Reproducibility of MALDI Imaging Based Tissue Classifications – Results of a Multi-Center Study

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Methods
Sections were prepared and measured as paraffinization and antigen retrieval. Serial (Bruker Daltonik GmbH), and underwent de-sectioned onto conductive glass slides (6 tumor types sampled at 3 sites) was used. Mouse intestine and a tissue microarray (TMA) showed that the spectra were clustered measured at five sites and two time points. Leave-one-site-out or a two-step Leave-one-TMA-out-leave-one-patient-out.

Results
Spatial segmentation on mouse gut samples measured at five sites and two time points showed that the spectra were clustered according to tissue type, not according to site or time of measurement (Figure 1). For the classification of the TMA data, the TMA was prepared and measured at three sites. A total of 407 monoisotopic mass spectral features were found in the average spectrum. A forward feature selection was used to identify the 25 most relevant features for the classification. The histological analysis of the H&E stained TMAs showed that some cores were heterogeneous with only small actual tumor areas (Figure 3). Some cores contained no tumor. The data were analyzed with and without histologic annotation of the tumor areas and feature selection taken into account. The results are shown in Table 1. A classification result of a leave-one-TMA-out-leave-one-subject-out classification.

Table 1. Accuracy of classification with and without cross validation and feature selection.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Leave-One-TMA-Out</th>
<th>Leave-One-Subject-Out</th>
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<tbody>
<tr>
<td>With histological annotation</td>
<td>78.3%</td>
<td>78.3%</td>
</tr>
<tr>
<td>Without histological annotation</td>
<td>84.5%</td>
<td>84.5%</td>
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</tbody>
</table>

Figure 1. Segmentation analysis of mouse intestine samples measured at five sites over three time points. (Scale: 3mm)

Figure 2. Layout of the Multi-Tumor-TMA

Figure 3. Histological annotation of tumor regions for one TMA section and classification map of a Leave-One-TMA-Out-Leave-One-Subject-Out Cross Validation

Introduction
Classification of tissues based on label-free mass spectrometric phenotypes measured directly from sections is a promising tool for clinical research. However, reproducibly measuring mass spectra can be challenging and comprehensive studies assessing the variation across different sites are largely lacking. In this work we have compared the reproducibility of Matrix-Assisted-Laser-Desorption/Ionization MALDI mass spectrometric imaging (MALDI-MSI) based tissue classifications measured at three different sites.

Methods
Mouse intestine and a tissue microarray (TMA) containing samples from 95 subjects (6 tumor types sampled at 3 sites) was used (layout shown in figure 2). Samples were sectioned onto conductive glass slides (Bruker Daltonik GmbH), and underwent deparaffinization and antigen retrieval. Serial sections were prepared and measured as previously published [1] at different sites. Samples were sprayed with trypsin and incubated. After digestion, matrix was applied and sections were measured at 50 μm step size with a rapiflex MALDI Tissuetype TOF mass spectrometer (Bruker). Data were analyzed using flexAnalysis (Bruker), SCILS Lab Pro (Bruker) and R (R-project).

The classification was calculated by linear discriminant analysis (R-package MASS). The performance was estimated as the accuracy of the classifications with different cross-validation-scenarios: Leave-one-sampling-site-out, Leave-one-TMA-out or a two-step Leave-one-TMA-out-leave-one-patient-out.

Results
Figure 2. Layout of the Multi-Tumor-TMA

Figure 3. Histological annotation of tumor regions for one TMA section and classification map of a Leave-One-TMA-Out-Leave-One-Subject-Out Cross Validation

Summary
We have shown that MALDI imaging of FFPE tissues can be performed reproducibly across different sites, operators and instruments. Although TMAs contain pre-selected tissue, a detailed histological annotation is necessary to obtain optimal results. Restricting the number of mass signals used for classification improves the performance of tissue based classifiers on new data. MALDI imaging based classifiers are able to generalize across sites.

Conclusions
- MALDI Imaging on FFPE-Tissue can be performed reproducibly
- Tissue classifiers are able to generalize across sites
- Feature selection improves the performance of classifiers
- Histological annotations are necessary even on TMA data

MALDI Tissuetype

For research use only. Not for use in clinical diagnostic procedures.

References