Diagnostic Tissue Spectroscopy and Its Applications to Gastrointestinal Endoscopy

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Optical spectroscopic methods are playing an increasingly important role in medicine, and gastrointestinal endoscopy is likely to be one of the most important areas of impact of the new technology. This article will familiarize endoscopists with basic technologies of diagnostic spectroscopies, discuss the types of information they provide, and describe how they can be incorporated in endoscopic instruments. The discussion focuses on 4 fundamental light-tissue interactions: elastic scattering, absorption, fluorescence, and Raman scattering. The physical basis of each technique and its mode of implementation in endoscopic applications are presented. We discuss the limitations and advantages of each technique, as well as research that uses spectroscopic approaches for the diagnosis of gastrointestinal disease. Finally, we discuss future directions in spectral diagnostic endoscopy.

The concept of the endoscope dates back almost 200 years. In 1805, Philip Bozzini first introduced the "lichtleiter," a rigid device for viewing the inner surfaces of the body. Although the concept of the instrument was sound, the technology did not exist to render it clinically viable. The device needed to be flexible enough to bend through the oral cavity into the esophagus, yet maintain its structural integrity for guidance. In addition, light sources at the time were limited to candlelight, not bright enough to properly illuminate the inner cavities of the body. It wasn't until the early 1930s, when Rudolf Schindler produced the semi-flexible Wolf-Schindler gastroscope, that the first useful images of the upper gastrointestinal (GI) tract were obtained. Although this was a great improvement, the device was difficult to manipulate and could only provide partial images of the stomach because of its lack of flexibility. The next major advance came in 1954 when Basil Hirshowitz at the University of Michigan read 2 important articles in the journal *Nature* on imaging with coherent fiber optic bundles.[1,2] Hirshowitz, realizing the importance of a technology capable of transmitting images through a flexible medium, immediately visited the British scientists responsible for the articles; however, he soon realized that given the current state of the technology, optical fibers could not transmit enough light to produce a reasonable image. Instead, with the help of his colleagues at the University of Michigan, he discovered that cladding (an insulating coat of an appropriate type of glass around the core optical fiber) could be used to transmit enough light through the fiber to produce a reasonably bright image. Finally, gastroenterologists were able to see parts of the body previously not accessible, and the endoscope evolved as a standard diagnostic tool in gastroenterology. The next major advance came with the advent of charged coupled device (CCD) imaging detectors by Bell Laboratories scientists Willard S. Boyle and George E. Smith in 1969. CCD technology developed quickly within the next decade, and the first video endoscope was made around 1980 by Welch Allyn Inc (Skaneateles Falls, NY). This new type of endoscope was pioneered by gastroenterologists Sivak and Fleischer at the Cleveland Clinic Foundation in the early 1980s.[3] The video endoscope allowed for "TV imaging" of the inner surfaces of the body. Instead of the physician bent over the eyepiece of the fiberscope, the physician and patient could view images on a monitor together. Images could also be recorded and processed digitally. Although these electronic endoscopes were more expensive, they eventually overtook the standard fiber optic based endoscopes; by the 1990s virtually all large diameter endoscopes were electronic (CCD based). Other improvements have lead to the reduction in size, increased resolution and magnification. Modern endoscopes also incorporate channels to pump air and water, deliver drugs, as well as to introduce and manipulate biopsy forceps and needles.

Although endoscopic technology has matured so that gastroenterologists are able to visualize and record high resolution images of the GI tract (and other internal parts of the body), there are still significant difficulties, particularly in regard to the diagnostic capabilities of current endoscopes. Many lesions are invisible and difficult to visualize with standard white light endoscopy. Random biopsy is still required in many surveillance applications. Even when lesions are visible, they must be removed from the body in order for a pathologist to make a final diagnosis, and there is a significant time delay (typically one day) between the removal of the tissue sample and its histological analysis. Perhaps, the next great stride that can be made for the endoscope is to advance its capability to visualize otherwise invisible lesions and hence, further improve its diagnostic potential.

Spectroscopic techniques offer the potential of such a technological advance. These techniques have the potential to make the invisible visible. They have been shown to provide detailed morphological and biochemical information about lesions

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without tissue removal, thus providing a "spectral diagnosis." They do not require biopsy, nor the fixation, processing and microscopic examination of tissue used by pathology. The weak beams of light used are noninvasive and do not alter the tissue in any way. Information can be provided in real time and in an objective and quantified manner.

Spectroscopic techniques can be incorporated in endoscopy in 2 general ways: contact probe and wide-field imaging. Contact probe measurements use a fiber optic probe to deliver and collect light from a small volume of tissue (~ 1 mm^3). This probe can be introduced into the biopsy channel of the endoscope and brought into gentle contact with the tissue. A light source, either white light or monochromatic light, is coupled into the fiber for delivery to the tissue. Collected light returning through one or more optical fibers is directed into a spectrograph, where it can be spectrally analyzed outside the body. Wide-field imaging applications generally illuminate a wide area of tissue (~ 1 cm^2) with a spectrally modified light source (ie, the incident light is made up of a narrow band of colors). The collected light is then imaged using a CCD. Thus, spectral information in wide-field imaging can be achieved through the light source.

Contact probe techniques have the advantage of providing detailed chemical and morphological information about the state of the tissue. Wide-field imaging, on the other hand, can create maps of disease entities for wide area surveillance, but generally lacks the detailed information needed for accurate diagnosis. The ideal spectral endoscopic system would incorporate the capability for wide area surveillance with the detailed information provided by a contact probe technique. Broad areas of tissue could be spectroscopically imaged, and suspect areas could then be studied in detail with a contact spectral probe. While emerging spectroscopic technologies show promise for reaching this end, none currently incorporates the capabilities of a contact probe and wide field imaging in one instrument.

These emerging spectroscopic technologies are based on the principles of light-tissue interactions. This article describes the principles of diagnostic tissue spectroscopy and the accomplishments of spectroscopy in diagnosing GI disease to date. We give examples of the state of the field at present and the directions in which it is likely to go. We describe the potential of "spectral endoscopy" and envision the endoscope of the future.

Light-Tissue Interactions in Diagnostic Tissue Spectroscopy

Light can interact with biological tissues in numerous ways. These interactions modify the characteristics of the re-emitted light, which can be measured to provide information about the tissue. One important measurable quantity of light is the wavelength, λ (where λ is measured in nanometers, nm, and one nanometer is one billionth of a meter). The wavelength describes the color of the light, so that blue light has wavelengths of approximately 450 nm, green light 520 nm, and red light 650 nm. These wavelengths make up the visible portion of the light spectrum. Ultraviolet (UV) and infrared (IR) light, not visible to the human eye, have wavelengths less than blue and greater than red light, respectively. Measuring the intensity of the re-emitted light as a function of its wavelength is the basis of spectral analysis.

Although it is not essential in understanding laser-tissue interactions to consider light as being made up of photons, the photon picture is sometimes useful. The energy of a photon is proportional to the frequency of the light wave, f, or inversely proportional to its wavelength ($f = c/\lambda$; c = speed of light). Photons of a shorter wavelength (higher frequency) have more energy than those of longer wavelengths (lower frequency). As light interacts with tissue structures, part or all of their energy may be transferred to the tissue, changing its energy (or wavelength) of the re-emitted photons.

Figure 1 illustrates a light ray incident on the tissue surface. A portion of the light will be reflected. The remainder will be transmitted into the tissue, where it may undergo one of various fundamental interactions. Here we consider 4 interactions most important for diagnostic tissue spectroscopy: 1) Elastic scattering (single scattering and diffuse scattering), 2) Absorption, 3) Fluorescence, and 4) Raman scattering. All of these interactions are dependent on the structural and biochemical composition of the tissue. The next 4 subsections describe these interactions in detail.

Elastic Scattering

An elastic scattering event redirects an incident light ray without changing its wavelength. Scattering in tissue can be caused by both cellular and extracellular constituents: cells, their nuclei, mitochondria, and collagen fibers. A light ray "sees" discrete structures within tissue as variations in refractive index.

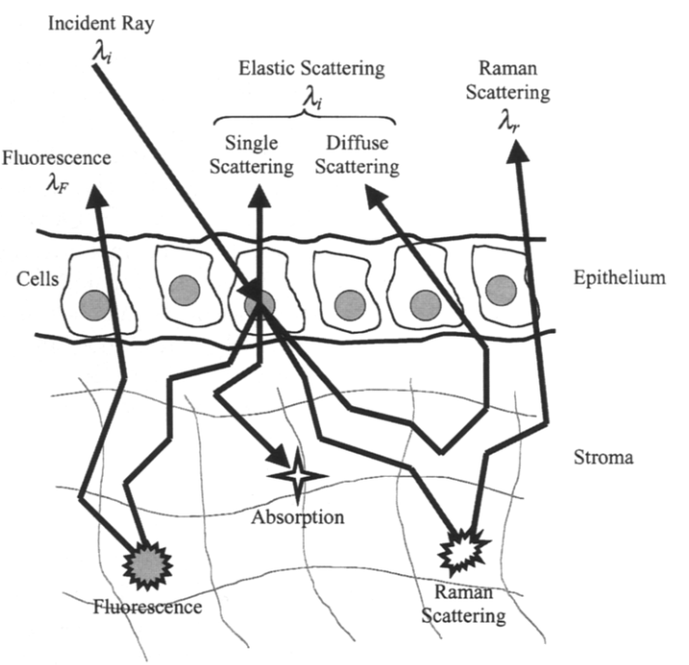

Fig 1. Fundamental light-tissue interactions. An incident ray that is transmitted into the tissue can interact with molecules in several different ways. The light may be elastically scattered, emerging with the same wavelength as the incident beam and providing information about the morphological structure of the scatterers in the tissue. It may be absorbed and either converted into internal energy or re-emitted as fluorescence. Monitoring this interaction provides chemical information about the chromophores and fluorophores in the tissue. The light can also undergo Raman scattering, transferring part of its energy to the scattering molecule and emerging with a longer wavelength (lower frequency). Raman scattering provides detailed molecular information about the tissue.

The extent to which light is scattered is a function of the density of particles, the particle size, and the ratio of the refractive indices of the particles relative to the medium. Thus, the scattered light provides information about structures within the medium, predominantly those structures that have the highest refractive index mismatch relative to their surroundings. In the case of tissue, important scattering structures are nuclei and mitochondria[4]; however, with the complex distribution of structures (refractive index variations) present, many structures ultimately contribute to the scattered light.[5]

Elastically scattered light emerges at the tissue surface after being scattered either once (single scattering) or many times (multiple scattering). Single scattered light results largely from scattering from the cell nuclei near the tissue surface (ie, within the epithelium). Multiple scattering is the predominate form of elastic scattering, and results from photons undergoing many scattering events within the tissue. Light emerging from the tissue surface that has been scattered multiple times is said to be diffusely reflected. Multiple scattering is the dominant light-tissue interaction, and it is responsible for the "turbid" appearance of biological tissue. Typically, under the influence of multiple scattering, light can penetrate several millimeters into the tissue. Thus, diffusely reflected light generally contains information about scattering structures deeper with the tissue (eg, collagen fibers within the stroma) than does singly-scattered light. The overall intensity of elastically scattered light diminishes with increasing depth. This is similar to the decrease in the intensity of sunlight as it travels through a thick layer of clouds on a foggy day.

Absorption

The intensity of this scattered light can be further reduced due to the presence of absorption. Molecules and atoms can absorb incident light, converting the light's energy into internal energy and ultimately heat. The energy of visible and UV light can elevate the electrons in molecules and atoms to excited (higher) energy states. These energy states exist as discrete levels, and can be excited only by photons of a frequency that corresponds to that energy level (Fig 2). The structure of the molecule determines which incident frequencies will be absorbed. For example, hemoglobin strongly absorbs blue light. Thus, the blue component will be removed from a beam of white light diffusely reflected from tissue containing hemoglobin, resulting in its characteristic red color.

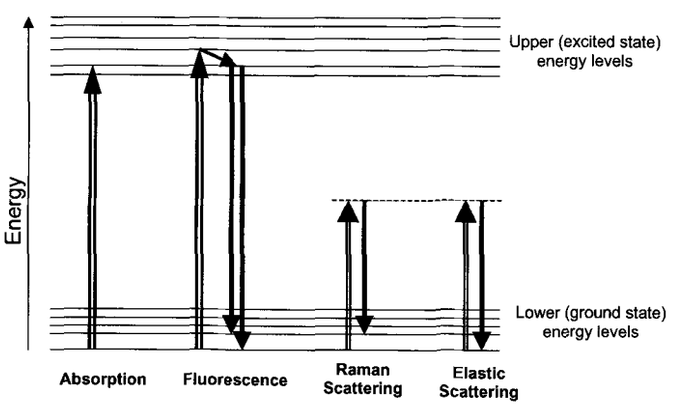

Fig 2. Energy level diagram of the fundamental light-tissue interactions. The lower energy levels represent the ground electronic state, while the upper levels represent the first excited electronic state. Within the ground and excited electronic states are vibrational and rotational energy levels. The open arrows represent the incident light, and the solid arrows the re-emitted light.

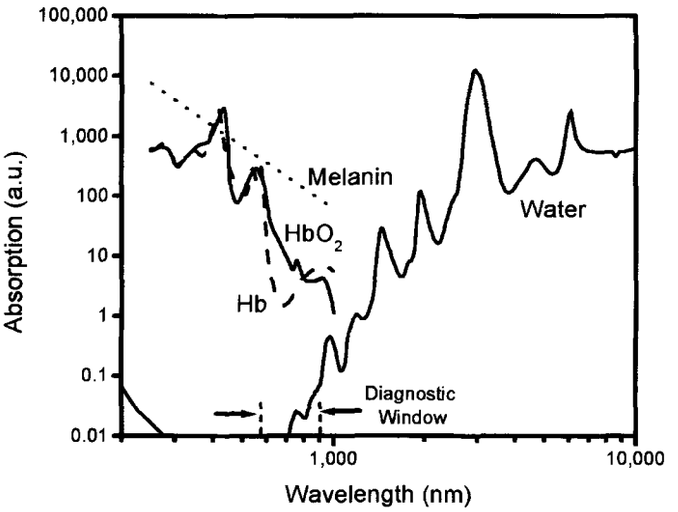

Fig 3. Absorption spectra for primary tissue chromophores (melanin,[59] oxyhemoglobin,[60] deoxyhemoglobin[60] and water[61]). Light can penetrate the tissue as deep as 1 cm in the diagnostic window (approximately 600-800 nm) due to the low absorption within that wavelength region. Water dominates tissue absorption within the infrared wavelength region (> 1000 nm), while hemoglobin and melanin dominate absorption within the visible region (300-600 nm).

The absorption spectrum of tissue depends on the type of predominant absorbing molecules or chromophores and therefore, provides information about its chemical composition. Figure 3 illustrates the absorption spectra of several tissue absorbers in the UV, visible and IR wavelength regions. Water is a strong tissue absorber in both the UV and IR regions. DNA and proteins absorb in the UV region, while hemoglobin, melanin, and β-carotene absorb in the visible region. Other tissue chromophores include porphyrin-like structures such as bilirubin, myoglobin, cytochrome c oxidase and protoporphyrin. Light is readily absorbed in the UV and infrared regions and thus, is able to penetrate only one or two cell layers deep into the tissue. Visible light of short wavelengths typically penetrates to a depth of 0.5 to 2.5 mm.[6] In the wavelength region of 600 to 1500 nm, known as the diagnostic window, scattering prevails over absorption, and light can penetrate as deep as 8 to 10 mm.[6]

Absorption-based techniques are widely used for the purpose of evaluating tissue oxygenation. For example, hemoglobin absorption spectra change with oxygenation. Oxyhemoglobin has a strong absorption peak in the blue region at 415 nm and a smaller double peak at 542 nm and 577 nm (Fig 3), while deoxyhemoglobin has a strong peak at 430 nm and a single peak at 560 nm (Fig 3). The pulse oxymeter is based on measuring these spectral changes in absorption of hemoglobin with various degrees of oxygenation.[7]

Fluorescence

In many cases light absorbed by a molecule is converted into heat; however, an absorbed photon can also be re-emitted as fluorescence. Fluorescence emission typically occurs at a lower energy (longer wavelength) than the absorption energy of the incident photons (Fig 3). The difference in wavelength between

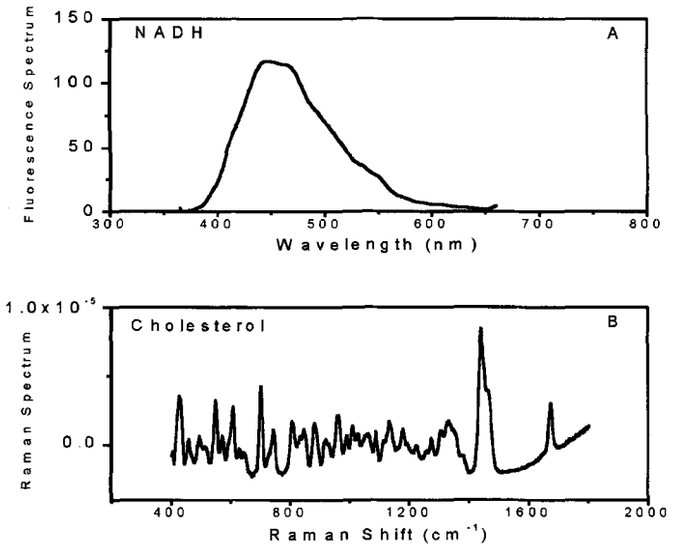

Fig 4. (A) Fluorescence emission spectrum of NADH, excited at 347 nm. (B) Raman spectrum of cholesterol. Notice the broad spectrum of the tissue fluorophore, whereas the Raman spectra contains narrow peaks. Note also the intensity scales for Raman scattering is normally orders of magnitude weaker than that for fluorescence.

the incident light and fluorescence is known as the Stokes shift. Fluorescence tends to be more dominant in the UV than in the IR.

Fluorescence spectra in tissue can be used to identify fluorophores and monitor their concentrations. Various tissue fluorophores include metabolic substances such as nicotinamide adenine dinucleotide (NADH) and flavins, aromatic amino acids such as tryptophan, tyrosine and phenylalanine, structural proteins such as collagen and elastin, several porphyrins, and lipids such as ceroid and lipofuscin. Each of these fluorophores has specific wavelengths for excitation and emission. Figure 4A shows the fluorescence emission spectrum of NADH excited with 347 nm light. Notice that the spectral width of the spectra is approximately 100 nm, typical of tissue fluorophores.

The fluorescence emitted by a molecule in tissue depends on the energy level difference between the electronic ground state and excited state and the distribution of vibrational and rotational levels within these states (Fig 2). These states can shift in the presence of different local environmental factors, such as pH, temperature, and oxygenation. In the progression of disease the local environment in which the fluorophores are embedded can change, as well as their relative concentrations, thus leading to changes in the observed fluorescence intensity and lineshape.

Most in vivo fluorescence spectroscopy studies to date have focused on monitoring the steady-state intensity of fluorescence emitted at one or more wavelengths (ie, the fluorescence induced by constant excitation light). An alternative approach measures the time-resolved fluorescence (the time decay of the fluorescence excited by a short pulse).[8] Typically, this time decay is on the order of nanoseconds. The method is technically more demanding than steady-state measurements, as it requires the use of a very short excitation pulse and special detectors. However, the time dependence of fluorescence emission can be very sensitive to changes in the environment of the fluorescent species. Furthermore, as time-resolved measurements are unaffected by tissue elastic scattering and absorption, they can provide diagnostic information that might otherwise be convoluted when measured with steady-state fluorescence. Another method of removing distortion effects due to absorption and scattering, known as intrinsic fluorescence spectroscopy, is discussed below.

Although there are many endogenous fluorophores (naturally occurring) within tissue, exogenous fluorophores (externally fabricated) can also be introduced to fluorescently label specific tissue constituents or processes. Exogenous fluorophores such as dyes can be tagged with ligands that bind to specific tissue proteins. Detection of the fluorescent dyes can then indicate the presence of specifically labeled proteins.[9]

Raman Scattering

Raman spectroscopy is a technique that measures the frequency shift and intensity of light inelastically scattered from molecules. A relatively new effect, it was discovered by the Indian physicist C. V. Raman in 1928.[10] As discussed earlier, in elastic scattering the energy of the scattered photon is the same as that of the incident photon (ie, the wavelength is unchanged); the light ray is merely redirected. In inelastic scattering, by contrast, a portion of the energy of the incident light is converted to internal energy of the scattering molecule. Hence, the energy of the scattered photon is reduced. In Raman scattering, a specific form of inelastic scattering important in probing biological tissue, the internal energy process is molecular vibration (and occasionally rotation). The incident wave sets the molecule into motion, and the energy of the scattered photons is reduced by this amount of energy. This change in energy manifests itself as a shift in frequency, which corresponds to the transfer of energy to or from the sample's vibrational or rotational modes. The intensity pattern of the scattered light produces a "fingerprint" of the scattering molecule. In the Raman effect, spectral information is encoded as frequency shifts in the scattered light; every molecule has its own distinct set of vibrations, hence its own characteristic frequency shifts. Raman frequency shifts are always the same, regardless of the wavelength of the incident light. This gives an important degree of flexibility in generating a Raman spectrum; UV, visible, or IR excitation light can all be used, and in each case the Raman fingerprints will be the same. The incident wavelength can thus be tailored to the specific application, in contrast to many spectroscopic techniques.

For biological samples, near infrared (NIR) radiation is typically used. The use of NIR excitation light confers 3 advantages to biomedical Raman spectroscopy: a reduction of background fluorescence, less laser-induced photothermal degradation, and deeper sampling depths. The large penetration depth in human tissue provides the opportunity to observe subsurface structures, regions that cannot be probed using conventional white light endoscopy. Further, eliminating thermal and photochemical damage while probing the maximum volume possible is critical for spectroscopic techniques used in medical procedures.

Because each molecule possesses a unique pattern of Raman shifts, the molecular composition of the tissue can be determined via Raman spectroscopy. Further, unlike fluorescence, reflectance, and absorption spectroscopies, Raman spectroscopy provides narrow spectral bands, with high information content, that can be assigned to specific molecular vibrations. Figure 4B shows these narrow bands in a Raman spectrum of

cholesterol. Furthermore, whereas there are relatively few endogenous biological fluorophores, many of whose spectra significantly overlap, there exist a multitude of Raman active chemicals in tissue; each has a unique spectral pattern and can be specifically related to healthy and diseased conditions. The data provide direct chemical information that is capable of characterizing a sample both qualitatively and quantitatively.

Despite its rich information content, Raman spectroscopy has not experienced as much clinical investigation as fluorescence. The Raman effect is weak. Typically only about one incident photon in 10 million is converted to a Raman-scattered photon. Hence, the Raman component can be easily overwhelmed by elastic scattering. Within the past few decades a variety of instrumental developments, primarily new lasers, detectors, and methods for rejecting elastically scattered light have expanded the utility of Raman spectroscopy for biomedical applications. Compact diode lasers can provide stable narrow-line excitation over a range of wavelengths in the NIR region. Silicon deep-depletion CCD detectors are best suited for tissue Raman spectroscopy because of their relatively high sensitivity and lack of fixed-pattern noise.[11] The application of holographic filter technology to Raman spectroscopy has also led to increased utilization of the technique in medical settings.[12]

Because the Raman effect is weak, the intense elastically scattered light must be removed so that it does not overwhelm the Raman signal. Holographic notch filters are devices for rejecting scattered light and are specific for the excitation wavelength used. These new technologies make rapid data acquisition as well as the design of compact, robust Raman instruments, needed for clinical applicability, a reality. However, in the past, the lack of availability of Raman spectral probes has severely limited clinical applications. Fortunately, this situation has changed (see discussion below).

Quasi-elastic light scattering[13] (also known as dynamic light scattering) is another example of inelastic scattering. In this process, the intensity of reflected light is studied as a function frequency or time. In quasi-elastic scattering of energy is conserved after a collision, but there is a shift in the frequency of the light due to differences in the direction of motion of the particles with respect to the detector (Doppler Shift).

Applications

The light-tissue interactions discussed above have been used to gain both structural and biochemical information about tissue. As discussed earlier, 2 types of technology can be used: contact probe and wide-field imaging. The following 2 subsections highlight some recent applications and their success in disease detection within the GI tract.

Contact Probe Applications

Contact probe applications use fiber optic probes for delivery and collection of light from tissue. These flexible probes, typically 1 mm in diameter, can be inserted into the biopsy channel of an endoscope, making them compatible with standard endoscope technology. The probe is brought into gentle contact with the surface of the tissue, and light is sampled from the volume surrounding the point of contact, typically around one cubic millimeter.

The technology to design and construct probes for the collection of in vivo fluorescence and scattering spectroscopic data is relatively well established.[14,15] A typical probe may consist of one optical fiber to deliver excitation light and one or more optical fibers to collect light and transport it to the detection system, such as a spectrograph. Single fibers are on the order of tens to hundreds of micrometers in diameter, roughly the size of a human hair. So, even probes consisting of 10 or more fibers can be manufactured with diameters less than one millimeter, which can pass through the accessory channel of the endoscope. These types of probes are used for implementing diffuse reflectance, fluorescence, and light scattering spectroscopies.

Diffuse reflectance spectroscopy (DRS) uses multiply scattered light emerging from the tissue to extract the morphological properties. The penetration depth of the diffusive light is 0.5 to 1.5 mm, and therefore, DRS predominately provides information about the morphology and absorption of the stroma. Diffuse reflectance spectra can be analyzed by using a physically based mathematical model to extract the hemoglobin concentration, oxygen saturation, average diameter, and density of scatterers. These physical parameters have been used to distinguish dysplasia in colon polyps[16] and Barrett's esophagus.[17]

Fluorescence spectroscopy can be used to extract biochemical properties. It is fast, easily implemented, and sensitive to small biochemical changes. It has been applied to detect atherosclerosis of the aorta and coronary arteries,[18] cervical dysplasia,[19-21] dysplasia in the bladder,[22-24] adenomatous colon polyps,[20,25] breast cancer,[26,27] dysplasia in the esophagus,[17,28] oral cavity dysplasia and cancer,[29-31] and dysplasia in the bronchi/lungs.[32]

Fluorescence photons are scattered and absorbed during their path to the tissue surface where they are collected via the optical fiber probe. Therefore, the spectral features of the collected fluorescence can be significantly distorted, making the extraction of biochemical composition of the tissue from the measured signal difficult. This is a particular problem in the presence of strong tissue absorbers such as hemoglobin. Intrinsic fluorescence spectroscopy (IFS) is a technique that extracts the fluorescence of the molecules unaffected by the absorption and scattering events from the bulk fluorescence.[31,33,34] Because diffuse reflectance undergoes similar absorption and scattering events as fluorescence, information contained within the reflectance spectra can be used to extract the intrinsic fluorescence from the collected fluorescence spectra. IFS has been used successfully in the GI tract to examine dysplasia in patients with Barrett's esophagus (BE).[34]

Light scattering spectroscopy (LSS) uses reflectance spectra to assess the morphology of the epithelium.[35] Eighty five percent of cancers originate in the highly cellular epithelium. The enlargement and crowding of cell nuclei is a primary indicator of dysplasia.[36] The spectral component of the singly backscattered light contains information on nuclear size distribution and density within the epithelium; however, singly backscattered light makes up only a small fraction (2%-5%) of the entire reflectance spectrum. The collected reflectance spectrum contains both diffuse reflectance (multiply scattered) and singly backscattered light. Extraction of the singly backscattered spectrum can be achieved by subtracting the diffuse reflectance fit[35] from the collected reflectance spectrum. The resulting singly backscattered spectrum exhibits an oscillatory behavior in wavelength (from blue to red light). The frequency of these oscillations corresponds to the nuclear size distribution within

the epithelium. LSS has been used to assess morphological changes in nuclei in patients with BE, showing a sensitivity and specificity for detecting dysplasia (all grades) of 90% and 90%, respectively.[37] LSS has also been used to determine grades of dysplasia in colon polyps.[38]

Recently, a new technique, tri-modal spectroscopy (TMS),[21,31,34] has been developed to combine 3 spectroscopic modalities (DRS, IFS, LSS) to determine biochemical, structural, and morphological information simultaneously to distinguish grades of dysplasia in patients with BE.[17] IFS is used to obtain the local contributions of fluorophores (eg, NADH and collagen). DRS provides information about the morphology and biochemistry of the stromal tissue. LSS determines nuclear size, density, and distribution of the epithelium. These spectral biomarkers represent biochemical and structural features, similar to those used by pathologists to make a diagnosis using histology, and just as with pathology, these spectral biomarkers can be used to make a diagnosis. TMS proved superior to any single spectroscopic technique alone (DRS, IFS, or LSS) in distinguishing grades of dysplasia in patients with BE. Of the 3 spectroscopic techniques, LSS was superior in separating dysplastic (low- and high-grade) from nondysplastic BE sites, whereas IFS was superior in distinguishing high-grade from low-grade and nondysplastic BE sites. However, the diagnoses were significantly improved by combining the results of all 3 modalities (TMS).

Another contact probe technique being explored is time-resolved fluorescence. Preliminary in vivo studies by Mycek et al[8] show a more rapid decay for adenomas than that from nonadenomas. The study used 10 ns pulses of 340 nm excitation light on a sample set of 17 patients with 24 polyps (13 adenomatous, 11 nonadenomatous). Although results established differences in the decay times of each tissue type, further investigations into the basis for these diagnoses are necessary, as fitting of the multiexponential decay curves to extract fluorophore lifetimes was not performed.

Several studies have investigated the utility of Raman spectroscopy in diagnosing GI disease. Most of these studies have focused on the examination of ex vivo tissues due to the lack of availability of Raman probes.[39] After showing the relevance of examining biopsied tissues, Shim et al[40] were able to differentiate intestinal metaplasia (BE) and dysplasia in esophageal biopsies with a sensitivity of 77% and a specificity of 93%. They next showed the feasibility of in vivo Raman spectroscopic point measurements of GI tissues, by using an early Raman probe, during routine clinical endoscopy.[40] Ex vivo studies by Stone et al[41] show the promise of Raman spectroscopy for discriminating several subclasses of esophageal cancer. Using statistical analysis, they were able to distinguish normal squamous epithelium, cardiac Barrett's, fundic Barrett's, intestinal metaplasia, high-grade dysplasia, adenocarcinoma, squamous dysplasia, and squamous cell carcinoma. Studies have also assessed the utility of Raman spectroscopy for the examination of stomach cancer. Ling et al[42] found differences in Raman peak position, relative intensity, and linewidth between normal and malignant stomach tissue biopsies.

As mentioned earlier, in vivo Raman spectroscopy of biological tissue has been limited by the lack of efficient Raman probes. While the technical difficulties in developing fluorescence and scattering spectroscopic probes have largely been solved, probes for Raman spectroscopy present technical difficulties, as Raman scattering is very weak and the probe fibers and adhesives can generate Raman signals that overwhelm Raman signals from the tissue. Development of successful clinical Raman spectroscopy techniques requires fabrication of optical fiber Raman probes that are small and flexible, exhibit high throughput and are free of background spectral features. Furthermore, these probes must be optimized to collect signals from tissue, a diffusive source. Recently, a new type of Raman probe has been developed, a small diameter, flexible device that is able to collect high quality spectra in clinically relevant timescales (1 s.).[43] The first clinical results have been obtained in breast and artery studies. These probes will prove instrumental in making the GI tract accessible to Raman spectroscopic analysis. The wealth of information obtainable from Raman spectra coupled with technical advances, which make the technique amenable to medical applications, will contribute to its widespread use in clinical diagnosis.

Although preliminary studies hold much promise for the use of Raman spectroscopy in detecting and characterizing GI abnormalities, further experiments that focus on understanding the origin of the spectroscopic changes responsible for disease differentiation are needed. Studies that monitor changes in a particular Raman peak are convoluted by the fact that in complex biological samples several distinct moieties may contribute intensity to a particular Raman band. Data analysis based on all of the spectral information will more accurately differentiate chemical species. Statistical analyses, such as principle component analysis and neural networks, use the full Raman spectrum for disease diagnosis. However, they only provide diagnostic information and do not elucidate the underlying chemical changes which are responsible for the separation. Alternately, models have been developed that provide chemical information as well as disease diagnosis.[44,45] These models fit tissue spectra with a linear combination of basis spectra derived from Raman microscopy of tissue components, such as an epithelial cell or a collagen fiber. To understand the relationship between a tissue sample's Raman spectrum and its disease state, we examine the contribution of each basis spectrum to a variety of pathologies. Morphologic modeling approaches have not yet been applied to GI tissues, but they are the key to a deeper understanding of the connection between tissue composition and Raman spectral features.

Several other contact probe techniques that have been applied to the GI tract, although not strictly spectroscopic, are optical coherence tomography[46,46-49] and confocal microscopy. Optical coherence tomography uses a contract probe to make microscopic images over a ~1 mm region of tissue. These are not wide-field images. Rather, they are cross-sectional images of the mucosa. Confocal microscopy can provide subcellular images deep within the tissue. This technology has been adapted to work within an endoscope and utilizes optical sectioning to reduce the effects of scattering.[50]

Wide-field Imaging Applications

While contact probe techniques provide detailed structural and biochemical information, they do not provide a means for wide area surveillance. Wide-field imaging techniques offer a method to monitor larger portions of the tissue; however, they generally offer less quantitative information. These techniques include chromoendoscopy, LSS imaging, fluorescence endoscopy, and photodynamic diagnosis.

Chromoendoscopy is a precursor spectroscopic technique

that uses standard video endoscopy along with dyes such as methylene blue and indigo carmine to image the mucosal surface. These dyes increase image contrast so that colorless features can be seen more easily. This has also been combined with magnification endoscopy, which allows the endoscopist to enlarge the image.[51]

Recently, a wide field imaging technique based on LSS has been shown to provide quantitative tissue information.[52] Previously, LSS had only been able to sample a small tissue area (~1 mm^2). As described in the previous sections, LSS provides quantitative information on the nuclear size distribution and density. LSS images are not conventional optical images. Rather, they are two dimensional maps of the spatial distributions of morphological parameters. Gurjar et al[52] created functional images of the percentage of enlarged nuclei and nuclear solid mass of separate colon polyps that grossly appeared identical. LSS images clearly distinguished the polyps as normal and dysplastic.

Fluorescence imaging endoscopy has recently been shown to successfully identify adenomatous colon polyps in vivo.[53-55] An argon laser was used to deliver excitation light to the tissue surface via a fiber optic probe placed within the accessory channel of the colonoscope. Two images were collected, one fluorescence and one white light image. The fluorescence image was digitized and processed to determine areas that had a high likelihood of dysplasia. A pseudo-color image was then superimposed on the white light image, indicating suspicious tissue areas. Figure 5 shows a typical fluorescence endoscopic image that correctly identifies two adenomatous polyps. This served as a real-time guide to biopsy for the endoscopist. Because the fluorescence signal is collected at the distal end of the colonoscope, this system does not require the use of expensive intensified CCD cameras.

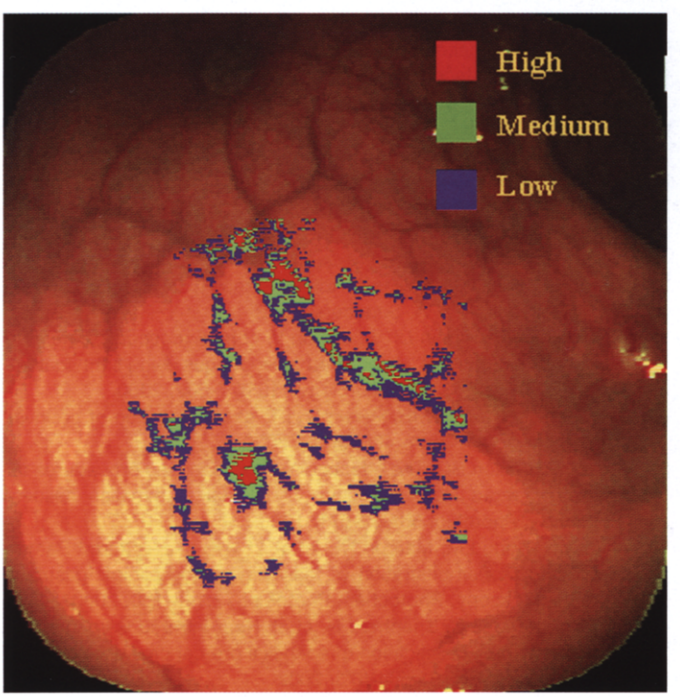

Fig 5. Fluorescence imaging endoscopy. The diagnostic map is overlaid on the conventional white light image. Three colors are used to indicate high, medium, and low likelihood of dysplasia. Two adenomas (red regions) are correctly identified.

Several other fluorescence endoscopy systems as well as photodynamic diagnosis systems utilize the fluorescence characteristics of tissue to create wide-field images. In fluorescence endoscopy, a light source excites the endogenous tissue fluorophores. It has been shown to distinguish normal and dysplastic tissue in real time,[54,56,57] and a commercial system is available (LIFE-GI; Xillex Technologies, Canada). Photodynamic diagnosis uses fluorescent drugs called photosensitizers such as 5-aminolevulinic acid that accumulate in dysplastic tissue where they are converted to protoporphyrin IX. Measurement of protoporphyrin IX fluorescence can thus be a guide to otherwise undetectable areas of dysplasia.[58]

Spectral Endoscopy: The Endoscope of the Future

This article has discussed the principles of spectral analysis of biological tissues and presented some examples of the current state of the art in gastrointestinal endoscopy. In this section, we discuss the likely prospects for spectral endoscopy—the endoscope of the future.

The recent application of TMS has shown the success of combining multiple spectroscopic modalities to determine a diagnosis. This technology is currently capable of characterizing a number of important physically relevant diagnostic parameters—contributions from collagen and NADH, nuclear size distribution and density, stromal scattering size and density, and hemoglobin concentration and oxygen saturation. As TMS further develops, identification of new diagnostic parameters can be envisioned—contributions from porphyrins, FAD, tryptophan, detection of goblet cells, as well as LSS methods to quantify distribution of sub-micron tissue structure, as well as cell nuclei.

The clinical use of Raman spectroscopy has up to now been limited by lack of availability of suitable Raman probes. As discussed above, an important breakthrough in Raman probe development has recently been achieved.[43] This opens the possibility of extending contact probe techniques to Raman spectroscopy, providing highly specific molecular and morphologic information. Perhaps a new contact probe instrument could be envisioned called quadra-modal spectroscopy (QMS !), combining four spectral modalities (Raman, DRS, IFS, LSS) and further increasing the sensitivity and specificity for characterizing disease.

These contact probe techniques are powerful, but at present not suitable for wide area surveillance. It may be possible to extend the methods of TMS to much wider regions, several centimeters in extent. Several technical hurdles will have to be overcome to accomplish this. For example, a 1-mm diameter probe provides a scale size that is well suited to characterize the absorption and scattering lengths characteristic of biological tissues. In a large field implementation, however, such a scale will not be present. If methods can be developed to impose an appropriate scale, then wide area TMS imaging endoscopes may become possible.

We have discussed 2 methods of implementation of spectroscopy in endoscopy: contact probe and wide field imaging. Spectral probes implemented via introduction of optical fibers in the biopsy channel of an endoscope, provide detailed morphological and biochemical information about the state of the tissues over a small (~1 mm diameter) region, from which an accurate diagnosis can be made. Wide area surveillance, by contrast, can

image the full endoscopic field, but the spectral diagnostic information provided is less specific and detailed. The 2 methods are complementary, suggesting the idea of endoscopic instruments that combine the 2. The wide area spectral diagnostic image could be superimposed on the white light image, and suspect areas could then be studied in greater detail with a contact probe incorporated in the endoscope. This type of integrated system could provide objective, detailed diagnostic information in real-time, without the need for tissue removal. In addition, spectroscopic information could be used as a guide to biopsy to reduce sampling errors. Spectroscopic data could be stored for comparison at post-therapy follow-up visits to help detect abnormalities or precancerous changes at a very early stage. While the last great stride in endoscopic technology came with the advent of the CCD to provide us with "TV images" of the internal surfaces of the body, perhaps optical spectroscopic technologies will provide the next great stride to add functionality to those images.

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