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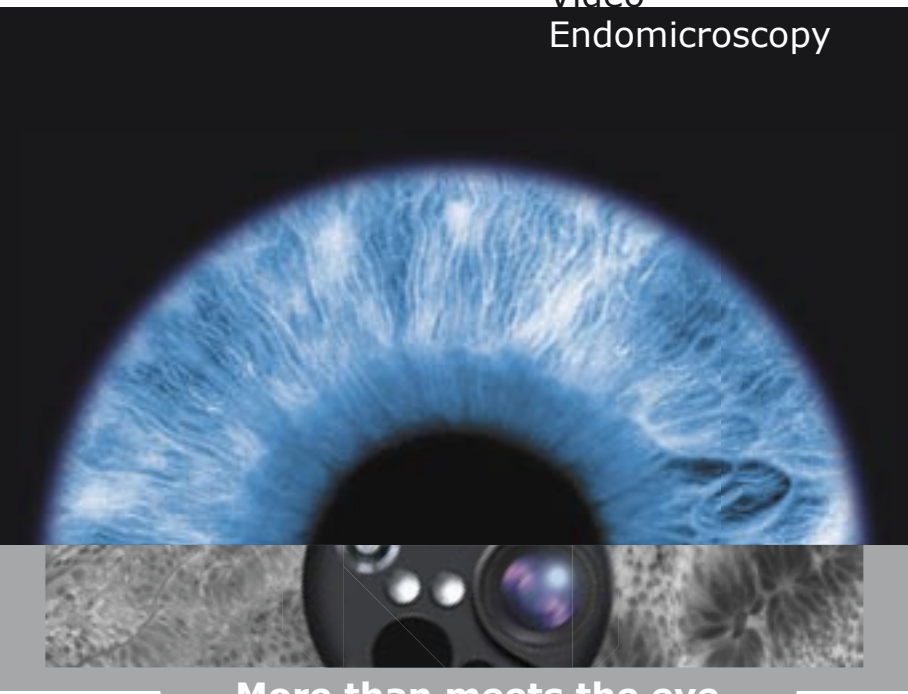

More than meets the eye

Mini-Atlas of Confocal Laser Endomicroscopy

PENTAX

Index

Preface

Dear colleagues,
It is a great pleasure to present you with this coloured mini-atlas as an introduction to confocal endomicroscopy. Confocal endomicroscopy is a newly developed endoscopic technique, where subsurface analysis of the gut mucosa and in vivo histology during ongoing endoscopy becomes possible in full resolution by point scanning laser analysis.

Within this mini-atlas we will present the technology, the diagnostic spectrum and the currently available clinical data about confocal laser endomicroscopy. The diagnostic spectrum of confocal endomicroscopy is currently expanding from screening and surveillance for colorectal cancer towards Barrett's oesophagus, Helicobacter pylori-associated gastritis and gastric cancer. The new detailed images seen with confocal laser endomicroscopy are unequivocally the beginning of a new era where this optical development will allow a unique look on cellular structures at and below the surface of the gut.

We would like to thank all contributors for their excellent input into this ongoing project. Furthermore we would like to thank PENTAX and OPTISCAN for their support and help. Especially we like to mention Daniela Janell, Steven Thomas and Peter Delaney for their enthusiastic and ongoing support.

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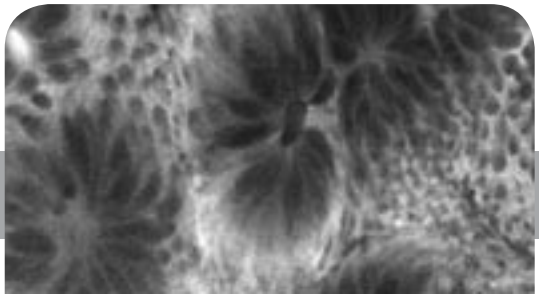
September 2005



Introduction

White-light endoscopy is used for gross examination of the mucosal surface in the gut. Different technologies like chromoendoscopy, magnifying endoscopy or narrow band imaging are being used to predict histology. Thus, the dream and goal of every endoscopist is real-time histology with surface and subsurface analysis of the mucosal layer at high resolution [1].

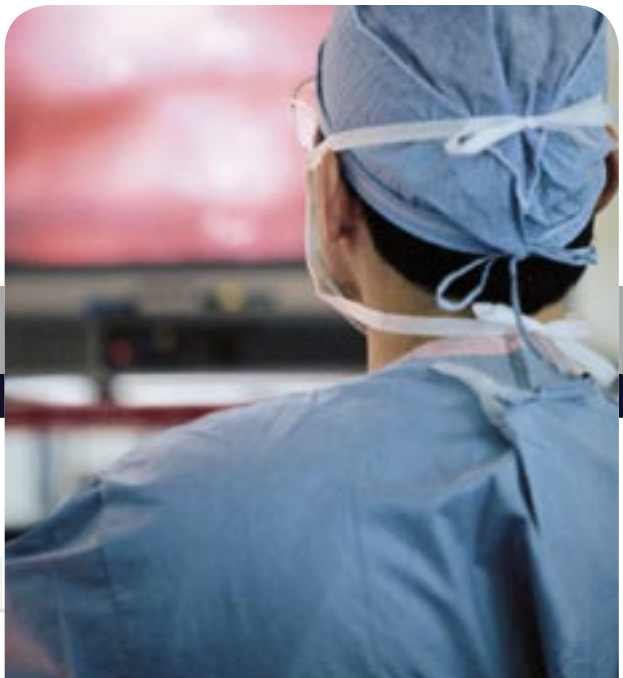
Confocal microscopy is an appropriate technology for the observation of cellular morphology at the time of examination (in vivo histology). Currently, a miniaturised confocal microscope was developed which could be integrated in the distal tip of a conventional colonoscope (joint venture between Pentax, Japan and Optiscan, Australia).

This technology allows white-light endoscopy and confocal microscopy simultaneously. More importantly, the working channel is free to perform endomicroscopic guided biopsies or immediate and targeted endoscopic therapy [4]. Previously, only limited performance has been demonstrated, mostly due to technical limitations imposed by the approaches taken to achieve miniaturisation [5, 6]. The newly discovered single optical fibre acting as both the illumination point source and detection pinhole [4] offers full resolution and was possible due to miniaturised scanning and optical systems.

With the newly developed technique, denoted confocal laser endomicroscopy, subsurface analysis of the gut mucosa and in vivo histology during ongoing endoscopy becomes possible in full resolution by point scanning laser analysis. This review describes the optical and diagnostic possibilities of confocal laser endomicroscopy.

Images in this mini-atlas were collected under study protocols approved by the Ethics Committee in Rheinland-Pfalz, Germany (no. 837.321.03).

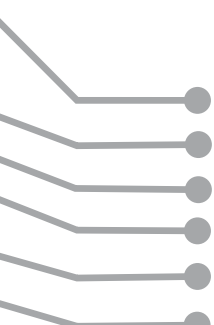
The confocal imaging feature has not been cleared for any specific diagnostic or therapeutic use by the FDA. Any application of this feature over and above the cleared intended use requires the filing of an IDE by the clinical investigator.



The confocal laser endoscope

The components of the confocal laser endoscope are based on the integration of a confocal laser microscope in the distal tip of a conventional video endoscope, which enables confocal microscopy in addition to standard video endoscopy. The diameter of both the distal tip and the insertion tube is 12.8 mm. The distal tip contains an air and water jet nozzle, two light guides, an auxiliary water jet channel (used for topical application of the contrast agent) and a 2.8 mm working channel. Actuation of imaging plane depth relative to the surface of the tissue is controlled using two additional buttons on the endoscope control body.

During laser endoscopy a single line laser delivers an excitation wavelength of 488 nm and the maximum laser power output is ≤1mW at the surface of the tissue. Confocal image data are collected at a scan rate of 0.8 frames/sec (1024x512 pixels) or 1.6 frames/sec (1024x1024 pixels). The optical slice thickness is 7 µm with a lateral resolution of 0.7 µm. The field of view is 500x500 µm. The range of the z-axis was 0-250 µm below the surface layer. Confocal images can be generated simultaneously with endoscopic images.



Confocal microscope

Air/water nozzles

Water jet

Objective lens

Instrument channel

Light guide

Contrast agents

A fluorescent contrast agent is used and needed to achieve high contrast images using confocal endomicroscopy. Potentially suitable agents in humans are fluorescein, acriflavine, tetracycline or cresyl violet. The contrast agents can be applied systemically (fluorescein, tetracycline) or topically (all others) by using a spraying catheter. In human studies most commonly intravenously given fluorescein (10%; colon, oesophagus, stomach) and topically applied acriflavine (0.2%; stomach, colon) were used. No data is so far available on the use of tetracycline and cresyl violet. Fluorescein is an inexpensive, non-mutagenic fluorescence contrast agent that has been frequently used in ophthalmology [7]. Since it is not enriched in the nuclei of intestinal epithelial cells upon systemic administration due to the pharmacokinetic properties, the nuclei are not readily visible in the confocal images. However, cells, vasculature and connective tissue can well be differentiated at high resolution. Intravenously applied fluorescein enables after less than 20 seconds a homogenous contribution of dye which lasts up to 30 minutes. Fluorescein allows to examine the whole spectrum of the z-axis (surface to 250µm depth).

In contrast, Acriflavine can be used topically to stain nuclei and cytoplasm. It becomes absorbed within seconds but is limited to the surface layers of the mucosa (0-100µm). It allows to judge nuclei architecture, which can be particularly important to graduate intraepithelial neoplaisa and cancer. However, the use of Acriflavine should be carefully considered because of the little but residual risk of possible mutagenic activity. Fluorescein and Acriflavine can also be used simultaneously which adds to the labeling properties.

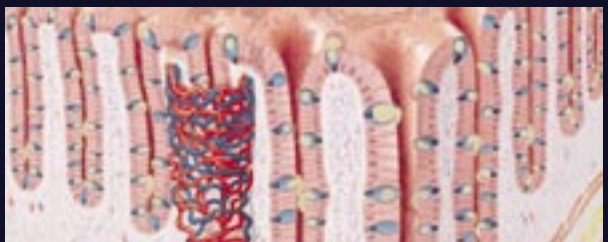
Procedure of confocal laser endoscopy

The confocal endoscope can be handled similarly to a standard endoscope. Initially white-light endoscopy is used to identify suspected lesions. Chromoendoscopy can be used in addition to unmask flat or diminutive lesions. Firstly, contrast dyes are applied. Then the distal tip of the endoscope is placed in gentle contact with the mucosa or lesion to obtain a stable position to reduce movement artifacts. Endomicroscopy should always be used in a targeted fashion due to the limited field of view (500x500µm).

In every area of interest images from the surface to deeper parts of the mucosal layer can be obtained and stored digitally in a specific folder associated with the site of collection. In vivo-histology becomes visible and an online diagnosis can be achieved. In addition, the digitally stored images can be re-inspected during the ongoing procedure or the images can be used later on for re-assessment or comparative studies.

Endomicroscopic diagnosis is only possible with a fundamental knowledge of the microarchitecture of the gut. Thus, the endoscopist is challenged with new microscopic details that require pathology training and an interdisciplinary approach with the pathologist. The pathologist is always a key partner for endomicroscopy. However, endomicroscopic images can be judged by the endoscopist with high accuracy after a distinct learning curve. Endomicroscopy will help to select and target biopsies and will help to avoid unnecessary biopsies. The time for random biopsies will rapidly decrease after the wide introduction of endomicroscopic systems in the field of endoscopy.

Targeted biopsies are easy to perform due to the proximity of the working channel and the endomicroscopic window at the distal tip of the endoscope [3].



It can be speculated that, based on the endomicroscopic diagnosis, more lesions will be immediately resected and sent to the pathologist for final confirmation.

However, there are relevant differences between conventional and in vivo histology. In vivo histology always provides transverse sections of the mucosa, whereas in conventional histology biopsies are cut preferably in a longitudinal direction. The image plane depth of in vivo histology is limited to 250µm. Thus, the mucosa can be examined but the submucosal layer is usually not displayed. In addition, graduation of neoplastic lesions (differentiation between low- or high-grade intraepithelial neoplasms) is currently not possible by using fluorescein guided endomicroscopy. Due to the pharmacokinetic properties nuclei are not readily visible.

However, the resolution of the endomicroscopic images is remarkably high. It allows a rapid differentiation between neoplastic and non-neoplastic lesions. The newly developed confocal pattern classification for colonoscopy differentiates three different types of tissue: normal epithelium, regeneration and neoplasia [see table 1]. This classification can be used for screening and surveillance for colorectal cancer. Similarly, the confocal Barrett classification based on cellular and vascular changes can be used to diagnose Barrett's oesophagus and Barrett's associated neoplasia [8].

At present, studies about the value of endomicroscopy are available for colorectal cancer screening, surveillance of patients with long lasting ulcerative colitis, in-vivo detection of Helicobacter pylori, diagnosis of gastric cancer and in-vivo detection of Barrett's oesophagus and Barrett's associated neoplasias [8-13].

Colorectal cancer: Screening with endomicroscopy

The confocal laser endoscope can be used routinely for screening of colorectal cancer. Suspected lesions can be examined in a targeted fashion by placing the endomicroscopic window onto the lesion.
27 patients underwent colonoscopy with the confocal endoscope using acriflavine or fluorescein sodium with blue laser illumination to evaluate the different staining patterns of acriflavine and fluorescein. Acriflavine and fluorescein both yielded high quality images. Whereas acriflavine strongly labeled the superficial epithelial cells, fluorescein offered deeper imaging into the lamina propria. Fluorescein was thus used for the prospective component of the initial study in which 42 patients with indication for screening or surveillance colonoscopy after previous polypectomy underwent colonoscopy with the endomicroscopic system. Confocal images were graded according to cellular and vascular changes and correlated with conventional histology in a prospective and blinded fashion.
13,020 confocal images from 390 different locations (256 inconspicuous areas; 134 circumscript lesions) were compared with histologic data from 1,038 biopsies. Subsurface analysis during confocal laser endoscopy allowed detailed analysis of cellular structures. The presence of neoplastic changes could be predicted by using the confocal pattern classification with high accuracy (Sensitivity 97.4%; Specificity; 99.4%; Accuracy: 99.2%) [4].

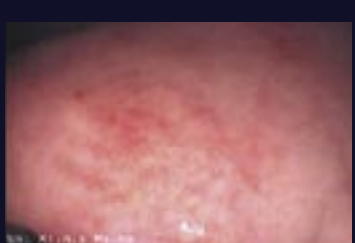
Ulcerative colitis: Surveillance with endomicroscopy

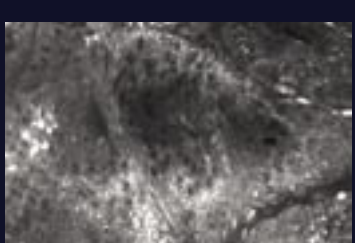
It is impossible to examine the whole surface of the colon in the endomicroscopic mode. Thus, it is important to combine endomicroscopy with chromoendoscopy in patients with ulcerative colitis (UC). Panchromoendoscopy with either methylene blue or indigo carmine is a valid diagnostic tool to improve the diagnostic yield of intraepithelial neoplasias by using the SURFACE guidelines [14]. Chromoendoscopy unmasks circumscript lesions [15] and confocal laser endomicroscopy can be used to predict intraepithelial neoplasias with high accuracy. Thus, targeted biopsies of relevant lesions can be performed and rapid confirmation of neoplastic changes by confocal laser endoscopy during colonoscopy may lead to significant improvements in the clinical management of UC patients. In 41 patients with long lasting ulcerative colitis in clinical remission endomicroscopy in conjunction with methylene blue aided panchromoendoscopy was used for surveillance. Chromoendoscopy with methylene blue did not lead to any interference with the laser scanning system.
14,596 confocal images from 464 different locations (369 inconspicuous areas; 95 circumscript lesions) were compared with histologic results from 1,392 biopsies. 67 of 95 circumscript lesions could only be seen after chromoendoscopy with methylene blue. Different cellular structures (epithelial and blood cells), capillaries and connective tissue limited to the mucosal layer could be identified by confocal microscopy. Due to the pharmacokinetic properties of fluorescein, nuclei could not be seen. However, the presence of neoplastic changes (Sensitivity: 94.4%; Specificity 95.6%; Accuracy 99.3%) and inflammation could be predicted with high accuracy [5].

In the first randomised trial concerning endomicroscopy 153 patients with long-term ulcerative colitis in clinical remission (SURFACE guidelines) were randomised at a 1:1 ratio to undergo conventional colonoscopy or panchromoendoscopy using 0.1% methylene blue in conjunction with endomicroscopy to detect intraepithelial neoplasia or colorectal cancer [10]. Circumscript lesions in the colonic mucosa detected by chromoendoscopy were evaluated with endomicroscopy for cellular and vascular changes according to the confocal pattern classification to predict neoplasia. Targeted biopsies of the examined areas were performed and histologically graduated according to the new Vienna Classification [16].

In the standard colonoscopy group randomized biopsies every 10 cm between the anus and cecum and targeted biopsies of visible mucosal changes were performed. Primary outcome analysis was the histological diagnosis of neoplasia. Using chromoendoscopy in conjunction with endomicroscopy (80 pts.; average examination time: 42 minutes) significantly more intraepithelial neoplasia could be detected (19 versus 4; p=0.007) as with standard colonoscopy (73 pts.; average examination time: 31 minutes). Endomicroscopy revealed different cellular structures (epithelial and blood cells), capillaries and connective tissue limited to the mucosal layer. 5,580 confocal images from 134 circumscript lesions were compared with histological results from 311 biopsies. The presence of neoplastic changes could be predicted with high accuracy (Sensitivity: 94.7%; Specificity: 98.3%; Accuracy: 97.8%).

In summary, chromoendoscopy unmasks circumscript lesions and confocal laser microscopy can be used to confirm intraepithelial neoplasias with high accuracy. Thus, biopsies can be limited to targeted biopsies of relevant lesions. In vivo histology with endomicroscopy may lead to significant improvements in the clinical management of patients with ulcerative colitis.





Barrett`s oesophagus

A prospective study on confocal laser endoscopy was conducted in patients with the indications long lasting reflux symptoms or surveillance endoscopy (known Barrett's oesophagus) as well as patients with suspected Barrett's associated neoplasias. Fluorescein aided endomicroscopy was performed by applying the endomicroscope over the whole columnar lined lower oesophagus (CLE). Images obtained within 1 cm of CLE were digitally stored and targeted biopsies or endoscopic mucosal resection of the examined areas was performed. In vivo histology was compared with the histological specimens. All digitally stored images were re-assessed by a blinded investigator using the newly developed Confocal Barrett Classification to predict histology. Intra- and interobserver variations of the involved endoscopists were evaluated by using kappa statistics. 42 patients fulfilled all inclusion criteria and were enrolled in this study. Endomicroscopy allowed to distinguish between different types of epithelial cells and detected cellular and vascular changes in Barrett's epithelium at high resolution during ongoing endoscopy. Barrett's oesophagus and associated neoplasias could be predicted with a sensitivity of 98.8%/91.7% and a specificity of 94.4%/99.0%, respectively (Accuracy: each 97.5%). The mean kappa for interobserver agreement for the prediction of histopathologic diagnosis was 0.843, whereas intraobserver agreement showed a mean kappa of 0.892. Fluorescence aided endomicroscopy is a new diagnostic tool for Barrett's oesophagus that allows in vivo histology of the mucosal layer during ongoing endoscopy. Gastric and Barrett's epithelium as well as Barrett's associated neoplastic changes can be diagnosed with high accuracy. Thus, endomicroscopy may be helpful for surveillance of patients with GERD [8]

Helicobacter pylori

Furthermore, the characteristic shape of Helicobacter pylori could be seen by confocal laser endomicroscopy. Specifically, it was found that acriflavine-aided confocal laser endoscopy can be used to detect Helicobacter pylori in vivo.
For this approach, the microscopic examination of the gastric mucosa was enabled by the systemic application of fluorescein, but in addition, acriflavine hydrochloride (0.05%; 25ml) was applied topically onto the surface of the tissue with the help of a spraying catheter. Acriflavine coated the surface and was absorbed into the upper part of the gastric mucosa. Acriflavine did not only stain the nuclei of the gastric epithelial layer but also led to detection of Helicobacter pylori at the surface and in deeper layers of the gastric epithelium if a Helicobacter infection is present [6]. Both focal accumulations and individual bacteria were revealed as bright dots by acriflavine staining. On close examination of the endomicroscope images by software zoom, the resolution was found to be sufficient to resolve the characteristic morphology of individual bacteria including both body and flagella [9].

Gastric cancer

Confocal laser endomicroscopy was also used to diagnose gastric cancer and precancerous conditions. Endomicroscopy was performed on five ex-vivo gastrectomy specimens and in upper GI endoscopies in vivo in eight patients. Acriflavine hydrochloride dye was used for ex vivo examinations and intravenous fluorescein sodium for in vivo examinations. A standard upper endoscopy was performed, upon which confocal images were obtained at standardized locations in the gastric antrum, body and cardia, before taking biopsy specimens from the same areas for histopathology. Confocal diagnostic criteria were established by comparison with histopathology as gold standard. Five endoscopists blinded to histology independently scored the confocal images on a template. Inter-observer correlation was analysed using kappa statistics.

2,766 confocal images from 132 different locations were obtained and compared with histology from 44 biopsy specimens. Diagnostic confocal features for normal gastric mucosa, chronic gastritis (presence of chronic inflammatory cells), intestinal metaplasia (goblet cells) and cancer (architectural atypia, increased nuclear- cytoplasmic ratio, chromatin condensation) were established. Using these diagnostic "features", a prospective blinded evaluation showed that the presence of gastric cancer could be predicted from confocal images with high accuracy (sensitivity 84%; specificity 95%; accuracy 80%). Inter-observer agreement by kappa statistics for sites (antrum, body, cardia), presence of intestinal metaplasia and cancer were 0.83, 0.89 and 0.63 respectively.

Confocal laser endoscopy enables immediate in vivo diagnosis of mucosal neoplasia and pre-neoplasia. Diagnoses of intestinal metaplasia and gastric carcinoma can be made using the described criteria with reliable inter-observer agreement. This enables cellular diagnoses during endoscopy as hitherto intestinal metaplasia could only be diagnosed from histopathology. In populations at risk of gastric cancer, confocal endoscopy has potential clinical applications in screening for gastric neoplasia and pre-neoplasia [12].

Conclusion

In vivo confocal laser endomicroscopy is a newly developed diagnostic tool enabling virtual in vivo histology of the mucosal layer during ongoing endoscopy. The new detailed images seen with confocal laser endomicroscopy are unequivocally the beginning of a new era where this optical development will allow a unique look on cellular structures at and below the surface of the gut.
Several prospective studies are currently available underlining the high diagnostic accuracy of confocal laser endomicroscopy. The diagnostic spectrum of confocal endomicroscopy is currently expanding from screening and surveillance for colorectal cancer towards Barrett's oesophagus, Helicobacter pylori associated gastritis and gastric cancers.
Furthermore, multiple other indications like celiac disease, microscopic colitis, MALT-lymphoma are under investigation. It is tempting to speculate that endomicroscopy will play an important diagnostic role in the future during gastrointestinal endoscopy. Further technical developments or improvements will surely enhance the optical possibilities. The door for functional and molecular in-vivo diagnosis will be opened.

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Confocal pattern classification for confocal endomicroscopy in the colon

Crypt architecture

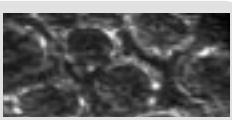
Normal: Regular luminal openings and distribution of the crypts covered by a homogenous layer of epithelial cells including goblet cells.

Regeneration: Star-shaped luminal crypt openings of focal aggregation of regular shaped crypts with regular or reduced amount of goblet cells.

Neoplasia: Ridged-lined irregular epithelial layer with loss of crypts and goblet cells. Irregular cell architecture with little or no mucin.

Vessel architecture

Normal: Hexagonal, honeycomb appearance that presents a network of capillaries outlining the stoma surrounding the luminal openings of the crypts.



Regeneration: Hexagonal honeycomb appearance with no or mild increase in the number of capillaries.

Neoplasma: Dilated and distorted vessels with elevated leakage. Irregular architecture with little or no orientation to adjacent tissue.

Normal colon

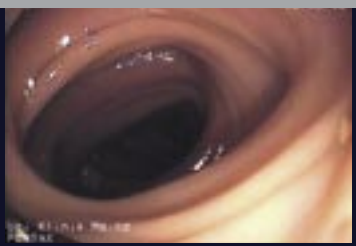


Figure 1

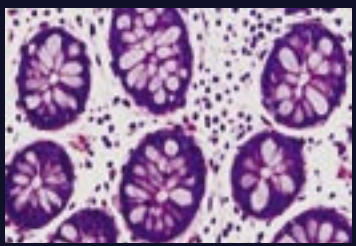


Figure 2

Figure 3

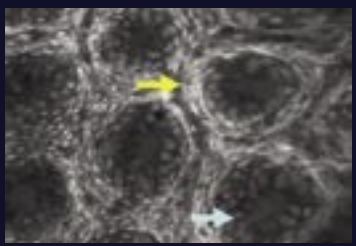


Figure 4

Figure 1: The endoscopy gives an overview of the mucosal surface and allows the observation of areas of interest.

Figure 2: The histology shows a normal architecture of crypts. (staining: Haematoxylin & Eosin)

Figure 3: For the endomicroscopy, flurescein (10%, 5ml) has been used as the contrast agent. (contrast agent: Fluorescein 10%, 5ml)

Figure 4: Acriflavine (0.05%, 15ml) can be used topically for endomicroscopy. Nuclei and collagen between the crypts are visible. (contrast agent: Acriflavine hydrochloride 0.05%, 15ml)

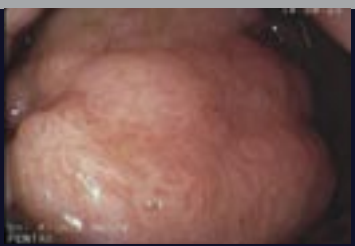


Figure 1

Figure 2

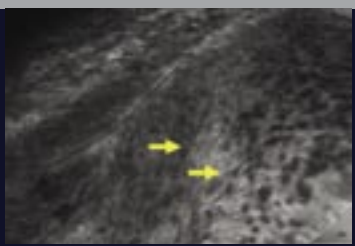


Figure 3

Figure 1: The endoscopy shows a large exophytic growing polyp with tubular and villous surface architecture.

Figure 2: The endomicroscopy shows increased density of capillaries and irregular capillaries. (contrast agent: Fluorescein 10%, 5ml)

Figure 3: The endomicroscopy predicts neoplasia. Mucin in the goblet cells is irregularly distributed. (contrast agent: Fluorescein 10%, 5ml)

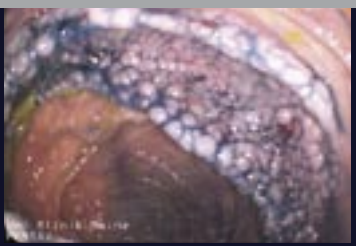


Figure 1

Figure 3

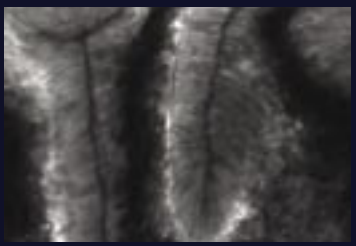


Figure 2

Figure 1: In the ascending colon, multiple polyps are present. (staining: Methylene Blue 0.1%, 10ml)

Figure 2: Tubular architecture of adenomas. (contrast agent: Fluorescein 10%, 5ml)

Figure 3: Loss of goblet cells and irregular arrangement of mucin in the cells indicating dysplasia. (contrast agent: Fluorescein 10%, 5ml)

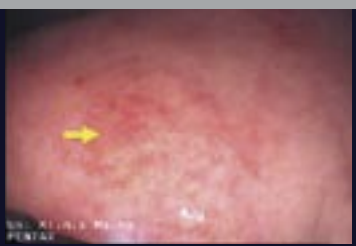


Figure 1

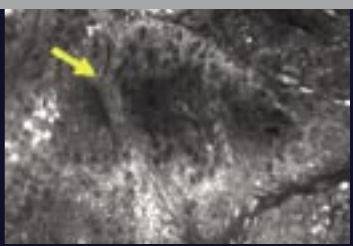


Figure 2

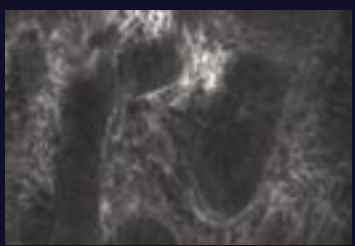


Figure 3

Figure 1: Endoscopic examination showed only slight alterations in the mucosa.

Figure 2: Distinct cellular infiltration. Enlarged space between the crypts indicating inflammation. Crypts show different shapes and sizes. (contrast agent: Acriflavine hydrochloride 0.05%, 15ml)

Figure 3: The characteristic distorted architecture in inflamed areas is clearly visible. (contrast agent: Fluorescein 10%, 5ml)

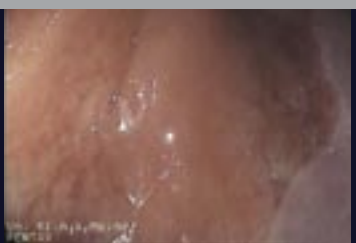


Figure 1

Figure 3

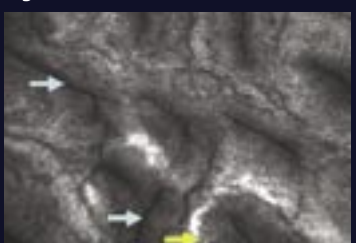


Figure 2

Figure 1: Upper endoscopy revealed a short segment of columnar lined lower oesophagus. Areas with mucosal changes are not visible.

Figure 2: Endomicroscopy of the suspected Barrett's oesophagus shows a villous like architecture with oesophageal glands and columnar epithelium. (contrast agent: Fluorescein 10%, 5ml)

Figure 3: The endomicroscopy shows goblet cells which are pathognomonic for specialised columnar epithelium that defines Barrett's epithelium.

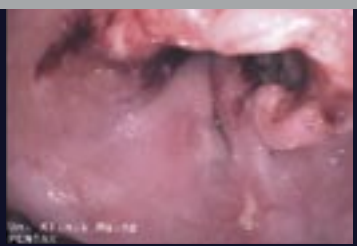


Figure 1

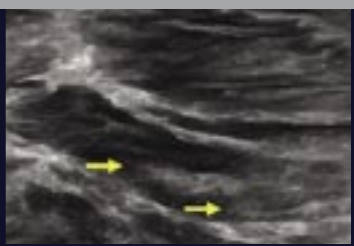


Figure 2

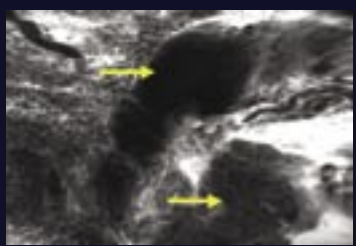


Figure 3

Figure 1: An exophytic and ulcerated cancer is visible in the distal oesophagus.

Figure 2: The endomicroscopy shows slightly irregular squamous cell epithelium at the apical border of the tumor. (contrast agent: Fluorescein 10%, 5ml)

Figure 3: Irregular cells predicting adenocarcinoma of the distal oesophagus. No regular vessels or gland architecture. (contrast agent: Fluorescein 10%, 5ml)

Figure 1

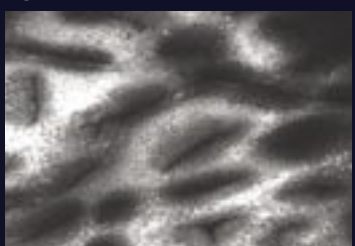


Figure 2

Figure 1 and 2: Foveolae gastricae are clearly visible. Connective tissue between the glands are shown in a typical cobble stone pattern. (contrast agent: Fluorescein 10%, 5ml)

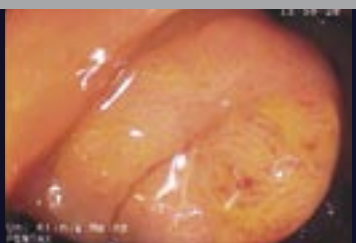


Figure 1

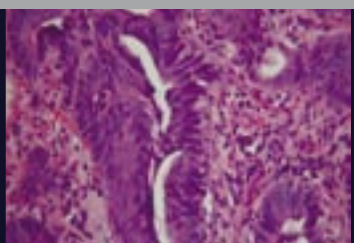


Figure 2

Figure 3

Figure 1: Upper endoscopy shows a circular growing cancer in the distal antrum with tubular surface architecture at the border of the tumour.

Figure 2: The histology presents moderately differentiated invasive tubules and malignant cytology. (contrast agent: Fluorescein 10%, 5ml)

Figure 3: Unstructured or highly irregular gastric glands. Lamina propria is displayed brightly and inhomogeneously due to neoangiogenesis and the leakage of the vessels. (staining: Haematoxylin & Eosin)

Figure 1

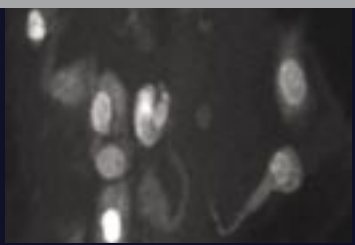


Figure 2

Figure 1: Fluorescein reveals the mucosal architechture whereas arciflavine helps to unmask Helicobacter pylori which are adherent at the mucosal surface or within the gastric glands. (contrast agent: Acriflavine hydrochloride 0.05%, 15ml and Fluorescein 10%, 5ml)

Figure 2: Further magnification (1,000 times) identify Helicobacter pylori with the characteristic shape and presence of flagella. (contrast agent: Fluorescein 10%, 5ml)

Multifocal squamous cell carcinoma of the stomach with limitation to the mucosal layer

Figure 1

Figure 2

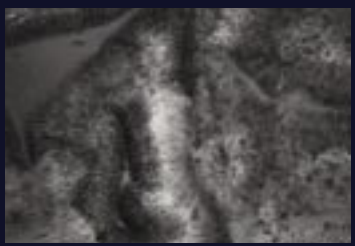


Figure 3

Figure 1: Chromoendoscopy with methylene blue delineated multiple areas with a distinct tubular staining pattern. (staining: Methylene Blue)

Figure 2: Distinct breaks of epithelial architecture with loss of basement membrane. Changes are limited to the mucosa and proximal parts of the lamina propria. (contrast agent: Fluorescein 10%, 5ml)

Figure 3: Endomicroscopic aided biopsies revealed malignant changes. (contrast agent: Fluorescein 10%, 5ml)

Gastric metaplasia in the duodenal bulb

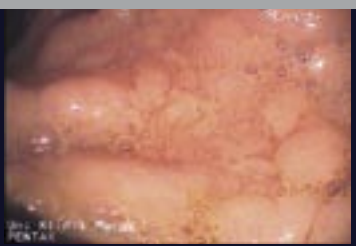


Figure 1

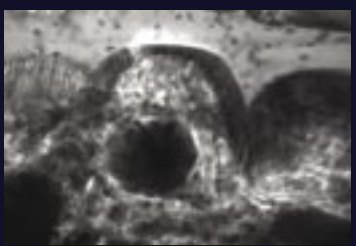


Figure 2

Figure 3

Figure 1: The endoscopy shows the typical surface architecture of gastric metaplasia.

Figure 2: Gastric glands covered by duodenal villi. (contrast agent: Fluorescein 10%, 5ml)

Figure 3: Disruption of the epithelium layer lead to a microscopic bleeding. The fluorescein-coloured blood flows into the duodenal lumen. (contrast agent: Fluorescein 10%, 5ml)

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