A New Compact Self-referenced Holographic Setup Tested on a Fluorescent Target

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Abstract: We propose a new self-referenced holographic microscope setup based on a special bifocal lens. This setup can detect and visualize fluorescent objects. The new principle and the experimental results of the imaging are also presented.
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1. Introduction

Digital holographic imaging creates the possibility of lens-less imaging [1] and the vision of a volume with large depths [2], because it is based on a wave front capturing technique. The holographic technique is based on the interference phenomena so it is a requirement that the applied holographic setup provide smaller optical path differences than the coherent length of the applied light. That is why the used coherent length determines the construction of the holographic optical setup. When light with short coherence length is used, (fluorescent holography [3], white light holography [4], incoherent holography [5] or self-referenced holography [6]) only a special optical design can ensure the needed optical path difference. We can categorize the special setups by their basic optical element, which can be e.g. an interferometer (Linnik interferometer [7], Hariharan-Sen interferometer [8], Mach-Zehnder interferometer, or Michelson interferometer) or a multi- or bifocal optical element (Fresnel zone-plate, spatial light modulator [9] or a bifocal lens [10, 11]).

The holographic setup presented in this article is a self-referenced fluorescence holographic microscope that is based on a bifocal lens. As far as the author knows, ring-shaped bifocal lens has not been used and presented before at the field of holography. This kind of bifocal lens in the optical setup creates a new shaped and a special structured in-line hologram. During the hologram reconstruction the twin-image, the image and the zero order will split in the real image plane. When an intensity target like a common fluorescent object is observed with this setup, phase information and twin image elimination are not needed. As there is no phase reconstruction requirement at this imaging setup the object can be reconstructed from a single-shot hologram.

In this paper I introduce the principle of this new self-referenced holographic imaging system by describing the bifocal lens and the not resolution optimized optical system design. With the reconstruction of a fluorescent target I demonstrate in practice the applicability of the ring-shaped bifocal lens at self-referenced holography. At last I discuss the disadvantages and the main benefits of this kind of self-referenced holographic construction.

2. The ring-shaped bifocal lens at self-referenced holography

A ring-shaped bifocal lens that is used for creating a self-referenced hologram is showed in Fig. 1A. This optical element has an axial symmetry and double focuses. The concentric regions of the lens near and far from the axis have different focuses. Therefore this bifocal lens separates the wave field of a single point source in the space, and focuses them to two different places with its central and ring areas. As in this setup the divergence of the central beam is bigger than the divergence of the ring-shaped beam, the two separated beams will overlap each other in a region during the propagation. The cross-section of the union of the two beams is also ring-shaped as it can be seen in Fig. 1B. Hence the optical path difference is smaller than the coherence length of the fluorescent light the interference phenomena can be observed in this union. Capturing this cross-section of the united beams with a detector we get the digital self-referenced hologram.

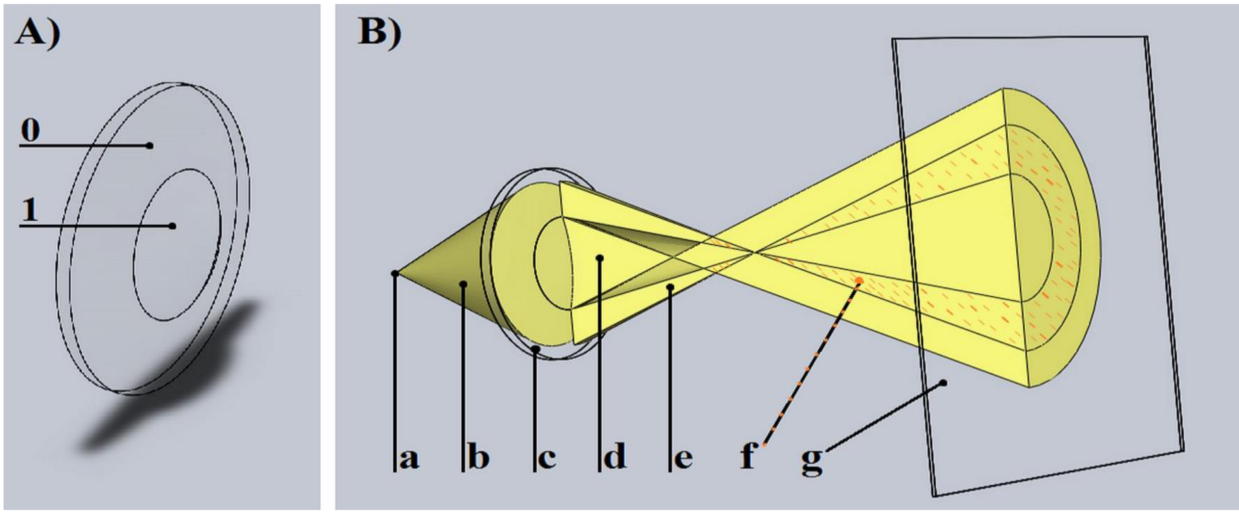


Fig. 1. A) A bifocal lens for self-referenced holographic purpose, where (0) is the ring area, and (1) is the central area. B) How the bifocal lens creates the self interference pattern of a single point. (a) The single target point. (b) Its emitted beam. (c) The bifocal lens. (d) The central beam. (e) The ring-shaped beam. (f) The union of the two ("d" & "e") beams, where the interference fringes appear. (g) Cross-section of the united beams where the hologram captured.

3. The built optical setup and the captured hologram

The bifocal lens in our self-referenced holographic setup has a zero dioptre (D_0) at the ring area and 2.5 1/m dioptre (D_1) at the middle area. The external radiuses of the ring (R_0) and of the central (R_1) areas are 5 mm and 3 mm respectively. This bifocal lens is placed at the back focal plane of an infinity corrected objective with a focus of 45 mm (NA=0.16). This is followed by an afocal optical system with an angular magnification of 0.2. The hologram is captured on the 1/4-th part of a Bayer-patterned CMOS sensor area that has a green color filter. The recorded hologram resolution is 640x480. The objective ensures the optical resolution of the object; the bifocal lens provides the self interference pattern, while the applied afocal optics fit the interference pattern to the detector. This self-referenced holographic setup is a tube mounted and compact device, as it can be seen in Fig. 2C.

The above shown self-referenced setup was tested on an USAF fluorescent test target. The emitted fluorescent light was filtered by a bandpass filter with a center wavelength of 530 nm and with a band with of 10 nm. This filter also eliminates the light used for the excitation. Due to the applied bandpass filter the coherence length of the light increases to ~28 μm. The self-referenced interferograms of many fluorescent object points add to each other in intensity, which can decreases the dynamic range. To avoid this effect only a small part of the USAF target was excited. The excitation light beam wavelength was 405 nm and its diameter was 50 μm. In Fig. 2A the observed area - the group number 4 of the USAF test target - can be seen. Fig. 2B shows the color photo of the observed fluorescent target. The test target was displaced from the focus. The captured self-referenced hologram as it can be seen in Fig. 2D contains the interference fringes in the bright ring-shaped area.

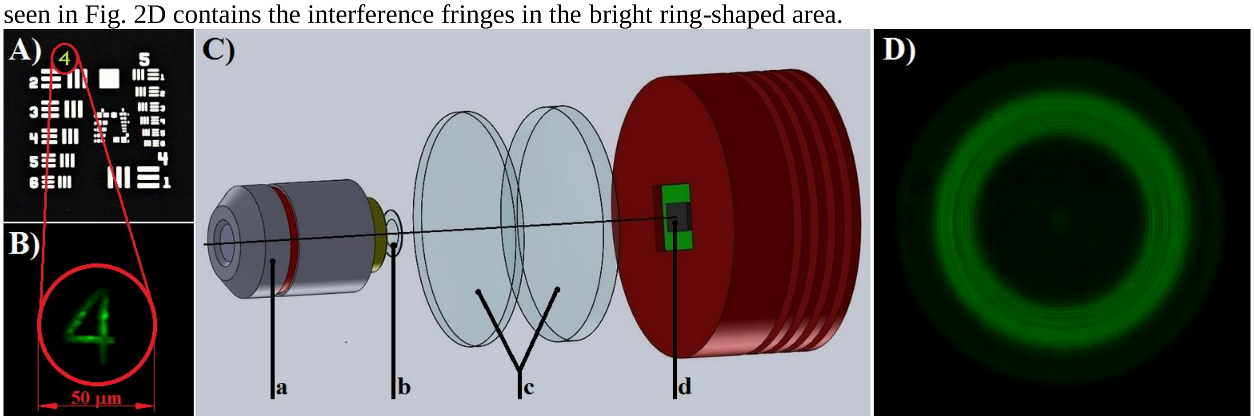
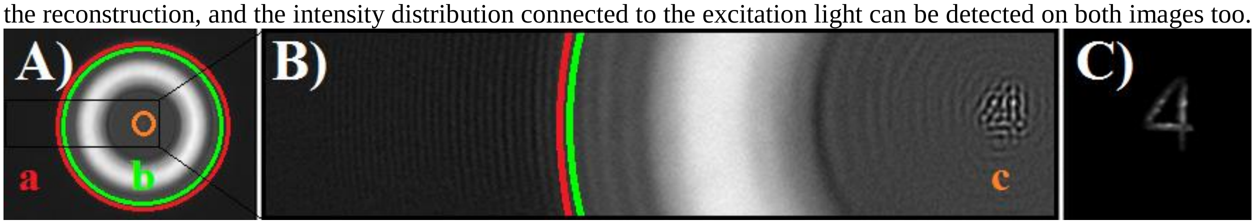


Fig. 2. A) USAF test target with the excited area. B) Fluorescent sample with a texture. C) Self-referenced holographic microscope based on the bifocal lens, where a) is the objective (f 45mm, NA 0.16); b) is the bifocal lens (f_0 inf, f_1 400 mm); c) is the afocal optical system; and d) is a detector. D) Image of the captured hologram.

4. The hologram reconstruction

Aring-shaped area contains the measured interference fringes in the captured hologram, therefore the central part of the hologram is produced by the intensity of the defocused middle beam and noise and does not contain any useful information for the reconstruction. In order to decrease the noise, the first step was to fill this part of the hologram with the averaged intensity value of this area. The hologram reconstruction was fulfilled by using the angular spectrum method [12]. Fig. 3A shows the image of the whole reconstruction. Fig. 3B delineates the separated orders (image, twin image and the zero order terms), while Fig. 3C shows the photo of the sample object so it can be compared with that of the reconstruction. The similarity can be clearly seen. The bright dots on the photo are also on the reconstruction, and the intensity distribution connected to the excitation light can be detected on both images too.



1. Fig. A) is the reconstructed hologram (640x480). B) is a part of the reconstruction (377x90). C) is the common photo of the sample. a) is the area of the twin image. b) is the area of the zero order. c) is the image.

Not only the similarity but the difference is also apparent. At this holographic imaging the low frequency components are missing. This creates this diffraction pattern around the image. A comparative measurement showed that the bifocal setup filters out about the 15 percent of low spatial frequencies.

5. Discussion and Conclusion

A new bifocal lens based self-referenced digital holographic microscope was introduced and constructed. This setup captured the self-referenced hologram of a fluorescent object. We got the image of the object from the hologram with reconstruction. This result demonstrates that the ring-shaped bifocal lens can be an alternative device to the interferometers, FZP, and SLM to build a self-referenced holographic setup. It is clear that its disadvantage is losing the low frequency components. But its benefits are the compact design, the insensitivity for vibrations, the advantages of the single-shot setups and its cheapness.

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7. References

[1.] Z. Gorocs and A. Ozcan, “On-chip biomedical imaging,” Biomedical Engineering, IEEE Reviews in **6**, 29–46 (2013).
[2.] M. Z. Kiss, B. J. Nagy, P. Lakatos, Z. Göröcs, S. Tőkés, B. Wittner, and L. Orzó, “Special multicolor illumination and numerical tilt correction in volumetric digital holographic microscopy,” Opt. Express **22**, 7559–7573 (2014).
[3.] B. W. Schilling, T.-C. Poon, G. Indebetouw, B. Storrie, K. Shinoda, Y. Suzuki, and M. H. Wu, “Three-dimensional holographic fluorescence microscopy,” Opt. Lett. **22**, 1506–1508 (1997).
[4.] D. Kim, J. W. You, and S. Kim, “White light on-axis digital holographic microscopy based on spectral phase shifting,” Opt. Express **14**, 229–234 (2006).
[5.] J. Rosen and G. Brooker, “Fluorescence incoherent color holography,” Opt. Express **15**, 2244–2250 (2007).
[6.] P. Gabolde and R. Trebino, “Self-referenced measurement of the complete electric field of ultrashort pulses,” Opt. Express **12**, 4423–4429 (2004).
[7.] A. Kozma and N. Massey, “Bias level reduction of incoherent holograms,” Appl. Opt. **8**, 393–397 (1969).
[8.] M. Kiss, Z. Gorocs, and S. Tokes, “Self-referenced digital holographic microscopy,” in “Cellular Nanoscale Networks and Their Applications (CNNA), 2012 13th International Workshop on,” (2012), pp. 1–4.
[9.] J. Rosen and R. Kelner, “Modified lagrange invariants and their role in determining transverse and axial imaging resolutions of self-interference incoherent holographic systems,” Opt. Express **22**, 29048–29066 (2014).
[10.] A. W. LOHMANN, “Wavefront reconstruction for incoherent objects,” J. Opt. Soc. Am. **55**, 1555_1–1556 (1965).
[11.] G. Martinez-Ponce, T. Petrova, N. Tomova, V. Dragostinova, T. Todorov, and L. Nikolova, “Bifocal-polarization holographic lens,” Opt. Lett. **29**, 1001–1003 (2004).
[12.] F. Shen and A. Wang, “Fast-Fourier-transform based numerical integration method for the Rayleigh-Sommerfeld diffraction formula,” Appl. Opt. **45**, 1102–1110 (2006).