Chapter 7

Conclusion and Future Scopes

The work described in this thesis centered on the development of digital holographic 3D microscopy techniques and its applications in quantitative phase imaging of biological specimen including plant and animal cells. Amplitude and phase information of the object wavefront obtained by the technique is useful in extracting various parameters of the biological sample under different conditions. Application of Digital holographic microscopy in the study of living cells, demonstrated its significance as it makes high contrast, label free imaging of such specimen possible. Time varying cell properties can also be studied using the developed techniques. Mach-Zehnder geometry based two beam setup of digital holographic microscope was constructed and used to extract static and dynamic parameters of the plant and animal cells. Onion skin cells were taken as sample for study. Effect of preservative treatment on plant cells was studied and change in the bio-physical parameters of the cells, such as optical thickness change before and after preservative treatment were quantified. Sugar solution as preservative solution was applied on onion skin cells. Due to concentration gradient inside and outside the cells, water from the cells moves out. Because of sugar solution higher concentration is generated outside the cells and that makes the cells to shrink as water comes out of the cell to balance the concentration moving lower concentration to higher concentration. Thickness of different epidermis of onion scales was also quantified and compared and inner epidermis was found thicker than the outer epidermis. Cell thickness in germinated onion cells were measured and found greater than the thickness of non-germinated onion cells as germinated

onion cells are juicier with enough amount of water inside the cells. Subcellular organelles such as nucleus, cell wall etc. were also imaged and their optical thickness were obtained using the microscope. Stomata in the plant tissue were also imaged. Using this microscope, static property like optical thickness and dynamic property such as osmosis in plant cells can be measured. Further in-depth investigation can be carried out using this device. Cell thickness and morphology at different depths in a layer might be of interest, since it will provide information on the growth of the cells from different position from the root. Study can be done in different seasons and under different environmental conditions; this may be useful to understand the growth procedure of plant cells in various conditions. To increase the storage life of vegetables and fruits various drying process are adapted for dehydration or preservative treatments are given to them. Various layers can be studied during or after this treatment and thickness of cells can be compared. Most of the plant leaves have upper and lower epidermis, growth stages of leaves of plants in different environment (change in temperature, light intensity, availability of water and atmospheric gases etc.) and in various types of soils, observing their upper and lower epidermis cell structure can be studied and compared for different conditions. For proper seed germination, parameters like temperature, moisture, air, and light conditions must be suitable. By varying these parameters, change in growth of plant and thus change in parameters of plant cells can be observed and analyzed by the use of this microscope. Also the effect of heavy metal treatment on germination of plant can be studied. Heavy metals may affect the plant physiology and may affect plant cells parameters. Thus plant cell growth and structure with various parameters at various stages can be observed. Presented in this thesis is just a basic application of MZDHM in study of plant cell nucleus. This can be used in advance study about nucleus. and altered nucleus shape is an important factor for investigating cell function. Shape and size of the nucleus may be altered due to changes in nuclear lamina, or by forces that from the cytoplasm. At different stages of growth of plant, size of nucleus also changes. Examination of structure and properties of cell wall during growth process of cells may be useful to gain information about its development as cell walls are present from the early stage of cell life. For example during ripening of fruits cell wall degrades,

that may change the size, shape or thickness of cell wall which can be studied using the developed microscope. Also effect of various treatments to cells may alter cell wall parameter that can also be measured. So, digital holographic microscope employing Mach-Zehnder configuration is ideal for life science applications.

Mach-Zehnder based DH Microscope is also ideal for quantitative imaging of animal cells. This was demonstrated by imaging and quantifying human red blood cells. A host of cell parameters based on the thickness profile of the cell were extracted. Static parameters like optical thickness, volume, surface area, diameter and shape were extracted from a single hologram. One of the biggest advantages of the developed imaging modality is that, it provides the thickness profile of the whole cell, from a single hologram, without the need of any scanning. This ability of the device was used to image the time varying optical thickness of the cells and to extract dynamic parameters of the cell such as amplitude and frequency of cell thickness fluctuations. High temporal stability (around 2nm over a time span of 30s) allowed this imaging of the temporal evolution of the cell thickness. The device was also be used to create spatial maps of these quantities. The developed microscope can provide several cell parameters based on its thickness as well as time variation of thickness. These parameters are very sensitive to the state of health of the cell and hence the parameters extracted using this device can be used to compare and classify different classes of cells leading to automatic identification of diseases affecting them. Also individual cell level study will be useful in correlating various cell parameters and change in parameters, which in turn may give new regime for disease diagnosis. Cell deformability with change in various pathophysiological conditions that may in turn change cell parameters like cell curvature, area expansion etc., can also be studied using this device. Also it will be interesting to investigate various bio-physical properties of the cells (especially red blood cells) and to correlate them to various conditions under which they are measured. Other components of whole blood such as white blood cells and platelets can also be quantified using this device, making it a compact hematology analyzer. Compact, portable, inexpensive digital holographic microscopes are of interest in point-of-care applications. So a digital holographic microscope using common path lateral shearing geometry was investigated. Purpose of the study was to develop a

stable microscope with simple configuration suitable to study dynamic property of biological samples. The developed device is simple as it comprises of only a few number of elements. The common path nature of the setup leads to high temporal stability of the device. Red blood cells were imaged and static and dynamic parameters were extracted using the common path microscope. Measured stability of the setup with a blank microscope slide was 0.9 nm, which is suitable for measuring red blood cell thickness fluctuations that are of order of few tens of nanometer. This common path setup was designed such a way that one of the object beams (generated by the lateral shearing element, which was a glass plate) gets filtered(sampled) by a pin-hole and behaves as a separate reference beam thereby providing full field of view for imaging. The setup was redesigned using a laser diode module and a webcam array to reduce the cost of the device. Self-referencing and common path digital holographic microscopes have the potential to revolutionize the area of quantitative phase imaging by providing compact and inexpensive off-axis holographic microscopes. These can be made into stand-alone portable devices as they require very few optical elements and area easy to implement. With the advent of miniature but powerful computational as well as display devices such as smart-phones, the recording as well as processing of holograms can be carried without the need of personal computers. In fact the holograms recorded by such devices can be sent to an off site server and processed and the results obtained can be sent back to the user. This will increase the field portability of the device as it becomes more compact and such devices will be extremely useful for point-of-care applications. Such a 3D microscope will not only be useful for cell imaging and parameter extraction but also for academic and other scientific purposes. Phase provided by the digital holographic microscope is the chord averaged phase from single projection (angle) of the probe beam. To extract information of the structures inside the cell or other optical inhomogeneity in the cells, it is necessary to differentiate between regions of different refractive indices inside it. A technique coupling self-referencing digital holography microscopy with phase tomography was developed to extract optical path length information inside the cell. Lateral self-referencing digital holographic microscope was modified to project the probe beam through the object at different angles and the extracted phase information at these

projection angles was used in back projection algorithm to achieve tomographic inversion to yield the cell optical thickness profile at different depths. Sections of object at different planes containing phase information were reconstructed. Two different methods were adopted for acquiring projections from various angles. In first method, a single laser diode module mounted on rotation and translation stage was used to achieve different projection angles. In second method, an array of laser diode modules (with the same configuration) fixed at different angles was used for obtaining multiple projection data. In first method number of projections is higher than the projections in the second method with the fixed array. Experiment was done with red blood cells as sample with laser diode array. Use of fixed array of laser diodes is advantageous as it eliminates unnecessary mechanical vibrations from the setup. But here only seven projections could be obtained with the array of laser diodes. The setup using an array of laser diode modules has the potential to measure local refractive index profiles of micro-objects, without the need for a mechanical scanning device to generate projection data. Next step is to use more number of laser diode modules, which will be turned on serially to create holograms at different projection angles, which in turn will increase the angle as well as number of projection for better quality reconstructed images.

The work presented in the thesis is an attempt to bridge the gap between the device (digital holographic microscope) and its application in the in life sciences. Digital holographic microscope configurations are presented and their applications has been demonstrated for quantitative imaging and study of plant and animal cells. These devices can be further explored for its application in many fields such as agriculture, food processing, biotechnology, medical sciences and clinical diagnosis.