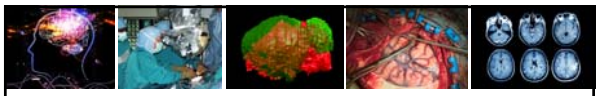


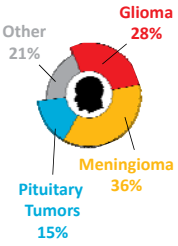
AACC/MSSS 2016

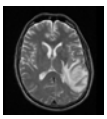
Ambient Ionization Mass Spectrometry for Brain Cancer Diagnosis

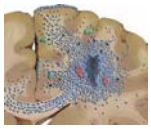
Valentina Pirro

Brain Cancer

- ***4 out of 5 malignant tumors are gliomas***
- ***5-year survival rate is 5% for glioblastoma***
- ***Brain tumors tend not to metastasize***
- ***Gliomas infiltrate into normal parenchyma***
- ***Unresected tumor is the primary cause of cancer reoccurrence***



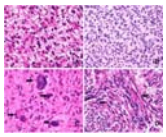




Main challenges

Accurate diagnosis

Combination of histology and molecular information



GFAP
IDH-1 / R132H
ATRX
P53
Ki-67
1p/19q

- Laborious and time demanding
- Discordant diagnosis
- Assessed post-operatively

Efficient treatment

Surgical resection

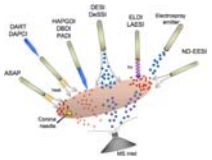
- Maximize tumor resection
- Minimize neurological damages
- No assessment of surgical margins

Luis et al. 2016 Acta Neuropathologica

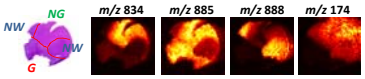
Can additional molecular information help?

- ***Provide new molecular-based metrics not accessible via morphological evaluation***
- ***Reproduce the metrics of current pathology using molecular information*** *(Tumor presence, tumor type, tumor grade)*
- ***Develop rapid and simple methodologies for intraoperative consultation***

Mass Spectrometry



- ***Specific for molecular identification***
- ***Sensitive***
- ***Adaptable***
- ***Ambient Ionization***
- ***Imaging***



Can ambient ionization MS become an integral technology for CNS tumor diagnosis?

Zhang et al. 2016 Clinical Chemistry

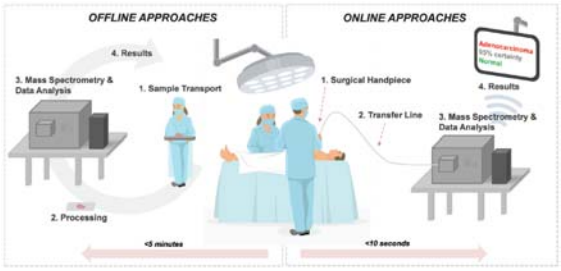
Outline

1. ***Describe ambient Ionization strategies developed for brain cancer diagnosis***
2. ***Discuss the diagnostic molecular information***
3. ***Introduce statistical approaches for mass spectral pattern recognition***
4. ***Present the first DESI-MS intraoperative results***

Brief History

Year	Topic	Author	Journal
2004	Development of ambient ionization	Takáts et al.	*Science*
2005	Tissue by ambient ionization	Wiseman et al.	*Angew. Chem.*
2009	Introduction of REIMS	Schäfer et al.	*Angew. Chem. Int. Ed.*
2010	DESI-MS analysis of human brain	Eberlin et al.	*Angew. Chem.*
2012	DESI brain tumor discrimination	Eberlin et al.	*Cancer Research*
2013	DESI imaging of surgical sample	Eberlin et al.	*PNAS*
2013	Introduction of iKnife	Balog et al.	*Sci. Transl. Med.*
2014	Study of 2HG by DESI-MS	Santagata et al.	*PNAS*
2014	Meningiomas by DESI-MS	Calligaris et al.	*IJMS*
2015	Spray-from-tissue ionization	Kononikhin et al.	*Anal. Bioanal. Chem.*
2016	NAA by DESI-MS to estimate TCP	Jarmusch et al.	*PNAS*
2017	DESI-MS (+) for tumor discrimination	Jarmusch et al.	*PLOS ONE*

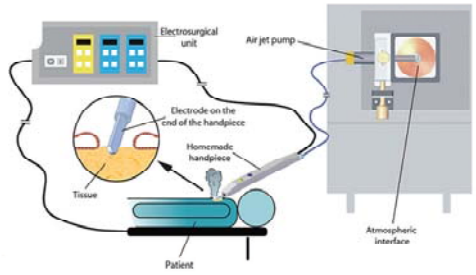
1. Ambient Ionization strategies



Ifa et al. 2016 Clinical Chemistry

REIMS or i-knife

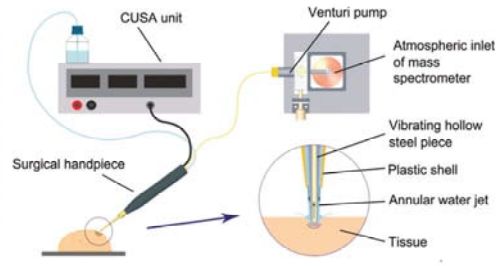
Online, in vivo, intrasurgical



Balog et al. 2013 Sci. Transl. Med.

CUSA/V-EASI

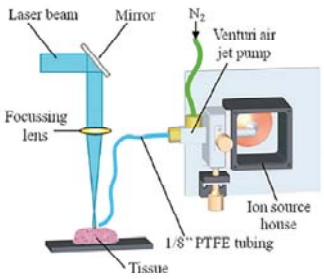
Online, ex vivo, intrasurgical



Ifa et al. 2016 Clin. Chem.; Schafer et al. 2011 Anal. Chem.

LDI-MS

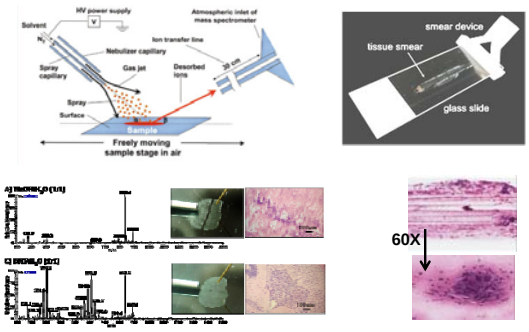
Online, ex vivo, intrasurgical



Schafer et al. 2011 Anal. Chem.

DESI-MS

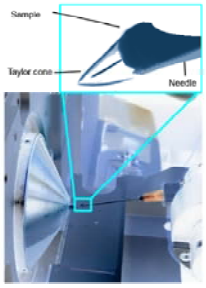
Offline, ex vivo, intrasurgical

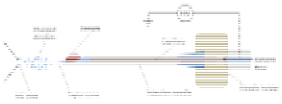


Takats et al. 2004 Science; Eberlin et al. 2011 Chembiochem; Jarmusch et al. 2016 PNAS

Tissue spray ionization MS

Offline, ex vivo





Kononikhin et al. 2015 Anal Bioanal Chem

2-3. Diagnostic molecular information

- ***Full scan mass spectral patterns are acquired***
- ***Use of low resolution MS to detect discriminatory patterns***
- ***Standardization protocols have not been established yet***

Schafer et al. 2011 Anal Chem; Kononikhin et al. 2015 Anal Bioanal Chem

Multivariate pattern recognition strategies are used to build classification models

Pathologically defined samples

↓

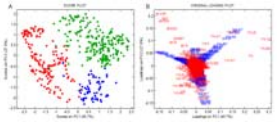
Classification methods

↓

Cross-validation

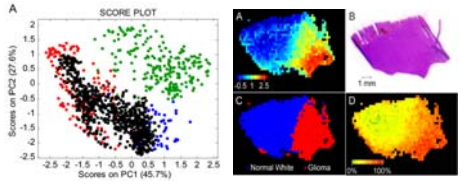
↓

Validation



	Predictions	
Belonging to	**Class 1**	**Class 2**
Class 1	**True**	**False**
Class 2	**False**	**True**

Use classification models for prediction of unknowns samples



Chemical results can be coregistered with stereotactic information

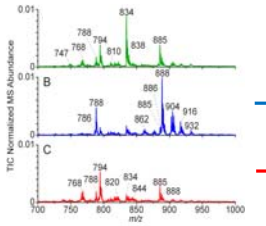
Agar et al. 2011 Neurosurgery; Jarmusch et al. 2015 PNAS

2-3. Diagnostic molecular information

❖ ***Glycerophospholipids***

- *Normal grey and white parenchyma have highly distinct lipid profiles*
- *Gliomas are known to infiltrate white matter more aggressively*

● Grey matter ● White matter ● Glioma

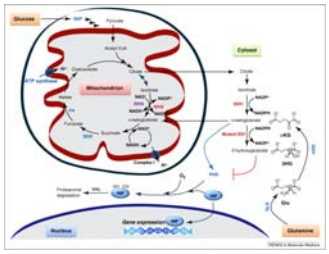


Increased abundance of sulfatides correlated with myelination of neurons

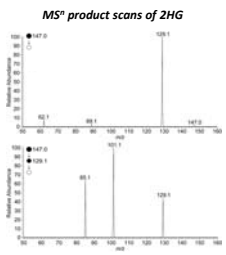
Increased abundance of PCs related to cell proliferation

❖ ***2-hydroxygluteric acid (2HG)***

- *2HG is a downstream product indicative of the presence of IDH mutation*
- *Presence of IDH mutation correlates with better patient outcomes*
- *IDH mutation is currently assessed post-operatively*



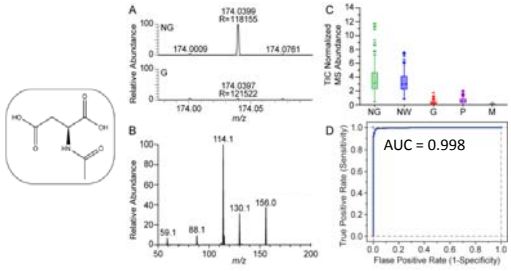
MSⁿ product scans of 2HG



Santagata et al. 2014 PNAS; Jarmusch et al. 2015 PNAS; Dang et al. 2010 Trends Mol. Med.

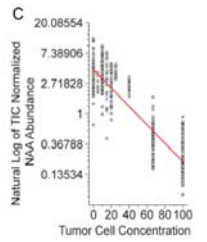
❖ ***N-acetyl aspartic acid, NAA***

- *NAA is an abundant molecule likely involved in neural metabolism*
- *NAA content in brain tissue decreases in several in neurological diseased and disorders*

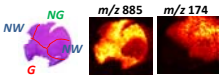


Jarmusch et al. 2015 PNAS

Exponential-like decay of NAA signal at the increase of tumor cell percentage



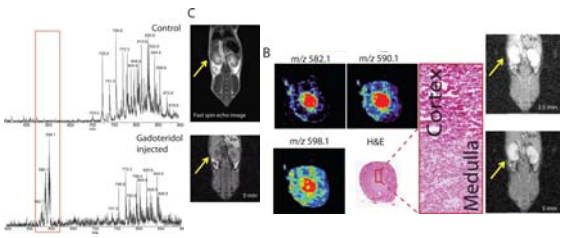
Distribution of NAA in a tissue section analyzed by DESI-MS



Estimated percentage of tumor cells in tissue smears

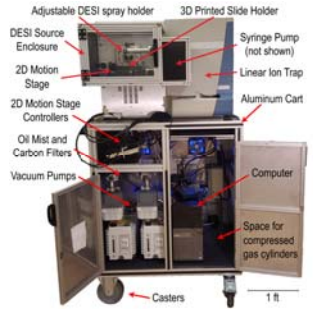
❖ ***Contrast agents***

Detect contrast agents by MS to map tumor heterogeneity and tumor boundaries



Tata et al. 2015 Anal. Chem.

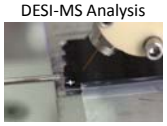
4. DESI-MS: clinical cases



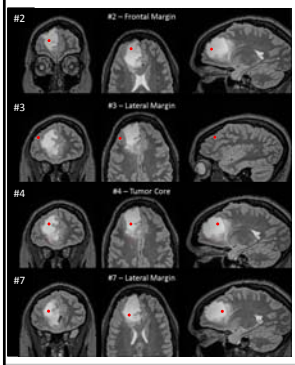
Stereotactic biopsy

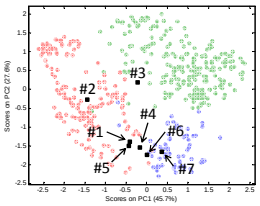
Smear Tissue

DESI-MS Analysis



Case 6 – diffuse infiltrating glioma, astrocytoma, IDH1 mutated



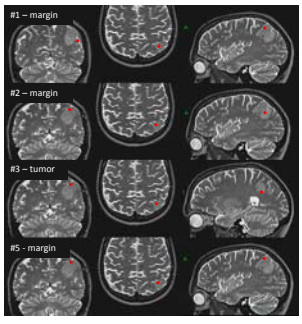


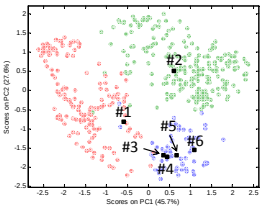
#	DESI-MS Prediction		Pathology	
	Diagnosis	TCP	Diagnosis	TCP
2	Glioma	93	Glioma	90
3	Grey Matter	11	Inf. Margin (Grey Matter)	15
4	Glioma	76	Glioma	70
7	White Matter	30	Inf. Margin (matter not specified)	15

unpublished data

23

Case 7 – glioblastoma, IDH wildtype





#	DESI-MS Prediction		Pathology	
	Diagnosis	TCP	Diagnosis	TCP
1	Glioma	70	Glioma (High grade)	90
2	Grey Matter	0	Inf. Margin (NOS)	10
3	White Matter	35	Glioma (High grade)	50
5	White Matter	12	Inf. Margin (NOS)	10

unpublished data

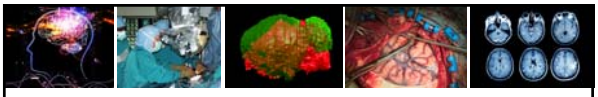
24

Conclusions

- Ambient ionization MS can be successfully integrated in the intrasurgical consultation both using online and offline approaches.

Metrics	Traditional Pathology	MS diagnostics
Tumor Presence	✓	✓
Type (Glial or non-glial)	✓	✓
Tumor Cell %	n/a	Regression of NAA Abundance
IDH mutant	n/a	MS/MS (2HG)
Primary or Secondary	Case History/Morphology	Future work
Grade (High/Low)	Morphology	Future work
Glioblastoma	Morphology	Future work

- Molecular information can assist tumor resection at surgical margins
- Clinical trials are necessary to better understand correlation between molecular and morphological information, between molecular information and patient outcomes (survival rate, etc)



Thanks for your attention