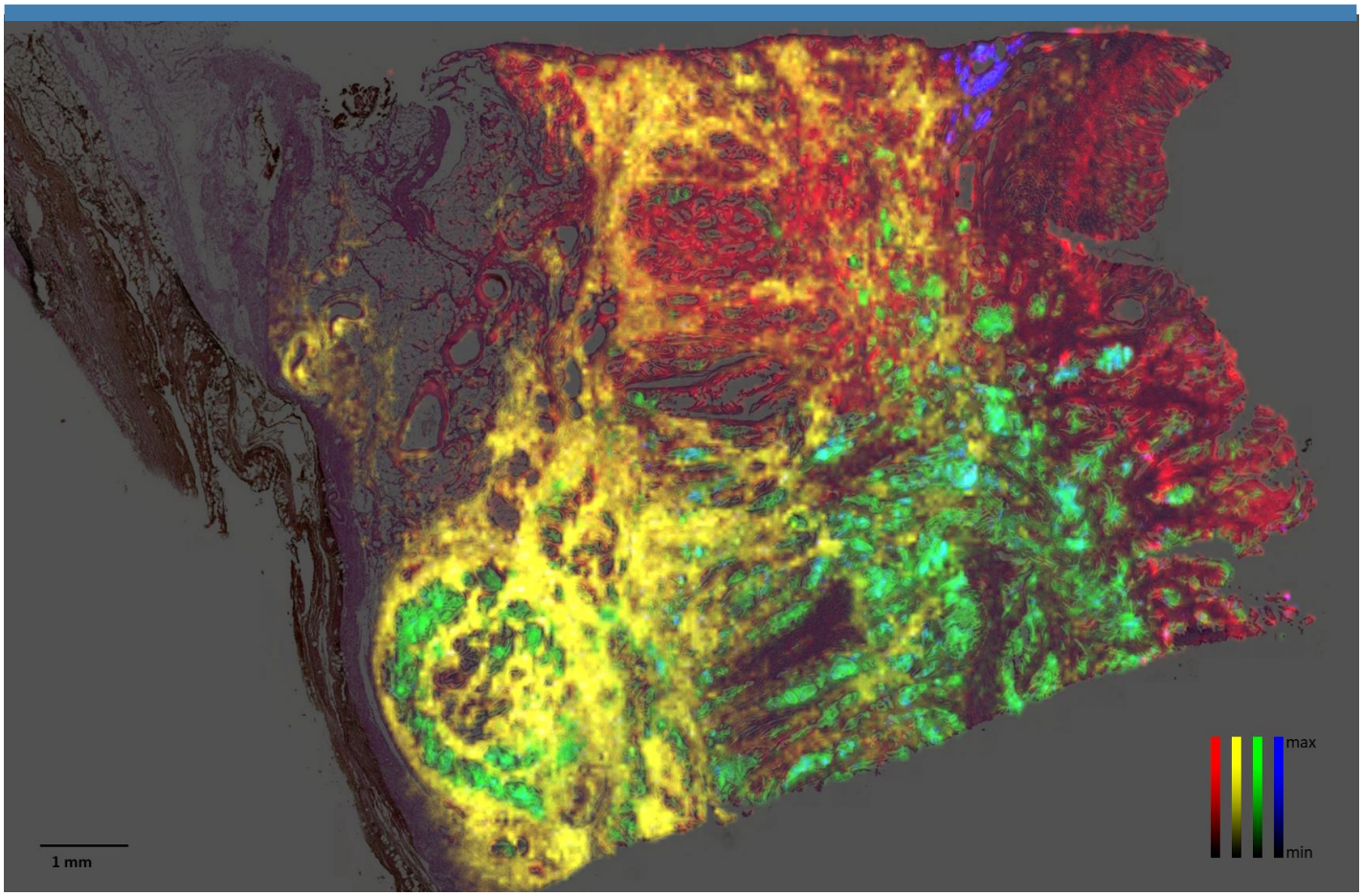
theratype
MOLECULAR LANDSCAPE IMAGING

Theratype's mass spectrometry imaging approach for the analysis of metabolites from FFPE tissue sections



Formalin-fixed, paraffin-embedded (FFPE) tissue samples possess great potential to provide metabolic insights into physiologically normal, predisposed and pathological processes. Building on established protocols and applications expertise in mass spectrometry imaging (MSI) of metabolites from FFPE tissues, multiple biomolecules can be analyzed label-free with high spatial resolution in the context of tissue morphology. Theratype's molecular imaging approach permits reliable high-throughput *in situ* metabolomics-based biomarker discovery and validation for research purposes and clinical application using prospective and retrospective FFPE sample collections.

theratype
Ingolstädter Landstrasse 1
D-85764 Neuherberg

t +49 (0)89 89084187
contact@theratype.com
www.theratype.com

Introduction

Frozen tissue specimens are a limited resource in clinical and biological applications, as these tissues are technically demanding in terms of storage and handling. Tissue processing by formalin-fixation and paraffin-embedding is routinely used in clinical practice to archive samples of various human cancers and diseased tissues, making these samples a valuable source in research. In addition, FFPE patient samples, amenable to indefinite storage at room temperature, are commonly clinically annotated in databases with long-term follow-up and outcome. In order to make FFPE tissue specimens accessible for the *in situ* analysis of small molecules, we developed a protocol using high-resolution matrix-assisted laser desorption-/ionization Fourier-transform ion cyclotron resonance mass spectrometry imaging (MALDI-FT-ICR MSI) requiring minimum FFPE sample amounts [1,2]. The metabolic imaging approach is suitable for samples of different sizes, from surgical resection specimens to small biopsies, and moreover, by analyzing tissue microarrays (TMAs), it can be applied to study patient tissue samples at a large scale and in a high-throughput fashion. A major advantage of the method lies in the parallel visualization of molecular content of tissues in conjunction with its histopathology allowing a 'virtual microdissection' (defined as regions of interest) of tissue compartments in order to generate cell-specific molecular signatures. The extracted molecular signatures can be linked with clinical data providing new opportunities for the investigation of disease mechanisms, the discovery of biomarkers, as well as mechanisms of therapeutic agents. Thus, deciphering the metabolic landscape from FFPE tissues sets new standards for clinical and translational research.

In this application note, we present our high-throughput workflow for metabolite imaging from different FFPE tissue sections. The application examples comprise the imaging of an individual human gastric cancer surgical resection specimen, different biopsy samples and a multipatient TMA. The resulting *in situ*-metabolomic data alone or merged with other omics and clinical data can be used for molecular correlation analyses, hierarchical clustering, pharmacometabolomics, pathway and machine learning classification tasks. In biomedical applications, metabolic profiles containing mass signatures can be used to identify tissue markers with implications in diagnostics, prognostics, or therapy response prediction.

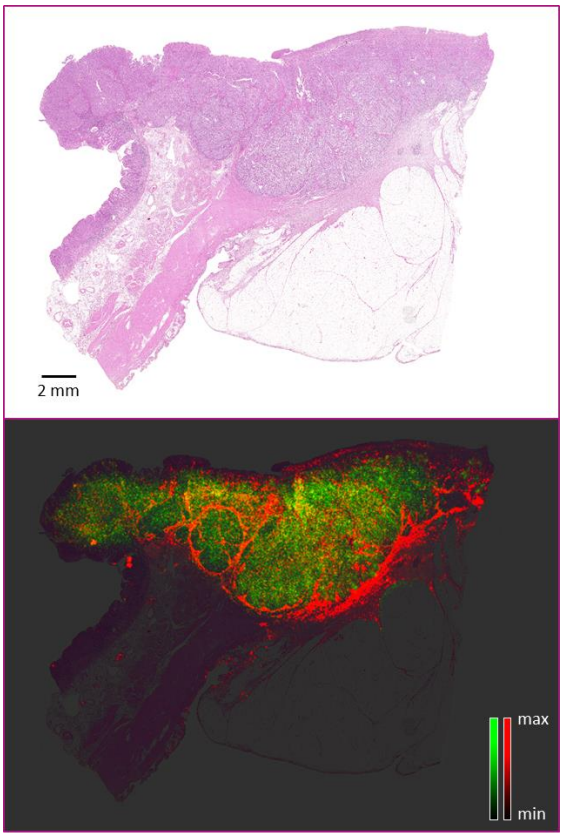


Figure 1: Whole tissue sections from a gastric cancer patient measured by MALDI FT-ICR MS imaging for the analysis of metabolites followed by hematoxylin and eosin staining. Ion maps showing glucose-6-phosphate (green: *m/z* 259.023) and N-acetylhexosamin sulfate (red: *m/z* 300.040) [4].

Method

FFPE tissue samples were sectioned with a thickness of 4µm and mounted onto indium–tin-oxide (ITO)-coated glass slides pretreated with 1:1 poly-L-lysine and 0.1% Nonidet P-40. Prior to MALDI matrix application FFPE sections were incubated for 1h at 70°C, deparaffinized in xylene

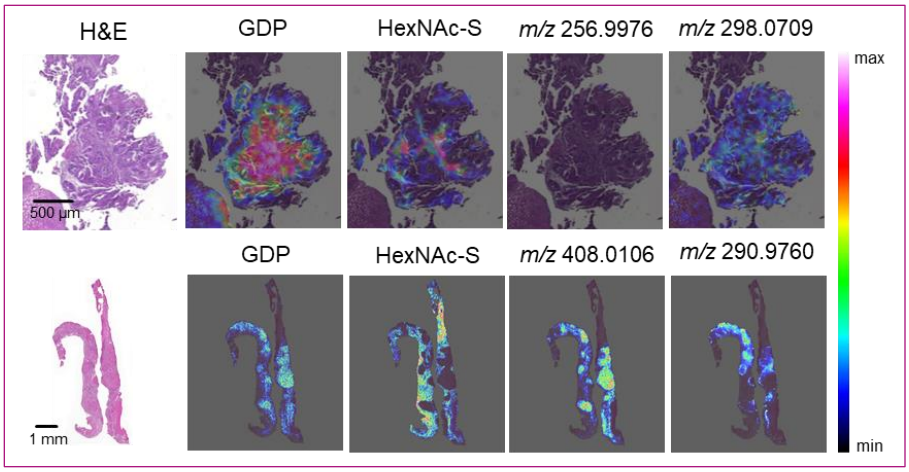


Figure 2: A gastric cancer biopsy and a liver biopsy featuring cirrhosis measured by MSI. Visualized is guanosine diphosphate (GDP), N-acetylhexosamin sulfate (HexNAc-S), and further unidentified metabolites [2,8].

and air-dried. FFPE samples were spray coated with 10mg/ml 9-aminoacridine hydrochloride monohydrate in 70% methanol. Metabolites were detected from tissue sections in negative-ion mode at 60µm lateral resolution on a 7T Solarix XR FTICR mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany) equipped with a dual ESI-MALDI source and a SmartBeam-II Nd: YAG (355 nm) laser. Digitized images of the hematoxylin and eosin stained tissues were coregistered to respective MSI data using FlexImaging (v. 5.0) (Bruker Daltonik).

Results and Discussion

The preservation of different classes of metabolites in deparaffinized FFPE tissue samples allows reliable targeted and non-targeted metabolic analyses by MSI. With Theratype's metabolite imaging approach for single FFPE tissue sections, a large number of metabolites (e.g. amino acids, saccharides, native glycans, hormones, vitamins, nucleosides, organic acids, and lipids) can be detected simultaneously. Figure 1 shows a FFPE gastric cancer patient tissue sample derived from surgical resection. Localization of metabolites such as N-acetylhexosamin sulfate (HexNAc-S) coincide with desmoplastic stroma and glucose-6-phosphate (G6P) reveal high abundance in tumor regions of the tissue section. Within cells, glucose is immediately phosphorylated to G6P to prevent diffusion of the metabolite out of the cells. G6P is an important intermediate in glucose metabolism and lies at the crossroads of different metabolic pathways including glycogen synthesis, glycolysis, and the pentose phosphate pathway.

We provided evidence that metabolites which are reproducibly detected in FFPE tissue sections with specific localization — e.g., tumor, tumor stroma or other compartments within tissue — are also found in corresponding regions of fresh frozen tissue samples [1-4]. A comparison of the molecular composition between frozen and FFPE tissue samples yielded recovery rates of *m/z* values in the range of seventy to eighty percent. This is in accordance with other mass spectrometry methods analyzing metabolites from FFPE tissues without imaging [5-7]. Given the small amount of tissue material required, metabolite imaging is a very resource-efficient approach. A single tissue section is sufficient for mass spectrometric analysis and subsequent staining allowing histological assignment. This makes the method particularly interesting for the molecular analysis of biopsies, which are typically limited for analytical tests due to their restricted volume (Figure 2).

Apart from studying metabolite distributions, the data generated by Theratype can be used to unlock metabolic information that contributes to the understanding of disease processes and the identification of new tissue markers and marker signatures that have implications for diagnosis, prognosis or therapeutic decision making. In this regard FFPE tissue collections, accompanied by patient information as well as other molecular determinants, represent invaluable resources for

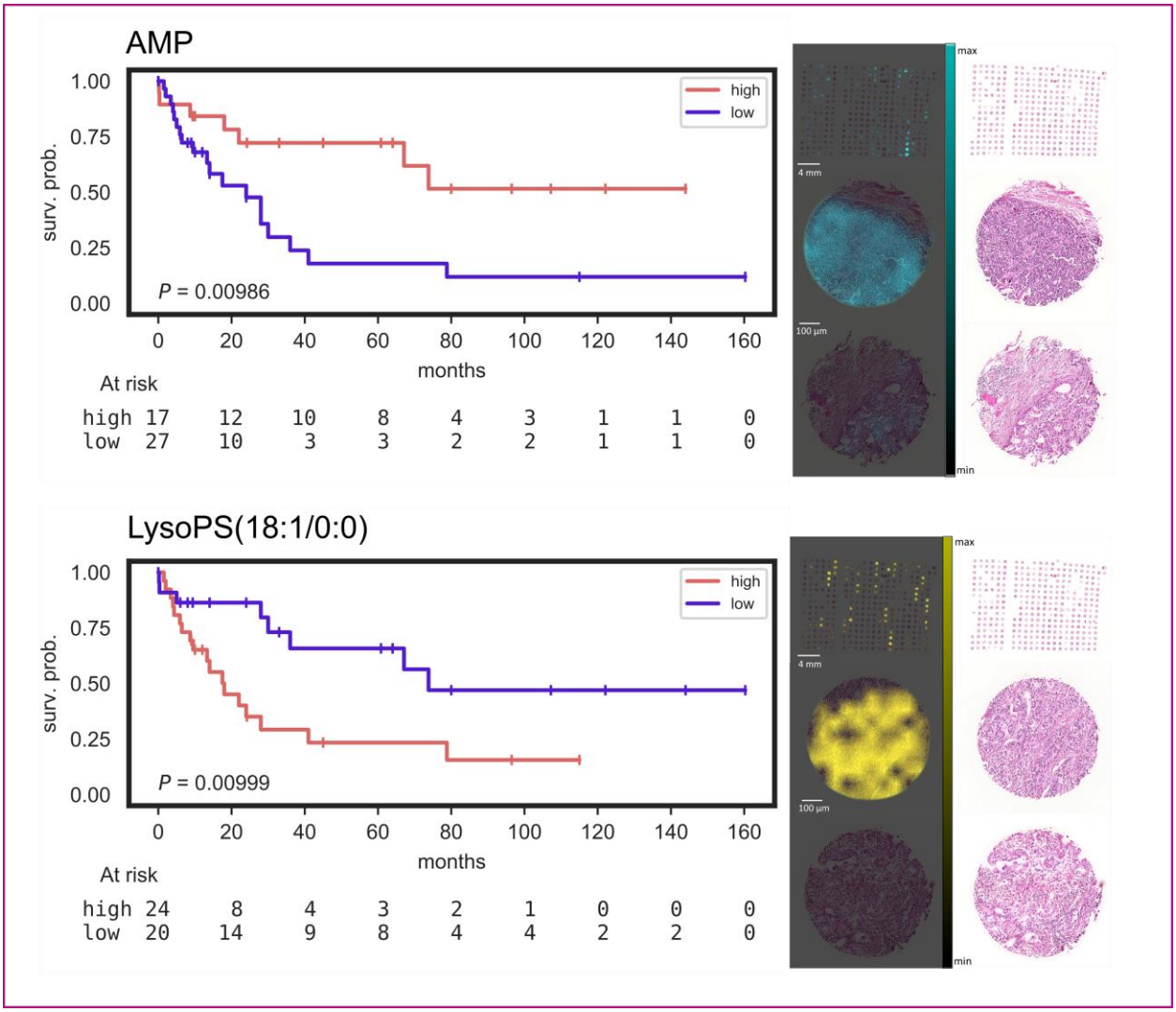


Figure 3: Kaplan-Meier survival curve for gastro-esophageal carcinoma patients stratified according to AMP and LysoPS(18:1/0:0) mass signals from MSI [8]. The statistical analysis of the log-rank test indicates P=0.0986 for AMP and P=0.00999 for LysoPS(18:1/0:0). Ion distribution maps on the right side showing localization of both prognostic factors in tumor tissue cores representative for low and high mass intensity.

translational studies in oncology and other fields. For instance, Figure 3 reveals that MSI data from tumor-specific regions of a multipatient gastroesophageal cancer TMA can be used to distinguish patients' outcome on basis of individual metabolites. Adenosine monophosphate (AMP) was found to be a prognostic factor of patient survival (P = 0.00986) [8]. The mass intensity was higher in the good prognosis group, while signal intensity was weak in the poor prognosis group. Furthermore, some specific lipid classes remain largely unaffected during deparaffinization and can therefore be identified using our biomarker discovery approach. The lysophospholipid LysoPS(18:1/0:0) was associated at high signal intensity with poor prognosis and at low intensity with a good prognosis (P = 0.00999) (Figure 3). Since disease-specific tissue metabolites are known to leak or secrete into body fluids, identified tissue markers can potentially be recovered in the plasma, serum, urine or saliva of patients. Additionally, this can drive the development of non-invasive assays for disease screening and monitoring.

Conclusion

The use of prognostic and predictive biological markers is gaining importance in the concept of a personalized, or precision medicine in cancer care. Theratype's mass spectrometry imaging approach for high-throughput *in situ* analysis opens the vast number of FFPE tissue collections to unlock metabolic information which can help us to understand disease processes and to identify new tissue markers with implications on diagnostics, prognostics, and for research purposes in general.

Advantages

- Reliable analysis of metabolites from FFPE tissue sections, tissue microarrays, and biopsies.
- Minimal sample requirements. A single tissue section is sufficient for MSI analysis followed by histological staining (e.g. hematoxylin and eosin)
- High mass resolution, spatial resolution and mass accuracy as prerequisite for clear assignment of metabolites to morphological tissue characteristics.
- Access to so far inaccessible metabolite species
- Application in the study of disease mechanisms and metabolic pathways
- Discovery and identification of new biological markers for diagnostics, prognostics, and therapeutic approaches

Literature:

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