Analysis and Simulation of Rare Cell Detection and Size Based Profiling Using Coupled Micro-Hall Detectors

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ABSTRACT

We present a comprehensive analysis and finite element method (FEM) modeling and simulations on using magnetic nanoparticles (MNPs) and Hall effect principles to detect, and estimate sizes of rare cells. In this model, rare cells labeled with MNPs are detected in-flow using coupled micro Hall voltage detectors (μHD). The voltage signals of μHDs are then analyzed to obtain size estimates. No physical contact, stressing, electrical excitation or immobilizing of cells is used. Voltage variations based on sensor dimensions, vertical separation distances and cell sizes were analyzed in simulations using finite element method. A signal processing algorithm was developed to determine cell size from detected voltages of bottom/top μHDs. This novel 2D dual μHD concept offers better accuracy when compared to conventional single μHD approach in cell count and also provide size profiling with 12.2% average error for cell diameters from 10 μm to 26 μm.

Keywords: rare cell detection, cell size profiling, single cell analysis, magnetic nanoparticles, hall effect sensors

1 INTRODUCTION

Detection of rare cells such as circulating tumor cells (CTC) in complex media such as blood is one of the most rapidly burgeoning research areas in biomedical research. CTCs are recently shown to carry valuable information on stages of disease [1]. In addition, counting CTCs in whole blood samples promises diagnostic as well as prognostic modalities. The most widely used method for counting CTCs is to fluorescently label cells and detect them using optical setups. This method uses complex and large optical instruments that inhibit portability and as such, this type of equipment are largely limited to advanced laboratories. Researchers around the world are working to develop new methods and devices for detecting cells that require less human intervention and are cost effective – enabling widespread diagnostic use. In these efforts, however, the focus is on the detection of cells and attaining absolute cell counts in whole blood. Differentiation of cells based on type and morphological qualities are mostly overlooked. However, important differences exist between myriad of cell types, and qualitative and objective characterization of these differences presents substantial insight into disease diagnosis and monitoring of therapy. One such differentiator is the cell size, and here, in addition to highly accurate cell counts, we propose a method to detect and differentiate cell sizes while cells are flowing through a microfluidic channel. This is achieved without using complicated biochemical immobilization steps.

Use of magnetic nanoparticles for detecting rare cells has been an extensive research topic in biomedicine for its potential to accurately profile numerous types of cells. The negligible magnetic noise of whole blood contributes much to this method's accuracy [2]. Prior research has mostly focused on using Hall Effect sensors in order to detect the concentration of rare cells circulating in blood [2], [3]. The typical procedure is to initially functionalize magnetic nanoparticles (MNP) so that they attach to specific target cells only. Then, the mixture of cells or whole blood is flowed slowly through a microfluidic channel containing magnetic sensors. A permanent magnetic field is required to magnetize the MNPs during this procedure.

Although previous research have addressed the matter of counting rare cells using MNPs, there is much space for research in obtaining the characteristics of individual cells such as size. In our work, we completed a conceptual analysis of using MNPs and coupled micro Hall detectors (μHD) to detect and estimate sizes of cells such as CTCs based on rare cell detection concepts that were initially proposed by Issadore et al. [2]. Our approach presents two improvements over prior work: 1. Use of coupled (top/bottom) μHD detector pairs for increased cell count accuracy. 2. Use of a signal processing algorithm to estimate the cell sizes.

2 METHODS

2.1 Modeling

Modeling of sensors, rare cells, MNPs and medium of microfluidic channel as well as simulations were completed in a commercial FEM tool, COMSOL Multiphysics software. In our model, the microfluidic channel height was fixed at 32 μm. This height is selected for detecting larger cells such as CTCs that generally have diameters ranging from 15 μm to 30 μm. MNPs were selected to have a diameter of 300 nm and MNP attached to a cell were

modeled as a thin (300 nm in thickness) shell with relative permeability of 500 covering the much larger cell body. This is an improvement from a previously published model that models attached MNPs as a bigger magnetic sphere at the center of the cell [2]. Two aligned µHDs were placed on the ceiling and the floor walls of the microfluidic channel as shown in Fig. 1. The cells were modeled as non-magnetic spheres. It has been shown that attaching a large number of (up to ~10^6) MNPs per cell is possible [2]; thus, assuming uniform bonding and treating the MNPs as a shell covering the cell is justified. The cell medium within the microfluidic channel is modeled as water. Although a real cell medium contains salts and a large amount of other material, this approximation is valid as a model for biological material.

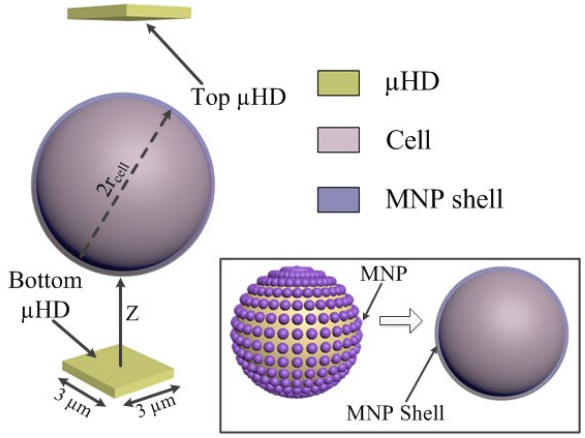

Figure 1: Model of a single cell passing between a µHD couple. Surrounding medium is modeled as water. Cell covered with a large number of MNPs, modeled as a 300 nm thick shell – inset. (not to scale)

The permanent magnetic field used to magnetize MNPs was modeled as a 0.5 T vertical magnetic field. Average magnetic flux density in the vertical direction (B_z) within µHDs were calculated using a commercial FEM tool, COMSOL Multiphysics. Extracting the effect of MNPs by removing the effect of fixed magnetic field is done at signal processing stage. The Hall voltage is calculated using (1). This method has been reported to give Hall voltage values that match experimental results as presented in [2].

$$V_H = -\frac{IB_z}{eNt} \quad (1)$$

Here, I represents the DC current through the sensor, B_z represents vertical flux density, e represents charge of an electron, N represents charge carrier density, and t represents the thickness of the sensor.

2.2 Simulation

Firstly the effect of cell position in horizontal plane was analyzed. Horizontal plane coordinates (x, y) of the cell were varied while keeping the vertical position (z) fixed. As expected, maximum Hall voltage was detected when the cell was aligned with the center of the sensor in x-y plane. It rapidly decreased when the cell is moved away from this position as shown in Fig. 2. Therefore, readouts for size estimation have to be taken when the cell is aligned with the sensors in x-y plane.

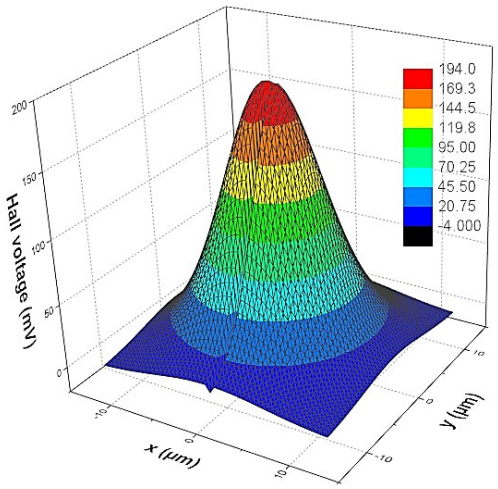

Figure 2: Hall Voltage variation with cell position in x-y plane. Vertical distance fixed at 3 µm and µHD centered at the origin in x-y plane. µHD size 3 µm × 3 µm. Cell diameter 15 µm with a 300 nm layer of MNPs.

The next simulation was carried out to observe the dependence of Hall voltage on cell diameter. It can be seen in Fig. 3 that there is an almost linear relationship between cell diameter and detected Hall voltage.

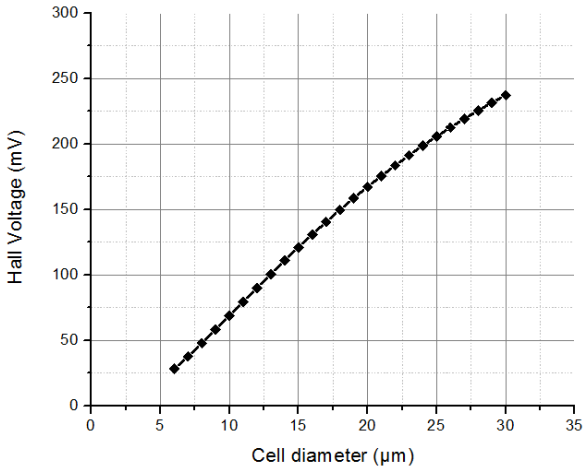

Figure 3: Hall Voltage plotted against cell diameter. Vertical distance fixed at 5 µm. µHD size is 3 µm × 3 µm.

Next simulation was performed to observe detected Hall voltages in both top/bottom µHDs when the vertical position of the cell is varied while keeping it aligned with µHD couple in horizontal plane. The results are plotted in Fig. 4, and it can be seen that detected signal exponentially decreases with distance from cell to sensor. Both µHDs generated signals that depend on the vertical position of the

cell (for a fixed cell size). It can be seen from this graph that for a conventional one sensor device, the cell has to be very close to µHD vertically for a detectable voltage signal to be generated. The maximum distance from sensor to cell for detection is dependent on cell diameter and µHD size, and the cells that flow farther away from µHD would skip detection. Using coupled top/bottom µHD pair design, our device increases this distance by more than twice; thus significantly reducing errors in cell detection and also allowing the device to work with cells of a vast size range.

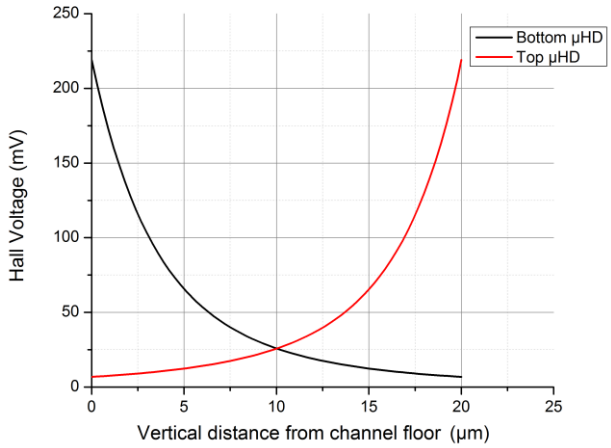

Figure 4: Hall voltages produced in a µHD couple when a cell bound with MNPs is moved from floor to ceiling of the microfluidic channel. µHD size is 3 µm × 3 µm. Cell diameter is 10 µm with a 300 nm layer of MNPs.

In the next study, the same simulation was performed for several sensor sizes, and results are plotted in Fig. 5. Sensor sizes varying from 1µm×1µm to 11µm×11µm were simulated. It has been previously shown that in µHD type sensors, sensor size should be close to the cell size for maximum performance in cell detection [2]. But as the main improvement of our research is to detect cell sizes, a different approach is justified. In our approach, a large variation of Hall voltage with the distance from sensor to cell is desired in estimating the cell size. Therefore, according to the results given in Fig. 5, smaller sensor sizes should give more accurate results. On the other hand, various fabrication issues can arise with very small sensor sizes. Also, covering the width of the cell flow channel would require a bigger array of sensors. These issues make it unpractical to reduce sensor size arbitrarily. Therefore, µHD size was selected to be 3µm x 3µm.

Finally, in order to generate a database for signal processing stage, the sensors were extensively characterized by executing a large number of simulations with a cell being moved from the floor to the ceiling of microfluidic channel in 0.5 µm steps. This is repeated for varying cell diameters between 6 µm and 30 µm. Cell position in x-y plane was kept aligned with the center of the sensor couple. The magnetic flux densities detected by the sensors for each simulation were saved into the database.

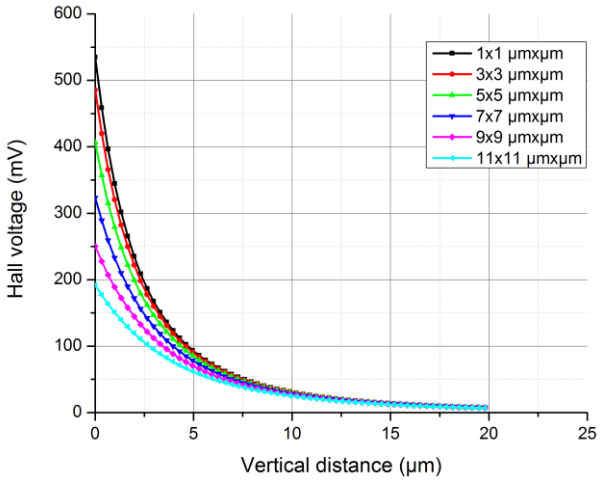

Figure 5: Plots for Hall Voltages vs. vertical distance from the sensor for different sensor sizes. The cell is aligned with the center of the µHDs in the horizontal plane. Cell diameter is 15 µm with a 300 nm layer of MNPs.

2.3 Signal Processing

The database populated by characterizing the sensor couple is used for estimating sizes of unknown cells. As the Hall voltage readouts are taken when cells are aligned with sensors, the Hall Voltage depends on verical distance to the cell from the sensor (z_{cell}) and the cell radius (r_{cell}) (2).

$$V_H = f(z_{cell}, r_{cell}) \quad (2)$$

The signal processing stage is implemented as a Matlab function. The algorithm that finds an estimate for the cell size first eliminates contradicting values and then selects the most likely value as the estimate. The accuracy of the estimates is affected by the thoroughness of the device characterization.

A set of (z_{cell}, r_{cell}) probable solutions exists for any Hall voltage readout from a single µHD. Two such sets of probable solutions exist for the top and bottom µHD sensors. As there is only a single actual cell present, the cell size actually seen by both sensors is same. The vertical distances to the cell seen by both sensors, when added to the actual cell diameter, has to be equal to the microfluidic channel height.

By analyzing the two sets of probable solutions and using the above two conditions, the algorithm first eliminates contradicting (z_{cell}, r_{cell}) solutions. Then, the most likely solution is selected and presented as the estimated cell diameter. Thus, both cell size and cell vertical position can be calculated using a couple of sensors. The algorithm can only estimate the cell size to the nearest (z_{cell}, r_{cell}) value pair available in the database. Therefore, extensive characterization of the devices with increased number of sample measurements yields better estimates. Signal processing stage is thus capable of analyzing the Hall voltage signal readouts from the coupled µHDs and producing an estimated cell size.

The correct size estimate can only be obtained if the cell aligns with the µHD couple while in flow. Our model also assumes cells flowing in single file with some distance between two adjacent cells. Therefore, it is important to reduce the width of the cell flow and to have a single file cell flow. This can be accomplished by hydrodynamic flow focusing in the direction of channel width with a sheath flow; a technique that has been perfected in the field of flow cytometry [4], [5].

Even with cell flow cross section reduced, a single sensor may not cover the whole cross section [2]. Therefore, an array of sensor couples can be used to cover the entire cross section. All or most of the sensor couples in the array would generate outputs when a CTC flows in the channel. The size estimate should only be made for the reading of the sensor couple that sandwiches the cell flow path correctly. This reading can easily be discriminated from the rest as it would be the largest Hall voltage (Fig. 2).

3 RESULTS AND DISCUSSION

The coupled µHD sensor and data analysis function were tested by running further simulations. Known random sized cells were placed at known vertical positions between the sensor couple and readings of top and bottom sensors were obtained by simulations. These readings were then passed to the signal processing function. Finally, the cell size estimates were compared against the actual cell size to verify results and plotted in Fig. 6. The average error and Pearson's r for cells with diameters from 10 µm to 26 µm were 12.2% and 0.87, respectively. As expected, accuracy increased with increasing cell diameter, and for cell diameters greater than 20 µm, average error was 4.14%.

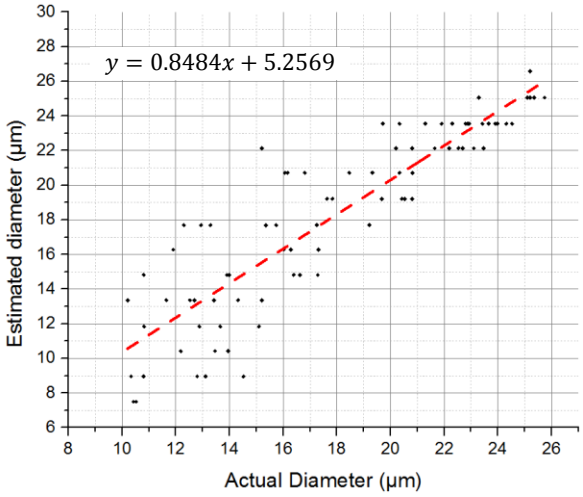

Figure 6: Comparison of actual and estimated cell diameters for random sized cells between 10 µm and 26 µm in diameter. The cells are placed at random vertical positions between µHD couple. Trend line and it's equation are also shown.

As cell size estimates were picked from the values in database, an inherent error is always present. The database used here was populated with data points relating to cell diameters increasing by 1.3 µm steps and vertical distances increasing by 0.5 µm steps. A better characterization of the device would increase accuracy. Estimates that are close to the actual sizes have been produced for different (z_{cell}, r_{cell}) values tested. However, when the cell diameter is very small (less than 10 µm), only detection could be done – accurate size estimations cannot be made.

Our method also provides better accuracy in cell detection and counting. However, this accuracy is hard to enumerate as it depends on multiple independent parameters such as actual cell size, electrical readout system, and flow conditions. Proposed coupled detectors operate without any physical contact, stressing, electrical excitation or immobilizing of cells. Also, the operating speed is far better compared to immobilization based systems as cells are detected and measured in-flow.

4 CONCLUSION

A conceptual analysis of a device capable of detecting and estimating sizes of rare cells at single cell level has been completed. Flow focusing is required in the direction of channel width, but is not required in the vertical direction. Voltage signals detected by a µHD couple are processed to estimate the cell size. Device was tested with cell with diameters between 6 µm and 26 µm. Cells with diameters below 10 µm were detected, but error in size estimation was large. Cells larger than 10 µm were detected and their sizes were estimated with an average error of 12.6%. The average error is reduced with increasing cell size, down to just 4.14% for cells with diameters greater than 20 µm. We believe overall average error could be brought below 5% with improved device design and signal processing algorithm in future work. No immobilizing or stressing of the cell is necessary for detection and size estimation.

This material is based upon work supported by the National Science Foundation under Grant No. ECCS-1349245

REFERENCES

[1] S. Sleijfer, J.-W. Gratama, A. M. Sieuwerts, J. Kraan, J. W. M. Martens, and J. A. Foekens, *Eur. J. Cancer*, vol. 43, no. 18, pp. 2645–50, 2007.

[2] D. Issadore, J. Chung, H. Shao, M. Liong, A. a Ghazani, C. M. Castro, R. Weissleder, and H. Lee, *Sci. Transl. Med.*, vol. 4, no. 141, p. 141ra92, 2012.

[3] A. Lapicki, H. Sanbonsugi, T. Yamamura, N. Matsushita, M. Abe, H. Narimatsu, H. Handa, and A. Sandhu, *IEEE Trans. Magn.*, vol. 41, no. 10, pp. 4134–4136, 2005.

[4] N. Sundararajan, M. S. Pio, L. P. Lee, and A. A. Berlin, *J. Microelectromechanical Syst.*, vol. 13, no. 4, pp. 559–567, 2004.

[5] G.-B. Lee, C.-C. Chang, S.-B. Huang, and R.-J. Yang, *J. Micromechanics Microengineering*, vol. 16, no. 5, pp. 1024–1032, 2006.