

# Incoherent digital holography with axial localisation by the rotating point spread function

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**Abstract:** In this study, the rotating point spread function, previously demonstrated in optical imaging, is for the first time implemented in incoherent holography. Theoretical concept of defocusing induced rotation supported by the experimental results is presented and used for a precise axial localisation.

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## 1. Introduction

Optical microscopy is essentially a 2D imaging technique covering wide range of applications in photonics and biophotonics. In many of them, the volumetric information has also been of growing importance. In non-destructive tracking and single molecule imaging, several methods using astigmatism [1], multi-plane detection [2] or two-photon processes [3] have been proposed to overcome limitations of standard imaging and to get information about volume samples. Lately, the optical vortex beams whose intensity profile rotates during free-space propagation were adopted for the 3D imaging and the rotating point spread function (PSF) engineering [4,5]. In this paper concept of the rotating PSF, originally developed for optical imaging [6,7], is implemented into the holographic system with digital image reconstruction. Based on the holographic nature of the image formation, the proposed method allowed the digital implementation of the discrete spiral modulation enabling a variable PSF engineering and an advanced depth estimation benefiting from the numerical image refocusing. Specifically, the rotating PSF was incorporated into the Fresnel Incoherent Correlation Holography (FINCH), that has recently emerged as a novel technique capable of the holographic imaging in spatially incoherent light [9]. In the FINCH experiments, the image is obtained in two independent steps including the hologram acquisition and its digital reconstruction. The proposed modification resulting in the image rotation and a depth estimation can be advantageously carried out by the post-processing of standardly acquired holograms without any additional requirements on the experimental system.

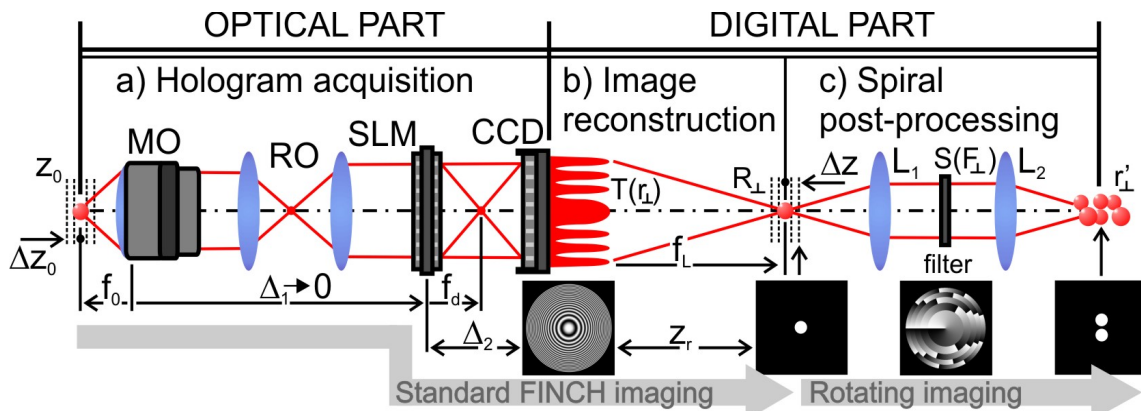


Fig. 1: Basic steps of the image formation in incoherent digital holography with the rotating PSF (MO-microscope objective, RO-relay optics, SLM-spatial light modulator, L<sub>1</sub>, L<sub>2</sub>-virtual Fourier lenses).

## 2. Basic theoretical concept of the image rotation in incoherent correlation holography

In order to implement the rotating PSF in incoherent holography, the novel theoretical concept including the standard FINCH imaging and the additional spiral post-processing has been created. The basic principle can be

explained by Fig. 1 illustrating both the optical and digital stages of the incoherent correlation imaging. In the optical part (Fig. 1a), the acquisition of holograms of incoherently illuminated or fluorescent 3D objects is realized. The spherical waves emitted by individual object points are collimated and through the relay optic system directed toward the spatial light modulator (SLM) [10]. The system represents a common-path interferometer in which the SLM is used to double impinging waves. By the SLM, all waves are divided into pairs of mutually correlated signal and reference waves creating interference point records. The total hologram of the 3D object captured by the CCD can be comprehended as an incoherent sum of the correlation records created for separate object points. Recording of the object is repeated three times with different phase shifts of the signal wave and the final hologram free from the nondiffracted light and the holographic twin image is created adopting the phase shifting process [11]. In the standard numerical reconstruction (Fig. 1b), the final hologram is illuminated by a plane wave and the transmitted light is propagated to the chosen reconstruction plane, where the image is conventionally reconstructed. In the subsequent processing proposed in this paper (Fig. 1c), the standard FINCH image is further treated. In a virtual 4-f Fourier system, the digital spiral modulation is carried out using a discrete phase mask composed of annular regions matching the Fresnel zones. By the helical phase with suitably chosen topological charges applied in the individual radial zones, the vortex modes are created, whose interference results in the azimuthally modulated PSF. The focus errors associated with the out of focus position of the object or the hologram reconstruction plane result in the PSF rotation.

### 3. Computational model of the image rotation in incoherent correlation holography

In order to explore and quantify the PSF rotation and clarify its connection with the experimental parameters, the analytical model describing the standard FINCH imaging, originally proposed in [12], was further extended by the digital spiral post-processing resulting in the defocusing image rotation. In the standard FINCH imaging of a point object, the complex function  $T$  representing the quadratic phase of a lens is obtained by the phase shifting of three successive taken records [12]. In  $T$ , both the lateral and axial position of the object is encoded and can be reconstructed using the Fresnel transform,  $FrT\{T\}$ . To reach a defocusing image rotation, the direct and inverse Fourier transform of the reconstructed image has to be further performed. This is performed in the virtual 4-f system with the digital spiral phase modulation applied at the Fourier plane. If the operation of the digital spiral mask is denoted as  $S$ , and the direct and inverse Fourier transform as  $FT$  and  $FT^{-1}$ , respectively, the complex amplitude of the signal after the spiral image processing can be symbolically written as

$$U = FT^{-1} \{S * FT \{FrT(T)\}\}.$$

In  $FrT\{T\}$ , information about experimental parameters and object and image space is encoded, while the parameters of the digitally implemented spiral mask  $S$  can be used for the PSF engineering and control of its rotation rate. The complex amplitude  $U$  or the intensity  $I=|U|^2$  thus can be used for a complete description of the system under different adjustments.

### 4. Experimental results

In the presented experiments, the main effort was devoted to the verification of the basic concepts briefly discussed throughout the paper. Using single point and two-point incoherent sources, the azimuthally shaped PSF was created and its rotation sensitivity to both the object and image space defocusing was examined.

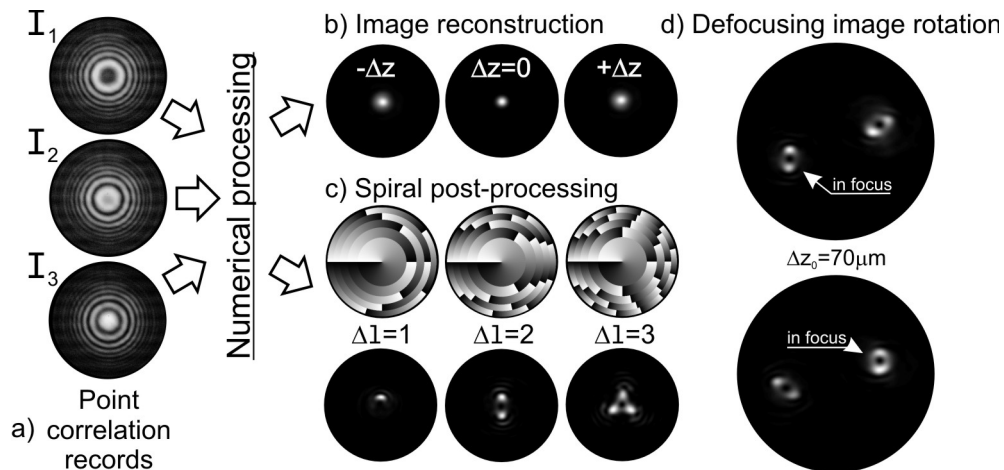


Fig. 2 Illustration of the standard reconstruction and the spiral post-processing of the point correlation records acquired in the FINCH experiment: a) phase shifted point correlation records, b) in focus and out of focus images obtained by the standard

reconstruction, c) spiral masks applied in digital spiral post-processing ( $\Delta l = 1; 2; 3$ ) and the related single-lobed and multi-lobed in focus PSF. d) Image of a pair of spirally processed point sources with the mutual axial distance  $\Delta z_0 = 70 \mu\text{m}$ .

In Fig. 2a, the point correlation records with different phase shifts of the signal wave are shown. The numerical processing of the correlation records enabled the image reconstruction free from the non-diffracted light and the holographic twin image (Fig. 2b). In Fig. 2c, the same correlation records were reconstructed with different parameters of the digital phase mask used in the spiral post-processing. With various values of the difference of the topological charges at the adjacent annular zones  $\Delta l$ , the PSF with different number of lobes can be obtained so that a multi-lobe PSF engineering is possible. The rotation sensitivity of the PSF to both the object and image space defocusing is demonstrated in Fig. 2d. In this case, two incoherent point sources with mutual axial distance  $\Delta z_0 = 70 \mu\text{m}$  in the object space were recorded by the standard FINCH set-up and reconstructed using the spiral post-processing. In the first snapshot, the point image on the left is in focus, while the other image is rotated as a result of defocusing. Due to the holographic nature of the proposed method, a reverse situation can be simply achieved in which the out of focus image is sharply reconstructed and vice versa (lower snapshot in Fig. 2d). The numerical refocusing performed with the same hologram clearly demonstrates the advantages of the rotating PSF in incoherent digital holography and verifies a possibility of the depth estimation in areas of a volume sample exceeding the depth of focus of the used microscope objective.

## 5. Conclusions

In the paper, the concept of the discrete spiral modulation was developed and used for the first demonstration of the rotating PSF in the incoherent digital holography. The image rotation was advantageously implemented by the spiral post-processing of the reconstructed correlation records acquired in the FINCH experiments. The advantages of the rotating PSF in inherently 3D resolving system were briefly discussed and possibilities of the further development of the method outlined.

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