Optical and Electrical Interfacing Technologies for Living Cell Bio-Chips

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Abstract: Whole-cell bio chips for functional sensing integrate living cells on miniaturized platforms made by micro-system-technologies (MST). The cells are integrated, deposited or immersed in a media which is in contact with the chip. The cells behavior is monitored via electrical, electrochemical or optical methods. In this paper we describe such whole-cell biochips where the signal is generated due to the genetic response of the cells. The solid-state platform hosts the biological component, i.e. the living cells, and integrates all the required micro-system technologies, i.e. the micro-electronics, micro-electro optics, micro-electro or magneto mechanics and micro-fluidics. The genetic response of the cells expresses proteins that generate: a. light by photo-luminescence or bioluminescence, b. electrochemical signal by interaction with a substrate, or c. change in the cell impedance. The cell response is detected by a front end unit that converts it to current or voltage amplifies and filters it. The resultant signal is analyzed and stored for further processing. In this paper we describe three examples of whole-cell bio chips, photo-luminescent, bioluminescent and electrochemical, which are based on the genetic response of genetically modified *E. coli* microbes integrated on a micro-fluidics MEMS platform. We describe the chip outline as well as the basic modeling scheme of such sensors. We discuss the highlights and problems of such system, from the point of view of micro-system-technology.

Keywords: Bio-chips, micro system technology (MST), biosensors.

1. INTRODUCTION

Whole-cell Bio-chips (WCBC) are micro-fabricated platforms that integrate living cells for screening, detection, sensing and monitoring of chemical substances. Such chips include few micro-system technologies and Micro-Electro-Mechanical Systems (MEMS) such as micro-fluidics, micro actuators, micro-magneto-mechanical systems (MMMS), microelectronics and micro-optics. Combining various micro-technologies, such as micro opto electro mechanical systems (MOEMS), and integrating a biological component allow forming a "lab on chip". Integrating cells on WCBC can either use the cell functionality or detect mal-functioning cells; both are unique features of such devices. One key consideration of WCBC is the design of a sensitive platform. Integrating living organisms on chip demands special technological solutions for the cells well-being and signal optimization. We will present in this paper highlights and problems of few WCBCs featuring optical, electrochemical and electrical (Impedance) detection.

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WCBC can be applied for environmental monitoring [1-11] and medical applications [12-14]. For example cancer cell monitoring and stem cell characterization [15-18] appeared recently. WCBC integrate few technologies from the life science and engineering disciplines. Here is short list of the key problems that require special technological solutions.

1. Cells sampling and deposition; i.e. cell harvesting procedures and cell placement on the chip. Whole-cell lab-on-chips can be used in the laboratory and in field deployable system. The complexity and the level of automation of the cell sampling and deposition depend strongly on the system size as well as over all cost per measurement.
2. Cell storage methods [19, 20], it depends on the cell type, longevity of use and the logistics involved in the specific application.
3. Cell maintenance; depends on the mode of use, type of cells and the application.
4. Cell replacement and disposal. Although there are no regulation for whole-cell bio chip we assume it follows existing cells disposal methods.

Whole-cell biochips offer an inherent advantage for functional sensors, i.e. probing general cell characteristics and behavior. WCBC provide answers to questions dealing with

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cell behavior and functionality such as "is the water safe to drink?" [9], "is the environment safe?" [7] or "is the cell cancerous?" [14]. In principle, cells integration on chip can emulate behavior of more complex living systems, thus providing a simple system answering very complex problems at relatively simple way and low cost. From the engineering point of view, it takes advantage of the MEMS research and development and the increasing improvement and advances in the field of sensitive optical sensors and Ultra Large Scale Integration (ULSI) Integrated Circuit (IC) technologies.

Several micro technologies platforms for basic whole-cell biosensor (WCBS) technologies are reviewed. In a previous paper [21] we classified those chips according to a. their mode of use and b. mode of sensing. The mode of use defines two classes of whole-cell biosensors: a. long term use whole-cell biosensors, and b. short term use whole-cell biosensors. The "mode of use" classification is based on the way the cells are handled prior to integration and their integration mode [19, 20]. Here are some details of the two classes of use [21].

1.1. Whole-Cell Bio Sensors for Long Term Usage

In this class of bio-sensors the cells are stored for a long period of time. When needed, the cells are activated and used and disposed. The cells can be used once or applied in a continuous sensing for days, weeks or even months. Those are bio-sensors where the biology is used for functional detection; i.e. the cell's functional response to external excitation is an intrinsic part in the detection process. The biology is the base of the detection mechanism and the electronics and photonic circuits are used to extract the signal. Integrating living cells on a chip for long term usage require life science and engineering technologies compatibility. It requires special attention since both technologies use different and in some cases incompatible, materials and processes. For example, during micro-fabrication there are highly toxic materials and aggressive cleaning and etching procedures and in some steps the chip is exposed to elevated temperature, far beyond the limits of the biological component. On the other hand, the biological materials include contamination, e.g. alkali metals etc., which is hazardous to semiconductor devices. Therefore, it is a good common practice to first process the semiconductor platform, second to deposit the cells and third, start the measurement. Most WCBC use prokaryotes with specific storage and handling procedures [19, 20]. It can be extended to eukaryotes, such as yeast or even mammalian cells. Eukaryote cell integration is challenging requiring better on-chip cell maintenance and support than is what available today. One solution is to develop an "Incubator on a chip" for long term use.

1.2. Whole-Cell Biosensors for Short Term Use

In some applications the cells are placed shortly before the measurement. In this case the storage, handling and care of the cells occur independently of the bio-chips process. The chips preparation and the cells procedures are in two parallel routes. The cells can be measured immediately after harvesting or stored under optimal conditions in proper facilities and measured at a later stage, as is or after chemical, or biological process.

The mode of sensing is based on the way the information generated by the cells is converted and translated to electronic information. The "mode of sensing" classification is complementary to that of "mode of use" classification. Historically, a variety of biological assays has been devised for cell based sensing; for example, colorimetry, fluorescence, bioluminescence, and electrochemical detection [12, 22, 23]. The challenge to sense the cell response, providing the desired information, without affecting the cells sensitivity and its viability and metabolism. Note, we define whole-cell biosensor as a system that includes living and functional cells, integrated with photonics and electronics subsystems. We exclude here invasive and destructive methods that break the cell membrane. Since typical enzymes and other large molecules do not penetrate the cell membrane, we need to use methods that extract the information form living cells without compromising their viability and functionality. Here is a short list of such methods.

1. Substrate mediated response using bio-chemical agents with smaller molecular weight as probes [11-14].
2. Delicate membrane penetration using small enough probes, e.g. carbon nano tubes, that do not cause damage [23].
3. Membrane interfacing in a way that will allow the desired signals to be transferred from the cell to the external world (e.g. deposit metal dots on the membrane [24-26]).
4. Examine molecules that are expressed at the membrane [17].
5. Use remote methods such as:
   a. Optical sensing detecting photo and bio luminescence effects [7, 9]
   b. Electrical methods, e.g. impedance spectroscopy, that are sensitive to changes of the dielectric characteristics inside the cells due to the cell response [15].

Note that there is another whole-cell bio chip family for neural sensing. Those chip integrate neural cells where the sensing is by coupling to conducting electrodes or to field effect devices [27] recording the spontaneous or triggered action potential of electrogenic cells as well as other processes, such as cell-acidification [28].

2. WHOLE-CELL BIOCHIP

2.1. General Platform Concepts

This paper is focused on whole-cell bio chips that are based on probing cell response to external excitation. The probing can be on the effect of the cell response on the system, such as at the impedance of cells attached to conducting electrodes [29]. It can be also due to the cell response itself. We present here a short description of a simple modeling method of the biological component in a method used in engineering for systems. We assume that that the biological and the electrical systems can be described by a set of state equations defining internal variables $\{q_i\}$, input x(t) and output y(t). This approach [22] allows modeling compatible with common electrical engineering simulators [30].

These methods are based on probing protein expression. The output of WCBC, y(t), depends on the excitation, x(r,t)

and on a set of internal variables. The input depends on position and time, and the chip generates a single response from each cell container which depends only on time. The system has internal variables, q_i , and their derivatives, q'_i are a function of the input and time and the internal variables and their derivatives:

$$\{q_i, q_i^{\cdot}\} = f(x(r,t), q_i, q_i^{\cdot}, t) \quad (1)$$

The input can be any signal that causes detectable protein expression. It can be an external excitation (e.g. toxicity, heat) or internal causing changes in the pattern of behavior of cells. We assume that the biological response function is determined by a small number of rate limiting equations that depend on the response mechanism of the cells. Typical whole-cell bio chip integrates 10^3-10^7 cells each one generates a signal. The simplest assumption is that the cell response is independent. More complex analysis can take into consideration collective phenomena, such as "quorum sensing".

The whole-cell biochips can be classified according to their output:

a. Optical Output – Either Photoluminescence or Bioluminescence

   i. Photoluminescence – A Photo Luminescent Protein is Generated in Response to the Excitation

   ii. Bioluminescence – the signal is generated due to the reaction between enzymes (luciferase) and substrates (luciferin) that are generated in response to the excitation. The generation of the proteins involves either direct genetic processes where the generation is proportional to the induction or indirect genetic processes where the response of a built-in inhibition mechanism is affected by the excitation. [9, 31]

b. Electrochemical output – the signal is generated due to the reaction between an externally applied substrate with an electrochemically active protein generated by the cell in response to external induction [13].

c. Electrical response – probing the electrical properties of the cells; for example the impedance of cells attached to conducting electrodes on a solid substrate [29] or the spectral impedance response of cells [15].

3. OPTICAL SENSING

Optical sensing is based on either photoluminescence or bioluminescent whole-cell bio-chip [4, 5, 9, 32-38].

3.1. Photo-Luminescent Whole-Cell Bio-Chip

Photo-luminescent biochips require external optical excitation at a wavelength shorter than the expected emission. We demonstrated emission from genetically engineered *E. coli* bacteria that express green fluorescent proteins (GFP) in response to the presence of acute toxic materials. In this case the optical signal output is proportional to the concentration of the fluorescent protein (C_{GFP}). The GFP generation rate depends on the type of the reporters, promoters, toxicant, and ambient conditions. As an example to this system we present here the response of genetically engineered *E. coli* to induction by nalidixic acid in water (Fig. **1**).

The system can be described by two state equations. The first one describes the rate of change in the concentration of the responding promoter, P_r as a function of G_0 ,the concentration of the bacteria (M), and C_{Tox} the concentration of the toxin:

$$\frac{dP_r}{dt} = P_r^{'}(G_0, C_{Tox}) \approx \mu \cdot G_0 \cdot C_{Tox} \quad (2)$$

Where μ is a constant.

The second state equation is derived from the assumption that the GFP generation rate is proportional to the responding promoter concentration. It is assumed that there is a one to one relation between the responding prompters and the triggered reporters expressing GFP [22]:

$$\frac{dC_{GFP}}{dt} = C_{GFP}^{'}(P_r) \approx \alpha \cdot P_r \quad (3)$$

This model is useful at low concentration and it fits nicely the response to various inducers, such as nalidixic acid. At higher concentration, which varies from one toxic material to another, there is typically a decline in the overall response with increasing inducer concentration. A simple expression of the light emission from the microbes assumes that it depends on the GFP concentration, C_{GFP}, and the excitation I_{ex}:

$$I_{emission} = \eta \cdot C_{GFP} \cdot I_{exitation} \quad (4)$$

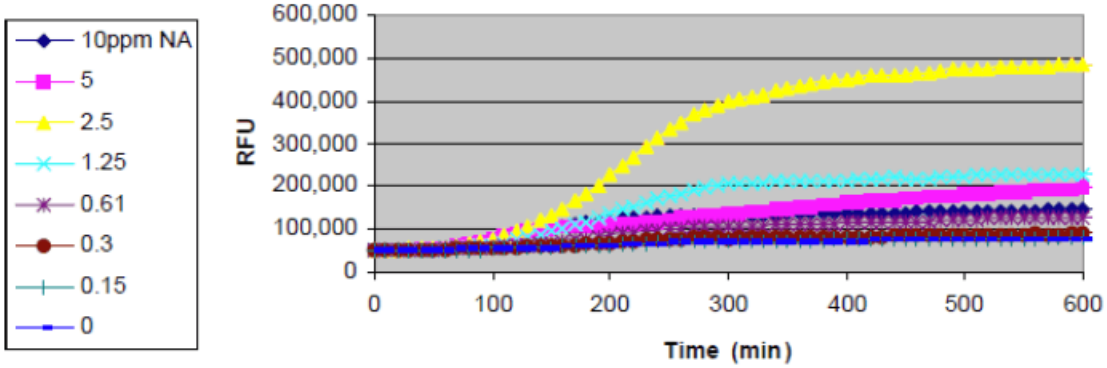

Fig. (1). Fluorescence emission from genetically engineered *E. coli* that were induced by the Nalidixic Acid (NA) [39] and measured by a Victor2 luminometer system. The microbes were engineered at the lab of Prof. S. Belkin at the Hebrew University in Jerusalem.

The excitation is per microbe, hence the over all emission should be the integral on the emission over the whole volume (v) containing the microbes:

$$Total\ emission = \iiint I_{emission} \cdot T \cdot dv = \eta \cdot \iiint C_{GFP} \cdot I_{exitation} \cdot T \cdot dv \quad (5)$$

Where T is the transmission function between the light emission at the coordinate (x,y,z) in the cell micro container and the output pupil of the cell container. Note that there is a further degradation in the luminescent signal due to the window and the optical filters transmission, the photon collection efficiency in the optical sensor entrance pupil and the sensor quantum efficiency.

3.2. Bio-Luminescent Whole-Cell Bio-Chip

First we describe the bio-chemical modeling of such devices. The mechanisms of bioluminescent whole-cell bio chips are well described in a recent review paper [9]. It is assumed that light is generated by the chemical reaction between the two enzymes, luciferase (E_L) and reductase (E_P) that are expressed in response to toxic material and a long-aliphatic aldehyde that acts as a substrate (S). The outcome of the chemical reaction is fatty acid with concentration P.

The rate of change of both enzymes, luciferase and reductase, depend on their expression, thus it is a function of the promoter's concentration, P_r [22]. However, they may have a limited lifetime thus the enzyme destruction rate (e.g. conversion to products in non-emissive pathways) depends on the enzymes concentrations.

$$\frac{dE_L}{dt} = \alpha_L \cdot P_R - \frac{E_L}{\tau_L}, \quad \frac{dE_P}{dt} = \alpha_P \cdot P_R - \frac{E_P}{\tau_P} \quad (6)$$

The promoters generation rate is similar to that given for photo-luminescent (1). This modeling predicts well the response to low concentration of toxic material. Better model, including higher concentration effects and more accurate state equations modeling is given by [30]. Typical response of such system appears in bioluminescent microbe's response to external inductions [22]. An example for such response of genetically engineered *E. coli* bacteria is shown in Fig. (**2**).

Next we describe the-optical modeling of the bioluminescent chip. The optical emission is assumed to be uniform in the exposed micro container. The light is emitted from each microbe over 4π spatial angle, reflected by the various interfaces, scattered by other bacteria and absorbed by other bacteria and the medium. An approximate modeling was given by [39] and more accurate modeling using ray tracing (ASAP™ software package) [40]. They tested various reaction container geometries and microbial cell concentrations as shown for example in the next Fig. (**3**).

A major problem in such modeling was the estimation of the absorption of the microbe dispersion in the micro containers. Ben-Yoav *et al.* [40] measured the absorbance of the *E. coli* cells in suspension by optical density (OD) measurements and a complete optical modeling of bioluminescence biochip is shown in [40]. We show an example for such modeling for the bio chip to the left and the ray tracing model to the right. Such modeling allows defining the system response as a function of the cell container shape. For example, we show in Fig. (**4**), the photon collection efficiency at the output pupil of a parabolic micro container filled with *E. coli*. The paraboloid sidewalls were coated with aluminum.

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.

4. ELECTROCHEMICAL BIO CHIP

The electrochemical signal is generated by substrate mediated response. Under stress, electrochemically active pro-

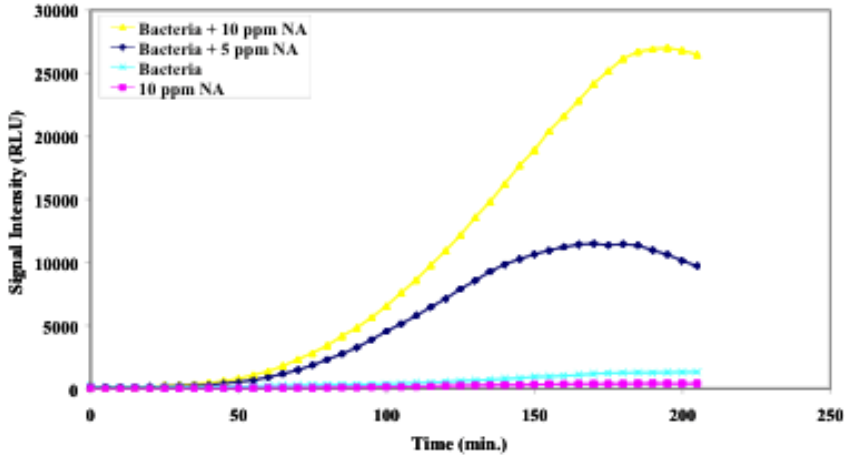

Fig. (2). Bioluminescence intensity vs. time from genetically engineered bacteria as function of time for Nalidixic Acid induction at various concentrations. (*E. coli*, *recA* promoter).

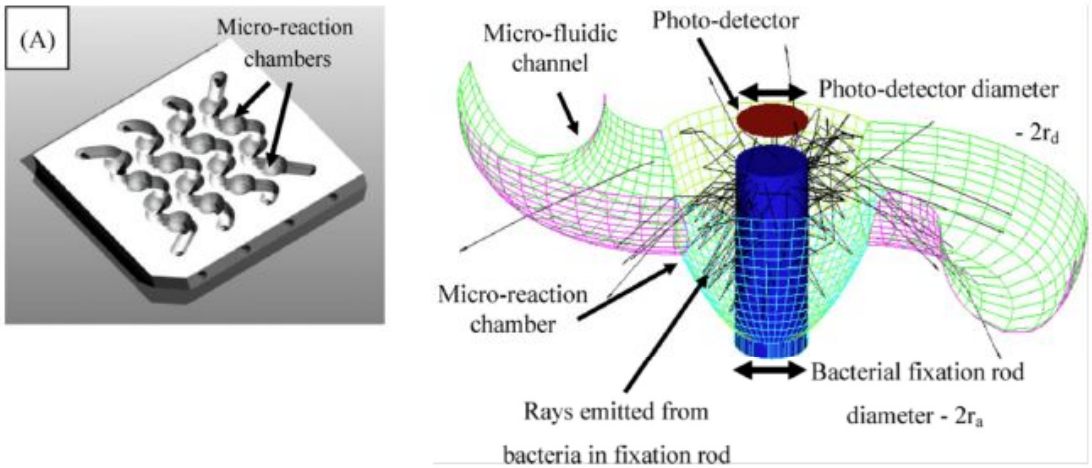

Fig. (3). **(A)** Bioluminescent chip and **(B)** its ray-tracing modeling.

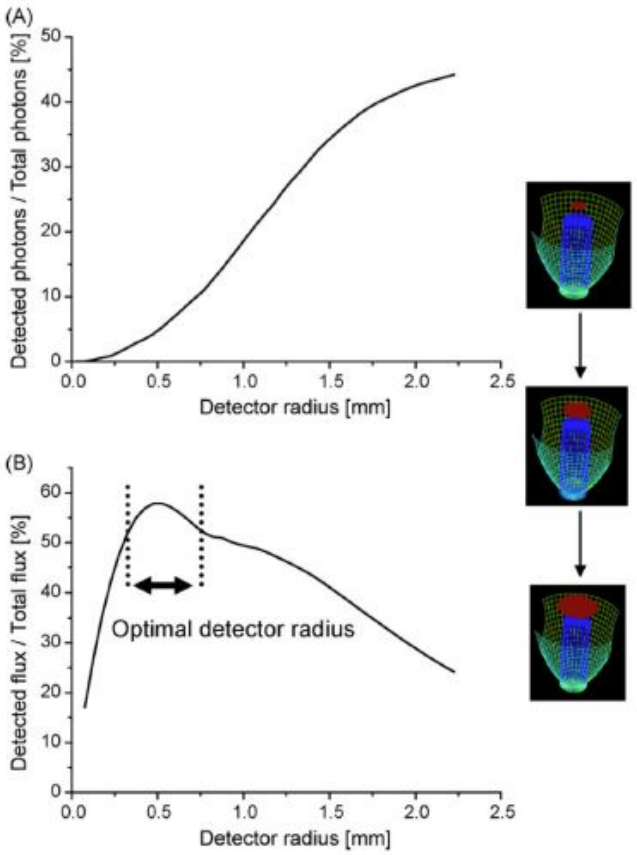

Fig. (4). The impact of the detector on the detection optical aspects: **(A)** detection yield and **(B)** detection efficiency.

tein (i.e. β-galactosidase, alkaline phosphatase) are expressed. Those proteins can interact with externally applied substrate (i.e. p-aminophenyl β-D-galactopyranoside (PAPG)) forming by-products that can be oxidized on specifically designed electrodes. Typical electrochemical bio chip is operated under constant voltage and the oxidation current is measured as indication to the electrochemically active protein expression. Typical on-chip electrochemical cell is shown in Fig. (**5**).

The electrodes are made of Au/Ti (working and counter electrodes) or Ag/AgCl on Au/Ti for the quasi-reference electrode. The chips are made either on thermally oxidized silicon or on polymeric substrates. The expressed protein are generated by the promoters, hence their total concentration, E_T , is proportional to the level of excitation by the toxic material.

$$\frac{dE_T}{dt} = k_0 \cdot C_{Tox} \tag{7}$$

The bio-chip solution contains substrate with concentration S which interacts with the enzyme forming a substrate-enzyme complex, ES [41]. The forward reaction constant of that reaction is K_1 and the reverse reaction constant is k_2. Therefore, the total enzyme concentration is split into free enzyme concentration - E, and bound enzyme concentration - ES:

$$E_T = E + ES \tag{8}$$

The bound enzyme-substrate complex can decompose to the by-product, P and free the enzyme for further activity. The byproduct generation reaction rate is k_3 and it is assumed that the reverse reaction is negligible. The intrinsic output variable here is the by-product concentration, P while the extrinsic output is the electrode current. A detailed analysis of such system appears in the works of Popovtzer et al [12-14, 42].

An integrated whole cell micro-fluidic biochip for electrochemical water toxicity detection was presented by Ben-Yoav *et al.* [11]. *E. coli* cells were genetically engineered to harbor a fusion of the promoter of the stress response gene of *recA* and the reporter gene of alkaline phosphatase (*phoA*). The bacterial whole cells were integrated onto a micro-chip that was manufactured by MEMS technology and various micro-chambers with volume ranging between 2.5 nl and 157 nl with electrode radius between 37.5 µm and 300 µm. The whole cell biochip was used to detected two different genotoxicants, nalidixic acid and 2-amino-3-methylimidazo [4,5-f]quinoline (IQ) (Fig. **7**). Its performance demonstrated minimal toxicant detection of 10 µg/ml for nalidixic acid using 30 minutes for induction and 0.31 µM for IQ using 120

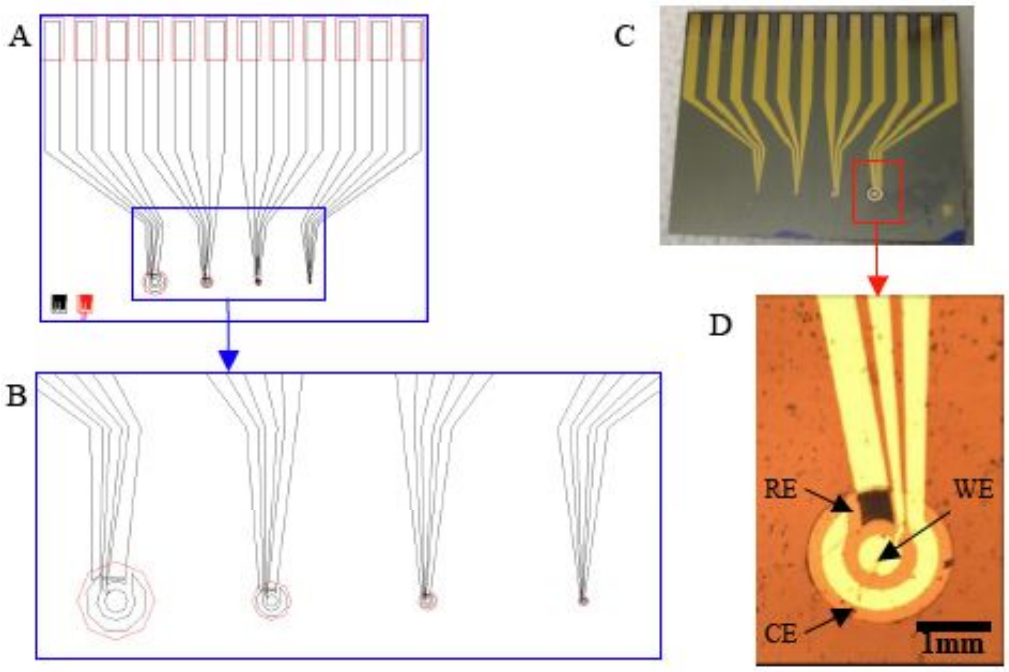

Fig. (5). Electrochemical chip comprises four electrochemical cells. **(A)** Chip masks formed using CAD software (Electrodes mask – Black, Chamber wall mask – Red). **(B)** Closer look on the electrochemical chambers, each contains three electrodes. **(C)** Photo of the electrochemical chip. **(D)** Closer look on the real electrochemical chamber with the three electrodes.

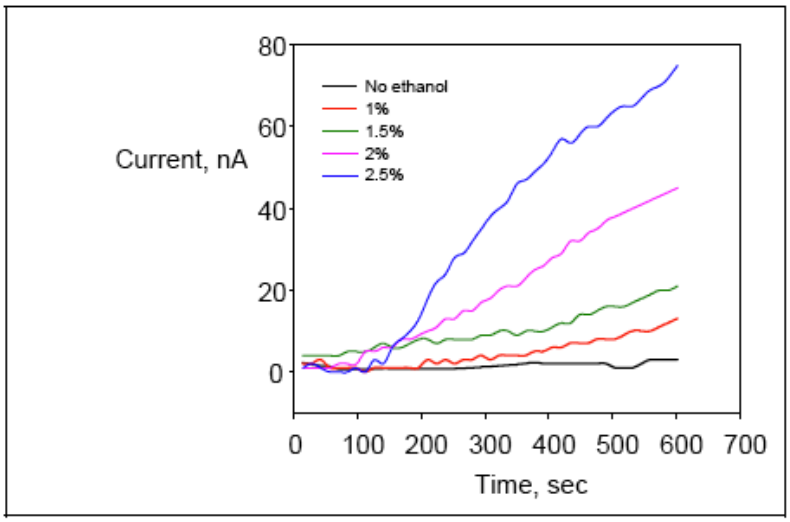

Fig. (6). Amperometric response curves for on line monitoring of ethanol using the nano-bio-chip. The recombinant *E. coli* containing a promoterless *lacZ* gene fused to promoter *dnaK* exposed to 0.5%- 2% concentration of ethanol. The bacteria cultures with the substrate PAPG and the ethanol were placed into the 100nL volume electrochemical cells on the chip immediately after the ethanol addition (~1 min) and where measured using the amperometric technique at 220mV [42].

minutes for induction, both 3 minutes after the addition of the substrate material.

SUMMARY AND CONCLUSIONS

Whole cell bio chips were demonstrated integrating microbe as bio sensors on micro-fluidic chips. The methods use very common micro fluidic system which serves as the biosensor container and the source and drain of the fluids under test.

The microbes are positioned in the cell-micro-containers, typically in an agar matrix or other methods. The fluids under test are in contact with the microbe containing media. The toxic materials are transported towards the bacteria and induce the sensing genetic reaction. In the optical system light is generated and is emitted from the cell micro containers. In the electrochemical sensors the products transport in the cell container. That transport may play an important role [13] in the overall system time response and sensitivity.

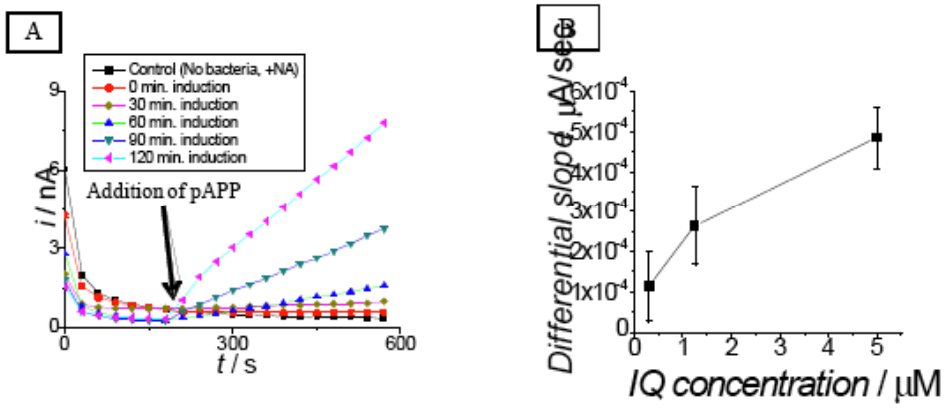

Fig. (7). (A) Chrono-amperometric results of bacterial cells following increasing periods of induction time with nalidixic acid. **(B)** Impact of the IQ concentration on the differential slope of the electrochemical current.

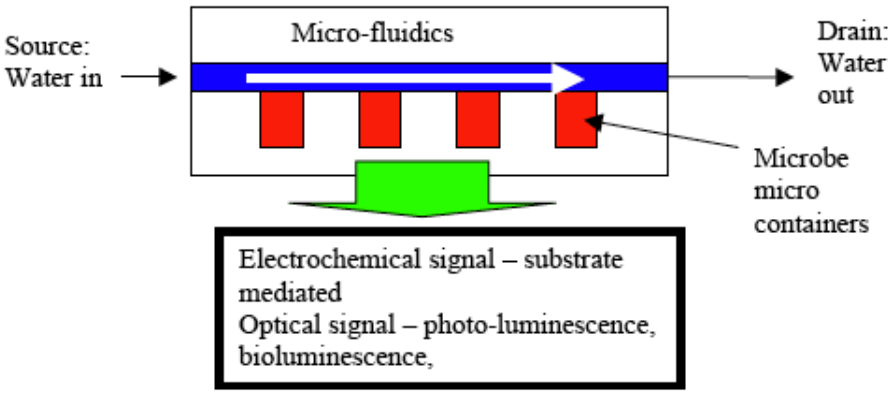

Fig. (8). General whole-cell bio chip system architecture.

The biological response is modeled using state equations. A more comprehensive description of that approach [21] shows system level modeling for the calculation of the Minimum Detectable Signal (MDS) and the Signal to Noise Ratio (SNR), note, that so far we assumed DC measurements where we follow the biological response by sample and hold of the information in few second intervals. This allows integration time of up to few minutes, increasing signal to noise ratio. However, it slows the response. Daniel et al [22] proposed using a low pass filter with a response similar to that of the system. This method works well experimentally, as it improves signal to noise ratio, however a more rigorous model of its theoretical background is required. An alternative method was proposed by Elman *et al.* [43, 44] where a light modulator on chip was built to generate an alternating photo-luminescent emission allowing heterodyne detection at a frequency near 1 KHz. This method, although more complicated, shifts the signal to higher frequency reducing low frequency excess noise. The bio-chip signal, either due to light or electrochemical reaction, is fed onto an electronics unit that amplifies filters or conditions the signal. All of this contributes noise, shape the signal and affects the overall signal to noise ratio.

In summary, we assume that the best modeling of such system should take into consideration all components: biological, fluidics, electronics, and/or optics. All should be integrated in one system model allowing full system modeling [30].

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REFERENCES

[1] Dalzell, D. J.; Alte, S.; Aspichueta, E.; de la Sota, A.; Etxebarria, J.; Gutierrez, M.; Hoffmann, C.C.; Sales, D.; Obst, U.; Christofi, N. A comparison of five rapid direct toxicity assessment methods to determine toxicity of pollutants to activated sludge. *Chemosphere*, **2002**, *47,* 535-545.

[2] Popovtzer, R.; Neufeld, T.; Biran, D.; Ron, E. Z.; Rishpon, J.; Shacham-Diamand, Y. Novel integrated electrochemical nano-biochip for toxicity detection in water. *Nano Lett.*, **2005**, *5,* 1023-1027.

[3] Lee, J. H.; Mitchell, R. J.; Kim, B. C.; Cullen, D. C.; Gu, M. B. A cell array biosensor for environmental toxicity analysis. *Biosens. Bioelectron.*, **2005**, *21,* 500-507 .

[4] Mitchell, R. J.; Gu M. B. An Escherichia coli biosensor capable of detecting both genotoxic and oxidative damage. *Appl. Microbiol. Biotechnol.*, **2004**, *64,* 46-52.

[5] Nivens, D.; Mcknight, T.; Moser, S.; Osbourn, S.; Simpson, M.; Sayler, G. Bioluminescent bioreporter integrated circuits: potentially small, rugged and inexpensive whole-cell biosensors for remote environmental monitoring. *J. Appl. Microbiol.*, **2004**, *96,* 33-46.

[6] Li, Y. F.; Li, F. Y.; Ho, C.L.; Liao, V. H. Construction and comparison of fluorescence and bioluminescence bacterial biosensors for the detection of bio-available toluene and related compounds. *Environ. Pollut.*, **2008**, *152,* 123-129.

[7] Belkin S.; Smulski D. R.; Dadon, S.; Vollmer, A. C.; Van Dyk, T. K.; Larossa, R. A. **()** A panel of stress-responsive luminous bacteria for the detection of selected classes of toxicants. *Water Res.*, **1997**, *31,* 3009-3016.

[8] Belkin S.; Van Dyk, T. K.; Vollmer, A. C.; Smulski D. R.; Larossa, R. A. Monitoring subtoxic environmental hazards by stress-responsive luminous bacteria. *Environ. Toxicol. Water Qual.*, **1998**, *11,* 179-185.

[9] Belkin, S. Microbial whole-cell sensing systems of environmental pollutants. *Curr. Opin. Microbiol.*, **2003**, *6,* 206-212.

[10] Elad, T.; Lee, J. H.; Belkin, S.; Gu, M. B. Microbial whole-cell arrays. *Microb. Biotechnol.*, **2008**, *1,* 137-148.

[11] Ben-Yoav, H.; Biran, A.; Pedahzur, R.; Belkin, S.; Buchinger, S.; Reifferscheid, G.; Shacham-Diamand, Y; A whole cell electrochemical biosensor for water genotoxicity bio-detection. *Electrochim. Acta*, **2009**, *54,* 6113-6118.

[12] Popovtzer, R.; Neufeld, T.; Ron, E. Z.; Rishpon, J.; Shacham-Diamand, S. Electrochemical detection of biological reactions using a novel nano-bio-chip array, *Sens. Actuators B Chem.,* 2006, *119*, 664-672.

[13] Popovtzer, R.; Natan, A.; Shacham-Diamand, Y. Mathematical model of whole cell based bio-chip: An electrochemical biosensor for water toxicity detection, *J. Electroan.. Chem.*, **2007**, *602*, 17-23.

[14] Popovtzer, R.; Neufeld, T.; Popovtzer, A.; Rivkin, I.; Margalit, R.; Engel, D.; Nudelman, A.; Rephaeli, A.; Rishpon, J.; Shacham-Diamand, Y. Electrochemical lab on a chip for high-throughput analysis of anticancer drugs efficiency, *Nanomedicine*, **2008**, *4,* 121-126.

[15] Ron, A.; Singh, R. R.; Fishelson, N.; Shur, I.; Socher, R.; Benayahu, D.; Shacham-Diamand, Y. Cell-based screening for membranal and cytoplasmatic markers using dielectric spectroscopy. *Biophys. Chem.*, **2008**, *135*, 59-68.

[16] Ron, A.; Fishelson, N.; Croitoru, N.; Shur, I.; Benayahu, D.; Shacham-Diamand, Y. Examination of the induced potential gradients across inner and outer cellular interfaces in a realistic 3D cytoplasmic-embedded mitochondrion model, *J. Electroanal. Chem.,* **2010**, *638* (1), 59-69

[17] Ron, A.; Fishelson, N.; Shur, I.; Croitoru, N.; Benayahu, D.; Shacham-Diamand, Y. The effect of irregularity on the dielectric dispersion characteristics of spherical cellular suspension. *Colloids Surf. B Biointerfaces*, **2009**, *74,* 127-135.

[18] Ron, A.; Fishelson, N.; Croitoriu, N.; Shacham-Diamand, Y. Theoretical examination of aggregation effect on the dielectric characteristics of spherical cellular suspension. *Biophys. Chem.,* **2009**, *140*, 39-50.

[19] Tessema, D. A.; Rosen, R.; Pedazur, R.; Belkin, S.; Gun, J.; Ekeltchik, I.; Lev, O. Freeze-drying of sol–gel encapsulated recombinant bioluminescent E. coli by using lyo-protectants. *Sens. Actuators B Chem.*, **2006.** *113*, 768-773.

[20] Bjerketorp, J.; Håkansson, S.; Belkin, S.; Jansson, J. K. Advances in preservation methods: keeping biosensor microorganisms alive and active. *Curr. Opin. Biotechnol.*, **2006**, *17,* 43-49.

[21] Shacham, Y.; Almog, R.; Daniel, R.; Rabner, A.; Popovtser, R. Nano-bio interfacing with living cell biochips, K. Sattler , editor, *Handbook of Nanophysics: Nanomedicine and Nanorobotics,* Taylor and Francis Group, LLC publication, New York. **2010**.

[22] Daniel, R.; Almog, R.; Ron, A.; Belkin, S.; Diamand-Shacham, Y. Modeling and measurement of a whole-cell bioluminescent biosensor based on a single photon avalanche diode. *Biosens. Bioelectron.,* **2008**, *24*, 882-887.

[23] English, B. P.; Min, W.; van Oijen, A. M.; Lee, K. T.; Luo, G.; Sun, H.; Cherayil, B. J.; Kou, S. C.; Xie, X. S. Ever-fluctuating single enzyme molecules: Michaelis-Menten equation revisited. *Nat. Chem. Biol.*, **2006**, *2*, 87-94.

[24] Vernick, S.; Moscovich-Dagan, H.; Porat-Ophir, C.; Rishpon, J.; Freeman, A. and Shacham-Diamand, Y. Directed metallization of single enzyme molecules with preserved enzymatic activity. *IEEE Trans. Nanotechnol.*, **2008**, *8,* 95-99.

[25] Ben-Yoav, H.; Freeman, A. Enzymatically attenuated in situ release of silver ions to combat bacterial biofilms: a feasibility study. *J. Drug Deliv. Sci. Technol.*, **2008**, *18*, 25-29.

[26] Dagan-Moscovich, H.; Cohen-Hadar, N.; Porat, C.; Rishpon, J.; Shacham-Diamand, Y.; Freeman, A. Nanowiring of the catalytic site of novel molecular enzyme-metal hybrids to electrodes. *J. Phys. Chem.*, **2007**, *C 111*, 5766-5769.

[27] Fromherz, P. Joining microelectronics and microionics: Nerve cells and brain tissue on semiconductor chips. *Solid-State Electron.,* **2008**, *52*, 1364-1373.

[28] Poghossian, A.; Schöning, M. J. Chemical and biological field-effect sensors for liquids-a status report, Handbook of Biosensors and Biochips, John Wiley & Sons. **2007**.

[29] Moore, E.; Rawley, O.; Wood, T.; Galvin, P. Monitoring of cell growth *in vitro* using biochips packaged with indium tin oxide sensors. *Sens. Actuators B Chem.,* **2009**, *139,* 187-193.

[30] Rabner A., PhD thesis, Tel Aviv University, **2010**.

[31] Polyak, B.; Mrks, B. Bioluminescent whole-cell optical fiber sensors, the handbook of biosensors and biochips. Edited by Robert S. Marksn et al, J. Wiley and Sons. **2007**.

[32] Sagi, E.; Hever, N.; Rosen, R.; Bartolome, A. J.; Premkumar, J. R.; Ulber, R.; Lev, O.; Scheper, T.; Belkin, S. Fluorescence and bioluminescence reporter functions in genetically modified bacterial sensor strains, *Sens. Actuators B Chem.,* **2003** , *90,* 2-8.

[33] Cho, J-C.; Park, K-J.; Ihm, H-S.; Park, J-E., Kim, S-Y.; Kang, I.; Lee, K-H.; Jahng, D.; Lee, D-H.; Kim, S-J. A novel continuous toxicity test system using a luminously modified freshwater bacterium. *Biosens. Bioelectron.,* **2004**, *20*, 338-344.

[34] Simpson, M. L.; Sayler, G. S.; Fleming, J. T.; Applegate, B. Whole-cell biocomputing. *Trends Biotechnol.*, **2001**, *19*, 317-323.

[35] Sørensen, S. J.; Burmølle, M.; Hansen, L. H. Making bio-sense of toxicity: new developments in whole-cell biosensors. *Curr. Opin. Biotechnol.*, **2006**, *17,* 11-16.

[36] D'Souza, S. F. Microbial biosensors. *Biosens. Bioelectron.,* **2001**, *16*, 337-353.

[37] Magrisso, S.; Erel, Y.; Belkin, S. Microbial reporters of metal bioavailability, *Microb. Biotechnol.,.* **2008**, *1*, 320-330

[38] van der Meer, J. R.; Tropel, D.; Jaspers, M. Illuminating the detection chain of bacterial bioreporters. *Environ. Microbiol.*, **2004**, *6,* 1005-1020.

[39] Rabner, A.; Belkin, S.; Rozen, R.; Shacham, Y. Whole cell luminescence biosensor-based lab-on-chip integrated system for water toxicity analysis. *Proc. SPIE*, **2006**, 6112.

[40] Ben-Yoav, H.; Elad, T.; Shlomovits, O.; Belkin, S.; Shacham-Diamand, Y. Optical modeling of bioluminescence in whole cell biosensors. *Biosens. Bioelectron.*, **2009**, *24,* 1969-1973.

[41] Michaelis, L.; Menten M. L. Die kinetik der invertinwirkung. *Biochemistry Z*, **1913**, *49,* 333-369.

[42] Popovtzer, R., PhD thesis, Tel Aviv University, **2004**.

[43] Elman, N. M.; Krylov, S.; Sternheim, M.; Shacham-Diamand, Y. Bioluminescence Detection using a Novel MEMS Modulation Technique. *IEEE Photonics Technol.. Lett.,,* **2006**, *18,* 2011-2013.

[44] Elman, N. M.; Ben-Yoav, H.; Sternheim, M.; Rosen, R.; Krylov, S.; Shacham-Diamand, Y. Towards toxicity detection using a lab-on-chip based on the integration of MOEMS and whole-cell sensors. *Biosens. Bioelectron.,* **2008**, *23,* 1631-1636.

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