International Journal of Physical Sciences Vol. 8(2), pp. 68-72, 16 January, 2013
Available online at http://www.academicjournals.org/IJPS
DOI: 10.5897/IJPS12.663
ISSN 1992 - 1950 ©2013 Academic Journals

Full Length Research Paper

Digital in-line holography for blood cell

Adnan Salih[1]* and Khawla J. Tahir[2]

[1]College of Science for Women, Baghdad University, Iraq.
[2]College of Science, Karbala University, Iraq.

Accepted 14 January, 2013

This paper investigates the application of Fresnel based numerical algorithms for the reconstruction of Gabor in-line holograms. A simple in-line digital holographic system was used to record blood cell. The Fresnel transformation (FS) method was used for reconstruction of the hologram. High contrast blood cell images were used to demonstrate numerical reconstruction process by Matlab. Sine, cosin, and amplitude holograms were reconstructed. This method provides new insights into the dynamics of the red blood cells and will be further used to investigate the effect of physiological and pharmacological effectors on RBCs. The spontaneous cell membrane fluctuations (CMF) of the red blood cell (RBC) was investigated, by calculating both thickness and refraction index of the cell blood.

Key words: Digital holography, blood cell, refractive index.

INTRODUCTION

Holography (Gabor, 1948; Leith and Upatnieks, 1964) is an imaging technique made up of two parts, recording and reconstruction. Digital holography differs from conventional holography in that a digital camera (Charge coupled device/complementary metal oxide semiconductor; CCD/CMOS) is used in place of photographic film or holographic plates. Reconstruction of the hologram is then performed numerically on a computer (Kreis, 2005; Schnars and Jüptner, 2004). The concept of digital holography emerged in the 1960s, as reviewed in Lesem et al. (1968). Numerical reconstruction techniques for optically recorded holograms had been applied. Digital holography greatly simplifies the hardware setup for cinematic holographic recording and reconstruction (Goodman and Lawrence, 1967). Despite these promising prospects, digital holography is inherently limited by the poor resolution of solid-state image sensors. Currently, the pixel size of most scientific CCD sensors is in the range of 6 to 10 µm, compared with the silver-halide holographic films with an equivalent pixel size down to 0.1 µm. Because the digital sensor elements cannot resolve interference fringes finer than the pixel size, the permissible angle between object wave and reference wave is limited to a few degrees (Pereira and Gharib, 2002; Murata and Yasuda, 2000). A digital hologram is created by the interference between a coherent object and reference beam, which is digitally recorded by CCD camera and processed by computational methods to obtain the holographic images. The digital hologram contains not only amplitude information of the object, but also phase (Myuny et al., 2006). Moreover, the ability of the CCD camera to quantify recorded light gives rise to a number of post processing methods that can for instance be used to calculate optical thickness or refractive index variations of an object provided knowledge of one of the other is available. Digital holography not only offers quantitative information phase but high fidelity and high resolution images (Peng et al., 2001).

This advance was facilitated by the availability of digital cameras with high spatial resolution and high dynamic range. The output of a numerical reconstruction is, in general, a complex two-dimensional representation of the wave front at a single distance from the camera plane, and so we refer to the digital capture combined with numerical reconstruction as an imaging system. The novel microscopic principle originally proposed by Gabor (1948) is the simplest realization of holography and has been coined digital in-line holographic microscopy (DIHM) (Coupland and Bera, 2008). Essentially, all past and present light microscopy of biological systems has been achieved through the lens of the compound microscope, which yields high spatial resolution at the cost of shallow depth of focus and has, in the process,

*Corresponding author. E-mail: dradnan_salih@yahoo.com.

condemned biological microscopy to a century of histological study made possible only through microtomy. Digital in-line holography (DIH) has come to represent a new tool for biological applications, supplementing conventional compound light microscopy, its simplicity of the microscope: In-line holography is microscopy without objective lenses. The hardware required is a laser, pinhole, and a CCD camera (Wenbo et al., 2001).

This configuration leads to a new application field, named as digital holographic microscopy (DHM), which takes the advantages of digital holography concerning the numerical manipulation of the complex object wave front in addition with the appealing properties provided by microscopy. In that sense, microscopy expands up the capabilities of digital holography in a similar way than digital holography, and also improves some aspects of microscopy by avoiding the limited depth of focus in high numerical aperture (NA) lenses and the high magnification ratios needed in conventional optical microscope imaging. Thus, DHM is coherent imaging methods allowing instantaneous and quantitative acquisition of both amplitude and the phase information of the objects have diffracted wave front. Imaging of phase distributions with high spatial resolution can be used to determine refractive index variations as well as the thickness of the specimen (Cuche et al., 1999).

The main aim of this study is to use in-line digital holography to record blood cell, investigate the spontaneous cell membrane fluctuations (CMF) of the red blood cell (RBC), and to demonstrate the numerical reconstruction process of holograms in different planes based on the Fresnel transformation (FS). This approach to digital hologram recording and numerical reconstruction not only eliminates wet chemical processing and mechanical scanning but also enables the use of complex amplitude information that is inaccessible in optical reconstruction.

METHODOLOGY

Fresnel transformation method

The FS is the most commonly used method in holographic reconstruction, because of the computational efficiency. The approximation of a spherical Huygens wavelet by a parabolic surface allows the calculation of the diffraction integral using a single Fourier transform. PSF can be simplified by the Fresnel approximation as (Montfort et al., 2006; Charri`ere et al., 2006):

$$S(x,y;z) = -\frac{ik}{2\pi z}\exp[ikz + i\frac{k}{2z}(x^2+y^2)] \quad (1)$$

Where s(x, y; z) Fresnel diffraction, z is distance and k is the wave number of light $K = 2\pi/\lambda$

And the reconstructed wave field is;

$$E(x,y;z) = -\frac{ik}{2\pi z}\exp[ikz + \frac{ik}{2z}(x^2+y^2)]$$
$$\times\iint E_0(x_0,y_0)\exp[\frac{ik}{2z}(x_0{}^2+y_0{}^2)]$$
$$\times\exp[-\frac{ik}{2z}(xx_0+yy_0)]dx_0dy_0$$
$$= \exp[\frac{ik}{2z}(x^2+y^2)]\xi[E_0S] \quad (2)$$

Where $E_0(x_0,y_0)$ is the modified wave field, x_o, y_o of the point object, and the spatial variation governed by a sine function with a quadratic spatial dependence. x, y and $\xi[E_0S]$ denote the spatial coordinates in the hologram and reconstruction plane, respectively

Fresnel diffraction of a beam during propagation. The resolution Δα of the reconstructed images determined directly from the Fresnel diffraction formula will vary as a function of the reconstruction distance z (Gabolde and Trebino, 2006).

$$\Delta\alpha = \frac{\lambda z}{N\,\Delta x_0} \quad (3)$$

Where N is the number of pixels and Δx_0 is the pixel width of the CCD camera, $\lambda = 532\ nm$ (wave length). As with the Huygens convolution method, there is a minimum z distance requirement set by Equation 4 (Schnars and Jueptner, 1994).

$$z_{\min} = \frac{a_x^2}{n_x\lambda} \quad (4)$$

Where $a_x = n_x\,\Delta x$ is the size of the hologram and n_x, Δx are the number and size of pixels, λ is wave length of light. At a very close distance, the spatial frequency of the hologram is too low and aliasing occurs. Normally, the object is placed just outside this minimum distance. Camera (1024 × 1024 pixels) resolution (Δα = 0.225 µm), (NA = 1.4),

Reconstruction in-line digital holography

This represents the reconstruction of sine-hologram, the sine-hologram and the cosine hologram will be expressed in terms of spatial frequencies. Therefore, we have (Lohmann and Rhodes, 1978; Poon, 2006; Poon and Kim, 2006; Yamaguchi and Zhang, 1997)

$$i_c(x,y) = Re\left[\int \mathcal{F}^{-1}\{\mathcal{F}|\Gamma_o|^2 OTF_\Omega\}dz\right]$$
$$= \text{Hsin}\,(x,y) \quad (5)$$

$$i_s(x,y) = Im\left[\int \mathcal{F}^{-1}\{\mathcal{F}\{|\Gamma_o(x,y;z)|^2 OTF_\Omega\}\,dz\right]$$
$$= Hcos\,(x,y) \quad (6)$$

Where

$$OTF_\Omega(k_x,k_y;z) = \exp\left[-j\frac{z}{2k_o}(k_x^2+k_y^2)\right]$$
$$= OTF\ \ (k_x,k_y;z) \quad (7)$$

$Im[.]$ denotes the imaginary part of the quantity within the bracket, Re[.] denotes the real part of the content inside the bracket, OTF_Ω optical transfer function, an amplitude transparency of $\Gamma_o(x,y;z)$

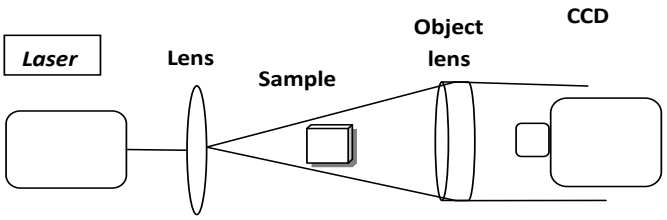

Figure 1. In-line digital holographic setup.

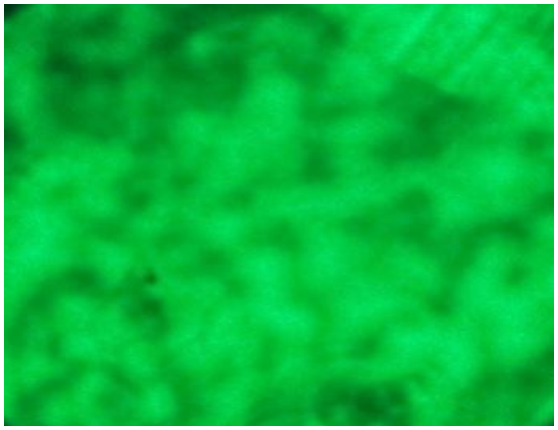

Figure 2. Recorded hologram.

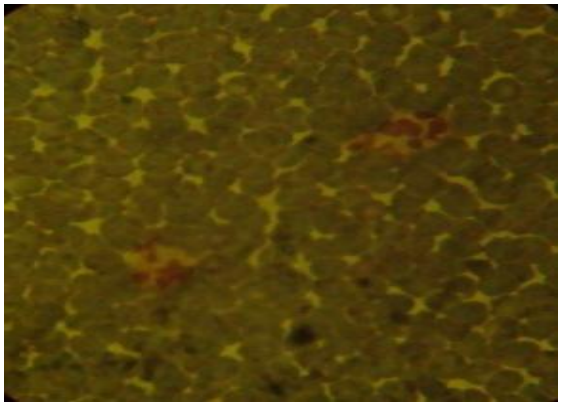

Figure 3. Reconstructed hologram (1024 × 1024) pixels.

located at a distance $\mathbf{z}$,,$\mathcal{F}^{-1}$. The spatial impulse response of the optical system shows the sine-hologram and the cosine-hologram. Since the two holograms can be stored digitally, we can also construct a complex digital hologram by using Equation 8.

$$H_{c\pm}(x,y) = H_{cos}(x,y) \pm jH_{sin}(x,y) =$$
$$\int\{|\Gamma_o(x,y;z)|^2 * \frac{k_o}{2\pi z}\exp\left[\pm j\frac{k_o}{2\pi z}(x^2+y^2)\right]\}dz \quad (8)$$

$H_{c\pm}(x,y)$ is called a complex hologram; $H_{cos}(x,y)$ is the cosine hologram, and $H_{sin}(x,y)$ is the sine hologram; the sine-hologram will be expressed in terms of spatial frequencies. To obtain real image reconstruction formed in front of the hologram, we will use the following equation:

$$H_{any}(x,y) * h(x,y;z_o) \quad (9)$$

Where $H_{any}(x,y)$ represents any holograms, that is, the sine-hologram, the cosine-hologram or the complex hologram.

For digital reconstruction, we will simply convolve the above holograms with the spatial impulse response in order to simulate Fresnel diffraction for a distance of Z_o. To obtain real image, reconstruction is formed in front of the hologram, a planar object at a distance of Z_o away from the x-y scanning mirrors, that is,

$$|\Gamma_o(x,y;z)|^2 = I(x,y)\delta(z-z_o)$$

Where $I(x,y)$ is the planar intensity distribution.

Reconstructed real image is

$$\boldsymbol{H_{C+}(x,y) = H_{cos}(x,y) + jH_{sin}(x,y)} \quad (10)$$

Note that the complex hologram is constructed as

$$\boldsymbol{H_{C-}(x,y) = H_{cos}(x,y) - jH_{sin}(x,y)} \quad (11)$$

Experiments

The experimental setup is shown in Figure 1. The sample (blood smear) illuminated with a green 532 nm Nd:YAG laser. A digital camera (1024 × 1024 pixels) resolution (Δα = 0.225 μm), (NA = 1.4), replaces the film. The unscattered part of the illumination wave serves as the reference wave to interfere with the waves scattered by the particles (object). The interference fringes, whose spatial frequency is proportional to the angle formed by the scattering direction and the reference beam direction.

In digital holography, an image sensor records the interference pattern between the light beam scattered by the object under study and a reference beam as shown in Figure 2. This interference pattern is processed in the computer to reconstruct an image of the diffracting object by simulating the reference and using Fresnel propagation.

Then it will have a reconstructed virtual image that is located at a distance of Z_o behind the hologram by computer simulation of digital holography which has been developed (Matlab).

RESULTS AND DISCUSSION

The recording hologram was reconstructed by using Fresnel propagation; the program was designed using Matlab. A reconstructed image for blood cell is shown in Figure 3. The color of the image is shown by Matlab program. To get a clear and magnifying picture, Equations 5 and 6 were solved using Matlab. The results are shown in Figure 4a and b.

Where sigma = $Z_o/2K_o$

We can also construct a complex hologram by using

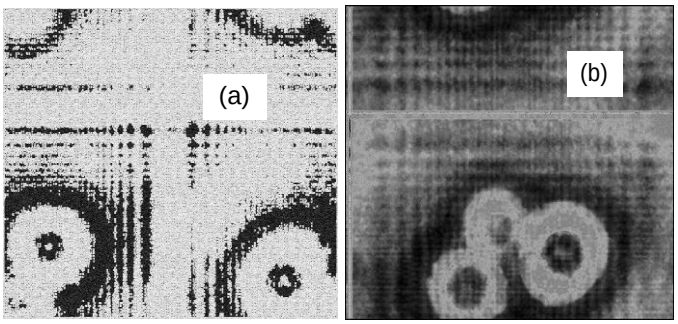

Figure 4. (a) Recorded sine hologram, (b) reconstruction sine hologram.

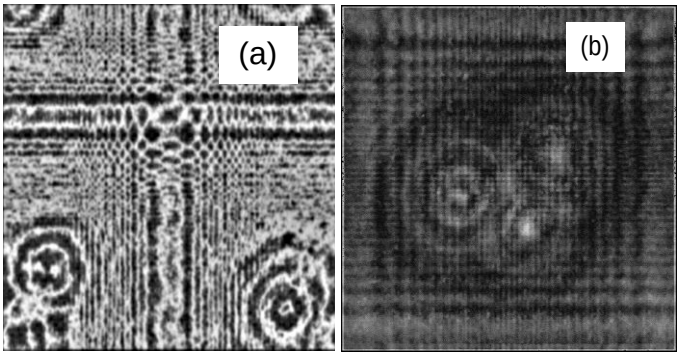

Figure 5. (a) Recorded cosine, (b) reconstruction cosine hologram.

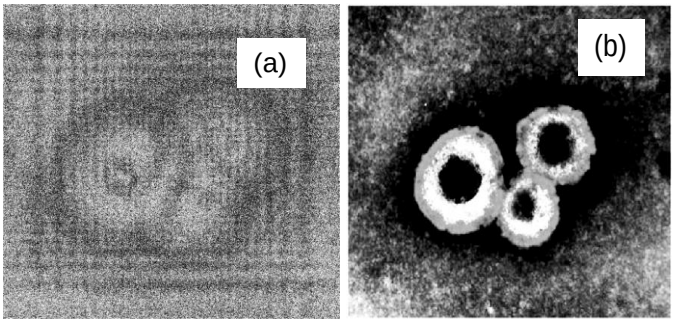

Figure 6. (a) Phase image, (b) amplitude image.

Equation 8. For digital reconstruction, we will simply convolve the above holograms with the spatial impulse response in order to Fresnel diffraction for a distance of Z_o. To obtain real image reconstruction formed in front of the hologram where we have used Equation 9 with the spatial frequency response, to obtain the last step (Figure 4b and 5b) which show the reconstruction of the sine-hologram and the cosine-hologram, respectively.

The complex hologram obtained and reconstructed virtual image that is located at a distance of Z_o behind the

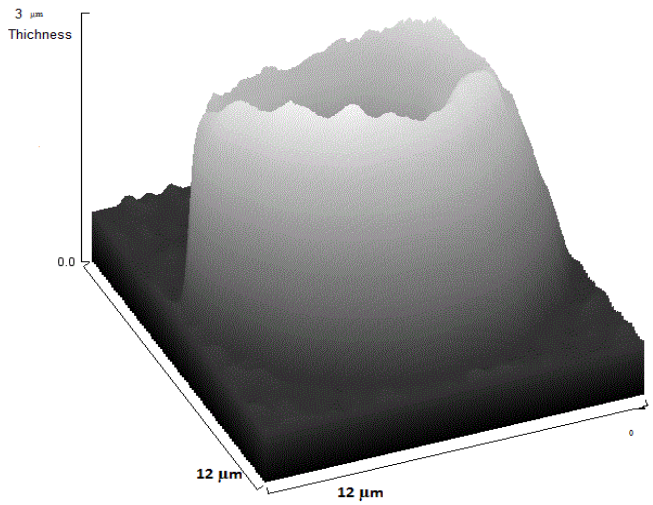

Figure 7. Draw surface of a red blood cell

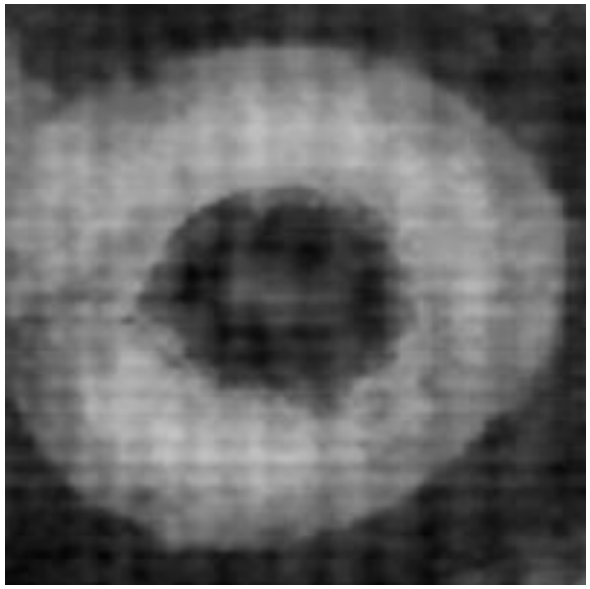

Figure 8. Red blood cell.

hologram by solving Equations 10 and 11 images was formed as shown in Figure 6a and b, respectively.

Thickness of the red blood cell was calculated by taking a picture shown in Figure 7, and it was plotted in a three-dimensional Matlab program and cell shape as shown in Figure 8, and the thickness of the cell was 2.2 µm.

From Figure 7, one can be able to know the aid in the diagnosis of malaria disease. The pathogenesis of malaria is largely due to stiffening of the infected RBCs, contemporary understanding ascribe the loss of RBC deformability.

The spontaneous CMF of the RBC has been investigated. DHM as an interferometric technique is able

to accurately provide the wave front deformation induced by a transparent specimen. After learning, thickness could account for refraction index by Equation (12) (Curl et al., 2005) as follows:

$$n_{c,i} = 1/h_i \int_0^{h_i} n_{c,i}(z)dz \quad (12)$$

Where z is the axial coordinate, hi is the cellular thickness, $n_{c,i}(z)$ is the function representing the value of the intracellular refractive index along the cellular thickness hi. The refraction index values ($n_{c,I}$ = 1.3847± 0.0003) and the mean refraction index measurements of different erythrocyte regions do not present statistically significant spatial variations. Such a spatially homogeneous intracellular refractive index is consistent with a homogeneous erythrocyte cytoplasm

Conclusion

This paper investigates the application of Fresnel based numerical algorithms for the reconstruction of Gabor in-line holograms. We focus on the two most widely used Fresnel approximation algorithms, the direct method. Both algorithms involve calculating a Fresnel integral, but they accomplish it in fundamentally different ways. The algorithms are performed differently for different physical parameters such as distance, CCD pixel size, and so on. We investigate the constraints for the algorithms when applied to in-line Gabor DHM. We show why the algorithms fail in some instances and how to alter them in order to obtain useful images of the microscopic specimen. We verify the altered algorithms using an optically captured digital hologram. This way was used to get a clear picture of the magnifying, as well as the erythrocyte CMF. This method provides new insights into the dynamics of the RBCs and will be further used to investigate the effect of physiological and pharmacological effectors on RBCs.

REFERENCES

Charri`ere F, Colomb T, Montfort F, Cuche E, Marquet P, Depeursinge C (2006). Shot-noise in flounce on the reconstructed phase image signal to noise ratio in digital holographic microscopy. Appl. Opt. 45(29):7667-7673.

Coupland J, Bera JL (2008). Optical tomography and digital holography. Meas. Sci. Tech. P. 19.

Cuche E, Marquet P, Depeursinge C (1999). Simultaneous amplitude-contrast and quantitative phase contrast microscopy by numerical reconstruction of Fresnel off-axis holograms. App. Opt. 38(34):6994-7001.

Curl CL, Bellair CJ, Harris T, Allman BE, Harris PJ, Stewart AG, Roberts A, Nugent KA, Delbridge LM (2005). Refractive index measurement in viable cells using quantitative phase-amplitude microscopy and confocal microscopy. Cytometry A65(1):88-92.

Gabolde P, Trebino R (2006). Single-shot measurement of the full spatial -temporal field of ultra short pulses with multi-spectral digital holography. Opt. Express 14:11460-11467.

Gabor D (1948). A new microscopic principle. Nature 16:777.

Goodman JW, Lawrence RW (1967). Digital image formation from electronically detected holograms. Appl. Phys. Lett. 11:77.

Kreis T (2005). Handbook of Holographic Interferometry. Wiley-VCH GmbH & Co. KGaAWeinheim.

Leith EN, Upatnieks J (1964). Wavefront Reconstruction with Diffused Illumination and Three-Dimensional Objects. J. Opt. Soc. Am. 54:1295.

Lesem LB, Hirsch PM, JA Jordan (1968). Computer synthesis of holograms for 3-D display. Commun. ACM 11:661.

Lohmann AW, Rhodes WT (1978). Two-pupil synthesis of optical transfer functions. Appl. Opt. 17:1141-1150.

Montfort F, Charri`ere F, Colomb T, Cuche E, Marquet P, Depeursinge C (2006). Purely numerical compensation for microscope objective phase curvature indigital holographic microscopy: Influence of digital phase maskposition. J. Opt. Soc. Am. A23(11):2944-2953.

Murata S, Yasuda SN (2000). Potential of digital holography in particle measurement. Opt. Laser Tech. 32:567-574.

Myuny KK, Lingfeng Y, Christopher JM (2006). Interference techniques in digital holography. J. Opt. A8:518-523.

Peng X, Miao J, Asundi A (2001). Studies of digital microscopic holography with applications to microstructuretesting. Appl. Opt. 40:5046-5051.

Pereira FP, Gharib M (2002). Defocusing digital particle image velocity and the three dimensional characterization of two phase flows. Meas. Sci. Tech. 13:683-694.

Poon TC (2006). Horizontal-parallax-only optical scanning holography, in chapter 10 of Digital Holography and Three-Dimensional Display: Principles and Applications, T.C. Poon ed., Springer, New York, USA.

Poon TC, Kim T (2006). Engineering Optics with MATLAB®. WorldScientific Publishing Co., Singapore.

Schnars U, Jueptner WP (1994). Direct recording of holograms by a CCD target and numerical reconstruction. Appl. Opt. 33:179-181.

Schnars U, Jüptner WPO (2004). Digital Holography, Springer. Ch. 7.

Wenbo Xu, Jericho MH, Meinertzhagen IA, Kreuzer HJ (2001). Digital in-line holographyfor biological applications. PNAS 98:20.

Yamaguchi I, Zhang T (1997). Phase-shifting digital holography. Opt. Lett. 22:1268-1270.