Space-Time Digital Holography enhances resolution and field of view for optical metrology and bioimaging applications

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We introduce STDH modality to record and process hybrid space-time representations. This allows improving resolution and Field of view with one single object scan. Different cells has been used as samples to verify this approach.

Keywords: Digital holography; Phase shift.

1. Introduction

Digital Holography (DH) is an optical 3D imaging technique able to retrieve the complex information scattered/diffracted from an object in the form of an interference pattern, where its amplitude and phase act as signals modulating a carrier system of fringes [1, 2]. Holographic techniques can yield a very high throughput thanks to an a posteriori refocusing capability. This allows recovering out-of-focus information that otherwise is lost in standard bright field microscopy. Space-Time Digital Holography (STDH) exploits the object motion to record the hologram in a hybrid space-time domain. This representation adds new capabilities to conventional Digital Holography, such as unlimited extension of the Field of View (FoV) and tunable phase shifting. This is the best candidate for imaging, both in amplitude and quantitative phase-contrast, biological samples flowing in microfluidic channels[3-5]. In addition, we showed that STDH is able to improve the spatial resolution as well. Differently from other super-resolution approaches, stitching between holograms or their spectra is no longer required [6]. Moreover, we introduced a new STDH modality to record and process hybrid space-time representations. This allows improving resolution with one single object scan.

We use a banded area of a CCD sensor to capture a set of observables while the object moves. For each object position, a column (or row) of pixels, which records a 1D signal, is acquired in the form of modulated fringes. Moreover, we use a diagonal pixel set to acquire 2D signals, which is an efficient way to scan that we recently introduced. The acquired sequence is then used to synthetize the STDH that corresponds to the modulation of a new carrier. The idea of STDH is to move complexity from the reconstruction to the data capture process, by imposing a specific constraint on the scanning velocity that has to be matched to the frame rate of the sensor. Thus, a synthetic STDH can be created maintaining all the features of a space-space DH, along with some novel ones.

2. Experiments

Recently, a series of STDH-based cell imaging experiments have been completed. the optical metrology of onion cells and Hela cells were preformed based on space-time holographic scanning. The holographic recording system is based on an off-axis Mach-Zehnder interferometer with coherent light source which wavelength is 532.8nm. The laser beam is divided into two beams by a polarizer beam splitter prism after simple filtering. The cells are placed on a 2D linear stage, it is used to achieve x-y plane scanning for STDH recording. Two 25× objective lenses are used for magnification imaging. A scientific-grade CCD camera is placed after beam splitter prism, the pixel size is 4.5μm × 4.5μm, and the screen size is 1280 × 960.

In the experiments, thanks to STDH, both resolution improvement and field of view expansion were achieved simultaneously. Differently from many other superresolution approaches proposed in literature, STDH avoids cumbersome stitching procedures, i.e. the object frequencies naturally self-assemble as a consequence of the fixed relation we impose between the scanning vector and the frame rate. We believe that resolution enhancement, FoV enlargement, flexible refocusing, phase retrieval and SNR improvement will make STDH a valuable tool for the high-throughput analysis of biological samples in microfluidic environment.

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