Estimating and Correcting Mie Scattering in Single Cell Infrared Microspectroscopy

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Strong Mie scattering signatures hamper the chemical interpretation and multivariate analysis of infrared microscopy spectra of single cells and tissues. During recent years, several numerical Mie scatter correction algorithms for the correction of Mie scattering in the FTIR spectroscopy of single cells have been published [1-4]. The algorithms employ different approximation formulae and are based on different approaches for retrieval of the pure absorbance spectra. Since it is not clear how the different algorithms are connected and how they perform in comparison, the user is at a loss to decide which algorithm to employ. In this paper, we connect the principles underlying the different algorithms employed. We further introduce a correction algorithm based on EMSC taking into account a complex refractive index. We then simulate spectra according to the exact Mie theory for the scattering of infrared light at absorbing spheres taking into account the high numerical aperture of infrared microscopes employed for the analysis of single cells and tissues. Based on the derived theory we show how the different algorithms perform by retrieving pure absorbance spectra by the different algorithms. The obtained corrected spectra are subsequently compared to the pure absorbance spectra used for simulating Mie scatter-distorted spectra.

References

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