Chapter 8

Conclusion and future scope

The work undertaken during the thesis aimed at implementing interferometric and non-interferometric techniques for three-dimensional imaging of phase objects (macro as well micro) using LED sources. The investigated objects included optical components, technical objects and biological cells. The thesis also focused on the development of low-cost Fourier domain optical coherence tomography (FDOCT) for performing sliced imaging of objects using LED sources.

The primary aim of the work was to demonstrate that Light emitting diodes (LEDs) can work as an alternative to a laser source in interferometric and non-interferometric thickness profiling of biological and technical samples. The presented work exploited various properties of LEDs such as its relative simplicity, compactness, robustness, low noise nature and cost effectiveness compared to laser sources.

Fringe projection technique (FPT), which is non-interferometric in nature, was employed to carry out surface mapping of optical components as well as in microscopy for three-dimensional imaging of RBCs. It was implemented utilizing an LED source and the optical thickness was quantified using Fourier Fringe Analysis Surface mapping of various optical components such as Fresnel biprism, cylindrical lenses and cylindrical rods etc were carried out to provide proof of concept of use of FPT in quantification of surface morphology of phase objects. The technique was then extended for 3D imaging of RBCs and also for quantifying its dynamics. FPT requires only few optical components and its compact nature provided a high temporal stability which is comparable to interferometric techniques. The technique also yielded bio-physical and bio-mechanical parameters of RBCs which were found to be consistent with the values reported in the literature. The future scope of the FPT for microscopy includes, projecting a grid like structure to obtain simultaneous horizontal and vertical shape measurement. Although FPT has many advantages, there are certain demerits of the technique such as it yields only gradient phase, and its phase sensitivity is less than that of interferometric techniques.

To overcome the demerits of FPT, Digital holographic microscopy was implemented using LED for thickness profiling of cells such as human RBCs and technical objects such as polystyrene beads. Conventionally laser is used as the illuminating light source to perform interferometry, due to its long coherence length, however it produces parasitic interference patterns as well as laser speckles which acts as noise consequently degrading the image quality. However, speckles are not very dominant in a smooth continuously varying biological smaples. Therefore, the developed digital holographic techniques used LED, which more than doubles the signal to noise ratio. However, owing to the low coherence of LED, it is a difficult task to generate interference fringes across large field of view. Hence, specific geometries (common path self-referencing configuration) had been implemented to perform digital holographic microscopy. Wavefront division interferometers using as Fresnel biprism and Lloyd's mirror and amplitude division interferometer based on Sagnac geometry had been used to develop digital holographic microscopes. Use of these interferometer geometries ensure that the path length between the object and reference beams are matched automatically leading to the generation of interference fringes. The spatial coherence of the LED was improved by creating a demagnified real image of the emitting area of the LED using a short focal length lens. In digital holographic microscopy, the field of view is determined by the region in which interference fringes results and owing to low coherence length (~15microteres) of LEDS, the achievable field of view was limited. Field of view improvement was achieved by optical arrangement involving multiple self-referencing modules for hologram multiplexing. This Since the investigated geometries are compact in nature and require lesser number of optical and imaging components (compared to a two-beam geometry such as Mach Zehnder), they are more immune to external mechanical vibrations and provided sub-nanometer temporal stabilities. All the developed microscopes were calibrated using a 15µm polystyrene beads and later used for examining bio-physical parameters such as (diameter, surface area, volume, projected area, eccentricity, sphericity, skewness, kurtosis, surface area/volume ratio) and bio-mechanical parameters (thickness fluctuation, frequency of thickness fluctuation, amplitude and frequency of volume fluctuation) of human RBCs. A comparative study was also undertaken using different light sources and sensors to explore which combination was the most optimized in terms of compactness, signal to noise ratio, and cost effectiveness. By using off the shelf optical components and a 3D printer, microscopes incorporating LED with Fresnel biprism and Lloyd's mirror have been converted into compact, portable and cost-effective devices which could find potential application in the field of biomedical imaging for point-of-care cell characterization. Presently further investigations are carried out using these microscopes for extraction of bio-physical and bio-mechanical features of blood cells. These studies will provide understanding on the effectiveness of the technique for its use in cell imaging, characterization, and classification.

Generally, lenses that are used in microscopes to image the samples under study, along with other optical components. However, use of lenses and may introduce chromatic as well as monochromatic aberrations which will distort the image. Lens also limit the field of view of the microscope. Therefore, we have demonstrated an off axis, lens-less self-referencing digital holographic microscope. The setup utilized only few components making it more immune to external mechanical vibrations and offered high temporal stability (0.6 nm). The microscope was tested for high resolution USAF target and numbers written with a marker pen on a microscope slide had been imaged. The data was recorded by a laser diode employing lateral shearing interferometer as well as LED employing Fresnel biprism interferometer. The future studies intend to investigate its use as a large field of quantitative phase microscopy system for view live cell imaging and characterization.

Low temporally coherent sources are ideal for sectional imaging using Optical Coherence Tomography (OCT). High power LEDs have the potential to replace the light sources presently used like Super Luminescent Diodes, which are costly and bulky. Also, usually high-end CCD arrays (one dimensional and two dimensional) are used for recording of the OCT interference patterns. OCT systems can be made compact and cost effective using LED as light source and low-cost digital arrays for recording of the interference patterns. A low-cost Fourier domain optical coherence tomography (FD-OCT) setup was developed by employing LED instead of the conventional Super-luminescent diodes (SLD) and a low-cost CMOS sensor (Webcam). Demonstration of a proof of concept, used mirrors kept at different axial positions as the object. Several parameters of the system are calculated such as optical detection sensitivity, lateral and axial resolution, and the maximum detectable depth. In the future the technique will be investigated for its use in sectional imaging of bio samples by using a high-power LED.

It can be inferred from the results obtained that using specific common path self-referencing geometries and by harnessing the temporal coherence of LED, employing clever optical arrangements, LED can be used as an alternative to laser in performing interferometric quantitative phase imaging of phase objects and other techniques. Moreover, portable, rugged and cost-effective devices can be made using these techniques. Also, effective algorithms and numerical processing using Machine Learning can make these microscopes yield quick and novel results in a single shot.