Optical coherence tomography (OCT) is a non-invasive imaging technique that provides real-time visualization of internal structures without the need for X-rays or other ionizing radiation. OCT images are generated by measuring the time it takes for light to travel through tissue and return to the detector. This is based on the principle that light is scattered by the microstructure of tissue, and the depth and nature of these scattering events can be translated into images of tissue architecture. OCT has been used in various medical fields, including gastroenterology, to detect and monitor abnormalities in the gastrointestinal tract and biliary system.

In the gastrointestinal tract, OCT can be used to image the mucosa, lamina propria, muscularis mucosae, and submucosa. OCT imaging allows for the detection of pre-neoplastic conditions such as Barrett's epithelium and dysplasia, and can be used to evaluate the depth of penetration of early-stage neoplastic lesions. OCT imaging of the pancreatic and biliary ductal system can improve the diagnostic accuracy for ductal epithelial changes and the differential diagnosis between neoplastic and non-neoplastic lesions.

Abstract
Optical coherence tomography (OCT) is an optical imaging modality that performs high-resolution, cross-sectional, subsurface tomographic imaging of the microstructure of tissues. The physical principle of OCT is similar to that of B-mode ultrasound imaging, except that it uses infrared light waves rather than acoustic waves. The in vivo resolution is 10-25 times better (about 10 µm) than with high-frequency ultrasound imaging, but the depth of penetration is limited to 1-3 mm, depending upon tissue structure, depth of focus of the probe used, and pressure applied to the tissue surface. In the last decade, OCT technology has evolved from an experimental laboratory tool to a new diagnostic imaging modality with a wide spectrum of clinical applications in medical practice, including the gastrointestinal (GI) tract and pancreatic-biliary ductal system. OCT images are generated from measuring the echo time delay and the intensity of back-scattered light. Wavelengths of optical coherence tomography (OCT) are in the near infrared region (700-1400 nm), where light is less scattered by tissue and can penetrate deeper. In the gastrointestinal tract, OCT imaging can be done in humans by using narrow-diameter, catheter-based probes that can be inserted through the accessory channel of either a conventional front-view endoscope, for investigating the epithelial structure of the GI tract, or a side-view endoscope, inside a standard transparent ERCP catheter, for investigating the pancreatico-biliary ductal system. Esophagus and the esophago-gastric junction has been the most widely investigated organ so far; more recently, also duodenum, colon and pancreatico-biliary ductal system have been extensively investigated. OCT imaging of the gastrointestinal wall structure is characterized by a multiple-layer architecture that permits an accurate evaluation of the mucosa, lamina propria, muscularis mucosae, and part of the submucosa. The technique may be, therefore, used to identify pre-neoplastic conditions of the GI tract, such as Barrett's epithelium and dysplasia, and evaluate the depth of penetration of early-stage neoplastic lesions. OCT imaging of the pancreatic and biliary ductal system could improve the diagnostic accuracy for ductal epithelial changes and the differential diagnosis between neoplastic and non-neoplastic lesions.

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Key words: Optical coherence tomography; Barrett’s epithelium; Dysplasia; Adenocarcinoma; Gastrointestinal tract; Pancreatico-biliary ductal system

INTRODUCTION
Optical coherence tomography (OCT) is an optical imaging modality, introduced in 1991[1], that performs high-resolution, cross-sectional, subsurface tomographic imaging of the microstructure in materials and biologic systems by measuring backscattered or backreflected infrared light.

The physical principle of OCT is similar to that of B-mode ultrasound imaging, except that the intensity of infrared light, rather than sound waves, is measured. OCT devices use a low-power infrared light with a wavelength ranging from 750 to 1300 nm in which the only limiting factor is the scattering of light. Scattering occurs when the light interacts with tissue surface and the image formation depends upon the difference in optical backscattering properties of the tissue. OCT images are generated from measuring the echo time delay and the intensity of back-scattered light[2,3]. Wavelengths of infrared light are used in OCT because they have lower scattering properties than visible light and can penetrate deeper into tissue.
the infrared light used in OCT are one to two orders of magnitude higher than ultrasound wavelength, so OCT technology can yield a lateral and axial spatial resolution of about 10 µm, which is 10 to 25 fold better than that of available high-frequency ultrasound imaging. The spatial resolution of OCT images is nearly equivalent to that of histologic sections. The depth of penetration of OCT imaging is approximately 1-3 mm, depending upon tissue structure, depth of focus of the probe used, and pressure applied to the tissue surface. Although the progressive increase in ultrasound resolution is accompanied by a corresponding decrease in depth of penetration, a similar trade-off between resolution and depth of penetration does not occur in OCT imaging.

In OCT, two-dimensional cross-sectional images of tissue microstructure are constructed by scanning the optical beam and performing multiple axial measurements of backscattered light at different transverse positions. The resulting data set is a two-dimensional array that represents the displayed as a grey-scale or false-color image.

Three types of scanning patterns are available for OCT imaging: radial, longitudinal, and transverse. The radial-scan probe directs the OCT beam radially, giving images that are displayed in a “radar-like”, circular plot. Radial scanning can easily image large areas of tissue by moving the probe back over the tissue surface and has the highest definition when the probe is inserted within a small diameter lumen, because the OCT images become progressively coarser when a large-diameter lumen is scanned, due to the progressive increase of pixel spacing with increasing the distance between the probe and the tissue. The linear and transverse probes scan the longitudinal and transverse positions of the OCT beam at a fixed angle, generating rectangular images of longitudinal and transverse planes at a given angle with respect to the probe. Linear scanning has the advantage that pixel spacing in the transverse direction is uniform and can better image a definite area of the scanned tissue, especially in presence of large-diameter and non-circular lumens, where maintaining constant distance from the probe to the surface over the entire circumferential scan may be impossible. Transverse scanning modality provides a better depth of field. Depth of field is the range of distances from the probe over which optimal resolution of scanning can be obtained; current OCT scans permit imaging depths of up to 2-3 mm in tissues, by using probes with different focuses.

In the last decade, OCT technology has evolved from an experimental laboratory tool to a new diagnostic imaging modality with a wide spectrum of clinical applications in medical practice, including the gastrointestinal (GI) tract and pancreatico-biliary ductal system.

OCT TECHNIQUE FOR GI TRACT AND PANCREATICO-BILIARY DUCTAL SYSTEM IMAGING

OCT imaging from the GI tract can be done in humans by using narrow-diameter, catheter-based probes. The probe can be inserted through the accessory channel of either a conventional front-view endoscope, for investigating the epithelial structure of the GI tract, or a side-view endoscope, inside a standard transparent ERCP catheter, for investigating the pancreatico-biliary ductal system.

OCT scanning can be done by maintaining the probe placed lightly or firmly on the wall of the GI tract. When the probe is placed lightly on the mucosal surface, the depth of penetration is limited mainly to the superficial submucosa; by this way superficial epithelium, lamina propria, and the upper part of submucosa are clearly visualized. When the probe is placed firmly against the mucosal surface, submucosa and muscularis propria can be clearly visualized, but details of the superficial layers of the mucosa are lost. When the OCT probe is held in strict contact with the tissue surface, as occurs when it is inserted across strictures of the pancreatico-biliary ductal system, the superficial epithelium may appear compressed and difficult to evaluate.

Several in vitro studies demonstrated the feasibility of OCT in the GI tract: In these studies the GI tract wall was identified as a multiple layer structure characterized by a sequence of hyper- and hypo-reflective layers, with a variable homogeneity of the back-scattered signal. Neoplastic and normal tissue also showed different light backscattering patterns.

Subsequent studies were, therefore, performed in ex vivo tissue specimens and aimed at comparing OCT imaging with histology, to assess the reliability of the OCT technique to identify and recognize the GI tract and pancreatico-biliary wall structure. OCT was shown to clearly differentiate the layer structure of the wall.

In vivo studies confirmed the possibility of OCT to recognize the multiple-layer structure of the GI wall, the possibility to introduce the OCT probe into a standard transparent catheter for cannulation during an ERCCP procedure permits the epithelial layers of the pancreatico-biliary ductal system and sphincter of Oddi to be investigated. From a clinical point of view, OCT imaging of the pancreatic and biliary ductal system could improve the diagnostic accuracy for ductal epithelial changes and the differential diagnosis between neoplastic and non-neoplastic lesions, since in several conditions, X-ray morphology obtained by ERCP and other imaging techniques may be non-diagnostic, and the sensitivity of intraductal brush cytology during ERCP procedures is highly variable.

In our studies, a near-focus OCT probe (Pentax, Lightlab Imaging, Westford, MA, USA) was used, with a penetration depth of about 1 mm and a resolution of approximately 10 µm. The probe operates at 1.2-1.4 µm center wavelength (nominal value: 1.3 µm), with a scan frequency ranging from 1000 to 4000 kHz (nominal value: 3125 kHz). Radial and longitudinal scanning resolutions have an operating range in tissue of 15-20 µm (nominal value: 18 µm), and 21-27 µm (nominal value: 24 µm), respectively. Infrared light is delivered to the imaging site through a single optical fiber 0.006 diameter. OCT probe is assembled in a catheter with an outer
diameter of 1.2 mm; the catheter-based probe consists of a rotating probe encased in a transparent outer sheath, which remains stationary while the rotating probe has a pullback movement of 1 mm/s, with an acquisition rate of 10 frames per second. Using this technique, a segment of tissue 5.5 cm long can be filmed over a 55-s period.

**OCT RECOGNITION OF GI TRACT AND PANCREATICO-BILIARY WALL**

**STRUCTURE IN NORMAL CONDITIONS**

**GI tract**

The esophagus and the esophago-gastric junction has been the most widely investigated organ so far. Esophago-gastric junction appears at OCT investigation clearly recognizable because the stomach wall shows a different OCT pattern, characterized by the presence of a vertical crypt-and-pit architecture of the mucosa that changes abruptly to the horizontal, layered tissue architecture of the esophageal squamous epithelium (Figure 1).

In normal conditions, OCT imaging of esophageal wall recognizes a multiple-layer structure characterized by a superficial weakly scattering (hypo-reflective) layer, corresponding to the squamous epithelium, a highly scattering (hyper-reflective) layer corresponding to the lamina propria, a weakly scattering layer corresponding to the muscularis mucosae, a moderately scattering layer corresponding to the submucosa, and a weakly scattering, deep layer corresponding to muscularis propria (Figure 2). The latter layer is not even recognizable in vivo, depending on the depth of penetration of the OCT probe used. Submucosal glands and vessels have also been identified in vivo. In a recent ex vivo study the muscularis mucosae was distinctly recognized during OCT investigation by using a Ti: Sapphire laser light source.

Overall, the normal esophageal wall architecture shows at OCT imaging a clearly recognizable, layered structure.

OCT images of the gastric mucosa are characterized by less contrast, depending upon the crypt-and-pit architecture of the glandular epithelium. Four layers can be identified from the surface: The glandular epithelium, muscularis mucosae, submucosa with blood vessels, and muscularis propria. Inflammation, as occurs in gastritis, has been reported to produce greater backscattering of the signal and a more pronounced crypt-and-pit pattern architecture, compared with normal tissue.

In the duodenum and small intestine OCT clearly recognizes the mucosa and submucosa with the vascular structure. OCT identified intestinal villous morphology and the degree of atrophy with 100% agreement compared to histology in a study by Hsiung et al., who analyzed OCT images ex vivo on fresh surgical specimens from the small intestine compared with histology. The ability of OCT imaging to recognize the villous pattern and its alterations could be used to identify celiac disease in real time during standard upper GI endoscopy in patients undergoing endoscopy for conditions often related to a misdiagnosed celiac disease, such iron deficiency anemia, osteoporosis, diabetes mellitus, or autoimmune disorders, and select dyspeptic patients who need biopsies for detecting the disease.

In the colon, mucosa and submucosa can also be seen with strong correlation with histology. Mucosa appears as a hyper-reflective layer; submucosa as a hypo-reflective layer with horizontal striations, and the OCT appearance is related to its composition, which could be of assistance in the diagnosis of chronic inflammatory conditions involving the submucosa. Dynamic application of pressure of the OCT probe on the tissue reveals compressibility of both mucosa and submucosa, that may be another criteria for identifying chronic inflammation and fibrosis. The detection of transmural inflammation serves to distinguish patients with Crohn's disease from those with ulcerative colitis; the detection of dysplasia may help in the follow-up of long-standing chronic inflammatory diseases. In presence of dysplasia, the detection of lower boundary of the mucosa may help in identifying the extension of dysplastic changes.
Pancreatico-biliary ductal system

To date, visualization of the epithelium of the main pancreatic duct has been obtained mainly post-mortem, in humans and ex vivo in animals, while in vivo it comes from one study in animal and another in humans. Normal biliary ductal system has been investigated in humans; ex vivo in a study and in vivo, in two ERCP-based studies. Sphincter of Oddi structure has also been investigated in normal and pathological conditions either in ex vivo or in vivo studies.

In a recent study by our group, OCT imaging of main pancreatic duct, common bile duct and sphincter of Oddi normal structure has been shown to be able to provide features that were similar to those observed in the corresponding histological specimens in 80% of sections; the agreement between OCT and histology in the definition of normal wall was good (81.8%). OCT images identified three differentiated layers up to a depth of about 1 mm. From the surface of the duct, it was possible to recognize an inner hypo-reflective layer corresponding to the single layer of epithelial cells close to the lumen, an intermediate homogeneous hyper-reflective layer corresponding to the fibro-muscular layer surrounding the epithelium, and an outer, less definite, hypo-reflective layer corresponding to the smooth muscular structure within a connective tissue in the common bile duct and at the level of the sphincter of Oddi, and connective-acinar structure in the main pancreatic duct (Figures 3-5).

The three different layers showed a linear, regular surface and each layer had a homogeneous back-scattered signal in every frame; however, the differentiation between the intermediate and outer layer appeared more difficult than between the inner and intermediate layer. The thickness of the inner and intermediate layers measured by OCT was similar to those measured by histology; the muscular and connective-acinar structure was visible until the working depth of penetration into the tissue of the near-focus probe (about 1 mm).

Smooth muscle structure appeared at OCT scanning as hyper-reflective, longitudinal strips within a context of hypo-reflective tissue and was particularly recognizable at the level of sphincter of Oddi. Veins, arteries and secondary pancreatic ducts were also identifiable by OCT, characterized by hypo- or non-reflective, well delimited areas.

The images acquired in this study provided information on tissue architectural morphology that could have only previously be obtained with conventional biopsy. These results suggest that OCT could become a powerful imaging technology, enabling high-resolution diagnostic images to be obtained from the pancreato-biliary system during a diagnostic ERCP procedure.
by using two parameters of tissue. OCT features predictive for the presence of intestinal metaplasia are: The absence of the layered structure of the normal squamous epithelium and the presence of the vertical crypt-and-pit morphology of normal gastric mucosa; a disorganized architecture with inhomogeneous backscattering of the signal and an irregular mucosal surface; the presence of submucosal glands characterized by a markedly hypo-reflective tissue below the epithelial surface.

**OCT RECOGNITION OF GI TRACT AND PANCREATICO-BILIARY WALL STRUCTURE IN DYSPLASTIC AND NEOPLASTIC CONDITIONS**

At present, the exact cause of the disorganized architecture and altered light-scattering associated with dysplastic tissue by OCT imaging is unknown. A number of factors have been suggested, including subcellular morphological changes, altered fibrovascular stroma and abnormal mucin content associated with neoplastic tissue change, proliferation of cells leading to a loss of epithelial and stromal orientation, and altered cytological features such as an increased nuclear-to-cytoplasm ratio that may alter infrared light backscattering.

In its current form and resolution, OCT will likely localize areas displaying architectural distortion to guide biopsy.

**GI tract**

Most of the so far published studies used OCT imaging to detect dysplasia and early cancer within Barrett's epithelium. Since the penetration depth of OCT does not exceed 1-2 mm, the technique could be useful, not only in detecting dysplasia, but also in staging superficial cancers that are difficult to stage accurately with ultrasound endoscopy. The technique appears, therefore, of crucial importance in the management of the disease.

Barrett's epithelium is characterized by the presence of specialized intestinal metaplasia within the esophageal mucosa. The hallmark histologic feature of specialized intestinal metaplasia is the presence of goblet cells. Identification of Barrett’s epithelium is of clinical relevance since the lesion requires endoscopic follow-up, being a recognized precancerous condition. In clinical practice Barrett’s epithelium is generally identified by performing multiple biopsies within the areas of gastric metaplasia, either in a random manner or after a previous vital staining with methylene blue (MB).

Although the inter-subject variability of OCT imaging of normal squamous epithelium and gastric mucosa appears to be low, the OCT imaging of Barrett's epithelium demonstrated a greater variability in the previous published studies. OCT features predictive for the presence of intestinal metaplasia are: (1) the absence of the layered structure of the normal squamous epithelium and the presence of the vertical crypt-and-pit morphology of normal gastric mucosa; (2) a disorganized architecture with inhomogeneous backscattering of the signal and an irregular mucosal surface; and (3) the presence of submucosal glands characterized at the OCT imaging as pockets of low reflectance below the epithelial surface (Figure 6). When these OCT criteria were applied to images acquired prospectively, the criteria were found to be 97% sensitive and 92% specific for specialized intestinal metaplasia, with a PPV of 84%. The presence of the crypt-and-pit architecture may render difficult to discriminate between intestinal metaplasia and normal or inflamed gastric mucosa.

Unfortunately, up to now, attempts to identify OCT patterns characteristic for dysplasia, mainly the high-grade type, have been substantially disappointing. The increased nuclear-to-cytoplasmic ratio occurring in dysplasia may alter the light reflection characteristics, giving a more inhomogeneous back-scattering of the signal (Figure 7). Because the degree of reflectivity depends upon nuclear size, a markedly homogenous and hypo-reflective back-scattering of the signal should indicate the presence of high-grade dysplasia; moreover, it is possible that by quantitating the OCT signal as a function of depth, OCT would be able to characterize high-grade dysplasia within intestinal metaplasia tissue. Poneros et al, by using two parameters of tissue reflectivity as an indicator of dysplasia, retrospectively diagnosed high-grade dysplasia with 100% sensitivity and 85% specificity. Such an accurate analysis of the degree of signal reflectivity requires to avoid areas with incorrect artifact signal properties: This may be obtained by the identification of a precisely defined area with homogeneous signal reflectance, an adequate catheter-tissue contact, and a reduction of motion.
artifacts. More recently, the morphological appearance of the OCT images, rather than the quantitative analysis of the OCT signal in the image, were used for the diagnosis and grading of dysplasia; for this purpose an endoscope fitted with an EMR standard cap was used, to stabilize the mucosal surface and avoid movement from esophageal peristalsis and transmitted cardiac and respiratory motion. In this study sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy for dysplasia were respectively, 68%, 82%, 53%, 89%, and 78%.

However, with the current available OCT devices, the recognition of dysplasia within intestinal metaplasia and mainly the differentiation between low- and high-grade dysplasia appears difficult. In a study by Pfau et al. on 24 patients, 30 dysplastic adenomas and 14 hyperplastic polyps were studied; the real-time OCT investigation showed that adenomas were more disorganized than the hyperplastic polyps, with a significantly more disorganized structure ($P = 0.0005$). Moreover, the infrared-light back-scattering of the adenomatous polyps appeared more hypo-reflective than hyperplastic ($P = 0.0007$). By using a computer-generated method to quantify the degree of scattering of individual pixels within a specified area in each image (60 × 60 pixels), it was found that the mean differences in light scattering were significantly greater between adenomatous and normal tissue (mean difference = 45.81), than between hyperplastic polyps and normal tissue (mean difference = 14.86). The real-time OCT infra-red light back-scattering score of polyps was also demonstrated to be a significant predictor of an adenomatous status. However, differently from the dysplasia occurring within Barrett’s epithelium, in the study done by these authors defined OCT parameters histologically proven to detect colonic dysplasia were not found.

Pancreatico-biliary ductal system
Pathological pancreatic ductal system has been investigated by our group in humans in two ex vivo studies performed on multiple surgical pancreatic specimens obtained from patients with pancreatic head adenocarcinoma.

In chronic inflammatory changes involving the main pancreatic duct, OCT still showed conserved three-layer
architecture. However, the inner, hypo-reflective layer appeared slightly larger than normal and the intermediate layer appeared more hyper-reflective than in normal tissue; this is probably because of the dense mononuclear cell infiltrate. The back-scattered signal was heterogeneous with marked hypo- or hyper-reflectance in some sections. The agreement between OCT and histology in the definition of MPD chronic inflammatory changes was poor (27.7%).

The OCT pattern in presence of dysplasia of the main pancreatic duct epithelium was characterized by an inner layer markedly thickened, strongly hypo-reflective and heterogeneous; this OCT finding is probably due to the initial structural disorganization (increased mitosis and altered nucleus/cytoplasm ratio). The surface between the inner and intermediate layers appeared irregular. As in chronic inflammatory tissue, dysplasia too gave strong hyper-reflectance of the intermediate layer, particularly in the part closest to the inner layer. The outer layer did not differ from other non-malignant conditions and appeared homogeneously hypo-reflective.

Overall, normal wall structure and chronic inflammatory or low-grade dysplastic changes cannot be distinguished in 38% of the sections because the architecture of the layers and surface light reflection did not show a characteristic OCT pattern.

In all sections with histologically proven adenocarcinoma, OCT showed a totally subverted MPD wall architecture. The three layers of the ductal wall and their linear, regular surface, normally giving a homogeneous back-scattered signal, were not recognizable. The margins between the connective-fibromuscular layer and acinar tissue were unidentifiable. The back-scattering of the signal appeared strongly heterogeneous, with minute, multiple, non-reflective areas in the disorganized pancreatic microstructure. Of sections with adenocarcinoma, OCT and histology were 100% concordant.

Figure 12 shows magnified OCT images from sections with either normal (A), tumor-associated chronic inflammation (B), low-grade dysplasia (C), and adenocarcinoma (D) tissue.
surveillance of Barrett's epithelium, in order to detect high-grade dysplasia and adenocarcinoma at early stage and identify cases in whom mucosectomy becomes a curative procedure.

In the pancreatico-biliary ductal system, OCT can be used to discriminate between non-neoplastic and neoplastic tissue when strictures of unknown etiology are identified during an ERCP procedure, being its diagnostic accuracy higher than reported for intraductal brush cytology.

However, despite the promising studies reported in literature, with the current available OCT devices the recognition of dysplasia within intestinal metaplasia and mainly the differentiation between low- and high-grade dysplasia appears difficult.

On the other hand, since OCT has a penetration depth that does not exceed the 2 mm, it has a greater capability of diagnosing adenocarcinoma confined within mucosa and submucosa and could, therefore, be useful in staging superficial cancers that are difficult to stage accurately by EUS.

Features characteristic for adenocarcinoma within Barrett's epithelium are the lack of the regular layered morphology of the esophageal wall and a markedly heterogeneous back-reflectance of the signal. However, further studies are needed to evaluate whether OCT can identify and stage the lesion at an early stage.

OCT appears more promising in the differential diagnosis between non-neoplastic and neoplastic lesions arising within the pancreatico-biliary ductal system, since the ductal wall layered structure can be recognized easier and clearer.

At present, it seems to be fairly premature to affirm that OCT plays a role in the real-time diagnosis of dysplasia in vivo. However, improvements in both axial and lateral resolutions to the subcellular level (< 5 µm) together with the development of better light sources and optics, may allow dysplastic cells to be better identified in the future. Doppler OCT could also offer a unique ability to provide detailed subsurface imaging of mucosal microvascular networks.

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