CONCENTRATION/SEPARATION OF CRYPTOSPORIDIUM OOCYSTS BY ON-CHIP HYBRID AC-ELECTROKINETICS FOR DIGITAL MICROFLUIDICS

Romuald Lejard[1], Jérôme Follet[2], Alexis Vlandas[1], Anne Follet[3], Vincent Thomy[1], Vincent Senez[1]

Université Lille Nord de France, France

[1] Institut d'Electronique, de Microélectronique et de Nanotechnologie (CNRS/IEMN), Villeneuve d'Ascq, France

[2] Institut Supérieur d'Agriculture (ISA), Lille, France

[3] Faculté Libre des Sciences et Technologies (FLST), Lille, France

ABSTRACT

This paper demonstrates the integration of droplet manipulation by electrowetting-on-dielectric (EWOD) with in-droplet manipulation of parasites (i.e. Cryptosporidium oocysts) by hybrid AC-electrokinetics (ACEK) [1]. While other techniques have been reported for in-droplet manipulation, this is the first time that ACEK (i.e. combined effect of dielectrophoresis, electroomosis and electrohydrodynamics) is used to concentrate/separate biological particles in droplet-based microfluidics. Parasites concentration/separation in a sub-volume of the mother droplet has been successfully performed with better efficiency than competing works reported in the literature.

KEYWORDS

Concentration/separation, digital microfluidics, AC-electrokinetics, parasites, water quality.

INTRODUCTION

Cryptosporidium is an Apicomplexan genus with many species that are pathogenic to humans and animals. Cryptosporidium species are the etiologic agent of cryptosporidiosis, a severe diarrheal disease which can be deadly for immunodeficient people like AIDS patients. The infectious Cryptosporidium oocyst is frequently transmitted via contaminated drinking water. Several miniaturized techniques have been developed to detect and quantify Cryptosporidium oocysts in water samples. They are based on surface plasmon resonance [2], impedance spectroscopy [3], microgravimetry [4] or piezoelectric cantilever sensor [5]. All these analysis used microliter volume of the samples. However, current methodologies for sampling and filtrating water lead to volume in the milliliter range. Microsystem approaches require therefore an additional ultra-concentration step with high recovery efficiency in order to release the constraints on the detection limit.

Our solution is to use digital microfluidics (DMF) by EWOD (in air) for sample preparation. Reasons for justifying this choice have been given previously [6-7]. In this context, one can find several authors that have integrated pre-concentration step in DMF. Wang proposed a system based on magnetic field [8]. Shah improved this system using interfacial forces [9]. Shah also used optoelectronic tweezers (combining optical tweezer and dielectrophoresis) to perform concentration/separation of Hela cells [10]. Valley and his colleagues used optoelectronic tweezers to manipulate particles. In their system, wettability of the active surface is modified by light [11]. Magnetic and optical methods have very high recovery efficiency (higher than 90%). However, their full integration in a lab-on-chip is not possible for time being. On the contrary, electrical methods can be integrated more easily, particularly with EWOD chip. A first system was introduced by Cho [12]. It uses electrophoresis and its recovery efficiency is 83% and concentration increase is 73%. Fan integrated dedicated interdigitated electrodes in EWOD chip (in oil) and used dielectrophoresis to concentrate cells [13]. Its recovery efficiency is 63% and concentration increase is 1.6x.

EXPERIMENT

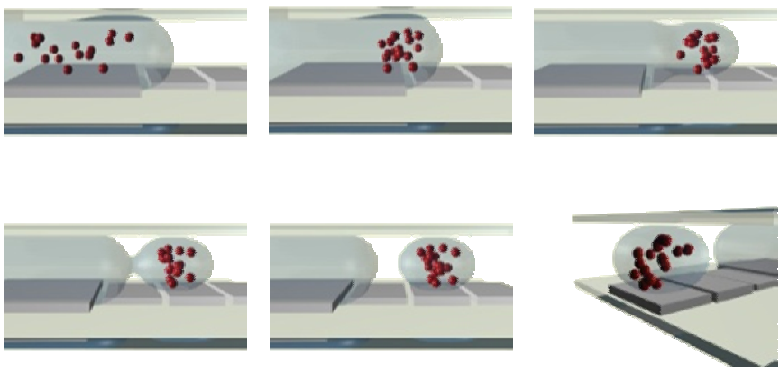

Figure 1: Schematic views of a-b) particle manipulation by ACEK in a droplet and subsequent c-d) droplet splitting and e-f) transporting by EWOD.

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

The concept of our device is described in Fig. 1 showing the two kinds of electrodes i) for concentration/separation of the micro-particles in an immobile mother droplet (Fig.1a-b), ii) for splitting into a daughter droplet and further displacement (Fig. 1c-f). We rely therefore on a single electrical control system to perform all the necessary functions on our device and the design of the concentration/separation electrodes is the key aspect of our work.

From a literature survey [14], we defined the best potential geometries (Fig.2) which were then optimized using the simulation strategy of Oh [15] but applying it in 3D instead of 2D. This is a crucial difference as, of the various forces acting on the micro-particles in the droplet, the equilibrium between negative dielectrophoresis and gravity forces defines a levitation height of the particles and thus a concentration in the Z-axis while the vortices formed by the electrothermal effects effectively traps them at defined locations in the XY plane. These phenomena can therefore only be seen in a full 3D simulation.

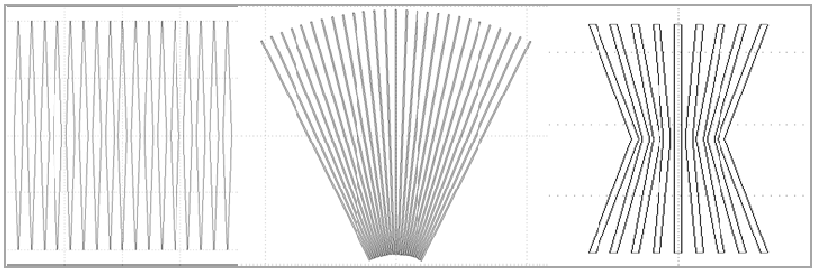

Figure 2: Schematic top view of the various geometries of the planar electrodes dedicated to the concentration/separation of the particle by ACEK: a) diamond, b) arrow, c) X shapes.

The various criteria for optimization such as the need to keep temperature in the liquid < 315°K to be bio compatible were assessed. Fig.3 gives the results of this 3D optimization in which the total force fields on each geometry clearly display vortices (in the dark circles) in which particles are gathered. By further adding as a constraint the drop splitting step we show that the X-shape electrode is the best choice for our system.

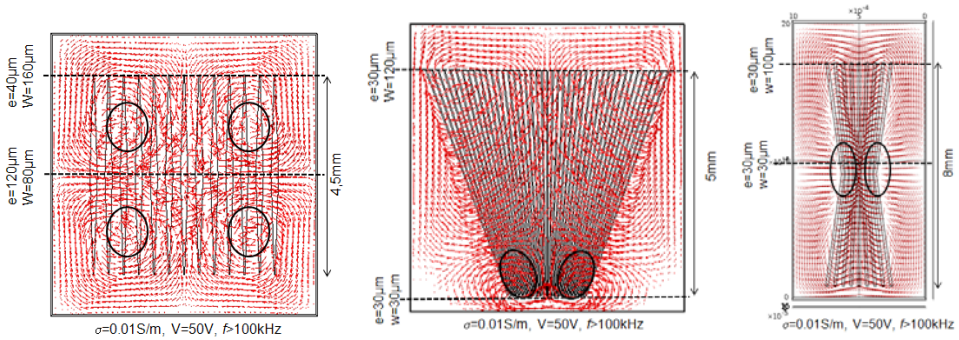

Figure 3: 2D cross sections (in the plane of the levitation height) of the 3D simulations of the total forces (dielectrophoresis, drag, gravity, buoyancy) obtained by solving charge conservation (Laplace), energy conservation (Fourier), momentum conservation (Stoke) equations with COMSOL MULTIPHYSICS for each electrode shape. Conductivity, potential drop and frequency were the same for all geometries. The vortices can be seen in the dark circles.

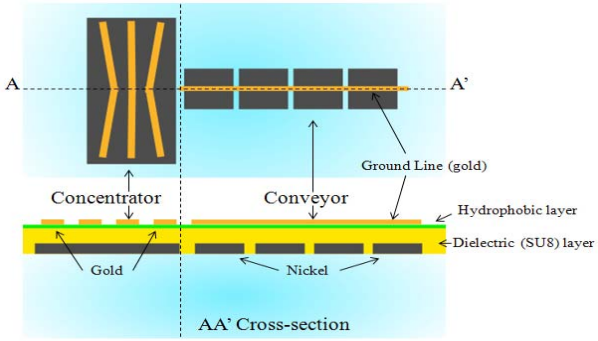

Figure 4: Schematic drawing (top & side views) of the topography of the micro-device showing the layering of the EWOD electrodes (Nickel), EWOD dielectric (SU-8) and ACEK and EWOD ground electrodes (gold). A superhydrophobic silicon cover is fixed on top of this structure to prevent evaporation and to reduce friction.

Fig.4 shows the top and cross-sectional views of the bottom part of the system. The resulting device was covered by a silicon superhydrophobic cover to perform all experiments in air.

We performed several concentration tests on Cryptosporidium parvum oocysts (spherical parasites with 5 µm diameter). We demonstrated clear concentration possibilities with high recovery rate (70%) in the vortices resulting in a 12x increase in local concentration (Fig.5).

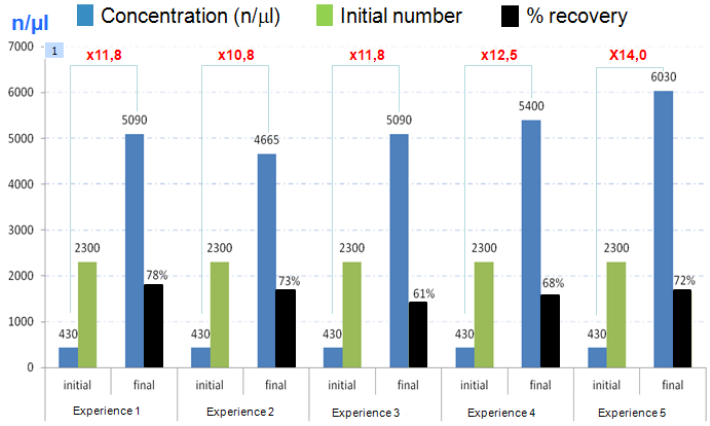

Figure 5: Results of the experiments (5) on the concentration of Cryptosporidium parvum oocysts. First column is the initial concentration, second column is the total number, third column is final concentration and last column is the percentage of recovery (number of gathered oocysts/total number of oocysts).

Extraction of this fraction in two daughter droplets (# 800nl) using EWOD resulted in droplets in which parasites concentration was multiplied by 4.5. This result is higher than the state of the art [13] which showed only a 1.6x increase. Finally, we demonstrated that using the same device, one can successfully distinguish and separate between two parasites (Cryptosporidium oocysts and Giardia cysts) differing in size and shape (5µm and 12µm respectively). These results of an integrated multi function electrical only system pave the way to lab on chip device for water quality control.

REFERENCES

[1] A. Castellanos, A. Ramos, A. Gonzalez, N.G. Green, H. Morgan, J. Physics D, 36, pp. 2584-2597, (2003).
[2] C.D. Kang, C. Cao, J. Lee, I.S. Choi, B.W. Kim, S.J. Sim, Water Research, 42, pp. 1693-1699, (2008).
[3] T. Houssin, J. Follet, A. Follet, E. Dei-cas and V. Senez, Biosensors Bioelectronics, 25, pp. 1122-1129, (2010).
[4] C. Poitras, J. Fatisson, N. Tufenkji, Water Research, 43, pp. 2631-2638, (2009).
[5] S. Xu, S. Mutharasan, Analytica Chimica Acta, 669, pp. 81-86, (2010).
[6] C. Wu, F. Bendriaa, F. Brunelle, V. Senez, Proc. Micro-TAS 2011, Seattle, USA, (2011).
[7] C. Wu, F. Brunelle, M. Harnois, J. Follet, V. Senez, Proc. MEMS 2012, pp. 777-780, (2012).
[8] Y. Wang, Y. Zhao, S.K. Cho, J. Micromech. Microeng., 17, pp. 2148-2156, (2007).
[9] G.J. Shah and C.J. Kim, J. of Microelectromechanical Systems, 18, pp. 363–375, (2009).
[10] G. J. Shah, A. T. Ohta, E. P. Chiou, M. C. Wu, C.J. Kim, Lab Chip, 9, pp. 1732-1739, (2009).
[11] J.K. Valley, S. Ningpei, A. Jamshidi, H. Y. Hsu, M. C. Wu, Lab Chip, 11, pp. 1292-1297, (2011).
[12] S.K. Cho, Y. Zhao, C.J. Kim, Lab Chip, 7, pp. 490-498, (2007).
[13] S.K. Fan, P.W. Huang, T.T. Wang, Y.H. Peng, Lab Chip, 8, pp. 1325-1331, (2008).
[14] K. Khoshmanesh and colleagues, Biosensors & Bioelec., 26, pp. 1800-1814, (2011).
[15] J. Oh, R. Hart, J; Capurro and H. M. Noh, Lab Chip, 9, pp. 62-78, (2009).

CONTACT

Vincent Senez 33-3-2019-7855 or vincent.senez@isen.fr