Evaluating Modulated Excitation and Conventional Methods for Background Correction in Raman Microscopy

M. Hermes[1,2], C. Beleites[1], R. Kiselev[1] A. Dathe[1], C. Krafft[1], J. Popp[1,2]

[1]Leibniz Institute of Photonic Technology Jena, Albert-Einstein-Str. 9, Jena, Germany
[2]Institute of Physical Chemistry and Abbe Center of Photonics, Friedrich-Schiller-Universität Jena, Helmholtzweg 4, Jena, Germany

Applying Raman microscopy to biological samples often suffers from broad spectral background. Sources of background can be found in a variety of physical phenomena such as florescence and scattering [1] as well as creeping currents in the electronics of a detector. Recent computational methods compensate background by fitting smooth baselines or try to model the effects which cause it. A more technical approach was first proposed by Funfschilling and Williams in 1976 [2] named modulated excitation Raman. This technique aims to separate background from signal by slightly shifting the excitation wavelength of the laser. The consequence being that the Raman signal also shifts but the background remains constant, which allows separating shifting components from background. This technique is proposed to be used for biomedical applications [3] but the literature lacs a faire comparison with conventional and more sophisticated techniques such as extended multiplicative signal correction [4].

This project tends to evaluate which of those methods is best for compensating broad spectral backgrounds. For this purpose a full factorial design of experiments is set up for a calibration problem with different levels of background. Furthermore limitations which apply to biological samples are taken into account as well. Therefore a tunable diode laser (DL Pro, center wavelength 785 nm, Toptica Photonics) is chosen and a HoloSpec RXN1 (Kaiser Optical Systems) Raman system is used for detection.

The experimental design is set up as calibration problem of urea in water with five concentration levels and a blank. Three levels of background are realized by adding indocyanine green as fluorescent dye. Furthermore each set of standards is replicated three times. The total of 54 samples is measured in a randomized order. Each sample is measured five times at five wavelengths (steps of 0.45nm ≈ 7cm^{-1} form 783.75 nm to 785.65 nm) which are also randomized in order. A second experiment with a fixed wavelength of 784.5 nm is performed for comparison. Data processing is set up in R. For calibration a Partial-Least-Squares regression is used. Results of modeling are benchmarked by their analytical figures of merit such as limit of detection and limit of quantification.

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