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Early diagnosis of tooth decay using fluorescence and polarized Raman spectroscopy

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Teeth fluorescence measurements are widely used in teeth decay researches, but less in clinical practice. The fluorescence spectrum strongly depends on many experimental factors. The tooth enamel must present a Raman spectrum with strong polarization anisotropy. Carious lesions of the enamel will produce an alteration of local symmetry and will increase much more scattering of light. This will reduce the anisotropy of the Raman spectra. The 959 cm^{-1} Raman peak has a high sensitivity to polarization in sound enamel and low sensitivity in carried enamel. Thus, Raman polarized spectroscopy could be a useful method to help fluorescence measurements in early detection of teeth caries.

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1. Introduction

Decay is not a problem in the first stage when it can be reversed. When enamel demineralization takes place, minerals will be replaced mainly by water. Thus the light path in the tooth substance will change. It will result in reduction of light absorption by enamel. Early detection of caries would enable the dentist to enhance remineralization and conservation of the tooth substance.

Many investigators have observed that human teeth fluoresce when irradiated with ultraviolet light. Fluorescence has earlier been used on patients for quantification of changes in early enamel caries lesion on teeth. The data on the spectroscopy of teeth are limited and very dispersive. Most of papers reports blue emission, few papers report also green[1] emission or yellow[1]. DIAGNOdent, the newest clinical system in caries detection by fluorescence, illuminates the tooth surface with pulses of red laser light and analyzes the emitted fluorescence. That means the most promoted instrument in dental clinics is measuring red fluorescence.

The patented laser fluorescence method of KaVo DIAGNOdent works on the basis of the differing fluorescence between healthy dental substance and diseased dental substance. Changes in the mineral content and porosity of the tooth surface result in a change (**increase**) in the pattern of fluorescence. Red wavelength of the laser used suggests the instrument measures the porphyrin fluorescence, which is a bacterial product.

Most of the investigations in the field of caries research have been related with quantitative laser-induced fluorescence-QLF[2]. Several studies in which an argon laser light source (488 nm!) was used to examine smooth enamel surfaces have shown a strong correlation between a **decrease** in fluorescence and the degree of enamel demineralization. QLF detects the decrease of the fluorescence radiance without identifying the spectral shape changes.

Many researchers measure blue emission under UV excitation, named autofluorescence. This fluorescence is "natural" fluorescence of healthy teeth. Variability of reported data comes from the variability in light sources used for fluorescence excitation (nitrogen laser, violet or blue DL, argon laser, Xe lamp with filters, blue LED) and from the variability in teeth used like sample.

Tooth enamel is the most mineralized tissue of human body. Its composition is 96 wt. % inorganic material and 4 wt. % organic material and water. In dentin, the inorganic material represents 70 wt. %. This inorganic material is mainly composed by a calcium phosphate related to the hexagonal hydroxyapatite, whose chemical formula is $[Ca_{10}(PO_4)_6(OH)_2]$[3]. Analysis of enamel and dentin also indicated the presence in small quantities of other elements such as Na, Cl and Mg.

Changes in Raman spectra were observed in PO_4^{3-} vibrations arising from hydroxyapatite of mineralized tooth tissue[4]. Examination of various intensities of the PO_4^{3-} vibrations (431 cm^{-1}, 590 cm^{-1}, 1043 cm^{-1}) showed consistent increase intensities spectra of carious lesions compared to sound enamel. The spectral changes are attributed to demineralization-induced alterations of enamel crystallite morphology and/or orientation. This

hypothesis is supported by reduced Raman polarization anisotropy derived from polarized Raman spectra of carious lesions.

Earlier polarized Raman spectroscopy was used to study the orientation of tooth enamel rods[5]. The majority of enamel rods have one orientation in a tooth and this orientation could be changed in the carried regions.

2. Methodology

2.1 Samples

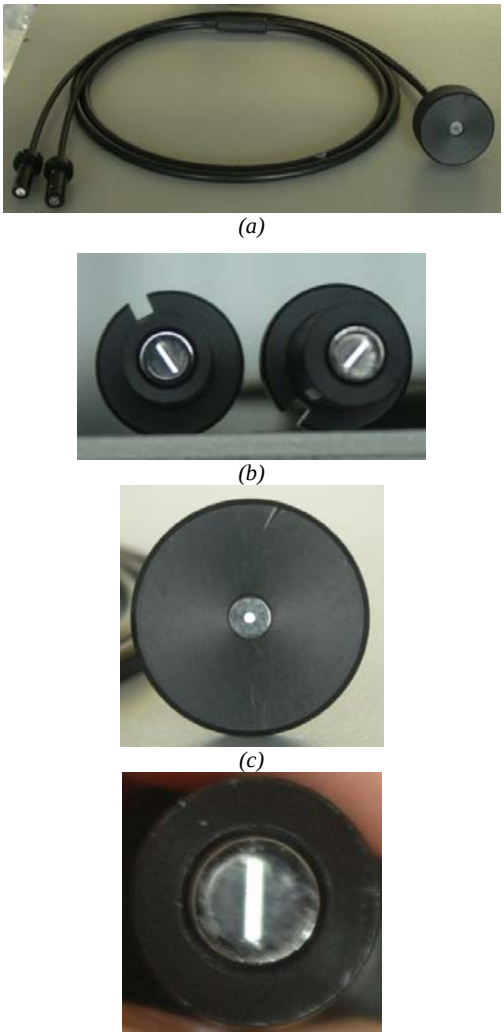
The measurements were made on extracted teeth (*ex-vivo* measurements). Teeth, without dental restorations, were selected to ensure the presence of questionable occlusal caries. Each tooth was tested for occlusal caries. The ten teeth were sectioned using a high speed dental handpiece and diamond bur, and then visually analyzed for the presence of caries. The presence or absence of dental caries was compared to the diagnoses of the dentist and Raman spectroscopy.

Teeth were visually examinated using an optical microscope.

Micro-Raman spectra were recorded on transverse sectioned teeth permitting subsurface evaluation of ultra-structural effects on enamel and dentin.

2.2 Fluorescence measurements

The teeth fluorescence data collected from literature are quite different depending of the geometry of the used system and of experimental conditions. Most of measurements are made *ex-vivo* using stationary ("classical") spectrofluorometers. Thus the excitation beam has a large enough size depending of the slit, but enough to irradiate a large area of the tooth. The collection of the emitted radiation implies also a larger area which covers different states of tissue, sound and carried enamel and dentin and a different relief. Many papers present measurements made by laser excitation. The high power of the laser focused in a small area has a deep penetration and produce a mixture of enamel and dentine light emission. The high power can also produce excitation of uncontrolled impurities present at the surface of the tooth. *In-vivo* measurements are most complex.



(a)

(b)

(c)

(d)

Fig. 1. The ends of the optical fibers bundle.

To perform *ex-vivo* teeth fluorescence it is reasonable to use a classical excitation (Xe lamp plus monochromator) of enough intensity and variable wavelength, a very sensitive detection system and a local irradiation using optical fibers. We have used a 2 way optical bundle of 200 μm each, from Skin Skan spectrofluorometer, coupled to spectrofluorometer FluoroLOG 3-22, from Jobin Yvon.

The bundle is presented in fig. 1.a. The optical coupling was done using a fiber platform as follow: one free end of the bundle (Fig. 1 (b)) to the excitation monochromator and the other end to the emission monochromator. The measuring termination of the bundle (Fig. 1 (c)) has mixed fibers (excitation and collection). The photo-irradiated area of sample is around 1 mm.

The optical fiber bundle was mounted in a special stage with micrometric screw (Fig. 2). This set-up was useful to perform measurements in different points situated on a straight line. It is like unidirectional scanning.

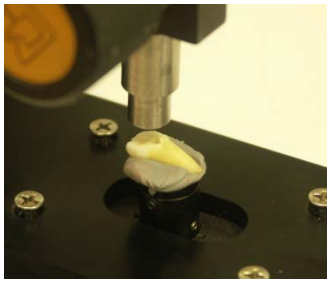


Fig. 2. The set-up for linear scanning of tooth fluorescence.

The excitation wavelength for all fluorescence measurements reported here was 390 nm.

2.3. Polarized Raman microscopy

The Raman measurements were performed with a Raman microspectrometer T64000 HORIBA Jobin Yvon, operating with the 647.1 nm excitation line from an Ar^+-Kr^+ laser (Coherent Innova-70) and confocal microscope Olympus (objective x50LF). The experimental set-up for polarization measurements was described in a previous paper[6]. The measurements were carried out only for two perpendicular directions of laser polarization plane, provided by a half-wave plate rotation.

The sectioned tooth sample was placed on the microscope slide looking for the laser beam to be approximately normal to the studied surface. The microscope stage XY displacements were accurately controlled with motorized micrometric precision screws. Thus we could realize a linear scanning of the analyzed surface (Fig. 3).

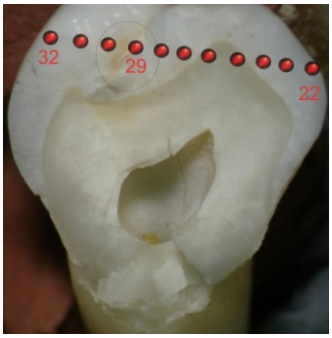


Fig. 3. Image of the sectioned 25 tooth reveals points used for Raman measurements in a linear scanning.

3. Data

3.1 Fluorescence spectra

Sample 56

It is a transversal section on a tooth with serious occlusal caries, depth penetrated in dentin. The measurement of fluorescence was done point by point unidirectional scanning with 1 mm step (Fig. 4). As can be expected the spectra are dramatically changed in various areas (spectra 3 and 4 of Fig. 4).

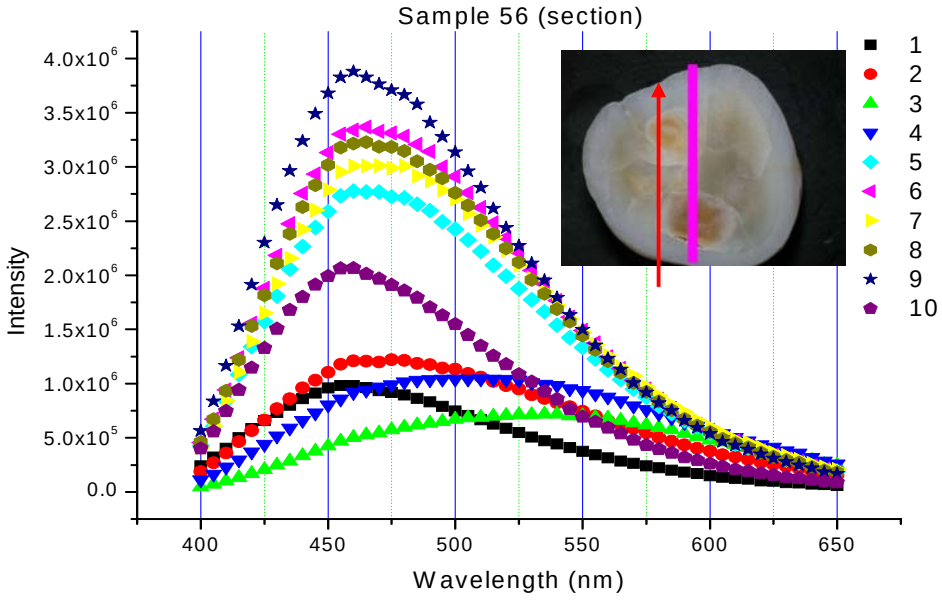


Fig. 4. Fluorescence spectra of sectioned sample 56 in different points of linear scanning.

The sound tooth spectrum consisted of a broad band (460 nm maximum) of fluorescence. The emission band has a large asymmetry which suggests (from deconvolution) presence of at least one secondary maximum at 490 nm. The carious tooth (brown area) exhibited a large emission band with green (500-530 nm) located maximum. This intensity peak decreased further for all carious areas depending on the carious stage too. More important is the half-bandwidth of the emission band is nearly double for cavity area than for health area, 175 nm versus 100 nm.

Sample 25 was measured with optical fiber by unidirectional scanning with 1 mm step too, in the arrow direction (Fig. 5). Scanning direction has passed through sound enamel, incipient decay, dentin and pulp. Spectra 7 and 8 has been measured on the external surface of the tooth, in crown enamel and, respectively, in pulp.

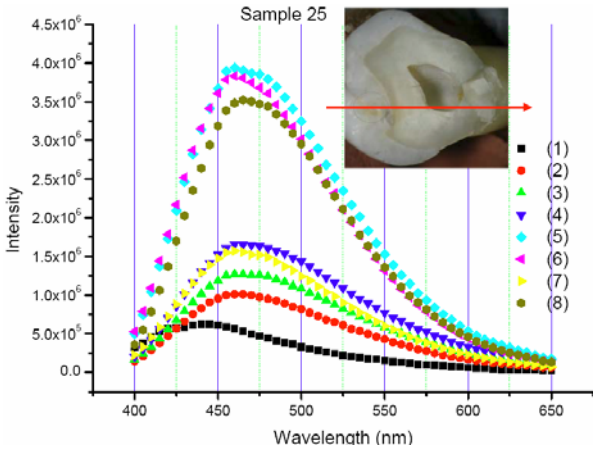


Fig. 5. The fluorescence spectra of different points of section in sample 25.

The maximum emission is 460 nm located too. The incipient decay in the circle indicated area (occlusal), weak yellow colored, presents a low intensity and large band of fluorescence. The green component (>500 nm) has also a major contribution reported to the blue component.

3.2 Polarized Raman spectrum of the tooth

Polarized Raman measurements were done on sample 25. The carious lesion appears like a weak yellow region deep in the enamel. Figs. 6, 7 and 8 show polarized Raman spectra of the sound enamel, dentin and carious lesion, respectively, of the same tooth. The carious lesion is deep subsurface at enamel-dentin interface and not easily observed.

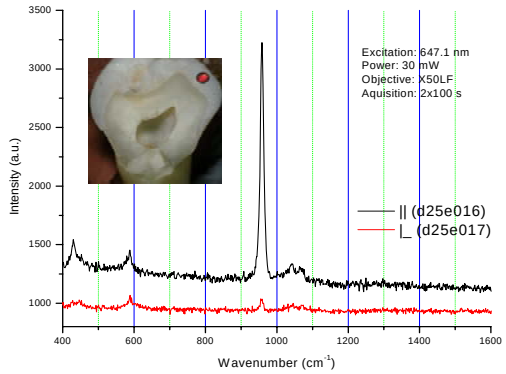


Fig. 6. Polarized Raman spectrum of sound enamel (D25 tooth).

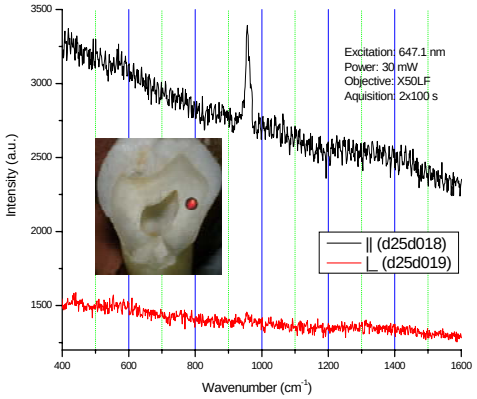


Fig. 7. Polarized Raman spectrum of dentine (sample 25). The background looks like a polarization sensitive fluorescence.

The background of the Raman spectrum measured on the dentine indicates fluorescence too. Raman peaks are hardly observed. The Raman spectrum of the carious enamel (Fig. 8) presents a high level background compared to normal enamel. This background is also many times higher than highest Raman peak of sound enamel. That means we have found at the same time two phenomena:

- decreasing of the Raman peaks because of the local disorder produced by decay process,
- strong increasing of the background because of the fluorescence process.

The increased red fluorescence in the carious enamel origins in the presence of organic waste of bacteria which produce the decay process. All the samples were cleaned and washed by standard procedure to be safely for manipulation. This process affected only external surface of the teeth. We suppose the cleaning solution did not penetrate the small hole of the occlusal caries.

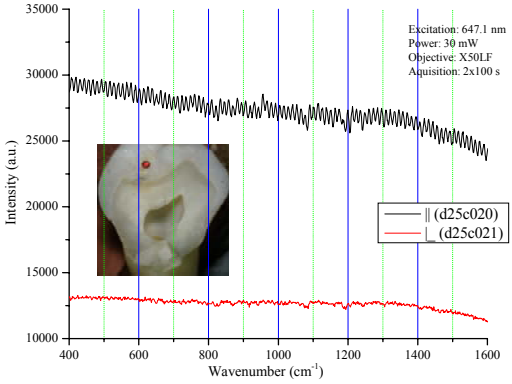


Fig. 8. Polarized Raman spectrum of carious enamel (sample 25).

4. Results

Micro-Raman spectral characteristics were compared for different positions on the tooth surface and for both polarization directions with peculiar attention to the mineral PO_4 most sensitive to polarization 959 cm^{-1} band. This vibration is characteristic of hard tissue integrity. In some conditions PO_4 ion could be substituted by CO_3 ion, decreasing the intensity of symmetric modes.

The ratio of depolarization of this band was calculated according the relation:

$$r = \frac{I_{per}}{I_{par}} \quad (1)$$

where I_{per} and I_{par} are the peak intensities of the 959 cm^{-1}, measured with the analyzer oriented perpendicular and parallel, respectively, to the polarization of the laser beam emitted by the sample.

We have calculated and compared the ratio of depolarization for the sound enamel, for the dentine and for the carious enamel. Fig. 9 shows the ratio of depolarization for *ex-vivo* measurements.

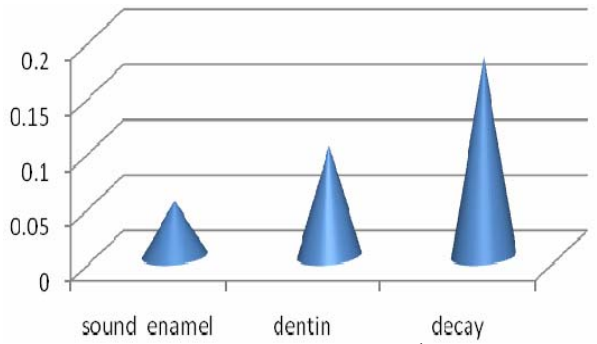


Fig. 9. Depolarization ratio for 959 cm^{-1} Raman peak (sample 25).

There is a normal difference between depolarization ratio of sound enamel and dentine. Dentine with a less crystallized structure has a stronger depolarization. Any change in the hard tissue of the enamel produced by caries will results in increasing the depolarization ratio. Other Raman frequencies do not exhibit significant alteration in the spectral profile with change in orientation.

5. Conclusions

The polarized Raman spectroscopy is a potential tool to help fluorescence in discover and characterize of early dental caries. The early stages of the decay alter the crystals orientation with results in polarization of the 959 cm^{-1} Raman band. Demineralization will increase the scattering of the light too. On the other hand, decay process increases the amount of unorganized material which generates fluorescence. As consequence, it is more difficult to measure the Raman spectrum.

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