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An IROA-based metabolomics protocols for accurate quantitation in metabolomics

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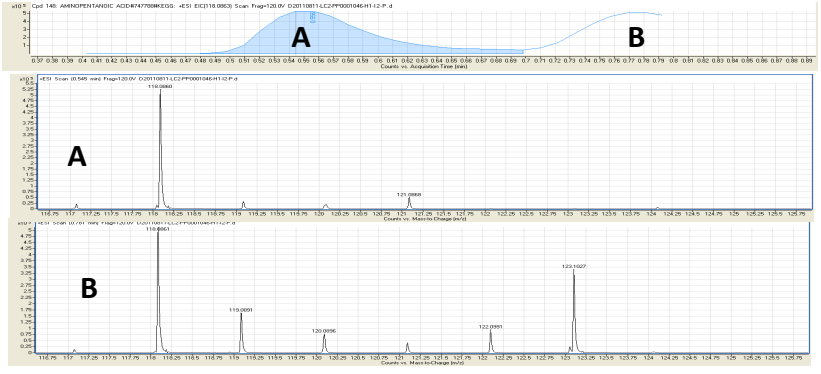
Artifact-based errors are common

When an authentic sample of a compound was injected it's retention time was within the time-range of peak A. In this run, two peaks A and B are found.

A normal assumption would be that A is the correct peak based on our past results; however, .



IROA identifies **A** as an artifact and **B** as a peak of biological origin, thus
Preventing an artifact from creating an error despite a "better time signature" on **A**.

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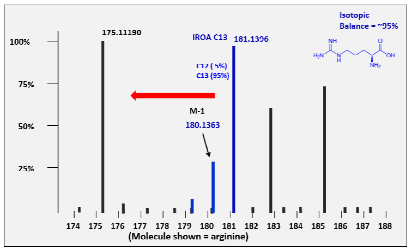
What is IROA?

The IROA (Isotopic Ratio Outlier Analysis) protocols embed additional chemical characteristics into the mass spectral data stream in the form of mathematically definable isotopic patterns.

This information is used to retrieve higher quality data, with reduced error, and lower overall experimental variance.



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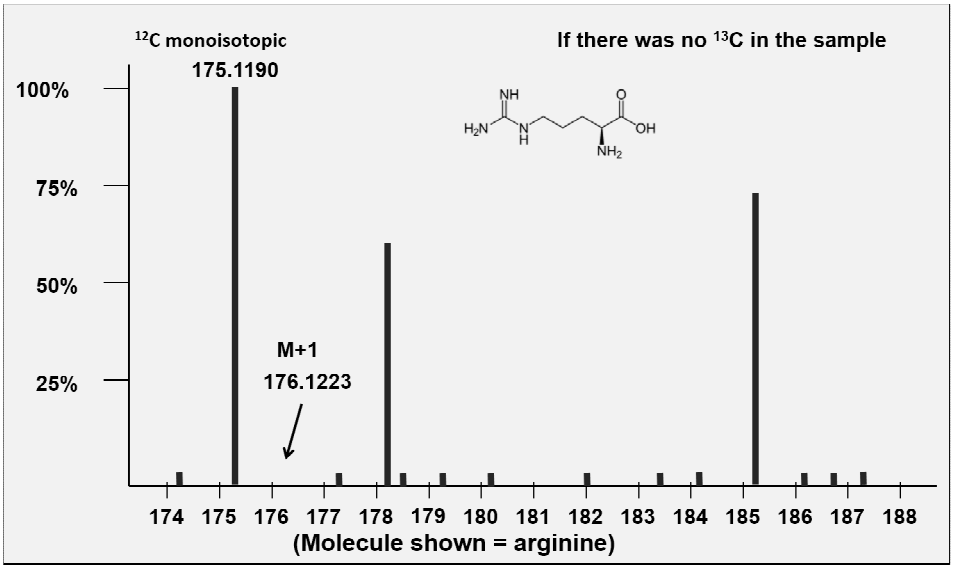
Creating Isotopic Patterns



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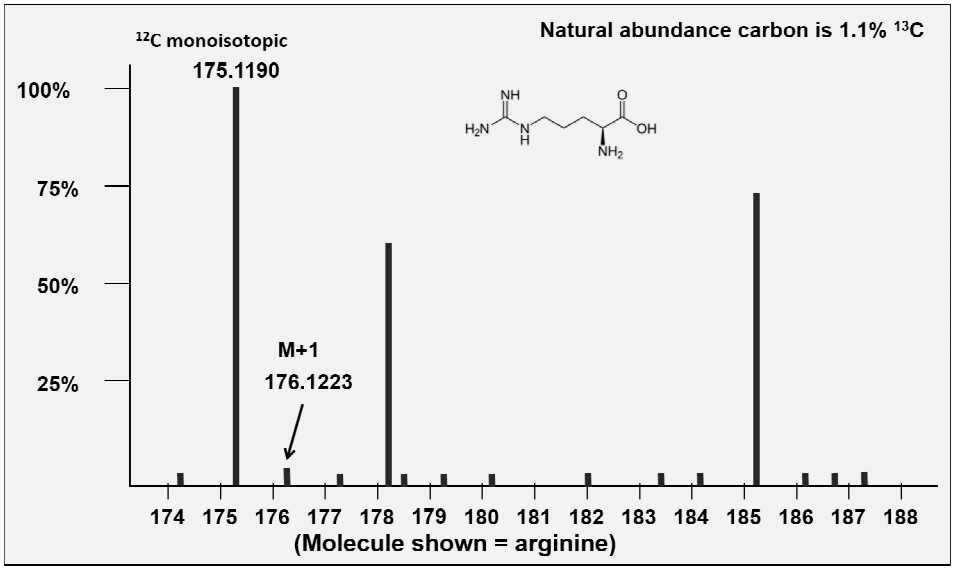
Creating Isotopic Patterns



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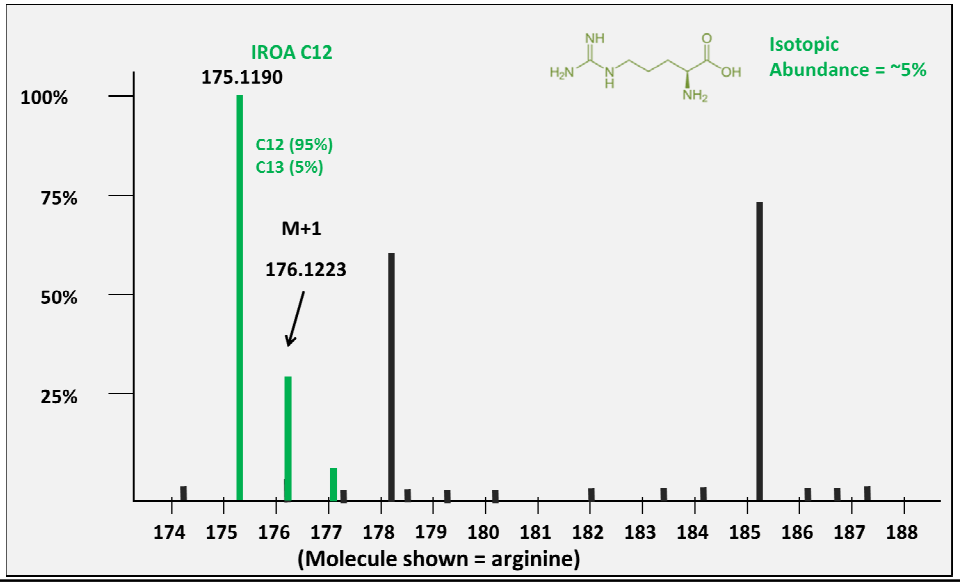
Creating Isotopic Patterns



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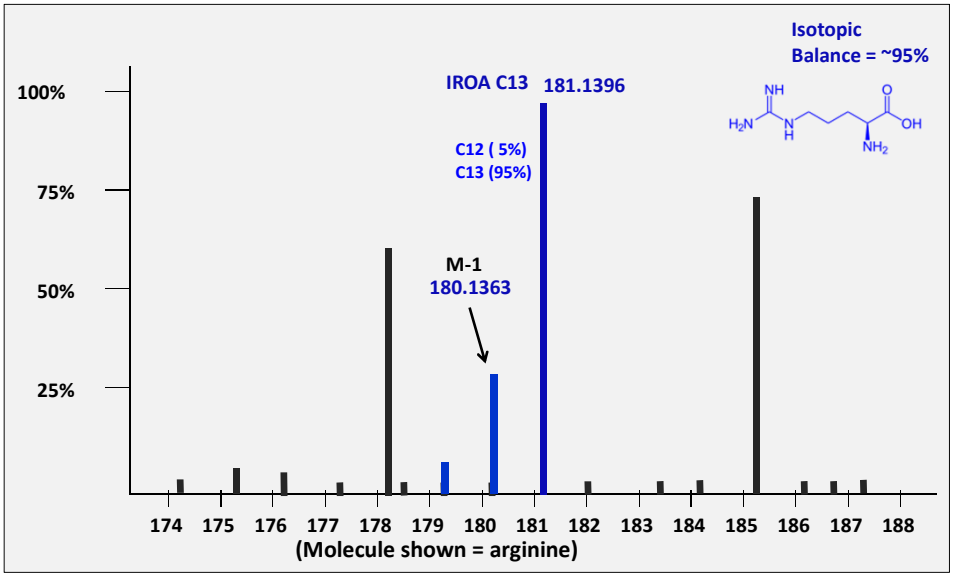
Creating Isotopic Patterns



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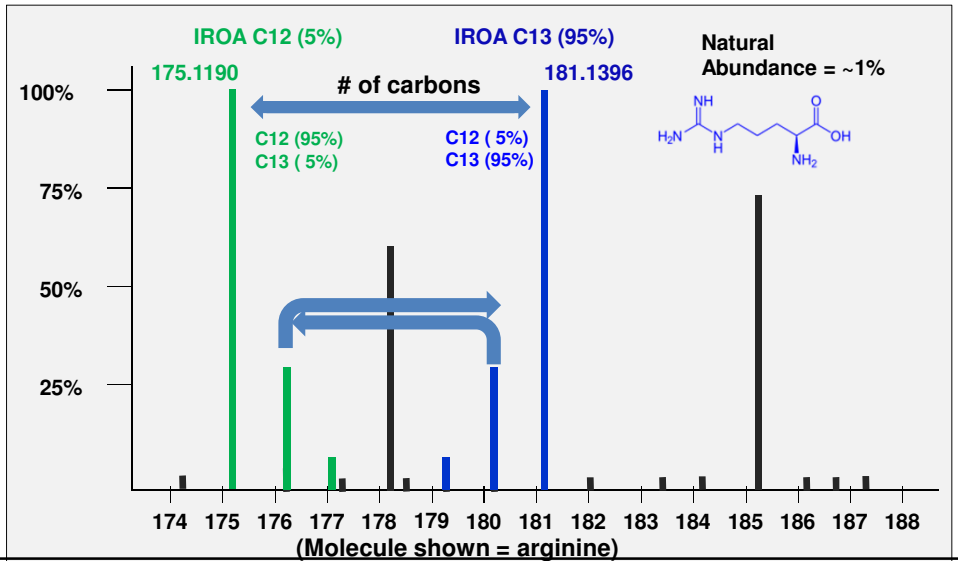
The IROA Peaks



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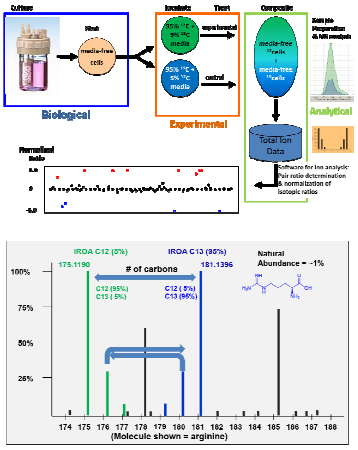
IROA Protocols

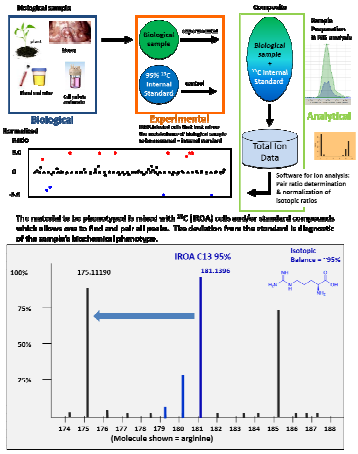
The Basic IROA Protocol

For R&D: to label control and experimental samples; untargeted analysis



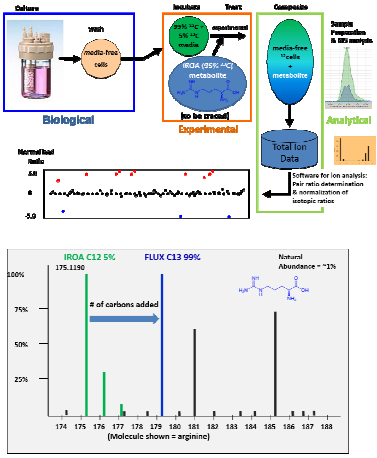
The Phenotypic IROA Protocol

For clinical and molecular diagnostics; targeted analysis for 100s of compounds



The Fluxomic IROA Protocol

Used for medical flux studies



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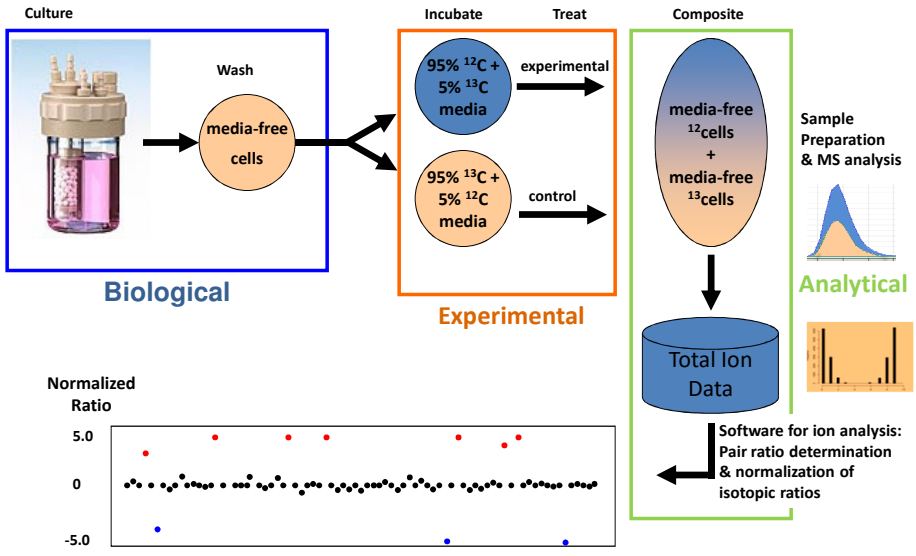
IROA®: Isotopic Ratio Outlier Analysis



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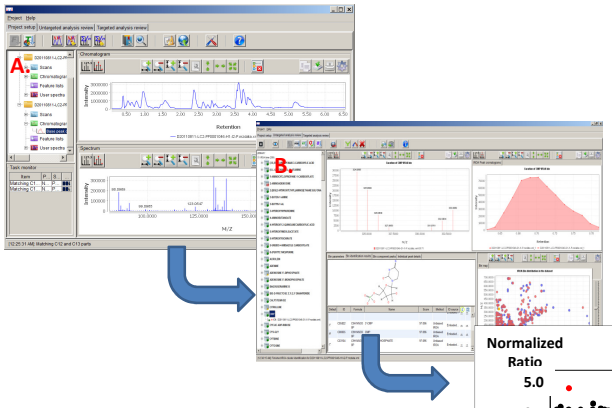
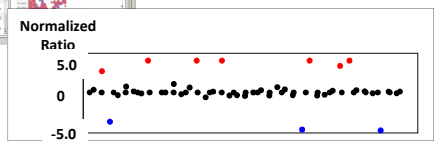
Automated Data Reduction



10^8 datapoints (mostly noise)

1 X 10^3 datapoints (pure biological)



Based on IROA embedded information the ClusterFinder program performs an automated Analysis, including:
Variance control, Data reduction, Noise removal, Data definition, Formula assignment

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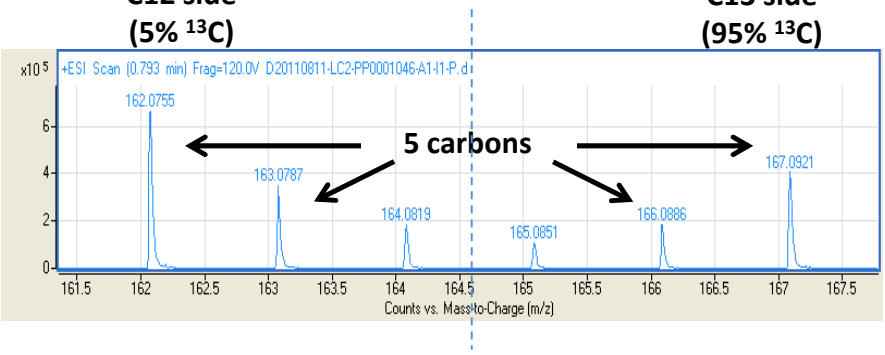
Anatomy of the IROA Signal

C12 side (5% ^{13}C)

C13 side (95% ^{13}C)



The IROA signal is made up of two halves.
Together they make a complete picture.
<u>Only a molecule of biological origin can show this signal.</u>

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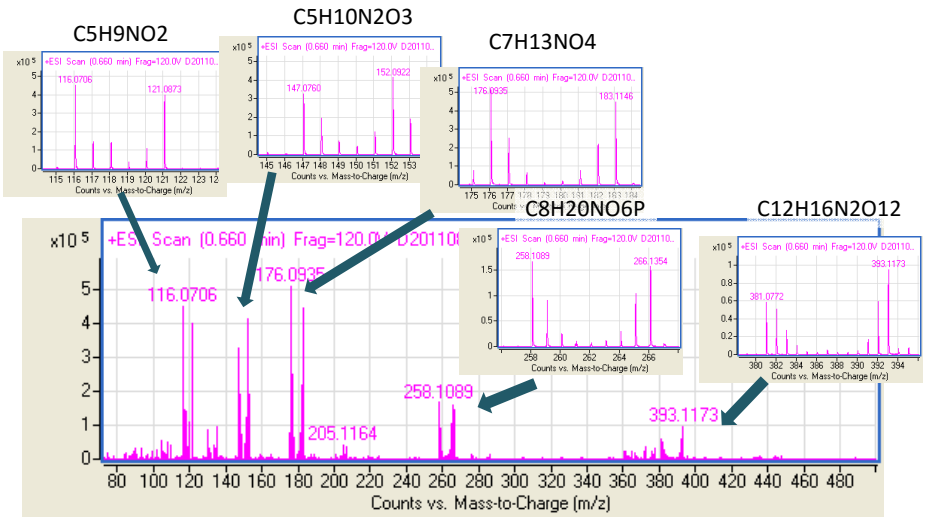
Paired Peaks



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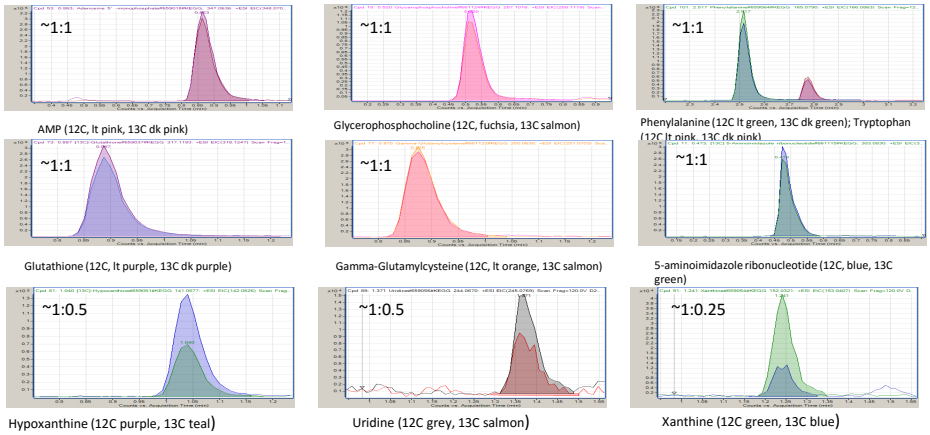
Overlaid C12/C13 Peaks



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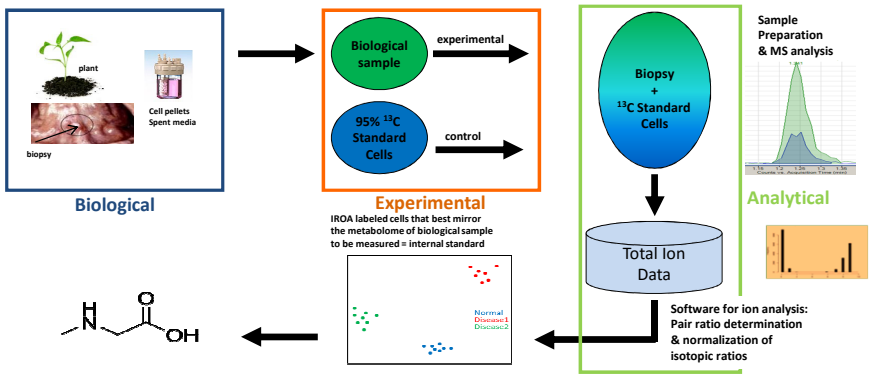
Phenotypic IROA Protocol



The material to be phenotyped is mixed with ^{13}C (IROA) cells and/or standard compounds which allows one to find and pair all peaks. The deviation from the standard is diagnostic of the sample's biochemical phenotype.

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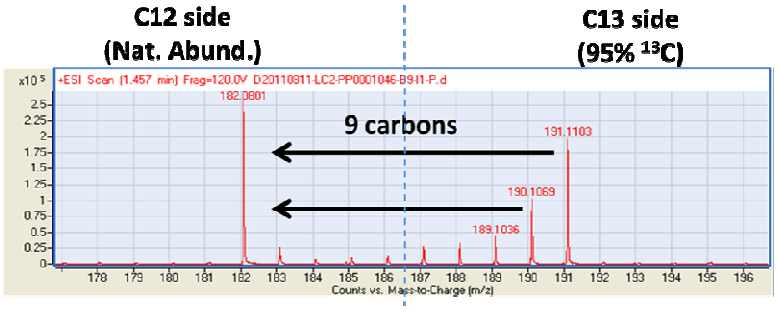
Phenotypic IROA peak pairs



The Phenotypic IROA signal is made up of two halves.
The C13 side tells you where to find the corresponding C12 peak.
The C12 side is unlabeled, i.e. it has natural abundance carbon.
The pairing makes the identity of all peaks measured unambiguous.

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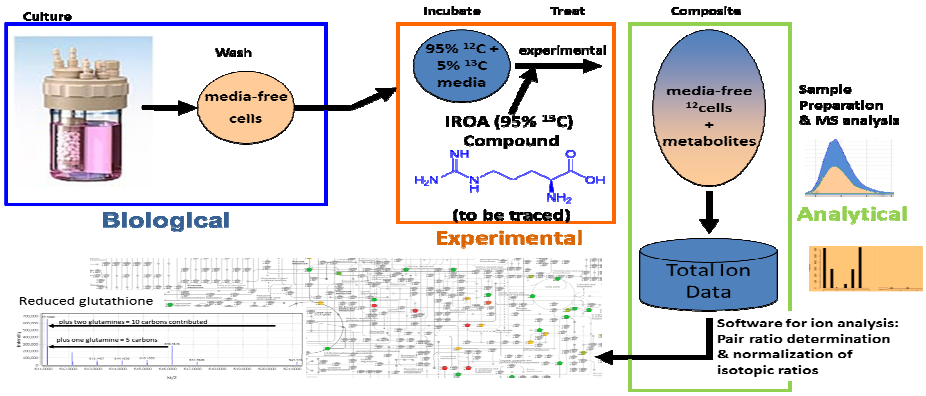
Fluxomic IROA Protocol



Every metabolite that is derivative of the traced compound, and only such metabolites, will have a truncated IROA pattern which indicates the number of carbons transferred and the percent of the overall metabolic pool that is labeled.

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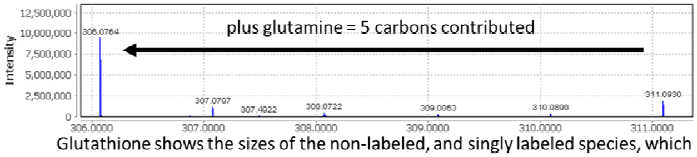
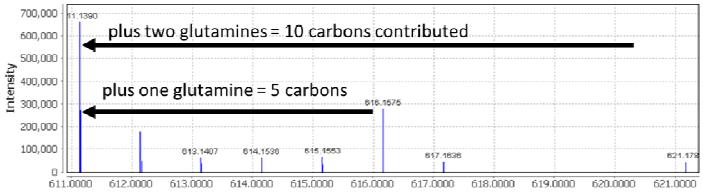
Fluxomic IROA Peak pairs



Glutathione shows the sizes of the non-labeled, and singly labeled species, which inherits a five carbon fragment from glutamine.



Oxidized glutathione shows the sizes of the non-labeled, singly labeled, and doubly labeled species. Each inheriting a five carbon fragment from glutamine.

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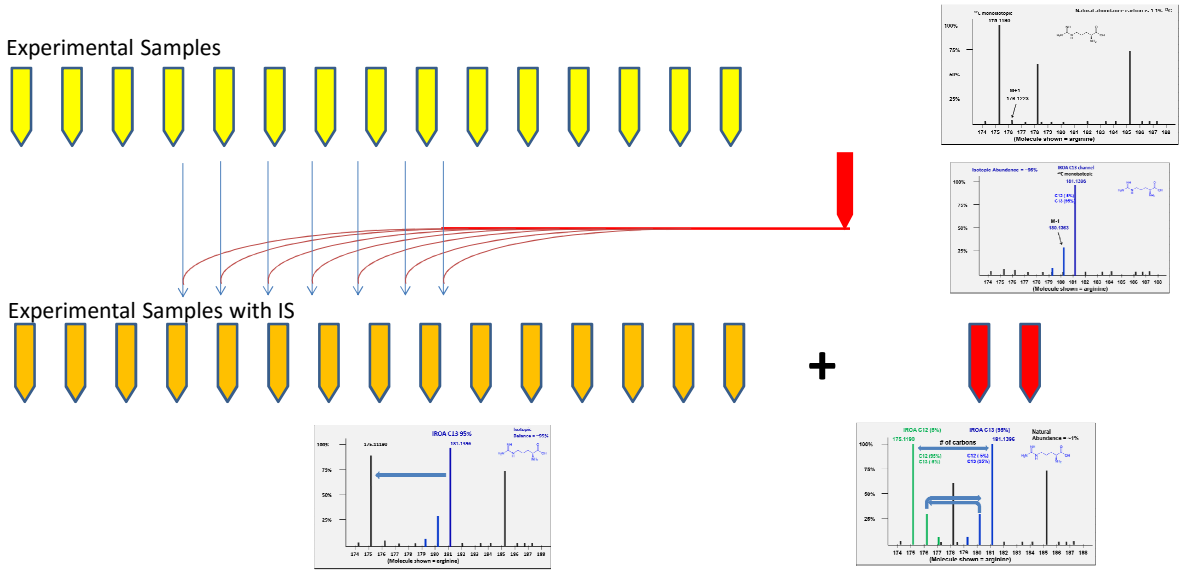
IROA-based Workflow



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IROA-based Workflow Uses

- The IS provides a standard concentration of 100's of **identified** compounds for **co-location**.
- It has enough compounds that it provides a Retention Time (RT) ladder that allows **alignment** of all peaks in the chromatogram.
- The total area under the curve of the IS may be used to **normalize** the experimental samples against one another.
- The same IS allows one to **overcome day-to-day, or even instrument-to-instrument variances**.

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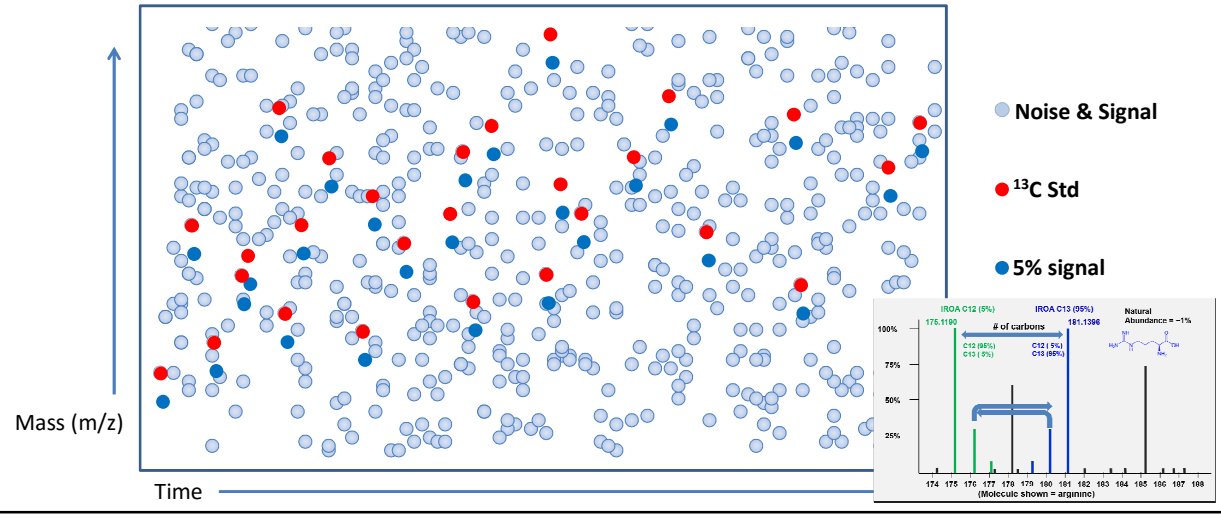
The Matrix samples are QA/QC samples

Identify

They are used to make "Dictionaries", and assess instrument condition.



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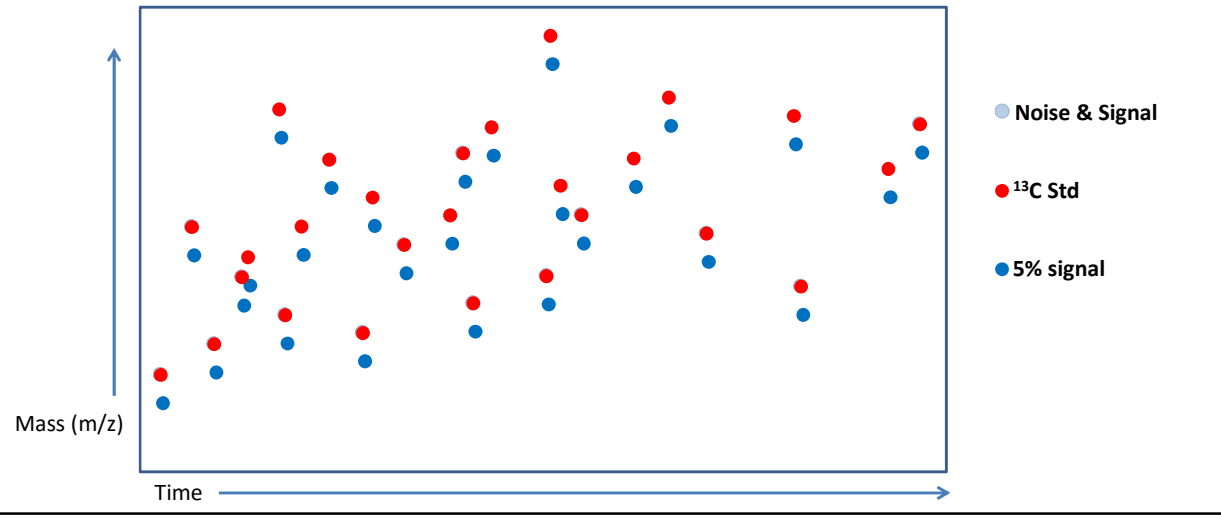
Dictionaries

Map & Export "Dictionary"



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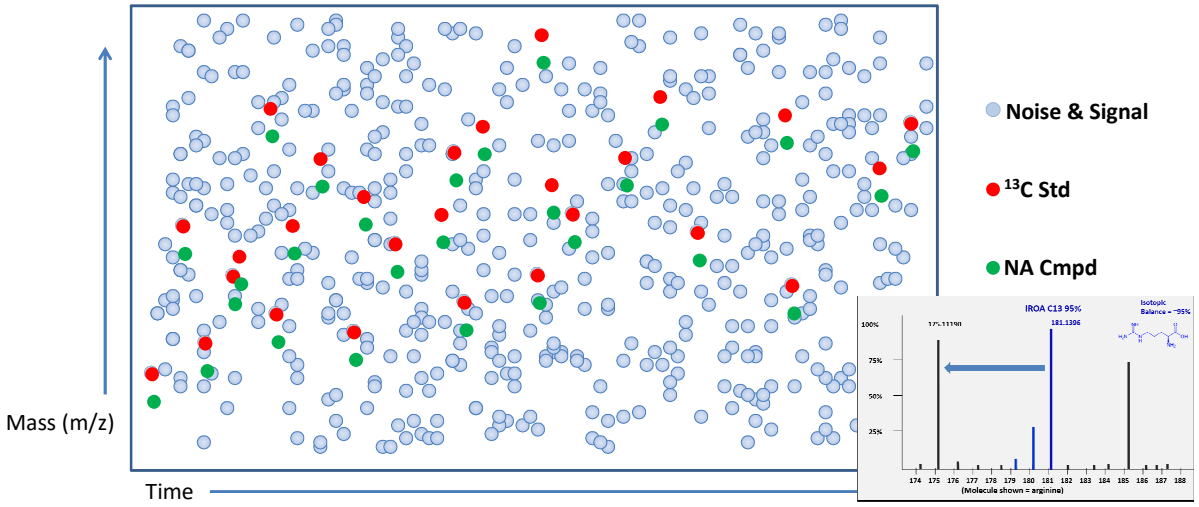
The Experimental Sample Process

Identify, Align, Co-locate, Measure & Normalize



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The Three IROA Protocols

- "Basic IROA protocol" - both the full C12 channel and the full C13 channel are used in the experiment.
  - the experiment is a completely **untargeted** analysis
  - **every** biological compound in either the C12 or C13 may be quantitated
- "Phenotypic IROA protocol" only the C13 channel is used in the experiment. Therefore,
  - the experiment is a completely complex **targeted** analysis
  - all compounds in the experimental (NA) sample may be quantitated against **every compound in the control sample (C13)**
- "Fluxomic IROA protocol" - single compound and the C13 channel is used in the experiment.
  - all **derivatives of that compound** will carry a unique signature which indicates the **number of carbons transferred**
  - the **relative size** of the pre and post metabolic pools is measured

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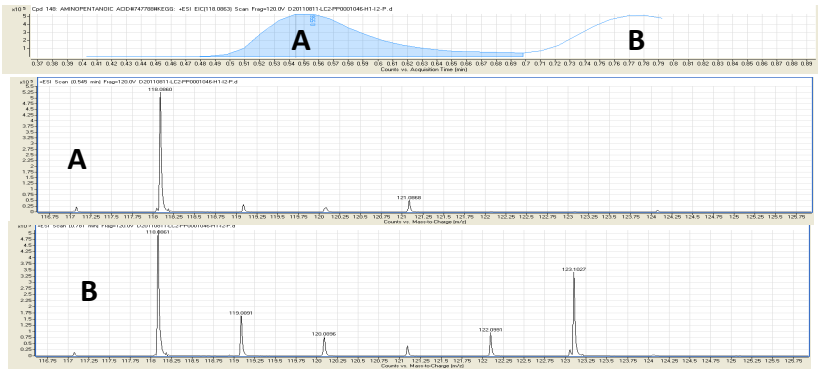
Artifact-based errors are avoided



IROA identifies **A** as an artifact and **B** as a peak of biological origin, thus
Preventing an artifact from creating an error despite a "better time signature" on **A**.

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Summary of IROA-based Workflow

1) Cost-effective simultaneous measurement of multiple biochemicals through the creation of IROA stable labeled Internal Standards (IS);
2) IS provides high level QC for accurate and reproducible results;
3) IS enables removal of false data (all noise and artifacts);
4) IS enables precise quantitation through complex software algorithms;
5) IS allows for normalization of samples to overcome sample-to-sample variation;
6) Once normalized, IS provides a map that can be used for compounds that are not in the IS.

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Thanks

Thanks to:

Tim Garrett & the SECIM Core 1 team.

Irwin Kurland for tests with GC/MS.

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Questions?