MetMSLine WorkFlow

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The following illustrates the MetMSLine workflow with example data:

1. Read in peak table and co-variate information and pre-process the data.

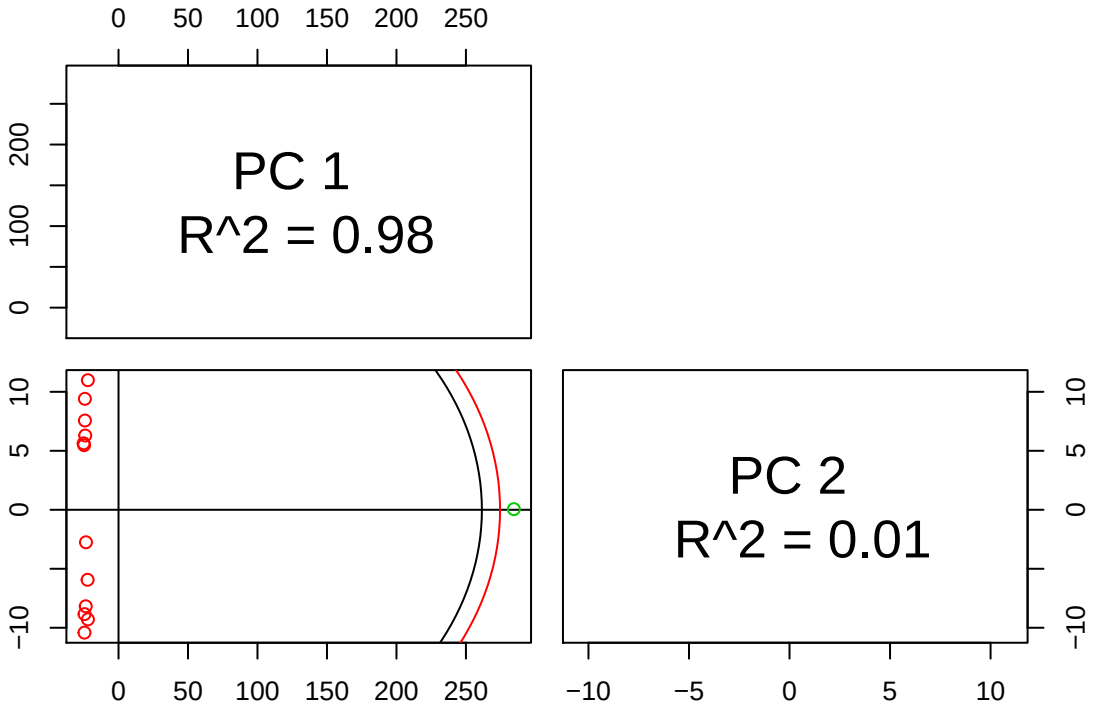
```
# file path example peakTable in comma delimited csv file
# (see ?example_mzXML_peakTable for details).
peakTable <- system.file("extdata", "MS1features_example.csv", package = "MetMSLine")
peakTable <- read.csv(peakTable, header=T, stringsAsFactors=F)


# load co-variates table in comma delimited csv file
coVariates <- system.file("extdata", "coVariates.csv", package = "MetMSLine")
coVariates <- read.csv(coVariates, header=T)
# observation names (i.e. sample names)
obsNames <- colnames(peakTable)[4:ncol(peakTable)]

# zero fill
peakTable <- zeroFill(peakTable, obsNames)
## zero filling...

# Normalize (median fold change/ probabilistic quotient), total ion signal
#  also available ?signNorm
peakTable <- signNorm(peakTable, obsNames, method="medFC")
## Median fold change normalization...

# data deconvolution based on retention time and interfeature correlation
# calculation of weighted mean (see ?weigthed.mean) within each pseudospectral
# cluster (i.e. the sum of mass spectral intensities across all samples are used
#          to weight the contribution of each feature to the average).
wMeanPeakTable <- rtCorrClust(peakTable, obsNames, rtThresh=2, corrThresh=0.9,
                              minFeat=1)
## hierarchical clustering peak group retention times...
##
## intra RT group correlation clustering 45 rt groups...
##
## Calculating weighted mean for 2516 pseudospectra accounting for 3720 of 3720 total features

# extract weighted mean pseudospectra table
wMeanPspec <- wMeanPeakTable$wMeanPspec

# log transform (base 2)
wMeanPspec <- logTrans(wMeanPspec, obsNames, base=2)
## log transforming to the base 2...
```

2. PCA projection, automatic outlier removal and score plot cluster identification.

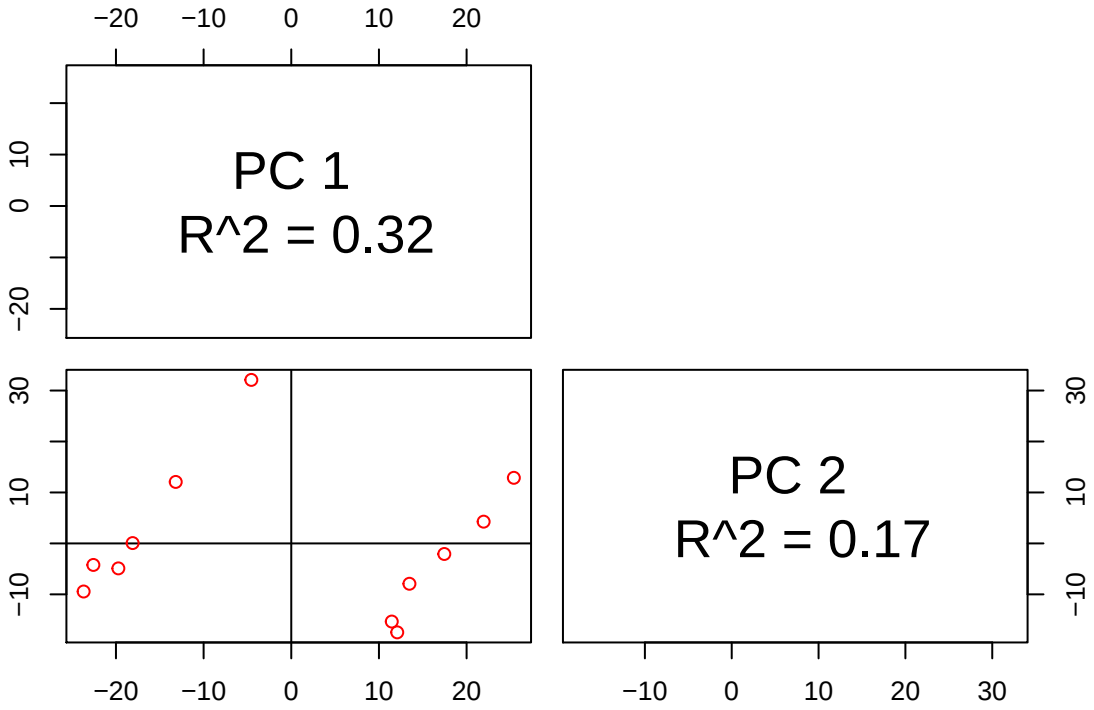
```
# add dummy blank to illustrate pca outlier detection
wMeanPspec$blank_1 <- 0.0001
# observation names (i.e. sample names)
obsNames <- colnames(wMeanPspec)[13:ncol(wMeanPspec)]

#  PCA projection using pca of pcaMethods and automatic outlier removal based
#  on proportional expansion of the Hotellings T2 ellipse
pcaOutResults <- pcaOutId(wMeanPspec, obsNames, cv="q2", outTol=1.05,
                          scale="pareto")
```

```
## Calculating PCA model 1...
## 1 outliers identified PCA model 1
## Calculating PCA model 2...
```

```
# Plot PCA displaying any outliers and expanded Hotelling's ellipse, colour according
# to any potential outliers detected. function modified from pcaMethods ?plotPcs.
plotPcsEx(pcaOutResults$pcaResults[[1]]$pcaResult,
          pcaOutResults$pcaResults[[1]]$exHotEllipse, type="scores",
          col=pcaOutResults$pcaResults[[1]]$possOut+2)
```



```
# plot second PCA model iteration after outlier removal
plotPcsEx(pcaOutResults$pcaResults[[2]]$pcaResult,
          pcaOutResults$pcaResults[[2]]$exHotEllipse, type="scores",
          col=pcaOutResults$pcaResults[[2]]$possOut+2)
```



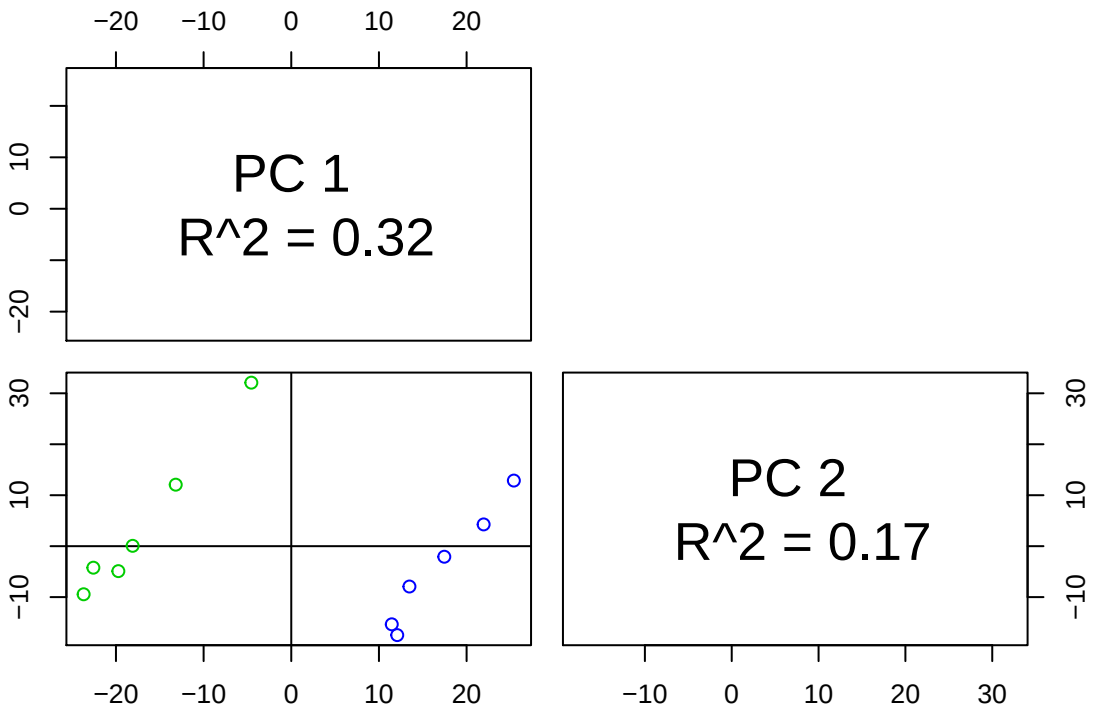

```
# show PCA results iteration 2
pcaOutResults$pcaResults[[2]]$pcaResult
```

```
## svd calculated PCA
## Importance of component(s):
##                    PC1    PC2
## R2              0.3188 0.1727
## Cumulative R2 0.3188 0.4915
## 2516     Variables
## 12   Samples
## 0    NAs ( 0 %)
## 2    Calculated component(s)
## Data was mean centered before running PCA
## Data was scaled before running PCA
## Scores structure:
## [1] 12  2
## Loadings structure:
## [1] 2516    2
```

```
# show Q2 cross-validation statistic
pcaOutResults$pcaResults[[2]]$pcaResult@cvstat
```

```
##      PC 1      PC 2
## 0.1623614 0.1814169
```

```
# label by extraction type using co-variates table
plotPcsEx(pcaOutResults$pcaResults[[2]]$pcaResult,
          pcaOutResults$pcaResults[[2]]$exHotEllipse, type="scores",
          col=as.numeric(as.factor(coVariates$extractionType)) + 2)
```



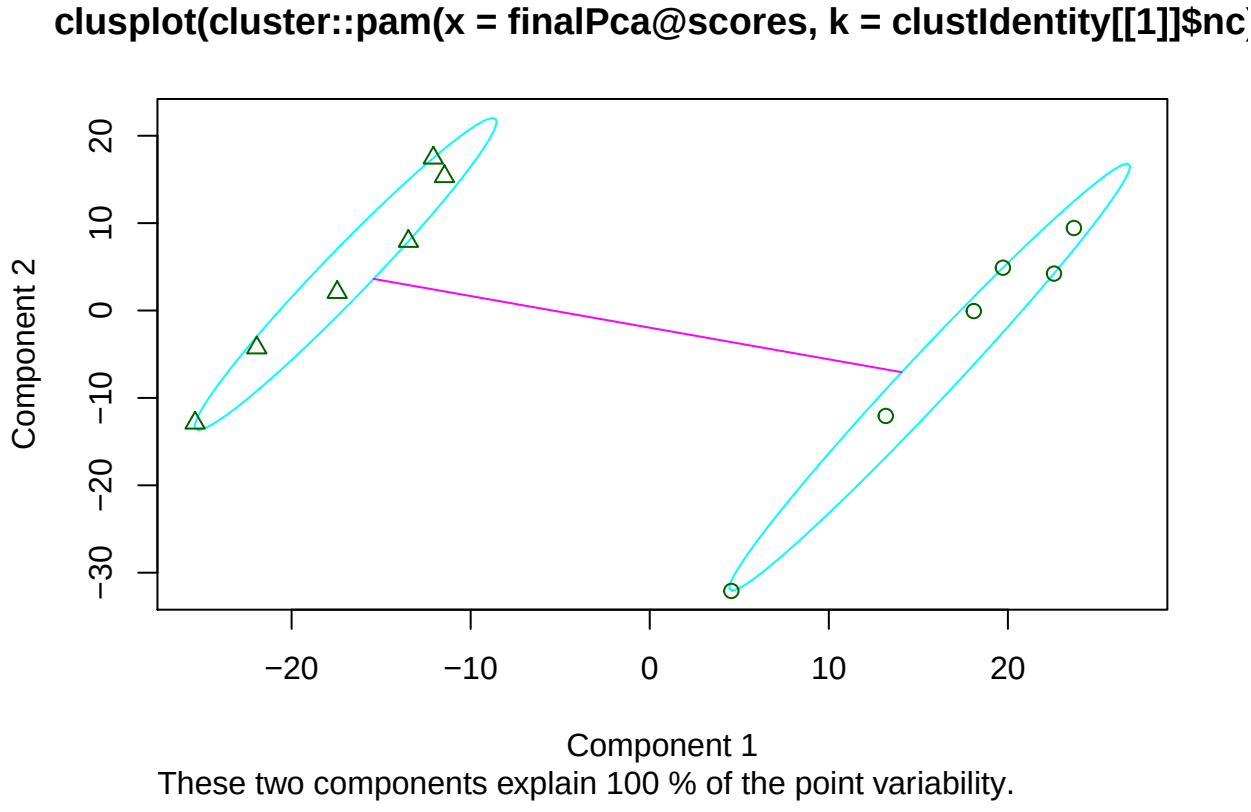

```
# Automatically identify potential cluster membership given the table of co-variates
finalPca <- pcaOutResults$pcaResults[[length(pcaOutResults)]]$pcaResult
clustIdentity <- pcaClustId(finalPca, coVarTable=coVariates)
```

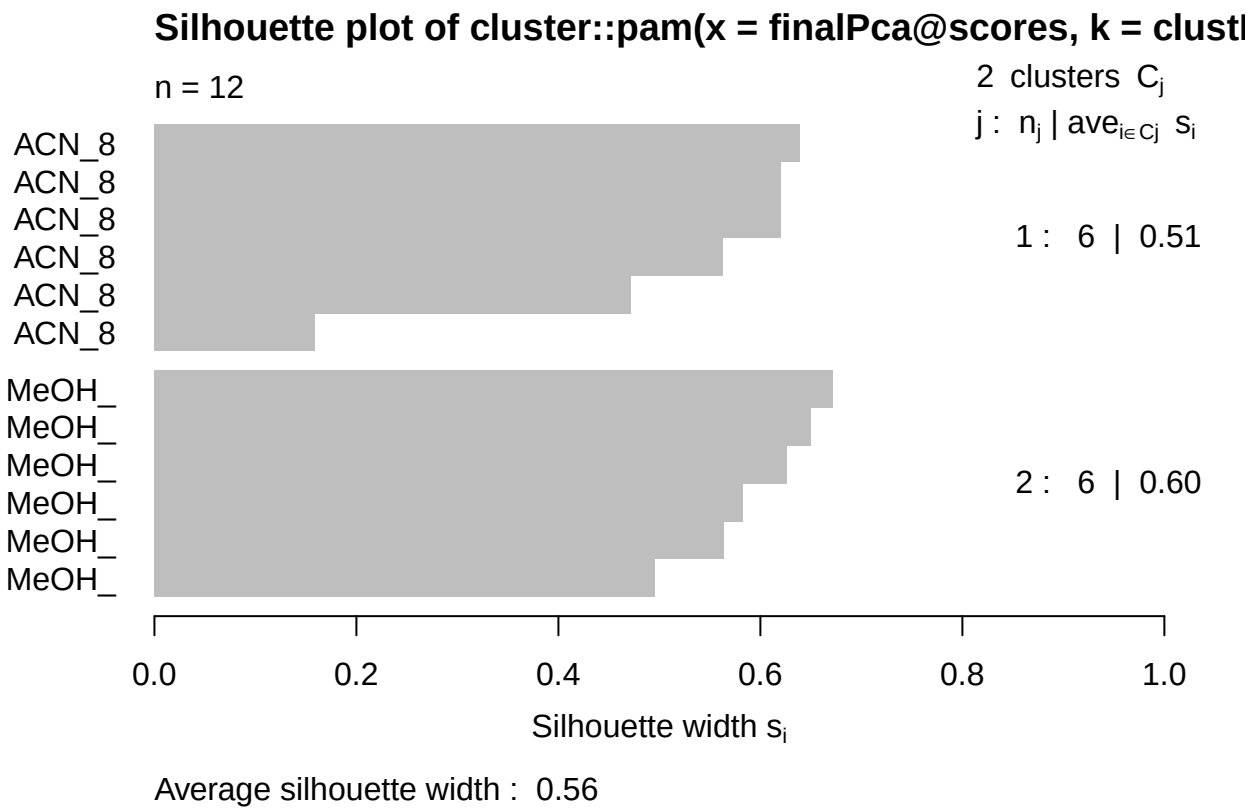
```
## 2 PCA scores clusters identified by PAM
```

```
## Warning in summary.lm(coVar): essentially perfect fit: summary may be
## unreliable
```

```
## largest coefficient of determination (r2) from linear modelling of all
##   co-variates to PCA scores clusters:
## extractionType
##       "1.0000"
```

```r
# plot pam cluster model (partioning around the medoids), minimisation of
# dissimilarities.
plot(cluster::pam(finalPca@scores, clustIdentity[[1]]$nc))
```

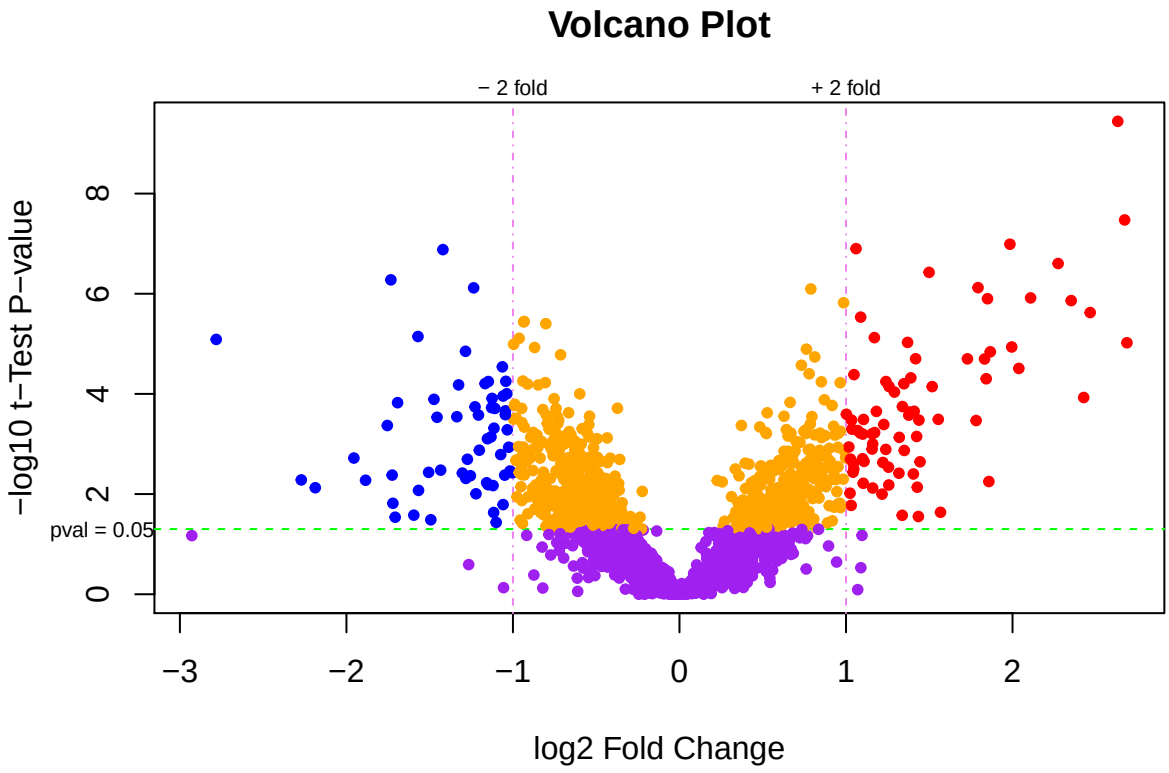




3. Univariate statistical analysis by co-variate based automatic test type selection.

The most appropriate univariate statistical method is selected based on frequency of factor levels of a co-variate (y-variable) supplied. This provides objective and automatable means of test selection. Multiple comparison adjustment can also be performed (e.g. Bonferroni).

```
# outliers removed peak table from pcaOutId output
outRemPeakTable <- pcaOutResults$outRem
obsNames <- colnames(outRemPeakTable)[13:ncol(outRemPeakTable)]

# automatic univariate statistical method selection and mean/median fold calculation
statResult <- coVarTypeStat(outRemPeakTable, obsNames,
                            coVariate=coVariates$extractionType,
                            Logged=T, base=2)
# volcanoPlot
volcanoPlot(log2(statResult[[5]]$FoldChange), statResult[[5]]$p.value)
```


Volcano Plot
− 2 fold
+ 2 fold
−log10 t−Test P−value
pval = 0.05
0
2
4
6
8
−3
−2
−1
0
1
2
log2 Fold Change