



DC Impedance-based Label-Free Cell Counter for Circulating Tumor Cell Detection



Hyungseon Choi¹, Kwang Bok Kim¹, Taek Dong Chung², and Hee Chan Kim^{3*}

¹Interdisciplinary Program, Bioengineering Major, Graduate School, Seoul National University, Seoul 110-744, Korea

²Department of Chemistry, Seoul National University, Seoul 151-747, Korea

³Department of Biomedical Engineering, College of Medicine and Institute of Medical & Biological Engineering, Medical Research Center, Seoul National University, Seoul 110-744, Korea

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

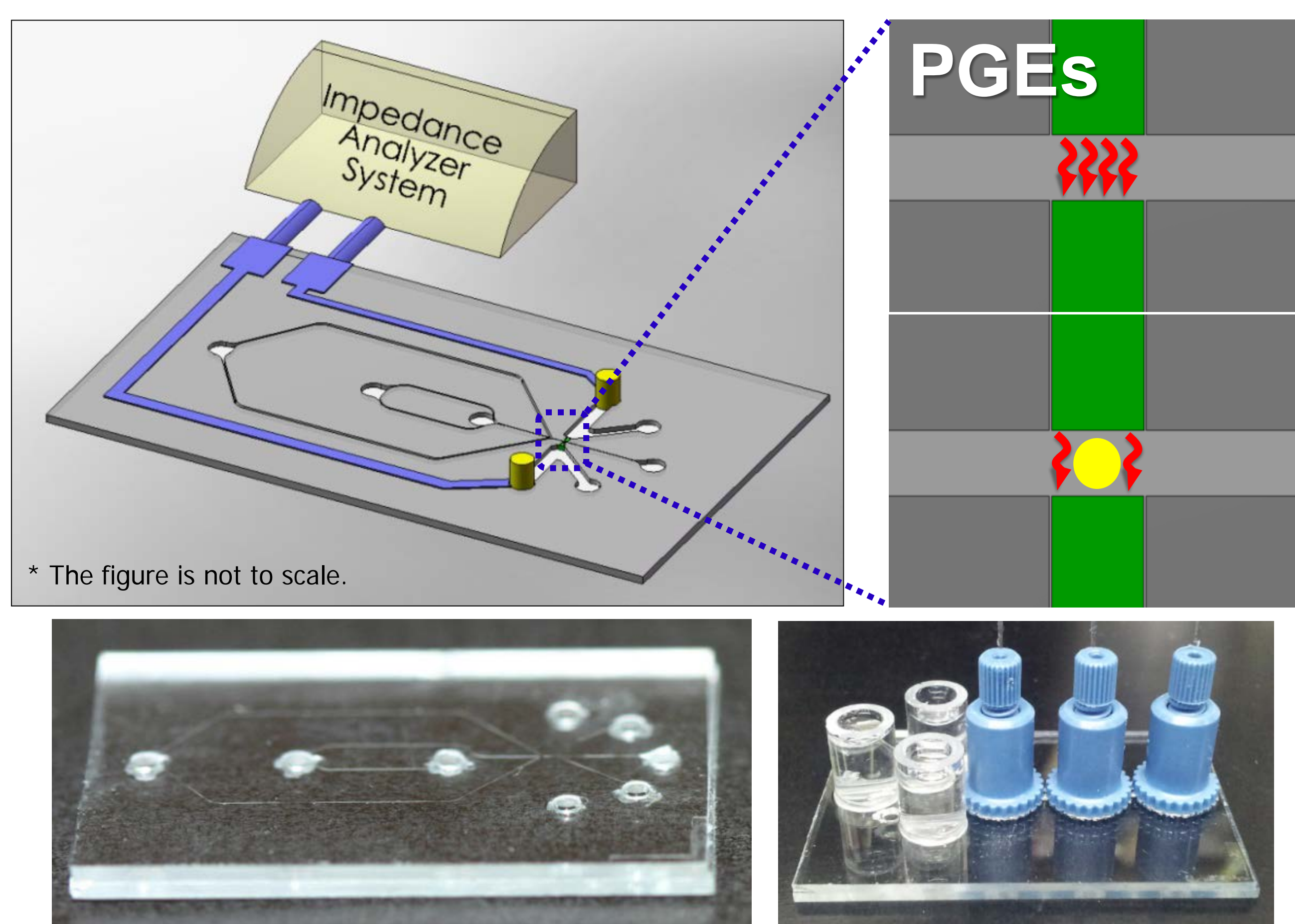
- PCR-based approaches
 - merit : very sensitive
 - demerit : low specificity (can result in false positives)
- immunoreaction-based techniques
 - merit : multi-parameter (EpCAM, CD45, CK, etc)
 - demerit : miss some CTCs (lack of exact antibody), low purity
- Size-based techniques using pores
 - merit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

◆ DC impedance-based CTC counting Method

- Size-based cytometry approaches
 - merit : independent with parameters, no physical stresses, potential to sort CTCs
no need a sample preparation

METHODOLOGY

◆ Schematic Diagrams of Developed System

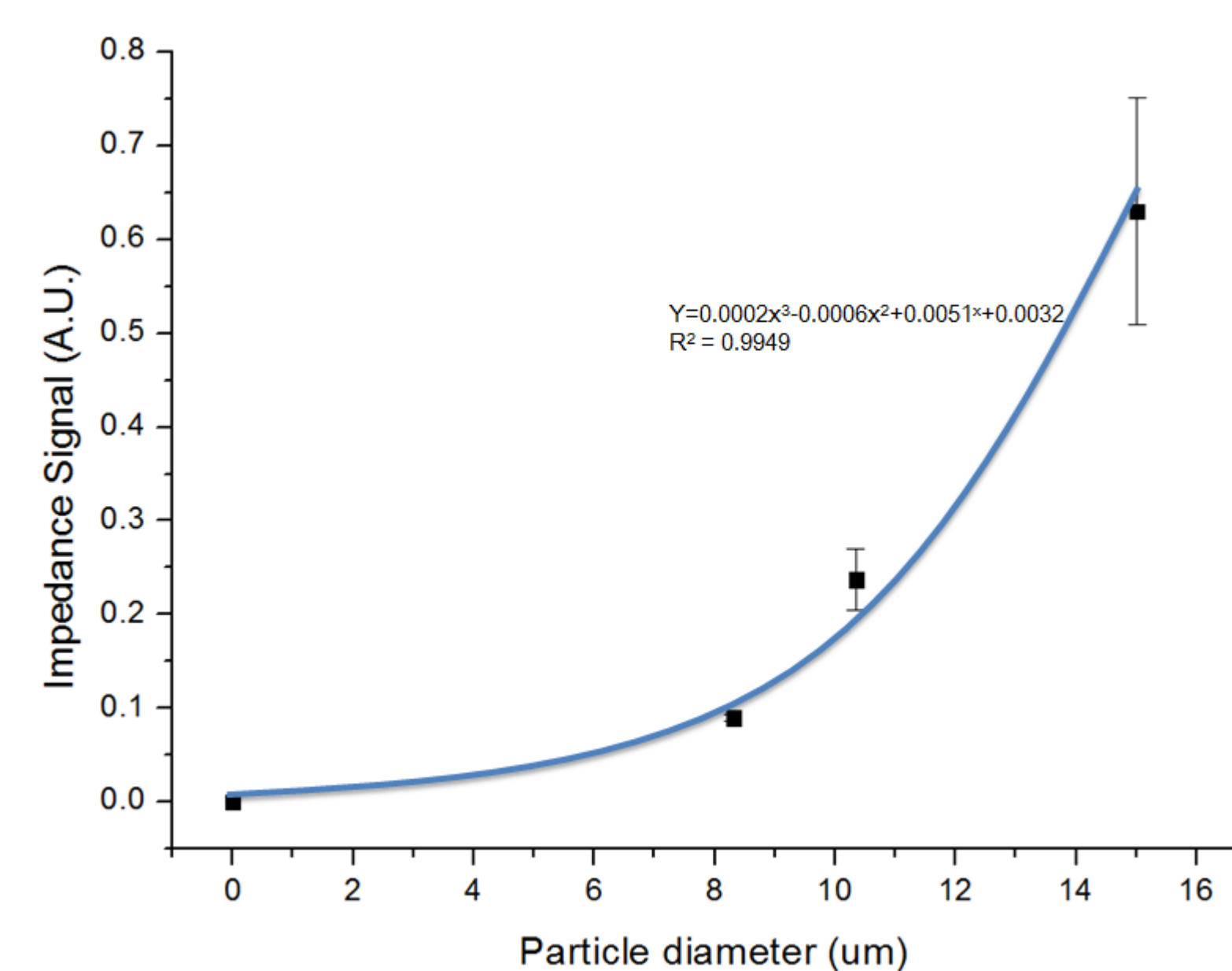


Impedance measurement of the PGE–microchannel–PGE series is done with the Ag/AgCl electrodes under external voltages.

- Double sheath flow for decreasing sample loss
- Flow rate : sheath flow : 2.5ml/h, sample flow : 0.8ml/h (By syringe pumps)
- Test sample
 - Micro-sized beads (8.31um, 10.35um, 15.02um; Bangs Laboratory)
 - CTC model : Ovarian Cancer Cell lines
 - Blood : breast cancer patients (in stage III or IV), obtained from National Cancer Center in Korea

RESULTS

◆ Calibration Curve of the DC impedance-based cytometer

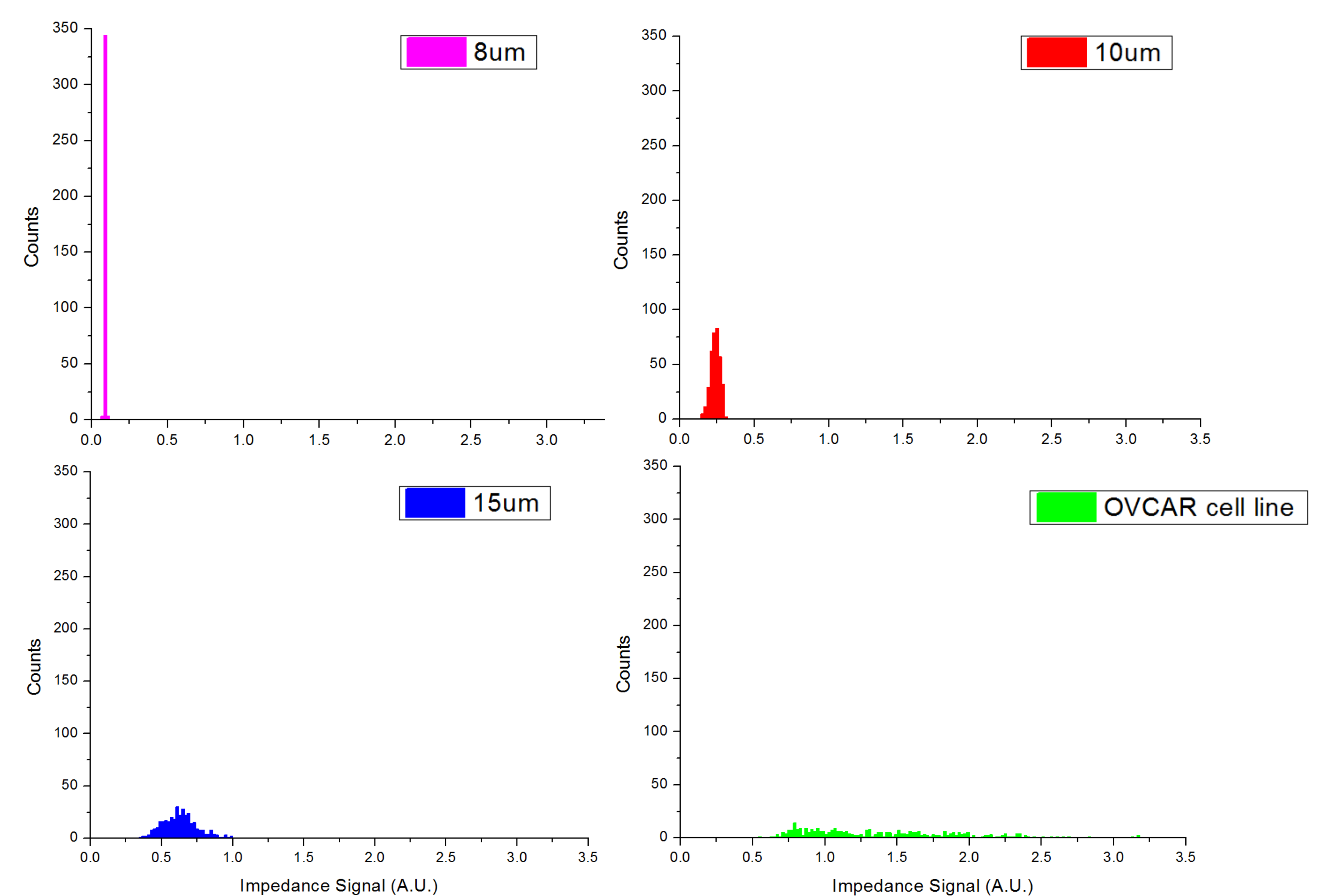


$$\Delta R = \frac{4\rho_m d_p^3}{\pi D_t^4}$$

ΔR : the resistance change of the ionic flow
 D_t : the cross area of detection region
 d_p : a diameter of a particle or a cell
 ρ_m : the resistivity of electrolyte

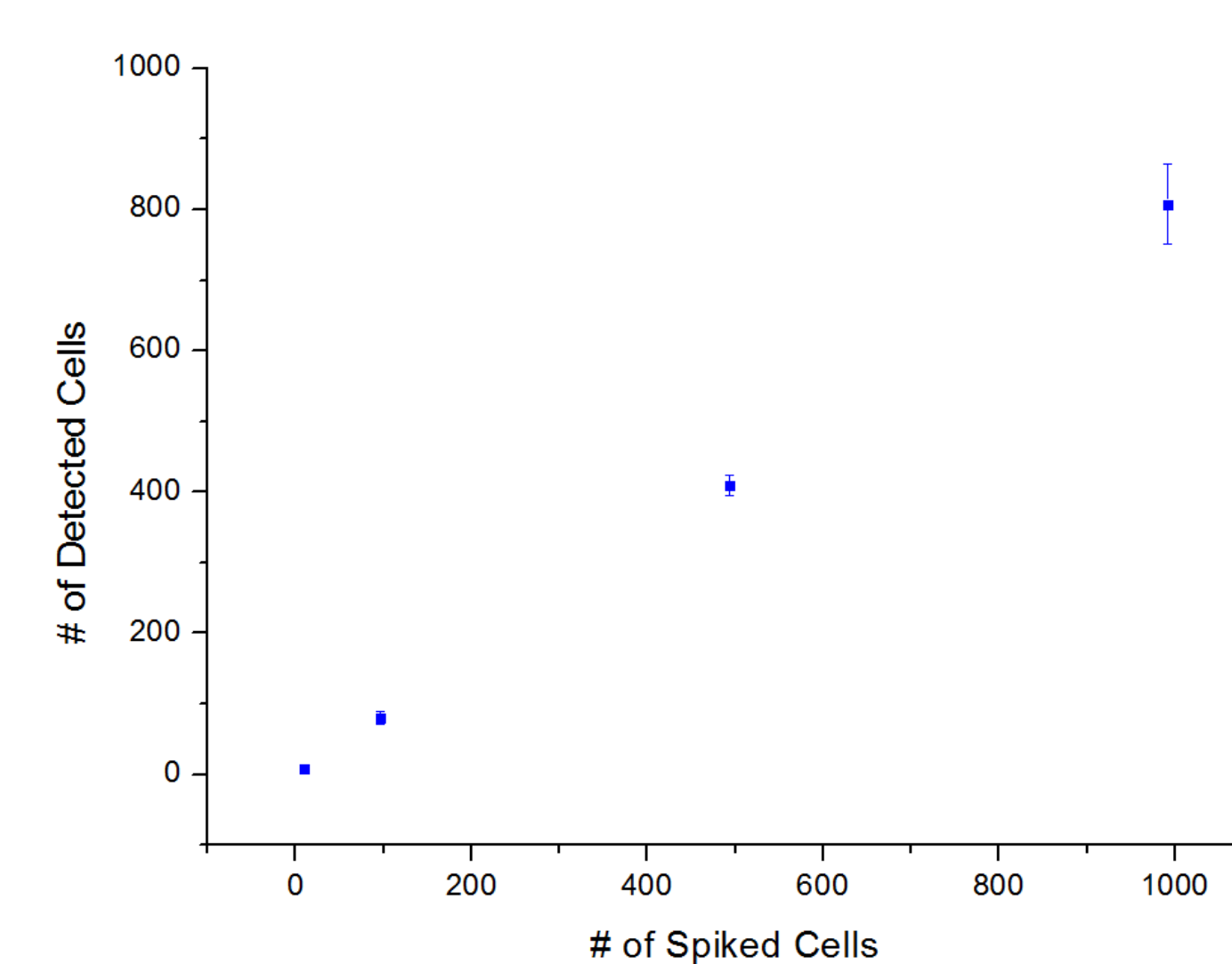
- Impedance Signal Peak amplitude depends on the third power of the particle diameter.
- The developed system is more suitable for the detection of larger cells.

◆ Ovarian Cancer Cell lines size distribution



- The peripheral blood cell : under 13um (Lara et al.) (13um => 0.46 in peak amplitude)
- Ovarian Cancer Cell lines : 16um to 24um in diameter (by calibration curve)

◆ Efficiency Test



- threshold over 0.46(peak amplitude)
- # of spiked Cells : 10, 50, 500, 1000
- Blood Volume : 500ul
- Efficiency : about 81.41%
- linear over the entire range

DISCUSSION

- PGEs function as not only separation between main microchannel and Ag/AgCl electrode chambers physically, but also passing ions for connection electrically between two Ag/AgCl electrodes.
- Impedance signal peak amplitude is linear to the volume of the cells, hence the DC-impedance based cell counter is more appropriate for the detection of larger cells such as CTCs.
- Size-based detection method is sufficient enough to count rare CTCs, and sensitive.

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