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In vivo pathology: microendoscopy as a new endoscopic imaging modality

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Basis for confocal microendoscopy

Earlier detection

Almost all cancers are easier to successfully treat if caught early. This principle is particularly true for the approximately 85% of cancers that are epithelial in origin. Most of these cancers do not originate fully malignant from normal tissue but progress through one or more pre-invasive pathologically recognizable steps. Currently for the majority of internal organs topologically connected to the outside environment, endoscopy is the primary method to screen for and diagnose pre-invasive and early (encapsulated or only locally invasive) cancers. The resolving powers of endoscopes used in current practice are limited to objects millimeters in size or in some cases submillimeter sizes (200 to 1000 microns). The visible contrast of early neoplastic lesions resolvable by current endoscopes is such that many to most of these lesions are not readily discernable to the human observer (eg, flat lesion in the colon) or are surrounded by a transformed field that makes their ready identification problematic (eg, Barrett's esophagus).

Improved sensitivity/specificity

To address these issues and in hopes of enhancing the role of endoscopy in detecting cancer and improving staging, treatment, and treatment follow-up, several research groups [1–8] and commercial enterprises (Optiscan, Victoria, Australia; Pentax, Japan; Olympus, Japan; Mauna Kea Technologies, Paris, France) [9–11] are developing instruments that significantly improve the resolv-

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ing power possible in endoscopic applications and improve the observable contrast between normal, transformed, and malignant tissue. These research and development efforts leverage novel in vivo confocal imaging techniques to achieve the improved gains in resolution and contrast. It is believed that these improvements in imaging will lead to improved sensitivity to disease detection and reduced false positives (improved specificity).

Point of care

In the developed world this could translate into reduced cancer care costs through the shifting of the stage at which cancer is detected. Examples include locally confined esophageal cancer, flat adenomas in the colon, as well as improved neoplastic margin delineation and the detection of multiple concurrent lesions in sites such as the esophagus and the colon.

In the developing world, much of the advanced medical infrastructure of the developed world is missing. Significantly improved sensitivity and, in particular, improved specificity could lead to see-and-treat strategies to extend the strategic use of scarce health care funds.

Relevance of pathologic analysis in vivo

Comparison to traditional endoscopy and other optical biopsy techniques

The targeted resolution of most of the existing in vivo confocal systems or those under development is approximately 1 to 3 microns in the lateral dimensions [3,12–14]. This resolution along with the optical sectioning capability of confocal imaging enables the acquisition of fields of histopathology like images of cells and nuclei in vivo without the need to excise the tissue. Confocal microendoscopy provides a means to visualize the size and arrangement of cells and nuclei and tissue structure (blood vessels, capillaries, crypts) in vivo with or without the use of complex contrast agents. Furthermore, using molecular-specific contrast agents, it is possible to expand the range of biomarkers that may be imaged. For many disease states observed in excised tissue collected during endoscopy, it is the observation of the size and spatial arrangement of nuclei and vascularization patterns within the tissue that enables the histopathologist to make a determination of the phenotype of the tissue (Fig. 1A, B).

This histopathological view is in contrast to traditional visual observation that infers the state of the tissue through changes in tissue color, intensity, saturation and hue, surface texture and topology, and vascular changes at the tissue surface. All of these observed changes are caused by: (1) changes in the arrangement and density of the cells in the tissue; (2) changes in the size and types of cells in the tissue; and (3) changes in the vascularization within the tissue. The degeneracy of the mapping of the underlying conditions to the observed characteristics (ie, multiple arrangements of cell structures, cell types, and vascular patterns can give rise to the same surface appearance) is what makes the visual assessment of

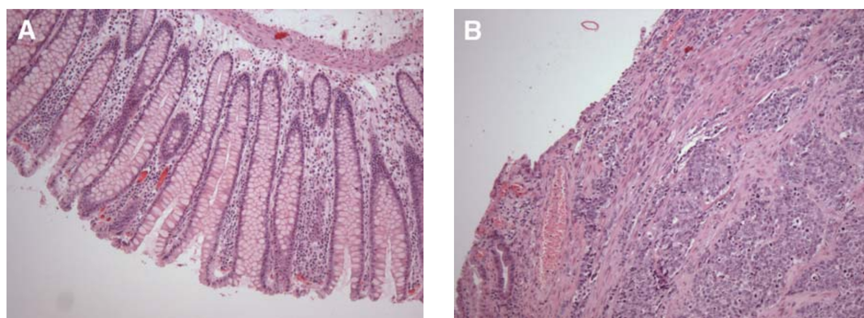

Fig. 1. Image of a hemotoxylin and eosin–stained 5-μm section of human colon. (*A*) On the left side is a normal area of colon in the section. (*B*) On the right side is an area of invasive cancer. Note the difference in the spatial arrangement of cells between the two images. (See also Color Plate 34).

endoscopic images so challenging for the skilled endoscopist. Similarly, most of the novel optical endoscopic tissue integration techniques described elsewhere in this edition to one degree or another infer histopathologic changes through their indirect effects on the bulk optical properties of collections of 10s (optical coherence tomography), 100s to 1000s (magnification endoscopy) [15], or millions of cells (fluorescence imaging, reflectance, or fluorescence spectroscopy). Some of these techniques also measure specific molecular changes in the tissue (fluorescence imaging or spectroscopy), which should also be measurable by spectral confocal microendoscopy [16].

Screening

The ability to visualize the spatial arrangement of cells in all three dimensions within tissue as well as the size of the nuclei and vascular structure will enable confocal microendoscopy to be useful for screening applications in the gastrointestinal tract, in particular screening the esophagus for Barrett's and its extent along the esophagus as well as for ulcerative colitis and adenomatous polyposis in the colon. One can envision confocal microendoscopy being used in two fashions in these instances: (1) for biopsy guidance for the selection and excision of the most relevant tissue for histopathologic interpretation and (2) once fully validated as a virtual biopsy device, reducing or removing the need to biopsy tissue. In addition to disease screening, localization of suspected dysplastic or cancerous tissue is an intended use of confocal microendoscopy devices in the gastrointestinal tract and other epithelial tissues. Another aspect of this is in assisting in the accurate staging of organs with cancer through the determination of the existence of squamous lesions or multiple primaries or localized metastasis.

Detection and monitoring

The ability to visualize changes in nuclear size and the density and organization of nuclei makes it possible to determine the margins of neoplastic lesions almost to the cell level. The ability to generate three-dimensional in vivo re-

presentations of tissue architecture makes it possible to monitor treatment in a minimally noninvasive fashion as well as monitoring for disease recurrence at the treatment site. Similarly, but applied to the noninvasive precancerous lesions, which commonly occur before the development of invasive cancer, confocal microendoscopy will likely find a home following the progression or regression of dysplastic tissue or atypical adenomous tissue in chemoprevention studies or cancer vaccine trials. Here the goal is to disrupt or halt the early carcinogeneous process through the use of agents (designed compounds, vaccines, natural products). Although this process can be studied through serial sample acquisition, the tissue excision process significantly disrupts this process. Thus, methods that can effectively monitor the success or failure of these tests noninvasively are particularly attractive to researchers and, eventually, clinicians.

The advantages of confocal microendoscopy are not limited only to the gastrointestinal tract but have significant advantages and possible uses for other epithelial tissues. A number of the groups developing these devices are currently primarily focused on some of these other epithelial tissue sites such as the cervix [17], lung [18], and oral cavity [19]. As these devices are developed, their applicability to the gastrointestinal tract will also develop further.

Confocal microendoscopy

Confocal principle

Enabled by advancing technology, confocal imaging [20] is an increasingly common form of microscopy that allows enhanced lateral resolution, improvements in axial resolution, and most importantly for in vivo confocal microendoscopy, improved rejection of out-of-focus information from material above and below the plane of focus (Fig. 2). For endoscopy, these benefits translate into cellular-level image resolution at depths of resolution up to hundreds of microns into epithelial tissue.

The key to a confocal microscope is the small aperture located at the conjugate image plane of the focal plane in both the illumination path and the light detection path. The effect of the illumination pinhole is to only illuminate effectively a single spot in the material being imaged. The effect of the detection pinhole is to block most of the returned light from out-of-focus objects above the focal plane (long dashed lines) and below the focal plane (short dashed lines). This narrows the depth of field considerably. These two pinholes are projected by the objective lens to the same point in the specimen: the two pinholes are confocal.

The disadvantage of confocal microscopy is the need to scan these pinholes across the sample to create a two-dimensional image from within the sample and the need to scan the pinhole across and up and down within the sample to create three-dimensional images from within the sample. How the various confocal microendoscopy systems scan these pinholes is one of the main differences between the types of confocal microendoscopy systems.

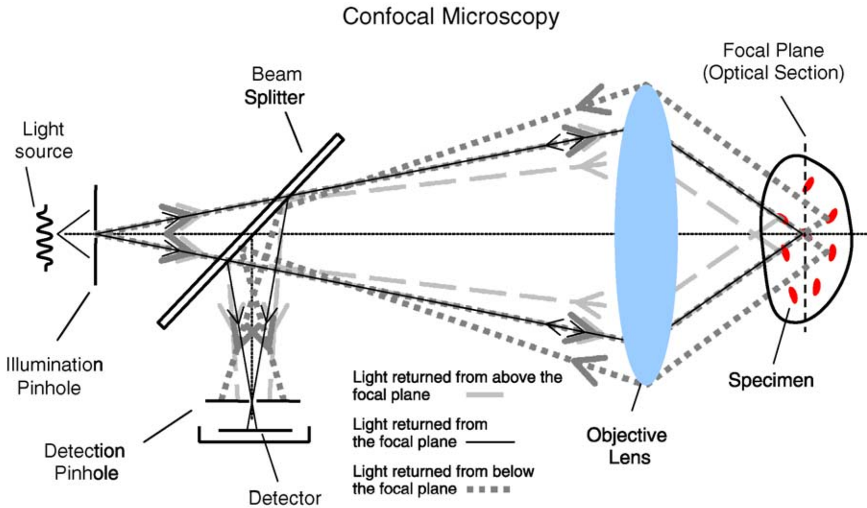

Fig. 2. Schematic diagram depicting a simplified epi-illumination (light source and detection on same side of objective lens) confocal microscope.

In addition to confocal imaging, confocal microendoscopy requires that the image acquisition system be coupled to the tissue for examination by a flexible light guide or light guides.

Basic methodologies

One can separate the major confocal microendoscopy efforts currently underway into two main categories based on their type of flexible light guide. Broadly the two basic methodologies are: (1) proximal scanning in which the scanning apparatus is coupled to a coherent fiber bundle that conducts the light back and forth from the imaged area at the distal end of the fiber bundle [2,3,21,22] and (2) distal scanning in which the light is conducted by a single fiber back and forth from the distal tip of the system, and the scanning of the illumination and detection pinhole is accomplished by miniaturized system at the distal end of the entire system [7,23–27].

Proximal scanning (coherent bundle)

For the proximal scanning systems, there are two main forms of scanning the pinholes across the coherent bundle. Similar to the majority of conventional confocal microscopes, these systems use one or more moving mirrors to scan the confocal pinholes in a raster fashion across the coherent fiber bundle [2,11] (Fig. 3). A modification of this method uses a single mirror to scan confocal slits (instead of pinholes) across the coherent fiber bundle (Fig. 4) [1]. This modification improves the speed of the imaging by making parallel the illumination and detection pathways (a slit versus a pinhole) at the expense of some loss of the ability to remove out-of-focus light, reducing the optical sectioning

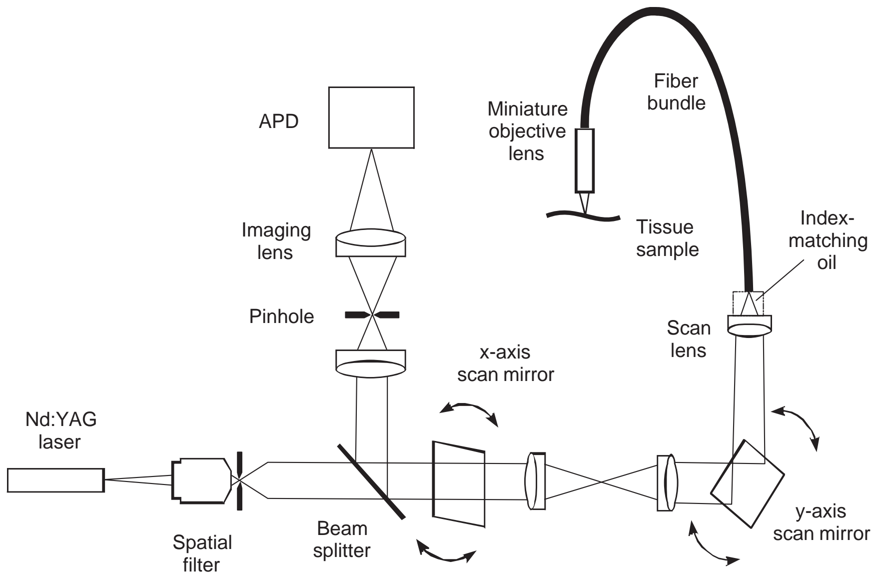

Fig. 3. Schematic diagram depicting a two-mirror galvanometer pinhole scanning coherent fiber bundle confocal microendoscope.

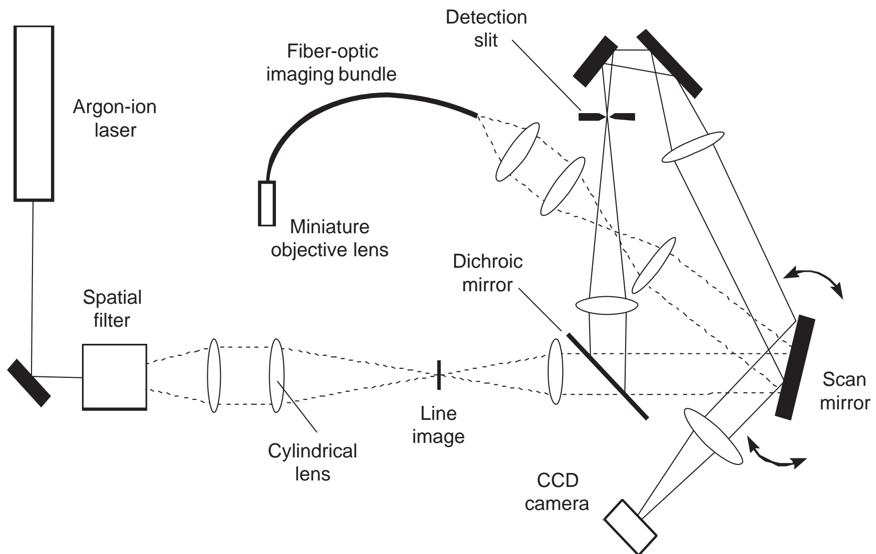

Fig. 4. Schematic diagram depicting a one-mirror galvanometer slit scanning coherent fiber bundle confocal microendoscope.

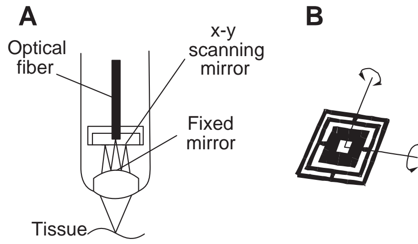

Fig. 5. (*A*) Schematic diagram depicting a one-micro mirror pinhole scanning single fiber confocal microscope. (*B*) An expanded view of the Z axis scanning envision to be used to scan the image plane.

ability of the system as a whole. An alternate method is to use a micro-electro mechanical system (MEMS) device to behave as an array of independently controllable illumination pinholes that can very rapidly sequentially illuminate one or more fibers of the coherent fiber bundle. The fiber bundle optically couples to a digital camera system in which the individual pixels of the camera behave as the detection pinholes [3].

Distal scanning (single fiber)

Three main forms of distal scanning are in use or under development. One uses a miniature mirror or mirrors to scan the pinholes (as represented by the single coupling fiber) across the tissue to be images (Fig. 5) [4,14]. Another method for distal scanning involves wiggling the coupling fiber within the optical system at the distal end of the system such that the fiber itself as the pinholes moves in a raster fashion and coupled by the optics at the distal end hence raster scans the tissue to be imaged (Fig. 6) [10]. A third method involves a combination of moving the coupling fiber only in one direction and using

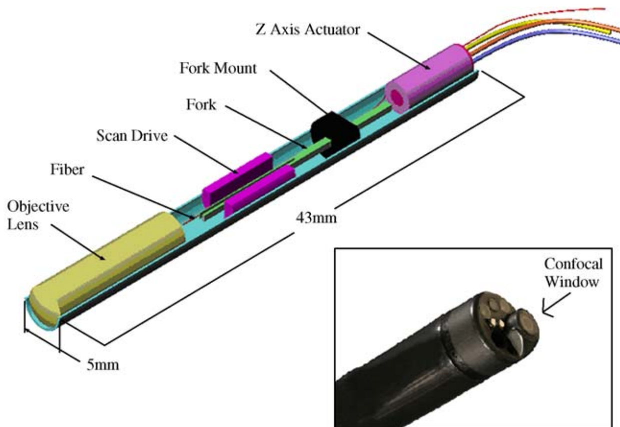

Fig. 6. Schematic diagram depicting a wiggling fiber pinhole scanning single fiber confocal microscope and image of system incorporated into a modified conventional endoscope. (*Illustration by* Peter Delaney. *Courtesy of* Optiscan Party, Ltd., Victoria, Australia.)

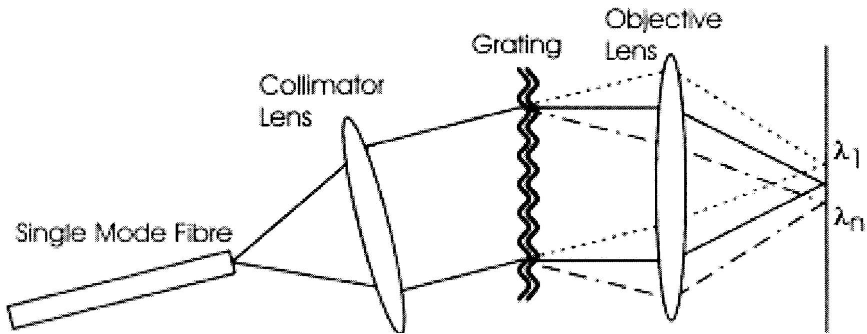

Fig. 7. SECM concept. Broad bandwidth light is delivered through a single-mode fiber and collimated onto a diffraction grating. The angularly dispersed light is then focused to different spectrally encoded locations in the focal plane of the objective lens. (*Courtesy of* G. Tearney, Boston, MA.)

wavelength-specific optical diffraction to create a line of pinholes, each of which conducts only a specific wavelength range of light to and from the targeted sample. The inventors of this technique at the Wellman Laboratories have called it spectrally encoded confocal microscopy (Fig. 7) [7].

Imaging modes

Confocal microendoscopy devices can confocally image in a variety of modes. The two most common are confocal reflectance imaging and confocal fluorescence imaging. In reflectance mode, imaging the same wavelength or color of light is used to illuminate the target as is detected from the target. This imaging mode makes use of differences in tissue component backscattering characteristics that either are native to the tissue (Fig. 8) or induced by a contrast agent (eg, acetic acid, Fig. 9) to modify the optical properties of the cellular components to be imaged. As with all versions of the confocal microendoscopy devices, the goal is to differentiate between the nuclear component of cells and the cytoplasmic component so as to be able to visualize the cell nuclei.

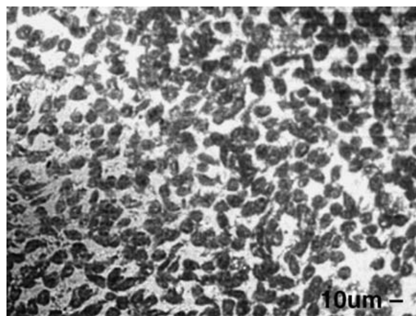

Fig. 8. Ex vivo bronchial tissue imaged confocally using tissue reflectance (450 nm light). No stains were used.

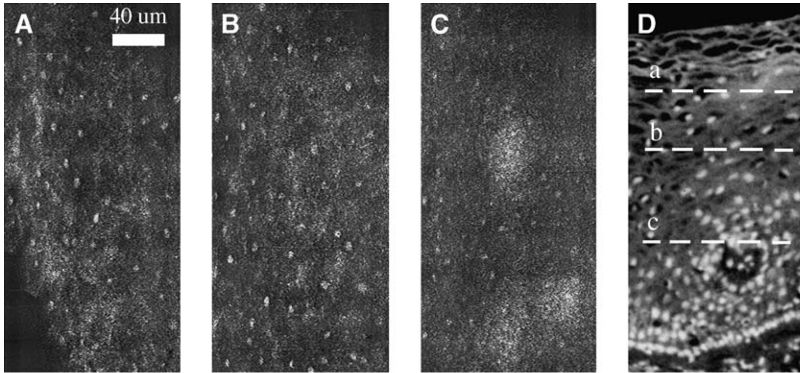

Fig. 9. En face images obtained ex vivo from cervical tissue after the application of acetic acid. (*A*) Image taken with confocal microscope with the image plane parallel to the epithelial surface and the focus below the surface. (*B*) Same as (*A*), but with the focus about twice as deep. (*C*) Same as (*B*), but with the focus about twice as deep again. (*D*) Image of hemotoxylin and eosin–stained transverse section using bright field microscopy. Contrast has been reversed in this black and white image to aid in comparing confocal and histologic images. Lines a, b, and c indicate the approximate depth at which the confocal images in (*A*), (*B*), and (*C*) were obtained.

For most fluorescence imaging of cell nuclei, an optically active fluorescence stain needs to be applied to the tissue to be imaged. What about autofluorescence? Most of the fluorescence staining methods currently in use label the nuclei of cells to one degree or another (Figs. 10–14). The unresolved issue with respect to applied fluorescence stains that allow cell nuclei to be distinguished is their possible carcinogenic effects and biologic activity, but this is an area of active research [29–31]. For the imaging of microvascularization and the local vascular system embedded within or under the epithelial structures, several fluorescence intervascular stains are currently approved for use in patients (Figs. 15 and 16). It is possible to confocally image only the endogenous (naturally occurring) fluorophore present in the tissue for light in the visible part of the spectrum.

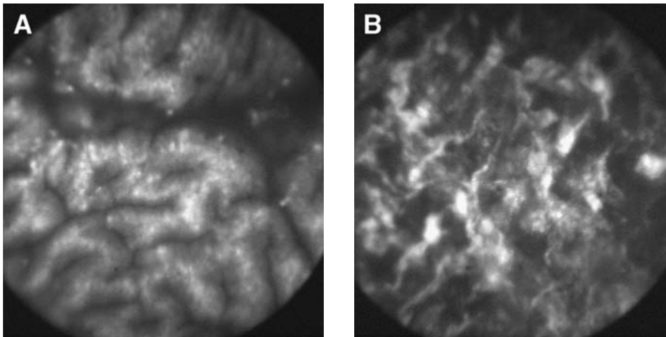

Fig. 10. Ex vivo rat tissue labeled with vital nucleic acid stain (acridine orange with 100:1 wash) obtained with slit scanning microendoscope catheter system. On the left (*A*) is an image of the colon and on the right (*B*) is an image of the esophagus. (*Courtesy of* A. Gmitro, Tuscon, AZ.)

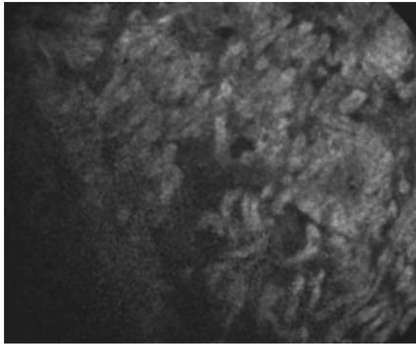

Fig. 11. Ex vivo mouse colon tissue with labeled with vital nucleic acid stain 30 μm (acridine orange) obtained with digital micromirror device-based microendoscopy system.

This restriction limits the systems to imaging mainly the connective tissues such as collagen and collagen crosslinks that make up the basement membrane and submucosa of most epithelial tissues (Fig. 17).

The minimum imaging requirements to be able to resolve individual cells in vivo are a lateral resolution approximately 3 microns or less (preferably approximately 1 micron) in the transverse directions and an optical sectioning ability on the order of 10 microns or better (Fig. 18). For resolving larger targets such as microvilli in the gastrointestinal tract or tissue microvascularization, substantially less resolving capability is required. Tightly coupled to this specification is the achievable field of view. Larger is better. However, resolution, field of view, and imaging time are all very tightly coupled, and it is difficult to improve one without having to sacrifice the others. For cellular level imaging, a field of view approximately 200 microns by 200 microns is likely sufficient to include enough structural and organization information for the visual assessment of the imaged tissue. The ability to perform optical sectioning in vivo (ie, resolve information from a specific level within the tissue) implies the need to be able to con-

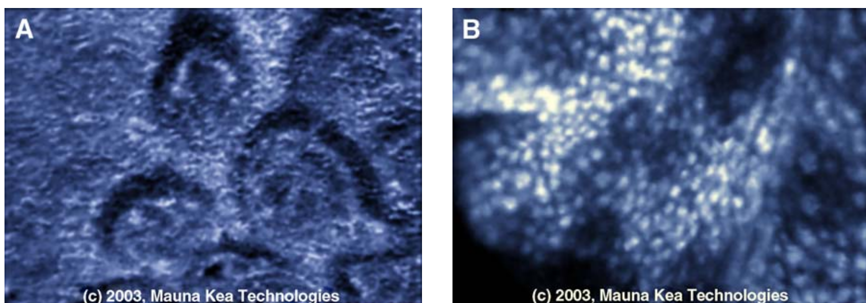

Fig. 12. Two in vivo mouse fluorescence images at a depth of 20 μm down from the surface. (*A*) On the left is an image of mouse intestine microvillosities with a field of view of 166 μm by 120 μm. (*B*) On the right is an image of the bladder via a catheter in the urethra. (*Courtesy of* Dr. Vicaut, Dr. Guillemin, and Mauna Kea Technologies, Paris, France). (See also Color Plate 35).

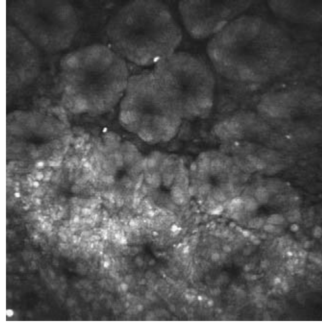

Fig. 13. In vivo fiber confocal image of rat colon. The single-channel images show the early stages of colitis following the oral consumption of 5% dextran sulfate sodium (Mr 40,000 Da; ICN Biochemicals, Ohio, USA) for 3 days (ie/ day 3 colitis). The mucosal surface of the colon was topically stained with acridine orange (0.01% w/v in saline; Sigma Chemical Company, St. Louis, MO, USA) for 2 minutes. Excess dye was removed with a 1-minute saline wash. (*Courtesy of* Wendy McLaren: Optiscan Imaging Limited and Monash University, Melbourne, Australia.)

trol from which specific level within the tissue to acquire the information. Among the systems currently under development, three primary methods have emerged:

1. Moving optical components within the distal lens system to change the optical path length to the focal plane in the tissue (Figs. 6 and 19).
2. Move the tissue relative to the distal optical system.
3. Spectrally select focal depth using a designed chromatically aberrated lens system (Fig. 20), such that the color of the imaging light determines the depth from which the image comes [18].

The confocal optical sectioning ability of all these systems depends on the numerical aperture (NA) (or the range of the angles of light used) of their distal

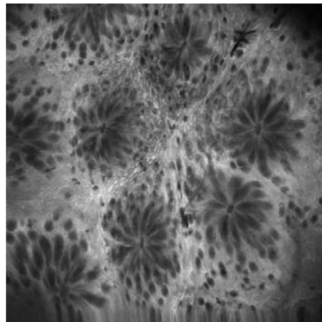

Fig. 14. Human mucosa in the sigmoid colon in vivo imaged using prototype confocal microscope equipped endoscope. Confocal image was obtained after administration of sodium fluorescein, 5 mL 10% w/v intravenously. (*Courtesy of* Ralf Kiesslich, University Hospital, Mainz, Germany and Optiscan Imaging Limited, Victoria, Australia.)

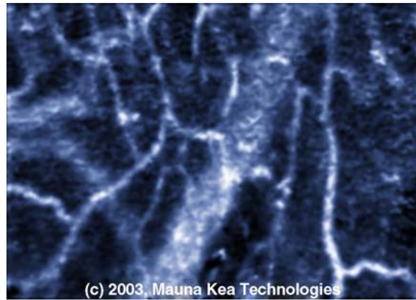

Fig. 15. Mouse intestine in vivo stained with fluorescein to visualize tissue microvascularization. (*Courtesy of* Dr. Vicaut and Mauna Kea Technologies, Paris, France). (See also Color Plate 36).

optics system and the size of the confocal pinhole. However, high NA optics tend to be larger than low NA optics and the size or diameter of the distal end of these confocal microendoscopes is critical to the organs and sites for which they may be used. For most of these systems, it is the size of the distal optics that limits how small the probes may be constructed. Current systems range in diameter from 0.4 microns up to 3 cm. Given the size of the objects being resolved relative to the size of the probe and organ in which they reside, it is surprising that, for most of these systems, motion artifacts are not an insurmountable problem. To work properly, these systems need to be locationally stable with respect to the tissue they are imaging to within a few microns over a substantial fraction of a second. Most of the systems in use or under development have targeted about four images per second or faster frame rates [1]. As a result, the probe tissue geometry needs to stay stable to within a few microns over a period of one-fourth of a second. Seemingly difficult, it turns out that the probes are flexible and, once in contact with the tissue, tend to move with the tissue as it moves and drag the

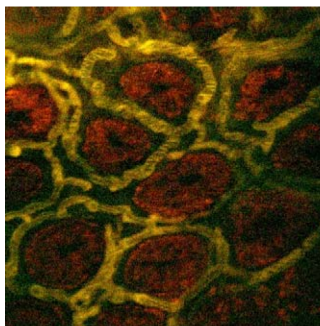

Fig. 16. The dual-channel images show the normal crypts and the vasculature in the distal region of the colon after the intravenous injection of 0.5 mL FITC-dextran (10 mg/mL in saline; average molecular weight dextran 167 kDa; Sigma, St Louis, Missouri, USA) and the topical application of tetracycline hydrochloride (20 mg/mL in saline; Achromycin; Lederle Laboratories, Baulkham Hills, NSW, Australia). (*Courtesy of* Wendy McLaren: Optiscan Imaging Limited, Victoria, Australia, and Monash University, Melbourne, Australia). (See also Color Plate 37).

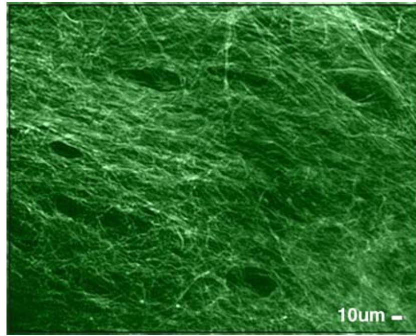

Fig. 17. Digital micromirror device confocal autofluorecsence submucosa images of ex vivo bronchial tissue from ~25–30 μm below the surface of the epithelium, 437 nm illumination and 525 nm emission. (See also Color Plate 38).

surface layer of the tissue in contact with the probe along with any motion of the probe. As most of these systems can only image from the surface to about 250 microns into the tissue, it is only the macroscopic surface layer that is being imaged (see Fig. 21 for an example of a prototype research system and Fig. 22 for an example of a prototype commercially available system).

Image analysis

Most confocal microendoscopy systems produce images of cell nuclei over hundreds of cells. The skilled observer with a familiarity of the histopathologic

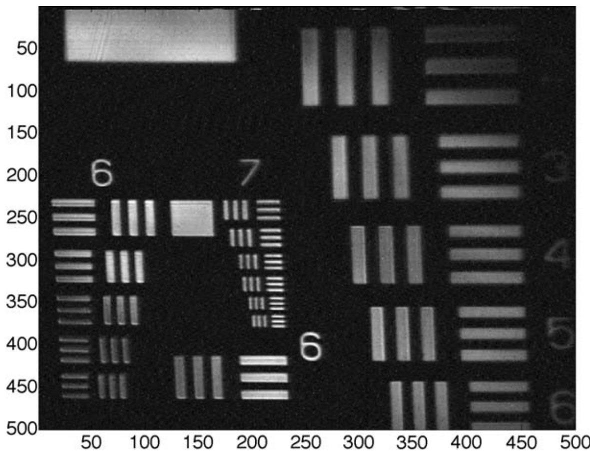

Fig. 18. A demonstration of the targeted resolution of confocal microendoscopy systems. This is a swept-source spectrally encoded confocal microscopy (SECM) image of a US Air Force resolution target. The bar spacing for the smallest element is 2.2 microns. The wavelength-encoded axis is along the x-axis. This image is 500 × 500 pixels and 400 (y-axis) × 440 microns (*Courtesy of* G. Tearney, Boston, MA).

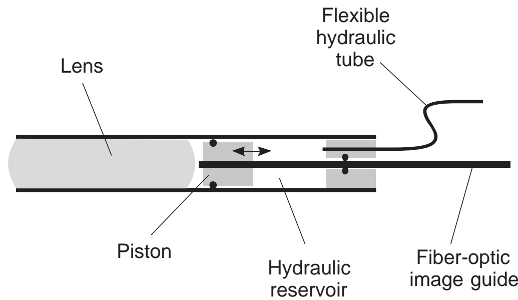

Fig. 19. Schematic example of distal lens and focus system in which the position of the fiber bundle is moved in a controlled fashion to affect focusing at different depths into the tissue being imaged as implemented by A. Gmitro et al [28].

changes associated with the disease states being examined can interpret this population of cells. Further, there are groups developing automated quantitative analysis tools to examine the spatial arrangement of cells in tissue to assist in the diagnostic evaluation of the images produced by confocal microendoscopy systems (Figs. 23 and 24). These tools are similar to those developed and used in automated image analysis of Pap smears.

Future developments

In the future, one can expect to see these systems demonstrate higher resolution, increased imaging speeds, and further reductions in the size of the

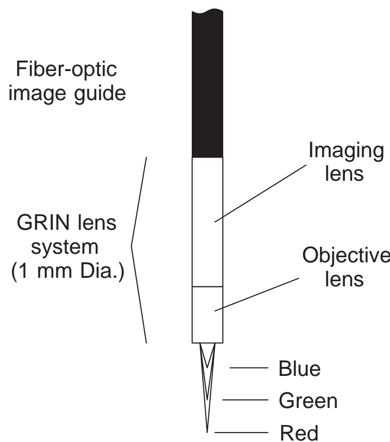

Fig. 20. Schematic depiction of GRIN (GRadient INdex) distal lens system in which the chromatic aberration of the system is such that different focal depths may be selected through the use of different illumination wavelengths (confocal reflectance mode imaging only).

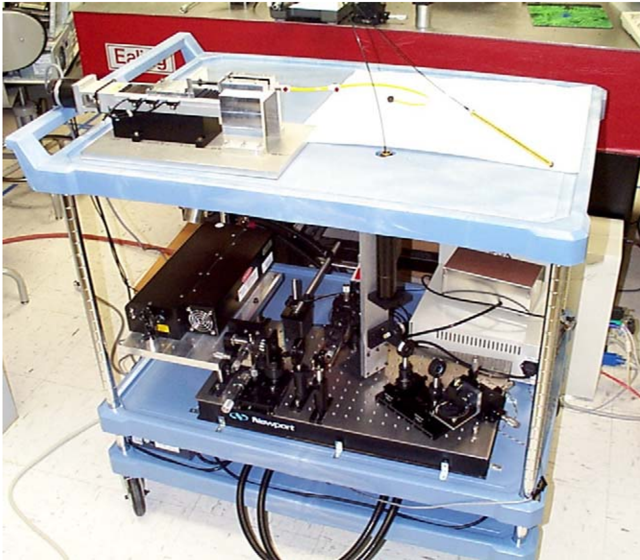

Fig. 21. Prototype in vivo confocal microendoscopy system for use in the cervix.

distal probes. In the near term as these systems are used in the clinical research setting, one can expect a number of correlative studies with conventional histopathologic assessment of excised tissue as started for the cervix [32] and as preformed for the new high magnification endoscopy systems [33]. Two of the commercial systems expect to be in limited clinical trials by the end of

Fig. 22. Prototype in vivo confocal microendoscopy system from Mauna Key Technologies, France. The system comprises a central unit, dedicated image processing and analysis software, and a handheld endomicroscopic probe (see insert). (*Courtesy of* Mauna Kea Technologies, Paris, France.)

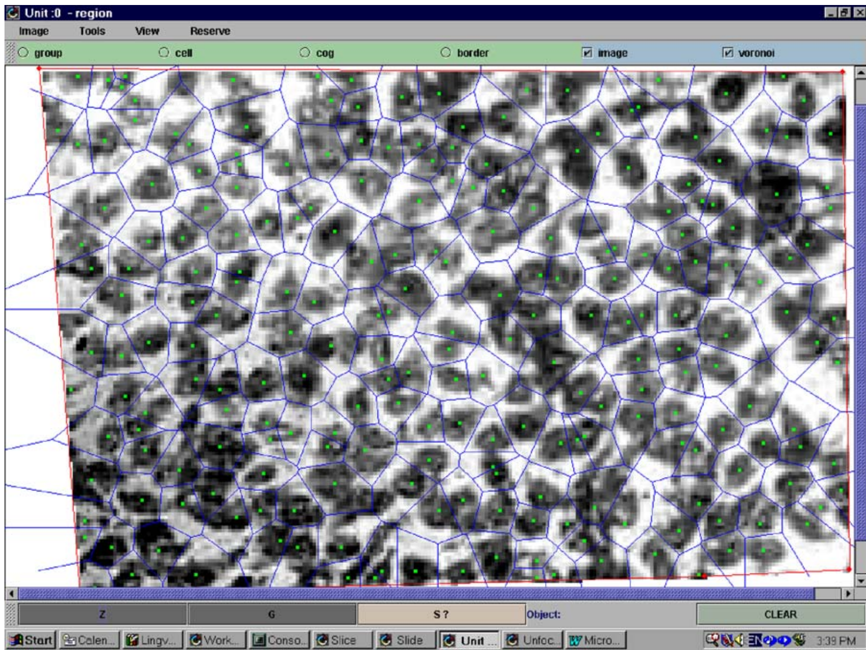

Fig. 23. Digital micromirror device confocal reflectance image automatically processed using quantitative architectural tools developed at the BCA Cancer Agency for stained histology sections. (See also Color Plate 39).

2003. As the developers and earlier adopters gain more experience with these systems and learn how to most optimally use them, on-line three-dimensional reconstructions of the tissue being imaged as well as quantification of the measurable tissue characteristics with in the images produced are likely to be available.

Looking further into the future as more is known about the molecular characteristics that identify the disease or conditions being studies, one can expect to see the emergence of specific molecularly targeted optical contrast agents used in conjunction with these in vivo cellular imaging systems generating in vivo cellular level functional imaging. The promise of the potential of this combination of emerging technologies can be seen in the recent work of the group at the University of Texas at Austin [34] (Figs. 25 and 26) and in the work by Anikijendo et al [35] and White et al [36] in melanoma mice models.

Most of the systems under development are either fluorescence imaging systems or reflectance imaging systems. In the future multimodal systems that combine multispectral reflectance and multispectral fluorescence imaging modes are likely to become available. Further, even given the formidable technical challenge associated with multiphoton pulse propagation through fiber, it is likely that future confocal microendoscopes will be able to make use of the increased

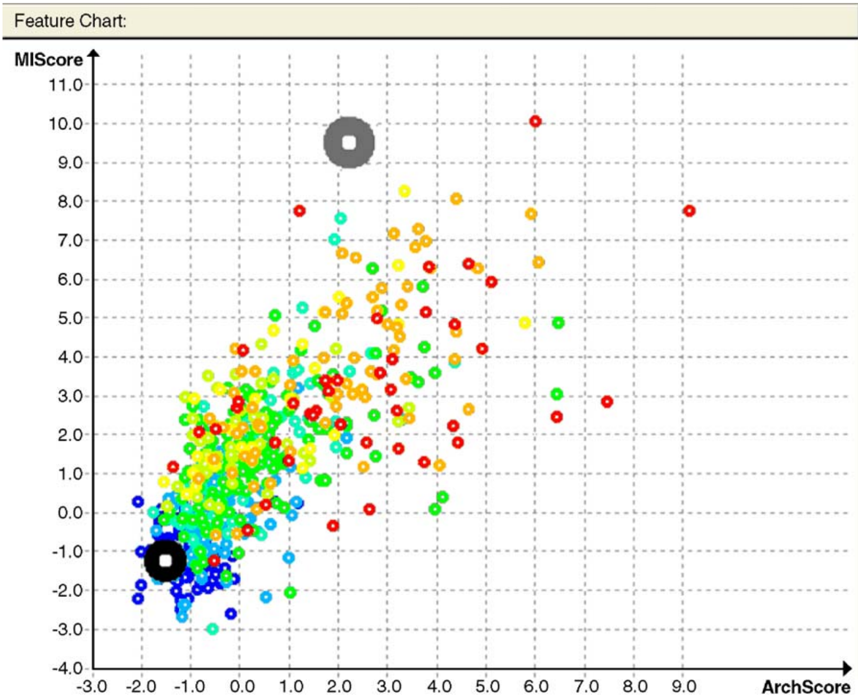

Fig. 24. Scatter plot of ArchScore (function based on architectural features) and MIScore (based on morphologic features) for a variety of tissues sections (lung, colon) in different stages of neoplastic progression (normal, hyperplasia, metaplasia, mild, moderate, severe dysplasia, carcinoma in situ, and invasive cancer). Large black donut represents score from normal colon; large gray donut represents score from invasive colon cancer. (See also Color Plate 40).

signal localization afforded by multi photon imaging as well as possible improvements in the imaging depth with multi photon systems.

Adoption issues

Numerous issues are likely to play a large role in the adoption of confocal microendoscopy by clinicians. The issues include the need to visually interpret a new level of in vivo images similar to pathology. Preliminary market research by the investigators suggest this clinical interpretation is not likely to present a significant problem in that many gastroenterologists are already familiar with the visual changes in the organization of tissue during the neoplastic progression from their own experience in examining excised tissue as part of their regular practice. Additionally, the adoption of ultrasound imaging in gastroenterology and other fields also indicates the flexibility of clinicians to adapt to new imaging modalities for clinical use. The capital cost of the equipment and associated disposables and software will need to be in line with current endoscopic

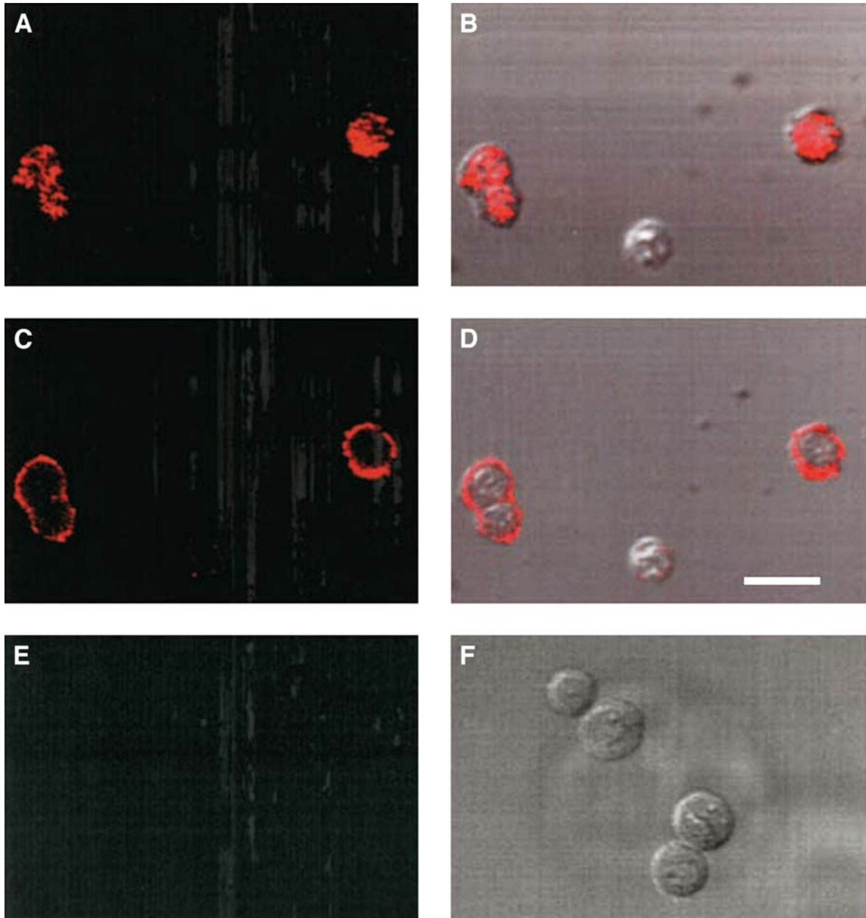

Fig. 25. Reflectance confocal microscopy (RCM) images of molecular-specific contrast agent–labeled cervical cancer cells. Excitation is at 647nm. The contrast agents are 20-nm colloidal gold particles conjugated to anti-epidermal growth factor receptor (EGFR) monoclonal antibodies. (*A*), (*C*), and (*E*) show RCM images. (*B*), (*D*), and (*F*) show RCM images overlaid on the corresponding transmittance images. (*A*) and (*B*) show the very top of the cell in focus. (*C*) and (*D*) show the equator of the cell in focus. (*E*) and (*F*) show the absence of signal when 20-nm nanoparticles are conjugated to a nonspecific protein (BSA) as a control. Scale bar is ~30 microns. (See also Color Plate 41).

instrumentation (endoscopic imaging systems) and biopsy forceps, and so forth. The greatest "cost" concern with respect to the adoption of new imaging or diagnostic instrumentation will be any incremental increase in the time required to perform a complete procedure. Over the last few years, reimbursement for endoscopic procedures has been declining, resulting in an effort to perform more procedures per given time period. Thus, substantial incremental increases in the time a procedure requires would likely have the effect of largely limiting the adoption to salaried endoscopists in tertiary care centers. And lastly, clinical trials

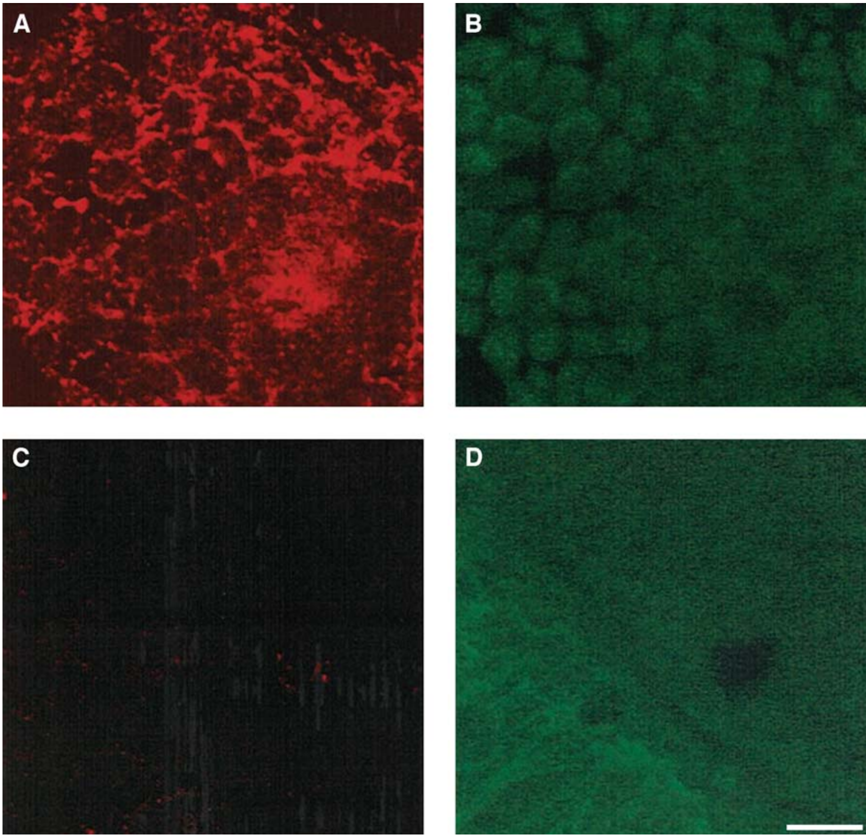

Fig. 26. Gold nanoparticle–antibody conjugate labeled fresh ex vivo tissue slices. (*A*) and (*B*) are abnormal tissue (cervical intraepithelial neoplasia), whereas (*C*) and (*D*) are histopathologically normal. Again, 20-nm gold particles were conjugated to anti-epidermal growth factor receptor (EGFR) antibodies. (*A*) and (*C*) are reflectance confocal microscopy images at 647nm, and (*A*) shows up-regulation of EGFR compared with (*C*). (*B*) and (*D*) are fluorescence confocal microscopy images of cellular auto-fluorescence (primarily from flavinadenine dinucleotide). They are added for morphologic comparison with (*A*) and (*C*). Scale bar is ~20 microns. (See also Color Plate 42).

providing compelling evidence of clinical and cost-effectiveness will need to be completed and accepted by clinicians.

Overview of selected microendoscopy applications in the gastrointestinal tract

Screening for Barrett's esophagus

Barrett's esophagus is a precursor lesion for cancer of the esophagus and is associated with a 30 to 125× increase in risk of getting cancer of the esophagus

[37–41]. In the United States, approximately 20 to 30 million individuals suffer from chronic (weekly) acid reflux and, therefore, are at high risk of cancer of the esophagus [37]. Approximately 10% of this population (two to three million individuals) will develop Barrett's esophagus [37–39,42]. Barrett's esophagus is a change in the esophageal epithelium of any length that can be recognized at endoscopy and is confirmed to have intestinal metaplasia by biopsy. Typical screening methodology is to perform an endoscopy of the esophagus for visual analysis and biopsy. Biopsies of suspected Barrett's esophagus are obtained to identify the pathology and rule out malignancy. Once Barrett's esophagus has been identified, the goal is to control acid reflux and symptoms (eg, proton pump inhibitors) and attempt regression of intestinal metaplasia.

Screening for Barrett's esophagus has generally been recommended for high-risk individuals [37]. The benefit and cost-effectiveness of screening has been the subject of increasing debate in recent years. A recent clinical update from the American Society of Gastrointestinal Endoscopy suggests that a less expensive endoscopic screening test for neoplastic Barrett's epithelium is necessary to gain acceptance of screening by gastroenterologists on the basis of clinical effectiveness and prudent clinical management [43]. Because of the small targeted size of confocal microendoscopy systems, one logical future use will be to screen for Barrett's esophagus by using a minimally invasive transnasal catheter for imaging of the esophagus in high-risk individuals. Most likely this test will combine macroscopic and microscopic imaging of the esophagus to identify intestinal metaplasia in the esophagus. A primary benefit of the procedure will be the combination of the traditional endoscopic and pathology aspects of the diagnostic requirements for Barrett's esophagus into one test. Thus, therapeutic recommendations could be made at the time of patient visit, as opposed to the delay required for pathologist review, in many instances.

For confocal microendoscopy to succeed at this application, preliminary market research suggests that the overall test sensitivity and specificity will need to be equivalent or better than current practice (estimated at approximately 90% sensitivity and 90% specificity). The ideal ease-of-use will be such that a procedure can be conducted on an unsedated patient on an outpatient basis in a hospital or clinic setting in a period of time not to exceed 20 minutes of clinician time and 45 minutes overall of patient time.

Screening for esophageal cancer

There is a well-recognized risk of developing adenocarcinoma in the esophagus of patients with Barrett's esophagus with specialized intestinal metaplasia. Most experts agree that surveillance endoscopy for patients with Barrett's esophagus and no history of dysplasia should be performed every 1 to 3 years [37,39,44,45]. For people with a history of dysplasia who have not been treated, endoscopy should occur more frequently. The goal of surveillance is the detection of dysplasia. High-grade dysplasia has been shown to significantly correlate with

the risk of progression to cancer. Typical screening methodology is to an endoscopy of the esophagus for visual analysis and biopsy. Recommended biopsy method is a four-quadrant biopsy every 1 to 2 centimeters for the length of the esophagus.

Confocal microendoscopy can be used to periodically examine individuals with Barrett's esophagus and those symptomatic for cancer of the esophagus as a means of identifying and treating cancerous and precancerous lesions at an early stage of development. Currently, no effective surveillance strategies have been identified. Dysplasia is not visible during routine endoscopy, and the current (random biopsy) detection method is complex, time consuming, and expensive. This test combines macroscopic and microscopic imaging of the esophagus to systematically and rapidly spot sample a large number of locations in the esophagus optically. The gastroenterologist takes biopsy samples from those sites under confocal microendoscopy that appear abnormal (dysplastic or cancerous) for pathologic analysis. The primary benefit of this technology is the more accurate identification of abnormal conditions, particularly earlier stage conditions, in a less invasive manner with fewer biopsies for the pathologist to review. Initial estimates suggest that 85% to 90% sensitivity and 90% specificity for dysplasia and cancer would be required to make the test effective.

Surveillance of inflammatory bowel disease

Inflammatory bowel disease (IBD), including ulcerative colitis and Crohn's disease, causes inflammation of the intestine. Ulcerative colitis occurs when sores, or ulcers, form on the inner lining of the colon (large intestine). Crohn's disease occurs when ulcers form throughout the entire tissue of the colon and other areas of the GI tract. Five percent of patients with ulcerative colitis are expected to die from colon cancer. Although the figure for Crohn's disease is not known, it is estimated to be comparable that of ulcerative colitis. In an effort to reduce mortality from colorectal cancer in patients with ulcerative colitis, surveillance to promote early detection has become a well-accepted and recommended practice. Although less researched, prevailing thought about screening for Crohn's disease is similar. Recommended IBD biopsy protocol is to take biopsy specimens every 10 cm for the length of the entire colon (eg, a random biopsy) taking up to an hour of the gastroenterologist's time and generating an extensive number of biopsy specimens for review by the pathologist.

Confocal microendoscopy could be used for in vivo imaging of colon tissue to monitor patients with long-term conditions of inflammatory bowel disease (IBD), including both ulcerative colitis and Crohn's disease. The initial test would be used in conjunction with conventional colonoscopes, where the microendoscopy bundle is inserted through the biopsy channel of the scope or built into the endoscope. Although not yet demonstrated, it is expected that the use of confocal microendoscopy will allow for the identification of earlier, more treatable precancerous and cancerous conditions and reduce the number of biopsy speci-

mens needed, while increasing the quality of the specimens taken, resulting in better outcomes and lower pathology costs.

Screening for colon cancer

There are a significant number of individuals whose risk for colorectal cancer is approximately twice that of the general population due to their personal or family history. Clinical guidelines suggest that these individuals should be screened periodically (typically ranging from every 1 to 5 years) for the detection of precancerous and cancerous conditions beginning at age of 40. Screening for all individuals is suggested at age 50 every 5 to 10 years. The number of individuals requiring screening may be reduced with the advent of pan-organ molecular and assay markers and other genetic tests for the identification of those at highest risk. Even so, there will exist a need for precise tools to identify and localize the lesions after a positive indication from a marker assay.

Approximately two thirds of colorectal cancers arise from adenomatous polyps; the origin of the other one third of cancers remains to be established. One primary theory, de novo carcinoma theory, relates to the transition from normal tissue to neoplastic tissue without the formation of a polyp due to rapid genetic changes. Most adenomas (lesions) are polypoid (polyps). Yet, a significant number, estimated between 5% and 30%, are flat and depressed lesions difficult to see by traditional endoscopy. Small polyps are also difficult to identify. One key study indicated that colonoscopy misses 25% of small polyps (< 5 mm) and 10% of large polyps (> 1 cm).

Confocal microendoscopy could be used for in vivo imaging of colon tissue for two primary screening functions: (1) systematic pan-colonic screening for flat and depressed lesions not easily observable by traditional endoscopy and (2) investigation of abnormally appearing tissue and polyps not removed by polypectomy (eg, hyperplastic polyps). The primary benefit would be the detection of high-grade dysplasia and cancer not detected by traditional colonoscopy means, particularly flat lesions and de novo carcinomas. Additionally confocal microendoscopy would enhance the clinician's ability to explore abnormally appearing areas and hyperplastic polyps when a biopsy or polypectomy is not feasible or prudent.

Additional applications

- As indicated, confocal microendoscopy offers benefits to a wide-ranging number of potential applications. The applications identified previously seem likely as initial applications. Other applications are likely to be pursued as researchers and clinicians become comfortable with the technology. Applications of particular promise for research and clinical relevance include: assessing/monitoring treatment and post-treatment conditions (eg, chemotherapy).

- Mid-surgery optical biopsy to limit time and improve assessment capabilities.
- Macro and micro imaging of tissue not easily accessible using traditional endoscopy (eg, biliary tree).

Summary

Confocal microendoscopy represents a compelling new imaging modality that brings pathologic information to the point of patient care. The technology permits direct observation of pathologic change at the microscopic level rather than traditional inference based on indirect changes at the macroscopic level. The targeted key benefit addresses earlier detection of precancerous and cancer conditions through improved biopsy selection and examination and more cost-effective solutions to screening and surveillance.

Numerous outstanding research and commercial groups with varying approaches to confocal microendoscopy are allocating significant efforts to making the technology commercially available, including two industrially sponsored clinical trials one already underway and one to commence in 2004 (Box 1).

Box 1. Confocal microendoscopy development programs: commercial and academic (researcher)

Distal scanning

- Microvision, Inc. / University of Montana - *Dickensheets*
- MEDEA Project: University of Athens, Eberhard-Karls-University of Tuebingen - *Knittel*, Fraunhofer Institute of Silicon Technology - *Hofmann*, Scuola Sueriore Sant'Anna – *Dario and Carrozza*, Laser-und-Medizin-Technologie, GmbH and Swiss Federal Institute of Technology
- Wellman Laboratories of Photomedicine < Massachusetts Institute of Technology - *Tearney*
- Olympus Optical Co. Ltd. / Tokyo Medical and Dental University - *Inoue*
- Optiscan Party, Ltd. / Asahi Optical Co. Ltd. (Pentax)
- Stanford University / Optical Biopsy Technologies Inc. – *Wang*

Proximal Scanning

- Digital Optical Imaging Corp. / British Columbia Cancer Agency - *MacAulay*
- Mauna Kea Technologies
- University of Arizona - *Gmitro*
- University of Texas at Austin – *Richards-Kortum*

The initial instruments will likely be geared toward applications relating to screening for and surveillance of esophageal and colon-related conditions. Other applications are likely to be identified and pursued as the technology becomes more accessible.

Future developments related to greater functionality, improved ease of use, and automated analysis are likely to facilitate adoption and use of the technology. Confocal microendoscopy is an enabling platform to accelerate the use and adoption of in vivo imaging through further evolution of the platform in combination with molecular markers and multimodality imaging. Clinical gastroenterologists should look forward to the potential of confocal microendoscopy as a logical and needed modality to advance the field of gastroenterology.

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