單一細胞阻抗量測分析

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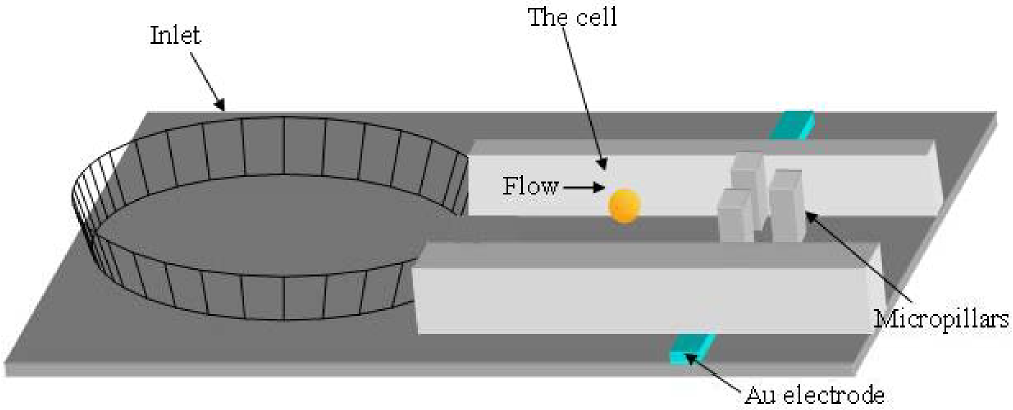
Outline

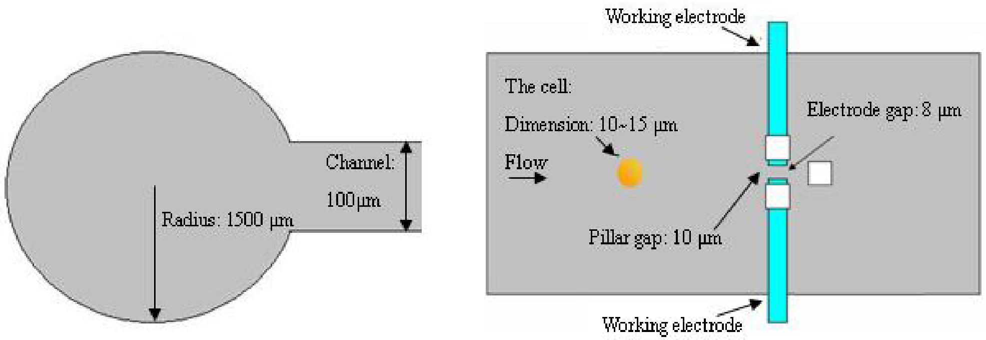
- Introduction
- Single cell impedance
  - Materials and Experiments
  - Modeling and FEM Simulation
  - Results and Discussion
- Portable impedance spectroscopy
  - Impedance system
  - Calibration
  - Results and Discussion
- Conclusions

Introduction

- Why single cells?
  - Small percentage of the cells exhibit symptoms of malfunction during the early stages of the disease's development
  - Description individual cells, not average information
- Why cell impedance?
  - Cell testing : Optical, medical and so on.
  - Provide electrical characterizations information of cell
  - To evaluate functional model of cells applying physiology and pathology information
- Is minimized impedance spectroscopy necessary?
  - Cell growth
  - Impedance analyzers are large and cumbersome

Materials and Experiments-Device

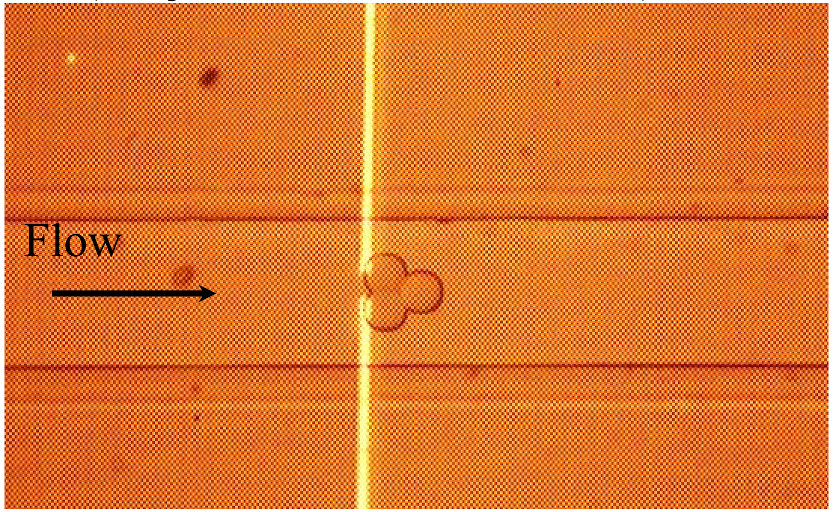




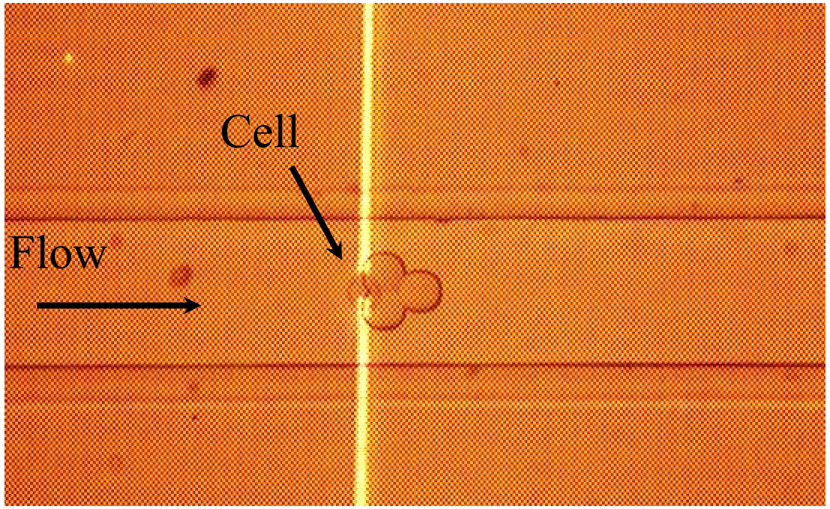
- **Glass substrate**
  - **Electrodes:**
    Au / Cr = 65/15 nm
- **PDMS channel**
  - **Microchannel:**
    100 x 25 μm
    (width x height)
  - **Micro-pillars**
    10 x10 x25 μm
    (width x length x height)
  - **Inlet / outlet ports:**
    1500 μm; radius

Materials and Experiments- Cell Capture

- HeLa cells (human cervical epithelioid carcinoma).
- Infusion pump (KD Scientific Inc., KDS100) with a flow rate of 5 ml/hr into the microfluidic channel.
- The impedance was measured by Precision Impedance Analyzer (Kayne Kerr Inc., 6440B)

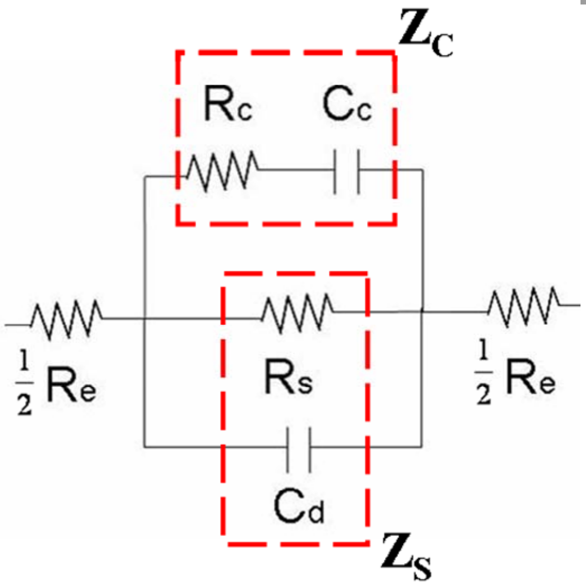


Cell-trapping structures before injection of cell solution.



Cell-trapping structures with the single cell of HeLa after injection.

Modeling



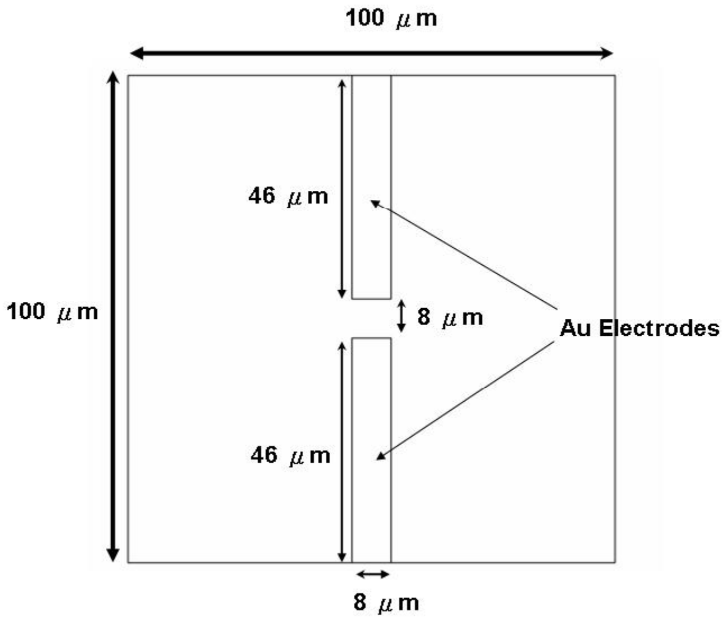
$$Z = \frac{1}{\frac{1}{R_c + 1/j\omega C_c} + (\frac{1}{R_s} + j\omega C_d)} + R_e = Z_R + jZ_I$$

$$Mag = \sqrt{Z_R^2 + Z_I^2}$$

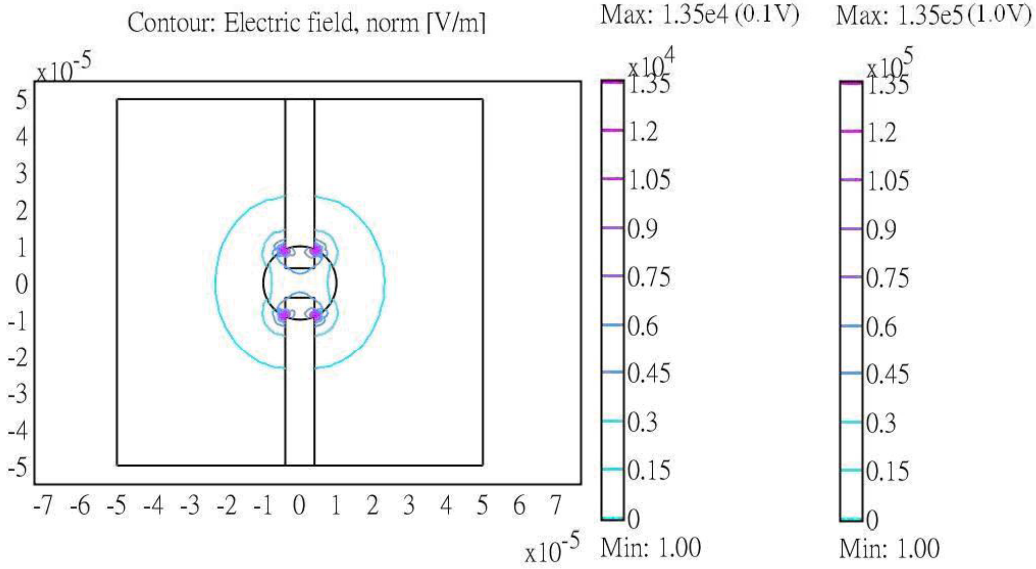
$$\theta = \tan^{-1}\frac{Z_I}{Z_R}$$

Zcell: cell impedance, include Cc and Rc
Zs: PBS solution impedance , include Rs and Cd
Cc: capacitance of cell membrane
Rc: resistor cytoplasm
Rs: solution resistor
Cd: soultion capacitance
Re: a pair of electrodes resistor

FEM Simulation



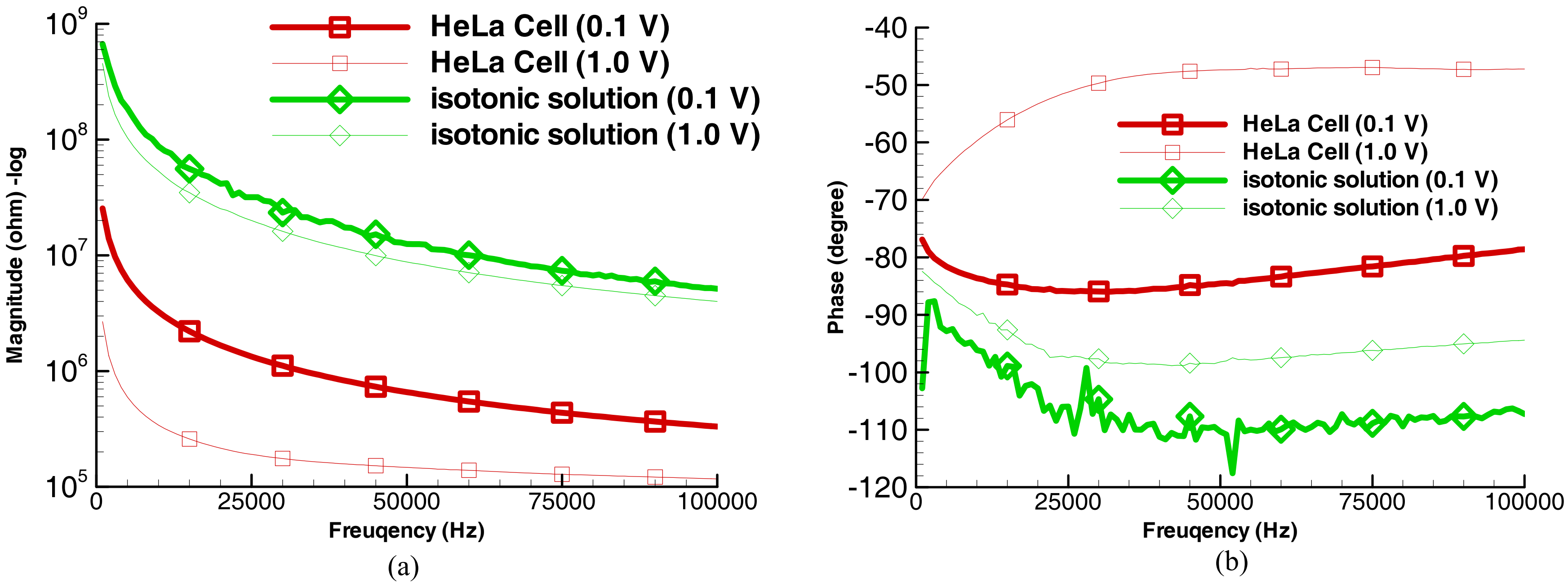
The structure of simulation



The distribution of electric field at 0.1V and 1V

1.The simulations is 2-dimensional and ignore Z-axis.
2.The maximum electric field intensity is located toward the boundary region between the cell and the electrodes.

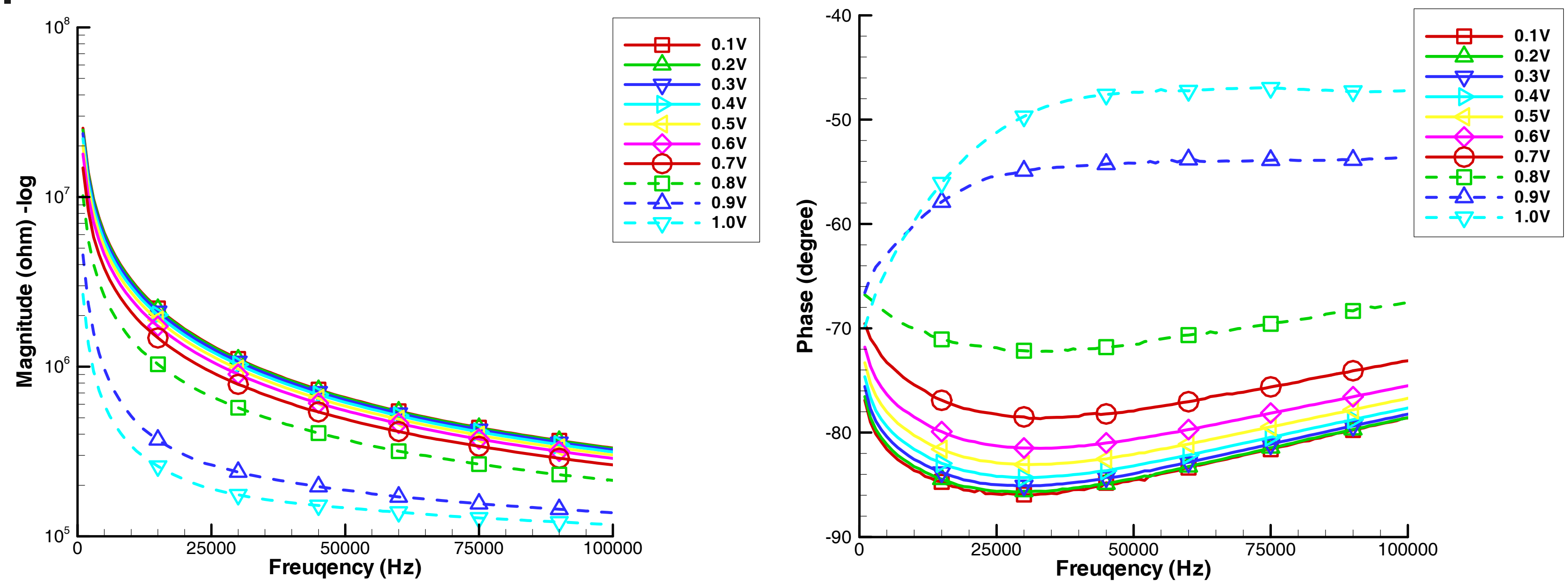
Results and Discussion-
The single HeLa cell & Isotonic solution



(a) Impedance magnitude and (b) phase of isotonic solution and the single HeLa cell at operating voltages of 0.1 V and 1.0 V

1. At 0.1V, the curves of the magnitude and phase were much rougher than those at 1.0 V
2. The impedance of isotonic solution at 0.1V were similar to those at 1.0V
3. The magnitude of the HeLa cell impedance was smaller than magnitude of isotonic solution about 1 ~ 2 orders in both cases

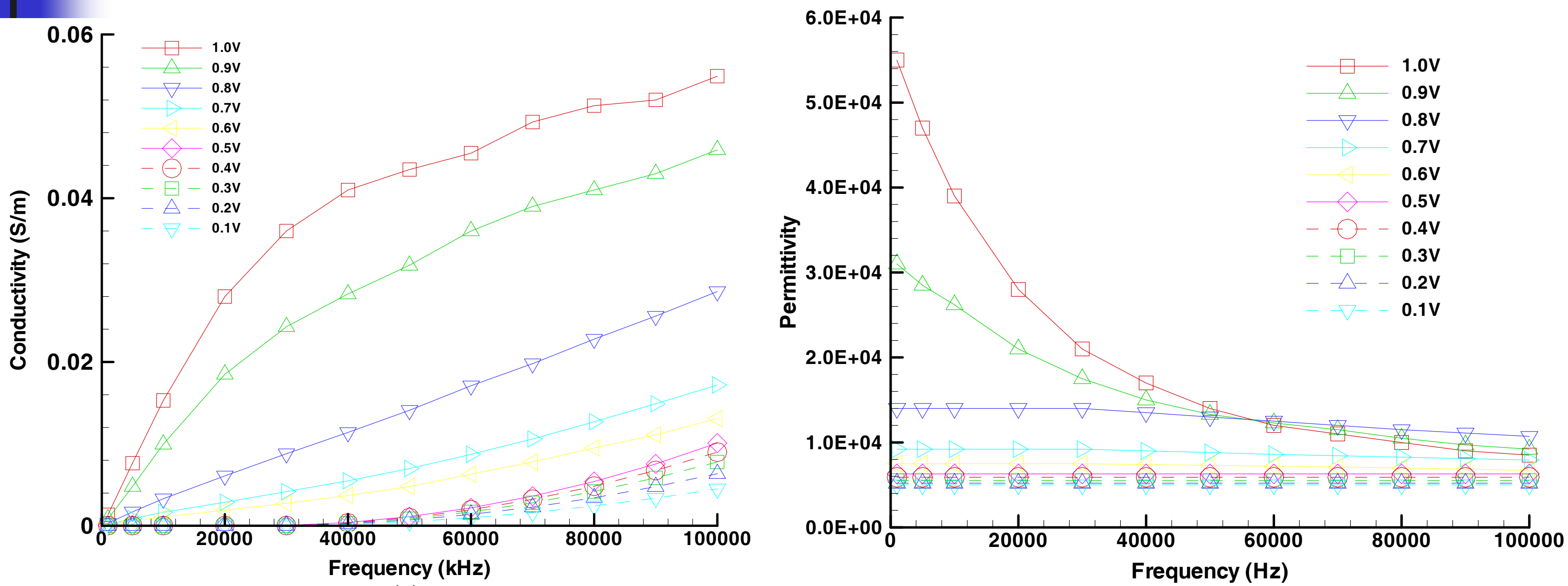
Results and Discussion-Impedance



The single HeLa cell impedance at different operation voltages (a) Magnitude (ohm) (b) Phase (degree)

1. The response of magnitude of single HeLa cell show dropped while frequency increased
2. Increasing operation voltage make HeLa cell magnitude decrease
3. The phase of the HeLa cell impedance is characteristics of a series circuit when the operating voltage exceeded 0.8V because Zcell became significant.

Results and Discussion-
Conductivity & Permittivity



(a) (b)

Variation of (a) conductivity and (b) permittivity of single HeLa cell with operational frequency as function of operational voltages in the range 0.1~1.0 V.

1.The conductivity and the permittivity of the HeLa cell increase with an increasing operational voltage at lower values of the operational frequency.
2. In frequency domain, increasing frequency increases the conductivity.
3.At high operational voltages at 0.9 and 1.0 V, the permittivity of the cell reduces rapidly with an increasing frequency.

Results and Discussion-
Empirical expressions

Conductivity

$$\sigma_V = \sigma_{V=0.1}\frac{\left(1+\sqrt{\frac{E_V/E_{V=0.1}}{f_{1000}}}\left(\frac{E_V}{E_{V=0.1}}-1\right)\times PC\right)}{CRIC}$$

$$\sigma_{V,f}(6k\sim 30k\ Hz)=\sigma_{V,f-\Delta f}\times\frac{\sigma_{V=0.1,f}}{\sigma_{V=0.1,f-\Delta f}}$$

$$\sigma_{V,f}(31k\sim 100k\ Hz)=\sigma_{V,f-\Delta f}\times\frac{E_{V=0.1}}{E_{V=0.1}+E_V/f_{100kHz}}\frac{\sigma_{V=0.1,f}}{\sigma_{V=0.1,f-\Delta f}}$$

Permittivity

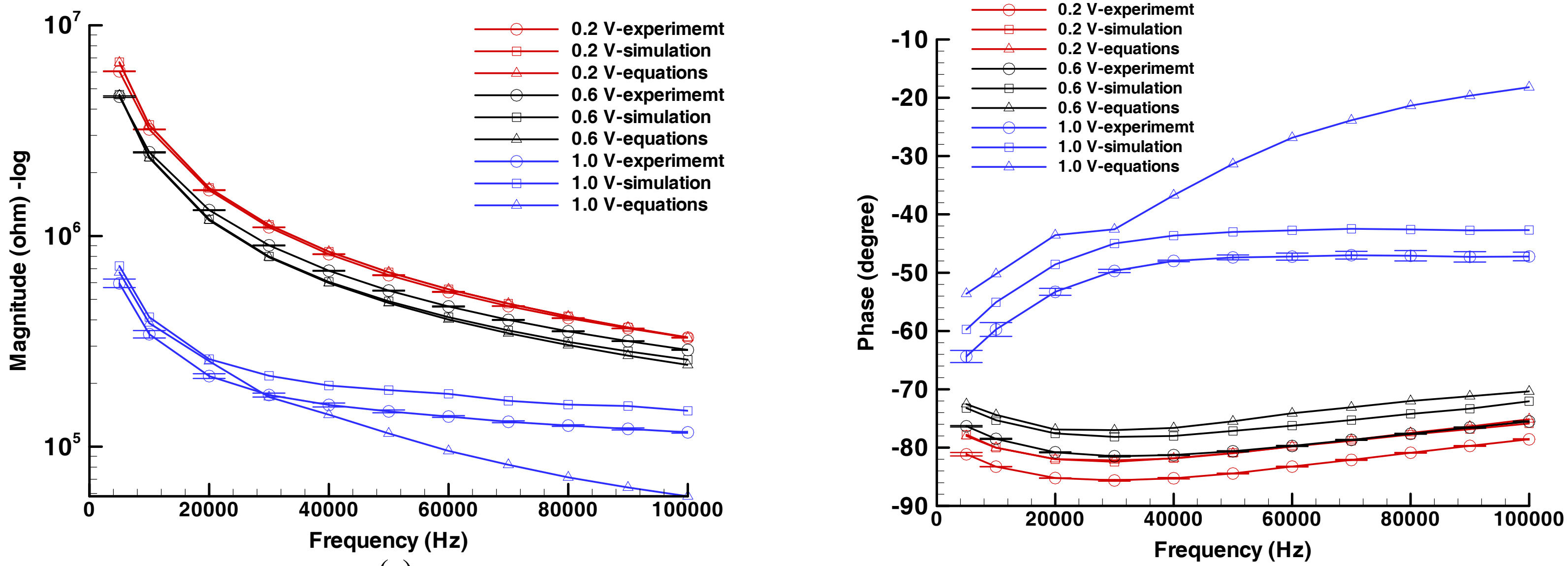
$$\varepsilon_V = \varepsilon_{V=0.1}\frac{\left(1+\sqrt{\frac{E_V/E_{V=0.1}}{f_{1000}}}\left(\frac{E_V}{E_{V=0.1}}-1\right)\right)}{CRIC}$$

$$\varepsilon_{V,f}(6k\sim 30k\ Hz)=\varepsilon_{V,1k}-\varepsilon_{V,f-\Delta f}\times PP\frac{f_f-f_{1k}}{f_{100k}}$$

$$\varepsilon_{V,f}(31k\sim 100k\ Hz)=\varepsilon_{V,30k}-\varepsilon_{V,f-\Delta f}\times PP\frac{f_f-f_{30k}}{f_{100k}}$$

f : frequency
PC : conductivity parameter whose value depends on the operational voltage
PP : permittivity parameter whose value depends on the operational voltage
$CRIC$: closing rate of the ionic channel
Δf : frequency step

Results and Discussion-
Experiment, Simulation & Equations

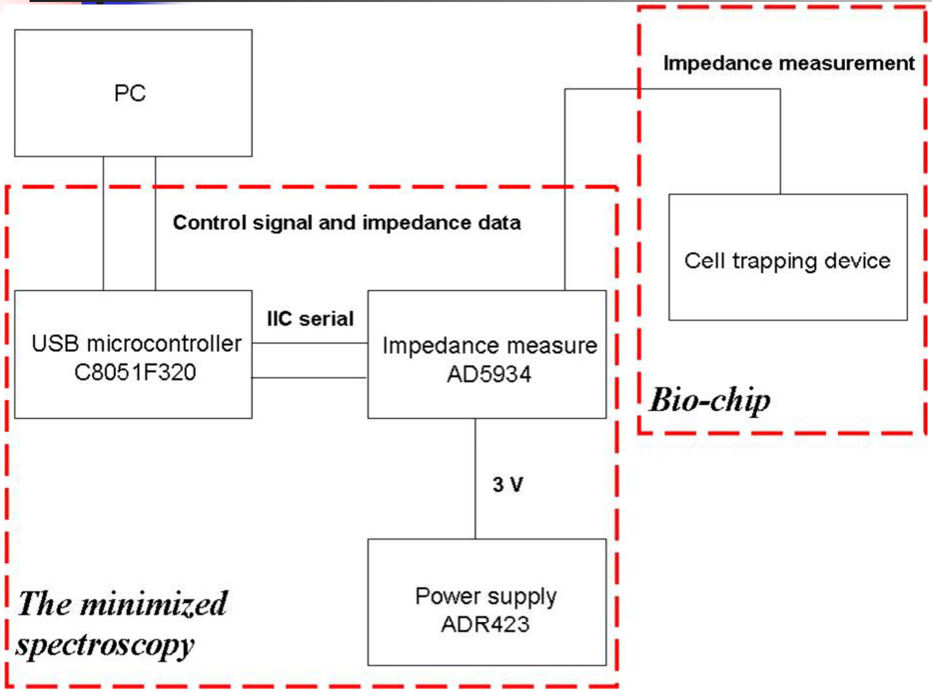


(a) (b)

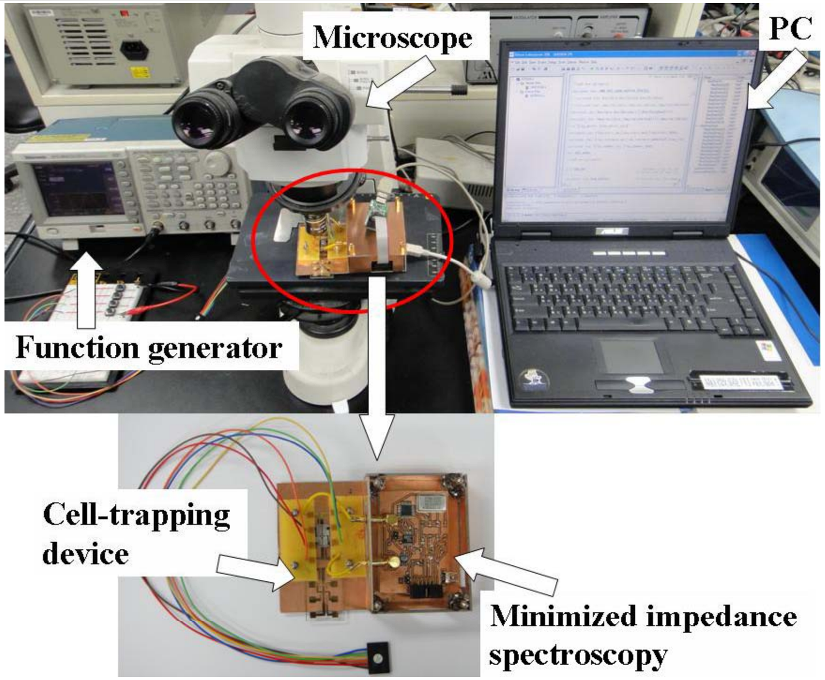
Variation of (a) magnitude and (b) phase of HeLa impedance signals as obtained experimentally, numerically and analytically for operational voltages of 0.2 V, 0.6 V and 1 V and operational frequencies in the range 5~100 kHz.

1.the simulated and predicted results for the magnitude and phase are in good agreement with the experimental observations at voltages of 0.2 V and 0.6 V.
2. At a higher voltage of 1.0 V, the predicted values deviate noticeably from the measurement results.

Portable impedance spectroscopy



Function block of impedance spectroscopy system



Photograph of impedance spectroscopy system.

1.The minimized impedance spectroscopy consists of a power supply chip, an impedance measurement chip, a USB microcontroller chip.
2. The portable system has dimensions of 10 × 8.5 × 3 cm (length × width × height)
3. The measurement accuracy and reliability obtained by the proposed system and a conventional precision impedance analyzer are compared.

Portable impedance spectroscopy - Calibration

Gain Factor

Step 1

$$Magnitude_{Known} = \sqrt{\left(R^2 + I^2\right)} = Impedance_{Known}$$

Step 2

$$Gain\ Factor = \frac{\left(\frac{1}{Impedance_{Known}}\right)}{Magnitude_{Known}}$$

Step 3

$$Impedance_{Unknown} = \frac{1}{Gain\ Factor \times Magnitude_{Unknown}}$$

Biquadratic fitting

Step 1

$$Magnitude_{Known} = \sqrt{\left(R^2 + I^2\right)} = Impedance_{Known}$$

Step 2

$$f\left(x = Magnitude_{1,\ 2.2,\ 3.3,\ 5,\ 6.8,\ 10,13\ \mathrm{k}\Omega}\right)$$

$$= ax^4 + bx^3 + cx^2 + dx + e$$

Step 3

$$Impedance_{Unknown} = f\left(Magnitude_{Unknown}\right)$$

Portable impedance spectroscopy - Accuracy & Reliability

Comparison of measurement results obtained from conventional precision impedance analyzer and the minimized impedance spectroscopy for 1.5 kΩ and 9 kΩ resistors at operating voltage of 0.5 V and frequency range from 11 to 101 kHz.

Frequency (Hz)	conventional precision impedance analyzer (1.5 kΩ)	Minimized impedance spectroscopy -Biquadratic Fitting (1.5 kΩ)	Minimized impedance spectroscopy - Gain Factor (1.5 kΩ)	conventional precision impedance analyzer (9 kΩ)	Minimized impedance spectroscopy -Biquadratic Fitting (9 kΩ)	Minimized impedance spectroscopy - Gain Factor (9 kΩ)
11000	1497.65	1460.42	2097.19	8931.60	9016.89	8303.65
21000	1497.65	1458.01	2094.07	8931.60	9008.27	8317.22
31000	1497.70	1462.01	2094.82	8931.80	9010.99	8289.20
41000	1497.75	1459.78	2092.72	8931.40	9007.56	8314.45
51000	1497.70	1457.23	2091.05	8931.20	9011.64	8323.90
61000	1497.75	1460.59	2092.44	8931.00	9016.34	8328.58
71000	1497.70	1460.75	2092.72	8930.80	9030.59	8297.89
81000	1497.75	1458.87	2094.35	8930.40	9018.34	8313.42
91000	1497.75	1459.33	2096.14	8930.40	9024.87	8311.38
101000	1497.75	1460.67	2099.14	8929.60	9005.22	8294.67

The reliability of the minimized impedance spectroscopy with 1 kΩ, 2.2 kΩ, 3.3 kΩ, 5 kΩ, 6.8 kΩ, 10 kΩ and 13 kΩ resistors at operating voltage of 0.5 V and frequency range from 11 to 101 kHz.

Frequency (Hz)	1 kΩ	2.2 kΩ	3.3 kΩ	5 kΩ	6.8 kΩ	10 kΩ	13 kΩ
11000	**99.84%**	**99.81%**	**99.93%**	**99.39%**	**99.88%**	**99.35%**	**99.01%**
21000	**99.84%**	**99.70%**	**99.44%**	**99.22%**	**99.50%**	**98.96%**	**99.50%**
31000	**99.71%**	**99.72%**	**99.33%**	**99.29%**	**99.70%**	**99.73%**	**99.45%**
41000	**99.81%**	**99.64%**	**99.57%**	**99.40%**	**99.92%**	**99.61%**	**100.00%**
51000	**99.99%**	**99.55%**	**99.40%**	**99.44%**	**99.92%**	**99.37%**	**99.27%**
61000	**99.87%**	**99.80%**	**99.46%**	**99.51%**	**99.18%**	**98.85%**	**98.54%**
71000	**99.59%**	**99.58%**	**99.00%**	**99.19%**	**99.50%**	**98.20%**	**99.53%**
81000	**99.91%**	**99.60%**	**99.55%**	**99.49%**	**99.84%**	**99.08%**	**100.00%**
91000	**99.90%**	**99.72%**	**99.59%**	**99.92%**	**99.95%**	**99.48%**	**99.25%**
101000	**99.71%**	**99.94%**	**99.80%**	**99.72%**	**99.64%**	**99.16%**	**99.22%**

1.After the calibration by “Biquadratic Fitting”, the minimum measurement accuracy of the minimized impedance spectroscopy with 1.5 kΩ is improved from 71.35% to 97.30%.
2.The minimum reliability of the proposed system for all resistors is 98.20% at an operating voltage of 0.5 V and in a frequency range of 11 to 101 kHz.

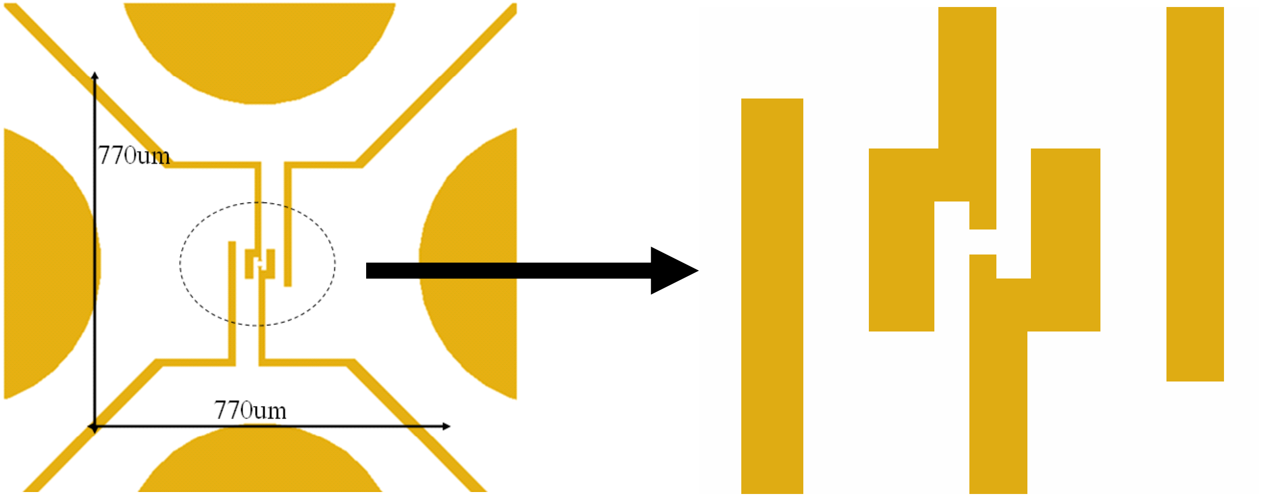
Portable impedance spectroscopy - Condition of portable impedance spectroscopy

- Function generator : Tektronix, AFG3022
  - Operation Voltage : 5 Vpp ~ 10 Vpp
  - Frequency :5 MHz

- Cell type: HeLa cell & MCF-7 cell
  - Cell concentration : 6×10^5 cells/cc

- Microscope : Nikon Eclipse 50i

Portable impedance spectroscopy - DEP cell-trapping device



Schematic view of the cell-trapping device with with Au plane electric microwell and measurement electrodes .

1.Voltages of 5 Vpp - 10 Vpp at 5 MHz produced by a function generator were applied to the quadrupole electrodes in the manipulation process.

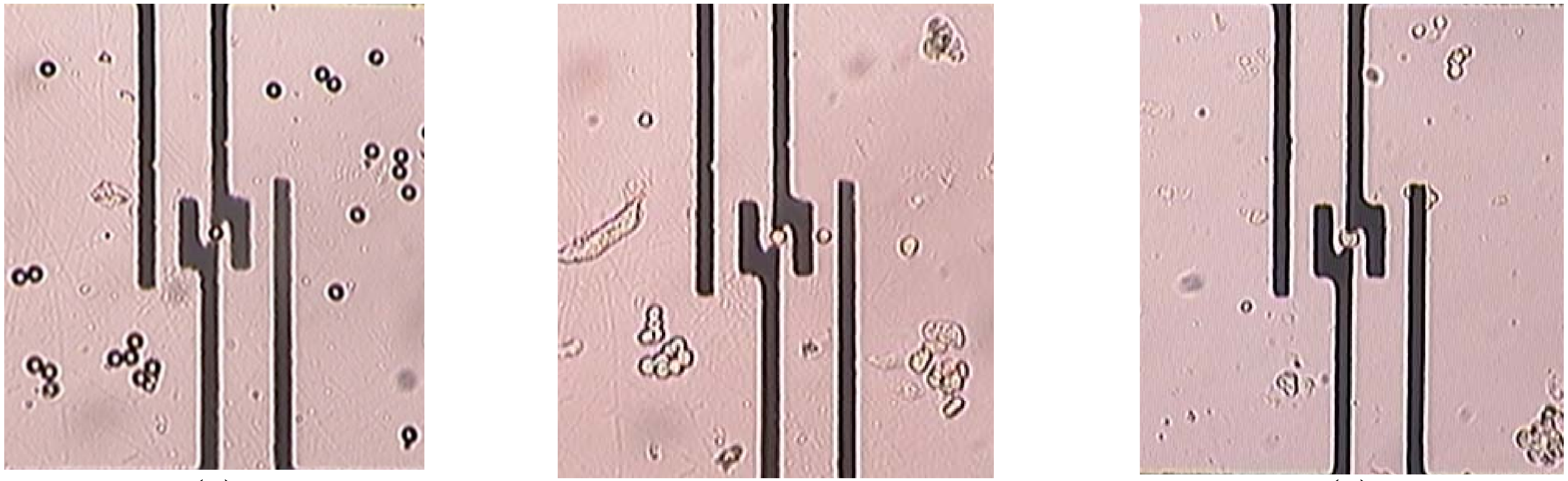
2.The quadrupole electrodes were energized to accumulate single latex beads or single cells at the center.

3.When a latex bead or single cell approached the microwell electrodes, the applied voltage was reduced to prevent the particle from passing through the microwell electrodes.

4.After a latex bead or single cell was positioned in the microwell electrodes, the energized electrodes were turned off.

5.Finally, the microwell electrodes trap was activated by applying an AC electric field of 5 MHz and 10 Vpp to capture the particle.

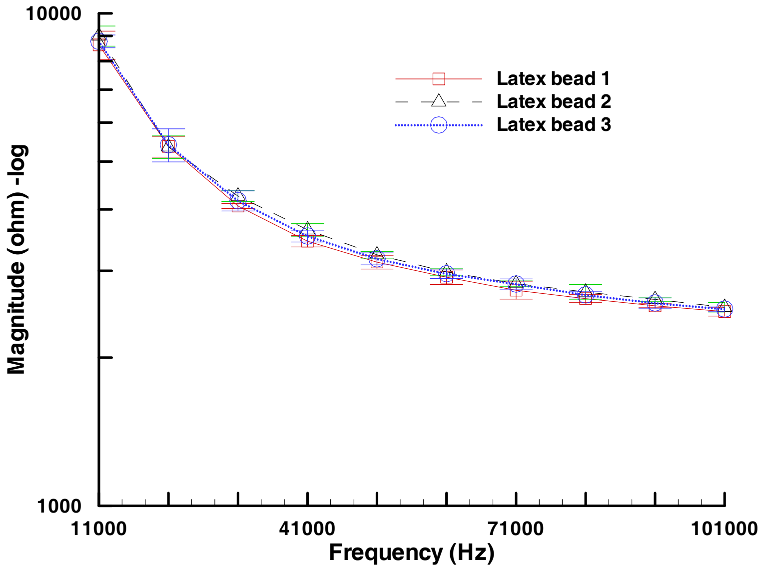
Portable impedance spectroscopy - DEP cell-trapping



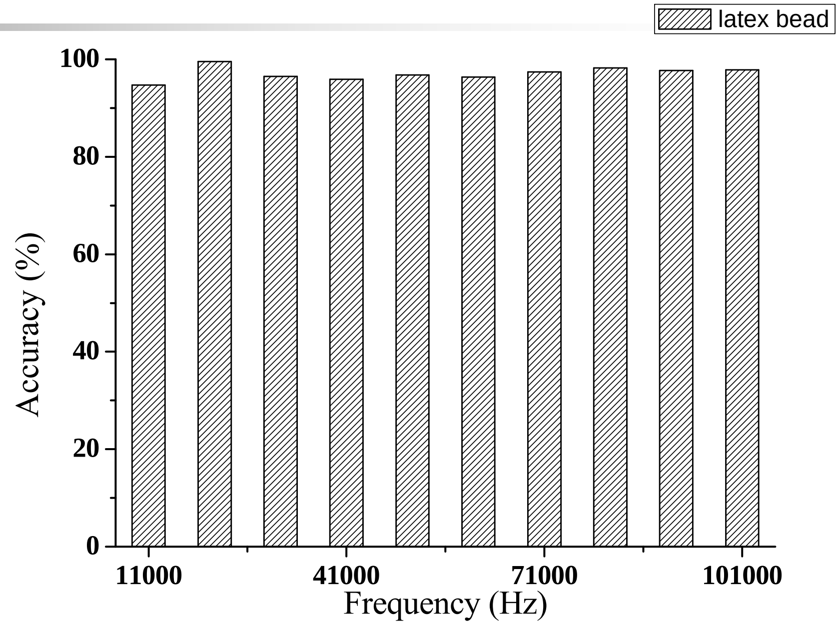
(a) (b) (c)

(a) The single latex bead, (b) the single HeLa cell and (c) the single MCF-7 cell trapping by plane electric microwell.

Results and Discussion- latex beads



(a)

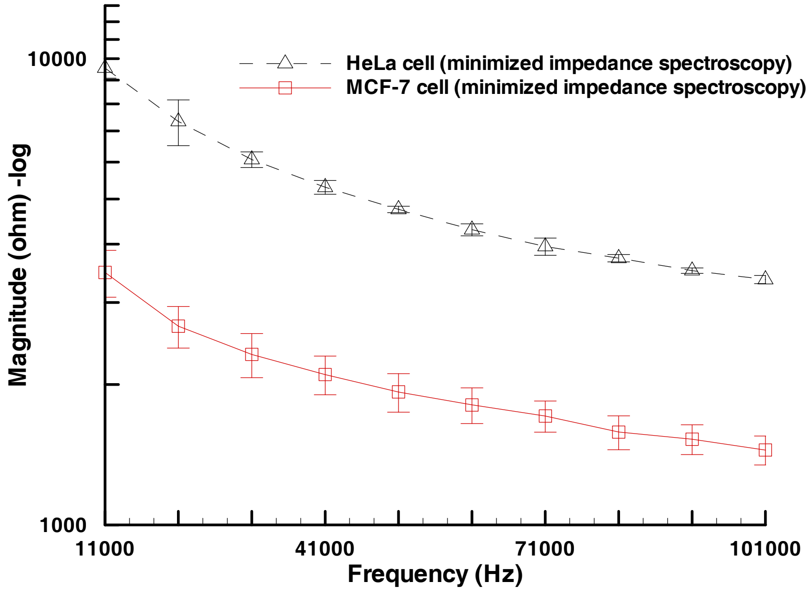


(b)

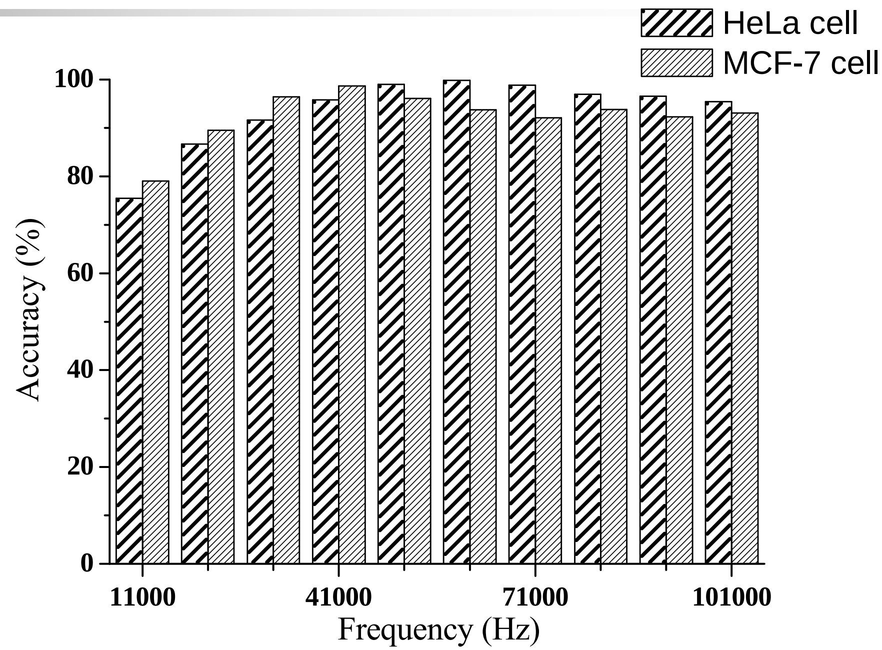
(a) Magnitude and (b) accuracy of three single latex beads using the minimized impedance spectroscopy at operating voltage of 0.5 V and frequency range from 11 to 101 kHz.

1. The certified mean diameter and size distribution Std. Dev of latex beads were 15.02 μm ± 0.08 μm and 0.15 μm (1.0%), respectively.
2. The percentage of impedance variation between the three single latex beads is 2.04% at operating voltage of 0.5 V and frequency of 101 kHz.

Results and Discussion-
HeLa & MCF-7



(a)



(b)

(a) Magnitude and (b) accuracy of single HeLa cells and single MCF-7 cells using the minimized impedance spectroscopy at operating voltage of 0.5 V and frequency range from 11 to 101 kHz.

1.. A significant difference was observed between the single HeLa cell line and the single MCF-7 cell line.
2.The magnitude of single MCF-7 cells is smaller than that of single HeLa cells about three times at operating voltage of 0.5 V and frequency from 11 to 101 kHz.
3. Increasing the frequency reduces the impedance of single HeLa cells and single MCF-7 cells

Conclusions

- The experiments reveal that the single HeLa cell is successfully captured by the micro pillars.
- The results of the HeLa cell experiments show that the magnitude of single HeLa cells declines as the frequency increases at all operation voltages
- Increasing the operating voltage reduces the magnitude of the HeLa cell impedance
- The phase of the HeLa cell impedance is characteristics of a series circuit when the operating voltage exceeded 0.8V because Zcell became significant.

Conclusions

- The conductivity of single HeLa cells increase with an increasing voltage.
- At high operational voltages, the permittivity of the cell reduces rapidly with an increasing frequency
- The experimental results show that the measurement accuracy and reliability of the minimized impedance spectroscopy is acceptable.
- Significant difference could be observed between the single HeLa cell line and the single MCF-7 cell line.

Furture work

- Electrical characterizations of cell growth
- Portable system
- Impedance analysis of protein

Thanks for your attention!