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Assessment of processing time and measurement accuracy of different phase compensation methods in Quantitative Phase Imaging via Digital Holographic Microscopy applied to Biological Specimens Holographic Microscopy

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Abstract: Six different methods for phase compensation in Digital Holographic Microscopy are compared using a calibrated test target and a *Toxocara canis* larva sample regarding processing time, measurement accuracy, and usefulness in biological imaging.

1. Introduction

Two phase artifacts must be computationally compensated to reconstruct accurate phase images using a single-shot off-axis Digital Holographic Microscope (DHM): (1) the spherical wavefront introduced by the imaging system operating in non-telecentric regime, (2) the linear phase ramp resulting from the required tilting angle between the two interfering waves to separate the sample's information from its twin image and the DC term in the Fourier space [1]. The first artifact can be compensated physically by setting the imaging system at the telecentric regime [2], leaving only the interfering linear phase ramp. The traditional method to compensate and remove this artifact involves manually determining the interfering angle until a phase reconstruction devoid of sawtooth fringes is achieved. This traditional method is time-consuming and heavily relies on the user's expertise. Automatic determination of this interfering angle requires precise knowledge of its spatial frequencies from the hologram's spectrum. Since 2000, several phase compensation algorithms and methodologies have been proposed to determine these carrier frequencies and provide accurate phase imaging. In this study, we perform a comparative study of six of the most prevalent algorithms to establish their primary characteristics and discern the scenarios wherein each algorithm exhibits optimal performance. This study evaluates the processing time and measurement accuracy for quantitative biological imaging using a sample of *Toxocara canis* larva.

2. The algorithms

The initial algorithm for the comparison is the Full ROI algorithm [3]. This grid-search algorithm is considered the ground-truth method as it extensively explores all carrier frequencies of the interfering tilt and their corresponding reconstructed phase images, guaranteeing optimal phase compensation [3]. Next, we use tuDHM [4], a full heuristic algorithm capable of rapidly finding the optimal reconstructed phase map at the expense of inconsistent performance depending on the sample. The Semi-Heuristic phase compensation (SHPC) method is also implemented and assessed [5]. This method uses a semi-heuristic search around intermediate optimal carrier frequency candidates between iterations to find the optimal compensation rapidly. Two additional variations of the traditional Fourier transform method are also explored. Whereas the first applies a zero-padding to the recorded hologram, the second approach comprises an unwrapped-fitting approach of the interfering parameters directly from the distorted phase image. In other words, the recovered phase map is unwrapped, and a linear fitting is performed to generate a second phase map with the same overall ramp. Finally, both phase maps are subtracted from one another to obtain a compensated phase map portraying only the sample's information. The last method is the reference hologram algorithm, in which a hologram without any sample in the field of view is recorded and then subtracted from the sample's hologram to eliminate the phase artifact related to the linear phase ramp [6].

3. Results

The first compensation is performed using a calibrated target (i.e., a USAF test target 1951) imaged in an off-axis telecentric DHM system. A 40x /0.65 NA infinity-corrected Microscope Objective (MO) lens and a tube lens (TL) of focal length 200 mm are used to collect the light scattered by the sample. In this first experiment, the goal is to identify the performance of each approach with a controlled sample regarding its capability to generate a plain background with the least phase jumps possible and its compensation time when running in a computer station hosting an Intel® Xe® running at 3.60 GHz. Also, the standard deviation with respect to the ground-truth method was measured to ensure the similarity of the reconstruction against the Full ROI phase map. The results are presented in Fig.1.

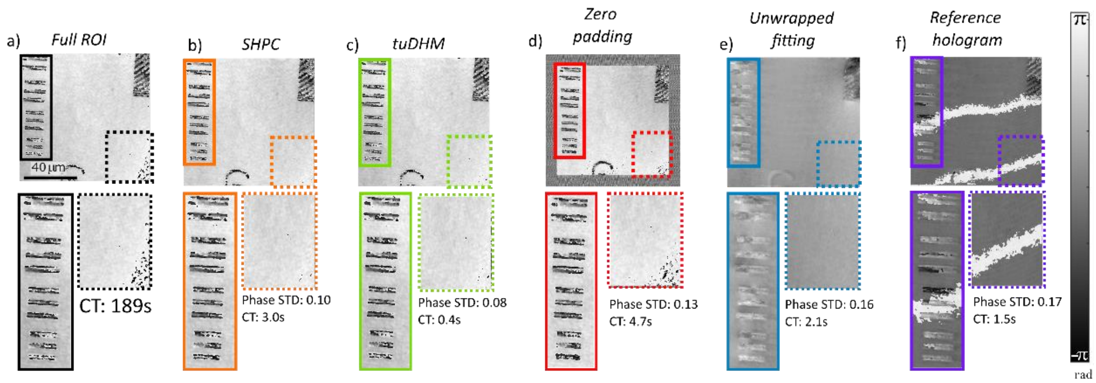

Figure 1. Comparison of the reconstruction algorithms: a) Full ROI. B) SHPC, c) tuDHM, d) Unwrapped fitting, e) reference hologram, and f) Zero padding algorithm. The STD is also included in each phase map.

From Fig. 1, one can conclude that tuDHM and SHPC methods exhibit the lowest STD value, while the zero-padding approach yields similar results as the SHPC method at a lower computational time. Conversely, the Unwrapped-Fitting and the Reference Hologram reconstructions provide the highest STD value. This discrepancy becomes apparent upon scrutinizing the quality of the resultant phase map. The subpar performance of unwrapped-fitting and reference hologram methods in generating quality images may be attributed to their unwrapping of phase maps, a process facilitated by the algorithm presented in [7,8]. Since the best results are those generated by the SHPC and tuDHM approaches, the second experiment with the biological sample has been conducted only with these two approaches. A *T. cani* sample is imaged using the same optical system. Considering its intricate details, lateral dimensions, and thickness, this sample has been selected to fully represent conventional biological imaging challenges in DHM. Results for the second experiment are presented in Fig 2.

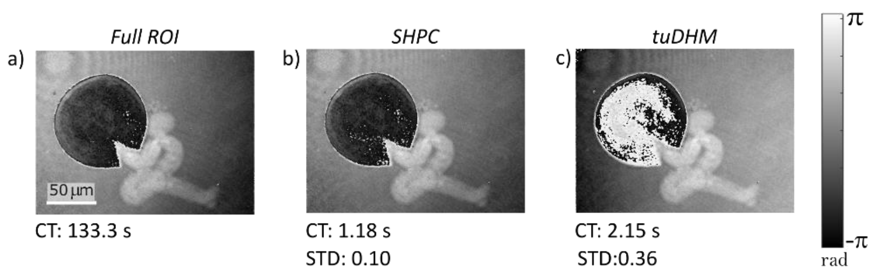

Figure 2. Comparison of the reconstruction algorithms with a biological sample of a T. canis larva: a) Full ROI. B) SHPC, c) tuDHM

From Fig 2. one can observe that the reconstructed phase image of the T. canis larva provided by the tuDHM method exhibits greater disparities compared to the ground truth method, as opposed to the SHPC approach. This is evidenced through qualitative evaluation of the images. In the tuDHM reconstruction, the larva exhibits a discernible phase jump, unlike the other two reconstructions where such a phase jump is absent. Moreover, this result is further underscored by the standard deviation of the phase maps. While the SHPC map displays a standard deviation of 0.10 rad, the tuDHM reconstruction yields a significantly higher standard deviation of 0.36 rad. This can cause one to conclude that the SHPC algorithm is as accurate and robust as the Full ROI method, being more than 100 times faster. In conclusion, among the evaluated approaches, the SHPC provides optimal performance regarding measurement accuracy, processing time, and its ability for biological imaging.

4. References

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