

# Rapid computational algorithm with minimum user input for reconstructing phase images in structured illumination Digital Holographic Microscopy

Sofía Obando-Vásquez,<sup>1</sup> Raúl Castaneda,<sup>1</sup> René Restrepo,<sup>1</sup> Carlos Trujillo,<sup>1,\*</sup> and Ana Doblas<sup>2,\*\*</sup>

<sup>1</sup>Applied Optics Group, School of Applied Science and Engineering, EAFIT University, Medellín, Colombia

<sup>2</sup>Electrical and Computer Engineering Department, University of Massachusetts – Dartmouth, U.S.A.

Author e-mail address: [\\*catrujilla@eafit.edu.co](mailto:catrujilla@eafit.edu.co), [\\*\\*adoblas@umassd.edu](mailto:adoblas@umassd.edu)

**Abstract:** A rapid computational algorithm is presented for Structured Illumination in Digital Holographic Microscopy. The proposed algorithm is based on the minimization of two cost functions to reconstruct improved resolution images with minimum user input automatically. © 2024 The Author(s)

## 1. Introduction

Structured Illumination Digital Holographic Microscopy (SI-DHM) combines the principles of Digital Holography Microscopy (DHM) and Structured Illumination (SI) to improve the resolution capability of the native DHM system. In SI-DHM, the sample under research is illuminated by a periodic pattern [1,2], transferring high frequencies of the sample that commonly are not transferred within the compact support of the native imaging system onto the sample. Consequently, SI-DHM systems have a higher resolution capability than DHM systems. The lateral resolution in SI-DHM is improved along the direction of the SI pattern, enabling the visualization of finer details within structures of biological specimens. In SI-DHM, the recorded hologram is the irradiance superposition between an object and reference wave like conventional DHM. Whereas the reference wave  $r(x, y)$  is a uniform tilted plane wave, the object wave is

$$u_i(x, y) = \left[ o\left(\frac{x}{M}, \frac{y}{M}\right) \cos\left(\frac{2\pi u_m}{M}x + \varphi_i\right) \right] \otimes_2 P\left(\frac{x}{\lambda f_{TL}}, \frac{y}{\lambda f_{TL}}\right), \quad (1)$$

where  $\otimes_2$  is the 2D convolution operator,  $\lambda$  is the source's illumination wavelength, and  $M = -f_{TL}/f_{MO}$  stands for the lateral magnification of the imaging system being  $f_{TL}$  and  $f_{MO}$  the focal length of the tube lens (TL) and the microscope objective (MO) lens, respectively. In Eq. (1), the first term before the convolution operator is the magnified complex distribution sample, illuminated by a sinusoidal pattern projected onto the sample with a modulation frequency of  $u_m$  along the x direction and initial phase shift value of  $\varphi_i$ . The second term in Eq. (1) corresponds to the 2D Fourier transform of the pupil aperture of the MO lens. Because the object information is mixed with the reference wave within the hologram distribution, one can filter the object distribution from the hologram's spectrum. In fact, the 2D spatially-filtered Fourier transform of the hologram is

$$H_F(u, v) = \frac{1}{2} e^{j\varphi_i} O\left(u - u_{\max} - \frac{u_m}{M}, v - v_{\max}\right) p\left[\lambda f_{TL}(u - u_{\max}), \lambda f_{TL}(v - v_{\max})\right] + \frac{1}{2} e^{-j\varphi_i} O\left(u - u_{\max} + \frac{u_m}{M}, v - v_{\max}\right) p\left[\lambda f_{TL}(u - u_{\max}), \lambda f_{TL}(v - v_{\max})\right] \quad (2)$$

where  $(u, v)$  are the lateral spatial frequencies, and  $(u_{\max}, v_{\max})$  are the spatial frequencies of the center of the +1 term, which are related to the interference angle of the DHM system [3]. In Eq. (2), the capital letters refer to the 2D Fourier transform distribution (i.e.,  $O(u, v) = \text{FT}[o(x, y)]$ ). In SI-DHM, the spectrum of the sample distribution in the +1 term is placed in two locations around the  $(u_{\max}, v_{\max})$  frequencies. Therefore, after the spatial filtering of the +1 diffraction term within the hologram's spectrum, the computational reconstruction framework in SI-DHM consists of the following steps: (i) the demodulation of each object spectrum to separate the high-spatial frequencies of the object; (ii) the tilt compensation for each individual super-resolved spectrum; and (iii) the final composition of the super-resolved spectrum, achieving super-resolved quantitative phase imaging.

## 2. Proposed Fast-blind algorithm

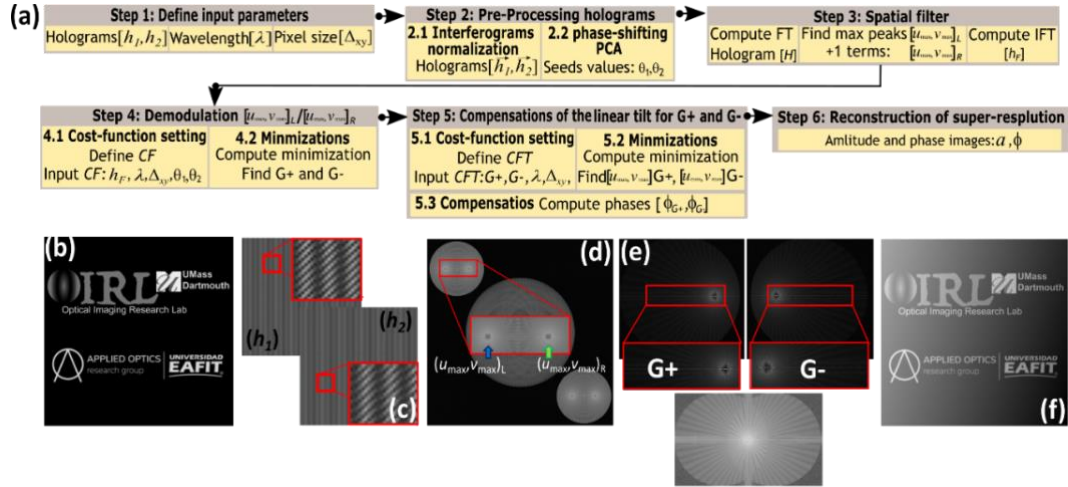
The block diagram of the proposed method is shown in Fig. 1(a). The block diagram comprises six stages: (1) Define input parameters, (2) Pre-processing holograms, (3) Spatial filter, (4) Demodulation, (5)

Compensation, and (6) Reconstruction. Figure 1(b) shows the performance of the proposed approach using a simulated phase object. The input parameters are two phase-shift holograms  $[h_1, h_2]$  in Fig. 1(c)], the wavelength ( $\lambda$ ), and the pixel size ( $\Delta_{xy}$ ). In the pre-processing step, the lateral shift between the sinusoidal fringes in the holograms is automatically found. The hologram's spectrums  $[H_{1,F}(u,v)$  and  $H_{2,F}(u,v)]$  are spatially filtered in step 3. Because the sample is illuminated using a SI pattern, the  $H_{1,F}(u,v)$  and  $H_{2,F}(u,v)$  terms present two maximum peaks, Fig. 1(d). The separation of the two maximum peaks in the spatially filtered holograms is decomposed into two individual spectrums [i.e.,  $G^-$  and  $G^+$  in Fig. 1(e)] by minimizing a cost function that quantifies the ratio difference between the true and residual maximum peaks [4]. The individual spectrums  $G^-$  and  $G^+$  can be derived as

$$G^+ = \frac{1}{2} O \left( u - u_{\max} - \frac{u_m}{M}, v - v_{\max} \right) p \left[ \lambda f_{TL} \left( u - u_{\max} \right), \lambda f_{TL} \left( v - v_{\max} \right) \right]$$

$$G^- = \frac{1}{2} O \left( u - u_{\max} + \frac{u_m}{M}, v - v_{\max} \right) p \left[ \lambda f_{TL} \left( u - u_{\max} \right), \lambda f_{TL} \left( v - v_{\max} \right) \right]$$
(2)

Once the  $G^-$  and  $G^+$  spectrums are correctly demodulated, they should be placed at the origin to compensate for the interfering angle and the phase shift of the SI pattern. This task is carried out by minimizing a second cost function that minimizes the phase jumps in each individual phase image [3]. The final stage combines these individual super-resolved distributions to provide the super-resolved phase image, see Fig. 1(f).



**Fig. 1.** Block diagram of the proposed fast-blind algorithm and simulated validation. The panels are: (a) block diagram; (b) simulates sample; (c) illustration of holograms  $[h_1, h_2]$ ; (d) Fourier Transform hologram  $H_1(u,v)$ ; (e) demodulated  $G^+$  and  $G^-$ ; and finally, (f) phase image reconstructed.

### 3. Conclusions

We have presented a rapid automatic algorithm for reconstructing phase images in SI-DHM systems. The novelties of the proposed algorithm are: (i) no prior knowledge about the characteristics of the structure illumination pattern is required; (ii) arbitrary phase shift between the two recorded holograms can be implemented; and (iii) reduced processing time, providing super-resolved phase images faster than a similar framework implemented using for loops.

### References

1. J. Ma, C. Yuan, G. Situ, G. Pedrini, and W. Osten, "Resolution enhancement in digital holographic microscopy with structured illumination," *Chinese Optics Letters* 11, 1328–1330 (2013).
2. E. Sánchez-Ortiga, M. Martínez-Corral, G. Saavedra, and J. Garcia-Sucerquia, "Enhancing spatial resolution in digital holographic microscopy by biprism structured illumination," *Opt Lett* 39, 2086 (2014).
3. R. Castaneda and A. Doblas, "Fast-iterative automatic reconstruction method for quantitative phase image with reduced phase perturbations in off-axis digital holographic microscopy," *Appl Opt* 60, 10214–10220 (2021).
4. A. Doblas, C. Buitrago-Duque, A. Robinson, and J. Garcia-Sucerquia, "Phase-shifting digital holographic microscopy with an iterative blind reconstruction algorithm," *Appl. Opt.* 58, G311–G317 (2019).