A simple dental caries detection system using full spectrum of laser-induced fluorescence

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ABSTRACT

Objectives: to develop an apparatus for the detection of early caries lesions in enamel using the full extent of the tooth fluorescence spectrum, through the integration of a laser diode, fiber optics, filters and one portable spectrometer connected to a computer, all commercially available; to evaluate the developed device in clinical and laboratory tests, and compare its performance with commercial equipment. *Methods*: clinical examinations were performed in patients with indication for exodontics of premolars. After examinations, the patients underwent surgery and the teeth were stored individually. The optical measurements were repeated approximately two months after extraction, on the same sites previously examined, then histological analysis was carried out. *Results*: the spectral detector has presented high specificity and moderate sensitivity when applied to differentiate between healthy and damaged tissues, with no significant differences from the performance of the commercial equipment. The developed device is able to detect initial damages in enamel, with depth of approximately 300 μm. *Conclusions*: we successfully demonstrated the development of a simple and portable system based in laser-induced fluorescence for caries detection, assembled from common commercial parts. As the spectral detector acquires a complete recording of the spectrum from each tissue, it is possible to use it for monitoring developments of caries lesions.

Keywords: Caries detection; enamel fluorescence; dental device development

1. INTRODUCTION

Conventional methods for detection of caries include visual inspection, tactile probing and radiographic images. In general, conventional clinical techniques present high specificity (the ability to detect healthy tissue), but low sensitivity and reproducibility, mainly because they are subjective methods [1,2,3]. The development of simple techniques to detect and quantify the fluorescence emitted by dental tissue has led to new medical equipment for the characterization of tooth tissue from its surfaces,[4, 5] a new class of devices for non-invasive clinical diagnosis of dental caries [6, 7]. As expected for optical methods, data interpretation can be affected by the presence of bacterial, plaque, stains, restorations, pigmentation, prophylactic toothpastes, the autoclaving procedure or plastic film protection at the probes, which can in some cases, lead to non-optimum occurrence of false positives or false negatives [8, 9, 10]. Some studies have reported that fluorescence-based methods do not necessarily outperform the traditional methods for generic caries detection, but it is accepted that they have the potential to present higher sensitivity and specificity [11, 12, 13]. It has been also recently demonstrated that careful observation of changes in the parameters of fluorescence can indicate lesion progression toward cavitation [14].

The carious process alters the optical properties of tooth tissues, such as absorption and emission spectra, scattering and polarization behavior, among other parameters. For example, it has been verified an increase in light scattering for incipient caries in enamel [15, 16]. Light-induced fluorescence varies with the wavelength of excitation and condition of the tissue. When tooth tissue is excited by blue light, healthy and carious regions exhibit similar fluorescence responses, so that differentiation can be performed only due to changes in optical scattering and light transmission [17]. However, when excited with red wavelengths (633 nm to 655 nm), dental tissues exhibit fluorescence in near infrared, with stronger fluorescent intensity coming from decayed tissues (attributed to bacterial metabolites) [18]. In addition, red light suffers less scattering in tooth tissues, thus being able to reach important fluorescent compounds that can identify caries, whether they are in enamel, dentin or at the amelo-dentinal junction [19].

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Devices for clinical diagnosis of caries based on light-induced fluorescence are commercially available [20]. Examples are the DIAGNOdent (KaVo, Germany), the Spectra Caries Detection Aid (Air Techniques, US), and the SoproLife (Acteon Group, France). Those products are based on the detection of the average intensity of a short range of wavelengths contained in the fluorescence signal. However, other types of detection could be carried out from the consideration of the whole fluorescence spectrum and its structure, and comparing it with that obtained from healthy tissue. In general, fluorescence spectroscopy applied to dentistry could even allow detection of mineral content changes with high sensitivity and specificity [17, 21, 22, 23].

In this study we aimed to develop and test a novel simple apparatus using light-induced fluorescence for the detection of early caries lesions in enamel, through the integration of a laser diode, fiber optics, filters and one portable spectrometer connected to a computer, all commercially available. As methodological goals, we sought to investigate, through in vitro and in vivo experiments using human teeth, a caries measurement technique using the full extent of the fluorescence spectrum and compare the performance of this technique with commercial equipment.

2. MATERIALS AND METHODS

Assembling the caries detection system

For the development of the caries detection system using a common portable spectrometer, we have chosen the USB2000 Miniature Fiber Optic Spectrometer (Ocean Optics, USA), configured to have a diffraction grating with 600 lines per mm, optical response in the range 530 nm to 1100 nm and nominal resolution of approximately 1.3 nm (model #4, from Ocean Optics Catalog). An embedded linear CCD array detector converts the optical signal into digital data. The calculated overall spectral resolution of the device is about 7.6 nm. We used an ordinary red laser diode as the excitation source ($\lambda \sim 657$ nm), with few milliwatts of maximum power (a device commonly used as a laser pointer), connected to a simple power and switching electronics, whose light beam was injected into an optical fiber.

The optical connection system for dental use (both delivery and collection) was composed by two optical fiber subsystems, concentric initially at one end and separated at the other end, i.e., a y-shaped fiber system. A handpiece integrates those two fiber sets in the concentric end, so that the peripherally disposed set of fibers delivers the excitation light (formed by six fibers with 400 μm) and one fiber in the center collects the resulting fluorescence (R400-7-VIS/NIR, Ocean Optics, USA). The handpiece presents a straight tip and diameter of 6.31 mm, as illustrated in Figure 1.

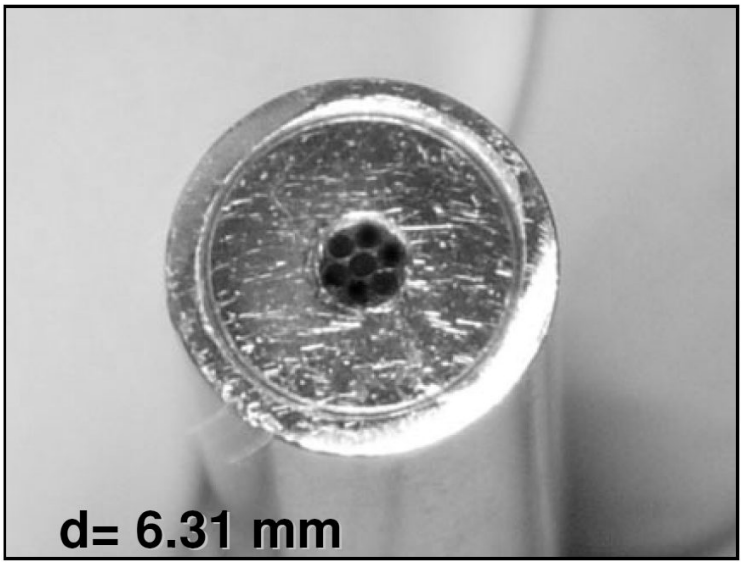

Figure 1: Tip of the handpiece used (R400-7-VIS/NIR, Ocean Optics, USA).

In order to block the passage of any light with wavelength below 700 nm, which can saturate the signal detection, we used an optical filter (LVF and LVFS, Ocean Optics, USA) connected to a coupling fiber (QP 600-025 VIS-NIR, Ocean Optics, EUA) and to the spectrometer. Both the coupling fiber and the optical connection fiber set were characterized using white light transmission. It was verified a monotonic, linear response as a function of wavelength in the region of interest (with low slope), which we considered in data analysis. In our system, the excitation laser power had to be above 2 mW in order to capture enough fluorescence. Before each measurement, we verified the optical power with a meter positioned at the output of the handpiece (LM10i, Fieldmaster, Coherent Inc., USA). We used the software that accompanies the spectrometer. It displays the signal in real time and allows configuration of parameters. We used integration time of 100 ms, spectra to average of 10, boxcar smoothing width of 5, in the "scope" mode. We performed the calibration of the spectrometer prior to get any spectral data, as recommended by the manufacturer. In Figure 2 we show the complete diagram of the developed system. It was assembled into a small transparent plastic box so as to allow transport between laboratory and clinical research.

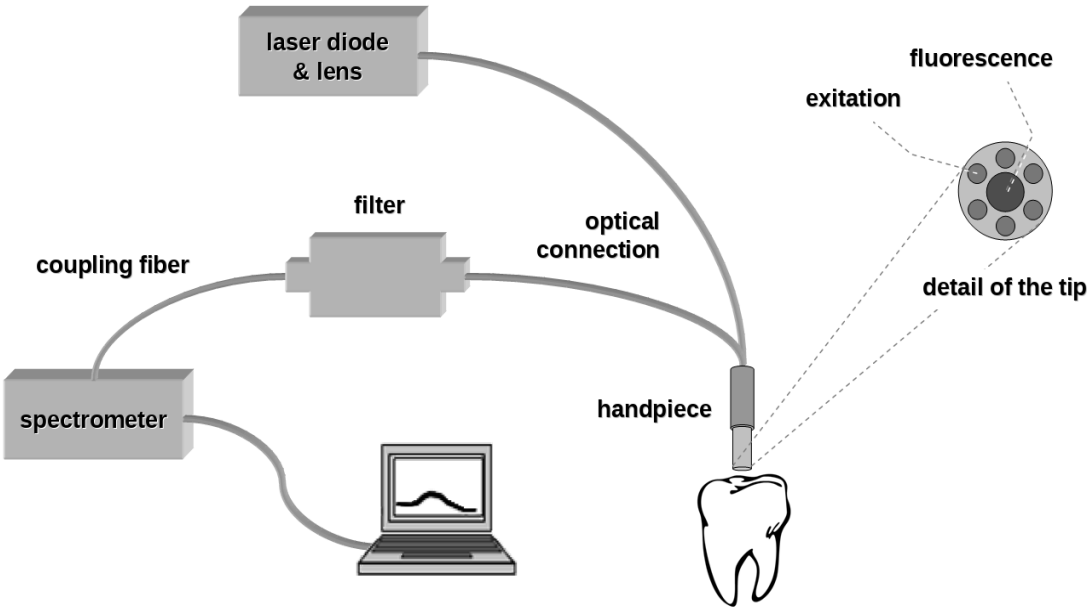

Figure 2: Setup for the caries detection system using a commercial spectrometer.

Measurements with the developed spectral detector

The handpiece was protected with a layer of PVC film to prevent cross contamination and always positioned perpendicular to each site. During measurements, the operator searches the most intense fluorescence, sweeping the entire lesion and its margins, and a single record for each site is then registered. It was also performed one measurement in a healthy region of tooth structure. Typical obtained spectra are shown in Figure 3. In our study, the recorded spectra were analyzed using spreadsheet software (Origin 7.0, OriginLab, USA).

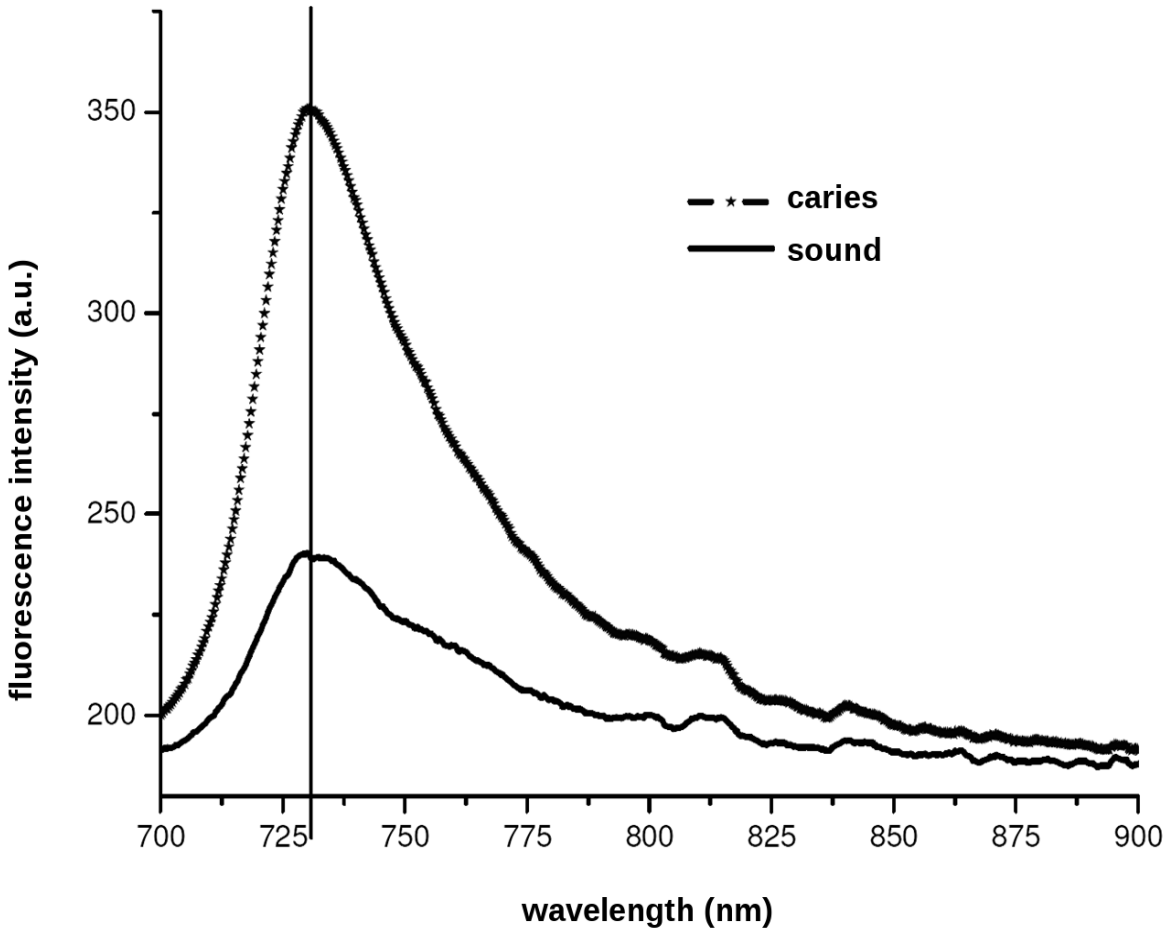

Figure 3: Typical spectra from a suspect site, using the developed spectral detector.

Measurements with a commercial system, DIAGNOdent

The DIAGNOdent model 2095 was operated by a sole trained operator, which followed manufacturer's recommendations. The handpiece was protected with one layer of PVC film to prevent cross-contamination. Every ten measurements the equipment was recalibrated using the ceramic standard that accompanies the product. We used the angled handpiece for occlusal surfaces, positioned perpendicular to each site, and performed three rotational movements so to sweep over the entire lesion and margins. The equipment displays the real-time intensity and the maximum value recorded during measurements, in a relative scale from 0 to 99. In our procedure, the maximum value found for each site was recorded.

Clinical and laboratory fluorescence tests

Clinical examinations were performed in patients between 18 and 35 years old. Patients with indication for exodontics of premolars were previously examined using the following exclusion criteria: restorations, caries visibly apparent, sealants and dental calculus. Cleaning was performed with nonfluorescent sodium bicarbonate jet (Prophy Jet, Kondortech,

Brazil) for 10 seconds on each tooth surface to be examined. After prophylaxis, the operative field was isolated with cotton rolls and the elements were dried with compressed air for 3 seconds. Each tooth was pre-examined visually using magnifying glass (2.5X) to verify sites of initial caries lesions. Occlusal surfaces of 42 premolars were chosen for this study. Non-contiguous sites on same tooth and different teeth in same patient were considered for the experiment. At the end of this selection process, we have determined 66 suspected points of lesion to be examined with both the developed spectral detector as with the DIAGNOdent. Immediately after both examinations, the patients underwent surgery by clinical professionals responsible for element removal. Immediately after surgery, the teeth were cleaned using toothbrush and running water, with no antibacterial compound. Then the mesial surfaces of the teeth were covered with nail varnish to facilitate later identification of the sites and ensure the same positioning during laboratory study. The roots were removed and the teeth were placed on sheets of wax. The teeth were stored individually in deionized refrigerated water (5° C, changed weekly) until their use in the study. The optical measurements were carried out approximately two months after extraction, on the same sites previously examined, with no additional cleaning and following the same measurement protocol.

Histological analysis

As reference standard method, we carried out histological analysis after the teeth sectioning. After the optical tests, all teeth were embedded in transparent orthophthalic resin (Resin 2110 Redelease, Brazil) and sectioned into 500-μm thick slices on a diamond blade cutter (Isomet-II80 II, Buehler, USA). Slides were prepared following the same protocol used by Mendes (2002) [24] and each tissue sample was reduced to a thickness of approximately 100 μm. The slides remained immersed in distilled water for at least 24 hours before the examination. Just before observation, a drop of deionized water was placed on the sample, being overlapped by a cover slip. The slides were examined with polarized light microscope (Leica DMLP, Germany). Lesions were classified on a scale of five scores: D0 = no caries; D1 = caries limited to the outer half of the enamel; D2 = caries limited to the inner half of the enamel without reaching the dentin; D3 = caries limited to the outer half of the dentin and D4 = caries involving the inner half of the dentin. All histological images were measured using image analysis software (Image J, http://rsb.info.nih.gov/ij/), thus we determined each lesion cross-sectional area and its deepest point.

Methods for analyzing fluorescence measurements

The following procedure was applied in order to analyze the data obtained with the spectral detector: (1) each spectrum was normalized, considering a baseline defined from its values in the range 850-900 nm; (2) the area under the curve, AUC, was obtained from 700 nm to 800 nm; (3) the ratio of the AUC for each pair of spectra, carious and healthy measured from the same tooth, was calculated. Those ratios of AUC, we will denominate RAUC, were classified against the results from histological analysis. Similarly, the results from DIAGNOdent measurements were also classified with the histological findings. The Kruskal-Wallis test was applied in order to correlate results obtained using each device with histological results, and cases with significant statistical difference were submitted to the Dunn test to find from what groups those differences arose. The Pearson's correlation coefficient analysis was also performed in order to study the correlation between data from the optical measurements, cross-sectional area and depth of the lesions.

We have also carried out the relative operating characteristic study, ROC. This requires dichotomized ensemble, so results were grouped following the classification from histology: those from D0 correspond to the absence of caries and those from D1+D2+D3+D4 formed the group of presence of caries. The diagnostic parameters, sensitivity, specificity and accuracy, were calculated for the spectral detector and DIAGNOdent, in the clinical and laboratory studies. ROC also provided the best cutoff point for the diagnosis of healthy or carious tissue, it was chosen as the position of the maximum of the sum of sensitivity and specificity [25]. The overall characteristics of each method (sensitivity, specificity, and accuracy) were then calculated using those cutoffs. For comparing the areas under ROC curves, a non-parametric approach was used [26]. For all statistical tests, the level of significance adopted was 5% ($p < 0.05$).

3. RESULTS

Spectral detector results and performance

The RAUC results from measurements using the spectral detector were categorized by the respective histology results, as shown in Figure 4, for clinical and laboratory studies. In those graphs, the circles represent the averages, the asterisks are extreme values, the boxes represent the most central data (percentile 25 to 75) and the line within the box is the median (which bisects distribution, considering the amount of data). The Kruskal-Wallis test has indicated statistically significant differences and the Dunn test demonstrated that group D0 in-vivo presented significant difference against all groups in the same study, and that D0 in-vitro presented significant difference from D2 and D3 in the same test. The results obtained with the spectral detector in the clinical study have presented positive correlation with the depth of the lesions, with a Pearson linear correlation of 0.42, as shown in Figure 5a. Similarly, the results from the spectral detector applied in vitro have shown a positive linear correlation of 0.43 with the depth of the lesions, as shown in Figure 5Figure5-b.

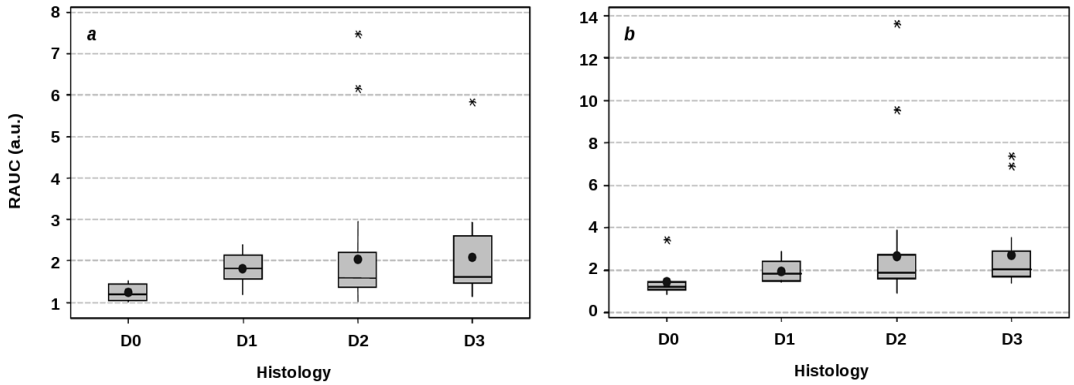

Figure 4: Box-plot for the spectral detector results in vivo (a) and in vitro (b), classified by histology. Circles represent averages, asterisks are extreme values, boxes represent central data (percentile 25 to 75) and the line within the box is the median.

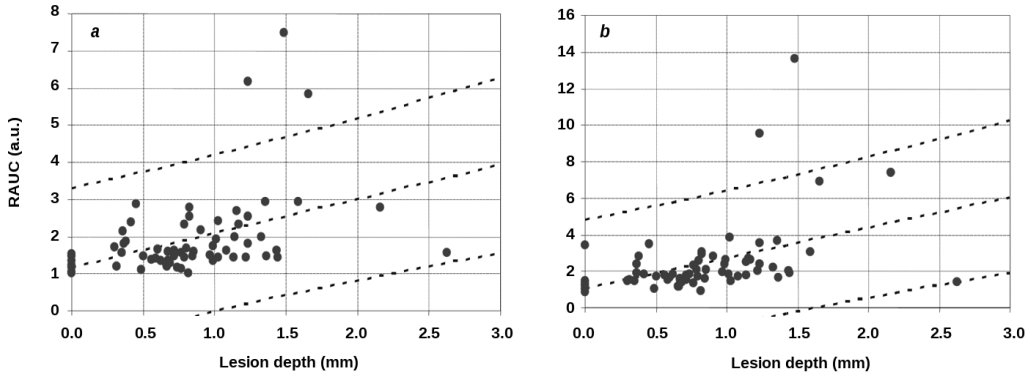

Figure 5: Scatter diagram of data from spectral detector (RAUC) in vivo (a) and in vitro (b) versus lesion depth (from histology). Also shown linear regression lines and prediction intervals.

In Figure 6 we show the calculated values of specificity, sensitivity and the sum of both parameters versus arbitrarily assigned cutoff points, considering D0 = sound and D1+D2+D3 = carious. The detection criterion must assume the best cutoff point able to provide a good balance between specificity and sensitivity. One approach is to choose the point of maximum in the curve of sum of sensitivity with specificity. Therefore, in our study, RAUC values greater than 1.55 for the in vivo measurements, and greater than 1.45 for the in vitro measurements, indicates the presence of caries.

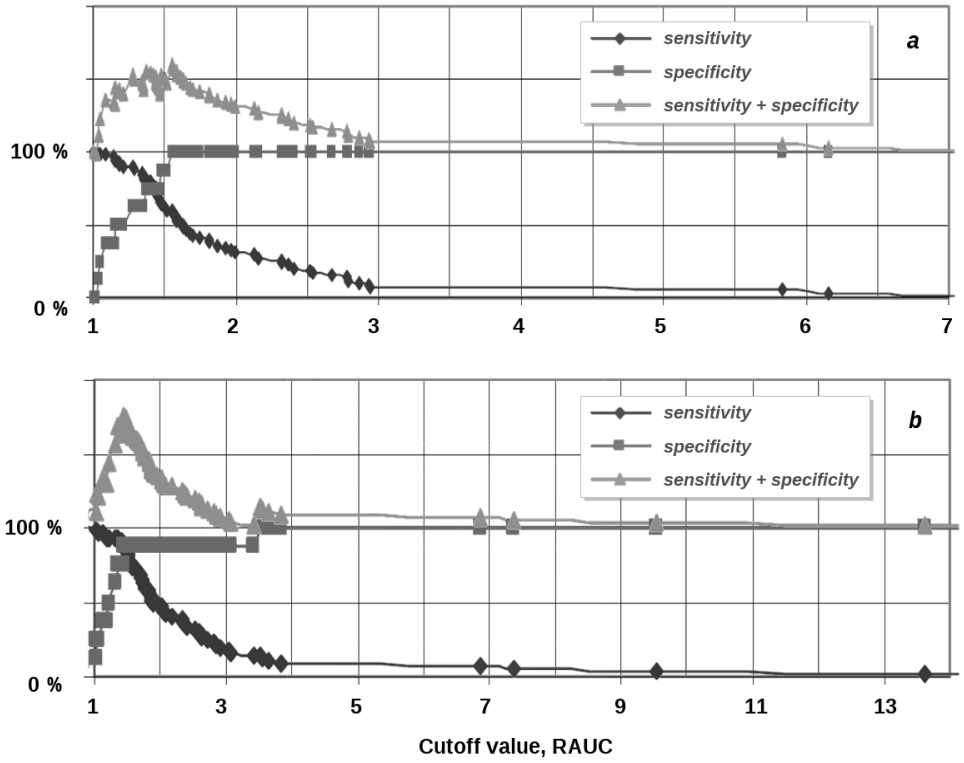

Figure 6: Specificity, sensitivity and their sum, as a function of hypothetical cutoffs, for the spectral detector (RAUC data) in the clinical study (a) and in the laboratory study (b).

Study with DIAGNOdent

Results from measurements with DIAGNOdent compared with reference standard method are represented in the box-plots of Figure7. The Kruskal-Wallis test has shown statistically significant differences, and by applying the Dunn test we verified that group D0 presents significant difference against all groups. The values obtained in the clinical study and in the laboratory study presented a positive correlation with the depth of the lesions, with a Pearson linear correlation of 0.41 and 0.45 respectively, as shown in Figure 8.

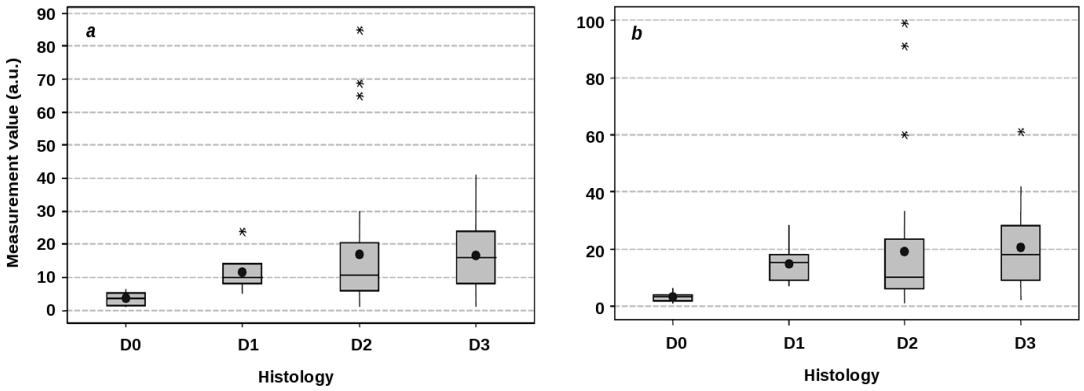

Figure 7: Box-plot for results obtained with DIAGNOdent in vivo (a) and in vitro (b), classified by histology. Circles represent averages, asterisks are extreme values, boxes represent central data (percentile 25 to 75) and the line within the box is the median.

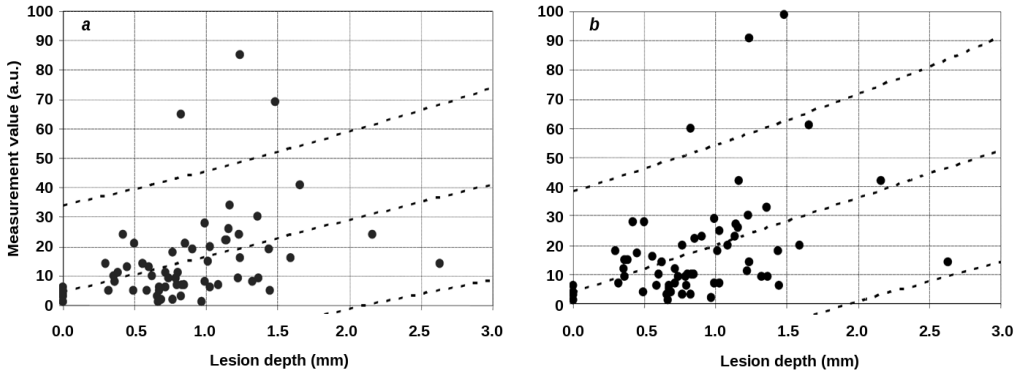

Figure 8: Scatter diagram of results from DIAGNOdent in vivo (a) and in vitro (b) versus lesion depth (from histology). Also shown linear regression lines and prediction intervals.

The specificity, sensitivity and their sum, calculated as function of assigned cutoff points (considering D0 = sound; D1+D2+D3 = carious), are presented in Figure 9 for the DIAGNOdent in the clinical and laboratory studies. Values greater than 7, in whatever study carried out, indicate the presence of caries.

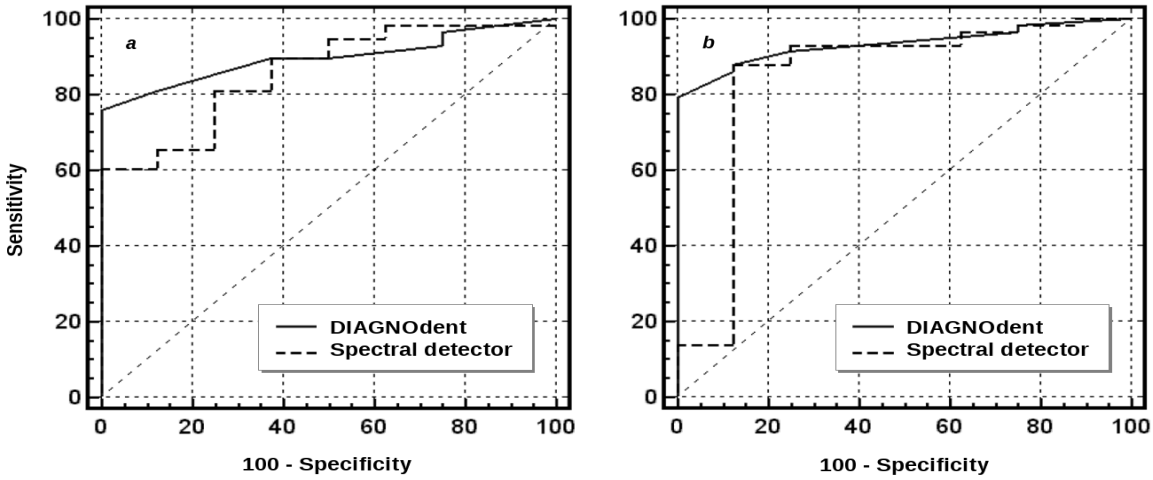

Figure 9: Comparison of relative operating characteristic curves (ROC) for the spectral detector (RAUC) and for DIAGNOdent, in the clinical study (a) and in the laboratory study (b).

In Figure10 one can see the relative operating characteristic curves, ROC, for each device and test. Considering the areas under those ROC curves, there were no statistically significant differences between the techniques, either in the clinical study or in the laboratory study. Table 1 and Table 2 present the parameters obtained in this work for the spectral detector and DIAGNOdent, respectively. The general values of sensitivity and specificity for each device are calculated with the best cutoff points determined. There were no significant differences among the parameters, considering clinical or laboratorial experiments or between the methods.

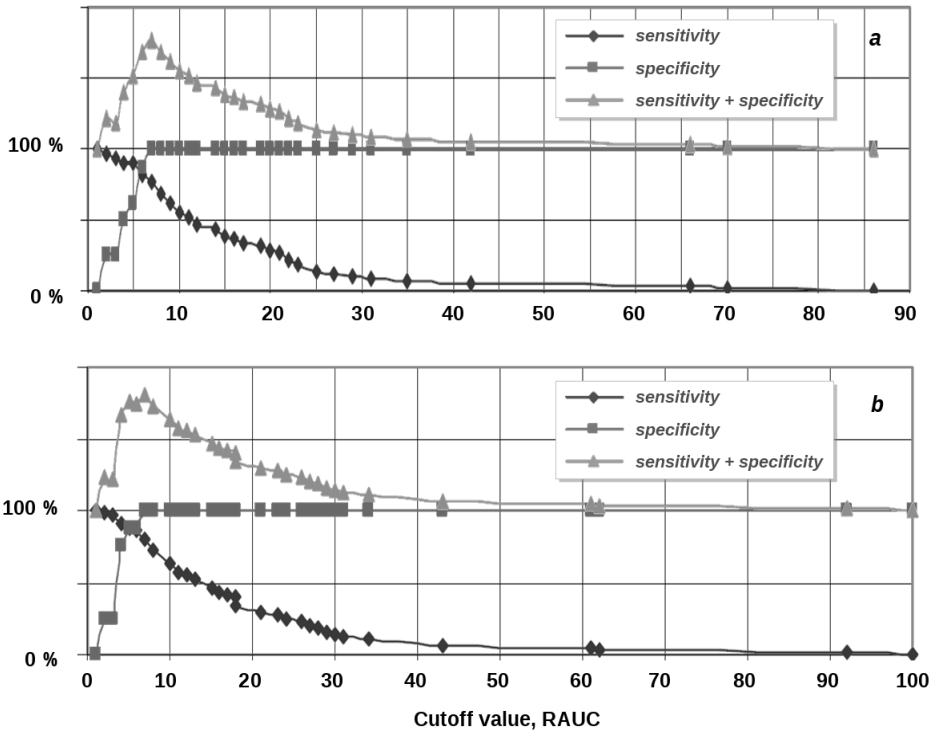

Figure 10: Specificity, sensitivity and their sum, as a function of hypothetical cutoffs, for DIAGNOdent in the clinical study (a) and in the laboratory study (b).

Table 1: Performance of the spectral detector in the studies carried out. It is shown the values of sensitivity, specificity and accuracy for the best cutoff point found as well as the area under the relative operating characteristic curve.

	Sensitivity	Specificity	Accuracy	ROC area
Clinical study	0.60	1.00	0.95	0.86
Laboratory	0.88	0.88	0.85	0.85

Table 2: Performance of DIAGNOdent in the studies carried out. It is shown the values of sensitivity, specificity and accuracy for the best cutoff point found as well as the area under the relative operating characteristic curve.

	Sensitivity	Specificity	Accuracy	ROC area
Clinical study	0.76	1.00	0.97	0.90
Laboratory	0.79	1.00	0.98	0.93

4. DISCUSSION

DIAGNOdent uses laser beam excitation (1 mW at 655 nm), however delivered by a single central optical fiber and the infrared fluorescence is collected back through nine peripheral fibers. There are interchangeable probes for the collection of the fluorescent light, with converging ends for occlusal surfaces or straight for smooth surfaces. In addition, DIAGNOdent is designed to simultaneously acquire the intensity of scattered excitation light and the fluorescence peak signal so as to display a normalized value of fluorescence intensity [23]. In our study, DIAGNOdent has presented sensitivity of 76% in vivo and 79% in vitro. The specificity and the area under the ROC curve showed no statistically significant differences in all essays, as expected considering previous works [27].

The spectral detector has presented sensitivity of 60% in our clinical test and 88% in the lab test. The lower sensitivity during the clinical test can be justified by considering the modest sophistication of the used handpiece and its not-so-optimized ergonomics, compared for instance with DIAGNOdent. Another meaningful difference between the techniques and procedures is the use of absolute values in our measurements with DIAGNOdent and relative values in our measurements with the spectral detector. In the latter, we considered the relationship between the area of spectra obtained from sound and carious tissues. Moreover those spectra were not obtained simultaneously. So, variations in the relative position of the fiber on the surfaces may have contributed to the lower sensitivity in our clinical study. In the laboratory tests, probably due to the better positioning in the measurements, the spectral detector has shown very high sensitivity. Other possible explanation is related to storage of the samples after teeth extraction. It has been demonstrated that storing solutions can cause a decrease in the fluorescence of caries lesions [28]. Nevertheless, no significant differences were observed in our study.

The obtained results in the clinical tests, for both techniques, should be interpreted with caution. Due to particularities of this study, the operator performed only a single measurement with each device. Similar research works have proposed an average of multiple records from the same site analyzed [9]. Moreover, in the present study the techniques have presented high specificity and moderate sensitivity, possibly influenced by the characteristics of the group of teeth selected (premolars) and the low severity of caries lesions in those teeth.

We have demonstrated the ability to differentiate between healthy and damaged tissues by using both optical methods studied. The minimum damage in enamel detected in the samples, which we verified by using the spectral detector, had a depth of approximately 300 μm. The correlations between optical measurements and the depths of the lesions were moderate however statistically significant (~ 0.4). This finding agrees with previous studies [29, 30].

By using the developed spectral caries detector, it is possible to store measured fluorescent spectra for future use, an interesting feature, since it has been reported that the fluorescence spectral signature from decayed tissues exhibit particular changes compared to sound tissues [18]. This ability could, for instance, be useful in monitoring caries lesions to investigate the efficacy of non-operative treatment in arresting initial caries lesions.

5. CONCLUSIONS

In this work, we have demonstrated the development of a simple and portable caries detection system for clinical use based in laser-induced fluorescence, from common commercial parts. Its design and methodology allows the use of a broad extent of spectral data coming from the tissue. We considered the ratio between the areas under the fluorescence spectra, from 700 nm to 900 nm, of sound and damaged tissues. Such methodology aimed to demonstrate the validity of measurements with the developed device considering the reference standard, histology. In our study using the developed apparatus, we have obtained same performance as that from a similar commercial device. As the spectral detector acquires a complete recording of the spectrum from each tissue, the device allows carrying out additional qualitative analyses and it could be used for monitoring the caries lesions.

6. ACKNOWLEDGEMENTS

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