Supplementary Information

for

A microparticle-labeled microfluidic impedance immunosensor array for enhancing sensitivity

Ali Khodayari Bavil[1,2], Drago Sticker[3], Mario Rothbauer[3], Peter Ertl[3], and Jungkyu Kim[1,2]*

[1] *Department of Mechanical Engineering, Texas Tech University, Lubbock, TX*

[2] *Department of Mechanical Engineering, University of Utah, Salt Lake City, UT*

[3] *Faculty of Technical Chemistry, Vienna University of Technology, Vienna, Austria*

* Address correspondence to Jungkyu (Jay) Kim

Department of Mechanical Engineering

University of Utah

Salt Lake City, Utah 84112, USA

Phone: (801) 583-3987

E-mail: jkim@mech.utah.edu

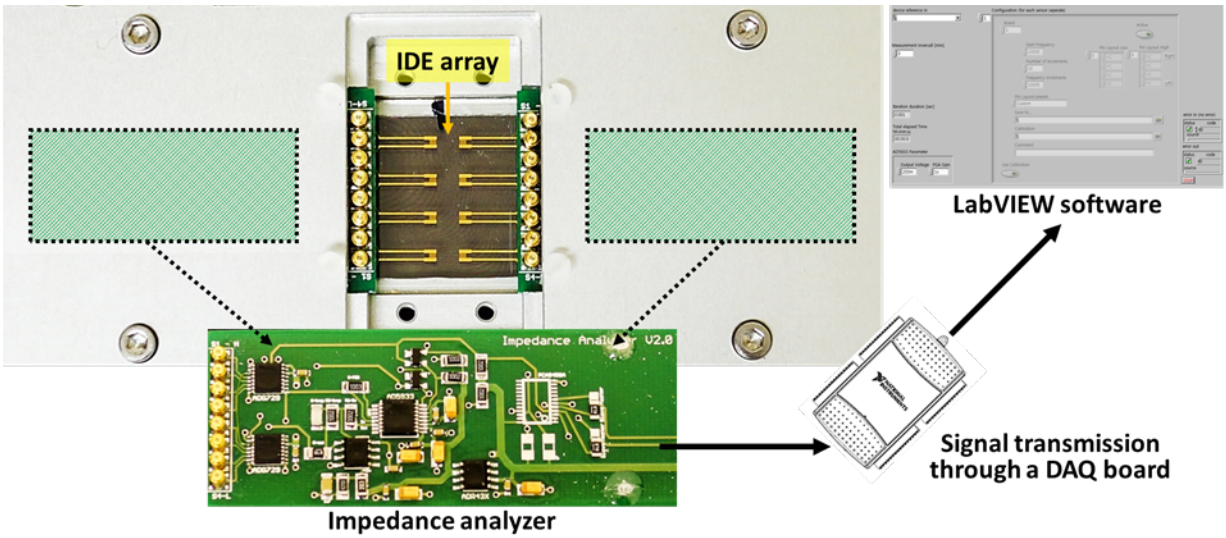

Figure S1.

The actual system setup consists of 1) a gold (Au) IDE array chip, 2) an impedance analyzing circuit, 3) a data acquisition (DAQ) board associated with a LabVIEW software and 4) a microfluidic channel on top of IDE array to deliver the analytes and buffer solutions and control the hydrodynamic forces for washing step.

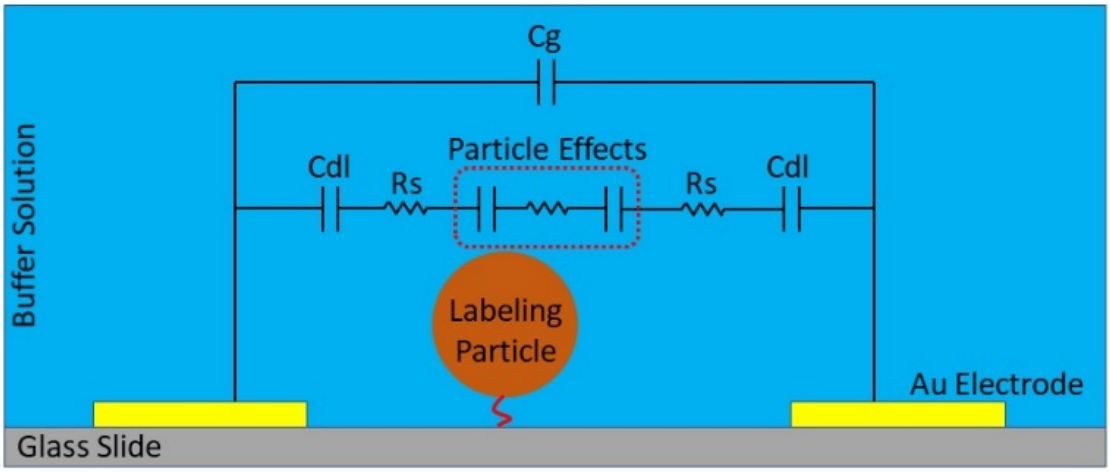

Figure S2.

Equivalent circuit of the impedance biosensor for microparticle labeled immunoassay. The analogous circuit is consisting of capacitance effects of electrodes, double layer capacitance of the electrode and microparticle surface, and the resistance of the solution.

The resistance of the solution between two electrodes is given by [1]:

$$R_{sol} = \frac{1}{nl}.\frac{1}{\kappa}.\frac{2K(\sin\frac{\pi w_{sp}}{2L})}{K(\cos\frac{\pi w_{sp}}{2L})} \quad \text{(S1)}$$

$$C_{eq} = nl\varepsilon \frac{K\left(\cos\frac{\pi\omega_{sp}}{2L}\right)}{2K\left(\sin\frac{\pi\omega_{sp}}{2L}\right)} \quad \text{(S2)}$$

Where n, l, κ, w_{sp}, and L are the number of electrodes, length of fringes, solution conductivity, the spacing between two electrodes, and center-to-center distance of two adjacent electrodes, respectively, and K(m) is the complete elliptic integral of the first kind of modulus and define as:

$$K(m) = \int_0^1 [(1-t^2).(1-mt^2)]^{-1/2} dt \quad \text{(S3)}$$

Buffer conductivity (κ) is measured by a handheld conductivity meter (Oakton CON 6+, Cole-Parmer, USA), as is plotted in **figure S3** for different concentrations of the buffer.

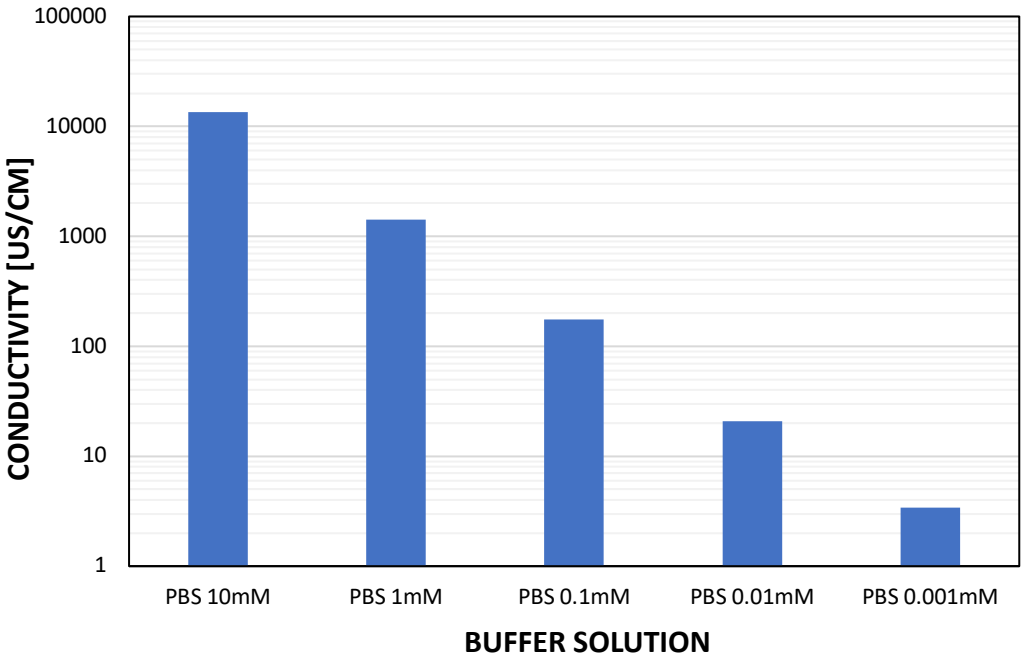

Figure S3.

Buffer conductivity for different concentrations measured by conductometer

Table S1.

Calculated solution resistance on the IDE by equation S1

	PBS 10mM	PBS 1mM	PBS 0.1mM	PBS 0.01mM	PBS 0.001mM
R(Solution)/Ohm	3.88e1	3.69e2	2.99e3	2.5e4	1.53e5

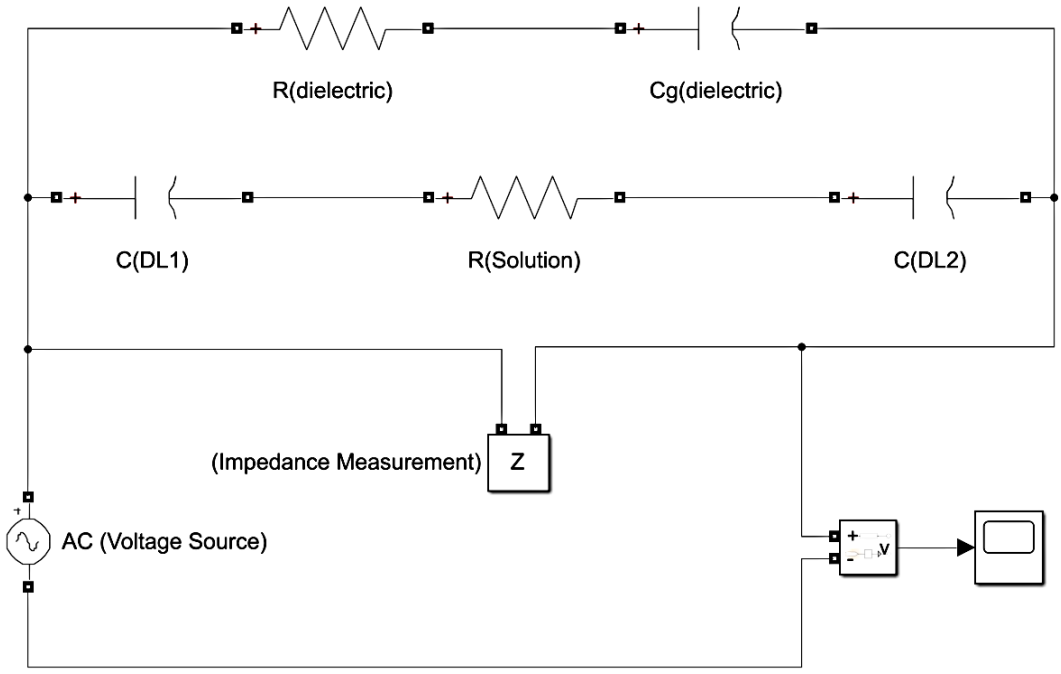

Figure S4.

Simulated equivalent circuit using Simulink, MATLAB

The two double layer capacitance of C(DL1) and C(DL2) are considered similar due to the symmetry of the electrodes and fixed to 30nF. The values for R(dielectric) and Cg(dielectric) are estimated 20kΩ and 0.6nF, respectively [2].

The impedance magnitudes for these solutions are also measured with the custom-made impedance analyzer and the same trend observed, as shown in **figure 2**. It should be noticed that the value from impedance analyzer is the reciprocal of the absolute value measure by the LCR

meter. From these measurements, the custom-made impedance analyzer is calibrated by equation (S4) and plotted for the desired range of frequency, as shown in **figure S5**.

$$G(f)=1/(Z_{LCR}(f) \times Z_{Analyzer}(f)) \qquad (S4)$$

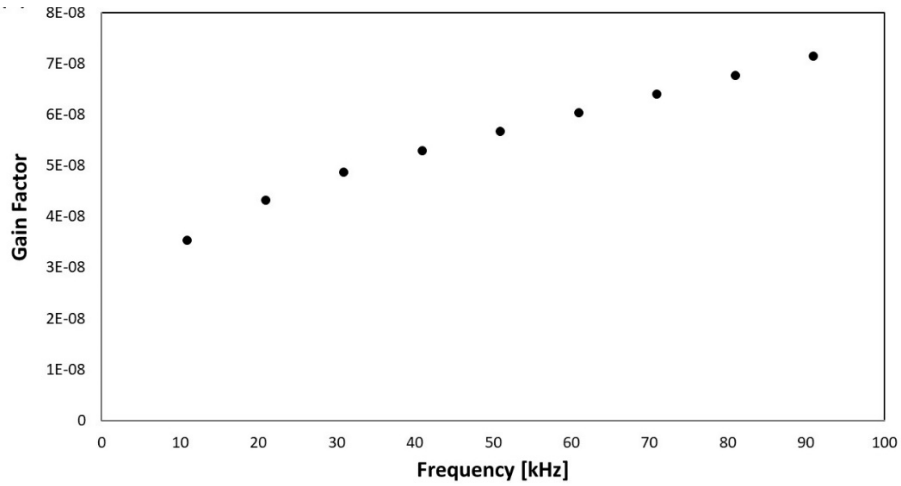

Figure S5.

The gain factor was calculated for different frequencies based on measurements by the impedance analyzer and LCR meter on different concentrations of the PBS buffer.

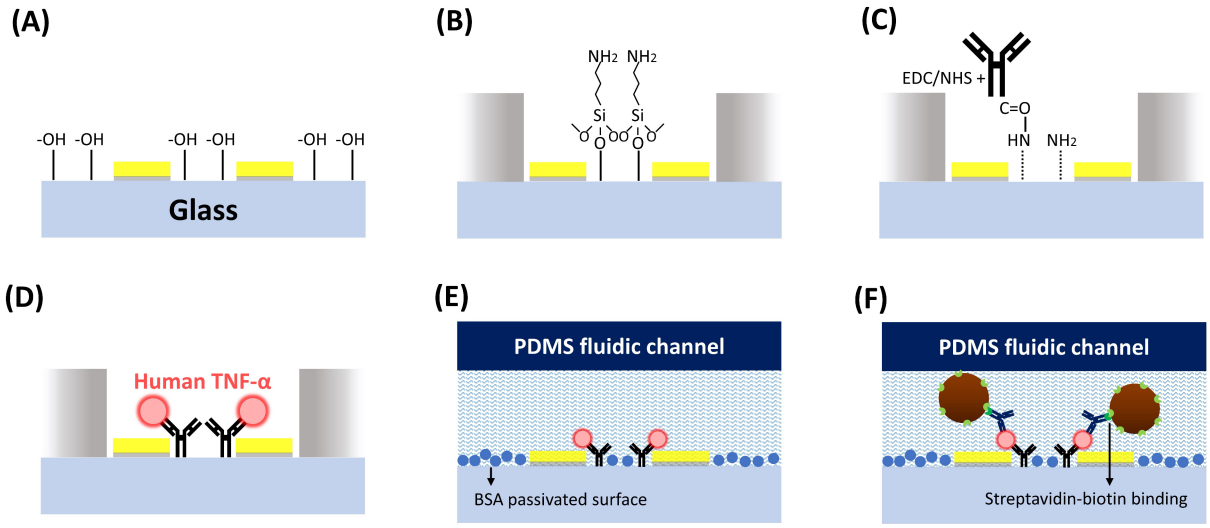

Figure S6.

Surface functionalization and human TNF-αImmunoassay with detection antibody conjugated microparticles, (A) Forming of hydroxyl groups by oxygen plasma cleaning, (B) 3% APTES incubation for 30 min after placing removable masking film, (C) immobilization of activated capture antibody by carbodiimide coupling method, (D) Incubation of different concentrations of the target, (E) Removing the mask and putting the PDMS fluidic channel, and (F) flowing detection antibody conjugated with magnetic microparticle and hydrodynamic washing

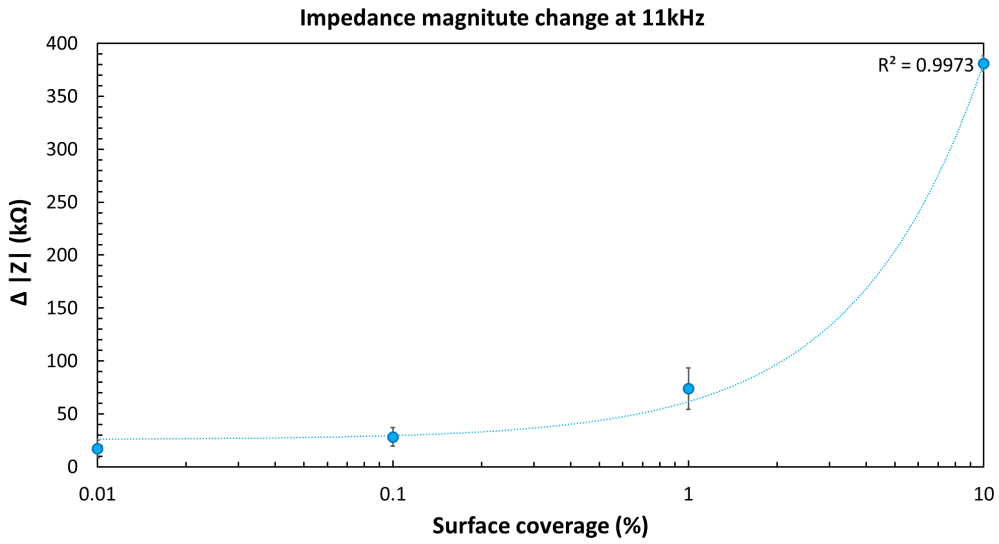

Figure S7.

Impedance differential magnitude for 2.8μm magnetic microparticles with respect to different surface coverage on the IDEs at 11 kHz.

The lowest concentration of the analyte that can be detected reliably is reported as [3, 4]:

$$LOD = [mean_{blank} + 1.645 \times (SD_{blank})] + 1.645 \times (SD_{low\ concentration\ sample}) \quad (S5)$$

References

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4. D. A. Armbruster and T. Pry, *Clin Biochem Rev*, 2008, **29 Suppl 1**, S49-52.