In Vivo Microscopy Resource Guide
CAP '13

The In Vivo Microscopy Resource Guide highlights resources that provide awareness and understanding of the technology.

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Feedback on this content is welcome, including suggestions for articles, webinars, or other resources. Please send comments, suggestions, and questions to capguides@cap.org.
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Section 1 In Vivo Microscopy: The Basics

1.1 Background

Several advanced optical imaging technologies have recently been developed that allow microscopic visualization of tissues in living animals or humans, including optical coherence tomography (OCT), confocal laser endomicroscopy (CLE) and others (such as multiphoton microscopy (MPM)). These new in vivo microscopy (IVM) technologies have in common the fact that they take advantage of changes in light (absorption, scattering, fluorescence emission, etc.) as it interacts with tissue to provide cellular (or near cellular) resolution images in real time at the patient bedside, without the need for tissue removal, fixation or staining. These IVM technologies are all tomographic, and thus can provide either 2-D images (like the en face images of colonic crypts above (right-OCT; center MPM; left CLE) or 3-D tomographic images with microscopic resolution. The data embedded in these images provides information regarding tissue architecture, cellular morphology and chemistry that can be used to render not only a tissue diagnosis, but also to predict prognosis and patient response to therapy. In some cases, the image data can be analyzed using statistical models to render an objective disease diagnosis without the bias inherent in visual image analysis. Details that should help in understanding each of these technologies are provided in Section 2.0, Understanding the Technology.

These early IVM imaging systems are the first steps toward higher resolution imaging systems of the future. Pathologists today have the opportunity to be collaborators in the development, validation and clinical trials of this technology, bringing their histopathologic expertise to the proper interpretation of the images being generated and establishing a future role for pathologists in image analysis and potentially as the interventional microscopist. Pathologists can also adopt these technologies as tools for ex vivo use in their own pathology practice, for intraoperative margin and sentinel lymph node assessment, directed sampling of surgical specimens in the grossing room, etc. Further discussion of the impact of IVM on pathology is provided below in Section 1.2, In Vivo Microscopy and Pathology. Insights from pathologists who are developers or early adopters of IVM technology are provided in Section 3.0, Insights from Early Adopters. And details of potential IVM applications in pathology practice can be found in Section 4.0, Ex vivo Pathology Applications of In Vivo Microscopy.

These new IVM technologies are also of great import to pathologists as IVM systems are now commercially available and have received FDA approval for clinical use. CPT billing codes have
also been approved to bill for clinical IVM procedures and IVM image analysis. Thus IVM is or will soon be used by our clinical colleagues in their day-to-day practice. In fact, OCT is currently the clinical gold standard in ophthalmology practice for imaging of the retina and anterior segment of the eye and is also being used clinically in cardiology, with little awareness by or impact on the practice of pathology. IVM is now coming into more widespread clinical use in gastroenterology and urology, with the integration of both OCT and confocal laser imaging systems into endoscopes for microscopic imaging of the epithelium and submucosa of accessible GI and GU tract tissues, such as the esophagus, stomach, colon, pancreas, biliary tract and bladder. IVM technology also can be applied using smaller fiberoptic probes, which can be used in conjunction with biopsy needles, in solid organs such as lung, breast, prostate or brain. And IVM can be performed on the skin without an endoscope or fiberoptic probe. Adoption of IVM in these clinical disciplines and organ systems will have a much greater impact on pathology practice.

While intracellular and individual cell image details are not yet equivalent to histopathology images, the architectural and cellular patterns seen in IVM images are interpretable by pathologists to either make differential diagnoses or to identify areas for biopsy with improved diagnostic yield. Such directed tissue biopsies might not only help more accurately target diseased tissues and thus limit the risk of sampling error, but also allow follow up of areas that underwent endoscopic removal of precancerous lesions and monitoring of pharmacological and other therapies. In addition to adoption of IVM for biopsy guidance, there is also a future goal of diagnosis without the need for a biopsy. The avoidance of a biopsy is particularly valuable in tissues such as the brain where biopsy can cause damage and dysfunction, in fragile patients such as patients with coagulopathies, or in cancers where disruption can cause unwanted cancer cell dissemination. IVM technology can also be configured to image entire mucosal surfaces or organs to screen for occult microscopic disease. These and other organ specific clinical applications of IVM are presented in detail in Section 5.0, In Vivo Clinical Applications of In Vivo Microscopy.

The following CAP documents provide a general introduction to the field of IVM and its impact on the practice of pathology. Links to IVM-related educational resources at CAP and in industry are provided in Section 7 and Section 8.

A) “Why IVM?” Flyer developed by In Vivo Microscopy Work Group

Free flyer available

B) In Vivo Microscopy: Illuminating the Future of Imaging in Pathology

Summary: This is a chapter in the CAP ebook, New Paths...New Choices: Pathology in an Era of Advancing Science and Disruptive Health Economic, created as part of the “Your Path. Your Choice” Promising Practice Pathways promotions plan.

Access the entire ebook

Access the chapter of the ebook on IVM

C) In Vivo Microscopy for the POET Report developed by CAP’s Technology Assessment Committee, posted December 20, 2010

Summary: Pathologists will soon share examination of tissue morphology at the architectural, cellular and molecular level with other physicians. In vivo microscopy technologies enable physicians to detect pathology by visualizing tissue through innovative tomographic methods. In vivo microscopy techniques provide additional data beyond that obtainable by histological methods, including volumetric data and time/flow data.

Developed by the Technology Assessment Committee (TAC), Perspectives on Emerging Technology (POET) reports and white papers are designed to provide pathologists with a high-level summary of a particular emerging technology that is likely to impact their practice in the reasonable future. POET reports help pathologists respond to clinician or patient inquiries about a technology. Its format includes a one-page summary plus select references (e.g., peer-reviewed articles, for further information and research). Although POETs deliver a short overview of a specific innovative technology, they are not a definitive technology assessment of the techniques used or a “how to” cookbook on implementing a test in a practice. Rather, they are intended to be used as an educational tool leading to a more detailed investigation by the Center, Council on Scientific Affairs, TAC or individual pathologists.

In Vivo Microscopy POET Report; POET Reports homepage

1.2 In Vivo Microscopy and Pathology

Pathologist Gary Tearney holding a tethered capsule developed in his research laboratory to perform IVM of the GI tract. Contributed by Tearney G, Massachusetts General Hospital, Boston, MA

The advent of new advanced IVM imaging technologies provides a unique opportunity for pathologists who are, after all, THE experts in interpretation of diagnostic microscopic images. We are also THE experts at establishing diagnostic criteria and analyzing diagnostic data sets, skills critical to bringing IVM and other advanced imaging technologies to widespread clinical use. Therefore, there is a definite role for pathologists in research on clinical applications of IVM, particularly in validating these new technologies for disease diagnosis.

However, whether or not pathologists play a role in development and validation of this technology, IVM will impact pathology practice. Our clinical colleagues will soon be using IVM to target their endoscopic and needle biopsies, if they are not already. We will be seeing these images clipped to our surgical pathology and cytology requisitions and projected at tumor boards and other clinical conferences, and will be asked to comment on them and to explain/resolve any discrepancies between the IVM images and pathology findings. Pathologists will also soon be using IVM technologies in their practice, during intraoperative consultations on margins of resection and sentinel lymph nodes and in the grossing room to direct sampling of
surgical specimens. So, it is critically important that pathologists be well versed in IVM and knowledgeable in the interpretation of IVM images.

The following are a few selected articles on IVM viewed from the pathologist’s perspective.

A) **In Vivo Microscopy: The Illuminating Future of Imaging in Pathology**


**Summary:** Webinar participants will discuss:
- Benefits and the challenges of using IVM
- Current and future applications of IVM
- Pathologists taking on a new role with IVM
- Opportunities for reimbursement

[Archived webinar recording]
Note: Webinar listed under Previous Sessions – New Roles of Pathologists

B) **Confocal Laser Endomicroscopy: A Primer for Pathologists**


**Summary:** The advent of new endoscopic optical techniques is likely to change pathologists' role in diagnosis. OBJECTIVE: To describe how confocal laser endomicroscopy (CLE) works, show its advantages and limitations compared to cytohistologic biopsy, and explore how it may affect the practice of pathology. DATA SOURCES: Literature review. CONCLUSIONS: Confocal laser endomicroscopy is proving its ability to provide histology-like images of tissues in vivo to help avoid risks and costs of conventional biopsies. Confocal imaging restricts light to 1 plane, emulating a paraffin section, and topical or systemic optical contrast agents allow subcellular resolution. New contrast agents could theoretically permit molecular characterization. In vivo imaging has begun to demonstrate novel, dynamic types of diagnostic features. Decreased histologic biopsies can be anticipated for a few scenarios. Significant limitations of CLE include the inability to create a tissue archive for broad molecular classification, suboptimal contrast agents, small fields of view and shallow penetration, paucity of clinical validation studies, and problems with reimbursement. Confocal laser endomicroscopy exposes new opportunities for pathologists: CLE technologies can be exploited in pathology, and diagnostic criteria expanded based on endoscopists’ discoveries. Potential synergy exists between CLE and cytology, allowing the low-magnification diagnostic architectural changes by CLE and cytomorphology to emulate the full diagnostic information in a histologic biopsy while providing an archive of material for molecular or immunohistochemical studies. Confocal laser endomicroscopy will decrease some types of biopsies, but offers an opportunity for pathologists to find new ways to provide value and improve patient care.

Free full text article available from the CAP’s [Archives]
PMID: 21970490

C) **In Vivo Optical Coherence Tomography: The Role of the Pathologist**

D) Validation of Novel Optical Imaging Technologies: The Pathologists’ View

Summary: Noninvasive optical imaging technology has the potential to improve the accuracy of disease detection and predict treatment response. Pathology provides the critical link between the biological basis of an image or spectral signature and clinical outcomes obtained through optical imaging. The validation of optical images and spectra requires both morphologic diagnosis from histopathology and parametric analysis of tissue features above and beyond the declared pathologic “diagnosis.” Enhancement of optical imaging modalities with exogenously applied biomarkers also requires validation of the biological basis for molecular contrast. For an optical diagnostic or prognostic technology to be useful, it must be clinically important, independently informative, and of demonstrated beneficial value to patient care. Its usage must be standardized with regard to methods, interpretation, reproducibility, and reporting, in which the pathologist plays a key role. By providing insight into disease pathobiology, interpretive or quantitative analysis of tissue material, and expertise in molecular diagnosis, the pathologist should be an integral part of any team that is validating novel optical imaging modalities. This review will consider (1) the selection of validation biomarkers; (2) standardization in tissue processing, diagnosis, reporting, and quantitative analysis; (3) the role of the pathologist in study design; and (4) reference standards, controls, and interobserver variability.

Full text article available from Journal of Biomedical Optics (USD $25.00)
PMID: 17994879
NOTE: Also cited in Sections 3.2 and 6.1.

1.3 Hot Topics: IVM in the News

As a rapidly evolving, transformative technology, IVM is in the news! The following are some of the recent press releases and media reports on clinical applications of IVM and other advanced imaging technologies.

A) Rapid, Label-free Detection of Brain Tumors with Stimulated Raman Scattering Microscopy

Summary: Surgery is an essential component in the treatment of brain tumors. However, delineating tumor from normal brain remains a major challenge. We describe the use of stimulated Raman scattering (SRS) microscopy for differentiating healthy human and mouse brain tissue from tumor-infiltrated brain based on histoarchitectural and biochemical differences. Unlike traditional histopathology, SRS is a label-free technique that can be rapidly performed in situ. SRS microscopy was able to differentiate tumor
from nonneoplastic tissue in an infiltrative human glioblastoma xenograft mouse model based on their different Raman spectra. We further demonstrated a correlation between SRS and hematoxylin and eosin microscopy for detection of glioma infiltration ($\kappa = 0.98$). Finally, we applied SRS microscopy in vivo in mice during surgery to reveal tumor margins that were undetectable under standard operative conditions. By providing rapid intraoperative assessment of brain tissue, SRS microscopy may ultimately improve the safety and accuracy of surgeries where tumor boundaries are visually indistinct.

Full text article available from Science Translational Medicine. (USD 20.00)
PMID: 24005159

B) Pill-sized Device Provides Rapid, Detailed Imaging of Esophageal Lining Novel System Could Allow Broader Screening for Esophageal Cancer, Other Conditions


Summary: Physicians may soon have a new way to screen patients for Barrett’s esophagus, a precancerous condition usually caused by chronic exposure to stomach acid. Researchers at the Wellman Center for Photomedicine at Massachusetts General Hospital (MGH) have developed an imaging system enclosed in a capsule about the size of a multivitamin pill that creates detailed, microscopic images of the esophageal wall. The system has several advantages over traditional endoscopy.

"This system gives us a convenient way to screen for Barrett’s that doesn’t require patient sedation, a specialized setting and equipment, or a physician who has been trained in endoscopy," says Gary Tearney, MD, PhD, of the Wellman Center and the MGH Pathology Department, corresponding author of the report receiving online publication in Nature Medicine. "By showing the three-dimensional, microscopic structure of the esophageal lining, it reveals much more detail than can be seen with even high-resolution endoscopy."

Free full text article available from EurekAlert
C) Improving the Accuracy of Cancer Diagnoses: New Spectroscopy Technique Could Help Doctors Identify Breast Cancers


**Summary:** Tiny calcium deposits can be a telltale sign of breast cancer. However, in the majority of cases these microcalcifications signal a benign condition. A new diagnostic procedure developed at MIT and Case Western Reserve University (CWRU) could help doctors more accurately distinguish between cancerous and noncancerous cases.

The new method, which uses a special type of spectroscopy to locate microcalcifications during the biopsy, could dramatically reduce the rate of inconclusive diagnosis, according to the researchers. In a study appearing in the *Proceedings of the National Academy of Sciences* the week of Dec. 24, they found that the spectroscopy technique had a success rate of 97 percent.


D) Laboratories Seek New Ways to Take a Look Inside


**Summary:** In a bioengineering laboratory at Stanford University, Christopher Contag, a microbiologist, is designing new approaches to "virtual" pathology. He has created a variety of instruments that can travel the esophagus, stomach and intestine, allowing pathologists to probe for cancers by peering in three dimensions below the surface of the skin.

Today a new wave of imaging technologies is again transforming the practice of medicine. They include new pathology tools — like the ones Dr. Contag’s team is developing — to give doctors an instantaneous diagnosis, as well as inexpensive systems, often based on smartphones, that can extend advanced imaging technologies to the entire world.

E) The Age of the Tricorder

The Medical Device of the Future is Already Here

Summary: The Star Trek television series and movies have proved remarkably prescient in anticipating future technologies – from flip phones to iPads – decades before they appeared in everyday life. Now, nearly half a century after the debut of the original series, the science fiction classic featuring Captain Kirk and the alien Mr. Spock, we are beginning to see modern-day equivalents of another iconic Star Trek device: the tricorder.

In the series, tricorders are handheld medical instruments used to detect, diagnose and treat any number of injuries or maladies, either terrestrial or alien. The design has evolved over the decades, but in all cases the device is portable, requires no external power source and provides some sort of sensing capability.

This description also can be applied to a new generation of sensing and imaging devices in our own time. Recent advances enabling miniaturization of the technologies, the advent of smartphones with cameras and data-transmission capabilities, and other factors have led to the development of a host of instruments that might be described as tricorders, or at least as tricorder-like. And there’s undoubtedly more to come.

Early this year, the X PRIZE and Qualcomm foundations announced the launch of the $10 million Qualcomm Tricorder X PRIZE, a global competition in which teams will leverage innovative technologies such as wireless sensing to develop a mobile platform that can make medical diagnoses independently of a physician or health care provider. The top prize will go to the team whose platform most accurately diagnoses a set of 15 diseases involving 30 consumers in three days, while also providing a compelling consumer experience.

Free full text article available from Biophotonics

F) World’s Smallest Microscope Improves GI Disease Detection and Speeds Treatment at Lankenau Medical Center in Philadelphia

Press release from Lankenau Medical Center announcing use of Confocal Laser Endomicroscopy, November 2, 2011.

Summary: Lankenau Medical Center physicians who specialize in gastrointestinal disorders are the first in the Philadelphia metropolitan area to use the world’s smallest flexible microscope to diagnose gastrointestinal and biliary diseases during regular endoscopy procedures so patients can be treated immediately. This new tool, known as probe-based Confocal Laser Endomicroscopy (pCLE) or “Cellvizio,” allows them to view living, moving tissue in real time at the cellular level so they can precisely pinpoint tissue that should be removed or treated.

Free full text article available from Marketwire
Section 2 Understanding IVM Technology

2.1 In Vivo Microscopy Technologies

In vivo microscopy (IVM) technologies currently approved by the FDA for clinical use in the United States are **confocal microscopy** and **optical coherence tomography (OCT)**. Newer technologies in the pipeline include: **multiphoton microscopy (MPM)** and **optical spectroscopy (fluorescence, reflectance and Raman)** and **spectroscopic imaging**. In addition, there are a multitude of optical imaging technologies, among them photoacoustic microscopy, molecular imaging microscopy, and functional microscopic imaging (e.g. Doppler and autofluorescence) in earlier stages of development.

The following are selected review articles that explain the basic principles behind, instrumentation used for and potential clinical applications of each of these IVM technologies.

To see a video of how an IVM procedure is performed go to this article in JOVE (the Journal of Visualized Experiments), which publishes articles teaching laboratory fundamentals using video demonstrations.

2.2 Confocal Microscopy

**Confocal microscopy** is a non-invasive, high resolution optical imaging technique that uses point illumination and a spatial pinhole to eliminate out-of-focus light, enabling ‘optical sectioning’ and tomographic imaging of specimens that are thicker than the focal plane. As such it has come into widespread use in cell biology and other biomedical research studies for 2-D and 3-D imaging of single cells.

Recent advances in instrumentation, including miniaturization to allow confocal microscopy to be performed through an endoscope, now allow 2-D and 3-D images (such as the en face confocal microscopy image of colonic crypts above) to be obtained in vivo, in real time, with cellular resolution, without the need for tissue removal, processing or staining. This has led to clinical application of confocal microscopy for in vivo imaging for disease diagnosis. **Confocal laser endomicroscopy (CLE)** – confocal microscopy performed through an endoscope – has many potential clinical applications from guidance of surveillance biopsies for dysplasia in the esophagus or colon to ‘optical biopsy’ in privileged sites in the brain, coronary arteries, etc. or in high risk patients such as those with coagulopathies where conventional biopsy may be contraindicated. Diagnostic confocal microscopy can also be performed directly – without the need for an endoscope – on the skin, where it can be used for such clinical applications as assessment of pigmented lesions and of margins at Mohs surgery for basal cell carcinoma.

The following are selected review articles that explain the basic principles behind, instrumentation used for and potential clinical applications of confocal microscopy and CLE.
A) Introduction to Confocal Microscopy

Summary: Conventional microscopy requires viewing a thin-cut “section” of fixed or frozen tissue, and therefore cannot be used to view thick tissue samples or for in vivo investigations. In vivo microscopy requires a virtual, rather than a physical, section of the specimen. Confocal microscopy, developed and patented by Marvin Minsky in 1955, uses optical imaging to create a virtual slice or plane, many micrometers deep, within the tissue. It provides very-high-quality images with fine detail and more contrast than conventional microscopy. In addition, the imaging technique allows for reconstruction of virtual 3-dimensional (3-D) images of the tissue when multiple sections are combined.

Full text article available from PubMed
PMID: 23187113
Note: Also cited in Section 5.3

B) Confocal Laser Endomicroscopy: A Primer for Pathologists

Summary: The advent of new endoscopic optical techniques is likely to change pathologists' role in diagnosis. Objective.—To describe how confocal laser endomicroscopy (CLE) works, show its advantages and limitations compared to cytohistologic biopsy, and explore how it may affect the practice of pathology. Data Sources.—Literature review. Conclusions.—Confocal laser endomicroscopy is proving its ability to provide histology-like images of tissues in vivo to help avoid risks and costs of conventional biopsies. Confocal imaging restricts light to 1 plane, emulating a paraffin section, and topical or systemic optical contrast agents allow subcellular resolution. New contrast agents could theoretically permit molecular characterization. In vivo imaging has begun to demonstrate novel, dynamic types of diagnostic features. Decreased histologic biopsies can be anticipated for a few scenarios. Significant limitations of CLE include the inability to create a tissue archive for broad molecular classification, suboptimal contrast agents, small fields of view and shallow penetration, paucity of clinical validation studies, and problems with reimbursement. Confocal laser endomicroscopy exposes new opportunities for pathologists: CLE technologies can be exploited in pathology, and diagnostic criteria expanded based on endoscopists' discoveries. Potential synergy exists between CLE and cytology, allowing the low-magnification diagnostic architectural changes by CLE and cytomorphology to emulate the full diagnostic information in a histologic biopsy while providing an archive of material for molecular or immunohistochemical studies. Confocal laser endomicroscopy will decrease some types of biopsies, but offers an opportunity for pathologists to find new ways to provide value and improve patient care.

Free full text article available from the CAP's Archives
PMID: 21970490
NOTE: Also cited in Section 1.1
C) Miami Classification for Probe-Based Confocal Laser Endomicroscopy


**Summary:** An essential element for any new advanced imaging technology is standardization of indications, terminology, categorization of images, and research priorities. In this review, we propose a state-of-the-art classification system for normal and pathological states in gastrointestinal disease using probe-based confocal laser endomicroscopy (pCLE). The Miami classification system is based on a consensus of pCLE users reached during a meeting held in Miami, Florida, in February 2009.

Full text article available from *Endoscopy* (USD 33.00)
PMID: 21818734
Note: Also cited in Sections 3.1, 3.3, 5.1.1, 6.2

2.3 Optical Coherence Tomography

Optical coherence tomography (OCT) is a noninvasive, high resolution optical imaging technique that provides real-time 2-D and 3-D images of tissue architecture in vivo (like the OCT image of colonic crypts above). OCT images map reflectivity (or bounce back) of light waves focused onto the tissue. Thus, OCT is an optical counterpoint to ultrasound imaging. However, OCT has near cellular resolution, which gives it a significant advantage over ultrasound and other conventional medical imaging techniques such as white light endoscopy, MRI and CT. As with confocal microscopy, recent advances in instrument miniaturization allow OCT to be performed through an endoscope. Further, like confocal microscopy, OCT can provide a specific disease diagnosis, such as a diagnosis of dysplasia in Barrett’s esophagus that cannot be obtained with conventional imaging techniques. OCT does have its limitations. Unlike ultrasound, OCT cannot image deep into tissue. So OCT is most often used to image superficial tissues like the skin, the mucosa of hollow viscera that can be accessed via endoscopes, or deep tissues such as the retina that can be imaged through a transparent window such as that provided by the lens.

The following are selected review articles that explain the basic principles behind, instrumentation used for and potential clinical applications of OCT.

To see a video of how an OCT procedure is performed go to this article in JOVE (the Journal of Visualized Experiments), which publishes articles teaching laboratory fundamentals using video demonstrations.
A) Optical Coherence Tomography-Current Technology and Applications in Clinical and Biomedical Research

**Summary:** Optical coherence tomography (OCT) is a noninvasive imaging technique that provides real-time two- and three-dimensional images of scattering samples with micrometer resolution. By mapping the local reflectivity, OCT visualizes the morphology of the sample. In addition, functional properties such as birefringence, motion, or the distributions of certain substances can be detected with high spatial resolution. Its main field of application is biomedical imaging and diagnostics. In ophthalmology, OCT is accepted as a clinical standard for diagnosing and monitoring the treatment of a number of retinal diseases, and OCT is becoming an important instrument for clinical cardiology. New applications are emerging in various medical fields, such as early-stage cancer detection, surgical guidance, and the early diagnosis of musculoskeletal diseases. OCT has also proven its value as a tool for developmental biology. The number of companies involved in manufacturing OCT systems has increased substantially during the last few years (especially due to its success in), and this technology can be expected to continue to spread into various fields of application.

Full text article available from SpringerLink (USD $34.95)
PMID: 21547430

B) Optical Coherence Tomography in Biomedical Research

**Summary:** Optical coherence tomography (OCT) is a noninvasive, high-resolution, interferometric imaging modality using near-infrared light to acquire cross-sections and three-dimensional images of the subsurface microstructure of biological specimens. Because of rapid improvement of the acquisition speed and axial resolution of OCT over recent years, OCT is becoming increasingly attractive for applications in biomedical research. Therefore, OCT is no longer used solely for structural investigations of biological samples but also for functional examination, making it potentially useful in bioanalytical science. The combination of in vivo structural and functional findings makes it possible to obtain thorough knowledge on basic physiological and pathological processes. Advanced applications, for example, optical biopsy in visceral cavities, have been enabled by combining OCT with established imaging modalities. This report gives an outline of the state of the art and novel trends of innovative OCT approaches in biomedical research in which the main focus is on applications in fundamental research and pre-clinical utilization.

Full text article available from SpringerLink (USD $34.95)
PMID: 21562739

C) Modified Full-Field Optical Coherence Tomography: A Novel Tool for Rapid Histology of Tissues
Summary: Here, we report the first use of a commercial prototype of full-field optical coherence tomography called Light-CT™. Based on the principle of white light interferometry, Light-CT™ generates quick high-resolution three-dimensional tomographic images from unprocessed tissues. Its advantage over the current intra-surgical diagnostic standard, i.e. frozen section analysis, lies in the absence of freezing artifacts, which allows real-time diagnostic impressions, and/or for the tissues to be triaged for subsequent conventional histopathology. MATERIALS AND METHODS: In this study, we recapitulate known normal histology in nine formalin fixed ex vivo rat organs (skin, heart, lung, liver, stomach, kidney, prostate, urinary bladder, and testis). Large surface and virtually sectioned stacks of images at varying depths were acquired by a pair of 10×/0.3 numerical aperture water immersion objectives, processed and visualized in real time. RESULTS: Normal histology of the following organs was recapitulated by identifying various tissue microstructures. Skin: epidermis, dermal-epidermal junction and hair follicles with surrounding sebaceous glands in the dermis. Stomach: mucosa with surface pits, submucosa, muscularis propria and serosa. Liver: hepatocytes separated by sinusoidal spaces, central veins and portal triad. Kidney: convoluted tubules, medullary rays (straight tubules) and collecting ducts. Prostate: acini and fibro-muscular stroma. Lung: bronchi, bronchioles, alveolar ducts, alveoli and pleura. Urinary bladder: urothelium, lamina propria, muscularis propria, and serosa. Testis: seminiferous tubules with intra-tubular sperms. CONCLUSION: Light-CT™ is a powerful imaging tool to perform fast histology on fresh and fixed tissues, without introducing artifacts. Its compact size, ease of handling, fast image acquisition and safe incident light levels makes it well-suited for various intra-operative and intra-procedural triaging and decision making applications.

Free full text article available from PubMed
PMID: 21773059
Note: Also cited in Section 4.3

D) Optical Frequency Domain Imaging of Ex vivo Pulmonary Resection Specimens: Obtaining One to One Image to Histopathology Correlation

Summary: This article includes a video demonstration of airway centered OFDI with a specialized custom built bronchoscopic 2.4 French (0.8 mm diameter) catheter. Tissue samples were marked with tissue dye, visible in both OFDI and histology. Careful orientation procedures were used to precisely correlate imaging and histological sampling locations. The techniques outlined in this manuscript were used to conduct the first demonstration of volumetric OFDI with precise correlation to tissue-based diagnosis for evaluating pulmonary pathology[24]. This straightforward, effective technique may be extended to other tissue types to provide precise imaging to histology correlation needed to determine fine imaging features of both normal and diseased tissues.

Free full text article available from Journal of Visualized Experiments
PMID: 23381470
Note: Also cited in Section 5.5
2.4 Multiphoton Microscopy

Multiphoton microscopy (MPM), also known as 2-photon, 3-photon or nonlinear microscopy, is a high resolution fluorescence imaging technique somewhat analogous to confocal microscopy. Both MPM and confocal microscopy can perform optical sectioning and, thus, produce 2-D images (like the MPM image of benign colonic crypts with mild nonspecific chronic inflammation above) or 3-D tomographic images. However, unlike confocal microscopes, multiphoton microscopes do not contain pinhole apertures. Rather, in MPM, optical sectioning is the result of the point spread function formed where 2 or more laser beams coincide – the multiphoton effect. Unlike a pinhole, the multiphoton effect also limits absorption of the excitation light by tissue outside of the plane of focus, increasing imaging depth and reducing photobleaching and phototoxicity. Thus, MM microscopy produces higher resolution (subcellular), higher contrast images at greater depth within the tissue than confocal fluorescence microscopy.

MPM is more instrument intensive than confocal microscopy, so work is only beginning now to adopt MPM microscopy for use during endoscopy. So, as of now, MPM is not as far down the path toward clinical use as confocal microscopy or OCT.

The following are selected review articles that explain the basic principles behind, instrumentation used for and potential clinical applications of MPM.

A) Live Tissue Intrinsic Emission Microscopy Using Multiphoton-Excited Native Fluorescence And Second Harmonic Generation


Summary: Multicolor nonlinear microscopy of living tissue using two- and three-photon-excited intrinsic fluorescence combined with second harmonic generation by supermolecular structures produces images with the resolution and detail of standard histology without the use of exogenous stains. Imaging of intrinsic indicators within tissue, such as nicotinamide adenine dinucleotide, retinol, indoleamines, and collagen provides crucial information for physiology and pathology. The efficient application of multiphoton microscopy to intrinsic imaging requires knowledge of the nonlinear optical properties of specific cell and tissue components. Here we compile and demonstrate applications involving a range of intrinsic molecules and molecular assemblies that enable direct visualization of tissue morphology, cell metabolism, and disease states such as Alzheimer’s disease and cancer.

Free full text article available from PubMed
B) **Nonlinear Magic: Multiphoton Microscopy In The Biosciences**  

**Summary:** Multiphoton microscopy (MPM) has found a niche in the world of biological imaging as the best noninvasive means of fluorescence microscopy in tissue explants and living animals. Coupled with transgenic mouse models of disease and ‘smart’ genetically encoded fluorescent indicators, its use is now increasing exponentially. Properly applied, it is capable of measuring calcium transients 500 micron deep in a mouse brain, or quantifying blood flow by imaging shadows of blood cells as they race through capillaries. With the multitude of possibilities afforded by variations of nonlinear optics and localized photochemistry, it is possible to image collagen fibrils directly within tissue through nonlinear scattering, or release caged compounds in sub-femtoliter volumes.

Full text article available from [Nature Biotechnology](https://www.nature.com/articles/nbt200311-1369) ($32.00 USD)  
PMID: 14595365

### 2.5 Optical Spectroscopy and Spectroscopic Imaging

![Diffuse Reflectance Image](image)


The term ‘optical spectroscopy’ refers to a number of optical techniques that assess the way in which the spectrum of light is changed by interaction with tissue. In addition to tissue architecture, optical spectroscopy techniques can provide quantitative measures of physically or physiologically meaningful tissue properties, such as chemical composition, tissue density, metabolism (NADH concentration), angiogenesis (hemoglobin concentration and oxygenation), nuclear/cytoplasmic ratio and fibrosis (collagen), some of which cannot be obtained by histology. These measures can then be translated into disease diagnoses or predictions of prognosis or response to therapy. Optical spectroscopy can be performed as a stand-alone technique, often through the use of hand-held fiberoptic probes, or piggy-backed onto IVM or other advanced imaging systems.

Optical spectroscopy techniques currently in development for disease diagnosis include diffuse reflectance, fluorescence and Raman spectroscopy. Diffuse reflectance spectroscopy measures the absorption or scattering of light by chemicals (such as hemoglobin) or particles (such as cell nuclei or collagen fibers) in the tissue. Fluorescence spectroscopy measures the fluorescence emitted by fluorescent chemicals (fluorophores) naturally occurring in tissue, such as collagen, elastin, NADH and calcifications. Raman spectroscopy provides a detailed chemical fingerprint of the tissue, by measuring spectral changes that occur when specific chemical bonds in tissue begin to vibrate when exposed to light. These spectroscopic measurements can be translated into false color spectroscopic images (such as the diffuse reflectance image of a cross section of bone and soft tissues of the forearm above). Like MPM,
optical spectroscopy and spectroscopic imaging are not as far down the path toward clinical use as confocal microscopy or OCT.

The following are selected review articles that explain the basic principles behind, instrumentation used for and potential clinical applications of these optical spectroscopy and imaging techniques.

A) **Advances in Quantitative UV-Visible Spectroscopy for Clinical and Pre-Clinical Application in Cancer**

   **Summary:** Methods of optical spectroscopy that provide quantitative, physically or physiologically meaningful measures of tissue properties are an attractive tool for the study, diagnosis, prognosis, and treatment of various cancers. Recent development of methodologies to convert measured reflectance and fluorescence spectra from tissue to cancer-relevant parameters such as vascular volume, oxygenation, extracellular matrix extent, metabolic redox states, and cellular proliferation have significantly advanced the field of tissue optical spectroscopy. The number of publications reporting quantitative tissue spectroscopy results in the UV-visible wavelength range has increased sharply in the past three years, and includes new and emerging studies that correlate optically measured parameters with independent measures such as immunohistochemistry, which should aid in increased clinical acceptance of these technologies.


   PMID: 19268567

B) **Book Chapter: Raman Spectroscopy Diagnosis of Breast Cancer and Atherosclerosis: A Primer**

   Book available from [Amazon.com](https://www.amazon.com)

   Note: Also cited in Section 3.2

C) **Diagnostic Applications of Raman Spectroscopy**

   **Summary:** Raman spectroscopy has been widely used in various fields of science. It has been successfully utilized to qualitatively and quantitatively determine the molecular compositions of solid, liquid, and gaseous samples. This review focuses on the diagnostic applications of Raman spectroscopy in the past 5 years, with specific emphasis on transplant allograft rejection and cancer detections. First we introduce the principle of Raman spectroscopy and associated surface enhancement techniques. Various recent biomedical and clinical applications of Raman spectroscopy are then reviewed in detail. Finally, we present the experimental and analytical techniques required to implement Raman spectroscopy in a laboratory. FROM THE CLINICAL EDITOR: This review focuses on evolving diagnostic applications of Raman spectroscopy with special emphasis on transplant allograft rejection and cancer detection.
### Section 3 Insights from Early Adopters

Opinions expressed in this section are the authors’ own and do not necessarily reflect an endorsement by CAP of any organizations, equipment, reagents, materials or services used by participating laboratories.

#### 3.1 Guillermo (Gary) J. Tearney, MD, PhD, FCAP

Guillermo (Gary) J. Tearney, MD, PhD, FCAP is a Professor of Pathology at Massachusetts General Hospital and Harvard Medical School. He is also the Associate Director of the Wellman Center for Photomedicine. Dr. Tearney is board certified in Anatomic Pathology. His research interests include the development and clinical validation of in vivo microscopy technologies. Dr. Tearney is the co-chair of the College of American Pathologists’ In Vivo Microscopy Work Group.

Dr. Tearney’s insights for the next wave of In Vivo Microscopy (and Diagnostic Spectroscopy) adopters (April 2012):

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<tr>
<td><strong>1</strong></td>
<td>IVM is an emerging field</td>
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<td><strong>2</strong></td>
<td>Huge opportunity for pathologists</td>
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<td><strong>3</strong></td>
<td>Keep learning</td>
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<td><strong>4</strong></td>
<td>Get involved</td>
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<td><strong>5</strong></td>
<td>Try it</td>
</tr>
<tr>
<td><strong>6</strong></td>
<td>Define your role</td>
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In Vivo Microscopy articles recommended by Dr. Tearney:

A) Miami Classification for Probe-Based Confocal Laser Endomicroscopy

Summary: An essential element for any new advanced imaging technology is standardization of indications, terminology, categorization of images, and research priorities. In this review, we propose a state-of-the-art classification system for normal and pathological states in gastrointestinal disease using probe-based confocal laser endomicroscopy (pCLE). The Miami classification system is based on a consensus of pCLE users reached during a meeting held in Miami, Florida, in February 2009.

Full text article available from Endoscopy (USD 33.00)
PMID: 21818734
NOTE: Also cited in Section 2.2, 3.3., 5.1.1, 6.2

B) Confocal Laser Endomicroscopy: Technical Advances and Clinical Applications

Summary: Since its introduction in 2004, confocal laser endomicroscopy (CLE) has emerged as a valuable tool for gastrointestinal endoscopic imaging. Endomicroscopy enables the endoscopist to obtain real time in vivo histology during ongoing endoscopy thereby creating “optical biopsies.” To date, numerous studies have shown potential applications of endomicroscopy in the clinical setting, including in vivo diagnosis of esophageal squamous cell carcinoma, Barrett’s esophagus, celiac disease, and colonic polyps. Moreover, recent data suggest the potential application of endomicroscopy in the field of molecular imaging. Additionally, in recent months new applications and developments in the field of confocal imaging were introduced including endomicroscopy of the liver, pancreatic, and bile ducts. Furthermore, by introducing a new needle-based confocal imaging system, which is small enough to be introduced through a 22-gauge puncture needle, a wide field for new applications of endomicroscopic imaging has been opened. Currently, 2 CE- and US Food and Drug Administration (FDA)-approved devices for endomicroscopy are available (Figures 1 and 2; Supplementary Table 1). In this review, we introduce both systems and discuss new technical advances and clinical applications of CLE.

Free full text article available from Gastroenterology
PMID: 20561523
Note: Also cited in Section 5.1.1

C) Consensus Standards for Acquisition, Measurement, and Reporting of Intravascular Optical Coherence Tomography Studies: A Report from the International Working Group for Intravascular Optical Coherence Tomography Standardization and Validation
**Summary:** The purpose of this document is to make the output of the International Working Group for Intravascular Optical Coherence Tomography (IWG-IVOCT) Standardization and Validation available to medical and scientific communities, through a peer-reviewed publication, in the interest of improving the diagnosis and treatment of patients with atherosclerosis, including coronary artery disease. **BACKGROUND:** Intravascular optical coherence tomography (IVOCT) is a catheter-based modality that acquires images at a resolution of ~10 μm, enabling visualization of blood vessel wall microstructure in vivo at an unprecedented level of detail. IVOCT devices are now commercially available worldwide, there is an active user base, and the interest in using this technology is growing. Incorporation of IVOCT in research and daily clinical practice can be facilitated by the development of uniform terminology and consensus-based standards on use of the technology, interpretation of the images, and reporting of IVOCT results. **METHODS:** The IWG-IVOCT, comprising more than 260 academic and industry members from Asia, Europe, and the United States, formed in 2008 and convened on the topic of IVOCT standardization through a series of 9 national and international meetings. **RESULTS:** Knowledge and recommendations from this group on key areas within the IVOCT field were assembled to generate this consensus document, authored by the Writing Committee, composed of academicians who have participated in meetings and/or writing of the text. **CONCLUSIONS:** This document may be broadly used as a standard reference regarding the current state of the IVOCT imaging modality, intended for researchers and clinicians who use IVOCT and analyze IVOCT data. Free full text article available from Journal of the American College of Cardiology. PMID: 22421299

**NOTE:** Also cited in Section 6.1

D) **Comprehensive Microscopy of the Esophagus in Human Patients with Optical Frequency Domain Imaging**


**Summary:** Optical coherence tomography (OCT) is a cross-sectional, high-resolution imaging modality that has been shown to accurately differentiate esophageal specialized intestinal metaplasia (SIM) from gastric cardia at the squamocolumnar junction (SCJ) and diagnose high-grade dysplasia and intramucosal carcinoma in patients with SIM. The clinical utility of OCT has been limited, however, by its inability to acquire images over large areas. **OBJECTIVE:** The aim of this study was to use recently developed high-speed OCT technology, termed optical frequency domain imaging (OFDI), and a new balloon-centering catheter (2.5 cm diameter) to demonstrate the feasibility of large area, comprehensive optical microscopy of the entire distal esophagus (approximately 6.0 cm) in patients. **DESIGN:** A pilot feasibility study. **SETTING:** Massachusetts General Hospital. **PATIENTS:** Twelve patients undergoing routine EGD. **RESULTS:** Comprehensive microscopy of the distal esophagus was successfully performed in 10 patients with the OFDI system and balloon catheter. There were no complications resulting from the imaging procedure. Volumetric data sets were acquired in less than 2 minutes. OFDI images at the SCJ showed a variety of microscopic features that were consistent with histopathologic findings, including squamous mucosa, cardia, SIM with and without dysplasia, and esophageal erosion. **LIMITATIONS:** Inability to obtain direct correlation of OFDI data and histopathologic diagnoses. **CONCLUSIONS:** Comprehensive volumetric microscopy of the human distal esophagus was successfully demonstrated with OFDI and a balloon-centering catheter, providing a wealth of
detailed information about the structure of the esophageal wall. This technique will support future studies to compare OFDI image information with histopathologic diagnoses.

Free full text article available from PubMed
PMID: 18926183
NOTE: Also cited in Section 5.1.1.2

3.2 Maryann Fitzmaurice, MD, PhD, FCAP

Maryann Fitzmaurice, MD, PhD, FCAP is an Adjunct Associate Professor of Pathology in the School of Medicine at Case Western Reserve University in Cleveland, Ohio, and Chief Medical Advisor to the Laser Biomedical Research Center at the Massachusetts Institute of Technology in Cambridge, Massachusetts. Dr. Fitzmaurice is a board certified surgical pathologist with expertise in cardiovascular, breast and transplant pathology. Her research focuses on development of novel, cutting-edge technologies for real time in vivo diagnosis at the bedside using optical spectroscopy and imaging.

She is also-a member of the College of American Pathologists' In Vivo Microscopy Work Group and chair of the IVM Resource Guide team.

Dr. Fitzmaurice’s insights for the next wave of In Vivo Microscopy (and Diagnostic Spectroscopy) adopters (April 2012):

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<tbody>
<tr>
<td>1</td>
<td>Educate yourself</td>
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<td>2</td>
<td>Don’t be intimidated</td>
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<td>3</td>
<td>Keep an open mind</td>
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<td>4</td>
<td>Identify opportunities</td>
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<td>5</td>
<td>Look in your own backyard</td>
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Get your feet wet
Get started by collaborating with other pathologists or clinical researchers at your (or nearby) institution(s). Pathologist input is greatly needed in bringing these technologies into clinical practice.

Share the wealth
Share what you have learned with your colleagues, especially residents who will likely use these new technologies in their future practice.

In Vivo Microscopy and Diagnostic Optical Spectroscopy articles authored by Dr. Fitzmaurice:

A) Portable Optical Fiber Probe-Based Spectroscopic Scanner for Rapid Cancer Diagnosis: A New Tool for Intraoperative Margin Assessment

**Summary:** There continues to be a significant clinical need for rapid and reliable intraoperative margin assessment during cancer surgery. Here we describe a portable, quantitative, optical fiber probe-based, spectroscopic tissue scanner designed for intraoperative diagnostic imaging of surgical margins, which we tested in a proof of concept study in human tissue for breast cancer diagnosis. The tissue scanner combines both diffuse reflectance spectroscopy (DRS) and intrinsic fluorescence spectroscopy (IFS), and has hyperspectral imaging capability, acquiring full DRS and IFS spectra for each scanned image pixel. Modeling of the DRS and IFS spectra yields quantitative parameters that reflect the metabolic, biochemical and morphological state of tissue, which are translated into disease diagnosis. The tissue scanner has high spatial resolution (0.25 mm) over a wide field of view (10 cm × 10 cm), and both high spectral resolution (2 nm) and high spectral contrast, readily distinguishing tissues with widely varying optical properties (bone, skeletal muscle, fat and connective tissue). Tissue-simulating phantom experiments confirm that the tissue scanner can quantitatively measure spectral parameters, such as hemoglobin concentration, in a physiologically relevant range with a high degree of accuracy (<5% error). Finally, studies using human breast tissues showed that the tissue scanner can detect small foci of breast cancer in a background of normal breast tissue. This tissue scanner is simpler in design, images a larger field of view at higher resolution and provides a more physically meaningful tissue diagnosis than other spectroscopic imaging systems currently reported in literatures. We believe this spectroscopic tissue scanner can provide real-time, comprehensive diagnostic imaging of surgical margins in excised tissues, overcoming the sampling limitation in current histopathology margin assessment. As such it is a significant step in the development of a platform technology for intraoperative management of cancer, a clinical problem that has been inadequately addressed to date.

Free full text article available from [PubMed](https://pubmed.ncbi.nlm.nih.gov/22303465) and [PLoS One](https://doi.org/10.1371/journal.pone.0030887)
PMID: 22303465
Note: Also cited in Section 4.1.1

B) Application of Raman Spectroscopy to Identify Microcalcifications and Underlying Breast Lesions at Stereotactic Core Needle Biopsy
Summary: Microcalcifications are a feature of diagnostic significance on a mammogram and a target for stereotactic breast needle biopsy. Here, we report development of a Raman spectroscopy technique to simultaneously identify microcalcification status and diagnose the underlying breast lesion, in real-time, during stereotactic core needle biopsy procedures. Raman spectra were obtained ex vivo from 146 tissue sites from fresh stereotactic breast needle biopsy tissue cores from 33 patients, including 50 normal tissue sites, 77 lesions with microcalcifications, and 19 lesions without microcalcifications, using a compact clinical system. The Raman spectra were modeled based on the breast tissue components and a support vector machine framework was used to develop a single-step diagnostic algorithm to distinguish normal tissue, fibrocystic change (FCC), fibroadenoma (FA) and breast cancer, in the absence and presence of microcalcifications. This algorithm was subjected to leave-one-site-out cross-validation, yielding a positive predictive value, negative predictive value, sensitivity and specificity of 100%, 95.6%, 62.5% and 100% for diagnosis of breast cancer (with or without microcalcifications) and an overall accuracy of 82.2% for classification into specific categories of normal tissue, FCC, FA or breast cancer (with and without microcalcifications). Notably, the majority of breast cancers diagnosed are ductal carcinoma in situ (DCIS), the most common lesion associated with microcalcifications, which could not be diagnosed using previous Raman algorithm(s). Our study demonstrates the potential of Raman spectroscopy to concomitantly detect microcalcifications and diagnose associated lesions, including DCIS, and thus provide real-time feedback to radiologists during such biopsy procedures, reducing non-diagnostic and false negative biopsies.

Full text article available from Cancer Research (USD 35.00)
PMID: 23729641
Note: Also cited in Section 5.2.2

C) Multimodal Spectroscopy In Vivo Detects Features of Vulnerable Atherosclerotic Plaque

Summary: Early detection and treatment of rupture-prone vulnerable atherosclerotic plaques is critical to reducing patient mortality associated with cardiovascular disease. The combination of reflectance, fluorescence, and Raman spectroscopy-termed multimodal spectroscopy (MMS)-provides detailed biochemical information about tissue and can detect vulnerable plaque features: thin fibrous cap (TFC), necrotic core (NC), superficial foam cells (SFC), and thrombus. Ex vivo MMS spectra are collected from 12 patients that underwent carotid endarterectomy or femoral bypass surgery. Data are collected by means of a unitary MMS optical fiber probe and a portable clinical instrument. Blinded histopathological analysis is used to assess the vulnerability of each spectrally evaluated artery lesion. Modeling of the ex vivo MMS spectra produce objective parameters that correlate with the presence of vulnerable plaque features: TFC with fluorescence parameters indicative of collagen presence; NC/SFC with a combination of diffuse reflectance β-carotene/ceroid absorption and the Raman spectral signature of lipids; and thrombus with its Raman signature. Using these parameters, suspected vulnerable plaques can be detected with a sensitivity of 96% and specificity of 72%. These encouraging results warrant the continued development of MMS as a catheter-based clinical diagnostic technique for early detection of vulnerable plaques.

Free full text article available from PubMed
D) **Principles and Pitfalls of Diagnostic Test Development: Implications for Spectroscopic Tissue Diagnosis**


**Summary:** Diagnostic spectroscopy has the potential to supplant the time-honored "gold standard" of light microscopy and herald an era of in vivo tissue diagnosis. However, the lessons in disease diagnosis learned by pathologists over the years should not be forgotten. This discussion will focus on the basic principles and pitfalls of diagnostic test development, and how they apply to optical spectroscopy tissue diagnosis.

Full text article available from *Journal of Biomedical Optics* (USD 25.00)

PMID: 10938775
Note: Also cited in Section 6.1

Dr. Fitzmaurice's suggestions for articles to read:

A) **Point-Of-Care Pathology with Miniature Microscopes**


**Summary:** Advances in optical designs are enabling the development of miniature microscopes that can examine tissue in situ for early anatomic and molecular indicators of disease, in real time, and at cellular resolution. These new devices will lead to major changes in how diseases are detected and managed, driving a shift from today's diagnostic paradigm of biopsy followed by histopathology and recommended therapy, to non-invasive point-of-care diagnosis with possible same-session definitive treatment. This shift may have major implications for the training requirements of future physicians to enable them to interpret real-time in vivo microscopic data, and will also shape the emerging fields of telepathology and telemedicine. Implementation of new technologies into clinical practice is a complex process that requires bridging gaps between clinicians, engineers and scientists. This article provides a forward-looking discussion of these issues, with a focus on malignant and pre-malignant lesions, by first highlighting some of the clinical areas where point-of-care in vivo microscopy could address unmet needs, and then by reviewing the technological challenges that are being addressed, or need to be addressed, for in vivo microscopy to become a standard clinical tool.

Free full text article available from PubMed
PMID: 21673433

B) **Validation of Novel Optical Imaging Technologies: The Pathologists' View**


**Summary:** Noninvasive optical imaging technology has the potential to improve the accuracy of disease detection and predict treatment response. Pathology provides the critical link between the biological basis of an image or spectral signature and clinical outcomes obtained through optical imaging. The validation of optical images and
spectra requires both morphologic diagnosis from histopathology and parametric analysis of tissue features above and beyond the declared pathologic "diagnosis." Enhancement of optical imaging modalities with exogenously applied biomarkers also requires validation of the biological basis for molecular contrast. For an optical diagnostic or prognostic technology to be useful, it must be clinically important, independently informative, and of demonstrated beneficial value to patient care. Its usage must be standardized with regard to methods, interpretation, reproducibility, and reporting, in which the pathologist plays a key role. By providing insight into disease pathobiology, interpretive or quantitative analysis of tissue material, and expertise in molecular diagnosis, the pathologist should be an integral part of any team that is validating novel optical imaging modalities. This review will consider (1) the selection of validation biomarkers; (2) standardization in tissue processing, diagnosis, reporting, and quantitative analysis; (3) the role of the pathologist in study design; and (4) reference standards, controls, and interobserver variability.

Full text article available from Journal of Biomedical Optics (USD 25.00)
PMID: 17994879
NOTE: Also cited in Sections 1.2 and 6.1

3.3 Gregory Y. Lauwers, MD, FCAP

Gregory Y. Lauwers, MD, FCAP is Vice Chairman of Pathology at Massachusetts General Hospital. He is Director of Gastrointestinal Pathology. Also, he is Professor of Pathology at Harvard Medical School. Dr. Lauwers’s research has focused on preneoplastic lesions of the gastrointestinal tract. Dr. Lauwers is also very active in development and interpretation of diagnostic features of IVM images.

Dr. Lauwers is a member of the College of American Pathologists’ In Vivo Microscopy Work Group.

Dr. Lauwers’ insights for the next wave of In Vivo Microscopy (and Diagnostic Spectroscopy) adopters (September 2013):

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<tr>
<th></th>
<th>It is important to realize that IVM is a new way of looking at microscopy</th>
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<tr>
<td>1</td>
<td>We are all neophytes still learning this technique.</td>
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<th>Pathologists are not in the driver’s seat in IVM</th>
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<td>The clinicians are the drivers in IVM for GI today. However, this presents new collaboration opportunities for pathologists. It’s up to the pathologists to convince the endoscopists that they and their patients are better served by pathologists being involved in the interpretation of these optical biopsies, whether at the time of the endoscopy or after the images have been captured.</td>
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Pathologists will have to modify their workflow

Pathologists must extend themselves beyond what they typically do - physically and technologically. For example, either pathologists must go to the endoscopy room where the images are being captured, or pathologists must have images beamed to them for viewing during the examination – modifying the typical workflow of pathologic diagnosis.

Learn about IVM

Read IVM articles and materials put forward by CAP, e.g. the CAP Pathology Resource Guide: IVM. Discuss IVM with leaders in the field. Get a sense of what IVM is and its value. Look at specifics of IVM in relation to various subspecialties (gastrointestinal, cardiovascular, and genitourinary, for example).

Learn IVM Techniques

We should make the effort to correlate images between typical histology and what is acquired by these new microscopy methods. Recognize that the features of standard microscopy are important, but pathologists will have to learn some new diagnostic features as acquired by IVM. For example, evaluating blood flow (and its modifications) in various pathological states that pathologists are not routinely evaluating.

Pathologists should not only help but also guide clinicians in evaluating in vivo images

In this role of collaborator/advisor, pathologists strengthen their position as specialists owning “microscopic” diagnoses. Then pathologists working closely with clinicians in evaluating IVM should determine what specimen is needed and what is not needed – thereby demonstrating added value in their collaboration.

In Vivo Microscopy articles recommended by Dr. Lauwers:

A) Miami Classification for Probe-Based Confocal Laser Endomicroscopy


Summary: An essential element for any new advanced imaging technology is standardization of indications, terminology, categorization of images, and research priorities. In this review, we propose a state-of-the-art classification system for normal and pathological states in gastrointestinal disease using probe-based confocal laser endomicroscopy (pCLE). The Miami classification system is based on a consensus of pCLE users reached during a meeting held in Miami, Florida, in February 2009.

Full text article available from [Endoscopy](http://www.endoscopyonline.com) (USD 33.00)
PMID: 21818734
NOTE: Also cited in Sections 2.2, 3.1, 5.1.1, 6.2
B) **Confocal Endomicroscopy for In Vivo Microscopic Analysis of Upper Gastrointestinal Tract Premalignant and Malignant Lesions**  

**Summary:** Confocal LASER endomicroscopy (CLE) is a new endoscopic technique which allows subsurface in vivo microscopic analysis during ongoing endoscopy, using systemically or topically administered fluorescent agents. It allows targeted biopsies to be taken, potentially improving the diagnostic rate in certain gastrointestinal diseases. Worldwide experience with CLE for upper gastrointestinal malignant and premalignant lesions is still reduced. Potential clinical applications are presented, including diagnosis of NERD, Barrett’s esophagus, atrophic gastritis, gastric intestinal metaplasia and dysplasia, gastric adenomatous or hyperplastic polyps, gastric cancer.

Free full text article available from [Journal of Gastrointestinal and Liver Disease](https://doi.org/10.2185/JGLD.2008.110)  
PMID: 18392254

C) **Diagnosis of Gastric Intraepithelial Neoplasia by Narrow-band Imaging and Confocal Laser Endomicroscopy**  

**Summary:** To evaluate the diagnosis of different differentiated gastric intraepithelial neoplasia (IN) by magnification endoscopy combined with narrow-band imaging (ME-NBI) and confocal laser endomicroscopy (CLE). METHODS: Eligible patients with suspected gastric IN lesions previously diagnosed by endoscopy in secondary hospitals and scheduled for further diagnosis and treatment were recruited for this study. Excluded from the study were patients who had liver cirrhosis, impaired renal function, acute gastrointestinal (GI) bleeding, coagulopathy, esophageal varices, jaundice, and GI post-surgery. Also excluded were those who were pregnant, breastfeeding, were younger than 18 years old, or were unable to provide informed consent. All patients had all mucus and bile cleared from their stomachs. They then received upper GI endoscopy. When a mucosal lesion is found during observation with white-light imaging, the lesion is visualized using maximal magnification, employing gradual movement of the tip of the endoscope to bring the image into focus. Saved images are analyzed. Confocal images were evaluated by two endoscopists (Huang J and Li MY), who were familiar with CLE, blinded to the related information about the lesions, and asked to classify each lesion as either a low grade dysplasia (LGD) or high grade dysplasia (HGD) according to given criteria. The results were compared with the final histopathologic diagnosis. ME-NBI images were evaluated by two endoscopists (Lu ZS and Ling-Hu EQ) who were familiar with NBI, blinded to the related information about the lesions and CLE images, and were asked to classify each lesion as a LGD or HGD according to the “microvascular pattern and surface pattern” classification system. The results were compared with the final histopathologic diagnosis. RESULTS: The study included 32 pathology-proven low grade gastric IN and 26 pathology-proven high grade gastric IN that were detected with any of the modalities. CLE and ME-NBI enabled clear visualization of the vascular microsurface patterns and microvascular structures of the gastric mucosa. The accuracy of the CLE and the ME-NBI diagnosis was 88% (95% CI: 78%-98%) and 81% (95% CI: 69%-93%), respectively. The kappa coefficient of agreement between the histopathology and the in vivo CLE imaging was 0.755; between the histopathology and the in vivo CLE imaging
was 0.615. McNemar's test (binomial distribution used) indicated that the agreement was significant (P < 0.05). When patients were diagnosed by ME-NBI with CLE, the overall accuracy of the diagnosis was 86.21% (95% CI: 73%-96%), and the kappa coefficient of agreement was 0.713, according to McNemar's test (P < 0.05). **CONCLUSION:** Higher diagnostic accuracy, sensitivity and specificity of CLE over ME-NBI indicate the feasibility of these two techniques for the efficacious diagnostic classification of gastric IN.

Free full text article available from PubMed
PMID: 23002348

**D) Functional Imaging of Colonic Mucosa with a Fibered Confocal Microscope for Real-time In Vivo Pathology**


**Summary:** Histologic interpretation of disease currently is performed with static images of excised tissues, and is limited by processing artifact, sampling error, and interpretive variability. The aim of this study was to show the use of functional optical imaging of viable mucosa for quantitative evaluation of colonic neoplasia in real time. **METHODS:** Fluorescein (5 mg/mL) was administered topically in 54 human subjects undergoing screening colonoscopy. Fluorescence images were collected with 488-nm excitation at 12 frames/s with the confocal microendoscopy system. Movement of fluorescein in the transient period (<5 s) and the lamina propria:crypt contrast ratio in the steady-state phase (>5 s) were quantified. **RESULTS:** Normal mucosa showed circular crypts with uniform size, hyperplasia revealed proliferative glands with serrated lumens, and adenomas displayed distorted elongated glands. For t less than 5 seconds, fluorescein passed through normal epithelium with a peak speed of 1.14 +/- 0.09 microm/s at t = 0.5 seconds, and accumulated into lamina propria as points of fluorescence that moved through the interglandular space with an average speed of 41.7 +/- 3.4 microm/s. Passage of fluorescein through adenomatous mucosa was delayed substantially. For t greater than 5 seconds, high sensitivity, specificity, and accuracy was achieved using a discriminant function to evaluate the contrast ratio to distinguish normal from lesional mucosa (91%, 87%, and 89%, respectively; P < .001), hyperplasia from adenoma (97%, 96%, and 96%, respectively; P < .001), and tubular from villous adenoma (100%, 92%, and 93%, respectively; P < .001). **CONCLUSIONS:** Confocal imaging can be performed in vivo to assess the functional behavior of tissue in real time for providing pathologic interpretation, representing a new method for histologic evaluation.

Free full text article available from PubMed
PMID: 17936692

**E) In Vivo Histopathology for Detection of Gastrointestinal Neoplasia with a Portable, Confocal Miniprobe: An Examiner Blinded Analysis**


**Summary:** Confocal fluorescence microscopy (CFM) has been mentioned to be a promising tool for in vivo histology. Recently, a portable confocal miniprobe has been developed. Our aim was to evaluate the potential benefit of CFM for detection of gastrointestinal neoplasia. **METHODS:** A total of 47 patients with known or suspected
neoplasia in the upper (n = 34) or lower gastrointestinal tract (n = 13) were examined with standard endoscopes. After mucolyis with 5-10 mL of acetic acid 1.5%, chromoendoscopy with 2-5 mL cresyl violet 0.25% was performed, with the substance also being used as a fluorophore for CFM. Real-time video sequences were recorded. Thereafter, biopsies were taken or mucosectomy/polypectomy was performed from the same examined area. All stored sequences were put into a random order and assessed by a pathologist and a gastroenterologist both blinded to any data. RESULTS: A total of 119 CFM video sequences were recorded of 85 benign or 34 neoplastic areas. Quality of CFM images was regarded too low in 24 (pathologist) and 14 sequences (gastroenterologist). For the pathologist, accuracy of CFM detecting neoplasia was 92.6% (suitable images) and 73.9% (intention to diagnose). The respective accuracy values for the gastroenterologist were 92.4% (suitable images) and 81.5% (intention to diagnose). Agreement between CFM and histopathology was excellent (kappa values, 0.821 and 0.817). CONCLUSIONS: We have demonstrated that CFM with a miniprobe has the potential to diagnose neoplasia during ongoing endoscopy. This system has the advantage that it can be used with standard endoscopes. Further studies are warranted for validation.

Free full text article available from Clinical Gastroenterology and Hepatology
PMID: 17689297
Section 4 Ex Vivo Pathology Applications of IVM

Reprinted from Keshtgar et al41, British Journal of Surgery, 2010;97(8):1232-1239, with permission from John Wiley & Sons, Ltd.

IVM technologies are being developed to address clinical diagnostic problems in a number of organ systems where more conventional imaging technologies, such as white light endoscopy, cardiac catheterization, x-ray mammography, CT and MRI, have diagnostic limitations. IVM technologies are being developed as clinical tools for in vivo use to allow our colleagues to better target biopsies to diseased tissue, screen entire organs for microscopic disease, etc. But IVM technologies are also being developed as tools to address problems frequently encountered by pathologists in their practice. Thus IVM technologies are also being developed for ex vivo use to perform more accurate intraoperative margin and sentinel lymph node assessments (as seen in the elastic light scattering (left) and H&E images (right) of an axillary lymph node metastasis above), assess adequacy of needle biopsies, and improve sampling of surgical specimens in the grossing room. Although confocal microscopy and OCT are being explored for these ex vivo pathology applications, optical spectroscopy and spectroscopic imaging are the leading technologies in development in this area at this time. In fact, the FDA has recently approved a hand held device for intraoperative margin assessment based on radio frequency, rather than optical, spectroscopy.

It is critically important that pathologists be well versed in IVM if they are to adopt these new optical imaging technologies in their day-to-day practice.

The following articles illustrate the current state-of-the-art for IVM for:

- Intraoperative assessment of margins of resection;
- Intraoperative assessment of sentinel lymph nodes;
- Specimen sampling in the grossing room; and
- Virtual histology
4.1 Surgical Margins of Resection

4.1.1 Breast Margins

A) Intraoperative Evaluation of Breast Tumor Margins with Optical Coherence Tomography


Summary: As breast cancer screening rates increase, smaller and more numerous lesions are being identified earlier, leading to more breast-conserving surgical procedures. Achieving a clean surgical margin represents a technical challenge with important clinical implications. Optical coherence tomography (OCT) is introduced as an intraoperative high-resolution imaging technique that assesses surgical breast tumor margins by providing real-time microscopic images up to 2 mm beneath the tissue surface. In a study of 37 patients split between training and study groups, OCT images covering 1 cm(2) regions were acquired from surgical margins of lumpectomy specimens, registered with ink, and correlated with corresponding histologic sections. A 17-patient training set used to establish standard imaging protocols and OCT evaluation criteria showed that areas of higher scattering tissue with a heterogeneous pattern were indicative of tumor cells and tumor tissue in contrast to lower scattering adipocytes found in normal breast tissue. The remaining 20 patients were enrolled into the feasibility study. Of these lumpectomy specimens, 11 were identified with a positive or close surgical margin and 9 were identified with a negative margin under OCT. Based on histologic findings, 9 true positives, 9 true negatives, 2 false positives, and 0 false negatives were found, yielding a sensitivity of 100% and specificity of 82%. These results show the potential of OCT as a real-time method for intraoperative margin assessment in breast-conserving surgeries.

Free full text article available from PubMed
PMID: 19910294
Note: Also cited in Section 5.2.3

B) Integrated Optical Coherence Tomography and Microscopy for Ex Vivo Multiscale Evaluation of Human Breast Tissues


Summary: Three-dimensional (3D) tissue imaging methods are expected to improve surgical management of cancer. In this study, we examined the feasibility of two 3D imaging technologies, optical coherence tomography (OCT) and optical coherence microscopy (OCM), to view human breast specimens based on intrinsic optical contrast. Specifically, we imaged 44 ex vivo breast specimens including 34 benign and 10 malignant lesions with an integrated OCT and OCM system developed in our laboratory. The system enabled 4-mum axial resolution (OCT and OCM) with 14-mum (OCT) and 2-mum (OCM) transverse resolutions, respectively. OCT and OCM images were compared with corresponding histologic sections to identify characteristic features from benign and malignant breast lesions at multiple resolution scales. OCT
and OCM provide complimentary information about tissue microstructure, thus showing distinctive patterns for adipose tissue, fibrous stroma, breast lobules and ducts, cysts and microcysts, as well as in situ and invasive carcinomas. The 3D imaging capability of OCT and OCM provided complementary information to individual 2D images, thereby allowing tracking features from different levels to identify low-contrast structures that were difficult to appreciate from single images alone. Our results lay the foundation for future in vivo optical evaluation of breast tissues, using OCT and OCM, which has the potential to guide core needle biopsies, assess surgical margins, and evaluate nodal involvement in breast cancer.

Free full text article available from Cancer Research
PMID: 21056988
NOTE: Also cited in Section 5.2.4

C) Portable Optical Fiber Probe-based Spectroscopic Scanner for Rapid Cancer Diagnosis: A New Tool for Intraoperative Margin Assessment

Summary: There continues to be a significant clinical need for rapid and reliable intraoperative margin assessment during cancer surgery. Here we describe a portable, quantitative, optical fiber probe-based, spectroscopic tissue scanner designed for intraoperative diagnostic imaging of surgical margins, which we tested in a proof of concept study in human tissue for breast cancer diagnosis. The tissue scanner combines both diffuse reflectance spectroscopy (DRS) and intrinsic fluorescence spectroscopy (IFS), and has hyperspectral imaging capability, acquiring full DRS and IFS spectra for each scanned image pixel. Modeling of the DRS and IFS spectra yields quantitative parameters that reflect the metabolic, biochemical and morphological state of tissue, which are translated into disease diagnosis. The tissue scanner has high spatial resolution (0.25 mm) over a wide field of view (10 cm x 10 cm), and both high spectral resolution (2 nm) and high spectral contrast, readily distinguishing tissues with widely varying optical properties (bone, skeletal muscle, fat and connective tissue). Tissue-simulating phantom experiments confirm that the tissue scanner can quantitatively measure spectral parameters, such as hemoglobin concentration, in a physiologically relevant range with a high degree of accuracy (<5% error). Finally, studies using human breast tissues showed that the tissue scanner can detect small foci of breast cancer in a background of normal breast tissue. This tissue scanner is simpler in design, images a larger field of view at higher resolution and provides a more physically meaningful tissue diagnosis than other spectroscopic imaging systems currently reported in literatures. We believe this spectroscopic tissue scanner can provide real-time, comprehensive diagnostic imaging of surgical margins in excised tissues, overcoming the sampling limitation in current histopathology margin assessment. As such it is a significant step in the development of a platform technology for intraoperative management of cancer, a clinical problem that has been inadequately addressed to date.

Free full text article available from PLOS One
PMID: 22303465
Note: Also cited in Section 3.2
D) Scatter Spectroscopic Imaging Distinguishes Between Breast Pathologies in Tissues Relevant to Surgical Margin Assessment

Summary: A new approach to spectroscopic imaging was developed to detect and discriminate microscopic pathologies in resected breast tissues; diagnostic performance of the prototype system was tested in 27 tissues procured during breast conservative surgery. EXPERIMENTAL DESIGN: A custom-built, scanning in situ spectroscopy platform sampled broadband reflectance from a 150-mum-diameter spot over a 1 x 1 cm(2) field using a dark field geometry and telecentric lens; the system was designed to balance sensitivity to cellular morphology and imaging the inherent diversity within tissue subtypes. Nearly 300,000 broadband spectra were parameterized using light scattering models and spatially dependent spectral signatures were interpreted using a cooccurrence matrix representation of image texture. RESULTS: Local scattering changes distinguished benign from malignant pathologies with 94% accuracy, 93% sensitivity, 95% specificity, and 93% positive and 95% negative predictive values using a threshold-based classifier. Texture and shape features were important to optimally discriminate benign from malignant tissues, including pixel-to-pixel correlation, contrast and homogeneity, and the shape features of fractal dimension and Euler number. Analysis of the region-based diagnostic performance showed that spectroscopic image features from 1 x 1 mm(2) areas were diagnostically discriminant and enabled quantification of within-class tissue heterogeneities. CONCLUSIONS: Localized scatter-imaging signatures detected by the scanning spectroscopy platform readily distinguished benign from malignant pathologies in surgical tissues and showed new spectral-spatial signatures of clinical breast pathologies.

Full text article available from Clinical Cancer Research (USD 35.00) PMID: 22908098
Note: Also cited in Section 5.2.3

E) Development of a Spatially Offset Raman Spectroscopy Probe for Breast Tumor Surgical Margin Evaluation

Summary: The risk of local recurrence for breast cancers is strongly correlated with the presence of a tumor within 1 to 2 mm of the surgical margin on the excised specimen. Previous experimental and theoretical results suggest that spatially offset Raman spectroscopy (SORS) holds much promise for intraoperative margin analysis. Based on simulation predictions for signal-to-noise ratio differences among varying spatial offsets, a SORS probe with multiple source-detector offsets was designed and tested. It was then employed to acquire spectra from 35 frozen-thawed breast tissue samples in vitro. Spectra from each detector ring were averaged to create a composite spectrum with biochemical information covering the entire range from the tissue surface to approximately 2 mm below the surface, and a probabilistic classification scheme was used to classify these composite spectra as "negative" or "positive" margins. This discrimination was performed with 95% sensitivity and 100% specificity, or with 100% positive predictive value and 94% negative predictive value.
F) MarginProbe(R): Intraoperative Margin Assessment During Breast Conserving Surgery by Using Radiofrequency Spectroscopy


Summary: In breast conserving surgery, the tumor should be removed with a clean margin, a rim of healthy tissue surrounding. Failure to achieve clean margins in the initial surgery results in a re-excision procedure. Re-excision rates are reported as being 11-46% for invasive carcinoma and ductal carcinoma in situ (DCIS). Re-excisions can have negative consequences such as increased postoperative infections, negative impact on cosmesis, patient anxiety and increased medical costs. Therefore, the surgical margin of invasive and intraductal (DCIS) breast tissue is a subject of intense discussion. Different options for intraoperative assessment are available, but all in all, they are unsatisfying. Frozen section margin examination is possible but is time consuming and restricted to the assessment of invasive carcinoma. In the case of DCIS, there is no procedure for intraoperative margin assessment. Thus, a solution for efficient intraoperative surgical margin assessment is needed. For this purpose, an innovative, real-time, intraoperative margin-assessment device (MarginProbe(R), Dune Medical Devices, Caesarea, Israel) was designed, and recent published clinical data reported a reduction of re-excisions by more than 50%.

Full text article available from Expert Review of Medical Devices (USD 86.00 for 24 hour access)
PMID: 23668703
NOTE: Also cited in Section 5.2.3

4.1.2 Skin Margins

A) Fast Evaluation of 69 Basal Cell Carcinomas with Ex Vivo Fluorescence Confocal Microscopy: Criteria Description, Histopathological Correlation, and Interobserver Agreement


Summary: Fluorescence confocal microscopy (FCM) represents a first step toward a rapid “bedside pathology” in the Mohs surgery setting and in other fields of general pathology. OBJECTIVE To describe and validate FCM criteria for the main basal cell carcinoma (BCC) subtypes and to demonstrate the overall agreement with classic pathologic analysis of hematoxylin-eosin-stained samples. DESIGN A total of 69 BCCs from 66 patients were prospectively imaged using ex vivo FCM. Confocal mosaics
were evaluated in real time and compared with classic pathologic analysis. SETTING Department of Dermatology, Hospital Clinic of Barcelona, Barcelona, Spain, between November 2010 and July 2011. PARTICIPANTS Patients with BCC attending the Mohs Surgery Unit. MAIN OUTCOMES AND MEASURES Presence or absence of BCC and histological subtype (superficial, nodular, and infiltrating) in the confocal mosaics. Eight criteria for BCC were described, evaluated, and validated. RESULTS Although there were minor differences among BCC subtypes, the most BCC-defining criteria were peripheral palisading, clefting, nuclear pleomorphism, and presence of stroma. These criteria were validated with independent observers (κ values >0.7 for most criteria). CONCLUSIONS AND RELEVANCE We herein propose, describe, and validate FCM criteria for BCC diagnosis. Fluorescence confocal microscopy is an attractive alternative to histopathologic analysis of frozen sections during Mohs surgery because large areas of freshly excised tissue can be assessed in real time without the need for tissue processing while minimizing labor and costs.

Full text article available from JAMA Dermatology (USD 30.00 for 24 hour access)
PMID: 23636776

B) Confocal Microscopy with Strip Mosaicing for Rapid Imaging Over Large Areas of Excised Tissue

Summary: Confocal mosaicing microscopy is a developing technology platform for imaging tumor margins directly in freshly excised tissue, without the processing required for conventional pathology. Previously, mosaicing on 12-x-12 mm(2) of excised skin tissue from Mohs surgery and detection of basal cell carcinoma margins was demonstrated in 9 min. Last year, we reported the feasibility of a faster approach called "strip mosaicing," which was demonstrated on a 10-x-10 mm(2) of tissue in 3 min. Here we describe further advances in instrumentation, software, and speed. A mechanism was also developed to flatten tissue in order to enable consistent and repeatable acquisition of images over large areas. We demonstrate mosaicing on 10-x-10 mm(2) of skin tissue with 1-mum lateral resolution in 90 s. A 2.5-x-3.5 cm(2) piece of breast tissue was scanned with 0.8-mum lateral resolution in 13 min. Rapid mosaicing of confocal images on large areas of fresh tissue potentially offers a means to perform pathology at the bedside. Imaging of tumor margins with strip mosaicing confocal microscopy may serve as an adjunct to conventional (frozen or fixed) pathology for guiding surgery.

Free full text article available from Journal of Biomedical Optics
PMID: 23389736

C) Rapid Screening of Cancer Margins in Tissue with Multimodal Confocal Microscopy

Summary: Complete and accurate excision of cancer is guided by the examination of histopathology. However, preparation of histopathology is labor intensive and slow, leading to insufficient sampling of tissue and incomplete and/or inaccurate excision of margins. We demonstrate the potential utility of multimodal confocal mosaicing microscopy for rapid screening of cancer margins, directly in fresh surgical excisions, without the need for conventional embedding, sectioning, or processing.
MATERIALS AND METHODS: A multimodal confocal mosaicing microscope was developed to image basal cell carcinoma margins in surgical skin excisions, with the resolution that shows nuclear detail. Multimodal contrast is with fluorescence for imaging nuclei and reflectance for cellular cytoplasm and dermal collagen. Thirty-five excisions of basal cell carcinomas from Mohs surgery were imaged, and the mosaics analyzed by comparison with the corresponding frozen pathology. RESULTS: Confocal mosaics are produced in about 9 min, displaying tissue in fields of view of 12 mm with x2 magnification. A digital staining algorithm transforms black and white contrast to purple and pink, which simulates the appearance of standard histopathology. Mosaicing enables rapid digital screening, which mimics the examination of histopathology. CONCLUSIONS: Multimodal confocal mosaicing microscopy offers a technology platform to potentially enable real-time pathology at the bedside. The imaging may serve as an adjunct to conventional histopathology to expedite screening of margins and guide surgery toward more complete and accurate excision of cancer.

Free full text article available from PubMed
PMID: 22721570
Note: Also cited in Section 5.3

4.1.3 GI Margins

A) Confocal Endomicroscopy for In Vivo Prediction of Completeness After Endoscopic Mucosal Resection

Summary: Endoscopic mucosal resection (EMR) is an alternative to surgery for removal of superficial gastric neoplastic lesions. Residual neoplastic tissue of the resection interface is difficult to detect by conventional endoscopy. The aim of this study is to assess the efficacy of confocal laser endomicroscopy (CLE) in predicting complete resection margins after EMR. METHODS: EMR was performed by using cap-assisted or “inject and cut” resection technique. Two weeks after EMR, the circumferential margins of the defect were inspected by using CLE, and completeness of excision was predicted from the CLE image. Additional EMR was performed if necessary. In vivo CLE diagnosis was validated against final histopathology. RESULTS: Twenty-seven lesions were removed by EMR in 27 patients. After excluding 3 patients for gastrectomy, a total of 24 patients underwent CLE assessment, of whom 9 with indefinite lateral margins underwent at least two consecutive CLE follow-ups. A total of 19 lesions were regarded as complete remission, and 5 lesions (21.7%) were incompletely excised according to final pathologic diagnosis. Accuracy of CLE in predicting incomplete resection for original lesions was 91.7%, with sensitivity and specificity of 100.0 and 89.5%, respectively. The residual lesions were treated by additional EMR guided by CLE. There was no recurrence on endoscopic biopsies at mean (range) follow-up of 8.3 (4-15) months. CONCLUSIONS: Confocal laser endomicroscopy has high accuracy for prediction of remnant tissue after EMR, and may lead to significant improvements in clinical surveillance after endoscopic resection.
4.2 Sentinel Lymph Nodes

A) Optical Scanning for Rapid Intraoperative Diagnosis of Sentinel Node Metastases in Breast Cancer

Summary: Intraoperative diagnosis of sentinel node metastases enables an immediate decision to proceed to axillary lymph node dissection, avoiding a second operation in node-positive women with breast cancer. METHODS: An optical scanner was developed that interrogated the cut surface of bivalved, but otherwise unprocessed, sentinel lymph nodes with pulses of white light by elastic scattering spectroscopy (ESS). The scattered light underwent spectral analysis, and individual spectra were initially correlated with conventional histology to develop a diagnostic algorithm. This algorithm was used to create false colour-coded maps of scans from an independent set of nodes, and the optimal criteria for discriminating between normal and cancer spectra were defined statistically. RESULTS: The discriminant algorithm was developed from a training set of 2989 spectra obtained from 30 metastatic and 331 normal nodes. Subsequent scans from 129 independent nodes were analysed. The scanner detected macrometastases (larger than 2 mm) with a sensitivity of 76 per cent (69 per cent including micrometastases) and specificity of 96 per cent. CONCLUSION: In this proof-of-principle study, the ESS results were comparable with current intraoperative diagnostic techniques of lymph node assessment.

B) Raman Spectroscopy--A Potential New Method for the Intra-Operative Assessment of Axillary Lymph Nodes

Summary: Sentinel Lymph Node Biopsy has become the standard surgical procedure for the sampling of axillary lymph nodes in breast cancer. Intra-operative node assessment of these nodes would allow definitive axillary surgery to take place immediately with associated benefits for patient management. Our experimental study aims to demonstrate that a Raman spectroscopy probe system could overcome many of the disadvantages of current intra-operative methods. 59 axillary lymph nodes, 43 negative and 16 positive from 58 patients undergoing breast surgery at our district general hospital were mapped using Raman micro-spectroscopy. These maps were then used to model the effect of using a Raman spectroscopic probe by selecting 5 and 10 probe points across the mapped images and evaluating the impact on disease detection. Results demonstrated sensitivities of up to 81% and specificities of up to 97% when differentiating between positive and negative lymph
nodes, dependent on the number of probe points included. The results would have concurred with histopathology assessment in 89% and 91% of cases in the 5 and 10 point models respectively. Using Raman spectroscopy in this way could allow lymph node assessment within a time-frame suitable for intra-operative use.

Full text article available from The Surgeon (USD 31.50)
PMID: 22525413
Note: Also cited in Section 5.2.4

4.3 Surgical Specimen Sampling

A) Optical Coherence Tomography for Rapid Tissue Screening and Directed Histological Sectioning


Summary: In pathology, histological examination of the tissue is the “gold standard” to diagnose various diseases. It has contributed significantly toward identifying the abnormalities in tissues and cells, but has inherent drawbacks when used for fast and accurate diagnosis. These limitations include the lack of in vivo observation in real time and sampling errors due to limited number and area coverage of tissue sections. Its diagnostic yield also varies depending on the ability of the physician and the effectiveness of any image guidance technique that may be used for tissue screening during excisional biopsy. In order to overcome these current limitations of histology-based diagnostics, there are significant needs for either complementary or alternative imaging techniques which perform non-destructive, high resolution, and rapid tissue screening. Optical coherence tomography (OCT) is an emerging imaging modality which allows real-time cross-sectional imaging with high resolutions that approach those of histology. OCT could be a very promising technique which has the potential to be used as an adjunct to histological tissue observation when it is not practical to take specimens for histological processing, when large areas of tissue need investigating, or when rapid microscopic imaging is needed. This review will describe the use of OCT as an image guidance tool for fast tissue screening and directed histological tissue sectioning in pathology.

Full text article available from IOS Press
PMID: 23542933

B) Modified Full-Field Optical Coherence Tomography: A Novel Tool for Rapid Histology of Tissues


Summary: Here, we report the first use of a commercial prototype of full-field optical coherence tomography called Light-CT™. Based on the principle of white light interferometry, Light-CT™ generates quick high-resolution three-dimensional tomographic images from unprocessed tissues. Its advantage over the current intra-surgical diagnostic standard, i.e. frozen section analysis, lies in the absence of freezing artifacts, which allows real-time diagnostic impressions, and/or for the tissues to be triaged for subsequent conventional histopathology. MATERIALS AND
METHODS: In this study, we recapitulate known normal histology in nine formalin fixed ex vivo rat organs (skin, heart, lung, liver, stomach, kidney, prostate, urinary bladder, and testis). Large surface and virtually sectioned stacks of images at varying depths were acquired by a pair of 10×/0.3 numerical aperture water immersion objectives, processed and visualized in real time. RESULTS: Normal histology of the following organs was recapitulated by identifying various tissue microstructures. Skin: epidermis, dermal-epidermal junction and hair follicles with surrounding sebaceous glands in the dermis. Stomach: mucosa with surface pits, submucosa, muscularis propria and serosa. Liver: hepatocytes separated by sinusoidal spaces, central veins and portal triad. Kidney: convoluted tubules, medullary rays (straight tubules) and collecting ducts. Prostate: acini and fibro-muscular stroma. Lung: bronchi, bronchioles, alveolar ducts, alveoli and pleura. Urinary bladder: urothelium, lamina propria, muscularis propria, and serosa. Testis: seminiferous tubules with intra-tubular sperms.

CONCLUSION: Light-CT™ is a powerful imaging tool to perform fast histology on fresh and fixed tissues, without introducing artifacts. Its compact size, ease of handling, fast image acquisition and safe incident light levels makes it well-suited for various intra-operative and intra-procedural triaging and decision making applications.

Free full text article available from PubMed
PMID: 21773059
Note: Also cited in Section 2.3

4.4 Virtual Histology

A) Confocal Mosaicing Microscopy of Human Skin Ex Vivo: Spectral Analysis for Digital Staining to Simulate Histology-Like Appearance

Summary: Confocal mosaicing microscopy enables rapid imaging of large areas of fresh tissue, without the processing that is necessary for conventional histology. Mosaicing may offer a means to perform rapid histology at the bedside. A possible barrier toward clinical acceptance is that the mosaics are based on a single mode of grayscale contrast and appear black and white, whereas histology is based on two stains (hematoxylin for nuclei, eosin for cellular cytoplasm and dermis) and appears purple and pink. Toward addressing this barrier, we report advances in digital staining: fluorescence mosaics that show only nuclei, are digitally stained purple and overlaid on reflectance mosaics, which show only cellular cytoplasm and dermis, and are digitally stained pink. With digital staining, the appearance of confocal mosaics mimics the appearance of histology. Using multispectral analysis and color matching functions, red, green, and blue (RGB) components of hematoxylin and eosin stains in tissue were determined. The resulting RGB components were then applied in a linear algorithm to transform fluorescence and reflectance contrast in confocal mosaics to the absorbance contrast seen in pathology. Optimization of staining with acridine orange showed improved quality of digitally stained mosaics, with good correlation to the corresponding histology.

Free full text article available from Journal of Biomedical Optics
PMID: 21806269
B) **Suitability of Infrared Microspectroscopic Imaging for Histopathology of the Uterine Cervix**


**Summary:** Infrared microspectroscopy (IR-MSP) has been proposed for automated histological tissue differentiation of unstained specimens based on chemical analysis of cell and extracellular constituents. This study aimed to determine the accuracy of IR-MSP-based histopathology of cervical carcinoma sections with complex tissue architecture under practically relevant testing conditions. **METHODS AND RESULTS:** In total, 46 regions of interest, covering an area of almost 50 mm\(^2\) on sections derived from paraffin-embedded tissue of radical hysterectomy specimens, were analysed by IR-MSP (nominal resolution ~4.2 mm). More than 2.8 million pixel spectra that were processed using fuzzy c-means clustering followed by hierarchical cluster analysis permitted image segmentation regarding different biochemical properties. Linear image registration was applied to compare these segmentation results with manual labelling on haematoxylin and eosin-stained references (resolution ~0.7 mm). For recognition of nine tissue types, sensitivities were 42-91% and specificities were 79-100%, mostly being affected by peritumoral inflammatory responses. Algorithmic variation of the outline of dysplasia and carcinoma revealed a spatial preference of false values in tissue transition areas. **CONCLUSIONS:** This imaging technique has potential as a new method for tissue characterization; however, the recognition accuracy does not justify a pathologist-independent tissue analysis, and the application is only possible in combination with concomitant conventional histopathology.

Full text article available from [Histopathology](https://www.histopathology.org) (subscription required)  
PMID: 22372426
**Section 5 In Vivo Clinical Applications of IVM**

IVM technologies are being developed to address clinical diagnostic problems in a number of organ systems where more conventional imaging technologies, such as white light endoscopy, cardiac catheterization, x-ray mammography, CT and MRI, have diagnostic limitations. IVM technologies are being developed as clinical tools for our colleagues to use in vivo to: better target biopsies to diseased tissue; minimize the need for blind biopsies such as those obtained during surveillance procedures; screen entire organs for occult microscopic disease; obtain microscopic diagnoses when tissues cannot be easily or safely excised; and assess prognosis and response to therapy. But no matter who uses the new IVM tools, pathologist input is needed both to develop and validate these new technologies and to interpret the diagnostic IVM images obtained clinically.

Below in a table illustrating the anticipated time line to full clinical adoption of IVM for a number of organ systems.

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<tr>
<th>Organ System</th>
<th>In Vivo Microscopy</th>
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<td>Eye</td>
<td>Standard of Care</td>
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<td>Cardiovascular</td>
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<td>Breast</td>
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Source: In Vivo Microscopy Work Group

The following articles illustrate the current state-of-the-art for IVM in various organ systems, including:

- the gastrointestinal tract, breast and skin, where IVM will have the greatest impact on the practice of pathology;
- the cardiovascular system, where IVM is in clinical use now, but with little impact on pathology practice;
- the lung, genitourinary and gynecologic tracts, and brain, where IVM is in earlier stages of development; and
- the eye, where IVM is currently the gold standard for the diagnosis of retinal diseases.
5.1 Gastrointestinal Tract and Pancreaticobiliary System

5.1.1 Gastrointestinal Tract

Possibly the most advanced clinical application area for in vivo microscopy is in the gastrointestinal tract. Two technologies, confocal microscopy and optical coherence tomography, are commercially available and FDA approved. Confocal microscopy, also known as confocal laser endomicroscopy (CLE) obtains images in the transverse plane with a resolution of about 1-2 µm. Most clinical forms of CLE detect exogenous fluorescence; typically fluorescein is injected IV and after a period of time, the confocal microscope is advanced to the luminal GI organ of interest where images are acquired. There are two main forms of CLE, probe-based CLE (pCLE) and endoscope-based CLE (eCLE). The pCLE device comprises a fiber bundle that transmits the confocal image. One advantage of pCLE is that the device can be inserted in the accessory port of any endoscope. With eCLE, the confocal microscope scanner is built into the endoscope, so this form of in vivo microscopy requires the acquisition of an eCLE endoscope. Images from both pCLE and eCLE have been used to diagnose Barrett’s esophagus, stomach cancer, small intestinal diseases, and colon polyps. A key feature of fluorescein is that it does not penetrate into nuclei and therefore conditions such as dysplasia show columnar cells that appear darker than non-dysplastic tissues.

As opposed to CLE, OCT provides cross-sectional images of tissue at a resolution of approximately 10 µm. OCT measures reflected light and therefore the contrast is based on differences in tissue properties that alter the reflectance of light. For instance nuclei scatter more light than cytoplasm and therefore dysplastic tissues appear darker than non-dysplastic tissues. OCT has been found to be capable of distinguishing Barrett’s esophagus from other upper GI tract tissues and diagnosing dysplasia based on architectural morphologic features. A newer form of OCT, termed volumetric laser endomicroscopy (VLE), images large regions of GI tract luminal organs using a balloon-centering catheter. For instance the entire distal esophagus (6.0 cm) can now be imaged at the microscopic scale in vivo using VLE (as shown in the image above). This technology may make it possible to forego random biopsy and instead perform IVM-targeted biopsy for more relevant tissue diagnosis. Recently, OCT has been implemented using a swallowable capsule. Capsule endomicroscopy opens up the possibility of screening for GI tract diseases based on comprehensive microscopic information obtained in vivo.
The following are selected articles on the application of IVM in the GI tract.

A) Confocal Laser Endomicroscopy in Gastrointestinal Diseases

**Summary:** Confocal laser endomicroscopy (CLE) is a novel endoscopic technique permitting in vivo microscopy (optical biopsies) of the gastrointestinal mucosa. CLE has been studied in a multitude of diseases of the upper and lower gastrointestinal tract, including Barrett’s esophagus, gastric inflammation and cancer, celiac disease, colorectal adenoma and carcinoma, and inflammatory bowel diseases. CLE has recently evolved and been studied for bile duct and liver imaging. CLE has shown overall high accuracy and enabled smart, targeted biopsies rather than untargeted sampling. Furthermore, the availability of real time microscopic information during endoscopy has immediate impact on therapeutic decisions and guides endoscopic interventions. CLE is also a unique tool for observation of (patho-)physiologic events in their natural environment (functional imaging) and has been linked to molecular imaging of gastrointestinal neoplasia in vivo, thereby broadening our understanding of mucosal pathology in clinical and basic science.

Full text article available from *Journal of Biophotonics* (USD 35.00 for 24 hour access)
PMID: 21567975

B) Confocal Laser Endomicroscopy: Technical Advances and Clinical Applications

**Summary:** Since its introduction in 2004, confocal laser endomicroscopy (CLE) has emerged as a valuable tool for gastrointestinal endoscopic imaging. Endomicroscopy enables the endoscopist to obtain real time in vivo histology during ongoing endoscopy thereby creating “optical biopsies.” To date, numerous studies have shown potential applications of endomicroscopy in the clinical setting, including in vivo diagnosis of esophageal squamous cell carcinoma, Barrett’s esophagus, celiac disease, and colonic polyps. Moreover, recent data suggest the potential application of endomicroscopy in the field of molecular imaging. Additionally, in recent months new applications and developments in the field of confocal imaging were introduced including endomicroscopy of the liver, pancreatic, and bile ducts. Furthermore, by introducing a new needle-based confocal imaging system, which is small enough to be introduced through a 22-gauge puncture needle, a wide field for new applications of endomicroscopic imaging has been opened. Currently, 2 CE- and US Food and Drug Administration (FDA)-approved devices for endomicroscopy are available (Figures 1 and 2; Supplementary Table 1). In this review, we introduce both systems and discuss new technical advances and clinical applications of CLE.

Free full text article available from *Gastroenterology*
PMID: 20561523
Note: Also cited in Section 3.1

C) Miami Classification for Probe-Based Confocal Laser Endomicroscopy
Summary: An essential element for any new advanced imaging technology is standardization of indications, terminology, categorization of images, and research priorities. In this review, we propose a state-of-the-art classification system for normal and pathological states in gastrointestinal disease using probe-based confocal laser endomicroscopy (pCLE). The Miami classification system is based on a consensus of pCLE users reached during a meeting held in Miami, Florida, in February 2009.

Full text article available from Endoscopy (USD 33.00)
PMID: 21818734
NOTE: Also cited in Sections 2.2, 3.1, 3.3, 6.2

5.1.1.1 Esophagus and Stomach

A) A Clinical and Histopathologic Focus on Barrett Esophagus and Barrett-Related Dysplasia

Summary: Barrett esophagus is a metaplastic, premalignant lesion associated with approximately 0.5% annual incidence of progression to esophageal adenocarcinoma. Diagnosis and screening of Barrett esophagus and Barrett-related dysplasia relies on histologic evaluation of endoscopic mucosal biopsies, a process that is burdened with interobserver variability. Objectives.—To review the histologic features and classification of Barrett esophagus and Barrett-related dysplasia, to discuss the underlying difficulties in diagnosis and pitfalls, and to provide a brief review of new developments related to therapeutic modalities for patients diagnosed with dysplasia. Data Sources.—Sources include a review of relevant literature indexed in PubMed (US National Library of Medicine). Conclusions.—In spite of interobserver variability, histologic assessment of dysplasia is currently the accepted method of surveillance, and subsequent patient management is dictated by this evaluation. Although not universal, endoscopic therapy is increasingly important in replacing esophagectomy for patients with high-grade dysplasia or early carcinoma.

Free full text article available the CAP’s Archives
PMID: 21970480

B) Endoscopic Evaluation and Advanced Imaging of Barrett's Esophagus

Summary: Enhanced visualization techniques are available for Barrett's esophagus and have promise in the detection of dysplasia and cancer. Several of these techniques, such as narrow band imaging and chromoendoscopy, are being applied clinically. These techniques will allow the endoscopist to screen the surface of the Barrett's esophagus to detect areas of neoplasia. Once detected, it is hoped that either magnification techniques, such as confocal laser endomicroscopy, or spectroscopic techniques can be of value in allowing in vivo real-time diagnostic capabilities.
5.1.1.1 Confocal Microscopy

A) Real-Time Increased Detection of Neoplastic Tissue in Barrett’s Esophagus with Probe-Based Confocal Laser Endomicroscopy: Final Results of an International Multicenter, Prospective, Randomized, Controlled Trial

Summary: Probe-based confocal laser endomicroscopy (pCLE) allows real-time detection of neoplastic Barrett’s esophagus (BE) tissue. However, the accuracy of pCLE in real time has not yet been extensively evaluated. OBJECTIVE: To compare the sensitivity and specificity of pCLE in addition to high-definition white-light endoscopy (HD-WLE) with HD-WLE alone for the detection of high-grade dysplasia (HGD) and early carcinoma (EC) in BE. DESIGN: International, prospective, multicenter, randomized, controlled trial. SETTING: Five tertiary referral centers. PATIENTS: A total of 101 consecutive BE patients presenting for surveillance or endoscopic treatment of HGD/EC. INTERVENTIONS: All patients were examined by HD-WLE, narrow-band imaging (NBI), and pCLE, and the findings were recorded before biopsy samples were obtained. The order of HD-WLE and NBI was randomized and performed by 2 independent, blinded endoscopists. All suspicious lesions on HD-WLE or NBI and 4-quadrant random locations were documented. These locations were examined by pCLE, and a presumptive diagnosis of benign or neoplastic (HGD/EC) tissue was made in real time. Finally, biopsies were taken from all locations and were reviewed by a central pathologist, blinded to endoscopic and pCLE data. MAIN OUTCOME MEASUREMENTS: Diagnostic characteristics of pCLE. RESULTS: The sensitivity and specificity for HD-WLE were 34.2% and 92.7%, respectively, compared with 68.3% and 87.8%, respectively, for HD-WLE or pCLE (P = .002 and P < .001, respectively). The sensitivity and specificity for HD-WLE or NBI were 45.0% and 88.2%, respectively, compared with 75.8% and 84.2%, respectively, for HD-WLE, NBI, or pCLE (P = .01 and P = .02, respectively). Use of pCLE in conjunction with HD-WLE and NBI enabled the identification of 2 and 1 additional HGD/EC patients compared with HD-WLE and HD-WLE or NBI, respectively, resulting in detection of all HGD/EC patients, although not statistically significant. LIMITATIONS: Academic centers with enriched population. CONCLUSIONS: pCLE combined with HD-WLE significantly improved the ability to detect neoplasia in BE patients compared with HD-WLE. This may allow better informed decisions to be made for the management and subsequent treatment of BE patients. (Clinical trial registration number: NCT00795184.).

Full text article available from Gastrointestinal Endoscopy (USD 31.50)
PMID: 21741642
NOTE: Also cited in Section 3.3
B) Reflectance Confocal Microscopy for the Diagnosis of Eosinophilic Esophagitis: A Pilot Study Conducted on Biopsy Specimens


Summary: Diagnosis of eosinophilic esophagitis (EoE) currently requires endoscopic biopsy and histopathologic analysis of the biopsy specimens to count intraepithelial eosinophils. Reflectance confocal microscopy (RCM) is an endomicroscopy technology that is capable of obtaining high-resolution, optically sectioned images of esophageal mucosa without the administration of exogenous contrast. OBJECTIVE: In this study, we investigated the capability of a high-speed form of RCM, termed spectrally encoded confocal microscopy (SECM), to count intraepithelial esophageal eosinophils and characterize other microscopic findings of EoE. DESIGN: A total of 43 biopsy samples from 35 pediatric patients and 8 biopsy samples from 8 adult patients undergoing EGD for EoE were imaged by SECM immediately after their removal and then processed for routine histopathology. Two SECM readers, trained on adult cases, prospectively counted intraepithelial eosinophils and detected the presence of abscess, degranulation, and basal cell hyperplasia on SECM images from the pediatric patients. A pathologist blinded to the SECM data analyzed the same from corresponding slides. SETTING: The Gastrointestinal Unit, Massachusetts General Hospital. RESULTS: Eosinophils by SECM demonstrated a higher reflectance than the surrounding cells and other inflammatory cells. There was good correlation between SECM and histology maximum eosinophil counts/high-power field (R = 0.76, P < .0001). Intra- and interobserver correlations for SECM counts were very good (R = 0.93 and R = 0.92, respectively; P < .0001). For the commonly used eosinophil count cutoff of 15 per high-power field, the sensitivity and specificity of SECM for EoE were 100%. The sensitivity and specificity for abscess, degranulation, and basal cell hyperplasia were 100% and 82%, 91% and 60%, and 94% and 80%, respectively. Intra- and interobserver agreements for these microscopic features of EoE were very good (κ = 0.9/0.9, 0.84/1.0, 0.91/0.81, respectively). LIMITATION: Ex vivo study. CONCLUSIONS: This study demonstrates that RCM can be used to accurately count intraepithelial eosinophils and identify other microscopic abnormalities associated with EoE on freshly excised biopsy samples. These findings suggest that RCM may be developed into a tool for assessing eosinophilic infiltration in the esophagus in vivo.

Free full text article available from PubMed
PMID: 21944314
NOTE: Also cited in Section 3.3

C) Comprehensive Imaging of Gastroesophageal Biopsy Samples by Spectrally Encoded Confocal Microscopy


Summary: Spectrally encoded confocal microscopy (SECM) is a high-speed reflectance confocal microscopy technique that has the potential to be used for acquiring comprehensive images of the entire distal esophagus endoscopically with subcellular resolution. OBJECTIVE: The goal of this study was to demonstrate large-area SECM in upper GI tissues and to determine whether the images contain microstructural information that is useful for pathologic diagnosis. DESIGN: A feasibility study. SETTING:
Gastrointestinal Unit, Massachusetts General Hospital. PATIENTS: Fifty biopsy samples from 36 patients undergoing routine EGD were imaged by SECM, in their entirety, immediately after their removal. RESULTS: The microstructure seen in the SECM images was similar to that seen by histopathology. Gastric cardia mucosa was clearly differentiated from squamous mucosa. Gastric fundic/body type mucosa showed more tightly packed glands than gastric cardia mucosa. Fundic gland polyps showed cystically dilated glands lined with cuboidal epithelium. The presence of intraepithelial eosinophils was detected with the cells demonstrating a characteristic bilobed nucleus. Specialized intestinal metaplasia was identified by columnar epithelium and the presence of goblet cells. Barrett’s esophagus (BE) with dysplasia was differentiated from specialized intestinal metaplasia by the loss of nuclear polarity and disorganized glandular architecture. LIMITATIONS: Ex vivo, descriptive study. CONCLUSIONS: Large-area SECM images of gastroesophageal biopsy samples enabled the visualization of both subcellular and architectural features of various upper GI mucosal types and were similar to the corresponding histopathologic slides. These results suggest that the development of an endoscopic SECM probe is merited.

Free full text article available from PubMed  
PMID: 19922916  
Note: Also cited in Section 3.3

D) Comprehensive Volumetric Confocal Microscopy with Adaptive Focusing

Summary: Comprehensive microscopy of distal esophagus could greatly improve the screening and surveillance of esophageal diseases such as Barrett’s esophagus by providing histomorphologic information over the entire region at risk. Spectrally encoded confocal microscopy (SECM) is a high-speed reflectance confocal microscopy technology that can be configured to image the entire distal esophagus by helically scanning the beam using optics within a balloon-centering probe. It is challenging to image the human esophagus in vivo with balloon-based SECM, however, because patient motion and anatomic tissue surface irregularities decenter the optics, making it difficult to keep the focus at a predetermined location within the tissue as the beam is scanned. In this paper, we present a SECM probe equipped with an adaptive focusing mechanism that can compensate for tissue surface irregularity and dynamic focal variation. A tilted arrangement of the objective lens is employed in the SECM probe to provide feedback signals to an adaptive focusing mechanism. The tilted configuration also allows the probe to obtain reflectance confocal data from multiple depth levels, enabling the acquisition of three-dimensional volumetric data during a single scan of the probe. A tissue phantom with a surface area of 12.6 cm(2) was imaged using the new SECM probe, and 8 large-area reflectance confocal microscopy images were acquired over the depth range of 56 μm in 20 minutes. Large-area SECM images of excised swine small intestine tissue were also acquired, enabling the visualization of villous architecture, epithelium, and lamina propria. The adaptive focusing mechanism was demonstrated to enable acquisition of in-focus images even when the probe was not centered and the tissue surface was irregular.

Free full text article available from PubMed  
PMID: 21698005
E) **Tethered Capsule Endomicroscopy Enables Less Invasive Imaging of Gastrointestinal Tract Microstructure**


**Summary:** Here we introduce tethered capsule endomicroscopy, which involves swallowing an optomechanically engineered pill that captures cross-sectional microscopic images of the gut wall at 30 mum (lateral) x 7 mum (axial) resolution as it travels through the digestive tract. Results in human subjects show that this technique rapidly provides three-dimensional, microstructural images of the upper gastrointestinal tract in a simple and painless procedure, opening up new opportunities for screening for internal diseases.

Full text article available from *Nature Medicine* (USD 32.00)  
PMID: 23314056

F) **Confocal Endomicroscopy for In Vivo Prediction of Completeness After Endoscopic Mucosal Resection**


**Summary:** Endoscopic mucosal resection (EMR) is an alternative to surgery for removal of superficial gastric neoplastic lesions. Residual neoplastic tissue of the resection interface is difficult to detect by conventional endoscopy. The aim of this study is to assess the efficacy of confocal laser endomicroscopy (CLE) in predicting complete resection margins after EMR. **METHODS:** EMR was performed by using cap-assisted or "inject and cut" resection technique. Two weeks after EMR, the circumferential margins of the defect were inspected by using CLE, and completeness of excision was predicted from the CLE image. Additional EMR was performed if necessary. In vivo CLE diagnosis was validated against final histopathology. **RESULTS:** Twenty-seven lesions were removed by EMR in 27 patients. After excluding 3 patients for gastrectomy, a total of 24 patients underwent CLE assessment, of whom 9 with indefinite lateral margins underwent at least two consecutive CLE follow-ups. A total of 19 lesions were regarded as complete remission, and 5 lesions (21.7%) were incompletely excised according to final pathologic diagnosis. Accuracy of CLE in predicting incomplete resection for original lesions was 91.7%, with sensitivity and specificity of 100.0 and 89.5%, respectively. The residual lesions were treated by additional EMR guided by CLE. There was no recurrence on endoscopic biopsies at mean (range) follow-up of 8.3 (4-15) months. **CONCLUSIONS:** Confocal laser endomicroscopy has high accuracy for prediction of remnant tissue after EMR, and may lead to significant improvements in clinical surveillance after endoscopic resection.

Full text article available from *Surgical Endoscopy* (USD 39.95)  
PMID: 21136097  
Note: Also cited in Section 4.1.3

G) **Mucosal Barrier Defects in Gastric Intestinal Metaplasia: In Vivo Evaluation by Confocal Endomicroscopy**

Summary: Helicobacter pylori infection and intestinal metaplasia (IM) are associated with gastric cancer. An impaired gastric mucosal barrier could be involved in this carcinogenesis. OBJECTIVE: To evaluate laser confocal laser endomicroscopy (CLE) for in vivo functional imaging of mucosal barrier defects in patients with IM. DESIGN: Prospective, controlled study. SETTING: A tertiary-care academic center. PATIENTS: This study involved patients with IM of the gastric mucosa who underwent CLE for surveillance. INTERVENTIONS: Specific IM mucosa and non-IM mucosa in patients were identified by CLE, and targeted biopsy samples were taken for histopathology and electron microscopy. MAIN OUTCOME MEASUREMENTS: Post-CLE assessment of paracellular fluorescein leakage was devised and validated by electron microscopy. We also evaluated the effect of H pylori eradication on the mucosal barrier. RESULTS: Forty-two patients were included. Of non-IM samples, the paracellular permeability was significantly increased in H pylori-positive samples compared with H pylori-negative controls (54 +/- 31% vs 3 +/- 6%, P < .05). Of IM samples, the permeability was significantly increased in both H pylori-negative and H pylori-positive samples (67 +/- 34% and 72 +/- 28% vs 3 +/- 6%, both P < .05). The results of post-CLE assessment correlated well with the electron microscopy findings (R(2) 0.834, P < .0001). After the eradication of H pylori, the paracellular barrier dysfunction of non-IM mucosa was significantly improved as shown by electron microscopy and CLE (both P < .001). However, there was no significant change in IM mucosa. LIMITATIONS: Single-center study. CONCLUSIONS: CLE allows functional imaging of mucosal barrier defects. Gastric IM is associated with an impaired paracellular barrier irrespective of H pylori eradication.

Full text article available from Gastrointestinal Endoscopy (USD 31.50) PMID: 22325805

5.1.1.1.2 OCT and OFDI

A) Comprehensive Microscopy of the Esophagus in Human Patients with Optical Frequency Domain Imaging


Summary: Optical coherence tomography (OCT) is a cross-sectional, high-resolution imaging modality that has been shown to accurately differentiate esophageal specialized intestinal metaplasia (SIM) from gastric cardia at the squamocolumnar junction (SCJ) and diagnose high-grade dysplasia and intramucosal carcinoma in patients with SIM. The clinical utility of OCT has been limited, however, by its inability to acquire images over large areas. OBJECTIVE: The aim of this study was to use recently developed high-speed OCT technology, termed optical frequency domain imaging (OFDI), and a new balloon-centering catheter (2.5 cm diameter) to demonstrate the feasibility of large area, comprehensive optical microscopy of the entire distal esophagus (approximately 6.0 cm) in patients. DESIGN: A pilot feasibility study. SETTING: Massachusetts General Hospital. PATIENTS: Twelve patients undergoing routine EGD. RESULTS: Comprehensive microscopy of the distal esophagus was successfully performed in 10 patients with the OFDI system and balloon catheter. There were no complications resulting from the imaging procedure. Volumetric data sets were acquired in less than 2 minutes. OFDI images at the SCJ showed a variety of microscopic features that were consistent with histopathologic findings, including squamous mucosa, cardia, SIM with and without dysplasia, and esophageal erosion. LIMITATIONS: Inability to obtain direct
correlation of OFDI data and histopathologic diagnoses. CONCLUSIONS: Comprehensive volumetric microscopy of the human distal esophagus was successfully demonstrated with OFDI and a balloon-centering catheter, providing a wealth of detailed information about the structure of the esophageal wall. This technique will support future studies to compare OFDI image information with histopathologic diagnoses.

Free full text article available from PubMed
PMID: 18926183
NOTE: Also cited in Section 3.1

B) Co-Registered Spectrally Encoded Confocal Microscopy and Optical Frequency Domain Imaging System

Summary: Spectrally encoded confocal microscopy and optical frequency domain imaging are two non-contact optical imaging technologies that provide images of tissue cellular and architectural morphology, which are both used for histopathological diagnosis. Although spectrally encoded confocal microscopy has better transverse resolution than optical frequency domain imaging, optical frequency domain imaging can penetrate deeper into tissues, which potentially enables the visualization of different morphologic features. We have developed a co-registered spectrally encoded confocal microscopy and optical frequency domain imaging system and have obtained preliminary images from human oesophageal biopsy samples to compare the capabilities of these imaging techniques for diagnosing oesophageal pathology.

Free full text article available from PubMed
PMID: 20629914
Note: Also cited in Section 3.3

5.1.1.1.3 MPM

A) Spectral Characterization and Unmixing of Intrinsic Contrast in Intact Normal and Diseased Gastric Tissues Using Hyperspectral Two-Photon Microscopy

Summary: Living tissues contain a range of intrinsic fluorophores and sources of second harmonic generation which provide contrast that can be exploited for fresh tissue imaging. Microscopic imaging of fresh tissue samples can circumvent the cost and time associated with conventional histology. Further, intrinsic contrast can provide rich information about a tissue’s composition, structure and function, and opens the potential for in-vivo imaging without the need for contrast agents. METHODOLOGY/PRINCIPAL FINDINGS: In this study, we used hyperspectral two-photon microscopy to explore the characteristics of both normal and diseased gastrointestinal (GI) tissues, relying only on their endogenous fluorescence and second harmonic generation to provide contrast. We obtained hyperspectral data at subcellular resolution by acquiring images over a range of two-photon excitation wavelengths, and found excitation spectral signatures of
specific tissue types based on our ability to clearly visualize morphology. We present the two-photon excitation spectral properties of four major tissue types that are present throughout the GI tract: epithelium, lamina propria, collagen, and lymphatic tissue. Using these four excitation signatures as basis spectra, linear unmixing strategies were applied to hyperspectral data sets of both normal and neoplastic tissue acquired in the colon and small intestine. Our results show that hyperspectral unmixing with excitation spectra allows segmentation, showing promise for blind identification of tissue types within a field of view, analogous to specific staining in conventional histology. The intrinsic spectral signatures of these tissue types provide information relating to their biochemical composition. CONCLUSIONS/SIGNIFICANCE: These results suggest hyperspectral two-photon microscopy could provide an alternative to conventional histology either for in-situ imaging, or intraoperative 'instant histology' of fresh tissue biopsies.

Free full text article available from PubMed and PLOS ONE
PMID: 21603623

5.1.1.2 Small Intestine, Colon and Rectum

A) Diagnostic Accuracy of Probe-Based Confocal Laser Endomicroscopy and Narrow Band Imaging for Small Colorectal Polyps: A Feasibility Study

Summary: Probe-based confocal laser endomicroscopy (pCLE) allows real-time in-vivo microscopic imaging of tissue. Narrow band imaging (NBI) can also classify colorectal lesions. Both systems may allow accurate optical diagnosis of small (6-9 mm) and diminutive (1-5 mm) polyps without histopathology. This study assesses the accuracy of pCLE and NBI for prediction of histology. METHODS: Participants underwent high-definition colonoscopy. The surface pit pattern of all polyps (1-9 mm) was determined in vivo using NBI. Confocal videos were obtained after administration of IV fluorescein. Recorded videos were subsequently analyzed offline, blinded to endoscopic characteristics, and histopathology. Confocal images were classified as neoplastic and non-neoplastic according to the Miami classification system. RESULTS: A total of 130 polyps (58 neoplastic, 72 non-neoplastic, mean size 4.6 mm) from 65 patients were assessed. Assuming histopathology as gold standard, pCLE had higher sensitivity than NBI (86% vs. 64%, P=0.008), with lower specificity (78% vs. 92%, p=0.027) and similar overall accuracy (82% vs. 79%, P=0.59). When 65 high-confidence cases were analyzed (polyps diagnosed identically with pCLE and NBI and with high-quality confocal videos), sensitivity and specificity were 94 and 97%. CONCLUSIONS: pCLE demonstrated higher sensitivity in predicting histology of small polyps compared with NBI, whereas NBI had higher specificity. When used in combination, the accuracy of pCLE and NBI was extremely high, approaching the accuracy of histopathology. Together, they may reduce the need for histological examination. However, further studies are warranted to evaluate the role of these techniques, especially in the population-based colon cancer screening.

Full text article available from American Journal Gastroenterology (USD 32.00)
PMID: 22068663
B) Interobserver Agreement and Accuracy Among International Experts with Probe-Based Confocal Laser Endomicroscopy in Predicting Colorectal Neoplasia


**Summary:** A recently developed probe-based, confocal laser endomicroscopy (pCLE) system provides images of surface colonic epithelium in vivo during any endoscopy. Our objective was to assess interobserver agreement, sensitivity, specificity, and overall accuracy in the diagnosis of neoplasia using pCLE. PATIENTS AND METHODS: 53 patients undergoing surveillance and screening colonoscopies were enrolled. A total of 75 lesions, were detected and all were inspected by pCLE prior to sampling or polypectomy. Intravenous fluorescein was used to optimize tissue contrast. Three pCLE users, blinded to histopathologic and endoscopic findings, reviewed the set of video sequences for crypt architecture, vessel architecture, and colorectal neoplasia diagnosis. Histopathologic diagnosis from the correspo

C) The Learning Curve, Accuracy, and Interobserver Agreement of Endoscope-Based Confocal Laser Endomicroscopy for the Differentiation of Colorectal Lesions


**Summary:** The endoscope-based confocal laser endomicroscopy (eCLE) system allows in vivo imaging of colorectal epithelium. Little is known about the learning curve for accurate interpretation of confocal images acquired with eCLE. OBJECTIVE: To determine the learning curve of eCLE, its diagnostic accuracy, and the intra- and interobserver agreement for the differentiation of colorectal lesions. DESIGN: Post hoc assessment of selected eCLE images. SETTING: Academic centers. PATIENTS: This study involved colonoscopic images from 47 patients. MAIN OUTCOME MEASUREMENTS: Learning curve of eCLE, accuracy, and intraobserver and interobserver agreement. METHODS: Three endoscopists received a short introduction to eCLE before evaluating 90 images. Observers assessed all eCLE images by using the Mainz classification. After each set of 30 images, the accuracy of each observer was assessed. The same procedure was repeated 6 months later by using the same set of images. LIMITATIONS: Post hoc assessment. RESULTS: There were no significant changes between the first set of 30 images and the 2 consecutive sets (P = .08 and P = .180, respectively). The overall
accuracy was 85.6%, 95.6%, and 92.2% for each observer. The kappa values of the intraobserver agreement were 0.68, 0.84, and 0.77 for each observer. The kappa value for interobserver agreement was 0.73 during the first and 0.72 during the second assessment. CONCLUSIONS: Accurate post hoc interpretation of eCLE confocal images can be learned quickly. High diagnostic accuracy was achieved by all 3 observers during the initial stage of the assessment, which remained high thereafter. Intra- and interobserver agreement was substantial for all 3 observers. Future studies should focus on the real-time assessment of eCLE images.

Full text article available from Gastrointestinal Endoscopy (USD 31.50)
PMID: 22459661
Note: Also cited in Section 6.3

D) Optical Diagnosis of Small Colorectal Polyps at Routine Colonoscopy (Detect InSpect ChAracterise Resect and Discard; DISCARD Trial): A Prospective Cohort Study

Summary: Accurate optical diagnosis of small (<10 mm) colorectal polyps in vivo, without formal histopathology, could make colonoscopy more efficient and cost effective. The aim of this study was to assess whether optical diagnosis of small polyps is feasible and safe in routine clinical practice. METHODS: Consecutive patients with a positive faecal occult blood test or previous adenomas undergoing surveillance at St Mark’s Hospital (London, UK), from June 19, 2008, to June 16, 2009, were included in this prospective study. Four colonoscopists with different levels of experience predicted polyp histology using optical diagnosis with high-definition white light, followed by narrow-band imaging without magnification and chroendoendoscopy, as required. The primary outcome was accuracy of polyp characterisation using optical diagnosis compared with histopathology, the current gold standard. Accuracy of optical diagnosis to predict the next surveillance interval was also assessed and compared with surveillance intervals predicted by current guidelines using histopathology. This study is registered with ClinicalTrials.gov, NCT00888771. FINDINGS: 363 polyps smaller than 10 mm were detected in 130 patients, of which 278 polyps had both optical and histopathological diagnosis. By histology, 198 of these polyps were adenomas and 80 were non-neoplastic lesions (of which 62 were hyperplastic). Optical diagnosis accurately diagnosed 186 of 198 adenomas (sensitivity 0.94; 95% CI 0.90-0.97) and 55 of 62 hyperplastic polyps (specificity 0.89; 0.78-0.95), with an overall accuracy of 241 of 260 (0.93, 0.89-0.96) for polyp characterisation. Using optical diagnosis alone, 82 of 130 patients could be given a surveillance interval immediately after colonoscopy, and the same interval was found after formal histopathology in 80 patients (98%) using British guidelines and in 78 patients (95%) using US multisociety guidelines. INTERPRETATION: For polyps less than 10 mm in size, in-vivo optical diagnosis seems to be an acceptable strategy to assess polyp histopathology and future surveillance intervals. Dispensing with formal histopathology for most small polyps found at colonoscopy could improve the efficiency of the procedure and lead to substantial savings in time and cost. FUNDING: Leigh Family Trust, London, UK.

Full text article available from Lancet Oncology ($31.50 USD)
PMID: 19910250
E) Multiphoton Tomographic Imaging: A Potential Optical Biopsy Tool for Detecting Gastrointestinal Inflammation and Neoplasia

Summary: Endoscopy is widely used to detect and remove premalignant lesions with the goal of preventing gastrointestinal (GI) cancers. Because current endoscopes do not provide cellular resolution, all suspicious lesions are biopsied and subjected to histologic evaluation. Technologies that facilitate directed biopsies should decrease both procedure-related morbidity and cost. Here we explore the use of multiphoton microscopy (MPM), an optical biopsy tool that relies on intrinsic tissue emissions, to evaluate pathology in both experimental and human GI specimens, using hematoxylin and eosin (H&E)-stained sections from these tissues for comparison. After evaluating the entire normal mouse GI tract, MPM was used to investigate disease progression in mouse models of colitis and colorectal carcinogenesis. MPM provided sufficient histologic detail to identify all relevant substructures in ex vivo normal GI tissue, visualize both acute and resolving stages of colitis, and show the progression of colorectal carcinogenesis. Next, ex vivo specimens from human subjects with celiac sprue, inflammatory bowel disease, and colorectal neoplasia were imaged by MPM. Finally, colonic mucosa in live anesthetized rats was imaged in vivo using a flexible endoscope prototype. In both animal models and human specimens, MPM images showed a striking similarity to the results of H&E staining, as shown by the 100% concordance achieved by the study pathologists’ diagnoses. In summary, MPM is a promising technique that accurately visualizes histology in fresh, unstained tissues. Our findings support the continued development of MPM as a technology to enhance the early detection of GI pathologies including premalignant lesions.

Full text article available from Cancer Prevention Research (USD 35.00) PMID: 22961775

5.1.2 Pancreaticobiliary System

With the creation and commercialization of confocal microscopy systems small enough to be used with flexible endoscopic probes, the engineering challenges required for the application of in vivo microscopy to the pancreaticobiliary system have now largely been overcome. These devices have enabled the burgeoning field of probe-based confocal laser endomicroscopy (pCLE), with numerous potential applications in the biliary system and other endoscopically accessible sites. Much interest lies in the potential of pCLE to assist in the evaluation of biliary strictures, which remain a diagnostic challenge with conventional endoscopic methods. As in other sites, confocal microscopy enables non-invasive in vivo visualization of biliary mucosa at cellular resolution, with the ability to obtain “optical sections” at varying depths. This allows for targeting areas of interest for further diagnostic workup, thereby greatly increasing the sensitivity of the endoscopic procedure. As in other organ systems, experience is limited with regard to interpretation of pCLE images, but diagnostic criteria are actively being proposed and validated against traditional histology.

The following are selected articles on the application of IVM in the pancreaticobiliary system.
A) **Optical Coherence Tomography in Detection of Dysplasia and Cancer of the Gastrointestinal Tract and Bilio-Pancreatic Ductal System**  

**Summary:** Optical coherence tomography (OCT) is an optical imaging modality that performs high-resolution, cross-sectional, subsurface tomographic imaging of the microstructure of tissues. The physical principle of OCT is similar to that of B-mode ultrasound imaging, except that it uses infrared light waves rather than acoustic waves. The in vivo resolution is 10-25 times better (about 10 microns) than with high-frequency ultrasound imaging, but the depth of penetration is limited to 1-3 mm, depending upon tissue structure, depth of focus of the probe used, and pressure applied to the tissue surface. In the last decade, OCT technology has evolved from an experimental laboratory tool to a new diagnostic imaging modality with a wide spectrum of clinical applications in medical practice, including the gastrointestinal (GI) tract and pancreatico-biliary ductal system. OCT imaging from the GI tract can be done in humans by using narrow-diameter, catheter-based probes that can be inserted through the accessory channel of either a conventional front-view endoscope, for investigating the epithelial structure of the GI tract, or a side-view endoscope, inside a standard transparent ERCP catheter, for investigating the pancreatico-biliary ductal system. Esophagus and the esophago-gastric junction has been the most widely investigated organ so far; more recently, also duodenum, colon and pancreatico-biliary ductal system have been extensively investigated. OCT imaging of the gastro-intestinal wall structure is characterized by a multiple-layer architecture that permits an accurate evaluation of the mucosa, lamina propria, muscularis mucosae, and part of the submucosa. The technique may be, therefore, used to identify pre-neoplastic conditions of the GI tract, such as Barrett’s epithelium and dysplasia, and evaluate the depth of penetration of early-stage neoplastic lesions. OCT imaging of the pancreatic and biliary ductal system could improve the diagnostic accuracy for ductal epithelial changes and the differential diagnosis between neoplastic and non-neoplastic lesions.


B) **Classification of Probe-Based Confocal Laser Endomicroscopy Findings in Pancreatico-Biliary Strictures**  

**Summary:** The accurate diagnosis of indeterminate pancreaticobiliary strictures presents a clinical dilemma. Probe-based confocal laser endomicroscopy (pCLE) offers real-time in vivo microscopic tissue examination that may increase sensitivity for the detection of malignancy. The objective of this study was to develop and validate a standard descriptive classification of pCLE in the pancreaticobiliary system. **PATIENTS AND METHODS:** A total of 102 patients undergoing endoscopic retrograde cholangiopancreatography (ERCP) with pCLE to assess indeterminate pancreaticobiliary strictures were enrolled in a multicenter registry; 89 of these patients were evaluable. Information and data on the following were collected prospectively: clinical, ERCP, tissue sampling, pCLE, and follow-up. A uniform classification of pCLE findings (“Miami Classification”) was developed, consisting of a set of image interpretation criteria.
Thereafter, these criteria were tested through blinded consensus review of 112 randomized pCLE videos from 47 patients, and inter-observer variability was assessed in 42 patients. RESULTS: A consensus definition of the specific criteria of biliary and pancreatic pCLE findings for indeterminate strictures was developed. Single-image interpretation criteria did not have a high enough sensitivity for predicting malignancy. However, combining two or more criteria significantly increased the sensitivity and predictive values. The characteristics most suggestive of malignancy included the following: thick white bands (>20 microm), or thick dark bands (>40 microm), or dark clumps or epithelial structures. These provided sensitivity, specificity, positive predictive value, and negative predictive value of 97%, 33%, 80%, and 80% compared with 48%, 100%, 100%, and 41% for standard tissue sampling methods. Inter-observer variability was moderate for most criteria. CONCLUSION: The Miami Classification enables a structured, uniform, and reproducible description of pancreaticobiliary pCLE. Combining individual characteristics improves the sensitivity for the detection of malignancy.

Full text article available from Endoscopy (USD 26.00) PMID: 22261749

5.2 Breast


In vivo microscopy techniques such as OCT and confocal microscopy, which have shallow tissue penetration, are best suited to in vivo clinical use in the skin and luminal viscera like the GI, GU and GYN tracts and cardiovascular system, and have not been widely applied in the breast. Rather, much of the work in the breast focuses on an alternate advanced optical imaging technology with deeper tissue penetration, diffuse optical tomography (DOT), also known as diffuse optical mammography. DOT can provide a 3-D image of the full thickness of the breast (similar to a conventional x-ray mammogram) using light rather than radiation, but does not yet have sufficient spatial resolution to challenge mammography for screening/early diagnosis of breast cancer. However, it is currently undergoing a large scale, multicenter clinical trial for monitoring/predicting clinical response to chemotherapy in breast cancer patients, where it shows considerable promise. A number of other advanced optical imaging technologies, including fluorescence, diffuse reflectance and Raman spectroscopy, are also in development as aids for the surgeon and the pathologist in assessing margins of resection and sentinel lymph
nodes during breast surgery (as in the above dual modality fluorescence-reflectance image (right) of invasive and in situ breast cancer (left)) or as aids to the radiologist in retrieving microcalcifications during stereotactic breast needle biopsies. As such, clinical application of these advanced imaging technologies in the breast will impact the everyday practice of surgical pathology. In fact, the FDA has recently approved a hand held device for intraoperative margin assessment based on radio frequency, rather than optical, spectroscopy.

The following are selected articles on the application of IVM in the breast.

5.2.1 Diffuse Optical Mammography

A) Assessing the Future of Diffuse Optical Imaging Technologies for Breast Cancer Management

Summary: Diffuse optical imaging (DOI) is a noninvasive optical technique that employs near-infrared (NIR) light to quantitatively characterize the optical properties of thick tissues. Although NIR methods were first applied to breast transillumination (also called diaphanography) nearly 80 years ago, quantitative DOI methods employing time- or frequency-domain photon migration technologies have only recently been used for breast imaging (i.e., since the mid-1990s). In this review, the state of the art in DOI for breast cancer is outlined and a multi-institutional Network for Translational Research in Optical Imaging (NTROI) is described, which has been formed by the National Cancer Institute to advance diffuse optical spectroscopy and imaging (DOSI) for the purpose of improving breast cancer detection and clinical management. DOSI employs broadband technology both in near-infrared spectral and temporal signal domains in order to separate absorption from scattering and quantify uptake of multiple molecular probes based on absorption or fluorescence contrast. Additional dimensionality in the data is provided by integrating and co-registering the functional information of DOSI with x-ray mammography and magnetic resonance imaging (MRI), which provide structural information or vascular flow information, respectively. Factors affecting DOSI performance, such as intrinsic and extrinsic contrast mechanisms, quantitation of biochemical components, image formation/visualization, and multimodality co-registration are under investigation in the ongoing research NTROI sites. One of the goals is to develop standardized DOSI platforms that can be used as stand-alone devices or in conjunction with MRI, mammography, or ultrasound. This broad-based, multidisciplinary effort is expected to provide new insight regarding the origins of breast disease and practical approaches for addressing several key challenges in breast cancer, including: Detecting disease in mammographically dense tissue, distinguishing between malignant and benign lesions, and understanding the impact of neoadjuvant chemotherapies.

Free full text article available from PubMed
PMID: 18649477

B) Diffuse Optical Imaging and Spectroscopy of the Breast: A Brief Outline of History and Perspectives
Summary: Breast cancer is the most common cancer among women in industrialized countries. At present, X-ray mammography is the gold standard for breast imaging, but has limitations, especially when dense breasts are imaged, as typically occurs in young women. Optical imaging can non-invasively provide information on tissue composition, structure and physiology that can be beneficially exploited for breast lesion detection and identification. In the last few decades optical breast imaging has been investigated, using different geometries (projection imaging and tomography) and measurement techniques (continuous wave, frequency resolved and time resolved approaches). Also, data analysis and display varies significantly, ranging from intensity images to maps of the optical properties (absorption and scattering), tissue composition, and physiological parameters (typically blood volume and oxygenation). This paper outlines the historical evolution of optical imaging and spectroscopy of the breast, highlighting potentialities and limitations, and presents an overview of the main applications and perspectives of the field.

Full text article available from *Photochemical and Photobiological Sciences*  
PMID: 22094324

### 5.2.2 Primary Breast Diagnosis

**A) Raman Spectroscopy: A Real-Time Tool for Identifying Microcalcifications During Stereotactic Breast Core Needle Biopsies**


**Summary:** Microcalcifications are an early mammographic sign of breast cancer and a target for stereotactic breast needle biopsy. We present here a Raman spectroscopic tool for detecting microcalcifications in breast tissue based on their chemical composition. We collected ex vivo Raman spectra from 159 tissue sites in fresh stereotactic breast needle biopsies from 33 patients, including 54 normal sites, 75 lesions with microcalcifications and 30 lesions without microcalcifications. Application of our Raman technique resulted in a positive predictive value of 97% for detecting microcalcifications. This study shows that Raman spectroscopy has the potential to detect microcalcifications during stereotactic breast core biopsies and provide real-time feedback to radiologists, thus reducing non-diagnostic and false negative biopsies.

Free full text article available from [PubMed](https://pubmed.ncbi.nlm.nih.gov/22025985/)  
PMID: 22025985  
Note: Also cited in Section 3.2

**B) Application of Raman Spectroscopy to Identify Microcalcifications and Underlying Breast Lesions at Stereotactic Core Needle Biopsy**


**Summary:** Microcalcifications are a feature of diagnostic significance on a mammogram and a target for stereotactic breast needle biopsy. Here, we report development of a Raman spectroscopy technique to simultaneously identify microcalcification status and diagnose the underlying breast lesion, in real-time, during stereotactic core needle biopsy procedures. Raman spectra were obtained ex vivo from 146 tissue sites from fresh
stereotactic breast needle biopsy tissue cores from 33 patients, including 50 normal tissue sites, 77 lesions with microcalcifications, and 19 lesions without microcalcifications, using a compact clinical system. The Raman spectra were modeled based on the breast tissue components and a support vector machine framework was used to develop a single-step diagnostic algorithm to distinguish normal tissue, fibrocystic change (FCC), fibroadenoma (FA) and breast cancer, in the absence and presence of microcalcifications. This algorithm was subjected to leave-one-site-out cross-validation, yielding a positive predictive value, negative predictive value, sensitivity and specificity of 100%, 95.6%, 62.5% and 100% for diagnosis of breast cancer (with or without microcalcifications) and an overall accuracy of 82.2% for classification into specific categories of normal tissue, FCC, FA or breast cancer (with and without microcalcifications). Notably, the majority of breast cancers diagnosed are ductal carcinoma in situ (DCIS), the most common lesion associated with microcalcifications, which could not be diagnosed using previous Raman algorithm(s). Our study demonstrates the potential of Raman spectroscopy to concomitantly detect microcalcifications and diagnose associated lesions, including DCIS, and thus provide real-time feedback to radiologists during such biopsy procedures, reducing non-diagnostic and false negative biopsies.

Full text article available from The Journal of Cancer Research (USD 35.00)
PMID: 23729641
Note: Also cited in Section 3.2

5.2.3 Margin Assessment

A) Development of a Spatially Offset Raman Spectroscopy Probe for Breast Tumor Surgical Margin Evaluation


Summary: The risk of local recurrence for breast cancers is strongly correlated with the presence of a tumor within 1 to 2 mm of the surgical margin on the excised specimen. Previous experimental and theoretical results suggest that spatially offset Raman spectroscopy (SORS) holds much promise for intraoperative margin analysis. Based on simulation predictions for signal-to-noise ratio differences among varying spatial offsets, a SORS probe with multiple source-detector offsets was designed and tested. It was then employed to acquire spectra from 35 frozen-thawed breast tissue samples in vitro. Spectra from each detector ring were averaged to create a composite spectrum with biochemical information covering the entire range from the tissue surface to approximately 2 mm below the surface, and a probabilistic classification scheme was used to classify these composite spectra as "negative" or "positive" margins. This discrimination was performed with 95% sensitivity and 100% specificity, or with 100% positive predictive value and 94% negative predictive value.

Free full text article available from PubMed
PMID: 21806286
Note: Also cited in Section 4.1.1
B) **Intraoperative Evaluation of Breast Tumor Margins with Optical Coherence Tomography**  

**Summary:** As breast cancer screening rates increase, smaller and more numerous lesions are being identified earlier, leading to more breast-conserving surgical procedures. Achieving a clean surgical margin represents a technical challenge with important clinical implications. Optical coherence tomography (OCT) is introduced as an intraoperative high-resolution imaging technique that assesses surgical breast tumor margins by providing real-time microscopic images up to 2 mm beneath the tissue surface. In a study of 37 patients split between training and study groups, OCT images covering 1 cm² regions were acquired from surgical margins of lumpectomy specimens, registered with ink, and correlated with corresponding histologic sections. A 17-patient training set used to establish standard imaging protocols and OCT evaluation criteria showed that areas of higher scattering tissue with a heterogeneous pattern were indicative of tumor cells and tumor tissue in contrast to lower scattering adipocytes found in normal breast tissue. The remaining 20 patients were enrolled into the feasibility study. Of these lumpectomy specimens, 11 were identified with a positive or close surgical margin and 9 were identified with a negative margin under OCT. Based on histologic findings, 9 true positives, 9 true negatives, 2 false positives, and 0 false negatives were found, yielding a sensitivity of 100% and specificity of 82%. These results show the potential of OCT as a real-time method for intraoperative margin assessment in breast-conserving surgeries.

Free full text article available from [PubMed](https://pubmed.ncbi.nlm.nih.gov/19910294/)
PMID: 19910294
Note: Also cited in Section 4.1.1

C) **Scatter Spectroscopic Imaging Distinguishes Between Breast Pathologies in Tissues Relevant to Surgical Margin Assessment**  

**Summary:** A new approach to spectroscopic imaging was developed to detect and discriminate microscopic pathologies in resected breast tissues; diagnostic performance of the prototype system was tested in 27 tissues procured during breast conservative surgery. **EXPERIMENTAL DESIGN:** A custom-built, scanning in situ spectroscopy platform sampled broadband reflectance from a 150-mum-diameter spot over a 1 x 1 cm² field using a dark field geometry and telecentric lens; the system was designed to balance sensitivity to cellular morphology and imaging the inherent diversity within tissue subtypes. Nearly 300,000 broadband spectra were parameterized using light scattering models and spatially dependent spectral signatures were interpreted using a cooccurrence matrix representation of image texture. **RESULTS:** Local scattering changes distinguished benign from malignant pathologies with 94% accuracy, 93% sensitivity, 95% specificity, and 93% positive and 95% negative predictive values using a threshold-based classifier. Texture and shape features were important to optimally discriminate benign from malignant tissues, including pixel-to-pixel correlation, contrast and homogeneity, and the shape features of fractal dimension and Euler number. Analysis of the region-based diagnostic performance showed that spectroscopic image features from 1 x 1 mm² areas were diagnostically discriminant and enabled quantification of within-class tissue heterogeneities. **CONCLUSIONS:** Localized scatter-imaging signatures detected by the
scanning spectroscopy platform readily distinguished benign from malignant pathologies in surgical tissues and showed new spectral-spatial signatures of clinical breast pathologies.

Full text article available from Clinical Cancer Research (USD 35.00)
PMID: 22908098
Note: Also cited in Section 4.1.1

D) MarginProbe((R)): Intraoperative Margin Assessment During Breast Conserving Surgery by Using Radiofrequency Spectroscopy

Summary: In breast conserving surgery, the tumor should be removed with a clean margin, a rim of healthy tissue surrounding. Failure to achieve clean margins in the initial surgery results in a re-excision procedure. Re-excision rates are reported as being 11-46% for invasive carcinoma and ductal carcinoma in situ (DCIS). Re-excisions can have negative consequences such as increased postoperative infections, negative impact on cosmesis, patient anxiety and increased medical costs. Therefore, the surgical margin of invasive and intraductal (DCIS) breast tissue is a subject of intense discussion. Different options for intraoperative assessment are available, but all in all, they are unsatisfying. Frozen section margin examination is possible but is time consuming and restricted to the assessment of invasive carcinoma. In the case of DCIS, there is no procedure for intraoperative margin assessment. Thus, a solution for efficient intraoperative surgical margin assessment is needed. For this purpose, an innovative, real-time, intraoperative margin-assessment device (MarginProbe((R)), Dune Medical Devices, Caesarea, Israel) was designed, and recent published clinical data reported a reduction of re-excisions by more than 50%.

Full text article available from Expert Review of Medical Devices (USD 86.00 for 24 hour access)
PMID: 23668703
NOTE: Also cited in Section 4.1.1

5.2.4 Sentinel Lymph Node Assessment

A) Raman Spectroscopy--A Potential New Method for the Intra-Operative Assessment of Axillary Lymph Nodes

Summary: Sentinel Lymph Node Biopsy has become the standard surgical procedure for the sampling of axillary lymph nodes in breast cancer. Intra-operative node assessment of these nodes would allow definitive axillary surgery to take place immediately with associated benefits for patient management. Our experimental study aims to demonstrate that a Raman spectroscopy probe system could overcome many of the disadvantages of current intra-operative methods. 59 axillary lymph nodes, 43 negative and 16 positive from 58 patients undergoing breast surgery at our district general hospital were mapped using Raman micro-spectroscopy. These maps were then used to model
the effect of using a Raman spectroscopic probe by selecting 5 and 10 probe points across the mapped images and evaluating the impact on disease detection. Results demonstrated sensitivities of up to 81% and specificities of up to 97% when differentiating between positive and negative lymph nodes, dependent on the number of probe points included. The results would have concurred with histopathology assessment in 89% and 91% of cases in the 5 and 10 point models respectively. Using Raman spectroscopy in this way could allow lymph node assessment within a time-frame suitable for intra-operative use.

Full text article available from The Surgeon (USD 31.50)  
PMID: 22525413  
Note: Also cited in Section 4.2

B) Optical Scanning for Rapid Intraoperative Diagnosis of Sentinel Node Metastases in Breast Cancer

Summary: Intraoperative diagnosis of sentinel node metastases enables an immediate decision to proceed to axillary lymph node dissection, avoiding a second operation in node-positive women with breast cancer. METHODS: An optical scanner was developed that interrogated the cut surface of bivalved, but otherwise unprocessed, sentinel lymph nodes with pulses of white light by elastic scattering spectroscopy (ESS). The scattered light underwent spectral analysis, and individual spectra were initially correlated with conventional histology to develop a diagnostic algorithm. This algorithm was used to create false colour-coded maps of scans from an independent set of nodes, and the optimal criteria for discriminating between normal and cancer spectra were defined statistically. RESULTS: The discriminant algorithm was developed from a training set of 2989 spectra obtained from 30 metastatic and 331 normal nodes. Subsequent scans from 129 independent nodes were analysed. The scanner detected macrometastases (larger than 2 mm) with a sensitivity of 76 per cent (69 per cent including micrometastases) and specificity of 96 per cent. CONCLUSION: In this proof-of-principle study, the ESS results were comparable with current intraoperative diagnostic techniques of lymph node assessment.

Full text article available from British Journal of Surgery (USD 35.00 for 24 hour access)  
PMID: 20593429  
Note: Also cited in Section 4.2

C) Integrated Optical Coherence Tomography and Microscopy for Ex Vivo Multiscale Evaluation of Human Breast Tissues

Summary: Three-dimensional (3D) tissue imaging methods are expected to improve surgical management of cancer. In this study, we examined the feasibility of two 3D imaging technologies, optical coherence tomography (OCT) and optical coherence microscopy (OCM), to view human breast specimens based on intrinsic optical contrast. Specifically, we imaged 44 ex vivo breast specimens including 34 benign and 10 malignant lesions with an integrated OCT and OCM system developed in our laboratory.
The system enabled 4-mum axial resolution (OCT and OCM) with 14-mum (OCT) and 2-mum (OCM) transverse resolutions, respectively. OCT and OCM images were compared with corresponding histologic sections to identify characteristic features from benign and malignant breast lesions at multiple resolution scales. OCT and OCM provide complimentary information about tissue microstructure, thus showing distinctive patterns for adipose tissue, fibrous stroma, breast lobules and ducts, cysts and microcysts, as well as in situ and invasive carcinomas. The 3D imaging capability of OCT and OCM provided complementary information to individual 2D images, thereby allowing tracking features from different levels to identify low-contrast structures that were difficult to appreciate from single images alone. Our results lay the foundation for future in vivo optical evaluation of breast tissues, using OCT and OCM, which has the potential to guide core needle biopsies, assess surgical margins, and evaluate nodal involvement in breast cancer.

Free full text article available from PubMed
PMID: 21056988
NOTE: Also cited in Section 4.1.1

5.3 Skin

The accessibility of the skin makes it an ideal organ for in vivo microscopy techniques, and methods and devices are now well developed and are being used in clinical practice. Reflectance confocal microscopy (RCM) has become the most widely used and studied modality, with the ability to achieve non-invasive “optical sections” at different anatomic levels of the skin with cellular-level resolution. The images thus obtained are oriented en face, and look very different than the traditional H&E stained sections that Pathologists are accustomed to interpreting. Nevertheless, diagnostic criteria have been formulated and validated for a number of neoplastic and non-neoplastic dermatologic conditions, with generally excellent correlation with traditional histology. Confocal microscopy techniques have also been used in the evaluation of margins (e.g., for Mohs specimens), and images very similar to H&E stained sections can be achieved with the use of fluorescent contrast agents (as in the confocal microscopy image (top) of a basal cell carcinoma (bottom) above). Limitations remain, most notably with regard to depth of imaging in vivo, but the application of in vivo microscopy techniques in Dermatology remains an area of intense interest, and will no doubt play a major role in diagnosis in the near future.

The following are selected articles on the application of IVM in the skin.
A) Introduction to Confocal Microscopy


**Summary:** Conventional microscopy requires viewing a thin-cut “section” of fixed or frozen tissue, and therefore cannot be used to view thick tissue samples or for in vivo investigations. In vivo microscopy requires a virtual, rather than a physical, section of the specimen. Confocal microscopy, developed and patented by Marvin Minsky in 1955, uses optical imaging to create a virtual slice or plane, many micrometers deep, within the tissue. It provides very-high-quality images with fine detail and more contrast than conventional microscopy. In addition, the imaging technique allows for reconstruction of virtual 3-dimensional (3-D) images of the tissue when multiple sections are combined.

Free full text article available from *Journal of Investigative Dermatology*
PMID: 23187113
Note: Also cited in Section 2.2

B) In Vivo Confocal Microscopy in Dermatology: From Research to Clinical Application


**Summary:** Confocal laser scanning microscopy (CLSM) represents an emerging technique for the noninvasive histomorphological analysis of skin in vivo and has shown its applicability for dermatological research as well as its value as an adjunct tool in the clinical management of skin cancer patients. Herein, we aim to give an overview on the current clinical indications for CLSM in dermatology and also highlight the diverse applications of CLSM in dermatological research.

Full text article available from *Journal of Biomedical Optics* (USD 25.00)
PMID: 23338938

C) New Directions in Dermatopathology: In Vivo Confocal Microscopy in Clinical Practice


**Summary:** In vivo confocal microscopy represents a new device that generates a virtual skin biopsy at cytologic resolution. This article describes the most relevant confocal findings and their histopathologic correlates in skin oncology and inflammatory diseases. The light and dark of confocal microscopy are briefly discussed in relation with its clinical applications.

Full text article available from *Dermatologic Clinics* (USD 31.50)
PMID: 23021059

D) Clinical Applicability of In Vivo Reflectance Confocal Microscopy in Dermatology


**Summary:** In vivo reflectance confocal microscopy (RCM) is a non-invasive diagnostic technique that offers the evaluation of the skin at real time with cellular resolution. In the past decade, multiple studies have been performed showing the clinical applicability of
RCM for the diagnosis of melanoma and non melanoma skin cancer (NMSC). In this regard, RCM has moved from a research tool to a valuable diagnostic technique being applied in daily clinical practice. In this regard, RCM aids in the diagnosis and differential diagnosis of various skin diseases and may also be used for selection of the biopsy site. Furthermore, RCM allows monitoring of a skin lesion over time without tissue alteration and thus represents a valuable method for treatment monitoring.

Full text article available from Dermatologic Surgery (USD 35.00 for 24 hour access)
PMID: 18261097

E) In Vivo Confocal Microscopy for Diagnosis of Melanoma and Basal Cell Carcinoma Using a Two-Step Method: Analysis of 710 Consecutive Clinically Equivocal Cases

Summary: We describe two algorithms to diagnose basal cell carcinomas (BCCs) and melanomas (MMs) using in vivo reflectance confocal microscopy (RCM). A total of 710 consecutive cutaneous lesions excised to exclude malignancy (216 MMs, 266 nevi, 119 BCCs, 67 pigmented facial macules, and 42 other skin tumors) were imaged by RCM. RCM features were correlated with pathology diagnosis to develop diagnostic algorithms. The diagnostic accuracy of the BCC algorithm defined on multivariate analysis of the training set (50%) and tested on the remaining cases was 100% sensitivity, 88.5% specificity. Positive features were polarized elongated features, telangiectasia and convoluted vessels, basaloid nodules, and epidermal shadowing corresponding to horizontal clefting. Negative features were non-visible papillae, disarrangement of the epidermal layer, and cerebriform nests. Multivariate discriminant analysis on the training set (excluding the BCCs) identified seven independently significant features for MM diagnosis. The diagnostic accuracy of the MM algorithm on the test set was 87.6% sensitivity, 70.8% specificity. The four invasive MMs that were misdiagnosed by RCM were all of nevus subtype. RCM is a highly accurate non-invasive technique for BCC diagnosis. Good diagnostic accuracy was achieved also for MM diagnosis, although rare variants of melanocytic tumors may limit the strict application of the algorithm.

Full text article available from Journal of Investigative Dermatology (USD 32.00)
PMID: 22718115

F) Rapid Screening of Cancer Margins in Tissue with Multimodal Confocal Microscopy

Summary: Complete and accurate excision of cancer is guided by the examination of histopathology. However, preparation of histopathology is labor intensive and slow, leading to insufficient sampling of tissue and incomplete and/or inaccurate excision of margins. We demonstrate the potential utility of multimodal confocal mosaicing microscopy for rapid screening of cancer margins, directly in fresh surgical excisions, without the need for conventional embedding, sectioning, or processing. MATERIALS AND METHODS: A multimodal confocal mosaicing microscope was developed to image basal cell carcinoma margins in surgical skin excisions, with the resolution that shows nuclear detail. Multimodal contrast is with fluorescence for imaging nuclei and reflectance for cellular cytoplasm and dermal collagen. Thirty-five excisions of basal cell...
carcinomas from Mohs surgery were imaged, and the mosaics analyzed by comparison with the corresponding frozen pathology. RESULTS: Confocal mosaics are produced in about 9 min, displaying tissue in fields of view of 12 mm with x2 magnification. A digital staining algorithm transforms black and white contrast to purple and pink, which simulates the appearance of standard histopathology. Mosaicing enables rapid digital screening, which mimics the examination of histopathology. CONCLUSIONS: Multimodal confocal mosaicing microscopy offers a technology platform to potentially enable real-time pathology at the bedside. The imaging may serve as an adjunct to conventional histopathology to expedite screening of margins and guide surgery toward more complete and accurate excision of cancer.

Full text article available from Journal of Surgical Research (USD 31.50)
PMID: 22721570
Note: Also cited in Section 4.1.1

G) Rapid Diagnosis of Two Facial Papules Using Ex Vivo Fluorescence Confocal Microscopy: Toward a Rapid Bedside Pathology

Summary: This article reports two papules on the face referred for Mohs micrographic surgery (MMS) as basal cell carcinomas (BCCs). Noninvasive in vivo reflectance confocal microscopy (RCM) could not be performed because of the difficult location (nose tip and jaw). A shave biopsy was performed in both cases. The two samples underwent rapid diagnosis with fluorescence-mode confocal microscopy (FCM); in each case, a 10-by-10-mm fluorescence confocal mosaic with high cellular morphologic resolution was obtained with high cellular morphologic resolution that enabled a precise diagnosis in less than 5 minutes. This report represents a first step toward a rapid bedside pathology.

Full text article available from Dermatologic Surgery (subscription required)
PMID: 22823541
Interventional cardiology has evolved over the last decade with percutaneous coronary artery procedures being the first line of treatment for symptomatic coronary artery disease. Today drug-eluting stents (DES) are the preferred treatment for coronary atherosclerosis as they have dramatically reduced the restenosis rates as compared to bare metal stents (BMS). However, concerns about the long-term safety of DES still exist. It has been demonstrated in patients who received first-generation DES (i.e., sirolimus-eluting stents and paclitaxel-eluting stents) that DES are associated with a steady increase in the cumulative incidence of very late (>1 year post implantation) stent thrombosis up to 4 to 5 years. It has been reported that the best method for the prediction of future events is the presence >30% uncovered stent struts, which can only be detected by optical coherence tomography (OCT) in living patients. Today interventional cardiologists are using OCT, and optical frequency domain imaging (OFDI) in clinical practice not only to determine stent coverage, but also to determine the nature of the neointimal growth (i.e., mature, proteoglycan and collagen rich; immature containing fibrin deposits; or inflamed neointima (hypersensitivity reaction)). Cardiologists have also used OCT and OFDI to study the nature of the underlying atherosclerotic plaque to determine not only the etiology of thrombosis, i.e., plaque rupture, plaque erosion and calcified nodule (as shown in the images above), but also the progression of plaque (adaptive intimal thickening, intimal xanthomas, pathologic intimal thickening, fibroatheroma and thin cap fibroatheroma) and the effects of treatment like statins on plaque progression.

It is clear that intervention cardiologists are beginning to learn the pathology of the disease and therefore it is important we pathologist also take interest and not only know pathology but how IVM imaging can help us understand disease progression in vivo.

The following are selected articles on the application of IVM in the cardiovascular system.

A) Intracoronary Optical Coherence Tomography, Basic Theory and Image Acquisition Techniques
Summary: Optical coherence tomography (OCT) imaging is showing great potential as an alternative or complementary tool to intravascular ultrasound (IVUS) for aiding in stent procedures and future diagnosis/treatment of atherosclerosis. Here, we describe the basic theory behind OCT imaging and explain important parameters such as axial resolution, lateral resolution and sensitivity. Also, we describe several image acquisition techniques that have been adopted for OCT imaging.

Full text article available from International Journal of Cardiovascular Imaging (USD 39.95) PMID: 21327912


Summary: Optical coherence tomography (OCT) is a novel intravascular imaging modality, based on infrared light emission, that enables a high resolution arterial wall imaging, in the range of 10-20 microns. This feature of OCT allows the visualization of specific components of the atherosclerotic plaques. The aim of the present Expert Review Document is to address the methodology, terminology and clinical applications of OCT for qualitative and quantitative assessment of coronary arteries and atherosclerosis.

Free full text article available from Oxford Journals PMID: 19892716

C) Expert Review Document Part 2: Methodology, Terminology and Clinical Applications of Optical Coherence Tomography for the Assessment of Intervventional Procedures

Summary: This document is complementary to an Expert Review Document on Optical Coherence Tomography (OCT) for the study of coronary arteries and atherosclerosis. The goal of this companion manuscript is to provide a practical guide framework for the appropriate use and reporting of the novel frequency domain (FD) OCT imaging to guide interventional procedures, with a particular interest on the comparison with intravascular ultrasound (IVUS).

Free full text article available from PubMed PMID: 22653335 NOTE: Also cited in Section 6.1

5.4.1 Vulnerable Atherosclerotic Plaque

A) Challenges on the Frontier of Intracoronary Imaging: Atherosclerotic Plaque Macrophage Measurement by Optical Coherence Tomography

Summary: Cellularity of the fibrous caps of coronary atheromas, manifested by the infiltration of macrophages (average size, 20 to 30 microm), is thought to weaken the structural integrity of the cap and predispose plaques to rupture. Therefore, an imaging technology capable of identifying macrophages within fibroatheroma caps in patients could provide valuable information for assessing plaque rupture risk. Recently, intravascular optical coherence tomography (OCT), a high-resolution coronary imaging modality, with an axial resolution of approximately 10 microm, has been introduced into the clinical setting. OCT images of the microstructure of the coronary artery wall enable accurate plaque-type characterization, supported by histopathological comparison data. Because of its high resolution, OCT may also be used to identify macrophages in vivo. In this paper we review recent developments in OCT for measuring macrophages in atherosclerotic plaques.

Full text article available from Journal of Biomedical Optics (USD 20.00)
PMID: 20210430

B) Conformational Change in Coronary Artery Structure Assessed by Optical Coherence Tomography in Patients with Vasospastic Angina

Summary: The aim of this study was to investigate the conformational change of arterial structure in the vasospastic lesion with optical coherence tomography. BACKGROUND: Coronary artery spasm plays an important role in the pathogenesis of ischemic heart diseases. The conformational change of each arterial layer during vasospasm has not been studied in detail. METHODS: We assessed 19 coronary arteries (10 spasm and 9 nonspasm lesions) with optical coherence tomography during the provocation test for coronary spasm. An intimal bump was defined as 1 or more intimal projections into the lumen that disappeared after the administration of nitroglycerine (NTG). Intimal gathering was defined as a folding/gathering of the intima, resulting in multiple kinks in the luminal contour that resolved after the administration of NTG.RESULTS: The spasm lesion more frequently showed an intimal bump at baseline and intimal gathering during spasm compared with the nonspasm lesion (spasm 80% vs. nonspasm 0%, p < 0.01, spasm 100% vs. nonspasm 0%, p < 0.01, respectively). The spasm lesion demonstrated a thicker maximum media thickness (spasm 0.24 ± 0.04 mm vs. nonspasm 0.12 ± 0.03 mm, p < 0.01) at baseline, whereas no differences were observed after the administration of NTG (spasm 0.13 ± 0.03 mm vs. nonspasm 0.13 ± 0.02 mm, p = 0.65).CONCLUSIONS: Our results suggest that medial contraction occurs even in an asymptomatic state and facilitates the formation of an intimal bump in patients with vasospastic angina. Luminal narrowing during spasm is associated with intimal gathering without alteration of intimal area.

Full text article available from Journal of the American College of Cardiology (USD 31.50)
PMID: 21958888

C) Atherosclerotic Tissue Characterization In Vivo by Optical Coherence Tomography Attenuation Imaging

**Summary:** Optical coherence tomography (OCT) is rapidly becoming the method of choice for assessing arterial wall pathology in vivo. Atherosclerotic plaques can be diagnosed with high accuracy, including measurement of the thickness of fibrous caps, enabling an assessment of the risk of rupture. While the OCT image presents morphological information in highly resolved detail, it relies on interpretation of the images by trained readers for the identification of vessel wall components and tissue type. We present a framework to enable systematic and automatic classification of atherosclerotic plaque constituents, based on the optical attenuation coefficient $\mu(t)$ of the tissue. OCT images of 65 coronary artery segments in vitro, obtained from 14 vessels harvested at autopsy, are analyzed and correlated with histology. Vessel wall components can be distinguished based on their optical properties: necrotic core and macrophage infiltration exhibit strong attenuation, $\mu(t)>or=10 \text{ mm}^{-1}$, while calcific and fibrous tissue have a lower $\mu(t)$ approximately 2-5 mm$^{-1}$. The algorithm is successfully applied to OCT patient data, demonstrating that the analysis can be used in a clinical setting and assist diagnostics of vessel wall pathology.

Full text article available from *Journal of Biomedical Optics* (USD 25.00)
PMID: 20210431

**D)** Coronary CT Angiographic Characteristics of Culprit Lesions in Acute Coronary Syndromes Not Related to Plaque Rupture as Defined by Optical Coherence Tomography and Angioscopy


**Summary:** Aims Pathological and clinical optical coherence tomography (OCT) studies have indicated that acute coronary syndrome (ACS) lesions have either ruptured fibrous caps (RFC-ACS) or intact fibrous caps (IFC-ACS). Although computed tomographic (CT) angiographic characteristics of RFC-ACS include low-attenuation plaques and positive plaque remodelling, features associated with IFC-ACS have not been previously described. The aim of this study was to assess the CT characteristics of IFC-ACS lesions. Methods and results Seventy-four patients with ACS/stable angina consented to multimodality imaging, of which 66 underwent CT angiography. Of these, 57 culprit lesions in 57 patients were evaluated with sufficient image quality from all four of OCT, angioscopy, intravascular ultrasound, and CT angiography. Intraluminal thrombus was assessed by OCT/angioscopy, and culprit lesions further classified by OCT-based demonstration of fibrous cap integrity. Of 35 culprit lesions with ACS, OCT revealed IFC with thrombus in 10 (29%) and RFC in the remaining 25 (71%); all 22 lesions with stable angina had intact fibrous caps. Fibrous caps were significantly thinner in RFC-ACS than IFC-ACS and stable angina (45 ± 12, 131 ± 57, and 321 ± 146 μm, respectively; $P = 0.001$). CT angiography revealed that low-attenuation plaques were more frequently observed in RFC-ACS than IFC-ACS and stable angina (88, 40, and 18%; $P = 0.001$) lesions. Similarly, positive remodelling was more predominantly seen in RFC-ACS than IFC-ACS and stable angina (96, 20, and 14%; $P = 0.001$). However, none of the specific CT angiography features clearly distinguished IFC-ACS from stable lesions. Conclusion In contrast to the situation with RFC-ACS, distinct culprit lesion characteristics associated with non-rupture-
related mechanisms are not identified by CT angiography. It will therefore not be possible to differentiate plaques likely to develop IFC-ACS from stable plaques.

Full text article available from Oxford Journals (USD 40.00)
PMD: 21719455

E) Imaging the Subcellular Structure of Human Coronary Atherosclerosis Using Micro-Optical Coherence Tomography
Liu L, Gardecki JA, Nadkarni SK, et al. Imaging the subcellular structure of human coronary atherosclerosis using micro-optical coherence tomography. *Nat Med*. 2011 Jul 10; 17(8): 1010-4. doi: 10.1038/nm. Summary: Progress in understanding, diagnosis, and treatment of coronary artery disease (CAD) has been hindered by our inability to observe cells and extracellular components associated with human coronary atherosclerosis in situ. The current standards for microstructural investigation, histology and electron microscopy are destructive and prone to artifacts. The highest-resolution intracoronary imaging modality, optical coherence tomography (OCT), has a resolution of ~10 μm, which is too coarse for visualizing most cells. Here we report a new form of OCT, termed micro-optical coherence tomography (μOCT), whose resolution is improved by an order of magnitude. We show that μOCT images of cadaver coronary arteries provide clear pictures of cellular and subcellular features associated with atherogenesis, thrombosis and responses to interventional therapy. These results suggest that μOCT can complement existing diagnostic techniques for investigating atherosclerotic specimens, and that μOCT may eventually become a useful tool for cellular and subcellular characterization of the human coronary wall in vivo.

Free full text article available from PubMed
PMD: 21743452

F) Intravascular Laser Speckle Imaging Catheter for the Mechanical Evaluation of the Arterial Wall

Summary: Laser speckle imaging (LSI) is a novel technique for measuring the mechanical properties of atherosclerotic plaques. In LSI, the decorrelation time constant of speckle intensity fluctuations provides an index of viscoelasticity that is closely related to plaque microstructure and composition. Here, we demonstrate for the first time, the feasibility of conducting LSI in vivo using a prototype 1.5 mm (4.5 Fr) diameter intravascular catheter. Investigation of the catheter performance using human arterial samples ex vivo shows that plaque time constants measured by the LSI catheter correlate well with those measured using a free-space bulk optics system. To demonstrate LSI in vivo, the catheter is interfaced with a portable console for intravascular evaluation in the aorta of a living rabbit. Distinct differences in arterial time constants are identified at normal aortic and stented sites in vivo with intravascular LSI.

Free full text article available from PubMed
PMD: 21361689

G) Multimodal Spectroscopy Detects Features of Vulnerable Atherosclerotic Plaque
Summary: Early detection and treatment of rupture-prone vulnerable atherosclerotic plaques is critical to reducing patient mortality associated with cardiovascular disease. The combination of reflectance, fluorescence, and Raman spectroscopy—termed multimodal spectroscopy (MMS)—provides detailed biochemical information about tissue and can detect vulnerable plaque features: thin fibrous cap (TFC), necrotic core (NC), superficial foam cells (SFC), and thrombus. Ex vivo MMS spectra are collected from 12 patients that underwent carotid endarterectomy or femoral bypass surgery. Data are collected by means of a unitary MMS optical fiber probe and a portable clinical instrument. Blinded histopathological analysis is used to assess the vulnerability of each spectrally evaluated artery lesion. Modeling of the ex vivo MMS spectra produce objective parameters that correlate with the presence of vulnerable plaque features: TFC with fluorescence parameters indicative of collagen presence; NC/SFC with a combination of diffuse reflectance β-carotene/ceroid absorption and the Raman spectral signature of lipids; and thrombus with its Raman signature. Using these parameters, suspected vulnerable plaques can be detected with a sensitivity of 96% and specificity of 72%. These encouraging results warrant the continued development of MMS as a catheter-based clinical diagnostic technique for early detection of vulnerable plaques.

Free full text article available from PubMed
PMID: 21280896
5.4.2 Coronary Stents

A) Optical Coherence Tomography: A New Imaging Modality for Plaque Characterization and Stent Implantation


**Summary:** Optical coherence tomography (OCT) is a novel, catheter-based, invasive imaging system based on near-infrared light with high image resolution (15-20 mum). The system allows for unparalleled imaging of the coronary artery lumen, plaque characterization, assessment of coronary stent strut apposition, neointimal coverage, vascular proliferative response, complications such as focal dissection or thrombus formation, and insight into the time course of stent endothelization. This review will describe the currently available developments in OCT technology and its application in both the clinical and research arenas.

Full text article available from *Journal of Interventional Cardiology* (USD 35.00 for 24 hour access)
PMID: 21198851

B) Feasibility and Safety of the Second-Generation, Frequency Domain Optical Coherence Tomography (FD-OCT): A Multicenter Study


**Summary:** This study sought to assess the effectiveness and safety of the second-generation frequency-domain optical coherence tomography (FD-OCT) system. **BACKGROUND:** The second-generation FD-OCT was recently developed, with simplified imaging technique and faster acquisition time compared to the first-generation time-domain OCT. However, the safety and effectiveness of the FD-OCT has not been evaluated, and this study was conceived as a preapproval study for Food and Drug Administration clearance for clinical use in the United States. **METHODS:** A total of 50 patients were enrolled from 3 institutions. Following stent implantation, the FD-OCT was performed with contrast injection through the guiding catheter to acquire pullback images with the pressure-triggered automatic pullback device. The primary endpoint was to achieve a median clear image length of more than 24 mm. Serious procedure-related or postprocedural adverse events (death, myocardial infarction, or ventricular arrhythmia) were recorded to assess safety of the device. **RESULTS:** The primary endpoint of obtaining >24 mm of median clear image length (CIL) was achieved in 94% of the subjects (47 out of 50), with measured CIL of 43.2 mm. In 5 patients (10.6%), a second attempt was necessary due to suboptimal image quality of the first pullback. In 36 patients (76.6%), a full stent length was obtained during the first attempt. There were no serious procedure-related or postprocedural adverse events. **CONCLUSIONS:** The new second-generation FD-OCT system provides fast and reliable resolution images of the coronary artery. The pullback can be safely performed over long segments of the artery without serious adverse events.

Free full text article available from *Journal of Invasive Cardiology*
PMID: 22562913
C) Ex Vivo Assessment of Vascular Response to Coronary Stents by Optical Frequency Domain Imaging

Summary: This study sought to examine the capability of optical frequency domain imaging (OFDI) to characterize various morphological and histological responses to stents implanted in human coronary arteries. BACKGROUND: A precise assessment of vascular responses to stents may help stratify the risk of future adverse events in patients who have been treated with coronary stents. METHODS: Fourteen human stented coronary segments with implant duration >/= 1 month from 10 hearts acquired at autopsy were interrogated ex vivo by OFDI and intravascular ultrasound (IVUS). Comparison with histology was assessed in 134 pairs of images where the endpoints were to investigate: 1) accuracy of morphological measurements; 2) detection of uncovered struts; and 3) characterization of neointima. RESULTS: Although both OFDI and IVUS provided a good correlation of neointimal area with histology, the correlation of minimum neointimal thickness was inferior in IVUS (R(2) = 0.39) as compared with OFDI (R(2) = 0.67). Similarly, IVUS showed a weak correlation of the ratio of uncovered to total stent struts per section (RUTSS) (R(2) = 0.24), whereas OFDI maintained superiority (R(2) = 0.66). In a more detailed analysis by OFDI, identification of individual uncovered struts demonstrated a sensitivity of 77.9% and specificity of 96.4%. Other important morphological features such as fibrin accumulation, excessive inflammation (hypersensitivity), and in-stent atherosclerosis were characterized by OFDI; however, the similarly dark appearance of these tissues did not allow for direct visual discrimination. The quantitative analysis of OFDI signal reflections from various in-stent tissues demonstrated distinct features of organized thrombus and accumulation of foamy macrophages. CONCLUSIONS: The results of the present study reinforce the potential of OFDI to detect vascular responses that may be important for the understanding of long-term stent performance, and indicate the capability of this technology to serve as a diagnostic indicator of clinical success.

Free full text article available from JACC: Cardiovascular Imaging
PMID: 22239896
5.5 Lung

Reprinted from Wang et al 106, 2010; 18(12):13027, with permission of the Optical Society.

In vivo microscopy (IVM) of the lung poses challenges due to the extensive branching and narrow diameter of the bronchial tree at risk for development of neoplastic diseases. There are also air-fluid interfaces beyond the airways that pose problems for IVM of interstitial diseases. Optical coherence tomography (OCT) has the potential to address these problems because the diameter of probes has been able to be decreased to about 600 microns (within the diameter of a 23 gauge needle), potentially allowing access to very small airways beyond the reach of standard bronchoscopes. OCT imaging is also sufficiently rapid to be able to scan an extensive surface of the airways within minutes. Unlike confocal laser microscopy, OCT is able to scan through air-fluid interfaces, potentially allowing interstitial lung diseases to be recognized. Preliminary studies are beginning to show promise, but controlled studies are lacking. OCT may have difficulty detecting dysplastic changes in the airway epithelium, since nuclear or cytoplasmic features cannot be resolved to show the cytology or architecture of the epithelium.

The following are selected articles on the application of IVM in the lung.

To see a video of how an IVM procedure is performed in the lung, go to this article in JOVE (the Journal of Visualized Experiments), which publishes articles teaching laboratory fundamentals using video demonstrations.

A) Optical Frequency Domain Imaging of Ex vivo Pulmonary Resection Specimens: Obtaining One to One Image to Histopathology Correlation

Summary: Lung cancer is the leading cause of cancer-related deaths(1). Squamous cell and small cell cancers typically arise in association with the conducting airways, whereas adenocarcinomas are typically more peripheral in location. Lung malignancy detection early in the disease process may be difficult due to several limitations: radiological resolution, bronchoscopic limitations in evaluating tissue underlying the airway mucosa and identifying early pathologic changes, and small sample size and/or incomplete sampling in histology biopsies. High resolution imaging modalities, such as optical frequency domain imaging (OFDI), provide non-destructive, large area 3-dimensional views of tissue microstructure to depths approaching 2 mm in real time (Figure 1)(2-6). OFDI has been utilized in a variety of applications, including evaluation of coronary artery atherosclerosis(6,7) and esophageal intestinal metaplasia and dysplasia(6,8-10). Bronchoscopic OCT/OFDI has been demonstrated as a safe in vivo imaging tool for
evaluating the pulmonary airways\(^{(11-23)}\) (Animation). OCT has been assessed in pulmonary airways\(^{(16,23)}\) and parenchyma\(^{(17,22)}\) of animal models and in vivo human airway\(^{(14,15)}\). OCT imaging of normal airway has demonstrated visualization of airway layering and alveolar attachments, and evaluation of dysplastic lesions has been found useful in distinguishing grades of dysplasia in the bronchial mucosa\(^{(11,12,20,21)}\). OFDI imaging of bronchial mucosa has been demonstrated in a short bronchial segment \((0.8 \text{ cm})\)\(^{(18)}\). Additionally, volumetric OFDI spanning multiple airway generations in swine and human pulmonary airways in vivo has been described\(^{(19)}\). Endobronchial OCT/OFDI is typically performed using thin, flexible catheters, which are compatible with standard bronchoscopic access ports. Additionally, OCT and OFDI needle-based probes have recently been developed, which may be used to image regions of the lung beyond the airway wall or pleural surface\(^{(17)}\). While OCT/OFDI has been utilized and demonstrated as feasible for in vivo pulmonary imaging, no studies with precisely matched one-to-one OFDI:histology have been performed. Therefore, specific imaging criteria for various pulmonary pathologies have yet to be developed. Histopathological counterparts obtained in vivo consist of only small biopsy fragments, which are difficult to correlate with large OFDI datasets. Additionally, they do not provide the comprehensive histology needed for registration with large volume OFDI. As a result, specific imaging features of pulmonary pathology cannot be developed in the in vivo setting. Precisely matched, one-to-one OFDI and histology correlation is vital to accurately evaluate features seen in OFDI against histology as a gold standard in order to derive specific image interpretation criteria for pulmonary neoplasms and other pulmonary pathologies. Once specific imaging criteria have been developed and validated ex vivo with matched one-to-one histology, the criteria may then be applied to in vivo imaging studies. Here, we present a method for precise, one to one correlation between high resolution optical imaging and histology in ex vivo lung resection specimens. Throughout this manuscript, we describe the techniques used to match OFDI images to histology. However, this method is not specific to OFDI and can be used to obtain histology-registered images for any optical imaging technique. We performed airway centered OFDI with a specialized custom built bronchoscopic 2.4 French \((0.8 \text{ mm diameter})\) catheter. Tissue samples were marked with tissue dye, visible in both OFDI and histology. Careful orientation procedures were used to precisely correlate imaging and histological sampling locations. The techniques outlined in this manuscript were used to conduct the first demonstration of volumetric OFDI with precise correlation to tissue-based diagnosis for evaluating pulmonary pathology\(^{(24)}\). This straightforward, effective technique may be extended to other tissue types to provide precise imaging to histology correlation needed to determine fine imaging features of both normal and diseased tissues.

Free full text article available from Journal of Visualized Experiments
PMID: 23381470
Note: Also cited in Section 2.3

B) Volumetric Optical Frequency Domain Imaging of Pulmonary Pathology with Precise Correlation to Histopathology

Summary: Lung cancer is the leading cause of cancer-related mortality. Radiology and bronchoscopy techniques do not have the necessary resolution to evaluate lung lesions on the microscopic scale, which is critical for diagnosis. Bronchial biopsy specimens can be limited by sampling error and small size. Optical frequency domain imaging (OFDI)
provides volumetric views of tissue microstructure at near-histologic resolution and may be useful for evaluating pulmonary lesions to increase diagnostic accuracy. Bronchoscopic OFDI has been evaluated in vivo, but a lack of correlated histopathology has limited the ability to develop accurate image interpretation criteria. METHODS: We performed OFDI through two approaches (airway-centered and parenchymal imaging) in 22 ex vivo lung specimens, using tissue dye to precisely correlate imaging and histology. RESULTS: OFDI of normal airway allowed visualization of epithelium, lamina propria, cartilage, and alveolar attachments. Carcinomas exhibited architectural disarray, loss of normal airway and alveolar structure, and rapid light attenuation. Squamous cell carcinomas showed nested architecture. Atypical glandular formation was appreciated in adenocarcinomas, and uniform trabecular gland formation was seen in salivary gland carcinomas. Mucinous adenocarcinomas showed alveolar wall thickening with intra-alveolar mucin. Interstitial fibrosis was visualized as signal-dense tissue, with an interstitial distribution in mild interstitial fibrotic disease and a diffuse subpleural pattern with cystic space formation in usual interstitial pneumonitis. CONCLUSIONS: To our knowledge, this study is the first demonstration of volumetric OFDI with precise correlation to histopathology in lung pathology. We anticipate that OFDI may play a role in assessing airway and parenchymal pathology, providing fresh insights into the volumetric features of pulmonary disease.

Full text article available from Chest Journal (USD 15.00 for 24 hour access) PMID: 22459781

C) In Vivo Optical Coherence Tomography: The Role of the Pathologist

Summary: Optical coherence tomography (OCT) is a nondestructive, high-resolution imaging modality, providing cross-sectional, architectural images at near histologic resolutions, with penetration depths up to a few millimeters. Optical frequency domain imaging is a second-generation OCT technology that has equally high resolution with significantly increased image acquisition speeds and allows for large area, high-resolution tissue assessments. These features make OCT and optical frequency domain imaging ideal imaging techniques for surface and endoscopic imaging, specifically when tissue is unsafe to obtain and/or suffers from biopsy sampling error. This review focuses on the clinical impact of OCT in coronary, esophageal, and pulmonary imaging and the role of the pathologist in interpreting high-resolution OCT images as a complement to standard tissue pathology.

Free full text article available from the CAP's Archives PMID: 23194041
Note: Also cited in Section 1.2

D) Differential Diagnosis of Lung Carcinoma with Coherent Anti-Stokes Raman Scattering Imaging

Summary: Aimed at bridging imaging technology development with cancer diagnosis, this paper first presents the prevailing challenges of lung cancer detection and diagnosis, with an emphasis on imaging techniques. It then elaborates on the working principle of
coherent anti-Stokes Raman scattering microscopy, along with a description of pathologic applications to show the effectiveness and potential of this novel technology for lung cancer diagnosis. As a nonlinear optical technique probing intrinsic molecular vibrations, coherent anti-Stokes Raman scattering microscopy offers an unparalleled, label-free strategy for clinical cancer diagnosis and allows differential diagnosis of fresh specimens based on cell morphology information and patterns, without any histology staining. This powerful feature promises a higher biopsy yield for early cancer detection by incorporating a real-time imaging feed with a biopsy needle. In addition, molecularly targeted therapies would also benefit from early access to surgical specimen with high accuracy but minimum tissue consumption, therefore potentially saving specimens for follow-up diagnostic tests. Finally, we also introduce the potential of a coherent anti-Stokes Raman scattering–based endoscopy system to support intraoperative applications at the cellular level.

Free full text article available from the CAP's Archives
PMID: 23194042

5.6 Genitourinary System

Although IVM is not yet widely adopted in Urology as it has been in Gastroenterology, the GU system also has luminal surfaces highly amenable to in vivo endomicroscopy. Specifically, the urinary tract can be examined for in situ, minute or recurrent lesions. IVM can also guide the acquisition of biopsies from the most clinically significant areas, such as areas of invasive urothelial carcinoma in a larger non-invasive lesion (as shown in the multiphoton microscopy (left) and H&E images (right) above). Therefore it is anticipated that IVM will be utilized for these purposes during cystoscopy in the near future. Margin assessment and preservation of periprostatic potency-associated nerves during radical prostatectomy is another application anticipated shortly. Also IVM can aid in sperm retrieval for fertility treatments. These applications of IVM in Urology are currently in research studies, but are expected to become clinical practice soon.

The following are selected articles on the application of IVM in the genitourinary system.
A) **Human Bladder Cancer Diagnosis Using Multiphoton Microscopy**  

**Summary:** At the time of diagnosis, approximately 75% of bladder cancers are non-muscle invasive. Appropriate diagnosis and surgical resection at this stage improves prognosis dramatically. However, these lesions, being small and/or flat, are often missed by conventional white-light cystoscopes. Furthermore, it is difficult to assess the surgical margin for negativity using conventional cystoscopes. Resultantly, the recurrence rates in patients with early bladder cancer are very high. This is currently addressed by repeat cystoscopies and biopsies, which can last throughout the life of a patient, increasing cost and patient morbidity. Multiphoton endoscopes offer a potential solution, allowing real time, non-invasive biopsies of the human bladder, as well as an up-close assessment of the resection margin. While miniaturization of the Multiphoton microscope into an endoscopic format is currently in progress, we present results here indicating that Multiphoton imaging (using a bench-top Multiphoton microscope) can indeed identify cancers in fresh, unfixed human bladder biopsies. Multiphoton images are acquired in two channels: (1) broadband autofluorescence from cells, and (2) second harmonic generation (SHG), mostly by tissue collagen. These images are then compared with gold standard hematoxylin/eosin (H&E) stained histopathology slides from the same specimen. Based on a "training set" and a very small "blinded set" of samples, we have found excellent correlation between the Multiphoton and histopathological diagnoses. A larger blinded analysis by two independent uropathologists is currently in progress. We expect that the conclusion of this phase will provide us with diagnostic accuracy estimates, as well as the degree of inter-observer heterogeneity.

Free full text article available from [PubMed](https://pubmed.ncbi.nlm.nih.gov/19360140/)

B) **Multiphoton Microscopy in the Evaluation of Human Bladder Biopsies**  

**Summary:** Multiphoton microscopy (MPM) is a nonlinear imaging approach, providing cellular and subcellular details from fresh (unprocessed) tissue by exciting intrinsic tissue emissions. With miniaturization and substantially decreased cost on the horizon, MPM is an emerging imaging technique with many potential clinical applications. Objectives: To assess the imaging ability and diagnostic accuracy of MPM for human bladder biopsies. Design: Seventy-seven fresh bladder biopsies were imaged by MPM and subsequently submitted for routine surgical pathology diagnosis. Twelve cases were excluded because of extensive cautery artifact that prohibited definitive diagnosis. Comparison was made between MPM imaging and gold standard sections for each specimen stained with hematoxylin-eosin. Results: In 57 of 65 cases (88%), accurate MPM diagnoses (benign or neoplastic) were given based on the architecture and/or the cytologic grade. The sensitivity and specificity of MPM in our study were 90.4% and 76.9%, respectively. A positive (neoplastic) diagnosis on MPM had a high predictive value (94%), and negative (benign) diagnoses were sustained on histopathology in two-thirds of cases. Architecture (papillary versus flat) was correctly determined in 56 of 65 cases (86%), and cytologic grade (benign/low grade versus high grade) was assigned correctly in 38 of 56 cases (68%). Conclusions: The MPM images alone provided sufficient detail to classify most lesions as either benign or neoplastic using the same basic diagnostic
criteria as histopathology (architecture and cytologic grade). Future developments in MPM technology may provide urologists and pathologists with additional screening and diagnostic tools for early detection of bladder cancer. Additional applications of such emerging technologies warrant exploration.

Free full text article available from the CAP's Archives
PMID: 22540300

C) Multiphoton Microscopy for Structure Identification in Human Prostate and Periprostatic Tissue: Implications in Prostate Cancer Surgery

Summary: Study Type - Diagnostic (exploratory cohort) Level of Evidence 2b What's known on the subject? and What does the study add? Prostate cancer surgery outcomes depend on an optimal balance of three aspects: complete removal of cancerous glands, preservation of nerves for sexual function and of sphincteric structures for urinary control. Current surgical techniques, even with the magnification provided by the robotic stereoscope, are insufficient to identify these structures in the surgical field. Multiphoton microscopy has been shown to produce high contrast images with subcellular resolution in fresh (unprocessed and unstained tissue) utilizing intrinsic tissue emission signals. We provide evidence that Multiphoton microscopy of freshly excised tissue from human radical prostatectomy specimens, without any processing or use of exogenous contrast, can identify all relevant prostatic and periprostatic structures. These include the prostatic acini, the stroma and the capsule, as well as periprostatic fascial structures such as loose connective tissue, nerves, blood vessels and fat, as well as areas of local inflammation. We also show that multiphoton microscopy is able to distinguish between normal prostate gland, those with benign hyperplasia, and those harboring cancer. OBJECTIVE: To test whether multiphoton microscopy (MPM) might allow identification of prostatic and periprostatic structures with magnification and resolution similar to gold standard histopathology. MATERIAL AND METHODS: The present study included 95 robotic radical prostatectomy patients who consented to participate in an Institutional Review Board-approved study starting in 2007. The types of specimens used for imaging were excised surgical margins and biopsies, and sections obtained from the excised prostate. The specimens were imaged with a custom-built MPM system. All images were compared with haematoxylin/eosin histopathology of the same specimen. RESULTS: MPM of freshly excised, unprocessed and unstained tissue can identify all relevant prostatic and periprostatic structures, such as nerves, blood vessels, capsule, underlying acini and also pathological changes, including prostate cancer. Histological confirmation and correlation of these structures and pathologies have validated the findings of MPM. CONCLUSIONS: MPM shows great promise as a tool for real-time intra-surgical histopathology without needing excision or administration of contrast agents. The results will, however, need to be confirmed in true surgical settings using a miniaturized MPM microendoscope.

Full text article available from BJU International (USD 35.00 for 24-hour access)
PMID: 21443651
D) Pilot Study of the Correlation of Multiphoton Tomography of Ex Vivo Human Testis with Histology

Summary: Although microdissection testicular sperm extraction has become first line therapy for sperm retrieval in men with nonobstructive azoospermia, there are challenges to the procedure, including difficulty differentiating between seminiferous tubules with normal and abnormal spermatogenesis. Multiphoton microscopy illuminates tissue with a near infrared laser to elicit autofluorescence, which enables real-time imaging of unprocessed tissue without labels. We hypothesized that we could accurately characterize seminiferous tubular histology in humans using multiphoton microscopy.

MATERIALS AND METHODS: Seven men with normal or abnormal spermatogenesis underwent testicular biopsies, which were imaged by multiphoton microscopy. We assessed these images in blinded fashion. The diagnosis rendered with multiphoton microscopy was then correlated with that of hematoxylin and eosin stained tissue. We evaluated the ability of multiphoton microscopy to differentiate normal from abnormal seminiferous tubules by examining autofluorescence characteristics and diameters, as imaged by multiphoton microscopy. Assessment was repeated with stained slides and results were compared. RESULTS: The overall concordance rate between multiphoton microscopy and stained slides was 86%. The seminiferous tubules of patients with nonobstructive azoospermia were smaller than those of controls when measured by multiphoton microscopy and staining (p <0.05). The proportion of normal tubules and the diameters obtained with multiphoton microscopy were not different from those obtained with hematoxylin and eosin (p >0.05). CONCLUSION: Multiphoton microscopy can be used to differentiate normal from abnormal spermatogenesis. Its characterization of seminiferous tubular architecture is similar to that provided by hematoxylin and eosin staining. Further investigation of the clinical applications of multiphoton microscopy may improve surgical sperm retrieval outcomes for patients with nonobstructive azoospermia.

Full text article available from Journal of Urology (USD 30.00)
PMID: 22704107
5.7 Gynecological System

Pap smear screening, colposcopy with acetic acid application, and the current addition of HPV viral testing have been very effective in decreasing cervical cancer. For these reasons, in vivo technology applications in GYN have lagged behind other fields. However, these technologies have great potential for imaging surfaces like the cervix, vagina, and even endometrium through a hysteroscope.

Recently, studies have also examined the feasibility of identifying HSIL cervical lesions in order to diagnose and treat at encounter, and thus reduce cost. The IVM technologies utilized for these purposes are confocal microscopy with Raman spectroscopy, optical spectroscopy (shown above) and multispectral digital colposcopy.

The following are selected articles on the application of IVM in the gynecological system.

A) **Effect of Anatomy on Spectroscopic Detection of Cervical Dysplasia**

**Summary:** Source It has long been speculated that underlying variations in tissue anatomy affect in vivo spectroscopic measurements. We investigate the effects of cervical anatomy on reflectance and fluorescence spectroscopy to guide the development of a diagnostic algorithm for identifying high-grade squamous intraepithelial lesions (HSILs) free of the confounding effects of anatomy. We use spectroscopy in both contact probe and imaging modes to study patients undergoing either colposcopy or treatment for HSIL. Physical models of light propagation in tissue are used to extract parameters related to tissue morphology and biochemistry. Our results show that the transformation zone, the area in which the vast majority of HSILs are found, is spectroscopically distinct from the adjacent squamous mucosa, and that these anatomical differences can directly influence spectroscopic diagnostic parameters. Specifically, we demonstrate that performance of diagnostic algorithms for identifying HSILs is artificially enhanced when clinically normal squamous sites are included in the statistical analysis of the spectroscopic data. We conclude that underlying differences in tissue anatomy can have a confounding effect on diagnostic spectroscopic parameters and that the common practice of including clinically normal squamous sites in cervical
spectroscopy results in artificially improved performance in distinguishing HSILs from clinically suspicious non-HSILs.

Free full text article available from PubMed
PMID: 19725732

B) Detecting High-Grade Squamous Intraepithelial Lesions in the Cervix with Quantitative Spectroscopy and Per-Patient Normalization

Summary: This study develops a spectroscopic algorithm for detection of cervical high grade squamous intraepithelial lesions (HSILs). We collected reflectance and fluorescence spectra with the quantitative spectroscopy probe to measure nine spectroscopic parameters from 43 patients undergoing standard colposcopy with directed biopsy. We found that there is improved accuracy for distinguishing HSIL from non-HSIL (low grade SIL and normal tissue) when we “normalized” spectroscopy parameters by dividing the values extracted from each clinically determined suspicious site by the corresponding value extracted from a clinically normal squamous site from the same patient. The “normalized” scattering parameter (A) at 700nm, best distinguished HSIL from non-HSIL with sensitivity and specificity of 89% and 79% suggesting that a simple, monochromatic instrument measuring only A may accurately detect HSIL.

Free full text article available in PubMed
PMID: 22025992

C) Multimodal Hyperspectroscopy as a Triage Test for Cervical Neoplasia: Pivotal Clinical Trial Results

Summary: To prospectively evaluate a new non invasive device that combines fluorescence and reflectance spectroscopy in a population in women at risk for cervical dysplasia. METHODS: A total of 1607 women were evaluated with multimodal hyperspectroscopy (MHS), a painless test with extremely high spectral resolution. Subjects who were referred to colposcopy based on abnormal screening tests or other referral criteria underwent the MHS test and also had a sample taken for additional cytology and presence of high risk human papilloma virus (HPV) prior to undergoing biopsy. RESULTS: Sensitivity of MHS for cervical intraepithelial neoplasia (CIN) 2+ was 91.3% (252/276). Specificity, or the potential reduction in referrals to colposcopy and biopsy, was 38.9% (222/570) for women with normal or benign histology and 30.3% (182/601) for women with CIN1 histology. Two year follow-up data, collected for a subgroup of 804 women, revealed 67 interval CIN2+ that originally were diagnosed at enrollment as normal or CIN1. MHS identified 60 of these (89.6%) as positive for CIN2+ prior to their discovery during the two year follow-up period. CONCLUSIONS: MHS provides an immediate result at the point of care. Recently, the limitations of cytology have become more obvious and as a consequence greater emphasis is being placed on HPV testing for cervical cancer screening, creating a need for an inexpensive, convenient and accurate test to reduce false positive referrals to colposcopy and increase the yield of CIN2+ at biopsy. MHS appears to have many of the attributes necessary for such an application.
D) Optical Technologies and Molecular Imaging for Cervical Neoplasia: A Program Project Update

**Summary:** There is an urgent global need for effective and affordable approaches to cervical cancer screening and diagnosis. In developing nations, cervical malignancies remain the leading cause of cancer-related deaths in women. This reality may be difficult to accept given that these deaths are largely preventable; where cervical screening programs have been implemented, cervical cancer-related deaths have decreased dramatically. In developed countries, the challenges of cervical disease stem from high costs and overtreatment. The National Cancer Institute-funded Program Project is evaluating the applicability of optical technologies in cervical cancer. The mandate of the project is to create tools for disease detection and diagnosis that are inexpensive, require minimal expertise, are more accurate than existing modalities, and can be feasibly implemented in a variety of clinical settings. This article presents the status and long-term goals of the project.

Free full text article available in *PubMed*
PMID: 21944317

E) Early Detection of High-grade Squamous Intraepithelial Lesions in the Cervix with Quantitative Spectroscopic Imaging

**Summary:** Quantitative spectroscopy has recently been extended from a contact-probe to wide-area spectroscopic imaging to enable mapping of optical properties across a wide area of tissue. We train quantitative spectroscopic imaging (QSI) to identify cervical high-grade squamous intraepithelial lesions (HSILs) in 34 subjects undergoing the loop electrosurgical excision procedure (LEEP subjects). QSI's performance is then prospectively evaluated on the clinically suspicious biopsy sites from 47 subjects undergoing colposcopic-directed biopsy. The results show the per-subject normalized reduced scattering coefficient at 700 nm (An) and the total hemoglobin concentration are significantly different (p<0.05) between HSIL and non-HSIL sites in LEEP subjects. An alone retrospectively distinguishes HSIL from non-HSIL with 89% sensitivity and 83% specificity. It alone applied prospectively on the biopsy sites distinguishes HSIL from non-HSIL with 81% sensitivity and 78% specificity. The findings of this study agree with those of an earlier contact-probe study, validating the robustness of QSI, and specifically An, for identifying HSIL. The performance of An suggests an easy to use and an inexpensive to manufacture monochromatic instrument is capable of early cervical cancer detection, which could be used as a screening and diagnostic tool for detecting cervical cancer in low resource countries.

Free full text article available from *Journal of Biomedical Optics*
PMID: 23843090
F) Diagnostic Applications of Raman Spectroscopy

Summary: Raman spectroscopy has been widely used in various fields of science. It has been successfully utilized to qualitatively and quantitatively determine the molecular compositions of solid, liquid, and gaseous samples. This review focuses on the diagnostic applications of Raman spectroscopy in the past 5 years, with specific emphasis on transplant allograft rejection and cancer detections. First we introduce the principle of Raman spectroscopy and associated surface enhancement techniques. Various recent biomedical and clinical applications of Raman spectroscopy are then reviewed in detail. Finally, we present the experimental and analytical techniques required to implement Raman spectroscopy in a laboratory. FROM THE CLINICAL EDITOR: This review focuses on evolving diagnostic applications of Raman spectroscopy with special emphasis on transplant allograft rejection and cancer detection.

Full text article available from Nanomedicine: Nanotechnology, Biology and Medicine (USD 31.50) PMID: 22024196

5.8 Brain


Currently, intraoperative diagnosis of brain tumors and other CNS tissue abnormalities relies on frozen section analysis. However, frozen section diagnosis presents with inherent challenges, including sampling error and frozen section artifact. An alternative intraoperative method for real-time tissue analysis, could serve as a useful adjunct to both the neuropathologist and neurosurgeon. In vivo confocal endomicroscopy (CEM), as well as other novel methods of optical imaging such as OCT, are emerging as new technologies with promising clinical and diagnostic potential in visualizing CNS neoplastic and nonneoplastic tissues (as in the above CEM image of glioma cells in a mouse model). These novel imaging modalities allow for visualization of tissues at cellular and subcellular levels in real time and can provide increased speed, as well as the potential to visualize margins of a resection cavity intraoperatively. Digital images can be stored for future retrieval by remote sites, or transmitted to distant pathologists for real time evaluation. Lastly, in vivo optical imaging devices can enhance the selection of cellular tissue for both molecular studies and tissue banking. The incorporation of these novel modes of imaging into the neurosurgical OR suite and the pathology laboratory have the potential to shape the future of surgical resection and
intraoperative diagnosis of brain tumors, thereby improving tumor resection and prolonging patient survival.

The following are selected articles on the application of IVM in the brain.

A) **Use of In Vivo Near-Infrared Laser Confocal Endomicroscopy with Indocyanine Green to Detect the Boundary of Infiltrative Tumor**


**Summary:** Object Infiltrative tumor resection is based on regional (macroscopic) imaging identification of tumorous tissue and the attempt to delineate invasive tumor margins in macroscopically normal-appearing tissue, while preserving normal brain tissue. The authors tested miniaturized confocal fiberoptic endomicroscopy by using a near-infrared (NIR) imaging system with indocyanine green (ICG) as an in vivo tool to identify infiltrating glioblastoma cells and tumor margins. Methods Thirty mice underwent craniectomy and imaging in vivo 14 days after implantation with GL261-luc cells. A 0.4 mg/kg injection of ICG was administered intravenously. The NIR images of normal brain, obvious tumor, and peritumoral zones were collected using the handheld confocal endomicroscope probe. Histological samples were acquired from matching imaged areas for correlation of tissue images. Results In vivo NIR wavelength confocal endomicroscopy with ICG detects fluorescence of tumor cells. The NIR and ICG macroscopic imaging performed using a surgical microscope correlated generally to tumor and peritumor regions, but NIR confocal endomicroscopy performed using ICG revealed individual tumor cells and satellites within peritumoral tissue; a definitive tumor border; and striking fluorescent microvascular, cellular, and subcellular structures (for example, mitoses, nuclei) in various tumor regions correlating with standard clinical histological features and known tissue architecture. Conclusions Macroscopic fluorescence was effective for gross tumor detection, but NIR confocal endomicroscopy performed using ICG enhanced sensitivity of tumor detection, providing real-time true microscopic histological information precisely related to the site of imaging. This first-time use of such NIR technology to detect cancer suggests that combined macroscopic and microscopic in vivo ICG imaging could allow interactive identification of microscopic tumor cell infiltration into the brain, substantially improving intraoperative decisions.

Full text article available from *Journal of Neurosurgery* (USD 15.00 for one day’s access) PMID: 21923240

B) **Intraoperative Confocal Microscopy in the Visualization of 5-Aminolevulnic Acid Fluorescence in Low-Grade Gliomas**


**Summary:** Greater extent of resection (EOR) for patients with low-grade glioma (LGG) corresponds with improved clinical outcome, yet remains a central challenge to the neurosurgical oncologist. Although 5-aminolevulnic acid (5-ALA)-induced tumor fluorescence is a strategy that can improve EOR in gliomas, only glioblastomas routinely fluoresce following 5-ALA administration. Intraoperative confocal microscopy adapts conventional confocal technology to a handheld probe that provides real-time fluorescent imaging at up to 1000× magnification. The authors report a combined
approach in which intraoperative confocal microscopy is used to visualize 5-ALA tumor fluorescence in LGGs during the course of microsurgical resection. METHODS: Following 5-ALA administration, patients with newly diagnosed LGG underwent microsurgical resection. Intraoperative confocal microscopy was conducted at the following points: 1) initial encounter with the tumor; 2) the midpoint of tumor resection; and 3) the presumed brain-tumor interface. Histopathological analysis of these sites correlated tumor infiltration with intraoperative cellular tumor fluorescence. RESULTS: Ten consecutive patients with WHO Grades I and II gliomas underwent microsurgical resection with 5-ALA and intraoperative confocal microscopy. Macroscopic tumor fluorescence was not evident in any patient. However, in each case, intraoperative confocal microscopy identified tumor fluorescence at a cellular level, a finding that corresponded to tumor infiltration on matched histological analyses. CONCLUSIONS: Intraoperative confocal microscopy can visualize cellular 5-ALA-induced tumor fluorescence within LGGs and at the brain-tumor interface. To assess the clinical value of 5-ALA for high-grade gliomas in conjunction with neuronavigation, and for LGGs in combination with intraoperative confocal microscopy and neuronavigation, a Phase IIIa randomized placebo-controlled trial (BALANCE) is underway at the authors’ institution. Comment in J Neurosurg. 2011 Oct;115(4):737-8; discussion 738-9.

Full text article available from Journal of Neurosurgery (USD 15.00 for 24 hour access)
PMID: 21761971

C) Intraoperative Confocal Microscopy for Brain Tumors: A Feasibility Analysis in Humans

Summary: The ability to diagnose brain tumors intraoperatively and identify tumor margins during resection could maximize resection and minimize morbidity. Advances in optical imaging enabled production of a hand-held intraoperative confocal microscope. OBJECTIVE: To present a feasibility analysis of the intraoperative confocal microscope for brain tumor resection. METHODS: Thirty-three brain tumor patients treated at Barrow Neurological Institute were examined. All patients received an intravenous bolus of sodium fluorescein before confocal imaging with the Optiscan FIVE 1 system probe. Optical biopsies were obtained within each tumor and along the tumor-brain interfaces. Corresponding pathologic specimens were then excised and processed. These data was compared by a neuropathologist to identify the concordance for tumor histology, grade, and margins. RESULTS: Thirty-one of 33 lesions were tumors (93.9%) and 2 cases identified as radiation necrosis (6.1%). Of the former, 25 (80.6%) were intra-axial and 6 (19.4%) were extra-axial. Intra-axial tumors were most commonly gliomas and metastases, while all extra-axial tumors were meningiomas. Among high-grade gliomas, vascular neoproliferation, as well as tumor margins, were identifiable using confocal imaging. Meningothelial and fibrous meningiomas were distinct on confocal microscopy-the latter featured spindle-shaped cells distinguishable from adjacent parenchyma. Other tumor histologies correlated well with standard neuropathology tissue preparations. CONCLUSION: Intraoperative confocal microscopy is a practicable technology for the resection of human brain tumors. Preliminary analysis demonstrates reliability for a variety of lesions in identifying tumor cells and the tumor-brain interface. Further refinement of this technology depends upon the approval of tumor-specific fluorescent contrast agents for human use.

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Full text article available from Neurosurgery Journal (USD 59.00)
D) **Neuroendovascular Optical Coherence Tomography Imaging and Histological Analysis**


**Summary**: Intravascular optical coherence tomography (OCT) is a recently developed optical imaging technique that provides high-resolution cross-sectional in situ images from intact tissue based on tissue reflectance of near-infrared or infrared light.

**OBJECTIVE**: To report on the feasibility of neuroendovascular OCT imaging and compare the neuroendovascular OCT findings with histology in nondiseased vessels in an animal, cadaveric, and clinical study.

**METHODS**: Catheter-based in vivo endovascular OCT imaging was performed in the common carotid arteries of 2 pigs and in the intracranial carotid arteries of 3 patients. The endovascular OCT device was delivered to the desired location via groin access and using standard endovascular procedures. Images were obtained via rotational and translational scanning using external motors. In vivo findings were reproduced using ex vivo OCT imaging in corresponding animal and human (cadaveric) harvested tissue segments. These segments underwent histological examination for comparison.

**RESULTS**: The structural compositions of the OCT-imaged segments of the common carotid arteries in pigs as well as the petrous and cavernous intracranial carotid arteries in patients were visualized at high resolution (8 mum). The in vivo images were identical to those obtained ex vivo, demonstrating the imaging capabilities of the endovascular OCT device. The OCT images correlated well with the images obtained after histological sectioning and visualized in vivo the laminar vascular structure.

**CONCLUSION**: Neuroendovascular OCT imaging is feasible for clinical use and can detect with high resolution the structure of arterial segments. Understanding OCT imaging in nondiseased arteries is important in establishing baseline findings necessary for interpreting pathological processes. This allows neuroendovascular optical biopsies of vascular tissue to be obtained without the need for excision and processing.

Free full text article available from [PubMed](PMID: 2135835)

5.9 Eye

Ophthalmology is the first clinical arena in which IVM has had a significant impact. The IVM technology in most widespread clinical use in ophthalmology at this time is optical coherence tomography (OCT). Since its inception in the early 1990s, OCT has rapidly progressed to become the gold standard for imaging of the anterior segment and retina, for the diagnosis of diseases such as macular degeneration.

The following is an article on clinical applications of IVM in the eye.

A) **Optical Coherence Tomography: History, Current Status, and Laboratory Work**


**Summary**: Optical coherence tomography (OCT) imaging has become widespread in ophthalmology over the past 15 years, because of its ability to visualize ocular structures
at high resolution. This article reviews the history of OCT imaging of the eye, its current status, and the laboratory work that is driving the future of the technology.

Free full text article available from PubMed and IOVS
PMID: 21493951
Section 6 Current Standards of Validation

IVM is a new and transformative technology. For there to be widespread clinical adoption of IVM, it must be clinically important, independently informative and of demonstrated beneficial value to patient care - by improving the accuracy of disease detection and diagnosis, as well as prediction of prognosis and response to treatment. And its usage must be standardized and validated with regard to clinical indications, technology of choice, procedure protocols, methods of image acquisition, nomenclature, image interpretation criteria, reproducibility (interobserver variability, etc.), and reporting of results, much as has been done for histology-based pathology diagnosis. Wide acceptance of IVM technology also requires training and documented competence prior to application in everyday clinical practice.

Even though IVM technologies are commercially available, are FDA approved and, for some procedures, have AMA CPT codes, standardization and validation of these technologies is still a work in progress. Our clinical colleagues are still in the process of reaching consensus as to the clinical indications and diagnostic thresholds for IVM, as well as the requirements for clinical trials (clinical endpoints, sample sizes required for adequately powered studies, etc.) that are used to make judgments as which IVM technologies to adopt and when. This is important in order to minimize the possibility that these potentially valuable innovations are prematurely abandoned due to lack of utilization and to avoid widespread use of IVM technologies before clinical studies documenting their effectiveness have been performed. Image classification schemes/criteria have been proposed for specific clinical applications, such as the diagnosis of vulnerable atherosclerotic plaque in the coronary arteries and the diagnosis of dysplasia in Barrett’s Esophagus. And a few early studies of interobserver agreement in the interpretation of IVM images for these same clinical applications have been reported. But much more remains to be done.

Pathologists should assume a leadership position in these IVM standardization and validation efforts. It is not only necessary for maintaining and enhancing our position in the medical care system, but also in the patients’ best interest.

The following are selected articles on:

- IVM validation and standardization
- IVM image classification schemes/criteria; and
- Interobserver agreement in interpreting IVM images

6.1 IVM Validation and Standardization

A) Validation of Novel Optical Imaging Technologies: The Pathologists’ View

Summary: Noninvasive optical imaging technology has the potential to improve the accuracy of disease detection and predict treatment response. Pathology provides the critical link between the biological basis of an image or spectral signature and clinical outcomes obtained through optical imaging. The validation of optical images and spectra requires both morphologic diagnosis from histopathology and parametric
analysis of tissue features above and beyond the declared pathologic “diagnosis.” Enhancement of optical imaging modalities with exogenously applied biomarkers also requires validation of the biological basis for molecular contrast. For an optical diagnostic or prognostic technology to be useful, it must be clinically important, independently informative, and of demonstrated beneficial value to patient care. Its usage must be standardized with regard to methods, interpretation, reproducibility, and reporting, in which the pathologist plays a key role. By providing insight into disease pathobiology, interpretive or quantitative analysis of tissue material, and expertise in molecular diagnosis, the pathologist should be an integral part of any team that is validating novel optical imaging modalities. This review will consider (1) the selection of validation biomarkers; (2) standardization in tissue processing, diagnosis, reporting, and quantitative analysis; (3) the role of the pathologist in study design; and (4) reference standards, controls, and interobserver variability.

Full text article available from *Journal of Biomedical Optics* (USD 25.00)
PMID: 17994879
NOTE: Also cited in Sections 1.2 and 3.2

B) **Consensus Standards for Acquisition, Measurement, and Reporting of Intravascular Optical Coherence Tomography Studies: A Report from the International Working Group for Intravascular Optical Coherence Tomography Standardization and Validation**

**Summary:** The purpose of this document is to make the output of the International Working Group for Intravascular Optical Coherence Tomography (IWG-IVOCT) Standardization and Validation available to medical and scientific communities, through a peer-reviewed publication, in the interest of improving the diagnosis and treatment of patients with atherosclerosis, including coronary artery disease. **BACKGROUND:** Intravascular optical coherence tomography (IVOCT) is a catheter-based modality that acquires images at a resolution of ~10 μm, enabling visualization of blood vessel wall microstructure in vivo at an unprecedented level of detail. IVOCT devices are now commercially available worldwide, there is an active user base, and the interest in using this technology is growing. Incorporation of IVOCT in research and daily clinical practice can be facilitated by the development of uniform terminology and consensus-based standards on use of the technology, interpretation of the images, and reporting of IVOCT results. **METHODS:** The IWG-IVOCT, comprising more than 260 academic and industry members from Asia, Europe, and the United States, formed in 2008 and convened on the topic of IVOCT standardization through a series of 9 national and international meetings. **RESULTS:** Knowledge and recommendations from this group on key areas within the IVOCT field were assembled to generate this consensus document, authored by the Writing Committee, composed of academicians who have participated in meetings and/or writing of the text. **CONCLUSIONS:** This document may be broadly used as a standard reference regarding the current state of the IVOCT imaging modality, intended for researchers and clinicians who use IVOCT and analyze IVOCT data.

Free full text article available from *Journal of the American College of Cardiology*
PMID: 22421299
NOTE: Also cited in Section 3.1


**Summary:** Optical coherence tomography (OCT) is a novel intravascular imaging modality, based on infrared light emission, that enables a high resolution arterial wall imaging, in the range of 10-20 microns. This feature of OCT allows the visualization of specific components of the atherosclerotic plaques. The aim of the present Expert Review Document is to address the methodology, terminology and clinical applications of OCT for qualitative and quantitative assessment of coronary arteries and atherosclerosis.


PMID: 19892716


**Summary:** This document is complementary to an Expert Review Document on Optical Coherence Tomography (OCT) for the study of coronary arteries and atherosclerosis. The goal of this companion manuscript is to provide a practical guide framework for the appropriate use and reporting of the novel frequency domain (FD) OCT imaging to guide interventional procedures, with a particular interest on the comparison with intravascular ultrasound (IVUS).

Free full text article available from [PubMed](https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3453088/)

PMID: 22653335

NOTE: Also cited in Section 5.4

E) The American Society for Gastrointestinal Endoscopy PIVI (Preservation and Incorporation of Valuable Endoscopic Innovations) on Real-time Endoscopic Assessment of the Histology of Diminutive Colorectal Polyps


**Summary:** The PIVI (Preservation and Incorporation of Valuable endoscopic Innovations) initiative is an ASGE program whose objectives are to identify important clinical questions related to endoscopy and to establish a priori diagnostic and/or therapeutic thresholds for endoscopic technologies designed to resolve these clinical questions. Additionally, PIVIs may also outline the data and or the research study design required for proving an established threshold is met. Once endoscopic technologies meet an established PIVI threshold, those technologies are appropriate to incorporate into clinical practice preserving the appropriate training in that endoscopic technology has been achieved.
The ASGE encourages and supports the appropriate use of technologies that meet its established PIVI thresholds. The PIVI initiative was developed primarily to direct endoscopic technology development toward resolving important clinical issues in endoscopy. The PIVI initiative is also designed to minimize the possibility that potentially valuable innovations are prematurely abandoned due to lack of utilization and to avoid widespread use of an endoscopic technology before clinical studies documenting their effectiveness have been performed. The following document, or PIVI, is one of a series of statements defining the diagnostic or therapeutic threshold that must be met for a technique or device to become considered appropriate for incorporation into clinical practice. It is also meant to serve as a guide for researchers or those seeking to develop technologies that are designed to improve digestive health outcomes. An ad hoc committee under the auspices of the existing ASGE Technology and Standards of Practice Committees Chairs develops PIVIs. An expert in the subject area chairs the PIVI, with additional committee members chosen for their individual expertise. In preparing this document, evidence-based methodology was employed, using a MEDLINE and PubMed literature search to identify pertinent clinical studies on the topic. PIVIs are ultimately submitted to the ASGE Governing Board for approval, as is done for all Technology and Standards of Practice documents. This document is provided solely for educational and informational purposes and to support incorporating these endoscopic technologies into clinical practice. It should not be construed as establishing a legal standard of care.

Full text article available from Gastrointestinal Endoscopy (USD 31.50)
PMID: 21353837

F) Principles and Pitfalls of Diagnostic Test Development: Implications for Spectroscopic Tissue Diagnosis

Summary: Diagnostic spectroscopy has the potential to supplant the time-honored “gold standard” of light microscopy and herald an era of in vivo tissue diagnosis. However, the lessons in disease diagnosis learned by pathologists over the years should not be forgotten. This discussion will focus on the basis principles and pitfalls of diagnostic test development, and how they apply to optical spectroscopy tissue diagnosis.

Full text article available from Journal of Biomedical Optics (USD 25.00)
PMID: 10938775
Note: Also cited in Section 3.2

6.2 IVM Image Classification Schemes/Criteria

A) Miami Classification for Probe-Based Confocal Laser Endomicroscopy

Summary: An essential element for any new advanced imaging technology is standardization of indications, terminology, categorization of images, and research priorities. In this review, we propose a state-of-the-art classification system for normal and pathological states in gastrointestinal disease using probe-based confocal laser
endomicroscopy (pCLE). The Miami classification system is based on a consensus of pCLE users reached during a meeting held in Miami, Florida, in February 2009.

Full text article available from *Endoscopy* (USD 33.00)
PMID: 21818734
NOTE: Also cited in Sections 2.2, 3.1, 3.3, 5.1.1

B) **New Classification for Probe-Based Confocal Laser Endomicroscopy in the Colon**


**Summary:** Probe-based confocal laser endomicroscopy (pCLE; Cellvizio, Mauna Kea Technologies, Paris, France) enables in vivo histology during colonoscopy and may allow endoscopists to make real-time diagnoses. A collaboration of five experts proposed a new pCLE classification for colonic use. The aim of this study was to assess interobserver agreement and accuracy of the new pCLE classification in the colon. **PATIENTS AND METHODS:** Eligible patients were prospectively investigated by pCLE. A subset of 13 pCLE video sequences was reviewed post hoc for the establishment of a new classification, which comprised three vessel categories and seven crypt categories. All five blinded observers then scored another set of 102 video sequences, using the new classification. Histopathology was used as a reference standard. **RESULTS:** The interobserver agreements on vessel and crypt architecture were ‘fair’ with kappa values of 0.29 and 0.27, respectively. When the classification was reduced to neoplasia vs. non-neoplasia (i.e. vessel or crypt type 3), overall agreement became ‘moderate’ (kappa = 0.56). Overall sensitivity and specificity for predicting neoplasia was 66 % and 83 %, respectively. When all observers agreed (69 % of videos), the corresponding figures became 80 % and 95 %. **CONCLUSION:** A new classification for pCLE in the colon had a ‘moderate’ interobserver agreement for differentiating neoplasia from non-neoplastic tissue in the colon. The overall accuracy (81 %) for predicting neoplasia was acceptable and became excellent (94 %) when all five observers agreed. Future research should focus on refinement and validation of the classification.

Full text article available from *Endoscopy* (USD 26.00)
PMID: 21971922

### 6.3 Interobserver Agreement in IVM Image Interpretation

**A) Interobserver Agreement and Accuracy Among International Experts with Probe-Based Confocal Laser Endomicroscopy in Predicting Colorectal Neoplasia**


**Summary:** A recently developed probe-based, confocal laser endomicroscopy (pCLE) system provides images of surface colonic epithelium in vivo during any endoscopy. Our objective was to assess interobserver agreement, sensitivity, specificity, and overall accuracy in the diagnosis of neoplasia using pCLE. **PATIENTS AND METHODS:** 53 patients undergoing surveillance and screening colonoscopies were enrolled. A total of 75 lesions, were detected and all were inspected by pCLE prior to sampling or polypectomy. Intravenous fluorescein was used to optimize tissue contrast. Three pCLE users, blinded to histopathologic and endoscopic findings, reviewed the set of video sequences for crypt
architecture, vessel architecture, and colorectal neoplasia diagnosis. Histopathologic diagnosis from the corresponding biopsies was the reference gold standard. RESULTS: Of the 75 colorectal lesions, 50 were neoplastic and 25 non-neoplastic. Interobserver agreement was moderate to good for the classification of neoplasia (kappa 0.55, 78% pairwise agreement), and moderate for vessel architecture (kappa 0.41, 67% pairwise agreement) and crypt architecture (kappa 0.49, 69% pairwise agreement). In distinguishing between neoplastic and non-neoplastic lesions, sensitivity, specificity, and accuracy were 76%, 72% and 75%, respectively. When videos of good or excellent quality only were considered, interobserver agreement for classification of neoplasia was higher (kappa 0.83, 92% pairwise agreement), as were sensitivity (88%), specificity (89%), and accuracy (88%). CONCLUSION: An international collaboration group had moderate to good interobserver agreement using a pCLE system to predict neoplasia, which is acceptable for this study.

Full text article available from Endoscopy (USD 33.00)
PMID: 20354938
Note: Also cited in Section 5.1.1.2

B) The Learning Curve, Accuracy, and Interobserver Agreement of Endoscope-Based Confocal Laser Endomicroscopy for the Differentiation of Colorectal Lesions


Summary: The endoscope-based confocal laser endomicroscopy (eCLE) system allows in vivo imaging of colorectal epithelium. Little is known about the learning curve for accurate interpretation of confocal images acquired with eCLE. OBJECTIVE: To determine the learning curve of eCLE, its diagnostic accuracy, and the intra- and interobserver agreement for the differentiation of colorectal lesions. DESIGN: Post hoc assessment of selected eCLE images. SETTING: Academic centers. PATIENTS: This study involved colonoscopic images from 47 patients. MAIN OUTCOME MEASUREMENTS: Learning curve of eCLE, accuracy, and intraobserver and interobserver agreement. METHODS: Three endoscopists received a short introduction to eCLE before evaluating 90 images. Observers assessed all eCLE images by using the Mainz classification. After each set of 30 images, the accuracy of each observer was assessed. The same procedure was repeated 6 months later by using the same set of images. LIMITATIONS: Post hoc assessment. RESULTS: There were no significant changes between the first set of 30 images and the 2 consecutive sets (P = .08 and P = .180, respectively). The overall accuracy was 85.6%, 95.6%, and 92.2% for each observer. The kappa values of the intraobserver agreement were 0.68, 0.84, and 0.77 for each observer. The kappa value for interobserver agreement was 0.73 during the first and 0.72 during the second assessment. CONCLUSIONS: Accurate post hoc interpretation of eCLE confocal images can be learned quickly. High diagnostic accuracy was achieved by all 3 observers during the initial stage of the assessment, which remained high thereafter. Intra- and interobserver agreement was substantial for all 3 observers. Future studies should focus on the real-time assessment of eCLE images.

Full text article available from Gastrointestinal Endoscopy (USD 31.50)
PMID: 22459661
Note: Also cited in Section 5.1.1.2
Section 7 IVM Billing/Reimbursement

The CAP has been closely involved in ensuring that pathologists are compensated fairly for pathologists’ work in the new area of IVM. For CPT® 2013, AMA established one new pathology CPT code to capture the work of optical endomicroscopic images interpretation and report. The new code is 88375 - Optical endomicroscopic image(s), interpretation and report, real-time or referred, each endoscopic session.

Another part of its continuing leadership role in the CPT to RUC (AMA/ Specialty Society Relative Value Scale Update Committee) process, CAP submits pathology code recommendations to the RUC for physician work, practice expense inputs and professional liability insurance crosswalks. The RUC sends its recommendations to CMS (Centers for Medicare and Medicaid Services) which are confidential until CMS publication.

For Medicare Physician Fee Schedule 2013, CMS listed new CPT code 88375 as carrier-priced; therefore, each Medicare contractor will provide a reimbursement amount for the service for 2013. However, code 88375 is anticipated to be an active service (non-carrier priced) for 2014. CMS will comment and/or make a decision on national pricing for new code 88375 by November 2013.

(CPT® is registered trademark of the American Medical Association)

Listen to the archive June 2013 webinar on the CAP’s ebook chapter, “In Vivo Microscopy: The Illuminating Future of Imaging in Pathology.”

Listeners will have the opportunity to hear expert advice and real examples, as well as learn the latest on how pathologists can take a leadership role in the field of advanced imaging, specifically In Vivo Microscopy (IVM).

Maria M. Shevchuk, MD, FCAP, chair of the IVM Workgroup, hosted the webinar and facilitated a discussion with Gary J. Tearney, MD, PhD, FCAP, vice chair of the CAP IVM Workgroup, and Jonathan L. Myles, MD, FCAP, chair of the CAP Economic Affairs Committee

The webinar is among a series of discussions to promote the ebook, New Paths...New Choices: Pathology in an Era of Advancing Science and Disruptive Health Economic, as part of the “Your Path. Your Choice” Promising Practice Pathways promotions plan. The webinars are designed to leverage specific chapters of the ebook to stimulate dialogue on topics designed to advance the specialty, incorporating and aggregating current CAP resources related to the webinar topic.

Archived webinar recording
Note: Webinar listed under Previous Sessions – New Roles of Pathologists
Section 8 Industry Education Resources

These conferences have been suggested by pathologists who are IVM early adopters.

8.1 Industry Conferences with IVM Topics

A) CAP13: Tuesday 6:30am Breakfast Workshop on “Advanced Imaging in Pathology: In Vivo Microscopy and Beyond”

Summary: In 2013, the Archive set up a special section called Advanced Imaging in Pathology. “Optical biopsies” represent new roles and opportunities in this rapidly evolving field for pathologists. Endoscopic microscopy is a new field where microscopic images are obtained from living patients. This capability opens up possibilities for obtaining histopathologic diagnoses from tissues that are difficult or unsafe to sample, screening entire organs for occult microscopic disease, and understanding disease mechanisms in vivo.

In this talk, we will describe some endoscopic microscopy techniques that are clinically available, including optical coherence tomography (OCT), optical coherence microscopy (OCM), and confocal microscopy (CM) and will discuss how these methods can potentially impact the practice of pathology and patient care. The focus will be on using these advanced imaging pathology tools in clinical cases for GI diseases.

Speakers: Gregory Y Lauwers MD, FCAP and Gary Tearney MD, PhD, FCAP

Visit the conference page

B) IVM Pathologist Speakers Presenting a Workshop at the Molecular Medicine Tri-Con Conference in February 10-12, 2014 in San Francisco

Molecular Medicine Tri-Con 2014 conference on February 10, 2014, in San Francisco IVM Workshop presented by CAP IVM Work Group pathologists includes:

• IVM diagnosis in surgical pathology, current and future
• IVM technologies, application, roles for pathologists
• In vivo imaging of cell and tissue dynamics

Speakers: Kamran Badizadegan, MD, FCAP
Gary Tearney, MD, PhD, FCAP
Maria Shevchuk, MD, FCAP
Richard Levenson MD, FCAP
Eric F. Glassy, MD, FCAP

Visit the conference page

C) CLEO 2014
San Jose, California
San Jose Convention Center
June 8-13, 2014

The biomedical track of CLEO 2012 has several presentations which relate to In Vivo Microscopy. Track session descriptions and presenter details are available here.
From early stage research to leading-edge applications, CLEO: 2014 builds on the long-established CLEO/QELS conference and its world-renowned peer-reviewed program. CLEO: 2014 featuring breakthrough research and applied innovations, has been redesigned to include the same high quality content under five core event elements: CLEO:QELS: Fundamental Science CLEO: Science & Innovation CLEO: Applications & Technology CLEO: Market Focus CLEO: Expo

Visit the conference page

D) SPIE Photonics West Conference, San Francisco
February 1-6, 2014
The Moscone Center
San Francisco, CA

Biomedical topics at the 2012 conference include:
- Photonic Therapeutics and Diagnostics
- Clinical Technologies and Systems
- Tissue Optics, Laser-Tissue Interaction and Tissue Engineering
- Biomedical Spectroscopy, Microscopy and Imaging
- Nano/Biophotonics

Visit the conference page

8.2 Biomedical Optics Education Resources

- **Beckman Laser Institute** at UC Irvine [http://www.bli.uci.edu/](http://www.bli.uci.edu/)
- **Laser Biomedical Research Center** at MIT [http://web.mit.edu/spectroscopy/lbrc.html](http://web.mit.edu/spectroscopy/lbrc.html)
- **Oregon Medical Laser Center** at the Oregon Health and Sciences University [http://omlc.ogi.edu/](http://omlc.ogi.edu/)
- **Wellman Center for Photomedicine** at MGH [http://www2.massgeneral.org/wellman/about.htm](http://www2.massgeneral.org/wellman/about.htm)
- **Cambridge Healthtech Institute** [http://www.healthtech.com/](http://www.healthtech.com/)
- **International Society for Optics and Photonics** [http://spie.org/](http://spie.org/)
- **Network for Translational Research in Optical Imaging (NTROI)**
The National Cancer Institute has established a Network for Translational Research in Optical Imaging (NTROI), the purpose of which is to develop, optimize and validate early stage optical imaging technologies through consensus, to enable rapid translation to clinical environments. [http://imaging.cancer.gov/programsandresources/specializedinitiatives/ntroi](http://imaging.cancer.gov/programsandresources/specializedinitiatives/ntroi)

A number of imaging centers of excellence are part of the NTROI:
- University of Michigan: [http://sitemaker.umich.edu/ntr/home](http://sitemaker.umich.edu/ntr/home)
- University of Washington St Louis: [http://ntroi.wustl.edu/](http://ntroi.wustl.edu/)
• University of Texas Houston: http://www.uthouston.edu/imm/centers/molecular-imaging.htm
• University of California Irvine (Beckman Laser Institute): http://www.bli.uci.edu/ntroi/
Section 9 CAP’s In Vivo Microscopy Education Resources

9.1 CAP Webinars

9.1.1 Upcoming Webinars

A) Ex Vivo Pathology Applications of IVM: Cooler than Frozen - Richard Levenson MD, FCAP
What is ex vivo and its clinical applications?

Tuesday, November 19 at 2 pm Central

Visit the registration page

9.1.2 Archived IVM Webinars

A) In Vivo Microscopy: The Illuminating Future of Imaging in Pathology

Summary:
• Benefits and the challenges of using IVM
• Current and future applications of IVM
• Pathologists taking on a new role with IVM
• Opportunities for reimbursement

Archived webinar recording
Note: Webinar listed under Previous Sessions – New Roles of Pathologists

B) Pathologists Opportunity in In Vivo Microscopy - Gary Tearney MD, PhD, FCAP

In vivo microscopy (IVM) involves imaging tissue microstructure from living patients. Potential applications of IVM include obtaining microscopic information from areas that are hazardous to sample (eye, brain, coronary arteries) and guiding biopsy sites so that the sampled tissue is more representative of the patient’s true disease state. Currently IVM devices are available in the fields of dermatology, ophthalmology, cardiology, and gastroenterology, with reimbursement and payment codes in place. Since pathologists are expert at interpreting microscopic images, it is important that pathology as a field play a role in how these technologies will be used in medicine in the future. In this talk, I will review the key, commercially available IVM technologies, will discuss up and coming techniques for imaging entire organs, and, looking towards the future, will describe new capsule approaches for microscopic screening and in vivo molecular imaging. I will furthermore speak about different opportunities for pathologists in the IVM and ways in which pathologists can get involved now in defining the future of this field.

Archived webinar recording
Note: Webinar listed under Previous Sessions – New Roles of Pathologists
9.2 CAP Annual Conferences

**CAP 13** – THE Pathologists’ Meeting™
October 13-16, 2013
Gaylord Palms Orlando
Orlando, Florida

**CWTB500  Advanced Imaging in Pathology: In Vivo Microscopy and Beyond**
Tuesday, October 15, 2013
6:30–7:45 AM
CME/CE NOT APPLICABLE

In 2013, the *Archives of Pathology & Laboratory Medicine* set up a special section called Advanced Imaging in Pathology. “Optical biopsies” represent new roles and opportunities in this rapidly evolving field for pathologists. Endoscopic microscopy is a new field where microscopic images are obtained from living patients. This capability opens up possibilities for obtaining histopathologic diagnoses from tissues that are difficult or unsafe to sample, screening entire organs for occult microscopic disease and understanding disease mechanisms in vivo. This session will describe some of endoscopic microscopy techniques that are clinically available, including optical coherence tomography (OCT), optical coherence microscopy (OCM), and confocal microscopy (CM); and it will discuss how these methods can potentially impact the practice of pathology and patient care. The focus will be on using these advanced imaging pathology tools in clinical cases for GI diseases.

**Presenters**
Gregory Y. Lauwers MD, FCAP
Gary Tearney MD, PhD, FCAP

9.3 CAP eBook and IVM

In February 2013, the CAP published an ebook with a chapter on IVM. This chapter highlights the New Roles of Pathologists in In Vivo Microscopy.

Pathologists will have many practice models to choose among in changing economic times. *New Paths... New Choices: Pathology in an Era of Advancing Science and Disruptive Health Economics* is an ebook created by the College of American Pathologists (CAP). The ebook is filled with the voices of pathologists who are taking control of their professional and economic destinies.

[Access the entire ebook](#)
[Access the chapter of the ebook on IVM](#)
9.4 CAP’s Archives and IVM

In January 2013, Archives set up four new Editorial Board Sections to focus on new and emerging fields. One of these is “Advanced Imaging in Pathology.” IVM related topics belong to the “Advanced Imaging in Pathology” section. The focus of this new area is to encourage authors for new and topical submissions.

Access the CAP’s Archives

9.5 CAPConnect and IVM

CAPConnect is your source for learning and sharing knowledge with peers. This community offers unique opportunities for: discussion and collaboration diagnostics, practice, management, emerging technologies, health care trends, and professional development.

In 2013, a discussion group on IVM was set up on CAPConnect. Post your questions to the IVM experts and join in the discussion.

Access CAPConnect

9.6 CAP IVM Information Flyers

These informational flyers provide “10 minute education” on aspects of IVM. Print them out and share them with others.
9.6.1 Why IVM?

The main technologies currently in clinical use and FDA-approved are confocal microscopy and optical coherence tomography (OCT). Many other technologies are in active development and in clinical trials, including spectroscopy-based techniques and multiphoton microscopy.

One common clinical application and the prototype for endoscopic use is in vivo imaging of the gastrointestinal tract. The architectural and cellular patterns generated by in vivo microscopy are interpretable by pathologists to make differential diagnoses and to identify areas for biopsy, improving diagnostic yield. Such directed biopsies decrease sampling errors resulting in fewer, less frequent biopsies, significantly decreased morbidity and significant cost-savings.

In vivo technology can also be used to screen whole organs (e.g., the entire esophagus or Barrett’s), to follow up on areas previously treated endoscopically, and to monitor pharmacologic, ablative, or surgical therapies. Other current applications of in vivo microscopy are the imaging of coronary vessels during interventional cardiology procedures, and in ophthalmology. In the future, in vivo microscopy may become widely used in certain applications, such as making some diagnoses in the brain or in other critical organs that cannot be safely biopsied, and in fragile patients such as those with coagulopathies.

Since in vivo microscopy is another method used for evaluating histopathology, pathologists, be they academic or community pathologists, should be integral members or organizers of the clinical teams, present in the endoscopy/treatment rooms, or diagnosing images remotely either in real time or off-line.

Another application of this technology, immediately accessible to pathologists, is its use ex vivo. Pathologists could evaluate needle biopsies intra-procedurally to determine if the required elements for diagnosis are present.

Ex vivo use of these technologies could potentially in some cases be used in a similar fashion to frozen sections, saving tissue, and potentially saving the patient another procedure. Also genomics medicine may require more tissue sampling from primary tumors and metastases and the sample size is likely to decrease.

Ex vivo imaging can help select tissues for genetic studies, without damaging or destroying minute samples. Additionally there would be a permanent digital record of the tissue submitted for analysis. Other ex vivo applications are margin determinations and sentinel lymph node evaluation/sampling.

Pathologists are the human tissue specialists and the experts in histopathologic diagnoses. Therefore pathologists’ involvement in ex vivo/in vivo microscopy is not only necessary for maintaining and enhancing our position in the medical care system, but it is also in the patients’ best interest.

To ensure that pathologists are compensated fairly for their work in the new era of IVM, the CAP worked with the AMA CPT and RUC to establish one new pathology CPT code 88375: Optical endomicroscopic image(s), interpretation and report, real-time or referred, each endoscopic session. For Medicare Physician Fee Schedule 2013, CMS listed the new CPT code 88375 as carrier-priced; but for 2014, national pricing is anticipated. Ex vivo and other applications do not yet have a CPT code, but the CAP is also very active in the CPT and RUC processes, and the CAP advocates for new codes as appropriate.

To promote in vivo microscopy awareness and education and to facilitate adoption, the CAP has formed an in Vivo Microscopy Work Group in January 2013. To date, the group has contributed to a chapter on IVM in the CAP ebook and IVM CAP webinars (6/13 and 8/13). Dr. Melissa Upton conducted a short course on optical biopsy at the ASCP meeting (9/13). At CAP ’13, an IVM Breakfast Workshop will be held on Oct. 15, featuring Drs. Gary Tearney and Gregory Lauwers, with IVM material at the CAP Booth. In 2014, pathologists will be presenting IVM workshops. In addition the CAP Pathology Resource Guide: In Vivo Microscopy, available for CAP members, will have an updated edition at CAP ’13. Color atlases of IVM images from various organs are planned.

In the near future, IVM and ex vivo applications of IVM will become an integral part of the practice of pathology, the CAP, along with the IVM Work Group, is actively promoting awareness and better understanding of IVM opportunities for pathologists.

What is an Optical Biopsy?

An optical biopsy is an in vivo microscopic assessment of tissue. Confocal laser Endomicroscopy, for example, enables visualization of microscopic tissue architectural and cellular morphology. It provides 2D images in a parallel tissue plane (en face) with 1-2 μm resolution at a depth of 10 μm. Traditional surgical biopsies are invasive and provide a transverse view of the mucosa. Optical biopsies impart endoscopic treatment and clinical-pathologic correlation.

9.7 IVM POET Report

A) In Vivo Microscopy


Summary: Pathologists will soon share examination of tissue morphology at the architectural, cellular and molecular level with other physicians. In vivo microscopy technologies enable physicians to detect pathology by visualizing tissue through innovative tomographic methods. In vivo microscopy techniques provide additional data beyond that obtainable by histological methods, including volumetric data and time/flow data.

Developed by the Technology Assessment Committee (TAC), Perspectives on Emerging Technology (POET) reports and white papers are designed to provide pathologists with a high-level summary of a particular emerging technology that is likely to impact their practice in the reasonable future. POET reports help pathologists respond to clinician or patient inquiries about a technology. Its format includes a one-page summary plus select references (e.g., peer-reviewed articles, for further information and research.) Although POETs deliver a short overview of a specific innovative technology, they are not a definitive technology assessment of the techniques used or a “how to” cookbook on implementing a test in a practice. Rather, they are intended to be used as an educational tool leading to a more detailed investigation by the Center, Council on Scientific Affairs, TAC or individual pathologists.

In Vivo Microscopy POET Report; POET Reports homepage
Section 10 Feedback

Feedback on this content is welcome, including suggestions for articles, webinars, or other resources. Please send comments, suggestions, and questions to capguides@cap.org.
The In Vivo Microscopy Resource Guide was updated by the CAP’s In Vivo Microscopy Work Group.
Bibliography


72. Prati F, Jenkins MW, Di Giorgio A, Rollins AM. Intracoronary optical coherence tomography, basic theory and image acquisition techniques. The international journal of cardiovascular imaging. Feb 2011;27(2):251-258. (5.4)


89. Testoni PA, Mangiavillano B. Optical coherence tomography in detection of dysplasia and cancer of the gastrointestinal tract and bilio-pancreatic ductal system. World journal of gastroenterology : WJG. Nov 14 2008;14(42):6444-6452. (5.1.2)


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