Optical modeling of bioluminescence in whole cell biosensors

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A B S T R A C T

Bioluminescence-based whole cell biosensors are devices that can be very useful for environmental monitoring applications. The advantages of these devices are that they can be produced as a single-chip, low-power, rugged, inexpensive component, and can be deployed in a variety of non-laboratory settings. However, such biosensors encounter inherent problems in overall system light collection efficiency. The light emitted from the bioluminescent microbial cells is isotropic and passes through various media before it reaches the photon detectors. We studied the bioluminescence distribution and propagation in microbial whole cell biochips. Optical emission and detection were modeled and simulated using an optical ray tracing method. Light emission, transfer and detection were simulated and optimized with respect to two fundamental system parameters: system geometry and bacterial concentration. Optimization elucidated some of the optical aspects of the biochip, e.g. detector radius values between 300 and 750 μm, and bacterial fixation radius values between 800 and 1200 μm. Understanding these aspects may establish a basis for future optimization of similar chips.

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1. Introduction

The combination of bioluminescent microbial sensor cells with electronic circuits provides an innovative option for a new class of devices, combining the environmental monitoring capabilities of genetically engineered microorganisms with optical application-specific integrated circuits (Nivens et al., 2004; Rabner et al., 2006; Eltoukhy et al., 2006; Simpson et al., 1998; Islam et al., 2007). Such bioreporters can be used to detect a very complex series of biochemical reactions, such as bioavailability, toxicity and genotoxicity by the emission of bioluminescence once exposed to the target chemicals they were “tailored” to detect (Belkin, 2003; Daunert et al., 2000; Hansen and Rensen, 2001). Such an integrated device should contain a light-tight enclosure, a bioluminescent bioreporter strain, a micro-environment that sustains the living bioreporter cells, and the integrated circuit for light detection and signal processing (Nivens et al., 2004). The advantages of this device, as demonstrated by Simpson and colleagues (Simpson et al., 1998) are that it can be produced as a single-chip, low-power, rugged, inexpensive device, and can be deployed in a variety of non-laboratory settings. Although there are many different detector types that could be used for this application, the most commonly used are based on a complementary metal oxide semiconductor (CMOS) detector. The key attribute of the device, and the one that will determine its applicability, is the minimum detectable signal (MDS) which is limited by thermally induced leakage current variations and readout noise in the front-end processing circuitry (Wells, 2006). Quite clearly, configuration aspects of the optical system may affect the optical path of the emitted light on its way from the bioreporter cells to the photo-detector.

The detection of light in optical biosensors encounters difficulties when the optical bio-signal intensity is weak, at or below the MDS of the photo-detector. In addition to the biological parameters determining the flux of photons emitted by each microbial cell, there are several physical issues that determine the measured signal: (1) optical properties of the media and the microbial cells, (2) optical design of the reaction container, i.e. geometry, reflection, and absorption, (3) optical path of the emitted light, and (4) the photo-detector quantum efficiency. Noise can be defined as any unwanted disturbance that obscures or interferes with a desired signal. The characteristics of the noise can be of either biological or non-biological origin. For instance, the performance of a photo-detector depends on inherent electrical noise in the detector, the noise in the optical pathway, and non-idealities of the photo-detector material. Major sources of electrical noise in photo-detectors include thermal noise (i.e. white noise), shot noise, and low-frequency noise (Yotter and Wilson, 2003). Different approaches have been applied to overcome these issues by enhancing the transmission of light in biochips, e.g. optical fibers (Horry et al., 2007; Polyak et al., 2001), waveguides (Ruano et al., 2003), thin layer coatings (Fouque et al., 2007), and total internal reflection (TIR)-based biochips (Chromis and Lee, 2004). Despite of these
solutions, there is no comprehensive knowledge on how the light distributed in these biochip systems, a knowledge which can contribute to improving biochips design.

In this paper we address several issues in bioluminescent-based biochips design and the optimization of the optical systems, using the ray tracing algorithms provided by the ASAP software. Stray light analysis is performed to optimize the total light collection efficiency and the system optical response. Various reaction container geometries and microbial cell concentrations are simulated and compared to experimental results. The optimization elucidated some of the optical aspects of the biochips. By understanding these aspects a more efficient and sensitive biosensor may be achieved.

2. Materials and methods

2.1. Absorbance assay of bacterial suspension

Absorbance of the *E. coli* cells in suspension was determined by optical density (OD) measurements (Spekol 1200, Analytik Jena, Germany). Different dilutions of suspended bacteria were measured for OD values in four wavelengths (475, 500, 525 and 600 nm) in 1 cm wide glass cuvettes (Sarstedt, Germany). Bacterial concentrations in the tested suspensions were determined by viable colony (CFU – colony forming unit) counts (Adams and McLean, 1999).

2.2. Optical modeling of bioluminescence

The total number of emitted photons by the genetically modified *E. coli* cells is a function of their interaction with the toxic chemicals they were designed to sense. The model assumed their bioluminescence as an incoherent, uniformly distributed, monochromatic light emitting media. The simulation assumed concentration of $10^7$–$10^9$ bacterial cells in 1 ml of solution, and a uniform non-directional emission distributed over 4π radians for every volume unit. Furthermore, a constant absorption coefficient, $\alpha$, due to intrinsic absorption by media and the bacterial cells was assumed. Internal scattering was neglected due its lack of effect on light intensity and spatial distribution. Furthermore, an experimentally derived absorption coefficient, which includes both light scattering and absorption due to bacterial cells, was fitted to the model. Finally ray reflectance and transmittance were specified by Fresnel’s equations for direct impact of incoherent light:

$$R = \frac{(n_1 - n_2)^2}{(n_1 + n_2)^2}$$  \hspace{1cm} (1)

$$T = 1 - R = \frac{4n_1n_2}{(n_1 + n_2)^2}$$  \hspace{1cm} (2)

when $R$ represents the reflectance, $n_1$ represents the refractive index of material 1, $n_2$ represents the refractive index of material 2, and $T$ represents the transmittance.

The 3D modeling of the bioluminescence in the commercial micro-reaction chamber (384-well micro-titer plate, Greiner Bio-One, Germany) and in the toxicity analysis biochip (Fig. 1) with all their physical properties was done using the optical Advanced Systems Analysis Program (ASAP, Breault Research Organization, Inc.) software. The model allowed a bioluminescent ray with a wavelength of 550 nm to split twice into daughter rays. Ray energy cutoff was $10^{-18}$ of the initial energy at the beginning of the simulation. The bacterial cells were modeled to constantly emit 1 photon as a function of their interaction with the toxic chemicals they were designed to sense.

![Fig. 1. Modeling of a toxicity analysis biochip: (A) The layout of the biochip; (B) the manufactured biochip and (C) simulation of the bioluminescence rays distribution in the toxicity analysis biochip.](image-url)
per second per bacteria, a value that is in the correct range of bacterial bioluminescence.

Ray tracing methods were used to model the light collection yield and efficiency by the solid state optical detector. The collection yield is defined as:

$$\eta_0 = \frac{N_c}{N_e} \quad (3)$$

when $\eta_0$ represents light collection yield, $N_c$ represents the total number of collected photons per second and $N_e$ represents the total number of emitted photons per second. The light detection efficiency is defined as:

$$\eta_1 = \frac{\phi_c}{\phi_e} \quad (4)$$

when $\eta_1$ represents light detection efficiency, $\phi_c$ represents the total flux of collected photons and $\phi_e$ represents the total flux of emitted photons by the bacterial cells in the medium.

2.3. Bioluminescence detection from E. coli cells in a commercial micro-reaction chamber

Genetically engineered E. coli cells harboring a recA::luxCDABE fusion (strain DPD2794) were grown overnight in Luria-Bertani (LB) broth (Domka et al., 2007) containing 0.1 mg/ml ampicillin with shaking conditions at 37 °C. The overnight culture was diluted ×1/100, regrown to an optical density of 0.15 (600 nm), and Nalidixic acid (NA), which served as the model toxicant, was added to a final concentration of 5 $\mu$g/ml. Following further 1 h incubation under the same conditions, aliquots of 10, 20, 40, 60, 80 and 100 $\mu$g/ml were introduced into individual wells of an opaque white 384-well micro-titer plate (Greiner Bio-One, Germany) and luminescence was immediately measured using a micro-titer plate luminometer (Victor2 1420, Wallac Finland).

2.4. Toxicity analysis biochip manufacturing

A model 16-well whole cell biochip for toxicity analysis was designed by using Computer Aided Design software (CAD) and manufactured by Computer Numerical Control (CNC) machining. The biochip contained parabolic micro-reaction chambers (1.75 mm in upper radius) and half sphere micro-fluidics (1 mm in radius) drilled in Polyvinylidene fluoride (PVDF) (Fig. 1(A) and (B)). A 500 nm thick aluminum coating was evaporated (E-beam evaporator Edwards-306) on the micro-reaction chambers to enhance reflectivity of the emitted bioluminescent rays (Fig. 1(B)). In these micro-reaction chambers, bacterial cells were fixed in agar and were deposited in a cylindrical shape.

3. Results and discussion

3.1. Optical absorbance in bacterial suspension

A conventional optical method was applied to measure bacterial concentration in suspension. The absorbance of the light due to the suspended bacterial cells was measured and correlated to bacterial concentration. Results of the absorbance due to different bacterial concentrations at different applied optical wavelengths are shown in Fig. 2(A). The absorbance increased linearly with the bacterial concentration in the range of 8 × 10⁶–7.53 × 10⁷ CFU/ml. These absorbance values are not entirely composed of the absorbance of the light by the bacterial cells. Light scattering is another phenomenon which occurs when the light reacts with the bacterial cells. Therefore, the absorbance values thus obtained include both absorbance and scattering parts, both increase with the bacterial concentration values and together they can be referred to as attenuation.

Beer–Lambert law relates the absorbance of light to the properties of the material through which the light is travelling. The absorbance is linearly dependent with the absorbance coefficient. Therefore, linear regression analysis was applied to extract the absorbance coefficient of the bacterial suspension for each different wavelength (Fig. 2(A)). It may be seen that the absorbance coefficient values decreased with the applied wavelength. The absorbance coefficient by definition is dependent on the way the material absorbs energy. Accordingly, a ray of light with longer wavelength and less
The impact of the bacterial concentration on the detection optical aspects: (A) photons detection rate; (B) detection yield (solid line) and total bacteria (striped line) and (C) detection efficiency.

energy, most likely to be less absorbed by the medium than a more energetic light. Furthermore, the energetic ray may react with more residual materials in the medium resulting in relatively higher absorbance.

3.2. Optical modeling of bioluminescence in commercial micro-reaction chamber

The bioluminescence emitted by an E. coli cell suspension in the chambers of a standard 384-well micro-titer plate micro-reaction chamber plate was studied. The impact of different E. coli suspension volumes on the bioluminescence distribution was modeled and compared to experimental measurements. Furthermore, the effect of the bacterial concentration on the bioluminescence detection yield and efficiency was simulated.

The comparison of the normalized bioluminescent light detection for different bacterial suspension volumes between the simulation results and the experimental measurements is shown in Fig. 3. The experimental results thus obtained exhibited a high correlation to the simulation results with a correlation coefficient of 0.98.

The impact of bacterial cell concentration on bioluminescence detection in the micro-reaction chamber is shown in Fig. 4. Photons detection rate values resulting from different bacterial cell concentrations clearly indicated the beginning of a saturation effect at $2 \times 10^9$ bacterial cells per ml (Fig. 4(A)). Fig. 4(B) represents the detection yield in terms of the ratio in percentage between the photons detected and the total photons emitted by the bacterial cells. Results obtained indicate that the detection yield decreased exponentially for higher bacterial concentrations. Furthermore, the detection efficiency in terms of the ratio in percentage between the detected flux and the total flux emitted by the bacterial medium depicted an exponential decrease from 20% to 3% for bacterial concentration range of $5 \times 10^7$–$10^9$ cells per ml (Fig. 4(C)).

3.3. Whole cell biochip for toxicity analysis based on bioluminescence detection

Bioluminescent-based whole cell biochip for toxicity analysis was developed. Its biosensing capabilities are based on genetically modified E. coli cells which biochemically react to the presence of genotoxicant materials by the emission of bioluminescence. The biochip harboring the sensing E. coli cells was modeled and simulated to study the optical distribution of the bioluminescent rays in the biochip.

The constructed model of the toxicity analysis biochip was simulated with the optical software ASAP™. Bioluminescent light distribution in the biochip is shown in Fig. 1(C). The software...
simulated 1000 rays emitted from the bioluminescent bacteria. Energy loss due to absorption as a function of the optical path was calculated and light detection optimization was simulated to result in two optical aspects: detection efficiency and detection yield. The effects of the detector radius (rd) and the fixation agar rod radius (ra) on the detection yield and the detection efficiency was investigated.

The detection efficiency and yield in the biochip in response to different detector radii (ra = 0.5 mm) are shown in Fig. 5. A positive correlation was resulted between the detection yield and the detector radius for 0–2.225 mm radius values (Fig. 5(A)). Although the total number of 3.5 × 10^8 bacteria in the system was constant, an optimal detector radius range was observed between 300 and 750 μm radius values of the detector (Fig. 5(B)).

The impact of shifting different fixation agar rod radii on the detection efficiency and yield in the biochip (ra = 0.5 mm) is shown in Fig. 6. A decrease with the detection yield as a function of the fixation agar rod radius for 0–1.725 mm radius values was observed (Fig. 6(A)). Although small agar rod radii yielded high detection yield values, the total number of bacterial cells in this region was negligible resulted in a limited number of emitted photons which may affect on the accuracy of these results. An optimal fixation agar rod radius range was shown between 800 and 1200 μm radius values of the fixation rod (Fig. 6(B)).

4. Summary and conclusions

A 3D optical model of a bioluminescence emitting bacteria localized in a whole cell biochip was developed, allowing an optimization of light collection and detection. The model provides a convenient tool for a comprehensive analysis of stray light in the biochip by simulating several optical aspects with a ray tracing program. The optical aspects were optimized in response to bacterial concentration and system geometry. The results thus obtained will help to design a more efficient light collecting biochip. In the future, comprehensive understandings of bioluminescence distribution in biochips will allow deploying for sensitive applications that require a rapid detection of environmental toxicity in water supplies.

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Appendix A. Supplementary data


References