Introduction

This document explains instructions on how to use the program **Elements for Metabolomics** to interrogate four different metabolomics datasets. We will highlight noteworthy features of the program as we examine each dataset.

Dataset Matrix

The datasets analyzed in this tutorial differ in important, relevant ways. The matrix below summarizes and compares each tutorial dataset.

Demo Dataset	Instrument	Study Type	Num Samples	Ionization Mode(s)	Peak Type	File Type
Demo1: OSU Standards	Sciex TripleTOF 5600	Standards	1	+	Profile	.wiff
Demo2: Debnath Standards Curve	Thermo Q Exactive	Standards	8	+	Profile	Thermo .RAW
Demo3: MTBLS87 Study	Thermo LTQ Orbitrap	Real Study	16	+ and -	Profile	.mzML
Demo4: MSE Xanthohumol in Zucker rats	Waters Xevo G2 QTof	Real Study	12	-	Centroid	Waters Raw

Rows highlighted in yellow indicate DDA datasets, rows highlighted in lime green indicate Waters MS^E datasets.

Loading the demo data

It is not necessary to load the data yourself to interrogate these datasets. Elements includes the results of these four demos packaged in the program. When you launch the program, a dialog will appear with buttons to create a new experiment, open saved experiment, or run a demo. Clicking the 'Run Demo' button will bring up a file chooser showing the four demo files. Alternatively, you can navigate to **Help -> Open Demo Files** in the main menu.

However, if you would like to load the demo data, you will need to download the demo data from our website: http://www.proteomesoftware.com/products/demo-data/ .

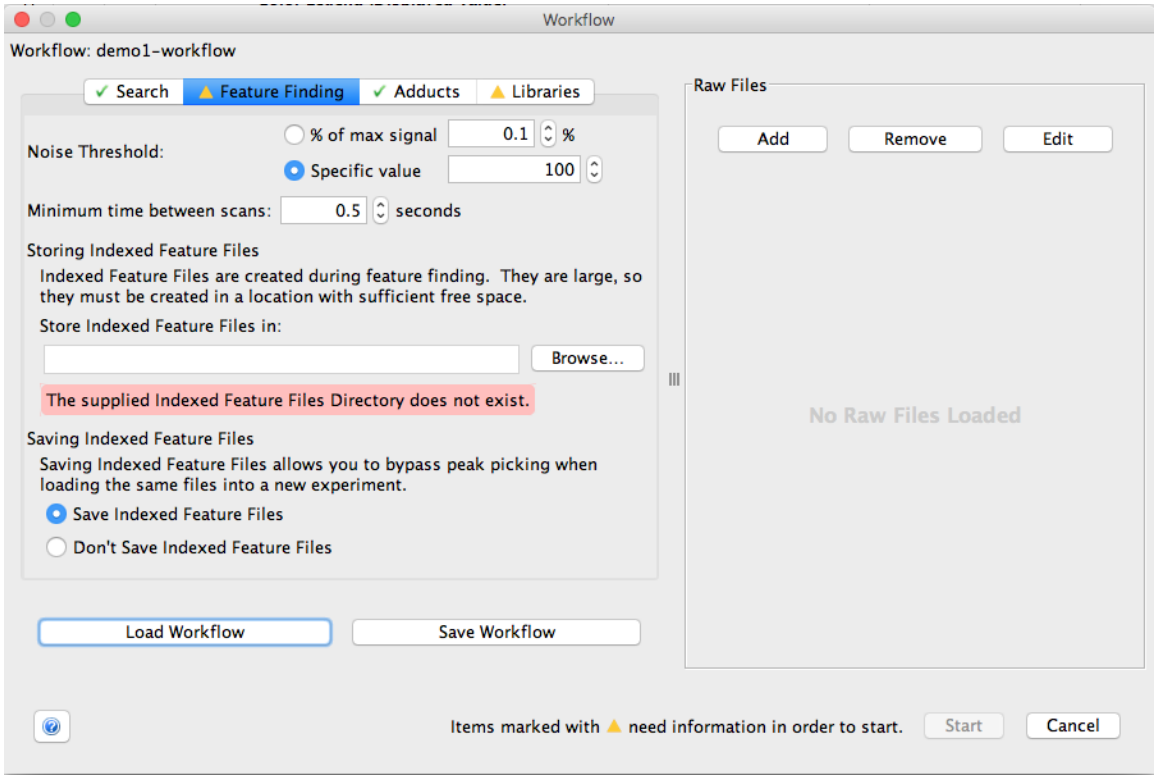
You may download either all of the demo data together, or the demo data associated with each individual file.

Inside this folder, you will find a subfolder named for each demo. Inside each of these demo subfolders, you will find a folder named 'data', and a .workflow file. The elements workflow files contain saved settings, which you can load directly into Elements.

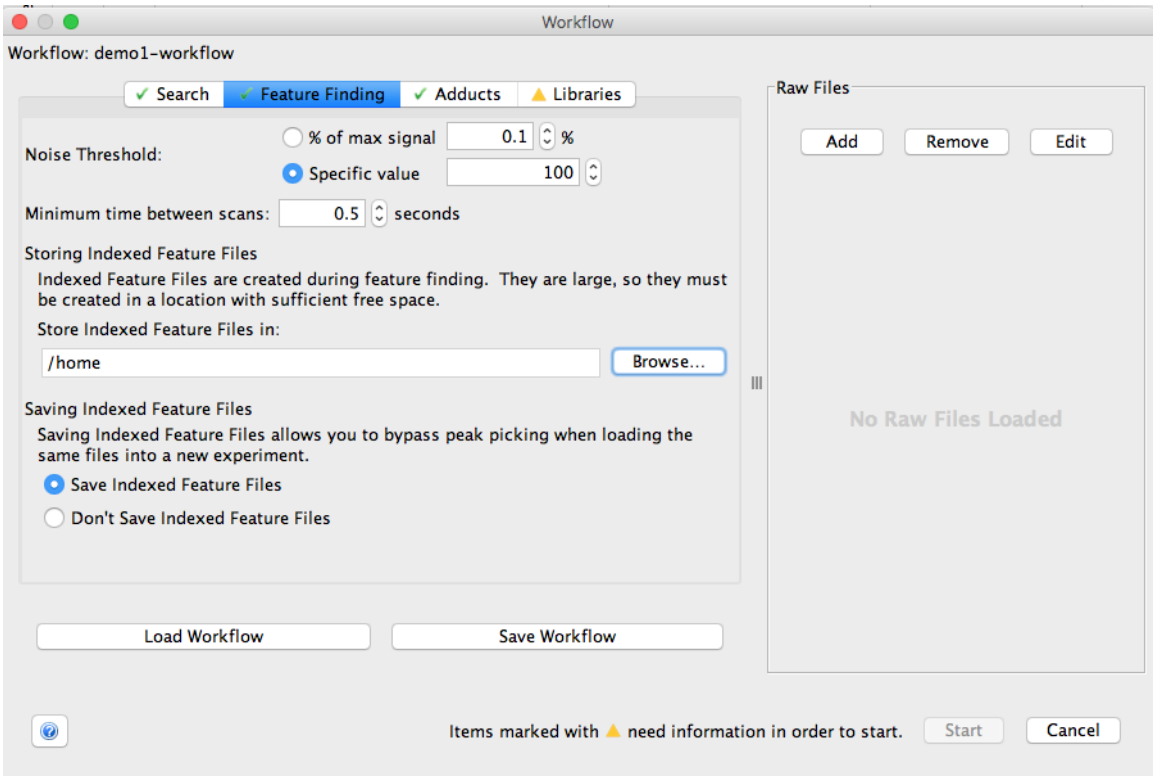
In Elements, create a new experiment, which will launch the workflow dialog. In this dialog, you will see a **Load Workflow** button in the lower left-hand part of the dialog. Click this button, and navigate to the saved workflow file corresponding to the demo which are you interested in loading.

Once you do, the dialog will change, and you will see yellow triangles on the **Feature Finding** and **Libraries** tabs, which indicate that you will need to specify additional information. This yellow triangle indicates that you will need to supply additional information.

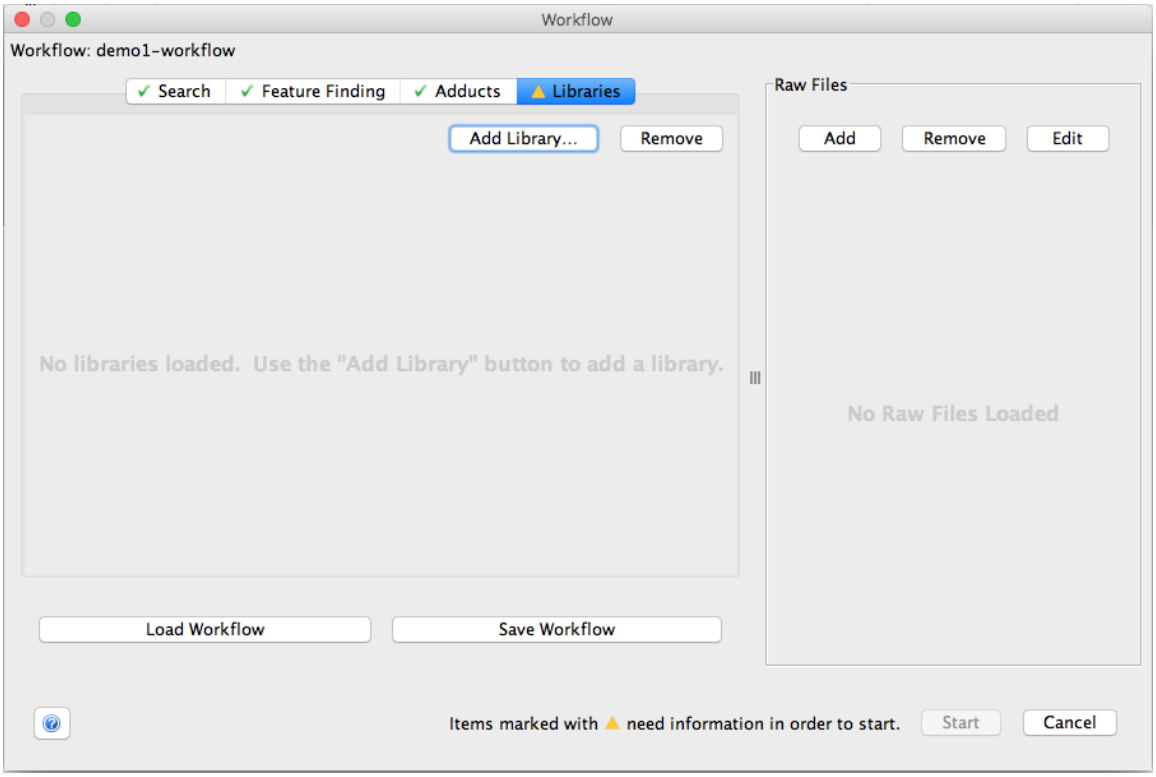
In the **Feature Finding** tab, you will need to specify the directory to which you will save your feature files:



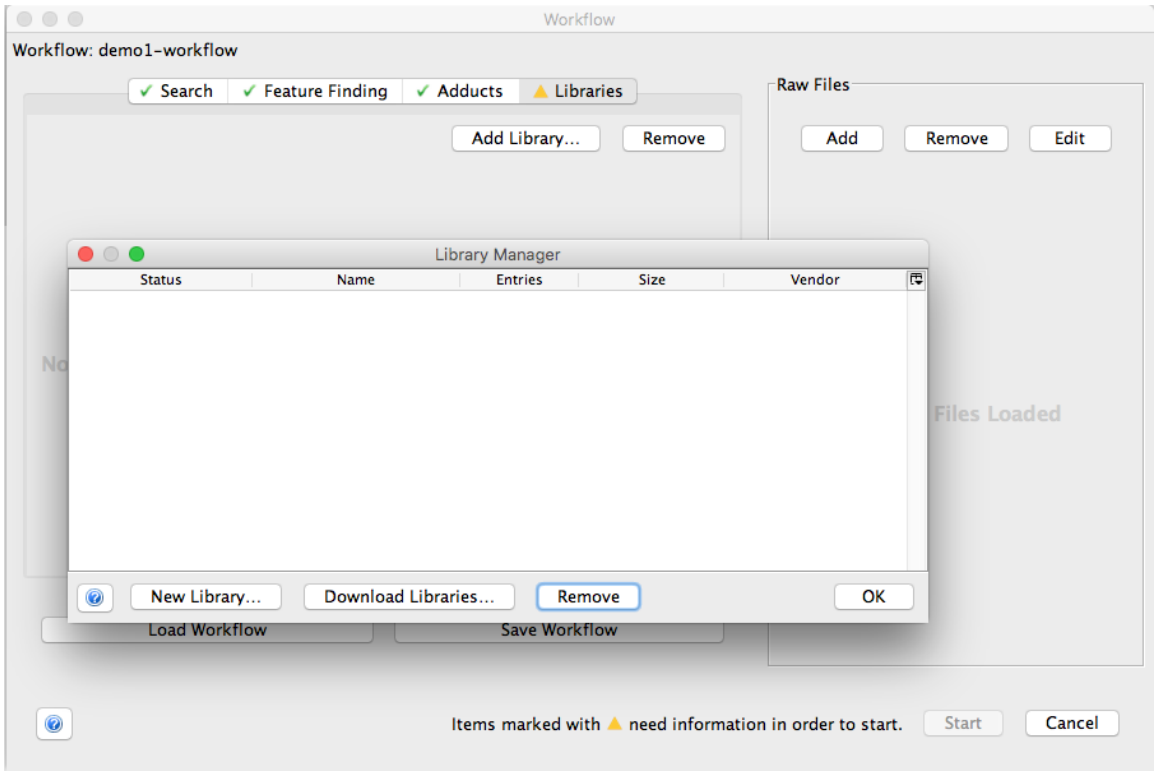
You will need to click the "Browse" button, and select a directory on your system. Please choose a directory that has both read and write access. Once you select a directory, the yellow triangle will change to a green check mark:



In the **Libraries** tab, you will see the following:



Click on the **Add Library…** button, which will launch the **Library Manager**. If this is your first time using Elements, the **Library Manager** will not contain any libraries:



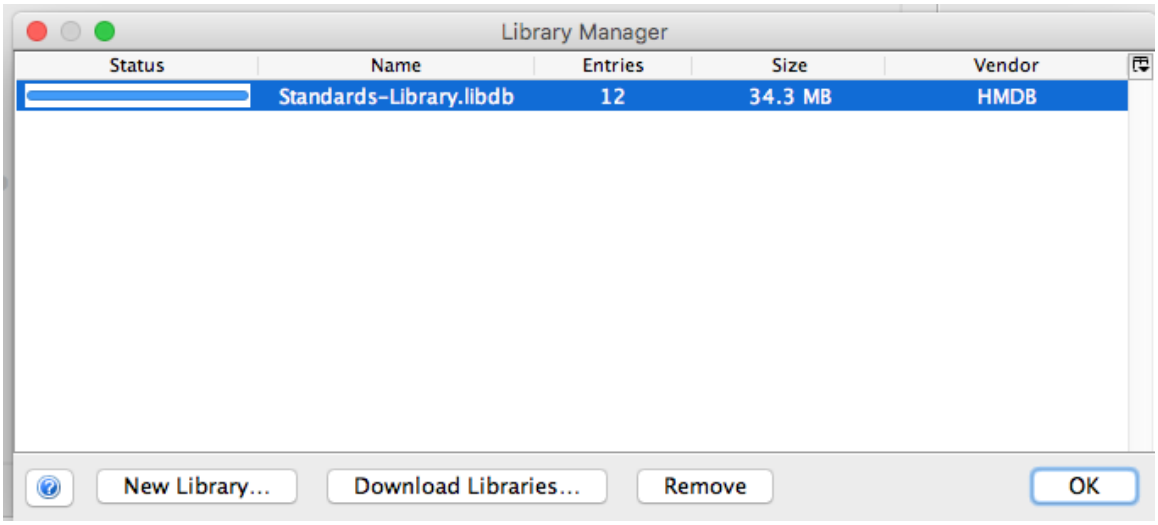
Now, you will need to add a library to Elements, by clicking the **New Library…** button. Once you do this, the library will be saved in the **Library Manager**, so you will not have to perform this step the next time you load data in Elements.

If you are loading demo 1, you will need to the "Standards-Library.libdb" file, which is included in the demo1 folder. Click the **New Library…** button, and navigate directly to the "Standards-Library.libdb" file.

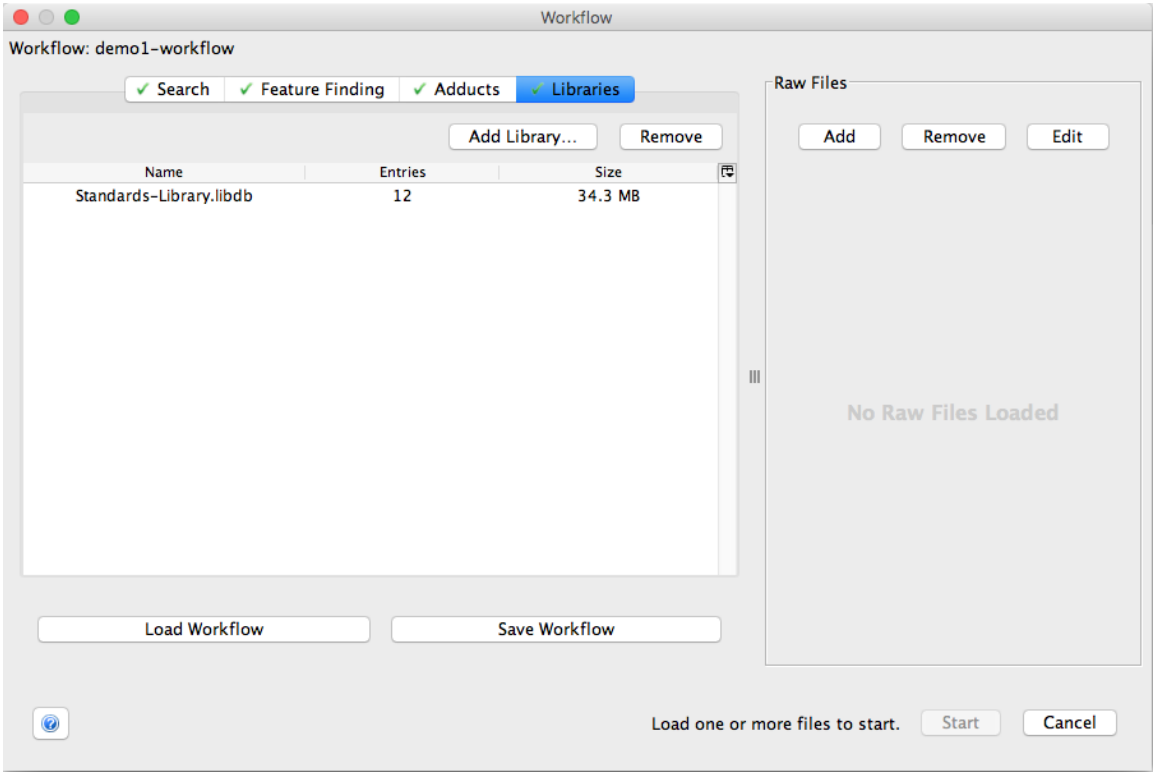
If you are loading demo 2, demo 3, or demo 4, then you will need to get the NIST spectral library.

To get the NIST spectral library, click the **Download Libraries…** button in the **Library Manager**. This will launch the proteome software website, to a page that will invite you to download the NIST spectral library. Read the instructions, and download this library. Make a note of the location on your computer where the NIST spectral library is downloaded. Finally, back in Elements, in the **Library Manager**, click the **New Library…** button, and navigate to the NIST spectral library file you have just downloaded.

Once you have added the appropriate library to the **Library Manager**, select the library, and click the OK button in the lower right-hand corner of the dialog.



Once you have performed this step, the library will appear in the loading dialog:

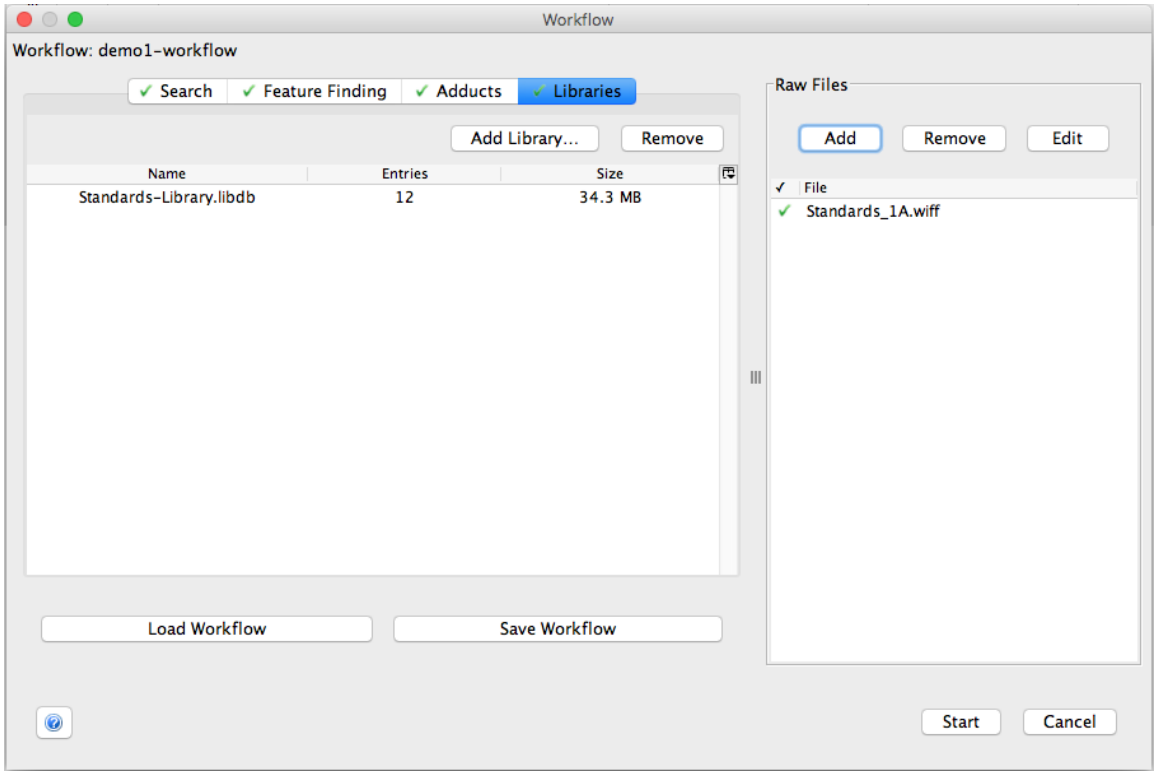


This will also cause the **Libraries** tab to be marked with a green check mark, indicating that all parameters have been appropriately specified.

At this point, you are now ready to load raw data files. Click the **Add** button in the **Raw Files** portion of the dialog on the right. This will bring up a file chooser. Navigate to the **data** folder within the demo folder of interest, and select all files.

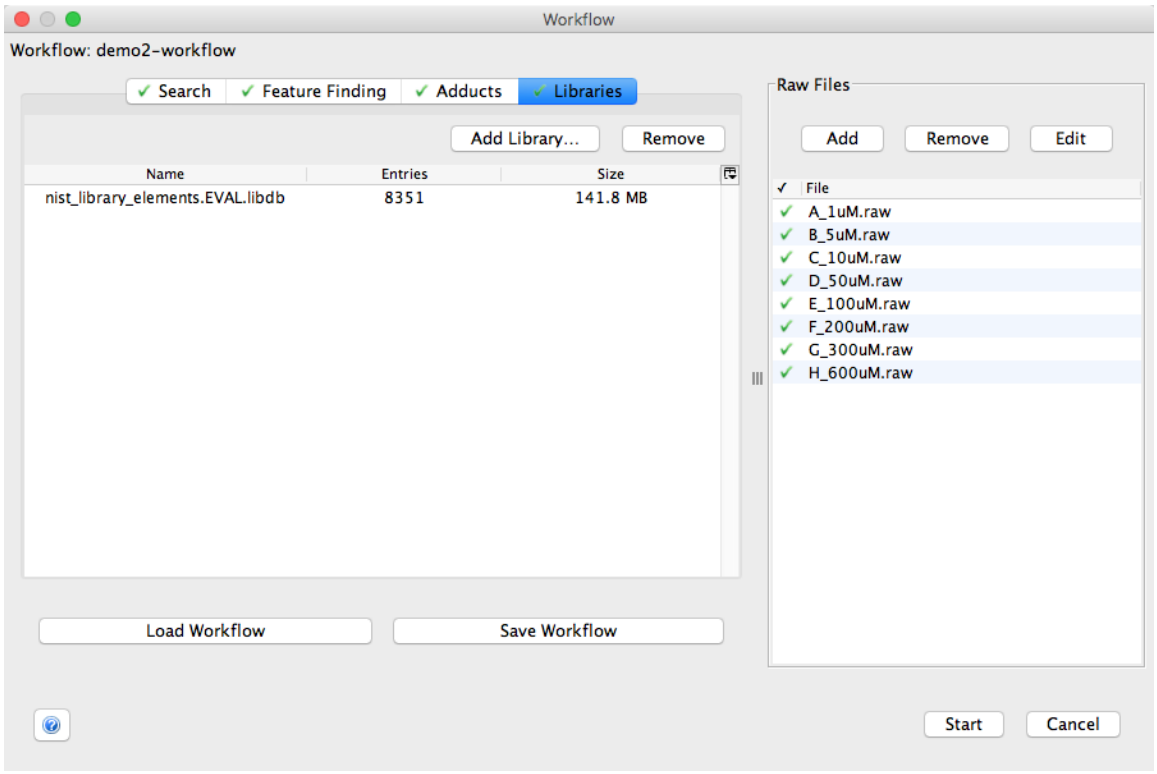
For demo 1, demo 2, and demo 3, the files will appear in the file chooser with a green check mark next to each file, as shown:

Demo 1:



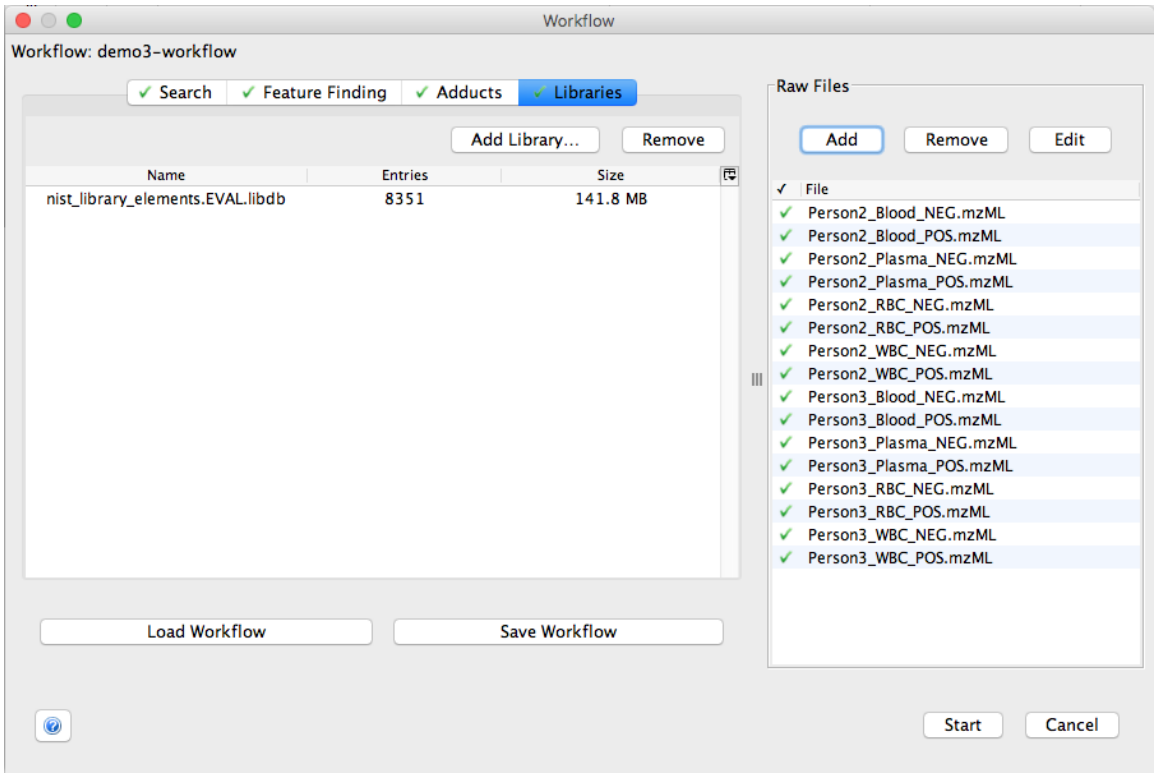
Note that demo 1 is using the Standards-Library spectral library.

Demo 2:



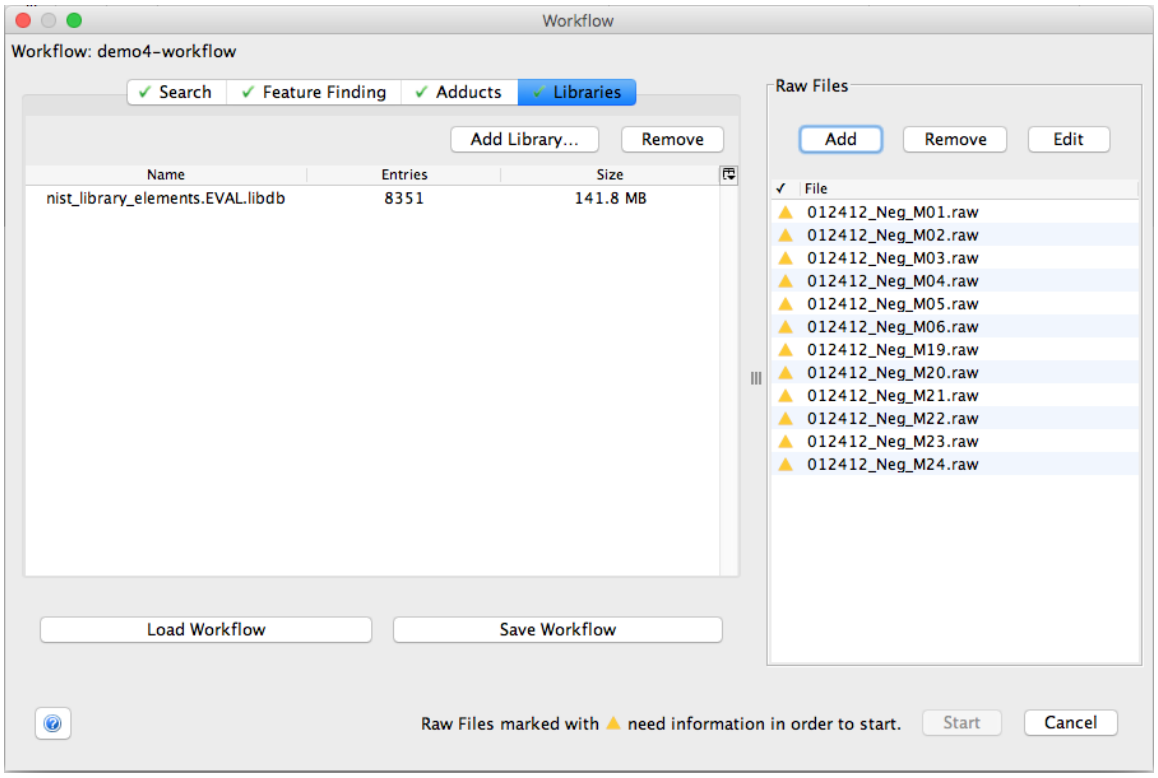
Note that the NIST spectral library is loaded. Here we show the EVAL library, which is appropriate for users evaluating Elements.

Demo 3:

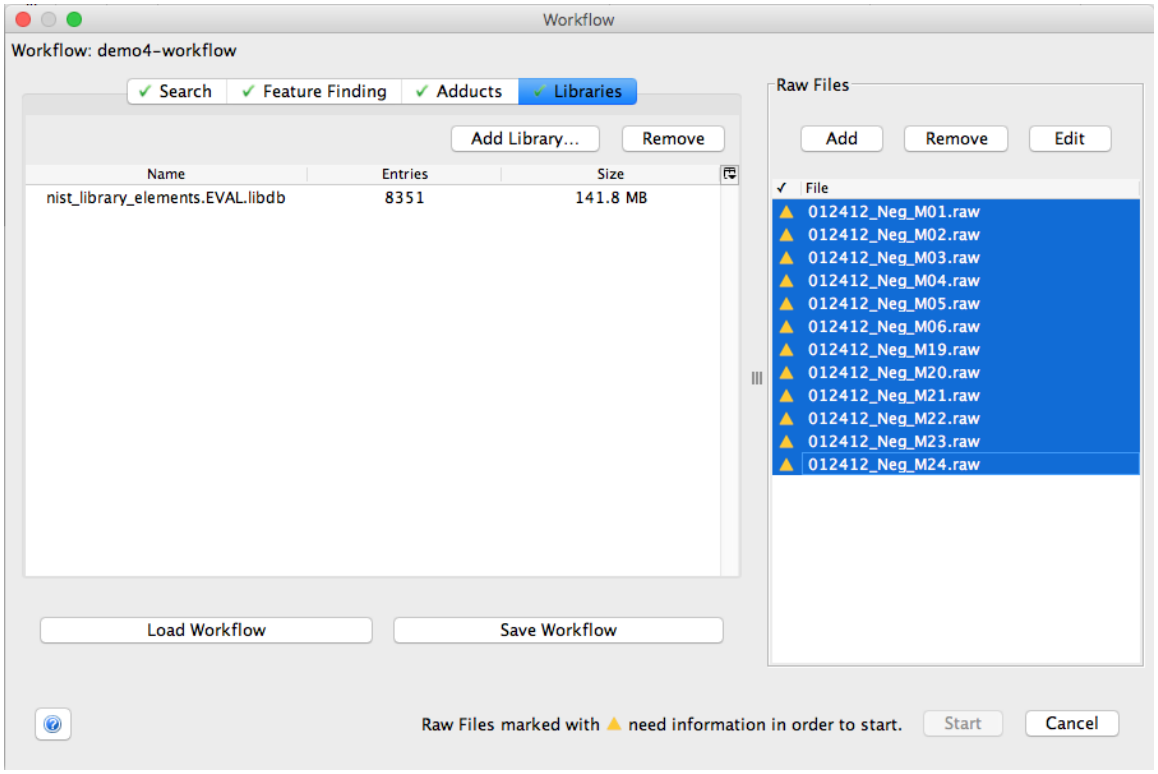


Note that the NIST spectral library is loaded. Here we show the EVAL library, which is appropriate for users evaluating Elements.

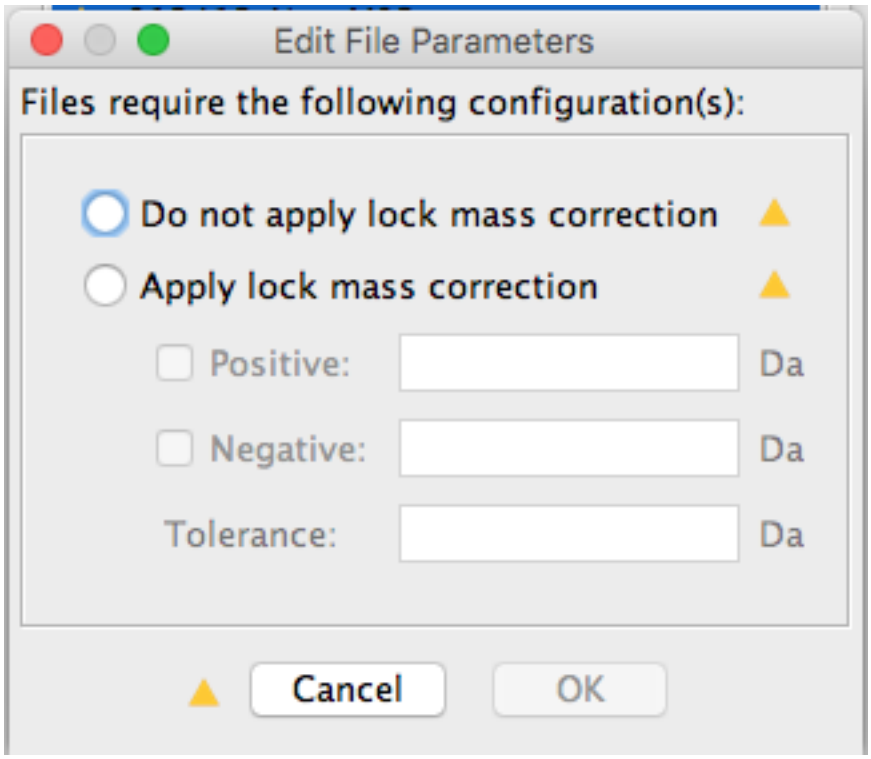
For demo 4, the files will appear with yellow triangles – indicating that more action is required:



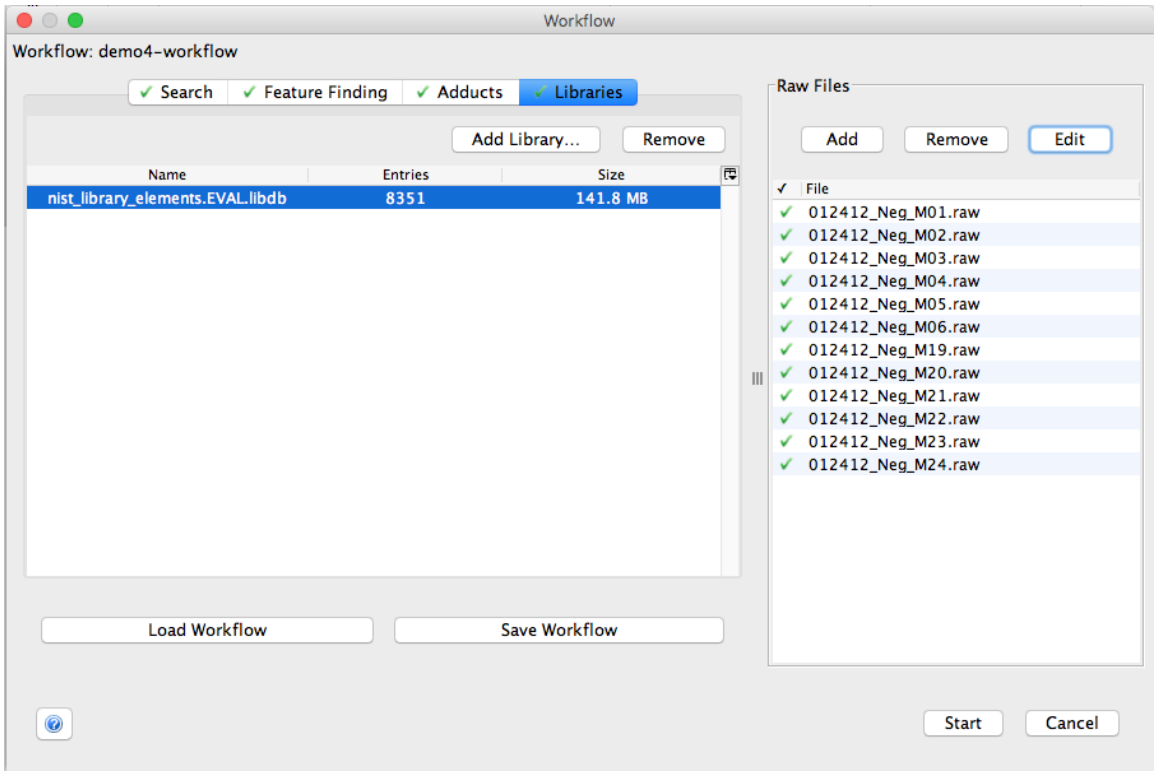
Select the first file in the **Raw Files** panel on the right, hold down the shift key, and select the last file in the list. This will select all files in the list:



While these files are selected, clicked the **Edit** button in the **Raw Files** panel. The following dialog will appear:



Select the **Do not apply lock mass correction** radio button at the top of the dialog, and click the OK button. The loading dialog will reappear, with all files ready for loading:



At this point, regardless of the demo you are working on, you should be able to now click the **Start** button, which will begin the analysis. At this point, please wait for Elements to load the data. While you wait, a loading dialog will appear, displaying the progress, as well as messages describing which stage of the analysis Elements is performing.

Post-processing adjustments

If you have loaded the data from raw files, you will need to make some minor adjustments for the resulting loaded file to match the demos packaged with the program. The adjustments are explained as follows, by demo:

Demo1-OSU-Standards Adjustments

By default, Elements thresholds metabolite identifications with an ID Score cutoff of 0.7. However, there are sometimes real identifications that exist below this threshold. This threshold can be changed in the upper right-hand corner of the main window. Adjust this value from 0.7 to 0.55.

Demo2-Debnath-Standards-Curve Adjustments

No adjustments are required for this demo.

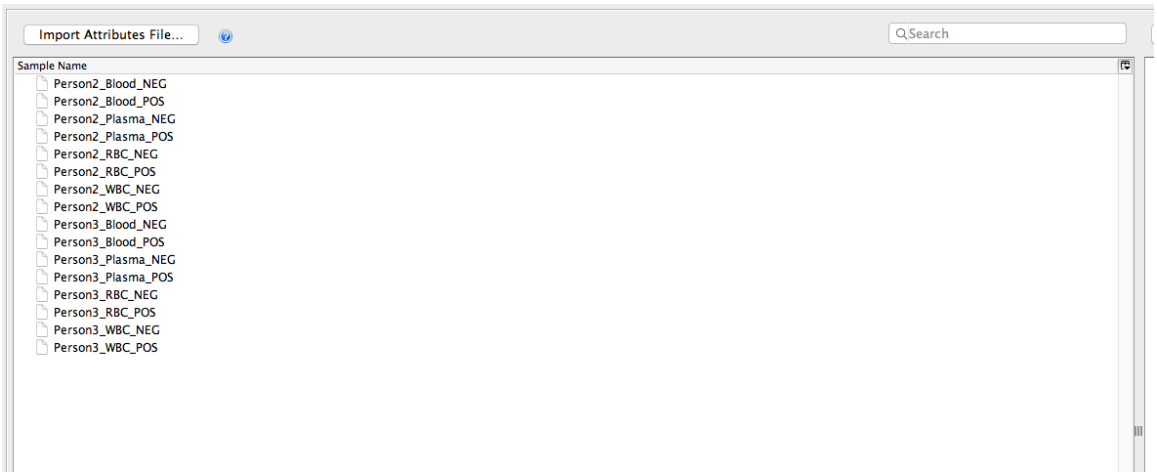
Demo3-MTBLS87 Adjustments

Adjust the ID Score to 0.9.

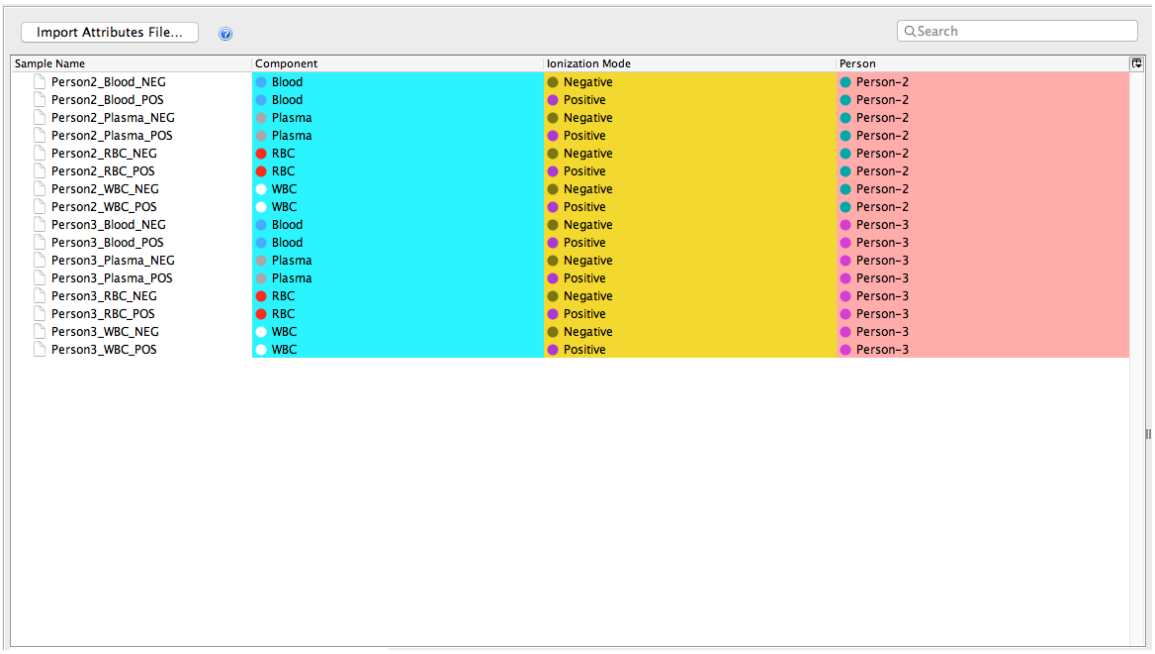
At this point, we are going to provide information about individual samples, and organize them into a hierarchy, based on the categories we specify. Provides a framework for flexible categorical organization, which can useful both in organizing sample information, and in searching for statistically overrepresented or underrepresented categories through interrogation of quantitative tests.

In this experiment, we have 3 layers of information: samples run in the mass spectrometer in either positive or negative ionization mode, samples deriving from one of four different blood components, and samples derived from two different people. We will show how to encode this information using Elements Summarization system.

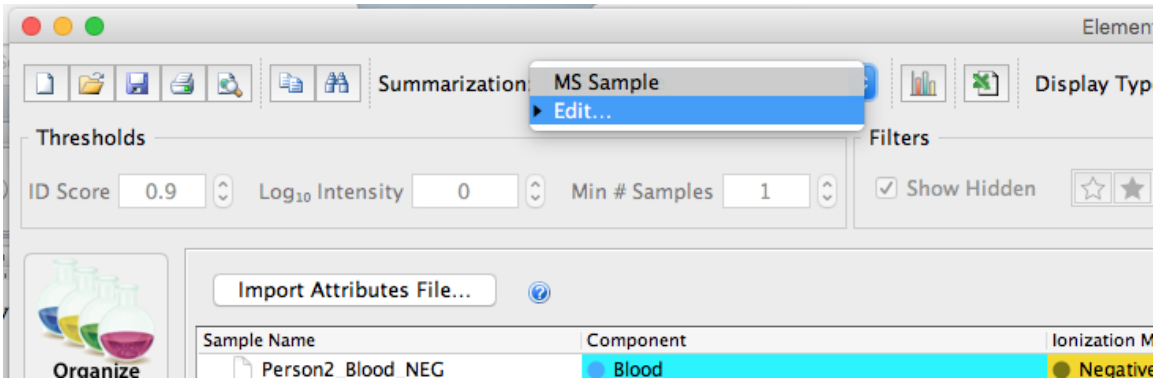
Switch to the **Organize View.** Initially, the files should look something like this:



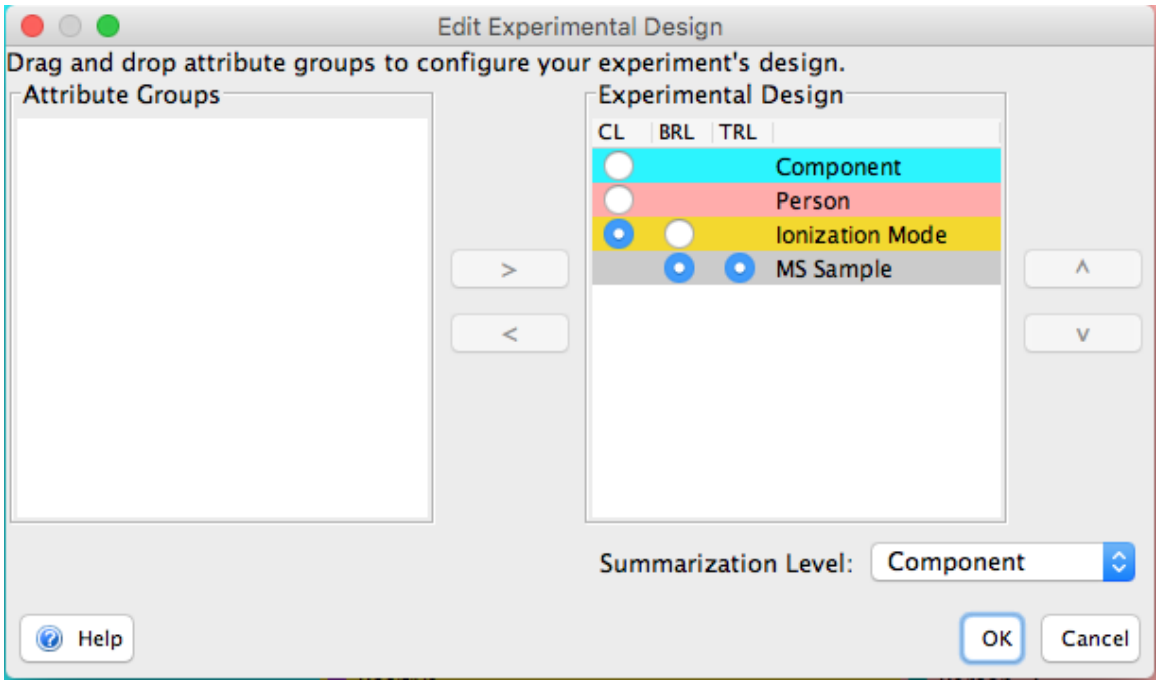
Now, click the **Import Attributes File…** button at the top of the view, and navigate to the attributes.csv file located in the demo 3 folder. Now, attributes will be associated with these samples:



Finally, create a hierarchy for these samples by navigating to the **Summarization**: drop-down menu at the very top of the main frame, and selecting the **Edit** option:



Within the dialog that appears, drag categories over to the right, and set up the following organization:



Click the OK button to return to the Organize View. Now, the samples will be organized like this:

Return to the samples view.

Now, we will star metabolites. "Starring" a metabolite tags the metabolite, which makes it sortable, filterable, and organizable with other starred metabolites of the same star type. In the Samples view, the 3rd column ,"Star" contains star icons. Find

the metabolite of interest, and single-click on the empty star next to the metabolite name. This will star the metabolite of interest.

To apply an orange star, click the star icon once.

Apply an orange star to the following metabolites:
Adenosine 5'-triphosphate_RT1
Adenosine 5'-diphosphate_RT1
Adenosine 5'-monophosphate_RT2

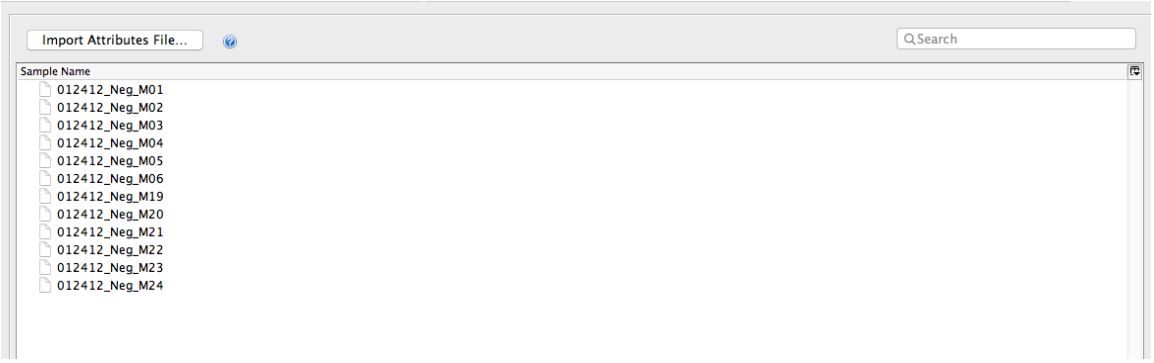
To apply a blue star, click the star icon twice.

Apply a blue star to the following metabolites:
Glycoursodeoxycholic acid
L-Cystine

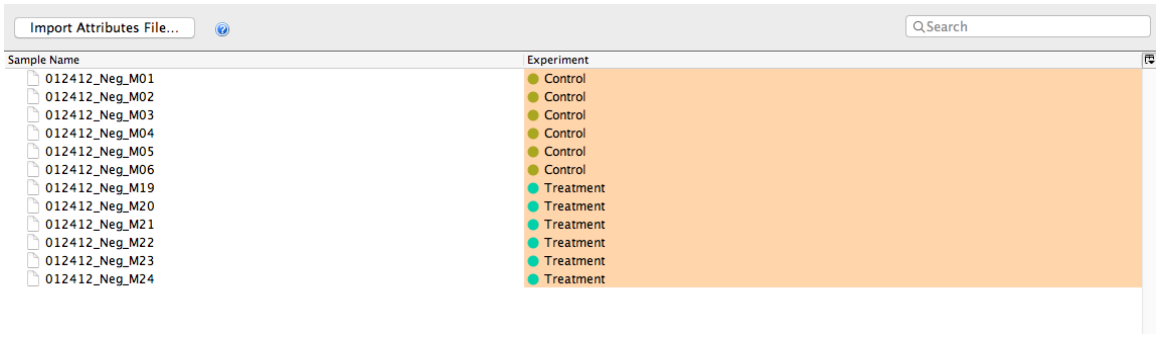
Demo4-MSE-Xanthohumol-in-Zucker-rats Adjustments

We will apply summarization information to this experiment just as we did for Demo 3.

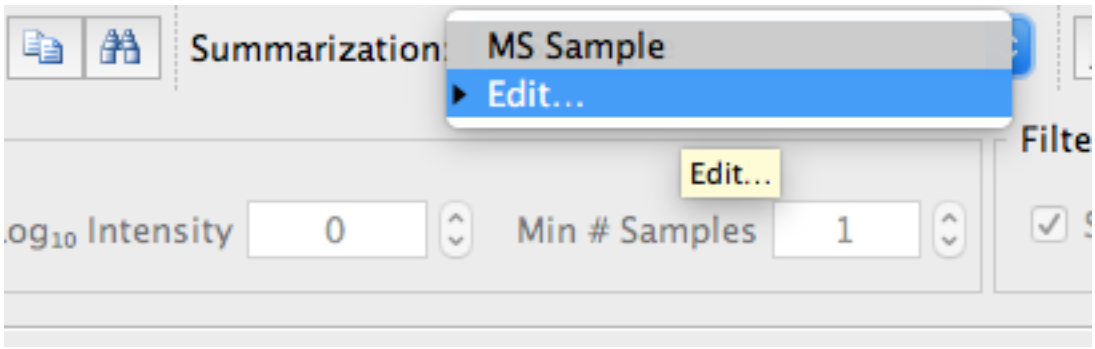
Initially, the samples should look like this:



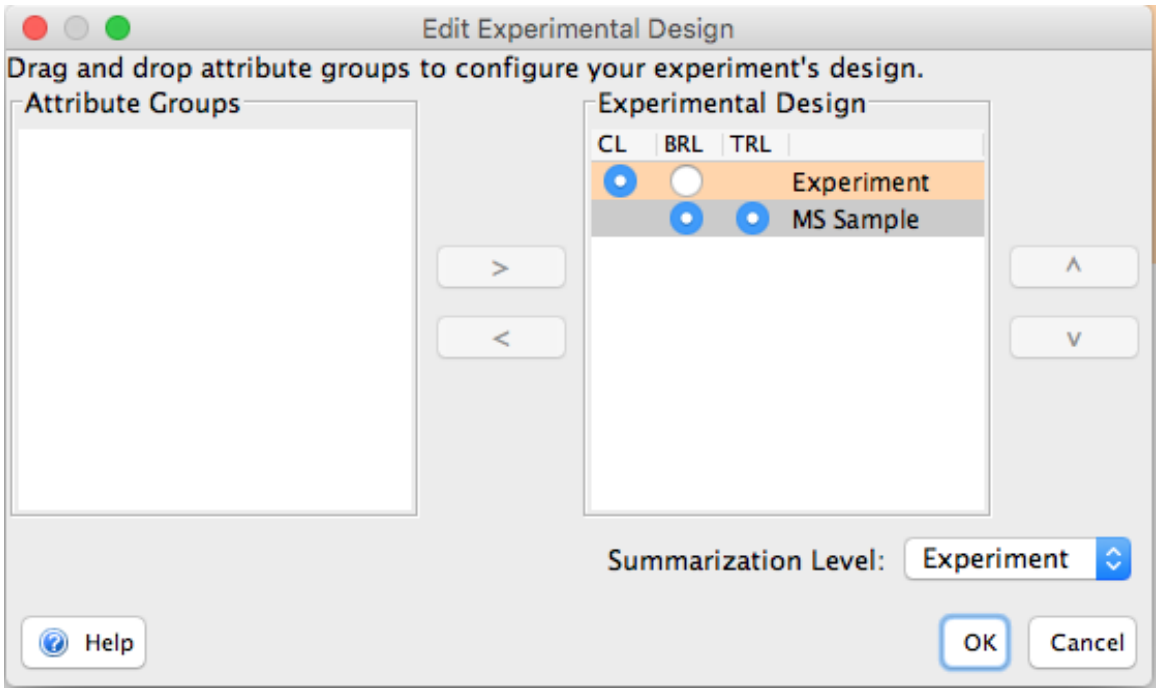
Now, click the **Import Attributes File...** button at the top of the view, and navigate to the attributes.csv file located in the demo 3 folder. Now, attributes will be associated with these samples:



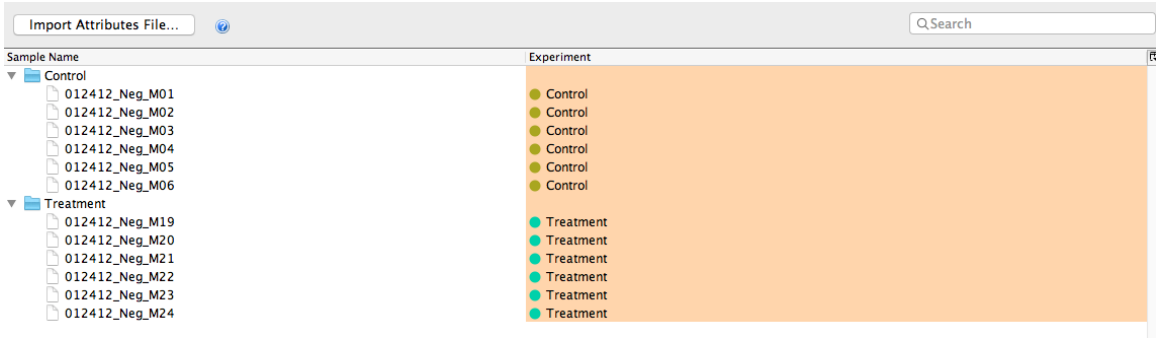
We will set up a very basic summarization of two categories, control vs. treatment. Navigate to the Edit Summarization drop-down menu in the main frame, and click "Edit Summarization":



This will launch the summarization dialog. Set up the following summarization:



Now click OK, which will return to the **Organize View**. The samples will appear summarized as follows:



Descriptions and Noteworthy Features

What follows is a description of each demo dataset, some overall remarks on the results, and a few Elements features highlighted using demo dataset results as examples.

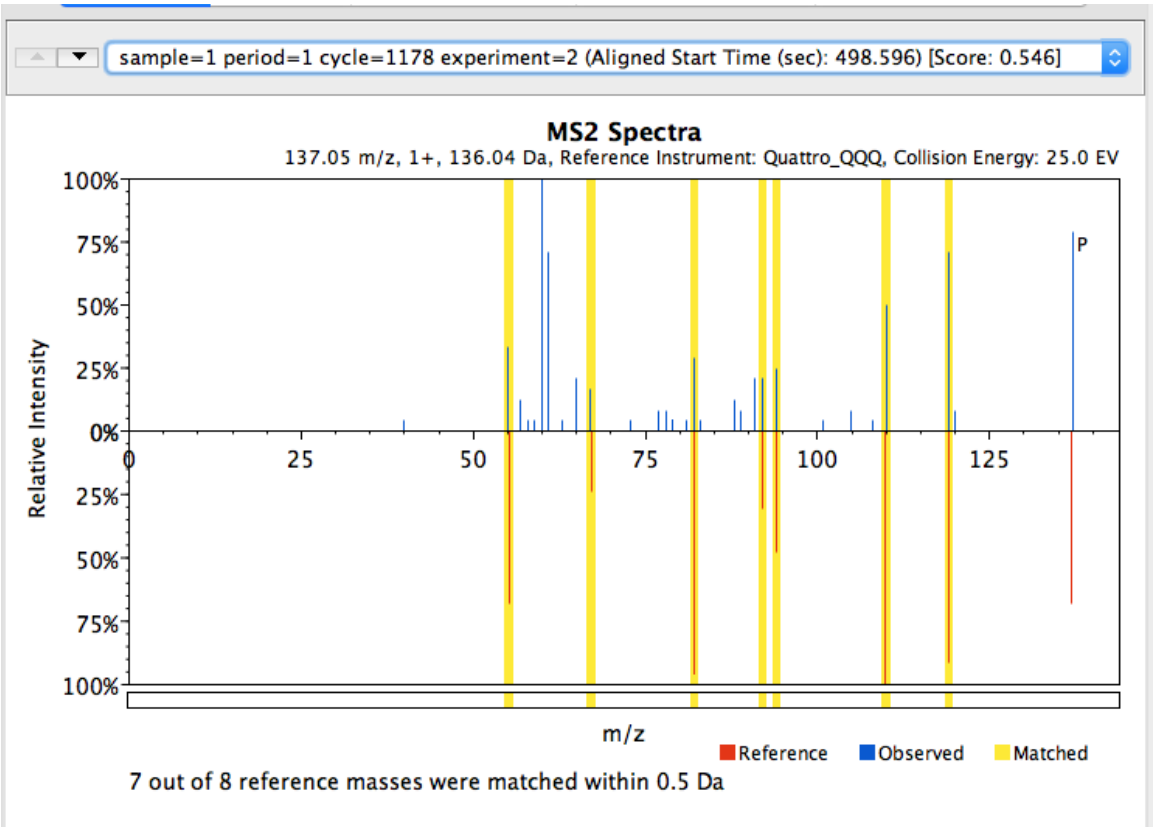
Demo1-OSU-Standards

12 known spiked-in standards are recovered, with high scores. These standards represent a wide variety of different classes of chemical compounds found naturally in the human body.

Note that a custom library was created for this analysis, and searched against the metabolites. There is a detailed discussion of how to create such custom libraries in the User's Guide.

Among the standards recovered, the known standard metabolite hypoxanthine has a lower quality score than the others. To see why this is so, select hypoxanthine_RT2 in the samples view, and click the **Metabolites** button on the left side of the frame to navigate to the **Metabolite View**.

In the **Metabolite View**, there is a head-to-tail plot showing the MS2s of the standard library metabolite, and the observed experimental metabolite. The plot shows many matches (highlighted in yellow), and designates the precursor with a "P" to the right of the spectrum:



Note that 7 out of 8 reference peaks match, yet the MS2 score is only 0.546. The low score is due to the presence of a lot of other peaks in the Observed spectrum which are not in the Reference spectrum, which suggests in this case that this hypoxanthine spectrum may be chimeric (contain fragments from more than one precursor).

In general, the **Metabolite View** facilitates in-depth exploration of individual metabolite identifications. While the ID Score is a useful guide, it is imperfect; this case highlights the utility of manually examining metabolite identifications.

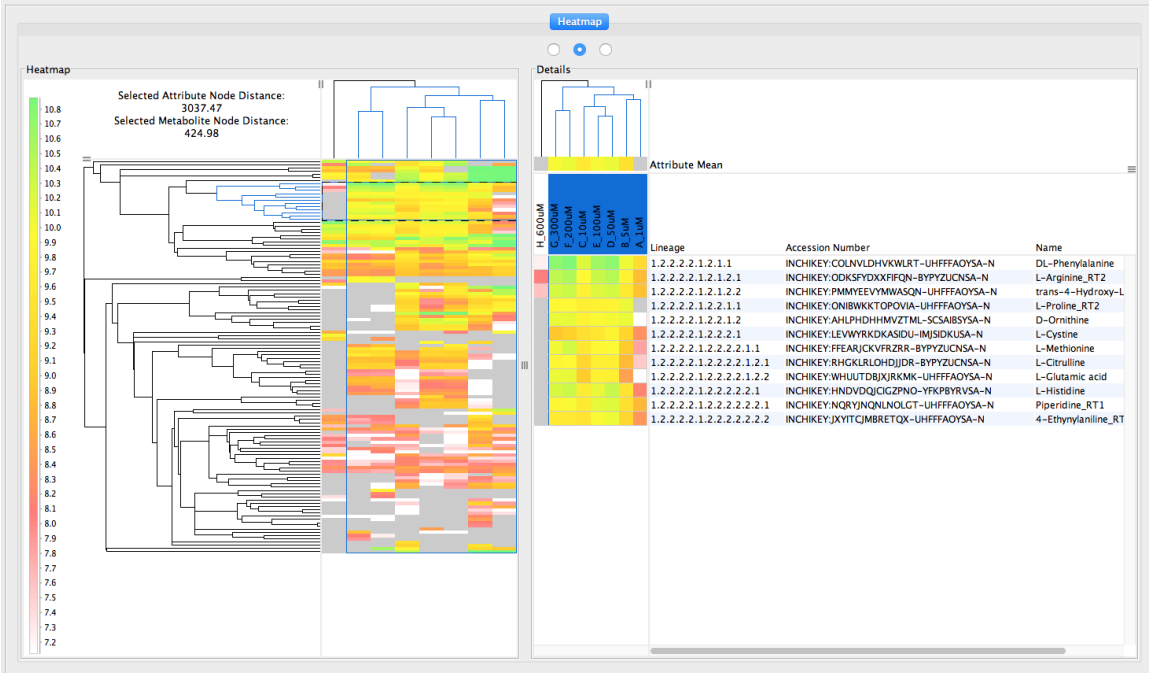
Demo2-Debnath-Standards-Curve

In this study, amino acid standards were spiked into solution with known concentrations (1 uM, 5 uM, 10 uM, 50uM, 100 uM, 200 uM, 300 uM, 600 uM). Amino acids were excluded from a given concentration if the amino acid is never observed at that concentration in
the human body.

We would expect the precursor intensity of these metabolites to increase as the concentration of injected standard increases. Looking at the Samples View, we first of all see a large number of our detected amino acid standards, and upon looking more closely, we see that the relative intensity of these standards does increase with

intensity – this is observed in the top-scoring metabolites, L-Lysine and DL-phenylalanine, and many others.

Switching to the Visualize view, there is an interactive heat map, where we see that many amino acids naturally group together based on their cross-sample intensity profile:



Demo3-MTBLS87

This study was taken from the public MetaboLights data repository, with metabolights number 87 (MTBLS87).

It is associated with the study:

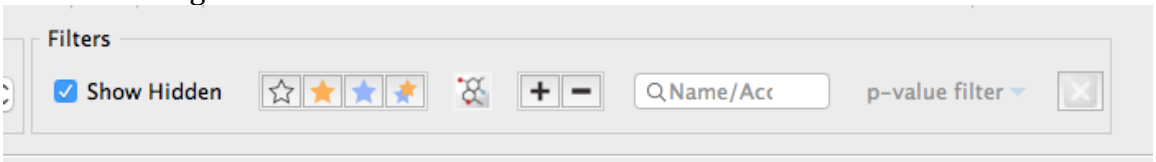
Chaleckis, Romanas, et al. "Unexpected similarities between the Schizosaccharomyces and human blood metabolomes, and novel human metabolites." *Molecular BioSystems* 10.10 (2014): 2538-2551.

In the samples we have analyzed, there were four blood components (blood plasma, red blood cells and white blood cells), taken from two different subjects, and run through both positive and negative ionization mode.

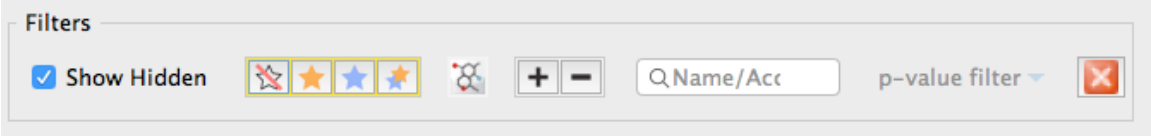
Elements finds a huge number of high-confidence metabolite identifications. Among these high-confidence metabolite identifications, Elements enables examination of the relative quantitation across categories. In the demo, information is organized to emphasize different blood components.

We have starred metabolites of potential interest. To hide all other metabolites from view except for those we have starred, in the "Filters" box at the top of the screen, click the empty star button:

Before clicking:



After clicking:



Two metabolites are marked with a blue star. These correspond to a metabolite found in every blood component except for one (the metabolite glycoursodeoxycholic acid, which is absent from the white blood cells), and the second to a metabolite found only in one blood component (the metabolite L-Cystine, found only in the plasma).

Three metabolites are marked with an orange star – adenosine tri, di, and mono-phosphate (ATP, ADP, and AMP). These metabolites are present in all 4 blood components in high concentration, however always significantly reduced in the Plasma as compared to the other components.

Demo4-MSE-Xanthohumol-in-Zucker-rats

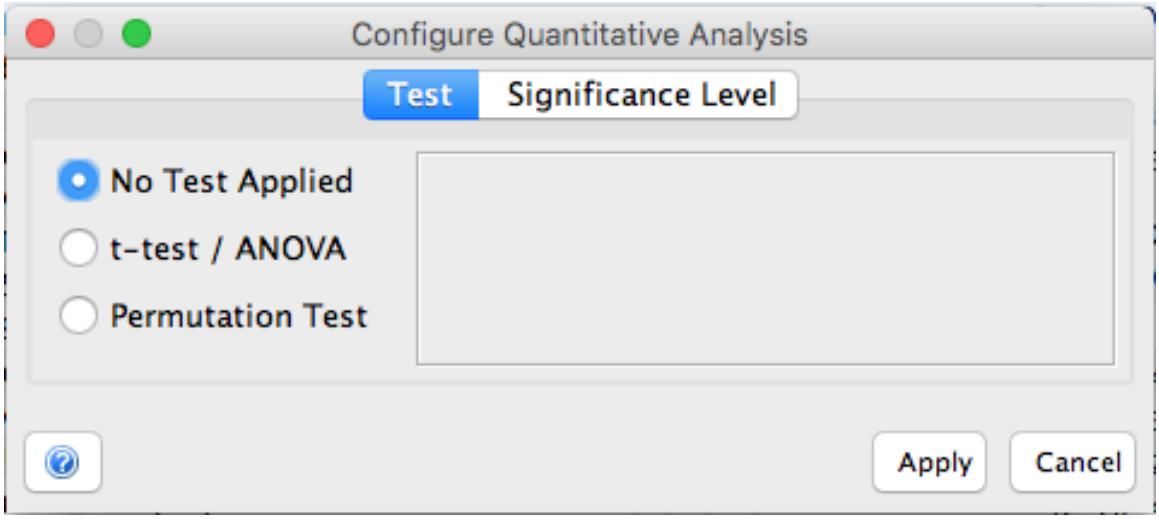
This dataset is associated with the recent study,

Wickramasekara, Samanthi I., et al. "Electrospray quadrupole travelling wave ion mobility time-of-flight mass spectrometry for the detection of plasma metabolome changes caused by xanthohumol in obese Zucker (fa/fa) rats."*Metabolites* 3.3 (2013): 701-717.

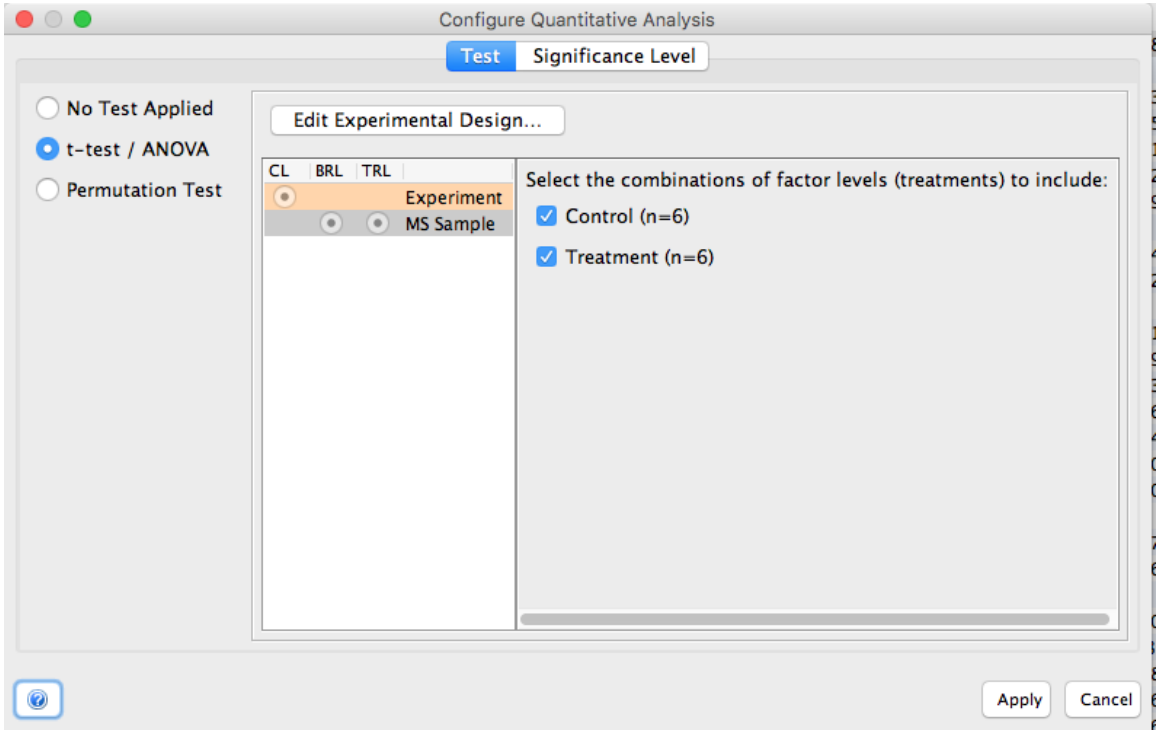
In that study, they emphasized the difference between obese Zucker rats fed a hop-derived dietary supplement called Xanthohumol, and rats that were not fed the supplement. Here, we have repeated a portion of the original analysis, focusing on male rat samples run in negative mode, comparing a high dose of treatment to control.

We can determine directly which metabolites differ significantly between categories by using Elements built-in statistical tests.

In the Experiment menu, navigate to **Experiment -> Quantitative Analysis**. The following dialog will appear:

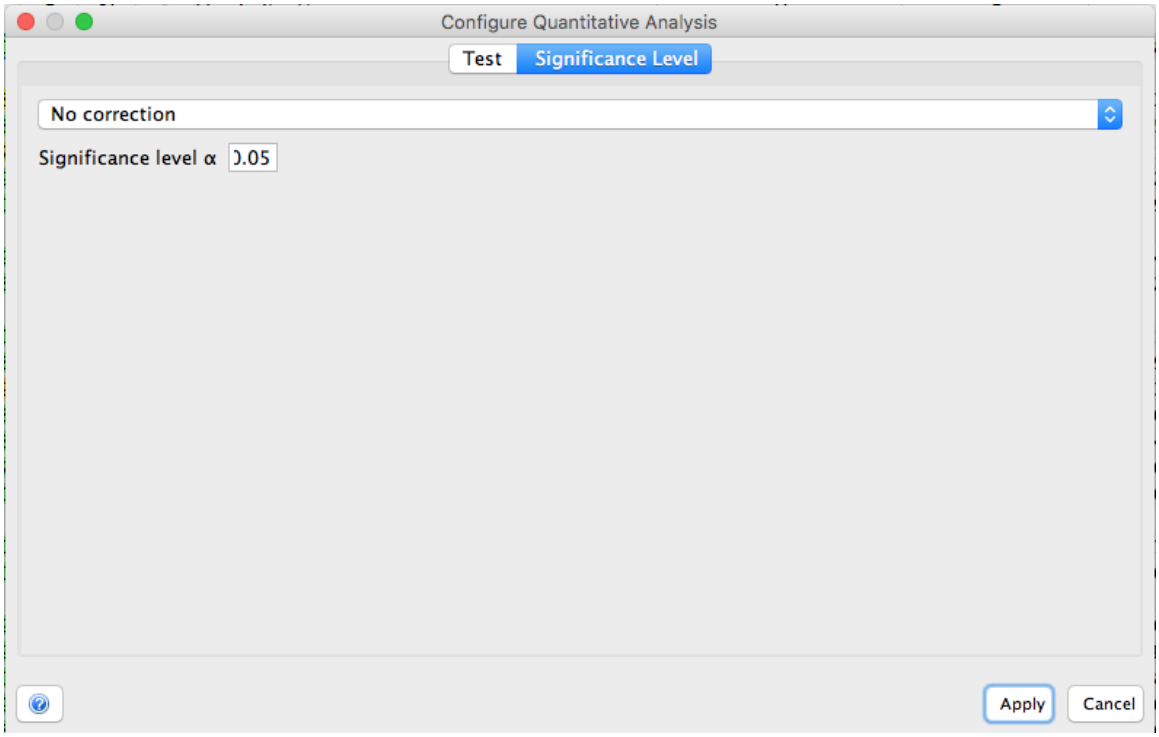


Change the radio button to 't-test / ANOVA'.



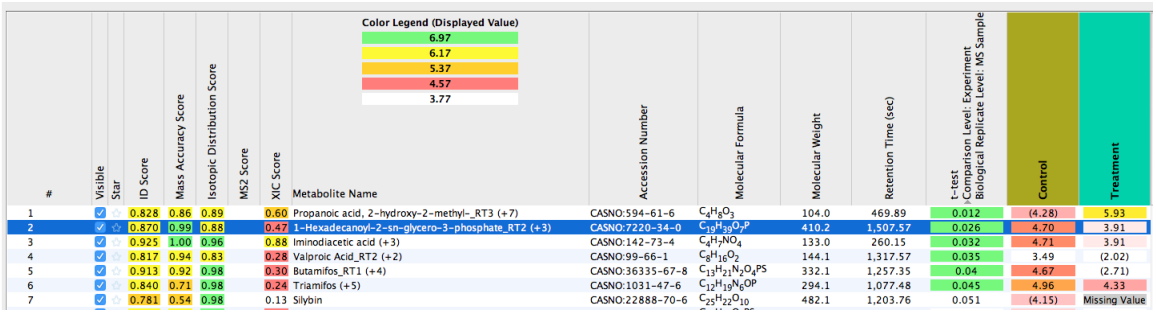
In the **Significance Level** tab, select **No correction** from the drop-down menu.

Then, click the 'Apply' button.



A new column will appear on the samples table. Click on the t-test column to sort the metabolite identifications by increasing significance. This may take a moment.

Once the file has finished, select the second metabolite in the list, 2-Hexadecanyl-2-sn-glycero-3-phosphate_RT2.



#	Visible	Star	ID Score	Mass Accuracy Score	Isotopic Distribution Score	MS2 Score	XIC Score	Metabolite Name	Accession Number	Molecular Formula	Molecular Weight	Retention Time (sec)	t-test Comparison Level: Experiment Biological Replicate Level: MS Sample	Control	Treatment
1	☑	☆	0.828	0.86	0.89		0.60	Propanoic acid, 2-hydroxy-2-methyl-_RT3 (+7)	CASNO:594-61-6	$C_4H_8O_3$	104.0	469.89	0.012	(4.28)	5.93
2	☑	☆	0.870	0.99	0.88		0.47	1-Hexadecanoyl-2-sn-glycero-3-phosphate_RT2 (+3)	CASNO:7220-34-0	$C_{19}H_{39}O_7P$	410.2	1,507.57	0.026	4.70	3.91
3	☑	☆	0.925	1.00	0.96		0.88	Iminodiacetic acid (+3)	CASNO:142-73-4	$C_4H_7NO_4$	133.0	260.15	0.032	4.71	3.91
4	☑	☆	0.817	0.94	0.83		0.28	Valproic Acid_RT2 (+2)	CASNO:99-66-1	$C_8H_{16}O_2$	144.1	1,317.57	0.035	3.49	(2.02)
5	☑	☆	0.913	0.92	0.98		0.30	Butamifos_RT1 (+4)	CASNO:36335-67-8	$C_{13}H_{21}N_2O_4PS$	332.1	1,257.35	0.04	4.67	(2.71)
6	☑	☆	0.840	0.71	0.98		0.24	Triamifos (+5)	CASNO:1031-47-6	$C_{12}H_{19}N_6OP$	294.1	1,077.48	0.045	4.96	4.33
7	☑	☆	0.781	0.54	0.98		0.13	Silybin	CASNO:22888-70-6	$C_{25}H_{22}O_{10}$	482.1	1,203.76	0.051	(4.15)	Missing Value

Now, double-click on this metabolite to move to the Metabolites View.

In the metabolites view, find the **Quantitative Charts** tab in the lower portion of the window. This chart visually confirms what the statistical test determined: this metabolite concentration differs significantly in precursor intensity between the two categories.

MS2 Spectra | References | Quantitative Charts | Isotopic Distribution | Feature 3D Plot Controls