Investigation of angular multiplexing and de-multiplexing of digital holograms recorded in microscope configuration

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Abstract: We investigated a method for the angular multiplexing and de-multiplexing of digital holograms recorded in microscope off-axis configuration. The multiplexing has been performed rotating numerically one hologram at different angles and adding all the rotated holograms to obtain a single synthetic digital hologram. Then the digital holograms were de-multiplexed thanks to the unique property of the digital holography to manage numerically the complex wavefields at different image planes. We show that it is possible to retrieve correctly quantitative information about the amplitude and phase maps. The obtained results can be useful to employ the multiplexing technique during the recording process by rotating the CCD array.

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1. Introduction

Digital Holograms can be multiplexed by encoding the information of two or more holograms in a single one. The multiplexing operation can be carried out either by optical or numerical techniques. Numerical Multiplexing (NM) is achieved by combining numerically digital holograms to obtain a single synthetic hologram, while, in Optical Multiplexing (OM), more than one digital hologram is recorded at the same time, i.e. in a single shot, by the array detector. OM of digital holograms has been implemented with the aim to measure various object properties, i.e. birefringence and three-dimensional mechanical deformations [1,2].

In the configuration proposed in ref. 1 two reference beams with orthogonal polarization states in combination with one object beam have been used. Instead in ref. 2 the spatial multiplexing is achieved by an incoherent mixing of two duplets of coherent waves that produce holograms carried with orthogonal polarized reference waves. The multiplexing technique for digital holograms is developed in order to obtain the simultaneous determination of the in-plane and the out-of-plane components of the displacement vector of an object submitted to some mechanical loading.

Multiplexing technique of digital holograms are also focussed on investigation of ultrafast events. In ref. 3 an holographic recording of nanosecond events on a conventional CCD camera was accomplished acquiring multiple pulsed holograms on a single CCD frame simultaneously. In ref. 4 an example of spatial angular multiplexing for recording ultrafast processes of the femtosecond order is proposed. Moreover, a measurement of the full spatio-temporal electric field of a femtosecond laser pulse on a single shot is obtained by wavelength multiplexed DH. The recorded digital hologram contains multiple spectrally resolved smaller holograms, each of one characterizes the spatial intensity and phase distributions of an individual frequency component of the pulse [5].

Multiplexing of digital holograms plays an important role in 3D imaging in full colours through multi-wavelength recording [6]. Besides, multiplexing has been used in multi-wavelengths DH to desensitize the interferometric gauge by means of an higher synthetic wavelength obtained by the beating of two wavelengths. [7,8,9]. Recently, optical angular multiplexing has been obtained using a single laser pulse divided into two trains of sub-pulses. Spatial multiplexing can be applied to both the reference beam for different carrier frequency and the object beam for off-axis and on-axis illuminations with the aim to get super-resolution [10]. Moreover, spatial multiplexing is a key element in DH experiments in which a diffraction grating is introduced into the set-up to get super-resolved images increasing synthetically the numerical aperture of the image sensor [11, 12].

Finally, efficient storage and/or transmission of a large number of digital holograms carrying 3D information in amplitude and phase about a live cell has been recently demonstrated by NM method [13].
As described above, the multiplexing of digital holograms has important implications in many fields of science and technology that stimulate deep investigation to develop new methods or to improve existing approaches.

Actually, in the most of the above mentioned approaches, spatial multiplexing of digital holograms is obtained optically, that means recording simultaneously more than one fringe pattern on the same sensor array. Depending on the different experiments, one or more reference beams with different angles are used to obtain interference fringe patterns with different spatial frequencies and oriented along different directions. All the holograms are superimposed in one composite CCD frame, and each of them can be independently reconstructed after the digital spatial filtering in the Fourier spectral domain of the multiplexed hologram. Each individual hologram is filtered out efficiently only if its bandwidth is sufficiently low and clearly separated by the others. The filtering operation is performed by selecting a pass-band for each of the multiplexed holograms in Fourier spectral domain. For each of the multiplexed signals a separate digital hologram is obtained by the inverse Fourier transform on the filtered spectrum. Obviously, the extension of the hologram spectrum in the Fourier plane determines how many digital hologram can be efficiently multiplexed. By the described techniques, up to three digital holograms have been multiplexed without decreasing the spatial resolution, that is using all the aperture of the CCD array [1,3,4,6].

On the other hand, the bandwidth of each single hologram depends on the optical configuration adopted for its recording. For hologram acquired in microscope configuration, usually named “image plane holograms”, the space-bandwidth product (SBP) of the hologram is inversely proportional to the focal length of the objective lens [14]. Therefore, if lenses with short focal length are used to obtain a large magnification, the hologram SBP is such that not more than two holograms can be separated in the Fourier plane and, therefore, only two holograms can be multiplexed.

Here we present a different approach to encode information from various digital holograms acquired in microscope configuration in a multiplexed one, through the angular rotation of the digital holograms around their optical axis. We investigate this novel approach by synthetically combining together several digital holograms, i.e. by NM method. In fact, each hologram is rotated numerically. Then, the combination is performed by the simple summation of the numerically rotated digital holograms. The multiplexing and demultiplexing operations and the obtained results are described and discussed in order to estimate the distortions introduced on the reconstructed amplitude and phase of each hologram. It is important to note that, alternatively, the same multiplexing method can be obtained through the rotation of the object as well as of the detector (i.e. CCD array) around its centre in the x-y plane, that is in a plane parallel to the hologram plane. In this case, different rotated digital holograms, sequentially recorded, can be multiplexed in synthetic digital hologram.

2. Multiplexing digital holograms by numerical angular rotation

By the proposed method we multiplex numerically up to five digital holograms. To demonstrate the feasibility of this technique, we acquire a single hologram by a Mach-Zehnder interferometer in microscope configuration. Then we create its five rotated replicas turning it around its centre by a fixed angle $\alpha=30^\circ$. The original hologram is recorded at laser wavelength of 532 nm, using a 20 X microscope objective having a focal length $f=9.0$ mm and numerical aperture NA=0.40. The CCD detector is made of 1024 X 1024 square pixel of 6.7 $\mu$m size. The specimen used is a particular type of MEMS (micro-electromechanical system).

Each hologram is padded with zeros [15] up to 1400 X 1400 square pixels to allow the whole five rotated holograms to be contained in the multiplexed hologram. Obviously the dimension of the padding window depends on the chosen angle of rotation. Then we add the five holograms in order to multiplexed them in a single synthetic hologram shown in Fig. 1.
Moreover, a reference hologram is acquired in a region of the sample far from the MEMS in order to calculate the phase retardation caused only by the MEMS subtracting the phase shift due to the interferometer. The reference hologram is multiplexed in the same way of the MEMS hologram. The multiplexing of the reference hologram is needed to avoid distortion effects in the reconstructed phase maps. In fact, the rotation of the hologram is obtained through a cubic interpolation algorithm that introduces some distorting effects. In order to avoid that these distortions affect the retrieved phase map, both the MEMS holograms and references holograms have to be processed in the same way. Therefore the two holograms experience the same distortion that is eliminated when the phase map is retrieved by calculating the difference between the two holograms.

The image of the MEMS obtained by reconstructing the amplitude from the multiplexed hologram in the image plane, obtained through Fresnel–Kirchoff integral, is shown in Fig. 2. The image corresponding to the five holograms are partially overlapped and therefore cannot be separated. The inset in Fig. 2 shows the Fourier transform amplitude of the multiplexed hologram. It is clear that the holograms cannot be separated in Fourier plane, since the holograms Fourier transform are superimposed because of their size.
3. De-Multiplexing digital holograms

In order to de-multiplex the five holograms, we exploit the digital holography property to manage numerically the complex wavefields at different image planes. In fact, we reconstruct the multiplexed hologram in the back focal plane (BFP) of the imaging lens.

The complex wave field in the BFP is proportional to the Fourier transform of the complex amplitude of the wave at an input plane of the imaging lens, therefore it corresponds to the spectrum of the object wave. It is centred around a carrier frequency that depends on some geometrical parameters of the experimental setup such as the angle between the reference and the object beams and on the reconstruction distance d. In Fig. 3 is shown the amplitude reconstruction of the multiplexed hologram in the BFP, at the distance \(d_r=400\) mm from the hologram plane.

![Figure 3](image_url)

Then, we choose a mask to filter each hologram in the BFP. The red frame in Fig. 3 indicates the shape and the dimension of the used filtering window, that is a circular window with a radius of 100 pixels, centred around the carrier frequency of the object spectrum. The transmittance of the mask is 1 within the frame and 0 outside. The dimension of the filtering windows is chosen with the aim to include the most of the useful spectrum of the object.

To extract all the five holograms, we rotate the mask with step of \(\alpha\), maintaining as pivot the centre of the reconstruction plane (as shown in Fig. 3). Then, each single wavefield is filtered and numerically back-propagated in the hologram plane.

At this point, we can proceed in two different ways. In the first case, the complex wavefields are rotated in the hologram plane by steps of \(-\alpha\) by means of the same interpolation routine used in the multiplexing process. Finally these filtered holograms are reconstructed in the image plane. In the second case, the complex wavefields are firstly reconstructed in the image plane and, then, the in focus images are rotated.

3.1 De-multiplexing by rotation in the hologram plane

Figure 4 shows the amplitude reconstruction for the hologram rotated by \(\alpha=30^\circ\) using the first de-multiplexing technique. Several replicas of the reconstructed image appear. They are caused by the interpolation routine used to rotate the filtered holograms.
To filter out this replicas, the complex wavefield is propagated again in the lens BFP where their overlapping is minimum (see Fig. 5). Then, the same mask employed in the de-multiplexing process is applied to filter the replicas in the complex wavefield that then is propagated back to the hologram plane and finally in the image plane. In this way we minimized the distortion caused by the interpolation routine.

It is worth to underline that, when \( \alpha \) is equal to 0° and 90°, the rotation become a simple permutation. Therefore no replicas are created by the rotating algorithm.

After the filtering of the replicas, the complex wavefield is propagated back to the hologram plane and then in the image plane to make the pixel of reconstruction in the image plane independent from the distance between the BFP and the image plane [14].

The final amplitude and phase reconstructions are shown in Figs. 6(a) and 6(b) for the hologram rotated by \( \alpha=30^\circ \). To obtain the phase reconstruction, the phase of the reference hologram rotated by the same angle has been subtracted.
We can compare the reconstructions obtained by the proposed technique to those one coming from the original hologram, showed in Fig. 7.

The noise present in the reconstructions of the de-multiplexed hologram (see Fig. 6) is caused by the overlapping of the objects spectra (see Fig. 3). Obviously, the smaller angle $\alpha$ is, that is the higher the number of multiplexed holograms is, the larger is the overlapping area between two close reconstructions in the BFP. Moreover, for a fixed $\alpha$, to decrease the effects of the overlapping we can diminish the radius of filtering window, but, in this case, also the spectral information of the filtered hologram and, therefore, the quality of the final reconstructed image are reduced.

Therefore, according to the shape of the holograms spectra, we have to find the right arrangement between the number of multiplexed holograms and the acceptable noise in the amplitude and phase reconstructions.

Finally, we show the reconstructions obtained using the second de-multiplexing technique, that is reconstructing firstly the complex wavefields in the image plane and, then, rotating the achieved in focus images.

3.1 De-multiplexing by rotation in the image plane

In Fig. 8 the amplitude and phase reconstructions of one filtered hologram de-multiplexed by rotation in the image plane are shown.
These reconstructions come out noisier than those obtained by the first de-multiplexing method. In fact, a sort of grid, caused by the interpolation routine, is present both in the amplitude and in phase reconstruction (this grid is well visible in the areas indicated by the arrows in Fig. 8).

![Fig. 8. Final amplitude and phase reconstructions of one filtered hologram after the de-multiplexing process by rotation in the image plane.](image)

To make evident the noise introduced by the second de-multiplexing technique, in Fig. 9 are plotted the wrapped phase profiles along the black lines of Figs. 6(b) and 8(b), respectively. The profile regarding the phase map retrieved by means of the second demultiplexing technique is clearly noisier than the other. However, this de-multiplexing method is faster than the first one that needs to propagate and filter twice the complex wavefield in the BFP.

![Fig. 9. Profiles concerning the phase reconstructions obtained by the first (a) and the second (b) de-multiplexing technique, respectively.](image)

### 4. Multiplexing by rotating the CCD and comparison with the numerical method

As discussed above, the proposed technique can be employed to multiplex digital holograms during the recording process by rotating the CCD array in five different angular positions. The acquired holograms are then multiplexed in single synthetic hologram. We show here the results obtained by means of this multiplexing approach versus the pure NM technique proposed in the previous sections.

Figure 10 shows the multiplexed holograms (a,b) and their amplitude reconstruction in the image plane (c,d) and in the BFP (e,f) obtained by rotating the CCD (left column) and by the NM (right column) technique, respectively. The specimen, used in this case, is a lithium niobate crystal with a reversed domain of hexagonal shape having an optical phase retardation in respect to the rest of the crystal. The main difference between the two set of images is the
shape of the five multiplexed holograms and of their respective reconstructions. On the contrary, the fringe orientation and then the position of the five images in BFP is the same in both cases (see Fig.10 (e) and 10(f)). Therefore, the holograms multiplexed by rotating the CCD can be de-multiplexed by the same procedure explained in the section 3.

![Fig. 10. The multiplexed holograms (a,b) and their amplitude reconstruction in the image plane (c,d) and in the BFP (e,f) obtained using the OM (a,c,e) and the NM (b,d,f) technique, respectively.](image)

The obtained amplitude and phase reconstructions are shown in Fig.11. Looking at the phase profiles, across the wall of the hexagonal domain, it results that the phase step value doesn’t change in the two cases. On the contrary the slope of the phase step is clearly different in the two profiles. This spreading is due to a decrease in the lateral resolution that we attribute to the use of the interpolation routine in the NM procedure.

![Fig. 11. Phase profiles across the wall of the hexagonal domain (along the line in phase reconstructions in the left-corner inset) for holograms multiplexed by rotating the CCD (a) and by the numerical multiplexing (b). In the right-corner inset are shown the amplitude reconstructions.](image)
5. Evaluation of the influence of the multiplexing on the phase retrieval

We discuss briefly the impact of the hologram on the phase reconstruction precision. In Fig. 12 we present a graph of the phase standard deviation (std) on an identical area of the phase reconstructions vs. the number of multiplexed holograms.

![Graph of phase standard deviation vs. number of multiplexed holograms](image)

Fig. 12. Graph of the phase standard deviation vs. the number of multiplexed holograms.

It results that the std increases slightly with the number of multiplexed holograms. We note that the phase std in the case of two multiplexed holograms is significantly increased compared with that of the initial hologram. On the contrary the std augments slowly while the number of multiplexing holograms increases from two to five.

6. Conclusion

We conclude that the studied angular multiplexing/de-multiplexing technique for digital holograms recorded in the microscope configuration allows to multiplex five holograms and to retrieve correctly quantitative information about the amplitude and phase maps through the numerical de-multiplexing. The results coming from this investigation can be also helpful in some super-resolution techniques where the object is rotated to collect the most possible of the spatial frequencies of its diffraction spectrum by a CCD array [16,17]. On the contrary, the method presented in Ref. 13, even if it’s more effective, it cannot be used for such kind of experiments. In fact, the angular multiplexing allows to multiplex the holograms in the direct domain (i.e. on the CCD plane) and can be obtained easily by rotating the CCD array, while in Ref. 13 the multiplexing is performed in the Fourier domain and is completely numerical.

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