The Status of Advanced Imaging Techniques for Optical Biopsy of Colonic Polyps

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The progressive miniaturization of photonic components presents the opportunity to obtain unprecedented microscopic images of colonic polyps in real time during endoscopy. This information has the potential to act as "optical biopsy" to aid clinical decision-making, including the possibility of adopting new paradigms such as a "resect and discard" approach for low-risk lesions. The technologies discussed in this review include confocal laser endomicroscopy, optical coherence tomography, multiphoton microscopy, Raman spectroscopy, and hyperspectral imaging. These are in different stages of development and clinical readiness, but all show the potential to produce reliable *in vivo* discrimination of different tissue types. A structured literature search of the imaging techniques for colorectal polyps has been conducted. The significant developments in endoscopic imaging were identified for each modality, and the status of current development was discussed. Of the advanced imaging techniques discussed, confocal laser endomicroscopy is in clinical use and, under optimal conditions with an experienced operator, can provide accurate histological assessment of tissue. The remaining techniques show potential for incorporation into endoscopic equipment and practice, although further component development is needed, followed by robust prospective validation of accuracy. Optical coherence tomography illustrates tissue "texture" well and gives good assessment of mucosal thickness and layers. Multiphoton microscopy produces high-resolution images at a subcellular resolution. Raman spectroscopy and hyperspectral imaging are less developed endoscopically but provide a tissue "fingerprint" which can distinguish between tissue types. Molecular imaging may become a powerful adjunct to other techniques, with its ability to precisely label specific molecules within tissue and thereby enhance imaging.

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INTRODUCTION

We present a review of the advanced imaging technologies under development in pursuit of real-time diagnosis of colorectal polyps. It is known that high-quality colonoscopy, with polypectomy of detected colorectal adenomas, can arrest the progression of adenoma to carcinoma at an early stage, thereby reducing the incidence of colorectal cancer. The gross morphological appearances of polyps are well described and hold clues to their likely nature, whether hyperplastic or adenomatous with varying degrees of dysplasia (1,2). An emerging generation of endoscopic imaging technologies is beginning to expand beyond the boundaries of visual lesion recognition and generates images of the cellular structure of polyps, often known as "optical biopsy" or "virtual histology" to aid clinical decision-making.

The benefits of real-time assessment of polyp histology could include avoidance of biopsy in low-risk hyperplastic lesions, with associated reduction in complication risks and histological costs. Alternatively, low-risk lesions could be resected and discarded without formal histological analysis. This could also provide early reassurance to patients with benign histology by giving them the result at the time of endoscopy, although it remains to be seen whether optical biopsy will be seen as an acceptable alternative to conventional biopsy by both patients and professional bodies. We review the current technologies showing potential to achieve the goal of accurate optical biopsy but which are not yet in routine or widespread clinical use. These technologies include confocal laser endomicroscopy (CLE), optical coherence tomography (OCT), multiphoton microscopy (MPM), Raman spectroscopy, hyperspectral imaging (HSI), and molecular imaging. This review discusses the endomicroscopic techniques showing the potential to produce virtual histology. Image enhancement techniques such as narrow band imaging or i-scan have not been included. A summary of the status of the included imaging modalities is presented in Table 1.

METHOD

A structured literature search was carried out to identify the current relevant studies. A series of scoping searches were performed, which included each of the discussion areas in this review. The results of these were screened for relevance using the title, then abstract, followed by review of the full text of the article. The Ovid MEDLINE database and the Cochrane Library database were searched in May 2019 for relevant meta-analyses, review articles, clinical trials, and preclinical research. Studies without peer review or not available in English were excluded. The studies included for review were scrutinized for results in humans; *ex vivo*

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Table 1. Summary of the characteristics of the advanced photonic technologies for optical biopsy of colorectal polyps

Technology	Principle of operation	Image type	Image features	Stage of development	Advantages	Limitations
Confocal laser endomicroscopy (CLE)	Laser light illuminates a plane of tissue at a preset depth, inducing fluorescence from the tissue which is then detected.	Two-dimensional *en-face* view of the polyp; the device can be scanned across the mucosal surface to visualize a larger area as a composite image.	Surface crypts and goblet cells have been demonstrated. Image depths from 0 to 250 μm are possible. Spatial resolution is about 0.7 μm.	A probe-based CLE system is commercially available (Mauna Kea technologies, Paris) Previously an endoscope-integrated CLE system was available, but this is no longer in production.	Prospective studies have demonstrated good diagnostic accuracy, and the technology could support a strategy of optical biopsy, when used by a suitably experienced operator (89).	Requires administration of fluorescein for optimal image quality.
Optical coherence tomography (OCT)	Similar to ultrasound, the reflection of a light wave within tissue is measured to produce an image.	A 2-dimensional image similar to a "B scan" of an ultrasound, showing good discrimination between mucosal layers.	Mucosal layers and glands can be identified. Shows progressive thickening and increasing irregularity of the mucosa during the development of adenoma. Image depth up to 2 mm is possible. Spatial resolution from 1 to 2 μm is reported.	OCT appearances in *ex vivo* colon tissue have been well characterized. Recently, endoscope-compatible OCT probes have been reported. No commercial system is available.	Exogenous contrast or fluorophore is not required. Could have uses in the detection of buried glands or residual tissue after polypectomy. Relatively fast image acquisition time.	Most probes developed to date use a spiral-scanning mechanism, which is suitable for upper GI applications but less so for the colon.
Multiphoton microscopy (MPM)	A near-infrared light source allows good tissue penetration of low energy photons. Two or more of these photons induce fluorescence in tissue fluorophores (endogenous or exogenous).	A 2-dimensional, *en-face* view below the mucosal surface. The depth is dependant on the focusing plane of the system.	Excellent resolution can be achieved comparable with conventional microscopy, demonstrating cells and subcellular structures.	Current studies have been performed *ex vivo*, although a miniature MPM probe has been developed with the aim of producing an endoscope-compatible system.	High-resolution images achieved with intrinsic sectioning into a single optical plane similar to conventional histology slides. Exogenous fluorophore may not be required.	Large components and difficult to miniaturize.
Raman spectroscopy	Measures the spectrum of photons returned from tissue with an altered wavelength. Spectra from an area of tissue can be visualized as an altered-color image.	A colored "overlay" of an image shows the relative abundance of different chemicals in a pixel of an image.	Precise labeling of cellular and tissue components with different colors dependant on the chemical constituents being labeled.	Laboratory-based systems exist for the *ex vivo* study of tissue samples and microscope slides. Insertable endoscopic probes have been described capable of producing a Raman spectrum at a single point in tissue, but not imaging.	Exogenous fluorophores do not appear to be required. Imaging shows good definition of both cellular and tissue features. Spectral differences between adenoma and hyperplasia are readily apparent.	Slow image acquisition times, meaning that a polyp could take several minutes to be scanned in its entirety. No miniaturized imaging systems available.
Hyperspectral imaging (HSI)	Produces reflectance spectra at a wide range of points within the visual spectrum, rather than the red, green,	An "image cube" is generated, in which each pixel has multiple color values applied to it. This can be processed into an altered-	Macroscopic or microscopic images are possible depending on the imaging equipment used.	Different groups have produced endoscopically insertable probes capable of discriminating between	Does not require administration of an exogenous fluorophore. Scanning times are several seconds, rather than minutes.	Still requires prospective validation of the different tissue signatures.

Table 1. (*continued*)

Technology	Principle of operation	Image type	Image features	Stage of development	Advantages	Limitations
	and blue perceived by the human eye.	color image to highlight features of interest.	Differences in reflectance spectra between different tissue types can be expressed visually.	adenomatous, hyperplastic, and normal tissue. No commercial system is available.		Miniaturization and development of a practical usable system is required.
Molecular imaging	Mucosal proteins are tagged with a fluorophore/antibody label. They may then be imaged in conjunction with other fluorescence-inducing methods, e.g., OCT or CLE.	The tagged portion of the sample produces greater signal intensity, highlighting the region or protein of interest.	These are dependent on the imaging modality being used, but with the addition of targeted contrast.	Currently in laboratory-based testing on *ex vivo* tissue and early animal studies. Multiple molecular targets have been identified to correspond with adenomatous tissue.	May augment the images acquired by optical techniques and provides precise labeling of the tissues of interest.	High cost of molecular labels. Labeled antibodies are potentially immunogenic.

results and laboratory data were included as appropriate dependant on the available research literature.

Confocal laser endomicroscopy

Of the advanced photonic techniques discussed in this review, CLE has been developed to the greatest degree with commercially available imaging platforms and a body of evidence to support clinical use. CLE obtains very high magnification and resolution images of the mucosal layer of the gastrointestinal (GI) tract by the use of a low-power laser to induce fluorescence or reflectance at a defined plane within tissue. The emitted fluorescent light is then detected; "confocal" refers to the alignment of the illumination and detection systems in the same focal plane. Typically, an exogenous fluorophore such as fluorescein is used (3).

Among the earliest clinical use of CLE was at Showa University in 2003 using the Olympus FluoView instrument, a CLE scanner inserted through the working channel of an endoscope (4). This demonstrated the ability of CLE to visualize colonic mucosal structures *in vivo*, including pits and goblet cells. Adenomatous tissue was shown to have a different appearance to normal tissue, with greater numbers of visible nuclei.

In 2004, the first endoscope with integrated confocal laser microscopy was demonstrated in Mainz, and the initial capabilities of endoscopic CLE were examined. A high degree of diagnostic accuracy was achieved, up to 99.4% for the prediction of neoplastic histology (5,6). Images could be obtained with a resolution of 0.7 μm and at a variable depth from 0 to 250 μm below the epithelial surface. Fluorescein and acriflavine were used as fluorophores, and fluorescein was found to produce improved labeling of the lamina propria.

The normal appearance of the bowel under CLE was further discussed by Odagi et al., (7) who conducted mapping studies of the small and large intestine from 45 patients and demonstrated good concordance between histological appearances and CLE images. Several studies have since described the features of normal bowel and of polyps seen using CLE (8–10). Adenomatous polyp tissue has been shown to demonstrate several abnormal features, including distorted and elongated glands, crypt budding, fusion of glands, lack of surface maturation, dark irregular epithelium, distorted vascular architecture, and mucin depletion. The transit of fluorescein through adenomatous tissue was also found to be measurably slower than through normal tissue (11). Hyperplastic polyps were shown to have stellate pits with enlarged, branch-like crypts (8). In 2011, the Miami consensus conference drew together a group of expert endoscopists with experience in CLE to define the criteria for diagnosis of different GI lesions, including colonic polyps. Furthermore, the CLE appearance of sessile serrated adenomas has been investigated to assess for characteristic appearances. These include the mucus cap, visible on CLE as a bright, clouded appearance, thin, branched crypts, increased goblet cells, and irregular crypts (12). Descriptions of normal colonic mucosa, hyperplastic polyps, and adenomatous polyps are presented in Figure 1 (13).

To date, 7 studies have attempted to assess the diagnostic accuracy of CLE for the *in vivo* diagnosis of adenomatous polyps vs nonadenomatous tissue (5,14–19). These include diagnoses made in real time during endoscopy and diagnoses made "off line" using the images obtained during endoscopy. The most recent meta-analysis in 2016 included 410 patients, who had a total of 1,074 lesions. This concluded that CLE demonstrated a pooled sensitivity of 83%, for the diagnosis of adenoma in

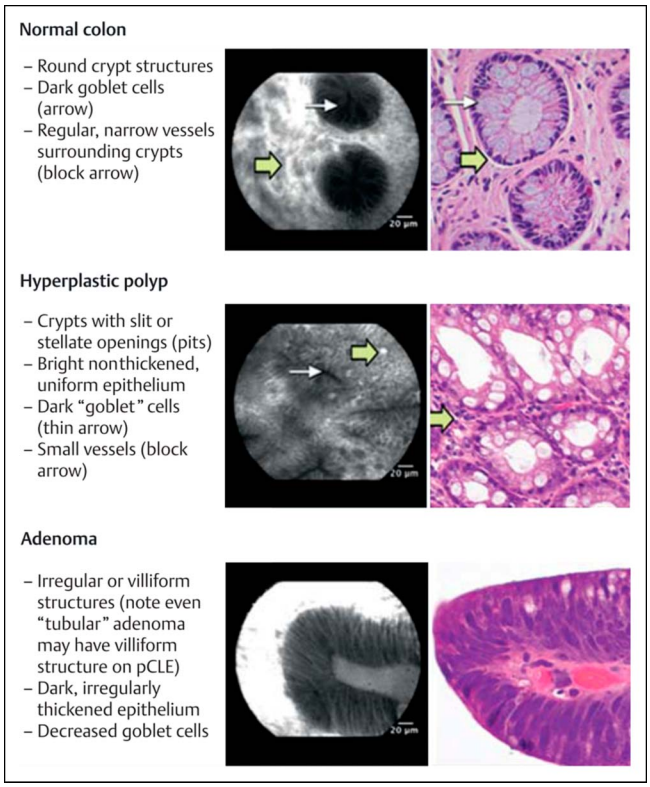

Figure 1. The Miami classification for probe-based confocal laser endomicroscopy.

a detected polyp, and specificity of 90% (20). It should be remembered that these diagnostic accuracies are produced by experts, familiar with CLE.

Other roles of CLE in the assessment of colonic polyps include assessments of lesion margins, depth, and residual tissue after polypectomy. Research in these areas is ongoing (19,21–23). In common with many of the optical biopsy techniques, CLE presents endoscopists with the challenge of interpreting the microscopic images. This skill is typically not frequently used by endoscopists, and the effects of training and experience on the accuracy of diagnosis are not fully understood. However, the learning curve of performing CLE and interpreting images does appear to be fast (24,25).

Optical coherence tomography

In clinical use since the 1990s for examination of the retina, the images acquired by OCT may be described as conceptually similar to ultrasound images, although using a measurement of reflected light rather than sound energy (26). OCT images can demonstrate subsurface tissue layers and structures with a depth of up to approximately 2 mm and resolution of 1–2 μm, suggesting the potential for assessment of mucosal thickness and lesion characterization (27,28). With advances in miniaturization of optical components, endoscopic OCT probes have become possible and have been produced either as forward-scanning or rotary-scanning devices (29). A rotary-scanning probe typically scans at 90° to the shaft of the endoscope and can be withdrawn or inserted to scan an area of mucosa. A forward-scanning probe directs the scanning beam in the direction of view, much like a forward-viewing endoscope. Typically, an optical fiber within the scanning tip is oscillated by a piezoelectric transducer to form a scanning pattern such as a spiral or grid (30). Both designs have been studied for potential application for polyp diagnosis in the colon.

Studies to define the capabilities of OCT have been successful in imaging murine and human colonic mucosa and demonstrated that the light-scattering properties of hyperplastic polyps and adenomatous polyps differed from healthy tissue (31–33). The layers of the colon have been well demonstrated in a rat model, showing clear demarcation of the mucosa, submucosa, and the muscularis propria. The appearances were also shown to vary, depending on the degree of pressure placed on the tissue (34). Studies of OCT for assessing adenomatous tissue have also demonstrated visible changes; in a rat model of adenoma and colorectal cancer development, serial OCT imaging shows a progressive mucosal thickening and attenuation of tissue boundaries as adenoma progresses (Figure 2) (35,36). Other studies in a mouse model have found that OCT can detect foci of abnormal crypts even in grossly normal mucosa (37). Further studies in humans by Alder et al. (38) have helped to define the OCT appearances of healthy human colonic tissue and in a variety of disease conditions, including ulcerative colitis and radiation proctitis. A spiral-scanning probe was used to acquire a three-dimensional image dataset, showing detail of the crypt patterns and close resemblance to traditional histology. This approach is a further refinement of the OCT technology using near-infrared light, volumetric laser endomicroscopy. Recent studies of volumetric laser endomicroscopy probes have demonstrated the ability to image colorectal polyps immediately after resection, showing definition of the mucosal layers and abnormal glandular architecture in dysplastic tissue (39).

The spiral-scanning probes for OCT allow the survey of large areas of mucosa and have been studied in the esophagus and biliary tree but may not be as applicable in the irregular lumen of the colon (40). Two groups have recently reported the development of forward-viewing OCT probes with rapid image acquisition times which could allow immediate assessment of visualized mucosa (41,42).

The images obtained by OCT require some interpretation, but this process could be simplified by the application of image analysis algorithms. Qi et al. (43) have demonstrated the ability to virtually reconstruct the OCT images into a 3-dimensional model of the mucosa, which illustrates crypt architecture and allows identification of aberrant foci. The role of OCT in the diagnosis of polyps remains an area of ongoing research interest. It is an attractive prospect for the generation of "virtual histology" because it does not necessarily require exogenous fluorophores or other markers and provides images at a useful level of tissue penetration and spatial resolution. OCT in the esophagus is being studied as a potential tool for Barrett's esophagus screening, using a spiral scanner (44). This approach seems less applicable to the colon, with its many folds and flexures. It seems more probable that the role of OCT could be for precise scanning of a detected lesion to assess the crypt architecture and mucosal layers. It could also have a role in margin and depth assessments of a larger polyp or to assess for the presence of residual tissue after polypectomy. An early clinically usable system is reported by Ding et al. (45) using a near-patient OCT microscopy system to image unprepared polyp tissue immediately after resection during colonoscopy before histological analysis. A high accuracy of prediction is achieved, with a short learning curve. Future developments may

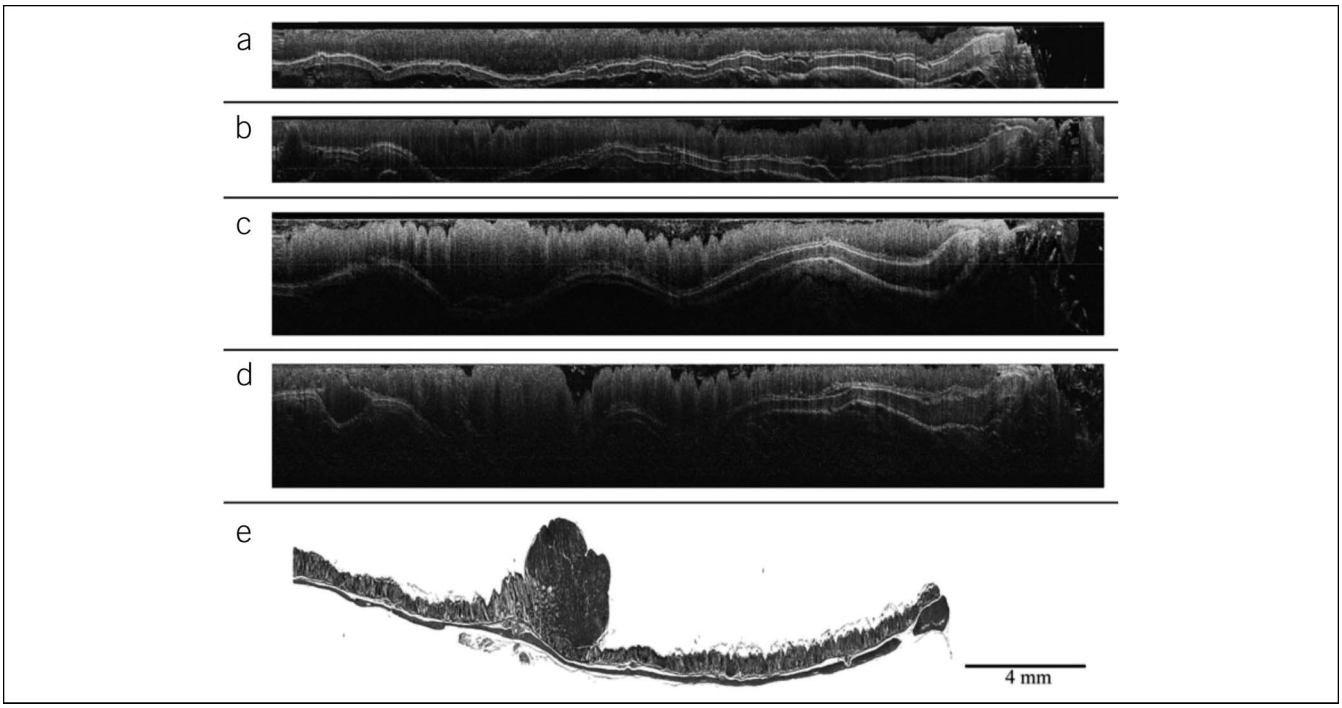

Figure 2. OCT time series images of the development of adenoma in an azoxymethane mouse model of colonic adenoma showing (**a**) mucosal thickening at 8 weeks post treatment, (**b**), moderate mucosal protrusion at 14 weeks, (**c**) further mucosal thickening at 22 weeks, (**d**) signal attenuation and absence of tissue boundaries at adenoma, 26 weeks, and (**e**) is the corresponding histological section, containing adenoma. Reproduced with permission from Dr Jennifer K. Barton (35). OCT, optical coherence tomography.

include insertable OCT probes suitable for colonoscopy or eventually integrated into the colonoscope.

Multiphoton microscopy

MPM relies on the absorption by a tissue fluorophore of 2 or more photons, followed by emission of a single photon. The fluorophore molecule may be endogenous, such as collagen, or exogenous, such as fluorescein. The technique demonstrates the ability to allow real-time observation of living tissue at a cellular level, including functional information implied by the fluorescence from metabolically active molecules (46). Typically a near-infrared laser light source is used; the photons are relatively of low energy and longer wavelength, and so are absorbed less in tissue than photons of bluer light, with consequent deeper tissue penetration (47).

MPM in the GI tract has been limited by the large size of many of the components, including lasers and scanning devices. In 2008, a miniaturized MPM probe was reported by Rogart et al. (48) and used to image rat colonic tissue *ex vivo*, as well as colonic tissue samples collected from volunteers. Collagen autofluorescence produced high-resolution images of cells and subcellular structures, comparable with conventional histological images.

A miniaturized forward-viewing probe was reported in 2012, using an oscillating optical fiber and capable of scanning a 110 × 110 μm field of view. The scanning device has a diameter of 3 mm, has a rigid length of 40 mm, and could be developed to allow insertion through the working channel of an endoscope for *in vivo* tissue imaging (49). This system has been used to produce reference images of the mouse GI tract, as well as to study the progression of adenoma to carcinoma in a mouse model of carcinogenesis. This demonstrated the ability of MPM to show progressive development of aberrant crypt foci and elongated, crowded nuclei (50). Other studies of adenoma progression have shown an increase in the volume of submucosal collagen and changes in its organization (51).

Other groups have performed comparison of human colonic mucosa against rectal adenocarcinoma in unstained resected tissue, demonstrating significant morphological differences between healthy mucosa and cancerous tissue under MPM and again showing images comparable with histological slides (Figure 3) (52). To date, these studies have been performed *ex vivo*, and no system capable of *in vivo* MPM is currently available (53–55).

MPM is an exciting imaging modality, allowing microscopic images to be produced from tissue, without the need for biopsy. In addition to structural information, the functional information obtained from fluorescence of metabolites has the potential to provide diagnostic information. A further interesting development is the report of a dual OCT and MPM imaging system, which could combine the information of structural and mucosal layers from OCT with the precise cellular imaging of MPT (56,57).

Raman spectroscopy

Raman spectroscopy relies on observing the interactions between photons and matter during light scattering. Using a Raman spectrometer, it can be observed that a tiny proportion of light reflected from an object has an altered color because of photons interacting with molecular vibrations and consequently shifting their wavelength. This is known as the Raman shift, and measurement of this altered spectrum can provide information about

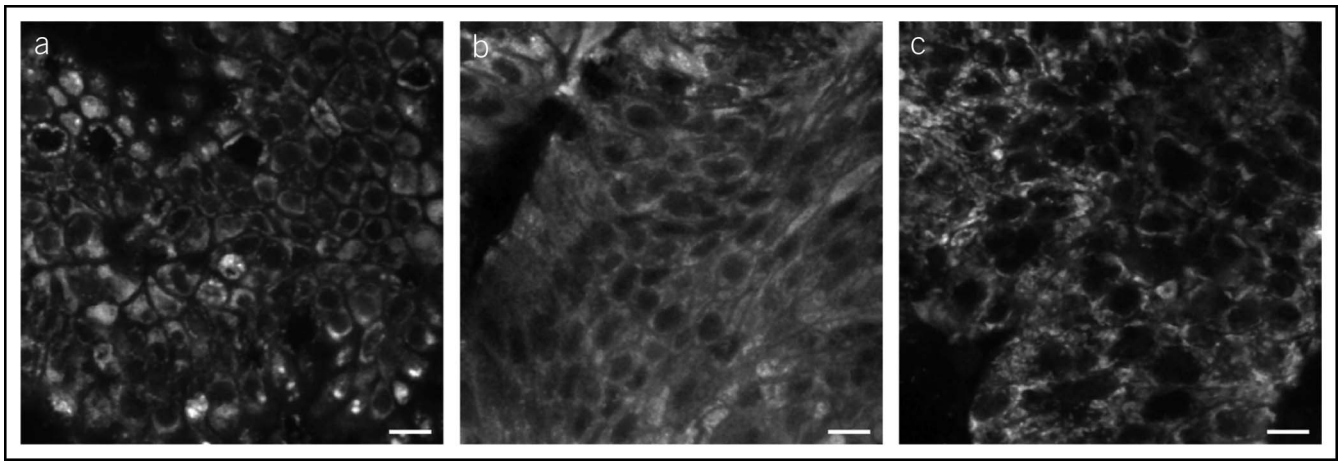

Figure 3. *Ex vivo* images of fresh biopsies from (**a**) healthy colonic mucosa, (**b**) adenomatous polyp, and (**c**) adenocarcinoma at a depth of 30 μm from tissue surface. Scale bar is 10 μm. Reproduced with permission from Dr Riccardo Cicchi (52).

the composition of the reflecting material (58). Raman spectral information may be combined with optical microscopy to produce an altered-color image of tissue, demonstrating cellular and subcellular structures labeled by their chemical constituents (59).

Early studies of Raman spectroscopy in the GI tract have shown the ability to produce Raman spectra from human GI mucosa, both *ex vivo* (60–63) and *in vivo* (64–66). The *in vivo* spectra were obtained by passing a fiber-optic probe through the working channel of an endoscope and placing the tip into contact with the mucosa. An insertable probe has been developed, capable of obtaining a Raman spectrum with an acquisition time of 1 second. Differences were observed between the spectral signal generated from hyperplastic and adenomatous polyps (Figure 4) (67). The spectra associated with each type of polyp can be distinguished by statistical analysis, increasing the prospect of automated or computer-aided diagnosis of polyp histology before resection (68).

Further developments in Raman spectroscopic imaging include the *ex vivo* scanning of normal colonic tissue and adenocarcinoma tissue. A Raman spectrometer attached to a scanning microscope has been used to produce pseudocolor maps of tissue sections, highlighting areas of collagen, protein, mucus and lipids, in normal and malignant tissue (69). A similar spectral microscope has demonstrated high resolution discrimination of tissue components in paraffin-embedded microscope slides (Figure 5) (70).

Raman endoscopic imaging is in its infancy but shows great potential for use in polyp discrimination. Limiting factors include

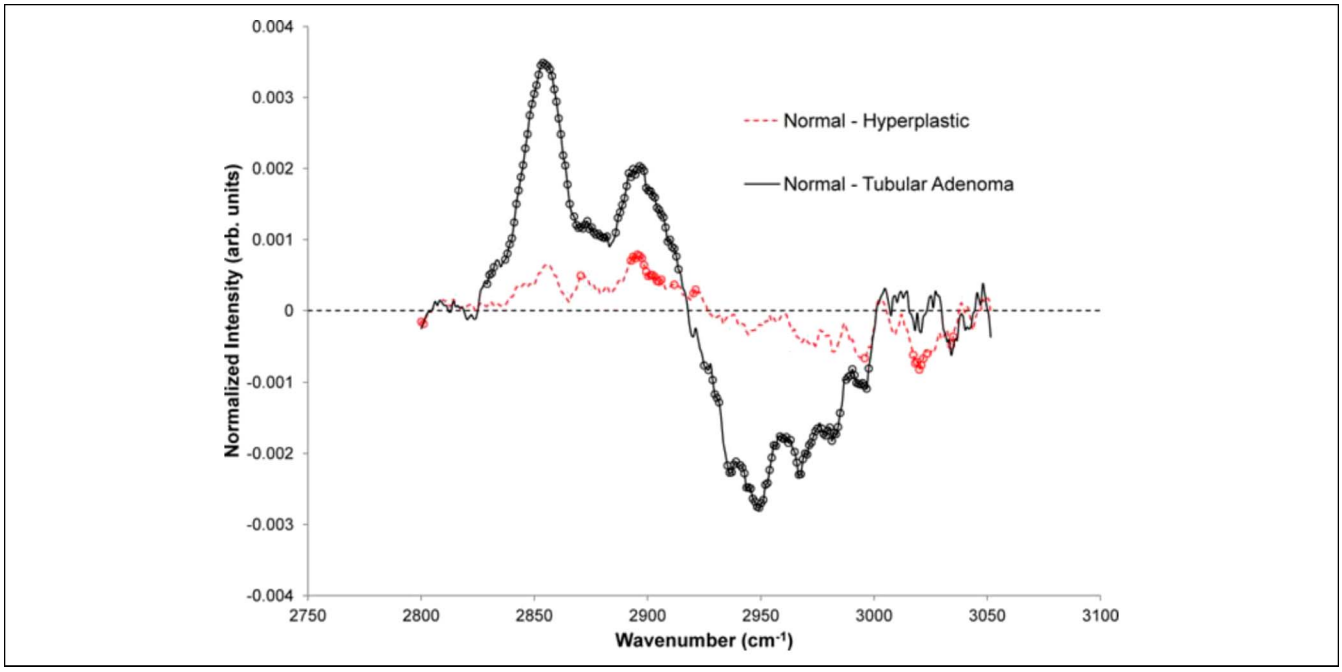

Figure 4. Average differences *in vivo* between the Raman spectra of hyperplastic (red dashed curve) and tubular adenoma (black curve) compared with normal tissue.

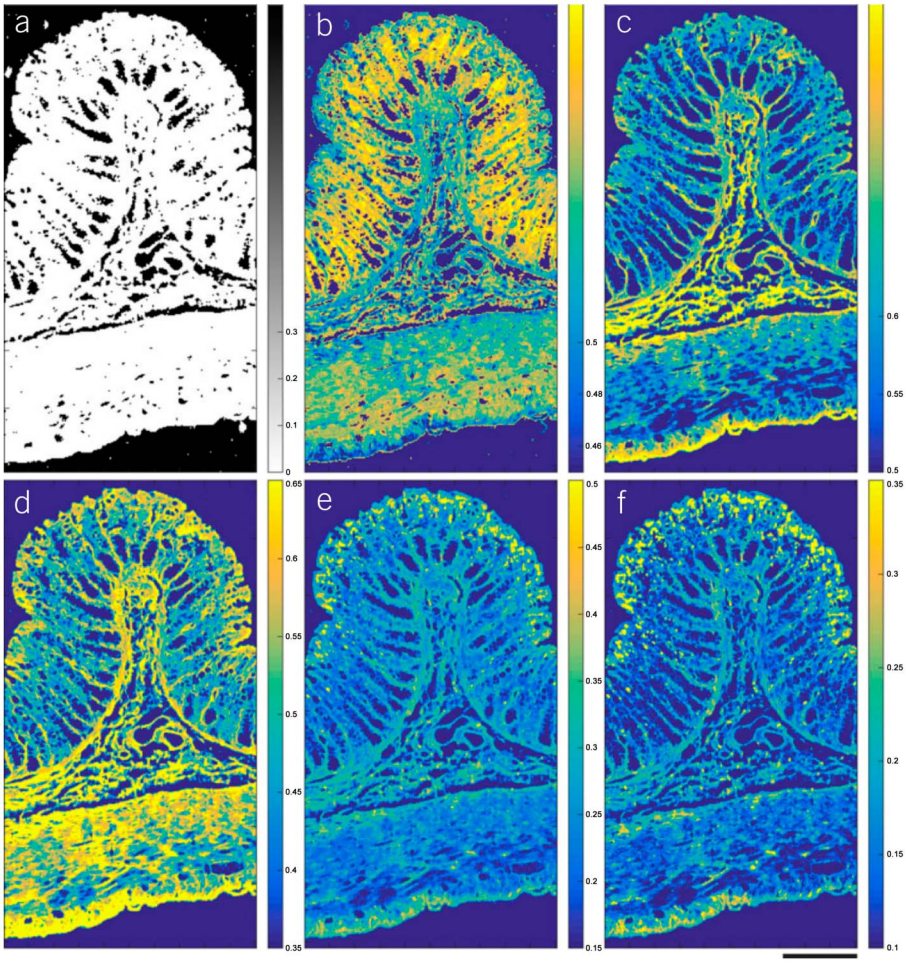

Figure 5. Maps of Raman spectral similarity in paraffin-fixed colonic tissue. Intensity indicates the similarity with the reference spectra of molecules anticipated to be present, including (**a**) background mask, (**b**) DNA, (**c**) collagen, (**d**) muscle acetone powder, (**e**) phosphatidylcholine, and (**f**) paraffin wax. Scale line is 200 μm.

the lack of miniaturized scanning devices and the long image acquisition times, up to several minutes (71).

Hyperspectral imaging

Originally developed for use in satellite imaging and geological surveys, HSI is an emerging field of medical diagnostic imaging, combining imaging with reflectance spectroscopy (72). Each pixel of an image contains information for many wavelength bands of light, rather than the red, green, and blue bands perceived by the human eye. This is expressed in Figure 6 (73). The additional spectral information may then be processed into a clinically useful format. Some current generation endoscopes (Fujifilm) include spectral estimation techniques (flexible spectral imaging color enhancement) which attempt to produce a high-contrast image from a white-light endoscopic image by enhancing the wavelengths of light associated with vascular structures and inflammation (74), True HIS endoscopy platforms are under development, and HSI has been studied in other medical applications including assessment of burns, melanoma, and cervical lesions.

Initial *in-vitro* studies of HSI and colonic polyp tissue demonstrated the ability of a hyperspectral microscope to distinguish between nuclei, cytoplasm, and lamina propria based on their spectral signatures; the resultant images were analyzed and could accurately distinguish between normal colonic mucosa, adenoma, and adenocarcinoma (75). More recently, there has been renewed interest in developing an endoscopic platform for HSI. One such system was described by Kumashiro et al. in 2016, using a fiber-optic "baby scope" through a conventional colonoscope and a hyperspectral camera to examine the resected polyp tissue. This identified significant spectral differences between normal mucosa and adenomatous tissue (76). The group also performed *in vivo* imaging and was able to produce an altered-color image, delineating areas of adenoma against normal tissue.

Another HSI imaging system described in 2016 also used a fiber-optic bundle to transmit the spectral data, inserted into the working channel of a colonoscope by a sterile sheath. This system combines the ability to record hyperspectral data and to induce tissue autofluorescence and was used to obtain images of small bowel in a porcine model, showing altered-color images of tissue surface structures, as well as a map of tissue oxygen saturation (77). Subsequently, a pilot study was undertaken in patients with colonic polyps, demonstrating the ability of the system to acquire real-time information during endoscopy. The clinical validation of this approach is ongoing.

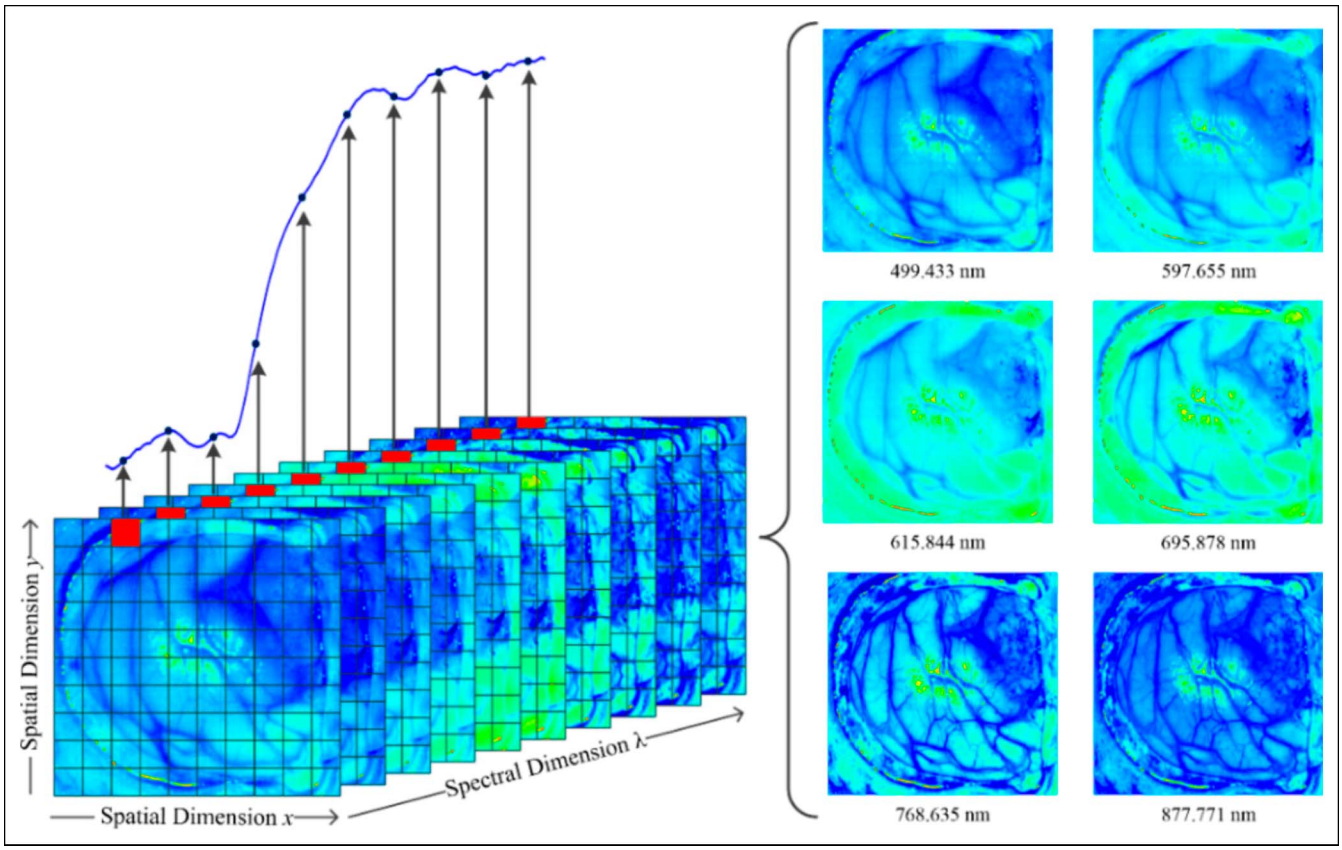

Figure 6. Illustrative example of the composition of a hyperspectral image (from the surface of the brain). The spectrum shown is obtained from the pixel highlighted in red. Multiple images will be obtained at different wavelengths (right) to compose the hyperspectral datacube. Reproduced with permission from Dr Anastasios Koulaouzidis.

Molecular imaging

Molecular Imaging is an emerging technique which uses fluorescently labeled antibodies to allow visualization of the biochemical processes taking place within tissue at a cellular level. This has the potential to provide precise, real-time diagnostic information about the detected lesions.

For the purposes of evaluating polyps, it is desirable to use antibodies directed against markers present on dysplastic tissue or against factors expressed by such tissues (78). For colonic polyps, epitopes of mutations in antigen-presenting complex, K-RAS, and p53 genes can be labeled (79) as can upregulated factors including cathepsin B, epidermal growth factor receptor (EGFR), vascular endothelial growth factor (VEGF), and carcinoembryonic antigen (CEA) (80–84).

In 2002, Keller et al. (81) described the topical application during colonoscopy of a fluorescein-labeled anti-CEA antibody and demonstrated that it was possible to induce fluorescence from adenomas. There have not, to date, been follow-up studies examining the diagnostic accuracy or use of this approach. Another target for cellular labeling is cathepsin B, a protease known to be upregulated in adenomatous tissue. This was investigated in 2002 by Marten et al., (80) who demonstrated that adenomas had high uptake of a labeled cathepsin B fluorophore, which could therefore be used to fluorescently label adenomatous tissue.

Further studies of molecular imaging have typically used fluorescent labeling in conjunction with a high-resolution photonics imaging techniques, such as OCT or CLE. A study by Yuan et al. (82) used a carbohydrate (α-l-fucose)-based fluorophore and performed OCT on adenomas in resected tissue from a mouse model. This demonstrated that fluorescent labeling of polyps in tissue is feasible and has the capability to produce high-resolution images with greater contrast than unlabeled OCT. Iftimia et al. (83) developed a labeled antibody to the integrin receptor, which is overexpressed in adenoma cells. This was administered topically to colon tissue in a mouse polyposis model, and the resected colons were examined using OCT. This approach also demonstrated increased fluorescence from abnormal tissue, with the potential to produce an enhanced OCT image.

The growth factors implicated in tumor progression are also potential targets for fluorescent labeling, and in 2010, Winkler et al. (84) produced a study in live mice, investigating the OCT images of polyps labeled with a VEGF antibody. This was delivered topically by colonic lavage, and the areas of high fluorescence correlated with histologically proven adenoma. In the same year, Foersch et al. (85) also studied VEGF molecular targeting using CLE to examine tissue from mouse polyposis models, xenograft models, and in surgical resections containing colorectal cancer. Strong fluorescent signal was achieved from areas of the polyp. These studies further demonstrate the potential for molecular imaging as an adjunct to advanced photonics microscopy techniques.

Another growth factor under active investigation is the EGFR, which may be overexpressed in human colonic neoplasms. Goetz et al. (86) have studied the anti-EGFR antibody cetuximab and

shown EGFR-specific fluorescence under CLE in tumors, correlating with the degree of EGFR expression. The same group has also demonstrated correlation between the intensity of fluorescence detected in mouse models and the progression of tumors (87).

In-vivo molecular-labeled imaging is a potential method of highlighting abnormal tissues, which could have great implications for the polyp detection. Early animal studies have demonstrated that labeled anti-CEA produces bright fluorescence from pancreatic and colonic tumors. The effect appears within 30 minutes of administration and could represent a useful adjunct to endoscopic polyp detection (88).

The role of molecular imaging is still emerging. It is an exciting technology, which may enable the development of a "molecular beacon" to detect and diagnose abnormal tissues in the colon. Potential limitations of the technology include its cost, a long half-life after injection of the molecular probe, and concerns about the potential for the labeled antibodies to induce immunogenicity.

DISCUSSION

An exciting era of endoscopy is developing with the emergence of several advanced optical technologies which show the potential for making accurate tissue diagnosis *in vivo*. This raises the possibility of an accurate optical biopsy, which could fundamentally change the way in which small colonic polyps are managed. Current recommendations suggest that a suitably accurate diagnostic method could allow the consideration of a "resect and discard" approach, in which low-risk polyps are diagnosed *in situ* and discarded without undergoing full histological analysis (89). This approach relies on a high sensitivity for adverse histology to avoid the possibility of misdiagnosing a diminutive cancer (90).

The technologies discussed in this article are relatively new and are at different stages of development; therefore, little analysis of their clinical effectiveness exists. CLE was reviewed by the ASGE as part of its review of real-time endoscopic prediction of polyp histology and 6 studies were considered informative. The variability and heterogeneity of these studies meant that meta-analysis could not be performed, although it is noted that CLE does show potential for accurate characterization of polyps (89). To date, the other advanced imaging techniques discussed in this article have not been widely studied in humans, but the animal model studies, the *ex vivo* human studies, and the early *in vivo* human studies demonstrate huge potential for near-histological quality images of tissue to be obtained. Further advances in miniaturization and the production of endoscopically compatible systems will allow studies to include real-time analysis of tissue in patients recruited prospectively, using histology as the reference standard. Robust studies are required, but this approach will ultimately characterize the clinical role of novel endoscopic imaging techniques.

Challenges facing the progress of virtual histology include the potential procedure delays associated with scanning of polyps; any system would have to be simple and easy to use to gain widespread acceptance from clinicians. A further consideration is the interpretation of virtual histology images. Although the learning curve of image interpretation has been shown to be fast, it is a new and unfamiliar skill to most endoscopists (24). The growing field of computer-aided diagnosis could help overcome this limitation by suggesting the correct interpretation of an image (52), although for accurate diagnostic results the image recognition algorithms require greater amounts of image data than are currently available for these new technologies.

The intended benefits of adopting an optical biopsy strategy include reduction of histology costs and laboratory burden, as well as avoiding the harm associated with unnecessary polypectomy, and allowing the patient to be reassured about low-risk polyps at the time of their endoscopy, rather than waiting for results. The technologies discussed above show the potential to achieve these aims. Further studies defining suitable conditions for their use will allow them to make a significant contribution to endoscopic practice.

CONFLICTS OF INTEREST

Guarantor of the article: Ben Glover, BM, BSc, MRCP.
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