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Phase microscopy using light-field reconstruction method for cell observation

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ABSTRACT

The refractive index (RI) distribution can serve as a natural label for undyed cell imaging. However, the majority of images obtained through quantitative phase microscopy is integrated along the illumination angle and cannot reflect additional information about the refractive map on a certain plane. Herein, a light-field reconstruction method to image the RI map within a depth of 0.2 μm is proposed. It records quantitative phase-delay images using a four-step phase shifting method in different directions and then reconstructs a similar scattered light field for the refractive sample on the focus plane. It can image the RI of samples, transparent cell samples in particular, in a manner similar to the observation of scattering characteristics. The light-field reconstruction method is therefore a powerful tool for use in cytobiology studies.

1. Introduction

It is difficult to observe cell samples using traditional microscopy without exogenous contrast agents, because the light-scattering and absorption properties of cell samples are usually so weak that the resulting image resolution is low. That is, merely increasing the value of the numerical aperture (NA) of the objective lens cannot improve the image quality sufficiently (Pluta, 1989; Shaked et al., 2009). Moreover, a clear refractive index (RI) distribution is vital for representing the biomedical features of a cell (Choi et al., 2007, 2010; Popescu et al., 2008). Multiple phase microscopy techniques have been developed and have proven quite useful in this regard, such as differential interference contrast (DIC) microscopy (Allen et al., 1981), phase contrast microscopy (Burch and Stock, 1942), Fourier phase microscopy (FPM) (Popescu et al., 2004) and spatial light interference microscopy (SLIM) (Wang et al., 2011). All of these techniques use the partial light-field interference method to obtain higher-contrast images, but these phase images are neither quantitatively equal nor proportional to the RI. Other techniques, such as Hilbert and diffraction phase microscopy (Bhaduri et al., 2014; Popescu et al., 2006), can yield quantitative phase images using a parallel reference light, while tomographic phase microscopy (TPM) applies a Radon algorithm and multi-angle illumination to generate 3D-phase images of the samples (Choi et al., 2007). However, the images acquired using these phase images are usually integrated along the illumination angle, which cannot reflect additional information about the RI distribution. In this paper, light-field reconstruction phase microscopy (LRPM) is proposed; it uses a light-field reconstruction method to map the RI near the objective lens focal plane, which is similar to observing a high-contrast scattered light field.

In traditional amplitude microscopy, even fluorescence microscopy, the sample can be regarded as a map of luminous particles. Each particle disperses countless light beams, but only those within the NA of the objective lens can be collected; they interfere on the image plane to form a point image, which is defined as the point spread function (PSF). The image of the object is simply the convolution of the object and system PSFs (Hell and Wichmann, 1994; Shroff et al., 2008; Xue et al., 2012). However, in RI microscopy, the sample, which is usually transparent to the working wavelength, has greater similarity to a collection of RI particles. Thus, when traveling through this type of particle, the light beam is delayed and bent rather than scattered, and therefore, the RI information cannot be imaged directly like a luminous point in amplitude microscopy. However, there are also similarities between RI imaging and scattering imaging. First, the light beam passing through RI particles on the object plane in any direction is focused on the point where the particles can be imaged using scattering. Second, if we record the quantitative phase image, the phase delay is proportional to the particle's RI, which is similar

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to the manner in which a more luminous point creates a brighter point image. Therefore, if the phase information of the sample at any illuminating angle can be recorded, the RI field can be reconstructed using the same method as that used in scattering imaging.

LRPM is similar to the synthetic aperture microscopy method (Kim et al., 2011), but these two techniques are not completely identical. The light-field reconstruction method records the transmitted phase delay along different angles within the aperture of the objective lens. It completely neglects the intensity information and the obtained image reflects the RI information directly. Both methods reconstruct a wider light field and can achieve a higher resolution than other techniques. However, the RI scattered field reconstructed in the LRPM approach covers a taper angle of 100◦, and the resolution is significantly higher than in traditional transmitted interference phase microscopy (Lee and Weiner, 2014; Lue et al., 2008).

2. Setup and control method

2.1. Setup

The experimental setup is based on a Mach–Zehnder heterodyne phase microscope (Choi et al., 2008) (Fig. 1), which is similar to the apparatus used in TPM, but the phase shifting component is replaced with a piezoelectric transducer (PZT). A helium-neon laser beam (wavelength, $\lambda = 633$ nm) is divided into sample and reference arms using a beam splitter. In the sample arm, a tilting 2D-galvanometer (GM) positioned in the image plane varies the illumination angle, so that the laser beam can traverse the sample covering a taper angle of 120°. The 2D-GM system consists of two lenses ($f = 75$ mm) and two 1D galvanometer-mounted mirrors. To ensure that the sample is illuminated at one fixed point, the lenses within the 2D-GM system are arranged as a 4f system with the two mirrors at the conjugate planes. The samples are clamped on a stage between an oil-immersion condenser lens (Olympus 1.4 NA) and an objective lens (Olympus UPLSA, 100×, 1.4 NA). A tube lens ($f = 200$ mm) images the sample onto the image plane with magnification $M = 110$. The four lenses in the LRPM system are scanning, condenser, objective, and tube lenses. Their arbitrary combinations can form a 4f system, and the second mirror of the 2D-GM system, sample, and a high-speed camera (MotionBLITZ® EoSens Cube7) are placed at the conjugate planes of this 4f system. In the reference arm, a mirror mounted on the PZT is used to change the optical path periodically to allow the phase information of the sample arm to be calculated. A polarization beam splitter (PBS) recombines the sample and reference beams, and a polarizer is used to adjust the contrast ratio of the interference pattern on the high-speed camera.

2.2. Control method

LRPM requires approximately 200 phase images at different angles for a scattered field to be reconstructed. These phase images are calculated using a four-step phase-shifting method (Choi et al., 2007); therefore, we must record approximately 800 interference images in order to reconstruct one phase image. When imaging live cell samples, the response time of the PZT and the imaging speed of the camera limit the system speed. The high-speed camera used in this system can record 512 × 512 pixel images at a speed of 5000 fps, but the PZT cannot shift the reference arm at the same frequency; therefore, the PZT response time is the limiting factor of the recording speed of the entire system. To solve this problem, we used the periodic phase-shifting method shown in Fig. 2.

The periodic phase-shifting method consists of three main steps. First, in the preparation step, the PZT is corrected and four voltages are chosen that correspond to four positions, which each extend the reference arm length by $\lambda/4$. The reference and sample beams are also adjusted to confirm that they are perfectly collinear after the PBS, when the light passes through the sample vertically. Second, in the imaging step, the PZT is moved to the original position and a serial number, $N = 0$, is set. Then, the GM scans steadily on a pre-established track, while the high-speed camera simultaneously records reference patterns at a fixed frequency. When the GM finishes scanning the track, the camera stops recording, the PZT voltage is changed, and N is incremented by one. After the

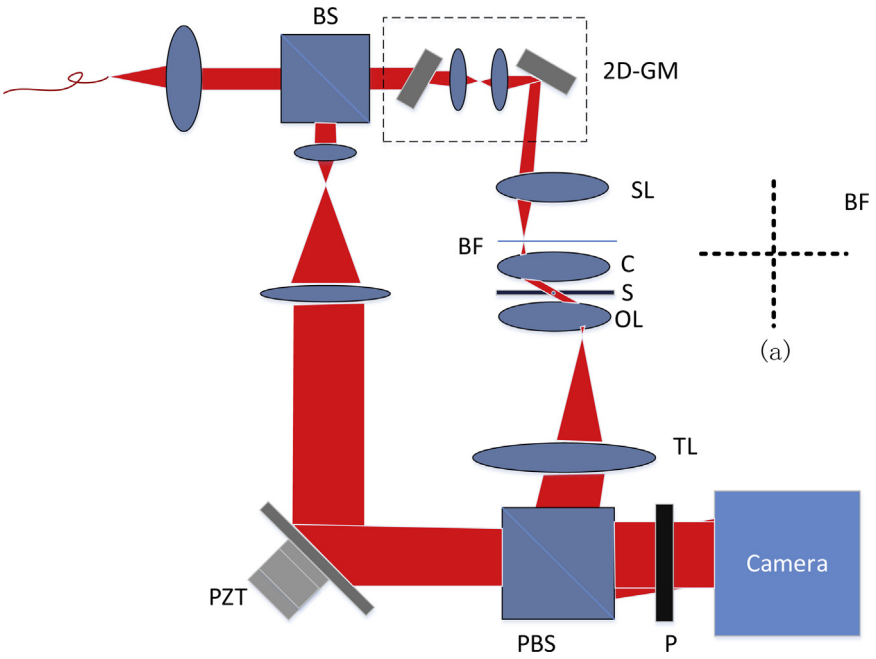

Fig. 1. Setup of LRPM system. BS, beam splitter; 2D-GM, two-dimensional galvanometer-mounted mirror system; SL, scanning lens with focal length $f = 150$ mm; BF, back focal plane of condenser lens; C, condenser lens; S, sample; OL, objective lens; TL, tube lens with $f = 200$ mm; PBS, polarization beam splitter; PZT, piezoelectric ceramics; P, polarizer; (a) scanning trace on BF.