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A Numerical Approach for Dielectrophoretic Characterization and Separation of Human Hematopoietic Cells

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***Abstract*—Rapid and reliable characterization of hematopoietic cells still remain the first step for precise medicine. Diagnosis of various diseases, ranging from infectious to cancer, relies on quantification of hematopoietic cells from blood. Therefore, there is an emerging need for label-free, low-cost, time-efficient, reproducible and quantitative characterization tools for the blood cells. Addressed herein is a numerical analysis for dielectrophoretic characterization of red blood cells, T-lymphocytes, B-lymphocytes and monocytes, which quantitatively incorporate with the membrane features of these cells to provide more insight into their dielectrophoretic responses.**

Keywords*— *Hematopoietic cells; Dielectrophoresis; Numeric Analysis; Characterization; Quantification

I. INTRODUCTION

Blood is the most used bodily fluid for diagnostics that comprises different types of cells circulated throughout the body with their complex interactions. Thanks to the developments in the lab-on-a-chip technologies, novel diagnostic methods intended to quantitatively characterize specific cell types in a fast and efficient way, directly from blood [1]. Integration of microfluidics with Micro-Electro-Mechanical Systems (MEMS) provided portable, low-cost, high-throughput, fast and precise tools for medical diagnostics and biological sciences [2]. Miniaturized dimensions in microfluidic systems allow mimicking natural interactions of cells either with other cells or their microenvironments while providing high-resolution data at single-cell resolution [3].

Contrary to recently emerged microfabricated tools, dielectrophoresis (DEP) is an old and well-established phenomenon, which is applied to biology in the 1950s [4]. As a method, DEP has the capability of quantifying intrinsic properties of cells in a label-free manner [5]. Therefore, DEP-isolated or DEP-characterized cells are both genetically and phenotypically preserved and reliably eligible to be used for downstream assays [6]. Precise, low-volume liquid handling features of microfluidics have been incorporated with sensitive and specific DEP manipulation techniques; as a consequence, DEP methods have again become one of the powerful methods for biological and clinical applications [7,8].

Fluorescent-activated cell sorters (FACS) or magnetic-activated cell sorters (MACS) are very much used high-throughput techniques today [9-14]. Both FACS and MACS use antibodies against a particular protein that might cause some phenotypic changes for the cells with metastable phenotypes such as drug-tolerant cells, persisters [6]. Moreover, other microfluidic-based cell sorting technologies still require optimization for cell recovery, throughput, and user-friendliness to move from bench side to diagnostics.

In this study, we focus on DEP-based numerical approaches for isolation and characterization of hematopoietic cells, particularly red blood cells and immune cells. Immune cells are highly heterogeneous cells and their heterogeneity increases with limited response time when they encounter abnormal changes in their microenvironments [15]. Furthermore, labeling surface antigens might alter their immune response via affecting cellular signaling. Considering all these limitations precise modeling and simulations are promptly required to provide reliable insights on their dielectrophoretic characterization. Most of the numerical methods use single-shell dielectric model with spherical surface area consideration for cells. Additionally, most of the simulations are performed to determine the gradient of the streaming dielectric field and flow [16-20] or to predict the interactions of cells [21] in the microfabricated devices. Nevertheless, Gascoyne and Shim obtained a dielectric model for the cells where they considered morphologic changes of circulating tumor cells through metastasis; however, they used smooth sphere assumption for the blood cells in this study [7]. One of the major limitations of developing precise and accurate numerical models underlines the lack of accuracy of measured cellular parameters. Most of the dielectric properties of cells were obtained from electrorotation, produced by rotating electric fields [22]. However, thanks to advanced imaging and recent measurement techniques, we can obtain more realistic

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dielectric models for the cells via introducing the surface structure of cells and variations of their microenvironment.

Herein we reported numeric analysis results for hematopoietic cells, including Red Blood Cells (RBC), T-Lymphocytes (T-cell), B-Lymphocytes (B-cell) and U937-Monocytes (U937-MC) incorporation with their surface morphology based on recent cell surface measurements. These results will be used for quantification of the dielectrophoretic behavior of these cells in biological and clinical characterizations.

II. MATERIALS AND METHODS

A. *DEP Theory and Numerical Method*

DEP has a well-established basic theory [23]. Characterization of cells is one of its applications, relies on its capability of generating specific dielectrophoretic forces (F_{DEP}) for different types of cells, in (1).

$$F_{DEP} = 2\pi\varepsilon_m r^3 \mathrm{Re}[f_{CM}]\nabla E_{rms}^2 \quad (1)$$

where, the radius of the spherical cell (r), applied electric field (E), the permittivity of the suspending medium ($\mathcal{E}_m$), and real part of Clausius-Mossotti factor $\mathrm{Re}[f_{cm}(\omega)]$ are used. Thanks to the real part of Clausius-Mossotti factor, $\mathrm{Re}[f_{cm}(\omega)]$, which creates specificity based on the conductivity and permittivity of the cells and their surrounding buffer as calculated in (2).

$$f_{cm} = \frac{\mathcal{E}^*_{eff} - \mathcal{E}^*_m}{\mathcal{E}^*_{eff} + 2\mathcal{E}^*_m} \quad (2)$$

where $\mathcal{E}^*_m$ is the complex permittivity of the medium, $\mathcal{E}^*_{eff}$ is the effective permittivity of the cell using (3) and (4), according to single-shell model using

$$\mathcal{E}^* = \mathcal{E} - \frac{j\sigma}{\omega} \quad (3)$$

$$\mathcal{E}^*_{eff} = \mathcal{E}^*_{mem} \frac{(\frac{r}{r-d})^3 + 2\frac{\mathcal{E}^*_{int} - \mathcal{E}^*_{mem}}{\mathcal{E}^*_{int} + 2\mathcal{E}^*_{mem}}}{(\frac{r}{r-d})^3 - \frac{\mathcal{E}^*_{int} - \mathcal{E}^*_{mem}}{\mathcal{E}^*_{int} + 2\mathcal{E}^*_{mem}}} \quad (4)$$

distinct dielectrophoretic properties of the cells such as permittivity ($\mathcal{E}$) and conductivity (σ), complex permittivity of the membrane ($\mathcal{E}^*_{mem}$), complex permittivity of the cytoplasm ($\mathcal{E}^*_{int}$), membrane thickness (d), the imaginary number (j) [24, 25].

These intrinsic dielectric properties determine the polarizability of the cells under the applied nonuniform electric field. In other words, each specific cell type will experience a specific DEP force. When the $\mathrm{Re}[f_{cm}(\omega)]$ is positive, positive DEP (pDEP) occurs, and strong electric field streams attract the cells. When the $\mathrm{Re}[f_{cm}(\omega)]$ is negative, negative DEP (nDEP) occurs, and electric field repels the cells. The frequency when the cells experience almost zero F_{DEP} and change their behavior form pDEP to nDEP, or vice versa, is defined as the crossover frequency (CF).

B. *Effect of membrane morphology on Dielectric Properties*

The single-shell cell models are widely employed mathematical models for dielectrophoresis owning to their simplicity and fast computation costs, Fig. 1.

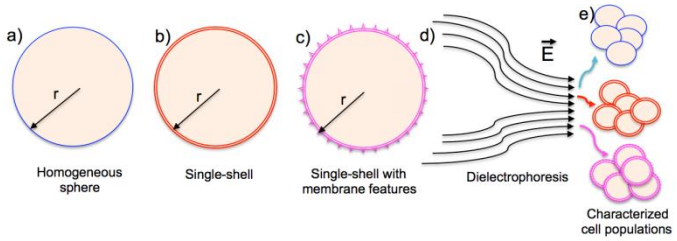

Fig. 1. Dielectric shell models for cells. a) Homogeneous sphere model, b) Single-shell model, shell thickness equals to cellular membrane thickness, c) Single-shell model with membrane features such as microvilli, filopodia, ruffles and folds, d) Applied nonuniform electric field (E), e) characterized subpopulations of cells due to their membrane differences

In this study, we used the single-shell spherical cell model developed by Pauly and Schwan [26] where cell membrane dielectric properties depend on $\mathcal{E}_{mem}$, σ_{mem}, r, and frequency of the applied electric field. However, different types of cells have different membrane morphologies. Gascoyne and Shim introduced membrane folding factor, Φ in (5) to link membrane differences to the dielectrophoretic responses of the cells to isolate cancer cells from blood due to their greater membrane folding factor compared to normal cells [7].

$$\Phi = \frac{A}{4\pi.r^2} \quad (5)$$

The effective permittivity of the cell using in (4) and (5) can be rewritten as in (6).

$$\mathcal{E}^*_{eff} = \mathcal{E}^*_{mem} \frac{(\frac{r\Phi}{r\Phi-d})^3 + 2\frac{\mathcal{E}^*_{int} - \mathcal{E}^*_{mem}}{\mathcal{E}^*_{int} + 2\mathcal{E}^*_{mem}}}{(\frac{r\Phi}{r\Phi-d})^3 - \frac{\mathcal{E}^*_{int} - \mathcal{E}^*_{mem}}{\mathcal{E}^*_{int} + 2\mathcal{E}^*_{mem}}} \quad (6)$$

For the cells based on the most recent surface area data using advanced measurement techniques from literature, we created Table I. Our homemade MATLAB script (Version R2016a, MathWorks) uses (4) and (6) and compares the dielectrophoretic responses of different types of blood cells in the absence and presence of membrane features.

III. RESULTS

Fig. 2 represents the dielectric properties of T-cell, B-cell, RBC and U937-MC using single-shell model based on the assumption that the membrane of these cells is homogeneous single shell. Different cell types exhibit different crossover frequencies under the same environmental conditions due to their intrinsic dielectric properties, extending frequencies from 0.1 kHz to 10 GHz, Table II. The crossover frequencies for RBC and U937-MC are 88.35 kHz and 19.59 kHz, respectively. Both T- cells and B-cells exhibit pDEP behavior for the whole frequency spectrum.

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Fig. 3 shows the dielectric properties of these cells when measured membrane surfaces or membrane folding factors (m-ff) are reflected. The real part of Clausius Mossotti factor did not change its trend. The crossover frequency for RBC remained 88.35 kHz while it increased 44 kHz for the U937-MC, Table II. Although T- cells with folding-factor 1.22 and B-cells with folding-factor 1.94 demonstrate pDEP behavior for the whole frequency range (Table II), their DEP-response curves are shifted, Fig. 3.

TABLE I. Dielectric parameters for hematopoietic cells.

Dielectric parameters (Symbol, unit)	RBC	T-cell	B-cell	U937-MC
Radius (r, µm)	2.8 [27]	3.29 [28]	3.29 [28]	7 [29]
Membrane thickness (d, nm)	4.5 [25]	7.5 [30]	7.5 [30]	7 [29]
Medium conductivity (σ_m, S/m)	0.01	0.01	0.01	0.01
Medium permittivity ($\mathcal{E}_m$, F/m)	$80\mathcal{E}_0$	$80\mathcal{E}_0$	$80\mathcal{E}_0$	$80\mathcal{E}_0$
Membrane conductivity (σ_{mem}, S/m)	10^{-6} [31]	$2.7x\ 10^{-5}$ [32]	$5.6x\ 10^{-5}$ [32]	$1x10^{-6}$ [33]
Membrane permittivity ($\mathcal{E}_{mem}$, F/m)	$4.44\mathcal{E}_0$ [31]	$8.89\mathcal{E}_0$ [28]	$10.67\mathcal{E}_0$ [28]	$12.5\mathcal{E}_0$ [29]
Cytoplasm conductivity (σ_{int}, S/m)	0.31 [31]	0.65 [28]	0.73 [28]	0.5 [29]
Cytoplasm permittivity ($\mathcal{E}_{int}$, F/m)	$59\mathcal{E}_0$ [31]	$103.9\mathcal{E}_0$ [28]	$154.4\mathcal{E}_0$ [28]	$50\mathcal{E}_0$ [29]
Measured surface area of the cells (A, µm^2)	-	-	265 [34]	280 [35]
Membrane folding factor (Φ)	1 [27]	1.22 [36]	1.94	0.45

$\mathcal{E}_0$ is the permittivity of the free space, 8.85×10^{-12} F/m.

TABLE II. Membrane features affect dielectrophoretic response of the cells

Membrane morphology	Crossover frequencies and dielectrophoretic responses			
	RBC	T-cell	B-cell	U937-MC
Smooth	88.35 kHz	pDEP	pDEP	19.59 kHz
Membrane features	88.35 kHz	pDEP	pDEP	44 kHz

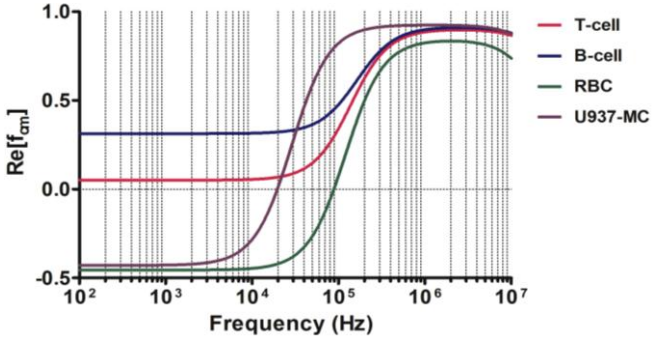

Fig. 2. The real part of Clausius-Mossotti factor (Re[f_{cm}]) vs. applied frequency shown for T-cell, B-cell, RBC and U937-MC in magenta, blue, green and purple, respectively.

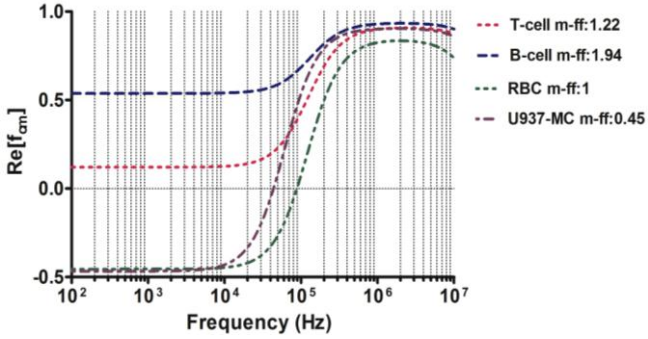

Fig. 3. The real part of Clausius-Mossotti factor (Re[f_{cm}]) including membrane features vs. applied frequency is shown for T-cell, B-cell, RBC and U937-MC in magenta, blue, green and purple, respectively.

IV. DISCUSSION

Mathematical models and numeric analysis methods provide rapid and powerful insights when they are capable of presenting necessary features of the required tasks in a clear, high-throughput and low computational complexity. According to obtained simulation results, both microfabricated device designs and experimental conditions might be determined in a short time, using fewer samples and less labor. Recently, precision medicine becomes one of the frontier research fields where fast, reliable, high-throughput and low-cost technologies will take place. In this area, dielectrophoretic tools will be widely used in separation, characterization and manipulation of clinical samples thanks to its label-free and quantitative nature [8,12,17,23].

In this study, we presented a simple and improved dielectric model of a cell using single-shell model [26]. Our model incorporates with the membrane features of a cell as Gascoyne and Shim reported for morphologic changes of circulating tumor cells through metastasis [7]. In their study, they introduced membrane folding factor for tumor cells while employing a smooth sphere assumption for blood cells, which successfully worked for isolation of tumor cells from blood. However, hematopoietic cells have different intrinsic and membrane properties that directly contribute their functional roles in blood circulation or immune defense. Likewise, the dielectrophoretic tools are sensitive enough to quantify these properties without modifying their genotype or phenotype [15,18,28,30,33]. In our model, we introduced the membrane folding factor using membrane surface area measurements from literature. Our results showed that membrane morphology of hematopoietic cells changed the crossover frequencies for U937-MC and RBC, while affecting the magnitude of pDEP responses of T-cells and B-cells.

Our model is clear enough to interpret the dielectric responses of the cells, it is accurate enough to distinguish biological features of hematopoietic cells, and last but not least it is computationally fast. In the era of precision medicine, the first step will be obtaining precise data from biological systems. Therefore, our model might be improved further to provide more realistic cellular dielectric models. The more accurate and precise cellular data gained, the smarter DEP devices and experimental conditions might be

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designed. Thus, complex biological interactions might be uncovered and applied to precision medicine in the near future.

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REFERENCES

[1] T. Yeo, S.J. Tan, C.L. Lim, D.P.X. Lau, Y.W. Chua, S.S. Krisna, G. Iyer, G.S. Tan, T.K.H. Lim, D.S.W. Tan, W.-T. Lim, C.T. Lim, Microfluidic enrichment for the single cell analysis of circulating tumor cells, Scientific Reports, 6 (2016) 22076.

[2] B. Çetin, D. Li, Dielectrophoresis in microfluidics technology, ELECTROPHORESIS, 32 (2011) 2410-2427.

[3] R.J. Kimmerling, G. Lee Szeto, J.W. Li, A.S. Genshaft, S.W. Kazer, K.R. Payer, J. de Riba Borrajo, P.C. Blainey, D.J. Irvine, A.K. Shalek, S.R. Manalis, A microfluidic platform enabling single-cell RNA-seq of multigenerational lineages, Nature Communications, 7 (2016) 10220.

[4] H.A. Pohl, J.S. Crane, Dielectrophoresis of Cells, Biophysical Journal, 11 (1971) 711-727.

[5] M. Elitas, N. Dhar, K. Schneider, A. Valero, T. Braschler, J.D. McKinney, P. Renaud, Dielectrophoresis as a single cell characterization method for bacteria, Biomedical Physics & Engineering Express, 3 (2017) 015005.

[6] M. Elitas, R. Martinez-Duarte, N. Dhar, J.D. McKinney, P. Renaud, Dielectrophoresis-based purification of antibiotic-treated bacterial subpopulations, Lab on a Chip, 14 (2014) 1850-1857.

[7] P.R.C. Gascoyne, S. Shim, Isolation of Circulating Tumor Cells by Dielectrophoresis, Cancers, 6 (2014) 545-579.

[8] R. Pethig, A. Menachery, S. Pells, P. De Sousa, Dielectrophoresis: A Review of Applications for Stem Cell Research, Journal of Biomedicine and Biotechnology, 2010 (2010).

[9] J. Chen, C. Xue, Y. Zhao, D. Chen, M.-H. Wu, J. Wang, Microfluidic Impedance Flow Cytometry Enabling High-Throughput Single-Cell Electrical Property Characterization, International Journal of Molecular Sciences, 16 (2015).

[10] V. Kiermer, FACS-on-a-chip, Nat Meth, 2 (2005) 91-91.

[11] D.W. Inglis, R. Riehn, J.C. Sturm, R.H. Austin, Microfluidic high gradient magnetic cell separation, Journal of Applied Physics, 99 (2006) 08K101.

[12] M.E. Warkiani, L. Wu, A.K.P. Tay, J. Han, Large-Volume Microfluidic Cell Sorting for Biomedical Applications, Annual Review of Biomedical Engineering, 17 (2015) 1-34.

[13] C.W. Shields, C.D. Reyes, G.P. López, Microfluidic Cell Sorting: A Review of the Advances in the Separation of Cells from Debulking to Rare Cell Isolation, Lab on a chip, 15 (2015) 1230-1249.

[14] N. Xia, T.P. Hunt, B.T. Mayers, E. Alsberg, G.M. Whitesides, R.M. Westervelt, D.E. Ingber, Combined microfluidic-micromagnetic separation of living cells in continuous flow, Biomedical Microdevices, 8 (2006) 299.

[15] M. Kirschbaum, M.S. Jaeger, T. Schenkel, T. Breinig, A. Meyerhans, C. Duschl, T cell activation on a single-cell level in dielectrophoresis-based microfluidic devices, Journal of Chromatography A, 1202 (2008) 83-89.

[16] E.B. Cummings, A.K. Singh, Dielectrophoresis in Microchips Containing Arrays of Insulating Posts: Theoretical and Experimental Results, Analytical Chemistry, 75 (2003) 4724-4731.

[17] H. Shafiee, J.L. Caldwell, M.B. Sano, R.V. Davalos, Contactless dielectrophoresis: a new technique for cell manipulation, Biomedical Microdevices, 11 (2009) 997.

[18] A. Salmanzadeh, H. Kittur, M.B. Sano, P. C. Roberts, E.M. Schmelz, R.V. Davalos, Dielectrophoretic differentiation of mouse ovarian surface epithelial cells, macrophages, and fibroblasts using contactless dielectrophoresis, Biomicrofluidics, 6 (2012) 024104-024104-024113.

[19] B. Yafouz, N.A. Kadri, F. Ibrahim, A numerical analysis of electric field strength over planar microarray dot electrode for dielectrophoretic lab-on-chip device, 2012 IEEE-EMBS Conference on Biomedical Engineering and Sciences, 2012, pp. 118-121.

[20] M.C. Yousuff, T.E. Ho, I. Hussain K, H.N. Hamid, Microfluidic Platform for Cell Isolation and Manipulation Based on Cell Properties, Micromachines, 8 (2017).

[21] M. Sancho, G. Martínez, S. Muñoz, J.L. Sebastián, R. Pethig, Interaction between cells in dielectrophoresis and electrorotation experiments, Biomicrofluidics, 4 (2010) 022802.

[22] D.G. Andrew, P.H.B. Julian, P. Ronald, A combined travelling wave dielectrophoresis and electrorotation device: applied to the concentration and viability determination of Cryptosporidium, Journal of Physics D: Applied Physics, 30 (1997) L65.

[23] R. Pethig, Review Article—Dielectrophoresis: Status of the theory, technology, and applications, Biomicrofluidics, 4 (2010) 022811.

[24] K.L. Chan, P.R.C. Gascoyne, F.F. Becker, R. Pethig, Electrorotation of liposomes: verification of dielectric multi-shell model for cells, Biochimica et Biophysica Acta (BBA) - Lipids and Lipid Metabolism, 1349 (1997) 182-196.

[25] A. Valero, T. Braschler, P. Renaud, A unified approach to dielectric single cell analysis: Impedance and dielectrophoretic force spectroscopy, Lab on a Chip, 10 (2010) 2216-2225.

[26] K.R. Foster, F.A. Sauer, H.P. Schwan, Electrorotation and levitation of cells and colloidal particles, Biophysical Journal, 63 (1992) 180-190.

[27] P.R.C. Gascoyne, X.-B. Wang, Y. Huang, F.F. Becker, Dielectrophoretic Separation of Cancer Cells from Blood, IEEE transactions on industry applications, 33 (1997) 670-678.

[28] J. Yang, Y. Huang, X. Wang, X.-B. Wang, F.F. Becker, P.R.C. Gascoyne, Dielectric Properties of Human Leukocyte Subpopulations Determined by Electrorotation as a Cell Separation Criterion, Biophysical Journal, 76 (1999) 3307-3314.

[29] K. Khoshmanesh, J. Akagi, S. Nahavandi, J. Skommer, S. Baratchi, J.M. Cooper, K. Kalantar-Zadeh, D.E. Williams, D. Wlodkowic, Dynamic Analysis of Drug-Induced Cytotoxicity Using Chip-Based Dielectrophoretic Cell Immobilization Technology, Analytical Chemistry, 83 (2011) 2133-2144.

[30] D.M. Vykoukal, P.R.C. Gascoyne, J. Vykoukal, Dielectric characterization of complete mononuclear and polymorphonuclear blood cell subpopulations for label-free discrimination, Integrative Biology, 1 (2009) 477-484.

[31] S. Bhattacharya, T.-C. Chao, N. Ariyasinghe, Y. Ruiz, D. Lake, R. Ros, A. Ros, Selective trapping of single mammalian breast cancer cells by insulator-based dielectrophoresis, Analytical and Bioanalytical Chemistry, 406 (2014) 1855-1865.

[32] P. Gascoyne, J. Satayavivad, M. Ruchirawat, Microfluidic approaches to malaria detection, Acta Tropica, 89 (2004) 357-369.

[33] Y. Polevaya, I. Ermolina, M. Schlesinger, B.-Z. Ginzburg, Y. Feldman, Time domain dielectric spectroscopy study of human cells: II. Normal and malignant white blood cells, Biochimica et Biophysica Acta (BBA) - Biomembranes, 1419 (1999) 257-271.

[34] A.M. Luke, D.B. Michael, R.W. Michael, Mechanical properties of mammalian cells in suspension measured by electro-deformation, Journal of Micromechanics and Microengineering, 20 (2010) 065007.

[35] D.L. Wiest, J.K. Burkhardt, S. Hester, M. Hortsch, D.I. Meyer, Y. Argon, Membrane biogenesis during B cell differentiation: most endoplasmic reticulum proteins are expressed coordinately, J Cell Biol, 110 (1990) 1501-1511.

[36] R.J. Sokol, G. Hudson, J. Wales, N.T. James, Morphometry of blood monocytes in malignant lymphoma, Journal of Clinical Pathology, 38 (1985) 904.

[37] L. Guillou, A. Babataheri, M. Saitakis, A. Bohineust, S. Dogniaux, C. Hivroz, A.I. Barakat, J. Husson, T lymphocyte passive deformation is controlled by unfolding of membrane surface reservoirs, Molecular Biology of the Cell, (2016).

[38] M. Herant, V. Heinrich, M. Dembo, Mechanics of neutrophil phagocytosis: behavior of the cortical tension, Journal of Cell Science, 118 (2005) 1789.