Neural Network for Blood Cell Classification in a Holographic Microscopy System

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ABSTRACT

Modern clinical laboratories are equipped with high-throughput flow cytometers for fast and accurate cell sorting. Most cytometers use selective biomarkers which often induce significant changes in the cell morphology, sometimes leading to cell death. However, for purposes like cell imaging there exist label-free techniques, for example digital inline holographic microscopy. Yet the image reconstruction algorithms needed to analyze the images do not scale up easily to large numbers of cells. We suggest an integrated, optical neural network to deal with the high-speed image classification with the promise of dense integration for ultrafast, cell sorting. A ternary classification task, distinguishing between monocytes, granulocytes, and lymphocytes resulted in 89% accuracy.

Keywords: digital inline holography, neural network, cell sorting, integrated optics.

1. INTRODUCTION

Tests on human blood samples are a cornerstone in modern clinical diagnostics. They provide important indicators of the human health condition and generally reveal infectious diseases as the respective proportions of human whole blood composition changes in this case. As a consequence the increased presence of white blood cells (WBCs) is often flagged by flow counters. Additionally, biomedical research faces regularly assessment of medical treatments and drug studies. Blood diagnostics play a major role in this respect. Therefore a reliable, accurate, and fast way of characterizing blood cells constitutes an ambitious, ongoing research goal.

Flow cytometers are the backbone of modern laboratory techniques. They work on small volumes of blood samples only, but require a specific preparation according to established protocols before being loaded into the cytometer. Within the cytometer, hydrodynamic focusing ingeniously aligns the blood particles, enabling accurate particle counting and characterization at appreciable throughput rates. Sometimes the sample preparation is rather cumbersome, involving lysis, the attachment of biomarkers, fluorescent dies, or even magnetic beads to the cells or their components. Consequently, most labeling techniques either change the cell morphology significantly or damage the cell integrity leading to apoptosis. These side-effects often prohibit additional investigations of the live cell embedded in its environment.

It is thus worth creating label-free techniques that integrate easily with flow cytometry. A promising one, which is also used in fast particle volume tracking, is digital inline holography microscopy (DIHM) [1]. In this article, we present a possible choice of an optical neural network that classifies cells at a much shorter process delay. This is possible because the neural network processes immediately the holographic interference pattern at its input. Neural networks have already been used in conjunction with cell type studies [2], [3], but very few is known about its application to WBC sorting in flow cytometers. It appears as an attractive approach to ultrafast, large-scale cell sorting (2 Mio. cells/sec) when densely integrated in a lab-on-chip configuration.

In the following we discuss the working principle of the on-chip flow cytometer as it is developed at imec [4]. Section III details the numerical implementation of the optical neural network. Simulation results are presented and discussed in Section IV.

2. ON-CHIP CYTOMETER DESIGN

The current prototype of an on-chip flow cytometer cleverly combines three crucial functions: asserting a microfluidic flow in a narrow channel, microscopic imaging, and sorting (see Fig. 1).

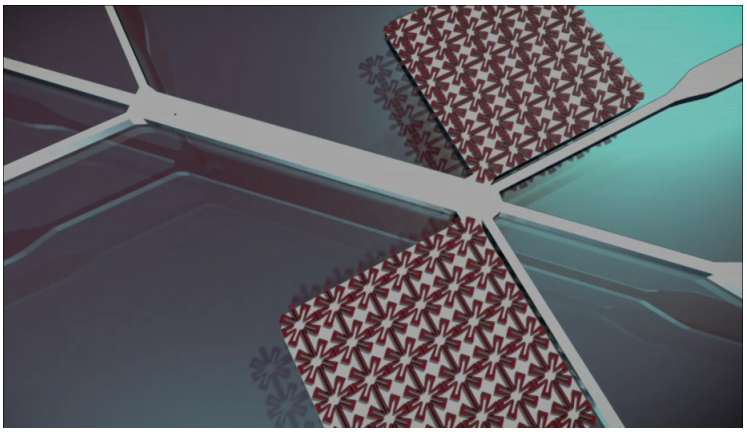

Figure 1. Typical design of a flow cytometer as proposed by imec [5]. Artistic view of the microfluidic channel (gray) and the steam bubble generating structure (red) next to the 3 outlets.

The microfluidic channel has an inlet where the loaded blood sample is dispensed. It aligns the cells so as to pass one by one the imaging area at a flow speed of > 1m/sec. At the other end the channel displays multiple outlets which ought to collect the different cell types.

The imaging is based on a holographic method named DIHM. DIHM is a lens-free imaging technique and as such does not suffer from aberrations and other phase-perturbing imperfections. It relies on the known fact that a hologram contains all the necessary information of the wave-front emitted by the object under study which is encoded in its fringe pattern. An easy way to create these interference patterns is to coherently superpose a reference beam and the diffracted object beam. As the name of DIHM suggests this is done in line, meaning that the reference beam is equal to the object beam. This technique has already been proposed by Gabor [6], the inventor of holography, but encountered difficulties in its practical realization due to the twin image problem. For the flow cytometer at hand, the reference beam is simply the spherical wave emanating from a small pinhole on top of the microfluidic channel which is illuminated by a green laser. When the reference beam impinges on the transparent cell it is scattered into different directions. The scattered light and the reference beam overlap coherently at the bottom of the microfluidic channel where an image sensor is positioned. Since the sensor consists of a CCD-array the image is subsequently processed in digital form.

Once the decision was taken, the appropriate signal is sent to the switching unit prior to the outlets. The underlying switching mechanism is achieved by a jet of steam bubbles that deflect the bypassing cell into the desired outlet.

3. METHODS

Since the flow cytometer is devised on a silicon-on-insulator CMOS platform, we target the implementation of a neural network using CMOS compatible silicon photonics. The optical network is designed to work in the feed-forward configuration, thus avoiding any back-action of the output on its input. This simplifies the training of the neural network because techniques such as error back-propagation exist. One hidden layer of optical neurons is fit in between an input layer and an output layer. Figure 2 shows a possible implementation.

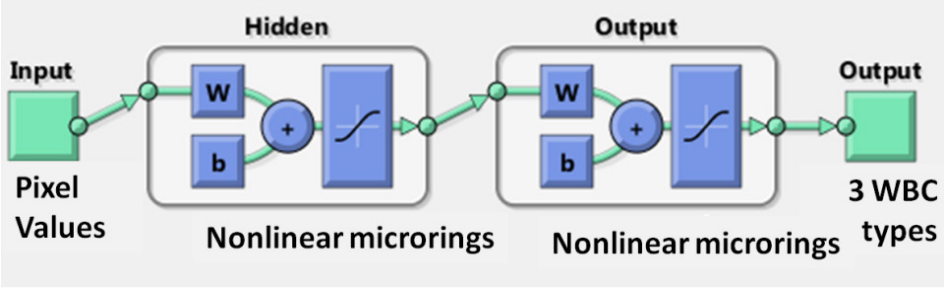

Figure 2. Schematic of the optical neural network [7] upon which the pattern detection depends. The pixel values from the input and can be realized by grating arrays. The nonlinear transmission function of each neuron in the hidden and output layer is realized by thermal microrings. Three different WBCs are distinguished at the output side.

The CCD image sensor is captures the diffracted as well as the reference field at the bottom of the microfluidic channel as a single cell is passing by. The weighting matrix combines the different input channels applying well designed weights to each of them. Each optical neuron receives input from a particular set of input nodes (gratings) and applies a nonlinear function to their sum. In our implementation we assume nonlinear

thermal microrings as optical neuron units. Microrings form a resonator cavity with high quality factor at optical frequencies. A high quality factor allows for a high build-up of the optical power density which enables occurrence of multiple nonlinear effects. Among all the nonlinearities we are most interested in the self-heating of the cavity due to two-photon absorption and subsequent free carrier absorption. Eventually all the responses of the microring resonators are again linearly combined and nonlinearly transformed before being read out by an integrated photodetector array.

We simulated this neural network on real data which was collected with the flow cytometer, as described in section II. The dataset (~7500 cells) consists of three types of WBCs: granulocytes, lymphocytes, and monocytes. Their proportions in normal human blood are 65 %, 30 %, and 5 %, respectively. We used 10-fold cross validation in order to average out random variations in the cell type distributions in training, validation, and test sets. A gradient-descent technique was implemented within the MATLAB™ Neural Network Toolbox [7] to update the network weights.

4. RESULTS

Choosing the best neural net instantiation out of a random set of initial weights, we obtained the following result on classification for the test set, shown below as a confusion matrix. Cross validation was used in order to achieve better error statistics and to select a specific model. For the resuts in Fig. 3, the data is averaged over 10 data folds. We notice that accuracies better than 90 % are achieved which is in agreement with typical errors of about 10 % (except for basophils) which are typical for standard cytometers in clinical laboratories [8]. An increased error weight was introduced to compensate the skew in the dataset; the monocytes population is relatively scarce when compared to the two dominant WBC populations, granulocytes and lymphocytes. This scarcity is also the main reason for the poor prediction accuracy of monocytes.

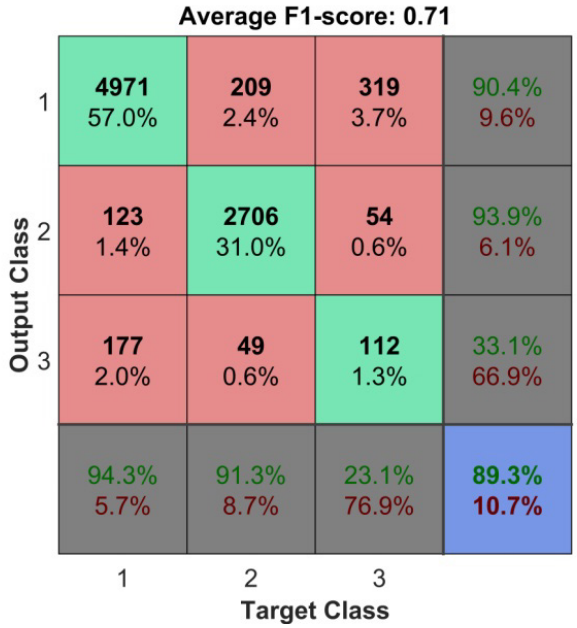

Figure 3. Confusion matrix for the three-part WBC classification. Target classes 1 to 3 represent granulocytes, lymphocytes, and monocytes. Total accuracy of nearly 90 % is achieved.

5. CONCLUSION

Employing numerical simulations and machine learning techniques, we confirmed successfully that it is possible to use neural networks in flow cytometer configurations, for example when selecting DIHM as imaging technique. Overall prediction accuracy close to 90% has been demonstrated, although the particular prediction accuracy for a single class may score lower when it is poorly represented in the sample.

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