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TERMINATION CRITERION FOR PCA WITH ANN FOR DETECTION OF NS1 FROM ADULTERATED SALIVA

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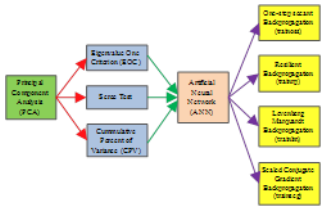
Graphical abstract



Abstract

Detection of Non-structural Protein 1 (NS1) in saliva has become appealing as it may lead to a non-invasive detection method for NS1-related diseases at the febrile phase, before complication developed. NS1 is found to have its unique molecular fingerprint from Surface Enhanced Raman Spectroscopy (SERS) technique. Our work here intends to investigate the effect of termination criterion of Principal Component Analysis (PCA) on the classification performance by the different Artificial Neural Network (ANN) learning algorithms. This will help in optimizing the automated classification of NS1 adulterated saliva, and hence detection of NS1-related diseases. Raman spectra of saliva (n=64) and saliva mixed with NS1 (n=64) are acquired using SERS obtained from the UiTM-NMRR 12868-NS1-DENV database. Large input data dimension of the raw [128 x 1801] are reduced according to the respective PCA termination criteria: Scree test [128 x 5], Cumulative Percent of Total Variance (CPV) [128 x 70] and Eigenvalues One Criterion (EOC) [128 x 115]. The reduced data dimensions are used as inputs to ANN algorithms. Performance of these algorithms, in term of [accuracy, sensitivity, specificity, and precision] from Levenbergh Marquardt (LM), Scale Conjugate Gradient (SCG), Resilient Backpropagation (RPROP) and One Step Secant (OSS) are investigated. The best performance [100%, 100%, 100%, 100%] are achieved from the integration of Scree test criterion and SCG learning algorithm, the highest expected of adulterated data.

Keywords: Nonstructural protein 1 (NS1), principal component analysis (PCA), artificial neural network (ANN)

1.0 INTRODUCTION

Nonstructural Protein 1 (NS1) is a biomarker for detection of diseases caused by Flaviviridae (Flavivirus genome) virus [1]. Amongst diseases caused by flavivirus are Japanese Encephalitis (JE), West Nile Encephalitis (WNE), Murray Valley Encephalitis (MVE), Dengue Fever (DF), Yellow Fever (YF), and Tick-borne encephalitis. Flavivirus genus is a single stranded ribonucleic acid (RNA) consisting of three structural protein (C, prM/M and E) and seven nonstructural protein (NS1, NS2A, NS2B, NS3, NS4A, NS4B and NS5). Of all the structures, NS1 is believed to play an important role that stimulates the viral antivirus in the immune system [2]. The virus is transmitted to human through infected mosquitoes or others arthropod bite. The flavivirus NS1 can be found in infected patient's blood serum from day 1 to 9 following the onset of disease. The amount of NS1 in blood serum is estimated to be in the range of 0.01 to

0.05 ppm [3]. NS1 antigen appears before IgM and IgG, making NS1 being one of the antigens with potential for early detection, early intervention that can prevent death.

In Malaysia, dengue fever outbreak is a national major concerns. Figure 1 illustrates a study on dengue cases and dengue deaths from 2010 to May 2015, based on statistic released to World Health Organization by the Malaysia Health Authority. The total of dengue death from January 2015 to May 2015 is 136, an increase of 100% compared to the same period in 2014, where only 68 death cases were reported [4][5][6][7].

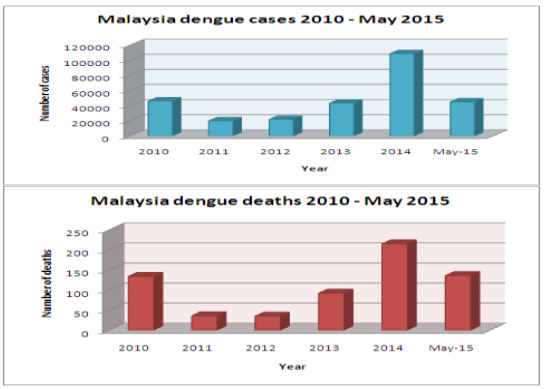


Figure 1 Statistic on dengue cases in Malaysia from 2010 to May 2015

Currently, the gold standard method used to diagnose dengue infection is Enzyme-Linked Immunosorbent Assay (ELISA) based on antibodies immunoglobulin IgM and IgG using blood sample [8]. The method is invasive and prone to blood-related infection. The procedure also requires repetitive steps which is time consuming. Furthermore, the antibodies (IgM, and IgG) are only detectable from day 5 after the onset of fever [3].

Saliva is an attractive alternative to blood for the detection of diseases biomarkers which is noninvasive and easily accessible. In addition, most of the molecules present in blood are also present in the saliva but at much lower concentration [9]. Of recent, NS1 is detectable in saliva but with much lower sensitivity and specificity via ELISA [10]. The encouraging finding permits further exploration for alternative low concentration NS1 detection in saliva. The new technique should also overcome the disadvantages of ELISA techniques as previously mentioned.

In this study, we are investigating a new method for detecting the presence of NS1 in saliva. The method integrates a highly sensitive and specific technique known as Surface Enhanced Raman Spectroscopy (SERS) with signal processing algorithms with Principal Component Analysis (PCA) for feature extraction and Artificial Neural Network (ANN) for classification. Explicit theoretical background of SERS, PCA and ANN are presented in Section 2. Different PCA termination criteria and ANN learning algorithms are implemented in this study are described in Section 3. Section 4 presents and compares the classification performance of the different combinations of termination criteria and learning algorithms.

2.0 THEORY

2.1 Raman Spectroscopy and Surface Enhanced Raman Spectroscopy

As molecules interact with laser light, its vibration produces light scattering consisting of elastic (Rayleigh) and inelastic (Raman) scattering. The amount of inelastic scattering is very small compared to elastic scattering, resulting in weak Raman signal. Raman scattering is an optical phenomenon discovered by Sir C. V. Raman, an Indian physicist in 1928 [11]. Raman spectroscopy is a spectrometer designed to capture the Raman scattering from samples of gases, solid, liquid, slurries, gel and powder. The spectrum produced by the spectrometer is known as Raman spectrum, which is unique for every molecule due to vibration of the molecular structure. SERS amplifies the feeble signals of Raman to a usable range, making it a popular technique amongst researchers to detect diseases [12]. Using SERS, the Raman signal is amplified, by binding target molecules to the surface of pure metals such as silver, gold, and copper [13]. It is reported to have detected up to a single molecule [14] and has been used in many applications including pathological biological molecule and disease detection.

2.2 Principal Component Analysis (PCA)

Principal Component Analysis (PCA) is a mature statistical technique surfaced about 100 years ago [15]. The founder of PCA was Pearson and the algorithm was developed formally by Hotelling in 1933 [16]. Since then, it has been widely applied to fields such as economy, ecology, psychology, meteorology to zoology. PCA is also used as a multivariate statistical feature extraction method to reduce the data dimension while preserving the important features. The multivariate data of interrelated variables are reduced by producing a new set of uncorrelated variables known as principal components (PC) [16]. The dimension of the transformed variables can be less than or equal to the dimension of the original data.

The first component (PC1) is obtained by finding the line of best fit with maximal variance through the original data. The second component (PC2) is orthogonal to PC1. PC1 preserves most of the variation present in the original variables while the subsequent PCs consist of the maximum variation present in the original variable, unexplained by the first PC [16].

Mathematically, principal components can be obtained by finding out the covariance matrices, eigenvalues and eigenvector of the data using equation (1) to (6), where "λ" is described as multiplier in eigenvalue.

$$\text{Mean } (\bar{x}) = \sum \frac{x}{n} \quad (1)$$

$$\text{Adjusted data} = x - \bar{x} \quad (2)$$

$$\text{Variance } (\sigma^2) = \frac{\sum (x-\bar{x})^2}{n-1} \quad (3)$$

$$\text{Covariance } (\sigma) = \frac{\sum (x-\bar{x})(y-\bar{y})}{\sqrt{1}\text{-}\lambda n-1} \quad (4)$$

$$\text{Eigenvalue} = \begin{bmatrix} cov(x,y) & cov(\dot{x},\dot{y}) \\ cov(x,y) & \sqrt{2}\text{-}\lambda \end{bmatrix} = 0 \quad (5)$$

$$\text{Eigenvector} = \begin{bmatrix} a & cov(x,y) \\ b & \sqrt{2}\text{-}\lambda \end{bmatrix} = 0 \quad (6)$$

In PCA implementation, it is important to choose PCs containing the most significant attributes of the original data and retained for subsequent analysis, using PCA termination criteria. In this study, three termination criteria, i.e. Eigenvalue-One-Criterion (EOC) [17], Scree test[18] and Cumulative Percent of Variance (CPV) are considered.

2.2 Artificial Neural Network (ANN)

Artificial Neural Network (ANN) is an Artificial Intelligent (AI) technique used for information processing by means of imitating the functions of a human brain. ANN perceptron concept was first introduced by Frank Rosenblatt in 1958. Frank Rosenblatt creates a mathematical model to simulate information processing in human neurons based on existent knowledge of neuro-physiology [19]. Over the years, ANN has become a useful tool to recognise patterns and to model complex relationship between input and output data [20-23]. There are two categories of ANN, feedforward and feedback network [24]. Feedforward network includes single layer perceptron, multilayer perceptron and radial basis function structure nets. Feedback network is more complex and competitive such as Kohonen's SOM, Hopfield network and Adaptive Resonance Theory (ART) models.

By using available data, ANN trains the network to learn about the data. The information gathered during the learning process are used by the network to organize itself for decision making. The learning process can be supervised and unsupervised training [25]. Of the two, supervised learning is more common in ANN development. With supervised training, the neural network is provided with actual output for every input [26]. The weight coefficient is adjusted based on the difference between the actual output and the predicted output values. Amongst the wealth of learning algorithms, Levenberg Marquardt (LM), Scaled Conjugated Gradient (SCG) Resilient Backpropagation (RPROP) and One Step Secant (OSS) are used to train MLP with backpropagation network for this study.

2.2.1 Multilayer Perceptron Backpropagation Learning Algorithm

Multilayer perceptron (MLP) also known as multilayer neural network. MLP trained with a backpropagation learning algorithm is widely used in neural networks [26]. MLP neural network consists of neurons that are divided into input layer, hidden layers and output layer [19]. In this section, the different learning algorithms used in this study are elaborated.

2.2.2 Levenberg Marquardt

Levenberg Marquardt (LM) algorithm is an approximation to Newton's method [27]. LM algorithm is a training function for a network that does not need to compute the Hessian matrix to approach second order training speed [28]. The Hessian matrix H can be approximate to (7) if the error function is squared sum and the gradient can be computed as (8) [29], where *J* is the Jacobian matrix that contains first derivatives of the network errors with respect to the bias and weight, while *e* is a vector of network errors [29].

$$H = J^T J \quad (7)$$

$$g = J^T e \quad (8)$$

LM is the fastest backpropagation algorithm but it requires more memory than other algorithms [29]. Equation (9) expresses the LM rule,

$$x_{k+1} = x_k - [J^T J + \mu I]^{-1} J^T e \quad (9)$$

where μ is a parameter that is used as scalar controlling behavior of the algorithm and I is a diagonal matrix identity [28]. When the value of μ increases, the step size gradient descent becomes smaller. For μ = 0, with the use of approximate Hessian matrix, LM follows Newton's method [29].

2.2.3 Scaled Conjugated Gradient

The Scaled Conjugated Gradient (SCG) algorithm is a network training function that has the ability to handle large scale problems effectively. It was developed by Moller [30][31] in order to reduce the computational time, by using LM algorithm way of scaling the step size to avoid the line search per learning iteration [27]. It is a good at dealing with training pattern recognition networks and large network [32].

The SCG algorithm is derived from quadratic minimization of objective function E within *N* iterations [33]. It utilizes second order information from neural network, similar to LM algorithm. Since the gradient information calculation is inexpensive, only a modest memory is required [30]. The vector sequence as in (10) and (11) is created with initial gradient $g_{initial} = \frac{\delta E}{\delta w}$ and direction vector $d_{initial} = -g_{initial}$, where $w = w_{initial}$, *t* is the initial time, *t+1* is the next iteration time,

d is conjugated direction and H is the Hessian matrix of the objective function E [29].

$$g(t+1) = g(t) + \lambda(t)Hd(t) \quad (10)$$

$$d(t+1) = -g(t+1) + \gamma(t)d(t) \quad (11)$$

$$\lambda(t) = \frac{g(t)^T g(t)}{d(t)^T Hd(t)} \quad (12)$$

$$\gamma(t) = \frac{g(t+1)^T g(t+1)}{g(t)^T g(t)} \quad (13)$$

2.2.4 Resilient Backpropagation

Resilient Backpropagation (RPROP) algorithm is a network training function based on independent technique to update the size of weight of the absolute value of the partial derivatives [33]. The learning rate adjustment of RPROP algorithm is unique because it depend only on the sign of the gradient but not the magnitude [33][34]. The gradient magnitude will become unpredictable if the error is highly complex and non-linear [35]. The size of weight update is determine by the update value of Δ_{ij} [35]. The direction of weight update is determine by the sign of partial derivative $\frac{\partial E}{\partial w_{ij}}$ [33]. The equation (14) and (15) is used to update the learning rate in RPROP, where $0 < \eta^- < 1 < \eta^+$.

$$\Delta_{ij}(t) = \begin{cases} \eta^+ . \Delta_{ij}(t-1), if \ \frac{\partial E}{\partial w_{ij}}(t-1).\frac{\partial E}{\partial w_{ij}}(t) > 0 \\ \eta^- . \Delta_{ij}(t-1), if \ \frac{\partial E}{\partial w_{ij}}(t-1).\frac{\partial E}{\partial w_{ij}}(t) < 0 \\ \Delta_{ij}(t-1), otherwise \end{cases} \quad (14)$$

$$\Delta_{wij}(t) = \begin{cases} -\Delta_{ij}(t), if \ \frac{\partial E}{\partial w_{ij}}(t) > 0 \\ +\Delta_{ij}(t), if \ \frac{\partial E}{\partial w_{ij}}(t) < 0 \\ 0, otherwise \end{cases} \quad (15)$$

From equation (14), it can be deduced that the learning rate is increased when no minimum is jumped over. Otherwise, the learning rate is decreased if the minimum is jumped over, which results in changing of the sign of error term [34]. The choice of the initial value $\Delta_{ij}(t)$ is important to avoid node saturation [34].

2.2.5 One Step Secant

The one step secant (OSS) algorithm calculates the derivative of performance using backpropagation technique. The calculation of the performance is dependent on the weight and bias variable X. The variable X behaves according to equation (16), where dX is the search direction and a is the parameter selected to minimize the performance along the search direction. [32].

$$X = X + a^* dX \quad (16)$$

The minimum point is located using the search function. The sign of gradient performance is negative for the first search direction. The new gradient and gradient of the previous step are used to compute the search direction using equation (17), where gX is the gradient, X_step is the change in weight on the previous iteration and dgX is the change in gradient from the last iteration.

$$dX = -gX + Ac^* X\_step + Bc^* dgX \quad (17)$$

3.0 METHODOLOGY

3.1 UiTM-NMRR 12868-NS1-DENV

Data for this work are Raman spectra of control group saliva and NS1 adulterated saliva, both from the UiTM-NMRR 12868-NS1-DENV database. The control group dataset was obtained from Raman analysis of saliva collected from healthy people aged between 23 to 34 years old in the morning, with reference to the published protocol [2][36]. On the other hand, the NS1 adulterated dataset was obtained from Raman analysis of saliva mixed with low concentration NS1 ranging from 0.2ppm to 1.0ppm. The purity of NS1 used in this study is 99%, at concentration of 1000 ppm from Abcam. It is then diluted based on the following dilution equation,

$$M_1 V_1 = M_2 V_2 \quad (18)$$

where M_1 is the initial concentration of NS1, V_1 is the initial volume, M_2 is the new concentration of the NS1 and V_2 is the new volume that required. Each dataset consists of 64 spectra and each spectrum consists of 1801 features (Raman shift-cm^{-1}). These two datasets together make the input vector, with dimension of (128 x 1801) to be fed into algorithm in Section 3.2.

3.2 Principal Component Analysis

Figure 2 shows the flowchart of the PCA algorithm for extracting feature. Prior to PCA, the input data are pre-processed through a 4 stages of algorithms to remove the unwanted features. The PCA algorithm is implemented by applying the 'princomp' command in the MATLAB environment producing the eigenvalues, eigenvectors and principle components. Then the algorithm will determine the number of PCs to be retained according to the termination criteria. As for CPV criterion the threshold is set to 90%

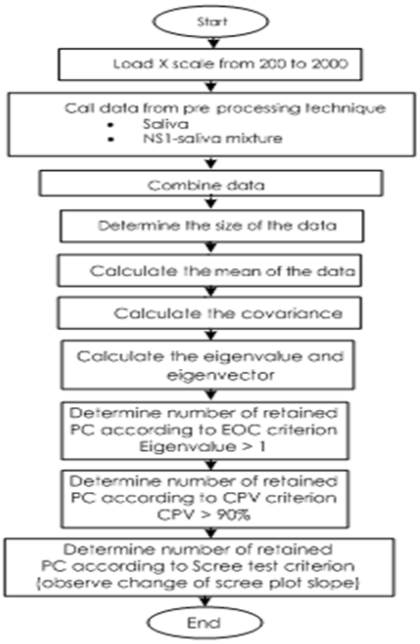


Figure 2 Flowchart to implement Principal Component Analysis to extract features from Raman spectra of saliva samples

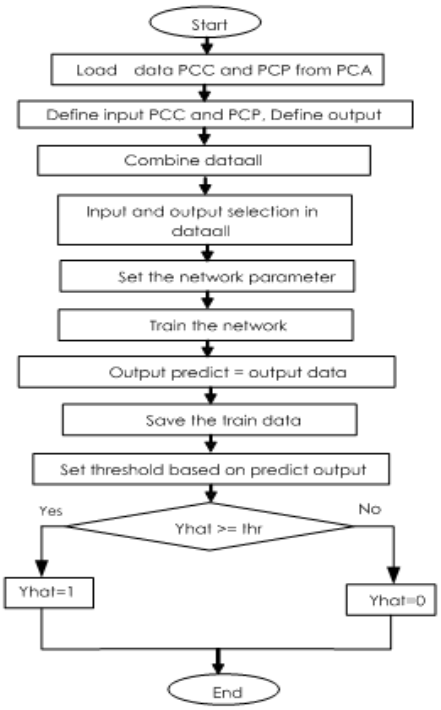


Figure 3 Flowchart to implement ANN for classification of saliva samples

3.3 Artificial Neural Network

Figure 3 shows the flowchart of ANN algorithm for classification. Output from PCA, which are PCs of the input data, are separated as PCC (PC of control group) and PCP (PC of NS1 adulterated saliva), before being fed to the ANN algorithm. Performance of the ANN is measured in term of accuracy, sensitivity, specificity and precision. The algorithm starts with loading the PCs to the network. Then the data is randomized by column using "RandStream" function. Next, the user is prompted on the number of PCs to be used as inputs, according to the termination criteria. Subsequently, the data will be trained by the network using the selected parameters. With the saved ANN architecture, the network then classify the test data into either control group or NS1 infected group.

4.0 RESULTS AND DISCUSSIONS

This Section presents results on performance of the classification system in response to variation in the termination criterion of PCA and learning algorithm of ANN. Section 3.1 depicts the result for PCA while Section 3.2 for ANN.

4.1 PCA Termination Criteria

Figure 4 shows the result for Eigenvalue One-Criterion (EOC) termination criterion, whereby the number of PCs to be selected is based on the eigenvalue. PCs with eigenvalue more than 1 are retained while those with eigenvalue less than 1 is discarded. From Figure 4, it can be observed that the number of PCs that satisfy the criterion is 115.

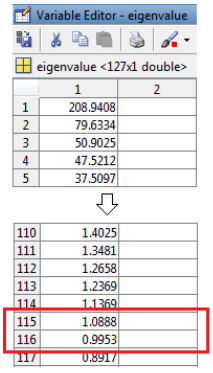


Figure 4 Ranking of PCs according to Eigenvalue One-Criteron (EOC)

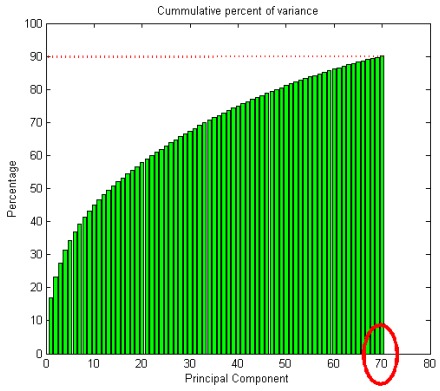


Figure 5 CPV barchart showing cumulative sum of PCs

Figure 5 shows the output from CPV termination criterion. From the graph, if a threshold of 90% is selected, the number of PCs required is 70. This reduces the data dimension from the original dimension of [128 x 1801] to only [128 x 70].

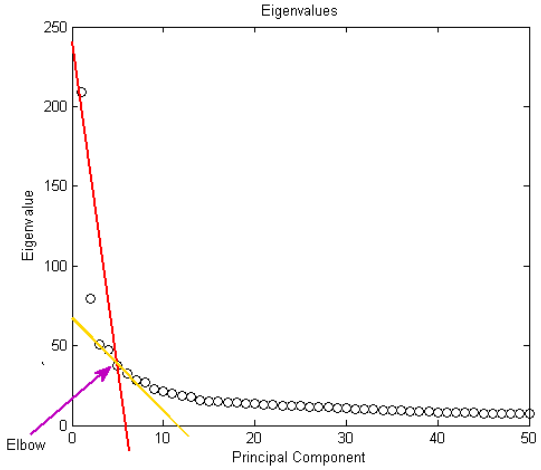


Figure 6 Scree plot for Scree test criterion

Figure 6 shows the Scree plot displaying eigenvalues of the PCs. It can be observed that the first inflection point, also known as *elbow*, occurs between the fourth and fifth PCs. Hence, the number of PCs retained is 5.

Table 1 summarizes the number of PCs that are retained in accordance to the three termination criteria. It is found that the number of PCs selected by Scree test is the least, i.e. it takes only the first five PCs to embody all the significant attributes of the original data.

Table 1 Number of PCs retained according to termination criteria

Termination Criteria	Number of PCs
EOC	115
CPV	70
Scree test	5

4.2 Performance of Classification by ANN

Table 2, 3, 4 and 5 tabulates the performance of LM, SCG, RPROP and OSS learning algorithms integrated with the different termination criteria. It can be observed that 100% across the board of performance, [Accuracy, Sensitivity, Specificity, Precision], is achieved with Scree test as the termination criterion, regardless of the learning algorithms.

With reference to Table 2, accuracy of 100% is only achieved using SCG algorithm, if EOC is used as the termination criterion. Accuracy of 94.7%, 89.5% and 63.2% are reported for OSS, LM and RPROP learning algorithm respectively. Accuracy with CPV as the termination criterion is observed as the lowest of the three criteria, regardless of the learning algorithms. The highest accuracy is 94.7% using OSS learning algorithm, while the lowest accuracy is 52.6% using RPROP learning algorithm.

With reference to Table 3, for EOC criterion, sensitivity of 100% is obtained with SCG learning algorithm, and less than 90% for the other learning algorithms. The highest sensitivity attained with CPV as the termination criterion is 88.9% with OSS learning algorithm.

With reference to Table 4, it is observed that SCG and OSS learning algorithms succeeded in reaching a specificity of 100%, regardless of the termination criterion.

With reference to Table 5, with EOC as the termination criterion, SCG and OSS learning algorithm are found to attain a precision of 100%, while LM and RPROP learning algorithm a precision of 83.3%. With CPV as the termination criterion, the precision is 100% for LM, SCG and OSS learning algorithm, whereas the precision of RPROP learning algorithm is 75%.

From the results, it can be perceived that Scree test criterion with only five PCs, is able to surpass the other two termination criteria, for all learning algorithms, in terms of accuracy, sensitivity, specificity and precision. This suggests that the five PCs embody sufficiently important attributes of the original signal for classification of NS1 adulterated saliva samples. Even though the number of PCs selected by EOC and CPV criterion are more, the performance of the classifier is found lesser, in addition to higher computational load and time.

Between EOC and CPV criterion, performance of OSS and SCG learning algorithms are found better than LM and RPROP. In terms of specificity and precision, both scored 100%. SCG learning algorithm

is found to co-ordinate well with EOC termination criterion, achieving accuracy and sensitivity of 100%. OSS is observed to work well with CPV termination criterion, however accuracy and sensitivity is less than100%. As for LM, the most popular learning algorithm, it is surprising to find that its accuracy with all the three termination criterion is more than 80%, but less than that of SCG and OSS. RPROP learning algorithm is found the most unsuitable for detection of NS1 molecule, its performance worsens with increase in number of PCs included in the termination criteria

Table 2 Accuracy for different PCA termination criterion and ANN learning algorithm

	LM	SCG	RPROP	OSS
Scree Test	10	100	100	100
EOC	89.5	100	63.2	94.7
CPV	84.2	89.5	52.6	94.7

Table 3 Sensitivity for different PCA termination criterion and ANN learning algorithm

	LM	SCG	RPROP	OSS
Scree Test	100	100	100	100
EOC	83.3	100	45.5	85.7
CPV	72.7	80	46.2	88.9

Table 4 Specificity for different PCA termination criterion and ANN learning algorithm

	LM	SCG	RPROP	OSS
Scree Test	100	100	100	100
EOC	92.3	100	87.5	100
CPV	100	100	66.7	100

Table 5 Precision for different PCA termination criterion and ANN learning algorithm

	LM	SCG	RPROP	OSS
Scree Test	100	100	100	100
EOC	83.3	100	83.3	100
CPV	100	100	75	100

5.0 CONCLUSION

The effect of termination criterion of PCA on the classification performance by the different learning algorithms of ANN is investigated. This is intended to optimize an alternative technique for detecting NS1 molecule in adulterated saliva, which will in turn benefit the detection of NS1 related diseases. To the best of our knowledge, this has been the first study to look into the detection of salivary NS1 with SERS, which offers advantages such as early, non-invasive and simple sample collection and less tedious sample preparation procedure. Of the PCA termination criteria, the Scree test criterion which transforms the original signal into 5 PCs, a reduction of 99.7% of data size, without sacrifice of useful information, is found to bring out the most optimized classification performance with SCG learning algorithm of ANN. The encouraging performance confirms a promising detection technique for salivary NS1 using SERS.

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