

Using NIST MS Search with Agilent OpenLab Software for Unknown Identifications

James Little

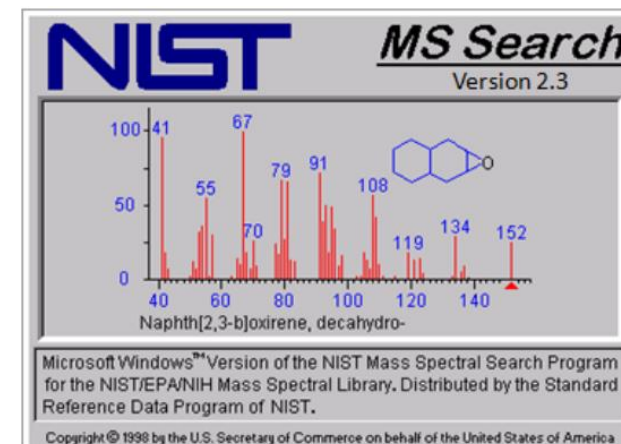
Mass Spec Interpretation Services

Kingsport, TN

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<https://littlemsandsailing.com/>

- Some tips on using NIST MS Search for unknown identifications
- Search with Agilent GC MSD/OpenLab CDS transferred to NIST MS Search
- Information supplied from Agilent (Emily Adams, Product Support Engineer)
- [YouTube Video](#)

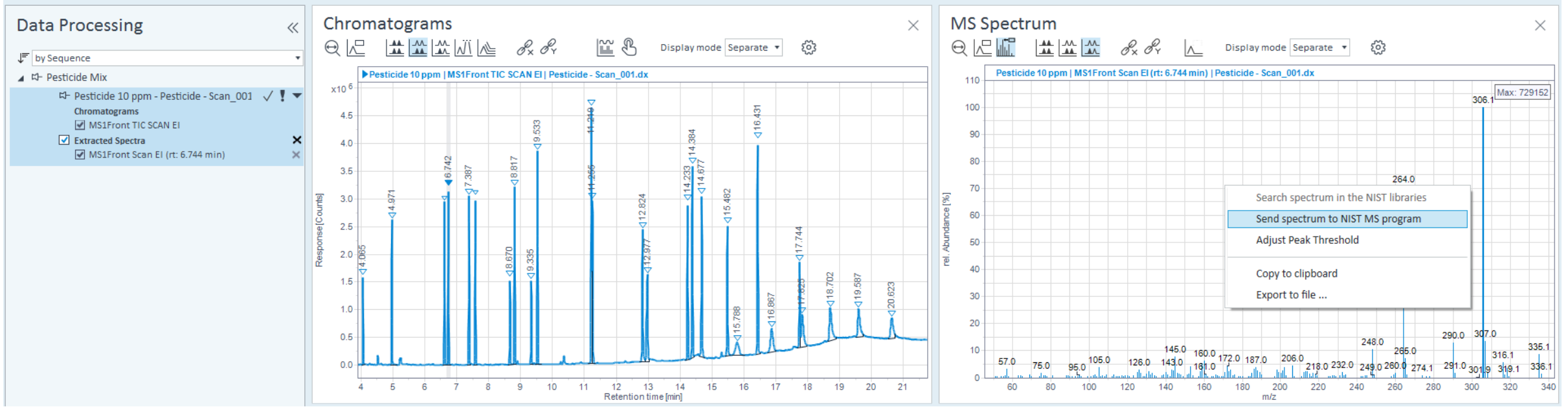


Topics Discussed

1. Sending spectra for search from OpenLab to external NIST MS Search Program
2. Processing OpenLab Files in Agilent MassHunter
3. Export files in netCDF (AIA) format for processing with AMDIS, Wiley KnowItAll, *etc.*
4. Data file locations in OpenLab file directories
5. Searching using NIST *dll* with results returned for internal display in OpenLab
6. Library format supported by OpenLab and interconversion of library formats with NIST Lib2NIST utility program
7. Adding an entry to existing user library or creating first entry in a new user library
8. Initial Settings for NIST Search V3.0 in EI and MSMS (tandem) modes
9. Extensive user help found on “Agilent OpenLab Help & Learning” website

1) Can you send spectra to NIST's MS Search program?

Yes! The simplest way is to right-click on the spectrum in OpenLab CDS and select Send spectrum to NIST MS Search program. (Note that this option is greyed out until NIST's MS Search package is installed on the same PC as OpenLab CDS.)



1) This will open NIST MS Search and load the spectrum you sent.

Note: When you click "Send spectrum to NIST MS program". The MSP text file is generated and saved to a temporary folder (C:\Users\\AppData\Local\Temp\SPECTRA.MSP), should you need to access it

The screenshot displays the NIST MS Search 3.0 interface. The top-left pane shows a list of spectra with the following entries:

#	Src.	Name
1	A	MS1Front Scan EI (rt: 6.744 min)
2	L	MS1Back Scan EI (rt: 10.951 min) Subtra...
3	L	MS1Front Scan EI (rt: 10.254 min)
4	L	MS1Back Scan EI (rt: 6.32 min)
5	L	MS1Front Scan EI (rt: 7.387 min)
6	L	MS1Front Scan EI (rt: 5.230 min)
7	L	Fentanyl Carbamate (EI, SWGDRUG libr...
8	L	Fentanyl Analog (EI, SWGDRUG library)
9	L	Amphetamine Analog (EI, SWGDRUG li...
1.	L	Methamphetamine Analog (EI, SWGDR...
1.	L	Cocaine (EI, SWGDRUG library)
1.	L	Cortisone (EI, SWGDRUG library)

The main plot area shows the mass spectrum of the sample peak at 6.744 min. The x-axis is m/z (50-350) and the y-axis is relative intensity (0-100). Key peaks are labeled at m/z 57.00, 105.00, 145.00, 206.00, 248.00, 264.00, 306.10, and 335.10.

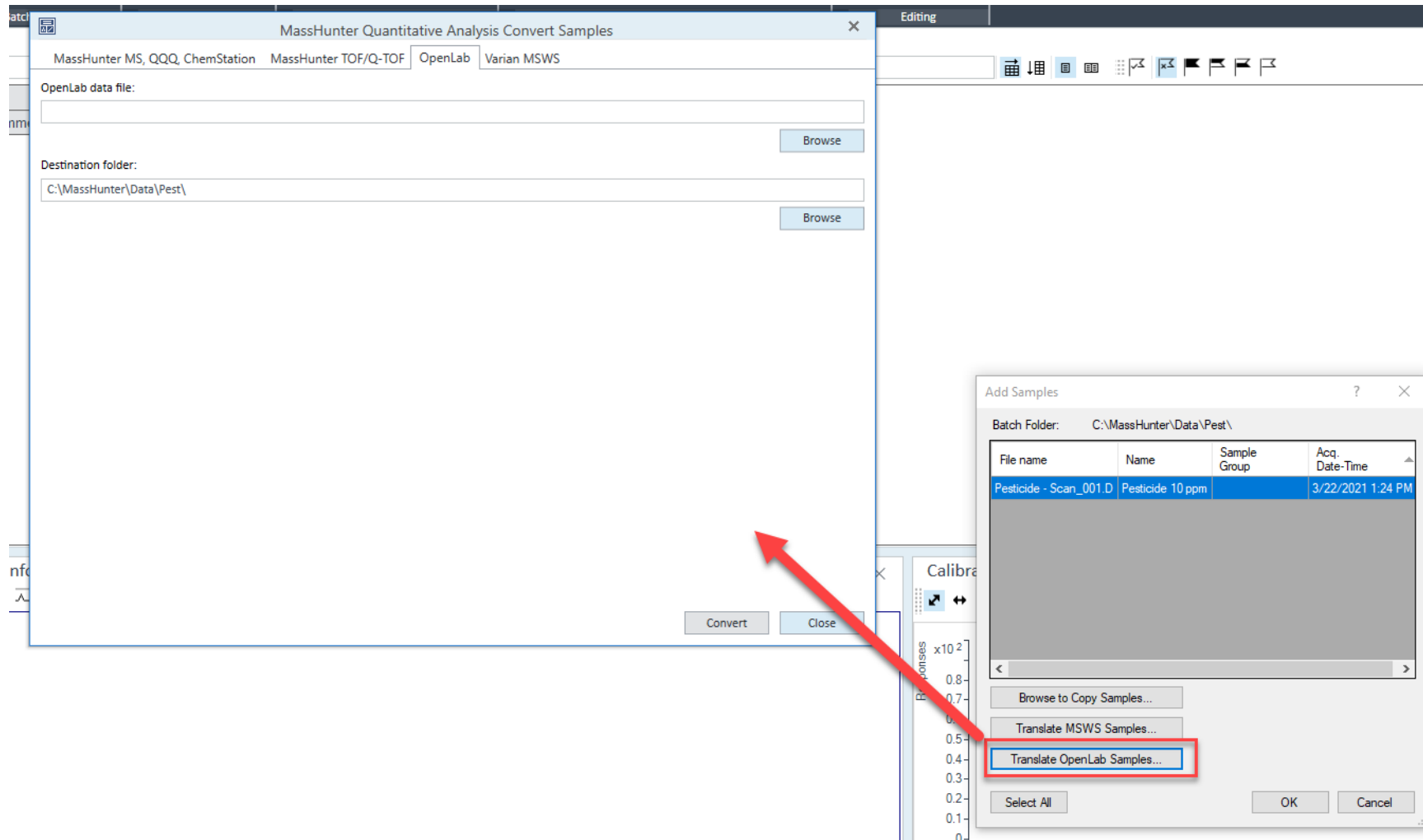
The search results table at the bottom left shows the following top matches:

#	Lib.	Match	R.Match	Prob. (%)	RI	PSS.Match	DBs	Name
1	M	943	943	96.9	1...	944	27...	Trifluralin
2	M	670	691	0.96	1...	820	17...	Fluchloralin
3	M	635	678	0.33	2...	648		6-Fluoro-4-oxo-1-propyl-
4	M	594	622	0.09	2...	606		6-Fluoro-4-oxo-1-propyl-
5	M	594	617	0.09	3...	668		(±)-Mahanine, acetate
6	M	592	696	0.09	2...	593		4-Iodo-3-nitroaniline, N-ε
7	M	586	687	0.07	2...	587		3-Iodo-5-nitroaniline, N-ε
8	M	581	624	0.06	1...	591		1-[3-Methoxy-4-(2,2,2-trifl...
9	M	575	595	0.05	1...	576	21...	Benfluralin
1.	M	570	736	0.05	2...	573	14...	5-Amino-2,3-dihydrophit
1.	M	568	589	0.04	3...	610		N-(4-Isopropyl-2-(3-oxo-
1.	M	561	665	0.03	3...	564		2-(4-amino-6-phenyl-1,3...
1.	M	560	644	0.03	3...	565	1M	m,p-Quaterphenyl

The reference mass spectrum for Trifluralin (mainlib) is shown at the bottom right. The x-axis is m/z (50-350) and the y-axis is relative intensity (0-100). Key peaks are labeled at m/z 43, 145, 264, 306, and 335. The chemical structure of Trifluralin is also displayed.

2) Can you process the OpenLab mass spec file in MassHunter?

Yes, you can convert OpenLab CDS data to MassHunter format. You can then analyze the converted data in MassHunter Quant, Qual, and Unknowns Analysis. You **cannot** open it in MSD ChemStation Data Analysis. There is a conversion tool you can access starting in MassHunter Quant 11.1.



3) Can you convert OpenLab to netCDF (AIA) files?

Yes. The AIA export functionality in Post Processing Plugins allows the export in NetCDF format. For example, if you wanted to use NIST's AMDIS program or Wiley KnowItAll, this is how you can export your data.

The screenshot displays the 'Processing Method' configuration interface. On the left, a tree view shows the navigation structure, with 'Post Processing Plugins' selected at the bottom. The main area is divided into a list of methods and a configuration panel for the selected 'AIA export' method.

Processing Method

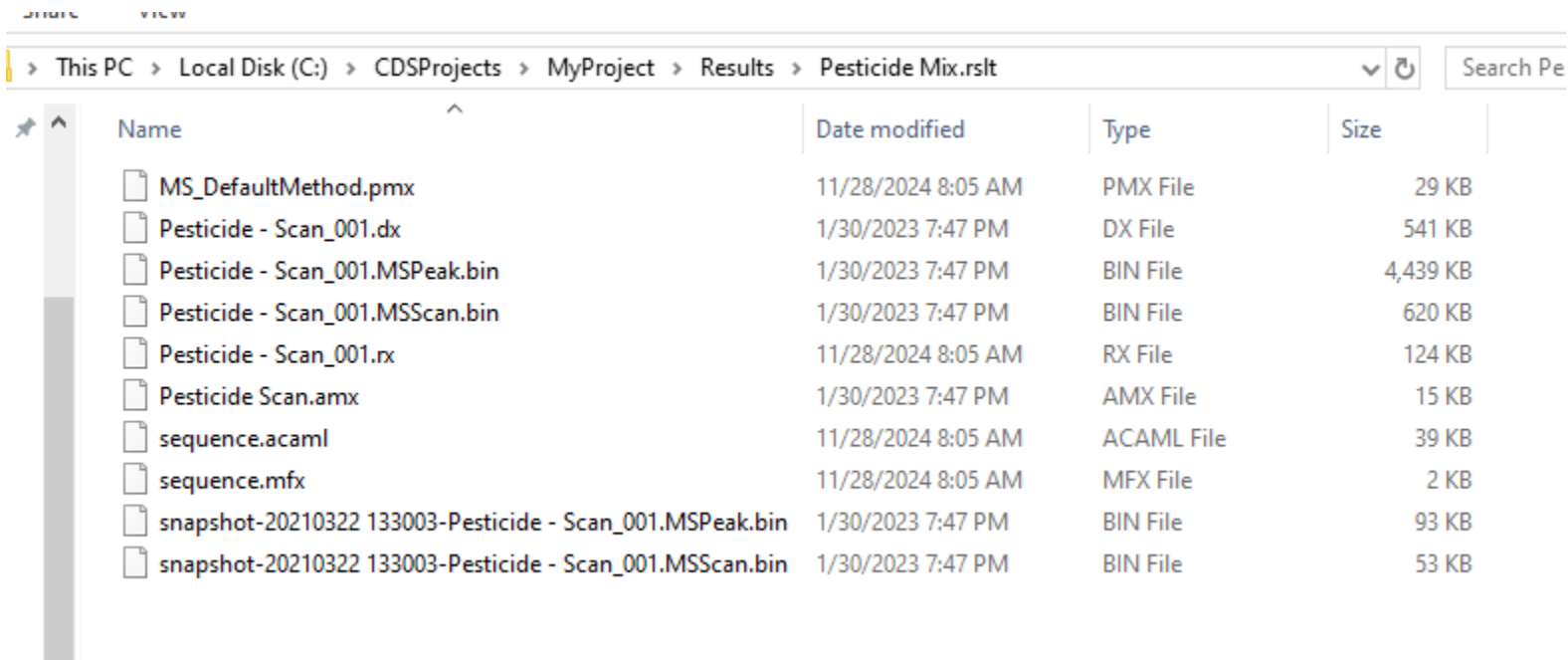
- Signals
 - Extraction
 - Chromatogram
 - Spectrum
 - Integration Events ChemStation
 - Standard
 - Advanced
 - Manual Integration
 - Compounds
 - Identification
 - Calibration
 - Spectra
 - System Suitability
 - Properties
 - Column
 - Spectral Analysis
 - MS Library Search
 - Reports
 - Injection Report
 - Tools
 - Custom Calculation
 - Post Processing Plugins

AIA export (AIA export)

Name	AIA export
Description	Exports OpenLab CDS2 raw data, MS spectra and peak results as netCDF format according to AIA Chromatography Data Standard Specification
Custom name	<input type="text" value="AIA export"/>
Run plugin	<input checked="" type="checkbox"/>
Export to	<input type="text" value="Windows file system"/>
Output path	<input type="text" value=""/> ... >
File name	<input type="text" value=""/> > Example:
Limit file name to	<input type="checkbox"/> <input type="text" value="15"/> characters
Allow to override existing CDF files	<input type="checkbox"/>

4) Data File Locations in OpenLab

- CDS is organized in result sets – files are grouped together based on the sequence they are run with into folders called <MyResultName>.rslt (or <MyResultName>.sirslt for single injections). This *.rslt or *.sirslt folder contains all the data and processing information needed to reconstruct the analysis (acquisition method, processing method, raw data, results, etc.)
- Raw data is stored in *.dx files. Results are stored in *.rx files. Each injection will have one *.dx and one *.rx file.
- The MS data is stored in the same format that MassHunter uses – namely for GCMS single quads there are two binary files which contain the raw data - *.MSPeak.bin and *.MSScan.bin.



The screenshot shows a Windows File Explorer window with the address bar set to 'This PC > Local Disk (C:) > CDSProjects > MyProject > Results > Pesticide Mix.rslt'. The search bar contains 'Search Pe'. The main area displays a list of files with columns for Name, Date modified, Type, and Size.

Name	Date modified	Type	Size
MS_DefaultMethod.pmx	11/28/2024 8:05 AM	PMX File	29 KB
Pesticide - Scan_001.dx	1/30/2023 7:47 PM	DX File	541 KB
Pesticide - Scan_001.MSPeak.bin	1/30/2023 7:47 PM	BIN File	4,439 KB
Pesticide - Scan_001.MSScan.bin	1/30/2023 7:47 PM	BIN File	620 KB
Pesticide - Scan_001.rx	11/28/2024 8:05 AM	RX File	124 KB
Pesticide Scan.amx	1/30/2023 7:47 PM	AMX File	15 KB
sequence.acaml	11/28/2024 8:05 AM	ACAML File	39 KB
sequence.mfx	11/28/2024 8:05 AM	MFX File	2 KB
snapshot-20210322 133003-Pesticide - Scan_001.MSPeak.bin	1/30/2023 7:47 PM	BIN File	93 KB
snapshot-20210322 133003-Pesticide - Scan_001.MSScan.bin	1/30/2023 7:47 PM	BIN File	53 KB

5) Can library searches be performed with NIST dll and then returned to OpenLab for Display?

Yes, see page 29 in the following [link](#) to Agilent documentation



Use MS Libraries Within OpenLab CDS

- 3 Under **MS Library Search > Properties**, select the desired library from the drop-down list and click the + icon to add it. For details, search for "Use the MS library search" in OpenLab Help and Learning.

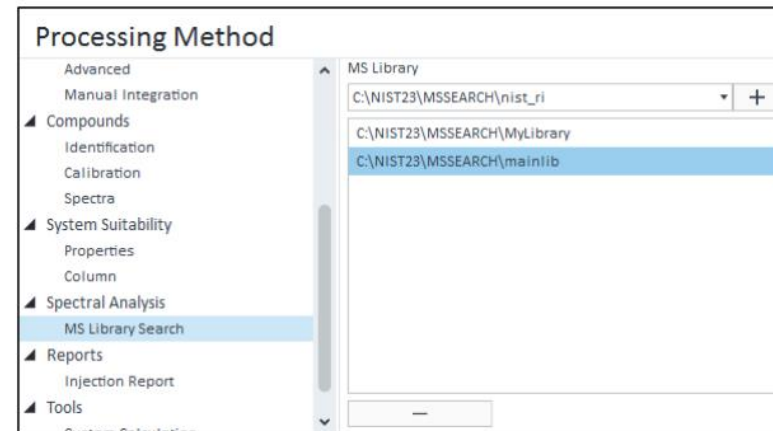


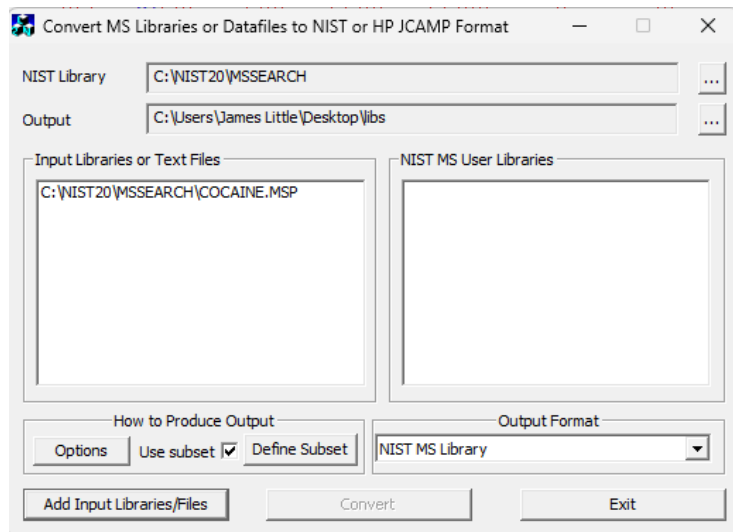
Figure 22 MS library properties

- 4 Save the processing method.
- 5 Load MS data and link it to the prepared processing method.
- 6 For details on performing an MS Library search, refer to OpenLab Help and Learning.

6) What library formats are used with OpenLab?

OpenLab CDS can only use libraries in NIST format – so for users with existing .L (PBM) libraries, we direct them to convert using NIST’s Lib2NIST utility. mslibrary.xml libraries can also be converted, if you first send them to JCAMP format, then use Lib2NIST.

Information can also be found in section 5 of the following manual, [here is the link](#)



OpenLab CDS

Setup and Configuration of MS Library Search

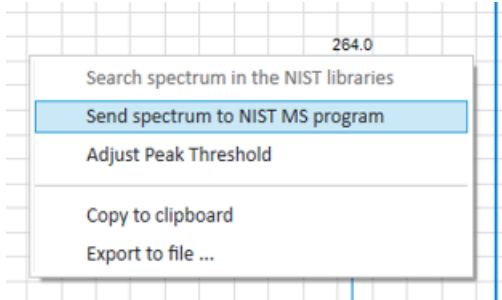
5 Library Maintenance

Modify or Create a Library	23
Modify a Library in a Shared Configuration	23
Create a New Shared Library	26
Convert an Existing Library to NIST Format	26

7) How do I add to or create a user library?

1. All libraries used in OpenLab are in NIST format
2. Thus, to add a library entry or to create the first entry in a new user library, first send the spectrum to NIST MS Search from OpenLab by using the “Send spectrum to NIST MS program”
3. Then the Librarian Tab is selected at bottom of the NIST MS Search Program
4. The remaining details are found in my videos with associated handouts on my web page
5. EI MS libraries, <https://littlesandsailing.com/2020/08/ms-master-class/>, Part V
6. MSMS libraries, <https://littlesandsailing.com/2020/12/lcms-unknown-identification-with-nist-search-using-msms-libraries/>, Part VII

1



2



8) Initial Settings for NIST Search V3.0 in EI and MSMS (tandem) Modes

- There are many settings to setup when first using NIST MS Search
- After setup, user saves as a configuration which makes easy to restore if changed during processing
- **EI** Quick Start Guide for Settings [Link](#)
- **MSMS (tandem)** Quick Start Guide for Settings [Link](#)

9) Agilent OpenLab Help & Learning

- Great resource for a large group of topics
- <https://openlab.help.agilent.com/>
- After choosing language, e.g., *one* can find information on library search
- <https://openlab.help.agilent.com/en/index.htm#t=mergedProjects%2FDataAnalysis%2FUseMSlibrarysearch.htm>
- Easily search for useful topics, e.g., look for information on AIA netCDF file export as shown below on right side of screen

