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The University of Georgia

®

Introduction to Metabolomics (2)



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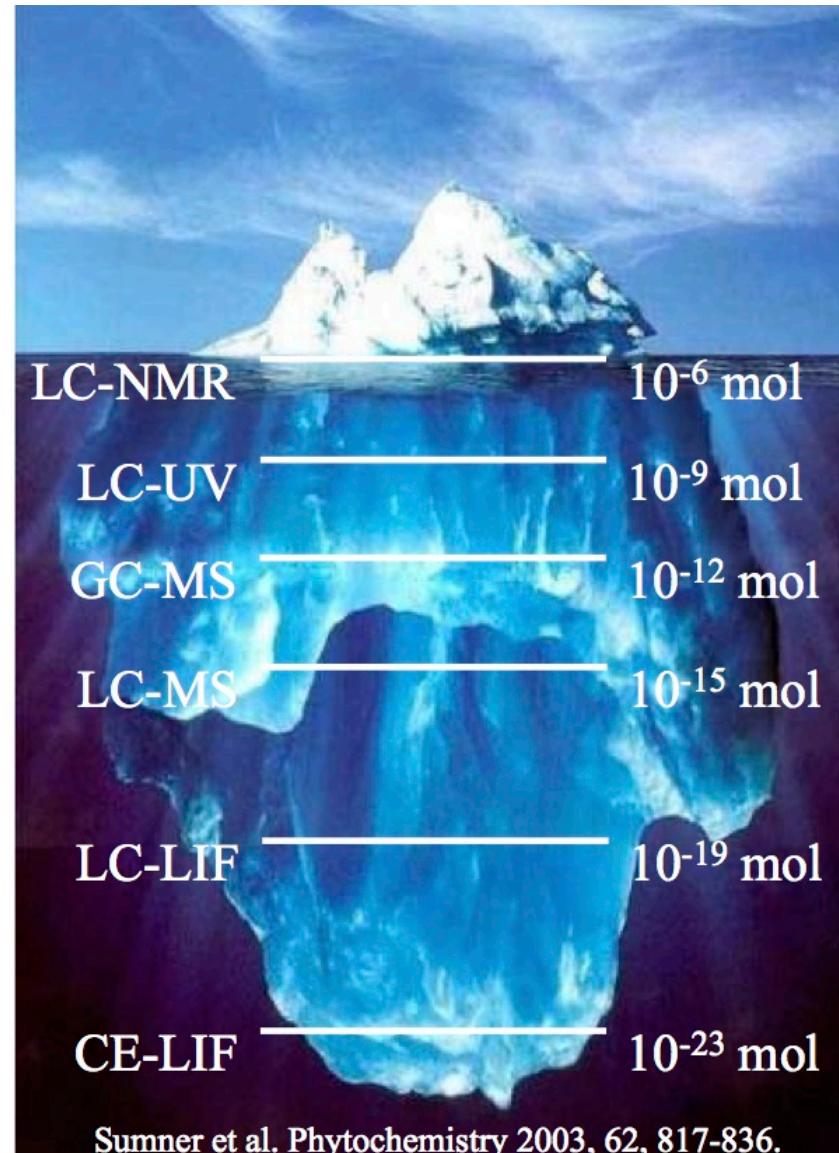
<http://edison.ccrc.uga.edu/>

NMR is not very sensitive

NMR

MS

Fluorescence



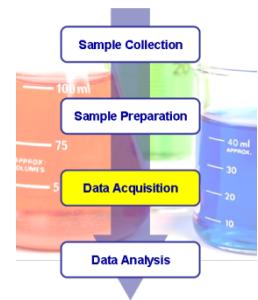
NMR versus MS

Mass Spectrometry (MS)

- Very fast
- Very sensitive
- Tens of thousands of “features”
- Excellent database libraries
- Not and quantitative
- Not great reproducibility
- Requires work-up
- Needs NMR for ID

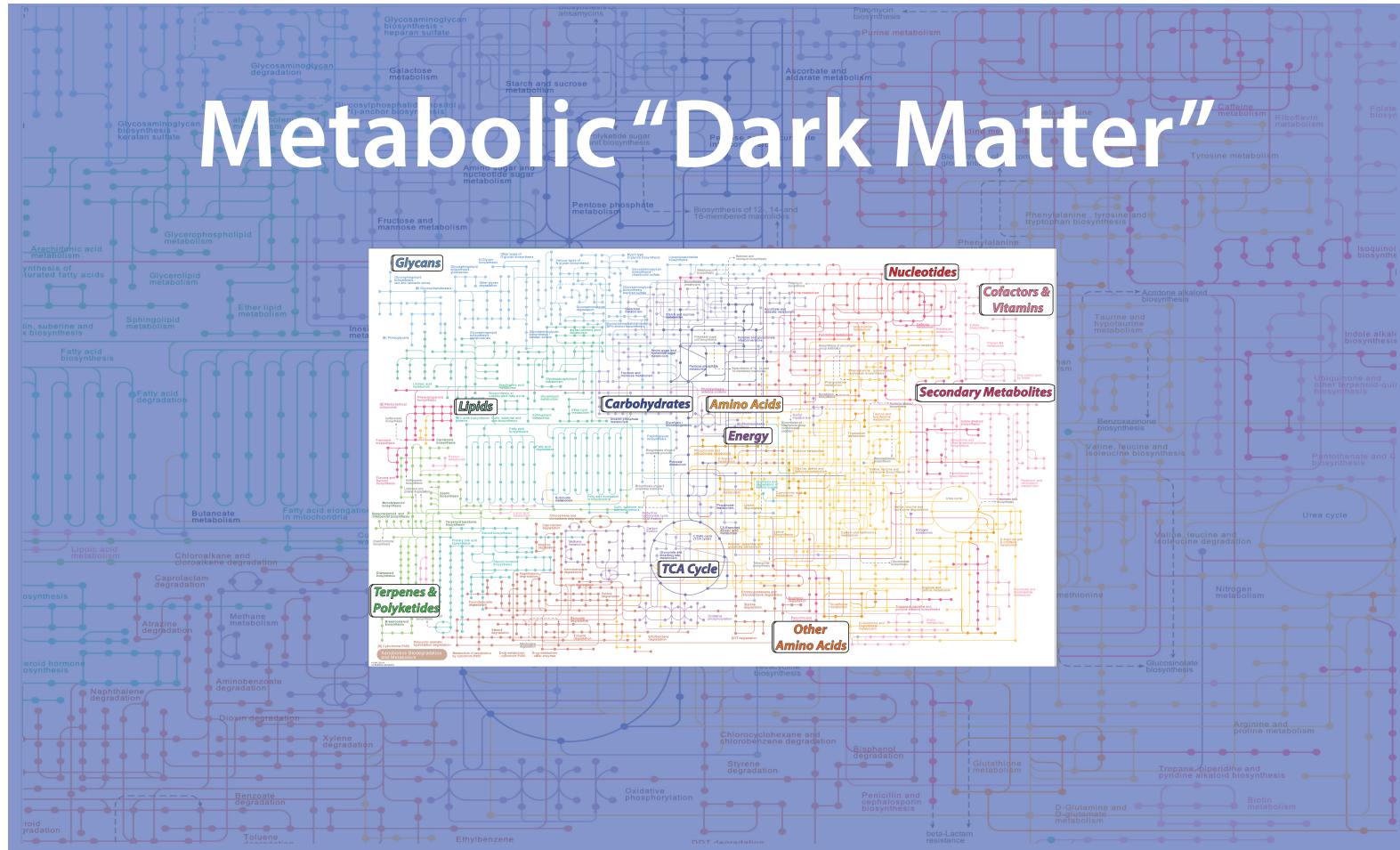
Nuclear Magnetic Resonance (NMR)

- Quantitative, fast
- Requires no work up or separation
- Hundreds of “features”
- Very reproducible
- Not as sensitive
- Needs MS or 2D NMR for positive ID
- Less developed database libraries



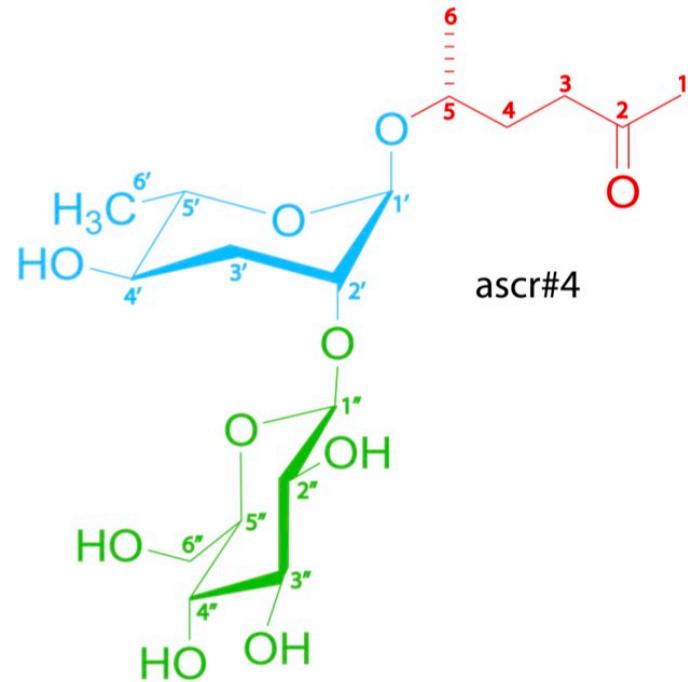
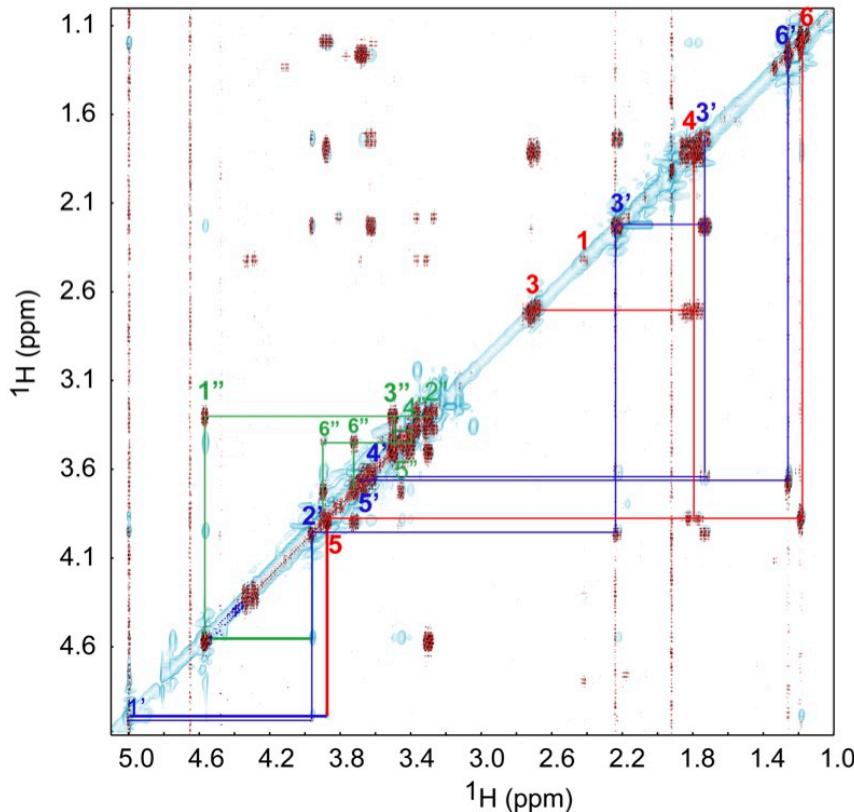
Adapted from Wolfender/Rudaz

There are more metabolites than currently mapped on known biochemical pathways



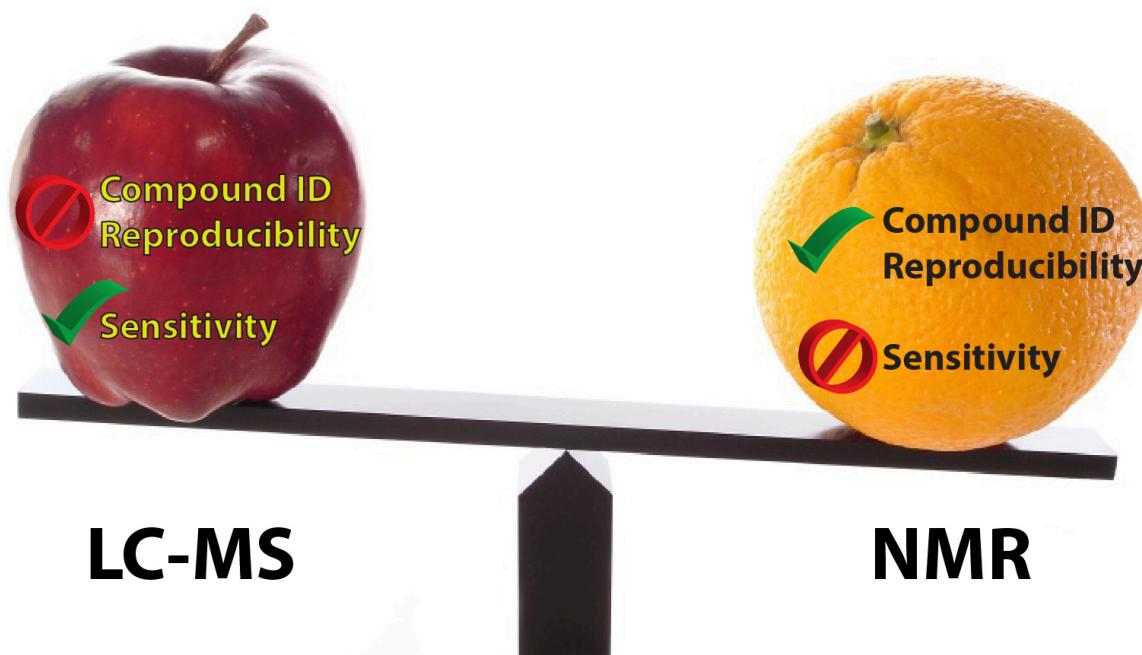
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Why use NMR at all?



“Unknown unknowns” require NMR. We had a known mass and fragmentation pattern by LC-MS but needed the atom-specific data that NMR can provide to determine the structure.

	Pros	Cons
LC-MS	Sensitivity	Metabolite ID
NMR	Metabolite ID	Sensitivity



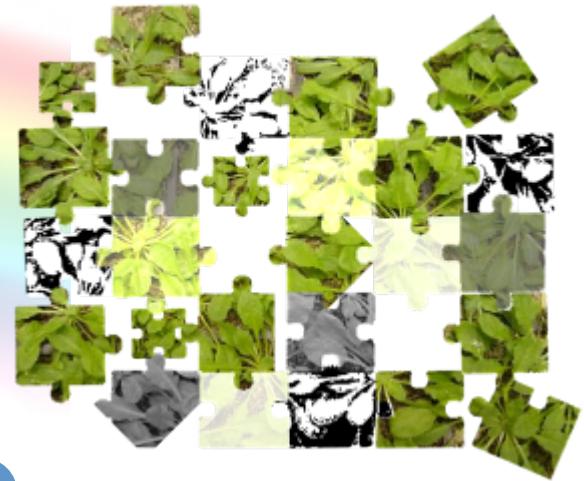
Analytical prism



Analytical
technologies



Biological
Phenomenon

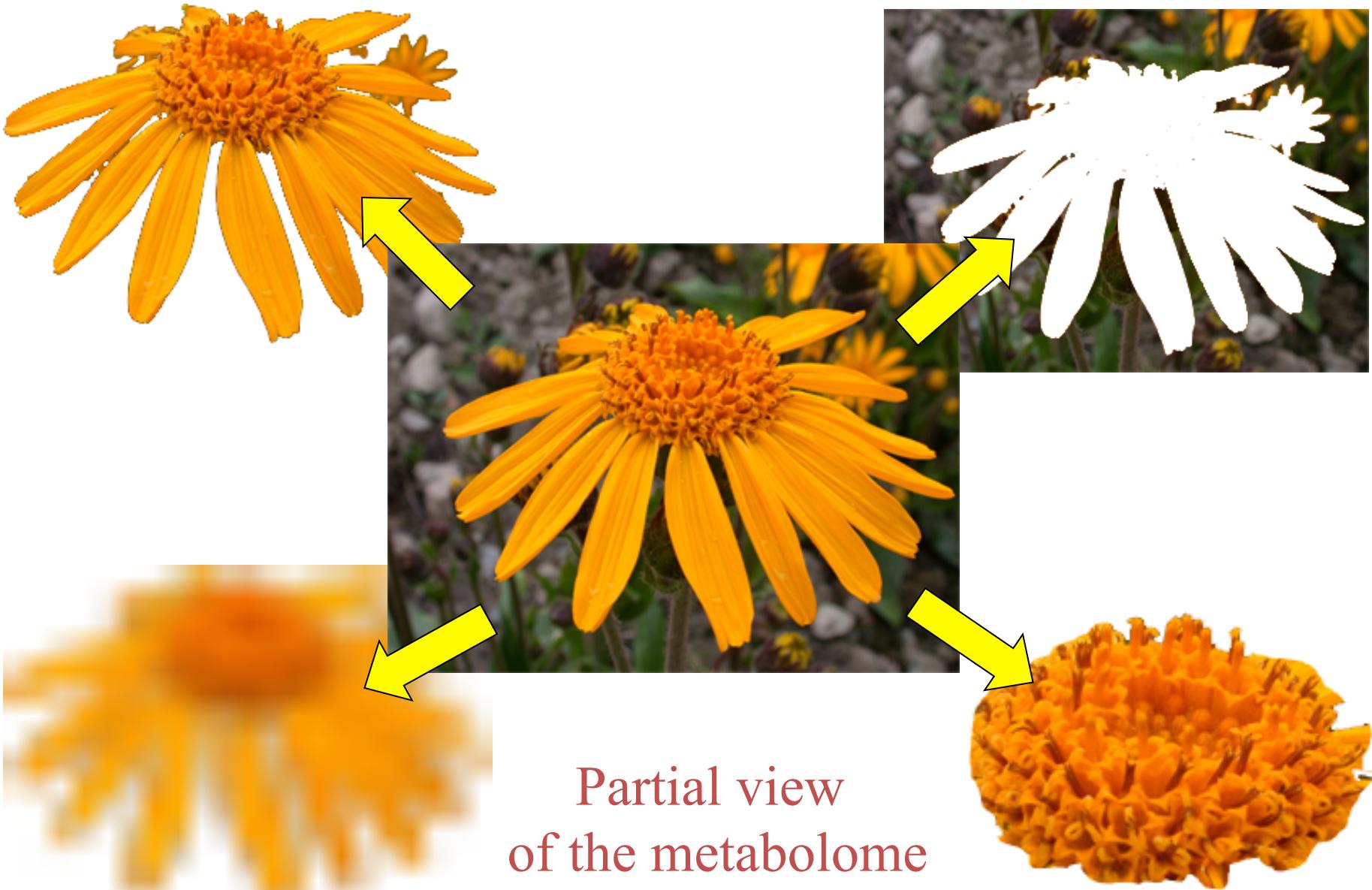


Today: We can access only a partial, noisy and deformed perception of the biological reality

Adapted from Wolfender/Rudaz

Observable Data

Selectivity and Sensitivity of Analytical Methods

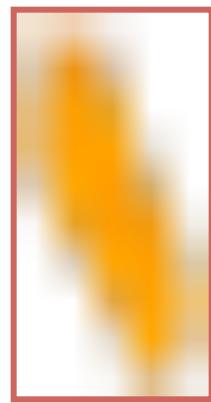
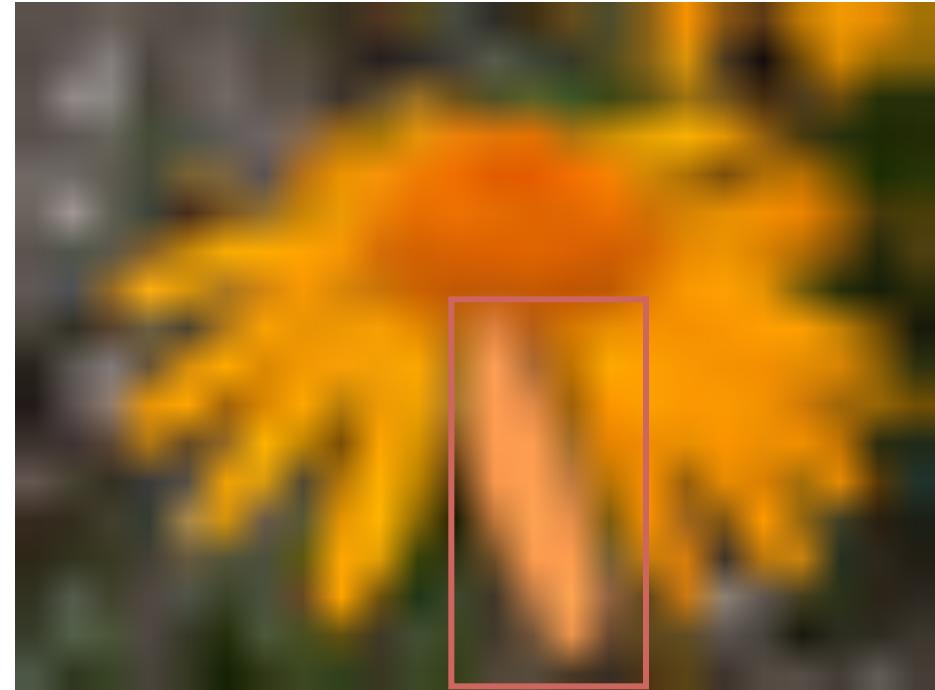
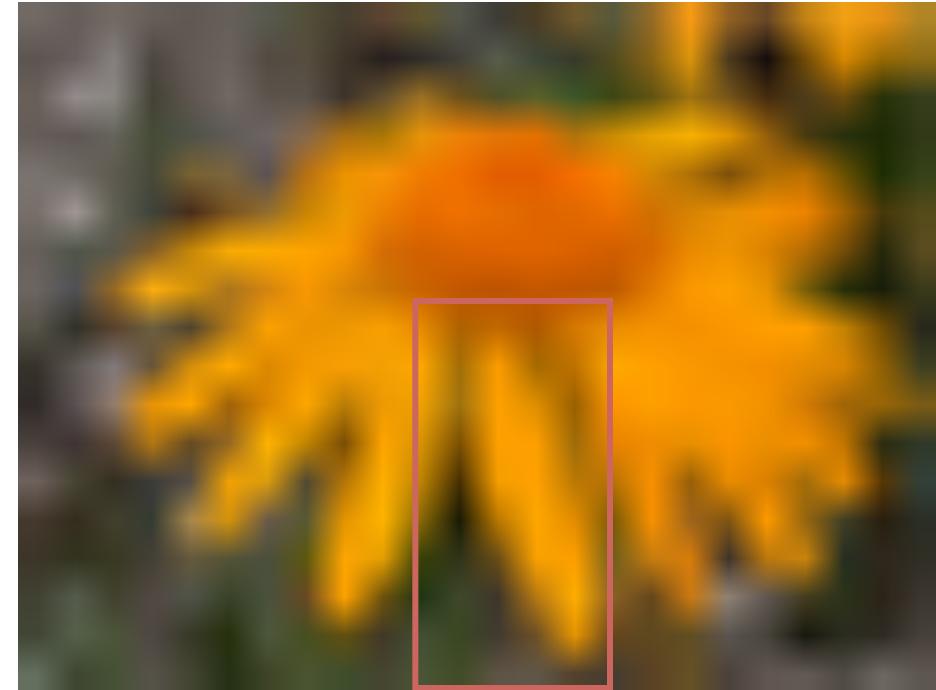


Partial view
of the metabolome

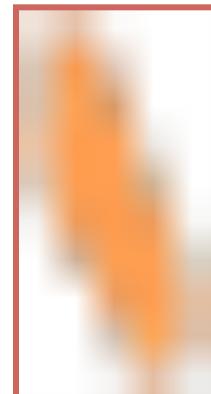
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Differences at low resolution: Case 1



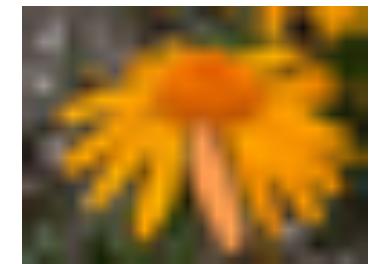
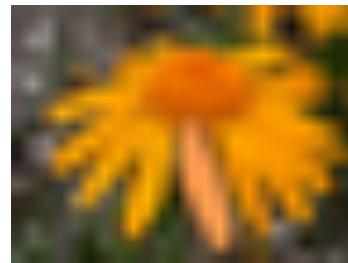
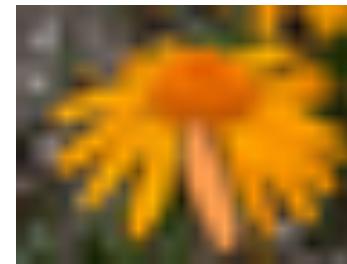
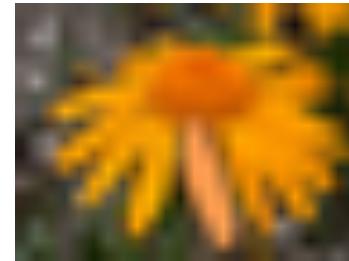
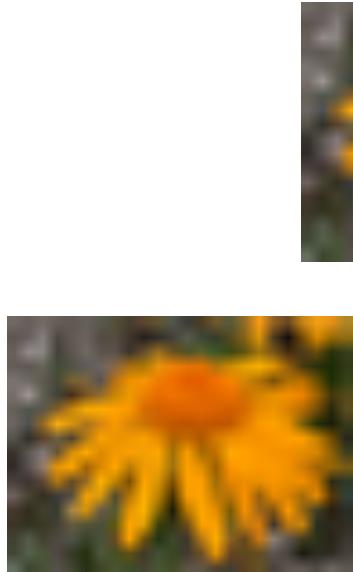
Good for NMR



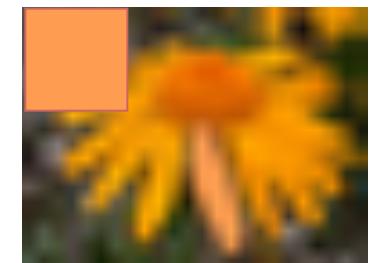
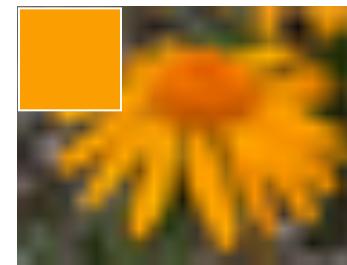
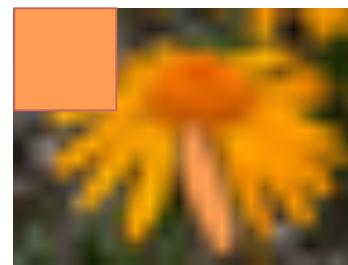
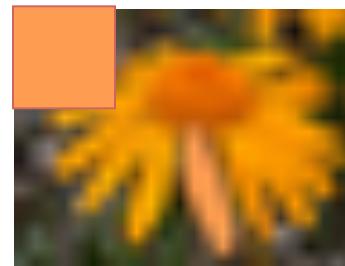
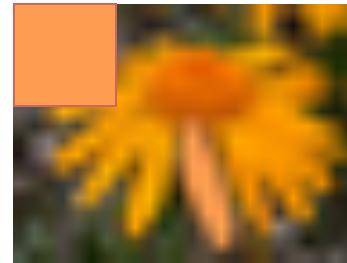
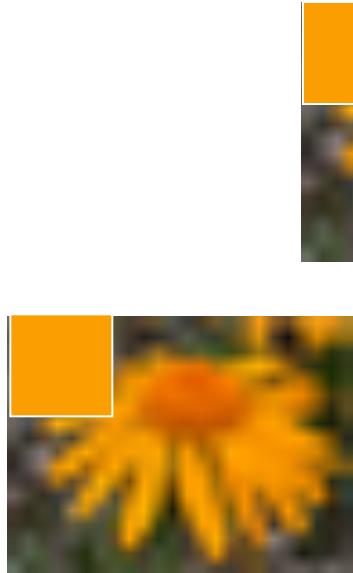
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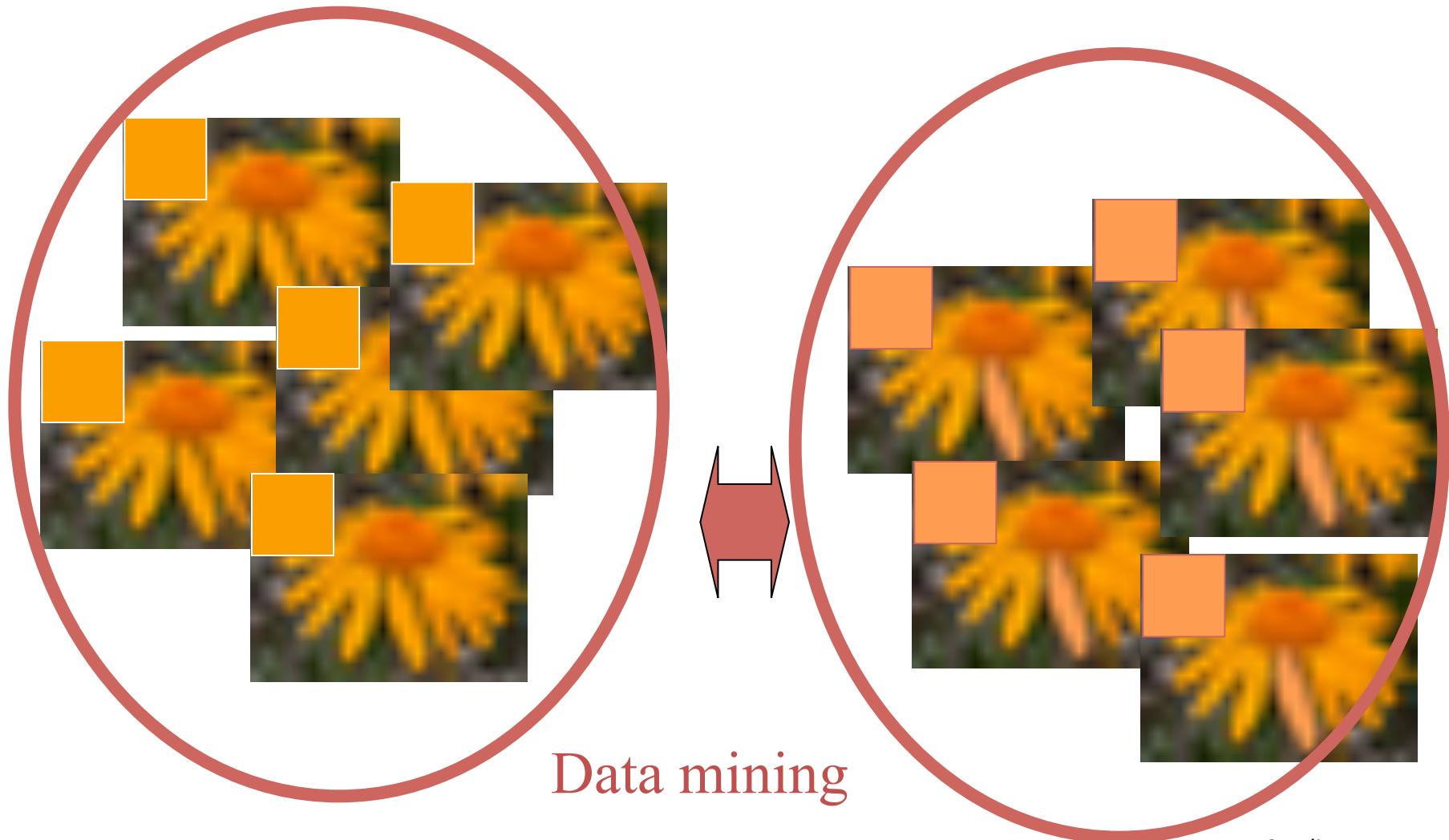
Is this difference statistically significant ?



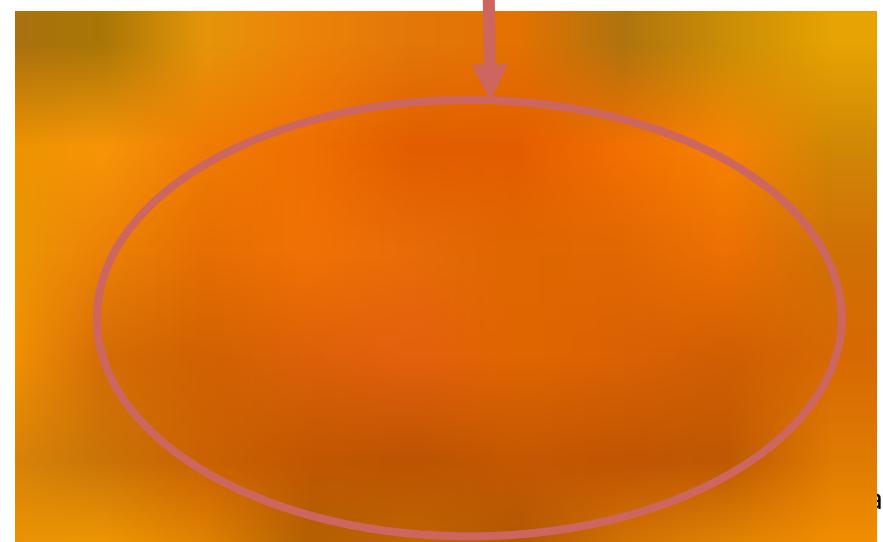
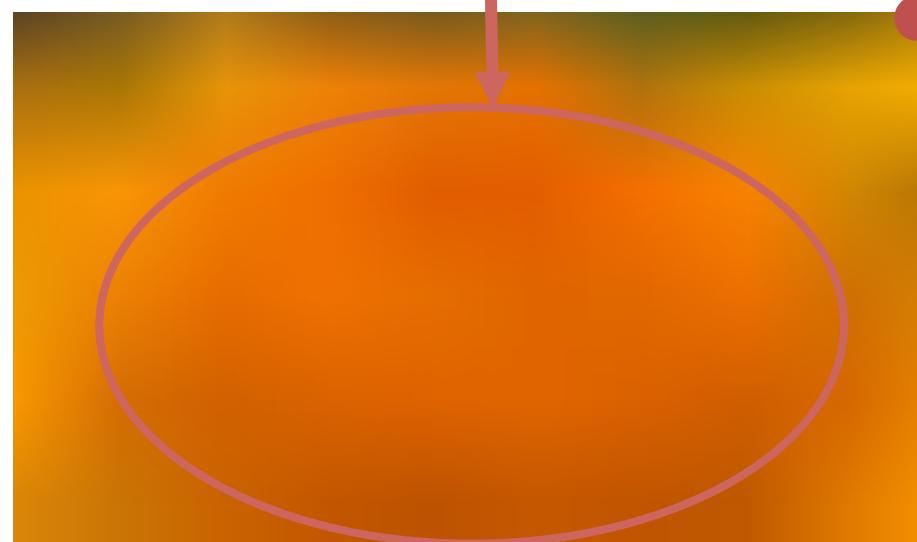
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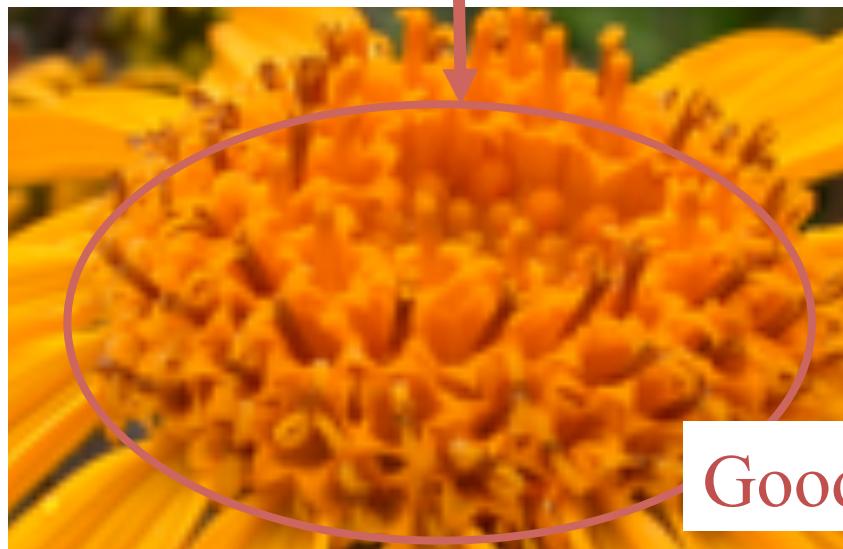
Is this difference statistically significant ?



Differences at low resolution: Case 2



Differences at high resolution: Case 2

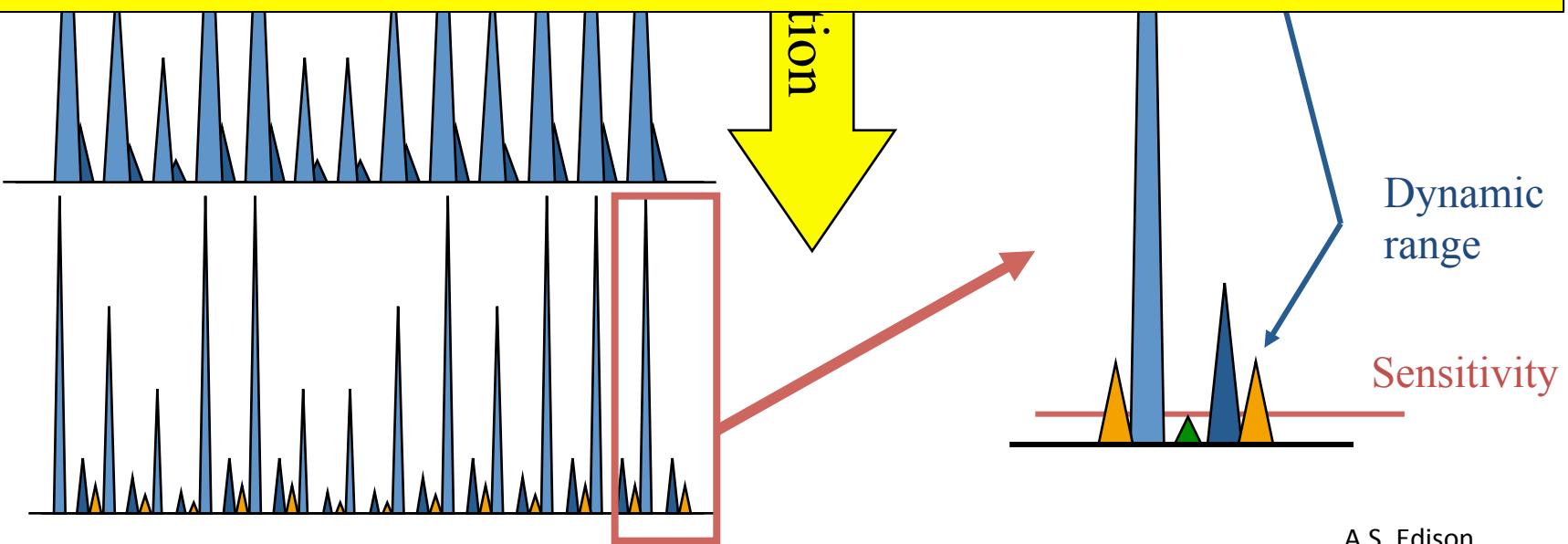


Resolution, Sensitivity and Dynamic Range

1D - Data

NMR has high dynamic range

Modern spectrometers can linearly quantify up to about 10^5



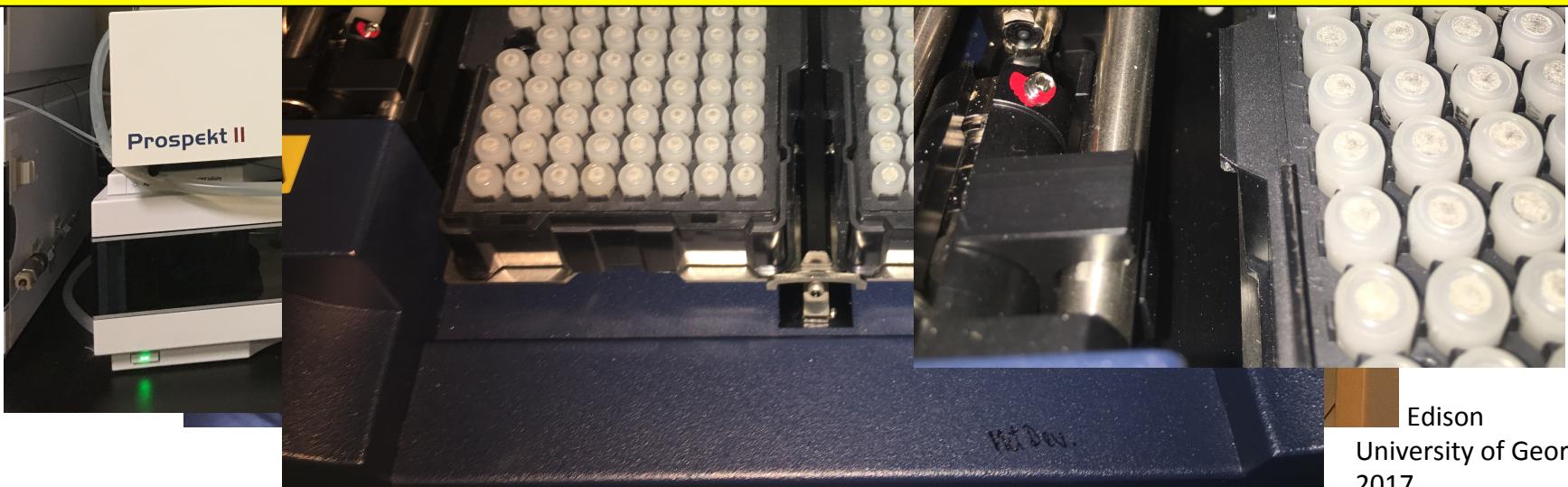
One way to combine NMR with MS is directly through HPLC and SPE



Isolate HPLC fractions by peak or time and analyze by NMR and MS.

Advantages: Can make multiple injections to boost NMR sensitivity

Weaknesses: Takes time and requires chemical separation



Next Lecture

Introduction to NMR in Metabolomics

- Approaches to analyzing mixtures
- Statistical correlations
- Database matching
- The use of 2D NMR in metabolomics
- PCA