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# Novel Imaging Techniques in Gastrointestinal Endoscopy in the Upper Gastrointestinal Tract

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Additional information is available at the end of the chapter

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## 1. Introduction

The advent of high definition endoscopy has transformed the management of pre-malignant and early malignant diseases of the esophagus and upper gastrointestinal tract. The ability to view the mucosa in detail whereby the cellular architecture can be viewed has enabled the endoscopist to make in-vivo histopathological diagnoses, which in turn will direct the management of the pathology instantly. In this chapter, we describe the various techniques available from high definition white light endoscopy, through chromoendoscopy and confocal endomicroscopy. We describe the characteristics and staging of lesions of the esophagus including Barrett's esophagus (BE) and associated esophageal adenocarcinoma. Furthermore, we describe how endoscopy can be used to define Barrett's and squamous dysplasia. Finally, we describe the classification and staging of early cancers of the esophagus and explore the role of endoscopic ultrasound. We also examine the role of emerging radiological techniques such as virtual colonography that act as adjuncts to current practice and will no doubt help to focus the expertise of skilled endoscopists towards interventional endoscopy rather than routine diagnostic procedures.

Accurate diagnosis and staging of benign and malignant lesions of the esophagus requires an in-depth understanding of current endoscopic techniques and the latest technology. The endoscopic optical technology has evolved rapidly in the last decade such that the resolution of the 'CCD' chip is up to 1.4 million pixels. The images are further enhanced by optical filters and post image processing technology allowing detailed views of the mucosal architecture. This in turn allows improved accuracy of diagnosis. We explore the roles of high definition white light endoscopy, chromoendoscopy, confocal endomicroscopy and EUS in the diagnosis and staging of esophageal neoplasia.

## 2. White Light Endoscopy (WLE)

This process has several limitations. White light endoscopy is not sensitive enough to detect neoplasia in pre-cancerous conditions in the upper GI tract. For example in Barrett's esophagus, the standard approach is to take one biopsy in every quadrant of the Barrett's segment every 1-2 cm and send for histopathological review [1]. If biopsies are taken every 2 cm, the average number per procedure is 12 and if taken every 1cm, this number will double. Even with an efficient endoscopy setup, it takes around 30 seconds per biopsy so the procedure takes up to 30 minutes to perform. It is also very time consuming for the pathologist, needing up to 30 minutes to evaluate a set of biopsies from a single endoscopy. Dysplasia and early BE neoplasia result in subtle changes that may not often be visible with WLE examination. In addition, random biopsies have significant sampling error since intestinal metaplasia and dysplasia have a patchy distribution and only a small fraction of the BE segment is sampled in this way. Even the most rigorous biopsy protocols including those using jumbo biopsy forceps survey less than 1% of the esophageal mucosa and still miss up to one third of cases with high-grade dysplasia (HGD) or early cancer [2-5].

## 3. Chromoendoscopy

The use of chromoendoscopy in the GI tract was first described in 1977 [6], and involves the topical application of stains or pigments to improve visualization of the mucosa during endoscopy. The basics of performing chromoendoscopy require only a small number of items: staining agents, spray catheters, water rinses, and mucolytic agents. There are three main types of stains that are used:

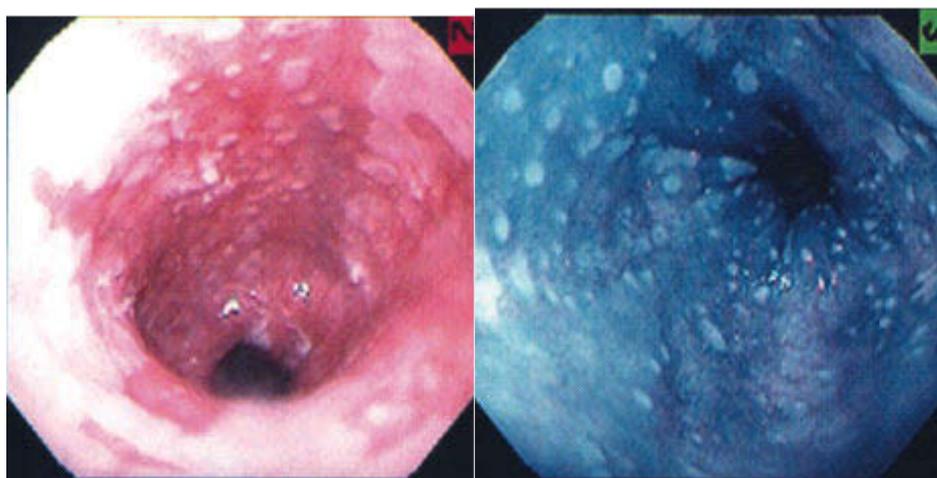
- i. Absorptive stains (methylene blue, Lugol's solution)
- ii. Contrast stains (indigo carmine, acetic acid)
- iii. Reactive stains such as congo red or phenol

There are two essential principles in chromoendoscopy: mucus removal and dye application. The former is achieved by using water, or occasionally some centers have advocated the use of a mucolytic agent; N-Acetylcysteine [7-9]. This can be achieved by flushing the agent through the working channel, using a spray catheter or even administering it as an oral solution before the endoscopic procedure. Once the mucus is cleared, the dye can then be applied.

### 3.1. Methylene Blue (MB) chromoendoscopy

MB, an absorptive dye, is probably the most investigated stain for evaluation of BE. MB is applied topically at a concentration of 0.5-1.0% and is absorbed by goblet cells present in metaplastic Barrett's epithelium. Much of the early work on MB has been performed by Canto's group [10]. The first series published in 1996 assessed 14 patients with Barrett's

esophagus and 12 control patients. Methylene blue stained specialized columnar epithelium in 18 of the 26 patients, including those with intramucosal carcinoma (1), high-grade dysplasia (1), and indefinite/low-grade dysplasia (6). The overall sensitivity of methylene blue staining for the biopsy finding of specialized intestinal metaplasia was 95%. The same group then went on to a prospective, sequence randomized, trial of MDMB versus standard surveillance endoscopy with 2cm quadrantic biopsy [11]. 41 patients were studied with each procedure performed by separate endoscopists within an interval of 3 to 4 weeks. The average number of biopsies was significantly lower with MBDB than 2cm quadrantic biopsy but the MB staining added a mean of 7 minutes (range 2 to 12 minutes) to the endoscopy procedure. Dysplasia or cancer was diagnosed in significantly more biopsy specimens (12% [12,13] vs. 6%,  $p = 0.004$ ) and patients (44% vs. 28%,  $p = 0.03$ ) by MBDB than by random biopsy technique.



**Figure 1.** Example of diffuse staining. Esophagus seen before and after staining with diffuse, uniform methylene blue staining [14].

The problems with MB in BE is that dysplastic areas do not stain. Furthermore even areas which do not harbor IM do not absorb the dye. This makes it difficult for the endoscopist to decide on which areas to target the biopsies during the procedure. There are also some issues with the uniformity of the dye. It has been examined in both long and short segment BE [12,13,15]. Two patterns of staining have been documented - diffuse and focal. Canto *et al.* [15] found that most patients with long segment BE exhibited diffuse staining, whereas Wo *et al.* [16] observed focal staining in their cohort of patients with long segment BE. Similar results have been found when examining short segment BE by Sharma *et al.* [17] who found that the majority of their patients with short segment BE stained diffusely. In contrast, in 30 patients with short segment BE assessed by Kiesslich's group [18], only 80% demonstrated staining in a focal pattern.

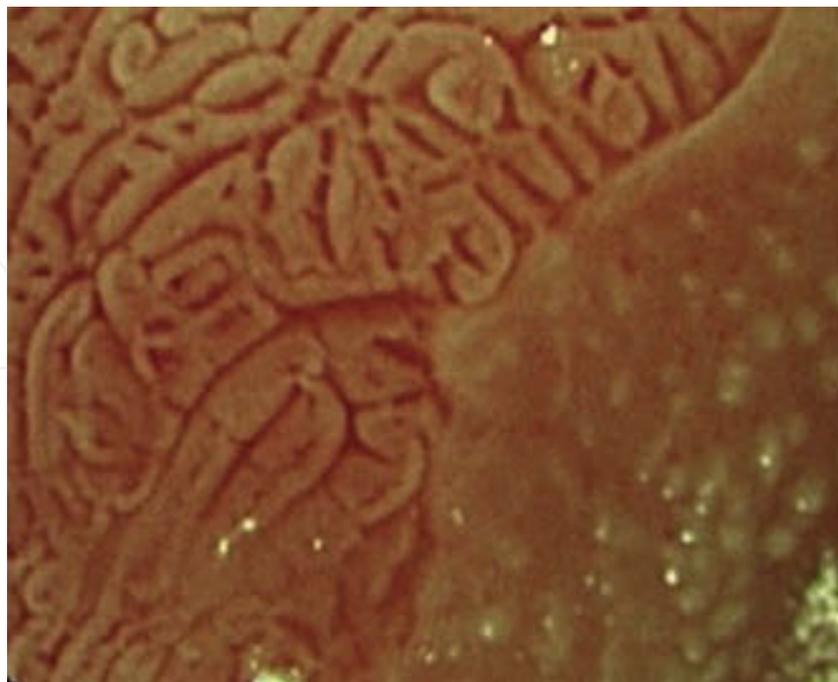
A recent meta-analysis assessing the diagnostic yield of MB in detecting intestinal and dysplasia in BE looked at 9 published studies that included 450 patients. Despite controlling for

differences in technique and quality of published data, the meta-analysis showed no significant benefit of MB chromoendoscopy compared with random biopsies in detecting SIM, dysplasia or early esophageal cancer [19].

Unfortunately MB is inconvenient to use. It must be left in contact with the mucosa for 3 minutes followed by vigorous washing to clear away excess dye. As a result the endoscopic appearances are unpredictable, subjective and not reproducible

### 3.2. Acetic acid chromoendoscopy

Acetic acid 2.5% (AA) when sprayed onto Barrett's mucosa causes a reversible acetylation of nuclear proteins to occur. This leads to an acetowhitening reaction, with increased opacity of the mucosal surface. It also causes vascular congestion and improves surface pattern evaluation. There is a growing body of evidence that magnification chromoendoscopy with acetic acid improves the diagnosis of specialized intestinal metaplasia. The technique is advantageous as it is both safe and inexpensive. When topically applied to multilayered squamous epithelium the acetic acid is progressively neutralized by mucus covering the epithelium and the underlying stroma and the vascular network are protected [20]. In single layered columnar lined esophagus the acetic acid reversibly alters the barrier function of the epithelium and reaches the stroma and vascular network. This leads to swelling of the mucosal surface and enhancement of the surface architecture. There is also enhancement of vascular pattern due to congestion of the capillaries. Transient changes to the structure of cellular proteins may also occur.



**Figure 2.** Acetic acid used to visualise Barrett's oesophagus, ridge pattern signifying Intestinal metaplasia

All of the studies using acetic acid have combined magnification endoscopy to study the pit pattern of the mucosa. Classification is based on Guelrud's description of four typical pit patterns; gastric patterns (pattern I = pits with a regular and orderly arranged circular dots; pattern II = reticular pits that are circular or oval and are regular in shape and arrangement); SIM patterns (pattern III = fine villiform appearance with regular shape and arrangement; pattern IV = thick villous convoluted shape with a cerebriform appearance with regular shape and arrangement).

In the first prospective cohort study of 49 patients, sensitivity for specialized intestinal metaplasia = 96.5%, specificity = 88.7% and overall accuracy was 92.2% [21]. Using modified criteria, a second study of 67 patients demonstrated sensitivity 88.5%, specificity 90.2% and diagnostic accuracy of 90% [22]. Reaud *et al.* studied 28 patients with a type III or IV pattern with sensitivity for SIM of 95.5%, specificity 42.9 % and diagnostic accuracy of 75% [23].

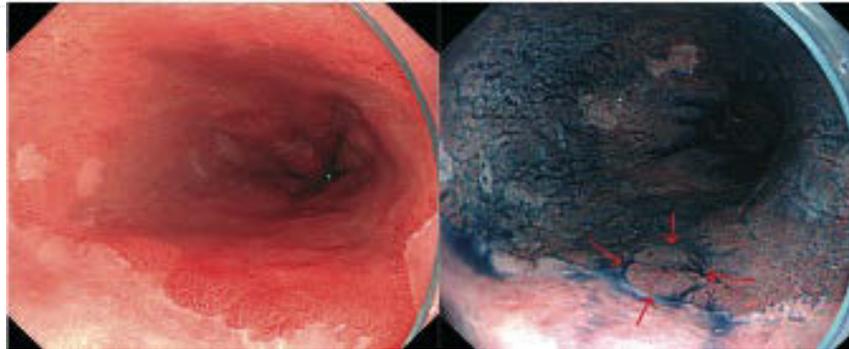
A further randomised crossover study using acetic acid for the detection of Barrett's metaplasia in 32 patients was performed by Hoffman *et al.* [24]. Patients were randomized to either standard video endoscopy with quadrantic biopsies or to magnifying endoscopy with acetic acid. All patients were re-examined after 14 days post initial endoscopy with the corresponding procedure. The investigators found that magnifying endoscopy enabled the prediction of Barrett's epithelium with a sensitivity of 100% and specificity of 66% and accuracy of 83.8%. The biopsies obtained following exposure to acetic acid yielded a significantly higher percentage of tissues containing Barrett's metaplasia (78%) compared to random biopsies (57%). Again this study had no dysplasia cases and the authors recognized this as a limitation of the study.

In a very recent landmark study by Longcroft-Wheaton *et al.* [25] from Portsmouth in the United Kingdom, the efficacy of acetic acid has been investigated in detecting Barrett's dysplasia. Data were collected from 190 patients with Barrett's esophagus examined over a 3 year period at a tertiary referral center from procedures performed by a single experienced endoscopist. Patients were first examined with white light gastroscopy and visible abnormalities were identified. Acetic acid (2.5%) dye spray was used to identify potentially neoplastic areas and biopsy samples were collected from these, followed by quadrantic biopsies at 2 cm intervals of the remaining Barrett's mucosa. The chromoendoscopic diagnosis was compared with the ultimate histological diagnosis to evaluate the sensitivity of acetic acid chromoendoscopy. Acetic acid chromoendoscopy had a sensitivity of 95.5% and specificity of 80% for the detection of neoplasia. There was a correlation between lesions predicted to be neoplasias by acetic acid and those diagnosed by histological analysis ( $r = 0.98$ ). There was a significant improvement in the detection of neoplasia using acetic acid compared with white light endoscopy ( $P = 0.001$ ).

### 3.3. Indigo carmine chromoendoscopy

Whilst Indigo carmine has been used in many studies looking at colonic neoplasia, it has not been studied to such a degree in the esophagus. As in the colon, it is not absorbed by esophageal and Barrett's mucosa, but accumulates in the pits and valleys between cells, highlighting the architecture. It is a contrast agent which can highlight mucosal irregularities and has been very helpful in the colon. However, results have been less encouraging in the esophagus.

Sharma *et al.* showed that using indigo carmine and high resolution endoscopy 3 distinct patterns can be recognized at endoscopy: ridged and/or villous, circular, and irregular and/or distorted [26]. Barrett's epithelium was most commonly identified in the ridged-villous pattern, whereas high-grade dysplasia was found entirely in the irregular/distorted pattern. An irregular/distorted pattern either throughout the entire segment of Barrett's esophagus or in combination with a ridge/villous or circular pattern had a sensitivity of 83%, a specificity of 88%, a positive predictive value of 45%, and a negative predictive value of 98% for high-grade dysplasia.



**Figure 3.** High-resolution white-light imaging (left) and indigo carmine chromoendoscopy (right) of a small mucosal lesion (type IIb) at the 6-o'clock position (arrows), detected in a patient with Barrett's esophagus. This area was regarded as suspicious after spraying of indigo carmine. High grade dysplasia was found in the corresponding biopsy specimens [27].

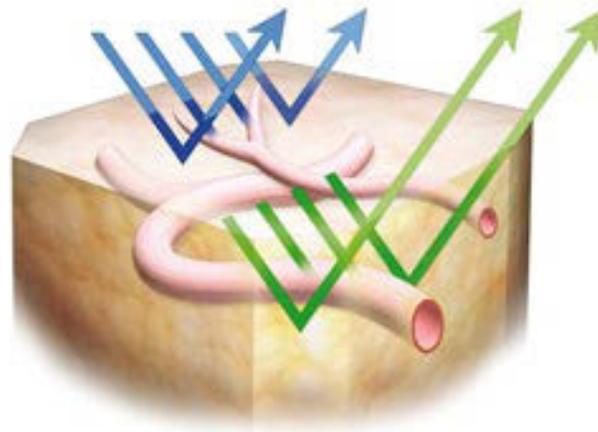
## 4. HD WLE & optical enhancements

Video endoscopy relies on a charge coupled device (CCD) chip to enhance image resolution and magnification. Standard definition (SD) WLE is rapidly being replaced by the introduction of high definition endoscopes. Video endoscopes use white light from a xenon or halogen source for illumination. The reflected light is captured by a CCD chip at the tip of the instrument in order to reconstruct the images. Conventional SD endoscopes are equipped with CCD chips that produce an image signal of 100,000 to 400,000 pixels which is displayed in SD format. The chips currently in use in HD endoscopes produce resolutions that range from 850,000 to 1.3 million pixels. In order to generate a true HD image, each component of the system (e.g. the endoscope CCD chip, the processor, the monitor, and transmission cables) must be HD compatible.

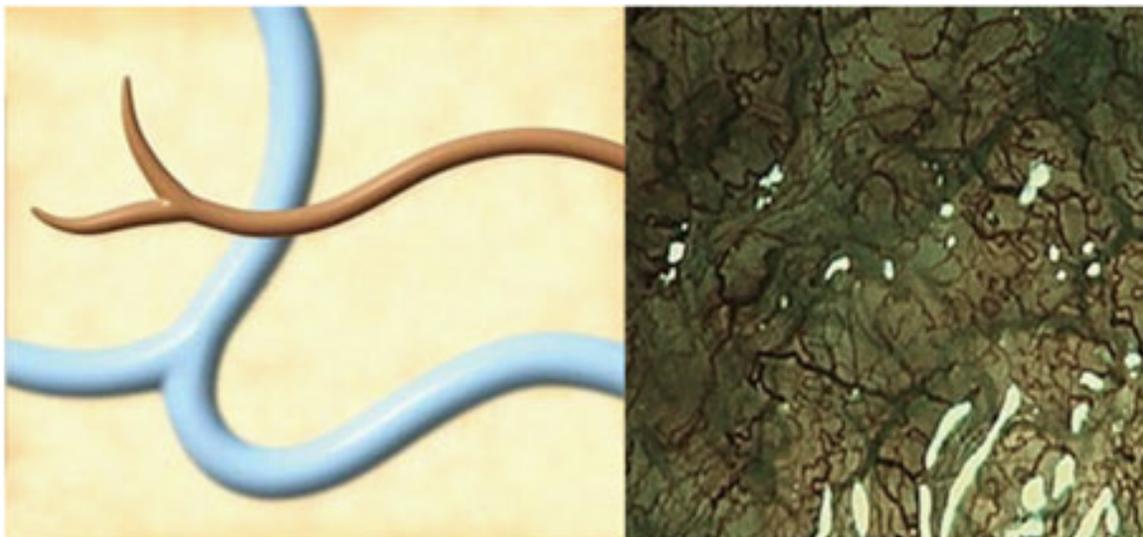
### 4.1. Enhanced imaging systems – Olympus Narrow Band imaging (NBI)

Conventional WLE uses the entire spectrum of visible light (400-700nm) to examine tissue. *Narrow band imaging (NBI)* developed by Olympus Medical Systems (Olympus, Japan) is a new advance in endoscopy that uses optic filters to isolate two specific bands of light: 415 nm blue and 540 nm green. By isolating these two bands of light and taking into account

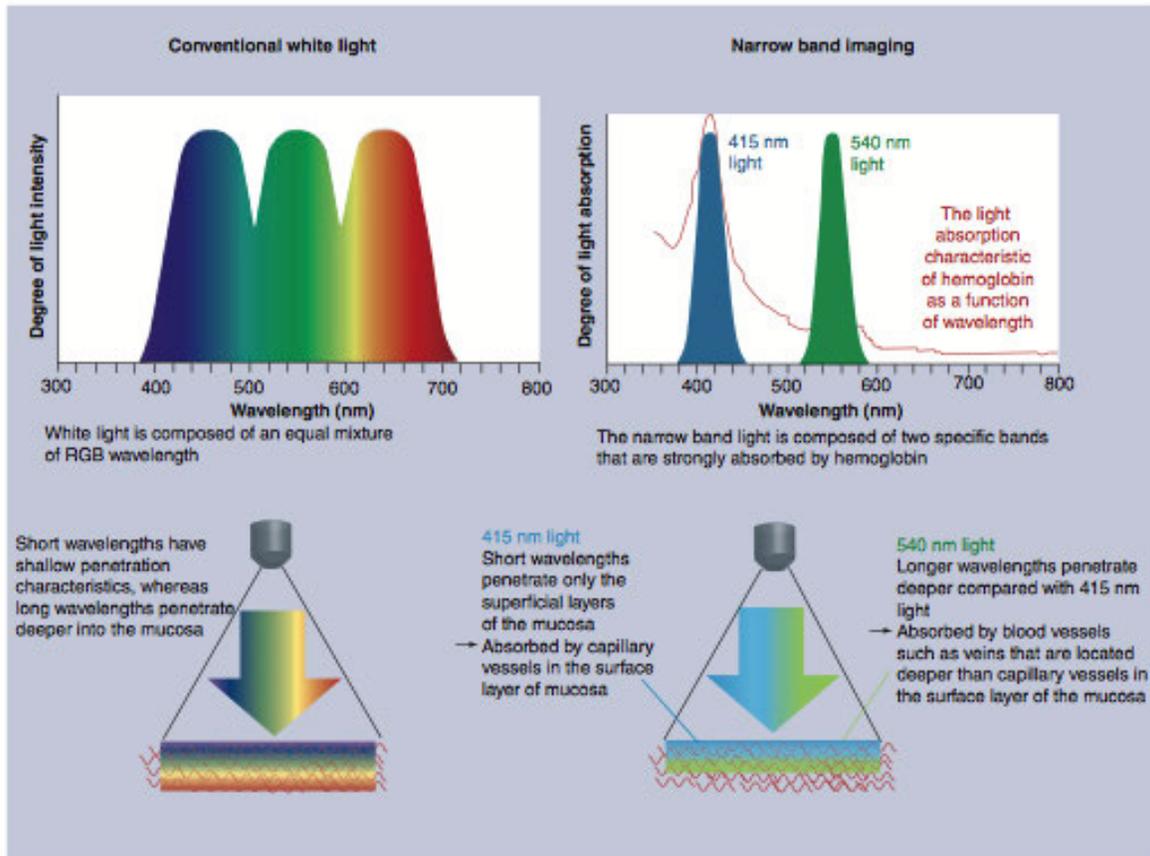
their absorptive and reflective properties on the mucosal surface, an image that enhances visualization of superficial mucosal and vascular structures is created. The quality of the surface pit pattern morphology is also clearly enhanced by this technology. It enables the endoscopist to switch between conventional white light and NBI views easily and quickly during the procedure, thus making the procedure itself less messy and cumbersome compared to chromoendoscopy. By depressing a lever on the endoscope, the focal distance of the lens at the tip of the endoscope can be adjusted electronically thus enabling the endoscopist to achieve a maximal magnification of 115X in real time.



**Figure 4.** NBI uses two discrete bands of light: One blue at 415nm and one green at 540nm. Narrow band blue light displays superficial capillary networks, while green light which penetrates more deeply into tissue displays subepithelial vessels and when combined offer an extremely high contrast image of the tissue surface.



**Figure 5.** NBI image on the monitor: Capillaries on the surface are displayed in brown and veins in the sub surface are displayed in cyan.



**Figure 6.** Light filtering in the narrow-band imaging system. The white light is split into two narrow bands: a blue narrow band of 415 nm and a green narrow band of 540 nm.

Although many studies have shown the benefit of NBI over conventional WLE in detecting HGD and early esophageal cancer, others have questioned whether NBI achieves any incremental improvement beyond that of HRE-WLE. Wolfsen *et al.* investigated whether NBI targeted biopsies could detect advanced dysplasia using fewer biopsy samples compared with conventional endoscopy using the four-quadrant biopsy method with a prospective, blinded, controlled tandem study [28]. The study revealed that NBI detected dysplasia in 57% of patients compared with 43% in the conventional endoscopy with four-quadrant biopsy group, with higher grades of dysplasia detected in the NBI group ( $P < 0.001$ ). In addition, more biopsies were taken in the four-quadrant biopsy group compared with narrow-band targeted biopsies (mean 8.5 versus 4.7;  $P < 0.001$ ). A study by Kara *et al.* investigated chromoendoscopy versus NBI, both in combination with high resolution endoscopy, in a prospective, randomized crossover study with 14 patients [29]. The sensitivity of chromoendoscopy and NBI was 93% and 86%, respectively, com-

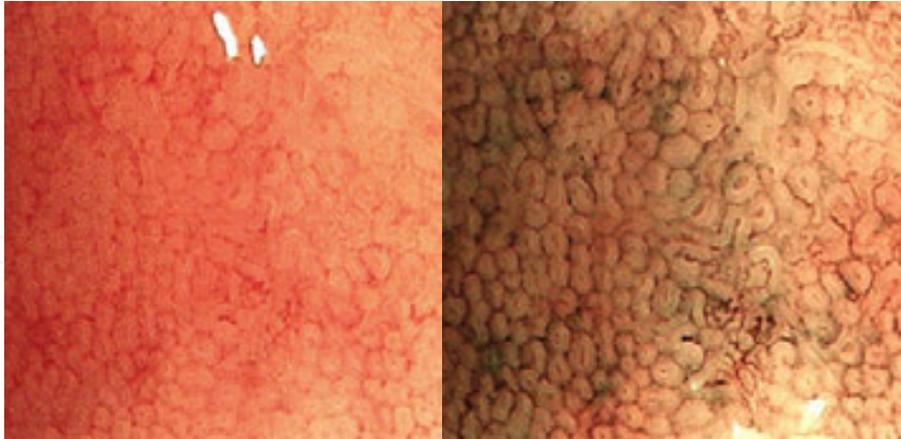
pared with 79% for four-quadrant biopsies with conventional endoscopy in the diagnosis of high-grade dysplasia or early cancer (EC) in patients with BE. Although chromoendoscopy and NBI identified additional lesions (chromoendoscopy two additional lesions in two patients; NBI four additional lesions in three patients), they did not increase per patient sensitivity for identifying HGD/EC.

Interestingly, in an inter-observer agreement study by Curvers *et al.* there was moderate inter-observer agreement for classification of mucosal morphology by NBI (0.40–0.56) [30]. Although there was improvement in image quality with NBI compared to HRE, NBI provided no significant improvement in inter-observer variability and yield for detecting neoplasia. The yield of HRE-WLE for neoplasia was 81%, 72% for NBI, and 83% for the HRE-WLE with NBI. The addition of enhancement techniques did not improve the yield of neoplasia in this series.

More recently Curvers *et al.* have performed a review of studies that analyzed NBI images for accuracy in differentiating HGD/cancer from low-grade dysplasia (LGD) or non-neoplastic Barrett's esophagus [31]. In a meta-analysis that included 149 areas with HGD/cancer and 607 areas with LGD or non-dysplastic Barrett's esophagus, NBI had a sensitivity for HGD/cancer of 97% (95% CI 89–99%) and a specificity of 94% (60–99%), and an accuracy of 96% (72–99%). Consequently, the use of 'targeted' biopsy techniques using image enhancement techniques has potential time and cost savings. They recognize however that these findings may not be generalizable as these studies were performed in high-risk populations.

#### **4.2. Comparing high definition WLE to enhanced imaging systems (NBI)**

The majority of studies looking at NBI compare its efficacy in relation to other endoscopic modalities such as chromoendoscopy or autofluorescence as well as HD-WLE. There are limited data directly comparing the efficacy of NBI versus HD-WLE in the diagnosis of dysplasia/early cancer in patients with Barrett's esophagus. A very recent randomized control trial from 2012 by Sharma *et al* [32] compared the use of HD WLE and NBI for detection of IM or dysplasia in patients with BE. Patients referred for BE screening or surveillance at three tertiary referral centers were prospectively enrolled and randomized to HD-WLE or NBI followed by other procedures in 3-8 weeks. During HD-WLE, four quadrant biopsies every 2 cm, together with targeted biopsies of visible lesions (Seattle protocol), were obtained. During NBI examination, mucosal and vascular patterns were noted and targeted biopsies were obtained. 123 patients with BE (mean age 61; 93% male; 97% Caucasian) with mean circumferential and maximal extents of 1.8 and 3.6 cm, respectively, were enrolled. Both HD-WLE and NBI detected 104/113 (92%) patients with IM, but NBI required fewer biopsies per patient (3.6 vs 7.6,  $P < 0.0001$ ). NBI detected a higher proportion of areas with dysplasia (30% vs 21%,  $P = 0.01$ ). During examination with NBI, all areas of HGD and cancer had an irregular mucosal or vascular pattern. This important study demonstrates that NBI targeted biopsies can have the same IM detection rate as an HD-WLE examination with the Seattle protocol while requiring fewer biopsies. In addition, NBI targeted biopsies can detect more areas with dysplasia. Regular appearing NBI surface patterns did not harbor high-grade dysplasia/cancer, suggesting that biopsies could be avoided in these areas.



**Figure 7.** High magnification white light endoscopy-round pits in keeping with columnar mucosa without intestinal metaplasia with corresponding area on right image seen with narrow band imaging (NBI) and magnification

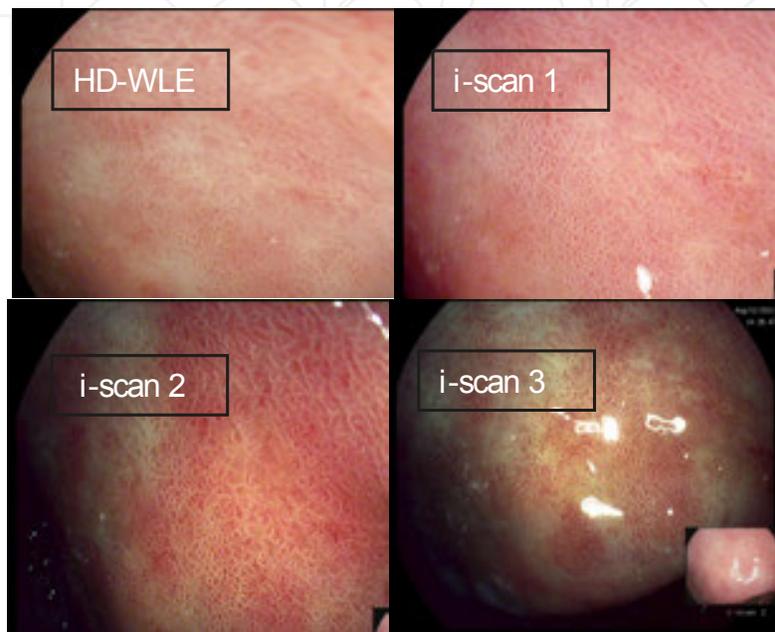
#### 4.3. Enhanced imaging systems – Pentax medical i-scan

A new endoscopic image enhancement technology, *i-scan*, has been developed by PENTAX (HOYA Corporation), Japan. *i-scan* uses the EPKi processor technology which enables resolution above HDTV standard, with distinct digital filters for special post processing online imaging, which can provide detailed analysis. *i-scan* is a novel endoscopic post processing light filter technology using sophisticated software algorithms with real time image mapping technology embedded in the EPKi processor. The computer controlled digital processing provides resolution of about 1.25 mega pixels per image. Different elements of the mucosa are enhanced by pressing a button on the hand piece of the high definition endoscope. *i-scan* can be used for surface analysis to recognize lesions using three modes of image enhancement. These are:

- i. *Surface enhancement (SE)/ i-scan 1* – enhancement of the structure through recognition of the edges
- ii. *Contrast enhancement (CE)/ i-scan 2* – enhancement of depressed areas and differences in structure through colors presentation of low density areas
- iii. *Surface and Tone enhancement (TE)/ i-scan 3*– enhancement tailored to individual organs through modification of the combination of RGB components for each pixel

*i-scan* images are as bright as conventional WLE images and therefore *i-scan* can observe larger areas in a distant view than NBI. *i-scan* does not need magnifying endoscopy to observe the demarcation between normal and abnormal tissue. *i-scan* can be switched on and effected quite simply and instantaneously by pushing a button, therefore it is an easy method for screening or detailed inspection, and may reduce both time and costs. The sensitivity and specificity of *i-scan* in detecting dysplasia in Barrett's patients is yet to be investigated.

There is as of yet no formal i-scan classification system for BE mucosal patterns. However using those devised for other modalities such as NBI, endoscopists are able to direct and target therapy to subtle anomalies based on these validated classification systems. There is however a growing body of work showing the increased accuracy of i-scan in the colon in detecting adenomatous polyp [33].



**Figure 8.** Pentax Images of an area of BE mucosa with IM using the various enhancement settings

#### 4.4. Flexible spectral imaging color enhancement (FICE)

Unlike NBI, which utilizes a physical filter, FICE (Fujinon, Japan) is a post processor technology which captures spectral reflectance by a color CCD video endoscope. This is sent to a spectral estimation matrix processing circuit contained in the video processor. The reflectance spectra of corresponding pixels that make up the conventional image are mathematically estimated. From these spectra, it is feasible to reconstruct a virtual image of a single wavelength. Three such single-wavelength images can be selected and assigned to the red, green, and blue monitor inputs, respectively, to display a composite color-enhanced multi band image in real time. In practice this can be used like narrow band imaging to remove data from the red part of the waveband and narrow the green and blue spectra

A prospective cohort study of 72 patients demonstrated that the identification of Pallisade vessels using FICE provided a clear demarcation between Barrett's mucosa and the gastric mucosa which was superior to standard white light endoscopy [34]. This study did not attempt to diagnose dysplasia and used transnasal Fujinon endoscopes. These are very small with a more limited field of view and no optical magnification.

In a small prospective cohort study of 57 patients which compared FICE with random biopsy in patients with suspected HGD or early cancer arising in BE, a sensitivity of 92% and specificity of 97% for FICE was achieved [35]. There was HGD or early cancer in 24/57 patients. However the investigators used acetic acid in addition to FICE.

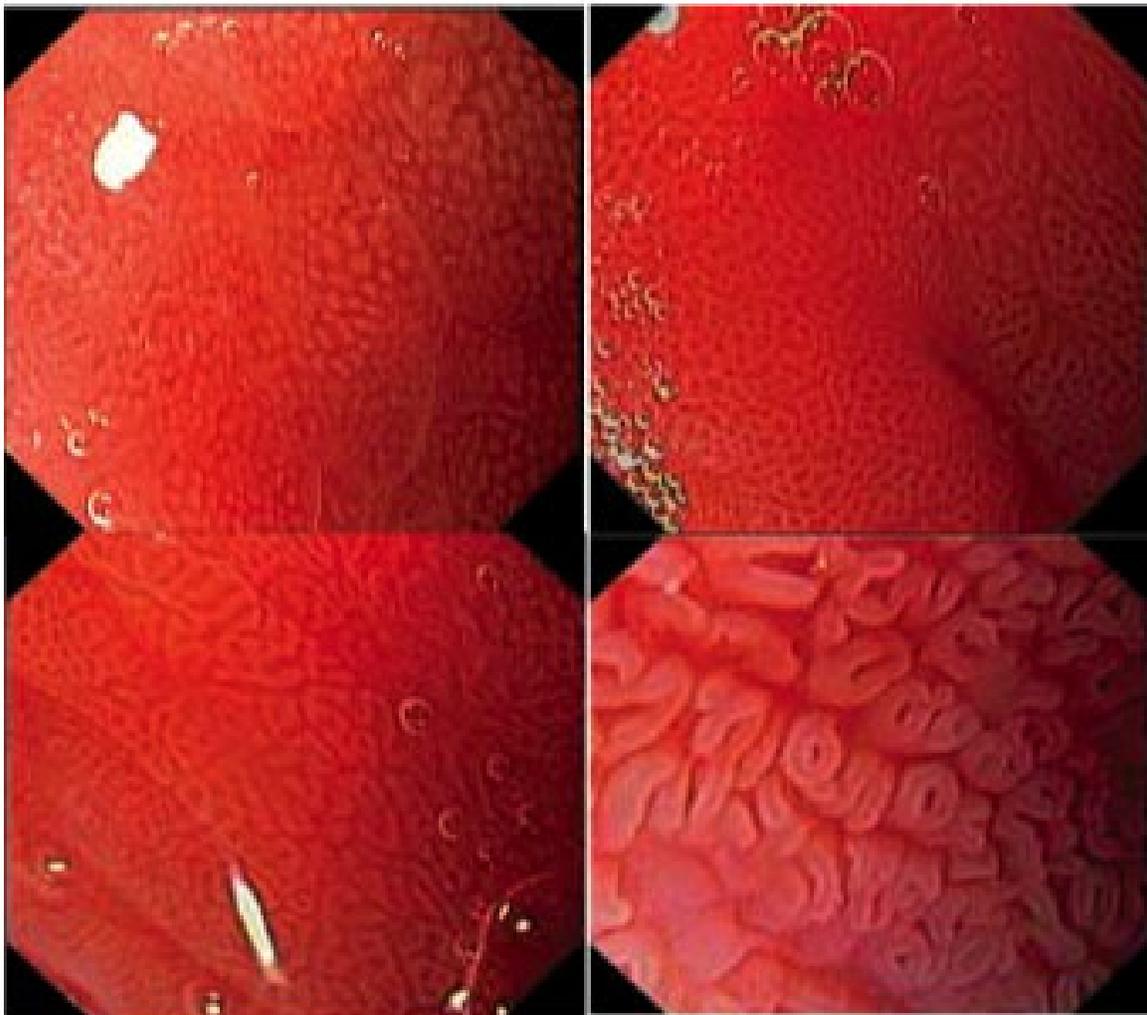
## 5. Mucosal classification systems for dysplasia in BE

There are now several recognized mucosal classification systems that have been described in the literature. They have formed the basis for further extensive work attempting to validate numerous optical enhancement modalities for neoplasia in BE. 2 of the very first systems were defined by Guelrud *et al.* and Endo *et al.* in 2001 and 2002.

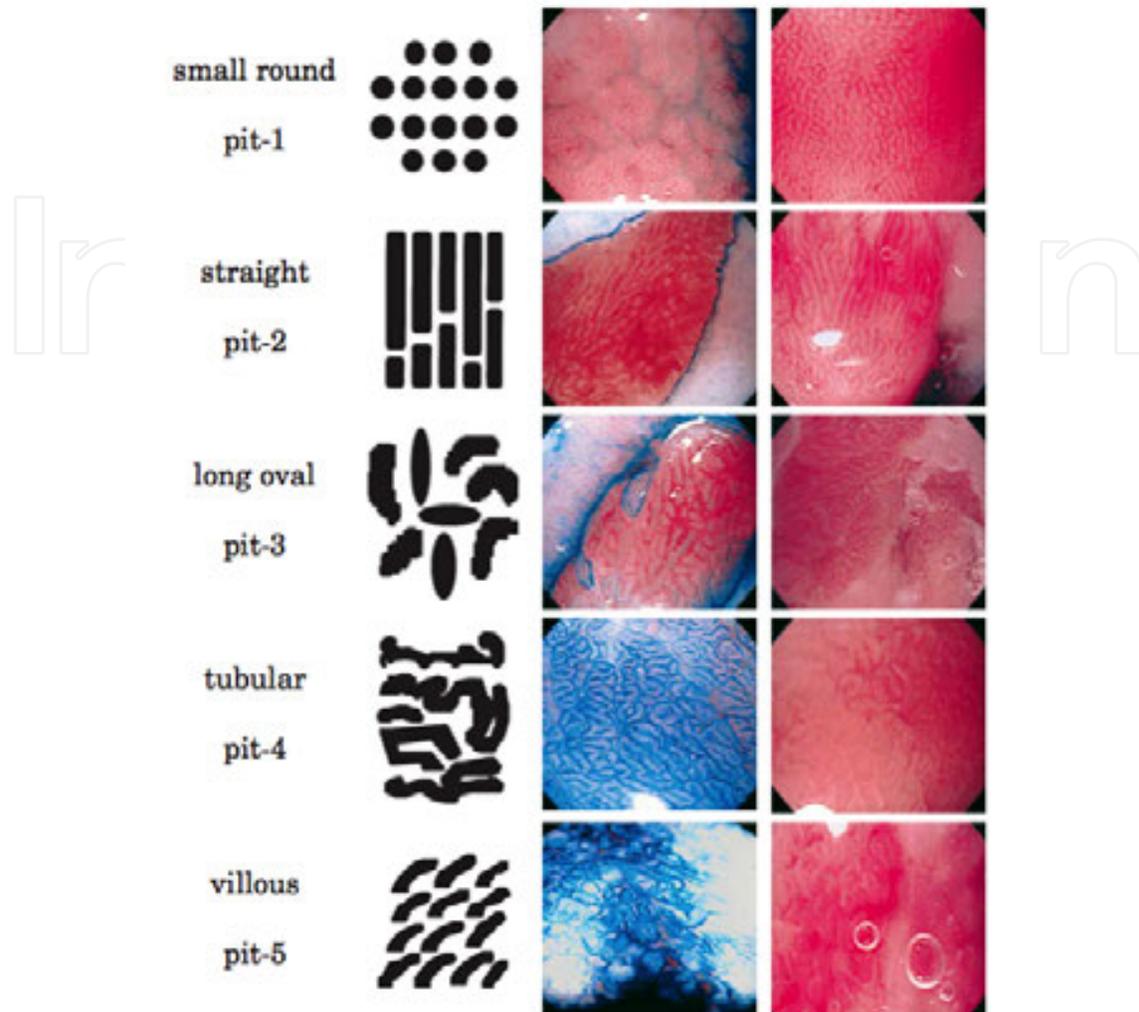
Guelrud *et al* [36] in 2001 described a technique they named enhanced-magnification endoscopy, which combines magnification endoscopy with instillation of acetic acid. They classified Barrett's mucosa into 4 patterns: I, round pits; II, reticular (circular or oval pits); III, villous (fine villiform appearance without visible pits); and IV, ridged (thick villi with convoluted cerebriform appearance without visible pits). This initial study by Guelrud *et al.* included 49 patients undergoing endoscopic surveillance for short-segment Barrett's esophagus. At the time the study was conducted, a magnification endoscope with standard resolution and a magnification power of 35x was used. A spray catheter was used to apply approximately 10 to 15 mL of 1.5% acetic acid onto the distal esophagus. This technique added an estimated 5 to 8 minutes to the length of a standard endoscopic examination. The 4 different mucosal surface patterns were observed, and one biopsy specimen was taken from each representative pattern. All patients with Barrett's esophagus had a villiform mucosal pattern that correlated with the finding of intestinal metaplasia on histopathologic evaluation of the biopsy specimens. The yield of biopsy specimens in the detection of intestinal metaplasia was correlated with the endoscopic pattern. Biopsy specimens from Pattern I mucosa revealed fundic epithelium, and this pattern served as a control for the analysis. Biopsy specimens from Pattern II mucosa revealed cardia mucosa in 90% of cases; intestinal metaplasia was found in two of 18 samples. Analysis showed that Pattern III and Pattern IV mucosa contained intestinal metaplasia in 87% and 100% respectively of biopsy specimens. The overall accuracy of enhanced magnification endoscopy for the detection of intestinal metaplasia was 92%; the positive predictive value of Patterns III and IV was 87.5%.

In 2002 Endo *et al.* [37] examined 67 regions in Barrett's mucosa and described 5 patterns. These patterns were classified into 5 categories. The dot type (pit-1) is characterized by small round pits of relatively uniform size and shape. The straight type (pit-2) consists of long straight lines. The long oval and curved type (pit-3) exhibits long extended pits, larger than those of the dot type (pit-1). The tubular type (pit-4) has a complicated and twisted pattern that is similar to a branch or gyrus-like structure. The fifth pattern (pit-5), villous type, has flat, finger-like projections. Methylene blue is applied topically at a concentration of 0.5-1.0% and is absorbed by goblet cells present in meta-

plastic Barrett's epithelium. Methylene blue stained 0% of the mucosa identified as types 1 and 2. However, intestinal metaplasia was found in 6% of biopsy specimens from mucosa that exhibited the type 1 pattern. The rate of positive methylene blue staining was 23% for type 3, and 40% of biopsy specimens from this type revealed intestinal metaplasia. Intestinal metaplasia was found in 100% of biopsy specimens from mucosa that exhibited either a type 4 or type 5 pattern. However, type 4 and type 5 mucosa were stained by methylene blue in only 60% and 50% of cases, respectively.

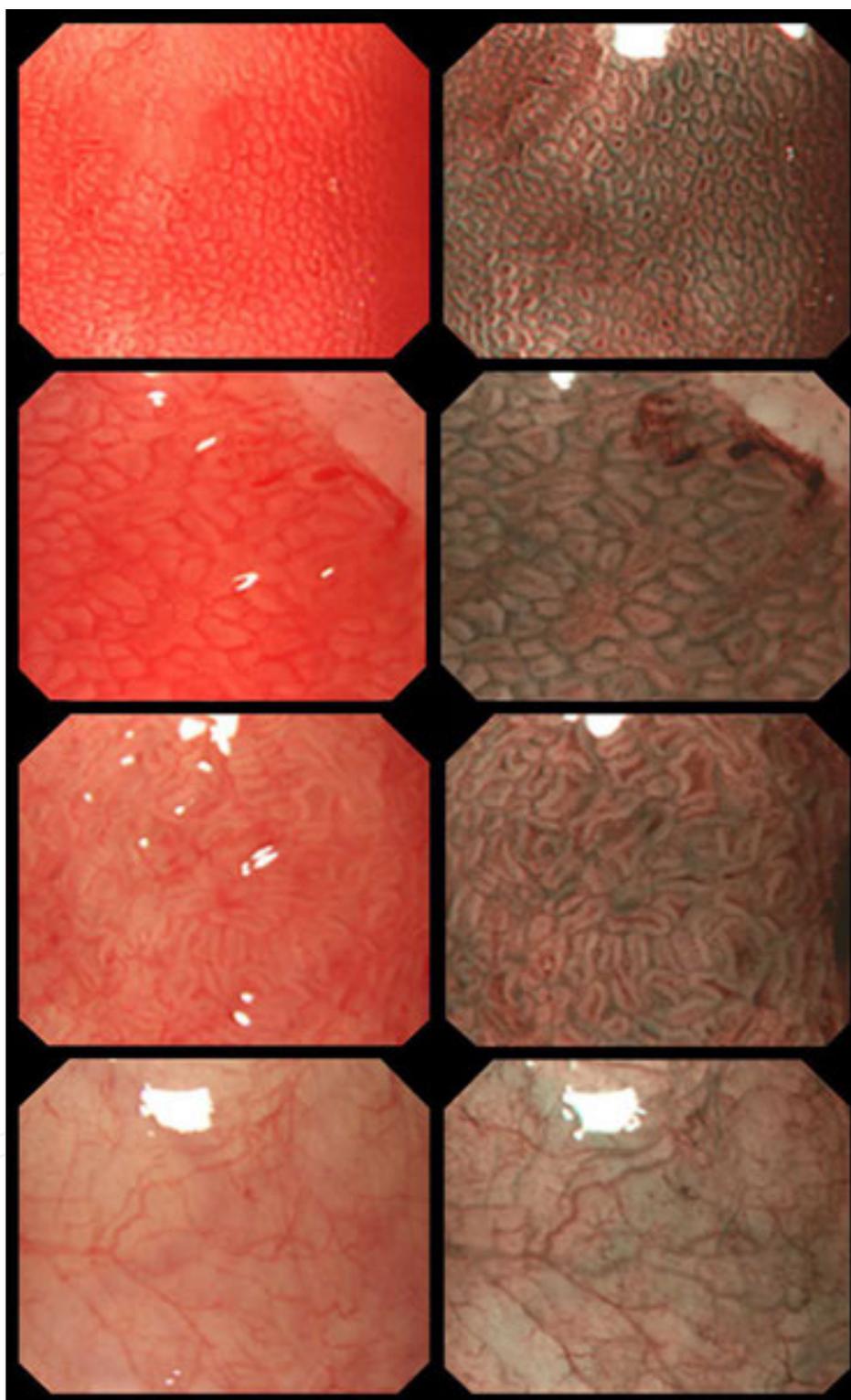


**Figure 9.** The First Guelrud classification (x35, 1.5% alcohol acetic acid); Pattern I: round pits with a regular and orderly arranged circular dots. Pattern II: reticular pits that are circular or oval and are regular in shape and arrangement. Pattern III: fine villiform appearance with regular shape and arrangement. Pattern IV: thick villous convoluted shape with a cerebriform appearance with regular shape and arrangement. Guelrud et al [36]

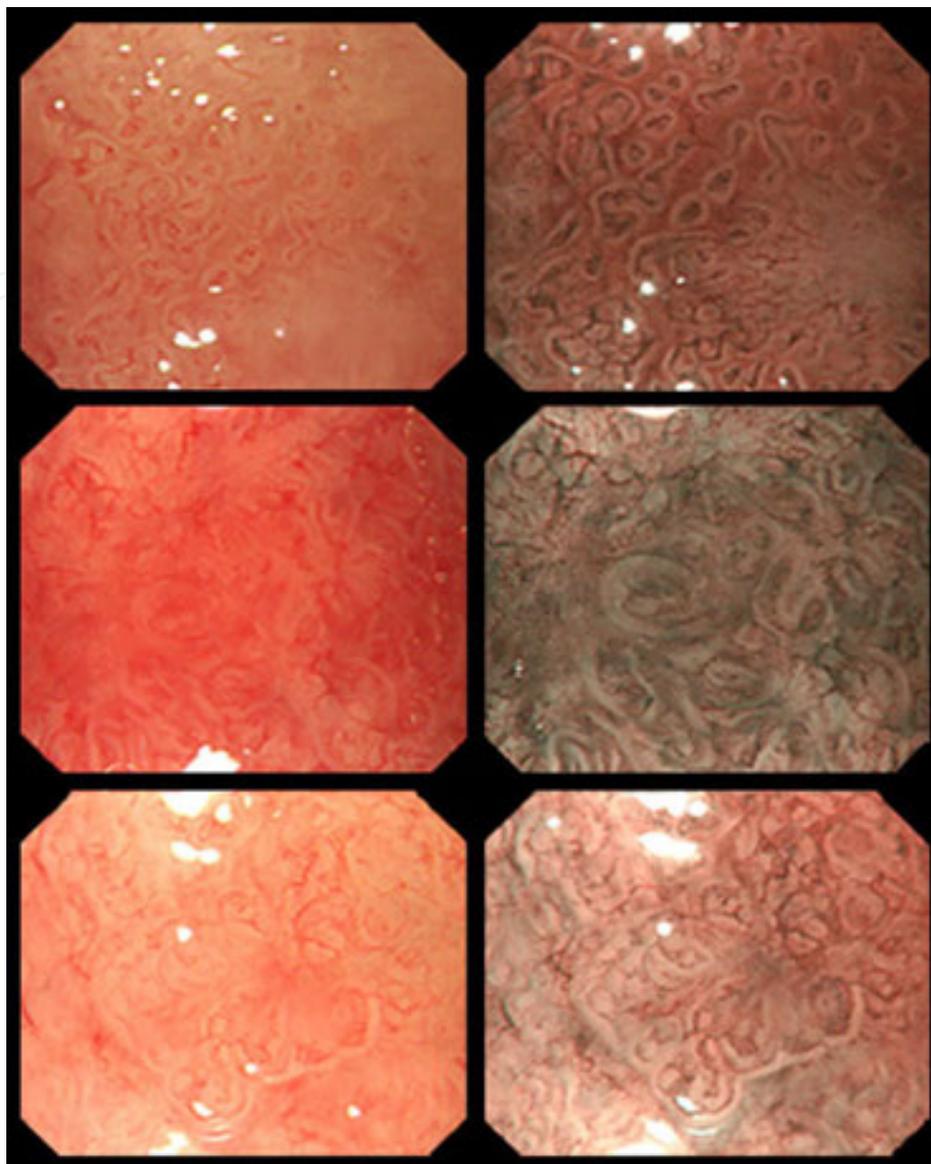


**Figure 10.** Endo Classification of pit pattern of Barrett's epithelium by magnifying endoscopy. Endoscopic views at right were obtained without methylene blue staining and those at left after application of methylene blue. Note that mucosa with pit-1, pit-2, and pit-3 patterns was not stained by the dye, whereas positive staining is evident within mucosa with pit-4 and pit-5 patterns Endo et al. [37]

Kara *et al.* in 2006 from Amsterdam [38] suggested a further classification system. They used NBI with magnifying endoscopy to image and then biopsy randomly selected areas in 63 patients with BE. Following this there was a formal review process of the images and biopsies. The relationship between mucosal morphology and presence of IM and HGD were evaluated. Areas of intestinal metaplasia were characterized by either villous/gyrus-forming patterns (80%), which were mostly regular and had regular vascular patterns, or a flat mucosa with regular normal-appearing long branching vessels (20%). HGD was characterized by 3 abnormalities: irregular/disrupted mucosal patterns, irregular vascular patterns, and abnormal blood vessels. All areas with HGIN had at least 1 abnormality, and 85% had 2 or more abnormalities. The frequency of abnormalities showed a significant rise with increasing grades of dysplasia. The magnified NBI images had a sensitivity of 94%, a specificity of 76%, a positive predictive value of 64%, and a negative predictive value of 98% for HGIN.



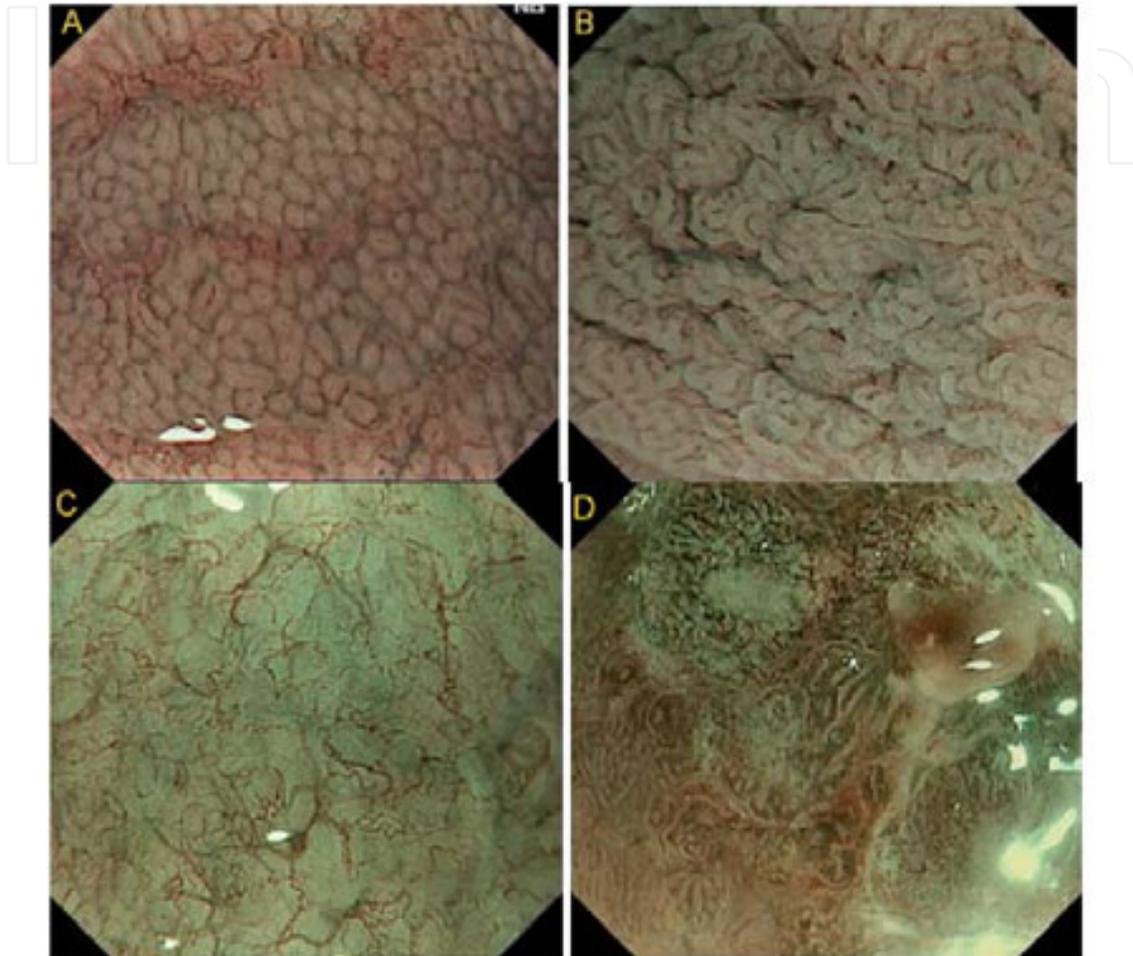
**Figure 11.** Magnified high-resolution white light (left) and NBI (right) endoscopic photographs of areas with nondysplastic BE (no staining, orig. mag.115). The upper 3 examples have regular villous/gyrus-forming mucosal patterns with regular vascular patterns; the villi are of various sizes and shapes but regular in all areas with blood vessels situated between the mucosal ridges. The lower image has a flat-type mucosa without pits or villi; the vasculature shows regular, normal-appearing long branching vessels. Kara et al. [38]



**Figure 12.** Magnified high-resolution white light (left) and corresponding NBI (right) endoscopic photographs of areas with HGIN in BE (no staining, orig. mag.  $\times 115$ ). These examples show irregular/disrupted mucosal patterns, with irregular vascular patterns with remnants of villous/gyrus-forming mucosal patterns. Kara et al. [38]

Singh *et al.* [39] from Nottingham in the United Kingdom have looked at an alternative simplified classification. In a prospective cohort study of 109 patients with Barrett's esophagus, mucosal patterns visualized with NBI were classified into four easily distinguishable types: A, round pits with regular microvasculature; B, villous/ridge pits with regular microvasculature; C, absent pits with regular microvasculature; D, distorted pits with irregular microvasculature. The NBI grading was compared with the final histopathological diagnosis. In 903 out of 1021 distinct areas (87.9%) the NBI grading corresponded to the histological diagnosis. The PPV and NPV for type A pattern (columnar mucosa without intestinal metaplasia) were 100% and 97% respectively; for types B and C (intestinal metaplasia) they were 88% and 91% respectively, and for type D (high grade dysplasia) 81% and 99% respectively.

With respect to inter- and intra-observer agreement, the mean k values in assessing the various patterns were 0.71 and 0.87 in the non-expert group; 0.78 and 0.91 in the expert group.



**Figure 13.** (A) Type A: round pits with regular microvasculature. (B) Type B: villous/ridge pits with regular microvasculature. (C) Type C: absent pits with regular microvasculature. (D) Type D: distorted pits with irregular microvasculature. Singh et al. [39]

A final endoscopic classification system for BE was described by investigators in Kansas again using narrow band imaging [40]. NBI images were graded according to mucosal pattern (ridge/villous, circular and irregular/distorted) and vascular pattern (normal and abnormal), and correlated with histology. Of 51 patients, 28 had IM without dysplasia, 8 had low-grade dysplasia (LGD), 7 had high-grade dysplasia (HGD), and 8 had cardiac-type mucosa. The sensitivity, specificity, and positive predictive value of ridge/villous pattern for diagnosis of IM without HGD were 93.5%, 86.7%, and 94.7%, respectively. The sensitivity, specificity, and positive predictive value of irregular/distorted pattern for HGD were 100%, 98.7%,

and 95.3%, respectively. If biopsies were limited to areas with irregular/distorted pattern, no patient with HGD would have been missed. However, NBI was unable to distinguish areas of IM from those with LGD.

## 6. Interobserver agreement of classification criteria

A potential drawback with all these classification systems has been the lack of intra-observer agreement between endoscopists when using the classification systems described above.

In order to compare the 3 above classification systems from Amsterdam, Nottingham and Kansas a recent comparative study was performed by Silva *et al.* [41]. They examined all 3 classification systems in 84 high quality video recordings collected on cases of BE using HD WLE and NBI. All assessors were blinded to the matched histology from these areas. The global accuracy was 46% and 47% using the Nottingham and Kansas classifications respectively and 51% with the Amsterdam Classification. Accuracy for detecting dysplastic lesions was 75% irrespective of classification system used. The inter-observer agreement ranged from fair (Nottingham  $\kappa=0.34$ ) to moderate (Amsterdam and Kansas,  $\kappa=0.47$  and  $\kappa=0.44$ , respectively). The authors concluded that all three systems revealed substantial limitations when assessed externally and that as a result, NBI could not replace random biopsies for histopathological analysis.

## 7. Confocal laser endomicroscopy (CLE)

CLE is a new technology that enables the endoscopist to perform a real time histological assessment of the upper gastrointestinal tract and in particular the esophagus. The most widely used CLE system is the 'endoscope with embedded CLE technology' (eCLE) made by Pentax, Tokyo, Japan and Optiscan, Melbourne, Australia. The eCLE enables visualization of both the epithelium and the subepithelial vascular structures with imaging at variable depths up to 250mm and a magnification power of up to 1000 $\mu$ m. A probe-based endomicroscopy system has been created by Mauna Kea Technologies in which the laser-scanning unit remains outside the patient, and the endomicroscopy probe is passed through the working channel of a standard endoscope. This probe-based CLE (pCLE) provides video sequence imaging at a rate of 12 images per second and allows for the compilation of images from a video sequence to create a composite video mosaic. The depth ranges from 50 to 150mm and is fixed based on the type of probe. These CLE systems use a wavelength of 48nm for excitation. CLE requires the use of contrast agent, most commonly intravenous fluorescein sodium, which is safe for imaging the gastrointestinal tract [42].

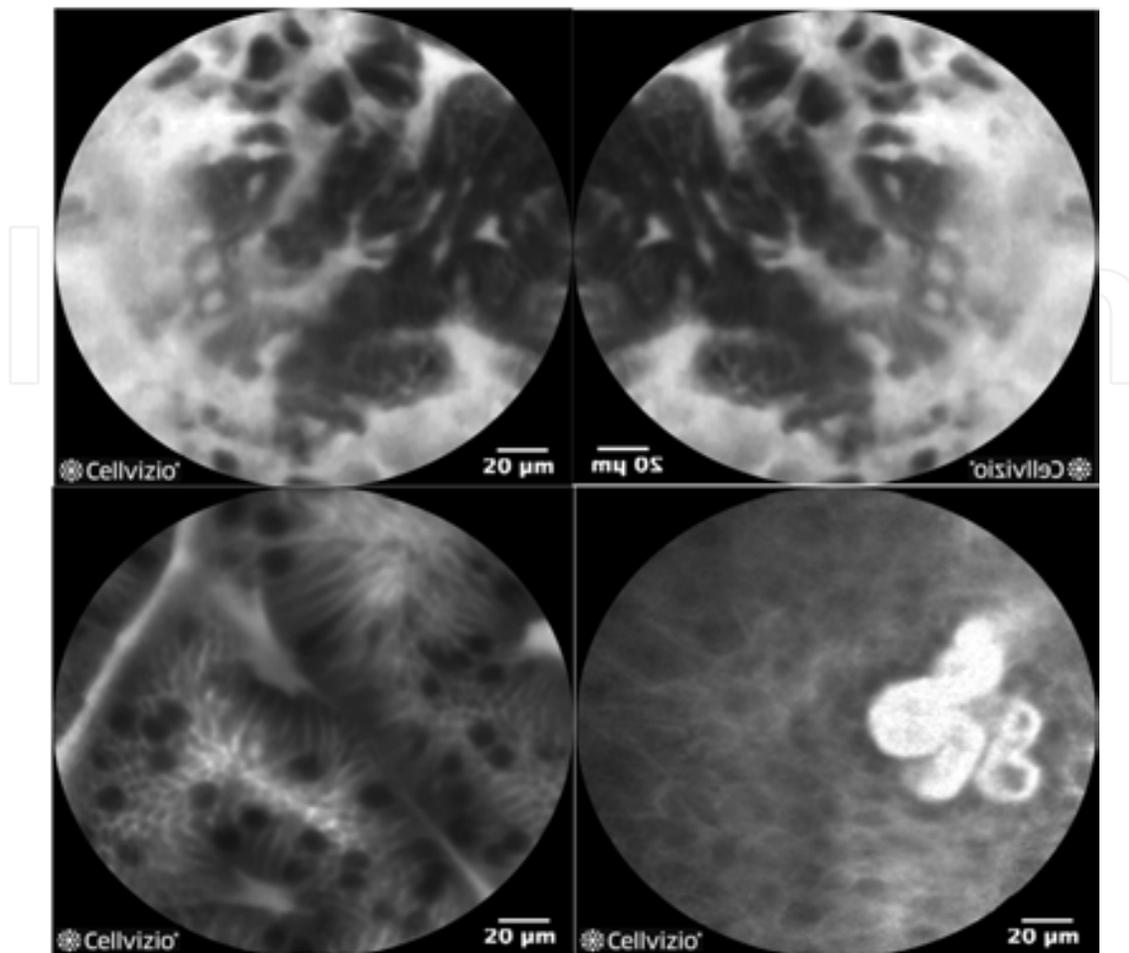
CLE classification systems for Barrett's esophagus with and without dysplasia have been described for standard endomicroscopy and probe-based endomicroscopy [43,44]. Signs of non-dysplastic Barrett's epithelium include a regular epithelial lining pattern, regular vascu-

lar pattern, presence of goblet cells, and preservation of the villous pattern of glands. Signs of dysplasia in Barrett's esophagus include irregular epithelial lining, fusion of glands, focal accumulation of dark cells with bright lamina propria, irregular vascular pattern, and disruption of the glandular pattern.

Kiesslich *et al.* [43] demonstrated that eCLE could diagnose Barrett's associated dysplasia during endoscopy with a sensitivity of 92.9% and a specificity of 98.4%. Dunbar *et al.* [45] conducted a prospective, double-blind, randomized crossover study comparing four-quadrant random biopsies with eCLE-targeted biopsy in 39 patients. They demonstrated that eCLE improved the diagnostic yield for detecting neoplasia in Barrett's esophagus. The yield of eCLE was 33.7% versus 17.2% with random biopsies ( $P = 0.01$ ). Furthermore, some patients undergoing eCLE would not have needed any random biopsies in order to diagnose neoplasia.

In 2011 Gaddam *et al.* [46] revised and validated a set of criteria for pCLE for dysplasia in BE using video recordings. Of multiple pCLE criteria tested in the first phase of their study, only those with  $\geq 70\%$  sensitivity or specificity were included in the final set. These were epithelial surface: saw-toothed; cells: enlarged; cells: pleomorphic; glands: not equidistant; glands: unequal in size and shape; goblet cells: not easily identified. Using these criteria overall accuracy in diagnosing dysplasia was 81.5% (95% CI: 77.5–81), with no difference between experts vs. non-experts. Accuracy of prediction was significantly higher when endoscopists were "confident" about their diagnosis (98% (95–99) vs. 62% (54–70),  $P < 0.001$ ). Accuracy of dysplasia prediction for the first 30 videos was not different from the last 45 (93 vs. 81%,  $P = 0.51$ ). Overall agreement of the criteria was substantial,  $\kappa = 0.61$  (0.53–0.69), with no difference between experts and non-experts.

In a very recent international prospective, multicenter, randomized controlled trial, Sharma *et al.* [47] investigated whether pCLE could allow for real time detection of neoplastic Barrett's esophagus. All patients with BE were examined by HD-WLE, narrow-band imaging (NBI), and pCLE, and the findings were recorded before matched biopsy samples were obtained. The order of HD-WLE and NBI was randomized and performed by 2 independent, blinded endoscopists. All suspicious lesions on HD-WLE or NBI and 4-quadrant random locations were documented. These locations were then examined by pCLE, and a presumptive diagnosis of benign or neoplastic (HGD/EC) tissue was made in real time after which biopsies were taken from all locations and were reviewed by a central pathologist, blinded to endoscopic and pCLE data. The sensitivity and specificity for HD-WLE were 34.2% and 92.7%, respectively, compared with 68.3% and 87.8%, respectively, for HD-WLE or pCLE ( $P = 0.002$  and  $P < 0.001$ , respectively). The sensitivity and specificity for HD-WLE or NBI were 45.0% and 88.2%, respectively, compared with 75.8% and 84.2%, respectively, for HD-WLE, NBI, or pCLE ( $P = 0.01$  and  $P = 0.02$ , respectively). However with the use of pCLE in conjunction with HD-WLE and NBI enabled the identification of 2 and 1 additional HGD/EC patients compared with HD-WLE and HD-WLE or NBI, respectively, resulting in detection of all HGD/EC patients, although not statistically significant. This may allow better informed decisions to be made for the management and subsequent treatment of BE patients.



**Figure 14.** Images of normal, dysplastic and cancer using probe based confocal endomicroscopy. The presence of goblet cells denoted intestinal metaplasia. (The images are courtesy of the DONT BIOPCE trial)

### 7.1. OCT – Optical Coherence Tomography

Optical Coherence Tomography (OCT) is an imaging modality that may have the ability to improve the current paradigm for endoscopic screening and surveillance that exists for patients with BE. OCT can be thought of as an analogous technique to ultrasound, however, instead of producing an image from the scattering of sound waves, it utilizes optical scattering based on differences in tissue composition to form a two-dimensional image [48]. The benefit of OCT over ultrasound is that it is capable of generating cross-sectional images of tissues with an axial-resolution of up to  $10\mu\text{m}$ , which is comparable to low-power microscopy.

Original OCT systems or time-domain OCT were limited to discrete locations or ‘point’ sampling due to slow acquisition rates. However, with the development of second-generation OCT, termed Optical Frequency Domain Imaging (OFDI), it is now possible to perform high-speed acquisition of large luminal surfaces in three-dimensions [49]. Due to its high-resolution and high-acquisition rates, utilizing this technique for screening and surveillance

of Barrett's esophagus may provide a means to evaluate pathologic states in long-segments of the esophageal lumen in real-time.

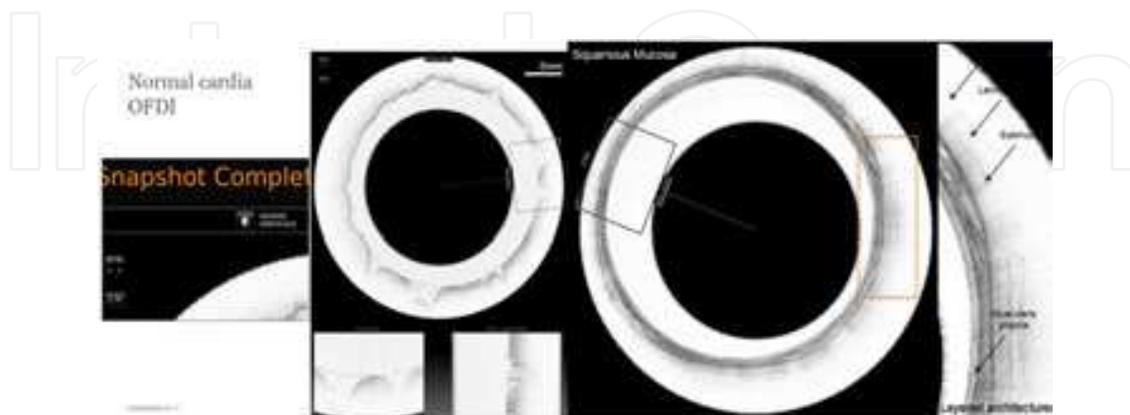
## 7.2. OCT in Barrett's esophagus

The first clinical application using *in vivo* endoscopic OCT for imaging of the human esophagus and stomach was performed by Bouma *et al.* in 2000 [50]. In this preliminary study, the ability of OCT to image normal esophageal mucosa and stomach, Barrett's esophagus, and adenocarcinoma was investigated. The authors concluded that they were able to differentiate the normal layered structure of the esophagus using OCT, including epithelium, lamina propria, muscularis mucosa, and submucosa. In addition, OCT was capable of differentiating between normal esophageal mucosa and Barrett's esophagus based on the lack of the layered structure found in Barrett's esophagus as well as a disorganized glandular morphology. Finally, esophageal adenocarcinoma was clearly differentiable by the presence of marked architectural disorganization. Several studies immediately followed this landmark study using *in vivo* OCT for the GI tract [51-54]. Similarly, they utilized a non-contact probe, approximately 2.5mm in diameter, introduced through the auxiliary channel of a standard endoscope. These studies all were significant in the contribution of development of OCT for GI imaging and played a major role in the potential clinical utility of OCT, however, they were limited to 'point' sampling and did not address diagnostic information relevant to dysplasia.

Subsequently, diagnostic criteria were developed for endoscopic OCT to diagnose specialized intestinal metaplasia (SIM), high-grade dysplasia (HGD), and intramucosal carcinoma (IMC). In prospective studies performed by Poneros *et al.* and Evans *et al.*, sensitivities from 81%-97% and specificities from 57%-92% for diagnosing SIM were reported [55,56]. Additionally, sensitivities and specificities for detecting HGD and IMC were reported in the ranges of 54%-83% and 72%-75%, respectively [55,57]. Unfortunately, similar to previous investigations, the studies were limited to 'point' sampling where a probe was placed at discrete locations and cross-sectional images were obtained. Although these studies made great strides in the diagnostic potential of OCT, the true clinical utility for Barrett's esophagus was not realized due to the potential for sampling errors analogous to biopsy.

More recently, technological advancements and the development of a second-generation OCT system, OFDI, has provided the ability to image long-segments of tissue with high-resolution and contrast identical to those obtained in OCT but at a rate approximately 100-times faster [58,59]. The first comprehensive imaging of the esophagus in human patients using OFDI was performed by Suter *et al.* In this study, a balloon-centering optical catheter was used to acquire long-segment (6cm) images of the esophagus during an endoscopic procedure (<2 minutes) [60]. During system and catheter development, a total of 32 patients were imaged prior to the design being unchanged. Once the final design had been established, a total of 10 patients out of 12 were successfully imaged using the comprehensive microscopy technique of OFDI, while 2 patients were not imaged due to imaging system malfunction. No adverse events or patient-related complications were reported in the study. Although the study presented promising case findings related to OCT diagnosis of normal

esophagus and cardia, ulcerated squamous mucosa, specialized intestinal metaplasia, and dysplasia, it was limited to image criteria established based on a non-contact OCT probe. Additional studies are needed to develop diagnostic criteria, intra-observer and inter-observer variability in diagnosis of OFDI imaging, and an OFDI-histopathologic correlative study using OFDI technology.



**Figure 15.** Optical Coherence Tomography images showing normal cardia and a cross sectional image through the squamous esophagus. (Courtesy of Ninepoint Medical)

### 7.3. Autofluorescence

When tissues are exposed to a short wave length light, endogenous biological substances (i.e., fluorophores) are excited, leading to emission of fluorescent light of a longer wavelength. This phenomenon is known as autofluorescence. Autofluorescence imaging (AFI) is a technique that can potentially differentiate tissue types based on their differences in fluorescence emission. Normal and neoplastic tissue have different autofluorescence spectra which may enable their distinction. This is due to the various different compositions of the endogenous fluorophores which includes collagen, NADH, aromatic amino acids and porphyrins in these tissues. This phenomenon was first utilized in Barrett's esophagus using spectroscopic point measurements. In brief, low collagen fluorescence and high NAD(P)H fluorescence characterize lesions with high grade dysplasia as opposed to non-dysplastic epithelia. Hence, with progression towards neoplasia one would typically observe a reduction in the intensity of green fluorescence, and a relative increase in red fluorescence.

In a 2006 60 patient study using a standard endoscope with an added AFI component, Kara was able to detect HGD in 22 patients, 14 of which were detected with AFI and WLE, and six of which were detected using AFI alone; thereby increasing the detection rate from 23% to 33% using AFI [61]. Only one of the patients was diagnosed using the standard four-quadrant biopsies alone. Results suggest that AFI may aid in the detection of additional HGD sites; however, it may not exclude the need for the standard four-quadrant biopsies. Sensitivity and specificity based on the 116 samples used for this study were 91% and 43%, respectively. Although no patient was diagnosed without AFI and four-quadrant biopsies, they cite a high rate of false positives using AFI alone, due in part to the loss of autofluorescence associated with acute inflammation.

## 8. VCE – Video Capsule Endoscopy

Wireless video capsule endoscopy (VCE) was approved by the Food and Drug Administration in 2001 as an adjunctive aid for the detection of small bowel disorders. Because patients ingest the capsule in the standing position and the small bowel VCE captures two frames per second, the traditional VCE often does not capture images of the esophago-gastric junction (EGJ).

Developed in 2004, the Esophageal capsule, or PillCam ESO (Given Imaging, Ltd., Duluth, GA, USA), captures 14 frames per second whereas the patient ingests the capsule in a supine position and then gradually resumes the sitting position during a 5-minute period. Usage of the first generation PillCam ESO demonstrated excellent sensitivity and specificity for the detection of erosive esophagitis and BE in a preliminary study of 106 patients (93 with GERD, 13 with BE). Sixty-six of 106 patients had positive esophageal findings, VCE identified oesophageal abnormalities in 61 (sensitivity, 92%; specificity, 95%). The per-protocol sensitivity, specificity, PPV, and NPV of VCE for Barrett oesophagus were 97%, 99%, 97%, and 99%, respectively, and for esophagitis 89%, 99%, 97%, and 94%, respectively. VCE was preferred over conventional upper GI endoscopy by all patients. There were no adverse events related to VCE. The investigators concluded that VCE is a convenient and sensitive method for visualization of oesophageal mucosal pathology and may provide an effective method to evaluate patients for oesophageal disease.

Following on from this initial landmark study a second generation esophageal capsule, ESO-2, was released by Given Imaging in 2007 with a 30% increase in the frame capture rate from 14 to 18 frames per second, advanced optics with three lenses instead of one lens, and expansion of field of view from 140° to 169°. To maximize visualization of the EGJ and reduce the presence of bubbles, the standardized ingestion protocol (SIP) was published by Gralnek *et al.* [62] and included having the patient lie on his/her right side during capsule ingestion while sipping 5–10 ml of water every 30 seconds. A subsequent clinical trial in 28 subjects using the SIP protocol and ESO-2 demonstrated visualization of the Z-line in 75% of subjects, and sensitivity of 100% with specificity of 74% for BE detection. The agreement between ESO-2 and EGD for description of the Z-line was 86% ( $k = 0.68$ ).

A 2009 meta-analysis [63] including nine studies with 618 patients undergoing primarily the first generation VCE demonstrated a pooled sensitivity and specificity of VCE for BE detection of 77% and 86% compared to 78% sensitivity and 90% specificity for upper GI endoscopy.

## 9. ESS - Elastic Scattering Spectroscopy

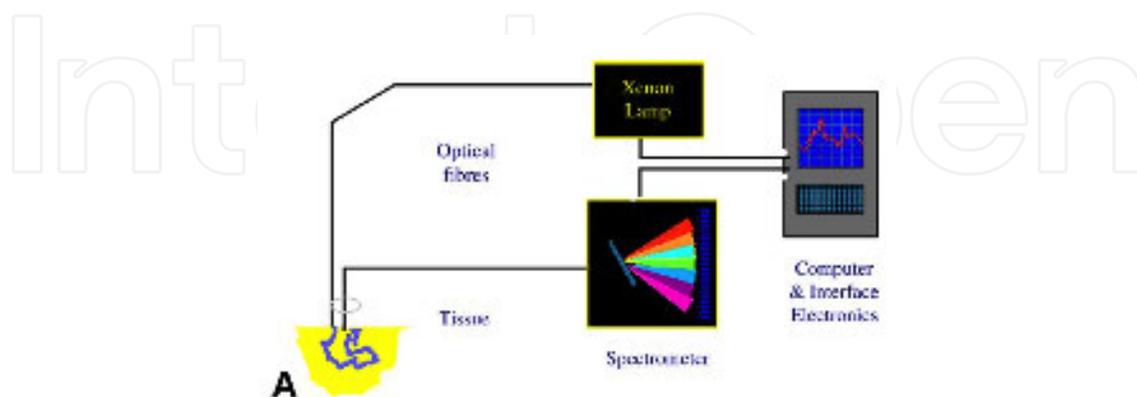
Elastic scattering spectroscopy (ESS) is based on white light scattering. In ESS, photons hit tissue and are backscattered without a change in wavelength. The relative intensity of this backscattering is influenced by the composition of the interrogated tissue, specifically the relative concentration of scatterers (i.e. nuclei, mitochondria, connective tissue) and absorb-

ers (i.e. hemoglobin). With the transition to dysplasia or neoplasia, tissues typically experience an increase in nuclear crowding and enlargement and a change in biochemical composition. All of these changes lead to characteristic alterations in light scattering, which can be used to delineate different tissue types.

ESS spectra relate to the wavelength-dependence and angular-probability of scattering efficiency of tissue micro-components. The sizes, indices of refraction and structures of the denser sub-cellular components (e.g., the nucleus, nucleolus, mitochondria) are known to change upon transformation to premalignant or malignant conditions. Indeed, histopathologists use these nuclear changes to grade dysplasia, i.e. the sizes and shapes of nuclei and organelles, the ratio of nuclear to cellular volume (nuclear:cytoplasmic ratio) and clustering patterns (nuclear crowding).

ESS is a point measurement. Mourant *et al.* analyzed elastic light scattering from isolated mammalian tumor cells and nuclei [64]. Using cells at different stages of growth in the cell cycle they demonstrated that light scattering at angles greater than about  $110^\circ$  was correlated with the DNA content of these cells. Based on model calculations and the relative size difference of nuclei from cells in different stages of growth, they suggest that this difference in scattering results from changes in the internal structures of the nucleus that are increased in the mitotic states.

In order to take optical measurements a flexible fibre-optic probe is passed down the working channel of an endoscope and normal white light is shone at the underlying tissue for a fraction of a second. The back-scattered light is collected and a spectral analysis performed. This scattering of light is sensitive to changes in the structure of the underlying cells and has already been shown to be able to detect HGD with a high degree of accuracy. Using pattern recognition techniques such as multivariate discriminate analysis, algorithms have been developed to classify spectra as premalignant or benign tissues. Optical measurements require less than a second to collect and during the collection of over 10,000 readings in previous studies no complications have occurred.



Our group at the National Medical Laser Centre at University College London have performed the largest clinical *in vivo* study of scattering spectroscopy to date, evaluating the value of ESS in discriminating dysplastic and non-dysplastic tissue in Barrett's esophagus

[65]. A total of 181 matched biopsy sites from 81 patients, where histopathological consensus was reached, were analysed. There was good pathologist agreement in differentiating high grade dysplasia and cancer from other pathology ( $\kappa = 0.72$ ). Spectral data was analysed by LDA + PCA to form a model which was then tested by leave one out cross validation (jackknife analysis). Elastic scattering spectroscopy detected HGD or cancer with 92% sensitivity and 60% specificity. If used to target biopsies during endoscopy, the number of low risk biopsies taken would decrease by 60% with minimal loss of accuracy. ESS had a negative predictive value of 99.5% for high grade dysplasia or cancer. These novel and very promising results show that ESS has the potential to target conventional biopsies in Barrett's surveillance. In order to validate this technique, a prospective study is required.

## 10. Raman spectroscopy

The Raman effect was first discovered and described in 1928 by Chandrasekhar Venkata Raman, for which he was awarded the Nobel Prize in 1930. It is an optical diagnostic technique that is a very valuable analytical tool. Only recently has it been used for biological and medical research. It exploits the frequency shift, which occurs when a sample is illuminated with laser light, due to the excitation of vibrational states in the constituent molecules. The most significant characteristic of Raman spectra is that the intensity of the individual peaks is linearly proportional to the concentration of the molecular constituents. Thus, a precise molecular fingerprint is obtained. It is a rapid, non-destructive, optical scattering technique that has the ability of objective identification of molecular markers such as protein conformation, nucleic acid and glycogen content. Thus the Raman spectrum is a direct function of the molecular biology of the tissue. Extensive work has identified typical peaks, in the region  $900\text{e}1800\text{ cm}_1$ , seen in the normal esophageal epithelium that changes during the process of malignant transformation. These may be useful for optical detection of neoplastic transformation during endoscopy.

## 11. FTIR spectroscopy

FTIR spectroscopy is an analytical technique that is based on the absorption of light in the region that excites molecular vibrations. Most spectrometers operate in the mid-IR range of approximately  $4000\text{ to }900\text{ cm}^{-1}$ . Molecular vibrations can be excited to higher levels by the absorption of radiation in this range. Absorption occurs when the energy of the IR radiation matches the energy difference of the 2 vibrational states. In this way, IR spectra gives information about the molecular vibrations in a given sample manifesting themselves in absorbance peaks at variable frequencies.

There is much literature outlining the success of FTIR spectroscopy in the identification of cancerous tissues from normal tissue. Donna *et al.* [66] found specific spectral changes in the Fourier Transform spectrum of esophageal cancers. Such changes were attributed to a range

of molecular alterations such as the decrease in glycogen level and the increase in DNA content. Limited work has however been done in the discrimination of the pre-malignant dysplastic states of Barrett's esophagus using FTIR spectroscopy.

In 2009 Wang *et al.* [67] studied the use of FTIR in tissue from patients with BE. In this series, spectra were collected from 98 excised specimens of the distal oesophagus, including 38 squamous, 38 intestinal metaplasia (Barrett's), and 22 gastric, obtained endoscopically from 32 patients. They demonstrated that DNA, protein, glycogen, and glycoprotein comprise the principal sources of infrared absorption in the 950- to 1,800-cm<sup>-1</sup> regime. The concentrations of these biomolecules were quantified by using a partial least squares fit and used to classify disease states with high sensitivity, specificity, and accuracy. Moreover, use of FTIR to detect premalignant (dysplastic) mucosa results in a sensitivity, specificity, positive predictive value, and total accuracy of 92%, 80%, 92%, and 89%, respectively, and leads to a better inter-observer agreement between two gastrointestinal pathologists for dysplasia (K 0.72) versus histology alone (K 0.52). This was the first study that demonstrated that the concentration of specific biomolecules can be determined from the FTIR spectra collected in attenuated total reflectance mode and can be used for predicting the underlying histopathology, which will contribute to the early detection and rapid staging of many diseases.

## 12. Virtual colonoscopy

Virtual colonoscopy (VC) is a diagnostic method enabling the generation of two-dimensional and three-dimensional images of the colon and rectum from the data obtained with relevant imaging modality, usually spiral computed tomography (CT). If CT is used, the method is also called CT colonoscopy, CT colonography, or CT pneumocolon. The main advantages of the VC which support its broader application in medical practice include: limited invasiveness, improved compliance of patients and value for screening for colorectal cancer.

The patient undergoing helical computed tomography with the intent of obtaining VC should undergo complete bowel preparation as for other procedures within abdomen, e.g. endoscopic colonoscopy. The priority is assigned to evacuation of the contents of the colon before CT. For this purpose, many agents are used including ethylene glycol electrolyte solution, magnesium citrate or oral sodium phosphate. Nevertheless, the quality of bowel preparation for VC varies considerably between different centres (Van Uitert *et al.*, 2008). The trend for the optimization of the diagnostic procedures and limitation of the burden to the patient resulted also in a strategy focusing on the performing of the optical colonoscopy just after VC, if it is positive for pathological lesions in the colon, in order to avoid repetition of the bowel preparation procedure. A strategy enabling identification of the artifacts resulting from fecal contents in the bowel in the process of generation of VC images was also proposed. This is achieved by labeling it with some type of contrast agent, e.g. barium or meglumine diatrizoate taken orally before the CT (Iannaccone *et al.*, 2004).

Growing use of the VC is supported by its lower invasiveness in the comparison to other diagnostic procedures and potential for higher compliance from patients. These features increase the value of the techniques as a screening test for disorders of the colon. The main indications for VC include screening for colonic polyps or cancer and failure or inadequate results of optical colonoscopy due to anatomical conditions or pathological lesions, e.g. obstruction of the colon lumen. Furthermore, the VC enables also for examination of extra-colonic structures not accessible during standard colonoscopy. This may be particularly important for these patients in whom pathological lesions were detected inside the colon lumen.

The Guidelines issued in 2008 by American Cancer Society, American College of Radiology and US Multi-Society Task Force on Colorectal Cancer included VC within recommended screening tests for colorectal cancer, which should be performed at 5 years intervals in population of at least 50 years or older (Levin et al., 2008). According to the Guidelines, VC should be performed after complete bowel preparation. The detection of a polyp of size >6 mm in VC necessitates the performance of optical colonoscopy, preferably the same day or second complete bowel preparation is needed

### **12.1. Patient preparation for VC**

The purpose of bowel preparation is to clean out the colon before imaging. Preparations can be the so-called wet preparations, such as polyethylene glycol and sodium phosphate preparations or the drier laxatives including magnesium citrate preparations, fleet enemas, bisacodyl tablets and LoSo Prep. Generally phospho-soda (fleet enema) is recommended for young and healthy patients while the polyethylene glycol preparation is preferable for the elderly.

Prepless techniques are currently also being investigated, requiring faecal tagging methods. This is achieved by the use of orally ingested agents, usually dilute barium or iodinated contrast medium that 'tag' or 'label' residual fluid or faecal matter.

A single dose of laxative together with three doses of 250 ml 2.1 % w/v barium sulphate the day before the scan may equal diagnostic performance in fully prepared patients. Image analysis requires a dedicated CT colonographic software package to 'subtract' the high attenuation labelled faecal residue from the colonic lumen, i.e. CT colonographic software package with a subtraction capability.

### **12.2. Data acquisition**

Thin-slice acquisition protocols with multi-detector row CT to cover the entire abdomen in a single breath hold should be used; 2.5 mm or 1.25 mm collimation is recommended.

### **12.3. Positioning**

Scanning usually begins in the supine position and is subsequently performed in the prone position if fluid is present in the colon. The second acquisition is to ensure that fluid-filled segments can be interpreted later.

#### 12.4. Premedication

The efficacy of administering Buscopan or glucagon before scanning to improve colonic distension is controversial. In a study of 240 patients who underwent virtual colonoscopy, Rogalla and colleagues found that glucagon improved distension significantly only when the results were analysed per segment; however Buscopan provided better volume distension and significantly reduced the number of collapsed colonic segments.

#### 12.5. Insufflation

Automated insufflation with carbon dioxide (CO<sub>2</sub>) using an automatic insufflation device is recommended as this maintains a constant CO<sub>2</sub> pressure during scanning. Where an automatic insufflator is not available, a hand pump may be used with insufflation of room air.

#### 12.6. Low-dose CT

Intrinsic high contrast between the colonic wall and insufflated gas allows dose-saving low MA protocols (e.g. 50 mAs). Recent data suggest that excellent sensitivity for cancer and polyps of over 6 mm can be achieved using a collimation of 2.5 mm and tube current of 10 mAs giving an effective dose of 2.15 mSV in men and 2.75 mSV in women.

#### 12.7. Intravenous contrast for problem solving

The use of contrast is also controversial in virtual colonoscopy. A study performed by Martina Morrin and colleagues found that sensitivity improved from 58% to 75% with the use of IV contrast. Intravenous contrast is helpful if there is poor preparation of the patient and should be used as a problem-solving tool.

#### 12.8. Interpretation of data

Interpretation of data can be performed by a 3D fly-through endoluminal approach, with simultaneous correlation with 2D axial images and 2D MPR images, software packages which include multiple imaging layout formats for adequate visualisation of the entire colon.

As CT colonography becomes more widespread, there is increasing inter-observer variation in interpretation. Computer-aided diagnosis (CAD) plays an important role in this regard. When applied to the colon, CAD relies on three main steps: (i) extraction of the colon from the 3D CT volume; (ii) identification of potential polyp candidates; and (iii) eliminating false positives as far as possible.

#### 12.9. Detection of lesions

In a study performed by Pickhardt *et al.* polyps greater than 6 mm were detectable using 1.25 – 2.5 mm collimation with multi-detector row CT. Sensitivity and specificity of CT colonography decreases as lesion size decreases below 5 mm.

The gastroenterological literature emphasizes the advanced adenoma as an appropriate target for screening. The advanced adenoma is classified by size or histology, with lesions greater than 10 mm or with villous histology being significant.

There has been debate about significance of small lesions in CT colonography screening or surveillance programmes; however practically this is not an issue for endoscopic screening techniques, as all lesions seen are removed. However with radiological screening, Pickhardt *et al.* have considered a cut-off size of 8 mm or greater as a recommendation for conventional colonoscopy, and recommend repeat follow-up scans for smaller lesions after 2 – 3 years.

### 13. Endoscopic ultrasound (EUS)

EUS combines 2 modalities: endoscopic visualization and high-frequency US. The ability to image the wall of the GI tract as a series of definable layers corresponding to histology, rather than as a single entity, is the basis for most indications for EUS. Other indications have emerged from the ability of EUS to provide detailed images of areas in immediate proximity to the GI tract and to guide needles precisely through the gut wall into surrounding structures.

The addition of endoluminal US offers a unique advantage over traditional endoscopy, allowing precise differentiation of the individual layers of the GI tract, and direct imaging of the surrounding organs and tissue. EUS allows assessment of submucosal GI lesions, loco regional staging of GI malignancy, tissue diagnosis, and staging of pancreaticobiliary lesions, non small-cell lung carcinoma, and mediastinal disease. In prospective trials, EUS has consistently been shown to have a significant impact on diagnosis and management. EUS-guided FNA has emerged as an adjunctive modality during standard endosonography, allowing tissue diagnosis of submucosal lesions, extraluminal lesions, and/or lymph nodes. Furthermore, therapeutic uses for EUS have been described and are used on a limited basis in some institutions.

EUS has become firmly established as an adjunctive endoscopic imaging study for patients with previously identified lesions of the GI tract and surrounding organs. Multiple studies suggest that EUS is superior to CT for tumor (T) and lymph node (N) staging of luminal and pancreaticobiliary malignancies. The ultimate choice of staging modalities is largely dependent upon patient selection and local expertise.

#### 13.1. The role of endoscopic ultrasound in staging of oesophageal cancer

Following on from a histological diagnosis of OAC it is imperative to complete a clinical TNM staging. This has recently been changed in the American Joint Committee on Cancer (AJCC) Seventh Edition.

TNM staging of esophagogastric junction (EGJ) adenocarcinoma

Primary tumor (T)*	
Tx	Primary tumor cannot be assessed
T0	No evidence of primary tumor
Tis	High-grade dysplasia
T1	Tumor invades lamina propria, muscularis mucosae, or submucosa
T1a	Tumor invades lamina propria or muscularis mucosae
T1b	Tumor invades submucosa
T2	Tumor invades muscularis propria
T3	Tumor invades adventitia
T4	Tumor invades adjacent structures
T4a	Resectable tumor invading pleura, pericardium, or diaphragm
T4b	Unresectable tumor invading other adjacent structures, such as aorta, vertebral body, trachea, etc.
Regional lymph nodes (N)†	
Nx	Regional lymph node(s) cannot be assessed
N0	No regional lymph node metastasis
N1	Metastasis in 1-2 regional lymph nodes
N2	Metastasis in 3-5 regional lymph nodes
N3	Metastasis in seven or more regional lymph nodes
Distant metastasis (M)	
M0	No distant metastasis
M1	Distant metastasis
Histologic grade (G)	
Gx	Grade cannot be assessed - stage grouping as G1
G1	Well differentiated
G2	Moderately differentiated
G3	Poorly differentiated
G4	Undifferentiated - stage grouping as G3 squamous

Table 1. 2011 AJCC 7<sup>th</sup> Edition TNM staging guidelines

The evidence suggests that EUS can successfully differentiate early (T1) from advanced intramucosal disease but it is poor at differentiating mucosal from submucosal lesions. Its role in managing patients with early disease therefore appears to be limited, although it does appear valuable for detecting associated malignant lymphadenopathy. Although the quality of computed tomography (CT) and magnetic resonance imaging (MRI) images has improved dramatically, EUS remains part of the standard algorithm for staging tumours [66;67].

Studies with small cohorts of cases have demonstrated that EUS can accurately predict submucosal invasion, especially when lesions are examined with high frequency EYS probes (20MHz) as this modality helps to delineate all 9 layers of the oesophagus compared to just the conventional 5 layers as seen by normal resolution EUS. Furthermore the role of EUS staging is limited by operator experience, location of the neoplasia and morphology of the lesion (flat versus elevated versus depressed).

In the last few years, endoscopic assessment of patients with early oesophageal cancers has come increasingly to rely on EMR to assess for submucosal invasion. In a prospective study of 64 patients by the Wiesbaden group, all subjects were carefully screened with conventional radial EUS at a frequency of 7.5 MHz and with HFUS if a visible lesion was present [68]. Pre-EMR staging was in agreement with the histological findings in 58 of the 64 patients (91%) evaluated. Two cases of EUS tumor over stage and 4 cases of EUS tumor downstage occurred.

In the largest trial to date, a prospective blinded trial by the same group compared staging of early oesophageal carcinoma using high resolution endoscopy (HR-E) with HFPUS [69]. There was no significant difference in diagnostic accuracy between the two techniques (83% for HR-E and 80% for HFPUS). Sensitivity for mucosal tumours was more than 90% for both modalities while sensitivity for submucosal tumours was lower, at 56% for HR-E and 48% for HFPUS. HFPUS was significantly more accurate at staging submucosal tumours in the tubular oesophagus (10/11; 91%) than those located at the oesophagogastric junction (2/14, 14%).

In a large study of 50 patients from Nottingham by Thomas et al. [70] the role of EUS in detecting depth of invasion and nodal involvement was investigated in patients with early Barrett's associated neoplasia of the oesophagus. Visible lesions in the Barrett's segment were described as Paris types 0-1 (n = 9), 0-IIb (n = 12), 0-IIa (n = 12), 0-IIa + IIc (n = 6), and 0-IIc (n = 5). Of the 50 patients, 46 (92%) had either EMR (n = 17), oesophagectomy (n = 23), or both (n = 6). All 12 patients (100%) with Paris 0-IIb lesions had T0/T1 m staging on EUS confirmed with resection histology. The sensitivity for EUS T-staging for Paris classification was 71.4% for type 0-I, 100% for type 0-IIb, 83% for type 0-IIa, 66.7% for type 0-IIa + IIc, and 66.7% for type IIc. Overall, 8 (17%) of the 46 patients were under staged and 2 (4%) were over staged. This study demonstrated that for detecting submucosal invasion, EUS has a sensitivity of 66%, a specificity of 93%, a negative predictive value of 85%, and a diagnostic accuracy of 84.4%.

## 14. Comparing EUS to CT and PET CT

EUS is also an important tool for evaluating malignant lymph nodes with a high sensitivity and specificity. The ability of EUS to detect lymph node involvement has been compared to computerised tomography (CT) in several studies. In a prospective study of 100 patients with confirmed early cancer in BE, Pech *et al.* compared the two modalities for accuracy of lymph node staging [71]. For the purposes of this study lymph nodes were considered as non-malignant when the pathological assessment was negative or the long-term follow-up showed no progression. EUS had a sensitivity of lymph node involvement of 75% and specificity of 97%, compared to 38% sensitivity and 100% specificity for CT.

In a more recent series by Choi et al. [72] where a total of 109 patients with respectable OAC were prospectively enrolled and retrospectively reviewed for evaluation of pre-operative EUS, PET and CT. The study showed that the overall accuracy of EUS for T-staging was 72%, and importantly it was the only method of delineating the oesophageal wall layers. The sensitivities for N staging were 42% for EUS, 49% for PET, and 35% for CT, and their specificities were respectively, 91.87 and 93%. The accuracy for N staging was 66% for EUS, 68% for PET and 63% for CT, and it did not differ across the 3 diagnostic modalities. This series shows that for loco-regional staging, EUS provides excellent T staging accuracy and similar accuracy for N staging compared with PET and CT scanning.

## 15. Conclusion

There have been significant efforts in recent years towards improving the ability to make real-time pathological diagnoses during endoscopy of the upper gastrointestinal tract. Decision making with respect to endoscopic treatment has been informed greatly as a result, meaning therapy can be delivered without delay obviating the need for repeated endoscopic procedures.

Practices have evolved greatly from the traditional approach of WLE and non-targeted biopsy. Today many more additional techniques are available, from chromoendoscopy and NBI to improved post-processing technology, through to the potential of OCT and ESS. These have prospective roles not only in detecting dysplasia in Barrett's esophagus, but also in the diagnosis and management of early esophageal squamous cell carcinoma and adenocarcinoma. The introduction of virtual colonoscopy with high sensitivity and specificity will allow skilled endoscopists to focus their time on interventional procedure and remove some of the growing burden of routine diagnostic colonoscopy. EUS continues to use the established radiological modalities in the GI tract to define tissue layers and stage neoplastic diseases accurately.

The challenge remains to clarify the exact roles of these techniques in clinical practice, to standardize their use (particularly with respect to classification systems) and to appropriately incorporate them into clinical practice on a large scale.

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