

NYSGE: Advances in Endoscopic Imaging: The Light at the End of the Tunnel

By Jacques Van Dam, M.D., Ph.D., Professor of Medicine, Stanford University
School of Medicine

The desire to see beyond the limits of one's own vision may be as old as sight itself. For the scientist, the development of the microscope made possible the study of worlds too small to observe with "the naked eye." For the astronomer, the telescope brought distant planets and stars seemingly within one's grasp. For the physician, the endoscope enabled the examination of the living body's previously dark and hidden cavities. However, the endoscope's ability to help make clinical diagnoses has always relied exclusively on the vision of its operator. A revolution in optics, powerful light sources, and exciting new technology has the potential to advance gastrointestinal endoscopy beyond its current limitations.

Optical biopsy is a term frequently used to describe the ability to make a diagnosis without the removal of tissue. There are many variations on this theme and all involve a "light-tissue" interaction of one form or another. The field of optical biopsy as it is currently being developed may be divided into many categories. Each is designed to enhance the clinician's ability to make a tissue diagnosis during an endoscopic procedure. This can take the form of images so highly magnified that individual cells and even some subcellular organelles may be observed. Another form of optical biopsy uses powerful light sources or lasers to increase the resolution of subcellular imaging. And in some instances, the very biochemical nature of cells can be exploited by light to differentiate the benign from the cancerous or precancerous cell.

Spectroscopy

Spectroscopy is the study of interactions between light and matter where the light is evaluated in its component wavelengths or colors. The use of color as a diagnostic or prognostic indicator has been documented since the time of Hippocrates, when it was thought that health involved the proper balance of four humors: yellow bile or cholera, red blood, white phlegm and black bile or melancholy. During the practice of modern day gastroenterology, endoscopy is the most commonly used technique that relies heavily on color differences for assessing the presence of disease. Several forms of spectroscopy exist and represent the analysis

of various fates of photons of light. When a photon strikes the surface of the gastrointestinal lumen, it can be absorbed or reflected by the tissue. This is the basis of *reflectance spectroscopy*. For instance, oxygenated blood appears red because oxygenated hemoglobin absorbs the wavelengths of light in the 400-600nm range, reflecting back wavelengths around 600 to 700nm (visible red light). Therefore colonic inflammation, accompanied by increased blood flow, appears red. Some photons of light excite tissue fluorophores, biochemical structures that emit longer wavelengths of light when excited by specific incident wavelengths of light. This is the basis of *fluorescence spectroscopy*. Some photons will scatter about within the tissue taking a course based upon the size and density of structures present such as collagen and cell nuclei. Some of these photons ultimately are emitted from the tissue and can be collected and analyzed. This is the basis of *light-scattering spectroscopy*. Finally, some photons will impart energy to molecules within the tissue, causing these molecules to vibrate at specific frequencies. This is the basis of *Raman spectroscopy*.

These various fates of photons are not random. Rather they reflect the biochemical and structural composition of the tissue upon which they are directed. Therefore the various forms of spectroscopy can reveal a multitude of information about a tissue. Investigators have begun using spectroscopy to discover information about the microscopic and molecular structures of tissue. For example, fluorescence spectroscopy can be used to determine the relative tissue content of a fluorophore such as NADH. Levels of NADH in a particular tissue may represent a particular rate of metabolism. Dysplastic epithelium might therefore contain relatively higher concentrations of NADH than non-dysplastic epithelium and thus fluoresces differently.

Fluorescence Spectroscopy

Laser-induced fluorescence (LIF) spectroscopy is the term used to describe the spectroscopic evaluation of light emitted from tissue after being excited by laser light. Lasers are a convenient source of light because very specific wavelengths can be obtained reliably. For instance, a nitrogen laser calibrated to emit light at wavelength 370nm, will emit only that wavelength. Light from a laser can be directed at a tissue surface and the resulting fluorescent light can be analyzed as it is emitted from that tissue. The difference in fluorescence intensities provides a spectroscopic

tool that has been shown to be successful in distinguishing between dysplastic and non-dysplastic epithelium.

Determining exactly what fluorophores within an epithelium are responsible for fluorescence differences between dysplastic and non-dysplastic tissue has remained speculative. Collagen is a major fluorophore in both normal and dysplastic colonic mucosa. The cytoplasm of dysplastic tissue contains a significantly increased amount of some unidentified fluorophore, perhaps of a porphyrin derivation. The intensity of tissue fluorescence correlates directly with the degree of dysplasia, suggesting the increasing presence of this fluorophore as cells became more poorly differentiated. While the exact causes of differences in fluorescence eludes us, the observed differences in fluorescence forms the basis for using fluorescence spectroscopy for distinguishing normal from dysplastic tissue.

Laser-induced fluorescence endoscopy (LIFE) takes fluorescence spectroscopy a step further. In LIFE, the light is not delivered through a single fiber, but rather through the light-source of an endoscope. This allows the endoscopist to shine specific wavelengths of light on a large field of tissue, just like the use of white light during standard endoscopy. Because of differences in content of fluorophores between dysplastic and non-dysplastic tissue, focal areas of dysplasia will be highlighted during LIFE and thus become visible to the endoscopist.

Light Scattering Spectroscopy

Light scattering spectroscopy (LSS) deals with light that is scattered in the backward direction after undergoing a single scattering event. This consists only of a small fraction (2-5%) of the overall amount of light that is eventually backscattered out of the tissue. The intensity of the singly backscattered light depends on the backscattering cross-section of the particle and it varies as a function of wavelength and scattering angle in a manner that is characteristic of the particle's size, shape, refractive index and number density. The origin of LSS signals depends on the geometry of collection. Particles that are large compared to the wavelength, such as nuclei, scatter predominantly in the near-exact forward and backward directions (even though backscattering is much weaker than forward scattering). In addition, the intensity of the backscattered light from large particles oscillates in intensity as a function of wave number with a frequency that is characteristic of the particle size and relative refractive index. In tissue, scattering from the outer cell membrane is not

significant because there is index matching between the membranes of neighboring cells. Thus, the nucleus is the major large cellular scattering center. Particles that are small compared to the wavelength, such as the tubules of the endoplasmic reticulum, scatter light in a nearly isotropic fashion over all angles. The intensity of the scattered light also varies smoothly as a function of wavelength. The angular and wavelength dependent distributions of the light intensity scattered by particles whose size is comparable to the wavelength, such as mitochondria and lysosomes, exhibit significantly broader features than the corresponding spectra of large particles, but not as broad as those of small particles. When particles of several sizes are present, the resulting signal is a superposition of these variations. In LSS, the size distribution and refractive index of the scatterers is determined from analysis of the spectrum of light backscattered by these particles.

Evaluation of the Spectroscopic Signal

Several algorithms have been developed to explore the diagnostic usefulness of the information that is present in measured reflectance spectra, fluorescence spectra, and light-scattering spectra from normal and diseased tissues. Such algorithms can be empirical, statistical or model-based. Empirical algorithms employ specific spectral features, such as the slope or the intensity of the spectrum within a specific wavelength region, to separate normal from diseased tissues. Such algorithms are easy to implement, but they do not provide any quantitative insights with regards to the origins of the observed changes. Statistical or pattern recognition algorithms, such as principal component analysis or artificial neural networks, require significant levels of data pre-processing and normalization, but they can be sensitive to very small differences that are useful in classifying spectra as normal or diseased. The results do not provide any information about the origins of the features that are used for classification either. Finally, model-based approaches describe the features of the measured reflectance spectra based on mathematical models of the relevant light-tissue interactions. Such models are not trivial to develop and they require assumptions that simplify the modeled system, but they provide quantitative information about specific changes in the optical properties of tissue that take place during disease development. Such information can be used to develop diagnostic algorithms, to further optimize instrument design and to gain important insights into the pathophysiology of diseases such as Barrett's esophagus dysplasia.

“Field Carcinogenesis” - Implications for Screening

Field carcinogenesis is the term used to describe the notion that the genetic or other environmental milieu that leads to carcinogenesis in one part of an organ, say the colon, can be detectable at a remote site. If so, an exquisitely sensitive method for detecting such subcellular or biochemical changes could be used to screen a population for cancer in a less invasive manner. Consider spectroscopic analysis of the rectum to detect concurrent neoplasia as a screening test. If positive, a colonoscopy would be indicated to remove the potential neoplasia. However, if negative, no colonoscopy would be indicated. The implications for such a minimally invasive screening test would be staggering. Such a screening test could be used to screen the population for lung cancer, esophageal cancer, pancreatic cancer, etc. The spectroscopic analysis of light-tissue interactions as described herein and in development is slowly approaching just such a possibility.