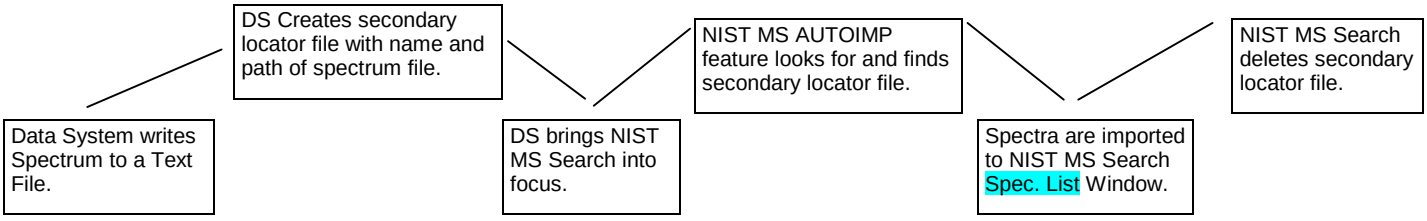
INTRODUCTION

This document describes how to start the NIST MS (Mass Spectral) Search Program Version 2.0 and higher or the AMDIS (Automated Mass spectral Deconvolution and Identification) program from another application. **NOTE: These instructions are different from those for previous versions of the NIST MS Search Program.** The **NAMES** of the Search program and its calling program **HAVE CHANGED FROM** those used with various releases of **Version 1.X**. Instructions for interfacing the NIST MS Search Program are on page 1. Instructions for interfacing AMDIS are on page 8.

USE OF NIST MS SEARCH PROGRAM WITH INSTRUMENT DATA SYSTEMS

The use of the automation features were implemented to enhance the performance of the NIST MS Search Program when used with proprietary mass spectral data systems (DS).



If your data system is capable of exporting spectra to the NIST format (described in **USER LIBRARIES** below) and you can call a Windows program from within your data system, these features are available to you. Implementation of these features has been provided on some manufacturers' data systems.

The command string used to start or bring into focus the NIST MS Search Program for Windows is:

C:\NIST98\MSSEARCH\NISTMS$.EXE <*space*> *possibility of several switches described below*

assuming the program is in the **C:\NIST98\MSSEARCH** directory (the default installation directory; if it is in some other directory, make the appropriate substitution (see **Location of the NIST MS Search Program** below).

Two locator files are required to use the automatic spectral import features, a primary loactor file AUTOIMP.MSD in the NIST MS Search Program's directory and secondary locator file which can be located in any desired directory and have any selected name and extension.

It is best if you write your linking routine so that it first looks to see if there is a file with name AUTOIMP.MSD in the NIST MS Search Program's directory. This primary locator file contains the name and location of the secondary locator file. If an AUTOIMP.MSD file is present, your program should examine its contents and use the contained name and location for writing your secondary locator file. It is NOT NECESSAY to rewrite the AUTOIMP.MSD file each time your program calls the NIST Program. Once put in place, the AUTOIMP.MSD file should not be modified.

WARRING: If your routine automatically creates the primary locator file AUTOIMP.MSD, it will overwrite the existing AUTOIMP.MSD file and can cause other programs to no long function properly with the spectral import feature of the NIST MS Search Program.

1. The first locator file MUST be in the SAME DIRECTORY as the NIST MS Search Program, have the name **AUTOIMP.MSD**. This file should be written only once and only in the case where there is no AUTOIMP.MSD file present in the NIST MS Search Program's directory. If an AUTOIMP.MSD file already exist, the secondary locator file name and loaction should be

used in the writting of the secondary locator file. The AUTOIMP.MSD contains the text string describing the location and name of the second locator file, i.e.:

C:\MYMSDS\DATA\FILESPEC.FIL

Where: **C:\MYMSDS\DATA** is the directory where the file, **FILSPEC.FIL (example name)**, resides.

The **AUTOIMP.MSD** file can be created with any ASCII text editor.

NOTE: If you use Microsoft NotePad, the default extension is .TXT even though you type AUTOIMP.MSD. The default settings for Windows Explorer will not show this .TXT extension. The file will not work unless the .TXT extension is removed.

2. The second locator file is written each time you save spectra to a separate file. This file contains the text string that describes the location and name of the text file containing the spectrum (or spectra) in the NIST text format described below. You can specify whether the spectra in the NIST MS Search Program's **Spec. List** Window (called MS Search Clipboard in ver 1.x) are added to or replaced by the automatic import. An example of the contents of the second locator file is:

C:\MYMSDS\DATA\DATAFILE.TXT <*space*> **Overwrite** *or* **Append**
23 62789

Where: **C:\MYMSDS\DATA** is the directory location of the spectra file, **DATAFILE.TXT** is name of the file containing the spectra, and **Overwrite** or **Append** is the instruction as to how the spectra are added to the **Clipboard** Window. If the Overwrite/Append argument is NOT part of the string, the user will be prompted as to whether or not to overwrite or append the contents of the NIST MS Search Program's **Spec. List** Window when the Program is brought into focus.

23 62789 are separated by a space decimal positive values of high-order and low-order words of your application's main window handle; they are necessary to enable **sWitchBack** Desktop Button feature (see below).

This secondary locator file is deleted each time the NIST MS Search Program is brought into focus and spectra are imported.

The **DATAFILE.TXT** is described below under **NIST Text Format of Individual Spectra**. This file can contain a single spectrum or multiple spectra.

Special Switches

Version 2.0 of the NIST MS Search Program allows **NISTMS$.EXE** to be called using five different command-line switches:

/Instrument
/PAR=2
/PAR=4
/PAR=8

Introduction

More than one switch can be used when **NISTMS$.EXE** is started:

Example: **C:\NIST98\MSSEARCH\NISTMS$.EXE /instrument /par=4**

This command string starts the NIST MS Search Program, activate the **sWitchBack** Desktop Button, (par=4) creates a file (**NISTLOG.TXT**) that contains the results of every search done. Upper- or lower-case characters can be used for these switches. Case is not an issue.

Prior to Version 1.7 of the NIST MS Search Program, a **/V1.5** switch was required for Versions 1.5 and later. This requirement has been eliminated in V1.7. **NOTE**: The command line to start Version 2 of the NIST MS Search Program is:

C:\NIST98\MSSEARCH\NISTMS$.EXE

Location of the NIST MS Search Program

Rather than hard coding a location for the NIST MS Search Program, it is better to determine the location each time the program is called from another application or, at least, the first time the Program is called. This is accomplished by looking in the WIN.INI section labeled NISTMS. Depending on what NIST/AMDIS programs you have installed, this section will appear as follows:

[NISTMS]
Path16=C:\NIST98\MSSEARCH\ *only used with Version numbers <2.0*
WorkDir16=C:\NIST98\MSSEARCH\ *only used with Version numbers <2.0*
Amdis32Path=C:\AMDIS32\ ***THIS IS NEW in V.2.0***
Path32=C:\NIST\NIST_VER2\ *only used with Version 2.0*
WorkDir32=C:\NIST\NIST_VER2\

Use Path16= for all versions before 2.0 and Path32= for Version 2 and higher.

Support of Structure Programs in the NIST MS Search Program, V2

Version 2 of the NIST MS Search Program provides more support of structures than previous versions. There is a Structure Search Command. If a structure rather than a spectrum is in the Spec. List pane of the Lib Search Tab, this structure can be searched to retrieve spectra of all compounds with similar structures by clicking on the Structure Search button (second button from the left) with the structure highlighted in the Spec. List pane.

When Version 2 saves a structure to the Windows Clipboard using the Right Mouse button (RMB) menu's command "Copy Structure to Clipboard", a file in MOL File format with the name Clipboard.MOL is written to the NIST MS Search Program's directory (by default, \NIST98\MSSearch). Other programs can either use the file as it appears on the Windows Clipboard or this file for structure importation.

If a spectrum containing a chemical structure is selected, the right mouse button menu's command "Send to" will result in a "Send to" menu that can have up to three additional items. These "Send to" menu items are a function of the first three lines in a text file in the NIST MS Search Program's directory (the WorkDir32 in [NISTMS] in the WIN.INI file) with the name AUTOIMP.STR. Each line contains three double-quoted strings:

1) A program name as it appears in the "Send to" menu
2) Program executable and path
3) Program startup parameters. %1 will be replaced by the mol file name.

The following is an example of the contents of an AUTOIMP.STR file:

"ChemSite" "C:\MolSuite\ChemSite\ChemSite.exe" "%1"
"ISIS Draw" "D:\ISIS Draw 2.2.1\IDraw32.exe" "%1"
"ACD/ChemSketch" "C:\acd40tl\chemsk.exe" "%1"

The last line of the above example results in ACD lab's ChemSketch starting with the contents of clipboard.mol being imported when ChemSketch opens.

When a program that is to be interactive with the NIST MS Search Program is installed, it must UPDATE or CREATE the AUTOIMP.STR file, or the AUTOIMP.STR file must be edited by the user before the third-party program will appear on and function from the "Send to" menu.

A third-party program can call the NIST MS Search Program by issuing the command:

C:\NIST98\MSSEARCH\NISTMS$.EXE /MOL=<molfile>

assuming the NIST MS Search Program is in the **C:\NIST98\MSSEARCH** directory; if it is in some other directory, make the appropriate substitution (see **Location of the NIST MS Search Program** above).

where <molfile> is a full pathname of the MOL file. If there is a space in the name, e.g., example c:\program files\a.mol, the name should be double quoted:

C:\NIST98\MSSEARCH\NISTMS$.EXE /MOL="c:\program files\a.mol"

Third-party program can obtain the path to NISTMS$ from Path32 in [NISTMS] in WIN.INI (see **Location of the NIST MS Search Program** above).

Explanation of Command Line Switches

/Instrument

The **/instrument** switch is used to make the **sWitchBack** Desktop Button active. If this switch is not used, the **sWitchBack** button is grayed. The **/instrument** also causes the program from which the NIST MS Search Program was called to automatically come back into focus after an automated search if the **Return Focus to Caller upon Completion** check box in the **Automation** dialog box has been selected. The second line of numbers describing the calling program's main window handle is required in the secondary locator file.

/PAR=2

The **/PAR=2** switch is used when the NIST MS Search Program is called from within another application. This allows spectra to be passed from the other application and searched with the NIST MS Search Program. The text results (spectrum header, match factor, reverse match factor, and probability) for a specified number of hits is stored in a text file, which can be accessed by the calling application for reporting purposes.

The **/PAR=2** switch causes spectra to be searched in background (if the **Automation** check box has been selected in the Automations dialog box) and produces the files **SCRESLT.TXT** and **SCREADY.TXT** in the NIST MS Search Program's directory each time the NIST MS Search Program is called with this switch. The NIST MS Search Program never comes into focus. If the NIST MS Search Program is not running, it starts iconized non-active and does not display the **User Spectrum Search** progress dialog box. The Program's main window is disabled while the search is in progress. If none of these two switches is present, then the NIST MS Search Program will search in background as if **Append** were added.

The **Automation**/**No Automation** button in the **User Search Options** dialog box has to be set to **Automation**.

The results of a specified number of hits from the search are written into **SCRESLT.TXT**. If more than one spectrum is imported, the search results for each spectrum will be in the file. The number of hits for each search that are stored is determined by the value in the "Number of First Hits to Print" entry box in the **Automation** dialog box, displayed by selecting the **Automation**/**No Automation** button in the **Library Search Options** dialog box.

If either or both **SCRESLT.TXT** and **SCREADY.TXT** exist, they will be deleted each time the NIST MS Search Program is called with **NISTMS$.EXE** and the **/PAR=2** switch before the search starts. **SCREADY.TXT** is created immediately after the search is completed and **SCRESLT.TXT** is closed.

The format for the **SCRESLT.TXT** file is in this example containing the first two hits for each of two imported spectra:

Unknown: Scan 493 (8.216 min): 50P0830C.D Compound in Library Factor = 197
Hit 1 : <<Methane, dibromo->>;<<CH2Br2>>; MF: 824; RMF: 856; Prob: 98.10; CAS: 74-95-3; Mw: 172; Lib: <<replib>>; Id: 10054.
Hit 2 : <<Methane, dibromo->>;<<CH2Br2>>; MF: 820; RMF: 853; Prob: 98.10; CAS: 74-95-3; Mw: 172; Lib: <<replib>>; Id: 10055.
Hit 3 : <<Methane, dibromo->>;<<CH2Br2>>; MF: 810; RMF: 865; Prob: 98.10; CAS: 74-95-3; Mw: 172; Lib: <<replib>>; Id: 17162.
Hit 4 : <<Methane, dibromo->>;<<CH2Br2>>; MF: 809; RMF: 839; Prob: 98.10; CAS: 74-95-3; Mw: 172; Lib: <<replib>>; Id: 17161.
Unknown: Scan 385 (6.416 min): 50P0830C.D Compound in Library Factor = -1187
Hit 1 : <<6-Chloromethyl-5-methylidene>>;<<C9H11ClO>>; MF: 613; RMF: 803; Prob: 23.43; CAS:66728-04-9; Mw: 170; Lib: <<mainlib>>; Id: 52538.
Hit 2 : <<Trichloronitromethane>>;<<CCl3NO2>>; MF: 612; RMF: 842; Prob: 22.52; CAS: 76-06-2; Mw: 163; Lib: <<replib>>; Id: 12645.
Hit 3 : <<Acetic acid, trichloro-, ethyl ester>>;<<C4H5Cl3O2>>; MF: 604; RMF: 624; Prob: 16.80; CAS: 515-84-4; Mw: 190; Lib: <<replib>>; Id: 242.
Hit 4 : <<Acetic acid, trichloro-, anhydride>>;<<C4Cl6O3>>; MF: 581; RMF: 590; Prob: 6.13; CAS: 4124-31-6; Mw: 306; Lib: <<mainlib>>; Id: 52533.

Extended character codes in chemical names in the file **SRCRESLT.TXT** should be interpreted as follows (codes are decimal numbers):

.alpha.	.beta.	.gamma.	.delta.	.epsilon.	.pi.	.sigma.	.mu.	.omega.	.+/-.	.eta.
224	225	231	235	238	227	229	230	234	241	252

The **SCREADY.TXT** file contains the number of spectra searched. This file can be used as an indicator for other programs that the NIST User Spectrum Search of the imported spectrum or spectra has completed. The contents of the **SCRESLT.TXT** file can be used in the preparation of reports that contain the results of library searches by other programs.

/PAR=4

The **/PAR=4** switch will cause User Spectrum Search results to be written to a user spectrum search log file, **NISTLOG.TXT**, which will be created in the NIST MS Search Program's directory, e.g., **C:\NIST98**. The **NISTLOG.TXT** file has the same format as the **SCRESLT.TXT** file, and its contents are determined in the same way as the **SCRESLT.TXT** file. Once NIST$.EXE has been called using the **/PAR=4** switch, User Spectrum Search results will be recorded in **NISTLOG.TXT** even if the NIST MS Search Program is brought back into focus without the switch, i.e., C:\NIST98\NISTMS$.EXE.

There is no facility within the NIST MS Search Program to remove the **NISTLOG.TXT** file. It must be removed with Windows Explorer. Once the NIST MS Search Program is exited and then restarted, the **/PAR=4** switch must be used to have the User Spectrum Search result recording resume in this subsequent session.

The **NISTLOG.TXT** file is always appended. It is the user's responsibility to delete it if necessary. This process of logging each search can be done concurrently with the background search (use of the **/PAR=2** switch).

/PAR=8

To stop recording User Spectrum Search results in **NISTLOG.TXT**, the NIST MS Search Program has to be brought into focus with **C:\NIST98\NIST$.EXE /PAR=8**. Stopping the recording of User Spectrum Search results can be done without exiting the Program.

USER LIBRARIES

One of the primary features of the NIST MS Search Program is the ability to do comparisons of user-generated mass spectra and create **User libraries** to search against. The Program will allow up to fourteen **User libraries**. These may be searched in conjunction with the NIST/EPA/NIH Mass Spectral Main and/or Replicates Libraries, or they may be searched by themselves. A maximum of sixteen libraries can be included for search at one time. The Program allows for the searching of combinations of libraries in any order. The inclusion of libraries and their order of search can be set from the "Libraries to Search and the Search Order" Window displayed with the **Libs** button from the different search dialog boxes.

User libraries are maintained as subdirectories under the Program's root directory. The name given to the **User library** is the name of the subdirectory. This subdirectory will contain a number of files that are used by the **User library**. These files exist in a proprietary format. The spectra and compound information are put in these files through the **Librarian** pane

The spectra and compound information can originate in a Text file in the NIST MS Text file format described in the **NIST Text Format of Individual Mass Spectra** on page 7. Spectra can also be entered through the program. This is done by using the "Spectrum Information" Window called with the **New Spectrum** button on the Librarian pane toolbar.

The NIST MS Search Program **User libraries** support **chemical structures**. Starting with V1.6 of the NIST MS Search Program, users may import their own chemical structures with selected user spectra. This is done in the **Librarian** pane by connecting a user-drawn structure in standard MOL-file format with a user spectrum. Such structure-drawing programs are widely available, e.g., ISIS/Draw may be freely downloaded from http://www.mdli.com/downloads/isisdraw.html and Advanced Chemistry Development's ChemSketch 4.0 may be downloaded from http://www.acdlabs.com/download/. As has always been the case, if a user spectrum is given its CAS registry number and the Main Library has a structure for it, this structure will automatically be shown with the associated spectrum unless an imported structure has been attached to the spectrum.

User libraries are very easy to create and maintain. You may want to create them using specific sets of spectra from the NIST/EPA/NIH Main or Replicates Library. Any spectrum in the **Spec. List** Window can be added to a **User library**. Any spectrum in the **Hit List** Window can be copied to the **Spec. List** Window.

The program can read user-submitted spectra in the JCAMP, U.S. EPA, or the NIST Text formats as well as formats of files submitted by various instrument manufacturers' mass spectrometer data systems. You should check with the manufacturer of your mass spectrometer's data system to see if there is file compatibility.

Instructions on building **User libraries** are included in the Librarian Help Screens. These also include the information necessary to add structures to **User library** spectra.

Utilities are provided that allow for the association of structures with spectra in the **User libraries**. There is also a utility that allows ChemStation user libraries with associated structures to be copied to the NIST **User library** format.

NIST Text Format of Individual Mass Spectra

The NIST Text file format is very simple. A file can contain as many spectra as you want. Each spectrum must start with the field title "Name:". There must be something in this field in order for the spectrum to be imported, i.e., the data file name and the scan number of the spectrum's fetus. The last required field title is "Num Peaks:". The contents of this field must be the number of mass/intensity pairs that make up the spectrum. Optional fields with the titles of "Comments:", "Formula:", "MW:", and "CAS#:" can be between the "Name:" and "Num Peaks:" fields.

When a spectrum is saved to a Text file from the NIST/EPA/NIH Main or Replicates Library, it will also contain fields with the names "NIST#:" and "DB#:". The field with the name "NIST#:" is on the same line as the "CAS#:" field and separated by a semicolon (;). The mass/intensity list begins on the line following the line with the field title "Num Peaks:". Lines of the mass/intensity list have no field title.

When you create a file with all the allowable fields, each field title should be on a separate line. Use the following format. The format for each spectrum in a Text file usable by the program should be as follows:

Line 1: NAME: Molecule (Required, up to 511 characters)
Line 2: COMMENT: Run 23, 8/8/88 (Optional, up to 511 characters)
Line 3: FORMULA: C6H6 (Optional)
Line 4: MW: 78 (Optional)

If the user spectrum contains a CAS registry number with its appropriate prefix (**CAS:**), the display of the spectrum in the **Plot**, **Compare**, and **Structure** Windows will have the structure of the compound with that CAS registry number in the NIST/EPA/NIH Main Library.

Line 5: CAS: 71-43-2 (Optional)

The actual mass spectral data (number of peaks in the spectrum) must begin on the next line. It does not actually matter what line this is, as long as it precedes the line that starts the mass/intensity pair data. The VERY NEXT line and subsequent lines MUST contain the paired mass/intensity values.

Line 6: Num Peaks: 18 (This prefix and the number of mass/intensity pairs is Required.)

Line 7: 26 430; 27 340; 28 40; 37 480; 38 611; 39 1411; 49 300; 50 1792; 51 2052; 52 1962; 63 340; 73 160; 74 480; 75 180;

Line n: 76 721; 77 1401; 78 9806; 79 651;

The peaks need not be normalized, and the masses need not be ordered. The exact spacing and delimiters used for the mass/intensity pairs are unimportant. For example, the peaks above could equally be presented as:

(26,430),(27,340),(28,40),(37,480),(38,611),(39,1411),
(49,300), (75,180),(50,1792),(51,2052),(52,1962),(63,340),
(73,160),(74,480),(75,180),(76,721),(77,1401),(78,9806),(79,651)

or each mass intensity pair could be on an individual line.

You can give the file any valid name. However, it is best to use the extension "MSP". This is the recognized default extension in the Program when you want to import user spectra.

Calling AMDIS from Within A Third-Party Program

AMDIS can be called from within a third-party program by starting AMDIS32$.exe. This ensures the communications happen with only one copy of AMDIS. AMDIS_32.EXE should be in the same folder as AMDIS32$.EXE. The format of the AMDIS call from a third-party program is:

C:\NIST98\AMDIS\AMDIS32$.EXE <*space*><datafile><*space*><*switches*>
where <datafile> is the full pathname of the data file to be loaded into AMDIS and <switches> is described below.

This example assumes that AMDIS is in the **C:\NIST98\AMDIS** directory; if it is in some other directory, make the appropriate substitution (see **Location of the NIST MS Search Program** above).

You can specify the type of analysis and the analysis mode for AMDIS with the switches.

For example, the command *c:\amdis\AMDIS32$.exe c:\data\grob.d\data.ms /x* will call AMDIS and use the RI Calibration data in the analysis.

The following switches work with **AMDIS32$.exe**

Switch	**Type of Analysis**
/S	Simple
/X	Use RI Calibration Data
/I	Use Internal Standards
/N	Use RI Calibration data + Internal Standards.
/C	RI Calibration/Performance
/K	Performance Check
/M	Manual (Loads in data file, does not perform analysis, leaves AMDIS in the forefront)
/Range=X:Y or /R=X:Y	This will cause the range bound by spectrum no. X to spectrum no. Y to be displayed in AMDIS

Only /M will bring AMDIS to the foreground. Using AMDIS32$ without /M will load the data file and run AMDIS in the background.
For example, the command *Run c:\amdis\amdis32$.exe /s d:\data\cal.d\data.ms* will open AMDIS, load in cal.d, and do a simple analysis. AMDIS will stay in the background.
The command *Run c:\amdis\amdis32$.exe /m d:\data\cal.d\data.ms* will open AMDIS and load cal.d. AMDIS will not do any analysis.

Additionally there are three switches that work with AMDIS_32.exe but do not function with AMDIS32$.exe. The format of the call for AMDIS_32.exe is the same as for AMDIS32$.exe. Note that running AMDIS_32.exe creates a new copy of AMDIS.

The following switches work with **AMDIS_32.exe**

Switch	**Analysis Mode**	**Action**
/A	Results	Loads in data file, analyzes the data file within the Results Window.
/D	Direct Confirm	Loads in data file in the Confirm Window., and analyzes the data file.
/E	Auto Exit	Loads data file, analyzes data file and shuts down.

For example, the command *c:\amdis\AMDIS_32.exe c:\data\grob.d\data.ms /e /x* will call AMDIS, use the RI Calibration data in the analysis, and close out of AMDIS.

Sending Spectra From AMDIS to a Third-Party Program

AMDIS can send deconvoluted spectra to the NIST MS Search Program. However, currently, there is no straightforward way to send AMDIS deconvoluted spectra to a third-party program. This feature is under development.

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