VIPER Tutorial

To change look of presentation, run either macro Make_Printable2 or macro Make_OnScreen2

For most useful form when printing, print as “Note Pages”

(If macros are not available, go to “Tools”, “Macros”, “Security”, “Security Levels” tab and set security to Medium. Then close and reopen this file.)

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Database Analysis Loading – Page 1

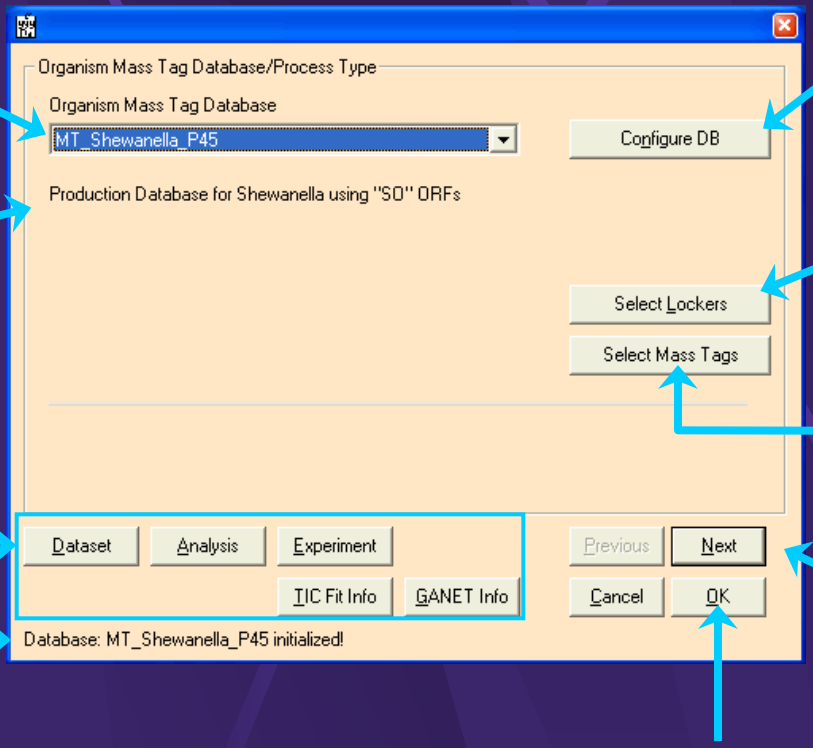
"File" menu, "New Analysis (Choose from DMS)"

Select database to load analysis from (and export results to).

Shows info about current database.

Buttons that have no function until a .PEK file is selected.

Database status.



Allows user to change exact connection details.

Brings up lockers selection form.

Brings up mass tag selection form (see next slide)

Advances analysis loading to second page.

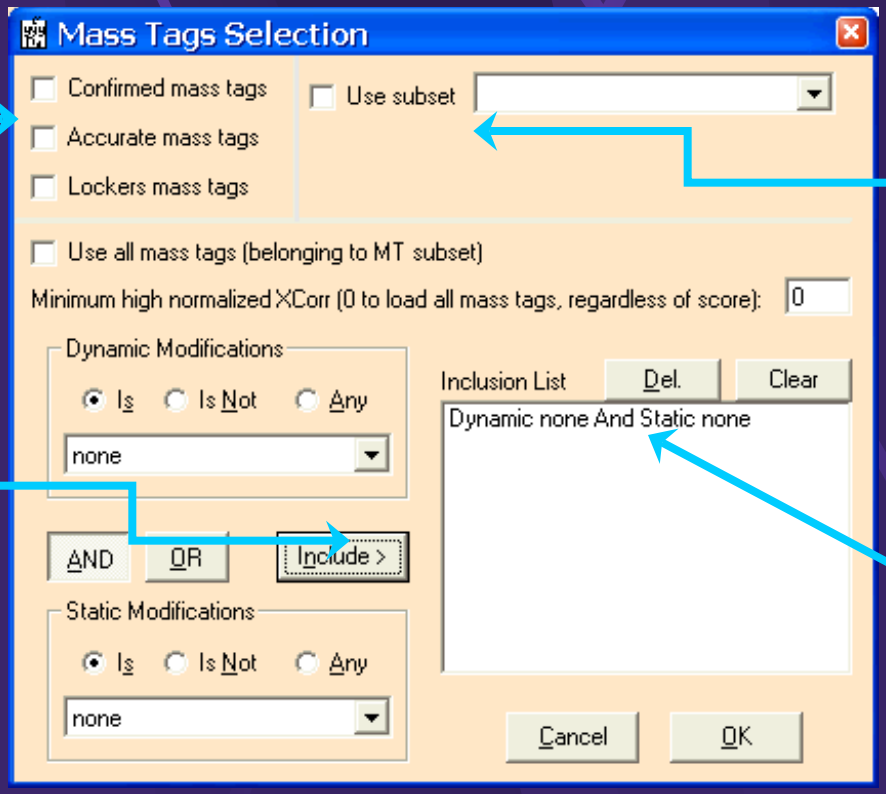
Completes loading (since no .PEK file has been selected yet, selecting this is the same as choosing cancel.)

Database Analysis Loading – Page 1

"File" menu, "New Analysis (Choose from DMS)"

To confine mass tags to only certain types, check one or more of these boxes.

Select mass tag characteristics and push "Include" to include it as a parameter.



Check the "Use subset" box and select a subset of mass tags to use. (Choose All to use from all mass tags in database.)

Inclusion list shows what parameters are used for mass tags loading. (Using more than 1 can sometimes cause odd results.)

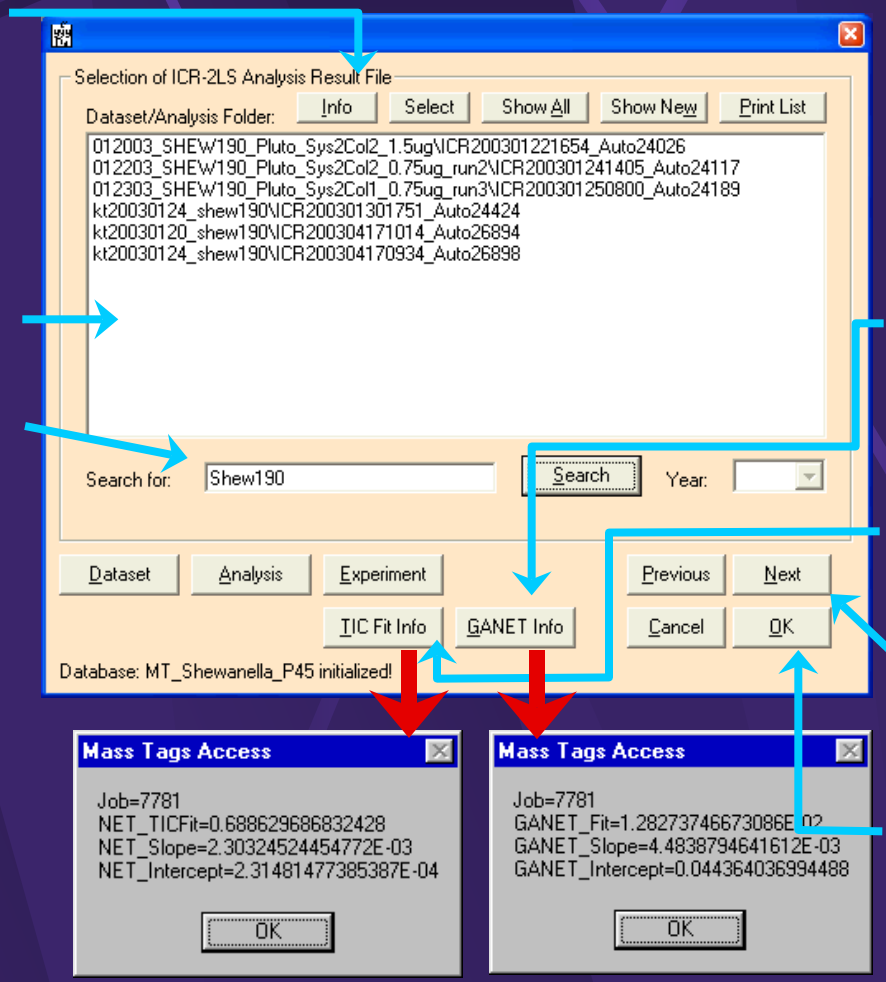
Database Analysis Loading – Page 2

"File" menu, "New Analysis (Choose from DMS)"

Displays info about selected analysis

Displays list of all analyses in database.

Search for analyses containing the search text.



Displays GANET information currently in database.

Displays TIC NET information currently in database.

Advances analysis loading to third page.

Completes loading (will not load unless file is selected on page 3).

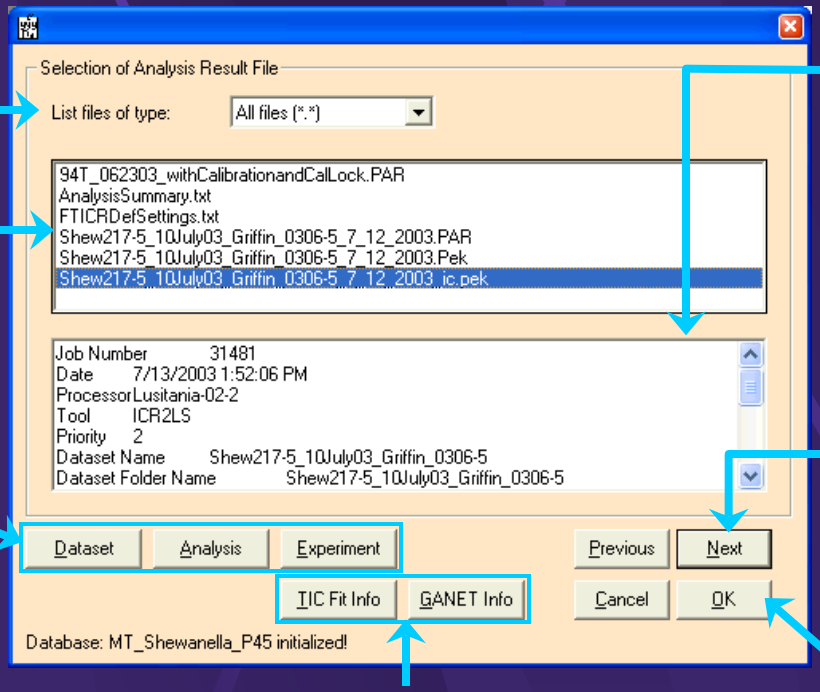
Database Analysis Loading – Page 3

"File" menu, "New Analysis (Choose from DMS)"

Selects type of files to display.

Shows files in analysis directory with correct file type.

Opens DMS pages for currently selected ICR-2LS analysis (currently doesn't work).



Displays contents of text file (drag and drop a file from the upper list to see it in this one).

Advances analysis loading to fourth page.

Completes loading (with default parameters shown on next page).

Displays TIC NET and GANET for currently selected analysis).

Database Analysis Loading – Page 4

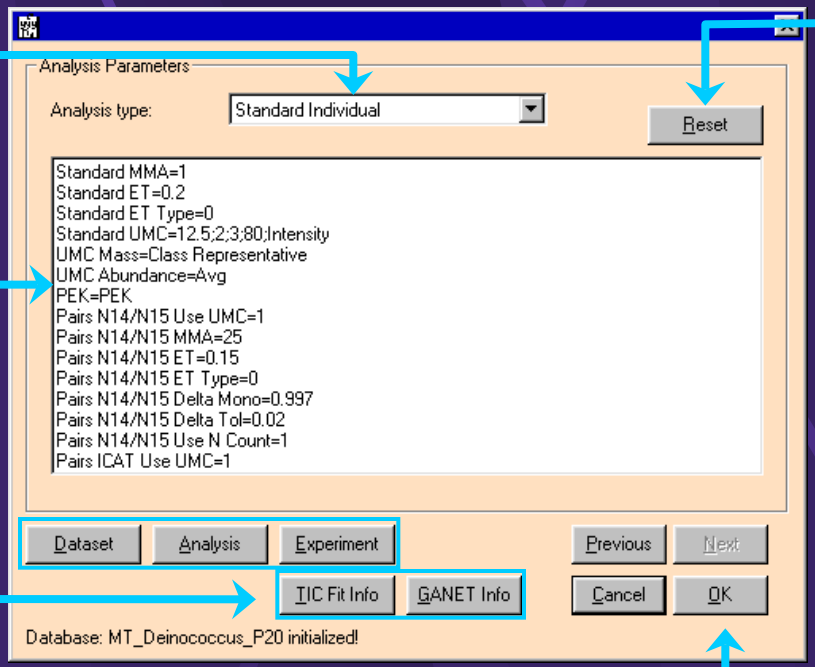
"File" menu, "New Analysis (Choose from DMS)"

Selects type of analysis to be done.

Sets various parameters for file analysis.

Opens DMS pages for currently selected analysis (currently doesn't work.)

Displays TIC NET and GANET for currently selected analysis.



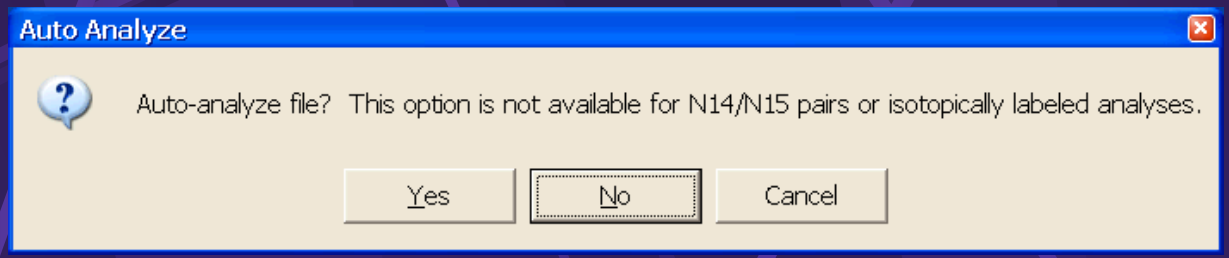
Resets parameters.

This information is stored to the database when exporting, but does not have to be defined.

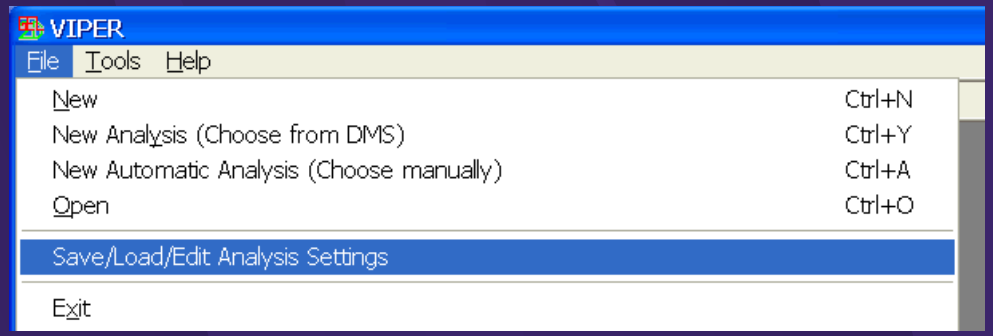
Completes selection of analysis to load.

Database Analysis Loading

"File" menu, "New Analysis (Choose from DMS)"



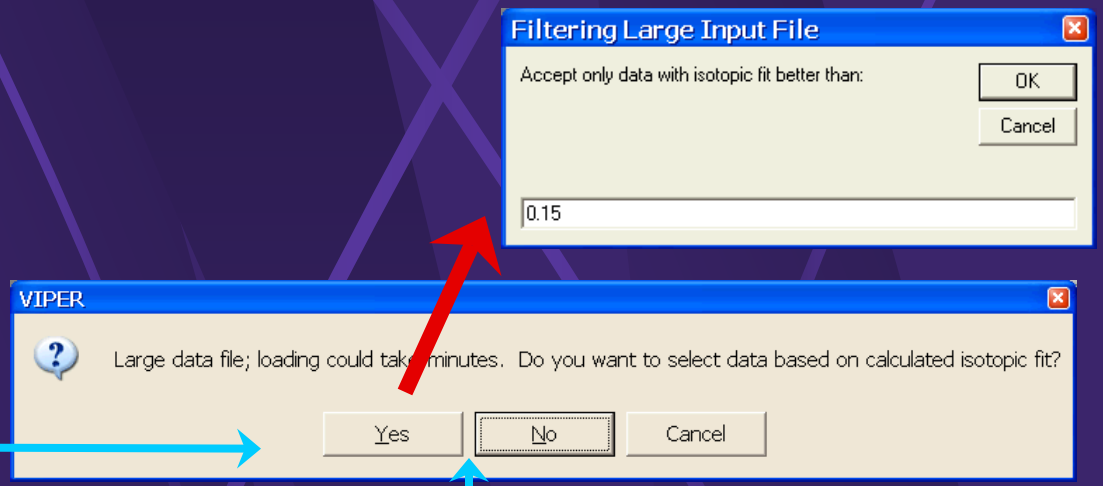
Auto-analysis is an advanced feature that allows you to process the data for a single analysis using the settings in a .Ini file. You can define the settings to use using File->Save/Load/Edit Analysis Settings. For manual analysis, simply choose "No".



Database Analysis Loading

"File" menu, "New Analysis (Choose from DMS)"

Loads analysis only with data having a calculated fit better than specified.



Loads analysis with all data.

After choosing the file to load, you will be asked if you would like to filter by isotopic fit. This is useful when loading large data files (> 5 Mb) and you plan on filtering by isotopic fit later on. Suggested filter values are 0.15 or 0.20 Alternatively, choose No to load all of the data; you may optionally filter by isotopic fit later on using the Filter form.

Manually Load PEK file

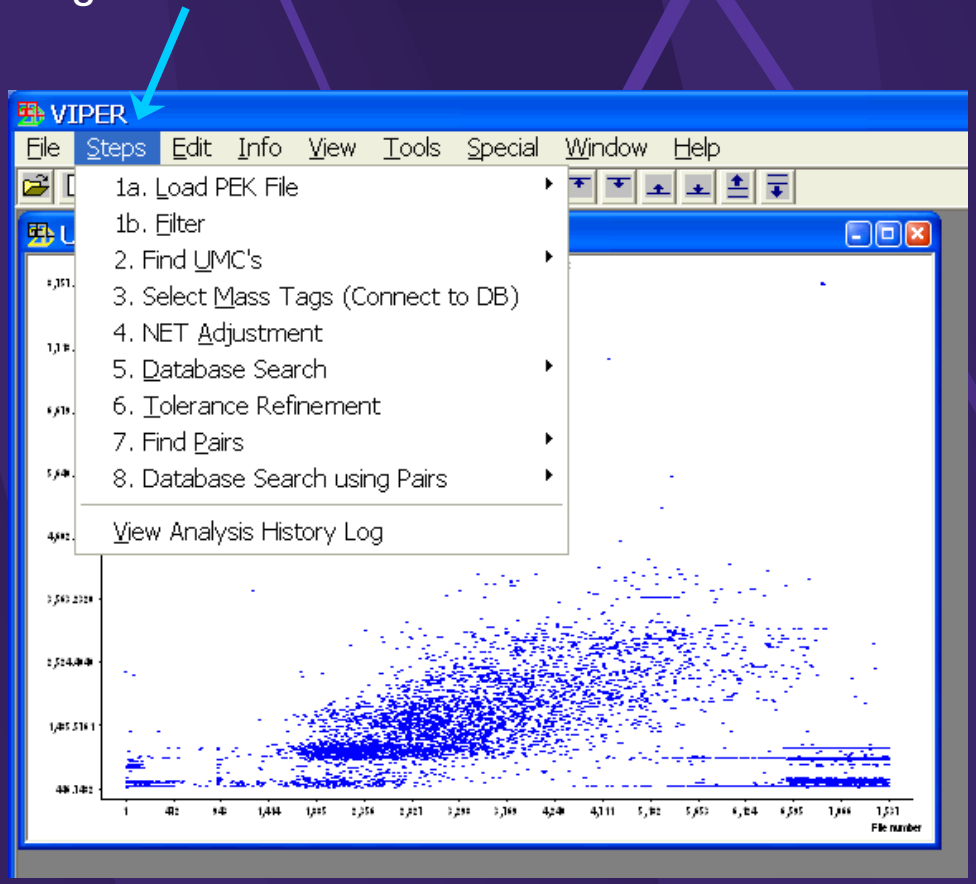
File menu, “New”

This is necessary if you want to look at the results of an experiment before they are incorporated into the database. To manually load a .PEK file from your computer, go to the File menu, “New”. Select the .PEK file from your computer or from a network share and choose OK. Generally, one should choose the calibrated _ic.PEK file or the calibrated _s.PEK file. See the previous two slides concerning the two Yes/No boxes that appear when manually loading a .PEK file.

“Steps” Menu Provides Guide

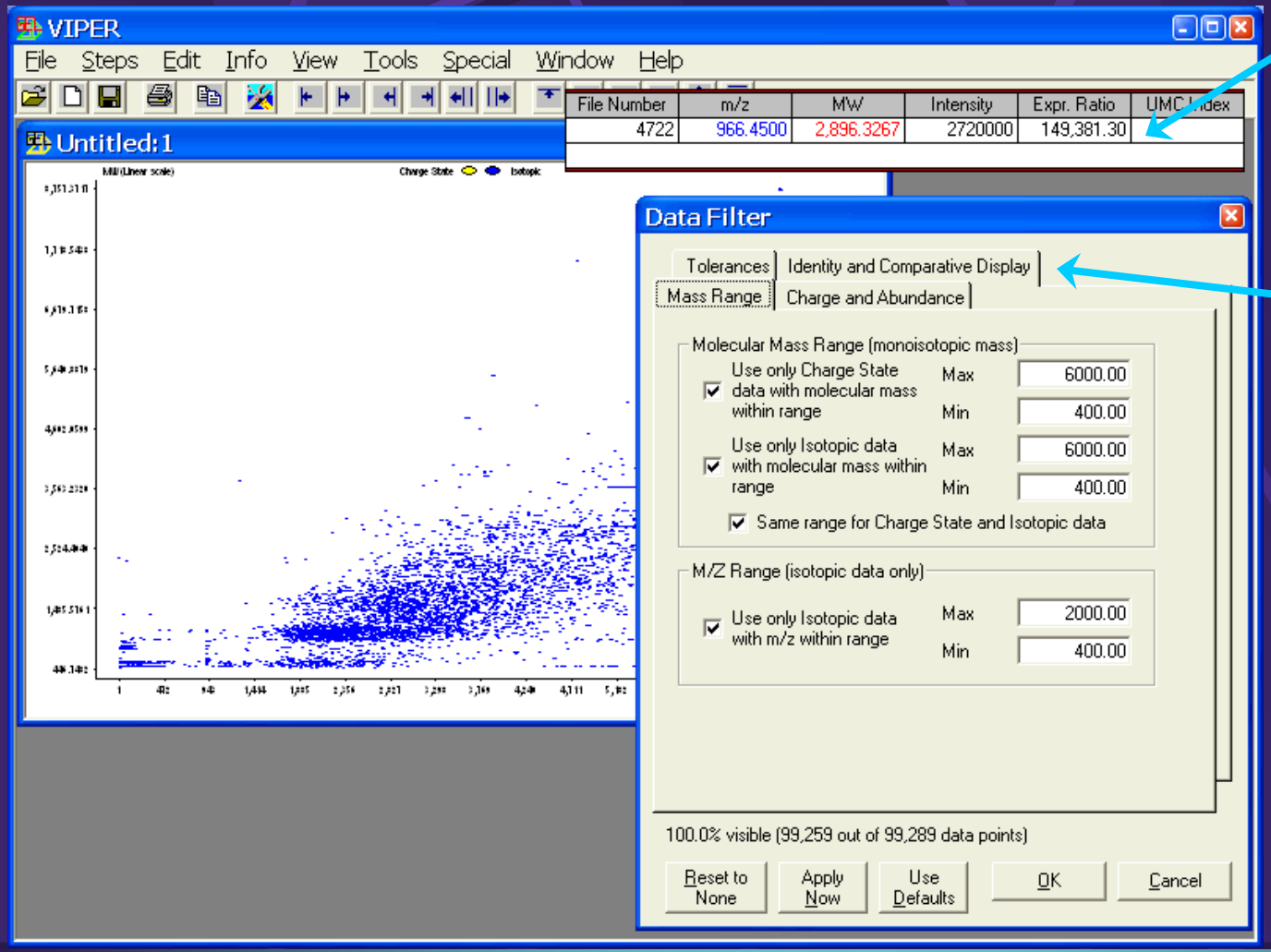
After loading the PEK file the “Steps” Menu will appear.

This menu guides one through the various steps required to match peaks to mass tags.



Step 1b. Filter Points

"Steps" menu, "Filter Points"



Coordinates display ("View" menu, "Coordinates") – Displays coordinate information when you move your mouse over a data point on the graph.

After searching the database, filter out all data that wasn't identified by selecting "Exclude unidentified data" on the "Identify and Comparative Display" tab.

Set parameters to filter on. Default parameters are:

1. Exclude data with isotopic fit < 0.2.
2. Mass range 400-6000.
3. m/z range 400-2000.
4. Charge state 1-6.

Click "Apply Now" to filter.

Click Reset for no filters.

Step 1b. Filter Points – Graph

"Tools" menu, "Filter Points – Graph"

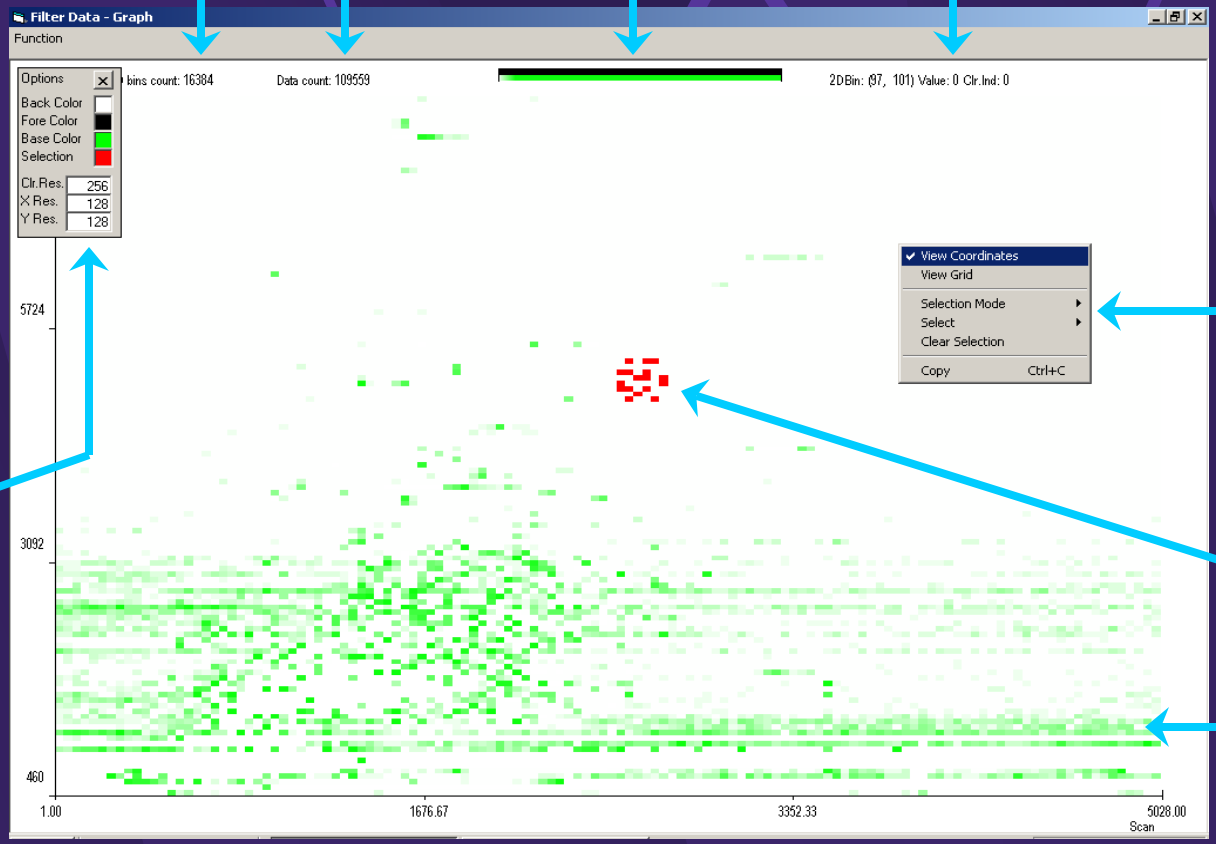
This function allows user to eliminate selected peaks from the current display.
The selected peaks are not actually removed, but are just made invisible.

2D Bins Count

Data Count

Color Scheme Bar

Coordinate Information



<u>Function menu</u>
Use to show various selections, exclude points, and display the options menu.

<u>Options menu</u>
Use to change color scheme and resolution of graph.

<u>Right-click on Graph</u>

Use to change extra viewing and selection options. You can also clear selections and copy the graph here.

Selected points on graph.

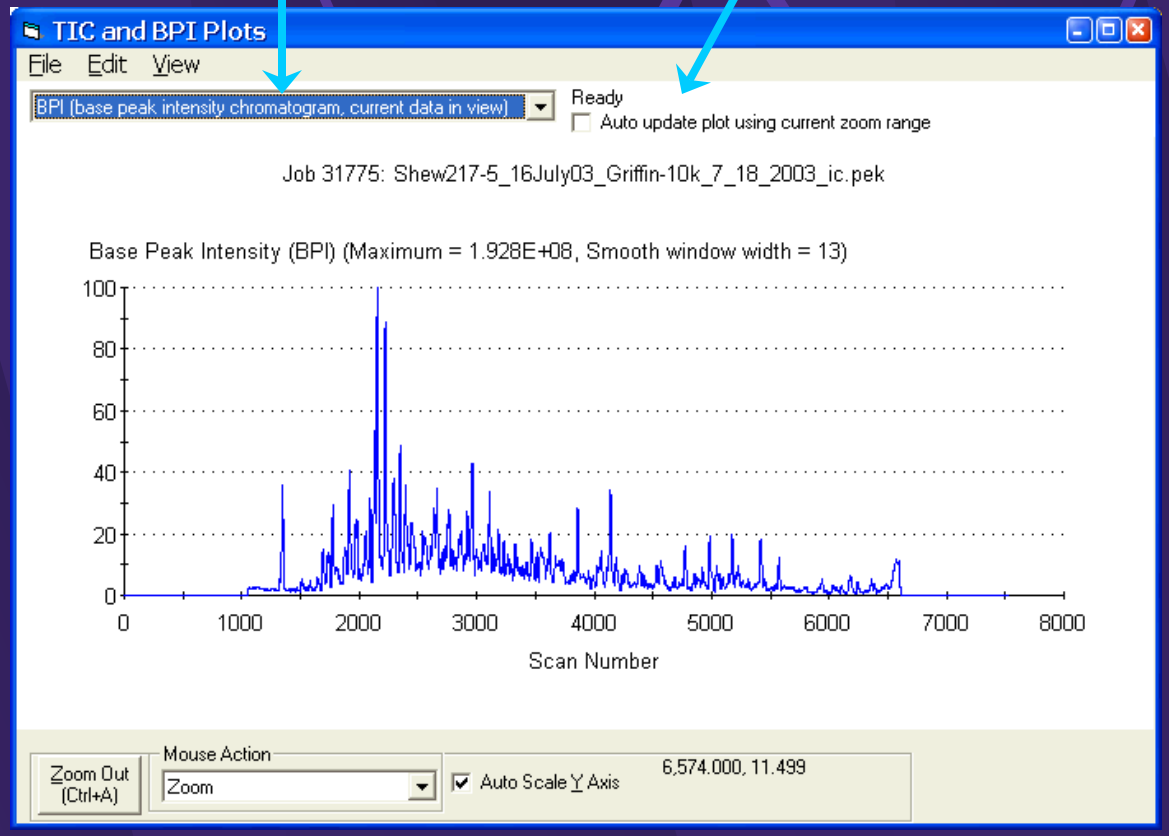
Data Points

Display TIC and BPI Plots

"View" Menu, "TIC and BPI Plots"

Choose between various TIC (total ion chromatogram) plots, or a BPI (base peak ion) plot.

Automatically update plot when checked.



Step 2. Find Unique Mass Classes

Steps Menu, “Find UMCs”

There are several ways to find the UMCs.
The two main options are:

“UMC 2003”

– "Favor Higher Intensity" is the fastest UMC search method, but creates the lowest quality UMC's.

–"Shrinking Boxes Favor Intensity" is the next slowest UMC search method, and creates better quality UMC's.

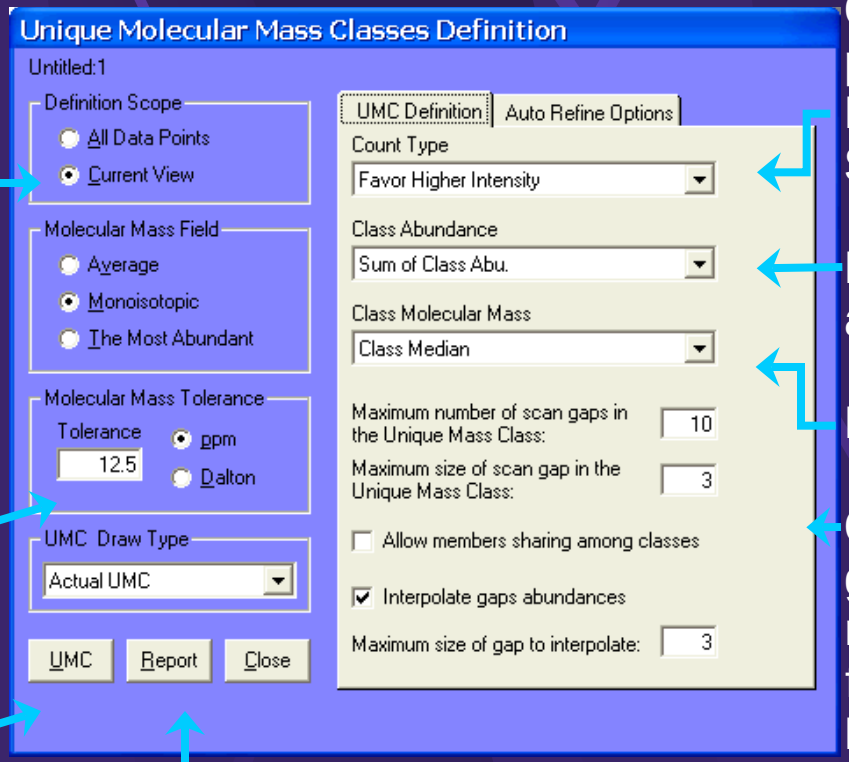
“UMC Ion Networks”

– This is the slowest UMC search method, but it creates the highest quality UMC's.

Step 2. Find UMC 2003 (Unique Mass Classes)

"Steps" Menu, "Find UMCs", "UMC 2003"

Select whether UMCs are done for all data points in display or only for the current view. (Use of Current View is useful if the user has filtered out data using Filter Points and you only want to search the filtered data.)



Controls function used to produce UMCs. Favor Higher Intensity and Shrinking Box are best.

Definition of class abundance

Definition of class MW.

Controls what type of gaps (scans in which no member of the UMC is found) are allowed. Normal settings are 10, 3, and 3.

Mass Tolerance, total range in UMC can be up to twice this number.

Start finding UMCs using current settings.

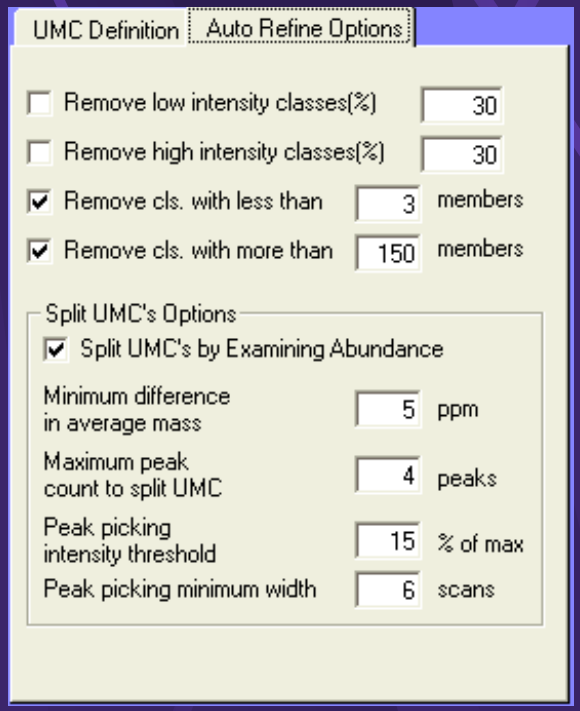
Displays UMCs (and parameters) as a text file. Can be used to save UMCs as a text file.

Step 2. Find UMC 2003 (Unique Mass Classes)

"Steps" Menu, "Find UMCs", "UMC 2003"

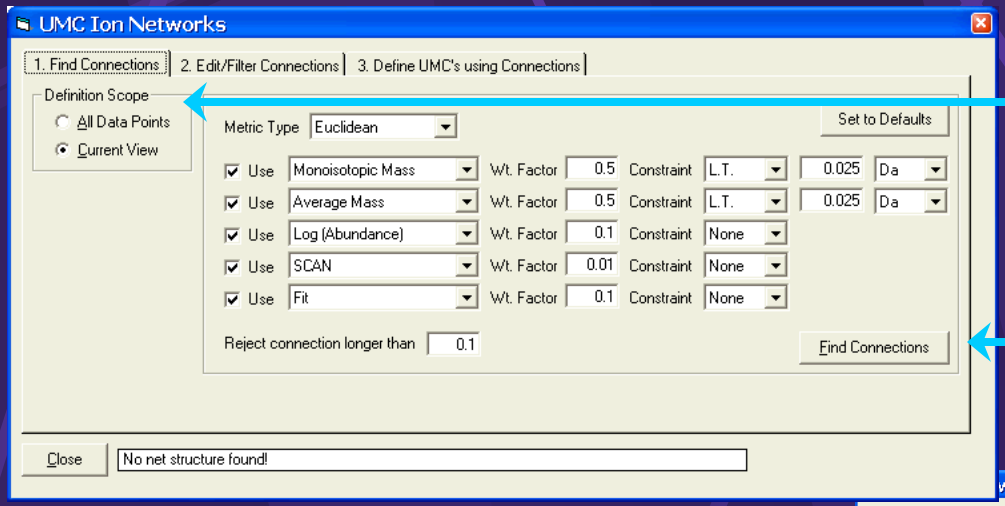
Auto-refine options can be used to filter out UMC's that have too few or too many members.

Split UMC's can be used to look for UMC's that contain 2 or more distinct populations of ions, based on abundance.



Step 2. Find UMC Ion Networks

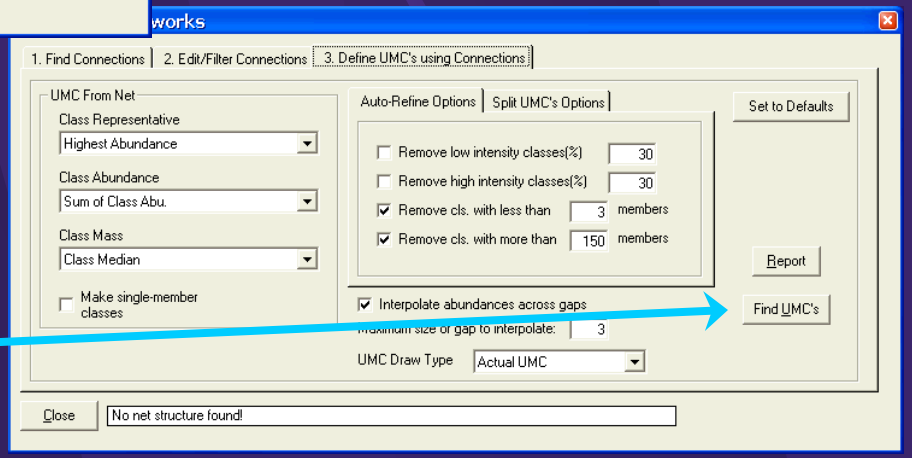
Use instead of UMC 2003 method. “Steps” menu, “Find UMCs”, “UMC Ion Networks”



Select the “All Data Points” or “Current View” option.

The default parameters should work fine.

Click “Find Connections”.



The options on Tab 2 can be used to filter the connections (typically skip this).

The parameters on Tab 3 are similar to the UMC 2003 Options.

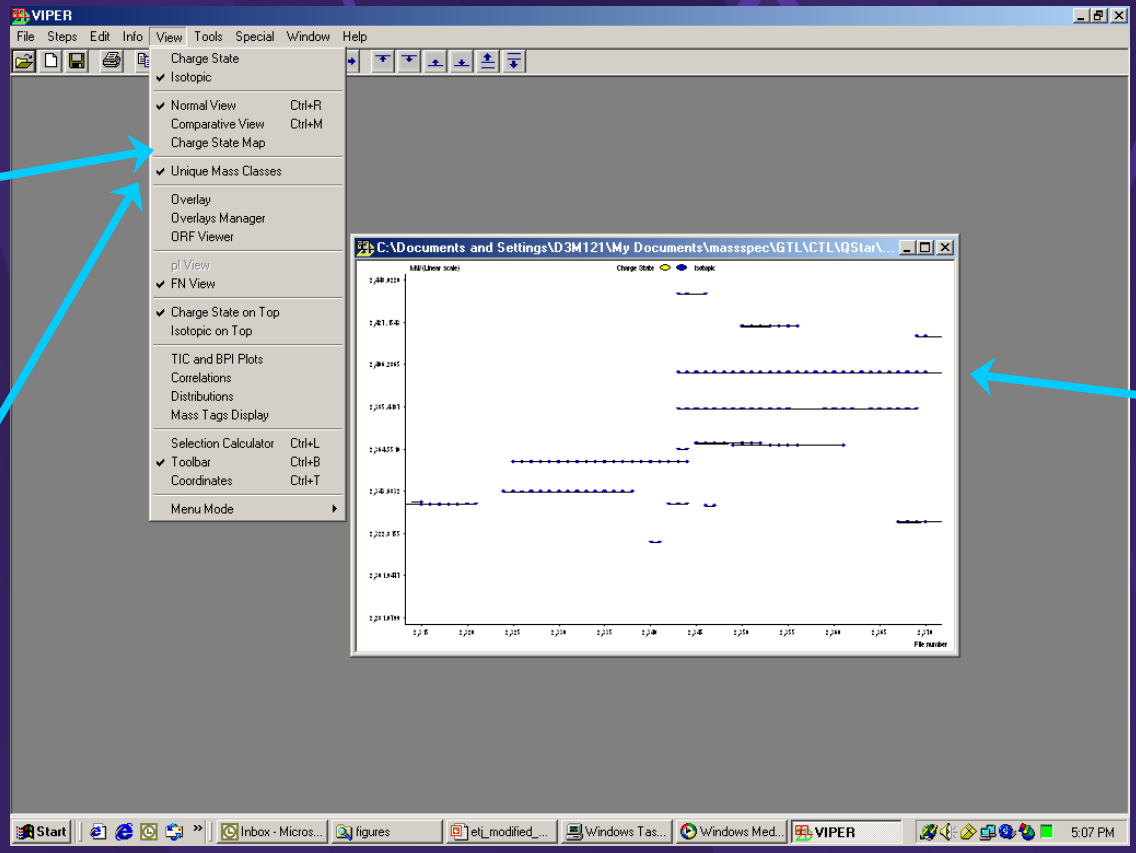
Click “Find UMC's” to identify the UMC's.

Display UMCs

"View" Menu, "Unique Mass Classes"

Select "Charge State Map" option if desired.

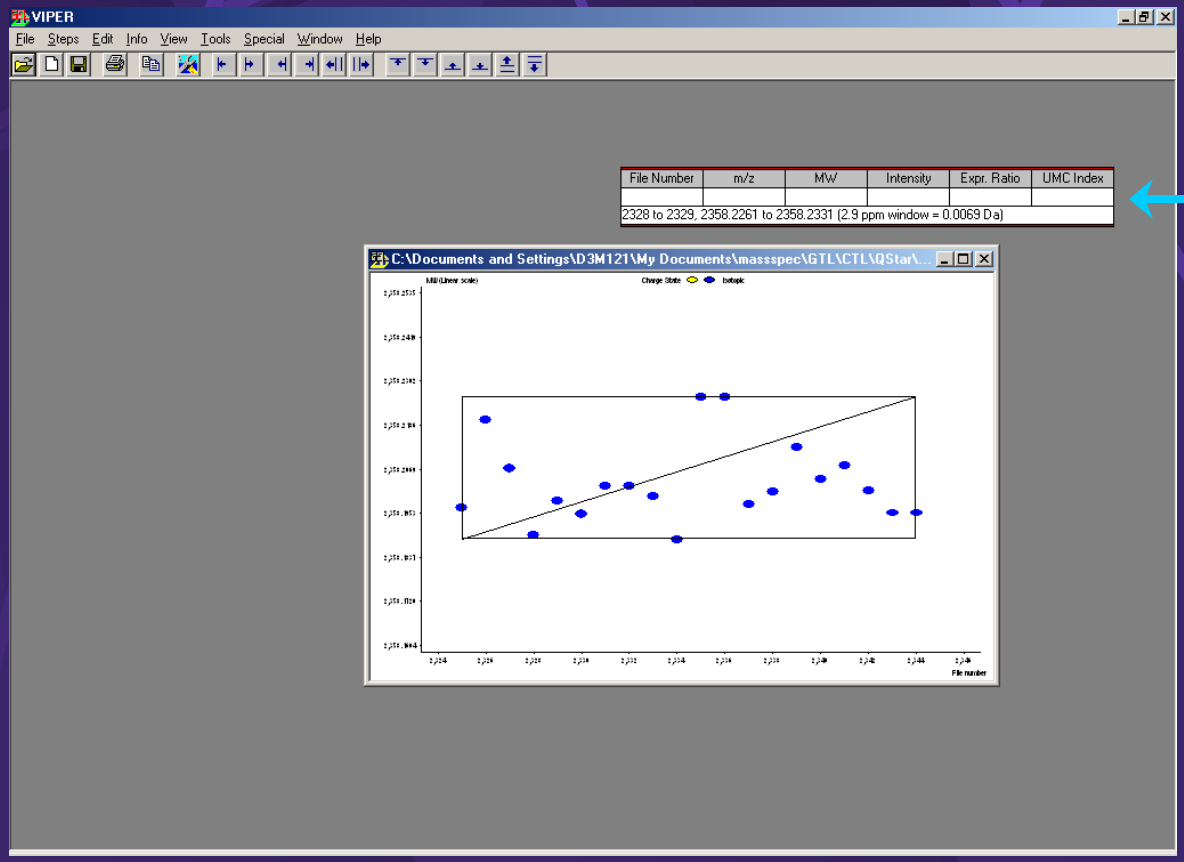
Select "Unique Mass Classes".



UMC's are represented as black lines

Zoom in on Single UMC

Use mouse to select rectangular region to expand.



"View", "Coordinates" Box.

Displays width in ppm of highlighted region.

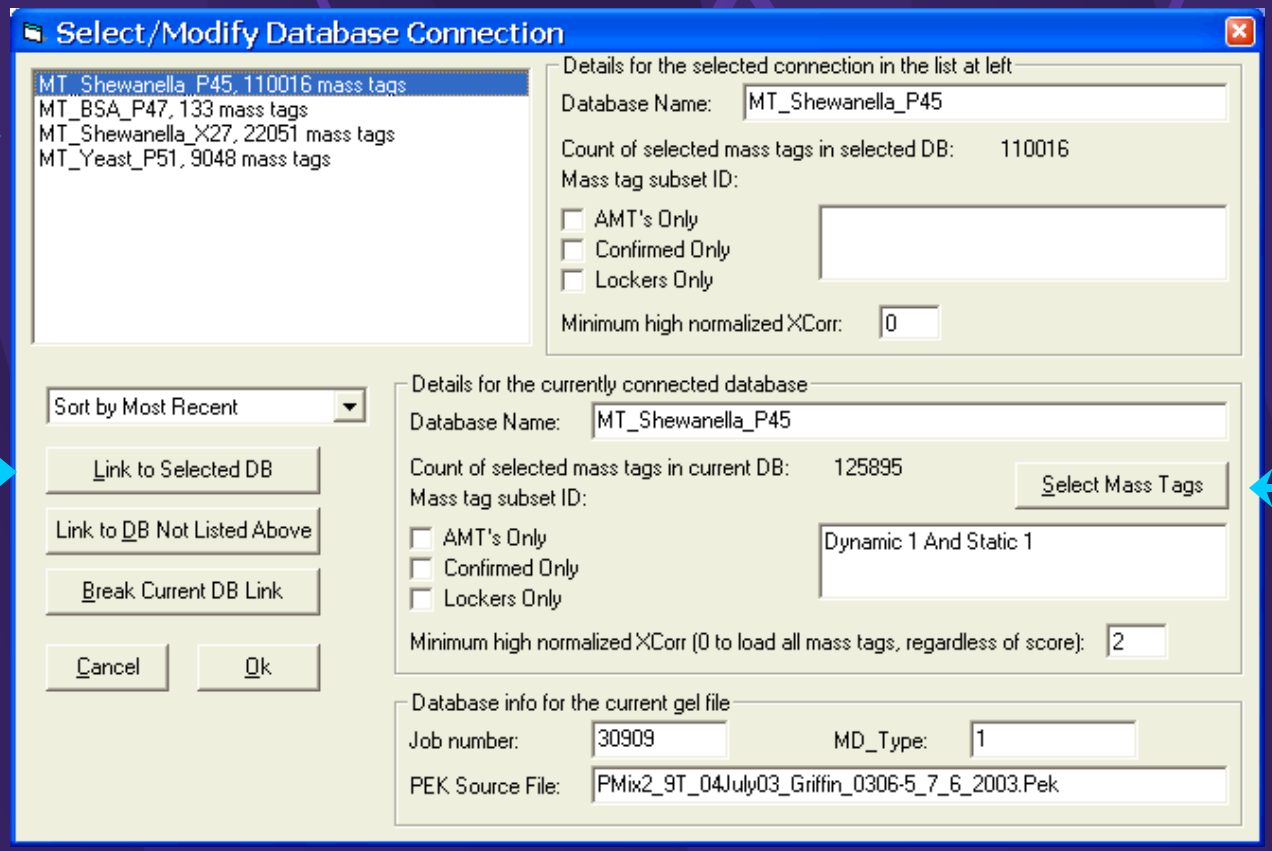
Step 3. Select Mass Tags (Connect to DB)

This step can be skipped if the data was loaded using the “New Analysis (Choose from DMS)” method to select a datafile.

1.Select the database.

2.Link to the database.

3.Select Mass Tags.



Step 3. Connect to Database: Details

If the datafile was selected using File menu, New, then this step cannot be skipped.

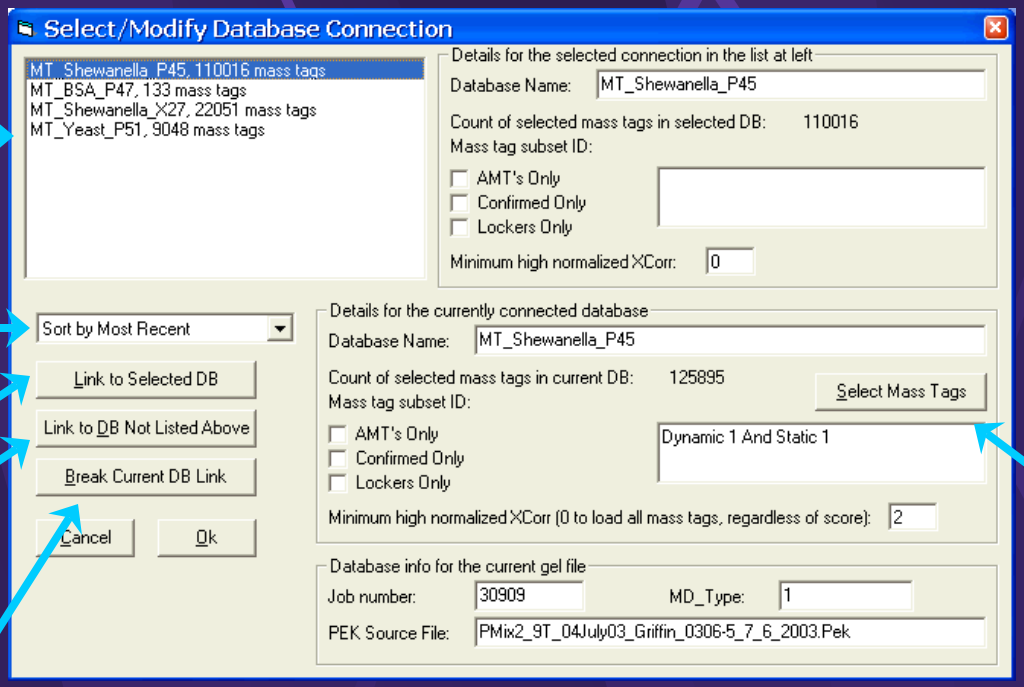
Recently selected databases are shown here (Double-click to connect to it).

Sort database list above by most recent or alphabetically.

Link with selected database from list above.

Link to a database not shown above.

Break the current link to a database (most often used to switch links from one database to another).



Information on selected database from the list on the left (such as modifications)

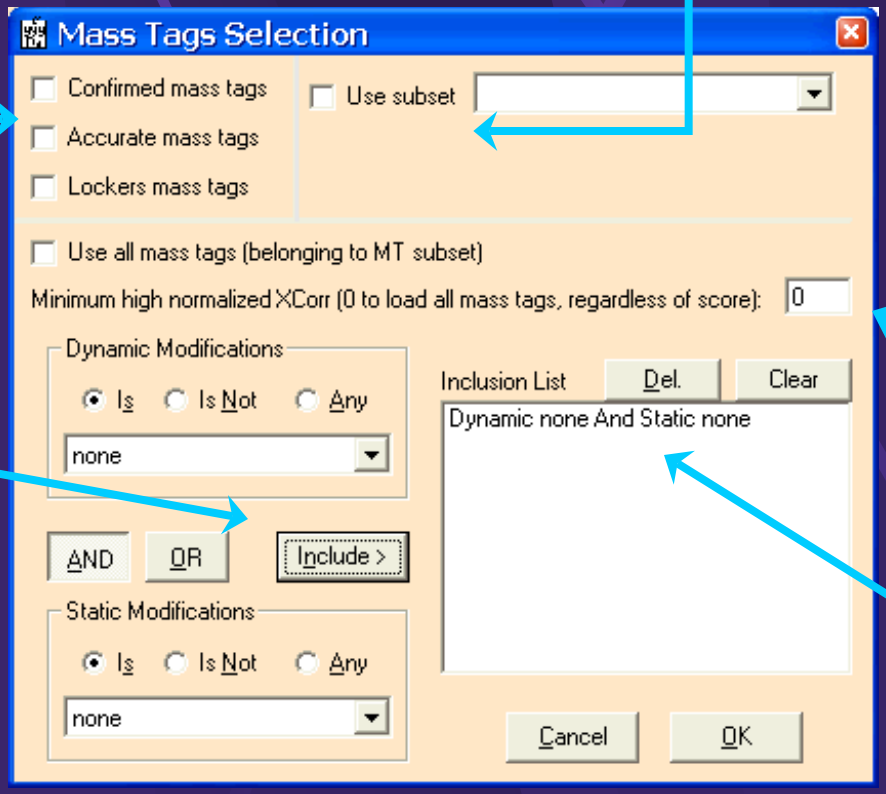
Information on the current database the file is connected (linked) to.

Select specific mass tags for the current database (See next slide for more details).

Step 3. Connect to Database: Details

To confine mass tags to only certain types, check one or more of these boxes.

Select mass tag characteristics and click “Include” to include it as a parameter.



Check the “Use subset” box and select a subset of mass tags to use. (Choose All to use from all mass tags in database.)

Filter by high normalized XCorr.

Inclusion list shows what parameters are used for mass tags loading. (Using more than 1 can sometimes cause odd results.)

Step 4. NET Adjustment

Edit Menu, “Net Adjustment” -> “UMC ID Peaks”

When opening, may take a few minutes for mass tags to load.

Function menu – Use to calculate NET and to view and export results as a text file.

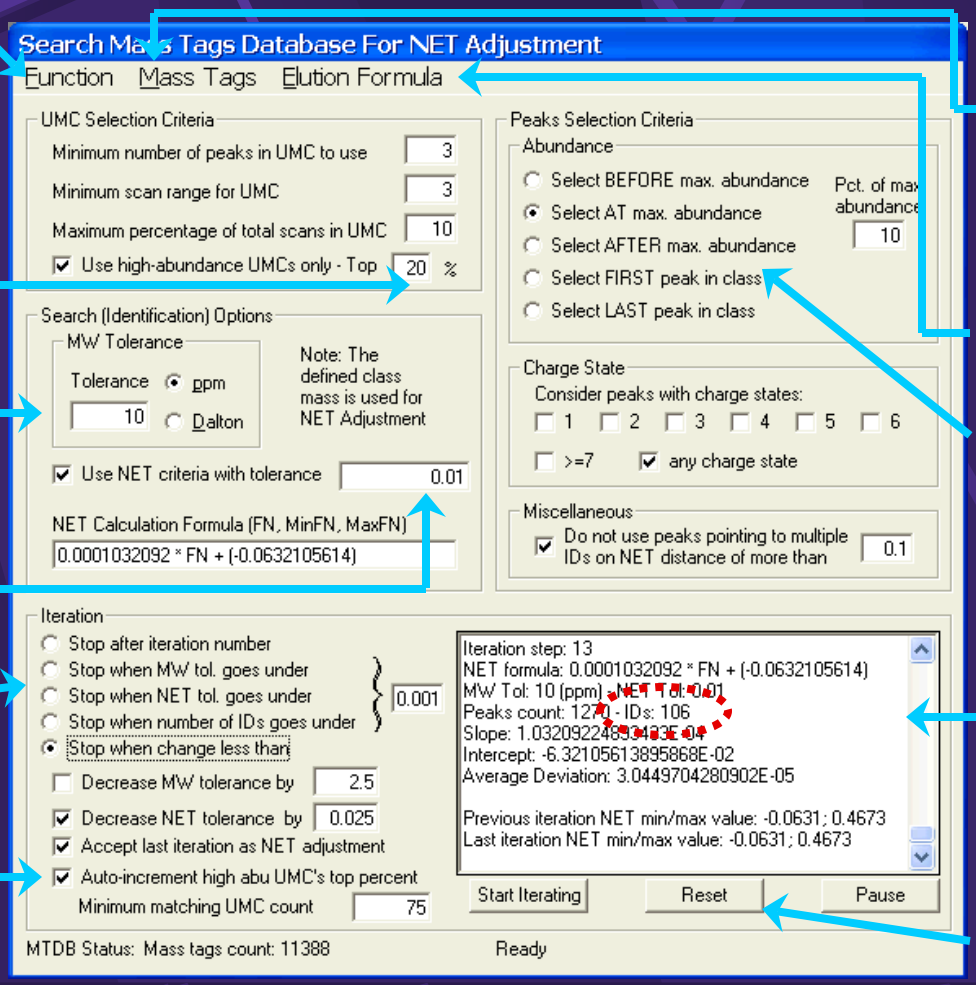
Use the lowest percentage of high-abundance UMCs (20%) while maintaining the number of IDs (from the iteration box) greater than the minimum matching UMC count.

Controls what MW tolerance is used to identify hits for calculating NET.

NET tolerance for selecting hits to calculate NET.

Define how to iterate the calculations.

Auto-adjust % of high abundance UMC's to use.



Mass Tags menu – Use to check the status of mass tags, reload mass tags, or load mass tags from a legacy database.

Elution Formula menu – Use to reset NET Calculation Formula back to Generic.

Define the criteria for selecting peaks when calculating NET.

Results from calculating NET after each iteration.

Reset to Generic NET equation and default start values.

Step 5. Database Search (for UMC to Mass Tag Identification)

"Steps" menu, "Database Search"

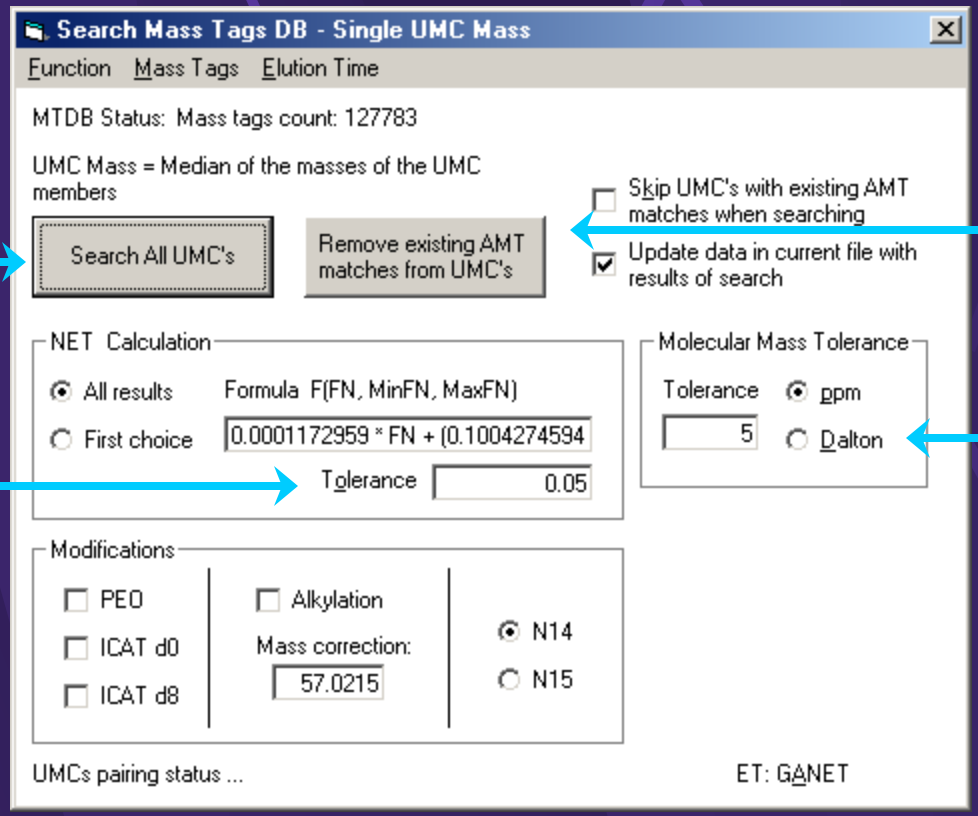
There are several ways to search the Mass Tag database. The two most common options are:

"UMC Single Mass" only uses the median mass of each UMC, and there is just 1 unique mass for the UMC and only one search of the database performed (for each UMC). If a match is found using the median mass, the result is stored in all of the ions for the given UMC. A time value (NET value) is also used for the search.

"UMC Ion-by-Ion" compares the mass of each ion (mass spec peak) in a given UMC with all of the mass tags in memory. If a match is found, then all of the ions in the given UMC are assigned the match. This is best when the UMC's have a large spread in mass, and the median mass may be too far away from the mass in the database. With this method, a UMC with 10 members will have 10 unique masses used for searching the database.

Step 5. Database Search (for UMC to Mass Tag Identification)

"Steps" menu, "Database Search", "UMC Single Mass"



1. Remove old matches (if searched before)

2. Set the Mass Tolerance

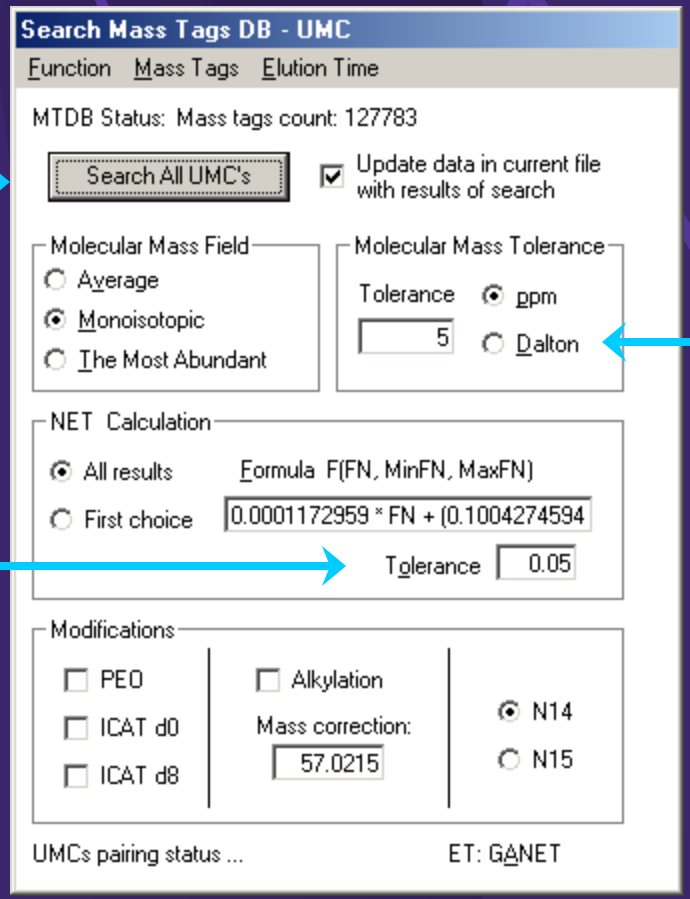
3. Set the NET Tolerance

4. Search All UMCs

Step 5. Database Search (for UMC to Mass Tag Identification)

"Steps" menu, "Database Search", "UMC Ion-by-Ion"

3. Search All UMCs



1. Set the Mass Tolerance

2. Set the NET Tolerance

Note: Old matches are automatically removed when a new search is performed.

Step 6. Tolerance Refinement

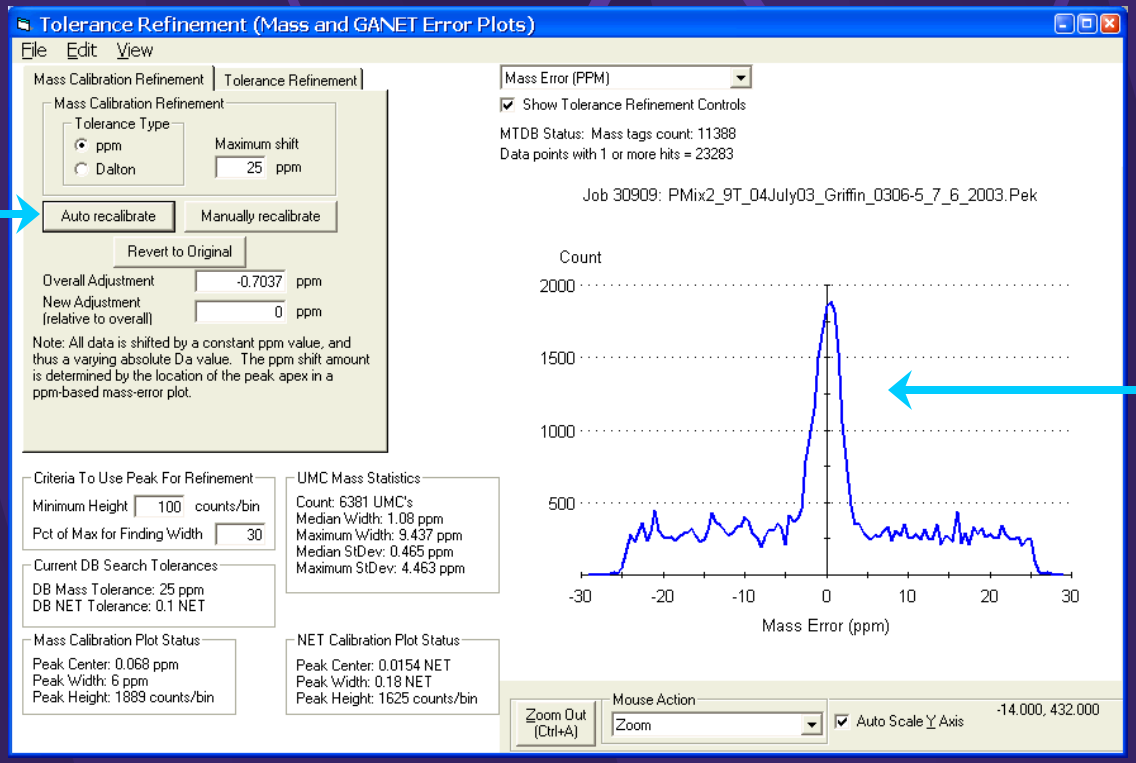
"Steps" menu, "Tolerance Refinement"

Improve mass accuracy by comparing the observed Mass Tags Molecular Weight to the value predicted by the database.

This function automatically calculates the histogram of the difference between the observed and predicted molecular weights.

To shift the peak of the histogram to a mass error of zero click "Start Re-calibration".

NOTE: After recalibrating the data search the Mass Tag database again using a more strict mass tolerance.



Before the recalibration the peak is usually not centered around zero.

After calibration the peak should occur at zero.

The width of this should be <10 ppm for FTICR data and may be >10 ppm for Qstar data.

Step 7. UMC Delta Pairs

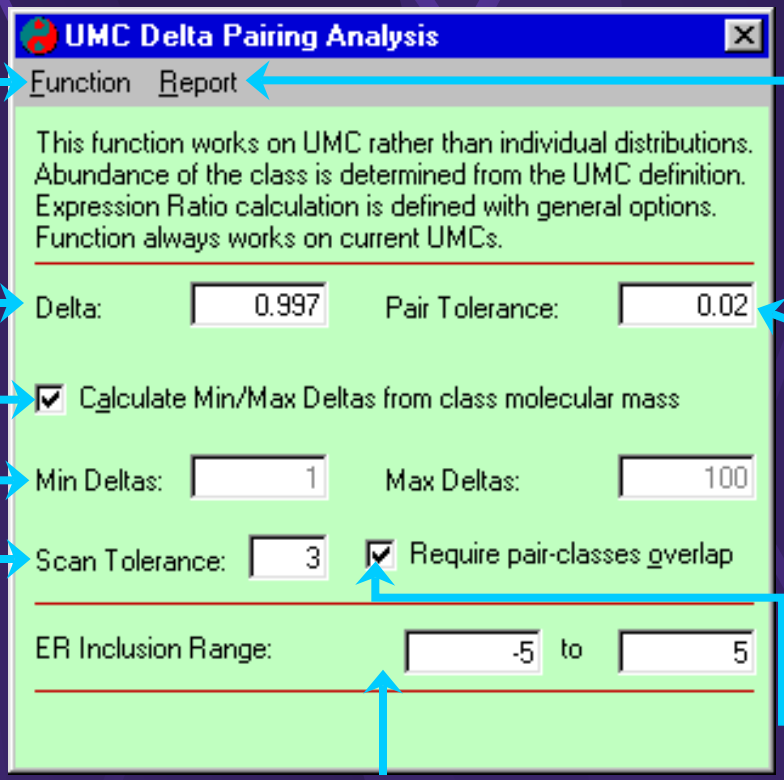
"Steps" menu, "Find Pairs" -> "Delta"

Function menu – Use to find pairs, clear pairs, recalculate ER, etc.

Delta MW used to find pairs. (in Daltons)

Max and min deltas to consider for a pair, or automatic calculation (easiest and most accurate thing to do).

Maximum scan difference that can occur between the starting scan of the low UMC and the starting scan of the high UMC.



ER Inclusion Range, used with some of the options in the "Function" menu.

Report menu – Use to export various pairs reports to text files.

Allowed difference between actual and calculated MW of high pair member (in Daltons).

Require high and low pairs to be found together in at least one scan.

Step 8. UMC N14/N15 Pairs Identification

"Tools" menu, "Search Mass Tags Database", "UMC N14/N15 Pairs"

Pairs menu – Use to eliminate certain kinds of pairs and export results to MTDB.

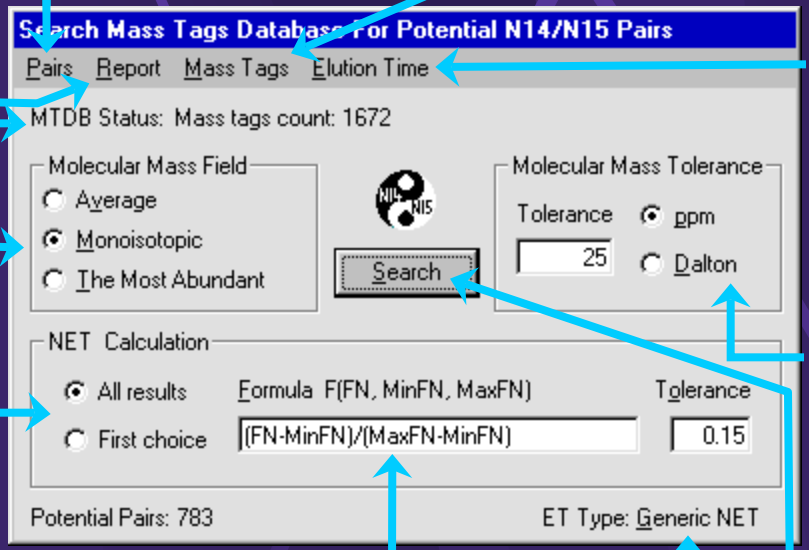
Mass Tags menu – Use to check status of mass tags or load tags from legacy DB.

Report menu – Use to export various pairs reports to text files.

Shows status of loaded mass tags.

Controls type of mass tag MW used for UMC identification.

Controls which identifications are output for each pair, all or only the best one.



Elution Time menu – Use to change elution time slope and intercept between Generic, TIC Fit NET and GANET.

Controls mass tolerance for identification

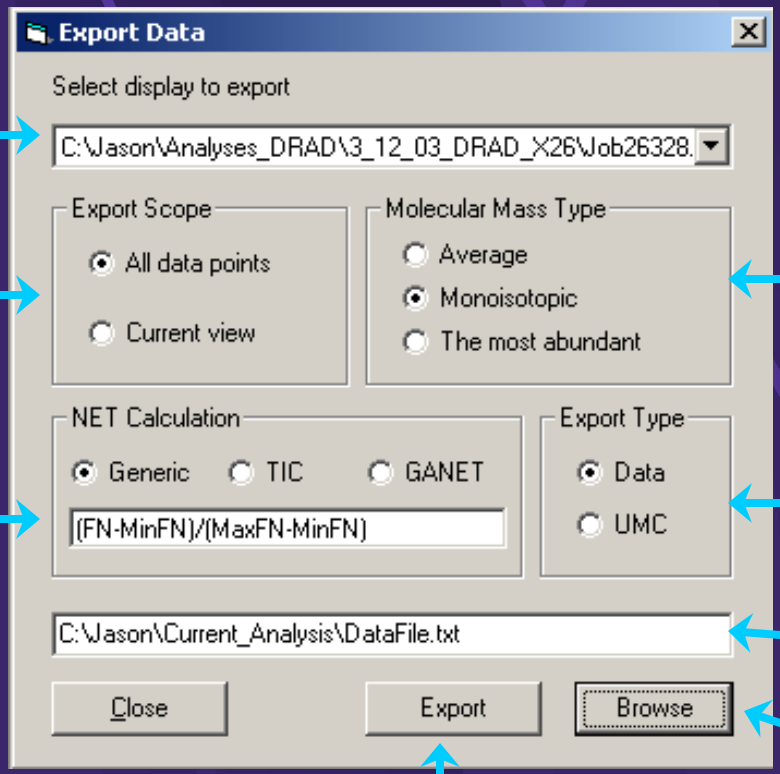
Searches with current settings.

NET formula and allowed tolerance for identification. (Formula can be changed either manually or by using the "Elution Time" menu.)

NET type as last selected in "Elution Time" menu.

“Export Data” function

“Special” menu, “Export Data”



Select from any of your open Gel files to export its data.

Choose whether to export all data points, or only those currently shown on the 2D Display (useful if you’ve filtered points and only want them exported).

Select whether you’d like the NET calculated using Generic, TIC, or GANET.

Select which molecular mass type you would like to be used when exporting data.

Select whether to export the data points or just the Unique Mass Classes (UMCs).

Export location

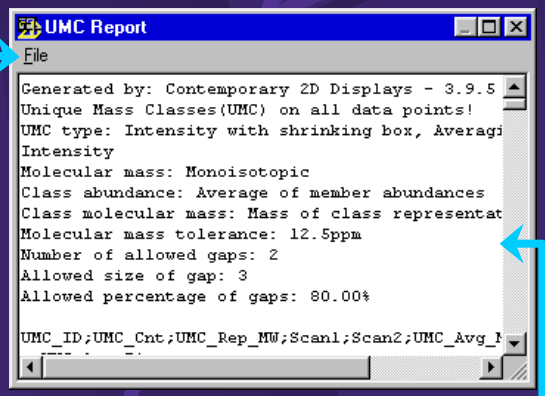
Click to choose where you want the data exported to.

Click to export data with selected parameters.

Report Saving and Excel Import

Microsoft Excel, “File” menu, “Open”, select a .txt file

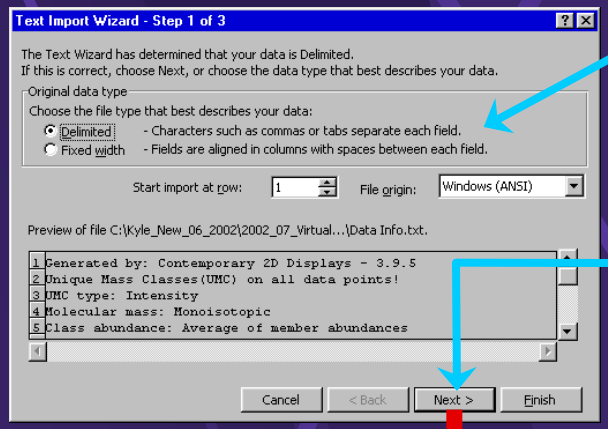
Sample Report



File menu – Use to save file, print, or close window

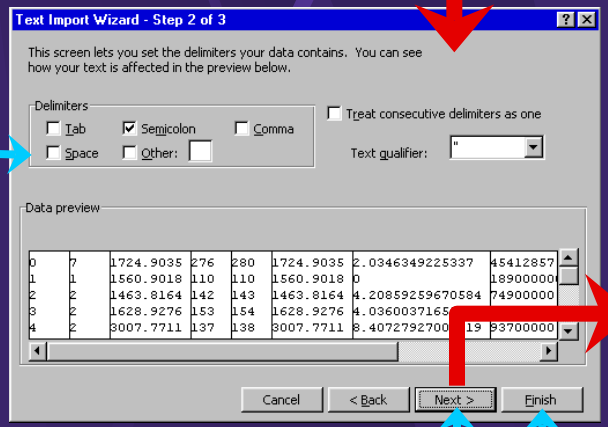
Report data.

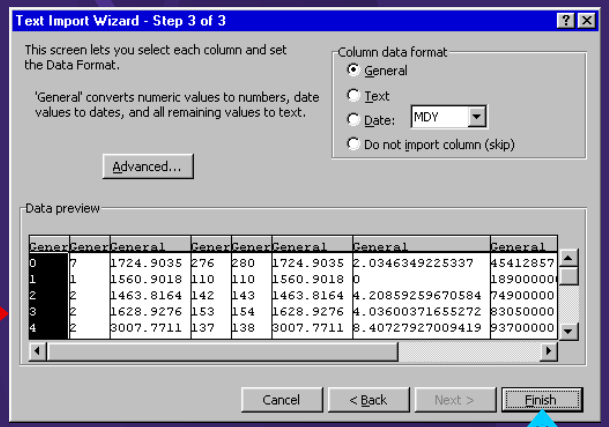
Sets delimiter(s) used for import.



Sets style of import file (delimited or fixed width.)

Advance import to second page





Advance import to third page

Finish import.

Copy Points (UMCs) in View to Clipboard Function

"Edit" menu, "Copy Points in View to Clipboard"

This function copies the Scan Number, GANET, UMC Index, Abundance, Charge state, m/z, monoisotopic mass, and any database match information for all of the data currently "in view" to the clipboard. Thus, if you zoom into a narrow region showing 10 spots, you can use this function to copy that data, where you can then open MS Excel and paste it in there.

Note: When "Include Empty Scans when Copying" is true, then empty lines are included in the copied list for scans with no ions. This can make it easier to line up the data between various analyses, or between different mass regions, but covering the same scan range.

"Edit" menu, "Copy UMCs in View to Clipboard"

This function copies the UMC index, ScanStart, ScanEnd, Average GANET, UMC monoisotopic MW (median of all UMC points), UMC MW standard deviation, UMC abundance, Range of charge states, Number of points in UMC, UMC average fit, Expression ratio, Number of mass tags the UMC matched, Mass Tag ID, Mass Tag monoisotopic MW, Mass Tag modifications, Number of points in UMC matching the Mass Tag of the UMCs currently "in view" to the clipboard.