Micro-Raman spectroscopy for assessment of periodontal disease follow-up

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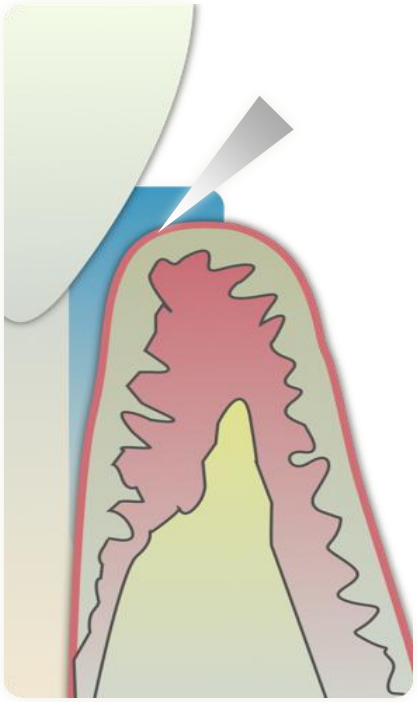
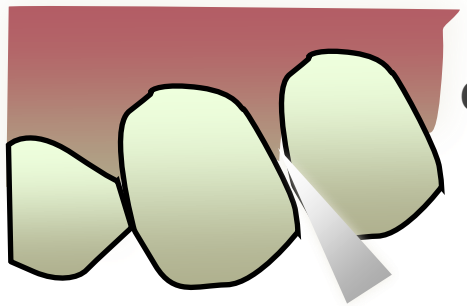
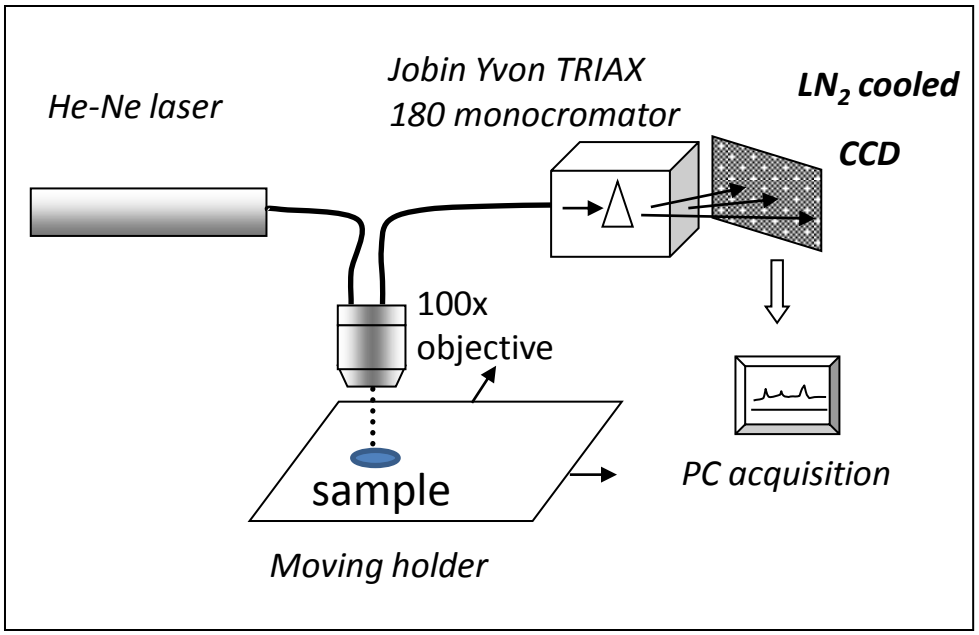
The aim of this study is to investigate the potentiality of the micro-Raman spectroscopy (m-RS) for the follow up of periodontal disease in a not invasive way.



μ-RS was performed on gingival crevicular fluid (GCF). The fluid was pooled from informed periodontal and health patients by using standardized sterile absorbent paper cones inserted 1 mm into the gingival crevice and left in situ for 30 s, without blood, saliva and plaque contamination.



Micro-Raman spectroscopy



Schematic view of experimental set up

The sample is excited by a *He-Ne* laser operating at a light wavelength λ = 633 nm, with a maximum nominal power of 17 mW. The signal is collected by a *Jobin-Yvon TriAx 180* monocromator, equipped with a liquid N_2 cooled CCD and a grating of 1800 grooves/mm, allowing a spectral resolution of 4 cm^{-1}. The laser light is focused on the sample surface by means of a 100 x (n.a. 0.90) optical objective on a excitation area of about 10 μm of size.

The spectra were obtained using accumulation times ranging in 60-300 seconds.

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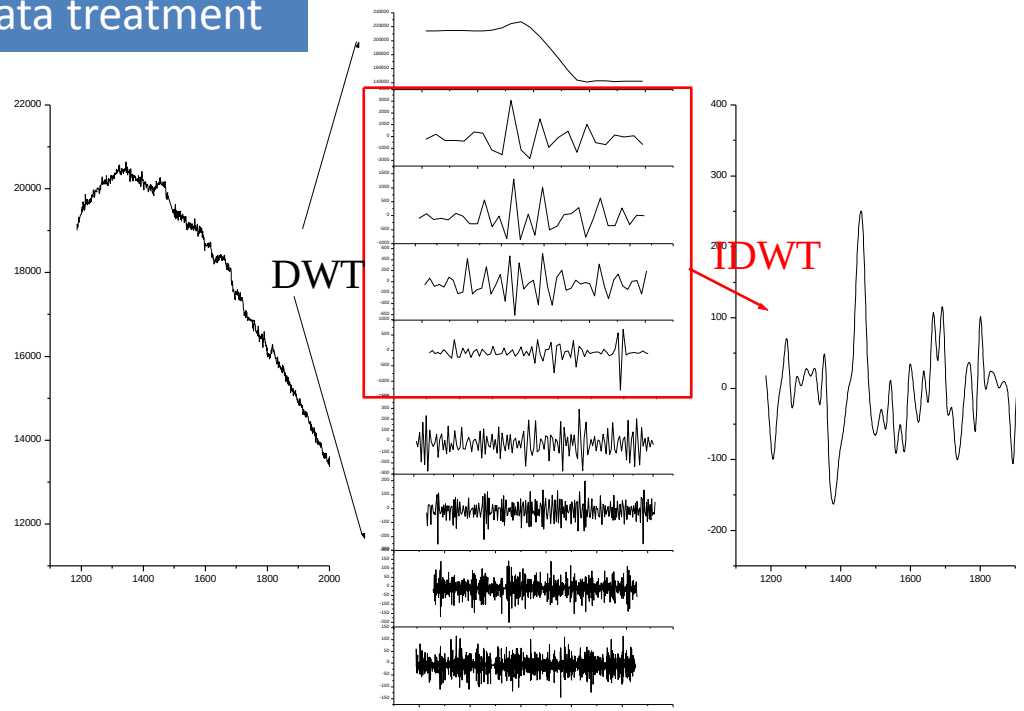
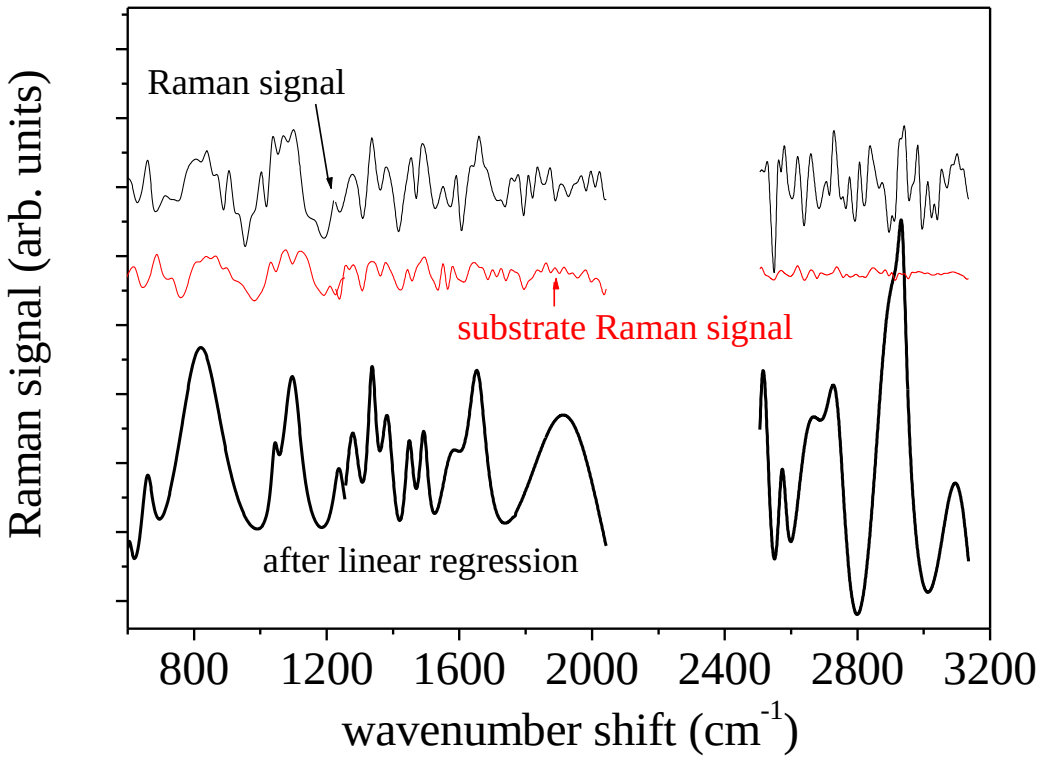
Wavelet based numerical data treatment

The spectra typically show a smeared background signal, with intensity of order of about 80% of the whole average intensity. In order to enhance signal readability and attenuate background and noise components we use a numerical data treatment based on wavelet algorithm [1]. The spectrum signal is cut up into different 'scale' components, by using spatially localized functions with average zero value (namely *wavelets*, small waves) instead of conventional Fourier transform sinusoidal functions.



This make possible to keep information on both frequency and spatial dependence of the signal. Basically the signal is represented in terms of the sum of elementary wavelets and decomposed in two signals, one containing the low frequency components (approximation A) and the other one the fluctuations (detail D). The algorithm is iteratively applied to the "approximated" part of the function and a higher level of A and D component pair is generated. A hierarchical representation of the data set is thus obtained allowing a multi-resolution analysis, know as discrete wavelet transform (DWT). Starting from the decomposed signal, the spectrum can be reconstructed (IDWT) removing low and high frequency components due to spurious background and non-correlated noise signal respectively. MATLAB 6.5 program (by MathWorks Inc.) was used for wavelet analysis with wavelet family of biorthogonal functions "bior6.8" .

[1] Camerlingo, C.; Zenone F.; Gaeta G.M.; Riccio R.; Lepore M.
Wavelet data processing of micro-Raman spectra of biological samples.
Meas. Sci. Technol. **2006** , *17*, 298-303.3



After the wavelet process the Raman signal of the substrate (paper cone) is subtracted from the raman signal of the sample by performing a linear regression of data.

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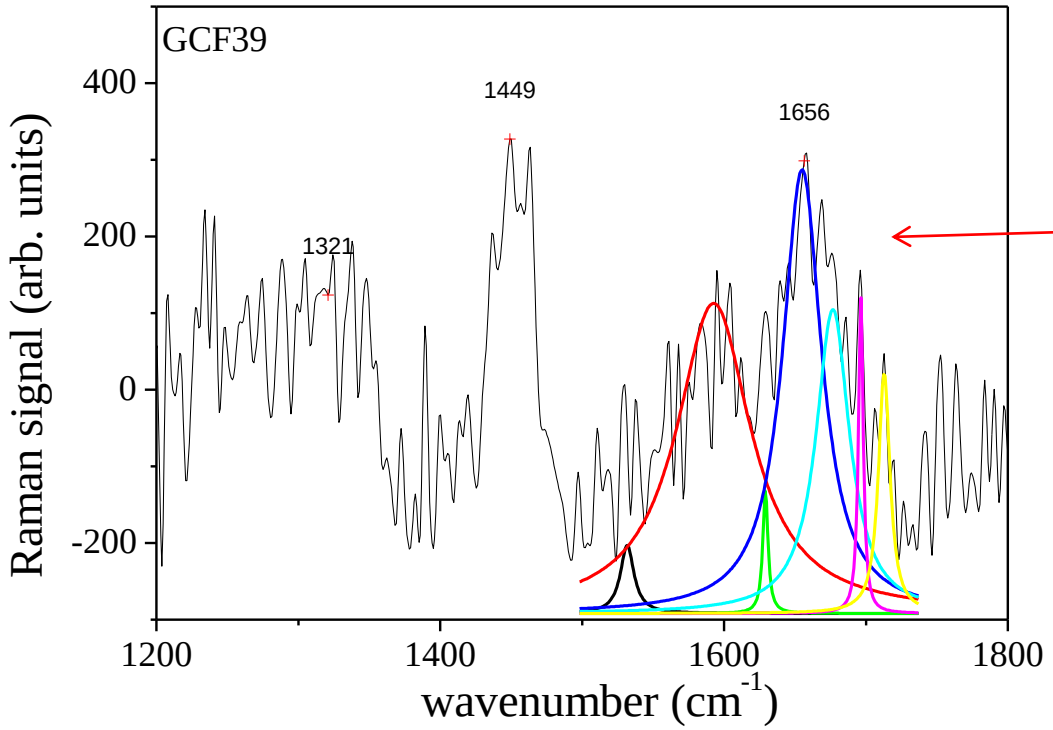


For signal interpretation the spectra were analyzed in terms of convoluted Lorentzian shaped vibration modes. Peaks constituting the spectrum were manually selected in order to define the starting conditions for a best-fit procedure based on the Levenberg-Marquardt nonlinear least-square method to determine convolution peaks with optimized intensity, position and width.

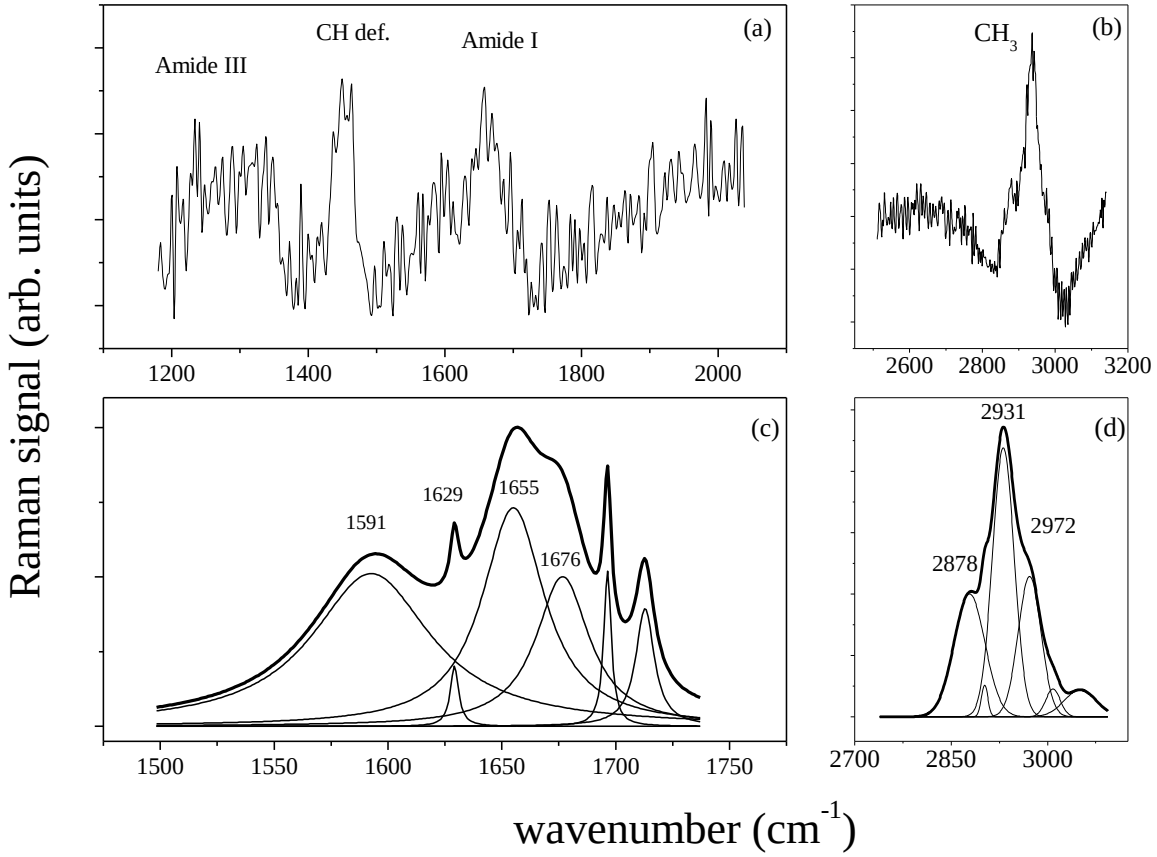
Raman spectrum of GCF from a health patient.
Protein contribution to spectrum are clearly evinced from the peaks at 1321 cm^{-1} (Amide III) , at 1449 cm^{-1} (CH_2), at 1580 cm^{-1} (C-C stretching) and at 1656 cm^{-1} (Amide I).

What we look for…

The level of osteopontin in gingival crevicular fluid has been found to correlate with clinical measure of periodontal disease [2]

Carotenoid concentration in GCF is expected to increase with the severity increase of the disease and in chronic periodontitis, with respect to healthy or gingivitis control. [2-3]

[2] Gonchukov, S.; Sukhinina A.; Bakhmutov D; Minaeva S. Raman Spectroscopy of saliva as perspective method for periodontitis diagnostics. *Laser Phys. Lett.* **2012**, *9*,73-77.
[3] Kim, S.C.; Kim, O.-S., Kim, O.-J.; Kim, Y.-J.; Chung, H.-J. Antioxidant profile of whole saliva after scaling and root planing in periodontal disease, J *Periodontal Implant Sci* **2010,** *40*, 164-171.



Raman spectrum of GCF from healthy patient for the 1200-2000 cm^{-1} (**a**) and 2500-3100 cm^{-1} wavenumber range **(b).** Deconvolution in Lorentzian peaks of the Amide I spectrum region (1500-1750 cm^{-1}) **(c)** and CH_3 region (2800-3000 cm^{-1}) **(d).**

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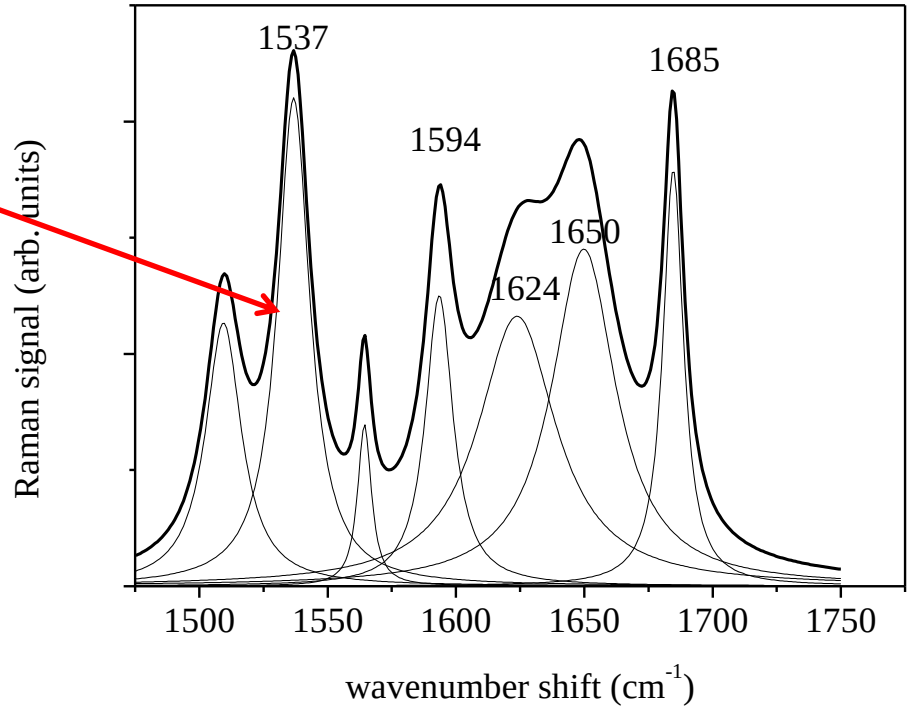
The intense peak at about 1537 cm^{-1} is likely due to the formation of isomerization products containing C=C groups [4] related to an increase of degraded carotene in GCF.



Raman spectrum of GCF from patient affected by chronic periodontitis: Deconvolution in Lorentzian peaks of the Amide I spectrum region (1500-1750 cm^{-1}).

4. Noda, I.; Marcott, C.; Two-dimensional Raman (2D Raman) correlation spectroscopy study of non-oxidative photodegradation of β-carotene. *J. Phys. Chem. A* **2002**, 106, 3371-3376.

Conclusions

☞A not invasive method based on μ-RS of GCF for follow up of periodontal diseases is proposed and tested.

☞ An automatic numerical data treatment based on *wavelet* algorithm was used in order to suppress the uncorrelated signal, to subtract the background signal and to increase the quantitative readibility of the Raman signal.

☞ An increase of degraded carotenoide content in GCF, as a mark of the inflammatory state, is inferred from the Raman spectra of chronic periodontitis affected patients.

☞ Even if a more systematic and wide investigation is necessary to validate the proposed methods, the preliminary results confirm the big potentiality of m-RS for specific molecular fingerprinting in medical applications.