Comparison of FTIR-imaging Measurements of Native Colon Tissue Prepared by Cryo-microtoming Using Transmission and Transflection Techniques.

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Recently, we reported on the infrared spectral histopathology of colon cancer tissue sections [1]. There have been several reports on differences of transmission and reflection measurements of cells and tissues dealing with "spectral abnormalities" as experienced from Fresnel reflection at layered interfaces, from scattering and from electrical field strength observed for tissues on IR-transparent or metallic reflective substrates [2-5]. In several papers, models and algorithms for correction of dispersive and Mie-scattering effects and normalisation have been presented, e.g. [6]. For explaining Fresnel reflection effects a clear interface must exist; however, our tissue slices prepared by cryo-microtoming show significant roughness after drying, so that also scattering at the interface tissue / air must be taken into account with scatter directed into a greater solid angle, so that the Fresnel reflection contribution is less than found with well-defined mirror-like interfaces.

Microtomed cryo-colon samples were prepared on CaF_2 or Kevley slides and dried at room temperature under dry air atmosphere to obtain water-free samples. The thickness and surface roughness of the prepared tissue layers was investigated by atomic force microscopy. For spectral histopathology, the pre-processed spectra using the "Resonant Mie Scattering (RMieS) correction" [6] were classified with a random forest classifier based on biopsy data previously annotated by a pathologist (spectral range 1800 – 950 cm^{-1} of spectra recorded from deparaffinised slices of colon tissue). Comparison of raw spectra and those normalised by RMieS correction, multiplicative scatter correction and standard normal variate transformation as well as minimum-maximum scaling for the classes of crypts, muscle and submucosa is presented. Surprisingly, the pre-processing of transmission and transflection spectra leads to a fine agreement of the spectral database used for classification of different tissue types and significant spectral differences as expected for the different substrates were not observed.

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