

Computational algorithm that enables synergetic phase compensation and automatic focusing for off-axis Digital Holographic Microscopy operating in telecentric mode

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Abstract: We have developed a joint phase compensation and autofocusing method for telecentric off-axis Digital Holographic Microscopy (DHM), providing in-focus reconstructed phase images without phase distortions. © 2023 The Author(s)

1. Introduction

One of the main hallmarks of Digital Holographic Microscopy (DHM) is the capability to focus different transverse planes within the sample's volume from a single off-axis hologram. This advantage makes DHM systems suitable to live-cell imaging applications, including particle tracking and motility studies of biological specimens. Despite the broad applicability of DHM technology in three-dimensional imaging, its application is commonly hampered by the inaccurate reconstruction of the phase images due to the presence of phase aberrations related to the incorrect estimation of the in-focus distance and the wrong compensation of the off-axis DHM configuration. Here, we present a computational method for automatically and accurately reconstructing out-of-focus pure phase objects in off-axis DHM operating at the telecentric mode to produce fully in-focus phase images of the samples. Our approach synergistically estimates the best focal plane and the parameters of the digital reference wave by minimizing cost functions.

2. Proposed framework for simultaneously autofocusing and phase compensation of off-axis holograms

The proposed autofocusing phase reconstruction method for off-axis telecentric-based DHM holograms contains three steps. The first step involves the definition of the input parameters: the out-of-focus hologram (h), the wavelength of the illumination source (λ), and the pixel dimensions of the camera (e.g., Δ_{xy}). The second step focuses on estimating the in-focus propagation distance by minimizing a cost function that measures a sharpness metric (e.g., a function that quantifies the grade of focusing on an image [1,2]). The common principle of any sharpness function is that in-focus images are those images in which the object's edges are well-defined. Therefore, one can estimate the optimal value of the propagation distance by analyzing the sharpness function on the reconstructed in-focus plane

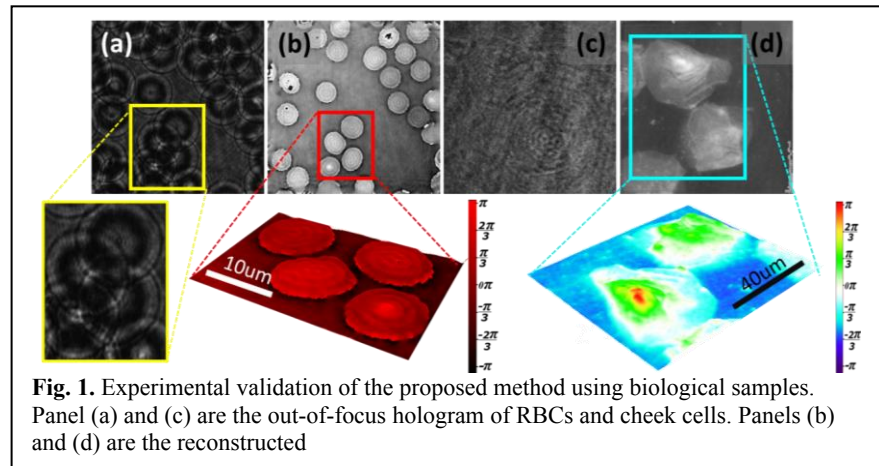
$$\hat{u}_{IP}(x, y) = \text{IFT} \left\{ \text{FT} \{ \hat{u}(x, y; z) \} \exp \left[-i \frac{2\pi z}{\lambda} \sqrt{1 - \lambda^2 (u^2 + v^2)} \right] \right\}, \text{ where } z \text{ is the propagation distance in the image}$$

space, (u, v) are lateral spatial frequencies, and $\text{IFT}[\cdot]$ and $\text{FT}[\cdot]$ are the 2D inverse Fourier transform and the Fourier transform, respectively, and $\hat{u}(x, y; z)$ is the complex distribution of the reconstructed object wavefront after filtering the +1 term from the off-axis hologram. A singularity of DHM is that the in-focus amplitude image of pure phase objects is uniform (e.g., $\hat{a}_{IP} = |\hat{u}_{IP}| = 1$), making only the edges of the objects on the amplitude images visible when the objects are out of focus [3]. We have chosen the Tamura coefficient (TC) [4] among the different sharpness metrics since its minimum value is found for the in-focus propagation distance. The TC metric is estimated from the reconstructed amplitude image, $\text{TC} = \text{sqrt} \left[\sigma(\hat{a}_{IP}) / \bar{\hat{a}_{IP}} \right]$, $\bar{\hat{a}_{IP}}$ and $\sigma(\hat{a}_{IP})$ are the mean and standard deviation values of the reconstructed amplitude image. The minimization of the TC metric is provided by the MATLAB built-in *fminunc* function, which finds a local minimum of an unconstrained multivariable function using the quasi-Newton algorithm [4].

After estimating the propagation distance for the in-focus plane based on the reconstructed amplitude image, the third step compensates for the interference angle introduced by the off-axis configuration. In this step, we have also implemented a minimization algorithm using the MATLAB built-in *fminunc* function. This second cost function tracks

the number of phase jumps in the reconstructed phase image after compensating the interference angle and refocusing the complex amplitude distribution [5,6].

The proposed method has been evaluated using two biological samples: red blood cells (RBCs) and cheek cells. In the RBCs experiment, the sample was located at $15\text{ }\mu\text{m}$ from the front focal plane of the MO lens. Figure 1(a) corresponds to the experimental out-of-focus RBC hologram. The yellow rectangle in Fig. 1(a) encloses a zoom-in region to ease the observation of the out-of-focus RBCs. Figure 2(b) is the best-reconstructed phase image found by the proposed method at a distance $z_{\text{obj}} = 15\text{ }\mu\text{m}$. Since the shape of the RBCs is well-defined in the reconstructed phase image, one can conclude that the proposal yields phase information without or with minimum phase distortions. The red rectangle in Fig. 1 (b) encloses a zoom-in region of the studied sample to display its three-dimensional (3D) pseudocolor phase distribution. On the other hand, Figs. 1(c) and (d) show the hologram and reconstructed unwrapped phase images of out-of-focus cheek cells. The unwrapping algorithm is based on the proposed method by Herraez et al., [7]. Unfortunately, we do not have any prior information on the axial location of the sample, mimicking a more realistic scenario in 3D particle tracking. Nonetheless, the proposed method found that the sample plane was located at a distance $z_{\text{obj}} = 0.037\text{ }\mu\text{m}$ from the front focal plane of the MO lens.



3. Conclusions

This research project presents a new method for autofocusing and removing phase aberrations from off-axis DHM. The method finds the best in-focus reconstruction plane by minimizing a sharpness metric, followed by a second minimization stage that reconstructs in-focus phase images with no aberrations due to the tilting angle between interfering wavefronts in an off-axis DHM system.

4. References

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