Phase Imaging of Human Cells Using Digital Holographic Microscopy

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***Abstract*—Digital holographic microscopy (DHM) is a 3D method for imaging cells without using labels. It gives detailed information about cell shape and behavior. This study has explored two applications of 3D phase imaging. Firstly, an image was made of an out-of-focus mono chromatically illuminated cheek cell with a conventional light field microscope. The resulting Fresnel diffraction pattern associated with the cell images has been numerically refocused at various positions. The images show details of the organelles. the changing shape of the cell nucleus and the chromosomes within it. Secondly, a simple in-line DHM system used phase imaging to analyze cancer cells. The system uses a 532nm laser, 10x and 40x microscope lenses, and a CCD camera to achieve clear phase images with 250nm resolution and 23.63x magnification. The system was used to view both the growth of cancer cells (SMMC-7721) and in a subsequent test the ability of fluorouracil, an anti-cancer drug, to destroy them. The DHM data displayed cell changes over at 24-hour intervals over a 24–96-hours period. Holographic Amplitude images show the shape of the cell and its nucleus, while phase images gave 3D cell structural details.**

Keywords—Digital holographic microscopy (DHM)

I. INTRODUCTION

A. Digital Holographic Microscope

Digital Holographic Microscopy (DHM) is widely used as a 3D imaging method in biomedical research. Its primary application is in the examination of minimally scattering microscopic organisms. It differs from conventional microscope in that a single holographic image retains the phase and amplitude information needed to reconstruct the whole field of interest. [1-4] The term 'In-line holography' describes a compact configuration with a shared pathway between the object and reference beams. DHM encodes a Fresnel diffraction pattern generated by the specimen. After numerically re-illuminating the hologram, the holographic image is reconstituted to regain the corresponding sample beam information. This is followed by numerical auto-focusing, a process that extracts both the preserved amplitude and the three-dimensional phase data. The amplitude portrayal delineates the object's profile, while the phase information signifies the optical path difference (OPD) of the specimen. [5-7] From amplitude and phase information the cell characteristics of the morphology, thickness, and three-dimensional spatial orientation of the sample. [8-11] DHM has a wide application across diverse domains. [12-14] However, these systems encountered limitations in optical resolution due to image resolution camera format size, and the available 3D reconstruction software. This paper described the use of conventional bright field microscopes without modification repurposed for DHM application to explore the resolution possible within the nucleus of human cheek cells. It also describes the creation of an in-line DHM devised for scrutinizing cancer cells. The DHM system consisted of 532 nm laser, mirror, 10× and 40× objective microscopes, and a CCD camera. This configuration yielded a resolution of 250 nm and an image magnification of 23.6×. Notably, this setup was effectively employed to quantitatively monitor the growth of SMMC-7721 cancerous cells and assess their response to an anti-cancer drug.

B. Recording Method

The DHM in-line interferometer encodes phase information within the Fresnel diffraction due to the interference between the reference and object beams at the recording plane of the camera [15]. The common propagation of the two beams retains the higher orders of diffraction overcoming the accepted Abbe limit, which is confined to the first order of diffraction. The digital recording process has performed previously [16-18].

C. Reconstruction Method

The amplitude and phase of the captured the optical diffraction pattern is recovered from the holographic reconstruction using dimensions of the microscope. Equation (1) outlines the numerical computation for reconstructing the complex amplitude and phase of the wavefront. [19, 20]. The arctangent operation is used to extract the phase information in equation(1):

$$\varphi(x,y) = arctan\frac{I_m[U_{hologram}(x,y)]}{R_e[U_{hologram}(x,y)]} \quad (1)$$

In equation (1), I_m is the real term of complex amplitude, R_e is the imaginary part. The arc-tangent function limits the phase values and is wrapped into [π, π].

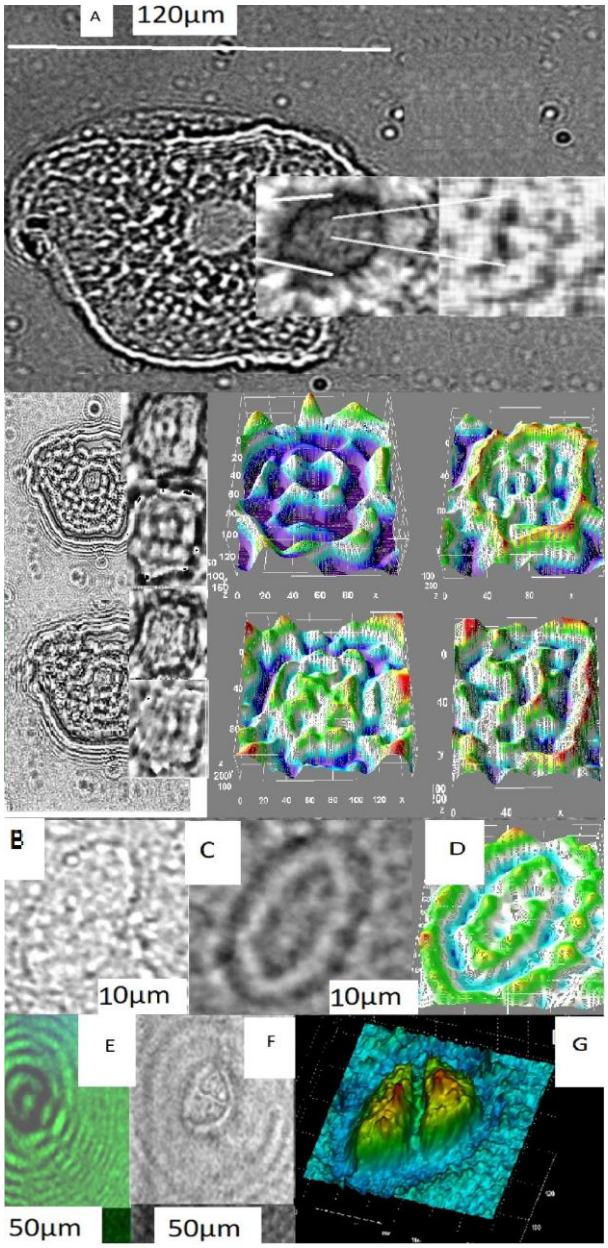

Fig. 1. Reconstruction of cell data.

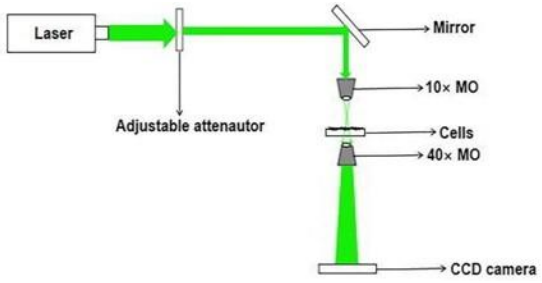

Fig. 2. Schematic diagram of in-line DHM system.

To extract the absolute phase, the arc-tangent function needs phase unwrapping as described by Judge [21] and then holographically reconstructed in 3 dimensions Chen [22].

D. Holographic Cell Imaging

As presented in Fig. 1A, the distribution of amplitudes in a sample is a measurement of the 2D shape of the cell. The reconstructed values of phase indicate the optical path difference (OPD), arising from the combination of thickness and refractive index distribution. This holographic process enables the visualization of the three-dimensional structure of the specimen, as illustrated in Figs. 1(B-D) and is expressed mathematically as follows:

$$\varphi(x,y) = \varphi_1(x,y) - \varphi_2(x,y) = \frac{2\pi}{\lambda} n h_{cell}(x,y) \quad (2)$$

In equation (2), φ is the object's unwrapped phase value is the difference between the phase φ_2 of light passing through the object and φ_1 and that of the undisturbed light. Further, λ is the wavelength of illumination source, n is the refractive index and the cell's thickness is h_{cell}. The combination of which represent the thickness of the cell and nucleus. For example, the phase change through a typical normal healthy nucleus in a cell as shown in Fig. 4a as 10 radians. However, the measurement contains a pedestal background created by the remaining contents of the cell estimated to be 4 radians. The refractive index of the normal cell nucleus as measured by [23] is 1.39. The cell is measured as essentially water with a refractive index of 1.33. This difference yields an estimate of nuclear thickness of 10 microns. The problem in estimating these values [24] is to be able to use phase measurement to identify the structures of individual organelles within the cell and to measure individual chromosomes within the nucleus. A first stage demonstration test was made on a cheek cell shown in Fig. 1A. The out-of-focus diffraction image was captured using a conventional microscope and refocused holographically to give an amplitude image with an optical resolution shown in an expanded region of 100 nm. Note that dust particles on the mounting slide surface have diffraction rings. Demonstrating they belong to a different plane in the holographic image. The lower part of Fig. 1A shows two different reconstruction planes of the image. Although hard to see, the nucleus reduces in size. The two image inserts are amplitude (upper) and phase (lower) of the nucleus. The color plots show amplitude left and phase of the nucleus right. It is of interest, but not performed here, to make a series of sequential images to reconstruct a model of the internal structure within the nucleus. A further series of tests have been performed on a different conventional microscope. Fig. 1B shows the conventional focused image of the nucleus of a cheek cell. The information within the nucleus is too complex to be understood. Fig. 1B is a holographically reprocessed image of the same nucleus. Fig. 1D shows the phase unwrapped image. The detail within the nucleus is now much clearer. A third set of images were made with the DHM microscope Figs. 1(E-G) shows the reconstruction of a SMMC-7721 cell during cell division. Fig. 1E is the initial holographically captured amplitude image, in this case there is a clear view of the Fresnel diffraction pattern resulting from the laser illumination. Fig.1F shows the reconstructed plane and Fig. 1G is the phase unwrapped map which now gives the height of the nucleus.

II. EXPERIMENT

A. In-Line DHM System

A 10× microscope objective is combined with a 40× microscope objective to magnify the informetric diffraction image (Fig. 2). The in-line arrangement increases system stability.

In Fig. 3(a-b), DHM was shown to have a resolution of 0.25μm/pixel when tested against group 7.6 of an USAF1951 optical test target.

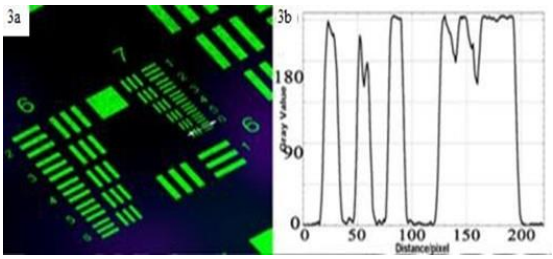

Fig. 3. System calibration based on standard USAF1951 target and line grating structure. (a) focused image of UASF1951 target. (b) intensity distribution in reconstruction along white arrow over group 7.6 in (a).

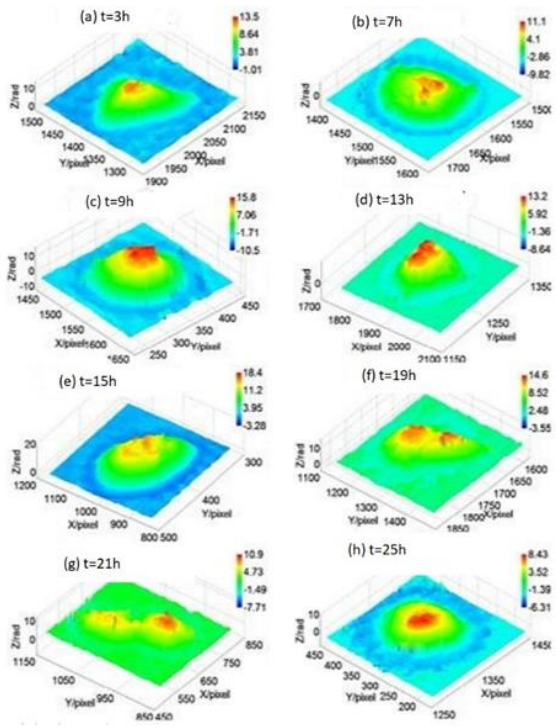

Fig. 4. Morphology analysis of cured 7721.

B. *Cell Experiments Performed Using Smmc-7721 Cells*

1) *Sample Preparation*

TABLE I. RECONSTRUCTED RESULTS DESCRIPTION DURING DIVISION PROCESS OF SMMC-7721.

Id	Cell Size (approx)	Time	Observations
4(a)	10 rad 10μ	3 h	Freshly attached SMMC-7721 cell
4(b)	10 rad 8μ	7 h	cell starts to copy chromatin and form a structure
4(c)	15.8 rad 11μ	9 h	””
4(d)	13.2 rad 8μ	13 h	The center of the original cell with a Linear cellular peak
4(e)	15.8 rad 14μ	15 h	The two chromosomal start to separate
4(f)	12 rad 14μ	19 h	””
4(g)	8.2 rad 8μ	21 h	""
4(h)	8.4 rad 9μ	25 h	The SMMC-7721 cell has replicated the original cell in 1(a)

The cured samples were made by adding 4% paraformaldehyde to cancer cells cultured in a petri dish for 15 to 20 minutes and then cleaned with deionized water three to four times.

2) *Morphology Analysis of Cured SMMC- 7721 Cells During a Growth Cycle*

The DHM system monitored the SMMC-7721 cells to analyze the changes within a growth cycle. A typical cycle is around 24 h and holograms were taken every two hours this period to capture the different stages of cell division and growth. The reconstructed data is presented in TABLE I and shown in Fig. 4.

TABLE II. RECONSTRUCTED RESULTS DESCRIPTION AFTER DRUG TREATMENT OF SMMC-7721

Id	Cell Size	Time	Observations
5(a,b)	13 rad	24 h	Higher peak than Fig. 4(h)
5(c,d)	10 rad	48 h	Lower peak start of cell disintegration
5(e,f)	7 rad	72 h	Decreased peak showing cellular apoptosis.
5(g,h)	6 rad	96 h	Cell fragmentation and the apoptosis ending.

Tests were also investigating changes in SMMC-7721 cells after drug treatment. They were cultivated for 24 h. Then treated with 1 mg/μL fluorouracil at 24 h intervals. Holograms taken at each treatment, the unwrapped phase images are presented in Fig. 5 and results listed in TABLE II.

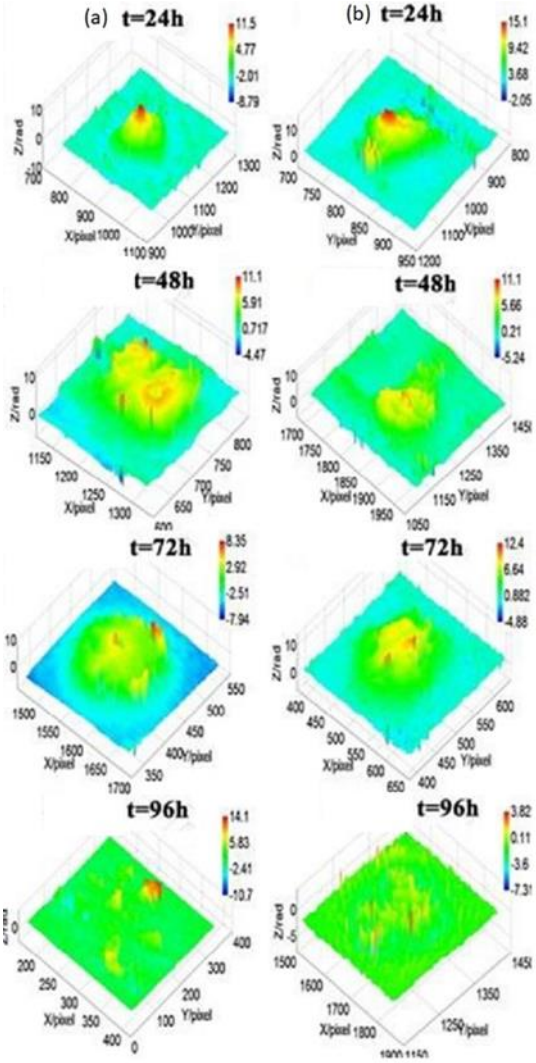

Fig. 5. Morphology analysis of cured 7721 cells after drug treatment.

III. Conclusion and Discussion

This paper presents two aspects of high resolution in-line Digital Holographic Microscopy monitoring of human cells.

In first case the shown in Fig. 2A simple low-cost version clearly defines the structure of cell and nucleus. A phase image digitally stored holographic reconstruction as illustrated in Fig. 1(B-D)demonstrates the ability to observe the growth and destruction of cancer 7721 cells Figs. 4-5. It also confirms the use of fluorouracil as an anti-cancer agent. The technique maps the dynamic structural morphology of human cells, their growth rate, cancer growth rate and the effects of anticancer drugs. Further the thickness of the nucleus has been calculated from holographic phase measurement. Although it is possible to measure a height and observe the changes in the nucleus' morphology; the accurate calculation of thickness of the nucleus is more complex. The holographic method integrates the total phase the value of both the nucleus and the surrounding cell. Which is a classical tomographic phase reconstruction problem. To simply solve the problem a value of the cell material has been subtracted. The results are presented in Figs. 4-5 and TABLEs I-II.

A second aspect of the work developed using a conventional bright field microscope. We used an out of focus position and a low coherence monochromatic source to create diffraction images of the human cell. The subsequent holographically reconstructed image yielded phase information and structural detail within the nucleus of a cheek cell shown in Fig. 1A. The resulting reconstruction was observed to be twice that of the conventional focused image. The conclusion from this result being that the diffraction pattern behaved in an analogous manner to other super-resolution microscopes which use structured light and confocal imaging to go beyond the Abbe limit. This many new opportunities. Firstly, by making a careful sequential holographic resolution of the nucleus it may be possible to recreate the structure of the chromosomes within it. Secondly, using fluorescent marking it could be possible to track the aging and renewal process of the DNA within the nucleus. Finally, because the holographic process stores 3D data of the nucleus it could be possible to watch the 'DNA' using basic optical bright field microscopes. The combined increases in camera resolution and sensitivity, evolution of software and computer processing power make this a remarkably interesting period for holography.

There has been an explosion in the amount of research development in high resolution microscopy. Fluorescent marking, particle tracking, and phase marking are now being combined to track how DNA (Deoxyribonucleic acid) combines to create and rebuild chromosomes within the human nucleus. Examples being, [25-32].

First, we gained knowledge of the chemical structure and now in this next decade will yield knowledge of the mechanisms and understand how the organelles create and exchange DNA with the chromosomes.

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