Extending the applicability of Raman microspectroscopy for biological samples using statistical analysis and plasmonic effects

M. Marro[1], S. Balint[1], S. Raj[1], S. Rao[1], D. Petrov[1,2]

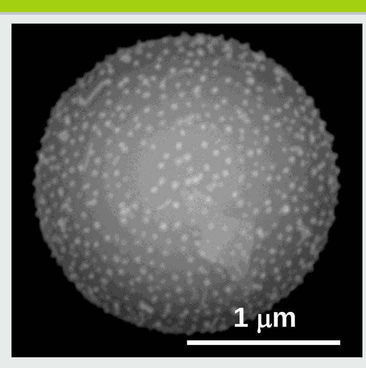
[1] ICFO – The Institute of Photonic Sciences, Mediterranean Technology Park, Castelldefels (Barcelona), Spain

[2] ICREA – Catalan Institute of Advanced Studies, Barcelona, Spain

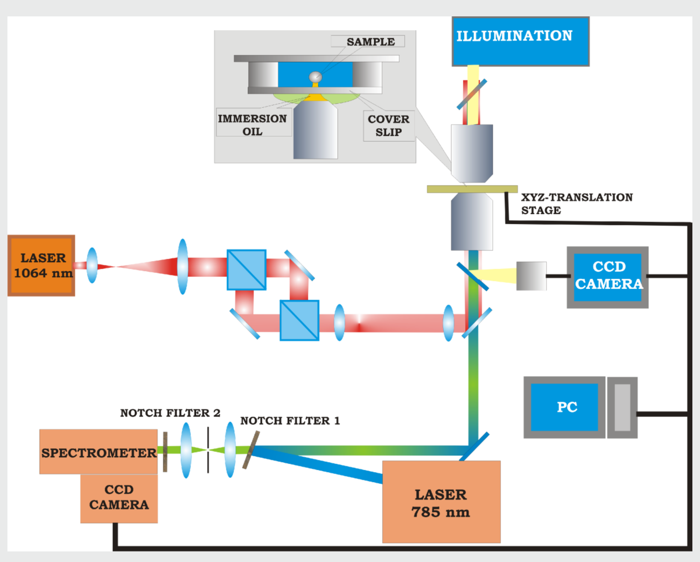
ABSTRACT

Raman spectroscopy is a promising technique in biomedical and biophysical studies due to its non invasive character and specificity. However, micro-Raman spectra of biomedical samples are inherently complex and weak. The combination of SERS with statistical techniques like 2D correlation analysis can improve the applicability of Raman spectroscopy in biomedicine and biophysics but it has yet not broadly investigated. Specifically, to the best of our knowledge, we present in this poster the first study where the 2D correlation analysis is applied to SERS obtained from biological samples. Two examples demonstrate the effectiveness of our approach.

BACKGROUND



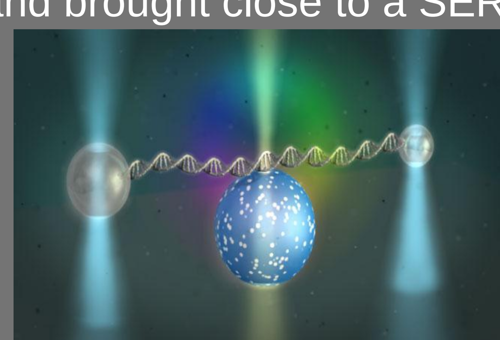
The SERS probes [1] are 2 μm silver coated silica beads covered with pMBA molecules that are embedded in cancer cells (U87-MG) through overnight incubation



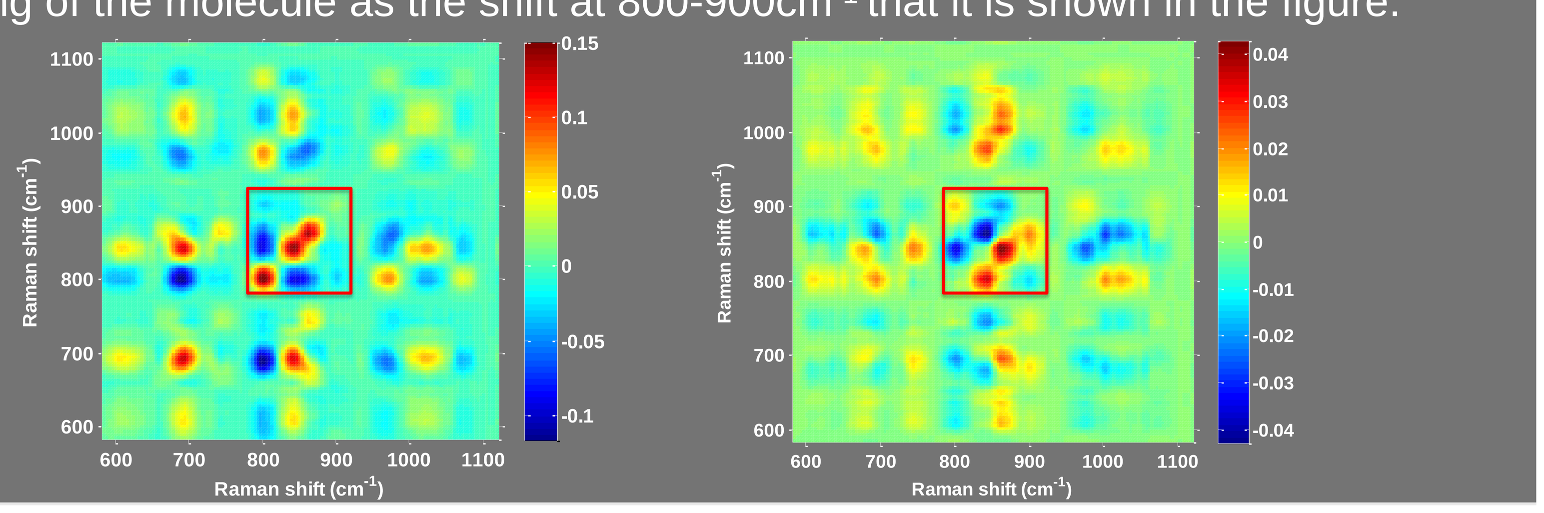
A confocal Raman system is used with a 785 nm (< 10 mW) beam passing through a 1.3 NA, 100X immersion oil objective, to excite the probes and collect the spectra. PDT treatment is achieved by a 40X objective with 532 nm laser beam.

SINGLE DNA MOLECULE STRETCHING

A DNA molecule that is anchored between two dielectric beads (one attached to the substrate and another optically trapped) is suspended in a solution following the procedure described in [2] and brought close to a SERS probe.



The Raman spectra are acquired for different stretching of the molecule by moving the optically trapped bead. By means of 2D correlation analysis of the data we are able to discover new correlated changes in intensity and position that appear during the stretching of the molecule as the shift at 800-900cm^{-1} that it is shown in the figure.



CONCLUSIONS

- The results showed great improvements in the studies and diagnosis of the biological samples with Raman microspectroscopy.
- The analysis revealed useful and other-wise inaccessible information about the dynamics of the processes and the composition of the samples investigated.

REFERENCES

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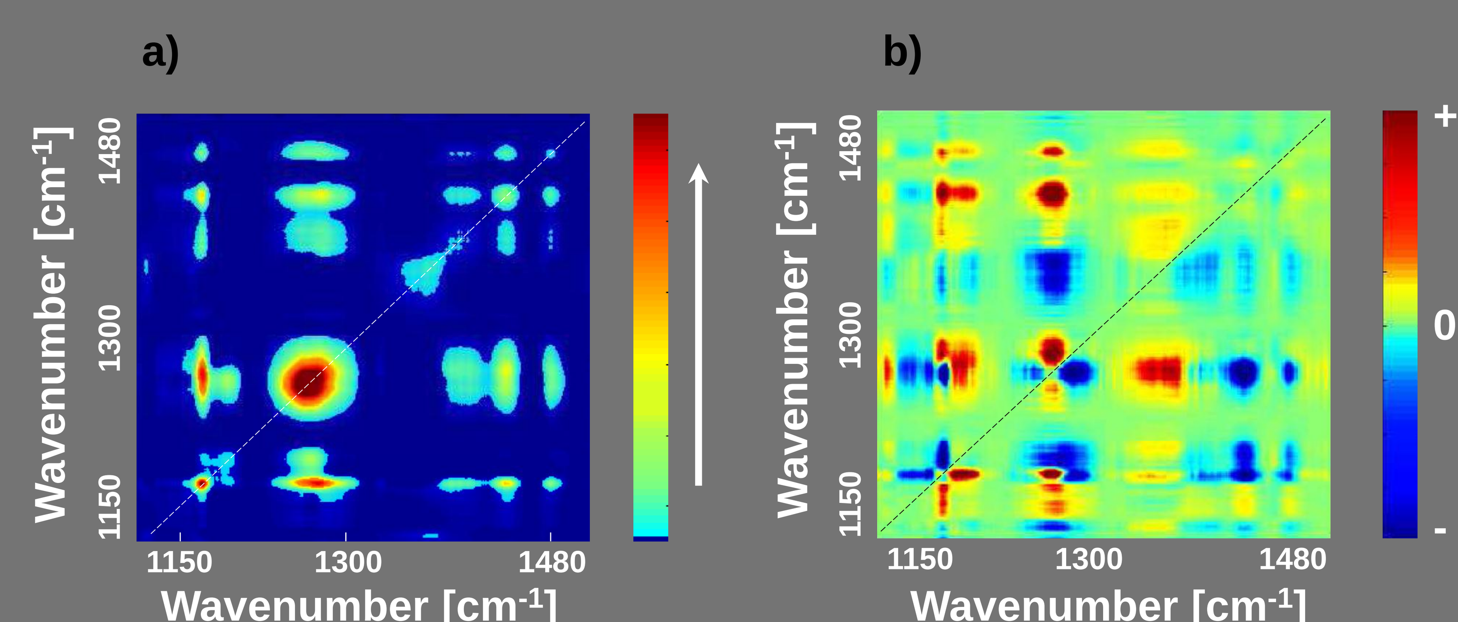
pH MONITORING IN LIVE CANCER CELLS

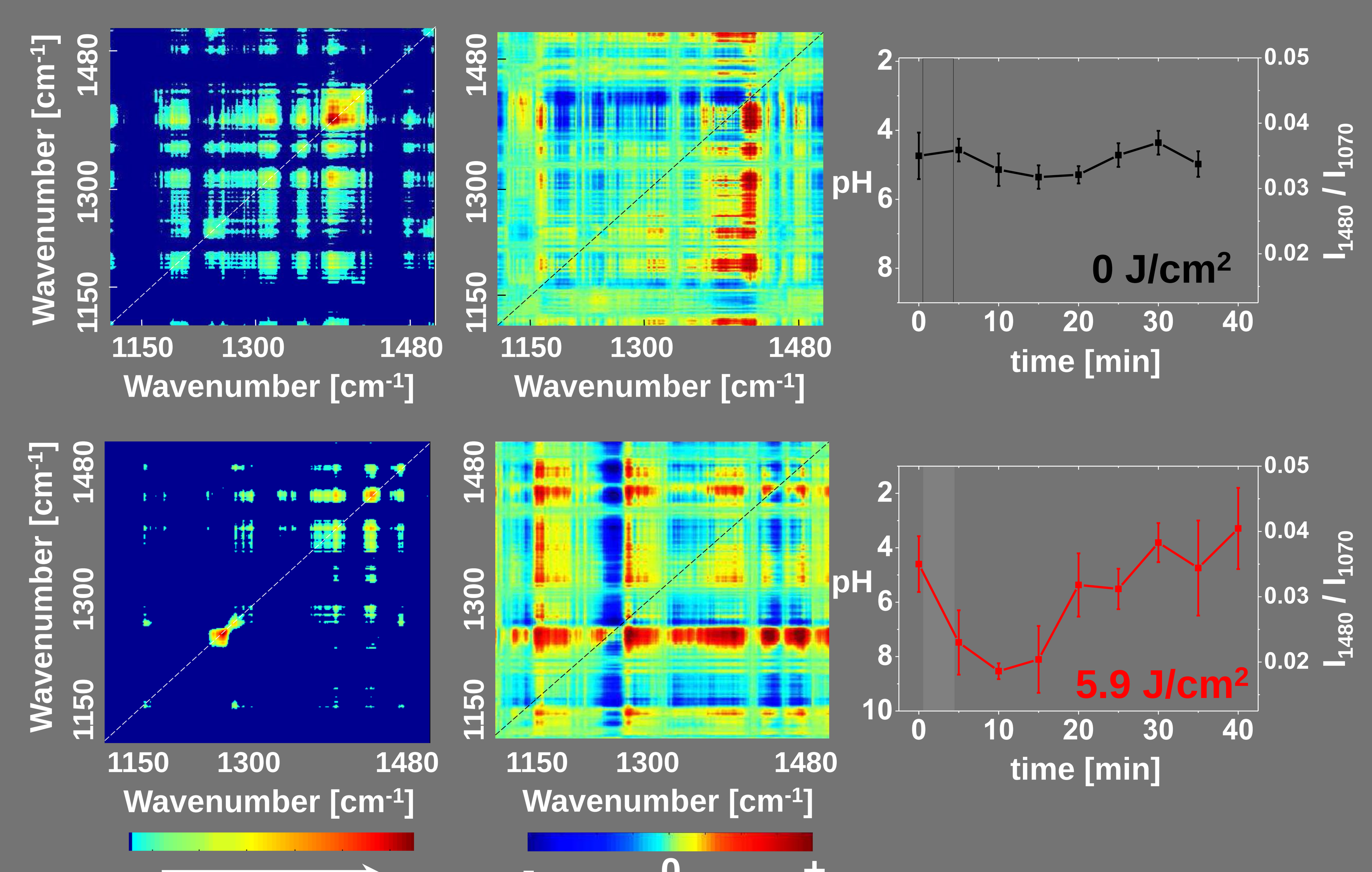
Monitoring the fluctuation of pH in a single cell before, during and after photodynamic therapy treatment plays an important role for characterization of cell death induced by photosensitizer molecules. We use surface-enhanced Raman scattering (SERS) probes covered with a pH sensitive linker molecule (4-mercaptobenzoic acid). Probes were embedded in a live cancer cell, to monitor the pH induced by emodin, a photodynamic therapeutic (PDT) drug. The results are analysed using 2D correlation spectroscopy [3].

To monitor the pH fluctuation inside the cancer cell, the calibration curve from SERS spectra of pMBA molecule at different pH need to be identified. Synchronous and asynchronous 2D correlation spectra were used to prove that SERS band at 1480 cm-1 is the best one to be used as a pH marker in the range of pH from 2 – 8.



Synchronous (a) and asynchronous (b) 2D correlation spectra were made from SERS spectra of pMBA molecule adsorbed on SERS probe. Spectra were recorded in pH range from 2 – 8 for one second acqusition time.

Photodynamic treatment was achieved by using the 532 nm laser beam to irradiate the cell with 5.9 J/cm2 light dose. Prior to the irradiation the cells were incubated with 10^{-6}M concentration of emodin for two hours.



From synchronous and asynchronous 2D correlation spectra it is clear that the pH inside the cell is fluctuating (pH ~ 5.5). After PDT treatment the pH drop and the cell dies with the pH increasing again. It is clearly seen from the 2D correlation spectra and also from the relative intensity of the carboxyl SERS band.

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