Abstract

Observation is the first and one of the most fundamental steps in scientific methodology. In the field of biomedical imaging, for observing and accessing information, light microscopy is still one of the least invasive and widely used technique. Brightfield light microscopes have several features that make them ideal for imaging biological cells, including high lateral resolution which matches the size of the sub-cellular structures of the cells. It is also relatively non-perturbing in nature enabling one to study the biological cells for a longer period to follow their dynamics. However, brightfield light microscopes only provide two-dimensional information about the sample (cells) under investigation. Moreover, such microscopes provide only low contrast images since most biological cells are transparent to visible light. Contrast is improved by staining the sample (chemical processing), which may deteriorate its life cycle. Hence, techniques that would provide high contrast images of low absorbing samples along with their thickness information without the need of staining (labelling) would be highly useful. The thickness information could provide knowledge about the state of the health of the cell under investigation, leading to its characterization and classification. The advancement in laser technology and digital sensors along with holography, extended the horizon of imaging techniques. Due to this evolution, the field of biomedical imaging has witnessed immense growth but there are still many low and middle-income countries that face major shortages of imaging equipment and diagnostic tools which becomes an obstacle for quick and affordable diagnosis and treatment of several diseases. Moreover, most of the present-day diagnostic tools are required to be operated under stringent conditions, by trained technicians. Therefore, the design and development of tools that are rugged, stand-alone, compact, inexpensive as well as field deployable, requiring minimum human intervention is essential to address these issues.

The work described in this thesis, details the efforts that has been made to investigate interferometric as well non interferometric techniques for the three-dimensional imaging of technical and biological samples using low coherent light emitting diodes (LEDs). The developed techniques can image and provide, bio-physical and bio-mechanical parameters of samples including human erythrocytes (red blood cells - RBCs), which will be useful in examining them for their characterization. LEDs offer certain advantages over laser such as they are small, rugged, bright, cost effective, and have a longer lifetime. Moreover, LEDs being low temporally coherent, minimizes the effect of speckles and parasitic interference patterns, which otherwise acts as a noise in the resulting images. These features of LEDs acted as a

driving force to use them as an alternative to laser in the developed imaging devices. The use of LEDs has also cut down the form factor and the cost of the developed systems. However, owing to their low spatiotemporal coherence, it becomes difficult to use LEDs in interferometric techniques as it does not generate high contrast interference fringes across a significant area (field of view). To tackle this issue, a sincere effort has been put forward to incorporate LEDs in interferometric techniques by means of special optical arrangement and geometries for performing quantitative phase contrast imaging. Furthermore, the investigated designs of digital holographic microscopes (interference microscopes) involving Lloyd's mirror and Fresnel Biprism have been converted into field portable, cost-effective devices using off the shelf components and 3D printing of the microscope structure. These devices can be used for point-of-care cell characterization, leading to assessment of the sample health. Apart from harnessing the low coherent property of LED, its intensity has also been exploited for retrieving phase information through Fringe projection technique (non-interferometric technique). Further, a Fourier domain optical coherence tomography (FDOCT) system have been developed by utilizing LED as the light source to obtain sample depth information. The technique is demonstrated as a proof of concept that a sufficiently high-power LED can be used as an alternative to super luminescent diodes which are conventionally used to perform OCT by making the system compact and cost-effective. The use of low-end CMOS sensors (Webcam) as a detector, further reduced the cost of the system.