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Technical validation of an ex vivo diagnostic device for colon polyp classification based on microwaves

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Abstract

Colorectal cancer (CRC) is a leading cause of death in developed countries, and early detection is crucial for a favorable prognosis of the patient. To promote and increase this early detection, screening program have been implemented in many countries, which has increased the amount of colonoscopies performed significantly. Consequently, the amount of colon samples and biopsies sent for histological analysis has also increased, which is creating bottlenecks in endoscopy and pathology departments at major public hospitals. A new technology to rapidly provide an initial diagnosis of the extracted polyps is under study at UPF in collaboration with Hospital Clínic and UPC. It is based on measuring the dielectric properties of the tissue using microwaves, in a non-ionizing and non-destructive manner. This device could potentially replace histological analysis of benign polyps, thus reducing the workload of pathologists.

In this thesis I intend to do a technical validation of this technology, in which the idea is validated with experts in the field, the state of the art is researched and the technological specifications are defined. To do so I carried out an extended bibliographic research, sent surveys to the medical professionals involved and went to Hospital Clinic to dive into the endoscopists and pathologists daily practice. I also investigated the different technological approaches for the device. Additionally, I analyzed the measurements obtained from a pre-proof of concept experiment and proposed a future experimental validation test. Finally, the milestones that have to be followed in order to launch this device into the market are explained.

The proposed device is a novel solution to an existing problem, which would be very beneficial for the medical community. However, there are still many steps to be performed before this device can reach the market.

Keywords

Colorectal cancer, anatomical pathology, colon polyp, tissue characterization, dielectric properties, microwaves, ex vivo diagnosis.

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1. INTRODUCTION

1.1. Clinical Need

1.1.1. Colorectal Cancer

Colorectal cancer (CRC) affects the colon and the rectum of the large intestine. It starts when there is an uncontrolled growth of cells in these areas, creating small noncancerous clumps of cells called polyps. Although these polyps are benign at first, they may become cancerogenous over time. CRC is the most commonly diagnosed cancer in Spain in 2017 with more than 34,000 new cases per year only considering the population between 50 and 69 years old. It is also the second leading cause of cancer-related deaths, with almost 16,000 deaths in 2017 [1].

1.1.1.1. Causes and Risk factors

CRC, as all cancers, is caused by a mutation in the DNA sequence that causes the cells to continuously divide even when they are not needed. This leads to accumulation of unnecessary cells, thus tumor formation. These mutations can be inherited or acquired, which are the most common causes of CRC. The risk factors for acquired CRC could in turn be modifiable, such as physical inactivity or obeseity, or non modifiable, such as age and race (Table 1).

INHERITED	Hereditary Nonpolyposis colorectal Cancer (HNPCC) Familial Adenomatous Polyposis (FAP)	
ACQUIRED	Not modifiable	Age, personal history, diabetes, race
	Modifiable	Physical inactivity, obesity, diet, smoking, alcohol abuse, diabetes

Table 1. Types and risk factors of colorectal cancer [2], [3].

1.1.1.2. Symptoms

CRC can take up to 15 years to develop [3], which explains why many patients exhibit no symptoms in the early stages. Some signs to be aware of are long lasting changes in one's bowel habits, such as diarrhea, constipation, or different consistency of the stool. Symptoms also include rectal bleeding,

abdominal discomfort, unexplained weight loss, a feeling that the bowel does not empty completely, weakness or even fatigue [2].

1.1.1.3. Diagnosis

Early diagnosis of CRC is crucial in obtaining a favorable outcome of the patient. About 90% of patients detected at an early stage survive the next 5 years, whereas less than 8% survive when detected at an advanced stage [4]. This is why screening programs have been implemented worldwide, in which they perform different tests to find cancer or precancer lesions in patients with no apparent symptoms. It is recommended to start regular screening at the age of 50. Screening tests for colorectal cancer include fecal occult blood test (FOBT), stool DNA test, sigmoidoscopy, standard (or optical) colonoscopy, and virtual colonoscopy (or computed tomographic colonography [2]–[4]. Many countries implement early detection programs based on colonoscopy or follow-up colonoscopies in case of a positive result of the primary test (usually FOBT). Colonoscopies are the most sensitive and specific detection test, as doctors can extract polyps in the entire colon and take biopsies for further analysis at the pathological anatomy unit. Nowadays all polyps found are extracted and sent for analysis.

1.1.1.4. Polyp and cancer classification

A critical step to correctly examine CRC in a patient is to analyze the polyps and determine its cancerogenicity. Polyps can be classified into neoplastic or nonneoplastic [5]. Nonneoplastic polyps can be hyperplastic, inflammatory, hamartomatous, among others, and in general do not have malignant potential. On the other hand, adenomas and adenocarcinomas are neoplastic polyps, and are considered a precancerous condition. The difference between them is mainly that adenomas are noninvasive and adenocarcinomas are invasive. In addition, adenomas can be subclassified into low grade dysplasia (LGD) and high grade dysplasia (HGD). Moreover, cancer development also depends on the polyp size, the quantity found and the dysplasia grade observed in the histologic analysis [6]. It is also important to stage the cancer, as it is an

indicator of its extension, and provides an insight to decide the best treatment (Table 2).

STAGE	CHARACTERISTICS
Stage I	The cancer is growing through the superficial lining, but has not passed beyond the colon wall or rectum.
Stage II	The cancer has reached the colon wall, but no lymph nodes are affected
Stage III	Lymph nodes are affected, but there is no metastasis to the rest of the body
Stage IV	There is metastasis to other organs

Table 2. Stages and characteristics of colorectal cancer

Polyp characteristics and the stage of the cancer are crucial in determining the therapeutic strategy to try to eradicate this cancer in the patient.

1.1.1.5. Treatment

The three main options to treat CRC are resection (endoscopy or surgery), chemotherapy and radiation [2]–[4]. Depending on the cancer stage and the patient's background doctors will recommend one or the other. Early stages of CRC can be treated with minimal interventions, such as polyp removal during colonoscopies, endoscopic mucosal resection, or minimally invasive surgery. When the cancer is invasive and has spread through the colon, part of the colon may be removed during partial colectomy, in which lymph nodes are also removed. Once the cancer is very advanced, surgery may be performed to alleviate the symptoms and improve lifestyle, not to cure the disease. Chemotherapy is usually done after surgery to prevent recurrence in the body, although it can also be performed before surgery to shrink the cancer and the area affected. Radiation therapy has a similar effect as chemotherapy, and sometimes they can be combined together for initial management of rectal cancer.

1.1.1.6. Incidence

As a consequence of the implementation of screening programs, and the increase of risk factors, CRC incidence is increasing (Figure 1) and will probably continue to increase. Currently it is the most common cancer in Spain [7].

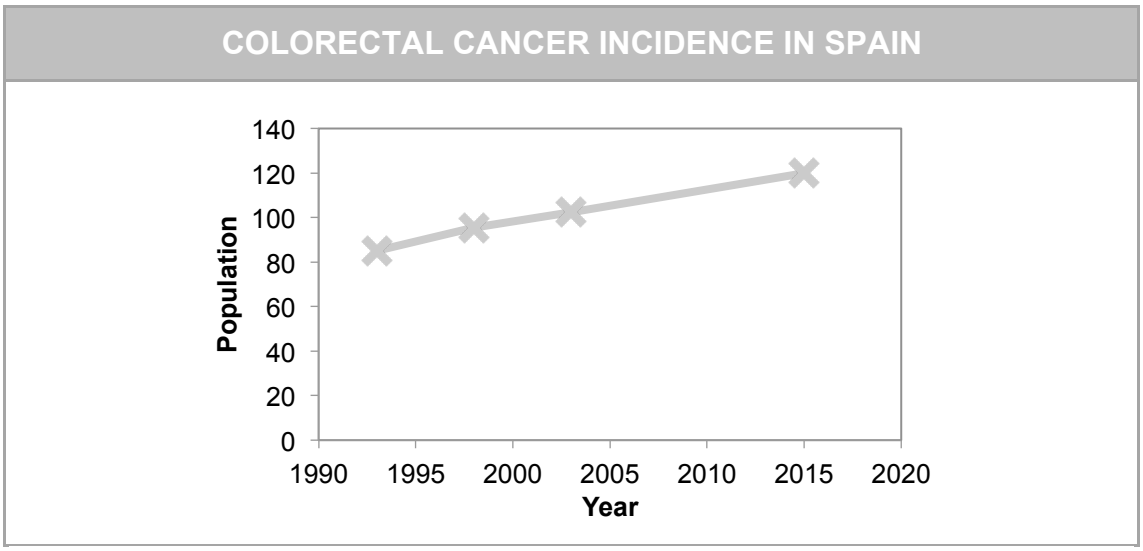

Figure 1. Colorectal cancer incidence in Spain over the last years. The population is expressed per 100,000 person-years [8]

1.1.2. Pathological anatomy

In order to stage the cancer and classify polyps, biopsies and resected polyps are sent to the pathological unit for a thorough analysis of the microscopic and molecular characteristics of the tissue. This unit is thus in charge of performing the complete diagnosis of the lesion, and it is considered the gold standard for tumor detection and classification. They do so by studying the macroscopic, microscopic, histochemical, immunological and molecular properties of the tissue by means of advanced microscopes, immunofluorescence and molecular pathology techniques [9].

Assessing the pathologic stage is the most accurate method for determining postoperative outcome. However, there are other pathologic features independent of stage that also have prognostic significance and aid in stratifying tumors within each stage [6]. This is why pathologist do not only look at the stage of the biopsy, but also the histologic grade, small vessel invasion, extramural venous invasion, perineural invasion, tumor border configuration, host lymphoid response and the status of surgical margins. The importance of each of these features will be discussed in section 2.2.2.

The workflow of a pathologic analysis can be seen in Figure 2 [10]. When receiving a colon polyp or biopsy, these are tagged with a specific code. This is crucial for tracking all the procedures and later relating the diagnosis with the

corresponding patient. Pathologists then carry out a macroscopic study of the sample, where they pay close attention to the morphological characteristics, specially size and shape. This process is called grossing. Then the samples are processed with alcohols for dehydration. To prepare the slides the samples are covered with paraffin, and then cut with a microtome. Once the cuts are on the slide, they are stained with hematoxylin-eosin (H&E). In some cases special stains are used when H&E cannot provide all the information pathologists are looking for [11].

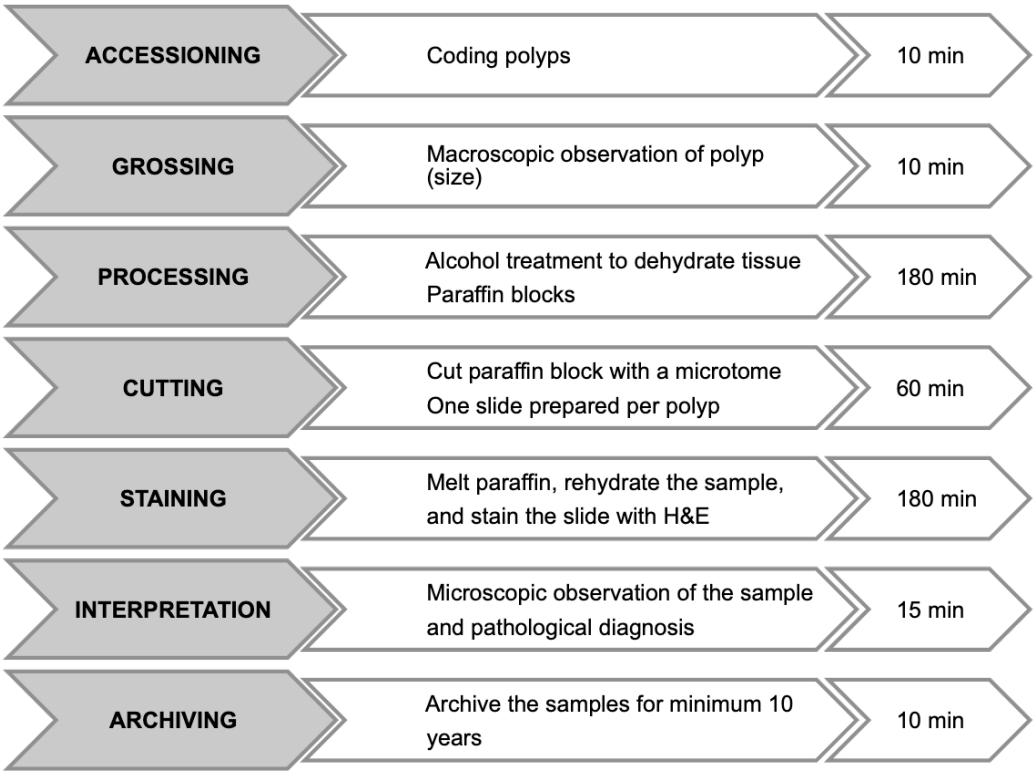

Figure 2. Pipeline of the pathological analysis of a polyp and the time taken for each task elaborated from the observation of the routine in the pathology department of Hospital Clinic de Barcelona.

Finally, the slides are sent to a pathologist that examines the microscopic properties of the tissue and elaborates a histopathological diagnosis taking into account the previous endoscopic information. All samples are stored a minimum of 10 years for legal reasons.

1.1.3. Clinical Problem

As mentioned previously, CRC is the malignant tumor with the highest incidence in Spain and an increasing worldwide problem. In Spain, the

Colorectal Cancer Screening Program was created in 2011, with the aim of being completely implemented by 2024 [12]. This program increased the amount of colonoscopies performed increased by more than 20.000 from 2015 to 2016 [13], [14], and a further increase is expected. Additionally, all the endoscopists surveyed agree that on average they extract more than 3 polyps in each colonoscopy, so the number of samples to be analyzed pathologically will also increase. This is already creating bottlenecks in endoscopy and pathology departments at major public hospitals (as all the polyps are biopsied and analyzed), so it is crucial to improve the efficiency of the current procedures.

Nowadays the pathological analysis has many manual processes that are labor and time consuming. It has been estimated that the cost of preparing an H&E slide is about 16€ [10], and takes more than 7 hours to process them (Figure 2), which proves the inefficiency of this process. Furthermore, there is an increasing demand that is hard to meet due to the increasing workload and the operational inefficiency. As a result, 57,5% of endoscopists stated that they wait more than 7 days to obtain results due to oversaturation of the pathology service.

Furthermore, although the histopathological evaluation is considered the gold standard, it has a limited prognostic value due to sampling limitations and the 2 dimensional analysis of a volumetric sample. Other controversies in colorectal cancer pathology reporting include the subjective nature of some of the elements assessed and the reproducibility. Surveyed pathologists state that only 1 slide is analyzed for a given polyp, which represents a small proportion of the whole polyp.

For these reasons, developing an easy and fast device capable of analyzing polyps in the same endoscopy room and giving quantitative information of the tissue characteristics, thus providing an initial diagnosis, would have a crucial clinical interest. The proposed solution is based on measuring the dielectric properties of polyps at microwave frequencies with a portable measuring station. Researchers from UPF in collaboration with Universitat Politècnica de Catalunya and Hospital Clínic de Barcelona demonstrated that the dielectric

properties are correlated with the degree of dysplasia of colorectal polyps [15]. The technique is nondestructive, does not require any tissue preparation and does not interfere with a possible subsequent pathological analysis. Therefore, the proposed solution will allow to prioritize samples for histological analysis according to the degree of dysplasia, and in the long term, elude histological analysis. This method can bring significant economic savings derived from CRC screening programs to our National Health System, and help contain the escalating health care costs prevalent in most developed countries.

1.2. Objectives

The objectives of this thesis are the following:

1. To explore the clinical need and the validity of the proposed diagnostic approach among health professionals specialized in this area.
2. To study the possible technological solutions for a device of rapid ex vivo diagnosis of colon polyps using microwaves, analyze its theoretical benefit over current diagnostic techniques, and design a validation experiment.
3. To explore the roadmap required for the proposed device to reach the market.

2. DIAGNOSTIC FRAMEWORK

In this section the current clinical setting for polyp diagnosis is described. Polyp diagnosis can be performed during colonoscopy and afterwards by the histological analysis of the resected samples. The use and the importance of each method on the final diagnosis depends strongly on each country. Treatment decision in the West is greatly based on size and location of the tumor, along with the histology of the biopsies. On the other hand, eastern endoscopists give a great role to the endoscopic classification to later decide therapeutic procedures [16]. The classification systems elaborated to classify the polyp during colonoscopies and the most important parameters during the histopathological examination are explored.

The information presented in this section is a result of an extensive bibliographic research, interviews with professionals, and surveys sent to pathologists and endoscopists.

2.1. Colonoscopic Diagnosis

Colonoscopies are endoscopic examinations used to detect anomalies in the inner lining of the colon or rectum. In this procedure a colonoscope, which is a large and flexible tube with a video camera at the tip that allows visual diagnosis, is inserted through the rectum. It also has a channel through which miniaturized tools can be inserted to extract biopsies and perform maneuvers to treat or diagnose [17].

A colonoscopy usually takes from 30 to 60 minutes, and the patient is under sedation. Doctors carry out colonoscopies to explore intestinal symptoms and find out the cause of these. They are also performed in the screening process, when the patient has a high risk of CRC, or he/she has positive results for other screening tests. During patient follow-ups doctors may also recommend regular colonoscopies [18].

2.1.1. Colonoscopic technologies

The endoscopic world has experienced a huge increase in the past years, and new technologies that allow examination of all details of the mucosa have been developed. The main advances achieved in colonoscopies directed to enhance tissue diagnosis are magnification, conventional chromoendoscopies and optical chromoendoscopies [19].

2.1.1.1. Currently in clinical practice

Optical magnification endoscopes enlarge the image up to 150 times whilst maintaining the image display quality. This allows a detailed visualization of the colorectal mucosa, and aids in the classification of polyps. Magnification can be used in combination with other techniques such as chromoendoscopies and NBI for better visualization of the mucosal pattern and better diagnostic results [20].

Conventional chromoendoscopy consists on spraying a chemical substance on the surface of the tissue to highlight specific areas to thus improve visualization of the surface pattern of the colon and contributes to the recognition of different types of epithelia, leading to an increase in the diagnostic potential of colonoscopies [21].

As an alternative to conventional chromoendoscopy, virtual chromoendoscopy is an electronic imaging technique that enhances visualization of the tissue without the need of dyes. These can be optical or digital. The optical technologies use optical lenses to filter out white light; whilst the digital ones post-process the video [22]. The main commercially available systems are NBI [23], FICE [24] and i-scan [25].

	ADVANTAGES	DISADVANTAGES
Magnification	Enlarges image Can be combined with other techniques	Not widely used Unclear diagnostic potential
Virtual Chromoendoscopy	Diagnostic potential No preparation required	Many different classification protocols Subjective
Conventional chromoendoscopy	Diagnostic potential	Useful for localized polyps Requires preparation and time Subjective

Table 3. Comparison of different techniques applied to colonoscopies to aid the visualization and diagnosis of colon lesions

In Table 3 the advantages and disadvantages of each technique are summarized. Virtual and conventional chromoendoscopy have similar diagnostic potentials, and high specificity in polyp classification. However, they depend on the experience and the subjective opinion of the endoscopists.

From the survey I found that virtual chromoendoscopies are the most used by endoscopists (72,5%), although most of them also use conventional chromoendoscopies and high definition (64,1% each). However, only 23,1% used magnification.

2.1.1.2. In development

Due to the importance that endoscopic tissue characterization can have, the search for an optimum diagnostic technique is being widely studied. A novel technique that is still in experimental phases is endocytoscopy. This technique providing optical magnification up to 1150 times and can be considered as an “optical biopsy” in vivo [26].

Another emerging technique is Confocal Laser Endomicroscopy (CLE). It consists on illuminating the tissue with a low-power laser and detection of the reflected light through a pinhole. This allows CLE to provide cellular images and thus evaluate the architecture of the tissue during endoscopy. Although the potential of this technology is apparent, studies to determine the clinical efficiency need to be carried out for it to be widely accepted [27].

2.1.2. Current Colonoscopic Tissue Characterization

With the techniques mentioned above, endoscopists are able to provide an initial diagnosis of the lesion and characterize the tissue. Some studies have been carried out with the purpose of trying to establish a relation between the endoscopic and histologic examination. For this purpose, many classification types have been elaborated.

A correct analysis of the morphology of superficial lesions can aid in predicting the degree of invasiveness in the submucosal tissue. Furthermore, the mucosal pattern of the colon is also crucial for a thorough study of the lesion.

From Table 4 we see that even though there are several classifications, they intend to distinguish between benign and malignant, and try to determine the invasiveness of the lesion. However, it seems clear that there is lack of consistency between protocol to classify polyps during colonoscopies, and many classifications systems depend on the technology used. Consequently, there is a great dependence on the human criteria and the experience of the colonoscopist to diagnose each polyp, leading to interobserver variability and poor reproducibility. More information on these classifications is displayed in appendix 2.

DIAGNOSIS	CLASSIFICATION	CHARACTERISTICS
Morphology	Paris [16]	i) Polypoids (0-I) ii) Non-polypoids: (0-II), (0-III).
Histology Prediction	Kudo [28]	i) Non-neoplastic ii) Noninvasive neoplasia iii) Neoplasia
	Sano [29]	i) Type I – Hyperplastic polyp ii) Type II – Adenoma iii) Type III – Adenocarcinoma
	NICE [30]	i) Type I – Hyperplastic polyp ii) Type II – Adenoma iii) Type III – Invasive Cancer

Table 4. Summary of the main endoscopic classification systems and their characteristics.

From the surveys, when endoscopists around Spain were asked whether they used any classification system for an initial diagnosis, 84,6% used the Paris classification, 59% used NICE and the Kudo system. The rest of the classifications were not widely used by the surveyed endoscopists.

2.2. Pathological Analysis

Nowadays, from the colonoscopy alone, it is very hard to predict the properties of the resected polyps, which is why they are sent to the anatomic pathology laboratory for further histopathological study. Following the macroscopic examination, the polyp is processed and prepared to be looked at through a microscope following the procedure described in section 1.1.2. Histopathological evaluation is crucial when managing patients with CRC, as it gives an initial diagnosis of the disease and is critical in determining the therapeutic strategy. Nowadays it is considered the most accurate method for determining the postoperative outcome for each patient.

2.2.1. Pathological Technologies

2.2.1.1. Currently in clinical practice

Many processes are carried out in the anatomical pathology laboratory in order to analyze the samples. Different equipment is required at each step, and many different technologies can be used. To prepare the slide, some processes are carried out by a machine and no supervision is needed. Such is the case of the tissue processing and the staining processes, where the samples are introduced into the corresponding machine and then picked up when the process is finished. However, there are still processes that require technicians to carry out, such as the macroscopic analysis. The most tedious process however is slicing the samples and preparing the slides, as it has to be done manually. Even though there are automatic microtomes, a technician is required to put the slice on the slide, making the process very laborious.

Once the slide is prepared, pathologists are in charge of analyzing it. Traditionally the slide has been put under a conventional light microscope and then analyzed. Pictures could be taken with dedicated microscope cameras. Recently, whole slide imaging (WSI) scanners has been implemented and are used in many hospitals. This scanning technique scans the entire slide and thus creates a "virtual slide". This has been an important improvement in slide analysis, as algorithms and machine learning can be applied to semi automatize the analysis [31].

2.2.1.2. In development

A need to automatize the pathological analysis is apparent, as it is a laborious process where many processes are carried out, which may lead to artifacts and limitations in the diagnosis. For this reason, many efforts have been inverted in developing techniques to automatize the process to reduce errors and optimize the process. Microscopy with ultraviolet surface excitation (MUSE) is an emerging non-destructive slide free technique based on fluorescent dyes that allows surface visualization and provides virtual H&E renderings [32]. Another non-destructive slide free technique proposed recently is light sheet fluorescence microscope, which optically sections the sample to provide a

volumetric image with the same level of detail as conventional pathology slides [33]. Finally, spectroscopic techniques based on stimulated Raman scattering can generate histology images of brain tumors, and its potential applications are being studied [34].

Even though these techniques look very promising, there is still a lot of research to carry out before they are implemented in the clinical workflow. Moreover, they still rely on subjective assessment of graphical information.

2.2.2. Current Histopathological Polyp Characterization

When polyps are sent to anatomical pathology, they are classified according to the Vienna classification, which looks at whether or not the polyp has neoplasia and its characteristics. The Japanese Gastric Cancer Association (JGCA) also intends to provide a pathological classification, but it is more complex, and less used (Table 5).

DIAGNOSIS	CLASSIFICATION	CHARACTERISTICS
Pathological Classification	Vienna [35]	i) Negative for neoplasia ii) Indefinite for neoplasia iii) Mucosal low grade neoplasia iv) Mucosal high grade neoplasia v) Submucosal invasion by carcinoma
	JGCA [16]	Type 0 – Type 6

Table 5. Summary of the main pathological classification systems and their characteristics

Many polyps appear to be benign when resected during the colonoscopy, but malignant polyps account for 5% [36] of all adenomas. An initial indicator of the risk of harboring malignancy is its size. It was found that in polyps that are 2 cm or greater in size, the incidence of invasive carcinoma increases to 35-53% [37]. Additionally, the texture is another indicator of malignancy; villous polyps have a greater tendency of being adenocarcinomas than tubular ones.

For polyps there are three main histopathological features that increase the risk of unfavorable outcomes, which are tumor grade, the existence of tumor tissue within 1 mm from resection margin, and lymphatic or venous vessel involvement

[38]. It has been estimated that presenting at least one of these features increases the risk of adverse outcomes to 10-25% [39], so further therapy is recommended. Contrary, if non are present, the risk is minuscule and the polypectomy itself is already considered the curative treatment.

There are several tumor grading systems that cause controversies. However, a two-tiered grading system has been accepted, which classifies the tumor into high grade or low grade. It has been demonstrated that high tumor grade is an indicator of unfavorable prognosis [40]. Many studies have demonstrated that vessel invasion is an independent adverse effect, although there are no standard guidelines for pathologic evaluation, and it is often underreported [41]. Finally, resection margins can predict local recurrence, as it is considered high when the tumor is within 1mm of the margin [42].

When health care professionals were asked about the importance of these features for each polyp type (hyperplastic, LGD adenoma, HGD adenoma, adenocarcinoma), 60% agreed that for hyperplastic polyps evaluating the resection margins is not indispensable, however 83% answered that it is an important feature to consider for adenocarcinomas. Additionally, 74% consider that the vessel involvement is not a necessary feature in hyperplastic polyps, and 88% consider it is in adenocarcinomas. This suggests that for adenocarcinomas, the evaluation has to be done with more detail, as they are potential cancerogenous tissues, but hyperplastic evaluation is not as rigorous.

The main problem pathologists face when diagnosing malignant polyps is that there is significant interobserver variability due to a lack of standardized evaluating systems when analyzing pathologic features, leading to poor reproducibility [6], [41]. Additionally, although considered the gold standard, the histopathological analysis has a limited prognostic value due to sampling limitations and the 2 dimensional analysis of a volumetric sample. The inefficiency of this process has been demonstrated, and it seems of great need to find a solution to reduce the workload of pathologists. The characterization of surgically removed colon tissue is explained in appendix 3. For now we are only focusing on polyp analysis, but extending the application of the device to surgeries in the future is not discarded.

2.3. Diagnostic problems

In Table 6 we see the main problems medical professionals face when trying to diagnose colon polyps through colonoscopies and through pathological analysis. A common problem is the relevance of the human criteria and the subjectivity of the clinician when determining the polyp type, which leads to interobserver variability. A need to reduce the subjective criteria seems apparent, which can be achieved with a qualitative measure of the dysplasia grade of the polyp.

COLONOSCOPIC DIAGNOSIS	PATHOLOGICAL ANALYSIS
No universal protocol	Time and labor consuming
Interobserver variability	Interobserver variability
Technology dependent	Sampling limitations
East vs West	2D representation

Table 6. Main problems existing in the current clinical practice to diagnose colon polyps.

3. THE PROPOSED SOLUTION: MICROWAVE BIOPSY

Microwaves are a fraction of the electromagnetic spectrum ranging from 300 MHz - 300 GHz. This range of frequencies is non ionizing and the technologies are non-invasive, low power and low cost. Microwaves are used in the medicine field for imaging and sensing in diagnosis and treatment applications. The first applications date to mid 1980s, and the first studies were performed in the late 1990s [43]. As a diagnosis tool, microwaves are used for monitoring human vital signs such as respiration or heartbeat and for imaging different conditions such as brain stroke [44], breast cancer [45] and bone density [46] among others. From a therapeutic perspective, microwaves are used in hyperthermia, radiofrequency ablation and microwave ablation systems [47].

All these techniques are based on illuminating the tissue with an incident electromagnetic field and measuring the scattered/reflected field with a proper sensor to obtain information about the object properties interacting with the illuminating field. The interaction of electromagnetic fields with the human body

is dependent on the inherent dielectric properties of each tissue. It has been demonstrated that the dielectric properties change between different organs and tissues and between healthy and diseased tissue. Based on these properties, electromagnetic waves are transmitted, absorbed, and reflected in different ratios. Hence, by collecting the received electromagnetic fields, microwave systems can infer the tissue properties.

3.1. Dielectric properties of colon tissues at microwave frequencies

Dielectric properties of a given material are molecular properties that indicate its capability of impeding electron movement [48] and describe their behavior in an electromagnetic field. It is expressed by means of the complex permittivity of the material:

$$\varepsilon_r(j\omega) = \varepsilon_r'(j\omega) + j\varepsilon_r''(j\omega)$$

Where $\varepsilon_r'(j\omega)$ is the relative permittivity and $j\varepsilon_r''(j\omega)$ refers to the conductivity.

Measuring this property on biological samples has been a great breakthrough, as it was found that it is a biomarker of several conditions such as edema, infraction or cancer. In case of breast cancer, it has been demonstrated that healthy tissue has different dielectric properties than cancerogenous tissue [49]. Healthy samples contain more adipose tissue type, which has lower water content, whilst malignant tissues have higher water content. This causes healthy and malignant tissue to have different dielectric properties. Lazebnik et. al [49] performed an extensive study demonstrating that the dielectric properties of breast tissue were tightly correlated with the composition of the tissue.

Recently a study carried out by researchers at UPF in collaboration with Hospital Clínic de Barcelona and UPC tested whether the same phenomena happened with colon tissue. They measured the dielectric properties of colon polyps and biopsies right after extraction during colonoscopies, and then sent the samples to the anatomical pathology unit for a further histological examination [15].

There are several models that can be used to parameterize the measured values of complex wideband permittivity of biological tissues at microwave frequencies, mainly the Debye and the Cole-Cole models. They used the one-pole Debye model to fit the dielectric properties of colon tissues over the 0.5-20 GHz band. The complex permittivity can be expressed and approximated with the Debye model as follows:

$$\varepsilon_r(j\omega) = \varepsilon_r'(j\omega) + j\varepsilon_r''(j\omega) = \varepsilon_\infty + \frac{\Delta\,\varepsilon_r}{1 + j\omega\tau} + \frac{\sigma_s}{j\omega\epsilon_0}$$

Where $\varepsilon_r'(j\omega)$ is the relative permittivity and $\varepsilon_r''(j\omega)$ is proportional the conductivity (σ) expressed in Siemens/m.

$$\sigma = \omega\varepsilon_0\varepsilon_r''$$

A total of 51 valid measurements were performed in order to analyze the relative permittivity and the conductivity of the samples. The samples were classified into 5 groups according to the histopathological report: adenocarcinoma, adenoma HGD, adenoma LGD, hyperplastic and healthy mucosa. Figure 3 shows the results obtained for both the relative permittivity and the conductivity of the samples.

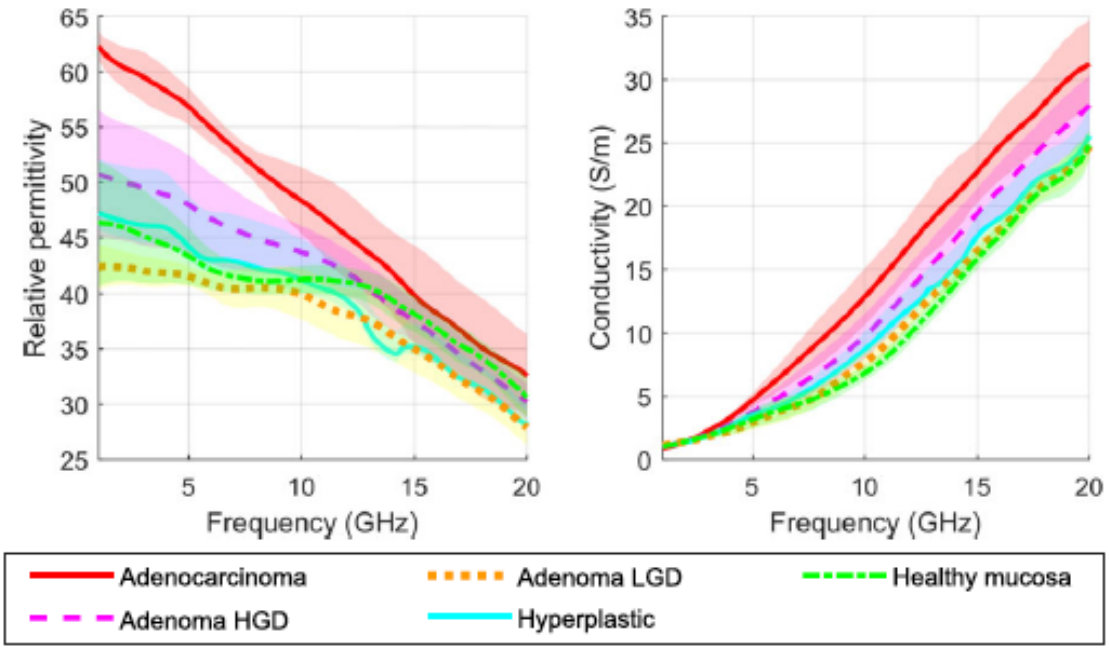

Figure 3. Measured relative permittivity and conductivity of colon tissues. The lines represent the median value and the areas around the median show the 95% confidence interval [15].

The results suggest a correlation between the complex permittivity and the grade of dysplasia, as we see higher values for both parameters in adenocarcinomas and adenomas HGD. The values also vary as frequency increases. With relative permittivity we see a clearer difference between the tissue types at low frequencies, whilst conductivity distinguishes tissues better at higher frequencies.

3.2. Proposed Device

Given the important dielectric contrast measured in colon tissues, a new device capable of automatically analyzing colon polyps once extracted to determine their grade of dysplasia in a rapid and nondestructive manner would be very useful. Results would be obtained within 2-5 minutes in the same room as the colonoscopy is performed, and no manipulation of the sample would be required.

3.2.1 Methods for dielectric properties' measurements

There are several techniques for dielectric property measurements, each one with different characteristics. The accuracy of these techniques depends on significant factors such as frequency, temperature, sample size or the nature of the material among others [50]. Below the main techniques considered for this device are explained and summarized in table 7.

3.2.1.1 Open ended coaxial probe

This technique consists on a metallic probe that senses the phase and magnitude of the dielectric properties of the sample. It covers a broad range of frequencies (0.5GHz-110GHz) and is a simple non-destructive method [50]. However, it assumes the sample is homogeneous under its sensing area, so the sample has to be large enough for accurate measurements, and is not accurate for low permittivity materials as it shows deflection. Additionally, air gaps can cause huge artifacts in the measurement, so the sample must be relatively flat under probe's sensing area (it is most suited for liquids or soft solids such as body tissues) and carefully placed under the probe to avoid air bubbles [51].

3.2.1.2 Resonant cavity

Resonant cavities have a specific frequency at which they resonate. When a sample is introduced in it, the resonance frequency and the quality factor is altered. From this, the complex permittivity of the sample can be measured at a single frequency [51]. It only requires calibration prior to the first measurement, the sample does not need further preparation, and small pieces can be measured. However it shows accurate results only in low dielectric loss materials, that is not the general case for body tissues, and it can only provide results at a single frequency [50].

3.2.1.3 Transmission line

In this method the sample is placed inside an enclosed transmission line, which can be a rectangular waveguide or a coaxial airline [50]. The coaxial line covers a broad frequency range, but is difficult to manufacture, whilst the waveguide line has a more limited frequency range, but is simpler. The sample must be carefully placed making sure it covers the entire cross-section of the line. This method assumes the sample is homogeneous and relatively flat [51].

METHOD	FREQUENCY	PREPARATION	SIZE	COMMENT
Coaxial probe	Broad band	Fast and simple	Small	Homogeneous and flat sample Calibration required
Resonant cavity	Single frequency	Non required	Very small	Homogeneous material Only for low dielectric materials
Transmission line	Broad band	Tedious	Small	Homogeneous and flat sample Resolution depends on sample length

Table 7. Methods for measuring the dielectric properties of colon tissue and their properties.

3.3. Validation with experts

A set of questions was designed for the survey sent in order to assess the opinions of professionals on this potential device (Appendix 1). The questions

were validated with both endoscopists and pathologists before being sent. Then these medical professionals helped me distribute the survey to a wider range of endoscopists and pathologists to obtain more answers. The surveys were sent via mail in a Google forms format to a list of endoscopists and pathologists separately from around Spain. I obtained 40 answers from endoscopists and 4 from pathologists. The fact that only 4 pathologists answered the survey could be an indicator of the amount of workload they have. The obtained results are commented below.

Almost 90% of professionals thought patients would benefit from this device, mainly because it would reduce anxiety and uncertainty, as doctors would be able to decide and recommend with more confidence the follow up procedure. Additionally, having initial diagnostic results could also avoid consequent visits for result discussion in some cases.

Similarly, 86,4% consider that this device would also help the health care professionals. This device would give them more autonomy and less dependence on the pathologists, allowing faster decision making and a better management of resources. They would be able to prioritize visits with patients with more complex and advances lesions, and minimize visits with those who are in a healthy condition. Furthermore, the proposed system would avoid personnel training and provide standardized and reliable results without having bias or human error. All this would lead to more efficient treatments and lower healthcare burden.

	PATIENT BENEFIT	MEDICAL BENEFIT
Yes	88,6%	86,4%
No	11,4%	13,6%

Table 8. Results showing the proportion of health-care professionals that think this potential device could benefit the patient and the healthcare professionals.

Furthermore, they were also asked in which lesions could the polyp be avoided sending to the anatomical pathology unit (Table 9). Around 75% believe that hyperplastic polyps would not need to be further sent for a pathological analysis, and 38,6% think that LGD adenomas would not need to be sent either.

However, all of the endoscopists surveyed answered that HGD adenomas and adenocarcinomas would have to be sent to the anatomical pathology unit.

HP	A-LGD	A-HGD	ADK
75%	38,6%	0%	0%

Table 9. Results showing the proportion of health-care professionals that think hyperplastic (HP), LGD adenomas (A-LGD), HGD adenomas (A-HGD) and adenocarcinomas (ADK) could be avoided sending to the anatomical pathology unit.

When asked if they considered this could be introduced in the current clinical practice, almost 90% marked yes as an answer, as it would provide more information in a quick manner, and thus aid in the interpretation of lesions. However, it should have a good specificity and sensitivity to give reliable results.

If this technology was tuned and reliable results were obtained, many endoscopists have proposed an in vivo application, without having to resect the polyp.

3.4. Experimental Validation Design

After performing a thorough analysis of the potential of this device and the great interest it may have in the day to day clinical practice, it is time to design a proof of concept experiment to validate the proposed method.

3.4.1. Proof of Concept

The design of the dielectric property measurement device is out of the scope of this work. However, a first approach to the achievable outcomes can be estimated using the dielectric property data already obtained with a commercial open ended coaxial probe in [15].

3.4.1.1. External factors

There are many external factors that could influence the measurements of this method, such as temperature, tissue dehydration or how the polyp was resected. The effect of size, injected solutions during polypectomies, resection method, polyp shape, tissue type, temperature and tissue dehydration was analyzed in [15]. Results can be seen in appendix 4. It was seen that polyps

had to be larger than 10 mm to be properly analyzed, and that the other factors explored did not significantly influence the measurements.

3.4.1.2. Initial results

According to the survey results and interviews most professionals would value a clear distinction between non-adenomas (hyperplastic polyps) and adenomas (adenocarcinoma, HGD and LGD adenomas). Additionally, as many professionals also consider that LGD adenomas could also benefit from this technology, a second classification separating benign and LGD adenomas from HGD adenomas and adenocarcinomas will also be carried out.

For this purpose, different thresholds for relative permittivity and conductivity were tried out to detect how well they classified the polyps. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) for each threshold were measured.

$$Sensitivity = \frac{TP}{TP+FN} \qquad Specificity = \frac{TN}{TN+FP}$$

$$PPV = \frac{TP}{TP+FP} \qquad NPV = \frac{TN}{TN+FN}$$

The sensitivity indicates the malignant polyps classified as malignant, also known as the true positive (TP) rate. A false negative (FN) is a malignant polyp classified as benign. The specificity represents the true negative (TN) rate, so the benign polyps classified as benign. A false positive (FP) is a healthy polyp classified as malignant. The positive and negative predictive values correspond to the correctly classified malignant and benign [52].

According to the survey, clinicians think that the PPV and the NPV are the most important factors for classifying polyps. When distinguishing between adenomas and non-adenomas (first classification), the relative permittivity threshold that showed best results for both PPV and NPV is 38 at 5GHz, with 92,5% and 74% respectively. Looking at the conductivity values a threshold of 7 gave PPV of 95% and NPV of 100 at all frequencies (8GHz, 7.5GHz and

5GHz). However, the results from this classification are not reliable, as the size of hyperplastic polyps was very limited, resulting in biased results.

When using the second classification separating benign and LGD adenomas from HGD adenomas and adenocarcinomas, the results were similar. The best thresholds for relative permittivity were 36 at 8GHz (PPV = 90%, NPV = 67%), and 38 at 5GHz (PPV = 94,44%, NPV = 61,54%). For conductivity the results were not satisfying, as the best outcome had PPV = 51,52% and NPV = 100% at 7.5GHz with a threshold of 9. This indicates that the conductivity values are not reliable for this classification. Results at 8GHz for the relative permittivity for the second classification are shown in Table 10. The rest of the results are shown in the appendix 5.

	RELATIVE PERMITTIVITY						
Threshold	**35**	**36**	**37**	**38**	**39**	**40**	**41**
Sensitivity	100	100	100	100	100	63,16	60,53
Specificity	3,70	11,11	14,81	37,04	59,26	100	100
PPV	39,53	41,46	42,50	50,00	60,71	100	100
NPV	100	100	100	100	100	30,00	28,57

Table 10. Sensitivity, specificity, PPV and NPV for different thresholds of the relative permittivity value at 8GHz for the second classification.

According to the obtained results, the best frequency to classify polyps would be 5GHz, as it shows high PPV and NPV for both classification systems in both the relative permittivity and the conductivity.

3.4.2. Future Experiment

Although the data from [15] shows that measuring the dielectric properties of polyps can quantitatively determine the grade of dysplasia and thus provide an objective polyp classification method, future experiments with the final device have to be performed. One of the main limitations of the experiment performed is that the probe used could only measure polyps larger than 10mm, consequently limiting the amount of hyperplastic polyps studied. Furthermore,

the three methods to measure dielectric properties explained in 3.2.1 should be tested to choose the most appropriate one.

To obtain statistically significant results we have to determine the sample size (N) such that it will give a certain degree of confidence. This is done with a power analysis study using G*power software (http://www.gpower.hhu.de/) [53], in which the effect size, the significance level and the power of the data are introduce [54]. The effect size is determined based on the variance of the data. In our scenario we would be considering 2 factors: frequency and tissue type. A non-paired ANOVA F-test was carried out (Appendix 6) on the data of 5 GHz. As a result, we found that at total of N=70 with homogeneous group sizes should be the sample size of the validating experiment.

4. FROM THE IDEA TO THE MARKET

The ultimate goal of any medical device is to reach the market to be used in the day to day clinical practice. In this section an overview of the roadmap to launch the medical device under study will be discussed.

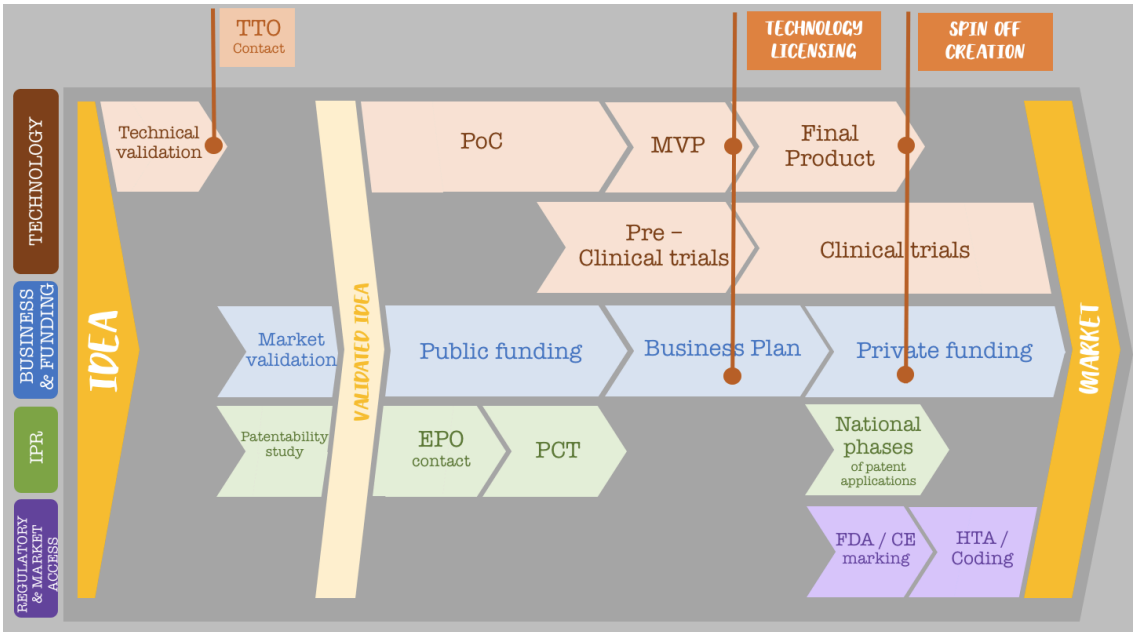

Figure 4: Roadmap to launch a device into the market. PoC: Proof of Concept. MVP: Minimum Viable Product. EPO: European Patent Office. PCT: Patent Cooperation Treaty). FDA: Food and Drug Administration. HTA: Health Technology Assessment. IPR: Intellectual Property Regulatory. [55]

In Figure 4 a general scheme is represented with the main activities that have to be carried out, organized by 4 main areas: technology, business & funding, patentability and regulatory & market access.

4.1. Technology

The first step is to perform a technical validation, in which the idea is validated with experts in the field, the technological specifications are defined, and the state of the art is researched. All these activities have been performed in this thesis, along with an evaluation of an initial pre-proof of concept experiment to demonstrate that microwave diagnostic can be a potential tool to classify colon polyps.

The next step to follow would be to design and implement an initial proof of concept (PoC) based on the selected method to measure the dielectric properties of polyps. In this process several prototypes will be constructed to gradually improve the previous one, so that at the end the final prototype would be obtained. From this point, the regulatory requirements for medical devices, which largely depend on the risk for the patient, define the scope of the further steps that are the clinical validation and industrialization.

With the final prototype the clinical trials can be started, which in this case aim to prove the efficacy of the device. Note that no preclinical trials are required, as the device acts on ex-vivo tissue without involving the patient. An initial experimental design has been conducted in this thesis

4.2. Business & Funding

Market validation consists on identifying the target market and exploring the way to enter it by exposing the benefits microwaves have over its competitors. Several interviews and surveys were carried out to do an initial primary research process. This allowed me to qualitatively understand the target market and thus be able to build a more focused product. We have seen that there is a clear distinction between the west and the east. The western countries (Europe) may be more interested on investing in our product, as there is an existing need to reduce the workload of the pathology department. Additionally, a secondary

research process to numerically identify the size and statistics of the market provided quantitative justification. The market size is quite large, as CRC has high incidence and the screening process implemented aims to increase the colonoscopies performed significantly in the next years. It is estimated that the global Colonoscopic market will increase with a Compound Annual Growth Rate of 5,25% over the period 2018 to 2024 [56].

Looking at the competitors, a position gap was built to find the market gap to position our device in relation to the technologies that are in market and emerging (Figure 5). On the right are leading companies that sell pathological equipment and on the left leading companies that sell colonoscopes.

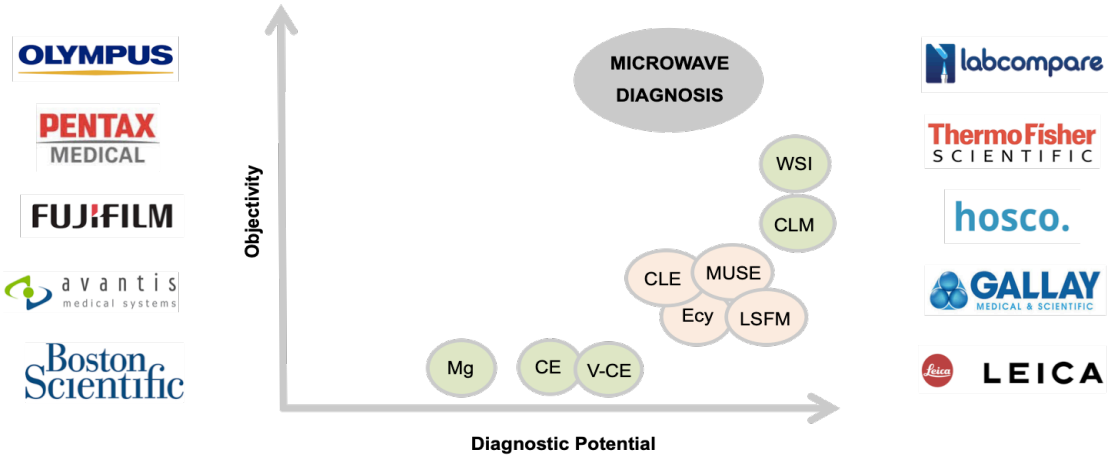

Figure 5. Positioning gap showing the main technological competitors of the device. CLM: Conventional Light Microscopy; V-CE: Virtual Chromoendoscopy; CE: Chromoendoscopy; WSI: Whole Slide Imaging; Mg: Manification; Ecy: Endocytoscopy; CLE: Confocal Laser Endomicroscopy. In green are the commercialized technologies, and in orange the emerging techniques.

To advance with the PoC we require public funding that can be obtained by applying to grants granted by public or private entities, such as AGUR, laCaixa or European Commission. During this process, the Business plan has to start being developed, which describes the business opportunity and how to implement it. It is a useful communication document that organizes the steps to follow and serves to convince possible investors and obtain blended loans (ENISA). These investors are usually private and cover the final steps of the project. They can be business angels, Venture Capital or crowd funding.

4.3. IPR

It is important to do an initial patentability validation to explore the legal intellectual protection mark. Patents are "*exclusive right granted for an invention, which is a product or a process that provides, in general, a new way of doing something, or offers a new technical solution to a problem*" [57]. There are three requirements an invention has to fulfill to be patentable: novelty, involve an inventive step and are susceptible of industrial application. This means that the product cannot be anticipated based on previous work, that it is sufficiently inventive, and that it provides a benefit. If possible, it is recommendable to patent products, as it gives protection of the technology and limits options for competitors. To assess whether microwave polyp diagnosis is patentable or not a patentability search was initiated (Appendix 7). Three relevant patents in the field have been found related to microwaves and dielectric properties. However, a further detailed patentability assessment and a written report on patentability should be carried out to explore what aspect of our device could be patented. This can be done with help of experts. Once it seems clear that the device can be somehow patentable, the patent should be filed to the National or European Patent Office (EPO), in order to obtain the protection, the countries of the Munich Convention and set the priority date. In case of wanting to patent the product internationally the Patent Cooperation Treaty (PCT) should be contacted.

4.4. Regulatory

One of the last steps to launch the device into the market is passing all the regulatory processes necessary to obtain the marketing authorization. Medical devices are regulated by national competent authorities such as the European Medicines Agency (EMA) in the European Union or the Food and Drug Administration (FDA) in United States. Since finding the regulatory information that applies to a specific medical device is cumbersome, generally manufacturers end up referring to consultant companies. These provide guidance and help in the regulatory process.

After an initial study based on the available data, this device shall fall under Directive 98/79/EC concerning in vitro diagnostic medical devices (IVD) [58]. IVDs are classified into 4 groups based on the level of risk associated with their use. More specifically, the device seems to fall into List B of Annex II [59] and thus may require the participation of a Notified Body in all aspects of the conformity assessment procedure.

5. DISCUSSION

It seems clear that nowadays there is an existing clinical need to reduce the workload of the pathological departments at hospitals. All sections send biopsies and tissue samples to be analyzed and obtain a histopathological diagnosis of the lesion, and they all want results as fast as possible. Consequently, pathological laboratories are saturated and overloaded with tissue samples. This could be reflected in the fact that only 4 pathologists answered the survey, which was sent to approximately 100 pathologists. One of the departments that sends most samples for pathological evaluation is the endoscopy unit. It seems of crucial importance to find a way to reduce the amount of samples this unit sends for histological analysis.

Currently there are classification systems that intend to provide an initial diagnosis of the polyps based on the characteristics found during the colonoscopy. However, these systems are based on the subjective criteria of the colonoscopist and do not have diagnostic relevance in western countries. The pathological analysis is considered the gold standard for polyp classification and cancer staging. However, it is a time and labor intensive process that also has flaws. The final diagnosis is also based on the personal experience of the pathologists, leading to interobserver variability. Additionally, it is based on analyzing a 2D image of a 3D volume, which is not representative of the whole lesion. Due to the problems and limitations of the current clinical practice, a technology that provides a quantitative diagnosis of the tissue characteristics is very attractive for pathologists and endoscopists. They think that it would pose a benefit to patients and health care professionals. Results

would be obtained real time, leading to faster treatment planning and decision-making. Additionally, follow-up visits could be avoided and fewer polyps would be sent to pathology, thus reducing workload of both pathologists and endoscopists. All this in turn would lead to significant savings to the healthcare system and help contain escalating costs.

A method to rapidly diagnose polyps ex vivo is being studied at UPF in collaboration with UPC and Hospital Clinic. This technique is based on measuring the dielectric properties of polyps with microwaves. Initial results show that this technique has the potential to distinguish adenomas from non-adenomas, and hyperplastic and LGD adenomas from HGD adenomas and adenocarcinomas, which are the two classifications clinicians would be interested in depending on the accuracy the final device. The least cancerogenous lesions could be rapidly diagnosed in site without the need of a further histological analysis. Since the great majority of polyps resected during screening colonoscopies are benign, the amount of polyps sent to pathological anatomy would be reduced significantly. Moreover, the polypectomy itself would already be considered as the treatment, so no future treatment would be required. Here again we see the importance of this proposed device in reducing the workload of healthcare professionals.

The relevance and potential of microwaves to detect the dielectric properties of polyps is clear. However, future experiments have to be carried out with the appropriate sample size to further validate the potential of this technology, and determine the best thresholds to classify polyps accordingly. The most important statistical parameter to take into account to establish the thresholds are the PPV and the NPV. The values for these parameters have to be high so that clinicians rely any medical decision on the results obtained.

In this work, a technical validation of the technology has been carried out in depth (with the measurements available), and an initial exploration of the current market has been done. What follows now are additional experiments to further validate the technology, clearly define the target market, search for public funding, and perform a thorough patentability analysis. All steps have to be carefully planned and carried out to be able to launch the device into the

market, as many factors have to be taken into account. It is a complicated and long process, but the benefits this carries can improve significantly the current clinical practice.

A main limitation of this work comes from the survey answers. Very few answers were recorded from health care professionals, especially from pathologists. As a consequence, we cannot be certain that the results obtained are representative of the opinion of the endoscopist's and pathologist's community, which can make the results inconclusive. However, the survey was sent to a limited number of professionals, as the technology is still in an early stage of development, and some degree of secrecy wanted to be preserved.

Furthermore, the statistical analysis to assess the classification potential of the device was based on a previous experiment that had several limitations. Polyps analyzed had to be larger than 10mm, the measurement method used may not be the method of the final device, and the sample number was not homogeneous for all groups. It served to detect whether microwaves were able to distinguish between tissue types, but further experiments have to be carried out to fully validate the technology.

6. CONCLUSION

All in all, the proposed device poses an interesting and novel solution to an existing problem. Pathologists, endoscopists and patients would benefit from this device, as well as the hospital budget. An initial technical validation has been carried out to appreciate the huge potential of this technology. However, future experiments have to be completed to fulfill specific accuracy requisites for clinicians to rely clinical medical decisions on this diagnosis. Future steps also include a thorough study of the market and the search for public funding to carry on with the proof of concept.

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8. APPENDICES

8.1. APPENDIX 1: Surveys

A survey was sent to pathologists and endoscopists to evaluate their current clinical practice and ask their opinion on the proposed solution. A total of 40 pathologists and 4 endoscopists answered the survey. Note that there are significantly less pathologists that answered, so the results are not at all representative of the pathological community

The survey was sent in Spanish to facilitate the response of local professionals, and thus obtain more answers. The first questions (1-13) are addressed to endoscopists to learn about their current practice with colonoscopies. Following are questions addressed to pathologists (14-30). Finally there are questions sent to both endoscopists and pathologists asking about the proposed device.

Significant results have been introduced throughout the thesis.

Encuesta sobre un dispositivo diagnóstico de clasificación ex vivo de pólipos del colon

SIMBIOsys y Physense, UPF

Soy una estudiante de ingeniería biomédica en la UPF, y para mi trabajo de fin de grado estoy explorando la viabilidad y el potencial de un dispositivo aún en fases de desarrollo en el campo de la colonoscopia. Se trata de un dispositivo capaz de analizar automáticamente pólipos de colon una vez extraídos y determinar su grado de displasia de manera rápida, no destructiva y sin requerir preparación. Los resultados se obtendrían entre 2-5 minutos en la misma sala que se realiza la colonoscopia. El dispositivo está siendo desarrollado por investigadores de la UPF en colaboración con la UPC y la Unidad de Endoscopia Digestiva del Hospital Clínic de Barcelona. Para validar la solución propuesta se precisa información de la práctica actual de los endoscopistas, así que te agradecería mucho si pudieras rellenar esta encuesta.

Si tiene cualquier duda o comentario puede mandarme un correo aquí: nerea.elosua01@estudiant.upf.edu

Colonoscopia

1. Cuánto tiempo llevas realizando colonoscopias?

☐ <5 años ☐ 5-15 años ☐ >15 años

2. Cuántas colonoscopias realizas a la semana?

☐ <10 ☐ 10-30 ☐ >30

3. De media, cuánto tardas en realizar una colonoscopia?

☐ <20 min ☐ 20-30 min ☐ 30-40 min ☐ >40 min

4.a. A veces los pacientes son recitados para repetir la colonoscopia, podrías estimar cuántas veces ocurre esto?

☐ <5 % ☐ 5-15 % ☐ >15 %

4.b. Cuáles son las razones y con qué incidencia?

	<10%	10-20%	20-30%	>30%
Mala preparación	☐	☐	☐	☐
Polipectomía complicada	☐	☐	☐	☐
Segunda opinión	☐	☐	☐	☐

5. Cuándo usas agua durante una colonoscopia y con qué frecuencia?

	<10%	10-20%	20-30%	>30%
Para limpiar el colon	☐	☐	☐	☐
Polipectomías bajo agua	☐	☐	☐	☐

6. Usas alguna tecnología durante las colonoscopias o algún accesorio para mejorar la calidad de imagen de los pólipos?

☐ Magnificación

☐NBI

☐ Cromoendoscopia

☐Alta resolución

☐Otro: _____

7.a. Siempre extraes los pólipos detectados?

☐ Sí ☐ No

7.b. Si no, en qué casos decides dejar el pólipo?

☐ Hiperplásticos en recto

☐ Polipectomía compleja

☐ Aspecto degenerado

8. Cuántos pólipos extraes de media en cada colonoscopia?

☐ <3 ☐ 3-7 ☐ >7

9. Qué porcentaje de pólipos no pueden ser recuperados después de polipectomías?

☐ <5% ☐ 5-10% ☐ >10%

10. Sigues alguna sistema de clasificación del tejido para hacer un diagnóstico inicial basado en el aspecto visual de la lesión?

☐ Clasificación JGCA (Japanese Gastric Cancer Association)

☐ Clasificación de Paris

☐ Clasificación Kudo

☐ Clasificación Sano

☐ Clasificación NICE

☐ No uso ningún sistema de clasificación endoscópica

☐ Otra: _____

11. Cuánto sueles esperar para obtener resultados de la unidad patológica?

☐ <24h ☐ 1-3 días ☐ 3-7 días ☐ >7 días

12. En tu opinión, es mucho tiempo?

☐ Sí ☐ No

13. Estarías satisfecho si pudieras obtener resultados fiables de la clasificación del pólipo inmediatamente después de su resección?

☐ Sí ☐ No

Patología

14. Cuáles son las razones y con qué incidencia?

	<10%	10-20%	20-30%	>30%
Digestivo	☐	☐	☐	☐
Cáncer de mama	☐	☐	☐	☐
Neumología	☐	☐	☐	☐
Urología	☐	☐	☐	☐

15. Cuánto tiempo llevas en la unidad de anatomía patológica?

☐ <5 años ☐ 5-15 años ☐ >15 años

16. Cuántos pólipos de colon se analizan al día en la unidad?

☐ <25 ☐ 25-50 ☐ >50

17. Siempre analizas los pólipos de la misma manera?

☐ Sí ☐ No

18. A veces, los portaobjetos se preparan de nuevo para un determinado pólipo. Por qué razones se hace esto y con que incidencia?

	<10%	10-30%	>30%
Error en el proceso de preparación	☐	☐	☐
Parte no representativa del pólipo	☐	☐	☐
Repetir análisis con otra tinción	☐	☐	☐

19.a. Con qué frecuencia pides una segunda opinión mientras analizas pólipos?

☐ <10% ☐ 10-30% ☐ >30%

19.b. Cuáles son las razones?

☐ Análisis de un parámetro subjetivo

☐ Opinión de un patólogo experimentado

☐ Presencia de parámetros contradictorios

☐ Otra: _____

20. Qué parámetros analizas en pólipos benignos?

☐ Tamaño

☐ Grado de displasia

☐ Tipo histológico

☐ Invasión de vasos

☐ Margen resecado

☐ Otra: _____

21. Qué tecnologías usas para analizar pólipos?

☐ Microscopio óptico

☐ Microscopio electrónico

☐ Light Sheet Mictorscopy (LSM)

☐ Otra: _____

22. Cuánto se suele tardar en realizar un análisis de pólipo (desde que entra a la unidad hasta que se entrega el resultado)?

☐ <24h ☐ 1-3 días ☐ 3-7 días ☐ >7 días

23. En tu opinión, es mucho tiempo?

☐ Sí ☐ No

24. Está saturado el servicio?

☐ Sí ☐ No

25. Hay demasiadas muestras?

☐ Sí ☐ No

26. Cuál es la parte del proceso que requiere más recursos (tiempo, personal, material...) y sería interesante reducir/evitar?

☐ Recepción de la muestra

☐ Análisis macroscópico

☐ Disección y preparación de portaobjetos con parafina

☐ Tinción

☐ Análisis patológico e interpretación

☐ Envío de resultados

☐ Otra: _____

27.a. Cuántos portaobjetos se preparan normalmente para un pólipo?

☐ 1 ☐ 2-5 ☐ >5

27.b. Crees que son suficientes para caracterizar la lesión? Por qué?

_____

28. Cuánto cuesta el procesado de la muestra hasta obtener una tinción H&E?

☐ <5€ ☐ 5-10€ ☐ 10-15€ ☐ >15€

29. Cuántas personas están involucradas en el análisis de un pólipo?

	1	2	3	4	5
Secretario	☐	☐	☐	☐	☐
Técnico	☐	☐	☐	☐	☐

Patólogo	☐	☐	☐	☐	☐

30. Crees que el análisis histológico actual tiene limitaciones? En caso afirmativo cita las principales.

_____

Dispositivo en desarrollo

31.a. Crees que los pacientes se beneficiarían de este dispositivo?

☐ Sí ☐ No

31.b. Cómo?

_____

32.a. Crees que el personal médico se beneficiarían de este dispositivo?

☐ Sí ☐ No

32.b. Cómo?

_____

33. Cuál crees que es el parámetro de calidad más significativo de diagnóstico diferencial de tejidos?

☐ Sensibilidad

☐ Especificidad

☐ Valor predictivo positivo (VPP)

☐ Valor predictivo negativo (VPN)

34.a. Si el dispositivo pretende sustituir el análisis patológico, que sensibilidad o valor predictivo positivo es necesario en el diagnóstico diferencial de:

	80-90%	90-95%	95-100%
Adenocarcinoma vs adenoma HGD	☐	☐	☐
Adenocarcinoma vs adenoma LGD	☐	☐	☐
Adenocarcinoma vs hiperplástico	☐	☐	☐
Adenocarcinoma vs sano	☐	☐	☐
Adenoma HGD vs adenoma LGD	☐	☐	☐
Adenoma HGD vs hiperplástico	☐	☐	☐
Adenoma HGD vs sano	☐	☐	☐
Adenoma LGD vs hiperplástico	☐	☐	☐
Adenoma LGD vs sano	☐	☐	☐
Hiperplástico vs sano	☐	☐	☐

34.b. Si el dispositivo pretende sustituir el análisis patológico, que especificidad o valor predictivo negativo es necesario en el diagnóstico diferencial de:

	80-90%	90-95%	95-100%
Adenocarcinoma vs adenoma HGD	☐	☐	☐
Adenocarcinoma vs adenoma LGD	☐	☐	☐
Adenocarcinoma vs hiperplástico	☐	☐	☐
Adenocarcinoma vs sano	☐	☐	☐
Adenoma HGD vs adenoma LGD	☐	☐	☐
Adenoma HGD vs hiperplástico	☐	☐	☐
Adenoma HGD vs sano	☐	☐	☐
Adenoma LGD vs hiperplástico	☐	☐	☐
Adenoma LGD vs sano	☐	☐	☐
Hiperplástico vs sano	☐	☐	☐

35.a. Si el dispositivo pretende priorizar los tejidos que se envían a anatomía patológica, que sensibilidad o valor predictivo positivo es necesario en el diagnóstico diferencial de:

	80-90%	90-95%	95-100%
Adenocarcinoma vs adenoma HGD	☐	☐	☐
Adenocarcinoma vs adenoma LGD	☐	☐	☐
Adenocarcinoma vs hiperplástico	☐	☐	☐
Adenocarcinoma vs sano	☐	☐	☐
Adenoma HGD vs adenoma LGD	☐	☐	☐
Adenoma HGD vs hiperplástico	☐	☐	☐
Adenoma HGD vs sano	☐	☐	☐
Adenoma LGD vs hiperplástico	☐	☐	☐
Adenoma LGD vs sano	☐	☐	☐
Hiperplástico vs sano	☐	☐	☐

35.b. Si el dispositivo pretende priorizar los tejidos que se envían a anatomía patológica, que especificidad o valor predictivo negativo es necesario en el diagnóstico diferencial de:

	80-90%	90-95%	95-100%
Adenocarcinoma vs adenoma HGD	☐	☐	☐
Adenocarcinoma vs adenoma LGD	☐	☐	☐
Adenocarcinoma vs hiperplásttico	☐	☐	☐
Adenocarcinoma vs sano	☐	☐	☐
Adenoma HGD vs adenoma LGD	☐	☐	☐
Adenoma HGD vs hiperplástico	☐	☐	☐
Adenoma HGD vs sano	☐	☐	☐
Adenoma LGD vs hiperplástico	☐	☐	☐
Adenoma LGD vs sano	☐	☐	☐

Hiperplástico vs sano	☐	☐	☐

36. Para qué lesiones es imprescindible tener información de que la base esté bien resecada?

	Es imprescindible	Con exactitud	Es deseable	No es necesario
Hiperplásticos	☐	☐	☐	☐
Adenoma LGD	☐	☐	☐	☐
Adenoma HGD	☐	☐	☐	☐
Adenocarcinomas	☐	☐	☐	☐

37. Para qué lesiones es imprescindible tener información de invasión de vasos?

	Es imprescindible	Con exactitud	Es deseable	No es necesario
Hiperplásticos	☐	☐	☐	☐
Adenoma LGD	☐	☐	☐	☐
Adenoma HGD	☐	☐	☐	☐
Adenocarcinomas	☐	☐	☐	☐

38. Qué lesiones crees que podrías evitar enviar la muestra a la unidad de anatomía patológica si puedes estimar su histología in-situ?

☐ Hiperplástico

☐ Adenoma LDG

☐ Adenoma HGD

☐ Adenocarcinoma

39.a. Crees que este dispositivo es compatible con la práctica clínica actual?

☐ Sí ☐ No

39.b. Comentario respecto a la pregunta anterior:

_____

40. Qué carencias ves en esta aproximación?

_____

41. Te gustaría que esta tecnología tuviera otras características?

_____

Detalles del dispositivo

42. Sería relevante la portabilidad del dispositivo, y poderlo usar en cualquier sala?

☐ Sí ☐ No

43. Hay algún otro aspecto que pueda ser importante para el desarrollo del dispositivo?

44. Otros comentarios:

8.2. APPENDIX 2: Colonoscopic tissue characterization classifications

8.2.1. Macroscopic appearance classification

The endoscopic appearance of colon lesions gives an initial assessment on the malignancy degree of the lesion, and plays an important role in posterior treatment assessment. According to the macroscopic appearance of the lesion, it can be classified into 6 main types according to the Japanese Gastric Cancer Association (JGCA) classification [16] (Table A1):

TYPE	CHARACTERISTICS
Type 0	Superficial polypoid in which the endoscopic appearance suggests a small cancer or a noninvasive neoplastic lesion. These polypoids can be morphologically classified with the **Paris classification** [16], [60]
Type 1	Polypoid carcinomas, usually attached on a wide base
Type 2	Ulcerated carcinomas with sharply demarcated and raised margins
Type 3	Ulcerated, infiltrating carcinomas without definite limits
Type 4	Nonulcerated, diffusely infiltrating carcinomas
Type 5	Unclassifiable advanced carcinomas

Table A1. JGCA classification of colon lesions according to their macroscopic appearance.

There is a debate between the West and the East regarding the relevance of this classification. Treatment decision in the West is greatly based on size and location of the tumor, along with the histology of the biopsies. On the other hand, eastern endoscopists give a great role to the endoscopic classification to later decide therapeutic procedures [16].

The Vienna classification intends to oversee these different points of view by proposing a histopathological classification of 3 main groups of neoplasia: i) noninvasive with low grade; ii) noninvasive with high grade; iii) cancer with invasion of lamina propria [35].

8.2.2. Morphologic Classification

A correct analysis of the morphology of superficial lesions can aid in predicting the degree of invasiveness in the submucosal tissue. To satisfy this purpose the Paris classification was designed. This classification classifies colon neoplasms into polypoids (0-I) or non-polypoids (0-II, 0-III), which can then be subdivided into flat (0-II) or depressed (0-III). Within the non-polypoid lesions it is important to notice the laterally spreading tumors (LST) [61], as a correct study of these may determine the probability to develop invasive carcinoma (Table A2).

TYPE	CHARACTERISTICS	SUBCLASSIFICATION
LST-G	Less than 7% are invasive carcinomas [62].	Homogeneous (0-IIa)
	Large nodules or with different sizes are associated to invasive carcinomas [5].	Mixed (IIa+Is, Is+IIa, IIa)
LST-NG	Higher risk of being invasive [62].	Elevated (IIa)
	Invasive crypts, large lesion size and depressed areas are associated to invasive carcinomas [5].	Pseudodepressed (IIc+IIa)

Table A2. Lateral Spreading Tumor classification and characteristics. LST-G corresponds to the granulated LST and LST-NG to the non-granulated.

8.2.3. Histologic prediction

The mucosal pattern of the colon is also crucial for a thorough study of the lesion. Some studies have been carried out with the purpose of trying to establish a relation between the endoscopic and histologic examination. As a result, several classifications have been established, which are able to predict in vivo the histology of epithelial lesions (Table A3). Using different technologies of endoscopies we are able to distinguish non-neoplastic lesions from neoplastic lesions, and predict the invasiveness of the neoplasia.

The Kudo classification analyses the pit pattern and classifies them into 7 main groups, but can be simplified into non-neoplastic, noninvasive neoplasia and invasive neoplasia [28]. It is applied when combining chromoendoscopy with magnification. The Sano classification is based on the histopathological characteristics of the capillary pattern (CP), as it was found that microcapillary vessels (MCV) increase their density as the lesion progresses. There are 3

main types and can distinguish between neoplastic and non-neoplastic polyps [29]. This classification works best in magnified NBI colonoscopies. A further classification, and the most common used is the NICE classification. It was developed to standardize the classification using NBI endoscopies. It looks at the color of the polyp, the vascular network and the superficial pattern to predict the histology of the lesion [30].

	RESULTS	TYPES
Kudo	Has shown a sensitivity of 92.3% and a specificity of 99.8% when differentiating neoplastic from non-neoplastic lesions [63].	Non-neoplastic
		Noninvasive neoplasia
		Neoplasia
Sano	Demonstrated 96.4% sensitivity and 92.3% specificity in classifying non-neoplastic and neoplastic lesions with [64].	Type I – Hyperplastic polyp
		Type II – Adenoma
		Type III – Adenocarcinoma
NICE	A meta-analysis showed that this classification has 91% specificity and specificity of 82.6% [65].	Type I – Hyperplastic polyp
		Type II – Adenoma
		Type III – Invasive Cancer

Table A3. Diagnostic accuracy and types of the main classifications of mucosal pattern.

From Table A3 we see that even though there are several classifications, they all intend to distinguish between benign and malignant, and try to determine the invasiveness of the lesion.

8.3. APPENDIX 3: Histopathological Colon Tissue Characterization

In case of surgically resected colon cancer tissues, the pathologic evaluation takes into account more histologic features, some of which have more prognostic significance than others. The most important prognostic factor is the pathologic stage, which indicates the extent of the disease. The best staging system is the TNM system [66]. The T refers to the primary tumor, N to the status of the regional nodes, and M distinguishes whether or not there is metastasis. To pathologically determine the stage, several tissue parts have to be carefully examined. The primary tumor has to be resected to evaluate the highest T category. To evaluate the N category, the nodes have to be removed and examined. It is suggested to examine 12 regional nodes for a more accurate classification. However, this process is problematic, as there is not a standard lymph node examination protocol, leading to variation between pathologists. Furthermore, to evaluate metastasis (M) distant tissues have to be analyzed microscopically [67].

Another pathologic parameter to take into account is the tumor grade. There are many tumor grading systems, causing interobserver variability. However, overall the tumor can be classified between high grade and low grade, and it has been demonstrated that high tumor grade is an indicator of unfavorable prognosis. In general this two-tiered grading system is accepted and advised, as it is simple, identifies tumors with poor prognosis, and reduces disagreement between pathologists [40].

Other histological features are not taken into account in the TNM staging, and may play an important role in the prognosis of the patient, but their association with patient outcome is not clear yet. Vessel invasion has been proven to be an independent adverse effect in 10 studies [6], although there are no standard guidelines for pathologic evaluation, and it is often underreported [41]. Resection margins may be useful to predict local recurrence, as it is considered high when the tumor is within 1mm of the margin [42]. Peritoneal involvement is also an adverse prognostic factor, but there is not a universally accepted definition, making the diagnosis difficult and subjective [41]. The presence of

tumor deposits, which are focal aggregates of adenocarcinoma that are discontinuous with the primary tumor, is associated with a poorer prognosis and a decreased survival rate. However, there are controversies with their classification, posing a further prognostic difficulty.

Contrarily, some studies suggest that the presence of a host lymphoid response is a favorable prognostic factor, as it indicates there is an immunological response, although there are some concerns regarding its significance [6]. There are also other pathologic parameters that have been found to not have prognostic significance. Tumor size was found to have no prognostic significance in 8 different studies [6]. Similarly, there is a controversial association between the tumor configuration and the prognosis. Moreover, the histologic type has not been proven to be significant either [68].

FEATURE	CHARACTERISTICS
Pathologic stage	Follows the TNM classification
Tumor grade	Classifies tumors into high or low grade
Vessel involvement	Has an adverse effect No standard pathological guidelines
Resection margins	Can predict local recurrence
Peritoneal involvement	Has an adverse effect The diagnosis is subjective
Tumor deposits	Indicator of poor prognosis Unclear classification system
Host lymphoid response	Favorable prognostic factor

Table A4. Summary of the main histologic features looked at in the pathological evaluation of a surgically resected colon sample.

All in all, staging the tumor using the TNM system is regarded as the gold standard for tumor classification, and it is the best method to estimate postoperative outcomes. However, it is time and labor consuming, as many samples have to be examined for accurate results. Moreover, there may be variability between clinicians due to a lack of standardized evaluating system when analyzing several pathologic features, leading to poor reproducibility [6], [41]. The inefficiency of this process has been demonstrated, and it seems of

great need to improve this process, and reducing the workload may seem an appropriate solution.

8.4. APPENDIX 4. ANOVA analysis of relevant factors

Several external factors that may influence the measurements were analyzed to detect if they significantly influenced the measurements or not. In [15] a one-way nested analysis of variance (ANOVA) F-test was carried out to study the effects that sample size, injected solutions, resection method and polyp shape had on the measurements (Table A4). This study was performed using the relative permittivity and conductivity measurements of the categorized LGD adenomas at 5GHz, because it was the group with most samples.

FACTOR	EFFECT
Size	Samples must be larger than 10 mm to cover completely the tip of the probe.
Injected solutions	The solutions used during polypectomies to facilitate the resection (indigo carmine dye, succinylated gelatin, adrenaline) did not result in a statistically significant effect.
Resection method	Resecting the polyp with cautery or cold snare did not present a statistically significant effect if the measurements are performed as far away from cauterization zone as possible (P= 0.8267 and P = 0.2587).
Polyp shape	Does not produce statistically significant differences (P= 0.582).
Tissue type	There were statistically significant differences between tissue types: healthy, hyperplastic, adenoma with LGD, adenoma with HGD, adenocarcinoma (P= 0,017 and P= 0,05) and non-statistical differences within groups.
Temperature	The temperature of the polyps should be controlled during measurements to avoid it influencing the result. For this aim the room temperature was regulated.
Tissue dehydration	By minimizing the time between resection and measurement we can minimize the effect of dehydration. The measurements were taken between 2 minutes and 1 hour, which is less than the recommended time agreed by the most recent papers [69].

Table A5. Factors that can influence the complex permittivity measurements and the effect they may have.

8.5. APPENDIX 5. Classification performance results

8.5.1. Adenoma vs non-adenoma classification

8.5.1.2. 5 GHz

	RELATIVE PERMITTIVITY					
Threshold	**35**	**36**	**37**	**38**	**39**	**40**
Sensitivity	97,37	97,37	97,37	97,37	89,47	89,47
Specificity	0	33,33	33,33	50,00	66,67	66,67
PPV	86,05	90,24	90,24	92,50	94,44	94,44
NPV	0	66,67	66,67	75,00	50,00	50,00

Table A6. Sensitivity, specificity, PPV and NPV for different thresholds of the relative permittivity value at 5GHz classifying benign and malignant polyps.

	CONDUCTIVITY			
Threshold	**5**	**6**	**7**	**8**
Sensitivity	100	100	100	86,84
Specificity	50,00	66,67	66,67	66,67
PPV	92,68	95,00	95,00	94,29
NPV	100	100	100	44,44

Table A7. Sensitivity, specificity, PPV and NPV for different thresholds of the conductivity value at 5GHz classifying benign and malignant polyps.

8.5.1.2. 7.5 GHz

	RELATIVE PERMITTIVITY						
Threshold	**35**	**36**	**37**	**38**	**39**	**40**	**41**
Sensitivity	100	97,37	94,74	84,21	78,95	71,05	57,89
Specificity	0	0	16,67	33,33	66,67	100	100
PPV	86,36	86,05	87,80	88,89	93,75	100	100
NPV	0	0	33,33	25,00	33,33	35,29	27,27

Table A8. Sensitivity, specificity, PPV and NPV for different thresholds of the relative permittivity value at 7.5GHz classifying benign and malignant polyps.

	CONDUCTIVITY				
Threshold	**6**	**7**	**8**	**9**	**10**
Sensitivity	100	100	89,47	81,58	71,05
Specificity	66,67	66,67	66,67	66,67	66,67
PPV	95,00	95,00	94,44	93,94	93,10
NPV	100	100	50,00	36,36	26,67

Table A9. Sensitivity, specificity, PPV and NPV for different thresholds of the conductivity value at 7.5GHz classifying benign and malignant polyps.

8.5.1.2. 8 GHz

RELATIVE PERMITTIVITY							
Threshold	**35**	**36**	**37**	**38**	**39**	**40**	**41**
Sensitivity	97,37	97,37	94,74	78,95	71,05	63,16	60,53
Specificity	0	33,33	33,33	33,33	83,33	100	100
PPV	86,05	90,24	90,00	88,24	96,43	100	100
NPV	0	66,67	50,00	20,00	31,25	30,00	28,57

Table A10. Sensitivity, specificity, PPV and NPV for different thresholds of the relative permittivity value at 8GHz classifying benign and malignant polyps.

CONDUCTIVITY					
Threshold	**6**	**7**	**8**	**9**	**10**
Sensitivity	100	100	100	84,21	73,68
Specificity	66,67	66,67	66,67	66,67	66,67
PPV	95,00	95,00	95,00	94,12	93,33
NPV	100	100	100	40,00	28,57

Table A11. Sensitivity, specificity, PPV and NPV for different thresholds of the conductivity value at 8GHz classifying benign and malignant polyps.

8.5.2. Hyperplastic and adenomas LGD vs HGD adenoma and adenocarcinomas classification

8.5.2.2. 5 GHz

	RELATIVE PERMITTIVITY					
Threshold	**39**	**40**	**41**	**42**	**43**	**44**
Sensitivity	100,00	100,00	94,12	94,12	88,24	52,94
Specificity	29,63	29,63	29,63	62,96	66,67	81,48
PPV	47,22	47,22	45,71	61,54	62,50	64,29
NPV	100,00	100,00	88,89	94,44	90,00	73,33

Table A12. Sensitivity, specificity, PPV and NPV for different thresholds of the relative permittivity value at 5GHz classifying hyperplastic and LGD adenomas vs HGD adenomas and adenocarcinomas

	CONDUCTIVITY			
Threshold	**7**	**8**	**9**	**10**
Sensitivity	100	94,12	82,35	82,35
Specificity	14,81	29,63	40,74	51,85
PPV	42,50	45,71	46,67	51,85
NPV	100	88,89	78,57	82,35

Table A13. Sensitivity, specificity, PPV and NPV for different thresholds of the conductivity value at 5GHz classifying hyperplastic and LGD adenomas vs HGD adenomas and adenocarcinomas

8.5.2.2. 7.5 GHz

RELATIVE PERMITTIVITY							
Threshold	**36**	**37**	**38**	**39**	**40**	**41**	**42**
Sensitivity	100	100	100	100	94,12	82,35	76,47
Specificity	3,70	11,11	29,63	44,44	59,26	70,37	74,07
PPV	39,53	41,46	47,22	53,13	59,26	63,64	65,00
NPV	100	100	100	100	94,12	86,36	83,33

Table A14. Sensitivity, specificity, PPV and NPV for different thresholds of the relative permittivity value at 7.5GHz classifying hyperplastic and LGD adenomas vs HGD adenomas and adenocarcinomas

CONDUCTIVITY				
Threshold	**7**	**8**	**9**	**10**
Sensitivity	100	100	100	88,24
Specificity	14,81	29,63	40,74	48,15
PPV	42,50	47,22	51,52	51,72
NPV	100	100	100	86,67

Table A15. Sensitivity, specificity, PPV and NPV for different thresholds of the conductivity value at 7.5GHz classifying hyperplastic and LGD adenomas vs HGD adenomas and adenocarcinomas

8.5.2.2. 8 GHz

RELATIVE PERMITTIVITY							
Threshold	**35**	**36**	**37**	**38**	**39**	**40**	**41**
Sensitivity	100	100	100	100	100	63,16	60,53
Specificity	3,70	11,11	14,81	37,04	59,26	100	100
PPV	39,53	41,46	42,50	50,00	60,71	100	100
NPV	100	100	100	100	100	30,00	28,57

Table A16. Sensitivity, specificity, PPV and NPV for different thresholds of the relative permittivity value at 8GHz classifying hyperplastic and LGD adenomas vs HGD adenomas and adenocarcinomas

CONDUCTIVITY					
Threshold	**6**	**7**	**8**	**9**	**10**
Sensitivity	100	100	100	100	75,00
Specificity	14,81	14,81	14,81	37,04	44,44
PPV	42,50	42,50	20,69	26,09	28,57
NPV	100	100	100	100	85,71

Table A17. Sensitivity, specificity, PPV and NPV for different thresholds of the conductivity value at 8GHz classifying hyperplastic and LGD adenomas vs HGD adenomas and adenocarcinomas

8.6. APPENDIX 6. Power Analysis

An F test is carried out to determine if the means between two groups are statistically significant or not. In figure A1 the settings of the G*power test can be seen.

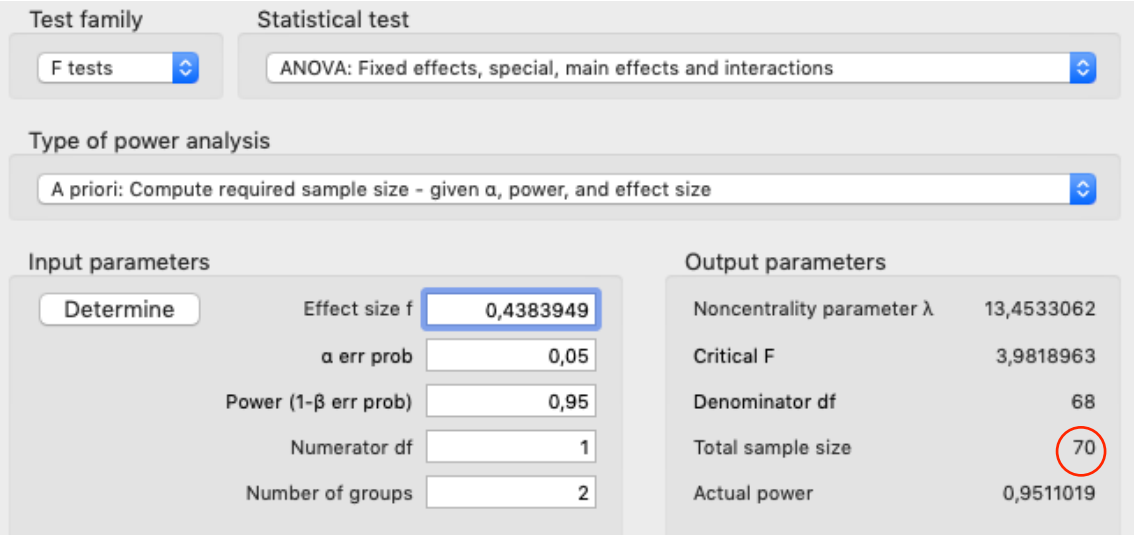

Figure A1. Power analysis specifications set into G*power.

PARAMETER	DEFINITION
Effect size	Is determined based on the variance of the data.
Alpha	This value is related to the confidence level. The lower it is the higher the confidence level.
Power	Is related with the error probability. It allows us to minimize false negatives.
Degrees of freedom	Levels - 1. In our case we have 2 levels.
Groups	Classification groups, in our case benign or malignant.

Table A18. Input parameters of the G*power test

8.7. APPENDIX 7. Patentability analysis

TITLE	INVENTOR	KEY CLAIMS	SEARCH TERMS	PATENT NUMBER	PUB DATE	FILE DATE
Metodo y sistema para examinar tejido segun las propiedades dielectricas del mismo	Dan Hashimshony	Sistema para examinar tejido con el fin de diferenciar el tejido examinado de otro tejido según las propiedades dieléctricas del tejido	Tissue characterization, Deielectric Properties	ES2302865T3	2008-08-01	2002-12-31
Microwave biopsy probe	Wendell Anderson	It enables analysis of lesions that is less invasive than excision biopsy techniques and provides analytical results of the biopsy	Microwave Biopsy	US2006007977 4A1	2006-04-13	2004-10-08
Electrical methods for detection and characterization of abnormal tissue and cells	Moshe Sarfaty Amir Lev	An apparatus for diagnosing a biological samples	Microwave Biopsy, Tissue characterization	US2010010604 7A1	2010-04-29	2009-12-30

Table A19. Relevant patents published