**Multimodal imaging of 3D cell cultures using MALDI MSI and fluorescent confocal microscopy**

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Multimodal imaging is emerging scientific field which combines several techniques to visualize analytes of interest. The main goal is to obtain additional information about the sample nature which allows accurate explanation of ongoing biological and chemical processes. Our biological models, 3D cancer cell lines or so called spheroids, were treated by potential cancerostatic perifosine. To visualize drug penetration within the spheroid, a matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI MSI) was used, and this was combined with fluorescent immunohistochemistry for determination of cell viability. These two methods were executed on the same tissue section, so the protocol for sample preparation had to be optimized. To co-localize images from particular modalities, the fiducial based coregistration was introduced. Subsequently, the quantification of signals was performed by so called peeling algorithm, which enabled to segment the spheroid from the boundaries to its core. Using the precise coregistration and signal quantification, we were able to reveal the effect of perifosine on adenocarcinoma spheroid proliferation, apoptosis and metastasis which are important characteristics of any cancer progression.