



Sub-surface quick imaging of typical blood cells based on orthogonal phase images by rotating method



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ABSTRACT

Due to the demands of the fast identifying method of the typical blood cells in scientific research and clinical examination, a new quick sub-structure imaging approach is presented. In this method, two orthogonal phase images of a cell's model are obtained by simulation experiment at first. According to the characteristics of the phase mutation which occurs on the boundary between nucleus and cytoplasm, the outlines of the model, including the ones of the sub-structure, could be extracted by edge detection. In view of the symmetry of the outlines, the geometrical model of the sample may be approximately segmented into several parts of revolving bodies. Furthermore, the revolving body could be built by rotating an outline along the perpendicular one. And then, the rest revolving bodies could be reconstructed by the same way. After all, the 3D sub-structure image is built based on their space structure characteristic. The feasibility of this method is demonstrated by a simulated monocyte, and the result agrees well with the real one. It shows that the method is very efficient and the characteristic of typical blood cells can be described.

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1. Introduction

Biological cells are mostly transparent, three-dimensional objects with absorbance and reflection characteristics similar to their surroundings. Thus, conventional intensity-based light microscopy approaches lack the required sensitivity. As a solution, contrast agents, such as fluorescent dyes, are used. However, fluorescent contrast agents tend to photobleach, reducing the available imaging time. Other concerns include potential cytotoxicity and the possibility that the agents will influence the cellular behavior. As an alternative, phase microscopy can provide label-free information on the cellular structure and dynamics. Traditional phase microscopy methods, such as phase contrast [1] and differential interference contrast microscopy [2], are widely used today, whereas these approaches also present drawbacks, including distinctive imaging artifacts. These approaches are not inherently quantitative methods.

Many advanced quantitative phase microscopy (QPM) techniques [3] have been developed to describe quantitative phase images of specimen-induced phase changes [4–8]. These

techniques can either provide average refractive index of cells or cell thickness [9–12]. However, 3D structure of the cell could not be demonstrated in detail.

These achievements have promoted the development of 3D imaging techniques. Several experimental implementations are capable of mapping the 3D structure of the specimen. For example, tomographic phase microscopy (TPM) [13] has been developed to reconstruct a 3D refractive index map of the cells and tissues. In this approach, multiple images from various angles of illumination with respect to the specimen are needed to reconstruct 3D structure with a set of angular images. If the phase of the transmitted field is interpreted as a line integral of the refractive index along the propagation direction, then the filtered back-projection algorithm based on the inverse Radon transform can be applied [14]. A more general approach, which takes the diffraction into account, is diffraction tomography first proposed by Wolf [15]. However, some blood cells have typical structural characteristics, such as five kinds of white blood cells. The identifications of these blood cells by means of TMP are complicated and time consuming. In practical application, a method of identifying the batch blood cells quickly and accurately is needed.

In this paper, we present a quick sub-structure imaging method to reconstruct the 3D surfaces of the nucleate blood cells. Only two orthogonal quantitative phase images are required. The outlines of

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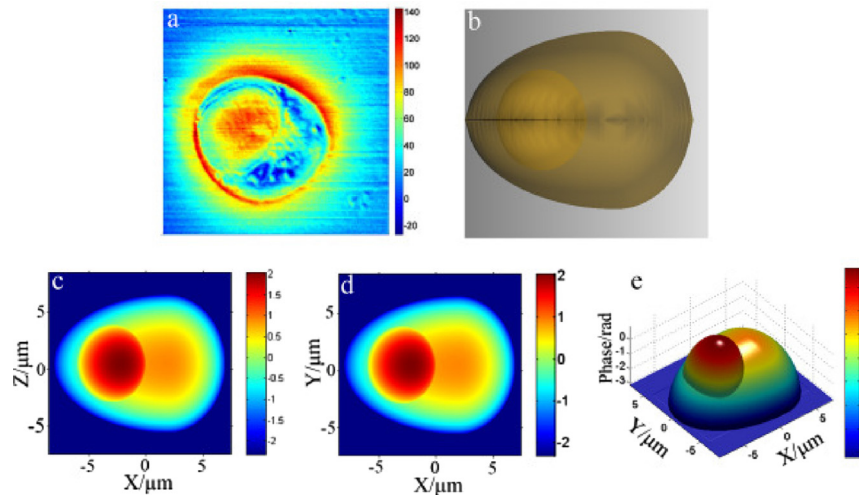


Fig. 1. The mononuclear cell. (a) the real phase image from Kert Edward’s work [17], (b) the simulation model, (c) the phase when light illuminates from y direction, (d) the phase when light illuminates from z direction, (e) 3D display of the phase image (d).

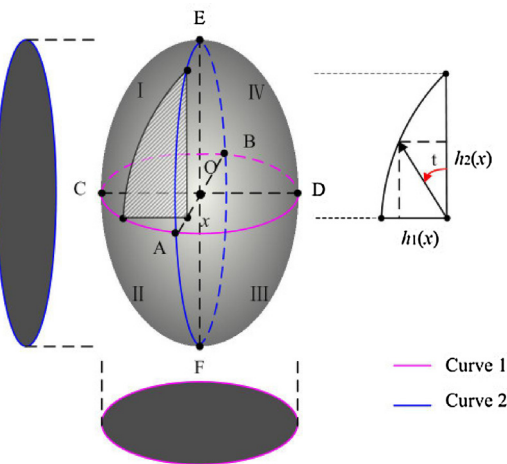


Fig. 2. The schematic diagram of the reconstruction method. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

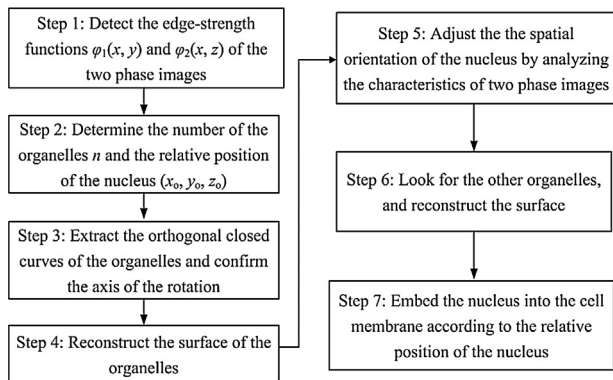


Fig. 3. The flowchart of numerical calculation.

the sample are extracted by detecting the edge-strength function of the phase images. The sub-structures could be segmented from edge-strength function. And the sub-surfaces of the sample could be reconstructed by rotating an outline along the other perpendicular outline. The method can be applied to fast identification of the batch complete blood cells with typical structural characteristics,

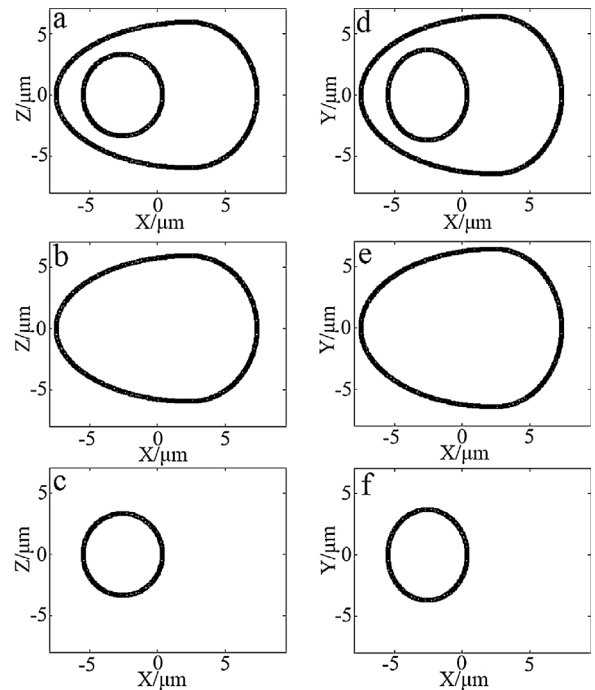


Fig. 4. The outlines of the cell. (a) the edge-strength of the phase image in x - z plane, (b) and (e) the outline of the cytomembrane, (d) edge-strength of the phase image in x - y plane, (c) and (f) the outline of the nucleus.

which avoids the need of scanning and calculating the complex refractive index and thickness information.

2. Simulation experiment of phase microscopy imaging

In clinical medicine, the blood test result is an important reference basis for the diagnosis and treatment of many diseases. Red blood cell (RBC) is the mainly kind of blood cell, playing an important role in the human body. Its 3D surface has been successfully reconstructed in our recently work [16]. Besides, there are white blood cells, such as neutrophil, eosinophil, basophil, lymphocyte and monocyte, with special structural characteristics. In which, monocyte is a common kind of white blood cell and its structure is shown in Fig. 1(a).

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