Remote Biomedical Spectroscopic Imaging of Human Artery Wall

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We discuss a general technique, laser spectroscopic imaging (LSI), remote acquisition of spectroscopic images of biological tissues and tissue conditions. The technique employs laser-induced spectroscopic signals, collected and transmitted via an array of optical fibers, to produce discrete pixels of information from which a map or image of a desired tissue characteristic is constructed. We describe a prototype LSI catheter that produces spectral images of the interior of human arteries for diagnosis of atherosclerosis. The diagnostic is based on the fact that normal artery wall and atherosclerotic plaque exhibit distinct fluorescence spectra in the 500–550 nm range when excited by 476-nm laser light; the fluorescence from blood is minimal. The catheter is composed of 19 optical fibers enclosed in a transparent, protective shield. Argon ion laser radiation is used for excitation, and an optical multichannel spectral analyzer is used for detection. Sequential sampling is used to minimize crosstalk among fibers and reduce blurring of the image. Computer-processed 19-pixel spectroscopic images are produced of fresh cadaver artery in vitro. Regions of normal tissue, plaque, and blood are identified, and the diagnoses are confirmed histologically and by direct spatial correlation. The results demonstrate the concept of using this laser catheter system for real-time imaging.

Key words: spectroscopy, imaging, laser, atherosclerosis

INTRODUCTION

Endoscopes and catheters with coherent optical fiber bundles have significantly improved the physician’s ability to view inaccessible parts of the body. As useful as this is, the resulting visual images, which result from surface reflectance of light, are limited in sensitivity, discernibility, wavelength range, and time response. We wish to discuss a different type of remote diagnostic imaging, which we term laser spectroscopic imaging (LSI), in which the images are constructed from optical spectroscopic signals rather than conventional reflected light. We present first results of a prototype LSI device, a multifiber laser catheter for imaging the arterial lumen, which uses laser-induced fluorescence (LIF) as a diagnostic probe and a sequential mapping process that quantitatively records the spectroscopic information in the field of view of each optical fiber. This approach has advantages compared to conventional angiographic viewing. The image provides a direct display of the distribution of normal and diseased tissue. Detection is electronic, and sensitivity can approach the quantum limit. Data can be acquired in times ranging from seconds to microseconds, and the quantitative spectra recorded are amenable to fast computer processing. Also, this capability for rapid quantitative "spectral diagnosis"[1,2] of tissue type and condition permits real-time feedback control of treatment processes. All these features are addressed in this paper.
tures apply to LSI in general and should allow a wide variety of tissue types and conditions, both physical and chemical, generally not visible to the eye, to be quantitatively mapped in real time.

Optical techniques for mapping and image formation are well established in such diverse fields as solar and planetary astronomy, aerial reconnaissance, industrial automation, and medicine [3-5]. Reflected light, thermal radiation, and spectral signals all have been employed. A relevant example is the work of Svanberg et al [6-8], in which multiple color filters are used to produce digitized or TV images displaying spectral information. Sartori et al [9] show an imaging technique whereby arterial samples are mechanically translated relative to the sensing fiber. The present work employs a related technique in which data are acquired via a multiple fiber array in which each fiber represents a single picture element (pixel), and mapping is performed sequentially.

In the LSI technique, spectra from many small neighboring sections or "fields" of an in situ tissue specimen are collected and analyzed, and the resulting information is displayed as a map. Spectra are remotely acquired via an array of optical fibers housed in a catheter, needle, or cannula, arranged so as to produce small spots of light, which illuminate adjacent fields of the specimen. Spectroscopic signals are obtained by transmitting laser light down individual fibers and analyzing the return light emitted from the tissue fields, all under computer control. This information is then assembled into a spectroscopic map or "image," with the location of each pixel determined by the position of the corresponding tissue field. The computer rapidly compares the data with known spectra to yield a spectral diagnosis for each pixel. The resulting LSI image describes the spatial distribution of tissue type, disease, or condition at the distal end of the device.

Since the display shows the pixels of diagnostic information in their correct relative location corresponding to the tissue surface contacting the distal end of the laser catheter, the term "spectroscopic image" has been chosen. This is of course not what a person would see by direct vision, as, for example, a fluorescent peak ratio cannot be "seen." The computer-processed data is displayed as a shaded (or perhaps false-colored) representation of tissue type, which may be quite unrelated to visual appearance. Any number of pixels may be used to form a spectroscopic image without changing the principles described here.

Unlike images seen with an endoscope or angioscope, where all fibers are viewed simultaneously, in LSI it is generally advantageous to illuminate and collect light from one fiber (or group of fibers) at a time. This sequential sampling minimizes the effects of light scattering, which is dominant in tissue at most optical wavelengths and tends to degrade the spectroscopic image. For simultaneous sampling, scattering causes diagnostic light emitted by a given illuminated field to enter other fields, thereby delivering erroneous diagnostic information to the receiving fibers. This crosstalk introduces uncertainty and error into diagnosis of individual tissue fields, and hence "blurring" of the overall spectroscopic image results. But with sequential sampling, crosstalk is reduced, since the incident light must then be scattered from its original field into another field, converted to diagnostic light there, and must then find its way back to the original field and be scattered up into the fiber, a less likely series of events. With computers, sequential sampling is fast and straightforward. A computer-controlled translator or beam-steering element can align fiber input ends in a fraction of a second, and an optical multichannel analyzer can acquire a spectrum equally rapidly. Once the spectra are collected, the computer can quickly analyze them. Sequentially sampled LSI images and diagnostic maps can thus be made in real time.

LSI is also ideally suited for combining treatment with diagnosis. The same device that produces a diagnostic image of a tissue specimen can also be used to treat it, for example, by delivering higher-power "therapeutic" light for ablating individual tissue fields. Compared to conventional endoscopy, there is no need to manipulate the treatment fiber nor to correlate separately the tissue field to be treated with the area viewed by an endoscope. For a given fiber, the diagnosed and treated tissue field are one and the same.

We demonstrate the use of LSI by creating spectroscopic images of the interior lumens of arteries to diagnose the presence of atherosclerosis. The diagnostic, LIF, is based on our discovery that normal artery wall and atherosclerotic plaque exhibit distinct fluorescence spectra in the 500- to 650-nm spectral range when excited by blue-green light [1].

In order to illustrate the LSI technique simply, we have limited the tissue types considered here to normal artery wall, early atherosclerotic plaque, and blood. However, studies in our laboratory have shown that it is also possible to differ-
entitate normal artery wall and complicated atherosclerotic plaque spectroscopically using LIP. [10] Others have shown that calcified plaque can be identified using spectroscopic techniques; [11] this could also be incorporated in an LSI system.

We have recently demonstrated that such spectra can be acquired with good signal-to-noise ratio using optical fibers to both transmit 476-nm exciting laser light to the tissue field and to collect the return fluorescence [12], a necessary step in implementing spectroscopic imaging.

The experiments used a flexible 2.5-mm-diameter catheter containing 19 glass optical fibers with a 105-μm core and a numerical aperture of 0.29 [13]. At the proximal end, the fibers were arranged in a line and mounted on a computer-controlled linear translator, and a 10× microscope objective focused the exciting laser beam onto a fiber input face. This arrangement permits rapid alignment of a chosen fiber. At the distal tip, the fibers were arranged at skew angles in a hexagonal close-packed array consisting of a center fiber surrounded by concentric rings of six and 12 fibers and enclosed in a transparent hemispherical quartz shield. The light spots on the output face of the shield were approximately 550 μm in diameter, and the fibers were positioned so that the spots covered the entire output face (inset, Fig. 1). Spectroscopic images were taken with the shield placed in contact with the tissue. The contact pressure distorts the tissue and brings it flush with the shield surface. Only tissue in contact with the shield is sampled because other areas are blocked with intervening blood. Even blood layers a fraction of a millimeter thick attenuate the signal to a negligible strength, as will be discussed below. Since the optical fiber is fixed in place inside the shield, it has an illumination spot and a field of view on the tissue that are well determined (in

![Diagram of catheter imaging system](image-url)

Fig. 1. Schematic diagram of catheter imaging system for diagnosing atherosclerosis.
this case 550 µm in diameter). With the geometry fully defined, intensity measurements are reproducible. Additional details about the laser catheter are given in reference [13].

Information was collected using sequential sampling, as described above. Argon ion laser light at 476 nm was directed onto a 10% reflective beamsplitter and focused with a microscope objective into the proximal end of the selected optical fiber (Fig. 1). Accurate positioning was required to minimize unwanted fiber fluorescence. Return fluorescence from the same fiber was collimated at the proximal end by the microscope objective, directed through the 90% transmissive beamsplitter and through filters to remove the scattered 476-nm laser beam, and focused into the core of a second optical fiber. The output end of this fiber was placed at the entrance slit of a spectrophotograph selected to analyze 450- to 700-nm light, which in turn was coupled to the intensifier of an optical multichannel spectral detector (OMSD). The signals were acquired on a small, dedicated microprocessor and transferred to a computer that processed and displayed each spectrum in the corresponding position of a 19-pixel map. The computer also controlled the optical fiber translator, sequentially illuminating the 19 tissue fields. The automated LSI mapping process required 5 minutes total, with about 2 minutes for microprocessor data acquisition and the rest of the time for data transfer and graphics display. The actual spectral sampling time for each field of view was 0.5 seconds.

For all spectra taken, tissue fields were illuminated with 640 µW/mm² of exciting light, delivered for an exposure time of 0.5 sec. Experiments in our laboratory show that this intensity-time combination is safely below that at which significant light-induced spectral alterations occur. Spectra were corrected for the nonuniform spectrometer response, using a calibrated tungsten filament lamp as a reference. Intensity variations in LIP response from fiber to fiber were less than 10%. Tissue samples were fresh cadaver aorta, ranging from normal to early uncomplicated atherosclerotic plaque. Blood was treated with ethylenediamine tetraacetic acid (EDTA) to prevent coagulation. For each image taken, the positions of the light spots on the tissue surface were carefully noted. The specimen was then evaluated by gross tissue inspection over this area, followed by histological examination. Histological sections were prepared by fixing the samples in formalin, embedding them on edge, sectioning them in a plane normal to the intimal surface, and staining them with hematoxylin and eosin.

Figure 2 shows fluorescence spectra of normal artery wall, early atherosclerotic plaque, and blood obtained from a single fiber; this provides a key for interpreting the images that follow. The three constituents are easily distinguished: the peak fluorescence intensity of normal tissue is about three times larger than for plaque, and its structure is more pronounced.

Studies in our laboratory have shown that although there is some sample-to-sample variability in the tissue peak fluorescence intensity, I maks and the peak-to-valley ratio, R = I(600)/I(550), in most cases these values are sufficient to differentiate between normal artery wall and early ath-

![Fig. 2. Characteristic fluorescence of normal artery wall, plaque, and blood excited with 476 nm light. The histology is the same as in Figure 3a.](image)

![Fig. 3. Test tissue configuration. a: Spectra. b: Geometry employed. c: LSI image (see key of Fig. 3). d: Sectioning diagram. e: Histological sections. The R-N section indicates normal tissue with a somewhat thickened intima; the P-P section indicates the presence of an early atherosclerotic lesion.](image)
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Figure 3

Spectra (a)

Orientation (b)

Image (c)

Top View (d)

Histology (e)
erotic plaque. For 38 total samples studied, we found that in normal tissue, $I_{\text{max}} = 844 \pm 258$ and $R = 1.10 \pm 0.09$; and in early atherosclerotic plaque, $I_{\text{max}} = 321 \pm 135$ and $R = 1.04 \pm 0.06$.

The characteristic normal artery wall fluorescence line shape of these cadaver samples can be interpreted as a broadband fluorescence with valleys created by reabsorption at certain wavelengths. These wavelengths match those of oxygenated hemoglobin absorption; however, they are also consistent with other metalloporphyrin complexes [14]. At the level of sensitivity of the imaging system no blood fluorescence was detected, so a tissue field filled with blood yielded a zero signal. Histological sections of the artery samples used are shown in Figure 3e.

Spectroscopic images were acquired for two configurations. Figure 3a shows the spectra and image produced by segments of normal and diseased tissue placed side by side in a petri dish and immersed in blood. The flat geometry enabled direct comparison of the image with the tissue in contact with the catheter. The catheter was positioned at an angle of 45 degrees to the surface of the tissue, with the tip in contact at the boundary and roughly half of it exposed to blood (Fig. 3b). Using Figure 2, pixels corresponding to a field consisting primarily of one tissue type can be readily identified as plaque, normal artery, or blood. However, when a field of view includes more than one tissue type, each contributes to the spectrum observed. The boundary between tissue types was determined by computing the relative fraction of the contributed spectrum using a linear combination algorithm, as described below; and alignment was estimated by comparison with the spectrum of adjacent pixels. The resulting image (Fig. 3c) clearly displays all three constituents in their respective positions.

The relative fraction estimate was based on the observed fluorescence intensities of the peaks at 520 and 600 nm and the valley at 550 nm. These three values can be expressed as a linear combination of characteristic intensities at these wavelengths for each tissue type. The coefficients give the relative portions of each tissue type in the field. If the field of view includes blood, which does not fluoresce, it absorbs so strongly (the penetration depth at 476 nm is about 40 $\mu$m [15]) that tissue beneath the blood does not contribute significantly to the spectrum. Therefore, the total intensity of the returning fluorescence is reduced in proportion to the fraction of the field of view occupied by blood.

The equations used to determine the relative fraction estimate are derived below. The variables used in the derivation are defined as follows: $H$ = intensity at 520 nm; $V$ = intensity at 550 nm; $T$ = intensity at 600 nm; $N$ = % healthy tissue; $P$ = % plaque; $B$ = % blood; $H_p$, $V_p$, $T_p$ = the characteristic intensities for normal tissue; and $H_p$, $V_p$, $T_p$ = the characteristic intensities for plaque.

Since blood has negligible fluorescence, it does not contribute to the signal but only attenuates it. The linear combination can be expressed as follows: $H = (H_p X N) + (H_p X P)$; $T = (T_p X N) + (T_p X P)$; $V = (V_p X N) + (V_p X P)$; and $N + B + P = 1$.

Solving for $N$, $P$, and $B$, we get

$$N = \frac{V(V_p X V)}{V_p}$

$$P = \frac{V(V_p X V)}{V_p}$

and

$$B = 1 - N - P.$$

The spectroscopic diagnosis of the distribution of normal tissue and plaque of Figure 3e was checked histologically. Tissue of each sample was sectioned along a line parallel and within 100 $\mu$m of the edge that had been in contact with the other sample (Fig. 3d). The resulting histological section is shown in Figure 3e. Comparison of Figure 3e and d confirms that the spectroscopic image gives accurate locations of the tissue fields in contact with the tip of the catheter.

A second experiment was performed to test the precision of the spectroscopic mapping process with the catheter inserted into an artery. In order to insure a known, well-defined distribution of tissue types, the blood vessel was simulated by assembling sections of diseased and normal artery wall around the catheter tip. The catheter was immersed in a blood field, as indicated in Figure 4.
4b. The resulting image (Fig. 4c) depicts a blood-filled lumen surrounded by areas of plaque and normal arterial wall. The three tissue types are clearly discernible, indicating that the catheter performs correctly in a configuration of the type to be encountered in situ. Histological evaluation was used to confirm the spectroscopic diagnosis of Figure 4c. This was accomplished by laying the tissue samples flat and obtaining microtome sections along the line corresponding to the position at which the tissue was in contact with the circumference of the catheter output shield (Figure 4d,e). The resulting histological sections are shown in Figure 4f. Comparison of Figure 4c and e again confirms that the spectroscopic image gives an accurate indication of distribution of the three tissue types at the catheter tip.

In conclusion, generation of a digitized spectroscopic image through an optical fiber catheter has been demonstrated. Each fiber views a different field of the specimen, and the image is composed of pixels corresponding to spectra from discrete fields, taken one at a time and assembled into a diagnostic map. This spectroscopic image is not the same as what would be viewed with an angiogram. In a conventional image, the intensity at each wavelength is determined by surface reflectance, and information is in the form of changes in the white light background of the incident beam. In contrast, a spectroscopic image is produced by the fluorescence response of the tissue. The signals are composed of wavelengths not present in the exciting light and are thus obtained on essentially zero background. They are therefore easy to detect, even if weak.

LSI mapping lends itself to a number of variations. Only one spectral diagnostic is utilized here, as this work is a study of the imaging process. But several LIF medical diagnostics have been discussed in the literature [1,6,7,16-28], and this system would be compatible with any of them that utilizes or can be adapted to optical fiber transmission. Biomedical mapping should also be compatible with the ultraviolet [22-27] and infrared [29] spectral diagnostics. It should also be amenable to Raman spectroscopy [28,30], which is sensitive to the more discrete vibrational "signature" of molecules, in contrast to the broader electronic spectra of LIF. Time-resolved spectroscopy [31,32] also can provide diagnostic information. Since such signals can be collected by optical fibers, this diagnostic could also provide a spectroscopic image, utilizing the dimension of time instead of wavelength. As a fluorescence decay constant cannot be "seen" but can be measured, the mapping process could allow an image to be viewed that is quite unlike that of a conventional endoscope image. Acoustic detection can be used to detect absorbing species that do not generate fluorescence [33].

Image acquisition time can be rapid and is not an intrinsic limitation; the current time, 5 minutes, was largely consumed by slow computer processing. Once this bottleneck is eliminated, the data acquisition rate in the present system will be limited by fiber alignment and shuttering, which takes about 200 msec; replacing the translator and shutter with an acousto-optic deflector and switch should reduce this to several μsec. Beyond this, the next limiting component is the OMSD, which has a minimum scan time of 20 μsec per pixel. Since only a few points on the spectrum need be taken to make a spectral diagnosis, the OMSD can be replaced with several photomultipliers and narrowband optical filters connected to DA converters, further reducing data collection and transfer time to a fraction of a millisecond per fiber. Clearly, with appropriate modifications real-time data acquisition is feasible.

The 19 pixel arrangement of the current system can be readily modified. More optical fibers can be added to enlarge the mapped area or increase resolution. Variable resolution can be achieved by illuminating the fibers in groups; coherent optic fiber bundles can be used in this case. If high-resolution, large-area spectral images are needed, requiring many optical fibers, then acousto-optic deflectors can be used to switch the laser beam among the fibers on a sub-microsecond time scale. Individual spectra from a single 10-nsec pulse have been acquired in our laboratory. In most practical applications resolution will be limited by scattering, as indicated earlier. Aside from scattering, the practical resolution limit is of the order of the dimension of a single mode optical fiber, about 5 μm. These smaller fibers would have the added benefit of being very flexible.

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