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# DIAGNOSIS OF TOOTH DECAY USING POLARIZED MICRO-RAMAN CONFOCAL SPECTROSCOPY*

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*Abstract*. The tooth enamel presents a Raman spectrum with strong polarization anisotropy. Carious lesions of the enamel will produce an alteration of local symmetry. This will reduce the anisotropy of the Raman spectra. Thus micro-Raman polarized spectroscopy could be a used in early detection of teeth caries.

*Key words*: Raman, tooth decay, polarization.

## 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].

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 $[Ca_{10}(PO_4)_6(OH)_2]$ [2].

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Changes in Raman spectra were observed in $PO_4^{3-}$ vibrations arising from hydroxyapatite of mineralized tooth tissue [3]. 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 [4]. 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 hand piece 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. 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 ×50LF). The experimental set-up for polarization measurements was described in a previous paper [5]. 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 (Fig. 1).

Fig.1 – Sample on the stage of the Raman microscope.

## 3. DATA

### 3.1. POLARIZED RAMAN SPECTRUM OF TOOTH

First polarized Raman measurements were done on sample 25. The carious lesion appears like a weak yellow region deep in the enamel. Figure 2 shows polarized Raman spectra of the sound enamel. The carious lesion is deep subsurface at enamel-dentin interface and not easily observed.

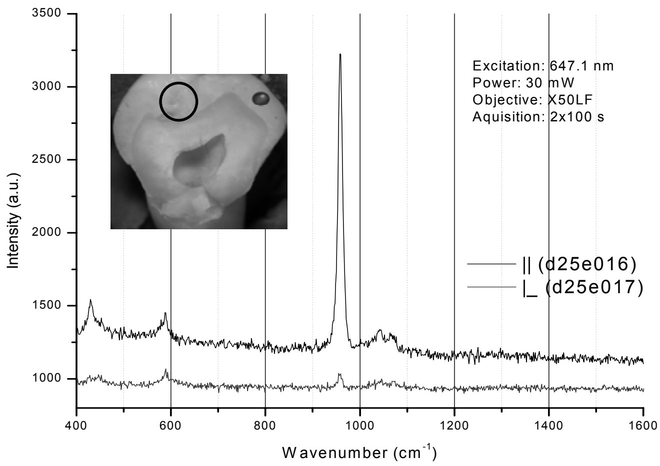


Fig. 2 – Polarized Raman spectrum of sound enamel (D25 tooth).

Micro-Raman spectral characteristics were compared for different positions on the tooth surface (dentin and carried enamel) 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. Using data reported in a previous paper [5] we have calculated the ratio of depolarization of this band like the ratio between $I_{perpendicular}$ and $I_{parallel}$ It means ration between 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 obtained the values showed in Table 1.

*Table 1*

Depolarization ratio for 959 $cm^{-1}$ Raman peak

| Sound enamel | Decay |
|---|---|
| 0.05 | 0.18 |

Any change in the hard tissue of the enamel produced by caries will results in increasing the depolarization ratio. Bands of regions $\nu_3$, $\nu_2$ and $\nu_4$ do not exhibit significant alteration in the spectral profile with change in orientation [6]. On the other hand, there are some changes in relative intensity of the bands which can be attributed to their complex structure (they have not a pure symmetry).

## 4. RESULTS

We have applied the method of depolarization ratio on the study of the surface of other teeth: sample D27 and sample D33. Both sample was sectioned to have easy access to small cavities difficult to be seen from outside.

Sample D33 (Fig. 3) was exposed to the laser beam with the external enamel, the plane sectioned part being fixed on the stage of the microscope. Because of the curved surface of the tooth we have tried to expose different points where the incident laser beam was normally to the surface. Unfortunately the Raman spectrum depends on the angle between the incident laser beam and the surface irradiated.

Sample 33 is an example of difficult decay diagnose because demineralization process developed more inside the tooth, under the free surface of enamel. On the tooth surface the presence of the decay could be seen as a small brown point.

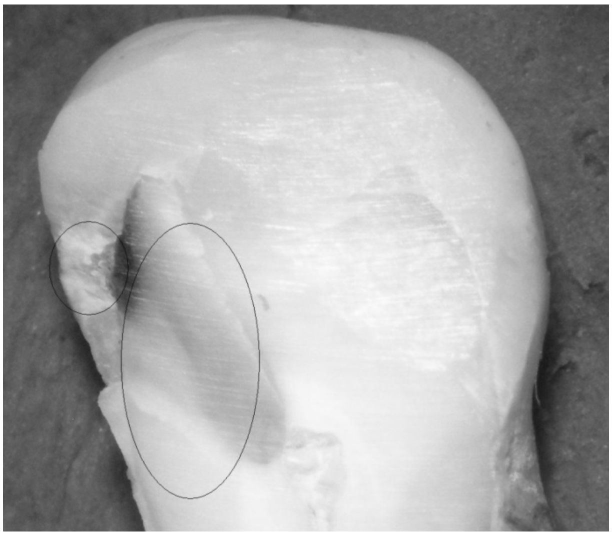

Fig. 3 – Sample D33 is a section in a pre-molar. The decay is deep in dentin under the enamel.

Raman spectrum of sound enamel, measured with the analyzer oriented parallel to the polarization of the laser beam, shows very fine peak profile of 959 $cm^{-1}$ vibration both at external surface enamel (045) and sectioned enamel (046). In the center of decay (small circle from Fig. 3) the luminescence was strong enough to cover all Raman peaks (047 in Fig. 4). Repeated measurement in the region of diffused decay (big circle from Fig. 3) shows yet a strong enough luminescence to block observing the Raman peaks, but smaller than in the center of tooth decay (048, in Fig. 4). We have repeated such measurement in points located far away and the luminescence signal decreases more and more.

The Raman spectrum of the carious enamel (Fig. 4) 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 that 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.

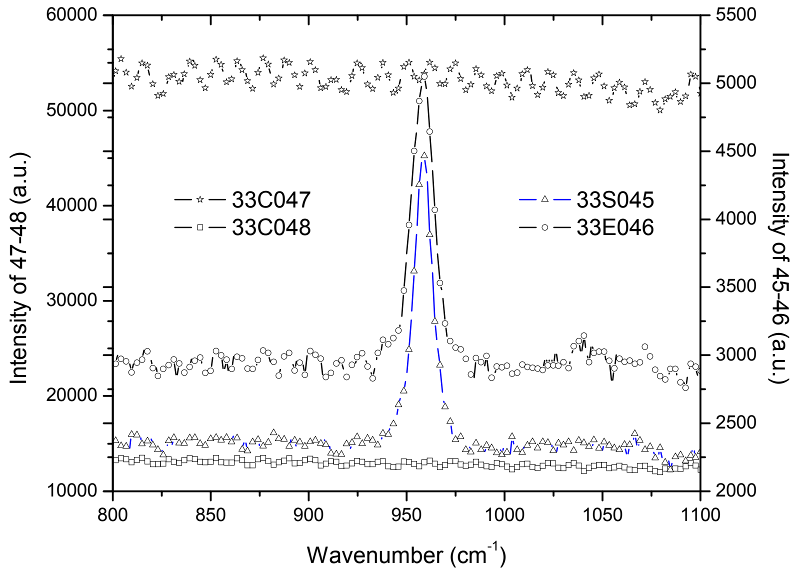


Fig. 4 – Polarized Raman spectra ($I_{\|}$) of sample D-33 measured in different points: 45 – external sound enamel, 46 – section on sound enamel, 47 – center of the decay, 48 – out of center of decay.

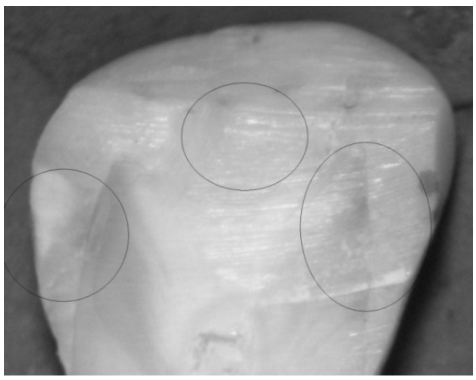

Fig. 5 – Sample D27 is a section in a pre-molar with deep decay.

Next measured sample was a similar premolar with deep decay (Fig. 5). First of all we have measured polarized spectrum of the sound enamel on the tooth longitudinal section. The appearance of spectrum is normal.

Then we have compared the polarized Raman spectrum of the sound enamel with polarized emission of brown area. The strong luminescence covers the possible Raman emission.

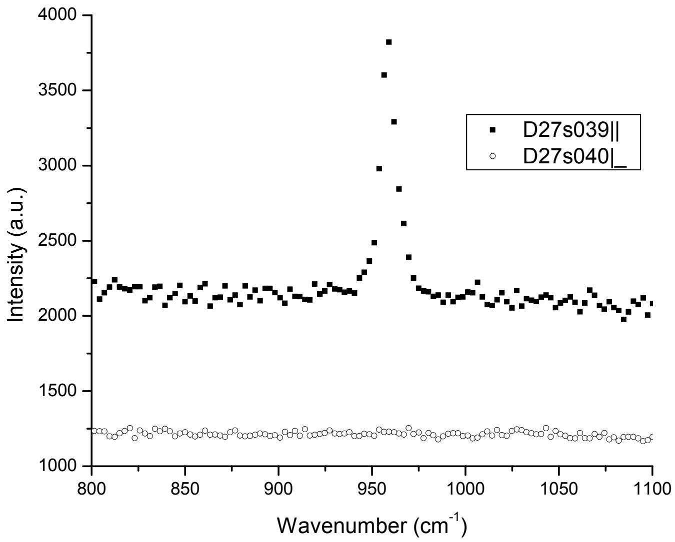


Fig. 6 – Polarized Raman spectra of sound enamel – D27 tooth.

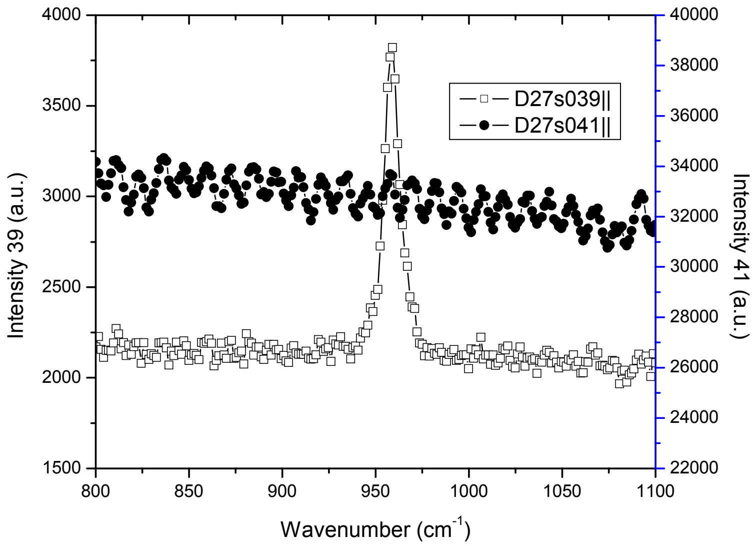


Fig. 7 – Polarized Raman spectra of the sound enamel (39) compared with brown spot enamel (41) – D27 tooth.

## 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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