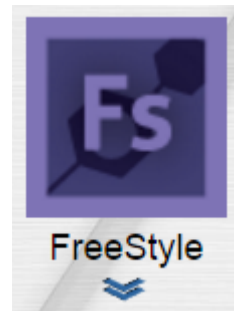


## Sending Accurate Mass EI Data from Orbitrap to NIST Search

- Nominal mass EI data can be sent to NIST Search using normal approach from Qual Browser in Xcalibur
- Accurate mass data cannot
- ThermoFisher FreeStyle sends accurate mass data to NIST Search
- FreeStyle delivered with Orbitrap Xcalibur software as separate stand-alone application
- Basic instructions to send from FreeStyle are demonstrated



## Overall Approach

- Initially process a raw format file chromatogram in FreeStyle and obtain an EI spectrum
- Perform a library search using ThermoFisher's internal search algorithm
- Change parameters in the internal search to employ accurate mass values in the internal search
- Changing to accurate mass values in internal search is also reflected in external search using NIST search software
- Enable accurate mass parameters in NIST external search algorithm

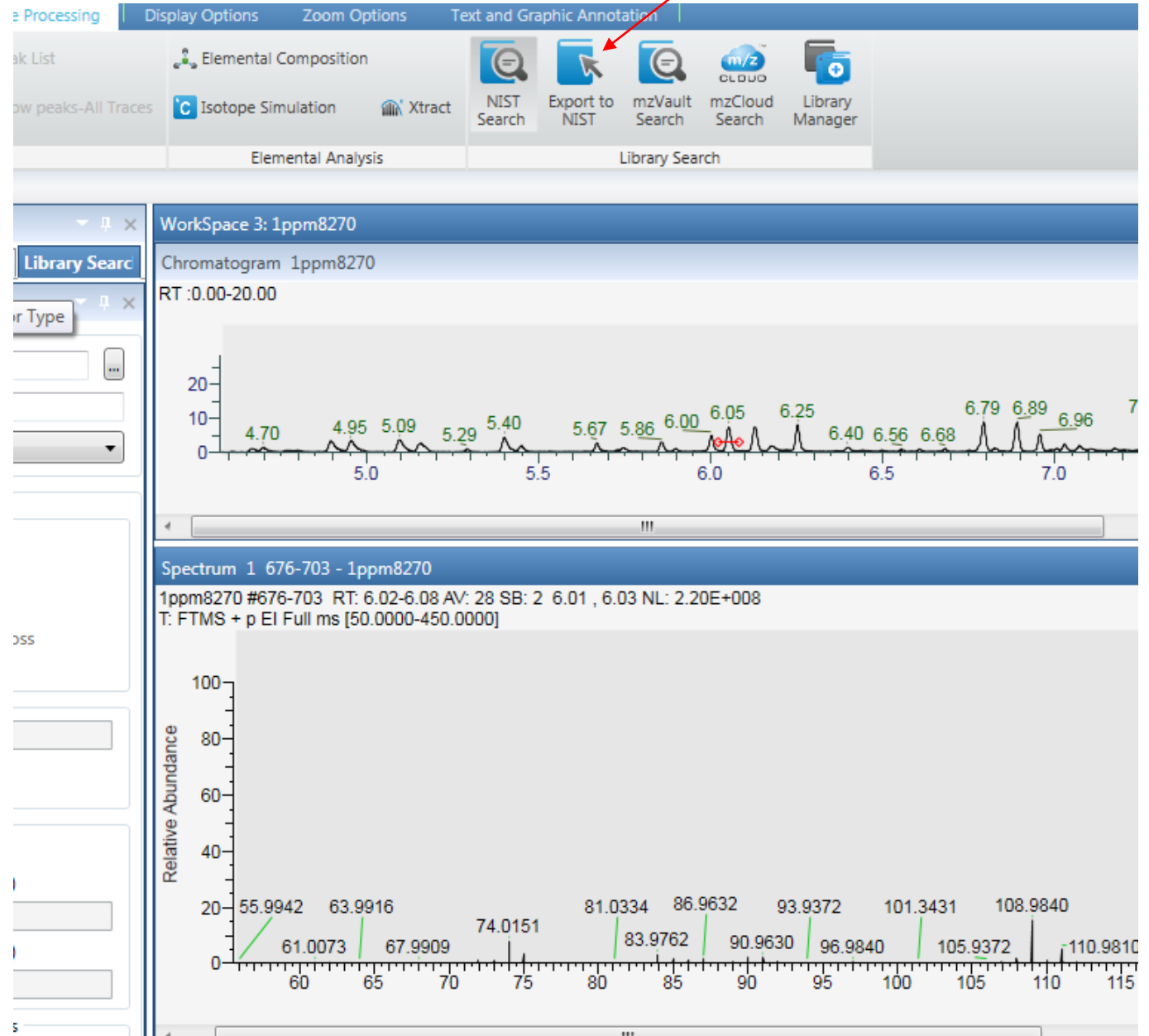
## Change Default Settings within FreeStyle Search

- *After* doing an internal search in FreeStyle, select “Library Search” tab in “InfoBar” Menu on left
- Select either MS/MS radio button or “In-source HiRes” button
- Then select “Make Default” at bottom of menu
- Then select “Apply” at

The screenshot displays the FreeStyle Search software interface. The 'Info Bar' on the left contains the 'Library Search' tab, which is highlighted with a red arrow labeled '1'. Below this, the 'Library Search' panel is open, showing search parameters. The 'Search Type' section has 'In-source HiRes' selected, indicated by a red arrow labeled '2'. At the bottom of the panel, the 'Make Default' button is highlighted with a red arrow labeled '3', and the 'Apply' button is highlighted with a red arrow labeled '4'. The main window shows a chromatogram with peaks at 7.71, 7.77, 7.82, and 7.85 minutes, and a mass spectrum with peaks at 55.0543, 79.0543, and 87.0441 m/z. A message at the top states: 'No Spectrum Trace for NistSearch: The search tolerance settings may be too low.'

## Send Accurate Mass Spectrum to External NIST Search

- Highlight spectrum of interest
- Click on “Export to NIST” at top of page



## Setup for NIST to Display and Utilize Accurate Mass Spectra Step 1

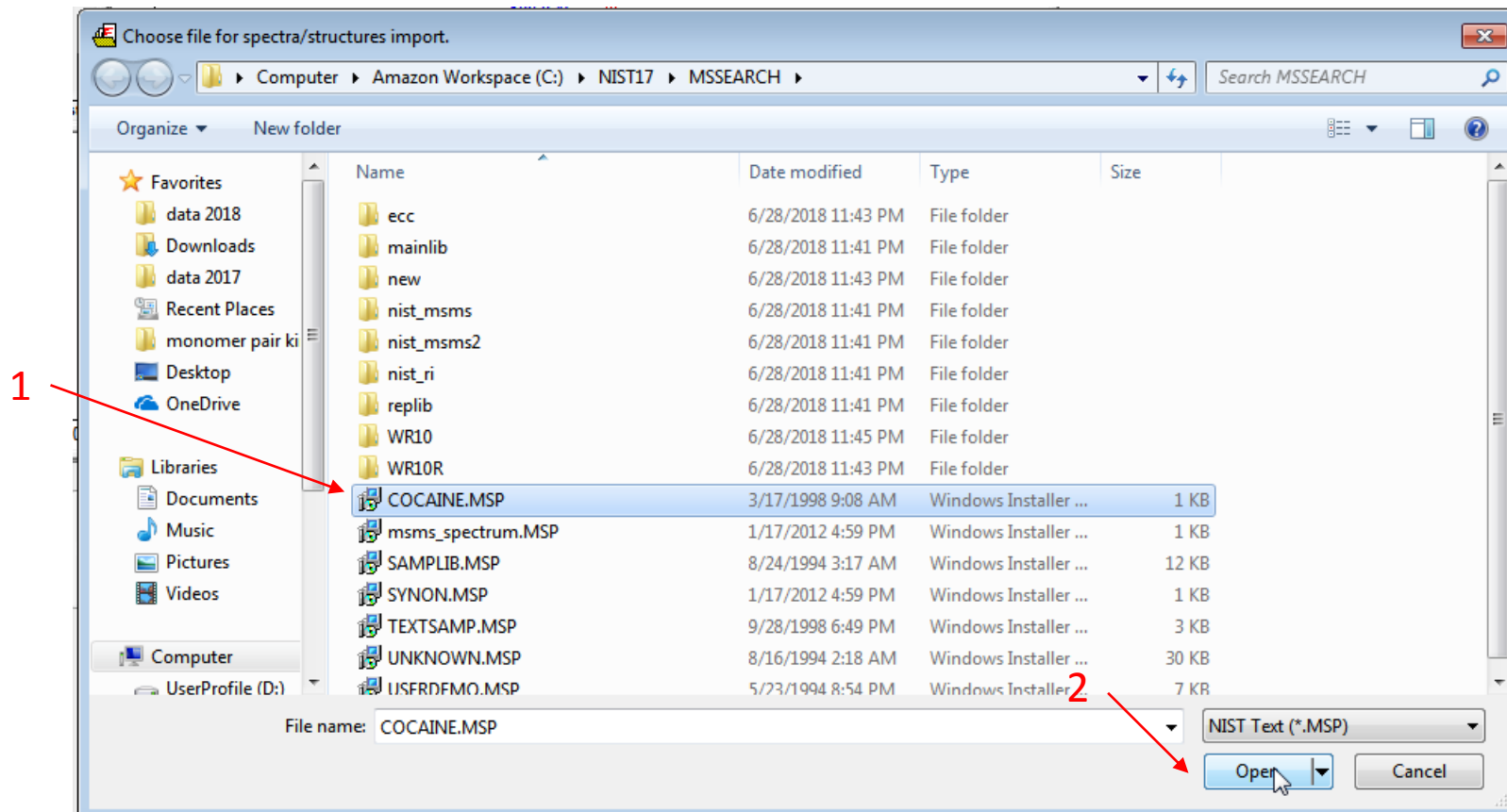
- Standard setup uses nominal mass values
- Change to accurate mass
- Import spectrum, then select an entry in Spec List and “Import” option

The screenshot shows the NIST MS Search 2.3 software interface. The title bar reads "NIST MS Search 2.3 - [Ident, Presearch Default - InLib = 552, 100 s". The menu bar includes "File", "Search", "View", "Tools", "Options", "Window", and "Help". The toolbar contains icons for various functions, including a search icon, a magnifying glass, and a question mark. The main window displays a table with columns "#", "Src.", and "Name". The first row is highlighted in blue and contains the text "1 A 1ppm". A red arrow labeled "1" points to this row. A context menu is open over the first row, with the "Import" option highlighted in blue. A red arrow labeled "2" points to the "Import" option. The context menu includes options such as "Library Search", "Structure Similarity Search", "Cut", "Copy", "Paste", "Select All", "Send To", "Import", "Export Selected", "Insert Clipboard Structure", "Copy Structure to Clipboard", "Insert Clipboard Spectra", "Copy Selected to Clipboard", "Print", "Print Preview", and "Properties". Below the table, there is a section for "Names" and "Structure" with a small plot showing a mass spectrum on a logarithmic scale from 1 to 1000 m/z.

#	Src.	Name
1	A	1ppm
2	A	1pp
3	E	1pp
4	E	1pp
5	A	1pp
6	A	1pp
7	A	1pp
8	A	1pp
9	A	1pp
10	A	1pp

## Setup for NIST to Display and Utilize Accurate Mass Spectra Step 2

- Select any MSP file and open it



## Setup for NIST to Display and Utilize Accurate Mass Spectra Step 3

- Select Import Options Button in window on right
- Change In-Source/EI Accurate ion m/z to either 4 or 5
- Enable Accurate m/z button
- *Then* import another entry from FreeStyle to update NIST display

Number of Spectra found: 1

#	The names of spectra retrieved
1	Cocaine

Buttons: Import All, Import Selected, Import Options, Search Options, Cancel, Help

Spectrum Import Options

EI Spectrum m/z Rounding  
Multiply m/z in imported spectra by  and round to the nearest integer  
Example: (CH<sub>2</sub>)<sub>n</sub> correction is 0.99888  
Optional  before rounding

Tandem Spectrum Accuracy  
Precursor ion m/z  decimal places  
Product ion m/z  decimal places

In-source/EI accurate ion m/z  decimal places

Adding spectra to Spec List  
 Prepend (add to the top)  
 Overwrite (replace)  
 Ask

Intensity threshold  
 % of max.   
 absolute

Spectra without precursor ion m/z value are  
Accurate m/z spectrum type  
 EI (nominal m/z)  In-source  Accurate m/z

RI type if unspecified

Include Synonyms

Buttons: OK, Cancel, Help

Chemical structure: CC(=C)Cl

# After Options Setup within NIST Import Menu and Another Entry Exported from FreeStyle

## Step 4

