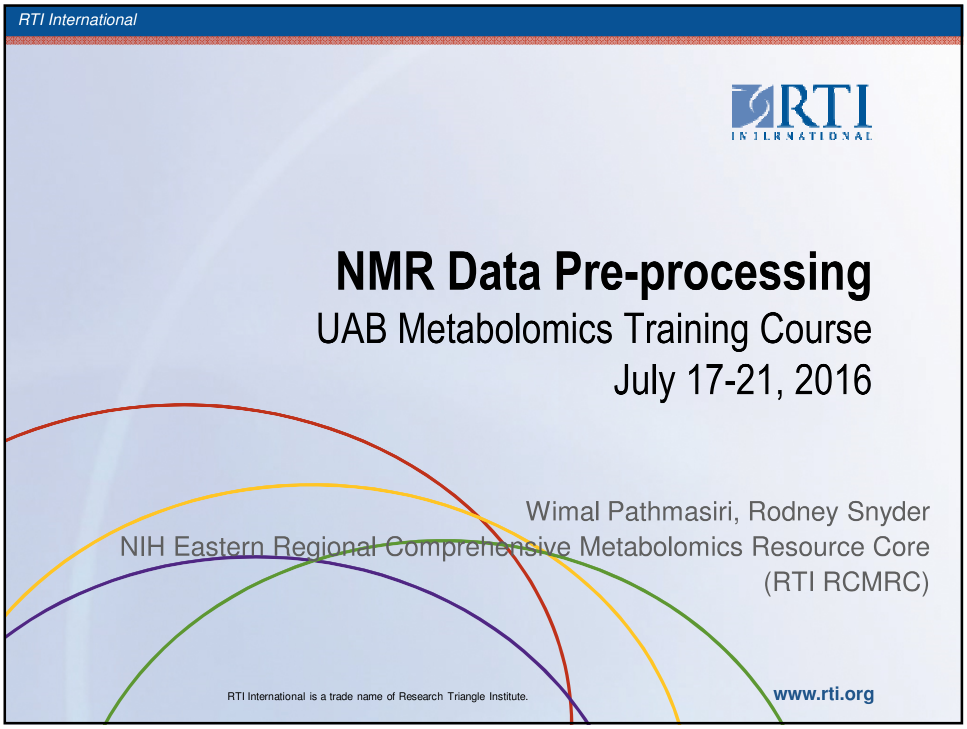
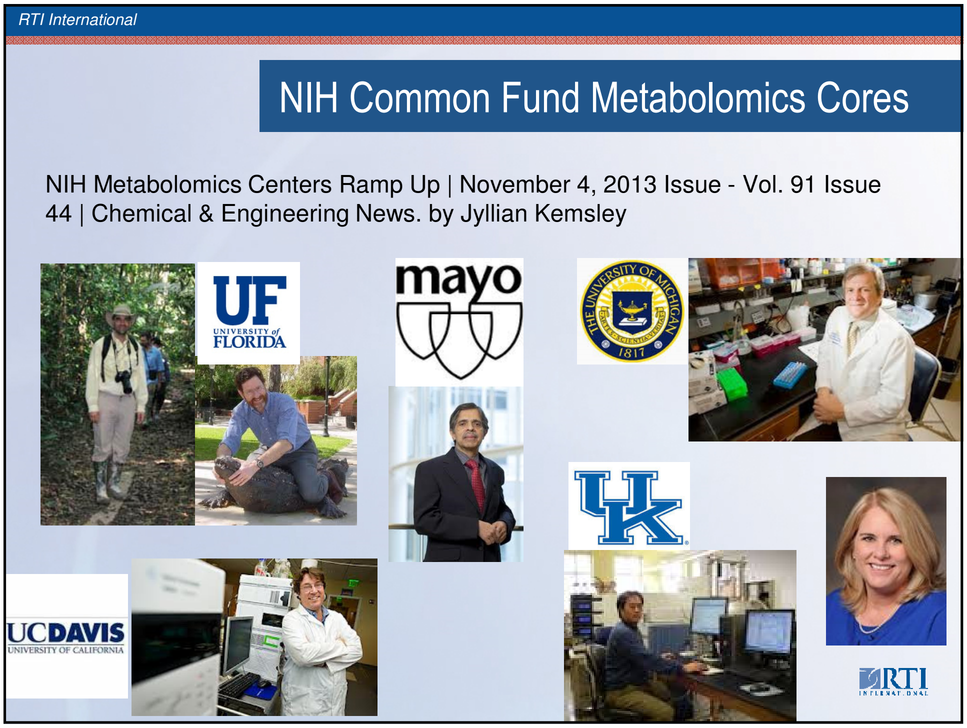


NMR Data Pre-processing

UAB Metabolomics Training Course
July 17-21, 2016

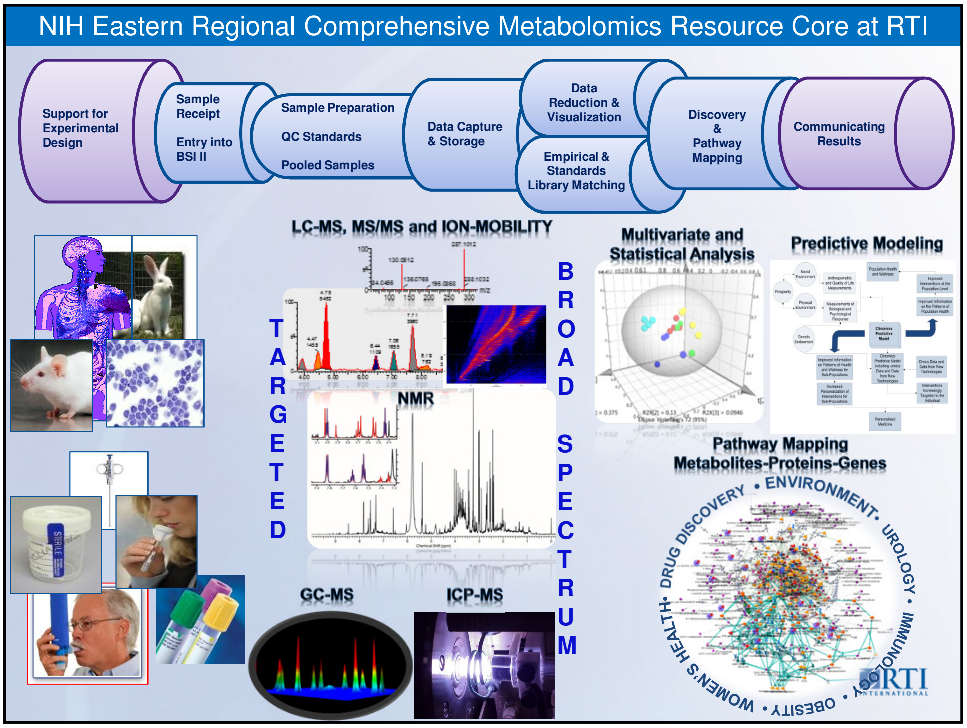
Wimal Pathmasiri, Rodney Snyder
NIH Eastern Regional Comprehensive Metabolomics Resource Core
(RTI RCMRC)

www.rti.org



NIH Common Fund Metabolomics Cores

NIH Metabolomics Centers Ramp Up | November 4, 2013 Issue - Vol. 91 Issue 44 | Chemical & Engineering News. by Jyllian Kemsley



NIH Eastern Regional Comprehensive Metabolomics Resource Core at RTI

Support for Experimental Design

Sample Receipt

Entry into BSI II

Sample Preparation

QC Standards

Pooled Samples

Data Capture & Storage

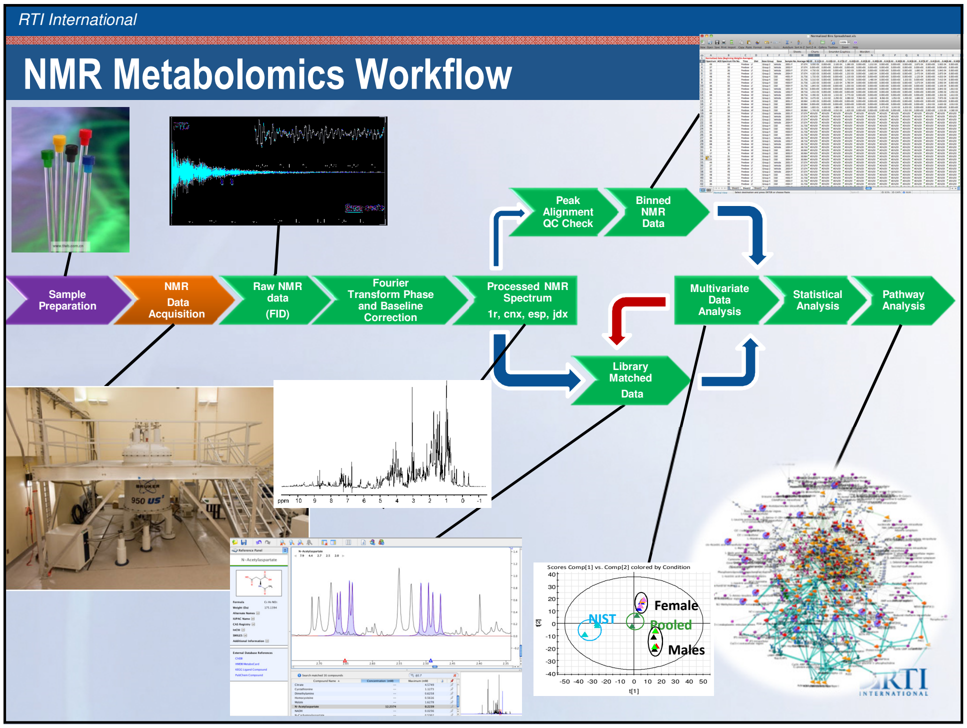
Data Reduction & Visualization

Empirical & Standards Library Matching

Discovery & Pathway Mapping

Communicating Results

LC-MS, MS/MS and ION-MOBILITY
TARGETED
BROAD SPECTRUM
NMR
GC-MS
ICP-MS
Multivariate and Statistical Analysis
Predictive Modeling
Pathway Mapping
Metabolites-Proteins-Genes
ENVIRONMENT • UROLOGY • IMMUNOLOGY • OBESITY • WOMEN'S HEALTH • DRUG DISCOVERY •
RTI INTERNATIONAL



RTI International

NMR Metabolomics Workflow

Sample Preparation

NMR Data Acquisition

Raw NMR data (FID)

Fourier Transform Phase and Baseline Correction

Processed NMR Spectrum 1r, cnx, esp, jdx

Peak Alignment QC Check

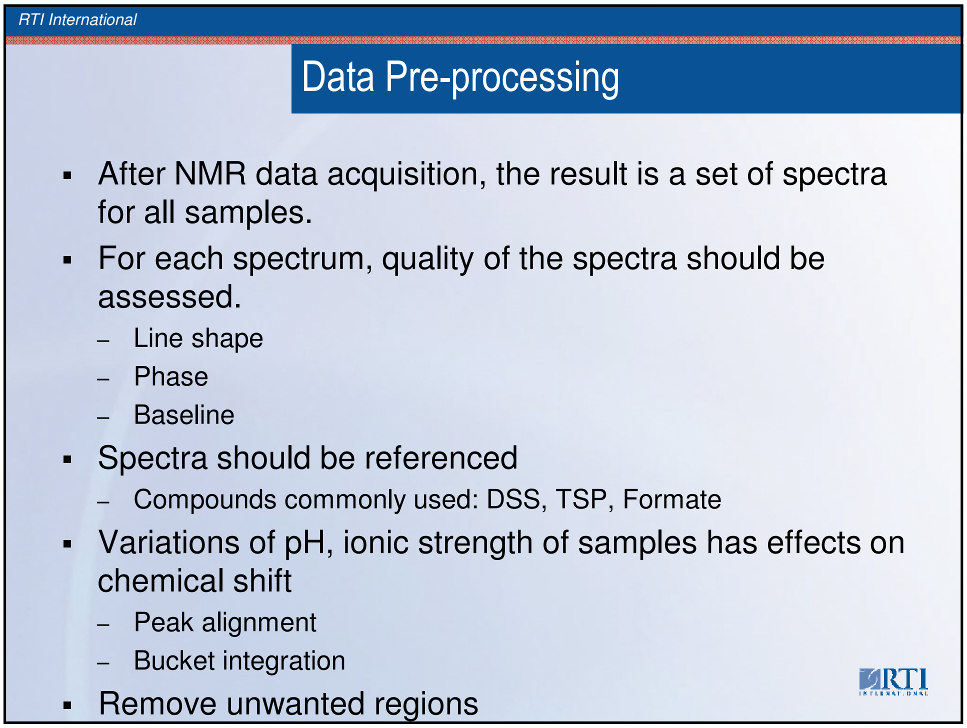
Binned NMR Data

Library Matched Data

Multivariate Data Analysis

Statistical Analysis

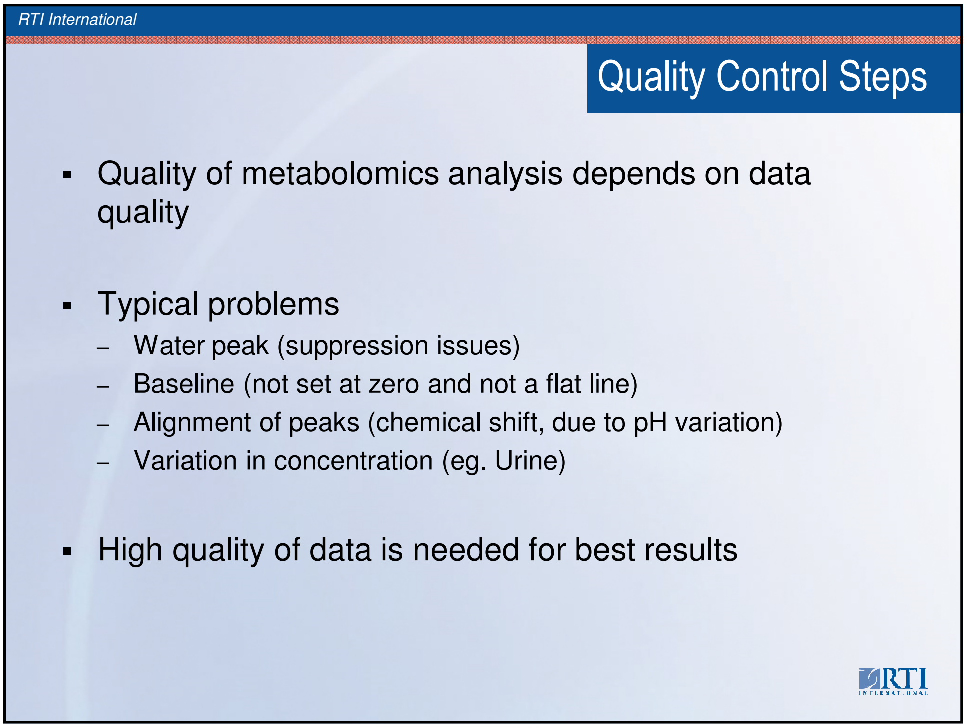
Pathway Analysis



RTI International

Data Pre-processing

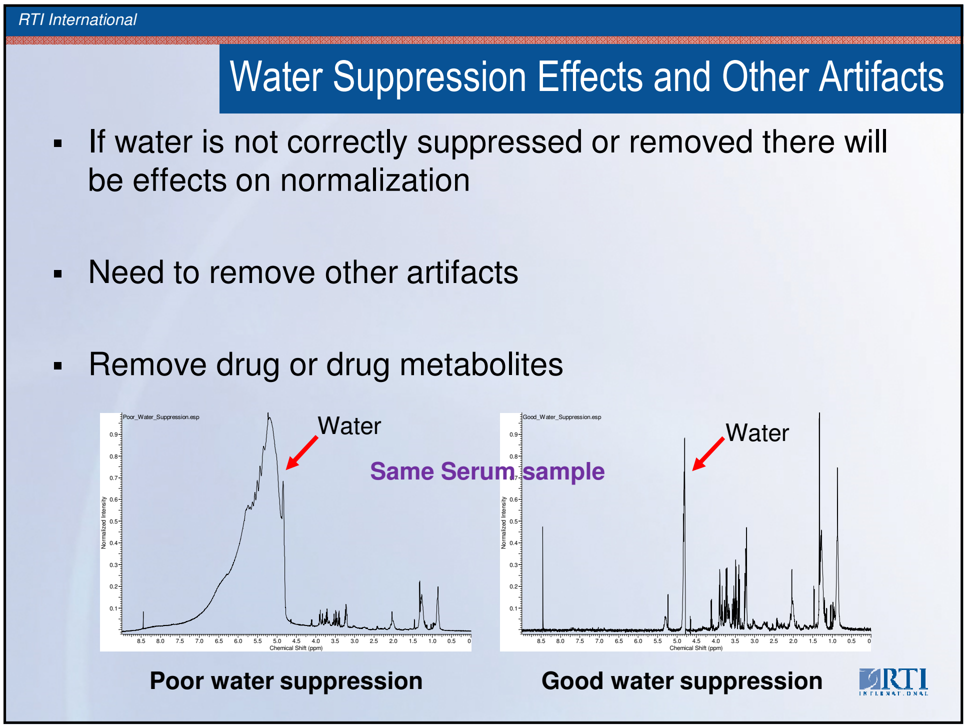
- After NMR data acquisition, the result is a set of spectra for all samples.
- For each spectrum, quality of the spectra should be assessed.
  - Line shape
  - Phase
  - Baseline
- Spectra should be referenced
  - Compounds commonly used: DSS, TSP, Formate
- Variations of pH, ionic strength of samples has effects on chemical shift
  - Peak alignment
  - Bucket integration
- Remove unwanted regions



RTI International

Quality Control Steps

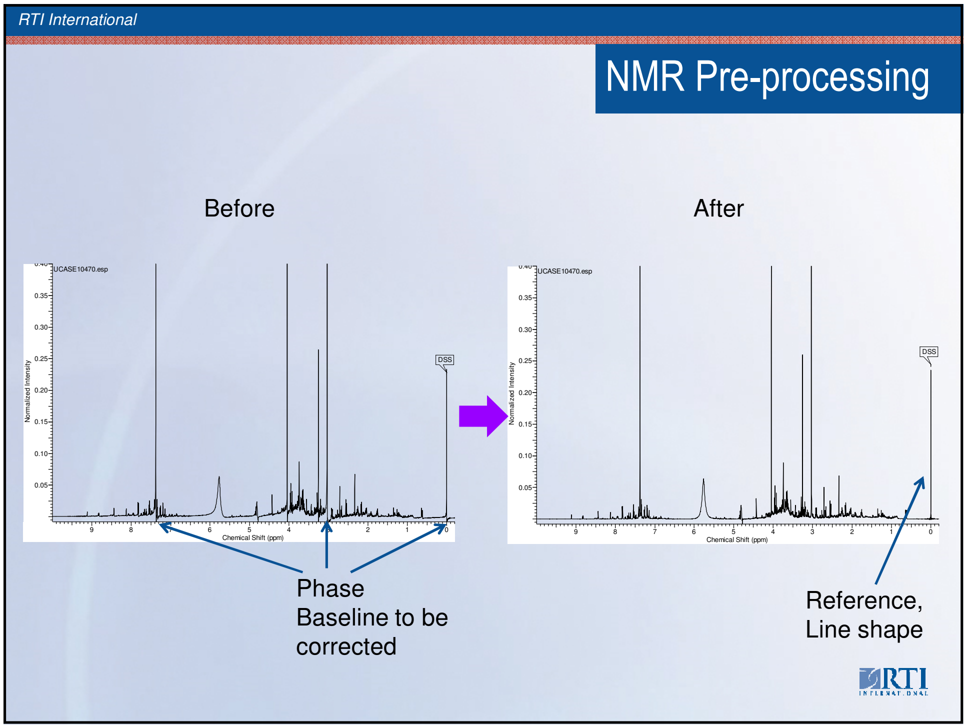
- Quality of metabolomics analysis depends on data quality
- Typical problems
  - Water peak (suppression issues)
  - Baseline (not set at zero and not a flat line)
  - Alignment of peaks (chemical shift, due to pH variation)
  - Variation in concentration (eg. Urine)
- High quality of data is needed for best results



Water Suppression Effects and Other Artifacts

- If water is not correctly suppressed or removed there will be effects on normalization
- Need to remove other artifacts
- Remove drug or drug metabolites

Poor water suppression **Good water suppression**

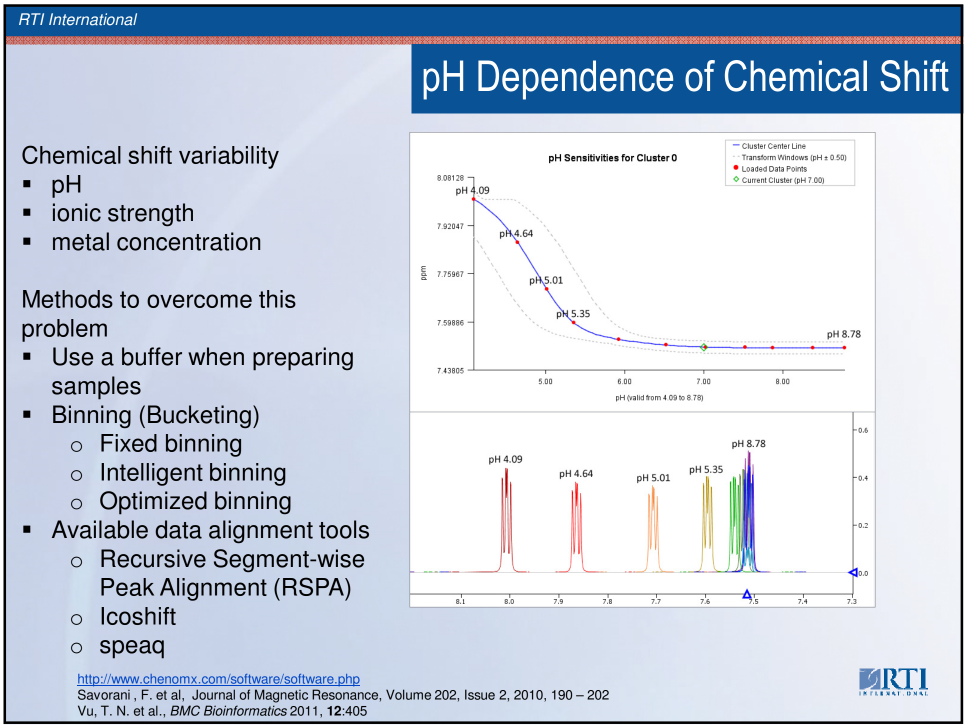


NMR Pre-processing

Before After

Phase
Baseline to be corrected

Reference,
Line shape



pH Dependence of Chemical Shift

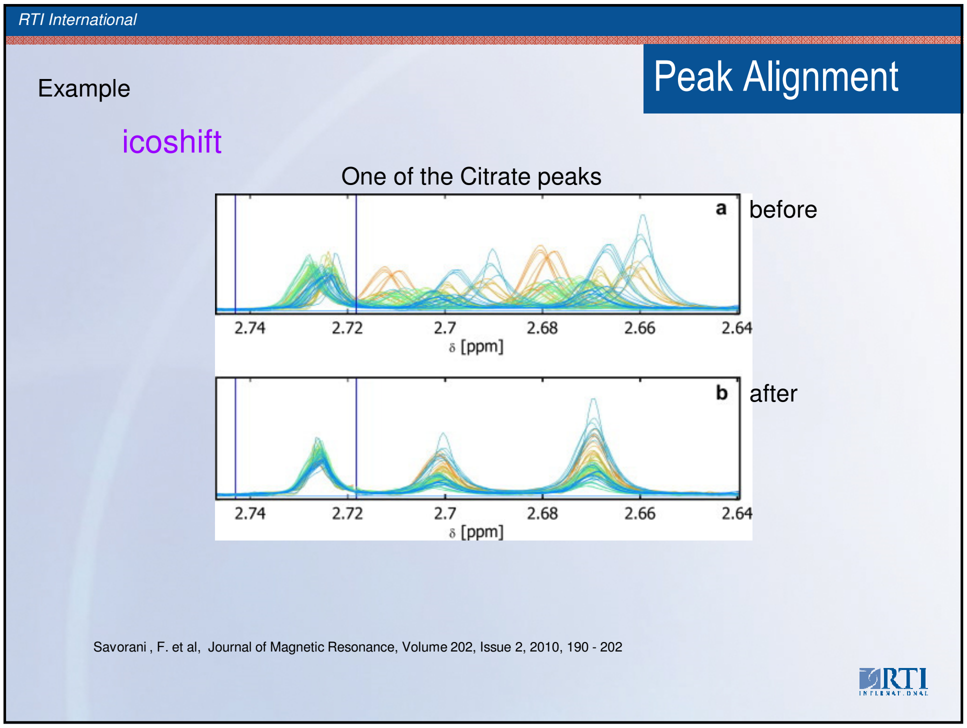
Chemical shift variability

- pH
- ionic strength
- metal concentration

Methods to overcome this problem

- Use a buffer when preparing samples
- Binning (Bucketing)
  - Fixed binning
  - Intelligent binning
  - Optimized binning
- Available data alignment tools
  - Recursive Segment-wise Peak Alignment (RSPA)
  - Icoshift
  - speaq

http://www.chenomx.com/software/software.php
Savorani , F. et al, Journal of Magnetic Resonance, Volume 202, Issue 2, 2010, 190 – 202
Vu, T. N. et al., *BMC Bioinformatics* 2011, **12**:405



Peak Alignment

Example

icoshift

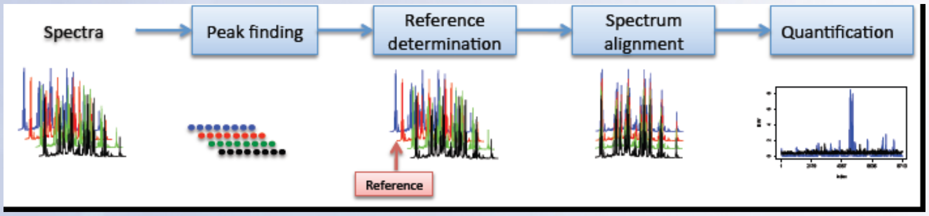
One of the Citrate peaks

Savorani , F. et al, Journal of Magnetic Resonance, Volume 202, Issue 2, 2010, 190 - 202

Peak Alignment

Example

speaq



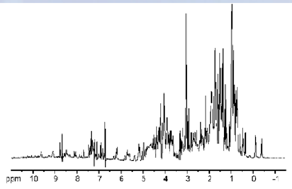
Spectra → Peak finding → Reference determination → Spectrum alignment → Quantification

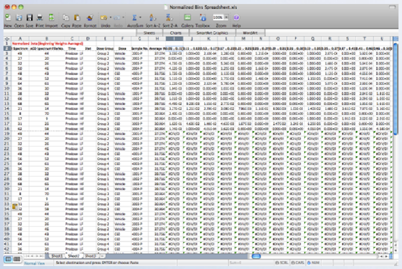
Reference

Vu, T. N. et al., *BMC Bioinformatics* 2011, **12**:405

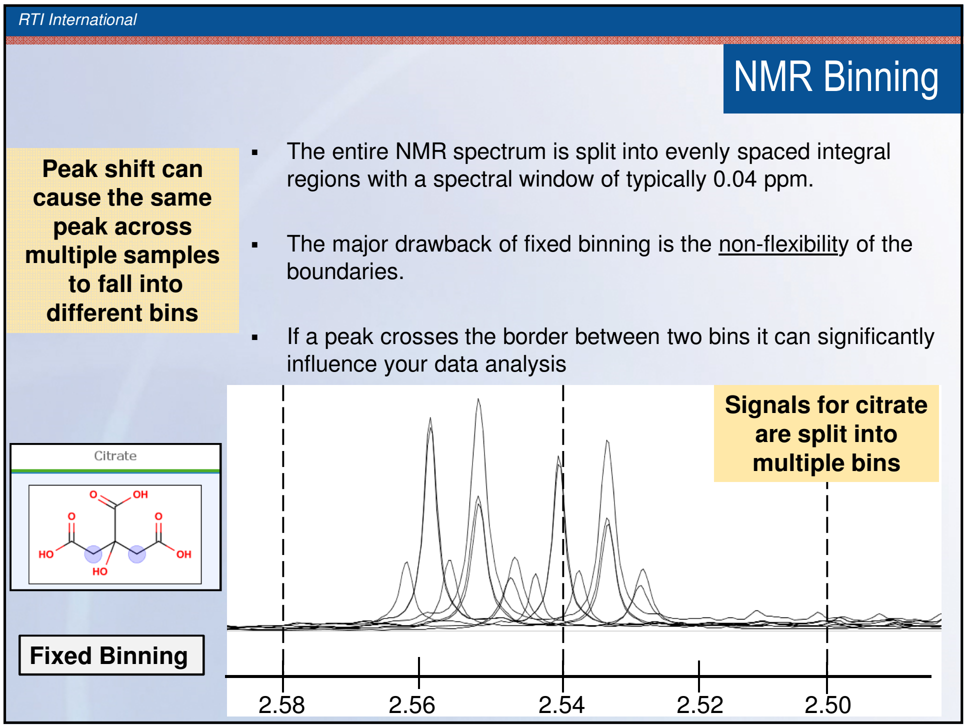
NMR Binning

- A form of quantification that consists of segmenting a spectrum into small areas (bins/buckets) and attaining an integral value for that segment
- Binning attempts to minimize effects from variations in peak positions caused by pH, ionic strength, and other factors.
- Two main types of binning
  - Fixed binning
  - Flexible binning





Normalized Data (Beginning Weights Averaged)



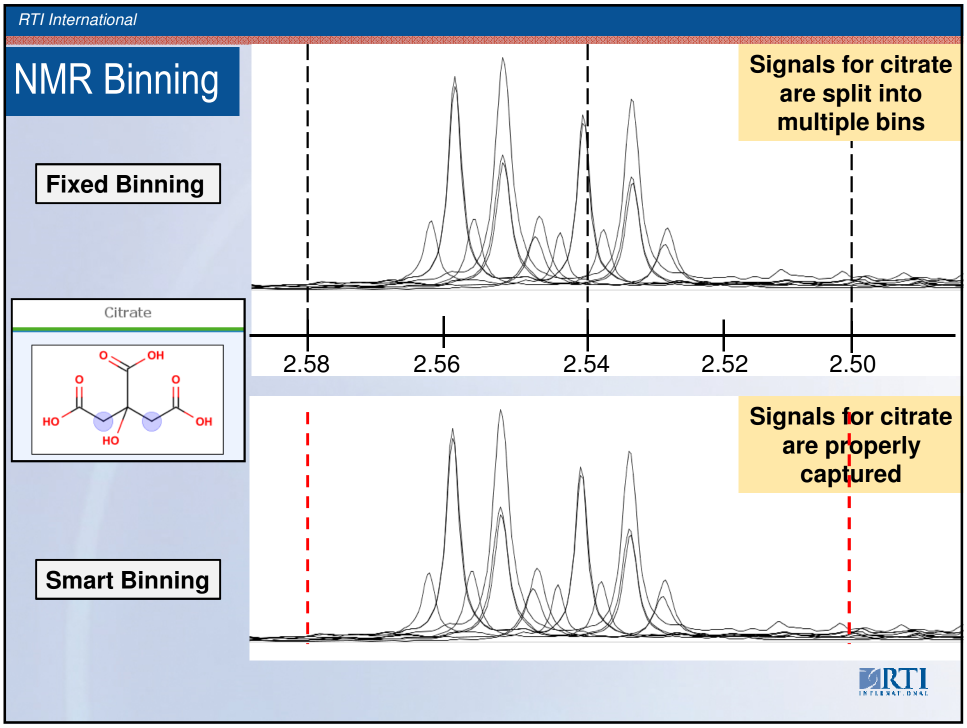
NMR Binning

Peak shift can cause the same peak across multiple samples to fall into different bins

- The entire NMR spectrum is split into evenly spaced integral regions with a spectral window of typically 0.04 ppm.
- The major drawback of fixed binning is the non-flexibility of the boundaries.
- If a peak crosses the border between two bins it can significantly influence your data analysis

Signals for citrate are split into multiple bins

Fixed Binning



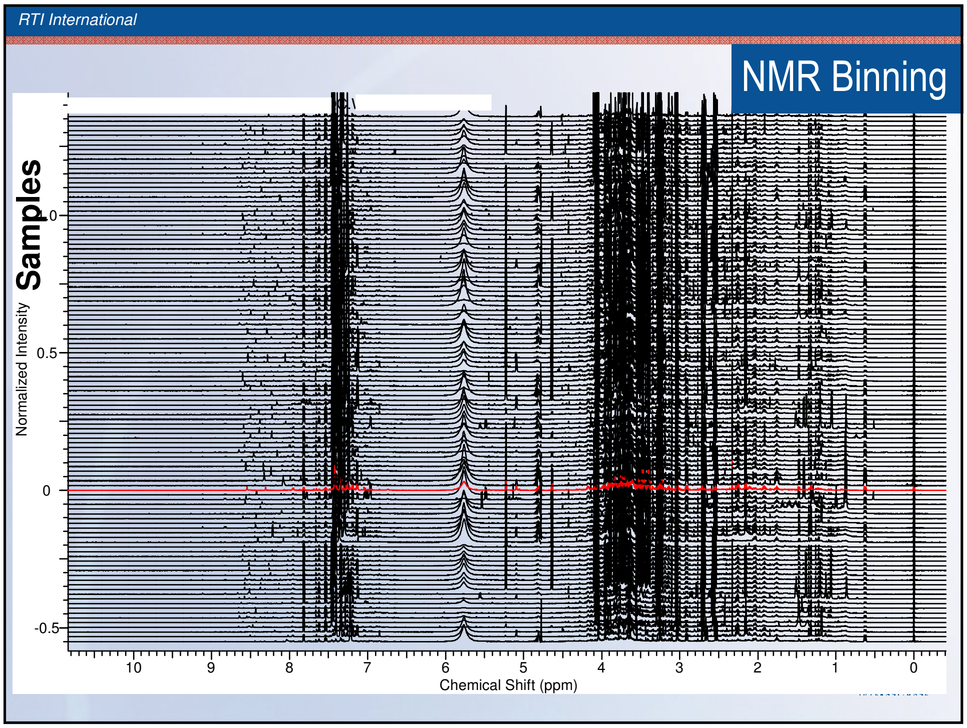
NMR Binning

Fixed Binning

Signals for citrate are split into multiple bins

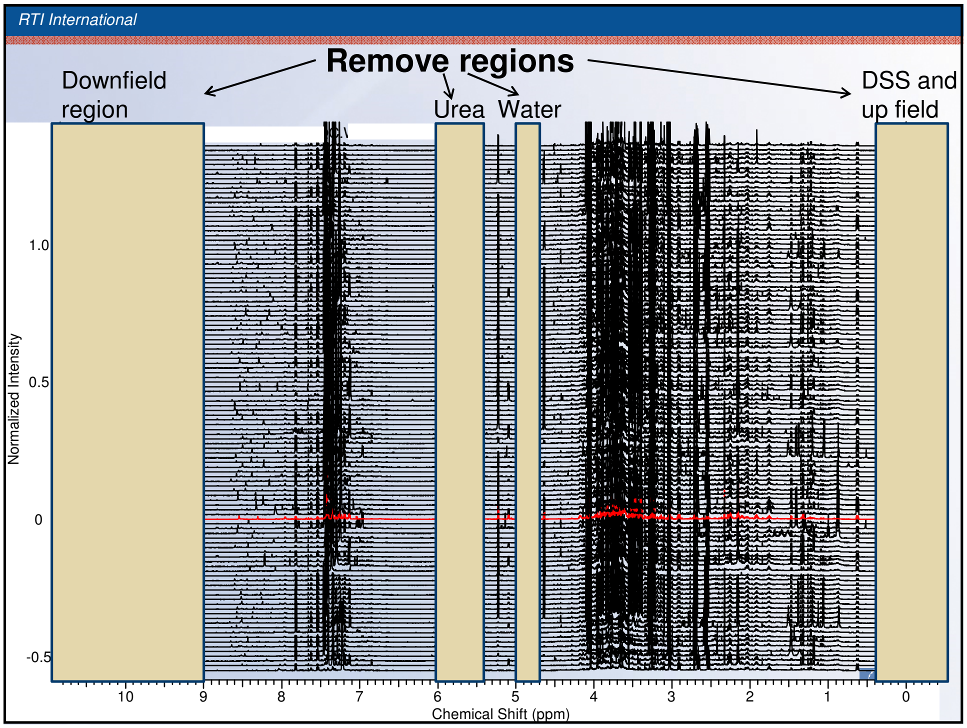
Smart Binning

Signals for citrate are properly captured



RTI International

NMR Binning



RTI International

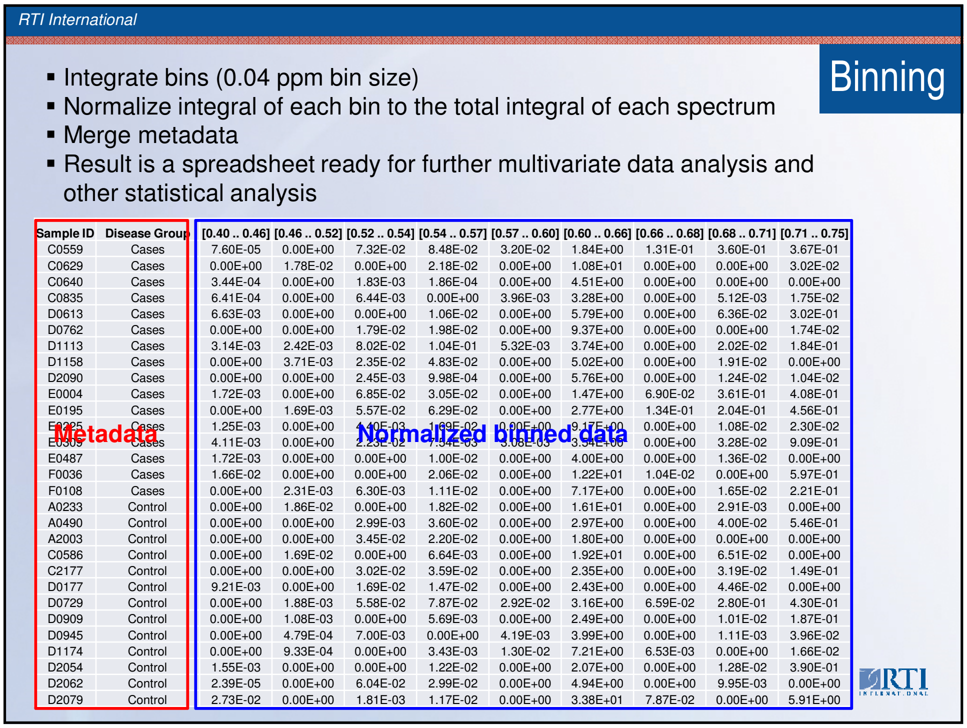
Remove regions

Downfield region

Urea

Water

DSS and up field



RTI International

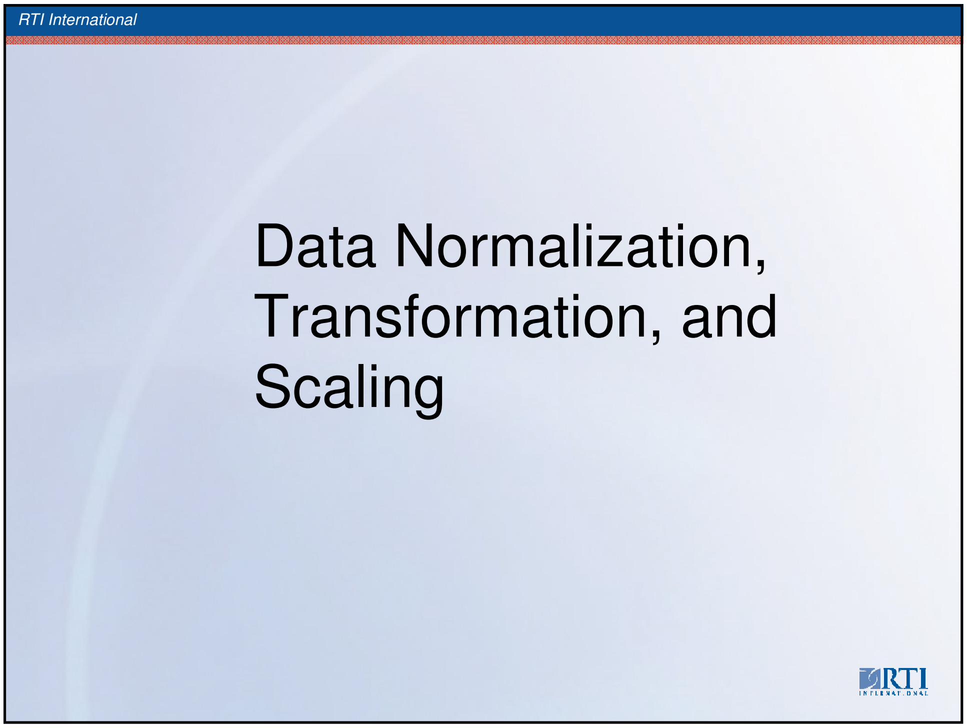
Binning

- Integrate bins (0.04 ppm bin size)
- Normalize integral of each bin to the total integral of each spectrum
- Merge metadata
- Result is a spreadsheet ready for further multivariate data analysis and other statistical analysis

Sample ID	Disease Group	[0.40 .. 0.46]	[0.46 .. 0.52]	[0.52 .. 0.54]	[0.54 .. 0.57]	[0.57 .. 0.60]	[0.60 .. 0.66]	[0.66 .. 0.68]	[0.68 .. 0.71]	[0.71 .. 0.75]
C0559	Cases	7.60E-05	0.00E+00	7.32E-02	8.48E-02	3.20E-02	1.84E+00	1.31E-01	3.60E-01	3.67E-01
C0629	Cases	0.00E+00	1.78E-02	0.00E+00	2.18E-02	0.00E+00	1.08E+01	0.00E+00	0.00E+00	3.02E-02
C0640	Cases	3.44E-04	0.00E+00	1.83E-03	1.86E-04	0.00E+00	4.51E+00	0.00E+00	0.00E+00	0.00E+00
C0835	Cases	6.41E-04	0.00E+00	6.44E-03	0.00E+00	3.96E-03	3.28E+00	0.00E+00	5.12E-03	1.75E-02
D0613	Cases	6.63E-03	0.00E+00	0.00E+00	1.06E-02	0.00E+00	5.79E+00	0.00E+00	6.36E-02	3.02E-01
D0762	Cases	0.00E+00	0.00E+00	1.79E-02	1.98E-02	0.00E+00	9.37E+00	0.00E+00	0.00E+00	1.74E-02
D1113	Cases	3.14E-03	2.42E-03	8.02E-02	1.04E-01	5.32E-03	3.74E+00	0.00E+00	2.02E-02	1.84E-01
D1158	Cases	0.00E+00	3.71E-03	2.35E-02	4.83E-02	0.00E+00	5.02E+00	0.00E+00	1.91E-02	0.00E+00
D2090	Cases	0.00E+00	0.00E+00	2.45E-03	9.98E-04	0.00E+00	5.76E+00	0.00E+00	1.24E-02	1.04E-02
E0004	Cases	1.72E-03	0.00E+00	6.85E-02	3.05E-02	0.00E+00	1.47E+00	6.90E-02	3.61E-01	4.08E-01
E0195	Cases	0.00E+00	1.69E-03	5.57E-02	6.29E-02	0.00E+00	2.77E+00	1.34E-01	2.04E-01	4.56E-01
E0225	Cases	1.25E-03	0.00E+00	4.40E-03	1.69E-02	0.00E+00	9.17E+00	0.00E+00	1.08E-02	2.30E-02
E0309	Cases	4.11E-03	0.00E+00	2.23E-02	7.54E-03	3.08E-03	3.54E+00	0.00E+00	3.28E-02	9.09E-01
E0487	Cases	1.72E-03	0.00E+00	0.00E+00	1.00E-02	0.00E+00	4.00E+00	0.00E+00	1.36E-02	0.00E+00
F0036	Cases	1.66E-02	0.00E+00	0.00E+00	2.06E-02	0.00E+00	1.22E+01	1.04E-02	0.00E+00	5.97E-01
F0108	Cases	0.00E+00	2.31E-03	6.30E-03	1.11E-02	0.00E+00	7.17E+00	0.00E+00	1.65E-02	2.21E-01
A0233	Control	0.00E+00	1.86E-02	0.00E+00	1.82E-02	0.00E+00	1.61E+01	0.00E+00	2.91E-03	0.00E+00
A0490	Control	0.00E+00	0.00E+00	2.99E-03	3.60E-02	0.00E+00	2.97E+00	0.00E+00	4.00E-02	5.46E-01
A2003	Control	0.00E+00	0.00E+00	3.45E-02	2.20E-02	0.00E+00	1.80E+00	0.00E+00	0.00E+00	0.00E+00
C0586	Control	0.00E+00	1.69E-02	0.00E+00	6.64E-03	0.00E+00	1.92E+01	0.00E+00	6.51E-02	0.00E+00
C2177	Control	0.00E+00	0.00E+00	3.02E-02	3.59E-02	0.00E+00	2.35E+00	0.00E+00	3.19E-02	1.49E-01
D0177	Control	9.21E-03	0.00E+00	1.69E-02	1.47E-02	0.00E+00	2.43E+00	0.00E+00	4.46E-02	0.00E+00
D0729	Control	0.00E+00	1.88E-03	5.58E-02	7.87E-02	2.92E-02	3.16E+00	6.59E-02	2.80E-01	4.30E-01
D0909	Control	0.00E+00	1.08E-03	0.00E+00	5.69E-03	0.00E+00	2.49E+00	0.00E+00	1.01E-02	1.87E-01
D0945	Control	0.00E+00	4.79E-04	7.00E-03	0.00E+00	4.19E-03	3.99E+00	0.00E+00	1.11E-03	3.96E-02
D1174	Control	0.00E+00	9.33E-04	0.00E+00	3.43E-03	1.30E-02	7.21E+00	6.53E-03	0.00E+00	1.66E-02
D2054	Control	1.55E-03	0.00E+00	0.00E+00	1.22E-02	0.00E+00	2.07E+00	0.00E+00	1.28E-02	3.90E-01
D2062	Control	2.39E-05	0.00E+00	6.04E-02	2.99E-02	0.00E+00	4.94E+00	0.00E+00	9.95E-03	0.00E+00
D2079	Control	2.73E-02	0.00E+00	1.81E-03	1.17E-02	0.00E+00	3.38E+01	7.87E-02	0.00E+00	5.91E+00

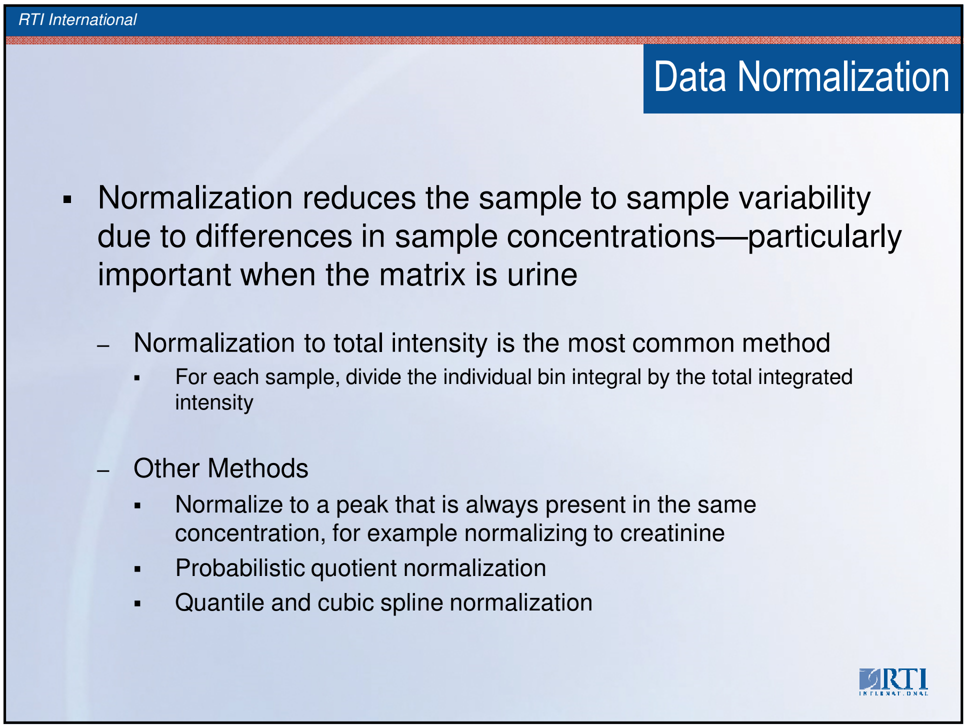
Metadata

Normalized binned data



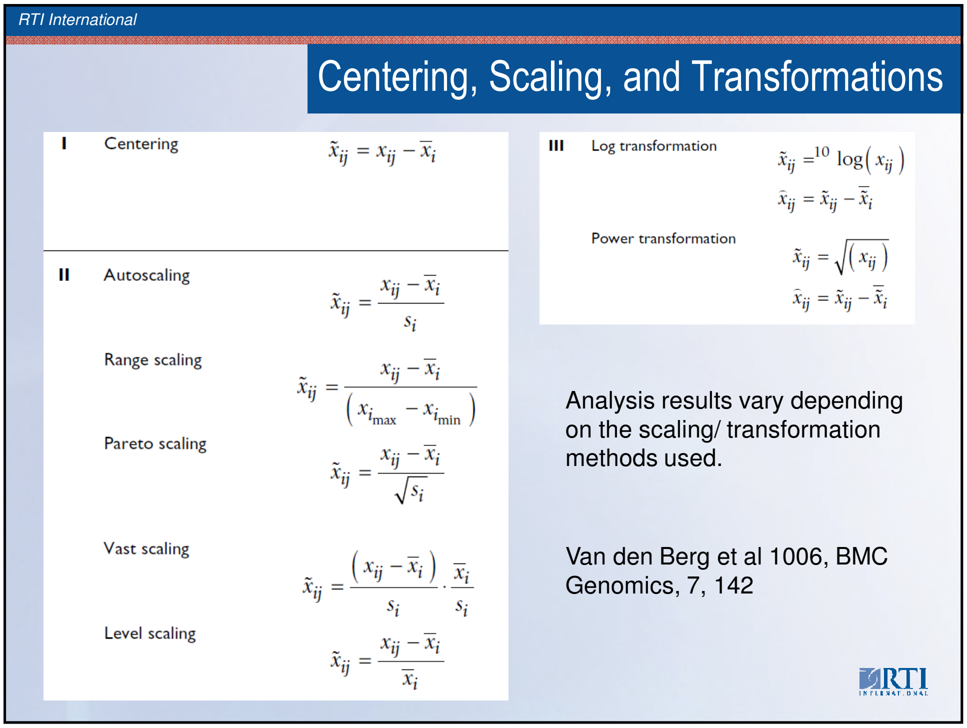
RTI International

Data Normalization, Transformation, and Scaling



Data Normalization

- Normalization reduces the sample to sample variability due to differences in sample concentrations—particularly important when the matrix is urine
  - Normalization to total intensity is the most common method
    - For each sample, divide the individual bin integral by the total integrated intensity
  - Other Methods
    - Normalize to a peak that is always present in the same concentration, for example normalizing to creatinine
    - Probabilistic quotient normalization
    - Quantile and cubic spline normalization



Centering, Scaling, and Transformations

I Centering

$$\tilde{x}_{ij} = x_{ij} - \bar{x}_i$$

II Autoscaling

$$\tilde{x}_{ij} = \frac{x_{ij} - \bar{x}_i}{s_i}$$

Range scaling

$$\tilde{x}_{ij} = \frac{x_{ij} - \bar{x}_i}{\left(x_{i_{\max}} - x_{i_{\min}}\right)}$$

Pareto scaling

$$\tilde{x}_{ij} = \frac{x_{ij} - \bar{x}_i}{\sqrt{s_i}}$$

Vast scaling

$$\tilde{x}_{ij} = \frac{\left(x_{ij} - \bar{x}_i\right)}{s_i} \cdot \frac{\bar{x}_i}{s_i}$$

Level scaling

$$\tilde{x}_{ij} = \frac{x_{ij} - \bar{x}_i}{\bar{x}_i}$$

III Log transformation

$$\tilde{x}_{ij} = {}^{10}\log\left(x_{ij}\right)$$

$$\hat{x}_{ij} = \tilde{x}_{ij} - \bar{\tilde{x}}_i$$

Power transformation

$$\tilde{x}_{ij} = \sqrt{\left(x_{ij}\right)}$$

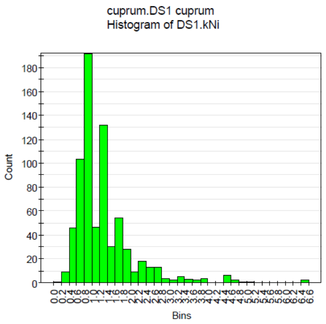
$$\hat{x}_{ij} = \tilde{x}_{ij} - \bar{\tilde{x}}_i$$

Analysis results vary depending on the scaling/ transformation methods used.

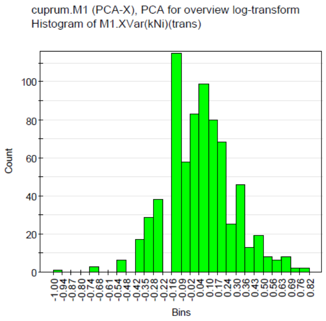
Van den Berg et al 1006, BMC Genomics, 7, 142

Data Transformation

- Before transformation
  - skew distribution



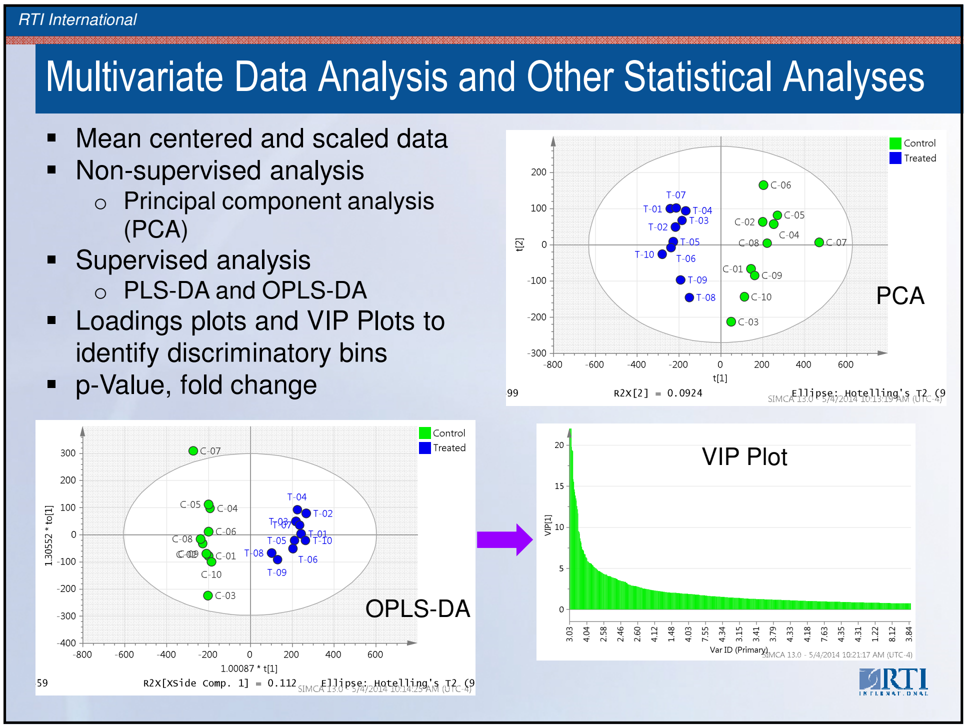
- After log-transformation
  - More close to normal distribution



Susan Wicklund, Multivariate data analysis for omics, Sept 2-3 2008, Umetrics training

Scaling

- Unit variance (autoscaling) divides the bin intensity by the standard deviation
  - May increase your baseline noise
  - Dimensionless value after scaling
- Pareto scaling divides the bin intensity by the square root of the standard deviation
  - Not dimensionless after scaling
- For NMR data, centering with pareto scaling is commonly used



RTI International

Multivariate Data Analysis and Other Statistical Analyses

- Mean centered and scaled data
- Non-supervised analysis
  - Principal component analysis (PCA)
- Supervised analysis
  - PLS-DA and OPLS-DA
- Loadings plots and VIP Plots to identify discriminatory bins
- p-Value, fold change

RTI International

STS Center

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Wimal Pathmasiri
NMR & GC-MS

Jim Carlson
LC- and GC-MS

Jessica Gooding
LC-MS

Kelly Mercier
NMR

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Biostatistics

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LC-MS/MS

Jocelin Spruill
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Metallomics

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Tim Fennell
Metabolism

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Rod Snyder
LC-MS

Sherry Black
In vivo and in vitro
Metabolism

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Systems

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In vitro metabolsm

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