Exploring the spectroscopic differences of Caki-2 cells progressing through the cell cycle while proliferating in vitro.

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Attempts to characterize the cell cycle phases that a cell may undergo while proliferating in vitro by means of vibrational spectroscopy have been realized previously; however, the FTIR spectra used for building the neural network capable to discriminate between different cell cycle phases were not corrected for scattering effect that the infrared light may experience while interacting with biological samples such as cells since the RMieS correction algorithm was not available at that time. In this project, a Renal Cell Carcinoma cell line, Caki-2, was used to study the spectroscopic differences of spectra from well-known cellular developmental stages. Two different approaches were used for revealing the cell cycle phase that each cell was undergoing at the point of fixation. The first approach consisted on the monochromatic staining of the DNA of all cells contained on the sample; thus, the assignment of a given cell cycle phase was performed based on the premise that the relative intensity of the fluorescent dye used (DAPI) is dependent upon the amount of DNA present in the cell, which is directly related to the progression of the cell through the cell cycle: e.g G2 cells contain the double amount of DNA than G1 cells. The second approach consisted of specific immunostaining of biological markers such as CDT1 and Geminin that are only present either in G1 or G2 phase cells. S phase cells were identified by intercalating an analogue of thymidine into the nascent DNA and subsequently detected by means of immunocytochemistry. The collected FTIR spectra were corrected using the RMieS algorithm and further data analysis was conducted in order to evaluate the possibility of building a deterministic computational model such a support vector machine for discriminating between the cell cycle phases of Caki-2 cells without the use of expensive and time consuming methods such as immunocytochemistry.

References