Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Evaluation of Agglutination Strength by Flow-Induced Cell Movement Assay Based Surface Plasmon Resonance (SPR) Technique

Krisda Sudprasert[a,b], Patjaree Peungthum[a,b], Apirom Vongsakulyanon[f], Ratthasart Amarit[d], Armote Somboonkaew[d], Boonsong Sutapun[e], Pimpun Kitpoka[f], Mongkol Kunakorn[f], Toemsak Srikhirin[a,b,c*],

[a]*Center of Intelligent Materials and Systems, Nanotec Center of Excellence at Mahidol University*

[b]*Materials Science and Engineering Programme, Faculty of Science, Mahidol University, Rama VI Rd., Phayathai, Bangkok, 10400, Thailand*

[c]*Department of Physics, Faculty of Science, Mahidol University, Rama VI Rd., Phayathai, Bangkok, 10400, Thailand*

[d]*Photonics Technology Laboratory, National Electronics and Computer Technology Center (NECTEC), Pathumthani 12120, Thailand*

[e]*School of telecommunication engineering, institute of engineering, Suranaree University of Technology, Muang, Nakhon Ratchasima, 30000, Thailand*

[f]*Department of Pathology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Rama VI Rd., Phayathai, Bangkok, 10400, Thailand*

[*]Corresponding author. Tel.:+66 2201 5855; fax: +66 2 201 5843.

E-mail address: sctsk@mahidol.ac.th, sctsk@yahoo.com (T. Srikhirin).

1. Simple model of cell movement response

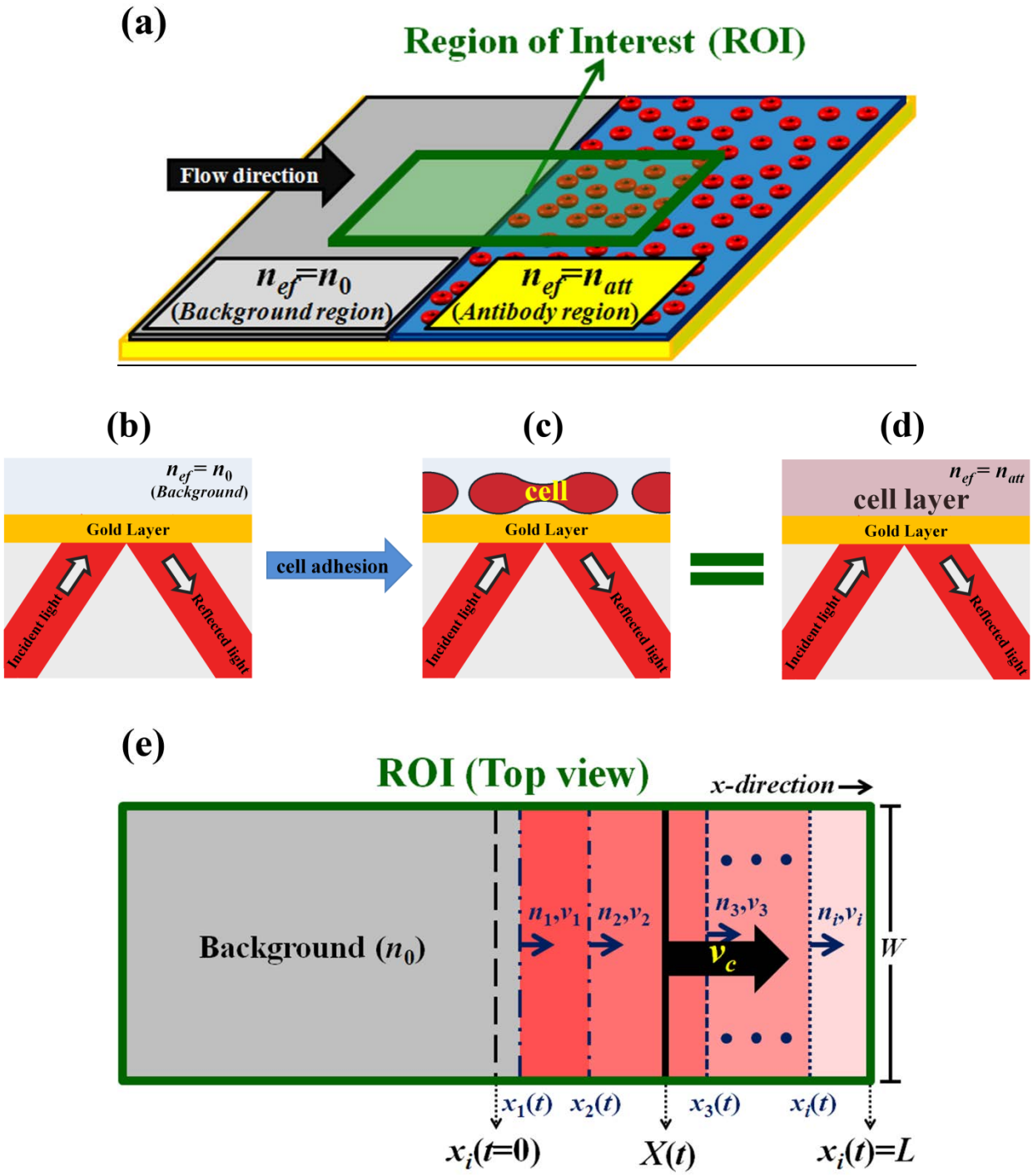


Fig. S1. A simple model of cell movement response

Based on the SPR technique, the adsorption and desorption of analytes or cells on the sensor surface was observed via the SPR signal, which represents a change of the effective refractive index (n_{ef}) from the background which is linearly proportional to the surface concentration of the cells in a narrow dynamic range. The SPR signal ($R(t)$) is given as follows:

$$R(t) = m\Delta n_{ef}(t). \tag{1}$$

m is the sensitivity coefficient that is used to convert the SPR signal into the refractive index unit (RIU). n_{ef} of the background (or running buffer) is n_0, as shown in Fig. S1a. After cell adhesion proceeds, adherent cells, which are uniformly distributed on the surface, can be assumed to be a cell layer where n_{ef} of the cell layer is n_{att}, as shown in Fig. S1b–d. A simple model of cell movement response is used which assumes that the cell layer is moving out of the region of interest (ROI) and being replaced by a background layer as shown in Fig. S1e. We assume that the cell layer consists of many sublayers that occupy the same space above the surface. These sublayers contain a group of cells having the same velocity. n_{ef} of the sublayer is n_i, where subscript i indicates the index of the sublayer corresponding to movement with velocity v_i. Therefore, n_{ef} or the average refractive index ($n_{avg}^{(i)}$) of the i^{th} sublayer in the ROI is given by

$$n_{avg}^{(i)} = \frac{A_1(t)n_0 + A_2(t)(n_i + \Delta n_i(t))}{A_0}, \tag{2}$$

where A_0 is the ROI area. A_1 is the initial area of the background and A_2 is the initial area of the sublayer. Because A_0 was fixed, $A_0 = A_1(t) + A_2(t)$. $A_0 = WL$, $A_1(t) = Wx_i(t)$, and $A_1(t) = W(L - x_i(t))$, where W and L are the ROI width and length. $x_{i(}t)$ is the position of the interface between the background layer and the i^{th} sublayer. $\Delta n_i(t)$ is the change in the refractive index of the sublayer due to the effect of the wall shear stress (WSS) acting on the cell, such as cell overlapping, lifting, or deformation. ($n_{avg}^{(i)} - n_0$) indicates the amount of cells per ROI area regarding to the i^{th} sublayer. The change of the refractive index due to cell attachment or detachment is the summation of ($n_{avg}^{(i)} - n_0$).

$$\begin{aligned}\Delta n_{ef}(t) &= \sum_{i=1}(n_{avg}^{(i)} - n_0) \\ &= \sum_{i=1}\left(1 - \frac{x_i(t)}{L}\right)\left(n_i - n_0 + \Delta n_i(t)\right)\end{aligned} \tag{3}.$$

Based on this assumption, only $x_{i(}t)$ and $\Delta n_i(t)$ are a function of time. If there are no effects of cell movement on n_i at an initial time ($\Delta n_i(t = 0) = 0$),

$$\begin{aligned}\Delta n_{ef}(0) &= \sum_{i=1}\left(1 - \frac{x_i(0)}{L}\right)\left(n_i - n_0\right) \\ &= \left(1 - \frac{x(0)}{L}\right)\left(n_{att} - n_0\right),\end{aligned} \tag{4}$$

where $n_{att} = \sum_{i=1}^{N} n_i - (N-1)n_0$. Because the cells adhering on the surface should be randomly distributed, the initial position of the interface between all i^{th} sublayers and the background layer starts at the same position, $x(0) = x_i(0)$, for all i. To remove the factor of the amount of the cells to this analysis and to focus on an average property of cell population, we propose to use the relative SPR signal (RS), defined as $\Delta n_{ef}(t)/\Delta n_{ef}(0)$, for description of the cell movement. From Eq. (1), the sensitivity factor can be omitted.

$$\mathrm{RS} \equiv \frac{\Delta n_{ef}(t)}{\Delta n_{ef}(0)}$$

$$= \sum_i \left(\frac{L - x_i(t)}{L - x(0)} \right) \left(\frac{n_i - n_0}{n_{att} - n_0} + \Delta R_i(t) \right), \qquad \text{subject to } x_i(t) \le L. \quad (5)$$

Therefore, the time derivative of the RS is

$$\frac{d\mathrm{RS}}{dt} = \sum_i \left(\frac{L - x_i(t)}{L - x(0)} \right) \frac{dr_i}{dt} - \sum_i \frac{v_i}{L - x(0)} \left(\frac{n_i - n_0}{n_{att} - n_0} + r_i(t) \right), \quad (6)$$

where $v_i = \frac{dx_i}{dt}$, $r_i(t) = \frac{\Delta n_i(t)}{n_{att} - n_0}$. If there is no further deformation, overlapping, or any change in cell–surface distance of a deformed cell at a static WSS, $\frac{dr_i}{dt}$ and $r_i(t) \to 0$. From Eq. (5),

$$\mathrm{RS} = \sum_i w_i \left(\frac{L - x_i(t)}{L - x(0)} \right)$$

$$= \left(\frac{L - X(t)}{L - x(0)} \right) \quad (7),$$

where $w_i = \left(\frac{n_i - n_0}{n_{att} - n_0} \right)$, $X(t) = \sum_i w_i x_i(t)$, and $\sum_i w_i = 1$. $X(t)$ is the average position of the interface between the sublayer and the background layer. w_i is the weight of the cell population of the i^{th} sublayer. From Eq. (6),

$$\frac{d\mathrm{RS}}{dt} = -\sum_i \frac{w_i v_i}{L - x(0)}$$

$$= -\frac{v_c}{L - x(0)} \quad (8),$$

where $v_c = \sum_i w_i v_i$. v_c is the average cell velocity. The v_c should be the average property of the cell population. The correct v_c is limited by the time period of movement of the cell with the highest velocity (v_m). Thus, the time period for analysis of v_c must not exceed the minimum limited time (t_l) that the sublayer with v_m takes to move out of the ROI. If v_i has a normal distribution, it is reasonable to estimate $v_m = v_c + 3\text{SD}$ (3 × standard deviation) with a general confidence limit of 1%. Therefore,

$$t_l = \frac{L - x(0)}{v_c + 3SD}. \tag{9}$$

If v_c is a constant at any time,

$$v_c = \frac{X(t_l) - x(0)}{t_l}. \tag{10}$$

Substituting Eq. (9) into Eq. (10)

$$\frac{L - X(t_l)}{L - x(0)} = 1 - \frac{1}{(1 + 3CV)}, \tag{11}$$

where $CV = SD / v_c$. CV is the coefficient of variation of v_c. From Eq. (7),

$$\text{RS}(t_l) = 1 - \frac{1}{1 + 3CV}. \tag{12}$$

This means that the analysis regime of the RS is confined by CV. For example, if $CV = 0.5$, $\text{RS}(t_l) = 0.6$. The analysis regime should be available at a value of the RS of more than 0.6.

2. Laminar flow in a rectangular channel and calculation of the WSS profile

To check the full development of laminar flow, we have to set the position of ROI over the hydrodynamic entry length (L_h). L_h is related to the diameter of channel (D) and the Reynolds number (Re), as following (Bhatti and Shah, 1987)

$$L_h \cong 0.05\,\mathrm{Re}\,D, \tag{13}$$

If we approximates that D is equal to the hydraulic diameter (D_h), given by

$$D_h = \frac{2WH}{W+H}. \tag{14}$$

where the height (H) and the width (W) of the channel are 235 and 493 μm, respectively. The dynamic viscosity (η) of the fluid is approximately 0.01 dynes·s/cm^2 (1 dynes/cm^2 = 0.1 Pa). We obtain the Re equal to 50 and L_h is less than 0.8 mm for the maximum flow rate 1000 μL/min that was used in the experiments. Because the critical Re for a rectangular channel is usually more than 1500 (Tosun et al., 1988) and the ROI was located at 7 mm from the inlet, it means that all our experiments deal with the laminar flow which was already fully developed.

However, the laminar flow may be perturbed due to the influence of the cell adhesion, which leads to a rough surface. The effect of the rough surface can be disregarded if the relative roughness (standard deviation of channel-surface height per hydraulic diameter of channel) is less than 0.01 for Re < 100 (Wang and Wang, 2007). Under the assumption that RBCs occupy 50% of the surface area (the most roughness), the relative roughness is about 0.003. Therefore, the RBC adhesions of our investigation did not interrupt the laminar flow in our experiment.

The WSS (τ_w) at any position is related to the gradient of fluid velocity, given by

$$\tau_w = \eta\left(\frac{\partial u_x}{\partial z}\right)_{z=0}. \tag{15}$$

Desired WSSs were controlled by adjusting the flow rate based on the Poiseuille–Hagen relation for a rectangular channel. x is the coordinate of fluid-flow direction. y and z are the coordinates of width and height, respectively. The position of the antibody surface is denoted as z = 0. The velocity profile (u_x) for the rectangular channel is given by(Natarajan and Lakshmanan, 1972; Purday, 1949)

$$u_x = \frac{Q}{WH}\left(\frac{m+1}{m}\right)\left(\frac{n+1}{n}\right)\left(1-\left(\frac{2z}{H}\right)^n\right)\left(1-\left(\frac{2y}{W}\right)^m\right), \tag{16}$$

where

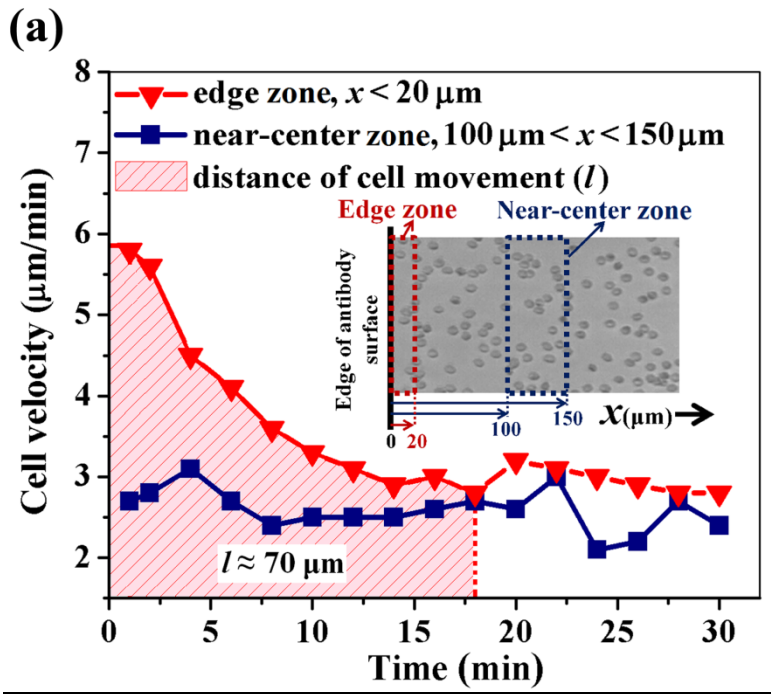
$$m = 1.7 + 0.5\left(H/W\right)^{-1.4} \tag{17}$$

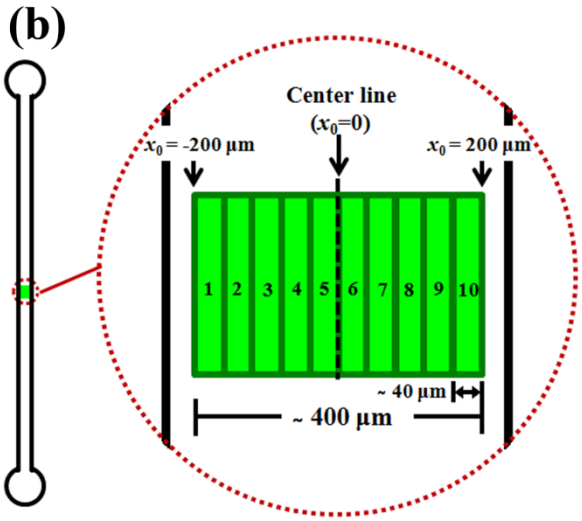
and

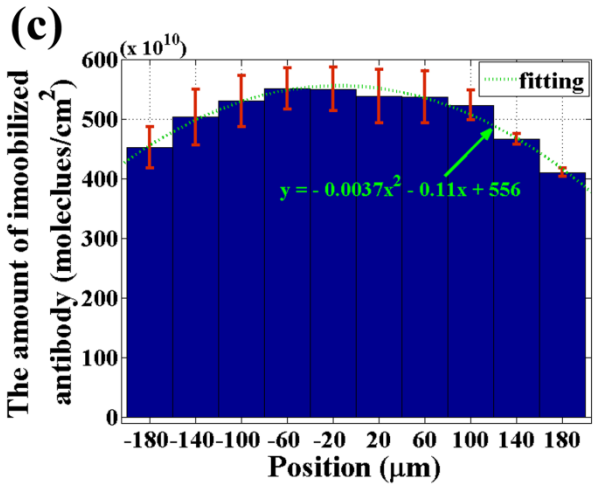
$$n = \begin{cases} 2 & \text{for } \dfrac{H}{W} \le \dfrac{1}{3} \\ 2 + 0.3\left(\dfrac{H}{W} - \dfrac{1}{3}\right) & \text{for } \dfrac{H}{W} \ge \dfrac{1}{3} \end{cases}. \tag{18}$$

Q represents the volume velocity of the fluid.

3. Effect of immobilized antibody distribution on flow-induced cell movement assay







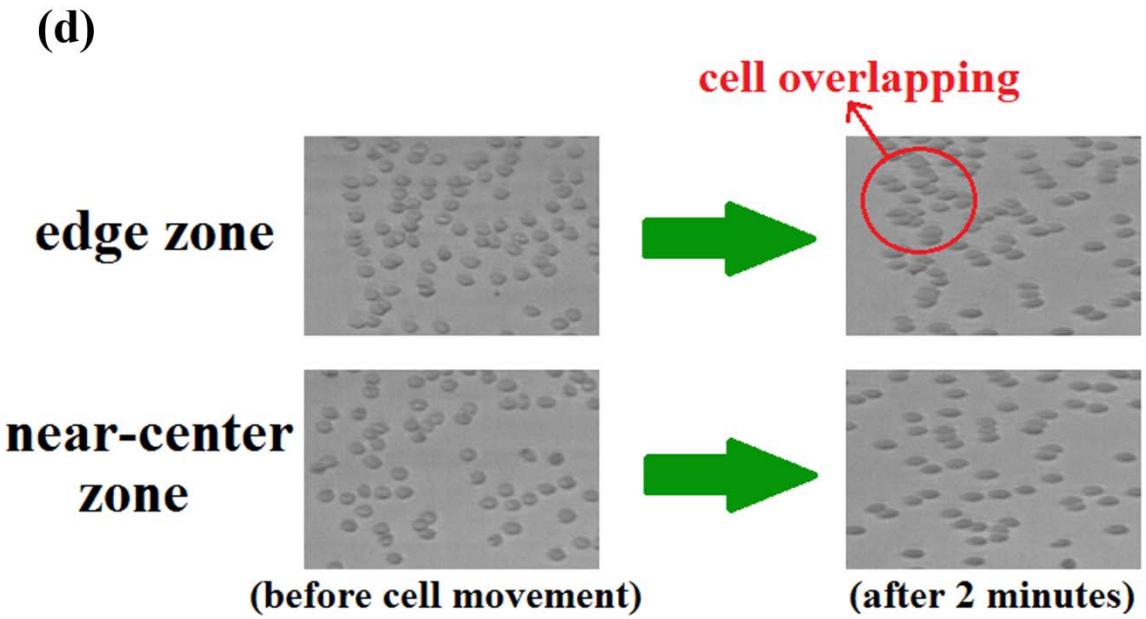


Fig. S3. Effect of antibody distribution on cell movement. (a) Comparison of the average velocity (n = 11) of RBCs initially attaching on the edge zone and the near-center zone of an antibody region. (b) The width and position of the ROI for investigating the amount of immobilized antibody. (c) The amount of immobilized antibody at any position corresponding to the ROI position of Fig. S3b. The highest immobilization was found in the central region of the channel and the amount of immobilized antibody decreased toward the channel wall in the form of a parabolic curve. (d) The aspect of cells initially attaching on the edge zone and near-center zone of the antibody region after applying the WSS.

The v_c observed from images showed that the RBCs that initially attached on the edge zone of the antibody region (eRBCs; x < 20 μm from the edge) started to move with higher velocity than the RBCs that initially attached on the near-center zone (cRBCs; 100 μm < x < 150 μm from the edge). The v_c of eRBCs decreased monotonically approaching to the v_c of the cRBCs, after the eRBCs migrated into the region more than 70 μm from the edge of the antibody region, as shown in Fig. S3a. In contrast, the v_c of the cRBCs was quite constant all the way. This means that the RBCs had a constant velocity after leaving the lateral zone (x < 70 μm from the edge). These results came from the lower antibody surface density in the lateral zone, which was influenced by the process of antibody immobilization. We measured the amount of immobilized antibody by sequentially placing 10 ROIs with widths of approximately 40 μm and heights of approximately 500 μm. The edge of the first ROI was placed at ~ 20 μm from the channel wall, as shown in Fig. S3b. The average response within 40 μm was detected. It confirmed that there was a lower surface density near the channel wall (Fig. S3c). We believe that these phenomena corresponded to the effect of mass transport limitation because the antibody surface density was fitted well by a quadratic function and had symmetry like the velocity profile of fluid. The different velocities of the fluid at any position influenced the different transportation rates of the antibody going onto the surface. Namely, a lower velocity of the antibody solution provided a lower diffusion rate of the antibody on the surface. Although the immobilization reaction is irreversible, the antibody near the channel edge was less

immobilized than that near the central region, according to the velocity profile, if the reaction time was insufficient. The essential effect of different antibody surface densities on the cell movement analysis is that it produces a situation of cell overlapping. The RBCs in the edge zone moved with higher velocity than those in the near-center zone. Consequently, more overlapping RBCs and more RBC density were found in the edge region for a few minutes, as shown in Fig. S3d. This phenomenon reduced the cell–surface contact area, which affected the first regime of the RS. In contrast, the RBCs in the near-center region kept a uniform distribution.

3. Reproducibility and stability of antibody surface after regeneration

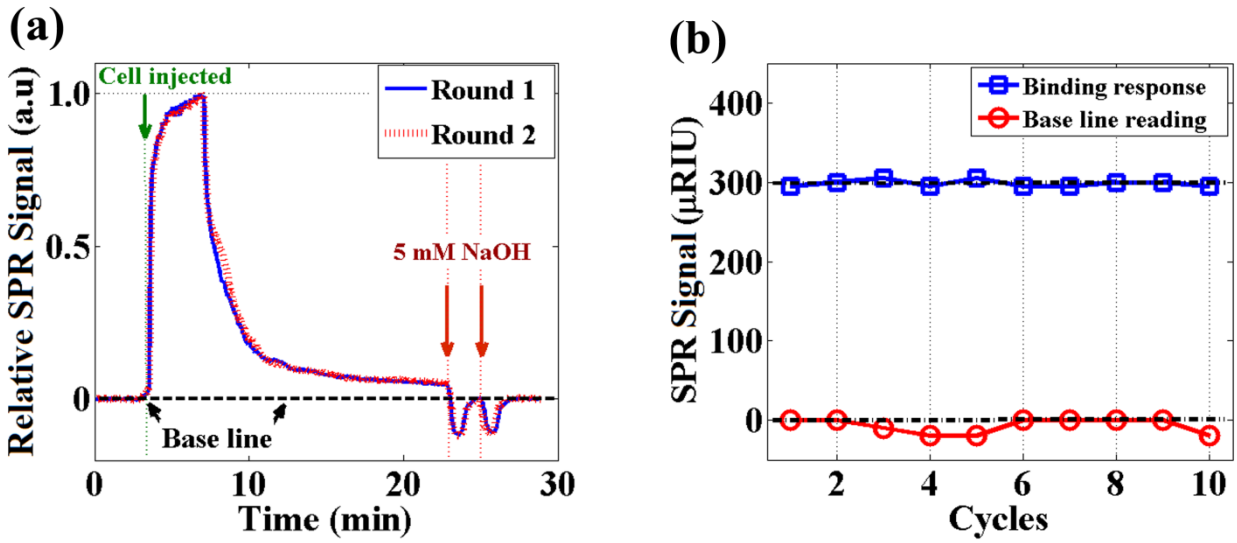


Fig. S4. Test of reproducibility and stability of the antibody surface after regeneration by 5 mM of NaOH twice. (a) The RS for the repeated analysis after the surface regeneration. The analysis showed good reproducibility of the RS. (b) The stability of the antibody surface after regeneration of the surface was observed using the 1% lysed RBCs. There is no statistical difference within 10 sequential-run times.

The antibody surface can be reused or regenerated by removing RBCs with 5 mM of NaOH for 1 minute twice. However, the antibody surface may lose activity during the regeneration due to the basic condition. Thus, we tried to examine the stability and reproducibility of the antibody surface after the surface regeneration.

The reproducibility was examined by monitoring the RS of the whole RBC analysis for the repeated analysis. It was found that the RS was reproduced well, as shown in Fig. S4a. The RSs of the first and second analyses are very similar. Moreover, there is no baseline shift. This means that RBCs and their fragments that were adsorbed on the surface in a previous run can be removed and the antibodies have no loss of function.

To reduce the effect of mass transport of the whole RBC, leading to a signal with low precision, a further investigation of the surface stability was carried out by 10 sequential rounds of lysed RBC analysis. The statistical difference was tested by Pearson's χ^2 test, as shown in Fig. S4b. The set of observed values is the binding signal from round 2 to round 10. The expected value was the binding signal of round 1. It was found that there was no statistically significant difference in the binding response after regenerating the antibody surface ($p > 0.05$) and the fluctuation of the signal of the base line and the binding response don't exceed the noise signal of the instrument (~20 μRIU). Thus, this confirms that the surface was available for repeated analysis within 10 sequential-run times.

References

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