

# Multiplatform mass spectrometry imaging analysis of aging endocrine system

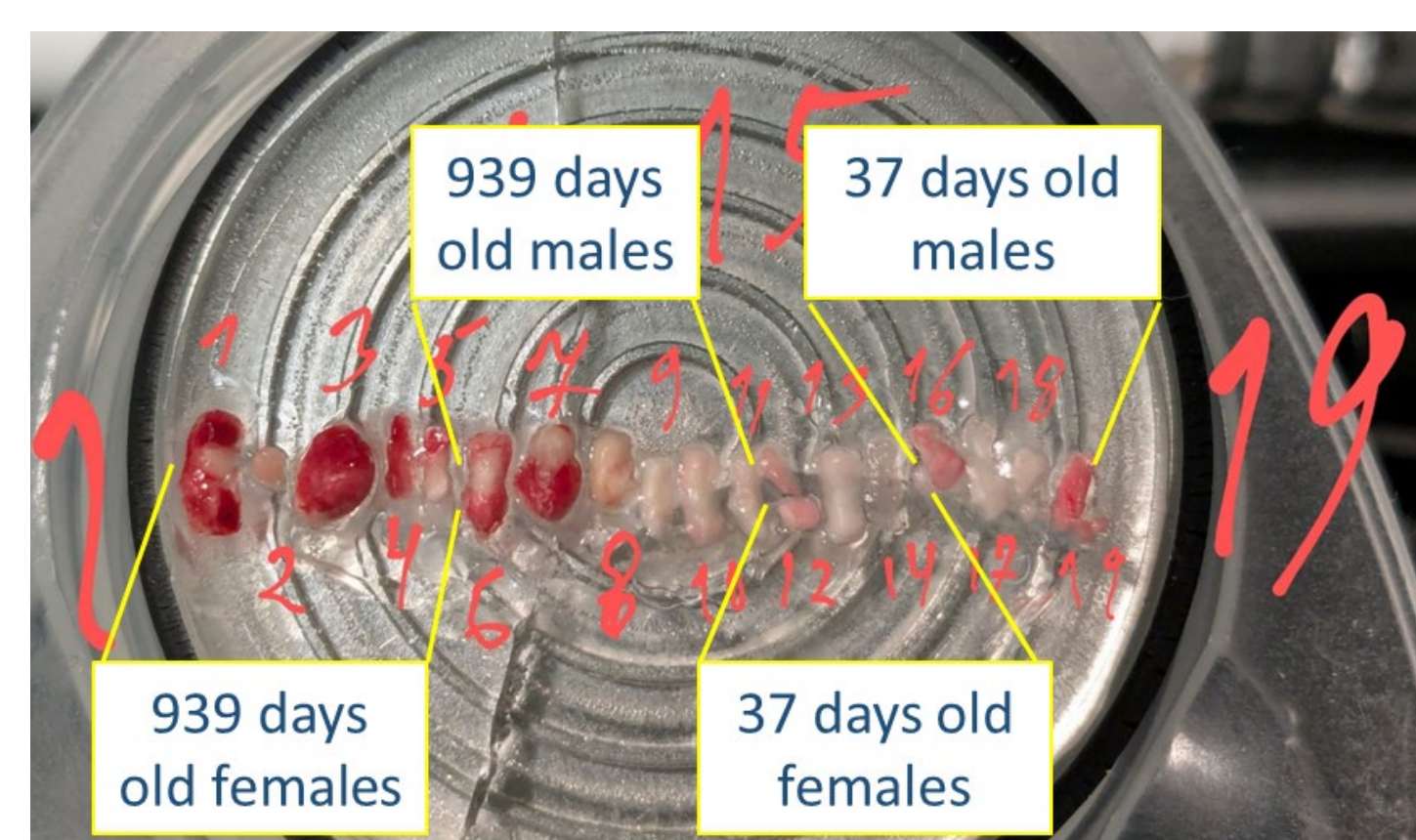
## Introduction

Significant biochemical changes occur as our organs age. Identifying these changes helps determine molecular targets for developing behavioral, dietary, and pharmaceutical strategies aimed at increasing longevity and improving quality of life. In this study, we demonstrate the use of multiplatform mass spectrometry imaging to investigate differences in metabolite, lipid, and peptide profiles in the pituitaries of 1-month-old and 31-month-old mice, with the latter being roughly equivalent to 80-year-old humans. This multiplatform approach utilizes a MALDI-trapped ion mobility spectrometry orthogonal time-of-flight (timsTOF) mass spectrometer alongside a MALDI-TOF/TOF mass spectrometer, enhancing analytical throughput, expanding molecular mass coverage, and generating compatible datasets while optimizing resource utilization and associated costs.

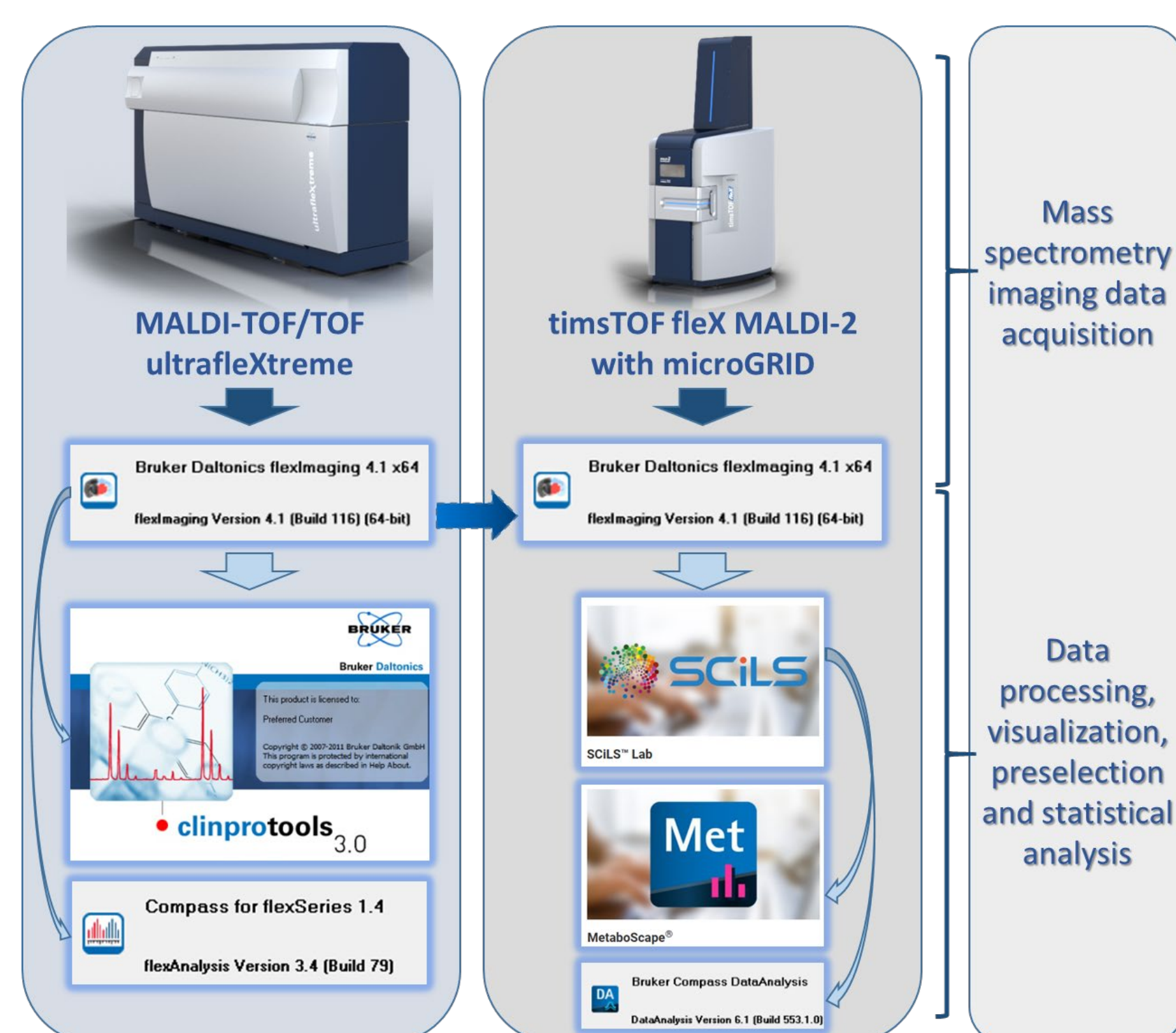
## Methods

Male and female C57BL/6 mice, aged 1 month and 31 months, were used in the experiments. Pituitaries were surgically dissected after euthanasia and rapidly frozen. Tissue sections, 12 micrometers thick, were cut and placed on ITO-coated glass slides (Delta Technologies). Various MALDI matrices were applied to the sections using an HTX M5 sprayer (HTX Imaging). Samples were analyzed using MALDI timsTOF flex and ultrafleXtreme mass spectrometers (Bruker). Data acquisition was performed in positive (both instruments) and trapped ion mobility modes (timsTOF flex) at spatial resolutions ranging from 5 to 250 micrometers. Different spatial resolutions facilitated either the rapid evaluation of a large number of samples or detailed spatiochemical investigation. Data processing and analysis were conducted using several software tools including SCIls Pro, DataAnalysis, and MetaboScape (Bruker).

Mouse pituitaries deposited on cryostat's chuck before sectioning

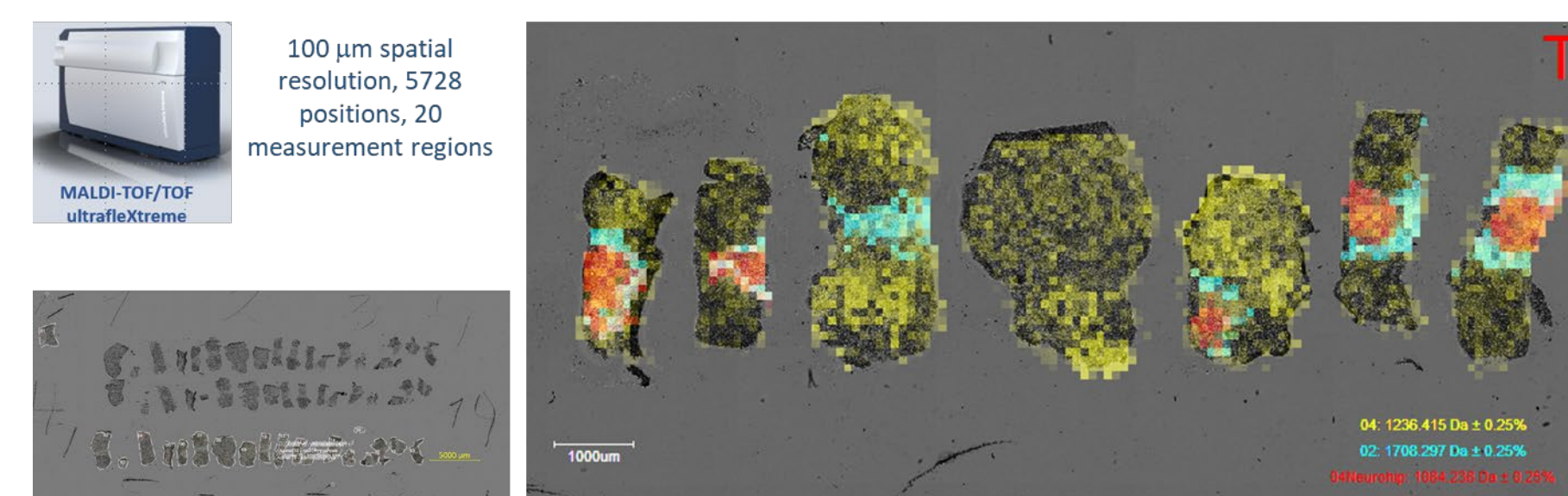


Experimental workflow for data acquisition and analysis

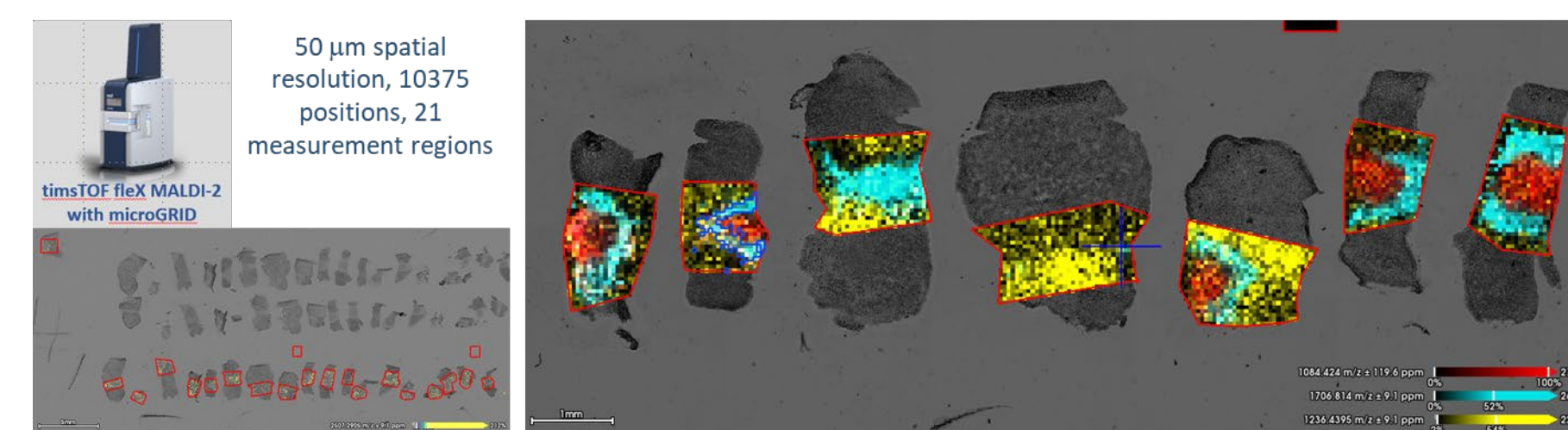


## Multiplatform analysis of mouse pituitary

Two stage determination of regions of interest for sample's comprehensive analysis using timsTOF flex

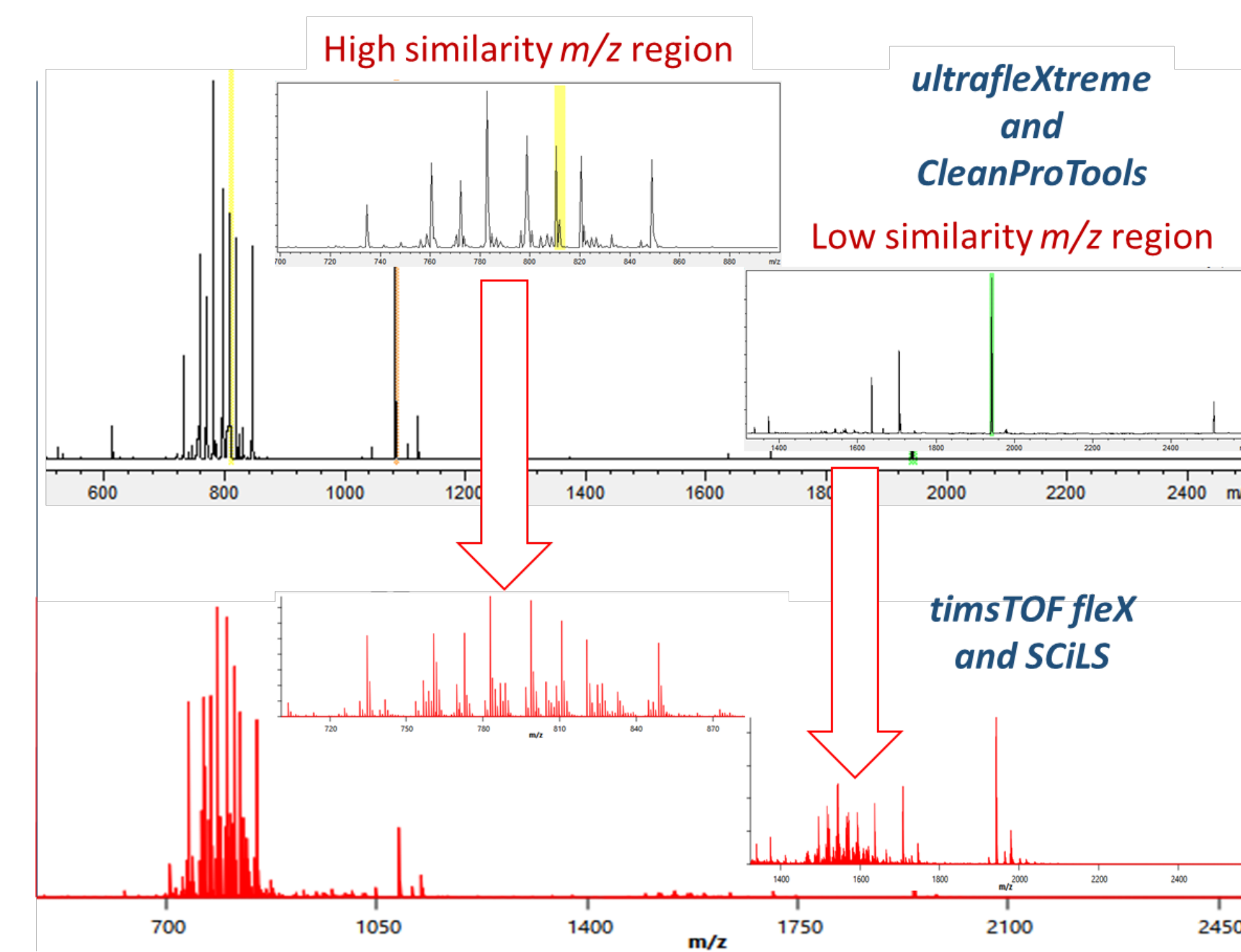


1<sup>st</sup> stage: full pituitary section MSI analysis using ultrafleXtreme and FlexImaging. Regions of interest are chosen.



2<sup>nd</sup> stage: MSI analysis of regions of interest of pituitary sections using timsTOF flex and FlexImaging

Average mass spectra generated by two workflows from data obtained from the same pituitary. Complimentary and partially repeatable data are generated.



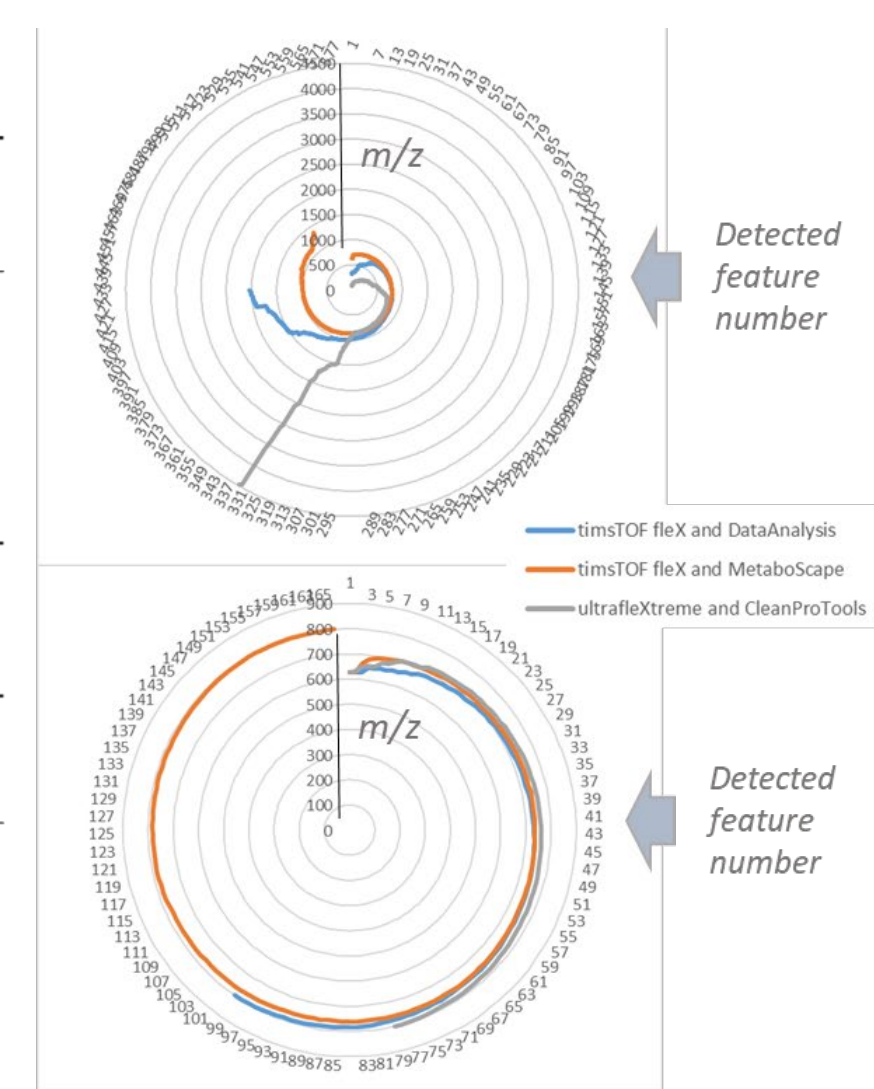
Different workflows are generating complimentary chemical information extending analyte coverage and allowing assessment of technical repeatability of measurements

Pearson's correlation coefficients for data obtained in full  $m/z$  range

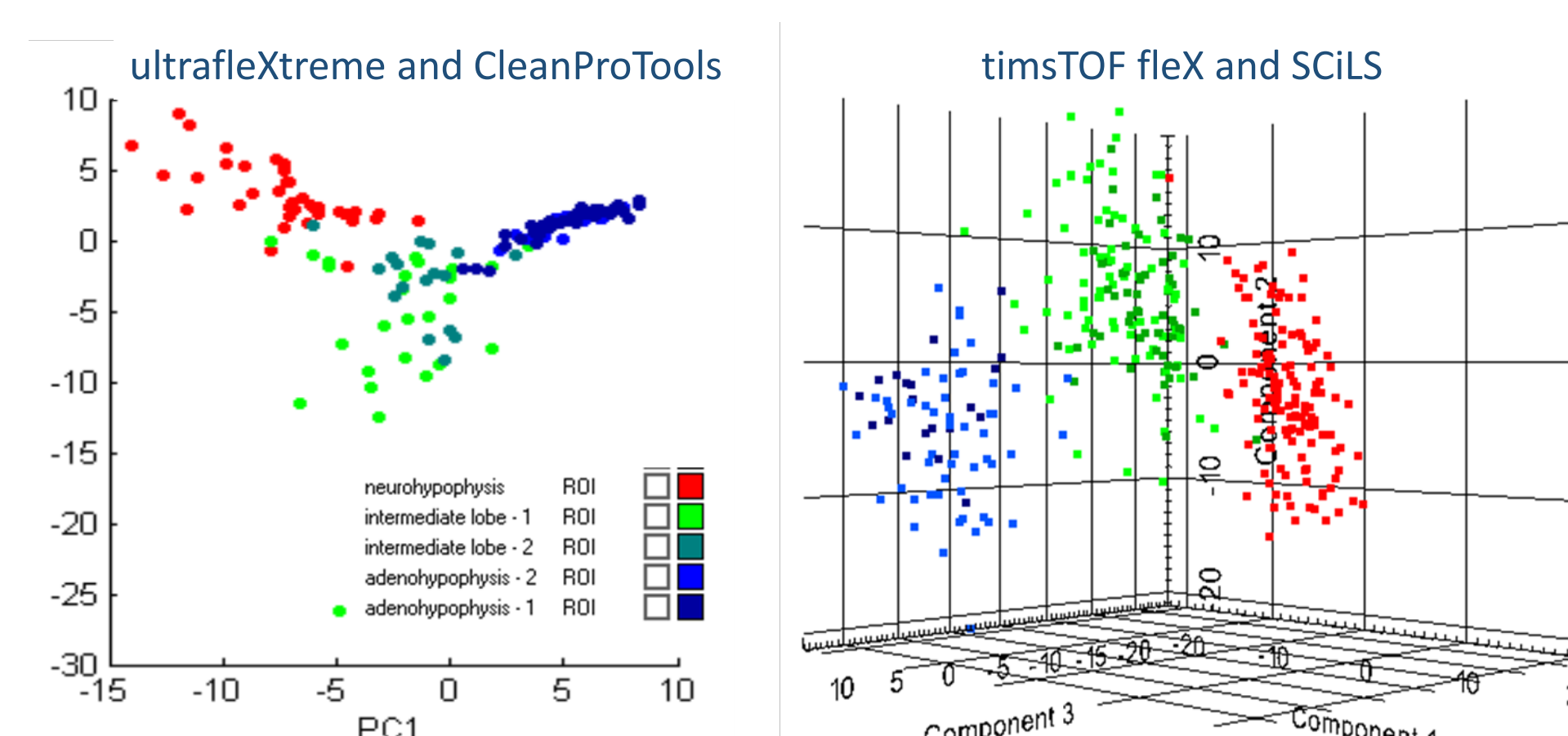
	timsTOF flex and DataAnalysis	timsTOF flex and MetaboScape	ultrafleXtreme and CleanProTools
timsTOF flex and DataAnalysis	1.00		
timsTOF flex and MetaboScape	0.95	1.00	
ultrafleXtreme and CleanProTools	0.75	0.82	1.00

Pearson's correlation coefficients for data obtained in 600-900  $m/z$  range

	timsTOF flex and DataAnalysis	timsTOF flex and MetaboScape	ultrafleXtreme and CleanProTools
timsTOF flex and DataAnalysis	1.00		
timsTOF flex and MetaboScape	0.96	1.00	
ultrafleXtreme and CleanProTools	0.98	0.98	1.00

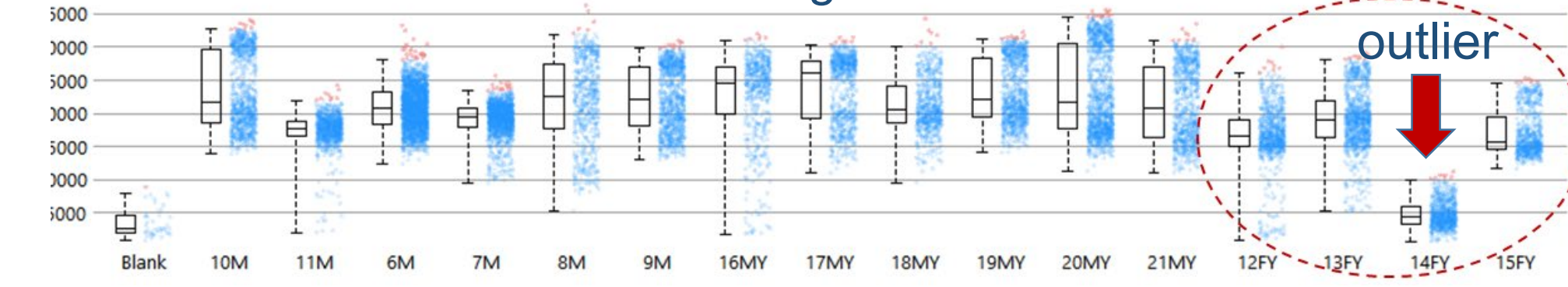


Principal component analysis of data generated by two workflows: three major pituitary anatomical regions are discriminated

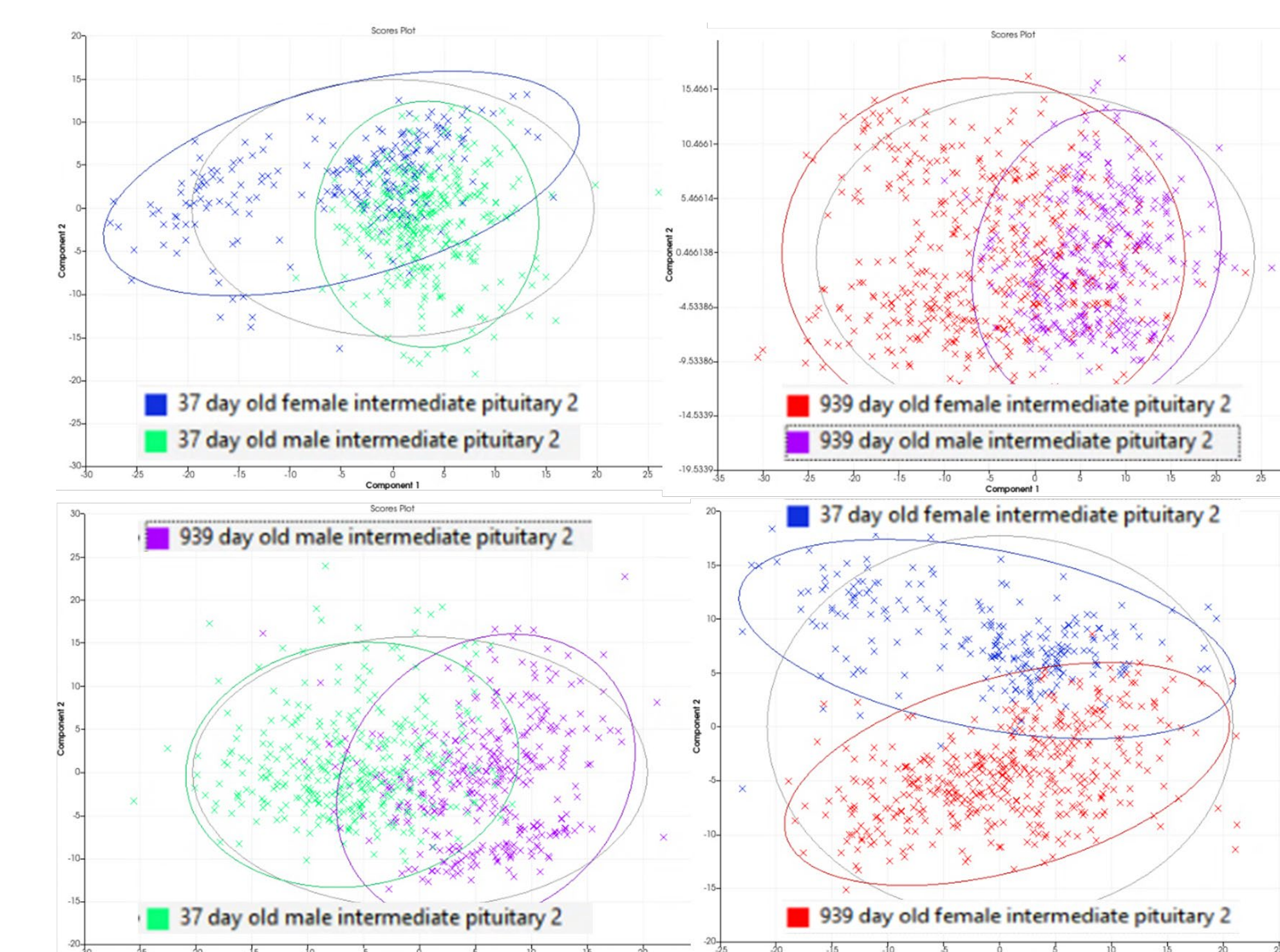


## Age and sex differences in chemical organization of mouse pituitary

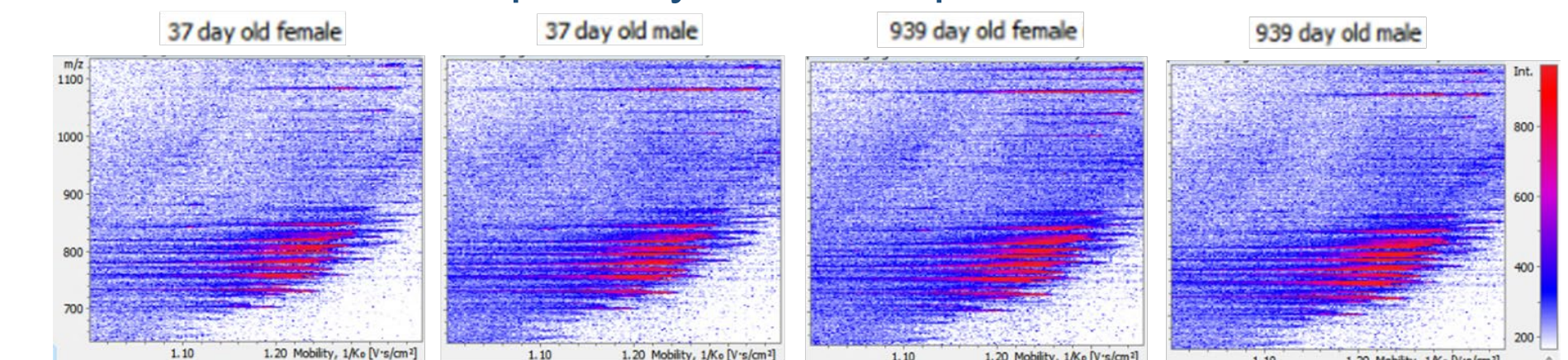
Variability of individual data sets has both biological and methodological causes



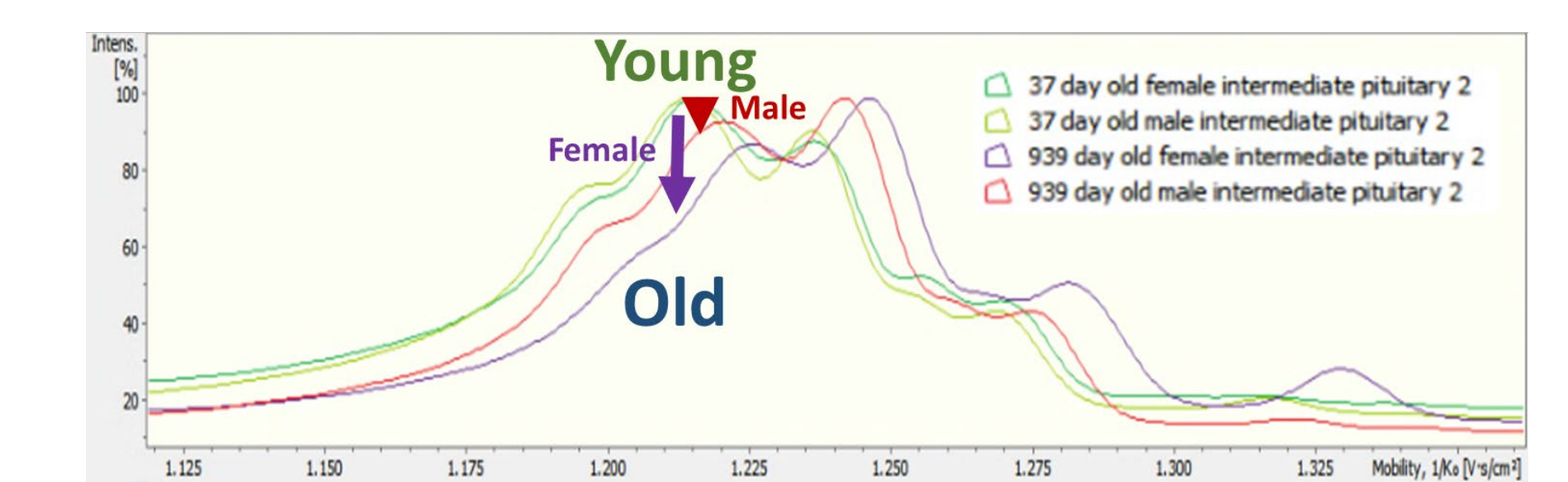
Principal component analysis uncovers chemical differences between intermediate pituitaries of different sex and age animals



Ion mobility separation improves analyte coverage and provides additional information on age and sex differences in intermediate pituitary chemical profiles

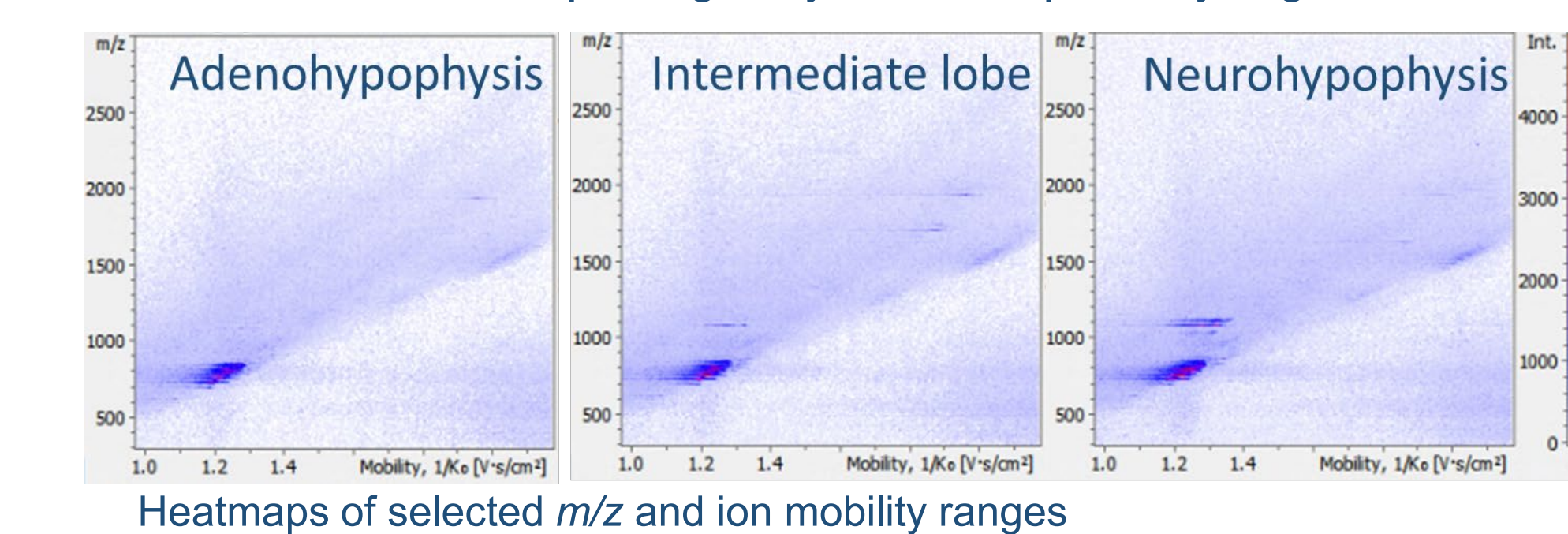


Heatmaps of selected  $m/z$  and ion mobility ranges

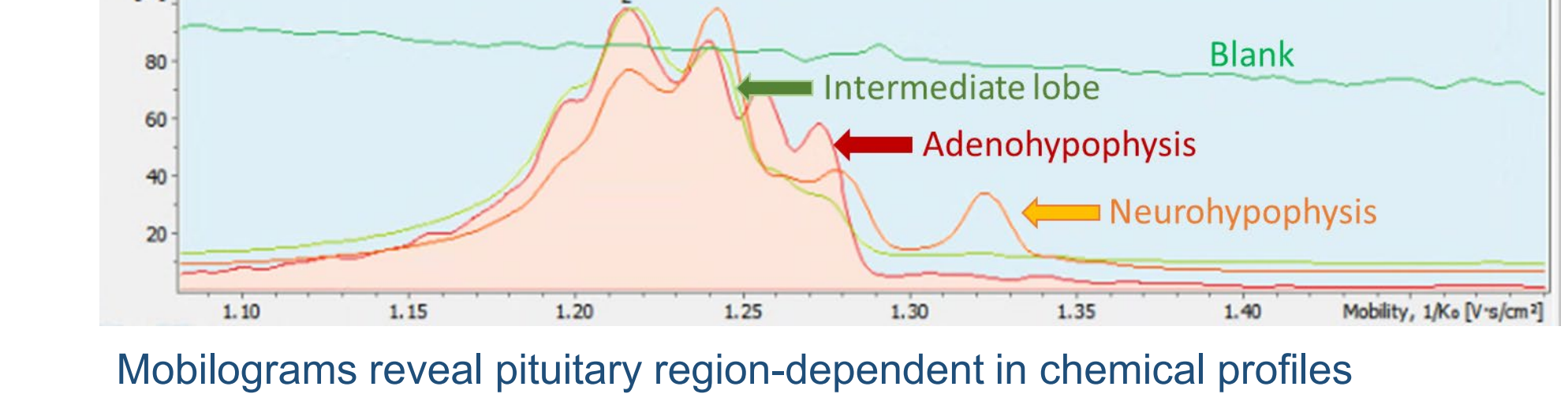


Mobilograms reveal age-dependent shifts in chemical profiles

Ion mobility separation improves analyte coverage and provides additional information on age and sex differences of chemical profiles in three morphologically-different pituitary regions

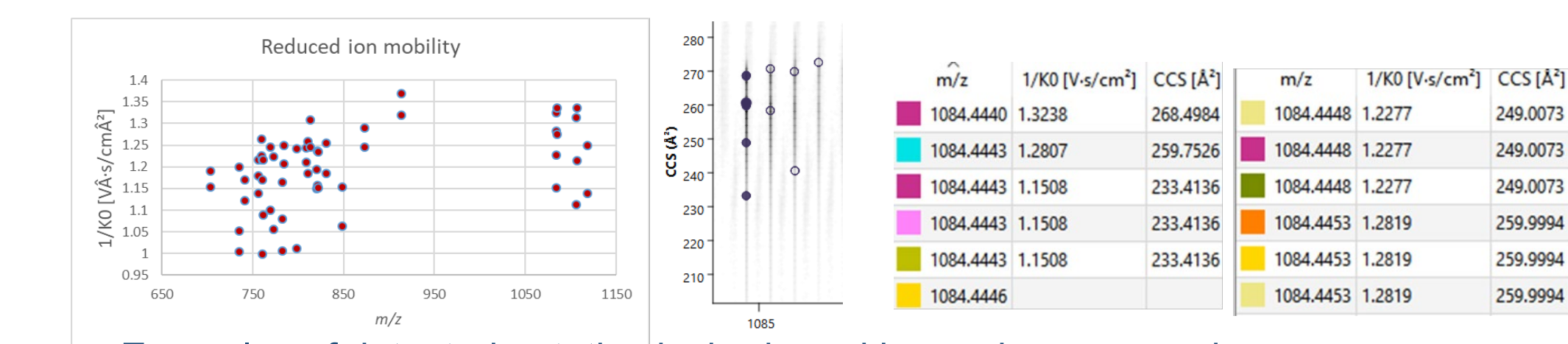


Heatmaps of selected  $m/z$  and ion mobility ranges



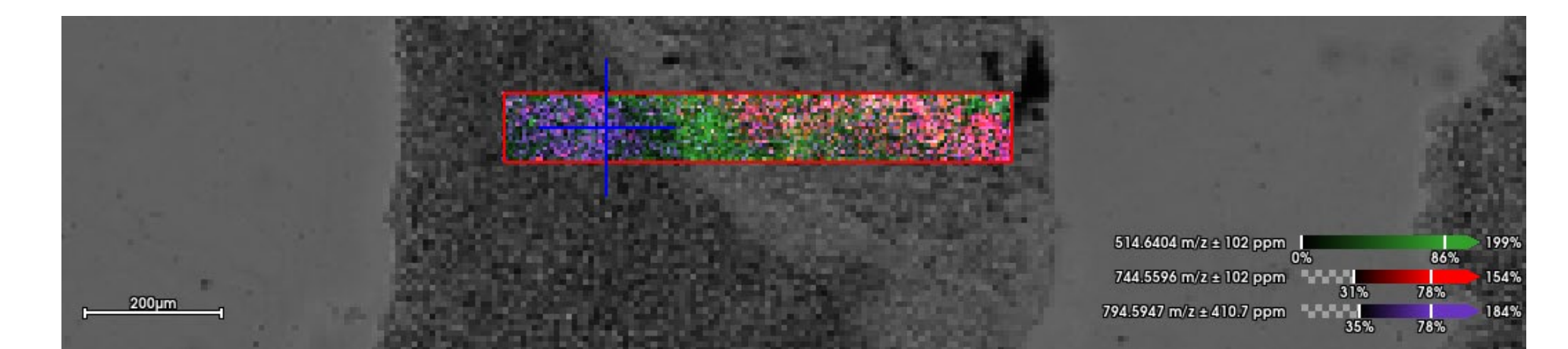
Mobilograms reveal pituitary region-dependent chemical profiles

Ion mobility separation allows detection of putative isomers. Among 526 features detected 11% are isobars.

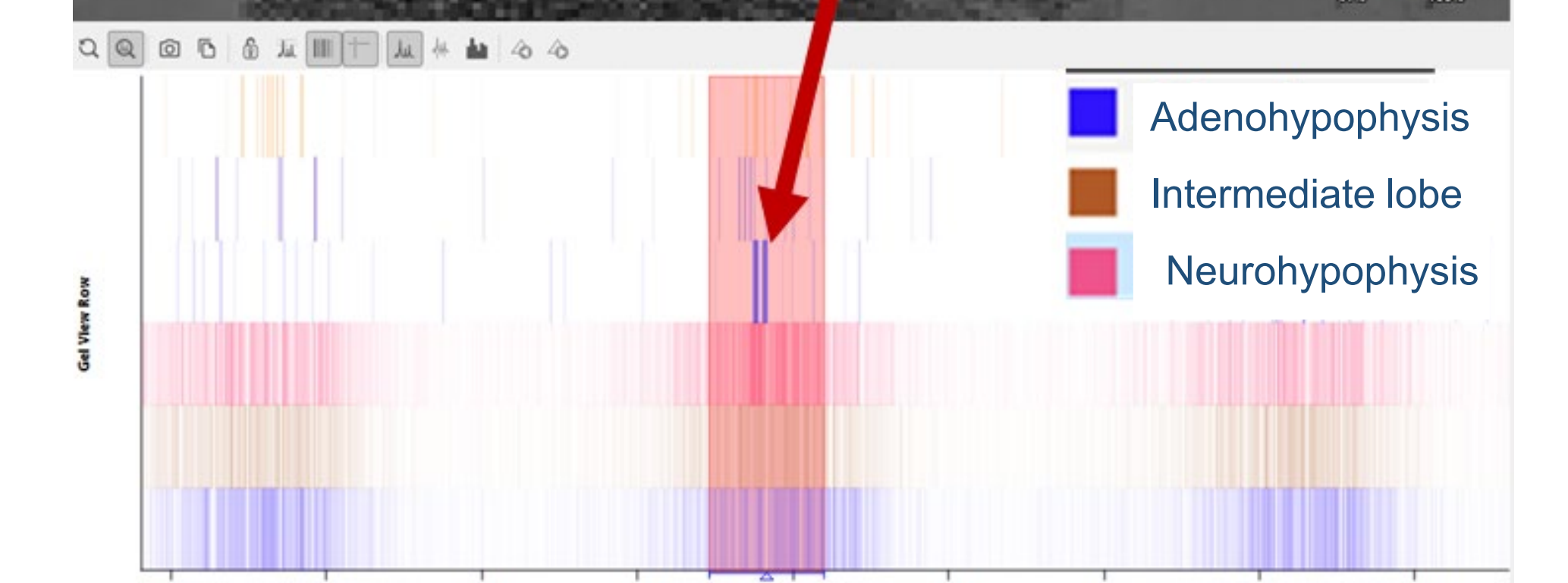
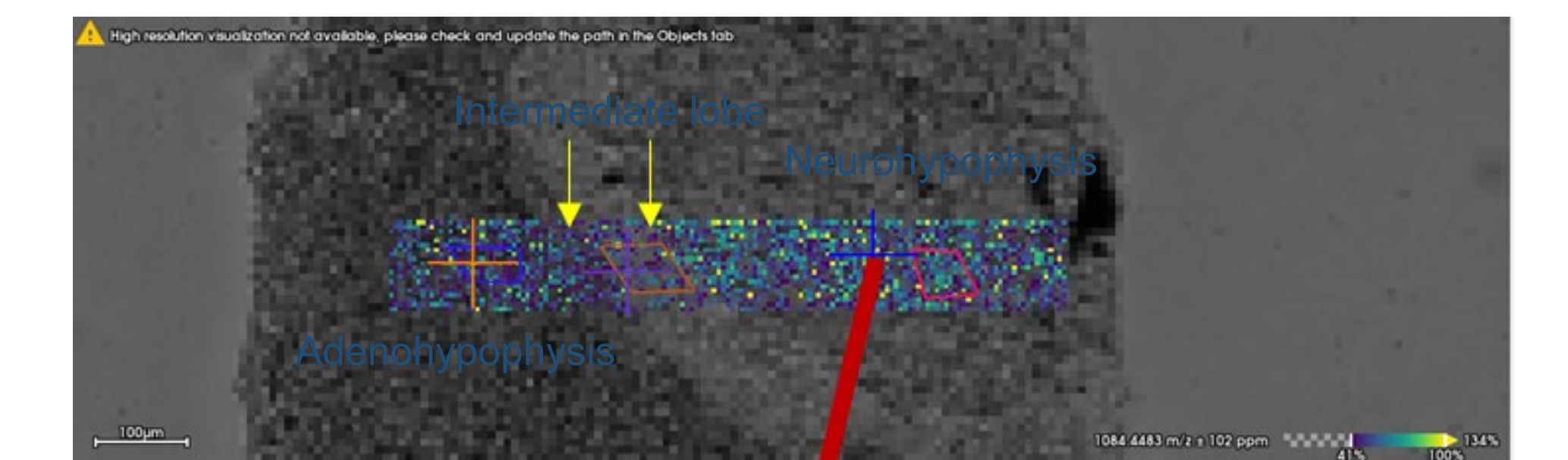


## High spatial resolution MSI analysis of pituitary using microGRID technology

MS imaging of mouse pituitary at 5  $\mu\text{m}$  spatial resolution

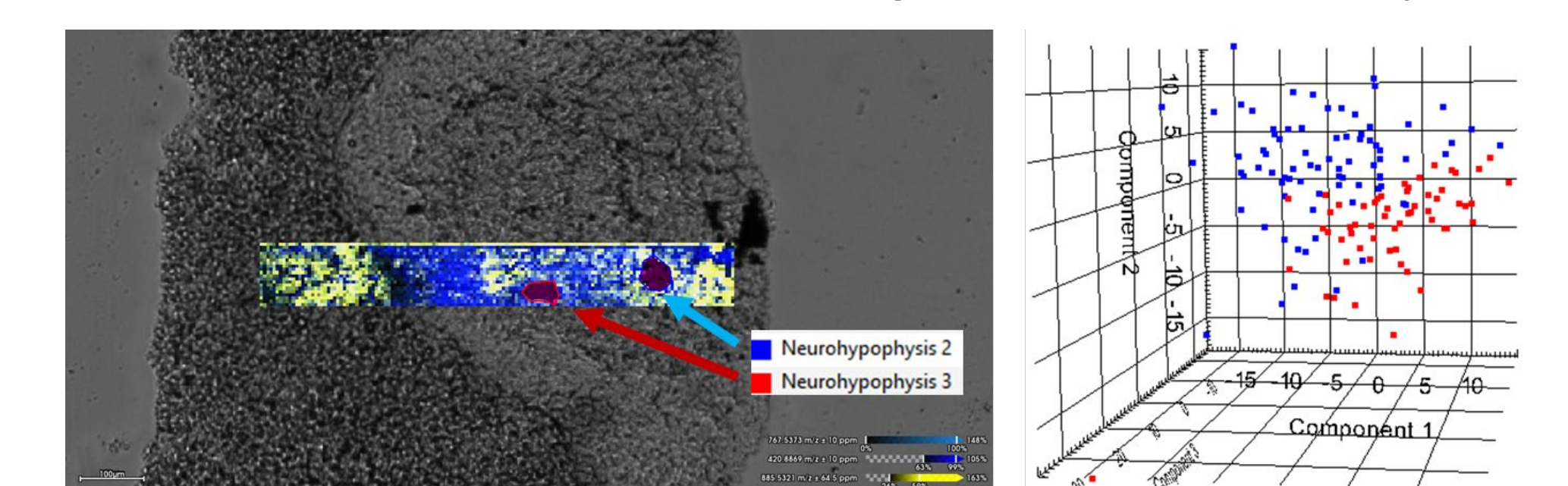


Pituitary region-specific biomarkers such as arginine-vasopressin are detected

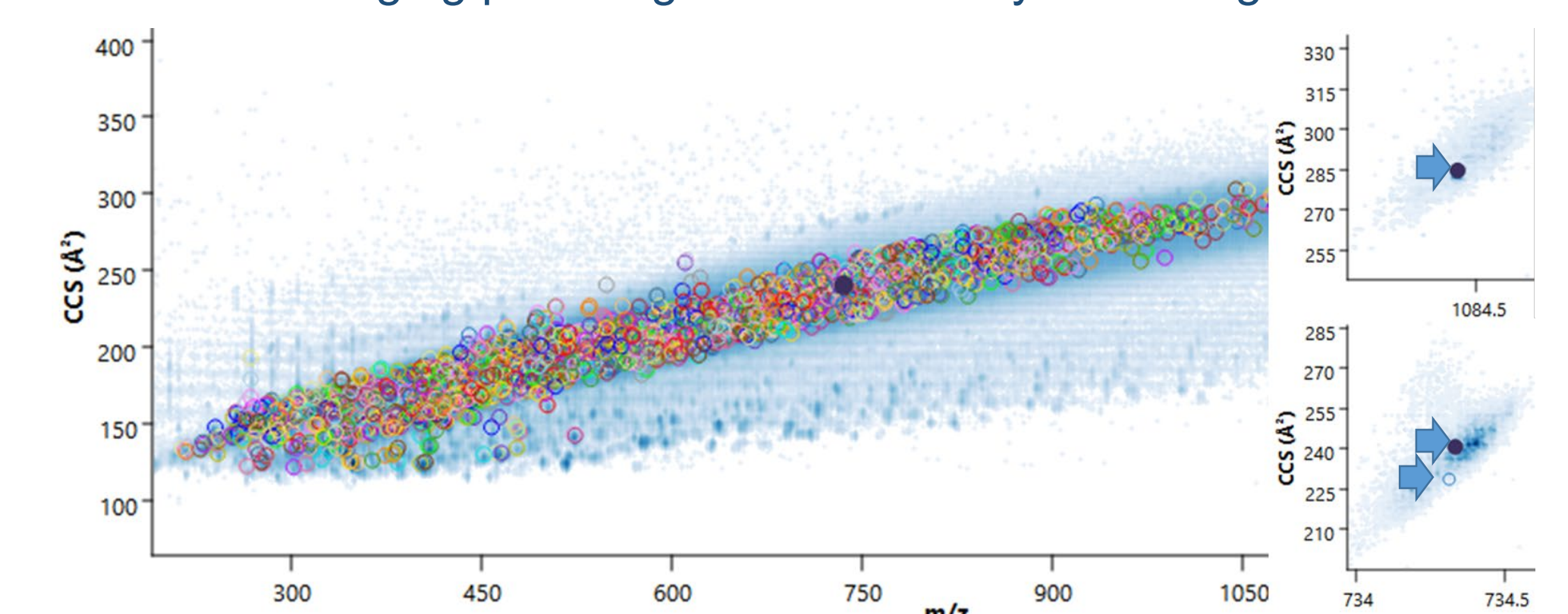


Gel view of selected  $m/z$  range: average mass spectra for three pituitary regions at the bottom and individual pixel mass spectra at the top.

Observation of intraregional chemical patterns is indicative of more complex than expected cellular organization of the pituitary



Ion mobility separation is compatible with high spatial resolution imaging providing enhanced analyte coverage



## CONCLUSIONS

- Multiplatform MSI analysis of spatiochemical differences in young adult and senescent rodent endocrine systems is demonstrated
- Our results revealed the fine chemical organization of the pituitaries, with unique molecular profiles distinguishing three functionally different pituitary regions—the anterior, posterior, and intermediate lobes
- Lipid and peptide profiles varied across these regions, with the posterior lobe (neurohypophysis) and the anterior lobe (adenohypophysis) displaying the most pronounced differences
- The timsTOF flex instrument, equipped with microGRID, allows high spatial resolution imaging using ion mobility at 5-micrometer steps. This enables the determination of subregional analyte distributions.

## ACKNOWLEDGEMENTS

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The authors declare no competing financial interest.