New Structure of a Special Pentaprism Designed by Simulation for Orthogonal Phase Microimaging

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Abstract. Quantitative phase imaging (QPI) has a very important application in the non-destructive imaging of biological cells. In view of the complex structure and various devices of imaging optical structure in traditional dual-channel orthogonal QPI, a new structure of a special pentaprism is designed as the main body of the orthogonal dual-channel phase microscopic imaging based on simulation design. In this dual-channel orthogonal QPI light structure, a hollow channel is designed as a sampling channel inside the pentaprism, a single light source through deflection of the pentaprism can form orthogonal dual-channel beams, with optical lens after diffraction modulation can form orthogonal dual-channel interference, the orthogonal dual-channel wrapped phase microscopic images are imaged on the sensors. The emulational and experimental results show that this design greatly simplifies the optical structure, uses very few optical devices, and the imaging path structure is compact and stable. With the use of single light source, the imaging quality of the dual light path has been significantly improved, which can provide a new technical basis for the improvement of biological cell (3-D) imaging technology and its instrument advancement.

Keywords: Phase imaging; Biological cells; Simulation design; Special pentaprism.

1. Introduction

Quantitative phase imaging (QPI) is a non-invasive, free-label method for detecting the morphological structure of cells that based on the relationship between the intracellular refractive index and the thickness of the cells. Generally, the physical thickness of cells and different refractive index distributions will cause different phase shifts of light waves, so this kind of technology can be utilized to observe the living cells[1]. QPI technique is mainly designed on the basis of the principle of diffraction and interference light path structure, to obtain interference image of a sample, the phase information of a sample can be gotten by using the corresponding phase recovery algorithm[2], which can be used to rebuild the shape of cells. Therefore, the phase microscopy imaging study with high contrast and high sensitivity and high resolution is very active.

In recent years, more and more scholars have paid attention to the research of QPI technology, this technology has developed rapidly in the direction of ultra-resolution, rapid and 3D structure reconstruction. A number of striking new technologies have emerged, such as: Nesici uses heterodyne scanning to quantitatively measure the amplitude and phase of the cells under the near-field optical microscope[3]; Iwai uses stable phase shift low coherent interference technology to carry out quantitative phase imaging[4]; Mann introduces the digital holographic quantification that with high resolution[5]. In the development and application of traditional technologies: Park propose a diffraction phase...
fluorescence microscope\cite{6}; Popescu propose diffraction microscopy to quantitatively study cell structures and dynamics\cite{7}; Kemper uses digital holographic microscopy to test the live cells\cite{8}; Mann propose a three-wavelength digital holographic quantitative phase imaging\cite{9}; Shaked uses a dual-interferometric channel quantitative microscope to observe the dynamic of living cells\cite{10}; Popescu introduces the white light diffraction chromatography technology of free label live cells\cite{11}; Chowdhury proposes a multi-modal 3D resolution and fluorescent sub-diffraction microscopy\cite{12}, and so on\cite{13}. From what has been discussed above, there have been several development of micro-technology for phase detection, including the axonomic hologram, optical diffraction tomography, phase shift interferometry and non-lens holographic imaging. Based on the optical path structure characteristics of interference microscopy, the interference microscopy is generally divided into two categories: one is the object light and the reference light in separation interference microscopy; the other is the object light and the reference in common optical path interference microscopy. The typical type of transmitted interference microscopy is Mach-Zehnder\cite{14}, which is widely used. In order to achieve the imaging of cell structure and morphology, there is usually dual-channel imaging\cite{15}. In which the basic optical path is still the commonly used optical path structure mentioned above.

Although phase chromatography solves the damage problem, 3D imaging requires scanning and complete data, which limits the imaging speed. The proposed dual-channel technology effectively reduces the data processing capacity and greatly increases the imaging speed\cite{16}. However, this technology, like the multi-beam chromatography technology, requires the imaging of samples by dual-beam or the rotation of samples\cite{17}. In this paper, it is used of special pentaprism refraction characteristics based on the dual channel imaging technical requirements, a single beam multiplexing imaging method is designed. The method is proved to be effective in theory and experiment, and their results show that the method is simple in structure, reliable in technology and good in effect.

2. Principle of Dual-channel Orthogonal Interference Phase Micro-imaging

In this method, the interference microscopic imaging of a biological cell was carried out along two orthogonal directions, and the corresponding phases can be obtained based on some recovery approach. The reconstruction method of orthogonal phase imaging is illustrated in Figure 1. The phase distribution $\phi_\perp$ and $\phi_\parallel$ corresponding to the orthogonal direction are labeled in Figure 1.

![Figure 1. A diagram of the phase shift produced along two orthogonal directions.](image)

The phase distribution of z direction imaging can be expressed as:

$$\phi_{(x,y)} = \frac{2\pi}{\lambda} \left( n_0 - 1 \right) h_0 + \left[ n_{(x,y)} - n_0 \right] h_{(x,y)}$$

(1)

Where, $(x, y)$ represents any point on the vertical plane of the optical axis, and $\phi_{(x,y)}$ represents the phase shift caused by the sample. $n_0$ represents the refractive index of the environmental liquid. If the refractive index of the homogeneous environmental liquid, $n_0$, is the same everywhere, it is a constant that does not change with space. $h_{(x,y)}$ is the thickness of the total grid in the direction of the optical axis.
n_i(x,y) represents the axial average refractive index of cells. For homogeneous cells, that is, non-nucleated cells, the internal refractive index is constant, that is \( n_i = n_1 \). For heterogeneous cells, there are \( \int_0^n n_i(x,y,z)dz \), such as monocytes, lymphocytes, etc., where \( h_{i(x,y)} \) represents the physical thickness of the cell along the axis. Equation (1) contains only two unknowns, namely \( n_1 \) and \( h_{i(x,y)} \). Because refraction distribution can reflect the spatial distribution of cell substructure, through the iterative method, the refractive index distribution of each layer of cells can be obtained, and then the 3D refractive index distribution of the cell can be obtained, that is, the 3D morphology distribution of the cell[18].

3. Double Channel Interferometric Phase Imaging Based on a Special Pentaprism
In this paper, an pentaprism double-channel sampling mirror can be used to realize the orthogonal sampling function, and off-axis and coaxial interference can be realized by using different model spectrosopes (LDBS2 and LDBS3). Based on the maximum entropy reconstruction method of 3D tomographic morphology, the 3D morphological reconstruction of the cell phase body can be realized by orthogonal phase distribution. In order to meet the requirement of beam quality, the phase micro-imaging of orthogonal double-channel are sampled by using the specular refraction and reflection characteristics of a special pentaprism as a device. As shown in Figure 2, the light coming from the laser beam go through the spectroscope LDBS1 to form parallel, such as quality of parallel beam, one of the single beam after the pentaprism form horizontal beam irradiation samples that in the sampling channel SC, after the pentaprism, part of the light transmission out, the other part of the light is reflected and refracted vertically out of the pentaprism, and then passes through the grating G2 and get the zero-order beam by using the filter SPF2, to form a reference beam with a vertical channel. Another beam of light after the spectroscopy LDBS1, part of it reflected and refracted from the pentaprism to form vertical beam irradiation samples, another part of it transmission from the pentaprism to form a horizontal beam, although the horizontal beam without passing samples, in order to match the another channel, still go through the grating G1 and get zero-order beam by using the filter SPF1, form the reference beam horizontal channel. Horizontal and vertical both channel object light and reference light after spectroscopy phase transfer LDBS2 and LDBS3 to produce interference, the interference beam passes the corresponding rear magnifying lens groups (BE1 and BE2) and image their interference figure on the corresponding CCD sensors (CCD1 and CCD2). The phase are recovered based on the interference images in order to get the corresponding phase distribution, it can be reconstructed of the sample 3D shape by the orthogonal phase diagrams. A channel with a square section is opened at the orthogonal point of the double beam of the pentaprism, which is used to pass through the sample. The imaging sampling method is simple and stable in structure because of one beam of light is used, the beam quality and dynamic change of the double channels are uniform, which overcomes the shortcomings of the dual laser sampling method. The coaxial and off-axis phase imaging can be realized by changing the model of LDBS2 and LDBS3 synchronously.

![Figure 2. Light path description of pentaprism sampler.](image-url)
4. Experimental Verification
In the experimental verification, the source of the light is a He-Na laser of 632.8nm, using the mode as the TE00 mode, the sample is a red blood cell, and the five inner angles of the special pentaprism are 90°, 117°, 117°, 108°, 108° corresponding to $\angle 1$ to $\angle 5$. The five surface properties are: the BC surface is the total reflection surface of the coating; the DE surface is semi-transparent and semi-reflecting, which accounts for 50%; the AB face, AE face and the CD face are the natural transmission surface. A tetrahedron hole (sample channel) within the pentaprism, where the two beams are orthogonal passing, is designed, which ensures that the incoming light does not have a second refraction, and can be passed through the sample, and the orthogonal beam intersects through the sample, which is located in the intersection of a vertical line 1/3 from C to B and a horizontal line 1/3 from E to D. Off-axis interference mode was selected in the experiment, and the angle of intersection between object light and reference light was 0.25 rad. The results of the experiment were shown in Fig.3, where (a), (b) are the orthogonal interference figures of the experiment, (c), (d) are the 3-D maps of the corresponding phase recovery from the (a) and (b) figures, (e) is the 3D morphological reconstruction of the sample, (f) is the open field micrograph of the red blood cell. Compared with the results of (e) and (f) in Figure 3, the morphology of the reconstructed red blood cell is highly consistent with their micrographs.

![Figure 3. The experimental results.](image)

5. Conclusion
In this paper, we propose a bi-channel orthogonal phase micro-imaging technique for biological cells based on pentaprism optical deflection. The optical path structure is simple and stable, which ensures the imaging quality with high efficiency. Its professional pentaprism development design has formed the following technical features: (1) The dual-channel interference optical path takes M-Z interference as the principle, the optical path structure is simple, compact, easy to obtain stable interference images; (2) The hollow tetrahedral hole opened in the pentaprism is used as the sampling channel, meanwhile, the regular tetrahedral hole avoids the cross-scattering when the two channels are orthogonal; (3) Single-beam optical diffraction ensures the quality of reference light and effectively overcomes the complex optical path structure of object light and reference light obtained from diffraction; (4) The design of rear light micro amplification solves the limitation of the prism on the imaging distance. Through the experimental verification, the technology has achieved good imaging quality and achieved the design goal, providing a new and practical technology with low cost for biological cell morphological reconstruction.

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