



## Combining digital holography and transport of intensity equation for quantitative phase imaging: a review

Ram Kumar<sup>1</sup>, Naveen K Nishchal<sup>1</sup>, and Kehar Singh<sup>2</sup>

<sup>1</sup>Department of Physics, Indian Institute of Technology Patna, Bihta, Patna-801 106, Bihar, India

<sup>2</sup>Optics and Photonics Center, Indian Institute of Technology Delhi, New Delhi-110 016, India

Dedicated to Professor Partha Banerjee for his enormous contributions to the advancement of research and education in holography through his unique vision and outstanding dedication

Recent advances in the field of digital sensors and computational facilities have turned digital holography (DH) into a powerful tool for many applications such as quantitative phase imaging, optical metrology, evaluation of cell parameters, optical cryptography, and optical pattern recognition among others. The transport of intensity equation (TIE) is one of the deterministic phase-retrieval methods, which does not use interferometric geometry. In this method, the phase is calculated directly from a set of intensities rather than iteratively approximating a solution. Recently, use of the TIE in the DH reconstruction process has been reported as a phase-retrieval method. Combining both DH and TIE provides better solution to the phase-retrieval. Refocusing property of the DH helps exploit the translation issue of digital sensor along the optical axis while capturing the intensity distributions at different depths. The issue of phase unwrapping is solved through the use of TIE. Hence, both the methods complement each other. In this paper, we review briefly the applicability of the combination of DH with TIE. © Anita Publications. All rights reserved.

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### 1 Introduction

The terms holograms and holography were first coined by Dennis Gabor who invented holography in 1948 and was awarded the Nobel Prize in 1971 for the discovery. Gabor's initial idea was to improve the resolution in electron microscopy using an 'optical corrector plate' to compensate the residual spherical aberration of an electron microscope [1]. However, Gabor did not succeed in his attempts because of the large difference between the optical-, and the electron wave lengths. To put it in very simple terms, holography is a two-stage process. The first stage consists of recording interference between two coherent waves called an 'object wave' and a 'reference wave'. In the second stage, the hologram is illuminated by a reconstructing wave (a replica of the reference wave). The process of diffraction involved in this stage is used to produce the 'object wave'. Since interference and diffraction are characteristics of waves, holography in principle can be performed with all waves in the electromagnetic spectrum, matter waves, and acoustic waves. The method of holography using optical wavelengths is termed as 'optical holography'. One of the disadvantages of optical holography is the requirement of optical recording medium, which requires high resolution photographic emulsions. This brings in severe inflexibility, long chemical processing, and relatively high running cost, limiting the number of possible applications. A detailed discussion of various aspects of optical holography (such as recording media, in-line and off-axis techniques, computer holography, incoherent holography, and

Corresponding author

e mail: [nkn@iitp.ac.in](mailto:nkn@iitp.ac.in) (Naveen K Nishchal). DOI: [10.54955/AJP.31.11-12.2022.1117-1128](https://doi.org/10.54955/AJP.31.11-12.2022.1117-1128)

various applications) is outside the scope of this article. A very simplistic description given above is only for the sake of continuity.

The advancements in charge-coupled device (CCD) and complementary metal oxide semiconductor (CMOS) technology-based detectors, and fast computational facilities have made possible the holographic operations digitally. Digital holography (DH) can be described as an electro-optical technique encompassing the abilities to capture an optical wavefield, an interferogram, on a digital sensor. The captured hologram can be stored in a computer and reconstruct the hologram numerically at any point in time and anywhere. Therefore, the DH overcomes the associated problems of the optical holography. Of late, DH has become a broad discipline of research with diverse applications [2-10]. The main objective of the holography in general, is to obtain the depth information that is retrieved in terms of phase.

Since DH allows capturing of hologram with a digital sensor, the reconstruction is carried out numerically. Computationally, diffraction of light is simulated from the re-illuminated hologram, on a computer screen. Principle of formation of hologram in DH is same as that of optical holography. It is the computation algorithm which provides measurement of phase and faithful reconstruction. The direct process of phase-retrieval from the DH uses the arc-tangent function, which is cumbersome and time consuming. In recent reports, with phase aberration compensation and coherent noise suppression, imaging quality improvement has been obtained in digital holographic microscopy [7,8]. Phase-retrieval algorithms have been developed in which the phase values are retrieved from intensity recordings [11]. The iterative phase-retrieval techniques are computationally intensive and hence are time-consuming. Their efficiency mainly depends on high computing capabilities. The phase-retrieval methods can be categorized into two types; interferometric and non-interferometric techniques. DH is an interferometric technique. Though interference-based techniques are well-established for phase-retrieval, they rely on perfect coherence of light source. Therefore, the phase aberration and coherent noise problems prevent accurate phase measurement and hence affect the quantitative phase imaging. However, Fresnel incoherent correlation holography [12] has been reported which avoids such problems. In this technique, the reflected white light from an object propagated through a diffractive optical element is recorded digitally. Three holograms are captured in sequence, each with a different phase factor of diffractive optical element and are superimposed in a computer. The reconstruction is carried out numerically.

Ptychography is a non-interferometric phase-retrieval method. It is based on diffractive imaging principle. It involves iterative phase-retrieval algorithm and has the ability to reconstruct complex optical fields beyond conventional hardware limits [13-15]. Ptychography reconstruction is mathematically closely related to that of recovering a signal from magnitude of its short-time Fourier transform. Though ptychography offers improved resolution, depth sectioning remains a challenge. A two-dimensional ptychographic forward model has been reported which assumes a thin object. In such a case, the interaction between the illumination and object can be factorized into a simple multiplication [15]. Recently, a Fourier ptychographic microscope has been reported, in which intensity data is recorded in spatial domain [14]. Fourier ptychography synthetically enlarges the numerical aperture of the system by capturing images corresponding to different parts of the spectrum and then they are assembled in the Fourier domain.

The transport of intensity equation (TIE) is a non-interferometric technique for phase-retrieval. It is relatively easy and computationally efficient process of phase-retrieval [16-25] as compared to the conventional phase-retrieval methods. In order to calculate the phase information, TIE requires two or more defocused intensity recordings. Of late, there are reports where phase-retrieval has been achieved from intensity patterns with partially coherent illumination [26]. In comparison to various methods of phase retrieval, TIE has been found as reliable and deterministic method and also cost effective [23,24]. For recording intensity patterns at two different neighbouring places, either the digital sensor or the sample needs to be mechanically translated. The mechanical displacement should be precisely controlled and accurately measured for quantitative phase

imaging applications. Gupta *et al* [26] demonstrated a technique in which instead of changing the propagating displacement, the variation in refractive index through liquid crystal variable retarder was achieved. Thus, the problem of mechanical translation of camera or sample was eliminated to capture the defocused image. Waller *et al* [27] used transport of intensity phase imaging in a volume holographic microscopy.

In 2013, the research group led by Asundi proposed a phase-retrieval technique after combining DH and TIE [28]. In this method, continuous phase information encoded in the DH was recovered. After numerical reconstruction and propagation of DH, TIE was applied. It was claimed that the recovered phase was free from the  $2\pi$  discontinuities; hence phase unwrapping problem was avoided. The combination of DH and TIE could also eliminate the tilt and quadratic phase aberration inherent with DH. Zuo *et al* [28] reported a direct continuous phase demodulation in DH employing TIE. Further, Zhou *et al* [29] proposed a technique, in which intensity images reconstructed from a DH were used as input for TIE for achieving unwrapped phase recovery. Phase imaging through DH and TIE was compared [30] for live cells and it was recommended to be combined for better accuracy. Of late, the combination has been reported [30-35] with better phase imaging capabilities and has been applied to varied types of applications.

In this paper, we review briefly the recent progress on the combination of DH and TIE techniques with the aim that it can be considered for newer applications. The combination overcomes the limitations of individual methods and brings out the useful features. The paper is organized as follows: the sections 2 and 3 discuss, respectively the principles of DH and TIE. Section 4 discusses the combination of DH and TIE techniques, its features, and usability. Section 5 discusses the potential applications and section 6 presents the conclusion.

## 2 Digital holography

As described earlier, DH consists of two processes: recording and reconstruction, like conventional holography. In DH, conventionally used photographic film is replaced by a CCD/CMOS camera for recording the hologram. The intensity of the interference pattern captured by a camera is transferred to the computer. Thus, the digital hologram is stored in the computer memory. For reconstruction of the hologram, the digitally stored hologram in the computer memory is accessed and numerically retrieved with the help of virtual reference wave. The virtual reference wave is simulated numerically, which is similar to the one used during recording. Usually, a plane or a spherical wave is used for the recording the digital hologram because these virtual reference waves can be synthesized easily and accurately, numerically. Since the recorded optical light field is in digital format, it is easy to apply digital signal processing techniques for improving the quality of reconstruction with various noise removal algorithms. The speed of the numerical reconstruction process depends only on the computer processor and efficiency of the algorithm.

Denoting  $E_0$  and  $R_0$  as the object and reference beams respectively, the resultant intensity can be expressed as,

$$H = |E_0 + E_R|^2 = |E_0|^2 + |E_R|^2 + E_0^* E_R + E_0 E_R^* \quad (1)$$

Here,  $H$  denotes the recorded intensity, which we call as the digital hologram. The 3rd and 4th terms on right hand side of Eq (1) contain the phase information. For reconstruction of the digitally captured hologram, a reference beam is simulated and applied to the hologram and it is propagated back to the distances used during recording. The first two real terms on the right-hand side of the Eq (1) present a central bright image, called as dc term. The 3rd and 4th terms contribute to the reconstructed images. On the computer screen the dc and both the images are reconstructed simultaneously. This reduces the available bandwidth. There are methods to remove the dc and one of the images [5,10]. The direct process of phase-retrieval from the DH uses the arc-tangent function. Depending upon the types of the object to be imaged, various recording geometries have been reported in the literature.

Figure 1 shows the schematic of a typical geometry for recording a digital hologram in the reflection geometry. In this set-up, a laser source is used to generate the coherent beam, which after collimation is allowed to illuminate the object. The reflected wavefronts from the object interfere with reference beam and the interference pattern is recorded by the camera. A neutral density filter is required to adjust the intensity of the incident beam. The captured interference pattern, termed as digital hologram is recorded by the CCD camera and stored in the computer memory for further processing.

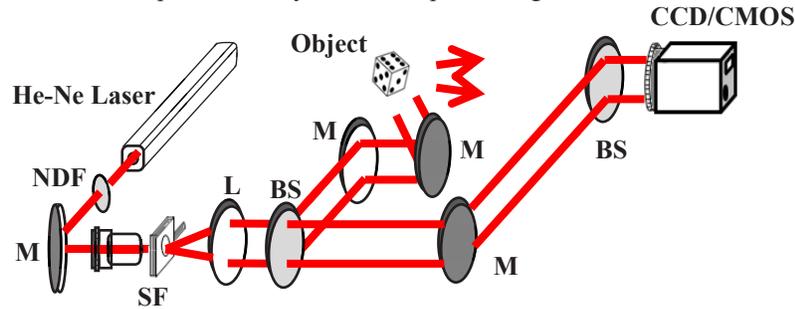


Fig 1. Schematic diagram for recording Fresnel digital holograms in reflection geometry. Figure adopted from Ref [41]. L: lens, M: mirror, SF: spatial filter, NDF: neutral density filter, BS: beam splitter, CCD: charge-coupled device, CMOS: complementary metal-oxide semiconductor. .

Considering Fresnel transform geometry, a digital hologram is simulated on MATLAB platform. An image of digit '1' has been used as the input object. The input image shown in Fig 2(a) is of size  $512 \times 512$  pixels. The used wavelength is 632.8 nm and the free-space propagation distance is 300 mm. The synthesized Fresnel digital hologram is shown in Fig 2(b). The numerically reconstructed image is shown in Fig 2(c). Here, no denoising and conjugate image removal techniques have been applied.

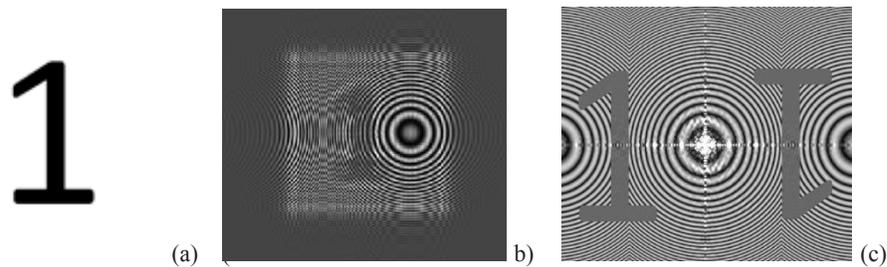


Fig 2. Fresnel hologram reconstruction. (a) Original image, (b) synthesized Fresnel hologram, and (c) reconstructed image [43].

The major concern with digital hologram reconstruction is the removal of undesired terms; dc and one of the conjugate images and phase wrapping. Another important aspect is that the method is cumbersome and time consuming. For improving the quality of reconstruction, phase unwrapping algorithms are applied.

### 3 Transport of Intensity Equation

The TIE is one of the deterministic phase-retrieval methods [16,17] in which the phase is computed directly from a set of intensity recordings. It is a non-iterative method. The TIE provides [16,38] an analytic relation between phase and intensity based on the Fresnel approximation in monochromatic light. The limitation with TIE technique is that it uses mostly pure phase objects. A pure phase object does not absorb much intensity so that the intensity across the observation plane remains constant [39]. This limitation of TIE restricts metrological applications to the non-transparent specimens illuminated by the monochromatic

light. The advantages of TIE are; it is computationally very efficient and requires only the acquisition of the intensities across two parallel planes separated along the principal axis of propagation [38-41].

The main assumption in the TIE is paraxial or Fresnel approximation. This means that the light beams have been assumed to travel along the principal axis of propagation. Considering z-axis as main direction of propagation, the complex wavefield of the monochromatic beam at a point  $r(x, y, z)$  in free-space can be written [40] as,

$$u(r) = f(r).exp(ikz) \tag{2}$$

where  $f(r)$  represents a slowly varying complex-valued amplitude function. The Eq (2) can be substituted into the Helmholtz equation because it should satisfy the Helmholtz equation as following,

$$(\nabla^2 + k^2)u(r) = 0 \tag{3}$$

The Eq (3) can be simplified as

$$\left(\frac{1}{2k} \nabla_T^2 + i \frac{\partial}{\partial z}\right) f(r) = 0 \tag{4}$$

where  $\nabla_T$  denotes Laplacian operator in the transverse plane ( $x, y$ ). This differential equation is also termed as the paraxial approximation of the Helmholtz equation. By means of parabolic equation, it can be expressed as,

$$\left(\frac{1}{2k} \nabla_T^2 + i \frac{\partial}{\partial z} + k\right) u(r) = \psi\{u(r)\} = 0 \tag{5}$$

For the sake of brevity,  $\psi$  has been substituted. Teague [16] used this parabolic equation and came up with the following statement,

$$u^*(r) \cdot \psi\{u(r)\} - u(r) \cdot \psi\{u^*(r)\} = 0 \tag{6}$$

$$k \frac{\partial}{\partial z} I(r) = - \nabla_T \cdot \{I(r) \nabla_T \phi(r)\} \tag{7}$$

Equation (7) convinces that TIE is an analytic differential equation that relates phase of the wavefield to the intensity. If the multiple intensity distributions across the observation plane are known, then the TIE can be solved by different methods such as orthogonal series expansion, multi-grid methods, and fast Fourier transform based method.

If an input object is a pure phase object or fully transparent to the incident intensity, the TIE can be solved by assuming the intensity  $I(x, y, z_0)$  to be constant across transverse plane as  $I_0$ . In this condition, the TIE can be converted into a single Poisson equation [41] as,

$$-\frac{k}{I_0} \frac{\partial}{\partial z} I(r) = \nabla_T^2 \phi(r) \tag{8}$$

The derivative of  $I(z)$  can be computed because intensity varies along the z-direction, the principal axis of propagation. The next step would be to solve the Poisson equation numerically. Applying fast Fourier transform, the Poisson equation can be solved, where the inverse of transfer function for the Laplacian operator can be used in the Fourier domain.

$$T(k_x, k_y) = -4\pi^2 (k_x^2 + k_y^2) \tag{9}$$

Computation of the intensity derivative along the z-axis is the most important step while solving TIE. It is because the intensity distribution is the only input to the TIE algorithm. TIE is calculated from two intensity recordings at  $z$  and  $-z$  positions after employing the finite approximation method.

$$\frac{\partial I(x, y, z_0)}{\partial z} \approx \frac{I(x, y, z_+) - I(x, y, z_-)}{\Delta z} \tag{10}$$

This approximation could serve better for phase-retrieval if three intensity measurements symmetric around the observation plane at  $z$ ,  $z_0$ , and  $-z$  are used. However, the choice of the distance is a trade-off in the TIE phase measurements. For better approximation of the difference quotient, small distance is suggested. But if the larger distance has to be chosen, the detection of the changes in the intensity would improve. It has been reported that the propagation distance in the range of the diffraction limited depth of focus is useful [41]. There are different approaches reported in literature on the improvement in approximation with intensity distributions at multiple planes.

A conventional TIE experimental set-up is shown in Fig 3. A light-emitting diode (LED) can be used as the light source for the experiment. Light from the LED source is allowed to pass through the biconvex lens (L1), here used as a condenser lens. The sample has been placed on a stage, after that microscopic objective, and a collimating lens is used to form a magnified image on the image plane. Here, lenses L2 and L3 are used as a 4f imaging processor, which relay the magnified image to the CCD plane. For recording two intensity distributions either the camera is mechanically displaced or the sample has to be displaced. The captured intensities are fed into the TIE algorithm for retrieving the phase.

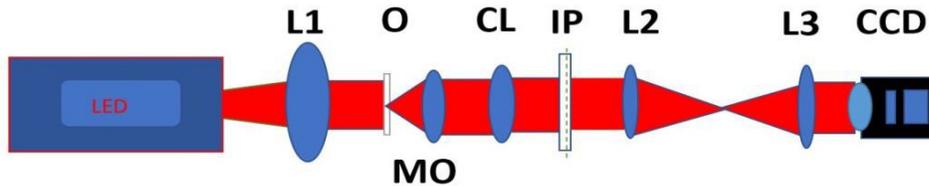


Fig 3. Schematic diagram of experimental set-up. Figure adopted from Ref [43]. LED: light-emitting diode, L: lens, O: sample, MO: microscopic objective, CL: collimating lens, IP: image plane, CCD: charge-coupled device camera.

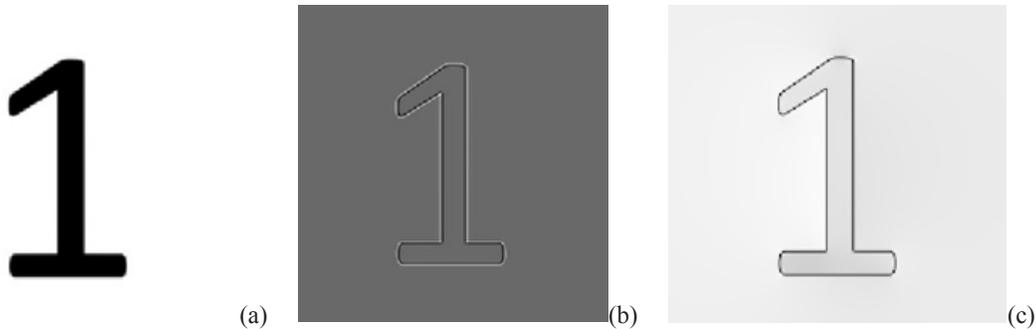


Fig 4. Simulation results of TIE. (a) Original image, (b) synthesized Fresnel hologram, and (c) retrieved phase image.

TIE simulation has been carried out on MATLAB platform where fast Fourier transform algorithm is applied. An image of digit '1' of size  $256 \times 256$  pixels, (Fig 4(a)), has been used as the input object. The input image is phase-encoded and its free space propagated images are reserved. Figure 4(b) shows the image recorded at intensity image after  $1.0 \mu\text{m}$  propagation. The retrieved phase image is shown in Fig 4(c). In this study, the wavelength has been used as  $600 \text{ nm}$ .

#### 4 Combining DH and TIE

DH is a mature technology for real-time and high-resolution three-dimensional phase imaging applications. In this method, the interference fringes convert phase changes into intensity variations. To get this phase back reconstruction process is followed. Only one digital hologram is required to retrieve

the light field, which is a great advantage of the DH. Although digital holography allows us to obtain the complex amplitude of the optical field, due to arc-tangent function its direct phase is wrapped. Therefore, it requires phase unwrapping algorithms for accurate phase measurement. Such a process is time-consuming, cumbersome, and computationally complicated especially for samples having complicated contours.

In 2010, Waller *et al* [27] proposed the idea of combining TIE with a volume holographic microscope for recording multiple intensity images in one frame. TIE is also a mature technology capable of directly extracting the quantitative phase without following the unwrapping algorithms. It is a non-interferometric technique, also called as self-interference method for phase measurement. It follows the method of propagation of a single beam in free-space. In comparison, the DH is affected by serious coherent noise and poor temporal stability, so DH needs stable and quiet condition for the measurements [28-32]. In contrast, the TIE also shows large error when the parameters are not selected properly, however, it can show better result if parameters are chosen appropriately. In case of quantitative phase measurement of transparent tiny samples, it can give good results for large-phase objects when the coherent noise can be ignored. For small phase objects, TIE may give better result. Such kind of comparative analysis can suggest a reference for application of two methods. However, in many cases we need to apply both the methods in succession according to the need and situation. For instance, Whittkopp *et al* [30] proposed the idea of a hybrid set-up combining digital holographic microscope (DHM) and TIE microscopic phase imaging system for three-dimensional data acquisition. The technique allowed the implementation of both methods in rapid succession for static target and live cells.

Zuo *et al* [28] reported the direct phase demodulation in DH with the use of TIE. TIE was applied following the numerical reconstruction and propagation of DH. In this technique, an electrically tunable lens and a 4f system was introduced into a conventional bright-field microscope to realize non-mechanical focus control for TIE. It has been claimed that the recovered phase is free from  $2\pi$  discontinuities that eliminates the phase unwrapping problem. It is possible to eliminate the tilt and quadratic phase aberration inherent in DH. But the method requires multiple recordings of intensity images. Further, in a study reported by Zhou *et al* in 2018, the intensity images reconstructed from a digital hologram were used as input to the TIE for unwrapped phase recovery. This method provided [29] elimination of physical movement of sample or imaging sensor. The proposed technique was tested for a phase object. Use of the TIE in DH reconstruction provides [31] ease for phase-retrieval applications. To summarize,

- (i) TIE requires physical movement of sample or imaging sensor for multiple intensity recordings. This can be avoided applying the refocusing property associated with DH.
- (ii) Use of TIE phase retrieval helps avoid phase unwrapping.
- (iii) Use of TIE would help eliminate tilt and quadratic phase aberration inherent with DH.

In 2021, Zhou *et al* [32] carried out the performance analysis of direct and unwrapped phase retrieval with DH and TIE combined technique. In this method, DH was used for numerically reconstructing the intensity distributions at different image planes. Following this physical movement of optical system for recording of multiple intensity images was eliminated. Phase-retrieval was achieved by the TIE technique and it was applied for both in-line and off-axis geometries for DH recording. Three basic steps for combining the DH and TIE have been suggested [32]:

- (i) recording of digital hologram of a sample,
- (ii) numerical reconstruction of intensity images at image plane and at defocused planes, and
- (iii) applying TIE for phase retrieval with three numerically reconstructed intensity images as input.

Phase imaging can be performed by TIE using sufficiently large defocusing distance in case of photon-starved illumination. Considering low-level illumination conditions for imaging such as in bio-

imaging and astronomy applications, TIE-based technique employing photon-counting phase imaging has been demonstrated by Gupta *et al* [33]. In low-level light situations, due to noise the imaging performance is degraded. It is because the magnitude of the wavefront curvature determines the quality of the retrieved phase for a given noise level and defocusing distance. In this study, a partially coherent light source was used where it was noticed that image misalignment issue comes into the picture due to the involved translation of imaging sensor for large distance. To solve this issue, Gupta and Nishchal [34] employed the digital hologram of the sample for required defocused intensities and then TIE was applied. For implementing the low-level light illumination while recording digital holograms, a variable neutral density filter was used.

Most recently, the combination of DH and TIE has been applied to digital holographic microscopy for eliminating the quadratic phase aberration [35]. Through numerical simulations, selection of regularization process under TIE has been suggested. Zhikhoreva *et al* [36] carried out a comparative analysis of TIE and off-axis DH for evaluation morphological parameters of cells. In a recent study, Tang *et al* [37] reported comparison of fast Fourier transform-based TIE method and common-path off-axis DH for quantitative phase measurements. They used some standard samples in the study and concluded that DH mainly suffers from coherent noise and poor temporal stability while TIE poses error when several factors are not selected properly.

DH and TIE both are phase-retrieval techniques and have been used in several applications. One of such applications is in optical image encryption [38]. The usability of the technique becomes significant if the input image/data to be secured is a phase-only function. Gupta *et al* [39,40] for the first time applied the concept of TIE in fully phase image encryption. Since TIE allows us to calculate the phase information from the defocused intensity, this phase information has been encrypted by spatially variant polarization encoding [40]. This asymmetric encryption method secures the phase information providing stronger security and higher encoding capacity.

## 5 Benefits of the combination of the DH and TIE and potential applications

As pointed out earlier, with emphasis and advances in TIE and DH, both the phase-retrieval tools have been combined [29,31,32]. The advantages of this combination are that multiple recordings of intensity distributions are automatically done numerically with a single digital hologram recorded and without the need of movement optical components such as sample holder and sensing device. Here, computed phase is naturally unwrapped and computational time is also much smaller than those using phase unwrapping algorithms. Whittkopp *et al* [30] proposed a digital holographic microscopy and TIE microscopic phase imaging systems for three-dimensional data acquisition. The technique allowed the implementation of both methods in rapid succession for static target and live cells. Another advantage of TIE over interferometric technique is that it is naturally compatible with commercial bright-field microscopes. Motion controller can be used for defocusing either by shifting the position of imaging camera or utilizing the microscope's own focus control. Physical defocusing may be avoided by utilising beam splitters, a programmed phase mask, or a tunable lens [28].

DH has already been reported for large number of applications and TIE has been used for phase-retrieval and imaging applications [22,25,33]. Combining both the tools enables ease and efficiency. Some of the applications already reported in literature include digital holographic microscopy, tomography, three-dimensional data acquisition, optical information security, elimination of quadratic phase aberration, and removing the artifacts caused due to low light conditions. Various optical geometries such as single-shot imaging have been reported for TIE where multiple acquisition is discarded with this technique [42]. A Michelson interferometer set-up with a light emitting diode as a light source has been used. The mirrors used in the set-up are slightly tilted for obtaining two laterally separated defocused images as a single-shot to the detector. Hence, the experimental set-up used in Ref [42] eliminates the requirement of any costly

optical device such as spatial light modulator. Further, a composite method of TIE-based phase imaging has been reported for broad range of spatial frequency recovery [43]. In TIE, the range of well-resolved spatial frequencies in the retrieved phase reduce with the increase in distance between imaging planes. Therefore, smaller image separation is required for recovering the higher spatial frequencies accurately. Similarly, for retrieving lower spatial frequencies accurately, larger separation is needed. A phase-image retrieved from the intensity distribution recorded at large defocus distance provides well-resolved low spatial frequencies. This is achieved at the expense of higher spatial frequencies. On the other hand, a phase image retrieved from small defocus intensity data provides higher spatial frequencies at the cost of lower spatial frequency content. To overcome this difficulty of high and low spatial frequencies with a single-phase image, Gupta and Nishchal [43] proposed a composite method.

## 6 Conclusions

The DH and TIE both are treated as excellent tools for retrieving phase information from the intensity data. DH requires single frame recording while TIE needs two or more intensity distributions for phase measurement. The results with TIE often lack reliability, while DH is regarded as a standard for phase measurements. There are benefits and limitations associated with both the tools. If both are combined and applied in a complementary way to each other, it serves better, as has been reported in some of the recent studies. Till date, there are very small numbers of research articles available in literature where DH and TIE both have been applied for some specific applications. This technology will be immensely helpful for biological scientists. It has been suggested that in future the combination of DH and TIE can be further developed by using higher-order intensity derivatives in TIE [29]. There are many possibilities for exploration of DH and TIE using partially coherent light sources with the help of optical scanning holography or Fresnel incoherent correlation holography. Such studies would open up newer dimensions of research with innovative solutions to research problems.

It may also be mentioned in passing that the use of TIE is a very active area of investigations, and a number of recent publications [44–52] may also be noted.

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