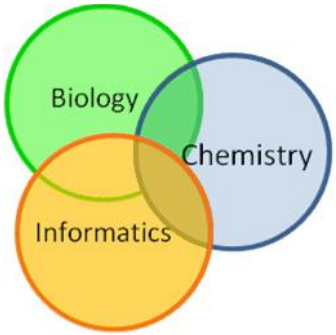


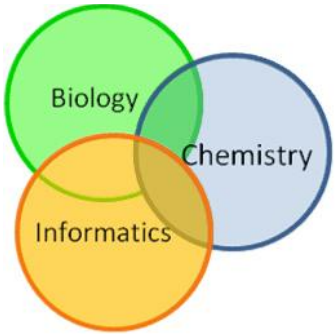
Normalization methods for analytical variance reduction

Statistics

Goals: Evaluate batch effects in replicated measurements and overview normalization methods

Topics:

1. Batch effects
2. Sample normalization
3. Variable transformation
4. Variable normalization



Identify the effects of sample drying

Statistics

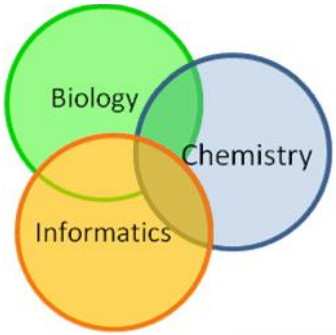
Use DATA: Normalization Data.csv

Visualize:

1. Batch effects in replicated measurements
2. The effect of normalization on samples and variables

Questions:

1. How can batch and outlier effects be mitigated?



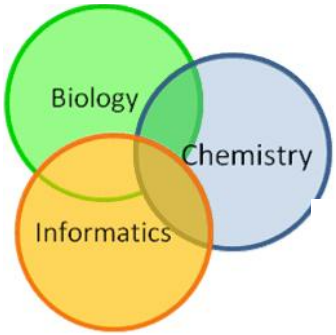
Question:

Are there any batch effects in this data?

Statistics

	Acquisition.order	Batch	Sample	z.C30.FAME
1	1	B1	train	14085
2	2	B1	test	17560
3	3	B1	train	15042
4	4	B2	test	12533
5	5	B2	train	18879
6	6	B2	test	19099
7	7	B2	train	18535
8	8	B3	test	17768
9	9	B3	train	20127
10	10	B3	test	21715

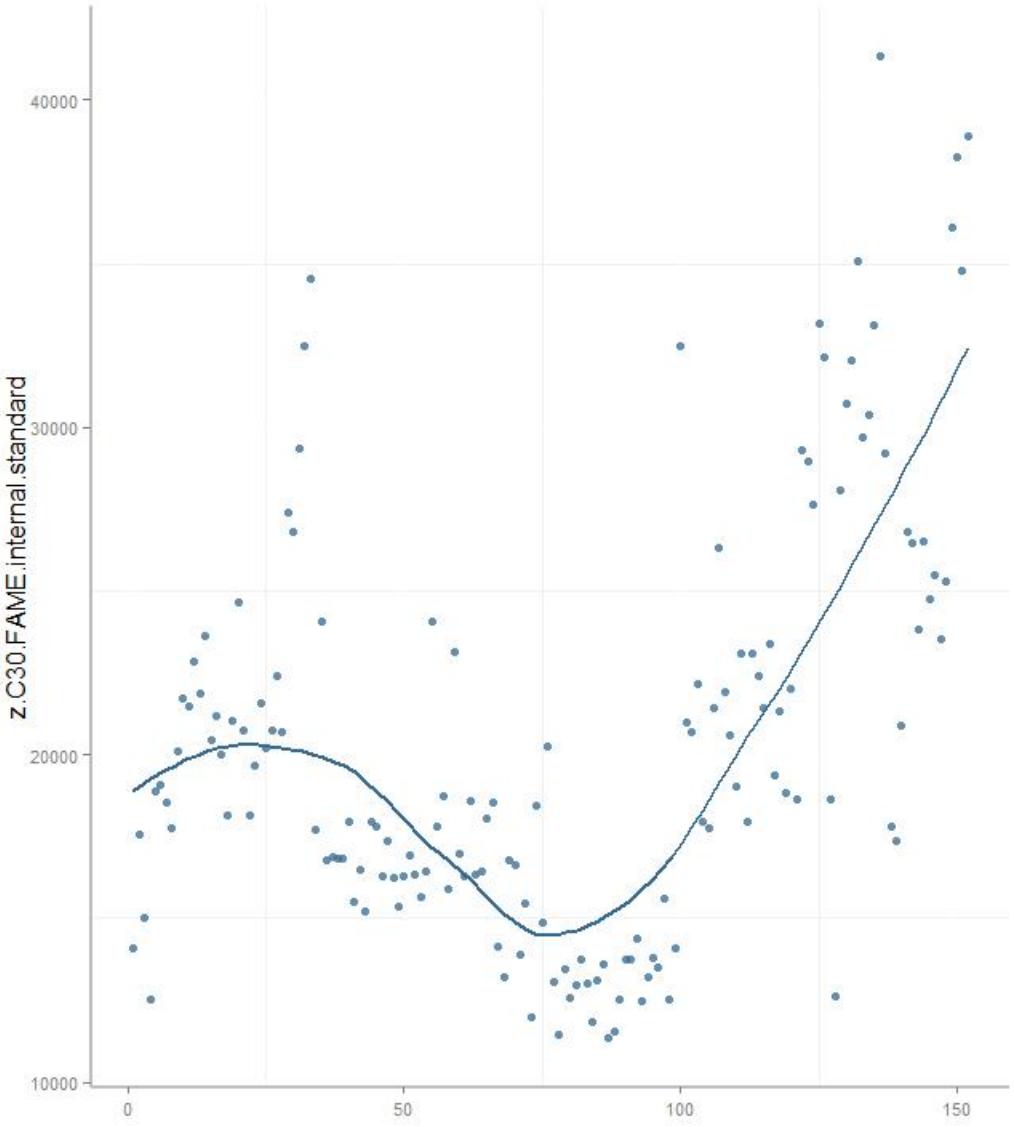
```
$dimensions
  rows columns
1  152      12

$factors
     Batch        Sample
 B15    :  5   test :76
 B24    :  5   train:76
 B26    :  5
 B11    :  4
 B12    :  4
 B13    :  4
```

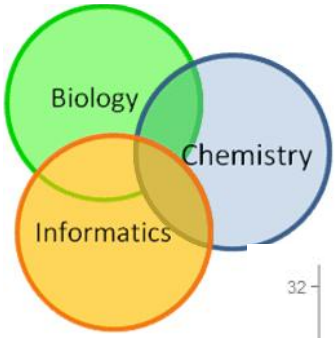


Answer:

Are there any batch effects in this data? (Yes!)

Statistics

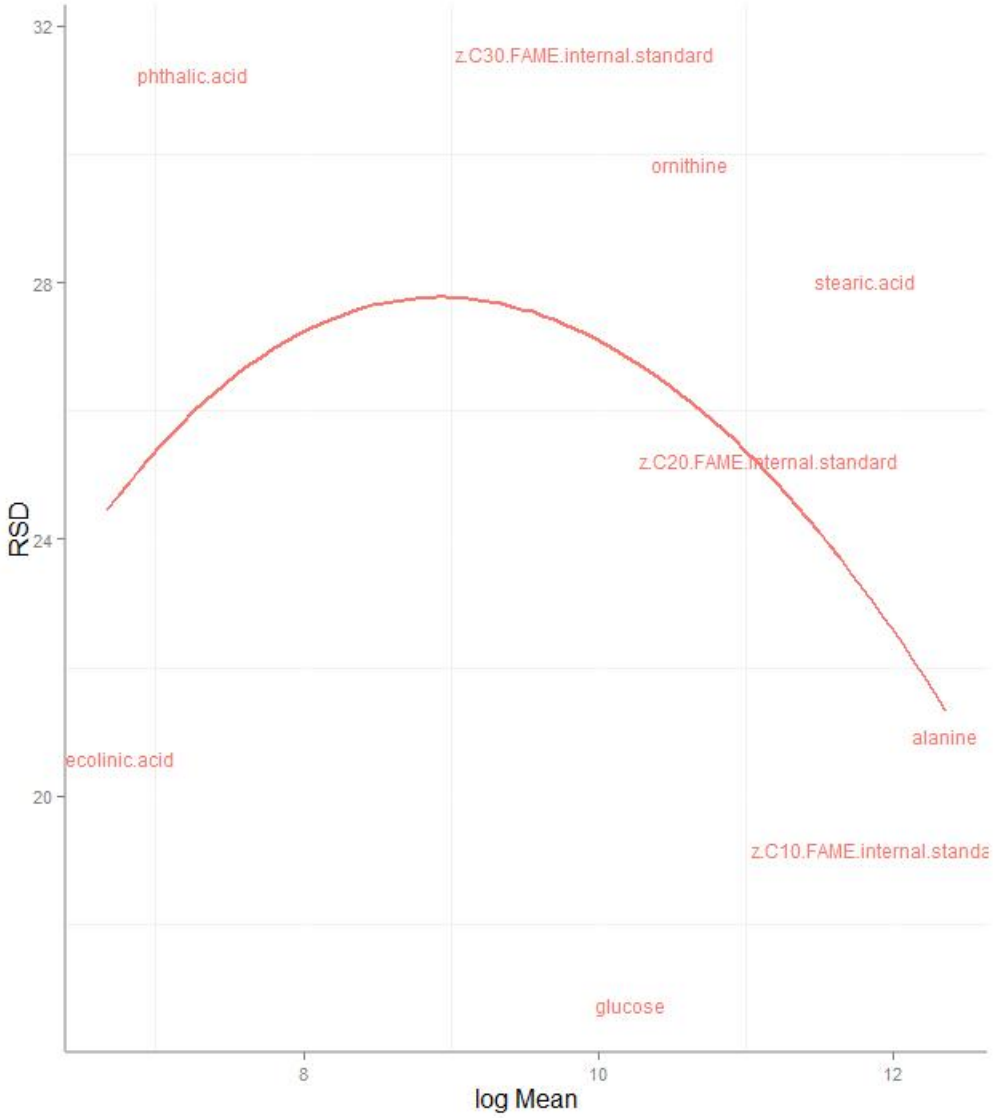




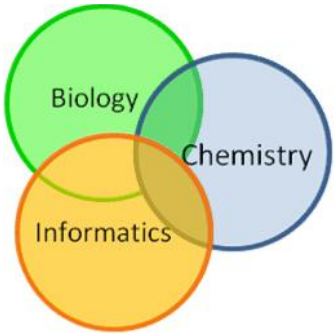
Answer:

Not all metabolites are similarly affected

Statistics



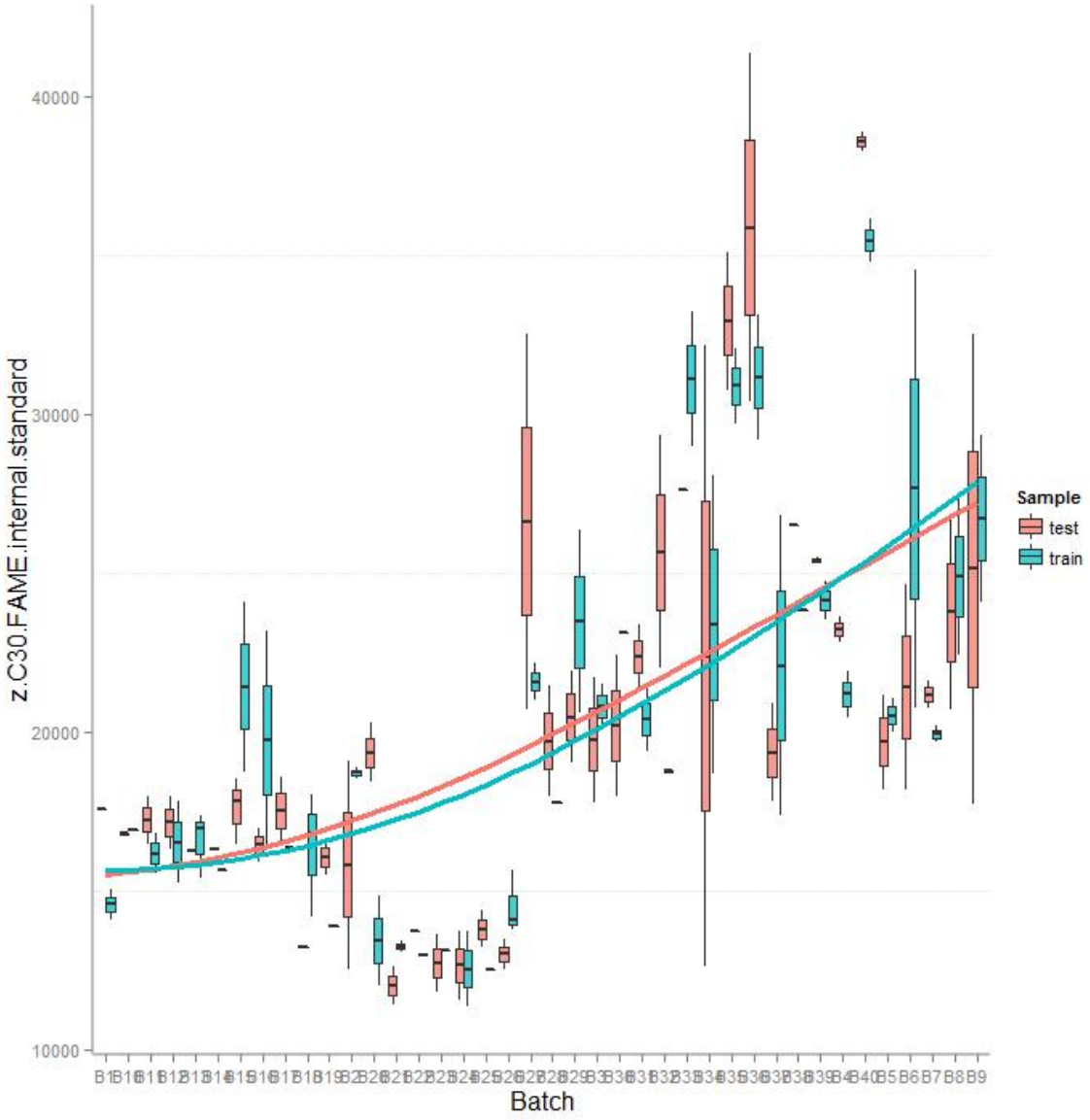

```
$methods
                                methods
sample.normalization               none
variable.transformation            none
variable.centering                 none
variable.normalization             none

$performance
$performance$batch
  median.RSD   range
1         13 5.9, 24

$performance$batch.summary
    RSD count percent cumulative.percent
1  0-10    10      25                  25
2 10-20    25      62                  87
3 20-30     5      12                  99

$performance$variable
  median.RSD   range
1         12 8.4, 19

$performance$variable.summary
    RSD count percent cumulative.percent
1  0-10     3      33                  33
2 10-20     6      67                 100
```

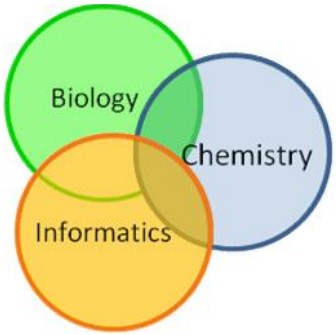


Question:

Can sample and variable normalizations reduce analytical variance and batch effects?

Statistics



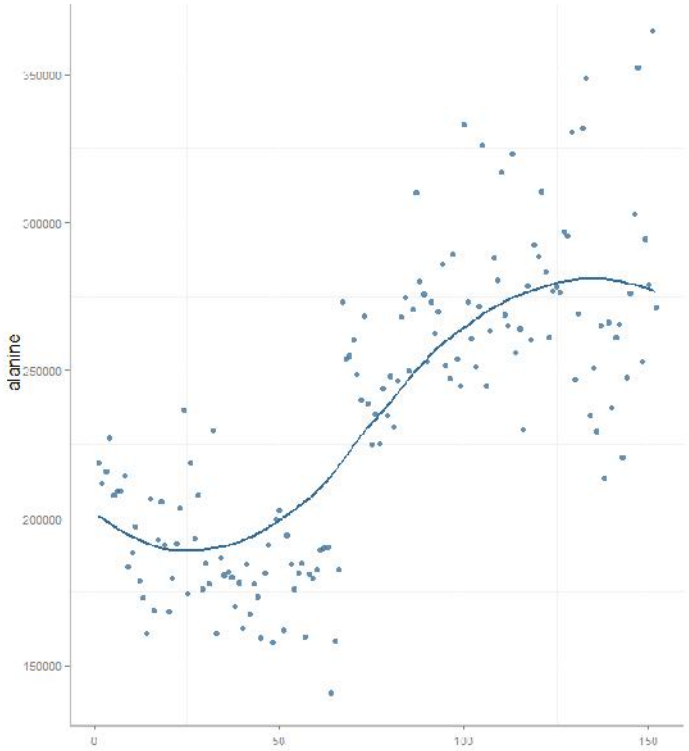


Answer:

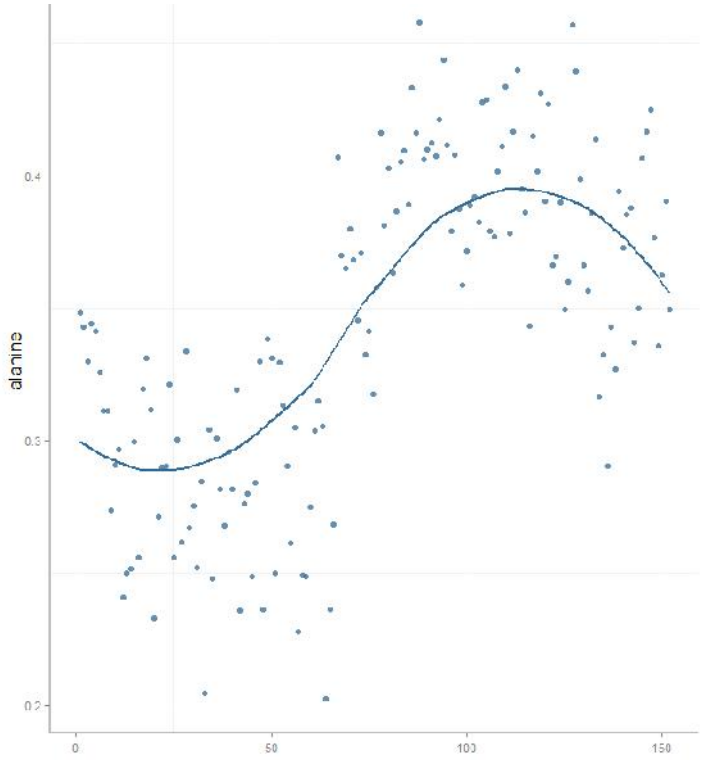
Sample and variable normalizations can reduce or increase analytical variance and batch effects.

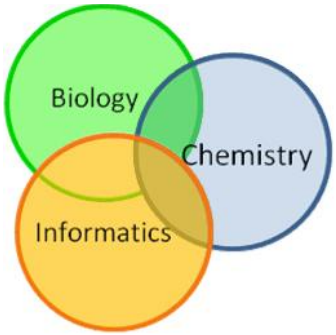
Statistics

raw



Sum normalization





Answer:

Sample and variable normalizations can reduce analytical variance and batch effects.

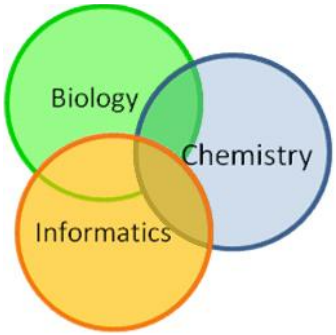
raw

Sum normalization

Statistics

```
$performance
$performance$batch
  median.RSD   range
1         13 5.9, 24

$performance$batch.summary
    RSD count percent cumulative.percent
1  0-10    10      25                 25
2 10-20    25      62                 87
3 20-30     5      12                 99

$performance$variable
  median.RSD   range
1         12 8.4, 19

$performance$variable.summary
    RSD count percent cumulative.percent
1  0-10     3      33                 33
2 10-20     6      67                100
```

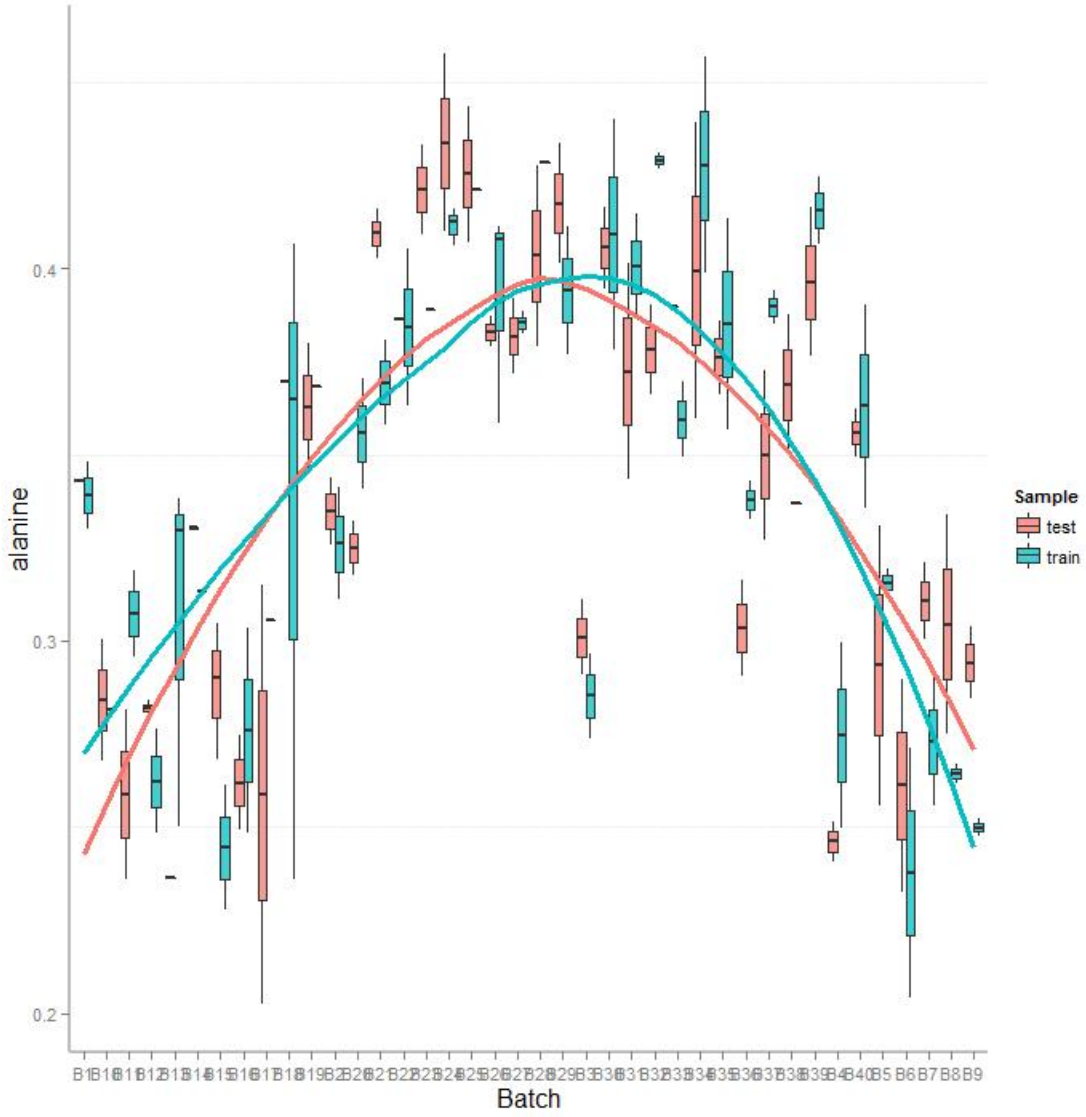
```
$performance
$performance$batch
  median.RSD   range
1         12 5.9, 23

$performance$batch.summary
    RSD count percent cumulative.percent
1  0-10    10      25                 25
2 10-20    28      70                 95
3 20-30     2       5                100

$performance$variable
  median.RSD   range
1         12 8.2, 19

$performance$variable.summary
    RSD count percent cumulative.percent
1  0-10     4      44                 44
2 10-20     5      56                100
```

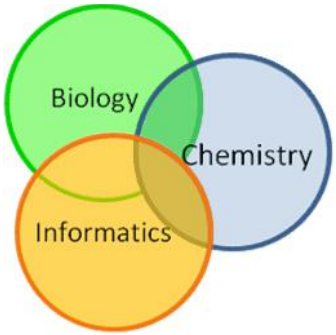


QC sample based normalizations

Can be useful for estimating and removing analytical variance

Statistics





Example: qcLOESS workflow

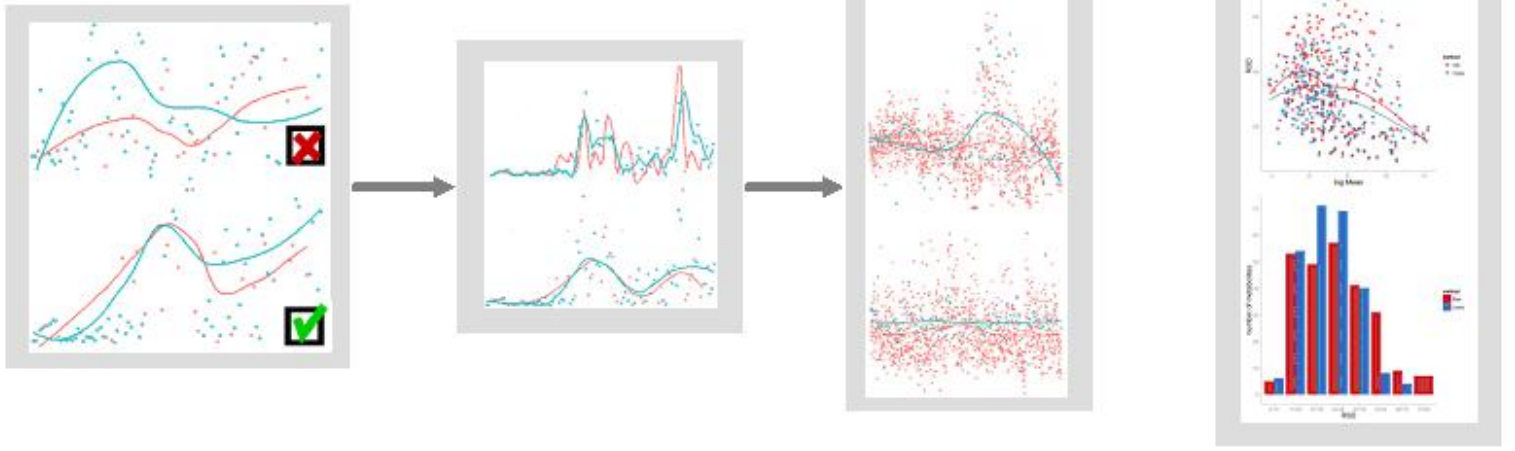
Statistics

1. Analysis of correlations between QC and sample trends

2. QC based variance model optimization

3. QC model based adjustment of sample variance

4. Normalization performance validation



Overview of Normalizations (PCA)