Quantitative phase imaging of Breast cancer cell based on SLIM

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Abstract We illustrated a novel optical microscopy technique to observe cell dynamics via spatial light interference microscopy (SLIM). SLIM combines Zernike's phase contrast microscopy and Gabor's holography. When the light passes through the transparent specimens, it could render high contrast intensity and record the phase information from the object. We reconstructed the Breast cancer cell phase image by SLIM and the reconstruction algorithm. Our investigation showed that SLIM has the ability to achieve the quantitative phase imaging (QPI).

1 Introduction

Phase contrast, discovered by Zernike in 1935, it provides the ability to observe transparent or phase objects that do not absorb or scatter light, especially living cell. Phase contrast is sensitive to optical path-length changes, so it can reveal inner details of cells without labeling and staining[1]. SLIM is based on the Zernike phase contrast which renders high contrast intensity images and reveals the intrinsic contrast of cell structure. The accuracy of resulting topography is comparable to the atomic force microscopy and the acquisition speed is higher[2]. There are various methods to observe phase objects, such as phase contrast, dark field image, and differential interference contrast. They are widely used in biomedical signal processing. However, they cannot provide quantitative measurement but qualitative measurement.

Quantitative phase imaging (QPI) has become a rapidly growing area of bioresearch which maps the phase distribution of image field[3]. And it has been widely used in imaging red blood cell[4,5,6], cell growth[5,7], cell refractive[8,9], optical properties of tissue[10]. However, QPI illumination often produced speckles and the experimental setup was too complex, which limits the deep observation of biological application, such as living cell imaging. To overcome the shortcomings of QPI mentioned above, a new method, SLIM, was demonstrated in this paper. It mainly consisted of a commercial phase contrast microscopy and a spatial light modulator. And the white light illumination of SLIM diminished speckle effects of traditional QPI method.

SLIM combined with Laplace operator can reveal a highly detailed quantitative phase image in cell structure without gradient artifacts, such as differential interference contrast microscopy or photo-bleaching and photo-toxicity limitation in fluorescence microscopy[11]. Combining SLIM with fluorescence imaging provides a unique method for studying cell cycle-dependent growth[12], and it is an easy method to measure the growth rate of individual adherent cells of various conditions. SLIM can be

used for instantaneous spatial light interference microscopy which combines the benefits of white light illumination in Zernike's phase contrast microscopy and phase stability associated diffraction phase microscopy[13]. And static iSLIM, Dynamic iSLIM, RGB iSLIM are all presented in the reference. SLIM could provide clinically relevant parameters for red blood cell analysis with unprecedented detail and sensitivity [14]. SLIM would set the basis for novel high-throughput topography and refractometry of man-made and biological nanostructures[15]. By measuring the system point-spread function and the modeling of coherent image formation in SLIM, it was found that the resolution and contrast of living cell images were significant improved[16].

The SLIM presented in this paper consisted of a commercial phase contrast microscopy and a spatial light modulator. We measured the phase of breast cancer cell to prove that SLIM could achieve quantitative phase imaging, and to lay a foundation for studying breast cancer cell dry mass. Female breast is composed of skin, fibrous tissue, mammary glands and fat composition. In the late 1970s the global breast cancer incidence showed linear growth trend. Breast cancer cells do not have some normal cell characteristics, and the connections between the cells are loose, which make the breast cancer cells easily to shed. Once the cancer cells shed, they can be transferred with the blood or lymph disseminated the whole body. At present, breast cancer has become one of the most severe threats to the health among common tumors. Thus studying breast cancer cells has a significance meaning.

2 SLIM Setup and Principle

2.1Experimental Setup

Fig.1 showed the schematic of instrument setup. The system combined Olympus IX73 commercial phase contrast microscopy with Holoeye spatial light modulator. The basic principle of SLIM is that the scattered light intervenes with unscattered light after the light passes through the sample. A phase ring provides a $\pi/2$ phase shift between scattered light and unscattered light, which is in the back focal plane of the objective. Then a liquid crystal phase modulator on the back focal plane was used to import phase shift. We recorded 4 intensity images with 4 different phases, which are 0, $\pi/2$, π, $3\pi/2$, respectively. Therefore the quantitative phase map of the sample can be uniquely determined by 4 intensity images.

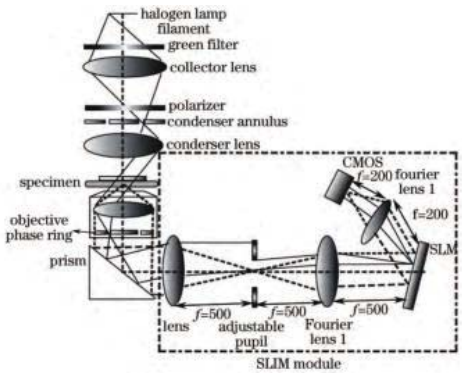

Figure.1 Experimental setup[17]. A lamp filament was projected onto a condenser annulus, which was located in the front of focal plane of the condenser. We added a Olympus color filter (431F550-W45) to eliminate the halo effects and improve the quality of image before the condenser. The central wavelength was 550nm. We used a lens and two 500mm lenses guide the light into SLM to make the phase grating modulate the scattered and unscattered light. A 20 ×/0.45 objective was used here. At last, a 200mm Fourier lens was used to guide the light into CMOS.

2.2 Principle

The principle of SLIM can be found in reference [2], and is summarized as follows. When light passes the sample, one portion of light remains scattered and contains the structure information of sample, and the other portion of light remains unscattered and forms a uniform background of the image. Here $U_1(x, y)$ denotes scattered light, U_0 denotes unscattered light, and $I(x, y; \varphi)$ denotes the light intensity accepted by the CCD, which can be expressed as equation (1).

$$I(x,y;\varphi) = |U_0|^2 + |U_1(x,y)|^2 + 2|U_0||U_1(x,y)|\cos[\Delta\varphi(x,y) + \varphi] \qquad (1)$$

$\Delta\varphi(x, y)$ in equation (1) is the phase difference between$U_1(x, y)$ and U_0, and φ is the additional phase modulation introduced by SLM. $\Delta\varphi(x, y)$ can be expressed as equation (2).

$$\Delta\varphi(x,y) = \tan^{-1}\left[\frac{I(x,y;3\pi/2) - I(x,y;\pi/2)}{I(x,y;0) - I(x,y;\pi)}\right] \qquad (2)$$

$\varphi(x, y)$ in equation (1) is the phase delay associated with the auto-correlation function, and can be expressed as equation (3), where $\alpha(x, y)$ is defined as the ratio of $|U_1|$ and $|U_0|$.

$$\varphi(x,y) = \tan^{-1}\left[\frac{\alpha(x,y)\sin(\Delta\varphi(x,y))}{1+\alpha(x,y)\cos(\Delta\varphi(x,y))}\right] \qquad (3)$$

Thus, together with equation (1) (2) (3), we can get the quantitative phase image associated with sample. Moreover, the local refractive index, used to calculate the sample mass, can be retrieved from equation (4), where n_0-n is the local refractive index contrast between surrounding culture medium and sample, h is the local thickness of the sample, and λ is the central wavelength of the illumination light.

$$\varphi(x,y) = \frac{2\pi}{\lambda}\int_0^{h(x,y)}[n(x,y,z) - n_0]dz \qquad (4)$$

3 Data Analysis

We placed the MCF-7 breast cancer cells with 10% FBS added in DMEM buffer and incubated for three days at 37 °c, 5% carbon dioxide incubator. The average size of breast cancer cells was 13μm. Figure 2 presented the MCF-7 breast cancer cells under the microscope. Figure 3 showed four phase rings, and the corresponding phase were 0, π/2, π, 3π/2. Figure 4 showed the images recorded by CCD corresponding to 0, π/2, π, and 3π/2 phase.

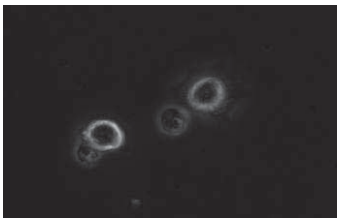

Figure.2 The MCF-7 breast cancer cells under the microscope.

Figure.3 The phase rings respectively were 0, π/2, π, 3π/2.

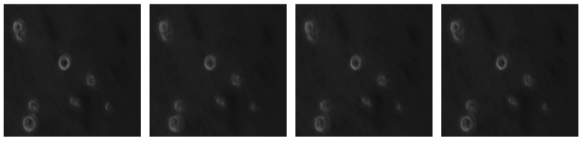

Figure.4 The images recorded by CCD corresponding to 0,π/2,π, 3π/2 phase.

Then the breast cancer cell phase can be reconstructed using equation (2), and the quantitative phase image of breast cancer cell was shown in Figure 5.

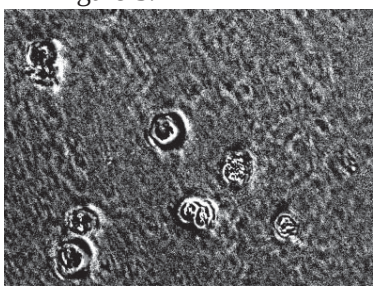

Figure.5 The quantitative phase image of Breast cancer cell.

4 Summary and Outlook

SLIM uses common interferometry, which enables temporally sensitive optical path-length measurement and provides speckle-free images. It will be widely used to extract the quantitative and nanoscale information about cell density, which can be used to demonstrate the physiological activity and status of individual cells, or reveal the heterogeneity between normal Breast cells. Meanwhile, SLIM also plays an important role in the studies on cellular differentiation and pathology, as well as early clinical diagnosis and treatment.

This paper took advantage of the ability of SLIM, which combines commercial microscopy and spatial light modulator, to reconstruct the quantitative phase image of Breast cancer cell, and obtained Breast cancer single-cell biophysical characterization. However, because of the noise introduced by the culture environment, the error of the system was larger than intrinsically allowed by the optical instrument. Thus more effort is needed to diminish the system error and improve the quality of quantitative phase image further.

Acknowledgment

This work was funded by the National Natural Science Foundation of China (No.61178089) and the Natural Science Foundation of Fujian Province (No.2014J01226).

Reference

[1] Zernike F 1955 *Sci*.**121** 345-349
[2] Wang Z, Millet L, Mir M 2011 *Opt. Express*. **19** 1016-1026
[3] Popescu G 2008 *Methods in Nano Cell Biology*. **Chap. 5, Elsevier**
[4] Paganin D and Nugent K A 1998 *Phys. Rev. Lett*. **80** 2586–2589
[5] Zicha D, Dunn GA 1995 *J. Microsc*. **179** 11–21
[6] Yang C H, Wax A, Dasari R R 2001 *Opt.Lett*. **26** 686–688
[7] Popescu G, Park Y, Lue N 2008 *Am. J. Physiol*. Cell Physiol. **295** C538–C544
[8] Rappaz B, Marquet P, Cuche E 2005 *Opt. Express*. **13** 9361–9373

[9] Lue N, Popescu G, Ikeda T 2006 *Opt.Lett*. **31** 2759–2761
[10] Ding H F, Nguyen F, Boppart S A 2009 *Opt.Lett*. **34** 1372–1374
[11] Wang Z, Millet L, Chan V 2011 *J Biomed Opt*. **16** 026019
[12] Mir M, Wang Z, Shen Z 2011 *PNAS*. **108** 13124-13129
[13] Ding H F and Popescu G 2010 *Opt. Express*. **18** 1569-1575
[14] Mir M, Tangella K, Popescu G 2011 *Bio. Opt.Express*. **2** 3259-3266
[15] Wang Z, Chun I S, Li X L 2010 *Opt.Lett*. **35** 208-210
[16] Haldar J P and Wang Z 2011 *IEEE T BIO-MED ENG*. **58** 2489-2497
[17] Chen D Q, Zheng M J, Li Z F,Wu H Q 2014 *Laser & Optoelectronics Progress*. **51** 121204