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Evaluation of healthy and infected cervical tissue using a LIFS system and a back-propagation neural network

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ABSTRACT

Laser Induced Fluorescence Spectroscopy (LIFS) is a technique that has been recently used to detect *in vivo* or *in vitro* cancer. The LIFS system has been used to analyze cervical tissue samples for histological evaluation. Because the fluorescence spectra are position and inter-probe dependent (albeit they were equally classified by histological evaluation) the relationship that exists in the data is complex. So, a back-propagation Artificial Neural Network is used to detect the relationships contained within them. The validation of the system was done with 5 different proves and a 100% classification coincidence between the neural network classification and a normal histological one was obtained. The LIFS-System works with a N_2 laser of 5 μJ pulse energy (t_{FWHM} = 3.8 ns at 337.1 nm wavelength) and spectra from 350 to 650 nm were processed and evaluated in a PC.

Key Words:
Laser induced fluorescence, Cervical cancer and neural network.

RESUMEN

La espectroscopia de inducción de fluorescencia con láser (LIFS) es una técnica recientemente usada para detectar cáncer *in vivo* o *in vitro*. En este trabajo se usa esta técnica para analizar y diagnosticar muestras de tejido cervical preparadas para su evaluación histológica. Debido a que los espectros de fluorescencia son muestra a muestra y punto a punto diferentes (a pesar de ser clasificados como iguales por medio de su evaluación histológica) no resulta simple determinar las relaciones básicas de los datos medidos. Por lo cual se usa una red neuronal artificial del tipo "back-propagation" para detectar estas relaciones. La validación del sistema se realizó con 5 diferentes muestras y se obtuvo una coincidencia del 100% entre la clasificación realizada por ésta y la realizada por el proceso histológico normal. El sistema LIFS trabaja con pulsos de 5 μJ (t_{FWHM} = 3.8*ns* a 227.1*nm*) provenientes de un láser de N_2 y los espectros de 350 a 650 nm fueron procesados y evaluados en una computadora personal.

Palabras clave:
Fluorescencia inducida con luz láser, cáncer cervicouterino y redes neuronales.

INTRODUCTION

In Mexico, cervical carcinoma is the most common cancer in women[1,2] even though widespread use of Pap smear and colposcopy programs are currently in place. In agreement to data existing the mortality in 2002 reaches 5,777 and in the same period approximately 12,516 new cases of cervical cancer were reported[3]. The Pap smear is unable to achieve a concurrently high sensitivity (the correct classification percentage on the precancerous tissue samples) and high specificity (the correct classification percentage on normal tissue samples). The accuracy of the Pap smear is limited by both sampling and reading errors. Approximately 60% of false negative smears are attributed to insufficient sampling; the remaining 40% are due to reading errors[4]. Colposcopy examination in expert hands maintains a high sensitivity at the expense of a significantly low specificity, leading to many unnecessary biopsies[5]. Biopsy followed by histology is the gold standard for definitive diagnosis of cancer, but a major drawback of this approach is that the diagnosis requires expert interpretation of the microscopically derived histopathological information and is prone to human errors[6].

Laser Induced Fluorescence Spectroscopy (LIFS) has the potential to improve the efficacy of cervical precancerous detection[7]. This technique has the capability to probe the biochemical and morphological changes that occur as tissue becomes neoplastic, through the spectral characteristics of the measured fluorescence. In this work, we perform preliminary testing on cervix tissue biopsies in order to develop a LIFS-system for the in vivo detection of precancerous state and cancer. The spectral information is correlated to tissue histopathology diagnosis through a back propagation neural network which is implemented in software in a PC. The system is fully automated in order to be handled by no experts. This spectroscopic approach may also provide an alternate biopsy diagnostic modality in clinical pathology setting.

MATERIALS AND METHODS

The cervical tissue samples from patients after resection at surgery were placed in 10% formaldehyde during 24 hours at room temperature and then embedded in paraffin for slicing. Sections of 4 μm were sliced for the microscope slide preparation. A standard hematoxylin and eosin protocol to stain the tissue samples was used. The study involved 15 tissue samples from 15 different patients and the histopathological report was taken as the correct diagnoses. Ten tissue samples were used as the training set of an artificial neural network to classify them in five different cases and 5 tissue samples were used as a validation of the neural network. All samples were between three and four years old.

The Figure 1 shows the experimental arrangement. A home made Blumlein N_2 Laser, 5 μJ, 250 W peak, at 337.1 nm ($\Delta\lambda_{FWHM}$ = 2.6 nm, Δt_{FWHM} = 3.8 ns) operated at 70 mbar N_2 is enough in all the measurements. A glass beamsplitter (a microscope slide) and a Motorola MRD500 photodiode were used to monitor the laser light and to trigger the detection system. The measured reflectance of the slide is 17%. A rectangular (1.7 mm x 0.9 mm) spot at the sample is obtained with a 2.54 cm diameter BK7 glass lens (15 cm focal length).

The cervix tissue samples (located between two microscope slides) were irradiated at 45º and the fluorescence was measured perpendicularly to the reverse side of the sample. In order to achieve a large signal, two BK7 ball lens (10 mm diameter, E32748, from Edmund Industrial Optics) forming a fiber coupler, were used. A E38956 quartz fiber optic light guide (6.2 mm core diameter, 91.4 cm length) from Edmund Industrial Optics was used to transmit the collected fluorescence to a 0.27 m Digital Scanning Monochromator (Spectra Pro 275 from Acton Research Co.), where the fluorescence is analyzed and measured by a Hamamatsu H957-08 PMT (Photo Multiplier Tube) module. The laser reference and scattered fluorescence pulses are registered by a digital oscilloscope.

PC is used to control the operation of the monochromatic and to save the signals measured by the oscilloscope through a GPIB interface. The Figure 2 shows the flow chart to obtain the fluorescence spectra. The average of 256 pulses is given by the oscilloscope every 5 nm from 350 to 650 nm. The peak value of the fluorescence intensity $I_{\ddot{e}}$ at each selected wavelength, using a National Instrument Data Acquisition LAB PC-1200 board, is stored in the PC. All these values are processed by an specific software to eliminate noise, to consider laser pulse variations and to graphic the spectra in different ways. The software was developed in a Lab-View (Ver. 6) graphic programming package.

RESULTS AND DISCUSSION

The experimental arrangement, (Figure 1) has been used previously to characterize the properties of Bond

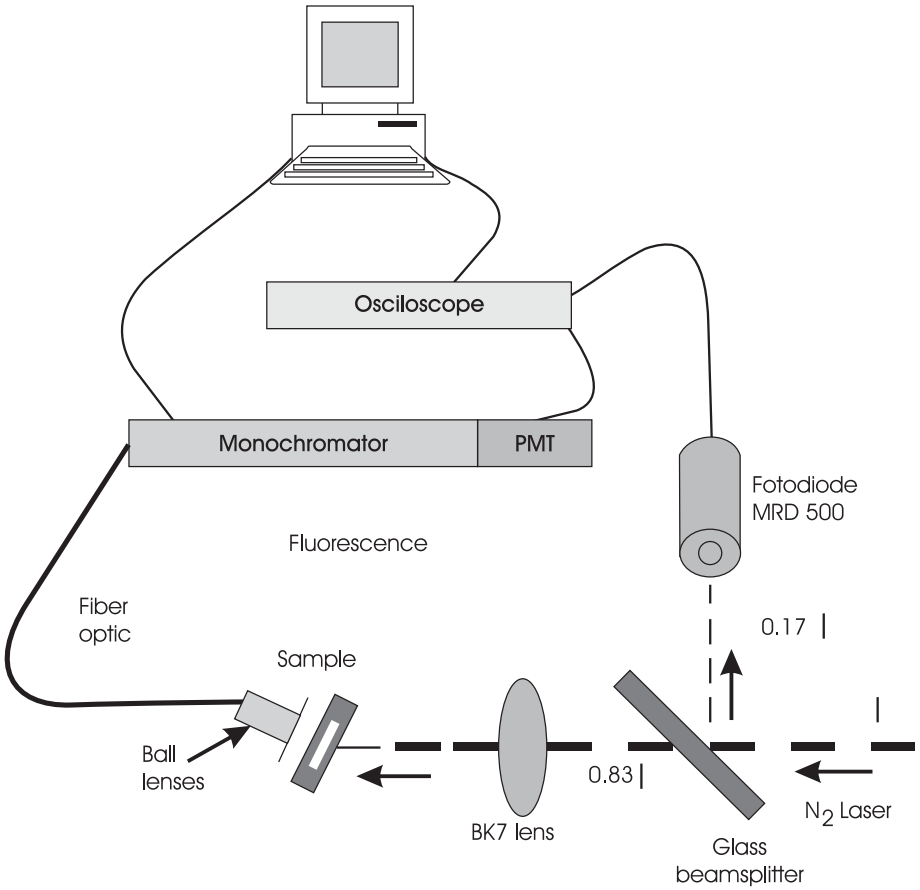

Figure 1. PC-Controlled LIFS System based on a N_2-laser and a quartz fiber optic light guide. The sample is located between two microscope slides.

paper at 337.1 nm wavelength[8] and shows a response time of 2 ns (given by the PMT). Fluorescence lifetimes in the order of 9 ns, which are in the order of the fluorescence lifetimes of human tissue[9], were measured.

The Figure 3 shows the average fluorescence intensity versus the wavelength from 10 different places each sample. The fluorescence of the healthy tissue sample is higher than the fluorescence of the others in the bands from 350 to 540 nm and from 575 to 650 nm, and that could be a criterion to separate malignant and non-malignant tissues. For 320 nm excitation some authors[10] have proposed to use only the ratio of the fluorescence intensities at 383 nm and 460 nm to classify malignant and non-malignant tissues for in-vitro cervical tissue obtaining a 33% of false negative diagnoses.

In order to compare all the intensity values of the fluorescence spectra in each tissue, the spectra in Figure 3 are normalized assigning the value of 1 to the maximal fluorescence intensity for each sample, see Figure 4. From (Figure 4) the differences between all fluorescence patterns are evident. For example, the maxima of HPV infection, invasive cancer and carcinoma in-situ spectra take place at around 560 nm, but they have different values between 350 to 500 nm. On the other hand, the maxima of healthy tissue and chronic cervicitis spectra takes place at 350 nm, but they have different values between 550 to 600 nm.

Multivariate statistical algorithms and neural network based algorithms have been successfully used to discriminate from between fluorescence spectra *in vivo* squamous (precancerous state) or non squamous intra epithelial lesions[4]. Here we propose the use of all the measured spectral intensities (49 per spectra), using a neural network, to classify the five tissue cases.

AUTOMATIC DIAGNOSES

A neural network is a good option to represent a complex relationship between any number of input parameters (like the ones presented in Figures 3 and 4) and an outcome of interest. The fact that an arti-

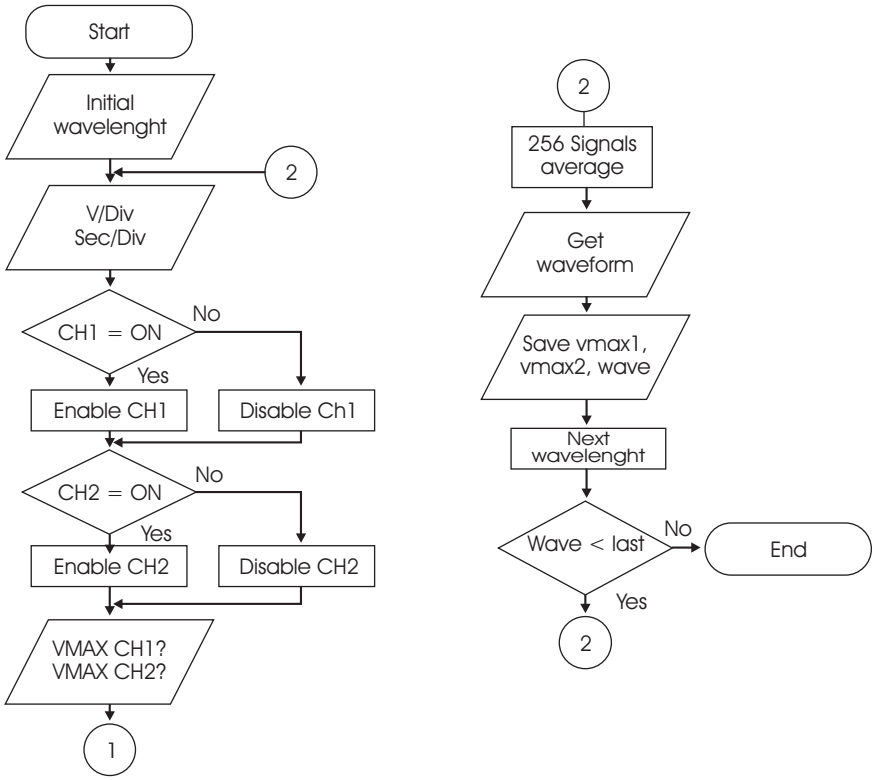

Figure 2. Fluorescence spectra measurements flow chart.

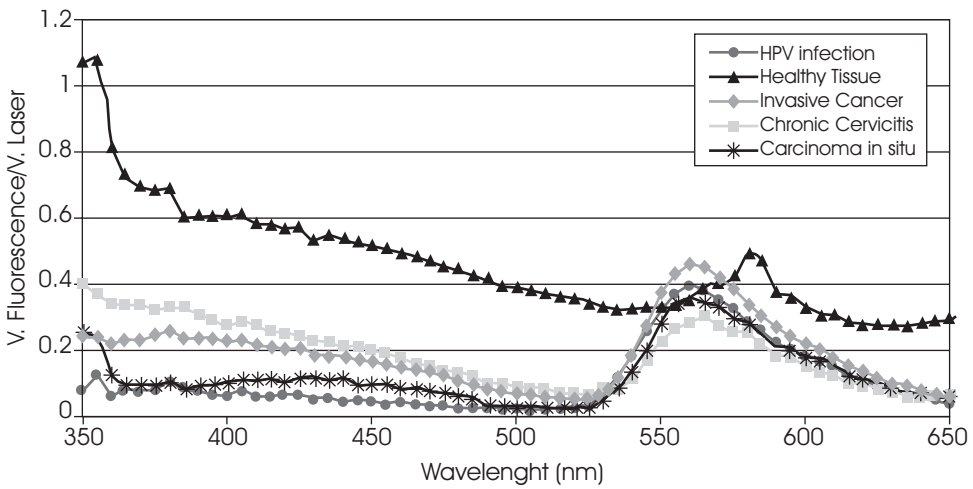

Figure 3. Average fluorescence intensity spectra different cervix tissue samples. (HPV-Human Papilloma Virus).

ficial neural network is largely independent of assumptions regarding the statistical distributions relating to the data makes them more reliant on large quantities of data[11]. Following the neural network theory[12-14] we propose two layers (with tansig and logsig transfer functions for the hidden and output layers) of adaptive weights network in a feed-forward diagram, see Figure 5. We consider 49 inputs, one for each (I_λ, λ) measurement (spectra from 410 to 650 nm), 49 hidden units and 5 outputs (one for each tissue classification in Figure 3). A fixed activation point b was used.

In our network each input site is connected to only one hidden unit through an associated weight $w_{i,i}$ (where i = 1, ..., 49 is the number of the input point). The hidden layer is connected to the output layer through the associated weights $w_{i,j}$ (where j = 1,..., 5 is the number of the output unit). The net-

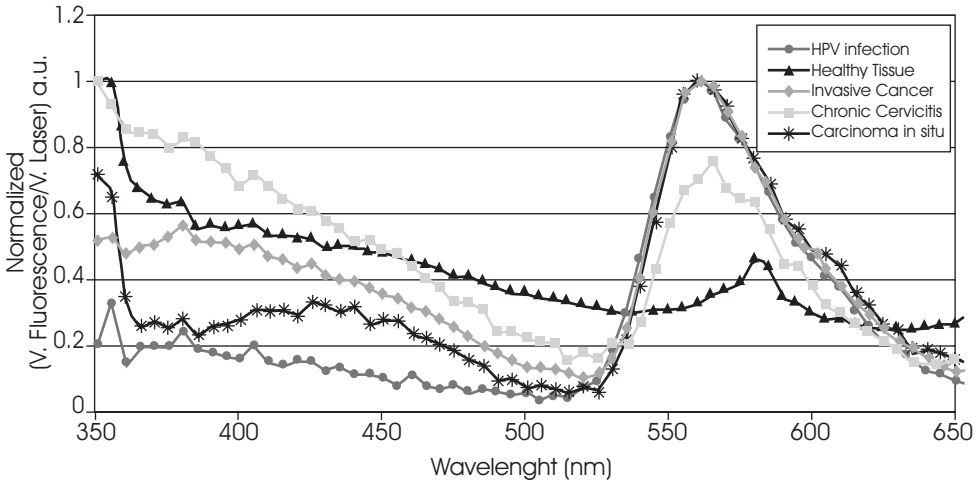

Figure 4. Fluorescence patterns different cervix tissue samples. Here the peak value each spectra in *Figure 2* have been normalized to 1.

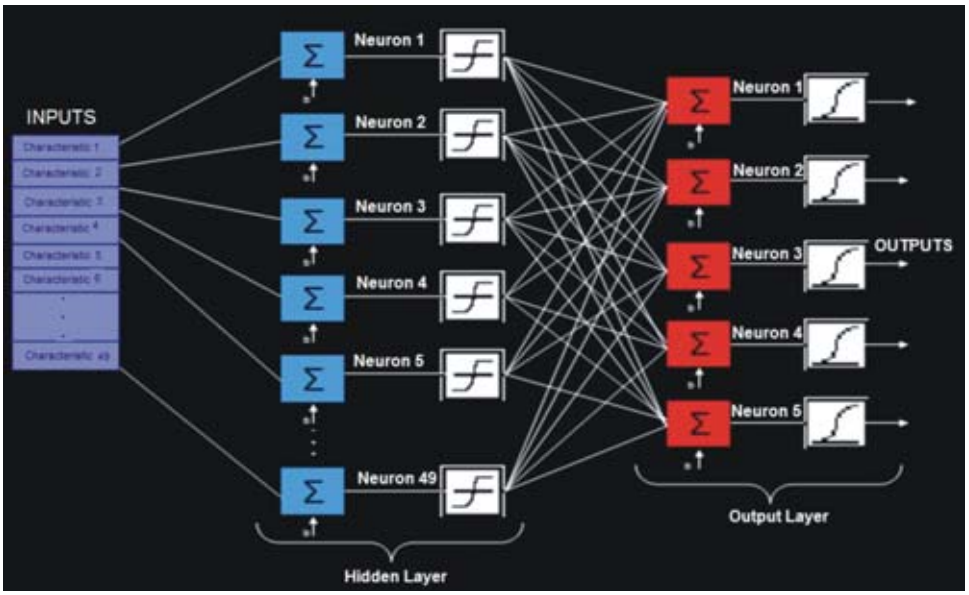

Figure 5. Proposed back-propagation neural network.

work starts with randomly proposed connection weights.

When an input spectra (I_λ, λ from Figure 4) is presented to this network a different diagnoses from the histological one results. A back-propagation learning algorithm is used to match both diagnoses. It minimizes the error function by changing all the connection weights. To find the initial weight values and the activation point b, the neural network was trained in Matlab with two tissue samples (each a histological diagnosed one). No pattern generalization problem was presented after 7,000 epochs. The complete code can be consulted elsewhere[15].

After minimizing the error function for the training set (finding the best weights) the input spectra of the validation set were presented to the network in order to recognize if these were similar to the learned spectra and produce a similar output. The neural network output layer produces only one value and the tissue classification is assessed as follows: Carcinoma *in situ* (0.98 ± 0.03); Healthy (0.3 ± 0.03); Chronic cervicitis (0.5 ± 0.03); Invasive cancer (0.7 ± 0.03); HPV infection (0.9 ± 0.03). When the value is out of these intervals the tissue is not classified. A 100% classification coincidence of the validation set data was obtained.

CONCLUSIONS

We have presented a time resolved N_2-laser induced fluorescence system that allows the measurement of cervical biopsies and diagnose healthy or can-

cerous tissue. The system is totally automatic and can resolve fluorescence spectra from 350 to 700 nm with a rise time of 5 ns. A back propagation neural network is used to process the spectra and classify the tissue in five different classes. Fifteen three to four year old pathologically classified healthy and ill cervix tissue which were prepared for histological evaluation in microscope slides were used in our investigation. Measured spectra were used to train a back propagation neural network, which yields then automatic diagnoses. The validation of the system was done with 5 different samples and a 100% coincidence between the neural network classification and the normal histological one has been obtained. So, the LIF spectroscopy approach may provide an alternative diagnostic modality in clinical pathology setting.

In order to reduce the measurement time from 20 minutes to the order of seconds, we are planning to use a UV-VIS-CCD based spectrometer. Also, the implementation of a reflection backscattering fiber optic light guide will allow us *in vitro* and *in vivo* diagnoses.

BIBLIOGRAPHY

1. Secretaría de Salud. México. Epidemiología 2006; 1(23): 1-7.
2. Lazcano-Ponce E, Herrero R, Muñoz N, Cruz A, Shah KVK, Alonso P, Hernández P, Salmerón J, Hernández M. Epidemiology of HPV infection among Mexican women with normal cervical cytology. International Journal of Cancer 2001; 91(3): 412-420.
3. International Agency for Research on Cancer, Globocan 2002 database. http://www-dep.iarc.fr/GLOBOCAN/table2.asp?cancer=142&region=99&sex=2&sort=1.
4. Tumer K, Ramanujam N, Ghosh J, Richards-Kortum R. Ensembles of radial basis function networks for spectroscopic detection of cervical precancer. IEEE Transactions of Biomedical Engineering 1998; 45(8): 953-961.
5. Mitchell MF. Accuracy of colposcopy. Clinical consultations in obstetrics and gynecology. 1994; 6(1): 70-73.
6. Majumder SK, Kumar R, Gosh NP, Gupta PK. Cancer diagnosis using N2 laser excited autofluorescence spectroscopy of formalin-fixed human tissue" Conference Proceedings of Photonics 2004. http://eel.inst.ac.ir/Sadr/Papers/bio4.pdf
7. Briokner CJ, Follen M, Boiko I, Galvan J, Thomsen S, Malpica A, Suzuki S, Lotan R, Richards-Kortum R. Autofluorescence patterns in short-term cultures of normal cervical tissue. Photochemistry and Photobiology 2000; 71(6): 730-736.
8. De la Rosa J, Bautista FJ. Optical properties of paper at 337.1 nm. Revista Mexicana de Física 2005; 51(1): 110-113.
9. Fang Q, Papaioannou T, Jo JA, Vaitha R, Shastry RK, Marcu L. Time-domain laser-induced fluorescence spectroscopy apparatus for clinical diagnostics. Review of Scientific Instruments 2004; 75(1): 151-162.
10. Glassman WS, Liu CH, Tang GC, Lubicz S, Alfano RR. Ultraviolet excited fluorescence spectra from non-malignant and malignant tissues of the gynecological tract. Lasers in the Life Sciences 1992; 5(1-2): 49-58.
11. Batuello TJ, Crawford ED. Artificial neural networks in prostate cancer. Lab. Medica International 2001; 18(3): 10-11.
12. Bishop CM. Neural networks for pattern recognition, Oxford University Press Inc., New York, 1995.
13. Hagan M, Demuth H, Beale M. Neural network design, PWS, Publishing Company, Boston, 1996.
14. Rojas R. Neural networks, a systematic introduction. Springer, Berlin, 1996.
15. Álvarez R, Franco EB, Torres R. Sistema auxiliar para el diagnóstico de carcinoma epidermoide invasor en tejido cérvix empleando tecnología láser. Trabajo terminal No. 633, ESCOM-IPN, 2005.