

Designing Aspects and Recent Advances in Digital Holographic Microscopy

Ritu Walia^{1,*}, Kamal Nain Chopra²

Abstract

Recent advances in Digital Holographic Microscopy have been discussed in this paper, besides throwing some light on the designing aspects of optical components like beam splitter and neutral density filter for the optimization of system performance. Some important related subtopics like spectral resolution of digital holograms, and Fourier-synthesis holography have been technically discussed. The optimization of acoustical holographic components for use in renewable energy production is presented in this study. The production of solar energy can be increased via holograms. It has been mentioned that the University of Michigan researchers in Arizona recently created a novel method for harnessing the excess solar energy utilized to light a solar panel. To achieve the most efficient light collecting, the holographic light collector's optical element must be symmetrically positioned in the middle of the photovoltaic module. There has also been a qualitative analysis of some significant research on the subject, particularly in the fields of biology and medicine. It is anticipated that the work will be beneficial to digital holographic microscopy researchers and designers. Furthermore, the study emphasizes the interdisciplinary nature of holography, showing its potential not only in optical instrumentation but also in advanced biomedical imaging, tissue engineering, and drug monitoring. By improving image reconstruction algorithms and enhancing hardware design, digital holography could revolutionize non-invasive diagnostics and provide real-time cellular monitoring. Future research directions may focus on integrating artificial intelligence and machine learning for automated hologram interpretation, which can significantly reduce human error and increase system efficiency.

Keywords: Digital holographic microscopy, spectral resolution of digital holograms, single element reflective digital holographic microscopy, and fourier-synthesis holography

INTRODUCTION

With the advent of holography, it has found many Applications in the field of optics. Digital holography (DH), which is predicated on the capturing and processing of holograms with digital cameras and computers, respectively, has grown significantly during the previous three decades. The three-dimensional surface or optical thickness distribution of an object may generally be ascertained by using the hologram to reconstruct all of the information of an optical wave, including its amplitude and phase.

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Increases in phase accuracy, measurement range, imaging speed, spatial resolution, and—most importantly—hologram recording and reconstruction of deep hyperspectral imaging (DH) have been the focus of numerous recent research investigations. In actuality, DH has undergone several new revolutions due to the quick advancement of computer science and associated fields, such as incoherent DH, compressive DH, DH

imaging over scattering medium, and entangled quantum holography. Digital holography has advanced thanks to a multitude of image processing methods, such as machine learning and deep learning technologies. At the moment, DH is widely used in industrial inspection, air and gas visualization, 3D imaging and display, and the biomedical industry. The latest developments in digital holography and some new technologies in quantitative phase imaging have led to the emergence of several novel and significant topics, including advances in digital holography techniques, 3D imaging and display systems, computer generated holograms, shearing and transport of intensity equation (TIE) based approaches, beam-propagation-based methods, and Fourier ptychography. Electronic

Holographic Microscopy (DHM) is an imaging-based interferometer technique that produces quantitative phase images related to the intracellular refractive index, which provide information about (i) the thickness and content of cells and (ii) their morphology. This technique makes it possible to visualize transparent cells without the need for specialized multi-well plates or labeling. Notably, digital holography has been applied in many different fields, mainly in holographic cryptography, optical metrology, remote sensing and inspection, and, more recently, quantum computing.

Numerous important studies (1-6) have been finished in the last few years. A label-free technology based on digital holographic microscopy (DHM) has been introduced by Kühnet al. (1) by automating a digital holographic microscope for image acquisition of cells using commercially available 96-well plates. This technology can be used for imaging-based screening and has been shown to be capable of cytotoxicity assessment using living mammalian cells. A Z'-factor of about 0.9 has been found, confirming the robustness of the DHM assay for phenotypic screening, and it has been asserted that the data produced by label-free DHM imaging and fluorescence-based techniques have been in good agreement for cell viability identification. [1-5]

Furthermore, a strong association has been found between known IC50 values for several dangerous substances and experimental cytotoxicity dose-response curves. It has been noted that the findings obtained are comparable, and that the main benefits of DHM over automated conventional fluorescence microscopy are that it is label-free and operates at a speed that is about an order of magnitude faster. It has been reported in the literature that the DHM is compatible with screening applications through the automation of a digital holographic microscope using standard 96 and 384 imaging plates. HCS research group, under Gerardo Turcatti, in collaboration with LyncéeTec S.A., After automating a DHM microscope and refining the technique, a spin-off business from EPFL that develops instruments for Digital Holographic Microscopy has verified screening applications. Label-free quantitative cellular imaging in cell viability screens, such as end-point primary screens and time-lapse hit clustering/validations for toxicological and drug development applications, has been made possible by their methodological approaches. This method has been proven to be better than other label-free

Interferometer microscopy for quantitative cellular analysis, and it is special in that it can be used for time-lapse imaging and high-throughput screening. Benjamin et al. (2) have used time-lapse videography to analyze and further analyze aspects linked to intracellular content and cell shape in endpoint measurements. The transmembrane water fluxes during the activity of the epithelial chloride channel, or CFTR, as determined by patch-clamp and iodide efflux approaches were quantified in situ by Jourdain et al. (3) using digital holographic microscopy (DHM), an interferometric technique. The findings showed that the specific CFTR blocker CFTRinh172 completely inhibits the water transport as determined by DHM, which is missing in cells lacking CFTR.

Actually, DHM is the application of digital holography to microscopy. It differs from other microscopy techniques in that it records the digital light wave front information emanating from the object as a hologram instead of the projected image of the object, and then uses a computer to compute the object image through a numerical reconstruction algorithm. It is evident from this that a computer algorithm takes the place of the image-forming lens in conventional microscopy in this method. DHM is comparable to other closely related microscopy techniques like as diffraction phase microscopy, optical coherence tomography, and interferometer microscopy in that they all rely on a reference wave

front to determine phase and intensity information. Remarkably, the data is captured on a digital Hence, it is different from the conventional microscopy, in which no reference wave front is used, and therefore, only intensity information is recorded, but other essential information about the object is not obtained.[6-8]

DESIGNING OF DIGITAL HOLOGRAPHIC MICROSCOPY

A number of experimental arrangements for the DHM have been suggested by various researchers. The DHM in real time using the graphics processing unit (GPU) card has been suggested by Shimobaba et al (7), and the working of this system can be easily understood from the following Figure1

The system is composed of two components: the GPU-based real-time computation system and the optical system for holographic recording. USB (Universal Serial Bus) is an industry standard that specifies the cables, connectors, and protocols used in a bus for connection, communication, and power supply between computers and other devices. MO is the microscope objective for improving the performance of the DHM. The charge-coupled device camera, mirror, neutral density filter, and beam splitter are denoted by the letters CCD, M, ND, and BS, respectively. Because the system has very little tolerance for changes in the incidence angle at different spots on the beam splitter, the collimator

Plays a critical function. This is why the design of the collimator in DGM is so vital. Up until now, difficulties with the intricate computing process have restricted this technique's potential. Under the direction of Dr. Ana Doblas and associates, the Optical Imaging Research Laboratory at the University of Memphis has developed algorithms that can automatically reconstruct 3D topography without the need for user experience or design knowledge, offering a new computational approach to Digital Holographic Microscopy [9-15]. Their efforts have created new avenues for applying the method in a variety of research fields, including biomedical imaging and materials physics. The schematic that this group has proposed is as follows Figure 2. Recently, Picazo Bueno et al (8) have given an advanced technique called Single-Element Reflective Digital Holographic Microscopy (Figure 3). The primary feature of digital holography is the preservation of the fundamental idea of classical holography, which states that the phase of the reference beam determines how the reconstructed image turns out. But unlike classical holography, where the image on the monitor screen appears to be three-dimensional to the human eye, this type of holography lacks volumetric information. Nevertheless, phase information can still be tallied and utilized in other ways, similar to interferometry.

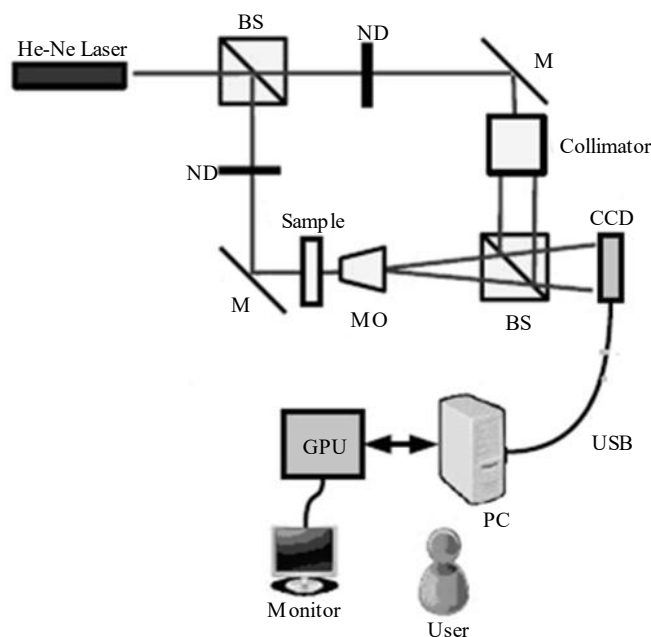


Figure 1. Outline of the real-time DHM system using the GPU. Figure slightly modified from the figure courtesy Shimobaba et al, Optics Express 16 (2008) pp. 11776-11781.

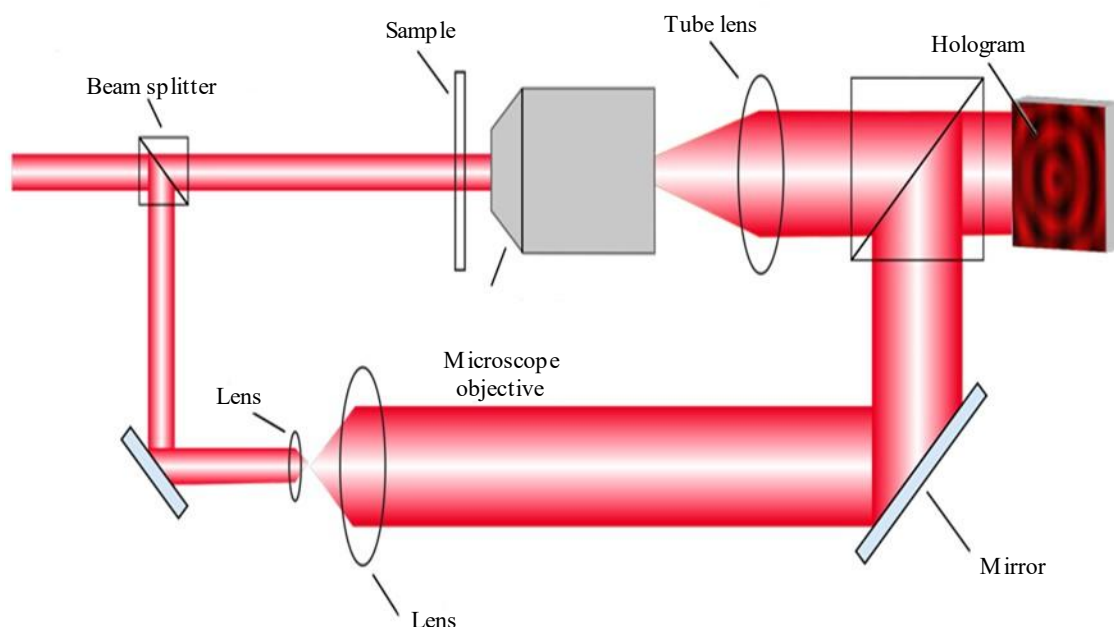


Figure 2. Schematic of Digital Holographic Microscopy adopted by Dr Ana Doblas, and collaborators. Figure courtesy researchoutresch.org.

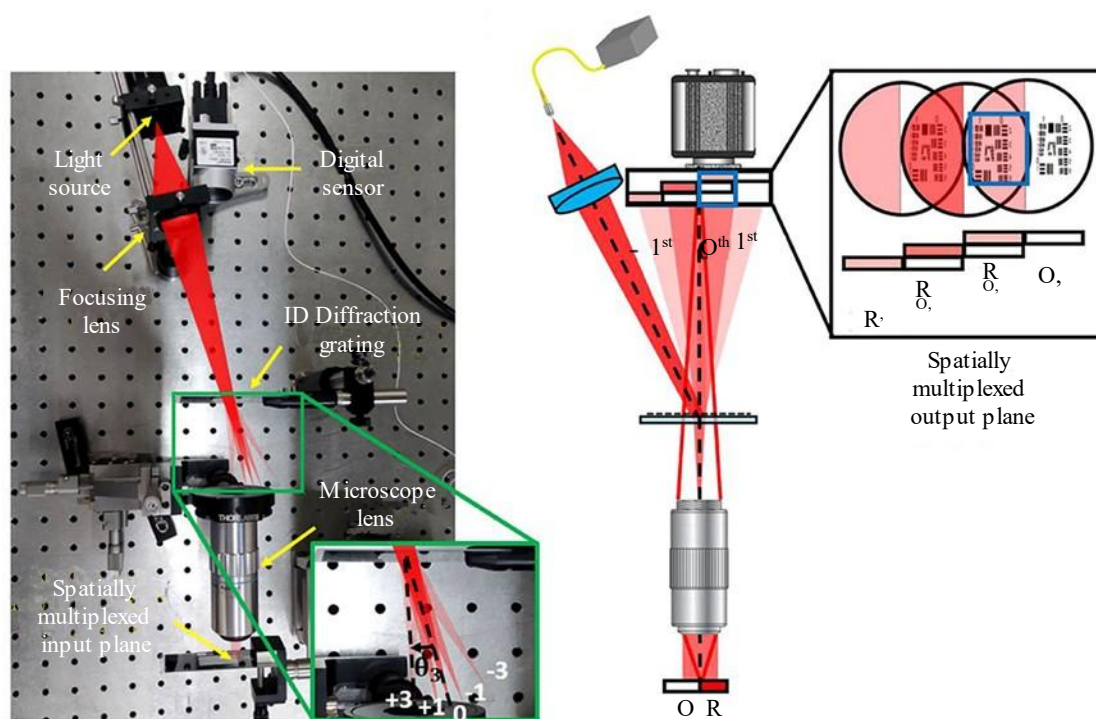


Figure 3. SER-DHM Schematic.

An image of the experimental setup with the lighting arm highlighted and the direction of the diffraction order magnified for clarity by the green rectangle. A frame containing the output plane (black rectangle) and the recording region (blue rectangle), where the digital sensor is positioned, is included in the optical scheme and ray tracing of the illumination and interferometric arms (B). Figure courtesy Front. Phys., 22 March 2021, Sec. Optics and Photonics, Brief Research

The angle of incidence (θ_i) that the lighting system must create in relation to the objective's optical axis in order to reroute light toward the microscope lens is given by the diffraction grating equation.

$$(m \lambda N) + \sin(\theta_i) = \sin(\theta_m) \quad (1)$$

Here, m is the diffraction order, λ is the light source's wavelength, and N is the grating's spatial frequency. Consequently, the diffracted beam of the m diffraction order is represented by θ_m .

The cut-off frequency for a coherent imaging system is described in the image spectrum domain as $f_c = NA/\lambda M$, where NA and M represent the imaging system's numerical aperture and magnification, respectively. As θ is the propagation angle of the 1st diffraction order, as determined by Equation (1), the minimal frequency N_{min} is equivalent to three times this cut-off frequency, as demonstrated by:

$$N_{min} = (3NA/\lambda M) \quad (2)$$

It is well known that digital holographic microscopy (DHM) is a potent technique that enables simultaneous observation of a specimen's phase and amplitude. Several computations for the Fresnel diffraction are needed to generate a reconstructed image from a hologram. According to Shimobaba et al. (7), the FFT (Fast Fourier Transform) algorithm can speed up Fresnel diffraction. They also stressed how challenging it is to reconstruct a hologram in real time, even when using a central processing unit (CPU) to compute the FFT algorithm's results. A real-time DHM system with several stream processors on a graphic processing unit (GPU) that can be used as a highly parallel processor has been reported by Shimobaba et al. (7). The optical equipment in the experimental setup is a traditional DHM setup with a neutral density filter, a beam splitter, and a low power (~ 5 mW) He-Ne laser serving as the reference light source. An objective lens, a test target serving as a sample, and a CCD camera with an excellent resolution of 1360×1024 and a pixel pitch of $4.65 \mu\text{m} \times 4.65 \mu\text{m}$. These holograms are then moved to a personal computer so that it can be used to control the GPU and the CCD camera through an interface. The GPU can compute Fresnel diffraction very quickly, which enables us to retrieve reconstructed images from holograms at a rate of about 24 frames per second. It is evident that the illumination with a coherent source like Laser creates the required interference pattern, which takes the shape of the hologram. The laser light is separated into an object beam and a reference beam using a beam splitter. [16-19]

An object wave front is formed when the object beam expands and illuminates the sample; this wave front is then captured by a microscope objective. A beam splitter then combines the object wave front and reference wave front to create an interference pattern that resembles a hologram. Using a numerical reconstruction algorithm, a computer operating as a digital lens uses the digitally recorded hologram to calculate a viewable image of the object wave front. It's vital to remember that digital holographic microscopy's lateral optical resolution is equivalent to that of traditional light microscopy. DHM shares the same diffraction limit as conventional light microscopy due to the numerical aperture. Of course, the DHM's excellent axial resolution of about 5 nm is a benefit.

It is important to know that the recording of the interference pattern can be done even at a very high sampling rate, which in fact depends on the wavelength λ and the angle θ between the propagation direction of O beam and the propagation direction of R beam. The sampling distance d can be calculated by using the following equation: [20]

$$d = \left\{ \frac{\lambda}{2 \sin \theta} \right\} \quad (3)$$

The hologram's maximum viewing angle is also influenced by the sample distance used during recording, therefore it needs to be carefully adjusted to attain the maximum viewing angle when

Placing a camera $\theta = \frac{\pi}{2}$, which can in fact be done by choosing the sampling distance d to be at least

equal to $\frac{\lambda}{2}$, so as to make sure that the Nyquist frequency of the The interference pattern's carrier frequency is equivalent to the hologram. Half of the sample rate of a discrete signal processing system is the Nyquist frequency, sometimes referred to as the folding frequency of a sampling system.

Simulations and experiments show that $\sim$ three orders of magnitude larger wavelengths are achievable to obtain good contrast images while maintaining a reasonable hologram size. This is because the resolution of the final image, rather than its physical size, is a crucial parameter in the screen setup. When designing digital holography, the designer must take into account two distinct equations: the on-axis and off-axis equations. This is because the holographic structure in on-axis digital holography is a low-pass spatial signal; so, the sampling criterion in the form of the subsequent equation must be applied:

$$f_{\max} \leq \frac{f_s}{2} = f_N \quad (4)$$

where $f_{\max}$ is maximum spatial frequency of the holographic pattern, f_s is sampling frequency of the digital image sensor, and f_N is the Nyquist frequency of the image sensor or Nyquist limit. However, in case of the off-axis digital holography, the holographic structure is a band-pass spatial signal, and therefore, sampling criterion in form of the following expression has to be used:

$$\Delta f = (f_2 - f_1) \leq \frac{f_s}{2} = f_N \quad (5)$$

where f_1 and f_2 are respectively the lowest and highest values pf the range of frequencies constituting any function $F(t)$, that can be transmitted continuously to any desired degree of precision.

Line scanning devices or a rectangular matrix of light detectors are used for image recording; the latter architecture is better for the deep learning environment. As a matter of fact, incident light uses the internal photo effect to separate charges. It then transforms into an electronic charge at each individual detector in the form of pixels. Finally, the charge transfer function transfers the charge packets within the Si semiconductor substrate to memory cells. Ultimately, the transferred charge is transformed into a voltage by the memory cells' capacitor matrix, and an amplifier then adjusts the voltage to meet the output specifications. This designing aspect has to be looked into by the Electronics design engineer, Another point to be taken care of while designing the optical components is that the surfaces of Beam splitter and Neutral density filter should be optically polished free from scratches and digs, and also having very good micro roughness, so that scattering is nearly indelible before coating. The coatings should preferable done with double ion beam sputtering technique (12), using dielectric materials – TiO₂ and SiO₂, to keep the scattering level to minimum possible levels, The most important point to be taken of, is that the beam splitting should be in same proportion of reflection and transmission, so that the contrast in the recorded fringes is maximum for the optimum performance of the DHM. Though, the aberrations are removed by software, still the optical components especially collimator should be free from aberrations.[22-25]

One of the key features of DHM is the formation of a phase shift image in addition to the standard bright field image (Figures.4 and5), As anticipated, in the case of the reflection DHM, the phase shift image yields an image of the object's topography.

This technique is unquestionably superior to differential phase contrast microscopy, which is used to view transparent objects such as living biological cells. In differential phase contrast microscopy, transparent objects are phase-shifted and visible by distorting the bright field image with phase shift

information. In contrast, in transmission DHM, a separate phase shift image is observed, providing valuable information about the object's optical thickness and enabling the visualization and quantification of transparent objects, which is why it is called quantitative phase contrast microscopy. It is now widely acknowledged that phase shift images (Fig. 4) can be easily segmented and analyzed by image analysis software using mathematical morphology. However, bright field or typical phase contrast photographs of living, unstained biological cells (Fig. 3) are extremely challenging to examine with image analysis tools, such as Cell Profiler. It's also significant that the DHM provides the 3-Dimensional data. A certain focal distance is used to compute an object picture, although the recorded hologram has all the necessary object wave front information. Consequently, by altering the focal distance parameter during the reconstruction process, the object at any focal plane can be recreated.

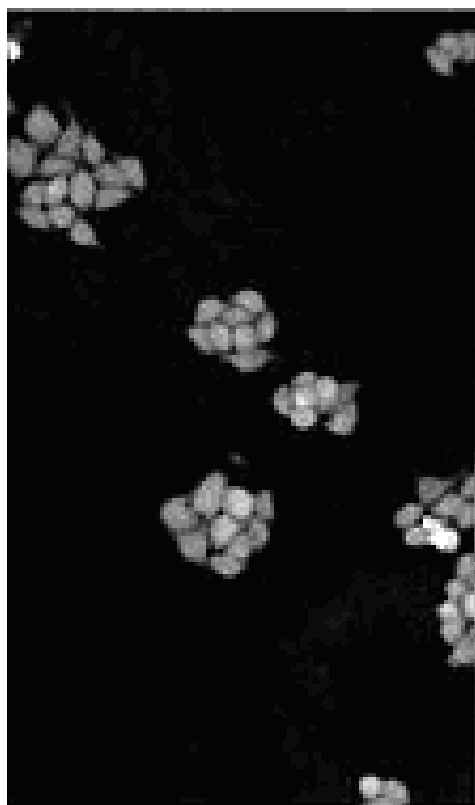


Figure 4. The ordinary bright field image produced by DHM.

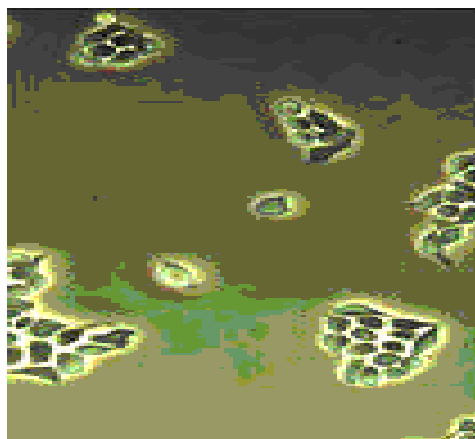


Figure 5. The phase shift image produced by DHM. Figure modified from the Figure courtesy phase-contrast, jpg file from wikimedia commons.

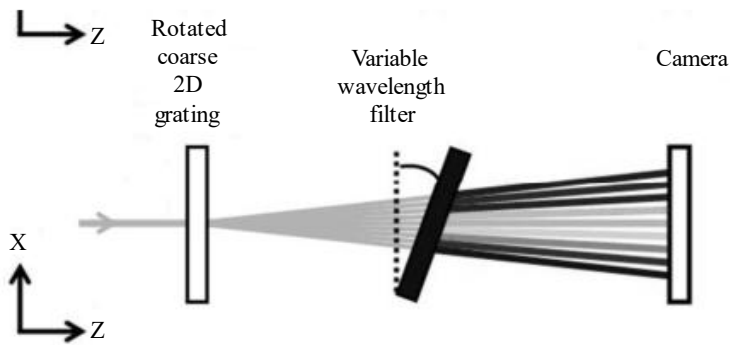


Figure 6. Spectral resolution of digital holograms; figure modified from the figure courtesy interferometry pdf, institute of optics, university of rochester, rochester, NY USA.

Theoretically, it is observed that the hologram has all the data required to construct an entire image stack. The DGM approach records the object wave front from various angles, allowing for the complete provision of the object's optical features and the creation of tomography images of the object. Another useful feature of the method is digital auto focus, which eliminates the need for vertical mechanical movement and enables incredibly quick scanning and imaging of surfaces. Actually, to produce a comprehensive and focused image of the object, a single hologram is first captured, and then sub-images computed at various focal planes are stitched together. The DHM systems are free from the common optical distortions since they lack an image producing lens. Nonetheless, the reconstruction algorithm's proper design compensates for the optical aberrations, accurately modeling the optical setup and preventing optical aberrations. Analyzing the Spectral Resolution of Digital Holograms is required to get complete information. [25-28]

On this subject, hardly many studies have been published. It is significant to remember that band pass filters can be used to spectrally resolve the digital holograms, as seen in the accompanying figure (viewed from the top):

For the vase of the computer generated holograms (CGHs), the most important numerical characteristics of recorders are (i) the total number of cells, which may be exposed, and (ii) their sampling interval, i.e. distances $\Delta\xi$ and $\Delta\eta$ between neighboring, separately and independently exposed resolution cells. The sampling interval defines the angular dimensions (θ_x, θ_y) of the reconstructed image, Figure 6 which are given by the sampling interval according to the following well known diffraction relation equations:

$$\theta_x = \frac{2\pi\lambda}{\Delta\xi_x}; \theta_y = \frac{2\pi\lambda}{\Delta\eta_y} \quad (6)$$

Interestingly, the computations show that to make the image's angular dimensions $\sim 2\theta_o$ or more with a reconstruction light wavelength of $\sim 0.5 \mu\text{m}$, $\Delta\xi$ and $\Delta\eta$ should $< 10 \mu\text{m}$. In the spatial light modulators and printing devices generally used for recording CGHs, sampling steps are from 1 to $10 \mu\text{m}$, and the total number of resolution cells is $\sim 103 \times 103$ to 104×104 . Thus, it has to be seen that the total number of cells, which may be exposed, and (ii) their sampling interval, have to be chosen so as to result in the optimization of the computer generated holograms. For this, both - theoretical calculations and experimental results have to be understood, and applied together. Resolving the frequency of the interference pattern created by the superposition of the reference wave with the wave scattered from the object is one of the primary requirements for the DH application of a camera. The maximum spatial frequency (θ_{max}) to be resolved between the waves, and can be computed by the approach (13), which is given by:

$$f_{\text{max}} = \left(\frac{2}{\lambda} \right) \sin \left\{ \frac{\theta_{\text{max}}}{\lambda} \right\} \quad (7)$$

Photographic emulsion has a resolution of about 5000 lines per millimeter, allowing for the recording of holograms with reference and object waves at angles larger than 180° . Nevertheless, since there is only around $5\text{ }\mu\text{m}$ separating two adjacent pixels, the highest resolution that can be achieved is about 100 lines per millimeter. It is crucial to remember that the designer must lower the Speckle Noise in order to obtain satisfactory outcomes. This is accomplished by adhering to method (Eqn 7) of reaching the Higher Resolution. The resolution for photographic emulsion is ~ 5000 lines per mm, which allows the recording of holograms with angles greater than 180° between the reference wave and the object wave. However, the distance between two neighboring pixels being only $\sim 5\text{ }\mu\text{m}$, the maximum achievable resolution is ~ 100 lines per mm. It is important to note that the designer, in order to get good results, has to reduce the Speckle Noise, which is done by following the approach (10) of achieving the Higher Resolution. Obviously, the speckle size δ_s depends on the distance X of two interfering light O and R waves having wavelength λ , at distance Z of the interference measurement, by the following expression:

$$\delta_s = \left\{ \frac{\lambda Z}{X} \right\} \quad (8)$$

Therefore the minimal speckle size δ_s , which is dependent on the aperture size W , can be computed as:

$$\delta_{s_{\min}} = \left\{ \frac{\lambda Z}{W} \right\} \quad (9)$$

Therefore, the designer has to choose the maximum possible value of the aperture size for minimizing the speckle size.

Since it produces the whole pulse in the space-frequency domain, holography employing a well-characterized very short pulse has attracted a lot of interest. Holograms are measured, one for each frequency component. When holography is carried out with a monochromatic beam, the entire spatial intensity and phase are obtained at the frequency of the beam.

$$E(x, y, \omega) \quad (10)$$

On the other hand, we obtain the whole pulse in the space-frequency domain if holography is carried out using a well-characterized very brief pulse and measuring a succession of holograms, one for each frequency component.

$$E(x, y, t) = \left(\frac{1}{2\pi} \right) \int_{-\infty}^{+\infty} d\omega e^{i\omega t} E(x, y, \omega) \approx \left(\frac{1}{2\pi} \right) \sum_{k=1}^n E(x, y, \omega_k) \delta\omega \quad (11)$$

It has to be understood that $E(x, y, t)$ is the initial condition in Maxwell's equations, giving the full spatio-temporal pulse field $E(x, y, z, t)$.

CONCLUDING REMARKS

As is evident, one of the primary benefits of deep learning (DH) is its ability to automatically do high-resolution 3-D quantitative sample imaging without the need for mechanical scanning. This is achieved through the numerical focussing of a 2-D image at various object planes. In addition, by using a single acquired image, it is possible (i) to reduce the size of the mass storage devices required for images saving, and (ii) to achieve a fast image transfer. High resolution images of bovine sperm are obtained by DHM through reconstruction of a single captured hologram. It's noteworthy to note that high resolution photos of morphological modification have also been obtained using the DHM approach. This is because the DHM allows for direct observation of the spermatozoa in their natural habitat. The DHM being so useful, many important studies (8-20) have been carried out during this

decade. There has been some intriguing research on patented technology for digital holographic microscopy, which uses a video (CCD) camera to capture a hologram created by the interference of a reference wave with a wave coming from the specimen. The technique's significance is found in the fact that the recorded image is sent to a computer, where numerical methods are employed to reconstruct a three-dimensional image of the specimen. This allows optical topography information to be retrieved from a single image grab without the need for scanning. This technique enables rapid, flexible, and cost-effective devices to photograph microscopic specimens in three dimensions at a resolution of less than one nanometer in the vertical direction, as shown by numerous application examples.

It is clear that this method creates high-resolution, real-time 3D digital images of a sample by utilizing the holographic principle. Holograms, which are produced by combining a coherent reference wave with the wave received from a specimen, are captured by a video camera and sent to a computer for real-time numerical reconstruction. A single hologram can be acquired and computed in a matter of microseconds.

One may compute the full wave front that comes from an object and generate phase and intensity images that offer quantitative data specified at a sub-wavelength scale that is helpful for accurate and consistent 3D measurements using the commercially available DHM® proprietary software. The contrast offered by intensity images is identical to that of traditional optical microscopy. When transmission occurs, the phase picture shows the phase shift caused by a transparent object; when reflection occurs, it can show the surface topography with a vertical resolution that is sub-nanometric. Numerous underlying biological processes can be used to analyze both of these findings.

The field is continually developing and discovering new uses for A heterodyne digital holography-based imaging microscopic approach that allows for the imaging of subwavelength-sized gold colloids in cell settings has been reported by Warnasooriya et al. (11). They have successfully used 40 nm gold nanoparticles to (i) mark the surface cellular receptors of 3T3 murine fibroblasts and (ii) use holographic microscopy to view the biological specimen in a total internal reflection configuration.. The higher scattering efficiency of the gold nanoparticles in comparison to cellular structures has been attributed to the accurate localization of a gold marker within a 3D mapping of the scattered field of the entire sample, with lateral precision of 5 nm and 100 nm in the x, y, and z directions, respectively. This demonstrates how holographic microscopy may be used to find nanoparticles in surroundings of living cells. Self-reference quantitative phase microscopy for microfluidic devices has been thoroughly examined by Jang et al. (12). Caprio and associates (13) have discussed the Digital self-referencing quantitative phase microscopy by wave front folding in holographic image reconstruction. Using numerical modification of the retrieved wave fronts, Miccio et al. (14) have shown the many elements of the Quantitative Phase Contrast in holographic microscopy.

Caprio et al. (15) have examined and talked about the use of digital holographic microscopy to assess the anatomy of human spermatozoa. In his thesis, Caprio (16) detailed his extensive research on Quantitative Label-Free Cell Imaging using Digital Holographic Microscopy, which is regarded as a comprehensive roadmap for the characterization of biological samples. According to Doblasi et al. (17), as the DHM enlarges the sample using microscope objectives (MOs), several associated effects that are absent from optical microscopy must be taken into account when quantitative phase imaging (QPI) is taken into account. They have clarified that the DHM's performance is significantly influenced by the residual phase curvature that the MO introduces, which has no effect on optical microscopy. Doblasi et al. (17) have examined the sample phase perturbations brought about by the MO phase curvature and have provided a comprehensive study of the physical parameters governing the proper use of MOs in DHM for the case of QPI.

The impact of coherent illumination on the precision of phase measurements in digital holographic microscopy has been explained by Lee et al. (18). By using the phase referencing and temporal averaging techniques together to suppress the phase noises produced by the laser source and image

sensor, their creative work has provided a way to increase net phase accuracy in a DHM system. To illustrate the coherence effect on fringe visibility and the reconstructed phase accuracy of digital holograms, a comparison between a laser diode operating in single- and multi-modes has also been provided.

Digital Holographic Microscopy, a label-free imaging approach that enables the viewing of transparent cells with standard imaging cell culture plates, has been discussed by Rappaz et al. (19). Furthermore, it has been noted that the quantitative DHM phase contrast image is associated with both cell thickness and the intracellular refractive index; (ii) DHM technology is appropriate for the creation of reliable and objective image-based assays, since digital holographic microscopy is a powerful tool for three-dimensional imaging and tracking; A review of the state-of-the-art in DHM for three-dimensional tracking and profiling has been presented by Yu et al. (20). The review focuses on DHM techniques, reconstruction criteria for three-dimensional tracking and profiling, and their applications in a variety of scientific fields, such as holographic tomography, particle imaging velocimetry, biomedical microscopy, and micro metrology. In addition to providing brief explanations of DHM procedures, they have compiled a number of representative DHM settings. In order to acquire three-dimensional profiles and four-dimensional trajectories of objects, they have also outlined and contrasted the reconstruction criteria. Furthermore, the specifics of the simulated and experimental proofs of DHM methods and associated reconstruction algorithms on variously shaped, sized, and conditional particles, biological cells, fibers, etc., have been given. It has been observed that the modern image sensors and lasers have given a useful tool for the cell biologists to observe and quantify cellular dynamics. In addition, the flexibility of a digital lens allow images to be refocused after recording, by doing the refocusing to compensate for focus drift completely with the help of software, which is done by creating images at several focal planes. It is important to understand that (i) from this temporary stack of images, the best in focus image is automatically selected to produce the final holographic image, or (ii) the focal distance is manually set to focus on a plane of interest.

One significant drawback of the self-interference DHM concept is that, although it has been proven to be especially helpful for simplified quantitative phase imaging of living cells, the coherent nature of the laser light causes scattering patterns that compromise the resolution for detecting changes in optical path length. Since Schubert et al. (21) have described a straightforward and effective method for reducing coherence disturbances in quantitative phase pictures, an electrically focus-tunable lens is used to adjust the amplitude and phase of the sample lighting. It has been underlined that the suggested approach works especially well with the self-interference DHM idea. It has been stated that the method's characterization results indicate that coherence-induced disturbances can be reduced by up to 70%. Furthermore, it has been shown to work well for improved quantitative imaging of living cells.

A digital holographic microscope can view both opaque and transparent specimens without staining them, according to İlhan et al. (22). However, a digitally recorded hologram must be mathematically reconstructed at the actual depth of the object in order to generate a focused image. They have developed a high-resolution digital holographic microscope that can autofocus to image objects with amplitude and phase. If the true depth of the item is unknown beforehand, the sharpness of several reconstructions at various distances is compared when the recorded hologram is huge. A substantial amount of computing power is needed for this process.

To evaluate the true focus depths of objects, they have proposed eleven different metrics of sharpness. The speed performance of focusing has been addressed, and a scaling technique has been introduced where the auto focusing speed increases on the order of square of the scale ratio.

Furthermore, the study examined the scaling performance on both computer-generated holograms and recorded holograms of biological samples. The findings indicate that the simulations closely match the practical results. A thorough review of the methods developed in the past ten years to achieve complete, quantitative, and absolute phase imaging, including phase-shifting, digital holographic

microscopy, Fourier phase microscopy, and Hilbert phase microscopy, has been provided by Colomb et al. (23). Even though these techniques are very similar, Colomb et al. (23) have explained that DHM offers certain benefits over phase shifting. For instance, DHM can capture holograms in a single frame, enabling absolute phase imaging in real time. Furthermore, unlike Fourier phase or Hilbert phase microscopy, DHM does not need taking in-focus pictures of the specimen on the digital detector (CCD or CMOS camera).

This is due to the fact that a numerical wave front propagation can numerically enlarge the depth of vision of high NA microscope objectives by performing a numerical focalization adjustment. Two distinct biological cells floating at different depths in a liquid can be numerically focalized from the same digital hologram, it has been emphasized; and (ii) the numerical propagation related to digital optics and automatic fitting procedures allows vibration-insensitive full-field phase imaging and full compensation for a priori any image distortion or/and phase aberrations introduced, for instance by flaws in holders or the perfusion chamber. It has also been shown that living cells may produce full field phase pictures in real time.

There is a wealth of information on the many facets and uses of digital holography in the recently released Special Issue on Digital Holographic 3D Imaging: Capture, Display, and Evaluation (24). Tatsuki et al. (25) reviewed digital holographic methods and their application to the most recent developments in multidimensional sensing. Digital holography is an interferometric imaging technology that, with a single-shot exposure, can produce a 3-dimensional image of incoherent light and a holographic image of nonlinear light, they have noted. It may be used to simultaneously image multidimensional information, including three-dimensional structure, dynamics, quantitative phase, various wavelengths, and the polarization state of light, and it doesn't require an imaging lens.

They have underlined how this technique's capacity for holographic recording has led to a wide range of applications. In recent times, there has been a surge in interest in the use of holographic optical elements for solar energy applications (26–28). These elements offer numerous benefits, including the potential to lower production costs, the capacity to choose specific solar spectrum bandwidths, and the ability to be integrated into architectural designs. Volume holograms are the most commonly used of the various holographic structure types because of their high efficiency (nearly 100%) and two crucial features: angular selectivity and chromatic selectivity. These features are crucial for designing lighting, solar shading, and solar concentrator systems.

It is evident from all of these recent groundbreaking discoveries that this discipline has been drawing more and more attention from researchers in quest of fresh applications.

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