Low-coherence off-axis digital holographic microscopy

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Usually, off-axis digital holographic microscopy requires a coherent light source in order to record a full-field hologram. Nevertheless, a LASER-based illumination leads to a non-negligible coherent noise, decreasing then the imaging quality. We hereby report a simple method to reduce the coherent noise contribution using a low-temporal-coherence illumination while maintaining a large interference area. A diffraction grating is hence introduced in the reference arm of the interferometer, allowing the coherence plane of the reference beam to be tilted following angular dispersion. The phase planes of the reference beam and the object beam appears to be coplanar. The principle and performance of low-coherence off-axis digital holographic microscopy are demonstrated. The three-dimensional reconstruction of a biological sample is performed.

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The layout of the off-axis DHM setup is shown in Fig. 1 and consists of a super-luminescent diode having a central wavelength $\lambda_0$ of 680 nm and a bandwidth $\Delta\lambda$ of 8 nm (SLD-26-HP, Superlum Diode). The incident beam is first spatially filtered before being divided in two by a beam-splitter cube. The reference beam $R$ of the interferometer is transmitted and passes through a diffraction grating (46-067, Edmund Optics) having a groove density of 70 lines/mm. According to angular dispersion, the coherence plane is tilted by an angle $\Phi(\lambda_0)$ of 2.7° with respect to the propagating vector [35]. Only the $+1$-order diffracted beam from the grating is required in the imaging system. Other order beams are removed using an obstruction which is not represented in Fig. 1. The beam reflected by the mirror $M$, i.e., the object beam $O$, is oriented by a mirror in order to illuminate the specimen to be measured. The microscope objective ($\times5$, NA = 0.17) collects the beam scattered by the sample and the tube lens forms the image on the CCD camera (TXG50, Baumer). The second beam-splitter cube allows to superimpose the reference beam $R$ and the object beam $O$ on the camera in the off-axis mode. Therefore, the mirror in the reference arm is able to adjust the incident angle $\theta$ between the two beams. The interferogram distribution is then recorded by the camera and, finally, an algorithm processes the full-field hologram and performs a numerical wavefront propagation using 2D Fast Fourier Transform (FFT) operators [3]. This numerical operation makes allows to retrieve the phase distribution of the sample.

Without the diffraction element in the reference arm, the width of interference area $L$ is limited by the coherence length of the light source $l_0$, and the angle $\theta$ between the two beams ($L = l_0 / \sin(\theta)$). Assuming a Gaussian-spectrum light illumination, the number of interference fringes $N$, having an interference contrast superior to 50%, can be expressed as [43]:

$$N = \frac{2 \ln(2) \lambda_0}{\pi \Delta\lambda} \frac{\sin(\theta)}{\sin(\Phi(\lambda) - \theta)} \tag{2}$$

Figure 2(c) shows the evolution of the number of fringes $N$ from Eq. 2 (blue dashed plot) as a function of the incident angle $\theta$. When $\theta$ equals $\Phi(\lambda_0)$, an infinity number of fringes should be recorded by the camera. However, in experiment (red solid plot), the 5-mm-size sensor limits the lateral field of view, yielding a linear evolution of $N$ between 2.2° and 3.2° (green area). For the measurements, the angle $\theta$ was implemented by rotating the mirror in the reference arm around the optical axis and the DHM system was free of object. Note that the incident angle $\theta$ depends also on the wavelength of light due to the bandwidth of the light source. This involves adjusting the grating-camera distance in order to record all the wavelength-dependent diffracted intensity profiles are plotted according to the white doted lines. (c) Evolution of both the calculated (in blue) and the measured (in red) number of fringes $N$ as a function of the angle $\theta$. Within the green area, $L$ is wider than the sensor size.
beams, i.e. the contributions from each spectral component. Not considering this chromatic effect could lead to a fringe wash-out and further a loss of the interference contrast. In addition, at the ideal angular position, the angle $\theta(\lambda)$ equals $\Phi(\lambda)$ regardless the wavelength.

Performance of the low-coherence interference imaging system was evaluated. Figure 3(a) shows the hologram of a 1951 USAF target, allowing the lateral resolution to be determined. It results in 2.19 $\mu$m of resolution limit (Element 6, Group 7). This allows to highlight that the diffraction grating has not impact on the imaging quality and the DHM system is thus assumed diffraction limited (the cut-off frequency of the optical transfer function of an aberration-free imaging system equals 2.00 $\mu$m [19]). Note that the resolving power of the imaging system would be around 4 $\mu$m using a 680-nm-wavelength monochromatic light source. Furthermore, a quantitative analysis of the phase deviation has been measured (Fig. 3(b)) through the re-

Fig. 3. Performance of low-coherence off-axis DHM system. (a) Hologram of a 1951 USAF target. The DHM system can resolve Element 6, Group 7. (b) Measurement of the spatial phase deviation using a flat mirror. The RMS of the phase deviation is 32 mrad within blue area, 30 mrad within black area and 27 mrad within green and red areas. Each square area consists of 100 $\times$ 100 pixels. $\lambda_0 = 680$ nm, NA = 0.17.

phased deviation measurement of the fixed mouse neuron recorded by the camera. The quan-
tative phase distribution was then calculated, enabling the morphology of the brain cell to be reconstructed (Fig. 4(b)). Assuming a mean refractive index of 1.375 along the propagation axis inside the mouse brain cell [7, 45], the height of at the center of the neuronal cell body (blue arrow in Fig. 4(a)), i.e. the soma, equals 12.9 $\mu$m. And, the height of the dendrites (green arrow in Fig. 4(a)) is around 3.8 $\mu$m.

This work presents the development of a low-coherence off-axis digital holographic microscope. Illuminated by a broadband light source, the parasitic coherent noise contributions which originate from multiple reflections between the optical component interfaces, are reduced. Despite, this type of illumination narrows the hologram width in off-axis configuration, a diffraction grating has been introduced in the reference arm of the transmissive-configuration interferometer in order to make parallel the reference and the object wave-packets. This method allows the hologram to cover the camera and, further, the standard deviation of the phase reconstruction to be lower than 30 mrad. The imaging technique has been applied for the reconstruction of a neuron cell. It can be mentioned that this method can be implemented in a reflective configuration.

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Fig. 4. 3D reconstruction of a mouse’s neuron using low-coherence off-axis DHM. (a) Width-field hologram. (b) reconstruction of the morphology. The soma, the dendrites and the axons of the neuron are recognized with the blue, the green and the red arrows, respectively. $\lambda_0 = 680$ nm, NA = 0.17. White scale bar represents 100 $\mu$m.
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