



PhD Thesis Defense MONICA MARRO SANCHEZ 'Statistical Analysis and Plasmonic Effects to Extend the Use of Raman Spectroscopy in Biochemistry'

MONICA MARRO SANCHEZ

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Friday July 12, 11:00. ICFO Auditorium

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Biophotonics

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Since its discovery in 1928, Raman spectroscopy (RS) has produced a revolution in the fields of analytical chemistry and molecular detection. Thanks to the latest technical advances, the expectations of the applicability of RS in biology have increased. Most recently, RS emerged

as an important candidate technology to detect and monitor the evolution of the biochemical content in biomedical samples non-invasively and with high specificity. However, the inherent properties of Raman scattering have limited its full exploitation for biomedical applications. In the past decade, Surface Enhanced Raman Scattering (SERS) and multivariate analysis have emerged as possible solutions for overcoming the low efficiency and the complexity of the Raman signals obtained from biological material. Until 2009, only a few studies had been reported using multivariate approaches, and these techniques were only employed to group different types of samples. Moreover, although the SERS effect was demonstrated for cells, SERS probes were not used in their full capacity to study complex biological processes inside cells.

This thesis is a step towards combining and using statistical analysis and SERS to expand the applicability of RS in biochemistry: from single molecule to cell and tissue level. This methodology could reveal novel insights, otherwise inaccessible using previous techniques.

Specifically, we began studying the changes in Raman spectra of a single DNA molecule and a RBC under stretching employing optical tweezers. SERS and statistical techniques such as 2D correlation and PCA were used to reveal important structural properties of those biological materials.

An experiment to study intracellular pH changes in glioma cells after Photodynamic Treatment (PDT) was performed by using SERS probes embedded in the cells. The evolution in the SERS spectra was analyzed using 2D correlation. To the best of our knowledge, this study represents the first use of the 2D correlation technique to study biological SERS spectra.

Furthermore, more complex systems were investigated, to reveal the molecular evolution of cells or tissues undergoing a biochemical process. PCA was used to study how lipid metabolism varied in different breast cancer cell lines depending on the degree of malignancy. However, PCA does not provide meaningful components that could be assigned directly to molecular Raman spectra. Consequently, Multivariate Curve Resolution (MCR) was

proposed and applied to extract physically and chemically meaningful molecular components that changed in cancer cells during the Epithelial to Mesenchymal transition (EMT).

We monitored the retina composition ex-vivo when neuroinflammation was induced. Our study was the first application of MCR to decompose and monitor the molecular content of biological tissue with RS. Biomarkers for the early detection of neuroinflammation processes were identified and monitored. This is the first step in establishing of a non-invasive and rapid screening technique for the early detection of multiple sclerosis or other neurodegenerative diseases in patients.

Finally, the flexibility of MCR-ALS algorithm was exploited to remove the presence of background signals in Raman spectra of cytological studies that mask and degrade the results of a statistical analysis. Application of MCR-ALS enabled identification of molecular components that play an important role in the progression of breast cancer cells towards bone metastasis.

This research demonstrated a powerful method that adds a new dimension to the field of analytical chemistry. Sensitive and highly specific information can be extracted non-invasively, rapidly, and without sample preparation. The samples can be monitored in vivo, quantifying molecular components difficult or impossible to obtain with current technology.

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