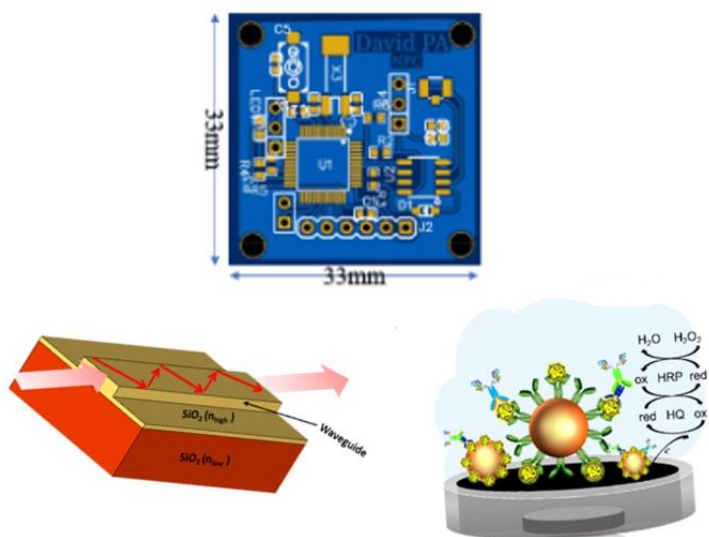


UM OLHAR SOBRE OS SENSORES NA PENÍNSULA IBÉRICA E AMÉRICA LATINA: ANO 2022

UNA MIRADA A LOS SENSORES EN LA PENÍNSULA IBÉRICA Y
AMÉRICA LATINA: AÑO 2022

A LOOK AT SENSORS IN THE IBERIAN PENINSULA AND LATIN
AMERICA: YEAR 2022



Coordenadoras
M. Teresa S. R. Gomes
Marta I. S. Veríssimo



universidade de aveiro
theoria potesis praxis

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M. Teresa S. R. Gomes e Marta I. S. Veríssimo

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MULTI-PHOTON MICROSCOPY SETUP FOR INTEGRATION IN COLONOSCOPES: AN OVERVIEW

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Introduction

Multi-photon microscopy (MPM) is considered one of the breakthrough inventions. This technique is based on the intracellular endogenous fluorescent signal, such as the two-photon excited fluorescence and second-harmonic generation (SHG) [1–3]. MPM provides optical sectioning from deep specimens with high scattering properties, restricting fluorophore excitation to the focal plane [4]. This type of microscopy can provide real-time information about cellular morphology and perform three-dimensional tomography on fresh tissues, being an excellent replacement for traditional laborious histopathology. MPM could be very sensitive in the diagnosis of colorectal cancer during conventional colonoscopy [1–3,5]. Among the endogenous fluorophores that can be imaged by MPM, nicotinamide-adenine dinucleotide (NADH) and flavine-adenine dinucleotide (FAD) are the most relevant. The fluorescent signal of these fluorophores can be acquired for cancer monitoring, because changes in the metabolism of glucose occur in cancerous cells, even before morphological changes appear in the tissues [6,7]. On the other hand, the SHG collagen signals can also be used to study tumor-induced changes in matrix organization [5].

The commercial MPM equipment is bulky and inadequate for miniaturization applications [1–3]. This work aims to integrate MPM in conventional colonoscopes (Figure 1), by developing a microfabricated MPM setup. The MPM prototype will be used complementarily to colonoscopy and will provide the *in-vivo* optical biopsy of colorectal tissues, being capable to detect human colon cancer in its early stage. Some studies related to the development of miniaturized probes with MPM technology have already been carried out. However, all of them focus only on the scanning system (probe) miniaturization, no study achieved the direct integration of the probe in a conventional colonoscope, and the probes are exclusively tested in ex-vivo tissues or animals. Thus, more studies are needed to successfully implement this technology in colonoscopy. This abstract presents an

overview of the MPM setup and some experiments already performed towards the final prototype implementation.

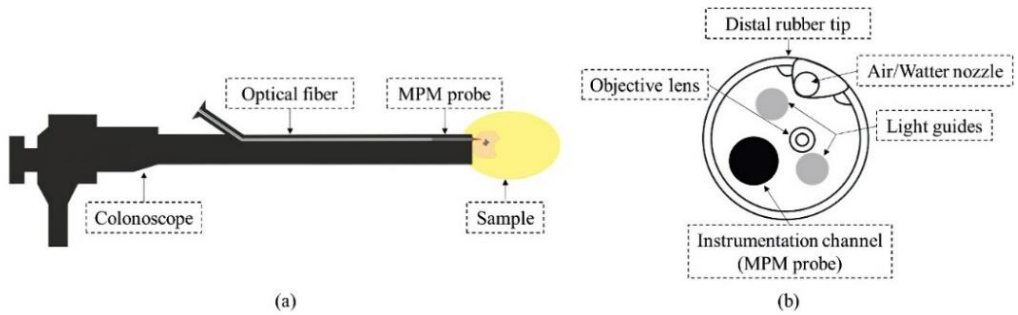


Figure 1. Colonoscope with a MPM probe: (a) side view; (b) front view.

Methods

This work will focus on the development of an MPM prototype based on the schematic presented in Figure 2. This setup includes the assembling of optical components, a microfabricated MPM probe (piezoelectric tube-based with GRIN lens assembly) for integration in the instrumentation channel of a commercial colonoscope, and optical filters for the MPM detection system.

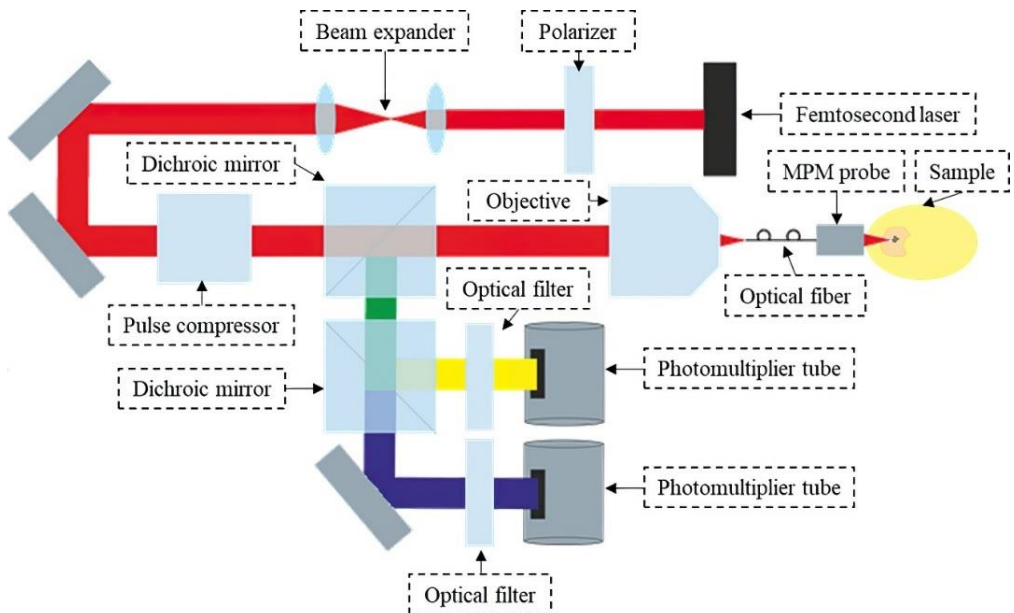


Figure 2. Schematic of the MPM prototype.

The microfabricated MPM probe should have a diameter smaller than the instrumental channel of conventional colonoscopes (3.8 mm). The piezoelectric tube to scan the tissue will be fabricated using conventional MEMS technologies for the deposition of the piezoelectric material (zirconate titanate), such as physical vapor deposition (PVD) and atomic layer deposition (ALD). The fabrication of GRIN lenses will be performed using photolithography, thermal reflow, and PDMS (polydimethylsiloxane) demolding. The optical filters will be implemented with a multilayer of dielectric thin-films (SiO_2 as the low-refractive index material and TiO_2 as the high-refractive index material). Before microfabrication using a PVD technique (RF-sputtering), the optical filters must be designed and simulated at a software tool (TFCalc 3.5). The optical filters will be used to ensure that only the signals emitted by the fluorophores will be acquired by the MPM detection system. Since NADH presents an emission wavelength peak at 460 nm, and FAD at 525 nm, at least two optical filters centered at those wavelengths should be designed and fabricated [6,7].

Experimental results

Ultrashort-pulse compressor: as presented in Figure 2, an important component of the MPM setup is an MPM laser ultrashort-pulse compressor. This component is useful for tissue imaging since allows the maintenance of ultra-short pulses, despite the optical path. The laser time compression improves the number of photons that reach the focal point at a given time, increasing the intensity of the generated signals. The compressor schematic based on the work developed by Akturk *et al.* in 2006 [8] is presented in Figure 3 (a). For this experiment, the prism-corner cube separation has varied a total of 15 cm in intervals of 1 cm using a rail. The output pulse full-width at half maximum (FWHM) was measured using an autocorrelator (APE Mini). The obtained values are presented in Figure 3 (b).

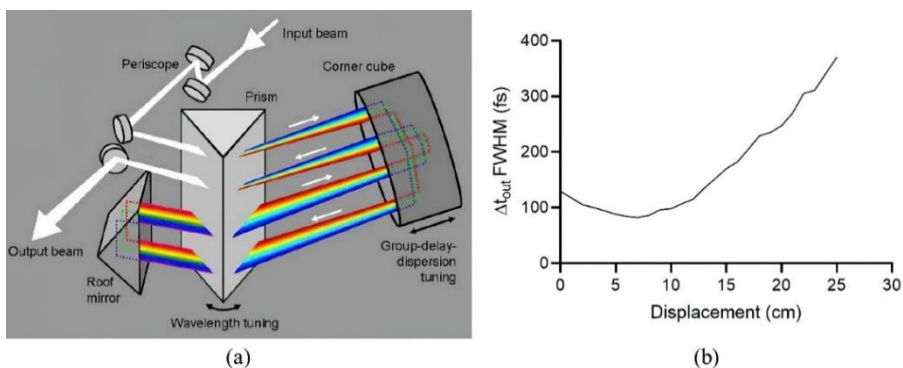


Figure 3. Ultrashort-pulse compressor: (a) schematic [8]; (b) variation of the output pulse FWHM with the prism-corner cube separation.

Optical filters: the optical filters simulation requires the use of the materials (SiO_2 and TiO_2) refractive indices. This task is crucial to obtain the thickness of each thin-film, which will form the narrow and high transmission filter at the wavelength of interest. Those refractive indices can be variable according to deposition method and microfabrication conditions. Thus, individual thin-films of SiO_2 (89.87 nm thick) and TiO_2 (91.36 nm thick) were deposited by RF-sputtering and characterized with profilometry (Veeco Dektak 150) and ellipsometry (J. A. Woollam alpha-SE). All the obtained results for the refractive indices are presented in Figure 4, where is also presented the theoretical refractive indices stated in the literature for comparison.

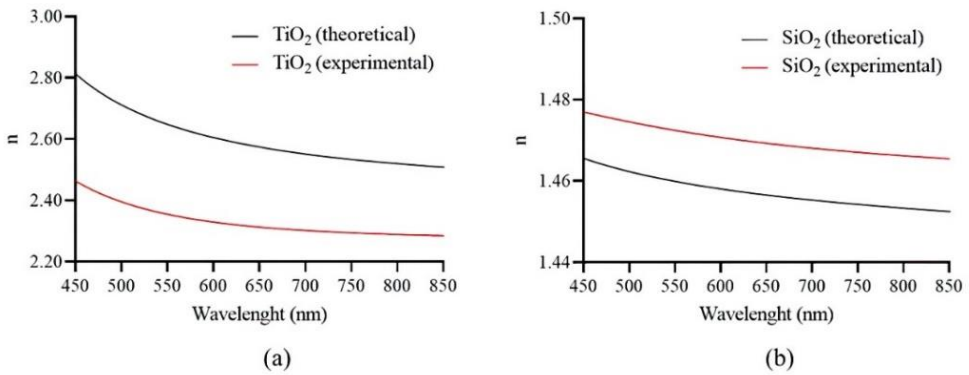


Figure 4. Theoretical and experimental refraction indices: (a) SiO_2 ; (b) TiO_2 .

Conclusions

This abstract presents some experimental work towards the implementation of a MPM setup for integration in conventional colonoscopes. Experiments with an ultrashort-pulse compressor for a MPM laser proved that the duration of the pulse varies with the prism-corner cube distance, and so the ultrashort-pulse compressor is working correctly. Regarding the optical filters, thin-films were deposited by RF-sputtering and characterized by ellipsometry. The results show that the deposited materials present refractive indices similar to the theoretical values, stated in the literature.

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