

Immunomagnetic Detection of Circulating Tumor Cells using a Microfluidic Chip: Cell Recognition and Analysis

Fraser Downing

Mechanical Engineering, University of Colorado at Boulder

NNIN REU Site: Microelectronics Research Center, The University of Texas, Austin, TX

NNIN REU Principal Investigator(s): Professor John X.J. Zhang, Ph.D., Biomedical Engineering, Stanford University

NNIN REU Mentor(s): Dr. Kazunori Hoshino, Department of Biomedical Engineering, University of Tokyo, Japan;

Yu-Yen Huang, Ph.D. Graduate Student, Department of Biomedical Engineering, University of Texas at Austin

Contact: fraser.downing@colorado.edu, john.zhang@engr.utexas.edu, hoshino@mail.utexas.edu, n9692416@gmail.com

Abstract:

Early recognition of cancer within a patient has proven to be a key component in successful treatment and survival. Given a blood sample containing circulating tumor cells (CTCs), these cells can be marked with ion oxide nanoparticles using a specific functionalization process. By running this prepared sample through a system that includes a microfluidic chip and a magnet, the labeled CTCs can be captured for analysis. Using three different dyes and exposure types, identification of a CTC is based on physical characteristics and appearances as they are observed under each exposure condition. Although CTCs possess a unique physical appearance in terms of fluorescence, size and shape, correct identification can be a challenging task. After working with several persons trained in cell identification, a tool was developed in MATLAB to facilitate the identification of CTCs based on the measurements and calculations for key characteristics as taken from the experiment images. By using explicit definitions for identification, a large volume of images can be analyzed quickly and efficiently while also ensuring judgment of each cell is based on the same criteria.

Introduction:

As new and innovative ways to fight cancer continue to develop, although an absolute cure has not yet been discovered, vast improvements have been made to where the disease has become treatable if the correct steps are made. Early recognition of cancer within the body can play a significant role in a patient's survival. One specific technique currently under investigation involves the positive selection of circulating tumor cells (CTCs) with an ion oxide nanoparticle.

Experimental Procedure:

By executing this process of marking using antigens such as cytokeratine, cancer cells within a sample were now bound with magnetic particles. The sample was then drawn through a microfluidic channel consisting of a polydimethylsiloxane (PDMS) mold sealed off by a glass capture slide (see Figure 1). The set up also possessed a permanent magnet aligned in parallel to the glass capture slide along the channel. Due to the magnetic field that was created, marked cells traveling through the channel became suspended on the glass capture slide. Upon trial completion, the captured cells were bonded permanently to the capture slide and the PDMS mold could be removed after a mild heat treatment. Once the slide had been isolated, a post-analysis process could begin, which was critical to determining the level of success achieved during the experiment.

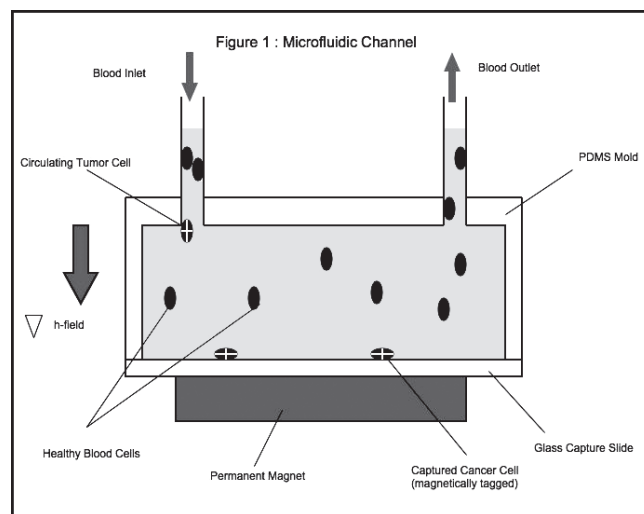


Figure 1: Microfluidic channel.

To begin this process, the slide was stained using three different substances. Cytokeratine (CK), an enzyme called CD45, and a stain referred to as DAPI were used as dyes due to a desired characteristic for binding. Each dye has unique fluorescent characteristics when exposed to different light conditions under a microscope. CTCs would appear bright green in CK, dim and red in CD45, and bright blue

in DAPI. Each exposure condition for a possible cell was photographed, as the images would be used to draw a conclusion on cell type.

Due to the ambiguity of parameter guidelines, identification could be a tedious and challenging task. Depending on the type of sample that was being utilized, there could be anywhere from 60 to 80 cells in need of investigation. Foreign particles, as well as wrongly tagged cells, were likely to exist, as a perfect experiment is impossible to achieve. Different dye concentrations caused variable brightness from slide to slide. Between these factors and the fact that the conclusion depended on a set of implicit definitions interpreted by the observer, a large possibility for misidentification existed.

In order to solve this issue, the implementation of an original program written in MATLAB to quantify the identification characteristics and express an opinion on cell type was explored. The defining properties obtained from the images could be used together to make a decision based on tangible quantities rather than user interpretations. In

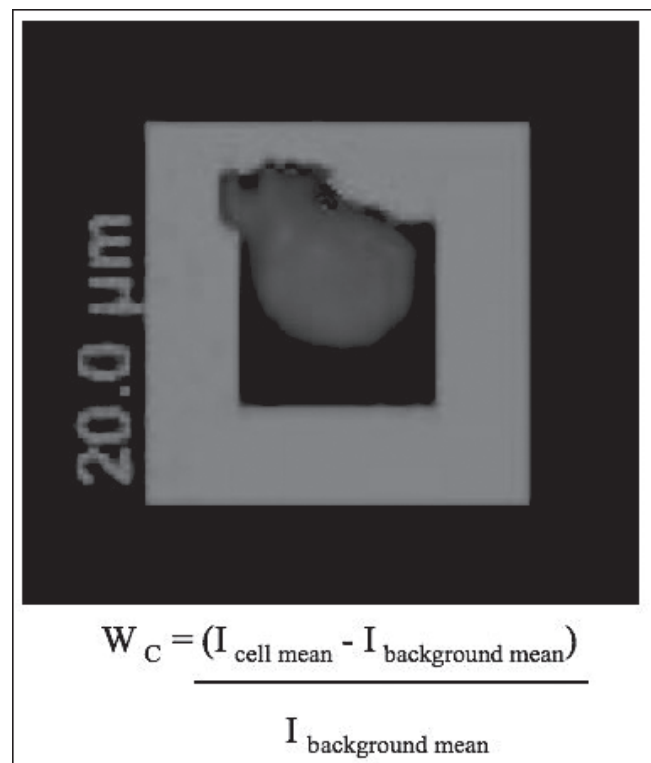


Figure 2: Weber contrast determination.

order to perform analysis, the location of the cell within the images needed to be obtained. This was done by the user adjusting a light-intensity threshold value or by manually selecting a region of interest if many visible bodies existed. Coordinate locations were obtained by finding the positions of the pixels within the selected regions. Once the locations were known, the Weber contrast ratio was taken in order to quantify color-specific light intensity.

As shown in Figure 2, the ratio took the mean value of the pixels for each color in the cellular region and an immediate background region, omitting any regions of high intensity. The Weber value was used as it normalized the light intensity measurements, eliminating the problem of variable brightness.

Results and Conclusions:

By implementing the previous steps into a graphical user interface, a user could easily explore the data for each cell. After running several image sets through analysis, a consistent result for the defining characteristics was determined. By saving these values to a database organized by cell type, normal distributions for the characteristic values could be generated and used to gauge cell type. The program also showed results consistent with the database for real patient samples as well as for different cancer types.

Future Work:

The explicit definitions used to define cancer cells show good promise, but much more research and testing needs to be carrying out in order to ensure consistency. Although uniform results were shown between cancer types, little is known about the reactions of other cancer types. However, if the consistency in results hold true, this program normalizes judgment while vastly increasing confidence in concluding cell type. This would be vital in a real-world setting, as a correct identification could mean a patient's survival.

Acknowledgements:

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References:

- [1] Lara-Velasco, O.; "Immunomagnetic Cell Separation: Further Applications of the Quadrupole Magnetic Cell Sorter"; Ohio State University, 2003.