Optics and Lasers in Engineering 80 (2016) 24–31

Contents lists available at ScienceDirect

Optics and Lasers in Engineering

journal homepage: www.elsevier.com/locate/optlaseng

Programmable aperture microscopy: A computational method for multi-modal phase contrast and light field imaging

Chao Zuo [a,b,*], Jiasong Sun [a,b], Shijie Feng [a,b], Minliang Zhang [a,b], Qian Chen [b]

[a] Smart Computational Imaging Laboratory (SCILab), Nanjing University of Science and Technology, Nanjing, Jiangsu Province 210094, China
[b] Jiangsu Key Laboratory of Spectral Imaging & Intelligent Sense, Nanjing University of Science and Technology, Nanjing, Jiangsu Province 210094, China

ARTICLE INFO

Article history:
Received 17 July 2015
Received in revised form
1 December 2015
Accepted 17 December 2015

Keywords:
Microscopy
Phase retrieval
Multi-modal imaging
Quantitative phase microscopy
Light field microscopy

ABSTRACT

We demonstrate a simple and cost-effective programmable aperture microscope to realize multi-modal computational imaging by integrating a programmable liquid crystal display (LCD) into a conventional wide-field microscope. The LCD selectively modulates the light distribution at the rear aperture of the microscope objective, allowing numerous imaging modalities, such as bright field, dark field, differential phase contrast, quantitative phase imaging, multi-perspective imaging, and full resolution light field imaging to be achieved and switched rapidly in the same setup, without requiring specialized hardwares and any moving parts. We experimentally demonstrate the success of our method by imaging unstained cheek cells, profiling microlens array, and changing perspective views of thick biological specimens. The post-exposure refocusing of a butterfly mouthpart and RFP-labeled dicot stem cross-section is also presented to demonstrate the full resolution light field imaging capability of our system for both translucent and fluorescent specimens.

1. Introduction

Computational microscopy is an emerging technology which extends the capabilities of optical microscopy with the combination of optical coding and computational decoding. It provides us with novel imaging functionalities or improved imaging performance which are difficult or impossible to achieve using a conventional microscopic system. For example, quantitative phase microscopy enables biological samples to be visualized and quantified without the need for specific staining or labelling by incorporating interferometry or phase retrieval algorithms with conventional microscopy [1–4]. Structured illumination microscopy and Fourier ptychographic microscopy utilize angularly varying oblique illuminations and synthetic aperture algorithms to bypass the resolution limit defined by the microscopic objective [5–7]. Light field microscopy captures 4D light fields that allow digital refocusing or changing perspective of images via post-processing [8,9].

Recent advance in LED lighting and digital display technology provide new opportunities for active digital illumination and imaging control for advancing microscopy. By integrating a spatial light modulator (SLM) or a video projector into the illumination or imaging path of the microscope, one gains the flexibility to produce sophisticated illumination patterns or dynamically switchable illumination sources with no moving components [10,11]. However, since the SLM is a technically complex device, these systems end up being heavy, bulky, and expensive. Alternatively, the active illumination control can be realized by replacing the condenser with a matrix of LEDs [12–14]. The LED-based condenser-free scheme provides a simple and cost-effective solution to realizing multi-modal contrast enhancement imaging but with a significantly reduced light collecting efficiency. Recently, instead of using a LED matrix, the use of a low cost liquid crystal display (LCD) to achieve programmable condenser illumination control has been reported [15,16]. In those methods, the condenser lens of a conventional microscope is kept unchanged but the condenser diaphragm was replaced a programmable LCD. The LCD-based programmable condenser techniques increase the photon budget and provide more flexibilities for controlling spatial coherence and microscope illumination over the LED-based condenser-free approaches.

In this paper, we present the programmable aperture microscope (PAM) to achieve multi-modal computational imaging. By integrating a programmable LCD as a low-cost transmissive spatial light modulator into the imaging aperture plane of the microscope, different spatial frequencies or angular components of the optical field can be directly manipulated. By properly choosing different binary patterns displayed on the LCD, the traditional methods of classic microscopy, including bright field, dark field,

* Corresponding author.
E-mail addresses: surpasszuo@163.com (C. Zuo), chenqian@njust.edu.cn (Q. Chen).

http://dx.doi.org/10.1016/j.optlaseng.2015.12.012
0143-8166/

differential phase contrast imaging can be realized without requiring specialized hardware components and any moving parts. Furthermore, it allows the quantitative phase of the sample to be measured non-interferometrically even with the use of an extended incoherent source from a standard microscope condenser. Finally, it achieves light field imaging at full sensor resolution through multiple exposures, without spatio-angular resolution trade-offs which are often inevitable in conventional microlens array based light field imaging. To the best of our knowledge, this is the first report of using a low cost LCD in the Fourier plane of the imaging path for multi-modal computational imaging and full sensor resolution light field microscopy. Compared with previously reported computational microscopic schemes based on programmable illuminations by using a LED array [12–14] or a programmable LCD [15,16], the reported PAM has the following advantages: first, the 4D dataset captured by angularly varying illuminations is an good approximation of the light field only in certain extremely simplified conditions (the sample should be angle-shift invariant respect to the incident light field, which in turn requires that the sample to be weak scattering or sufficiently thin) [17], while our approach gives one direct access to the precise 4D light field, which is independent of sample characteristics. Second, not just limited to conventional amplitude or phase specimens, our light field imaging approach can also be applied to fluorescent or self-luminous specimens. Finally, the programmable aperture module can be designed as a passive add-on that can be incorporated into most standard bright field or fluorescence microscopes with no hardware modifications.

2. System setup

The layout of the PAM is shown in Fig. 1. The whole system is built based on a commercial inverted infinity-corrected microscope (Olympus IX83), which itself is composed of a collector lens, condenser associated with aperture diaphragm, objective, reflective mirror (M_1), and tube lens, producing a magnified image of the specimen at the camera output port (image plane). The light from the built-in halogen lamp passes through a green interference filter (central wavelength $\lambda = 550$ nm, 45 nm bandwidth) to create quasi-monochromatic illuminations. To gain access to the rear aperture of the microscope objective, an additional 4f relay system is introduced, with the LCD located at the central Fourier plane. In this case, the rear focal plane of the objective is imaged onto the LCD surface, and then modulated by the binary displayed pattern (to pass or to be blocked). The LCD is an Electronic Assembly DOGXL160-7 display with a resolution of 160 × 104 and 20 Hz frame rate. The panel size of the LCD is 78 × 61 mm (3.3 in.), which is sufficient to cover the entire spectral spread at the rear aperture of the microscope objective. The LCD is controlled by computer software via USB with an Atmel ATmega88 microcontroller. An AVI format movie clip (Media 1) is included to demonstrate different patterns shown on the display under our software control.

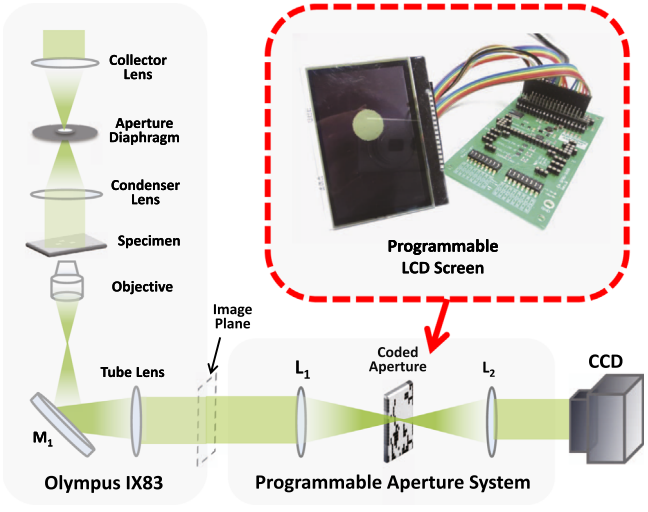

Fig. 1. Schematic setup for the PAM system. M1, mirrors; L_1, L_2, lenses (focal length $f_1 = 250$ mm, focal length $f_2 = 150$ mm). The boxed inset shows a photograph of the LCD, which allows dynamic attenuation mask patterns to be encoded under software control (Media 1).

3. Results

3.1. Multi-modal contrast-enhancement imaging

We first demonstrate the flexibility of our PAM to realize contrast-enhancement imaging of unstained transparent samples. Fig. 2 clearly illustrates the various imaging modalities images of unstained cheek cells and the corresponding binary patterns displayed on the LCD. The sample is imaged by standard Olympus objective with magnification 10 × (UPLFLN10X, $NA_{obj} = 0.3$) in the Köhler illumination configuration with illumination numerical aperture $NA_{ill} = 0.1$. For bright field imaging, we just display a clear background so that all light arriving the rear focal plane can pass through the LCD. As shown in Fig. 2(a), the sample is difficult to visualize under conventional bright field microscopy because the absorption and scattering are too weak to produce any contrast. However, once we block out the central part of light within the circle, allowing only scattered light goes on, a dark field image can be obtained. The increase in contrast is evident in Fig. 2(b), where the edges or boundaries of the cells are clearly highlighted in white against a dark background. It should be noted that in conventional dark field imaging, the illumination NA overfills the objective NA by using specialized condenser or diaphragm. While our approach can achieve similar effect by active controlling the light at the detection path without additional optics, albeit with a reduced illumination aperture.

3.2. Differential phase contrast and quantitative phase imaging

Our PAM can also be used for phase contrast imaging and quantitative phase imaging. By far, the most popular phase contrast imaging techniques employed in commercial microscopes are Zernike phase contrast and differential-interference contrast (DIC). However, both of them require specialized optics and provide images where phase and amplitude information is mixed. As an alternative to DIC, differential phase contrast (DPC) can produce an phase contrast image that looks similar to DIC, but with better linearity with respect to the phase gradient. DPC was originally proposed in the field of scanning electron or optical microscopy based on a split-detector [18], and recently has been adapted to wide-field optical microscopy based on asymmetric illumination [19,13]. By taking two images with two opposite but complementary illumination directions, the DPC image can be computed as their normalized difference. In this work, instead of changing the illumination directions, we can arrive another wide-field equivalent of the DPC based on split-aperture. By displaying two complementary (left-half and right half) patterns as shown in Fig. 2(c), the DPC image can be recovered by

$$D_x = \frac{I_R - I_L}{I_R + I_L}. \quad (1)$$

The similar process may be repeated for realizing top-bottom DPC, with top half and bottom half patterns sequentially displayed, as