Spatio-Temporal Processing for Multichannel Biosensors Using Support Vector Machines

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***Abstract*—Rapid-response biosensing systems are necessary to counteract theats due to foreign and high-consequence pathogens. A yes/no multichannel biosensor is an important tool that enables simultaneous detection of different pathogens, independent of their relative concentration level. This paper proposes a novel multichannel biosensing technique, which combines multiclass support vector machines (SVMs) with multichannel immunosensors. The method combines spatial and temporal information generated by the multichannel immunosensor for rapid and reliable discrimination between pathogens of interest. This paper demonstrates that by including temporal and cross-reactive spatial signatures, the accuracy of the system can be improved at low pathogen concentration levels and for discrimination between closely related strains of pathogens. Compensation of systematic and biosensor fabrication errors is achieved by the use of a supervised SVM training which is also used in system calibration. Experimental results, with a prototype multichannel biosensor used for discriminating strains of *E. coli* (K12 and O157 : H7) and *Salmonella enterica serovar* Thompson, show an accuracy of 98% for concentration levels, 10^0-10^8 colony forming units per milliliter, and total detection time of less than 6 min.**

***Index Terms*—Biosensors, conductometric immunosensor, electrochemical immunoassay, machine learning, polyaniline, support vector machines (SVMs).**

I. Introduction

NATURALLY occurring foodborne-disease outbreaks have caused an estimated 76 million illnesses, which includes 325 000 hospitalization and 5000 deaths in the United States every year [1], [2]. The United States Department of Agriculture estimate indicates a loss of $2.9–$6.7 billion due to medical costs and lost productivity due to these outbreaks [2]–[6]. One of the keys in controlling such outbreaks is rapid detection of the target organisms to ensure efficient implementation of preventive or corrective measures.

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Biosensors have emerged as highly promising for rapid diagnosis. Biosensors are analytical instruments possessing a biomolecule as a reactive surface in close proximity to a transducer, which converts the binding of an analyte with the biomolecule into a measurable signal [7], [8]. Immunosensors (biosensors that use antibodies as biomolecule) are of great interest because of their applicability (any compound can be analyzed as long as specific antibodies are available), specificity (selectivity of the antigen–antibody reaction), and high sensitivity. Immunosensors with electrical readouts offer several advantages over their optical counterparts for their reduced cost, reduced form factor, and ease of signal acquisition. Application areas of immunosensors include pathogen detection in food matrices. A quartz-crystal-microbalance-based biosensor was used to detect *Salmonella spp.* with detection limits around 10^6 colony forming units per milliliter (CFU/ml) [9]. Shah *et al.* [10] have developed an amperometric immunosensor with a graphite-coated nylon membrane serving as a support for antibody immobilization and as a working electrode. This approach was used for detecting *E. coli* with a low detection limit of 40 CFU/ml [11]. Additionally, a portable flow-through amperometric immunoassay system has been developed for detecting *E. coli* as low as 50 cells/ml in 22 min [12]. By careful optimization, the linear working range for that immunoassay was found to be 50–1000 cells/ml [13]. An antibody-based conductometric biosensor using porous filter membranes, developed by Muhammad-Tahir and Alocilja, has shown a detection limit of 80 CFU /ml for bacteria and 10^3 cell culture infective dose per milliliter of Bovine Viral Diarrhea Virus antigens in about 6 min [13]–[15]. The biosensor is relatively inexpensive to fabricate and easy to operate, which makes it an attractive candidate for designing multichannel biosensors. As is commonly observed in biosensor devices, the response of that biosensor is nonlinear and shows a hook effect between 10^1 and 10^8 CFU/ml, which makes it unsuitable for quantitative analysis [16]. However, for field deployable applications, a "yes/no" biosensor that detects the absence or presence of pathogens, independent of their concentration levels, is sufficient. This is useful for zero-tolerance pathogens where ingesting even a small amount has proven dangerous due to its virulence and pathogenicity.

The U.S. Food Safety inspection Service has established a zero-tolerance threshold for *E. coli* O157 : H7 contamination in raw meat products [17]. The infectious dosage of *E. coli* O157 : H7 is ten cells; the Environmental Protection Agency standard in water is 40 cells/L [6]. The U.S. also has a

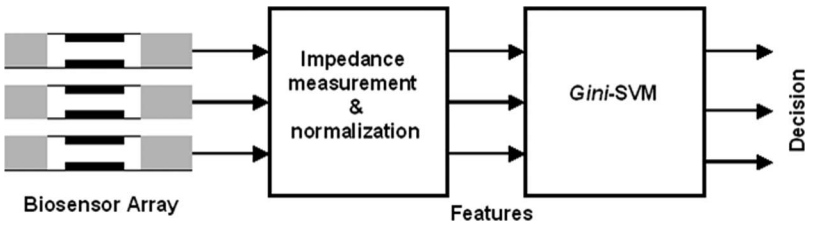

Fig. 1. Architecture of the simulated multichannel biosensor.

zero-tolerance rule for *Salmonella*, *L. monacytogenes* [6], [13], [18], [19]. Thus, any detection method must be very sensitive at very low-concentration levels [20].

In this paper, we use a multiclass support vector machine (SVM) for implementing a simulated multichannel biosensor to identify and discriminate different classes (types) of pathogens, especially at low-concentration levels. SVMs are an attractive choice for implementing classifiers for biosensing application, because they generalize well even with relatively few data points in the training set and bound on the generalization error, which can be directly estimated from the training data [21]. This is important because of the limited amount of data that is usually available for calibration of the biosensors. The learning ability of the classifier can be controlled by a regularizer in SVM training, which determines the tradeoff between its complexity and its generalization performance. In addition, the SVM training algorithm finds, under general conditions, a unique classifier topology that provides the best out-of-sample performance [21]. Since the architecture is feed forward, it is amenable to parallel hardware implementation [22], [23]. Gini-SVM is a multiclass SVM that uses quadratic entropy in its training formulation [24]. Like any other kernel machine, Gini-SVM operates by mapping input vectors into a high-dimensional feature space where a maximal margin hyperplane can be found that linearly separates the training data. The relevance of high-dimensional feature processing is evident in this paper, where Gini-SVM is able to extract nonobvious features from multichannel biosensor data. With the help of SVM, this paper not only demonstrates the importance of temporal information for detecting pathogens at low-concentration levels but also shows that by including cross-reactive information, the discrimination between related strains of pathogens can be improved.

II. Architecture and Principle of Operation

The "multichannel" biosensor in this paper is a proof of concept demonstration for SVM as a signal classifier in a multichannel design. It consists of independent single-channel biosensors, each is specific to pathogens of interest. Fig. 1 shows the architecture of the proposed system. An impedance measurement system senses the electrical signals produced by the biosensor array and normalizes them before classification. A Gini-SVM classifier uses the spatio-temporal impedance features to identify the presence or absence of target pathogens in the biological sample. In this paper, three target pathogens were chosen (*E. coli* O157 : H7, *E. coli* K-12, and *Salmonella* Thompson).

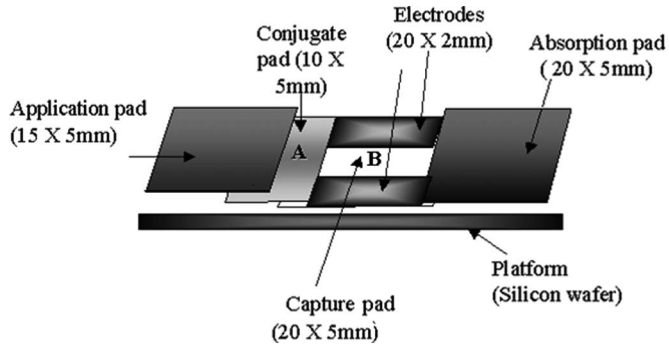

Fig. 2. Schematic diagram of the biosensor. (A) Conjugate pad for polyaniline-labeled antibody absorption. (B) Capture pad coated on each side with silver electrodes. Gap between electrodes is the site for antibody immobilization.

A. Immunosensor Architecture

The architecture of the single-channel biosensor in [14] is shown in Fig. 2. It is composed of four different pads: sample application, conjugate, capture, and absorption. Details about fabrication of the biosensor can be found in [14], and its principle of operation is described briefly. The biosensor uses a capillary action based on lateral flow technique to move the liquid sample from one pad to another. Fig. 3 illustrates the principle of operation of the immunosensor. Before the sample is applied, the gap between the electrodes in the capture pad is open [Fig. 3(a)]. Immediately after the sample is applied to the application pad, the solution carrying the antigen flows to the conjugate pad, dissolves the polyaniline-labeled antibody (Ab-P), and antigen–antibody binding occurs, forming a complex. The antibody–antigen binding occurs at the conjugate pad and forms a complex [Fig. 3(b)].

This complex is carried into the capture pad containing the immobilized antibodies. A second antibody–antigen reaction occurs and forms a sandwich [Fig. 3(c)]. Polyaniline in the sandwich forms a molecular wire and bridges the two electrodes. The polymer structures extend out to bridge adjacent cells and lead to impedance change between the electrodes [14]. The impedance change is determined by the number of antigen–antibody bindings, which is related to the antigen concentration in the sample. The unbound nontarget organisms are subsequently separated by a capillary flow to the absorption membrane. The impedance change is sensed as an electrical signal, which is then processed by a multiclass SVM.

B. Multiclass SVM Architecture

Even though SVMs were originally developed for binary classification problems [21], they have been extended to

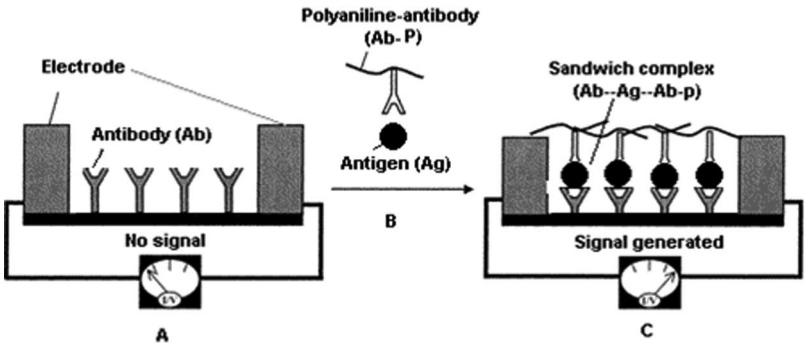

Fig. 3. Cross section of a capture pad (A) before and (C) after analyte application.

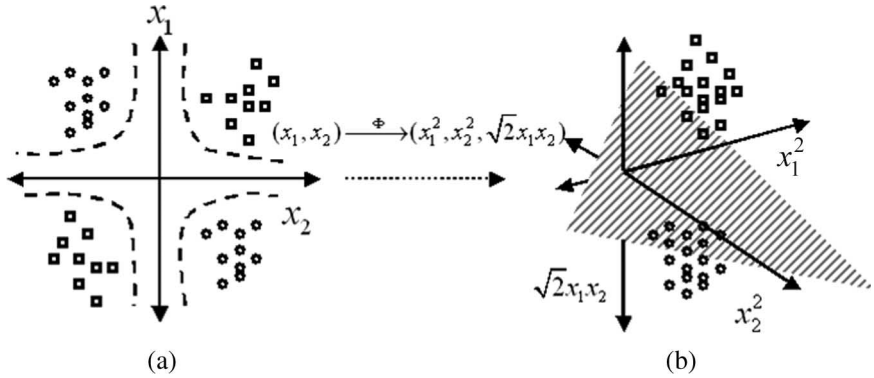

Fig. 4. Plot showing mapping of (a) a nonlinearly separable classification into (b) a linearly separable problem using a nonlinear mapping $\Phi(\cdot)$.

several multiclass formulations [25], [26]. In this paper, we use Gini-SVM framework [24] to design multiclass SVMs. The advantage of Gini-SVMs over other multiclass methods is that it produces normalized output scores that can be combined with other probabilistic models (e.g., Gaussian mixture models, hidden-markov models). Similar to other kernel machines, Gini-SVMs map the input vectors $\overline{z} \in \Re^D$ to a higher dimensional feature space by using a nonlinear transformation $\Phi(\cdot)$. Fig. 4 illustrates an example of the mapping operation from a two-dimensional feature space to a three-dimensional space. In the feature space, the data points corresponding to the binary classes (denoted by "circles" and "squares") are nonlinearly separable. In the higher dimensional space, the data points are linearly separable and can be classified correctly by a linear hyperplane. A multiclass SVM comprises of M linear hyperplanes constructed in the higher dimensional space and is given by

$$f_k(\overline{z}) = \langle \overline{w}_k, \Phi(\overline{z}) \rangle + b_k, \qquad k = 1, \ldots, M \tag{1}$$

where $\langle \ldots, \rangle$ defines an inner product in the higher dimensional space, and $\overline{w}_k$, b_k are the parameters of the kth hyperplane. As with SVMs [21], the hyperplane parameters $\overline{w}_k$ are obtained as linear expansion over training features $\Phi(\overline{x}_n), n = 1, \ldots, N$ as $\overline{w}_k = \sum_{n=1}^{N} \lambda_k^n \Phi(\overline{x}_n)$, where $\lambda_k^n \in \Re$ are the expansion coefficients. Accordingly, the inner products in the expression for $f_k(\overline{z})$ convert into kernel expansions over the training data $\overline{x}_n$, $n = 1, \ldots, N$ by transforming the data to feature space according to

$$\begin{aligned} f_k(\overline{z}) &= \langle \overline{w}_k, \Phi(\overline{z}) \rangle + b_k \\ &= \sum_{n=1}^{N} \lambda_k^n \langle \Phi(\overline{x}_n), \Phi(\overline{z}) \rangle + b_k \\ &= \sum_{n=1}^{N} \lambda_k^n K(\overline{x}_n, \overline{z}) \rangle + b_k \end{aligned} \tag{2}$$

where $K(\ldots,) : \Re^D \times \Re^D \to \Re$ denotes any symmetric positive-definite kernel that satisfies the Mercer condition and is given by $K(\overline{x}, \overline{z}) = \langle \Phi(\overline{x}), \Phi(\overline{z}) \rangle$, which is an inner product in the higher dimensional feature space. For example in Fig. 4, the kernel function corresponding to $\Phi(\cdot)$ is given by $K(\overline{x}, \overline{z}) = (\langle \overline{x}, \overline{z} \rangle)^2$. The use of kernel function avoids the curse of dimensionality by avoiding direct inner-product computation in higher dimensional feature space. Some other examples of valid kernel functions are radial basis functions $K(\overline{x}, \overline{z}) = e^{(-\sigma(\overline{x}-\overline{z})^{\mathrm{T}}(\overline{x}-\overline{z}))}$ or polynomial functions $K(\overline{x}, \overline{z}) = [1 + \langle \overline{x}, \overline{z} \rangle]^{\mathrm{P}}$.

The parameters λ_k^n, b_k in equation are obtained using a supervised training procedure.

Given a set of labeled training data $\{(\overline{x}_n, \overline{y}_n) \in \Re^D \times \Re^M, n = 1, \ldots, N\}$, where $\overline{y}_n$ is a label vector denoting the class membership of the nth data, such that $\sum_{i=1}^{M} y_{ni} = 1$, the supervised Gini-SVM training involves solving a linearly

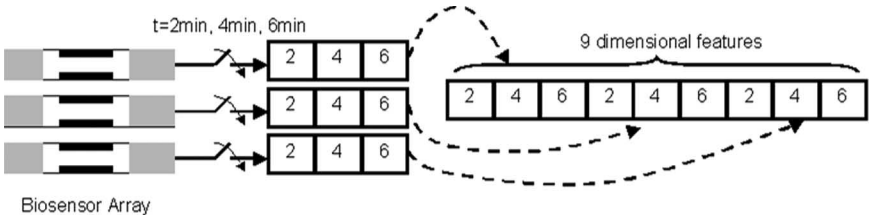

Fig. 5. Method for generating training and test data for SVM using the multichannel biosensor.

constrained quadratic programming problem [24] and is given by

$$H_{\mathrm{g}} = \min_{\lambda_i^m} \sum_{i}^{M} \left[\frac{1}{2} \sum_{i}^{N} \sum_{m}^{N} \lambda_i^m Q_{lm} \lambda_i^m + \gamma C \sum_{m}^{N} \left(y_{im} - \lambda_i^m / C \right)^2 \right] \tag{3}$$

subject to constraints

$$\begin{aligned} \sum_{m=1}^{N} \lambda_i^m &= 0 \\ \sum_{i=1}^{M} \lambda_i^m &= 0 \\ \lambda_i^m &\leq C y_{im} \end{aligned} \tag{4}$$

where C, γ are hyperparameters controlling SVM optimization [21]. Here, $Q_{lm} = K(\overline{x}_l, \overline{x}_m)$ represents the kernel image matrix, and the hyperparameters γ and C are obtained by tuning the performance of Gini-SVM on a cross-validation dataset. The constrained optimization problem (3) can be solved by several standard quadratic programming techniques, and details can be found in [27] and [28]. Fortunately, at the solution of the optimization problem, only a few of the parameters λ_k^n corresponding to the training vectors $\overline{x}_n$ (also known as support vectors) are nonzero and need to be stored for implementing the multiclass classifier.

III. Experimental Methods

A. Biosensor Preparation

The biosensors were prepared based on the specificities of antibodies: *E. coli* biosensors were prepared from affinity purified polyclonal goat anti *E. coli*, which is reactive to all *E. coli* species in immunogen (Biodesign Intl., Saco, ME); *E. coli* O157 : H7 biosensors used lyophilized affinity purified goat polyclonal anti *E. coli* serotype O157 : H7, whose cross reactivity to other *E. coli* strains has been minimized through extensive adsorption using non-O157 : H7 serotypes of *E. coli* (Kirkegaard & Perry Laboratory Gaithersburg, MD); *Salmonella* biosensors were prepared with lyophilized affinity-purified polyclonal IgG antibody isolated from a pool of serum collected from goats immunized with different strains of *Salmonella* (Kirkegaard & Perry Laboratory Gaithersburg, MD). The application and absorption pads were made of cellulose membrane, and the conjugate pads were made of fiberglass membrane grade G6. The capture pad was constructed using nitrocellulose (NC) membrane. The electrodes were fabricated with silver paste to provide an electrical connection between the NC membrane and the data-acquisition system. The conjugate pad was designed to allow the adsorption and flow of polyaniline-conjugated antibodies. Antibody concentration used was 150 μg/ml, and the polyaniline concentration used was 1 mg/ml. The capture pad consists of pores, which allow nontarget antigens to flow through and yet provide good adsorption properties for immobilized antibodies. The antibody concentration on the capture pad was set to 500 μg/ml. These values were found to be optimal, resulting in the highest ratio between the number of captured cells and the actual cell concentration tested [14], [15]. The membranes were attached to an etched copper printed circuit board, which was used to connect to the measurement system. For signal measurement, the prepared biosensor was connected to a BK multimeter Model AK-2880 A (Worchester, MA) with the RS-232 interface and BK software. The signal measured by the multimeter was in the form of change in resistance. Before sample application, the resistance across the electrodes was infinite.

To begin the detection process, 0.1 ml of the pure culture sample was added onto the sample application pad. Measurements were taken for 6 min at 2-min intervals. Three replications were performed for each experiment. Two sets of data were generated. For blank (or control) measurements, the biosensor was calibrated with the noninoculated sample. A normalized and unnormalized set of impedance measurement was generated for experiments. If the measured resistance across the biosensor electrodes, when a control solution is applied, are denoted as R_b, the normalized resistance was computed by $N = R_s/R_b$, where R_s is the impedance measurement across the biosensor electrodes at different time intervals. The other dataset directly used the unnormalized scores but inversely proportional to R_s as $N = 1/R_b$. This expression is obtained with a current sensing method for measuring impedance and is popular in many potentiostatic applications. For each model pathogen (*E. coli* K12, *E. coli* O157 : H7 and *Salmonella* Thompson), nine sets of measurements, including three replications of each set, were obtained for concentrations $10^0 - 10^8$ CFU/ml. Twenty seven additional sets of measurements were made with blank (solution without any pathogens), resulting in a total of 108 data points. Each measurement was organized according to Fig. 5. A sample containing specific pathogen and at a specific concentration was applied to the corresponding biosensor. Resistance across the biosensor electrodes was measured after 2, 4, and 6 min, which were concatenated according to Fig. 5 to form a nine-dimensional vector. The vector captures not only the information about

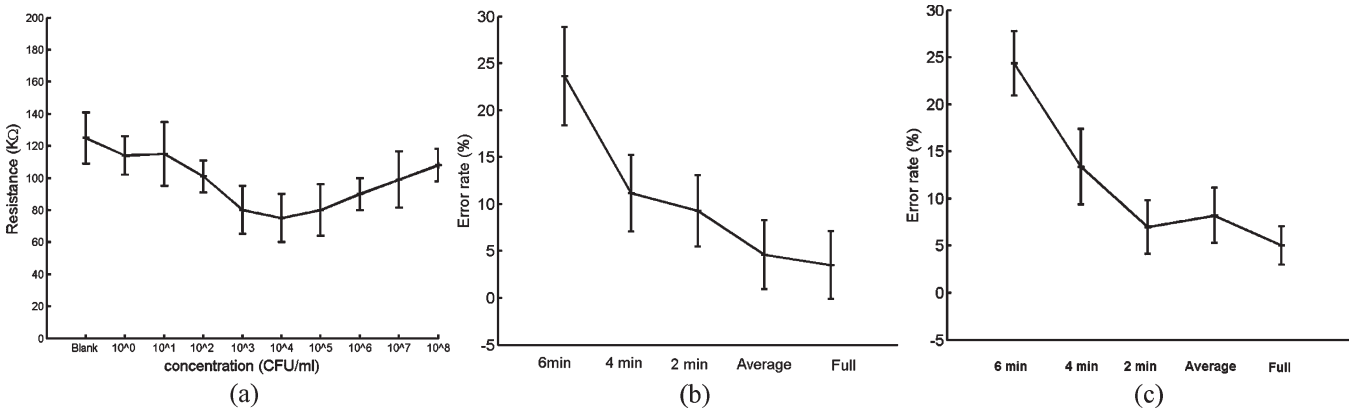

Fig. 6. (a) Measured change in resistance for *E. coli* O157 : H7 biosensor for different pathogen concentrations. (b) Comparison of error rates and variance for different methods with unnormalized biosensor data. (c) Comparison of error rates and variance for different methods for normalized biosensor data.

reaction of pathogen with its antibodies (self-information) but also information about reaction of pathogens with other antibodies (cross-information). Each of the 108 data points (nine-dimensional) was labeled and used for SVM training, cross validation, and testing.

B. SVM Training and Testing

A 100-fold cross-validation procedure [21], [29] was used to evaluate the performance of the biosensor. For each cross-validation procedure, 14 data points per class (*E. coli* K-12, *E. coli* O157 : H7, *Salmonella* Thompson, and control) were randomly chosen out of 27 data points available per class (three replicates of 2-, 4-, and 6-min measurements). This resulted in 56 training points and 52 test points. A Gaussian kernel was chosen for SVM training and testing. A multiclass Gini-SVM was trained according to the procedure in Section II-B, using the Gini-SVM MATLAB toolkit [30]. For each cross-validation step, the performance of the trained SVM was computed on the held-out set. The cross-validation procedure was repeated 100 times, and the mean error rate and variance were computed. Fig. 6(a) shows an example of a measured impedance across the electrodes of the single *E. coli* O157 : H7 biosensor when the concentration of pathogens is varied from 10^0 to 10^8 CFU/ml. The plot demonstrates the nonlinear response of the biosensor, which makes the quantitative measurement of the biosensor difficult. In addition, it can be seen from Fig. 6(a) that a large overlap between error bars for blank and for low concentrations $(10^0-10^1$ CFU/ml) makes the detection based on a simple threshold prone to false positives.

Fig. 6(b) compares the average error rate and its standard deviation computed for 100 cross-validation steps for five different methods labeled as "6 min," "4 min," "2 min," "average," and "full." For the procedure labeled as "6 min," "4 min," and "2 min," only the unnormalized resistance measured after 6, 4, or 2 min were used for SVM training and testing. Fig. 6(b) also shows that the resistance measured after 2 min contains more discriminatory information than other time intervals. The procedure labeled "average" used the mean of "6 min," "4 min," and "2 min" as the feature for SVM training and testing. Procedure "Full" utilized all the nine dimensions for training and testing according to the procedure in Fig. 5. Fig. 6(b) shows that the procedure "full" exhibits the lowest error rate, indicating that combining high-dimensional temporal features at 2, 4, and 6 min leads to better discrimination and demonstrates an average error rate of 2.5%. The temporal signature is especially effective in detecting pathogens at relatively low-concentration levels $(10^0-10^1$ CFU/ml). When similar analysis was performed using measurements normalized with resistance corresponding to the blank, it can be seen in Fig. 6(c) that the detection rate degrades. This shows that inclusion of blank information does not improve the performance of the biosensor. However, for this experiment, the procedure "full" still gives the best performance with an error rate of 5%. The remaining analysis in this paper is based on the unnormalized resistance data.

Fig. 7(a)–(c) shows the error rates and variance computed using five different procedures for each pathogen. The plots indicate that for *E. coli*, the most discriminatory information is present in the measurement at 2 min, whereas for *Salmonella* Thompson, the best performance is obtained at 4 min. This illustrates another drawback of using a simple thresholding scheme for implementing multichannel biosensor. Such a scheme would require different time alignment for different biosensors. Fig. 7(a)–(c) also shows that for all three biosensors, the procedure "full" which utilizes all the temporal information achieves the best performance.

Fig. 8 shows the error distribution for the procedure "full" for different pathogen concentration levels. As expected, most of the errors (73% of 2.5%) occurred at low-concentration levels, indicating that the performance of the biosensor can be improved significantly by reducing the error at the low concentrations. Fig. 9 shows the benefits of using cross-reactive information in improving the detection performance of the multichannel biosensor. The error rate and variance labeled by "self" correspond to the biosensor trained with only a self-reactive information, and the rate and variance labeled by "a cross" correspond to the biosensor trained with both self-and cross-reactive information. In all cases, the entire temporal signatures at 2, 4, and 6 min were used for discrimination. The figure shows that for *Salmonella* Thompson, spatial or cross-reactive information does not affect the detection performance. However, for the closely related strains *E. coli* K12 and *E. coli* O157 : H7, the detection rate improves significantly, indicating

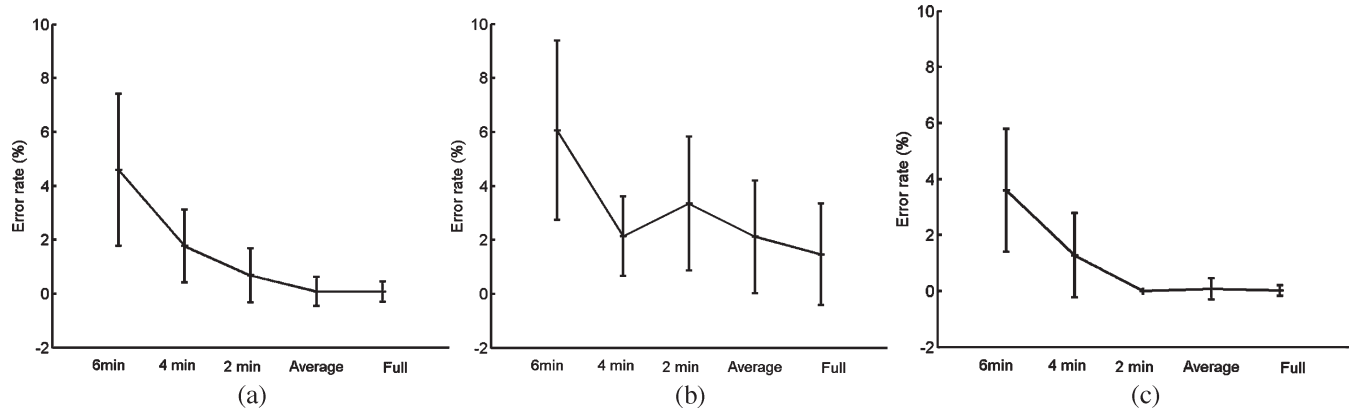

Fig. 7. Comparison of error rates and variance for different methods obtained using 100-fold SVM cross validation. (a) *E. coli* O157 : H7, (b) *Salmonella* Thompson, and (c) *E. coli* K-12.

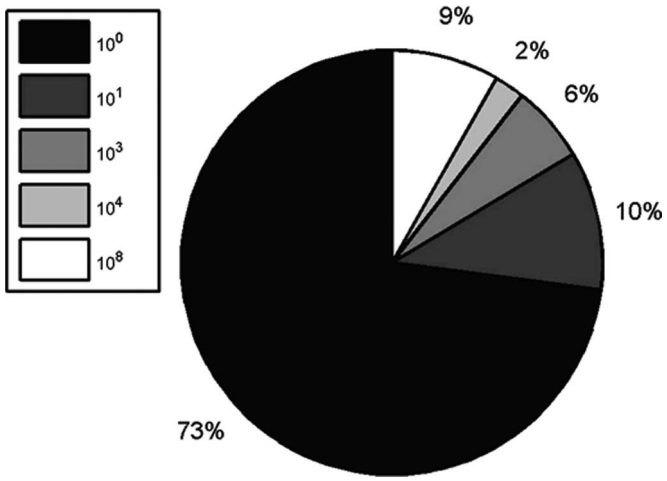

Fig. 8. Distribution of error rates for the method labeled "full" for different pathogen concentration levels.

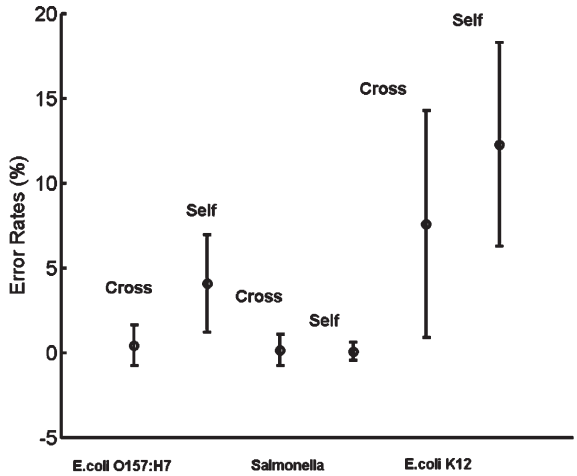

Fig. 9. Plot showing the comparison of error rates when cross-channel features are included ("cross") and when cross-channel features are excluded ("self") for *E. coli* O157 : H7, *Salmonella* Thompson, and *E. coli* K12.

the benefits of the multichannel processing. Improvement in detecting *E. coli* O157 : H7 by including cross-reactive information is expected as it has been shown in [13] that a generic *E. coli* K12 biosensor can be used to detect *E. coli* O157 : H7 pathogens, but it was also claimed in [13] that generic *E. coli* K12 cannot be detected by an *E. coli* O157 : H7 biosensor. Our results show, as can be seen in Fig. 9, that the detection of generic *E. coli* also improves by including its interaction with *E. coli* O157 : H7 antibodies.

IV. Discussions

As shown in this paper, the biosensor is a viable tool for detecting pathogens without quantitatively differentiating the concentration levels. The nonlinear signatures, which are generated by the biosensor, can be utilized by a semiparametric machine learning technique. SVMs are ideal for discriminating high-dimensional features and sparse training data. The superior generalization ability of SVMs with high-dimensional features is due to the regularization and smoothing principles. High-dimensional biosensor features are generated by embedding temporal information, which captures the reaction between antigen–antibody–polyaniline complex with antibodies immobilized on the capture pad. The time-varying signal variation pathogen specifies signatures that can be used by an SVM for discrimination. The signal was measured for 10 min, after which the biosensor response degraded due to the ineffectiveness of polyaniline [31].

Additional features are generated by incorporating cross-reactive information, which is the signal generated by biosensors when used with pathogens not specific to the biosensor. Muhammad-Tahir and Alocilja have already shown in [13] that nonpathogenic *E. coli* K-12 biosensor can be used to detect *E. coli* O157 : H7 pathogens. It was also shown in [13] that the response of biosensor specific to *E. coli* O157 : H7 did not respond to *E. coli* K-12 and generated signals very similar to that of the blank. We have shown in this paper that by incorporating cross-reactive information between *E. coli* K-12 and *E. coli* O157 : H7 in the SVM training, the detection accuracy for *E. coli* K-12 increases. This indicates that the SVM is able to extract small discriminatory features that could be buried in the noise when using a multimeter to measure the signal, as shown in [14]. Cross reactivity of the *E. coli* O157 : H7 antibody to other *E. coli* strains "has been minimized" (Kirkegaard & Perry Laboratories product data sheet) during production but obviously not completely eliminated, according to our data. The cross-reactive features along

with time-varying features are used to form high-dimensional features.

An interesting outcome of analysis in this paper was to show irrelevance of calibration based on the control solution (solution without any pathogens). Due to high variations in the biosensor response to different blank solutions, the normalization with blank impedance degrades the performance of the SVM classifier. When used with unnormalized features, the SVM learning can automatically calibrate itself to different testing conditions. This feature is useful, as the variance in response of the biosensor is predominant because of fabrication errors and bimolecular mutability in constructing the device. Several kernel functions and parameters were evaluated for SVM training and testing. For each choice of kernel functions, the classifier error rate showed a similar trend as when using a Gaussian kernel.

V. Conclusion

We have presented a novel model for multichannel biosensor structure, which is sensitive to multiple pathogens and can detect low concentration of pathogens by exploiting spatio-temporal signatures using the SVM-based classifier. SVMs, with their excellent generalization ability, are an appropriate tool for signal processing for a conductometric multichannel biosensor. This paper has shown benefits of embedding spatio-temporal information for efficient discrimination, which suggests that obtaining fine time-varying signatures using an automated data-acquisition system may improve the discriminatory performance of the SVM. We are currently integrating a multichannel potentiostatic integrated circuit with electrochemical sandwich immunoassay, which will enable simultaneous real-time impedance measurement and detection. The multichannel biosensor would have great potentials to be used in rapid detection of foodborne pathogens, livestock diseases, and other biological hazards against agriculture and food systems.

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