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Structured Illumination Digital Holographic Microscopy via two integrated Mach-Zehnder interferometers

Sofía Obando-Vásquez[a,1], René Restrepo[a,3], Carlos Trujillo[a,4], and Ana Doblas[b,5]
[a] Applied optics group, School of Applied Science and Engineering, Universidad EAFIT, Medellín, Colombia
[b] Department of Electrical and Computer Engineering University of Massachusetts Dartmouth, MA, USA
[1]sobandov@eafit.edu.co ,[2] racastaneq@eafit.edu.co,[3] rrestre6@eafit.edu.co, [4] adoblas@umassd.edu, [5]catrujilla@eafit.edu.co

Abstract: We propose two Match-Zehnder interferometers coupled to create a structured illumination digital holographic microscope with tunable modulation frequency capability, expanding the system's numerical aperture regardless of the microscope objective lens used.

1. Introduction

Structured illumination (SI) enables the object spectrum's lateral displacement within the transfer function's compact support, allowing unobserved high spatial frequencies to pass through the optical imaging system. In other words, the spatial bandwidth of the optical transfer function can be doubled by illuminating a sample with a structured illumination pattern whose modulation frequency is equal to the cutoff frequency of the imaging system. Diffraction gratings [1], Fresnel biprisms [2], Wollaston prisms [3], and spatial light modulators [4] are common specialized optical elements that generate such "structured patterns. This work uses an additional Mach-Zehnder interferometer to illuminate a sample with high-contrast sinusoidal fringes in an off-axis digital holographic microscope (DHM). Compared to other reported structured illumination systems, the Mach-Zehnder interferometer produces patterns with a tunable modulation frequency and a large field of view, producing two-dimensional super-resolved phase images for microscope systems. Without requiring specialized optical elements, our proposal relies solely on additional mirrors and beam splitters to generate the required modulating fringe pattern, easing its broad implementation within our community.

2. Proposed optical setup

Figure 1a) shows the optical setup of the proposed structured illumination digital holographic microscopy (SI-DHM) coupling two Mach-Zehnder interferometers. A collimated beam emerging from a laser is expanded using a beam expander (BE). The expanded beam illuminates a beamsplitter (BS1) that splits the original wavefront into two beams with the same intensity. The reflected wavefront emerging from the BS1 is then reflected by a mirror (M2) and transmitted through a second beamsplitter (BS3) to reach the sample space. The collimated beam transmitted through BS1 is split into two beams with the same intensity by a linear density filter (LDF). The reflected plane beam emerging LDF is also reflected by BS3, generating an interference pattern between these two plane beams at the sample plane. This generated interference pattern illuminates the sample to generate the structured illumination pattern. The light scattered by the modulated sample (i.e., sample + structured illumination pattern) is then collected by an infinity-corrected microscope objective (MO) lens and transmitted through the tube lens (TL). Assuming that the sample is located at the working distance of the MO lens (i.e., the front focal plane of the principal MO lens), the in-focus image of the sample distribution is located at the back focal plane of the TL lens. To simplify the computational framework of the SI-DHM, the MO and TL lenses are set up following a telecentric configuration system [5].

The off-axis hologram of the DHM system is generated by superposing the plane beam emerging from the TL lens with the plane wave transmitted by the LDF, which is consequently reflected by the M1 mirror and the BS2 beamsplitter. A CCD camera, located at the back focal plane of the TL lens, records the off-axis structured illumination holograms. Figure 1b) and 1c) show, respectively, an experimental hologram and its Fourier spectrum of a USAF test target. The inset of the hologram [Fig. 1b)] clearly shows two interference patterns encoded in the hologram. Whereas the interference pattern with the highest spatial modulation frequency is related to the off-axis DHM system (i.e., the diagonal pattern), the one with the lowest spatial modulation frequency is related to the structured illumination system (i.e., the vertical pattern). The main difference between DHM and SI-DHM systems is that two laterally displaced copies of the object spectrum are encoded within the ±1 terms in the hologram's spectrum [Fig. 1c)].

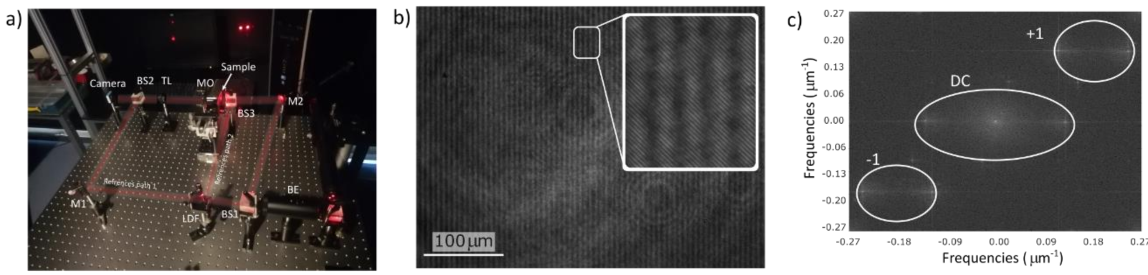

Figure 2. Proposed SI-DHM configuration: a) optical setup coupling two Mach-Zehnder interferometers; b) experimental hologram, c) power spectrum of the experimental hologram.

A key feature of the proposed SI-DHM system is that the spatial modulation frequency of the SI pattern can be adjusted by tilting one or more optical elements, such as M2, LDF, or BSE. This adjustment enables fine-tuning of the spatial modulation frequency of the interference pattern, matching the cutoff frequency of any microscope objective lens. Figure 2 shows the experimental holograms for three modulation frequencies: low, medium, and high. The smaller the difference between the spatial modulation frequency and the cutoff frequency of the DHM system, the greater the resolution improvement. In fact, the higher the spatial modulation frequency, the more separated the spectral components within the ±1 terms, transferring more of the object's high-frequency content inside the system's transfer function.

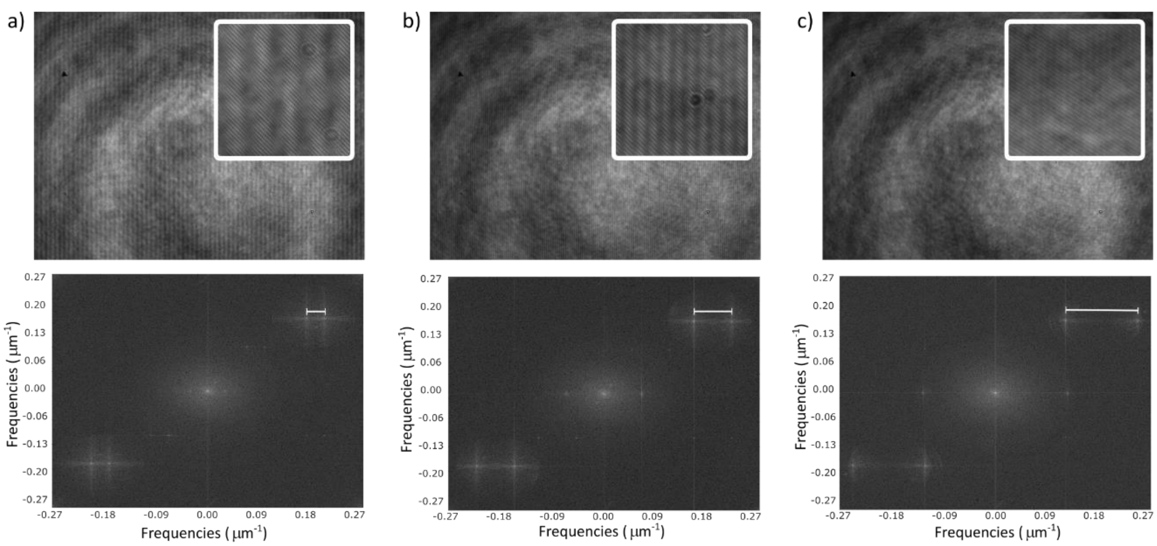

Figure 1. Demonstration of the frequency tunability capability of the SI-DHM system.

In summary, the proposed SI-DHM system generates tunable-frequency structured patterns via the easy alignment of non-diffractive optical elements, being capable of generating super-resolved phase images for any MO lens.

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