

MultiAlign Tutorial 04 – Reviewing An Analysis

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About this tutorial

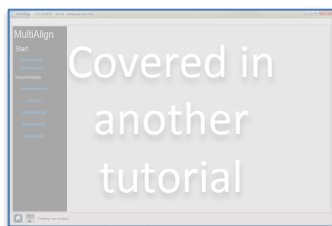
- ▶ This tutorial will describe the graphical user interface (GUI) after an analysis has been performed in great detail
- ▶ You will learn
 - Shortcuts for drilling through the data
 - How to interact with plots
 - Details about every view

Analysis overview

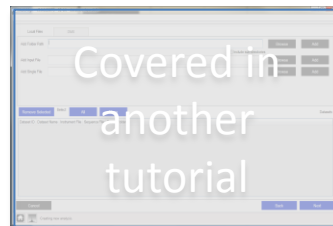
THIS SECTION PROVIDES A SET OF BACKGROUND INFORMATION TO HELP GUIDE YOU THROUGH THE REST OF THE TUTORIAL

Previous tutorials covered this material

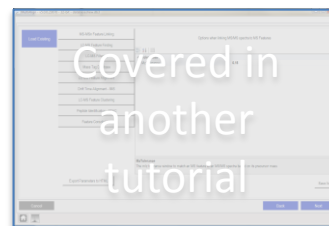
Wizard



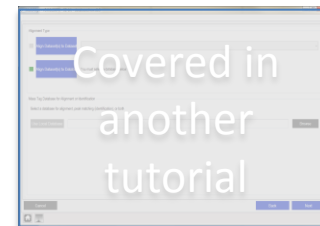
1. Home Screen



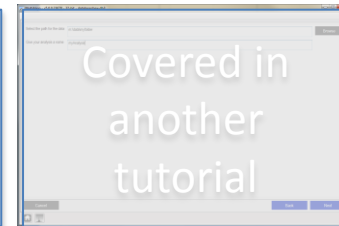
2. Select Data



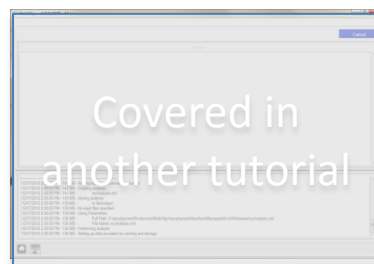
3. Set Parameters



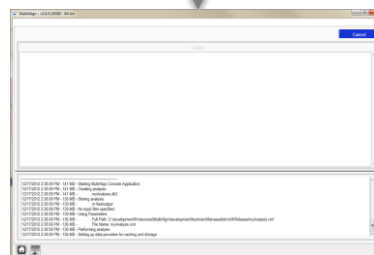
4. Select Baseline
and Mass Tag
Database



5. Set Analysis
Path and Name



6. Running
Analysis



7. Analysis View
Window



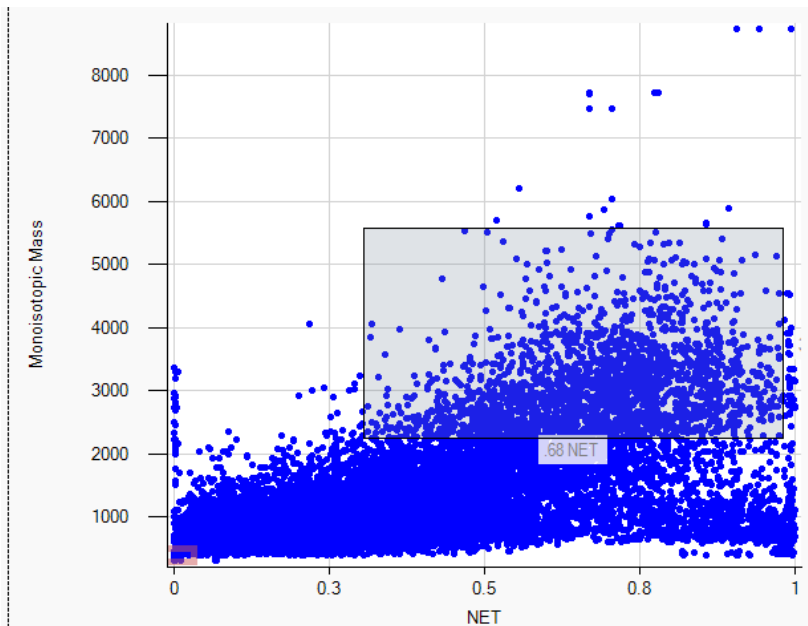
GUI Basics

UNDERSTANDING HOW TO INTERACT WITH PLOTS



Interacting with Plots

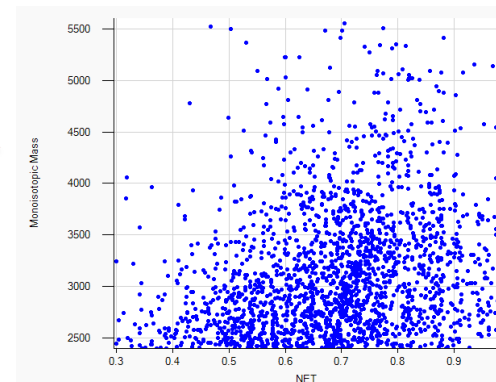
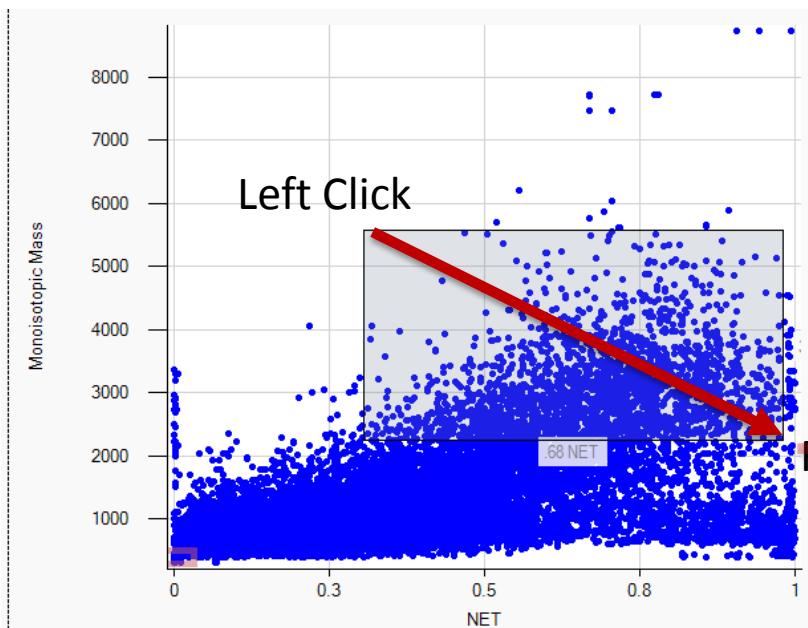
- ▶ This section describes how to interact with each plot.
- ▶ It is intended to provide basic to advanced functionality of the user interface





Plot Zooming

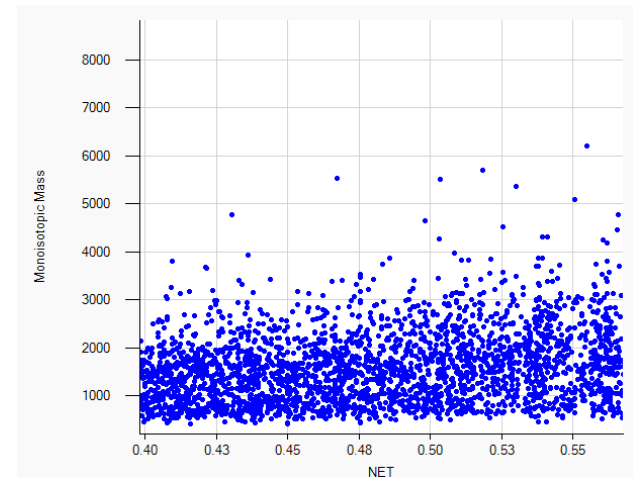
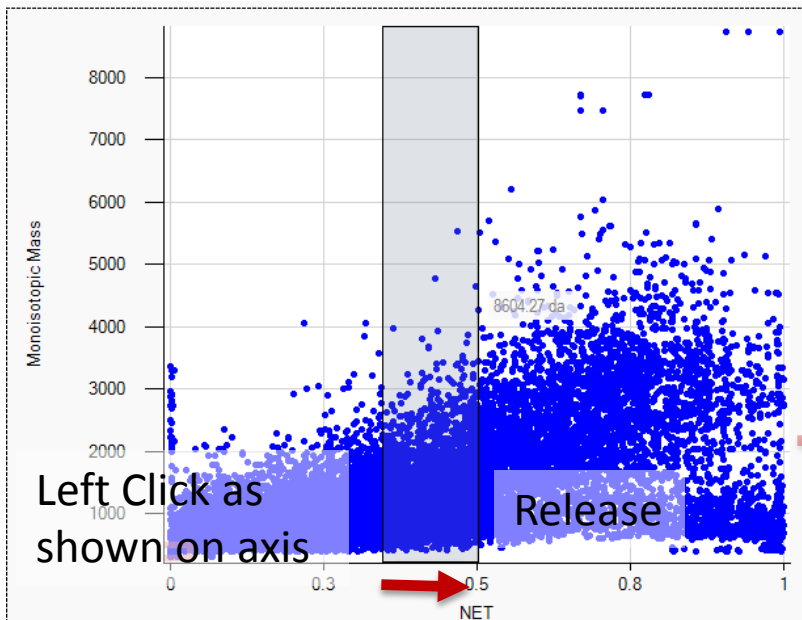
- ▶ Zooming can be done by
 - Left Click on plot
 - Hold
 - Drag
 - Release to complete zoom





Axis only Plot Zooming

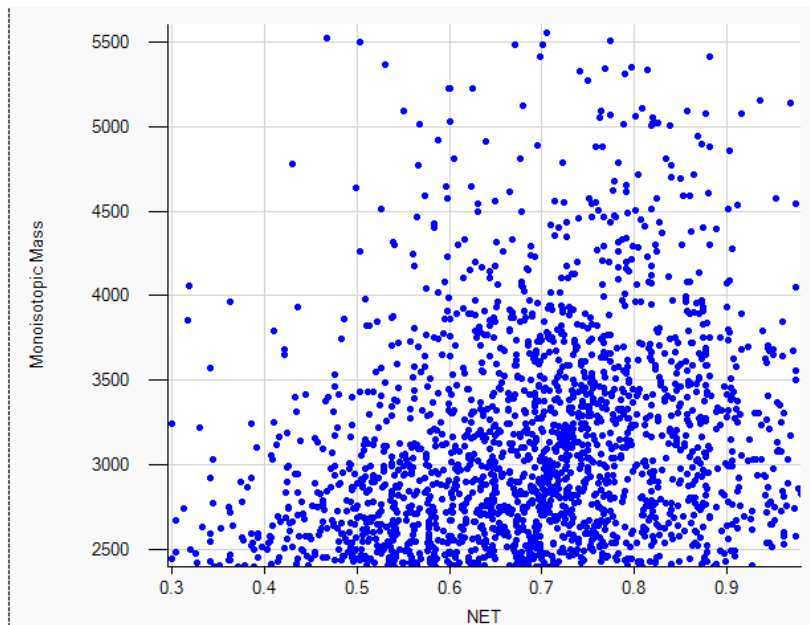
- ▶ Zooming can be done by
 - Left Click on axis
 - Hold
 - Drag
 - Release to complete zoom



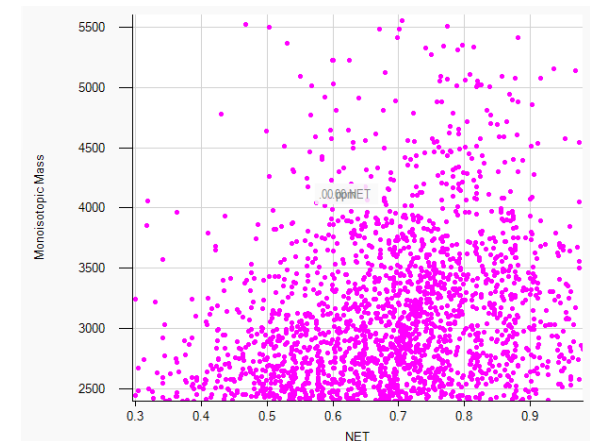


Selecting a Series

- ▶ A series is a set of points
- ▶ Clicking on a dot will select the series of points it is a part of

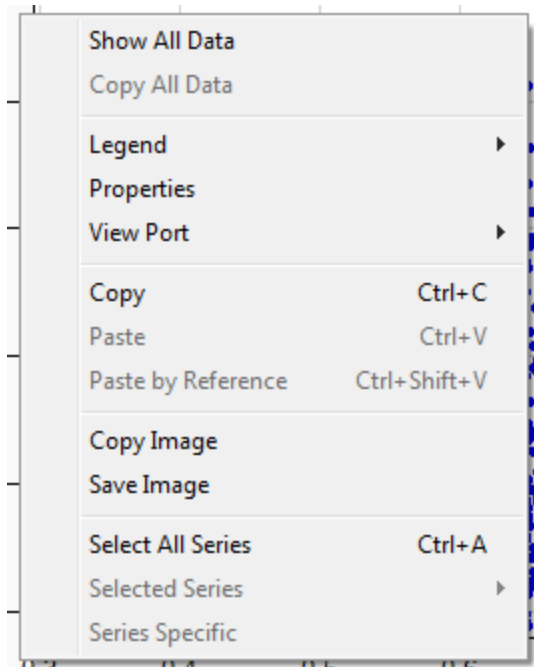


Left Click on a
dot in the
series





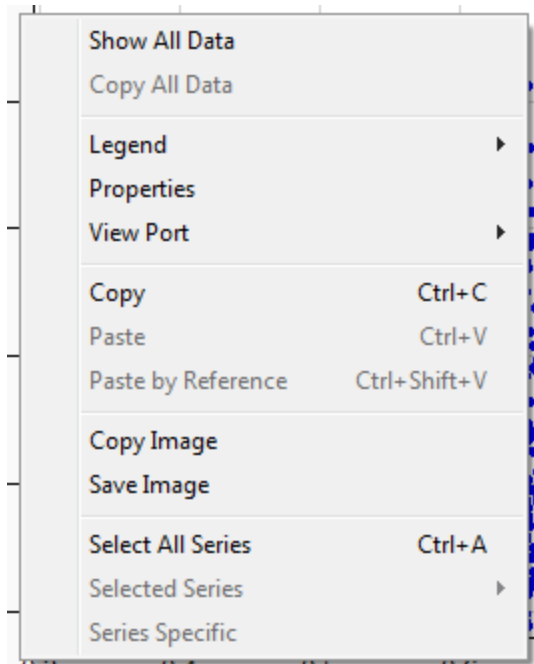
Context Menu (1)



- ▶ Right Click on the plot
- ▶ A context menu will appear
- ▶ Show All Data
 - Auto-zooms the plot to show all data points
- ▶ Copy All Data
 - Copies data from the plot to system clipboard for pasting into other software applications, e.g. Excel
- ▶ View Port
 - Allows you to step back to a previous zoom range



Context Menu (2)

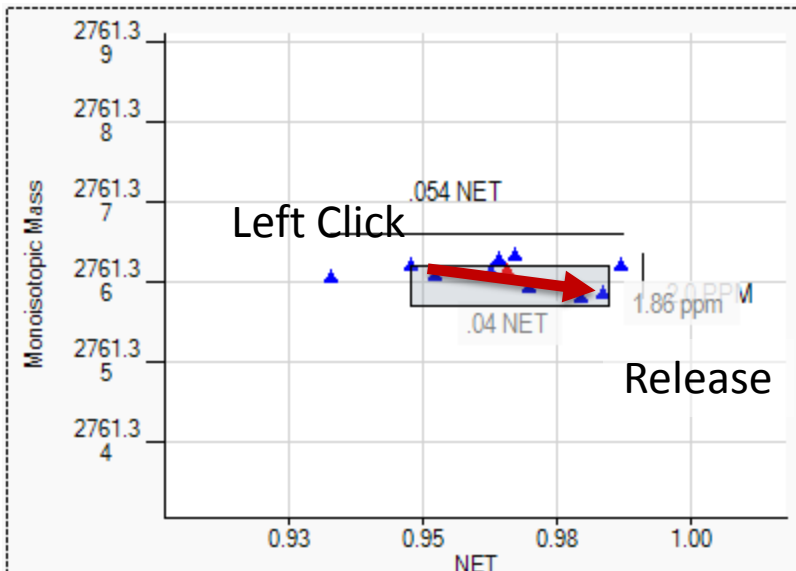


- ▶ Copy
 - Saves a WMF version of the plot, with the plot data still in raster form
- ▶ Copy Image
 - Will copy a rasterize image to the system clipboard
- ▶ Save Image
 - Saves the plot as an image to file
- ▶ Select All Series
 - Selects all series of points
- ▶ Selected Series
 - Details what series of points are selected



Measuring Distances

- ▶ Some plots will allow you to measure the distance between points
 - e.g. ppm and NET differences
- ▶ To Measure:
 - Hold Shift
 - Left Click
 - Drag
 - Release left mouse button
- ▶ The distance in the left window shows:
 - 1.86 ppm mass difference
 - .04 NET difference

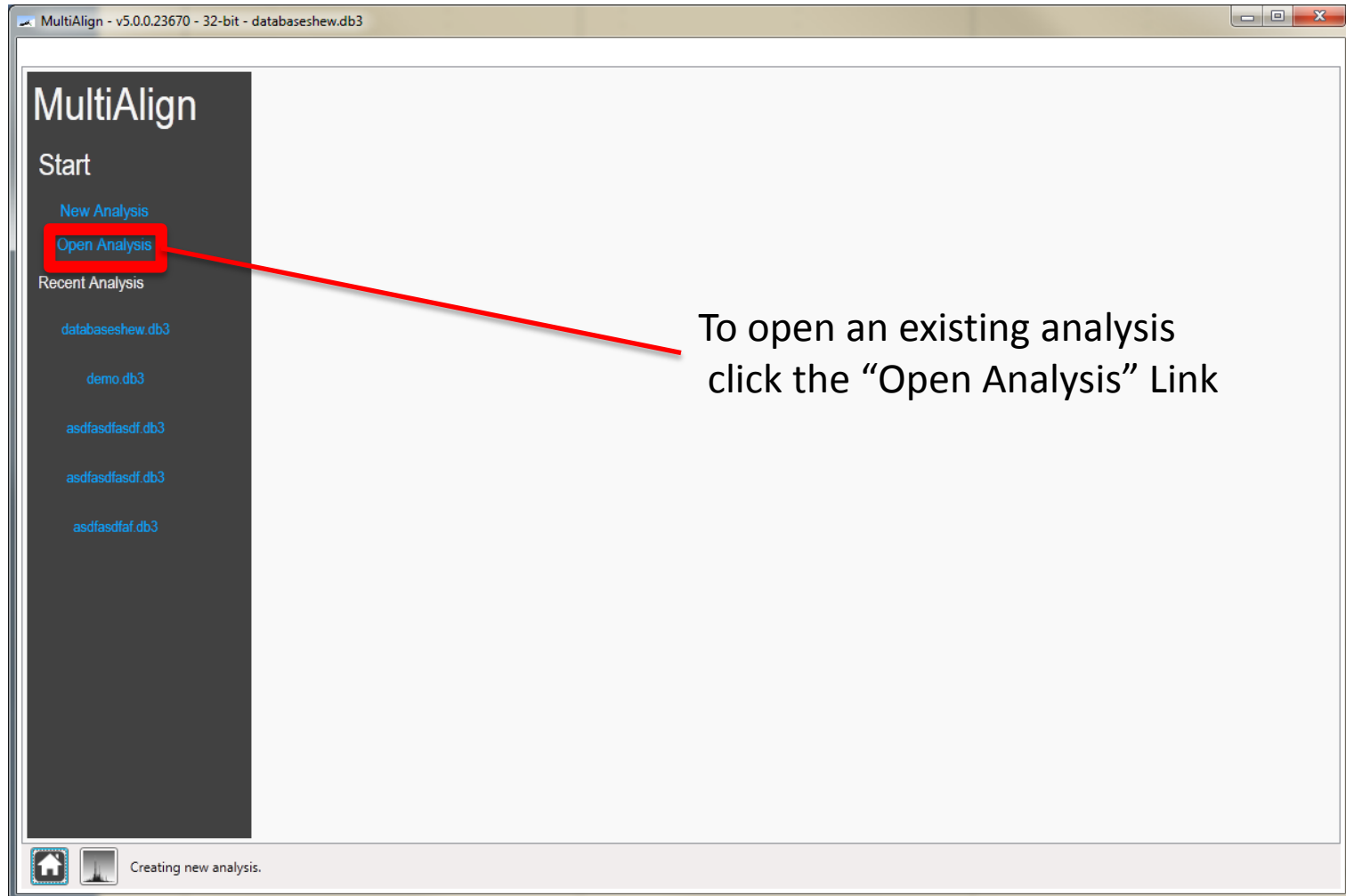


Reviewing An Analysis

GLOBAL VIEW OF ANALYSIS

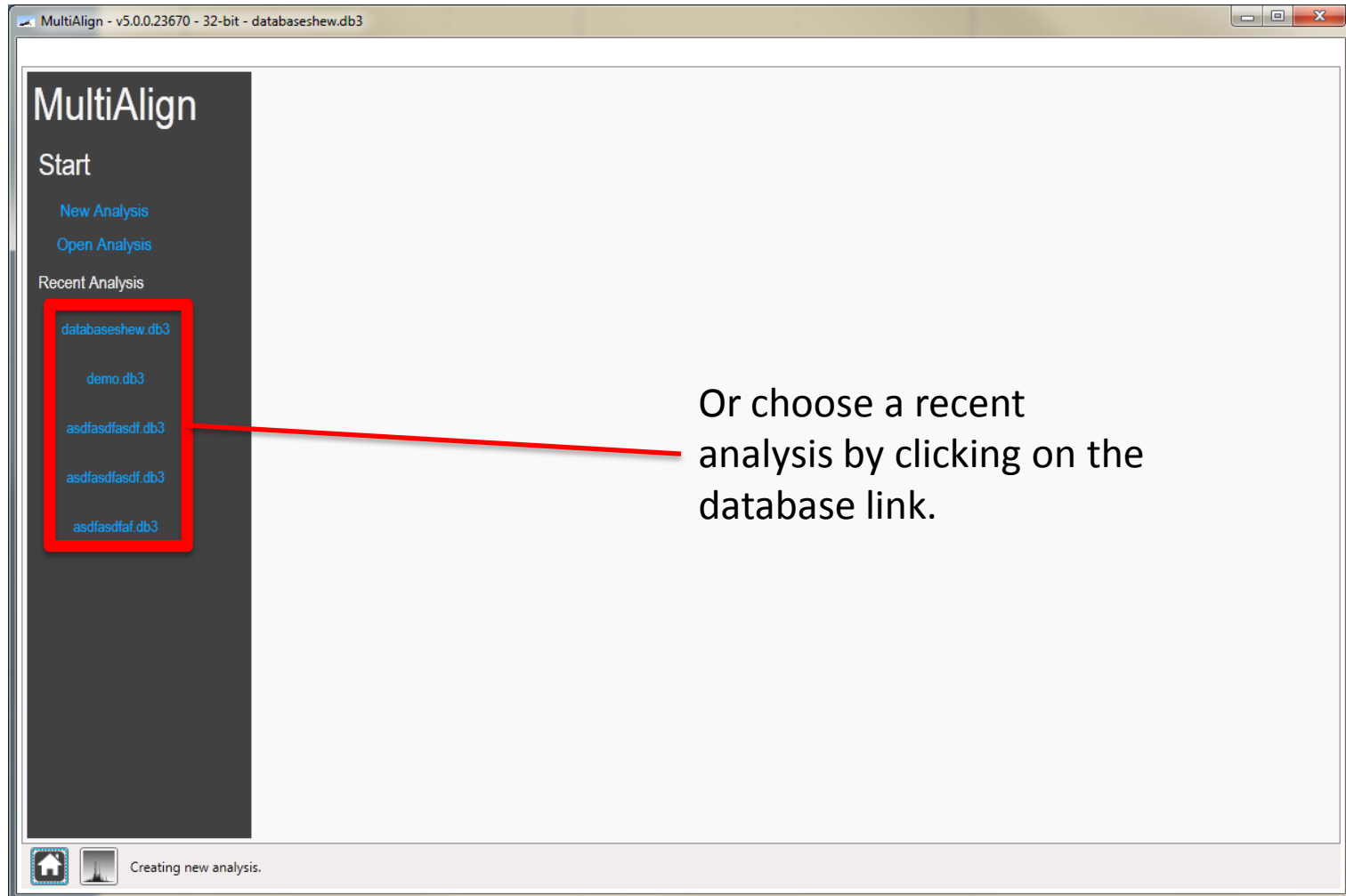


Open the analysis





Load Recent

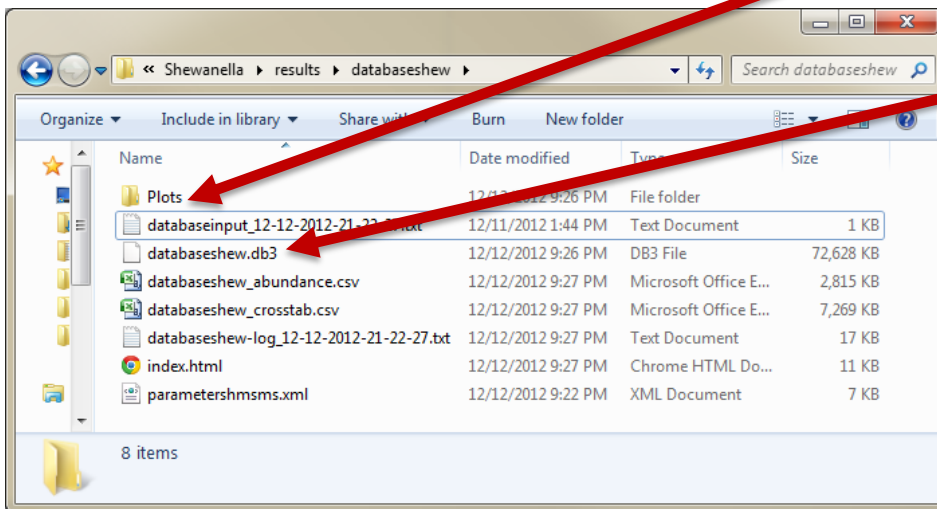


Or choose a recent analysis by clicking on the database link.

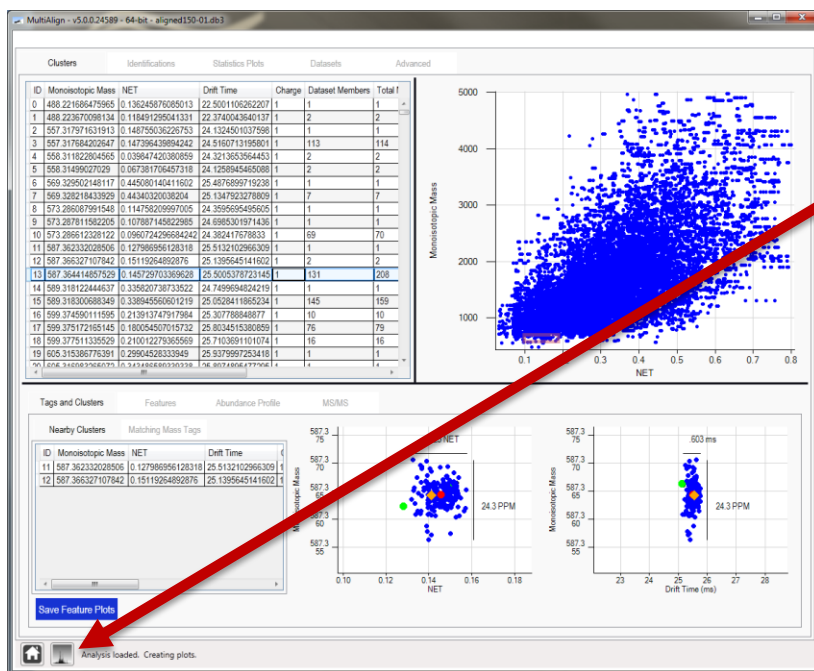


Review of output

- ▶ Remember that MultiAlign stores its results in a directory.
- ▶ Alignment related plots must exist in the Plots subfolder.
- ▶ The analysis database file should also exist.
- ▶ This is important later on because MultiAlign will search for these plots to create visualization elements in the GUI



- ▶ This is the main window that should appear
- ▶ If it does not, click on the **analysis button** at the bottom of the screen.





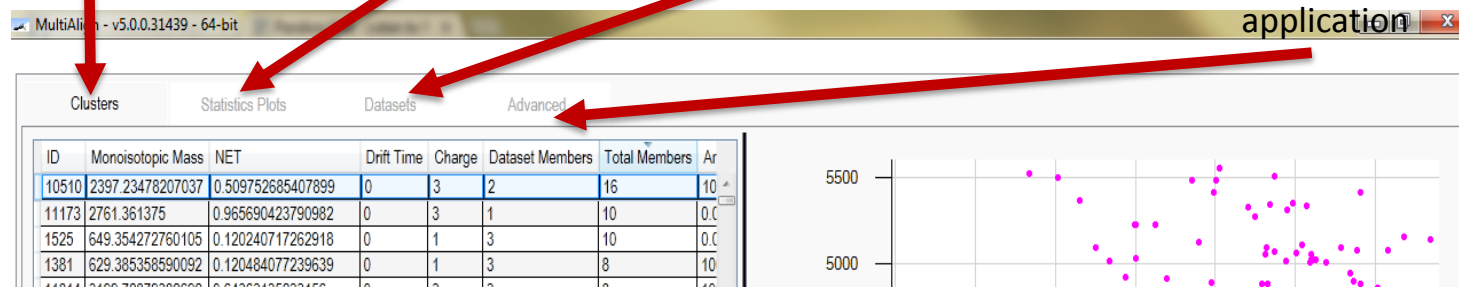
Analysis View – Major Views

Investigation of individual clusters

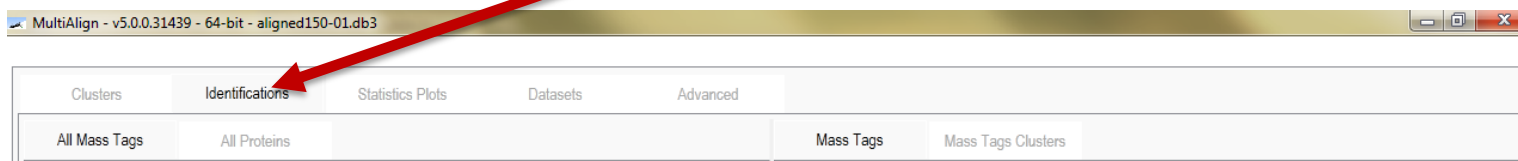
Global analysis statistics window

Dataset information

Configuration window for application



If a mass tag database is used in the analysis, this tab allows investigation of each mass tag





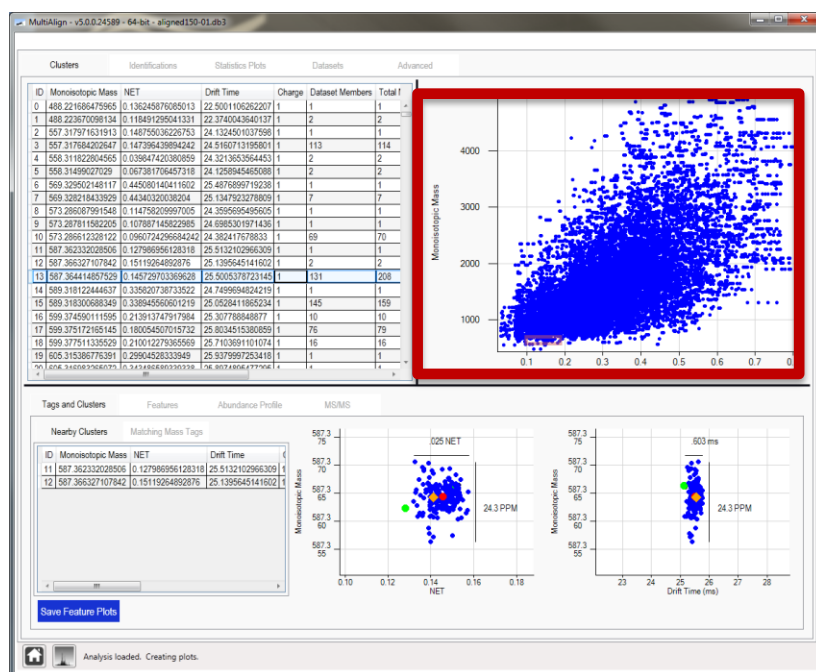
Investigating clusters

THE CLUSTER TAB AND SELECTED CLUSTER VIEWS



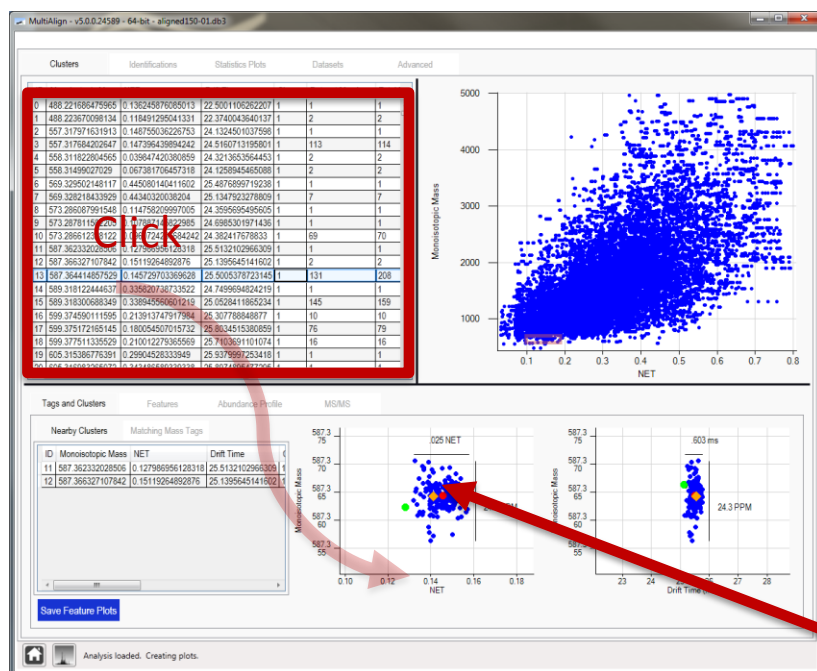
Cluster View

- ▶ The clusters scatter plot window displays all of the clusters found in the analysis.
- ▶ Each **blue** dot represents a feature found across multiple datasets.
- ▶ This plot is interactive





Cluster View



- ▶ The clusters data grid shows all detailed information about each cluster.
- ▶ The columns are sort-able by clicking on the column header
- ▶ You can also reorder the column orders by clicking on a column header and dragging to its new location
- ▶ Left clicking on a row in the cluster data grid will display the details about the cluster below



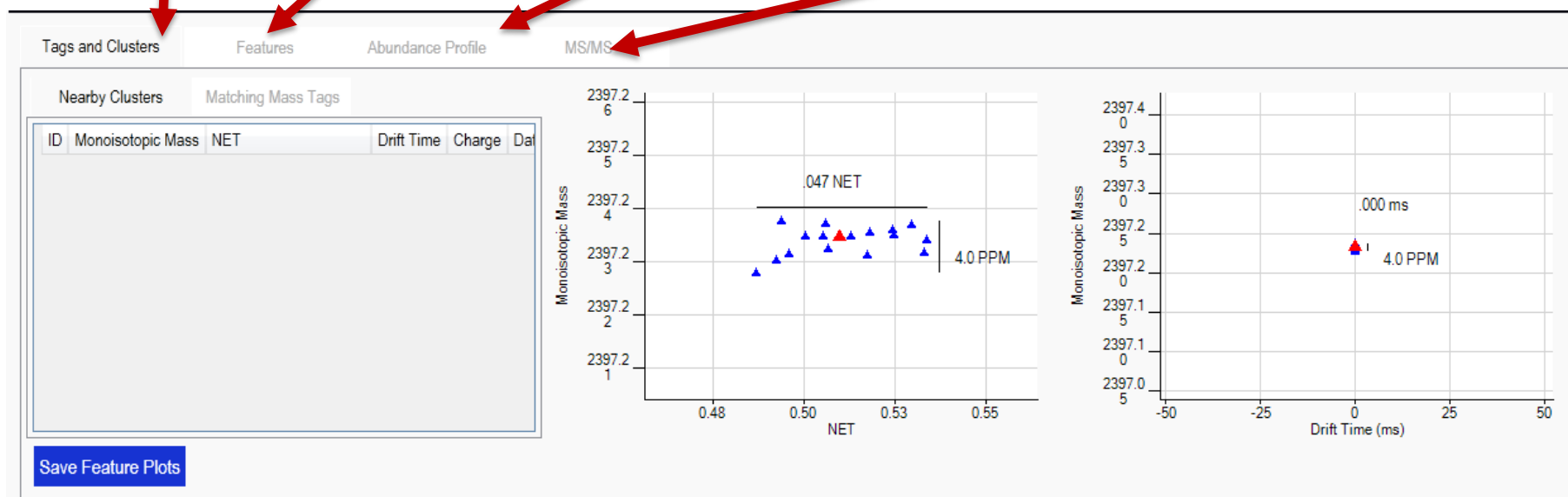
Selected Cluster View Basics

Visualization of features within cluster, matching mass tags, and nearby clusters

Detailed Information about each feature in the cluster

Abundance profile plots of cluster

Any MS/MS spectra plots associated with cluster



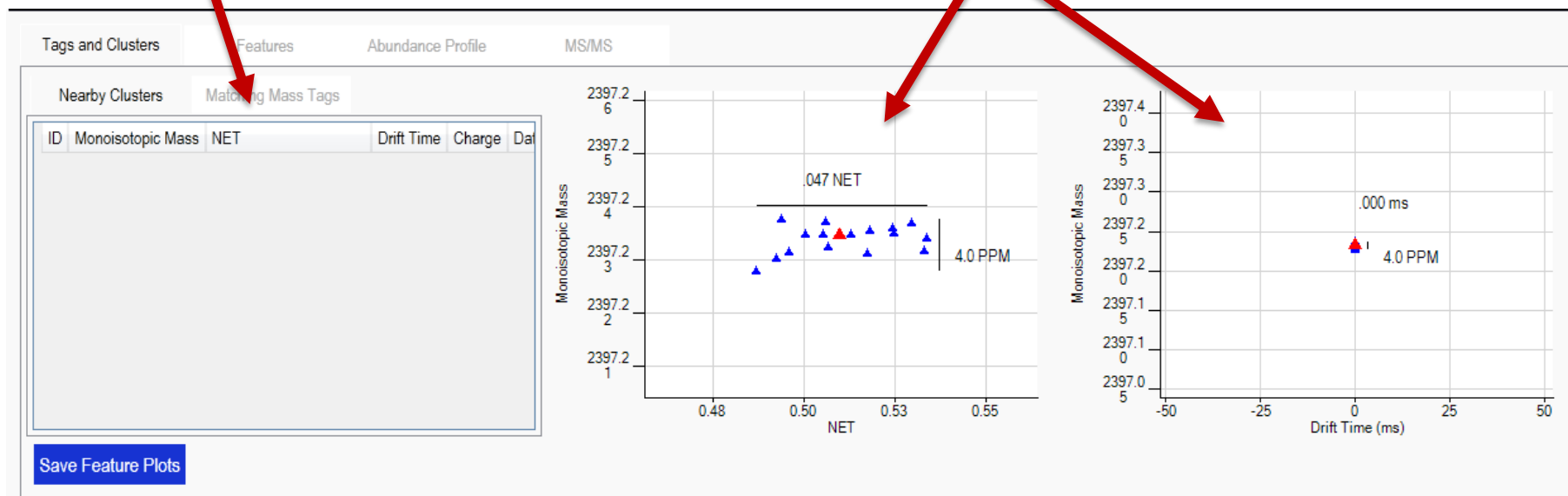


Tags and Clusters (1)

Data grid that shows any
matching mass tags or tags
that are nearby within 6 ppm
or .03 NET

Scatterplot that shows a cluster (**red**) and the features
that comprise the cluster (**blue**)

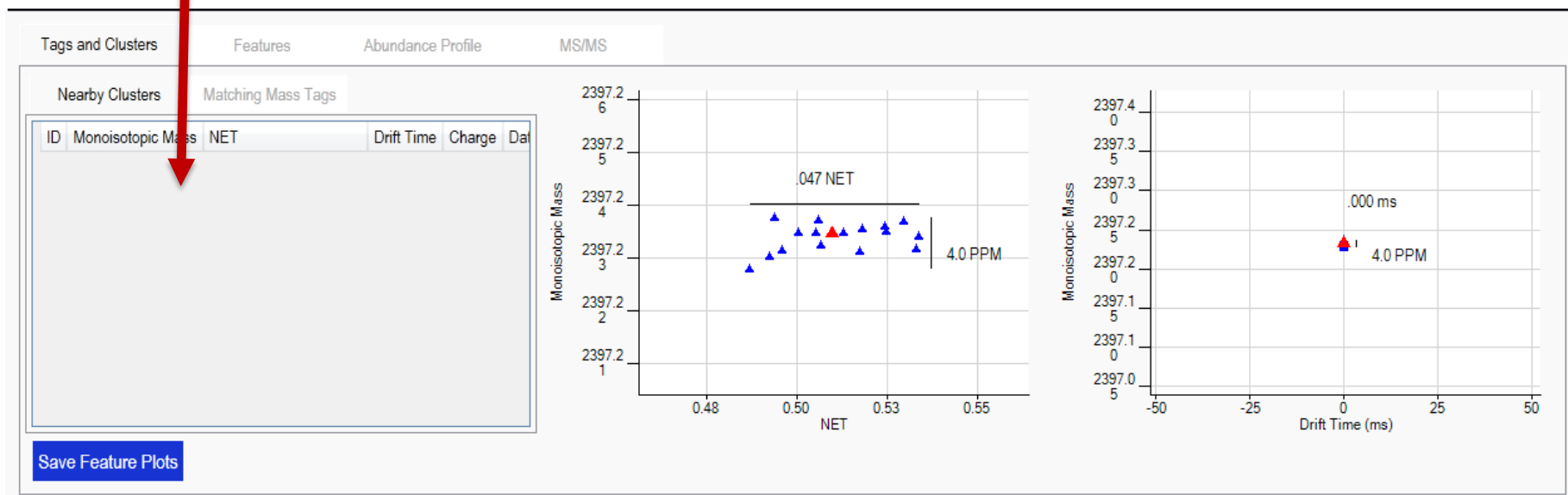
NOTE: Shape is based on charge state
NOTE: Drift time plot can be hidden in the analysis
Advanced Tab





Tags and Clusters (2)

Data grid that shows any
nearby clusters within a 6
ppm and .03 NET



Shape Charge

●	+1
■	+2
▲	+3
⛶	+4
✕	+5

LC-MS Features from selected cluster

LC-MS Features from nearby cluster
(hollow)

Selected Cluster Centroid

Nearby Cluster Centroid

Features and Cluster Centroids



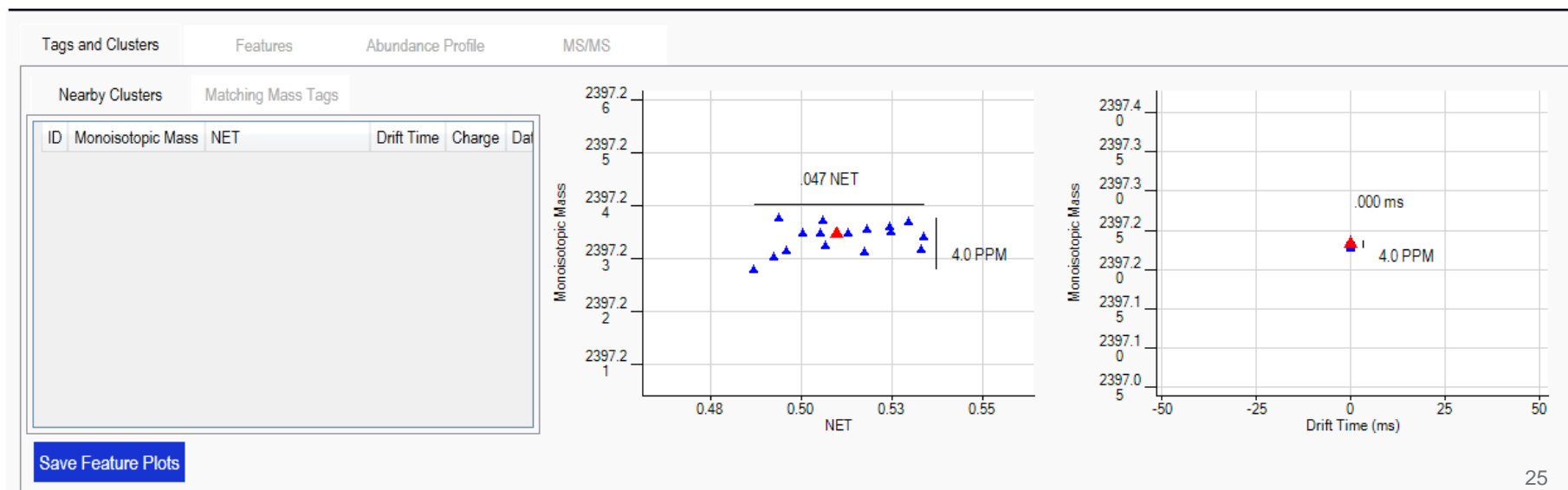
Matched mass tag

Unmatched mass tag nearby

Matched Mass Tag

LC-MS Features from nearby cluster
(hollow diamond) – nearby mass tag
unmatched

Mass Tags



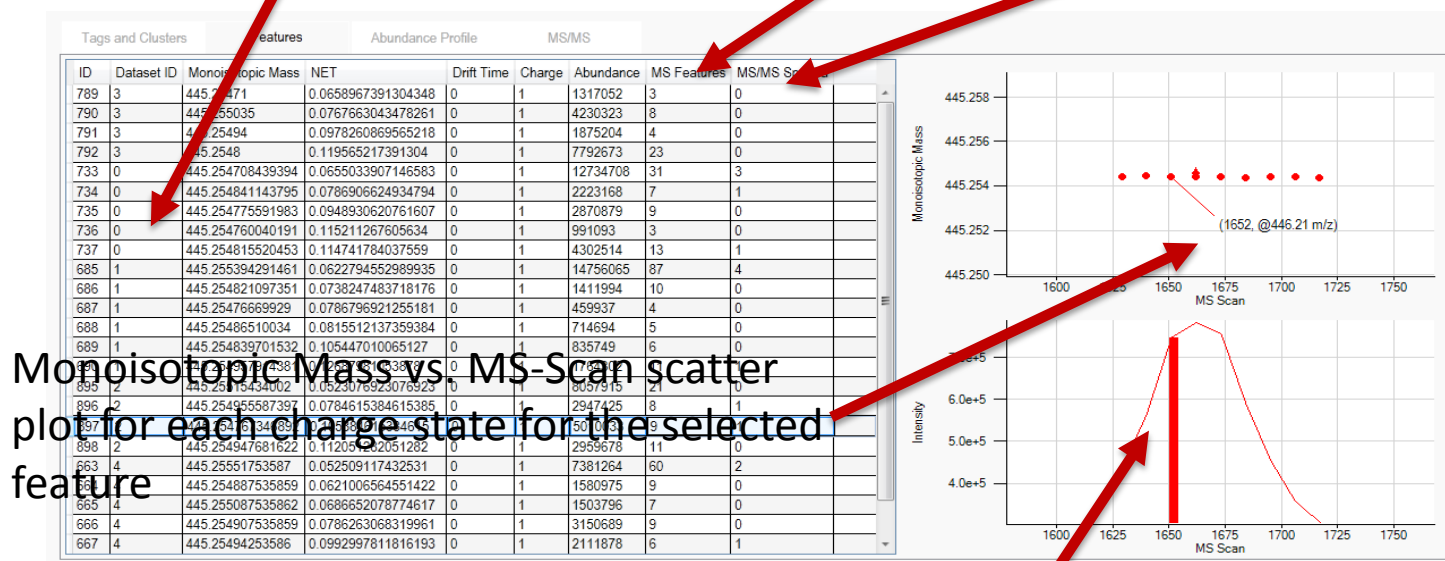


Features (1)

Detailed information about each feature in cluster (consensus feature)

Number of MS Features (i.e. number of parent scans) feature appeared in

Number of MS/MS spectra associated with feature



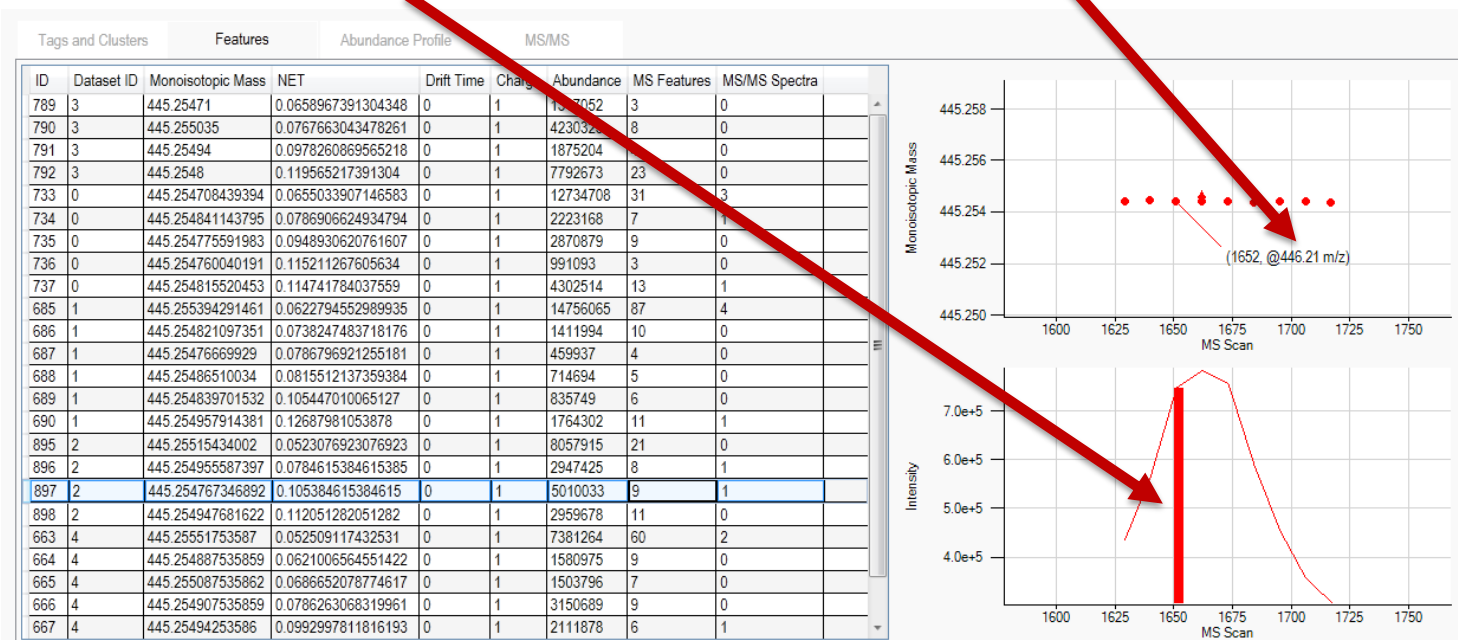
LC-chromatogram of each charge state (depicted by color) for the selected feature



Features (2) – MS/MS

Red bar displays
where on the LC-
chromatogram the
fragmentation
occurred

Pre-cursor m/z and
MS/MS scan



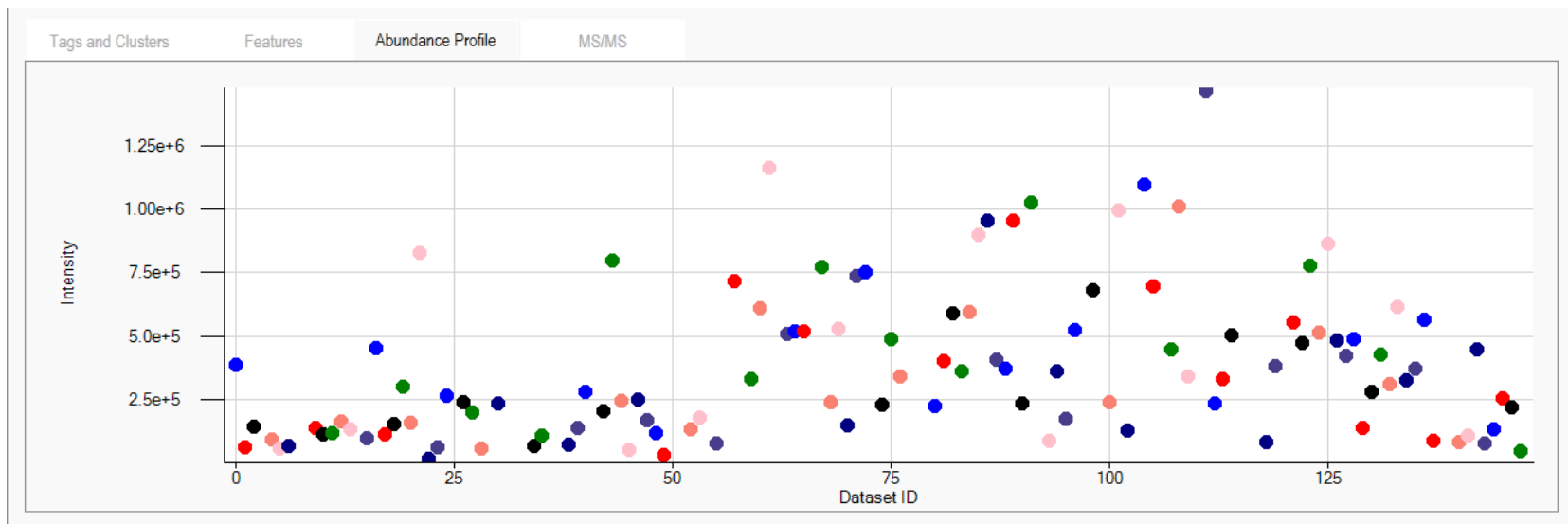


Abundance Profile

This slide currently shows the abundance (raw) from each dataset.
Each dot represents the abundance for a feature from the dataset id below.

This plot is interactive

Future feature: organize each dataset by factor (e.g. age or time point)

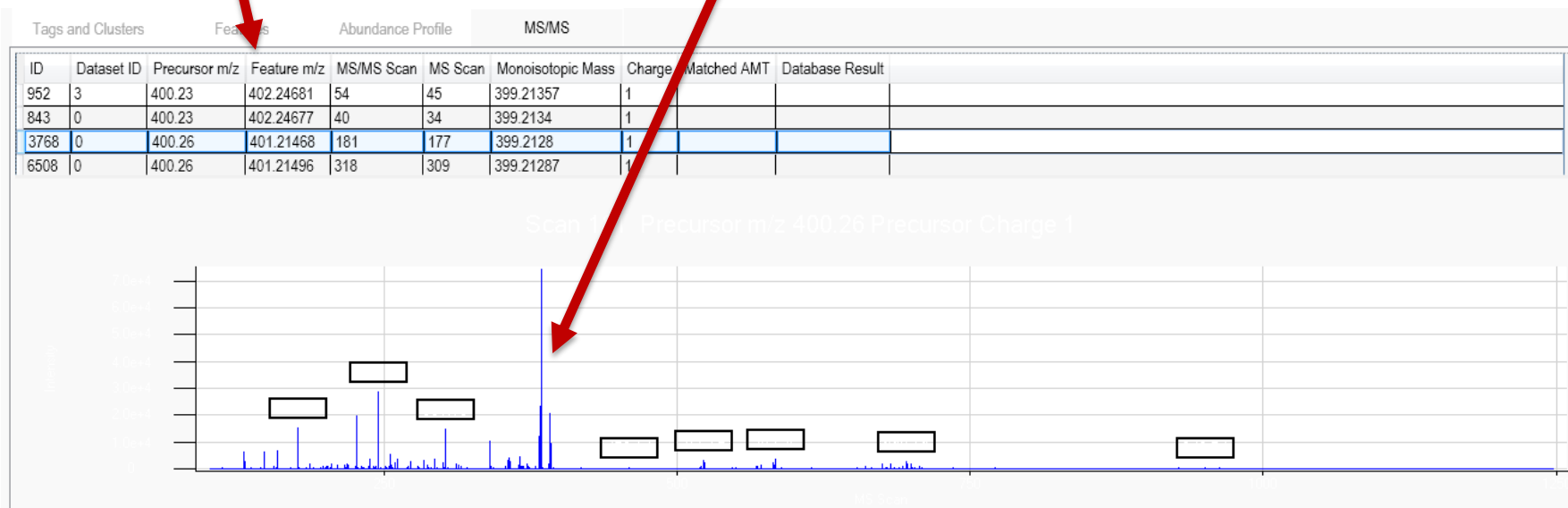




MS/MS Spectra

Information about all
MS/MS spectra
related to selected
cluster

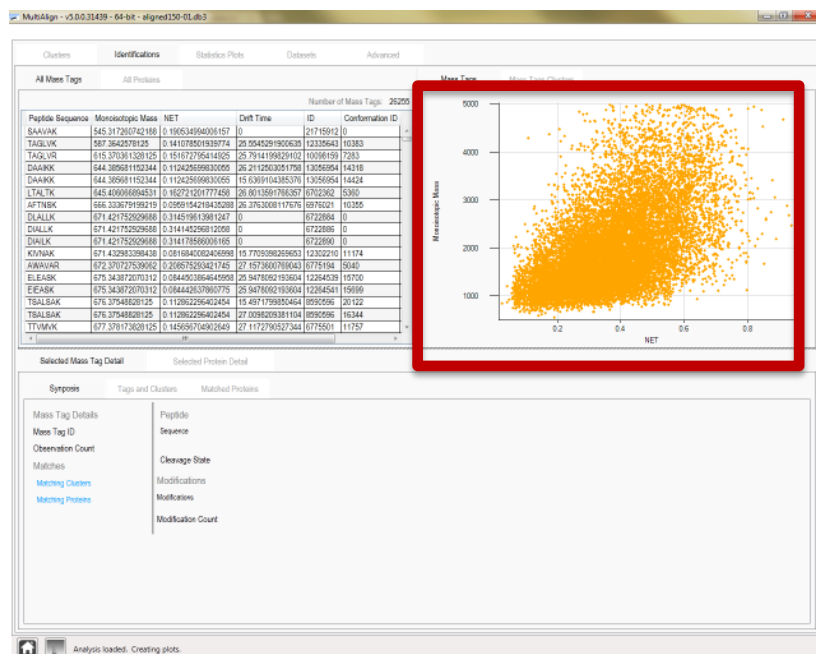
Full MS/MS spectra



Investigating mass tag matches

USING A MASS TAG CENTRIC APPROACH, FINDING HOW MANY CLUSTERS MATCH

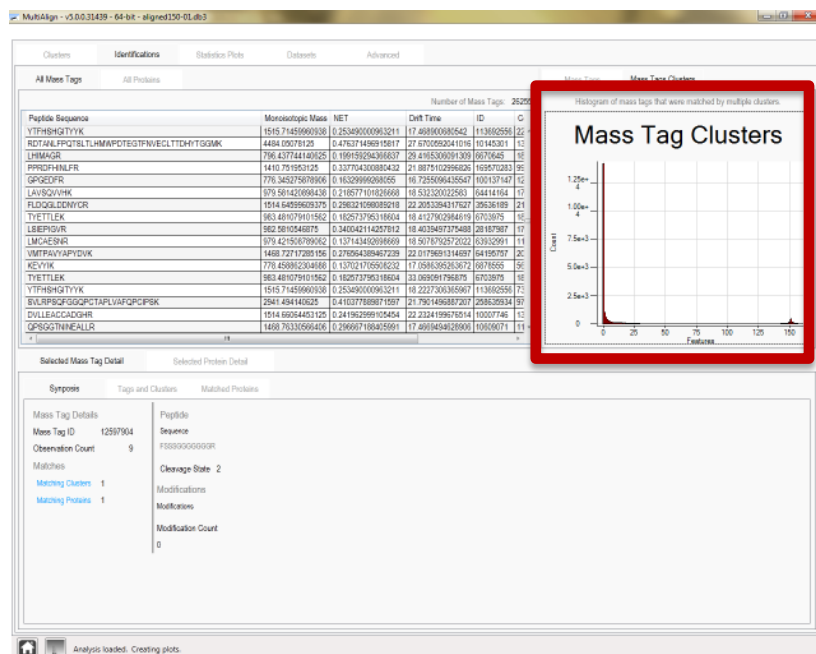
Mass Tag View (1)



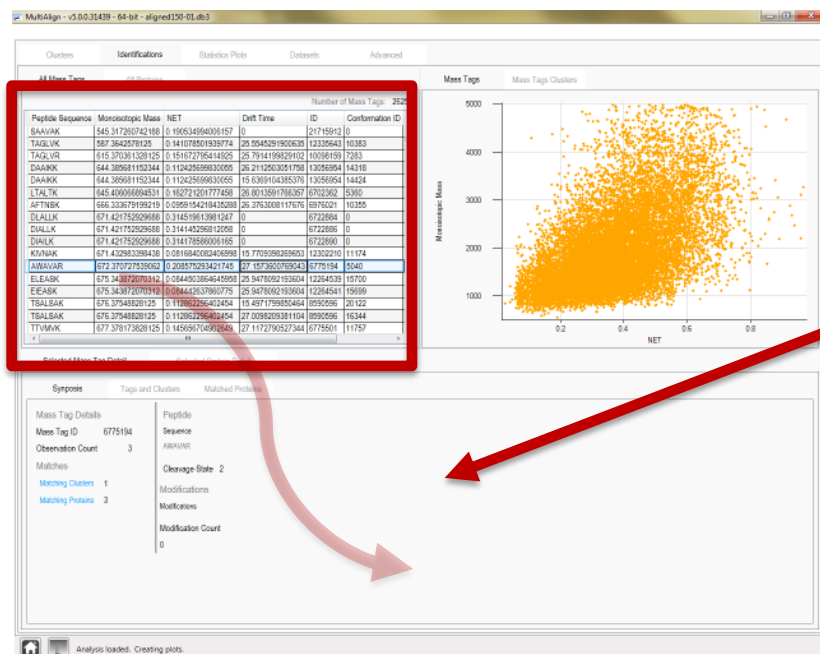
- ▶ The mass tag plot shows all mass tags present in the AMT Tag database used for alignment and/or peptide identification via STAC.
- ▶ Mass Tags are represented by orange diamonds.
- ▶ This plot is interactive

► The mass tag histogram shows how many features (e.g. a peptide from an individual dataset) to the mass tag.

► This plot is interactive



► The mass tag data grid shows detailed information about each mass tag.

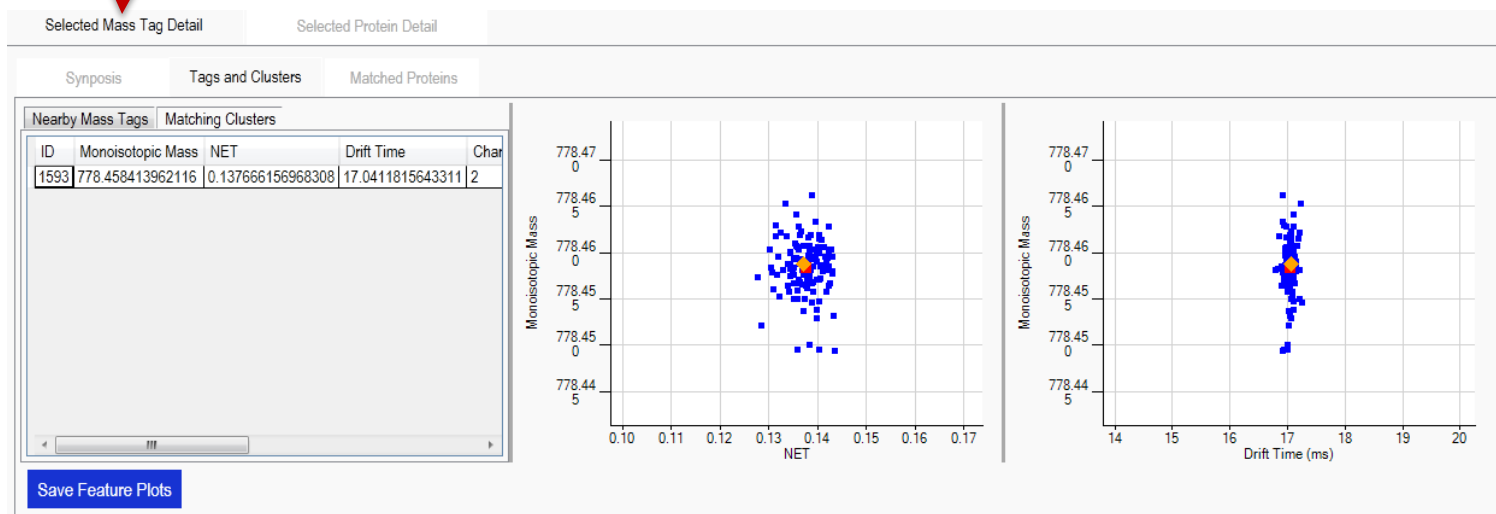


► Left clicking on a row in the mass tag data grid displays detailed information about the mass tag



Selected Mass Tag View

Detailed information about
the mass tag



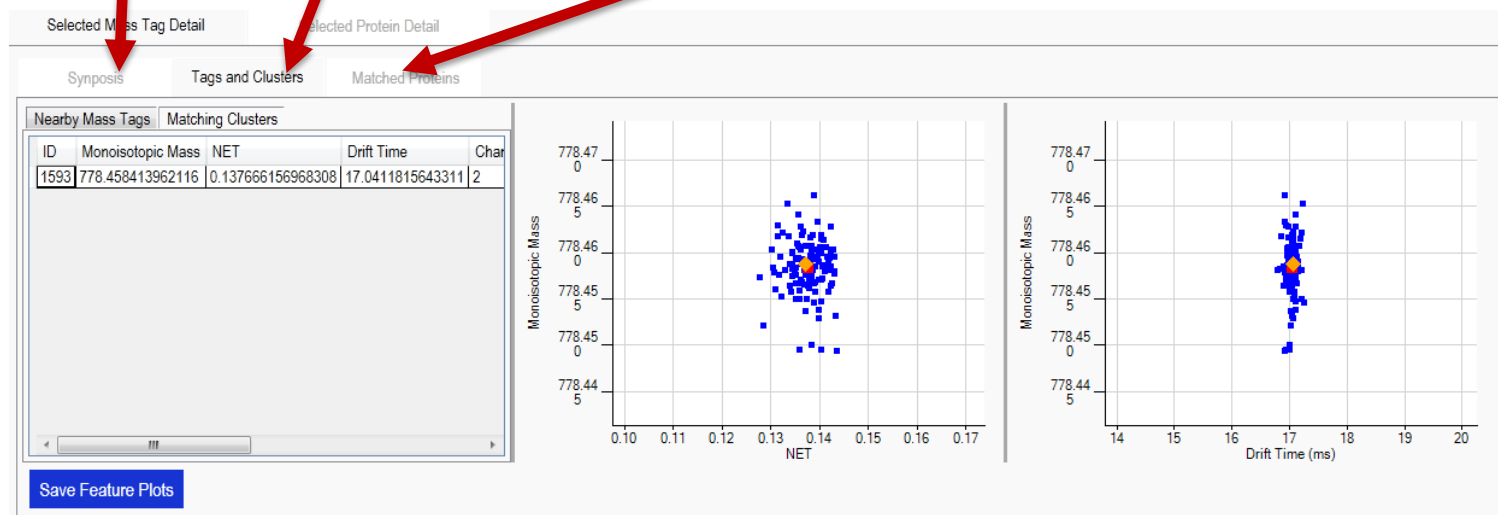


Selected Mass Tag Detail View

Synopsis about the mass tag

Visualization of mass tag and
matching clusters (shown
below)

Detailed information about
the protein the mass tag is
part of (proteomics only)





Mass Tag Synopsis

Quick information about the
mass tag

Peptide sequence information, cleavage states (2 =
fully tryptic, 1 = partially tryptic), and modification
detail

Selected Mass Tag Detail		Selected Protein Detail	
Synopsis		Tags and Clusters	Matched Proteins
Mass Tag Details		Peptide	
Mass Tag ID	6878555	Sequence	KEVYIK
Observation Count	9	Cleavage State	2
Matches		Modifications	
Matching Clusters	1	Modifications	
Matching Proteins	1	Modification Count	0

Matched protein and cluster detail. *A future feature is under development that allows users to drill back into cluster and protein detail.*



Mass Tag - Tags and Clusters (1)

Clusters that match to this
mass tag

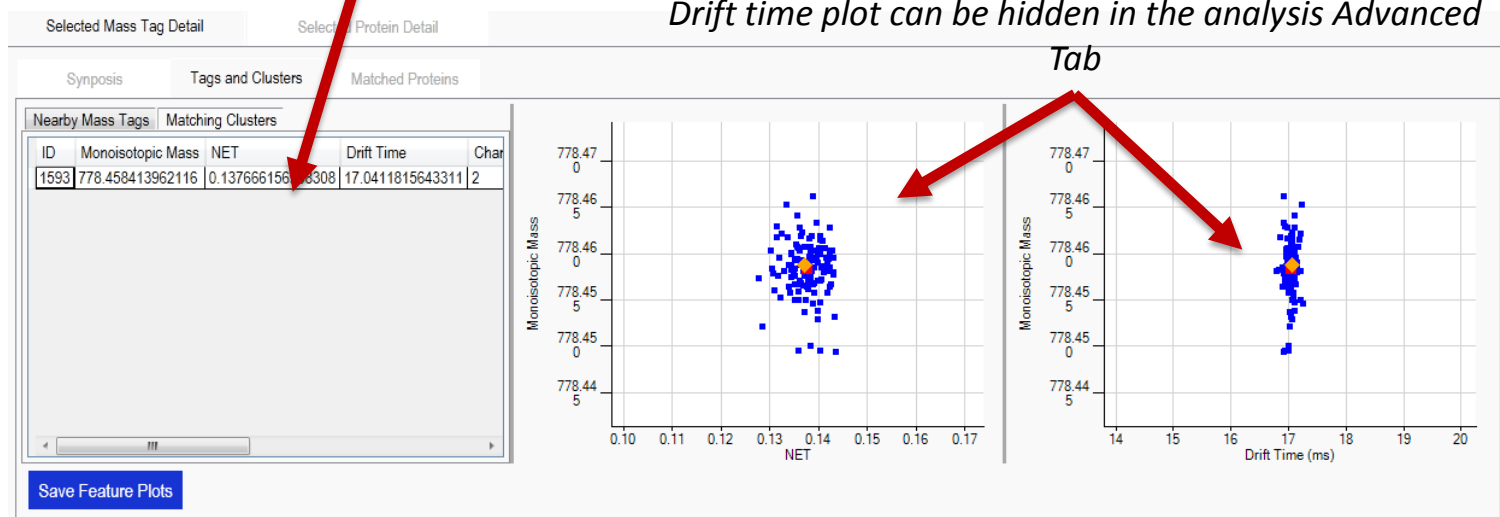
And on other tab, nearby
mass tags

Scatter plot that shows all matching clusters (**red**) and
their features that comprise the cluster (**blue**)

Selected mass tag is displayed as **orange** diamonds.
Nearby mass tags are displayed as hollow diamonds

*NOTE: Feature and Cluster shapes are based on charge
state*

Drift time plot can be hidden in the analysis Advanced



●	+1
■	+2
▲	+3
⛶	+4
✕	+5



LC-MS Features of matched clusters

LC-MS Features from nearby cluster
(hollow)

Matched Cluster Centroid

Nearby Unmatched Cluster Centroid

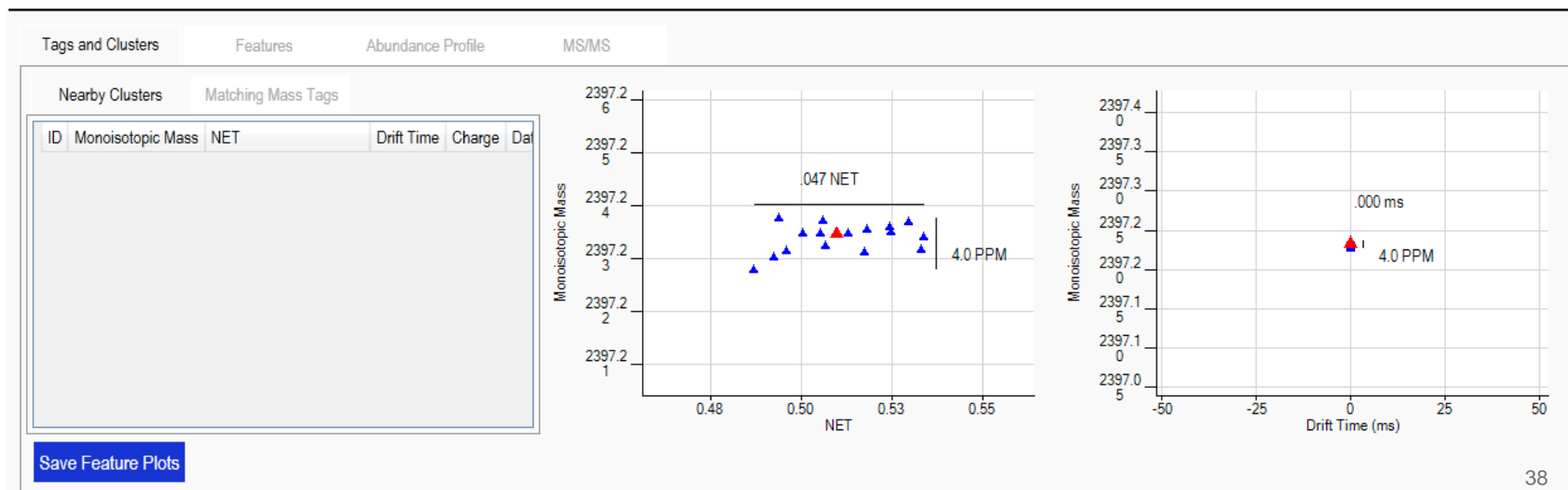
Features and Cluster Centroids

 Selected mass tag
 Nearby mass tags

Selected Mass Tag

LC-MS Features from nearby cluster
(hollow diamond) – nearby mass tag
unmatched

Mass Tags



Mass Tag – Matched Proteins

Details about the proteins that this mass tag rolls into. Shown here is multiple mass tags because the mass tag (peptide in this case) selected has multiple conformations.

Selected Mass Tag Detail				Selected Protein Detail	
Synopsis				Tags and Clusters	Matched Proteins
Protein ID	Ref ID	Description	Sequence		
5555720	2831		CO6_HUMAN		
5555720	2831		CO6_HUMAN		
5555720	2831		CO6_HUMAN		

Global Statistics Plots

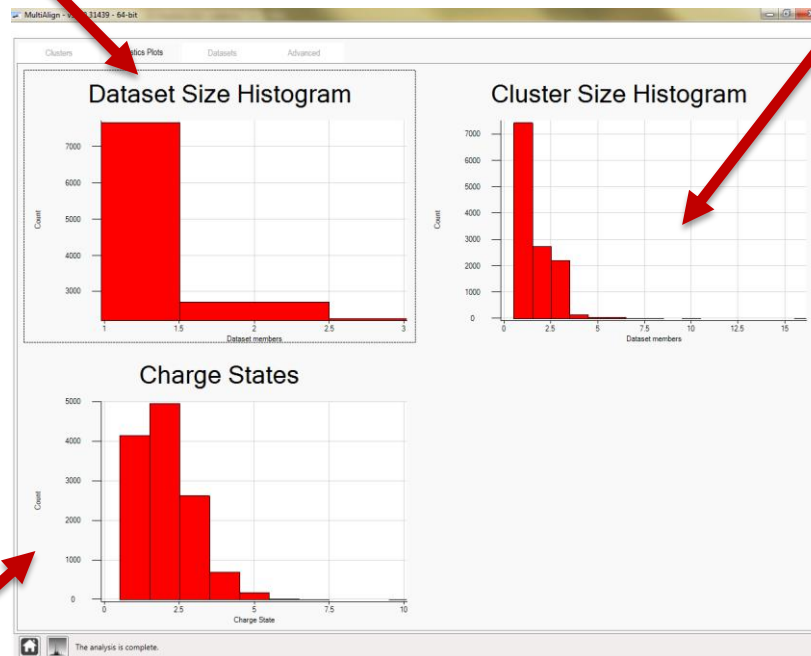
UNDERSTANDING THE GLOBAL STATISTICS



Global Statistics Plots (no AMT tag database)

Histogram of the number of datasets represented by each cluster

Histogram of the cluster sizes
(number of features per cluster)



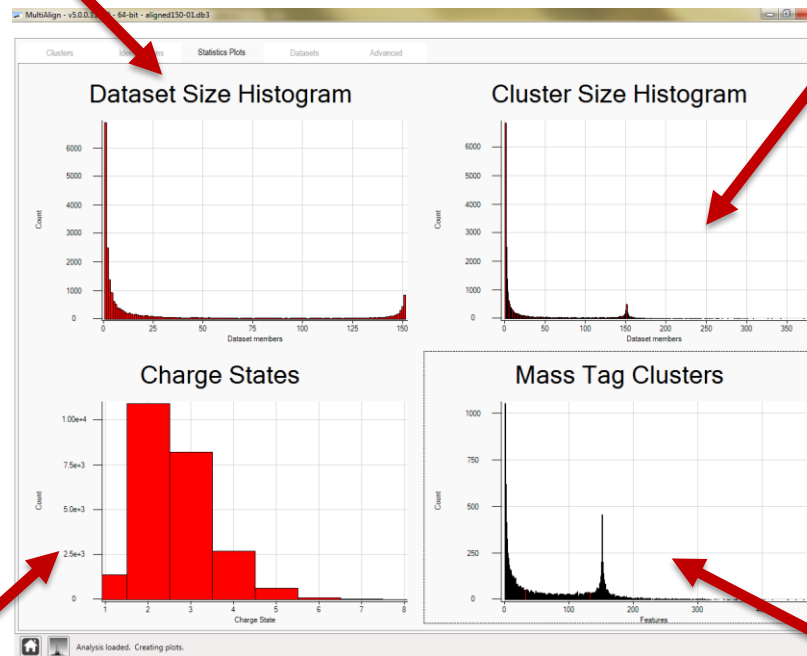
Histogram of charge states



Global Statistics Plots (no AMT tag database)

Histogram of the number of datasets represented by each cluster

Histogram of the cluster sizes (number of features per cluster)



Histogram of charge states

Histogram of mass tags that match to individual features (sum of cluster sizes for clusters that match to a mass tag)

NOTE: The differences in the plots is from analyzing two different datasets and the total number of datasets (150 for this slide vs. 3 for the previous)

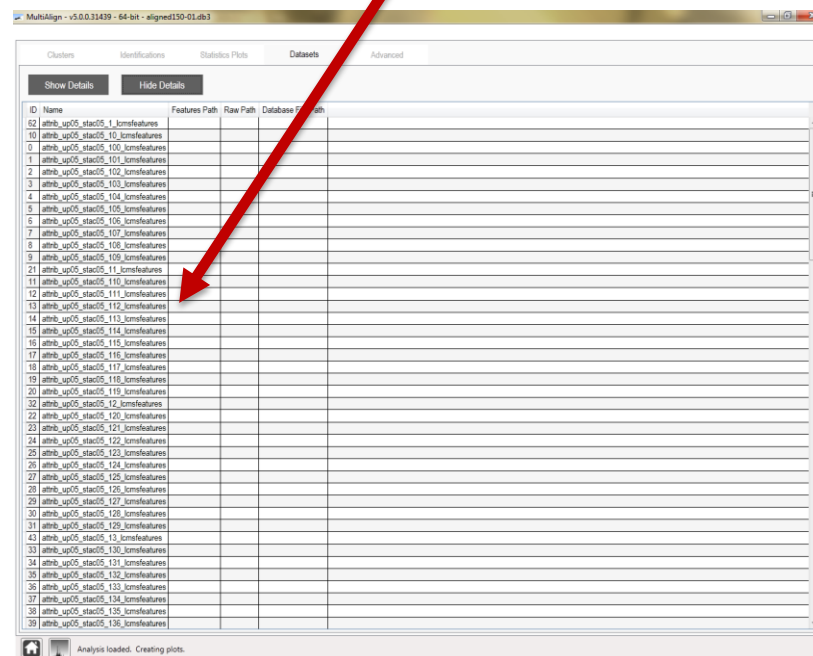
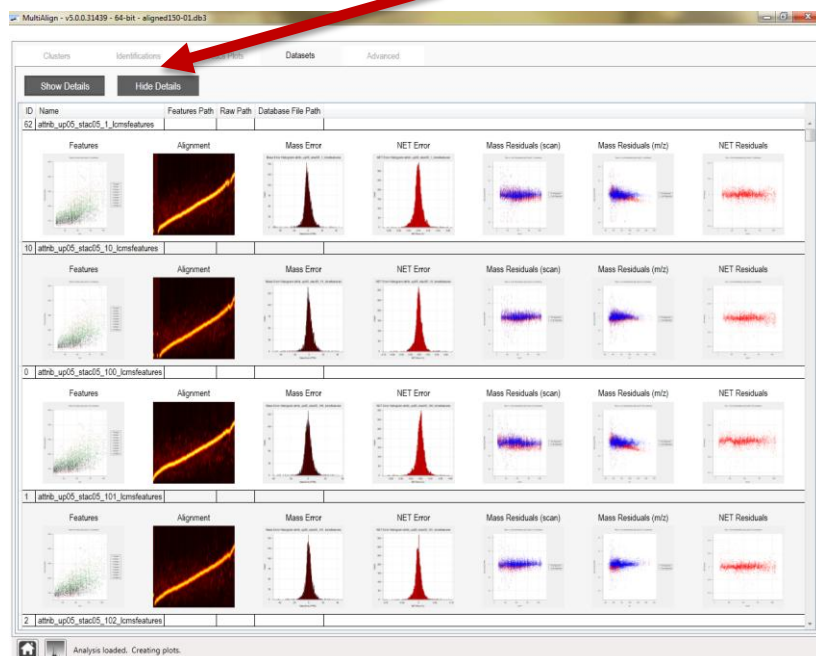


Datasets Plot

DATASET INFORMATION

Allows the user to toggle
between dataset plot view and
data grid mode

Collapsed view of each dataset

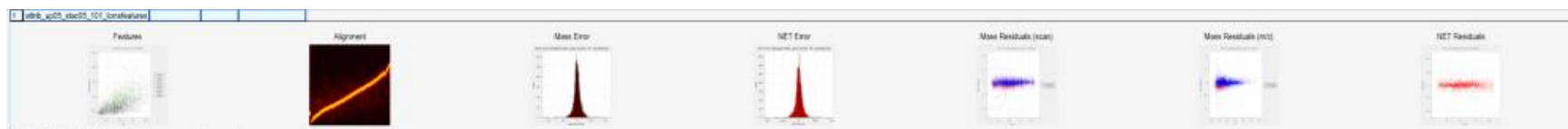




Dataset Plot View

These plots are created from the high resolution images stored in the Plots directory where the database was created.

Future feature will be to make these plots interactive





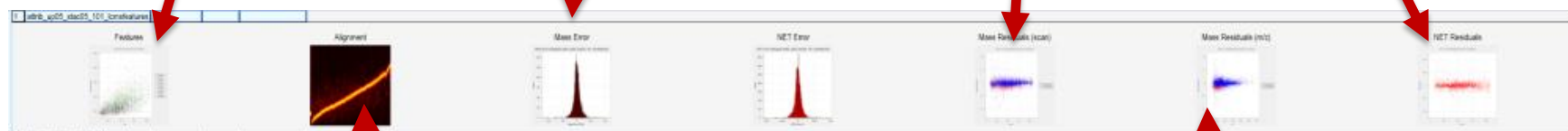
Dataset Plot View

LC-MS Feature Scatter plot

NET vs. scan residuals

Mass error histogram

Mass vs. Scan Residuals



LCMSWarp alignment heatmap

Mass (m/z) vs. scan residuals

NET error histogram



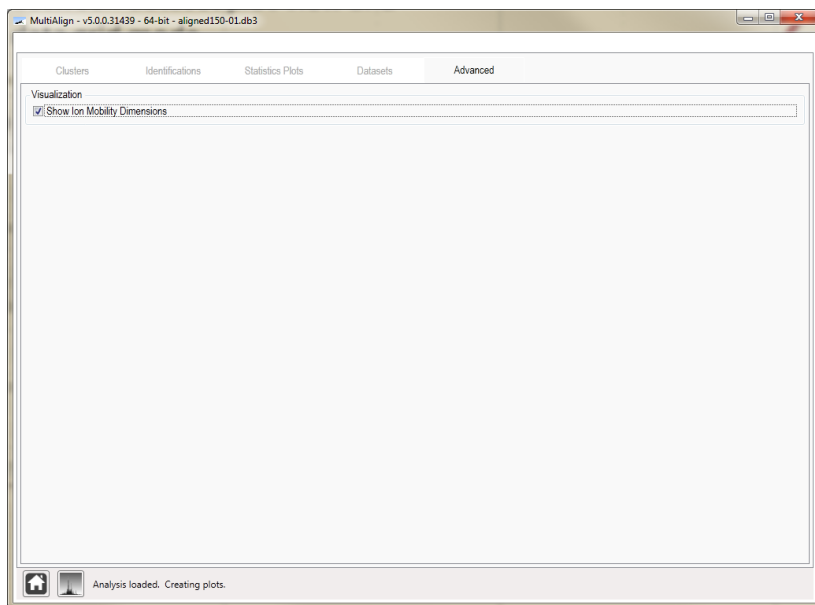
Advanced Tab

APPLICATION CONFIGURATION



Advanced View

- ▶ This view allows you to customize the application and visualization defaults.
- ▶ This view currently has a minimal set of features displayed.



► For more information see the MultiAlign website:

<http://omics.pnl.gov/software/MultiAlign.php>