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Study of spatial lateral resolution in off-axis digital holographic microscopy

Ana Doblas [a,*], Emilio Sánchez-Ortiga [a], Manuel Martínez-Corral [a], Jorge Garcia-Sucerquia [b,*]

[a] University of Valencia, 3D Imaging and Display Laboratory, Department of Optics, E-46100 Burjassot, Spain
[b] Universidad Nacional de Colombia Sede Medellin, School of Physics, A.A. 3840, Medellin 050034, Colombia

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ABSTRACT

The lateral resolution in digital holographic microscopy (DHM) has been widely studied in terms of both recording and reconstruction parameters. Although it is understood that once the digital hologram is recorded the physical resolution is fixed according to the diffraction theory and the pixel density, still some researches link the resolution of the reconstructed wavefield with the recording distance as well as with the zero-padding technique. Aiming to help avoiding these misconceptions, in this paper we analyze the lateral resolution of DHM through the variation of those two parameters. To support our outcomes, we have designed numerical simulations and experimental verifications. Both the simulations and the experiments confirm that DHM is indeed resolution invariant in terms of the recording distance and the zero-padding provided that it operates within the angular spectrum regime.

1. Introduction

Digital holographic microscopy (DHM) is a well-established technique for MEMS evaluation [1–3], living cell screening [4–7] and particle tracking [8–13]. Based on the original Gabor's idea [14], DHM allows the retrieval of the complex wavefield scattered by samples from variety of fields [15–18]. The capacity of retrieving scattered complex wavefields powers DHM with the possibility of performing quantitative phase imaging (QPI). As in any microscopy technique, the lateral resolution has been a matter of great interest; since the onset of DHM many works have been published to master the spatial resolution of DHM and to find ways to improve it [4,19–24].

DHM is a hybrid imaging technique that can be understood as the application in cascade of two processes. The first stage is the optical recording of a digital hologram. In this stage the sampling frequency, the wavefield propagation, and interference phenomena determine which spatial frequencies are recorded. The second stage is the numerical recovery of the wavefield scattered by the object. The combined performance of these two stages, determines the spatial frequencies that compose the retrieved image, namely the spatial resolution of the technique. According to the classical definition in microscopy, the spatial resolution of a DHM is defined as the minimum distance between two point-objects such that they are distinguishable in the image retrieved from the hologram.

Although the conditions that allow DHM to operate in the diffraction limit regime [19] have already been established, many DHM systems do not operate in such regime and still remains some controversy about their resolution limit. Two parameters have been particularly studied: the recording distance [4,21,25] and the zero-padding of the digital hologram prior to the numerical reconstruction [21,26–29]. For the former it has been claimed [25] that out-of-focus holograms produce reconstructed images with better resolution than in-focus holograms. For the latter, zero-padding has been proposed as a method for controlling the resolution of reconstructed images [26,27].

In this paper, we assess the spatial resolution of DHM in terms of the recording distance and the zero-padding while the DHM operates in the angular spectrum domain [30], in off-axis architecture and at non-diffraction limit regime [19]. Our study confirms that DHM is indeed resolution invariant in terms of the recording distance and the zero-padding.

The paper is organized as follows: Section 2 reviews the basic theory that is behind the recording and reconstruction stages in an off-axis DHM. In Section 3, we define the resolution limit in DHM and present a model for the evaluation of the lateral resolution. The evaluation of the spatial lateral resolution as a function of the recording distance is presented in Section 4. In Section 5 the effects of the zero-padding on the spatial lateral resolution of the

* Corresponding authors.
E-mail addresses: a.isabel.doblas@uv.es (A. Doblas), jigarcia@unal.edu.co (J. Garcia-Sucerquia).

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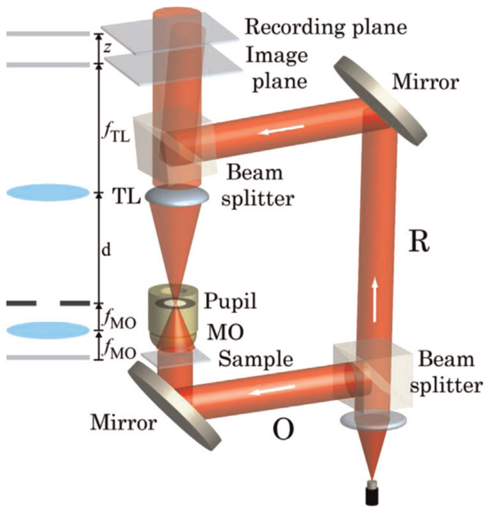

Fig. 1. Scheme of an off-axis DHM. In a general case, the MO and the TL are arranged in non-telecentric mode.

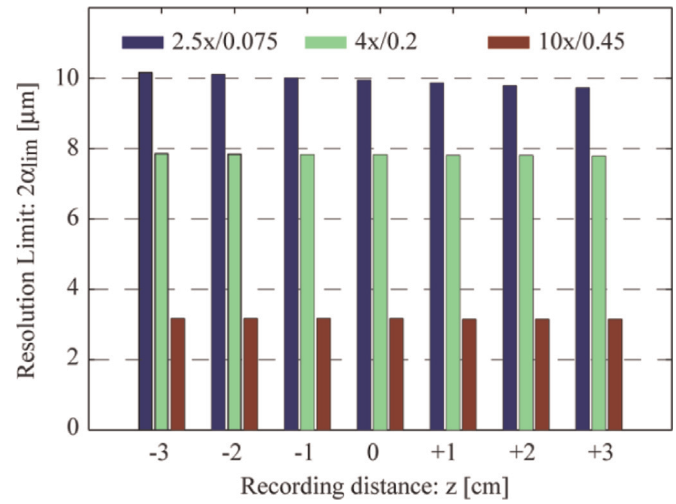

Fig. 3. Numerically-evaluated resolution limit vs. the recording distance for an off-axis DHM system.

DHM are evaluated. The studies in Sections 4 and 5 are performed both numerically and experimentally. Finally, Section 6 is dedicated to summarize the main achievements of our research.

2. Fundamental of off-axis DHM

DHM is a hybrid imaging technique based on two stages: the optical recording of hologram and its numerical reconstruction. In the case of the off-axis architecture, the reconstruction stage can be performed after a single shot capture. As illustrated in Fig. 1, an optical microscope, known here as the host microscope, is inserted in one of the arms of a Mach–Zehnder interferometer. The light-beam emitted by a laser of wavelength λ_0 impinges on a beam splitter cube. One of the split beams illuminates the sample, $O(x, y)$, which is set at the front-focal-plane (FFP) of the microscope objective (MO). The image $O(x, y)$ is then obtained at the back-focal-plane (BFP) of the tube lens (TL). Commonly, this plane is named as the image plane (IP) of the optical microscope.

The complex wavefield $U_{IP}(x, y)$ produced by the microscope at the IP can be computed by application in cascade of ABCD transformations [31,32]. After regular algebra it is possible to obtain

$$U_{IP}(\boldsymbol{x}) = \frac{1}{M^2} e^{ik_0(2f_{MO}+d+f_{TL})} exp\left(i\frac{k_0}{2C}|\boldsymbol{x}|^2\right) \times \left\{O\left(\frac{\boldsymbol{x}}{M}\right) \otimes_2 \tilde{p}\left(\frac{\boldsymbol{x}}{\lambda_0 f_{TL}}\right)\right\}, \quad (1)$$

where $\boldsymbol{x} = (x, y)$ are the transverse coordinates, $k_0 = 2\pi/\lambda_0$ is the wave number, and $\tilde{p}(\boldsymbol{x})$ is the Fourier transform of the aperture transmittance of the imaging system. The lateral magnification, $M = -f_{TL}/f_{MO}$, does not depend on the distance, d, between the MO, the BFP and the TL.

The distance d, however, is a relevant parameter in performance of DHM, as shown recently [33,34]. In Eq. (1) we find a quadratic phase term whose radius of curvature

$$C = \frac{f_{TL}^2}{f_{TL} - d}, \quad (2)$$

appears due to the use of the microscope in non-telecentric regime ($d \neq f_{TL}$). As direct consequence of this phase term, the DHM becomes a shift-variant imaging system [22,33,34], with important ruining effects in the QPIs.

The irradiance pattern recorded on digital camera is the result of the interference between a tilted plane wave

$$R(\boldsymbol{x}) = \sqrt{I_R}\, exp(i\mathbf{k}\cdot\boldsymbol{x}), \quad (3)$$

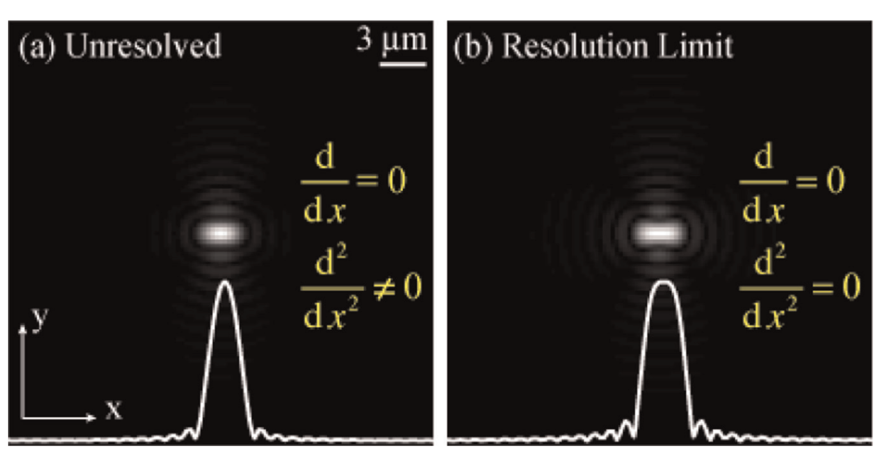

Fig. 2. Numerical test of the lateral spatial resolution. (a) Reconstructed image calculated from a simulated hologram of two points spaced $2\alpha = 0.6$ μm. (b) The same for two points separated $2\alpha_{lim} = 0.7$ μm. For the calculations we assumed a setup in which $\lambda_0 = 633$ nm, $M = -50$, $NA = 0.55$, $f_{TL} = 200$ mm, $d = 180$ mm, $z = +3$ cm and $N = 1024$ pixels.