Full-field optical coherence tomography: novel imaging technique for extemporaneous high-resolution analysis of mucosal architecture in human gut biopsies

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MESSAGE

Full-field optical coherence tomography (FFOCT) is an imaging technique of biological tissue based on tissue light reflectance analysis. We evaluated the feasibility of imaging fresh digestive mucosal biopsies after a quick mounting procedure (5 min) using two distinct modalities of FFOCT. In static FFOCT mode, we gained high-resolution images of general gut tissue-specific architecture, such as oesophageal papillae, gastric pits, duodenal villi and colonic crypts. In dynamic FFOCT mode, we imaged individual epithelial cells of the mucosal lining with a cellular or subcellular resolution and identified cellular components of the lamina propria. FFOCT represents a promising dye-free imaging tool for on-site analysis of gut tissue remodelling.

Digestive epithelial cells and their cellular microenvironment are key players in gut health, as well as in initiation and evolution of digestive diseases.[1 2] Characterisation of mucosal remodelling has been improved by the use of high-resolution endoscopic technologies, such as high-definition endoscopy and confocal endomicroscopy.[3 4] However, they remain limited by their resolutions and field of view, and their inability to perform functional or dynamic tests.

Full-field optical coherence tomography (FFOCT), a variant of optical coherence tomography, allows direct capture of 'en face' or cross-sectional images without contrast agent at a micrometric spatial resolution within a depth of a few hundred microns.[5] Recently introduced time-dependence analysis of the FFOCT signal, that is, dynamic full-field optical coherence tomography (D-FFOCT), allows acquisition of sequential images with high temporal resolution, providing novel information on dynamic changes at the subcellular level.[6]

Therefore, the goal of this study was to determine the ability to image mucosal structures in normal endoscopic biopsies from various regions of the gut by using both static full-field optical coherence tomography (S-FFOCT) and D-FFOCT.

Standard endoscopic biopsies were collected at different GI locations in normal-appearing mucosa and were placed in Hank's balanced salt solution. Individual fresh biopsy was placed with the surface of interest facing upward in the biopsy holder of the FFOCT set-up and covered with a dedicated glass slide. This holder was placed in the imaging set-up and silicone oil optical fluid was applied onto the glass slide prior to imaging. Time required between the initiation of the mounting procedure and first image acquisition was less than 5 min. Next, a macroscopic image of the whole biopsy was obtained using the wide-field camera mode in order to screen for regions of interest. S-FFOCT and D-FFOCT images of the selected region were acquired at multiple depths. At the end of the FFOCT imaging procedure, fresh biopsies were fixed in paraformaldehyde solution, cut and stained to obtain 'en face' pathology slides.

Using the S-FFOCT acquisition mode, we were able to gain high-resolution images of biopsies to a depth of around 100 µm, allowing identification of general organ-specific architecture such as oesophageal papillae (figure 1), gastric pits (online supplementary fig 1), duodenal villi (figure 2) and colonic crypts (online supplementary fig 2), as well as highly backscattering structures, probably indicating conjunctive tissue in all biopsied gut segments. In addition, in the oesophagus but not in other gut segments, pericellular lining of epithelial cells could also be detected. Second, using D-FFOCT, we were able to identify cellular and intracellular structures, characteristics of epithelial cells in the oesophagus (figure 1), gastric fundus and antrum (online supplementary fig 1), duodenum (figure 2) and colon (online supplementary fig 2) to a depth of around 100 µm (see online supplementary video). We were also able to visualise probably immune cells not observed in S-FFOCT, as well as other cells of the lamina propria, in the duodenum and colon (figure 2 and online supplementary figure 2). In addition, structures corresponding to conjunctive tissue previously observed in S-FFOCT were also detected in D-FFOCT.

A first major result of the study was the ability to identify distinct cellular structure within fresh gut biopsies using two different FFOCT acquisition modalities. In particular, images obtained using

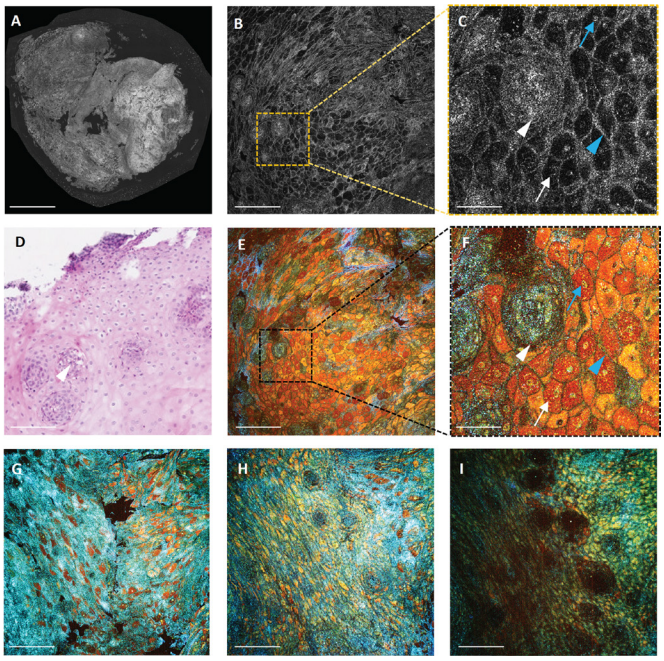

Figure 1 FFOCT images of a normal oesophageal mucosa in S-FFOCT and D-FFOCT modes compared with the 'en face' H&E-stained slide. The whole S-FFOCT image (A), the selected region of interest as obtained in S-FFOCT (B) and D-FFOCT (E), magnified (×4) S-FFOCT (C) and D-FFOCT (F) at a 10 µm depth, en face histological specimen after H&E staining (×100) (D), D-FFOCT imaging of the epithelium from the surface (G), at a 40 µm depth (H) and at an 80 µm depth (I). Epithelial cell cytoplasm (white arrows) and nuclei (blue arrows), pericellular lining (blue arrowheads), lamina propria papillae (white arrowheads) (see Results section in the online supplementary material). Scale bars: 1.25 mm (A), 320 µm (B,E,G,H,I), 80 µm (C,F), 155 µm (D). D-FFOCT, dynamic full-field optical coherence tomography; S-FFOCT, static full-field optical coherence tomography.

S-FFOCT are solely based on the differential reflectance of cell membrane, collagen, adipose and muscle tissue, as different tissues have different reflectance properties.[7] Therefore, conjunctive tissue composed of highly backscattering structures such as collagen was the most intense detected structure.[8]

Another major finding of the study was that by changing FFOCT modality to D-FFOCT, we were able to identify individual epithelial cells in all gut segments studied. In D-FFOCT, variation of interferometric signal caused by intracellular movements or vibrations of cells is measured over time. Because imaging of the metabolic activity allowed various constituents with low reflectance to be revealed from the surrounding tissue, D-FFOCT provided detailed images of a wide range of features that were barely visible with S-FFOCT, improving overall contrast of the images. D-FFOCT takes advantage of the stability of the supporting tissue with time to observe only the weak time-dependent signals from the cells due to the dynamic recording mode.

Histology associated to immunohistochemistry is the standard technique to analyse tissues at a subcellular resolution. However, it requires that the tissue be fixed, subsequently preventing from using the tissue for another technique, and does not allow functional analysis. On the other hand, high-resolution endoscopy provides in vivo images of the surface mucosa but fails to show subcellular structures. Therefore, FFOCT may be able to fill a gap between a precise examination of fixed tissue and a resolution-limited examination of living tissue. Currently, FFOCT only enables imaging of ex vivo specimens in human due to the benchtop nature of the device. In particular, the necessity of still specimen during acquisition currently challenges the development of an in vivo device for applications in gastroenterology, prior to the development of software for correction of motion artefacts.

FFOCT might be an alternative to extemporaneous pathology, as the machine could easily be installed in or near an endoscopy unit. S-FFOCT imaging is fast—30 s for 3D acquisition of a region of interest of 1.3×1.3 mm over a 100-micron depth with 0.5-micron-spaced stacks—and could be used to guide for regions of interest to be more precisely imaged using D-FFOCT, which requires a longer acquisition time, about 12 s per stack of the same region of interest. In a pilot study, S-FFOCT was successfully performed on tissue obtained using endoscopic ultrasound-guided fine needle aspirations.[9] Another study demonstrated the feasibility of ex vivo S-FFOCT for the characterisation of colonic polyps.[10] The development of artificial intelligence for pathology reading might also offer new perspectives for this technology, which generates a considerable number of images requiring rapid and reproducible (observer independent) analysis of data.[11]

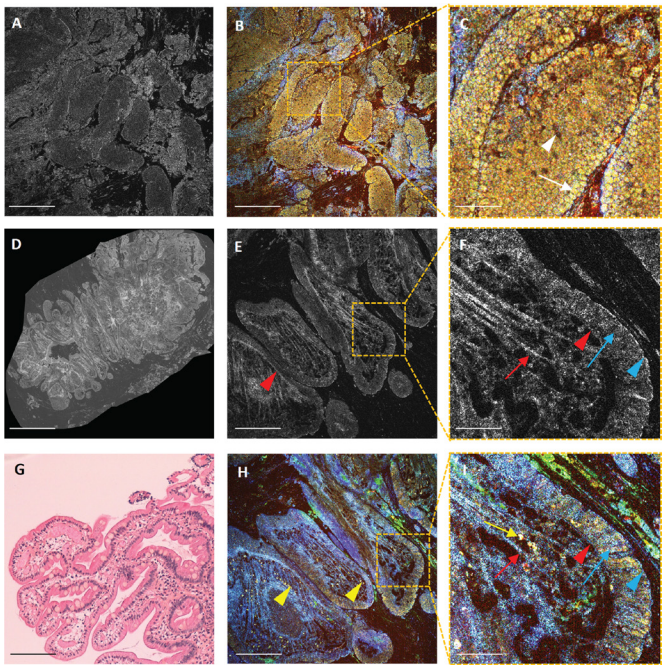

Figure 2 FFOCT images of a normal duodenal mucosa in S-FFOCT and D-FFOCT modes compared with the 'en face' H&E-stained slide. Selected regions of interest as obtained in S-FFOCT (A,E) and D-FFOCT (B,H), magnified (×4) S-FFOCT (F) and D-FFOCT (C,I), at a 0 µm depth (A–C) and a 10 µm depth (E,F,H,I), the whole S-FFOCT image (D) and en face histological specimen after H&E staining (×100) (G). Different behaviours of epithelial cell in D-FFOCT (white arrow, white arrowhead). From outside to inside the villus in S-FFOCT and D-FFOCT: a lining of the proximal side of the epithelium (blue arrowheads), the single-layer epithelium (blue arrows), a delineation of the basal side of the epithelium (red arrowheads) and finally the lamina propria containing linear highly scattering structures (red arrows), thought to be collagen fibres. In D-FFOCT, rich lymphocytic infiltration (yellow arrowheads) and vascular structures containing cells (yellow arrow) within the lamina propria (see the Results section in the online supplementary material). Scale bars: 1,25 mm (D), 320 µm (A, B, E and H), 80 µm (C, F and I), 155 µm (G). D-FFOCT, dynamic full-field optical coherence tomography; FFOCT, full-field optical coherence tomography; S-FFOCT, static full-field optical coherence tomography.

FFOCT imaging allows a precise morphological assessment of the digestive mucosa. On one hand, S-FFOCT enables the analysis of the refractive index fluctuations of the different tissues imaged, which is a key parameter as it characterises how the light interacts with the tissue. As fibrosis mechanisms are still poorly understood in eosinophilic oesophagitis or in Crohn's disease, conjunctive tissue remodelling could be explored using FFOCT. On the other hand, D-FFOCT mode should enable the analysis of mucosal functions, provided that a better understanding of the biological origin of the signals is achieved. Such knowledge could be applied to use D-FFOCT for risk stratification, screening or early diagnosis of epithelial cancers.

In summary, the analysis of standard endoscopic biopsies using static and dynamic FFOCT enabled visualisation of cellular and subcellular structures of the healthy GI mucosa. This promising technology has to be tested in pathological conditions and opens up prospects for the understanding and diagnosis of digestive mucosal diseases.

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