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G. Filippidis[a], G. Zacharakis[a], A. Katsamouris[b], M. Kouktzela[a], S. Montan[c], S. Andersson-Engels[c], Theodore G. Papazoglou[a]

[a] Foundation for Research and Technology-Hellas, Institute of Electronic Structure and Laser, Laser and Applications Division, P.O. Box 1527, 71110 Heraklion, Crete, Greece

[b] University of Crete, Medical School, University Hospital, Department of Vascular Surgery, GR-71409 Heraklion, Crete, Greece

[c] Lund Institute of Technology, Department of Physics, Box 118, SE-221 00 Lund, Sweden

ABSTRACT

In order to investigate the effects of conservation in liquid nitrogen and formalin on peripheral vascular tissue (abdominal aortic, femoral and flank artery tissue) laser-induced fluorescence spectra were recorded during the exposure of these tissues to He-Cd radiation (442 nm). The spectral distribution of tissue fluorescence allowed the development of simple algorithms based on the intensity difference in order to discriminate the tissue samples when they were fresh and after they were stored for 24 and 48 hours in liquid nitrogen or formalin.

Keywords : Conservation, fluorescence, algorithms, discrimination.

INTRODUCTION

In the last few years several researchers have attempted to incorporate laser-induced fluorescence for the characterization of arterial wall structure. A number of groups [1-4] have used a variety of surface fluorescence detection schemes to improve tissue discrimination. The data analysis was based on the recording of the relative intensity of two or more regions of the spectrum and the comparison of their absolute values of ratios for normal and atherosclerotic samples. Results have shown high predictive accuracy (~ 85%). In addition to these methods fluorescent probes have been used as positional markers in a wide field applications [5-6].

In this paper we evaluate whether He-Cd laser-induced fluorescence may assist in discrimination of fresh tissue samples from tissues which were stored in liquid nitrogen or formalin for 24 and 48 hours.

METHODS

1) Tissue samples.

Tissue from nine patients was investigated. Two abdominal aortic, three flank artery and four femoral artery samples from by-pass operations were obtained in an unfixed state, within half-hour after the excision. The vessels were opened longitudinally to expose the intimal surface. Following gross inspection one of these speciments was classified as normal and the rest as of various degrees of atherosclerosis (fibrous plaque, calcified plaque, aneyrysmal thrombus). In some specimens there was both calcified and fibrous plaque. The specimens were cleaned and rinsed in saline solution. They were then fixed on a saline moistured sponge in order to maintain humidity. After the end of each experiment the samples were stored in formalin (10%) or in liquid nitrogen. The samples were irradiated again after 24 and 48 hours. The frozen samples were first allowed to reach room temperature before irradiation.

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2)   Experimental apparatus

The experimental apparatus is shown in Fig. 1. A He-Cd laser emitting at 442 nm was used for excitation. The average power used during the experiments was of the order of 15-20 mW. The output of the laser was reflected at right angles by a dichroic mirror (99.3% at 45°) and was focused on the input of a step index multimode fiber (560 μm core diameter). Fluorescence emission was collected by the same fiber. Then it passed through the dichroic mirror and was focused at the entrance slit (100μm) of a 0.25 m spectrograph employing a 450 grooves mm^{-1} holographic grating. Data acquisition and analysis were performed via an optical multi-channel analyzer (OMA) employing a diode array detector. The signal from the diode array detector was fed to a 486 PC for A/D conversion and further processing. Wavelength calibration was performed with a mercury lamp. A high pass filter (Schott CG 490 nm) was placed in front of the spectrograph entrance in order to isolate the fluorescence signal from reflected laser light. The detection system (including the spectrograph, detector and fiber) was not corrected for uniform spectral response. This did not, however, affect the validity of our algorithms since all of them were based on relative intensities. The spectrum obtained during the experiment represented the time-integrated fluorescence of the underlying tissue. The OMA was operated in the free scanning mode.
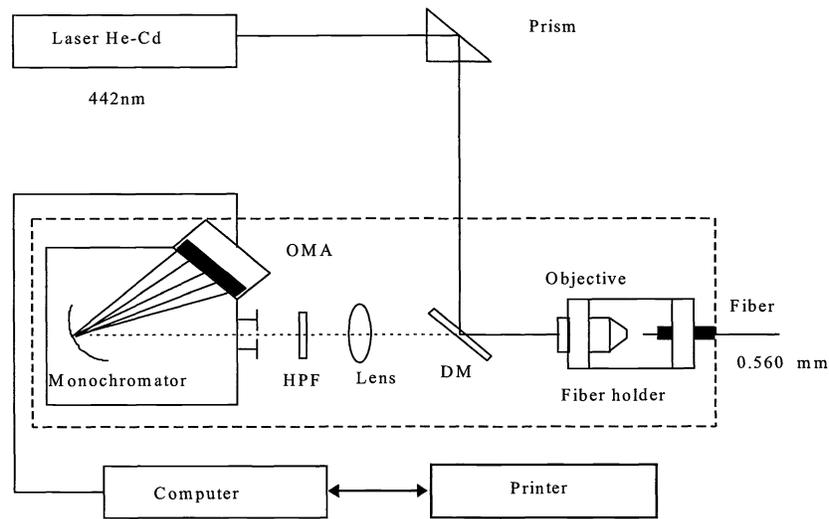
Fig. 1: Experimental apparatus for laser induced fluorescence measurements of normal and atherosclerotic tissues. All optical components, except the pumping laser (He-Cd, emitting at 442 nm), are located in a diagnostic module. HPF: high pass filter; DM: dichroic mirror; OMA: optical multi-channel analyzer.

3)   Data acquisition processing

The acquisition strategy was to acquire background corrected fluorescence spectra from each tissue sample. The background was recorded during laser shots on the saline moistured sponge. This accounted for the fluorescence of the fiber, saline and other external sources of noise. This background spectrum was subtracted automatically from each spectrum that obtained from the tissue sample. Each spectrum was processed in the following manner: the average intensities of the spectral regions 534-538 nm (PE 1), 542-546 nm (VA 1), 548-552 nm (PE2), 577-581 nm (VA 2), 591-595 nm (PE 3), 608-612 nm (VA3), and 663-667 nm (PE4) were calculated. Using these parameters twelve different simple algorithms were constructed. These twelve algorithms were implemented on the acquired spectra. For each tissue sample three or four spectra were obtained each time thus all the algorithms have a standard deviation around the mean value.

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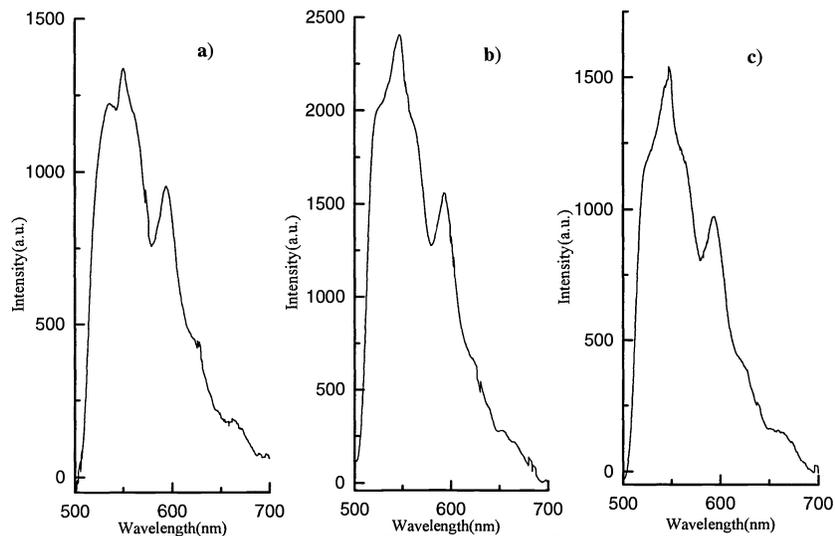
Fig. 2: Fluorescence spectra from fibrous plaque (from femoral artery tissue) a) when it was fresh, b) and c) after it was stored for 24 and 48 hours in liquid nitrogen respectively.
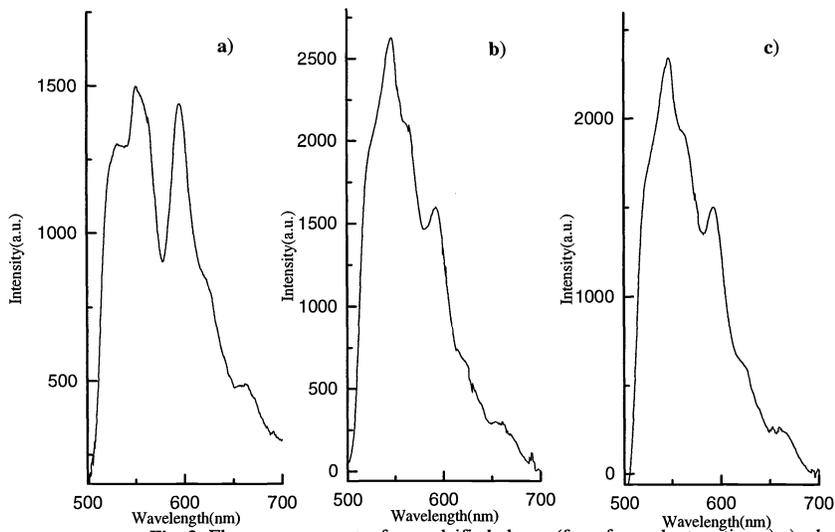
Fig. 3: Fluorescence spectra from calcified plaque (from femoral artery tissue) a) when it was fresh, b) and c) after it was stored for 24 and 48 hours in formalin respectively.

RESULTS

Tissue samples that were placed in formalin seem to have more changes in their spectral distribution than those placed in liquid nitrogen. This is obvious from the spectra that were taken after 24 and 48 hours (Fig. 2, Fig. 3). Furthermore, it was observed that most changes took place in the first 24 hours. Important changes between 24 and 48 hours were not observed either for formalin nor for liquid nitrogen conservation.

The algorithms that seemed to have a good rate of success are the same for formalin and for liquid nitrogen storing. In most cases algorithms succeeded to distinguish the fresh tissue samples from those placed in liquid nitrogen or in formalin for 24 hours but they failed in doing so for the time interval between 24 and 48 hours. Better results were obtained for fibrous than for calcified plaques. The algorithms which have more success in discrimination of the tissue samples were A11, A10,

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A8, A12, A6, A9 (Table 4) which contain mostly information related to the red part of the fluorescence spectrum (right side slope)

Algorithms	Fresh fibrous plaque	After 24 hours in formalin	After 48 hours in formalin	Fresh calcified plaque	After 24 hours in liquid nitrogen	After 48 hours in liquid nitrogen
A1=PE1-PE2/VA1	-0.124 ± 0.016	-0.123 ± 0.004	-0.130± 0.006	-0.141± 0.040	-0.130±0.022	-0.110±0.008
A2=PE1-PE2/VA2	-0.213 ± 0.040	-0.198±0.008	-0.203±0.006	-0.192±0.051	-0.215±0.037	-0.167±0.014
A3=PE3-PE4/VA3	1.222 ± 0.187	1.543±0.098	1.509±0.070	0.938±0.054	1.336±0.028	1.146±0.029
A4=PE1/ PE2	0.889±0.013	0.884±0.003	0.882±0.003	0.873±0.031	0.870±0.020	0.889±0.008
A5=PE1/ PE3	1.223±0.062	1.423±0.009	1.442±0.006	0.907±0.095	0.916±0.053	0.962±0.004
A6=PE2 / PE3	1.374±0.079	1.609±0.009	1.634±0.002	1.037±0.076	1.052±0.036	1.082±0.004
A7=PE3 / PE4	3.981±1.057	5.746±0.895	5.846±0.224	2.886±0.186	5.336±0.160	3.564±0.139
A8=PE2 /PE4	5.528±1.812	9.257±1.498	9.557±0.368	2.998±0.360	5.616±0.277	3.860±0.165
A9=PE1-PE3 / VA2	0.313±0.097	0.450±0.001	0.467±0.007	-0.144±0.159	-0.134±0.090	-0.051±0.007
A10=PE2-PE4/VA3	1.847±0.364	2.686±0.152	2.664±0.114	0.993±0.148	1.423±0.088	1.278±0.037
A11=(PE1-VA1)/ (PE2 -VA2)	0.003±0.018	-0.124±0.007	-0.056±0.029	-0.084±0.011	-0.314±0.018	-0.322±0.007
A12=(PE2-VA2)/ (PE3-VA3)	1.675±0.083	1.441±0.068	1.532±0.078	1.154±0.031	1.066±0.022	0.981±0.013

Table 4 The twelve algorithms, their mean values and the standard deviation for
fibrous and calcified plaques corresponding to the three cases described in the text.

DISCUSSION

The purpose of this study was to achieve the discrimination of tissue samples when they were fresh and after they were conserved in liquid nitrogen or formalin for 24 and 48 hours. All experiments were performed on abdominal aortic, flank and femoral artery samples taken from by-pass operations. Oxyhemoglobin exhibits an absorption peak at 550 nm. Due to this effect we would expect some alteration of the spectrum in this region. In our measurements the small "valley" between the 542-546 nm (VA1) and 577-581 nm (VA2) regions represents this absorption peak. Atherosclerotic samples, such as those used in our study (except the normal one), absorb less hemoglobin than normal post-mortem aortic samples[8]. In addition in ex vivo (no hemoglobin) and in vitro (hemoglobin diffusion) conditions similar spectral features have been observed[7]. This indicates that reduced spectral interference of hemoglobin is expected for the samples used in this study. Moreover, when the tissue is stored in formalin for 24 and 48 hours further minimization of the hemoglobin effect is expected. It has also been shown that fluorescence excitation and collection via the same fiber minimizes the effect of hemoglobin on the spectra[8]. Since this particular collection scheme was used in this work, the role of the hemoglobin was expected to be limited.

CONCLUSIONS

Laser induced fluorescence spectra were recorded during the exposure of normal and atherosclerotic tissue samples, which were obtained during by-pass operations, to He-Cd laser irradiation. The samples were conserved in formalin and in liquid nitrogen and they were irradiated again after 24 and 48 hours. Twelve simple algorithms were constructed in order to discriminate the tissue samples when they were fresh and after 24 and 48 hours.

The algorithms seemed to have better results for formalin conservation and for fibrous plaques than for calcified plaques. Some of them succeeded to discriminate the tissue samples obtained directly after resection from those stored in liquid nitrogen or formalin for 24 hours, but they usually failed to do so between 24 and 48 hours. Our results seemed to suggest that the methodology described in this paper, combined with the further development of our experimental module, and the continuation of these kind of experiments may serve a step towards the production of a computer-controlled laser angioplasty system.

ACKNOWLEDGEMENTS

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