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Artificial neural network modeling and optimization of an electrochemical biosensor for plasma miR-155-based breast cancer detection

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MicroRNA-155 (miR-155) is a clinically important biomarker involved in cancer progression, immune regulation, and inflammatory diseases, highlighting the need for sensitive and reliable detection methods. Conventional biosensor fabrication often relies on labor-intensive trial-and-error optimization, which delays the development of practical diagnostic tools. In contrast to most previous studies that focus on predicting analyte concentration from biosensor signals, this work develops a data-driven framework for modeling the nonlinear relationships between fabrication parameters and biosensor output. Artificial neural networks (ANN) and adaptive neuro-fuzzy inference systems (ANFIS) were proposed to model a voltammetric biosensor for plasma miR-155 detection. A dataset containing the biosensor output current and six fabrication parameters was used. The optimal parameter values were determined using a genetic algorithm (GA). The results show that the ANN approach outperforms ANFIS, achieving an R^2 value of 0.9845. The optimal fabrication parameters were 7.12 nM, 85.22 min, 6.54 min, 118.02 min, 0.12 mM, and 93.39 min for detection probe concentration, detection probe incubation time, MCH incubation time, hybridization time, OB concentration, and OB incubation time, respectively, resulting in an output current of 223 nA. The ANN-GA framework offers a practical and efficient strategy for biosensor development by reducing experimental iterations, thereby lowering material consumption and enabling rapid parameter optimization. These findings demonstrate that ANN-assisted optimization can accelerate the development of cost-effective, high-performance biosensors, supporting their translation into clinical diagnostics for early and accurate miR-155 detection.

Keywords Artificial neural networks, ANFIS, Electrochemical biosensor, Modeling, Optimization, MiR-155

Biosensors are analytical devices used to detect or quantify various analytes, such as medical or biological markers. They are applied in fields ranging from early cancer detection to measuring environmental toxins^{1–3}. A typical biosensor consists of several key components: a bioreceptor, a transducer, and a signal processor. The transducer plays a crucial role and is often used to classify biosensors into categories such as electrical, electrochemical, optical (surface plasmon resonance, Surface-enhanced Raman scattering, fluorescent, colorimetric), mechanical, and thermal biosensors^{4,5}.

Electrochemical biosensors are the most widely used due to their precision, simplicity, low cost, and relatively straightforward operation⁶. A well-known example is the blood glucose meter, which demonstrates these advantages. In electrochemical biosensors for DNA or RNA detection, the electrode (made of materials such as carbon or gold) serves as the core component of the transducer. Its surface provides a platform for immobilizing nanomaterials, nanocomposites, and ultimately the bioreceptor⁷.

To achieve optimal sensitivity in electrochemical biosensors, fabrication parameters must be carefully evaluated and optimized. This process typically requires numerous experiments to identify the best combination

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of materials, concentrations, incubation times, and other variables. These experiments are resource-intensive, consuming significant time and materials, and increasing the risk of human error. However, they are essential to lower the detection limit and extend the linear range of the sensor. To address the limitations of traditional experimental methods, researchers have explored alternative strategies such as design of experiments, computer simulation, and modeling⁸. Among these, model-based approaches are particularly attractive because they can potentially reduce the number of costly experiments required^{2,8–11}.

The goal of biosensor modeling is to identify the underlying mathematical function that characterizes the relationship between the output signal and the input fabrication parameters. One approach is to use mathematical techniques to solve the problem analytically^{12–14}. However, a major challenge with these methods is that the inherent nonlinearity of biosensor behavior often leads to computationally intensive problems for which analytical solutions may not exist, especially for high-dimensional sensor structures¹⁵. Therefore, these methods usually require strict limitations on the number of fabrication parameters to remain computationally feasible.

An alternative approach is to use machine learning (ML) techniques to model the relationship between the input and output parameters of a biosensor. ML has attracted considerable attention in various scientific disciplines due to its ability to learn complex non-linear relationships from data^{16,17}. Numerous ML-based approaches, including artificial neural network (ANN)^{18–21}, support vector machine (SVM)^{22,23}, adaptive neuro-fuzzy inference system (ANFIS)²⁴, and extra trees regressor (ETR)²⁵ have been applied in various fields, such as electronic tongues²⁶, smartphone-based imaging²⁷, fluorescent array-based sensing²⁸, and chemiluminescence²⁹.

Akbari et al.²⁴ developed an ANFIS model to analyze the current-voltage characteristics of a graphene-based bacterial biosensor in order to reduce the number of experiments required for the detection of *E. coli*. Their model, which consists of a single input, five rules, and five membership functions, outperformed conventional mathematical models in estimating bacterial concentration. De et al.³⁰ used a deep neural network to improve dopamine detection by attenuating interference from molecules such as ascorbic acid and uric acid. The model was trained with square-wave voltammetry data and achieved a classification accuracy of up to 98%. Similarly, Challhua et al.³¹ improved rabies virus detection using graphene-based electrochemical biosensors and reported that SVM outperformed principal component analysis (PCA) in classification accuracy and generalizability. Wekalao et al.³² applied a gradient boosting algorithm to predict resonance frequency shifts and sensitivity metrics as a function of biosensor design variables. They achieved an R^2 value of 1.0 and reduced the iterative design cycle by 85%.

Recently, several studies have developed ML-based approaches for modeling biosensors^{33,34}. Comprehensive reviews of these works are available in Cui et al.³⁵, Hassan et al.³⁶, and Ma et al.³⁷. Although ML has become increasingly prominent in biosensing applications³⁸, most existing studies focus on predicting analyte concentration from electrochemical signals, with far fewer investigations dedicated to miRNA-based biosensors. Current ML–miRNA studies typically use ML for biomarker classification, miRNA expression analysis, or concentration estimation, rather than fabrication modeling^{16,39}. As a result, the modeling and optimization of fabrication conditions in electrochemical miRNA biosensors remain largely unexplored, and when addressed, are usually limited to only one or two experimental factors^{32,40}.

To address these challenges, this study integrates machine learning with evolutionary optimization to improve the fabrication of a voltammetric biosensor for plasma miR-155 detection. Unlike previous ML-based biosensor research, which primarily focuses on predicting analyte concentration, this study models the nonlinear interactions among six fabrication parameters. We used a dataset collected from a voltammetric biosensor developed for plasma miR-155 detection⁴¹. The dataset included results from 51 laboratory experiments in which the output current of the biosensor was recorded under varying conditions: the concentration and incubation time of the detection probe, the MCH incubation time, the hybridization time of the target miRNA, and both the concentration and incubation time of the label molecule (OB). An ML–GA framework was used to determine optimal fabrication conditions. This combined approach for miRNA biosensors enables *in silico* optimization that reduces experimental workload, minimizes material consumption, and accelerates the development and translation of biosensors into clinical practice. The main contributions of this study are (1) the development and comparison of ML-based approaches to model the nonlinear relationship between fabrication parameters and output current, and (2) the application of a GA to identify fabrication conditions that maximize output current while reducing time and cost.

Materials and methods

Data acquisition

The dataset used in this study was obtained from a voltammetric biosensor developed to detect the miR-155 biomarker associated with early-stage breast cancer¹⁰. The biosensor operates through the hybridization of a thiolated DNA capture probe immobilized on a gold electrode, followed by Oracet Blue (OB) intercalation and differential pulse voltammetry (DPV) for electrochemical readout. Six fabrication parameters were selected as inputs because they represent the most influential steps governing surface chemistry, probe packing density, duplex formation, and electroactive labeling: probe concentration, probe time, MCH time, hybridization time, OB concentration, and OB time. Other factors were kept constant to avoid variability. Gold electrodes (2 mm diameter) were modified with thiol capture probes (SH-Probe) at 8.0 μM for 120 minutes. MCH was then added to the electrode surface at 0.15 mM for 5 minutes to fill the areas between the capture probes. The target miRNA was subsequently applied to the electrode surface to hybridize with high selectivity to the capture probes attached to the electrode surface (120 minutes). Finally, OB was introduced to the electrode for 60 minutes as an electrochemical label to generate the final readout signal (Fig. 1). All experiments were conducted under

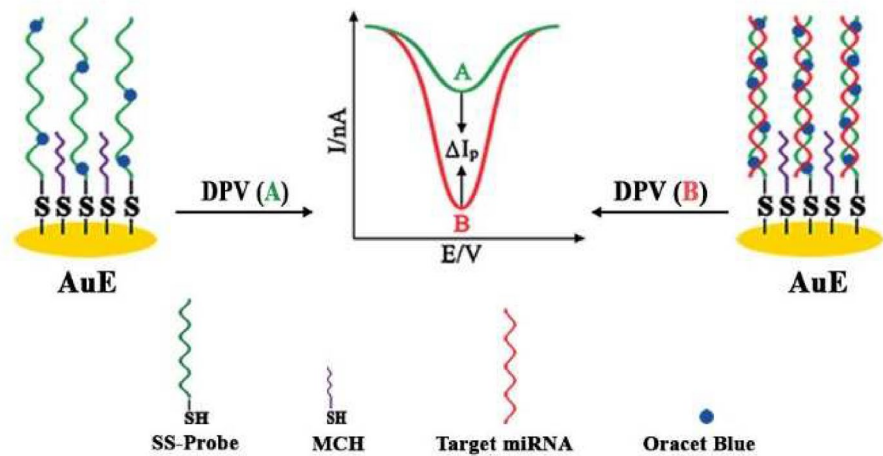


Fig. 1. Schematic illustration of biosensor preparation for detecting miRNAs¹⁰. Gold electrodes were modified with thiol-modified probes, MCH was added between the probes, the target miRNA was hybridized, and Oracet Blue (OB) served as the electrochemical label for DPV measurement.

Variable	Unit	Range	Experimental conditions
Probe Concentration	μM	0.5 - 14	Probe time = 120 min, MCH time = 10 min, Hybrid time = 90 min, OB conc = 0.10 mM, OB time = 90 min
Probe Time	min	30 - 240	Probe conc = 8 μM , MCH time = 10 min, Hybrid time = 90 min, OB conc = 0.10 mM, OB time = 90 min
MCH Time	min	1 - 30	Probe conc = 8 μM , Probe time = 120 min, Hybrid time = 90 min, OB conc = 0.10 mM, OB time = 90 min
Hybridization Time	min	15 - 210	Probe conc = 8 μM , Probe time = 120 min, MCH time = 5 min, OB conc = 0.10 mM, OB time = 90 min
Oracet Blue Concentration	mM	0.01 - 0.2	Probe conc = 8 μM , Probe time = 120 min, MCH time = 5 min, Hybrid time = 120 min, OB time = 90 min
Oracet Blue Time	min	1 - 120	Probe conc = 8 μM , Probe time = 120 min, MCH time = 5 min, Hybrid time = 120 min, OB conc = 0.15 mM

Table 1. Summary of experimental design and parameter ranges used for dataset generation.

controlled conditions (25°C, phosphate buffer pH 7.0, 0.3 M NaCl, and DPV performed in 0.1 M PBS) to ensure reproducibility and eliminate confounding chemical or thermal effects.

A total of 51 distinct fabrication conditions were tested, each representing a unique combination of the six parameters (Table S1). For each condition, three replicate measurements were recorded, and the mean DPV peak current (nA) was used as the response variable. Table 1 summarizes the ranges explored for each parameter. After data collection, all replicate values were averaged, and the resulting samples were randomly shuffled. All six input variables and the output current were normalized to the range [0, 1] using min–max scaling. No data augmentation, smoothing, or filtering was performed, and all experimentally measured values were retained.

Modeling

A suitable way to describe the behavior of biosensors is through mathematical and computational modeling. The purpose of modeling is to develop effective algorithms for describing, analyzing, and predicting scientific problems. For biosensors, physical and chemical processes can be expressed by equations and mathematical relationships. However, due to the nonlinear components of the governing equations, analytical solutions in many mathematical models are valid only under certain conditions⁴². In general, computer modeling is the only effective solution to these problems⁴³. The high complexity of solving partial differential equations analytically and the high degree of uncertainty and error in numerical results have led to growing interest in ML-based systems. These systems allow computers to learn without explicit programming. ANN and ANFIS methods are ML algorithms used to improve system performance.

The dataset used in this study includes 51 fabrication conditions, which is typical for electrochemical biosensor studies. However, this may limit the amount of training data and increase the risk of overfitting. To ensure robust model development, several strategies were implemented. First, the dataset was randomly shuffled and divided into training, validation, and test subsets to provide an unbiased estimate of generalization performance. Second, input variables were normalized to a common scale to improve training stability. The strong agreement between predicted and experimental responses across all models indicates that the learned relationships reflect the underlying physical behavior of the biosensor rather than artifacts of overfitting. Sections 2.2.1 and 2.2.2 use ANN and ANFIS to model sensor output, reducing the cost of required testing and increasing design efficiency.

Artificial Neural Network (ANN) model

Theoretically, ANNs simulate the neuronal networks of the human brain. ANNs attempt to estimate a function based on sample data. This means these computer models can learn the processes that generated the data, given

sufficient data. ANNs can model complex input-output relationships without prior knowledge. The multi-layer perceptron (MLP) is one of the most commonly used types of ANNs. Perceptrons, the basic computational units of a neural network, were created by simplifying biological neurons. The ability of MLPs to approximate arbitrary functions is one of their advantages in mapping capabilities. An MLP network contains an input layer, one or more hidden layers, and an output layer. In addition to the input layer, each layer contains 'neurons' (processing nodes) with non-linear activation functions. One of the most important parameters affecting ANN performance is the size of the hidden layer. In this study, an MLP with a single hidden layer was used to model the output current of the biosensor. This network can be modeled as a function $F: R^n \rightarrow R^m$ that maps the input variables $x \in R^{(n \times 1)}$ into outputs $y \in R^{(m \times 1)}$. If we consider a hidden layer with h neurons, it can be written as follows:

$$y = F(x) = f_2(b_2 + W_2(f_1(b_1 + W_1x))) \quad (1)$$

where $W_1 \in R^{(h \times n)}$ is the weight matrix which connects the input layer to the hidden layer, $W_2 \in R^{(m \times h)}$ is the weight matrix connecting the hidden layer to the output layer, $b_1 \in R^{(h \times 1)}$ is the bias vectors for the hidden layer, $b_2 \in R^{(m \times 1)}$ is the bias vectors for output layer, and f_1 and f_2 are the transfer functions for hidden layer and output layer, respectively.

It is important that the learning algorithms make certain predictions for the dataset. Therefore, during the training phase, the algorithm improves its predictions based on the current true values until it achieves an acceptable level of accuracy. This method is commonly used for classification and regression and has been extensively developed, especially in the field of biosensors⁴⁴. The weight vector of a properly trained neural network represents the knowledge that the network has gained about the problem. In this study, the goal of training the neural network is to find the parameter $\theta = \{W_1, b_1, W_2, b_2\}$. As a result, the values of the network outputs are close to the desired outputs and the predefined loss function is minimized. In most cases, the following loss function ($J(\theta)$) is used for MLP networks:

$$J(\theta) = \frac{1}{N} \sum_{i=1}^N \frac{1}{2} \|y - \hat{y}\|^2 \quad (2)$$

where y is the real output values, $\hat{y}$ is an estimated output value, and N is the total number of data points.

An optimization algorithm called gradient descent is used to minimize error and find the absolute minimum of the cost function. The key step in gradient descent is calculating the gradient of the cost function with respect to the parameters. However, finding this gradient is challenging because artificial neural networks (ANNs) have inputs and outputs that are not directly connected; hidden layers provide the connections between them. To address this, ANNs use iterative back-propagation algorithms. The back-propagation algorithm calculates the gradient of the error function using the chain rule for derivatives. In this study, the well-known gradient descent-based Levenberg-Marquardt (LM) algorithm was used to train the MLP neural network. According to the dataset, the neural network included six inputs (probe concentration, probe time, MCH time, hybridization, OB concentration, and OB time) and one output current (the response of the biosensor) (Fig. 2).

To prevent data leakage, the dataset was shuffled and then randomly divided into training, testing, and validation subsets before normalization or model configuration. The test subset remained isolated throughout model development and was not used during training. For training and testing the network, 75% and 15% of the data were randomly selected, respectively. The remaining 10% of the data was used as validation data to assess the generalizability of the model.

The results were evaluated using three different criteria, including the coefficient of determination (R^2), the root mean square error (RMSE), and the mean absolute percentage error (MAPE) (Eqs. 3–5):

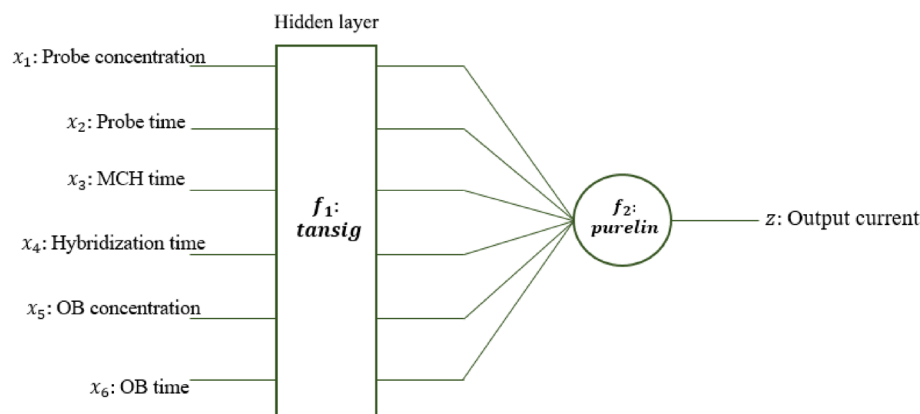


Fig. 2. The ANN model with a hidden layer for predicting biosensor current. The six input variables correspond to the biosensor fabrication parameters: probe concentration, probe time, MCH time, hybridization time, OB concentration, and OB time. The output is the biosensor current.

$$R^2 = 1 - \frac{\sum_{i=1}^N (\hat{y}_i - y_i)^2}{\sum_{i=1}^N (y_i - \bar{y})^2} \quad (3)$$

$$\text{RMSE} = \sqrt{\frac{\sum_{i=1}^N (\hat{y}_i - y_i)^2}{N}} \quad (4)$$

$$\text{MAPE} = \frac{1}{N} \sum_{i=1}^N \left| \frac{\hat{y}_i - y_i}{y_i} \right| \quad (5)$$

where, y and $\hat{y}$ are experimental and predicted output current, respectively. The total number of data is N and $\bar{y}$ represents the mean value of the experimental output current.

On the other hand, the number of neurons in the hidden layer is a critical parameter for achieving optimal model performance. It is currently a challenge to choose a suitable ANN structure for a typical biosensor application. The reason is that there are no reliable theories to determine the appropriate network structure for a given problem. As a result, most ANN models have been developed using the trial-and-error method^{45–48}. In this study, six neurons were initially selected for the hidden layer, and this number was subsequently increased stepwise to achieve the best network structure. At each step, MAPE and R^2 were calculated for the training, test, and evaluation data. Since the network weights are randomly determined for each training, the output also varies for each training. Therefore, 100 iterations were performed for each structure to compensate for the effect of random selection of neural network weights at each step. Finally, the network structure with the lowest MAPE at each step was selected as representative of that structure.

Adaptive Neuro Fuzzy Inference System (ANFIS) model

Neuro-fuzzy refers to the combination of neural networks and fuzzy inference systems, where the neural network determines the fuzzy parameters. In this context, “determination of fuzzy system parameters by neural networks” refers to the automatic determination of fuzzy parameters such as fuzzy rules or fuzzy set membership functions⁴⁹. This combination leads to a hybrid model that leverages the advantages of both methods while minimizing their limitations. ANFIS integrates low-level ANN computations with the reasoning capabilities of fuzzy logic. It benefits from the architecture and training capabilities of artificial neural networks to increase the generalizability of the fuzzy inference system (FIS). ANFIS aims to discover mathematical patterns that align with both human reasoning and natural patterns. The fuzzy inference system is based on if-then rules to determine the relationship between multiple input variables and the output variable. The ANFIS model can be used to solve complex problems for which there is no algorithmic solution or where input data is incomplete or unclear. This model has shown promising results in solving several ill-posed engineering problems⁵⁰.

Fuzzy logic is a generalization of Boolean logic, which has defined limits for each class. Instead of Boolean input values, ANFIS uses membership functions for each input. In this study, an ANFIS model with a first-order Sugeno fuzzy inference system was used to establish a correlation between the design parameters of the miRNA biosensor and its output current. For example, to illustrate the model in Figure 3, the system takes the following form when considering two inputs, one output, and two rules:

$$\text{Rule1: if } x_1 \text{ is } A_1 \text{ and } x_2 \text{ is } B_1 \text{ then } y_1 = p_1x_1 + q_1x_2 + r_1 \quad (6)$$

$$\text{Rule2: if } x_1 \text{ is } A_2 \text{ and } x_2 \text{ is } B_2 \text{ then } y_2 = p_2x_1 + q_2x_2 + r_2 \quad (7)$$

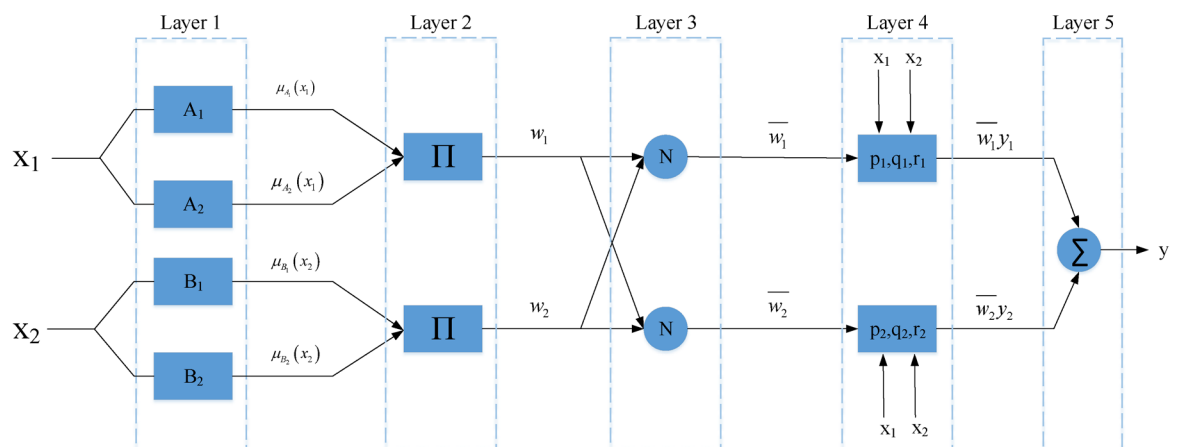


Fig. 3. The first order takagi-sugeno adaptive neuro-fuzzy system (ANFIS) with two inputs and two rules.

where x_1 and x_2 are the inputs and y_1 and y_2 are the outputs of rules. A and B are linguistic terms defined for x_1 and x_2 , respectively. Variables p , q and r denote the parameters of the first-order output function. Figure 3 shows a typical ANFIS architecture that consists of five layers:

Layer 1 – Fuzzification: The nodes in this layer are adaptive nodes with functions that may be generalized s-shaped membership functions, triangular membership functions, bell-shaped membership functions, or any other membership functions. The membership functions denoted as $\mu_{A_1}(x_1)$ and $\mu_{A_2}(x_1)$ for x_1 , and $\mu_{B_1}(x_2)$ and $\mu_{B_2}(x_2)$ for x_2 , fuzzify the input variables by mapping them to a fuzzy membership degree.

Layer 2 – Implication: The nodes in this layer are all fixed (labeled II). This layer calculates the firing strength of each rule by using Eq. 8 and Eq. 9:

$$w_1 = \mu_{A_1}(x_1) \times \mu_{B_1}(x_2) \quad (8)$$

$$w_2 = \mu_{A_2}(x_1) \times \mu_{B_2}(x_2) \quad (9)$$

where w_1 and w_2 are the firing strength of rule 1 and rule 2, respectively.

Layer 3 – Normalization: The nodes in this layer are all fixed as well (labeled N). It represents each rule's firing strength that is normalized by using Eq. 10 and Eq. 11:

$$\bar{w}_1 = \frac{w_1}{w_1 + w_2} \quad (10)$$

$$\bar{w}_2 = \frac{w_2}{w_1 + w_2} \quad (11)$$

Layer 4 – Adoption: This layer is comprised entirely of adaptive nodes with node functions. It is also similar to a linear neuron. Additionally, it indicates the contribution of each rule in the output by using Eq. 12 and Eq. 13:

$$\bar{w}_1 y_1 = \bar{w}_1 (p_1 x_1 + q_1 x_2 + r_1) \quad (12)$$

$$\bar{w}_2 y_2 = \bar{w}_2 (p_2 x_1 + q_2 x_2 + r_2) \quad (13)$$

Layer 5 – Combining: This layer is composed of only one fixed node (labeled Σ), which calculates the output as the sum of all the inputs by using Eq. 14.

$$y = \bar{w}_1 y_1 + \bar{w}_2 y_2 \quad (14)$$

In this study, a structure called PCA-FIS is proposed to model the biosensor response. Principal component analysis (PCA) is a widely used statistical tool for data analysis and dimensionality reduction. By applying PCA to the input data, PCA-FIS models the resulting principal components using three ANFIS sub-models. It performs an orthogonal transformation on the data and maps the correlated input variables to a space where they are uncorrelated. These new variables are called principal components (PCs). PCs are defined as orthogonal directions with the highest variance. In this work, we used three ANFIS sub-models to predict the output current of the biosensor. Figure 4 shows a block diagram of the proposed method.

To remove correlated features and reduce overfitting, PCA was applied to the input dataset in the first step. The concentration of the detection probe (Probe CONC), the incubation time of the detection probe (Probe Time), the MCH incubation time (MCH Time), the hybridization time of the target miRNA (Hybrid Time), the concentration of the label molecule (OB CONC), and the incubation time of the label molecule (OB Time) were considered, and six principal components (PCs) were generated. Because six principal components collectively

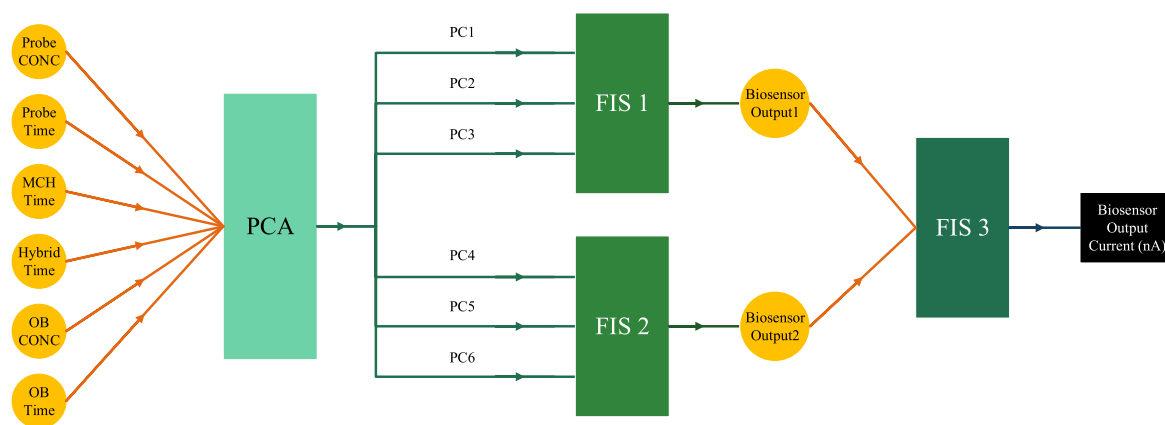


Fig. 4. Structure of the proposed PCA-FIS model. The six biosensor fabrication parameters were first reduced to principal components (PCs) using PCA, which were then used as inputs to fuzzy inference systems (FIS). The final output is the predicted biosensor current.

retained 100% of the variance, all components were used to avoid information loss and to ensure orthogonal inputs for stable fuzzy model training. In the second step, two sub-networks, FIS1 and FIS2, were trained. The six PCs from the first step were divided into two groups: the first three PCs were fed into FIS1, and the remaining three into FIS2. Finally, the outputs of these two sub-models were used as input for a third sub-model (FIS3), which generates the predicted current value. This ANFIS-based model is referred to below as the PCA-FIS model.

The trial-and-error method was used to determine the optimal structure of the model using “different membership functions”, “different numbers of these functions”, and “different combinations of inputs”. The structure of the FIS1 sub-model is shown in Fig. 5a. All three input variables of FIS1 have Gaussian membership functions (Eq. 15).

$$f(x; \sigma, c) = \exp\left(-\frac{(x - c)^2}{2\sigma^2}\right) \quad (15)$$

where a , c , and σ are the characteristics of membership functions that are modified during training.

The number of membership functions for PC1, PC2, and PC3 was 6, 7, and 3, respectively, so 126 rules ($6 \times 7 \times 3$) were used to estimate the biosensor response based on the first three PCs. The membership functions of all PCs are shown in Fig. 5. Similarly, Fig. 6a shows the structure of the FIS2 sub-model with PC4, PC5, and PC6 as inputs. In this sub-model, 5, 7 and 3 Gaussian membership functions were used for PC4, PC5 and PC6, respectively. Thus, 105 rules ($5 \times 7 \times 3$) were used to estimate the response of the biosensor (Fig. 6).

The FIS3 sub-model uses the outputs of FIS1 and FIS2 to generate the final estimate of the biosensor output current (Fig. 7a). The output of FIS1 provides a general estimate of the biosensor output (Fig. 7b), which FIS3 then refines using FIS2 to obtain the final estimate. FIS1 contains 16 membership functions, and FIS2 contains 6 membership functions, resulting in 96 fuzzy rules (16×6) in FIS3. The membership functions for the first input (output of the FIS1 sub-model) are triangular (16), while those for the second input (output of the FIS2 sub-model) are Gaussian (Fig. 7c):

$$f(x; a, b, c) = \max\left(\min\left(\frac{x - a}{b - a}, \frac{c - x}{c - b}\right), 0\right) \quad (16)$$

where a , b , c , and σ are the characteristic of membership functions that are modified during training.

In this study, MATLAB software (R2019b) was used to develop the ANFIS model. The statistical criteria of coefficient of determination (R^2), root mean square error (RMSE), and mean absolute percentage error (MAPE)

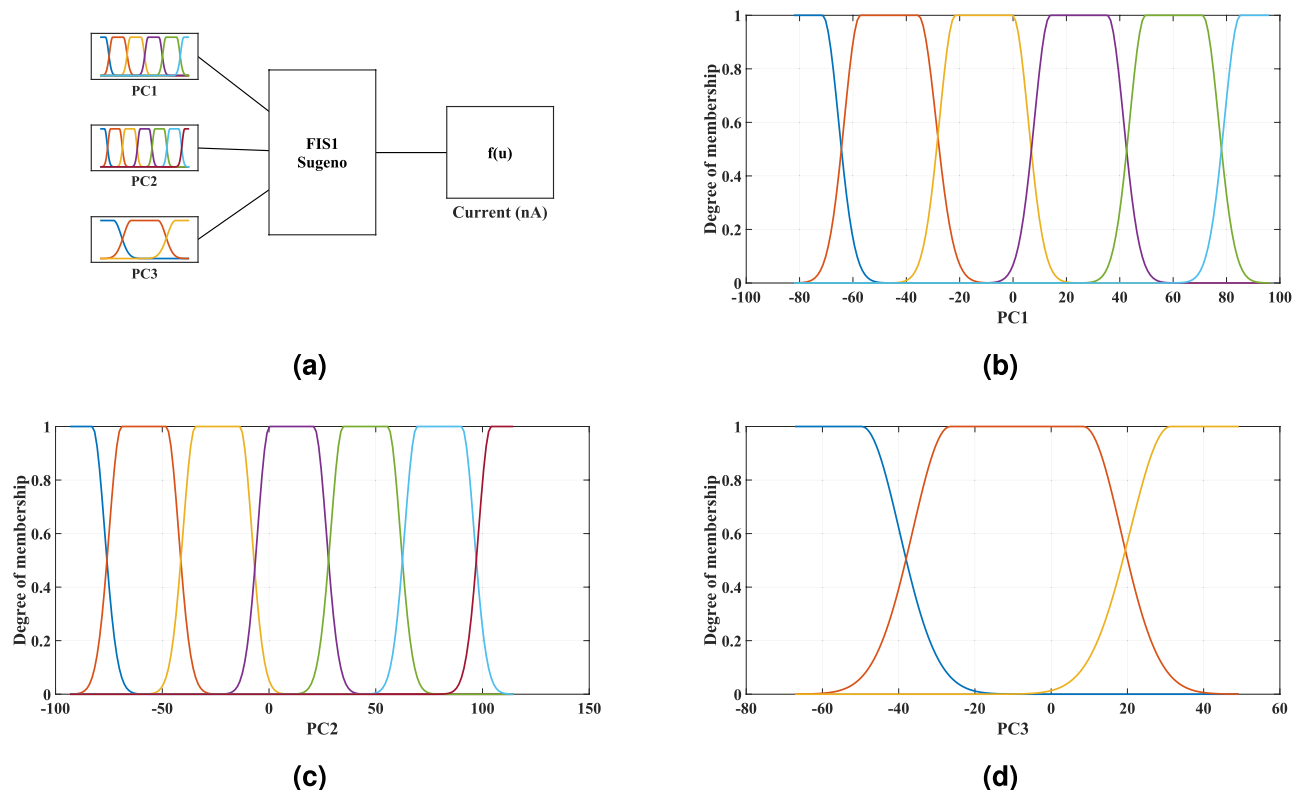


Fig. 5. FIS1 sub-model. (a) Structure of FIS1 with 126 rules using PCs as inputs. (b) Membership functions of PC1. (c) Membership functions of PC2. (d) Membership functions of PC3.

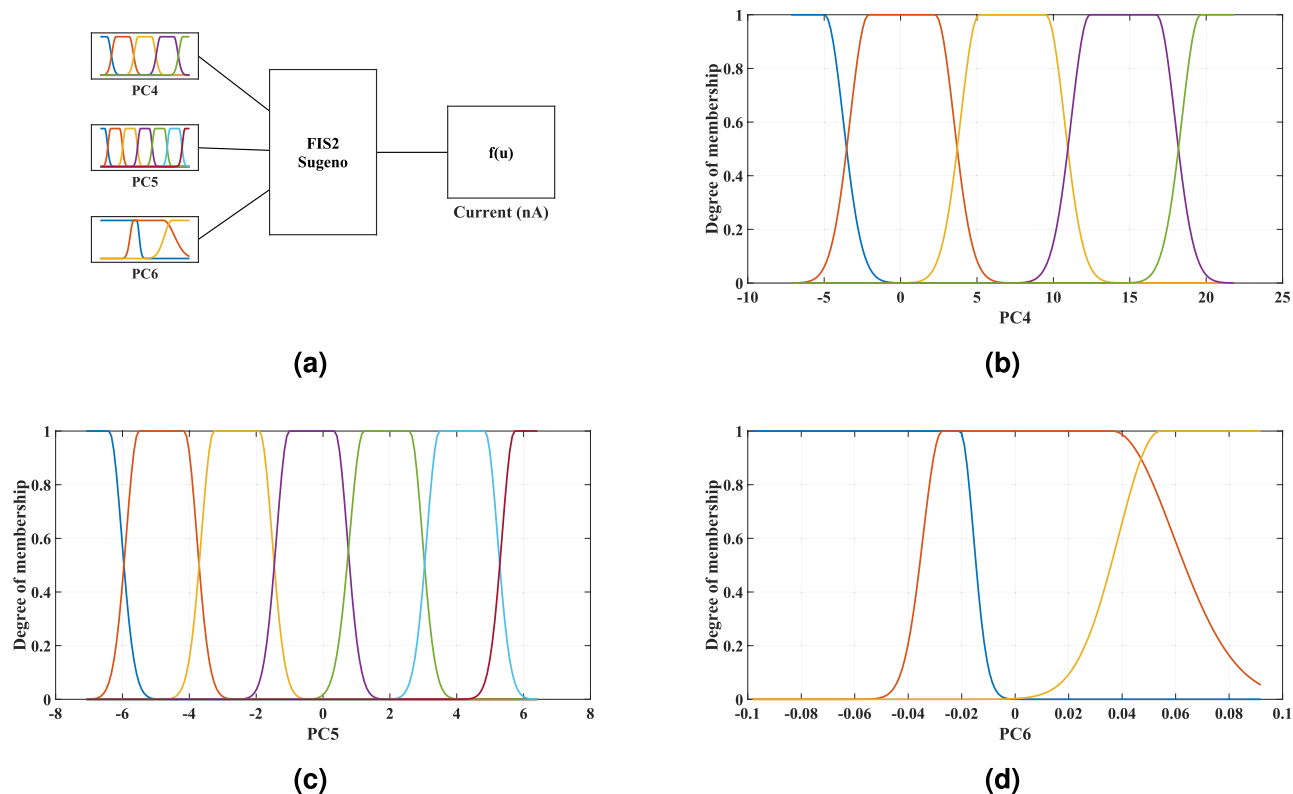


Fig. 6. FIS2 sub-model. (a) Structure of FIS2 with 105 rules using PCs as inputs. (b) Membership functions of PC4. (c) Membership functions of PC5. (d) Membership functions of PC6.

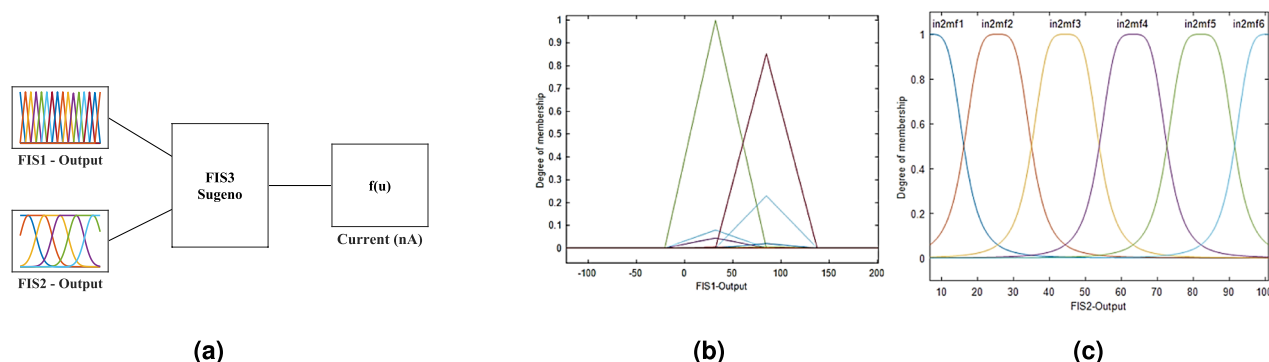


Fig. 7. FIS3 sub-model combining outputs of FIS1 and FIS2. (a) Structure of FIS3 with 96 rules. (b) Triangular membership functions for the first input (FIS1 output). (c) Gaussian membership functions for the second input (FIS2 output). The combined model produces the final prediction of the biosensor current.

(Eqs. 3–5) were used to evaluate the PCA-FIS model. A comparison between the ANN and PCA-FIS models was conducted to determine the most suitable model.

Optimization of the biosensor using genetic algorithm

The biosensor fabrication space is highly nonlinear, multidimensional, and contains potential interactions. The optimization aims to improve biosensor performance while reducing costs. Therefore, after developing a model to predict the biosensor output current, an optimization procedure is required to determine the optimal values of the independent variables. In other words, the values of the independent variables that yield the maximum output current must be calculated. These variables are: probe concentration (x_1), probe time (x_2), MCH time (x_3), hybrid time (x_4), OB concentration (x_5), and OB time (x_6). To achieve this, the following optimization problem must be solved:

$$\begin{aligned} & \underset{x_1, x_2, \dots, x_6}{\operatorname{argmax}} && F(x_1, x_2, \dots, x_6) \\ & \text{subject to} && x_i^{\min} \leq x_i < x_i^{\max}, i = 1, \dots, 6. \end{aligned} \quad (17)$$

where $\{x_1, x_2, \dots, x_6\}$ are the independent variables, and $F(x_1, x_2, \dots, x_6)$ is the output current. The parameters $x_i^{\min}$ and $x_i^{\max}$ represents the minimum and maximum values of each variable, respectively.

In this study, the optimization problem was solved using GA. GA is an iterative method based on Darwin's theory of natural selection, in which the most suitable solution survives in each generation (iteration). It is highly effective for solving complex problems, whether they are constrained or unconstrained. GA efficiently explores nonlinear, multidimensional design spaces without requiring gradient information or an explicit mathematical model⁵¹. Compared with local search or classical experimental optimization methods, GA performs global exploration and is less likely to become trapped in local optima⁵².

In GA terminology, each possible solution to the problem is called an *individual*, and the set of individuals is called a *population*. Each individual is described by a group of variables called *genes*. The genes are encoded into a string of bits, numbers, or objects to form a *chromosome*. The encoding scheme depends on the nature of the problem. The GA uses a population-based search in the simulated model to find the optimal values of the desired parameters. Figure 8 illustrates the main steps of the GA algorithm.

In the first step, called the initial generation, the algorithm starts with a set of initial values for $\{x_1, x_2, \dots, x_6\}$. These initial values are considered as the initial solution for Eq. 17. In the fitness step, a fitness function is defined to assign a fitness score to each individual. This score indicates how well-fitted an individual is to solve the problem. The obtained fitness scores are used in the selection step to select the fittest individuals to produce the next generation of solutions. A crossover operator is then applied to the two selected individuals to produce offspring. Finally, the mutation operator is used to preserve population diversity and expand the search space. Mutation allows the GA to avoid similar solutions and prevent convergence to a local minimum. The result of the mutation step generates the next generation of solutions to the optimization problem. These solutions (individuals) go through the same steps, and the algorithm continues until it reaches the maximum number of iterations or the desired fitness. Table 2 shows the configuration of the GA algorithm used in this study.

Result and analysis

In this section, the results of the biosensor modeling and optimization have been investigated and discussed. First, the results of the ANN model and the model development approach are provided to determine the optimal structure. Next, the results of the PCA-FIS model and its optimal structure are discussed. We then evaluate the ANN and ANFIS models developed to predict the output current of the miRNA biosensor based on probe concentration, probe time, MCH time, hybrid time, OB concentration, and OB time as inputs. The modeling results for the two approaches are then compared. Finally, the results of optimization with GA based on the best model are presented.

ANN model

To estimate the response of the electrochemical biosensor with bare gold electrodes, a two-layer neural network with a hidden layer of 13 neurons and an output layer was used. The Levenberg-Marquardt algorithm was used to train the neural network and minimize learning errors. The hyperbolic tangent sigmoid function (tansig) and the linear function (purelin) were used as transfer functions in the hidden layer and output layer, respectively. The number of neurons in the hidden layer is the most important tuning parameter in an MLP neural network model, as it can affect the model's accuracy and efficiency. Therefore, the number of neurons was gradually increased from 6 to 20. Figure 9 shows the obtained values of MAPE and R^2 as a function of the number of neurons in the hidden layer for the training (black line), test (red line), and all data sets (blue line). For each

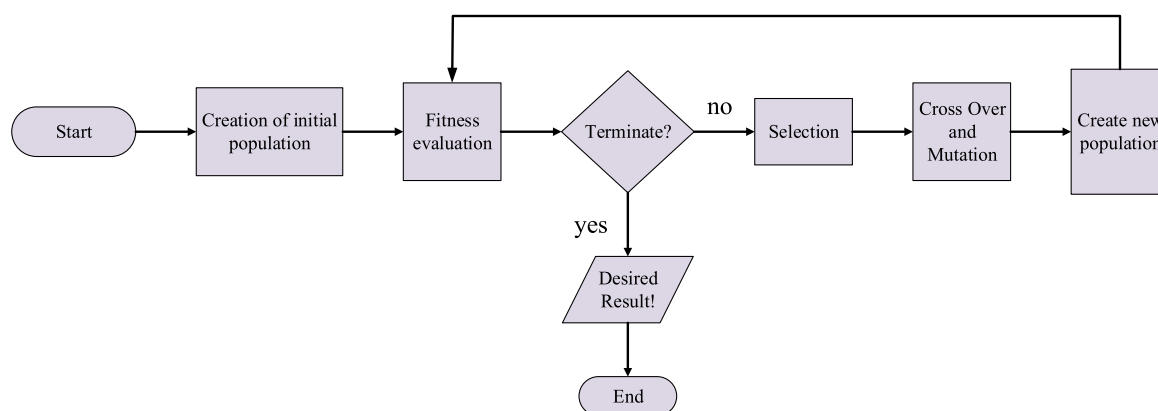


Fig. 8. Flowchart of the genetic algorithm approach used for optimization. The fitness function was defined to maximize the biosensor response current based on fabrication parameters.

Parameter	Value
Population type	Double vector
Population size	200
Creation function	Constraint dependent
Fitness scaling function	Rank
Selection function	Stochastic uniform
Elite count	10
Crossover fraction	0.8
Mutation function	Constraint dependent
Migration direction	Forward
Migration fraction	0.2
Nonlinear constraint algorithm	Augmented Lagrangian
Initial penalty	10
Penalty factor	100
Stopping criteria	Generations: 600
	Stall generations: 50
	Function tolerance: 1×10^{-6}
	Constraint tolerance: 1×10^{-3}

Table 2. Genetic algorithm (GA) configuration used for optimizing the biosensor fabrication parameters.

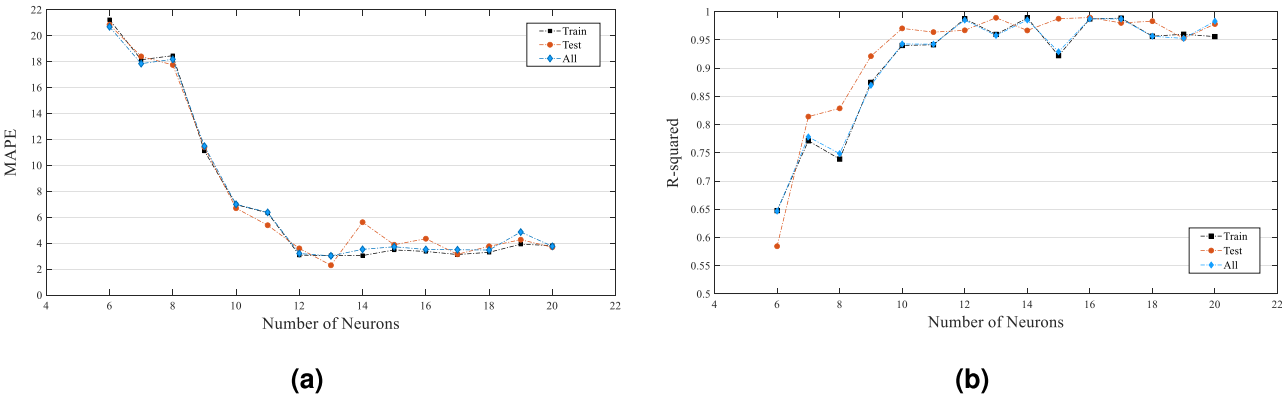


Fig. 9. Variation of ANN performance with the number of neurons in the hidden layer for train, test, and all datasets. **(a)** Mean absolute percentage error (MAPE). **(b)** Coefficient of determination (R^2).

ANN type	Hidden layer		Output layer		Training function	Topology
	Number of neuron	Transfer function	Number of neuron	Transfer function		
Multi layered perceptron	13	Tan-sigmoidal	1	Pureline	Levenberg-Marquardt	6–13–1

Table 3. Architecture and training characteristics of the developed artificial neural network model.

ANN structure, the training procedure was performed with 100 iterations. The model with the highest R^2 and lowest MAPE was considered the best.

In this study, the final ANN architecture consisted of an input layer with six nodes, a single hidden layer with 13 neurons, and one output neuron (6–13–1 topology). The hidden layer used a hyperbolic tangent sigmoid (tansig) activation function, and the output layer used a linear (purelin) activation function. Training was performed in MATLAB (R2019b) using the Levenberg–Marquardt (LM) optimization algorithm with default training settings. Overfitting was controlled by using a held-out validation subset and confirming that prediction errors were similar across the training, validation, and test subsets. The structure and characteristics of the ANN developed in this section are summarized in Table 3.

Figure 9a shows that the MAPE values decrease as the number of neurons increases to 12, 13, and 12 for the train, test, and all data, respectively. Further increasing the number of neurons had no significant effect on the MAPE values. Figure 9b shows that the R^2 values increase as the number of neurons increases to 12, 13, and 13 for the train, test, and all data, respectively. Similar to the MAPE plot, further increasing the number of neurons

did not lead to a significant change in the R^2 values. Thus, based on these results, especially considering the $MAPE$ values of the test data, it can be concluded that the MLP ANN model provides a more accurate estimate when the number of neurons in the hidden layer is fixed at 13. Moreover, the lower $MAPE$ value shows that the ANN model was able to predict the experimental data satisfactorily.

Figure 10 shows the output current compared to the predicted values of the optimal ANN model for the training, testing, and all datasets. As shown in the figure, all data points are concentrated around a line with a slope of 45° . This indicates that the prediction of the experimental output current with the developed ANN model was successful. Thus, the data predicted by the model are reasonably consistent with the experimental data. Furthermore, the small number of off-line points indicates that the model has a low error for the training, testing, and all data. The model performs slightly better for output currents between 0 and 60 nA than for 60–100 nA. This can be explained by the biosensor response: in the lower current range, changes in fabrication parameters produce relatively smooth, quasi-linear variations in current, making these regions easier for the network to approximate. In contrast, in the high-current range (60–100 nA), the biosensor operates in a more nonlinear region, where small changes in parameters such as “OB time” can trigger sharp increases in current. Another reason may be the limited information available to learn these abrupt transitions, resulting in larger prediction errors at high currents. Similarly, the ANN model achieved better results for output currents in the range of 0 to 60 nA than in the range of 60 to 100 nA. In the 0 to 60 nA range, changes in input variables did not lead to abrupt changes in output current, while in the 60 to 100 nA range, slight changes in input variables had a dramatic effect on output current. In some experiments, changing an input such as “OB time” led to a sharp increase in the biosensor response. In some cases, the combination of these highly unpredictable factors led to errors in the model learning process.

PCA-FIS model

Three ANFIS sub-models, namely FIS1, FIS2, and FIS3, were developed in a specific configuration to design the PCA-FIS model. Various membership functions and their combinations were tested through a trial-and-error procedure to determine the optimal specifications for FIS1, FIS2, and FIS3 in the PCA-FIS model, considering the $MAPE$ and R^2 criteria. To evaluate the performance of the developed model, the output currents predicted by the PCA-FIS model for the training, test, and all datasets were plotted against the experimental values of the output current (Fig. 11).

Considering the configuration steps, the data clustered around a line with a slope of 45° for the training (Fig. 11a), test (Fig. 11b), and all datasets (Fig. 11c), clearly demonstrating the model's effectiveness in estimating the output current of the biosensor. In other words, the figure shows acceptable agreement between the experimental and predicted data. Similar to the ANN model results, the model produced more misestimates for current values above 60 nA than for values below 60 nA. For the PCA-FIS model, $MAPE$ values of 4.35%, 4.17%, and 4.31%, and R^2 values of 0.9345, 0.9041, and 0.9318 were determined for the train, test, and all data, respectively. Although the PCA-FIS model has relatively large rule bases, the predictive results did not indicate severe overfitting. Across all three datasets, prediction accuracy on the test data remained comparable to that on the training data.

Comparing the ANN and the PCA-FIS models

Table 4 presents the $RMSE$, $MAPE$, and R^2 values for both ANN and PCA-FIS models across train, test, and all datasets. To ensure a fair comparison, the same combination of train and test data was used for both models. In all data groups (train, testing, and all data), the ANN model showed lower $RMSE$ and $MAPE$ values compared to the PCA-FIS model. The ANN results more closely matched the experimental data, challenging the common assumption in the literature⁵⁶ that ANFIS-based approaches generally outperform ANN models. This difference likely arises because ANN performance does not degrade when the number of input variables moderate and the input-output space has high cardinality. In this study, six fabrication parameters created a multidimensional

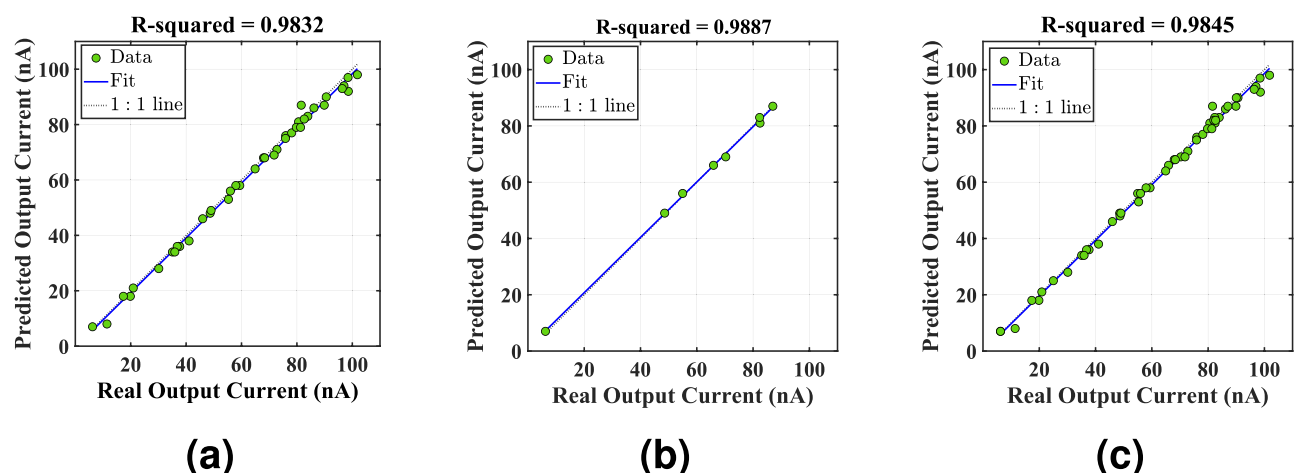


Fig. 10. Experimental versus predicted biosensor output current obtained from the optimal ANN model for (a) train data, (b) test data, and (c) all data.

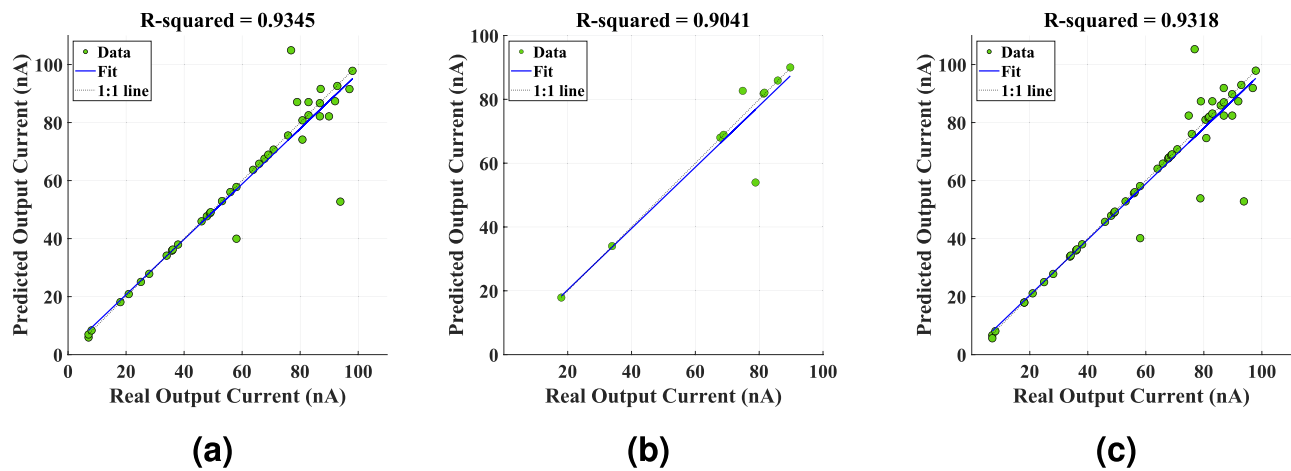


Fig. 11. Experimental versus predicted biosensor output current using the PCA-FIS mode for (a) train data, (b) test data, and (c) all data.

	Train Data			Test Data			All Data		
	RMSE	MAPE	R^2	RMSE	MAPE	R^2	RMSE	MAPE	R^2
ANN Model	2.15	3.88	0.9832	2.30	3.87	0.9887	1.94	3.41	0.9845
PCA-FIS Model	8.67	4.35	0.9345	8.26	4.17	0.9041	8.59	4.31	0.9318

Table 4. Performance comparison of the ANN and PCA-FIS models for predicting the biosensor output current. Root mean square error (RMSE), mean absolute percentage error (MAPE), and the coefficient of determination (R^2) are reported for the train, test, and all dataset. The same train–test split was used for both models to ensure a consistent and fair evaluation.

input space with nonlinear interactions. The ANN was able to capture these interactions through its layered structure, while the ANFIS relied on fuzzy rules that became less efficient as dimensionality increased and the number of data points remained limited. In contrast, the inability to handle input spaces with high dimensionality is a disadvantage of the ANFIS model. Although a modified three-ANFIS structure was used to address this limitation, the ANN still consistently performed better. Another possible factor is that ANFIS may be more prone to overfitting with small datasets.

Compared with PCA-FIS, the ANN model has a more compact architecture with fewer trainable parameters and much faster training. This enables efficient approximation of the fabrication–response relationship while maintaining high predictive accuracy. However, ANN offers limited interpretability regarding the influence of individual fabrication parameters on the output. In contrast, PCA-FIS uses fuzzy rules that provide a semi-interpretable, linguistically meaningful mapping, but this interpretability increases complexity. The need to apply PCA to decorrelate inputs and the resulting large rule base significantly increase computational burden and reduce practical interpretability. Overall, while PCA-FIS offers a more transparent modeling structure, the ANN model achieves a better balance of predictive accuracy, model simplicity, and computational efficiency. As further evidence of the suitability of the ANN method proposed in this study, a statistical comparison was performed with the most relevant and highly cited studies on biosensor modeling using ANN and fuzzy techniques (Table 5). The results showed that the ANN model proposed in this study outperformed the models described in the literature. As shown in Table 5, the ANN paradigm could potentially replace mathematical constitutive biosensor models.

To further clarify the relative influence of fabrication parameters on biosensor response, a SHAP-based feature-importance analysis was performed (Figure S1). As shown in Figure S1, OB incubation time had the greatest impact on the predicted current. This highlights the dominant role of the electroactive labeling step in determining signal intensity. Probe concentration and OB concentration were also highly influential, indicating that both surface loading and label availability govern hybridization efficiency and electron-transfer density. In contrast, probe incubation time, hybridization time, and MCH incubation time contributed more modestly, suggesting that once baseline hybridization and surface-blocking conditions are met, further variations have smaller effects.

Optimization

Based on the results from the previous section, the ANN model was selected as the best model to determine the optimal values of the input variables using the GA algorithm. Table 6 presents the optimal values of the input variables obtained from the GA algorithm, along with the experimental values. The goal of the optimization process was to maximize the biosensor current output, subject to the values of the independent variables (Eq. 17),

Number of inputs	Best model	Statistical performance parameters	Reference
6	MLP ANN model with a single hidden layer, Levenberg–Marquardt (LM) back-propagation (BP) algorithm, and linear and sigmoid transfer functions in the output (4 nodes) and hidden layers (12 nodes), respectively.	True interval prediction percentage: 99.75%	53
65	MLP ANN model with a single hidden layer, back-propagation (BP) algorithm, and purelin and sigmoid transfer functions in the output (3 nodes) and hidden layers (21 nodes), respectively.	$R^2 = 0.9995, 0.9989, \text{ and } 0.9987$; $RMSD = 0.1227, 0.1368, \text{ and } 0.1405$; $REP(\%) = 3.110, 3.3509, \text{ and } 3.8519$	29
2	MLP ANN model with two hidden layers, back-propagation (BP) algorithm, and linear and tangsing transfer functions in the output (2 nodes) and hidden layers (10 nodes), respectively.	$R^2 = 0.958$	54
160	MLP ANN model with a single hidden layer, and satlins and purelin transfer functions in the output and hidden layers (7 nodes), respectively.	R^2 of 0.960, 0.952, and 0.967; $RMS E = 2.570$; $NRMSE = 0.032$	26
3	FNN model with a single hidden layer, and linear and sigmoidal transfer functions in the output (one node) and hidden layers (16 nodes), respectively.	$R^2 = 0.961$; $MSE = 0.0356$	27
2	Fuzzy-logic model (Mamdani-type) for dopamine biosensor; modeling effect of temperature and substrate concentration using fuzzy membership functions and rule-based inference.	Relative error < 5%; validated against experimental data with high agreement	13
2	ANFIS model for dopamine biosensor; hybrid learning algorithm (least-squares + gradient descent) used to tune membership functions.	Relative error $\approx$ 3–5%; improved accuracy compared to standalone NN and fuzzy models	56

Table 5. Summary of representative studies that have applied machine learning for biosensor modeling. The table presents the number of input variables, architecture, training algorithm, and statistical performance metrics used in each study. Abbreviations: R^2 – correlation coefficient, MSE – mean square error, RMSD - root mean square difference, RMSE - root mean square error, NRMSE - normalized root mean square error, REP – relative error of prediction, VAF - value adjustment factor, MAPE - mean absolute percentage error, ANFIS - Adaptive neuro-fuzzy inference system.

Variable	Laboratory values	GA values
Probe Concentration (nM)	8	7.12
Probe Time (min)	120	85.22
MCH Time (min)	5	6.54
Hybrid Time (min)	120	118.02
OB Concentration (mM)	0.15	0.12
OB Time (min)	60	93.39
Biosensor Output Current (nA)	98	223

Table 6. Comparison of experimentally tested fabrication parameters and GA-optimized values, with the corresponding biosensor output currents.

including the concentration and incubation time of the detecting probe, MCH incubation time, target miRNA hybridization time, and the concentration and incubation time of the label molecule (OB). This optimization achieves the lowest cost in terms of both money and time while maximizing the biosensor current output. Since the manufacturing process for biosensors generally aims to be cost-effective and time-efficient, it is necessary to use less material (probe concentration and OB concentration) and less time (probe time, MCH time, hybrid time, and OB time) in constructing biosensors. As shown in Table 6, the optimal values of probe concentration and OB concentration obtained from the GA algorithm were 7.12 nM and 0.12 nM, respectively, indicating that the GA application resulted in 10% and 20% reductions in materials compared to their respective laboratory values. Similarly, the optimal values of probe time and hybrid time obtained from the GA algorithm were 85.22 min and 118.02 min, respectively, representing 28.98% and 1.65% reductions in time compared to their respective laboratory values. The optimal biosensor output current values obtained from the GA algorithm and the experiments were 223 nA and 98 nA, respectively. This shows that the optimal biosensor output current increased significantly (127.55%) compared to the best value obtained from the experiments. An increase in the optimal values of MCH time and OB time obtained from the GA algorithm was also observed; however, the optimal values presented by the GA algorithm were generally better in terms of both material and time consumption, as well as output current, compared to the laboratory values. This demonstrates that the GA optimization effectively achieved its objective. Consequently, in this study, optimizing the biosensor output current led to a reduction in material usage and detection time. This could be attributed to the interpolation capability of the ANN model and the search capability of the GA algorithm to find the globally optimal point from the interpolated dataset. Beyond statistical improvements, the results indicate benefits for clinical translation. ANN can predict biosensor response under specific conditions and identify optimal conditions without extensive experimental trial-and-error, accelerating biosensor fabrication. Reduced material consumption and shorter processing times can lower costs and make fabrication more efficient. These improvements are important for adapting biosensors to different biomarkers and applications in clinical practice, which is essential for translating biosensors into clinical use.

Conclusion

In this study, the applicability of artificial neural networks (ANN) and adaptive neuro-fuzzy inference systems (ANFIS) was evaluated for predicting biosensor response. The dataset was obtained from a biosensor designed for plasma miR-155 detection and included six fabrication variables: the concentration and incubation time of the detection probe, MCH incubation time, hybridization time of the target miRNA, and the concentration and incubation time of Oracet Blue (OB). Comparative statistical analysis showed that the ANN model produced lower prediction errors and higher R^2 values than ANFIS, indicating greater robustness and predictive accuracy. Although ANFIS can perform well for low-dimensional problems with limited interactions, its performance in this study was likely affected by the multidimensional input space and relatively small sample size. Therefore, the ANN model was selected as the preferred modeling approach for subsequent optimization.

After modeling, a genetic algorithm (GA) was combined with the trained ANN to identify fabrication parameter settings that maximize the biosensor output current. This framework provides a practical alternative to traditional trial-and-error optimization, which is often expensive, time-consuming, and difficult to reproduce. By exploring the multidimensional design space in silico, the GA identified a combination of probe, MCH, hybridization, and OB conditions that yielded a substantially higher predicted current than the best experimental value. This integrated strategy could reduce the number of required laboratory experiments, decrease material consumption, and shorten development time.

Despite these promising results, several limitations should be acknowledged. This work focuses on a single voltammetric miRNA biosensor platform and a relatively small dataset of 51 fabrication conditions, which may limit the generalizability of the models to other biosensor types or operating regimes. Additionally, the GA-optimized parameter set was evaluated only at the model level and has not yet been confirmed experimentally, so it should be considered a high-value candidate for future laboratory validation. Future studies should expand the experimental dataset, include external validation on newly fabricated sensors, and extend the proposed framework to other transduction modes (e.g., potentiometric, conductometric) and biosensor formats (e.g., optical, thermal, or paper-based devices). Incorporating model uncertainty analysis and cost-constrained optimization would further enhance the practical utility of machine learning-driven biosensor design.

Data availability

The dataset used in this study is available in the Supplementary Information.

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AI : Conceptualization, methodology, investigation, formal analysis, validation, data curation, software, visualization, writing – original draft, writing – review & editing. SH : Conceptualization, Methodology, project administration, supervision, resources, data curation, validation, writing – review & editing. MA : Conceptualization, Methodology, supervision, resources, validation, visualization, writing – review and editing. AS : writing – review and editing.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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