Advances in Using JMP and JMP Pro for Analysis of High Spatial Resolution Mass Spectrometry Images

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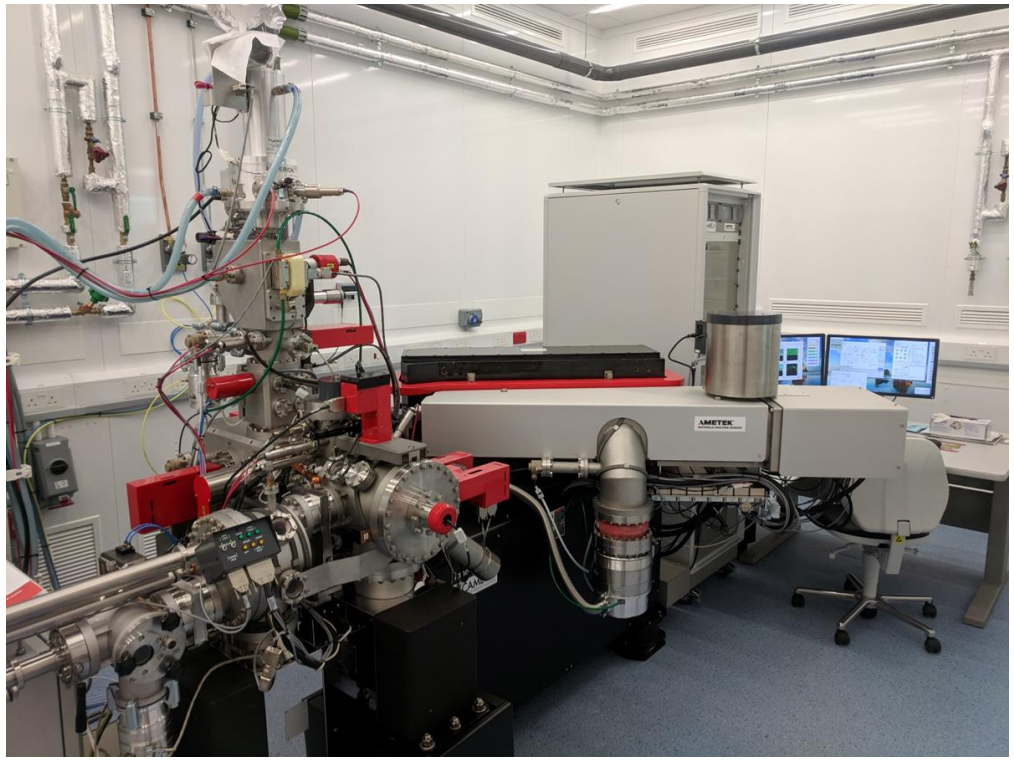
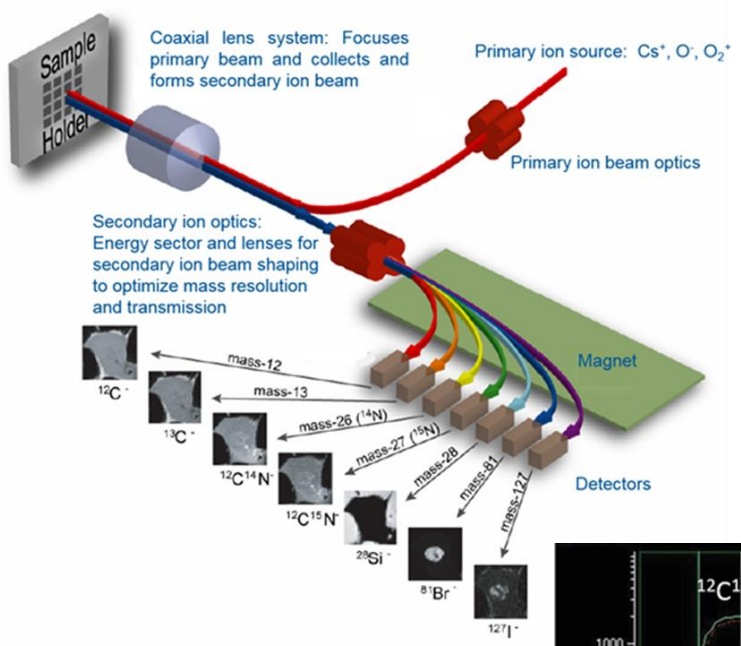
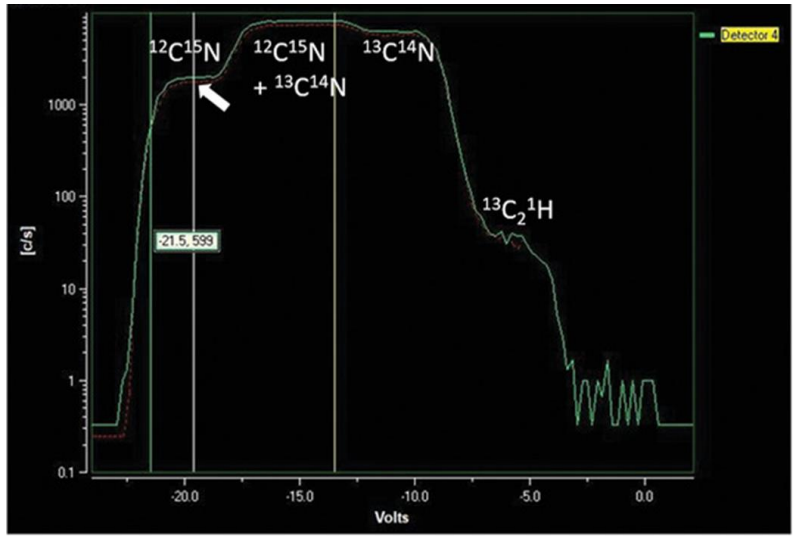
NanoSIMS for high spatial resolution mass spectrometry imaging







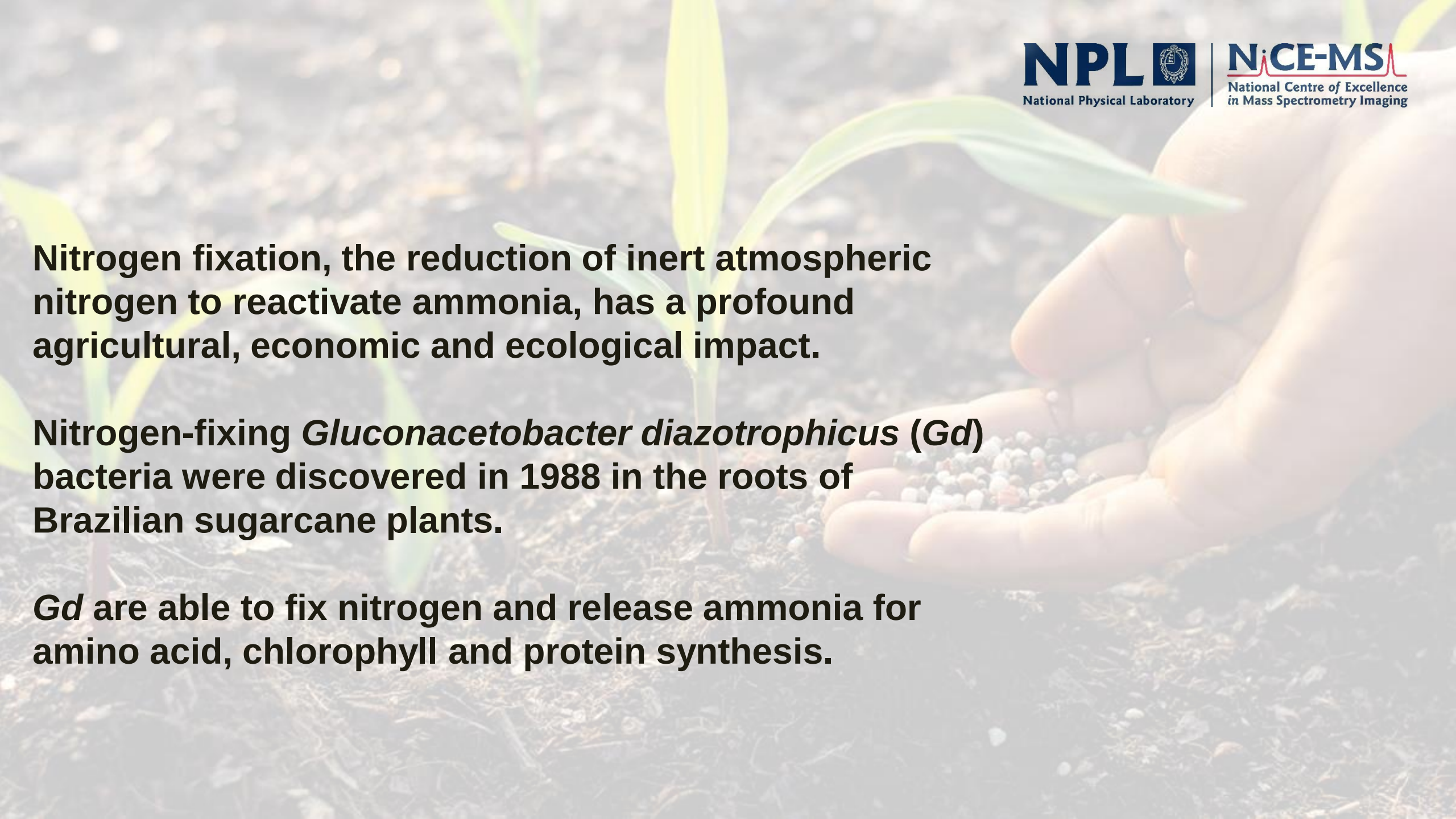
1. High spatial resolution (~35 nm) 2. High mass resolution (m/Δm ~ 15-20k) 3. High sensitivity (ppm-ppb)

Case Study 1

The availability of fixed nitrogen frequently limits agricultural production throughout the world.

Extensive global use of synthetic nitrogen fertilizers has resulted in:

- atmospheric pollution by ammonia and nitrous oxide
- smog, fine particulate pollution
- ecosystem acidification
- climate change



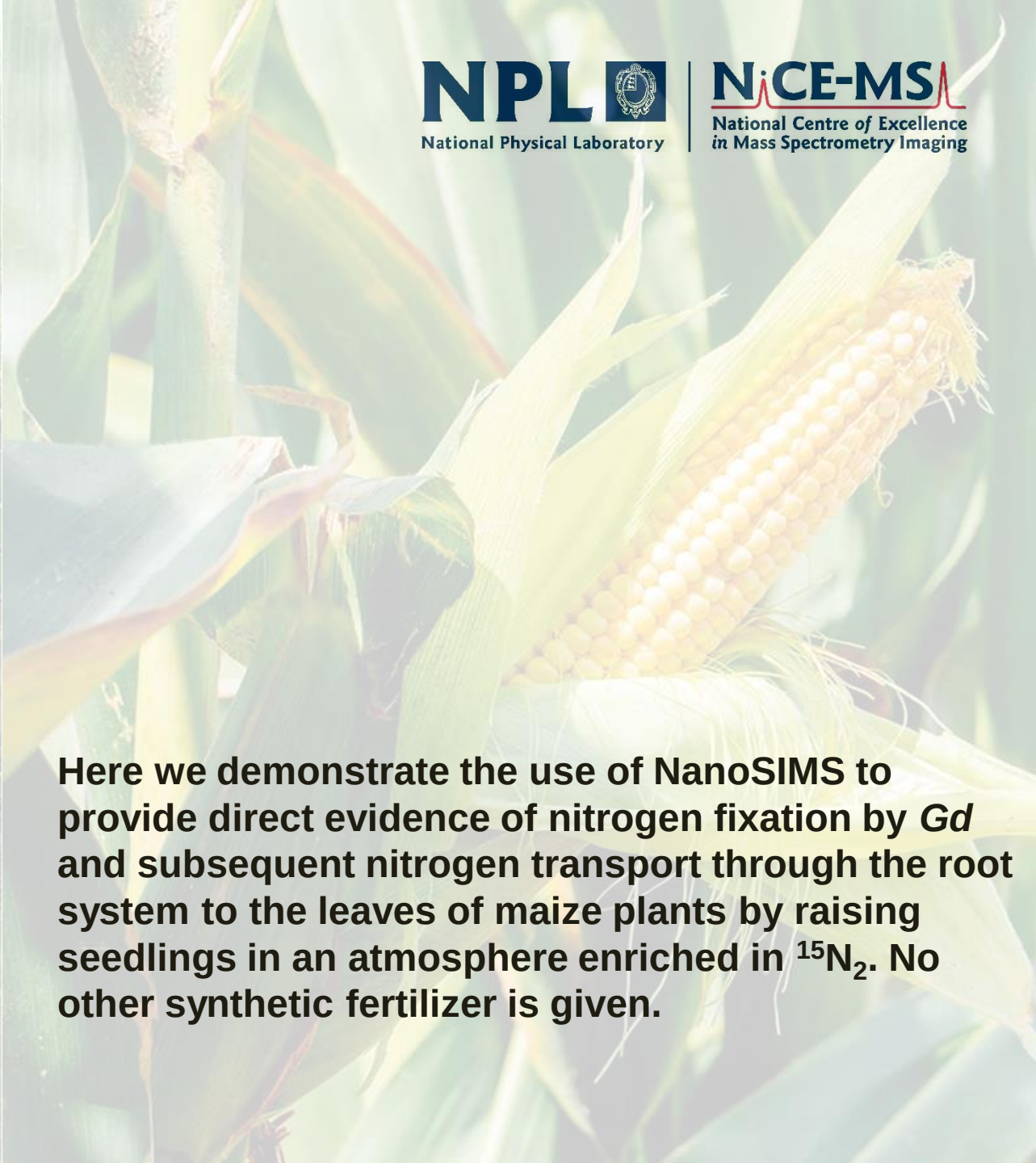
Nitrogen fixation, the reduction of inert atmospheric nitrogen to reactivate ammonia, has a profound agricultural, economic and ecological impact.

Nitrogen-fixing *Gluconacetobacter diazotrophicus* (*Gd*) bacteria were discovered in 1988 in the roots of Brazilian sugarcane plants.

Gd are able to fix nitrogen and release ammonia for amino acid, chlorophyll and protein synthesis.

There have been several reports of enhanced growth of sugarcane and other non-legume crops inoculated with *Gd*.

In no case has it been proven that significant combined or fixed nitrogen has been transferred from *Gd* to its plant partner.



Here we demonstrate the use of NanoSIMS to provide direct evidence of nitrogen fixation by *Gd* and subsequent nitrogen transport through the root system to the leaves of maize plants by raising seedlings in an atmosphere enriched in $^{15}N_2$. No other synthetic fertilizer is given.

JMP Workflow Builder Increases Efficiency of Basic First Level of Analysis

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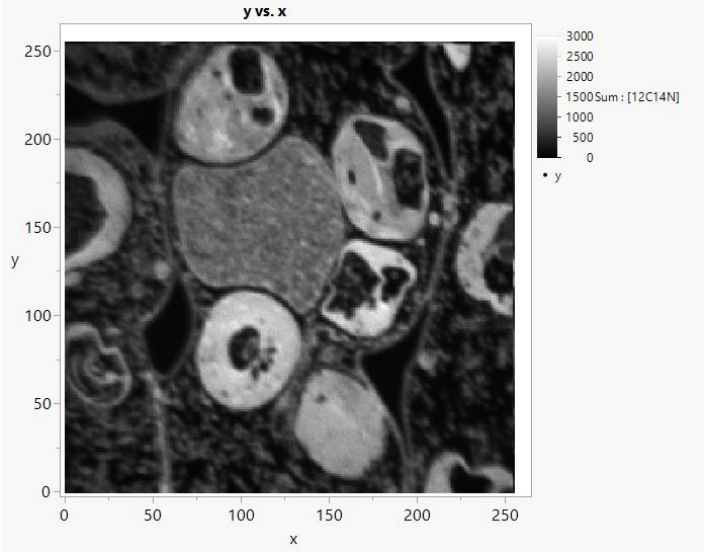
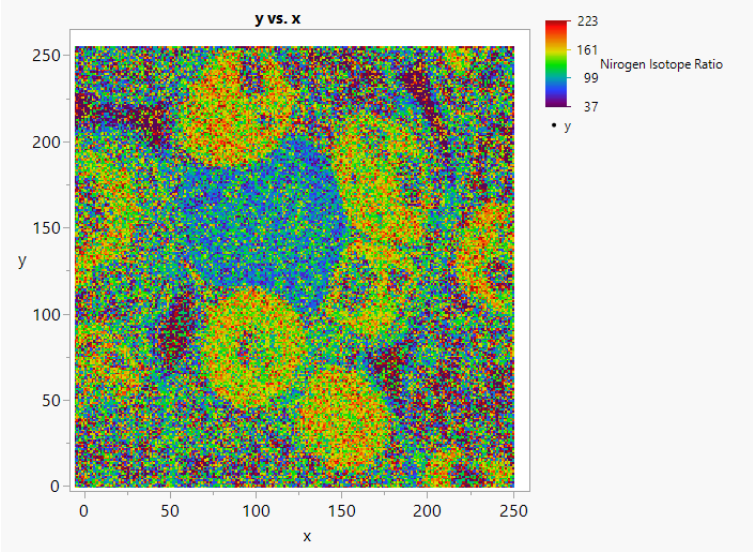
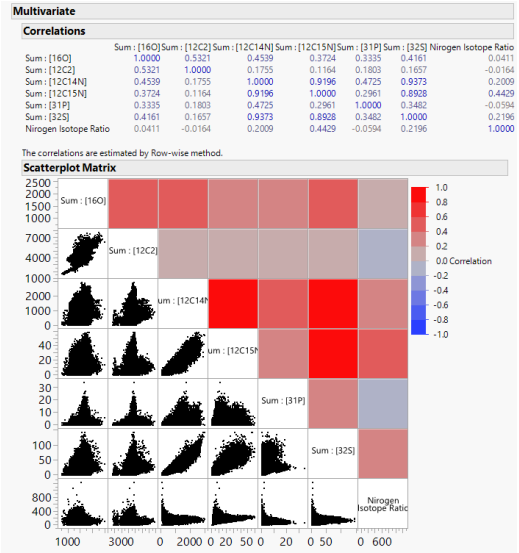
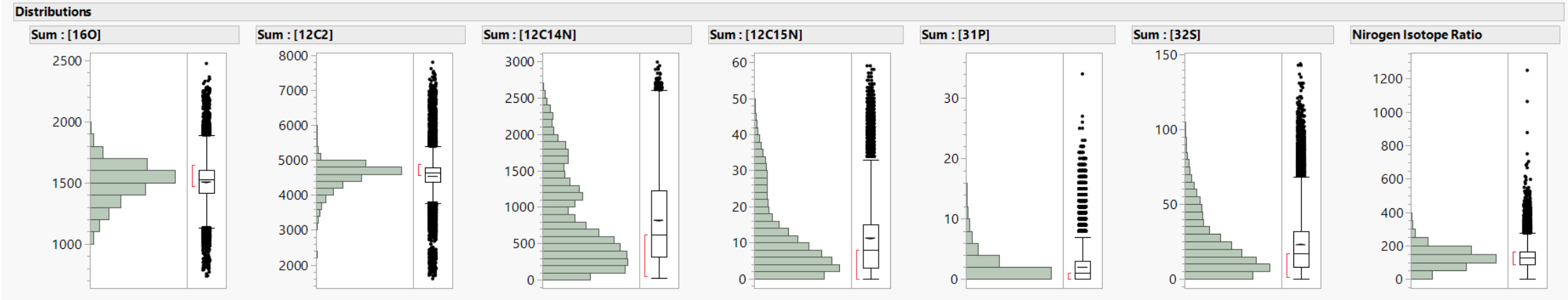






~180 seconds manually; ~ 6 seconds with Work Flow Builder

Time savings up to 1 hour per day



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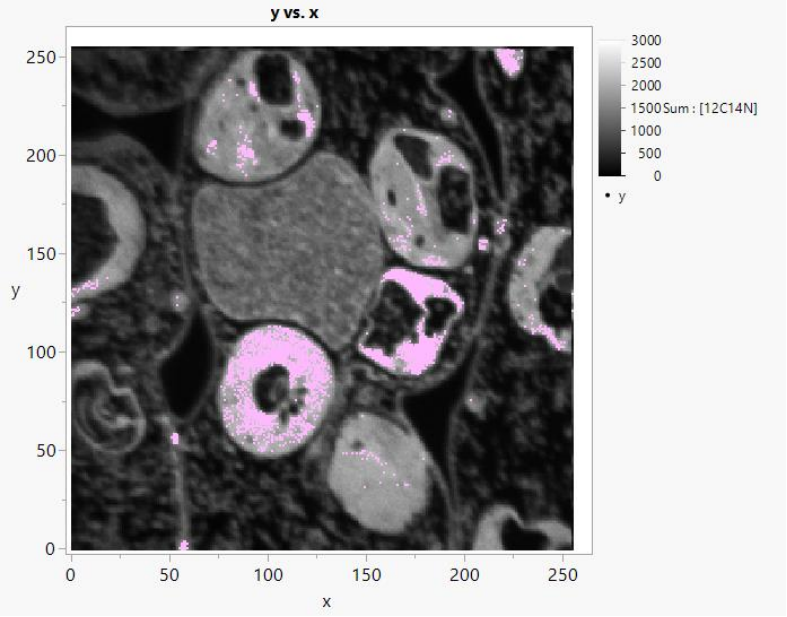
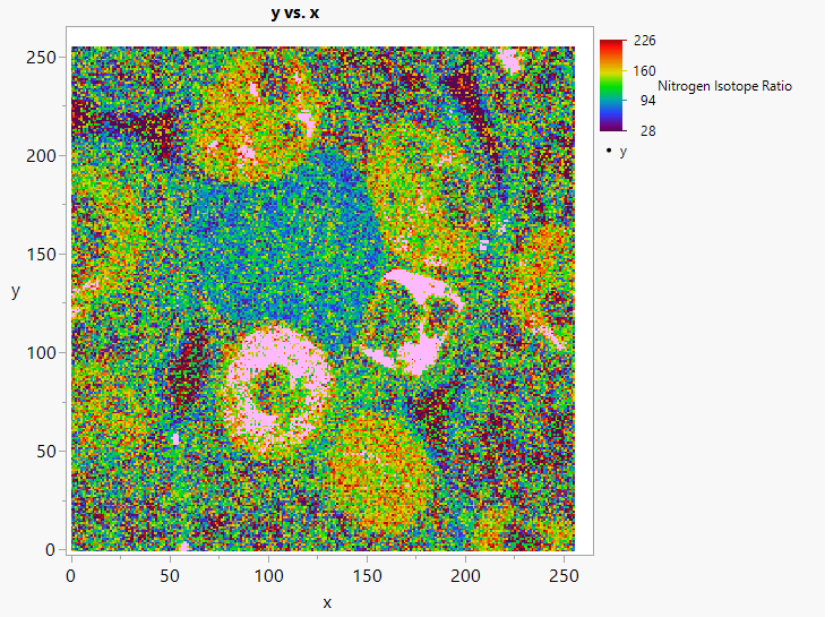
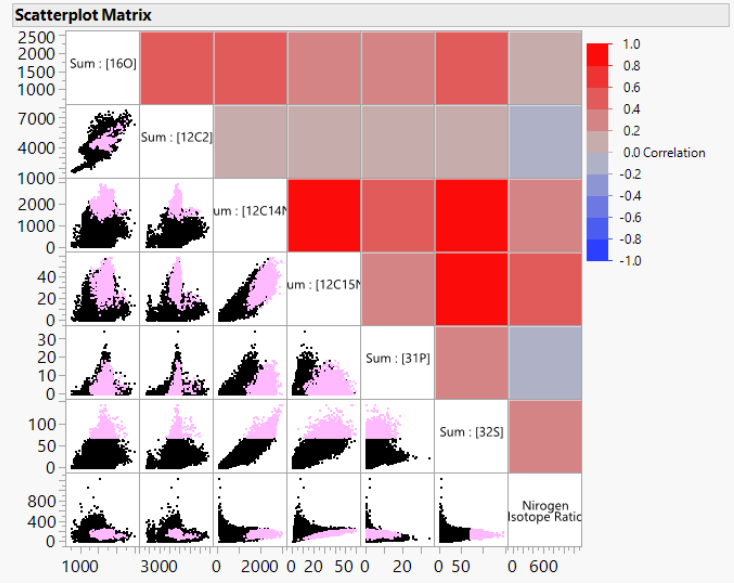
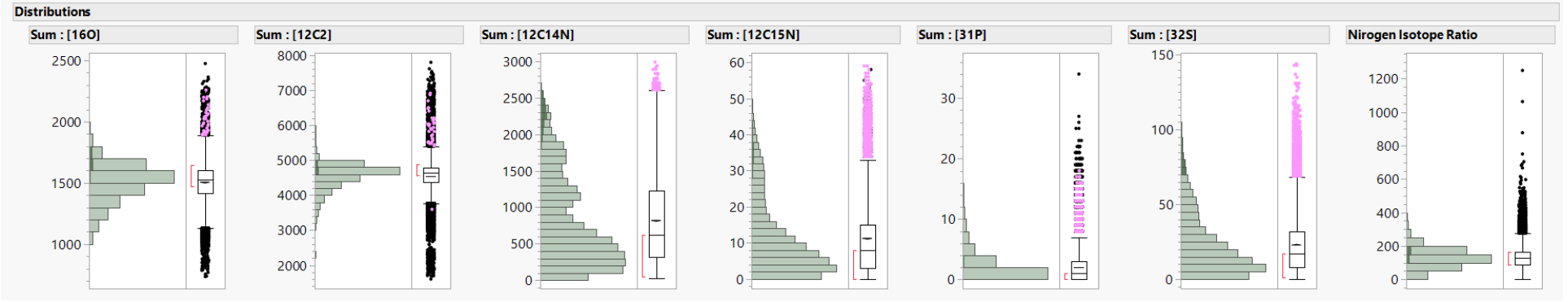


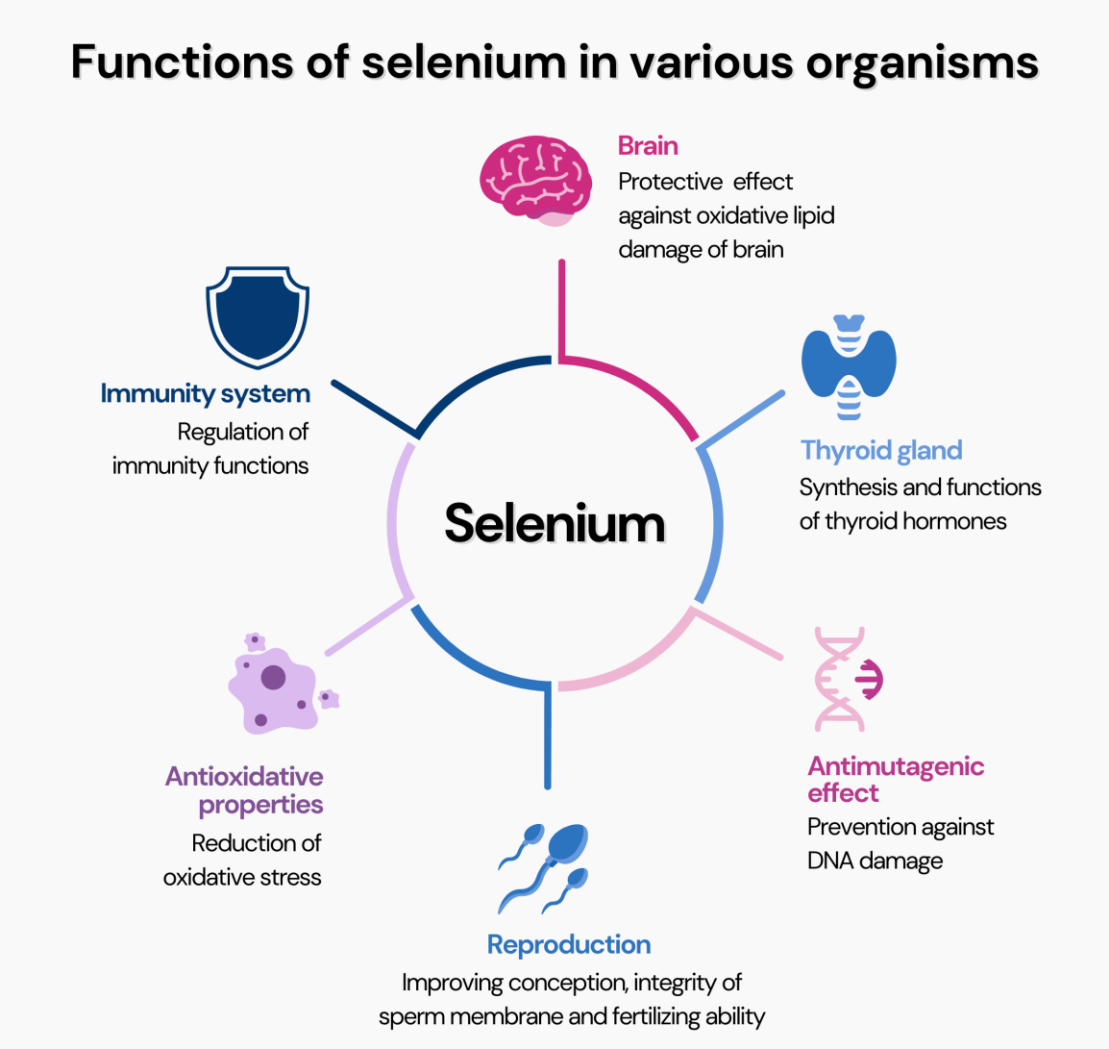






For N fixation to occur, the help of sulfur containing enzymes is required.

Case Study 2: Adding more complexity to JMP Workflow Builder for specific projects

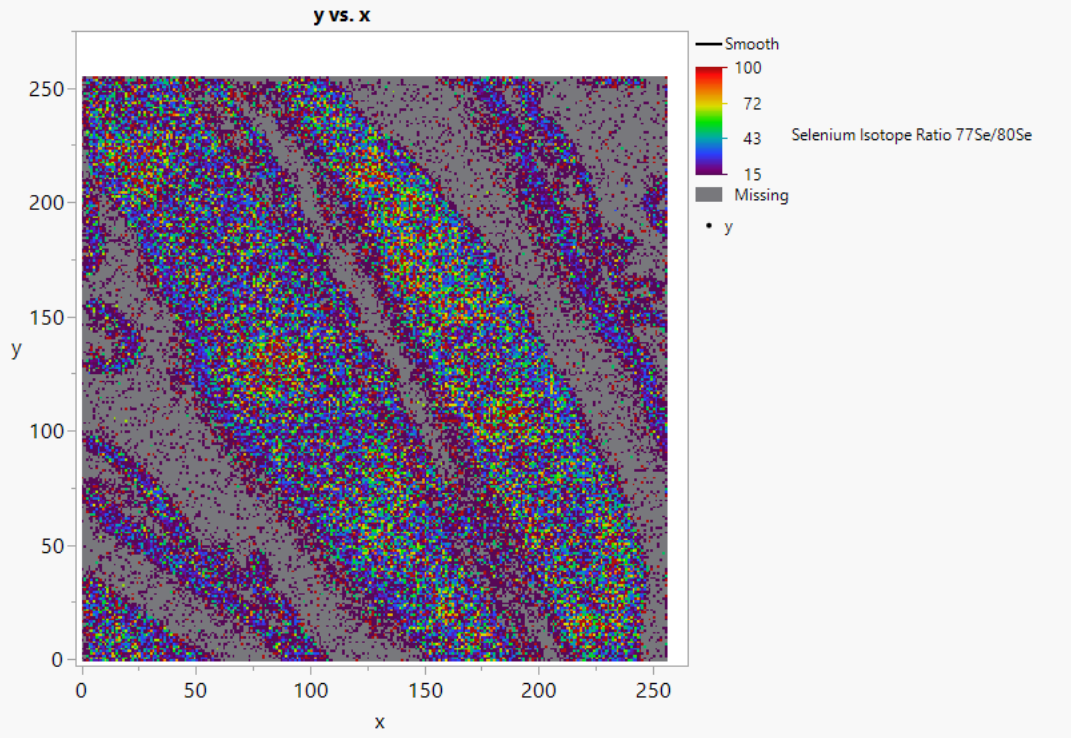


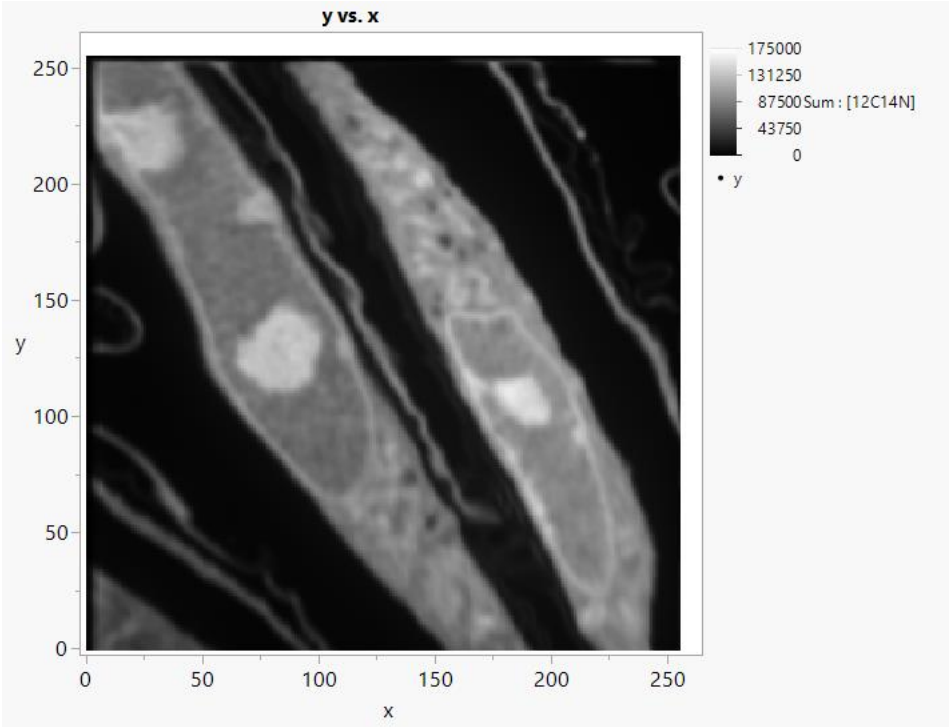
Studying metabolism of selenium precursors (supplements) to selenoproteins in cells. *Do they associate to help protect the integrity of DNA?*

Need to analyze at concentrations relevant to the body – too much can be toxic.

Cells were cultured in media with 300 nM concentrations of stable isotope labeled ^{76}Se-methylselenocysteine, ^{77}Se-seleno-L-methionine or ^{82}Se-Na-selenite at isotopic purities of 99.9%, 99.8% and 98.9%, respectively as well as 2 μm ^{15}N-thymidine to label DNA.

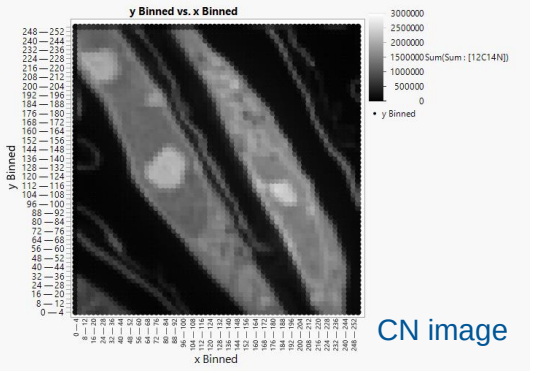
Adding more complexity to JMP Workflow Builder



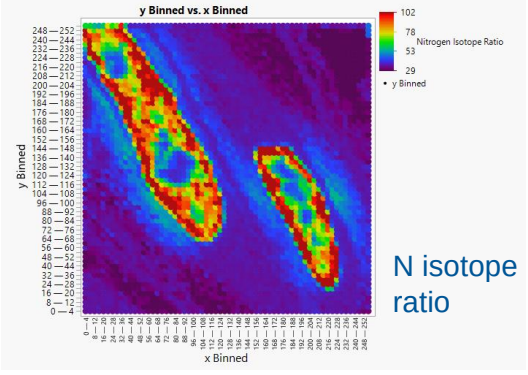


Low selenium counts leads to an abundance of missing data. Therefore, we might be better off trading spatial resolution for more counts by binning the image.

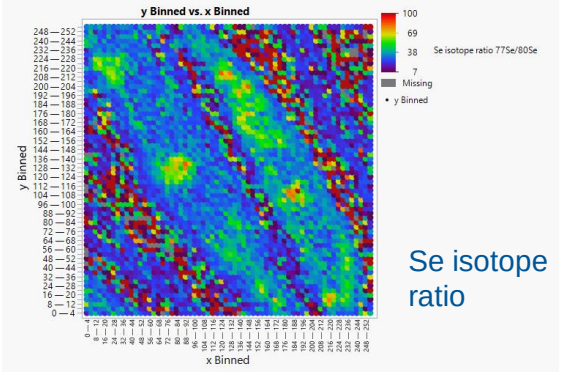
Adding more complexity to JMP Workflow Builder



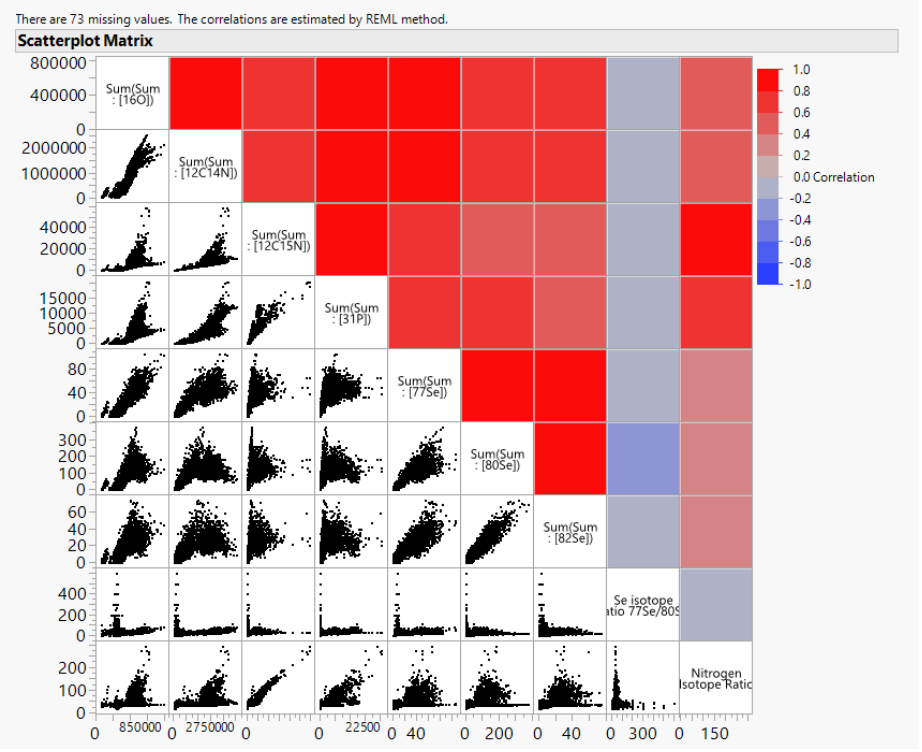
CN image



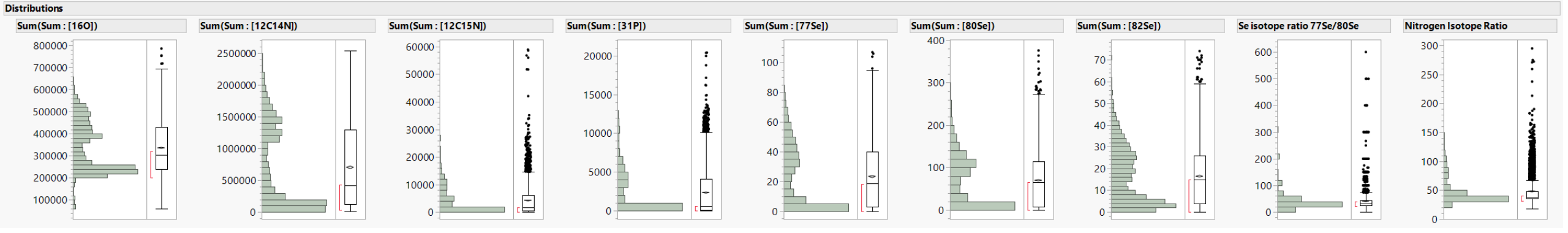
N isotope ratio



Se isotope ratio



Negative correlations between ^{77}Se, ^{80}Se and $^{77}Se/^{80}Se$??



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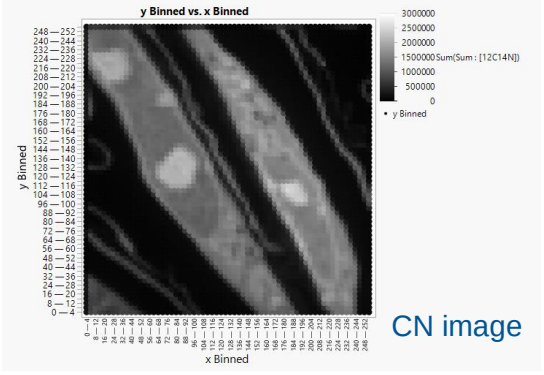

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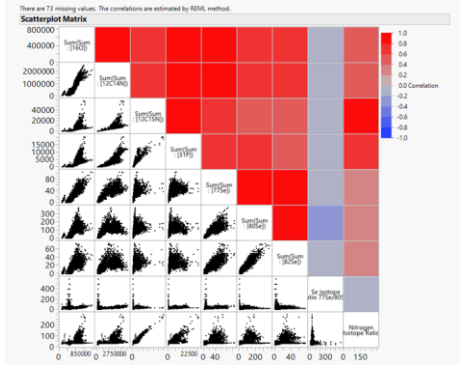
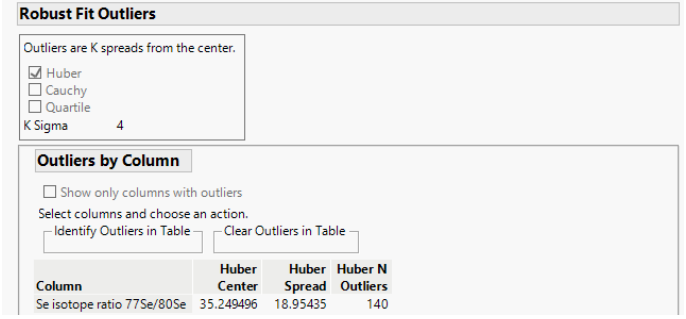
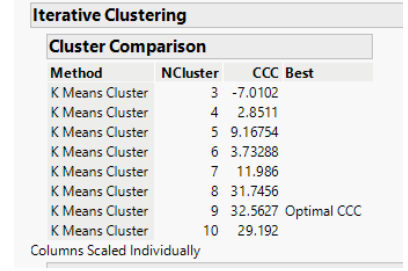
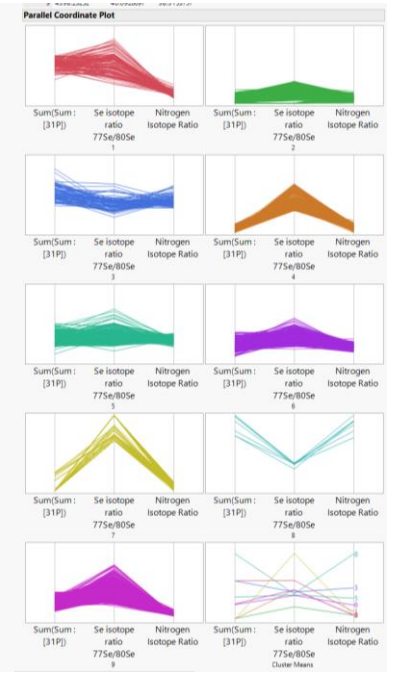
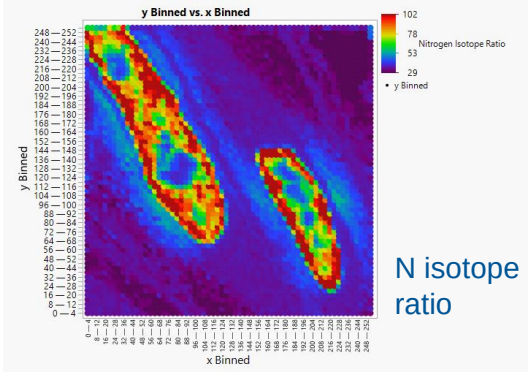


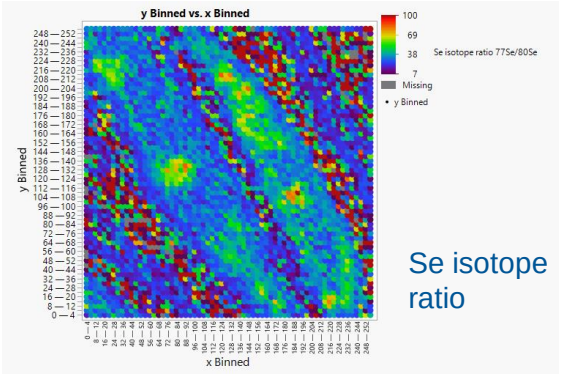




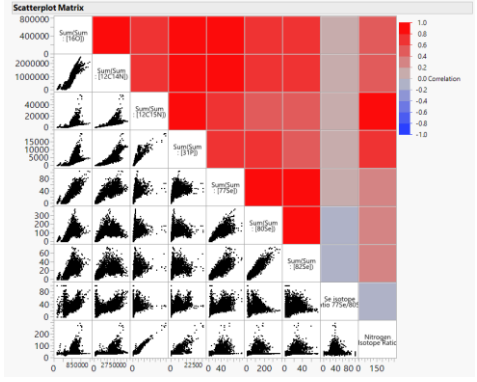


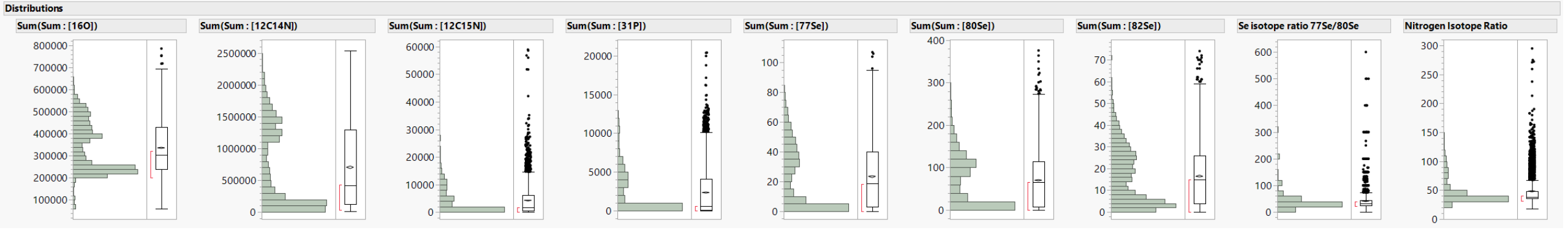


N isotope ratio



Se isotope ratio





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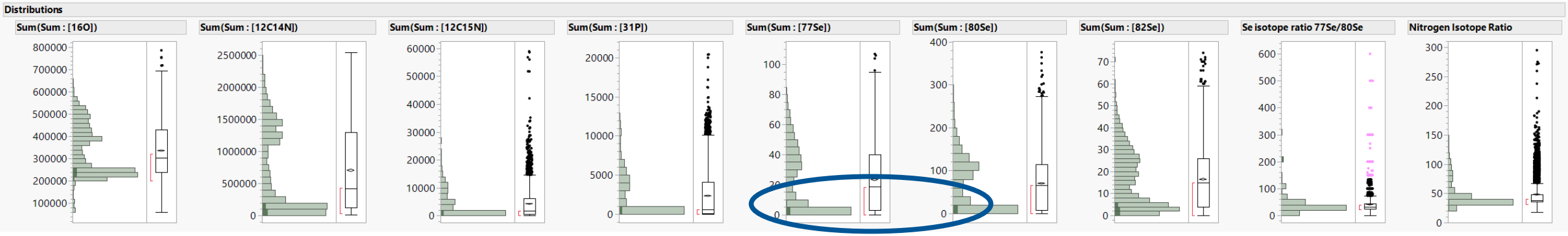

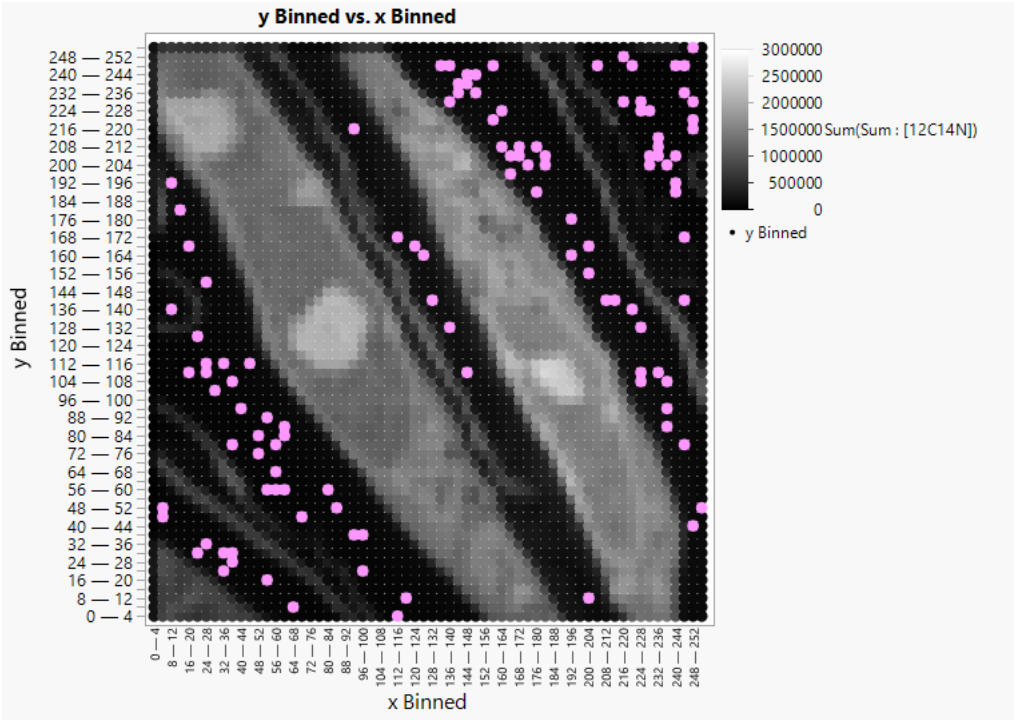

Outlier issue related to low Se counts coming from embedding resin

Adding more complexity to JMP Workflow Builder

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Iterative Clustering

Cluster Comparison

Method	NCluster	CCC	Best
K Means Cluster	2	1.3581	Optimal CCC

Columns Scaled Individually

K Means NCluster=2

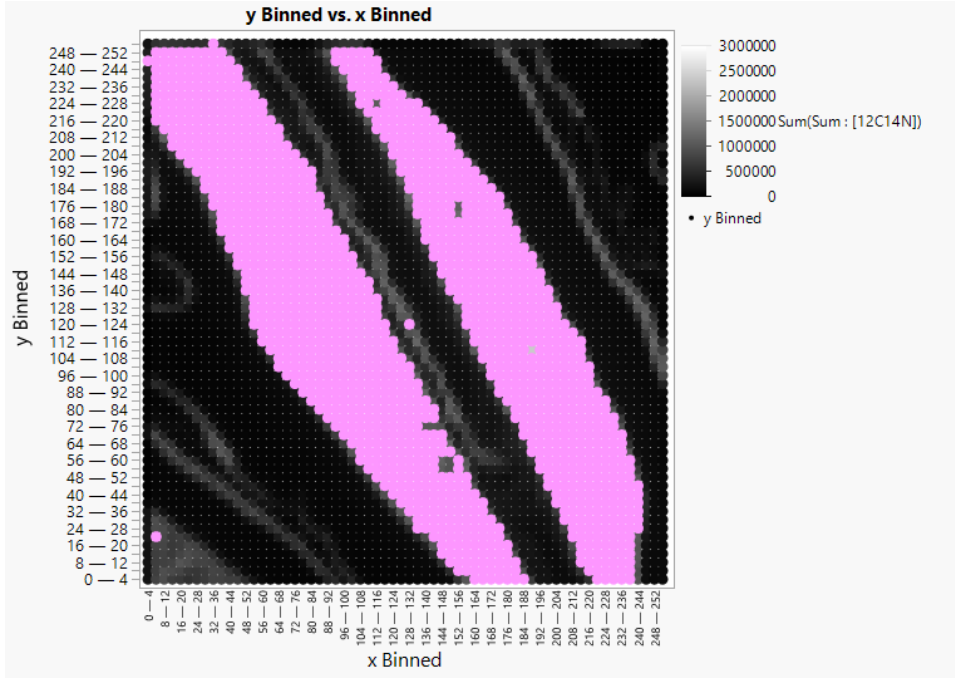
Columns Scaled Individually

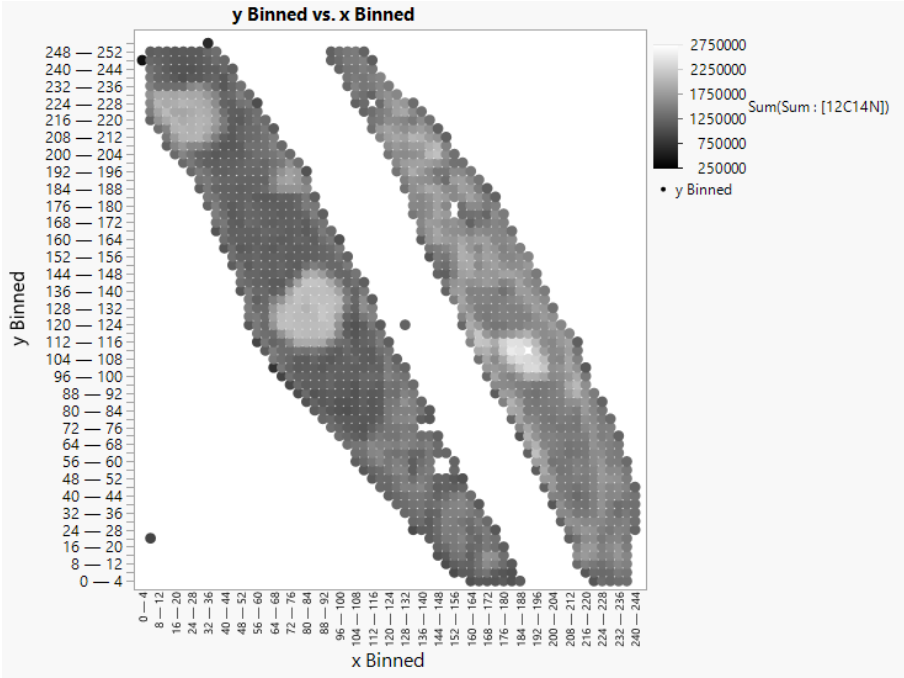
Cluster Summary

Cluster	Count	Step	Criterion
1	1492	12	0
2	2391		

Cluster Means

Cluster	Sum(Sum : [31P])	Se isotope ratio 77Se/80Se	Nitrogen Isotope Ratio	Sum(Sum : [12C14N])
1	5646.91689	38.3031031	66.5420533	1469999.46
2	437.84358	32.9258357	37.6174782	284603.046





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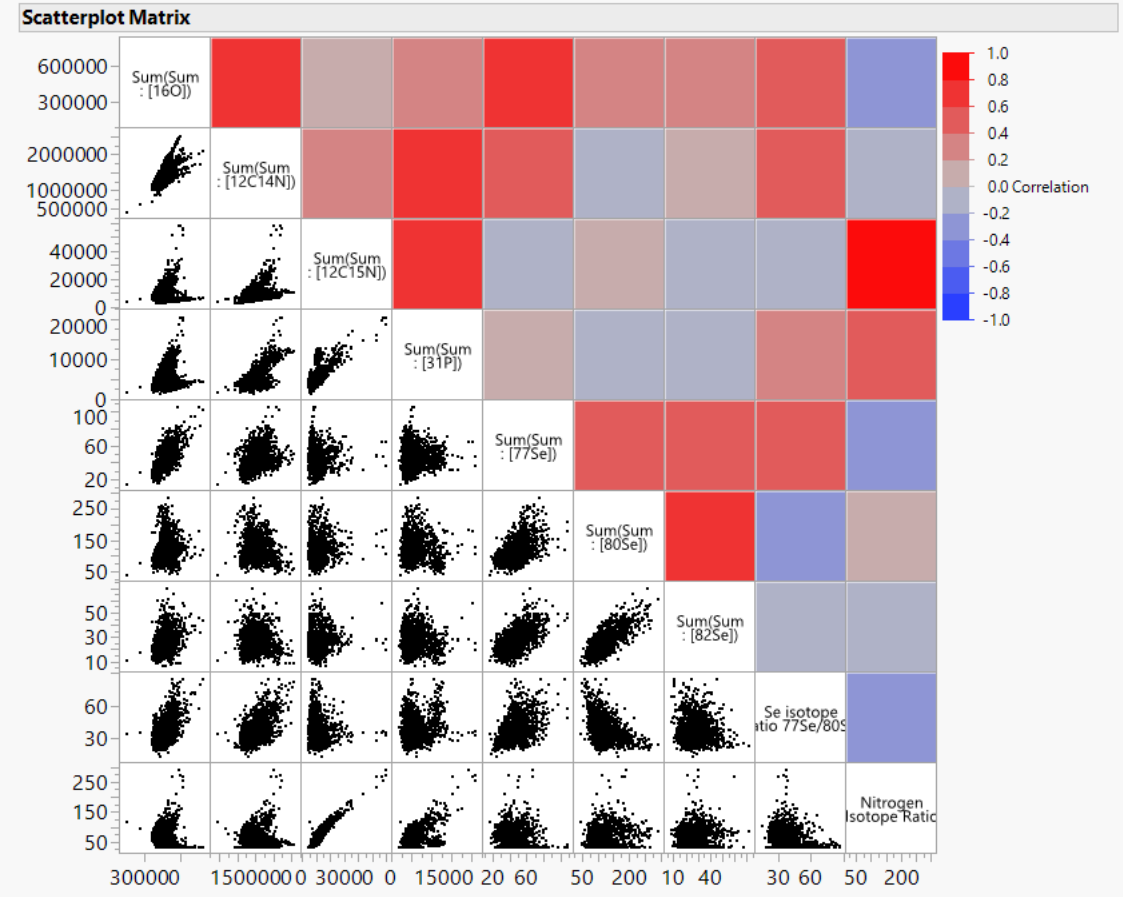
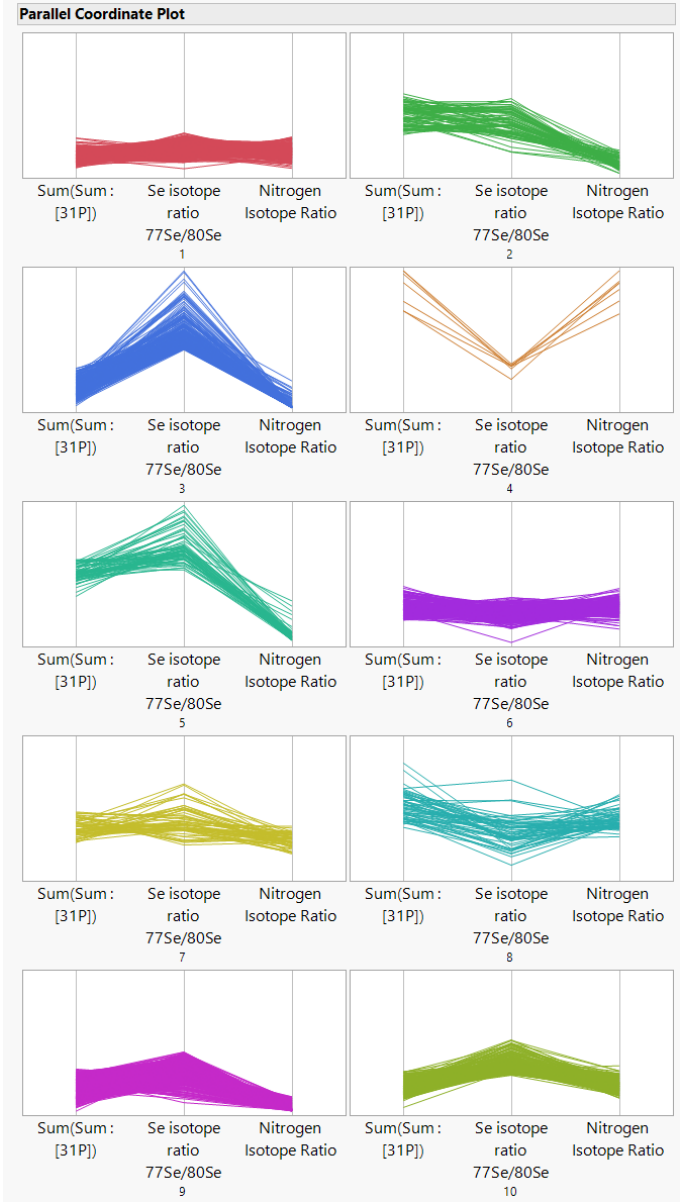


Work Flow Builder:

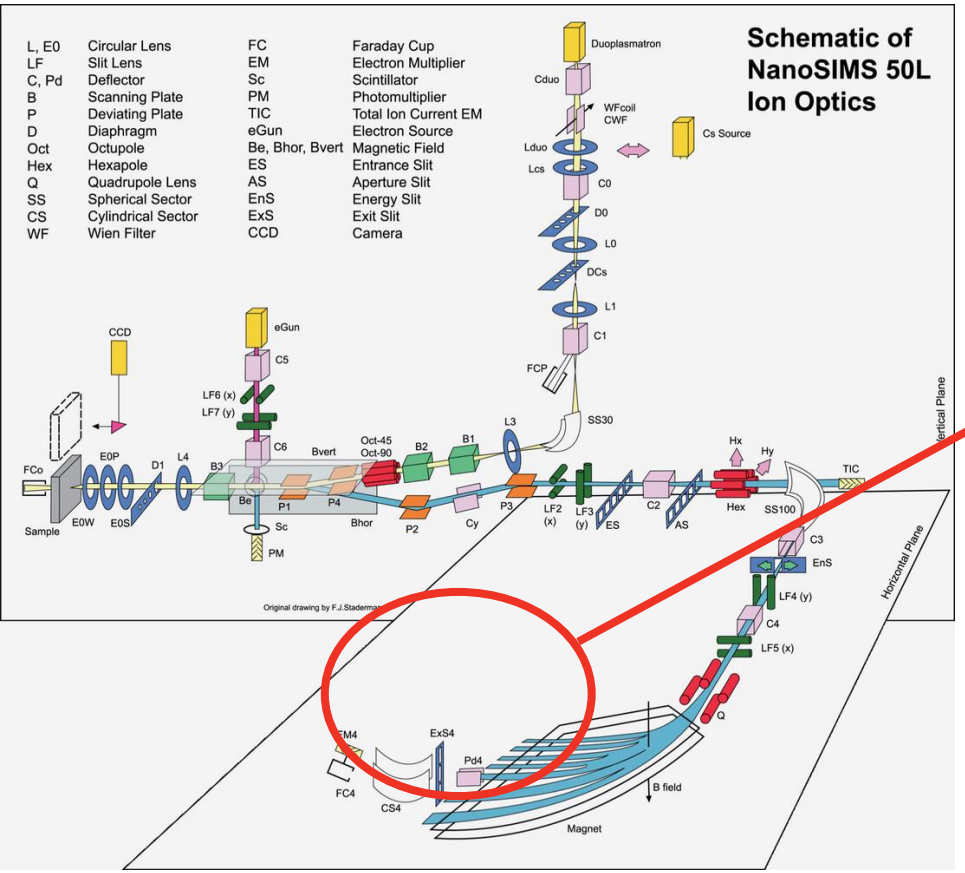
1. 4x4 bin of the image
2. Graph builder x3 (project specific)
3. Distributions of all mass counts and isotope ratio values
4. Multivariate statistics – scatterplot matrix/heat map
5. Outlier analysis on Se isotope ratios
6. K-Means clustering – parallel coordinate plots
7. Use k-means to segment out the cells
8. Create new subset data table with just this data
9. Graph builder again
10. Multivariate statistics

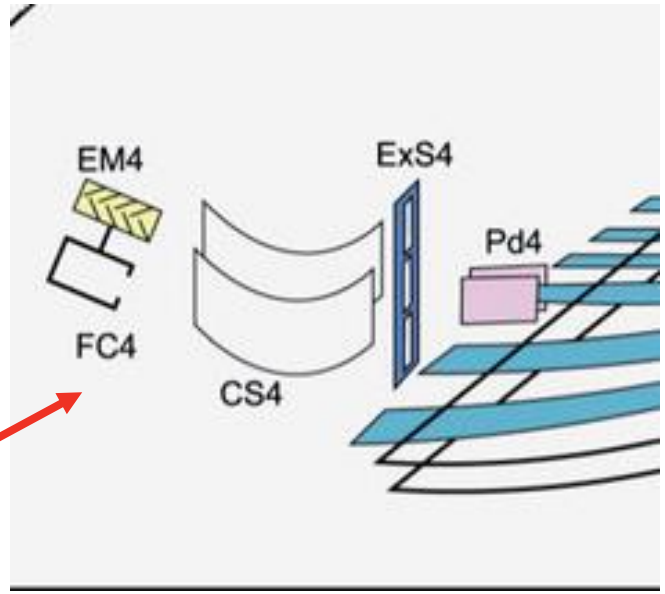
Adding more complexity to JMP Workflow Builder



No real strong evidence of high Se isotope ratios with high N isotope ratios.

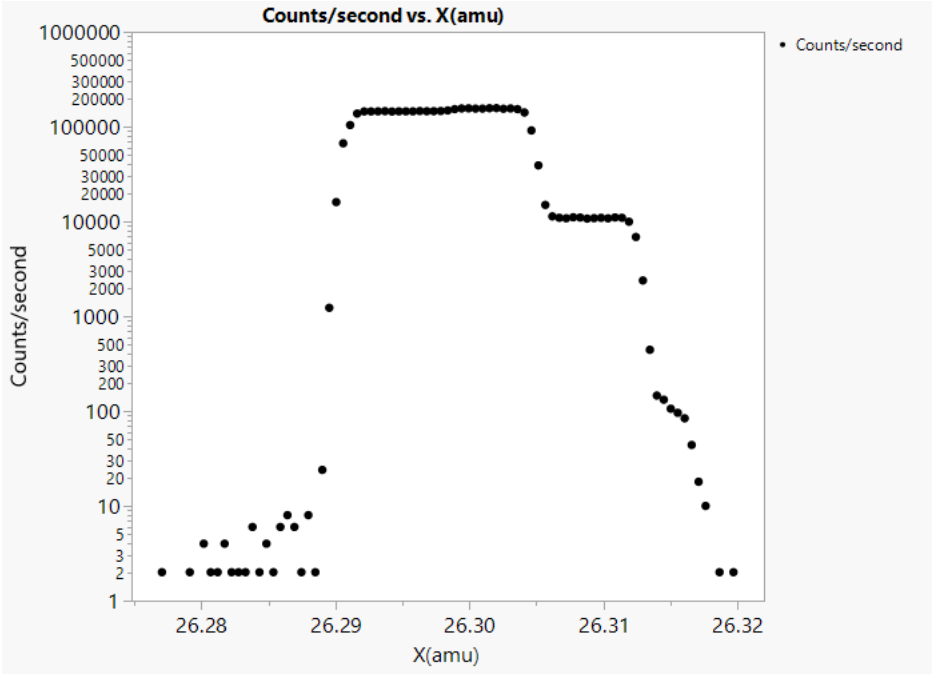
Next Steps – Functional Data Explorer





Mass spectrum:

- Three distinct peaks, but what masses do they represent?
- At low masses, generally easy to figure out, typically using mass differences and/or isotope ratios.
- But if not, we usually analyze a standard of the elements of potential interest – the peak positions should not move as the magnetic field is exquisitely controlled
- Width of a single mass peak will always be the same
- Haar wavelet
- Can we develop “virtual standards” by shifting peaks in the data table and using FDE?



Acknowledgements

Dr. David Dent – The Sustainable Nitrogen Foundation, UK

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Colleagues at NiCE-MSI

Department for Science, Innovation, & Technology