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Multimodal FTIR Microscopy-guided MALDI Mass Spectrometry Imaging for Tumor Analysis

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Abstract
MALDI-Mass spectrometry imaging (MSI) is an enabling technology for label-free spatially resolved molecular analysis of biological tissues and of drug distribution in life science and pharmaceutical research (1). The rich molecular information content evaluated by high-resolution magnetic resonance MSI opens new horizons for biomarker as well as clinical pharmacology research (2, 3). However, translation of high-resolution MSI into clinical practice is restricted due to key limitations like low throughput (multiple hours per section) and computationally challenging interpretation. Therefore, multimodal data acquisition techniques are gaining increasing importance, as the analysis of tissues by multiple sensors hold the potential to greatly improve tissue evaluation (4).
Here, we present an integrated approach that utilizes non-destructive Fourier transform infrared (FTIR) microscopy, which requires virtually no sample preparation, and MSI for the spatial detection of tissue features, such as tumors. A multi-step data processing workflow was developed in MATLAB to enable simultaneous tissue segmentation of FTIR images and targeted extraction of MS spectra belonging to tumor-related regions. We apply FTIR imaging as an upstream modality to improve accuracy of tissue-morphology detection and to retrieve diagnostic molecular signatures in an automated, unbiased and spatially aware manner. Here, we show the general applicability of multimodal FTIR-guided MALDI-MSI by demonstrating precise tumor localization in human primary gastrointestinal stromal tumors (GIST).

I. Guided imaging provides throughput and analysis of predefined regions

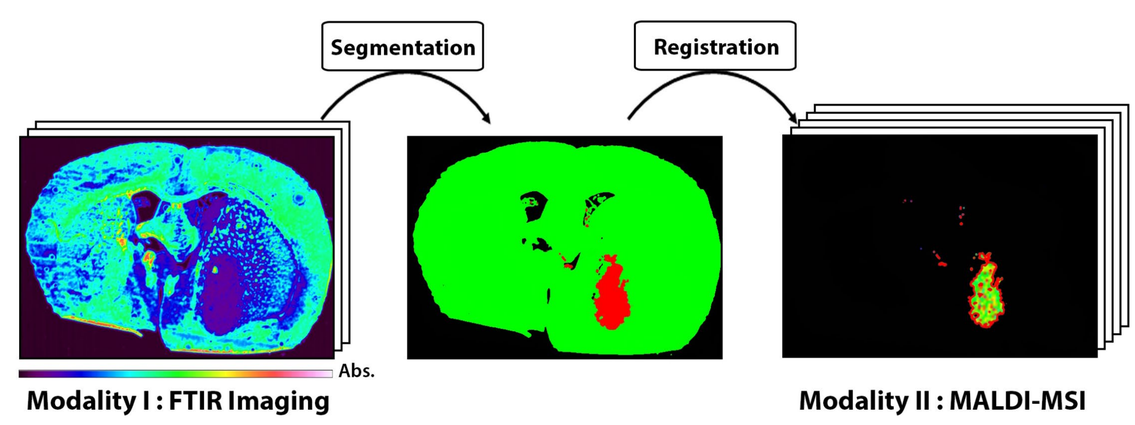

Fig. I: Concept of multimodal FTIR-guided acquisition and interpretation of MALDI MSI. Spatial contours are derived from fast, non-destructive FTIR measurements as the first modality and registered to the subsequently acquired second modality, MALDI-MS images, to allow segment-specific mass comparison.

II. Evaluation of FTIR-derived spatial tumor contours

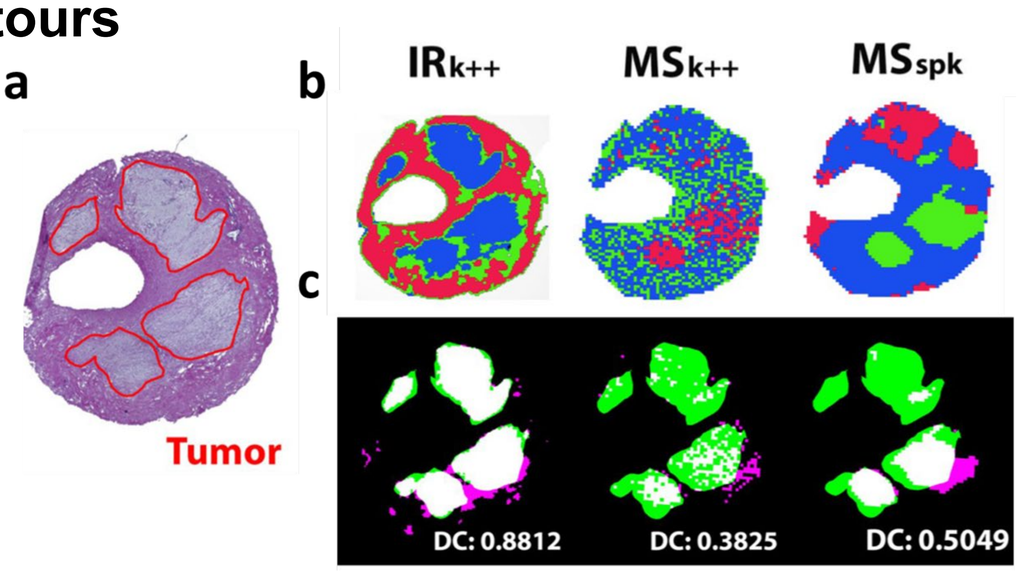

Fig. II: Accuracy of tumor contour assignment derived from FTIR-and MS image segmentation. (a) H&E stain of human patient-derived GIST tissue were used to evaluate. (b) Comparative segmentation of FTIR images using kmeans++ clustering and MALDI-MS images using both kmeans++ and spatially aware clustering (spk). FTIR segmentation exhibits clear tissue contours and is less sensitive to noise when compared to MSI. (c) Segmentation results for FTIR image matched tumour boundaries identified in H&E histopathology of adjacent GIST section with higher accuracy (Dice similarity coefficient = 0.88) when compared to segmentation results of MALDI-MS image.

III. Case Study: Tumor identification by means of FTIR cluster analysis

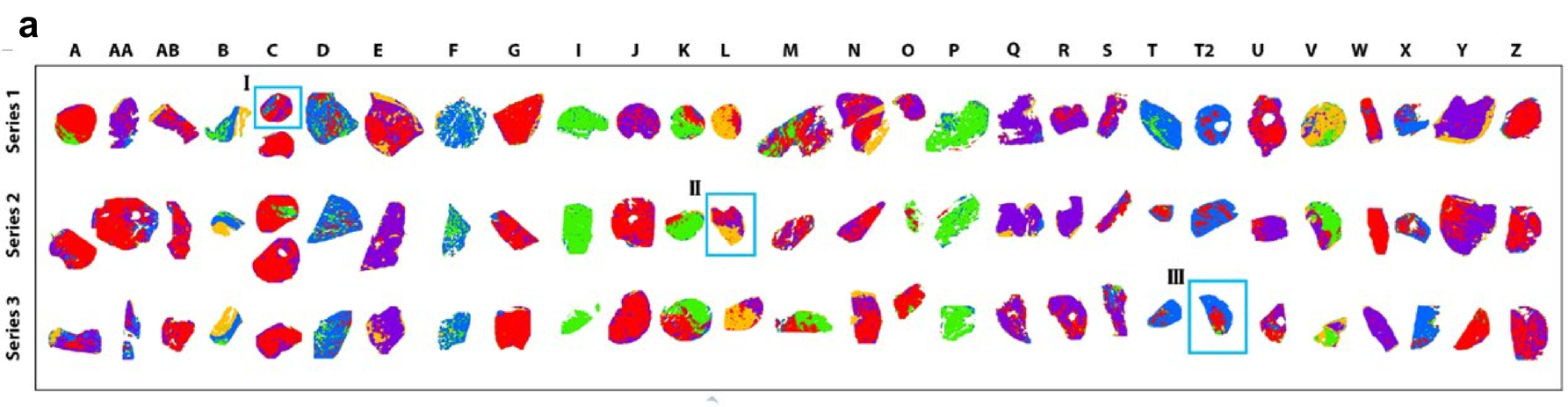

Fig. III A: Simultaneous FTIR image segmentation of 89 tissue sections from 27 GIST patients by means of k-means++ cluster analysis reveals intra- and inter-section dependencies. A strong correlation between segmentation and the occurrence of tumour cells was observed, as a separation between tumorous and non-tumorous areas (i.e. fibrosis, necrosis) was rapidly (~90 s) done. Red and purple areas were found to represent the occurrence of tumour after comparison to histopathological annotation of subsequent H&E stains.

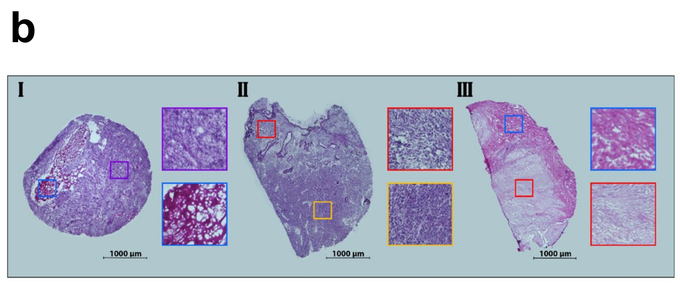

Fig. III B: Magnified subsequent H&E images of patient C, patient L and T2 exemplify FTIR-based distinction of tumorous areas and cancer-free tissue in accordance with histologically observed differences.
Color coding: red and purple = tumor,
blue = fibrosis and edema;
yellow = sectioning artefacts like folds and freezing damage

Conclusion
The workflow presented here for FTIR-guided MALDI MSI offers:
- Automated morphology-related mass feature screening
- Predefinition of subpopulations within large tissue cohorts
- Assessment of tissue heterogeneities prior to MSI acquisition
- Fast, targeted high-resolution MSI of predefined morphologies
- Superior multi-section identification of cancerous outlines in the basis of a single H&E stain

References
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[4] Rabe et al., Sci Rep. 2018; 8(1):313