# *The Inherent Problem of Transflection-mode Infrared Spectroscopic Microscopy and the Ramifications for Single Cell Analysis*

P. Bassan[1,2], J. Lee[2], J. Pissardini[2], P. Gardner[1,2]

[1] Manchester Institute of Biotechnology, University of Manchester, 131 Princess street, Manchester, M1 7DN, UK
[2] School of Chemical Engineering and Analytical Science, University of Manchester, Oxford Road, Manchester, M13 9PL, UK

Transflection-mode FTIR spectroscopy has become a popular method of measuring spectra from biomedical and other samples due to the relative low cost of substrates compared to transmission windows, and a higher absorbance due to a double pass through the same sample approximately doubling the effective path length. Generally the reflecting substrate is either a metallic coating on a glass slide (Au or Al are most popular) or a low-*e* microscope slide that has a metal doped tin (IV) oxide coating which is ~80% transparent to visible light but highly reflecting in the infrared region. The optical properties of the reflective substrates have largely been taken for granted since in all cases the reflectivity varies smoothly and is generally featureless in the region of interest. Recently, however, the optical phenomenon of the electric field standing wave (EFSW) has been investigated and shown to exhibit a wavenumber-dependent modulation in spectral intensity as a function of sample thickness [1,2]. In this work the influence of the EFSW on thin-film samples deposited on low-*e* slides is shown experimentally and show how the EFSW effect could influence the classification of cell and tissue [3]. Given these finding the practice of using translation spectroscopy in a field where we attempt to tease out the most subtle difference in spectra must be brought into question.

References:

[1] H. Brooke, D. L. Perkins, B. Setlow, P. Setlow, B. V. Bronk, M. L. Myrick, *Applied Spectroscopy* 62, 881-888, (2008).

[2] J. Filik, M. D. Frogley, J. K. Pijanka, K. Wehbe, G. Cinque, *Analyst* 137, 853-861, (2012).

[3] P. Bassan, J. Lee, A. Sachdeva, J. Pissardini, K. M. Dorling, J. Fletcher, A. Henderson, P. Gardner, *Analyst* 138 (1), 144 - 157, (2013).