A Polarized Digital Holographic Approach in Biological and Medical Research

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A new, simple digital holography-based polarization microscope for birefringence imaging of biological cells is presented. This approach could open the way to a new class of label-free diagnostic tool in biological and medical research and diagnosis.

Keywords: Birefringence, Leukemia cell, Polarization sensitive digital holographic imaging.

1. Introduction

When the refractive index (RI) of a material depends on the polarization and propagation direction of light, the material is called birefringent. Not only anisotropic crystals but also biological cells and tissues, where proteins are regularly organized in oriented filaments, can show a birefringent behaviour, such has been reported in myocardial tissues, red blood and sperm cells [1-3]. With the aim to label-free and non-invasively characterize birefringence in tissues and cells, polarization sensitive digital holographic imaging (PSDHI) is an emerging technique based on the analysis of amplitude and phase of the diffracted beam by means of two orthogonally polarized reference waves [4] allowing to obtain not only morphology information, 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 discriminate three different B-leukemia transformed cell lines based on their birefringence and morphological features.

2. Results

Cell lines used were RS4;11, REH and MN60 B-leukemia cells (from Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Germany). The first two cell lines were both classified as the L2-blast (i.e., B-cell precursor leukemia) subtype, while the third cell model was classified as the L3-blast (i.e., B-cell leukemia) subtype [5]. The PSDHI setup was realized for transmission imaging and comprises two Mach-Zender interferometers, one for each orthogonal reference beam [4,6]. Amplitude and phase maps for the two components of the object field can be retrieved from the acquired hologram, which consists in two different sets of fringe patterns, due to the interference of the object field with the two orthogonal reference waves. Then, to characterize the birefringence pattern of the sample, two parameters were evaluated: the amplitude ratio β, related to the different transmitted intensities for the two orthogonal components, and the phase difference $\Delta\varphi$, linked to the different optical paths due to the anisotropy of the RI [4,6]. With the aim to obtain a birefringence analysis independent on the cells size, that increases a lot in the MN60 cells, a characteristic vector was generated for each cell by combining the vectors obtained considering the histograms representing the number of pixel with a specific grey level for both β and $\Delta\varphi$ maps, the $\Delta\varphi$ maximum value, the total $\Delta\varphi$ (birefringent volume), and the birefringent area normalized to the total area of each cell. The principal component analysis (PCA) was performed on this set of data to evaluate the discrimination efficiency. 3D scatter plot for the first three principal components is reported in Fig.1. The leave-one-out method was used for cross validation of the model [5]. The resulting confusion matrix, that specify the correct and incorrect cells classifications, is reported in Tab.1.

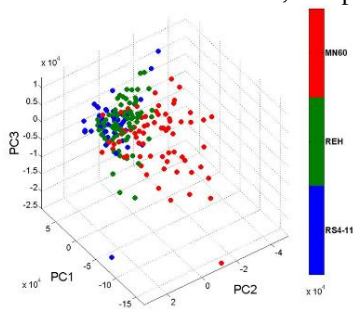

Fig. 1. 3D PCA scatter plots of the first three principal components for datasets obtained by analyzing the characteristic vectors.

Table 1 Confusion matrix

	RS4;11	**REH**	**MN60**
RS4;11	**56%**	36%	8%
REH	15,7%	**66,3%**	18%
MN60	7,1%	18,3%	**76,6%**

3. Conclusion

In conclusion, PSDHI permits to discriminate among three B-leukemia transformed cell lines, making it in principle usable for diagnosis of acute lymphoblastic leukemia type B, a cancer with a high mortality rate that affects B lymphocytes.

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