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Photorefractive digital holographic microscopy applied in microstructures analysis

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A B S T R A C T

In this work, we present a Photorefractive Digital Holographic Microscopy (PRDHM) technique based on the writing–reading holographic process in photorefractive $Bi_{12}TiO_{20}$ (BTO) crystal and the obtainment of phase and amplitude of the object wave were performed by a digital holographic method. We demonstrate that the wave diffracted by a photorefractive hologram recorded in a BTO crystal can be combined with a reference wave to record a second hologram in a CCD sensor in a configuration of digital holographic microscopy. The experimental measurements were performed on samples like red blood cells and a thin film structure, and were obtained quantitative values of amplitude and phase of the object wave, as well as 3D graphs, of the analyzed samples by the digital reconstruction holographic method. This technique presents a new method in replacement of the usual methods for reconstruction of holograms recorded in a photorefractive crystal and also presents the possibilities to obtain 3D phase images for surfaces characterization and applications in dynamic holography.

1. Introduction

Photorefractive holography has been increasingly used for measuring deformations, vibrations, stress analysis, optical metrology and for quantitative measurements of amplitude and phase of the object waves in static and dynamic processes [1–12]. In photorefractive holography, the holographic recording medium is a photorefractive material (PRM), in which the holographic grating recording is characterized by the following light-induced mechanisms: photorefractive, thermo-optic or photo-chromic effect [1,2]. The main phenomenon that characterizes the photorefractive crystals (PRCs) are: the photorefractive effect, which consists on the refractive index modulation through charge carriers photo-induction and linear electro-optic effect; the refractive index modulation by thermo-optic effect, due to gradient of temperature on the crystal surface; and, the photo-chromic effect, that generates absorption coefficient modulation at high light intensities. These holographic recording materials present many advantages, like in situ self-processing of recording medium, indefinite reusability, high resolution and low response time. Moreover, PRCs do not present fatigue in dynamic and reversible processes.

Particularly, the fast photorefractive sillenite crystals as $Bi_{12}SiO_{20}$ (BSO), $Bi_{12}TiO_{20}$ (BTO) and $Bi_{12}GeO_{20}$ (BGO), have been used in holographic interferometry, since many interesting properties qualify them as recording media for real-time holographic interferometry (or dynamic holographic interferometry) [3–13]. These crystals, in diffusive regimen, present a much faster hologram recording process when compared with other types of PRCs and provide less noisy holographic images due to their anisotropic diffraction property. This feature consists of an orthogonal polarization state between the transmitted object wave across the crystal and the wave diffracted by the hologram, enabling the removal or control of the transmitted object wave intensity, which is one of the major noisy sources [3,4]. Usually, in photorefractive holographic interferometry, for phase determination, is applied the phase-shifting technique [5,6], where the phase of each point (pixel) of the image is calculated using a sequence of intensity interferograms acquired and combined to generate the phase interferogram. Many works contributed to development of the holographic interferometry by phase-shifting techniques using photorefractive crystals [5–11]. Despite the widely application of this method, it can contribute for noisy images and represent a slower reconstruction process when compared with the numerical one, because it is necessary three or more intensity images for realize the reconstruction of the object image [5–12], while the numerical uses a single hologram [14–22].

On the other hand, in digital holography, the amplitude and phase of the optical field are available for direct manipulation of

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digital hologram recorded in a CCD sensor, holographic detector, by calculating the complex optical field of the image [14]. The applications of digital holography to microscopy are particularly advantageous, because a single hologram enables to obtain the phase and intensity information of a three-dimensional object. These advantages have been noted since early in the development of digital holography and applied to imaging analysis of microstructures and biological microscopy [15–22,27]. In off-axis holography, the angle of incidence of the reference beam is chosen so that, when the hologram is reconstructed, the zero order light may be spatially separated from the reconstruction diffracted beam. The method of off-axis holography involves a high spatial-frequency fringe pattern that must be sufficiently sampled by the CCD.

Recently, some works have presented the combination of the photorefractive crystals and the digital holography: Zhao et al. [28] used digital holography, with a Mach-Zehnder interferometer and a CCD camera, for refraction index change measurements in photorefractive crystals using two holograms (with and without crystal illumination) as usual in holographic interferometry; de Angelis et al. [29] studied photorefractive crystals characterization by means of reflection setup for holographic recording based in interferometric techniques. But until now, the photorefractive crystals had not been used as holographic recording medium for digital holographic microscopy, the goal of this work, with direct phase image reconstruction.

In this work we present, by the first time in our knowledge, a new method of double holography, where a holographic recording medium, the photorefractive Bi12TiO20 (BTO) crystal, has high resolution and high rate of recording holograms, in diffusive regimen, and numerical reconstruction by means of a digital holographic microscopy method allowing us to obtain amplitude and phase images of the micro-structured objects. This technique can replace the usual method of reconstruction of holograms recorded in photorefractive crystals, the phase-shifting method, joining the advantages of the optical recording and numerical reconstruction. The methods of optical reconstruction and obtaining amplitude and phase images of photorefractive holography are mostly phase-shifting techniques, which require 3, 4 or 5 phase-shifted holographic interferograms to generate the phase maps and a series of noises are inserted in results (especially, in holographic microscopy, reference [30]), consequently, are several mechanisms needed to remove the ambiguity of phase characteristics of these techniques, references [5–12]. Moreover, the digital holography methods allow the numerical reconstruction of holograms to obtain the amplitude and phase images using a single hologram, references [14–22]. Thus, the method proposed in this manuscript, we have a system of photorefractive holography which gives the amplitude and phase holographic images using a single hologram. Specifically in the case of holographic microscopy, this is an advantage of this approach over the photorefractive holographic microscopy, see reference [30].

The use of photorefractive process in replacement of the digital recording of holograms was studied by Gu et al. [31] and acquisition rates of 470 frames per second were obtained in their work. This high rate of recording holograms photorefractive crystal is a great advantage of this method, as it is known that CCDs have high resolution with low acquisition rate and vice versa, and this method we can register angularly multiplexed holograms of fast events in BTO photorefractive crystal ('fast' sillenite crystal, see references [1,3,13]) and make your optical reconstruction (diffracted beam) more slowly controlling the intensity of light or laser wavelength to be compatible with the acquisition rate of the CCD. Thus, the prospects of this method for dynamic holographic microscopy are very promising and it was called Photorefractive Digital Holographic Microscopy (PRDHM).

The experimental measurements were performed on samples like a red blood cells culture and a thin film structure, and were obtained quantitative values of amplitude and phase of the object wave, as well as, 3D graphs.

2. Theoretical considerations

2.1. Holographic recording process in photorefractive BTO crystal.

In photorefractive holography using sillenite BTO crystal, the photorefractive BTO crystal is used as the recording medium of the holograms [1]. The holographic recording occurs by a refractive index modulation given by $\Delta n = -(1/2)n^3 r_{41} E_{sc}$, via photorefractive effect in diffusive regimen. And, the holographic reconstruction of the object wave (this wave carries the phase information of the object surface) occur in quasi real-time, where the optical reconstruction of the holographic image is the made trough diffraction of the reference wave by the hologram recorded in the PRC, diffracted wave. If λ is the recording wavelength, ρ is the crystal rotator power, L is the crystal thickness, m is the modulation of the incident interference pattern and 2θ is the angle between the interfering beams, the diffraction efficiency of a hologram grating recorded in a PRC is given by [3–6]:

$$\eta = \left(\frac{\pi \Delta n}{\lambda cos\theta} \frac{\sin \rho L}{\rho L}\right)^2 m^2 \tag{1}$$

The intensity, I_0 at a point (x,y) resulting from the superposition of the diffracted $(I_{0,D})$ and the transmitted$(I_{0,T})$ intensities is given by [6]:

$$I_0(x,y) = I_{0,T}(x,y) + I_{0,D}(x,y)[1-e^{(-t/\tau)}]^2 \tag{2}$$

where, τ is the hologram response (writing or erasure) time. The holographic reconstruction of the object wave, $I_{0,D}(x,y)$, is written as [6]:

$$I_{0,D}(x,y) = I_{0,O}(x,y) + I_{0,R}(x,y)[1-\eta] + 2gYcos\Delta\Phi \tag{3}$$

where, $I_{0,D}(x,y)$ and, $I_{0,R}(x,y)$ are object and reference beam intensities, respectively, g is a parameter of the polarization coupling of beams, $Y = [\eta(1-\eta)I_{0,O}(x,y)I_{0,R}(x,y)]^{1/2}$ is the interference term and $\Delta\Phi$ is the phase shift on the object beam [6].

2.2. Numerical reconstruction by digital holographic microscopy.

For digital reconstruction, we used the Double Propagation Method (DPM) [20,21] which consists in two steps for digital hologram reconstruction: first, is made the numerical calculus of propagation until the focal plane, Fig. 1(a); following, the complex amplitude of the diffraction pattern in this plane is calculated and a new calculus of propagation until the image plane, Fig. 1(b).

This method allows performing the image reconstruction begin from holograms focused in the CCD plane, or out of it, and with only one hologram can be calculated the phase field with curvature correction caused by microscopic objective.

The description of this method can be made by: the first step, the Angular Spectrum $A(k_\xi,k_\eta;z=0)$ of the hologram is obtained. The Angular Spectrum is defined as the Fourier Transform of the digital hologram:

$$A(k_\xi,k_\eta;z=0) = \iint E_H(\xi_0,\eta_0,0) exp[-i(k_\xi \xi_0 + k_\eta \eta_0)] d\xi_0 d\eta_0, \tag{4}$$

where, k_ξ and k_η are the spatial frequencies of ξ and η. $E_H(\xi_0,\eta_0,0)$ is the complex amplitude of the digital hologram. Then, the virtual image and the zero order are removed digitally and the field correspondent to this new spectrum on the hologram plane is calculated. Is performed a propagation until the focal plane of the image $z=D$ and a new Angular Spectrum is calculated in the

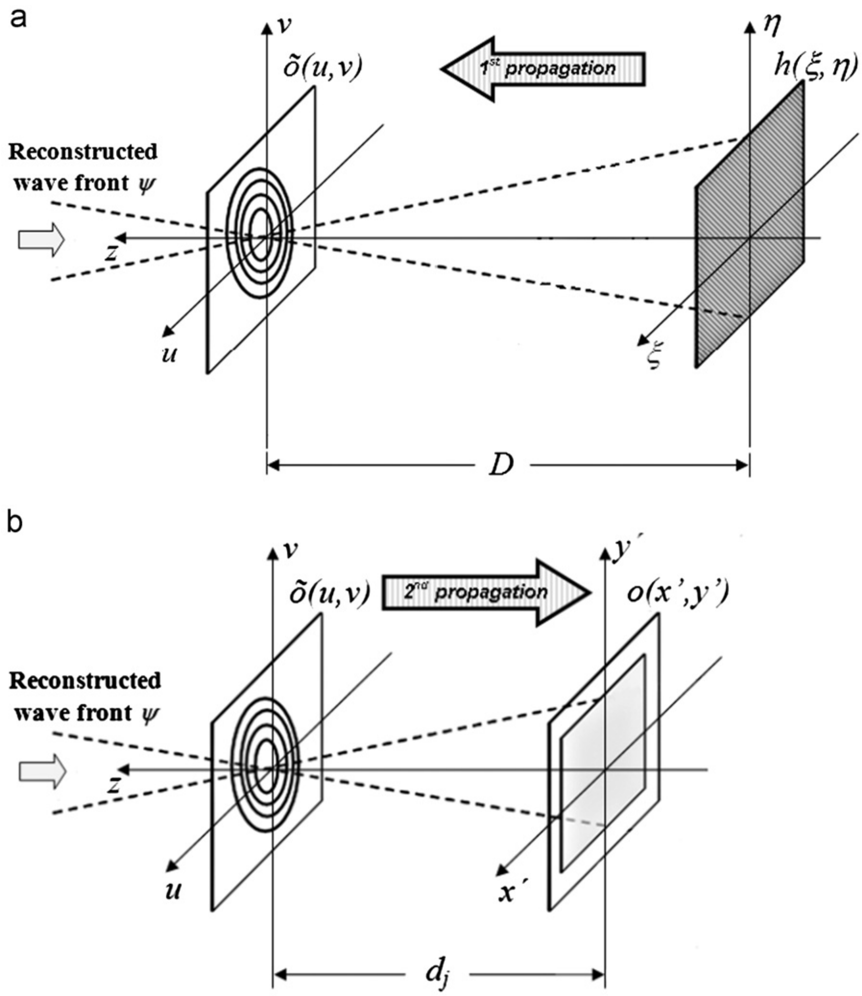

Fig. 1. Double Propagation Method for image reconstruction, where (a) first propagation until the focal plan, and (b) new propagation process until the image plane.

position $z : A(k_\xi, k_\eta; z)$.

$$A(k_\xi, k_\eta; z=D) = A(k_\xi, k_\eta; z=0)\exp(ik_z D), \quad (5)$$

where $k_z = \sqrt{k^2 - k_\xi{}^2 - k_\eta{}^2}$.

Through an inverse Fourier Transform, the field correspondent to this plane is calculated $E_H(u,v,z)$.

The next step of this method is based on a second propagation until the image plane (d_j). The field is determinate on this plane:

$$A(k_\xi, k_\eta; z=d_j) = A(k_\xi, k_\eta; z=D)\exp(ik_z d_j), \quad (6)$$

$$E_H(x',y',d_j) = \iint A(k_\xi, k_\eta; z=d_j) exp[i(k_\xi x' + k_\eta y')] dk_\xi dk_\eta, \quad (7)$$

This calculation results in a matrix of complex numbers and the amplitude and phase of the object wave can be determined:

$$I(x',y') = Re[E_H(x',y')]^2 + Im[E_H(x',y')]^2. \quad (8)$$

$$\Phi(x',y') = \arctan\frac{Im[E_H(x',y')]}{Re[E_H(x',y')]}. \quad (9)$$

The relation between the phase $\Phi(x',y')$ and high of the object $h(x',y')$ is given by:

$$\Delta\Phi(x',y') = \frac{2\pi}{\lambda} h(x',y')(n_{object} - 1). \quad (10)$$

where, n_{object} is the refraction index of the transparent sample. The phase map is calculated and through this relation the topography of the surface analyzed can be obtained.

The compensation of aberrations is fundamental when quantitative phase determination and several approaches have been proposed to remove the aberrations: a method proposed by Cuche et al. [23]; a double-exposure technique proposed by Ferraro et al. [24]; the paper of Colomb et al. [25]; a method was proposed by Miccio et al. [26] who performed a two-dimensional fitting with the Zernike polynomials of the reconstructed unwrapped phase. In our formulation for imagem reconstruction is specific for digital holographic microscopy; this way the objective of the first stage is the calculation of the objects' Fourier transform plane, where the complex wavefield contains all the information about the phase and intensity of object wavefield. Then, the second step is to reconstruct the complex amplitudes of the image wavefield starting from the objects' Fourier transform plane [22].

3. Experiments and results

Fig. 2 displays experimental setup for PRDHM. The Fig. 2(a) shows the photorefractive holographic recording process in BTO crystal in transmission configuration. The He–Ne laser beam (λ=632.8 nm), after passing through a beam-splitter BS1, is divided in reference beam 1, $I_{0,R1}(x,y)$, and object beam, $I_{0,O(x,y)}$, then these beams are expanded by spatial filters SF's and pass through the lens L's to correct their parallelism. In the photorefractive holographic recording process, a microscopic objective (Zeiss model 50X, 0.95NA) directs the image of the micro-structured sample into

BTO crystal, object beam, which combines it with the reference beam with a 30 degree angle between them and gives the volume hologram recording of the microscopic object.

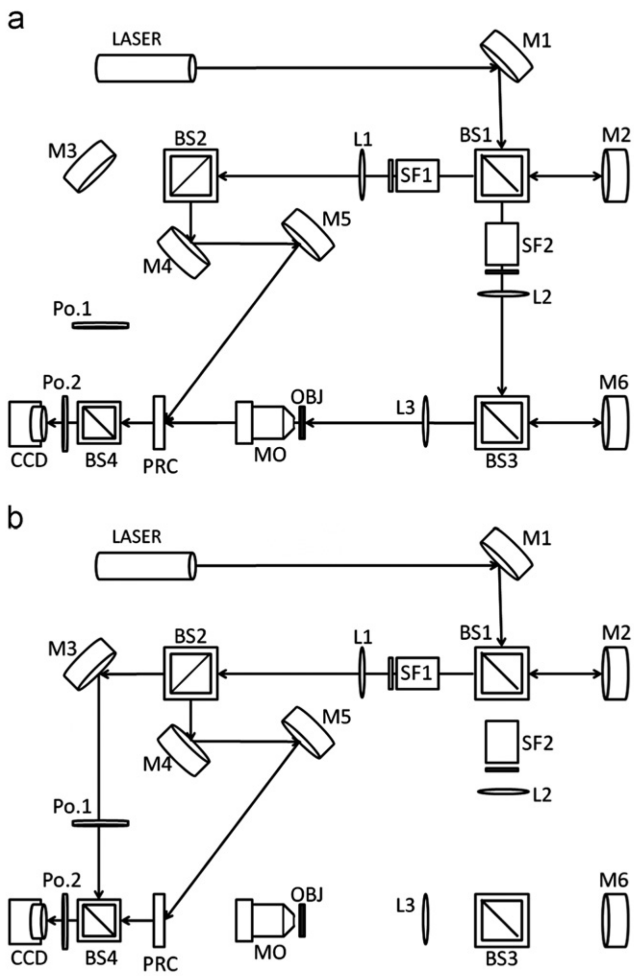

Fig. 2. Experimental setup for Photorefractive Digital Holographic Microscopy (PRDHM) with photorefractive sillenite $Bi_{12}TiO_{20}$ (BTO) crystal as recording medium, where M's are mirrors, BS's are beam-splitters, SF's are spatial filter, L's are lens, Po's are polarizer, MO is a microscopic objective, PRC is a BTO crystal, OBJ is a transparent object and CCD is camera: (a) photorefractive holographic writing-reading process in BTO crystal; and (b) holographic reading for digital holographic reconstruction.

The photorefractive holographic reconstruction process, reading hologram, is displaying in Fig. 2(b), where, the image is optically reconstructed by diffraction of reference beam 1 in holographic grating recorded in BTO crystal (Eq. (3)), diffracted beam $I_{0,D}(x,y)$.

In this technique, PRDHM, the diffracted beam interfere with reference beam 2,$I_{0,R2}(x,y)$, producing an digital hologram in the CCD sensor:

$$I_1(x,y) = I_{0,R2}(x,y) + I_{0,D}(x,y)[1-e^{(-t/\tau)^2}] + 2\sqrt{I_{0,R2}(x,y)I_{0,D}(x,y)[1-e^{(-t/\tau)^2}]}\cos\Delta\Phi \quad (11)$$

where $\Delta\Phi$ is the phase-shift of the holographic pattern $I_1(x,y)$ recorded on the CCD. We use the CCD camera (ImagingSource Inc., model DMK41BU02.H, Sony sensor 1024×768 pixels selection, pixel size $\Delta x = 4.65\ \mu m$, acquisition rate of 15 fps at full resolution) and, by digital reconstruction, the amplitude and phase image can be finally obtained. A slight angle is introduced between the diffracted beam and reference beam 2 for off-axis holography. An USB-2 cable connects the camera to the microcomputer, where image reconstruction is performed by the HOLODIG program supported in MatLab© [18–22] using double propagation reconstruction algorithms. In this software, the phase and amplitude of the object wave are reconstructed and the removal of the phase discontinuity (unwrapping process) is realized directly.

The calibration process of the coordinate axis system was realized through the relation between the image of the object and the image of a calibration reticule target. The images of the object have 640×480 pixels and the calibration reticule target has divisions of 10 μm size. The Fig. 3 demonstrates the relation made by the software among the images. Using this process, the calibration of the lateral axis was performed.

The axial resolution is realized through the relation of the phase of the object surface and the high of the object. This relation is given in Equation 12 and is applied in the software.

The hologram of each object image is recorded in BTO crystal, in according with Fig. 2(a). In following, the diffracted beam in hologram grating that carried the holographic image (amplitude and phase) and the reference beam 2 produce a hologram which will be digitalized by CCD camera. This hologram erasure due to the interaction of the diffracted beam with the PRC, what creates a uniform pattern over the previously recorded hologram. Finally, the image reconstruction (phase and amplitude) was performed by mean of the DPM method using only one hologram, Fig. 1a and b.

For the PRDHM setup were used a thin film structure and a red blood cell as micro-structured samples. In Fig. 4(a–d) are displayed the interferogram image of the thin film structure,

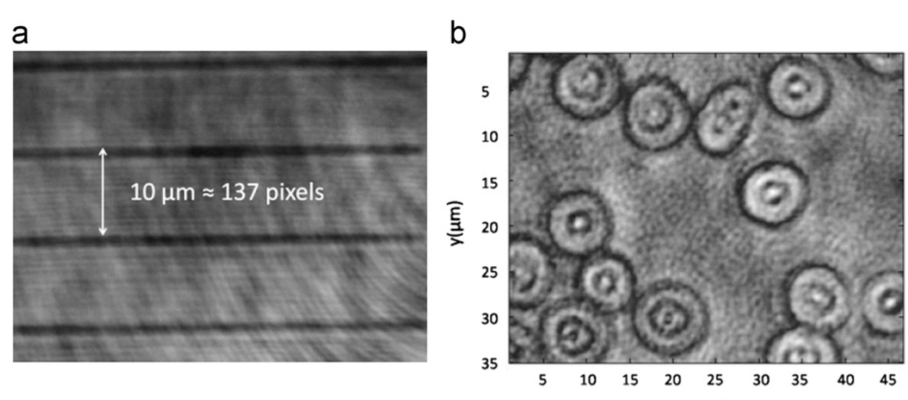

Fig. 3. Image used for calibrating the system. The figure on the left (a) shows the calibration slide image and the figure on the right (b) shows the red blood cells image captured by the CCD.

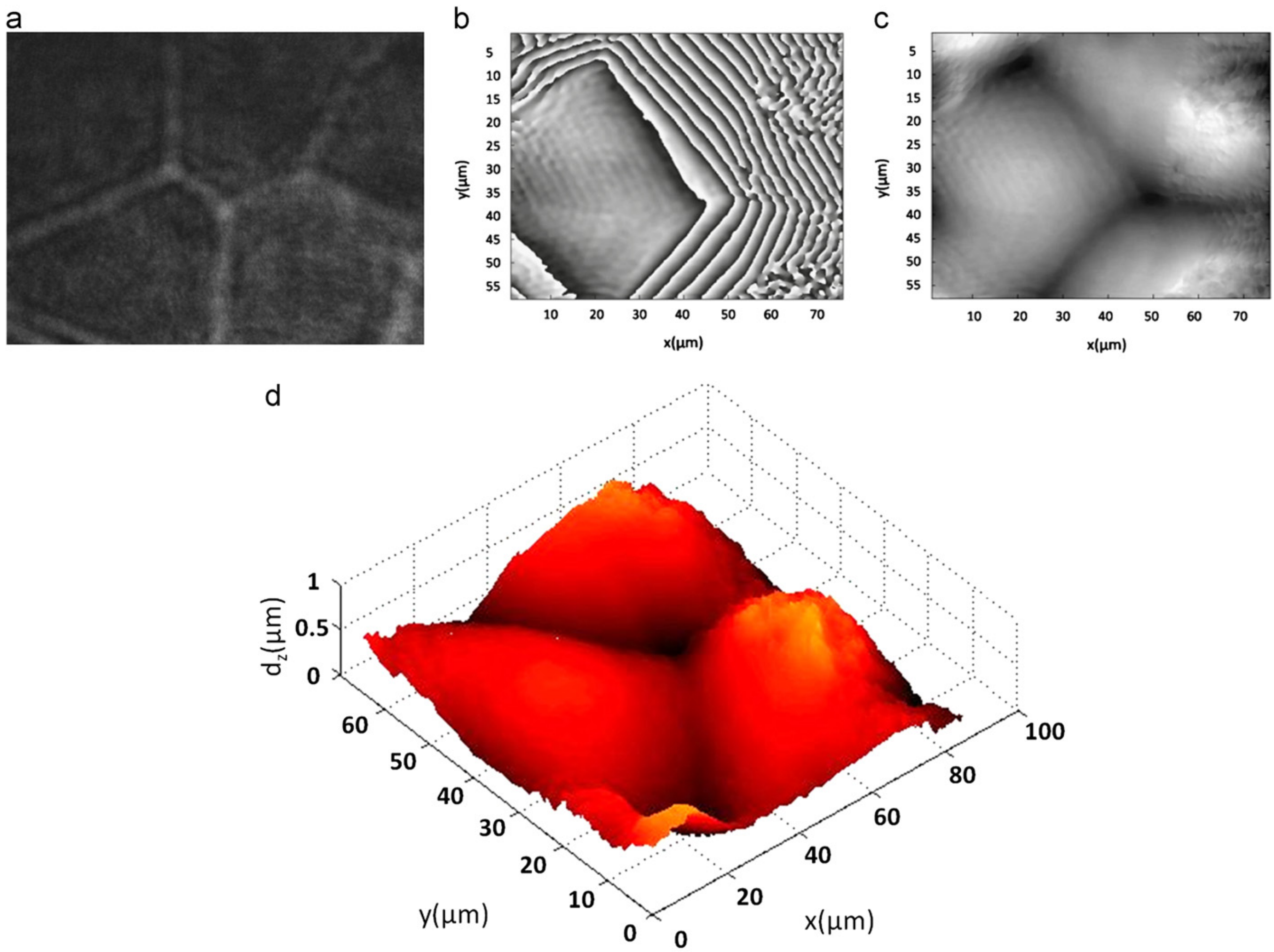

Fig. 4. Results of a thin film structure by PRDHM: (a) phase map of the object; (b) unwrapped phase map; and (c) 3D representation of the surface of the object.

the phase image, the phase map after removing the discontinuity and the 3D graphic, respectively.

In Fig. 5, the red blood cells as micro-structured samples are analyzed: (a) is displayed the hologram digitalized by a CCD camera of a sample of red blood cells, (b) shows the reconstructed amplitude image, (c) the reconstructed phase image, and (d) shows the 3D graphic. The Fig. 6 shows a single red blood cell image obtained by the PRDHM process. This result demonstrates that is possible to analyze single cells, i.e. analyze structures individually.

The results showed that our measurements are in good agreement with the expected results. Besides the characterization of the tridimensional morphology of surfaces, the technique allows the dimensions measure of the samples through calibration analysis. The literature diameter value of a red blood cell is in the range 7–9.5 μm [32], which is in accordance with the result obtained by PRDHM, Fig. 6(b). And, the method as a whole this is a system of double holography, where both the methods individually digital holographic microscopy (references [14–22]) and photorefractive holography (references [5–12,30]), have been tested and validated by countless studies by our group and others. And based on these studies and the results obtained we can say that this method has the same characteristics as the valid holographic techniques.

Additionally, when we compare with the phase-shifting holographic microscopy technique using photorefractive sillenite crystal [30], the PRDHM enables the obtainment of the phase of the object wave with only one hologram recorded in the crystal and reconstructed numerically via digital holography techniques, while using the phase-shifting techniques are required 3, 4 or 5 holographic interferograms to obtain the phase information of the object being analyzed. In the phase-shifting holographic microscopy technique using photorefractive sillenite crystal medium, the holographic recording occurs by a refractive index modulation, via photorefractive effect in diffusive regimen and the holographic reconstruction of the object wave front is made by phase-shifting technique [30]. Fig. 7 illustrates results of red blood cells using the phase-shifting technique. In this image is possible to notice the eminent presence of errors due to the PZT device and the process of the acquisition. The high quality of the results obtained with the technique of PRDHM (Fig. 5) compared to our results obtained with the photorefractive holographic microscopy using phase-shifting technique (Fig. 7 and reference [30] in manuscript) can be evaluated in the phase maps modulated obtained by both the techniques. Comparing the phase maps obtained by the two techniques, we see that in Fig. 5(b), the phase map has a much smaller amount of noise relative to the phase maps of Fig. 7(b). This is due to the intrinsic characteristics of the phase-shift technique, where the process of generating the optical phase map is a phase variation the reference beam (in this case the 4-frames technique) caused by PZT device attached to the mirror M2 (see Fig. 2 and references [5–12]). Therefore, the PRDHM presents a more efficient tool for photorefractive phase maps reconstruction in holographic microscopy.

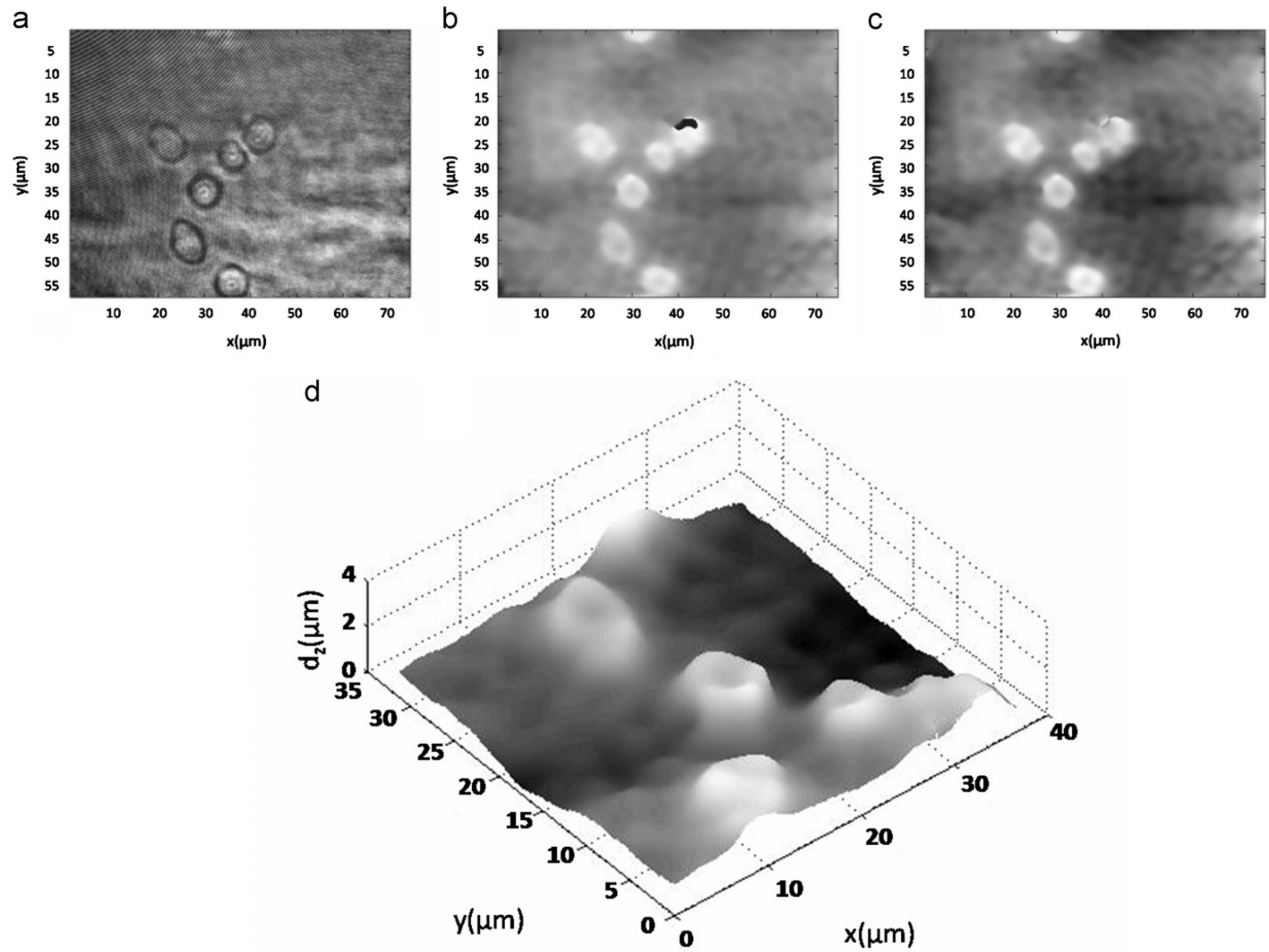

Fig. 5. Results of red blood cells sample by PRDHM: (a) hologram; (b) phase map; (c) phase map after unwrapping; and (d) image 3D representation.

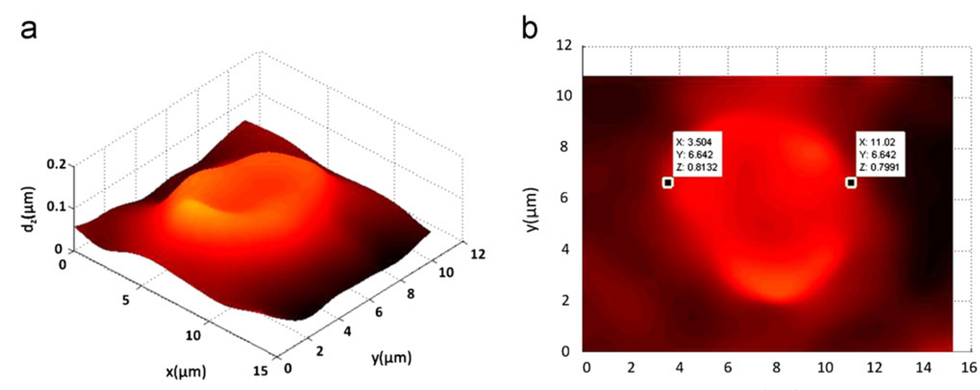

Fig. 6. (a) Individual red blood cell (erythrocyte) image obtained by PRDHM and (b) analysis of the diameter of a single red blood cell image obtained by PRDHM.

4. Conclusions

We reported an innovative method for holographic microscopy that uses an integrated system of photorefractive recording and digital holographic reconstruction, so-called Photorefractive Digital Holographic Microscopy. This system demonstrates, for the first time to the best of our knowledge, through the combination of the hologram recording on a fast photorefractive BTO crystal and reading-reconstruction hologram by digital holographic microscopy method that is possible to obtain the amplitude, phase and 3D imaging of several micro-structured samples. These images can be used in morphological characterization, displacement mapping and analysis of biological processes, among other applications. The presented results showed topographic images of samples like a thin film structure like a frost glass slide and red blood cells. These results demonstrate the advantages of this technique when compared with the phase shifting technique: one photorefractive holographic recording and high quality of results. On the other

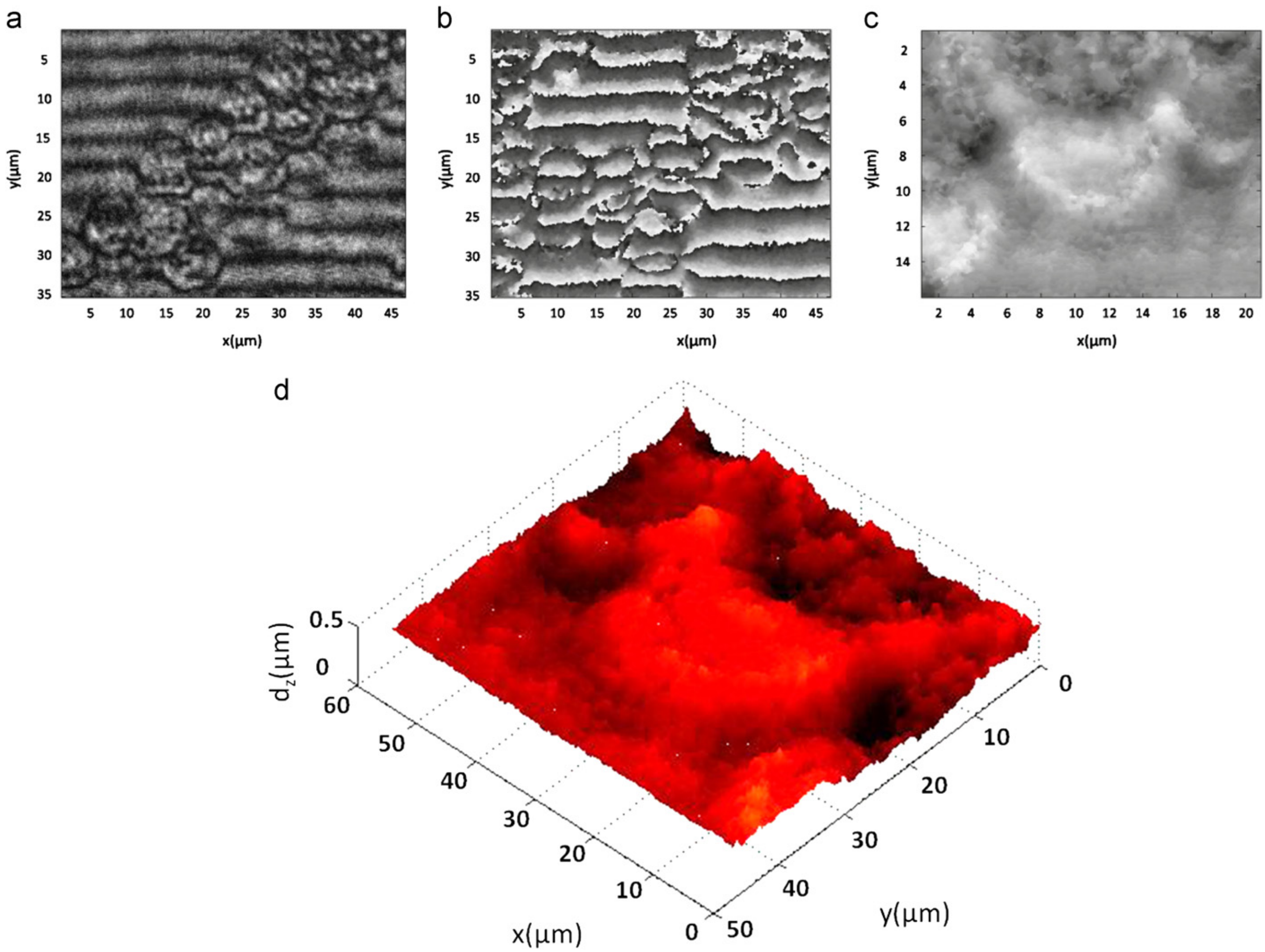

Fig. 7. (a) Sequence of interferograms of a sample of red blood cells obtained by 3 set phase-shifting technique; (b) phase map without filtering; (c) phase map after filtering; and (d) pseudo-3D image of a red blood cell.

hand, the application of photorefractive fast crystals as a holographic recording medium indicates this system can be a potential tool for dynamic holographic process recording.

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