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Single-shot two-channel Fresnel bimirror interferometric microscopy for quantitative phase imaging of biological cell

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ABSTRACT

A simple slight-off-axis, single-shot, and two-channel interferometric microscopy is demonstrated for the quantitative phase imaging of a biological sample, only using a cube beamsplitter and a Fresnel bimirror interferometer. The incident beam is replicated into two parallel beams using a tilted cube beamsplitter, while the transmission beam is the replica and the reflection beam is the mirror-reverted replica. The two replicas encounter different mirrors of the Fresnel bimirror interferometer, and are reflected and brought to interfere with each other at the image plane of a digital camera. Subsequently, two interference channels with a relative π (rad) phase shift in one interferogram are acquired simultaneously using only one digital camera. The proposed method is capable of obtaining two-channel, slight-off-axis interference in a single shot. The use of small amount of ready-made and low-cost optical components renders the system compact, stable, and easy to implement. In addition, two identical mirrors of the Fresnel bimirror interferometer are symmetrically arranged with a very small angle. Their reflected beams vary in direction over a wide space range with the rotation of the Fresnel bimirror interferometer and the interference always occurs. Based on this characteristic, we are able to capture the interferograms over a large space range and obtain the quantitative phase information. This is a suitable method for the quantitative phase imaging of biological samples.

1. Introduction

Many applications, such as cell analysis, are fundamentally based on the morphology of a biological cell, and quantitative phase measurement as an inevitable trend for transparent biological cell has become very popular in modern biomedical and life science recently. As a kind of non-invasive and non-destructive quantitative phase measurement technique, interferometry such as digital holographic microscopy has been used in some practical biological detection procedures [1–7]. The typical optical setup of a digital holographic microscopy typically involves a Mach–Zehnder layout to separate a beam of light into two parts initially. One part is modulated by the sample as an object beam and another clean part as the reference beam; subsequently, the object beam and reference beam are combined by a beam splitter to produce an interferogram. However, this approach is sensitive to the external environment such as air fluctuation and mechanical vibration, and different external vibrations arising out of the two separated beams along different paths may greatly affect the temporal stability.

To eliminate the effects of external factors and improve the system stability, the common-path geometry setup [8–14] and self-referencing mode [10,11,15–19] can be applied. For these methods, one part of the incident beam that does not carry any sample information is regarded as the reference beam and the other part is modulated as the object beam based on the object information. In addition, using fewer conventional optical elements to achieve the interference between the two halves of the collimated beam has become increasingly popular and important. It reduces the cost and improves the system stability. For example, Arun Anand et al. described a common-path and self-referencing digital holographic microscopy technique using only a Lloyd's mirror (a single-plane mirror) [10]; they also introduced another method using a single glass plate to achieve the lateral-shearing digital holographic imaging of small biological specimens [18]. In Ref. [20], Weijuan Qu et al. presented an optical configuration for transmission digital holographic microscopy based on a cube beamsplitter interferometer. In addition, Zahid Yaqoob et al. used two square mirrors, each with an independent tip–tilt control, to present an imaging modality capable of providing the high-speed optical dispersion measurement of live cells [21]. All

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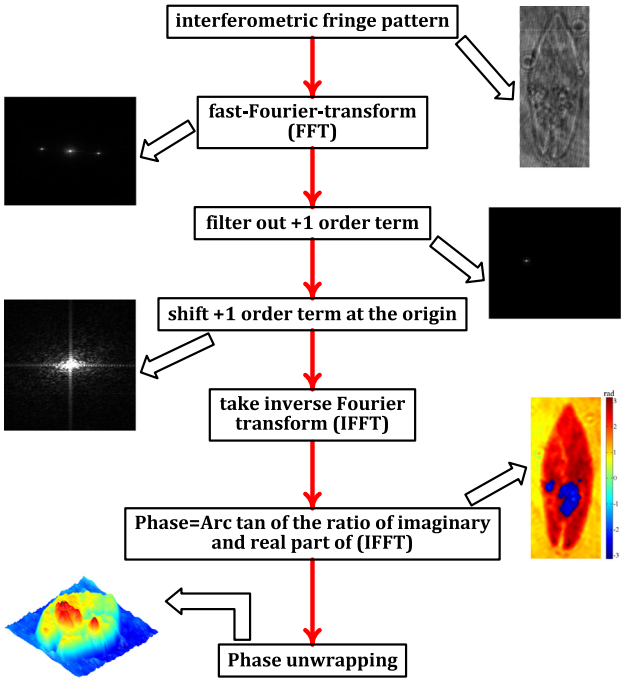

Fig. 1. Flow diagram of fringe analysis for quantitative phase imaging.

the design methods mentioned above are practical and promising to improve the performance of quantitative phase measurements.

Herein, a new near-common-path, slight-off-axis, two-channel, and single-shot interferometric microscopy is proposed for the quantitative phase imaging of a transparent biological sample. It combines a cube beamsplitter (BS) [20,22,23] with a Fresnel bimirror interferometer (FBMI) [21,24–27], which consists of two plane mirrors slightly angled with respect to each other. The suggested system is simple, and relatively low cost because of the few optical elements used. Compared with previous methods, this proposed configuration can not only reduce the number of optical elements, but also require no rigorous optical-path difference matching between the different interferometric arms, because it allows the reference beam and object beam to propagate in parallel along the same optical path. Therefore, this method is easy to implement, and may have a wider range of applications.

2. Data processing method

To acquire the phase information quantitatively, the classical fast-Fourier-transform (FFT) method [28,29] is used to process the captured interference fringe pattern according to the flow diagram of fringe analysis shown in Fig. 1.

In the proposed experiment system, the FBMI changes the light transmission direction; therefore, different coordinate systems should be defined in the object plane and image plane for expression convenience. The transmission direction of light is defined as the Z-axis, in which the coordinates (x', y', z') represent the object plane before the FBMI, and the image plane is represented by the coordinates (x, y, z). To simplify, we only considered the image plane. The intensity distribution $I(x, y)$ of the interference fringe pattern on the image plane can be expressed by

$$I(x,y) = a(x,y) + b(x,y)\cos[\phi(x,y)], \tag{1}$$

where $\phi(x, y)$ contains the desired phase information, $a(x, y)$ represents the unwanted background information and other irradiance variations, and $b(x, y)$ is the modulation factor related to the local contrast of the pattern. $a(x, y)$ and $b(x, y)$ carry the additive and multiplicative disturbances in general, respectively. If the visible light source intensity is equally distributed to the transparent cells, both $a(x, y)$ and $b(x, y)$ follow the uniform distribution.

The interference fringe pattern can be rewritten in the following form:

$$I(x,y) = a(x,y) + c(x,y) + c^*(x,y), \tag{2}$$

where

$$c(x,y) = (1/2)b(x,y)\exp[i\phi(x,y)], \tag{3}$$

and * denotes the complex conjugate, $i = \sqrt{-1}$.

Further taking the two-dimension Fourier-transform of Eq. (2) with respect to both x and y, the following expression can be obtained:

$$I(\mu,\nu) = A(\mu,\nu) + C(\mu,\nu) + C^*(\mu,\nu). \tag{4}$$

Where (μ, ν) is the coordinate in the frequency domain corresponding to the spatial coordinate (x, y). Therefore, we can obtain the separated trimodal distribution of the spectrum with the zero-order term $A(\mu, \nu)$ at the origin, +1-order term $C(\mu, \nu)$, and −1-order term $C^*(\mu, \nu)$ placed symmetrically approximately the origin. To eliminate unwanted background variations and the effect of the conjugate image, in the next step, the zero-order term $A(\mu, \nu)$ and one of the two symmetrical parts, say $C^*(\mu, \nu)$, are filtered out. Because this remaining frequency spectrum is no longer symmetrical, the +1-order term $C(\mu, \nu)$ is shifted to the origin:

$$I'(\mu,\nu) = \mathrm{BPF}[I(\mu,\nu)] = C(\mu,\nu). \tag{5}$$

Here, BPF[ ] is the bandpass filter operator. Subsequently, $c(x, y)$ can be obtained by applying the inverse Fourier transform to Eq. (5). The phase calculated from $c(x, y)$ can be expressed as

$$\phi(x,y) = \arctan\frac{\mathrm{Im}[c(x,y)]}{\mathrm{Re}[c(x,y)]}, \tag{6}$$

where "Re" denotes the real part and "Im" the imaginary part.

It is noteworthy that the arctangent result known as the wrapped phase is confined to a particular range $[-\pi, \pi]$; therefore, the phase obtained from Eq. (6) is indeterminate to a factor of 2π. The discontinuous phase should be appropriately unwrapped to convert to the continuous phase. Furthermore, a linear relationship exists between the phase information of the transparent sample and its three-dimensional (3D) profile information. The thickness distribution $t(x, y)$ of the sample can be calculated based on the relationship: $\Delta\phi(x, y) = (2\pi/\lambda)\cdot(n_{obj} - n_m)\cdot t(x, y)$, where $\Delta\phi(x, y)$ represents the above calculated unwrapped phase difference and λ is the light wavelength in vacuum. n_{obj} and n_m are assumed as the constant refractive index of the sample and the medium approximately the sample, respectively.

3. System configuration and principle of measurement

The proposed interferometric microscopy system and the corresponding interference schematic diagram of the FBMI are shown in Fig. 2(a) and (b), respectively. To enable this method to be integrated into a common microscope product, as shown in Fig. 2(a), the essential configuration of the conventional inverted microscope is first constructed. A continuous helium–neon laser (wavelength $\lambda = 632.8$ nm; minimum output power = 2 mW) is used as the illumination source for a less phototoxic imaging approach of the biological samples [30]. Behind the illumination source, a spatial filter (L_1, 40× microscope objective lens with NA = 0.65; P, 25-μm pinhole) and a beam expander lens (L_2, focal length = 20 mm) are used to obtain a high-quality laser. In the following experiment, because the size of the sample (paramecium) is relative large, a 5× microscope objective lens with 0.12 NA is utilized to magnify the sample. Based on the specification of the objective lens, the system's theoretical resolution, $r \approx 3.22$ μm can be calculated according to $r = 0.61\lambda/\mathrm{NA}$. Further, the system's field