# Phase retrieval of microscope objects using the Wavelet-Gabor transform method from holographic filters

Martín Hernández-Romo[a], Alfonso Padilla-Vivanco[a], Myung K. Kim[b] and Carina Toxqui-Quitl[a]

[a]Universidad Politécnica de Tulancingo, 100 Calle Ingenierías, Tulancingo, Hgo. México.
[b]University of South Florida, 4202 E. Fowler Avenue, Florida, United States

## ABSTRACT

An analysis of an optical-digital system based on the architecture of the Mach-Zehnder interferometer for recording holographic filters is presented. The holographic recording system makes use of one microscope objective in each interferometer arm. Moreover, the Gabor Wavelet Transform is implemented for the holographic reconstruction stage. The samples studied of this research are selected in order to test the retrieval algorithm and to characterize the resolution of the holographic recording system. In this last step, some sections of an USAF1951 resolution chart are used. These samples allow us to study the features of lighting in the recorded system. Additionally, some organic samples are used to proven the capabilities of the method because biological samples have much complex morphological composition than others. With this in mind, we can verify the frequencies recovered with each of the settings set in the retrieval method. Experimental results are presented.

**Keywords:** Digital Holography Microscopy, Wavelet transform, Three-Dimensional microscopy, Numerical Diffraction

## 1. INTRODUCTION

Holography is a technique that produces a three dimensional image of objects, having all of the information from the scene including its depth, which is commonly recorded by highly-photosensitive means. Because all registration materials respond only to the intensity of the image, it is necessary to convert the phase information from intensity variations. The amplitude and phase of the scattered wavefront, which is reflected from the objects are retrieved afterwards by displaying the hologram. The process for the recovery of the amplitude and phase of the object is divided into two stages. In the first stage, it is obtained the recorded interference pattern also known as holographic filter. The second stage is called the reconstruction of optical field, which is obtained by the diffracted light passing through the holographic filter. With the technological advancement has been possible to leave behind the analog holography giving way to digital holography in which the conventional photochemical process of holography is replaced by electronic images, bringing a new range of capabilities. Digital holography offers numerous advantages such as the rapid acquisition of holograms, the availability of full amplitude and phase information of the optical field, plus the versatility of the image processing techniques.[17] Efficiency of Digital Holography strongly depends on the resolution of the electronic sensor used for recording the holographic filters.[19] The further development of optoelectronic systems and data processing prompted the Digital Holography into new perspectives: it applies to optical distortions,[10] measurements,[14] microscopy[15] and for investigations of fluids in liquids and gases.[3] Due to the importance that the digital holography has been gaining in different areas such as in optical microscopy, where it is helpful not only to be able to see the different organisms in a plane but also to be able to observe and measure in three dimensions, it is of interest here to analyze the theoretical basis and techniques for the reconstruction of holographic filters based on the use of the Gabor Wavelet Transform. This owing to, in classical techniques such as the method of the Fresnel diffraction integral where is necessary to perform an additional processing of spatial filtering for either zero order or twin images, or even noise on interference pattern. Contrary, in the Gabor Wavelet coefficients method is possible to reconstruct the wavefront of the object from the hologram plane eliminating the spatial filtering process.

Corresponding author: E-mail: alfonso.padilla@upt.edu.mx, Tel.: +52 775 755 82 02
E-mail: martin.hernandez@upt.edu.mx

Current Developments in Lens Design and Optical Engineering XV, edited by R. Barry Johnson, Virendra N. Mahajan, Simon Thibault, Proc. of SPIE Vol. 9192, 91921G · © 2014 SPIE
CCC code: 0277-786X/14/$18 · doi: 10.1117/12.2062666

This work is organized as follows: Section 2 gives a brief review of a hologram reconstruction method based on the Gabor Wavelet transform. A system for recording hologram filters is presented in Section 3. Section 4 gives a detailed description of the experimental results applied to some biological samples. Finally, the conclusions of the work are presented in Section 5.

## 2. RECONSTRUCTION METHOD

As stated by Zhong,[6] the Gabor Wavelet namely the *mother wavelet* is defined as,

$$\psi(x,y) = \frac{1}{\sqrt[4]{\pi}}\sqrt{\frac{2\pi}{\gamma}}\exp\left[-\frac{(2\pi/\gamma)^2(x+y)^2}{2} + j2\pi(x+y)\right] \tag{1}$$

where $\gamma = \pi\sqrt{2/\ln 2}$ and $j$ is the imaginary unit. A series for analyzing wavelets namely *daughter wavelets*, which are built up by shifting, rotation and scaling of the *mother wavelet*, through the next expression,

$$\psi_{s,\theta}(x,y,a,b) = \frac{1}{s}\psi\left(\frac{x-a}{s},\frac{y-b}{s},\theta\right), \tag{2}$$

therefore the *daughter wavelets* are expressed as,

$$\psi\left(\frac{x-a}{s},\frac{y-b}{s},\theta\right) = \frac{1}{\sqrt[4]{\pi}}\sqrt{\frac{2\pi}{\gamma}}\exp\left\{-\frac{(2\pi/\gamma)^2[(x-a)^2+(y-b)^2}{2s^2}\right\} \cdot \exp\left\{j2\pi\frac{(x-a)\cos\theta+(y-b)\sin\theta}{s}\right\}. \tag{3}$$

If the hologram is created by the interference of two coherent waves in off axis geometry, where the first one is called reference wave $R(x,y)$ which has the information of the recording media and the second one called object wave $O(x,y)$ that comes from the object, then the next expressions define the two beams as follows

$$O(x,y) = o(x,y)\exp[j\phi(x,y)], \tag{4}$$

$$R(x,y) = R_0\exp\left[j\frac{2\pi}{\lambda}(x\cos\alpha+y\cos\beta)\right], \tag{5}$$

where $(x,y)$ are the coordinates of the hologram plane, $o(x,y)$ and $R_0$ are the amplitude of the waves, $\phi(x,y)$ is the phase of the object wave, $\lambda$ is the wavelength, and $\alpha$ and $\beta$ are respectively the angles between the propagation directions of the object and reference waves on the $x$ and $y$ directions. Therefore, the intensity of the hologram $I(x,y)$ can be written as

$$I(x,y) = |R_0|^2 + |o_{x,y}|^2 + R_0O(x,y)\exp\left\{j\left[-\frac{2\pi}{\lambda}(x\cos\alpha+y\cos\beta+\phi(x,y)\right]\right\}, + R_0O(x,y)\exp\left\{-j\left[-\frac{2\pi}{\lambda}(x\cos\alpha+y\cos\beta+\phi(x,y)\right]\right\}. \tag{6}$$

Hence, the 2D-Gabor Wavelet Transform for the hologram can be written as

$$\begin{aligned} W(s,\theta,a,b) &= \int\int_{-\infty}^{\infty} I(x,y)\psi^*_{s,\theta}(x,y,a,b)dxdy, \\ &= \int\int_{-\infty}^{\infty} \{A + R_0o(x)\exp[j\varphi(x,y)] + R_0o(x)\exp[-j\varphi(x,y)]\}\,\psi^*_{s,\theta}(x,b,a,b)dxdy, \end{aligned}$$

where the modulus $|W(s,\theta,a,b)|$ researches its maximum at $s = T$ and $\theta = \alpha$. Consequently, the wavelet coefficient at the peak of the 2D-GWT are described by,

$$W_p(a,b) = \frac{\sqrt{\gamma^3}}{\sqrt[4]{4\pi^3}}R_0o\exp\left\{j\left[-\frac{2\pi}{\lambda}(x\cos\alpha+y\cos\beta)+\phi(x,y)\right]\right\}. \tag{7}$$

Multiplying the $W_p$ by an ideal wave corresponding to a replica of the reference wave, then the reconstructed wave $U_p(x, y)$ at the hologram plane is obtained as follows

$$\begin{aligned} U_p(x, y) =& W_p(x, y) R_0 \exp\left[j \frac{2\pi}{\lambda}(x \cos\alpha + y \cos\beta)\right] \\ =& \frac{\sqrt{\gamma^3}}{\sqrt[4]{4\pi^3}} R_0^2 \exp[\phi(x, y)] \end{aligned} \tag{8}$$

As it can be seen, the reconstructed wave $U_p$ from $W_p$ is equal to the object wave at the hologram plane multiplied by a constant coefficient.

## 3. SYSTEM FOR RECORDING HOLOGRAM FILTERS

The Holographic Microscopic System is depicted in the Figure 1, this system is based on Mach-Zehnder interferometer. A laser beam followed by a spatial filter (SF) and illumination control system (P1, P2, Cl) is separated into two beams by a beam splitter BS1. One beam serves as a reference wave and another beam illuminates the samples. A objective lens MO1 produces a magnified image of the sample and another objective lens MO2 is placed in the reference arm to get matching of the wavefront curvatures. The beam splitter BS2 placed in front of the CCD sensor combines the two beams before they are recorded by the CCD camera.

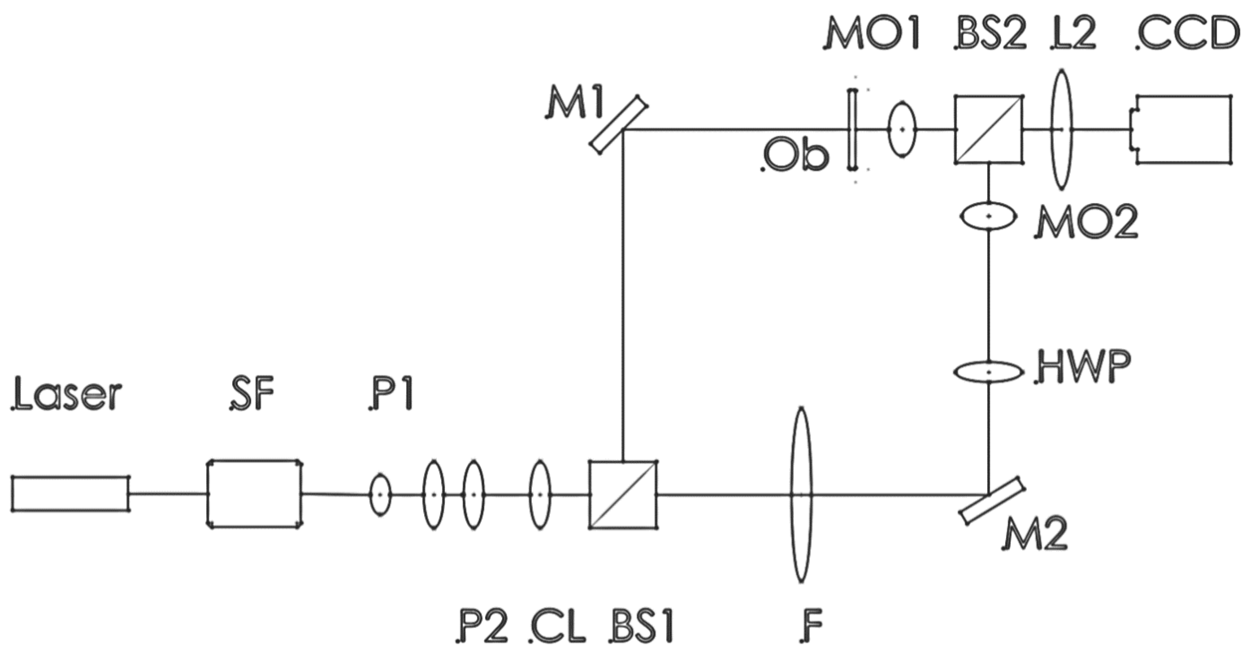


Figure 1. Digital Holographic Microscopic System Scheme

A photograph of the setup is presented in Figure 2. The objective lenses have an amplification of 10X, a numerical aperture of 0.3, a resolution of 1.198$\mu m$, and a work distance of 5.2$mm$. The Figure 2.b shows a section of the setup.

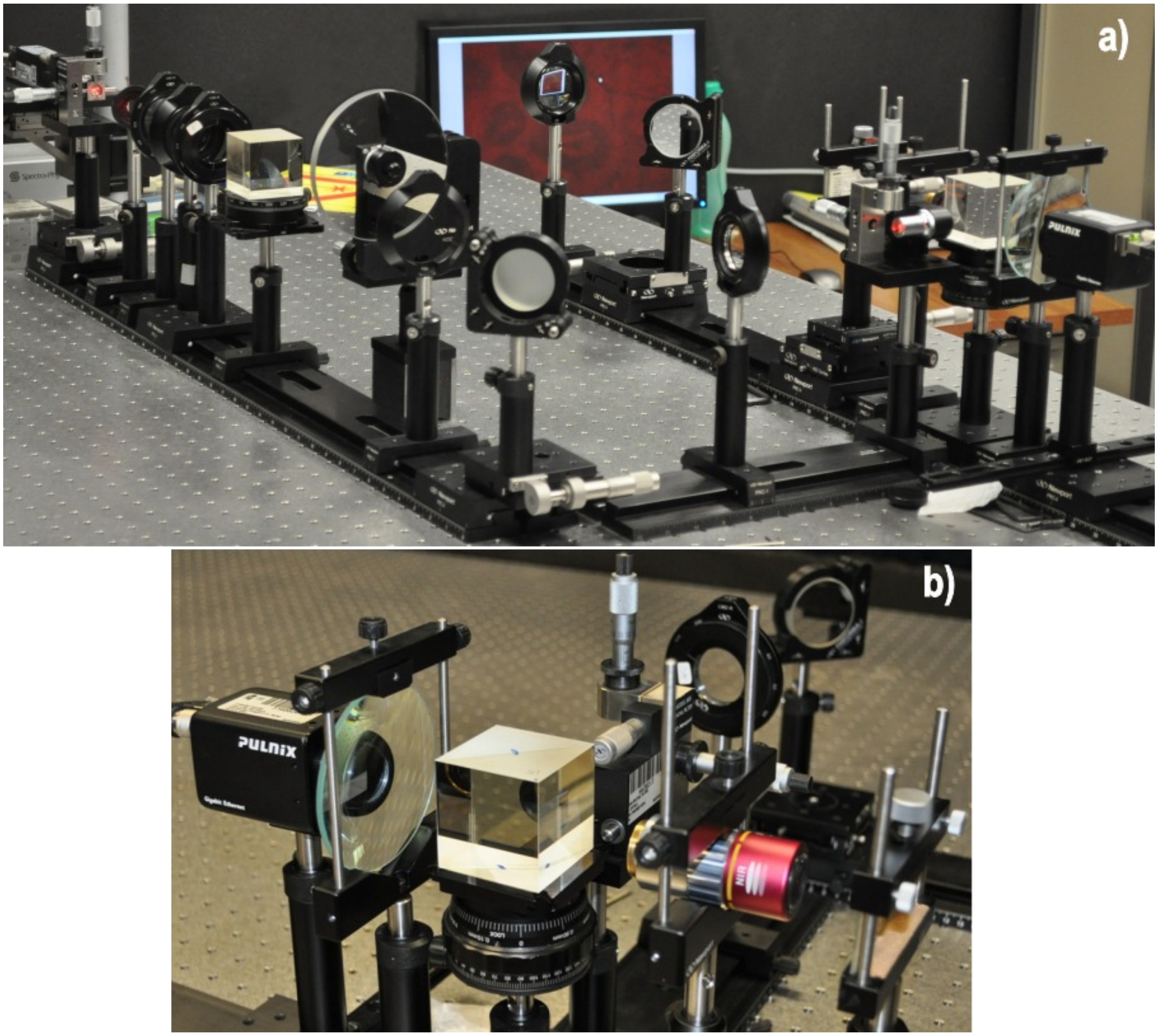


Figure 2. Digital Holographic Microscopic System

## 4. EXPERIMENTAL RESULTS

An example of the reconstruction process is shown in the Figure 3. We can observe here how the hologram is obtained from a resolution chart USAF1951. Also, we can see the amplitude and phase reconstructed in the peak of the wavelet coefficients as shown in the Figures 3.b and 3.c. In order to compensate for phase aberrations and the image distortion, a reference hologram without the presence of any specimen is also recorded. The compensate results are shown in the Figure 4, also in the Figure 4.d is shown a section of the phase retrieval. Finally, a 3D representation of the hologram is shown in the Figure 4.e.

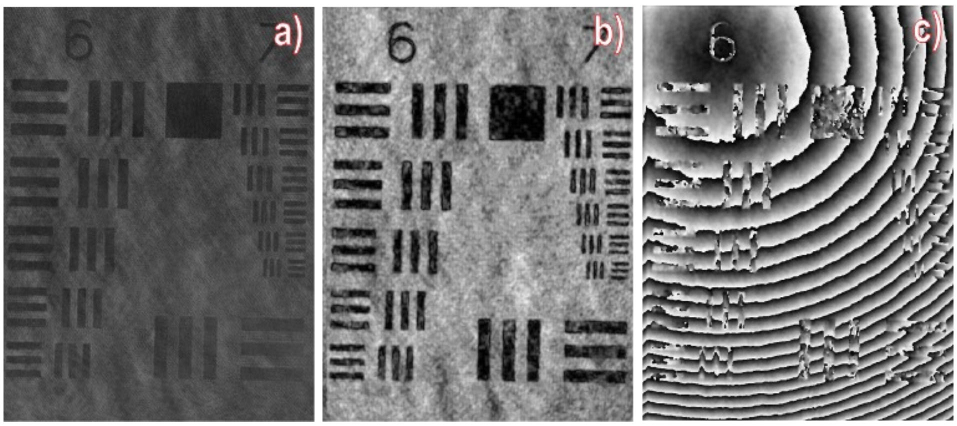


Figure 3. Resolution chart USAF 1951 a) Hologram; b) the amplitude; c) The wrapped phase

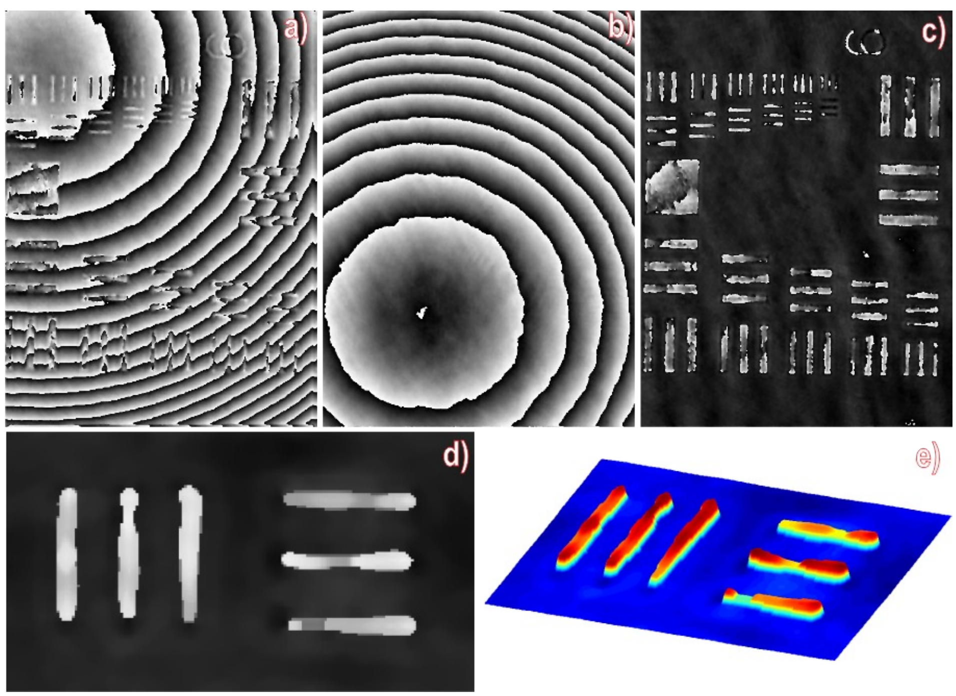


Figure 4. Correction of the phase aberration a) Wrapped phase; b) phase of the reference hologram; c) the unwrapped phase; d) the unwrapped phase and e) 3D reconstruction of the hologram

Another reconstruction case is the Elderberry pith cells which are shown in the Figure 5a. The Figures 5.b and 5.c show the reconstruction of both amplitude and the phase of the hologram. The unwrapped phase is shown in the 5.d. The 3D view of the phase is shown in the Figure 5.e. As we can be seen in the last Figure some peaks of singular values are produced, they are caused by the numerical compensation that was done to the phase.

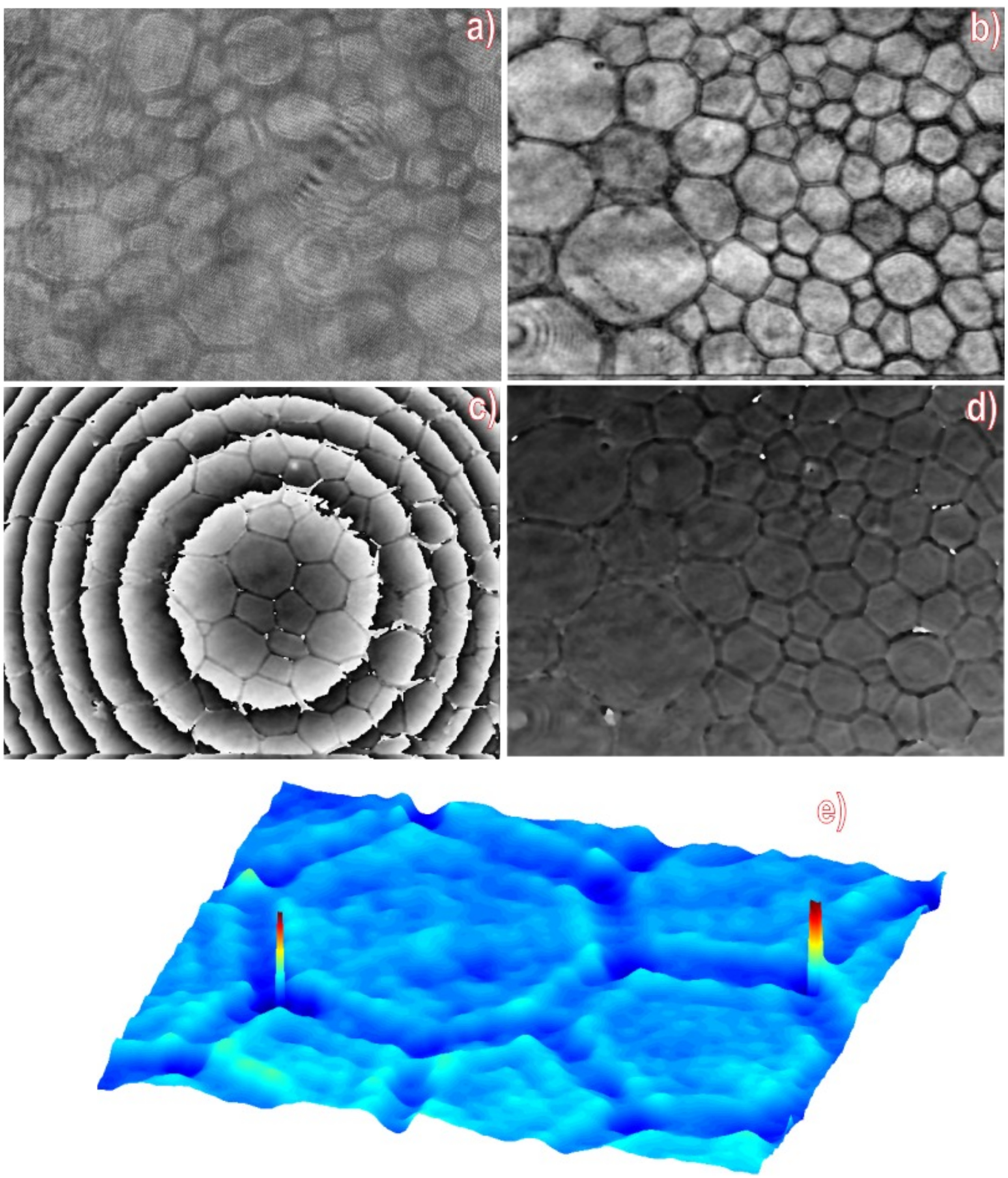


Figure 5. Elderberry pith cells specimen a) Hologram; b) the amplitude; c) the wrapped phase; d) the unwrapped phase and e) 3D reconstruction of the hologram

In the Figure 6, a second reconstruction of a biological sample is shown. In this case, a rabbit intestine section is used as input object, this sample has interesting absorption characteristics which permit to obtain a three-dimensional visualization of the instenstine. In the Figures 6.b and 6.c are respectively shown the reconstruction of the amplitude and phase of the hologram. The unwrapped phase is shown in the 6.d. Finally, the Figure 6.e shows the 3D view of the phase.

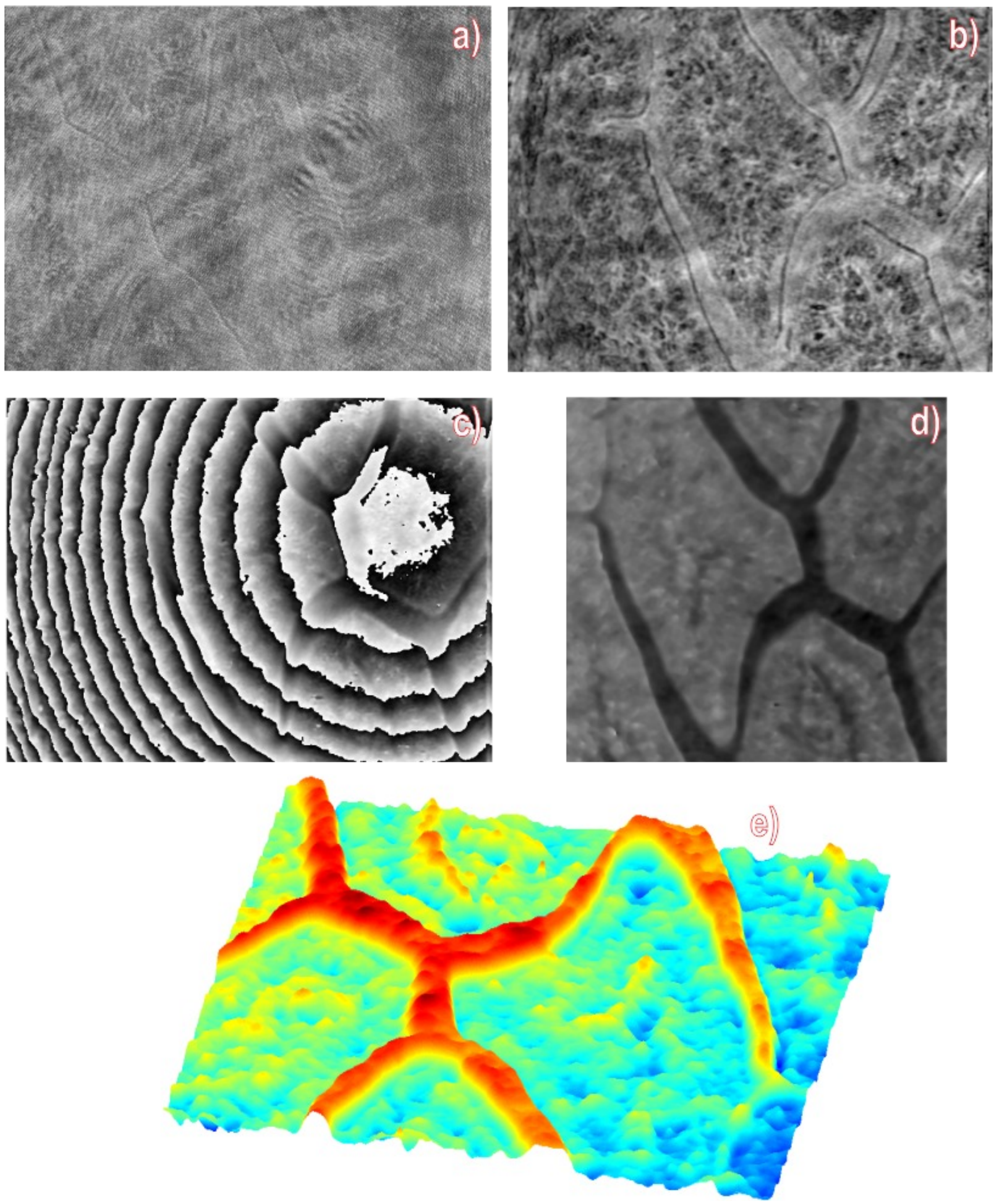


Figure 6. Rabbit intestine a) Hologram; b) the amplitude; c) the wrapped phase; d) the unwrapped phase and e) 3D reconstruction of the hologram

Additionally, the amplitude image is improved by using different reconstruction planes as shown in the Figure 7. In the Figure 7.b is shown the reconstructed in the peak of the wavelet coefficients. Some different planes are used for the reconstruction as the maximum and minimum values as shown respectively in the Figures 7.c and 7.d. In the Figure 7.e, is shown the calibration of the amplitude reconstructed without noise.

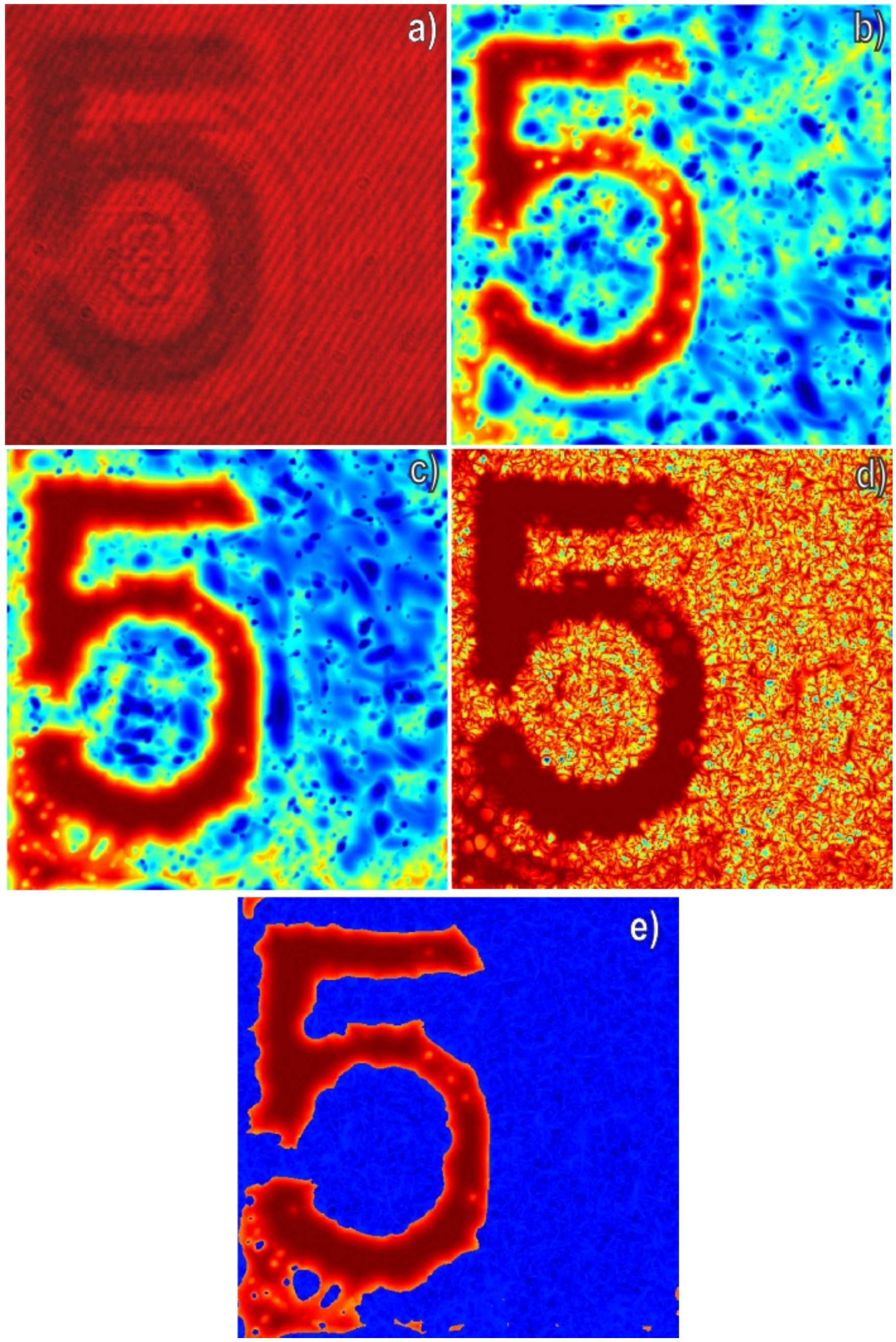


Figure 7. Resolution chart USAF 1951 a) Hologram; b) Peak plane; c) Maximum value plane; d) Minimum value plane and e) Amplitude correction

## 5. CONCLUSIONS

In this paper, it has been described an off-axis transmission Digital Holographic Microscope for measuring the phase of a specimen. We have combined a holographic reconstruction algorithm based on the Gabor wavelet transform and an aberration compensation method through a reference hologram, of the optical devices used during the recording of the hologram filters. All these procedures are usefull in order to have the amplitude and phase of the specimen. With the reconstruction algorithm, the zero order and the twin holographic image are not arise, so that as it can be seen some interesting results of high quality for holographic reconstruction are obtained. To achieve high axial resolution in the phase image we have applied a reference hologram to correct parasitic phase aberrations. This methodology should be useful for inspecting biological structures and and other industrial specimens.

## ACKNOWLEDGMENTS

This research was supported by National Council for Science and technology (CONACyT) under the Grant No. 303531.

## REFERENCES

[1] Denisyuk, Y. N. Holographic art with recording in three-dimensional media on the basis of Lippman photographic plates, Optics Applied to Metrology (pp. 365-368), (1978).
[2] Yu, F., Yang, X., Introduction to Optical Engineering, Cambridge, (1997).
[3] Gu, C., Holographic memory for high-density data storage and high-speed pattern recognition, In Photonics Asia 2002 (pp. 198-207). International Society for Optics and Photonics, (2002).
[4] Chen, N., Zhong, J., Application of wavelet transform to 3D shape measurement, Chinese Optical Letters, Vol 1, No. 11, (2003).
[5] Hariharan, P., Optical Holography, Principles, techniques, and applications., second Edition, Cambridge Studies in Modern Optics.(2004).
[6] Zhong, J., Weng, J., Phase retrieval of optical fringe pattern form the ridge of a wavelet transform. Optics Letters, Vol. 30, No. 19, 2560-2562, (2005).
[7] Jones, K., Wavelet Image Processing Applied to Optical and Digital Holography: Past Achievements and Future Challenges, Wavelets XI, Proc. of SPIE Vol. 5914 591423-1, (2005).
[8] Poon, T.C., Digital Holography and Three Dimensional Display: Principles and Applications, Springer, ISBN 0-387-31340-0, (2006).
[9] Colomb, T., Cuche, E., Charrire, Florian., Automatic procedure for aberration compensation in digital holographic microscopy and applications to specimen shape compensation, Applied Optics, 0003-6935/06/050851-13, (2006).
[10] Malacara, D., Óptica tradicional y moderna, Fondo de cultura economica USA, (2007)
[11] Poon, T.C., Optical Scanning Holography with MatLab, Springer, (2007).
[12] Kühn, J., Charrière, F., Digital holographic microscopy for nanometric quality control of micro-optical components,Integrated Optoelectronic Devices, (2007).
[13] Khmaladze, A., Kim, M.k., Lo, C., Phase imaging of cells by simultaneous dual-wavelength reflection digital holography, Optics express, 16(15), 10900-10911, (2008).
[14] Yoshikawa, T., Handbook of optical metrology principles and applications, CRC Press, (2009).
[15] Weng, J., Zhong, J., Phase reconstruction of digital holography with peak of two-dimensional Gabor wavelet transform, Appl.Opt. 48 (2009).
[16] Mundt, J., Kreis, T., "Digital holographic recording and reconstruction of large scale objects for metrology and display", Optical Engineering, 49(12), 125801-125801. (2010).
[17] Kim, M.k., Principles and techniques of digital holographic microscopy, Journal of Photonics for Energy, 018005-018005, (2010).
[18] Voelz, D., Computational Fourier Optics a *MatLab*® tutorial, SPIE PRESS, (2011).
[19] Picart, P.,Li, J., Digital Holography, Wiley, (2012).
[20] Brooker, G., Modern Classical optics, Oxford,(2013).