

MICROFLUIDIC ELECTRIC IMPEDANCE SPECTROSCOPY FOR MALARIA DIAGNOSIS

Sungjae Ha¹, Monica Diez-Silva¹, E Du¹, Sung Jae Kim²,
Jongyoon Han¹, Ming Dao¹, and Anantha P. Chandrakasan¹

¹Massachusetts Institute of Technology, USA, ²Seoul National University, South Korea

ABSTRACT

This paper presents a label-free and noninvasive cell-counting assay system for electrical diagnostic tests of *Falciparum malaria* using electric impedance spectroscopy (EIS). Our system enabled a high-throughput test in a compact form-factor by the integration of a microfluidic device and a custom circuit board. Based on the differentiation of dielectric properties between cells and container medium, the target cells were individually detected by surface charge density and disease state. [1] This method suggests a potential electrical diagnostic test for malaria in portable electronics.

KEYWORDS

Malaria, electric impedance, spectroscopy, label-free, diagnosis.

INTRODUCTION

Malaria is an infectious disease of humans caused by *Plasmodium* parasites, with an estimation of 1,238,000 deaths in 2010. [2]. While five different species of *Plasmodium* can infect humans, *P. falciparum* causes the most severe forms of the disease being responsible for more than 90% of the deaths. [2] While *P. falciparum* develops in a host red blood cell (RBC), the RBC undergoes pathological modifications of its optical, mechanical, magnetic properties, and also the electrical property classified as electric impedance. [3-6]. Any of these changes may be utilized for possible malaria diagnosis. However, an effective diagnostic test requires high throughput due to the low parasitemia (the proportion of infected RBCs of malaria). [7]

Electric impedance spectroscopy (EIS) is a technique to measure the electric impedance of biological cells under continuous flow condition, thus enables faster test compared to the trapped cell analysis that has limited detection throughput. [8] While RBCs insulate current flow at low frequency, high frequency AC current flows through the cell membrane and cytoplasm, so the electric impedance is affected by the intracellular change of RBCs. Thus, there was a report on the labeled detection of *Babesia Bovis*-infected bovine RBCs using EIS at 8.7-MHz. [9] However, the use of chemical label made it unclear whether the observed difference was resulted by the parasites or the chemical. For on-site diagnostics, direct and label-free detection by EIS would be highly desirable.

EXPERIMENT

Our proposed system, shown in Figure 1, consists of two parts: a microfabricated probe and a reader circuit board. The probe was designed to investigate tiny human RBCs, of which diameter is less than 10 μ m, by one at a time in a channel of 30 μ m x 5 μ m cross-sectional area and 20 μ m electrode spacing. The circuit board, shown and described in Figure 2, was connected to the probe and continuously measured the electric impedance at the microfluidic channel. This integrated single cell analysis system based on electric impedance is small and capable of high throughput up to 500 cells per second, thus potentially capable of detecting low parasitemia samples.

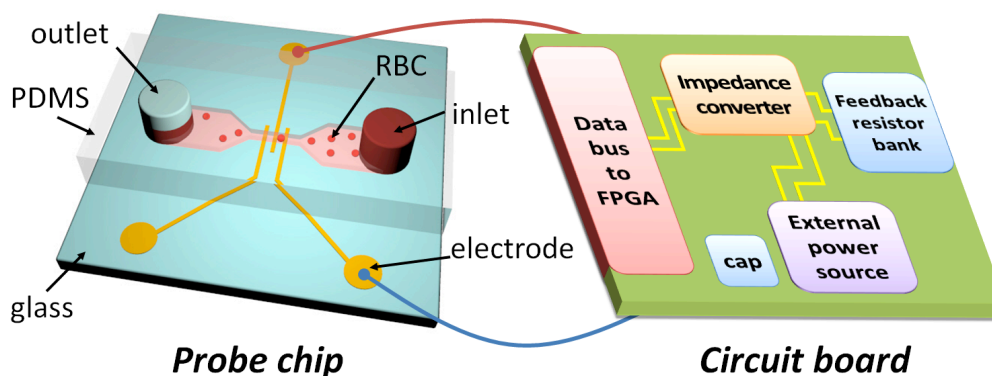


Figure 1. Proposed Microfluidic EIS system for malaria diagnosis.

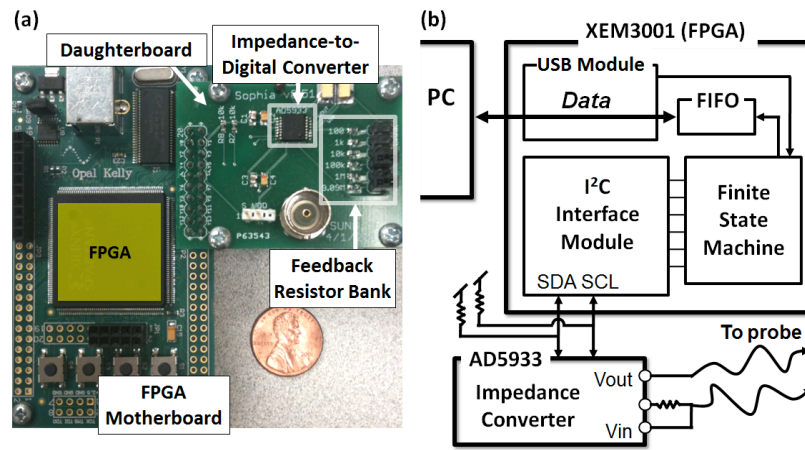


Figure 2. Custom circuit boards (a, left) and the controller block diagram (b, right).

The miniaturized EIS system was first verified by polystyrene bead assays. Figure 3 shows that EIS at 50-kHz could clearly differentiate isometric polystyrene beads of different surface charge density. Plain polystyrene beads ($\sigma = 10^{-16}$ S/m) in relatively conductive PBS solution ($\sigma = 1.2$ S/m) impede electric current, thus generate positive peaks in the magnitude plot of impedance. However, carboxyl functionalized beads produce smaller peaks than the plain beads by a factor of 1.85, due to the surface conduction around the beads. The size dependency must be considered to clarify the effect of surface charge. By Maxwell's approximation equation, 10% mismatch in the diameter of the beads can only contribute by a factor of 1.33. Therefore, the difference resulted from the attracted mobile charge near the surface of the carboxyl microbeads rather than from size mismatches.

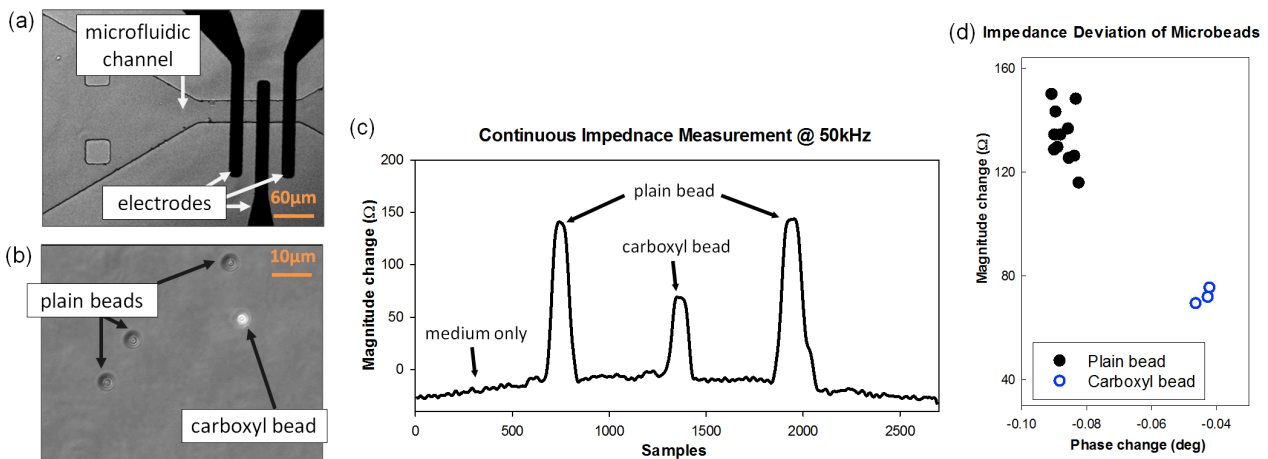


Figure 3. Microfluidic channel and electrode for EIS (a), visual differentiation of microbeads (b), and EIS results of plain and carboxyl-functionalized microbeads (c, d).

Since parasitic proteins change the surface charge density of an infected RBC, it was also possible to differentiate a malaria-infected RBC from healthy RBCs without any chemical label attached (Figure 4). The separation at 50-kHz appeared at the late stage of malaria when the effect of parasitic proteins became considerable. However, the separation between live cells suffered overlaps between each disease stage group. The overlaps are natural considering that electric impedance of live cell is strongly affected by cell size as well as disease stage of which both are continuous and hardly controllable.

To further investigate the intracellular change of infected RBCs for the early stage malaria detection, EIS test at a higher frequency was conducted. In Figure 5, EIS at 2-MHz could separate them by the impedance phase transition curves when a cell flow over the pair of electrodes. The selectivity on early stage malaria is expected to increase at even higher frequencies, as the intracellular resistance more contributes in the overall cell impedance at higher frequencies. These experimental results suggest the possible separation of early stage malaria and potential applications of EIS in diagnosis of malaria and other parasitic diseases.

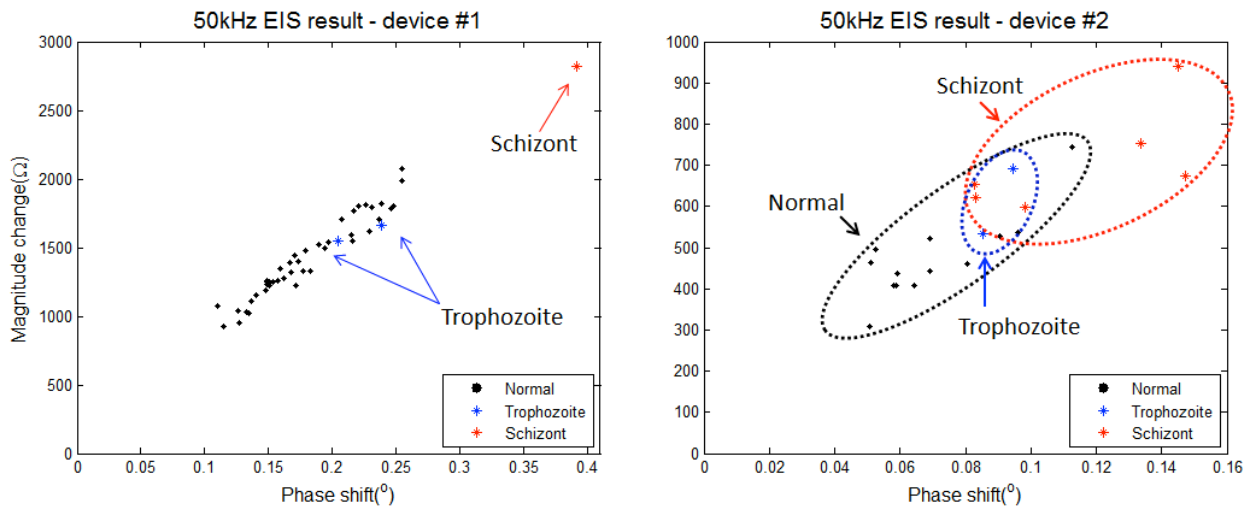


Figure 4. EIS results at 50kHz in two different devices show separation of Schizont stage cells.

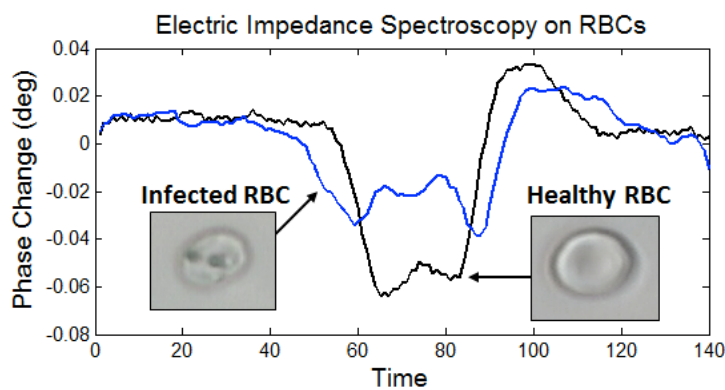


Figure 5. Phase transition of impedance measured for a healthy RBC and an infected RBC at 2MHz EIS.

ACKNOWLEDGEMENTS

This work was funded by Center for Integrated Circuits and Systems (CICS) and Fulbright Science and Technology Fellowship.

REFERENCES

- [1] Sungjae Ha, *A malaria diagnostic system based on electric impedance spectroscopy*, S.M. Thesis, MIT, Cambridge, MA, USA (2011).
- [2] World Health Organization, *World Malaria Report: 2010*, Geneva, Switzerland (2010).
- [3] Y. Park, M. Diez-Silva, G. Popescu, G. Lykotrafitis, W. Choi, M. S. Feld, and S. Suresh, *Refractive index maps and membrane dynamics of human red blood cells parasitized by plasmodium falciparum*, PNAS, **105**, 13730–13735 (2008).
- [4] H. Bow, I. V. Pivkin, M. Diez-Silva, S. J. Goldfless, M. Dao, J. C. Niles, S. Suresh, and J. Han, *A microfabricated deformability-based flow cytometer with application to malaria*, Lab Chip, **11**, 1065–1073 (2011).
- [5] S. Hackett, J. Hamzah, T. M. Davis, and T. G. S. Pierre, *Magnetic susceptibility of iron in malaria-infected red blood cells*, Biochim. Biophys. Acta, **1792**, 93–99 (2009).
- [6] C. Ribaut, K. Reybier, O. Reynes, J. Launay, A. Valentin, P. L. Fabre, and F. Nepveu, *Electrochemical impedance spectroscopy to study physiological changes affecting the red blood cell after invasion by malaria parasites*, Biosens Bioelectron, **24**, 2721–2725 (2009).
- [7] Thomas Hanscheid, *Diagnosis of malaria: a review of alternatives to conventional microscopy*, Clin Lab Haematol, **21**, 235–245 (1999).
- [8] H. Morgan, T. Sun, D. Holmes, S. Gawad and N. G. Green, *Single cell dielectric spectroscopy*, J. Phys. D: Appl. Phys, **40**, 61-70 (2007).
- [9] A. Valero, T. Braschler, and P. Renaud, *A unified approach to dielectric single cell analysis: Impedance and dielectrophoretic force spectroscopy*, Lab Chip, **10**, 2216–2225 (2010).

CONTACT

Sungjae Ha sungjae@mit.edu