Spectroscopy microsystem for the detection of early cancer

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Abstract— **The research team is focused on the development of a chip-sized spectroscopy microsystem for the detection of gastrointestinal (GI) early cancerous lesions. The paper will briefly cover the outstanding properties of diffuse reflectance and fluorescence spectroscopy detection and the concept of co-integration of highly selective thin-films optical interference filters on silicon photodiodes, in order to meet the growing clinical demands for highly sensitive and reliable tools for the detection of early cancer in the GI tract. It will be also described some of the developments on-going by the research team.**

Index Terms— Gastrointestinal early cancer, Diffuse reflectance and fluorescence, spectroscopy microsystem.

I. CONTEXT

Gastrointestinal (GI) cancers are among the fifth most common cancers worldwide, representing a significant burden on society with a major impact in economy and quality of life [1-2]. Epithelial cancers are usually preceded by pre-cancerous or dysplastic changes that affect both the epithelium and connective tissue. An early identification of cancer, preferably before macroscopically visible changes occur, is a major goal in medicine since the chances of an effective treatment considerably increase when the disease is detected at the dysplastic stage, improving the survival rate [3-5]. However, early cancerous lesions are difficult to detect using standard screening methods, i.e, early stage lesions are not readily identifiable by visual inspection during routine endoscopy. For this reason, physicians often take a large number of unnecessary biopsies to increase the chances of detecting these invisible lesions, which results in sampling errors, high costs associated with the procedure, and physical and psychological discomforts for the patient [4-7]. Therefore, more sensitive endoscopic screening methods and tools, which enable differentiation between premalignant and normal tissue, are of scientific as well as of clinical interest.

Spectroscopy techniques have the potential to overcome current screening methods limitations and considerably improve the ability to detect early stage lesions, reducing costs and morbidity to the management of cancer. These techniques are based on the relative differences in the way light interacts with normal and abnormal tissues, which during disease transformation acquire altered optical properties. Specifically, diffuse reflectance and fluorescence spectroscopy have the potential to provide microscopic morphological and biochemical information of normal and diseased tissue in real-time, which can be used to establish a diagnosis.

Several groups have applied diffuse reflectance and fluorescence spectroscopy for an accurate detection of GI early cancerous lesions (i.e. dysplasia) [3-5, 8]. In these studies, spectroscopy is performed using invasive and uncomfortable endoscopes connected to costly, sophisticated, and bulky illumination equipment, i.e.: high efficiency detectors, as charge coupled device cameras - CCDs; light sources, as xenon arc lamps or UV lasers; spectrograph; optical fibers for light delivery and collection. Overall, those set-ups are not portable and compact, making it cumbersome in the clinical environment.

Therefore, a chip-sized device that includes spectroscopy techniques (diffuse reflectance and fluorescence) without the need for expensive light sources, optical fibers, and spectrograph or CCDs, while keeping the same throughput as conventional instruments, will be developed within this project. Due to the on-chip integrated optics, the device will feature more reliable results once the losses associated with the coupling of the different device parts, required in a macro-scale (fiber-sensor-electronics-fiber), are significantly reduced. It could be integrated in endoscopes making it more portable and adaptable to the clinical setting. It is important to note that its small size will enable future integration within an ingestible pill for GI diagnosis.

II. GOALS

From the previous context description it has become clear that the key to successful treatment of GI cancer is the ability to detect the cancer at its earliest stages using spectroscopy techniques, preferably in a chip-sized spectroscopy platform. Therefore, the principal objective of this research project is the development of a chip-sized spectroscopy microsystem for the detection of morphological and biochemical tissue changes that intent to overcome the above drawbacks. The device will combine the outstanding properties of diffuse reflectance and fluorescence spectroscopy detection and the concept of co-integration of highly selective thin-films optical interference filters on silicon photodiodes, in order to meet the growing clinical demands for highly sensitive and reliable tools for the detection of early stage cancer in the GI tract. Miniaturized ultraviolet and white LEDs will be incorporated on-chip, featuring illumination sources for fluorescence and diffuse reflectance measurements, respectively (see Figure 1). The

major technological innovation of the project is the take-up of microdevices on-chip integration combined with spectroscopy techniques, all in a single system. Such a small-scale system will be portable and compact, which makes it adaptable to clinical settings. Moreover, optical radiation is non-ionizing and, thus, not harmful for the tissues. For these reasons, if successful, a microdevice with all these integrated functionalities will be a boom in the sector of technologies for early cancer diagnosis.

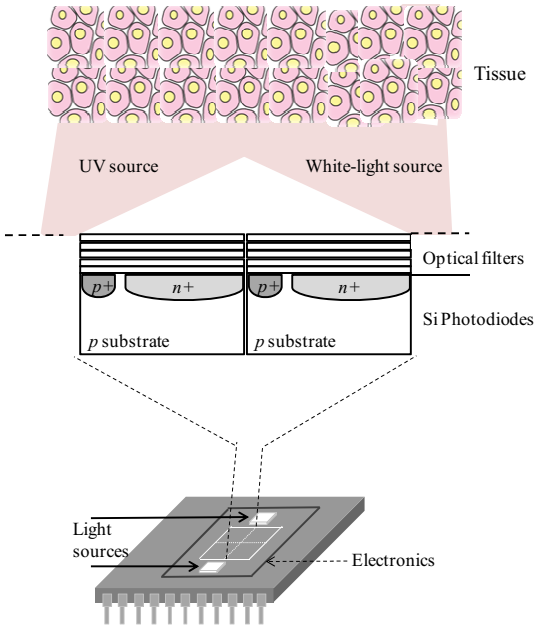

Figure 1. Schematic overview of the spectroscopy microsystem (not scaled).

III. Research Team

The research is being developed mainly in the Algoritmi Centre, where PI belongs and in the Centre of Biological Engineering, both from University of Minho. TUDelft, Holland, is also cooperating in this project. Information about the research group can be found in http://www.dei.uminho.pt/~gminas/AreasInv.htm/ResearchGroup.htm.

IV. Methodology

For the spectroscopy microsystem, two optical techniques are being implemented: diffuse reflectance and fluorescence spectroscopy. In diffuse reflectance, white-light delivered to the tissue is absorbed and multiple scattered and part of it returns, carrying information about tissue scattering and absorption properties. Scattering is originated mainly by collagen fibers in connective tissue. In the visible range, absorption in epithelial tissues is mainly due to hemoglobin, which absorbs light without emitting a fluorescent photon. The major hemoglobin absorption peaks are nearly the 420 nm and 540-580 nm regions. A decrease in scattering, and consequently in the diffuse reflectance signal, is associated with increasing stages of early cancer. An increased hemoglobin concentration, which also translates into a reduced reflectance signal, may be associated with angiogenesis that is proved to be linked with early cancers [3,5,7,9].

GI tissues produce fluorescence when excited by ultraviolet or short-wavelength visible light. The dominant fluorescent tissue layer is the submucosa, where collagen and elastin emit around the 400 nm spectral band. NAD(P)H and collagen were identified as *in vivo* fluorescent biomarkers of precancerous changes in the GI tract. An increase in NAD(P)H is associated to an increase in cellular metabolic activity and proliferation, both of which occur with the progression of cancer. A decrease in collagen can be an indicator of loss of structural integrity, and is translated by a low fluorescence intensity. Thus, in general, fluorescence intensity of GI cancerous tissue is lower compared to healthy tissue [3-5,7,9-12]. This concept, together with diffuse reflectance, can be used to distinguish the presence and absence of malignancy.

Using these techniques, a quantitative analysis can also be performed, with the huge benefit of having values to objectively characterize tissues. Diffuse-reflectance spectra can be analyzed to obtain tissue information, such as hemoglobin total concentration. Fluorescence spectra can be analyzed to extract the relative contributions of tissue fluorophores, such as NAD(P)H and collagen. These parameters can then be analyzed using statistical methods to develop quantitative diagnostic algorithms [3-5,7].

Before the full integration of those techniques in a chip-sized device, measurements on normal and on several malignant stages of GI tissues must be performed in order to identify and validate the most important wavelengths for diagnosis. This knowledge is crucial to design the optical filters for the selection of specific wavelength bands, and to project photodiodes with increased quantum efficiency in particular spectral ranges. In a first approach, it is used a bench-top spectrophotometric setup for *ex-vivo* tissue assessment that is calibrated using tissue "phantoms". After achieving those spectral bands, thin-films optical filters should be designed, in order to replace the spectrograph function in conventional systems, and post-processed on top of the photodiodes for signal detection. CMOS microelectronics circuits for the photodiodes signals readout and data acquisition, and commercial available miniaturized ultraviolet and white LEDs for tissues illumination, should also be integrated in a further stage.

The filtering system is based on Fabry-Perot thin-film optical resonators [13]. These filters will be deposited by IBD (Ion Beam Deposition) on top of photodiodes. The integration of optical filters, photodiodes and other functions on a single-chip requires the system to fit in a microelectronics process, preferably CMOS. However, for wavelengths below 480 nm, standard CMOS photodiodes have low quantum efficiency. Therefore, photodiodes with improved quantum efficiency at low visible wavelengths will be designed in a non-standard, but compatible, CMOS process. For the remaining visible spectral bands a standard CMOS process will be used [14-15].

V. WORK DEVELOPED

The most important wavelengths for diagnosis of early stage cancer are in the 350-750 nm region of the electromagnetic spectrum [3-5,7]. As an initial step, for the development and for testing the feasibility of using the optical filters for the selection of narrow spectral bands, three spectral bands were chosen centered at 540, 560 and 580 nm. It is important to note that the filters centered at 540 and 580 nm will be able to select spectral bands that correspond to two of the tissue hemoglobin absorption peaks. The thin-film optical filters consist of two flat parallel mirrors separated by a specific distance, with a resonance cavity in the middle, and are designed to yield a narrow pass-band around those specific central wavelengths. Their structure is a multilayer composed by 11 layers (see Figure 2). The mirrors are dielectric mirrors composed of TiO_2 and SiO_2 thin-films. These materials offer good optical performance characteristics with low absorption losses.

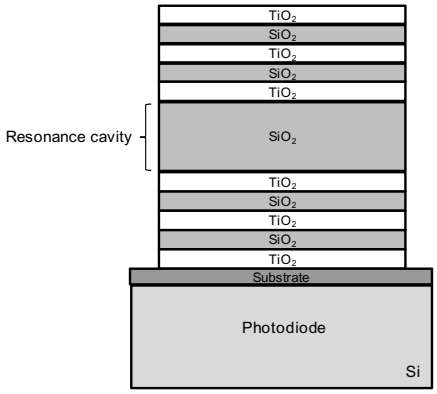

Figure 2. Structure of the Fabry-Perot optical filters.

Analytical calculations were performed to determine the thicknesses of the filters' layers for the selection of the different spectral bands. Those three optical filters were designed with TiO_2 and SiO_2 thicknesses of 52 and 95 nm, respectively. The resonance cavity thicknesses were 192 nm for the 540 nm filter, 206 nm for the 560 nm filter, and 227 nm for the 580 nm filter.

Thin-film optics software TFCalc™ 3.5 was used for the structural optimization of the optical filters. Simulation results, presented in Figure 3, show that each filter is sensitive to its specific spectral band, as desired, with FWHM (Full Width at Half Maximum) less than 15 nm, and with a ratio of maximum transmittance to background noise greater than 90/15. The FWHM translates the quality of the filter in terms of its ability to select a narrow band of the spectrum, and therefore, should be as low as possible. On the other hand, the maximum transmittance peak should be high, with at least twice the intensity of any background noise that might appear in the considered spectral range. Work is on-going to design more optical filters for the selection of narrow spectral bands in the 350-750 nm spectral range.

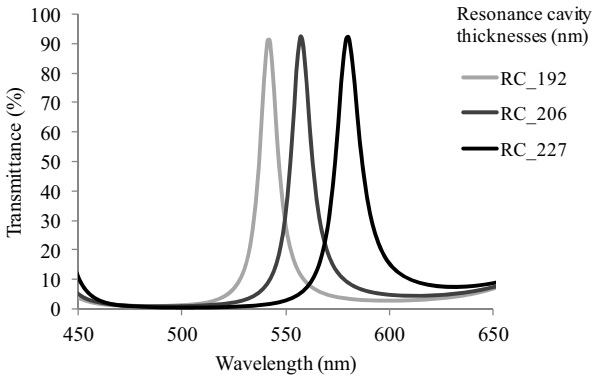

Figure 3. Simulated spectral transmittances of the optical filters.

VI. RESULTS AND DISCUSSION

After the simulations, and given the obtained satisfactory results, the three optical filters were deposited, by IBD, on glass substrates. The filters' transmittance performance was then evaluated (Figure 4).

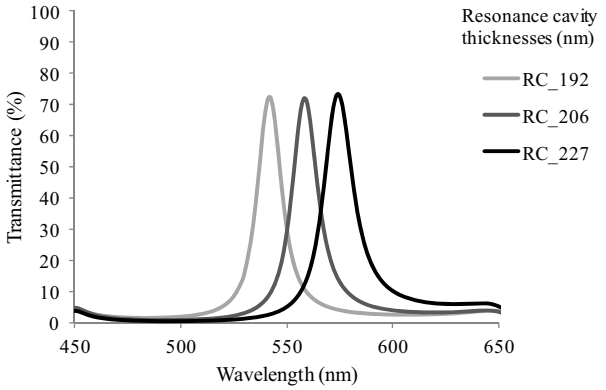

Figure 4. Measured transmittance spectra for the fabricated optical filters.

It is possible to conclude from the previous results that each filter is sensitive to a single spectral band, with FWHM less than 20 nm and with transmittances higher than 70%. The results are, thus, in good agreement with the simulations. The lower transmittances, compared to the simulated ones, are due to the detector used in the measurement that, in spite of being a commercial one, is not an ideal one, whereas during simulations an ideal detector was considered. The performance of the optical filters could be improved by increasing the number of layers, but the fabrication process complexity would also increase.

Furthermore, a structural characterization was performed, using cross section SEM (Scanning Electron Microscope) in order to assess layer thicknesses and interface quality between the different layers (Figure 5).

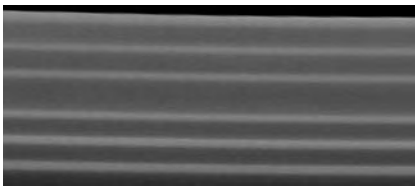

Figure 5. SEM photograph showing the cross-section of one optical filter (magnification 100,000 times)

From Figure 5 it is noticeable a clear separation between the SiO_2 and TiO_2 layers, with very low interface mixing. Also, it is shown very good film flatness along the entire area. This feature allows guarantee the parallelism of the mirrors relative to the resonance cavity, which is crucial for reproducible optical measurements.

With these results, it was proved the feasibility of fabricating thin-film optical filters for the selection of narrow spectral bands. These filters and the remaining selected as the work is progressing, will be, in the future, deposited on top of silicon photodiodes, to constitute the core element of the spectroscopy microsystem.

VII. Future Developments

Diffuse reflectance measurements were performed on tissue phantoms, in an initial stage, using the fabricated thin-film optical filters for the extraction of tissue information, such as the hemoglobin concentration. After the fabrication of the remaining optical filters in the 350-750 nm spectral range, both diffuse reflectance and fluorescence measurements will be performed. These spectra will be then analyzed, using statistical methods, to develop quantitative diagnostic algorithms.

Overall, at the end of the project a chip-sized device with integrated spectroscopy and multi-sensing functions will be obtained. The dimensions of the optical microsensors (thin-film optical filters and photodiodes) and LEDs can be scaled down to 50×50 μm^2 and 1×1 mm^2, respectively. Due to its small size, it can be used for *ex-vivo* tissue assessment (biopsies), for example, at the physician's office, allowing immediately results. Additionally, it will also have the potential to be integrated within an ingestible pill for *in-vivo* GI tissue assessment. The microsystem will fulfill important requirements, such as: multidiagnostic functions; high throughput; reduced cost; and disposability. A huge clinical impact of this device in early stage cancer management is envisioned.

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