A combination of single-cell MALDI-MS imaging and fluorescence microscopy to explore molecular heterogeneity in cell cultures

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Motivation

- MS-analysis of extracts from pooled cells provide a general molecular profile but is blind to heterogeneities within a culture
- Single-Cell heterogeneity is an inherent property of every cell population
- Its analysis is fundamental to understanding the development, function, and role of specific cells that share the same genotype but may display different phenotypical properties

Experimental Set-up

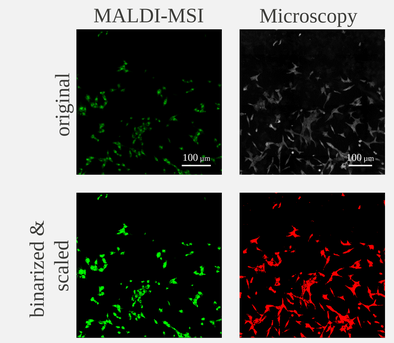
Sample Preparation & Data Aquisition:

- Cell Culture: cells (Vero-B4 and Caki-2) were grown on 8-well chamber slides; fixed in 4% formaldehyde for 5 mins; stained with Hoechst and WGA; washed; sublimated with matrix (2,5-DHAP).
- Microscopy: digital slide scanner (SLIDEVIEW VS200, Olympus); 20x objective; bright-field and fluorescence.
- MALDI-2-MSI: timsTOF fleX (Bruker) with 8 µm pixel size and Orbitrap Q Exactive Plus (Thermo Fisher Scientific) with 2 µm pixel size.

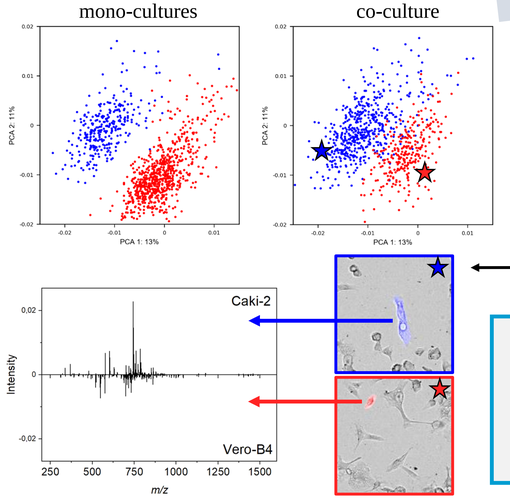
Data Processing:

- SCiLS Lab (Bruker): spectra reduced to signals correlating to cell regions using the co-localization function. Python: Single-Cell Segmentation (otsu-thresholdand watershed) on the three microscopy channels.
- Co-registration: binarized MALDI-MSI and microcopy data were overlaid using 2-D- and rotation-correlation.
- Machine learning: Single-Cell mass spectra were normalized to the TIC. A support-vector machine was trained on mono-cultures (5-fold cross-validation) and then used to classify co-cultures.

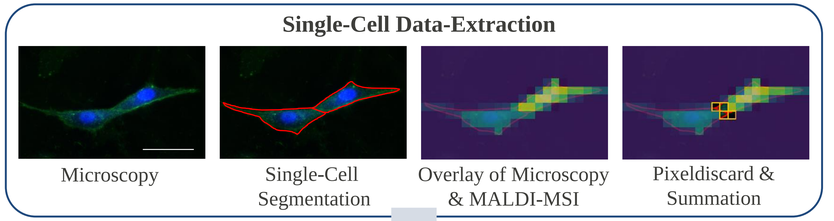
Co-registration

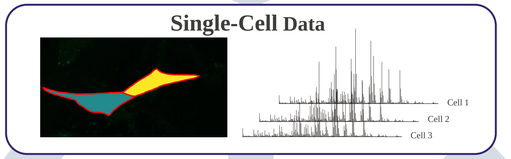


principal component analysis



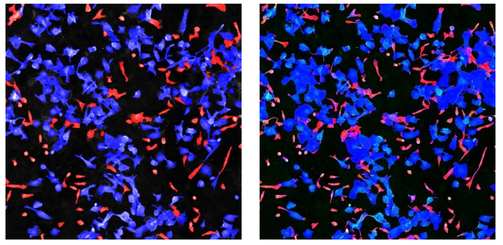
Workflow





Model system: Co-culture of Vero-B4 and Caki-2 cells

Machine learning-based classification



based on *m/z*-values

based on morphology (AI algorithm provided by Olympus)

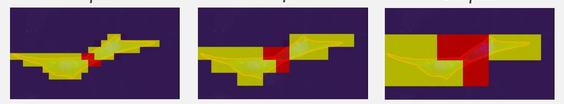
identified by *m/z*-based classification

Conclusion

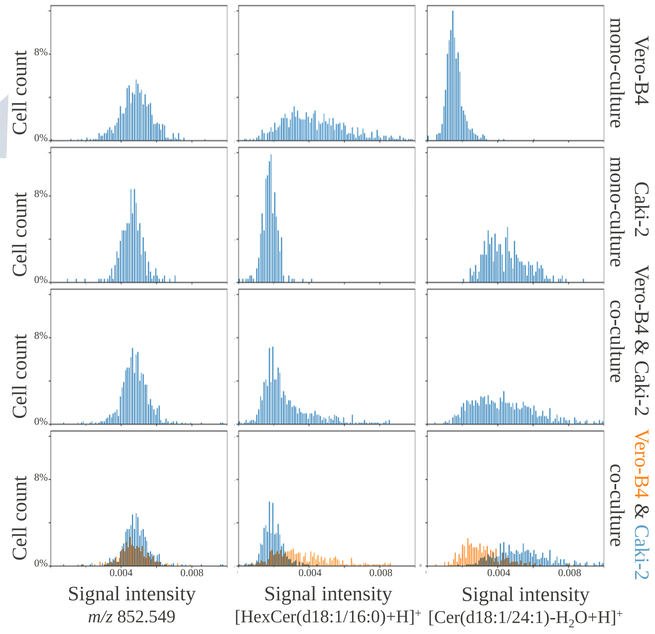
- Versatile tool to investigate heterogeneity in cell cultures
- Combines molecular and morphological information
- Enables statistical and machine learning-based data analysis

Pixel-discard

	2 µm	4 µm	8 µm	16 µm	32 µm
1 cell, 100 %	83.5%	57.9%	26.6%	4.3%	0%
1 cell, 50-99 %	9.5%	19.0%	28.0%	24.3%	9.1%
1 cell, 1-50 %	4.8%	17.3%	34.6%	54.0%	61.9%
2 cells	2.3%	5.8%	10.7%	17.4%	29.0%



Signal-intensity histograms



Acknowledgements

DFG IZKF OLYMPUS