Laser Induced Breakdown Spectroscopy Coupled with Neural Networks for the Identification of Fingernails of Smokers and Non-smokers

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Abstract. Laser Induced Breakdown Spectroscopy (LIBS) coupled with Neural Networks (NN) was used for the identification of biological samples. LIBS spectra were measured by Echelle type spectrometer in the spectral range from 200 to 1000nm using ICCD camera. Fingernails from smokers and nonsmokers were taken as sample. The advantages of the technique such as being fast, simple and real- time analysis with no or small preparation of the sample make LIBS suitable for this analysis. Due to the similar elemental composition of the samples and large amount of data, the classification by conventional chemometric methods was difficult therefore the use of advanced chemometric methods such as NN was necessary.

Introduction

The determination of biomarker in human nail has been studied by numerous research groups using different techniques and approaches [*Evjen,* 2011, *Pathak et al.,* 2010, *Roduskin et al.,* 2000]. The goal of these procedures was to find a correlation between the biomarker with some medical condition. The composition of nails is based on a structural protein, keratin, which is also found in other biological tissues such as hair and skin. Nails can act as a reservoir to xenobiotic or their degradation products. Therefore, the presence of high amount of certain element is often related to the exposure level of heavy metal and its study can lead to determination of some illnesses. One advantage of using nail instead of other biological tissues is their isolation from metabolic processes, whereas in blood or urine the concentration of heavy metal can be interfered by metabolic process.

Changes in elemental composition of nails are directly related e.g. oesophageal cancer has been reported in some studies [*Nouri et al.,* 2008]

LIBS is an appropriate technique to carry out the monitoring of biomarkers in qualitative or quantitative analysis due to its advantages such as no sample preparation was required in this case, its high speed of data acquisition and being cost-effectiveness. In several studies LIBS technique has been used to the determination of biomarkers in nail, for instance, the discrimination between opium consumer and control subject [*Shadman et al.,* 2012] or other diseases such as hyperthyroidism and hypothyroidism [*Bahreini et al.,* 2012].

LIBS is able to analyse a sample by direct measurement of the plasma emission generated by a high energy laser pulse providing a immediate spectral fingerprint which contains spectroscopic information of the elemental composition from the analysed sample [*Cremers et al.,* 2006].

In this study, LIBS technique in combination with Neural Networks (NN) was applied to the discrimination between smoker and non-smoker subjects.

The use of several chemometric methods in the LIBS data processing has been evaluated in other researches [*Sirven et al.,* 2006, *Myakalwar et al ,* 2011, *Caceres et al.,* 2013]. The most used methods are Principal Component Analysis (PCA), Soft Independent Modelling of Class Analogy (SIMCA), and Partial Least-Squares Discriminant Analysis (PLS-DA), however, these methods are not able to give satisfactory results in some applications. For this reason NN was selected combining a high identification ability with a simple implementation.

Experimental

Apparatus

The scheme of the used experimental set-up is shown in Fig. 1. A Q-switched Nd:YAG laser (Brilliant Eazy, Quantel) operating at 532nm with the maximum energy per pulse of 165mJ and the laser pulse duration of 4ns was used for plasma generation. Plasma emission was collected by the optical components such as lens (Thorlabs, BK7), mirrors, and optical fiber (Ocean optics, quartz) and analyzed by the Echelle spectrometer (Mechelle ME 5000) with intensified CCD camera (iStar, Andor Technology). The range of recorded wavelengths was 200–975 nm. Spectral resolution of the spectrometer is $\lambda/\Delta\lambda = 4000$.

The delay between the acquisition and plasma collection was set to 0.8 μs, using a gate width of 1 μs. These setups were selected from previous testing measurements, where different gate delay and gate width were studied for obtain the best ratio signal to noise.

All measurements were corrected with the spectral response of Echelle spectrometer.

Samples

A total of twenty-six human fingernails were measured. Samples were divided into two groups, smoker and non-smoker, 13 nails for each group. For this study nails from 10 men and 3 women were taken. Moreover, in order to reduce differences between individual, samples were chosen in pairs, smoker and non-smoker, in the same range of age. Finally 10 samples were chosen belonging to people in the age range of 18–27, 6 samples in the age range of 27–36 and 10 samples from people between 45–55 years old. Moreover, studies show that the elemental composition of the nail is influenced by age, sex, and geographical location [*Li et al.,* 2012, *Favaro,* 2013], therefore samples from healthy subject, with regular diets, non-lacquered nail and with profession where their nails were not in contact with chemicals were selected.

Fingernails were taken from free edge of nail plate and kept separately in individual plastic bags. In order to reduce any surface contaminations, all samples were washed in an ultrasonic cleaner (Digital Ultrasonic Cleaner PS-10A) for 5 minutes and then rinsed once in acetone. The samples were left to dry at the room temperature before the LIBS measurement. For each sample 10 spectra were recorded.

Table 1 gives the description of nails samples selected for the study. Samples were designated with letters followed by a number. The first letter represents the type of sample (S:smoker, NS: non-smoker) and the number indicates the ID of the individual.

The elemental composition of samples was determined according to the spectral lines. For identification of samples as smoker or non-smoker, Neural Networks were used.

Neural Networks model

Home-made Neural Network (NN) software based on Matlab was specifically developed to deal with the problem of smoker classification from LIBS data. NN model was based on a multilayer perceptron, feedforward, supervised networks. In a NN model, the processing unit are called neurons that are arranged in two or three layers, more frequently in three layers (input, hidden and output layers). In this network topology each neuron receives information from all of the neurons of the previous layer [*Maren et al.*, 1990]. In present work, 20,000 neurons (spectral data) were taken as input, 10 neurons in the hidden layer and two in the output layer.

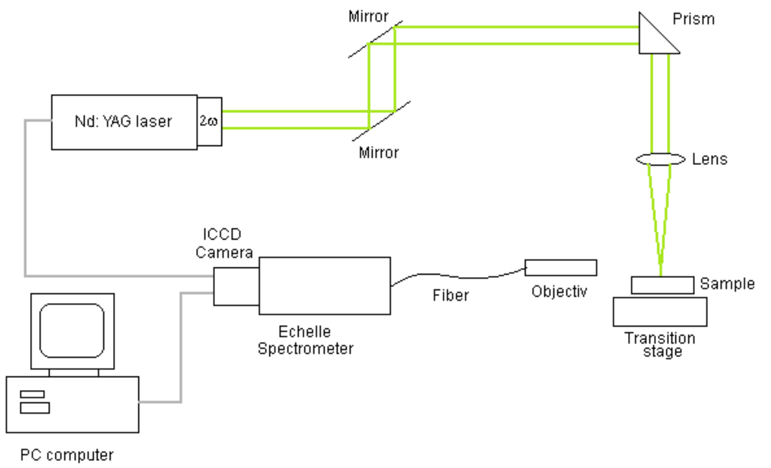

Figure 1. Scheme of LIBS apparatus.

Table 1. Samples of smokers and nonsmokers using in the study.

	Nomenclature	Age	frequency
Smokers	S-1*	18	6/day
	S-008*	29	5/day
	S-83*	49	20/day
	S-86*	49	20/day
	S-34	27	5/day
	S-201	22	38/day
	S-6*	24	7/day
	S-15*	52	20/day
	S-80*	55	10/day
	S-105*	30	1–2/day
	S-18	55	10/day
	S-106	22	6/day
	S-110	25	20/week

*used for training

	Nomenclature	Age
Nonsmokers	NS-003*	28
	NS-8*	21
	NS-9*	22
	NS-16*	54
	NS-17*	55
	NS-31*	23
	NS-47*	23
	NS-52*	46
	NS-82	54
	NS-87	44
	NS-99	25
	NS-100	27
	NS-103	33

*-used for training

Results

26 fingernails samples, 13 for smokers and 13 for nonsmokers were analyzed using LIBS. A qualitative analysis of samples was carried out using the LIBS spectra coupled with a NN analysis. Fig. 2 shows a typical LIBS spectrum for a smoker (gray line) and non-smoker (black line). A total of 45 spectral lines of 12 different elements such as Ca, Mg, Na, K, Si, Ti, Al, Fe, C, H, O, N were assigned based on NIST atomic spectra database [*Ralchenko et al.,* 2013]. No lines corresponding to toxic metals such as Cd, As, Pb, Cr were found in the spectra despite their direct exposure to tobacco. In the elemental analysis of fingernails between smokers and nonsmokers, no visual differences were observed. Therefore, in order to achieve the classification of highly similar spectra, NN model was created. For the NN analysis data were divided into two spectral sets, training set and validation set. The NN training was carried out with 8 samples of smokers and 8 of non-smokers, 160 spectra (ten for each sample) were used. The NN model was validated with 5 samples of smokers and 5 of non-smokers, not included in the training, therefore were completely unknown for the model of Neural Network. Table 2. and Table 3. give the NN classification results for all individuals, smokers (Table 2.) and non-smokers (Table 3.). The samples marked with asterisk were used for training process and as well as for validation. Remaining samples were only used for the testing process.

The prediction results obtained by the NN analysis were assessed by means of the parameter "correlation value" (CV). A CV value equal to one indicates a perfect match between the predicted NN value and the actual category (smoker or non-smoker) whereas CV value of zero indicated no match at all. The CV limit for the correct classification of a sample was taken to be higher than 0.8.

Results in Table 3 show that NN was able to predict correctly non-smoker subject, except in only two cases (NS-82, NS-87) where the samples were considered as "unclassified."

In case of smoker subjects (Table 2), the results show that only one to five smokers were correctly classified, whereas two were incorrectly classified as non-smoker and two were unclassified. These results can be caused by some other outside influences connected with smoking such as different smoking frequency, different brand of cigarettes (with several type of quality of the filters), different inhalation depth and also by the physiological condition of each individual.

Therefore, LIBS is able to produce a characteristic spectral fingerprint for each individual even though there are no differences in the elemental composition of the nails from smokers and non-smoker. However, the NN results demonstrate that there are not clear variations between smokers and non-smoker that might conduct to a successful classification.

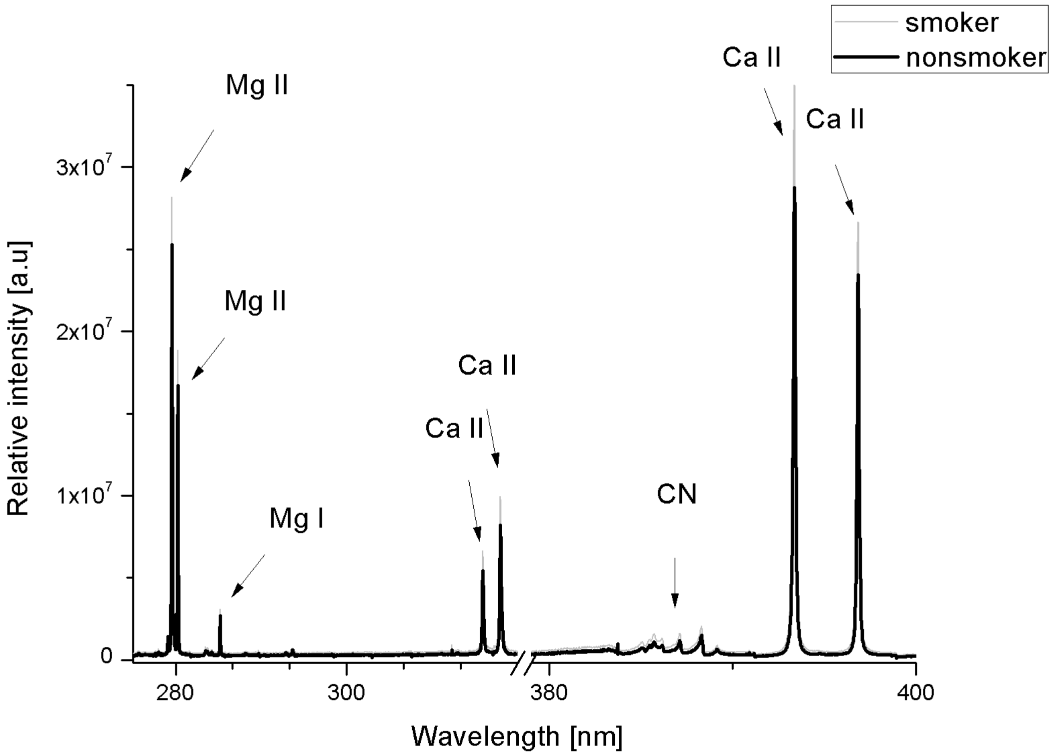

Figure 2. Spectral comparison between samples from smokers (gray) and non-smokers (black).

Table 2. Results of the fingernail analysis by Neural Network, samples of smokers.

Sample	Correlation Value Smoker	Correlation Value Non-smoker	Correctly Classified
S-1*	0.995	0.006	✓
S-008*	0.938	0.046	✓
S-34	0.489	0.493	Unclassified
S-83*	0.856	0.132	✓
S-86*	0.996	0.005	✓
S-201	0.020	0.980	✗
S-6*	0.993	0.023	✓
S-15*	0.994	0.006	✓
S-18	0.690	0.395	Unclassified
S-80*	0.906	0.096	✓
S-105*	0.993	0.008	✓
S-106	0.008	0.992	✗
S-0110	0.878	0.206	✓

Table 3. Results of the fingernail analysis by Neural Network, samples of nonsmokers.

NS-003*	0.030	0.964	✓
NS-8*	0.006	0.995	✓
NS-9*	0.092	0.921	✓
NS-16*	0.005	0.995	✓
NS-17*	0.010	0.986	✓
NS-31*	0.027	0.968	✓
NS-47*	0.131	0.898	✓
NS-52*	0.060	0.943	✓
NS-82	0.397	0.637	Unclassified
NS-87	0.483	0.500	Unclassified
NS-99	0.059	0.931	✓
NS-100	0.009	0.994	✓
NS-103	0.076	0.939	✓

Conclusion

This work focuses on the classification of fingernails from smokers and nonsmokers using LIBS coupled with Neural Networks. 13 samples of smokers and 13 of nonsmokers were classified based on their characteristic spectral fingerprint. In spite of differences in the elemental composition between smoker and non-smokers a qualitative analysis revealed that no toxic elements such as Pb, Cd, As, Cr were present in smoker samples and therefore a visual classification was not possible. The NN classification showed a potential for non-smoker subjects, however, in smokers individual other parameters as smoking frequency and the sort of tobacco should be taken into account to achieve a better classification results.

The no preparation samples and its cost-effective performance make LIBS-NN methodology suitable for the monitoring of biomarkers in biological samples, helping the current procedure to the detection of diseases.

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