Label-free Characterization and Sorting of Human Pathogens

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Microfluidic Impedance Cytometry

Microfluidic Impedance Cytometry (MIC) measures the dielectric properties of single cells as they pass through a microchannel with integrated electrodes. An electrical field is applied by the microelectrodes, with the electrical impedance of single cells being retrieved from their interaction with the field. A measurable change in the differential signal, obtained by the microelectrodes, is proportional to particle impedance [1].

The application of numerous different frequencies can be used as a cell-characterization tool, being able to discriminate various cell types. Examples of this discrimination are the differentiation of the three main populations of white blood cells [2], stem cells [3] or parasite-infected cells [4]. A high throughput (up to 1000 cells per second) or the capability of performing measurements using a physiological medium are some of advantages of the system.

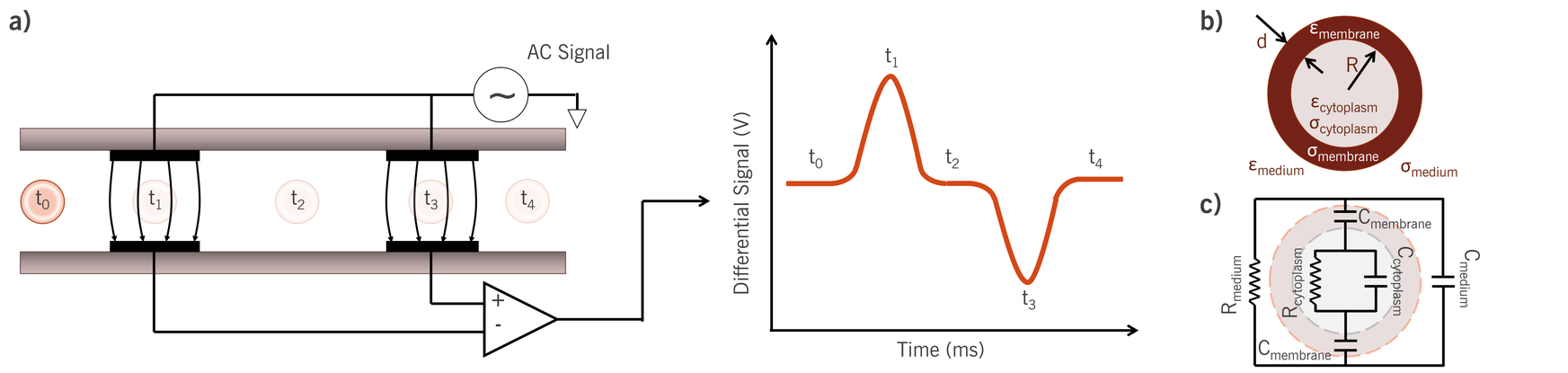

Figure 1 – a) Schematic representation of the MIC system structure and operation. Suspended cells are driven through the microchannel by a pressure driven flow. An AC signal is applied on both top electrodes, generating an electric field. A differential signal between the measurements of the bottom electrodes is measured. After the cell entrance in the channel (t_0), it will flow until it reaches the electrode area (t_1). At this point, given the usually insulating properties of polymer beads or cell membranes, the signal in the first set of electrodes drop, resulting in a positive differential peak in the differential signal. The opposite occurs when the cell passes through the second set of electrodes (t_3), where a negative peak is observed in the differential signal. This differential signal is then used to identify cells and later perform the dielectric characterization. **b)** Single-shell model used to model and characterize non-nucleated cells (such as red blood cells). Other models can be constructed to better represent the cells/particles under study. **c)** Equivalent circuit model also used to characterize the cells under study.

Water-borne pathogens

From the different water-borne pathogens, protozoan parasites, as *C.Parvum, C.Muris* and *G. Lamblia* still remain a problem, presenting resistance to water treatment methods and a low infectious dose [5].

Another remaining problem is related to the differentiation of viable and non-viable oocysts of the protozoan parasite in the standard detection method. An automated process could reduce detection time, reduce the level of human intervention required, aid in better assessing the risk posed to human health and contribute to the saving of numerous resources.

Preliminary results show good differentiation between viable and non-viable samples.

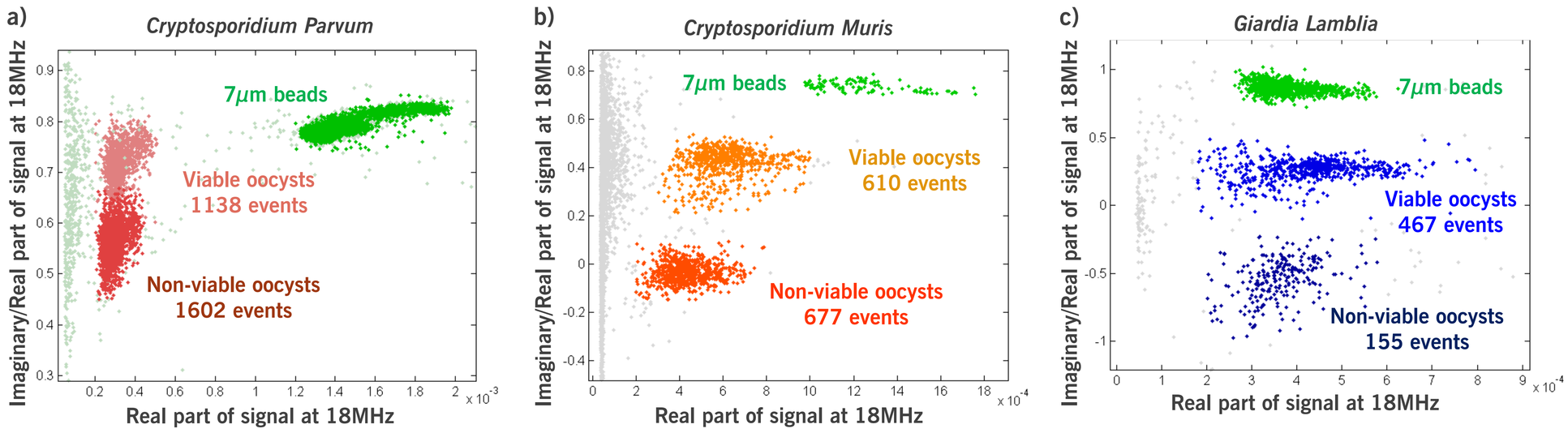

Figure 2 – Scatter plots of the ratio between Imaginary and Real parts over the Real part of the signals measured by the MIC for different water-born pathogens **a)** *Cryptosporidium Parvum,* **b)** *Cryptosporidium Muris* and **c)** *Giardia Lamblia.* Scatter plot represent two experiments, overlapped in the plot, performed at 18MHz, with samples containing polystyrene 7μm beads and viable or non-viable oocysts of the pathogens in samples with different concentration of PBS: **a)** 4x, **b)** 1x and **c)** 0.25x.

Malaria Infected Red Blood Cells

Human malaria is the world's most important disease caused by parasites. More than 40 % of the world population lives where malaria is endemic, where about 207 million cases and 627 000 malaria deaths have been estimated in 2012 [6].

A dielectric characterization of the parasite could help develop a better diagnostic tool, aimed at low-concentration causative parasites. Previous dielectric studies [7] showed that the parasite's presence causes the cell to lose is ability to control its cytoplasmic ion concentration.

Preliminary results are in accordance with this notion. After a fitting process, the dielectric properties of the healthy and infected red blood cells (iRBCs) can be retrieved. As expected, the cytoplasm conductivity of iRBCs matches that of the medium due to changes in the membrane.

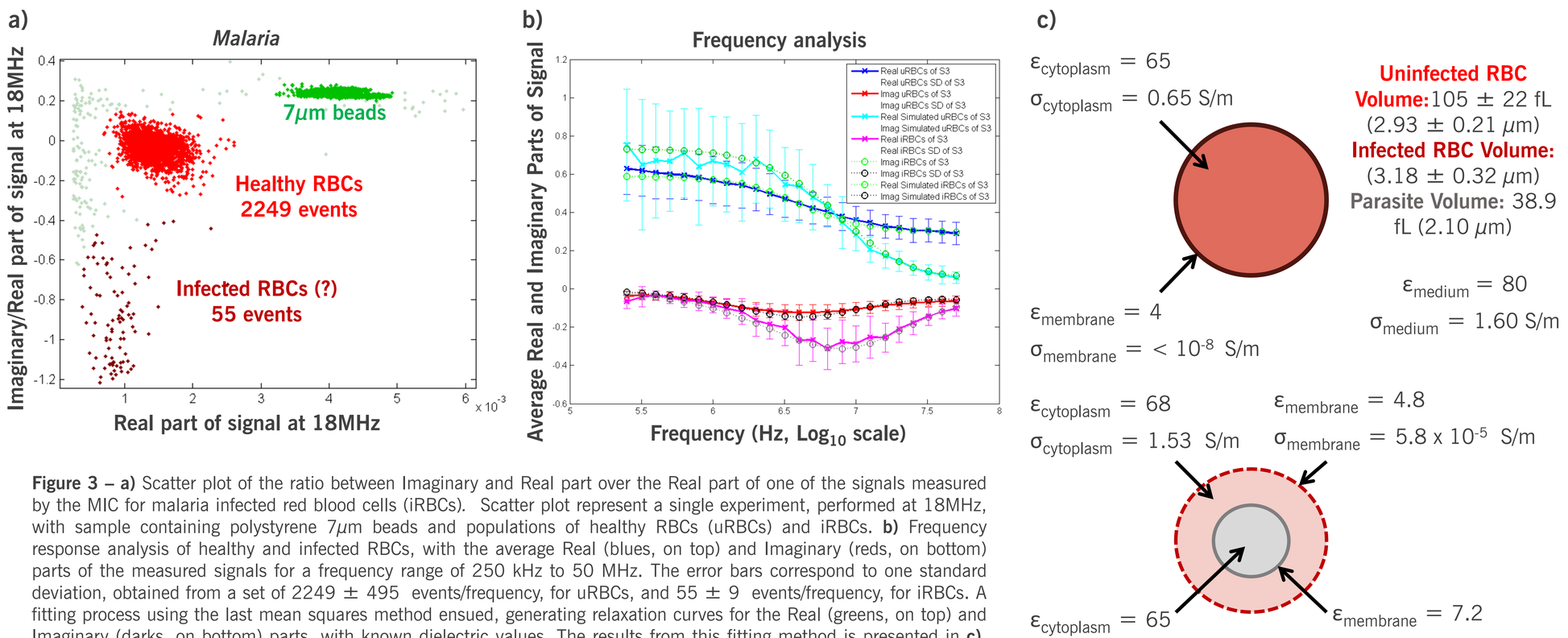

Figure 3 – a) Scatter plot of the ratio between Imaginary and Real part over the Real part of one of the signals measured by the MIC for malaria infected red blood cells (iRBCs). Scatter plot represent a single experiment, performed at 18MHz, with sample containing polystyrene 7μm beads and populations of healthy RBCs (uRBCs) and iRBCs. **b)** Frequency response analysis of healthy and infected RBCs, with the average Real (blues, on top) and Imaginary (reds, on bottom) parts of the measured signals for a frequency range of 250 kHz to 50 MHz. The error bars correspond to one standard deviation, obtained from a set of 2249 ± 495 events/frequency, for uRBCs, and 55 ± 9 events/frequency, for iRBCs. A fitting process using the last mean squares method ensued, generating relaxation curves for the Real (greens, on top) and Imaginary (darks, on bottom) parts, with known dielectric values. The results from this fitting method is presented in **c)**, where the permittivity, ε, and conductivity, σ, for the different cell components and medium can be found.

References

[1] T. Sun, H. Morgan, "Single-cell microfluidic impedance cytometry: a review", Microfluidics and Nanofluidics 8(4), 423-443 (2010).
[2] D. Holmes, D. Pettigrew, C. H. Reccius, J. D. Gwyer, C. V. Berkel, J. Holloway, D. E. Davie, and H. Morgan, "Leukocyte analysis and differentiation using high speed microfluidic single cell impedance cytometry," Lab Chip 9, 2881–2889 (2009).
[3] H. Song, Y. Wang, J. M. Rosano, B. Prabhakarpandian, C. Garson, K. Panta, and E. A. Lai, "A microfluidic impedance flow cytometer for identification of differentiation state of stem cells," Lab Chip 13, 2300–2310 (2013).
[4] C. Kuttel, E. Nascimento, N. Demierre, T. Silva, T. Braschler, P. H. Renaud, and A. G. Oliva, "Label-free detection of Babesia bovis infected red blood cells using impedance spectroscopy on a microfabricated flow cytometer," Acta Trop. 102, 63–68 (2007).
[5] H. Bridle, B. Miller, M. Desmulliez, "Application of microfluidics in waterborne pathogen monitoring: A review", Water Research 55, 256-271 (2014).
[6] World Health Organization, "World Malaria Report" (2014).
[7] P. Gascoyne, C. Mahidol, M. Ruchirawat, J. Satayavivad, P. Watcharasit, F. Becker, "Microsample preparation by dielectrophoresis: isolation of malaria", Lab. Chip 2 (2), 70–75 (2002).

SEVENTH FRAMEWORK PROGRAMME

LAPAS