CHAPTER 8: SUMMARY

In this thesis we have described the development of OCT setups and their utilization for applications in biomedical imaging like imaging of Zebrafish organs (eye and brain), monitoring of wound healing and discrimination of pathologies of tumour tissues. Towards this objective, we first developed a TDOCT setup. It uses a mechanical scanning reference arm mirror using motorized translation stage for axial or depth scan. Major advantage with this linear translation based scanner is that it facilitates large imaging depth range suitable for imaging transparent samples like eye. The TDOCT setup was used for imaging various ocular structures of Zebrafish eye such as cornea, iris, eye lens and retina. Several ocular parameters such as corneal thickness and retinal thickness were measured from the acquired ocular images. OCT imaging provides direct measurement of optical path length of the light travelled inside the medium. This information was used to estimate the effective or integrated refractive index of the lens for formalin fixed Zebrafish. The study was also extended for in-vivo measurement of the refractive index profile of Zebrafish eye lens. The gradient refractive index profile was retrieved by iterative fitting of optical path calculated by ray tracing method with that experimentally measured using OCT. We have also investigated the use of chemically etched tapered fiber and demonstrated the improvement in resolution by visualization of chloroplasts in Elodea densa plant leaf.
The slow imaging speed (~ one minute per image) of the TDOCT setup hampered its use for \textit{in-vivo} imaging of biological tissues. To overcome this, a high-speed OCT system was developed where a rapid scanning FDODL was introduced in reference to achieve scan rates of ~ 4000 scans/s which leads to imaging at 8 frames per second with 500 A-scans per frame. The setup was used for 3D optical imaging of brain with resolution (~20 µm) significantly better than obtained by other techniques like MRI and computed tomography. The setup was also used for monitoring the healing of wounds non-invasively without sacrificing the animal and demonstrated the applicability of the technology for rapid assessment of the wound healing to facilitate timely treatment planning. The high speed image acquisition using resonant scanning FDODL requires large detection bandwidth that compromises the sensitivity of the system. To implement fast image acquisition without significantly compromising the detection sensitivity OCT system based on Fourier domain approach was also developed. This approach eliminates the need for scanning in reference arm and thus facilitates enhanced image acquisition speed with better signal to noise ratio. The FDOCT setup was used to image \textit{in-vivo} samples like skin and nail with 10 frames per second with 1000 A-scans per image. The A-scan rate can further be increased up to ~29 kHz (limited by the read out rate of the LSC) that is sufficient to achieve video rate (32 fps) imaging. The standard OCT setup is only sensitive to the refractive index variation of the tissue. Many biological tissues exhibit birefringence which can modify the polarization state of the incident light. Therefore, we developed a time domain PSOCT setup, which measures orthogonal polarizations of the scattered light, to monitor the birefringent constituents (collagen, tendon, etc.) of the tissue. The PSOCT setup was used for monitoring of collagen remodelling during wound healing progression in the resected tissues of bacterial infected and uninfected mice models. The setup was also used to monitor the changes in the
collagen that take place in malignancy and based on these measurements normal, benign and malignant breast tissue samples were discriminated. Further, OCT based system was also used to measure mechanical properties of breast tissues and significant differences in the stiffness coefficients of normal, benign and malignant tissue were obtained.

In addition to the polarization and elastography information, phase sensitive measurements of OCT interference was also explored. The phase sensitive measurements help to retrieve optical path length changes beyond the restriction of the coherence length of the source. The nanometer scale optical path lengths, obtained using phase sensitive measurements of the interference fringes, were used for the measure of refractive index of biomimetic materials and single biological cell (keratinocyte cells).

The present work can be further extended along the following directions:

We used PSOCT setup for monitoring wound healing. However, for the ease of imaging in a clinical environment, a fiber based high speed PSOCT imaging setup would be better. It will also be interesting to incorporate the Doppler measurements[153] with PSOCT as it would also provide information regarding micro-circulation and angiogenesis of wound healing in addition to the collagen remodelling and morphological changes. Since, formation of new blood vessels is essential for several physiological and pathological events, e.g. embryogenesis, wound healing and tumor growth and metastasis, it would also be a useful tool to visualize the tumor microvasculature and developmental studies on Zebrafish embryos also.

We have used the polarisation sensitive and elastography based measurements with OCT imaging to discriminate the normal, benign and malignant tissue pathologies. Another interesting line of research can be to explore the use of optical contrast agents like nanoparticles to further enhance the diagnostic imaging capabilities of OCT. These
contrast agents may enable site-specific labelling of tissue structures, and thus improve the visualization of early-stage and metastatic tumors and tumor margins to help image-guided surgical resections. Incorporation of the OCT with other imaging modalities such as confocal fluorescence microscopy, laser induced fluorescence, Raman spectroscopy, multi photon microscopy, etc. will also be a worthwhile objective as it will help simultaneous characterization of the 3-D tissue morphology and its biochemical composition [154,155]. These biochemical and morphological information would be useful for fast screening of the normal and diseased tissue at an early stage.

We made use of phase sensitive measurement of the interferometric fringes to measure the refractive index of bio-mimetic materials and single cells. By incorporation of 2D galvo-scanner (for raster scanning of beam) in the setup, it will be possible to measure the two dimensional distribution of the optical path lengths across the biological cells. This would help high resolution imaging of the microscopic objects like cells without staining or affecting them [156]. The studies could be performed to assess the variation in optical path length of the cellular objects affected by different treatments or by the environmental factors such as temperature or pH. It can also be used for label free quantitative and qualitative apoptosis studies which begin with a variety of morphological changes that differs from viable cells.