# Optical coherence tomography imaging of colorectal neoplastic polyps developed in genetically modified rats

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## Summary

*The high incidence and mortality of colorectal cancer requires the need to develop new technologies for the early detection and diagnosis of disease in early stages. In this way, cure rates of patients could be increased. Despite the imaging techniques used today for the detection and diagnosis of colorectal cancer, there is still an unmet need for a technique that could provide images of tissue below the visible surface with a higher resolution, and with the possibility of distinguish adenomatous polyps and allow rapid diagnosis in situ previous to the histopathological examination. One of these emerging techniques with great potential is Optical Coherence Tomography (OCT). In this work, a developed neoplastic polyp in the colon of a genetically modified PIRC F344/NTac-Apcam rat has been imaged using OCT in order to observe its subsurface structure. The polyp was imaged with a Thorlabs commercial OCT system at 1300 nm obtaining an axial and lateral resolution of 3 μm and 4 μm respectively. Results show that the neoplastic polyp demonstrated irregular glands with enlarged nuclei in the epithelial layer showing different morphology that affect the underlying homogenous layer structure of healthy colon tissue. 3D rendering of the sample provided by the OCT system allows obtaining an "optical biopsy" for better analysis of the surface and subsurface structure of the colonic polyp. The inclusion of a scanning OCT probe through an endoscopic working channel could be used to perform in-vivo imaging of polyps during colonoscopy as a support for an improved diagnosis.*

## 1. Introduction

Colorectal cancer (CRC) is the third most common cause of cancer death, and represents about 10% of all cancer cases [1-2]. The majority of CRC develops from adenomatous polyps, which are premalignant lesions that develop over a period of years. These polyps are identified using colonoscopy, the gold standard method for detection of polyps. It is an endoscopic examination that provides direct visualization of the colonic mucosa using white-light and narrow-band imaging (NBI) based on light absorption of hemoglobin [3]. Detected polyps are removed by endoscopic polypectomy for further histological analysis to determine the classification of polyps and depth of invasion. However, such a process is invasive and the detection of small polyps (< 5 mm) has a miss rate between 15% and 26% [4-5].

In order to complement the information provided by colonoscopy, optical coherence tomography (OCT) has been progressing towards an 'optical biopsy' of lesions as it provides subsurface polyp image information regarding thickness of mucosal layers and its optical properties [6-7]. With the purpose of developing this research field, ex-vivo colon OCT imaging studies have been performed to determine parameters that differentiate between benign and adenomatous polyps [8-9].

In this sense, the PICCOLO project [10] aims to design and develop a new compact, hybrid and multimodal photonics endoscope probe based on OCT and Multi-Photon Tomography (MPT) for improving in vivo diagnosis and clinical decision support. By combining the outstanding structural information from OCT with the precise functional information from MPT, this innovative endoscope would provide gastroenterologists immediate and detailed in situ identification of colorectal neoplastic lesions and facilitate accurate and reliable in vivo diagnostics, with additional, grading capabilities for colon cancer as well as in-situ lesion infiltration and margin assessment. For that, human representative animal models are used to generate imaging biomarkers that allow automated detection, assessment and grading of disease.

Although the above-mentioned PICCOLO project aims to use jointly MPT and OCT photonics technologies, only OCT technology is addressed in this work. Concretely, a first study has been carried out where neoplastic polyps developed in genetically modified rats have been imaged with the purpose of observing its development as well as proposing the use of OCT as a complementary imaging modality for the analysis of ex-vivo colon samples.

## 2. Methods

### 2.1. Optical coherence tomography

OCT is a non-invasive, high-resolution imaging technology capable of providing cross-sectional and three-dimensional images to visualize the structure of a biological sample [7]. OCT systems are based on partially coherent light interferometry (PCLI) by measuring the magnitude and echo time delay of backscattered light from a sample using a broadband light source with a short coherence length. The time delay represents the longitudinal location of reflection points. The axial and lateral resolution define image quality and for OCT imaging both resolutions are independent from each other.

The basic setup for an OCT is shown in Figure 1. It basically consists of: 1) a light source at a center wavelength λc, 2) a wavelength range δλ, 3) two optical arms created by the beam splitter including the reference and sample. The light travels through both arms creating an interference pattern if the optical distance difference between both arms is within the coherence length of the light source. The interference pattern is then detected with a photodetector and finally digitized with an acquisition card.

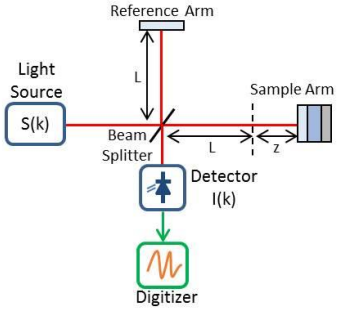


***Figure 1.*** *OCT basic setup*

The samples imaged in this article were imaged using a commercial Thorlabs OCT system Ganymede GAN620 with an OCT-LK2-BB lens at a λc = 900 nm, with an axial resolution of 3 μm and lateral resolution of 4 μm.

### 2.2. Rodent models

Rodent models are used to simulate disease progression for the validation of optical imaging tools due to their accurate modeling of human pathology. In this case in particular, the large intestine of rodents and humans are similar in mural structure [11]. For this paper, ex-vivo tissue samples were sections of the colon of "polyposis in rat colon" (PIRC F344/NTac-Apcam [12-13]) rats that developed neoplastic polyps. Ethical approval for the samples was provided by the CCMIJU Animal Welfare Committee and by the Regional Government of Extremadura.

For the ex-vivo sample preparation, the colon from the rat was excised and a protocol for sample preservation was followed. First, the rat colon was divided in three regions following the ascending, transverse and descending anatomical structures of the colon. The regions were then immersed in 4% formaldehyde overnight at a temperature of 4°C for 14 hours. Finally, the samples were submerged in phosphate buffered saline (PBS) and 0.1% of sodium azide after three washes with PBS each 30 minutes after at least 14 hours and kept in refrigeration at 4°C.

OCT is used to obtain cross-sectional images to measure mucosal thickness at the site of adenoma (2x normal mucosal thickness) and a mild protrusion above the basal mucosal height (<50% total thickness). Signal attenuation with depth has also been observed, and the underlying tissue boundaries are fainter as compared to healthy colon tissue [14].

## 3. Results

A neoplastic polyp (Figure 2A) was imaged across the indicated arrow in order to differentiate the subsurface structure of healthy against the polyp regions. Figure 2B,C shows four regions of the sample with different optical and morphological properties. These include regions where the polyp has affected the homogenous structure and regions that were not affected.

Regions 1 and 4 are normal tissue next to the developed polyp. Mucosal and submucosal layers can be clearly identified where the muscularis mucosa appears as a scattering band separating each layer for both regions. Both layers show a homogeneous structure, however in the orthogonal direction in region 1 polyp development has affected the substructure. The layering is not as homogeneous as well as presenting areas with either higher or lower backscattered light, hence highlighting the relevance of analyzing different transversal cuts when classifying a polyp. Region 4 presents the most homogenous layering in both directions as it has not been fully invaded by the polyp growth.

Regions 2 and 3 show the developed neoplastic polyp whose total length across the horizontal axis is ≈ 0.4 mm. Compared to regions 1 and 4, the subsurface structure homogeneous layering has been lost, and instead there is a heterogeneous structure distribution of the crypt pattern. The polyp has developed invasive irregular glands with enlarged nuclei in the epithelial layer that have different morphology as well as different optical scattering properties compared to the homogeneous mucosal and submucosal layering.

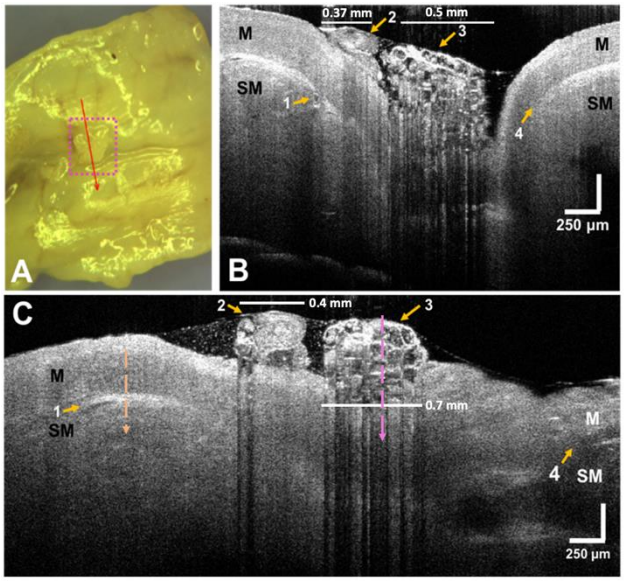


***Figure 2****. Neoplastic polyp imaging. A) Neoplastic colon sample. B) Orthogonal B-scan of the polyp. C) B-scan across the red arrow. Four regions are identified: (1) Homogenous subsurface structure layering has been affected. (2-3) Large polyp showing a heterogeneous substructure presenting glands of different shapes and different scattering properties. (4) Healthy tissue with the most homogenous subsurface structure showing a clear layering. M: Mucosa; SM: Sub-mucosa*

A B-scan is obtained by stacking adjacent A-scans next to each other. Two examples of an A-scan are taken in the orange and pink lines from Figure 2C. With the A-scan it is possible to measure the thickness of the different layers as well as the microstructures. Figure 3A (orange) shows the A-scan of healthy tissue with two layers of 75 and 60 µm separated by a distance of 125 µm. Compared to the healthy tissue, the polyp structure (Figure 3B) presents irregular glands with enlarged nuclei in the epithelial layer showing different morphology with thicknesses that vary between 13 and 45 µm. This feature makes OCT different from colonoscopy and it is comparable to histology in terms of axial resolution without the need of slicing the tissue.

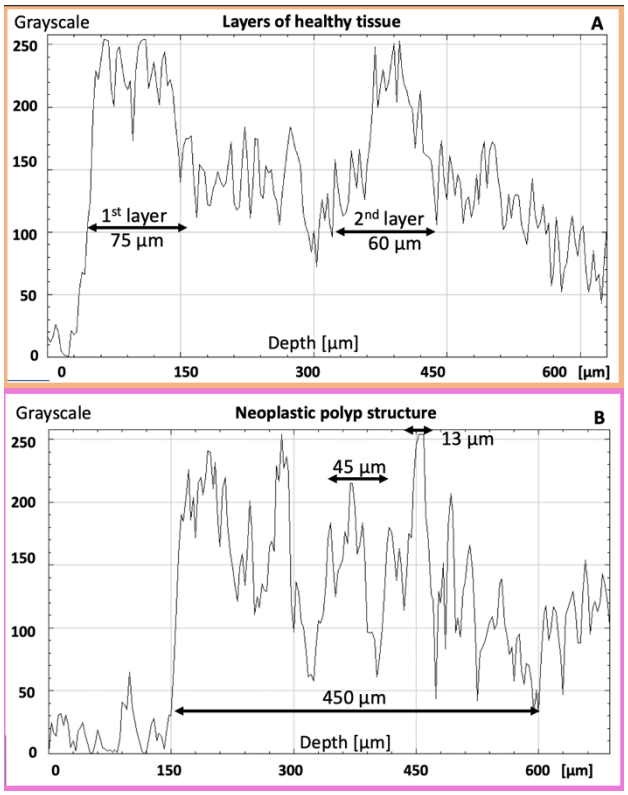


***Figure 3.*** *A-scans A) Two layers identified in healthy tissue. B) Several micro features are observed in the neoplastic polyp*

With the obtained cross-sectional images it is possible to render a volume of the sample by stacking adjacent B-scans. With this, it is possible to retrieve 'virtual cuts' or 'optical biopsies' where the sample can be sliced at a specific depth in order to observe its internal layering and homogeneity. This term is a C-scan. Two examples are shown in Figure 4A,B including the surface of the polyp and an internal cut which is equivalent to an 'optical biopsy' as the cut is made in depth of the sample.

An interesting aspect of OCT imaging compared to other imaging modalities can be seen when comparing region 1/4. When observed at the top region 1 shows a clear structure with 'stripes' as well as a homogeneous structure, however for an internal slice it can be seen that their homogeneity is different since region 1 has been affected by the polyp growth.

Regions 2 and 3 show an irregular crypt structure in terms of its shape and heterogenous distribution on the surface, compared to the 'stripes' of healthy tissue. The subsurface rendering of the polyp shows regions with clear high backscattered properties, as well as inhomogeneous distributions, with glands of different shapes and sizes.

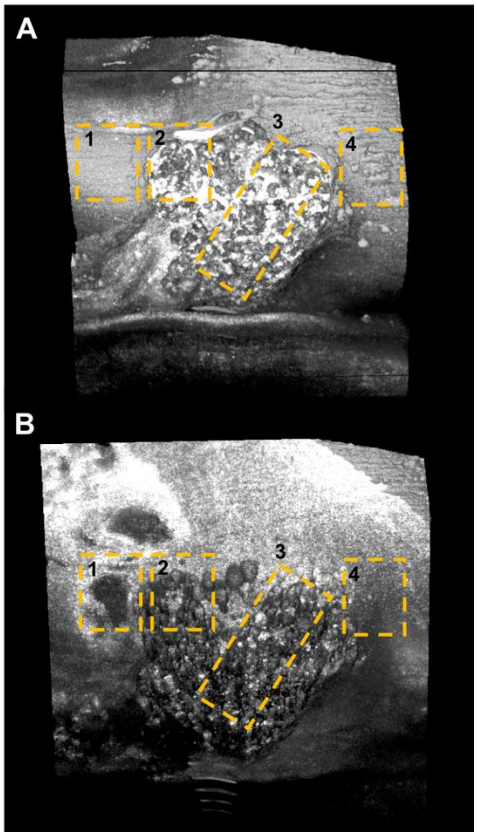


***Figure 4.*** *OCT C-scan 'optical biopsy' A) Reconstruction of the surface of the sample. B) Sub-surface C-scan showing internal structure distribution. The same regions as in Figure 2 are identified.*

## 4. Discussion and conclusion

The proposed method for sample preparation by extracting the rat colon and then dividing it into three regions with healthy and polyp tissue across the transversal plane provided clear imaging regions in order to differentiate the sub-surface morphology and observe how a developed polyp affects normal tissue.

The developed neoplastic polyp has caused an abnormal growth of healthy tissue in terms of a heterogeneous structure with irregular glands of different sizes, altered backscattering properties and an epithelium that no longer has a symmetrical curvature. 3D rendering of the sample provides a better picture of the surface and subsurface structure of the sample, as well as comparing internal cuts that have shown heterogeneity of neoplastic crypt structures.

OCT is an imaging modality with a great potential that provides subsurface image data allowing ex-vivo polyp samples studies in order to observe the abnormal growth and compare it against healthy tissue. Compared to other diagnostic methods, OCT could be considered as a generator of a "virtual histology", since the use of

exogenous fluorophores or other markers is not necessary to support the obtaining of a diagnosis, providing highly useful images of tissue penetration and spatial resolution [15]. In contrast to other optical image modalities, OCT is able to image non-invasively and without the need for tissue preparation [16]. Surface imaging modalities, such as chromoendoscopy, virtual chromoendoscopy, magnification endoscopy and endocytoscopy, or autofluorescence imaging, are limited to superficial lesions and improving magnification would mean restricting the field of view, making thus more difficult the screening and surveillance for multi-focal lesions. And within the subsurface imaging modalities, such as endoscopic ultrasonography, confocal endomicroscopy or OCT, the latter combines nearly microscopic resolution with volumetric and subsurface real-time imaging capabilities [17]. Further analysis to a broader sample of polyps should be performed as future works to strengthen the conclusions drawn in this paper.

## 5. Acknowledgment

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