Supplementary Material

On-Chip Latex Agglutination Immunoassay Readout by Electrochemical Impedance Spectroscopy

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A. Characterization and optimization of agglutination conditions

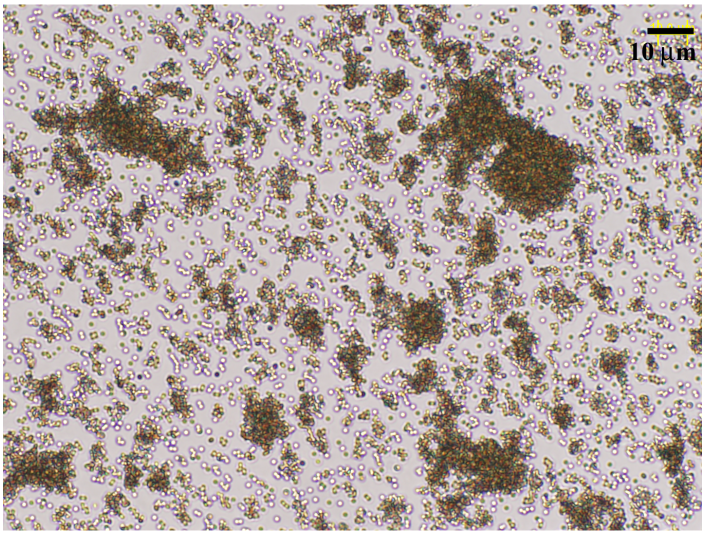

Fig. S1 A typical optical image of a 3.25 % w/v latex-IgG particle suspension of size 1 μm after 4.5 h of incubation with 1 μg ml^{-1} of antigen.

Table S1. Analytical calculations for obtaining the optimal antigen concentration required for maximal latex-IgG particle agglutination.

Number of goat anti-rabbit IgG molecules conjugated per latex particle (as supplied by the vendor)	4.47×10^4 molecules particle^{-1}
Experimentally determined concentration of rabbit IgG required for maximally agglutinating 3.25 % w/v latex-IgG suspension	1 μg mL^{-1}
Molecular weight of rabbit IgGs	2.56×10^{-19} g molecule^{-1}
Observed ratio of IgGs : antigens required for agglutination (approx.)	620 : 1

Assuming that the amount of antigen required to maximally agglutinate any other latex-IgG concentration decreases/increases proportionally, we determined the optimal antigen concentrations for the following latex IgG suspensions –

Table S2. Analytically derived optimal antigen concentrations for different latex-IgG suspensions.

Latex-IgG concentration (% w/v)	Optimal antigen concentration (μg mL^{-1})
3.25	1
2.05	0.631
1.25	0.385
0.58	0.179
0.44	0.135
0.06	0.018

B. Calculations for solution resistance

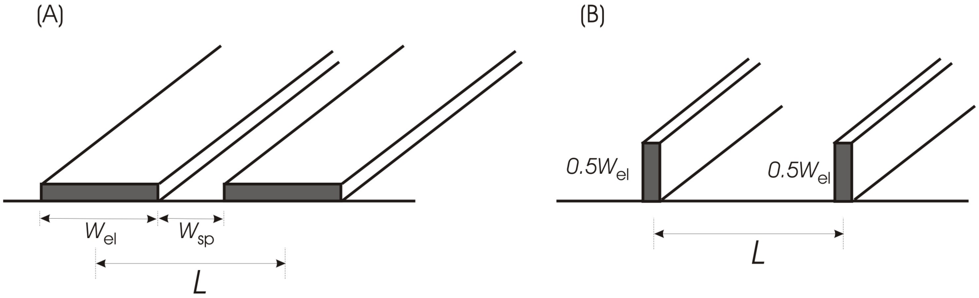

Fig. S2 (A) The spacing between the interdigitated electrodes in our experimental setup. (B) The over-simplified layout of the electrodes to estimate the total resistance of the chip.

1. Solution resistance of ultrapure DI water

Using ionic theory. The resistance of ultrapure DI water between each interdigitated electrode pair is given by, $R_{pair} = \frac{\rho L}{A} = 303.33\ \text{M}\Omega$ where, ρ is the resistivity of DI water (18.2 MΩ cm), L is the distance between the electrodes (25 μm), and A is the area of each electrode (15 μm × 1000 μm). The dimensions of the electrode pair are approximated from the actual layout as shown in Figure S2. For 2 sets of 24 interdigitated electrode pairs in parallel, the total resistance of the chip results as $R_{sol} = \frac{R_{pair}}{2 \times 24} = 6.3\ \text{M}\Omega$.

Using numerical calculations. The magnitude of R_{sol} using the numerical formula proposed by Gerwen *et al.* is calculated as, $R_{sol} = \frac{\rho}{nl} \frac{2K\left(\sin \frac{\pi w_{sp}}{2L}\right)}{K\left(\cos \frac{\pi w_{sp}}{2L}\right)} = 4.73\ \text{M}\Omega$. Here, n is the number (2 × 25) and l is the length of the electrodes (1000 μm), $K(k)$ is the complete elliptic integral of the first kind with k modulus, and w_{sp} (10 μm) and L (40 μm) are as shown in Figure S2.

2. Solution resistance of phosphate buffer saline (PBS)

Using ionic theory. The resistivity of PBS solution determined from Faraday's laws of electrolysis is given by, $\rho = \frac{1}{F c z (\mu_{Na} + \mu_{Cl})} = 58\ \Omega$ cm, where F is the Faraday's constant (9.65×10^4 C mol^{-1}), c is the concentration of the free salt ions (0.137 M), z is the valence number of ions in the salt (1), and μ_{Na} (5.19×10^{-8} m^2 V^{-1} s^{-1}) and μ_{Cl} (7.91×10^{-8} m^2 V^{-1} s^{-1}) are the ionic mobilities of sodium and chloride ions, respectively. Substituting the value of PBS resistivity for DI water in the above equation, R_{sol} is obtained as 20.05 Ω.

Using numerical calculations. Substituting the value of PBS resistivity for DI water in the above numerical formula, the solution resistance of PBS is obtained as, $R_{sol} = 15$ Ω.

C. Effect of experimental parameters on impedance

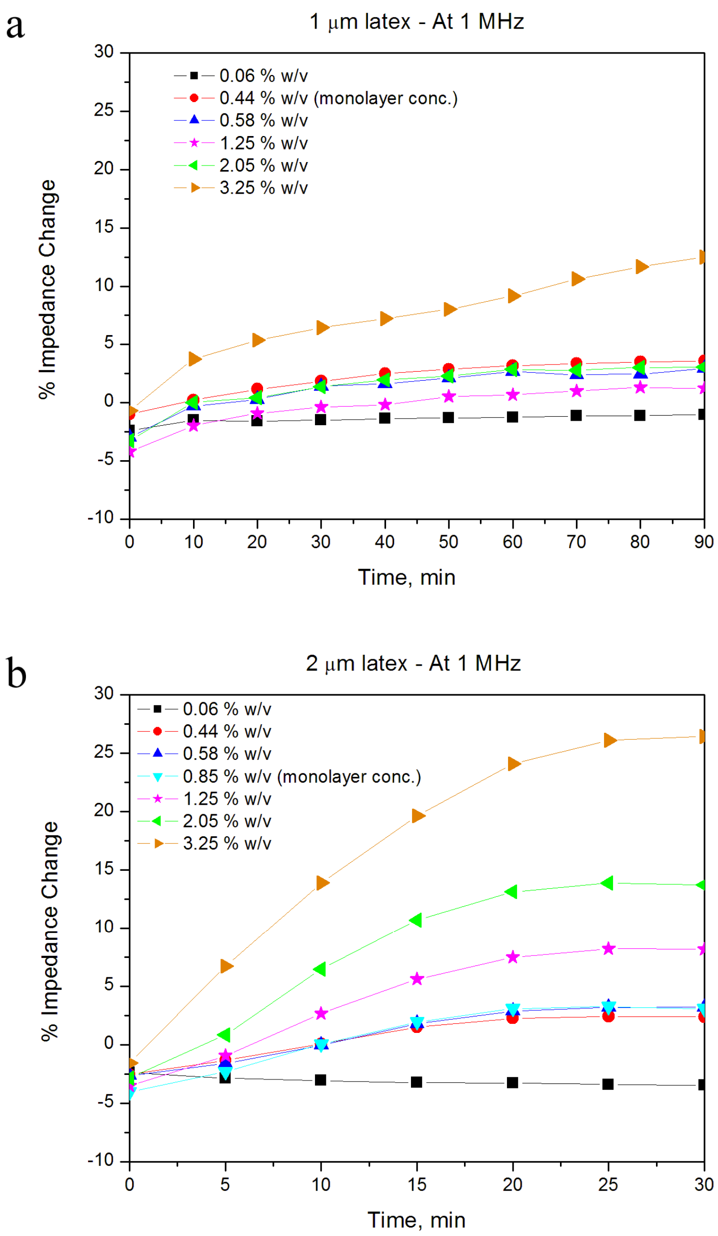

Fig. S3 Effect of sedimentation time on the impedance response at 1 MHz for plain sulfate-stabilized latex particles of size (**a**) 1 μm and (**b**) 2 μm.

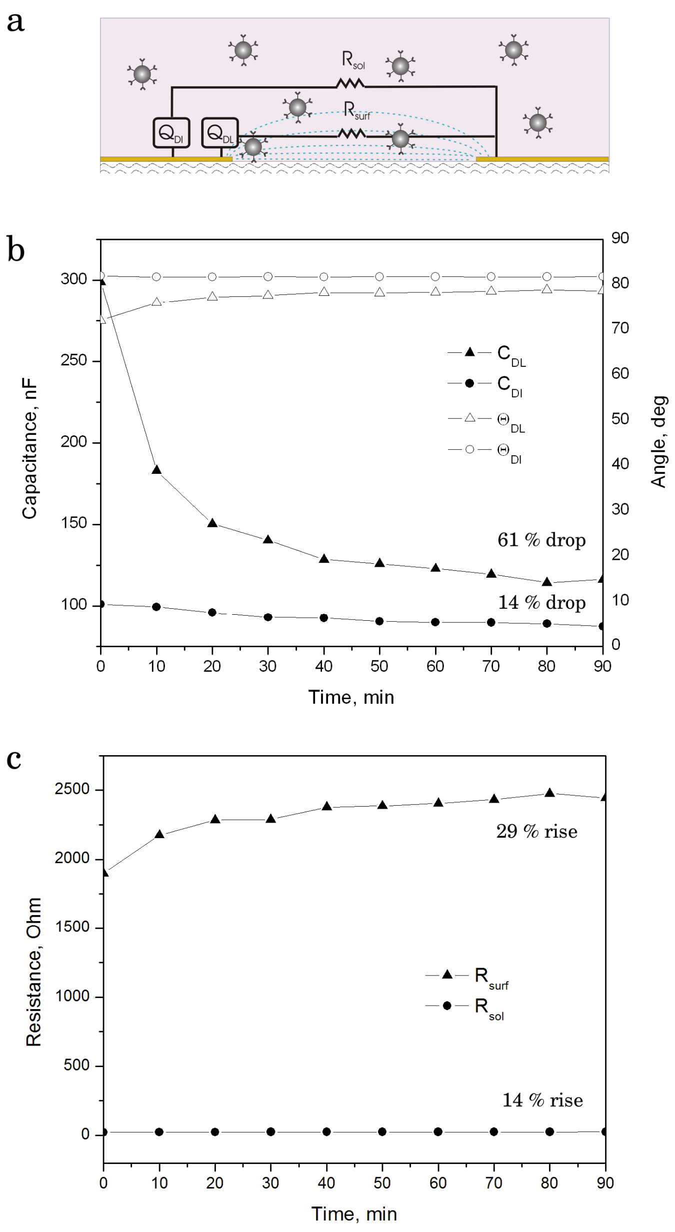

Fig. S4 Time dependent impedance response of sedimenting 1.0 μm latex-IgG particles fitted to the PBS model. (**a**) Schematic of the equivalent circuit model. (**b**) Evolution of the angles and capacitive parts of the constant phase elements for the electric double layer and the dielectric. (**c**) Evolution of the solution and surface resistances. Latex-IgG concentration was 3.25 % w/v and the fitting was performed for data collected over frequencies 1 Hz to 1 MHz.

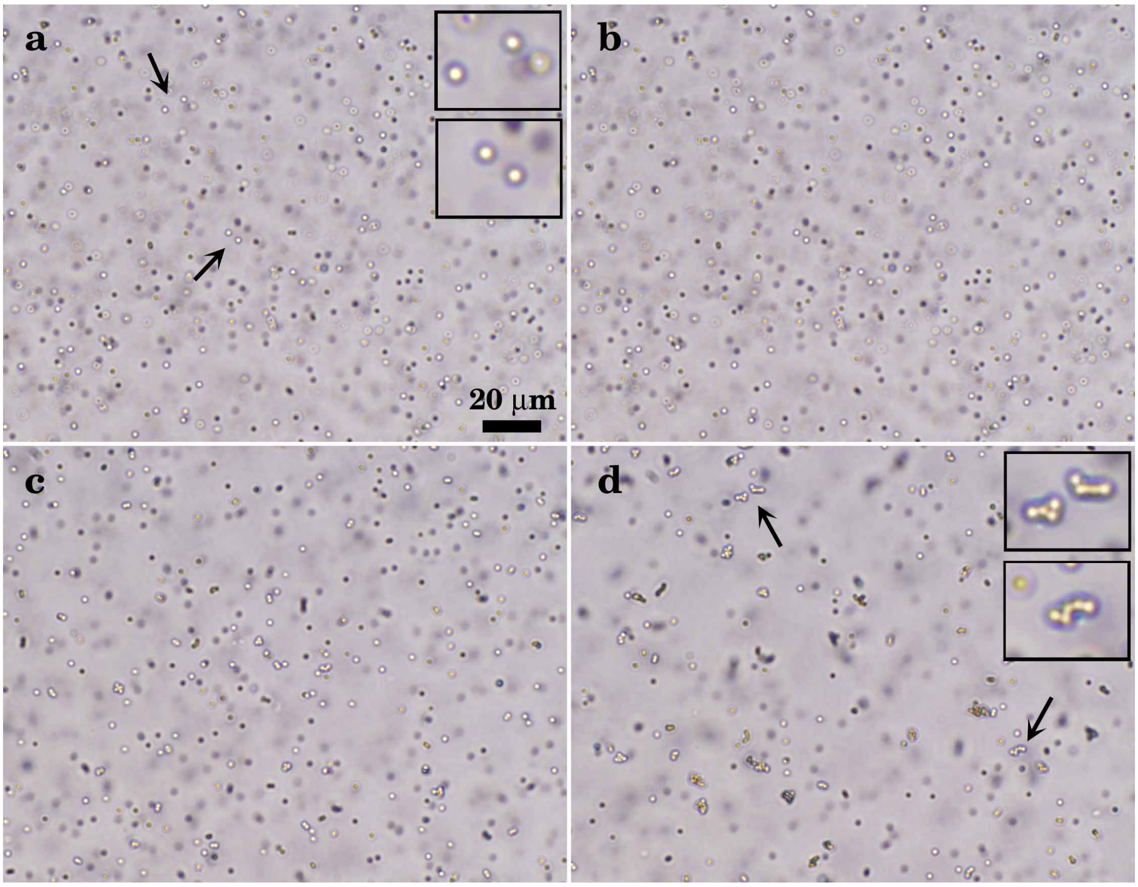

Fig. S5 Time-lapsed optical microscopy images of 3.25 % w/v latex-IgG suspension of 1 μm particles agglutinated using 1 μg mL^{-1} antigen: (**a**) 1 min, (**b**) 30 min, (**c**) 60 min, and (**d**) 90 min. The images were acquired after diluting the sample 100× and dispensing 10 μL of it on a glass slide under a glass cover slip. Insets show enlarged views of the areas pointed with arrows.

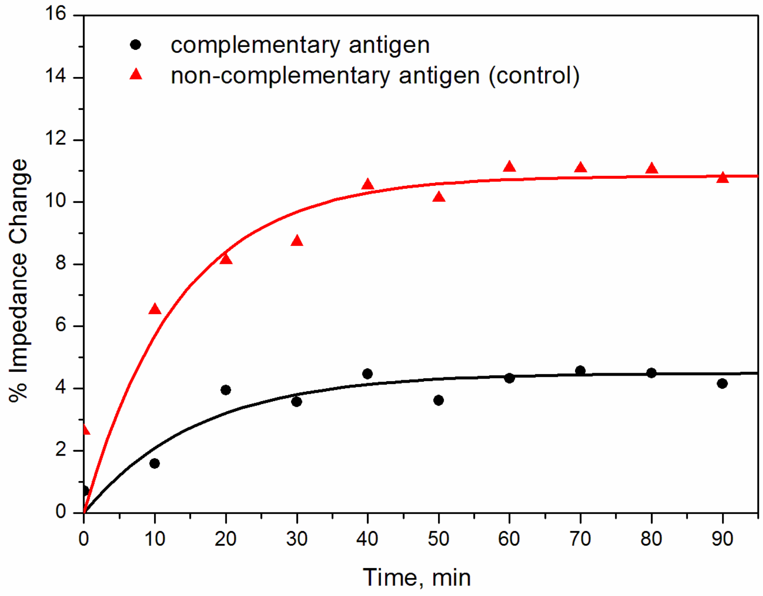

Fig. S6 The effect of 1 μg mL^{-1} of rabbit (●) and 1 μg mL^{-1} of mouse (▲) antibodies on the time-dependent impedance response of 3.25 % w/v latex-IgG suspension at 1 MHz. The lines are a guide to the eye.

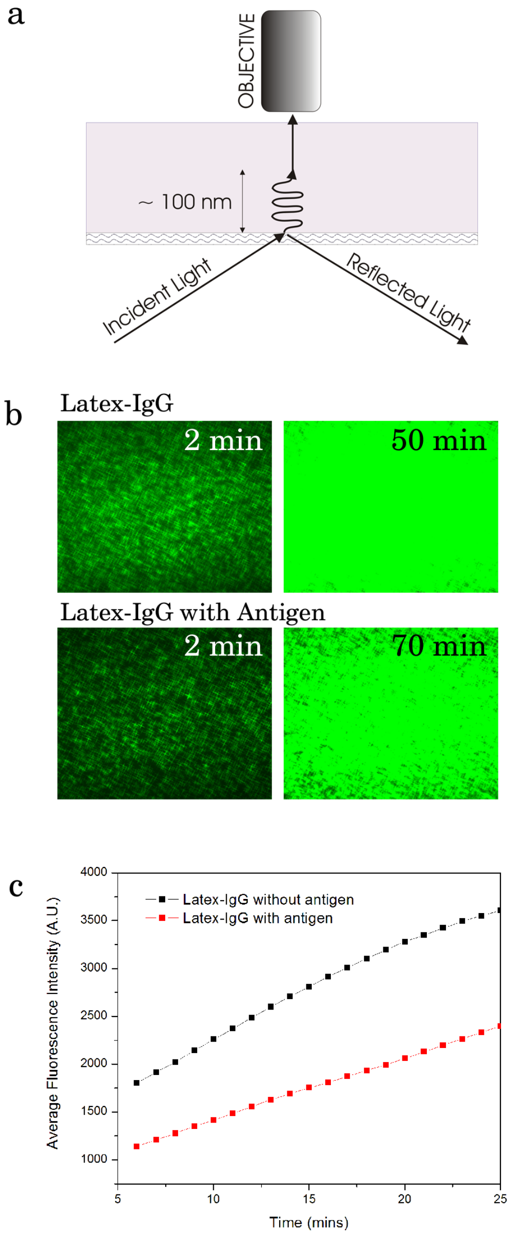

Fig. S7 Detection of latex agglutination using TIRF microscopy. (**a**) Schematic illustrating the signal generation in TIRF microscopy. The excitation is produced by using evanescent waves that are generated only when the incident light is totally reflected at the glass-water interface. (**b**) Fluorescence intensity comparison for a 0.06 % w/v latex-IgG particle suspension before and after 0.018 μg mL^{-1} antigen addition. The intensity saturated more rapidly without antigens. (**c**) Change in fluorescence intensity as a function of time. Greater slope in the absence of antigen indicates more rapid saturation.

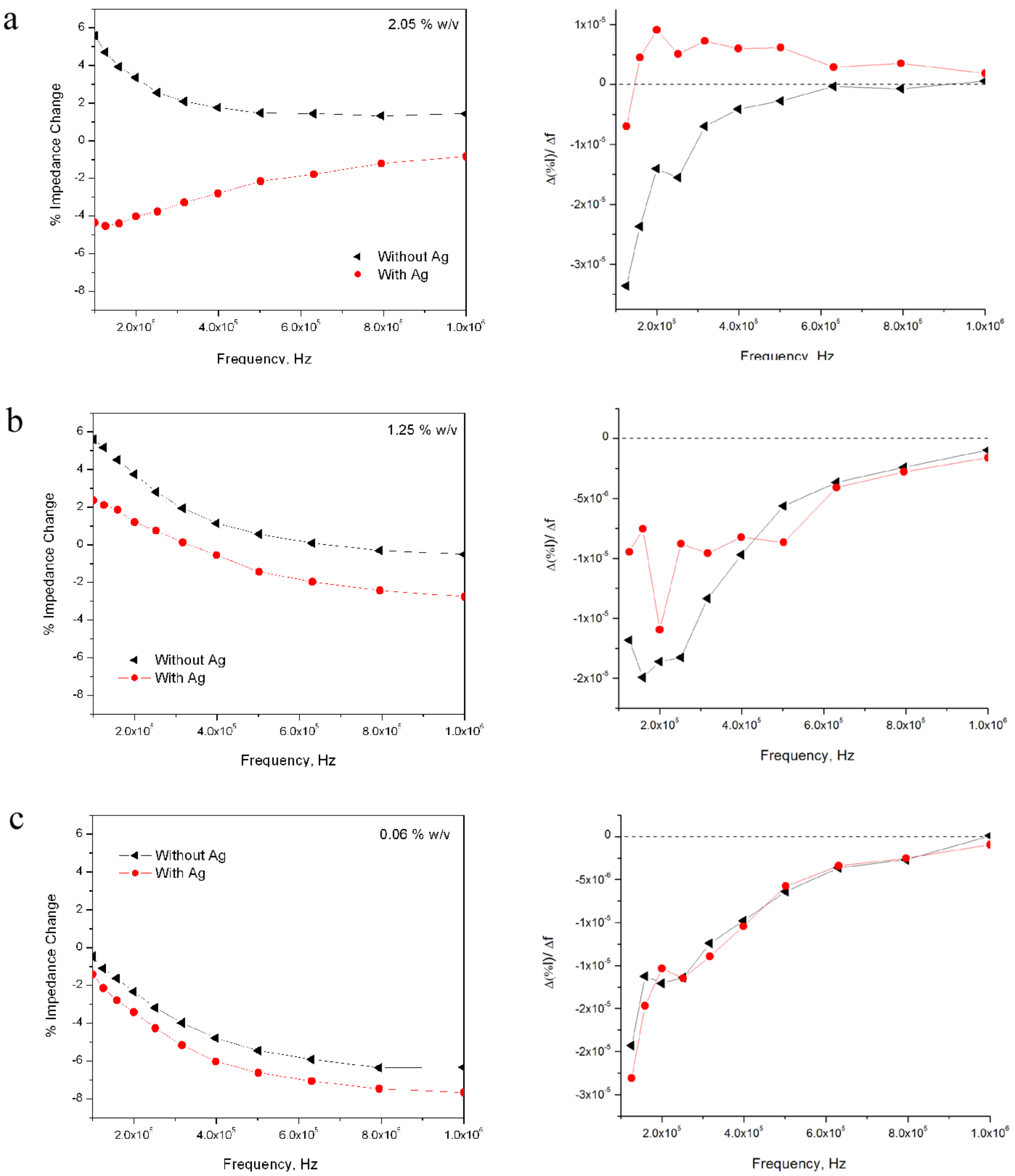

Fig. S8 Performance of the impedance-based immunosensor at 10 min for three different concentrations of latex-IgG suspensions: (**a**) 2.05 % w/v, (**b**) 1.25 % w/v, and (**c**) 0.06 % w/v. The corresponding antigen concentrations were 0.631, 0.385, and 0.018 µg mL^{-1}, respectively. Chip configuration was effective only at higher particle concentrations. (See also Figure S6 for lower concentrations).

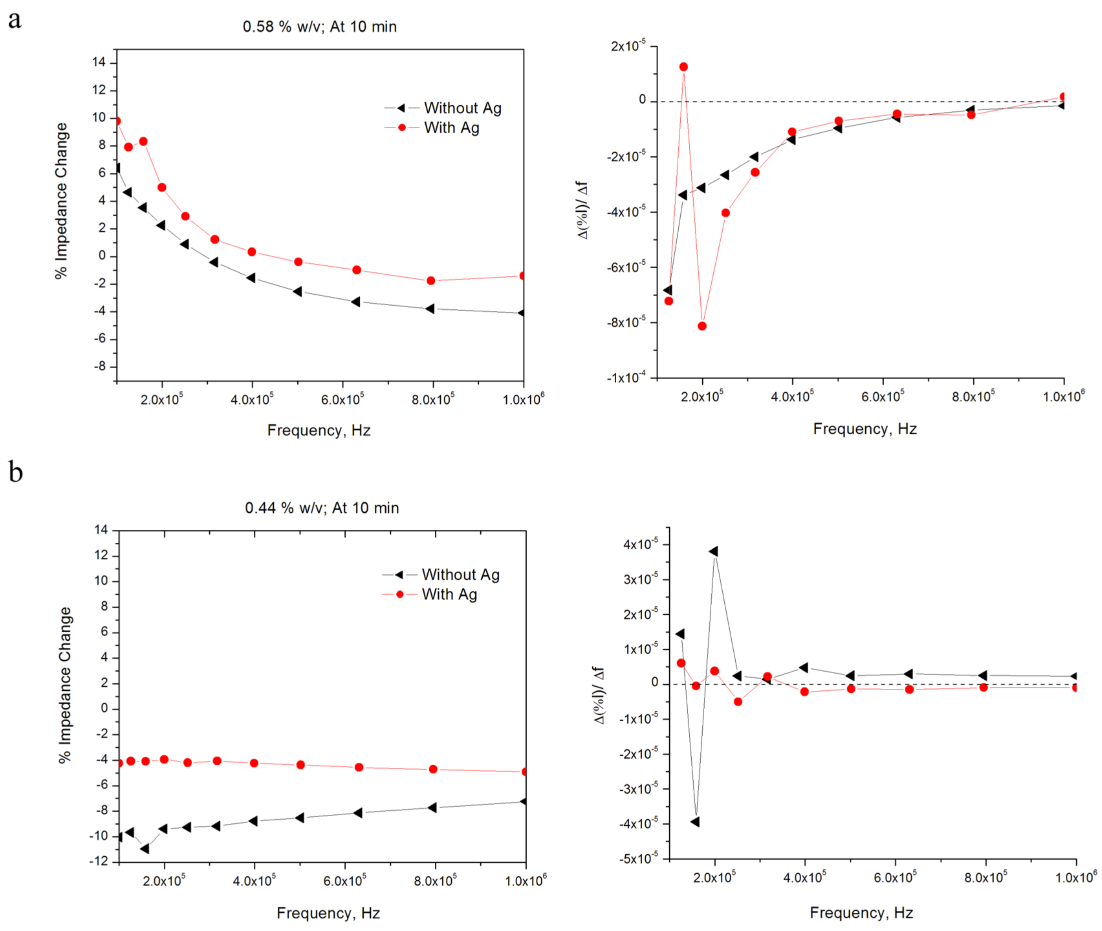

Fig. S9 Output of the immunosensor for low antigen concentrations. (**a**) 0.58 % w/v latex-IgG agglutinated with 0.179 μg mL^{-1} of antigen. (**b**) 0.44 % w/v latex-IgG agglutinated with 0.135 μg mL^{-1} of antigen. The antigen could not be detected at these concentrations in the first 10 min.

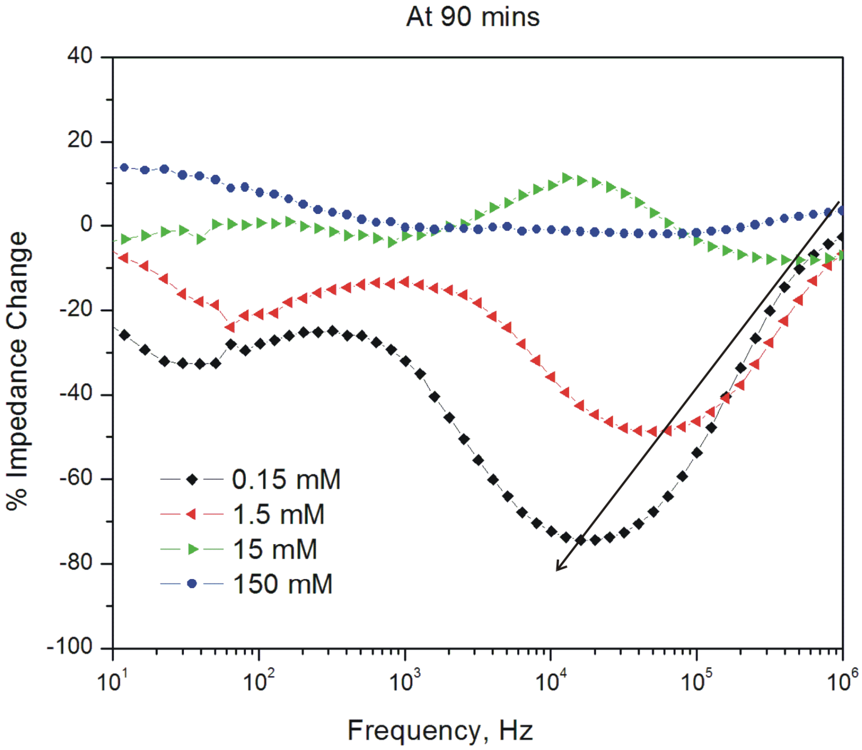

Fig. S10 Effect of electrolyte concentration on impedance. All readings were taken 90 min after 1.0 μm sulfate-stabilized latex particles of 0.44 % w/v concentration were allowed to sediment freely under gravity. The peaks shifted with decreasing salt concentration as a result of the increasing double layer thickness and a stronger electrostatic interaction between the particles.

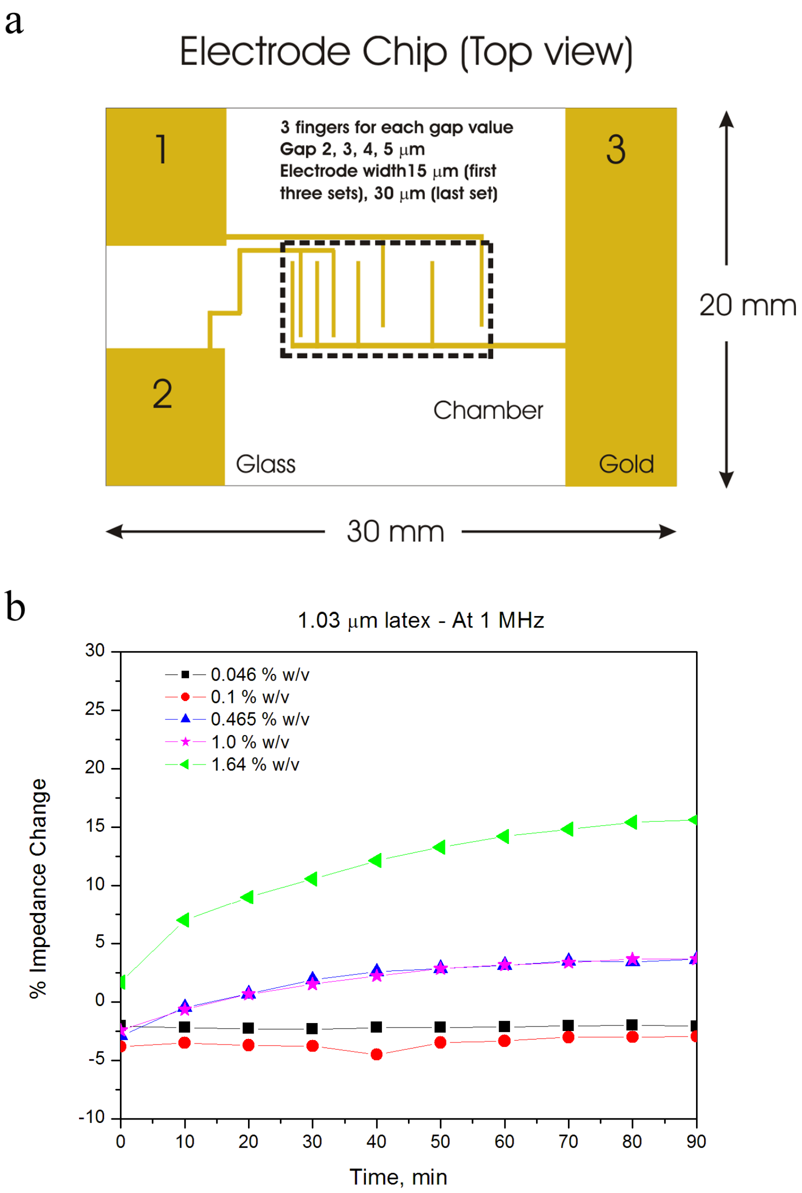

Fig. S11 Effect of electrode geometry on impedance. (**a**) Layout of the electrodes. (**b**) Impedance response for various latex concentrations at 1 MHz and as a function of time. This electrode geometry does not appear to have a major effect on the signal.

D. Calculations for monolayer concentration of sedimented particles on a chip

Area of chamber-enclosed chip, A_c = length of chamber (l) × width of chamber (w)

$$A_c = 0.5 \text{ cm} \times 0.2 \text{ cm} = 0.1 \text{ cm}^2$$

Projected area of one particle, $A_p = \pi/4 \times$ [diameter of particle (d_p)]^2

$$A_p = \pi/4 \times [1\times10^{-4} \text{ cm}]^2 = 7.85\times10^{-9} \text{ cm}^2$$

Number of particles required to form a monolayer, $N = \left(\frac{A_c}{A_p}\right) \times$ particle packing efficiency for a hexagonally closed packed lattice (ϕ)

$$N = \left(\frac{0.1}{7.85\times10^{-9}}\right) \times 0.91 = 1.15\times10^{7} \text{ particles}$$

Mass of one particle, m_p = density of latex (ρ_p) × volume of one particle (V_p) = $\rho_p \times \pi/6 \times d_p{}^3$

$$= 1.05 \text{ g cm}^{-3} \times \pi/6 \times [1\times10^{-4} \text{ cm}]^3 = 5.50\times10^{-13} \text{ g}$$

Volume of chamber-enclosed chip, V_c = Area of chamber (A_c) × height of chamber (h)

$$V_c = 0.1 \text{ cm}^2 \times 0.015 \text{ cm} = 0.0015 \text{ cm}^3$$

Particle monolayer concentration, $c = \frac{m_p \times N}{V_p} = 0.0044 \text{ g cm}^{-3} = 0.44$ % w/v

Similarly, repeat calculations for 2 µm particles to obtain 0.85 % w/v