Biosensing for Commercial Applications

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A biosensing platform based on the principle of real-time unlabeled impedimetric detection has been developed. The platform detects bio-targets by measuring impedance changes in real-time. This biosensor is aimed at multiple segments of the life sciences market and is planned for commercialization in the near future. These products will leverage monolithic integration concepts, unique device technology, proprietary user interface software, and smart algorithms for interpreting data generated during the assay.

1 Introduction Motivation

World markets for chemical and biological analytical tools exceed $40 billion with the annual growth rate over 20% in some segments. This market is characterized by high profit margins typically exceeding 50%. The current trend in the bio-analytical markets is a move towards democratization of the technology into the areas of point of care testing, including medical diagnostics, drug development, food safety, environmental monitoring, as well as applied and fundamental research. Such a trend favors an approach like that of SLA that will create high performance and low cost platforms.

A significant fraction of bio-analytical instrumentation is based on the biosensing principle coupling specific biological molecular recognition with physical signal transduction. Most contemporary progress in biosensor technology is due to advances in transduction technology, signal processing, and assay automation. These include improvements in micro-fabrication, micro-electronics, computational data analysis algorithms, system integration, micro-fluidics, and robotics.

2 Real-time, Unlabeled Biosensing

Nearly all biosensor-based assays today involve three or more stages. First, a specific *target* bio-molecule is captured (or bound) by a probe molecule affixed to a sensor surface. Next, a *labeled marker* molecule is attached to the probe-target complex leading to the formation of a labeled molecular complex. This stage typically involves washing excess labeled molecules from the test chamber after the labeled complex is formed. Last, the labeled complex is detected by a transducer using either optical or electrical means. The main purpose of labeling is to increase the sensitivity of transduction to the bound target. A prime disadvantage is the inability to "watch" the binding process in real-time, for labels are typically added only at the endpoint of the binding process.

Trends in biosensing technology are toward the development of faster, cheaper, smaller, and easier to use assay instruments and methods. These, in turn, are driven by market demands for point-of-care diagnostics, field analyses, and consumer home applications. All of these trends are well-served by the development of real-time, unlabeled detection methods. Deleting the molecular labeling stage substantially simplifies the assay protocol, and this leads to reduced processing time and reduced assay and instrument cost. More importantly, signal transduction and analysis of an unlabeled assay can proceed while binding occurs. In other words, an unlabeled assay can be performed in real time, and analysis of the binding kinetics can therefore be done. The ability to analyze binding kinetics leads to increased assay confidence.

3 Impedimetric Platform

A portable, low-cost instrument can be achieved by means of unlabeled electrochemical detection. Within this context, a real-time biosensor platform based on impedimetric detection of specific biological binding has been developed [2-5]. [http://www.sharplabs.com/biosensor.php]. The principal ideas behind impedimetric detection are depicted in

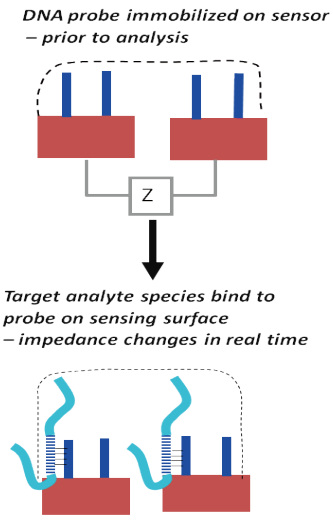


Fig. 1 Impedimetric detection: principle of action. Analyte binding gives a clear change in impedance. At a given frequency, binding can be measured as a function of time (real-time measurement).

Fig. 1 .

An interdigitated electrode surface is functionalized with a biological molecular probe (oligonucleotide sequence, antibody, etc.) that are capable of specific recognition and capture of target analyte molecules. The capture induces a change in the electrical double-layer capacitance in the vicinity of the electrode surface, and thus, a change in the electrode impedance that is detected in real time.

Analyte binding produces impedance changes over a substantial frequency range. Detection is performed while binding occurs since no molecular labeling is used. Although the impedimetric bio-assay has been well-established for decades [1], it is still primarily confined to laboratory research because no common integrated platform exists that allows for development of commercial bio-assays for the broad market. Such a platform requires multi-stage integration, including a micro-array of transducers, supporting circuitry, real-time signal analysis, assay control software, microfluidics, and supporting biochemistry. This impedimetric platform has been developed to include all of these components, and to provide versatility for a broad range of biological assays.

Platform flexibility together with established guidelines for assay development and optimization [2] opens opportunities for application in a variety of different bio-analytical areas. Versatility serves to mitigate the risk of a new product offering in the very competitive biotech market, due to its potential for immediate market penetration. In addition, the platform offers a high performance, low cost paradigm that will allow rapid access to new markets, which have been previously out of reach for this type of instrumentation. Areas of high market potential include research and development, food safety, environmental monitoring, medical diagnostics, and drug development.

4 Platform Features

The core of the platform is the IA-2 impedance analyzer instrument (Fig. 2).

The instrument has a stimulus frequency range of 10-3000 Hz. (This will soon be upgraded to 10-10,000 Hz.) The stimulus voltage range is 10-212 mV. The instrument measures load impedances of up to 500 KΩ. (This will be increased to 10 MΩ in the upgraded version.) A unique and important feature of the instrument is its ability to read up to 15 independent electrodes simultaneously, thus permitting synchronous detection of several bio-analytes on one sensor array. Another important instrument characteristic is the high speed of impedance scanning that performs up to eight impedance measurements per second per electrode. Additional features include sensor temperature control and high bandwidth communication with the host computer.

The impedimetric array (Fig. 3) is comprised of fifteen gold interdigitated electrode (IDE) pairs fabricated on glass with 40 µm gaps (the gap can easily varied from 10 to 100 µm).

The electrodes are enclosed in a micro-fluidic chamber consisting of three separate measuring chambers, each enclosing five individual electrode pairs. If necessary, the fluidic chamber configuration can be altered to create a single measuring chamber for all fifteen IDEs. The IDE surface can be functionalized using various biochemical techniques; thus far, several modifications of the common sulfhydryl chemistry have been tested [5].

Array quality control (QC) after manufacturing and functionalization

Fig. 2 SLA Impedance Analyzer.

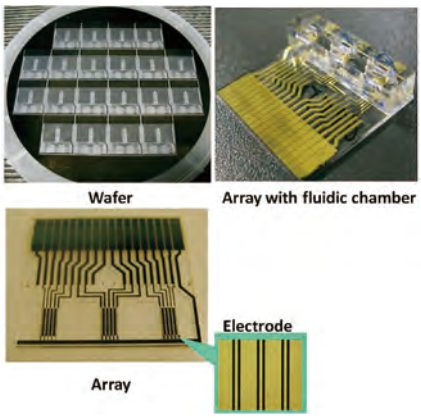
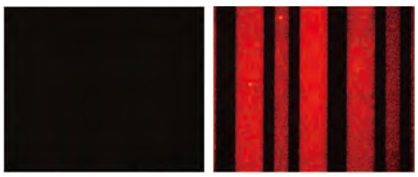


Fig. 3 SLA Impedimetric Array.



Negative control: 0.5 μM of non-specific target

0.5 μM specific target

Fig. 4 Surface Preparation and Fluorescent QC test. The QC test is based on specific capturing of biotinylated target followed by Incubation with streptavidin modified fluorescent beads. A fluorescent signal is then detected microscopically. For the negative control a biotinylated target that does not bind with the probe on the array surface is employed. Fluorescent QC test confirms the quality of the array to capture a specific target.

is critical to ensure assay consistency and accuracy. Accordingly, a protocol for fluorescent QC testing is applied to two randomly selected arrays from each manufactured set. The test is based on specific capturing of a biotinylated target followed by incubation with streptavidin-modified fluorescent beads. A fluorescent response is then observed by optical microscopy. For a negative control a non-complementary biotinylated target is used that does not bind the probes on the array surface. Images obtained for both specific and non-specific test arrays provide a definite indicator of manufacturing and functionalization quality. This is illustrated in Fig. 4.

5 Signal Analysis and Parameter Estimation

A key feature of the SLA platform is a fully integrated data analysis capability. The analysis firmware is based on algorithms described in [4] and [5], and is incorporated into the IA-2 instrument user interface. The instrument allows both real-time and offline signal analysis. Real-time mode provides quantitative results for binding kinetics in a short time as impedance signals are evolving. Offline analysis provides the means for batch processing of assays that have already been performed.

Ideally, probe-target binding is described by an impedance signal model (Langmuir isotherm) that follows a rising exponential function in time after analyte is introduced into the biosensor chamber and is represented by the formula:

$$|Z(t)| = B - Ae^{-st} + v(n)$$

Eq 1

Here, s, A, and B, are positive constants, and noise is represented by $v(n)$.

Ignoring noise and drift, $B-A$ is the constant offset at which the response starts, and represents the biosensor baseline impedance shown in Fig. 5.

The baseline is dependent on sensor structure, buffer concentration, temperature, and stimulus frequency. Shown in grey is a typical noisy impedance signal during target binding. The estimation algorithm takes such a signal as input and extracts the exponential time constant, s, amplitude, A, and the equilibrium (endpoint) amplitude, B. From these, an estimate of the underlying model function can be computed from Eq. 1. The red curve in the figure shows such a model function. The graphical effect of variation in s is also shown. The green curve depicts a larger value of s, and the blue curve a smaller value. Both s and A hold important biochemical information connected with target concentration, sensor surface coverage, and target-probe hybridization kinetics. Amplitude

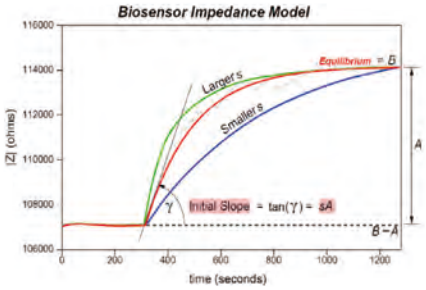


Fig. 5 Grey curve: noisy acquired impedance signal. Red curve: model function (according to Eq. 1) computed using algorithm. Green and blue curves: examples of model functions for larger and smaller (respectively) s values.

A is proportional to the percentage of sensor surface covered by bound target and is related to the maximum binding capacity at the target concentration used. It is also indicative of molecular target-probe affinity. The time constant, s, is proportional to the binding rate constant, and is independent of A. Other quantities may be derived from these fundamental ones. For example, the initial binding rate is trivially given by

$$\frac{d}{dt}|Z|_{t=0} = As.$$

This is illustrated in Fig. 5. Another derived parameter is the area between the baseline impedance and the response curve. The analysis software interface makes all of these available to the operator as well as their mean and variance across the electrodes within each chamber. All of these parameters are useful for assay quantification, classification, and optimization [2].

The estimation algorithm falls into the category of State-space Signal Modeling approaches that have wide applications in signal array processing, medical MR spectrum analysis, speech processing, and now impedance biosignal analysis. The tutorial paper [6] contains an excellent discussion

of the basic ideas, although much development has occurred since its publication.

These methods assume a general signal model composed of uniformly spaced samples of a sum of *p complex* exponentials corrupted by noise, $v(n)$, and observed over a time aperture of N samples. This is described by the formula:

$$x(n) = \sum_{k=1}^{p} a_k e^{s_k n} + v(n),$$

Eq 2

where $n = 0, 1, \ldots N-1$. The parameters of the model are complex amplitudes, $\{a_k\}$, and complex frequencies (poles), $\{s_k\}$. The signal model of Eq. 1 is a special case of Eq. 2 having two *real* poles, $s_1 = 0$ and $s_2 = -s$ in the complex s-plane, and two *real-valued* amplitudes, $a_1 = B$ and $a_2 = -A$.

All state-space algorithms break the general non-linear estimation problem into two steps. The first estimates the $\{s_k\}$ and the second estimates the linearly involved $\{a_k\}$ in Eq. 2, given the poles. In addition, all of the techniques involve a Principal Component Decomposition in step 1 that dramatically reduces the effects of noise on the final estimates.

This methodology is superior to a simple exponential curve fit because a simple curve fit is highly sensitive to the effects of noise. This is readily demonstrated by application of simple curve fitting and the above described method to a noisy synthetic exponential signal. The nominal value of s was 0.002 sec^{-1} and the amount of added noise was such that the signal-to-noise ratio was 30 dB. One thousand iterations were run and the results are summarized in Fig. 6.

The black graph shows s values returned by curve fitting, and the red graph shows those returned by the estimation algorithm. A similar result was obtained for parameter A. It is clear from this that, although more computationally expensive, the more sophisticated algorithm extracts much more stable parameters than does simple curve fitting. Second, the algorithm is naturally extensible to the case where more than one decaying exponential is present in the signal. This may occur, for example, when the sensor is functionalized with more than one probe so that multiple binding processes occur simultaneously.

6 Current Technology Development

Applicability of the platform for development of genomic DNA assays has also been demonstrated. DNA targets have been amplified from three different common commensal and pathogenic *E. coli* strains, and Fig. 7 demonstrates differences in exponential binding curve parameters obtained for these different targets using the estimation algorithm (2).

In the near future the platform will be tested for detection of RNA targets, for direct detection of microorganisms in agricultural produce, as well as in the general environment.

7 Flow-through Module and Whole-cell Studies

Currently under development is a flow-through and sample injection module for the impedimetric array platform. Along with affording a high degree of assay automation, this module will open exciting new opportunities for cell sorting and cell adhesion studies that will be useful in general medical research, immunology, new drug development,

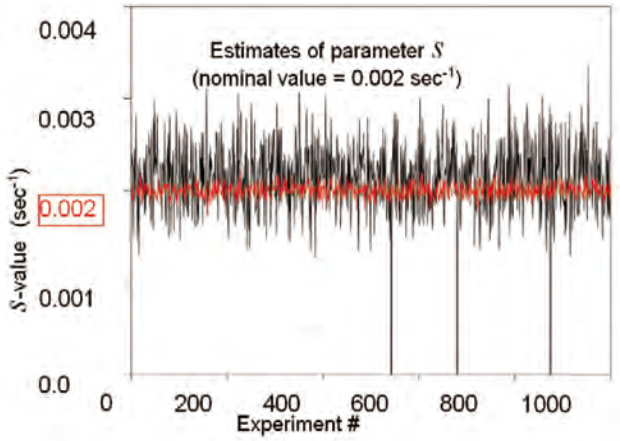
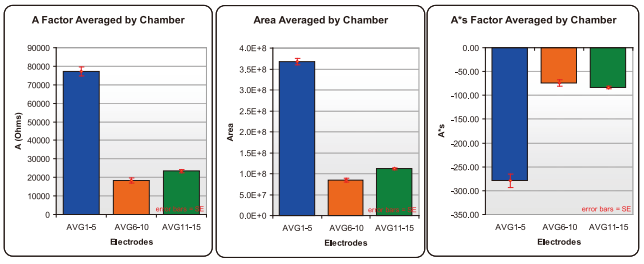


Fig. 6 Statistical variation of estimated s-values over 1000 trials using simple curve-fitting (black) and a sophisticated estimation algorithm (red).



Blue: Specific DNA target
Orange: DNA Negative control 1
Green: DNA Negative control 2

Fig. 7 Estimation algorithm averages for impedance responses to specific and non-specific DNA sequences. Parameter *A* averaged by chamber (left), Area averaged by chamber (center), and *A**s factor averaged by chamber (right).

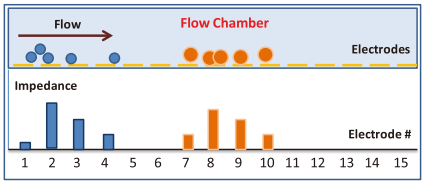


Fig. 8 Diagram of impedimetric analyses of cell population under flow conditions. A snap-shot at a single time point is demonstrated.

and cell biology. The life of multi-cellular organisms, such as humans, is built on cell-cell adhesion and the interactions of various molecules with cell surfaces. These events determine the normal development of the whole organism, its organs and tissues, and may also be responsible for abnormal or pathological processes. In many cases, cell adhesion and interactions with the intercellular matrix occur under flow conditions as, for example, in blood vessels, lymphoid system, urinary tract, reproductive, and digestive systems. The various shear forces affecting cell adhesion in these systems can be simulated using the flow-through module. In addition, impedimetric array electrodes in the flow-through chamber can be functionalized with various biochemical capturing agents, including cell recognition receptors, antibodies, etc., with different affinities toward specific cells under study. Subsequent specific binding of cells to the electrodes will increase the measured impedance. A flow of a cell suspension sample through a chamber with an array of functionalized electrodes will produce a specific signal signature indicative of a particular cell type (Fig. 8).

This signature will be a function of several parameters including: (i) affinity of the cells toward the capturing agent, (ii) cell shape and size, and (iii) flow rate. Thus, varying the array functionalization chemistry and flow rate will allow "fingerprinting" of the cell population in terms of the presence and proportion of specific cell types, and analysis of cell properties in detail.

8 Future Development

Further advanced development will proceed along two major lines. The first will incorporate localized microheaters for individual temperature control of array electrodes, as well as introducing different transducer types, such as bio-functionalized field effect transistors (FET's), in conjunction with IDE's. This allows for wide extension of applications, e.g., DNA melting curve studies, and multiple different assays performed simultaneously on the same chip. The second direction is associated with development of a fully automated portable instrument wherein all assay stages, including sample preparation and target amplification, will be performed 'on the chip'.

9 Conclusions

Commercialization of the developed platform is planned in the near future. Detailed market studies indicate several opportunities including (i) initial market penetration with a platform targeted for R&D users (planned for the beginning of 2013), (ii) development and commercialization of a flow based platform for whole cell assays targeting drug development, food safety, and medical diagnostics, (iii) development and commercialization of 'on- chip' DNA assay targeting food safety and medical diagnostics. In summary, development of the biosensor platform is aimed at creating a pathway for entrance and further expansion into the life sciences market in general. Indeed, Sharp's competitive advantage in system integration and low cost mass manufacturing (especially fabrication of arrays on glass) provides a unique opportunity.

References

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