Chapter 7

Future scopes and road ahead

Need of the hour in the medical and health care field is the development of low-cost, field portable diagnosis devices that can reach masses and can be used for point-of-care applications. Work done during the course of this thesis was aimed at that. Most commonly used bright field microscopes were modified using off the shelf optical and imaging components to convert it into a compact device that could be attached to smart phone. The advantage of using smart phones is that it can be used as an image acquisition, image transmission and in sometimes image processing device, which is stand alone. The images acquired using the compact microscope and recorded in a smart-phone were transmitted to an off-site computer for processing. Simple image processing algorithms were used to extract features from the images, which were rich in information about the samples (blood smears). The developed image processing algorithms were able to identify and classify blood cells with high accuracy. Of course a staining process was required to identify between the cells. Presently work is being conducted by the use of multi-colored mono-chromatic LED sources to identify between different cells. This may lead to label free cell identification with bright field microscopes. Also presently the bright field microscope is being converted into a fluorescent microscope by the introduction of quasi-monochromatic LEDs and gel filters. This will provide further information on the state of health of the sample. The image processing algorithms are also being modified such that it can be converted into a smart-phone application so that, the images need not be transmitted to off-site computers for processing.

The disadvantage of bright field microscopes is that owing to low absorption of visible light by most of the living cells, use of bright field imaging leads to low contrast images. Also these images do not provide any information on the shape (morphology) of the cells and its time variation, which is a very sensitive parameter depending upon the health of the cells. Quantitative phase contrast imaging provides high contrast images of low absorbing samples as well as their morphological information. Two-beam digital holographic interference microscope employing Mach-Zehnder interferometer configuration was designed and developed especially for imaging of blood cells and automatic identification of malaria. The device was used in 3D imaging of red blood cells in thin smears. By the use of correlation algorithms which were based on the extracted cell parameters (obtained from the cell morphology of healthy and diseased samples) automatic identification of malaria became possible. This shows the potential of DHIM as an intelligent microscope that can provide an educated guess on the state of health of the cells, based on the vast amount of information (in the form of cell parameters) which is available while using this microscope. A device based on Mach-Zehnder DHIM was also fabricated. This can be used as a laboratory version of the microscope in which different laser wavelengths can be coupled from outside. Presently this microscope is being used to gather data on other diseases (such as thalassemia and sickle cell anemia) that could be identified by observation of blood cells. Researchers working in the area of cell biology and life sciences have shown tremendous interest in this microscope and in the near future a version of the microscope for applications in life and health sciences will also be developed, intended for use in the study of cell migration and cell tracking.

But the drawback of MZ-DHIM is its bulkiness due to use of multiple optical elements for beam steering. Also the temporal stability of the device is affected by its two-beam nature. This is overcome by the use of self-referencing of axis DHIM. The simplest version of this category of microscopes uses a glass plate to create holograms. The main advantage is that such a device requires very few optical elements and hence is very compact and highly temporally stable making it suitable to study nanometer level cell thickness fluctuations. Hand-held version of the lateral Shearing DHIM was fabricates using low cost optics and imaging array. It is a stand-alone version, which could be attached to smart-phones. The captured holograms were processed in an off-site PC. Cell parameters as well as a guess on the state of health of the sample based on the extracted cell parameters are transmitted back to the user for further course of action. A web based application was developed for this. Presently work is progressing in the use of this microscope for extraction of physical and mechanical parameters of cells and identifying the changes in these parameters for healthy and diseased samples. Clinical trials are also progressing. In the near future the device will be field tested in harsh environments. Also the device has the potential to be converted into a color DHIM system by coupling lasers of various wavelengths. This can be achieved quite easily. Also the use of multiple wavelengths will provide spectroscopic information of cells under investigation.

Self-referencing DHIM, even though compact, low cost and easy to use, has the disadvantage that only a part of the field of view is usable (especially for large imaging arrays). The device also requires sparse object distribution. To overcome this disadvantage a wavefront division DHIM was designed employing a two-lens system. The device comprised of two lenses of same configuration (same focal length and clear aperture) placed side by side with a separation between them. This acted as the wavefront division module. Sample was placed under one of these lenses and an unmodulated illuminating beam passes through the other. This creates a separate, clean reference beam. Further a prototype of the device was fabricated by 3D printing its structure. The device was used for imaging of blood smears. Presently optimization of the device is being carried out by trying out different combination of lenses, which can make the device more compact.

In its natural state, cells are normally surrounded by fluids. But in fluids, the cells move around. So in order to study them in their natural condition, they need to be immobilized. Optical tweezers can be used for it. By integrating DHIM to optical tweezers, morphology changes occurring to trapped cells can be studied and quantified. A low cost optical tweezer was developed using DVD optical pic-up unit. A very low cost quadrant photodiode and related image acquisition system was also developed in-house for measurement of corner frequencies of trapped particles. The front end of the QPD comprised of four optical fibers which were coupled to four photodiodes at the back. It worked as good as a commercially available QPD and was used to measure corner frequencies of trapped micro-spheres. The corner frequency distribution was used to identify and classify the micro-spheres. A LS-DHIM device was integrated on to the low-cost optical tweezer to extract cell morphological information. Presently work is progressing by using the trap and the LS-DHIM on healthy and diseased red blood cells for cell identification and cell sorting.

The devices presented in the thesis has the potential to be used in the health care sector especially for disease diagnosis purpose. Also researchers in the area of biological and health sciences can also benefit from the use of these devices. But the adaptation of these devices in health care industry as well as in life and health sciences require successful conveying of the advantages and improvements in imaging and measurement by the use of these devices to people working in these areas and also on the feedback and inputs from them.