Overview of cell motility-based sickle cell disease diagnostic system in shearing digital holographic microscopy

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Abstract

We overview a previously reported low-cost, compact, and 3D-printed shearing interferometer system for automated diagnosis of sickle cell disease based on red blood cell (RBC) bio-physical parameters and membrane fluctuations measured via digital holographic microscopy. The portable quantitative phase microscope is used to distinguish between healthy RBCs and those affected by sickle cell disease. Video holograms of RBCs are recorded, then each video hologram frame is computationally reconstructed to retrieve the time-varying phase profile of the cell distribution under study. The dynamic behavior of the cells is captured by creating a spatio-temporal data cube from which features regarding membrane fluctuations are extracted. Furthermore, the Optical Flow algorithm was used to capture lateral motility information of the cells. The motility-based features are combined with physical, morphology-based cell features and inputted into a random forest classifier which outputs the health state of the cell. Classification is performed with high accuracy at both the cell level and patient level.

1. Introduction

Digital holographic interference microscopy (DHIM) is an emerging optical technology capable of measuring both amplitude and phase information of phase objects such as living cells [1]. Given its ability for label-free and non-invasive quantitative phase imaging, DHIM has found recent success in the study of morphological cell properties in biological samples and for identifying diseases [2–14]. Example diagnostic applications using DHIM include screening for diseases such as malaria [9], diabetes [14], and sickle cell disease [2]. Furthermore, off-axis single-shot digital holographic methods, are capable of video rate data capture enabling the measurement and monitoring of spatio-temporal dynamics in live biological cells [2, 15, 16]. Various arrangements are available for the implementation of DHIM systems, however, most of the existing off-axis arrangements are complex, costly, bulky, and temporally not stable. Common-path shearing interferometers provide a highly stable arrangement for digital holographic microscopy while offering greater stability than two-beam systems [8], enabling the design of compact and field portable medical diagnostic instruments. Quantitative phase information obtained by the numerical processing of the recorded holograms provides important bio-physical information of the samples, which could be used for identification of diseases affecting them. In this manuscript, we overview our previously reported system for identification and diagnosis of sickle cell disease by examining cellular motility information of red blood cells (RBCs) in a compact, field-portable, 3D-printed shearing DHIM system [2]. After numerical phase reconstruction of each cell, morphological and cell membrane fluctuation features are extracted to characterize the RBCs. These features are used along with a random forest classifier to discriminate between healthy and disease-state cells.
2. Materials and methods

The developed DHIM system uses a shearing geometry to provide a compact and highly temporally stable device [2, 8, 11]. From the recorded holograms, the cell thickness distribution (cell morphology) is extracted. By studying the variations in the cell thickness over time, in coordination with other measured cell bio-physical parameters, highly accurate cell identification may be achieved.

2.1. Shearing-based digital holographic microscope

The field-portable device is 3D-printed and uses only a few optical components which results in a compact, and low-cost diagnostic device. A laser diode (λ = 633 nm) emits a collimated beam to illuminate the sample under inspection. Following the sample, a microscope objective (40X, 0.65 NA) magnifies the sample. The object beam then falls upon a fused silica glass plate (5 mm thickness), placed at a 45° angle to the incoming beam. The shear induced by the glass plate enables a simple implementation of off-axis digital holography wherein the glass plate is responsible for the generation of two laterally sheared beams. Portion one of the laterally sheared beams, not containing any object information, will act as the reference beam and generate an interference pattern (hologram) by superposing with the section of the other beam that contains the cellular information. The hologram is recorded by the sensor placed at the image plane of the objective lens. The fringe frequency for a shearing system is determined as $f_s = S/r\lambda$ where $S$ is the lateral shear induced by the glass plate, $r$ is the radius of curvature of the wavefront, and $\lambda$ is the vacuum wavelength of the laser source [17]. The lateral shear produced by the glass plate is a function of the glass plate thickness ($t_g$), and the incidence angle upon the glass plate ($\beta$). This relationship is defined by $S = t_g \sin(2\beta)/\sqrt{n^2 - \sin^2(\beta)}$ where $n$ is the refractive index of the glass plate [17, 18]. The schematic of the system is illustrated by figure 1(a).

The digital sensor used in this system was a Thorlabs DCC1545 M with sensor dimensions 6.66 mm $\times$ 5.32 mm and pixel pitch of 5.2 $\mu$m (8-bit dynamic range). The resultant field-of-view for this device was approximately 165 $\mu$m $\times$ 135 $\mu$m, and the theoretical resolution limit was calculated as 0.6 $\mu$m by the Rayleigh criterion. The main advantages of a shearing configuration are that its off-axis geometry simplifies the numerical processing in comparison to inline digital holographic systems, and its common-path set-up reduces the form factor of the system over two-arm systems such as the Mach–Zehnder interferometer while also removing effects of uncorrelated phase changes caused by noise differences between the object and reference arms. These advantages make the shearing configuration an ideal implementation for compact and highly temporally stable interferometric systems. The total dimensions of the compact system as shown in figure 1(b) are 90 mm $\times$ 85 mm $\times$ 200 mm.

2.2. Suitability of the system for measurement of cell thickness

Digital holographic microscopy provides a straightforward mechanism for measuring the thickness of phase objects. Following recovery of the object phase, the thickness can be computed from the optical path length using refractive index values of the cell and the surrounding medium. The off-axis geometry allows for easy separation of the real and conjugate images from the DC term in the Fourier domain by spectral filtering. After spectral filtering, the inverse transform provides the complex amplitude distribution of the sample under study. From the complex amplitude ($\tilde{U}(f_x, f_y)$), the object phase is given as $\Phi = \tan^{-1}\{\text{Im}[\tilde{U}] / \text{Re}[\tilde{U}]\}$, where $\text{Im}[-]$ and $\text{Re}[-]$ represent the real and imaginary functions, respectively. The extracted phase is then unwrapped by Goldstein’s branch-cut method [19] to provide the unwrapped phase distribution. Additionally, the phase distribution measured from a region of the sample containing only blood plasma is used as a reference and subtracted from the object phase to reduce the effects of system aberrations [20]. The optical path length (OPL) is computed from the unwrapped phase ($\Phi_{\text{un}}$) according to $\text{OPL} = \Phi_{\text{un}}[\lambda/(2\pi)]$. Furthermore, when the refractive indices of the object and backgrounds are known, the height can be computed as $h = \text{OPL}/\Delta n$, where $h$ is the height and $\Delta n$ is the refractive index difference. In the case of RBCs, $\Delta n = n_\text{RBC} / n_\text{plasma}$ with average values for RBC and plasma of 1.42 and 1.34, respectively [21]. However, in the case of sickle cell disease, since the presence of abnormal hemoglobin may alter the average refractive index of diseased cells, the analysis in this study was performed directly on the phase distributions [2].

2.2.1. Temporal stability

Capturing the nanometer-scale membrane fluctuations of an RBC requires a system to maintain suitable temporal stability over the duration of measurements. The temporal stability can be measured by considering the phase fluctuations of a blank glass slide without sample for a predetermined time period. Here, the temporal stability of the system was evaluated accordingly by recording a video hologram of a blank glass slide without sample for 20 s at 30 frames per second. From this recorded fringe pattern, the standard deviation of the pixel-wise optical path length differences between each frame and a reference frame.
Figure 1. (a) Schematic of the overviewed shearing digital holographic microscope. (b) 3D-printed compact system. Reprinted with permission from [2] © The Optical Society.

Figure 2. Temporal stability testing results for the 3D printed system, providing a mean standard deviation value of 0.76 nm [2]. Reprinted with permission from [2] © The Optical Society.

was calculated and used as a metric of system stability. A histogram of the computed standard deviation values, which have a mean value of 0.76 nm, is shown in figure 2. Given that the expected magnitude of membrane fluctuations is on the order of tens of nanometers, this system with sub-nanometer stability is well suited to study cell membrane fluctuations [2].

3. Relation between health-state of cells and spatio-temporal cell behavior

The health-state of a cell and the effect it may have on the morphology of the cell has been well reported, including work on malaria [9], sickle cell disease [2, 10], and diabetes [14], to name few. Moreover, in [22], the morphological changes of RBCs were studied over time following exposure to increased concentrations of calcium. This study showed the increased calcium levels change the morphology, likely due to degradation of the cellular membrane and decreased membrane rigidity. Furthermore, there is growing interest in the study of spatio-temporal cell behavior as a function of cell health. In [15], time-lapse digital holographic microscopy was used to evaluate the cell membrane fluctuations of RBCs and it was observed that healthy RBCs become stiffer over long storage times. DHIM-based spatio-temporal evaluation of cells has also been used for the label-free monitoring of live yeast cells [16]. By measuring the time-dynamic behavior of cells, we may gain insight into the function of the cell, rather than simply its structure as provided by morphological features alone. As the physiology of healthy and diseased cells differ, both the morphological and mechanical properties will vary, which can result in unique spatio-temporal behavior for a given health state.

3.1. Sickle cell disease

Sickle cell disease (SCD) is an inherited blood disease defined by the presence of an abnormal hemoglobin known as sickle hemoglobin, with hemoglobin being the protein responsible for oxygen transport [23]. Cells affected by this disorder are misshapen during deoxygenation (with a sickle structure) and hinder oxygen transport throughout the body. Figure 3 depicts reconstructions of RBCs taken from healthy volunteers (figure 3(a)) and individuals with sickle cell disease (SCD) (figure 3(b)). Despite the presence of sickle and rod-shaped cells in SCD patients, not all of the patients’ cells are misshapen, and chemical tests rather than visual inspection are required for diagnosis. Standard protocol for diagnosis of SCD relies on either gel
electrophoresis or high-performance liquid chromatography to analyze the hemoglobin content in the red blood cells. These assays are not uniformly available and blood samples are often required to be sent for specialized commercial laboratory testing and interpretation. Once received, specimen processing takes several hours and is often performed in batches to save costs which may extend the time for results to up to 2 weeks from the initial blood draw. The overviewed system \([2]\) uses only a small drop of blood for analysis and measures the spatio-temporal cellular activity in addition to cell morphology, providing results in near real-time. This point of care testing generates a bedside diagnosis and improves both the efficiency and quality of care delivered.

This analysis reveals the presence of the abnormal hemoglobin not only effects cell shape but also cell membrane fluctuation rates. This is in agreement with rationale that that SCD-RBCs may be stiffer than healthy RBCs as a result of the abnormal hemoglobin \([23]\). Video representation of a healthy RBC has been provided as supplementary material (available online at stacks.iop.org/JPPHOTON/2/031002/mmedia) in Visualization 1.

4. Classification

To distinguish between healthy cells and sickle cell disease cells, we use a machine learning approach and extract features to be inputted into a random forest classifier. The use of morphological features which describe the overall shape of the cell through measures such as mean optical path length, coefficient of variation, optical volume, projected area, ratio of projected area to optical volume, skewness, and kurtosis has previously shown success for cell identification and classification \([11]\). In the overviewed work \([2]\), we extend the abilities of the system to include measurements of the spatio-temporal cell information in order to provide additional useful information regarding the cell behavior to the classifier.

In total, 150 cells from six healthy volunteers (4 female, 2 male) and 150 cells from eight patients with sickle cell disease (2 female, 6 male) were used in the classification experiments. No differences in the presentation of the disease are expected based on gender. All data was collected at the UConn Health Center in accordance with UConn Health and UConn Storrs IRB policy standards. All participants were at least 18 years of age. Healthy volunteers did not have any hemoglobinopathy trait. No subjects were included if they had received a blood transfusion in the preceding 3 months. Approximately 6 to 8 ml of blood was drawn from each subject, then a thin blood smear was prepared for analysis in the DHIM system. Video holograms were recorded for 20 s at 30 frames per second. Following data acquisition, the individual cells are segmented and numerically reconstructed to allow for feature extraction and classification.

4.1. Spatio-temporal feature extraction

After numerical reconstruction of each frame in the video sequence for a cell, the timeframes are stacked sequentially to form a spatio-temporal data cube which contains the time-varying cellular information. Formation of the data cube is depicted in figure 4. Columns of the data cube represent pixel locations, and rows represent time points of the video.

After construction of the data cube, we compute a 2D mean map and a 2D standard deviation map by taking the means and standard deviations, respectively, over time at each pixel location. Examples of these maps are shown by figures 5(a) and (b) respectively. Next, we then take the standard deviation of the data to provide a single numerical value from each of the two computed 2D maps for use as spatio-temporal features in the classification scheme. These two spatio-temporal features are referred to as the standard deviation of the 2D mean map (STD_MEAN) and the standard deviation of the 2D standard deviation map (STD_STD), respectively. Furthermore, we use the Optical Flow (OF) algorithm \([24]\) to capture lateral motility information. The OF algorithm computes vectors which correspond to movement between successive frames. An example of OF vectors can be seen by figure 5(c). The standard deviation of a cells OF vector magnitudes is used as our third spatio-temporal feature.
Figure 4. (a) Sequential stack of the 3D optical path length reconstructed frames for a segmented RBC. (b) Spatio-temporal data cube. The red box indicates a single pixel stack representative of a single pixel location. Reprinted with permission from [2] © The Optical Society.

Figure 5. (a) 2D mean map and (b) 2D standard deviation map extracted from the spatio-temporal data cube. (c) Optical flow vectors for lateral movement between successive frames of a healthy RBC. Reprinted with permission from [2] © The Optical Society.

Table 1. Confusion matrices for classification of healthy and SCD-RBCs [2].

|                   | Spatio-temporal-based features | Morphology-based features | Combined features |
|-------------------|-------------------------------|--------------------------|------------------|
|                   | PN   | PP   | PN   | PP   | PN   | PP   |
| AN                | 56   | 19   | 67   | 8    | 65   | 10   |
| AP                | 14   | 61   | 3    | 72   | 0    | 75   |

AN = Actual Negative; AP = Actual Positive; PN = Predicted Negative; PP = Predicted positive; Negative = healthy RBCs; Positive = sickle cell disease RBC.

Table 2. Summary of classification accuracy outputs for disease diagnosis of patients based on features used [2].

|                    | Healthy patient 1 | Healthy patient 2 | SCD patient 1 | SCD patient 2 |
|--------------------|-------------------|-------------------|---------------|---------------|
| Spatio-temporal    | 20%               | 30%               | 72%           | 96%           |
| Morphological      | 90%               | 80%               | 92%           | 96%           |
| Combined           | 100%              | 100%              | 100%          | 100%          |

4.2. Random forest classification model

The extracted morphological and spatio-temporal features were input into a random forest classifier [25] with 100 decision trees. Classification was performed at both the cell and patient level. For cell level classification, all cells were randomly split such that 75 healthy and 75 SCD RBCs were used in training and 75 of each class remained for testing. Results of the cell-level classification are shown by the confusion matrices in table 1. Using only the spatio-temporal or morphology-based features alone results in classification accuracies of 78% and 92.6%, respectively. When all features are used, an accuracy of 93.3% is achieved. Patient level classification was performed by removing two patients’ cells for each class from the training of the classifier for use in testing.

Results of patient level classification are shown by table 2 where the percentages show the percentage of a patient’s cells that were classified correctly. From table 2 it is evident we achieve the best performance when we include all features (bio-physical and mechanical), and that the inclusion of the spatio-temporal features has provided additional information to our classifier resulting in improved performance.
5. Discussion

The overviewed system provides several advantages over the standard methods of sickle cell diagnosis as a low-cost, highly accessible system that can reduce the time to diagnosis. The DHIM-based system can provide results in just a few minutes compared to standard methods which take hours to complete and may be costly to run. Moreover, the compact, and field portable system requires only a small sample of blood for analysis and has suitable temporal stability to operate in the field, removing requirements for dedicated lab facilities.

6. Conclusion

In conclusion, we have overviewed a system for highly accurate cell and disease identification using spatio-temporal features related to cellular motility in a compact shearing-based digital holographic microscope. Video holograms of thin blood smears were recorded, individual cells were segmented and reconstructed, followed by feature extraction and classification. The inclusion of motility-based features improves performance over the use of morphological features alone. Future work includes continued research into using dynamic cell behavior in 3D imaging systems for highly accurate cell identification and disease diagnosis.

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