



A Label-Free Size-based Micro Coulter Counter System for Circulating Rare Tumor Cells



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INTRODUCTION

◆ Circulating Tumor Cells

- Cells that have detached from a primary tumor and circulate in the bloodstream
- Powerful tool for medical application
 - cancer prognosis, diagnosis of minimal residual disease
 - assessment of tumor sensitivity to anticancer drugs
 - personalization of anticancer therapy
- Characters
 - rare cells (about 1 – 100 cells per mL of whole blood)
 - epithelial type (EpCAM)
 - bigger size than peripheral blood cells

◆ Conventional CTC detection method

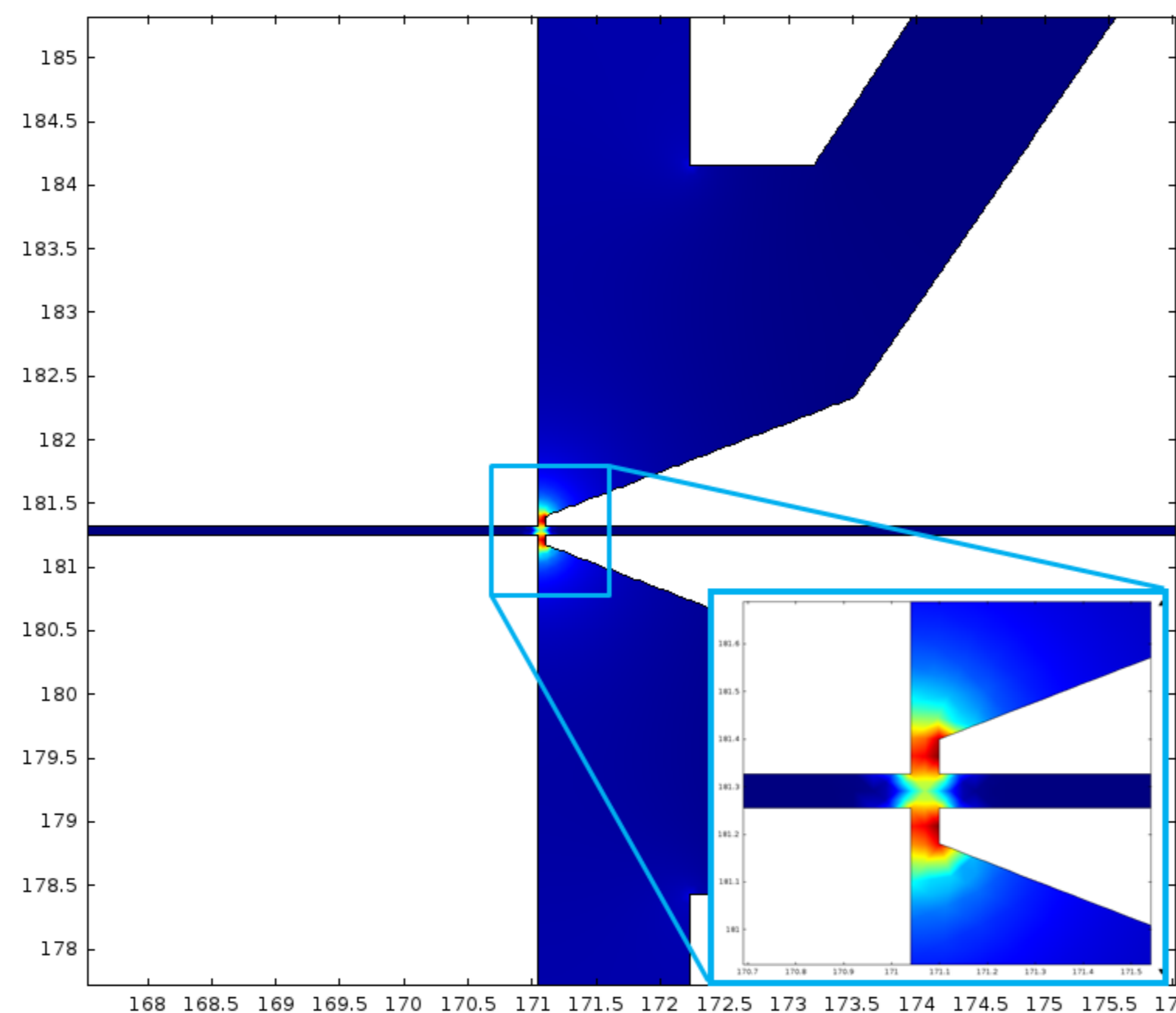
- PCR-based approaches
 - merit : very sensitive
 - demerit : low specificity (can result in false positives)
- immunoreaction-based techniques
 - merit : multi-parameter (EpCAM, CD45, CK, etc)
 - demerit : miss some CTCs (lack of exact antibody), low purity
- Size-based techniques using pores
 - merit : independent with parameters(like antibodies)
 - demerit : physical damages by high pressure

◆ Coulter counter-based CTC counting Method

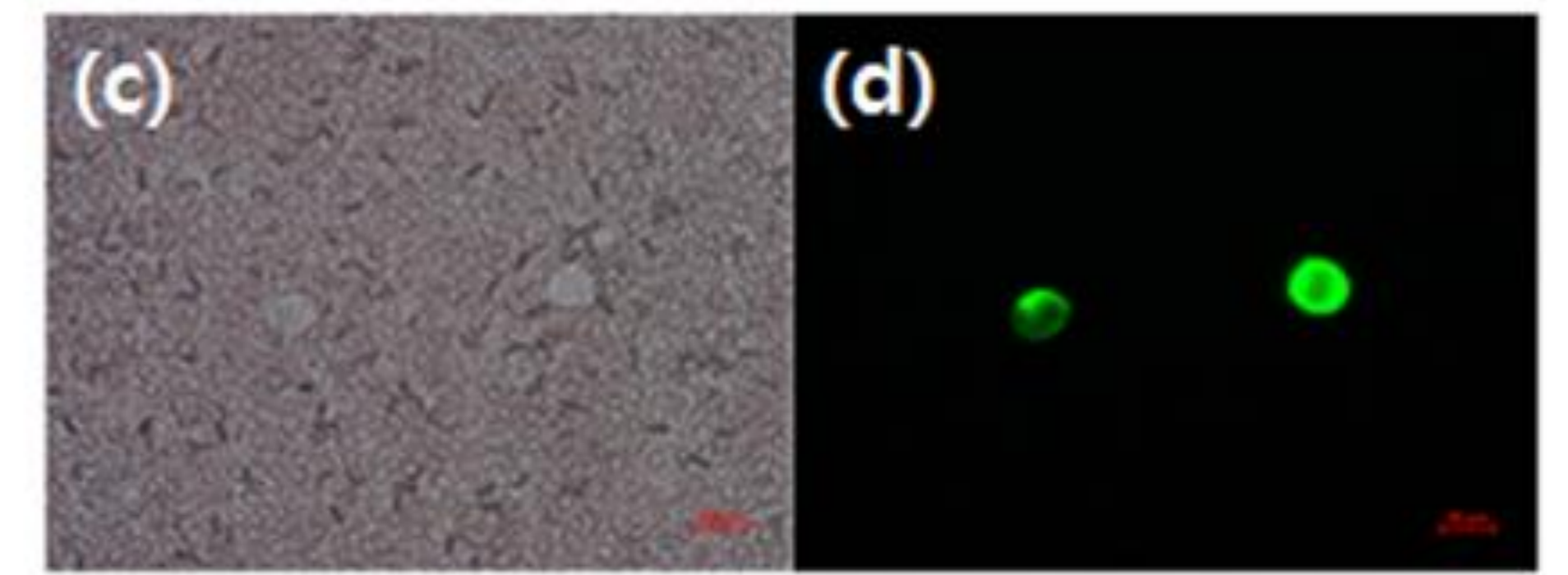
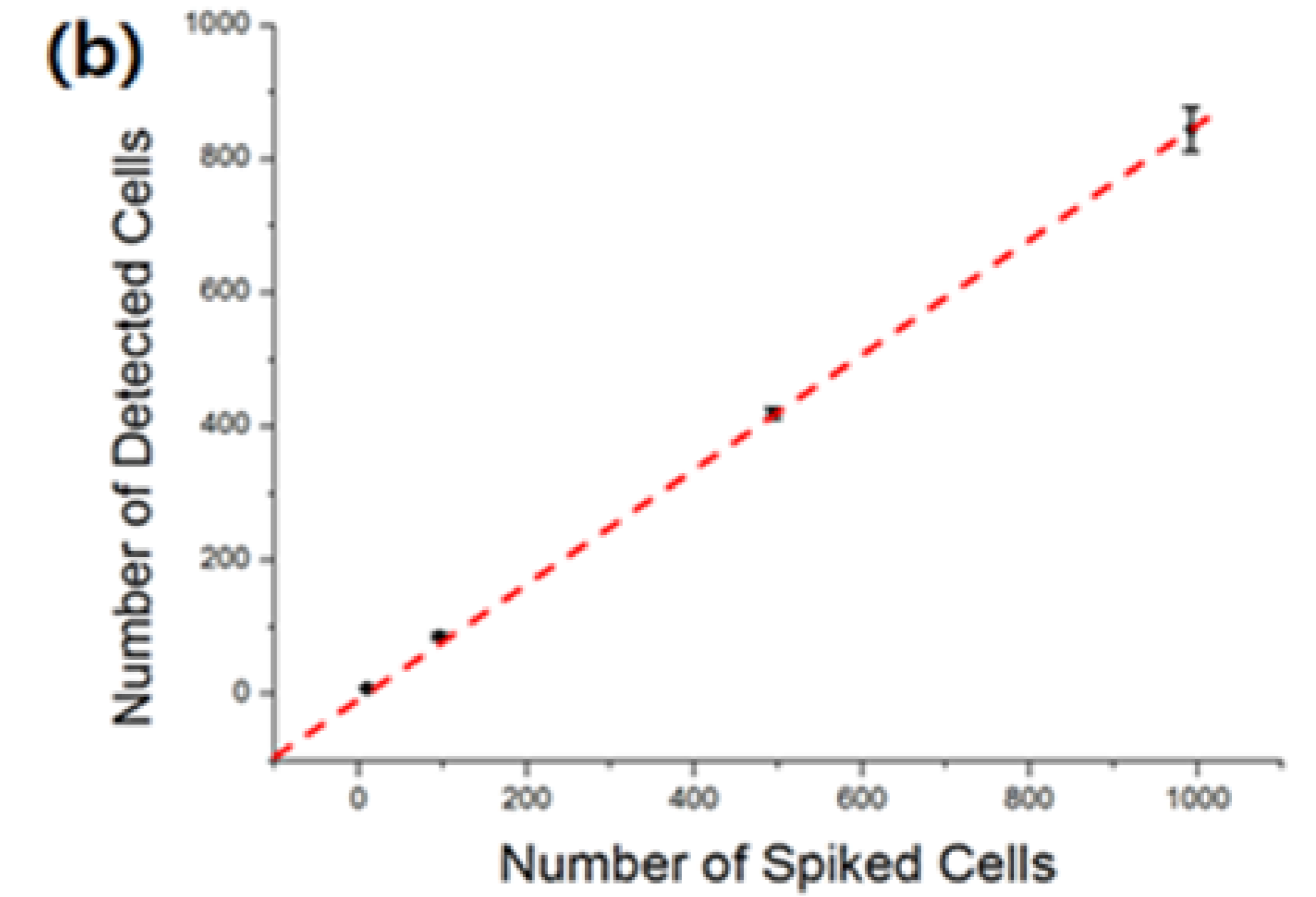
- Size-based cytometry approaches
 - merit : independent with parameters, no physical stresses, potential to sort CTCs without need a sample preparation

- Resistance measurement of the PGE–microchannel–PGE series is done with the Ag/AgCl electrodes under external voltages.
- When a cell placed in detection region, resistance is increasing, and the amplitude of the resistance change is proportional to the volume of the cell.
- Test sample
 - Normal blood : Blood samples of healthy volunteers.
 - CTC model : Ovarian cancer cell line (OVCAR-3).
 - Patient blood : Breast cancer patients from National Cancer Center (NCC).
- Experimental setup
 - Customized detection circuit
 - Signal Acquisition : DAQ card (NI USB-6009) at 10kHz
 - Self-programmed LabView software (National Instrument)

◆ Simulation of Detection Region

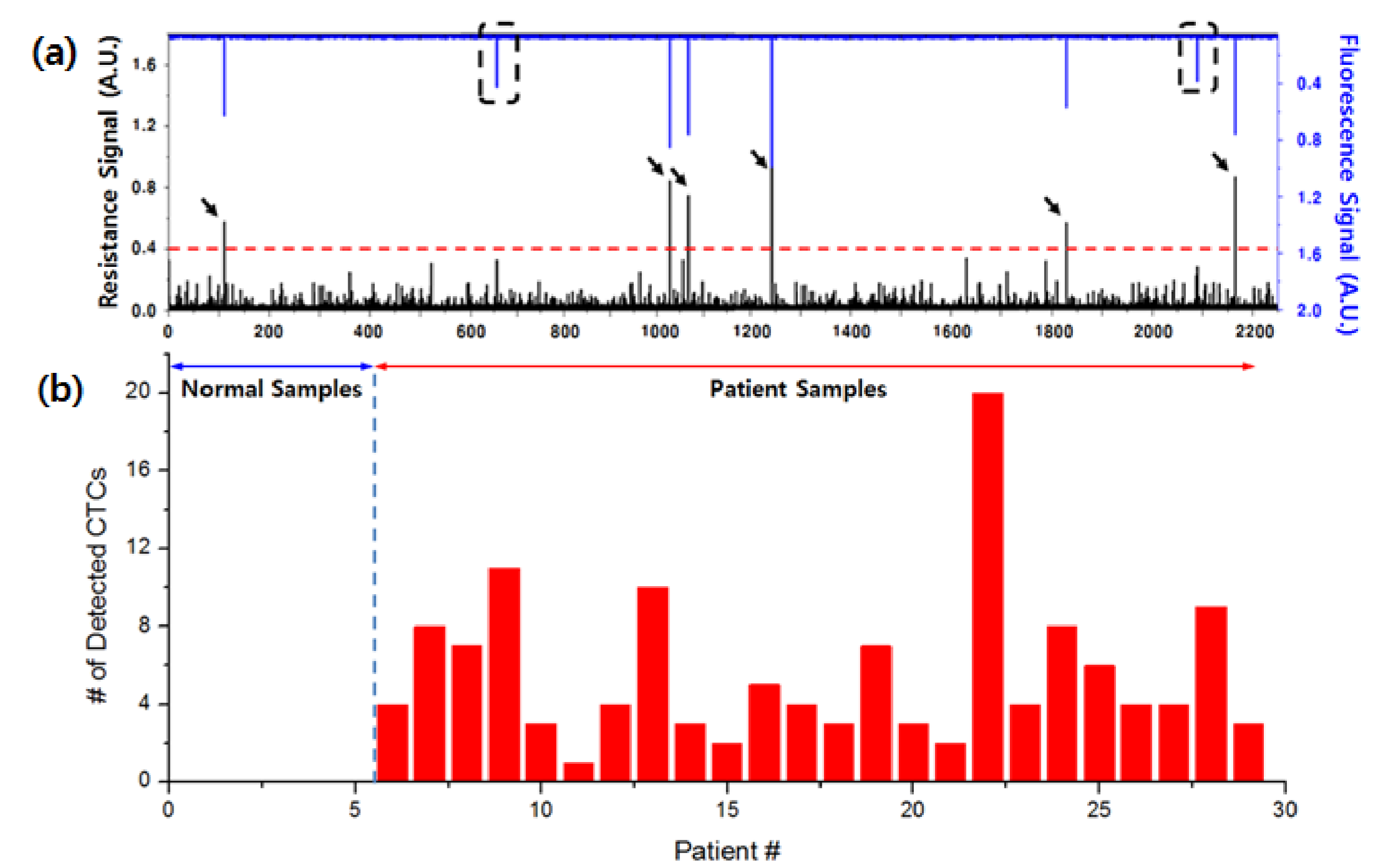


- Simulation Program : COMSOL
- Images express the electric displacement field norm (C/m^2) and electric fields are concentrated on the detection region.



- (a) Resistance change signals from PBS, normal blood samples from healthy volunteers, and OVCAR-3 cells-spiked normal blood samples.
 - Tall peak over threshold are appeared only in cancer cell spiked-blood samples.
- (b) The number of detected cells when a various number of cells (10, 100, 500, and 1000 cells) was spiked in healthy normal blood samples. (efficiency : 88%)
- (c) Images of EpCAM labeled OVCAR-3 cells in a blood sample.

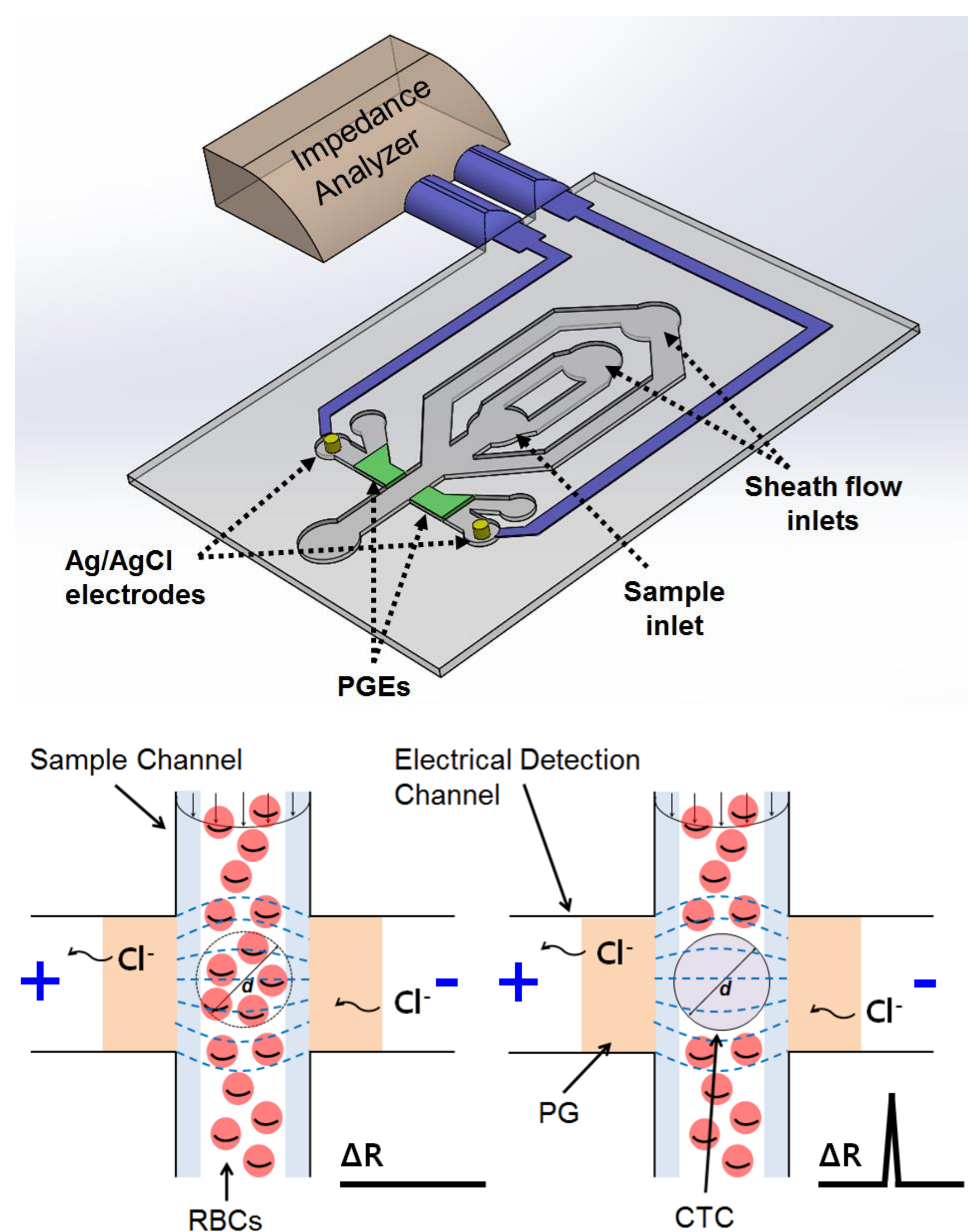
◆ Cancer patient blood samples Test



- (a) Detection of CTCs in breast cancer patient samples with resistance change and fluorescence.
 - Some fluorescence peaks were not detected by resistance change : small CTCs.
- (b) The number of tall peaks by CTCs from 5 healthy donors and 24 breast cancer patients.

METHODOLOGY

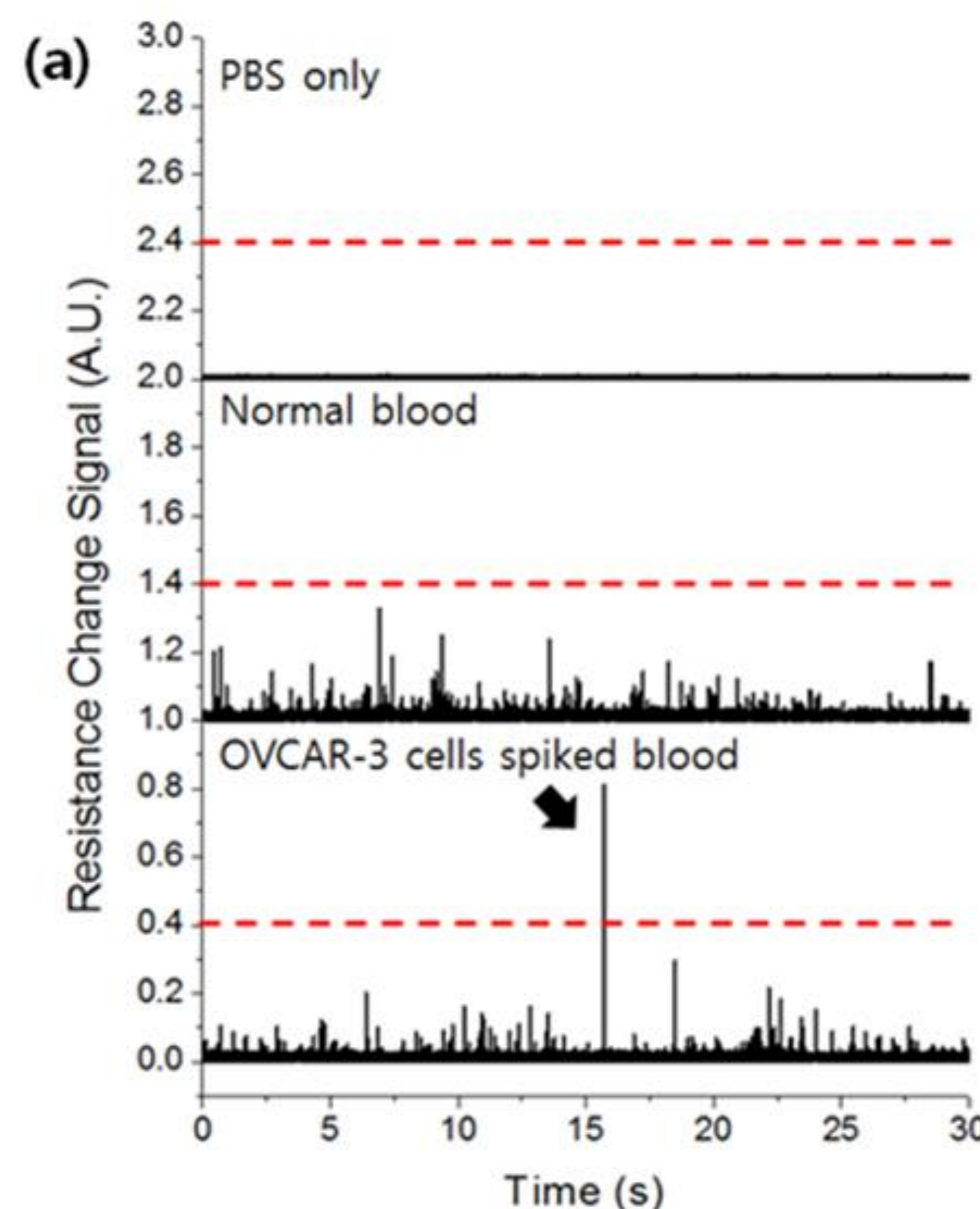
◆ Schematic Diagrams of Developed System



• Chip Dimension : 100 μm width and 30 μm height

RESULTS

◆ OVCAR-3 cells spiking Test



DISCUSSION

- Although test time for larger volume of blood is long, the simplicity of the microchannel and the system enables the system to parallelize easily.
- The proposed system has a sufficient potential to detect CTCs in the blood.
- Flow cytometry-based detection system is expected to separate CTCs from blood.

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