Polarization Sensitive Digital Holographic Imaging in Biology

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Abstract. A new, simple digital holography-based polarization microscope for quantitative birefringence imaging of biological cells is presented. As a proof of concept, two different class of cells have been characterized by polarization sensitive digital holographic imaging (PSDHI). These two cases study reported are: differentiation of leukaemia cells and identification of reacted sperm cells. Although further experimentation is necessary, the suggested approach could represent a prospective label-free diagnostic tool for use in biological and medical research and diagnosis.

1 Introduction

The main motivation of this work is to fast label-free characterize a birefringent material, i.e. a material with refractive index (RI) dependent on the polarization and propagation direction. There are several paper in literature that demonstrate birefringence in some cells and tissues, where proteins are regularly organized in oriented filaments. Just to cite a few, a large reduction in birefringence in myocardial tissues has been measured in the infarcted regions compared to the healthy tissue values [1]; moreover, birefringence pattern of human breast cancer cells increased when highly invasive cancer cells are considered respect to weakly invasive cells [2]. Therefore, there is a great interest in the study of birefringence distribution in cells and tissue in a label free and non-invasively way.
With this aim, polarization sensitive digital holographic imaging (PSDHI) is an emergent technique based on the study of amplitude and phase of the diffracted beam through two orthogonally polarized reference waves [3]. Thus, not only morphological information is available, such as the classic digital holography imaging, but also intrinsic information about the polarization state of the sample through the phase change quantification. In this work we apply PSDHI to two cases study: leukemia cells differentiation and human sperm cells characterization based on their birefringence and morphological features.

2 Results

2.1. Experimental setup

A polarization sensitive digital holographic imaging system has been implemented by combining two Mach-Zender interferometers obtained by splitting the reference beam in two linearly and orthogonally polarized beams. These two reference beams interfere with the object beam polarized at 45°, producing two different sets of fringe patterns, i.e. two holograms related to the orthogonal components of the object field (Obj_x and Obj_y). By applying the standard reconstruction algorithm on each selected and filtered hologram, amplitude and phase maps for the two components of the object field can be recovered, as detailed descripted in Ref. [4]. Then, to characterize the birefringence pattern of the sample, two characteristic parameters have been evaluated: the amplitude ratio β, associated to the different transmitted intensities for the two orthogonal components, and the phase difference $\Delta\varphi$, connected to the different optical paths due to the anisotropy of the RI [4]:

$$\beta = \arctan\left(Obj_x / Obj_y\right) \quad (1)$$

$$\Delta\varphi = \varphi_x - \varphi_y \quad (2)$$

2.2. Acute lymphoblastic leukemia type B (B-ALL) cells differentiation

As first application, we considered the possibility to differentiate acute lymphoblastic leukemia type B (B-ALL) cells based on their birefringence and morphological features. This differentiation process could be very useful for timely and accurate diagnosis. Three different B-leukemia transformed cell lines have been considered: RS4;11, REH and MN60 B-leukemia cells [5,6]. The first two cell lines were both classified as the L2-blast subtype (i.e., B-cell precursor leukemia), while the third cell model was considered as the L3-blast subtype (i.e., B-cell leukemia). Since MN60 cells are much greater in size respect to the other two cell lines and with the aim to perform a birefringence analysis independent on the cells size, a characteristic vector was generated for each cell. It was made up by combining the vectors achieved considering the histograms representing

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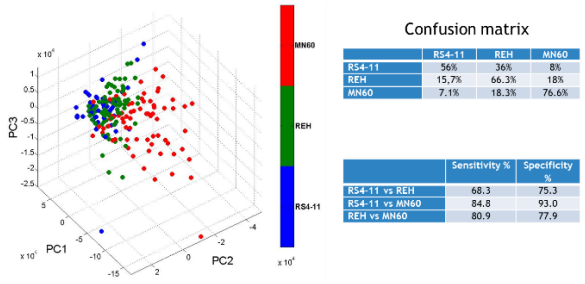
the number of pixel at a specific grey level for both β and Δφ maps, the Δφ maximum value, the total Δφ (birefringent volume), and the birefringent area normalized to the total area of each cell [5,6]. The principal component analysis (PCA) was performed on this set of data to evaluate the discrimination efficiency. The leave-one-out method was used for cross validation of the model. The obtained 3D scatter plot for the first three principal components is reported in Fig.1 as well as the resulting confusion matrix, that identify the correct and incorrect cells classifications.



Confusion matrix

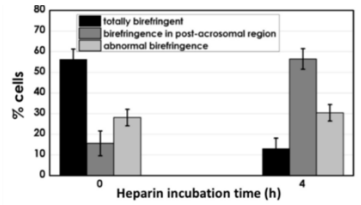
	RS4-11	REH	MN60
RS4-11	56%	36%	8%
REH	15.7%	66.3%	18%
MN60	7.1%	18.3%	76.6%

	Sensitivity %	Specificity %
RS4-11 vs REH	68.3	75.3
RS4-11 vs MN60	84.8	93.0
REH vs MN60	80.9	77.9

Fig. 1. 3D PCA scatter plots of the first three principal components for datasets obtained by analysing the characteristic vectors and the corresponding confusion matrix. Sensitivity and specificity are reported, too.

2.3 Sperm cells acrosome reaction

As a second example, we studied the birefringence in sperm cells. Indeed, it has been demonstrated that healthy sperm cells show a head totally birefringent due to longitudinally oriented protein filament, while when acrosome reaction occurs, that means that the sperm cell is ripe to enter into the oocyte, birefringence is typically in the post-acrosome area [7,8]. Thus, birefringence could be considered a method to distinguish between reacted and non-reacted spermatozoa and could be used in intracytoplasmic sperm injection (ICSI). Acrosome reaction was induced by exposing the cells to heparin for 4 hours, then, distribution of birefringence patterns of sperm from 3 donors was carried out on control and reacted samples. Analysing the retrieved phase difference maps, we observed a total birefringent head in control semen, while in reacted cells birefringence was present only in the post-acrosomal region [4]. Precisely, when reacted sperm cells are characterized, we note a very high increase in the percentage of analyzed samples that show birefringence localized in post-acrosomal region accompanied by a marked decrease of sperm with the whole birefringent head respect to the control sample (intact acrosome), as showed in the histogram reported in Fig. 2. Additionally, we witnessed a lower number of samples with abnormal birefringence, all of which are associated to the loss of motility and to the existence of abnormal morphology. Moreover, since PSDHI is a quantitative phase microscopy, it allows to retrieve the average phase difference Δφ obtained for control and reacted samples. These values show a decrease of about fourfold of the mean value of Δφ when the acrosome reaction occurs (see Fig. 2).



	0h in heparin	4h in heparin
Mean(Δφ) [rad]	1.39	0.36

Fig. 2. Distribution of birefringence patterns of sperm from 3 donors exposed to heparin for 0 h (control sample), and 4 h (reacted sample); evaluated mean phase difference Δφ for the two groups of samples.

3 Conclusions

In conclusion, PSDHI technique has been presented and the results obtained from the birefringence tests performed with the developed holographic system showed a good discrimination between three different lines of leukemia and a good discrimination between intact and reacted sperm cells.
The unique potentialities of holography have allowed to carry out not only the three-dimensional reconstruction of the analyzed samples, allowing an evaluation of their morphological characteristics, as richly reported in the literature, but also birefringence distribution. Results demonstrated that PSDHI can be effectively used to perform a non-invasive and label-free selection on cells and thus could be a valuable label-free tool in biological and medical research.

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