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Rigid and Conductive Dual Nanoprobe for Single Cell Analysis

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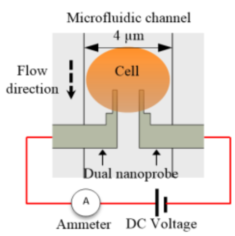
Graphical abstract



Abstract

Electrical property characterization of a single cell can be used to infer about its physiological condition, e.g. cell viability. Due to that, a dual nanoprobe-microfluidic system for electrical properties measurement of single cells has been proposed. This paper is concerned about the mechanical and electrical characterizations of the dual nanoprobe. Electrical and mechanical characterizations were conducted to measure the resistance and the strength of the dual nanoprobe for five different metals i.e. Aluminium, Copper, Silver, Tungsten, and Zinc using finite element approach. From the findings, Tungsten's nanoprobe has the highest strength while the resistance values for the five materials are not significantly different. Therefore, Tungsten is selected as the most recommended metal for the dual nanoprobe. We also performed single cell electrical measurement to test the functionality of the sensor. This work provides general information of the nanoprobe which can be used as a framework in other applications involving Nano devices i.e. cell surgery and drug delivery.

Keywords: Electrical Nano probe; finite element method; microfluidic; single cell analysis

1.0 INTRODUCTION

Single cell analysis has gained researcher attention in microbiological studies thanks to the rapid development of nanotechnology. The cells are now being studied individually and not based on population of cells. Each cell type 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. For an 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. In recent years, studies on single cell analysis have been focusing on characterizing the cells [1-6]. One of the approaches for cell characterization is through single cell electrical properties, i.e. impedance, dielectric, and conductivity measurement. In general single cell electrical measurement can be divided into four techniques i.e. impedance spectroscopy [5-14], electro kinetic [15-17], patch-clamp [18], and probing [19, 20]. Our previous work based on probing technique using dual Nano probe has shown its positive capability in practical application of detecting cell viability based on electrical property measurement [21].

The conventional method of cell viability detection is using colorimetric or fluorescent dyes [22]. This method lacks the capability to produce instantaneous and quantitative cell viability information which is important in the study of dynamics of cell death [23]. Alternative method for cell viability detection is through the cell's electrical properties characterization. Even though the cell viability detection based on electrical measurement has already been reported, most of the devices have several disadvantages, i.e. current leakage [23, 24], labour intensive, bulky system, and slow throughput rate [21]. Therefore, further improvement is needed. We proposed a new dual Nano probe design to be integrated in a microfluidic chip for single cell electrical properties measurement.

This paper presents two characterizations, i.e. mechanical characterization and electrical characterization of a dual Nano probe design using finite element method for possible single cell analysis application. Section two in this paper explains the proposed method of the dual Nano probe for measuring electrical property of single cells. Section three describes the process for both electrical and mechanical characterizations. Section four highlights simulation configurations for characterization and section five for single cell electrical measurement using Abaqus FEA software. Section six describes the verification and validation of the simulation results. Section seven discusses the results obtained from the simulation.

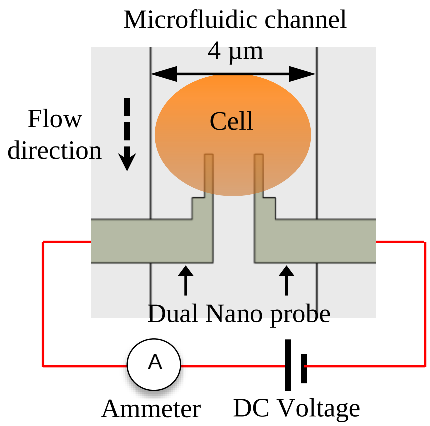


Figure 1 Schematic diagram of proposed system

■2.0 PROPOSED METHOD

Using the dual Nano probe technique for measuring single cell electrical properties have several advantages, i.e. instantaneous, quantitative, and small damage to the cell [21]. In general, the target single cell will be penetrated by the dual Nano probe up to the intracellular part of the cell. Then, a small voltage source is applied to the cell through conductive Nano probe and current flow will be measured for analysis. Even though this technique is invasive but due to small size (200 nm) probe the wounded cell able to recover. Our previous experiment used an active approach where single cell was in a static position on a substrate and dual Nano probe was moved by Nano manipulator to penetrate the single cell [21]. On the other hand, in our new system, a passive approach will be used. The Nano probe will be in a fixed position while the cell moves towards the Nano probe. Figure 1 showed the schematic diagram of the proposed method. The dual Nano probe will be placed in a fixed position at the middle of a micro channel. Cell movement is been manipulated and guided to be penetrated by the dual Nano probe. This manipulation is achieved by integrating with microfluidic chip. Figure 2 shows the proposed design for dual Nano probe-microfluidic integration. Microfluidic system which consist of micro channel and pump, will aid the measurement process by delivering only one single cell to the Nano probe for a fast and efficient electrical measurement. The small size of micro channel only allows one cell to flow to the dual Nano probe at a time. Pump is used to control the fluid flow in the microfluidic chip and by manipulating the flow rate single cell is been forced to be penetrated by the dual Nano probe at a certain depth. Voltage will be applied and current flow will be measured for analysis. After the measurement the cell will be flush out and the system ready for the next measurement. The measurement process inside the chip can be observed under microscope due to chip's transparency. Figure 3 shows the new Nano probe design for microfluidic integration.

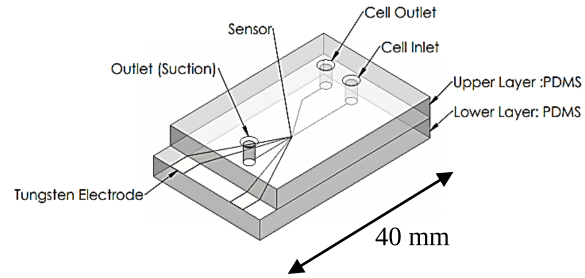


Figure 2 Dual Nano probe-microfluidic integrated system

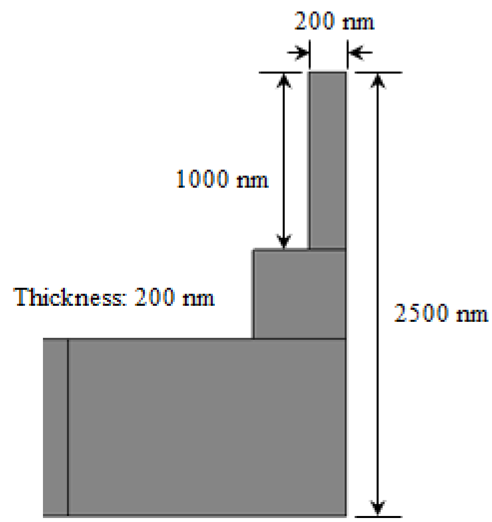


Figure 3 Nano probe dimension

■3.0 ELECTRICAL AND MECHANICAL CHARACTERIZATION OF DUAL NANOPROBE

The main component of the system is the dual Nano probe. It needs to be designed, characterized, and optimized before it can be used for single cell analysis. There are two characterizations which have been performed; electrical and mechanical characterizations. In electrical characterization the objective is to characterize the electrical property, i.e. resistance of the dual Nano probe. The probes must have a low resistance value in order to measure the electrical property of a single cell. During measurement, the probe will be connected to an ammeter for current measurement on single cell. Therefore, the probe that acts like an interface between the ammeter and the single cell should have minimum effect on the measurement and also does not limiting the ammeter measurement range. Based on previous findings, intracellular part (cytoplasm) of the cell is a good electrical conductor [19, 20]. In theory, cytoplasm is an electrolyte solution that contains ions and its electrical conductivity depends on the concentration and type of ions [25]. The resistance of the dual Nano probe can be considered sufficient if its value does not exceed the resistance of the cell's cytoplasm. This criterion will ensure significant current measurement of the single cell.

In the mechanical characterization, the objective is to measure the strength of the dual Nano probe. The Nano probe needs to be rigid and small in size in order to penetrate a cell. Smaller Nano probe size is required in order to produce a small wound as possible to the cell and lower the penetration force. Five different metals, i.e. Aluminum, Copper, Silver, Tungsten, and Zinc have been studied for both electrical and mechanical characterizations. We also consider the failure factor of the dual Nano probe. We obtained the maximum applied force before the Nano probe break. However, this additional study was carried out only on Tungsten Nano probe due to demanding computational resources for this kind of simulation.

4.0 CHARACTERIZATION VIA SIMULATION

Simulation approach has been commonly used in many research area and the findings are significant [26]. This approach has been vastly implemented in other microstructures, e.g. carbon nano tube [27-30]. There are several benefits of using simulation, i.e. faster development time, reduce cost, and safe. The key to a successful simulation is based on how realistic the tested model would be. Therefore, they are many parameters involved and one must be able to define the most relevance parameters as not all parameters are necessary to be acknowledged. There are cases where some parameters will be ignored, so the same thing was done in this research, i.e. noise and temperature. Noise and temperature depend on the environment of the measurement. The actual experimental measurement will be performed in a well-ventilated room for keeping the temperature constant and the noise will be filtered from the measurement. The simulation work was done using finite element analysis software (ABAQUS FEA) which is capable to perform multi-physics analysis.

4.1 Electrical Characterization of the Dual Nanoprobe Using Finite Element Approach

For electrical characterization, the dual Nano probe was modeled as 3D solid deformable DC3D4E and a 4-node linear coupled thermal-electrical element was used. This type of element commonly used to simulate the multi-physic analysis of electrical and thermal distributions. Even though the element is being coupled but analysis can be made independently for electrical or thermal by omitting non relevance parameters. Only electrical conductivity of the material was defined for five different metals; Aluminum, Copper, Silver, Tungsten, and Zinc.

For meshing the model, hexahedron was used due to higher accuracy. Figure 4 shows the simulation setup for electrical characterization. Dual Nano probes were positioned in such a way that the probes touching the probe tip one another. A voltage applied in one Nano probe and grounded in another. Resistance was calculated based on current measurement through the probe using Ohm's Law. The simulation was done repeatedly using five different materials.

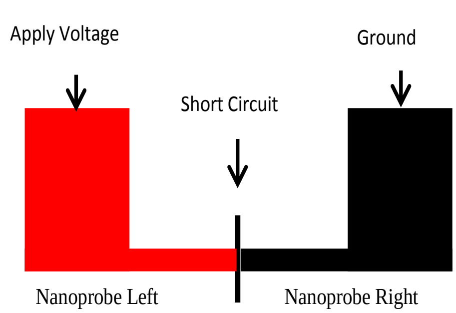


Figure 4 Simulation setup for the dual Nano probe's electrical characterization

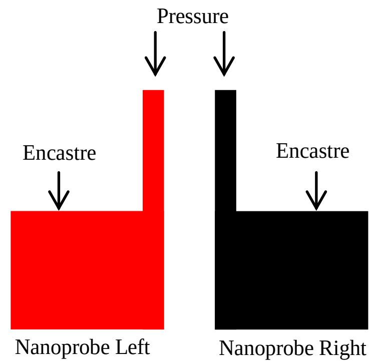


Figure 5 Simulation setup for the dual Nano probe's mechanical characterization

4.2 Mechanical Characterization of the Dual Nanoprobe Using Finite Element Approach

In mechanical characterization, the dual Nano probe was modelled as 3D solid deformable C3D8R and 8-node linear brick 3D stress element was used. Density and elastic properties i.e. Young's modulus and Poisson's ratio, were defined for five different metals; Aluminium, Copper, Silver, Tungsten, and Zinc. The model was meshed using hexahedron mesh type. Figure 5 showed the simulation setup for dual Nano probe mechanical characterization. Both Nano probes were encastred, i.e. fixed, and pressure was applied to the probe tip. The pressure applied displaced the probe tip and the value obtained is used to evaluate the Nano probe rigidity. The same simulation was done repeatedly using different metals. Another configuration has been setup for Nano probe damage or failure test simulation. The objective is to determine the maximum force the Nano probe can hold before failure. This simulation is also important in measuring Nano probe strength. We performed simulations using the Tungsten material with additional damage parameters i.e. ultimate stress and present elongation. These parameters were obtained from [31].

5.0 SINGLE CELL ELECTRICAL MEASUREMENT

One of the applications of our Nano probe is single cell viability detection. The same concept of measurement has been done experimentally using dual Nano probe in our previous study [21]. Based on the results obtained, single cell viability can be detected based on electrical measurement. Previous design also has been characterized but limited to electrical property of dual Nano probe [32]. In order to test the performance of new sensor design, we performed a single cell electrical measurement via simulation on a single cell model. This measurement was simulated to ensure the performance and functionality of the dual Nano probe. The Nano probe should be able to measure the current flow in the cell.

5.1 Simulation Setup

Figure 6 shows the simulation setup for single cell electrical measurement. The type of element for the Nano probe in this simulation is similar with dual Nano probe electrical characterization, i.e. 3D solid deformable DC3D4E and a 4-node linear coupled thermal-electrical element. The same type of element was applied to the single cell model. Single cell has been modelled as a one layer solid sphere shape based on Yeast cell with the size of 4 µm in diameter [33]. The cell model is defined with cell cytoplasm's electrical conductivity of 0.5 S.m-1 [34, 35]. A voltage of 2 Volts was applied to the first Nano probe and grounded in another. The same voltage value was applied in the previous experimental study [21]. The final output, i.e. electrical current, was calculated based on simulation results. Results validation was made by comparing the current value obtained from simulation with experimental data [21].

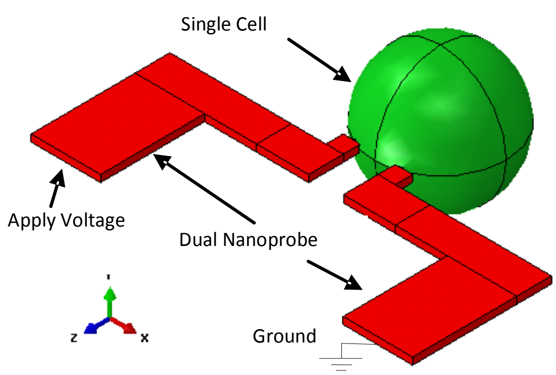


Figure 6 Simulation setup for the single cell electrical measurement

■6.0 VERIFICATION AND VALIDATION

Another important step is verification and validation of the simulation results. Electrical and mechanical characterizations were performed only in the simulation and have yet to be experimented. Therefore this step is to ensure the simulation results are correct and the results obtained were reasonable in term of value range. Comparison between simulation and calculation were done for results validation. Verification was done for each electrical and mechanical characterization simulation by creating simple simulations and compares the results obtained by calculation. In electrical characterization, the electrical resistance of Nano probe was calculated using electrical resistance given by Eq. 1

$$R = \rho\, L/A \qquad (1)$$

where ρ is the electrical resistivity, L is the length and A is the cross section area. In mechanical characterization, the maximum beam deflection or tip displacement was calculated using Eq. 2

$$\delta_B = q/24EI(3L^4-4\alpha^3\, L-\alpha^4) \qquad (2)$$

where, q is the force per meter, E is the Young's Modulus, I is the moment of inertia, L is the beam length, and α is the partial length of unapplied force. The simulation setup and approach was verified based on comparison of the values obtained from both simulation and calculation. Next, the same verified configuration was applied to the desired simulation setup. Again the simulation results were compared with the calculated values.

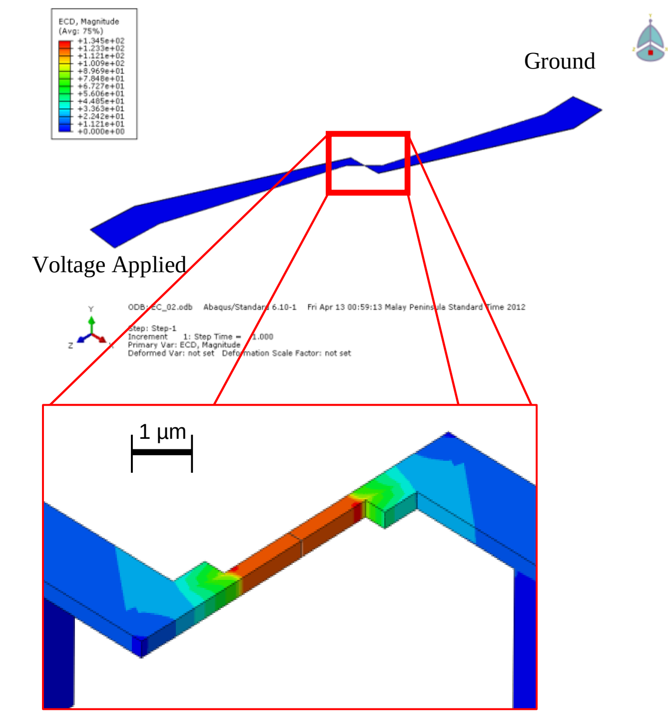


Figure 7 Current density on the dual nanoprobe. Inset image shows the magnified view of the dual nanoprobe

■7.0 RESULTS AND DISCUSSIONS

7.1 Result of Electrical Characterization of the Dual Nanoprobe

Figure 7 showed the simulation results in running mode. Colour on the components indicates current density, (ECD) that flow throughout the system. From the result, the dual Nano probe has higher current density than other components. This shows that the highest resistance is at the dual Nano probe. Current, I is obtained by using Eq. 3

$$I = ECD \times Ap \qquad (3)$$

where Ap is the Nano probe cross section area. Resistance, R is calculated by using Ohm's Law Eq. 4 given as

$$R = V/I \qquad (4)$$

where V is the applied voltage. Table 1 summarizes the dual Nano probe resistance for different metals. Silver has the lowest resistance, i.e. 1.939 Ω due to its high electrical conductivity under room temperature. However, there are insignificant differences of resistance between five other metals. Therefore, all the metals tested pass the first criteria as a conductive Nano probe and any of them can be selected as the dual Nano probe material in term of Nano probe.

Calculation of dual Nano probe resistance using Ohm's Law shows the adequacy of the simulation results which in a reasonable value range. There a few factors influence the accuracy for both calculation and simulation results, i.e. complex geometry, calculation approximation, and simulation setting. Complex geometry of the Nano probe makes the current path non-uniform. Therefore, assumption was made for calculating resistance. We assume the current path is uniform through the entire geometry. In simulation, the results can be affected by the simulation setting, i.e. meshing size. Smaller mesh size has higher accuracy but it will increase the computing resources required for processing.

Table 1 Resistance of the dual Nano probe

Metal	Price, USD/LB	Young's Modulus, GPa	Resistance,Ω		Electrical Conductivity , S/m
			Simulation	Calculation	
Silver	550.56	83	1.939	1.452	6.30E+07
Copper	4.45	128	2.028	1.535	5.96E+07
Aluminium	1.16	70	3.163	2.614	3.50E+07
Tungsten	16.25	411	5.507	4.840	1.89E+07
Zinc	1.11	108	6.075	5.381	1.70E+07

7.2 Result of Mechanical Characterization of the Dual Nanoprobe

Figure 8 shows the simulation result for mechanical characterization. Colors on the model indicate the displacement of the Nano probe and highest displacement occur at the tip of the Nano probe where the force of 1 μN was applied. Table 2 summarizes the Nano probe displacement for five different metals. Nano probe second criteria are rigidity. For electrical measurement on a single cell, the Nano probes not only need to be conductive but rigid as well. During single cell penetration, the dual Nano probe will exert impact force due to the momentum of a moving single cell. The force exerted will tend to deform or bend the Nano probe and this will require Nano probe calibration before proceeding with each single cell electrical measurement. This will complicate the measurement process. The worst case is the dual Nano probe touches each other which will create a short circuit in the system. From the characterization results, Tungsten has the smallest displacement of 0.65 nm as compared to the other metals. Therefore, Tungsten has the highest rigidity which satisfies the second criteria for a rigid Nano probe. Based on the results from the electrical and mechanical characterizations, the most preferred metal for the Nano probe is Tungsten. Tungsten has been used in fabricating a dual Nano probe and has shown its biocompatibility in single cell electrical characterization measurement [21].

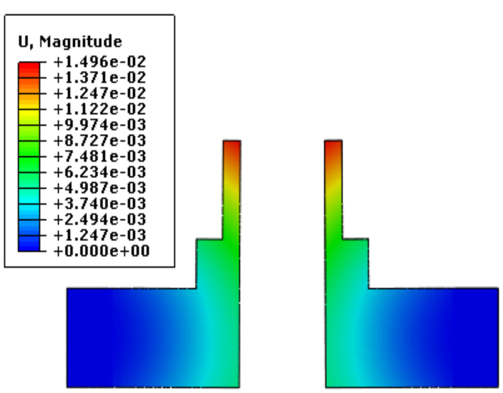


Figure 8 Displacement of the dual nanoprobe

Table 2 Deformation of the dual Nano probe for applied force at 1μN

Metal	Price, USD/LB	Young's Modulus, GPa	Displacement, nm
Silver	550.56	83	4.70
Copper	4.45	128	2.05
Aluminium	1.16	70	6.55
Tungsten	16.25	411	0.65
Zinc	1.11	108	2.42

Failure factor on the Nano probe was also being considered in this study. The simulation setup is basically the same with mechanical characterization but with the addition of failure parameters in the material properties. However, only Tungsten's Nano probe was tested due to demanding computational resources. Figure 9 shows the simulation results of the damage on the Tungsten's Nano probe. As result, the maximum force that can be applied on the Tungsten's Nano probe is 35.6 μN and if the force applied exceeds this value, the probe will break or damage. This result helps to keep the user from breaking the Nano probe accidentally. Once we know the Nano probe limit we can prevent damage to the Nano probe. As for the single cell penetration, the Nano probe should not break since the penetration force a single cell approximately below 1 μN [2]. Other user or researcher might want to use the same Nano probe design for other application and this result will give them a rough idea what it can do. The Nano probe not only limited for electrical measurement but also can be used as an actuator for other application, i.e. single cell surgery, drug single cell delivery, and single cell thermal measurement. Figure 10 shows the strain energy whereas Figure 11 shows force applied on the Nano probe. The graph explains the occurrence of energy drop at the point where the Tungsten Nano probe breaks or damage. To validate the results, we do a comparison with stress-strain graph for Tungst en, and it shows that this value is reasonable. The maximum stress or point of break obtained in the simulation (880 MPa) close to the ultimate stress of Tungsten metal (980 MPa).

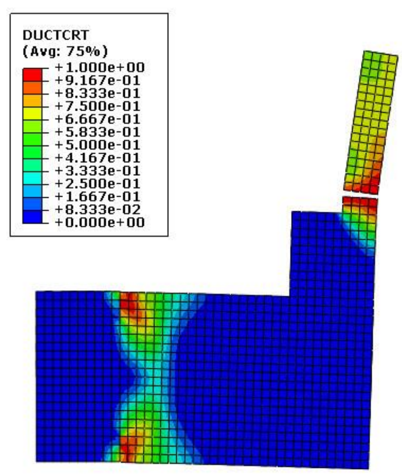


Figure 9 Nano probe damage simulation

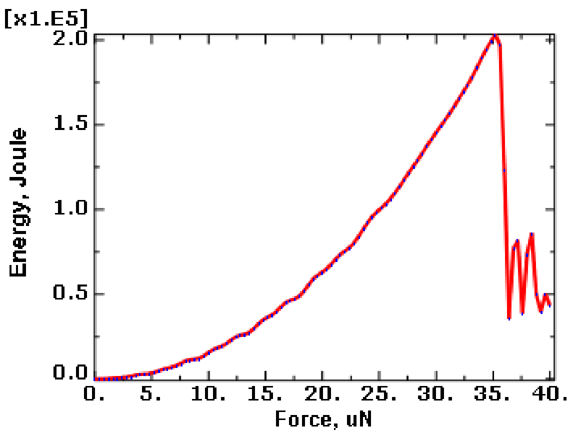


Figure 10 Strain energy of Tungsten Nano probe

7.3 Result of Electrical Measurement on a Single Cell Model

Figure 11 shows the electrical potential distribution across the simulated part and indicates a complete electrical connection from the voltage source to the ground. Figure 12 shows the current density of the simulated model. Based on these results, the current value was obtained. The current obtained from the simulation is 1.12 µA while experimental measurement reported a current value of 262 pA [21]. Obviously, the current value of the simulation is higher than experimental results.

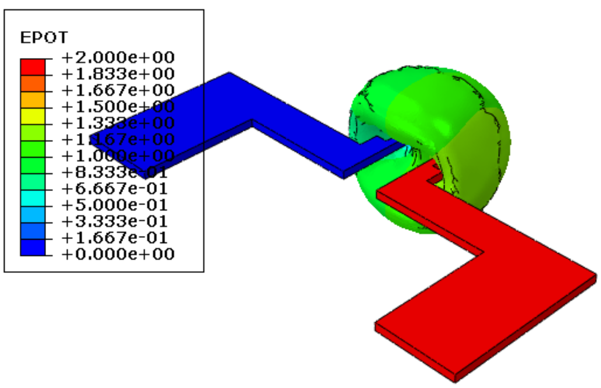


Figure 11 Electrical potential distribution when applying 2 Volts

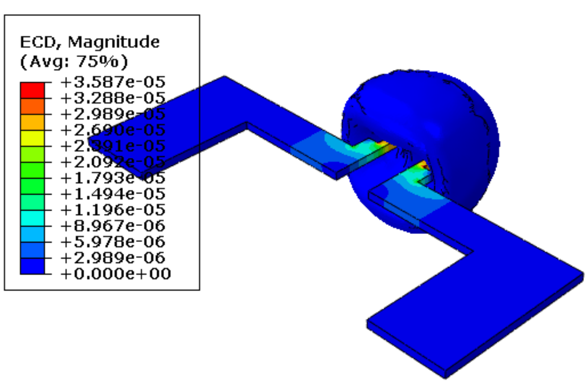


Figure 12 Current density distribution

There are several factors that lead to the diversity of current value. 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 Nano probe, the positive and negative ions attracted to the Nano probe accordingly. Positive ions will attracted to negative Nano probe and negative ions attracted to positive Nano probe. Accumulation of ions around the Nano probe 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. Besides that, the depth of penetration between simulation and experiment is different. New Nano probe is designed to penetrate a cell at deeper depth (1 µm) but previous experiment only slightly penetrate the cell (300 nm depth) [21] enough to pass through the cell wall (200 nm thickness) and cell membrane layers (7 nm thickness) [36] to reach the cell's cytoplasm. Deeper penetration will have a wider contact area between cell intracellular and Nano probe. Therefore, resistance is reduced as the current has wider area to flow. We also test for 300 nm depth and the current obtained is 566 nA. However, this simulation able to shows the new Nano probe design ability in measuring the single cell electrical property. The result can improve with a better single cell model but the current results design shows a promising functional sensor.

■8.0 CONCLUSION

This paper deals with the characterization process on a microstructure called dual Nano probe. We performed electrical and mechanical characterizations in search for a conductive and rigid dual Nano probe for intracellular single cell analysis using finite element analysis. In addition, we also performed single cell electrical measurement to test the Nano probe functionality. This Nano probe is designed to be integrated with microfluidic system. The electrical characterization studied on the resistance of five different metals. While mechanical characterization studied on the dual Nano probe strength. Material for the dual Nano probe was chosen based on the characterization results that fulfill the criteria for a conductive and rigid Nano probe. Simulation on single cell electrical measurement results shows promising results in term of the dual Nano probe's functionality. We validate the measurement results with an experimental data. This work results can also be used in other applications involving nano devices, e.g. cell surgery and drug delivery [37].

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