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Published in:
Gastroenterology

DOI:
10.1053/j.gastro.2018.01.070

Publication date:
2018

Gastroenterology 2018;154:1876–1886

Novel Developments in Endoscopic Mucosal Imaging



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Endoscopic techniques such as high-definition and optical-chromoendoscopy have had enormous impact on endoscopy practice. Since these techniques allow assessment of most subtle morphological mucosal abnormalities, further improvements in endoscopic practice lay in increasing the detection efficacy of endoscopists. Several new developments could assist in this. First, web based training tools could improve the skills of the endoscopist for enhancing the detection and classification of lesions. Secondly, incorporation of computer aided detection will be the next step to raise endoscopic quality of the captured data. These systems will aid the endoscopist in interpreting the increasing amount of visual information in endoscopic images providing real-time objective second reading. In addition, developments in the field of molecular imaging open opportunities to add functional imaging data, visualizing biological parameters, of the gastrointestinal tract to white-light morphology imaging. For the successful implementation of abovementioned techniques, a true multi-disciplinary approach is of vital importance.

Keywords: Endoscopy; Computer Aided Detection; Molecular Imaging.

Over the last decades, endoscopic imaging has evolved from fiberoptic endoscopes to video endoscopy. Important advancement in imaging quality has taken place with the introduction of charge-coupled devices and more recently complementary metal-oxide-semiconductors in current state-of-the-art endoscopes. This has resulted in the introduction of high-resolution high-definition (HD) endoscopy systems that offer a spatial resolution of less than 10 μm. This allows high-quality imaging for detection and evaluation of most subtle morphologic abnormalities of the mucosa. In addition, these systems can be equipped with zoom endoscopes, with magnification abilities up to more than 120 times, and near focus, allowing detailed visualization of the mucosal and vascular patterns of areas of interest throughout the gastrointestinal tract.

In addition, technical improvements in optics and digitalization have led to the development of novel endoscopic enhancement techniques (such as, optical chromoendoscopy) (see Figure 1). Currently, all major endoscopy companies have developed their own version of optical chromoendoscopy. In general, these techniques can be divided in so called *pre*-processing or *post*-processing techniques. In pre-processing techniques, such as narrow band imaging (Olympus, Tokyo, Japan), or Blue Laser Imaging (Fujifilm, Tokyo, Japan), the mucosal imaging is enhanced by using blue light that only penetrates superficially into the tissue and causes less scattering.[1,2] In addition, blue light encompasses the maximum absorption wavelength of hemoglobin, which results in better visualization of vascular structure. Post-processing techniques, such as Fujifilm intelligent chromo-endoscopy (Fujinon) and i-SCAN (Pentax, Toyko, Japan), use standard white light excitation.[3,4] A proprietary algorithm reprocesses the reflected image, resulting in different image settings that can be utilized for different indications. Most often, pre-processing techniques have a better signal-to-noise ratio, resulting in images with a higher resolution and brightness compared with post-processing techniques.[5] In addition, pre- and post-processing techniques can be combined (eg, Optical Enhancement [Pentax]).

Conventional mucosal enhancement techniques using topical administrations of dyes (eg, chromoendoscopy) are generally considered as cumbersome procedures that are

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Abbreviations used in this paper: CAD, computer aided diagnosis; CLE, confocal laser endomicroscopy; FME, fluorescence molecular endoscopy; HD, high definition; HIS, hyperspectral imaging; i.v., intravenous; NIR, near infrared; OCT, optical coherence tomography; SSAP, sessile serrated adenomas/polyp; TCE, tethered capsule endomicroscopy; VLE, volumetric laser endomicroscopy.

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0016-5085
https://doi.org/10.1053/j.gastro.2018.01.070

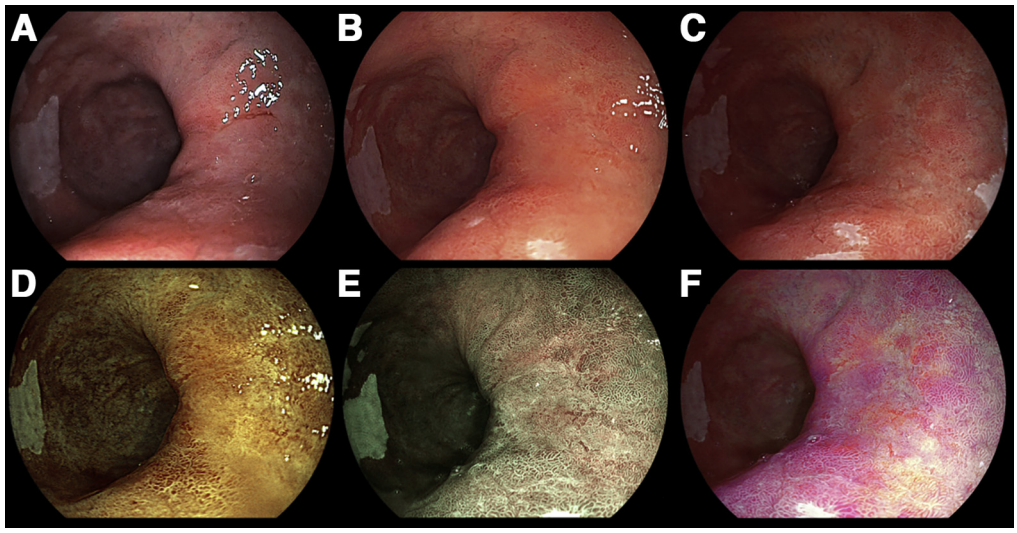

Figure 1. Images of flat (type 0-IIb) early carcinoma in Barrett's esophagus obtained with different generation endoscopes (Fujinon): standard video endoscope (EG-530) white light image (*A*), first generation HD endoscope (EG-600) white light image (*B*), Fujifilm intelligent chromo-endoscopy image (*D*), the new generation endoscope (EG-760) white light (*C*), Blue Laser Imaging (*E*), and Linked Color Imaging (*F*).

highly operator dependent with respect to the correct application of dyes and interpretation of the images. Optical chromoendoscopy, on the contrary, can be easily switched back and forth between white light imaging and optical chromoendoscopy by a simple push on a button off the endoscopic handle, providing a reproducible image each time.

The widespread availability, easy applicability, and reproducibility of these mucosal enhancement techniques will ultimately lead to the replacement of conventional chromoendoscopy by optical chromoendoscopy for most indications. This implementation should be guided with proper research because Preservation and Incorporation of Valuable endoscopic Innovations thresholds have not (yet) been surpassed for optical chromoendoscopy in several indications. Although for certain applications it will be difficult to provide scientific proof (ie, delineation), optical chromoendoscopy is here to stay.[5–7]

In the past, several other interesting and potentially promising advanced endoscopy techniques have been proposed for improving endoscopic detection and/or classification (eg, spectroscopy, endocytoscopy, confocal laser endomicroscopy [CLE], autofluorescence imaging). However, most of these techniques have not been implemented into general endoscopy practice.

In this review we will describe the most recent developments in endoscopic imaging that have the potential to change endoscopy in the near future, and envision the next steps and challenges in endoscopic mucosal imaging.

Training Modules

Background

As previously mentioned, current imaging quality with superimposed optical chromoendoscopy provides the endoscopist with high-resolution imaging containing far more details than ever seen in endoscopy. This means that all morphologic information for the detection and differentiation of subtle abnormalities is present in the provided images, it only has to be recognized. As such, in most cases, the endoscopist has become the rate-limiting step in endoscopic imaging for detection of lesions and interpretation of results. Therefore, the challenge in further improving endoscopic imaging in the near future lies not in improving the actual visibility of lesions by new imaging technology, but in creating training tools to help the endoscopist to recognize subtle clinically relevant lesions.

Current Status and Future Applications of Training Modules

Recently, the International Working Group on the Classification of Oesophagitis has developed an interactive Web-based teaching tool for the detection and delineation of early neoplastic lesions in Barrett's esophagus.[8] High-quality HD videos were obtained from patients with early neoplasia and from non-dysplastic patients. Three experts independently delineated lesions off-line frame-by-frame at 1-second intervals throughout all videos using specifically developed software. The experts were blinded for each other's delineations. To validate this training tool, 189 assessors (ranging from trainee to junior and senior endoscopists) from Northern America and Europe scored 4 sets of videos. The order of sets and the order of videos within each set were randomized for each assessor. After each set, assessors were guided through mandatory, tailored feedback based on their scores and the experts' delineations. Detection and delineation scores significantly increased over the 4 sets, with no significant differences between trainees, juniors, and seniors. This study showed that interactive structured Web-based training tools can play an important role in improving endoscopic quality. Such tools enable exposure to a vast amount of examples, with structured feedback hereby increasing experience in endoscopic recognition much more quickly than in the past. In the future, such learning tools could be integrated into endoscopy systems with real-time delineation and

direct feedback from a computer algorithm, based on large database of endoscopic images (see below). National endoscopic societies, health care programs, and governments should start working on and investing in such standardized training programs.

Volumetric Laser Endomicroscopy

Background

Volumetric laser endomicroscopy (VLE), which is based on optical coherence tomography (OCT), is a novel advanced imaging technique that is able to perform a complete scan of the esophagus wall, including subsurface layers, with a resolution comparable to low-power microscopy.[9] OCT works analogous to ultrasound, utilizing light waves instead of sound waves to form 2-dimensional images based on differences in optical scattering of tissue structures. With the development of second-generation OCT, it is now possible to perform high-resolution, high-speed acquisition of large luminal surfaces, and ultimately creating 3-dimensional imaging of the esophagus wall.[10] VLE provides a 6-cm long circumferential scan of the esophagus with a depth of 3 mm in 96 seconds. VLE, therefore, has the potential to improve the detection and delineation of early neoplastic lesions in Barrett's esophagus, improving the cost-effectiveness of Barrett's surveillance by eliminating the problem of sampling errors of random biopsies.

Current Status and Future Applications of VLE

To investigate the potential of VLE and validate results for detection of early Barrett's neoplasia, it is important that structures identified on VLE can be correlated with histology. Recently, a unique VLE–histology image database was created; aligning VLE data one-to-one with freshly cut endoscopic resection specimens.[11] Based on this database, VLE prediction score was developed showing a sensitivity and specificity for early neoplasia of 83% and 71%, respectively.[12] One other recently published study also using endoscopic resection specimens as gold standard, though a less stringent VLE pathology correlation protocol and used a slightly different 'diagnostic algorithm,' reported a sensitivity and specificity of 86% and 88%, respectively, for scoring early neoplasia.[13] Both studies used ex vivo data of fresh endoscopic resection specimens as gold standard, which may have several limitations, such as resection artefacts and lack of vascularization. Therefore, in vivo scans may provide different information than ex vivo still images. Hence, validation of VLE features and the VLE prediction score for neoplasia in larger in vivo datasets is mandatory. In addition, current results are promising but do not exceed the Preservation and Incorporation of Valuable endoscopic Innovations criteria that have been developed by the American Society of Gastrointestinal Endoscopy.

Recently, a VLE laser marking system has become available that is capable of applying VLE-guided superficial temporary cautery marks on the esophageal mucosa.[14] The marked area can be located accurately on the esophageal surface using white light endoscopy, allowing the endoscopist to obtain reliable correlated histologic samples. Furthermore, laser marking could facilitate and improve endoscopic treatment by aiding in lesion delineation.

Nevertheless, during explorative studies in in vivo scans, it was found that because of the large amount of data consisting of subtle gray-scales, VLE interpretation was complex and will likely remain challenging for the human eye,[15] especially if an endoscopist has to scrutinize a large full VLE scan for quick, real-time diagnosis during endoscopy. Objective tools such as automated computer-aided analysis would be a valuable aid in VLE interpretation.[16] For further details see below.

An alternative could be tethered capsule endomicroscopy (TCE), a small device incorporating the same technology as VLE but in the size of a capsule that is swallowed by the patient, which images the entire esophagus during descending and during pull-back of the tether.[17] TCE provides a 3-dimensional scan of the entire esophagus without the need for endoscopy. This non-invasive, low-cost device can be operated by a nurse or in a general practitioner's setting.[18] A TCE computer algorithm could indicate the (risk of) presence of disease in the scan (ie, Barrett's esophagus, neoplasia, eosinophilic esophagitis); subsequently, patients testing positive on the TCE scan would be referred for upper endoscopy.

Computer Aided Detection

Background

More and more, the growing abundance and complexity of medical data exceeds the human capability for analysis. In some cases, it is the sheer volume of the data that renders it infeasible for manual processing, while in other cases the data can be too complex, such that patterns that indicate disease elude the human eye. As previously mentioned, a potential tool that may facilitate the improvement of endoscopic detection and classification in the near future is computer-aided detection (CAD) and computer-aided diagnosis (CADx). Exploiting the ample computational power provided by modern-day computers, machine learning offers a technique to automatically recognize informative patterns in large sets of data.

A typical CAD algorithm (sequence of steps that is followed by a computer) consists out of several stages, like that schematically depicted in Figure 2. First, it can be necessary to prepare the data for adequate analysis in a so-called pre-processing stage. For instance, in CAD systems for endoscopic video, it is beneficial to first identify and remove reflections because they might impede further analysis. Next, descriptive information is extracted from the data that enables discrimination between different cases of interest. This information is captured in so-called "features." For endoscopic images, these features typically aim at capturing the shape, color, and texture of the image content. Features represent this information by numbers, meaning a set of numbers is computed for each data sample, which

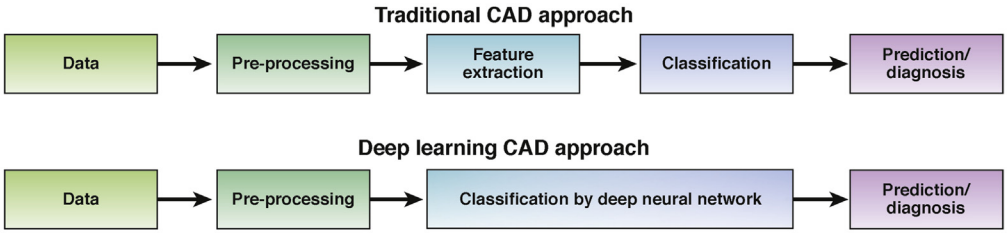

Figure 2. General sequence of stages followed by a CAD algorithm. *Top*: traditional approach. *Bottom*: deep learning approach, which has enjoyed a large wave of renewed interest over previous years.

can be a small part of an image, for example. Clinicians can greatly contribute to the design of such features by providing visual clues for analysis, leading to an optimal CAD performance.[16,19] Finally, given these features for a large set of data points, a machine learning algorithm exploits advanced mathematical optimization methods to *learn* a model that best fits the available data. Once computed, this model can be used to predict aspects of new samples based on its corresponding features; for example, specific color and texture patterns captured by these features might indicate disease.

Fueled by the exponential increase in computational power and the vastly increasing amount of data, deep learning has emerged as an alternative approach to the traditional machine learning methods. This technique uses an artificial neural network of multiple layers to simultaneously learn the most discriminative features *and* the optimal classifier, blurring the distinction between these 2 stages (see bottom diagram of Figure 2). Because of the enormous amount of data that is typically required to train these deep neural networks – millions of examples – its application for CAD was initially limited. However, recent developments have shown that the knowledge contained in these networks can be transferred between different application domains (referred to as *Transfer Learning*). Transfer learning offers a way to address the lack of sufficient training data in the medical domain, by re-using networks trained on large data sets from other domains (such as the popular ImageNet database,[20] containing millions of categorized images of, for example, animals, food, buildings, and tools). By tuning these pre-trained networks with medical data, promising results can be achieved. Two studies performed by Tajbakhsh et al[21] and Shin et al[22] demonstrated the broad applicability of transfer learning by successfully using it for polyp detection in colonoscopy videos, pulmonary embolism identification in computed tomography, and boundary detection in ultrasound images for carotid intima-media thickness estimation. We expect that transfer learning will be a very useful tool for moving forward CAD systems for endoscopy.

Like the enhanced visualization introduced by optical chromoendoscopy and training of endoscopists in recognizing subtle lesions, CAD could also offer some powerful additions to the toolset of the endoscopist. One major advantage of such a system is that it can yield an objective second opinion during endoscopic surveillance, generating the exact same prediction, regardless of the endoscopist that carries out the procedure. Especially so because studies have shown a considerable intra- and inter-observer variability, even among expert endoscopists.[23,24] In other fields, similar quantitative approaches have shown to be very useful for addressing this variability, using a CAD system as an additional reader.[25–28]

Another notable advantage of CAD is that it can quickly process very large amounts of data. This allows CAD algorithms to learn from a huge amount of examples, far exceeding any number of training cases that human observers encounter in a lifetime. Additionally, a CAD algorithm does not experience fatigue or lack of concentration.

Moreover, given the ongoing developments in imaging sensors, novel types of data can be endoscopically collected. For example, a large number of specific wavelengths, even outside the visible spectrum (hyperspectral imaging [HSI]) with, for instance, addition of molecular targeted fluorescent tracers or structural information of the submucosal layers (OCT). While these new modalities potentially enhance diagnosis by yielding additional information, the acquired signals are growing in complexity and we expect that their interpretation will be unfeasible for human observers. CAD systems provide an attractive solution here, performing an initial analysis on the raw signal and providing a compact visualization that enables the best real-time interpretation by the endoscopist.

Current Status and Future Applications of CAD in Endoscopy

The first CAD algorithms for endoscopic video analysis were proposed over a decade ago,[29–31] mainly focusing on the detection of colorectal lesions. Factors such as better imaging quality, more processing power, and larger data sets have led to a considerable improvement in the success of automated polyp detection for white-light colonoscopy.[32] Furthermore, also for the automatic selection of interesting wireless capsule endoscopy frames, a large range of algorithms have already been proposed; however, only marginal progress is reported regarding their clinical implementation.[33]

For the classification of colonic polyps, a wide variety of CAD systems have been proposed. These systems typically exploit the pit-patterns identified by Kudo et al,[34] to develop discriminative features for the analysis of magnifying endoscopy in combination with narrow band imaging[35–39] or iScan.[39] Although 2 of these studies reported a diagnostic concordance between the CAD system and the experts exceeding 97%, a 2010 study showed that human observers still demonstrate a superior performance.[37] However, a recently proposed CAD approach for characterization of

colorectal lesions by Misawa et al,[40] for the analysis of narrow band imaging endocytoscopy images, has demonstrated very promising results, meeting the Preservation and Incorporation of Valuable endoscopic Innovations criteria for high-confidence predictions. This result was achieved with a relatively straightforward CAD approach, fueling the expectation that further improvements may soon facilitate the adoption of "diagnose-and-leave" and "resect-and-discard" strategies, even for novice endoscopists.

In a recent study, Quang et al[41] developed an algorithm for the automated interpretation of high-resolution microendoscopy image, aiming to detect squamous cell carcinoma. While the algorithm demonstrated a sensitivity and specificity of 95% and 91%, respectively, the authors also implemented their approach on a tablet, on which it could run in real-time. This latter aspect is very appealing to bridge the gap to clinical practice. Moreover, because squamous cell carcinoma primarily affects patients in developing countries, a low-cost solution is key to its clinical applicability. Likewise, a system for automated interpretation of high-resolution microendoscopy images for the detection of esophageal adenocarcinoma has been proposed.[42] Although limited by the slow execution time and the small field of view of the high-resolution microendoscopy system, the algorithm identified neoplastic lesions in Barrett's esophagus with a sensitivity of 88% and a specificity of 85%. Recently, a CAD system for the analysis of white-light HD endoscopic images in Barrett's patients achieved a comparable performance, with a patient-level sensitivity and specificity of 0.86 and 0.87, respectively.[43] In addition, this method also produced annotations of the suspected lesions in the images (see Figure 3); when compared with experts, the system performed considerably worse in determining the boundaries of the lesion. However, as an add-on detection tool, it may have a far greater impact on endoscopic practice.

One of the most promising emerging applications for CAD is the analysis of signals that are not easily interpretable by human observers. For example, as the previously mentioned VLE system, these high-resolution, grayscale OCT images may contain subtle grayscale variations that are hard for clinicians to identify by visual inspection, moreover, given the large amount of images to be analyzed. For this reason, Swager et al[16] have investigated an algorithm for the automated analysis of VLE scans, demonstrating a considerable detection rate compared with human observers (a maximal area under the curve of 0.95 vs 0.81, respectively). Furthermore, the algorithm classified VLE frames in the order of milliseconds, enabling the analysis of a full scan (1200 frames of 4096 $\times$ 2048 pixels) in under a minute. Using a similar image acquisition method, with a tethered capsule for capturing the VLE signal, Ughi et al[44] presented a CAD system, automatically segmenting and characterizing the esophageal wall, while discriminating between normal squamous and Barrett's tissue.

Another modality that captures more data than could be easily visualized for a human observer is HSI. Instead of 3 color bands of the visual spectrum, HSI divides the electromagnetic spectrum in many more bands and is not

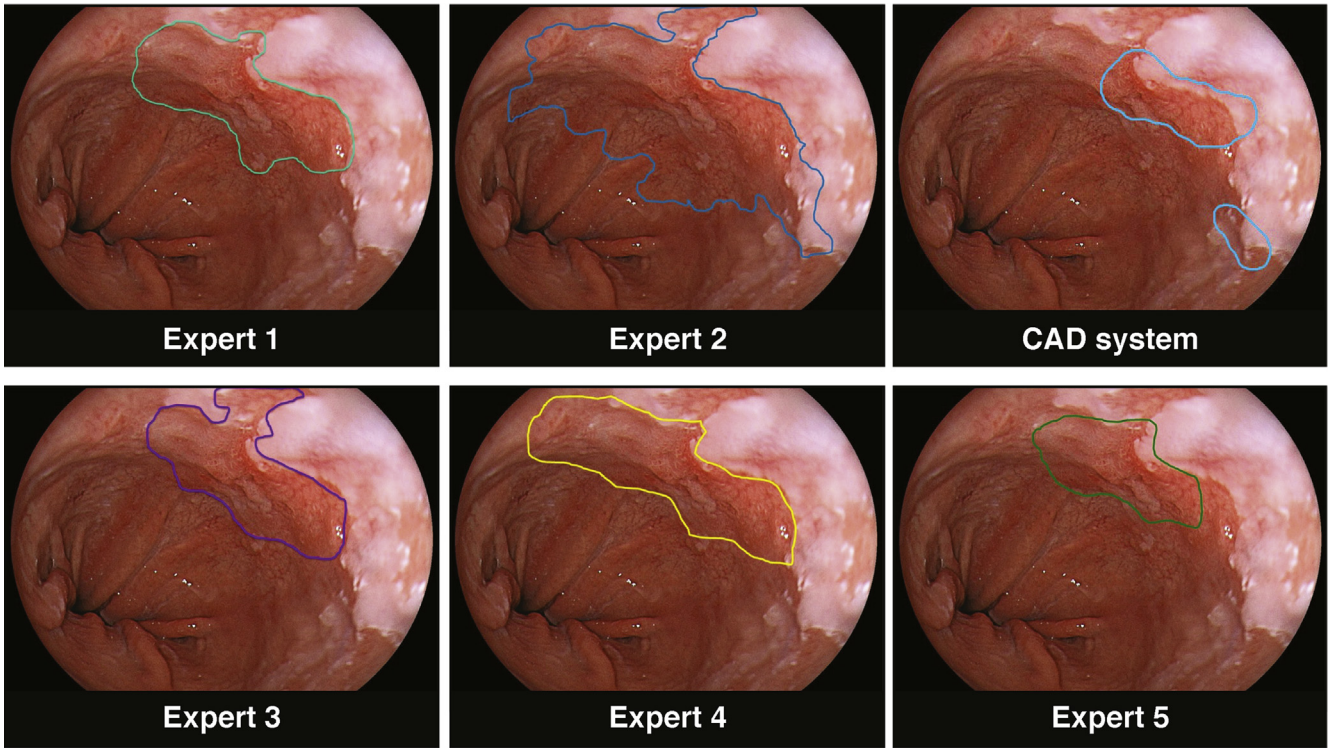

Figure 3. CAD annotation (*top right*) of an early cancerous lesion in Barrett's esophagus compared with the annotations of 5 experts on Barrett cancer.

limited to the part that is visible to humans. This imaging modality is potentially a very useful tool to distinguish between normal and cancerous cells.[45] Because the architecture of these neural networks can naturally deal with images that have more than 3 color channels, we believe convolutional neural networks offer a very attractive technique for HSI image analysis.

Pitfalls and Challenges for Implementation of CAD in Endoscopy

Although the presented results of the majority of the feasibility studies described are promising, none of these algorithms have thus far penetrated to clinical practice. This observation can be attributed to a number of challenges that need to be overcome for the field to move forward. First, most algorithms are evaluated on relatively small datasets, with very specific inclusion criteria, that do not reflect the conditions encountered during endoscopy and are, therefore, prone to selection bias. Second, an annotated ground truth is required for the final evaluation of an algorithm, which are very laborious and costly to produce. Finally, the interaction between the clinician and a CAD system is not optimal. Currently, 2 distinct lines of research are co-existing, but hardly cooperating. A joint effort between clinicians and engineers in a true multi-disciplinary approach is essential for the development and implementation of clinical CAD systems.

Molecular Imaging

Background

Another evolving field within endoscopic imaging is the molecular imaging approach. Molecular imaging targets "highlights" areas of disease based on pathologic characteristics, such as enhanced intracellular processes, changes in vascularization, up-regulation of excreted cytokines or peptides, or increased receptor expression on cell membranes in the inflammatory or tumor microenvironment (Figure 4). In the clinic, the most used molecular imaging technique is positron emission tomography, which most often uses fluordesoxyglucose to visualize enhanced glucose uptake and increased metabolism in tumors, dysplastic tissue, and inflammatory lesions. Recently, real-time optical molecular imaging that can be used during surgical and endoscopy procedures have been developed.[46–51] Whereas positron emission tomography relies on radioactive compounds, these optical imaging methods use fluorescent light to visualize biological processes in which fluorescent dyes absorb light with specific wavelengths and subsequently emit fluorescent light in a longer wavelength.[52] Fluorescent dyes can be attached or incorporated in, for instance, small molecules, affibodies, antibodies. or small peptides.

Fluorescence molecular imaging is most commonly performed using dyes in the visible light spectrum (wavelength 300-700 nm) or in the near infrared (NIR) (wavelength 700-900 nm) range. NIR wavelengths

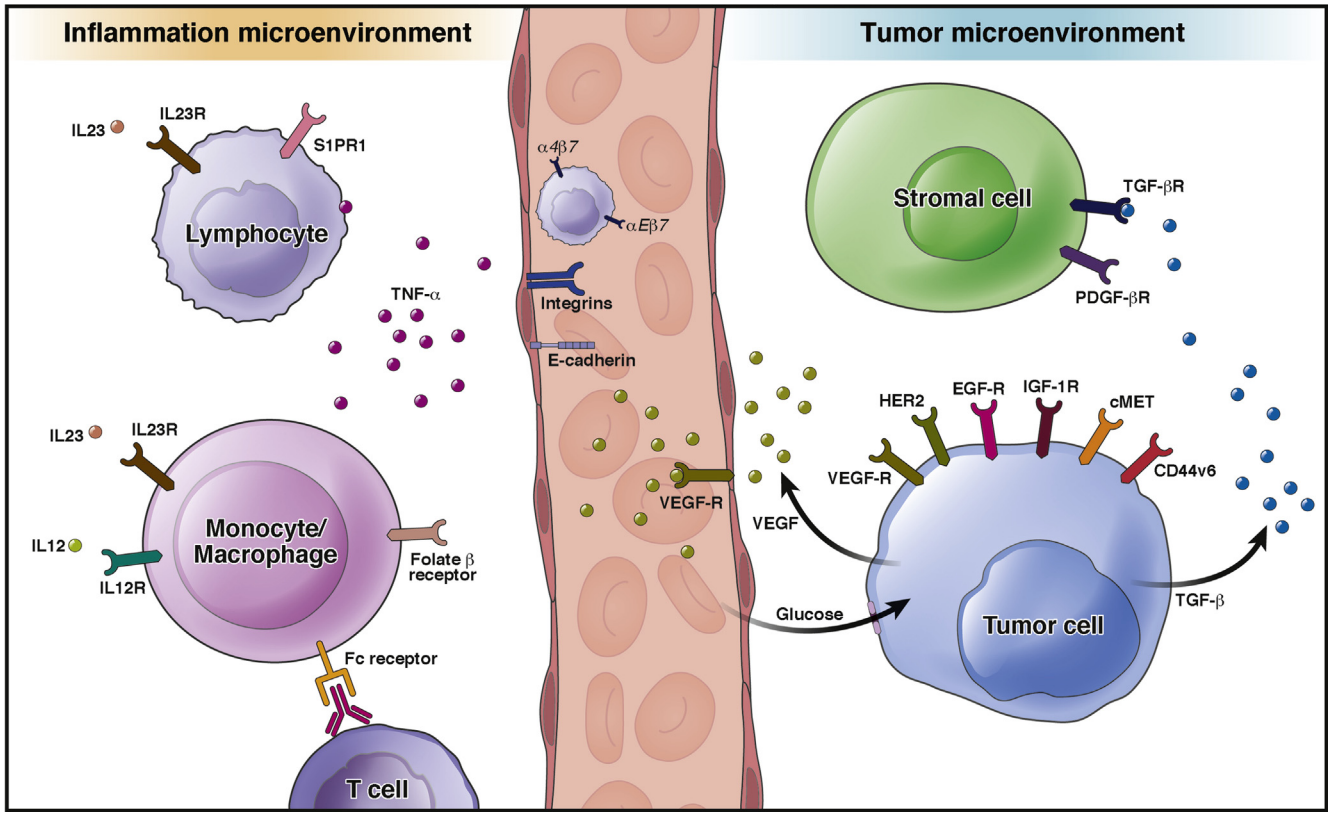

Figure 4. Overview illustration of potential molecular imaging targets in the microenvironment of inflammation and tumor tissue.

penetrate deeper into the target tissue and are less subjected to interference by hemoglobin absorption, autofluorescence signals, and tissue scattering.[53] However, NIR fluorescence leads to significant technical challenges in the design of clinical endoscopes for the detection of fluorescence.

To date, fluorescence molecular endoscopy (FME) has been evaluated following intravenous administration (i.v.) and topical administration of the fluorescent tracers. Most likely, i.v. administration results in more specific and homogeneous accumulation of the tracer in the target area compared with topical administration, no mucus barriers needed to be passed. However, i.v. administration requires an interval between the administration of the tracer and the FME procedure. This interval is dependent on the pharmacokinetic profile and the pharmacodynamic properties of the tracer. Moreover, in most cases, dose escalation studies are necessary to identify the optimal dose for novel tracers to maximize target uptake with minimal background uptake.

In contrast, topical administration of fluorescent tracers is easy, fast, and comparable to, for example, administration of indigo carmine during chromoendoscopy or lugol staining. Challenges include homogeneous tracer distribution and non-specific binding of the tracer to mucus. Therefore, this approach seems feasible for the esophagus and stomach but remains a challenge in the colon.

As with CAD imaging, molecular imaging could offer powerful additional information to the endoscopist. Molecular imaging provides real-time biological information and offers supplementary data that can be superimposed over to standard morphology imaging. Because fluorescence signals are easy to recognize, it will provide the endoscopist a "red-flag" method. Moreover, software algorithms like we described for CAD could automatically alert the endoscopist and thereby even reduce the human factor, which might lead to an improved detection of, for instance, colonic polyps or dysplasia in Barrett's patients.

Current Status and Future Applications of Molecular Imaging

Despite numerous published preclinical endoscopy studies on molecular imaging, existing clinical data is limited. However, several promising translational and early stage clinical studies have shown the potential of molecular imaging for the detection of dysplasia and treatment monitoring.

Detection of Dysplasia in Barrett Esophagus

Bird-Lieberman et al[54] investigated ex vivo the use of lectins, cheap and non-toxic peptides that bind to glycans which expression alters during the progression of Barrett to esophageal adenocarcinoma, as contrast agent and demonstrated reduced binding of the fluorescent tracer in the context of dysplasia in Barrett's patients. Two papers from the Wang's group[55,56] showed the potential of topical administration of ASY-fluorescein isothiocyanate (FITC), a synthetic peptide, ASYNYDA, identified by phage display. CLE highlighted areas of dysplasia after topical administration of ASY-FITC.[55] However, CLE can only sample small areas (50 μm) and is not suitable for surveillance of an entire Barrett segment. Therefore, Joshi et al[56] used the same peptide for wide-field detection of dysplasia following topical administration. Areas of high grade dysplasia and EAC were identified with a sensitivity of 76% and 94% specificity. To optimize fluorescence signals, post-processing was used limiting real-time detection of lesions. This is partly caused by concurrent white light and fluorescence endoscopy in the visible light spectrum, which limits the sensitivity and contrast because of endogenous auto-fluorescence. Nagengast et al[57] evaluated the monoclonal antibody bevacizumab targeting vascular endothelial growth factor A labeled to a NIR fluorophore, CW800, for dysplasia detection after both i.v. and topical administration. In a small feasibility study, FME topical administration of the tracer showed a promising 33% improved dysplasia detection over HD white light endoscopy, signifying the role of angiogenesis as hallmark of cancer.

Detection of Colorectal Polyps

Detection of polyps relies, besides bowel cleaning, on specific morphologic tissue signatures and thus focus and experience of the endoscopist, which results in reported adenoma miss rates of up to 50% in high-risk Lynch Syndrome patients.[58] In the general population, especially flat and right-sided sessile serrated adenomas/polyps (SSAPs) are notoriously difficult to detect. Three recent studies have been published that address this problem. Burggraaf et al[47] reported high fluorescence uptake in all adenomas using a cMET tracer, with increased adenoma detection compared with white light imaging alone. However, fluorescence imaging and white light imaging was performed with an outdated fiber endoscope, pointing out the developmental status of this study. Hartmans et al[59] evaluated bevacizumab-800CW for the detection of polyps. In a dose escalation study in patients with familial adenomatous polyposis, NIR-FME using 25 mg of bevacizumab-800CW highlighted very small dysplastic adenomas with high in vivo fluorescent contrast at video rate (Figure 5*A*). To improve detection of SSAPs, Joshi et al[60] developed a small fluorescence peptide for detection of SSAPs. In 38 patients, specific binding to identified SSAPs could be demonstrated in vivo, with subsequent ex vivo quantification and correction methods (Figure 5*B*).

Treatment Monitoring

Next to lesions detection, FME holds great potential for assessment of treatment responses, for instance in stratification of inflammatory bowel disease patients toward targeted therapies. A proof of principle was demonstrated using fluorescently labeled adalimumab to detect mTNF-expressing cells in the gut of 25 patients with active Crohn's disease (CDAI >150).[61] During colonoscopy, fluorescent adalimumab was topically administrated on the inflamed tissue. Subsequently, mTNF-positive cells were quantified by CLE by counting the number of cells per confocal image. Interestingly, patients with a high amount of

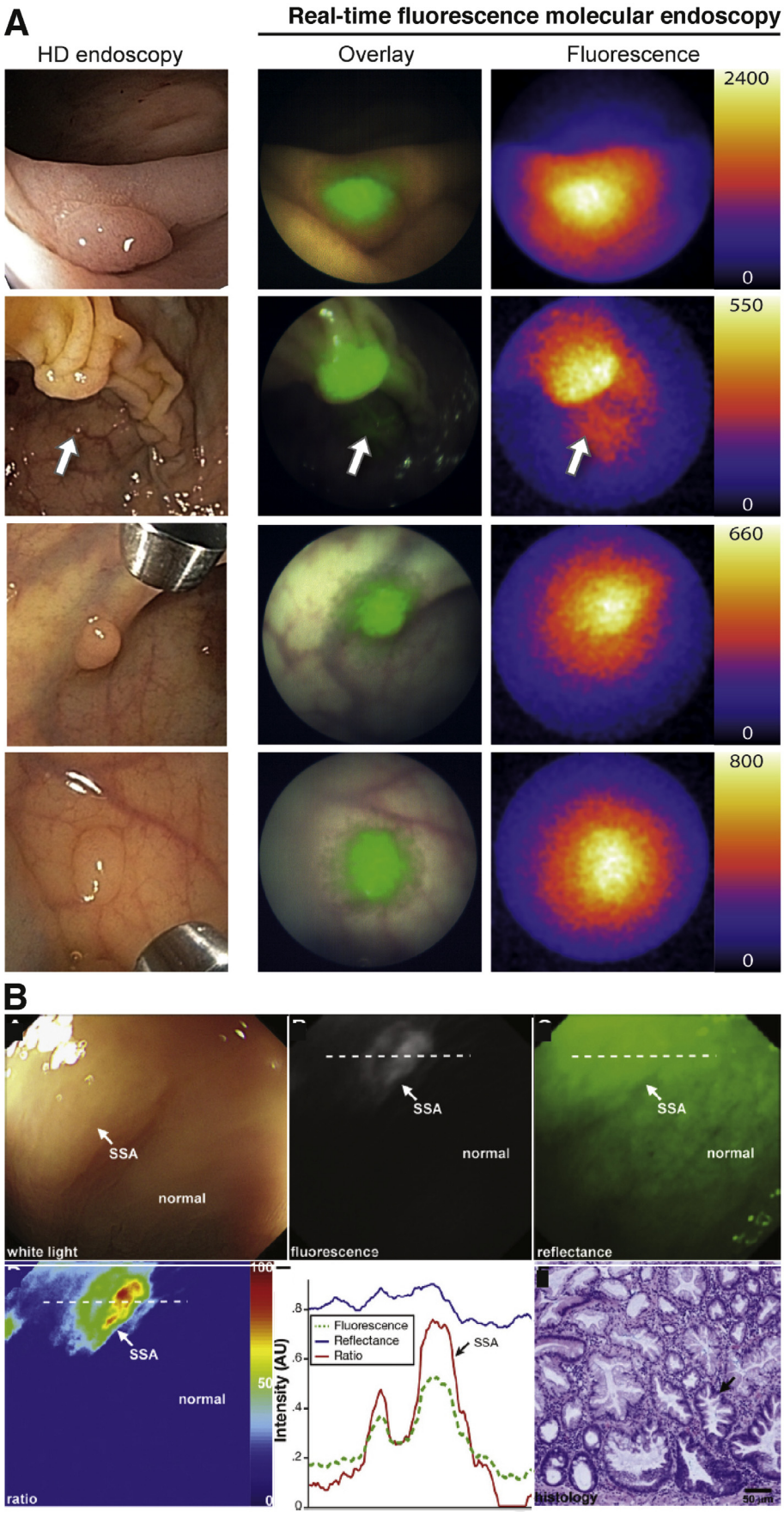

Figure 5. (*A*) The images show the ability of the near infrared fluorescence molecular endoscopy system to visualize small adenomas at video frame rate (10 frames per second). The white arrow indicates a second small adenoma. The overlay images are automatically generated by the software, showing the highest fluorescence intensities in bright green and the very low fluorescence intensities as absent. Reprinted with permission from Hartmans et al.[59] (*B*) Targeted in vivo images of flat SSA in proximal colon. (*Upper left panel*) Endoscopic image with white light illumination (Video 1) shows limited visualization of SSA (*arrow*) with flat morphology. (*Upper middle panel*) Fluorescence image (Video 2) shows increased intensity and high contrast from lesion (*arrow*), while normal colonic mucosa shows minimal background. (*Upper right panel*) Reflectance (Video 3) and co-registered fluorescence images are combined in a ratio to correct for differences in distance over the image field of view. (*Lower left panel*) Ratio image shown in pseudocolor enhances signal from SSA (*arrow*). (*Lower middle panel*) Intensities from fluorescence, reflectance, and ratio images along horizontal dashed line in (BD) show a peak at location of SSA (*arrow*). (*Lower right panel*) Corresponding histology shows pathologic features of SSA (*arrow*). Reprinted with permission from Joshi et al.[60]).

mTNF-positive cells (mean, >20 cells/focal image) had a significantly higher probability of clinical response, namely 92%vs 15%, compared with patients with low mTNF-positive cells, independent from MAYO score or C-reactive protein levels. With multiple biologicals becoming available for inflammatory bowel disease patients, this approach could offer upfront patient stratification. Moreover, when these fluorescently labeled drugs are administered intravenously, it can give detailed insight in local drug distribution and could assist in drug development by determine optimal dosing during phase I/II studies and offer us better understanding of the mechanism of action in our patients.

Pitfalls and Challenges for Implementation of Molecular Imaging in Endoscopy

The development of fluorescent tracers is costly and requires a multidisciplinary team of chemists, biologists, pharmacists, and clinicians.[62] It requires a reproducible labeling method, extensive binding, formulation, and stability studies, with often additional toxicity studies. For large-scale production, involvement of the pharmaceutical industry is often mandatory. In clinical trials, challenges lie in the standardization of operating procedures, image interpretation, and quantitative measurements. Because fluorescence signals are dependent on the optical properties of tissue, ie, scattering and absorption of light, these properties can have significant influence on fluorescent signals and, thus, implementation in the clinic. Corrections have been attempted by collecting reflectance images in addition to the white light and fluorescence images and performing multi-diameter single-fiber reflectance and single-fiber fluorescence (MDSFR/SFF) spectroscopy.[53,56,59] This latter technique can correct for the absorption and scattering of tissue and thus accurately quantify the intrinsic fluorescence signals, which is most likely essential for individualized treatment decisions.

Conclusions

The enormous improvements in endoscopic imaging quality in recent years, and the development of optical chromoendoscopy have widely impacted endoscopic practice. Because these techniques now allow assessment of most subtle morphologic mucosal abnormalities, further improvements in endoscopic practice lay in increasing the detection efficacy of endoscopists. Most likely, in the near future the development of (Web based) training tools for enhancing the detection and classification of lesions, the addition of CAD, and molecular imaging will play an important role in raising endoscopic quality. Next to education and training, CAD raises endoscopic quality of the captured data. It will aid the endoscopist in interpreting the increasing amount of visual information in endoscopic images, subsequently providing objective second reading during ongoing endoscopy. In addition, CAD will be helpful in data analyses of more advanced and complex imaging techniques, such as molecular imaging, VLE, and HSI. The addition of molecular imaging to current HD morphology imaging using targeted fluorescent tracers adds contrast and provides the endoscopist with a "red-flag" technique. This can be used not only for lesions detection, but also for the assessment of treatment responses. Integrated with CAD, it will further decrease weakness of the human factor. For the successful implementation of the abovementioned techniques, a true multi-disciplinary approach is of vital importance.

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Received September 7, 2017. Accepted January 6, 2018.

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Conflicts of interest
The authors disclose the following: WBN, the UMCG received an unrestricted research grant for molecular imaging development from surgVision BV (the Netherlands). The remaining authors disclose no conflicts.