# Phase-Shifting Gabor Holographic Microscopy

Vicente Micó, Javier García, Zeev Zalevsky, and Bahram Javidi, Fellow, IEEE

Abstract—A new lensless microscopy method able to recover the complex wavefront diffracted by a sample from a set of inline recorded holograms is presented. It is based on a modified Gabor-like setup where a condenser lens and a spatial light modulator (SLM) are inserted in a classical Gabor configuration. The condenser lens provides the sample's spectrum at the system Fourier plane while the SLM allows phase shifting modulation of the central spot (DC term) of the sample's spectrum. As consequence, the proposed imaging system recovers the complex amplitude distribution of the diffracted sample wavefront without an additional reference beam. Experimental results validate the proposed method and expand the Gabor method applicability beyond cases of weak diffraction assumption.

*Index Terms*—Holography, microscopy, optical image processing, spatial light modulators (SLMs).

## I. INTRODUCTION

ENSLESS microscopy started as early as Dennis Gabor proposed a new method to achieve imaging in electron microscopy working without lenses [1]. In its basic architecture, Gabor's setup proposed an in-line configuration where two waves interfere at the output plane: the imaging wave caused by diffraction at the sample's plane and the reference wave incoming from the non-diffracted light passing through the sample. Using this in-line configuration, it is possible to recover the object's complex wavefront by means of classical holographic tools with proper reconstruction process. However, this procedure is restricted to weak diffractive samples, that is, the process must be ruled by holography. Only under this assumption, the sample's diffracted light can be considered as a perturbation of the reference beam and the underlying Gabor's principle becomes true. Otherwise, the sample excessively blocks the reference beam and diffraction dominates the process preventing the accurate recovery of the sample's complex wavefront.

This dichotomy can be easily removed by inserting an external reference beam at the recording plane. Thus, holography dominates the process independently if the sample should or should not be considered as a weak diffractive one. In this case, the sample information is placed in one interferometric beam

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V. Micó and J. García are with the Department of Optics from the Universitat de Valéncia, C/ Doctor Moliner, 50, 46100, Burjassot, Spain (e-mail: vicente. mico@uv.es; javier.garcia.monreal@uv.es).

Z. Zalevsky is with the School of Engineering from the Bar-Ilan University, Ramat-Gan, 52900 Israel (e-mail: zalevsz@macs.biu.ac.il).

B. Javidi is with the Electrical and Computer Engineering Department, University of Connecticut, Storrs, CT 06269-1157 USA (e-mail: bahram@engr. uconn.edu).

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and the reference beam in a different one and both are brought together to produce an interference pattern. One can find different ways to reinsert the reference beam. Thus, Leith and Upatnieks reported on different schemes based on an off-line holographic architecture and, thus, avoided the distortion caused by overlapping, in the observation direction, of the three holographic terms incoming from the in-line scheme [2]–[4]. On the other hand, Yamaguchi *et al.* [5], [6] proposed an in-line architecture where the reference beam is phase-shifted in order to remove the image distortion incoming from the twin image while optimizing the space-bandwidth product of the detector due to the absence of carrier fringes in the recorded hologram in the off-line configuration. Many other applications of digital holography have been proposed, including object recognition, microscopy, and identification of microorganisms [7], [8].

Nowadays, digital lensless in-line holographic microscopy (the original idea proposed by Gabor with digital capabilities) combines an optical implementation and a digital reconstruction incoming from the development of modern solid-state image sensors with numerical processing capabilities provided by computers. Several applications have risen up from this kind of microscopy allowing three-dimensional (3D) imaging with micrometer resolution. Some examples include underwater observations, tracking of moving objects and particles, identification of microorganisms, as well as the study of erosion processes in coastal sediments [8]-[19]. But many approaches are restricted to weak diffraction assumptions as the original Gabor's concept was. Otherwise, the reconstructed images will undergo higher or lower distortion depending on both the density and the profile depth of the sample's volume under study [20]. In that sense and in a similar direction as Leith and Upatnieks pointed out, digital in-line holography considering an external reference beam has been also reported extensively in the literature [21], [22]. Another way to provide a reference beam in the interferometric recording is based on a lensless Fourier transform holographic architecture where the sample and the reference beams are generated from the same plane following a common path until reaching the charge-coupled device (CCD) [23], [24].

In this paper, we present a new method capable of recovering the complex amplitude wavefront diffracted by the sample in a digital in-line holographic architecture without the need to add an external reference beam and by removing the Gabor's limitation concerning weak diffractive samples. It is based on a Gabor-like setup but having two additional elements. The first one is a condenser lens added between the input sample and the CCD to provide focusing of the illumination beam at an intermediate plane (Fourier plane). The second one is an SLM placed just at the Fourier plane to perform phase-shifting procedure. Since the DC term of the sample's spectrum is related with the light non-diffracted by the sample, a phase-shift can be introduced in the reference beam by modulating the SLM pixels corresponding with the DC term. Although we propose here the use of an SLM, a single optical element able to modulate the DC term of the focused spectrum can also be considered instead of an array of  $N \times N$  pixels. This kind of modulation is a known method widely used for wavefront sensing and aberration compensation in imaging systems [25]-[32] and some examples of such single optical element can be found in [31] and [32]. Now, we apply it to the field of digital holographic microscopy where several advantages are derived from the use of the phase-shifting method. On one hand, both zeroth-order and twin image terms become removed in the reconstruction process. Since the holographic nature of the method allows sample imaging at different depths by digital propagation algorithms, the reconstructed images will present a better signal-to-noise ratio. Also, due to phase distribution recovery, there is no need to perform coordinate transformation for high numerical apertures (NAs) and magnifications because the complex amplitude distribution can precisely be propagated. Moreover, since magnification in an in-line hologram is related to the distance between the illumination pinhole and the sample, the smaller the distance the higher the magnification. As high magnifications are pursued in microscopy, the separation between twin and real images of the reconstructed hologram becomes small because both of them are also related with the distance from the source to the sample. The proximity of the twin image will severely affect the quality of the reconstructed image. In our method, we are avoiding the twin image, and the reconstruction will not be distorted by it.

## **II. SYSTEM DESCRIPTION**

The proposed experimental setup is depicted in Fig. 1, and can be implemented in both transmissive and reflective configurations. Basically, a collimated laser beam is focused by a condenser lens at its image focal plane. As the input object is placed between the condenser lens and the image focal plane, the lens will provide the Fourier transformation of the object's complex amplitude distribution (object's spectrum) at its image focal plane. We place the SLM just at this plane. Finally, a CCD records the Fresnel pattern that is propagated at short distance from the SLM. In addition, a beam splitter is needed to allow recording of the in-line diffracted patterns in the reflective case.

The motivation of such a configuration is the following. Since the central part of the object's spectrum is responsible for the DC term of the image, or in other words, for the non-diffracted light (reference beam) in the Gabor's concept, it is possible to record a sequence of Fresnel patterns in which the background is shifted with respect to the diffracted components. That is, it is possible to phase-shift the recorded in-line hologram in time sequence by modulating the SLM pixel which spatially coincides with the DC term. The only restriction is that the pixel size of the SLM will be smaller than the central lobe of the object's spectrum, which is given by the object extent and its distance to the CCD [31]. Thus, conventional phase-shifting algorithms can be applied by previously calibrating the SLM in order to know the phase-step to be introduced by the SLM for each modulation.



Fig. 1. Two different possibilities to experimentally validate the proposed method: (a) transmissive and (b) reflective configurations.

The proposed method can be applied without the need for weak diffraction assumption as required for conventional Gabor holograms and provided that there is a DC term in its spectrum. Thus, an in-line hologram is recorded by the CCD and stored in the computer's memory for each of the phase-steps originated at the SLM. Once the whole process is performed, the set of recorded images is processed using a conventional phase-shifting algorithm [22]. In essence, if the arriving amplitude distribution at the CCD can be considered as the addition between a reference beam (incoming from the DC term) and an imaging beam (incoming from the diffracted light at the object's plane), the intensity distribution recorded by the CCD at a given instant t is

$$I_{\text{CCD}}(x, y; t) = |O(x, y) + R(x, y; t)|^{2}$$
  
=  $\left|O(x, y) + R_{0} \exp\left\{i\frac{k}{2z_{0}}(x^{2} + y^{2})\right\}\right|^{2}$   
 $\times \exp\left\{i[\phi_{n}(x, y) + \phi(t)]\right\}^{2}$  (1)

O(x, y) and R(x, y; t) being the amplitude distributions representative of the imaging and reference beams respectively, k is the wavenumber,  $z_0$  is the distance between the SLM and the CCD (see Fig. 1),  $R_0$  is the amplitude of the reference beam,  $\phi(t)$  is a linear phase variable in time according to the phase-shifting procedure, and  $\phi_n(x, y)$  is the initial phase difference between imaging and reference beams. The spherical phase factor is due to the divergence of the reference beam.

Assuming that the time dependence of the different recorded intensities is a function of the intensity image number p multiplied by the phase step between two consecutive images ( $\phi(t) = p\phi_K$ ), (1) can be rewritten as follows, when capturing the different intensity images in time sequence:

$$I_{\rm CCD}(x, y; t) = |O(x, y)|^2 + R_0^2 + 2R_0 \\ \times \operatorname{Re} \left[ O(x, y) \exp \left\{ -ik \frac{(x^2 + y^2)}{2z_0} \right\} \right] \\ \times \cos[p\phi_K + \phi_n(x, y)].$$
(2)

where Re takes the real part of the term between square brackets. Now, the phase-shift algorithm computes the different intensity distributions stored in time sequence by the CCD and recovers the phase distribution of the diffracted wavefront [22]. Naming m as the number of images that integrate a full phase-shifting cycle, the applied algorithm permits the recovering of the initial phase distribution according to

$$\phi_n(x,y) = \arctan \frac{-\sum_{i=1}^m I_i(x,y) \sin \left[\frac{2\pi}{m}(i-1)\right]}{\sum_{i=1}^m I_i(x,y) \cos \left[\frac{2\pi}{m}(i-1)\right]}.$$
 (3)

This phase distribution can be combined with the amplitude distribution (square root of one of the captured intensity images) to recover full complex amplitude distribution of the diffracted wavefront. Notice that this reconstructed wavefront excludes the part of the DC term that is used for phase modulation. This missing component can be neglected if the size of the modulated pixel is small as compared to the DC lobe size or can be simply added digitally.

Once the phase-shifting method is applied, the recovered complex amplitude distribution is digitally propagated, until the object plane, using the convolution method applied to the diffraction Rayleigh–Sommerfeld integral [22]. In this way the diffraction integral is numerically computed exactly by using three Fourier transformations through the convolution theorem, that is,

$$RS(x, y; d) = FT^{-1} \{ FT\{U(x, y)R(x, y)\} FT\{h(x, y; d)\} \}$$
(4)

where RS(x,y) being the propagated wave field, U(x,y)is the processed amplitude distribution resulting from the phase-shifting algorithm, R(x,y) is the reference wave, h(x,y)is the impulse response, (x,y) are the spatial coordinates, FT is the numerical Fourier transform operation realized with the FFT algorithm, and d is the propagation distance. Since we directly define the Fourier transformation of the impulse response as  $H(u,v;d) = FT\{h(x,y;d)\}$ , with the spatial-frequency coordinates (u,v), the calculation of the propagated wave field to an arbitrary distance d is simplified to  $RS(x,y;d) = FT^{-1}\{\hat{U}(u,v)H(u,v;d)\}$ , where  $\hat{U}(u,v)$  is the Fourier transformation of U(x,y).

### **III. EXPERIMENTAL IMPLEMENTATION**

A reflective configuration has been selected for experimental validation. A doublet lens (80-mm focal length) focuses the laser beam (532-nm wavelength) onto a reflective SLM (Holoeye HEO 1080 P, 1920×1080 pixel resolution, 8  $\mu$ m



Fig. 2. Low transmittance object case: (a) obtained images under Gabor approach and (b) obtained images using the proposed method.

pixel pitch). The SLM is connected to a computer where the modulation is controlled by changing the gray level of the central pixel of the image that is transferred to the SLM. Finally, a beam splitter cube ( $20 \times 20$  mm size) is used to reflect the light onto a CCD camera (Basler A312f,  $582 \times 782$  pixels, 8.3  $\mu$ m pixel size, 12 bits/pixel). After calibration, the SLM provides 64 phase levels covering the required full  $2\pi$  range for the phase-shifting process.

In the experimental validation, we have studied two different types of objects: synthetic resolution tests and leukocytes biosample. In all the cases, the results provided by our approach are compared with those ones obtained when assuming Gabor's approach. Notice that the weak diffraction assumption needed for Gabor's holography is defined by the transmittance degree and leukocytes concentration for the resolution test and biosample cases, respectively.

Fig. 2 shows the case of a negative USAF test target. This case corresponds with an object that blocks a high amount of light and, thus, violates the Gabor's condition. In Fig. 2(a) we can see the image obtained when considering the Gabor's concept, that is, recording of one hologram and digital back propagation to the input plane. This image must be compared with the image obtained using the proposed approach that is depicted in Fig. 2(b).

We can see that the imaging under the classical Gabor in-line holographic approach is not successful because the object is highly non-transmissive. But, also in the case that the input object should be considered as a Gabor-like object, the proposed method provides better image quality. To highlight this fact, Fig. 3 depicts the cases of the central part of a positive USAF test and a biosample composed by leukocytes in low concentration. Now, the objects are essentially transparent and will block only a small part of the transmitted light. So, Gabor's approach can be assumed. However, we can still see that the images obtained using the proposed method [Fig. 3(b) and (d)] have a more uniform background (less noise and better contrast) than those ones obtained assuming Gabor's principle [Fig. 3(a) and (c)]. This fact is because the twin image and zeroth-order term are removed by the phase-shifting process applied in our approach.

Obviously, the lower the transmittance of the objects is, the worst the image quality we will obtain. Fig. 4 depicts the case of a spoke test target and an area of the leukocytes biosample having higher concentration. Now, we are in an intermediate



Fig. 3. High transmittance object cases: (a)–(c) images obtained without using the reported method for a positive USAF test and a leukocytes biosample; and (b)–(d) images obtained with using the reported method for a positive USAF test and a leukocytes biosample.



Fig. 4. Medium density object cases: (a)–(c) images obtained without using the reported method for a spoke test target and a leukocyte biosample; and (b)–(d) images obtained with using the reported method for a spoke test target and a leukocyte biosample.

case between objects presented in Figs. 2 and 3, and we can see as imaging capabilities come uniquely from the use of the proposed method. Once again, (a)-(c) and (b)-(d) are the obtained images without and with using the proposed method, respectively.

Finally, since the proposed method recovers the complex amplitude distribution of the diffracted wavefront, we can use it to digital post-process the image. Just as example, Fig. 5 depicts the case of another area of the leukocytes biosample. Images depicted in Fig. 5(a)–(b) corresponds with the cases where the proposed approach is not and is considered, respectively. Cases Fig. 5(c)–(d) depicts the phase unwrapped distribution of images presented in Fig. 5(a)-(b), respectively, and cases



Fig. 5. (a)–(b) Recovered images without and with, respectively, using the reported method. (c)–(d) Phase-unwrapped images of (a) and (b), respectively. (e)–(f) 3D representation of the solid and dashed white line rectangles depicted in cases (a) and (b), respectively. Scale bars in gray level represent optical phase in radians.

Fig. 5(e)-(f) shows a 3-D representation of the solid and dashed white line rectangles of cases Fig. 5(c)-(d), respectively. Once again, not only imaging but also phase quantification is only possible from the use of the proposed method.

## IV. CONCLUSION

We have implemented and experimentally validated a novel procedure in the field of lensless digital in-line holography that improves the capabilities and extends the applicability of the Gabor-based configuration in microscopy. The proposed method is based on the phase-shift produced in the non-diffracted light (DC term) of the object's wavefront by using a single pixel of an SLM. This modulation can also be performed by simpler optical elements involving a single pin-hole architecture plate [31], [32]. The whole procedure implies the recovery of the wavefront's complex amplitude (both phase and amplitude distributions) and allows the digital backpropagation into the object's plane using numerically computed algorithms. Several different objects have been presented validating the proposed method.

The reported method is similar to the phase modulation implemented in point-diffraction interferometry for wavefront sensing and aberration compensation [25]–[32] but now it is expanded to digital in-line holographic microscopy with imaging purposes. The proposed method has unique advantages coming from the in-line configuration (simplicity, robustness and optimization of the space-bandwidth product adaptation of the CCD), from the phase-shifting method (image without distortions due to the twin image removal and applicability to strongly diffracting objects), and from its holographic nature (the recovery of the phase distribution enables additional digital image processing tools).

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**Vicente Micó** received the M.S. degree in physics in 1999, the B.S. degree in optics and optometry in 2000, and the Ph.D. degree in physics in 2008, all from the Universitat de Valencia, Valencia, Spain.

From 2000 to the beginning of 2009, he has been with AIDO (Technological Institute of Optics, Color and Imaging), and the last two years he was the head of the Optical Design Unit at the Optical Engineering Department. During that period, he acted also as external collaborator of the Optics Department at the University of Valencia. He is currently an Assistant

Professor at the Optics Department at the University of Valencia. His research interests are in the areas of optical metrology, digital holography, digital holographic microscopy and optical superresolution, including vibration and deformation metrology, interferometry, speckle applications, and lensless coherent imaging.



**Javier Garcia** received the B.Sc. and Ph.D. degrees in physics from the Universitat de Valencia in 1989 and 1994, respectively.

Currently, he is full Professor in Optics at the Universitat de Valencia, Spain, since 2008. His activity roots in optical image processing and interferometry. He has published over 100 papers on peer reviewed journals and several patents. The research lines have been on speckle interferometry, holography and optical pattern recognition. At present the main activities are centered on remote vibration measurements,

3D image description, and optical superresolution imaging.



Zeev Zalevsky received the B.Sc. and direct Ph.D. degrees in electrical engineering from Tel-Aviv University, Tel-Aviv, Israel, in 1993 and 1996, respectively.

He is currently an Associate Professor in the School of Engineering in Bar-Ilan University, Israel. His major fields of research are optical super resolution, nano-photonics, in-fiber devices, fiber optics, optical data processing, diffractive optical elements and beam shaping, 3-D estimation and RF-photonics. He has published 2 books, more than

10 book chapters, more than 200 refereed papers and holds about 15 issued patents.

In 2007 Dr. Zalevsky received the Kril prize given by the Wolf foundation. In 2008, he was awarded with the International Commission for Optics (ICO) prize for his contribution to the field of optical super resolution. In 2009, he received the Juludan prize for advancing technology in medicine.



**Bahram Javidi** (S'82–M'83–SM'96–F'98) received the B.S. degree from George Washington University, Washington, DC, and the M.S. and Ph.D. degrees from the Pennsylvania State University, University Park, all in electrical engineering.

He is the Board of Trustees Distinguished Professor at the University of Connecticut which is the highest rank and honor bestowed on a faculty member based on research, teaching, and service. He has over 630 publications. He has completed 8 books and 44 book chapters. He has published over 250 technical articles in major peer reviewed journals. He has published over 330 conference proceedings, including over 110 Plenary Addresses, Keynote Addresses, and invited conference papers. His papers have been cited over 5500 times according to the citation index of WEB of Science.

Dr. Javidi is Fellow of seven scientific societies, including Institute of Electrical and Electronics Engineers (IEEE), American Institute for Medical and Biological Engineering, Optical Society of America, and Institute of Physics. In 2008, he received a Fellow award by John Simon Guggenheim Foundation. In 2010, he was the recipient of GeorgeWashington Universitys Distinguished Alumni Scholar Award, Universitys highest honor for its alumni in all disciplines. He received the 2008 IEEE Donald G. Fink prized paper award among all (over 180) IEEE Transactions/Journals, and Magazines. In 2007, The Alexander von Humboldt Foundation awarded him the Humboldt Prize for outstanding US scholars. In 2005, he received the Dennis Gabor Award in DiffractiveWave Technologies from the International Society for Optical Engineering (SPIE). Early in his career, the National Science Foundation named him a Presidential Young Investigator, and he received The Engineering Foundation and the Institute of Electrical and Electronics Engineers Faculty Initiation Award. Dr. Javidi was the recipient of the IEEE Lasers and Electro-optics Society Distinguished Lecturer Award twice in 2003-2004 and 2004-2005. Early in his career, the National Science Foundation named him a Presidential Young Investigator. He was selected in 2003 as one of the nation's top 160 engineers between the ages of 30-45 by the National Academy of Engineering to be an invited speaker at The Frontiers of Engineering Conference. He is on the Editorial Board of the PROCEEDINGS OF THE IEEE (ranked #1 among all IEEE Journals and Transactions), and is currently the Editor in Chief of the Springer-Verlag series on Advanced Science and Technologies for Security Applications. He is on the editorial board of the IEEE/OSA JOURNAL OF DISPLAY TECHNOLOGY and the SPIE Optics Reviews Journal.