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Title	Optical spectroscopy for biological and biomedical applications: potentially impacting future of healthcare with research, clinical translation and education
Authors	Saito Nogueira, Marcelo
Publication date	2021-11-10
Original Citation	Saito Nogueira, M. 2021. Optical spectroscopy for biological and biomedical applications: potentially impacting future of healthcare with research, clinical translation and education. PhD Thesis, University College Cork.
Type of publication	Doctoral thesis
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Chapter 2

Colorectal cancer pathophysiology, screening and diagnostics

Marcelo Saito Nogueira

This chapter is part of the thesis "Optical spectroscopy for biological and biomedical applications: potentially impacting future of healthcare with research, clinical translation and education" by Marcelo Saito Nogueira

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2. Colorectal cancer pathophysiology, screening and diagnostics

Now that cancer epidemiology aspects have been described, chapter 2 covers biochemical and microstructural changes associated with colorectal carcinogenesis (with concepts extendable to other types of cancers such as OC), and discusses challenges and opportunities of cancer detection and management. Based on these challenges and opportunities, the aim of this chapter is to clarify the motivation of applications optical techniques to bridge the knowledge gaps on carcinogenesis-associated topics and propose solutions for limitations in (1) early cancer detection, (2) cancer patient stratification, (3) disease monitoring, (4) prediction of treatment outcome, and (5) treatments with good cosmetic effect.

2.1 CRC pathophysiology: causes and clinical features

As previously mentioned, CRC risk factors mainly involve genetic and environmental factors. While genetic factors are extensively discussed in terms of multiple genes associated with inherited familial CRC syndromes such as familial adenomatous polyposis and Lynch syndrome (a.k.a., hereditary non-polyposis CRC) [1], environmental factors are related to the accumulation of alterations on genetic material of a single individual (sporadic CRC). This accumulation has a range of causes including exposure to ionizing radiation, unhealthy diet, tobacco smoking, heavy alcohol use, excessive body weight, lack of physical activity [2].

CRC can be originated through diverse pathways, which hinders establishing fixed criteria for its identification during surveillance. These criteria include:

1. malignant potential of the abnormal tissue protruding into the colorectal lumen (i.e., types of neoplastic and non-neoplastic polyps)
2. long-standing inflammatory bowel disease (IBD) [3,4] such as ulcerative colitis (UC) and Chron's disease (CD).
3. evolution through grades of dysplasia [5]
4. size and number of potentially malignant (i.e., neoplastic) lesions [6]

Other criteria being considered for new tests include genetic tests, assessment of intestinal microbiome [7]. However, CRC screening is performed by:

1. Fecal sampling tests such as the guaiac-based fecal occult blood test (gFOBT) and the immunochemical quantitative detection of human hemoglobin (iFOBT)
2. Radiologic examinations such as the double-contrast barium enema (DCBE) and the computed tomographic colonography (CTC)
3. Endoscopic screening through endoscopy, sigmoidoscopy, colonoscopy

The results of these tests are used to design the treatment plan that optimizes the patient prognosis. A simplified schematic of the clinical steps to identify CRC and decide the best treatment procedure is shown in figure 2.1:

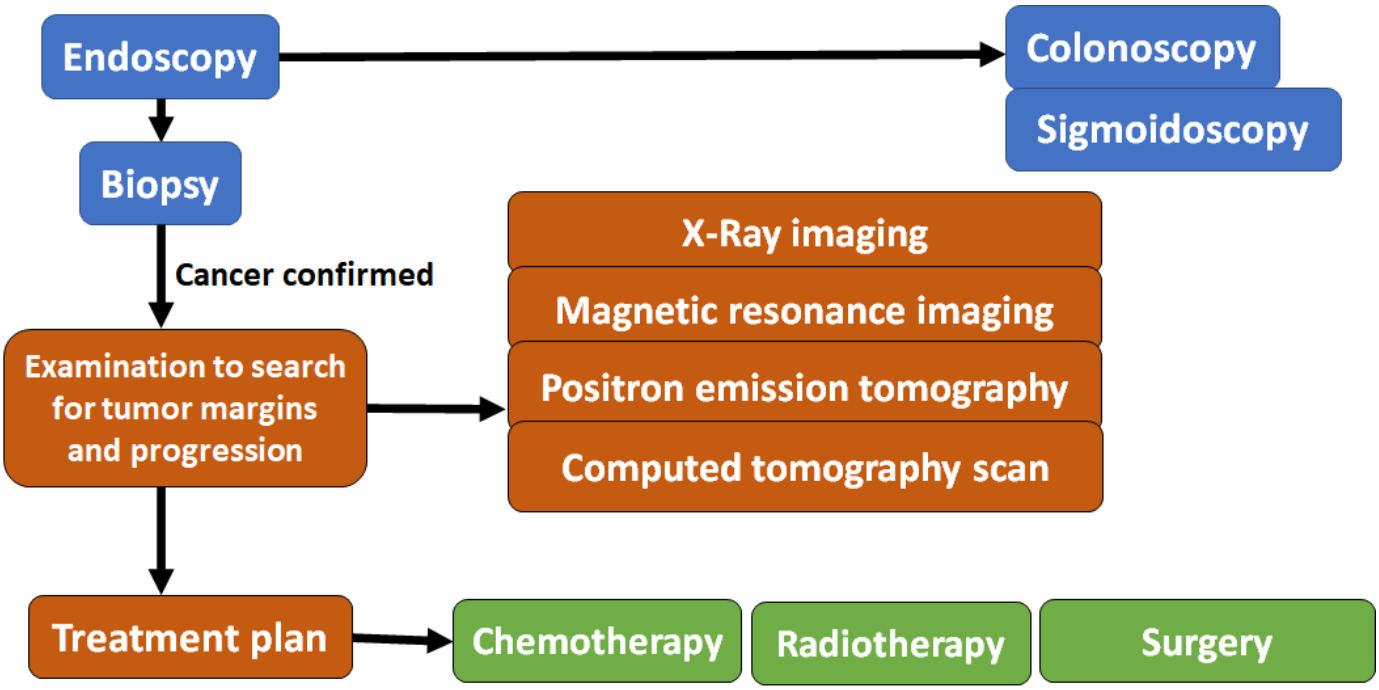


Figure 2.1: Schematic of the clinical steps adopted for CRC endoscopic screening, diagnosis and treatment.

In order to understand the current logic behind the criteria of surveillance and screening tests as well the factors influencing clinical decision-making based on results of these tests, this thesis includes basic CRC term definitions to be considered by the readers (Appendix 1: Clinical term definition and considerations).

2.2 Clinical need on early CRC detection: screening and diagnostic

Currently, clinicians rely on screening and diagnostic tests in order to identify patients with premalignancies. Screening programs for the early CRC detection are designed to maximize primary prevention by identifying potential problems and advising patients to suppress habits linked to CRC risk factors before cancer develops. Primary prevention eliminates the cause of diseases with routine immunization programs such as vaccination. If primary prevention is not possible or effective, secondary prevention measures follow with routine screening and early-stage diagnosis allowing for timely interruption of disease progression. Tertiary prevention focusses on treatment and recovery to avoid the complication of diseases. Early CRC detection is most relevant in secondary prevention measures.

Secondary prevention consists of a screening test and a diagnostic (confirmatory) test used to determine the treatment for improved patient prognosis. By using risk stratification based on patient history, physical examination, laboratory tests (e.g., biofluid/biosolid analysis, radiologic examinations, ultrasound, magnetic resonance imaging), screening programs allow the detection of early diseases by selecting asymptomatic patients with high risk to develop a certain condition. Once patients are selected, follow-up diagnostic tests and optimized treatment is expected. Otherwise, screening tests are insignificant [8].

In premalignant- and malignant-related CRC conditions, diagnostic tests are essential to improve the treatment plan and ensure optimum patient prognosis. Accurate diagnosis and surgical removal of CRC and premalignancies relies on their successful localization during conventional procedures. However, real-time visualization methods used in conventional procedures (mentioned in section 2.1) have a limited sensitivity for this type of localization, as they are primarily based on tissue morphological changes which happen in relatively late stages in terms of the progression from genetic mutations to CRC development (section 2.3 Colorectal carcinogenesis: from genetics to tissue morphology and

biochemistry). Moreover, the gold standard histopathological analysis of resected tissue samples is time-consuming and at times cannot be performed for all suspect lesions on the allocated time of the endoscopic procedure.

In this context, the clinical need for CRC diagnosis, surgery and therapy is accurately locating and completely removing premalignant lesions and surrounding compromised tissue. Since clinical procedures have limited time, tools providing endoscopy and surgical guidance must enable real-time detection of CRC and premalignancies.

Surgical guidance for full removal of CRC is only effective if cancer margins and metastatic lymph nodes are correctly identified. In terms of premalignancy identification, most of CRCs progress from adenomas and the challenge comprises of removing only those (less than 5% of all adenomas) which progress into CRC [9]. In addition, removal of premalignant serrated polyps is required, as they represent one-third of all colonic polyps associated with CRC [10,11]. Still, avoid missing potentially malignant lesions remains a challenge for endoscopists, but has increasing importance with populations growth and ageing, especially considering that 40-50% of the developed-countries populations will develop one or more polyps in their lifetime (most of them benign) [12,13] and adenoma prevalence is 1-4% for individuals between 20-30 years old and 50% for those older than 70 years [13,14].

Increasing the sensitivity to premalignancies and the accuracy to completely remove CRC and potential metastasis requires detecting signs of potential malignancy. In order to understand the underlying biological processes leading to CRC and which cancer stage can be identified with conventional clinical methods, these processes and its implications are described in sections 2.3 and 2.4 of this thesis.

2.3 Colorectal carcinogenesis: from genetics to tissue morphology and biochemistry

Traditionally, CRC is separated into sporadic (cumulative genetic alterations due to environment causing 75%-80% of all CRC) and familial (hereditary) cases. Although previous studies have shown that environmental causes/ CRC risk factors (e.g., tobacco smoking and alcohol consumption) are related to increased risk of CRC, irregular follow up periods and inaccurate quantitative monitoring of these causes over time (e.g., how many cigarettes were smoked or how many alcohol units were consumed per week) hinder the temporal correlation between the intensity of those causes and the concentration of cancer biomarkers that cannot be monitored in real-time. Since the quantitative correlation between CRC risk factors and accumulation of premalignancies over time is unclear, the time when CRC lesions arise becomes unpredictable. Due to this unpredictability and the long time that normal colorectal tissue can take to develop into cancer, past DNA sequencing studies have primarily sought to understand the origin of CRC by investigating alterations on individuals genetically predisposed to CRC [15], who are easier to identify in the population and do not experience changes in the root cause of premalignancies. Also, these studies predominantly analyzed adenomatous polyps, which are the most common type of neoplastic polyp.

2.3.1 Genetic carcinogenic factors

In the canonical view of colorectal carcinogenesis [16], most of CRCs are originated from adenomatous polyps following the “adenoma-carcinoma sequence”. In the “adenoma-carcinoma sequence”, molecular pathways based on methylation of CpG island, chromosomal instability, and microsatellite instability [17,18] lead to carcinogenesis over a period of for 5 -10 years [19]. It is estimated that 80-85% of the transformation of adenomas into carcinomas can be associated with chromosomal instability [15,20]. In this case, histologic alterations arise primarily from genomic changes including “activation of proto-oncogenes (K-Ras) and inactivation of at least three tumor suppression genes” [1]. This inactivation is associated with the loss of “adenomatous polyposis coli (APC, chromosome region 5q21), p53 (chromosome region 17p13), heterozygosity for the long arm of chromosome 18 (18q LOH, DCC/SMAD4; DCC: deleted in colorectal cancer)” [21], as well as mutations in genes TGFBR and PIK3CA [1].

When colorectal carcinogenesis involves microsatellite instability, it follows the "mutator" pathway [15], which happens in 15%-20% of sporadic CRCs [22]. This pathway is based on the accumulation of mutations in short repetitive DNA sequences throughout the genome (microsatellite sequences) [23,24], which damage the mismatch repair genes (such as hMLH1, hMLH3, hMSH2, hMSH3, hMSH6, hPMS1, hPMS2 and others [15,25]) and consequently lead to mutation rates 100- to 1000-fold higher in tumor cells compared to normal cells [15,26].

In general, genetic carcinogenic factors are present at very low concentrations within cells and are difficult to detect with optical techniques with no contrast agents (label-free techniques). Typically, detection sensitivity is achieved in combination with contrast agents (labels) amplifying signals by at least 1000 times (nucleic acids present at pM concentrations, and it is currently possible to detect biomolecules at nM concentrations), and/or biochemical techniques involving antibody conjugation to extract only relevant molecules from samples (e.g., biofluids or smeared tissue). Label-free optical techniques covered in this thesis do not have sufficient sensitivity to detect tissue genetic changes, but future methods using systems built as part of this thesis will use labeled techniques to potentially amplify signals up to 1000000 times (chapter 8, section 8.2.3 Raman spectroscopy). Furthermore, understanding the development of cancer from its initial (genetic) stages is required to design new screening/diagnostic techniques with improved sensitivity.

2.3.2 Ultra- and micro-morphological alterations during carcinogenesis

The genetic changes described above culminate into tissue biochemical and morphological alterations. Well-known morphological alterations occur in the colonic epithelium. In normal states (figure 2.2), the epithelium of colonic mucosa consists of many cryptal cells including differentiated cells close to the tissue surface and stem cells on the bottom of the crypt [27,28]. Stem cells proliferate in order to produce cells to regenerate the epithelium [29]. The balance between production of new cells and elimination of excessive mature cells is controlled by apoptosis during a 2-3 day cell renewal process [29,30]. In abnormal conditions (figure 18), the mucosa attempts to repair its integrity by increasing cell replication to eliminate potentially malignant cells, i.e., mutated cells experiencing dysplasia, metaplasia, neoplasia, and ulcer-associated effects due to DNA damage. This increase in cell replication has been associated with higher risk of CRC, which happens mostly in patients older than 65 years, with adenomatous polyps of the large bowel, familial adenomatous polyposis, long-standing inflammatory bowel disease and those who previously went through polypectomy or partial colonic resection for CRC [29,31–35]. Although cell replication may work to "peel off" differentiated cells in the colonic epithelium once they undergo apoptosis to keep the balance of cell turnover in normal conditions, potentially malignant cells are not affected by signaling pathways triggering apoptotic cell death [29]. The failure in eliminating potentially malignant cells leads to increased probability of generating CRC over time. One of the consequences of this failure is the formation of aberrant crypt foci, which is considered a biomarker of colon carcinogenesis [36,37].

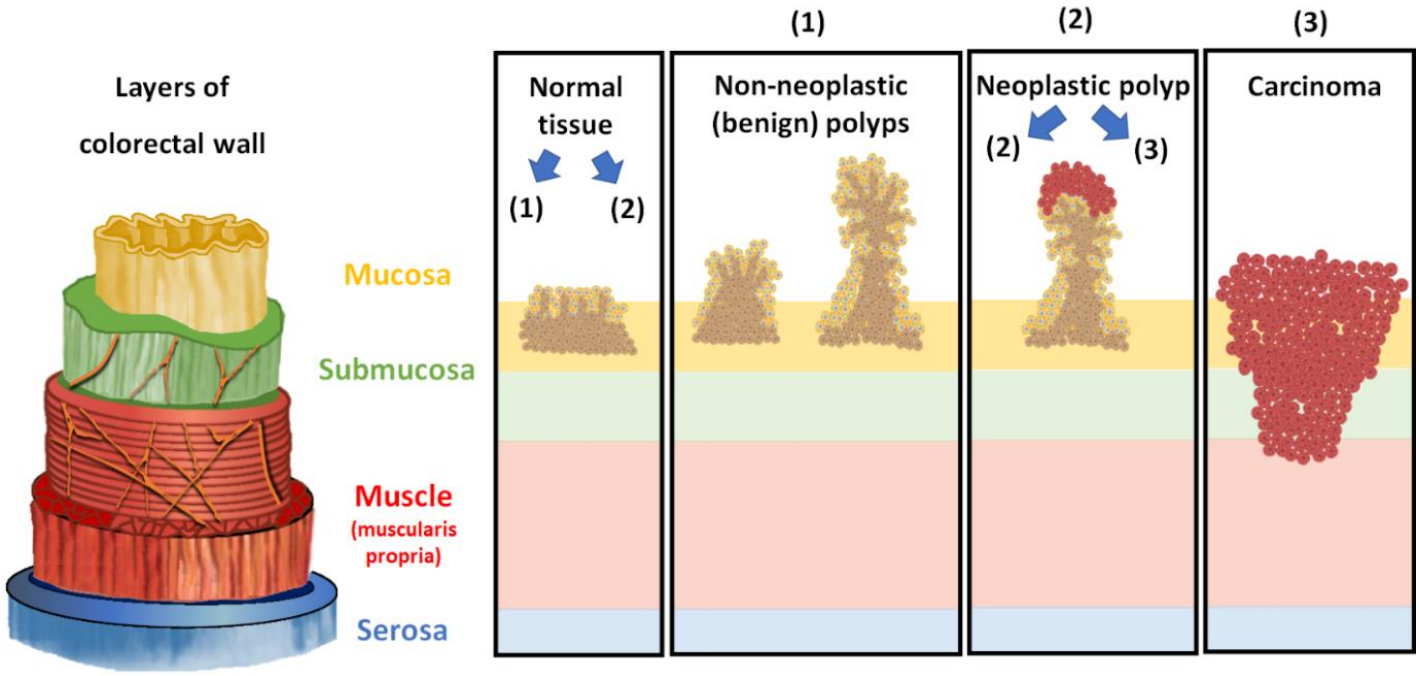


Figure 2.2: Layers of colorectal wall and morphological changes happening at each CRC stage, where (1) represents the transition between normal tissue to non-neoplastic polyps, (2) represents the transition between normal tissue to a neoplastic polyp without malignant mutations or the non-development of a neoplastic polyp into carcinoma, (3) represents the transition between a neoplastic polyp with malignant mutations and invasive carcinoma.

2.3.3 Biochemical changes during carcinogenesis

In previous studies, tissue biochemical changes leading to carcinogenesis through diet differences have been described to happen due to formation of N-nitroso compounds through bacterial decarboxylation of amino acids in the presence of a nitrosating agent and secondary-bile-acids-induced increase cell proliferative activity [38]. N-nitroso compounds are responsible by the production of DNA-binding chemicals which can mutate tumor suppressor genes and oncogenes [39,40]. These compounds are believed to be generated by bacterial degradation or fermentation of undigested dietary protein and other nitrogenous compounds and production of ammonia, phenols and hydrogen sulfide. The produced substances raise the intraluminal pH, induce inflammation, and increase cell proliferation rates [38,41–43]. Secondary bile acids rise cell proliferation by "expanding the proliferative compartment of the colonic crypt up to the middle third". This rise combined with the induction of formation of reactive free oxygen radicals by the same bile acids [38,44] increase the risk of mutation and malignant transformation [38]. Secondary bile acids are produced through enzymatic deconjugation and dehydroxylation of primary bile acids by anaerobic bacteria in the colon [38] and found two times more concentrated in individuals having a diet containing high animal fat [45].

Other tissue biochemical changes are related to tobacco smoking, alcohol consumption and inflammation [38]. Tobacco smoke is known to contain carcinogenic substances such as aromatic amines, N-nitrosamines, benz-pyrenes and acetaldehyde, which produce DNA adducts that disrupt normal gene function and replication [46]. A similar process happens for alcohol consumers, as acetaldehyde is the primary metabolite of alcohol [38]. In the case of inflammatory bowel diseases (IBD), all carcinogenesis stages (initiation, promotion, progression and metastasis) are influenced by immune mediators (such as immune cells and cytokines) and dysbiosis of the microbiome [47,48]. Inflammation is initiated by diet factors including the previously mentioned fatty acids and N-nitroso compounds and becomes more common as individuals age, as less anti-inflammatory properties are provided by gut microbiome with larger amounts of opportunistic facultative anaerobes, and immune system functionality is compromised by age-induced chronic immune and inflammatory disturbances [38,48]. Repeated inflammation events "mediated by cytokines and prostaglandins cause increased proliferation

of inflammatory cells and aberrations in their phagocytosis and apoptosis" [38]. Consequently, inflammatory cells and cytokines not only generate chronic inflammation and tissue damage, but also facilitate angiogenesis (through production of fibroblast growth factor (FGF) and vascular endothelial growth factor (VEGF) [49]) and protect malignant cells by suppressing the organism immune response [38,42]. The cytokine- and prostaglandin E2 (PGE2)-induced increase in cell proliferation is associated with the higher metabolism of arachidonic acid, which is a fatty acid forming major part of biomembranes. Arachidonic acid end-product is PGE2 and can produce pro-inflammatory cytokines by cyclooxygenase enzymes (COX) [38]. Thus, arachidonic acid plays an important role in "inflammatory pathways where direct contact is made between pro-inflammatory microorganisms and luminal carcinogens or between the bowel wall and fecal water irritants" [38].

With the above in mind, colorectal carcinogenesis been mostly investigated with genetic and biofluid tests. However, studies of non-genetic tissue biomolecular changes associated with colorectal carcinogenesis are scarce and necessary for a complete understanding of mechanisms normal colorectal tissues undertake to culminate in CRC, especially in the sporadic cases, which tend to be more heterogeneous due to multiple environmental causes that can be involved with genetic mutation.

2.4 Field effect carcinogenesis (FEC)

Initial genetic mutations predisposing normal colorectal tissue to CRC are not visible through conventional screening and diagnostic tests. Due to the increased cell proliferation caused by these mutations, higher predisposition to CRC can be spread over a wide distance. The process generating this predisposition is called field effect carcinogenesis (FEC), field cancerization or field defect. Even though evidence of field effect is still scarce for CRC, second primary cancers have been frequently observed in adjacent normal tissues of urinary tract and head and neck [50] and it is believed that FEC occurs in a similar way for CRC. Potentially malignant mutations change the gene expression and, thus, the molecular microenvironment inside the cells. Upon sufficient accumulation of molecular alterations, ultra- and micro-morphological changes become apparent. Finally, the progress of micro-morphological changes culminates into macroscopic tissue alterations that are visible in conventional tests.

Since the progression from genetic mutations to tissue morphological changes occurs over time (about 5-10 years for the adenoma-carcinoma sequence [19], the feasibility of early detection of premalignancies is dictated by the sensitivity to elements in early stages of potential CRC. These elements are shown in figure 2.3.

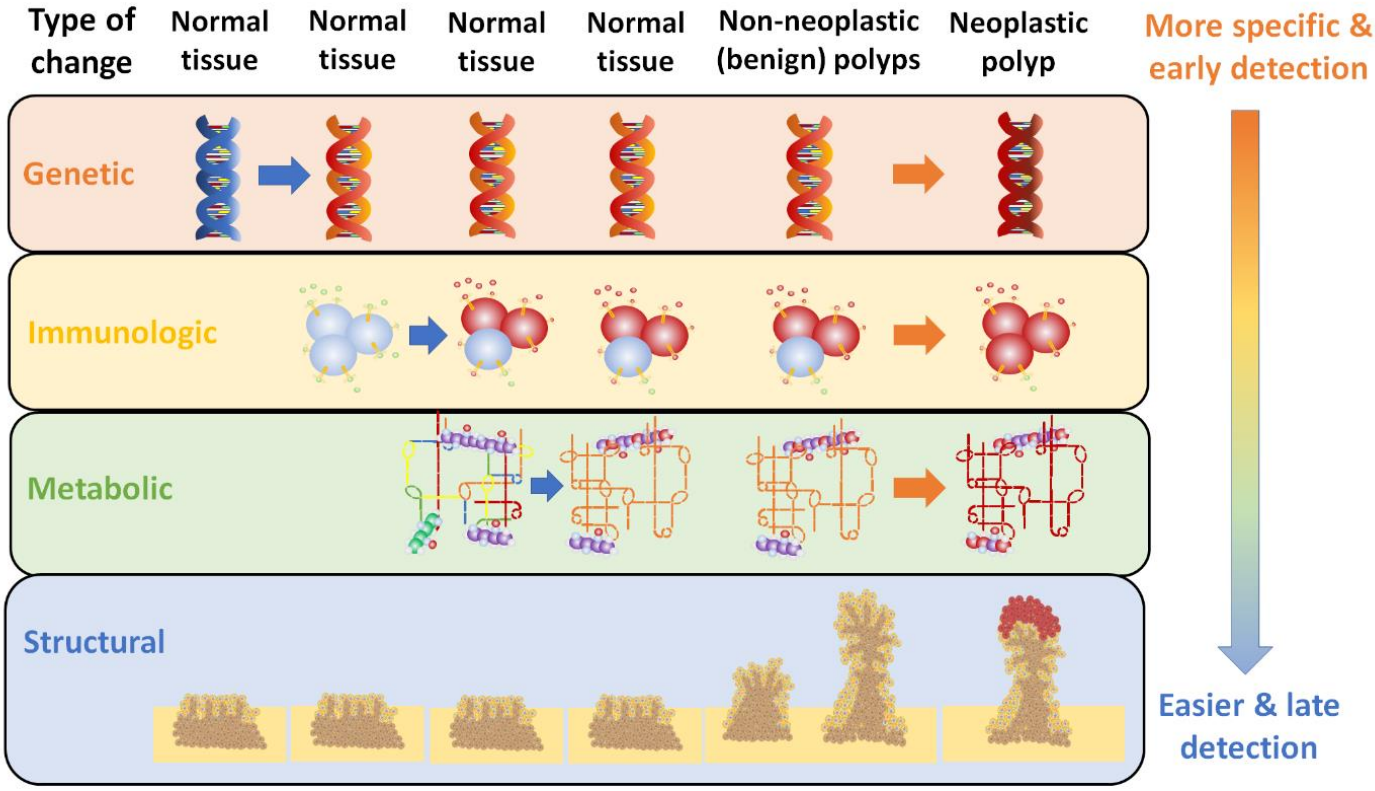


Figure 2.3: Progression of genetic, immunologic, metabolic and structural changes during the adenoma-carcinoma sequence (time passes from the left to the right side of the figure). Blue arrows show the first changes which prevail in the next stages but are not necessarily neoplastic. Genetic alterations result in the expression (production through RNA transcription and translation) of different proteins which change the immunologic system. These changes culminate in metabolic changes. Orange arrows indicate neoplastic alterations, which follow the same sequence of genetic, immunologic, metabolic and structural changes but with other groups of mutations associated with malignancy. Light-based (optical) technologies discussed in this thesis probe are potentially capable of detecting biochemical changes at immunologic and metabolic level, as well as microstructural alterations (e.g., increased number of mitochondria) occuring concurrently and after metabolic changes (i.e, before and during structural changes).

The dissemination of the initial mutation which predisposes the tissue to CRC can occur over a wide distance (3-10 cm for head and neck cancer [29]), which may not be removed during surgery because, even when biopsy is requested within the cancerization field, conventional microscopes cannot identify predisposed cells which did not show microstructural changes [51–53]. This field has been observed in several organs and anatomical sites such as colon and rectum, stomach, esophagus, head and neck, prostate, lung, breast, and skin [54] and has major implications for approaches using "localized" surgical cancer margins (also known as circumferential resection margin (CRM) for CRC surgery), which may not be sufficient to remove all traces of cancer from the patient [29]. Limitations of non-identification of potentially malignant tissue with conventional techniques and the need of precisely delineating CRC for its complete removal while preserving important surrounding tissues (nerves, blood vessels, lymph nodes) during surgery will be discussed in the next sections along with optical techniques which can provide colonoscopic, laparoscopic and surgical guidance.

2.5 Current CRC screening and diagnostic tests and their effectiveness

As already shown in previous sections CRC is mostly diagnosed in its late stages (around 75% of all CRC in Ireland are diagnosed at stage 2 or higher) and survival rates dramatically improves with early-state CRC detection (from 11.6 % survival rates for diagnosis at CRC stage IV to 94.8 % survival rates at stage I). Improving CRC screening and diagnostic are

extremely important to decrease the risk of CRC incidence and mortality, especially if based on tests which can be made affordable (low cost) [55], preferentially non-invasive or minimally invasive (for patient adherence to screening), repeated at regular intervals tailored by the risk of patient subgroups [56]. Obviously, CRC survival rates can only be reduced upon patient compliance to screening followed up by confirmatory diagnostic tests and appropriate treatment. This section will focus on screening and diagnostic tests.

2.5.1 Endoscopy

Gastrointestinal (GI) endoscopy is one of the most extensively used procedure for diagnostic and therapy of GI disorders. Endoscopy allows imaging, assessment of disease status, and treatment. GI endoscopy is divided into upper (esophagus, stomach, duodenum, jejunum) and lower (rectum, colon, and terminal ileum) endoscopy. Lower GI endoscopy consists of inserting an imaging device into the anus, inspecting the lumen of the large bowel, and treating bleeding (hemostasis) with heating treatment, clipping, or injecting medications, as well as strictures with stents inflated inside the colon (which allow decompression and recanalization of intestinal obstruction). Other therapeutic applications of colonoscopy include foreign body extraction, and lesion resection/ablation. Treatment is only possible after screening and diagnosis have been performed by assessing the disease status based primarily on tissue shape and color. This assessment can be made by sigmoidoscopy and colonoscopy.

Sigmoidoscopy is a minimally invasive procedure (less invasive than colonoscopy) which allows examination of the large bowel until the sigmoid colon. Rigid sigmoidoscopy is used to image the anorectum until the recto-sigmoid junction, whereas flexible sigmoidoscopy can inspect up to the splenic flexure. Flexible sigmoidoscopy has been effective in decreasing CRC mortality up to 53% in 16 years of follow-up [57] and 31% with single flexible sigmoidoscopy procedures [58]. Advantages include the less specialized training required to execute the procedure (can be performed by nurses and general practitioners (GPs)) [59], cost-effectiveness compared to FOBT programs [55] and its minimal invasiveness (possibility of application without sedation [60] and increased patient adoption as a result). Disadvantages are the limited colonic areas which can be examined and the need of concurrent colonoscopy in case of positive findings (polyps, abnormal tissue or cancer) [60].

In contrast with sigmoidoscopy, colonoscopy allows examination of the entire large bowel (colon) and the distal part of the small bowel. Colonoscopy not only has the advantage of visualizing the whole colonic mucosa and potential lesions of asymptomatic or symptomatic patients, but also has up to 98% sensitivity for detection of lesions greater than 6 mm (white-light colonoscopy) [61] and has been responsible for a 65% and 48-67% decrease in relative risk of CRC mortality and CRC incidence, respectively, on average-risk patients who underwent the procedure between 1989 and 1993 (“benefit extended for up to 18 years after screening has occurred”) [62]. With this in mind, colonoscopy remains as the gold standard screening and diagnostic test for potential colon malignancies and CRC as well as the greatest method of wide availability for colorectal adenoma detection and removal. The conventional type of colonoscopy applied in medical centers is the white-light colonoscopy, which has the aforementioned benefits and relatively low compliance and uptake, possibly due to the requirements of bowel preparation and procedural sedation [60]. Other reasons for the limited compliance and uptake are disadvantages such as invasiveness, complication rates of 0.08-0.1% [60], risk of perforation of ≤ 0.7% [63], and high cost of sedation and post-operative care [60]. Even though compliance is still a challenge, colonoscopy is an emerging technique which is increasingly more employed for CRC screening [55], especially with the growth in the number of screening programs worldwide. Colonoscopy has been shown to effectively decrease the CRC mortality risk by 60-70% for the left colon [64,65], but still misses 2.1% of adenomas ≥10 mm, 13% of adenomas between 5–10 mm, and 26% of adenomas between 1–5 mm, as well as 22.4% of polyps of any size [66].

2.5.2 Fecal laboratory tests

One of the routine CRC screening tests most intensively and extensively studied worldwide is the fecal occult blood test (FOBT), which are based on the detection of bleeding in the intestines and can be divided into guaiac-based FOBT and immunochemical FOBT. The guaiac-based FOBT is based on the detection of the heme group of Hb (which makes it sensitive to ingestion of meat and analgesics, leading to false-positive results in case they are ingested before the test), while the immunochemical FOBT senses the globin component of Hb [67] and, thus, is more specific and does not require special diet.

Although guaiac-based FOBT is non-invasive, cheap [55], and able to reduce the relative risk of CRC mortality by 16-25%, it may generate up to 80% of false positives due to sensitivity to bleeding at any segment of the gastrointestinal (GI) tract [68]. The low specificity of this test may expose healthy patients to unnecessary risk of complications, since invasive tests are prescribed in case of positive findings. Also, guaiac-based FOBT has a low compliance (between 53-67% of the people who offered to do the test [55]). Immunochemical FOBT tends to have a higher compliance and has better accuracy, which accounts for 76.5% higher sensitivity and 95.3% higher specificity for CRC and adenomas compared to the guaiac-based FOBT [69]. Still, accuracy may depend on following a strict protocol for sample refrigeration to not generate false-negatives [70].

In general, immunochemical FOBT can be a potentially better test compared to guaiac-based FOBT [67], but its real value is still questionable, especially considering its application did not decrease the overall risk of CRC incidence or mortality[71] and sensitivity is low compared to colonoscopy (27.1% and 65.8% sensitivity for advanced neoplasia and invasive cancer upon one-time immunochemical FOBT [72]).

2.5.3 Radiologic imaging tests

Radiologic examinations for colorectal cancer screening and diagnostic include single-contrast barium enema, double-contrast barium enema (DCBE), computed tomographic (CT) colonography (also known as virtual colonoscopy), and magnetic resonance imaging (MRI) [73]. DCBE is a very safe procedure based on abdominal fluorescence images for examination of the entire colon and rectum with contrast generated by a solution of gas and liquid barium applied into the colonic lumen [74]. Yet, the equipment used, labor intensive procedure and contrast agent costs make DCBE expensive, and patient uptake and compliance can be a challenge due to the bowel preparation requirements and patient discomfort of the procedure [75]. Uptake and compliance tend to be better with CT colonography [76], which is able to minimally-invasively (due to cumulative exposure to ionizing radiation rarely leading to complications [77]) visualize the entire colon with sensitivity to polyps >1 cm comparable to colonoscopy [78] by using combining CT scanning with digital subtraction of intraluminal fluids and stool which absorbed contrast medium [74]. Still, CT colonography requires significant bowel preparation and definitive diagnosis requires colonoscopy and biopsies in case of positive results.

In terms of diagnosis, the most used examinations for colonic cancer are DCBE and CT colonography, whereas magnetic resonance imaging tends to be more used in rectal cancer cases due to the limited applicability of DCBE and CT colonography in these cases [79] and to the accuracy provided to determine the circumferential resection margin (CRM), which is more important in rectal cancer [79,80]. On the other hand, MRI is more expensive and may not be available in many medical centers. In terms of screening, DCBE and CT colonography are predominant. CRC sensitivity of DCBE is 87-97% [75], whereas CRC miss rates are estimated to be 15-22% [81] and adenoma sensitivity is significantly lower (50% for adenomas > 1 cm and maximum 73% for adenomas >7 mm [75,82]).

2.6 Limitations of colonoscopy (gold standard screening and diagnostic test)

The goal of colonoscopy is not only to detect cancer, but also to detect and remove benign neoplastic polyps with malignant potential, thus reducing the subsequent incidence of cancer. However, the sensitivity and specificity for the detection of premalignant polyps during conventional white-light colonoscopy are 82.9% and 80.0%, respectively [83], whereas, even small cancers can be overlooked especially with flat lesions in the right colon [84–89].

Colonoscopy is mostly limited by non-identification of premalignancies, flat lesions and interval CRC (i.e., "CRC diagnosed between screening and post-screening surveillance examinations" [13,90], estimated to account for 3% of the all diagnosed CRCs [89]) as well as patient-dependent or endoscopist-dependent procedural factors. As discussed in the section 2.3 Colorectal carcinogenesis: from genetics to tissue morphology and biochemistry, potentially malignant genetic, immunologic and metabolic changes are not visible to the naked eye. Then, identifying premalignancies with conventional medical instruments using white-light illumination (such as conventional colonoscopy). These premalignancies may include neoplastic tissue which can be overlooked (e.g., flat lesions) or even normal colorectal tissue predisposed to CRC, which can spread over a wide distance and affect colorectal mucosa diffusely due to field effect carcinogenesis (FEC). Although removing potentially malignant tissue would be ideal, identifying tissues with predisposed cells without microstructural alterations is not possible even if biopsy is requested within the cancerization field [51–53].

In terms of resection of neoplastic tissue, flat lesions pose a substantial challenge on detection within the time and equipment constraints during colonoscopy, as their flat shape is highly associated with miss rates of 42% for flat adenomas and 32% for flat polyps [91]. Flat lesions could be nonpolypoid or sessile serrated adenomas-polyps (SSAP) [84]. About 50 % of proximal dysplastic or large SSAP are flat [92,93], and it is estimated that only 3-9% of all SSAP are actually detected [94]. In addition, nonpolypoid CRC are mostly located in the right colon [95], which may explain higher rates of missed lesions in this colon segment [84–89]. In fact, missed lesions can lead to major implications when patient compliance and uptake is low during screening programs. For instance, 23-58% of interval CRC are estimated to be generated from missed lesions [96] and interval CRCs have 2.4 times more chance to be found in the right colon [97]. Lesions may be missed due to their flat shape, but also due to procedural factors including as patient inadequate bowel preparation and comorbidities (pulmonary, cardiovascular and diverticular diseases [98]), as well as use of suboptimal equipment [99], limited endoscopist skills at times [100], time constraints of the procedure [101]. With all the above in mind, real-time and accurate identification of potentially malignant tissues could help overcoming the limitations of colonoscopy, which may lead to lower risk of CRC incidence and mortality due to more effective procedures.

Currently, the gold standard for the characterization of polyps is histopathology, but this is not available in real time. False classification of innocuous polyps as benign or malignant neoplasia at colonoscopy will lead to unnecessary polypectomy with associated risk to the patient [102,103]. Furthermore, with increasing emphasis on local excision of early colorectal cancer, polypectomy may not remove a malignant polyp completely without accurate detection of field change, where neoplasia is not only localized to a small portion of colonic mucosa but may affect colorectal mucosa more diffusely [19,104,105]. Then, even apparently uninvolved, readily accessible mucosa which is part of the field will contain biomarkers of carcinogenesis [106], as already discussed in the section 2.4 Field effect carcinogenesis. With this in mind, techniques which can detect tissue biomarkers at molecular level are particularly useful to probe changes occurring before or simultaneously as structural alterations at cellular and tissue scales. In this case, CRC detection could be performed in its early stages, and tumor delineation may become more precise [106]. Optical spectroscopy and imaging methods have shown to be sensitive to nano-structural alterations [51–53] and to molecular changes at micromolar, nanomolar ranges [107], and could be suitable for disease detection and surgical guidance (to be discussed in chapters 3, 7 and 11).

2.7 CRC treatment and implications of precise cancer delineation

Surgery is the most common treatment for CRC. Since CRC is found in its late stages treatment in most cases (about 75% of Irish cases, as previously described), surgery is combined to neoadjuvant chemotherapy in order to downstage the tumor [108], and adjuvant chemotherapy to reduce the risk of recurrence [109] and distant metastases [108]. Colon cancer is likely to recur at distant sites, whereas rectal cancer tends to recur locally. Due to this tendency to local recurrence and the repercussions of this recurrence to patient's prognosis (potential complications include bowl obstruction and uncontrollable pain), radiotherapy is usually included in rectal cancer treatment [108]. Typically, surgery is combined with neoadjuvant chemotherapy for advanced-stage colon cancer, and combined with neoadjuvant chemoradiotherapy for advanced-stage rectal cancer [110]. Conventional neoadjuvant treatment options for rectal cancer include:

- Long-course radiochemotherapy (LCRT): radiotherapy of 45–50 Gy with 1.8–2 Gy fractions over 5–6 weeks [110].
- Short-course radiotherapy (SCRT): radiotherapy of 5 Gy in 5 fractions) without preoperative chemotherapy [110].
- Total neoadjuvant therapy (TNT): systemic chemotherapy applied before or after neoadjuvant LCRT or SCRT [110] (used in patients with metastases or increased risk to have metastases).

Once chemotherapy and radiotherapy have weakened cancer cells, chances of success depend on the surgical procedure. Rectal cancer surgery is more challenging in terms of the balance between complete resection of the tumor (and reduction of the risk of recurrence) and sparing normal surrounding tissue, as extending cancer margins may damage of vital surrounding structures (e.g., hypogastric plexus and bladder). Therefore, the precise determination of tumor margins through the shortest distance between tumor and resected surface is essential to improve patient prognosis. This distance is known as circumferential resection margin (CRM), as it is measured over the entire circumferential resection plane, and is one of the most important factors related to overall survival and local recurrence [111–113].

The European Society for Medical Oncology (ESMO) Clinical Practice Guidelines for rectal cancer currently defines an involved CRM as CRM ≤1 mm from the tumor-free margin [114], which corroborates with the fact that CRM ≤1 mm is strongly associated with disease-free survival compared to CRMs ≤0.5, ≤2.0, and ≤3 mm [115]. Still, 20% of the rectal cancer surgeries have positive margins which increase the risk of stage I tumors by 2%, stage II tumors by 14% and stage III tumors by 33% as well as tumors lower in the rectum by 10% [113]. Although positive resection margins have been reduced to <10% after the introduction of the total mesorectal excision [112,116,117], and LCRT further decreased from 13 to 4% the of remainder 10% [112,118], correctly determining the optimal resection plane is still a challenge. Difficulties during the surgical procedure include limited tissue visualization (small space in the pelvic cavity) and impaired haptic feedback with the increase of the number of laparoscopic surgeries over the years. With this in mind, surgical devices providing intraoperative and real-time tissue identification could improve the accuracy of the determination of CRM and potentially the surgery outcome.

This thesis will discuss optical technologies that could be used for endoscopic, surgical and laparoscopic guidance. In particular, these technologies could potentially:

- decrease the number of premalignancies (e.g., neoplastic lesions and normal mucosa predisposed to cancer) and flat lesions (e.g., sessile serrated polyps) overlooked during endoscopic procedures.
- delineate cancer margins more precisely by either providing real-time surgical guidance or obviating the need for multiple biopsies or polypectomies of normal mucosa.
- assess the microstructural and biochemical status of normal and cancerous/pre-cancerous tissues, which is currently limited to time-consuming biochemical tests. This status can be associated with tumor staging, histological and regression grades, degree of spread to regional lymph nodes and parameters influencing the treatment for optimal patient prognosis.

Since previously described diagnostic/surgical approaches are either invasive and time-consuming, or require expensive equipment (and/or maintenance of such equipment such as in MRI), methods enabling real-time, cost-effective

and accurate tissue identification are missing for the applications listed above. These methods are enabled by optical technologies developed in this thesis [119–209]. To design instruments applying such optical technologies, one needs to have the big picture of technologies available and which system components can be combined to meet the clinical need at the lowest possible cost and manufacturability. Such big picture is discussed in chapter 3 of this thesis.

2.8 References

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