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Four channels multi-illumination single-holographic-exposure lensless Fresnel (MISHELF) microscopy

Martín Sanz, Jose Ángel Picazo-Bueno, Luis Granero, Javier García, Vicente Micó*

Departamento de Óptica y de Optometría y Ciencias de la Visión. Facultad de Física. Universidad de Valencia, C/ Doctor Moliner 50, Burjassot 46100, Spain

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A B S T R A C T

MISHELF microscopy [Opt. Express 23, 21352 (2015)] has been recently reported as the background technology of a new concept of compact, cost-effective and field-portable lensless microscope [Sci. Rep. 7, 43291 (2017)] based on wavelength multiplexing and a fast and robust algorithm for twin image minimization and noise reduction. In this manuscript, MISHELF microscopy is expanded beyond its actual configuration by considering 4 illumination/detection channels while retaining its working principle concerning single-shot, twin image mitigation and noise averaging. Proof of principle validation of the proposed improvement is conducted through experiments with a resolution test target and a micro sphere sample.

1. Introduction

Lensless microscopy emerges as a discipline of digital holographic microscopy where lenses are removed from the optical layout. Lensless microscopy originally derives from a digital implementation of the Gabor's invention [1,2] where a point source of coherent light illuminates the sample and the diffracted wavefront is recorded by a digital sensor [3]. Under certain approximations [4,5], the recording process is ruled by holography and image reconstruction coming from the recorded Fresnel diffraction pattern is achieved by applying numerical methods in the digital domain [6]. Lensless microscopy has found a wide variety of applications such as, just to cite a few (the list is very large), submersible imaging [7,8], tracking particles/cells evolution [9–11], spectacle lens inspection [12,13], optofluidic approaches [14–16], and telemedicine and global health [17–19].

Nowadays, lensless microscopes have experienced a strong development because of the new achievements in optical components in the optoelectronic field as well as their price reduction. Thus, there are available solution of low cost, portable and miniaturized versatile devices for biomedical applications [8,18,20–28], some of them working in a lensfree scheme [8,18,20–23] while others operating as smartphones implementations [24–28].

Recently reported [29], MISHELF (initials coming from Multi-Illumination Single-Holographic-Exposure Lensless Fresnel) microscopy proposes a new concept of compact, cost-effective and field-portable lensless microscope prototype based on wavelength multiplexing and a fast and robust algorithm for twin image minimization and noise reduction. MISHELF microscopy is based on the illumination of the sample with three different wavelengths and the recording of the different Fresnel diffraction patterns. Different implementations of this technique have been reported at lab level (bench-top system within a well-controlled environment) where the process is conducted sequentially in time using three laser diode at 685, 785, and 940 nm wavelengths [30] or in a single snap-shot with RGB multiplexing in the illumination/detection stages [31,32]. MISHELF microscopy concept has been validated as a prototype in the field-setting [29] and newly merged with wide field of view lensfree microscopy to create a novel imaging platform based on dual mode imaging in real time with different magnifications and resolution capabilities in lensless microscopy [33].

In this contribution, we report on the extension from 3 to 4 useful illuminations/detection channels in MISHELF microscopy. This new approach retains the single-exposure working principle (useful for imaging dynamic samples) of MISHELF microscopy while improves twin image mitigation, halo reduction and noise averaging since there are now 4 available channels with valuable information about the inspected sample. Proof of principle validation is provided in virtue of a new laboratory prototype and two different calibration samples (resolution test target and micro spheres) are used for demonstration of the proposed method. The manuscript is organized as follows. Section 2 presents the optical layout and the manufactured prototype as well as the general algorithmic/processing to be performed. Section 3 includes the experimental validation. And Section 4 concludes the paper.

2. Experimental layout

A scheme of the proposed 4-channels MISHELF microscopy concept is presented in Fig. 1(a) while some views (front, lateral and perspec-

* Corresponding author.
E-mail address: vicente.mico@uv.es (V. Micó).

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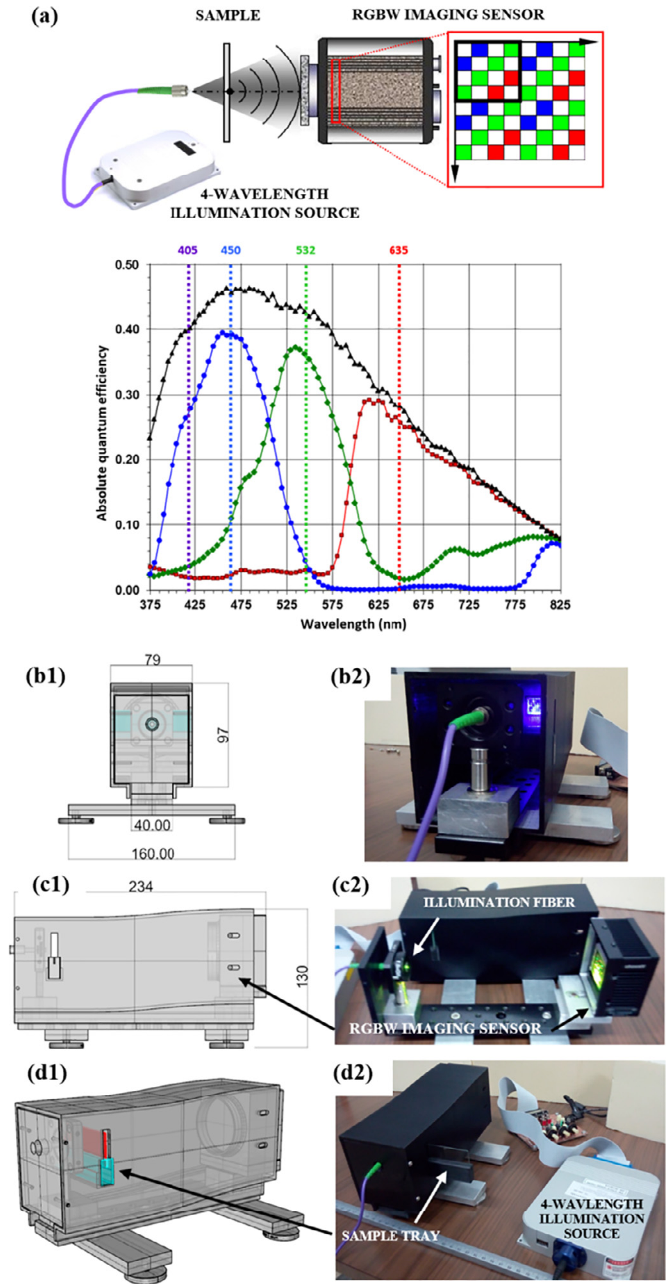

Fig. 1. (a) Optical layout of the proposed 4-channels MISHELF microscopy including the spectral sensitivity of the 4 different camera channels where black graph corresponds with the W channel; from (b) to (d) different views of the prototype concerning its design (left column) and pictures (right column). Units are in mm.

tive) of the designed (left column) and manufactured (right column) prototype are included through Figs. (b)–(d). The microscope prototype has been designed using commercially available CAD software platform and built using Fused Deposition Material with ABS for most of the components and mechanization process for the rest. As it can be seen, the illumination fiber and the imaging sensor are placed on both ends of the prototype while the sample is inserted by a lateral slot in the proximity of the illumination source using a sample tray. Approximately, the distances between source/sample (z) and sample/camera (d) are 15 and 175 mm, respectively, defining a theoretical magnification of $M = (d + z)/z \cong 12.6X$.

To implement the 4-channles MISHELF microscopy configuration, we have used a 4 wavelength source of fiber optic coupled diodes (Blue Sky Research, SpectraTec 4 STEC4-405/450/532/635 nm). The RGBV diodes are coupled to single mode fibers, so the numerical aperture (NA) of the illumination becomes very limited (~0.1 range). As imaging sensor, we have used a 4 channels RGBW camera (Viewworks VA-8MG2-C10CA0, 3296 × 2474 pixels, 5.5 µm pixel size, 10 fps, GigE interface). This camera contains a Kodak Truesense sensor with a color filter pattern (see Fig. 1(a)) that builds upon the standard Bayer pattern by adding panchromatic (W) pixels to the RGB pixels present on the sensor. As a result, the basic cell of the RGBW filter array is integrated by a 4 × 4 pixels (see Fig. 1(a)) which is replicated along the sensor area. Originally, these W pixels are aimed to increase sensitivity in the black and white, so the final image is improved in terms of luminance. The spectral sensitivity of the different filters is also included in Fig. 1(a).

But our goal is to use the 4 different sensitivities of the imaging sensor to retrieve 4 images coming from the 4 different illuminations. Because of the fact we are not using a tuned scheme for illumination/detection as it happens in Refs. [31,32], we need to extract real intensities from the detection channels. An in-depth description of the algorithm is presented in Refs. [29,32] but a few words about the new parts are included here.

In essence, MISHELF algorithm is divided in two main blocks: the first one is related with a pre-digital preparation of the holograms before entering into the second block which performs phase retrieval by applying a fast convergence algorithm [32]. The first block starts with the recording of a RGBW in-line hologram in a single snap-shot. After that, demosaicing is implemented to extract separately the RGBW information provided by the camera since we know the pixel ordination in the basic cell of the filter array (Fig. 1(a)). This procedure is needed in order to eliminate the crosstalks and to improve image quality in the reconstruction process. Thus, the real RGBV holograms corresponding with the true RGBV illuminations can be computed by two different ways. First, one can theoretically know the amount of a given wavelength entering into the different channels by looking at the spectral sensitivity graph included at Fig. 1(a). This is a rough procedure for obtaining a set of 4 × 4 factors regarding the contribution of each illumination wavelength into each detection channel. So, it is possible to roughly retrieve the real RGBV holograms by a weighted subtraction operation guided by this 4 × 4 factors. And second, the retrieval of these real RGBV holograms can be also computed using calibration. We have preferred to use calibration since it is a more precise way to know the exact contribution of the 4 × 4 factor's set rather read it from the graph. Calibration means that, without placing the object and fixing the intensity per each illumination source, each laser diode is sequentially turned on and 4 different images are recorded. Then, the 4 channels from each recorded image are obtained and a relative weight factor is computed from the global intensity distribution of each channel. We have selected the mean intensity value of the whole image as key metric for computing the 4 × 4 factor's set.

Essentially, the intensities at each camera channels (I_R, I_G, I_B, I_W) result as a combination of the 4 illumination intensities (I_R, I_G, I_B, I_V) and can be written as

$$\begin{pmatrix} I_R^{CCD} \\ I_G^{CCD} \\ I_B^{CCD} \\ I_W^{CCD} \end{pmatrix} = \begin{pmatrix} a_1 & b_1 & c_1 & d_1 \\ a_2 & b_2 & c_2 & d_2 \\ a_3 & b_3 & c_3 & d_3 \\ a_4 & b_4 & c_4 & d_4 \end{pmatrix} \cdot \begin{pmatrix} I_R \\ I_G \\ I_B \\ I_V \end{pmatrix} \Rightarrow \vec{I}_{\text{det}} = M_{cal} \cdot \vec{I}_{\text{em}} \tag{1}$$

The matrix elements (a_i, b_i, c_i, d_i) represent the detector response of each RGBW channel to the RGBV illuminations, being i = 1, 2, 3, 4. Those coefficients can be obtained theoretically or experimentally as previously stated and this procedure needs to be done once as preliminary calibration of the prototype. After the detector response matrix (M_{cal}) is experimentally obtained, it is possible to obtain the real intensities by computing the inverse matrix of M_{cal} in the form of

$$\vec{I}_{em} = M_{cal}^{-1} \cdot \vec{I}_{\text{det}} \tag{2}$$