Pudding the Cell in Celebrate: Cell Classification by mid-IR Spectroscopy and Photothermal Imaging

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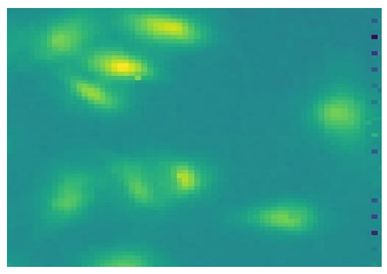
Introduction
01

The label-free detection of cell types and characteristic cell features is in high demand in medical and biological research. FTIR is the state-of-the art chemical imaging method for (cancer) tissues and cells [1, 2, 3]. However, it has limited spatial resolution (~ 3 µm) and is not suitable for measurements in water-filled channels (e.g., microfluidic chips). Mid-infrared photothermal (MIP) spectroscopy offers increased resolution (~0.5-1 µm) at the same specificity as FTIR. Relying on a visible beam for detection, MIP bypasses the problem of water absorption at IR wavelengths [4].
We are using IR to distinguish between healthy, cancerous and metastasizing cells in tumor-on-a-chip systems. We report on our initial experiments using machine learning and IR spectroscopy for cell classification, employing conventional FTIR microscopy and MIP imaging.

Samples
02

Three different cell lines were analyzed. MDA-MB-231 WT, a highly invasive breast cancer cell line, was used as parent cell line for a modified non-invasive cell line, while healthy lymphatic endothelial cells served as a control. The cell samples were fixed with paraformaldehyde on CaF_2 coverslips.

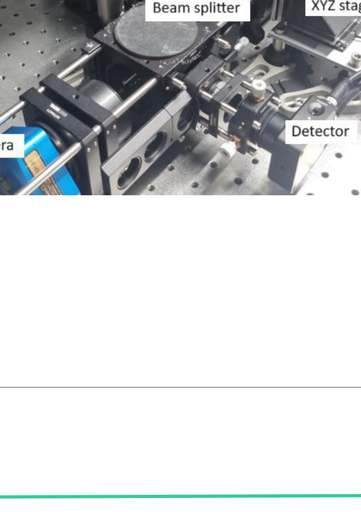
Sample	Cell line
A	MDA-MB-231 WT
B	MDA-MB-231 Jag1KO
E	HDLEC



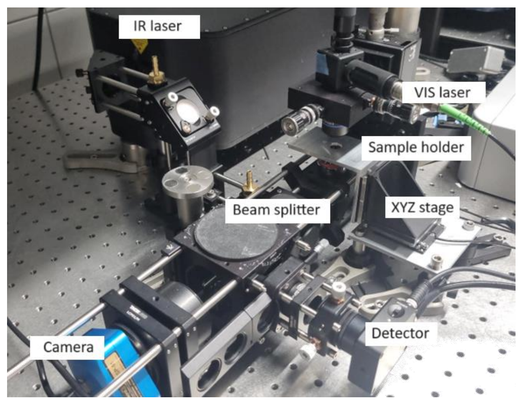
IR-image of cell line A

Setups
03

A commercial FTIR (Bruker Hyperion 3000) was used to obtain infrared hyperspectral images. The data was collected with an FPA detector, yielding 64x64 pixels per image, with each pixel representing the average of 64 IR spectra. The spatial resolution was 2.7 µm.



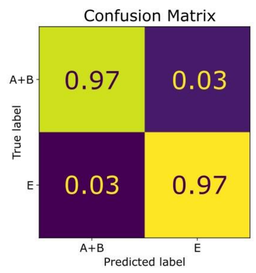
The FTIR spectra were compared to our mid-IR photothermal (MIP) instrument, which is equipped with a MIRcat-QT (Daylight Solutions) external cavity quantum cascade laser to illuminate the sample with IR light. Local IR absorption is detected using a 633 nm visible laser with a spatial resolution of ~1 µm.



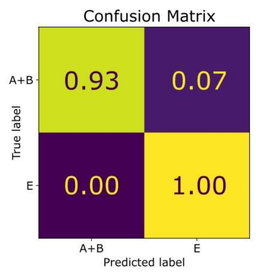
FTIR Spectral analysis
04

Cancer vs. Non-Cancer

PCA-LDA of cells A&B vs E



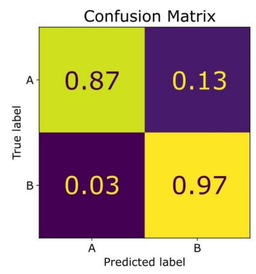
PLS of cells A&B vs E



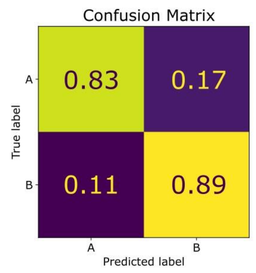
Prediction accuracy: 97 % (PCA-LDA) and 95 % (PLS)

Invasive vs. Non-Invasive

PCA-LDA of cells A vs B

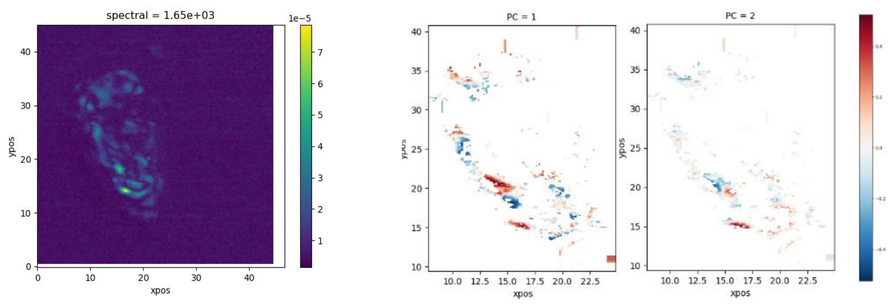


PLS of cells A vs B



Prediction accuracy: 91 % (PCA-LDA) and 86 % (PLS)

MIP imaging
06



MIP images at 1456 cm^{-1}, 1549 cm^{-1} and 1650 cm^{-1}

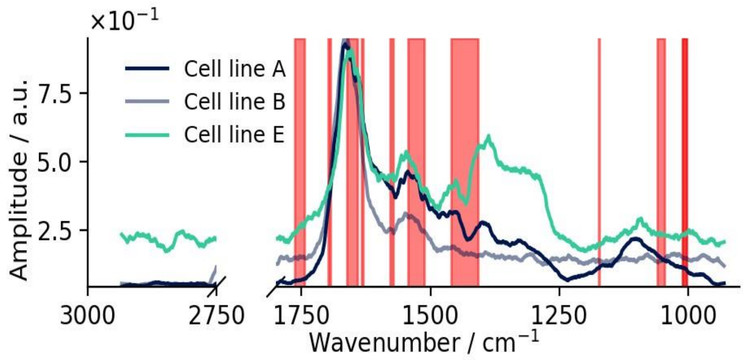
PCA score image

LASSO
05

LASSO algorithm to identify regions of interest for further analysis and imaging

Identified ROIs

Cells	Regions of interest / cm^{-1}
A vs. E	1446-1477, 1549-1579,1631-1655, 1745
A vs. B	1003-1011, 1045-1076, 1408-1473, 1641-1699, 1736



Measured spectra from the MIP-module with marked regions of interest from the LASSO-analysis

Conclusions & Outlook
06

In this study, we evaluated several chemometric techniques to distinguish between cancerous/non-cancerous and invasive/non-invasive human cells using different machine learning models. Moreover, spectral regions of interest were identified for subsequent MIP imaging. The potential of MIP hyperspectral imaging in the analysis of subcellular structures has been demonstrated. Further experiments will be conducted for quantitative analysis and label-free imaging.

[1] Liu Dong et al., 'Evaluation of Fourier Transform Infrared (FTIR) Spectroscopy with Multivariate Analysis as a Novel Diagnostic Tool for Lymph Node Metastasis in Gastric Cancer', Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy 289 (2023) 122209.
[2] Allison Derenne et al., 'Lipid Quantification Method Using FTIR Spectroscopy Applied on Cancer Cell Extracts', Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids, 1841 (2014) 1200–1209.
[3] Hersh K. Bhargava et al., 'Computationally Derived Image Signature of Stromal Morphology Is Prognostic of Prostate Cancer Recurrence Following Prostatectomy in African American Patients', Clinical Cancer Research 26, no. 8 (2020).
[4] Jiaze Yin et al., 'Video-rate mid-infrared photothermal imaging by single-pulse photothermal detection per pixel', Science Advances 9, no. 24 (2023).

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