Intraoperative-DESI-MS-based Biopsy Analysis Platform for Glioma Diagnosis, Extent of Tumor Cell Infiltration Estimation, and IDH Subtyping

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Background and introduction

Gliomas

- Surgical resection is primary treatment
- Grade IV five-year survival rate is 5%
- Residual tumor is primary cause of recurrence

Our goal: Develop and evaluate an intraoperative DESI-MS-based tool to assist neurosurgery by providing rapid diagnostic information

1) Isocitrate dehydrogenase (IDH) mutation status
2) Disease status
3) Tumor cell percentage (TCP)

Intraoperative MS at IUSM

Pirro et al. PNAS, 2017, 114(26): 6700-6705
Desorption electrospray ionization (DESI)

Detects metabolites and lipids directly from fresh tissue

Workflow of the intraoperative DESI-MS experiment

1) Tissue Smearing

2) Sample Loading on DESI Source

3) Sample raster under DESI Spray

4) MS Data Acquisition
Workflow of the intraoperative DESI-MS experiment

*Tissue Smearing*

*Sample Loading on DESI Source*

*Sample raster under DESI Spray*

*MS Data Acquisition*
2-hydroxyglutarate for assessing isocitrate dehydrogenase mutation status

\[
\text{α-ketoglutaric acid} \\
\text{Mutant IDH} \\
\text{2-hydroxyglutaric acid (2HG)}
\]

2HG DESI Signal Correlates with 2HG Concentration

\[y = 0.0619x - 2.4573 \quad R^2 = 0.986\]

Histopathologically Correlated DESI-MS Database was created

Lipid and metabolite profiling with DESI-MS imaging

Pathology-based Region-Of-Interest (ROI) Selection

41 Tissue Sections

447 ROIs Selected

Assessing disease status using PCA-LDA

Intraoperative (unknown) sample

Met. profile

Multiply by training set lipid and metabolite loading matrices

Lip. profile

Multiply by training set fused loading matrix

Combined PC Scores → Fusion PC Scores → LDA Prediction and Classification

Multiply by training set lipid and metabolite loading matrices

Met. PC Scores

Lip. PC Scores

Fusion PC Scores

Standard normal variate normalized metabolite and lipid profiles

Data fusion

SCORE PLOT

Project unknowns onto fused PC score space and predict disease state with LDA

Scores on PC1 (45.7%)

Scores on PC2 (27.6%)

Grey matter

White matter

Glioma
Assessment of tumor infiltration

What about mixtures (real samples)?
Do dynamics of lipid profile reflect tumor infiltration?

Glioma

White Matter

Grey Matter

Assess tumor infiltration using the lipid signature of the tissue

Regression using PC scores and ternary mixture model

\[ Y = \beta_{GM} X_{GM} + \beta_{WM} X_{WM} + \beta_{G} X_{G} \]

\[ [PC1 \ PC2] * [%GM \ %WM \ %GL] \]

\[ \sum [%GM \ %WM \ %GL] = 100\% \]

### Summary of intraoperative patient cohort

| Subjects                  | 58 recruited  
|                          | 6 Clinical Screenfails  
|                          | 3 DESI Outliers  
| Biopsies                 | 247 Total  
| Smears                   | 334 Total  
|                          | 62 excluded  
|                          | 272 smears included in statistics  
| Gender                   | 24 F  
|                          | 25 M  
| Age (years)              | 46 ± 16  
| IDH Status               | 23 Mutant  
|                          | 25 Wild-Type  
|                          | 1 Unknown  
| Glioma Grade             | 1 Grade I  
|                          | 17 Grade II  
|                          | 6 Grade III  
|                          | 25 Grade IV  

![MRI images](image-url)
Diagnosis of tumor cores and surgical margins-example from a single subject

Biopsy 155: Glioma, High TCP, IDH Mutant

Biopsy 154: Infiltrative margin (white matter), Low TCP, IDH Mutant
IDH Mutation status was classified with 92.4% accuracy

158 smears, 29 subjects

Sensitivity 86.6%
Specificity 97.5%

Decision line = 6.18
Comparison of histopathology diagnoses with DESI-MS diagnoses

<table>
<thead>
<tr>
<th>Lipid Deconvolution TCP Category</th>
<th>Low</th>
<th>Medium</th>
<th>High</th>
<th>Total</th>
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</thead>
<tbody>
<tr>
<td>Low</td>
<td>92</td>
<td>23</td>
<td>11</td>
<td>126</td>
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<tr>
<td>Medium</td>
<td>15</td>
<td>9</td>
<td>14</td>
<td>38</td>
</tr>
<tr>
<td>High</td>
<td>8</td>
<td>20</td>
<td>80</td>
<td>108</td>
</tr>
<tr>
<td>Total</td>
<td>115</td>
<td>52</td>
<td>105</td>
<td>272</td>
</tr>
</tbody>
</table>

Mixed tissue samples are challenging for our current data and statistical methods.
Conclusions

Intraoperative DESI-MS system was developed and utilized over a 4-year period to analyze brain tissue biopsies from 49 human subjects.

IDH Mutation assay performed the best and provided accuracy of 92.4%.

Accurate prediction of mixtures was difficult.

Few low-TCP samples were misclassified as high-TCP; few high-TCP samples were misclassified as low-TCP.