

Data Processing Using Thermo Freestyle Software with NIST MSMS Search for Compound Identifications

NIST Search Type Demonstration

James Little

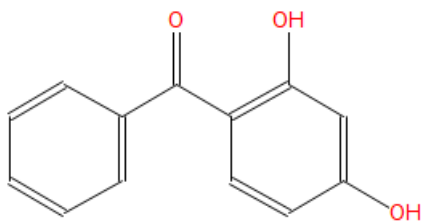
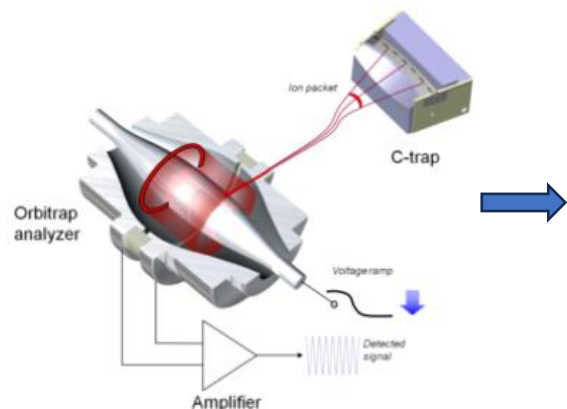
Mass Spec Interpretation Services

<https://littlesandsailing.com/>

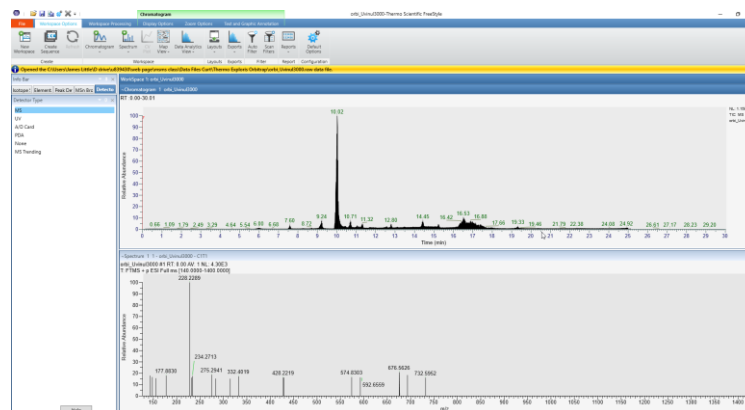
11/06/2025

Thermo Freestyle

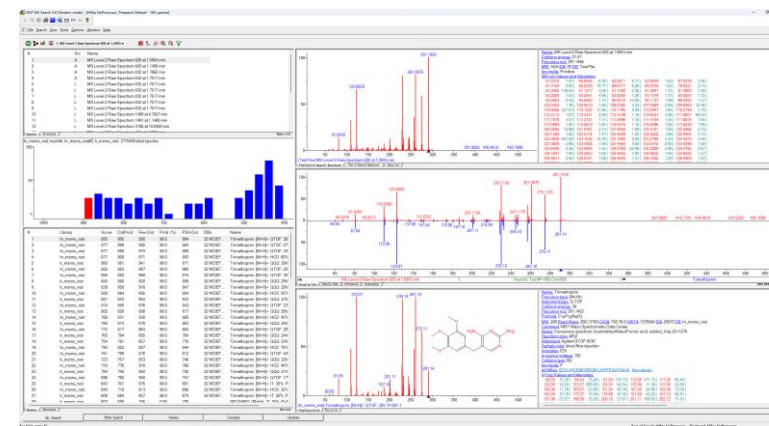
Thermo Orbitrap MSMS



Uvinul 3000
MW 214



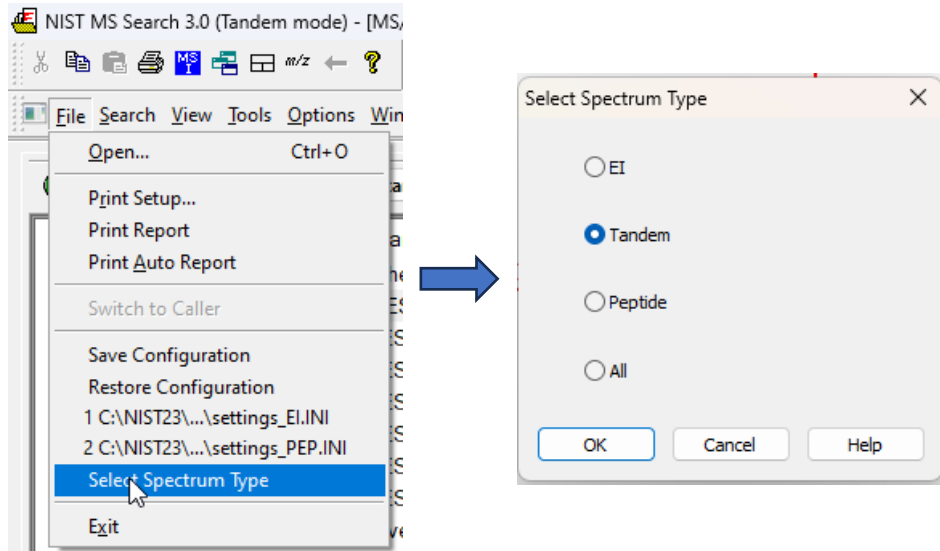
NIST MSMS Search



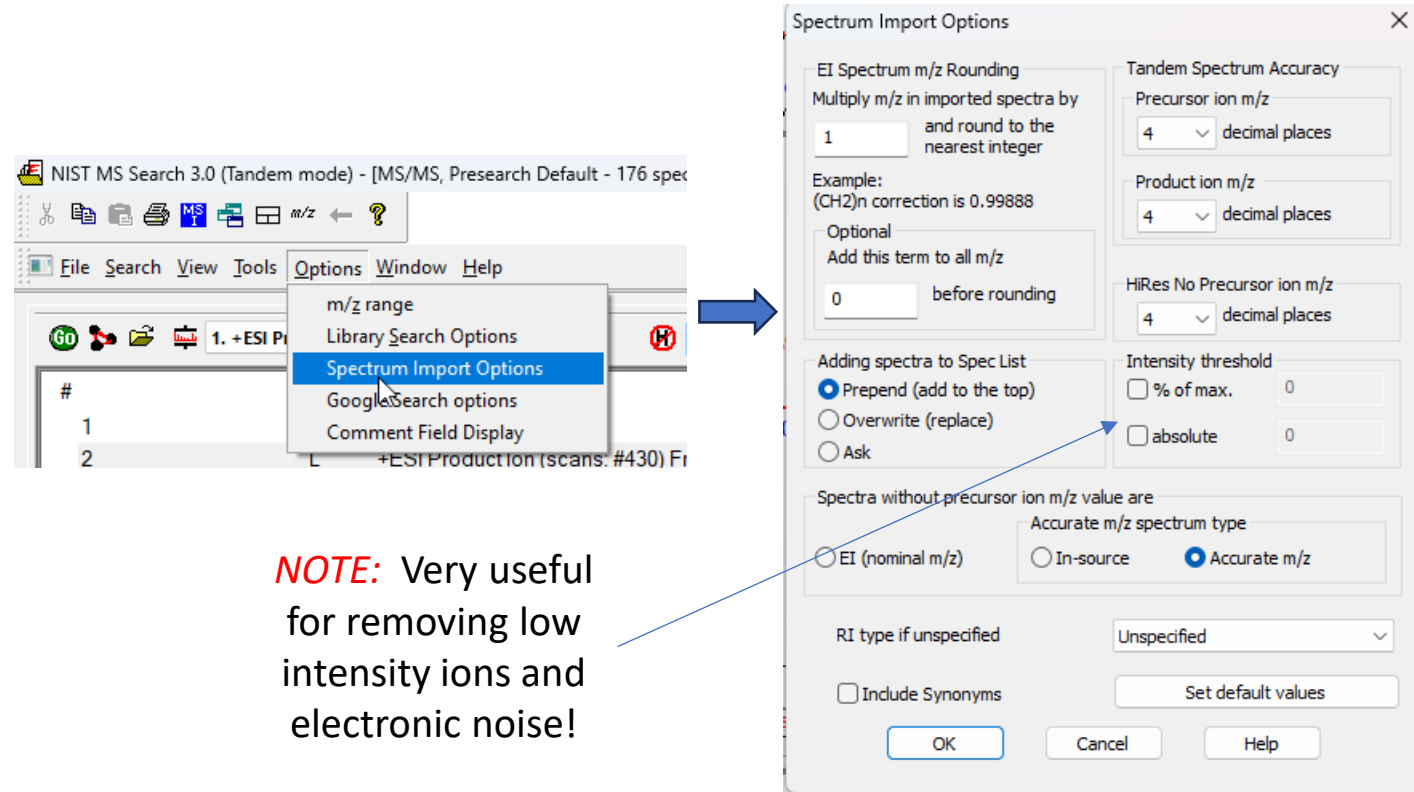
- More information in **my** “Advanced Course for NIST MSMS” on my website!
- Also, more information in this handout

Typical Settings for NIST MS Search 3.0 (Tandem Mode) And Information on Selecting Types of NIST Searches

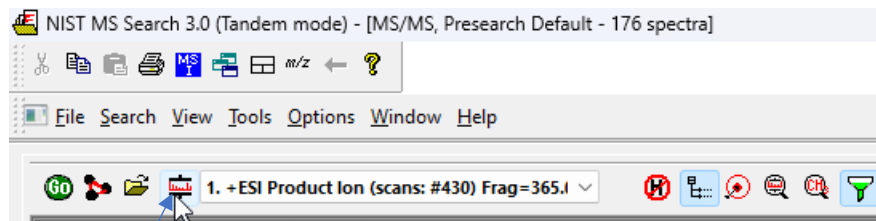
v.3.0 NIST Search *Simplifies* Menus for Different Modes



Typical Spectrum Import Options



Library Search Options for NIST MS Search 3.0 (Tandem Mode)

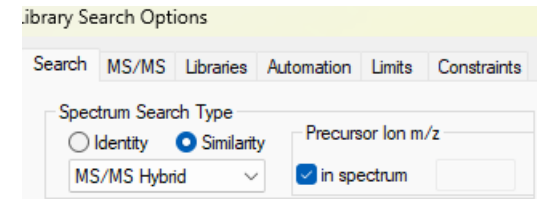
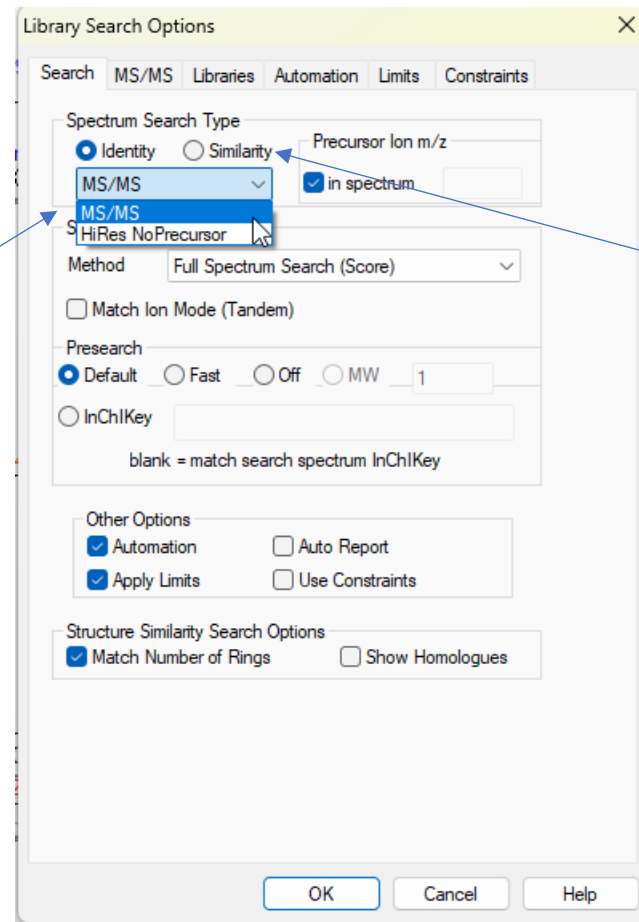


Click here

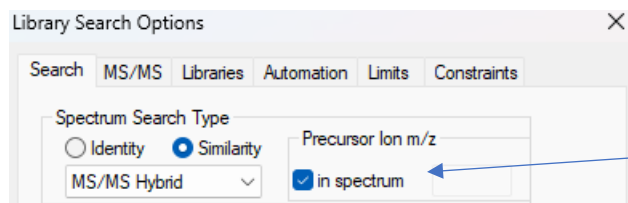
Can do Identity MS/MS which searches spectra restricting by the precursor ion and its specified m/z Tolerance set in MS/MS tab (this one returns fewer but likely more useful results if compound's spectrum is present in accurate mass format)

Or

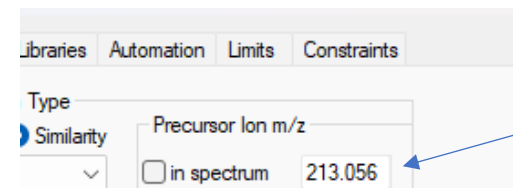
Identity/High Resolution No Precursor, just compares the overall fit of unknown spectra to reference spectra



- Another type search is Similarity/Hybrid MS/MS which can be **very** useful if a related compound is present in the reference libraries, but the actual compound is **not** present
- See NIST MSMS **Full** Course for details on Hybrid Search



If precursor m/z imported correctly with spectrum, will have Precursor Ion m/z "in spectrum" selected

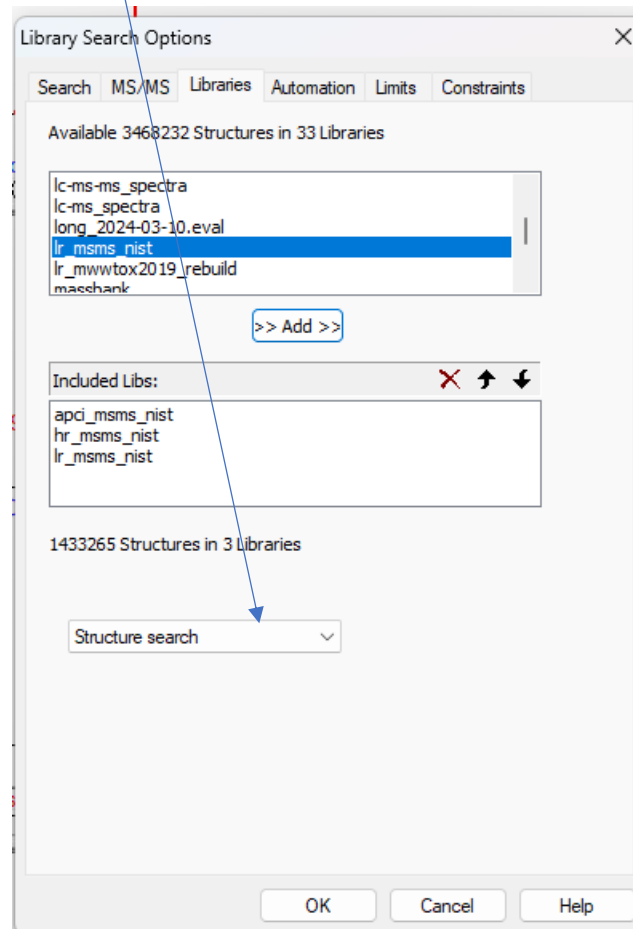
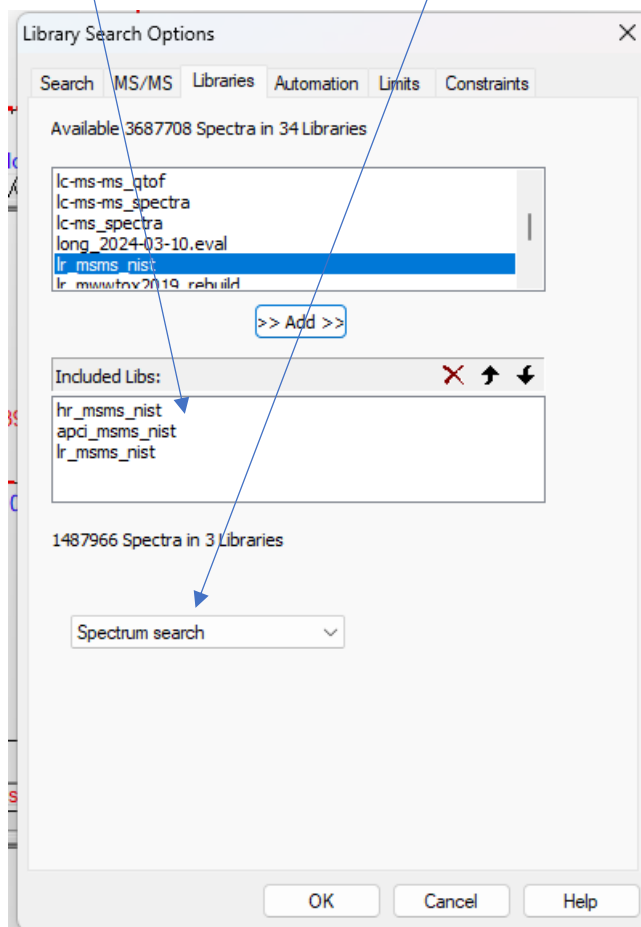


If precursor m/z **not** imported properly, user can deselect "in spectrum" and then enter value manually

Other Settings for NIST MS Search 3.0 (Tandem Mode)

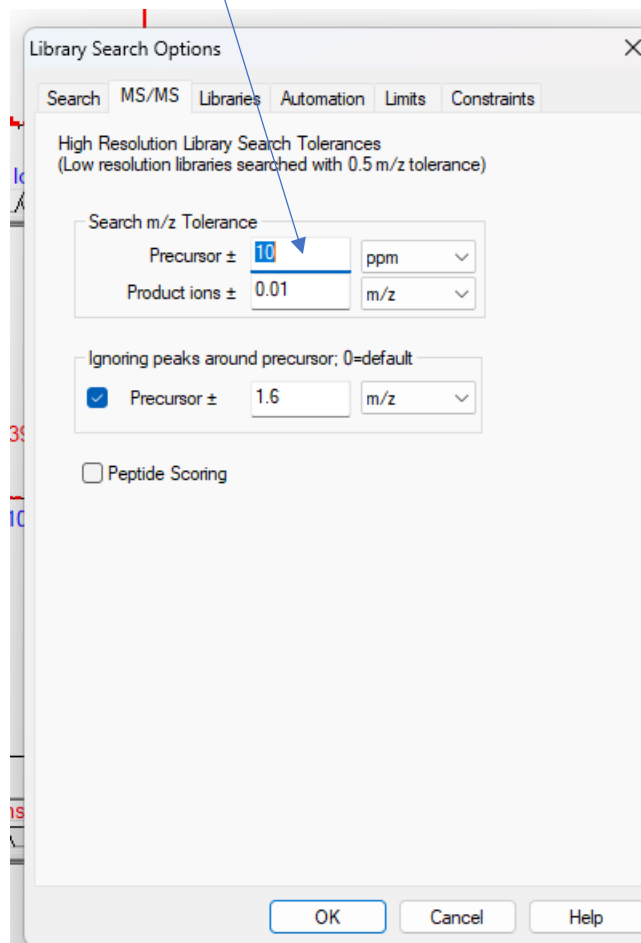
Select whichever MSMS Libraries you want to search, the ***standard ones*** with NIST 2023 Software are selected below for ***spectrum search***

Libraries to search by Structure are also selected after toggling to ***Structure Search***

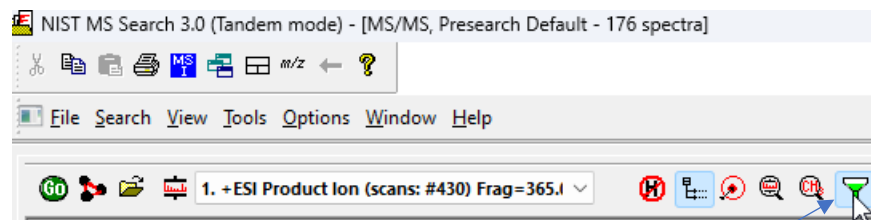


Check the settings in Library Search Options/MS/MS Tab

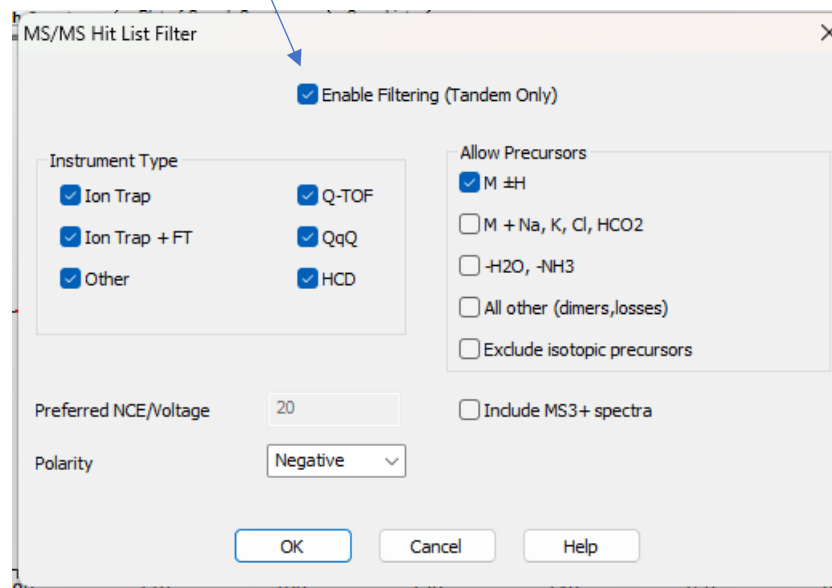
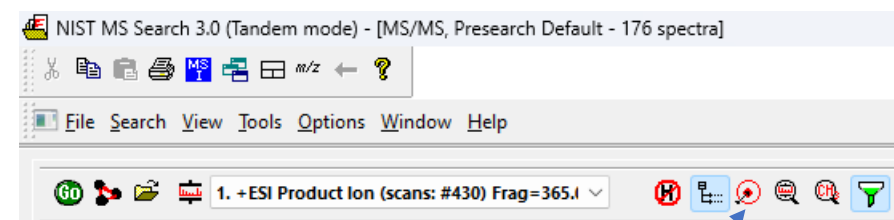
Set the tolerance for precursor m/z which is appropriate for your instrument



Other Settings for NIST MS Search 3.0 (Tandem Mode)



The “funnel” settings affect the results displayed from MSMS search such as polarity of MSMS spectrum and many others if “*Enable Filtering (Tandem Only)*” is selected



The “Best Matching” only can greatly simplify the results, removing all but the best matching spectrum using the CAS Number as a filter

Important!! Save Your Settings for Future Sessions!!

- After initially setting up search preferences, most normally do not change significantly
- *All Settings* can be saved for future searches by **Save Configuration** with a unique name
- User can then easily **Restore Configuration**

