This notebook provides a template for the comparison of replicate datasets using the MathDAMP package. It is intended for datasets acquired by capillary electrophoresis coupled to a time-of-flight mass spectrometer (CE-TOFMS). The datasets are modified to have a 0.02 m/z units resolution along the m/z axis and saved in a binary format as *bdt datafiles.

### Optional: Exploring the data, locating the peak of the internal standard in the reference dataset

For a preliminary exploration, the loaded data may be visualized on density plots. The dimensions of the signal intensity matrix of the datasets are too large to fit on the screen, so some signals may not be visible. For a high resolution view, save the density plot as a postscript file, and then convert it to PDF using ghostscript or similar tools.

Because of significant differences between datasets acquired by quadrupole and time-of-flight mass spectrometers, different default appearances of the density plots are desirable for CE-TOFMS data (e.g., denser tickmarks and gridlines along the m/z axis, etc.).

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**Step 1: Loading the Data**

First, the MathDAMP package must be loaded. Please assign the path leading to MathDAMP files to the MathDAMPPath variable. Due to the size of the datasets and the global variable $HistoryLength, it is set to 1 to save memory. 1GB of physical memory may be necessary to execute this notebook.

```mathematica
$HistoryLength = 1;
MathDAMPPath = "~/baran/math/ms/MathDAMP.1.0.0/";
<< (MathDAMPPath <> "MathDAMP.m")

MathDAMP version 1.0.0 loaded (2006/04/26)
```

Datasets acquired by capillary electrophoresis coupled to a time-of-flight mass spectrometer (CE-TOFMS) will be used for the demonstration in this notebook. The *bdt datafiles were generated using a separate in-house software from *csv datafiles exported by the Analyst QS software (for Agilent TOFMS). The csv data were binned to a 0.02 m/z units resolution along the m/z axis, baselines were subtracted from the individual electropherograms (as by the DAMPSubtractBaselines function with default options), noise was removed (as with the DAMPRemoveNoise function with default options), the data were binned to 1 m/z units resolution and saved in a binary format as *bdt datafiles.

```mathematica
fnames = FileNames["~/baran/data/examples/*", All, "*bdt"] & /@ ("1h", "4h");
(ctr1, ampl1) = DAMPImportBDT[#, StringReplace[#, __ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <> ___ <
Performing the Differential Analysis

The function DAMPTwoGroups performs the comparison of two groups of replicate datasets. DAMPNormalizeGroup function is used internally to align and normalize the datasets along with the annotation tables. Please refer to the MathDAMP.nb notebook for more details about the implementation of functions DAMPNormalizeGroup and DAMPTwoGroups. Execute FunctionName to list a brief description of the respective function's available options.

### Step 2: Performing the Differential Analysis

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#### DAMPNormalizeGroup

DAMPNormalizeGroup[mdatas,options] aligns mdatas [a list of datasets] and normalizes them according to the areas of the peaks of the internal standard and external normalization coefficients [optional]. The results are returned as a list of rules: [NormalizedDatasets=..., AlignedAnnotationTables=...].

- **Reference** - position of the reference dataset within mdatas to which the remaining mdatas will be normalized [default: 1].
- **AlignmentTimeRange** - peak picks from the reference dataset falling within this time range only (specified as [starttime,endtime] in minutes) will be used for alignment [default: All].
- **RepresentativeWeakOptions** - options to be passed to the DAMPSelectRepresentativePeaks function to filter the initial peak picks [default: PeaksPerChromatogram=5, PeaksPerInterval=0, Intervallize->0].
- **PeakPickingOptions** - options to be passed to the DAMPPickPeaks function [default: Threshold=5000, PeakLayoutPlotOptions=...].
- **FitShiftFunctionOptions** - options to be passed to the DAMPFitShiftFunction function [default: None].
- **AnnotationTables** - a list of annotation tables to be aligned to the sample mdatas [default: None].
- **OutputTimeRange** - time range to which the resulting normalized datasets should be cropped [default: All].
- **ExternalNormalizationCoefficients** - list of coefficients by which the signal intensities in mdatas will be multiplied. The number of coefficients in the list must equal the number of datasets in mdatas [default: None].
- **Resolution** - resolution to which the datasets were binned along the m/z dimension. The annotation tables passed through the AnnotationTables option will be binned the same way [default: 1].
- **InternalStandard** - the internal standard for signal intensity normalization may be specified in one of two ways: 1) short name (3rd column) from the first annotation table in the list passed via the AnnotationTables option. In this case the position of the internal standard will be extrapolated from the aligned annotation table and the vicinity blindly integrated 2 specifying the m/z and integration time range [as in the reference dataset] explicitly: [ms, [starttime,endtime]] [default: None].
- **Auto2IntegrationVicinity** - if the location of the internal standard is extrapolated from the aligned annotation table, this option determines the vicinity [in minutes] of the predicted retention/migration time to be blindly integrated [default: [-25,25]].
- **SaveMemory** - if set to true, signal intensities are rounded to integers in internal calculations and results [default: True].

#### DAMPTwoGroups

DAMPTwoGroups[mdatas1,mdatas2,options] generates datasets representing the absolute, relative, and absolute/relative differences between the averaged datasets of mdatas1 and mdatas2 datasets. The results are returned as a list of rules: [AveragedDatasets=..., AveragedGroup1=..., AveragedGroup2=..., GroupCounts=..., GroupNames=...].

- **ThresholdForRelative** - relative difference in the relative result will be set to 0 if neither of the two corresponding signal intensities from the averaged datasets is equal to or greater than this threshold [default: 0].
- **GroupNames** - names to assign to groups [will be combined into the SampleName of the results].

Most of the options for the DAMPTwoGroups and DAMPNormalizeGroup functions are specified explicitly in the command below to allow easy editing of the options. The annotation table for the cation mode CE−MS analysis is used. This table was assembled according to the CE−TOFMS analysis of a mixture of standard compounds. Methioninesulfone is used as the internal standard.

The short name (in the annotation table) 363 is passed to the DAMPNormalizeGroup function via the InternalStandard option. The location of the peak of the internal standard will be extrapolated from the aligned annotation table. Overlaid electropherograms of the vicinities of the expected peaks of the internal standard are plotted along with indicators of the beginning and the end of blindly integrated regions for visual confirmation. To specify the location of the peak explicitly, use the notation \([m/z, [starttime,endtime]]\) instead of the short name. In this case it would be \((182,[14,14.6])\) according to the electropherogram at the end of the optional section.

The first dataset from the cth list will be used as the reference dataset (as specified by the optional name Template). The DAMPTwoGroups function returns the absolute, relative, and absolute/relative difference between the averages of the datasets of both groups. Additionally, a t-score map is generated by performing a t-test for the groups of corresponding signal intensities. In the case below, only peaks picked in the migration time range 8−23 min in the reference sample are used for the alignment. This leads to a better alignment of the peaks in this range at the expense of the alignment of the stack of peaks with migration times around 25 min.

Clear[clf];
clf = DAMPTwoGroups[ctrls, smpl, NormalizeGroupOptions -> Reference=1, AlignmentTimeRange=All, InternalStandard=363, Auto2IntegrationVicinity=→.-2,2, PeakPickingOptions = [Threshold=5000], RepresentativeWeakOptions = [PeaksPerChromatogram = 5, PeaksPerInterval = 5, Intervallize->0], FitShiftFunctionOptions = [GapPenalty = [3, 5]], PeakLayoutPlotOptions = DAMPCETOFMSPeakLayoutOptions, AnnotationTables = [DAMPLoadAnnotationTable["MatchDAMPPath\leftrightarrow\"lab\_cens\_nation.cvs\"]], OutputTimeRange = [4, 27], ExternalNormalizationCoefficients=Non, ThresholdForRelative=0, GroupNames=Automatic, AbsRelTrendFilter=2];
Picked Peaks

Representative Peaks
Step 3: Exploring the Results, Listing the Candidates

The normalized datasets may be explored on density plots (annotated). The averaged normalized datasets from the first group are shown on the plot below.

IS normalization coefficients: \{1.00000, 1.02771, 1.04268, 1.16882, 1.18705, 1.15713, 1.24596\}
DAMPDensityPlot[AveragedGroup/.salt, MaxScale -> 20000, AnnotationTables -> (AlignedAnnotationTables/.salt), Sequence @ DAMPCETOFMSDensityPlotOptions];
The $t$-score map or any of the result datasets calculated using the averages of the groups may be plotted to show differences between the groups. Additionally, the result datasets may be combined or used as filters against each other. For example, the absolute relative difference (of the averages) result may be filtered to allow only those signals for which the $t$-score is above certain threshold.

```plaintext
filtset = DAMPFilter[AbsRel /. rslt, DAMPSmooth[TScores /. rslt], 5];
DAMPDensityPlot[filtset, MaxScale -> 20000, AnnotationTables -> (AlignedAnnotationTables /. rslt), Sequence @@ DAMPCETOFMSDensityPlotOptions];
```
Overlaid electropherograms in the vicinities of the most significant differences (according to a particular result) may be plotted in descending order of significance for visual confirmation (and for the rejection of false positives). Below are the electropherograms of the top 50 candidates from the filtered absolute-relative difference result. The vertical dashed line indicates the position of the most significant difference according to the result dataset. (Further below are the top 50 candidates from the $t$-test result.)
Guanidoacetic Acid

\[5\text{-Amino-5-valeric acid}\]
Different results datasets provide different ranking. For example, differences in small peaks usually do not score high in the absolute/relative difference result. On the other hand, presence of an outlying peak (in terms of signal intensities) in one dataset causes the drop of the difference in t-score ranking. Since different results have their strengths and weaknesses, it may prove beneficial to generate the lists of candidates based on multiple results. Below are electropherograms of candidate differences ranked according to the t-test result. Apart from the different ranking, smaller peaks achieve higher ranking in the list below when compared to ranking according to the absolute/relative result. However, the difference for L-Alanine (ranked second according to the absolute/relative result) is not among the first 50 candidates from the t-test result. Also note, that the vertical dashed line indicating the most significant difference often does not correspond to the peak top for the t-test result.