The influence of hydrodynamic conditions and chamber geometry on the analytical signal from integrated biochips

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Abstract. The integration of a biochip with a PDMS fluidic chamber was accomplished. The advantage of dynamic analyte flows, versus static ones, was experimentally demonstrated. This was further clarified by numerical simulation including the approximate mathematical solutions modelling the convective motion and the biotinylated antibody-streptavidin interaction. The possibility of influenza virus A/California/07/09 (H1N1)pdm09 detection by the proposed method was demonstrated.

1. Introduction
In recent years, protein biochips have become widespread. The duration of the analysis, due to diffusion processes during the interaction of the analyte with the matrix of sensor elements, is one of the disadvantages of biochips. It is possible to reduce the analysis time by organizing convective flows of the sample, and microfluidic devices can be used for this purpose. The combination of a biochip with a microfluidic device enables new sample analysis capabilities. The aim of the work was: 1) to study the possibility of accelerating the analysis on the biochip by integrating a microfluidic device and 2) to simulate the convective diffusion transfer of reagents and its impact on the immunoreaction taking place on the chip. Biotin-streptavidin interactions, widespread in indirect immune analysis, were used as a model reaction.

2. Experiment
For experimental studies, we used biochips (Research Institute of Influenza, Russia) with a 20x3 matrix of sensor elements (spots) with a diameter of 400 µm each. Spots were obtained by applying drops of biotinylated antibody (AT) 4H1b solution with a concentration of 500 µg/ml for the first row, 250 µg/ml for the second row, and 125 µg/ml for the third row. The biochip was connected to a single-channel 7 µl polydimethylsiloxane (PDMS) flow chamber (obtained by the method of "soft" lithography) by a mechanical support (Fig.1). The flow chamber was filled with individual samples through a tube (internal diameter of 0.2 mm) by peristaltic pump, after which the free end of the filled tube was connected to the outlet of the chamber. Then, samples (25 µl of fluorescent labelled str-Cy5 at different concentrations) were pumped cyclically at different sample flow rates and durations of circulation. After washing the matrix with a PBS buffer solution (pH 7.5 with 0.01% Tween 20) in the same chamber, the results of the 4H1b antibody’s interaction with Str-Cy5 were measured by a ScanArray Express fluorescent reader (Perkin Elmer, USA).
The obtained results confirmed the possibility of reducing the duration of the analysis by the implementation of a flow system. When the flow rate was 22 µl/min and the duration of circulation was 15 min, the fluorescence intensity of the spots was 39000±700 e. In the static mode for the same sample after 30 min incubation, the signal intensity was at the level of 15000±1400 e. (PMT 55%, the concentration of Str-Cy5 0.5 µg/ml).

The influence of the sample flow rate (at a sample volume of 25 µl) on the fluorescence intensity, i.e. protein binding, was investigated. When the sample flow rate was increased to 22 µl/min, the analytical signal intensity increased. Further increasing of the sample flow rate led to signal decreases, probably due to the effect of the flow on the antibodies attached to the substrate.

Chip scans, after the reactions with Str-Cy5 (concentrations of 1, 10, 100, and 1000 ng/ml for 10 min at the sample flow rate of 22 µl/min), are shown in Fig. 2. In addition, the feasibility of influenza virus A/California/07/09 (H1N1)pdm09 detection, by the proposed method, was demonstrated (Fig. 3). A three-stage reaction (the "sandwich": bonded 4H1 – virus H1N1 - 4H1b - Str-Cy5) was carried out; virus concentration was 60÷500 ng/ml.

3. Mathematical modeling
Two methods for simulating analyte binding with the matrix-bound sensor elements were used: 1) a numerical simulation by COMSOL MULTIPHYSICS, taking into account the underlying physically-based processes, reactions, and boundary conditions, and 2) a search of the approximate analytical solutions.
In numerical simulation, the biotin–streptavidin binding reaction is simplified as streptavidin’s adsorption onto the reaction surface of the spot matrix with the same concentration of active binding sites. This approach allows, in particular, one to check the adequacy of the physical and mathematical modelling conditions. In some cases, we will assume that the qualitative coincidence of the results with the known theoretical laws is sufficient.

The observed effect of a significant increase in the mass transfer rate at the inlet (outlet) of the reaction chamber in comparison with its central part (Figs. 2,4) can be caused by a discrepancy between the convective velocity $V_x$ profiles at the inlet (outlet) of the flow from the reaction chamber and the steady-state parabolic profile in the chamber.

![Figure 4](image)

**Figure 4.** The results of numerical simulation of the transverse component of the convective flow rate at the central section of the chamber (a) and the bound protein concentration (b) after 10 min. of sample pumping. The original sample concentration was 1000 ng/ml

The entrance of substances into the linearly expanding channel allows one to use the hydrodynamics of the diffuser for a steady flow, or a parabolic poiseuille profile, as approximations; both variants allow for well-known analytical solutions [1]. Changing the axial component of the velocity according to the continuity equation will cause additional convective motion of the substance in the vertical section of the reaction chamber. So a convective transport, the intensity of which decreases in the central part of the chamber due to the stabilization of the velocity profile, is added to the diffusion transport.

Near the walls and the axis of the channel, the velocity values corresponding to the diffuser and the poiseuille (parabolic) profile practically coincides; in the intermediate sections their divergence is significant. The section area with the greatest divergence of velocity profiles is shown in the Fig. 5.

![Figure 5](image)

**Figure 5.** Profiles of the longitudinal component of the convective velocity in the chamber
The expanding channel entering the chamber is considered as a diffuser with an angle $\pm \pi/4$. The velocity $V(\varphi)$ is calculated as:

$$V(\varphi) = 6v_0(\varphi)/r,$$

where $\varphi$ - the angle from the axis of the diffuser and the limited by $\pi/4$ (wall), $v$ -- kinematic viscosity coefficient, $r$ -- radial displacement. The $u$ function is calculated using an integral:

$$2\varphi = \int_u^{u_0} \frac{du}{[(u_0-u)(u^2+u+u_0u+q)]^{1/2}}.$$

Here with

$$\pi/2 = \int_u^{u_0} \frac{du}{[(u_0-u)(u^2+u+u_0u+q)]^{1/2}}$$

and

$$R/6 = \int_u^{u_0} \frac{udu}{[(u_0-u)(u^2+u+u_0u+q)]^{1/2}},$$

that allows one to determine the two constants of integration – $q$ and $u_0$. In particular, for the two curves in Fig. 5, the value $u_0=0.25$ corresponds to $q=5.62$; $u_0=0.18$ – $q=8.28$. The continuity equation has the form:

$$\frac{\partial V_x}{\partial x} + \frac{\partial V_y}{\partial y} = 0.$$

Since the component of velocity $V_x$ varies depending on the distance $x$ passed by the front of the sample (according to Fig. 5), then $V_y$ is also changing. Due to the condition of adhesion on the wall ($V_y=0$), there is a non-zero convective component of the velocity in the cross section of the channel, which provides additional mixing of reagents. Thus, the diffusion component of the mass transfer is supplemented by a convective component. Closer to the middle of the chamber, the rate of change of the convective profile $V_x$ is reduced and becomes a constant; this component become negligible. Experiments and numerical calculations confirm the high intensity of the fluorescent signal (more intensive mass-transfer) in the area of the entrance of the substance into the chamber (see Figs. 2, 4).

The additional convective movement of the substance can be estimated by averaging the velocities in the continuity equation

$$V_y = -2HV_x/L.$$

Here, $H$ is the half of width (1.5 mm) and $L$ is the chamber length (10 mm). In order to evaluate the average cross-section, let’s assume that the chamber’s profile is almost parabolic. Using $V_x$, we can understand the average change in velocity in the axial direction in the transformation of the profile from the diffuser to parabolic. The relative divergence of the velocity at $u_0=0.18$ will be near 0.099.

The average absolute value of $V_x$ will be $3.55 \cdot 10^{-5}$ m/sec. For 10 seconds, the movement perpendicular to the axis of $X$ will be approximately of $1.07 \cdot 10^{-4}$ m/sec.

In order to estimate the diffusion distance traveled by streptavidin particles, we use the geometric approximation of the spherical particle and the Stokes-Einstein formula for the diffusion coefficient. Based on the size of the small globular protein of at least 40 nm, we used an estimated radius of 20 nm. As such, the diffusion distance, for the considered time of 10 sec, will be about $1.2 \cdot 10^{-4}$ m. The additional convective movement of particles in the plane perpendicular to the axis of the input channel is at least comparable to the diffusion movement; this practically doubles the flow of the substance. Thus, in the areas of input (output) from the reaction chamber, the total (convective and diffusion) mass-transfer compared to diffusion only, at least, doubles.

Fig. 6 shows the dependence of the averaged concentration of the bound streptavidin on the time of contact with 6, 9, 12, 15, 20 spots of the matrix middle row, obtained by numerical simulation. After analyzing the steady-state value (A) of the informative signal for different dot locations, we
determined that the A dependence on the spot number N, or almost on the analysis time, which is directly proportional to the number, is adequately approximated by the decreasing exponential function. According to the methodology outlined in the paper [2], the dependence

\[ A = \alpha \exp(-\beta t), \beta > 0 \]

has a high adequacy. It is characteristic for the concentration dynamics during a reaction of the first (pseudo first) order.

The shape of the curves is adequately described by the logistic growth curve (the determination coefficients exceed 0.92). This type of dependence is typical for the processes of population development (exponential growth) with saturation. In particular, the biotin-streptavidin reaction, while limiting the number of sites, is fully consistent with similar dynamics.

![Figure 6](image_url)

**Figure 6.** Dependences of the concentration (averaged over the area) of the reacted protein on the time of interaction with spots 6, 9, 12, 15, 20 of the matrix middle row, obtained by numerical simulation (curves from top to bottom).

4. Discussion and conclusion

The advantage of dynamic analyte flows, versus static ones, was experimentally demonstrated. The value of the critical sample flow rate (corresponding to a flow rate of 22 µl/min) was determined. Values exceeding this cause decreases in sample signals for the analytes. This is probably due to the washing out of some of the antibodies bonded onto the substrate and/or insufficient Str-Cy5 residence time near the binding antibodies.

The analytical signal value depends on the number of bound complexes of immobilized antibodies and their captive antigens. The concentration of antibodies in spots is proportional to the concentration of the protein solution used for printing biochips. It was shown that the dependence of analytical signal on the concentration of the 4H1b solution to obtain dots in the range of 125-500 µg/ml has a power-law form. For different conditions, the exponent varies from 0.39±0.06 to 0.59±0.02. In the range of Str-Cy5 concentrations from 1 to 1000 ng/ml with a reaction duration of 10 min, and with a sample flow rate of 22 µl/min, the dependence of the analytical signal can be approximated as

\[ I = 1936.3 \sqrt{C}; [C] \text{ ng / ml (correlation coefficient 0.87-0.98).} \]

For uniform distribution of an analyte on a sensor’s surface, the sample must be continuously mixed during the analysis. The role of the diffuser can be played by the extension when the input channel is switched to the chamber. Under the considered chip geometry, such convective movement of particles with radius ~20 nm in the plane perpendicular to the axis of the input channel is at least comparable to the diffusion movement (1.2·10^-4 m); this practically doubles the "flow" of the substance.

The results of numerical simulation (Fig. 6) show that with the given chamber geometry and sample flow rate, 1 minute is sufficient for biotin-streptavidin binding. This renders possible the performance of accelerated, less time-consuming, immunoreaction-based sample assays in the
immobilized surface (chip) format. As an additional proof-of-principle, detection of the influenza a/California/07/09 (H1N1)pdm09 virus (concentration of 60–500 ng/ml, using a three-stage ‘sandwich’ reaction) was demonstrated.

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