Quantitative microscopy and nanoscopy of sickle red blood cells performed by wide field digital interferometry

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Abstract. We have applied wide-field digital interferometry (WFDI) to examine the morphology and dynamics of live red blood cells (RBCs) from individuals who suffer from sickle cell anemia (SCA), a genetic disorder that affects the structure and mechanical properties of RBCs. WFDI is a non-contact, label-free optical microscopy approach that can yield quantitative thickness profiles of RBCs and measurements of their membrane fluctuations at the nanometer scale reflecting their stiffness. We find that RBCs from individuals with SCA are significantly stiffer than those from a healthy control. Moreover, we show that the technique is sensitive enough to distinguish classes of RBCs in SCA, including sickle RBCs with apparently normal morphology, compared to the stiffer crescent-shaped sickle RBCs. We expect that this approach will be useful for diagnosis of SCA and for determining efficacy of therapeutic agents. © 2011 Society of Photo-Optical Instrumentation Engineers (SPIE). [DOI: 10.1117/1.3556717]

Keywords: red blood cells; erythrocytes; sickle cell disease; sickle cell anemia; cell imaging; quantitative phase microscopy; interferometry.

In this paper, we have applied, for the first time to our knowledge, WFDI techniques to quantitatively image sickle RBCs and to measure the nanometer-scale fluctuations in their thickness as an indication of their stiffness. The technique is capable of simultaneously measuring the fluctuations for multiple spatial points on the RBC and thus can yield a map describing the stiffness of each RBC in the field of view. Using this map, the local rigidity regions of each cell can be quantitatively evaluated. Furthermore, since the technique is basically a quantitative imaging technique rather than one-point measurement, we can use it to simultaneously evaluate cell transverse morphology plus thickness in addition to its stiffness profile. This yields various physical properties for live RBCs in a noninvasive, label-free manner, providing a sensitive tool for diagnosis and research.

WFDI measurements were performed by utilizing an interferometric microscopy setup, reported in detail in our previous publication.8 In this previous paper, we have used the system for quantitative microscopy of cardiomyocytes, in which thickness and refractive index cannot be easily decoupled. As shown next, this is not the case for RBCs, in which thickness and stiffness measurements can be easily performed by WFDI. In the optical setup, a coherent laser light (17 mW HeNe) is spatially filtered and split to reference and object beams. The object beam is transmitted through the sample and magnified by a microscope objective (40×, 0.66 numerical aperture). The reference beam passes through a similar compensating microscope objective and combined with the object beam at a small angle. A tube lens projects the combined fields on a digital camera (640×480, 7.4 μm×7.4 μm pixels, 120 full frames per second), where an off-axis interferogram of the sample is created. Using a single interferogram, the fully quantitative phase profile of the sample...
We have acquired phase profiles of 24 RBCs obtained from two different persons with SCA and 12 RBCs obtained from a healthy person. For each RBC, phase profiles were collected at a frame rate of 120 frames per second during 10 s and converted into thickness profiles. For each cell, we have calculated the standard deviation of the thickness fluctuations $\sigma_h$, which is inversely proportional to the stiffness map of the RBC.

Averaging $\sigma_h$ over the entire RBC area, marked as $\langle \sigma_h \rangle$, gives an indication of the cell flexibility, since less rigid RBCs are expected to fluctuate more than stiffer RBCs.

Figure 1 presents the dynamic quantitative phase profile and the associated thickness scalebar for one of the analyzed RBCs obtained from the healthy person. This specific cell yielded $\langle \sigma_h \rangle = 64.12$ nm. For comparison, Video 2 presents the dynamic quantitative phase profiles and the associated thickness scalebar of two RBCs obtained from a person with SCA. As can be seen in Video 2, the right cell has a regular round morphology, whereas the left cell has a crescent morphology. These sickle RBCs yielded $\langle \sigma_h \rangle = 28.73$ nm for the round-morphology RBC and $\langle \sigma_h \rangle = 13.54$ nm for the crescent-morphology RBC. Thus, even though the sickle RBC on the right has a visibly normal morphology, it is found to be more than twice as stiff as the healthy RBC.

Figure 2 presents the $\langle \sigma_h \rangle$ values obtained for RBCs of three groups: 12 round-morphology RBCs from a healthy person, 12 round-morphology RBCs from two persons with SCA, and 12 crescent-morphology RBCs from two persons with SCA. Each
of the two groups of 12 sickle RBCs was composed of 5–7 RBCs from the first person with SCA and 5–7 RBCs from the second person with SCA, where no significant difference was seen between the \( \langle \sigma_h \rangle \) values of the RBCs from the two individuals with SCA. The healthy RBCs yielded \( \langle \sigma_h \rangle = 51.07 \pm 12.02 \text{ nm} \) (which compares favorably with the values obtained for healthy RBCs by Park et al.\(^{13}\)), the round-morphology RBCs from SCA individuals yielded \( \langle \sigma_h \rangle = 21.76 \pm 7.64 \text{ nm} \), and the crescent-morphology RBCs from SCA individuals yielded \( \langle \sigma_h \rangle = 13.82 \pm 3.92 \text{ nm} \). These results demonstrate that the healthy RBCs are two to three times less stiff than the round-morphology sickle RBCs, and the latter are approximately half as stiff as the sickle crescent-morphology RBCs. Greater statistical difference, indicated by the lower \( p \)-values (\( p < 0.001 \)), is obtained between the group of healthy RBCs and each group of the sickle RBCs than between the two groups of sickle RBCs (\( p < 0.05 \)). The high statistical significance of the difference between the round-morphology RBCs from SCA individuals and the healthy RBCs demonstrates that although the sickle RBC shape might visibly appear to be the same as healthy RBCs, analyzing their thickness fluctuations by WFDI gives a clear indication that they are sickle RBCs.

In summary, we have demonstrated that WFDI is able to obtain dynamic quantitative phase profiles of sickle RBCs in a noncontact noninvasive manner. Based on these profiles, we have calculated the nanometer-scale thickness fluctuations of the RBCs and obtained a metric of their stiffness. Sickle RBCs were found to be significantly stiffer than healthy RBCs. Furthermore, we have demonstrated that it is possible to differentiate between sickle RBC morphologies taken from the same subjects by analyzing their thickness fluctuations, where crescent-morphology RBCs are more rigid (fluctuate less) than round-morphology RBCs. We anticipate that this technique will find uses for diagnosis and monitoring of SCA, as well as usefulness as a research tool, since therapeutic agents that decrease sickling can be expected to improve the abnormal cell rigidity described here.

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