Synopsis of the thesis entitled

LOW COHERENCE INTERFEROMETRIC TECHNIQUES FOR IMAGING AND OPTICAL CHARACTERIZATION OF CELLS AND TISSUES

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Abstract

This work can be broadly classified into two sections depending on the applications; one dealing with cells imaging (averaged information) and the other with the tissue imaging (sectional information). The initial part of the work basically will capitalize on projecting LED as an alternative to Lasers and develop stand-alone miniaturized digital holographic interference microscopes. The developed microscopes are materialized using 3D printing technology and are employed for studying physical and mechanical properties of Red Blood Cells, resulting in cell characterization which may lead to disease diagnosis. The emphasis will be on imaging Red Blood Cells (RBCs) by employing LED to perform digital holographic interferometric Microscopy. In the later part of the work, low cost and compact Optical Coherence Tomography (OCT) systems will be developed by employing broadband source such as LED. Biological tissue as well its phantoms will be studied using these techniques. Thus, Low coherent source will be incorporated in various systems to image living cells as well as tissue which will lead to their characterization. This characterization may further help in disease diagnosis. The final part of the work will focus on employing lensless modalities for the examining biological samples. Employing lensless geometry will enhance the structural aesthetics and can further decrease the size of the digital holographic interference microscopes.

Keywords

Medical imaging, Low coherence interferometry, Digital Holographic Interference Microscopy, Optical techniques, Optical coherence tomography, Lensless holography

Introduction

In many developing and under developed countries, infectious diseases still continue to be the major cause of enormous number of deaths every year due to diseases such as Malaria, Tuberculosis, Sickle Cell Disease out of which many of the fatalities are children under the age of five. People residing in the low-income countries are still deprived of well-resourced diagnostic laboratories for initial disease diagnosis due to several economic or geographical factors which is detrimental to the socio-economic development. Failure of timely and correct diagnosis of disease is a serious impediment in improving the health of a nation [1-5]. Proper treatment of the diseases requires quick and affordable diagnosis which further requires deployment of large number of stand-alone and inexpensive diagnostics devices for on field application which requires minimum human intervention and could be operated even by untrained non-technical personnel. Therefore, low cost, rapid and automated detection and identification of biological samples can play a significant role in the future point of health care solutions.

Studying the bio-mechanical properties of human erythrocytes also known as Red Blood Cells (RBC) can help in their characterization. It has been reported that parasitization of RBC by parasites such as Plasmodium Falciparum leads to structural and mechanical modifications in

it and therefore, studying these properties such as membrane fluctuation lead to disease diagnosis. The structure of the RBC undergoes essential change in shape in the case of Sickle cell disease therefore the quantification of its bio-mechanical properties can also lead to its diagnosis since it deforms the shape of the RBC [6-12]. Therefore, characterization and identification of human RBC might be useful for disease diagnosis by studying its properties such as surface area, diameter, volume, surface area to volume ratio, thickness, membrane fluctuation, volume fluctuation etc. Moreover, the correlation analysis of these parameters might provide unique information for distinguishing and understanding various diseases [13,26,35]. Similarly, it is important to study how the tissues are structured and how they function since it helps in studying various fatal diseases such as cancer. It helps in figuring out the cause and designing the efficient plan of action for its treatment. A review of the treatment can be carried out which can decide its success rate.

Most biological specimens do not absorb light passing through them; they do diffract the light and causes a phase shift of the light. Thus, they are called phase objects. It is the diffraction of light at the specimen that allows objects to become visible. Phase objects are nearly invisible because the amplitude differences in the image are generally too small to reach the critical level of contrast required for visual detection [13]. The contrast of such specimen can be improved by staining the sample or its fixation but it might bring undesirable alteration in the structure of the cell or its constituent molecules [14]. Therefore, unstained objects present a unique problem for the light microscopist because their images are low contrast and essentially invisible in ordinary bright field microscope. Thus, conventional microscopic systems suffer from loss of quantitative information regarding the morphological properties of the transparent or semi-transparent biological samples.

Phase objects induce change in the phase of the light but human eye cannot detect differences in phase. The optical method which transforms differences in the relative phase of object waves to amplitude differences in their images is called phase contrast microscopy. Phase contrast microscopy produces high contrast images of transparent specimen such as cells and micro-organisms without any need of chemically staining it [15-19] by featuring an optical design that transforms differences in the phase of object-diffracted waves to differences in the intensity, making objects appear as if they had been optically stained.

As a result of advanced computer and photonic devices, Digital holography (DH) [16-36] has emerged as a promising technique of a new paradigm for performing quantitative phase contrast imaging which renders the three-dimensional images of the object. Unlike, conventional bright field microscopy, which gives high-resolution information about an object only in a fixed focal plane with the depth of field less than a micron, digital holography provides a rapid and efficient approach to reconstruct high contrast 3D images of samples from a single hologram [34]. It is a full field, marker-free, non-contact, non-invasive imaging technique along with the added advantage of numerical focusing. Therefore, the demand for 3D imaging of the objects is increasing day by day especially in the fields of life science, information science, medical diagnosis, robotics, defense and security [33]. Digital holography is an interference technique which allows for real time three-dimensional imaging of cells due to its capability of non-invasively visualizing and quantifying transparent biological cells in a single

shot. It has been recently demonstrated that Digital Holographic microscopy has been utilized in examining various kinds of blood cells, nerve cells, protozoa, bacteria, without labelling [35,37].

One of the limitations of interferometric technique in the transmission mode is that it cannot be employed in imaging thick samples or biological tissues as optical radiation passing through the tissue gets scattered, which leads to image blurring. This restricts the use of this technique only in imaging thin biological samples where the thickness is much less than the mean free path for scattering. To tackle this problem Optical Coherence Tomography (OCT) is employed for the three-dimensional sub-surface, non-invasive imaging of biological tissues which makes use of a broadband source. It is capable of producing high-resolution cross-sectional images of inhomogeneous samples, such as biological tissue and it finds applications in the fields like ophthalmology, gastroenterology, dermatology, and cardiovascular imaging, etc. [38-43]. Optical Coherence Tomography has emerged as a rapid, non-invasive and non-contact optical imaging technique which offers cross sectional images of biological tissues and it makes use of low coherence interferometry [43].

Generally, Interferometric techniques require a light source with both high spatial and temporal coherence to guarantee the formation of the interference fringes [44]. But using high coherent sources lead to parasitic interferences and coherent noise thereby degrading the image quality [44-47]. The introduction of these parasitic interference or coherent noise significantly corrupts the spectrum of the cross-correlation term. Moreover, these noise factors also introduce blurring around the borders of the cross-correlation term which makes it difficult to fit a spatial filter for the numerical reconstruction process. To overcome this a light source with low coherence can be employed [48-50]. However, it is difficult to employ such a source in a two beam off axis geometry since it requires a rigorous and time-consuming optical path length matching between both the arms to generate interference pattern. If fringes are to be produced using a such a source, great care is to be taken to equalize the optical paths travelled in both the arms over all wavelengths and to match the beam ratio, otherwise no fringes will appear [50-53]. In addition to this, two-beam off axis geometries are highly prone to external mechanical vibrations due to their space-separated two arm architecture. This requires a stable and sterile environment such as floating optical tables which further increases the cost of the system and restricts its portability. Therefore, common path self-referencing geometries will be employed in order to perform quantitative phase contrast microscopy of biological samples using LED.

The work consists of two parts. One deals with cell imaging, while the other deals with tissue imaging. For cell imaging we have employed digital holographic interference microscopy and for tissue imaging Fourier Domain Optical Coherence tomography (FD-OCT) is employed. Both these techniques will be performed using LED as the light source. Following are the objectives of the work presented in this synopsis.

1 (a) Development of digital holographic interference microscopes employing LED as a light source using common path self-referencing geometries for examining human RBC

(b) Employing Lensless geometries for imaging biological samples using both Laser and LED

2 Development of low-cost Fourier Domain Optical Coherence Tomography (FD-OCT) employing LED and a webcam for examining tissues

Digital Holographic interference techniques for imaging biological samples

The following work involves development of digital holographic interference microscopy using common path self-referencing geometries employing LED as the light source for examining human RBC. Since LED is a quasi-monochromatic source it has a lower temporal and spatial coherence compared to Laser. Therefore, the use of LED does not generate interference fringes over the whole field of view of the sensor. Therefore, the spatial coherence area of the LED needs to be enhanced. Efforts have been put by taking in account all the positive aspects of using a low coherent source (LED) and to incorporate it in a specific geometry which can harness and enhance the restricted coherence of LED to design a digital holographic microscope. To enhance the spatial coherence of the LED without effecting the output intensity, instead of a spatial filtering assembly, a Microscope objective lens (MO, de-magnifying lens) (10X, NA=0.25) is used, which reduces the effective emitting area of the source. Geometries such as Sagnac interferometer, Lloyd's mirror and Fresnel Biprism have been exploited for performing interference microscopy.

i. Development of Self-Referencing Sagnac Digital Holographic Microscopy exploiting the coherence properties of LED

In the present case, a Sagnac interferometer based self-referencing digital holographic microscope with LED as a source is used to study the static and dynamic properties of RBC and their three-dimensional imaging using a webcam. The two object beams encounter the same set of optical elements, while propagating in opposite directions. Both the beams are folded into each other by reflection off the two mirrors and self-referencing configuration is obtained where one of the beams acts as a reference beam while the other acts as an object beam. Holograms recorded using an optoelectronic device, are reconstructed using numerical methods. The advantage of using this geometry is that there is no flux loss, the fringe density is variable and the setup has high stability. The use of LED and webcam reduces the cost of the system compared to using a LASER source and a CCD camera.

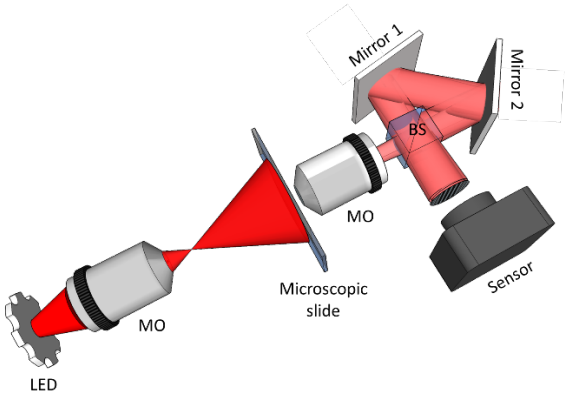

Fig. 1. Schematic of the setup employing Sagnac interferometer

The Sagnac interferometer works on the principle of amplitude division. The working of this interferometer is based on the fact that the beam travels two identical but oppositely directed paths before uniting to produce interference. A path length difference is produced when a deliberate slight shift is given to the orientation of one of the mirrors leading to fringe pattern. Since the beams are superimposed and therefore inseparable, the interferometer cannot be put to any conventional uses. These in general depend on the possibility of imposing variations on only one of the constituent beams. A compact, easy to implement common path digital holographic microscope based on Sagnac interferometer geometry is implemented using a LED and the holograms are recorded using a webcam sensor. The increased temporal stability of the device makes it compatible to use it to study the cell fluctuations by imaging the oscillations of the cell membrane of human RBC.

The microscope is used to image red blood cells to investigate its ability for 3D imaging of biological specimen. A thin smear of blood was formed on a microscope slide which was placed on a sample holder mounted on a translation stage for focusing the sample. Holograms with and without the object in the field of view were recorded and phase difference was calculated. The reconstructed phase profile was converted into optical thickness information. Fig. 2. (b) shows the reconstructed 3D thickness profile using webcam from which the doughnut shaped thickness profile of RBC is evident.

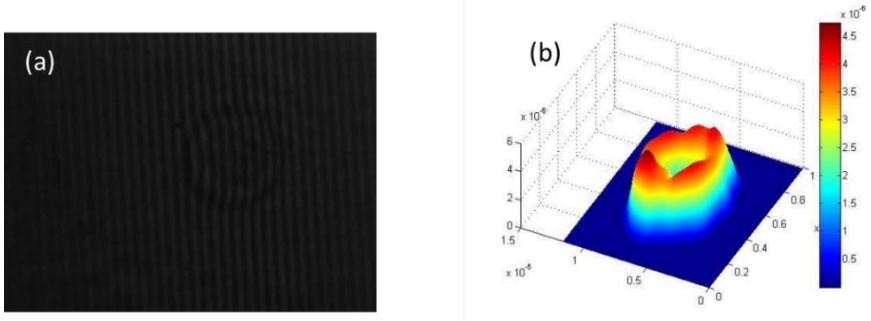

Fig.2. (a) shows the hologram of human RBC recorded with the developed microscope employing LED and Webcam (b) shows the 3D rendering of the recorded RBC

ii. Field of view enhancement in low coherent Digital Holographic Microscopy using Lloyd's Mirror

Yet another geometry that has been exploited is Lloyd's mirror interferometer [54] which is a common path and self-referencing configuration that can employ LED as a source for performing quantitative phase contrast imaging of biological specimen. The objective of the work is to enhance the field of view by tackling LED's low spatial coherence by skillfully adjusting optical components so as to increase the area over which the fringes are obtained. Moreover, the use of LED provides experimental simplicity making the system compact, inexpensive, portable and easy to use. Experiments are carried out by preparing a thin smear of human erythrocytes on a microscope glass slide and their static and dynamic properties are studied. Data is also acquired using LASER to carry out a comparative study.

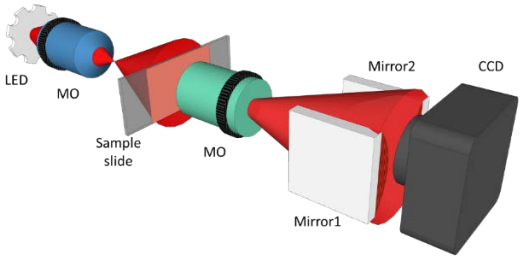

Fig.3. Schematic of the setup employing Lloyd's mirror interferometer and LED

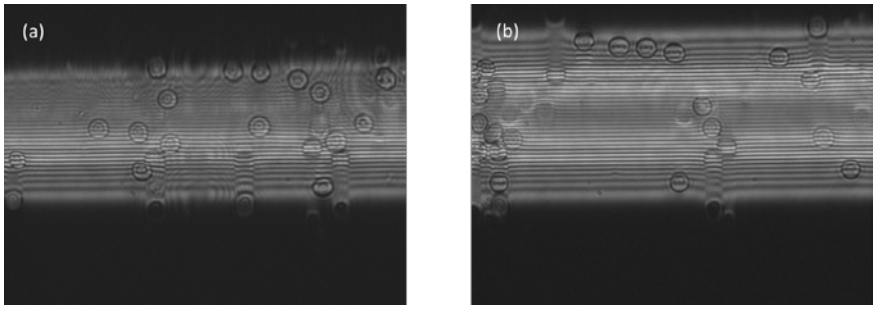

Fig.4. (a) shows hologram recorded with a single mirror (b) shows hologram recorded with two mirrors which shows enhanced field of view.

A Lloyd's mirror interferometer is exploited for performing quantitative phase contrast interference microscopy schematic of which is shown in the Fig. 3 where a LED emitting 627nm wavelength having maximum output power 2W with emitting area of $1mm^2$ is employed as a source. The source illuminates the micro-objects which are imaged using a microscope objective lens (20X, 0.40NA). A mirror is placed after the magnifying lens in such a way that a portion of the expanding beam (spherical wavefront) is folded back onto itself. Interference pattern obtained by superposition of direct beam and the reflected beam is recorded at the recording plane. A CCD sensor (Thorlabs, 8bit dynamic range, 4.65µm pixel pitch) is used to record the hologram. Using a single mirror does not generate interference

pattern in the whole field of view of the camera. Therefore, two mirrors are used to fold the incoming beam which generates fringes over a larger area which allows us to examine more number of samples in a single shot. Fig. 4(a) and (b) shows the holograms recorded with a single and two mirrors respectively. Fig. 5(a) shows the hologram recorded with the developed microscope employing Lloyd's mirror interferometer and a pair of mirrors while Fig. 5(b) shows the three-dimensional rendering of the object wavefront of the RBC.

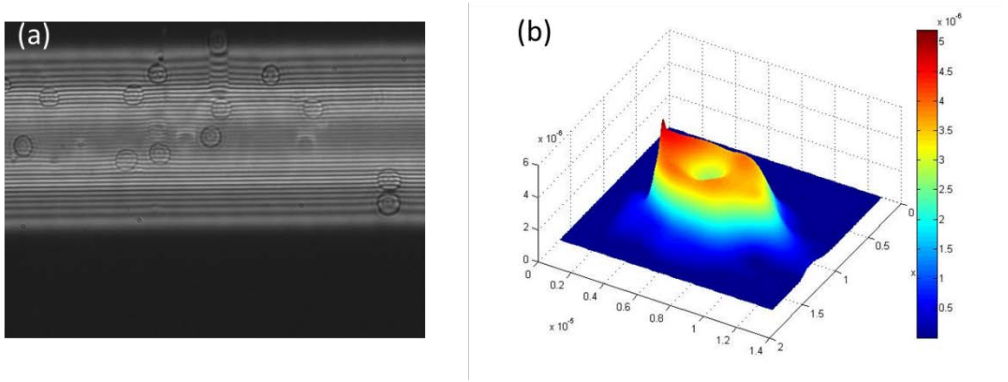

Fig.5. (a) shows the hologram of human RBC recorded with the developed microscope employing LED and CCD (b) shows the 3D rendering of the recorded RBC

iii. Wide field of view Off axis digital holographic microscopy exploiting the coherence properties of LED using a pair of Fresnel Biprism

Here we report the development of a wide field of view off axis self-referencing digital holographic microscope employing a LED source. Efforts have been put by taking in account all the positive aspects of using a low coherent source (LED) and to incorporate it in a specific geometry which can harness and enhance the restricted coherence of LED to design a digital holographic microscope. To enhance the spatial coherence of the LED without effecting the output intensity, instead of a spatial filtering assembly, a Microscope objective lens (MO, de-magnifying lens) (10X, NA=0.25) is used, which reduces the effective emitting area of the source. The experiment can also be performed using exotic light sources with short wavelengths like ultraviolet LED (Luxeon star LED, λ=385nm, maximum output power 475mW) which leads to improved resolution.

The schematic of the table top version of the microscope setup is as shown in Fig.6. LED (Lumilux, λ=627nm, maximum power output of 2W, Δλ=20nm) with emitting area 1mm^2 is employed as a source of light. This size of the source is reduced with the help of 10x MO in order to increase the spatial coherence area of the source. The de-magnified source illuminates the sample (a thin smear of human RBC on a microscope slide) which is imaged using a microscope objective (20X, 0.40NA). A pair of Fresnel biprism (176°, 4cmx5cm) with the same specification is placed which splits the incoming light and recombines it to form multiple holograms on the same sensor. These holograms are recorded using a CCD sensor (Thorlabs, 8bit dynamic range, 4.65 µm pixel pitch) at the rate of 10Hz for 30 sec. The recent boost in the field of 3D printing and the simplicity of this geometry has also facilitated the fabrication of

portable and sturdy printed device as shown in Fig.9. Similar work has been done by using laser source and a biprism [55].

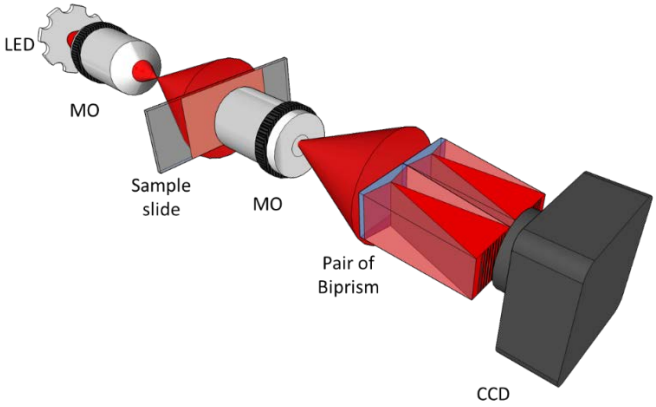

Fig.6. Schematic of the developed microscope employing LED and a pair of biprism

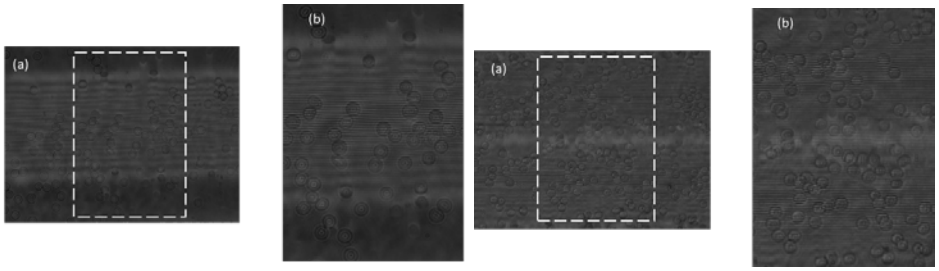

Fig. 7. (a) Hologram recorded with a single bi-prism. (a) Portion inside the region of interest, (a) Hologram recorded with a two bi-prisms. (a) Portion inside the region of interest

The use of biprism allows path length matching leading to high contrast fringes even using LED sources. As discussed, due to low spatial coherence the interference fringes do not cover the whole field of view leading to small usable region. The field of view increment was achieved using two parallel biprism leading to two separate field of views on the same sensor. This is equivalent to having two separate self-referencing field of views. Fig.7. (a) shows the hologram recorded using single bi-prism whereas Fig.7. (a) shows the hologram recorded using two bi-prisms. From both the figures it is quite evident that the field of view is increased using set of two parallelly placed bi-prisms.

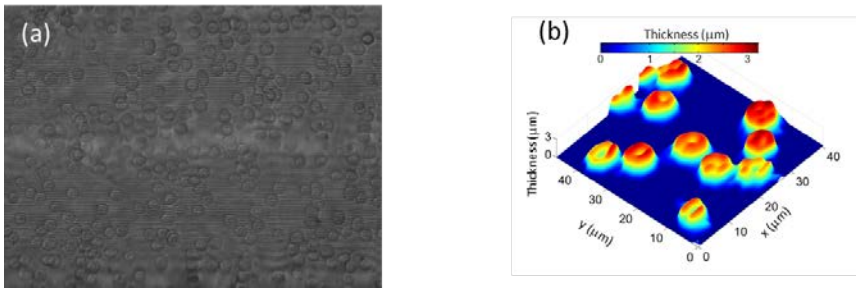

Fig.8. (a) shows the recorded hologram employing a pair of biprism and LED (b) shows the three-dimensional rendering of the RBC

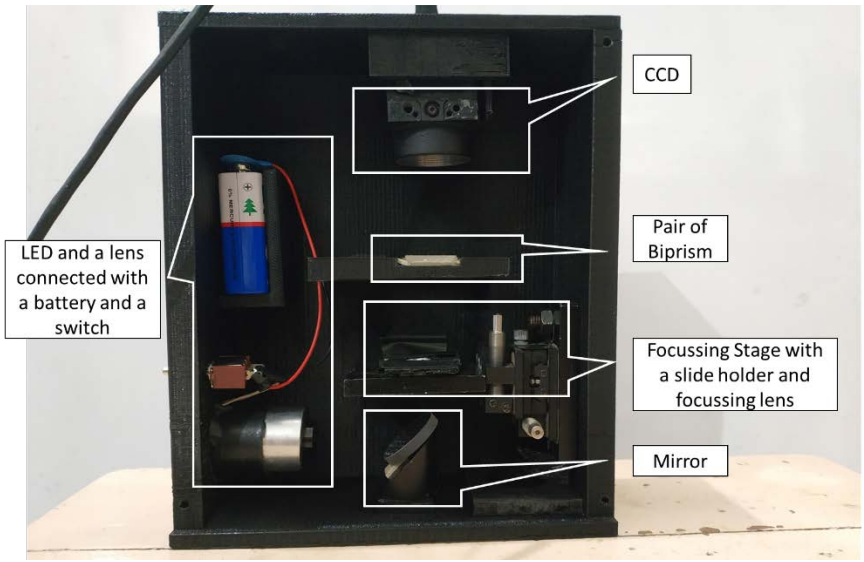

Fig.9. The developed 3D printed handheld portable microscope

Common path self-referencing off axis Lens-less imaging modalities with increased field of view for imaging biological samples

In this work we have proposed the employment of Lens-less geometries for performing interference microscopy. The use of lens-less geometries ensures the elimination of limitations pertaining to aberration and the field of view introduced due to the objective lens [65-62]. The lens-less modality is the most compact, portable, lightweight and robust [60] and comes with performance trade-off for e g. If the sample is placed very near to the detector array it ensures a large Field of view (FOV) and yields well separated diffraction patterns from individual cells but at the cost of resolution capability. On the other hand, if the sample is places further from the detector it yields finer imaging but at the cost of FOV [58]

Several geometries have been developed for performing digital holographic microscopy with differing advantages and disadvantages out of which the “in-line” geometry is the most compact and robust. It allows fabrication of ultra-compact devices but in order to extract phase information, but with the limitation of image overlap. Furthermore, to extract the phase

information of the object under study it requires capturing several in-line holograms obtained by either changing the illumination wavelength, angle of illumination or a combination of both. Moreover, computationally exhaustive algorithm is to be used to extract the phase information [63-64]. This limitation can be overcome by employing “two beam off-axis” geometries such as Mach Zehnder but it comes with the limitation of providing lower temporal stability which is essential in studying dynamic properties of the sample. Therefore, “off-axis common path” geometry is proposed to be employed in this case for performing lensless holography for imaging biological samples. We will be employing lateral shearing interferometer along with a laser source. In order to employ a low coherent source such as LED, Fresnel Biprism will be used as an interferometer.

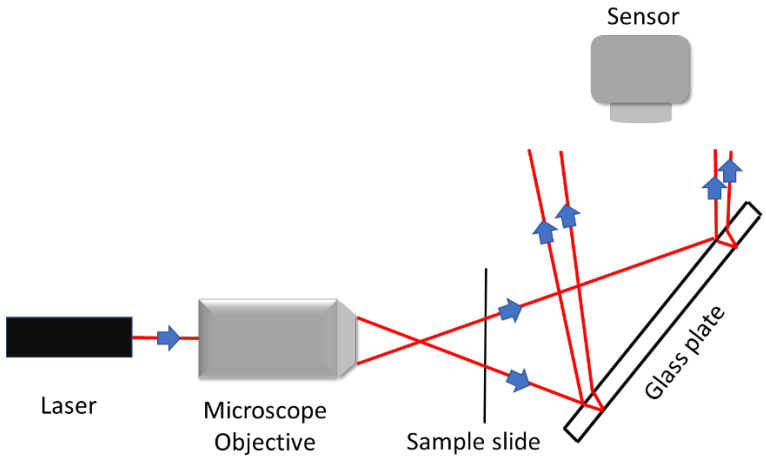

Fig.10. Schematic of common path self-referencing off axis lens-less imaging system

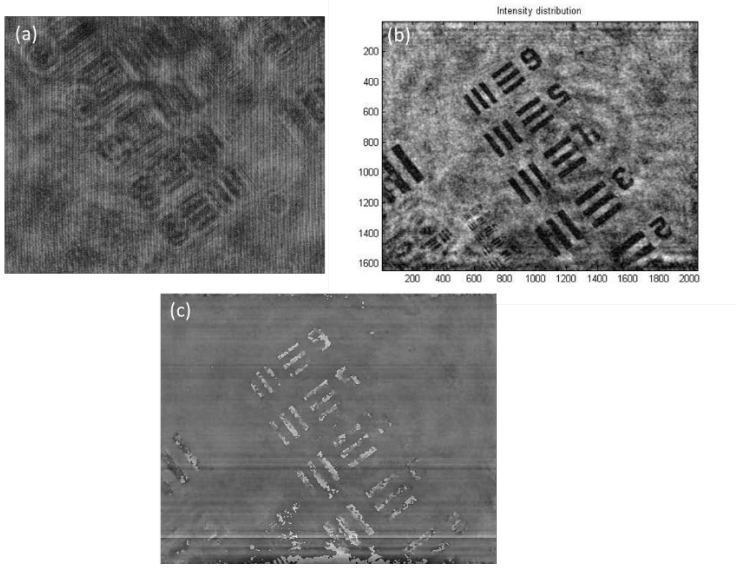

Fig.11. (a) USAF target used as an object (b) shows the numerically focused image of the USAF target (c) shows the phase image of the USAF target

Low-cost Optical Coherence Tomography employing LED for tissue imaging

In recent years the need of 3D optical imaging techniques has been increased for imaging scattering materials including biological tissues. For depth information of the objects like biological tissues, reflecting materials, need of optical depth imaging has been raised. Here, we consider one of the depth imaging techniques called Optical Coherence Tomography (OCT). It finds variety of application in the field of Bio-medicine, tissue engineering and biomaterials, Nanotechnology etc. [43]

Optical coherence tomography is an emerging type of imaging modality which performs high resolution, cross sectional tomographic imaging of internal structures in materials and biological system. OCT has revolutionized the field of medical imaging and has evolved over the past decade as one of the most important imaging techniques.

Optical Coherence Tomography (OCT) has grown into a rapid, non-contact and non-invasive optical imaging method which provides depth resolved cross-sectional images with micrometer scale axial resolution without the need for contrast agent [65,66]. OCT and ultrasound techniques both are majorly governed by the same principle of measuring time delays of the probe wave except the fact that OCT works in EM regime while ultrasound techniques makes use of sound waves [67]. Since the speed of light (~ 3 × 108 m/s) is significantly higher than the speed of sound, its direct time of flight imaging is not possible with the present electronic devices in case of OCT [68]. Low coherence interferometry serves as the basis of OCT for indirect measurement of depth of the sample.

An archetypal OCT system is based on the splitting of a broadband optical field and its recombination to get interference pattern. A typical Time Domain OCT (TDOCT) is a system where a single broadband optical field gets split into two parts out of which one travels in a reference path, reflecting from a reference mirror, and the other travels in a sample path where it is reflected from multiple layers within the sample. As the source used has low temporal coherence, to achieve interference between the two optical fields it is mandatory to have optical path length difference less than the coherence length of the source. The variation in refractive index between different layers of the sample is encoded as variation in intensity peaks in the interference pattern. By translating the reference mirror, the reference path length is adjusted to match multiple optical paths arising due to different layers within the sample to produce time domain interference pattern. Depth information can also be derived from frequency domain measurements by Fourier transforming the output spectrum. In such an arrangement the requirement for translating the reference mirror is eliminated and component frequencies of the OCT output are detected using a spectrometer. There are variants of OCT systems such as Time Domain OCT (TDOCT), Fourier Domain OCT (FDOCT), Swept source OCT (SSOCT) etc. [43]

To overcome the limitations of TDOCT which includes mechanical scanning of the sample, Fourier domain OCT (FDOCT) is employed for imaging the tissue. In FDOCT, the imaging is done by looking sequentially at different wavelengths of a broadband optical source. The speed

in FDOCT system is increased owing to the fact that each depth scan is acquired in a single snapshot, which makes it extremely useful for the study of in-vivo ophthalmic applications. Moreover, functional parameters of the object can also be studied along with the structural information [43,69]. FDOCT approach uses a fixed reference arm and the interferometric signal is spectrally dispersed on a linear detector array with the help of a diffraction grating. The Fourier transform of the measured interference spectrum is used to retrieve the axial (depth) information from all the depths simultaneously. Thereby, the Fourier domain approach not only improves signal to noise ratio but also eliminates the need for scanning in the reference arm and facilitates enhanced image acquisition speeds.

LED based inexpensive FDOCT systems

Development of a low cost FDOCT system is being proposed by replacing the conventional light source like SLD and fiber coupled diodes with LED and the high-end sensor such as CCD with a CMOS sensor (Webcam). Unlike TDOCT system, FDOCT does not require any moving parts for axial scanning. The output light is split into its component frequencies with the help of a diffraction grating and the spectrum is studied for extracting crucial information about the sample as shown in Fig.11. For validating the device various biological tissues or phantom objects which has properties comparable with an actual tissue will be studied.

To test the depth imaging capability of the developed OCT setup initially we scanned mirror in axial direction (similar to that used for TDOCT systems) with steps of 10µm up to 150µm and acquired the spectral interference patterns. In this case object is not scanned in lateral direction, position of illuminating cylindrical linear source is same with respect to lateral direction. The spectral interference patterns at each 20µm step size are as shown in Fig.12. Fourier transform of those spectral interference patterns reconstructs the image of the slit at corresponding depth.

The next part of the work will focus on scanning two mirrors at different depths and the developed low cost compact FDOCT system will be implemented for imaging of biological tissues.

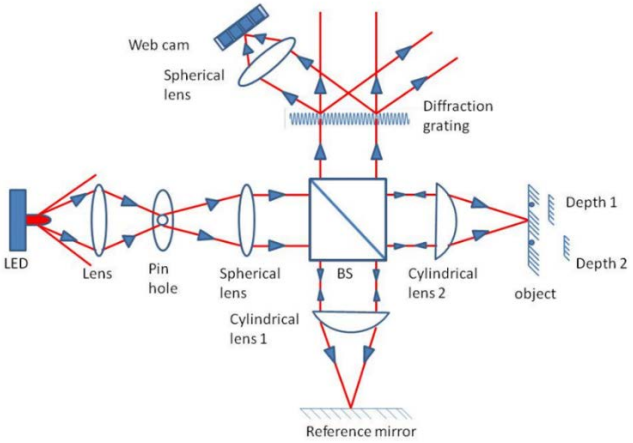

Fig.12. A low cost and compact FDOCT system using LED and Webcam

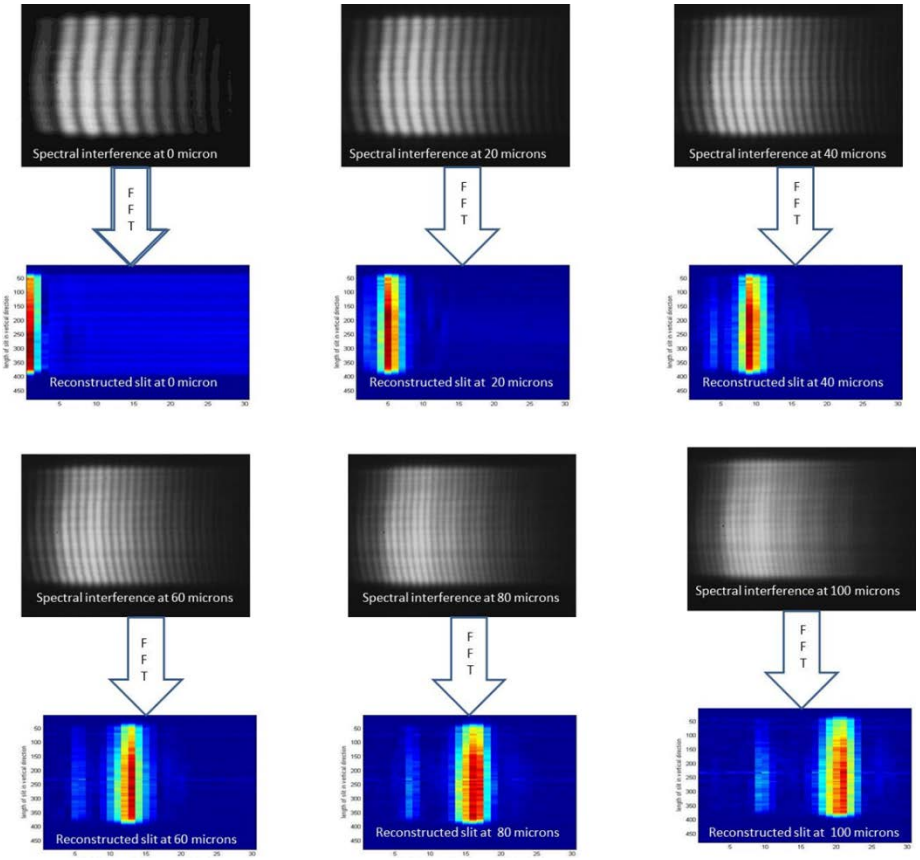

Fig.13. Spectral interference and the reconstructed slits by taking FFT of intensity profile along each pixel (linear beam focused on the mirror) at each 20μm translation of mirror in axial direction particular depth

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