Biological variance and statistical variation in the Raman spectra of human cells depending on the origin and the collection time

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Due to its many advantages, such as inherently label-free and very specific chemical contrast, its non-invasiveness and non-destructiveness, Raman spectroscopy is gaining more and more importance for the application to biological and medical problems, such as the classification of healthy and cancerous cells and tissues, the fast and reliable identification of pathogens from body fluids or the identification of metabolites for therapy control. The evaluation of the spectroscopic analysis often requires the use of statistical classification models. When dealing with biological and medical samples some problems arise due to the high biological variety of the samples originating from different donors, patients or cultivation batches. Furthermore, variations may be introduced into the spectral data by different time spans between sample collection and measurement or by the use of different instruments.
In this contribution we present an example from the research for new, label-free and non-destructive methods for optical tumor diagnostics focusing on circulating tumor cells. These circulating tumor cells from peripheral blood which are very rare and difficult to detect offer a high potential for tumor diagnostics, cancer therapy selection and monitoring and therefore are of utmost interest. Previously, we could show how Raman spectroscopy can be used in combination with statistical classification methods to distinguish the different cell types that are present in peripheral blood such as leukocytes, leukemic cells and solid tumor cells using dried cells [1], cells captured in solution [2] as well as manipulated in a microfluidic chip [3]. Here, we present Raman data of the four cell types (primary leukocytes, leukemic cells and two breast cancer cell lines) excited at 785 nm from nine different measuring days originating from different donors and from different cultivation batches. Spectral variations due to these biological variations as well as due to different time spans after cell collection and two different excitation lasers are shown. However, statistical methods, in particular linear discriminant analysis, enable the successful classification of healthy and cancerous cells from different patients and from different cultivation batches measured on different days. Furthermore, marker bands which proved to contribute significantly to a successful classification have been identified.

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