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Fluorescence and diffuse reflectance spectroscopy for early cancer detection using a new strategy towards the development of a miniaturized system

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***Abstract*—This paper describes the design of a miniature, cost-effective spectroscopy system for assessing tissue biochemical and morphological information using a few wavelengths. This instrument will integrate thin-film optical filters and silicon photodiodes, avoiding the use of a spectrograph and optical fibers. The components in the set-up design are described. The feasibility of using only 16 wavelengths to accurately extract tissue properties is confirmed on physical tissue models. Also, the suitable spectral performance of several optical filters for the selection of these wavelengths is demonstrated. The reduced size of this device will make possible its implementation in an endoscopic capsule.**

I. Introduction

CANCERS of epithelial origin usually progress through increasing grades of dysplasia. Early detection of dysplasia is important for managing cancer, since the chances of successful treatment considerably increase when the disease is diagnosed at an early, non-invasive stage. However, dysplastic lesions are not always endoscopically visible, thus requiring several random, unnecessary tissue biopsies to be taken. Optical methods may overcome some limitations of current screening methods [1]-[3]. Diffuse reflectance spectroscopy (DRS) and intrinsic fluorescence spectroscopy (IFS) have shown great ability for the detection of dysplasia, by exhibiting different spectral features that can be correlated with normal and diseased tissue. These modalities provide quantitative information about biochemical and structural tissue attributes (tissue parameters), from which objective diagnostic algorithms are developed [3].

Diffuse reflectance spectra from tissues are used to extract information about hemoglobin concentration and saturation, light scattering parameters, and other tissue characteristics, using a well-developed model based on the diffusion approximation of light propagation in tissue. This method is known as DRS and provides information about the morphology and biochemistry of bulk tissue [4]. Intrinsic fluorescence is the fluorescence unaffected by tissue scattering and absorption, and is obtained using the diffusely reflected light to remove spectral distortions. The relative contributions of endogenous tissue fluorophores (e.g., NADH and collagen) can be extracted from the intrinsic fluorescence, being the method known as IFS. The concentrations of these fluorophores are dependent on the tissue disease state. Frequently, biochemical changes precede morphological changes within the tissue, which opens the possibility for a very early detection of dysplasia [5].

Clinical instruments intended to perform DRS and IFS usually employ optical fibers for light delivery and collection. This can be a drawback since regular optical fibers are not able to collect a huge portion of the reemitted signal, thus requiring high quantum efficiency detectors, such as CCD's (charge coupled device cameras). Also, they integrate costly and sophisticated light sources (xenon arc lamps, UV lasers). For this reason, the development of a miniature, less costly spectroscopy system without using optical fibers, spectrograph or CCD cameras could possibly increase the collection efficiency and improve throughput. A few groups have attempted to develop instruments with some of these features using LEDs for illumination and photodiodes for detection [6]-[8].

As a long-term goal we propose the development of a miniaturized spectroscopy device to be integrated on an endoscopic capsule for the detection of dysplasia in the gastrointestinal tract. This system includes LEDs for illumination (UV LEDs for IFS and white-light LEDs for DRS) and thin-film optical filters and silicon photodiodes for the selection and detection of the most significant wavelengths for diagnosis.

This report describes the design of the instrument. As a first step towards the final goal, simulations on wavelength reduction are performed to investigate the feasibility of replacing the spectrograph by several thin-film optical filters, and the use of a few wavelengths to accurately quantify tissue parameters. Physical tissue models with known optical properties are used for the feasibility study. The design and performance of thin-film optical filters is illustrated.

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II. INSTRUMENTATION

A miniaturized spectroscopy system will be designed to be integrated within the endoscopic capsule. The instrument is based on white-light and UV LEDs, and optical microsensors (thin-film optical filters together with silicon photodiodes). The firsts will be used as the illumination sources for DRS and IFS measurements, and the second to select and detect specific light wavelengths. The use of such components obviates the need for costly, bulky and sophisticated illumination and detection equipment (xenon arc lamp, UV laser, CCD camera, spectrograph), and optical fibers for light delivery and collection.

This microsystem will add important diagnostic functions to the current endoscopic capsules, taking advantage of the commercial available capsule platform (such as the battery, wireless data transmission, antenna, etc.). Fig. 1 illustrates the several components of the miniaturized spectroscopy system.

A. Wavelength Reduction Simulations on Physical Tissue Models

The replacement of the spectrograph by a series of optical filters was investigated in the 350 to 750 nm spectral range. The capability of a spectroscopy system to extract tissue information with accuracy using just a few wavelengths was evaluated with a series of experiments on physical tissue models ("phantoms") with known scattering and absorption parameters. The phantoms consist of mixtures of 20% intralipid and human hemoglobin (Sigma Aldrich Co.) at various concentrations, and water. The mass concentrations for intralipid were 0.5% and 1%, whereas the concentrations of hemoglobin were 0.5 and 1mg/mL. The intralipid and the hemoglobin are used for scattering and absorbing, respectively.

Two groups of phantoms were measured by DRS: the first with different concentrations of absorber and a fixed intralipid concentration; the second with a variable scatterer concentration and a fixed hemoglobin amount. A sample of $BaSO_4$ was used as a reflectance standard. The UV-3101PC spectrophotometer, from Shimadzu, was used to measure the diffuse reflectance spectra.

DRS is used for the extraction of tissue quantitative information. By fitting the reflectance spectrum to the model described by Zonios *et al.* [4], four tissue optical parameters are extracted: A, the reduced scattering coefficient at the reference wavelength; B, related to the average scatterer size; cHb, the concentration of hemoglobin; and α, the oxygen saturation of hemoglobin.

Several combinations of 16 wavelengths were simulated. The combination that provided the best results, in terms of tissue parameters extraction, comprises the following discrete data points: 350, 370, 380, 400, 420, 450, 480, 510, 540, 560, 580, 600, 620, 650, 700, and 750 nm. These 16 wavelengths will be selectively detected using one stack of TiO_2 and SiO_2 thin-films, placed on top of the photodiode, as will be detailed on the next subsection. The remaining spectral values will be obtained by interpolation within the range of the discrete data set.

B. Thin-film Optical Filters

A thin-film optical filter array (composed by 16 filters) will be deposited by ion beam deposition on top of photodiodes. The filtering system, based on Fabry-Perot thin-film optical resonators, should be designed to yield a narrow pass-band around the selected wavelengths. With this optical filter array only a white light and a UV source for illumination will be needed, thus avoiding a full spectrograph. The filters consist of two flat parallel mirrors composed of a stack of TiO_2 and SiO_2 thin-films, with a SiO_2 resonance cavity in the middle. TiO_2 and SiO_2 have been selected because of the IC compatibility and the well characterization of their deposition process. In addition, the refractive index of SiO_2 is almost wavelength independent for the spectral band between 350 nm and 750 nm.

III. RESULTS

A. Tissue phantoms study

In order to experimentally determine the accuracy of a spectroscopy system to extract tissue properties using only 16 wavelengths, several DRS measurements were performed on tissue phantoms, in a range of absorbing and scattering concentrations. The collected spectra were normalized by the reflectance spectra measured from the standard to correct for the wavelength dependent response of the system.

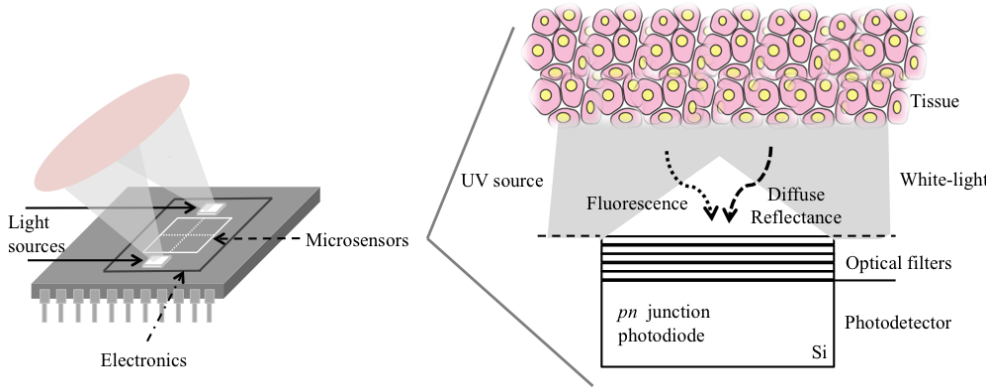

Fig. 1. Miniaturized spectroscopy system with LEDs as light sources, optical filters and photodetector for wavelength selection and detection, respectively (not scaled).

The diffuse reflectance spectra taken from different phantoms using the full wavelength range, and using only 16 selected wavelengths are shown in Fig. 2. These 16 intensities are the signal that would be read by the photodiode array. DRS is then used to extract the tissue optical parameters by fitting each measured reflectance spectrum (solid lines) to the model described by Zonios *et al.* [4] (dashed lines).

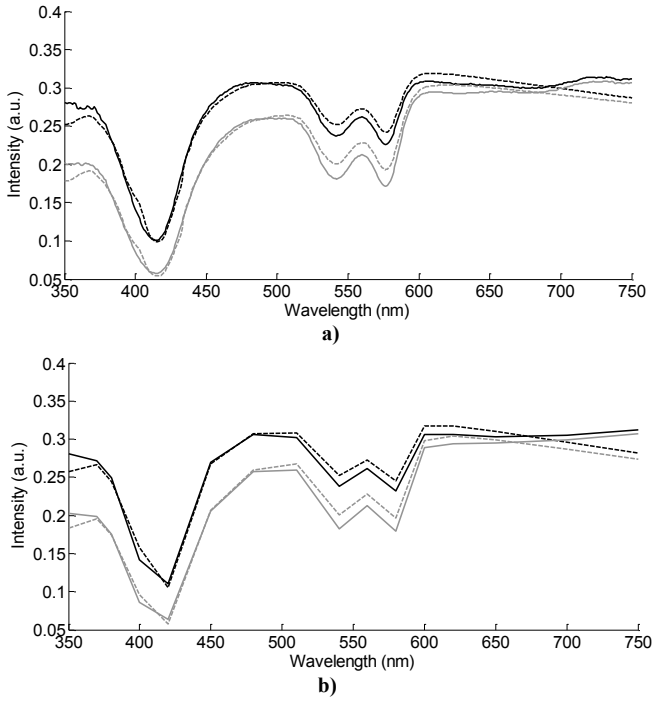

Fig. 2. Diffuse reflectance spectra measured from two different phantoms (phantom 1: black line; phantom 2: grey line): (a) using the full wavelength range; (b) and only 16 wavelengths. The best fit spectra according to the model of Zonios *et al.* are also plotted (dashed lines).

The extracted values for parameters *A*, *B*, *α,* and *cHb*, from different phantoms are presented in Table 1. As expected, the value of *A* increases with intralipid concentration. The concentration of hemoglobin (*cHb*) is very similar to the expected value with less than 10% difference.

The use of the full wavelength range (400 wavelengths) to measure diffuse reflectance or the use of only 16 wavelengths seems to provide comparable results in terms of optical properties in tissue physical models. These results point out that it is not necessary to use the full 350-750 nm spectrum to extract tissue optical properties with sufficient accuracy.

The composition of the phantoms presented in this study is simplified compared to human tissue. However, hemoglobin, which is a very important parameter to differentiate normal from malignant tissues, is considered the most important absorber in the visible region of the spectra for human tissue, and its concentration can be extracted with good accuracy using just a few wavelengths. In the same way, other physiological parameters, such as NADH and collagen, may also be quantified using just a few wavelengths.

B. Optical Filtering System

The previous results have shown the possibility of using only 16 wavelengths to extract tissue optical properties. These wavelengths can be selected using an optical filter array. This array is composed by four groups of four optical filters to cover different ranges: 350-400 nm; 420-510 nm; 540-600 nm; and, 620-750 nm.

Thin-film optics software TFCalc^{TM} 3.5 was used for the structural optimization of the optical filters. Simulation results show that a multilayer stack of TiO_2 and SiO_2 thin-films for the dielectric mirrors with a SiO_2 resonance cavity is the best option in terms of optical characteristics and fabrication process. For each of the mentioned groups, the optical filters are composed by 11 layers of TiO_2 and SiO_2, and can be easily tuned to a different wavelength by adjusting only the thickness of the 6th layer (the SiO_2 resonance cavity). This SiO_2 thickness, in each group, increases 9 nm to move the wavelength peak 10 nm.

Meanwhile, only 8 of the required 16 optical filters are being fabricated. In Fig. 3 the simulated transmittances of these 8 Fabry-Perot optical filters are presented, with the layer stacks described in Table 2. The presented results show that each filter is sensitive to a single spectral band, with FWHM < 15 nm, and with a ratio of maximum transmittance to background noise greater than 80/25 (enough in terms of optical characteristics).

TABLE 1
REFLECTANCE PARAMETERS MEASURED FROM TISSUE PHANTOMS WITH DIFFERENT HEMOGLOBIN (HB) AND INTRALIPID CONCENTRATIONS.

			Full Spectrum				16 Wavelengths			
Phantoms	Intralipid mass concentration	Hb concentration	*cHb* (mg/mL)	*A*	*B*	*α*	*cHb* (mg/mL)	*A*	*B*	*α*
1	0.5%	0.5	0.498	0.914	0.673	1.0	0.519	0.903	0.771	1.0
2	0.5%	1.0	0.944	0.887	0.538	1.0	1.013	0.873	0.704	1.0
3	1%	0.5	0.480	1.787	0.546	1.0	0.499	1.759	0.648	1.0
4	1%	1.0	0.988	1.782	0.559	1.0	1.075	1.752	0.742	1.0

TABLE 2
MAXIMUM TRANSMISSION PEAK AND LAYER THICKNESS OF 8 FABRY-PEROT OPTICAL FILTERS.

		Wavelength transmission peak (nm)							
		420	450	480	510	540	560	580	600
		Layer thickness (nm)							
	TiO_2	42	42	42	42	58	58	58	58
	SiO_2	74	74	74	74	92	92	92	92
Mirror	TiO_2	42	42	42	42	58	58	58	58
	SiO_2	74	74	74	74	92	92	92	92
	TiO_2	42	42	42	42	58	58	58	58
Cavity	SiO_2	**127**	**154**	**181**	**199**	**166**	**184**	**202**	**220**
	TiO_2	42	42	42	42	58	58	58	58
	SiO_2	74	74	74	74	92	92	92	92
Mirror	TiO_2	42	42	42	42	58	58	58	58
	SiO_2	74	74	74	74	92	92	92	92
	TiO_2	42	42	42	42	58	58	58	58

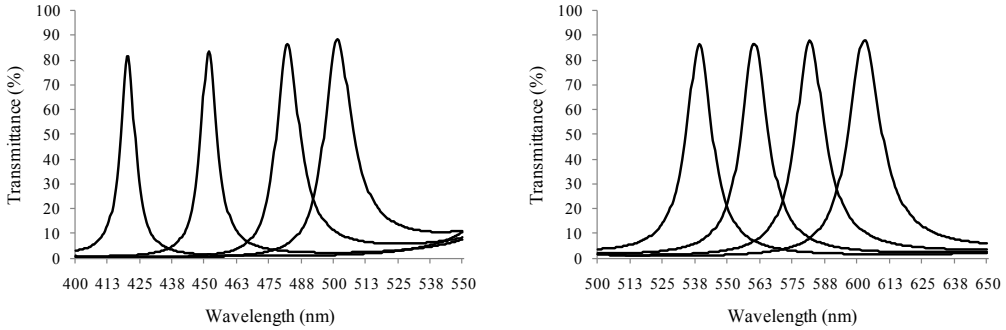

Fig. 3. Simulated transmittance spectra for 8 Fabry-Perot optical filters, with the Fabry-Perot layer stacks described in Table 2. The SiO_2 layer thickness changes from 127 to 220 nm in 9 nm increments.

IV. CONCLUSION

This study demonstrated that it is possible to extract tissue information accurately using a small number of wavelengths. These results are a very important step towards the development of a much smaller, high throughput system, compared with the conventional clinical spectroscopy instruments.

Overall, the results showed the feasibility of replacing the spectrograph by a series of thin-film optical filters. Also, with UV and white-light LEDs as illumination sources and photodiodes as the detector, the device would then be completely miniaturized, whereas still achieving good performance in the extraction of tissue optical properties. The fabrication of the entire system and its preliminary results will be discussed elsewhere.

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