Inverted Microscope for Raman-based Cell Identification in Microfluidic Chip Environment

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Scope of the research initiative "Jena Cell Identification Group" (JenZIG) is the combination of Raman spectroscopy with optical trapping and microfluidic chips for cell identification and sorting. Using upright microscopes, fluidic connections were realized at the bottom side [1]. Cells tend to sediment in the tubing at low flow rates which is problematic for a continuous and stable injection of single cells. The contribution presents an in-house built inverted microscope to solve the problem. The performance of the new microscope was characterized for the distinction of breast carcinoma derived tumor cells (MCF-7, BT-20).

The inverted microscope consists of fiber ports for coupling the excitation laser and the spectrograph with CCD detector. This enables to connect the microscope to a commercial Raman system, here HoloSpec RXN1 (Kaiser Optical Systems). Laser line filter, beamsplitter, objective holder and notch filter were installed in a 30×30 mm cage system (Thorlabs). Optical components were selected for high throughput. Comparison with the standard Leica microscope (Microprobe, Kaiser) showed almost same signal intensities.

Cells were transferred into a small sample chamber with a quartz coverslip. 150 Raman spectra were collected per cell type with the inverted microscope and 50 Raman spectra per cell type with the Leica microscope using an oil immersion objective 100×/1.25. Data sets were analyzed R [2] using the package hyperSpec [3]. Classification models were developed based on partial least squares projection followed by linear discriminant analysis (PLS-LDA). Out-of-bootstrap [4], a resampling scheme comparable to iterated cross validation, was used for validation. Classification performances are discussed for data sets collected on four days. Data from both microscopes gave similar accuracies for distinction of MCF-7 and BT-20 cells. Further experiments will be performed in quartz microfluidic chips using more different cell types [1].

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