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Sony Corporation
Tokyo Medical and Dental University
Japan Science and Technology Agency (JST)

New technology to analyze and sample single cells without using labeling material

— Discrimination of different cancer cells through an original electric measurement technology —

In the framework of JST's program "Development of Advanced Measurement and Analysis Systems", Sony Corporation and Tokyo Medical and Dental University have developed the world first technology enabling discrimination of single cells without labeling material by using the difference of their electric properties.

Recently, analysis and sampling of single cells have become more important for studies of regenerative medicine or cell transformations induced by diseases, for example. To bring regenerative medicine into clinical practice, precise sorting of stem cells from other cells is critical. Also, to understand the mechanisms of diseases like cancer, pathological change of normal cells must be thoroughly traced. The instruments commonly used for analysis of single cells are flow cytometers, which Sony, among other manufacturers, is developing and selling. Flow cytometers enable fast, accurate analysis of cells, color-coded with labeling material such as fluorescent dyes, and sampling of only targeted ones. There is another demand, however, for analysis and sampling of a certain number of cell types, for which no color-coding reagent is available. Thus, recently, a few instruments that discriminate single cells through analysis of their electric impedances (*1) have been developed. With conventional technologies, however, impedances at only two frequencies can be analyzed, and the information obtainable from them is limited to the size and density of a cell.

Sony original technology enables simultaneous analysis of impedances at 16 frequencies for single cells passing through the detector in one millisecond. In addition, by optimizing the fluidic structure and electrode configuration of the microfluidic chip (*2), in which cells flow, measurement noises have been drastically reduced. With these realized, the information on the difference or change of the electric properties of the membrane and interior of a cell is obtainable through analysis of the dielectric spectrum (*3) calculated from the frequency dependent impedance for each cell.

In collaboration with Professor Shuki Mizutani's group at Tokyo Medical and Dental University, we succeeded in discriminating two types of cancer cell lines solely on the basis of the difference in dielectric spectrum.

Furthermore, for sorting and sampling of cells after they are analyzed, the technology to control the flow direction of cells by applying an electric field upon the fluidic channel has been developed. This has been achieved by flow simulations and optimizations of electrode structure and other parameters in the fluidic channel. The simulations were used to precisely predict the motion of cells flowing in the electric field, according to a technology developed in collaboration with Professor Kazuyoshi Nakabe's group at Kyoto University.

In the future, on the basis of these achievements, it is expected that a variety of cells analyzed, sorted, and sampled without using labeling material can be practically used asneeded in life science researches such as regenerative medicine, immunology, and so on.

These achievements are presented at the international conference "μTAS 2011" in Seattle, USA from October 2nd (PDT), 2011. Also, a prototype of the instrument is exhibited at "Bio Japan 2011" in Pacifico Yokohama from October 5th (Japan Time).

These achievements have been obtained in the following framework.

Program : Development of Advanced Measurement and Analysis Systems
Project : Development of dielectric spectro-cytometer
Team leader : Shinji Omori
(Life Science Laboratory, Advanced Materials Laboratories, Sony Corporation)
Period : 2009 – 2013 (as scheduled)
Supervisor : Yuzuru Fushimi
(Research Professor at Saitama University Research Management Bureau)

In this program, JST aims to develop measurement and analysis systems and related systems that meet requirements from leading-edge researches.

<Background>

Recently, in medical and biological researches, some leading-edge studies focus on realization of regenerative medicine, cell therapy, and genetic diagnosis. For cell/gene-based researches and diagnoses, the information from cell analysis is important. One of the instruments commonly used for cell analysis in medical and biological researches is a flow cytometer. it analyzes with high throughput cells labeled with fluorescent dyes flowing in a narrow channel by irradiating laser light. Because this method requires labeling of cells with dyes specific to the cell types in advance, analysis and sampling of cells in native states is impossible.

Sony has been developing, since 2009, a new analysis system named dielectric spectro-cytometer in collaboration with Tokyo Medical and Dental University in the framework of JST's program "Development of Advanced Measurement and Analysis Systems". In this project, we aim at developing the technology to analyze and sample single cells without labeling material like fluorescent dyes by using the difference of their electric properties (Figures 1 and 2).

<Technological Achievements>

□ Development of ultra-fast impedance analyzer and microfluidic chip

In this project so far, analysis of dielectric spectra of single cells has been achieved for the first time in the world by measuring their impedances over multiple frequencies. On the basis of the dielectric spectra from cells, two types of cancer cells can be discriminated (Figure 3).

This was realized by the combination of two original technologies described below.

1. Simultaneous measurement and analysis of impedances over multiple frequencies

In conventional technologies, impedances are measured only at two frequencies for single cells passing through the detector in one millisecond. In this program, we have developed an original analyzer that enables fast, accurate measurement and analysis of impedances at 16 frequencies at most. This provides the information on the difference and change of the electric properties of the membrane and interior of a cell, in addition to the information on the size and density of the cell obtainable by conventional methods.

2. Optimization of the microfluidic structure and electrode configuration

In conventional methods, the relevant dielectric spectrum cannot be accurately measured, because the measured impedance is greatly affected by parasitic signal from ions in the sample solution. On the other hand, in our system, the accuracy in determining the dielectric spectrum of the cell is increased, because the signal from ions is suppressed by optimizing the structure and electrode configuration of the microfluidic chip in which cells are flowing cells are detected (Figure 2).

 Construction of a prototype

We have developed the ultra-fast impedance analyzer and detector with embedded electrodes inside the microfluidic chip and constructed the prototype of an automated analysis system (Figure 4). The microfluidic chip consists of polymer films originally used for flexible substrates for circuitries in electronics products. This allows low-cost, large volume production through use of industrial fabrication processes for circuit substrates. Therefore, the chip is cheap and thus disposable for each sample to avoid sample contamination and other problems (Figure 2).

This prototype is exhibited in the JST booth at Bio Japan 2011 in Pacifico Yokohama from October 5th to 7th.

 Development of cell sorter

We are also developing the technology to sort single cells according to the difference or change of their electric properties (Figure 1). Namely, after a certain cell passed through the detector, the flow direction of the cell is controlled by applying a voltage.. For the design of the cell sorter, we needed to perform numerical simulation, taking into account various factors such as fluidic structure, flow, electric force and heat originating from the electrodes, and so on.

In collaboration with Professor Kazuyoshi Nakabe's group at Kyoto University, Sony has developed the simulator that precisely predicts the motion of a cell and confirmed that the simulation and experimental results agree well (Figure 4). This leaded to the design for integration of the detector and cell sorter into a single microfluidic chip (Figure 2).

<Perspectives>

We will complete a new prototype that integrates cell analysis and sorting functions. Taking the advantage that cells are analyzed and sorted without labeling material, this instrument is applicable to a wide area of medical and biological researches including regenerative medicine, cell therapy, and drug screening.

In this project, the location of Sony's hardware development is placed in the open laboratory inside the campus of Tokyo Medical and Dental University, a project partner, to establish tight collaboration between academia and industry and between medicine and engineering. In this scheme, we are not only developing the instrument but also exploring applications for dielectric spectro-cytometer. We will disclose the results of evaluation tests and call for opinions about possible applications of the instrument from a broad society of related experts.

<Figures>

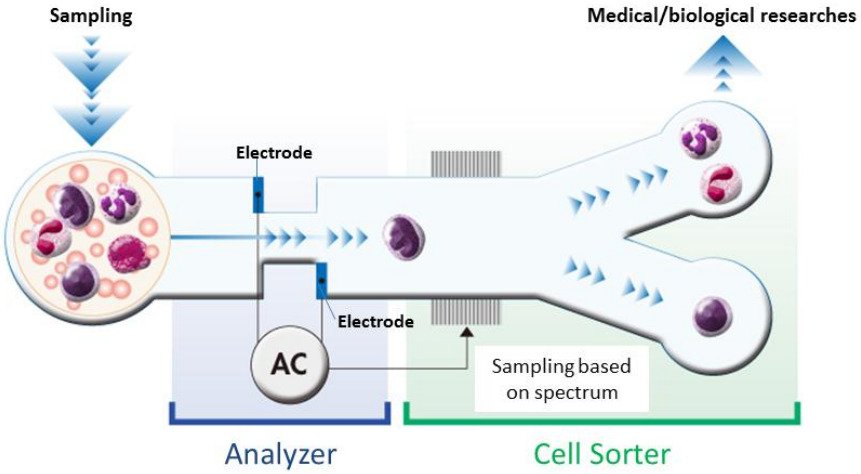

Figure 1. Principle of dielectric spectro-cytometer.

Solution containing cells of different types are flown into the fluidic channel, and the dielectric spectra of single cells passing through the gap between two electrodes are analyzed with the impedance analyzer (labeled "AC" in the figure). On the basis of the analysis, they are judged to be either necessary or unnecessary and sorted into different chambers (the branched part labeled "Cell Sorter" on the right-hand side of the figure).

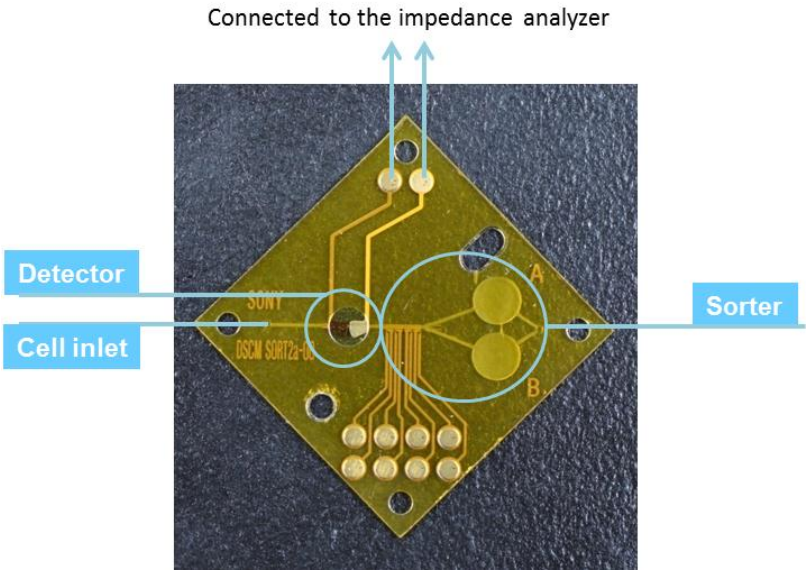

Figure 2. Microfluidic chip that integrates cell analysis and sorting functions.

Fluidic channels as thin as or even thinner than hairs are combined to provide a pathway for cells. In the middle of the channel, the detector that consists of two electrodes is embedded for impedance measurement. After the detector, there are electrodes for cell sorting. Cells flow into either chamber A or chamber B according to their cell types.

By using industrial fabrication processes for flexible substrates of electric circuits, chips can be produced in large volume with low cost and are thus disposable for different samples.

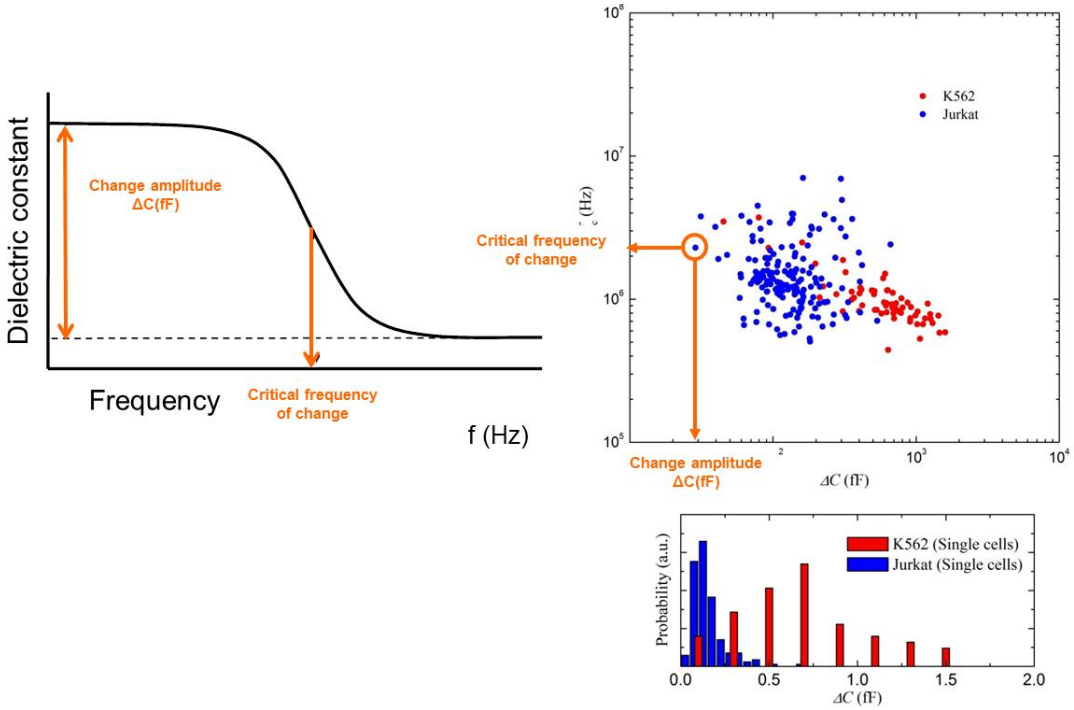

Figure 3. Discrimination of different cancer cells.

(A) Typical dielectric spectrum for a cell. It is characterized by parameters such as the amplitude and critical frequency of the spectrum. These parameters are extracted from the dielectric spectra for cells and analyzed to discriminate different cells.

(B) Distributions of two parameters for two different cancer cells shown in red and blue, respectively.

(C) Histogram to show the distributions of the amplitude. Two cancer cells have different distributions.

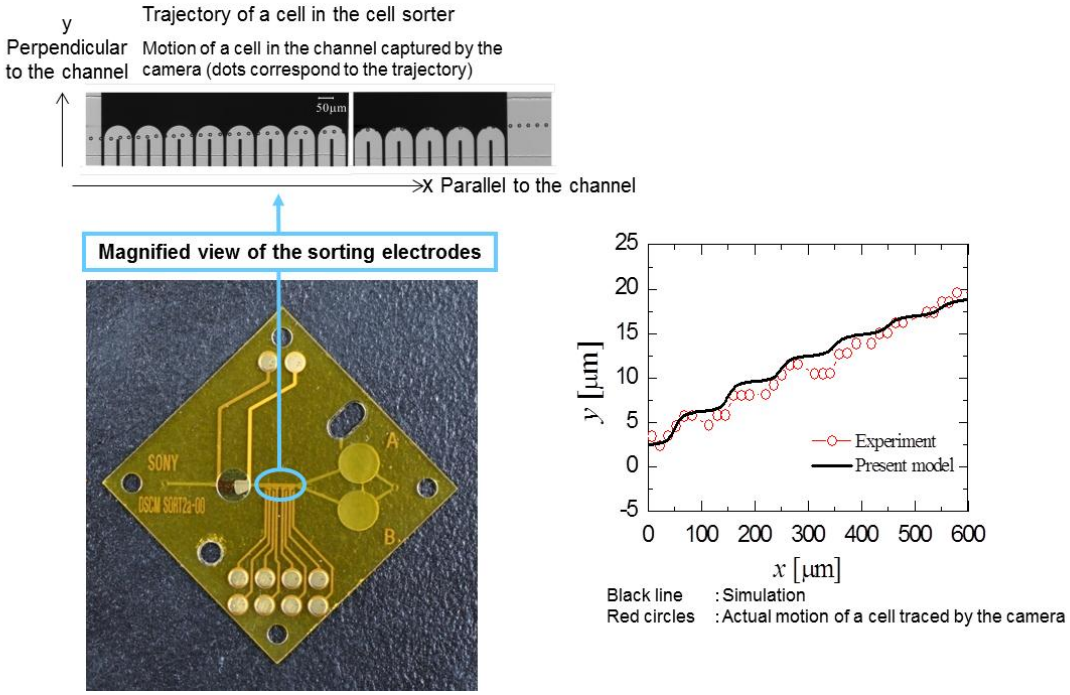

Figure 4. Cell sorter design

(A) Picture showing the trajectory that a cell is following under the electric field from the sorting electrodes in the fluidic channel.

(B) Comparison of the simulated trajectory with the experimental one. Actual motion of a cell is reproduced by simulation.

Figure 5. Picture of the prototype of dielectric spectro-cytometer.

<Terminology>

*1: Impedance

When an alternative current voltage (periodically varying voltage with a certain frequency) is applied upon a material and the current is measured, the quantity obtained by dividing the voltage by the current is called the impedance. Even though it is also called the electric resistance especially for the direct current voltage, since it is commonly called the impedance for the alternative current voltage, the term “impedance” is used throughout the text. The dielectric constant is calculated from the impedance.

*2: Microfluidic chip

A microfluidic chip is a part that allows reactions and analysis of extremely small amount of material in fluidic channels as thin as a hair.

*3: Dielectric spectrum

When a material is placed in the electric field, positive and negative charges are separated or moved by the electric force. The dielectric constant of the material represents the degree of charge separation or mobility. If measured over the wide range from low frequencies to high frequencies, the dielectric constant varies. This frequency-dependent variation is termed the dielectric spectrum. The dielectric spectrum is one of the cell’s characteristics, which depends on the size, structure, and electric properties of the constituents of the cell.

<Publication>

Conference name
The 15th International Conference on Miniaturized Systems for Chemistry and Life Sciences (μTAS 2011)

Presentation titles
“Single cell dielectric spectroscopy in a micro-channel”, coauthored by Sony and Tokyo Medical and Dental University

“Numerical model for microparticle and lymphocyte motions in dielectrophoretic manipulation device”, coauthored by Kyoto University and Sony

<References>

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