Wiley Spectral Webinar
Part IV: Advanced NIST Hybrid Search of EI and MS/MS Spectra

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- *Retired* Research Fellow, Eastman Chem. Co.*
- *42 years experience unknown identification*
- *Now Consultant, MS Interpretation Services*
- *Specialties[1] EI GC-MS, LC-MS/MS, Chemical Ionization,[7] Accurate Mass, Derivatization,[8,9] MS library management, SciFinder,[10] Chemspider,[10] Surfactant ID,[11] NMR, GC-IR, organic synthesis, matrix ionization effects,[21] etc.*

Eastman Chemical Company, Main Site, Kingsport, TN
50 Manufacturing Sites Worldwide, ~14,500 Employees

>50 Mass Specs Networked
Worldwide

* https://en.wikipedia.org/wiki/Eastman_Chemical_Company

Wiley Webinar Series on Effective Use of Mass Spectral Libraries

Part I: Spectral Searches[2] with NIST MS Search
Part II: Structure Searches[2] with NIST MS Search and Using MS Interpreter[2,13-15]
Part III: AMDIS[3,4,12] (NIST) for Processing EI Mass Spectral Data Files
Part IV: Advanced NIST Hybrid Search[16-19,22] of EI and MS/MS Spectra
Part V: Creating and Sharing[5] User EI and MS/MS Libraries

Note:[20] Handouts for ***All*** Sessions ***Now Online***!
Google Search "little mass spec and sailing"

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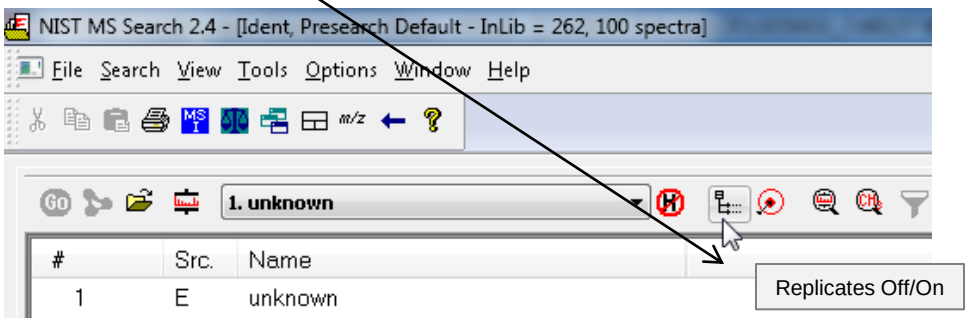
Part IV: Advanced NIST Hybrid Search of EI and MS/MS Spectra[16-19,22]

Webinar Topics:

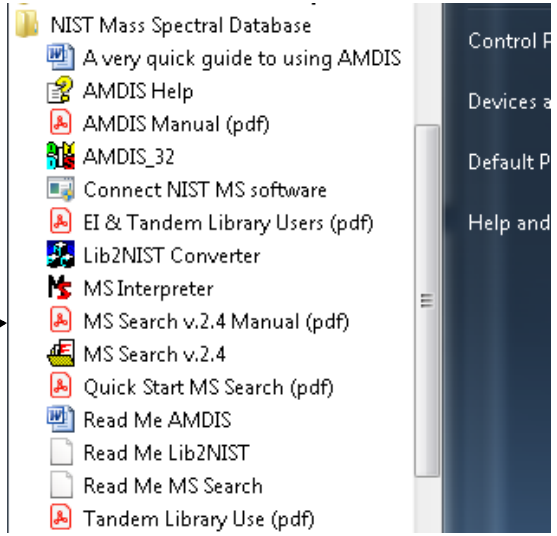
- Description
- Setting up search parameters, indices, and displays
- Example of unknown identification with hybrid
- Comparison of “hybrid” EI to “Simple (standard) Identity Search
- DeltaMass table and examples
- Use of additional information in process
- Information on NIST MS/MS (Tandem) “hybrid” search
- Wiley MS/MS (Tandem) Library for Identity MS/MS searches

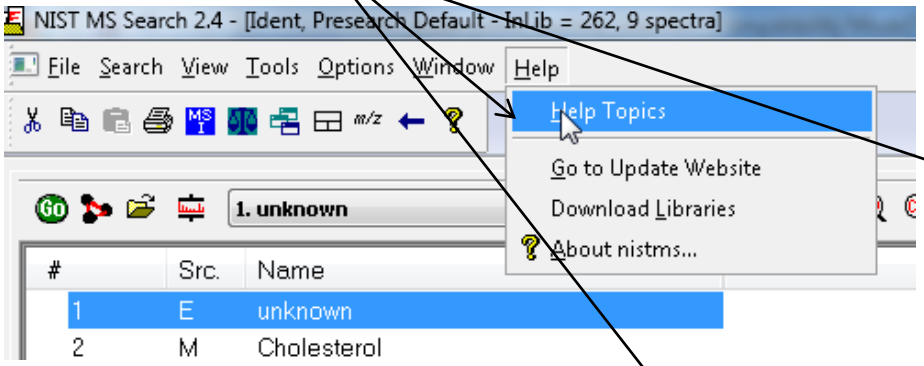
Help Files for NIST Search

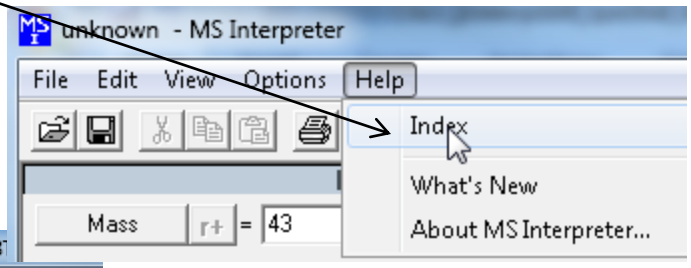
-"Hover" over Program Icon with mouse and function description displayed

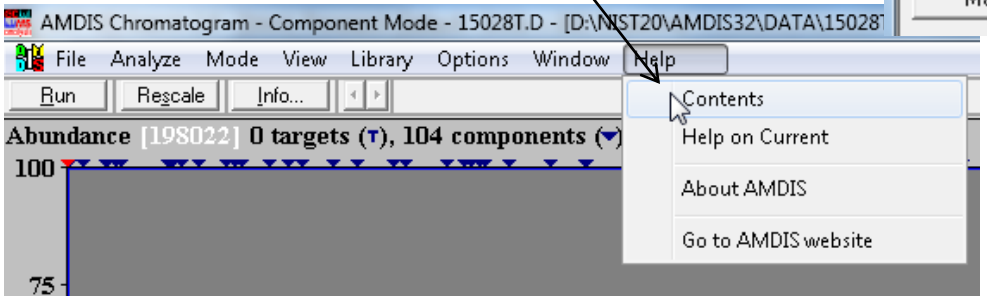


-Detailed documentation for NIST Search[2] and AMDIS[3,4,12]
-MS Interpreter included in NIST manual[2] and in posters[13-15]
-Windows Program Group
-"In program" assistance for all three programs



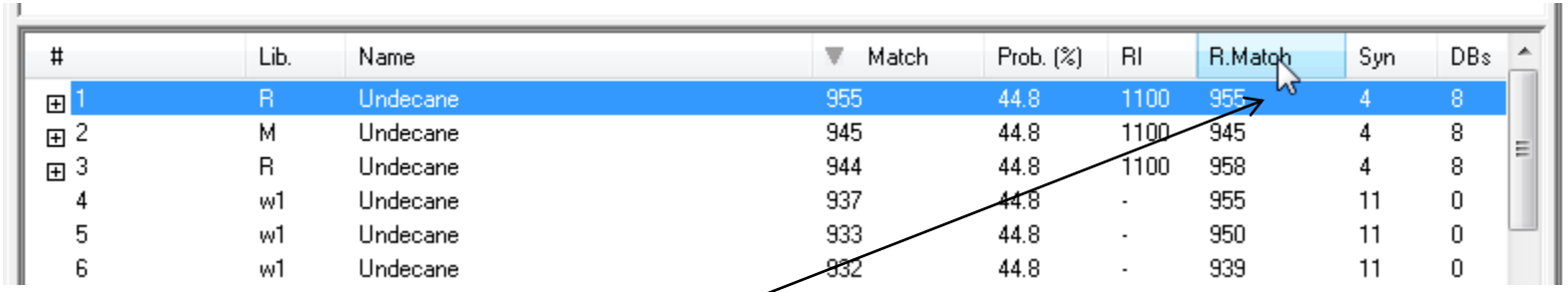






NIST Software in General is “Windows Compliant”

-***left click (LMB)*** to select an item, ***double LMB*** on that item to perform operation
-***right click (RMB)*** in area or item to see operations that can be performed or to change properties of window
-***LMB*** on first item and last item to select group ***while*** holding ***shift key***
-***LMB*** to select/deselect individual items ***while*** holding ***Ctrl button***
-use up and down arrows ***on keyboard*** to step between entries
-some NIST windows such as librarian have no delete button to delete ions, ***must*** use delete key on keyboard!
-***control a*** (select all), ***control x*** (delete selected), ***control c*** (copy); ***control v*** (paste)
-***control k*** copies entries into windows in tab-separated text format, e.g., ***paste*** into Excel
-F1 MS Search help
-F9 send spectrum to MS Interpreter
-***LMB*** and ***zoom*** mass spectral windows, ***RMB*** then ***LMB*** to ***zoom out***



#	Lib.	Name	Match	Prob. (%)	RI	R.Match	Syn	DBs
1	R	Undecane	955	44.8	1100	955	4	8
2	M	Undecane	945	44.8	1100	945	4	8
3	R	Undecane	944	44.8	1100	958	4	8
4	w1	Undecane	937	44.8	-	955	11	0
5	w1	Undecane	933	44.8	-	950	11	0
6	w1	Undecane	932	44.8	-	939	11	0

-***LMB*** on column of interest
-Can sort in lower value first or higher
-Will show use in mixtures in example later in presentation

Part IV: Advanced NIST Hybrid Search of EI and MS/MS Spectra[16-19,22]

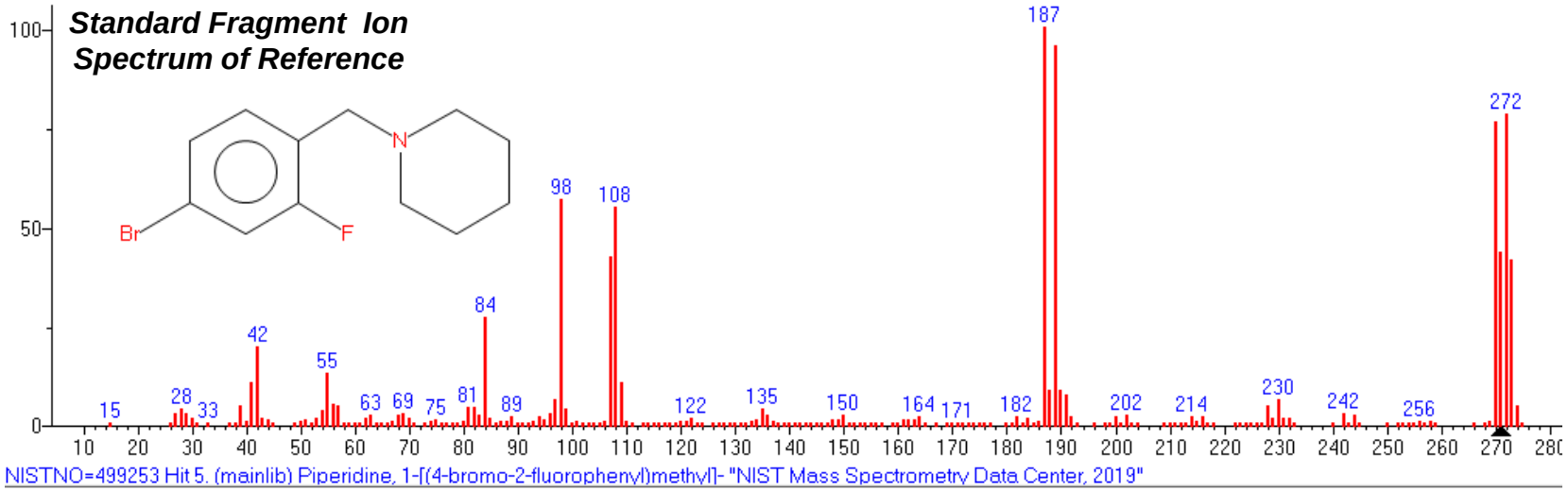
Program Description:

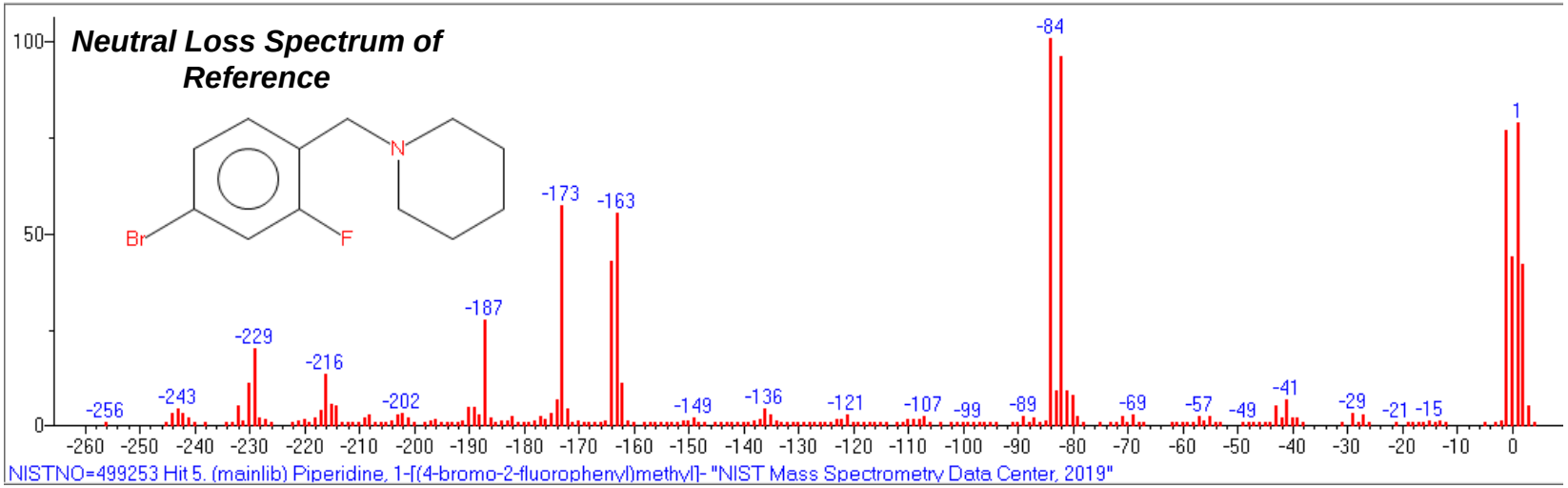
- Hybrid search generates a similarity score matching fragments ***and*** neutral losses
- Extends the scope of the library by including “nearest neighbor”
- Success requires the presence of similar compounds in the library
- Mass difference must be confined to a single region of molecule and no significant alteration of fragmentation behavior
- *DeltaMass* is the mass difference between query and library compound and reflects the modification of the molecule

My Personal Experience:

- Used for over 20,000 searches in 2 years
- *Routinely amazed* by the types of similar compounds with high match factors
- Very frequently useful results not noted in “simple” (standard) similarity search
- Utility in finding similar model compounds, support for fragmentation mechanisms, and possible identification of unknowns

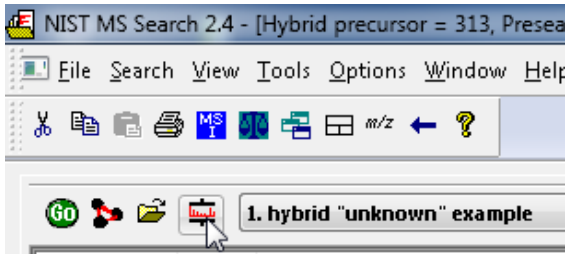
Hybrid Generates a Similarity Score on Matching *Both* Fragment Ions and Neutral Losses of Unknown to Library Reference Spectra[16-19,22]

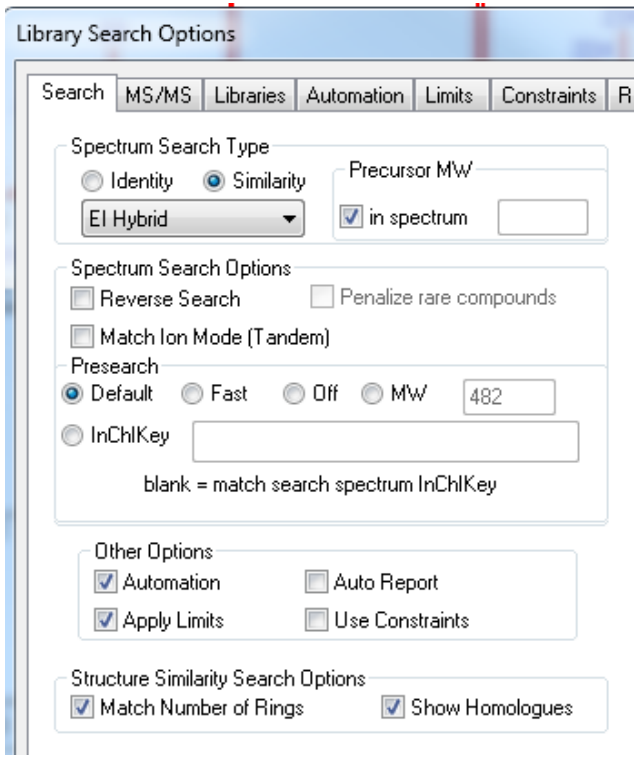


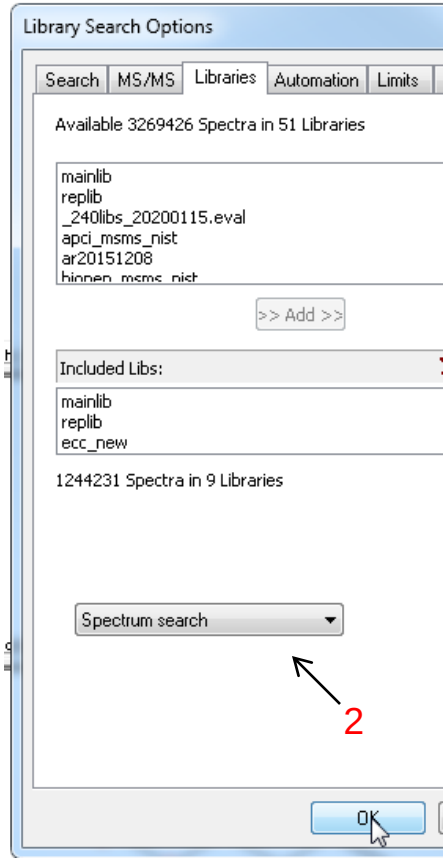


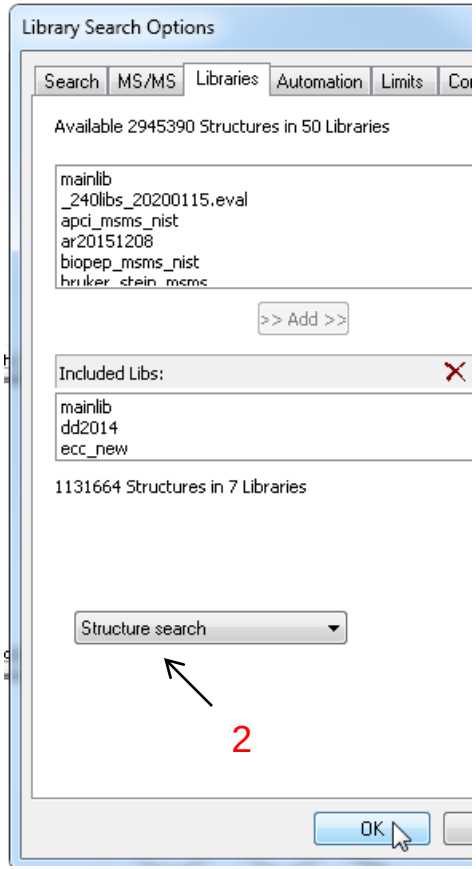
Setting Up Search Parameters for EI Hybrid Search (pg 1)
Very Similar to Those in "Simple" EI Similarity

1) LMB Library Search Options
2) Setup ***both*** Structure and Spectrum search





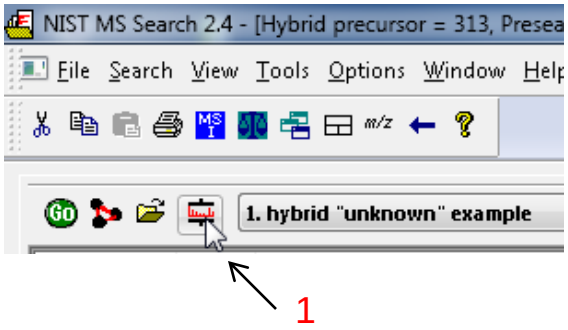


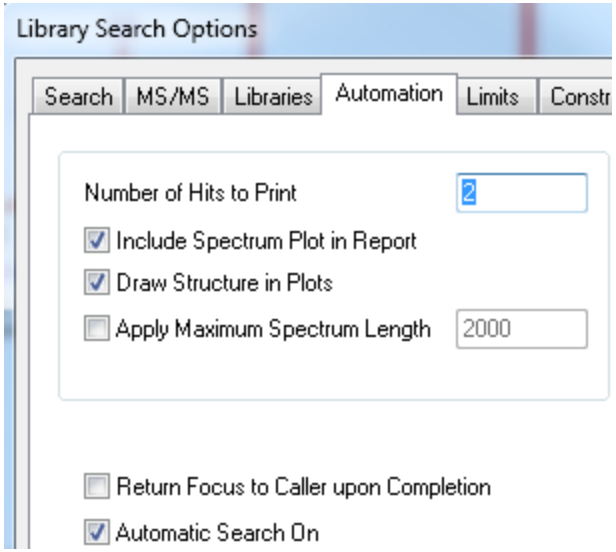


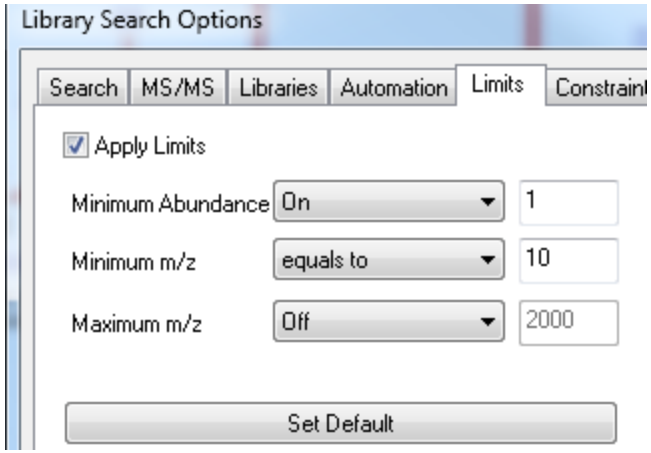
Note: In previous Version 2.3 (2017), "EI Hybrid" was just "Hybrid"**!**

Setting Up Search Parameters for EI Hybrid Search (pg 2)
Very Similar to Those in "Simple" EI Similarity

1) ***LMB*** Library Search Options

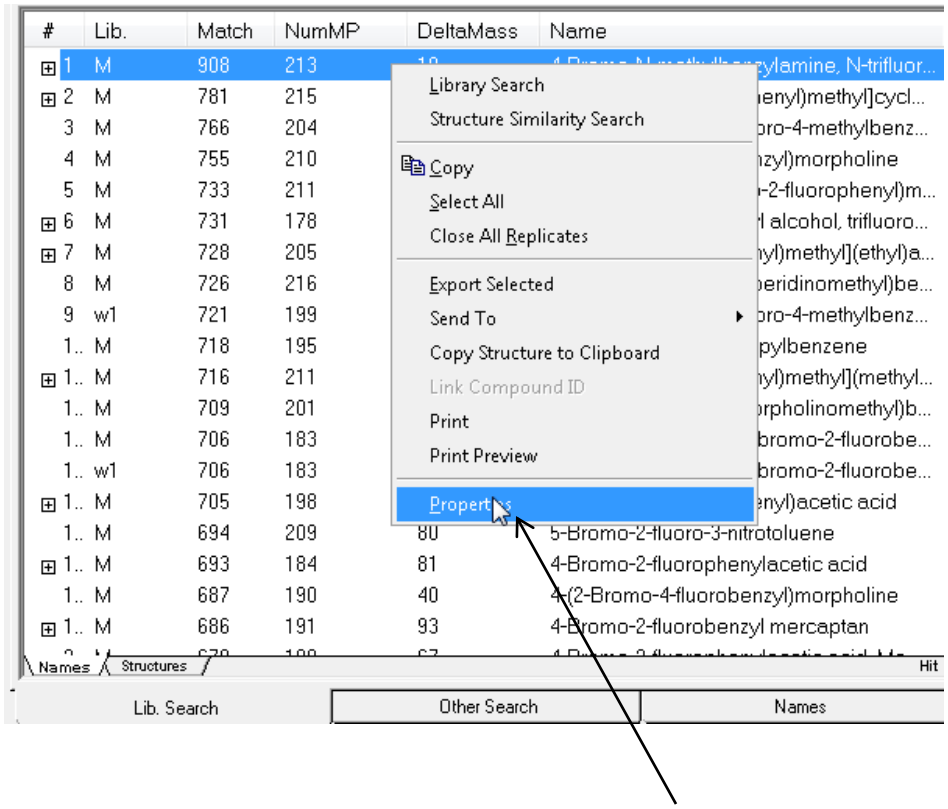


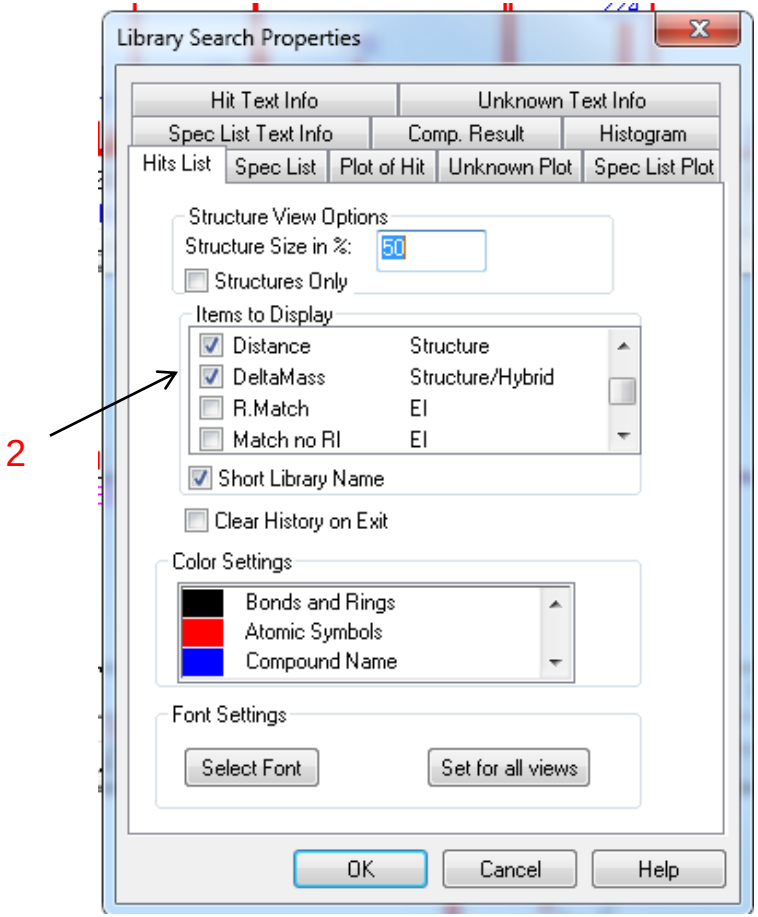




Setting Up Display Parameters for EI Hybrid Search
Critical to Include DeltaMass Column to Hits List

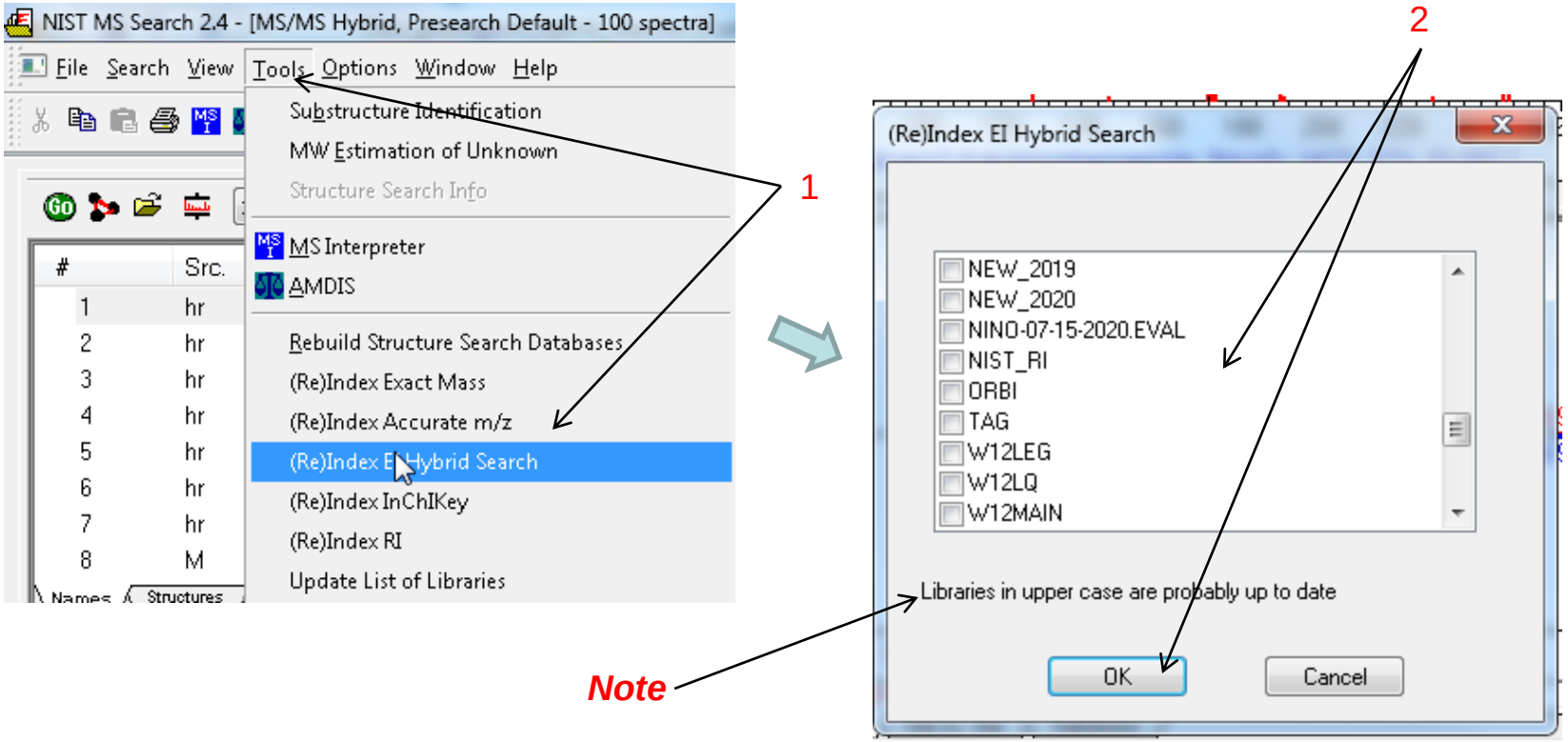
1) ***RMB*** in Hits List Window, then ***LMB*** Properties
2) ***LMB*** Check DeltaMass in Items to Display





Setting Up Indices for EI Hybrid Search
Must Index Wiley and User Libraries

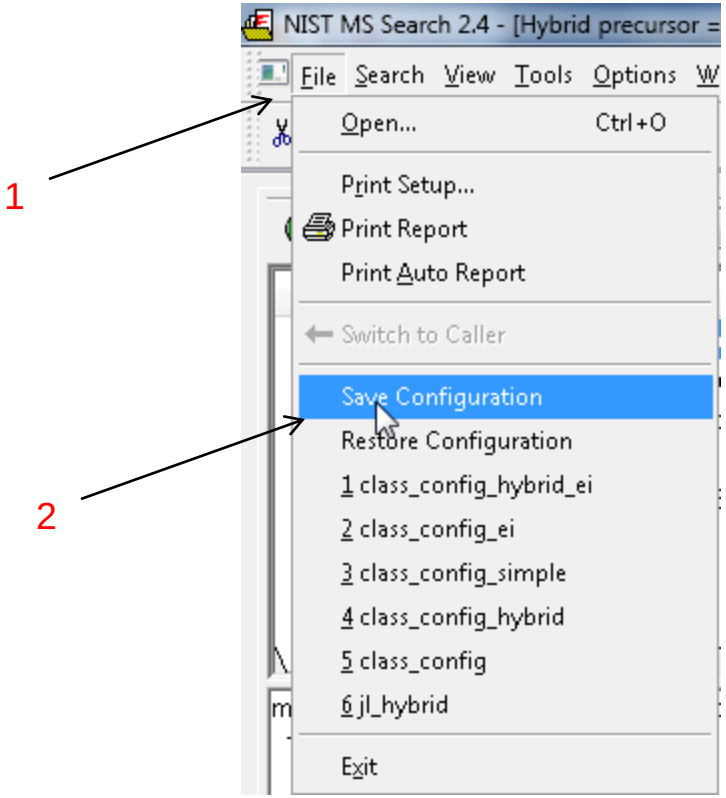
1) Wiley and User EI libraries need to be indexed for Hybrid Searches
2) After new additions to user library, “re-indexing” required
3) Select ones to (Re)Index and then OK



Save Configuration for EI Hybrid Search

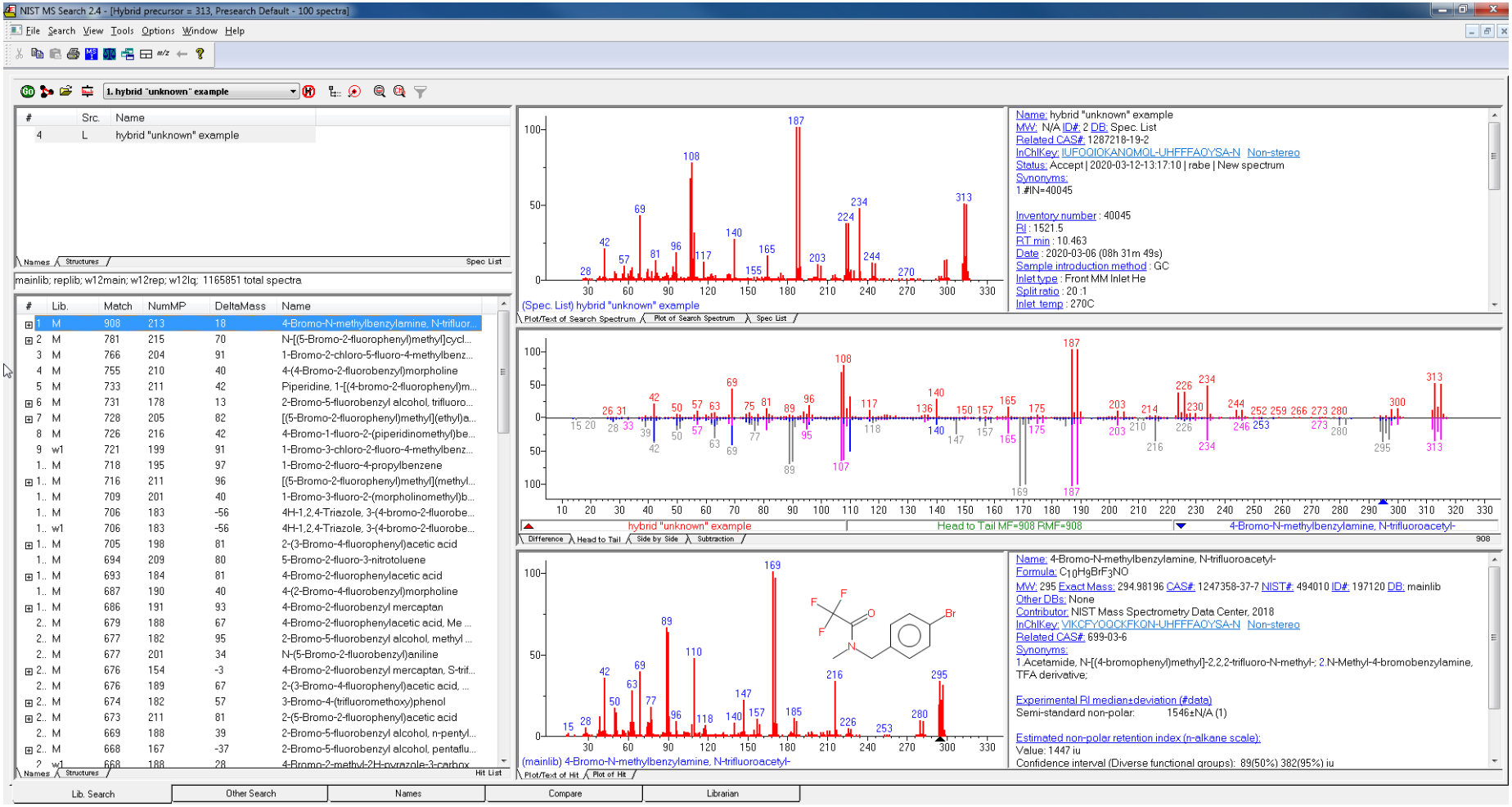
e.g., *Named class_config_hybrid_ei*

1) **LMB File**
2) **LMB Save Configuration** as **class_config_hybrid_ei**
3) Simple to switch between “simple” EI search and “hybrid” search
4) Example below called “simple” EI search, **class_config_simple_ei**



Hybrid Search Results for Unknown

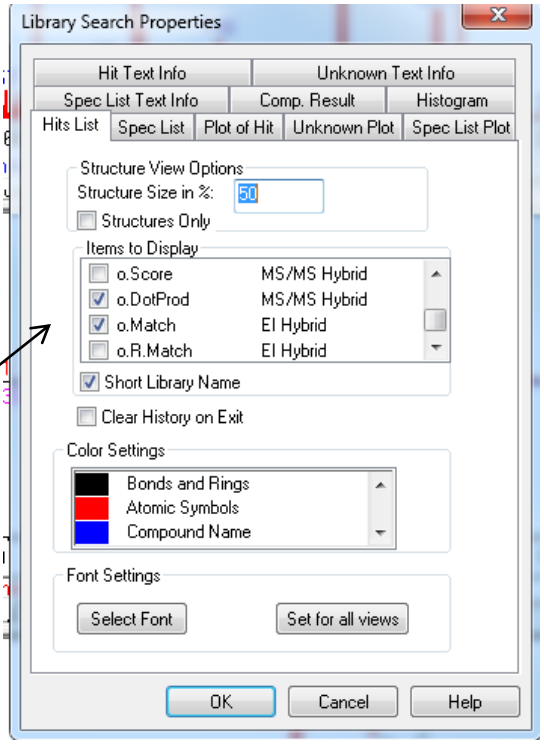
1) Best match factor is 908, next closest is 781
2) ***DeltaMass*** is 18, common for fluorine (mass 19) replacing hydrogen (mass 1) on ring, i.e. 19-1=18
3) See list later for common DeltaMasses

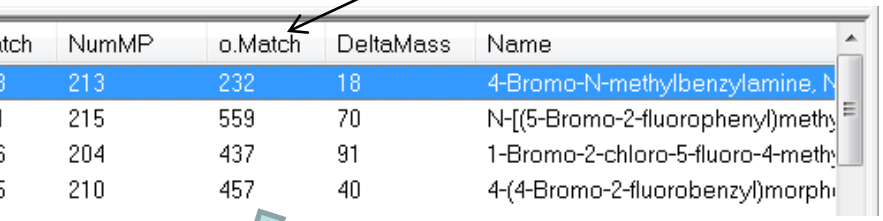


Tip: See Normal EI Identity Search Results in Hybrid Search Hit List

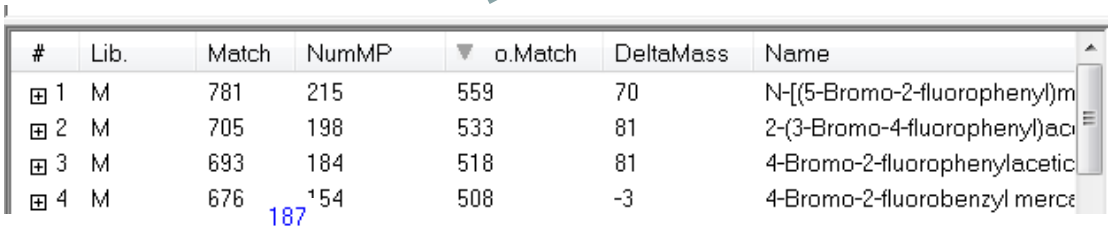
Add o-match Column and Sort

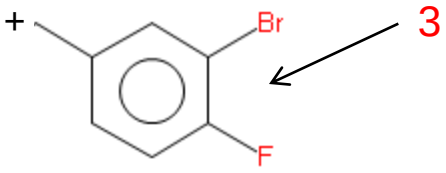
1. ***RMB*** in Hybrid search list menu, ***LMB*** select “Hits List Tab”, select “Items to Display”, o-match
2. **LMB** on o-match header to resort by “simple” (standard) search [EI Normal Identity]
3. ***Top 24*** hits contain the substructure with F and Br on a benzyl group, *m/z* 187





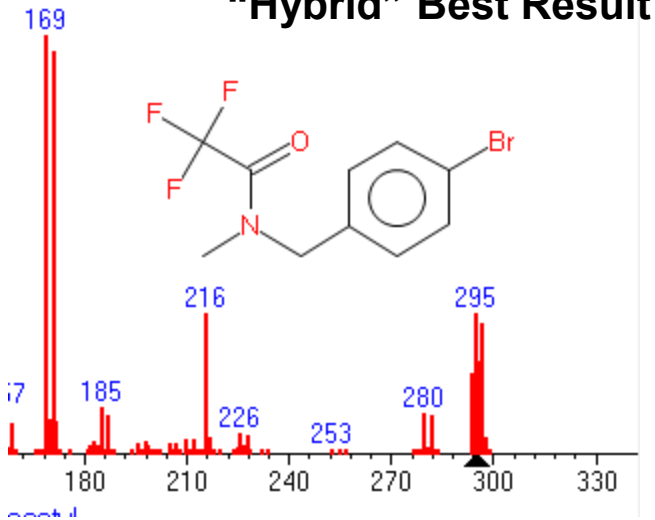
Resorted by o-match [EI Normal Identity





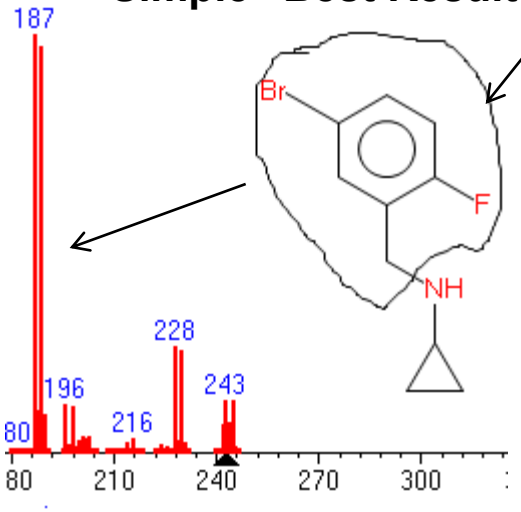
“Mentally” Merge Information of “Hybrid” and “Simple” Identity EI Search

“Hybrid” Best Result

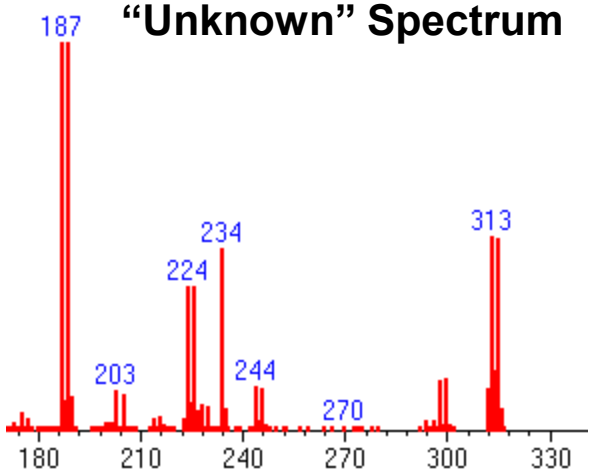


“Simple” Best Result

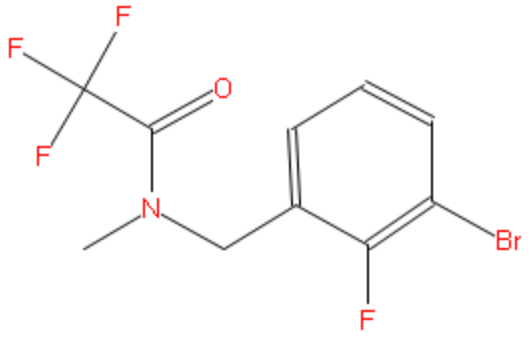
m/z 187



“Unknown” Spectrum



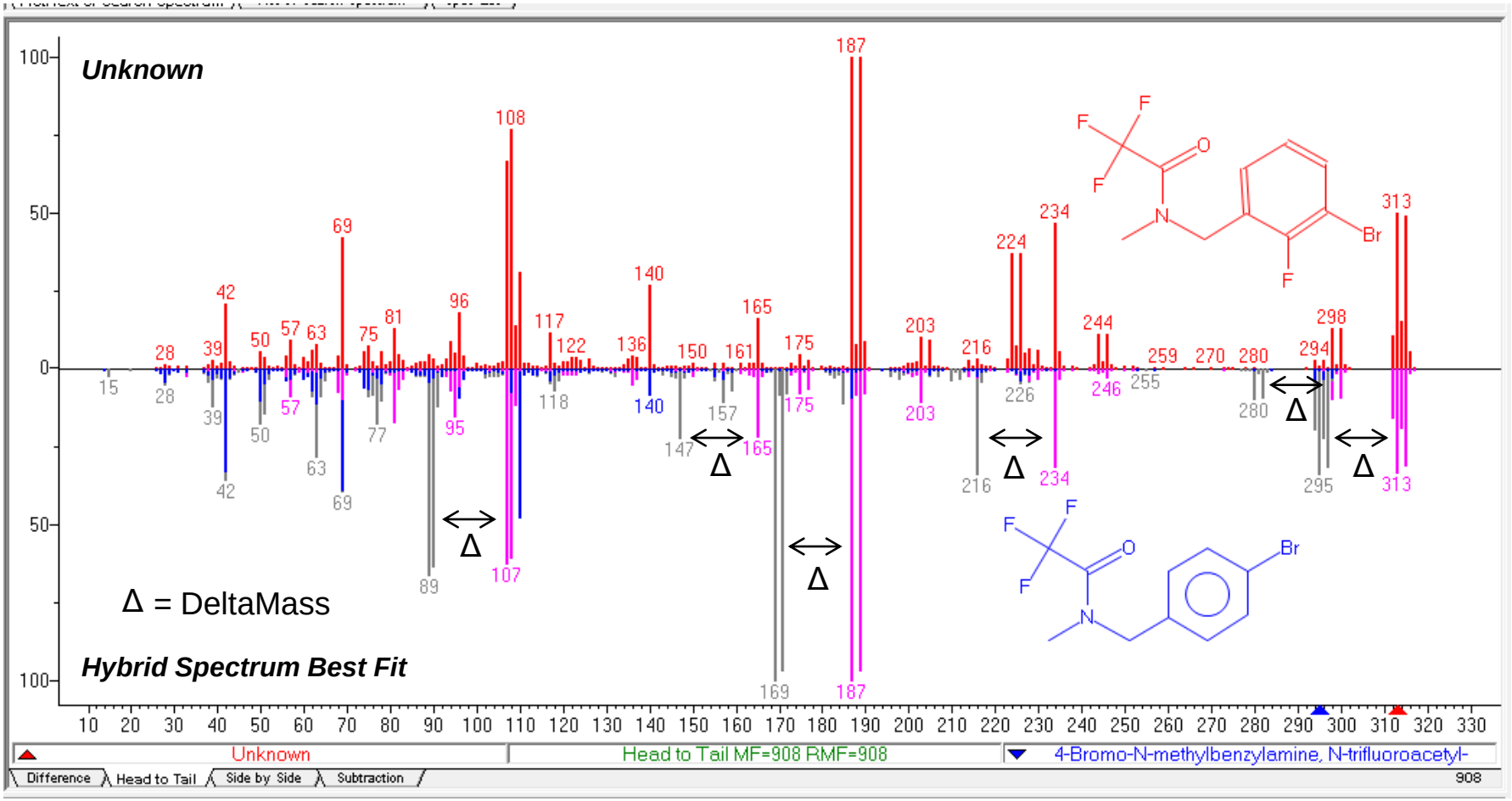
“Unknown Identity”



Closer Look at Middle Display[16]

Top is Unknown, Bottom is "Hybrid" Spectrum

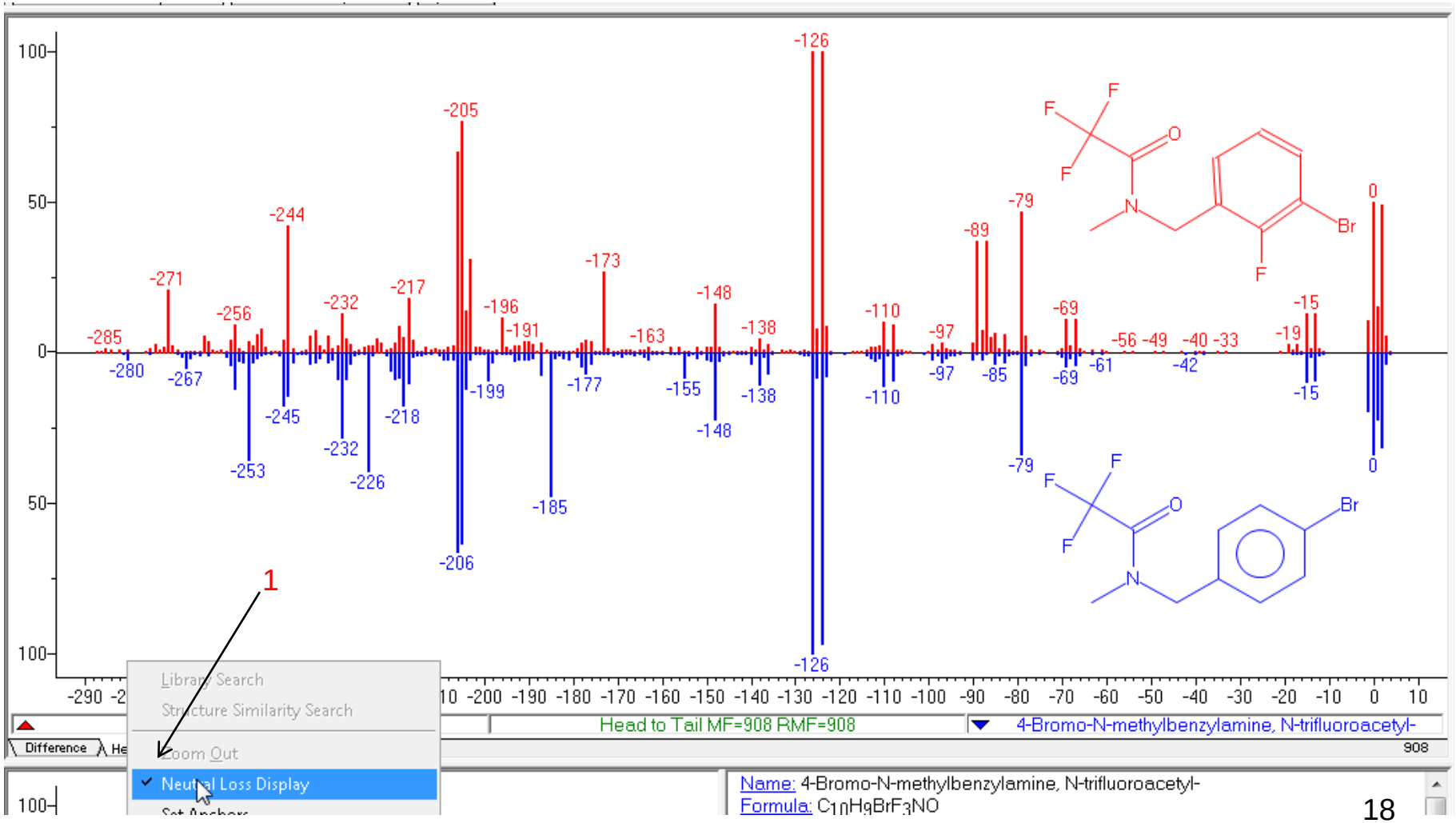
- In bottom spectrum, original ions in grey shifted by DelaMass (Δ) 18 for user visual comparisons
- Can take a while to adjust to this view versus standard "Head to Tail" views



Alternate Comparison of Hybrid Spectrum: Neutral Loss Display

Top is Unknown, Bottom is "Hybrid" Spectrum

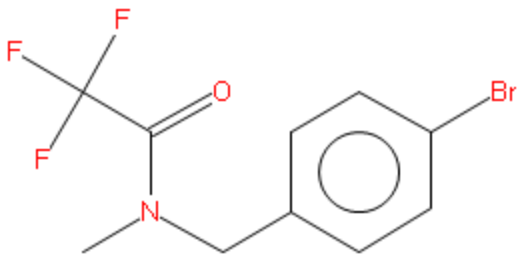
1. **RMB** in spectrum window, **LMB** select Neutral Loss Display
2. Shows whole spectrum of reference shifted by DeltaMass of 18
3. ***Easier*** and ***more efficient*** to ***look at "Hybrid" display*** with experienced eye!



Use Available Accurate Mass Data to Support Nominal Mass Hybrid Search

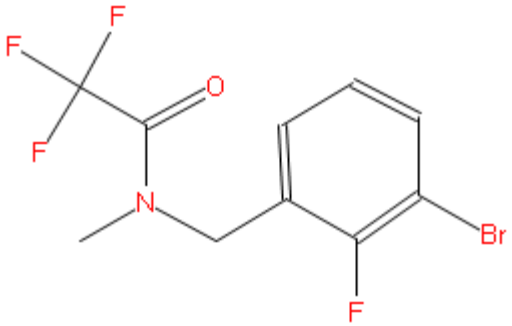
Accurate Mass EI Data Readily Available in Some Labs !

Sub-/Low- ppm mass accuracy!



Formula: $C_{10}H_9BrF_3NO$

Best Hybrid Fit



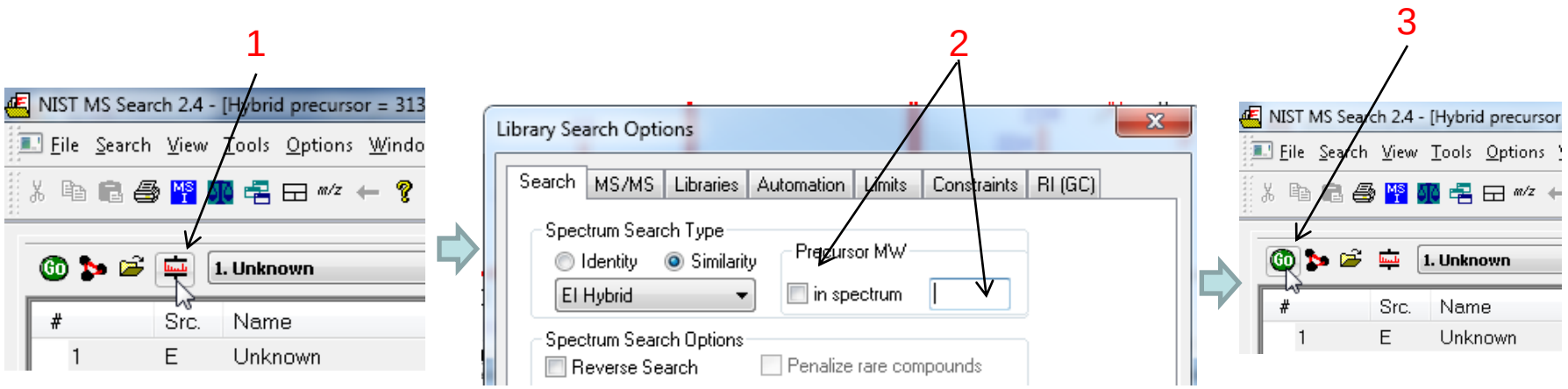
Formula: $C_{10}H_8BrF_4NO$

Unknown

	Accurate Mass	Accurate DeltaMass Element	Nominal Mass	DeltaMass Element
Unknown	312.9725	18.9984	313	19
Best Hybrid Fit	294.9820	1.0078	295	1
DeltaMass	17.9906	17.9906	18	18

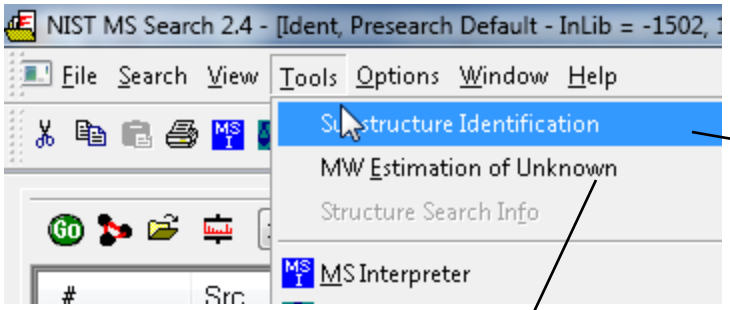
*Hybrid Search **Needs** Nominal MW of Species to Work Properly!*

- Many EI spectra do not have molecular ions (~10-15%)
- ***User*** must determine by:
  - Letting program determine automatically
  - User proposing from logical losses at higher mass in spectrum
  - Chemical Ionization[7]
  - NIST Software
- If different than automatically determined
- ***LMB*** Library Search Options (1)
- ***Uncheck*** “in spectrum” (2)
- ***Enter*** proposed Nominal MW/Save, ***then search (3)***

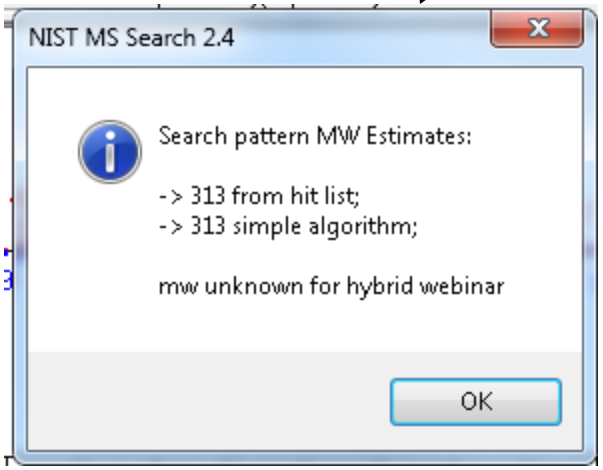


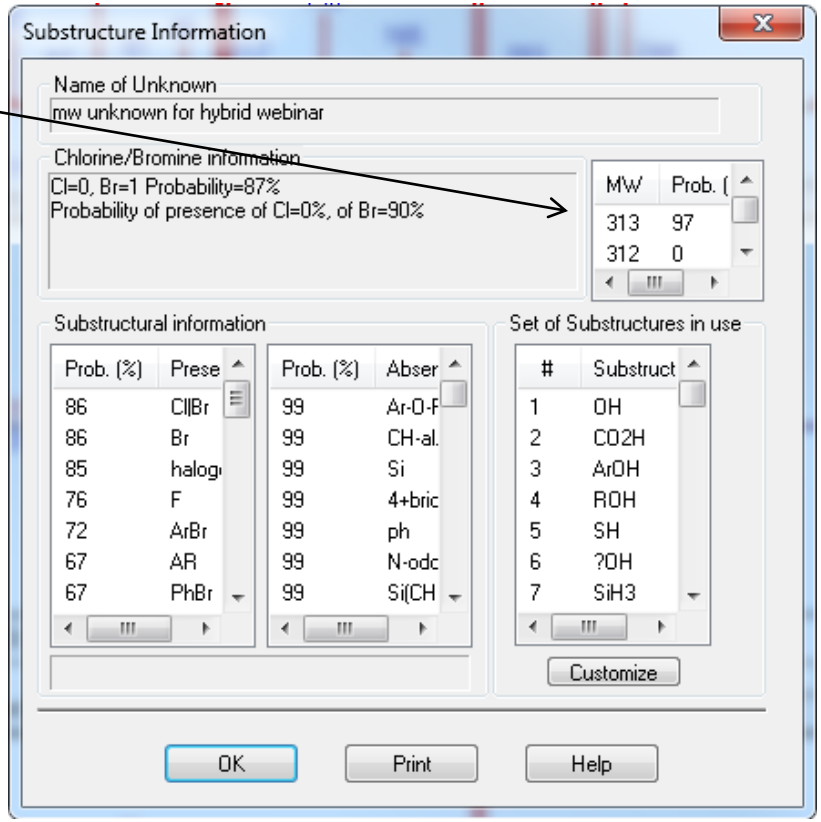
*Using NIST Software to Obtain **Proposed** Nominal MW*

-***After*** (***critical qualifier***) *library searching an unknown spectrum, uses hits and "rules-based-systems" to propose molecular weight and* substructural information
-***Two different ways*** of estimating MW from *either* "Substructure Identification" (1) *or* "MW Estimation of Unknown" (2)
-"Simple" algorithm ***does not use hit list*** information



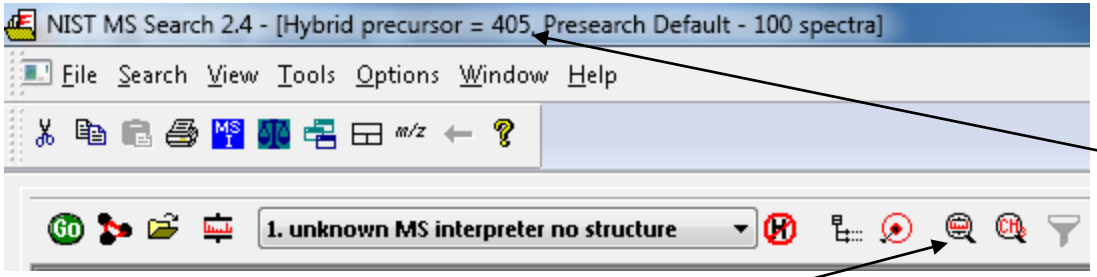
1
2

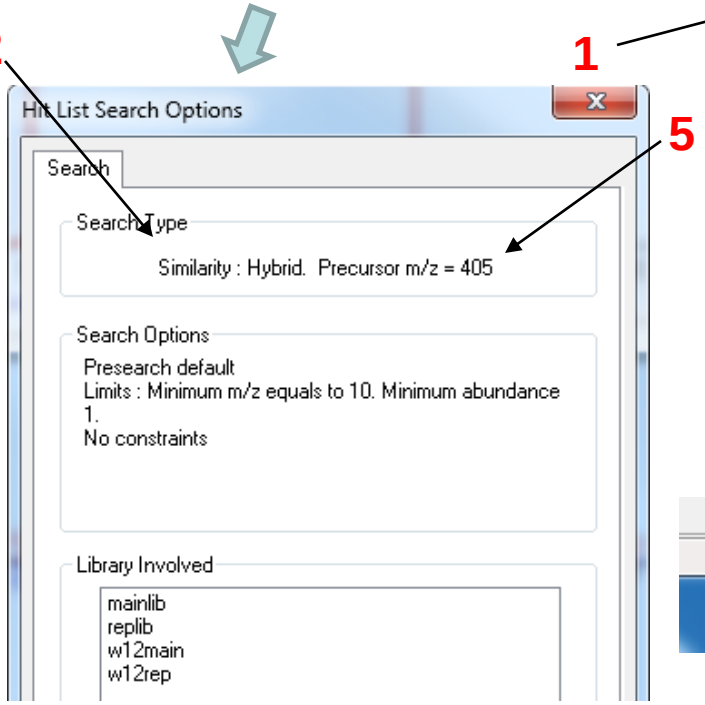


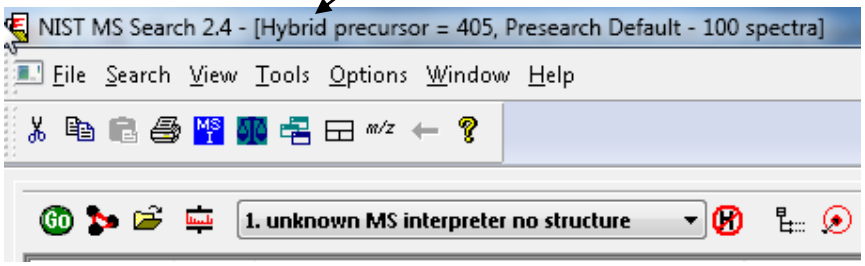


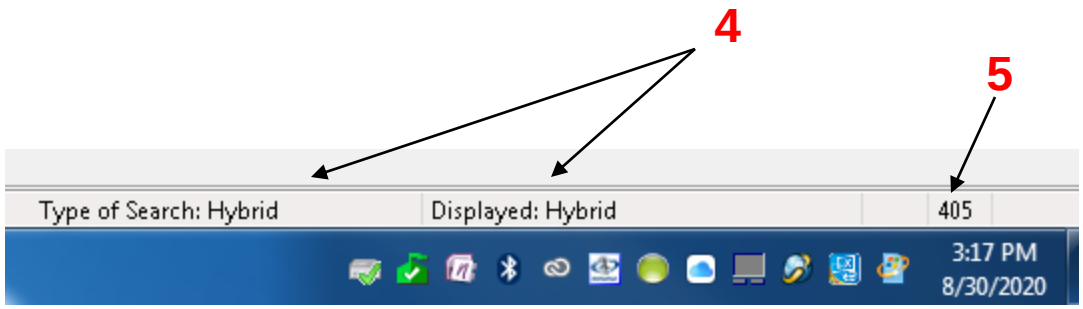
View of Hit List Structure Hybrid Search Options *Utilized*

1. ***LMB*** the "View Hit List Search Options"
2. See details of the last search performed
3. Also, an abbreviated description of the last search noted at ***top*** of NIST search window
4. Furthermore, at the ***bottom*** of the screen, *respectively* displayed, are the types of searches that will be performed and the type of results currently displayed
5. The ***MW guessed*** by the hybrid search algorithm, ***if not picked correctly***, the search will not be done properly! See slides 19-20









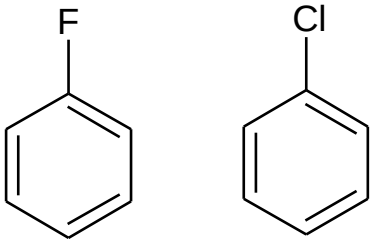
DeltaMass Table

- Hybrid DeltaMass values noted in spectral evaluation
- Value note can be + /–, depending only species present/absent
- ***Continually updated*** on web page as time permits*

**Link to Most Current Hybrid DeltaMass Updates*

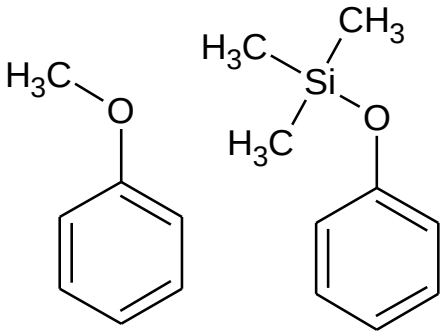
Associating Some *Simple* Structures with DeltaMass Values

- Some simple ***small*** MW compounds to illustrate types of substructural information
- Of course, these substructures can be a part of ***much larger*** molecules
- ***Note:*** Odd values of DeltaMass contain one nitrogen change in structure, thus ***"Nitrogen Rule"***
- Isotope ratios and/or accurate mass helpful with redundancies



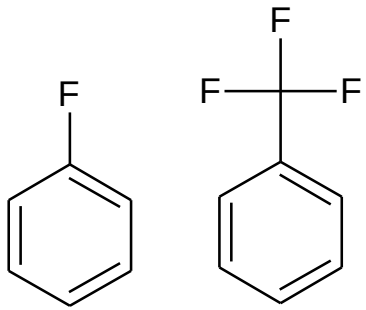
MW 96 MW 112

DeltaMass 16



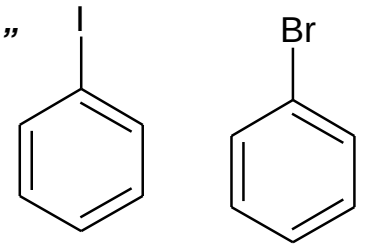
MW 108 MW 166

DeltaMass 58



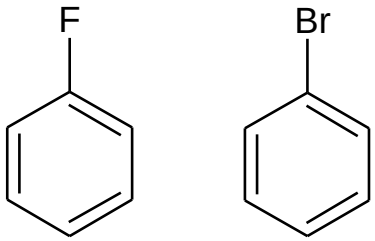
MW 96 MW 146

DeltaMass 50



MW 204 MW 156

DeltaMass 48



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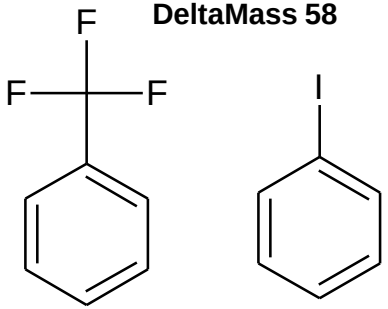
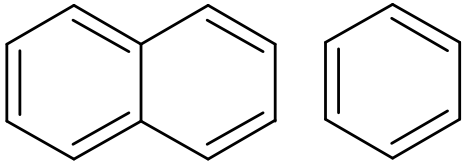
DeltaMass 60



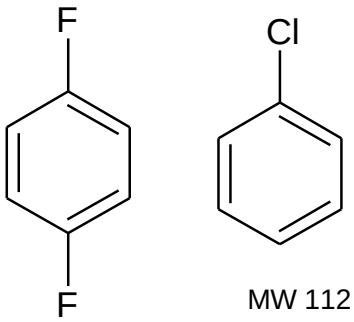
MW 146 MW 204

DeltaMass 58



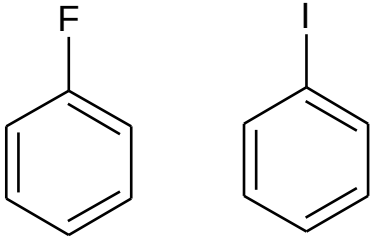
MW 128 MW 78

DeltaMass 50



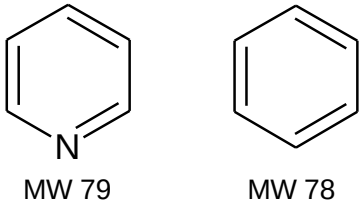
MW 114 MW 112

DeltaMass 2



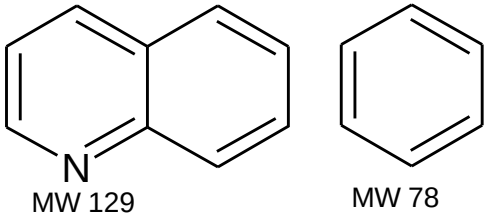
MW 96 MW 204

DeltaMass 108



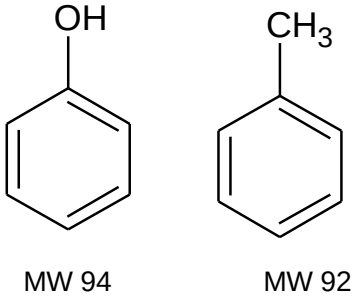
MW 79 MW 78

DeltaMass 1



MW 129 MW 78

DeltaMass 51



MW 94 MW 92

DeltaMass 2

Other Things to Consider Using Hybrid Search for Unknown Identification

- Accurate mass data
- Chemical ionization data[7]
- Sample history
- Isotope ratios (can be modeled in MS interpreter)
- Logical fragments using MS interpreter
- Other identified/related components found in sample
- Information from organic chemist/sample submitter
- Other techniques such as NMR, IR, elemental analysis, deuterium exchange CI[7], derivatization, etc..

NIST MSMS (Tandem) Hybrid Searches[17-19]

- Hybrid formatted NIST libraries*
- Identification of compounds not in the library
- Change search from EI identity or EI Hybrid to MS/MS or MS/MS Hybrid, respectively

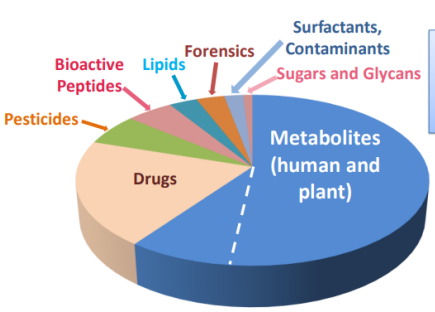
NIST Tandem Mass Spectral Library

2020 Release

31K Compounds, 2X More than 2017
186K Precursor Ions - 1.3M Spectra

Fragmentation Methods	Precursor Ion Types
27,840 HRAM (High Res Accurate Mass) Compounds	26,575 Protonated
29,890 QTOF, HCD, IT-HRAM, QqQ Compounds	12,589 Deprotonated
29,444 Ion Trap Compounds (Low Res., up to MS^4)	10,032 Water/Ammonia Loss
246 APCI HRAM 'Extractables and Leachables'	24,167 Other In-Source Generated

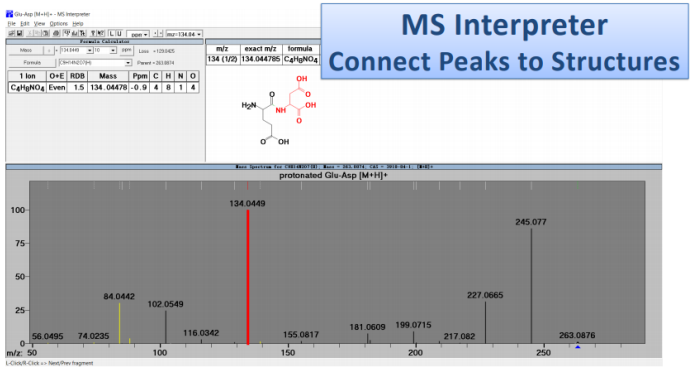
Types of Compounds and Spectra



75%(+) 25%(-)
32% MS^2 in-source
8% MS^3 and MS^4
Over wide energy range

6,000 human metabolites

MS Interpreter
Connect Peaks to Structures

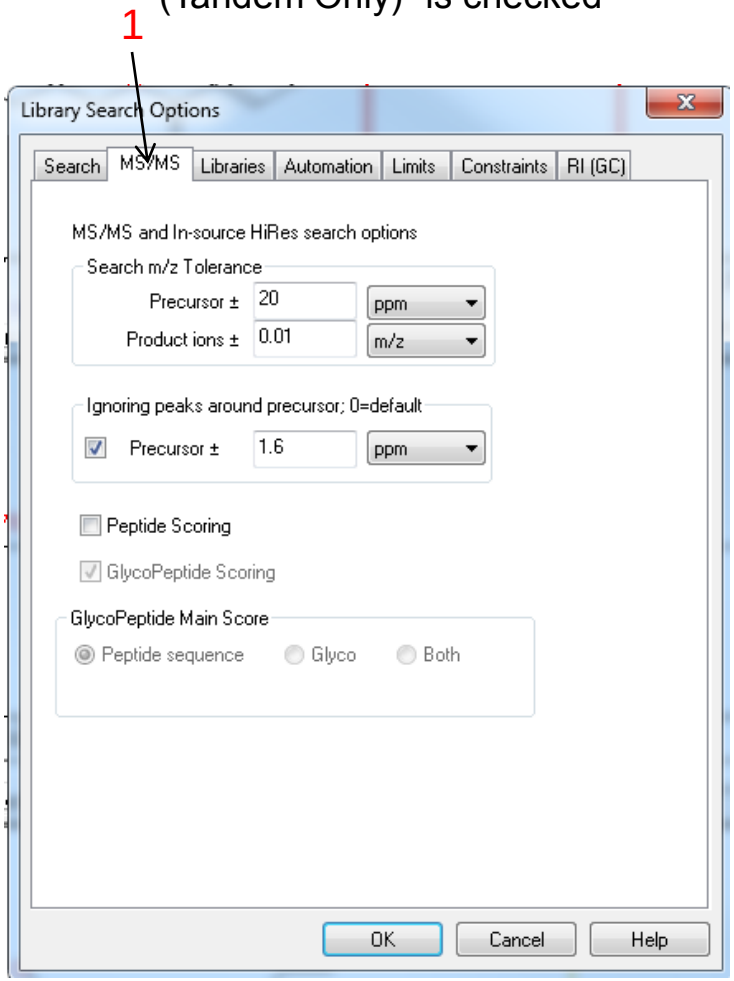


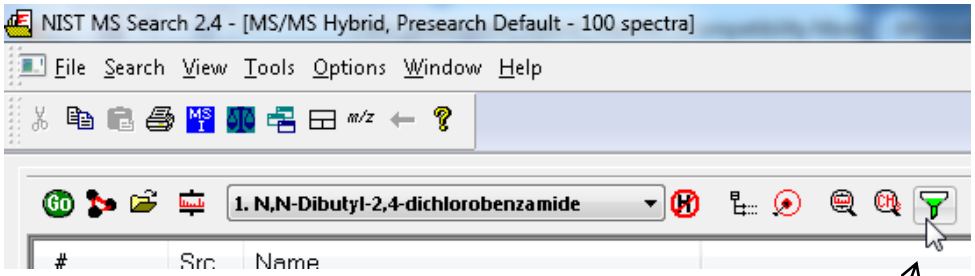
Hybrid Search - Identify Compounds Not in the Library

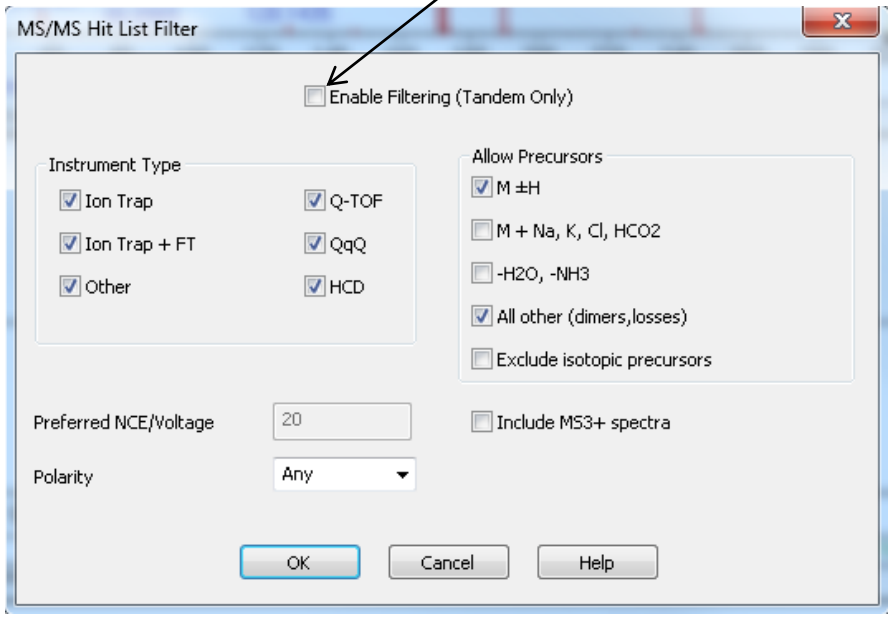
*NIST MSMS Database link

Additional NIST MSMS (Tandem) Hybrid Search Menus

1) ***Initial*** search limited by mass accuracy of precursors and fragments
2) **LMB** "MS/MS Hit List Filter Options"
3) Filters applied to hit list ***after*** the search and ***only*** affect hit list display when "Enable Filtering (Tandem Only)" is checked



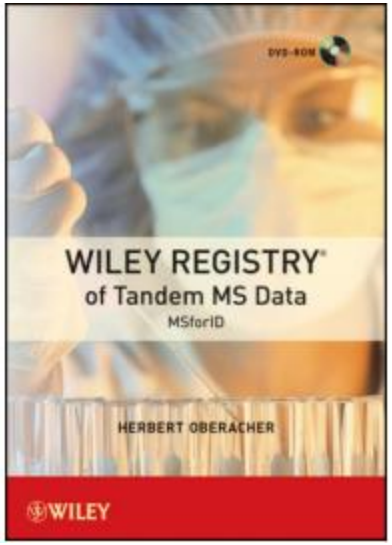




Wiley MSMS (Tandem) Libraries

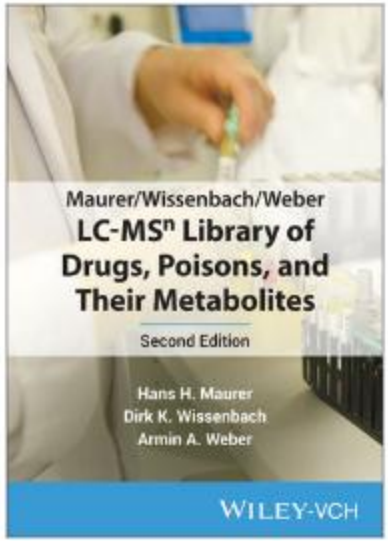
Wiley Registry of Tandem Mass Spectral Data, MS for ID by Herbert Oberacher

- 12,048 positive and negative spectra of 1,163 unique compounds
- Compounds of interest for forensics, toxicology, and pathology
- Covering areas of illicit drugs, pharmaceuticals, pesticides, and other small bioorganic molecules
- Measured by the Oberacher team at the Institute of Legal Medicine
- High mass accuracy LC-MS/MS library
- Compatible with various vendor formats
- ***Not currently*** supported in hybrid format
- *Click here for link*



Maurer/Wissenbach/Weber LC-MSn Library of Drugs, Poison, and Their Metabolites, 2nd Edition

- MS 2 and MS3 spectra of over 2,270 parent compounds and 3,600 of their metabolites
- Produced for clinical research and routine labs
- Confirm and identify drugs, poisons, and/or their metabolites
- 13,000 spectra, 10,787 structures
- Toxilogically relevant, metabolites/artifacts, endogenous molecules/impurities
- Compatible with various vendor formats
- ***Not currently*** supported in hybrid format
- *Click here for link*



Webinar References (*Internet Links*)

1. *James Little Mass Spectral Resource Website*
2. *NIST Search Software Detailed Manual*
3. *AMDIS Program for Data Processing Detailed Manual*
4. *Basic Instructions for Using AMDIS with NIST Search*
5. *Nightly Automatic Update of Users' Libraries*
6. *Using NIST Search from Instrument Manufacturers' Software*
7. *Chemical Ionization for MW Determination*
8. *Trimethylsilyl Derivatives for GC-MS*
9. *Methyl Ester Derivatives for GC-MS*
10. *SciFinder/ChemSpider and Accurate Mass LC-MS Data for Unknown ID's*
11. *Surfactant Identification*
12. *QuickGuide.rtf Supplied with AMDIS Software Installation for Retention Indices*
13. *New Developments in the Modeling of Ion Fragmentation by MS Interpreter Software*
14. *Enhancements to NIST MS Interpreter for Modeling High Mass Accuracy Tandem Mass Spectra*
15. *An Automated Method for Verifying Structure-Spectral Consistency Based on Ion Thermochemistry*
16. *Combining Fragment-Ion and Neutral-Loss Matching during Mass Spectral Library Searching: A New General Purpose Algorithm Applicable to Illicit Drug Identification*
17. *The Hybrid Search: A Mass Spectral Library Search Method for Discovery of Modifications in Proteomics*
18. *Hybrid Search: A Method for Identifying Metabolites Absent from Tandem Mass Spectrometry Libraries*
19. *Structure Annotation of All Mass Spectra in Untargeted Metabolomics*
20. *Most Current Handouts for Webinar Series, Parts I-V*
21. *Lipid Matrix Ionization Effects in LC-MS*
22. Mass Spectral Similarity Mapping in Hybrid Searches Applied to Fentanyl Analogs

Acknowledgements

Wiley Webinar Production:

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