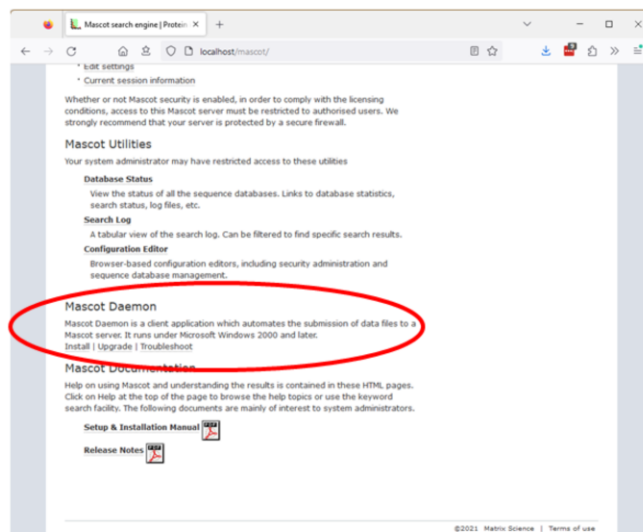


Automation with Mascot Daemon

MASCOT



Installing Mascot Daemon



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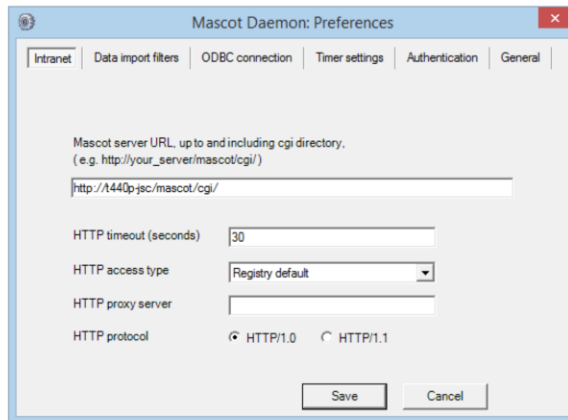


Mascot Daemon is our automation client. It's a part of the Mascot package that is only available when you have a Mascot server in-house.

It is only available for Windows because all of the mass spectrometry data systems are Windows packages, so this is always the platform where the data files originate.

Links to install and update Daemon can be found on your local Mascot home page, so that anyone with access to the server can easily get a copy of Daemon. You should try and make sure that you have the same version of Mascot Server and Mascot Daemon installed. An older version of Daemon will probably work, but it won't support features in later versions of Mascot Server.

Installing Mascot Daemon



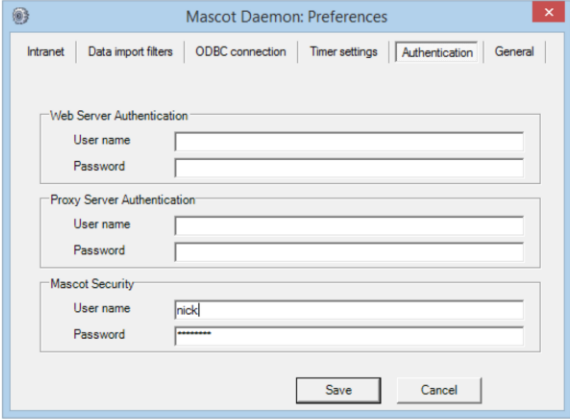
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When Mascot Daemon is first launched, this dialog will be displayed and you have to enter the URL of your local Mascot server to proceed. Note that you cannot enter the public web site URL ... it simply won't work. If Mascot security is enabled, you will also need to enter a valid user name and password on the authentication tab.

Installing Mascot Daemon

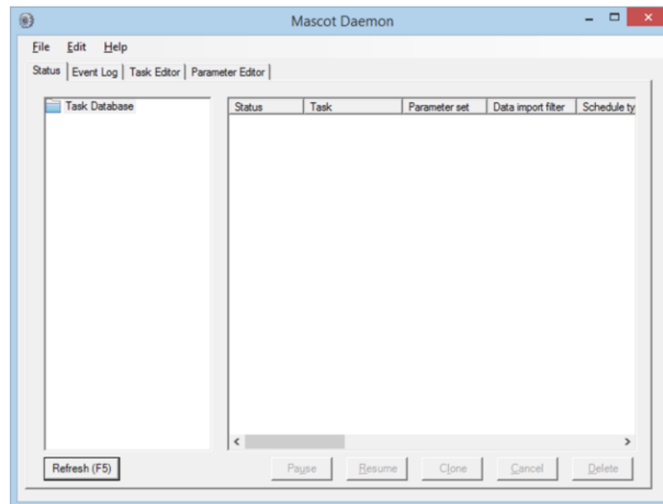


The screenshot shows the 'Mascot Daemon: Preferences' dialog box with the 'Authentication' tab selected. The dialog has a title bar with a close button. Below the title bar is a tabbed interface with tabs for 'Intranet', 'Data import filters', 'ODBC connection', 'Timer settings', 'Authentication', and 'General'. The 'Authentication' tab is active and contains three sections: 'Web Server Authentication', 'Proxy Server Authentication', and 'Mascot Security'. Each section has 'User name' and 'Password' input fields. The 'Mascot Security' section has 'nick' entered in the 'User name' field and asterisks in the 'Password' field. At the bottom right are 'Save' and 'Cancel' buttons.

Section	User name	Password
Web Server Authentication		
Proxy Server Authentication		
Mascot Security	nick	*****

Mascot security is not the same as web server security or having to tunnel through a firewall or authenticated proxy server. There are separate fields for each of these.

Installing Mascot Daemon



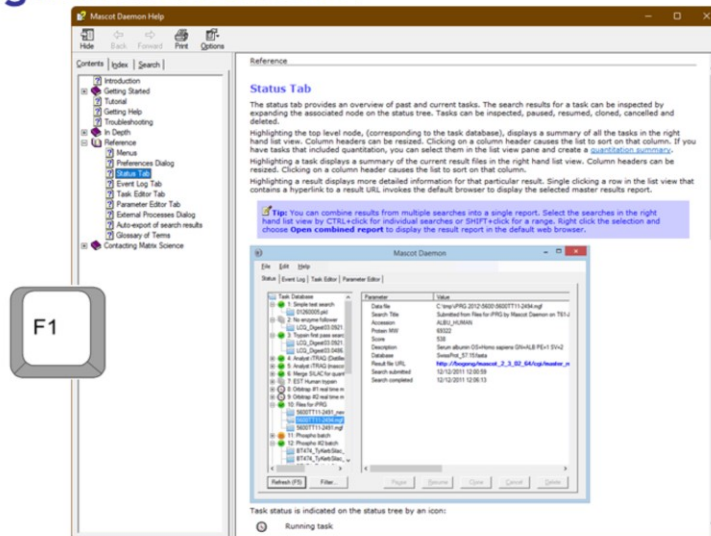
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Once Daemon has connected to the Mascot server, you will see a display like this. The user interface is a simple, tabbed dialog box.

Installing Mascot Daemon



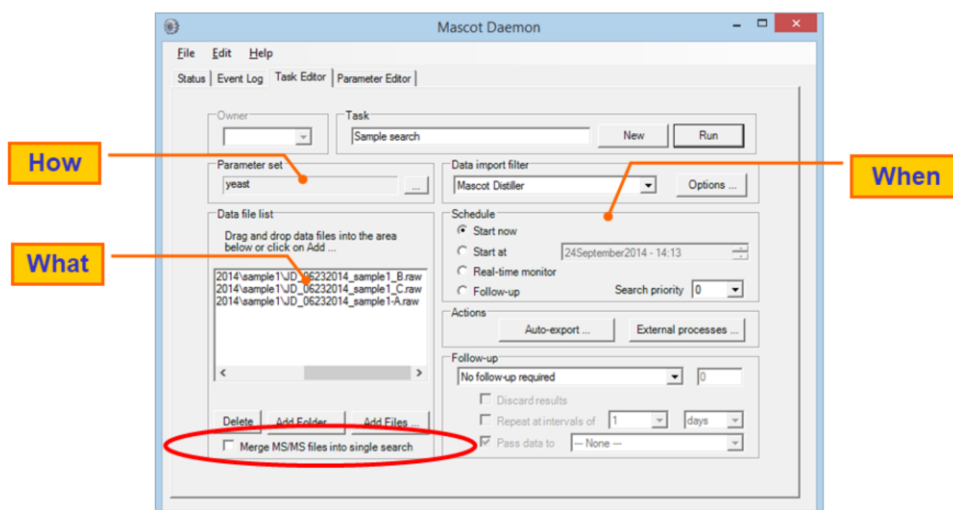
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Mascot Daemon has comprehensive, context sensitive help. Just press F1. There is a tutorial to get you up to speed and detailed reference material.

Task Editor tab



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Lets look at the tabs in more detail.

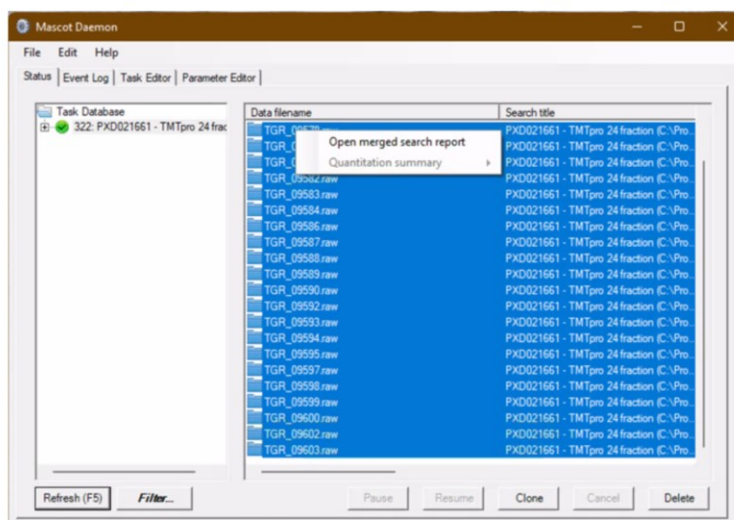
The Task Editor tab is used to define each task. A task defines *what* data will be searched and *when* the search will take place. A Parameter set is selected that contains the search parameters. This determines *how* the data files will be searched.

Here we have a very simple batch task. A single data file has been selected, a parameter set has been chosen, and the task will run the search as soon as we press run.

The data files can be simple peak lists. In this case, we are using Mascot Distiller as a data import filter and the data file is a RAW file. More about data import filters later.

Note the checkbox at the bottom left. Merging a batch of files into a single search can be very useful. MudPIT fractions, for example.

Merging multiple results into a single report



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However if you are using the Windows operating system for your Mascot Server and the default IIS web server, you can hit a 4 GB upload limit.

There are a number of ways around this limit such as using Mascot Daemon on the same computer as Mascot Server, so the searches are submitted on the command line, moving from the IIS web server to Apache or using Mascot Distiller for peak picking and merging the results there.

You can also merge results post search. Select the task to display the search results and select multiple entries, right click and select “Open the merged search report”.

The combined search will open in a web page and list the results files that have been merged at the top of the report. This will work with searches that have been processed by any peak picking software including Mascot Distiller.

Search Parameter Editor

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The parameter editor allows sets of search parameters to be defined and saved to disk, so that they can be used over and over again by tasks. The search parameters define *how* the data will be searched. As you can see, the fields are very similar to those on the web browser search form.

The search title includes some tokens, or variable, that will be replaced when the task runs. Here, for example, <taskname> will be replaced by the name of the task and <localhost> will be replaced by the name of the computer on which Daemon is running. This will make it easy to identify where the searches came from when looking at the Mascot search log on the server. There are many such tokens. You can find details in the help file.

The screenshot displays the Mascot Daemon application window. At the top, there is a menu bar with 'File', 'Edit', and 'Help'. Below the menu bar is a status bar with 'Status', 'Event Log', 'Task Editor', and 'Parameter Editor'. The main window is divided into two panes. The left pane, titled 'Task Database', shows a tree view of tasks. The right pane, titled 'Parameter', shows a table of parameters for the selected task.

Task Database

- 1: Project PXD029062
 - K030_8.raw
 - R00_1.raw
 - R00_2.raw
 - R00_3.raw
 - R00_4.raw
 - K030_5.raw
 - K030_6.raw
 - K030_7.raw
- 2: Project PXD029062 old style
- 3: DW AR 1
- 4: DW AR All
- 5: DW AR rest
- 6: PhoX BSA
- 7: PhoX BSA
- 8: PhoX BSA
- 9: PhoX BSA
- 10: DW AR last

Parameter

Parameter	Value
Data file	\\ELECTUS\MSData\XPD029062\K030_8.raw
Search Title	Project PXD029062 (C:\ProgramData\Matrix Science\Mascot Daemon\paran
Accession	P00761
Protein MW	25078
Score	4116
Description	SWISS-PROT:P00761 TRY_PIG Trypsin - Sus scrofa (Pig).
Database	contaminants_20160129.fasta, UP5640_H_sapiens_20202022.fasta
Result file URL	http://electus/mascot/cgi/master_results_2.pl?file=.../data/202
Database	D:\MGFV1 Project PXD029062\1aae44a50f3d4cb___K030_8.ra
Cache folder	D:\MGFV1 Project PXD029062
Search submitted	6/4/2022 11:19:38 PM
Search completed	6/4/2022 11:20:15 PM
Last event	6/4/2022 11:21:05 PM (MDS1129) Mascot Distiller exited with code -1073741

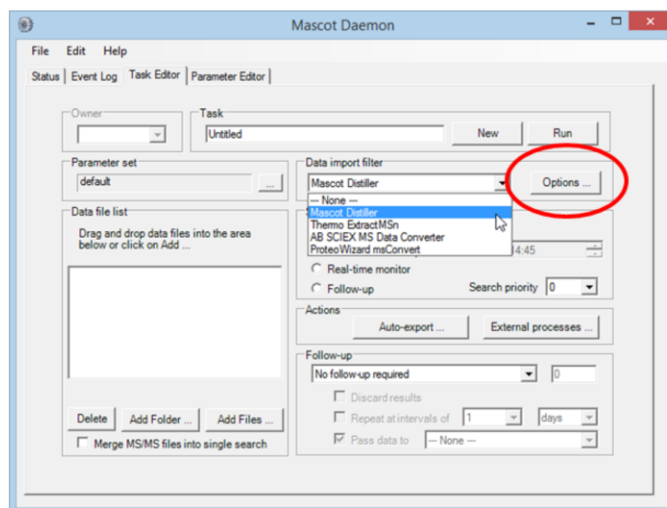
At the bottom of the window, there are buttons for 'Refresh (F5)', 'Filter...', 'Pause', 'Resume', 'Clone', 'Cancel', and 'Delete'.



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Data files within a particular task are run serially, but multiple tasks can be set running at the same time. This means that several data files can be processed and search the same time.

Data Import Filters



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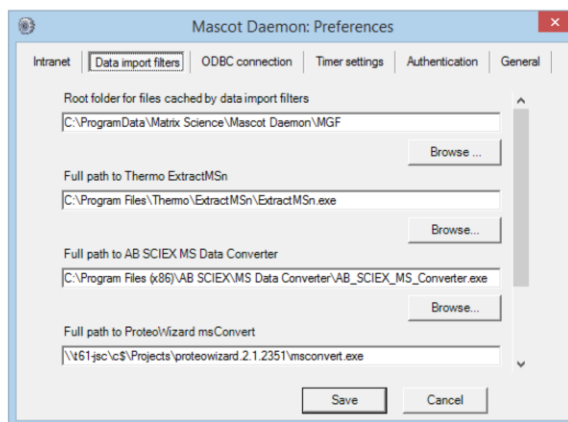
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Going back to the task editor tab. For some file formats, we have data import filters. These allow Daemon to generate peak lists directly from the binary files created by the MS data system.

Next to the drop down list, you have a button to view the options for the selected data import filter.

For Mascot Distiller, you need to have a licence for the Daemon Toolbox. If present on the Daemon PC, Mascot Distiller will be added to the list of import filters automatically.

Data Import Filters



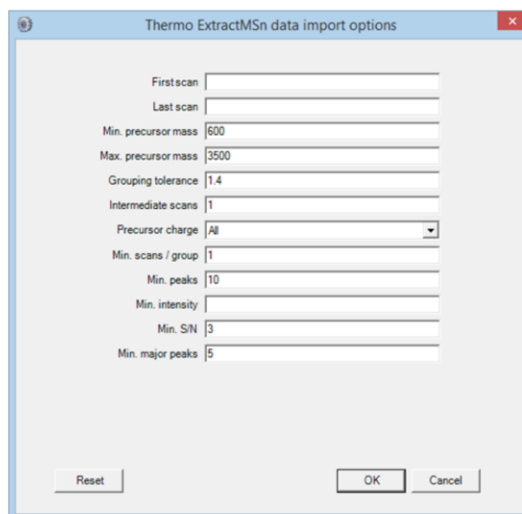
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All other data import filters must be configured on the Data import filters tab in Preferences. Install the package, browse to the executable, and save.

Data Import Filters



The screenshot shows a dialog box titled "Thermo ExtractMSn data import options". It contains several input fields for configuring data import filters. The fields and their values are as follows:

Field	Value
First scan	
Last scan	
Min. precursor mass	600
Max. precursor mass	3500
Grouping tolerance	1.4
Intermediate scans	1
Precursor charge	All
Min. scans / group	1
Min. peaks	10
Min. intensity	
Min. S/N	3
Min. major peaks	5

At the bottom of the dialog box, there are three buttons: "Reset", "OK", and "Cancel".

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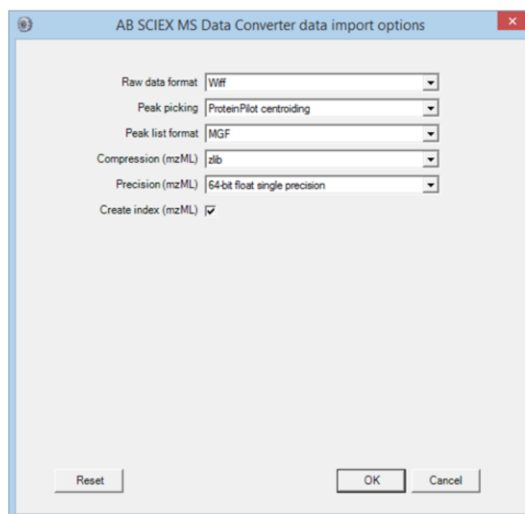
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A number of data import filters are pre-configured and their options are described in the Mascot Daemon help - just press F1

Thermo ExtractMSn is a utility that creates DTA peak lists from Thermo Xcalibur .RAW files. However, it is no longer actively developed by Thermo and considered obsolete as it is not able to process data from newer instruments like the Thermo Q-Exactive.

For reference Mascot Daemon calls the utility using the parameters specified in this dialog, then merges the DTA files into a single MGF file. ExtractMSn can be downloaded from the Thermo web site.

Data Import Filters



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The AB Sciex MS Data Converter supports both Wiff files and TOF/TOF data. Output can be an MGF peak list file or an mzML file containing a more complete representation of the data. For rapid peak picking, the converter exports the centroids determined by the instrument software during acquisition. For Wiff files, there is also an option to use the peak picking algorithm from ProteinPilot, which gives good results on TripleTOF data.

Data Import Filters

ProteoWizard msConvert data import options

Peak list format: MGF

Default binary precision: 64-bit precision

Precision m/z: 64-bit precision

Precision intensity: 32-bit precision

Omit index (XML): ☐

Use compression (XML): ☒

Filter options: -filter "peakPicking true 1-" -filter "threshold count !"

Reset OK Cancel

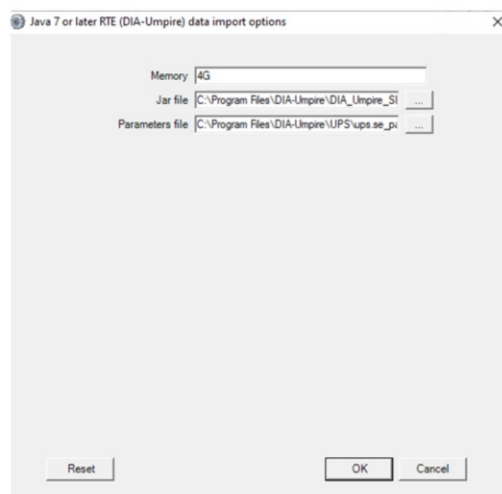
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ProteoWizard msconvert is a command line tool for converting between various file formats. It has a large number of options. Note that the input file format is not specified; msconvert auto-detects the format.

Data Import Filters



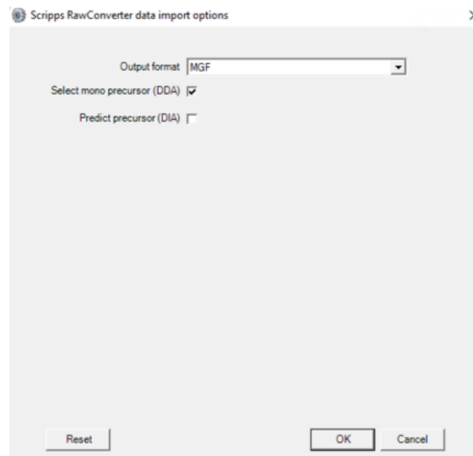
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DIA-Umpire is an open source Java program that enables untargeted peptide and protein identification and quantitation using DIA data. The DIA-Umpire signal extraction module deconvolutes the DIA data to create a conventional DDA-type peak list, suitable for database searching.. The only options in Mascot Daemon are the path to the java jar file and the processing parameters.

Data Import Filters



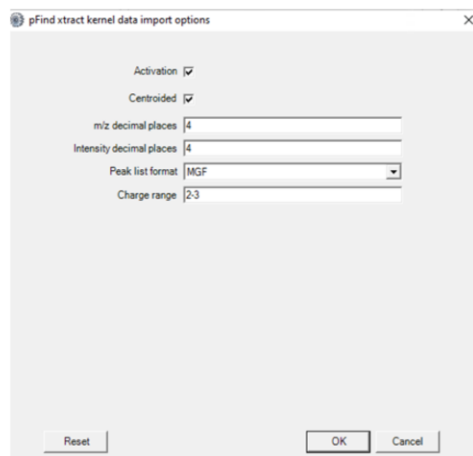
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Scripps RawConverter is a command line tool for converting Thermo Raw files into peak lists. It accepts RAW data generated by either data-dependent acquisition (DDA) or data-independent acquisition (DIA).

Data Import Filters



A screenshot of the 'pFind xtract kernel data import options' dialog box. The dialog has a title bar with a close button (X). Inside, there are several settings: 'Activation' and 'Centroided' are both checked with checkboxes. Below these are two text input fields: 'm/z decimal places' and 'Intensity decimal places', both containing the value '4'. There is a dropdown menu for 'Peak list format' currently showing 'MGF'. At the bottom, there is a 'Charge range' input field containing '2-3'. At the very bottom of the dialog are three buttons: 'Reset', 'OK', and 'Cancel'.

Activation	<input checked="" type="checkbox"/>
Centroided	<input checked="" type="checkbox"/>
m/z decimal places	4
Intensity decimal places	4
Peak list format	MGF
Charge range	2-3

The pFind application also has a command line peak picking application xtract that can be used by Mascot Daemon. There are versions for various common file formats.

Data Import Filters

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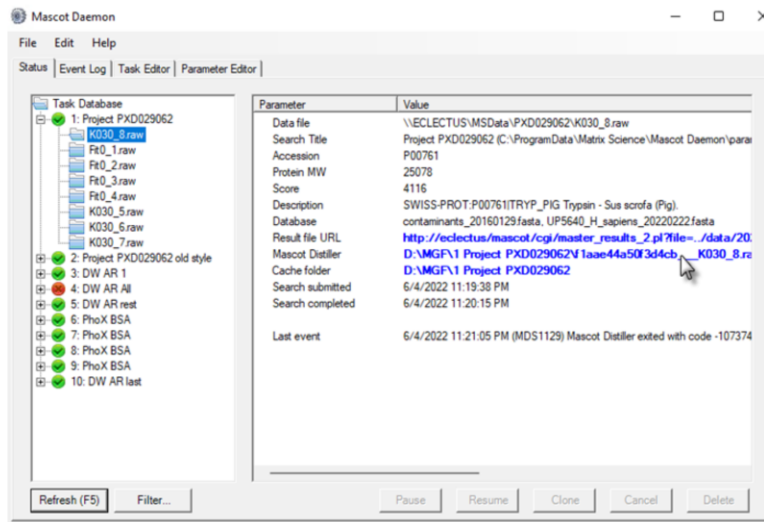
The most powerful data import filter is Mascot Distiller, because it can import all of the major raw file formats. The dialog is relatively simple because the peak picking options are contained in the selected processing options file.

The idea is that you adjust the processing options by working interactively with a typical data file in Mascot Distiller Workstation. You then save these options to a file and select it here.

You can ask Daemon to save a project file containing the Peak lists and Mascot search results.

If you have the search and quantitation toolboxes, you can also batch automate quantitation. We'll be discussing quantitation in detail in another presentation.

Data Import Filters



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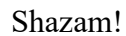


If Distiller is used for peak picking, once the search has completed, you get a hyperlink to open the project in Mascot Distiller. Click on the link and ...

The screenshot displays the Mascot Distiller software interface. At the top, the title bar reads "Mascot Distiller - def8a8377381999..._ID_06232014_sample2.Braw1". The menu bar includes File, Edit, View, Processing, Analysis, Tools, Windows, and Help. Below the menu is a toolbar with various icons for file operations and analysis. The main window is divided into two panes. The left pane, titled "Proteins", shows a list of protein search results. The right pane, titled "Quantitation Table", displays a table with columns for Ref, Accession, Score, Mass, #, Avg. Intensity, Amount, and Description. Below the table, a mass spectrum plot is shown, with the x-axis labeled "m/z" and the y-axis labeled "Relative Intensity". The plot shows a series of peaks, with the base peak at m/z 1000. The plot is titled "ID_06232014_sample2.Braw1".

Ref	Accession	Score	Mass	#	Avg. Intensity	Amount	Description
1.1	YALC1309	3635	54910.44	44	647498150	1.000	CD14 (P1) S100-000000000000000000, Chr 1 from 11317-71289, Verified OMP, "Pyruvate 11"
1.2	Y000476	295	55641.7	6	10377068	6.150	PV-2 S100-000000000000000000, Chr 10 from 3604545-3604539, reverse complement, Y000476
2.1	Y001749	3761	40934.26	36	62977633	1.613	INSD2 S100-000000000000000000, Chr 10 from 41357-42346, Verified OMP, "Threonine"
2.2	Y000476	1976	55641.7	6	69471633	1.747	INSD1 S100-000000000000000000, Chr 10 from 1000002-1000002, Verified OMP, "Threonine"
3.1	Y001749	3761	40934.26	36	62977633	0.042	INSD2 S100-000000000000000000, Chr 10 from 41357-42346, Verified OMP, "Threonine"
4.1	Y001749	3690	41680.30	39	63095219	1.027	PC1 S100-000000000000000000, Chr 10 from 24902-25281, reverse complement, Y001749
4.2	Y001749	442	42152.9	9	34440353	0.529	PC2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.1	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.2	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.3	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.4	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.5	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.6	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.7	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.8	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.9	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.10	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.11	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.12	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.13	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.14	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.15	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.16	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.17	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.18	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000, Chr 10 from 41724-41715, Verified OMP, "Threonine"
5.19	Y001749	3690	41680.30	39	63095219	0.019	INSD2 S100-000000000000000000,

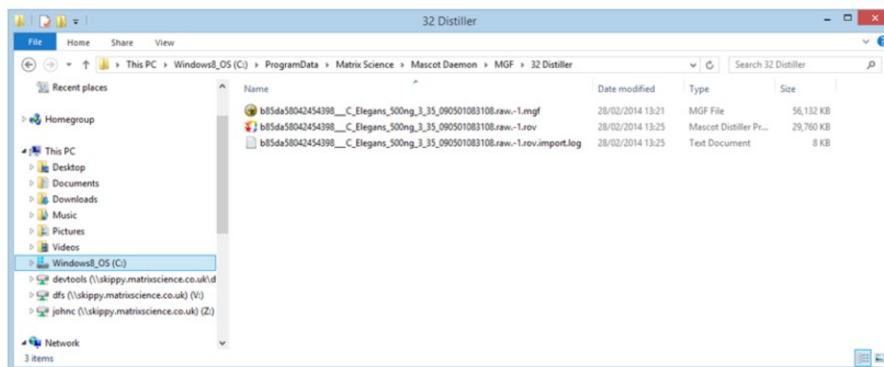
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Data Import Filters

- Peak lists and Distiller projects created by Daemon are saved to disk:

```
%allusersprofile%\Matrix Science\Mascot  
Daemon\MGF\<task name>
```



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Peak lists created by Daemon are always saved to disk. This is the default path, which can be changed in preferences.

When you delete a task, any peak lists are also deleted. If you have used Mascot Distiller as in import filter, then the distiller project file is also deleted. The raw data files are never deleted.

You may also find Distiller project files, log files and result exports in the same folder.

Types of task

Batch task

- A batch of data files to be searched immediately or at a defined time

Start on competition task

- Wait for a running task to finish before starting the next

Real-time monitor task

- New files on a defined path are searched as they are created

Follow-up task

- Accepts data files from another task. For example, to repeat a search against a different database

Lets talk in more detail about tasks. Mascot Daemon supports three kinds of task:

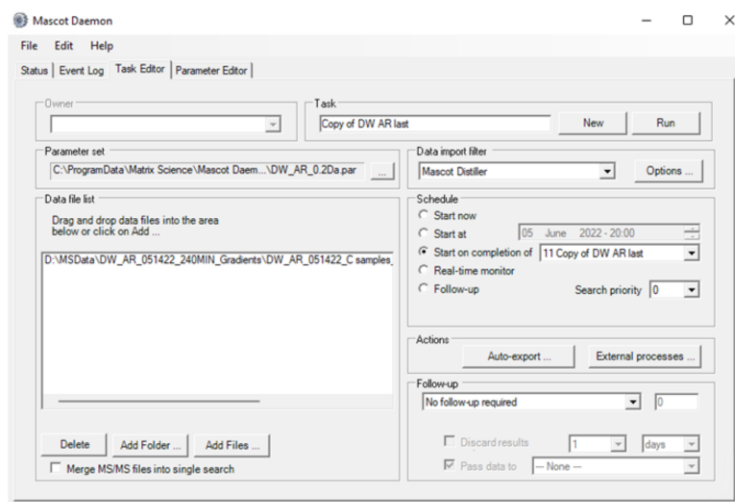
A batch task allows you to search one or more specified files immediately or at a defined date and time.

A start on competition task waits for a running task to finish before starting the next.

A real-time monitor task is the key to real automation. You define a task using a file path for the data file which includes wild cards. Any file that matches this path is picked up and searched.

A follow-up task is a task where the data files are passed to it by another task. This is very powerful because it allows searches to be chained together to implement complex workflows. For example, a batch of data files might be screened against a contaminants database containing entries for keratins, BSA, trypsin, etc. Those data files which fail to find a match can then be automatically searched against a non-redundant protein database. Spectra which are still unmatched can then be searched against an EST database, etc., etc.

Start on completion of <task name>



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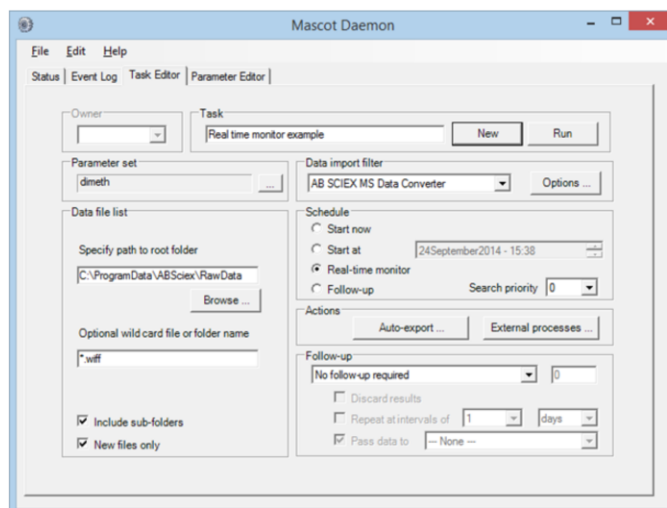
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When you select start on completion of in the schedule frame, the task editor tab looks like this. Any tasks that are currently running will be listed in the dropdown box.

If an active task is selected, then Daemon will wait for that task to complete before starting the next.

This means that you can queue up tasks to run serially rather than in parallel. You can run tasks in an order each one starting after the end of the earlier one. Or have multiple tasks start after the first one has finished etc.

Real-time Monitor Tasks



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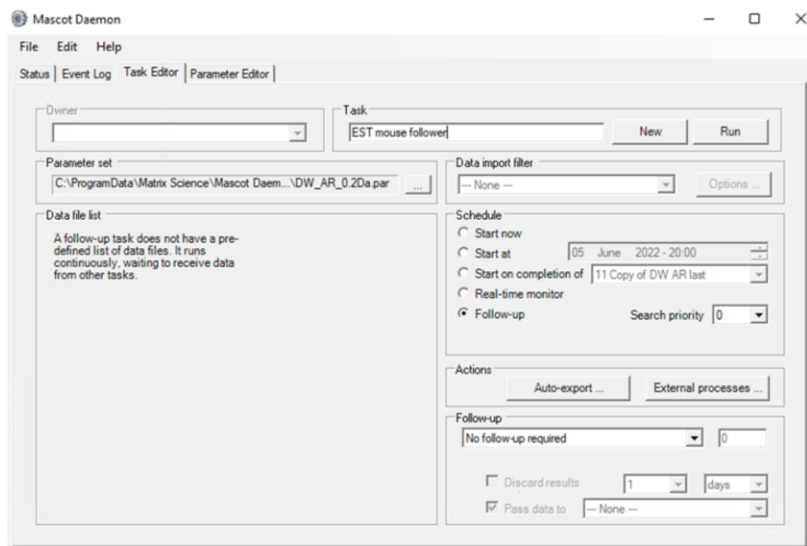
When you select real-time monitor in the schedule frame, the task editor tab looks like this. Here, we are looking in the specified directory and all its subdirectories, for any file called with the extension wiff.

Note that the final part of the path says “file or folder name”. This is because some raw files are actually folders, not files.

If Include sub-folders is checked, then Daemon will search down through all sub-directories of the root folder.

If new files only is checked, then any files that exist when the task is started will be ignored. A common problem when using real-time monitor like a batch task is to have this checked and then wonder why nothing is happening.

Follow-up Tasks



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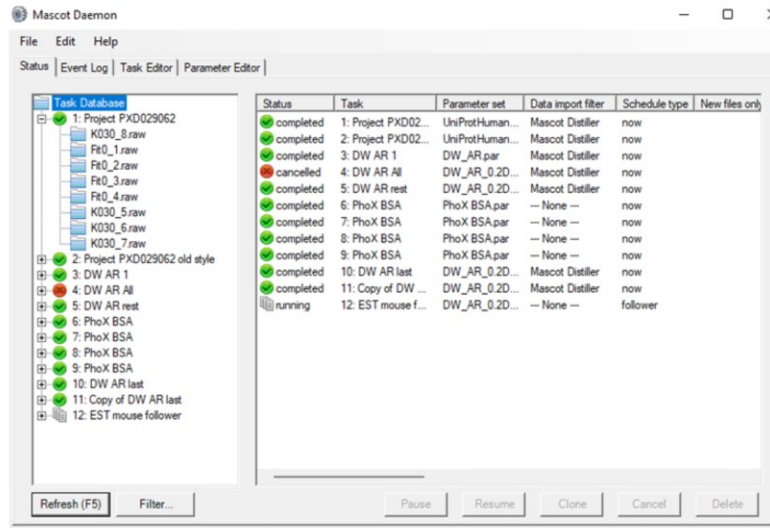
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Follow-up tasks are a little more difficult to explain. Any task can pass data to a follow-up task to be searched again in a different way. Only follow-up tasks can receive data from other tasks in this way.

Let's illustrate with an example. Imagine we want to search our MS/MS data files against the mouse proteome as a first pass search. We then want to search all spectra that fail to get a significant match against a database of mouse EST sequences.

We start at the end of the chain, and define the EST search as a follow-up task. We just choose Follow-up in the schedule frame and a parameter set, created earlier in the parameter editor, that contains the required search parameters.

Follow-up Tasks



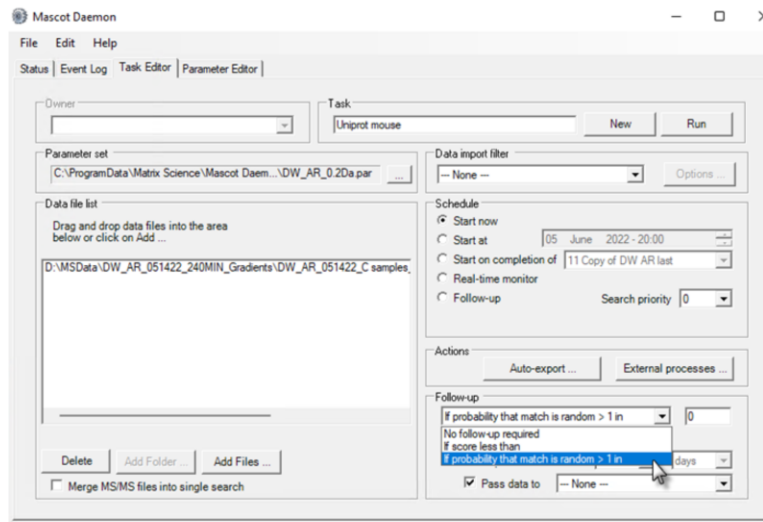
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We press run, and we see our Follow-up task running. Although the task is running, nothing will happen until we feed some data into it.

Follow-up Tasks



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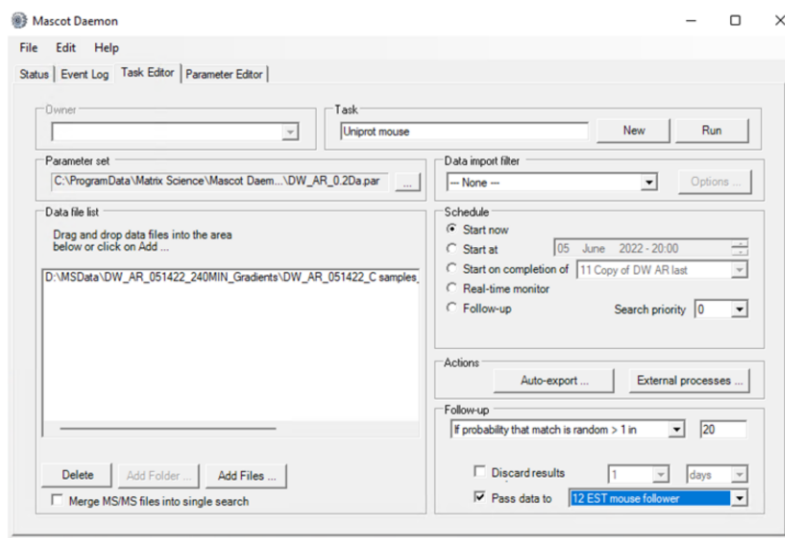
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We go back to the task editor and create a simple batch task. There is one data file and the search parameters are a different set, in which the databases are the Uniprot complete mouse proteomes plus second database of contaminant sequences.

Before pressing Run, we have to link this task to the follow-up task. We need to specify a condition for passing the data to the follow-up task. This can be score based or probability based.

Follow-up Tasks



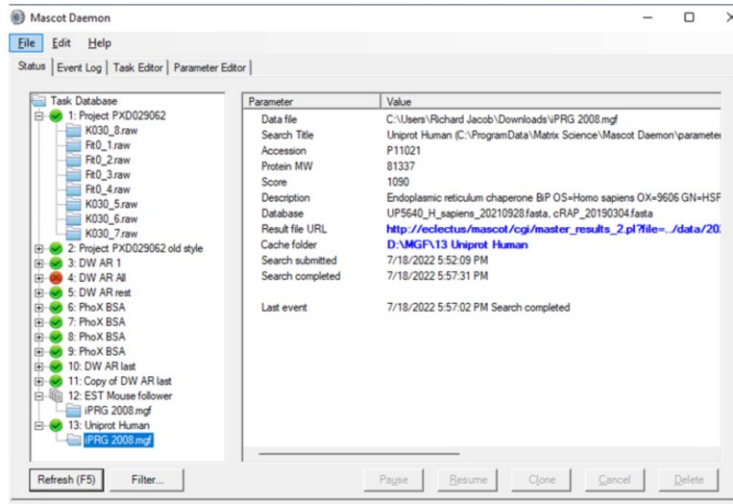
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For this example, we will use the default significance threshold of 5%. If the score a peptide match in the first search is below a 5% significance threshold, then the spectrum will be passed to the follow-up task we created earlier. Note that you also have the option to repeat the search at intervals, maybe to see whether a better match can be found in some future update to the sequence database. The task is now configured, and we can press Run.

Follow-up Tasks



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Some time later, the status tab looks like this. The results for the first search appear under task 13. Those spectra that failed to obtain a significant match were passed to the follow-up task, task 12, where the result information appears once the search has completed. There is a link to the cache directory in the status tab for the search.

Follow-up Tasks

- Can chain follow-up tasks to create complex workflows
- To create a chain, start at the end and work back to the beginning
- Multiple batch or real-time monitor tasks can feed into a follow-up task
- Can also drag and drop result nodes onto follow-up task nodes

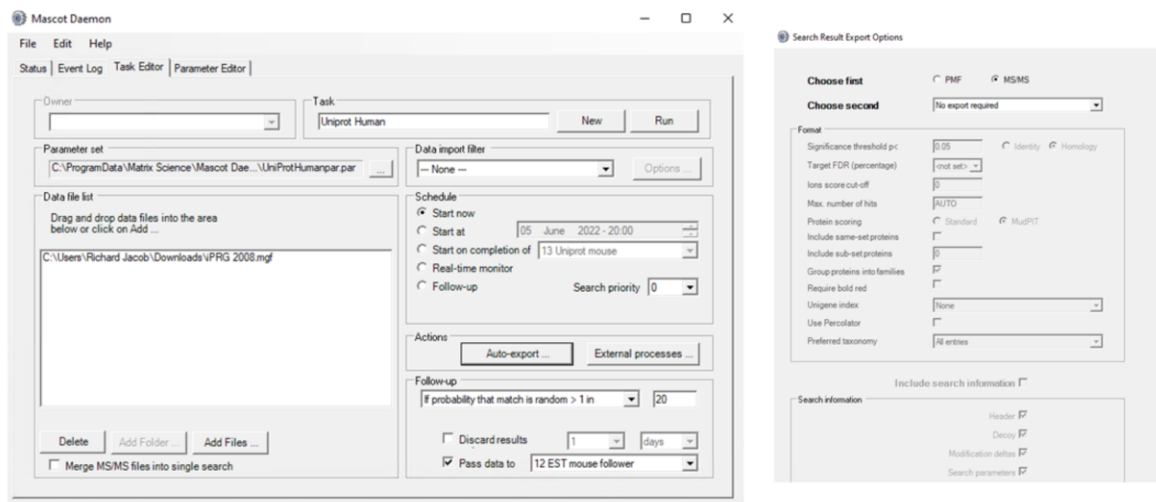
One follow-up task can pass data to another follow-up task, creating a chain of tasks to implement a complex workflow. Thresholding applies to individual spectra. If there are 1000 spectra, and 100 get a good match, we take the 900 that didn't get a match and pass them to the follow-up task. You can think of the chain of tasks as a series of finer and finer sieves.

Remember that, when you define the chain, you have to start at the end of the chain, with the last task, and work forwards to the first task in the chain.

Having created a follow-up task, or a chain of follow-up tasks, multiple batch or real-time monitor tasks can feed into it. It just sits there, waiting.

Note that any result node can be dragged and dropped onto a follow-up task node to repeat the search using a different parameter set.

Auto Export



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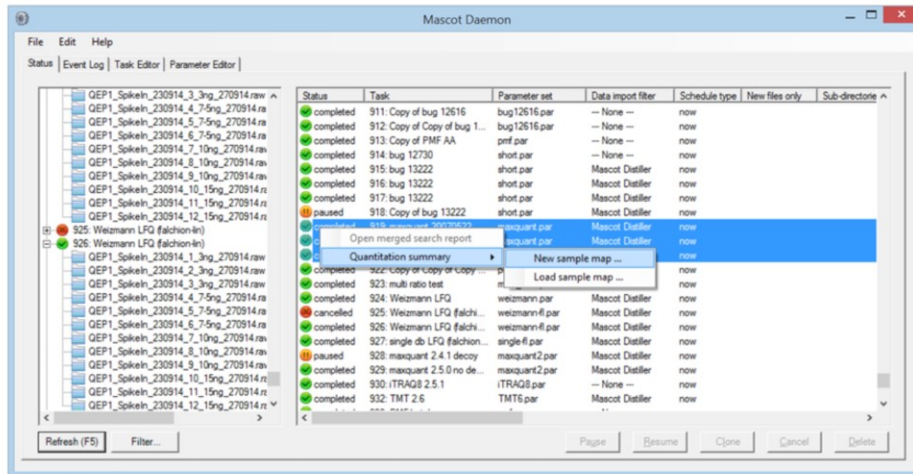
We'll briefly touch on a few other features of Daemon.

Search results can be exported to a file automatically, at the end of the search, in any of the formats supported by the Mascot Server. Each file is saved to the cache directory for the task.

You must set the controls according to your knowledge of the data and search parameters. First choose whether the results are PMF or MS/MS ions search, then choose the export format. For more information on the other controls, refer to the *Exporting result reports* help page on your Mascot server. If you choose incompatible settings, for example using Percolator scoring when the auto-decoy option is not selected in the search parameters, this may generate a warning or, in some cases, a fatal error when the export job is submitted to the server.

If an export is selected and the dialog closed, the label on the button uses a bold italic font.

Exporting protein expression with Quantitation Summaries



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The Quantitation Summaries feature has been added to Mascot Daemon in version 2.7. Searches run through Daemon that include label or label-free quantitation, including reporter methods such as iTRAQ and TMT, can be combined and annotated to create a Quantitation Summary.

The analyses can be spread across any number of Daemon tasks and can include existing results from earlier versions of Daemon. For reporter ion experiments, you can use Distiller for peak picking, but this is not a requirement. For MS1 quantitation methods, such as SILAC and label-free, the raw files must be peak picked and quantified by Mascot Distiller.

In the left hand panel select the “Task Database” node at the very top in order to view the different tasks in the right hand panel.

You can now select one or more tasks and right click to create a sample map. There is also a filter button that can be used to find specific tasks.

Sample map

Sample map - *

<input checked="" type="checkbox"/>	Task	Task name	Raw file path	Raw file name	Parameter set	Fastq(s)	Submitted	Fraction	Intensity LFQ
<input checked="" type="checkbox"/>	924	Weizmann LFQ	\\kippymatrix...	GEP1_Spikeln_230914_1_3ng_270914.raw	weizmann.par	ecoli_proteome_...	06/08/2019 17:2...	1	3ng
<input checked="" type="checkbox"/>	924	Weizmann LFQ	\\kippymatrix...	GEP1_Spikeln_230914_2_3ng_270914.raw	weizmann.par	ecoli_proteome_...	06/08/2019 18:0...	2	3ng
<input checked="" type="checkbox"/>	924	Weizmann LFQ	\\kippymatrix...	GEP1_Spikeln_230914_3_3ng_270914.raw	weizmann.par	ecoli_proteome_...	06/08/2019 18:4...	3	3ng
<input checked="" type="checkbox"/>	924	Weizmann LFQ	\\kippymatrix...	GEP1_Spikeln_230914_4_7.5ng_270914.raw	weizmann.par	ecoli_proteome_...	06/08/2019 19:2...	1	7.5ng
<input checked="" type="checkbox"/>	924	Weizmann LFQ	\\kippymatrix...	GEP1_Spikeln_230914_5_7.5ng_270914.raw	weizmann.par	ecoli_proteome_...	06/08/2019 20:0...	2	7.5ng
<input checked="" type="checkbox"/>	924	Weizmann LFQ	\\kippymatrix...	GEP1_Spikeln_230914_6_7.5ng_270914.raw	weizmann.par	ecoli_proteome_...	06/08/2019 20:4...	3	7.5ng
<input checked="" type="checkbox"/>	924	Weizmann LFQ	\\kippymatrix...	GEP1_Spikeln_230914_7_10ng_270914.raw	weizmann.par	ecoli_proteome_...	06/08/2019 21:1...	1	10ng
<input checked="" type="checkbox"/>	924	Weizmann LFQ	\\kippymatrix...	GEP1_Spikeln_230914_8_10ng_270914.raw	weizmann.par	ecoli_proteome_...	06/08/2019 22:0...	2	10ng
<input checked="" type="checkbox"/>	924	Weizmann LFQ	\\kippymatrix...	GEP1_Spikeln_230914_9_10ng_270914.raw	weizmann.par	ecoli_proteome_...	06/08/2019 22:4...	3	10ng
<input checked="" type="checkbox"/>	924	Weizmann LFQ	\\kippymatrix...	GEP1_Spikeln_230914_10_15ng_270914.raw	weizmann.par	ecoli_proteome_...	06/08/2019 23:2...	1	15ng
<input checked="" type="checkbox"/>	924	Weizmann LFQ	\\kippymatrix...	GEP1_Spikeln_230914_11_15ng_270914.raw	weizmann.par	ecoli_proteome_...	07/08/2019 00:0...	2	15ng
<input checked="" type="checkbox"/>	924	Weizmann LFQ	\\kippymatrix...	GEP1_Spikeln_230914_12_15ng_270914.raw	weizmann.par	ecoli_proteome_...	07/08/2019 00:4...	3	15ng

Contaminant DB: None | Average [MD] | Settings ... | Save sample map ... | Save quantitation summary ... | Close

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The Sample Map is used to annotate the list of result files with recognisable sample identifiers.

LFQ-merge-yabby-924.txt - Excel

John Cottrell

Family index	Member index	Protein IDs	Peptide counts (all)	Peptide counts (unique)	Fasta headers	Peptide XICs [3ng]	Unique peptide XICs [3ng]	Peptide XICs [7.5ng]	Unique peptide XICs [7.5ng]	Peptide XICs [10ng]	Unique peptide XICs [10ng]	Peptide XICs [15ng]	Unique peptide XICs [15ng]	Mol. weight [kDa]	Sec. structure
2	1	1:3:P06733	37	33	Alpha-enolase OS=Homo sapien	139	119	147	129	141	123	136	117	47481	
3	1	2:3:P13929	7	3	Beta-enolase OS=Homo sapien	21	1	20	2	19	1	22	3	47299	
4	2	1:3:P07900	61	43	Heat shock protein HSP 90-alpha	193	138	185	132	184	132	182	127	85006	
5	2	2:3:P08238	54	34	Heat shock protein HSP 90-beta	182	118	175	113	173	112	172	108	83554	
6	2	3:3:P14625	35	33	Endoplasmic OS=Homo sapien	85	76	84	75	80	71	83	74	92696	
7	2	4:3:Q12931	12	11	Heat shock protein 75 kDa, mit	19	16	19	16	23	19	18	13	80345	
8	3	1:2:P05787	52	44	SWISS-PROT:P05787 Tax_id=966	163	137	159	137	157	132	162	137	53671	
9	3	2:3:P08670	46	42	Vimentin OS=Homo sapiens O	133	121	124	113	125	114	132	120	53676	
10	3	3:2:Q3KNV1	36	2	TREMBL:Q3KNV1:Q96GE1 Tax_id	94	5	87	5	93	2	86	3	51411	
11	3	4:2:P08729	35	1	SWISS-PROT:P08729 Tax_id=966	92	3	82	0	94	3	83	0	51443	
12	3	5:3:K7EPT8	7	4	Glial fibrillary acidic protein (Fr	18	7	19	8	20	9	19	7	8373	
13	3	6:2:Q6NXH9	6	1	TREMBL:Q6NXH9 Tax_id=10090	15	0	13	1	17	0	16	1	59502	
14	3	7:3:K7EPI4	3	1	Glial fibrillary acidic protein (Fr	10	2	10	2	9	0	9	0	14086	
15	3	8:2:Q5XKES	6	2	SWISS-PROT:Q5XKES Tax_id=96	13	1	10	2	13	2	11	1	58059	
16	3	9:2:Q01546	5	1	SWISS-PROT:Q01546 Tax_id=96	12	0	10	1	10	0	10	0	66400	
17	4	1:3:P21333	78	72	Filamin-A OS=Homo sapiens O	189	177	187	174	190	180	192	183	283301	
18	4	2:3:O75369-8	83	77	Isoform 8 of Filamin-B OS=Hom	158	146	158	145	167	157	157	148	283626	
19	5	1:3:P13639	61	60	Elongation factor 2 OS=Homo s	163	163	170	169	169	168	167	166	96246	

LFQ-merge-yabby-924

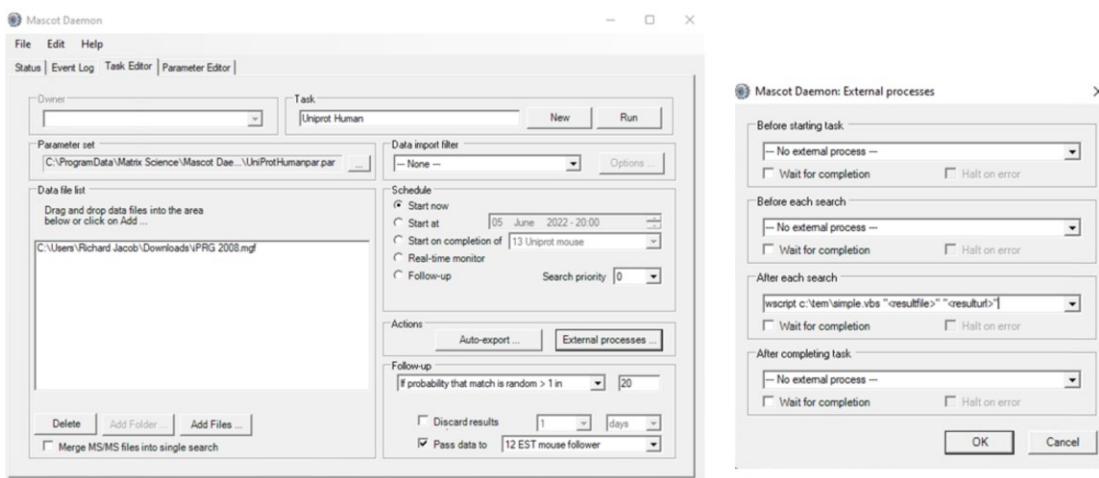
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And Daemon creates and saves the Quantitation Summary, a tab delimited text file which is the input file for statistical analysis. This can then be used with anything from Excel to Perseus or a programming environment like R.

There is more about this in the Quantitation talk.

External Processes



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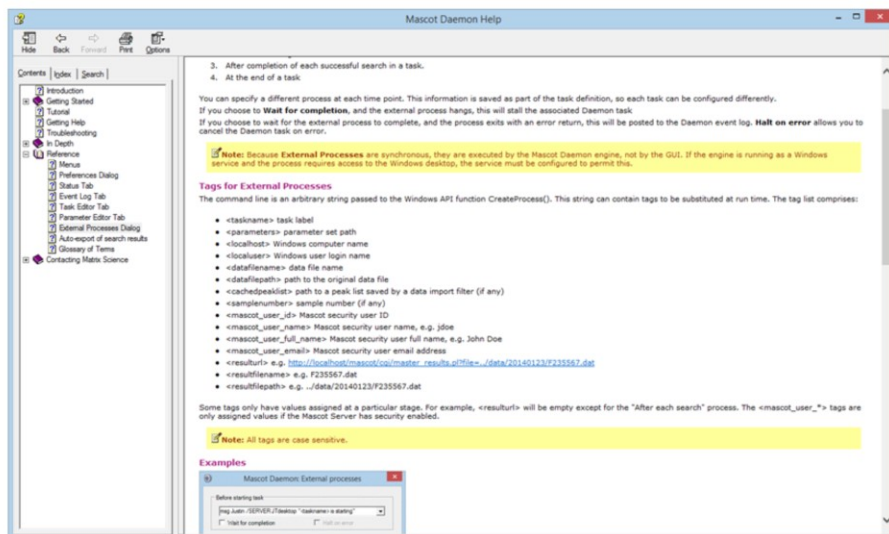
The task editor tab includes a button labelled 'External processes'. A Mascot Daemon task can execute external processes at one or more of the following time points:

- At the start of a task
- Before submitting each search in a task
- After completion of each successful search in a task.
- At the end of a task

A process is a one line command. This could execute a Windows program, or it could be a batch file or script to perform some more complex procedure. You can pass arguments to the commands by including tokens, such as `<taskname>`.

If an export is selected and the dialog closed, the label on the button uses a bold italic font.

External Processes



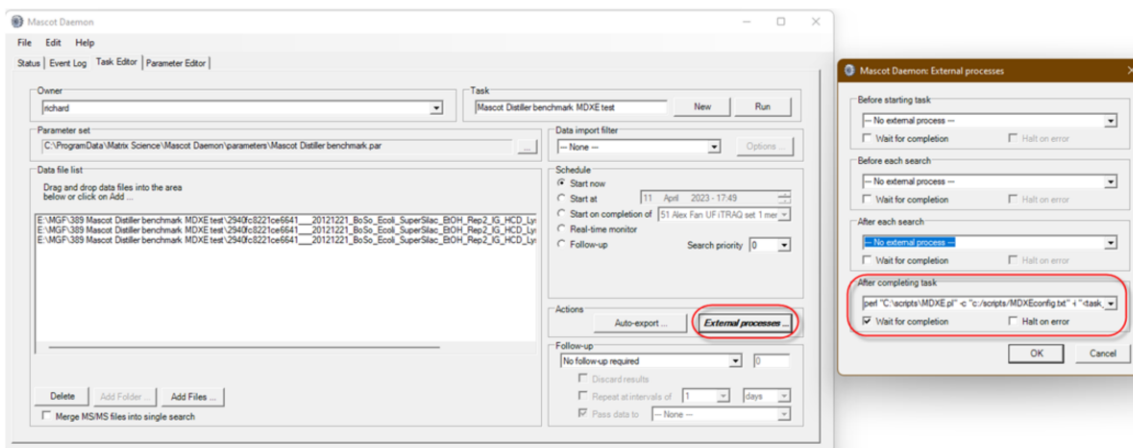
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The help file contains a list of all the available tokens. It also gives examples of how to use External processes, such as a script to build an HTML page in real-time that contains hyperlinks to the search results.

Mascot Daemon Export Extender

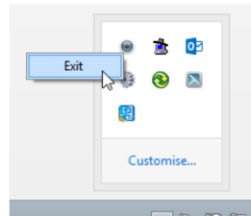


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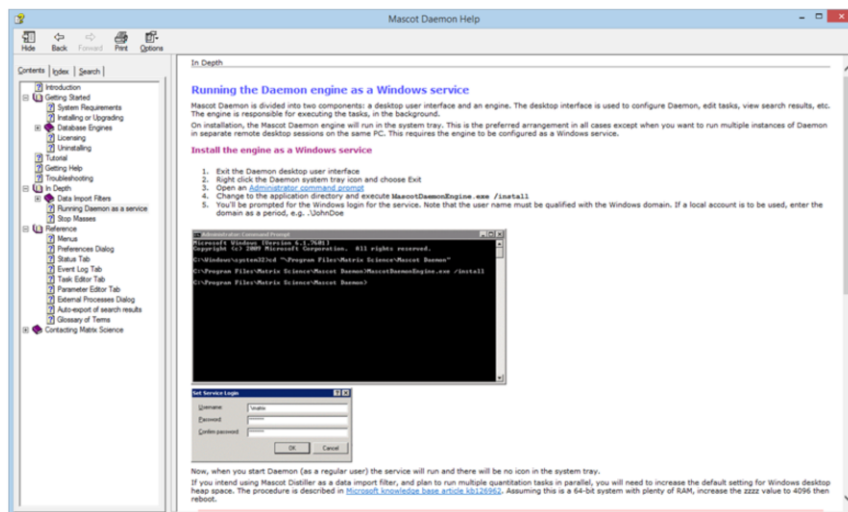
The only real limitation for External Processes settings is that you can only call one external task. However, we have an easy to use helper script that allows you to call multiple processes in one go. Mascot Daemon eXport Extender (MDXE) will process all the files in the batch and can create multiple reports in one call. We tried to build a bit more flexibility into the script so that it can call any command line program. There are example command lines to export Mascot Distiller quantitation reports automatically. More information of its usage can be found at <https://www.matrixscience.com/blog/mascot-daemon-export-extender.html>

Mascot Daemon Engine



Mascot Daemon is divided into two components: a Graphical User Interface (GUI) and an engine. We have been looking at the GUI, which runs on the desktop and is used to configure Daemon, edit tasks, view search results, etc. The engine is responsible for executing the tasks, in the background, whether the GUI is running or not. If something goes horribly wrong, you may need to stop and start the engine to clear the problem. Exit Daemon on the desktop then locate the Daemon icon in the system tray, right click it, and choose exit.

Mascot Daemon Engine



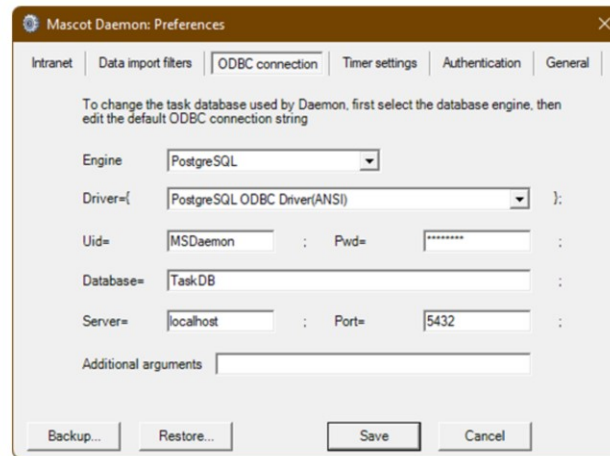
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Running the engine in the system tray is the preferred arrangement in all cases except when you want to run multiple instances of Daemon in separate remote desktop sessions on the same PC. This requires the engine to be installed as a Windows service. You'll find full instructions in the help.

The Mascot Daemon Database



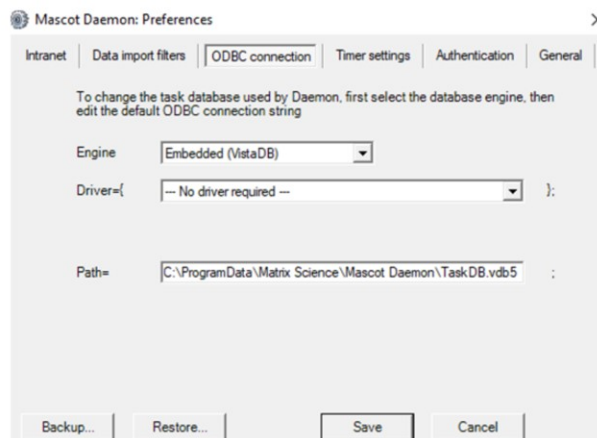
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Under the hood, Mascot Daemon stores information in a relational database. The installation default is to use an embedded database engine called VistaDB. This is the recommended choice unless you wish to share a single task database between multiple instances of Daemon running on different PCs, which requires a high performance database engine. You can choose from Microsoft SQL Server, mySQL, Oracle, and PostgreSQL. These are configured on the ODBC connection tab in Daemon preferences. When multiple instance of Mascot Daemon share the same task database, each task is "owned" by the instance that created it. You can only Pause, Resume, Cancel, or Delete your own tasks. However, any instance can Clone any task and can transfer data files to any follow-up task, either by dragging and dropping the result node or by specifying the task in follow-up criteria.

Backing up and restoring the database



Backup saved to C:\ProgramData\Matrix Science\Mascot Daemon

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Mascot Daemon includes a backup and restore feature for the Task Database. It works for all database formats and is often a simpler option when using an SQL database.

Press the back up button to save an XML dump of the database to the “C:\ProgramData\Matrix Science\Mascot Daemon” directory. We recommend copying the back up files to a different disk so they are not lost if the C disk fails.

To restore the database click on the Restore button and navigate to the directory where the backups are stored in the file dialog and select the xml file you wish to restore.