High throughput imaging of blood smears using white light diffraction phase microscopy

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ABSTRACT

While automated blood cell counters have made great progress in detecting abnormalities in blood, the lack of specificity for a particular disease, limited information on single cell morphology and intrinsic uncertainly due to high throughput in these instruments often necessitates detailed inspection in the form of a peripheral blood smear. Such tests are relatively time consuming and frequently rely on medical professionals tally counting specific cell types. These assays rely on the contrast generated by chemical stains, with the signal intensity strongly related to staining and preparation techniques, frustrating machine learning algorithms that require consistent quantities to denote the features in question. Instead we opt to use quantitative phase imaging, understanding that the resulting image is entirely due to the structure (intrinsic contrast) rather than the complex interplay of stain and sample. We present here our first steps to automate peripheral blood smear scanning, in particular a method to generate the quantitative phase image of an entire blood smear at high throughput using white light diffraction phase microscopy (wDPM), a single shot and common path interferometric imaging technique.

Keywords: peripheral blood smear, hematology, quantitative phase imaging, microscopy, interferometry, label-free imaging, wDPM

1. INTRODUCTION

In spite of the recent advances in automated blood analyser technologies such as flow cytometers and impedance analysers, blood smear analysis remains an indispensable technique for diagnosis of several hematological disorders.[1,2] The reason is that the complete blood count (CBC) reading generated by these blood analysers is does not provide disease specific and high resolution information on individual cell morphology which needs to be obtained through a blood smear test. Blood smear tests are carried out in pathology labs by chemically staining a smear of blood on a glass slide (typically a Giemsa stain) and observing the shape and morphology of each cell under a microscope in order to aid in the diagnosis of diseases such as malaria, anaemia, thrombocytopenia and leukaemia among others. The diagnosis requires staining of the cells and relies on human investigation which provides information that is qualitative and hence subjective.[3,4]

Quantitative phase imaging (QPI) refers to a set of microscopy techniques where contrast in the images is generated endogenously by measuring the optical path length difference across a cell or tissue sample. This optical path length difference is proportional to the phase $\phi(x,y)$ of the imaging field and is given by

$$\phi(x,y) = \frac{2\pi}{\lambda}[n_{cell}(x,y) - n_m]t(x,y) \quad (1)$$

where n_{cell} is the refractive index of the cell, n_m is the refractive index of the medium surrounding the cell, λ is wavelength

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of light and $t(x, y)$ is the thickness of the cell.[5-8] The refractive index difference $[n_{cell}(x, y) - n_m]$ is related to the dry mass content of the cell and, hence, the phase of cell not only changes with its thickness but also with variations in its intracellular contents.[9-14] A QPI image or phase map quantifies physical and biochemical properties of cells and provides a means for carrying out analysis that is free from the subjectivity of the investigator and lends itself to automation.

White light Diffraction phase microscopy (wDPM) has been reported previously in literature as a QPI technique that is implemented as an add-on module to a commercial microscope. As an interferometric technique that is both common path and single-shot, wDPM provides a stable, repeatable imaging modality for high throughput acquisition. Using white light illumination, a wDPM system also has high spatial phase sensitivity and low speckle noise.[15-17]

We show here a quantitative label-free method for obtaining the optical path length image of an entire blood smear at 100x magnification by using wDPM. Using a slide scanning and mosaic stitching software platform developed in-house we are able to obtain the quantitative phase image of the entire smear at high throughput.

2. EXPERIMENTAL PROCEDURES

2.1 Sample

We obtained our blood smear microscope slide sample from our collaborating pathologist Dr. Krishnarao Tangella at Presence Covenant Medical Center in Urbana, IL (IRB approved). The smear was unstained and was cover-slipped with methanol as the mounting medium. Methanol was chosen as the mounting medium instead of more conventional media such as xylene owing to its lower refractive index (n = 1.3282 at 0.550 nm), providing higher contrast in the image according to Eq. (1).

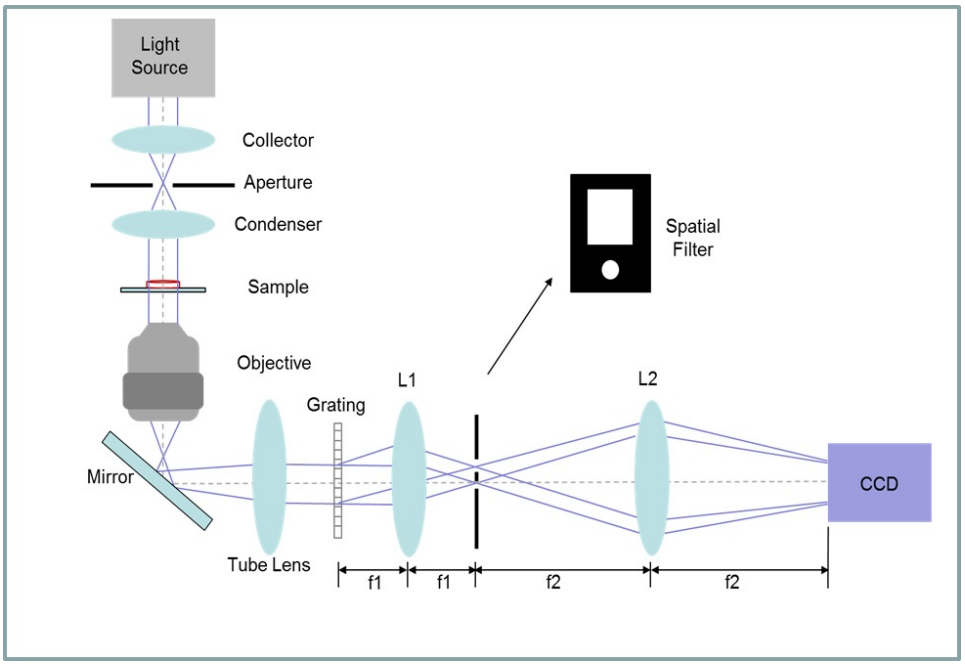

Figure 1. The wDPM setup used for blood smear scanning constructed as an add-on module to a commercial microscope

2.2 Optical Setup

The optical setup for the wDPM system is illustrated in Figure 1. As shown in the figure, a diffraction grating (Edmund Optics 110 lp/mm) is placed at the conjugate image plane at the output port of a commercial microscope Zeiss Axio Observer Z1 (represented by the optical train from the light source to the tube lens). A 4f system formed by lenses L1 (f1 = 60 mm) and L2 (f2 = 150 mm) forms another conjugate image plane on the CCD camera (Andor Zyla). At the Fourier plane of lens L1 a spatial filter is placed. As illustrated, the spatial filter low pass filters the zeroth diffraction order such that only its dc component is allowed through, forming a reference plane wave at the camera plane. The first order is allowed to go through unaffected. The raw DPM image is generated by the interference of the full imaging field of the first order $I_1(x, y)$ and the zeroth order plane wave I_0 at the camera plane and is given by

$$I(x, y) = I_0 + I_1(x, y) + 2\sqrt{I_0}\sqrt{I_1(x, y)}\cos[qx + \phi(x, y)] \quad (2)$$

where q is spatial frequency shift caused by the grating in the first order.

A 40x/0.75 NA bright field objective was using for imaging whereas the external optics added a further 2.5x magnification, resulting in a total magnification of 100x. The condenser aperture was closed to 0.09 NA for maximum spatial coherence and interference between the two orders.

2.3 Slide scanning, stitching and phase reconstruction

A slide scanning software, developed in-house in Visual C++ was used for scanning the entire sample microscope slide. The total scanning time for the entire smear area (23 mm x 26 mm, mosaic of 47970 images of 1776 x 1760 pixels each) was around 6 hours. A typical raw wDPM image generated by the system is shown in Figure 2 (a) with the fringes, formed as the result of the off-axis interference between the zeroth and first orders, clearly visible. The procedure for the reconstruction of the phase image is schematically illustrated in Figure 2. A MATLAB based code was used for this reconstruction. After reconstruction of the phase, the images for each field of view were stitched together using a stitching code written in Python.

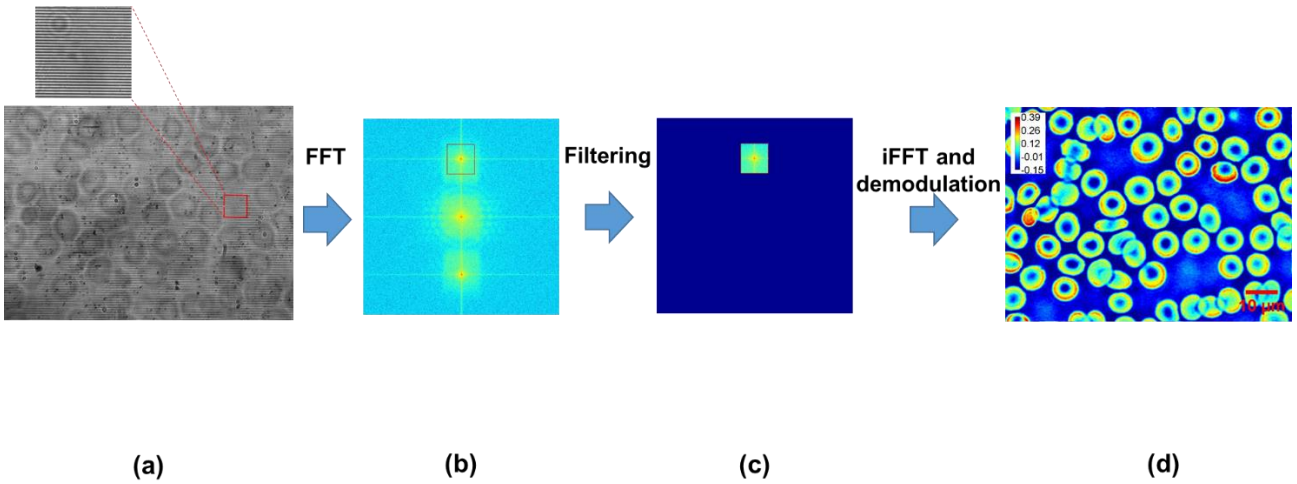

Figure 2. The Fourier transform of the raw image (a) is taken, as shown in (b). Using a band pass filter, the +1 order in the Fourier transform is filtered (c) and an inverse Fourier transform is taken. The resulting complex field is demodulated to remove the carrier frequency q. Taking the phase of the result gives us the $\phi(x, y)$ image (d). Color bar shows phase value in radians

3. RESULTS

Figure 3 shows the phase image obtained using the wDPM system and slide scanning software. As shown in the figure, a large scanning area of around 6 cm^2 can be efficiently imaged for a comprehensive determination of the numbers and morphologies for both red and white blood cells at high throughput. As described in previous publications from our group, the resolution of the wDPM system is diffraction limited provided that grating period satisfies the sampling criterion. Our grating was chosen bearing this in mind and the diffraction limited resolution was calculated to be approx. 0.83 μm.[18]

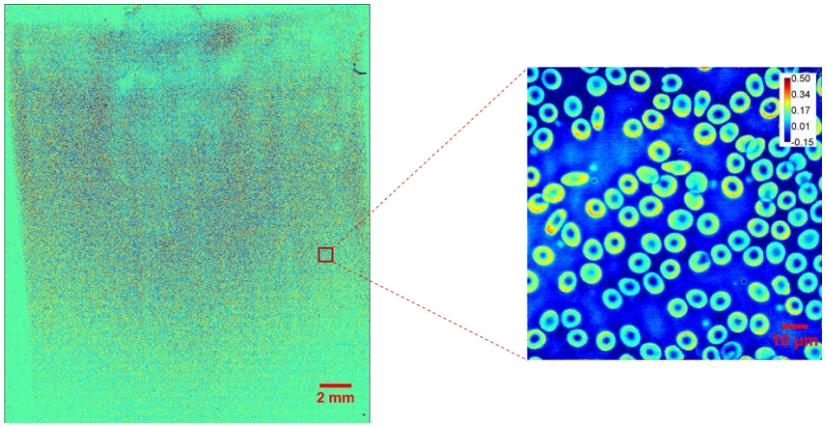

Figure 3. Stitched phase image of the entire 6 cm^2 scanning area spanned by the blood smear with a zoomed in image showing the phase of cells within the smear. Color bar shows phase in radians.

4. FUTURE WORK

As part of future efforts, we propose to develop algorithms for automatic segmentation of red and white blood cells from the phase image of the entire smear. Such segmentation will allow the determination of the total cell numbers and 2-d morphological parameters of each cell such as sphericity and projected area of each cell. Since the phase value contains information about the product of the refractive index and thickness (Eq. 1), by using published refractive index values for red and white blood cells, thicknesses of these cells may also be determined. The combination of segmentation and measurement of thickness can provide access to 3-d morphological parameters of cells that are not only indicative of cell type but can also diagnose the presence of any diseases that lead to symptomatic morphological changes in cells such as sickle cell anemia, haemolytic anemia, anisocytosis etc. Since our system has the capabilities of scanning large areas and generating their phase images as high throughput, our vision for the future is to provide a comprehensive single cell level automated morphological analysis of blood smears that addresses the shortcomings of current methods in terms of the speed and objectivity of the test.

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REFERENCES

[1] Bain, B. J., "Diagnosis from the Blood Smear," New England Journal of Medicine, 353(5), 498-507 (2005).

[2] Peterson, P., McNeill, S. and Gulati, G., [Laboratory Hematology Practice], Wiley-Blackwell, 10-25, (2012).

[3] Mir, M., Ding, H., Wang Z. *et al.*, "Blood screening using diffraction phase cytometry," Journal of Biomedical Optics, 15(2), 027016-027016-4 (2010).

[4] Mir, M., Wang, Z., Tangella, K. *et al.*, "Diffraction Phase Cytometry: blood on a CD-ROM," Optics Express, 17(4), 2579-2585 (2009).

[5] Popescu, G., [Quantitative Phase Imaging of Cells and Tissues] McGraw Hill, New York (2011).

[6] Park, Y., Popescu, G., Badizadegan, K. *et al.*, "Diffraction phase and fluorescence microscopy," Optics Express, 14(18), 8263-8268 (2006).

[7] Mann, C., Yu, L., Lo, C.-M. *et al.*, "High-resolution quantitative phase-contrast microscopy by digital holography," Optics Express, 13(22), 8693-8698 (2005).

[8] Kim, M. [Digital Holographic Microscopy: Principles, Techniques, and Applications] Springer, New York (2011).

[9] Cooper, K. L., Oh, S., Sung, Y. *et al.*, "Multiple phases of chondrocyte enlargement underlie differences in skeletal proportions," Nature, 495(7441), 375-378 (2013).

[10] Park, Y., Diez-Silva, M., Popescu, G. *et al.*, "Refractive index maps and membrane dynamics of human red blood cells parasitized by Plasmodium falciparum," Proceedings of the National Academy of Sciences, 105(37), 13730-13735 (2008).

[11] Park, Y., Yamauchi, T., Choi, W. *et al.*, "Spectroscopic phase microscopy for quantifying hemoglobin concentrations in intact red blood cells," Optics Letters, 34(23), 3668-3670 (2009).

[12] Cho, S., Kim, S., Kim. Y. *et al.*, "Optical imaging techniques for the study of malaria," Trends in Biotechnology, 30(2), 71-79 (2012).

[13] Memmolo, P., Miccio, L., Merola, F. *et al.*, "3D morphometry of red blood cells by digital holography," Cytometry Part A, 85(12), 1030-1036 (2014).

[14] Seo, S., Isikman, S. O., Sencan, I. *et al.*, "High-Throughput Lens-Free Blood Analysis on a Chip," Analytical Chemistry, 82(11), 4621-4627 (2010).

[15] Bhaduri, B., Pham, H., Mir, M. *et al.*, "Diffraction phase microscopy with white light," Optics Letters, 37(6), 1094-1096 (2012).

[16] Popescu, G., Ikeda, T., Dasari, R. R. *et al.*, "Diffraction phase microscopy for quantifying cell structure and dynamics," Optics Letters, 31(6), 775-777 (2006).

[17] Pham, H., Bhaduri, B., Ding, H. *et al.*, "Spectroscopic diffraction phase microscopy," Optics Letters, 37(16), 3438-3440 (2012).

[18] Bhaduri, B., Edwards, C., Pham, H. *et al.*, "Diffraction phase microscopy: principles and applications in materials and life sciences," Advances in Optics and Photonics, 6(1), 57-119 (2014).