INTEGRATED DUAL NANOPROBE-MICROFLUIDIC SYSTEM FOR SINGLE CELL ELECTRICAL PROPERTY CHARACTERIZATIONS

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This thesis is dedicated to my beloved parents,

Hajah Zakinah Binti Ibrahim and Haji Mat Sulaiman Bin Jaya.

Thanks for always being there for me.

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ABSTRACT

This thesis presents the simulation of an integrated dual nanoprobe-microfluidic system for single cell electrical characterizations. Recent cell studies have shown a possible early disease diagnosis at a single cell level by characterizing its electrical properties. One of the studies uses a dual nanoprobe showing its ability in quantitatively detecting single cell viability. However, this method has low throughput rate, high skilled labour requirement and bulky system. We propose an improved system that overcomes these limitations. This study is divided into five stages. The first stage focuses on deciding the system concept and nanoprobe design. The second stage involves nanoprobe characterization which is based on electrical and mechanical properties of five different materials: Silver, Copper, Aluminium, Tungsten and Zinc. The third stage is a single cell modeling of *Saccharomyces cerevisiae* for mechanical and electrical model. The fourth stage is nanoprobe integration with microfluidic system. The final stage is single cell electrical property characterizations. From the study, several findings were obtained and concluded. First, the most preferred material for nanoprobe is Tungsten which has low electrical resistance of 5.5 Ω and can withstand an external force up to 35.6 µN before failure. Second, the two layers cell model was validated by displaying a close agreement in terms of penetration force (640 nN) with experimental data. Third, successful cell penetration was achieved at 5.1 pl/min flow rate in 4 µm diameter micro channel. Lastly, insulating the nanoprobe reduces the effect of penetration depth on the current measurement and enables the characterization of single cell cytoplasm electrical conductivity to be realized. Currently the developed system is suitable for cell viability detection application. Furthermore, this system has a potential to be used in single cell thermal measurement, single cell drug delivery and early disease diagnosis.

ABSTRAK

Tesis ini membentangkan penyelakuan sistem dwi-nanoprob mikrofluidik bersepadu bagi pencirian elektrik sel tunggal. Kajian terbaru menunjukkan penyakit boleh dikesan pada peringkat awal hasil daripada pencirian sifat elektrik sel tunggal tersebut. Salah satu kajian tersebut menggunakan dwi nanoprob dual dalam mengesan daya maju sel tunggal secara kuantitatif. Walau bagaimanapun, kaedah ini mempunyai kelemahan daripada segi kadar pengesanan yang rendah, keperluan buruh yang mahir, dan sistem yang sukar digerakkan. Kami mencadangkan satu sistem yang dapat mengatasi batasan-batasan ini. Kajian ini dibahagikan kepada lima peringkat. Peringkat pertama memberi tumpuan kepada menentukan konsep sistem dan reka bentuk nanoprob. Peringkat kedua melibatkan pencirian nanoprob berdasarkan sifat-sifat elektrik dan mekanikal lima bahan yang berbeza: Perak, Tembaga, Aluminium, Tungsten dan Zink. Peringkat ketiga adalah memodelkan sel tunggal *Saccharomyces cerevisiae* untuk model mekanikal dan elektrik. Peringkat keempat ialah persepaduan nanoprob dengan sistem mikrofluidik. Peringkat akhir adalah pencirian sifat elektrik sel tunggal. Dari kajian ini, beberapa penemuan telah diperolehi dan disimpulkan. Pertama, bahan yang paling sesuai untuk nanoprobe adalah Tungsten kerana mempunyai rintangan elektrik yang rendah iaitu sebanyak 5.5 Ω dan boleh menahan daya beban sehingga 35.6 μN. Kedua, model sel lapisan telah disahkan dengan memaparkan kuasa penembusan yang sama dengan data eksperimen iaitu 640 nN. Ketiga, penembusan sel berjaya dilakukan pada kadar aliran 5.1 pl / min dalam 4 μm diameter saluran mikro. Akhir sekali, nanoprob yang disaluti penebat dapat digunakan untuk mencirikan kekonduksian elektrik sitoplasma sel. Pada masa ini sistem yang dibangunkan sesuai bagi aplikasi pengesanan daya maju sel. Sistem ini juga mempunyai potensi untuk digunakan dalam pengukuran haba sel tunggal, penghantaran ubat sel tunggal, dan mengesan penyakit diperingkat awal.

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LIST OF SYMBOLS

U, V	–	Voltage
I	–	Current
R	–	Resistance
Re	–	Real part of a complex number
Im	–	Imaginary part of a complex number
ω, f	–	Frequency ($\omega = 2\pi f$)
Z	–	Impedance
C	–	Capacitance
G	–	Conductance
$\boldsymbol{F}$	–	Force
E	–	Electric field
$\boldsymbol{r}$	–	Radius
η	–	Viscosity
$\boldsymbol{K}$	–	Scaling factor
$\boldsymbol{\sigma}$	–	Conductivity
ρ	–	Resistivity
ε	–	Permittivity
Φ	–	Volume fraction
f_{CM}	–	Claus-Mossotti factor
$\boldsymbol{k}$	–	Spring constant
$\boldsymbol{\delta}$	–	Deflection
$\boldsymbol{Q}$	–	Flow rate
$\boldsymbol{v}$	–	Velocity
ϑ	–	Cell constant

LIST OF ABBREVIATIONS

HNC	–	Head and neck cancer
AC	–	Alternating current
DC	–	Direct current
ECM	–	Equivalent circuit model
ROT	–	Electrorotation
DEP	–	Dielectrophoresis
pDEP	–	Positive Dielectrophoresis
nDEP	–	Negative Dieletrophoresis
MEMS	–	Micro-Electrical Mehanical System
E-SEM	–	Environmental-Scanning Electron Microscopy
FEM	–	Finite Element Method
FEA	–	Finite Element Analysis
LOC	–	Lab on chip
FIB	–	Focus ion beam
PDMS	–	Polydiemethylsiloxanne
EIS	–	Electrochemical Impedance Spectroscopy
ECD	–	Current density

CHAPTER 1

INTRODUCTION

1.1 Background of Research

Single cell analysis has gained researchers attention in microbiological studies thanks to the rapid development in nanotechnology. The cells are now being studied individually and not only based on populations of cells. The main advantage of single cell analysis over population analysis is accuracy. Population studies unable to characterize individual cell accurately as the result obtained is based only on average data. Each cell may have unique properties which could be used as a marker for cell type classification. Each cell type is expected can be differentiated from one another if their individual properties, i.e. mechanical, electrical, and chemical, can be characterized. This information is important in early disease detection applications. Beside mechanical properties [1-9], cells can also be characterized based on the electrical properties [10].

1.2 Single Cell Characterization based on Electrical Properties

In recent years, studies on single cell analysis have been focusing on characterizing the cells electrical properties, i.e. resistance, capacitance, dielectric constant, and conductivity [11-16]. Some of the researches have shown their potential in a practical applications i.e. single cell viability detection and single cell cancer detection.

1.2.1 Single Cell Viability Application

One of the applications of single cell electrical property measurement can be seen in single cell viability detection [17]. Figure 1.1 shows a single cell electrical measurement results in detecting single cell viability. Cell viability is a determination of living or dead cells. It is very important in biological studies, especially when researchers try to manipulate cells in a cellular suspension which either the cells grown on a substrate or cells that have been removed from the body. This unnatural environment can cause certain types of cell to die.

Without knowing the cell viability a research cannot determine a valid result. In other words, before cell manipulation is carried out the cell need to be known in terms of viability for the result to be valid. During cell manipulation, the cell viability could also be affected. This cell manipulation can be categorized into different types such as mechanical, chemical, and electrical. So, it is also important to know the cell viability after cell manipulation.

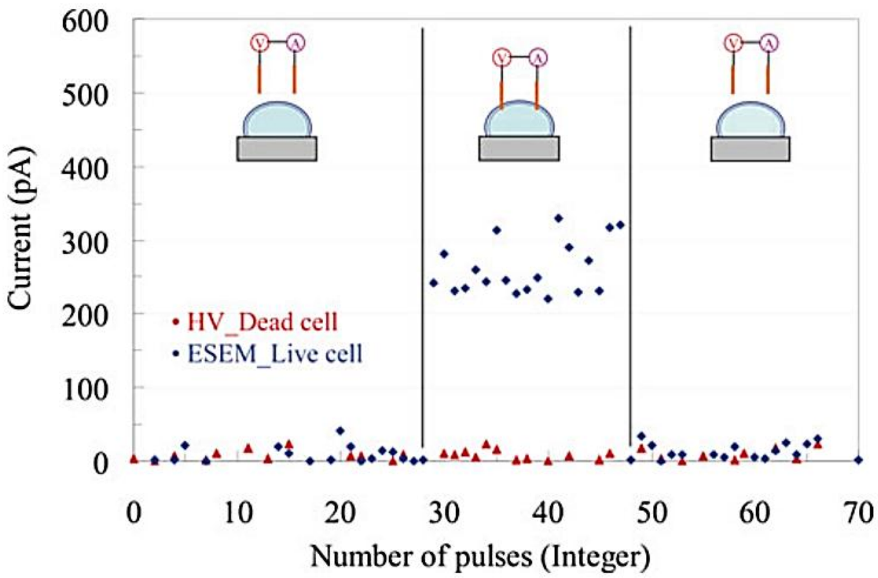


Figure 1.1 Single cell viability detection [17].

The conventional method of cell viability detection uses colorimetric or fluorescent dyes. This method lacks the capability to produce instantaneous and quantitative cell viability information which is important in the study of dynamics of cell death [18]. Thanks to the new technology, cell viability is no longer determined through chemical reaction but based on the electrical properties. This method is much better in terms of producing instantaneous and quantitative information.

1.2.2 Single Cell Cancer Detection Application

A more practical study has shown a potential application of single cell electrical properties characterization in detecting cancer cells [19, 20]. The human body is made of several types of cell and each one of them has their own function. Figure 1.2 shows several types of cell in the human body. However, certain cell may mutate into another form of cell type; cancer cell, and affect the human health and may lead to death without treatment. Hence, it is important to detect and cure the sick cells or remove them before they can give harm to the human body.

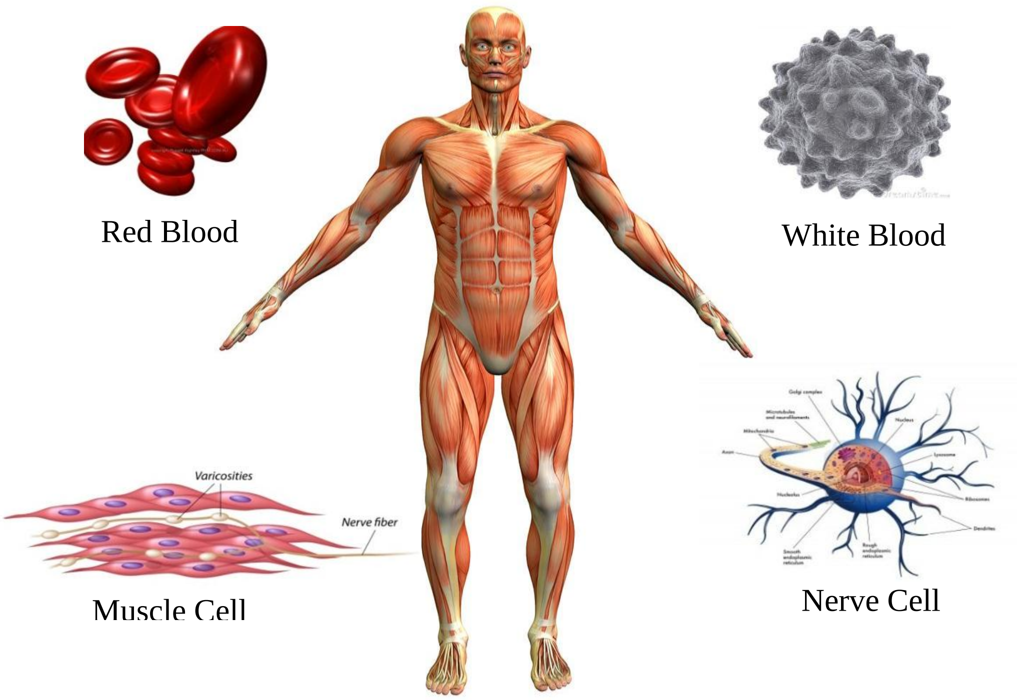


Figure 1.2 Cell types in human body.

For example, patients with blood cancer are fortunate if their illness is detected at an early stage. However, chance for patient survival decreases when the illness is detected too late. Blood cancer is caused by abnormal growth and function of blood cells, i.e. red blood cells and white blood cells. It is possible to detect cancer if we are able to characterize the cell's properties and used the information obtained to differentiate between cancer cells with normal cells.

Figure 1.3 shows the electrical measurement results for head and neck cancer (HNC) cell for different cancer phase [19]. The results showed significant changes in electrical properties of a cancer cells which may hold the answer for early disease detection for HNC.

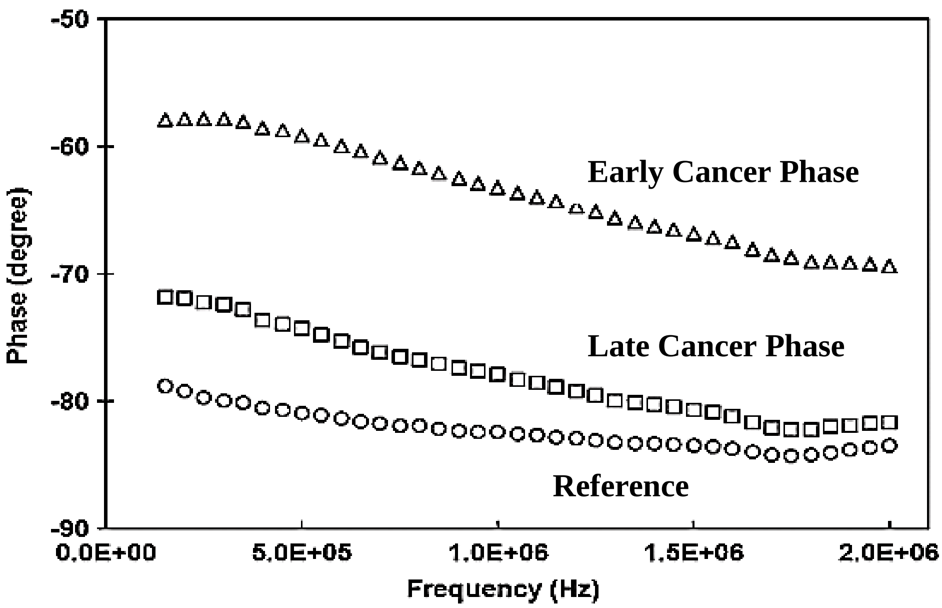


Figure 1.3 Single cell electrical measurements for different cancer phase [19].

1.3 Problem Statement

Even though the single cell electrical measurement devices have already been invented, most of the devices have several disadvantages, i.e. labour intensive and bulky system. These disadvantages make the single cell electrical measurement ineffective, low throughput rate (inefficient), and costly.

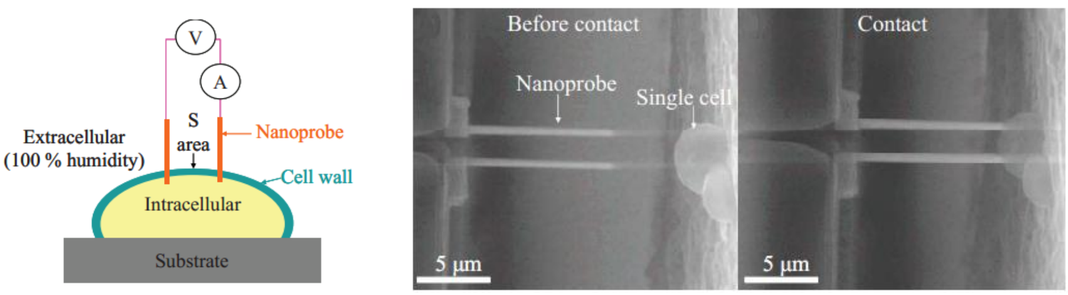
Operator skills play an important role when manual measurement is being conducted especially for the type of instrument that requires a direct contact with the single cell in micrometer scale, e.g. dual nanoprobe [3, 13, 17, 21]. When a new operator is needed, they need to be intensively trained and familiarize with the technique in order to ensure an accurate and reliable measurement results. Operators skills can be gained through experience or sending them to a training program but both of them take times and costly. Easy equipment handling or minimum operator needs is favourable to those who need to focus more on the study rather than spending more time to learn new equipment or technique.

High number of data can provide convincing and accurate results to a study finding. However, low throughput rate measurement require more time in obtaining more data and it is become a disadvantage for a study on a single cell type that has short life cycle, i.e. yeast cell [22]. Currently, the throughput rate is depending on the operator ability. Therefore, measurement throughput rate improvement is limited. In a repetitive measurement, the operator may not be able to keep their instrument handling consistency at a long period of time due to fatigue and induce human error. For high sensitivity measurement type, even a slight error may pose a significant effect to the measurement results.

Bulky system makes the single cell measurement less portable and costly to be built. The general type system or bulky system may suitable to be use at initial stage of the research but for other user to be able to use the same technique, the system need to be transform into a specific type measurement system. The transformation will improve the system by reducing the size of the system, cost, and more portable. This way, more users will be interested to use them as one of their research tools. Therefore, there is a need to reduce the operator role in single cell measurement for a consistent measurement accuracy and higher throughput rate and also improve the device from bulky system to a more portable system. In the end, single cell electrical measurement can be conducted not only by inexperienced operator but also under none specific lab environment.

1.4 Purpose of the Study

The purpose of the study is to improve the single cell electrical measurement based on dual nanoprobe technique proposed by M. R. Ahmad et al. in 2009 [13]. Figure 1.4 shows how the dual nanoprobe is used for single cell electrical measurement. Generally, our research aims to integrate the dual nanoprobe with a microfluidic system for reducing the requirement for labour skills, higher throughput rate and portable system. The new platform will replace the unnecessary bulky system. Our proposed system will be used to gain new findings or aid other research regarding cell studies, and researchers will be able to focus more on the study without the need to use complex equipment for advanced measurement. There are many applications prior to this study such as cancer cell detection, drug delivery, cell manipulation and others. Hence, more and more research can be done in the same period of time than before.



(a) **(b)**

Figure 1.4 Single cell electrical measurements using dual nanoprobe [13] (a) schematic diagram of the technique (b) experimental measurement.

1.5 Objectives of the Study

The objectives of this research are;

1) To design, characterize and optimize the dual nanoprobe for single cell analysis.
2) To design a microfluidic system to be integrated with the dual nanoprobe.
3) To implement the integrated dual nanoprobe-microfluidic system for single cells electrical property measurement.

1.6 Scope of the Study

1. The research will be carried out mostly through simulation using finite element analysis software, ABAQUS.
2. In the simulation the cell model is based on Yeast cell and the parameters involved will be obtained from experiment and journal papers.
3. Only the cell's cytoplasm electrical properties will be measured.

1.7 Organization of Thesis

This thesis is divided into 7 chapters. First chapter discusses on the significance of the research including research background, problem statement, objectives, and scopes. In the second chapter, single cell electrical measurement techniques and theory are being thoroughly discussed. Third chapter discusses on the research methodology been used in the study and explains on the system concept and nanoprobe design. The forth chapter discusses on the nanoprobe characterization for both electrical and mechanical properties using five different materials i.e. Silver, Copper, Aluminium, Tungsten, and Zinc. The fifth chapter explains on single cell simulation model of *Saccharomyces cerevisiae* for mechanical and electrical model. The sixth chapter discusses on nanoprobe integration with microfluidic system and single cell electrical property characterization.

The last chapter is the conclusion and future recommendations for this research. Each chapter has its own objective and the results obtained are directly discussed.

1.8 Summary of Works

Work flow on system development during research is summarized in the flow chart as shows in Figure 1.5.

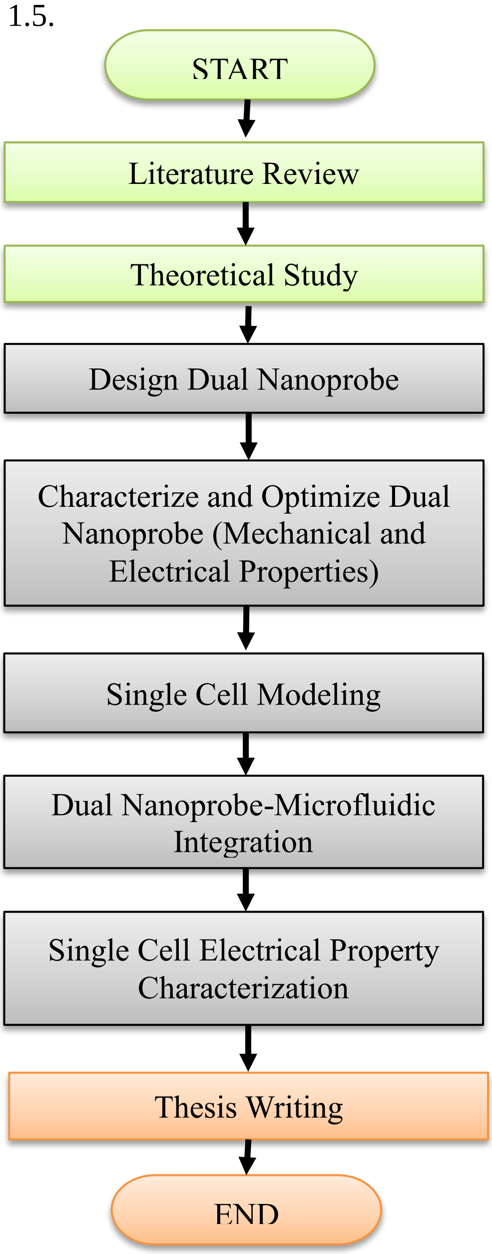


Figure 1.5 Research development work flow.

REFERENCES

[1] M. R. Ahmad, M. Nakajima, M. Kojima, S. Kojima, M. Homma, and T. Fukuda, "Nanofork for Single Cells Adhesion Measurement via ESEM-Nanomanipulator System," *IEEE Transactions on NanoBioscience,* vol. 11, pp. 70-78, 2012.

[2] M. R. Ahmad, M. Nakajima, S. Kojima, M. Homma, and T. Fukuda, "Buckling nanoneedle for characterizing single cells mechanics inside environmental SEM," in *Nanotechnology Materials and Devices Conference, 2009. NMDC '09. IEEE,* 2009, pp. 27-32.

[3] M. R. Ahmad, M. Nakajima, S. Kojima, M. Homma, and T. Fukuda, "Nanoindentation Methods to Measure Viscoelastic Properties of Single Cells Using Sharp, Flat, and Buckling Tips Inside ESEM," *IEEE Transactions on NanoBioscience,* vol. 9, pp. 12-23, 2010.

[4] D. J. Müller and Y. F. Dufrêne, "Force nanoscopy of living cells," *Current Biology,* vol. 21, pp. R212-R216, 2011.

[5] A. Suchodolskis, V. Feiza, A. Stirke, A. Timonina, A. Ramanaviciene, and A. Ramanavicius, "Elastic properties of chemically modified baker's yeast cells studied by AFM," *Surface and Interface Analysis,* vol. 43, pp. 1636-1640, 2011.

[6] E.-Y. Kwon, Y.-T. Kim, and D.-E. Kim, "Investigation of penetration force of living cell using an atomic force microscope," *Journal of Mechanical Science and Technology,* vol. 23, pp. 1932-1938, 2009.

[7] I. Obataya, C. Nakamura, S. Han, N. Nakamura, and J. Miyake, "Mechanical sensing of the penetration of various nanoneedles into a living cell using atomic force microscopy," *Biosensors and Bioelectronics,* vol. 20, pp. 1652-1655, 2005.

[8] A. E. Pelling, S. Sehati, E. B. Gralla, J. S. Valentine, and J. K. Gimzewski, "Local Nanomechanical Motion of the Cell Wall of Saccharomyces cerevisiae," *Science,* vol. 305, pp. 1147-1150, 2004.

[9] A. David, D. Vincent, E. Kevin Mc, W. Linda, J. G. Hermann, and F. D. Yves, "Structure, cell wall elasticity and polysaccharide properties of living yeast cells, as probed by AFM," *Nanotechnology,* vol. 19, p. 384005, 2008.

[10] H. P. Schwan, "Electrical and Acoustic Properties of Biological Materials and Biomedical Applications," *Biomedical Engineering,* vol. 31, pp. 873-877, 1984.

[11] Y. Zheng, E. Shojaei-Baghini, C. Wang, and Y. Sun, "Microfluidic characterization of specific membrane capacitance and cytoplasm conductivity of single cells," *Biosensors and Bioelectronics*, vol. 42, pp. 496-502, 2013.

[12] J. Chen, Y. Zheng, Q. Tan, Y. L. Zhang, J. Li, W. R. Geddie, *et al.*, "A microfluidic device for simultaneous electrical and mechanical measurements on single cells," *Biomicrofluidics*, vol. 5, p. 014113, 2011.

[13] M. R. Ahmad, M. Nakajima, T. Fukuda, S. Kojima, and M. Homma, "Single cells electrical characterizations using nanoprobe via ESEM-nanomanipulator system," in *9th IEEE Conference on Nanotechnology*, 2009, pp. 589-592.

[14] K. Asami, "Characterization of biological cells by dielectric spectroscopy," *Journal of Non-Crystalline Solids*, vol. 305, pp. 268-277, 2002.

[15] J. L. Sebastian Franco, A. Sanchis Otero, J. R. Madronero, and S. M. S. Martin, "Dielectric Characterization of the Yeast Cell Budding Cycle," *Progress in Electromagnetics Research*, vol. 134, pp. 1-22, 2013.

[16] L. Chia-Feng, J. Jen-Yu, C. Ming-Kun, C. Ya-Chun, W. Pin-Chian, and J. Ling-Sheng, "Single cell impedance analysis and electrical characterization in micro-fluidic device," in *2011 IEEE International Conference on Nano/Micro Engineered and Molecular Systems (NEMS)*, 2011, pp. 121-126.

[17] M. R. Ahmad, M. Nakajima, M. Kojima, S. Kojima, M. Homma, and T. Fukuda, "Instantaneous and Quantitative Single Cells Viability Determination Using Dual Nanoprobe Inside ESEM," *IEEE Transactions on Nanotechnology*, vol. 11, pp. 298-306, 2012.

[18] B. Rubinsky and Y. Huang, "Cell Viability Detection using Electrical Measurement," US Patent US 6,927,049 B2, 2005.

[19] Y. Cho, K. Hyun Soo, A. Bruno Frazier, Z. G. Chen, S. Dong Moon, and A. Han, "Whole-Cell Impedance Analysis for Highly and Poorly Metastatic Cancer Cells," *Journal of Microelectromechanical Systems*, vol. 18, pp. 808-817, 2009.

[20] Y. H. Cho, T. Yamamoto, Y. Sakai, T. Fujii, and K. Beomjoon, "Development of microfluidic device for electrical/physical characterization of single cell," *Journal of Microelectromechanical Systems*, vol. 15, pp. 287-295, 2006.

[21] M. R. Ahmad, M. Nakajima, S. Kojima, M. Homma, and T. Fukuda, "In Situ Single Cell Mechanics Characterization of Yeast Cells Using Nanoneedles Inside Environmental SEM," *IEEE Transactions on Nanotechnology*, vol. 7, pp. 607-616, 2008.

[22] A. K. Bryan, A. Goranov, A. Amon, and S. R. Manalis, "Measurement of mass, density, and volume during the cell cycle of yeast," *Proceedings of the National Academy of Sciences*, 2009.

[23] J. Chen, J. Li, and Y. Sun, "Microfluidic Approaches for Cancer Cell Detection, Characterization, and Separation," *Lab on a Chip,* vol. 12, pp. 1753-1767, 2012.

[24] H. Fricke, "The Electric Capacity of Suspensions with Special Reference to Blood," *The Journal of General Physiology*, vol. 9, pp. 137-152, 1925.

[25] M. R. Ahmad, M. Nakajima, S. Kojima, M. Homma, and T. Fukuda, "The Effects of Cell Sizes, Environmental Conditions, and Growth Phases on the Strength of Individual W303 Yeast Cells Inside ESEM," *IEEE Transactions on NanoBioscience*, vol. 7, pp. 185-193, 2008.

[26] K. Asami, T. Hanai, and N. Koizumi, "Dielectric properties of yeast cells," *Journal of Membrane Biology,* vol. 28, pp. 169-180, 1976.

[27] M. Hywel, S. Tao, H. David, G. Shady, and G. G. Nicolas, "Single cell dielectric spectroscopy," *Journal of Physics D: Applied Physics,* vol. 40, pp. 61-70, 2007.

[28] T. B. Jones, *Electromechanics of Particles*: Cambridge University Press, 1995.

[29] M. Thein, F. Asphahani, A. Cheng, R. Buckmaster, M. Zhang, and J. Xu, "Response characteristics of single-cell impedance sensors employed with surface-modified microelectrodes," *Biosensors and Bioelectronics,* vol. 25, pp. 1963-1969, 2010.

[30] H. Fricke, "A Mathematical Treatment of the Electrical Conductivity of Colloids and Cell Suspensions," *The Journal of General Physiology* vol. 6, pp. 375-384, 1924.

[31] T. Noll and M. Biselli, "Dielectric spectroscopy in the cultivation of suspended and immobilized hybridoma cells," *Journal of Biotechnology,* vol. 63, pp. 187-198, 1998.

[32] R. Hölzel, "Electrorotation of Single Yeast Cells at Frequencies Between 100 Hz and 1.6 GHz," *Biophysical Journal,* vol. 73, pp. 1103–1109, 1997.

[33] C. Dalton, A. D. Goater, J. P. H. Burt, and H. V. Smith, "Analysis of parasites by electrorotation," *Journal of Applied Microbiology,* vol. 96, pp. 24-32, 2004.

[34] G. Fuhr and P. I. Kuzmin, "Behavior of Cells in Rotating Electric Fields with Account to Surface Charges and Cell Structures," *Biophysical Journal,* vol. 50, pp. 789-795, 1986.

[35] Y. Huang, R. Holzel, R. Pethig, and B. W. Xiao, "Differences in the AC electrodynamics of viable and non-viable yeast cells determined through combined dielectrophoresis and electrorotation studies," *Physics in Medicine and Biology,* vol. 37, p. 1499, 1992.

[36] J. Gimsa, T. Müller, T. Schnelle, and G. Fuhr, "Dielectric spectroscopy of single human erythrocytes at physiological ionic strength: dispersion of the cytoplasm," *Biophysical Journal,* vol. 71, pp. 495-506, 1996.

[37] J. P. Huang and K. W. Yu, "First-principles approach to electrorotation assay," *Journal of Physics: Condensed Matter,* vol. 14, p. 1213, 2002.

[38] M. Sancho, G. Martínez, and C. Martín, "Accurate dielectric modelling of shelled particles and cells," *Journal of Electrostatics,* vol. 57, pp. 143-156, 2003.

[39] P. R. C. Gascoyne, F. F. Becker, and X. B. Wang, "Numerical analysis of the influence of experimental conditions on the accuracy of dielectric parameters derived from electrorotation measurements," *Bioelectrochemistry and Bioenergetics,* vol. 36, pp. 115-125, 1995.

[40] S.-I. Han, Y.-D. Joo, and K.-H. Han, "An electrorotation technique for measuring the dielectric properties of cells with simultaneous use of negative quadrupolar dielectrophoresis and electrorotation," *Analyst,* vol. 138, pp. 1529-1537, 2013.

[41] N. G. Green and H. Morgan, "Dielectrophoresis of Submicrometer Latex Spheres. 1. Experimental Results," *The Journal of Physical Chemistry B,* vol. 103, pp. 41-50, 1998.

[42] F. F. Becker, X. B. Wang, Y. Huang, R. Pethig, J. Vykoukal, and P. R. Gascoyne, "Separation of human breast cancer cells from blood by differential dielectric affinity," *Proceedings of the National Academy of Sciences,* vol. 92, pp. 860-864, 1995.

[43] S. K. Mohanty, S. K. Ravula, K. L. Engisch, and A. B. Frazier, "A micro system using dielectrophoresis and electrical impedance spectroscopy for cell manipulation and analysis," in *12th International Conference on Transducers, Solid-State Sensors, Actuators and Microsystems,* , 2003, pp. 1055-1058.

[44] R. Pethig, "Review Article---Dielectrophoresis: Status of the theory, technology, and applications," *Biomicrofluidics,* vol. 4, pp. 022811-35, 2010.

[45] Z. Zhu, O. Frey, D. S. Ottoz, F. Rudolf, and A. Hierlemann, "Microfluidic single-cell cultivation chip with controllable immobilization and selective release of yeast cells," *Lab on a Chip,* vol. 12, pp. 906-915, 2012.

[46] P. R. C. Gascoyne and J. Vykoukal, "Particle separation by dielectrophoresis," *ELECTROPHORESIS,* vol. 23, pp. 1973-1983, 2002.

[47] K. G. Klemic, J. F. Klemic, M. A. Reed, and F. J. Sigworth, "Micromolded PDMS planar electrode allows patch clamp electrical recordings from cells," *Biosensors and Bioelectronics,* vol. 17, pp. 597-604, 2002.

[48] A. Stett, V. Bucher, C. Burkhardt, U. Weber, and W. Nisch, "Patch-clamping of primary cardiac cells with micro-openings in polyimide films," *Medical and Biological Engineering and Computing,* vol. 41, pp. 233-240, 2003.

[49] S. Pandey, R. Mehrotra, S. Wykosky, and M. H. White, "Characterization of a MEMS BioChip for planar patch-clamp recording," *Solid-State Electronics,* vol. 48, pp. 2061-2066, 2004.

[50] R. Pantoja, J. M. Nagarah, D. M. Starace, N. A. Melosh, R. Blunck, F. Bezanilla, *et al.*, "Silicon chip-based patch-clamp electrodes integrated with PDMS microfluidics," *Biosensors and Bioelectronics*, vol. 20, pp. 509-517, 2004.

[51] C. Ionescu-Zanetti, R. M. Shaw, J. Seo, Y.-N. Jan, L. Y. Jan, and L. P. Lee, "Mammalian electrophysiology on a microfluidic platform," *Proceedings of the National Academy of Sciences of the United States of America,* vol. 102, pp. 9112-9117, 2005.

[52] C. Chen and A. Folch, "A high-performance elastomeric patch clamp chip," *Lab on a Chip,* vol. 6, pp. 1338-1345, 2006.

[53] B. Matthews and J. W. Judy, "Design and fabrication of a micromachined planar patch-clamp substrate with integrated microfluidics for single-cell measurements," *Journal of Microelectromechanical Systems,* vol. 15, pp. 214-222, 2006.

[54] W.-L. Ong, J.-S. Kee, A. Ajay, N. Ranganathan, K.-C. Tang, and L. Yobas, "Buried microfluidic channel for integrated patch-clamping assay," *Applied Physics Letters*, vol. 89, p. 093902, 2006.

[55] T. Lehnert, D. M. T. Nguyen, L. Baldi, and M. A. M. Gijs, "Glass reflow on 3-dimensional micro-apertures for electrophysiological measurements on-chip," *Microfluidics and Nanofluidics,* vol. 3, pp. 109-117, 2007.

[56] S. Li and L. Lin, "A single cell electrophysiological analysis device with embedded electrode," *Sensors and Actuators A: Physical,* vol. 134, pp. 20-26, 2007.

[57] W.-L. Ong, K.-C. Tang, A. Agarwal, R. Nagarajan, L.-W. Luo, and L. Yobas, "Microfluidic integration of substantially round glass capillaries for lateral patch clamping on chip," *Lab on a Chip,* vol. 7, pp. 1357-1366, 2007.

[58] A. H. M. Sulaiman and M. R. Ahmad, "Modeling and simulation of novel method of single cell viability detection via electrical measurement using dual nanoprobes," in *2012 International Conference on Enabling Science and Nanotechnology (ESciNano)*, 2012, pp. 1-2.

[59] A. H. Mat Sulaiman and M. R. Ahmad, "Electrical and mechanical characterizations of dual nanoprobe for potential application of single cells analysis," in *2012 IEEE EMBS Conference on Biomedical Engineering and Sciences (IECBES),* , 2012, pp. 404-407.

[60] S. Yajing, M. Nakajima, S. Kojima, M. Homma, and T. Fukuda, "Nano knife fabrication and calibration for single cell cutting inside environmental SEM," in *2010 International Symposium on Micro-NanoMechatronics and Human Science (MHS),* 2010, pp. 316-320.

[61] M. Rahman, A. Mat Sulaiman, M. Ahmad, and T. Fukuda, "Finite Element Analysis of Single Cell Wall Cutting by Piezoelectric-actuated Vibrating Rigid Nanoneedle," *IEEE Transactions on Nanotechnology, ,* vol. 12, pp. 1158-1165, 2013.

[62] E. Zampetti, S. Pantalei, A. Pecora, A. Valletta, L. Maiolo, A. Minotti*, et al.*, "Design and optimization of an ultra thin flexible capacitive humidity sensor," *Sensors and Actuators B: Chemical,* vol. 143, pp. 302-307, 2009.

[63] Ganesh Thiagarajan, Kavita Deshmukh, Yong Wang, A. Misra, J. Lawrence Katz, and P. Spencer, "Nano finite element modeling of the mechanical behavior of biocomposites using multi-scale (virtual internal bond) material models," *J Biomed Mater Res,* vol. 83, pp. 332-344, 2007.

[64] E. Dintwa, P. Jancsók, H. K. Mebatsion, B. Verlinden, P. Verboven, C. X. Wang*, et al.*, "A finite element model for mechanical deformation of single tomato suspension cells," *Journal of Food Engineering,* vol. 103, pp. 265-272, 2011.

[65] I. Cimrák, M. Gusenbauer, and T. Schrefl, "Modelling and simulation of processes in microfluidic devices for biomedical applications," *Computers & Mathematics with Applications,* vol. 64, pp. 278-288, 2012.

[66] I. Romero and J. J. Arribas, "Modeling and Simulations of the Dynamics of Growing Cell Clusters" in *Advances in Cell Mechanic.* S. Li and B. Sun Eds. Berlin Heidelberg: Springer, 2011, pp. 1-25.

[67] A. E. Smith, K. E. Moxham, and A.P.J. Middelberg, "On uniquely determining cell–wall material properties with the compression experiment," *Chemical Engineering Science,* vol. 53, pp. 3913-3922, 1998.

[68] E. H. Zhou, C. T. Lim, K. S. W. Tan, and S. T. Quek, "Finite element modeling of the micropipette aspiration of malaria-infected red blood cells," in *Third International Conference on Experimental Mechanics and Third Conference of the Asian Committee on Experimental Mechanics,* 2005, pp. 763-767.

[69] E. H. Zhou, C. T. Lim, and S. T. Quek, "Finite Element Simulation of the Micropipette Aspiration of a Living Cell Undergoing Large Viscoelastic Deformation," *Mechanics of Advanced Materials and Structures,* vol. 12, pp. 501-512, 2005.

[70] W. Fichtner, "Overview of Technology Computer-Aided Design Tools and Applications in Technology Development, Manufacturing and Design," *Journal of Computational and Theoretical Nanoscience,* vol. 5, pp. 1089-1105, 2008.

[71] K. E. Drexler, "Toward Integrated Nanosystems: Fundamental Issues in Design and Modeling," *Journal of Computational and Theoretical Nanoscience,* vol. 3, pp. 1-10, 2006.

[72] J. S. Rathore, R. Majumdar, and N. N. Sharma, "Planar Wave Propagation Through a Tapered Flagellated Nanoswimmer," *IEEE Transactions on Nanotechnology,* , vol. 11, pp. 1117-1121, 2012.

[73] A. Adamo and K. F. Jensen, "Microfluidic based single cell microinjection," *Lab on a Chip,* vol. 8, pp. 1258-1261, 2008.

[74] C. Yi, C.-W. Li, S. Ji, and M. Yang, "Review: Microfluidics technology for manipulation and analysis of biological cells," *Analytica Chimica Acta,* vol. 560, pp. 1-23, 2006.

[75] J. P. Beech, S. H. Holm, K. Adolfsson, and J. O. Tegenfeldt, "Sorting cells by size, shape and deformability," *Lab on a Chip,* vol. 12, pp. 1048-1051, 2012.

[76] J. M. Gere, *Mechanics of Material*, 5 ed. USA: Brooks/Cole, 2001.

[77] H. Bianchi and R. Fernández-Prini, "The conductivity of dilute electrolyte solutions: Expanded lee and wheaton equation for symmetrical, unsymmetrical and mixed electrolytes," *Journal of Solution Chemistry,* vol. 22, pp. 557-570, 1993.

[78] K. A. Siggers and C. F. Lesser, "The Yeast Saccharomyces cerevisiae: A Versatile Model System for the Identification and Characterization of Bacterial Virulence Proteins," *Cell Host & Microbe,* vol. 4, pp. 8-15, 2008.

[79] G. M. Walker, "Yeasts," in *Encyclopedia of Microbiology (Third Edition)*, S. Editor-in-Chief: Moselio, Ed., ed Oxford: Academic Press, 2009, pp. 478-491.

[80] R. Cheong, S. Paliwal, and A. Levchenko, "Models at the single cell level," *Wiley Interdisciplinary Reviews: Systems Biology and Medicine,* vol. 2, pp. 34-48, 2010.

[81] W. Bai, K. Zhao, and K. Asami, "Effects of copper on dielectric properties of E. coli cells," *B, Biointerfaces Colloids and surfaces.,* vol. 58, pp. 105-115, 2007.

[82] R. G. Sargent, "Verification and Validation of Simulation Models," in *Winter Simulation Conference*, 2007, pp. 124-137.

[83] R. Hölzel and I. Lamprecht, "Dielectric properties of yeast cells as determined by electrorotation," *Biochimica et Biophysica Acta (BBA) - Biomembranes,* vol. 1104, pp. 195-200, 1992.

[84] V. Raicu, G. Raicu, and G. Turcu, "Dielectric properties of yeast cells as simulated by the two-shell model," *Biochimica et Biophysica Acta (BBA) - Bioenergetics,* vol. 1274, pp. 143-148, 1996.

[85] A. J. Bard and L. R. Faulkner, *Electrochemical Methods: Fundamentals and Applications*: Wiley, 2000.