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Quantitative phase imaging with single shot digital holography

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ABSTRACT

We demonstrate quantitative phase imaging using single shot digital holography for a calibrated spiral phase object. A single frame of near on-axis digital hologram of a spiral phase plate is recorded and the complex object field in the hologram plane is retrieved using a constrained optimization approach. Experimental results show the feasibility of a quantitative phase imaging technique which has superior performance to conventional Fourier filtering methods. Single shot capability suggests that this method is suitable for holographic imaging of dynamic objects such as live biological cells.

1. Introduction

Optical imaging of transparent objects like biological cells, tissues, and micro-organisms is of great interest in the field of biomedical sciences. The absorption of light in this type of samples is very low; however, their thickness or refractive index variations over illumination area can modify the phase profile of a spatially coherent beam of light passing through the sample. Early phase sensitive microscopic imaging techniques e.g. Zernike phase contrast [1] provide powerful visual tools for qualitative analysis of transparent samples but are incapable of giving quantitative phase information. Quantitative phase imaging requires an interferometric approach such as digital holography (DH). In DH the interference data is typically recorded on a digital array detector (CCD or CMOS) and the image recovery (amplitude and phase) at the desired plane is performed computationally. With the advancement of digital detector arrays and ready availability of fast computational hardware, DH has become an important tool for marker free, non-destructive, quantitative phase imaging applications [2–5]. The most commonly used configuration in DH is the off-axis setup where a plane reference beam is used at an angle with respect to the object beam for providing the carrier frequency. The off-axis configuration is simple in the sense that the dc and cross terms in the recorded hologram are typically separated in Fourier space and the quantitative phase information may be recovered by Fourier domain filtering. One of the main limitations of this configuration is that the object resolution in single shot DH experiments is limited by the minimum reference beam angle condition for non-overlap of the dc and the cross terms. This condition states that in order to obtain holographic reconstruction of an object having highest spatial frequency of B, the minimum reference beam angle required is given by $\sin^{-1}(3B\lambda)$ (Ref. [6], Section 9.4.3). At visible light wavelengths and with typical pixel sizes of 2–5 μm for digital detector arrays, this non-overlap requirement severely limits the achievable lateral resolution for dynamic imaging applications, e.g. live cells moving in liquid medium. The current single shot DH techniques cannot fully utilize the lateral resolution capability offered by the digital detector array [5]. The object resolution in DH imaging may be improved by using phase shifting digital holography [7] where multiple holograms are recorded corresponding to different phase steps of the reference beam. The requirement of multiple frames however prevents truly dynamic DH imaging.

Recently, we described a new computational approach for image recovery from a single digital hologram frame [8,9]. In this approach the complex object field in the hologram plane is obtained by solving a constrained optimization problem which is unlike the physical hologram replay process that is usually mimicked in the conventional DH numerical processing. This procedure was shown to overcome some of the current resolution limitations on single shot digital holography. In particular, experimental results using an amplitude object (USAF resolution chart) on visual inspection showed high quality image recovery even when the dc and the cross terms in the recorded hologram overlapped substantially in the Fourier domain. However, the accuracy of phase recovery was not considered in the initial demonstration of our method [8]. In this paper we demonstrate the effectiveness of this single shot DH technique for quantitative phase imaging by using a calibrated phase object for illustration. Establishing the quantitative phase accuracy of this method may

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be considered as a starting point for enabling multiple applications of this single shot technique. The outline of this paper is as follows. In Section 2, we briefly review our constrained optimization approach to DH numerical processing for extracting the complex object field from a single hologram frame. The experimental results are described in Section 3 where we compare the phase recovery for a spiral phase object using both the conventional Fourier filtering approach and our constrained optimization method. Finally in Section 4 we provide concluding remarks.

2. Constrained optimization approach for single shot digital holography

We model the recovery of complex object information from a single digital hologram frame as a constrained optimization problem. The hologram pattern recorded due to interference between the object beam $O(x,y)$ and the reference beam $R(x,y)$ at the camera plane is given by

$$H=|O|^2+|R|^2+OR^*+O^*R \tag{1}$$

The reference beam R is given as $R = R_0 \exp(i\ kx\ \sin\theta)$ where θ is the reference beam angle relative to the nominal object beam direction, R_0 is the constant amplitude of the reference plane wave, and $k=2\pi/\lambda$ is the wave vector where λ is the wavelength of laser illumination used. The recovery of the complex object function O is achieved by solving an optimization problem with smoothness constraint on the object function O. We define a cost function $C(O,O^*)$ to be minimized using the recorded hologram H, the unknown object function O and the known reference beam R.

$$C(O,O^*)=\frac{1}{2}\left|\left|H-(|O|^2+|R|^2+OR^*+O^*R)\right|\right|^2+\alpha\,\psi(O,O^*) \tag{2}$$

In Eq. (2) the first term represents the L2-norm error and $\psi(O,\ O^*)$ is a penalty function for imposing a smoothness constraint on the complex object function O. In a Fresnel zone off-axis configuration as used by us, the smoothness constraint is justified as O is a diffracted field and is expected to have local smoothness. Further, an appropriate use of smoothness constraint guarantees the absence of any modulation due to carrier fringe frequency or its harmonics in the solution of the minimization problem for O. We solve the minimization problem above using a gradient descent scheme outlined below [8]. The complex gradient of the cost function with respect to O^* is represented as

$$\nabla_{O^*}C(O,O^*)=-[H-(|O|^2+|R|^2+OR^*+O^*R)].(O+R)+\alpha\,\nabla_{O^*}\psi(O,O^*) \tag{3}$$

The iterative update scheme for the solution is given as:

$$O^{(n+1)}=O^{(n)}-t[\nabla_{O^*}C]_{O^{(n)}} \tag{4}$$

here ∇ is the functional gradient with respect to O^* and 't' is a positive constant denoting the step size in the negative gradient direction which is selected by standard line search in each iteration [10]. The actual implementation of Eq. (4) is carried out in each iteration by first, setting $\alpha=0$ in Eq. (3), and then applying the smoothening constraint. In particular, the smoothness constraint is implemented simply by convolution of the L2-norm updated guess solution with an averaging square shaped filter G [8]. The overall iteration scheme is as shown in Eq. (5).

$$O^{(n+1)}=G\otimes\{O^{(n)}+t[H-(|O^{(n)}|^2+|R|^2+O^{(n)*}R+O^{(n)}R^*)\times(O^{(n)}+R)]\} \tag{5}$$

As explained in Ref. [8], we choose the spatial extent of G to be approximately equal to half the carrier fringe period which prevents any modulation of the resultant solution. As long as the spatial extent of the averaging mask G is much smaller than the expected Fresnel blur at the hologram plane, one can expect minimal loss in resolution. The complex object function O is back propagated [6] to the image plane by angular spectrum method. The phase of the resultant image O_{image} may then be obtained as

$$\Phi(x,y)=\tan^{-1}\frac{\mathrm{Im}(O_{image}(x,y))}{\mathrm{Re}(O_{image}(x,y))} \tag{6}$$

here, O_{image} represents the back propagated object field to form the image, 'Re' represents the real part and 'Im' represents the imaginary part, respectively. Since the object function O recovered using the optimization method has the same size as the sensor which is also the same as the size of the image O_{image}.

3. Experimental setup for quantitative phase imaging

While first demonstration of the optimization procedure with some experimental result was demonstrated in Ref. [8], the object used was an amplitude object and further the quantitative phase accuracy was not tested in that work. To verify the possibility of quantitative phase imaging using this optimization approach, we used charge 1 spiral phase plate (RPC Photonics) as an object. A Fresnel hologram of this phase object was recorded using the Mach–Zehnder configuration in Fig. 1.

The path BS1-M1-BS2 is the reference arm whereas the path BS1-M2-BS2 is the object arm. The mirror M1 is tilted to provide a suitable carrier fringe frequency. A CMOS camera (Imaging Source, DMK 72BUC02, CMOS, 1944 × 2592 pixel array, 2.2 μm square pixels) was used for recording the hologram. While there are several choices for phase objects we selected the spiral phase

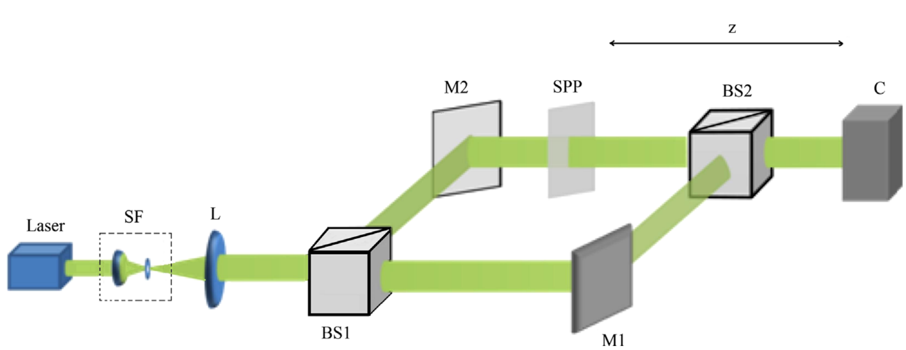

Fig. 1. Experimental setup for single shot quantitative phase imaging. SF: spatial filter, BS1, BS2: Beam splitters, M1, M2: Mirrors, SPP: spiral phase plate (phase object), C: CMOS array. Laser wavelength is 532 nm, distance between SPP and sensor C is $z=13$ cm.