Lasers in the Life Sciences 3(4), 1990, pp. 205–215
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Printed in the United States of America

Excimer Laser Induced Autofluorescence from Atherosclerotic Human Arteries

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(Received October 5, 1989; in final form February 20, 1990)

Excimer laser induced fluorescence has been studied *in vitro* as an effective diagnostic aid in laser angioplasty. Calcified plaque can be distinguished from nonatherosclerotic tissue based on autofluorescence spectra elicited by excimer laser radiation. Calcified tissue consistently displays a broader fluorescence profile than healthy arterial wall. Spectra from lipid-rich plaque precursor regions also display variation relative to those of normal tissue. These spectral differences have been observed with both dual and single optical fiber fluorescence collection systems. Spectra taken from diseased sites after ablative penetration of the calcified plaque are quite similar to spectra obtained at healthy sites after ablation into the wall tissue. Fluorescence spectra obtained during ablation in air at calcified sites reveal distinct sharp lines not seen upon ablation of healthy vessel wall. Refinement of these analytical techniques may permit precise monitoring of excimer laser angioplasty in real time and without the need of a second laser source.

KEYWORDS: *autofluorescence, angioplasty, spectral analysis*

I. INTRODUCTION

Laser angioplasty requires precise control of the ablation process so that the atherosclerotic plaque is targeted and removed without causing damage to the adjacent healthy wall tissue. Using tissue autofluorescence as a diagnostic aid was proposed by Kittrell *et al.* in 1985 [1]. Since then several studies have been reported using visible laser sources to obtain autofluorescence signatures from arterial tissue [2–7]. Spectral analysis of such signatures can yield much information about diseased arteries, including two-dimensional maps of the luminal surface delineating plaque regions [8].

Arterial wall autofluorescence can be utilized for purely diagnostic purposes with low excitation laser intensities. However, the ablation procedure for recanalization of occluded arteries requires a real-time feedback control mechanism to terminate the powerful ablation laser automatically before the structural integrity of the wall is compromised. Excimer lasers are promising candidates for ablative vessel recanalization due to their photoablative

fluorescence response elicited by ultraviolet sources can be used for diagnosis of atherosclerosis and automatic control of the ablation process. Therefore this paper examines the possibility of using the ultraviolet excimer laser to evoke useful autofluorescence spectra. Fluorescence work utilizing ultraviolet sources has previously been reported [11–13]. Clarke *et al.* found little variation in autofluorescence spectra from diseased and healthy tissues excited by 351 nm light [13], while others did find consistent differences in autofluorescence response using a variety of ultraviolet sources [11, 12]. A reexamination of the 351 nm fluorescence response using a XeF excimer laser is presented here.

This particular laser is studied for several reasons. As with other UV excimer lasers, it results in the desirable ablative tissue removal described above. In contrast to excimer lasers operating further in the ultraviolet wavelength region, this source is believed to have minimum mutagenic potential [14, 15]. Also, short, high energy pulses can be relatively easily transmitted through optical fibers.

In this work the autofluorescence response of arterial wall is treated as a macroscopic property of the tissue. The wall tissue contains many different molecular species in a complex biologic environment, and the complete spectral response is due to the simultaneous photochemical action of many agents. Detailed analysis of this photochemistry has been shown to be useful in analyzing laser-induced tissue spectra [11]. However, much information can be gleaned using a macroscopic approach, as will be shown.

II. EXPERIMENTAL SETUP AND PROCEDURE

The experimental apparatus was designed to mimic an *in vivo* clinical system. A single fiber autofluorescence excitation and collection system is shown in Figure 1. An XeF excimer laser (351 nm wavelength) was used as the excitation light source. The laser delivered 50 mJ pulses of energy at a repetition rate of 10 Hz. For simple tissue fluorescence measurements the laser pulse energy was attenuated so that the value delivered to the tissue was approximately 0.01 mJ. For experiments involving laser ablation of tissue higher delivered pulse energies were needed (5–15 mJ) and the attenuator was removed. The laser light passed through a quartz flat and was coupled by a lens into an optical fiber leading to the tissue sample. The arterial sample was pinned flat and positioned so that the fiber tip was approximately 2 mm above the sample and aimed at the site of interest on the endothelial surface. The tissue fluorescence generated by the ultraviolet laser pulse was collected by the same fiber and transmitted back to the quartz flat, which served as a beamsplitter. Part of the fluorescence signal was reflected off this flat surface and was focused into a second fiber leading to the spectrometer. A color glass filter (Corning CS 0-51) was placed in front of the spectrometer entrance slit to suppress the laser light reflected off the tissue sample. A Tektronix computer connected to the optical multichannel analyzer (OMA) was used to analyze the data.

For some experiments a slightly different experimental set-up involving two fibers at the tissue site was used. One fiber delivered excimer laser light to the arterial sample, while a second carried the induced fluorescence spectrum to the spectrometer. The fluorescence collection fiber could be placed parallel and adjacent to the laser delivery fiber to examine tissue fluorescence, or could be placed parallel to the tissue surface and near the point of intense laser irradiation during ablation experiments to collect light emitted from the plume

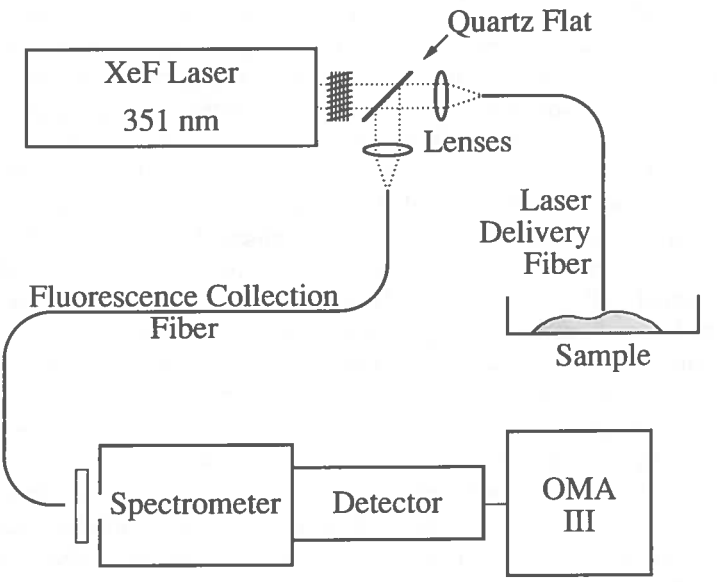

FIGURE 1 The single-fiber experimental setup. The quartz flat serves as a beamsplitter. Most of the laser light passes through this window and is delivered to the sample to induce fluorescence and/or ablation. A fraction of the resultant fluorescence is reflected off the quartz surface and sent to the spectrometer.

of removed material. Intimal surface autofluorescence spectra from both healthy and diseased wall sites were compared for both collection schemes.

All fibers used in both configurations were made of UV grade quartz and had core diameters of 800 μm. Two fiber tip geometries were used on the fiber end at the tissue sample. For low laser intensity fluorescence measurements, this tip was polished flat to collect as much fluorescence as possible. The spot size on the tissue surface generated by such a tip was approximately 1 mm in diameter. This corresponded to a fluence of approximately 1 mJ cm^{-2} on the arterial surface. For ablation experiments the tip was reformed into a spherical lens. This lens increased the ablation rate by focusing the laser light to a spot size of 0.7 mm diameter and increasing the delivered fluence to 1–10 J cm^{-2} at the tissue surface. The spherical tip was formed by heating the fiber end briefly in the flame of an acetylene torch. This technique resulted in reproducible, uniform spherical lenses with approximately 0.5 mm radius of curvature. Beam uniformity and spot-size were measured for both tip geometries using a beam profiling system. All other fiber ends in the set-up were polished flat for maximum collection of light.

The color glass filter was necessary in both the single and dual fiber schemes because reflected laser light was transmitted to the spectrometer along with the fluorescence signal. The filter used allows 50% transmission at 390 nm, meaning that the autofluorescence response at wavelengths shorter than 420 nm was significantly attenuated. Although this

terized by macroscopic properties as either normal endothelium, lipid-rich tissue (early stage atherosclerosis) or calcified plaque. Spectra were obtained for each site with the samples exposed to air except for a few experiments described below where the tissue was submerged in isotonic saline. Immediately following the fluorescence experiment, each site was marked with India ink and the samples were immersed in 10% formalin solution for preservation and subsequent microscopic histologic analysis. The relative thickness of the intimal and medial wall layers as well as the degree of lipid and/or calcium salt deposition were measured to confirm the initial pathologic characterization.

Before comparison, all the acquired spectra were normalized to the same relative intensity scale. The absolute intensity of an acquired fluorescence spectrum will vary sensitively with changes in the collection geometry of the optical fiber tip relative to the arterial wall, a feature difficult to control *in vivo*. In contrast, the curve shape of the fluorescence profile depends much more sensitively on the chemical composition of the tissue at the targeted site. Normalization removes differences in absolute magnitude between spectra, and allows direct comparison between spectral profile shapes. The normalization procedure used in this work consists of multiplying each obtained curve by a constant so that the intensity at 470 nm is 10000 counts. This wavelength is chosen because it is near the peak of the fluorescence response, where the signal to noise ratio is maximal.

Subtraction analysis was performed on luminal fluorescence spectra as a means of comparing the profile shapes. First a normal spectrum was generated by averaging the spectra of all samples identified as healthy or normal tissue. Other fluorescence spectra were then subtracted from this normal spectrum and the area under the resultant difference spectrum was used as an index of the degree of disparity between the two spectra.

To study the effects of an aqueous environment on fluorescence, and to search for a soluble component to the fluorescence response, autofluorescence spectra were obtained at sites on two samples from each tissue category. The samples were then covered with saline and repeat spectra immediately taken. Subsequent spectra were taken repeatedly every 5 minutes over a 30 minute interval in an effort to see any evolution of the spectral response.

Whether fluorescence analysis could be used to monitor the progress of ablative plaque removal was also studied. The single fiber configuration was used to deliver high energy pulses (5–15 mJ) of excimer laser light and cause localized ablation of tissue in air. Low intensity fluorescence profiles were obtained periodically during ablative excavation to a certain depth in the sample. This involved acquiring a spectrum using low fluence pulses (1 mJ cm^{-2}), then removing a small volume of tissue with high fluence pulses (1 J cm^{-2}), and repeating the process until penetration to some depth of interest at the tissue site was reached. Both normal and diseased sites were studied in this fashion.

Spectra were also acquired during ablation at tissue sites. The dual optical fiber system was used in two different configurations for these studies. This was necessary because the relatively intense laser pulses required for ablation caused noticeable fluorescence in the optical fiber material, and hence would have distorted the tissue spectrum obtained with the single fiber approach. Fiber fluorescence was not observed in the low laser intensity studies. In one arrangement the fluorescence collection fiber was in parallel with the laser delivery fiber to acquire tissue fluorescence spectra. In the other arrangement the collection fiber was repositioned to collect light selectively from the plume of ablation products.

III. RESULTS AND DISCUSSION

A. Endothelial Surface Fluorescence

Initially the two fiber collection scheme was used to obtain fluorescence profiles from 38 arterial sites in air. This group consisted of 8 normal adult sites, 14 fatty or "lipid-rich" sites, and 16 sites of gross calcification. Typical fluorescence profiles obtained are shown in Figure 2A. Normal tissue invariably exhibited fluorescence spectra very similar in shape to the solid trace shown, with a peak intensity around 450 nm and a "shoulder" close to 410 nm. Spectra from calcified samples consistently contained broader peaks than normal profiles, with relatively stronger fluorescence especially at shorter wavelengths. Several calcified tissue spectra (5 of 16) revealed a distinct second peak near 410 nm. Fluorescence spectra from lipid-rich sites also showed variation from the normal signature, also manifested as a generally broader fluorescence profile. Given the filter attenuation noted above, these results concur with those given in [14].

While the curves of diseased tissue consistently exhibited a broader peak than the normal, the difference was not striking. All spectra show similar profile shape and have peak intensities in approximately the same wavelength region. Subtle variations can be enhanced if a healthy trace is subtracted from the diseased, as is done in Figure 2B. Difference profiles were then obtained for each of the 38 sites by subtracting this composite healthy spectrum

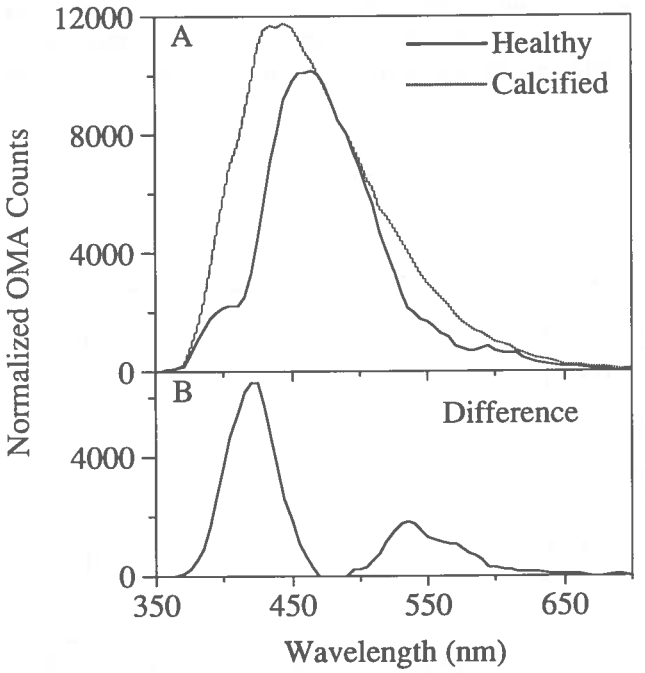

FIGURE 2 A: The fluorescence spectrum from healthy arterial wall, obtained with two fiber scheme, is shown as

As shown, upon spectral subtraction both lipid-rich and calcified plaque tissues exhibited significant disparity in fluorescence response relative the standard healthy wall spectrum. While the average difference profile area for the healthy group was essentially zero, with a moderate standard deviation, the spectra from diseased wall areas resulted much larger residual curve areas and much larger variation. The wide range of results for the diseased tissue types is to some extent attributable to the heterogeneous nature of these histologic divisions. Microscopic examination of the tissue sites following spectroscopy, while confirming the initial macroscopic classifications, found great diversity within the diseased groups. In particular, lipid-rich tissue showed wide variation in the degree of fatty infiltration and fibrosis (intima/media thickness ratio). One site in this group was found to contain microscopic calcium deposits, and the difference curve area associated with this site was one of the largest in the lipid-rich class. The variation within the healthy tissue group constitutes one standard deviation of the area distribution. Establishment of such a standard, by acquiring spectra at normal arterial sites from a large patient population may prove essential in developing a clinical diagnostic system. The results presented here support others in the literature that *in vivo* spectral diagnosis of atherosclerosis should be possible [8, 11, 13, 16].

One factor which must be considered when studying these fluorescence spectra is the possible light-absorptive role of hemoglobin within the wall tissue. The absorption spectrum for hemoglobin contains peaks near 425 nm, 540 nm, and 575 nm wavelength, and this is thought to contribute significantly to the characteristic structure of visible laser-induced arterial spectra. In addition, there may be significant postmortem alteration of the wall fluorescence, as additional hemoglobin diffuses into the tissue [16]. Examination of the

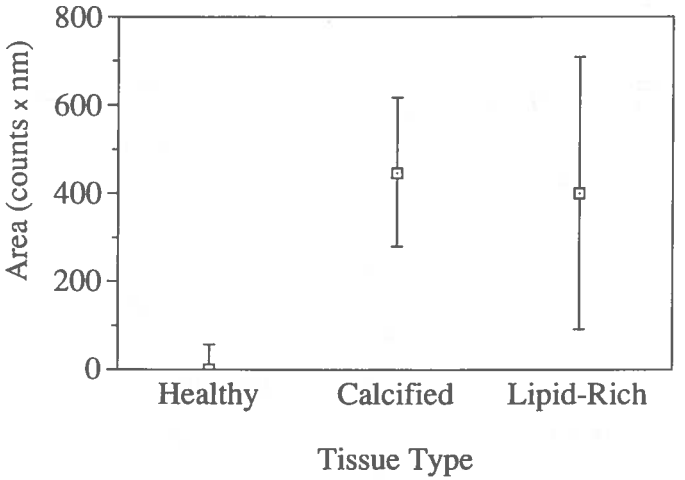

FIGURE 3 "Difference curve" areas, following substraction of a composite healthy fluorescence profile, averaged for the three histologic groups. One standard deviation bracketing is shown for each group.

difference spectrum in Figure 2B shows that the greatest disparity between healthy and diseased tissue UV laser-induced fluorescence profiles also occurs in the wavelength regions of high hemoglobin absorption, suggestive of a higher hemoglobin content at the normal wall site. Some of this difference may be due to a higher permeability of normal tissue to hemoglobin diffusion postmortem. In an attempt to wash out a diffusible component to the fluorescence response, two samples from each tissue category were examined with the two-fiber setup in air, then covered with saline. Immediate repeat spectra showed no change with respect to those taken in air. The samples were then allowed to incubate for 30 minutes with repeat spectra taken every 5 minutes. During this time no discernable change in fluorescence profile shape was seen. This does not rule out the possibility of postmortem changes, however, and the issue illustrates the need for follow-up *in vivo* studies. In addition hemoglobin absorption in all likelihood will distort *in vivo* wall spectra acquired within a blood-filled artery, and may dictate the need for vigorous saline flushing of vessels in clinical procedures.

Fluorescence spectra were also obtained from arterial tissue using the single fiber configuration. Spectra from healthy sites were quite similar to those seen with the dual fiber system, while calcified regions exhibited the same spectral characteristics as noted above. It has been reported by Richards-Kortum *et al.* using a visible laser source that the spectral shape obtained, using a visible laser excitation source, depends significantly on the geometry of the collection system used, due to tissue filtering [17]. This may be less relevant for an ultraviolet source due to the shallow penetration depth of the laser light.

Following the low laser intensity spectroscopy experiments the luminal surface of each sample was inspected for changes in appearance at the irradiated sites. No visible changes in color or morphology were observed. This fact combined with the constancy of the fluorescence response during repeated spectrum acquisition indicated that no significant photobleaching of the samples was occurring during these experiments.

B. Subsurface Fluorescence

Low intensity fluorescence measurements were also taken in conjunction with high laser intensity ablative excavation into the arterial wall. Example results are shown in Figure 4. (The dual intense lines near 700 nm wavelength are an artifact due to back scattered laser light passage through the spectrometer in second order.) Ablation at healthy tissue revealed significant change in spectral shape (Fig. 4A, solid trace versus dashed) after very shallow penetration into the wall, beyond which the curve remained essentially constant until the sample was nearly perforated. Results similar to those shown were obtained at 3 other normal arterial sites. Four sites containing luminal calcified plaque with a significant amount of underlying grossly normal wall tissue were studied in the same manner. Ablative excavation into a site containing calcified plaque produced little change in the fluorescence response until the plaque layer was penetrated. The spectrum obtained at a diseased site after penetration of the calcified plaque but prior to wall perforation (Fig. 4B, solid trace) exhibits similarities to that taken at depth in the normal tissue (4A, solid curve). Since atherosclerotic changes are usually confined to the intimal arterial layer, this result is to be expected. These results suggest spectroscopic changes may have utility in determining the appropriate endpoint to a laser angioplasty procedure. However, in cases of advanced calcified plaque

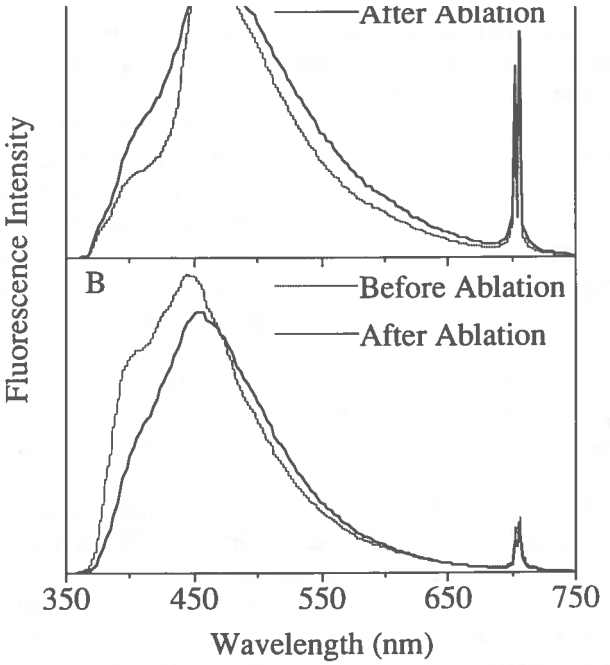

FIGURE 4 A: Single fiber fluorescence profile from the healthy arterial endothelial surface is shown dashed. The solid curve represents the profile taken from same site after excimer laser ablation to a depth of approximately 0.7 mm into a 2.0 mm sample. B: Fluorescence profiles at a calcified site at the endothelial surface (dashed) and after ablative penetration of hard plaque material (solid). Note the similarity between the two spectra taken at depth in the wall.

C. Fluorescence During Ablation

To look at the possibility of real time monitoring of the ablation process, spectral profiles were also obtained during tissue removal at high laser fluence. Ablation at calcified plaque sites was accompanied by an audible snap and brief formation of a bright plume extending away from the sample surface. Figure 5A shows a spectrum obtained under these conditions. Note the sharp lines superimposed on the broad fluorescence curve in the calcified site spectrum. (The dual intense lines near 700 nm wavelength are again an artifact due to back scattered laser light.) Analogous lines were not seen at any healthy site during ablation. Clarke *et al.* reported seeing similar sharp lines during ablation [13] and Prince *et al.* found similar lines in the ablation plume when using a different laser source [18]. These groups attribute the phenomenon to calcium emissions from the ablated plaque material.

Using the dual fiber collection scheme with the collection fiber repositioned parallel to the sample surface and close to the ablation site, it was possible to collect an emission spectrum exclusively from the plume, shown in Figure 5B. Arterial plaque is known to contain phosphate salts of magnesium in addition to calcium compounds [19]. Many of the lines seen can be associated with ionic calcium transitions [20], as is done in Table I.

Since the sharp lines only appear during plasma formation in calcified tissues, plume

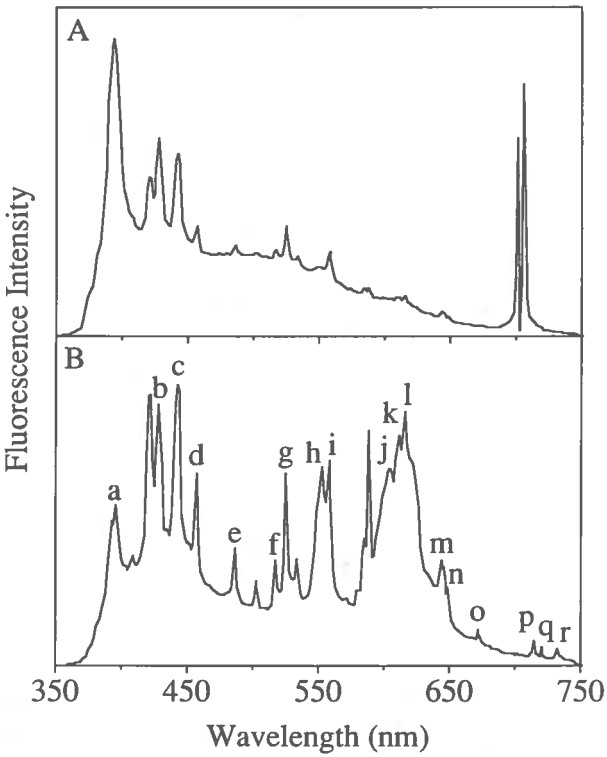

FIGURE 5 A: Spectrum obtained at calcified site during ablation in the air. B: Fluorescence response form plume during ablation at same site. Discrete lines in the fluorescence response were seen only at calcified sites and only during ablative plume formation.

spectroscopy may have some utility as an in vitro method of determining with high spatial resolution the pathologic calcium deposition in tissue. Currently such procedures require electron microprobe or atomic absorption analysis. Moreover, this would also be a very useful real-time indicator of plaque ablation during a recanalization procedure in vivo. The effect could not be reproduced with samples submerged in saline. The initial pulse fluence (1 J cm^{-2}) was not sufficient to any cause significant ablation in the aqueous environment. Increasing the pulse fluence (10 J cm^{-2}) did cause ablation and the occurrence of a small flash believed to be a plasma, but did not evoke sharp lines in the spectral profile. This was presumably due to quenching of the discrete emissions in solution. The deleterious effect of saline submersion was also seen by Clarke *et al.* Considerably higher energy pulses would perhaps produce the lines seen in air, through the formation of a dynamic cavity at the ablation site, but these were not attainable with the experimental system used.

IV. SUMMARY

		Known Emissions	
Peak	λ (nm)	λ (nm)	Element
a	395.7	395.7	Ca (I)
b	428.4	428.3	Ca (I)
c	442.1	442.5	Ca (I)
d	456.8	457.1	Mg (I)
e	486.8	486.4	P (II)
f	517.3	517.2	Mg (I)
g	525.2	525.4	P (II)
h	553.0	552.8	Mg (I)
i	558.2	558.3	P (II)
j	604.1	604.3	P (II)
k	611.9	612.2	Ca (I)
l	616.1	616.2	Ca (I)
m	643.6	643.9	Ca (I)
n	649.2	649.4	Ca (I)
o	671.4	671.8	Ca (I)
p	714.5	714.8	Ca (I)
q	720.1	720.2	Ca (I)
r	732.3	732.6	Ca (I)

deviations from normal. The diagnosis can be made with either a dual or single fiber scheme. The fluorescence response is unaffected by arterial immersion in saline. Spectra taken after ablation into normal tissue differ slightly from those taken at the endothelial surface, and closely resemble spectra taken at diseased sites after ablative penetration of the calcified plaque. Spectra taken at calcified sites during the ablation process in air reveal sharp spikes not seen with ablation of noncalcified tissue. These are attributed to calcium and magnesium salt components in the ablation plasma. With further refinement, excimer laser fluorescence can be a useful tool in diagnosing arteriosclerosis and monitoring the progress of ablative vessel recanalization.

Acknowledgments

This research was supported by the National Institutes of Health under grants numbered 1 RO1 HL 36894-01 and F 32-HL 0730801. The authors also gratefully thank Diaguide Corporation for their assistance in supplying the UV-grade optical fibers used.

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