A model for enhanced chemiluminescence reactions in microsystems

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ABSTRACT

Detection of hybridized targets—like DNA strands—in microsystems for biotechnology is usually done by fluorescence. However, recent investigations have shown that chemiluminescence could be a more effective and sensitive tool for detection under the condition that dispersion of light due to diffusion is not too important. In this study, a model for an enhanced chemiluminescence reaction using luminols is proposed and the resulting light emission and absorption by the CMOS APS (active pixel sensor) is calculated.

Keywords: luminol, diffusion, Michaelis-Menten model, APS (Active Pixel Sensor).

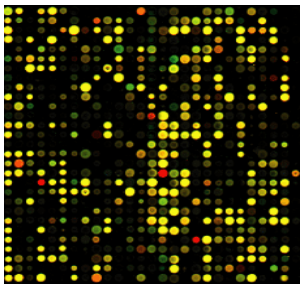

Fig.1. View of a DNA microarray.

INTRODUCTION

Biorecognition of targets—like DNA strands—is the basis of DNA chips. Conventionally detection is done by a hybridization step followed by fluorescence marking of the targets (fig.1) [1-3]. This type of detection, however, usually requires an external and expensive scanner in order to read one by one the signal of each active areas of the chip (sometimes more than 10000). Recently, with the development of microelectronic sensors like the APS, investigations have been conducted to obtain a total integration on a chip [4]. A more powerful light signal is then required and enhanced chemiluminescence could be the solution (fig.2). Enhanced chemiluminescence is based

Fig.2. Chemiluminescence of luminal molecules in presence of hydrogen peroxide (H_2O_2)

on a reaction between a chemical species like luminol or luciferase, in presence of hydrogen peroxide (H_2O_2), and an activator (here p-iodophenol), with a catalyzor (HRP, Horse Radish Peroxidase) [5,6].

In this work, a model for the chemiluminescence reaction is proposed that includes diffusion of the luminols, their reaction with an active substrate hybridized with immobilized HRP molecules, the diffusion of r-luminols and their recombination resulting in a photon emission. The model makes use of the COMSOL numerical software. The results are exported to a MATLAB model that predicts the light radiation and the response of the APS.

1 CHEMILUMINESCENCE REACTION

The reaction is sketched in figure 3. Schematically it can be decomposed by the following equations

$$HRP + L \leftrightarrow HRPL \rightarrow HRP + L^* \quad (1)$$

and

$$2L^* \rightarrow L + AP^* \quad (2)$$

where L, L^*, HRP and AP^* denote respectively luminols, r-luminols, horseradish peroxidase and aminophtalate. The species AP^* is an aminophtalate that emits immediately a photon.

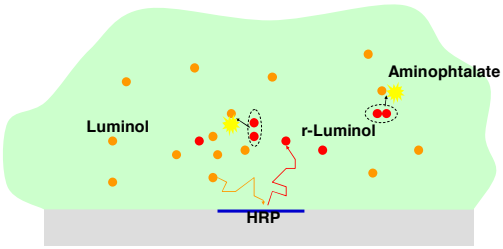

Fig.3. Sketch of the enhanced chemiluminescence reaction

The luminols and r-luminols diffuse and react in the liquid volume according to

$$\frac{\partial c_i}{\partial t} = \nabla.\left(D_i \nabla c_i\right) + R_i \tag{3}$$

where indices i=1, i=2 and i=3 correspond to the luminols, r-luminols and aminophtalates. In (3) c is the concentration, D the diffusion coefficient and R the reaction rate. According to (1) and (2), the reaction rates are given by

$$\begin{aligned} R_1 &= k\,c_2^2 \\ R_2 &= -2\,k\,c_2^2 \\ R_3 &= k\,c_2^2 \end{aligned} \tag{4}$$

where k is the reaction speed.

At the wall, at the contact of the active surface grafted with the HRP molecules, the Michaelis-Menten relation produces the boundary conditions

$$D_1\,\nabla c_1 .\,\vec{n} = -\frac{V_{\max}}{1+\dfrac{K_M}{c_1}} = -\,D_2\,\nabla c_2 .\,\vec{n} \tag{5}$$

The constants V_{max} and K_M have been experimentally determined. Solving (3) with the reaction rates (4) and using the boundary conditions (5) produces the concentration of r-luminols c_2 as a function of the concentration of luminols c_1.

2 LIGHT RADIATION MODEL

It is assumed that r-luminols/aminophtalates emit photons isotropically, i.e. the emitted photon has an equal probability of being emitted in any solid angle from the molecule. However, only the photons emitted inside a cone of angle 40° are detected by the sensor; indeed, outside this cone, photons are reflected by the APS surface (fig. 4).

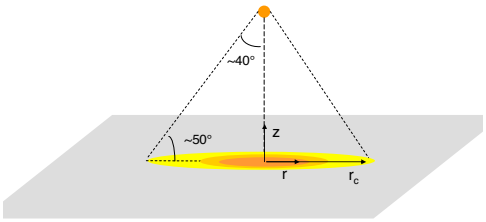

Fig.4. Sketch of light emission from the r-luminols/aminophtalates.

It can be shown that the transfer function is close to a truncated Gaussian, and its expression is

$$A(r,z) = \exp\left(-\frac{1}{2}\left(\frac{r}{r_c}\right)^2\right)\frac{I}{4\pi z^2}H(r_c - r) \tag{6}$$

where H is the Heaviside function, r_c the critical radius given by $r_c = z\tan(40°)$ and I is an intensity value. The value of I is not important since the signal on the sensor will be analyzed relatively to the maximum pixel intensity.

In the case where the fluid is not transparent, we make the assumption that the light is absorbed proportionally to the traveled distance. By denoting z_0 the distance above which all the light intensity is absorbed (z_0~50 μm for water), the transfer function (6) can be rewritten under the form

$$A(r,z) = \exp\left(-\frac{1}{2}\left(\frac{r}{r_c}\right)^2\right)\frac{I}{4\pi z^2}H(r_c - r)\left(\frac{z_0 - z}{z_0}\right)H(z_0 - z) \tag{7}$$

The light captured by the APS result in the superposition of all the radiated light by the r-luminols/aminophtalates (fig. 5). A coupling with the r-luminol concentration is then needed.

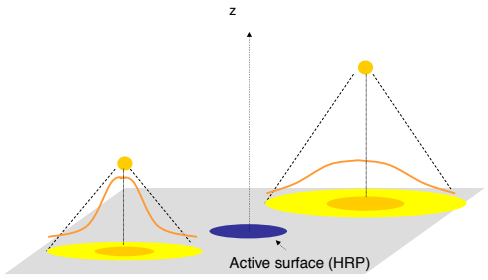

Fig.5. Schematic of the light arriving on the APS surface. R-luminols close to the surface will produce a sharp, powerful Gaussian peak while distant r-luminols will only produce a diffuse, smeared peak.

3 COUPLING CHEMILUMINESCENCE REACTION AND RADIATION MODEL

The detected signal will be the superposition of the light emitted by each elementary volume of the spatial domain. Hence the volume is decomposed in planes of thickness Δz and volume elements $\{\Delta x, \Delta y, \Delta z\}$ (fig.6).

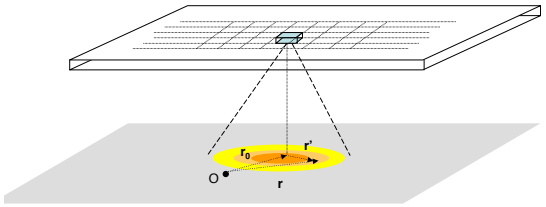

Fig.6. Schematic of the light emission of each elementary volume element towards the sensor.

The algorithm then necessitates downloading the r-luminol concentration c_2 calculated with COMSOL into a MATLAB program. The MATLAB program calculates successively the contribution of each horizontal elementary plane. The contribution S_i of the elementary plane I can be written formally under the convolution form

$$S_i(\vec{r}) = \Lambda_i(\vec{r}) \otimes c(\vec{r}) = \int_{plane\,i} \Lambda_i(\vec{r}_0 - \vec{r})\, c(\vec{r}_0)\, ds \qquad (8)$$

Using the relation $F(f \otimes g) = F(f)F(g)$ where F is the Fourier transform [7], the luminescence signal is obtained by

$$S_i(\vec{r}) = IF\left[F(\Lambda_i(\vec{r}))\, F(c(\vec{r}))\right] \qquad (9)$$

where IF is the inverse Fourier transform. On a numerical standpoint, FFTs (Fast Fourier Transform) and IFFTs (inversed Fast Fourier Transform) of the MATLAB toolbox are used. The final signal S is given by the summation of all the Δz planes contributions

$$S = \frac{1}{\sum_i \Delta z_i} \sum_i \Delta z_i\, S_i \qquad (10)$$

4 MODELING RESULTS

4.1 Concentration

The r-luminol concentration is calculated by the COMSOL model. Due to r-luminols recombination (2) and to a relatively low diffusion speed of luminols compared to the reaction speed, the r-luminol concentration has the flat shape shown in figure 7.

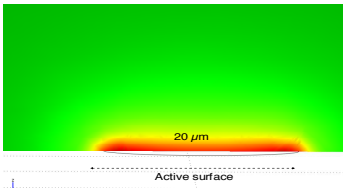

Fig.7. Map of r-luminol concentration at t= 500 seconds.

4.2 Radiative transfer function

A typical radiative transfer function is shown in figure 8.a. Depending on the z-location of the considered horizontal plane, the signal differs: close to the APS surface the signal has a "rectangular profile" closely linked to the concentration profile. Further from the APS surface, the signal is smeared—as expected—and has a Gaussian shape (fig.8). However, because the radiative transfer function has a $1/z^2$ dependency, the contribution of the distant planes is small.

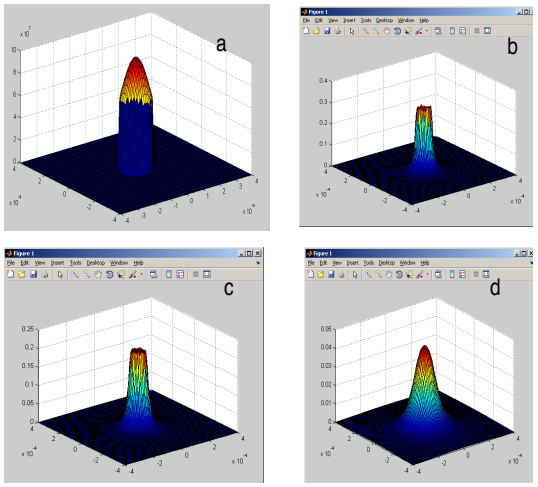

Fig.8. (a) transfer function for a source at 10 μm from the APS surface; (b) image of the horizontal plane at 5 μm; (c) plane at 10 μm; (d) plane at 20 μm.

4.3 Light signal on the APS

Using the coupling algorithm of section 3, the resulting light signal is shown in figure 9 where the figure 9.a is a contour plot of the r-luminol concentration, figure 9.b the calculated light signal and figure 9.c the experimental observation at t=500 s. The ring of maximum light signal at the periphery of the circular active surface corresponds to

the ring of maximum concentration. This ring is experimentally observed in fig.9.c (limited by the dark contour).

In the case where a spurious small convective motion occurs in the micro-chamber—for example under the action of a Marangoni convection [8], the model can easily be adapted by changing the reaction diffusion model by a convection-diffusion-reaction model based on the equation

$$\frac{\partial c_i}{\partial t} + U.\nabla c_i = \nabla.\left(D_i \nabla c_i\right) + R_i \qquad (11)$$

where U is the flow velocity. Figure 10 compares the results for a flow of 20 μm/s in a 300 μm deep micro-chamber. For larger velocities of the fluid (U_x = 50 μm/s), corresponding to a shear rate of $\partial V/\partial z = 6V/d \approx 0.6 \quad s^{-1}$ a luminescent wake forms, that can spread on a relatively long distance, as shown in figure 11.

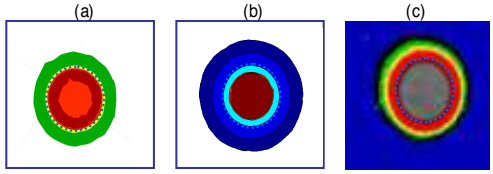

Fig.9. (a) concentration (COMSOL), (b) light signal (COMSOL+MATLAB), (c) experimental observation. Unfortunately the color map is different in each figure due to the three different visualization tools. The outer contours are defined by $c=c_{max}/20$ for (a) and $I=I_{max}/100$ for (b) and (c). Dotted line corresponds to the active surface limits.

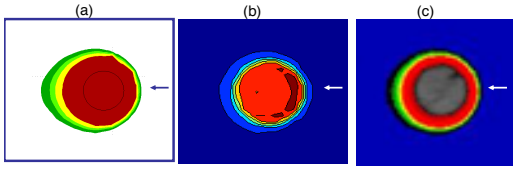

Fig.10. (a) concentration (COMSOL), (b) light signal (COMSOL+MATLAB), (c) experimental observation, in the case of a mean convective velocity U_x = 20 μm/s.

5 CONCLUSION

Enhanced luminol chemiluminescence reaction for detection and bio-recognition in microsystems for biotechnology can be satisfactorily modeled by a diffusion reaction model based on the Michaelis-Menten approach coupled to a light transmission model.

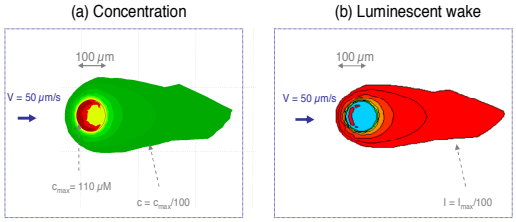

Fig.11. (a) concentration (COMSOL), (b) light signal (COMSOL+MATLAB) for U_x = 20 μm/s.

The halo formed by the diffusing concentration of r-luminols produced by the chemiluminescence reaction is determined by the model. In the case where the dimensions of the system are adapted and the liquid at rest, the coupling of enhanced chemiluminescence and APS detection can be an interesting solution [9]. The model shows that uncontrolled motion of the liquid should be avoided.

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