Agilent G2722AA Spectrum Mill Extractor for Thermo Fisher Scientific Ion Trap Data Files

Quick Start Guide

What is the Spectrum Mill Data Extractor for Thermo Ion Trap Data?

The Agilent Spectrum Mill MS Proteomics Workbench is a collection of tools for high-throughput processing of MS and MS/MS spectra to provide protein and peptide identifications and relative quantitation. The Agilent G2722AA Spectrum Mill Extractor for Thermo Fisher Scientific Ion Trap Data Files enables use of the time-saving Spectrum Mill workbench with LCQ, LTQ, LTQ FT, and LTQ Orbitrap *.raw files. It prepares raw data for further Spectrum Mill processing by doing the following:

- Extracts and merges nearby MS/MS spectra from the same precursor ion (supports numerous modes, such as CID and ETD)
- Optionally merges MS^2 and MS^3 spectra from the same precursor ion
- Assigns precursor charges where possible
- Calculates spectral features used in other Spectrum Mill programs
- Filters MS/MS spectra by quality
- Enables quantitation by calculating extracted ion chromatograms from the intervening MS precursor scans

Installation

To activate the license for this software, see the *Spectrum Mill MS Proteomics Workbench Installation Guide*. To process *.raw data files, a copy of Xcalibur equivalent to or newer than the version that was used to acquire the data needs to be co-resident on the server.

Agilent Technologies

Spectrum Mill Help - core product documents and online help

See the following to learn the core Spectrum Mill workbench.

Scientists

Quick Start Guide Get a quick overview of the Spectrum Mill workbench.

Familiarization Guide Follow step-by-step instructions to process example Agilent ion trap, Q-TOF, and TOF data.

Application Guide Learn step-by-step details to use all functions of the software.

Online Help Consult the online help for in-depth information not given in the *Application Guide*. Display online help in one of three ways:

- Click help links on the left-hand side of the Spectrum Mill home page.
- Click the **Help** button at the top of a Spectrum Mill form to get complete instructions for that form.
- Click links on the blue dividing bars in the forms to get field-level help.

Quick Reference Card After you are familiar with the software, consult this card for an overview of the steps to process MS/MS data.

System Administrators

Installation Guide Use this guide to install the Spectrum Mill workbench, and to activate the license for the Spectrum Mill Data Extractor for Thermo Ion Traps.

Application Guide See the following chapters:

- **Chapter 8: System Administration**

  Get an overview to install databases and perform other system administration tasks.

- **Chapter 9: Files Created during Spectrum Mill Data Processing**

  Refer to this chapter to troubleshoot data processing, to selectively remove parts of the processing, or to decide which files to archive.

Online Help From any **Help** page, click links under **For System Administrators:**

- **Protein Databases** (link to **millhtml\SM_instruct\faman.htm**)

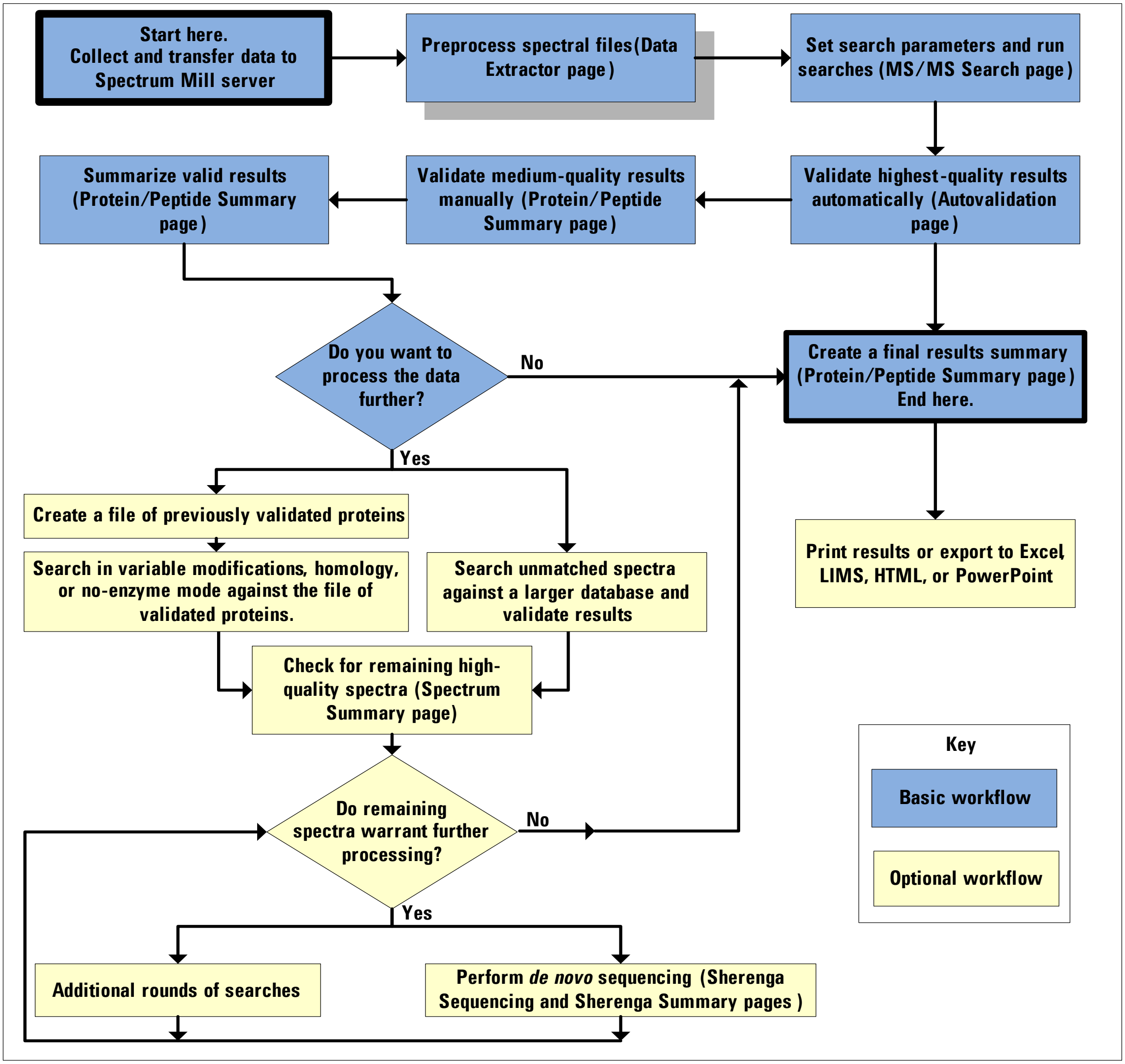
  Learn details to install databases, create indices, and create subset databases.

- **Server Administration** (link to **millhtml\SM_instruct\servadmn.htm**)

  Learn details to perform other system administration tasks.

Roadmap for MS/MS data processing

This diagram shows the overall Spectrum Mill work flow for MS/MS data. The Thermo Data Extractor accomplishes the part of the work flow with the shadow.



Familiarization tutorial

Exercise 1. (Optional) Transfer LCQ data file to the Spectrum Mill server

To process Thermo Fisher Scientific ion trap *.raw data files with the Spectrum Mill workbench, you first move or copy the files into the appropriate directory on the Spectrum Mill server.

To make it easy to compare data sets, it is important that you set up the appropriate directory structure for your files on the Spectrum Mill server. Whenever you want to compare samples in a set, you need to set up a subdirectory for each sample. This directory may contain data files from multiple sample fractions or gel slices.

NOTE The LCQ example file may have been installed on the server at the time the Spectrum Mill software was installed. If so, you may either skip this exercise or (if you want the practice) delete the example data from the server and install it again with this exercise.

Steps	Detailed Instructions	Comments
1 Copy the example data file, **X:\ExampleData\msdataSM\ExampleData\ThermoFinnigan\LCQ_Mix.RAW**, from the *Spectrum Mill Example Data CD* onto your client PC.	• Copy **LCQ_Mix.RAW** from the *Spectrum Mill Example Data CD* to any directory on your client PC.	• You do this exercise outside the Spectrum Mill workbench. Use your normal file management utilities. • This exercise simulates the likely laboratory scenario of file transfer from a client or instrument PC to the server; you may transfer files directly from the CD to the server if you prefer.

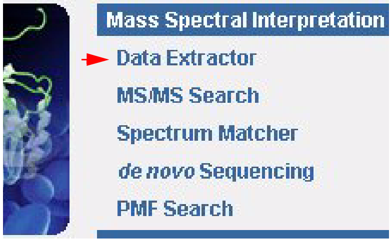
Steps	Detailed Instructions	Comments
2 Create the **msdataSM\ExampleData\ThermoFinnigan** folder on the Spectrum Mill server.	**a** On your server, find the **SpectrumMill** folder. **b** In this folder, click to open the folder **msdataSM**. **c** In **msdataSM**, create **ExampleData\ThermoFinnigan**. You need only one folder because this exercise uses a single sample.	• If you don't know how to find your Spectrum Mill file system, ask the person who installed your software. • Do not include spaces or parentheses in your directory name. • When you process your own samples, remember to set up a separate folder for each sample. Each folder should contain all sample fractions. • You may create up to ten folders between **msdataSM** and your data files, but shorter path lengths reduce memory usage, especially for large data sets.

3 Copy the LCQ example data file to the new directory.	• Copy or move **LCQ_Mix.RAW** from your client PC to the new folder on the server PC.	• When you process your own data, remove any spaces or parentheses in the data file names. For example, change the file **my sample.d** to **my_sample.d.**
		• For best results, you should always process raw data files with the Spectrum Mill workbench. However, it is possible to process *.dta files:
		  • For *.dta files that contain multiple spectra, copy the file to the same directory as you would a *.raw file.
		  • For *.dta files that each contain a single spectrum, create a **cpick_in** subdirectory and copy the individual spectral files there.
		  • Note that *.dta files use the generic extractor for peak list files rather than the raw file extractor that is the topic of this Quick Start Guide.

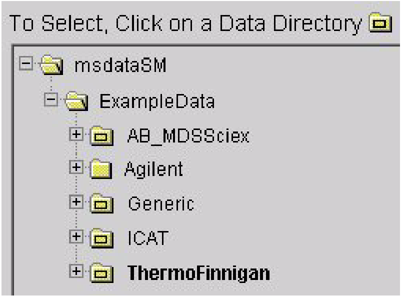
Steps	Detailed Instructions	Comments
4 Make sure you have both read and write permissions for the data folder you just created on the server.	**a** Right-click the **ThermoFinnigan** folder and select **Properties**. **b** Clear the **Read-only** check box if it is marked. **c** In the **Confirm Attribute Changes** dialog, click **Apply changes to this folder, subfolders, and files**. **d** Click **OK**. **e** If necessary, repeat step **a**. **f** Click the **Security** tab. **g** Make sure all user groups have full permissions. **h** Click **OK**.	If the Spectrum Mill workbench cannot write to the folders that contain your data files, you may encounter errors.

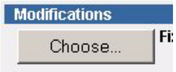
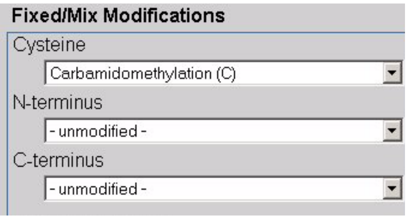
Exercise 2. Run the Data Extractor

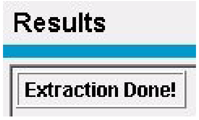
The Spectrum Mill Data Extractor preprocesses raw data files to extract high-quality spectra for database searches. The Data Extractor automatically detects which type of raw file you have and then invokes the appropriate extraction program. For LCQ data, it invokes the Spectrum Mill Data Extractor for Thermo Ion Trap Data. This program extracts and merges nearby MS/MS spectra from the same precursor ion, optionally merges MS^2 and MS^3 spectra from the same precursor ion, assigns precursor charges where possible, calculates spectral features, filters MS/MS spectra by quality, and calculates extracted ion chromatograms (EICs) for the intervening MS precursor scans. The latter are used for quantitation.

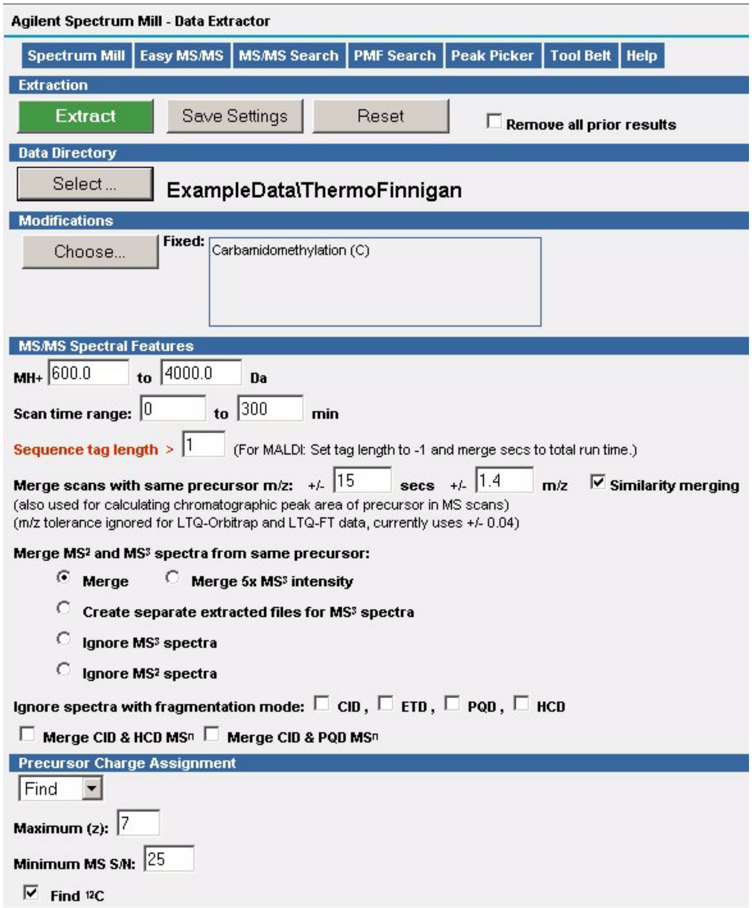
Steps	**Detailed Instructions**	**Comments**
1 Start the Spectrum Mill workbench on your PC.	• Double-click the desktop icon to launch the Spectrum Mill workbench. 	You will see the Spectrum Mill home page.
2 If you don’t see the Spectrum Mill icon, launch your web browser and type the URL for the Spectrum Mill home page.	**a** Click your Internet Explorer icon. **b** In the web browser window, type the URL for the Spectrum Mill server.	If you don’t know the URL, ask the person who installed the software.
3 Check that you see the Spectrum Mill home page.		
4 If you don’t, check that the server is booted up and that your URL is correct.		
5 Navigate to the Data Extractor page.	• From the Spectrum Mill home page, click the Data Extractor link. 	

Steps	Detailed Instructions	Comments

6 Select the **msdataSM\ExampleData\ThermoFinnigan** data directory.	**a** Click the **Select...** button near the middle of the form.
	
	
	b Expand the directory tree and click the word **ThermoFinnigan** to select that data file directory.
	
	
	c Make sure that the name of the **ThermoFinnigan** directory changes to a **bold** font. This indicates that it has been selected.
	d Click **OK.** The data directory appears as in Figure 1 on page 10.
		• Directories are identified by different types of icons:
		  • Plain folders indicate directories that do not have data files directly beneath them.
		  • Folders with rectangles indicate data directories.
		  • Folders with line spectra (bar graphs) indicate data files.
		• Try clicking the names of each type to see which turn **bold**, indicating that they are selectable.
		• The software remembers your data file selection when you go to other Spectrum Mill forms.
		• If you mark the **Make Default** check box in this dialog, the software remembers your data directory even after you close your web browser.

7 Choose the appropriate cysteine modification.	**a** Click the **Choose...** button near the middle of the form.  **b** Under the **Cysteine** heading, select **Carbamidomethylation**.  **Fixed/Mix Modifications** Cysteine Carbamidomethylation (C) N-terminus - unmodified - C-terminus - unmodified - **c** Click **OK.** The name of the modification appears as in Figure 1 on page 10.	• Carbamidomethylation is the default when you first install the Spectrum Mill workbench, so you may be able to skip this step. • To view details about the modifications that are currently available on your server, click the **Details** button at the bottom right of the **Choose Modifications** dialog. • For more information about choosing modifications, see the online help. • Your system administrator can configure custom modifications.

Steps	Detailed Instructions	Comments
8 Set other parameters as shown in Figure 1 on page 10.	**a** Keep the defaults whenever appropriate. This example uses all default settings, except for **Data Directory**. **b** Examine the items in red text carefully, since these are the ones you may need to change when you process your own samples. **c** Click a blue section divider bar to display help for that section of the form.	• You use the **Sequence tag length** to filter out noisy spectra. This is the longest sequence of amino acids that is represented by the fragments in a spectrum. • If you set this to **>1**, you ensure that all possible good spectra are extracted. You have opportunities to set more stringent requirements later when you perform the database search.
9 Start the extraction.	**a** Click the **Extract** button.  **b** View extraction progress in the **Results** area to the right of the Data Extractor form. **c** Scroll to the *top* of the **Results** area to see the message that indicates that extraction is finished.  Results Extraction Done!	• The Data Extractor processes all files in the directory. • Extraction time varies depending on the number and size of the files. • You can use your client PC for other tasks while the extraction is taking place. • If the extraction fails to progress, check that you have the appropriate Thermo Fisher Scientific software installed. For details, see the *Spectrum Mill MS Proteomics Workbench Installation Guide*. • If you want to stop the extraction, click the red **Stop Extraction PID: xxx** link at the top of the Results section. Then see the Tool Belt chapter in the *Spectrum Mill MS Proteomics Workbench Application Guide* for further instructions.
10 (Optional) View the **cpick_in** subdirectory to see the files Data Extractor has created.	**a** Navigate to the folder **SpectrumMill\msdataSM\ExampleData\ThermoFinnigan\cpick_in**. **b** Notice the new files created there. Each one represents an extracted spectrum from your raw data file.	File names are in the format: Data_File_Name.aaaa.bbbb.c.pkl, where • aaaa = first merged scan • bbbb = last merged scan • c = assigned precursor charge (0 means charge was ambiguous)



☐ Remove all prior results

ExampleData\ThermoFinnigan

Fixed: Carbamidomethylation (C)

MH+ 600.0 to 4000.0 Da

Scan time range: 0 to 300 min

Sequence tag length > 1 (For MALDI: Set tag length to -1 and merge secs to total run time.)

Merge scans with same precursor m/z: +/- 15 secs +/- 1.4 m/z ☑ Similarity merging
(also used for calculating chromatographic peak area of precursor in MS scans)
(m/z tolerance ignored for LTQ-Orbitrap and LTQ-FT data, currently uses +/- 0.04)

Merge MS^2 and MS^3 spectra from same precursor:

● Merge ○ Merge 5x MS^3 intensity
○ Create separate extracted files for MS^3 spectra
○ Ignore MS^3 spectra
○ Ignore MS^2 spectra

Ignore spectra with fragmentation mode: ☐ CID , ☐ ETD , ☐ PQD , ☐ HCD

☐ Merge CID & HCD MS^n ☐ Merge CID & PQD MS^n

Find

Maximum (z): 7

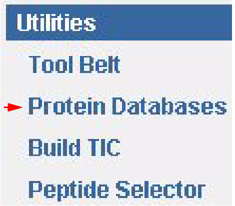
Minimum MS S/N: 25

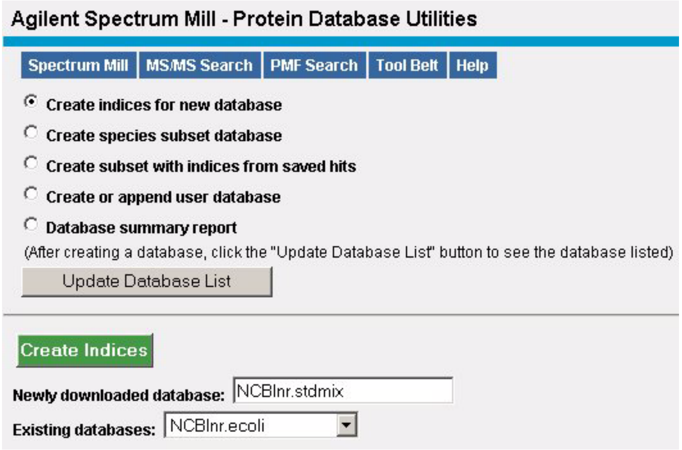
☑ Find ^{12}C

Figure 1 Settings for Data Extractor

Exercise 3. Create indices for user-created database

The Spectrum Mill workbench allows you to create and search user databases. In this exercise, you create indices for a user database so you can later search it.

Steps	Detailed Instructions	Comments
1 If necessary, copy the user database **NCBInr.stdmix** to your Spectrum Mill server.	**a** If the **NCBInr.stdmix** database is already installed in your Spectrum Mill database directory, skip to step 2. **b** If not, find the file **X:\Example Databases\NCBInr.stdmix** on your *Spectrum Mill Installation CD*. **c** Copy it to your database directory on your Spectrum Mill server.	• The database directory likely has a name like **X:\seqDB**. • If you are not sure, ask your system administrator.
2 Make sure security permissions are set properly for the folder that contains the file **dbname.js**.	**a** Right-click the **SpectrumMill\millhtml\SM_js** folder and select **Properties**. **b** Click the **Security** tab. **c** Make sure all user groups have full permissions. **d** Click **OK**.	If the Spectrum Mill workbench cannot write to this folder, you may encounter errors.
3 Navigate to the Protein Databases page.	• From the Spectrum Mill home page, click the Protein Databases link. 	
4 Fill in the form as shown in Figure 2.	**a** On the Protein Databases page, click the **Create indices for new database** option. (This is the default.) **b** Set **Newly downloaded database** to **NCBInr.stdmix.**	For **Newly downloaded database**, you type the exact database file name.
5 Click the **Create Indices** button.	• After a short wait, check the bottom of the page for text that indicates that the software is creating the database indices.	These indices bear no resemblance to those used by another popular database search program.


Agilent Spectrum Mill - Protein Database Utilities

● Create indices for new database
○ Create species subset database
○ Create subset with indices from saved hits
○ Create or append user database
○ Database summary report

(After creating a database, click the "Update Database List" button to see the database listed)

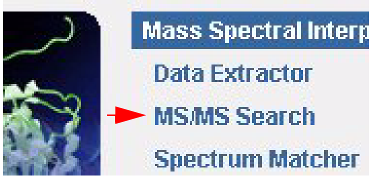
Newly downloaded database: NCBInr.stdmix

Existing databases: NCBInr.ecoli

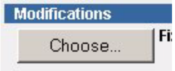
Figure 2 Create indices for user database

Exercise 4. Run database searches

After you have extracted your spectra and created indices for your user database, you are ready to search each spectrum against a protein or DNA database. As you process data with the Spectrum Mill workbench, you may iterate through multiple rounds of database search and results validation, with the goal of identifying as many spectra as possible. These exercises illustrate only a single identity mode search. In identity mode, the spectra must be consistent with the database sequence. When you process your own data, you might also search in variable modifications or homology mode, where spectra may show modifications relative to the database sequence.

Steps	Detailed Instructions	Comments
1 Navigate to the MS/MS Search page.	• Do one of the following: • From the Protein Databases page, click the **MS/MS Search** button.  • From the Spectrum Mill home page, click the link to MS/MS Search. 	Since Spectrum Mill pages have buttons to take you to the next processing tasks, you can navigate directly from the Protein Databases page to the MS/MS Search page.
2 Check that your **Data Directory** is set to **ThermoFinnigan**.	• If you have just performed the data extraction, your **ThermoFinnigan** data directory should already be set correctly. If not, click the **Select...** button to select the **ThermoFinnigan** folder.	
3 Select the “standard mix” database.	• For **Database** (under **Search Parameters**) select the **NCBInr.stdmix** database for which you just created indices.	If the database name does not appear, reload or refresh the MS/MS search form.

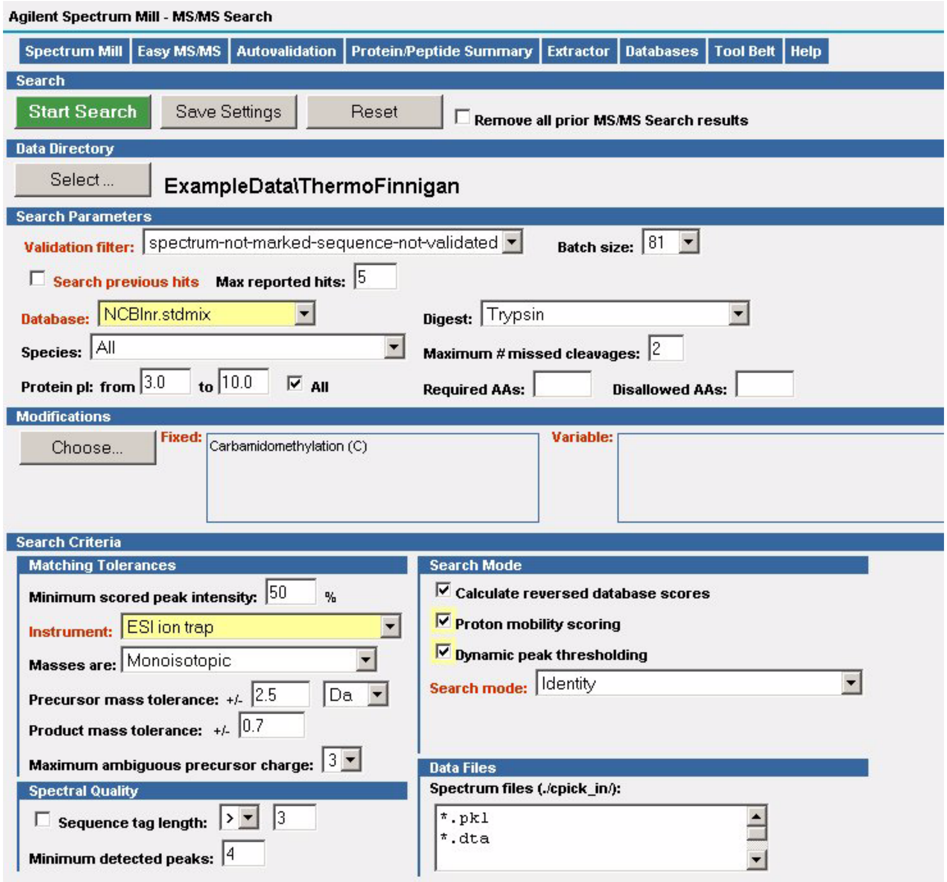
Steps	**Detailed Instructions**	**Comments**

4 Choose the appropriate cysteine modification.	**a** Click the **Choose...** button near the middle of the form. 
	b Under the **Cysteine** heading, select **Carbamidomethylation**
	c Click **OK.** The name of the modification appears as in Figure 3 on page 17.
		• The modification is likely already set.
		• Three types of modifications are available for MS/MS Search:
		  • The **fixed** modifications are assumed to apply universally and are searched in a single search cycle.
		  • The **mix** modifications trigger cyclic MS/MS searches, where a different form of the modification is searched in each cycle.
		  • The **variable** modifications allow for both modified and unmodified forms within a peptide. All variable modifications are searched in each cycle.
		• For more information about choosing modifications, see the online help.
		• Your system administrator can configure custom modifications.

5 Set **Instrument** to **ESI ion trap**.		• If you process LTQ data, for **Instrument**, select **ESI linear ion trap**. • If you process Orbitrap or LTQ FT data, select your instrument based on where the MS/MS occurs and set the appropriate **Precursor mass tolerance**. For details, see the section on selecting Thermo Fisher Scientific instruments in the online help for MS/MS Search.

Steps	Detailed Instructions	Comments
6 Set up the **Search Mode**.	**a** Make sure that the check box for **Calculate reversed database scores** is marked. (This is the default setting.). **b** Mark the check box for **Proton mobility scoring**. **c** Mark the check box for **Dynamic peak thresholding**. **d** Verify that the **Search mode** is set to **Identity**.	• When you **Calculate reversed database scores**, you search against peptide sequences in their forward and inverted directions. If you obtain similar scores for both searches, you likely have a false positive. • **Protein mobility scoring** is a scoring enhancement for ion trap data. It applies a bonus or penalty based on the expected relative intensities for MS/MS fragments from a given peptide sequence. When you process your own ion trap data, it is best to use this scoring, except with modifications that significantly alter the fragmentation patterns of peptides. • **Dynamic peak thresholding** is a scoring enhancement that enables identification of more low-abundance and short-chain peptides. For each extracted spectrum, the software calculates the search scores as the number of spectral peaks varies from n=4 up to the maximum set by the variable **peakLimitCount** in **instrument.txt**. It then displays the best score from the set.

Steps	Detailed Instructions	Comments
7 Make sure other parameters are set as shown in Figure 3 on page 17.	**a** Keep the defaults whenever appropriate. **b** Examine the items in red text carefully, since these are the ones you may need to change when you process your own samples. **c** Click a blue section divider bar to display help for that section of the form.	
8 Start the search.	**a** Click the **Start Search** button **b** View search progress in the **Results** area to the right of the MS/MS Search form. **c** Scroll to the *top* of the **Results** area to see the message that indicates that the search is finished.	• MS/MS Search processes all spectral files in the directory. • Search time varies depending on the size of the database. This search goes fairly quickly because you search a small user database. • You can use your client PC for other tasks while the search is taking place. • If you want to stop the search, click the red **Stop Search PID: xxx** link at the top of the Results section. Then see the Tool Belt chapter in the *Spectrum Mill MS Proteomics Workbench Application Guide* for further instructions.



☐ Remove all prior MS/MS Search results

ExampleData\ThermoFinnigan

Validation filter: spectrum-not-marked-sequence-not-validated

Batch size: 81

☐ Search previous hits

Max reported hits: 5

Database: NCBInr.stdmix

Digest: Trypsin

Species: All

Maximum # missed cleavages: 2

Protein pI: from 3.0 to 10.0 ☑ All

Required AAs:

Disallowed AAs:

Fixed: Carbamidomethylation (C)

Variable:

Minimum scored peak intensity: 50 %

Instrument: ESI ion trap

Masses are: Monoisotopic

Precursor mass tolerance: +/- 2.5 Da

Product mass tolerance: +/- 0.7

Maximum ambiguous precursor charge: 3

☑ Calculate reversed database scores

☑ Proton mobility scoring

☑ Dynamic peak thresholding

Search mode: Identity

☐ Sequence tag length: > 3

Minimum detected peaks: 4

Spectrum files (./cpick_in/):

```
*.pkl
*.dta
```

Figure 3 MS/MS Search settings

Exercise 5. Run Autovalidation

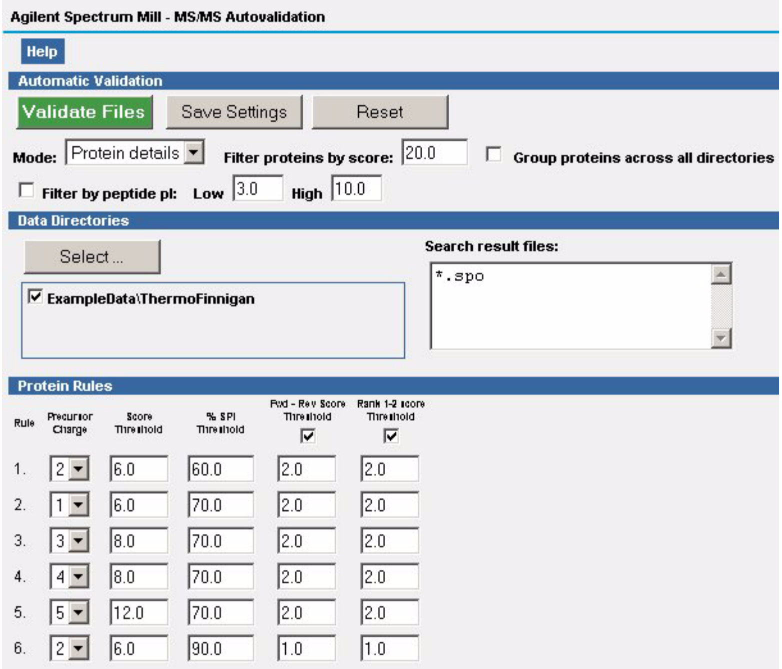
After you have completed a database search, you validate the good results. Validation means that you accept that the matches are correct.

The Spectrum Mill workbench provides a means for segregating search results that contain a valid interpretation of an MS/MS spectrum from those that do not. Results that are *not* validated can then be subjected to subsequent rounds of searches (against other databases or in variable modifications mode, for example). Results that *are* validated can be summarized in a results table.

The Spectrum Mill workbench provides two ways of validating results. One way uses the Autovalidation page, and is totally automated. You use this method only to validate the highest-scoring results—those that do not require manual review. The other method of validating uses the Protein/Peptide Summary page for manual review and validation. This exercise describes autovalidation. To learn more about manual validation, see the *Spectrum Mill MS Proteomics Workbench Application Guide*.

Steps	Detailed Instructions	Comments
1 Navigate to the Autovalidation page.	• Navigate to this page from one of two other pages: • MS/MS Search • Protein/Peptide Summary	You will see the form shown in Figure 4 on page 20.
2 Check that your **Data Directory** is set to **ThermoFinnigan**.	• If you have just performed data extraction and MS/MS search, your data directory should already be set correctly. If not, click the **Select...** button to select the **ThermoFinnigan** folder.	
3 Validate first in the **Protein details** mode.	**a** For **Mode**, keep the default of **Protein details**. **b** Keep the default scoring presets. **c** Click the **Validate Files** button. **d** Watch for a Spectrum Mill Validation Summary that lists the hits and spectra that have been validated.	• In this mode, the software summarizes results by protein, and considers all the peptides that belong to a given protein. • Using the default scoring, individual peptides must have scores greater than 6 to 12 (depending on charge state), and the cumulative protein score must be greater than 20.

Steps	Detailed Instructions	Comments
4 Validate second in the **Peptide** mode.	**a** For **Mode**, select **Peptide**. **b** Keep the default scoring presets. **c** Click the **Validate Files** button. **d** Watch for a Spectrum Mill Validation Summary that lists the hits and spectra that have been validated.	• In this mode, the software summarizes results by peptide. Even if it finds only a single peptide corresponding to a protein, it validates the corresponding search results provided that the peptide score is high enough. • Using the default scoring, individual peptides must have scores greater than 11 to 15 (depending on charge state). This score threshold is higher than in the **Protein details** mode, where you have the additional assurance of knowing you have identified more than one peptide per protein.
5 Close the Autovalidation form.		



Agilent Spectrum Mill - MS/MS Autovalidation

Automatic Validation

Mode: Protein details

Filter proteins by score: 20.0

☐ Group proteins across all directories

☐ Filter by peptide pI: Low 3.0 High 10.0

Data Directories

☑ ExampleData\ThermoFinnigan

Search result files:

`*.spo`

Protein Rules

Rule	Precursor Charge	Score Threshold	% SPI Threshold	Fwd - Rev Score Threshold ☑	Rank 1-2 score Threshold ☑
1.	2	6.0	60.0	2.0	2.0
2.	1	6.0	70.0	2.0	2.0
3.	3	8.0	70.0	2.0	2.0
4.	4	8.0	70.0	2.0	2.0
5.	5	12.0	70.0	2.0	2.0
6.	2	6.0	90.0	1.0	1.0

Figure 4 Autovalidation form

Exercise 6. Display valid database search results

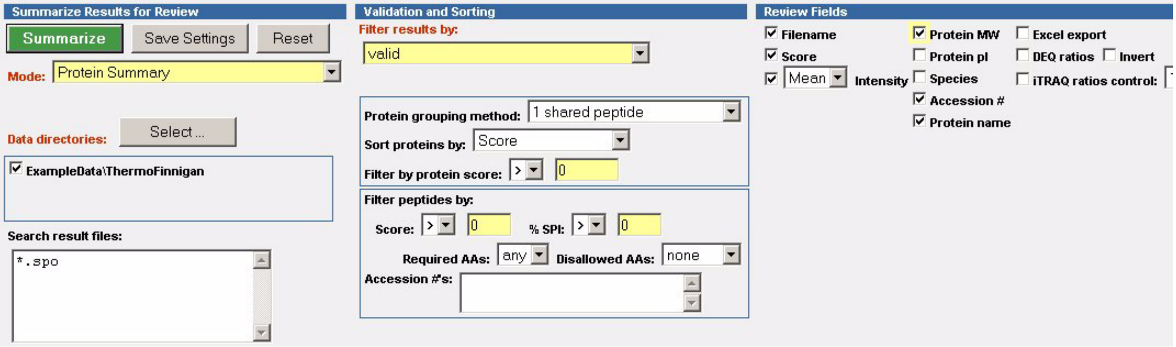
In this exercise, you summarize the results that you just automatically validated.

Steps	Detailed Instructions	Comments

1 Navigate to the Protein/Peptide Summary page.	• If the Protein/Peptide Summary page is already open, do nothing.
	• If not, do one of the following:
	  • From the MS/MS Search page, click the **Protein/Peptide Summary** button.
	  • From the Spectrum Mill home page, click the link to Protein/Peptide Summary.
		There are links to this page from many other pages.

2 Check that your **Data Directory** is set to **ThermoFinnigan**.	• If you have just performed autovalidation, your data directory should already be set correctly. If not, click the **Select...** button to select the **ThermoFinnigan** folder.	
3 Set the **Mode** to **Protein Summary**.		Notice that the summary input form changes to correspond with the new display mode.
4 Set the **Filter results by** to **valid**.		• The **Filter results by** setting selects from your data only the results that match your setting. • In this case, it selects only the results that match the **valid** setting (which was designated during autovalidation).
5 Leave **Sort proteins by** set to the default of **Score**.		• The **Sort** parameters determine how the data are sorted in the results display. • When you process your own data, select the setting that is most helpful to you.
6 Set the **score** and **% SPI** filters to **> 0**.	**a** Set **Filter by protein score** to **> 0**. **b** For **Filter peptides by**, set both **Score** and **% SPI** to **> 0**.	When you set this to **> 0**, you are sure to display all the valid results.

Steps	Detailed Instructions	Comments
7 Set the **Review Fields**.	**a** Mark the **Protein MW** check box. **b** Keep the other default settings. **c** Check to see that your form looks like that in Figure 5 on page 23. Note that changes from defaults are highlighted in yellow.	• **Review Fields** determine what information you see in the final results summary. • The default settings are shown in the online help. Click the **Review Fields** blue dividing bar to access the online help.
8 Click the **Summarize** button.		
9 Examine the overall summary report.	• Check that your results are similar to those in Figure 6 on page 23.	Note the colored cells in the summary report. • The color-code indicates relative quantities. Dark red is highest, orange is intermediate, and yellow is lowest. • The **Distinct Peptides** column gives the number of peptides detected for each protein, • The **Mean Peptide Spectral Intensity** is an average of the intensities for all peptides detected for that protein. These intensities are calculated from extracted ion chromatograms from the precursor ions. • For more accurate quantitation, under **Review Fields**, for **Intensity**, select **Total** rather than **Mean**. • These intensity results are sufficient for studies where you are interested in differences of two-fold or more.



Summarize Results for Review

Mode: Protein Summary

Data directories:

☑ ExampleData\ThermoFinnigan

Search result files:

```
*.spo
```

Validation and Sorting

Filter results by: valid

Protein grouping method: 1 shared peptide

Sort proteins by: Score

Filter by protein score: > 0

Filter peptides by:

Score: > 0 % SPI: > 0

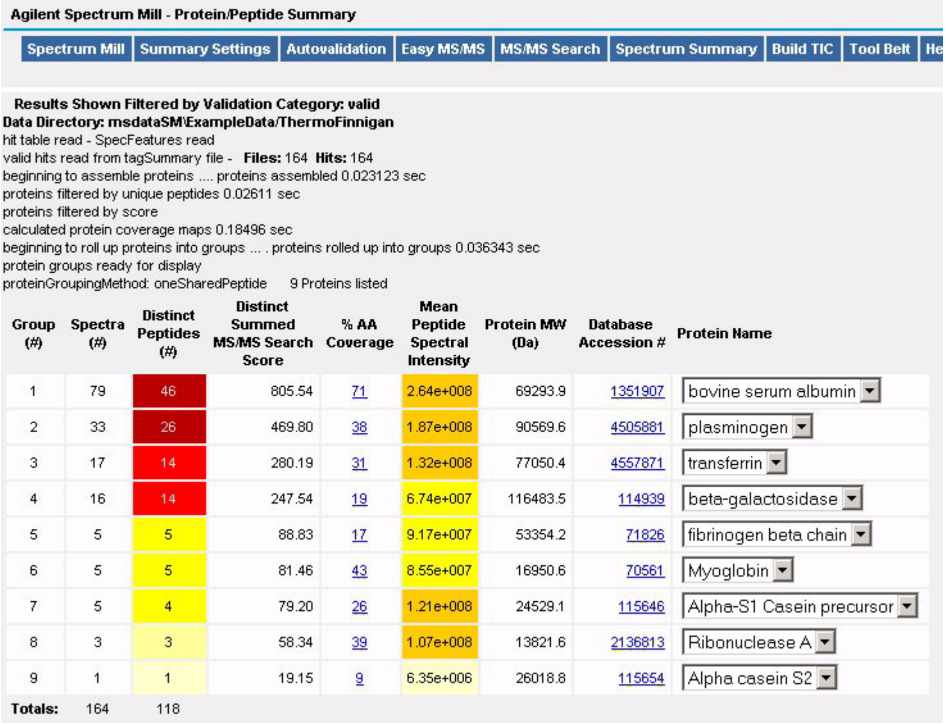
Required AAs: any Disallowed AAs: none

Accession #'s:

Review Fields

☑ Filename
☑ Score
☑ Mean Intensity
☑ Protein MW
☐ Protein pI
☐ Species
☑ Accession #
☑ Protein name
☐ Excel export
☐ DEQ ratios ☐ Invert
☐ iTRAQ ratios control:

Figure 5 Settings to summarize valid results - Protein Summary mode



Agilent Spectrum Mill - Protein/Peptide Summary

Results Shown Filtered by Validation Category: valid
Data Directory: msdataSM\ExampleData/ThermoFinnigan
hit table read - SpecFeatures read
valid hits read from tagSummary file - **Files:** 164 **Hits:** 164
beginning to assemble proteins .... proteins assembled 0.023123 sec
proteins filtered by unique peptides 0.02611 sec
proteins filtered by score
calculated protein coverage maps 0.18496 sec
beginning to roll up proteins into groups ... . proteins rolled up into groups 0.036343 sec
protein groups ready for display
proteinGroupingMethod: oneSharedPeptide 9 Proteins listed

Group (#)	Spectra (#)	Distinct Peptides (#)	Distinct Summed MS/MS Search Score	% AA Coverage	Mean Peptide Spectral Intensity	Protein MW (Da)	Database Accession #	Protein Name
1	79	46	805.54	71	2.64e+008	69293.9	1351907	bovine serum albumin
2	33	26	469.80	38	1.87e+008	90569.6	4505881	plasminogen
3	17	14	280.19	31	1.32e+008	77050.4	4557871	transferrin
4	16	14	247.54	19	6.74e+007	116483.5	114939	beta-galactosidase
5	5	5	88.83	17	9.17e+007	53354.2	71826	fibrinogen beta chain
6	5	5	81.46	43	8.55e+007	16950.6	70561	Myoglobin
7	5	4	79.20	26	1.21e+008	24529.1	115646	Alpha-S1 Casein precursor
8	3	3	58.34	39	1.07e+008	13821.6	2136813	Ribonuclease A
9	1	1	19.15	9	6.35e+006	26018.8	115654	Alpha casein S2
Totals:	164	118						

Figure 6 Protein summary of valid search results

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In this Book

The *Quick Start Guide* presents first steps to use the Spectrum Mill Extractor for Thermo Fisher Scientific Ion Trap Data Files.

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