Off-axis digital holographic camera for quantitative phase microscopy

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Abstract: We propose and experimentally demonstrate a digital holographic camera which can be attached to the camera port of a conventional microscope for obtaining digital holograms in a self-reference configuration, under short coherence illumination and in a single shot. A thick holographic grating filters the beam containing the sample information in two dimensions through diffraction. The filtered beam creates the reference arm of the interferometer. The spatial filtering method, based on the high angular selectivity of the thick grating, reduces the alignment sensitivity to angular displacements compared with pinhole based Fourier filtering. The addition of a thin holographic grating alters the coherence plane tilt introduced by the thick grating so as to create high-visibility interference over the entire field of view. The acquired full-field off-axis holograms are processed to retrieve the amplitude and phase information of the sample. The system produces phase images of cheek cells qualitatively similar to phase images extracted with a standard commercial DHM.

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OCIS codes: (050.1950) Diffraction gratings; (050.7330) Volume gratings; (330.6110) Spatial filtering; (090.1995) Digital holography; (110.0180) Microscopy; (180.3170) Interference microscopy.

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Digital holographic microscopy (DHM) is a quantitative optical imaging technique that is able to capture the complex wavefront (amplitude and phase) of the light interacting with a sample [1–3]. Capturing the wavefront is performed by recording the spatial interference pattern of the beam that interacts with the sample (i.e. object beam) and a mutually coherent reference beam using a digital camera. With a highly coherent source, the interference can be easily obtained, with the drawback of reduced image quality due to parasitic interference and coherent noise. Parasitic interferences are significantly reduced by using short coherence light sources [4–6]. On-axis digital holographic microscopy works with broadband light, hence parasitic interference is reduced but requires acquiring multiple interferograms which limits the ability to perform real-time imaging and adds complexity to the system. Conversely, off-axis interferometry provides an opportunity for dynamical quantitative phase measurement because only one digital hologram is sufficient to recover phase and amplitude [5–7]. However, under broadband illumination and in order to be able to exploit the whole field of view of the camera in off-axis geometry, the coherence planes of the two beams must be parallel. We and others have shown that coherence plane manipulation on the reference beam allows for full field imaging in off-axis geometry [5,6].

Instead of generating interferograms using an isolated reference beam from object beam, through separate optical paths, the reference beam can be generated by illuminating the sample in the first place and applying low-pass filtering on the signal beam containing the sample information [8,9]. A major advantage of this configuration is that such a system can be attached to the output of a conventional microscope (image plane) and generate interferograms of the sample from which the phase and amplitude information can be extracted. In [8], spatial filtering has been done using an optical pinhole. Pinhole spatial filter uses the principles of Fourier optics to alter the spatial structure of the beam. However, the requirement that the reference beam be passed through a pinhole, makes the alignment a non-trivial task, especially for a non-specialist in microscopy. If the beam illuminating the sample is angularly deviated, it misses the pinhole and no reference is generated hence not interferogram is obtained. In [9] pinhole spatial filtering has been applied by use of a spatial light modulator (SLM). An amplitude diffraction grating is placed at the image plane of a microscope. The zeroth order beam is spatially filtered using an SLM placed in the Fourier plane of a 4f imaging system, to generate the reference beam. The first diffracted order is transmitted through a rectangular opening mask on the SLM to generate the object beam. Both beams are interfered and the interference is captured by a CCD camera at the image plane.

A filtering approach which is applied directly on the beam propagation angle would alleviate the problems related to pinhole alignment. Holographic spatial filtering [10,11], operates with an unfocused beam and relies on high angular selectivity of thick holographic gratings to remove spatial frequencies. Therefore, it can be used as a replacement for pinhole to produce a reference beam from a sample beam. Since the angle of the Bragg diffracted
beam is dependent on wavelength, one can create an element which generates a spatially filtered reference beam that changes wavelength when an angular deviation occurs (e.g. in the case of a wedged sample holder). Hence a spatial filtering system based on Bragg diffraction should be less susceptible to misalignment than a pinhole-based system.

The thick holographic grating introduces a large coherence plane tilt in the reference beam. This tilt must be compensated in such a way that reference beam coherence plane is coplanar with the one of the object beam while interfering on the camera in off-axis configuration. This can be achieved using a thin holographic grating as an angular dispersive element to introduce a tilt in the other direction and compensate the unwanted tilt in the reference beam coherence plane. In [5] we have introduced a method based on a holographic grating recorded on a photopolymer (BAYFOL HX) from Bayer MaterialScience AG, to correct the coherence tilt in off-axis DHM.

The paper is organized as follows: section 2 describes holographic spatial filtering in two dimensions using thick holographic gratings. Section 3 introduces the holographic camera system to obtain phase and amplitude information. In section 4, single-shot phase and amplitude measurements are performed on human cheek cells using the proposed setup. Results are compared with the measurements obtained from a commercial DHM in transmission configuration.

2. Holographic spatial filtering

The holographic spatial filter used throughout the experiment is a phase grating recorded on a thick photosensitive glass [12–14]. The grating is fabricated by interfering two coherent beams from a HeCd laser on a 3 mm thick glass. Subsequent heat treatment is performed to reveal and fix the grating.

Bragg selectivity \( \Delta \theta_{\text{FWHM}} \) of a transmission grating is defined as the angular deviation from the Bragg condition for which the diffraction efficiency is reduced by half [15].

\[
\Delta \theta_{\text{FWHM}} = \frac{\Lambda}{d}.
\]

where \( d \) is the thickness and \( \Lambda \) the period of the grating. From Eq. (1), the angular selectivity of a grating is inversely proportional to its thickness, therefore a very high angular selectivity can be achieved using thick gratings. For example with a grating with 0.9 \( \mu \)m period recorded on a 3 mm thick glass, an angular selectivity of 0.3 milliradian is achievable. This high angular selectivity enables the thick grating to filter out the spatial frequencies above this value in the incident beam and generate a cleaned-up beam. Experimental results are presented in the next section. The thick grating used in these experiments is recorded on a 3 mm glass (0.9 \( \mu \)m grating period) in our lab using two-beam interference.

2.1 Filtering in one and two dimensions using thick holographic gratings

A single thick grating spatially cleans the beam in one dimension, owing to its high angular selectivity in one plane (plane formed by the grating vector and the incident beam). Filtering in one dimension is demonstrated using a USAF target in the setup illustrated in Fig. 1. The USAF target is illuminated by 633 nm light having 10 nm bandwidth. The beam is then diffracted by a thick grating and imaged on a CCD camera (1392x1040 px, 6.45x6.45 \( \mu \)m from Baumer).

#207189 - $15.00 USD Received 26 Feb 2014; revised 24 Apr 2014; accepted 27 Apr 2014; published 1 May 2014 (C) 2014 OSA 1 June 2014 Vol. 5, No. 6 | DOI:10.1364/BOE.5.001721 | BIOMEDICAL OPTICS EXPRESS 1723
The intensity image of the USAF target and its Fourier transform are shown in Figs. 2(a) and 2(b). Figure 2(c) and 2(d) show the intensity image of the USAF target after diffraction by thick grating and its Fourier transform. One-dimensional filtering effect is shown in the intensity image (blurred edges in one direction) and in the Fourier domain (removed spatial frequencies in one direction).

To obtain a clean reference beam, the signal beam needs to be filtered in two dimensions. Filtering in two dimensions with phase gratings was demonstrated by Ludman et al. [16]. He showed that two-dimensional spatial filtering is possible using two thick gratings with grating vectors perpendicular to each other, carefully oriented with respect to each other, so that the Bragg effect from the first grating cleans up one direction and then the second grating cleans up the orthogonal direction, as illustrated in Fig. 3.
However, there are practical difficulties with this configuration including sensitivity to the mutual alignment of the gratings and a clean-up beam which is inconveniently not in the same horizontal plane as the incident beam on the first grating.

A much simpler design using a single thick grating was shown in [17]. The holographic clean-up system is illustrated in Fig. 4. It is comprised of a single thick grating and a right angle prism mirror as a retro-reflecting element placed in the path of the diffracted order of the grating. In Fig. 4, the beam transmitted through the beam splitter BS is diffracted by the grating. After the first pass through the grating, the beam is cleaned-up in only one direction. The prism mirror is placed after the grating and rotated by 45° in order to rotate the beam by 90° and send it back to the grating. The second pass through the grating cleans the second direction of the beam.

Figure 5(a) shows the intensity image of the unfiltered beam whose spatial frequencies are shown in the Fourier transform image [Fig. 5(b)]. The intensity image of the beam after double passing through the thick grating and the corresponding Fourier transform presented in Fig. 5(c) and 5(d), respectively.

The bright diagonal line in the intensity image shown in Fig. 5(c) is due to the diffraction loss occurring in the corner of the prism mirror.
3. Experimental setup

To demonstrate the operation of our proposed digital holographic camera, we have constructed the setup illustrated in Fig. 6. The sample is illuminated by a supercontinuum fiber laser source (SC400-6, Fianium) filtered at 633 nm with 10 nm bandwidth, to generate a beam containing the sample information. The sample beam is then collected by a 20x, 0.4 numerical aperture, infinity-corrected microscope objective (Olympus plan achromat). The tube lens (effective focal length of 200 mm) then produces an intermediate image plane. The beam splitter splits the sample beam in two arms. The beam reflected by the beam splitter, which is a replica of the sample beam, serves as the object beam. The reference beam is optically processed by low-pass filtering the object beam in two dimensions using the thick grating/retro reflector arrangement described in section 2.1.

The thick grating introduces a large coherence plane tilt in the reference beam in both dimensions. This means that the coherence plane after the second path through the thick grating makes an inconvenient angle with the coherence plane of the object beam and requires an out of plane propagation of the object beam to achieve the full field interference of the beams on the CCD. To solve this problem, the thick grating is rotated in such a way that the diffracted beam propagates out of plane with an angle equal to the coherence plane tilt introduced in the beam after the first path through the grating. In the fabricated thick grating, this coherence plane tilt is equal to 45°. The retro-reflecting prism mirror placed after the thick grating is rotated by 45° for two purposes: 1) rotating the beam by 90° and sending it back to the thick grating for the second direction filtering, 2) rotating the prism with an angle equal to the coherence plane tilt, results in a coherence plane which is effectively tilted by 45° only in one direction after the second path through the thick grating.

A second grating, thin this time, is placed in the reference path to compensate a large portion of the 45° tilt angle and leave out a residual 2.5° tilt in the reference beam coherence.
plane, which enables full field off-axis interference with the object beam (making an angle of 2.5° with the reference). The object beam is only transmitted through the thin grating. Thus the function of thin grating is to correct the coherence plane tilt of the reference beam and recombine the object and reference in an off-axis geometry. Two conditions have to be satisfied to have interference in short coherence: 1) equal optical path in both arms of the interferometer, 2) spatial coherence. To satisfy the first condition, a delay is introduced by a right angle prism mirror in the object arm mounted on a linear translation stage. For the second condition to be satisfied, a dove prism is introduced in the object beam and rotated by 45°. The dove prism flips the beam orientation and rotates it by 45° which results in rotation of the beam by 90°. This introduces the same rotations in the object beam as introduced by the retro-reflecting prism mirror in the reference arm and insures that the corresponding regions of the reference and object beams interfere with each other and thus preserving spatial coherence. This allows to use any low spatial coherence light source (e.g., LED, halogen lamp) as the illumination source.

L1 and L2 are achromatic lenses. While L2 images the intermediate image on the CCD, L1 is placed in the path of the reference beam to compensate for the wavefront curvature.

The generated reference and object beams finally interfere over the whole area of the CCD in an off-axis configuration.

4. Experimental results

4.1 Phase image of human cheek cells

To demonstrate quantitative phase imaging using the proposed digital holographic camera system, we used human cheek cells (~50 µm) on a microscope slide as sample. Figure 7(a) and 7(b) show the recorded hologram and reconstructed phase in the proposed system respectively. Figure 7(c) and 7(d) show the recorded hologram and the reconstructed phase of the same sample measured by traditional DHM in transmission.
Fig. 7. 50 µm human cheek cells: (a) hologram, (b) reconstructed phase, measured in our setup, (c) hologram, (d) reconstructed phase, measured in a commercial DHM.

Figure 8(a) displays the quantitative phase along the line shown in Fig. 8(b), measured in proposed setup in comparison with measurement in a traditional DHM (DHM-T1003 from Lyncée Tec). Figure 7 and Fig. 8 show that the morphology and reconstructed phase are well in agreement, considering the fact that the measurements setups (traditional DHM and the proposed setup) were in different locations.
4.2 Angular deviation sensitivity

An attractive feature of the setup depicted in Fig. 6 is its relaxed alignment sensitivity to angular displacement of the signal beam. With this system, we show that obtaining interference with a short coherence source is significantly less sensitive to misalignments compared to conventional systems employing pinholes. To demonstrate this, we placed a 2° wedge prism before the sample to introduce an angular deviation in the beam illuminating the sample. This angular deviation is translated into approximately 300 µm displacement of the
focal point in Fourier plane. This displacement is large enough to miss a hypothetical pinhole which would be placed in the Fourier plane of a lens. However, since in our system the wavelength band diffracted depends on angle, an angle change in the beam illuminating the grating (0.09° in this case) corresponds to a different wavelength band diffracted. Thus the reference beam is slightly shifted in wavelength. Therefore, we are still able to generate a reference beam and extract the sample phase with our proposed setup. Figure 9(a) shows the phase of cheek cells measured when the sample illumination is parallel to the microscope optical axis. Figure 9(b) shows the measured phase when the beam incident on the sample is deviated by 2°.

Fig. 9. Phase reconstruction of cheek cells: (a) measured with aligned illumination (b) measured with 2° misalignment.

5. Conclusion

We have developed a digital holographic camera system which can potentially be retrofitted to the camera port of any standard microscope to obtain off-axis holograms of the sample over the entire field of view, under low coherence illumination. The proposed system splits and merges the reference and object beams after the output of the microscope, while using spatial filtering to erase the information from one of the beams before it merges with the other beam. We employed holographic spatial filtering based on thick gratings which replaces conventional spatial filtering based on pinholes with the advantage of less sensitivity to the angular deviation resulting from misalignment in the incident beam on the sample. The phase image measurements on human cheek cells prove that it is possible to obtain results comparable to those obtained with a commercially available, stand-alone digital holographic microscope.

Acknowledgments

The authors would like to acknowledge the support from CTI (Commission for Technology and Innovation) 13366.1 PFFLE-NM in Switzerland.