Fast Resonant Mie-scatter Correction Algorithm: Parameter Choice and Validation

J. Solheim[1], T. Konevskikh[1,2], A. Kohler[1]

[1]Department of Sciences and Technology (IMT), Norwegian University of Life Sciences, 1430 Ås, Norway.
[2]MWT Analytics, 1430 Ås, Norway

Infrared spectroscopy of micrometer-sized and approximately spherical structures, such as single cells, are affected by Mie scattering. During recent years, several methods have been proposed for retrieving pure absorbance spectra from such measurements.

In 2008 a method based on extended multiplicative signal correction (EMSC) was proposed, where an approximation formula for the Mie extinction was implemented in an EMSC model by a meta-model using principal component analysis (PCA) [1]. The approach was further developed to handle the so-called resonant case, where the real part of the refractive index undergoes fluctuations due to absorption [2]. This model is called resonant Mie Scatter EMSC (RMieS-EMSC). Recently, Konevskikh et. al. suggested a further improvement of the Bassan algorithm, as it could be shown that the corrected spectra are not strongly affected by the reference spectra used: the algorithm shows much clearer chemical features of the measured spectrum [3.].

In this work, the algorithm by Konevskikh et. al. was further refined. We present a program for correcting absorbance spectra, where the RMieS-EMSC algorithm is implemented in MATLAB. In order to test the stability of the code, a set of apparent absorbance spectra was simulated. The pure absorbance spectra were based on the matrigel spectrum, and chemical information was altered by changing absorbance peak heights. Mie scatter contributions were estimated from experimentally obtained measurements [1]. Apparent absorbance spectra with similar Mie scattering features as the experimental data were simulated using the simulated pure absorbance spectra as input for the imaginary part of the refractive index. The obtained simulated apparent absorbance spectra have the following advantages: (1) the underlying pure absorbance spectra are known and corrected spectra can be directly compared to the pure absorbance spectra used for simulation, (2) the scattering features of the simulate apparent absorbance spectra resemble scattering features observed in experimentally obtained spectra. The simulated spectra were used to test the stability of the RMieS-EMSC algorithm and to refine the algorithm further. Results demonstrate high stability of the algorithm for a variety of parameter settings. Sensitivity towards the number of principal components used in the correction is reviewed. To some extent this number is affecting the correction in the amide region, but the impact is shown to be generally low. As the algorithm is an iterative process, both the stop criterion and measures to decrease computational time are reviewed. The algorithm's ability to retrieve the true peak position of the amide I band is discussed.

References

[1] A. Kohler, J. Sulé-Suso, G. Sockalingum, M. Tobin, F. Bahrami, Y. Yang, J. Pijanka, P. Dumas, M. Cotte, D. G. van Pittius, G. Parkes and M. Høy, Appl. Spectrosc. 62, 259-266 (2008).
[2] P. Bassan, A. Kohler, H. Martens, J. Lee, H. Byrne, P. Dumas, E. Gazi, M. Brown, N. Clarke and P. Gardner, Analyst 135, 268-277 (2010).
[3] T. Konevskikh, R. Lukacs, R. Blümel, A. Ponossov and A. Kohler, FaradayDiscuss. 187, 235-257 (2016).