CHAPTER 7

Thesis Summary and Future Scope

In this research report, we have proposed suitable image processing algorithms for cytopthological analysis with recently developed/custom built portable, low-cost high-throughput microscopy, and microfluidics based imaging flow cytometry. We have presented a framework for focus stack patch based cell analysis using a custom designed convolutional neural network for malarial parasite detection due to protozoan of type falciparum and have shown that the detection accuracy outperformed the methods without using the focus stack. As part of the system, we have proposed a cascaded segmentation strategy to facilitate quantitative analysis moving in a direction to facilitate automated malaria diagnostic platforms for resource-poor settings.

We have also presented a completely automated cell analysis platform for a very cost-effective high-throughput microfluidic based imaging flow cytometry. The cell analysis platform includes feasible pre-processing methods, non-iterative, graph-based accurate cell segmentation strategy, hand-engineered feature extraction and a classification framework. We have explored cell signature (advanced PCA signature) based classification, hand-engineered morphologic feature based classification as well as deep learning based classification for leukaemia cell-lines; K562, MOLT and HL60. In our investigation, we have found that deep learning methods outperformed the conventional systems in the classification of these cell lines. We hope that imaging flow cytometers equipped with the proposed frameworks for image processing would enable cost-effective, automated and reliable disease screening in over-loaded facilities, which cannot afford to hire skilled personnel in large numbers. Such platforms would potentially facilitate screening camps in low income group countries; thereby transforming the current health care paradigms by enabling rapid, automated diagnosis for diseases like cancer.

However, all the works presented in this research report have used cultured cells. This includes both the malaria samples used in focus stack based classification and the leukaemia cell-lines used in the microfluidic microscopy setup.

The cultured cell-line helped us to generate the correct ground truth enabling quantitative analysis. A natural extension to this work could be testing the proposed methods on samples from real patients. Also, the proposed cell classification method in mIFC could naturally be extended for differential WBC counting. The method can be extended for a 3 part classification of WBCs. However, the 5 part differential classification requires the usage of an appropriate stain. Thus, for differential cell counting, one interesting work could be to bring up with a suitable stain at right concentration so that it can be used in flow.

We have addressed the leukemia cell-line classification mainly using the morphological features extracted from cell images. However, there are methods that address the problem based on the biomechanical properties of cells (Otto et al. (2015a)). These properties such as cell's deformability, cytoadherance, and time-dependent response to an applied stress change on the onset and progression of cancer (Suresh (2007)). There are quite a few design modifications in microfluidics channels (Yavuz (2009)) to study these parameters. One straight forward approach could be to include tiny constriction in the channel allowing the cells to squeeze through. The collected images can be used to extract required deformability parameters. For example, Kim et al. (2015) uses a node-pore sensor and a contraction channel to measure a deformability index of the cells thereby enabling differentiation of particular cancer-cell types within a heterogeneous cell population. There is also the concept of pinched flow microfluidics (J.S. Dudani (2013)) which uses flow channels to pinch and release the cells. Cells having different elasticity and viscosity might have separate lateral dynamic equilibrium position when they pass through the pinch. This fact is used to selectively mark cancer cells against healthy cells (Hur et al. (2011)). More recently Gangadhar et al. (2015) has studied extraction of deformability index of cells even with a straight channel microfluidic device and has validated the method by studying the change in deformability index of healthy, diabetic and sphered red blood cells. Thus, the study on the changes of biomechanical properties of leukemia cells for cell differentiation could be an important extension to the research problem that we were addressing. Also note that, we have addressed leukaemia cell-line classification problem from cultured cell-lines. For its applicability to end goal, leukaemia has to be identified from naive blood samples containing WBCs, RBCs, platelets, etc.

The methods discussed need to be extended/adapted for other types of diseases such as cervical cancer (Du et al. (2006)). This includes the modification that has to be brought on both microfluidics channel construction and on the processing framework. First, the modifications required to be brought in the design of microfluidics channels. Herein, the channel widths and depths have to be modified appropriately to incorporate the largest possible cervical cells (squamous cell can be as big as 1618 μm). Also, it would be essential for incorporating other strategies like micro-pillar arrays(Kim et al. (2017); Kaminaga et al. (2015)) or obstacle based methods (Huang et al. (2004)) to ensure that the cells are isolated without forming clumps in microfluidic channels. Further, in accordance with the modifications made to the channels, the optics have to be modified. Like the magnification might have to be increased or decreased and the depth of field of imaging has to be extended if the channel depth is increased. Coming to the question of image processing, in order to identify duplicate cells, we can make use of image processing algorithms like digital image correlation (McCormick and Lord (2010)). Further downstream processing might remain fairly the same, except for the parameters like circularity, which will have minimal relevance in case of cervical cells as they are largely irregular. Other measures of internal complexity like texture, variance etc., would still remain relevant and the developed framework would be directly applicable with minimal modifications. Also, the mIFC experiments that we had conducted could be tried for a larger dataset unlike the relatively smaller dataset (618 cells) used in this study.

Note that, the parameters used in the algorithm for processing cells used in this research work are explicitly defined. Most of these parameters as explained in relevant sections are defined based on the size of cell in image plane. When we are using the same processing framework for the analysis of different type of cell images, these hard-wired parameters need to be changed taking the cell image size into consideration. The size of cell in image plane is decided by the parameters of optics & hardware components used (Wayne (2014)). These include resolution of microscope objectives, magnification employed, and pixel size of the sensor. Given that the hardware components are standardized in the final design iteration and their arrangement remains fairly constant, the algorithms may not need any modifications for the type of cells analysed in this research. However, the

changes in the implementation may lead to changes in the illumination intensity, which affects the hard-wired parameters such as intensity thresholds used in focus stack slide scanner. In such a case, one can include an intensity calibration step to decide these parameters. This calibration can be performed, when no microfluidic device is placed, using the Gray level intensity recorded at the sensor. Coming to the case of pixel to real distance (magnification), the channel width is a known dimension, which can be used to assess this parameter from the video itself and set all the relevant parameters which are defined based on the cell size in image plane. The prototypes developed as part of this research work are still in the final stages of commercialization. At this point in time, the beta prototype is soon to go into clinical evaluation and would be brought to market soon after that. The bill of materials of both the microfluidics system and the focus stack collecting microscope as discussed in Chapter 1 is around 1500 US $. In general, the cost of the final prototype tends to be 4 - 5 times the bill of materials (Chaturvedi (2016)). So, even with that into consideration, this would still be a cheaper alternative, when compared to the current industry standards like Amnis 199,000 US $ (Amn (2016)).