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Color-coded LED microscopy for quantitative phase imaging: Implementation and application to sperm motility analysis

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ABSTRACT

Color-coded light-emitting diode (LED) microscopy (cLEDscope) is a novel computational microscopy technique capable of multi-contrast and quantitative phase imaging of biological specimens using color-multiplexed illumination. Using specially designed LED patterns, it is capable of recording multiple differential phase contrast (DPC) images in a single exposure and employs a computational algorithm to retrieve the phase distribution of the specimens. Herein, we describe the detailed procedures in the cLEDscope implementation for quantitative phase imaging. Several notable features and caveats in the cLEDscope setup and image processing are also outlined. The imaging model is derived for our specific configuration, and the associated phase-retrieval algorithms are presented on the basis of a weak-object transfer function. As an illustrative application of the quantitative cLEDscope, we demonstrate its utility as a sperm-motility analyzer by exploiting its real-time quantitative imaging capability.

1. Introduction

Quantitative phase microscopy is one of the rapidly evolving microscopy techniques for measuring the phase distribution of transparent biological and material specimens. The phase information encodes the optical thickness of the specimen, which is a product of the refractive index and physical thickness of the specimen [1], and can thus be used to perform quantitative biological studies, such as the measurement of cell volume alterations [2], membrane fluctuations [3], cell mass and growth dynamics [4,5], rapid contraction of cardiomyocytes [6], and intracellular vesicle trafficking [7–9] under various stimuli. The optical phase information can also be utilized to detect infectious disease [10,11], genetic disease [12,13], and cancer [14,15]. The phase distribution can be measured via diverse optical means. The use of interferometry is a common strategy for such purposes. Over the past years, many quantitative phase imaging (QPI) methods based on interferometry have been developed, including QPI techniques employing a phase-shifting interferometer [16–21], digital holography [22–26], Hilbert phase microscopy [27,28], diffraction phase microscopy [8], and low-coherence interferometry [29–31]. Additionally, the interferometric configurations have been miniaturized and modularized to be compatible with conventional microscopes; the QPI modules can simply be connected to the camera port of the microscope, transforming the microscope into a QPI system [32]. Interferometric QPI methods typically exhibit the capability of high-precision phase detection with precision of a few milliradians [30]. However, these methods require interferometric optical arrangements with coherence light sources, which suffer from coherent artifacts (e.g., speckles). Alternative QPI methods involve the non-interferometric measurement of the phase distribution. These approaches do not require a complicated optical setup but typically require multiple images in different imaging configurations, followed by computation. Exemplary methods include QPI based on the Transport of Intensity Equation [33–36] and various types of wavefront sensors (e.g., pyramid [37] and partitioned aperture wavefront sensors [38]).

QPI can also be realized in light-emitting diode (LED) microscopy [39–43]. LED microscopy has recently been highlighted as a versatile platform for computational microscopy. In LED microscopy, a programmable LED array is positioned in the source plane of a microscope, and through the controlled illumination of LEDs, various label-free imaging modes can be realized. LED microscopy as a multi-contrast microscopy (i.e., bright-field, dark-field, and differential phase contrast) has been demonstrated [39,44,45]. High-resolution imaging over a large field of view has also been demonstrated via Fourier ptychographic approaches [46,47] in the platform of LED microscopy. For phase imaging, Tian et al.

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[41] presented quantitative phase-gradient and phase imaging using multiple images acquired with four different illumination patterns. To improve the imaging speed, Lee et al. [39] performed QPI imaging based on two color-multiplexed LED patterns configured to produce differential phase contrast (DPC) images along two orthogonal axes. Subsequent two-dimensional integration in the Fourier domain enables QPI [39]. These methods are effective for generating QPI, but their applications require synchronous operation between the image sensor and the LED light source, which may compromise the simplicity of implementation. To address the drawback, Philips et al. [48] and Lee et al. [49] demonstrated simple and robust imaging methods capable of single-shot QPI via color-multiplexed illumination, albeit with different color-encoding and computation schemes.

We term the method used in [49] as quantitative color-coded LED microscopy (q-cLEDscope), simply to distinguish its operation from its previous demonstration, i.e., multi-contrast cLEDscope. In q-cLEDscope, the LED pattern is trisected in equal angles, and each region is assigned to red, green, and blue colors, respectively. A computational model based on the weak-object transfer function (WOTF) has been derived, and the corresponding phase retrieval based on Tikhonov regularization enables single-shot QPI without any modification of the hardware in LED microscopy. Herein, we present detailed instructions regarding the implementation, alignment, and color-leakage correction procedures for the microscope, as well as a detailed discussion on the phase-retrieval algorithm. As an exemplary application of the q-cLEDscope, we perform a motility analysis of human sperm cells.

2. q-cLEDscope implementation and image reconstruction

2.1. Optical setup

The optical setup for the q-cLEDscope is identical to that for the cLEDscope, as illustrated in Fig. 1. A color LED array (32 × 32 RGB LED Matrix Panel with 4-mm pitch, Adafruit 607, USA) is placed at a certain distance from the specimen plane, such that it is approximately located in the Fourier plane of the specimen. In theory, this condition is met if the source is positioned at $z > 2D^2/\lambda$ [50], where D is the source size, and λ is the illumination wavelength. However, this configuration is not practical, as the illumination light intensity would be extremely small in the specimen plane. We note that operating the cLEDscope with the LED light source positioned at ~100 mm from the specimen plane is suitable for our purposes. We numerically calculated the phase distortion with the LED array at 100 mm from the specimen plane. The phase distortion within the field of view (FoV) of 300 μm × 300 μm was found to be smaller than 0.2 rad. This phase distortion can be corrected via diverse calibration methods, as described in [24].

For quantitative phase imaging, the LED pattern is configured as a circle composed of three regions at equal angles, i.e., 120°. Each region is assigned a color: red, green, or blue. The LED array can be operated using an inexpensive microprocessor (Arduino UNO R3, Arduino, Italy). The illumination LED pattern should be larger than the numerical aperture (NA) of the objective. The effect of the illumination size on the phase measurement is detailed in Section 2.5. The light transmitted through the specimen is collected by a microscope objective (NA 0.45, 20×, Nikon, Japan) and delivered to a color camera (IOI Flare 2M-360CL, IO Industries, Canada). The camera can be installed in the output camera port of a conventional microscope.

For cLEDscope operation, the use of microscope objectives with high transmittance over a broad spectral range is desired. Color-dependent intensity inhomogeneity across the image FoV and wavefront distortion result in a non-uniform phase background, which could overwhelm the phase information of a specimen. The objectives with chromatic and spherical aberrations, along with flat-field correction, may alleviate the issue. In our case, the objective with a high spectral transmittance in the visible light range (Plan Fluor 20×/0.45, Nikon, Japan) was utilized, and it could produce high-contrast quantitative phase images. Other more sophisticated and expensive objectives can be employed, which correct for high-order aberrations. Even with the aberration-corrected objectives, residual systematic aberration could still be present, deteriorating the image quality. In such cases, the correction method described in [41] can be utilized. A reference phase image is obtained in the absence of a specimen. The image is then subtracted from the phase image of specimens to eliminate non-uniform phase background caused by systematic aberration.

The LED array can be aligned with respect to the optical axis of a microscope in multiple ways. One approach is to use relay optics to image the objective back focal plane to the color camera. In the absence of a specimen, an image of the illumination source is then recorded by the image sensor. By varying the pattern and size of the LED illumination, one can position and determine the size of

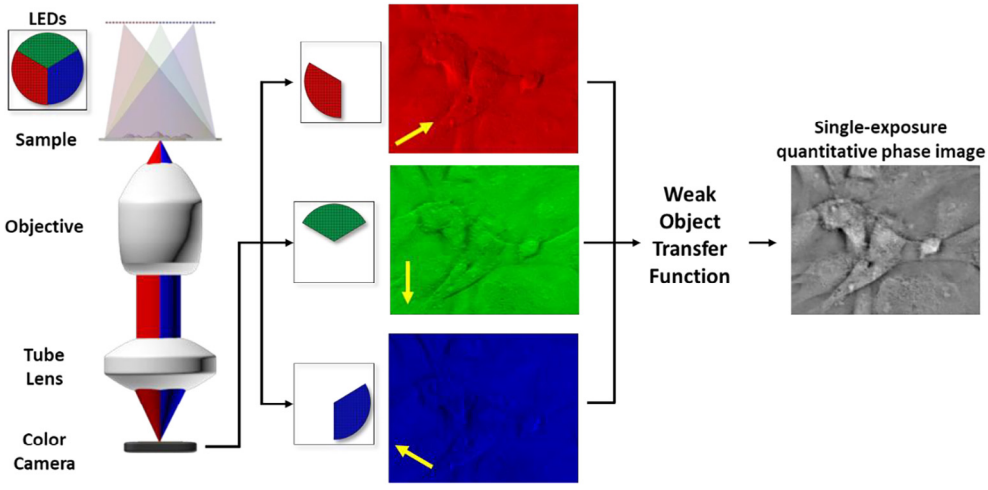

Fig. 1. Schematic of q-cLEDscope. The illumination LED circle is trisected into three regions—red, green, or blue—in equal angles. The light transmitted through the specimen is then collected by an objective and measured by a color image sensor. The RGB images are extracted from the acquired color image and used to generate DPC images along the three different directions. The DPC images are used for QPI reconstruction based on the WOTF. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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