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Endoscopic Image Analysis of Aberrant Crypt Foci

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"Cá dentro inquietação, inquietação,
É só inquietação, inquietação…
Há sempre qualquer coisa que está pr'acontecer
Qualquer coisa que eu devia perceber
Porquê não sei, porquê não sei,
Porquê não sei – ainda!"

José Mário Branco

Abstract

Currently, cancer is one of the most problematic diseases, which cause approximately 1.7 million deaths per year. This work is focused on colorectal cancer, a disease that affects equally men and women and its incidence ranks second among all cancers, in Portugal. However, if early detected, it can be avoided.

It is believed that aberrant crypt foci (ACF) could have a crucial role in the sequence adenoma-carcinoma, being a precursor of colorectal cancer. As such, its recognition through endoscopic images could potentiate the detection and diagnosis of this type of cancer.

The most prominent techniques used in colon and rectum scanning are colonoscopy or, more recently, capsule endoscopy. However, the medical doctors spend substantial time observing the resultant images. As such, image processing is a potential tool that could be used to diminish that period of time.

The aim of this Master thesis project is to develop computational methodologies that will allow the detection and quantification of ACF of images captured *in vivo* by endoscopy. It would be very useful and helpful for medical doctors to have a reliable computerized and fast method for assessing the ACF's pattern images.

Resumo

Actualmente, o cancro é uma das doenças mais problemáticas, sendo responsável pela morte de cerca de 1.7 milhões de pessoas por ano. Este trabalho estuda, em particular, o cancro colorectal, uma doença que afecta igualmente homens e mulheres e que representa a segunda maior taxa de incidência de cancro em Portugal. No entanto, se for detectada cedo, a morte pode ser evitada.

Pensa-se que os focos de criptas aberrantes (ACF) podem ter um papel importante na sequência adenoma-carcinoma, sendo um percursor do cancro colorectal. Como tal, o seu reconhecimento através de imagens endoscópicas pode potenciar a detecção e diagnóstico deste tipo de cancro.

A principal técnica utilizada para a exploração do cólon e recto é a colonoscopia ou, mais recentemente, a cápsula endoscópica. Contudo, o processo de observação das imagens é muito demorado. Como tal, o processamento de imagem é uma ferramenta importante que pode ser usada para diminuir este tempo.

O objectivo deste projecto de Mestrado é desenvolver metodologias computacionais que permitirão a detecção e quantificação de ACF em imagens capturadas *in vivo* através de endoscopia. Tal será muito útil para os médicos pois permitirá a existência de métodos rápidos e fiáveis no processo de avaliação de padrões de ACF em imagens.

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List of abbreviations

ACF – Aberrant Crypt Foci

ASIC – Application Specific Integrated Circuit

CE – Capsule Endoscopy

CMOS – Complementary Metal Oxide Semiconductor

CRC – Colorectal Cancer

DNA – Deoxyribonucleic acid

FDA – Food and Drug Administration

HSI – Hue Saturation Intensity

LED – Light Emitting Diode

RGB – Red Green Blue

1. Introduction

Cancer is one of the most problematic diseases in the modern world. In the United States, one in four deaths is due to cancer (Jemal, Siegel et al. 2008). In Europe, is estimated that 3.2 million people were diagnosed with cancer in 2006 and 1.7 million people died from this disease. Since 2004, the annual number of new cases has increased by 300,000 and due to ageing of the European population, these numbers will increase over the next few decades (Gouveia, Coleman et al. 2008).

This project is focused on a particular type of cancer: Colorectal. In Portugal, this is a growing problem since the latest cancer registries showed that 14.6 % of cancer deaths in 2005 were due to Colorectal Cancer (CRC). Moreover, its incidence ranks second among all cancers in men and women and, from 2000 to 2005, the number of CRC deaths increased at an annual average growth rate of 3% (Pinto, Paquete et al. 2010), Figure 1.

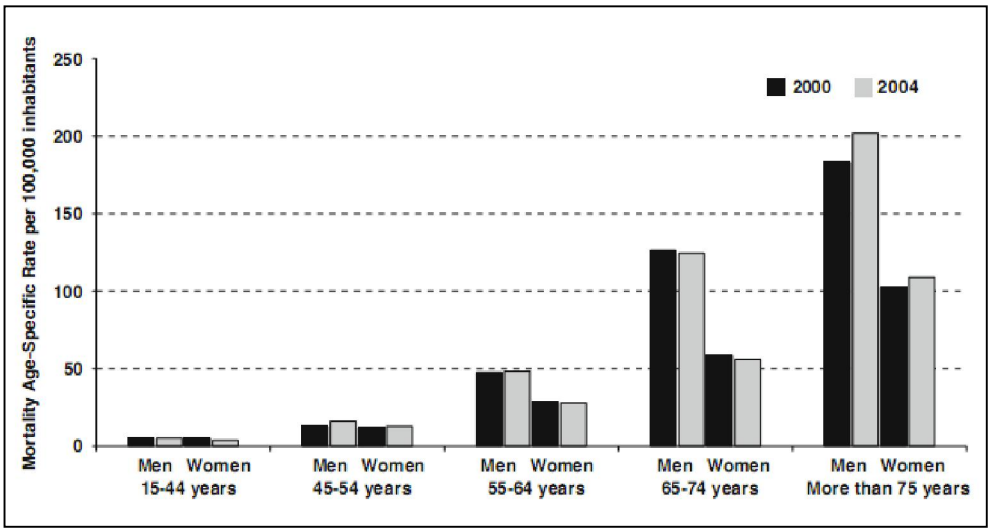

Figure 1 – CRC mortality: age-specific rate per 100,000 inhabitants (from (Pinto, Paquete et al. 2010)).

Nevertheless, it is possible to prevent CRC since it develops over 10–20 years, providing time for disease identification and interruption, long before it poses a clinical threat (Hawk, Umar et al. 2004).

It is generally accepted that most CRC arise from preexisting adenomas (Shpitz, Bomstein et al. 1998). In the large intestine, adenomas are polypoid dysplastic foci that are thought to be precursors of cancer. In the last few years the early events of human colorectal tumorigenesis have been extensively investigated. Among these, aberrant crypt foci (ACF) have been

described topologically as clusters of abnormally large colonic crypts identified on the mucosal surface of the human colon after staining with methylene blue (Roncucci, Modica et al. 1998).

Since 1987, when Bird first described ACF in rodents, it is thought that they may have a crucial role, since they are thought to be a possible precursor of colorectal cancer (Bird 1987). Considering this, the identification of ACF in endoscopic images will allow the diagnoses and detection of this cancer (Adler, Gostout et al. 2002). In this context, the regular analysis through endoscopy or colonoscopy will contribute to the early detection of these structures, usually known as polyps, and their removal will prevent the appearance of CRC.

The aim of this project is to develop computational methodologies that will allow the detection and quantification of ACF of images captured *in vivo* by endoscopy. It would be very useful and helpful for medical doctors to have a reliable computerized and fast method for assessing the ACF's pattern images. Image processing methods have the potential to achieve this, and, as such, they will be the basis of this work (Figueiredo, Figueiredo et al. 2010).

This report has been divided into 6 principle sections. In section 2, it will be detailed the anatomy, physiology and histology of the colon and rectum in order to understand the characteristics of these organs. Section 3 is focused on the processes known about colorectal cancer, being an introduction to section 4 that explores with more detail the aberrant crypt foci and summarizes the research work that has been done about this subject. Then, section 5 explores the endoscopic technique through which will be obtained the images to analyze and detect ACF. Finally, section 6 is constituted by a review of literature about several image processing techniques applied to endoscopic images.

2. Anatomy, Physiology and Histology of the Colon and Rectum

The biological mechanisms of the colon and rectum are intimately related with its anatomy, physiology and histology. In this section, these three topics will be detailed to characterize the functionality of the colon and rectum in the human organism, providing initial information about these structures. In this way, it will be easier to further understand the mechanisms involved in CRC and the characteristics of ACF to be detected by image processing techniques.

2.1. Anatomy and Physiology of the Colon and Rectum

The large bowel, located in the abdominal cavity, can be seen as a pipe with a 6 cm diameter and approximately 1.20 cm length. It is subdivided into: the caecum and appendix, the ascending colon, hepatic flexure, transverse colon, splenic flexure, descending and sigmoid colon and the rectum and anal canal, Figure 2. Although the large bowel has a bigger diameter than the small one, the epithelial surface area is much smaller, since the colon is about half the length of the small intestine, lacks villi in its mucosa and has a sacculated shape (Widmaier, Raff et al. 2004; Ellis 2011).

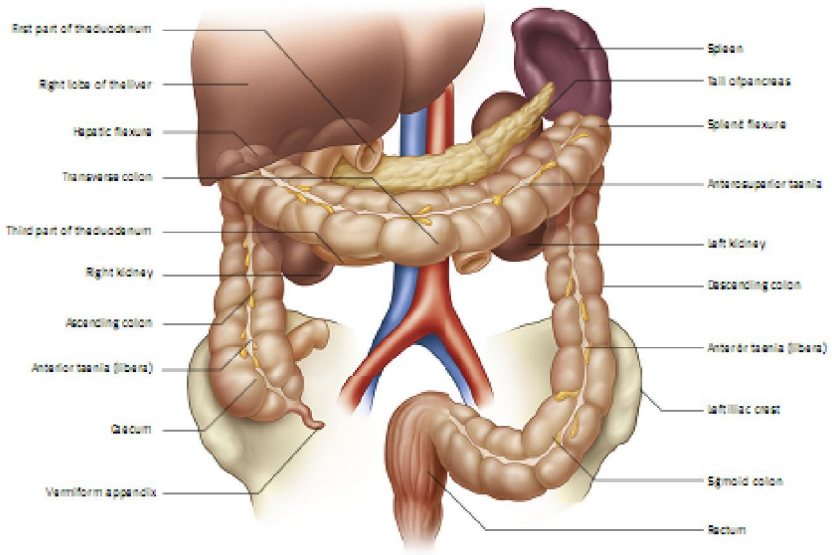

Figure 2 – The large bowel (from (Ellis 2011)).

The colon represents the terminal segment of the digestive tract and is approximately 1.50 m in length (Moreira Jr and Wexner 2005). However, the human colon should not be considered as one functional unit since the proximal and distal colon differs in many properties. There are differences in the anatomy, neural and blood supply, and absorption characteristics as the length of the colon is traversed. The motility patterns, residence time and properties of luminal contents also differ (Edwards 1997). From the caecum to the sigmoid

portion, the size decreases gradually, starting with 7 cm in diameter and finishing with 2.5 cm. The colon has been identified as an organ of importance for the nutrition and health of man having a large metabolic activity, through its intestinal micro flora, and a significant ability to absorb water and electrolytes from fecal material (Edwards 1997). It can absorb up to 5 l of water in 24 hours. The motility of the colon is more complex than observed in either the stomach or the small bowel, and is divided into two functional units: rhythmic peristaltis and tonic contractions (Moreira Jr and Wexner 2005). Moreover, it houses a variety of bacteria, known as commensals, which ferment carbohydrates and release hydrogen, carbon dioxide and methane gas. They also synthesize vitamin K and some B vitamins and are responsible for breaking down the bilirubin into urobilinogen (Porrett and McGrath 2005).

Although the human colon has a lower absorption capacity than that of the small intestine, the material remains in the colon for much longer. Colonic residence time is 2-3 days, whereas food is in the small intestine for as little as 5 h. This long colonic residence time provides a significant opportunity for the slow absorption of drugs and other materials, either targeted specifically at the large intestinal mucosa or designed to act systemically (Edwards 1997).

The Colon vascularization is dependent on the superior and inferior mesenteric arteries (Mazzucchelli and Maurer 2004).

The rectum, situated at the end of the sigmoid colon, is approximately 13 cm in length and begins where the colon loses its mesentery. It is divided into an upper third, middle third and lower third, lies in the posterior aspect of the pelvis and ends 2-3 cm anteroinferiorly to the tip of the coccyx, where it bends downwards to form the anal canal (Moreira Jr and Wexner 2005; Porrett and McGrath 2005). The rectum exhibits a different mode of motor activity as rhythmic peristalsis is absent most of the time. The mechanism of evacuation is complex and involves voluntary and involuntary muscle activity, mediated by mucosal and pelvic receptors. The interior iliac arteries supply the rectum and anus, and the venous drainage is manly by internal iliac veins. The nerves supplying the large bowel are via the sympathetic and parasympathetic nerves (Porrett and McGrath 2005).

2.2. Histology of the Colon and Rectum

The large bowel consists of a mucosal membrane with no folds except in its distal (rectal) portion. No villi are present in this portion of the intestine. The intestinal glands are long and characterized by a great abundance of goblet and absorptive cells and a small number of enteroendocrine cells. The absorptive cells are columnar and have short, irregular microvilli (Junqueira and Carneiro 2004).

Analysing the colon in particular, it consists of a series of concentric layers. Starting in the lumen, these layers are: columnar mucosa, basement membrane, lamina propria, muscularis mucosae, submucosa, muscularis propria, inner circular layer, outer incomplete longitudinal layer (*taenia coli*) and serosa (Ellis 2011), Figure 3.

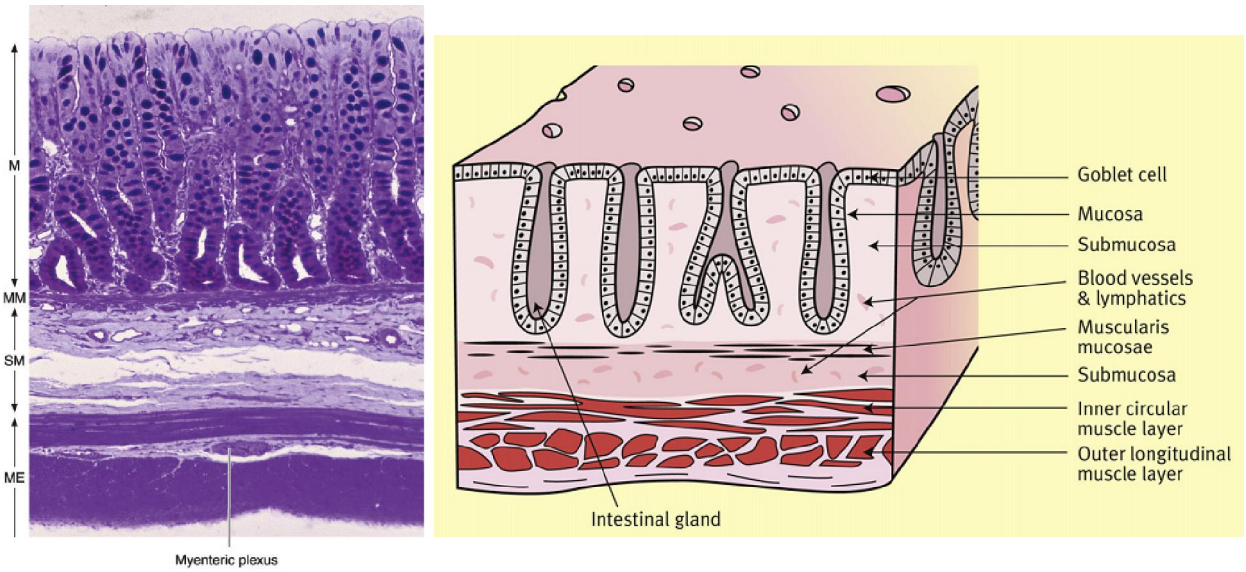

Figure 3 – Photomicrograph of a section of large intestine with various structures (left) and a diagram of the histological features of the colon (right). (M - Mucosa, MM - muscularis mucosae, SM -submucosa, ME - muscularis externa (from (Junqueira and Carneiro 2004; Amerongen 2010))

The outer layer of the large bowel, serosa, is over the colon and is covered by adventitia. The muscularis propria in the colon has the longitudinal muscle layer arranged into three longitudinal bands, the teniae coli. The mucosal surface of the colon at birth is similar to that of the small intestine but rapidly changes with the loss of the villi leaving a flat mucosa with deep crypts. There are stem cells aligned along the crypt wall and they are believed to reside in the bottom of the crypt, while transit cells are disposed along the middle part of the crypt axis and the differentiated cells are at the top. As such, stem cells present at the base of crypts divide and the progeny migrates upward, enter cell cycle arrest, and differentiate before finally reaching the luminal surface epithelial layer, where they undergo apoptosis and shed into the lumen (MacFarlane and Stover 2008), Figure 4.

As the gut ages there is a decrease in the number of non-goblet crypt cells and this is related to an increase in faecal water. Moreover, in normal colonic crypts, the cells renew completely each three to four days, through a programmed mechanism. If this programmed mechanism changes, disease may appear: the shapes of the crypts change, they become aberrant crypts, aggregate in clusters, and thus aberrant crypt foci (ACF) appear (Figueiredo, Figueiredo et al. 2010).

The mucosa has a simple columnar epithelium composed of the same cell types as are found in the small intestine, except that Paneth cells are present only in the proximal part of the large intestine, goblet cells are more numerous and absorptive cells are correspondingly fewer. The colonic crypts in the distal colon are longer than those in the proximal colon. At the rectoanal junction, there is an abrupt transition from simple columnar to stratified squamous epithelium. Lymphoid follicles are common, especially in the distal colon and the rectum (Edwards 1997; Amerongen 2010).

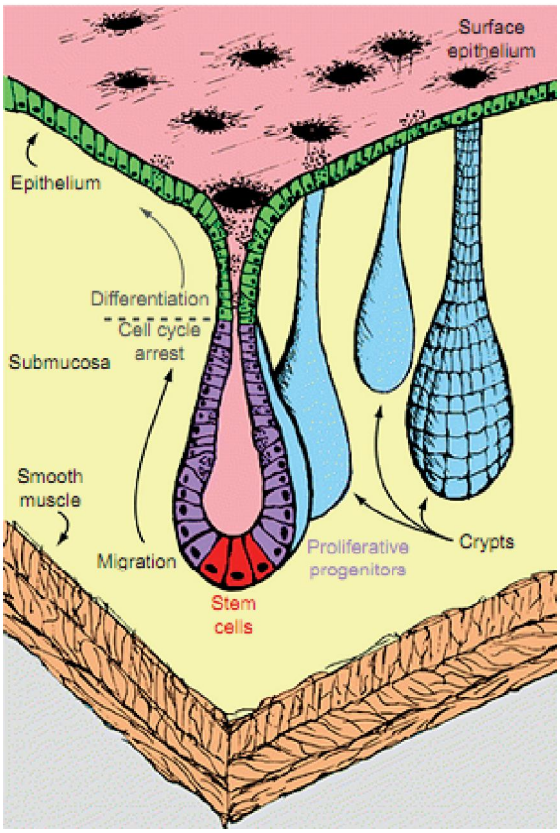

Figure 4 – Anatomy of colon and stem cells cycle (from (MacFarlane and Stover 2008)).

3. Colorectal Cancer

The complex process of cancer formation is characterized by alterations in the morphology and behavior of normal cells. In a balanced organism, all cells have molecular mechanisms regulating their growth, differentiation and death. However, cells can accumulate a succession of genetic mutations leading to corrupted DNA information (Grady 2004; Doucas and Berry 2006). This information leads to abnormal patterns of gene expression and, as a result, the effects of normal genes that control cell growth, survival and spread are enhanced and those of genes that suppress these effects are repressed. Even after a cancer has been formed, the genetic instability of the malignant cell means that changes in the nature of the cancer continue to occur, creating difficulties regarding treatment strategies. Most cancers result from a series of genetic errors occurring over a prolonged period; hence, the incidence of most cancers increases with age. Aberrant gene expression leads to a number of key changes in fundamental biological processes within cancer cells (Doucas and Berry 2006; Harrington 2008).

Cancer is fundamentally a molecular and a genetic disease, characterized by two classes of genes, oncogenes and tumor suppressor genes, which each provides an essential function in normal cells. Oncogenes are the mutation result of the proto-oncogenes, which control cell proliferation, survival and spread. In this way, after the occurrence of mutation, the cancer cell is capable of uncontrolled cell division, enhanced survival and dissemination. Tumor suppressor genes code for inhibitory proteins that normally act to prevent cell growth. When gene mutation occurs, this function is lost, allowing cancer cells to grow uncontrollably and contributes to tumor formation (Doucas and Berry 2006; Harrington 2008).

The cancer formation is not a simple process, since it involves an initial mutation that must be coupled to cell proliferation and must promote the clonal expansion of the cells. From studies of colon cancer, it appears that at least five mutations in stem cells are needed to trigger the cancer formation (Grady 2004).

The colorectal cancer, so called since it occurs at the colon and rectum, is characterized by these general mutations and processes, but it has several particularities that are going to be detailed henceforward.

Ninety-eight percent of all malignancies that develop within the large intestine arise from glandular mucosa and are thus classified as adenocarcinomas by histology (Limburg and Ahlquist 2004). Malignant transformation of the colorectal epithelium typically occurs as a

multistep, multipath, multifocal process that requires sequential or concomitant damage to several genes within and across cellular generations (Hawk, Umar et al. 2004).

The carcinogenesis multistep process begins with the clonal expansion of genetically altered epithelial cells, followed by the formation of clusters of these abnormal cells. The resulting cluster formation is generally denominated Aberrant Crypt Foci (ACF) and represents the earliest stage of dysplasia that can be recognized using current technology. In response to poorly understood molecular signals, a subset of ACF advances to become adenomatous polyps, which are often referred to simply as adenomas (Limburg and Ahlquist 2004), Figure 5.

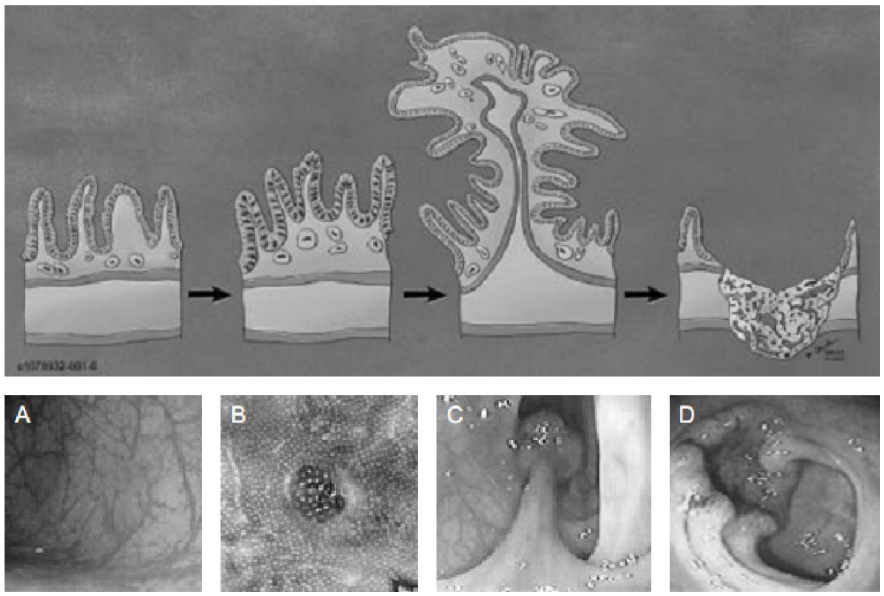

Figure 5 – The process of colorectal carcinogenesis, as represented by schematic (upper image) and endoscopic images: A) Normal colorectal mucosa, B) Aberrant Crypt Foci, C) Adenomatous polyp, D) Adenocarcinoma (from (Limburg and Ahlquist 2004)).

Therefore, ACF are thought to have a crucial role in the multistep process, the adenoma-to-carcinoma sequence, and it is going to be explored in the next section.

The colorectal cancer screening can reduce substantially the number of deaths, and it is suggested to be performed every 10 years, after completing 50 years old. Colonoscopy remains the gold standard for colorectal exploration. It has the advantage of allowing assessment of the entire colon with the possibility of simultaneous biopsy and removal of any polyps (polypectomy) (Mitry 2008).

Concluding, the progressive findings about CRC and its screening could provide new ways of control cancer dissemination, since at early stages, there are no symptons or they are non-specific. An effort at detection through screening programs is essential and, in the future, it certainly will contribute to the reduction of the death number (Labianca, Beretta et al. 2004).

4. Aberrant Crypt Foci

Aberrant Crypt Foci (ACF) has been studied since 1987, when Bird first identified these structures in the colon of carcinogen treated rodents. In that study, the mucosal surface of whole rodent colons was examined microscopically after stained with methylene blue (Bird 1987). In the next years, several studies have been done using animal models, in an attempt to understand the role of ACF as possible putative preneoplastic lesions, i.e. as an indicator of tumor formation. In these studies, animals were injected repeatedly with a colon specific carcinogen and sequential analyses of histological sections of colonic tissues were performed. The findings have been promising and generally favor the use of ACF as end points in assessing the risk of colon cancer incidence or as biomarkers in the identification of cancer modulators (Pretlow, Barrow et al. 1991; Bird 1995). Considering these facts, the next steps involved studies in human colons.

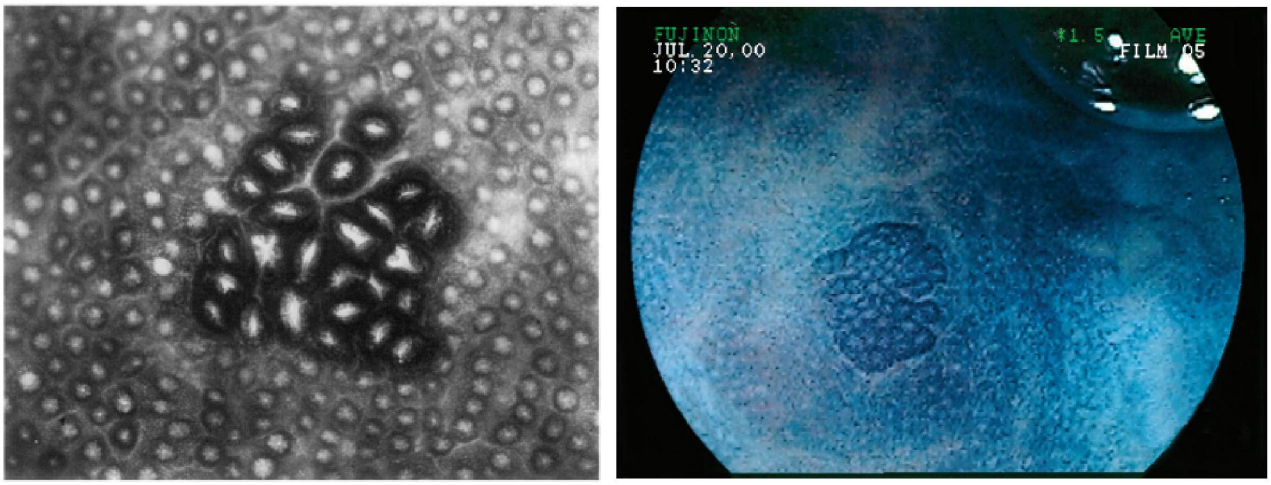

Figure 6 – En face photomicrograph of sporadic aberrant crypt focus in sheet of colonic mucosa stained with methylene blue (left) (from (Nucci, Robinson et al. 1997)) and typical endoscopic appearance of an ACF (right) - This image was acquired with a Fujinon EC-410 CM magnifying colonoscope after the rectal mucosa had been stained with 0.2% methylene blue (right) (from (Rudolph, Dominitz et al. 2005)).

In 1998, (Shpitz, Bomstein et al. 1998), (Roncucci, Modica et al. 1998) and (Takayama, Katsuki et al. 1998) studied several aspects related to the hypotheses that ACF may be an indicator of colon malignancy. The main conclusions achieved by these authors showed that significantly more ACF were observed in patients with carcinoma than in patients with benign colonic disease and that experimental evidence supports the view that density of ACF is strictly related to initiation in colon carcinogenesis. As such, the large ACF large and those who have dysplastic features may be precursors of adenoma and cancer. Presently, several studies indicate that the number of ACF increases when in presence of patients with colon carcinoma, or that there are more rectal ACF in persons with adenomas (Adler, Gostout et al. 2002;

Rudolph, Dominitz et al. 2005). However, It is also known that a number of compounds with the ability to reduce the occurrence of ACF, e.g. 2-(carboxyphenyl)retinamide or genistein, may actually enhance the development of colon cancers (Mori, Yamada et al. 2004). Moreover, (Rudolph, Dominitz et al. 2005) suggests that neither ACF size nor number has been shown to be predictive of progression. Therefore, neither of these ACF characteristics can be considered valid intermediate end points for cancer prevention studies at this time. As such, a better description of the distribution of ACF in the general population, and the relation of ACF to demographic, dietary, and personal habits will be necessary to advance our understanding of the biological meaning of these lesions (Stevens, Swede et al. 2007).

Regarding the influence of age, different studies suggested that the number of ACF in a colon varies with time. In (Shpitz, Bomstein et al. 1998) was observed that the overall prevalence of ACF increased slightly with age, although these differences were not statistically significant. However, (Roncucci, Modica et al. 1998) and (Takayama, Katsuki et al. 1998) did not observe significant differences that could confirm a relation between the number of ACF and age in patients with colon carcinogenesis. Nevertheless, (Takayama, Katsuki et al. 1998) observed an increased prevalence of ACF after 40 years old, and suggested that after this age, periodic endoscopic surveillance of patients is recommended.

Another relevant aspect verified was the existence of gradient of ACF number along the colon. According to (Shpitz, Bomstein et al. 1998) and (Roncucci, Modica et al. 1998), the number of ACF per square centimeter of mucosal surface increased gradually from proximal colon to distal. This fact is consistent with the usual incidence of colorectal cancer, normally situated at the distal colon and rectum.

According to heredity, (Stevens, Swede et al. 2007) observed a higher mean number of ACF in patients with a family history of CRC than in those without this risk factor. Furthermore, ACF does not appear to be dependent on patient gender, as (Roncucci, Modica et al. 1998) suggested.

The detection of ACF in the colon mucosa is usually performed by magnifying colonoscopies, an instrument exceptionally well suited for determining the presence or absence of ACF in humans, according to (Adler, Gostout et al. 2002), Figure 6. To perform this detection, it is essential the definition of ACF. In this way, according to (Mori, Yamada et al. 2004), ACF are defined as single or multiple crypts that: (1) have altered luminal openings; (2) exhibit thickened epithelia; and (3) are larger than adjacent normal crypts. Additionally, (Figueiredo, Donato et al. 2009) suggested that ACF were lesions in which the crypts were more darkly stained with methylene-blue than normal crypts and that they are often elevated from the focal plane of the microscope, Figures 6 and 7.

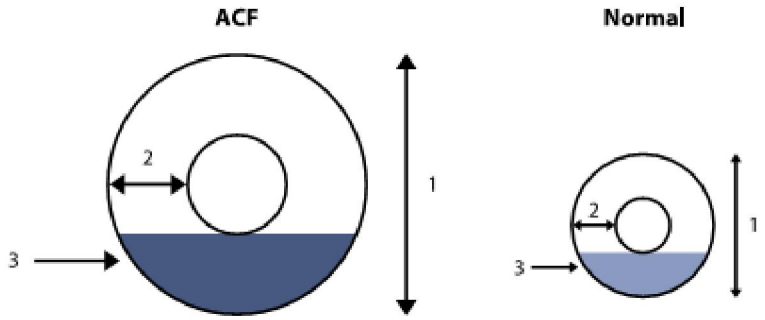

Figure 7 – Graphic detection of ACF: 1 – Crypt diameter; 2 - epithelial thickness; 3 – epithelial staining intensity (from (Schoen, Mutch et al. 2008)).

Finally, different studies were performed using only rectal mucosa, since it is easier to use in experimental studies with magnification chromoendoscopy. In (Figueiredo, Donato et al. 2009) was used this technique to investigate the possibility of ACF being a carcinogenesis precursor, and found that rectal ACF could be an indicator of a carcinoma elsewhere in the colon.

In conclusion, ACF behavior is still uncertain, but several steps have been made in order to understand its role on the sequence adenoma-carcinoma. Moreover, (Akshay, Paul et al. 2009) concluded that there are considerable variability among endoscopists about whether or not a lesion is ACF, and about the presence of endoscopic criteria in the lesion under observation. However, digitally enhanced images, as used by some investigators, may be a solution, but whether they can be implemented for use in large-scale studies is unclear.

5. Endoscopy Technique

The concept of examining the body's interior and its organs dates back to ancient times. In 1806, Philipp Bozzini crated his first light conductor in an effort to study hollow organs and human body cavities (Natalin and Landman 2009). Since that time, endoscopy has evolved to the traditional endoscopy present, nowadays, in almost every Hospital. However, despite being a significant advance in digestive tube diagnostic, there are still some problems to solve. One of the major concerns was related to the inaccessibility of exploring the small bowel, since there are only 2 types of endoscopes using probes which are introduced into the oral or rectal cavities by means of insertion tubes. Such techniques permit detailed and reliable analyses and represent the best nonsurgical tool available today to manage some diseases of the digestive tube. Nevertheless, both gastroscopy and colonoscopy are considerably invasive and frequently ill tolerated by patients. Moreover, they have to be performed by skilled personnel (Carpi, Galbiati et al. 2007). Consequently, several advances were pursued leading to the appearance of the capsule endoscopy (CE), Figure 8.

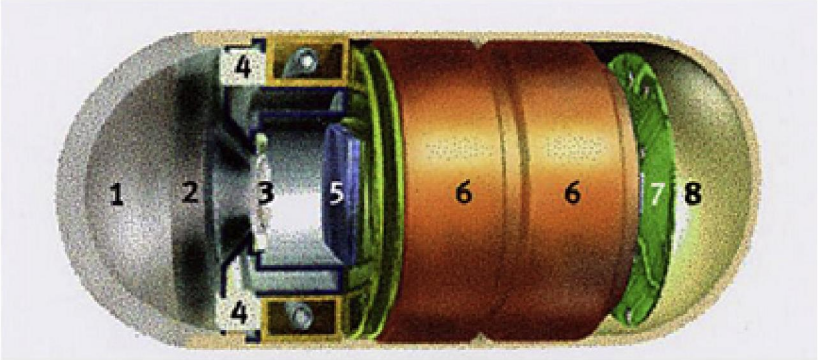

Figure 8 – Internal view of an endoscopic intestinal capsule showing the different technical elements: 1 – Optical Dome; 2 – Lens Holder; 3 – Optical Lens; 4 – Light Emitting Diodes (LEDs); 5 – CMOS Imager; 6 – Batteries; 7 – ASIC Transmitter; 8 – Antenna (from (Michel and Gérard 2008).

Since 2000, when (Iddan, Meron et al. 2000) first reported the CE, it has developed rapidly with important technical improvements. Initially, CE was created to explore the small bowel, but currently there are other solutions where it is applied to esophagus and the colon (Michel and Gérard 2008). The system consists of 3 components: (1) a capsule endoscope, (2) a sensing system composed of sensing pads attached to the trunk, a data recorder, and a battery pack, and (3) a personal computer workstation with proprietary software that reviews and interprets the images (Mishkin, Chuttani et al. 2006). With ethical committee approval, the first human volunteer study was performed in August 1999. The wireless capsule endoscope has received a

CE mark and FDA approval in August 2001 for use in patients (Swain 2003). The main specifications of the capsule endoscope can be found in (Mishkin, Chuttani et al. 2006).

The intestinal endoscopic capsule was designed to be swallowed and to go through the digestive tube, capturing approximately 50000 frames during 8 hours of examination. After that time, it is normally expelled by the organism and the resulting data are downloaded, processed and finally viewed on a monitor (Michel and Gérard 2008). Considering the large amount of information captured by a capsule endoscopy and the posterior time of analysis by a physician (usually, between 40 and 60 min, depending on the experience), several solutions have been created to reduce this time, and to process automatically the resultant images in an attempt to identify some pathologies, like small bowel inflammation, ulcers, and cancer cells (Dias, Correia et al. 2007; Natalin and Landman 2009; Iakovidis, Tsevas et al. 2010). Another innovative investigation with CE is trying to control its navigation, since the impossibility of any motion control of the capsule makes the visceral exploration not accurate enough (Carpi, Galbiati et al. 2007).

Despite the advantages of the endoscopic capsule, it can cause some problems such as obstruction, particularly in presence of some diseases as Crohn's, or influence on electrical devices as pacemakers (Adler and Goustout 2003).

In this Master thesis project, it will be performed endoscopic image analysis acquired by endoscopy or capsule endoscopy, in an attempt to automatically detect aberrant crypt foci, since they are thought to be colorectal cancer precursors. This analysis will also contribute to help physicians in their process of diagnosis.

6. Methods of Computational Image Analysis

The detection of pathologies through endoscopic images has been performed by medical doctors, usually trained for that. However, in spite of being good in their tasks, they are also slow, could be affected by fatigue, boredom, and environmental factors, are susceptible to committing errors and they are regularly subjective and qualitative (Rangayyan 2005). Hence, there are several computational techniques of image processing capable of detecting patterns, edges, clusters, etc. that can aid medical doctors in their diagnosis.

Concerning to endoscopic images, acquired by capsule endoscopy, there are approximately 50000-60000 images per examination and it takes experienced medical clinician over an hour to view and analyze all the video data. Moreover, the physicians might miss some abnormalities if they were present only in one or two frames of the video, or if that cannot be detected by the naked eyes due to their size, color, texture and distribution. Furthermore, distinct clinicians may have different findings when come to the same image data. All these problems motivate the researchers to develop reliable and uniform assisting approaches to reduce the great burden of the physicians (Baopu and Meng 2007).

An overview of the principal techniques used in biomedical image processing is going to be detailed in this section and, later, it will be presented a review about what is specifically applied to endoscopic images.

At the most basic level, a digital image is represented by a rectangular array of numbers, divided into small regions: pixels. The intensity number inserted in each pixel reflects the brightness of the image at the corresponding point. (Castleman 1996) The digital image processing can be divided in fundamental steps. According to (Gonzalez and Woods 2002), the fundamental steps are image enhancement, image restoration, compression, morphological processing, segmentation, representation and description, and recognition. However, not all of these steps are important to the proposed aim of this work and, as such, only the relevant will be briefly detailed.

6.1.Pre-processing

Image pre-processing is usually performed in order to reduce or eliminate noise. In (Yu and Tan 2009) is suggested that the preprocessing stage consists of several subtasks including image enhancement, noise reduction, gradient magnitude estimation and preliminary LOI (layer of interest) extraction.

Hence, this first step in image processing should be adjusted according to the algorithms applied and in function of the application.

6.2. Image Segmentation

One of the most critical steps in the process of reducing images to high-level information is segmentation. It subdivides an image into its constituent regions or objects and the level to which it is applied depends on the desired result, that is, segmentation should stop when the objects of interest in an application have been isolated (Gonzalez and Woods 2002).

There are several algorithms of segmentation and the most relevant will be detailed.

6.2.1. Thresholding

The process of thresholding is used to separate an object from the background. As such, one simple way is to define a range of brightness values in the original image, select the pixels within this range as belonging to the foreground, and reject all the other pixels to the background, dividing an image into two colors: usually black and white (binary image) (Russ 2002). Mathematically, it could be defined as:

$$g(x,y) = \begin{cases} 1, & f(x,y) < T \\ 0, & f(x,y) \geq T \end{cases}$$

where *g(x,y)* is the output image, *f(x,y)* the input image and *T* the defined threshold value.

Moreover, the definition of the threshold value could be manual, requiring previous knowledge or iterative experiments, or automatically, which combines the image information to get adaptive threshold. (Otsu 1979) suggested a method to automatically find the value of the threshold based on the image histogram.

According to the information used to define the threshold values, algorithms can be further classified as edge-based ones, region-based ones and hybrid ones (Ma, Tavares et al. 2009).

6.2.2. Edge-based

An edge is generally defined as a boundary between two regions with different grey-level properties. The algorithms of segmentation based on edge detection try to separate the interested regions according to its boundaries. Hence, Wavelet transform, Canny edge

detection, Sobel edge detection and Laplacian edge detection are the most preeminent examples of this type of segmentation.

The wavelet transform provides a progressive encoding of the image at various scales and its functions are localized. As such, the wavelet transform tends to deal better with the edges of the regions and of the image. There are two main functions generally used: Haar and Daubechies. The first one is the simplest, but they are both based in the same principle of subdivide the image into quadrants according to average values of intensity (see, for example, (Gonzalez and Woods 2002) for more details).

The Laplacian edge detection is based on the calculation of the second derivative of the image that will produce an abrupt zero-crossing at an edge (Davis 1975). It is commonly implemented digitally by a convolution kernel that generally is a 3x3 matrix composed of different numbers, according to its application. This kernel will convolve with every pixel of the image, resulting in an output image with the edges detected.

The Sobel edge detector uses two kernels with horizontal and vertical direction. It calculates the magnitude and direction gradient of each pixel and its neighborhood. Since an edge is characterized by a higher magnitude value when compared to its neighborhood, this detector will be able to detect every edge and construct an output image with a black background and white edges.

Finally, Canny edge detector is based on finding the zero-crossing of the laplacian of Gaussian (LoG), a bandpass filter that calculates the second derivative of a smoothed image (Canny 1986; Russ 2002).

6.2.3. Region-based

Algorithms based on regions are used to separate objects from the background, taking into account the pixels properties of a region. The principal method cited is region growing.

Region growing, initially proposed by (Adams and Bischof 1994), is a procedure that groups pixels with similar characteristics as gray level, texture or color. The simplest approach starts with a “seed” point, that could be a single pixel, and according to the properties of the seed, the group is formed through attachment of other pixels with the same characteristics. The process of growing should stop when no more pixels satisfy the criteria for inclusion in that region (Gonzalez and Woods 2002; Jun 2010).

6.2.4. Watershed

Watershed is a type of threshold algorithm, but instead of simply thresholding the image at the optimum gray level, it uses gradually different values of threshold until it segment the image into the desired regions.

The algorithm starts with a threshold at a low grey level that segments the image into the proper number of objects, but with boundaries that are too small. Then the threshold is raised gradually, one gray level at a time. The boundary of the objects will expand as the threshold increases; however, when two or more objects get in touch, they cannot merge and, as such, the algorithm stops its execution. If no objects contact, the process is terminated before the threshold reaches the grey level of the background.

The threshold values should be well defined in order to avoid initial objects merged or, on the other hand, not present in the beginning (e.g. threshold too low) (Castleman 1996).

6.2.5. Deformable models-based

The algorithms included in this class can be viewed as a modeling of curve evolution. They could be divided into parametric and geometric.

Starting with parametric models, they track the evolution through sample contour points. The moving equation for the contour can be derived through energy functions or defined directly through dynamic forces (Ma, Tavares et al. 2009)

One of the most common deformable models is the snake method. It was developed by (Michael, Andrew et al. 1988), who described this method as a matching of a deformable model to an image by means of energy minimization. From any starting point, the snake deforms itself into conformity with the nearest salient contour. Normally, it is used an edge detector based on gradient to detect the boundary of the desired object. As such, a snake is an energy-minimizing spline that pulls it toward features such as edges, and constrained by internal spline forces that impose piecewise smoothness (Rangayyan 2005).

Concerning to geometric deformable models, there are two principal methods described: Level-set and Chan and Vese model.

The Level-set method was fists introduced by (Osher and Sethian 1988) and is based on a partial differential equation (PDE), the Hamilton Jacobi equation. The main idea is to implicitly embed the moving contour into a higher dimensional level set function and view the contour as its zero level set. Then, instead of tracking the discrete contour points, one can track the zero level set of the level set function. Formally, the evolution of the interface is driven by a

time-dependent PDE where the so-called velocity term reflects the image features characterizing the object to be segmented. The level set methods have been successfully applied to structural shape and topology optimization problems (Gelas, Bernard et al. 2007; Wang, Lim et al. 2007; Ma, Tavares et al. 2009).

The Chan and Vese model can be described as an active contour without edges. As such, contrary to snakes, this method is not based on an edge function to stop the evolving curve on the desired boundary. It is based on Mumford–Shah segmentation techniques (Mumford and Shah 1989) and the level set method and is capable of detecting objects whose boundaries are not necessarily defined by gradient or when they are very smoothness, for which the classical active contour models are not applicable (Chan and Vese 2001). The algorithm extracts the desired object through simultaneously minimizing the intensity variations inside and outside the contour. The most appreciable advantage of Chan and Vese model is that it can obtain a boundary of discrete points, which is quite useful when the objects of interest are represented by discrete pixel clusters and have no clearly defined boundaries (Ma, Tavares et al. 2009).

6.2.6. Neural Networks

Usually, the final step in image segmentation is attributed to region classification. Neural networks have a crucial role in this process and (Aleksander and Morton 1990) proposed a definition:

A neural network is a massively parallel distributed processor made up of simple processing units, which has a natural propensity for storing experimental knowledge and making it available for use. It resembles the brain in two respects:

1. *Knowledge is acquired by the network from its environment through a learning process.*
2. *Interneuron connection strengths, known as synaptic weights, are used to store the acquired knowledge.*

Generically, neural networks mimic the processes found in biological neurons, and they are used to predict and learn from a given set of data. As such, giving information about the image (some of this information is a result from the processes explained at section 6.3.), it could predict the presence of same pathology as, for example, cancer. In this way, neural networks are widely used in medical image analysis (Baopu and Meng 2007).

6.3. Features Extraction

The process of segmentation allows obtaining an output image with regions well defined, or objects separated from background. Further, it is possible to extract some features, as color or texture, to later classify the input images. However, these methods are sometimes used as segmentation algorithms too. In this point, it will be briefly explained the most common features extracted due to its relevance in the study of endoscopy images (Tjoa and Krishnan 2002; Tjoa and Krishnan 2003; Baopu and Meng 2007; Charisis, Hadjileontiadis et al. 2010).

6.3.1. Texture

Many images contain regions characterized not so much by a unique value of brightness, but by a variation in brightness that is often called texture. If the grey level is constant everywhere in the object, or nearly so, we say that the object has no texture. However, if the gray level varies significantly within the object then the object has texture. In this way, the measurement of the grey level variance is a good indicator of texture (Castleman 1996).

The texture analysis could be performed in four different approaches, called: statistical, co-occurrence matrix, spectral and structural.

Statistical approaches describe texture using moments of the grey level histogram of an image or region. It measures the standard deviation, variance, skewness (measure of asymmetry) and kurtosis to characterize the texture of the image.

Concerning to co-occurrence matrix, it is constructed based on direction (vertical, horizontal, etc.) and distance (one pixel, 2 pixels, etc.) of an image. The elements of the matrix are defined according to the mean number of times that a grey level occurs in two pixels separated by a distance and direction predetermined. There are 3 types of matrix that could be constructed defined as entropy, inertia and energy (Castleman 1996).

The spectral features are based on the two-dimensional Fourier transform, since it contains complete information about image's texture. There are 3 features of the Fourier spectrum that are useful for texture description: (1) Proeminent peaks in the spectrum give the principal direction of the texture patterns. (2) The location of the peaks in the frequency plane gives the fundamental spatial period of the patterns. (3) Eliminating any periodic components via filtering leaves nonperiodic image elements, which can then be described by statistical techniques (Gonzalez and Woods 2002)

Finally, the structural approach to texture analysis assumes that the texture pattern is composed of a spatial arrangement of "texture primitives". These are small objects that

constitute, for example, one unit of a repeated pattern. As such, the features extraction is based on finding these primitives and quantify their spatial arrangement (Castleman 1996).

6.3.2. Color

The color image processing can be performed in different formats. The two principal formats referred in the literature are RGB (Red, Green and Blue) and HIS (Hue, Saturation and Intensity). In the RGB model, each color appears in its primary spectral components of red, green and blue. As such, an image is represented by 3 matrixes, each one representing the intensity level in the red, green and blue domain.

The HSI model is composed of three main characteristics: Intensity, Saturation and Hue. Intensity is the average of the R, G and B grey-levels, although different schemes with unequal weighting of the colors are also used. However, this intensity does not have any information about color. As such, Hue and saturation are responsible for that information. Hue is expressed as an angle and it refers to the spectral wavelength that most closely matches with the original image. The saturation parameter is described as the radius of the point from the origin of a color circle. Around the periphery of the circle, fall the saturated color and at the center lie neutral (gray) shade (zero saturation) (Castleman 1996).

Generally, the color feature extraction is based on histogram. The color histogram is obtained by computing the number of pixels having the same color and it provides a very useful clue for image indexing and object recognition (Wu, Kankanhalli et al. 2002). In (Swain and Ballard 1991) was proposed a new method based on histogram intersection through a similarity measure. That is, given a pair of histograms, the result of the intersection of a model histogram with an image histogram is the number of pixels from the model that have corresponding pixels of the same color in the image. Another strand of research about color has focused on moment invariants under different kinds of geometric and photometric changes (Gevers and Stokman 2004; Gevers, Voortman et al. 2005).

6.4. Application to Endoscopy Images

Different investigation work has been made to detect the presence of colon cancer from 2 perspectives: in histological colon tissues, or through detection of polyps or ACF in endoscopic images. In this Master thesis project, the aim is to detect colorectal cancer using endoscopic images.

In the literature, there are several algorithms applied to segmentation and feature extraction. Concerning to segmentation applied to endoscopy images, (Tjoa, Krishnan et al. 2002) based their approach on color feature hue measure, since colonoscopic images contain rich color information, and it can provide better results for the segmentation of the colonoscopic images than approaches using merely intensity information.

In the 90's decade, (Krishnan and Goh 1997) and (Krishnan, Xin et al. 1999) based their work in a procedure including 2 steps: image segmentation and labeling. The color image segmentation was based on the histogram analysis using scale-space filter. From these segmented regions it was possible to extract some features and attribute a label based on fuzzy rule, i.e. attribute some conditions to the features measured in order to aggregate them. For example, if area is middle and the mean value of intensity and saturation is low, then the region is lumen.

After an image is segmented into regions, there are several features that could be extracted, in particular, texture and/or color in the wavelet domain and patch-based Support Vector Machine (SVM) classifier.

In (Tjoa and Krishnan 2002) and (Maroulis, Iakovidis et al. 2003) was proposed a novel method for extracting new texture-based quantitative parameters from the texture spectra both in chromatic and achromatic domains of the colon images obtained by endoscopy. The textural features obtained were later used as inputs to a neural network. However, several authors proposed a hybrid of texture and color features, and found that it gives a better approach on colon status classification than the 2 features separated (Karkanis, Iakovidis et al. 2003; Tjoa and Krishnan 2003).

The color features extraction could be based on Wavelet covariance (Karkanis, Iakovidis et al. 2003), Wavelet transformation (Barbosa, Ramos et al. 2008) or RGB color space using BEEMD (Bidimensional Ensemble Empirical Mode Decomposition) (Charisis, Hadjileontiadis et al. 2010).

Finally, (Peng, Kap Luk et al. 2004) proposed a new method to detect abnormal regions in colonoscopic images by patch-based classifier ensemble. The multiple sizes of patches provide

multiple level representation of the image content, which can help improve detection results. Several fusion criteria are explored to determine the final output of the ensemble. Experimental results showed promising performance of their proposed method.

Concerning to ACF detection, in (Figueiredo, Figueiredo et al. 2010) was proposed a method to detect ACF in endoscopic images based on partial differential equations, more exactly, active contours without edges (Chan and Vese model). However, there is still much work to do, since there are few articles in the literature reporting image segmentation to detect these structures in endoscopic images *in vivo*.

7. Conclusion

Cancer is one of the most concerning diseases in the modern world, and this fact motivates the research for solutions in the detection, diagnosis and treatment.

Considering the mechanisms involved in this pathology, several advances have been made and, over time, more accurate imaging techniques have been used, as computed tomography and magnetic resonance. Concerning to gastrointestinal tract, endoscopy and colonoscopy are the most important forms of diagnosis. Recently, it was developed the capsule endoscopy allowing the investigation of the small bowel and diminishing the discomfort associated with this type of exams.

Regarding colorectal cancer, it is thought that ACF could have a crucial role in the sequence adenoma-carcinoma, although this fact is not certain and several studies are still in course with an attempt to clarify this concept.

The detection could be performed through endoscopy/colonoscopy or capsule endoscopy; however, the time spent during analysis by medical doctors could be substantially reduced using computational image processing techniques.

There are computational algorithms that could be applied to endoscopic images, such as those presented as image segmentation methods. In this context, future development will be based on finding the best algorithm to accurately segment, detect and quantify ACF structures, from endoscopic images.

8. Work Plan

Completed this general review, the future work will be divided into the follow main steps:

1. Selection of the computational platform to use and the experimental cases to consider;
2. Development of computational methodologies to perform endoscopic image processing of aberrant crypt foci;
3. Application, tests and analysis of the developed methodologies in experimental cases, synthetic and real;
4. Writing of the thesis report.

The planned tasks are distributed over 4 months, according to the next table:

Task	Date
1	End of March
2	End of May
3	Middle of June
4	End of June

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