Optics Communications 422 (2018) 3–7

Contents lists available at ScienceDirect

Optics Communications

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

# Highly stable lens-less digital holography using cyclic lateral shearing interferometer and residual decollimated beam

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ARTICLE INFO

*Keywords:*
Quantitative phase imaging
Digital holography
Phase measurements
Interferometric imaging
Interferometry

ABSTRACT

Lens-less digital holography for quantitative phase contrast imaging is established with a cyclic lateral shearing interferometer and non-collimated light beam. In a self-reference type configuration, the object and reference beams are generated from the same spherical wavefront after the light beam has passed through the sample plane. The experimental configuration is implemented with simple optical components involving beam splitter and mirrors. This proposed architecture has the advantage that the system does not require any special optical element such as pinhole, gratings, and other customized optical components and hence can be easily realized with significantly simplified alignment procedure. Experimental results for both amplitude and phase objects are presented as a proof of concept.

## 1. Introduction

Interferometry and digital holography based quantitative phase contrast imaging (QPCI) method has grown into a very effective tool for imaging of transparent microscopic and macroscopic samples [1–14]. The most common experimental approach for the implementation of digital holography based QPCI is the realization of an off-axis holographic recording geometry using a two-beam setup, wherein, the object beam and reference beam traverse different optical paths. Thus, there is requirement of a separately generated coherent reference beam. This two beam interferometry based QPCI technique enables high resolution imaging and hence have been extensively applied in biological research areas such as label free minimally invasive live cell analysis etc. [15,16]. However, a major weakness of this off-axis recording configuration, is the requirement of a separately generated coherent reference wave, where the splitting into object and reference beams is carried before the light beam interacts with the sample. Consequently, they are subjected to differential environmental noise which results in reduced phase stability. Furthermore, in order to record good contrast interference fringes, this geometry also demands precise adjustment of beam intensity ratio, which is dependent on the absorption properties of the sample under study and also on the magnification (10×, 20× etc.) of the system through the use of microscope objectives (MO). The contrast of the recorded interferogram directly affects the fidelity of the reconstructed quantitative phase contrast image data. Thus, a manual adjustment of beam intensity ratio is required. Moreover, to ensure the stability of the recorded interference pattern, the interferometric study is required to be performed on a vibration isolation optical table to avoid the mechanical perturbations. Such vibration isolation tables are in general not available in most of the pathological research laboratories which limits the usage of the already developed QPCI instruments that are available commercially [17,18].

In order to circumvent these issues, the concept of in-line/common-path configurations were explored by many research groups [2,4,6,7,9–11,13,14,19–21]. In the in-line/common-path configuration, the two interfering beams are generated after the light beam has passed through the sample which then follows almost the same optical path while encountering the same set of optical components. Since both object and reference beams are derived from the same wavefront, the experimental configuration becomes much more immune to mechanical vibrations and in some cases eliminates the need of positioning the system on a vibration isolation optical table. Another advantage of this approach is that the beam ratios as well as the path lengths of the two beams are almost the same, thus inherently generating high-contrast interference fringes. However, it has been observed that almost all the existing in-line/common-path configurations require specialized optical elements (e.g. modified Hartmann mask [1], diffraction grating/grating-pair [14],

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https://doi.org/10.1016/j.optcom.2018.01.014
Received 20 July 2017; Received in revised form 1 November 2017; Accepted 5 January 2018
Available online 30 January 2018
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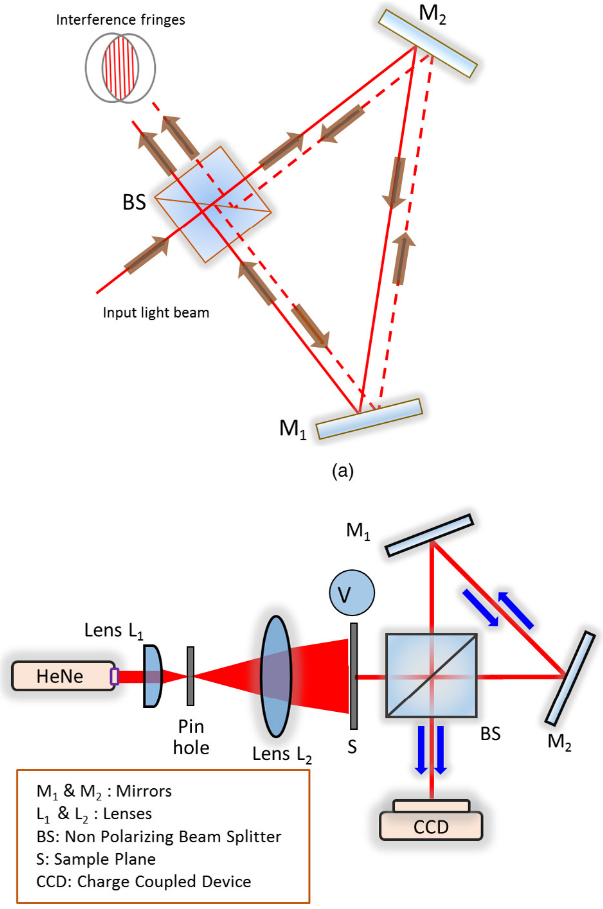

(a)

(b)

**Fig. 1.** (a) Schematic of a cyclic lateral shearing interferometer. The arrows show the central ray paths. (b) Schematic of the experimental setup for QPCI using a cyclic interferometer. In the sample plane, the object is placed at one-half of the optical-field-of-view (FOV). The interferometer section creates two laterally shifted and collinearly propagating non-collimating wavefronts for interference at the CCD plane. CCD: Charge-Coupled Device.

a second MO [2], spatial light modulators [2,3,13], customized reflective surfaces [5,7], wedge-plate [6], polarization optics [8], pinhole [9,10], retro-reflector [11] etc.) to convert one of the split beams into a reference beam. Similarly, in Ref. [20], Lue et al. has established a quantitative dispersion phase microscopy technique by using a quasi-common path approach in which the sample plane is partitioned into two halves. The extent of the sample is limited to one half and the beam passing through the other half is used as the reference beam. Two specially designed mirrors with independent tip-tilt controls are used to form an interference pattern at the image plane. Common-path interferometers are also used in other applications such as the recording of incoherent-object hologram as complex spatial coherence function [21].

In this paper, we propose a rather simplified approach for realizing digital holographic QPCI devoid of any specialized optical components. We implement the concept of a single-pass cyclic lateral shearing interferometer (CLSI) in which the spherical beam size is much larger than the sample dimension, in order to simultaneously possess regions with and without sample information [22]. This light field is split into two that follow practically the same optical path but in opposite directions. CLSI is originally developed for testing of optical surfaces and beam collimation [23–25]. A schematic of this interferometer is shown in Fig. 1(a) which involves basic optical elements such as beam splitter and mirrors. CLSI is much more stable to mechanical vibrations than other multi-optic interferometers such as Mach–Zehnder or Michelson interferometer [3–5,16]. Moreover, as the two counter propagating beam paths are nearly identical, CLSI could also be easily implemented with low coherence sources. CLSI further has the ability to introduce variable amounts of shear between the two interfering beams in a rather flexible way such as simple rotation or translation of the associated mirrors and beam splitter [22]. Another important point to note is that in our CLSI based QPCI, we have used non-collimated beam in a lens-less configuration to record the interferogram as opposed to many previous QPCI architectures wherein collimated light beams are used [2,5,10,11]. This makes the practical realization of the experimental configuration more comfortable and compact thus improving the portability issues. Since, no imaging lens is used after the sample, hence the possibility of introducing aberrations due to redundant optical components can be avoided. It is worth mentioning here that the optical layout of a cyclic interferometer and a Sagnac interferometer are very identical. However, we make use of lateral shearing of two counter propagating beams in the triangular cyclic interferometer in contrast to conventional common path Sagnac interferometer.

## 2. Methodology

Fig. 1(b) shows the schematic of the proposed configuration for performing QPCI with CLSI [22]. Light beam from a coherent source (He–Ne, $\lambda = 632.8$ nm) is spatial filtered and magnified with the help of the pinhole and lenses $L_1$ & $L_2$. The sample under investigation is illuminated by a divergent wavefront. In CLSI, the spherical beam, comprised of with and without sample regions, is initially split into two using a non-polarizing cube beam splitter (BS). The two mirrors ($M_1$ and $M_2$) are then adjusted in such a way that the two split beams are collinearly propagating as it travels towards the camera plane as well as they are laterally shifted with respect to each other. In our configuration, the lateral shear is realized in the horizontal direction. However, it is possible to implement the lateral shear in any other orientation on the transverse plane by suitably adjusting the mirrors of the cyclic interferometer. Note that the lens $L_2$ in general is used to project a collimated optical beam onto the sample and CCD (charge-coupled device) sensor through the intervening interferometer [16]. However, here, we have realized a more generalized architecture wherein divergent wavefronts that differ from the collimated arrangement are used. Additionally, due to the cyclic interferometer configuration, the two interfering wavefronts at the CCD sensor plane has nearly identical curvatures and intensity. Thus, the CCD sensor records the interference of two collinearly propagating laterally shifted divergent wavefronts as depicted in Fig. 2. In addition, as both the split beams contain the sample information, further adjustment is needed such that an area of the sample that contains no object information is superimposed with the image of the specimen.

The two beams interfere with each other at the sensor plane to create an interferogram which is then digitally captured by CCD sensor (pixel resolution: 1040×1392, pixel size: 6.45 μm). This gray scale hologram can be represented as:

$$I = \left| R_1(x,y) + O_1(x,y) \right|^2. \tag{1}$$

Where $R_1(x,y)$ is wavefront of the reference wave and $O_1(x,y)$ is that of the object wave. $x$ and $y$ are transverse spatial coordinates at the CCD sensor plane. The reference wave is a laterally shifted copy of the light beam that has passed through the sample but does not contain any sample information. Thus for numerical analysis purpose, the reference wave can be treated as a divergent spherical wave emanating from a point source $(x_{p0}, y_{p0})$ such that

$$R_1(x,y) = \left| R_1(x,y) \right| \exp\left( ikr_{R1} \right). \tag{2}$$

Where $k$ is the wave number and $r_{R1} = \sqrt{(x - x_{p0})^2 + (y - y_{p0})^2 + Z^2}$ and $Z$ is the longitudinal distance of the sensor plane from the point reference source.