and short axes [Fig. 2(d)].

We then retrieved the scattering of sickle RBCs in different

dissymetric light scattering signals along those two axes. These anis-

The values of \( \omega_0 \) for all types of sickle RBCs are approximately
two times greater than those for the healthy RBCs

Between different types in sickle RBCs, \( \omega_0 \) is not statistically different. The \( \Gamma \) values of sickle

RBCs are significantly smaller than those of the healthy RBCs

The increase of \( \omega_0 \) in all types of sickle RBCs indicates the loss of deformability [Fig. 4(a)], which is consistent with pre-

The loss of deformability in the sickle RBCs can be explained by alterations in RBC metabolism and membrane structure including loss of membrane
phospholipid symmetry, uncoupling of the lipid bilayer from the sub-membrane structure, and abnormal membrane phosphorylation. Abnormal membrane phosphorylation may seriously affect the enhanced membrane fluctuations in the presence of ATP. The self-assembly of HbS may also decrease the deformability in the sickle RBCs; polymerization of HbS could transform the viscous Hb solution in the sickle RBC cytoplasm into viscoelastic material.

Whereas elasticity characterizes resistance to deformation, viscosity characterizes resistance to a rate of deformation. For the RBC, the effective viscosity is associated with the recovery time after large deformation, and it is primarily determined by the shear modulus of the spectrin network as well as the membrane surface viscosity as . The significant decrease in effective viscosity of ISCs, implying a decrease in recovery time, suggests a decrease in or an increase in , or both. This is also explained by alterations in composition or structures of the RBC lipid bilayers or decreased binding of glyceraldehyde phosphate dehydrogenase to the membrane. These altered viscoelastic properties of the sickle cell can explain the significantly decreased dynamic fluctuations in the sickle cell membrane.

In conclusion, we present anisotropic light scattering of sickle RBCs. The aFTLS technique, a variation of FTLS, precisely and systematically measures anisotropic light scattering of asymmetric small objects. Using aFTLS, we study the light scattering from sickle RBCs and demonstrate anisotropic static light scattering patterns with respect to the elongated shape of sickle RBCs. The dynamic light scattering analysis reveals alternations in mechanical properties depending on the morphological type of sickle RBCs. In the future, the aFTLS technique could be used in combination with other existing optical imaging techniques to better study other RBC related diseases, for example, to understand the protective mechanism of sickle RBCs against infection of malaria parasite.

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References