

# Wiley Spectral Webinar

## Part IV: Advanced NIST Hybrid Search of EI and MS/MS Spectra<sup>23</sup>

12/27/20

**Note:**<sup>23</sup> Created a **Separate** Webinar Series on MS/MS Tandem Searches<sup>23</sup>

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Kingsport, TN

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



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

\* [https://en.wikipedia.org/wiki/Eastman\\_Chemical\\_Company](https://en.wikipedia.org/wiki/Eastman_Chemical_Company)



>50 Mass Specs Networked  
Worldwide

## Wiley Webinar Series on Effective Use of Mass Spectral Libraries

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

**Note:**<sup>20</sup> 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<sup>16-19,22</sup>

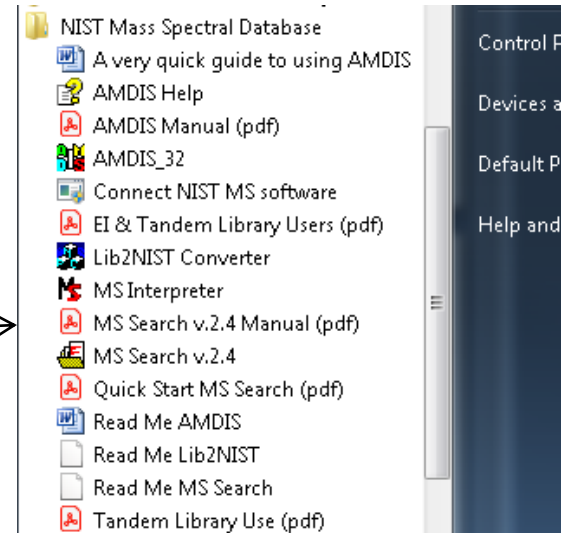
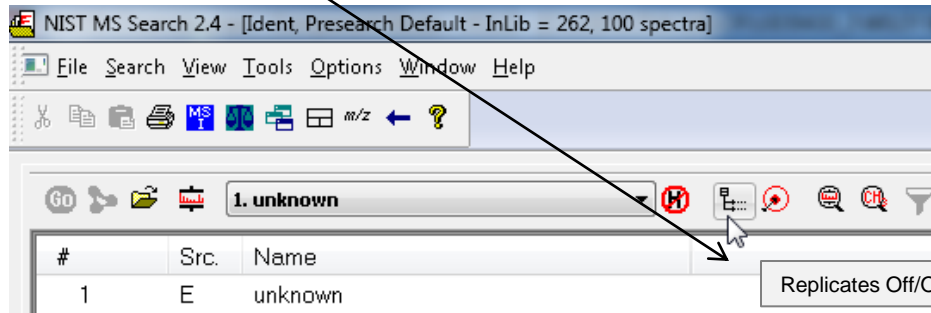
### Webinar Topics:

- Description
- Setting up search parameters, indices, and displays
- Example of unknown identification with hybrid
- Comparison of “hybrid” EI to “Identity EI Normal” Search
- DeltaMass table and examples
- Use of additional information in process
- Information on NIST MS/MS (Tandem) “hybrid” search<sup>23</sup>
- Wiley MS/MS (Tandem) Library for Identity MS/MS searches

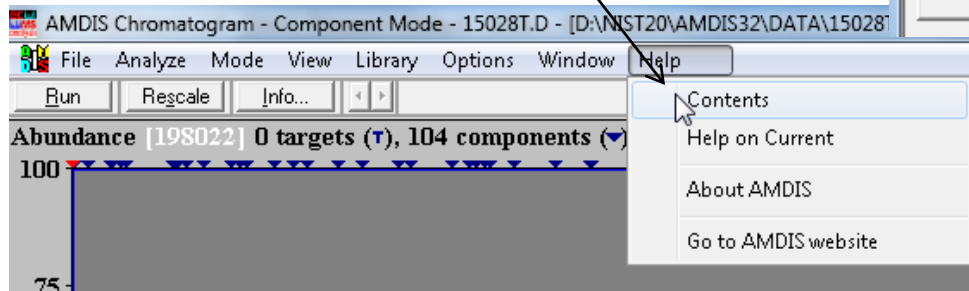
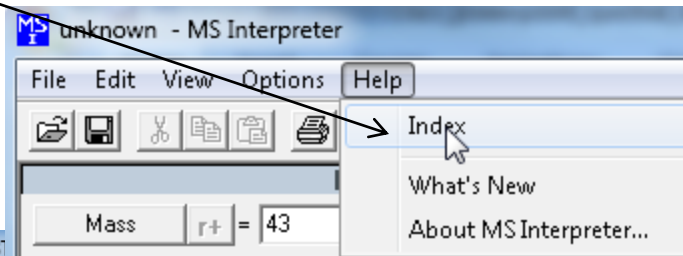
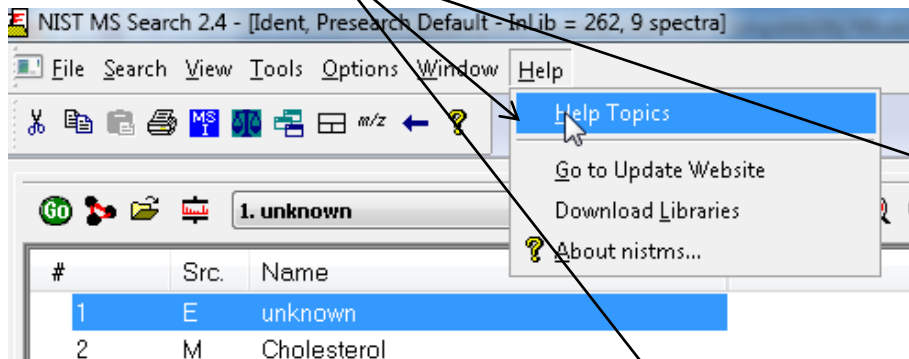
**Note:**<sup>23</sup> Created a **Separate** Webinar Series on MS/MS Tandem Searches<sup>23</sup>

# Help Files for NIST Search

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



- Detailed documentation for NIST Search<sup>2</sup> and AMDIS<sup>3,4,12</sup>
- MS Interpreter included in NIST manual<sup>2</sup> and in posters<sup>13-15</sup>
- 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**

**Tip 3:** LMB and drag to rearrange order of column headers

#	Lib.	Name	▼ Match	Prob. (%)	RI	B. 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

**Tip 1:** When reviewing search results, use up and down arrows on keyboard to quickly step through results!



**Tip 2:** When viewing structures in MS Interpreter, use left and right arrows on keyboard to quickly review results!



## Part IV: Advanced NIST Hybrid Search of EI and MS/MS Spectra<sup>16-19,22</sup>

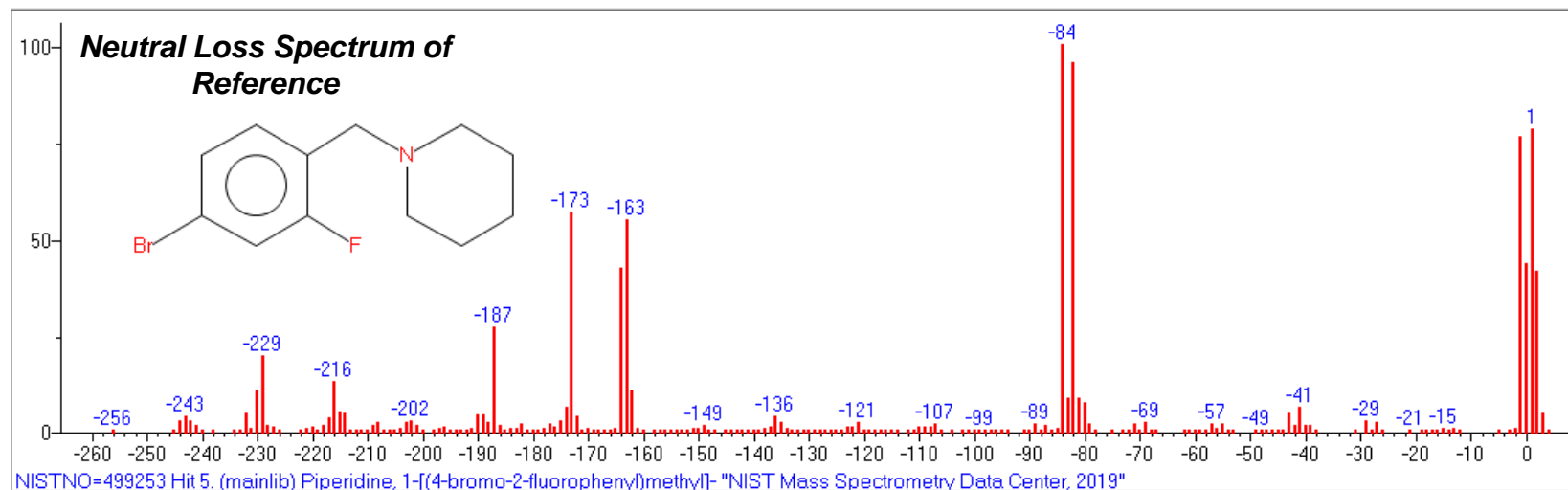
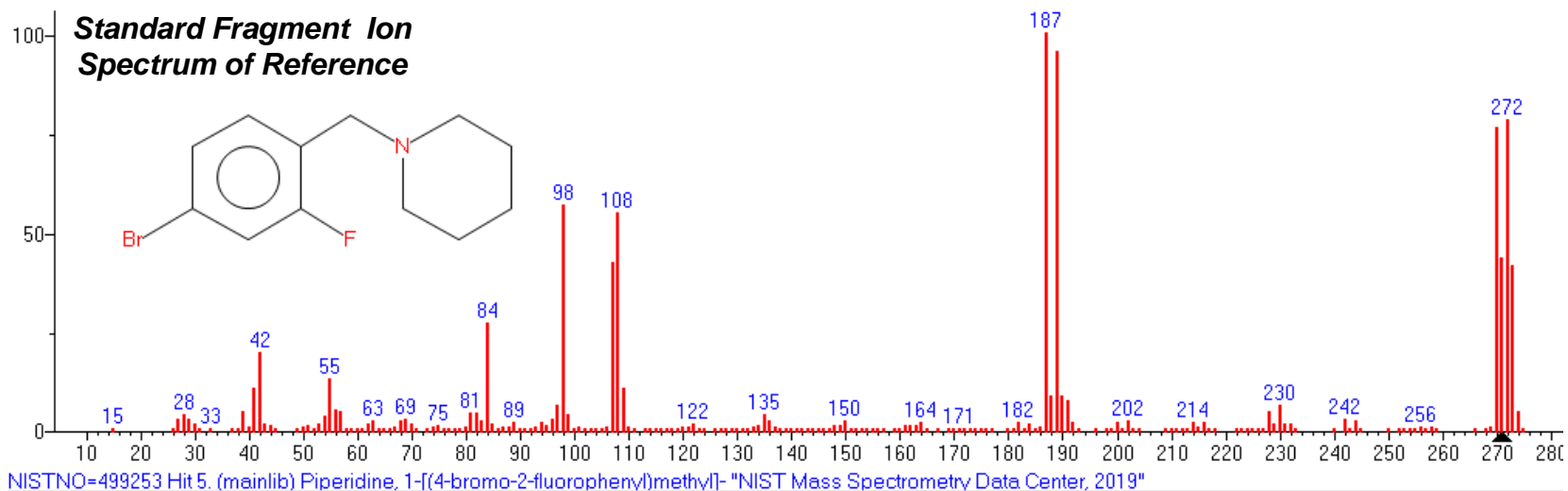
### Program Description:

- 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<sup>16-19,22</sup>

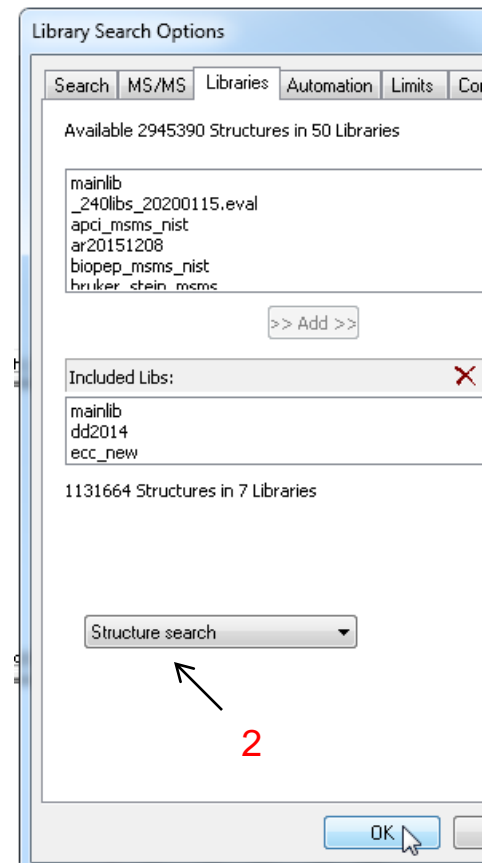
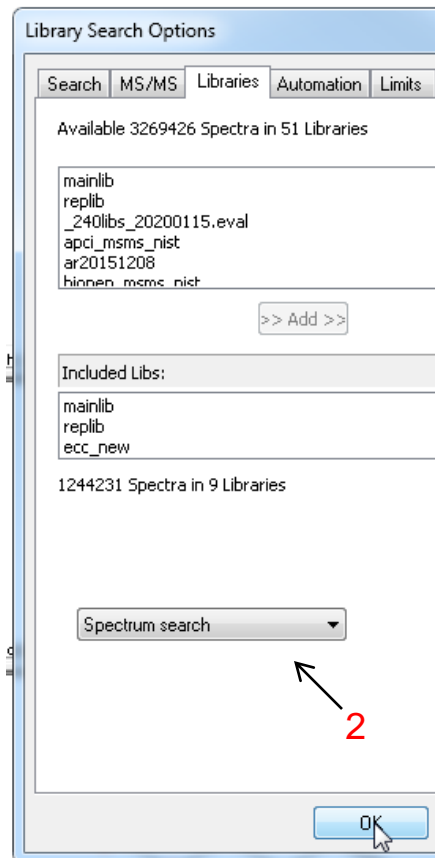
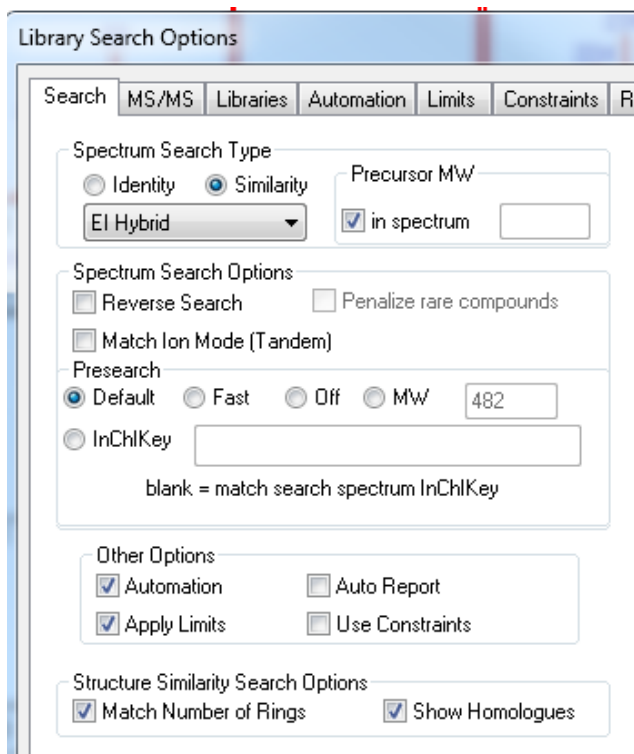
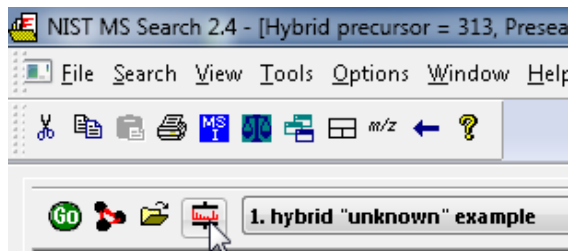




# Setting Up Search Parameters for EI Hybrid Search (pg 1)

## Very Similar to Those in "Identity" EI Normal

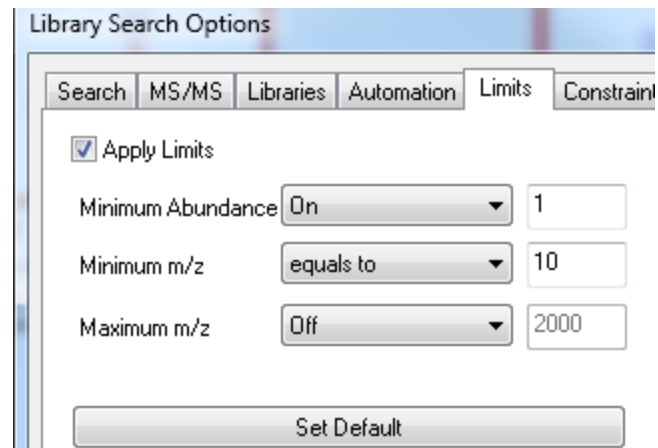
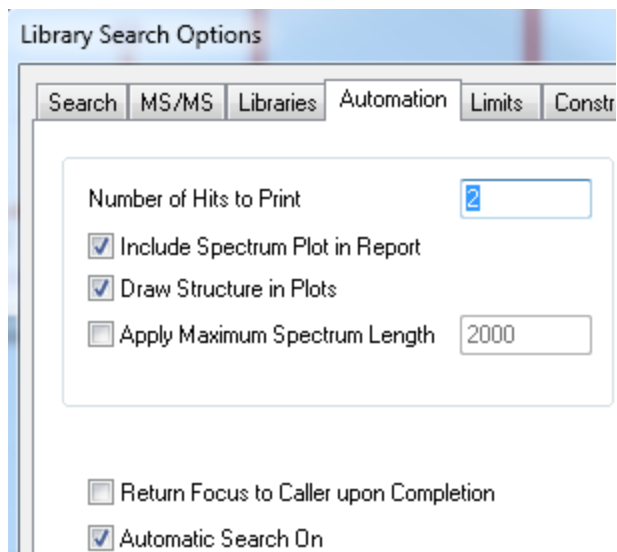
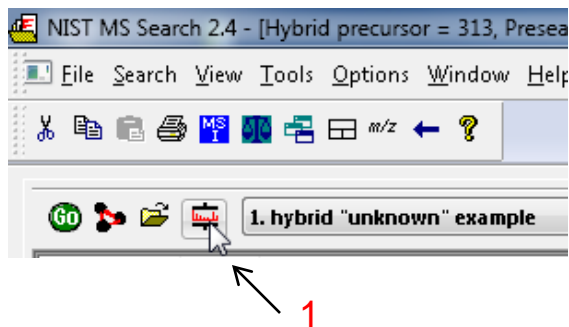
- 1) LMB Library Search Options
- 2) Setup **both** Structure and Spectrum search



**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 "Identity" EI Normal

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

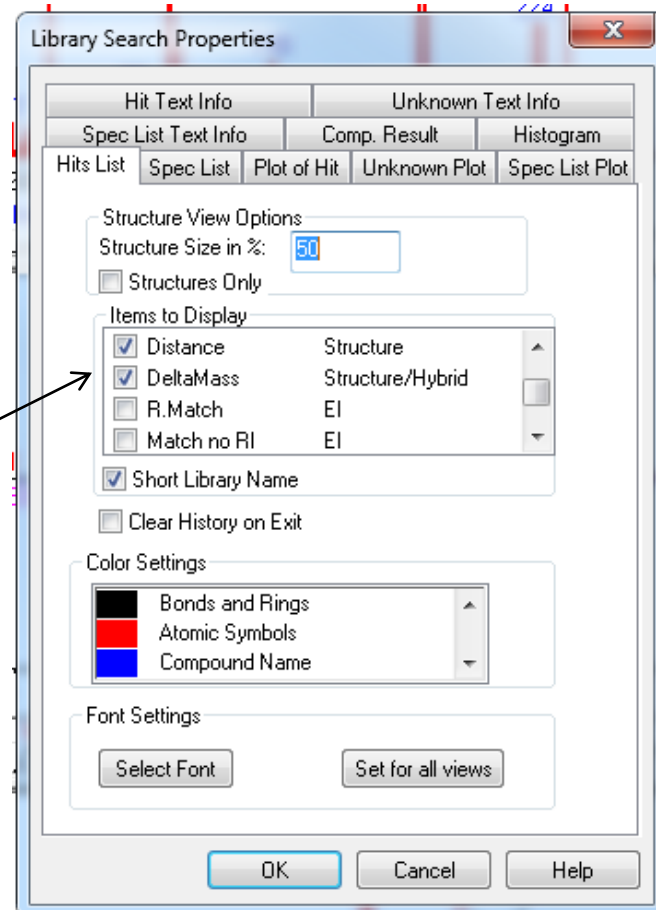


# Setting Up Display Parameters for EI Hybrid Search

## *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

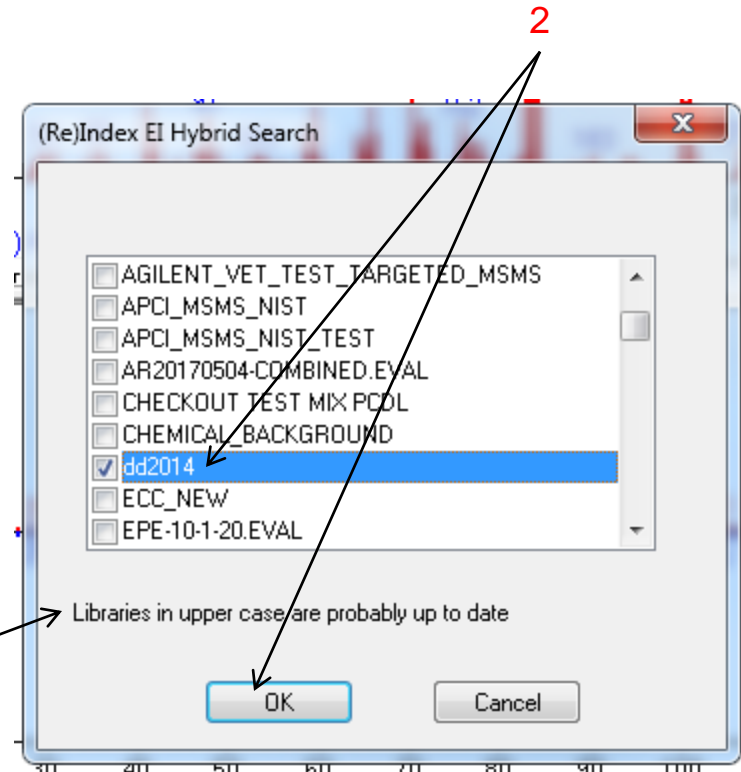
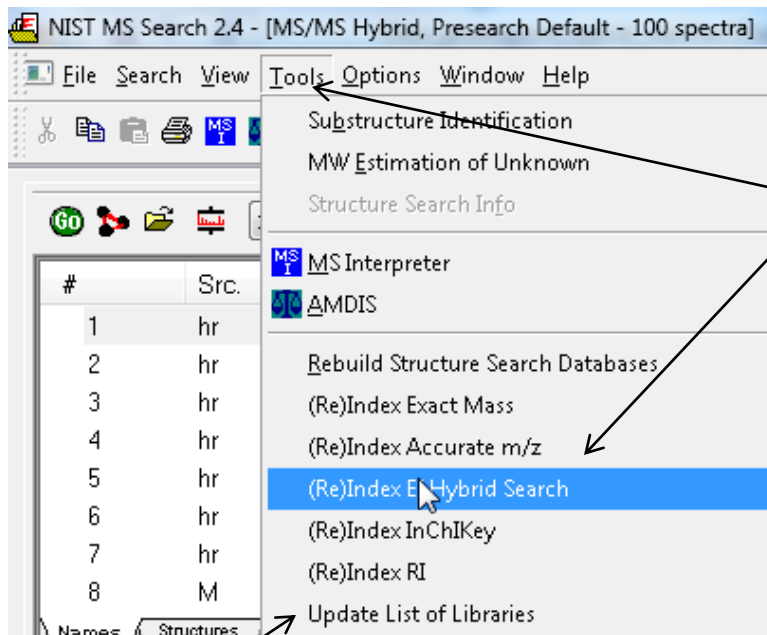
#	Lib.	Match	NumMP	DeltaMass	Name
1	M	908	213	10	4-Bromo-2-fluorobenzylamine, N-trifluor...
2	M	781	215		(methyl)cycl...
3	M	766	204		pro-4-methylbenz...
4	M	755	210		zyl)morpholine
5	M	733	211		-2-fluorophenyl)m...
6	M	731	178		l alcohol, trifluoro...
7	M	728	205		yl)methyl](ethyl)a...
8	M	726	216		peridinomethyl)be...
9	w1	721	199		pro-4-methylbenz...
1..	M	718	195		pylbenzene
1..	M	716	211		yl)methyl](methyl...
1..	M	709	201		rpholinomethyl)b...
1..	M	706	183		bromo-2-fluorobe...
1..	w1	706	183		bromo-2-fluorobe...
1..	M	705	198		nyl)acetic acid
1..	M	694	209	80	5-Bromo-2-fluoro-3-nitrotoluene
1..	M	693	184	81	4-Bromo-2-fluorophenylacetic acid
1..	M	687	190	40	4-(2-Bromo-4-fluorobenzyl)morpholine
1..	M	686	191	93	4-Bromo-2-fluorobenzyl mercaptan
2..	M	679	199	67	4-Bromo-2-fluorobenzylacetic acid Me...



1

## 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
- 4) Also, “Rebuild Structure Search Databases” to **include new structure** in similar search



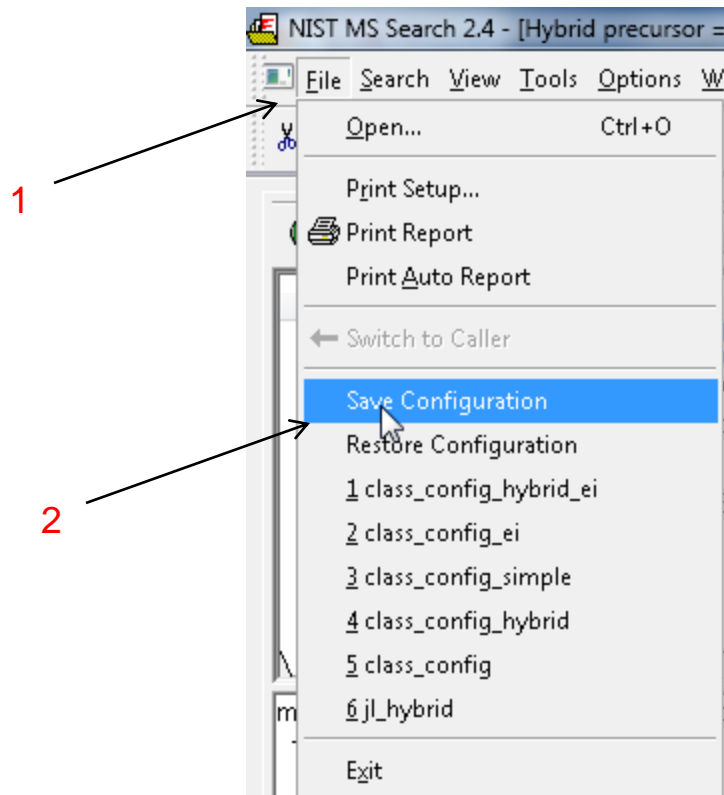
**Note**

**Note:** If you create a new user library, **Update List of Libraries** or close NIST Search program and reopen

## Save Configuration for EI Hybrid Search

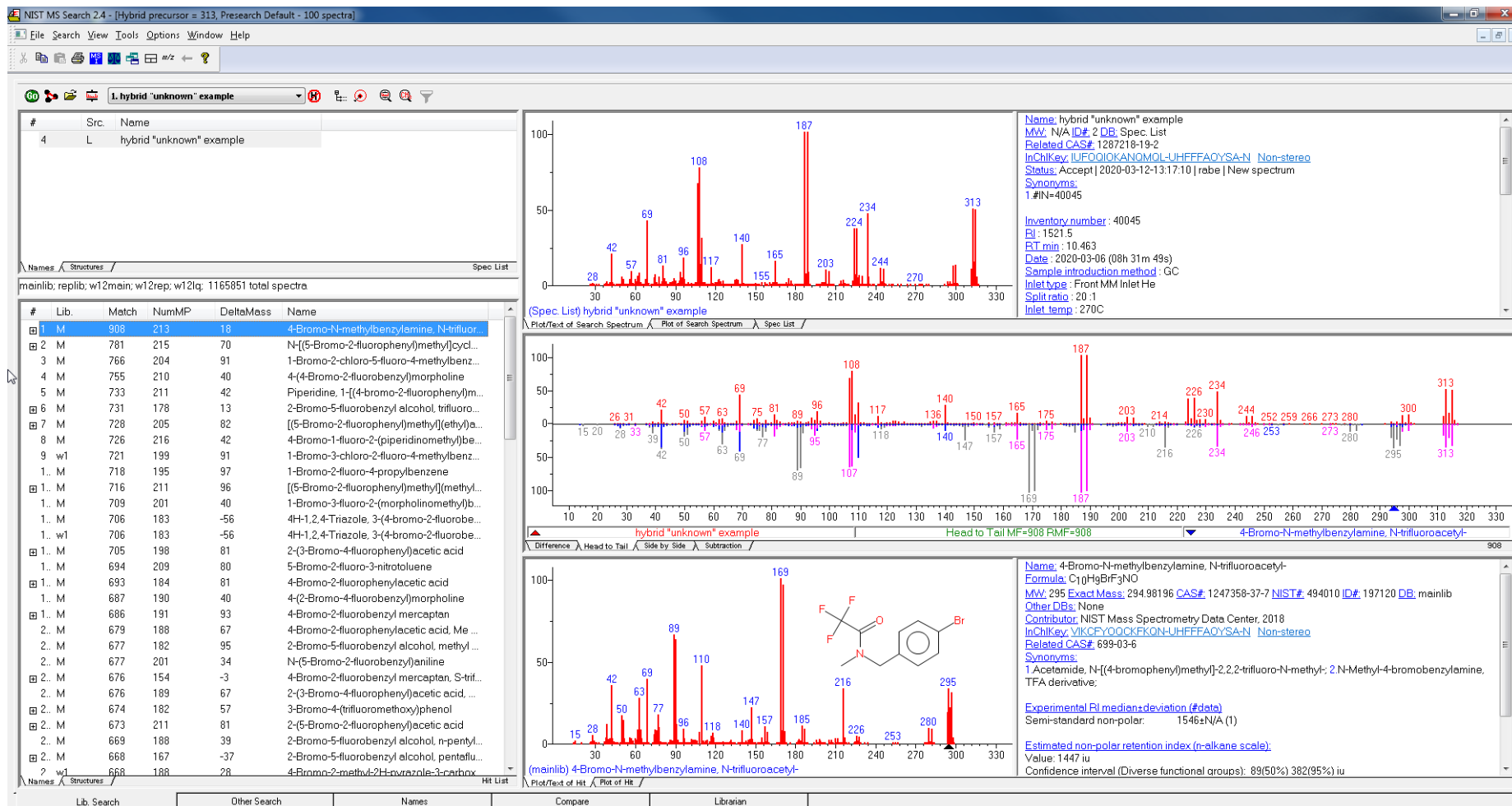
*e.g., Named class\_config\_hybrid\_ei*

- 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**



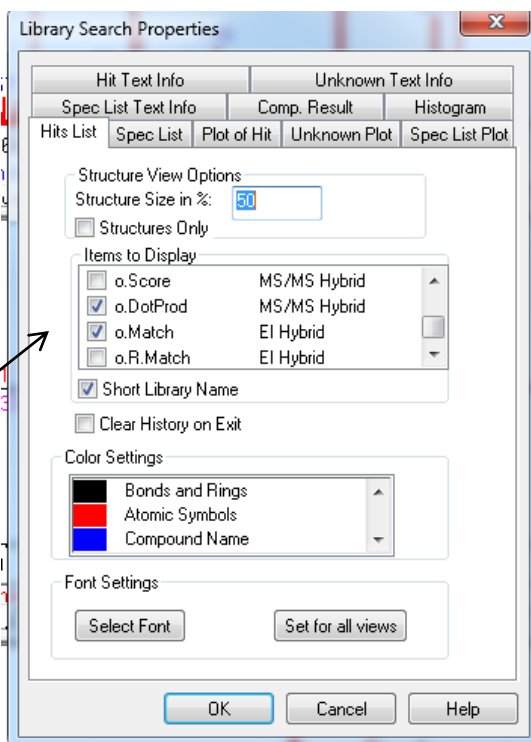
# Hybrid Search Results for Unknown

- 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

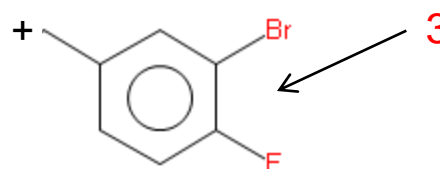
1. **RMB** in Hybrid search list menu, **LMB** select “Hits List Tab”, select “Items to Display”, *o-match*
2. **LMB** on *o-match* header to resort by results “*similar, but not identical*” to standard Identity EI Normal results
3. **Top 24** hits contain the substructure with F and Br on a benzyl group, *m/z* 187



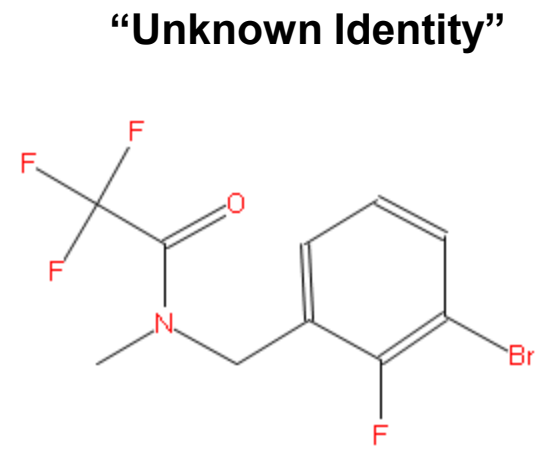
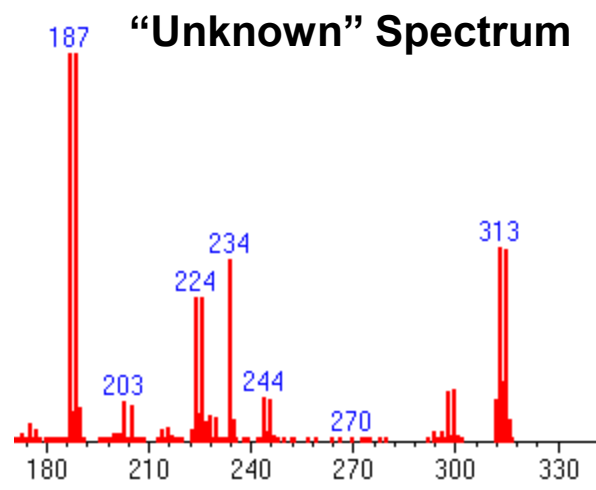
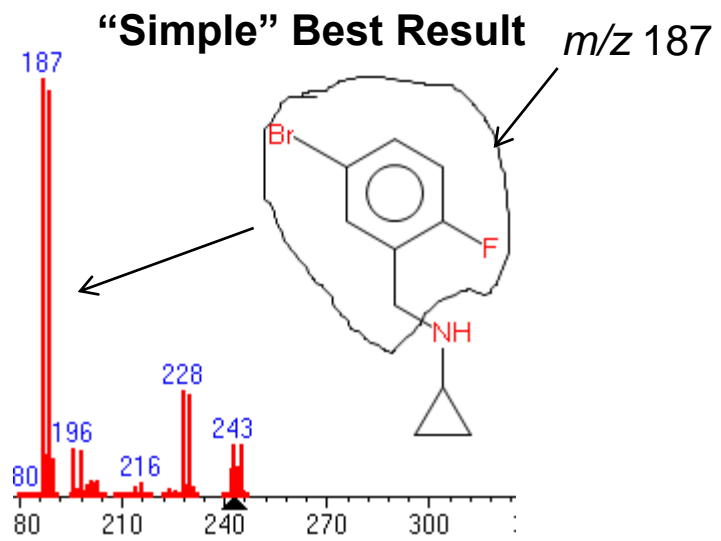
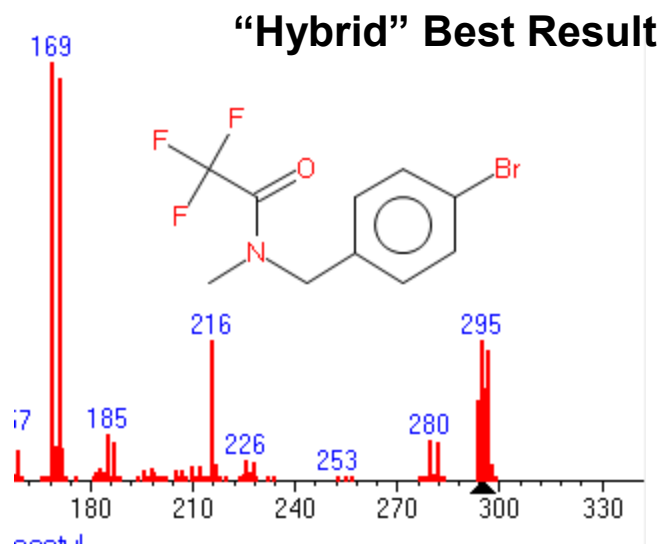
#	Lib.	Match	NumMP	<i>o.Match</i>	DeltaMass	Name
1	M	908	213	232	18	4-Bromo-N-methylbenzylamine, N
2	M	781	215	559	70	N-[(5-Bromo-2-fluorophenyl)methy
3	M	766	204	437	91	1-Bromo-2-chloro-5-fluoro-4-methy
4	M	755	210	457	40	4-(4-Bromo-2-fluorobenzyl)morphi

**Resorted by *o-match*  
[EI Normal Identity]**

#	Lib.	Match	NumMP	<i>o.Match</i>	DeltaMass	Name
1	M	781	215	559	70	N-[(5-Bromo-2-fluorophenyl)m
2	M	705	198	533	81	2-(3-Bromo-4-fluorophenyl)ac
3	M	693	184	518	81	4-Bromo-2-fluorophenylacetic
4	M	676	154	508	-3	4-Bromo-2-fluorobenzyl merc



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

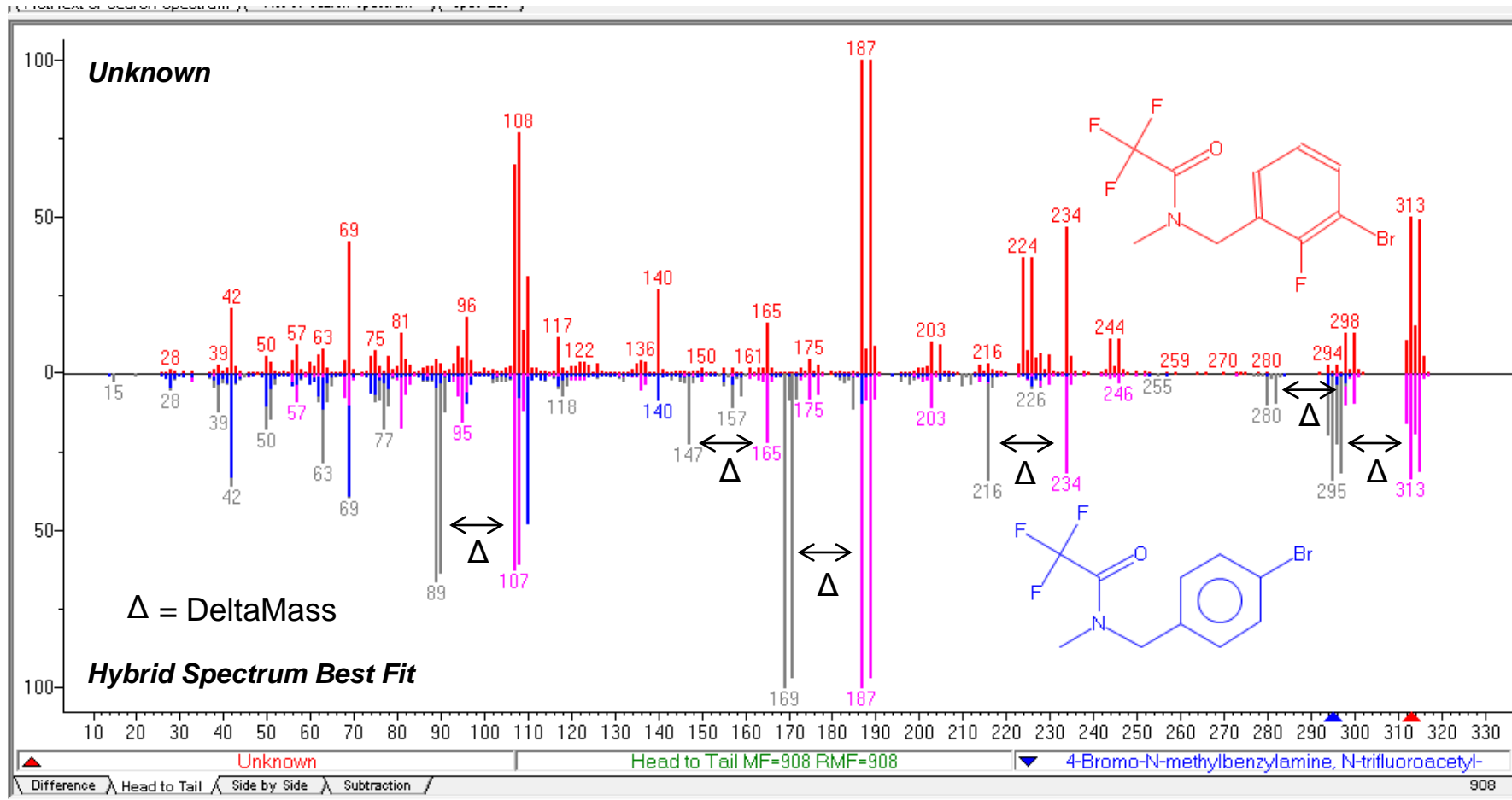




## Closer Look at Middle Display<sup>16</sup>

### Top is Unknown, Bottom is "Hybrid" Spectrum

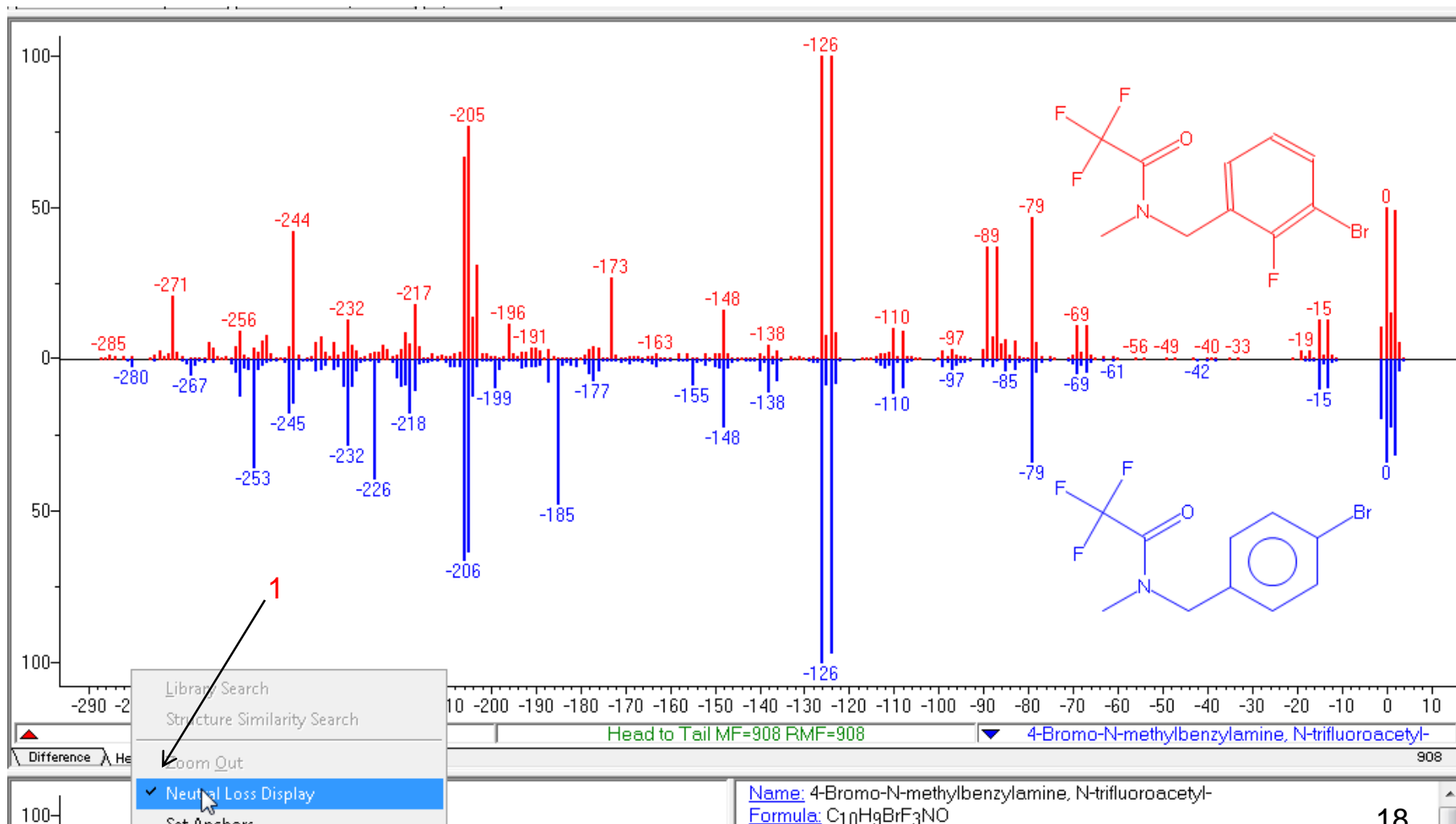
- In bottom spectrum, original ions in grey shifted by DeltaMass ( $\Delta$ ) 18 for user visual comparisons
- Can take a while to adjust to this view versus standard "Head to Tail" views



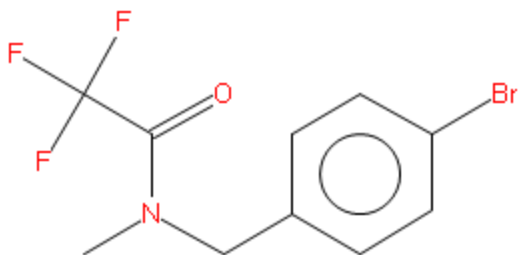
# Alternate Comparison of Hybrid Spectrum: Neutral Loss Display

## 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!

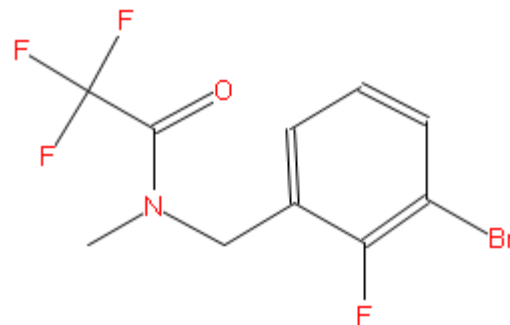


**Use Available Accurate Mass Data to Support Nominal Mass Hybrid Search**  
**Accurate Mass EI Data Readily Available in Some Labs !**  
**Sub-/Low- ppm mass accuracy!**



Formula: C<sub>10</sub>H<sub>9</sub>BrF<sub>3</sub>NO

**Best Hybrid Fit**



Formula: C<sub>10</sub>H<sub>8</sub>BrF<sub>4</sub>NO

**Unknown**

	Accurate Mass	Accurate DeltaMass of Element	Nominal Mass	DeltaMass of Element
<b>Unknown</b>	312.9725	18.9984	313	19
<b>Best Hybrid Fit</b>	294.9820	1.0078	295	1
<b><i>DeltaMass</i></b>	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<sup>7</sup>
  - NIST Software
- If different than automatically determined
- **LMB** Library Search Options (1)
- **Uncheck** “in spectrum” (2)
- **Enter** proposed Nominal MW/Save, **then search** (3)

The image illustrates the workflow for hybrid search in NIST MS Search 2.4. It consists of three sequential screenshots:

- Screenshot 1:** The main interface shows the 'Go' button (labeled 1) and a search results table with one entry: 

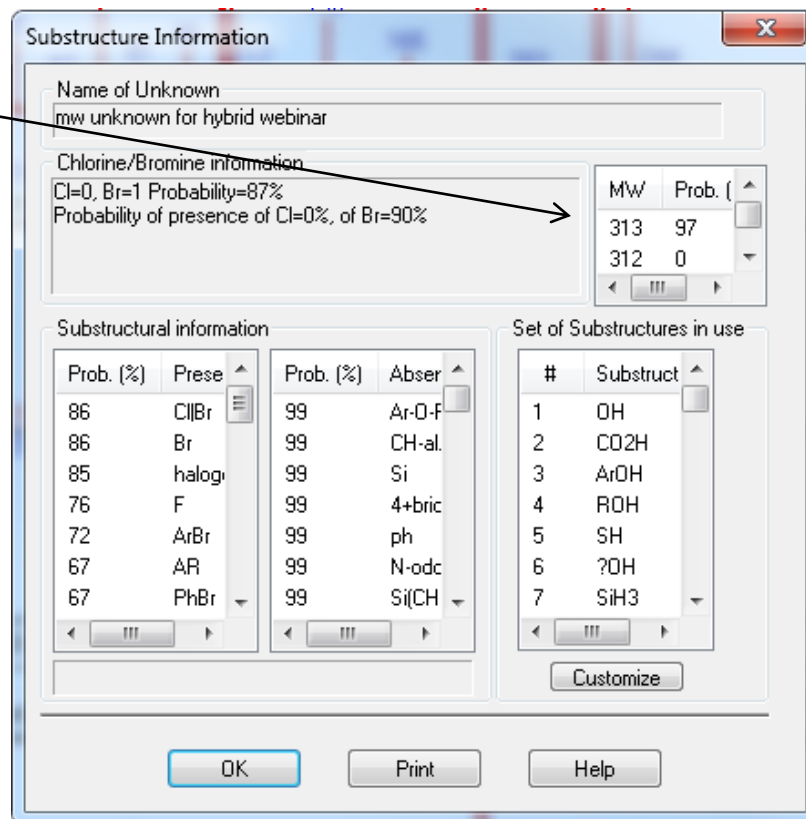
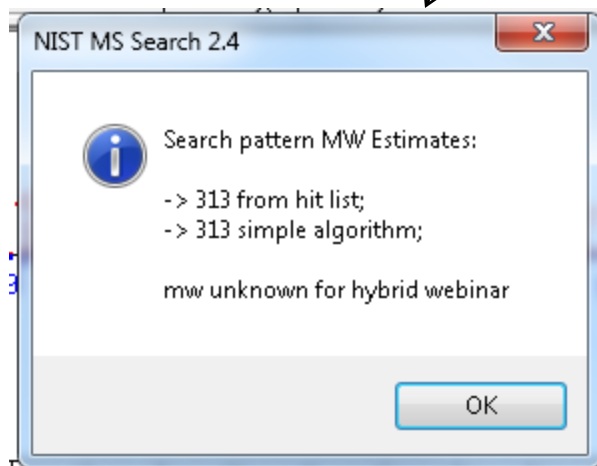
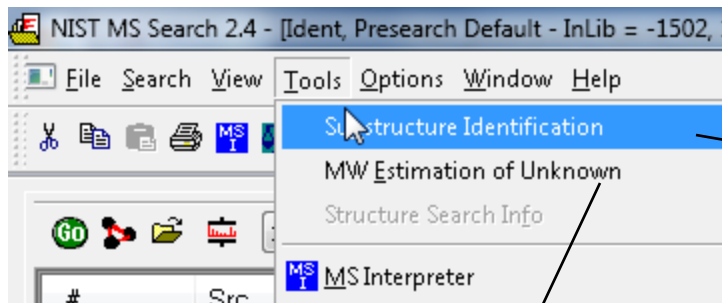
#	Src.	Name
1	E	Unknown
- Screenshot 2:** The 'Library Search Options' dialog box is open. The 'Spectrum Search Type' is set to 'EI Hybrid'. The 'Precursor MW' section has the 'in spectrum' checkbox unchecked (labeled 2).
- Screenshot 3:** The main interface shows the search results table with one entry: 

#	Src.	Name
1	E	Unknown

 (labeled 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



# 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

The image displays two screenshots of the NIST MS Search 2.4 software interface. The top screenshot shows the main application window with the title bar indicating a hybrid precursor search at m/z 405. The bottom screenshot shows the 'Hit List Search Options' dialog box, which provides detailed search parameters. Red arrows and numbers (1-5) link the numbered list items to specific UI elements.

**1.** Points to the search type field in the 'Hit List Search Options' dialog, which is set to 'Similarity : Hybrid. Precursor m/z = 405'.

**2.** Points to the 'Hit List Search Options' dialog box.

**3.** Points to the search type dropdown menu in the main application window, which is set to '1. unknown MS interpreter no structure'.

**4.** Points to the 'Type of Search: Hybrid' and 'Displayed: Hybrid' fields in the hit list structure table.

**5.** Points to the precursor m/z value '405' in the hit list structure table.

Type of Search	Displayed	m/z
Hybrid	Hybrid	405

## DeltaMass Table

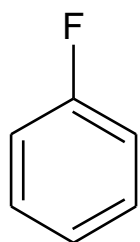
- Hybrid DeltaMass values noted in spectral evaluation
- Value note can be + /—, depending only species present/absent
- ***Continually updated*** on web page as time permits\*



\* [Link to Most Current Hybrid DeltaMass Updates](#)

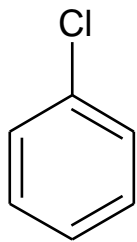
# Associating Some *Simple* Structures with DeltaMass Values

- 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

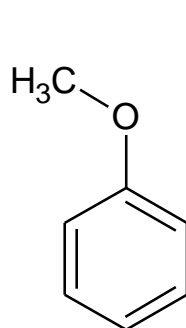


MW 96

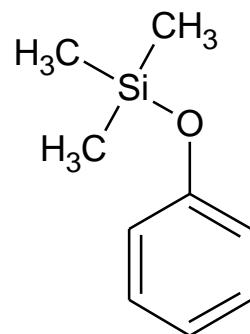
**DeltaMass 16**



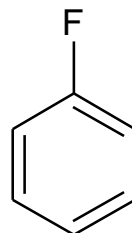
MW 112



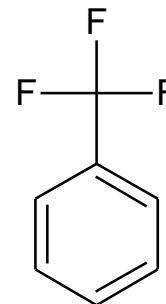
MW 108



MW 166

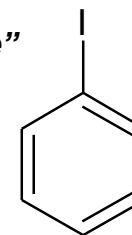


MW 96

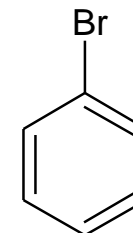


MW 146

**DeltaMass 50**

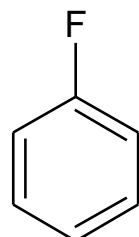


MW 204



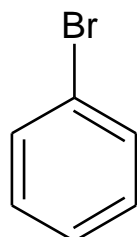
MW 156

**DeltaMass 48**

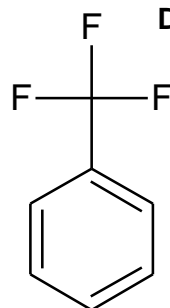


MW 96

**DeltaMass 60**

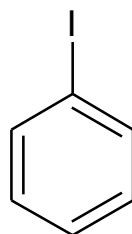


MW 156

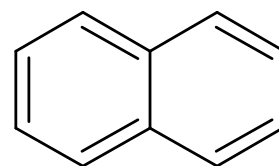


MW 146

**DeltaMass 58**

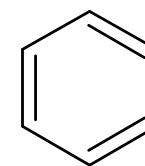


MW 204

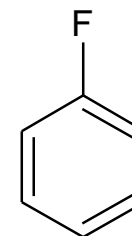


MW 128

**DeltaMass 50**

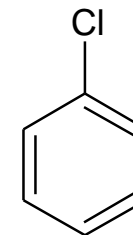


MW 78

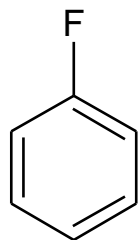


MW 114

**DeltaMass 2**

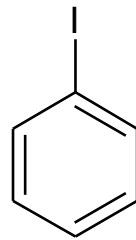


MW 112

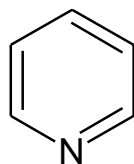


MW 96

**DeltaMass 108**

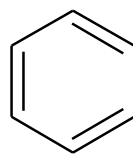


MW 204

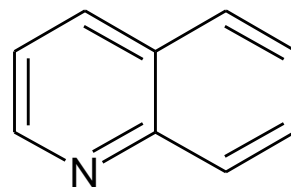


MW 79

**DeltaMass 1**

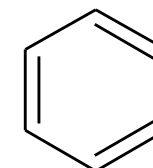


MW 78

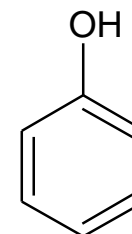


MW 129

**DeltaMass 51**

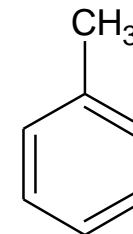


MW 78



MW 94

**DeltaMass 2**



MW 92



## Other Things to Consider Using Hybrid Search for Unknown Identification

- Accurate mass data
- Chemical ionization data<sup>7</sup>
- 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<sup>7</sup>, derivatization, etc..

# NIST MSMS (Tandem) Hybrid Searches<sup>17-19</sup>

**Note:**<sup>23</sup> Created a **Separate** Webinar Series on MS/MS Tandem Searches<sup>23</sup>

- 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

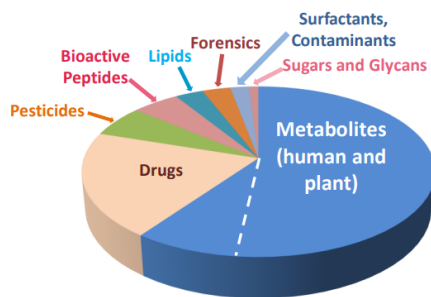
### Fragmentation Methods

27,840 HRAM (High Res Accurate Mass) Compounds  
29,890 QTOF, HCD, IT-HRAM, QqQ Compounds  
29,444 Ion Trap Compounds (Low Res., up to MS<sup>4</sup>)  
246 APCI HRAM 'Extractables and Leachables'

### Precursor Ion Types

26,575 Protonated  
12,589 Deprotonated  
10,032 Water/Ammonia Loss  
24,167 Other In-Source Generated

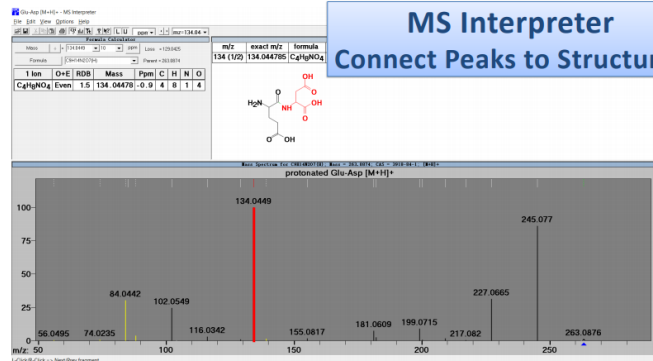
### Types of Compounds and Spectra



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

6,000 human metabolites

### MS Interpreter Connect Peaks to Structures



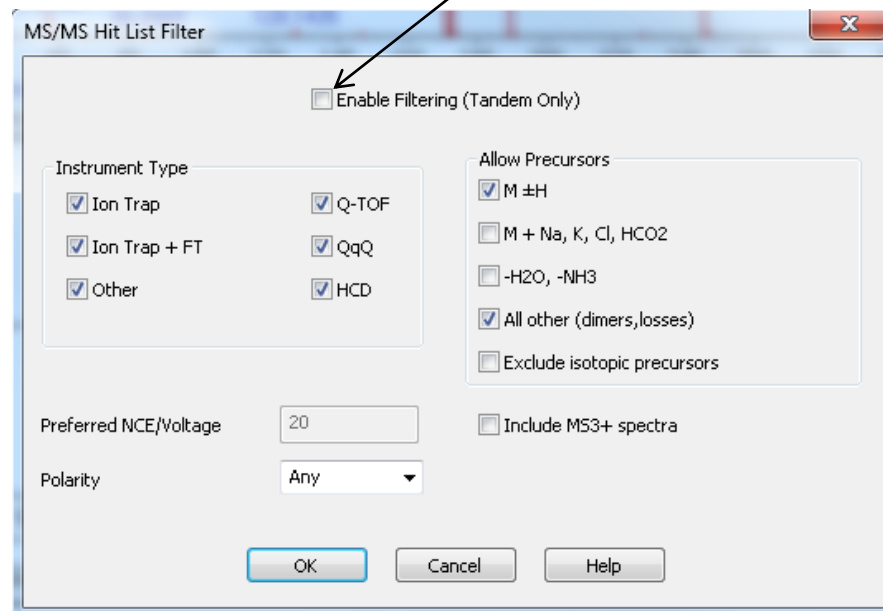
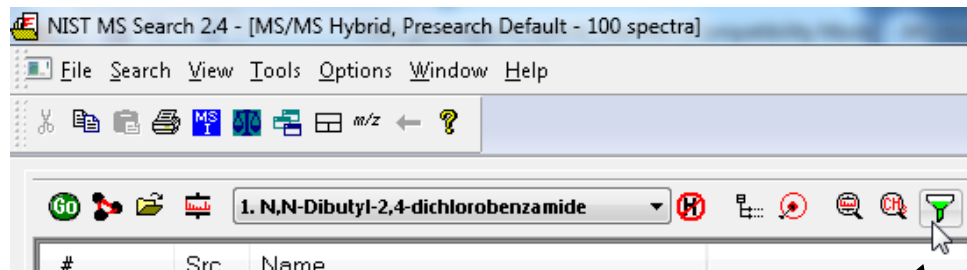
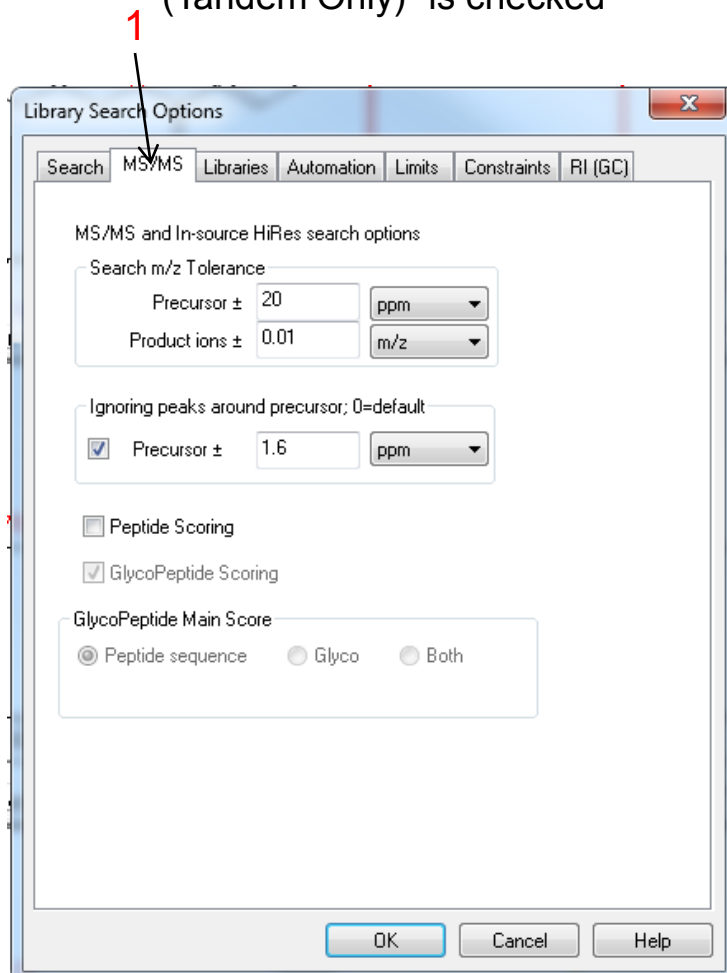
Hybrid Search - Identify Compounds Not in the Library

[\\*NIST MSMS Database link](#)

# Additional NIST MSMS (Tandem) Hybrid Search Menus

**Note:**<sup>23</sup> Created a **Separate** Webinar Series on MS/MS Tandem Searches<sup>23</sup>

- 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

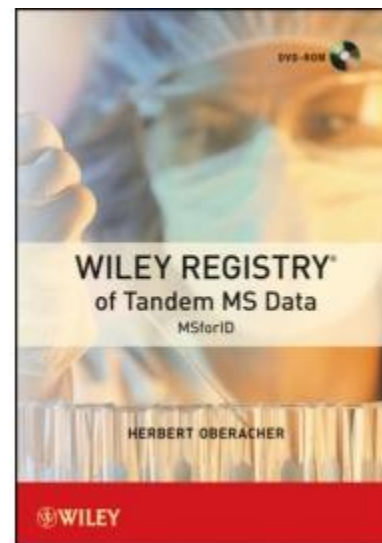


# Wiley MSMS (Tandem) Libraries

**Note:**<sup>23</sup> Created a **Separate** Webinar Series on MS/MS Tandem Searches<sup>23</sup>

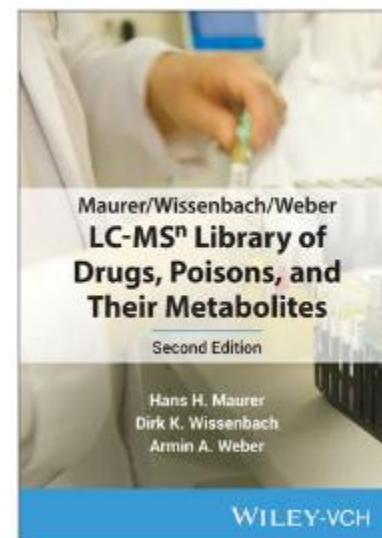
## Wiley Registry of Tandem Mass Spectral Data, MS for ID by Herbert Oberacher

- 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-MS<sup>n</sup> Library of Drugs, Poison, and Their Metabolites, 2<sup>nd</sup> 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
- Toxicologically 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](#)
23. [Identification of Unknowns by MS/MS \(Tandem\) Spectra with NIST Search](#)

## Acknowledgements

### **Wiley Webinar Production:**

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