Use of Information Visualization Methods Eliminating Cross Talk in Multiple Sensing Units Investigated for a Light-Addressable Potentiometric Sensor

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Letters to *Analytical Chemistry*

Use of Information Visualization Methods Eliminating Cross Talk in Multiple Sensing Units Investigated for a Light-Addressable Potentiometric Sensor

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The integration of nanostructured films containing biomolecules and silicon-based technologies is a promising direction for reaching miniaturized biosensors that exhibit high sensitivity and selectivity. A challenge, however, is to avoid cross talk among sensing units in an array with multiple sensors located on a small area. In this letter, we describe an array of 16 sensing units of a light-addressable potentiometric sensor (LAPS), which was made with layer-by-layer (LbL) films of a poly(amidomine) dendrimer (PAMAM) and single-walled carbon nanotubes (SWNTs), coated with a layer of the enzyme penicillinase. A visual inspection of the data from constant-current measurements with liquid samples containing distinct concentrations of penicillin, glucose, or a buffer indicated a possible cross talk between units that contained penicillinase and those that did not. With the use of multidimensional data projection techniques, normally employed in information visualization methods, we managed to distinguish the results from the modified LAPS, even in cases where the units were adjacent to each other. Furthermore, the plots generated with the interactive document map (IDMAP) projection technique enabled the distinction of the different concentrations of penicillin, from 5 mmol L^{-1} down to 0.5 mmol L^{-1}. Data visualization also confirmed the enhanced performance of the sensing units containing carbon nanotubes, consistent with the analysis of results for LAPS sensors. The use of visual analytics, as with projection methods, may be essential to handle a large amount of data generated in multiple sensor arrays to achieve high performance in miniaturized systems.

Silicon-based multiple sensors have fostered the development of multiparameter systems within a single chip, which is advantageous for producing miniaturized devices.[1] The use of field-effect devices (FEDs), for example, is suitable for on-chip integration of biosensing arrays in biological recognition systems.[1,2] Light-addressable potentiometric sensors (LAPS) represent a typical example of such FED structures and a promising platform for multisensors.[1−3] The sensor signal of LAPS consists of an induced ac photocurrent, where the area of the sensing surface is defined by illumination of a modulated light beam. With light generated by 16 infrared light-emitting-diodes (IR-LEDs),[3] an enzymatic biosensor array was obtained by modifying the gate surface with appropriate materials.[4] This device was also employed to evaluate the cell metabolism and to detect the growth of bacteria.[1,2,5,6] However, the phenomenon of cross talk[7−9] may occur during the signal detection for multiple sensing units on the LAPS chip, which may be related to the small distance among the 16 IR-LEDs.

The difficulties in distinguishing similar samples in sensing and biosensing tasks are normally obviated with the use of statistical methods, including principal component analysis (PCA),[10] as it is the case for biosensors and taste sensors.[11−15]

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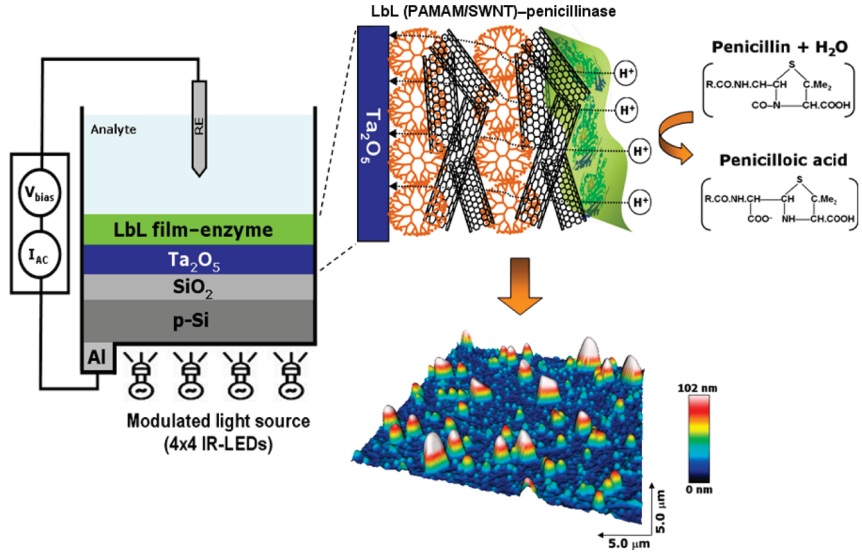
Scheme 1. Experimental Setup of the LAPS Sensing Unit (Left);[a] LbL Film Architecture (Middle, Zoomed Figure);[b] Reaction Involved in the Detection Principle (Far Right); and AFM Image of the LbL Film Coated with the Penicillinase (Bottom)[c]



[a] A FED device is adapted with the analyte in solution and the reference electrode replacing the gate electrode. The unit is comprised of a six-bilayer LbL film made with alternating layers of SWNT and PAMAM, on which a layer of penicillinase was adsorbed. This film was deposited on a p-Si chip coated with a double-layer insulator of SiO_2–Ta_2O_5 and an Al ohmic contact on the rear side. Part of the aluminum was removed for illumination with an array of 16 IR-LEDs. [b] SWNTs are interwoven with PAMAM layers, with the penicillinase layer adsorbed on top. [c] This image was obtained with Bio-Mat Workstation (JPK Instruments, Germany) AFM microscope, in the tapping mode.

Another possibility, which has been overlooked so far, is the use of information visualization methods that are ideal for handling large amounts of high-dimensional data. Among such methods one may single out multidimensional projection techniques, where a graphic visualization is provided based on neighborhood relationships among the recorded data, as defined by a distance or dissimilarity measure.[16]

In this letter, we employ projection techniques implemented in a suite of tools, referred to as PEx,[17] to exemplarily treat the data from current-constant measurements in penicillin biosensors based on LAPS devices. The LAPS array consists of 16 units, where eight of them were not covered by the enzyme penicillinase. We show that the cross talk among sensing units, which seemed to occur by a mere visual inspection of the data, can be entirely eliminated in the projections, when suitable techniques are applied.

METHODOLOGIES

The materials employed to fabricate the PAMAM/SWNT LbL films as well as the solutions of polymix buffer, enzyme penicillinase (PEN), and penicillin were described in previous papers,[4,18] and details are included in the Supporting Information. The procedure to prepare the LAPS chip with a structure of p-Si–SiO_2–Ta_2O_5 and the specifications of the electronic unit implemented for the LAPS devices with 16 IR-LEDs were reported in refs 3 and 4. The detection experiments were carried out by recording the temporal change of the ion concentration in the solution owing to the enzymatic reaction in the constant-current (CC) mode, where the bias voltage was controlled by a feedback loop to maintain a fixed photocurrent.[3,4] Scheme 1 illustrates the operation principle of the LAPS setup and the modified chip structure with the LbL film and enzyme. Also shown are an idealized scheme of the PAMAM/SWNT LbL film covered with a layer of penicillinase and a height atomic force microscopy (AFM) image from the LbL film–enzyme surface. The latter displays that the adsorbed enzyme is covering the film surface, which is represented by the white and red spots. For biosensors based on field-effect devices, such as LAPS, it is important to obtain a stable membrane atop the gate insulator (Ta_2O_5). Therefore, the use of dendrimers and COOH-functionalized carbon nanotubes allows the formation of a stable, porous film, which is suitable for enzyme immobilization and enhances the permeation of ions and hence the sensitivity.

Multidimensional projection techniques map data from a high dimensional space $\mathcal{R}^m$ ($m > 3$) into a visual space $\mathcal{R}^p$ ($p = \{1,2,3\}$) seeking to preserve, up to an extent, the similarity or neighborhood relationships between the data instances.[16] Given $\delta(x_i,x_j)$, a distance between a pair of instances, and $d(f(x_i),f(x_j))$, a distance, normally Euclidean, between their projections onto $\mathcal{R}^p$, the projection technique is a function f: $\mathcal{R}^m \rightarrow \mathcal{R}^p$, which aims at minimizing $|\delta(x_i,x_j) - d(f(x_i),f(x_j))|$, $\forall\, x_i, x_j \in \mathcal{R}^m$.[19] With

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the use of the human visual ability, it is possible to explore the m-dimensional data in order to verify if the groups of similar instances match with the user expectation. That is, if the original m-dimensional data defines space groups of what is known to be similar and separates what is known to be dissimilar.

Several projection techniques exist (see also ref 16 for a list of such techniques) with various approaches to minimize the difference $|\delta(x_i,x_j) - \mathrm{d}(f(x_i),f(x_j))|$. In this work, we employ the interactive document map (IDMAP) technique.[20] Initially devised to create visual representations of document collections, IDMAP comprises two major steps: First, the m-dimensional instances are projected into a two-dimensional space using a very fast dimension reduction approach, called Fastmap.[21] Second, the resulting initial placement is improved with a very precise strategy, called Force Scheme.[19] Fastmap maps the m-dimensional data into a p-dimensional space projecting the instances into p mutually orthogonal directions. The idea is to recursively project the m-dimensional instances into p hyper planes, where the coordinates of each dimension p are given by the projection of the m-dimensional data into straight lines belonging to these hyper planes. The Force Scheme mimics the behavior of attraction and repulsion forces to improve an initial placement. The rationale behind this strategy is as follows: given $Y = \{y_1,y_2,...,y_n\} \in \mathscr{R}^p$ as an initial projection of an m-dimensional data set $X = \{x_1,x_2,...,x_n\} \in \mathscr{R}^m$, for each projected instance $y_i \in Y$, a vector $\vec{v}_{ij} = (y_j - y_i)$, $\forall\, y_i \neq y_j$ is calculated; then, y_i is moved in the direction of $\vec{v}_{ij}$. The amount of movement is proportional to the difference between the current distance $\mathrm{d}(y_i,y_j)$ between the projected instances and the desired ideal distance $\delta(x_i,x_j)$. An iteration of Force Scheme occurs when this process is applied to all instances. By successively iteration of this algorithm, the difference $|\delta(x_i,x_j) - \mathrm{d}(y_i,y_j)|| \,\forall\, y_i,y_j \,\mathscr{R}_p$ is gradually reduced, resulting into a more precise projection.

In the experiments reported in this letter, $\delta(x_i,x_j)$ is defined as the Euclidean distance function, so we can assess on the visual layout the magnitude of the difference between the multidimensional instances. The Force Scheme was executed either until the system of attraction and repulsion forces reached a stable state or when a fixed number of iterations (normally 50) were performed.

RESULTS AND DISCUSSION

We adopted the LbL technique[22−25] to modify the surface of the LAPS chip with carbon nanotubes (CNTs).[26−28] The aim was to investigate possible effects that this nanomaterial could have on the biosensing properties of the system. CNTs have been widely studied in various types of sensors, being advantageous for reaching a high sensitivity and performance when integrated with biomolecules.[4,18,29−32] Figure 1a depicts dynamic constant-current mode measurements for a bare LAPS and a modified LAPS

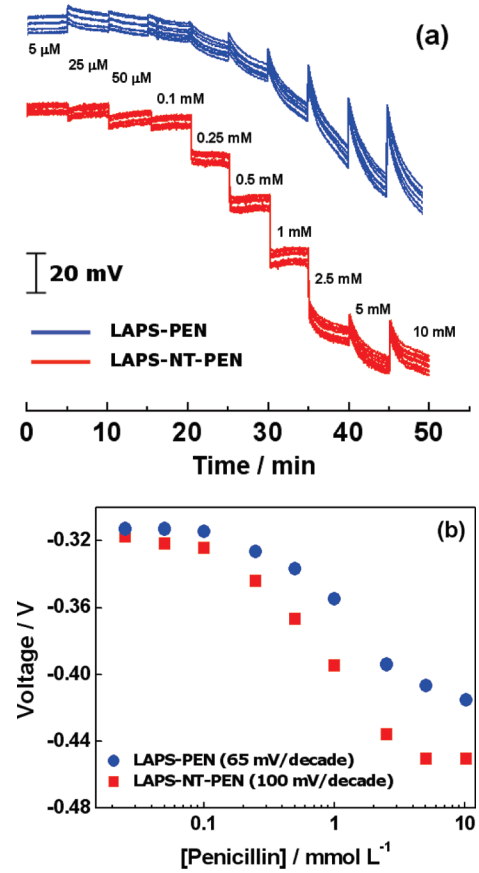

Figure 1. Constant-current (CC) mode response at different penicillin concentrations for a bare LAPS and a LAPS-NT sensor (a), and corresponding calibration curves and sensitivity (b). To set the ideal parameters for CC-mode measurements, the LAPS sensors were characterized using current−voltage (I/V) measurements, whose photocurrent signal was monitored as a function of the external bias voltage and used to define the working point for the CC-mode measurement of each of the 16 IR-LEDs. The bias voltage was controlled by a feedback loop to maintain a fixed photocurrent to obtain the temporal change of the ion concentration in the solution from the enzymatic reaction of penicillin to penicilloic acid.

with an LbL film (LAPS-NT), which are used in detecting penicillin G within a large concentration range from 5 μmol L^{-1} to 10 mmol L^{-1}. A significant enhancement in the signal was observed for the LAPS chip containing the CNTs, in addition to a faster response and better stability with a smaller drift, in comparison with the LAPS chip without CNTs. The sensitivity was 100 mV/decade in the linear range reaching from 0.25 to 5 mmol L^{-1}, while for the bare LAPS chip it was ~65 mV/decade in the

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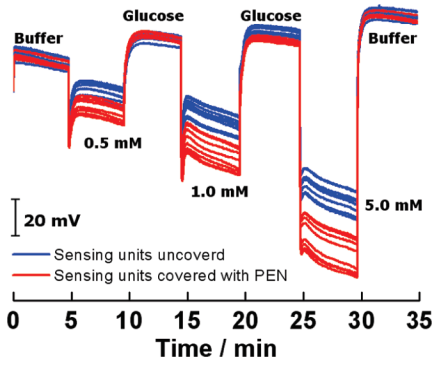

Figure 2. Constant-current mode response of the LAPS-NT sensing units for the detection of different samples. The selectivity of the penicillin biosensor is demonstrated as no potential changes are detected for buffer and glucose samples, respectively.

same range, as shown in the calibration curves in Figure 1b. These effects are associated with the LbL film architecture, which might provide an optimized condition for adsorption and distribution of the enzyme on the sensor surface facilitating the penetration of H^+ ions through the film.[4,18] The advantages of using the LbL method are therefore clear. The control of the molecular architecture with alternating layers of PAMAM and SWNTs led to a large surface area for the enzyme immobilization. Furthermore, we have found in subsidiary experiments that other film-forming techniques, viz. casting or spin-coating, could not be used, as the resulting films were not as homogeneous and stable as the LbL films.

Data from Figure 1 have been typical of sensing units containing a top layer of penicillinase. We also performed experiments where only eight units of the LAPS chip have been covered by the enzyme, while the CC-mode measurements were performed with all the 16 spots illuminated. Figure 2 shows that for the LAPS-NT-penicillinase biosensor, no significant change in potential was noted in the control experiments with the polymix buffer and 50 mmol L^{-1} glucose solutions, respectively, which were used alternately with penicillin concentrations of 0.5, 1.0, and 5.0 mmol L^{-1}. This demonstrates, on the one hand, the selectivity of these biosensing units, as changes in potential were observed only with addition of penicillin G. However, in contrast to what one should expect, on the other hand, the results for the units containing no penicillinase showed a response signal, as if the units could detect changes in the H^+ ion concentration from the enzymatic reaction.

Possible reasons for this unexpected result might be (i) an abrupt pH change in the penicillin solution immediately after the enzymatic reaction, (ii) diffusion of the H^+ ions from the area containing the enzyme to others where there should be no enzyme, or (iii) cross talk among the sensing units, especially for those that are adjacent to each other. The first hypothesis was discarded because no change in pH was observed for the penicillin solutions after the detection measurements. The second hypothesis cannot hold either because the diffusion of ions would take distinctly longer to give a measurable signal under the experimental conditions employed, according to the calculations of Schöning and co-workers.[33] Therefore, the unexpected signal for the units that did not contain penicillinase can only be attributed to a cross talk among sensing units,

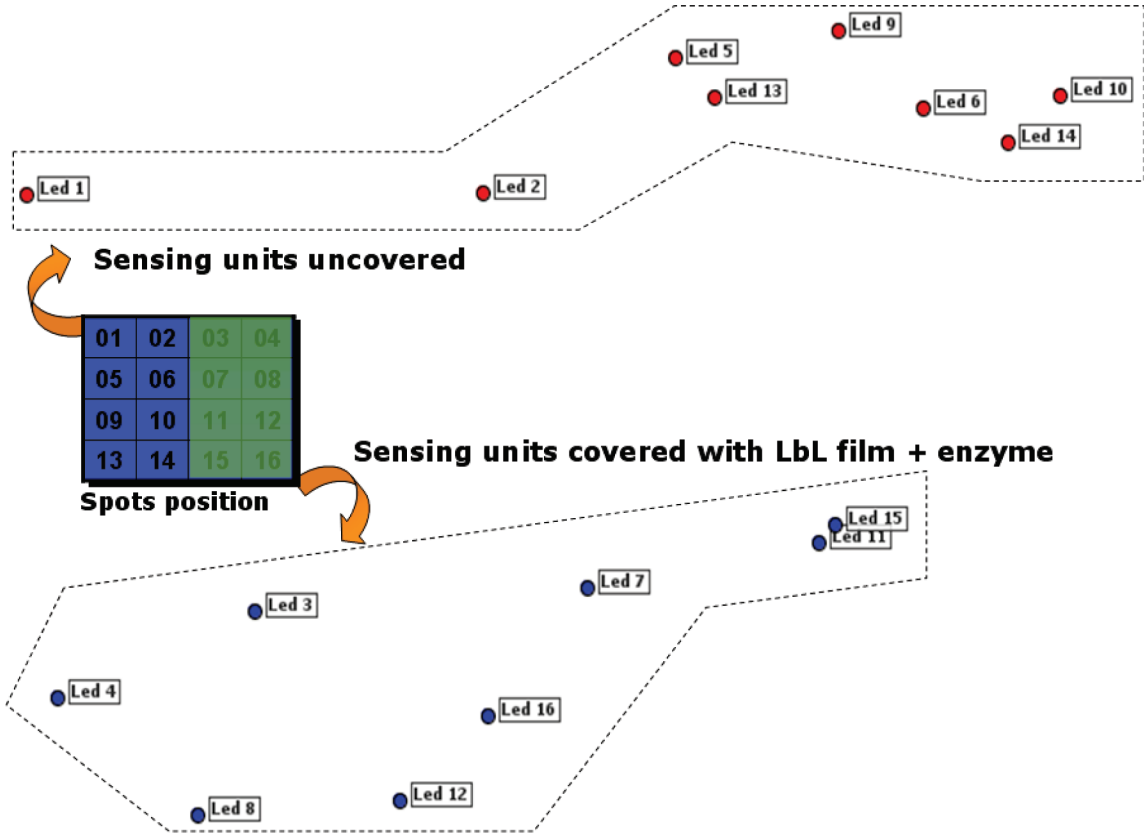

Figure 3. IDMAP visualization of the samples from distinct sensing units covered and uncovered with PAMAM/SWNT LbL film + enzyme. The clear separation between the two groups demonstrates the elimination of the cross talk among the sensing units, confirming the applicability of projection methods to evaluate data in (bio)sensing.

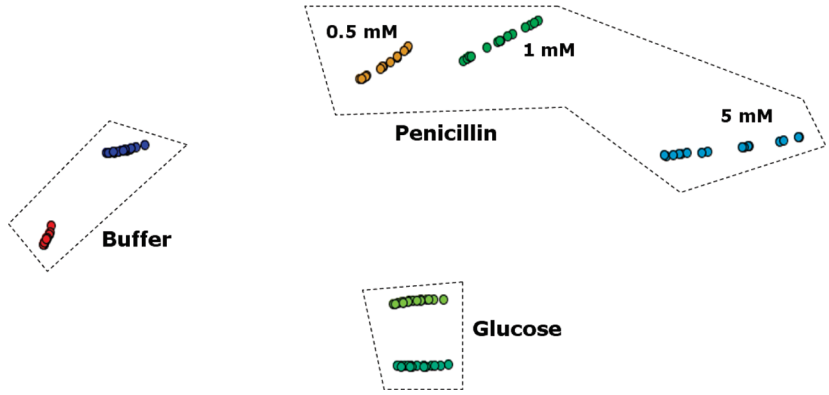

Figure 4. IDMAP visualization with distinct separation of buffer, glucose, and penicillin samples, respectively, used during the detection experiments for a LAPS-NT biosensor. The data groups presented in the visualization demonstrate the selectivity shown in Figure 2 and also the improved performance of the modified biosensor for distinguishing different samples.

which could hamper the simultaneous detection of distinct samples of penicillin G using the LAPS multiple sensor system.

It is known that statistical methods may help to distinguish between very similar samples, in cases where a visual inspection of data fails. We have performed a systematic study with various statistical methods to eliminate the cross talk, making use of the techniques implemented in the PEx platform.[17] The widely used linear method principal component analysis,[10] for instance, could not solve the problem. Among the several projection techniques tried, projections of the data created using the IDMAP technique[20] produced the best results. Figure 3 shows an IDMAP projection obtained from the CC-mode measurements to distinguish the sensing units containing the enzyme from those that did not. The numbering of the spots in the scheme represents the real sequence in which the spots are activated during the measurements. The visualization displays a large distance between the two groups with a clear distinction in the response of the sensing units containing penicillinase. Therefore, the cross talk among sensing units that was apparent in the raw CC-mode data was entirely eliminated when one observes the data using the appropriate projection technique.

The same projection technique also allows distinguishing between penicillin samples with different concentrations and from buffer and glucose solutions, as shown in Figure 4. Moreover, a better distinction is attained with the units containing CNTs, when compared with the biosensor with penicillinase deposited on the bare LAPS (see Supporting Information, Figure S1). For the latter, the visualization displayed data samples with a poor separation, hampering the identification of distinct clusters. Such a result demonstrates the enhanced performance of the modified LAPS in the response signal, as observed in Figure 1. For the sake of illustrating the wide applicability of projection techniques, the responses from all the sensors employed (bare LAPS, LAPS-NT, and also a third LAPS-polyelectrolyte) could be distinguished, as indicated in Figure S2 in the Supporting Information.

The results obtained with the projections pave the way for further experiments, in which one can propose an approach with different enzymes immobilized on the sensing units and obtain multiselective sensors in the same LAPS chip. This will amount to the combination of visualization methods with the concept of electronic tongues that also employed sensor arrays.[11,14]

CONCLUSIONS

To our knowledge, this is the first report of usage of information visualization methods to eliminate the effects of cross talk in sensing units. These effects were investigated in CC-mode measurements with 16 sensing units to detect penicillin G in a single LAPS device. The device had an unexpected response being obtained for some spots that did not contain the enzyme penicillinase. With the use of the IDMAP projection technique, with the Euclidean distance measure, it was possible to distinguish the data from units containing the enzyme penicillinase from those that did not. Moreover, plots could be generated indicating that the distinction was better for the units made with LbL films incorporating CNTs; this is also consistent with the improved sensitivity of ~100 mV/decade toward penicillin G. Sample visualizations with IDMAP also demonstrated that the sensing units allowed distinction of penicillin at different concentrations and from buffers and glucose solutions as well.

It should be mentioned that the cross talk in sensor arrays may be minimized by increased control of the experimental procedures, as in a refined alignment of the LED beams in this study. In any case, the application of projection techniques may help increase performance. For LAPS devices, in particular, it can be fruitful for the development of new multisensing approaches in the same chip, where a fine control of the data is required to obtain an improved selectivity, in addition to high sensitivity.

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SUPPORTING INFORMATION AVAILABLE

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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