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Dual Nanoprobe for Single Cell Viability Detection: Method Characterization

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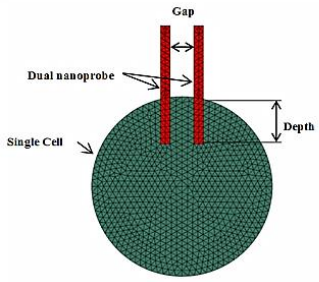
Graphical abstract



Abstract

This paper presents characterization results of the dual nanoprobe technique for single cell viability detection. Characterization is one of the steps in improving single cell viability detection technique in term of dual nanoprobe sensitivity, design and measurement configuration. The characterizations were focused on improving dual nanoprobe sensitivity and design by studying the effect of different material types, cross sections and measurement configuration, i.e. penetration depth and the gap of the dual nanoprobe on the measurement result. From the findings, the most preferred material is Tungsten and different cross section shapes do not give significant differences in dual nanoprobe sensitivity. It was also found that the current flow increases significantly with deeper penetration depth and narrower probes gap. Therefore, penetration depth and gap need to be constant during measurement in order to get reliable single cell viability detection result. The dual nanoprobe also has the potential to be used in single cell surgery, single cell thermal measurement, single cell drug delivery, and early disease detection applications.

Keywords: Microstructure; cell viability; microbiology; modelling; simulation

1.0 INTRODUCTION

In microbiology field, single cell studies have aggressively increased in recent years. One of the studies is cell viability detection. In single cell analysis, it is important to differentiate between live and dead cells [1]. Basically, cell has two features that have been normally used for viability detection, i.e. function and membrane properties. One of the methods to check the cell viability through cell function is by measuring the cell metabolism. For instance, the cell function to process glucose can be measured using positron emission tomography (PET) [2]. PET measurement requires a skilled operator, high tech equipment and risk of harmful exposure to toxic radiation.

Due to the limitations faced by the PET approach, most conventional method utilizes the membrane properties. This approach is better in term of simple procedure and requires less equipment. The cell membrane functions as a protector to prevent substance or medium enters the intracellular of the cell. It is known that the integrity of the cell wall decreases when a cell is dying [1]. Conventional methods manipulate this condition by using colorimetric dyes, e.g. trypan blue and fluorescent, to detect cell viability [3, 4]. However, this technique has a few drawbacks. The detection is based on optical observation and can only produce qualitative results. Even though the procedure is simple, the detection is a slow process and cannot produce instantaneous results. Hence, the technique does not suitable for certain cell types that have a short life span.

Previously, we have proposed a novel method for single cell viability detection to overcome the limitations by the conventional method [1]. The novel viability detection is based on electrical measurement on a single cell by using a dual nanoprobe. The advantages of this method are the ability to produce quantitative and instantaneous results. Experimental measurement results showed a significant difference between alive and dead cell. However, the method is still at an early stage of research and there are plenty of improvements that can be done.

Realizing this notion, this paper was written in order to publish the characterization results in an effort of improving the existing technique in term of dual nanoprobe sensitivity, design and measurement configuration. These studies were performed using commercial finite element analysis software (Abaqus FEA). Most of the parameters, i.e. dimension and material properties, required for the simulation were obtained from our previous experimental data [1].

■2.0 NOVEL METHOD FOR SINGLE CELL VIABILITY DETECTION

The details of the proposed method have been discussed thoroughly in our previous paper [1]. Figure 1 shows the schematic diagram of the proposed method and scanning electron microscopy (SEM) image during measurement. The nanoprobe has been tested on *W303*, wild-type yeast cell.

Basically, yeast cell consists of three layers, which is known as the cell wall, membrane, and cytoplasm but only the last layer is the area of interest. In theory, cytoplasm is an electrolyte solution that contains ions and its electrical conductivity depends on the concentration and type of ions [5]. The conductivity of the cytoplasm reduces due to decreasing ion concentration when the membrane integrity of the cell is deteriorated or dying. By realizing this phenomenon, cell viability can be detected quantitatively via electrical measurement.

In order to perform viability detection, the dual nanoprobe must be able to penetrate the cell until it reaches the cytoplasm layer. After the cell had been penetrated successfully, a pulse voltage of 2 volts is applied to the probe. Then, pulse current that flow through the cell will be measured using Femto-ammeter. The measurement was conducted on a group of yeast cells with known viability, i.e. alive and dead cells. From the results obtained, current measurement showed a significant difference between alive and dead cell.

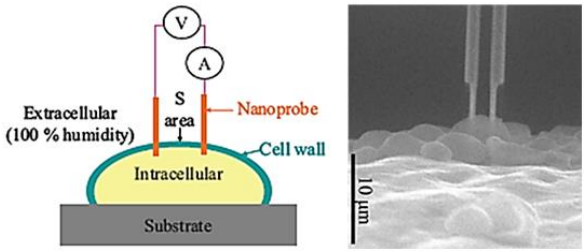


Figure 1 Schematic diagram of the single cell viability method and experimental SEM image [1]

■3.0 METHODOLOGY

The nanoprobe has been fabricated and tested experimentally but has limited information for further improvement. Figure 2 shows the fabricated dual nanoprobe attached to the modified commercial Olympus AFM cantilever. Characterization is one of the approaches that help the user to have a detailed understanding of the nanoprobe from different aspects, i.e. electrical, chemical and mechanical. The information obtained will be useful in foreseeing technique improvement in specific areas, i.e. sensitivity, accuracy, repeatability, system costing, and applications. This study has been divided into two parts, i.e. the dual nanoprobe characterization and single cell electrical measurement configuration characterization.

Before that, we simulated the exact measurement configurations in the experiment. Figure 3 shows the proposed method configurations in a simulation environment. Basically, there are three main components in the simulation, i.e. dual nanoprobe, single cell, and the base. The base is one of the parts of the Olympus AFM cantilever that is connecting dual nanoprobe and Femto-ammeter in the experimental setup. These components are sufficient to perform the same function for single cell viability detection although the real experiment has more components, e.g. chip holder, femto ammeter, nanomanipulator, and an observation chamber system called Environmental-Scanning Electron Microscope (E-SEM). Simulation result was being compared with experiment data.

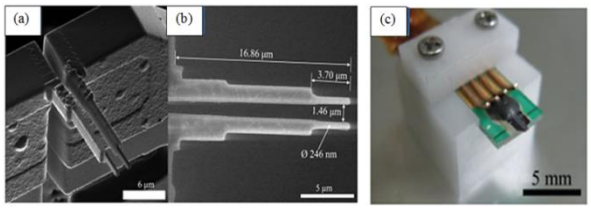


Figure 2 Dual nanoprobe (a) side view, (b) top view, and (c) cantilever holder [6]

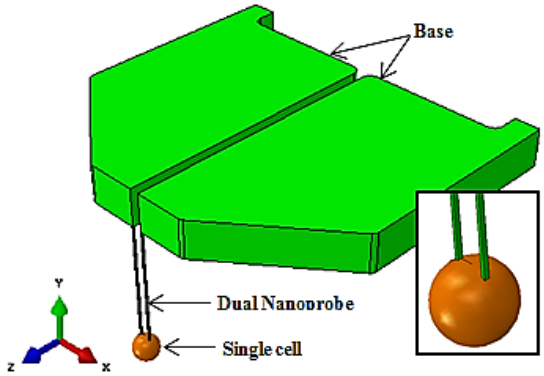


Figure 3 Simulation setup on the method

3.1 Dual Nanoprobe Characterization

First, the dual nanoprobe was characterized for five different materials, i.e. Silver, Copper, Aluminum, Tungsten and Zinc. These metals have high electrical conductivity and commonly used in electronic systems. The purpose is to investigate the effect of different material on the dual nanoprobe sensitivity. This will help to decide the most preferred material for dual nanoprobe and provide a list of potential material replacement.

Besides material, we also characterized the nanoprobe shape or cross section. Similarly, the purpose is to investigate the effect of dual nanoprobe shape whether it will give significant effect on the nanoprobe sensitivity and help to determine which shape is the best for a nanoprobe. Three types of cross sections, i.e. rectangular-shaped, square-shaped and circular-shaped were investigated. Figure 4 shows the cross section of the dual nanoprobe with different shapes. In the previous experiment, dual nanoprobe was fabricated as a rectangle-shaped probe using Tungsten due to its high tensile strength and electrical conducting capability.

In dual nanoprobe characterization studies, i.e. material and probe shape, we omitted single cell model in the simulation. Figure 5 shows the dual nanoprobe orientation in simulation. In this setup, the nanoprobes were connected together at the tip and usually called as short-circuit position in electrical terms. A voltage of 2 volts was applied to the base A and grounded at base B. The characterizations were performed by replacing the material definition and shape of dual nanoprobe.

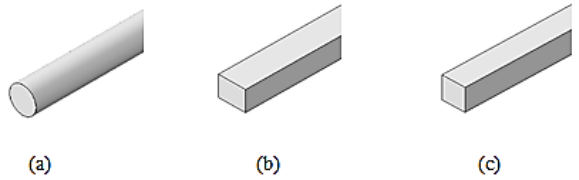


Figure 4 Different types of cross section (a) circular, (b) rectangle, and (c) square

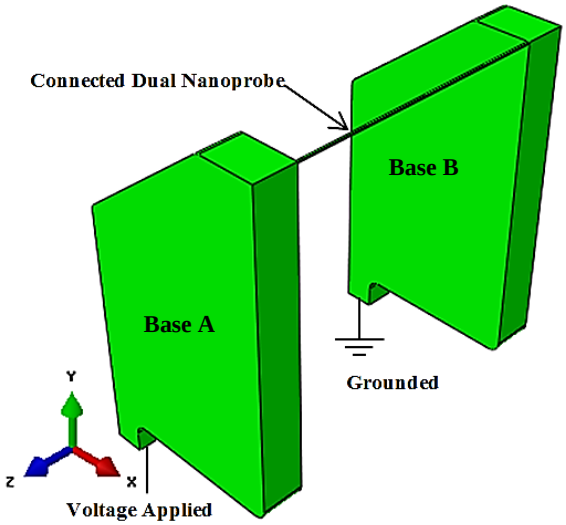


Figure 5 Short circuit setup for dual nanoprobe characterization

3.2 Measurement Configuration Characterization

The second part of the study emphasized on single cell electrical measurement configuration characterization. The purpose is to study the effect of different measurement configurations, i.e. penetration depth and probe gap, on the measurement result. In an experiment, it is difficult to control the length of nanoprobe which is in contact with the cell's cytoplasm without prior determination on the cell wall and membrane thickness.

Previously, the dual nanoprobe was being forced to penetrate the cell approximately at 300–350 nm penetration depth without cell burst. The depth is assumed sufficient for the nanoprobe to reach the cytoplasm of yeast cell since the reported cell wall and membrane of the cell is approximately 207 nm thick [7] but the thickness vary for each cells due to several factors, i.e. age and type. SEM image can only confirm the nanoprobe length inside a cell but unable to specifically measure the actual length of a nanoprobe in contact with the cytoplasm. This investigation is difficult to achieve in experiment since the existing measurement system is unable to observe the nanoprobe inside a cell without cutting the cell open.

As an alternative, the investigations are done via simulation. In simulation, we have better control of the penetration depth on cytoplasm by excluding cell wall and membrane in the cell model. In addition, the dual nanoprobe gap is also being studied in preparation for smaller cell measurement that may require a narrow gap.

There are two main components were used in the single cell electrical measurement simulation, i.e. dual nanoprobe and single cell model. The base was omitted to reduce computational resources. The components were assembled in a way where the dual nanoprobe already penetrated the single cell in a certain depth. Then, the components were merged together into one solid part. This approach was necessary as the limitation of the software that requires only one solid part for electrical analysis. Similar to the previous setup, we applied a voltage of 1 volts to the first nanoprobe and grounded at the second nanoprobe using the boundary condition definition. The characterizations were performed by changing the penetration depth and probe gap. Figure 6 shows nanoprobe gap and penetration depth. Ten different gaps and depths were studied in the range of 0.2-2.0 µm.

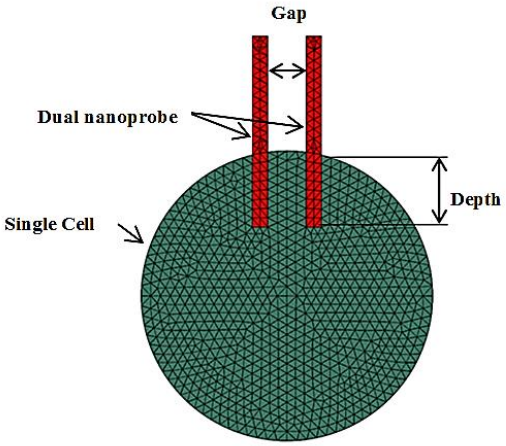


Figure 6 Nanoprobe gap and penetration depth

3.3 Cell Model

The cell model used in this study was a model based on W303 wild-type yeast cell, which is the same yeast type that was used in our previous experimental work. Yeast is a sphere shape cell with a diameter ranging from 4 µm to 6 µm [8]. The cell was modelled as a single layer solid sphere with a diameter of 3.8 µm which represent only the cell cytoplasm layer. The electrical conductivity of yeast cytoplasm is 0.55 S.m-1 for live cell and approximately 0 S.m-1 for dead cells [9, 10].

3.4 Simulation Validation

The simulation setup was validated by comparing the simulation result with calculated values. We performed a simple simulation test for resistance measurement on a solid square block of Tungsten material. Table 1 shows the comparison results. It was found that the resistance value from the simulation has the same value with calculated resistance using Equation (1) given as

$$\text{Resistance, } R = \rho L/A \qquad (1)$$

where ρ is the material resistivity, L is the material length, and A is the cross section area. This result shows that the element type, material definition, and boundary conditions were correctly configured in the simulation.

Table 1 Simulation validation

Method	Dimension, µm	Material	Resistance, Ω
Simulation	1x1x1	Tungsten	5.29E-2
Calculation	1x1x1	Tungsten	5.29E-2

4.0 RESULTS AND DISCUSSION

For a start, we compared the results obtained from simulation of a single cell viability detection using dual nanoprobe with experimental data. In the experiment we measured the current using Femto-ammeter while in simulation current was calculated by using Equation (2) given as

$$\text{Current, } I = ECD \times A \quad (2)$$

where ECD is current density and A is the cross section area. Figure 7 shows the simulation results. Colour contour on the components indicates current density throughout the system. From the result, nanoprobe region has the highest current density. Therefore, the current was calculated using dual nanoprobe cross section and the ECD value at that region. Table 2 shows the summarize comparison between experimental and simulation results. It was found that simulation current value is higher than experiment measured current.

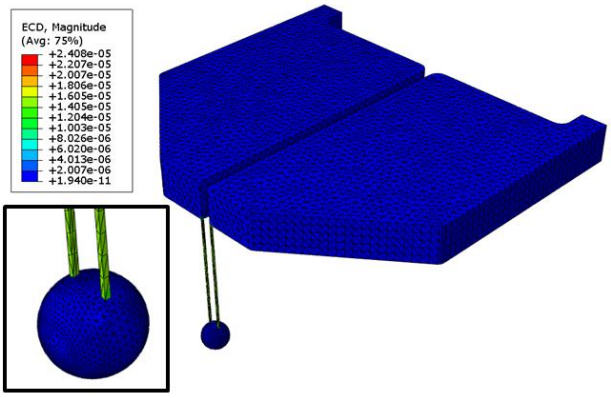


Figure 7 Simulation of single cell viability detection using dual nanoprobe

Table 2 Result comparison between simulation and experimental data

Properties	Experimental		Simulation	
Resistance, Ohm	1000		37.46	
Sensitivity, mA/V	1		26.7	
Voltage, V	2		2	
Probe Gap, µm	1.46		1.46	
Penetration Depth, µm	0.35		0.35	
Current	Alive	Dead	Alive	Dead
	262 pA	2 pA	395 nA	10 nA

There are several factors that lead to the diversity of current value i.e. electrochemical resistance and measurement configuration. In the experiment, electrochemical reaction occurs which create additional resistance known as electrode polarization resistance. Beside organelles, cytoplasm is full of ions, i.e. Sodium ions, Potassium ions, and others. When DC voltage is applied to dual nanoprobe, the positive and negative ions attracted to the nanoprobe accordingly. Positive ions will attracted to negative nanoprobe and negative ions attracted to positive nanoprobe. Accumulation of ions around the nanoprobe creates a layer of ions which increase the total resistance to the current flow. In simulation, we could not simulate electrochemical reaction due to software limitation and can only perform electrical analysis on solid parts.

Another possible explanation is the measurement configuration. The actual experiment could not determine the exact contact area size or penetration depth with the cell's intracellular (cytoplasm) due to inaccurate nanoprobe penetration depth measurement which obtained by optical image analysis on 2-Dimensional images. Image capturing angle and orientation may influence the analysis accuracy. Therefore, the contact area or penetration depth in the simulation may not able to represent the actual experimental configuration.

Plus, the cell model in this study only been developed to simulate cytoplasm electrical conductivity for different cell viability stage and assuming the other cell part, e.g. cell wall and membrane are not affecting the measurement. Cell wall for example, is more to capacitive type material [11]. Therefore, it is assumed that cell wall will not affect the measurement conducted using DC source. Nevertheless, the simulation shows a functional single cell viability detection between alive and dead cells same as the experiment.

4.1 Dual Nanoprobe Characterization Results

Dual nanoprobe performance was measured based on measurement sensitivity. Higher sensitivity provides better sensing capability but it will increase noise vulnerability. However, noise can be filtered through software programming or hardware filtering circuit. Sensitivity was calculated using Equation (3) given as

$$\text{Sensitivity, } \upsilon = \Delta\text{Current}/\Delta\text{Voltage} \quad (3)$$

Table 3 shows the dual nanoprobe characterization results based on five different materials. Based on the results, the highest sensitivity was achieved by Silver nanoprobe. However, the sensitivity for other materials is also sufficient for single cell viability detection as long as the nanoprobe resistance is relatively low compared to the measurement subject resistance which in this case is the single cell. Therefore, low sensitivity differences between the materials shows that the nanoprobe can be made from any of them. In stiffness aspects however, dual nanoprobe needs to be strong to penetrate the cell wall and membrane. Fabricated dual nanoprobe was made from Tungsten and selected due to its high strength capability. Material strength can be evaluated based on Young's modulus value. High Young's modulus value indicates high strength material. Tungsten has the highest Young's modulus compare to other materials in this study and had been tested experimentally. Therefore, out of five materials Tungsten is the most preferred material for nanoprobe due to its sensitivity and high strength capability.

Table 3 Dual nanoprobe characterization results for five materials

Material	Young's Modulus, GPa	Sensitivity, υ
Silver	83	0.175
Copper	128	0.165
Aluminium	70	0.099
Tungsten	411	0.055
Zinc	108	0.050

Dual nanoprobe also been characterized based on cross section shape. Table 4 shows the sensor performance comparison between three cross section shapes, i.e. circular, square, and rectangle. From the results, different probe shapes do not significantly affecting the dual nanoprobe performance. Hence, the current dual nanoprobe design using rectangle cross section is still suitable for future design.

4.2 Measurement Configuration Characterization Results

In single cell electrical measurement configuration characterization, the relationship between the probe gap and the penetration depth of the dual nanoprobe with the current flow in the cell is investigated. Figure 8 shows the simulation results for ten different gaps in the range of 0.2-2.0 µm. From the results, we found that the current measured reduce at wider gap. This result supported by Equation (1) where the resistance increase when the length of the measured medium increase. The contact area between nanoprobe and the cytoplasm was kept constant in this study. In electrolytic conduction theory, the ions passing through the cell will have greater resistance when travel at longer distance. This information helps the user to calibrate the nanoprobe for different gap to suite new requirement, e.g. smaller cell size. However, a too narrow gap will create a short circuit connection between the probes and cannot be used for single cell viability detection.

Table 4 Dual nanoprobe characterizations of cross section shape

Probe Shape	Current, A	Sensitivity, ʊ
Circular	0.1063	0.05315
Square	0.1064	0.05320
Rectangle	0.1065	0.05325

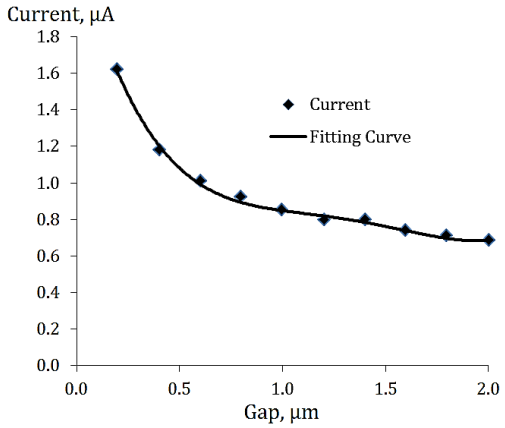


Figure 8 Characterization results of dual nanoprobe gap

Figure 9 shows the simulation results for ten different penetration depths in the range of 0.2-2.0 µm. Based on the findings, the measured current increase in deeper penetration. This is because the contact area between dual nanoprobe and cytoplasm increase as the probes submerge deeper at constant gap. Wider contact area allows more ions to move from dual nanoprobe to cytoplasm and vice versa. However, deep penetration will cause damage to the internal organelles of the cell, i.e. nucleus [12]. Through experimental studies, minimum penetration depth was at 300-350 nm where the nanoprobe needs to break the cell wall and membrane to reach the cytoplasm area. Cell wall and membrane were reported to have a thickness of 100-200 nm and 5-10 nm respectively [13]. This information is important to ensure a functioning sensor while minimizing the cell damage. The same cells can be used to perform other measurement if the cell viability can be maintained.

For reliable single cell viability detection, the probe gap and penetration depth need to be kept constant since they are significantly affecting the current measurement. Future works will involve integration with a microfluidic system for a higher throughput rate measurement.

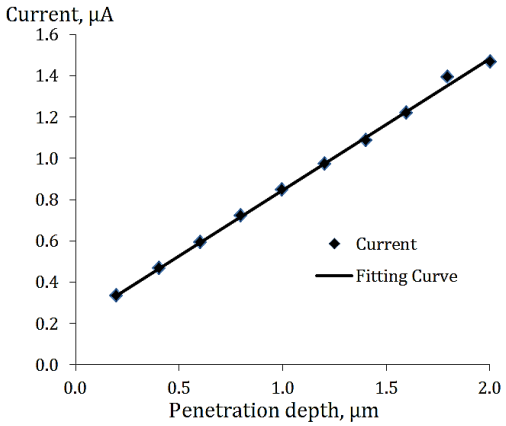


Figure 9 Characterization results on penetration depth

5.0 CONCLUSION

We performed electrical characterizations on the novel method of single cell viability detection based on electrical measurement using a dual nanoprobe. The characterizations were performed to study the effects of the different materials (Silver, Copper, Aluminium, Tungsten, and Zinc), probe shape (circular, square, and rectangle), and measurement configuration (probe gap and penetration depth) on the method for detecting single cell viability. There are several conclusions can be made. Tungsten is considered as the preferred material based on strength and sensitivity. Secondly, the current nanoprobe shape is still suitable for future nanoprobe design. Thirdly, different nanoprobe and penetration depth are significantly affecting the current measurement and need to be kept constant during the measurement for reliable single cell viability detection. In the future, this novel single cells viability detection will be improved by integrating the dual nanoprobe with a microfluidic chip for a portable, faster, and efficient measurement.

Acknowledgement

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References

[1] Ahmad, M. R., Nakajima, M., Kojima, M., Kojima, S., Homma, M., and Fukuda, T. 2012. Instantaneous and Quantitative Single Cells Viability Determination Using Dual Nanoprobe Inside ESEM. *IEEE Transactions on Nanotechnology*. 11(2): 298–306.

[2] Melder, R. J., Elmaleh, D., Brownell, A. L., Brownell, G. L., and Jain, R. K. 1994. A Method for Labeling Cells for Positron Emission Tomography (PET) Studies. *Journal of Immunological Methods*. 175(1): 79–87.

[3] Kucsera, J., Yarita, K., and Takeo, K. 2000. Simple Detection Method for Distinguishing Dead and Living Yeast Colonies. *Journal of Microbiological Methods*. 41(1): 19–21.

[4] Bonora, A. and Mares, D. 1982. A Simple Colorimetric Method for Detecting Cell Viability in Cultures of Eukaryotic Microorganisms. *Current Microbiology*. 7(4): 217–221.
[5] Bianchi, H. and Fernández-Prini, R. 1993. The Conductivity of Dilute Electrolyte Solutions: Expanded Lee and Wheaton Equation for Symmetrical, Unsymmetrical and Mixed Electrolytes. *Journal of Solution Chemistry*. 22(6): 557–570.
[6] Ahmad, M. R., Nakajima, M., Fukuda, T., Kojima, S., and Homma, M. 2009. Single Cells Electrical Characterizations Using Nanoprobe via ESEM-nanomanipulator System. In *9th IEEE Conference on Nanotechnology*. 589–592.
[7] Pelling, A. E., Sehati, S., Gralla, E. B., Valentine, J. S., and Gimzewski, J. K. 2004. Local Nanomechanical Motion of the Cell Wall of Saccharomyces cerevisiae. *Science*. 305(5687): 1147–1150.
[8] Bai, W., Zhao, K., and Asami, K. 2007. Effects of Copper on Dielectric Properties of E. Coli Cells. *B, Biointerfaces Colloids and Surfaces*. 58(2): 105–115.
[9] Asami, K., Hanai, T., and Koizumi, N. 1976. Dielectric Properties of Yeast Cells. *Journal of Membrane Biology*. 28(1): 169–180.
[10] Raicu, V., Raicu, G., and Turcu, G. 1996. Dielectric Properties of Yeast Cells as Simulated by the Two-shell Model. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*. 1274(3): 143–148.
[11] Zheng, Y., Shojaei-Baghini, E., Wang, C., and Sun, Y. 2013. Microfluidic Characterization of Specific Membrane Capacitance and Cytoplasm Conductivity of Single Cells. *Biosensors and Bioelectronics*. 42(1): 496–502.
[12] Ahmad, M. R., Nakajima, M., Kojima, S., Homma, M., and Fukuda, T. 2008. The Effects of Cell Sizes, Environmental Conditions, and Growth Phases on the Strength of Individual W303 Yeast Cells Inside ESEM. *IEEE Transactions on NanoBioscience*. 7(3): 185–193.
[13] Walker, G. M. 2009. Yeasts. In *Encyclopedia of Microbiology*. Third Edition. S. Editor-in-Chief: Moselio, Ed. Oxford: Academic Press. 478–491.