

“Análises Químicas por Espectrometria de Massas: uma nova ferramenta para a patologia”

Andréia de Melo Porcari
Outubro, 2016

Espectrometria de Massas

Espectrometria de Massas (MS)

- Seletividade / Especificidade
- Sensibilidade
- Análises Múltiplas (vários analitos)
- Confiabilidade Analítica
- Elucidação Estrutural
- Quantificação



Introdução
de
amostras

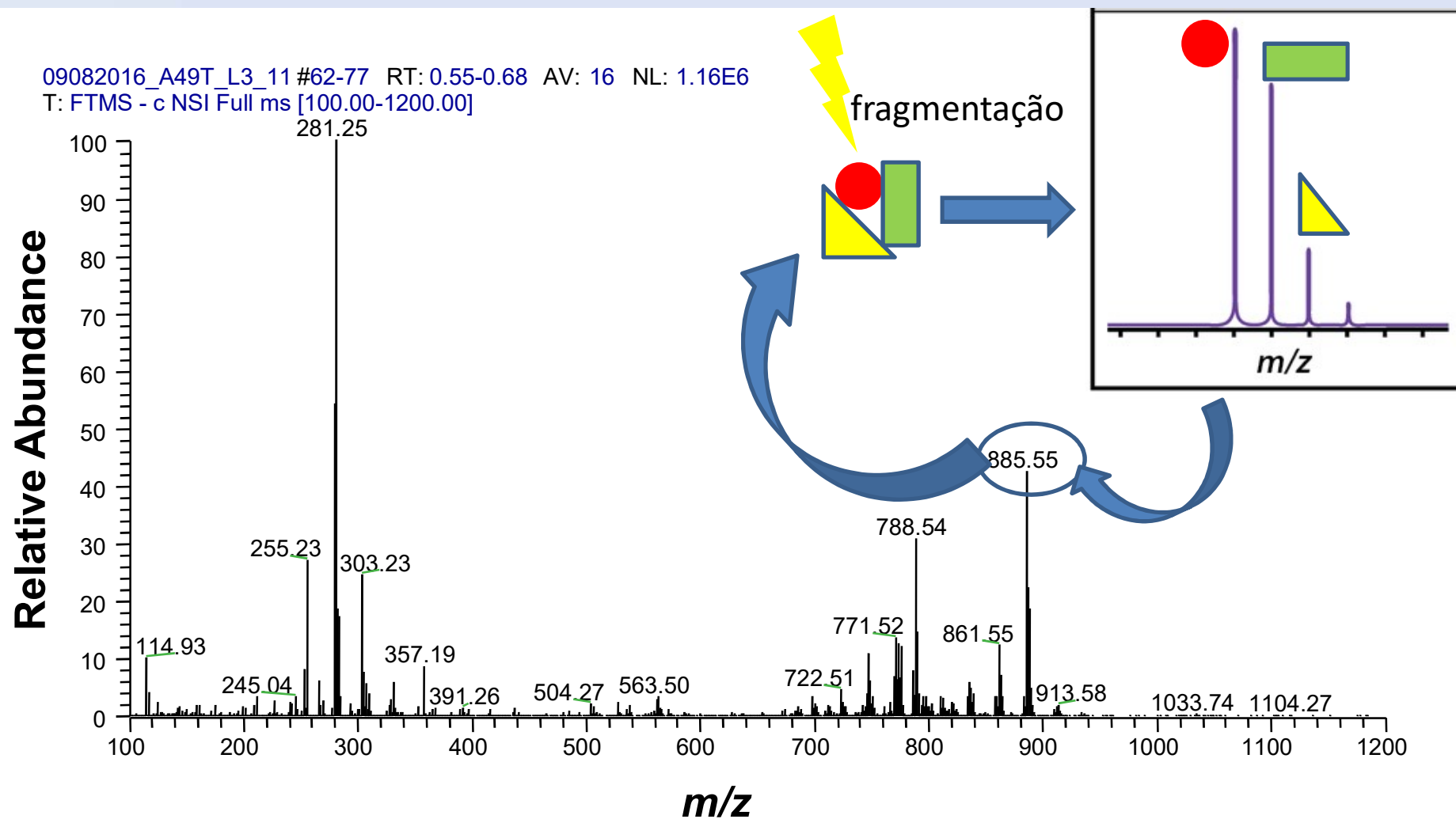
Fonte de
ionização

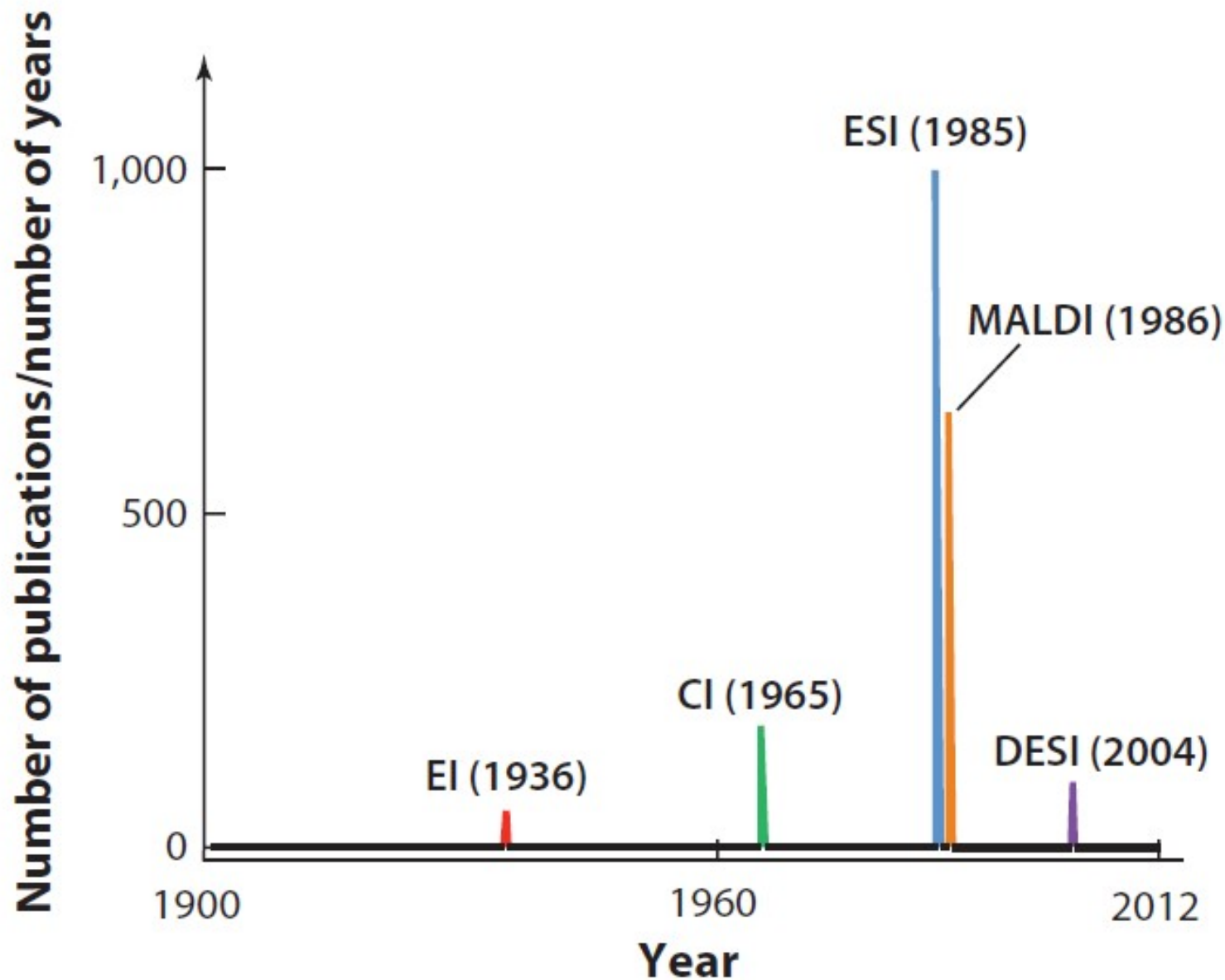
Analizador
de m/z

Detector

Vácuo

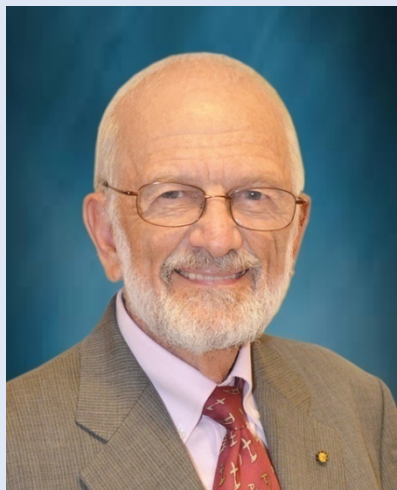
Espectro de Massas





Annu Rev Phys Chem. 2013;64:481-505

Ambient Ionization



Professor Graham Cooks – Purdue University – USA -2004

Mass Spectrometry Sampling Under Ambient Conditions with
Desorption Electrospray Ionization

Science, 306(5695), pp.471-473.



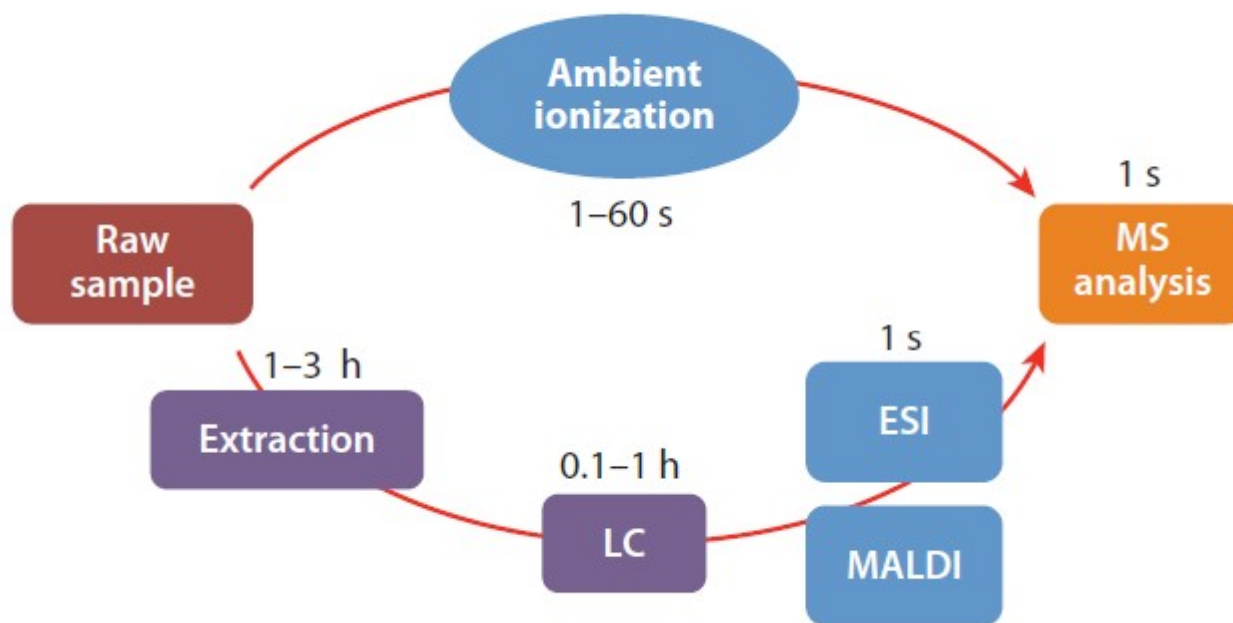
Preparo de
amostra

Science 306, no. 5695 (2004): 471-473



Ambient Ionization

Analysis of complex condensed-phase samples



Annu Rev Phys Chem. 2013;64:481-505

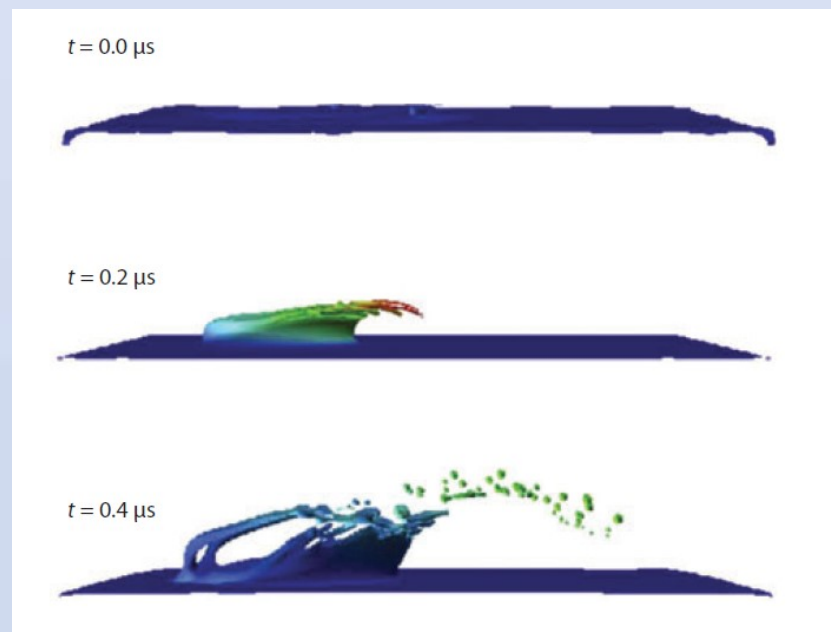
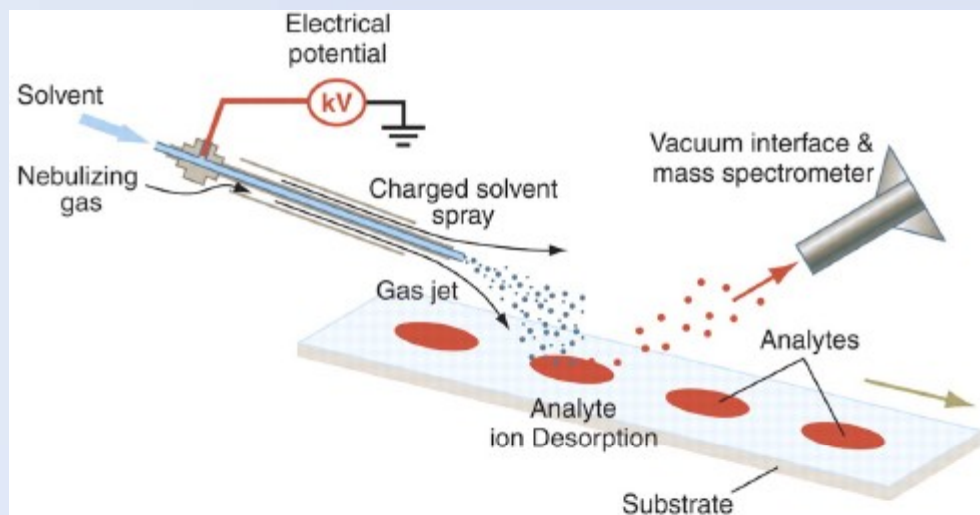
III Fórum de Ensino Superior da Área Química:
"Avanços na Identificação e Determinação de Substâncias"
21 de outubro de 2016

Conselho Regional de Química – IV
Comissão de Ensino Superior

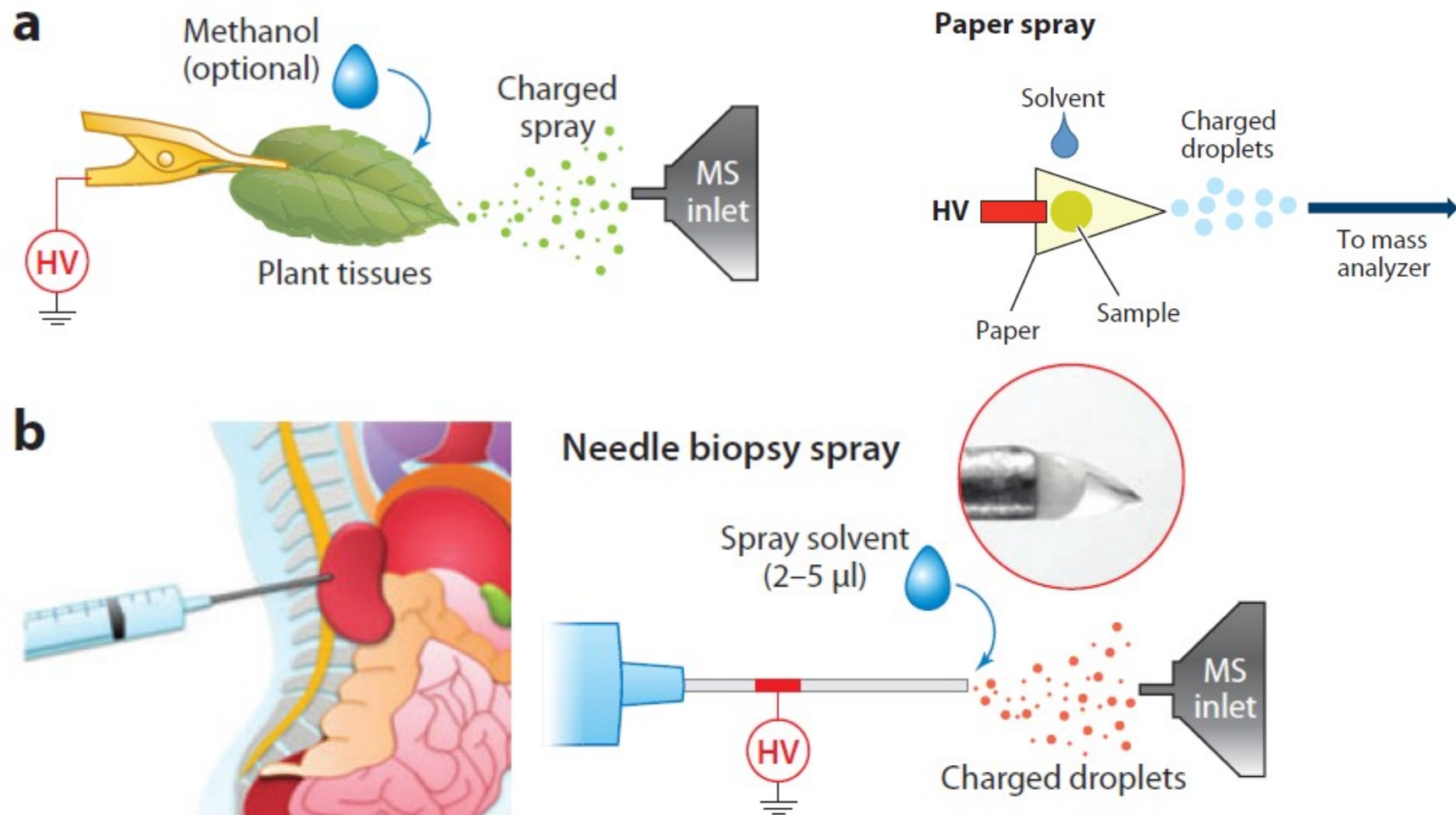


DESI-MS

Desorption Electrospray Ionization - MS



Annu Rev Phys Chem. 2013;64:481-505x
Science 306, no. 5695 (2004): 471-473

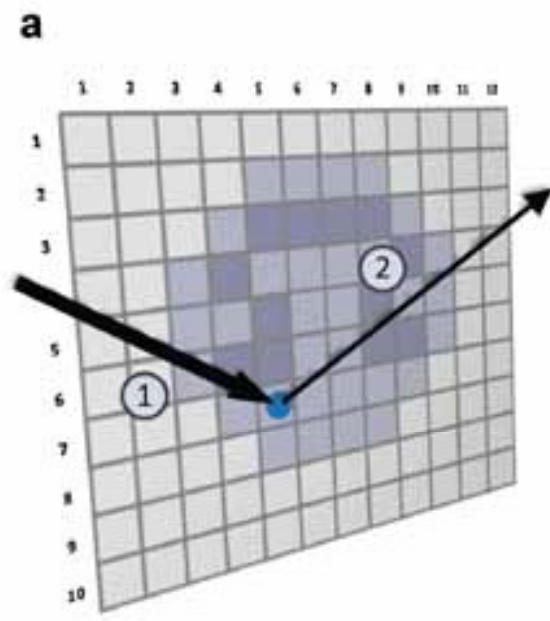


Spray ambient MS features:

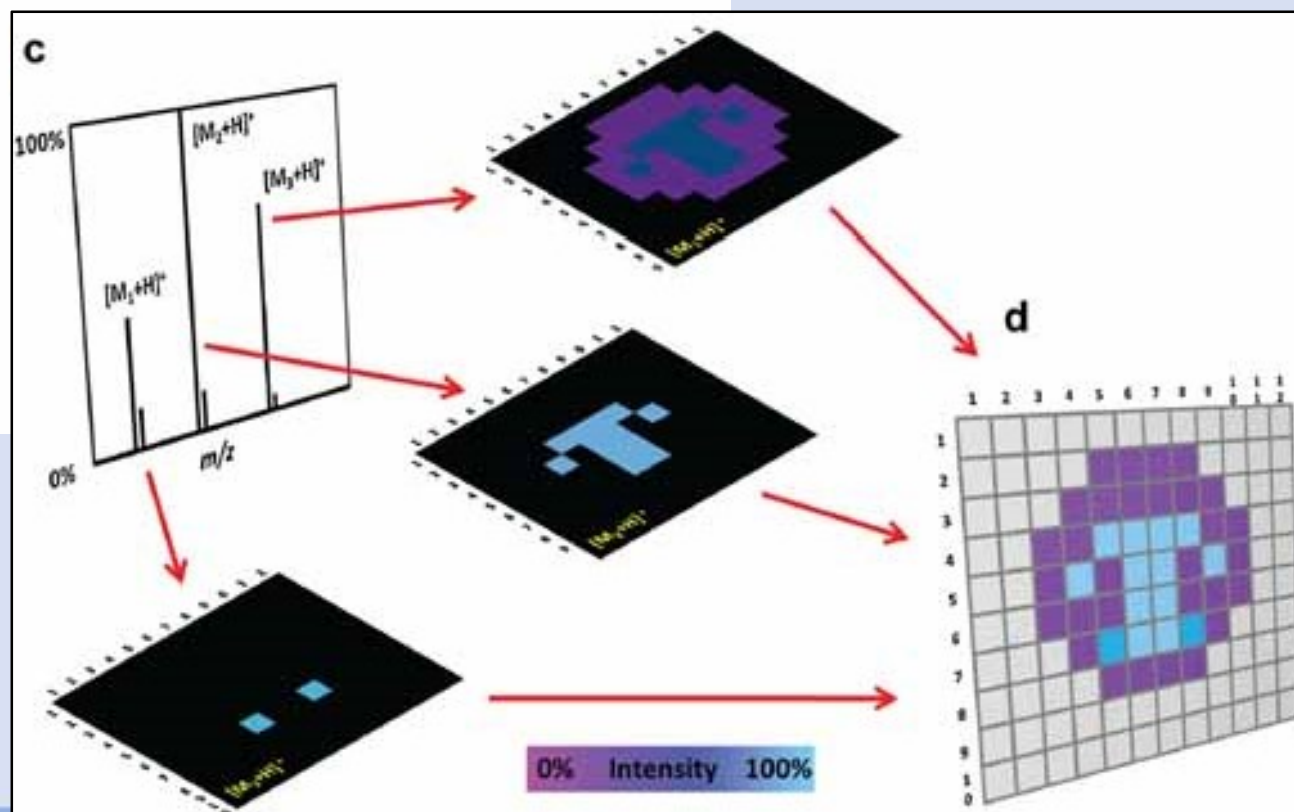
- Direct ionization
- Minimum sample pretreatment
- Ambient environment
- Microextraction of analytes

DESI-MS Imaging

Mass Spectra linked to
Spatial Coordinates (X,Y)

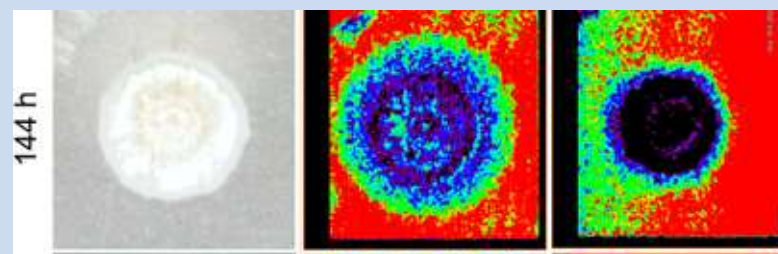
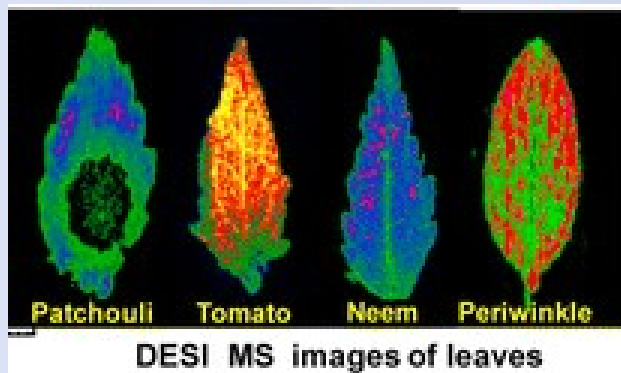
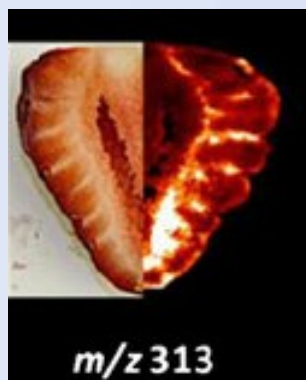
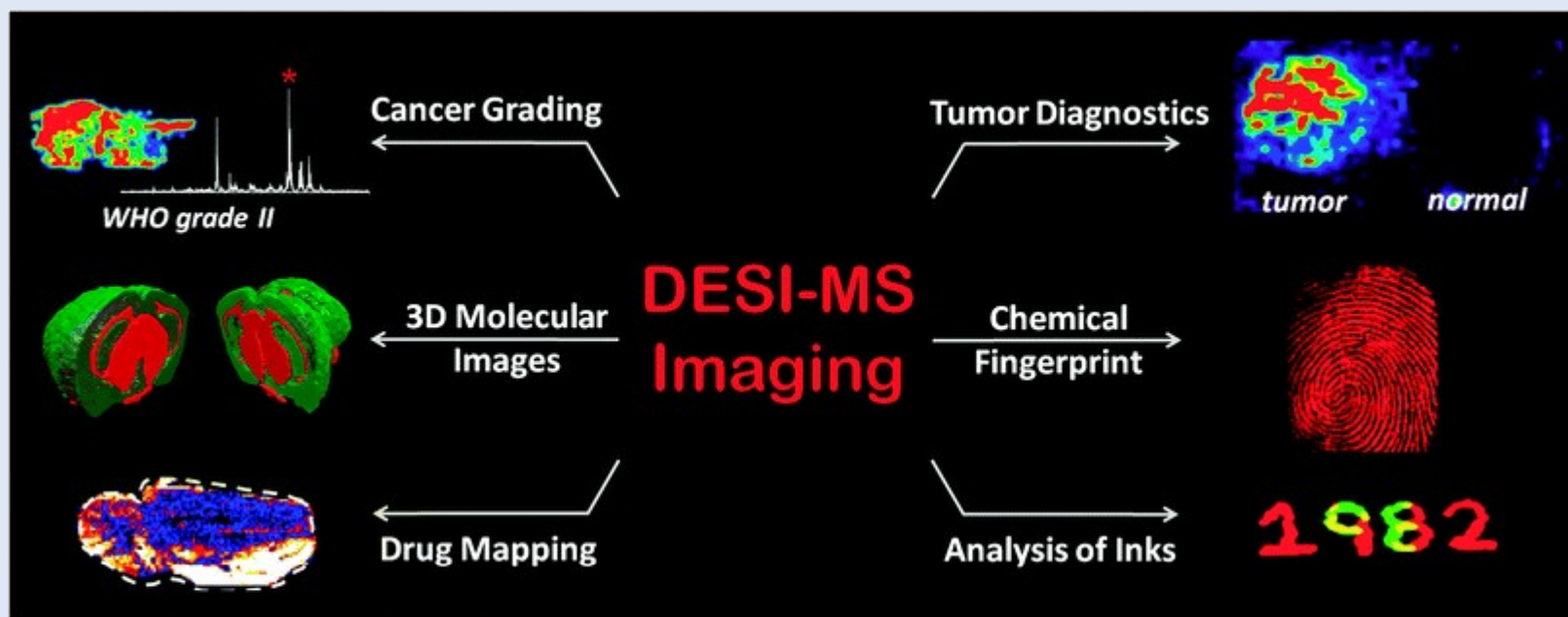


Resolução espacial
de 100 – 200 μm



Mass Spectrom Rev. 2013;32(3):218-43

DESI-MSI



J. Agric. Food Chem., **2013**, 61 (31); *Chem Commun (Camb)*, **2011**; 47(10)

III Fórum de Ensino Superior da Área Química:
"Avanços na Identificação e Determinação de Substâncias"
 21 de outubro de 2016

Conselho Regional de Química – IV
 Comissão de Ensino Superior



Imaging Mass Spectrometry: Enabling a New Age of Discovery in Biology and Medicine Through Molecular Microscopy

Richard M. Caprioli^{1,2}

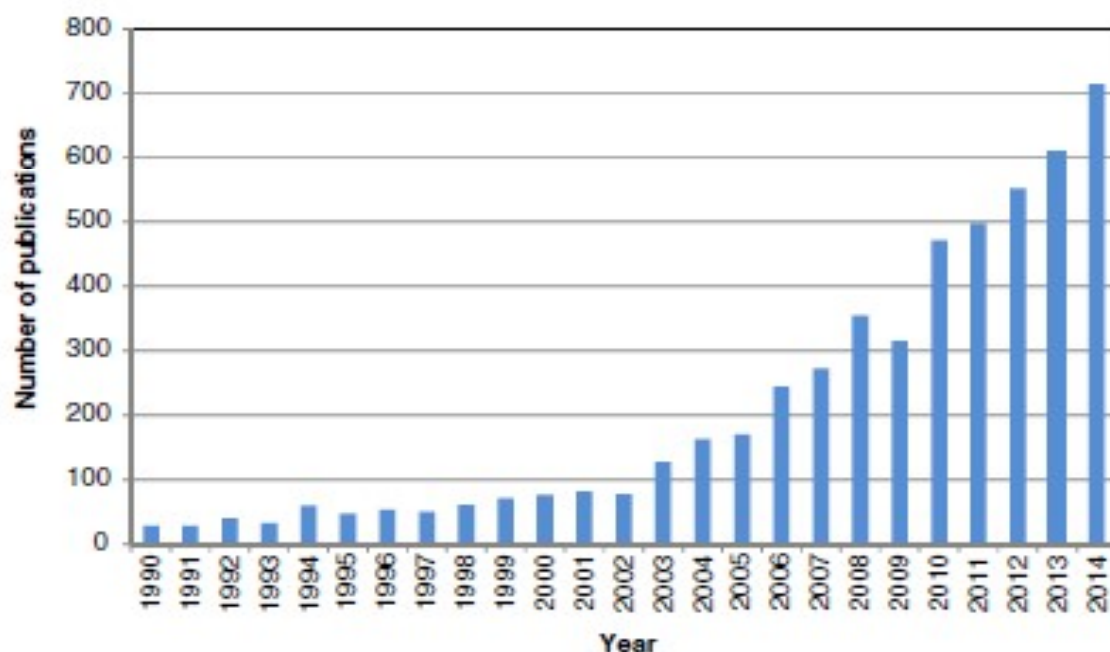


Figure 1. Number of publications by year from data obtained from a search with SciFinder Scholar for imaging mass spectrometry

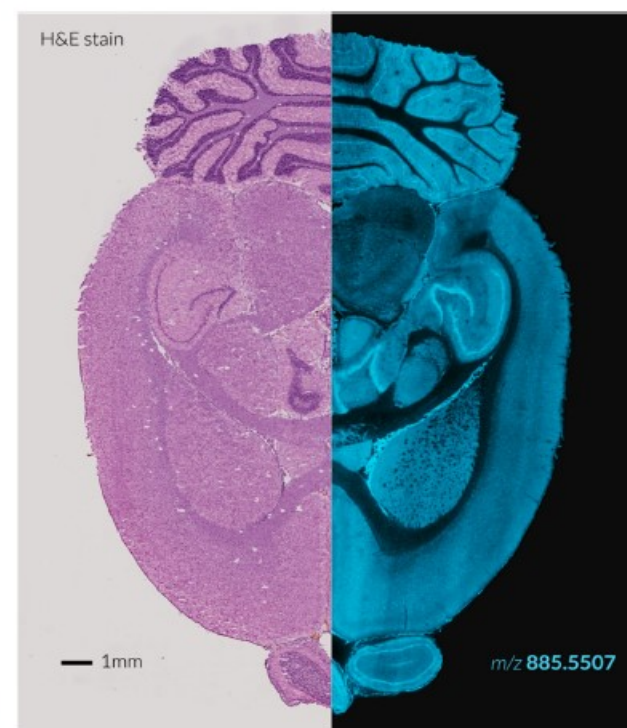
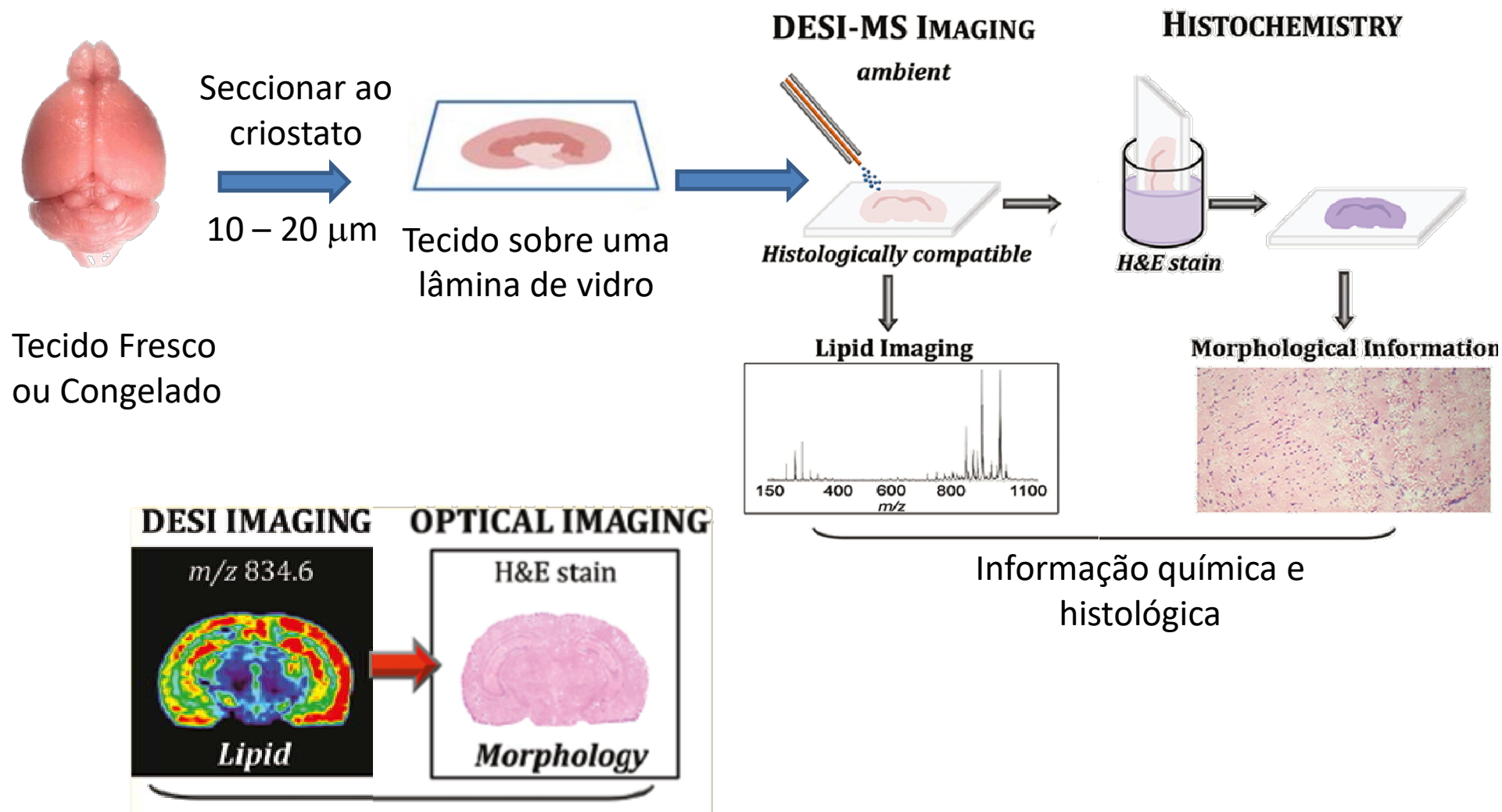


Figure 2. (left panel) – Partial microscopic image of a transverse section of mouse brain stained with hematoxylin/eosin. (right panel) – MALDI MS image of a partial brain section of the ion at m/z 885.5507 – [PI(38:4)] taken at a spatial resolution of 15 μm on a Bruker Solarix 15 T FTICR MS in the negative-ion mode and with $>100,000$ resolving power at m/z 400. These data were acquired at a laser repetition rate of 2 kHz and an acquisition speed of ~ 0.35 s/pixel. The image dataset has a total of 364,740 pixels and a file size of ~ 800 GB

DESI-MSI workflow para tecidos



Solvente Histocompatível

Eberlin et al.

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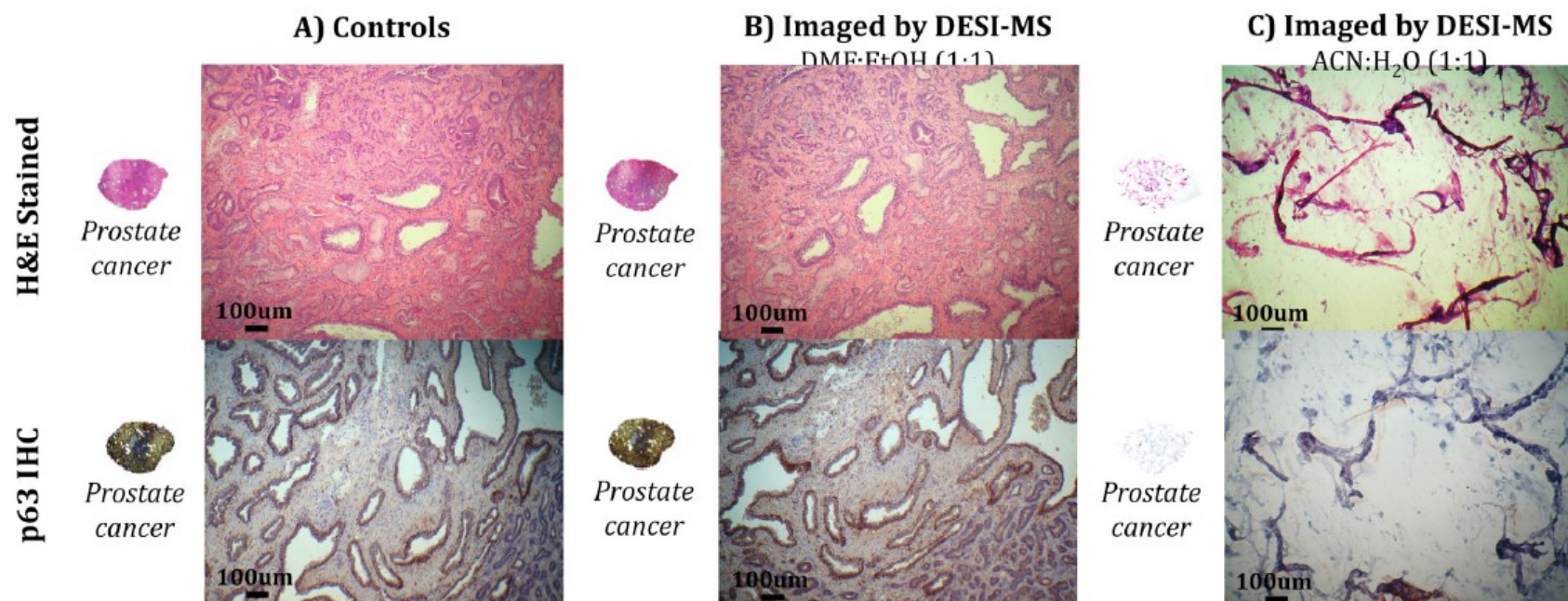


Figure 3.

Magnified brightfield optical images of prostate cancer tissue sections subjected to H&E stain or p63 IHC. Optical images of the entire tissue sections are shown to the left of each magnification. When compared to controls (A) no effect on tissue morphology was observed in tissue sections first imaged by DESI-MS using (B) DMF:EtOH (1:1). Tissue destruction occurs using conventional solvent systems, such as (C) ACN:H₂O (1:1).

Desorption Electrospray Ionization then MALDI Mass Spectrometry Imaging of Lipid and Protein Distributions in Single Tissue Sections

Livia S. Eberlin,[†] Xiaohui Liu,[‡] Christina R. Ferreira,[†] Sandro Santagata,[§] Nathalie Y.R. Agar,^{*,‡} and R. Graham Cooks^{*,†}

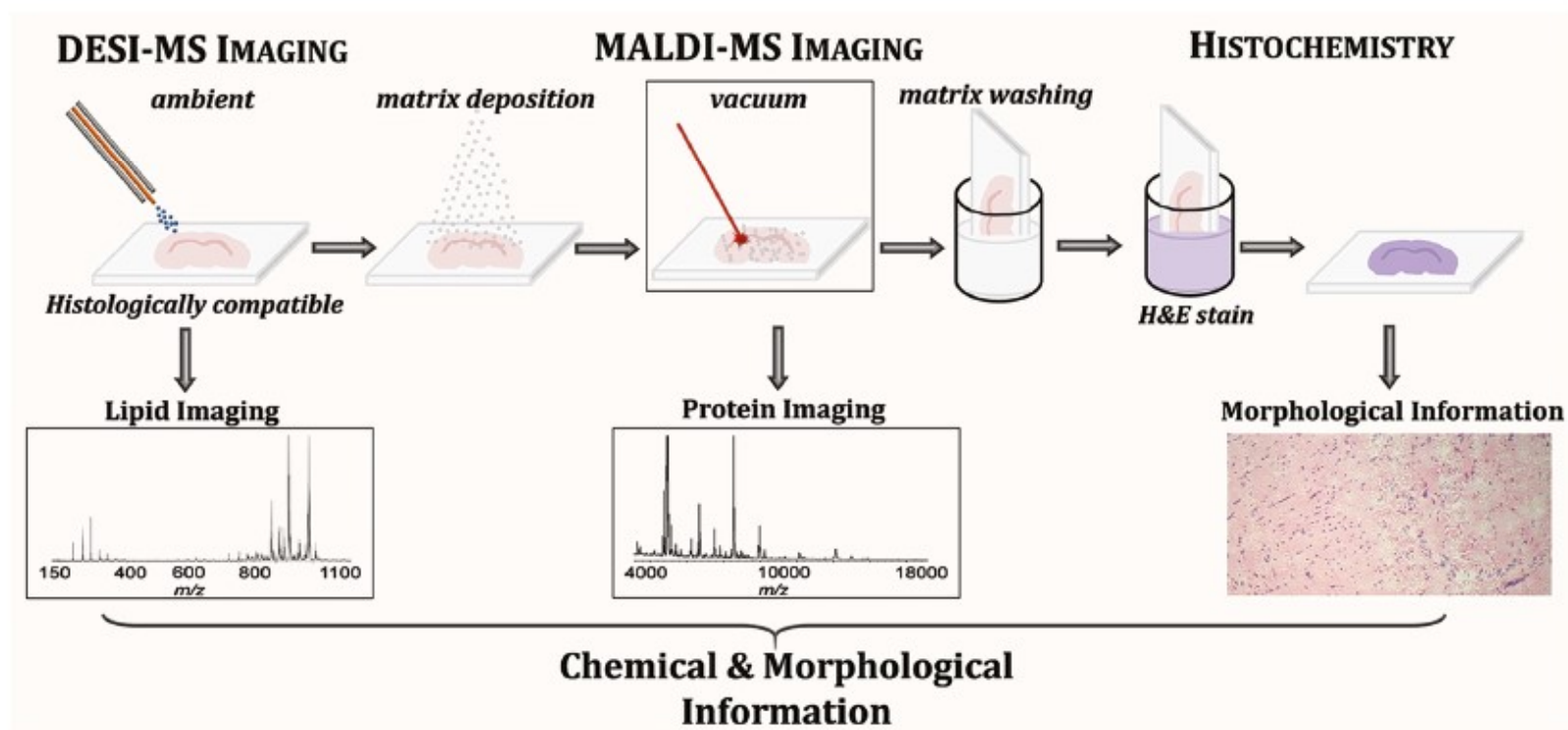
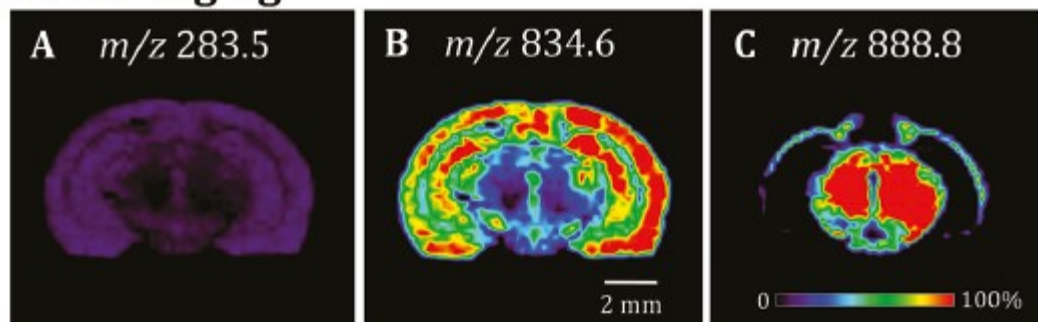
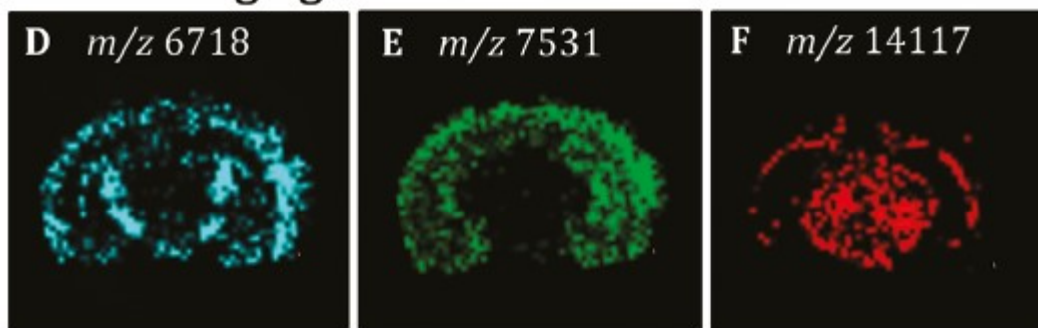


Figure 1. Schematic of the workflow used to perform DESI-MS and MALDI-MS imaging on the same tissue section thaw mounted onto an ITO glass slide, followed by H&E staining.

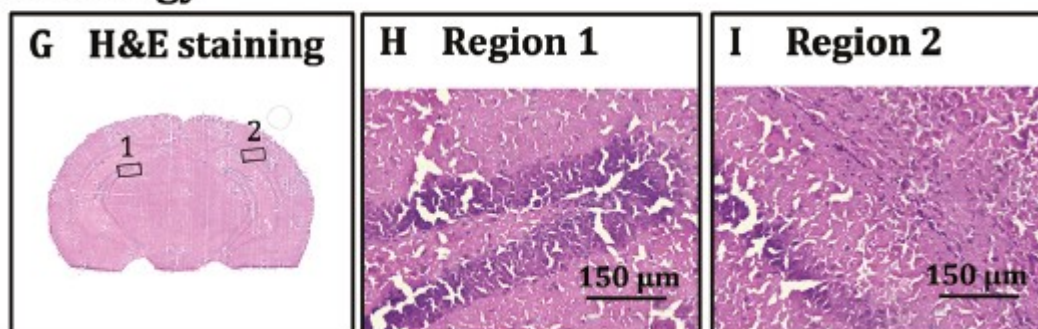
DESI imaging



MALDI imaging



Histology



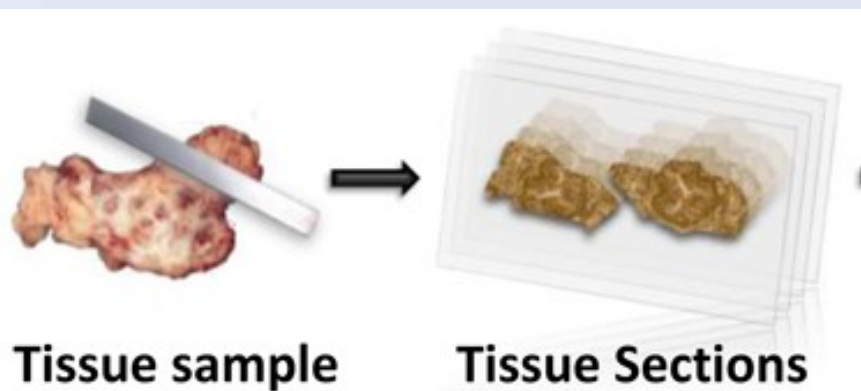
(A) m/z 283.5, FA(18:0), stearic acid;
(B) m/z 834.6, PS(18:0/22:6), and
(C) m/z 888.8, ST(24:1).

After DESI-MS imaging, the same tissue section was subjected to MALDI-MS imaging. Ion images are shown for ions

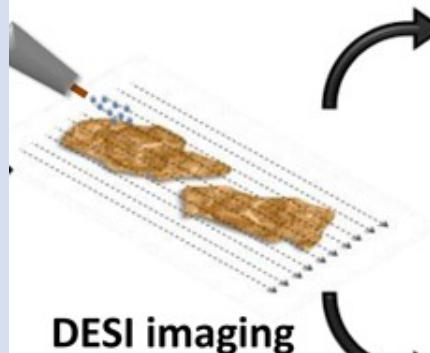
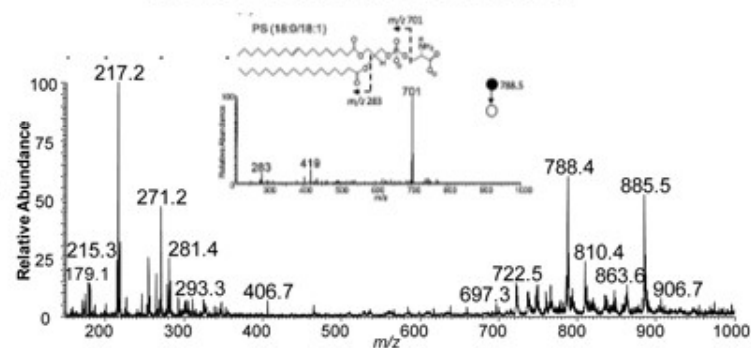
(D) m/z 6718, PEP19;
(E) m/z 7531, neurogranin;
and (F) m/z 14117, MBP (myelin basic protein) isoform 8.

Optical image of the same tissue section after removal of the MALDI matrix and H&E staining is shown in (G). High magnification views of different morphological regions are shown in (H) and (I).

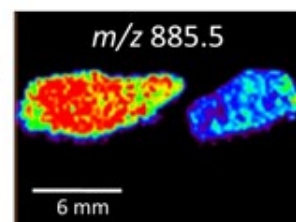
Como encontrar o marcador?



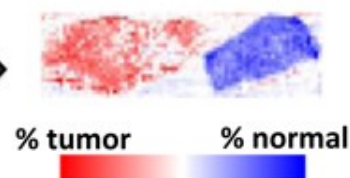
Tissue Characterization



DESI ion image



Statistical Analysis

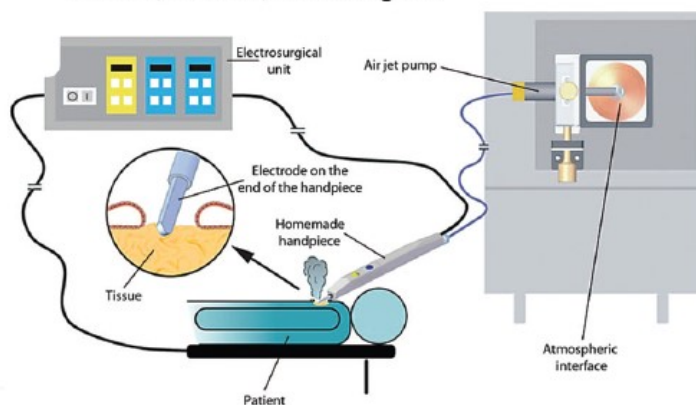


Biochim Biophys Acta. 2011;1811(11):946-60

Análise Online vs. Offline

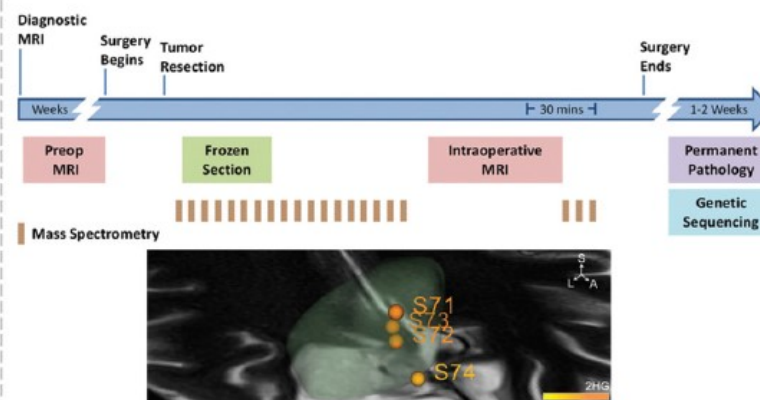
A) REIMS - iKnife

Online, in vivo, intrasurgical



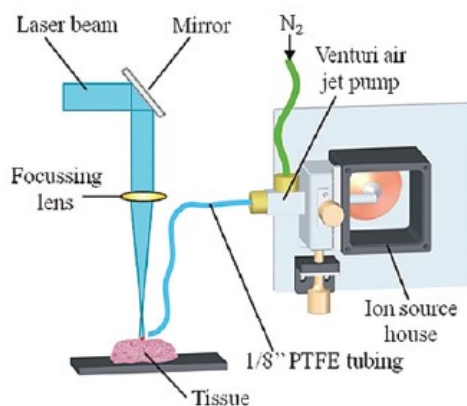
B) DESI-MS

Offline, ex vivo, intrasurgical



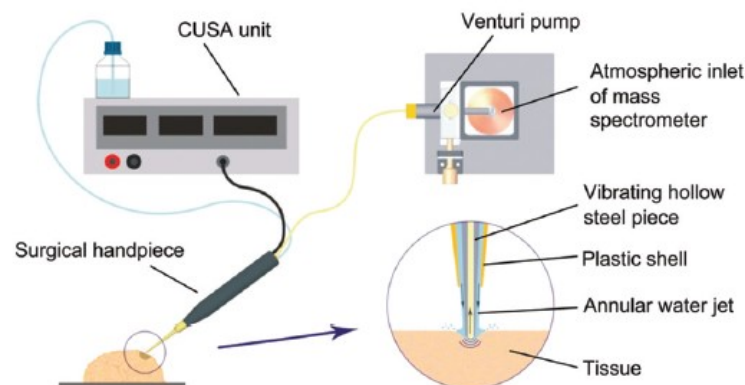
C) LDI-MS

Online, ex vivo



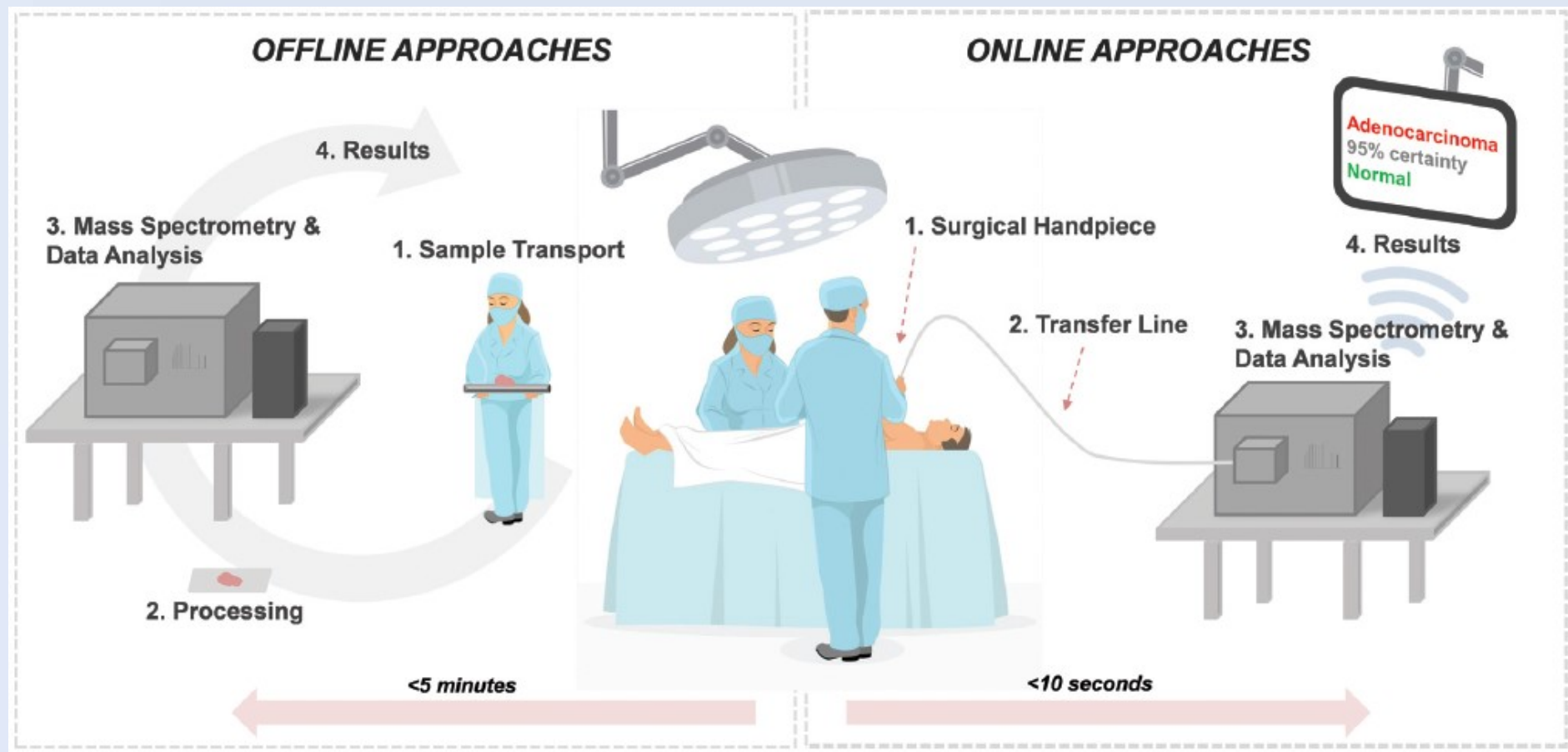
D) CUSA/EASI

Online, ex vivo



Clin Chem. 2016; 62(1):111-23

Análise Online vs. Offline



Clin Chem. 2016; 62(1):111-23

DESI-MSI para diagnóstico de câncer



Livia S. Eberlin – University of Texas

Molecular and Cellular Pathobiology

**Cancer
Research**

Classifying Human Brain Tumors by Lipid Imaging with Mass Spectrometry

Livia S. Eberlin¹, Isaiah Norton², Allison L. Dill¹, Alexandra J. Golby², Keith L. Ligon³, Sandro Santagata³, R. Graham Cooks¹, and Nathalie Y.R. Agar²



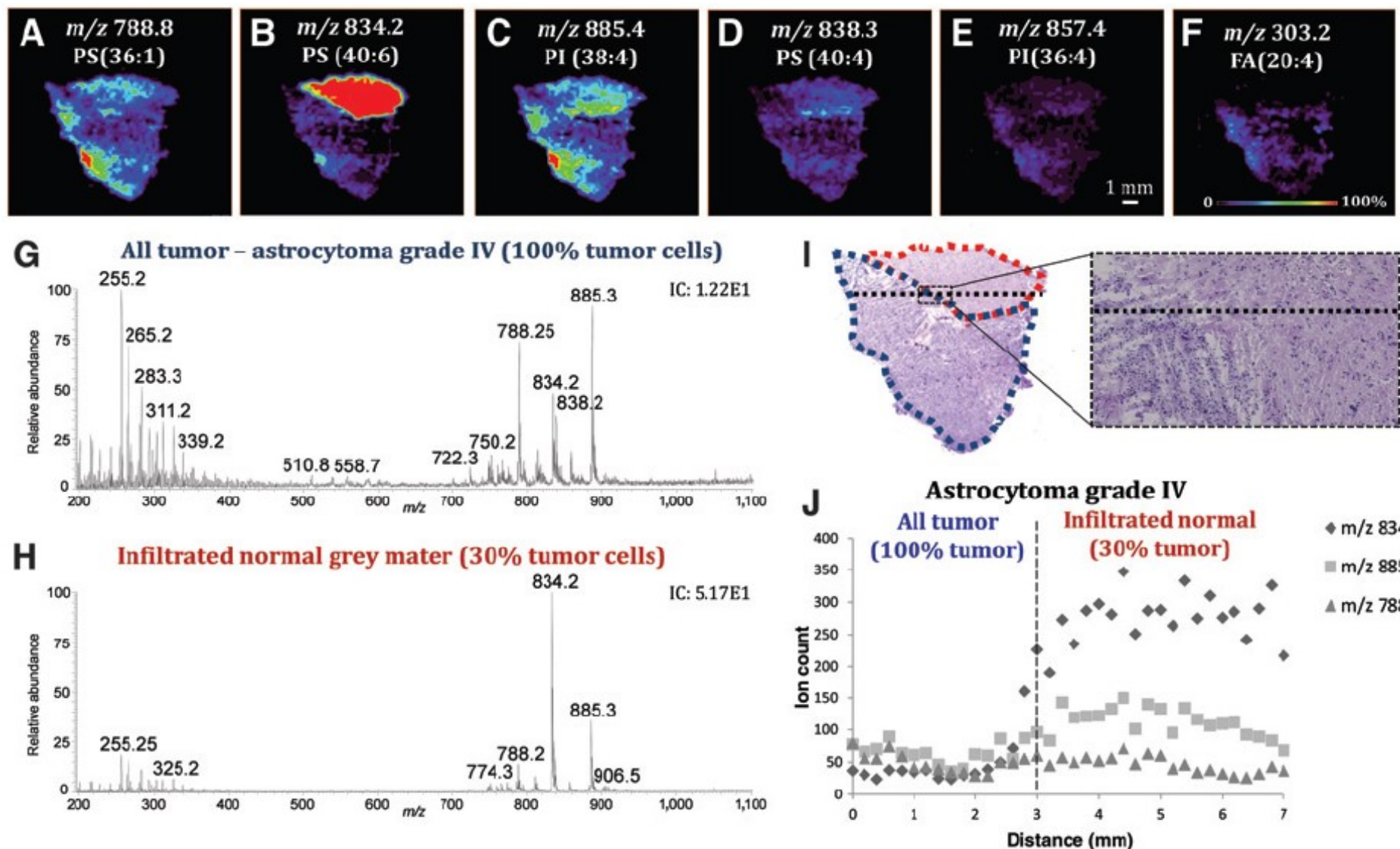
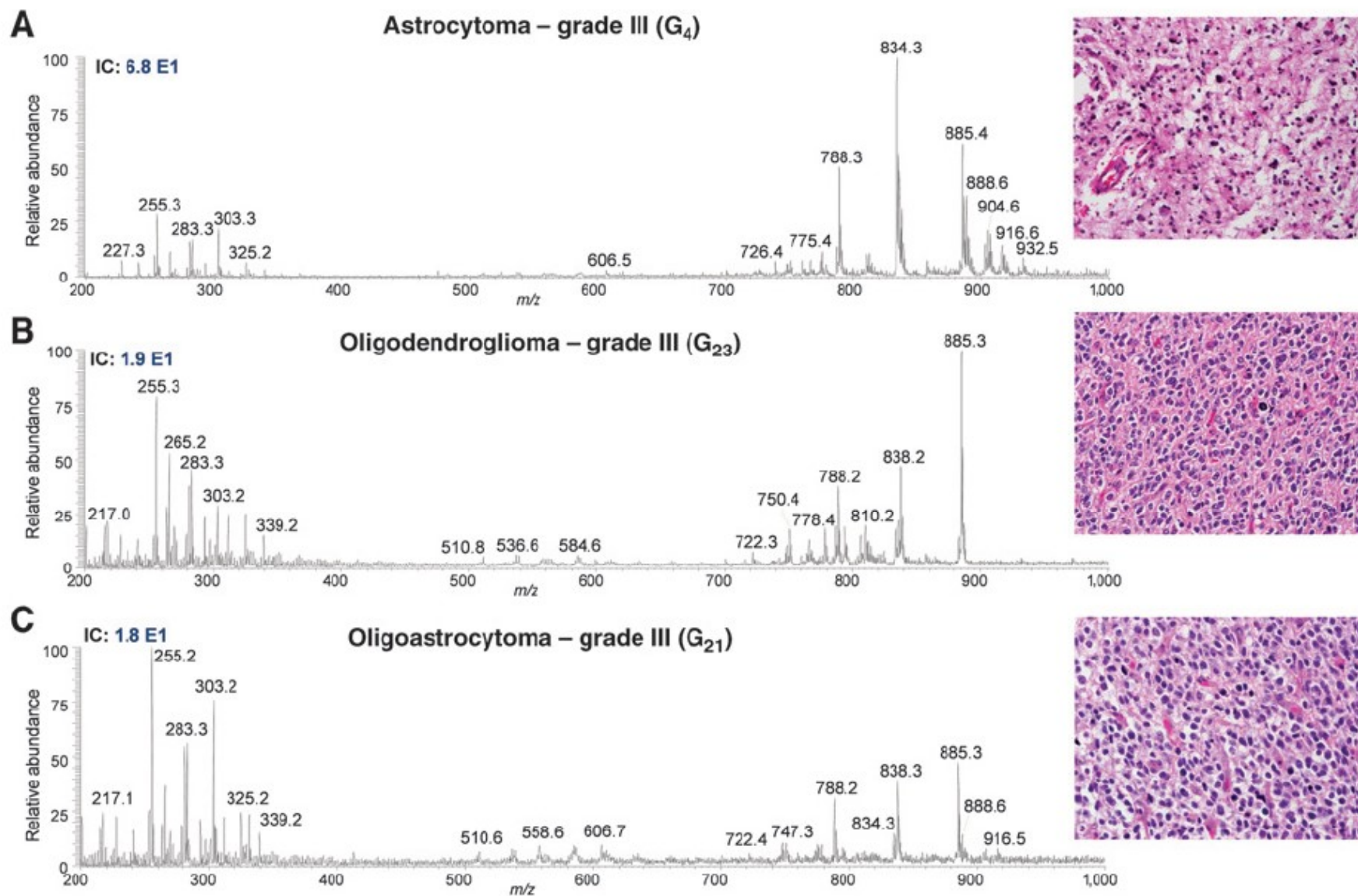


Figure 3. Tumor heterogeneity and infiltration is assessed by DESI-MS imaging. Negative ion mode DESI-MS ion images of glioblastoma G₃₃ showing the distribution of (A) m/z 788.8, PS(36:1); (B) m/z 834.2, PS(40:6); (C) m/z 885.4, PI(38:4); (D) m/z 838.3, PS(40:4); (E) m/z 857.3, PI(36:4), and (F) m/z 303.3, FA(20:4). The color scale used in ion images is shown in F, which is normalized to the most intense (100% relative intensity, red color) peak in the mass spectra. Mass spectra are shown from regions delineated with (G) blue and (H) red dotted lines, diagnosed as a region of 100% tumor cell concentration and infiltrated gray matter (30% tumor cell), respectively. Optical image of the H&E-stained adjacent section is shown in I, showing heterogeneous regions and the border between those magnified. In J, a plot of the total abundance of the ions m/z 834.2, m/z 885.4, and m/z 788.8 by the distance (mm) throughout the dotted black line marked in H&E-stained optical image of I is shown.



Quimiometria aplicada a DESI-MSI

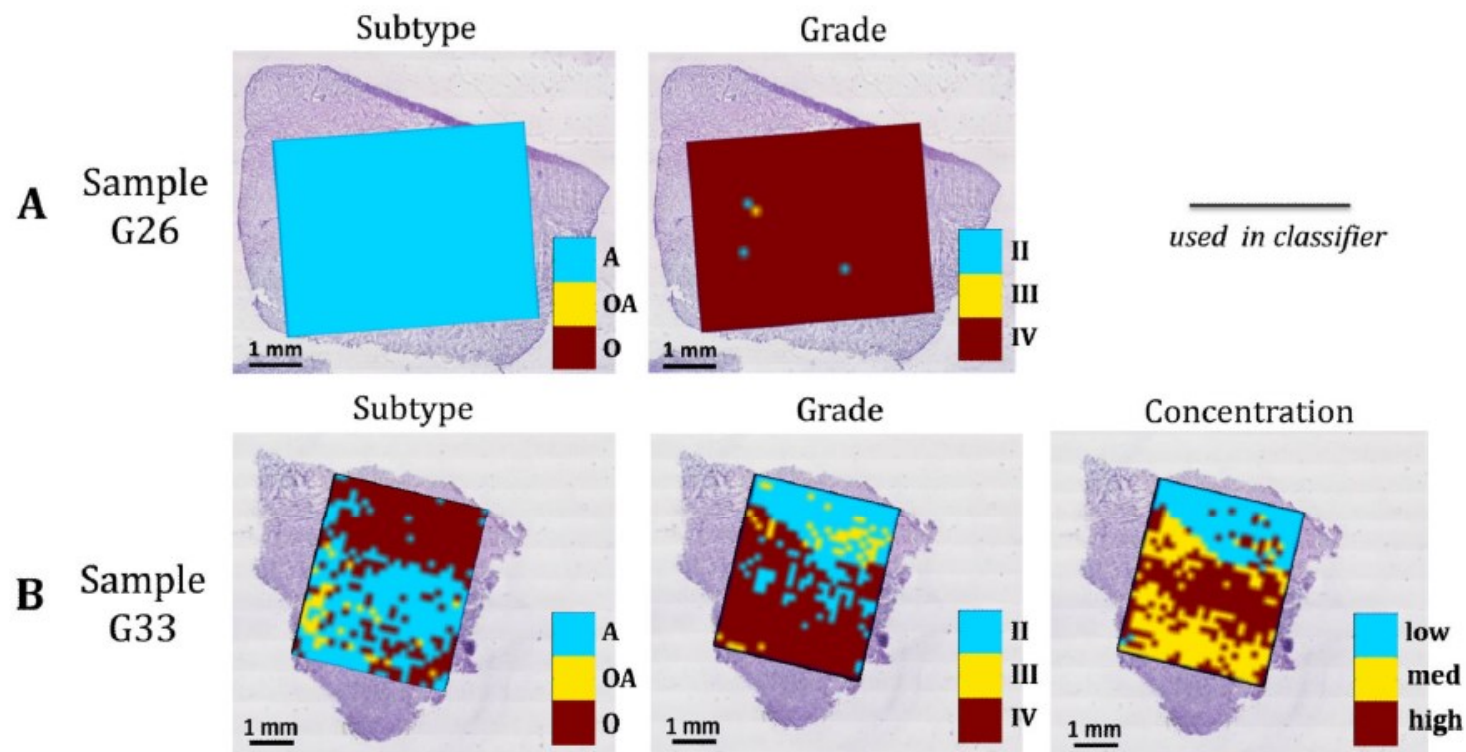


Figure 5.

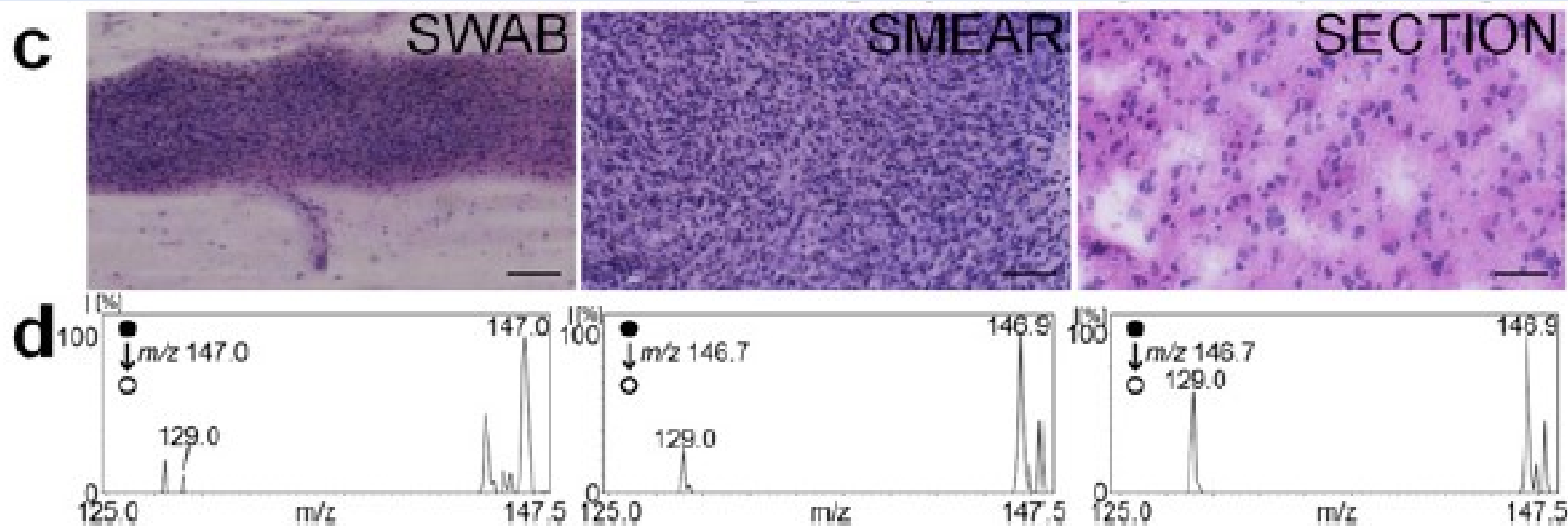
Classification results can be visualized as a class image using a color code corresponding to each of class and registered to the optical image of an adjacent section. Subtype, grade, and concentration classification for (A) Sample G33, (heterogeneous) and (B) Sample G26 (homogenous).

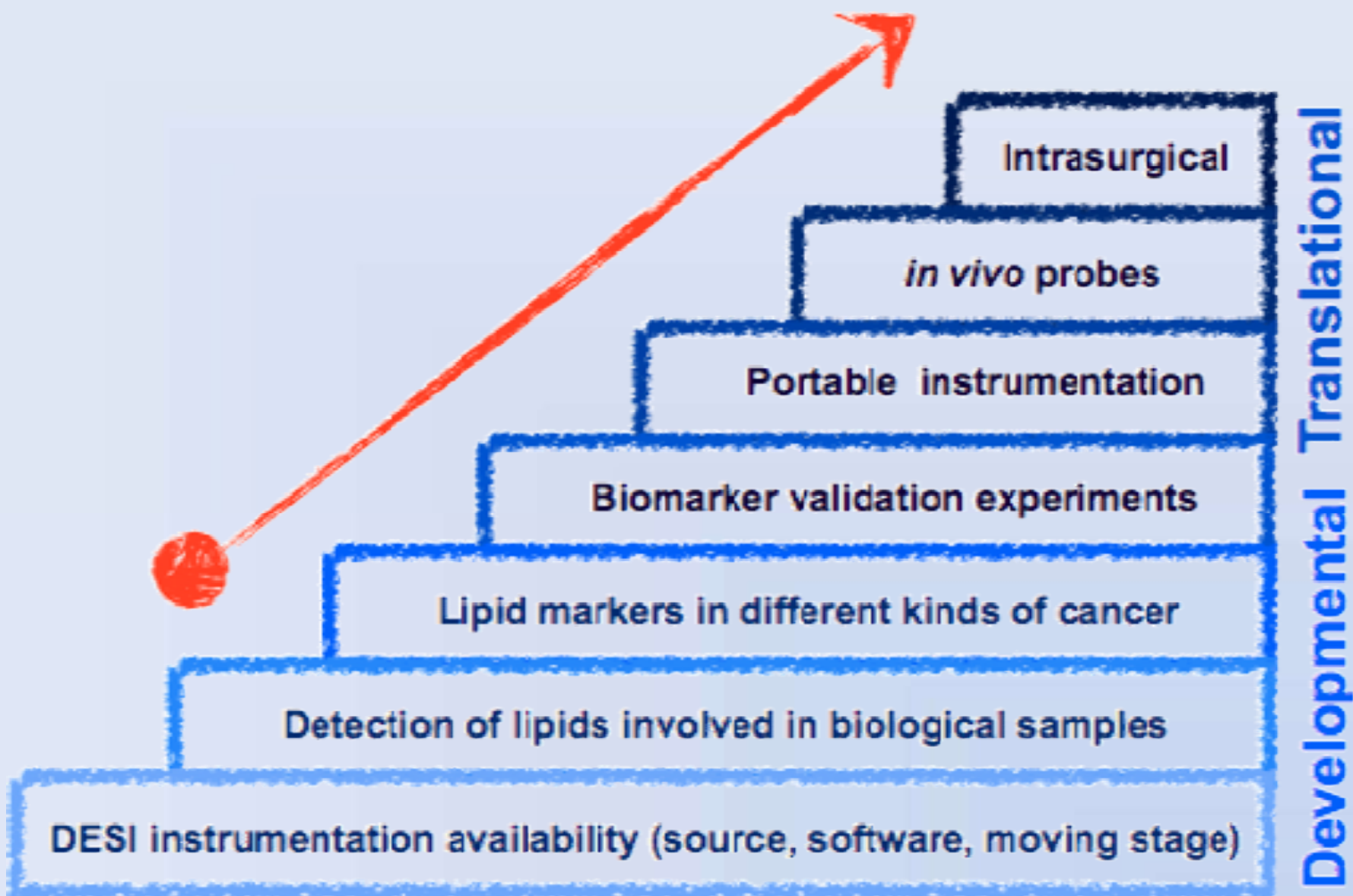
Intraoperative mass spectrometry mapping of an onco-metabolite to guide brain tumor surgery

Sandro Santagata^{a,b,c,1}, Livia S. Eberlin^{d,e,1}, Isaiah Norton^f, David Calligaris^f, Daniel R. Feldman^{a,f}, Jennifer L. Ide^f, Xiaohui Liu^f, Joshua S. Wiley^{d,e}, Matthew L. Vestal^f, Shakti H. Ramkissoon^{a,b}, Daniel A. Orringer^f, Kristen K. Gill^a, Ian F. Dunn^f, Dora Dias-Santagata^g, Keith L. Ligon^{a,b,h}, Ferenc A. Joleszⁱ, Alexandra J. Golby^f, R. Graham Cooks^{d,e,2}, and Nathalie Y. R. Agar^{c,f,i,2}



Intraoperative – real time analysis

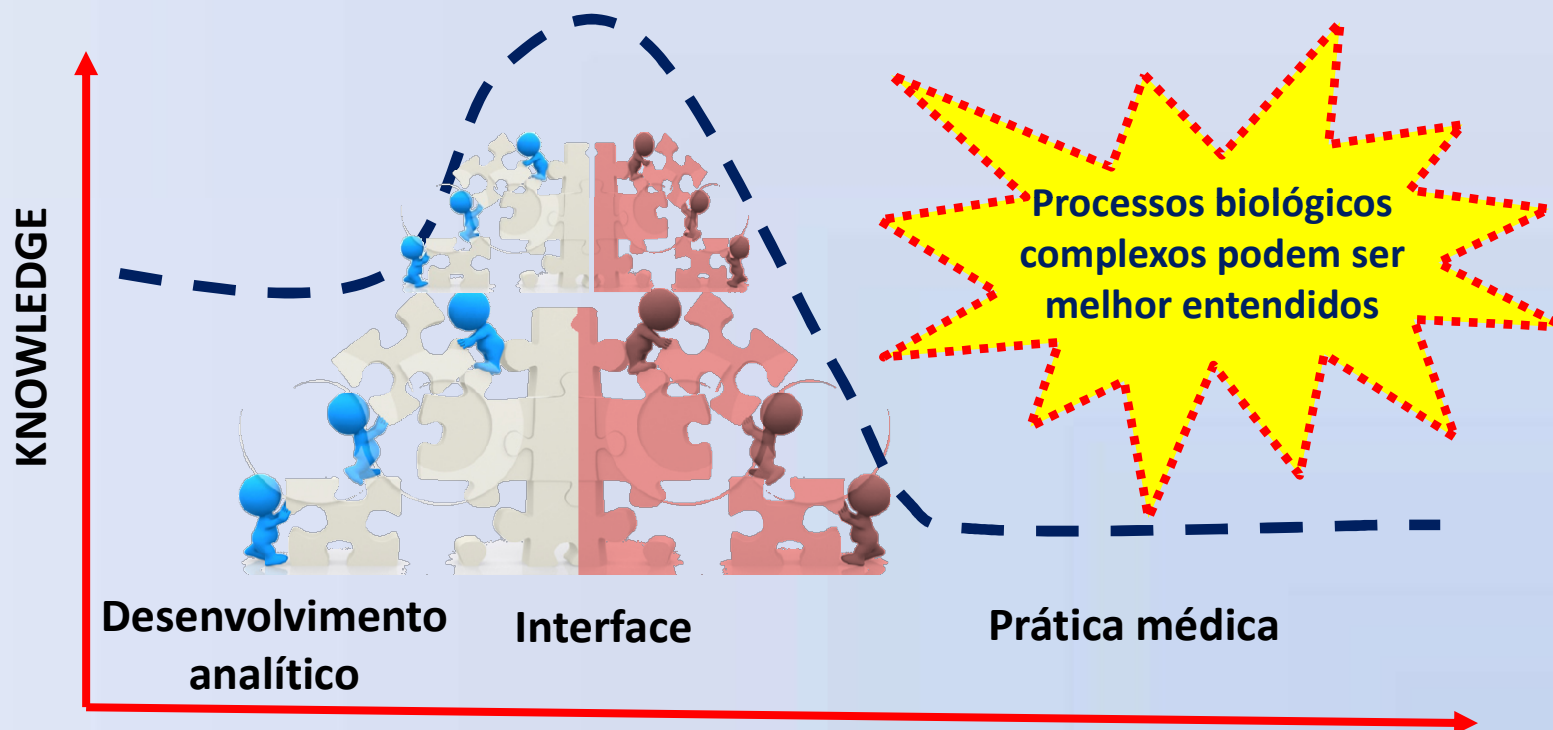




Biochim Biophys Acta. 2011;1811(11):946-60

Conclusões

- A espectrometria de massas vem ganhando destaque em análises clínicas
- Estamos atuando na interface do conhecimento





1st IBERO-AMERICAN
CONFERENCE ON
MASS SPECTROMETRY
BrMASS - 2016

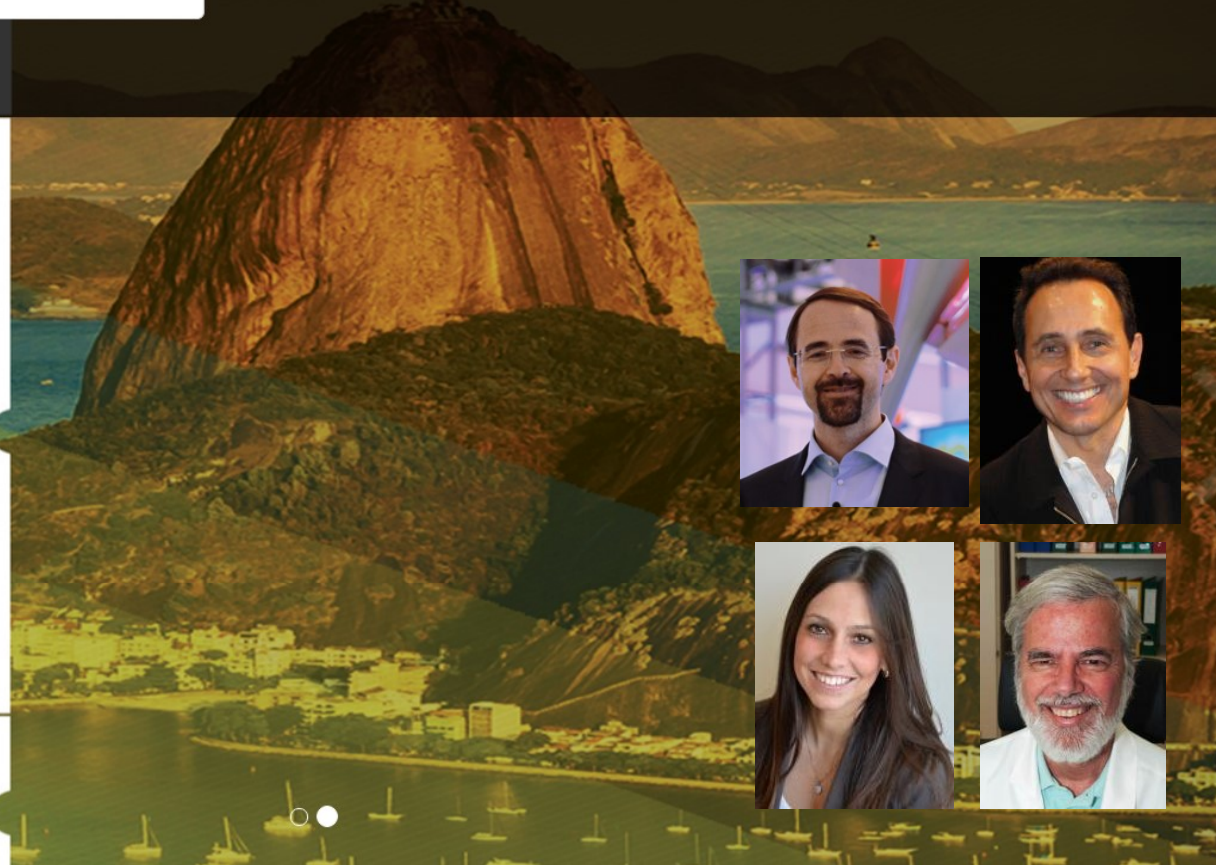


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