Chapter 1

Introduction

Work presented in this thesis is based on the designing and application of digital holographic interference microscope (DHIM) for quantitative phase contrast imaging of micro objects. In this chapter basic concept of holography and its evolution from conventional method (film based) to digital method is briefly described. Applications of various digital holographic configurations in microscopy are discussed in general including outline to the configurations described in the thesis.

1.1 On-axis and off-axis holography

In 1948, Dennis Gabor introduced concept termed 'Holography' as 'a new microscopic principle'. In the word holography 'holo' means 'the whole', representing recording of complete information, which is both amplitude and phase, about the wave field. This recorded hologram (which is nothing but an interference pattern) then used to regenerate the wavefront scattered by the object under investigation [1].

Holography is the consequence of Gabor's effort started with the interest to improve the resolving power of electron microscope to image atomic lattices. Idea dawned in his mind of recording electron micrograph which contains whole information and correcting it by optical means, as the picture gets blurred due to severe spherical aberration with the limitation of aperture of electron lens. This became a base for 'A new microscopic principle' with two stages process, recording and reconstruction. Physical foundation of holography lies in the science of waves, the interference and diffraction. Diffraction was first noted by F. M. Grimaldi as deviation from

rectilinear propagation and interference generated by thin film was first observed by R. Hooke. Mathematical basis of wave theory describing these effects was founded by C. Huygens. T. Young introduced interference principle and A.J. Fresnel calculated diffraction patterns for different objects. Thus, sufficient knowledge was given by these scientists to formulate the fundamental principle of holography. G. Kirchhoff, Rayleigh, E.Abbe, Lippmann, M. Wolfke, H.Boersch and W. L. Bragg were very near to the principle of holography. But practical realization of holography was done by D. Gabor in 1948 [1]. He presented holography as lensless process for image formation by reconstructed wavefront. He was awarded Nobel Prize in Physics in 1971, for the invention and development of the holographic method [1, 2, 3, 4]. Holography described by is a two stage process. In the first step record of the phase and amplitude information of the scattered wave field (object wavefront) from the object under investigation is constructed by combining it with the unscattered wave field (reference wavefront) on the photographic plate with concept of comparing phases of object wavefront with phase of 'coherent background' or 'reference wavefront' (Fig.1.1a). Recorded pattern is a spatially varying intensity distribution (interference pattern) generated by the superposition of the two fields (Fig1.1b). Thus information about the full complex wave field of the object is contained in the resulting interference pattern. This recorded pattern containing information about intensity and phase of wave field is called hologram.

Second step is reading information contained in the hologram by illuminating the hologram as if hologram is new object. Reference wave (without the object) is used to illuminate microstructures of interference pattern and image of the object is reconstructed by diffracted (scattered) reference beam which mathematically is the product between the illuminating wave and the transmission function of the hologram [3, 4]. But the reconstructed image resulting from this inline setup is corrupted by the DC terms as well as the twin image (Fig.1.2). Reconstruction generates 'virtual image' and 'real image' of the object both overlapping each other [2, 3, 4]. This experiment predates the invention of laser by more than 12 years. It was not possible to produce good quality coherent light, which is essential to create high quality holograms, so

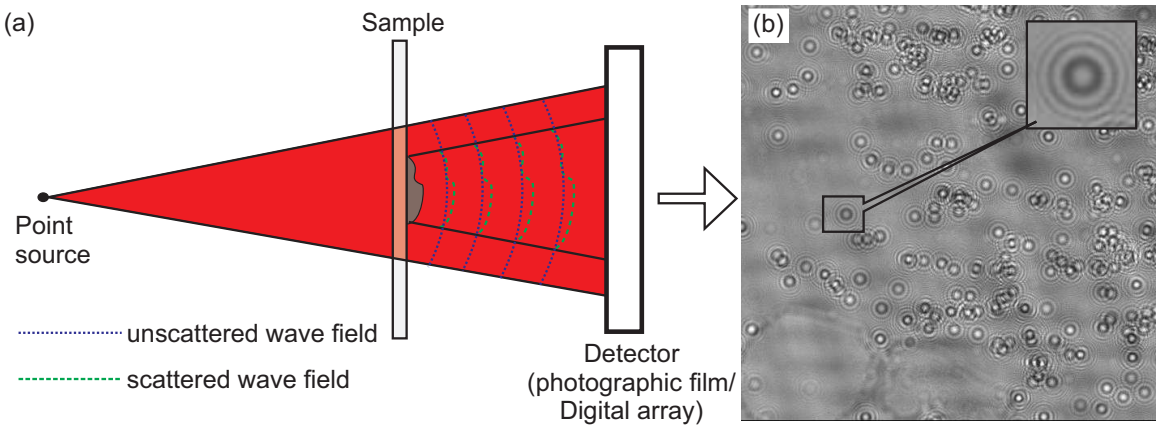

Figure 1.1: (a) Recording geometry for Gabor holograms (on-axis geometry). (b) Hologram resulting from superposition of scattered and unscattered wavefronts at the detector plane (object was a random distribution of transparent microspheres).

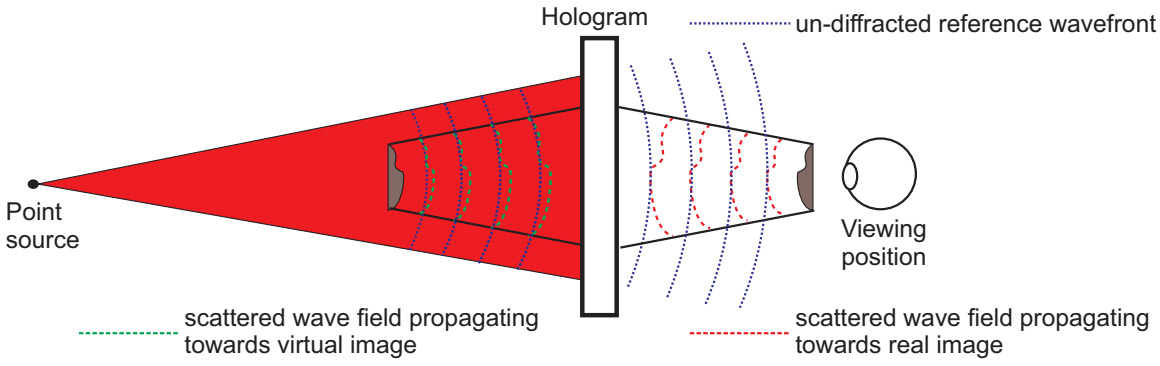

Figure 1.2: Image retrieval from Gabor (on-axis) holograms

Gabor used Mercury arc lamp as source. Also the short coherence length of Mercury-arc lamp source was the reason for implementing on-axis geometry, in which the object and the reference wavefronts propagate along the same path and interfere with zero angle between them. After invention of laser, E. Leith and J. Upatnieks [5] introduced off-axis holography (Fig.1.3). In their experimental setup, Helium-Neon (He-Ne) laser with high coherence length was used and reference beam was separated by an angle with respect to the object beam. In reconstruction two angularly separated images were obtained (Fig.1.4). This method is also called split beam or two-beam method. This was a considerable improvement in Gabor's original arrangement. 3D media can also be used for recording of holograms as shown by Denisyuk [6].

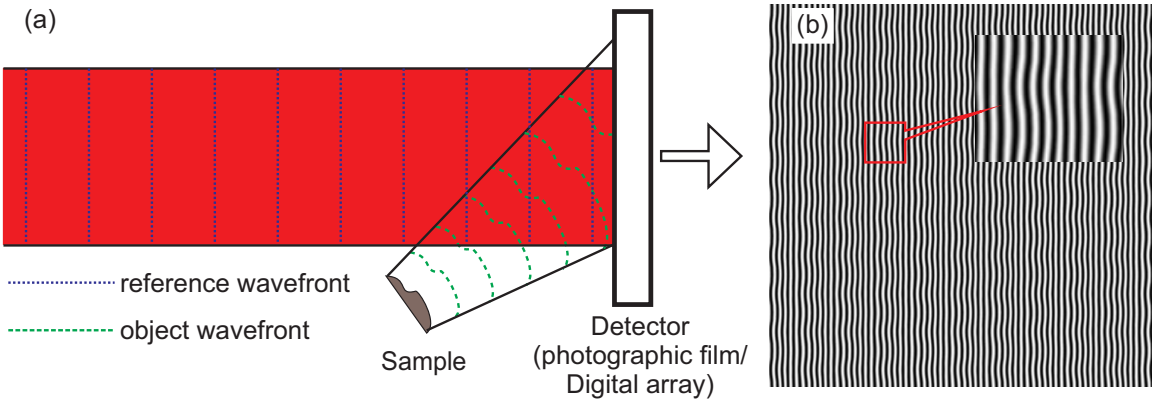

Figure 1.3: (a) Recording of off-axis holograms (b) Hologram resulting from superposition of object and reference wavefronts (object was a distribution of transparent microspheres).

1.2 Conventional to digital holography

Any medium sensitive to photons can be used for recording of holograms. This includes digital arrays consisting of micrometer sized diodes. These arrays make the direct recording of digital holograms possible. With the digitally recorded holograms, image reconstruction can be achieved numerically on a computer instead of physically reconstructing the hologram by illuminating it with the reference wavefront. They have also advantages over traditional holograms as no photographic films are

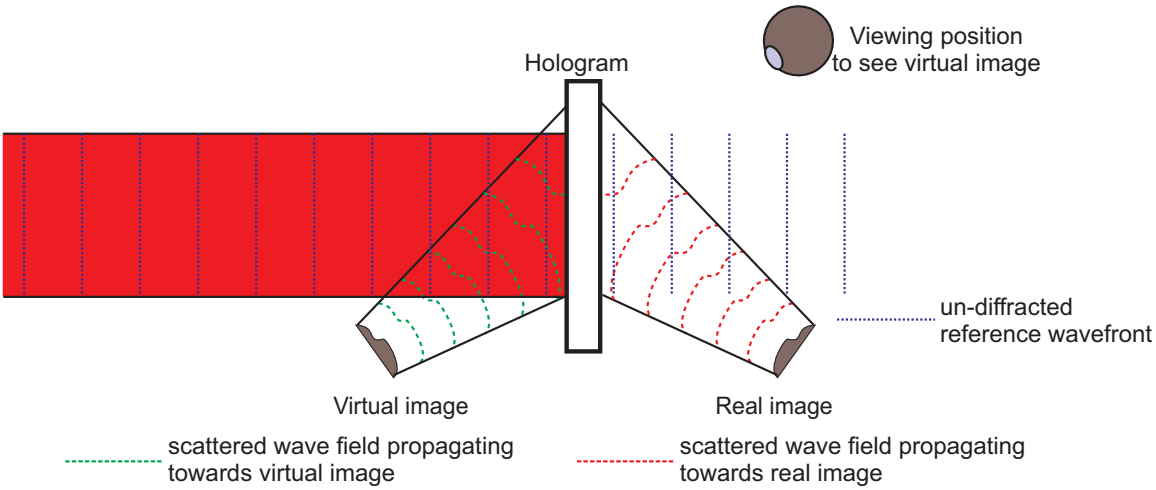

Figure 1.4: Image reconstruction from off-axis holograms

required for recording and hence no chemicals or physical development is necessary for reconstruction. The use of an electronic sensor allows video rates of capture and the recorded holograms can be processed at a later time to retrieve the time varying object profile. In fact the first significant step towards digital holography was demonstrated by Brown and Lohmann, who used a computer-guided plotter to generate a hologram [7]. They generated binary digital holograms and reconstructed them optically. Their purpose was to study its use in pattern recognition. The following year, Goodman and Lawrence [8] recorded a hologram on a vidicon camera with the lens removed. They then used a digital computer to reconstruct these holograms numerically [8]. Numerical reconstruction of holograms was also demonstrated by Yaroslavskii, Merzlyakov and Kronrod [9]. T. S. Huang [10] also demonstrated possibility of computer generated holograms and computerized reconstruction of Fourier and Fresnel holograms [10]. The field of digital holography was growing with the accessibility to compact digital arrays and computers with high computational abilities. In 1994, Schnars and Jauptner [11] successfully recorded and numerically reconstructed Fresnel holograms using a CCD array, by numerically simulating light propagation from the hologram to the image plane. In single shot digital holography, a plane reference wave and the wave scattered from the object interfere at the surface of electronic sensor at an

angle. The resulting hologram is electronically sampled and stored. Reconstruction of the recorded digital holograms is achieved by simulating diffraction of the reference beam occurring at the microstructures of the recorded hologram by numerical implementation of scalar diffraction integral.

1.3 Application of holography

Since the discovery of holography, many applications of holography and then application of digital holography are established. Various parameters responsible for changing phase of wave field can be images and quantified using holography, as holography is based on principle of phase change. Deformation measurement [12], shape measurement or contour measurement [13], determination of refractive index change [14], measurement of vibration modes [15, 16] etc., are different applications reported in the literature. Measurement of change in refractive index is an important parameter to study micro objects, especially biological cells or samples [17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45]. Digital holography with microscopy can obtain quantitative phase information of micro-objects that in turn provides information on the optical thickness variation in the object. Digital holographic interference microscopy has immense potential for application in biomedical imaging. Imaging of various biological cell types and their dynamics is possible using DHIM. It can also be used for tomographic imaging either using multi-wavelngth approach [46] or by acquiring the optical field transmitted from numerous different directions [47, 48, 49, 50]. It can also be used for microscopy and metrology of microstructure such as MEMS which are of relatively smooth and well defined surface profile [51, 52]. Nonlinear optics processes can be monitored with DHIM [53, 54]. Many other techniques like optical scanning holography, total internal reflection holographic microscopy, particle field holography, heterodyne holography, digital interference holography are developed for various applications. The main focus of this thesis is the development of digital holographic interference microscopes and their application.

1.4 Digital holographic interference microscopy (DHIM)

It has always remained great area of interest to explore the microbes or microorganisms, which are said to be the first forms of life on our planet and they have been influencing our lives since time immemorial. With the aid of various microscope techniques, it has become possible to look into the world of these micro objects. Study of static and dynamic properties of these objects, their form, structure, reproduction, physiology, metabolism and classification has become practicable with development of different imaging techniques such as bright field microscopy, fluorescence microscopy, dark field microscopy, phase contrast microscopy etc., [55]. Since many living cells are transparent to light in the visible regime, their observation by recording the light intensity transmitted by the cells (bright field imaging techniques) becomes a bit difficult without the use of tagging or labelling agents. So observation and identification of cell with microscopes which relies on intensity profiles requires staining of the specimen to improve the contrast. But the process of staining may change the morphology and life cycle of the cell. An easy way to increase image contrast without tagging agents is to use phase imaging techniques, where phase of the object wavefront (wavefront interacting with the specimen) instead of its intensity is imaged. In optical phase contrast microscopy, two most widely used techniques are Zernike and Nomarski methods [55, 56]. But quantitative phase information cannot be obtained by Zernike phase contrast microscopy due to shading-off effect. In Differential interference contrast (DIC) technique that is in Nomarski technique, accurate quantitative phase measurement can be done but some sophisticated techniques for signal acquisition and processing are required. Invention of holography made imaging of object viable in its three dimensional and in realistic nature by recording entire field information that is amplitude and phase [1, 2, 3, 4, 5]. With the invention of lasers (coherent source of light) providing high quality interference patterns and with the concept of off-axis illumination which eliminates the problem of twin images and zero-order, full potential of holographic interferometric technique was realized [2, 3, 4].

Digital holographic microscope can be constructed using various geometries depending on the application, stability, compactness, cost effectiveness and field portability of the setup.

1.4.1 On-axis DHIM with Gabor lens-less geometry

In Gabor lens-less holographic microscope set up no lens is used for imaging the object. Source beam is a spherical beam and this diverging beam is used to illuminate the object (Fig.1.1a). Here, single beam is used for illumination and no separate reference beam is used. Object in this case should be transparent. Illuminating wave field emerging out from the object is partly scattered and partly un-scattered and this wave field behaves as coherent background and the field scattered by the object carrying object information is object beam [19, 57, 58]. Both are allowed to interfere at detector plane. Reference beam and object beam both travel along the same direction with no angle difference between them. It is called inline or on-axis geometry. Also all the components are kept inline. Fig.1.1 a shows the schematic diagram of inline DH microscope with Gabor's lens-less geometry. It is more suitable for small objects, as reference should not be perturbed excessively and most of the beam reaching the detector should be unperturbed. This makes only sparse object distributions recordable. This technique is also useful in particle image analysis [59, 60, 61].

1.4.2 Inline DH Microscopy with Gabor's geometry using lens

Introducing lens in Gabor's original setup of holographic microscopy that is recording hologram with imaging lens, as shown in the Fig.1.5 [62].

This lens is used to get magnified image of the object and also increases the numerical aperture of the system compared to lens-less geometry [57]. Lens can be introduced for illuminating the object with plane wave. This makes reconstruction easy, as there is no restriction on propagation distance of plane wave. On other hand in lens-less setup, numerical aperture and resolution can be increased by keeping object closer to the sensor, but in reconstruction aberration may get introduced if Nyquist frequency criterion is not achieved.

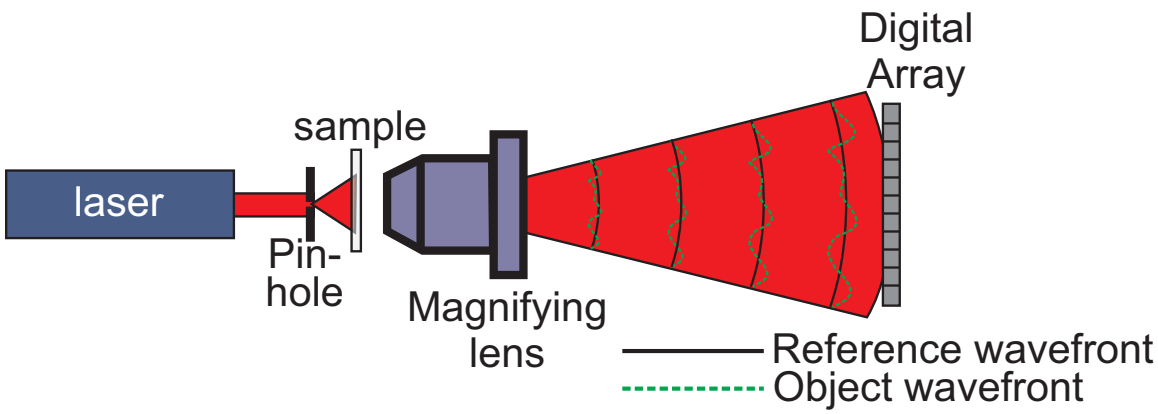

Figure 1.5: Gabor DHIM using a magnifying lens. The detector plane is situated near the image plane of the magnifying lens.

1.4.3 Inline DH Microscopy with separate reference beam

Gabor setup can be modified by introducing a separated on-axis reference beam (Fig.1.6), making it useful to record non-sparse object distributions also [21, 24, 28, 35, 63]. Here object beam and reference beam both are separated and are allowed to travel in the same direction without making any angle between them. Source beam is split into two beams using beam splitter or other optical component. Mach-Zehnder and Michelson interferometers are commonly used interferometer geometries for this type of set up. In this, two beams travel along different path and then both beams are allowed to interfere at detector plane. Hologram is recorded and stored digitally. Reconstruction is done numerically, simulating the diffraction of reference beam from the recorded hologram. In inline setup space bandwidth product (SBP) is higher, that ensures capability of system for recording of entire object information. Thus inline DHIM is easier to implement due to its simple structure so a compact, simple and cheap microscope can be constructed using this geometry. But this method suffers from poor reconstructed image quality due to the presence of the DC term (bright spot due to reference wave intensity) and the out-of-focus twin image. As reconstruction beam form a focused real image of the object and an out-of-focus virtual image. These two images are directly overlapped and are not separable with conventional inline techniques. This is called the 'twin image problem' in holography.

The result is that the phase of the object cannot be unambiguously reconstructed because of an unwanted artifact term from the virtual image [2, 3, 4, 5]. This could potentially lead to greater error in localizing objects along the direction of the optical axis.

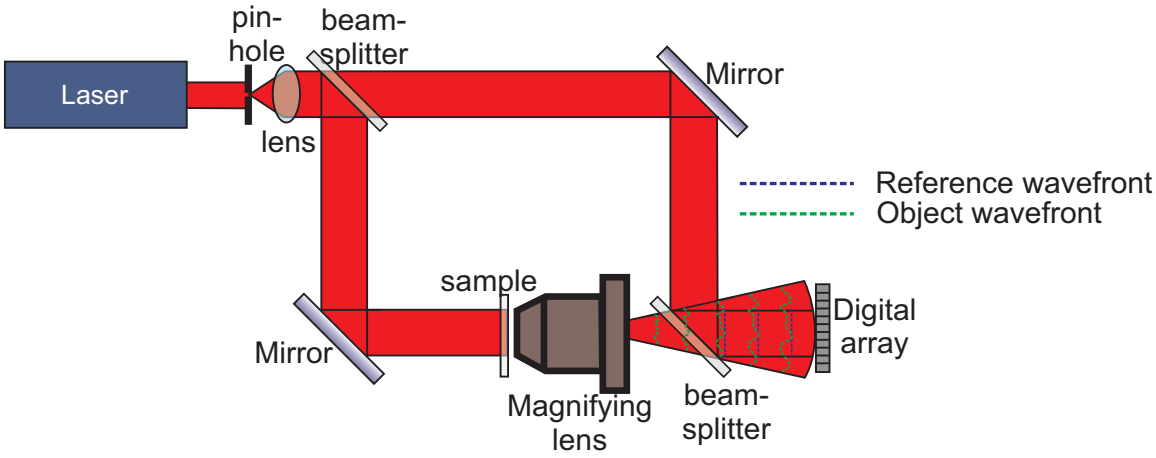

Figure 1.6: On-axis DHIM with a separate reference beam. Here also the detector plane is situated near the image plane of the magnifying lens.

For removal of the 'twin image problem' from on-axis digital holography, various algorithms and number of techniques are mentioned in the literature [64, 65, 66, 67, 68, 69, 70]. Phase shifting interferometry is one of the best solutions for eliminating the twin image problem. It is almost similar to inline setup, with the only difference of two wave retardation plates or other phase shifting arrangement. The linearly polarized reference beam allowed to pass through half-wave plate and quarter-wave plate. By rotating the plates one can achieve four phase shift permutations of 0, $\pi/2$, π and $3\pi/2$. Holograms are recorded with a shift in phase between the object field and the reference field, and these may be combined to obtain the object field amplitude and phase at the recording plane [68]. Applying suitable reconstruction algorithm real image of object can be retrieved suppressing DC term and the twin image. But drawback of this technique is that dynamic object cannot be studied, as during capture of four interferogram object needs to be static. This method is

not suitable for studying living biological specimens. However, method for capturing multiple interferograms in parallel [66], phase shifting array device is developed. Considering all these, off-axis geometry for holographic microscopy offers advantages over on-axis geometry, such as spatially separated virtual and real images and correct phase retrieval from a single hologram without phase stepping.

1.4.4 DHIM based on Off-axis geometry

Off-axis DHIM is the setup with geometry that makes the reference beam and object beam interfere at some angle at detector plane as shown in Fig.1.7 [17, 18, 20, 22, 23, 25, 26, 27, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54].

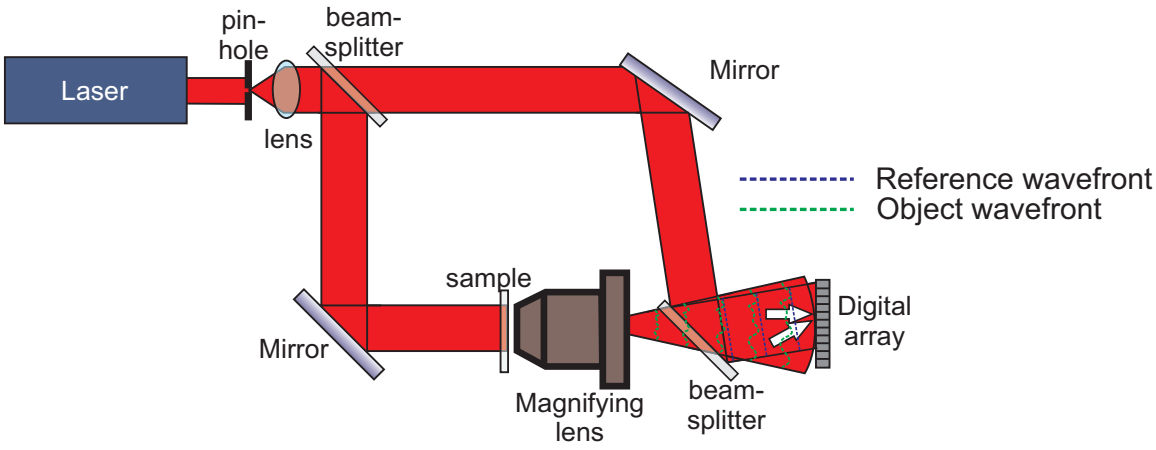

Figure 1.7: Off-axis two-beam DHIM. Detector plane is situated near the image plane of the magnifying lens making the phase retrieval possible by the use of a filtered Fourier transform.

This eliminates twin image issue, as this configuration separates out real image, DC term and virtual image in space in spatial frequency domain [69, 70, 71]. This angle offset adds a carrier frequency to the signal making unambiguous reconstruction of the amplitude and phase of the light from the original object possible from a single hologram and the twin image problem of inline holography can be removed. Fourier transform of digitally recorded hologram can be taken numerically and desired part of it can be spatially filtered to extract the real image of the object [69, 70]. For

off-axis DHIM, laser beam is split into two identical beams. One of these beams transilluminate the object under investigation and hence carries object information and another beam behaves as reference beam. These two beams superpose to create the interference patterns (holograms) at sensor array, situated at the image plane of the lens used to magnify the object. Optical path travel by two beams must be almost equal (it should be exactly same in the case of low temporally coherent sources) to get high contrast interference pattern. Since, in this case, the phase information can be reconstructed from a single hologram; the method may be used to study time evolving path length changes in the case of living cells. Most commonly used two-beam or off-axis setup is Mach-Zehnder setup [17, 18, 20, 22, 23, 25, 26, 27, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54].

In this thesis, Chapter 3 and Chapter 4 present application of Mach-Zehnder (MZ) geometry based two-beam digital holographic microscope to study biological samples. In Chapter 3, Digital holographic microscope with Mach-Zehnder geometry is presented and application of microscope in the study of plant cells in various conditions is shown. Plant cells are larger in size (few tens of micrometers). Use of full field of view in MZ-DHIM is one of the reasons for choosing the setup for plant cell study. Flexibility in construction of DHM with MZ geometry allows it to be used for studying dynamic properties of cells in various conditions. We have chosen onion skin cells as sample, and effect of preservative treatment on onion cells applying sugar solution to it, comparison of cell thickness for different epidermis layers of onion scales, comparison of thickness for germinated and non-germinated onions etc. is presented in the chapter. 3D reconstruction thickness profile of subcellular components such as cell wall, nucleus is also shown. Application of MZ-DHIM for obtaining static and dynamic properties of human erythrocytes (here Red blood cells) is discussed in Chapter 4. Cell membrane fluctuations are measured using the technique. The main disadvantage of DHIM employing off-axis (Mach-Zehnder) configuration is that it is prone to mechanical vibrations due to the two-beam geometry. When two beams travel along different paths, they can acquire phases (due to vibration), which are uncorrelated, leading to low temporal stabilities. But temporal stability is utmost importance while measuring fluctuations cell membranes. To overcome this hurdle,

one would like to have a single path setup (like that of Gabor on-axis DHIM) while providing direct access to phase information (like that of off-axis DHIM). Unwanted fluctuations arising due to two-beam geometry can be greatly reduced by using common path geometry [72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83].

1.4.5 Common path off-axis DHIM

In DHIM with common path geometry, two beams travel along the same path and encounter same set of optical elements. And that is the reason, one can construct a simple, compact (with less number of components) and temporally stable 3D microscope for quantitative study of static and dynamic properties of biological specimen. In the configuration of common path geometry a portion of the object beam is converted into reference beam and object and reference beam propagate along the same direction as in inline geometry but interfere at detector at some angle, thus forming off-axis holograms. One of the ways to implement common path DHIM is by spatially filtering one of the two object beams [72, 73, 74, 75, 76, 77, 78]. An easier way to implement common path geometry is using self-referencing geometry, where a portion of the object beam, which is not modulated by object information, acts as the reference [79, 80, 81, 82, 83, 84].

Common path DHIM with spatial filtering

In this geometry a separate reference beam is created from one of the two identical versions of the object beam by spatial filtering (Fig.1.8).

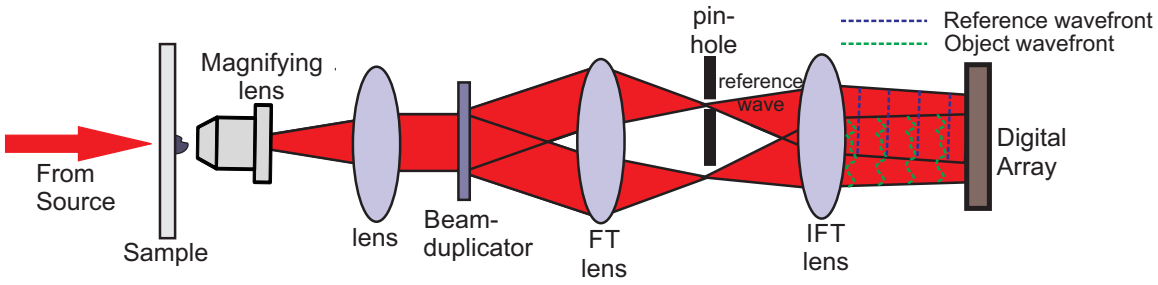

Figure 1.8: Common path Off-axis two-beam DHIM. A separate reference wave is created by spatial filtering of one of the duplicated object wavefronts.

Two versions of the object beam are created by a beam duplicating element, like a beam splitter, diffraction grating or even a diffuser [72, 73, 74, 75, 76, 77, 78]. One of these beams is converted into a reference beam, usually by Fourier transforming both the object beams and filtering one of the beams by the use of a pin-hole to remove any object information from it. This pin-hole is kept at the front focal plane of the Fourier transforming lens. Diffraction phase microscope with common path geometry including diffraction grating to split the output beam was represented by G. Popescu [72, 73, 74, 75], in which at the image plane of inverted microscope, an amplitude diffraction grating is used to get multiple diffraction orders containing full spatial information of the object. This grating is kept at the beginning of a 4f system. Zeroth- and first-order beams are focused at Fourier plane by a lens and then isolated by spatial light modulator. Only DC component of the zeroth-order beam is passed and behaves as a reference beam and first order is fully passed that behaves as imaging field [72]. Both beams then interfere after passing through another lens (inverse Fourier transform lens) and spatially modulated interference fringes are formed and captured by digital array. Quantitative phase image of the object is then retrieved by numerical processing (usually a filtered Fourier transform of the resulting fringe pattern) and cell morphology is measured [72, 73, 74, 75]. N. Shaked also described common path geometry using Michelson interferometer geometry by spatially filtering one of the object beams [76]. In his experiment an inverted microscope is combined with τ interferometer [76]. Magnified output beam from the inverted microscope is Fourier transformed by a lens and then allowed to pass through a beam splitter. One of the resulting two object beams is then filtered by a pinhole kept at Fourier plane. This beam behaves as a reference beam which exhibits almost no object information because only the dc part of the spatial frequency spectrum of the image is allowed to pass. Second beam behaves as object beam. Both these beams are made to interfere at detector plane after inverse Fourier transform by another lens in a 4f system [76]. Another reported geometry, uses the advantage of counter propagating beams in Sagnac geometry to create two object beams travelling along the same path, albeit in opposite directions [77]. Here also one of the beams is Fourier filtered to act as the reference beam. These common path geometries provide highly temporally stable

configurations which are suitable to measure dynamics of cells with sub nanometer temporal resolution [72, 73, 74, 75, 76, 77, 78].

Chapter 5 provides the description of development of a common path DHIM using lateral shearing geometry and its applications in imaging of human erythrocytes. Its geometry is based lateral shearing configuration employing a thick glass plate to create two laterally shifted versions of the object wavefront. One of the sheared wavefront is used to obtain separate reference beam by sampling the portion of wavefront by a pinhole. The use of a laser diode module as the source, a webcam sensor as the detector and simple glass plate as beam duplicating device make the set up cost effective. Less number of components makes the setup temporally stable, compact and simple. Static and dynamic properties of Red blood cells are obtained and presented.

Common path DHM with Self-referencing

An easier implementation of common path two-beam DHIM can be achieved by the use of self-referencing geometry [79, 80, 81, 82, 83, 84]. In this configuration, a portion of the object beam, that is un-modulated by object information, acts as the reference wave field (Fig.1.9) for the modulated portion of the same wavefront. Here no special optical elements are needed to convert object beam to reference beam. Optical components like grating or other beam splitting elements can be used to obtain two identical beams from a source. As shown in the Fig.1.9, source beam passes through the object and the magnifying lens, is converted into two identical beams by a beam duplicator (grating, beamsplitter, glass plate, mirrors etc). These two object beams are angularly shifted and are made to superpose at the sensor plane for creation of holograms, that could be reconstructed numerically just like a normal off-axis two-beam holograms [82, 83, 84]. The easiest and common way to construct self-referencing DHM is by lateral shearing of the object beam using glass plate [82, 83, 84]. The phase information from the hologram of the background (recorded without the object, but with the surrounding medium present) is subtracted to get object phase information.

In the beginning of Chapter 5 self-referencing DHM using lateral shearing geometry is discussed. The most important advantage of this technique is its immunity

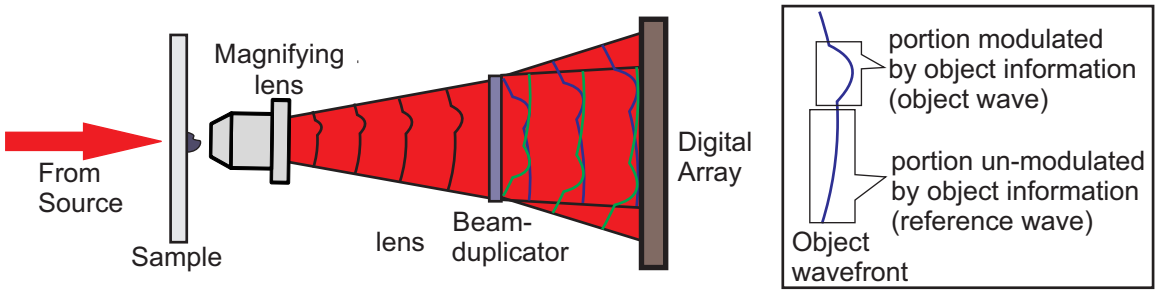

Figure 1.9: Common path off-axis self-referencing DHIM. Portion of the object wavefront un-modulated by the object information act as the reference wave field and the modulated portion act as the object wave field. Angular separation between them is introduced by the beam duplicator.

to external vibrations. Also only few optical components are required to build such a microscope [82, 83]. One can make it simple, compact, field portable and also cost effective setup with such geometry. However, they have the drawback that only half the field of view is usable and that it can image only sparse object distributions [82, 83]. In addition, the reference beam may contain some undesirable information about the object structure, which could be solved by spatial filtering of the one of the object beams to convert it into reference beam. In Chapter 6, efforts made to construct a self-referencing digital holographic phase tomographic microscope using lateral shearing interferometer configuration are detailed. The microscope consists of an array of laser diode modules illuminating the micro-objet at different angles. The holograms are reconstructed to yield object phase information at different projection angles. This phase information is then used in the back projection algorithm to yield local values of object optical path length change.

1.5 Outline of the thesis

This thesis focuses on development and application of DHIM for quantitative phase contrast 3D imaging and quantification of micro objects. It is an effort towards development of various digital holographic 3D imaging techniques by implementing different geometries and applying them for extracting static and dynamic parameters

of biological samples like plant cells as well as human erythrocytes. With background of development from holography to digital holography, an introduction to DHIM as quantitative phase contrast imaging technique in its various forms such as Gabor's lens less DH Microscope, inline DHIM, off-axis DHIM and common path DHIM etc., has been discussed in the present chapter. Chapter 2 provides the basic theory of digital holography, recording of hologram using digital sensors and numerical reconstruction methods with angular spectrum propagation approach. Chapter 3 is based on application of Mach-Zehnder off-axis DHIM (as mentioned in section 1.5.4) in study of plant cells. Static and dynamic properties of plant cells in various conditions are studied. Onion skin cells are chosen to study the process of osmosis as well as its texture change with preservative solution. Thickness changes in cells under various conditions are compared. This work is intended to show application of digital holographic microscope in study of plant cell physiology, which in turn is useful in food processing, agriculture, botanical and biotechnological research. Chapter 4 includes application of same setup in study of static and dynamic parameters of human red blood cells. The parameters like cell thickness profile, cell membrane fluctuations may further be useful in identifying and analyzing healthy and diseased cells. Chapter 5 represents a cost effective, compact, simple and temporally stable common path DHM with a spatial filter using lateral shearing geometry, with its application in the study of human red blood cells. This common path digital holographic microscope is configured with a separate reference beam as discussed in section 1.5.5A. In chapter 3, 4 and 5, obtained results for biological cells from single hologram basically provides a path integrated or average output of path length change. Under- or overlying structures inside cells, or other optical inhomogeneity cannot be differentiated by above mentioned techniques. Phase tomography with digital holographic microscope is useful in extracting phase information from various directions to the object, and thus extracting spatially separated structure profile inside the cells. Chapter 6 introduces technique of phase tomography of micro objects using self-referencing digital holographic microscopy. Multiple projections of object from various angular directions (projection) are taken and are used in back projection algorithm to yield the local optical path length values. Finally in Chapter 7, summarizing contribution

of the thesis in development of various techniques for phase contrast imaging and their application in study of micro objects, future directions that could be pursued are advocated.