Learning-based method for full phase reconstruction of biological samples in digital holographic microscopy

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***Abstract*—A convolutional autoencoder for complete phase reconstruction in Digital Holographic Microscopy is reported. After proper training, this computationally efficient method reconstructs DHM holograms accurately when compared to the traditional approaches. This learning-based method is trained and validated with experimental samples of red blood cells.**

Keywords—Digital Holographic Microscopy, compensated phase maps, convolutional autoencoder, video rates rendering.

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I. Introduction

One of the most common tools to recover the phase information of microscopic translucent samples is Digital Holographic Microscopy (DHM) [1], [2]. This imaging technique has a broad number of applications in biology and biomedicine [3]–[5]. Nonetheless, to reconstruct an aberration-free phase image from a DHM hologram, a computationally demanding numerical spatial filtering process must be precisely executed. This spatial filtering process involves i) a proper selection of the frequency components carrying the information of the sample in the Fourier domain of the acquired hologram, and ii) some phase compensation method for the tilting angle between the interfering waves in this off-axis technique. Although different numerical approaches have been proposed to fully compensate and reconstruct DHM holograms in phase [6], [7], the computational complexity of these proposals still restricts the proper recovery of phase maps at video rates[8]. In this contribution, we propose a convolutional autoencoder-based method to fully compensate and reconstruct DHM holograms without the need for any spatial filtering process. Thus, once the model is appropriately trained, there is a reduction of the computational complexity in the hologram reconstruction. This learning-based model is trained with a dataset composed of 24,024 sections of phase images reconstructed from experimentally acquired DHM holograms. Our proposal is validated with biological samples of red blood cells.

II. Digital Holographic Microscopy

In this proposal, a typical Mach-Zehnder type DHM setup is used, Figure 1. In this setup, the light source is a 532nm He-Ne laser, which is expanded and collimated by a beam expander and then divided into two waves by a first beam splitter. The object wave illuminates the sample after being reflected by a plane mirror, and then the light scattered by the specimen is collected by a 40X/0.65NA infinity-corrected microscope objective (MO). A tube lens generates a magnified image of the sample at its back focal plane To avoid spherical aberrations due to the use of the MO, the tube lens is placed in telecentric regime [4]. The reference wave propagates with no perturbations to a plane mirror and the second beam splitter, which recombines both the object and reference waves to generate the interference pattern (i.e., the DHM hologram). The DHM hologram is recorded by a CMOS sensor (1920x1200 square pixels with side 5.86 μm) located at the back focal plane of the tube lens, i.e., image-plane conditions are met. In this image-plane DHM setup, the off-axis angle between the interfering waves is adjusted by tilting the mirror in the reference arm and/or the second beam splitter. The off-axis configuration ensures that both amplitude and phase images can be reconstructed from a single hologram, being the DHM system more suitable for dynamic imaging.

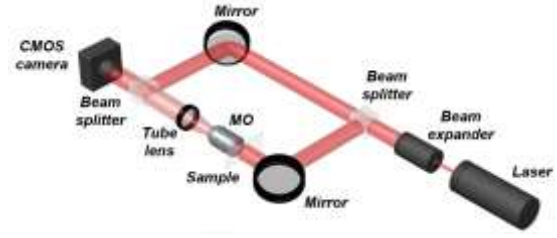

Fig. 1. Typical off-axis Digital Holographic Microscopy setup.

The following procedure was executed to create a dataset that consists of pair of DHM holograms and their corresponding reconstructed phase images. Firstly, we recorded the holograms from unstained red blood cells using our off-axis DHM system. In

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total, we recorded 858 different holograms. Secondly, these holograms were reconstructed using an optimized version of [6] to provide fully-compensated phase images. Finally, each pair of hologram and reconstructed phase images was divided into small images with 256x256 pixels to generate our dataset of 24,024 images.

III. Proposed Learning-Based Method for Full Phase Reconstructions

The experimental dataset was used to train a convolutional autoencoder whose structure is depicted in Figure 2. In this model, 80% of the dataset was used to train the network and the remaining dataset was used to validate its performance. The encoder part of this model comprises three convolutional layers, with 256, 128, and 64 filters, each having a stride of 2 pixels. Finally, a flatten layer is added to transform the bi-dimensional information obtained by the stack into a one-dimensional vector, which is further summarized by a latent layer of 512 neurons. As expected, the decoder part of the model is a mirrored version of the autoencoder, resulting in an output grayscale image of 256x256 pixels. The training of this model was performed via a logarithmic loss function with a batch size of 16 images, a gradient descent optimizer, and during 30 epochs.

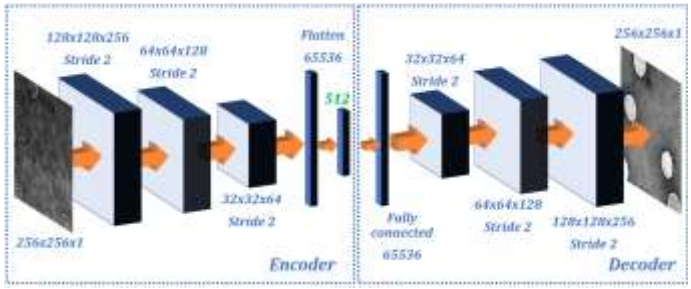

Fig. 2. Convolutional autoencoder for full phase reconstructions in DHM.

Figure 3 shows the results of the proposed learning-based model after proper training. The experimental DHM holograms are shown in panels (a) to (d). Whereas the reconstructed phase images using the traditional method are depicted in panels (e) to (h), the reconstructed phase images obtained by our convolutional autoencoder are shown in panels (i) to (l). Although the proposed convolutional model introduces some noise in the cells' edges, it presents several advantages over the traditional method. Firstly, the proposed convolutional autoencoder provides additional detailed features inside some cells that cannot be recovered using the traditional method, see green circles in Figs. 3(g) and (k). Our approach also reduces the background noise in the reconstructed phase images, as the red circles show. Finally, our learning-based model reconstructs some cells that are incorrectly reconstructed by the traditional method, as shown by the blue circles. This setback of the conventional approach is due to the illumination inhomogeneities for different regions of the recorded DHM holograms, as shown by comparing Fig. 3(a) and (b). After training the model, the proposed method requires 5 ms to compute a phase map of 256x256 pixels running on a personal computer powered by an Intel Core i7-8700 @ 3.20GHz. The traditional method requires 35 ms to reconstruct the same image and use the same laptop, leading to a 7-fold reduction in time achieved by the proposed convolutional autoencoder. In summary, the proposed learning-based method recovers the phase information of biological samples accurately from a single DHM hologram with reduced computational complexity.

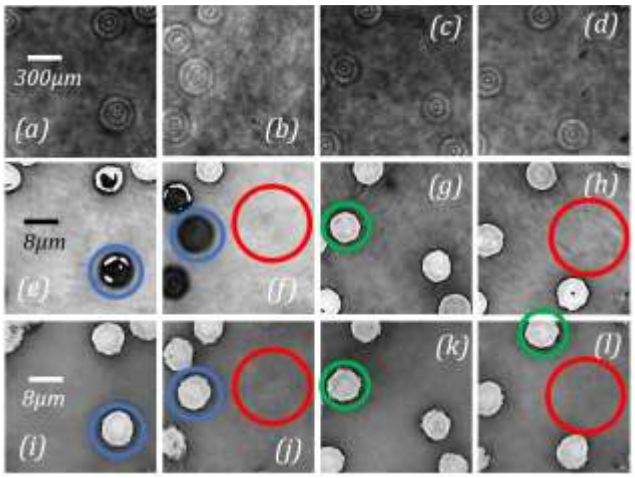

Fig. 3. Result of the proposed method for phase reconstruction in DHM.

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