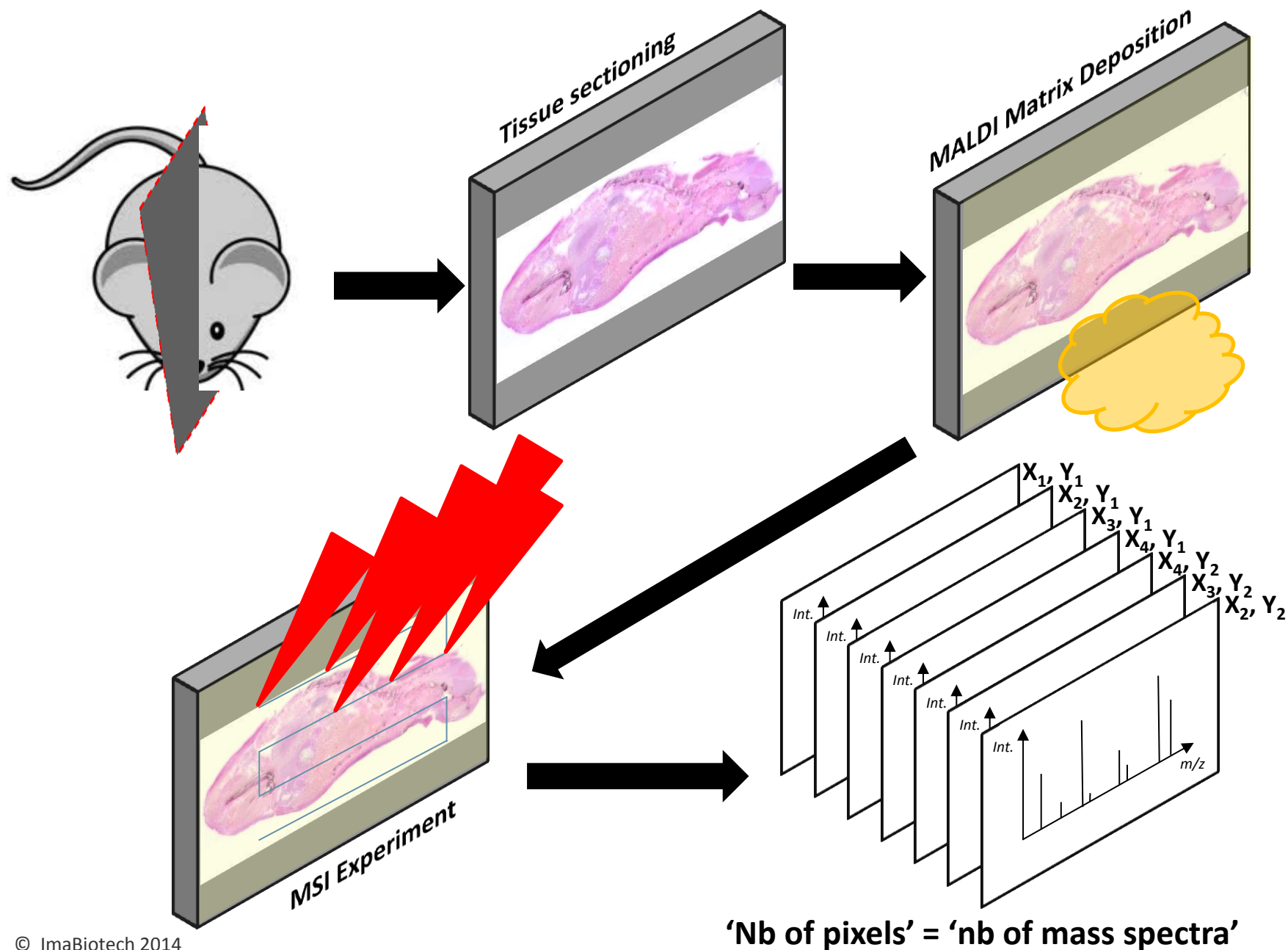


Applications of Quantitative Mass Spectrometry Imaging in drug development

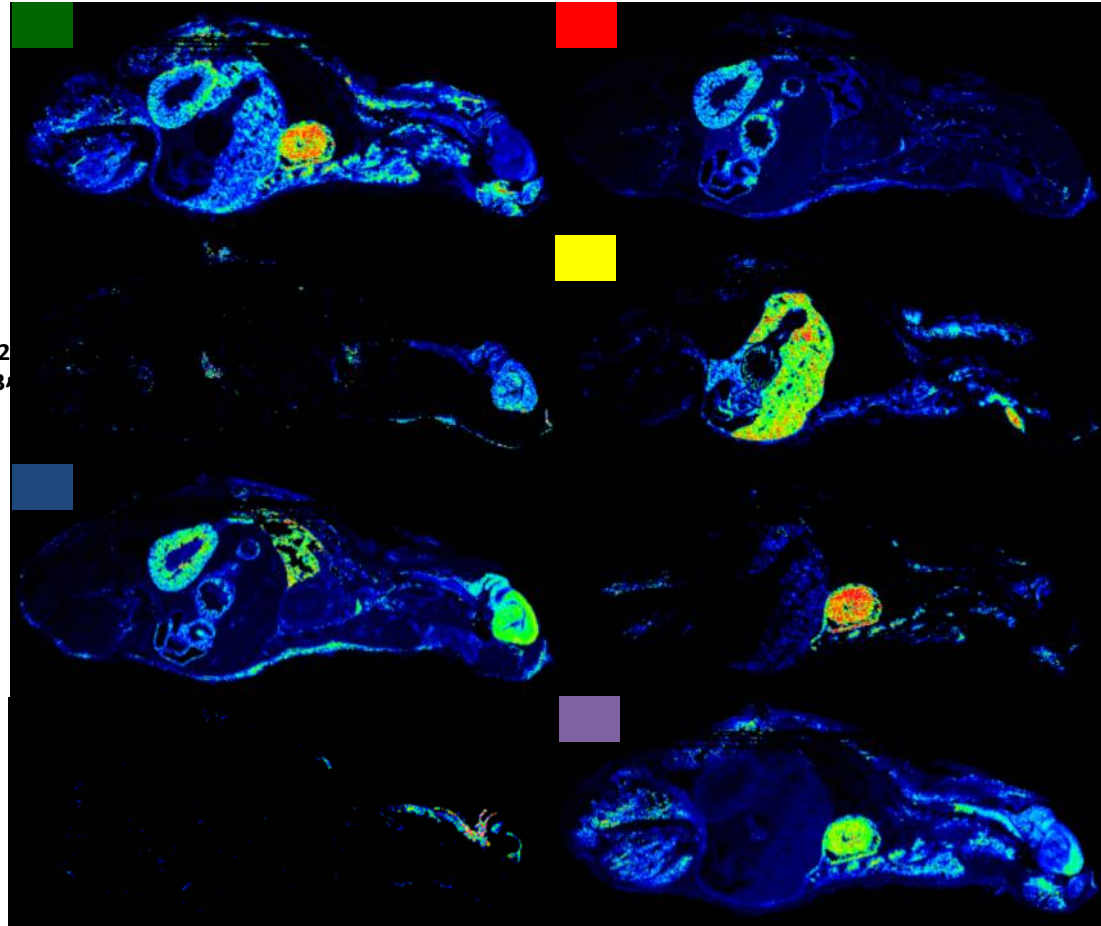
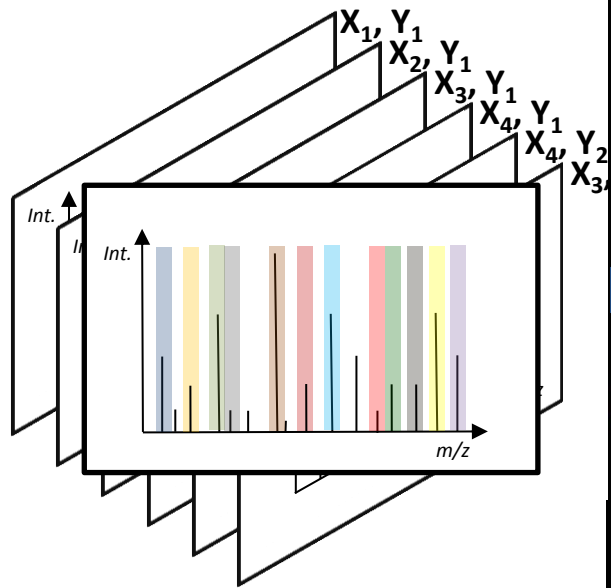
David BONNEL, PhD
ImaBiotech, France

EBF 7th Open Meeting, Barcelona, 2014

Mass Spectrometry Imaging (MSI)?

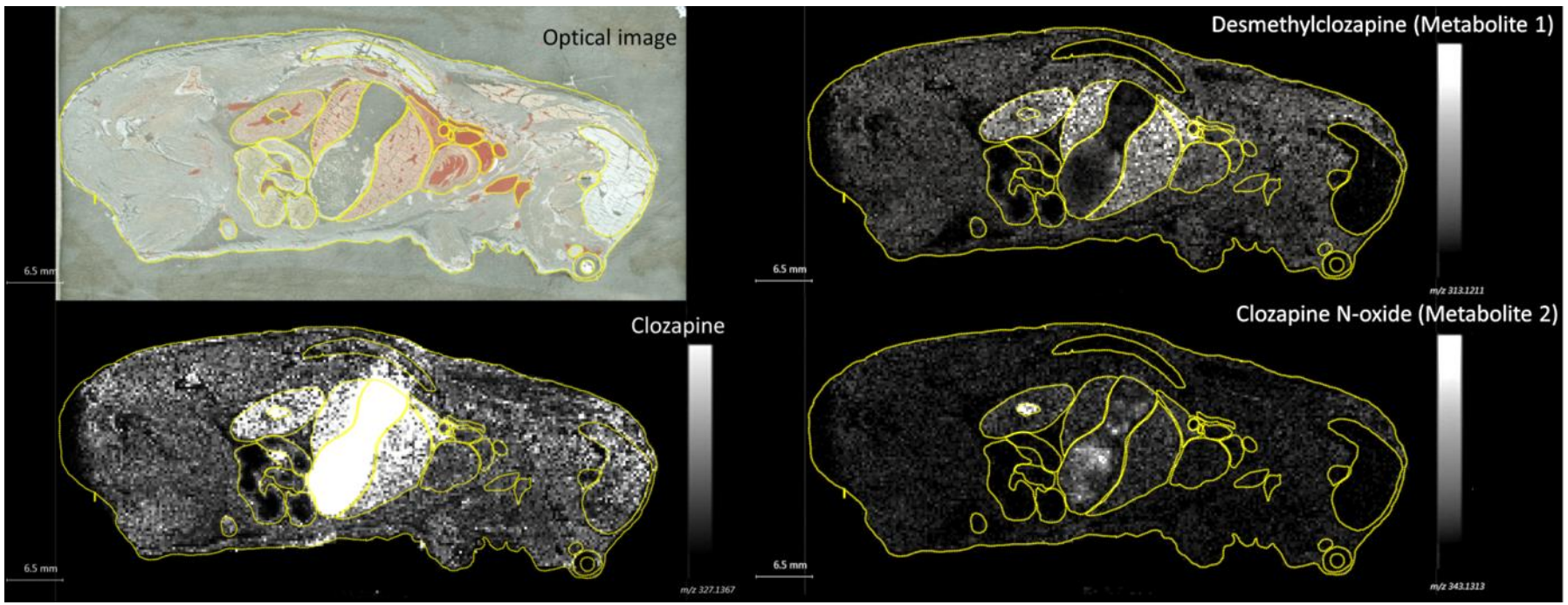


Mass Spectrometry Imaging (MSI)?



Each m/z signal = One specific MS image

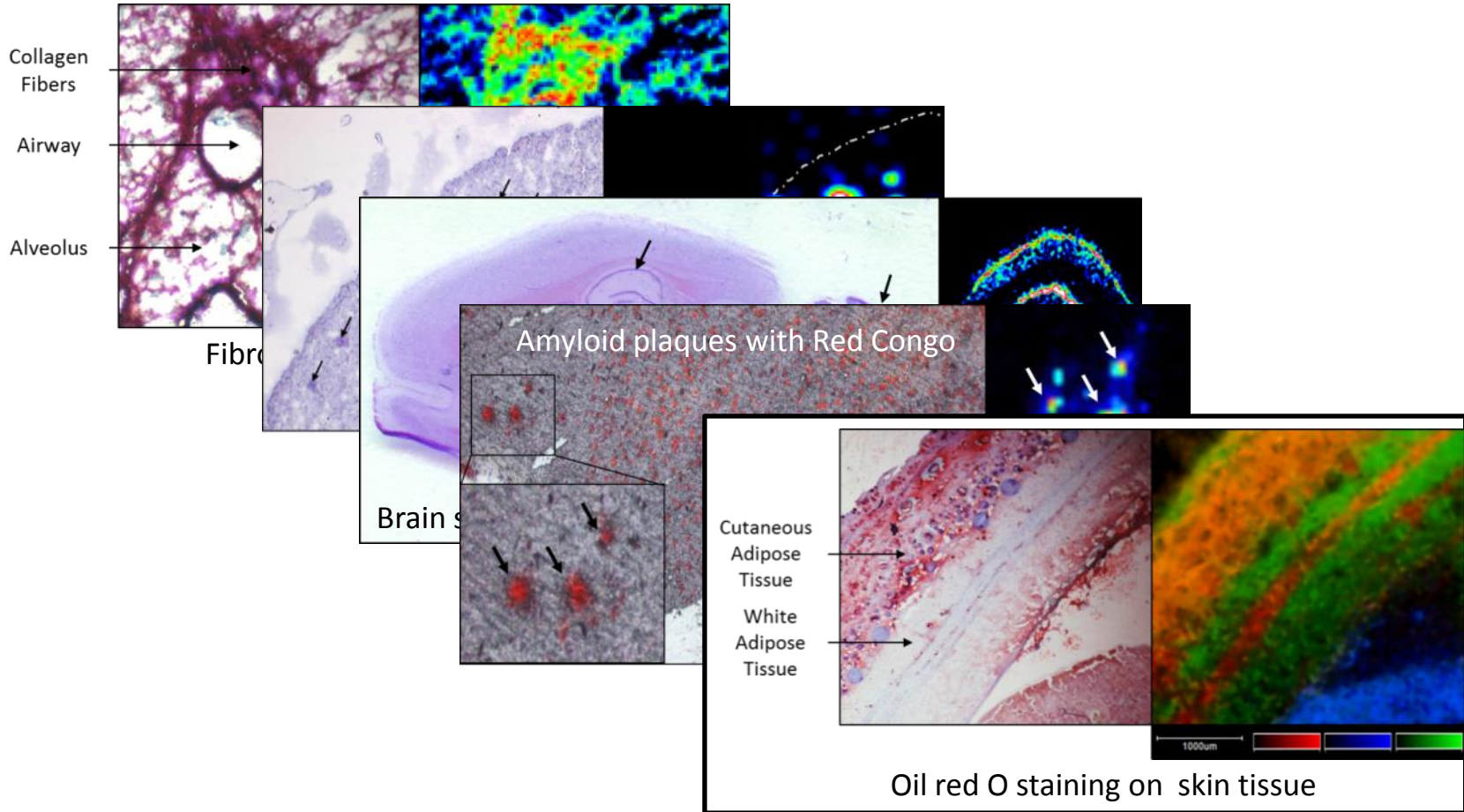
WB Clozapine and its related metabolites distributions using MSI

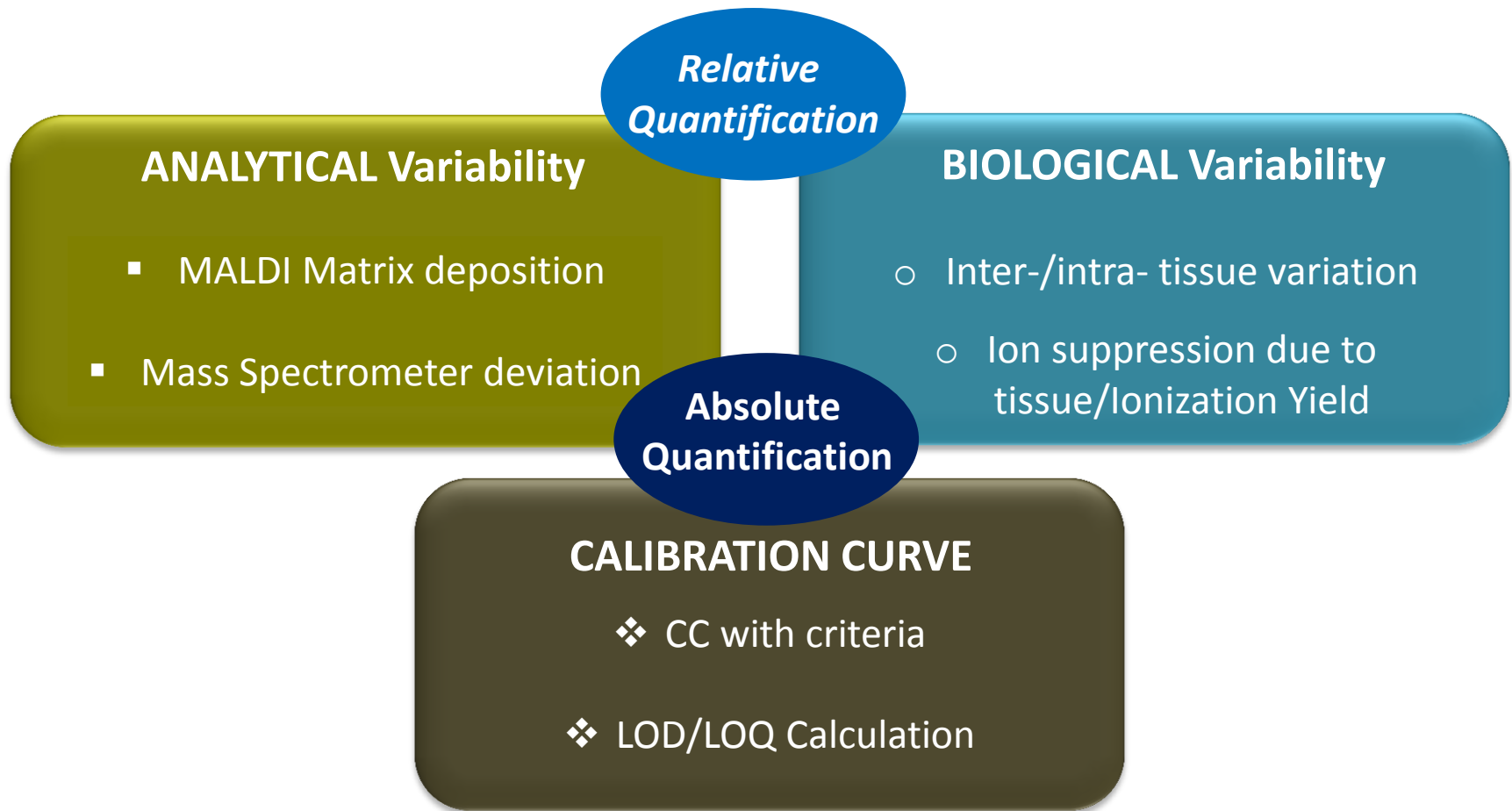




MSI and Histology

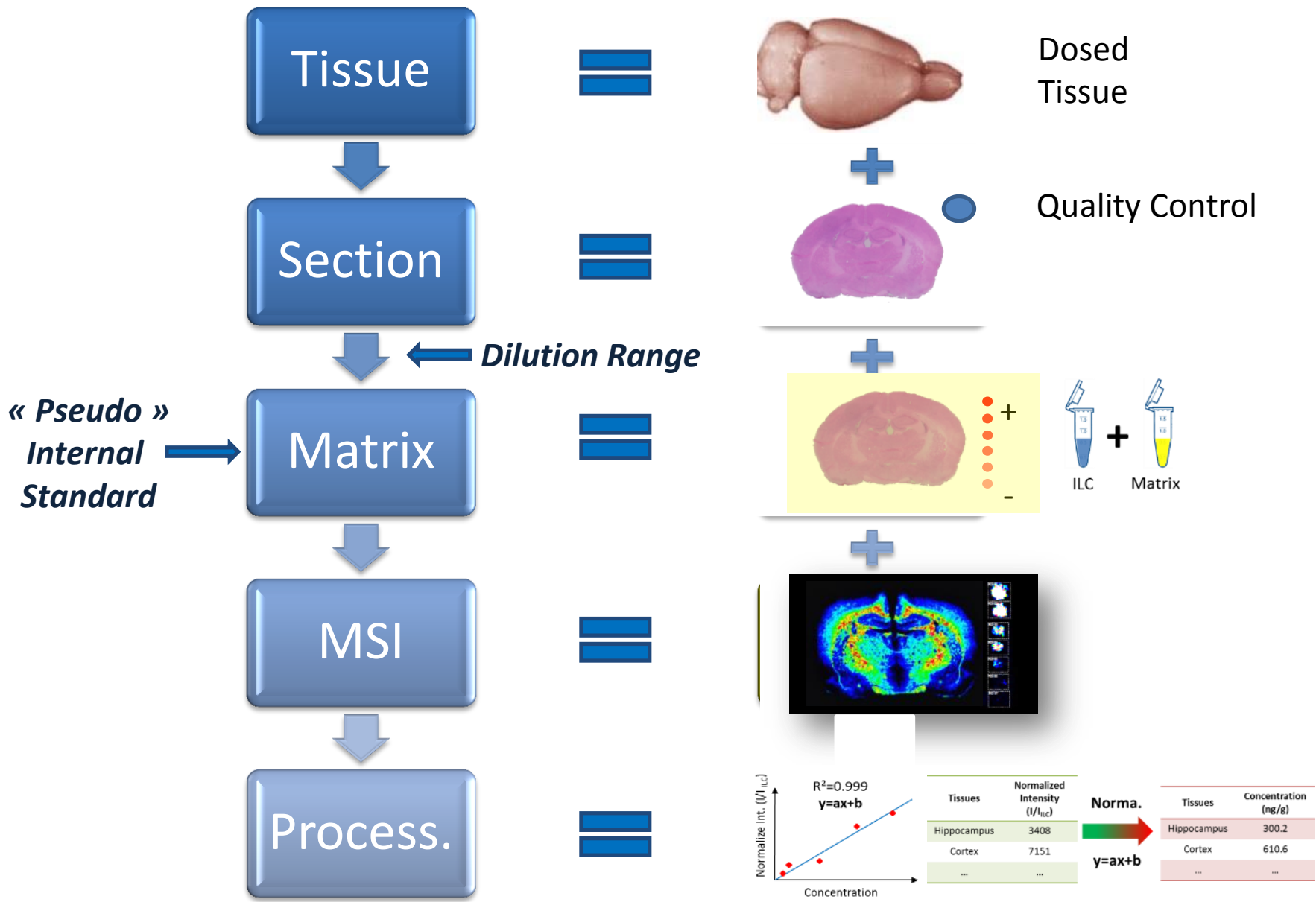
MSI is a complementary technique to support histology







QMSI workflow





Applications of QMSI

<i>Applications ILC</i>	Small Molecule
<i>Target molecule</i>	Diazepam
<i>Model</i>	Dosed Rat (15 mg/kg, i.v., 15 min)
<i>Sample</i>	Brain
<i>Therapeutic area</i>	Anxiolytic, anticonvulsant
<i>Preparation</i>	Cryosection (10 μm)
<i>Matrix</i>	DHB
<i>ILC</i>	d5-Diazepam (30 μM , m/z 290)
<i>Ion images</i>	$[\text{M}+\text{H}]^+$, m/z 285
<i>Raster size</i>	200 μm

Instrumentation:

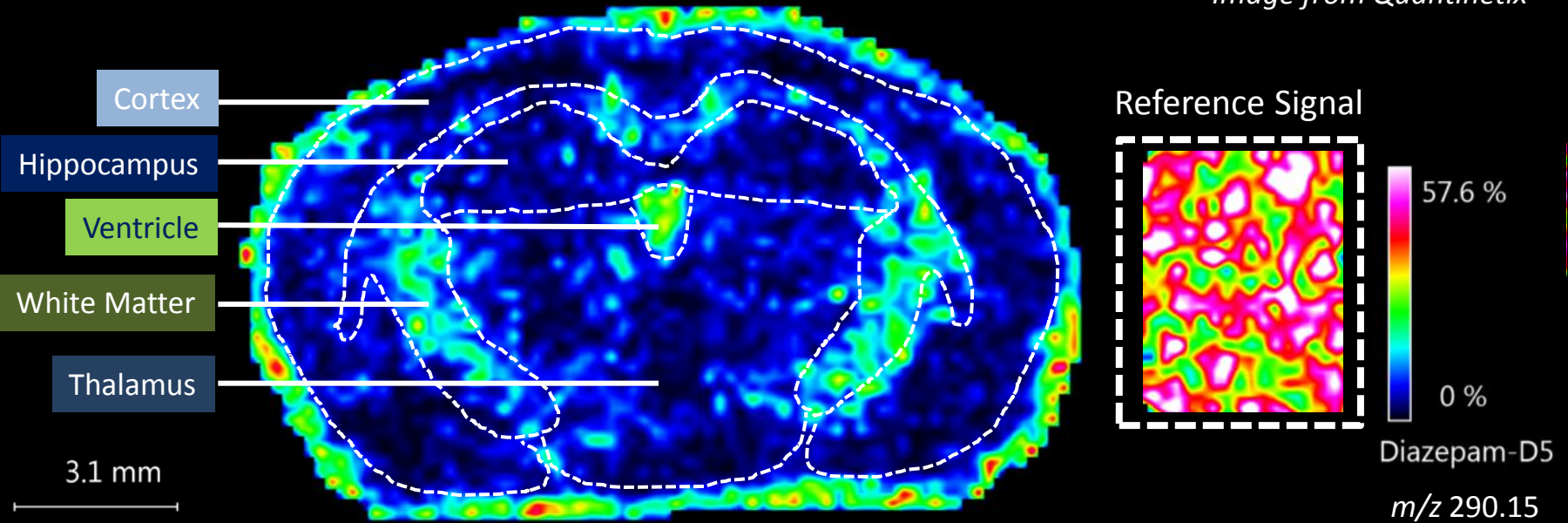
- MALDI-TOF Mass Spectrometer Autoflex Speed (Bruker Daltonics)
- Matrix Automated Deposition Device SunCollect (SunChrom)



Biological variability: Ion suppression on tissue

Distribution of d5-Diazepam deposited on the brain & on the slide (200 μm)

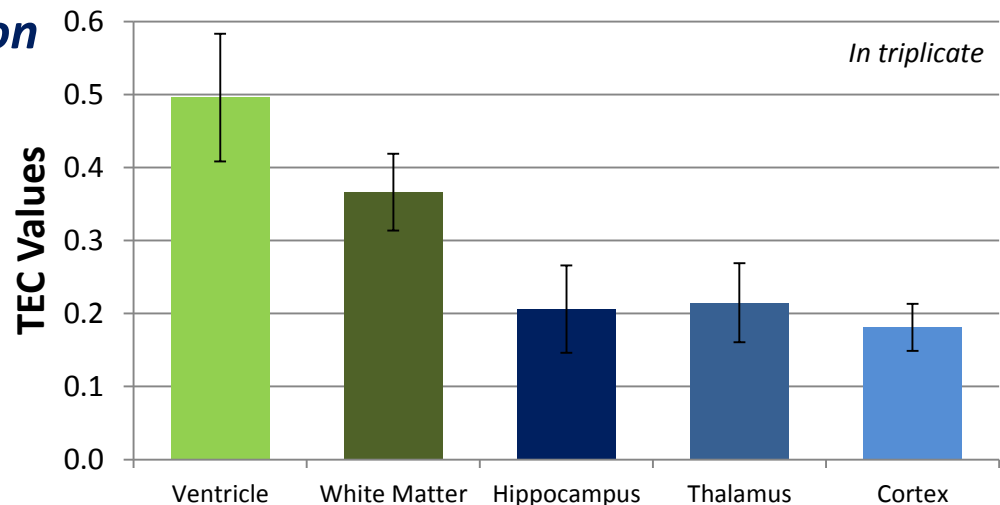
Image from Quantinetix™



Evaluate the specific ion suppression of each kind of tissues

- ✓ Tissue type specific
- ✓ Molecule specific

$$\text{TEC} = \frac{\text{Int (Tissue)}}{\text{Int (Slide)}} < 1$$



Histological tissue effect: Intra-Normalization & “Real” Image

Distribution of Diazepam within dosed brain section (200 μm)

Unnormalized image

Normalized image/d5-Diazepam

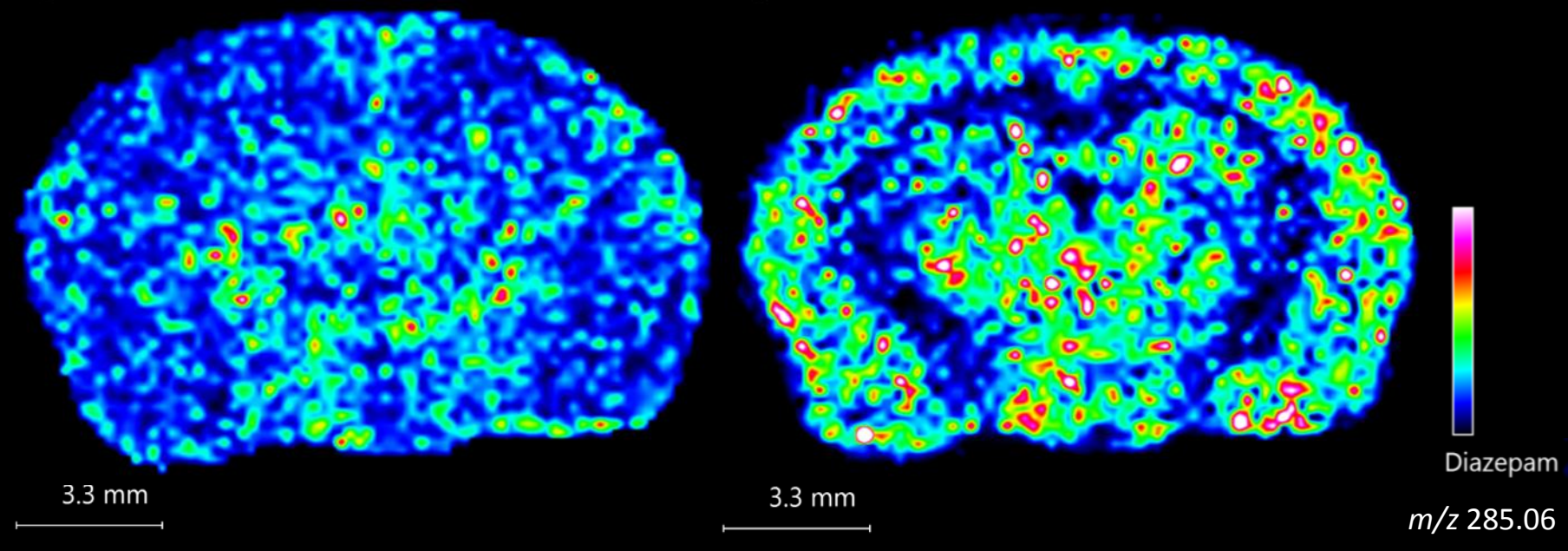


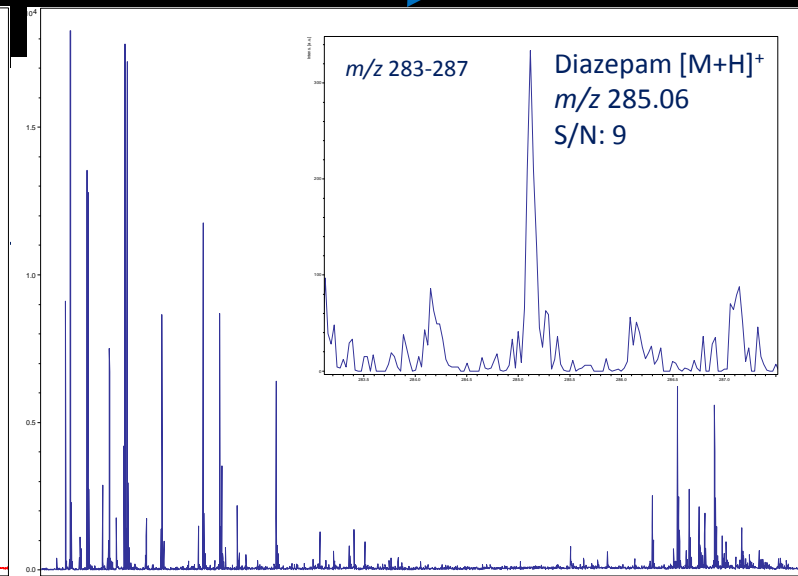
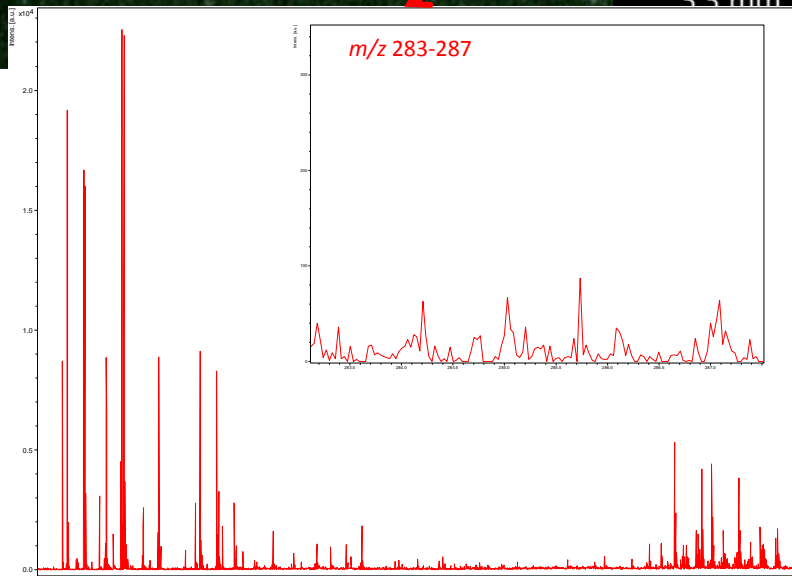
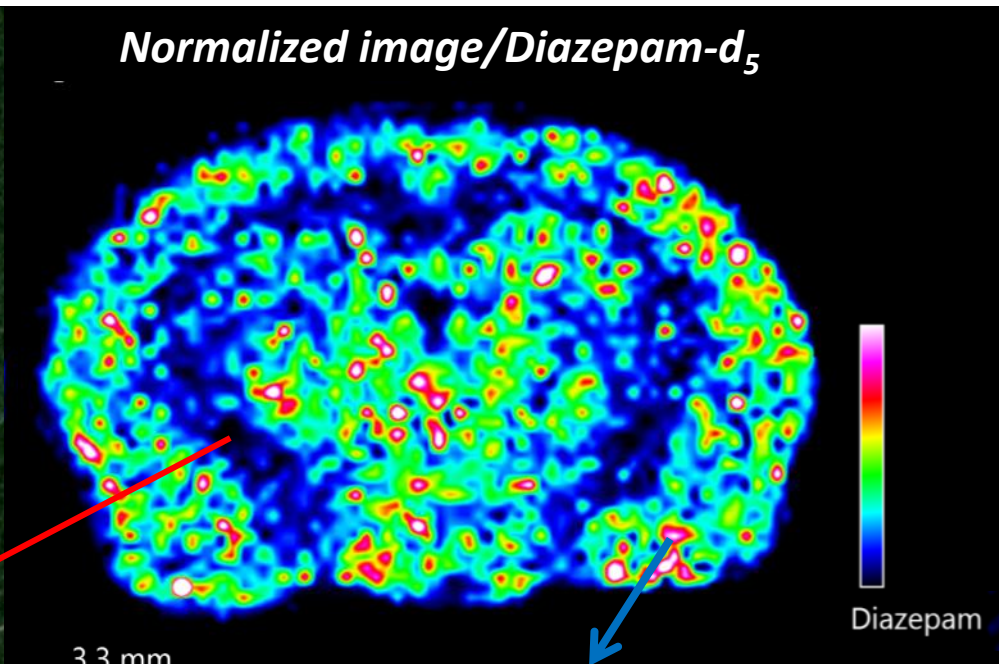
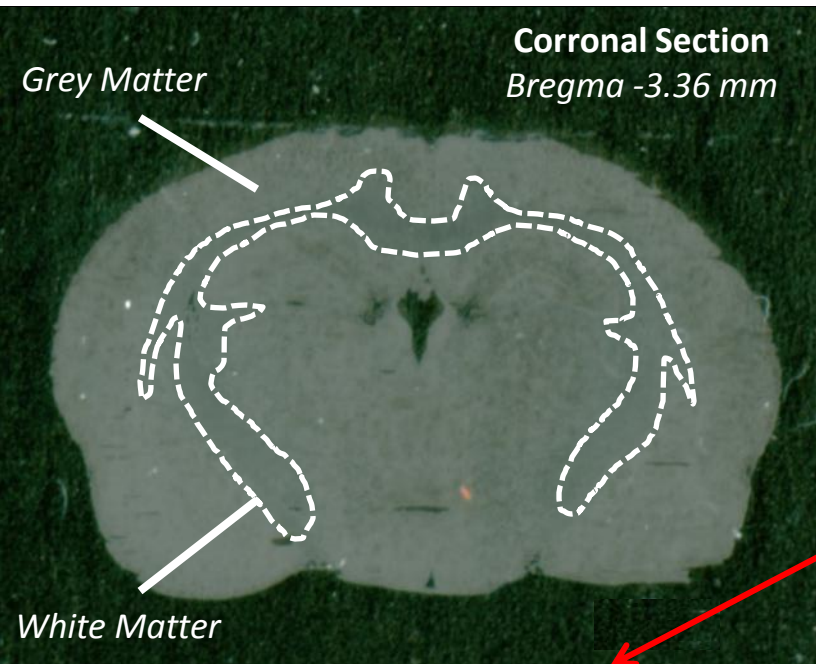
Image from Quantinetix™

- Normalization of molecular image using ILC
- See the “real” distribution of the analyte¹



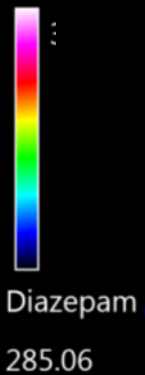
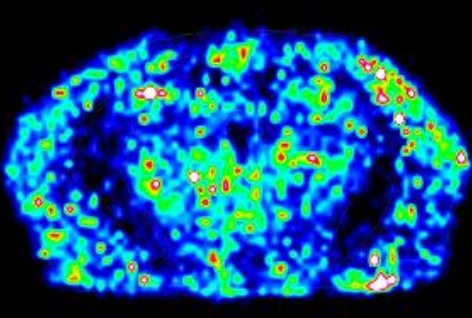
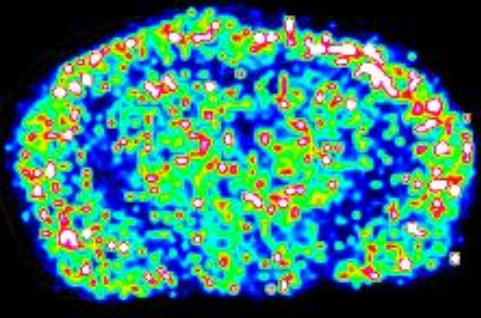
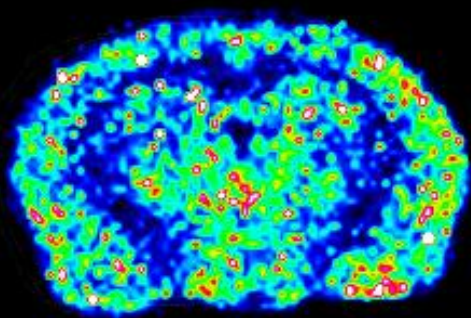
Histological tissue effect: Intra-Normalization & "Real" Image

Distribution of Diazepam within dosed brain section (200 μm)





Analytical variability: Inter-samples normalization



Replicate/Day 1

Replicate/Day 2

Replicate/Day 3

QC's Sample preparation:



+

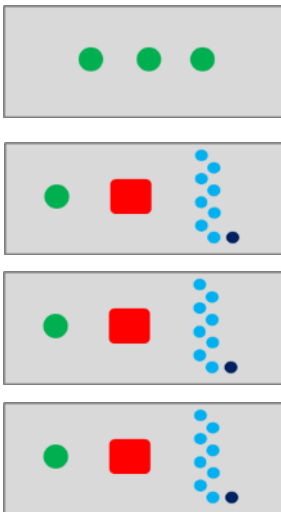


Diazepam



Homogenate
(50 µg/g)

Frozen
Section



Mean 3 QC's
value

1 QC/image

1 QC/image

1 QC/image



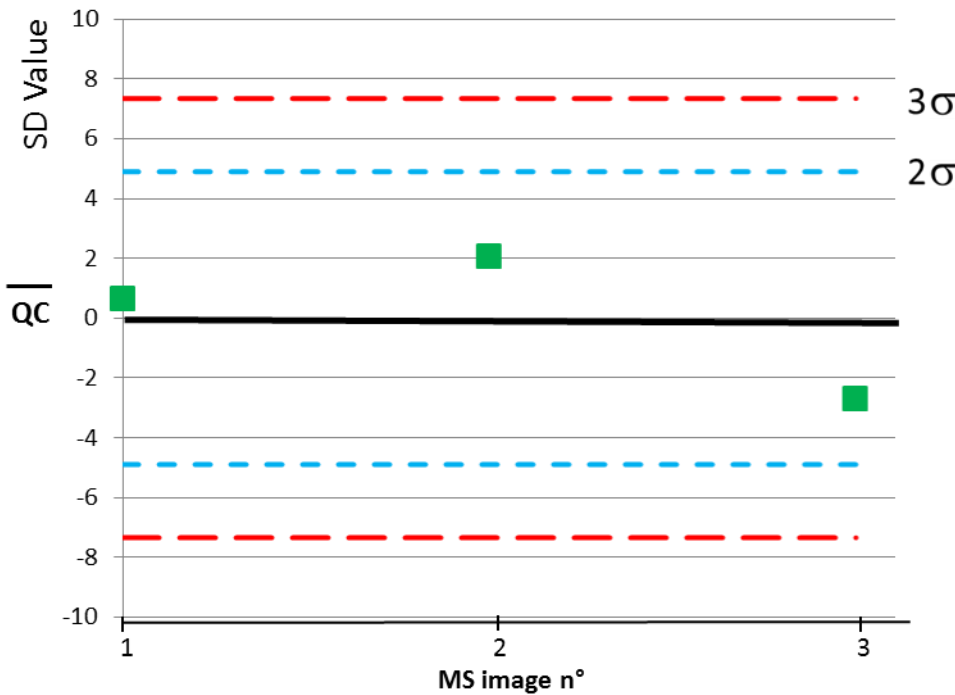
MSI





Analytical variability: Inter-samples normalization

QC's Control chart:

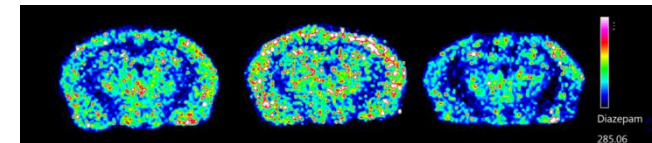


QC's Normalization Factor:

$\overline{QC} = 1.02E+04$

MS image n°	QC _i (Int.)	Coeff. = QC _i / \overline{QC}
1	1.09E+04	1.1
2	1.23E+04	1.2
3	7.52E+03	0.7

Normalization of
MS images



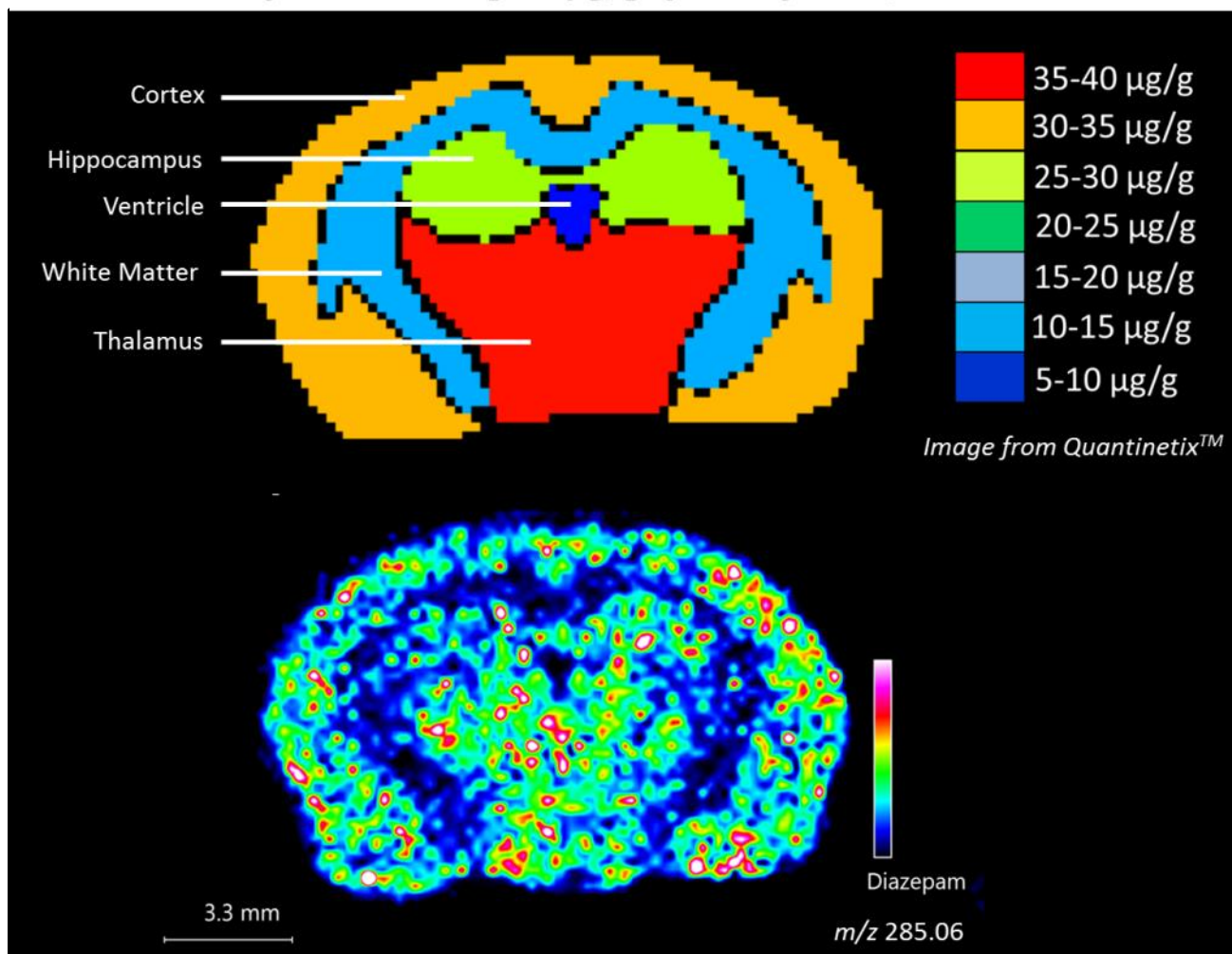
For inter-days/samples normalization, QCs...

- ✓ Increase the reproducibility of MSI experiments
- ✓ Provide better confidence into data interpretation.



Quantitation of Diazepam in Rat Brain using ILC

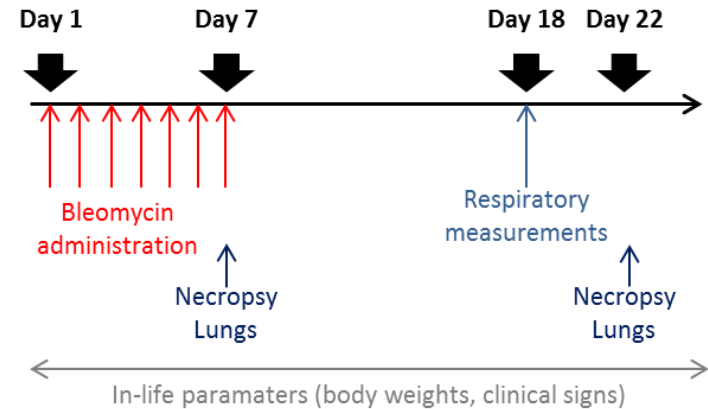
Generation of a Virtual Image in $\mu\text{g/g}$ of tissue from QMSI data





Applications of QMSI

	Small Molecule
Target molecules	markers
Model	IPF Rat Model (Bleomycin)
Sample	Lung
Therapeutic area	respiratory
Preparation	Cryosection (10 µm)
Matrix	DHB
ILC	Matrix ions
Raster size	20 µm



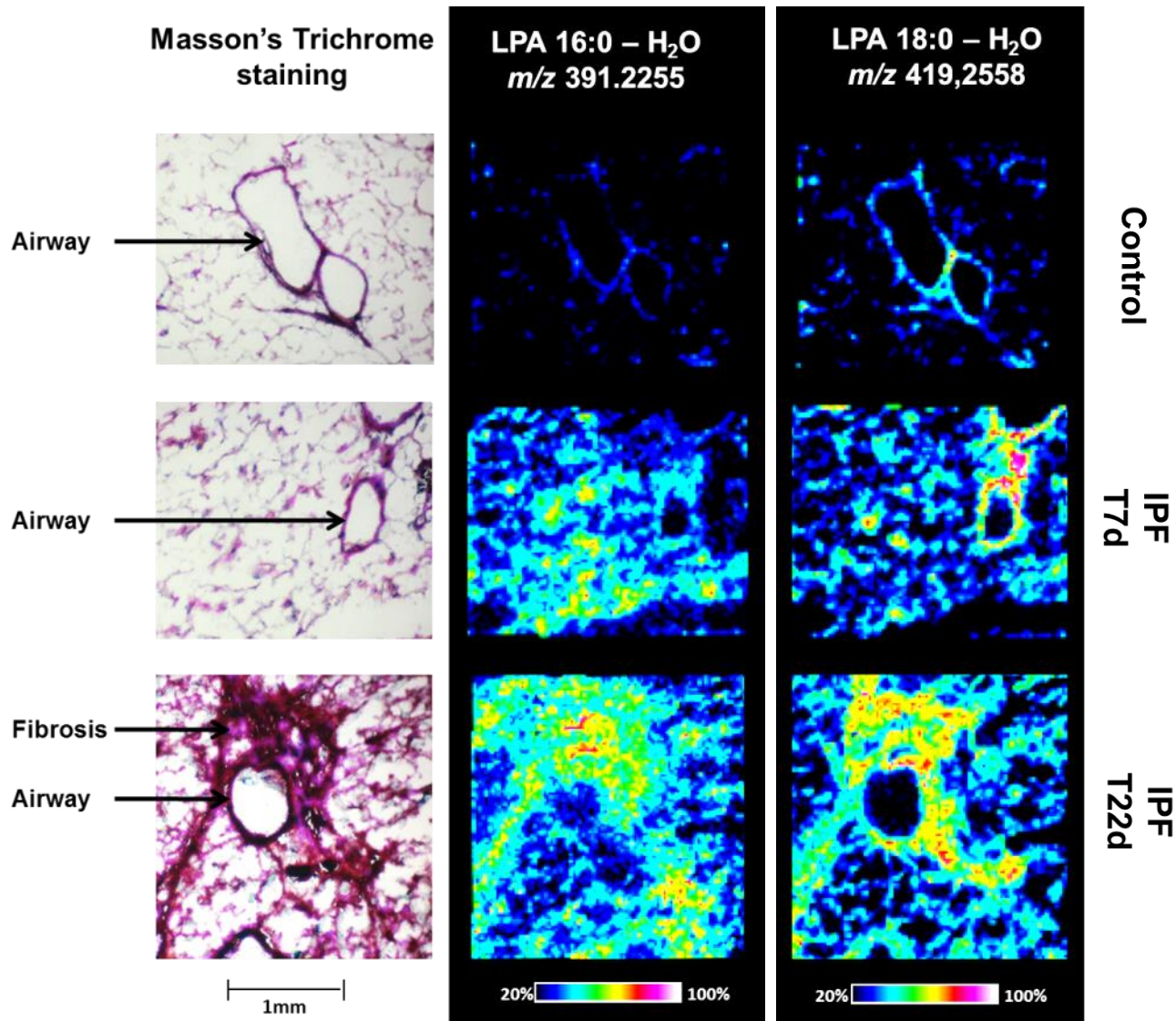
Instrumentation:

- MALDI-FTICR Mass Spectrometer Solarix (Bruker Daltonics)
- Matrix Automated Deposition Device SunCollect (SunChrom)



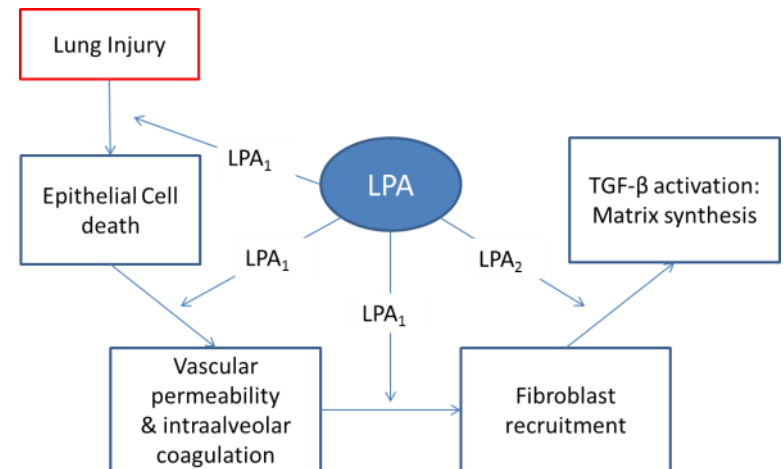
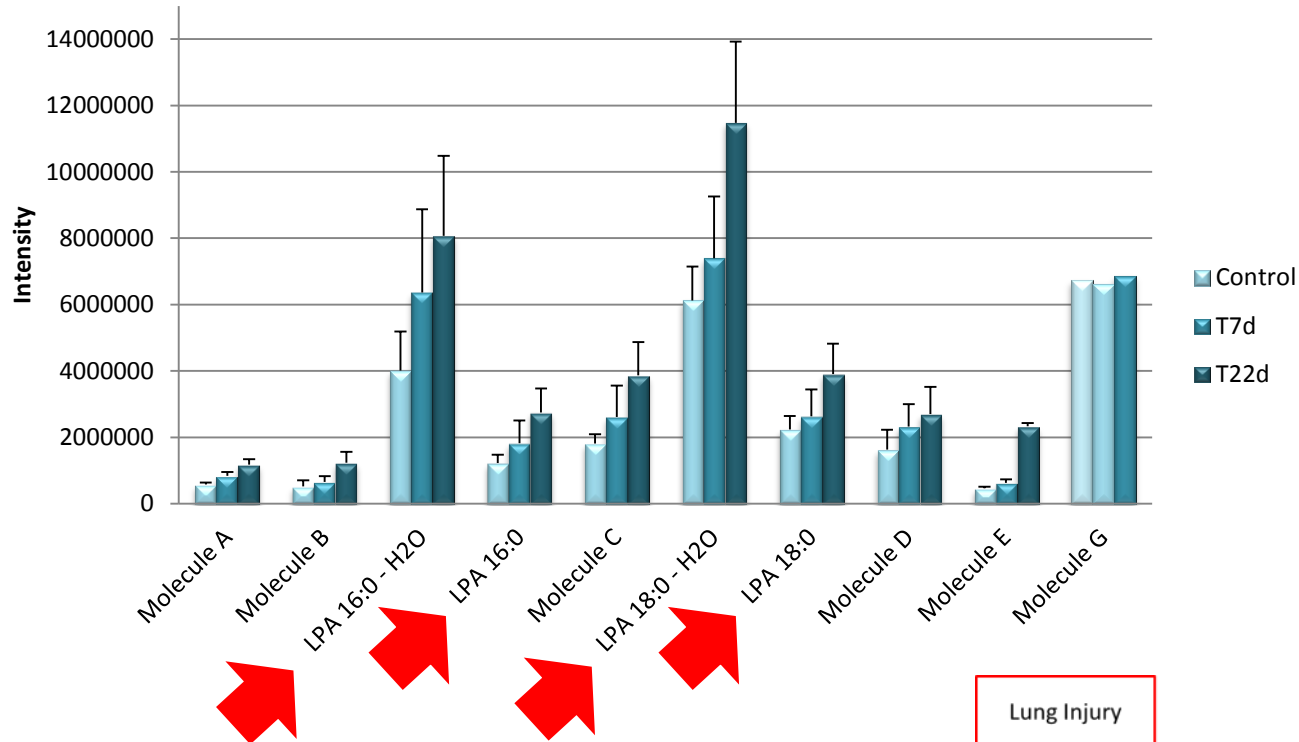
Readout Discovery: IPF Disease

Aim: identify some early readout markers of the IPF in a Bleomycin model



Readout Discovery: IPF Disease

Fibrosis biomarkers intensity comparison
between control (N=3) and IPF (N=6) tissues





Conclusion

Analytical Variability = Quality Control (QC)

- ↑ Reproducibility/repeatability.
- ↓ Variation coeff.
- Inter-samples comparison
- ↑ Confidence

*Multimaging
Software*

Histological Variability = Internal standard (ILC)

- ✓ Tissue Suppression Effect Normalization (Intra-)
- ✓ See “real image”

*Quantinetix
Software*

ImaBiotech's expertise: 200 QMSI projects

- ✓ Success: 89% (limitation: sensitivity)
- ✓ Average inter-sample variability: 15%-20% (based on the QCs)
- ✓ Linearity: 2.5-3 Log
- ✓ Best Limit of detection: 5ng/g
- ✓ Average Limit of detection: 150ng/g



Acknowledgement



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Dr. Gregory Hamm
Dr. Jean-Philippe Ebran
Fabien Pamelard
Alain Héron
Raphaël Legouffe*

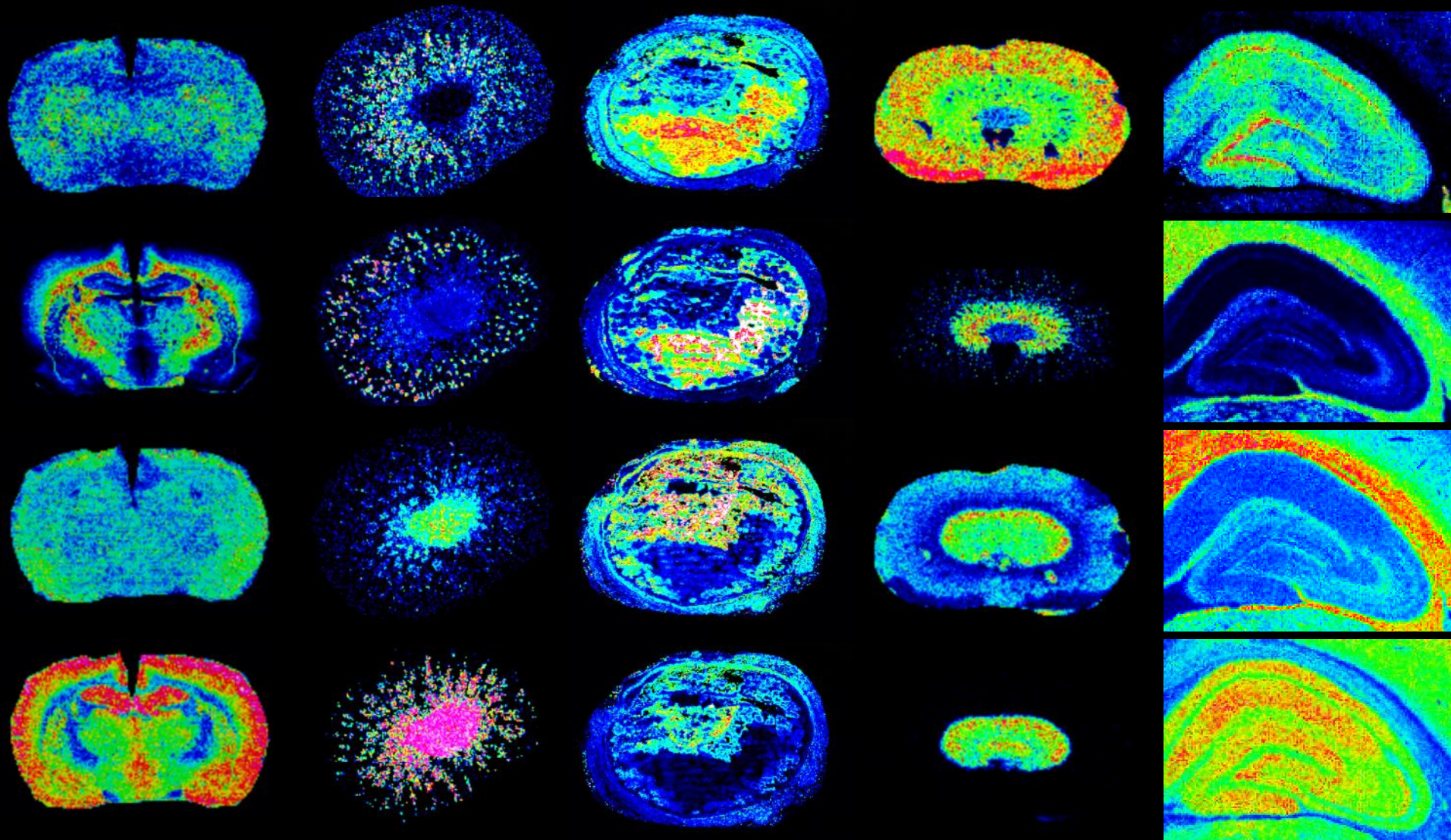
*Constance Buissart
Guillaume Hochart
Gael Picard-de-Muller
Julien Stauber
Emeline Falaux*



Discovery Research Services,
Edinburgh, United-Kingdom
*Dr. Mary McELROY
Dr. Stephen MADDEN*

“A picture is worth a thousand words”

Confucius (551–479 BC)



Thank you for your attention