

# Information for NIST 20 Tandem Library Users

This document describes special features of the **NIST Mass Spectral Search Program v.2.4** (NISTMS.exe) of interest to the **Tandem Library** users. General software features are presented in separate documentation.

## Libraries:

There are four separate **NIST** reference libraries containing tandem spectra obtained by tandem mass spectrometry.

1. **hr\_msms\_nist** – contains all high resolution – accurate mass (HRAM) spectra of ‘small molecules’
2. **lr\_msms\_nist** – contains all low resolution (unit mass accuracy) spectra of ‘small molecules’. When searching, the fragment ion tolerance is always set to  $\pm 0.5$  *m/z* units, regardless of the value set in the MS/MS search tab. The precursor ion tolerance set in the **MS/MS** tab of the **Library Search Options** dialog box is used for all libraries. Use of this library is not recommended for high resolution hybrid searches.
3. **apci\_msms\_nist** – contains about 4K HRAM spectra from atmospheric pressure chemical ionization (targeted for *extractables* and *leachables*)
4. **biopep\_msms\_nist** contains all spectra of peptides of biological interest (mostly HRAM)

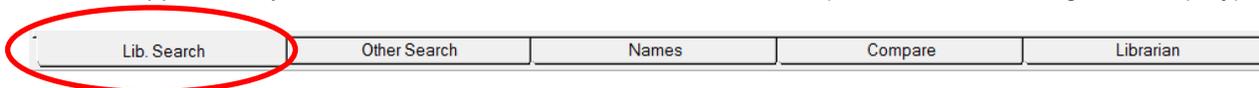
## Library Searching:

Library search settings are specified in the dialog box opened by selecting the fourth button in the **Button Bar** shown below:

**Button Bar** (at the top of the **Lib Search** tab view)



This bar appears only when the **Lib. Search** tab view is selected (at bottom of the Program's display)



**Search Tab** (shown below in the **Library Search Options** dialog box) selects the type of search and relevant controls. The tandem searches are:

### 1. Identity: MS/MS

Searches only library spectra whose precursor-ion's *m/z* value matches that of the query spectrum (this value should be in the **PrecursorMZ:** field of submitted spectrum or may be manually entered in the **Precursor ion *m/z*** field of the dialog box).

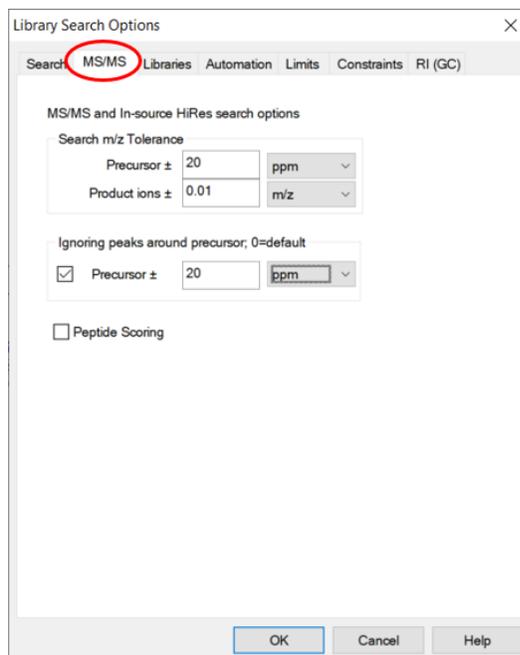
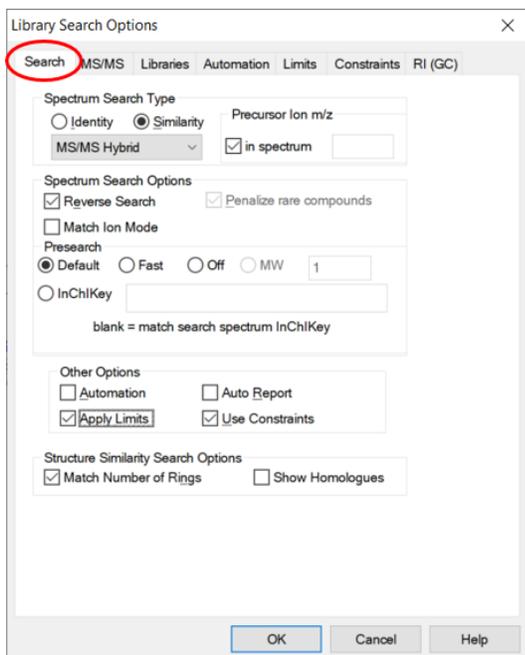
### 2. Identity: In-source HiRes

Same as MS/MS search except does not use the precursor mass to constrain searches.

### 3. Similarity: Hybrid MS/MS (Shown selected in figure below)

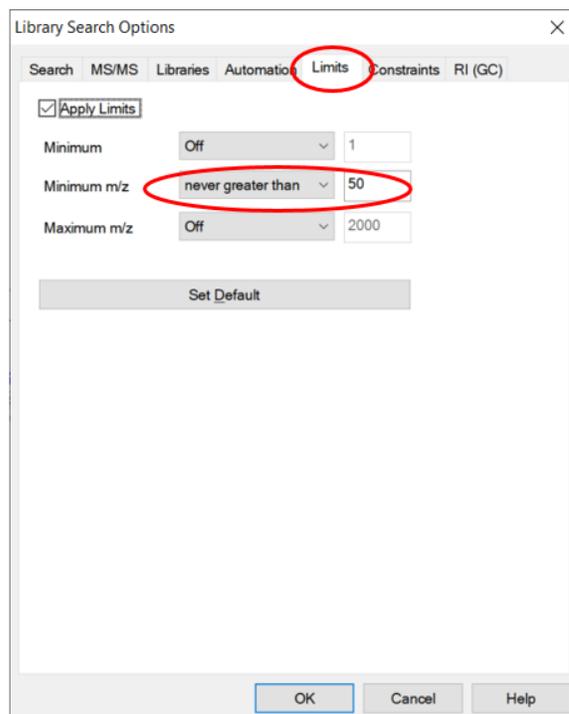
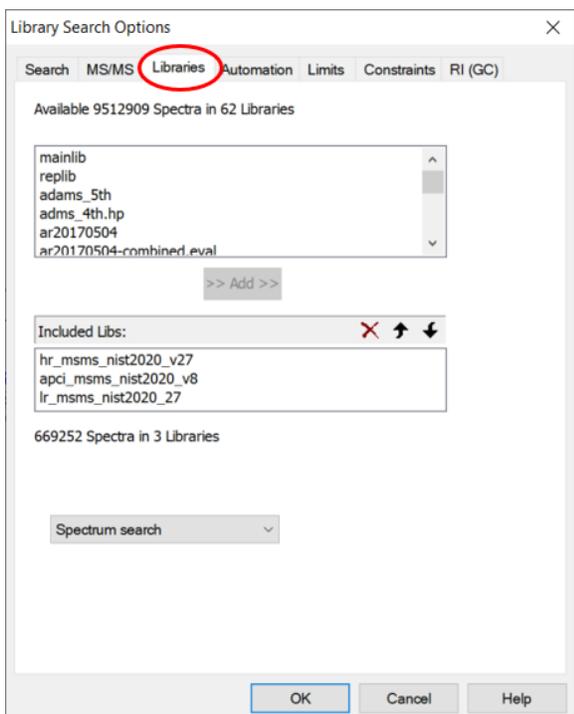
This finds all spectra having matching product ion and neutral loss peaks. Can aid the identification of compounds not present in the library.

Other searches are intended for unit mass resolution electron ionization (EI) spectra as is common for GC/MS analysis using quadrupole mass analyzers. Other settings are associated with all MS libraries and are described in the main documentation.



### **MS/MS Tab** (above right):

The precursor mass tolerance should be set to reflect the accuracy of your instrument - NIST Tandem Library spectra always have the exact mass value for the precursor ion. It is generally recommended that the product-ion tolerance be set at 0.01  $m/z$  units to ensure that lower mass peaks are matched. Due to the fact that spurious peaks commonly appear near the precursor ion, a setting of 20 ppm is recommended for the **Ignoring peaks around precursor** specification. **DO NOT** select **Peptide Scoring** unless using peptide libraries.



### **Libraries Tab** (shown above left):

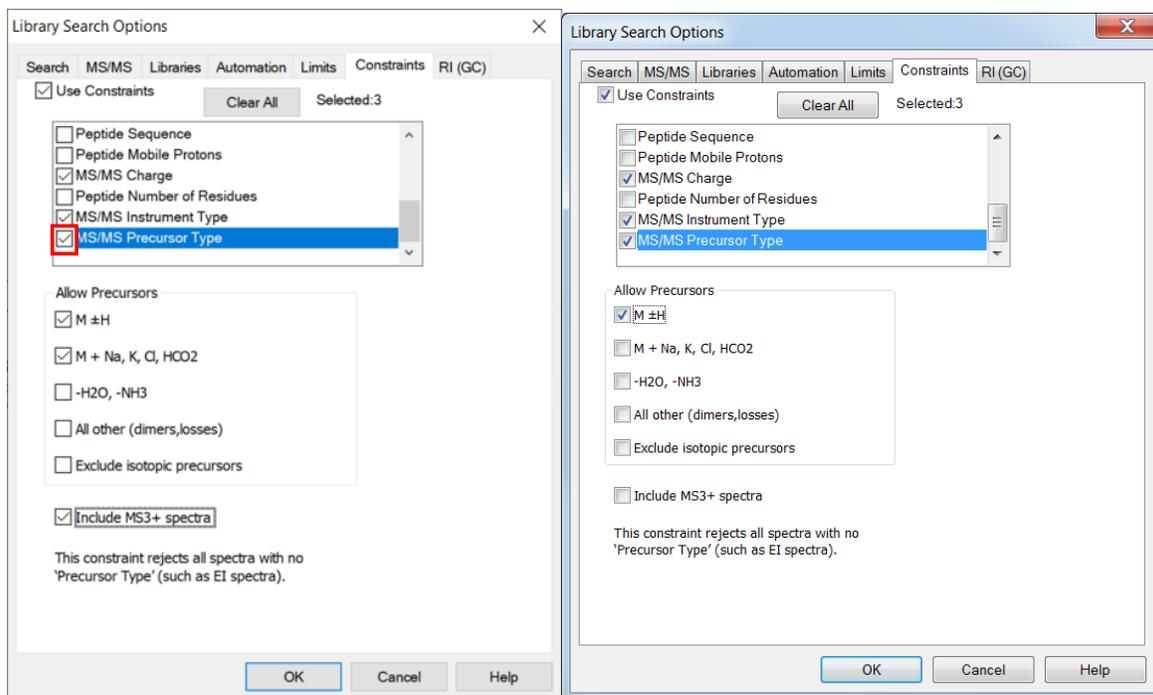
Select libraries for searching by double clicking on the library in the list in the upper window. Your libraries may have different names than shown here. Libraries with 'lr' (low-resolution) prefix are always searched using a wide mass tolerance for product ion masses. Libraries can be added by double-clicking on the name in the upper list or removed by double clicking on its name in the lower list.

### **Limits Tab** (shown above right):

It is recommended that **Minimum  $m/z$** : box be set in the **Limits** tab in the **Library Search Options** search dialog. This should correspond to the lowest  $m/z$  value reported by your instrument. The default is 50  $m/z$ . Unless set, spectrum comparison begins at the higher of the lowest  $m/z$  value in the two spectra being compared. Make sure there is a check in the check-box next to the **Apply Limits** label. Selecting this check-box will also be reflected in the box with the same label in the **Search** tab of the **Library Search Options** dialog box.

### **Constraints tab** (shown next page):

This shows a variety of filters that reduce the number of spectra in the Hit List. Three filters are of special interest for tandem library users: **MS/MS charge**, **MS/MS Instrument Type** and **MS/MS Precursor Type**. These filters have no effect on EI (GC/MS) searches. When any constraint is selected, a check is automatically placed in check-box next to **Use Constraints** label. Selecting this check-box will also be reflected in the box with the same label in the **Search** tab of the **Library Search Options** dialog box.



## Scores (reported in the **Lib Search** tab view's **Hit List** window)

When matching two product-ion mass spectra, **Scores** are reported as well as the **Dot Product**. The **Dot Product** is a simple mathematical measure widely used in reporting spectrum similarity. An exact match is reported as 999. The **Score** is adjusted for spectra with few peaks and is intended to adjust for the reduced selectivity when matching small numbers of peaks. For a single peak spectrum for example, a match will report a 999 for the **Dot Product** and significantly lower the **Score** depending on abundance. The difference between the **Dot Product** and **Score** will reflect an estimate of the uncertainty in identification due to the small number of peaks (generally less than four). This **Score** is simply a rough measure of identification confidence and has no well-defined statistical meaning.

## Hit List Filtering (rightmost button on button bar in Search tab – see below)



The **NIST Tandem Libraries** contain product-ion spectra formed over a range of energies, precursor-ion types, and fragmentation methods. This means that a search of the **Tandem Libraries** can produce many Hits generated for a single compound, which can be tedious to sort through. To facilitate this task, a filtering process is available that readily controls the types of spectra shown. This is available for both the **Lib Search** and **Other Search** tab views. The Filter is invoked by pressing the filter button at the end of the **Button Bar** shown above for the **Lib Search** tab view. (it is at the top of **Hit Lists** in the **Other Search** tab view). The filtering process may be turned on and off with the **Enable Filtering** check-box at the top of the **MS/MS Hit List Filter** dialog box (below). When the filter is enabled, the filter button will appear to be pressed. Clicking on the **OK** button in this dialog box causes the Hit List to conform to the selections made. These controls match corresponding controls in the **Constraints** section, except they do not permanently remove Hits – all original Hits can be seen by turning off the **Enable Filtering** check-box. The **Preferred NCE/Voltage** is not active for spectrum searches – it limits **Other Search** results to the value closest to the entered **Preferred** value.

MS/MS Hit List Filter

Enable Filtering (Tandem Only)

Instrument Type

Ion Trap       Q-TOF

Ion Trap + FT       QqQ

Qother       HCD

Allow Precursors

M ±H

M + Na, K, Cl, HCO<sub>2</sub>

-H<sub>2</sub>O, -NH<sub>3</sub>

All other (dimers, losses)

Exclude isotopic precursors

Preferred NCE/Voltage: 20

Include MS<sub>3</sub>+ spectra:

Polarity: Any

OK      Cancel      Help

**Hit List Display tab:** (of the **Library Search Properties** dialog box)

It is recommended that when examining Hit Lists of spectra from Tandem Libraries, three Tandem-specific additional columns be selected for display. They are **Prec. Type** (precursor-ion type) **Instr. Type** (instrument type) and **Energy**. The first, **Prec Type**, identified the ion that was selected for fragmentation, which include in-source generated ions. **Instr. Type** gives the fragmentation method Q-TOF, QqQ, HCD, Ion-trap (IT) and high-resolution Ion-trap (IT-FT). For all **Hybrid searches** the **DeltaMass** selection should also be made.

The **Library Search Properties** dialog box is displayed placing the Mouse Pointer on the Hit List window (lower left panel of the display in the **Lib. Search** tab) and pressing the Right Mouse Button (RMB); then select **Properties** from the RMB menu, the last item in the list. The **Hits List** tab is one tab in the display, as shown in the figure on the right).

