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Dual-mode optical microscope based on single-pixel imaging

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ABSTRACT

We demonstrate an inverted microscope that can image specimens in both reflection and transmission modes simultaneously with a single light source. The microscope utilizes a digital micromirror device (DMD) for patterned illumination altogether with two single-pixel photosensors for efficient light detection. The system, a scan-less device with no moving parts, works by sequential projection of a set of binary intensity patterns onto the sample that are codified onto a modified commercial DMD. Data to be displayed are geometrically transformed before written into a memory cell to cancel optical artifacts coming from the diamond-like shaped structure of the micromirror array. The 24-bit color depth of the display is fully exploited to increase the frame rate by a factor of 24, which makes the technique practicable for real samples. Our commercial DMD-based LED-illumination is cost effective and can be easily coupled as an add-on module for already existing inverted microscopes. The reflection and transmission information provided by our dual microscope complement each other and can be useful for imaging non-uniform samples and to prevent self-shadowing effects.

1. Introduction

Optical microscopy techniques have become essential tools in basic and applied research. For instance, in the biological realm, fluorescence microscopy is the method of choice for imaging proteins and their nano-scale dynamic organization. Despite impressive improvements in optical resolution during the last decades [1–3], there is still room to improve a range of other features such as the trade-off between the field of view and the depth of field [4,5], 3D imaging of specimens [6], or the design of cost-effective, light-weight platforms that can operate in resource-limited settings for global health challenges [7,8], among others.

Structured illumination microscopy (SIM) stands out among the different optical microscopy techniques because it allows us to retrieve spatial frequency information inaccessible with standard uniform illumination schemes [9,10]. It is based on a standard wide-field optical microscope where the specimen is illuminated with a set of known sinusoidal patterns with different phase shifts. The object is reconstructed from multiple acquisitions by using dedicated algorithms. Additionally, this fringe-projection method is attractive because of its intrinsic optical sectioning capability that enables whole-field optically sectioned images [11,12].

In the conventional implementation of a SIM, a focal plane array detector detects variations in light intensities scattered by the sample with spatial resolution. Other structured illumination approaches have also been implemented for microscopy during the past few years. The single-pixel imaging (SPI) scheme [13] has gained considerable attention as a very effective sensing mechanism and has triggered diverse applications where conventional cameras equipped with millions of pixels fail to give an adequate response, including optical microscopy [14]. As a matter of fact, SPI allows imaging at previously undeveloped spectral bands [15,16], at the photon-counting regime [17], in presence of strong turbulence or scattering [18,19], and also through multimode optical fibers [20,21]. The combination of wide-field structured illumination altogether with a bucket detector has made hyperspectral imaging across the visible spectrum possible in a fluorescence microscope [22]. Also, a prototype microscope system based on SPI to image simultaneously in the visible and the short-wave infrared has been recently demonstrated [23].

In SPI the problem of spatial resolution is shifted away from the sensor to a set of a microstructured spatial masks that are codified onto a programmable spatial light modulator. The masks are optically projected onto the sample through the microscope objective and the whole intensity is collected onto a bucket (single-pixel) sensor. Measurements are sequential by changing of the spatial mask. If many different masks are used, their shapes and the intensity signals are combined to retrieve the sample. This generalized mask scanning offers several advantages, like signal-to-noise ratio enhancement and the possibility to reduce the

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acquisition time through compressive sensing, over the traditional raster scan used in confocal microscopy where a single bright pixel is scanned to build up the image [24,25].

The nature of SPI enforces a reciprocal relationship between the frame rate and image size as the time required to capture an image scales with the number of pixels in the image. Two different approaches can be employed to deal with this issue. On the one hand, given some reasonable assumptions about the sparsity of the signal, compressive sensing dramatically reduces the number of measurements well below the number of pixels of the sample [26,27]. What is remarkable here is that with the only "a priori" knowledge about the sparsity of the sample it is possible to get rid off the measurement of the full-length signal, so it saves time. More recently, adaptive sensing has been introduced as a way to circumvent the computational complexity in convex optimization or greedy algorithms used in compressive sensing [23,28,29]. On the other hand, SPI usually relies on the use of fast spatial light modulators such as the digital micromirror device (DMD) to codify the projecting masks. DMDs permit highly flexible codification of binary masks at frame rates above 20 kHz. Extensive application of the DMD to microscopy has been reported in the past few years including conventional SIM microscopy with fringe projection [30], super-resolution and optical sectioning microscopy [31,32] and the programmable array microscope [33–35]. Interestingly, fast DMD and pattern illumination is at the core of optogenetics, a tool for noninvasive activation and silencing of neurons and muscles [36,37].

Along the same lines than SPI microscopy, here we demonstrate a dual-mode microscope that can image specimens in reflection and transmission modes simultaneously. Many specimens, such as biological samples, are weak reflectors but produce transmission images with good contrast. Inversely, other samples are very dense and provide poor transmission images or generate self-shadowing effects. Conventional optical microscope designs make the simultaneous collection of transmitted and reflected light inefficient, restrictive, or even impossible. In general they need different light sources for transmission and reflection, thereby preventing that both images be simultaneously measured in a single sensor. Alternatively they can work with several digital cameras, but then a careful calibration and geometric adjustment of both sensors is necessary. The usefulness of dual-mode microscopy for histopathology studies of skin tissue has been recently reported based on a lensless holographic setup [38,39] and near-field scanning optical microscopy [40].

Here, we demonstrate that the SPI architecture is particularly well-fitted for this dual operation recording both reflection and transmission information simultaneously with a single light source and a simple light sensor configuration constituted by two single-pixel detectors. The field of view, the optical resolution, and the focused plane are determined by the light projection system. Therefore, the reflection and transmission images obtained by each light detector are automatically adjusted geometrically with no need of calibration procedures. Furthermore both images are focused unequivocally to the same plane of the sample. In summary, in our microscope, reflection information complements the transmission one very efficiently, which can be very useful for imaging non-uniform samples and to prevent self-shadowing effects.

For the practical implementation, instead of using a high-end DMD as previous single-pixel microscopy techniques, we use of an off-the-shelf DMD from a cost-effective digital light projector. These devices utilize an offset diamond pixel layout which generates geometrical artifacts. In addition to prove the dual mode operation, in this work we demonstrate an algorithm to precisely allocate pixels in memory to deal with this problem.

2. Dual-mode single-pixel imaging

In a dual-mode SPI microscope, the DMD plane is relayed by a convenient optics onto the sample plane and the forward and backscattered light components are simultaneously collected onto dual single-pixel photodetectors located at the transmission and reflection ports of the microscope, respectively (see Fig. 1). A sequence of M sampling patterns is codified onto the DMD so that the irradiance striking the photodetector at the transmission port in the ith timestep is

$$Y_i = \langle \Psi_i(\vec{x}), T(\vec{x}) \rangle, \tag{1}$$

where $T(\vec{x})$ is the transmittance distribution of the sample, $\Psi_i(\vec{x})$ denotes the ith measurement pattern, and $\langle \Psi_i(\vec{x}), T(\vec{x}) \rangle$ represents the inner product between both functions. The state of the sampling projector $\Psi_i(\vec{x})$ is changed from one timestep to the next to implement a full set of measurements

$$\mathbf{Y} = \mathbf{ST}, \tag{2}$$

where $\mathbf{S}$ is a sensing matrix whose ith row is a one-dimensional reshaping of the ith sampling mask $\Psi_i(\vec{x})$, and $\mathbf{T}$ and $\mathbf{Y}$ are N-dimensional vectors representing a one-dimensional reshaping of the unknown transmittance distribution and the result of the measurements at the transmission port, respectively.

Equivalently, the measurements at the reflection port are concisely represented by the series of linear equations

$$\mathbf{Z} = \mathbf{SR}, \tag{3}$$

where $\mathbf{R}$ and $\mathbf{Z}$ are N-dimensional vectors representing a one-dimensional reshaping of the unknown reflectance distribution and the result of the measurements at the reflection port, respectively,

The problem of the measurement can be stated as: given the result of the measurements $\mathbf{Y}$ and $\mathbf{Z}$ derive the set of values $\mathbf{T}$ and $\mathbf{R}$ that best represent the transmittance and reflectance distributions $T(\vec{x})$ and $R(\vec{x})$ of the sample.

In the easiest implementation of the retrieval algorithm, which is possible for well-conditioned measurements systems, a number of measurements M equal to the number of pixels of the sample is required and both the transmission and the reflection images are retrieved through the inverse matrix as

$$\mathbf{T} = S^{-1}\mathbf{Y}, \quad \mathbf{R} = S^{-1}\mathbf{Z}. \tag{4}$$

Concerning the measurement patterns, various matrices can be employed. For instance, raster-scan style masks stem from the well-known raster-scan technique in which spatial pixels are measured sequentially. Random matrices can also be used in

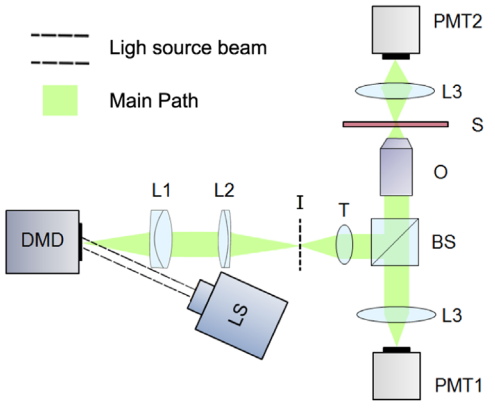

Fig. 1. Experimental set-up. (LS) Light source. (DMD) Digital micromirror device. (L1, L2) Relay lenses. (I) Intermediate image of the DMD. (T) Tube lens. (BS) Beam splitter. (S) Sample plane. (L3) Condenser lenses. (O) Projection objective. Reflection and transmission photomultiplier tubes (PMT1 and PMT2, respectively).