READ ME FIRST!

Please read the notes for specific Windows platforms starting on page 3 before following the installation instructions in Chapter 3 of the Installation and Setup Manual. A complete, up-to-date copy of the Installation and Setup Manual can be found on the Mascot installation CD, (*manual.pdf*).

New: Quantitation

Mascot 2.2 contains all of the infrastructure needed for a wide variety of quantitation experiments.

New: Configuration Editor

Web browser based configuration editor can be used to view and edit `quantitation.xml`, `unimod.xml`, and the other Mascot configurations files.

New: Automatic Error Tolerant Searches

New: Automatic Decoy Database Searches

And lots more

- Mass values are now selected, iteratively, by peak intensity for PMF searches.
- A new search parameter to handle cases where the 13C peak was picked without having to open out the mass tolerance.
- A new utility, TS2Mascot, for importing peak lists from an Applied Biosystems 4000 series databases.
- Mascot Daemon has a reduced memory footprint and can now handle raw files and peak lists larger than 2 GB.

Changes since last release

Details of individual changes since the previous major release of Mascot are listed on page 8 of these notes.

Last minute additions to documentation

See page 2

Last minute additions to documentation

- The installation program creates a file containing configuration settings for Apache 2.x web server. If you use Apache, ensure that ForkForUnixApache in the Options section of mascot.dat is set to 1

- On page 34 of the Installation and Setup Manual, the IIS web server configuration page has a checkbox labelled 'Use SSL/TLS to access this web site'. This checkbox has since been removed. If you want to run Mascot on a secure (https:) IIS server, you must configure IIS for non-SSL (http:) operation, perform the Mascot installation, then switch back to secure mode.

- When performing an upgrade, any text files that have been modified since the last installation are copied to a directory called _install_backup. This includes configuration files, Perl scripts, and HTML pages.

- Setup.exe is the Mascot installation program for 32 bit (x86) Windows platforms. Setup64 exe is for 64 bit (x64) Windows platforms. Similarly, there are two installers for ActivePerl, one with x86 in the filename, for 32 bit Windows, and one with x64 in the filename, for 64 bit Windows.

- MSDB has become too large to fit onto the installation CD, and the installer now uses SwissProt as a test database. Additional sequence databases, including MSDB, are supplied on a separate DVD.

- Some third party applications require helper scripts to be installed on the Mascot web server. If Mascot security is enabled, you should be aware that such scripts may create security holes.

- Some compressed sequence database files are now larger than 2 GB. Even with the latest release of wget, (1.10.2), there are bugs that interfere with Time-Stamping. Probably best to update very large databases by hand until these issues are resolved.

- If your web browser is Microsoft Internet Explorer 7, you may find it very difficult to distinguish between normal and bold face type in a Mascot result report. This is a side effect of Clear Type. To fix, in Internet Explorer, click the Tools button, and then click Internet Options. Click the Advanced tab, scroll to the Multimedia section, clear the Always use Clear Type for HTML check box, and then click OK. The changes will take effect the next time you start Internet Explorer.

- If you are using Applied Biosystems GPS Explorer to perform iTRAQ or ICAT quantitation, the modification names for the tags must be the ones defined within GPS, e.g. Lysine(K)_iTRAQ. This is a problem, because Mascot now uses the PSI standard names defined in Unimod. A new configuration file, called mod_aliases, in the Mascot config directory, can be used to "alias" the old GPS names to the new PSI standard names. On installation, the entries in mod_aliases are commented out. To enable this feature, edit the file in a text editor to remove the comments and add any additional aliases that you require. Simply follow the link on the Windows Start menu (Programs; Mascot; config; Mascot mod_aliases file).

Notes for specific Windows Platforms

Windows NT4

Microsoft ceased to support Windows NT4 in 2004. Mascot 2.2 will be the last Mascot release to support NT4. Now would be a very good time to upgrade to a more recent version of Windows.

If you wish to continue with NT4, the new Mascot installer requires Windows Installer 3, which is not available for Windows NT4. Please email support@matrixscience.com to request a CD with the old installer.

To upgrade Mascot using the old installer

1. Service pack 6 or 6a must be installed

2. If your version of Perl is less than 5.6, it must be upgraded. Uninstall Perl, then install Perl 5.8 from the Mascot CD. More details on page 29 of the manual. You may need to update Windows Installer. To do so, change to the unpacked\html\downloads directory on the Mascot CD, and execute the file InstMsiW.exe

3. Ensure that any other users are aware that they will not be able to perform searches while the upgrade is taking place.

4. Check that you are logged in with administrator privileges, and execute setup.exe on the Mascot CD

5. If anything on the system information page is incorrect, refer to the troubleshooting section of the manual, beginning page 44.
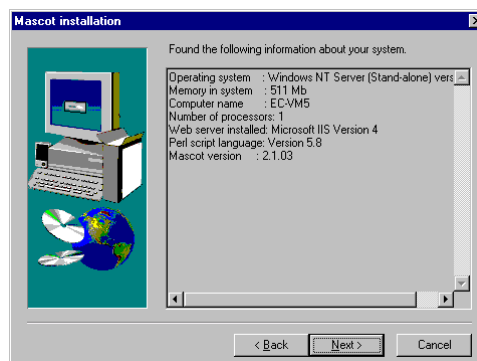
6. Choose to upgrade unless the previous version was not working properly, or if you want to install Mascot onto a new drive, or install it into a new Web Server. Note that it is not possible to run both the old and the new version of Mascot on the same system.
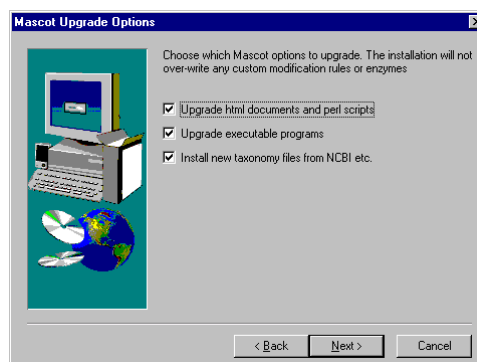
7. You should leave all options selected unless you understand the system very well, and have customised some Perl scripts or HTML pages. Any additional enzymes that you have added will be unaltered.
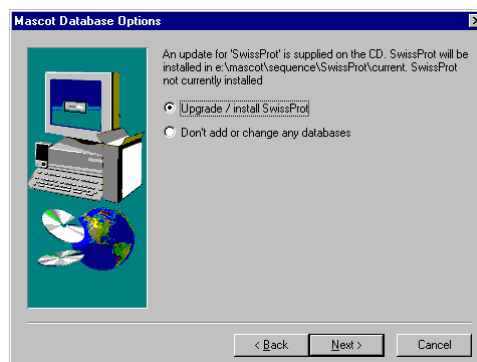
8. Choose the appropriate option for sequence database installation.

9. In Mascot 2.2, modifications are taken from a new XML file, unimod.xml. Your existing mod_file will be backed up. You will need to add any custom modifications that were in your mod_file to unimod.xml after the installation has completed using the new, browser-based configuration editor.

NOTE: It is not always possible for the installation program to update your own additions and changes in mascot.dat. It is a good idea to compare your mascot.dat file with the latest template file, not.mascot.dat, to judge whether further changes should be made. Mascot.dat entries are fully described in Chapter 6 of the Setup and Installation manual.

Windows 2000

Service pack 4 must be installed

A clean installation of Windows 2000 SP4 includes Windows Installer 2. It is very likely that the installer will already have been upgraded to version 3 in the course of installing other software packages. If not, you will see a message when you
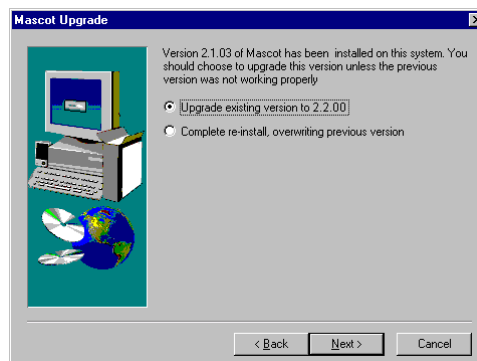
try to install Mascot stating that the installer must be upgraded. For Windows Installer 3.x download details, go to http://support.microsoft.com/?kbid=893803

Windows XP

The latest service pack should be installed

64 bit support

XP Professional x64 edition uses IIS 6.0. See the notes about this version of IIS under Windows Server 2003, below.

There are no repositories for 64 bit ActiveState Perl modules. If you plan to use Perl for applications besides Mascot, you may find it more flexible to install 32 bit Perl.

To install Mascot on XP Professional x64 edition, execute setup64.exe instead of setup.exe

Windows Firewall

Remember to open port 80 (HTTP) if you want to access the Mascot server from other computers

Windows Server 2003

The latest service pack should be installed

IIS 6.0

If a search is submitted from a browser and the connection is broken before the search is complete, the search will be killed. The only known workaround is to use Apache 2.x as your web server.

IIS 6 does not serve files with unknown MIME types. Its default list of MIME types does not include XML schema documents. See Microsoft Knowledge Base article Q326965 for the procedure to add *.XSD to the IIS 6 list of MIME types
http://support.microsoft.com/default.aspx?scid=kb;en-us;326965

64 bit support

There are no repositories for 64 bit ActiveState Perl modules. If you plan to use Perl for applications besides Mascot, you may find it more flexible to install 32 bit Perl.

To install Mascot on a Server 2003 x64 platform, execute setup64.exe instead of setup.exe.

Windows Firewall

Remember to open port 80 (HTTP) if you want to access the Mascot server from other computers

Windows Vista

IIS 7.0

A default installation of IIS 7.0 is not sufficient for running a CGI application such as Mascot. From the Control Panel, choose 'Programs and Features'. Choose 'Turn Windows features on or off'. Expand the node for Internet Information Services and ensure that the checkboxes shown below are checked, in addition to any default selections. Then, choose OK.
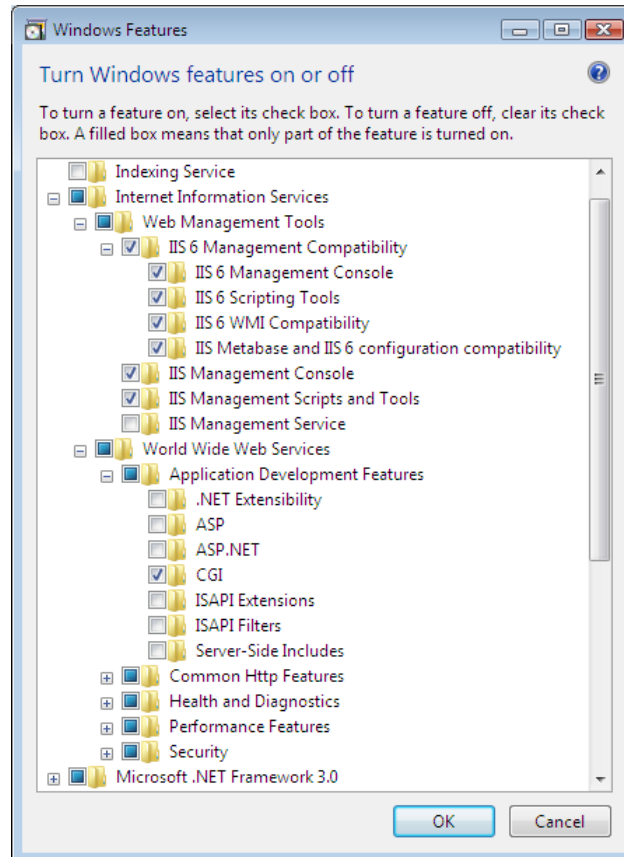
With IIS 7.0, there will be no progress reports during a search unless you edit C:\Windows\system32\inetsrv\config\ApplicationHost.config.

In a text editor, find

    <location path="Default Web Site">

then find

```
<add name="CGI-exe" path="*.exe" verb="*"
modules="CgiModule" resourceType="File"
requireAccess="Execute" allowPathInfo="true" />
```

add responseBufferLimit="0", so that it becomes

```
<add name="CGI-exe" path="*.exe" verb="*"
modules="CgiModule" resourceType="File"
requireAccess="Execute" allowPathInfo="true"
responseBufferLimit="0" />
```

Apache

Mascot 2.2 has been tested with Apache 2.2.4 on Vista. It is not known whether earlier releases of Apache are also compatible with Vista. The procedure is as follows:

1. Download the latest Apache MSI installer from http://httpd.apache.org/download.cgi. You must install Apache as Administrator. It is not sufficient to install as a user with administrator privileges. The easiest way to do this is to open a command window by right clicking the start menu item (under accessories) and choosing 'Run as administrator'. Verify that the title bar says 'Administrator: Command prompt'. Execute the installer by typing the filename in this command prompt and pressing return. Accept all defaults.

2. At the end of the installation, you will be prompted by Windows firewall. Choose to unblock port 80 (HTTP). Don't be concerned if you see an empty error message at the end of the installation. This is because the installer is unable to start the Apache service.

3. Install ActiveState Perl from the Mascot installation CD. Accept all defaults

4. Install Mascot

5. Copy the Apache configuration settings from the httpd.conf file created in the Mascot config directory and paste them at the end of the Apache httpd.conf file, (in C:\Program Files\Apache Software Foundation\Apache2.2\conf). Save the changes.

6. In Windows Explorer, go to the Apache bin directory, (C:\Program Files\Apache Software Foundation\Apache2.2\bin). Right click ApacheMonitor.exe and choose properties. On the compatibility tab, check 'Run this program in compatibility mode for' with 'Windows XP SP2' in the drop-down list, then choose OK.

7. Re-start Vista

64 bit support

There are no repositories for 64 bit ActiveState Perl modules. If you plan to use Perl for applications besides Mascot, you may find it more flexible to install 32 bit Perl.

To install Mascot on a 64-bit Vista platform, execute setup64.exe instead of setup.exe.

Windows Firewall

Remember to open port 80 (HTTP) if you want to access the Mascot server from other computers

Changes since Version 2.1.04

ID	Title
21	select mass values in a peptide mass fingerprint by intensity
335	Report should display errors in the units chosen in the search form
1244	Support for metabolic labeling, e.g. N15
2045	Exclusive (binary) modifications. E.g.ICAT where all Cys in one peptide are heavy or all light.
2046	Limit number of variable mods on a single peptide
2047	Report emPAI Quantitation
2057	Help text needs to describe expectation value threshold
1504	Add table of links to Peptide View report to display reports for other matches
1752	Warning for 'impossible' tags
1777	db_gui.pl doesn't allow multiple wildcards
1803	Database maintenance utility should check limits: 1. Database name > 19 chars 2. Parse rule > 128 chars 3. Any Path > 256 chars 4. Number of threads > 1024
1822	Integrated (automatic) error tolerant search
1828	Add a note about MaxAccessionLen to the varsplic help page
1911	Add a help pages for quantitation
1975	Automatic decoy database search
2035	Would like a "Spoof as " drop down list in the search form for user names and emails
2038	Would like option to export the protein sequence in export utility
2039	Show the precursor neutral loss peak in Peptide View
2061	MudPIT switch point should be ratio of queries to entries, not a fixed number of queries
2070	IT_MODS should be available in local scope of peak list
2078	Daemon fails if peak list > 2GB
2083	Seq tags cannot extend to full length of sequence
2096	Database maintenance test of ref file fails for Celera CDS databases
2098	Help text for error tolerant search needs improving
2109	Give database name with error M422
2111	Mascot Daemon needs to pass through user parameters in follow on searches
2114	Integra Daemon needs sort by options in dialog box to choose search parameter set
2158	Control characters can appear in Mascot email field
2159	Improve memory footprint of Daemon. Need to make intermediate field into disk file
2160	Limit to 4 cores for licensing.
2161	Database maintenance should recommend num threads based on HT or dual core
2162	Change case of database name, db_gui.pl doesn't realise need new _SEQ
2163	In cluster mode, spurious error message about changed service binary file location for service MascotNodeService at each startup. (Case sensitivity problem)
2164	Want option to export the query title from export_dat.pl

2166	xml output from ms-getseq to speed up retrieval of batches of sequences
2167	xml output for ms-gettaxonomy to speed up retrieval of batches of queries
2169	ms-monitor.exe exits with error M00012 if problem with database file permissions
2170	If "ACCESSION" field is specified and the accession is a secondary accession for an entry in db, then it will never be found
2171	embedded user parameters are not handled properly in cluster mode
2116	Support for ETD/CID mode by allowing allow INSTRUMENT in local scope of spectrum
2121	Modifications syntax needs ResiduesProteinCterm and ResiduesProteinNterm
2125	Select summary – remove redundant protein check boxes.
2127	Specifying y-ions will make repeat search fail
2129	Add modification position to the custom CSV/XML export script
2131	Too easy to overwrite existing definition in database maintenance when trying to add new database
2134	Daemon limited to data files < 2 GB
2141	Remove error message from database maintenance about accession too long
2143	daemon desktop application crashes if try to run task where follow-up task has been deleted
2145	Daemon "repeat at interval" searches not working correctly
2151	Get duplicate key in results file if there are (legitimate) duplicate analyzerType attributes in mzData
2152	Pass through the mzData accession to the results files
2156	Daemon fails to try guest login
2157	Remove Protein Mass (SEG) from the Mascot ms-ms search form
2172	Need to enable multiple mascot executables co-exist in the same environment in cluster mode
2173	Masses close to 16 kDa can cause problems
2175	MGF clarification & documentation on Data Format help page
2176	Misleading error message when using ITOL= in BEGIN IONS
2177	Allow for poor peak detection, picking a C13 peak with accurate mass data (PEP_ISOTOPE_ERROR)
2181	Cluster mode fails to bootstrap if gethostbyname_r fails
2185	To make result file completely self-contained, need to add information for enzyme, taxonomy, quantitation, and modifications
2186	Daemon status tab can display wrong task label
2187	Finer control of parser params for client.pl xmlresults2 output
2188	ShowSubSets should be a number between 0 and 1 which represents the maximum fraction of the peptide matches in the primary hit that can be missing in the sub-set hit. 1 would be equivalent to Show Sub-sets true, 0 would be equivalent to Show Sub-sets false.
2189	If export includes a "masses" section, XML from export utility may not validate against mascot_search_results_1.xsd if there are fixed mods
2190	Problems with conflicts between variable mods and error-tolerant mods
2192	client.pl should return version numbers for individual binaries.
2194	Crash when submitting a fasta file instead of a peak list
2198	ms_getseq post request for NA sequence with showsequence = true and showlen = false fails to translate
2200	add support for quantitation.xml to ms_status.exe for getparams.pl
2201	Support 64 bit Linux on Intel/AMD
2217	Change lcq_dta_shell.pl to have default Min. Scans / Group of 1
2221	Further changes for "BIG" mascot – need mass range limits for internal fragments

2224	master_results.pl needs to use expectation value instead of 1/probability (to avoid underflow)
2225	Store part of event log in Integra version of Task DB so that Integra is guaranteed access to remote Daemon event log
2227	Daemon will mess up Distiller import if scan numbers are not consecutive in data file.
2230	Add second error graph in peptide view for ppm errors
2203	In Daemon, change default setting for "Save Distiller project" to true
2204	Database maintenance utility allows & in database name. Should be illegal
2205	Daemon gives Run-time error '35601' Element not found on start up if there are multiple tasks which have New in the action_request field
2206	If the date directory is created for a task id, then it doesn't have correct rights
2208	Daemon parameters editor tab; instrument combo box not disabled when MS/MS unchecked
2210	MinPepLenInSearch is being incorrectly applied to PMF
2211	Daemon 2.1.04 will not work with Mascot 2.0 (Was OK in 2.1.00)
2212	Help not clear about SCANS and RTINSECONDS (Can only go between BEGIN IONS and END IONS)
2213	File not found error in Daemon if using import filter and task name has trailing space(s)
2214	Add the expectation value to the yellow popup
2215	lcq_dta_shell.pl needs to have security login
2231	getseq.pl should give an error when user doesn't have access to a database
2232	Export script failing to escape double quotes in attributes
2235	ms-getseq and ms-gettaxonomy need detailed error info in xml output
2237	protein masses are large negative numbers for error tolerant search of NA database
2238	Wrong mass values being reported for substitutions found in error tolerant search of NA database
2247	In error tolerant search, need to make it clear in pop-up which matches are ET and which are standard
2248	Status screen reports too many processors!
2249	Add support for minInternalMass, maxInternalMass to peptide_view.pl
2252	XML export should output the number of ions matched - getNumIonsMatched()
2254	Add mzData format as peak list option for Mascot Distiller import filter in Mascot Daemon
2255	gi_taxid_nucl.dmp is too large for Linux. Problem is memory fragmentation.
2256	When multiple fixed mods are specified for same residue or terminus, this should be a fatal error. At the moment, the duplicates are converted to variable
2257	Add z+2 ions for ETD
2259	Items missing from export_dat.pl output: QUANTITATION, query_intensity, query_charge, all peptide matches for a query. At query level: RTINSECONDS, SCANS, TOL, TOLU, IT_MODS, INSTRUMENT
2261	Small ions score discrepency in error tolerant
2264	Bug 1495 is back. If one mod name is a sub-string of another, it is ignored.
2266	Daemon spelling mistake: monisotopic in mascot daemon (parameters page)
2268	Cannot use the -f option or move the data directory if the input file is mzData file
2273	Mods string will use characters A-W to support more than 9 mods
2276	ms-monitor exits without error with:SubClusterSet 7 14
2279	add support for unimod_2.xml to ms-status.exe for getparams.pl
2280	Note in Daemon help about mascot.dll memory leak

2281	ms-monitor should create mod_file, substitutions, and masses for third party apps
2282	implement multiple NL and pep NL for exclusive modification groups (even though they are otherwise treated as choice of fixed mods)
2284	Overflow problems with variable mods and mass values over 9865 Da
2288	Cannot POST searches > 2 GB
2289	protein mass is incorrect when using quantitation if there are exclusive mods
2293	Allow the delimiter for CSV export to be changed
2297	Cannot search more than 1000 sequence tags without adding an ms spectrum
2298	Remove error M000345 (pI) from the category mailed to sysadmin
2299	Add support to Daemon for TS2MASCOT
2300	peptides with X at different positions are merged in result file. For example, protein1 with a peptide XABCD with the best ion score on WABCD-substitution and protein2 with WACBX with the best score on WACBD-substitution
2301	browscap.ini updated
2302	extract_msn -P parameter (MS^n) not working in Daemon
2304	For ET search, don't try substitutions for a residue that was an X
2307	File extension of "lastquery.asc.mas" causes weird behaviour on Windows when Access is installed
2308	Mascot Daemon should write its own version number to event log
2309	column alignment problem in CSV export
2312	Misleading error message: M00012 Can't open .unsort for "rb" when there is no peak list
2319	Scripts must use Parser to access config information from mascot.dat, unimod.xml, enzymes, taxonomy, fragmentation_rules.
2320	Can get very low qmatch and hence score threshold due to MinPepLenInSearch
2321	Add section to update script to create decoy database automatically
2322	http://unxutils.sourceforge.net/ is dead, change links to http://gnuwin32.sourceforge.net/packages.html
2327	error tolerant pop-ups broken when choose suppress pop-ups
2328	increase limit on number of parse rules from 64 to 256
2331	We need to make the following parameters configurable through security session object: 1) maxnumber of variable mods in a standard search 2) maxnumber of variable mods in ET search (old and new) 3) maxnumber of accession strings for old type ET search
2334	SECTASK_MAXJOBPRIORITY not being used anywhere
2336	Cross-site scripting vulnerability. It is possible to inject rogue scripting code into a submitted search form
2337	Installer will use SwissProt instead of MSDB
2347	maxnumber of accession strings for old type ET search to be read from security
2355	Export should include flag for whether match would be bold in summary report
2357	export_dat.pl not overriding mascot.dat file when RequireBoldRed=1
2362	Daemon needs to support error tolerant & decoy searches
2367	Remove references to trembl_new
2380	Export for PMF mixture gives invalid csv file
2383	Update Unix installation script to use parser
2398	Improve usage instructions for db_update.pl
2399	ms-monitor passes flags no longer used to load_node.pl