Evaluating a correction algorithm for resonant Mie scattering (RMieS) in single cell spectra

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Recent theory has shed light on the origins of the 'dispersion artefact' which has been attributed to resonant Mie scattering (RMieS)[1,2]. RMieS is observed as a sharp decrease in intensity on the high wavenumber side of absorption bands, namely the amide I (1655cm^{-1}), also causing a downward shift of the true peak position. A preliminary algorithm for correcting RMieS is presented here with focus on the quality of the corrected spectra. An ideal correction should output a spectrum which is representative of the true absorbance spectrum of the input with no distortion of the biochemically information. The true spectrum of a sample such as a single cell is unknown, making it difficult to conclude if the corrected spectrum is representative of that cell's biochemistry.

In order to test the algorithm, two data sets were created artificially by adding together many Gaussians, using a random number generator to vary peak height, width and position of each spectrum. The second data set was made sufficiently different from the first such that PCA would show that there are two groups of data. Theoretical RMieS curves were constructed using random number generators to vary scatterer size and refractive index properties within realistic ranges. These RMieS curves were linearly added to the artificial spectra to create a data set of artificial scattered spectra. The algorithm is built on a previous algorithm, the extended multiplicative signal correction (EMSC)[3] which successfully removed the baseline oscillations from Mie scattering, however the dispersion artefact remained present. The new physics of RMieS was incorporated into the program, and the artificially scattered spectra were corrected.

The new algorithm including RMieS correction was an improvement upon the previous EMSC as seen from the corrected spectra, the dispersion artefact is no longer visible. The PCA results show that the two groups separate more clearly with the new model, but also show that the spectra are not identical to their true absorbance spectra. An iterative process was tested where the corrected spectrum was used as the new reference spectrum, this resulted in a further improvement. The corrected spectra were not identical to their true absorbance spectra as shown by the PCA, and were distorted slightly such that they shared 'similarity' to the reference spectrum. This result is important as it gives an idea as to how spectra of single cells might be somewhat distorted after correction. On a positive note, even with the distortion the two groups have become separable via PCA when before RMieS correction they were not.

References:

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