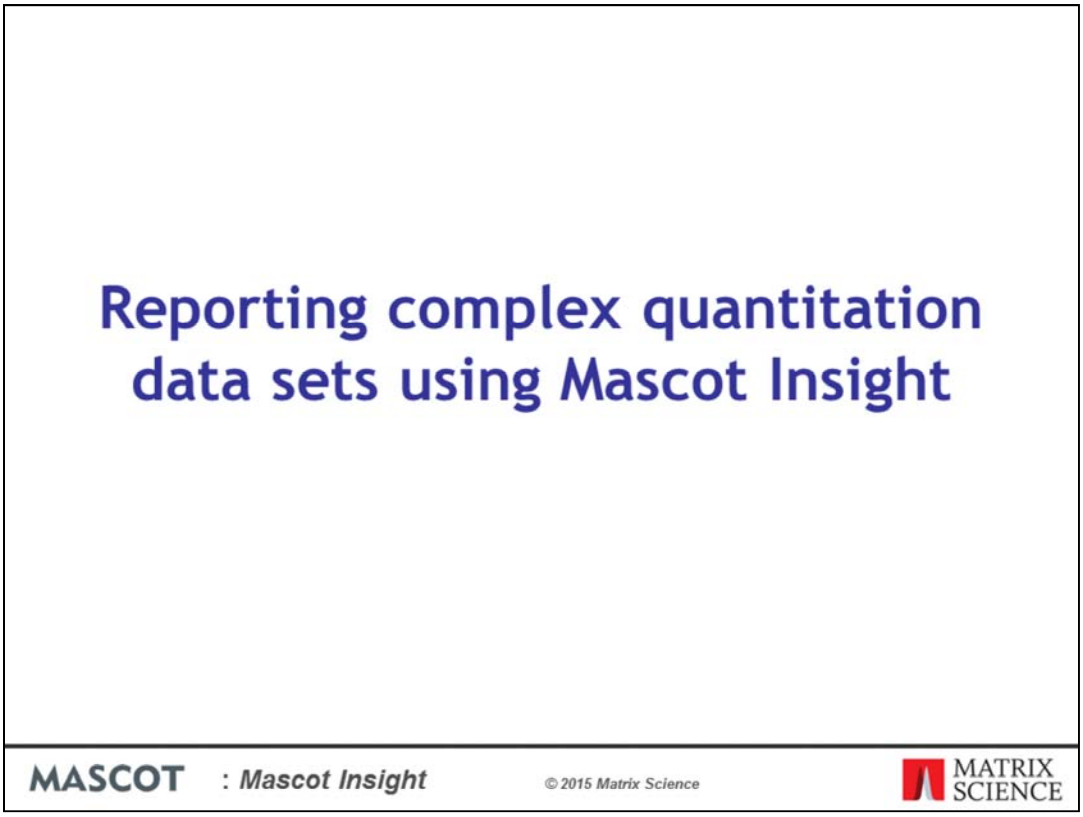
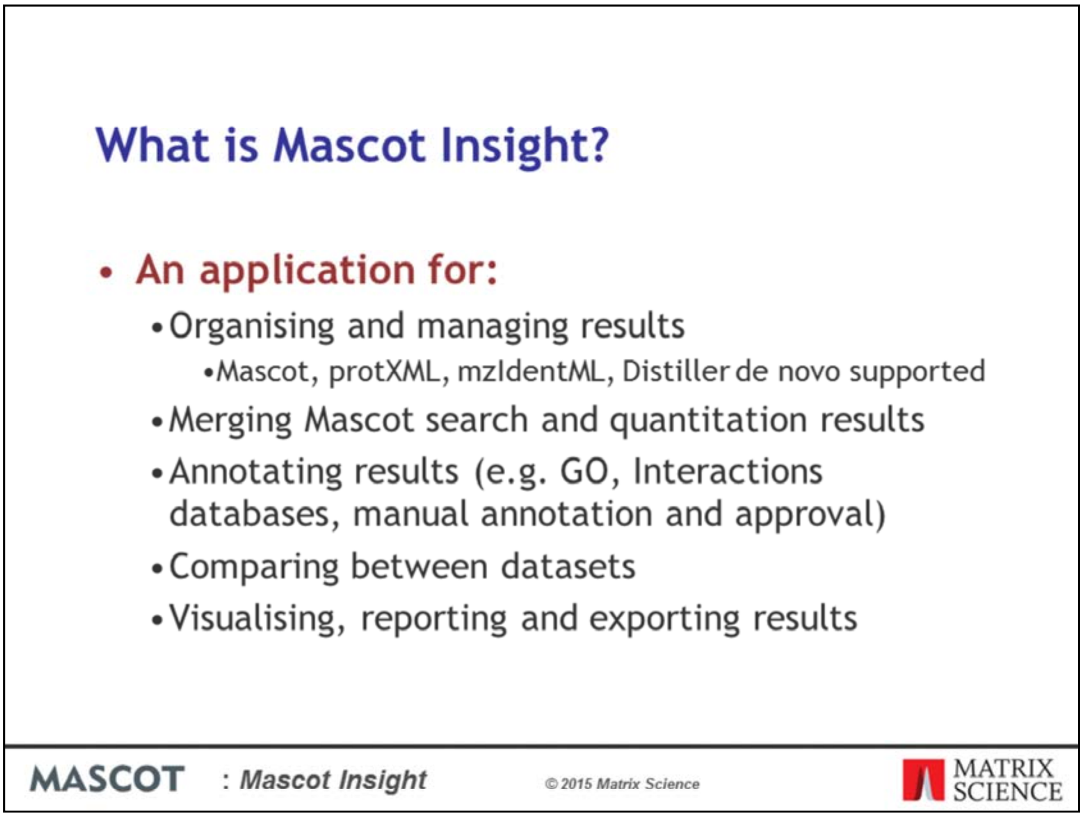


Reporting complex quantitation data sets using Mascot Insight



What is Mascot Insight?

- An application for:
  - Organising and managing results
    - Mascot, protXML, mzIdentML, Distiller de novo supported
  - Merging Mascot search and quantitation results
  - Annotating results (e.g. GO, Interactions databases, manual annotation and approval)
  - Comparing between datasets
  - Visualising, reporting and exporting results

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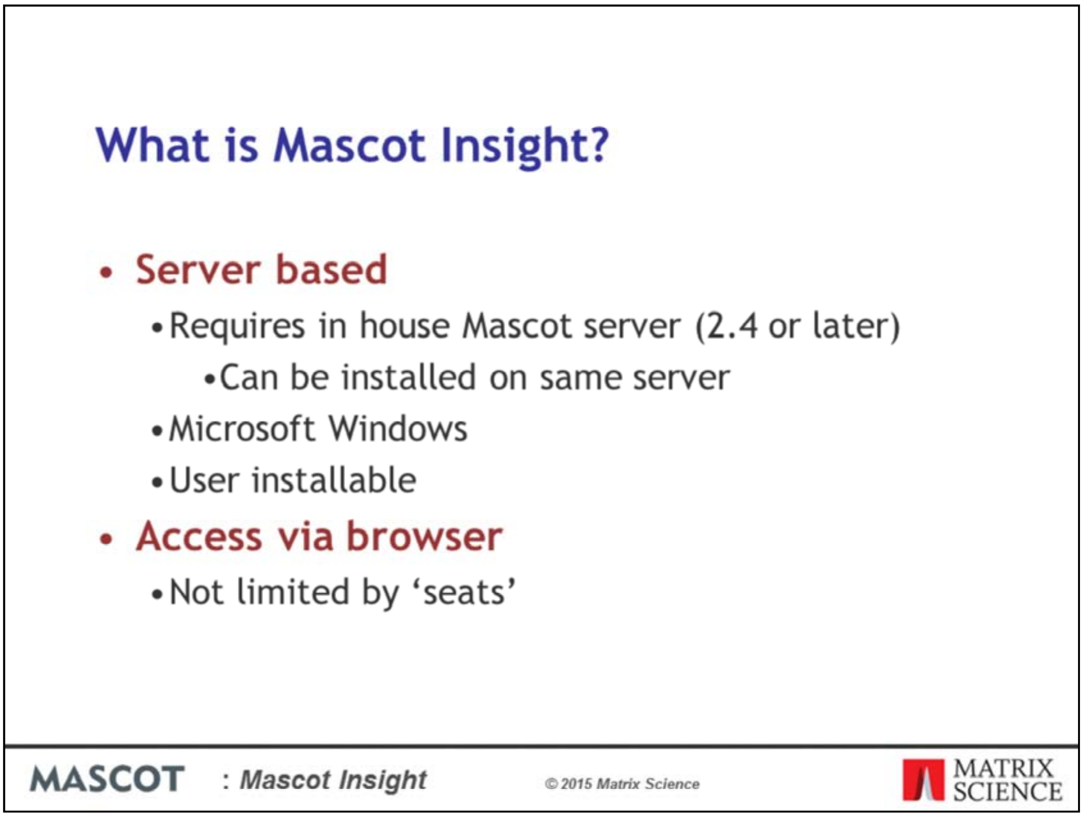
Mascot Insight is a new application designed to help you to organise and manage your Mascot search and quantitation results.

Mascot Insight provides ways to flexibly merge your Mascot search and quantitation results, including MS-1 based quantitation data from Mascot Distiller, such as SILAC and Carbon 13 data, and MS2 based quantitation such as iTRAQ and TMT.

Mascot Insight allows you to further annotate your results, including areas such as Gene Ontology annotation of results, use of molecular interactions databases and manual annotation and approval of protein hits

And Mascot Insight provides a wide range of reports covering areas such as comparing datasets, quantitation analysis, plotting charts etc, and provides exports for these to allow you to easily share those results and to export data in machine readable formats

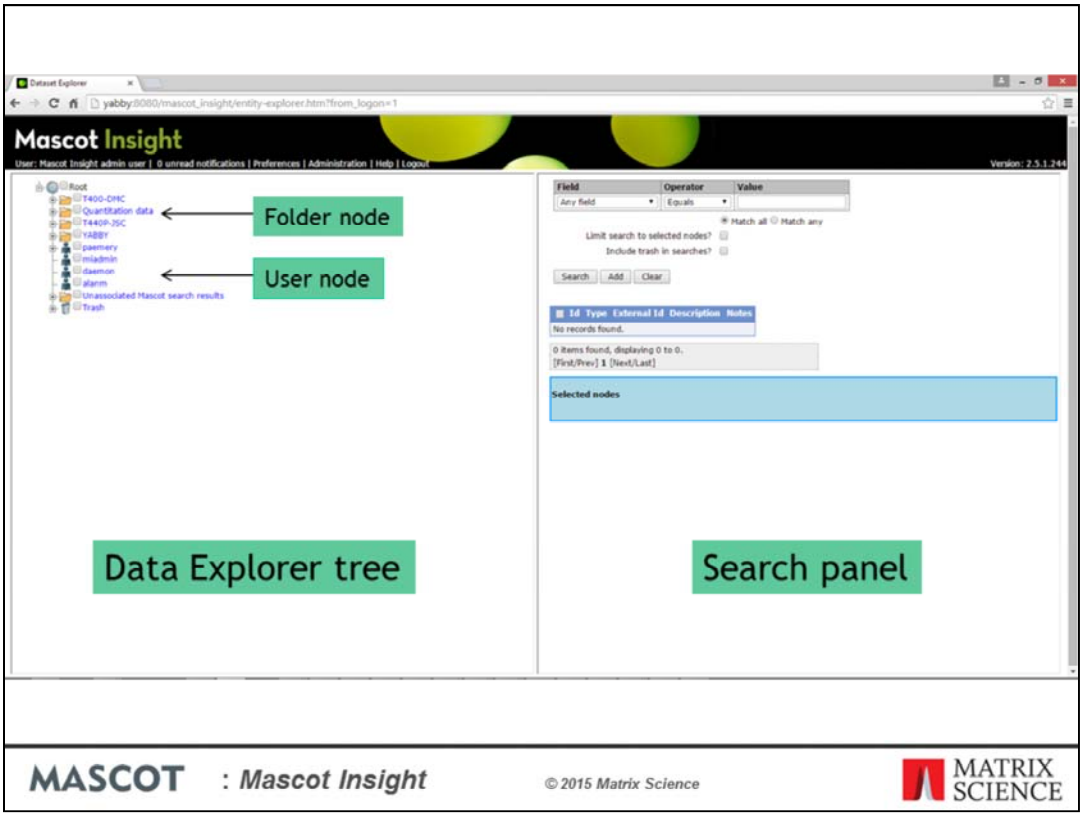


What is Mascot Insight?

- Server based
  - Requires in house Mascot server (2.4 or later)
    - Can be installed on same server
  - Microsoft Windows
  - User installable
- Access via browser
  - Not limited by ‘seats’

MASCOT : *Mascot Insight* *© 2015 Matrix Science* MATRIX SCIENCE

Mascot Insight is a server based system. It requires you have an in house copy of Mascot, version 2.4 or later. It can also be installed on the same system as your Mascot server, provided you have plenty of spare resources and it is a Microsoft Windows server. You access Mascot Insight via a browser and there are no limits on the number of users that can be connected to the system at any one time.



Limit search to selected nodes? □

Include trash in searches? □

No records found.

0 items found, displaying 0 to 0.
[First/Prev] 1 [Next/Last]

Folder node

User node

Data Explorer tree

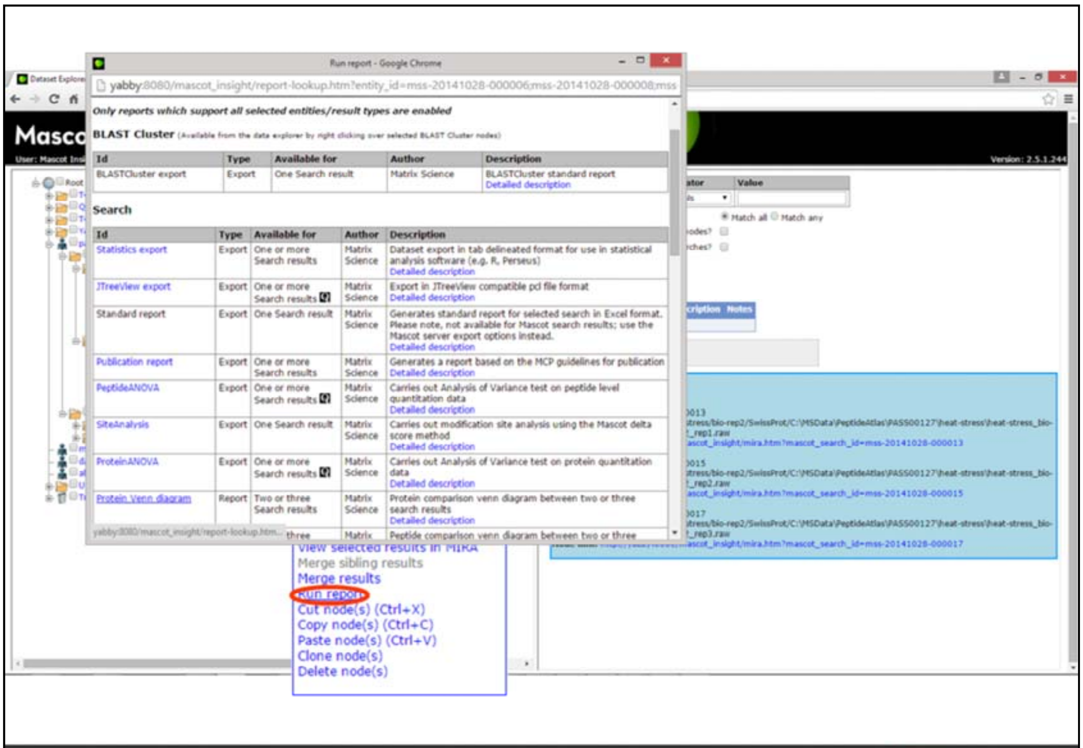
Search panel

MASCOT : *Mascot Insight* *© 2015 Matrix Science* MATRIX SCIENCE

Once you've logged in, this is the home page of the application. On the left we have the Data explorer tree. This is where the data available in the system is organised and displayed, with different types of node for storing different types of information. Data can be manually position on the tree, or structures can be automatically generated to your own specifications

On the current view, we can see two main types of node – User nodes and Folder nodes. Each user is given a User node automatically, and this is for them to organise their own data and experiments, and you can also have shared group nodes.

On the right hand side of the home page, we have the 'Search panel'. The data explorer tree may contain many thousands of search results, and the search panel allows us to rapidly search the tree to find a particular result or set of results. Security is role-based and handled by Mascot security. Exactly what you see on the dataset explorer tree when you log in depends on your user's roles, and on what access settings have been given to particular nodes on the tree by their owners.



Run report - Google Chrome

yabby:8080/mascot_insight/report-lookup.htm?entity_id=mss-20141028-000006;mss-20141028-000008;mss

Only reports which support all selected entities/result types are enabled

BLAST Cluster (Available from the data explorer by right clicking over selected BLAST Cluster nodes)

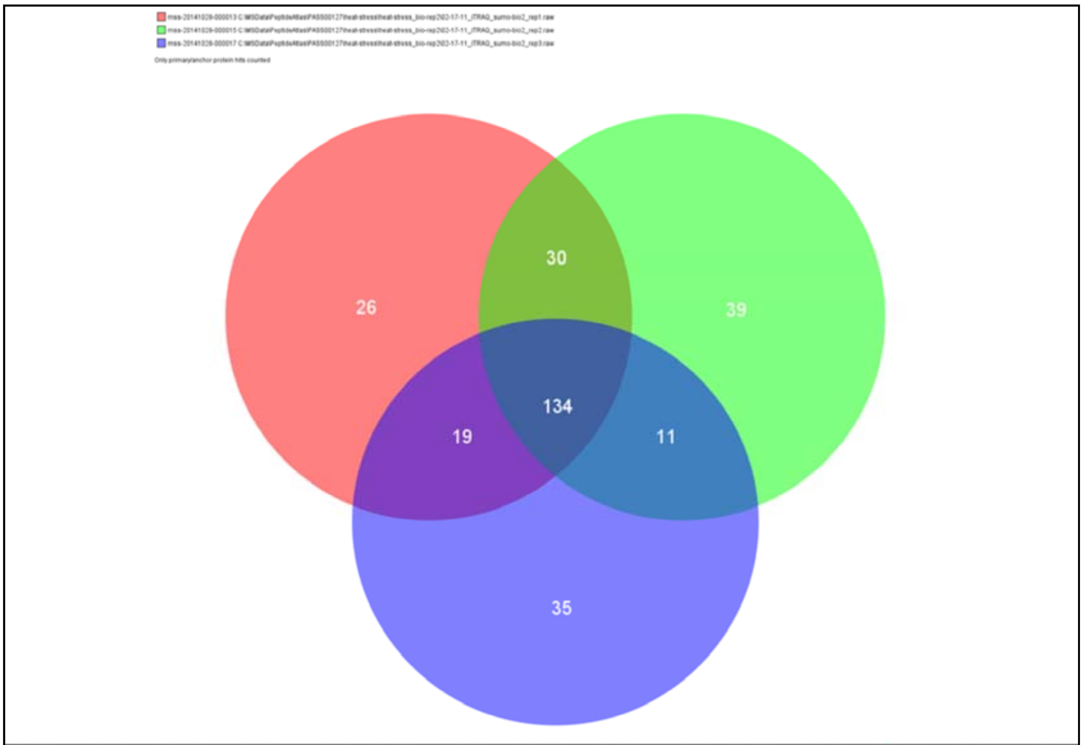
Id	Type	Available for	Author	Description
BLASTCluster export	Export	One Search result	Matrix Science	BLASTCluster standard report Detailed description

Search

Id	Type	Available for	Author	Description
Statistics export	Export	One or more Search results	Matrix Science	Dataset export in tab delineated format for use in statistical analysis software (e.g. R, Perseus) Detailed description
JTreeView export	Export	One or more Search results	Matrix Science	Export in JTreeView compatible pcl file format Detailed description
Standard report	Export	One Search result	Matrix Science	Generates standard report for selected search in Excel format. Please note, not available for Mascot search results; use the Mascot server export options instead. Detailed description
Publication report	Export	One or more Search results	Matrix Science	Generates a report based on the MCP guidelines for publication Detailed description
PeptideANOVA	Export	One or more Search results	Matrix Science	Carries out Analysis of Variance test on peptide level quantitation data Detailed description
SiteAnalysis	Export	One Search result	Matrix Science	Carries out modification site analysis using the Mascot delta score method Detailed description
ProteinANOVA	Export	One or more Search results	Matrix Science	Carries out Analysis of Variance test on protein quantitation data Detailed description
Protein Venn diagram	Report	Two or three Search results	Matrix Science	Protein comparison venn diagram between two or three search results Detailed description
		three	Matrix	Peptide comparison venn diagram between two or three

Here I've expanded out the Data Explorer tree under my user node to show you how it can be used to group data. Under the user node I've got data from two experiments which were examining SUMO interacting proteins in Arabidopsis thaliana after either heat stress or multiple chemical stresses. Each experiment had two biological replicates. The data for each biological replicate was searched first against a contaminants database, and then MS/MS spectra which failed to give a match were automatically researched against SwissProt using a Mascot Daemon follow-up task. Each biological replicate had three technical replicates run. As you can see, everything is logically laid out on the Data Explorer tree, in a structure which was created automatically by the system when the results were imported – I'll be going into more detail about that later in the presentation.

If I want to manipulate a set of results, I do that by selecting the result nodes as I've done here. Right clicking then opens a context menu which allows me to carry out a number of operations, such as viewing the results or changing the default result display conditions. I can also run reports directly from here. As an example of that, we'll generate a three-way Venn diagram of the protein matches from these three technical replicates. Simply click on the 'Run report' menu option and the select the 'Protein Venn Diagram' link on the window that opens.



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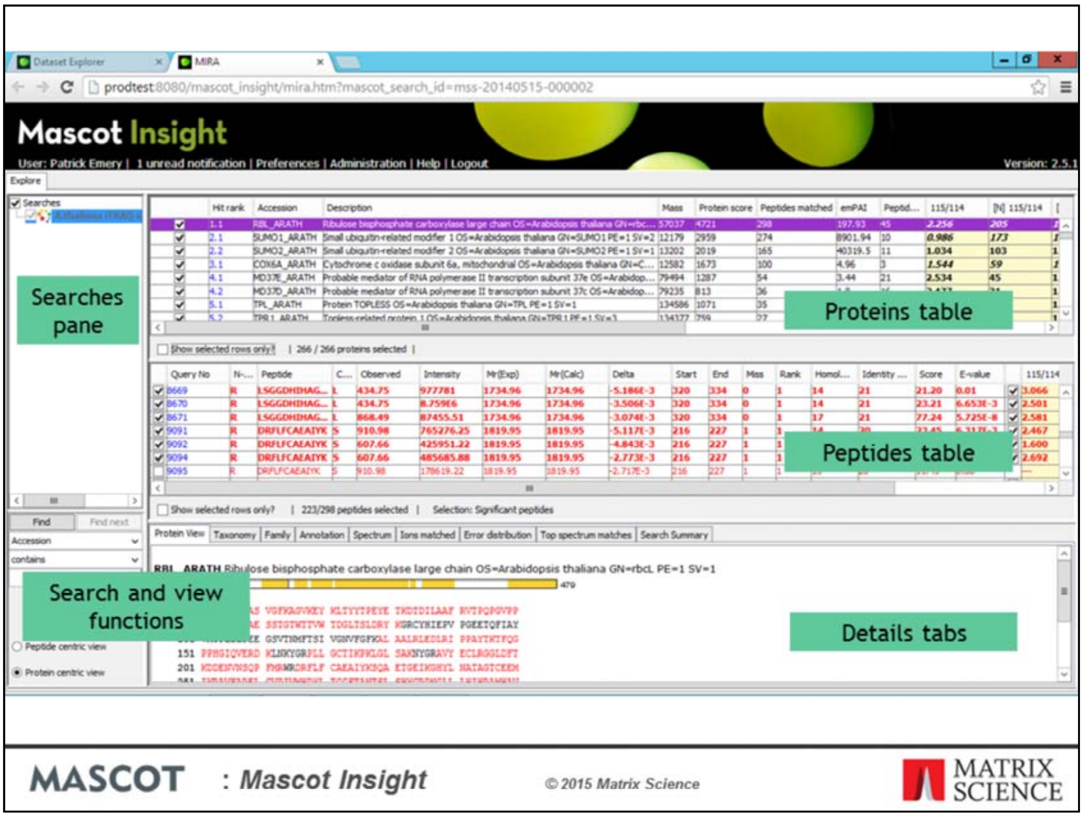
And the system will generate the report.



	Hit rank	Accession	Description	Mass	Protein score	Peptides matched	emPAI	Peptd...	115/114	[N] 115/114
☑	1.1	RBL_ARATH	Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana GN=rbc...	57037	4721	298	197.93	45	2.256	205
☑	2.1	SUMO1_ARATH	Small ubiquitin-related modifier 1 OS=Arabidopsis thaliana GN=SUMO1 PE=1 SV=2	12179	2959	274	8901.94	10	0.986	173
☑	2.2	SUMO2_ARATH	Small ubiquitin-related modifier 2 OS=Arabidopsis thaliana GN=SUMO2 PE=1 SV=1	13202	2019	165	40319.5	11	1.034	103
☑	3.1	COX6A_ARATH	Cytochrome c oxidase subunit 6a, mitochondrial OS=Arabidopsis thaliana GN=C...	12582	1673	100	4.96	3	1.544	59
☑	4.1	MD37E_ARATH	Probable mediator of RNA polymerase II transcription subunit 37e OS=Arabidop...	79494	1287	54	3.44	21	2.534	45
☑	4.2	MD37D_ARATH	Probable mediator of RNA polymerase II transcription subunit 37c OS=Arabidop...	79235	813	36				
☑	5.1	TPL_ARATH	Protein TOPLESS OS=Arabidopsis thaliana GN=TPL PE=1 SV=1	134586	1071	35				
☑	5.2	TPR1_ARATH	Topless-related protein 1 OS=Arabidopsis thaliana GN=TPR1 PE=1 SV=3	134377	759	27				

☐ Show selected rows only? | 266 / 266 proteins selected |

	Query No	N-...	Peptide	C...	Observed	Intensity	Mr(Exp)	Mr(Calc)	Delta	Start	End	Miss	Rank	Homol...	Identity ...	Score	E-value		115/114
☑	8669	R	LSGGDHIHAG...	L	434.75	977781	1734.96	1734.96	-5.186E-3	320	334	0	1	14	21	21.20	0.01	☑	3.066
☑	8670	R	LSGGDHIHAG...	L	434.75	8.759E6	1734.96	1734.96	-3.506E-3	320	334	0	1	14	21	23.21	6.653E-3	☑	2.501
☑	8671	R	LSGGDHIHAG...	L	868.49	87455.51	1734.96	1734.96	-3.074E-3	320	334	0	1	17	21	77.24	5.725E-8	☑	2.581
☑	9091	R	DRFLFCAEAIYK	S	910.98	765276.25	1819.95	1819.95	-5.117E-3	216	227	1	1					☑	2.467
☑	9092	R	DRFLFCAEAIYK	S	607.66	425951.22	1819.95	1819.95	-4.843E-3	216	227	1	1					☑	1.600
☑	9094	R	DRFLFCAEAIYK	S	607.66	485685.88	1819.95	1819.95	-2.773E-3	216	227	1	1					☑	2.692
☐	9095	R	DRFLFCAEAIYK	S	910.98	178619.22	1819.95	1819.95	-2.717E-3	216	227	1	1						—

☐ Show selected rows only? | 223/298 peptides selected | Selection: Significant peptides

RBL_ARATH Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana GN=rbcL PE=1 SV=1

```
    AS VGFKAGVKEY KLTYYTPEYE TKDTDILAAF RVTPQPGVPP
    AE SSTGTWTTVW TDGLTSLDRY KGRCYHIEPV PGEETQFIAY
    ZE GSVTNMFTSI VGNVFGFKAL AALRLEDLRI PPAYTKTFQG
151 PPHGIQVERD KLNKYGRPLL GCTIKPKLGL SAKNYGRAVY ECLRGGLDFT
201 KDDENVNSQP FMRWRDRFLF CAEAIYKSQA ETGEIKGHYL NATAGTCEEM
```

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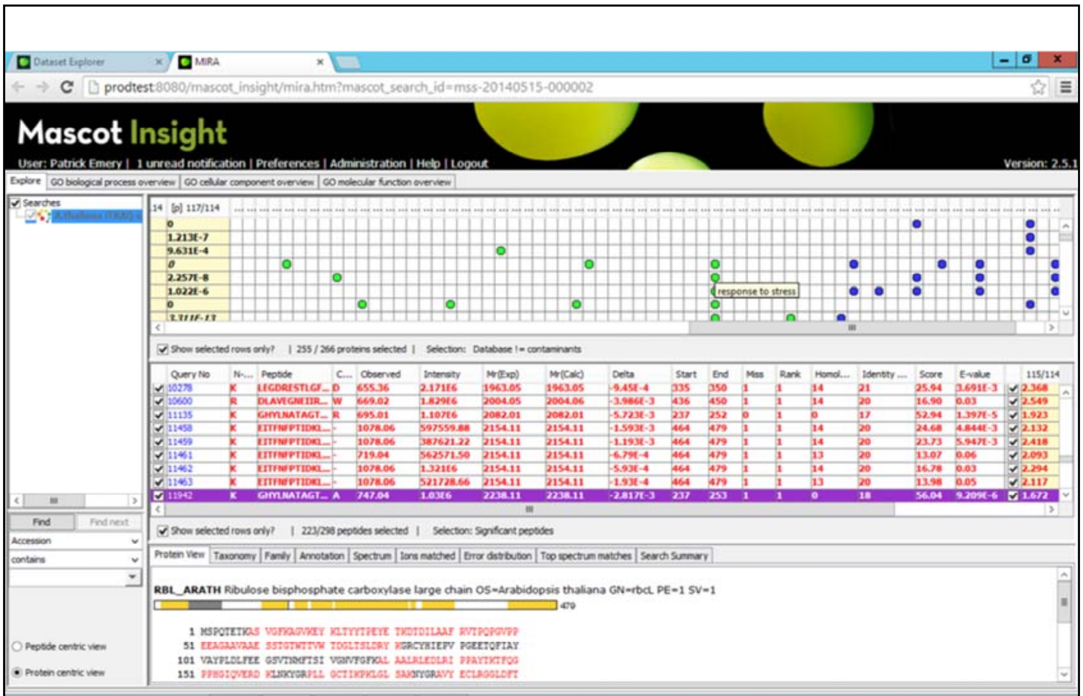
The main tool used to view results and generate reports in Mascot Insight is a Java applet called MIRA (for Mascot Insight Results Applet).

MIRA provides a tabular view of a single result or merged set of results. You can view results in either the Protein centric mode (as we are doing here), or in a peptide centric view. On the upper left we have the searches pane, which is used to change between the selected results, changing which result is being displayed in the main body of MIRA. It is also used for running search level reports from within MIRA and for setting result display parameters across multiple searches. The upper panel on the right contains the proteins table. This is the protein hit list for the currently selected result on the searches pane, and resembles the 'Report builder' view in the standard Mascot protein family report. If we click on a row in the Proteins table:

The peptides table, containing the list of matching peptide hits for the selected protein, and various details tabs (such as a protein view) are populated. Clicking on a table column header for either the proteins or peptides table sorts the table on that field. You can also re-order the columns by dragging them and also show and hide columns as you wish – you can save your default column selection, order and column widths if you wish; your choices are saved to the Mascot Insight database, so are available on any client PC you log in from. In the bottom left of the main pane is a panel with search functions, and options to switch between this view and a peptide (or spectrum) centric view.



☑ Show selected rows only? | 255 / 266 proteins selected | Selection: Database != contaminants

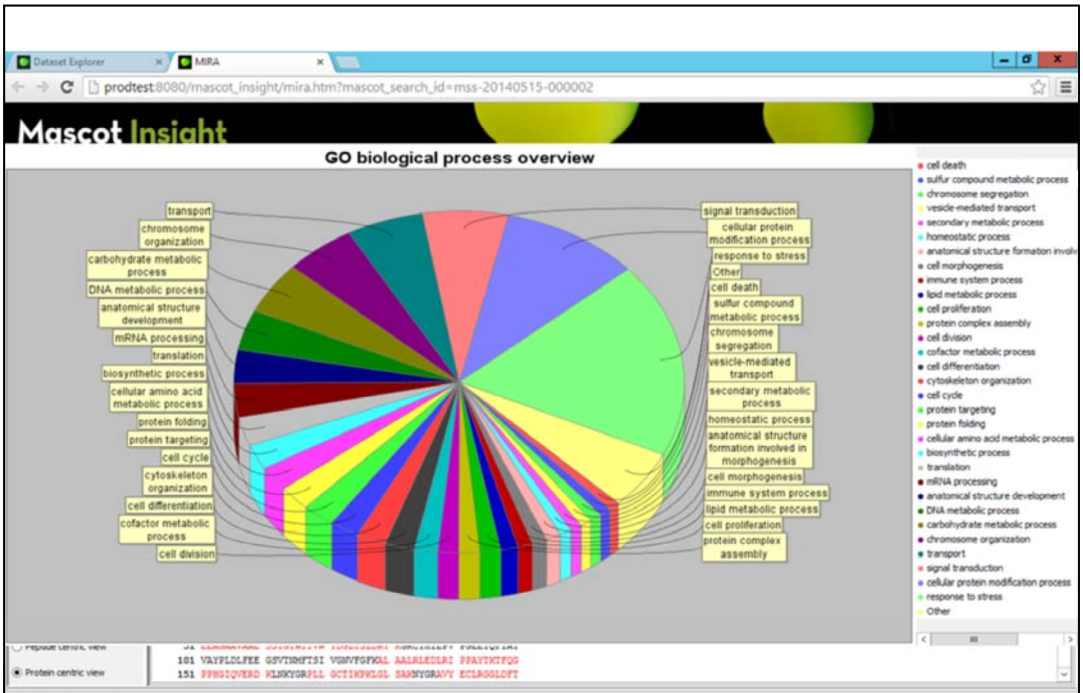
	Query No	N-...	Peptide	C...	Observed	Intensity	Mr(Exp)	Mr(Calc)	Delta	Start	End	Miss	Rank	Homol...	Identity ...	Score	E-value		115/114
☑	10278	K	LEGDRESTLGF...	D	655.36	2.171E6	1963.05	1963.05	-9.45E-4	335	350	1	1	14	21	25.94	3.691E-3	☑	2.368
☑	10600	R	DLAVEGNEIIR...	W	669.02	1.829E6	2004.05	2004.06	-3.986E-3	436	450	1	1	14	20	16.90	0.03	☑	2.549
☑	11135	K	GHYLNATAGT...	R	695.01	1.107E6	2082.01	2082.01	-5.723E-3	237	252	0	1	0	17	52.94	1.397E-5	☑	1.923
☑	11458	K	EITFNFPTIDKL...	-	1078.06	597559.88	2154.11	2154.11	-1.593E-3	464	479	1	1	14	20	24.68	4.844E-3	☑	2.132
☑	11459	K	EITFNFPTIDKL...	-	1078.06	387621.22	2154.11	2154.11	-1.193E-3	464	479	1	1	14	20	23.73	5.947E-3	☑	2.418
☑	11461	K	EITFNFPTIDKL...	-	719.04	562571.50	2154.11	2154.11	-6.79E-4	464	479	1	1	13	20	13.07	0.06	☑	2.093
☑	11462	K	EITFNFPTIDKL...	-	1078.06	1.321E6	2154.11	2154.11	-5.93E-4	464	479	1	1	14	20	16.78	0.03	☑	2.294
☑	11463	K	EITFNFPTIDKL...	-	1078.06	521728.66	2154.11	2154.11	-1.93E-4	464	479	1	1	13	20	13.98	0.05	☑	2.117
☑	11942	K	GHYLNATAGT...	A	747.04	1.03E6	2238.11	2238.11	-2.817E-3	237	253	1	1	0	18	56.04	9.209E-6	☑	1.672

☑ Show selected rows only? | 223/298 peptides selected | Selection: Significant peptides

RBL_ARATH Ribulose bisphosphate carboxylase large chain OS=Arabidopsis thaliana GN=rbcL PE=1 SV=1

```
  1 MSPQTETKAS VGFKAGVKEY KLTYYTPEYE TKDTDILAAF RVTPQPGVPP
 51 EEAGAAVAAE SSTGTWTTVW TDGLTSLDRY KGRCYHIEPV PGEETQFIAY
101 VAYPLDLFEE GSVTNMFTSI VGNVFGFKAL AALRLEDLRI PPAYTKTFQG
151 PPHGIQVERD KLNKYGRPLL GCTIKPKLGL SAKNYGRAVY ECLRGGLDFT
```

You can enable GO Analysis in MIRA. GO annotations are shown in the proteins table, as different coloured dots. Hover over a cell for a tooltip.


Dataset Explorer
MIRA
prodtest:8080/mascot_insight/mira.htm?mascot_search_id=mss-20140515-000002
Mascot Insight
GO biological process overview
transport
chromosome organization
carbohydrate metabolic process
DNA metabolic process
anatomical structure development
mRNA processing
translation
biosynthetic process
cellular amino acid metabolic process
protein folding
protein targeting
cell cycle
cytoskeleton organization
cell differentiation
cofactor metabolic process
cell division
signal transduction
cellular protein modification process
response to stress
Other
cell death
sulfur compound metabolic process
chromosome segregation
vesicle-mediated transport
secondary metabolic process
homeostatic process
anatomical structure formation involved in morphogenesis
cell morphogenesis
immune system process
lipid metabolic process
cell proliferation
protein complex assembly
● cell death
● sulfur compound metabolic process
● chromosome segregation
● vesicle-mediated transport
● secondary metabolic process
● homeostatic process
● anatomical structure formation involv
● cell morphogenesis
● immune system process
● lipid metabolic process
● cell proliferation
● protein complex assembly
● cell division
● cofactor metabolic process
● cell differentiation
● cytoskeleton organization
● cell cycle
● protein targeting
● protein folding
● cellular amino acid metabolic process
● biosynthetic process
● translation
● mRNA processing
● anatomical structure development
● DNA metabolic process
● carbohydrate metabolic process
● chromosome organization
● transport
● signal transduction
● cellular protein modification process
● response to stress
● Other
○ Peptide centric view
● Protein centric view

```
101 VAYPLDLFEE GSVTNMFTSI VGNVFGFKAL AALRLEDLRI PPAYTKTFQG
151 PPHGIQVERD KLNKYGRPLL GCTIKPKLGL SAKNYGRAVY ECLRGGLDFT
```

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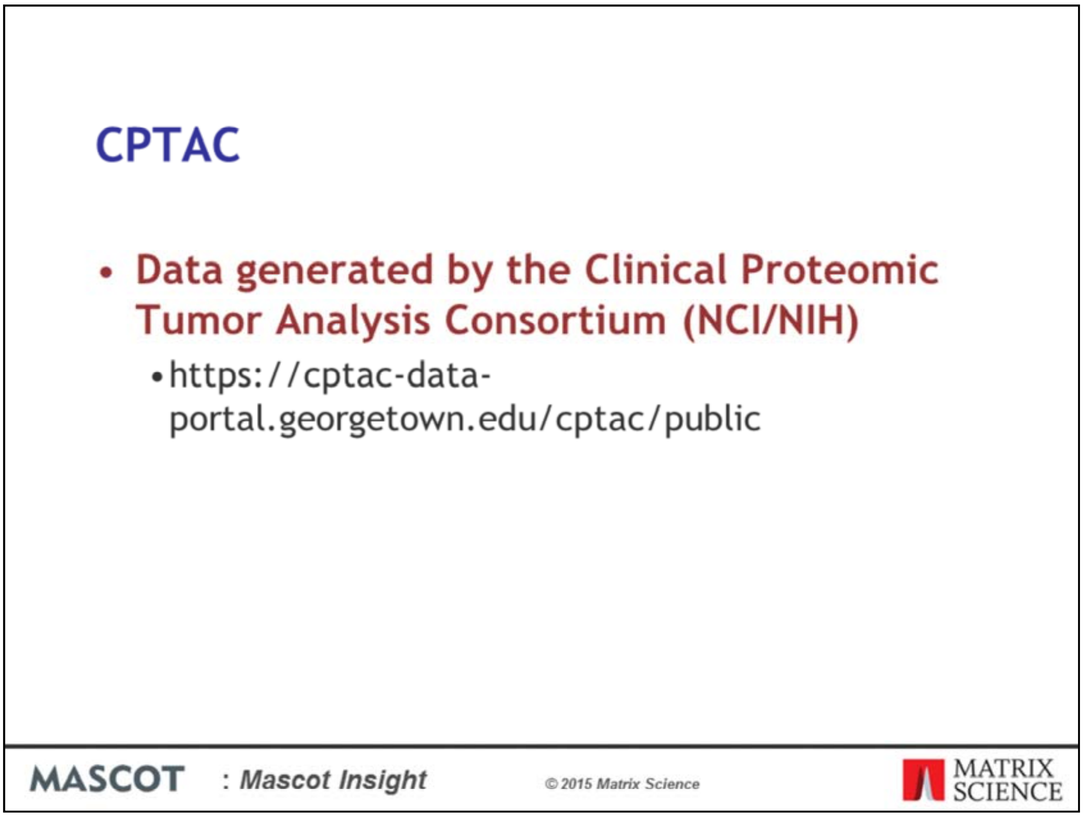
GO assignments are also presented in additional tabs as piecharts. Click on a wedge of the pie-chart, and the protein table is filtered on that GO assignment



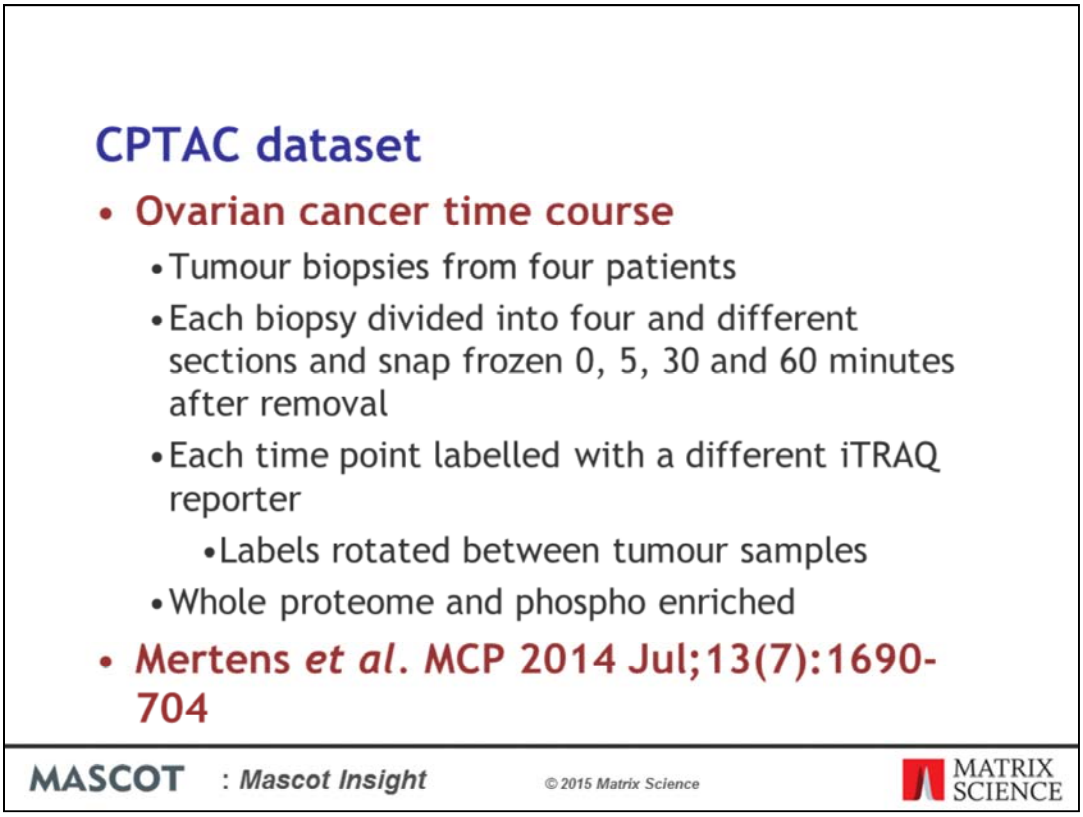
CPTAC

- Data generated by the Clinical Proteomic Tumor Analysis Consortium (NCI/NIH)
  - https://cptac-data-portal.georgetown.edu/cptac/public

MASCOT : *Mascot Insight*

MATRIX SCIENCE

To give you a realistic picture of how Insight can be used to organise and report a complex dataset, I’ve chosen a dataset taken from the NCI/NIH Clinical Proteomic Tumor Analysis Consortium – the data are publically available and can be downloaded from https://cptac-data-portal.georgetown.edu/cptac/public



CPTAC dataset

- Ovarian cancer time course
  - Tumour biopsies from four patients
  - Each biopsy divided into four and different sections and snap frozen 0, 5, 30 and 60 minutes after removal
  - Each time point labelled with a different iTRAQ reporter
    - Labels rotated between tumour samples
  - Whole proteome and phospho enriched
- Mertens *et al*. MCP 2014 Jul;13(7):1690-704

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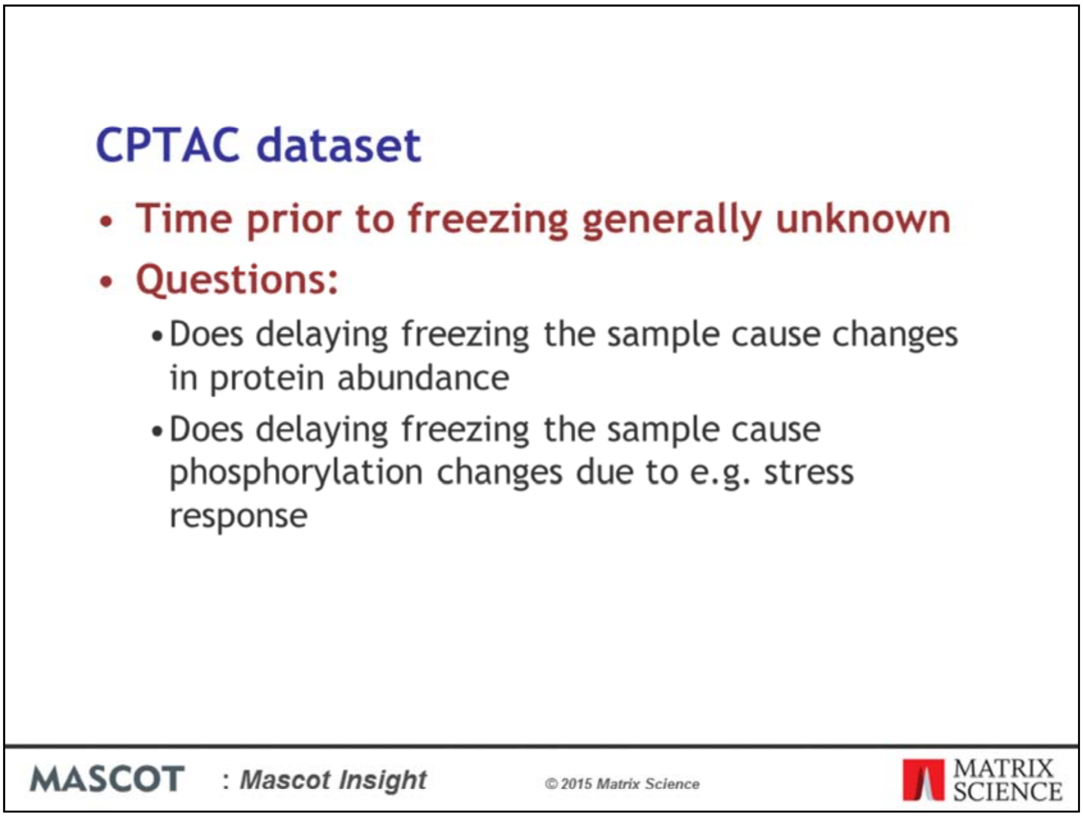
The specific dataset used in an Ovarian cancer time course, which is a subset of the data used in the Mertens *et al.* MCP 2014 Jul;13(7):1690-704 paper. In this case we're looking at four biological replicates from four patients. For a really well designed experiment you'll also have technical replicates of course – if you go to the CPTAC website you'll find data files and studies looking at technical replicates and reproducibility.



CPTAC dataset

- Time prior to freezing generally unknown
- Questions:
  - Does delaying freezing the sample cause changes in protein abundance
  - Does delaying freezing the sample cause phosphorylation changes due to e.g. stress response

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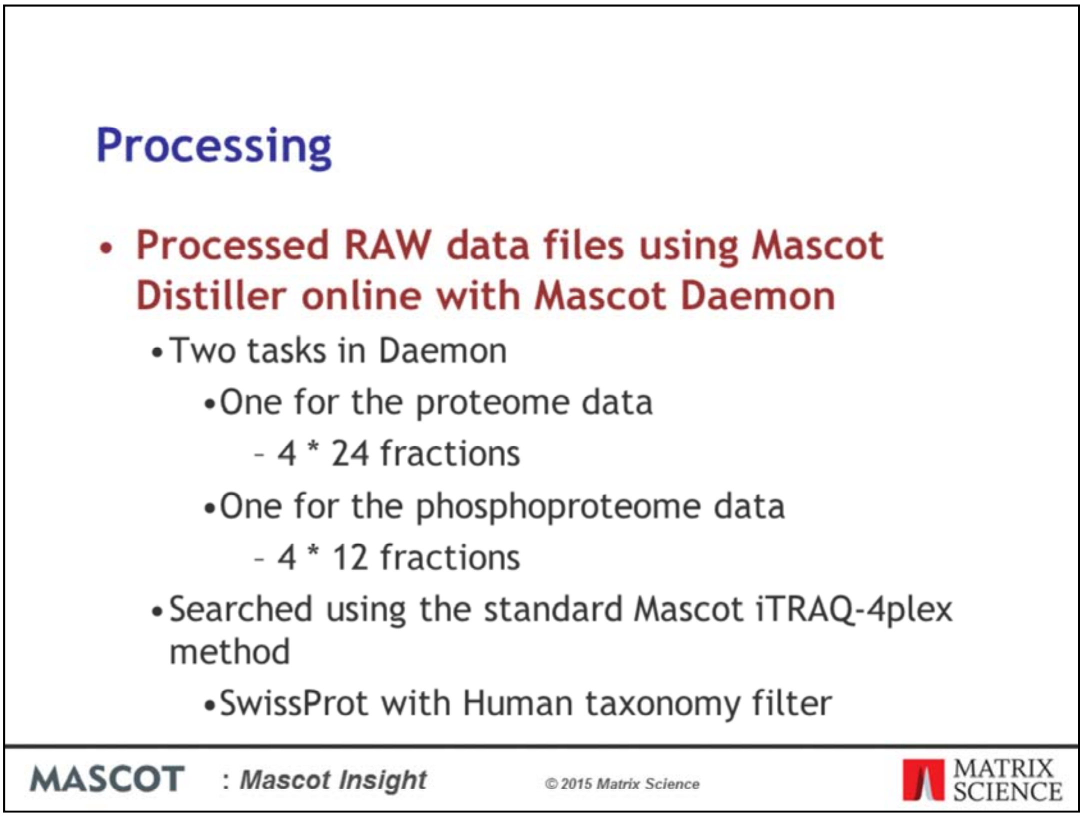
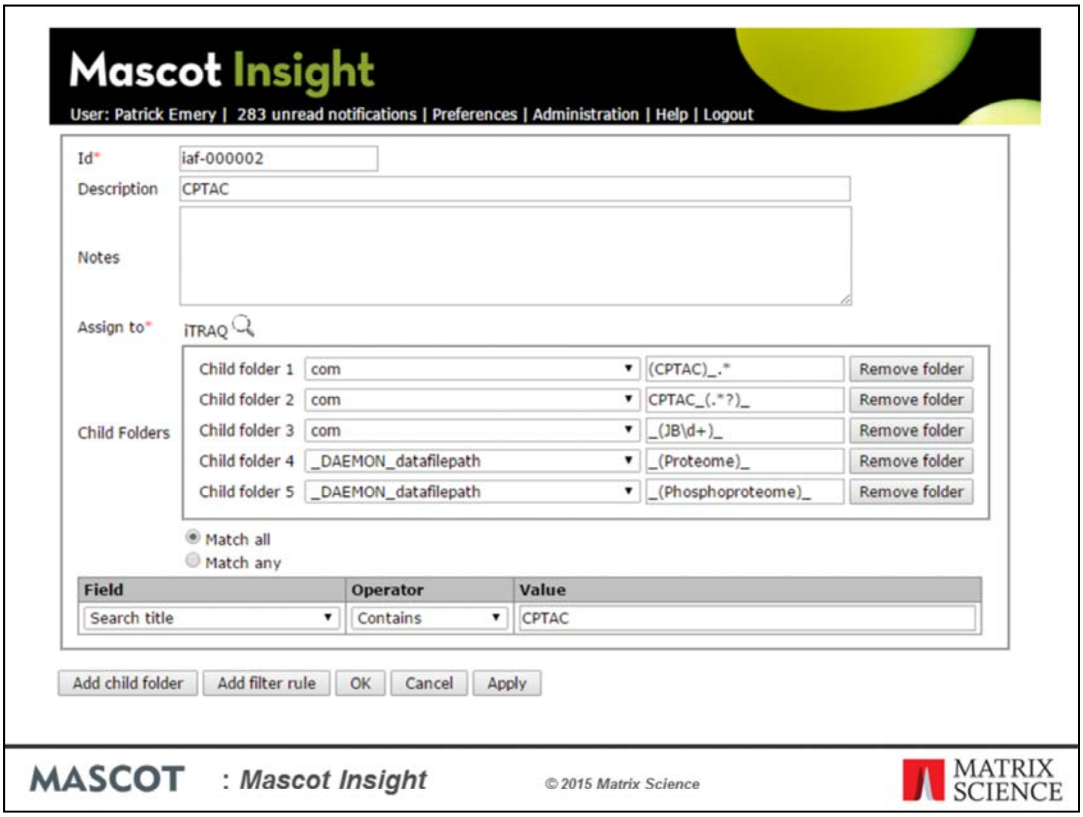
CPTAC are carrying out proteomics characterisation of tumour samples originally collected as part of the Cancer Genome Atlas (TCGA) project. However, these samples were not collected with proteomics studies in mind and the time between the tumour tissue being collected and the sample being frozen is generally not known for the samples - in general these tissues are collected without any tight regulation or documentation of this time period. The time course experiments were designed evaluate the impact of this ischemic time.



Processing

- **Processed RAW data files using Mascot Distiller online with Mascot Daemon**
  - Two tasks in Daemon
    - One for the proteome data
      - 4 * 24 fractions
    - One for the phosphoproteome data
      - 4 * 12 fractions
  - Searched using the standard Mascot iTRAQ-4plex method
    - SwissProt with Human taxonomy filter

The datasets are from Orbitraps. The raw data was reprocessed using Mascot Distiller online with Mascot Daemon in two tasks. One for the whole proteome datasets and one for the phosphoproteome datasets. Data were searched against SwissProt with a human taxonomy filter using the standard Mascot iTRAQ 4-plex quantitation method.



Mascot Insight
User: Patrick Emery | 283 unread notifications | Preferences | Administration | Help | Logout

Id* iaf-000002

Description CPTAC

Notes

Assign to* iTRAQ

Child Folders

Child folder 1	com	(CPTAC)_.*	Remove folder
Child folder 2	com	CPTAC_(.*?)_	Remove folder
Child folder 3	com	_(JB\d+)_	Remove folder
Child folder 4	_DAEMON_datafilepath	_(Proteome)_	Remove folder
Child folder 5	_DAEMON_datafilepath	_(Phosphoproteome)_	Remove folder

● Match all
○ Match any

Field	Operator	Value
Search title	Contains	CPTAC

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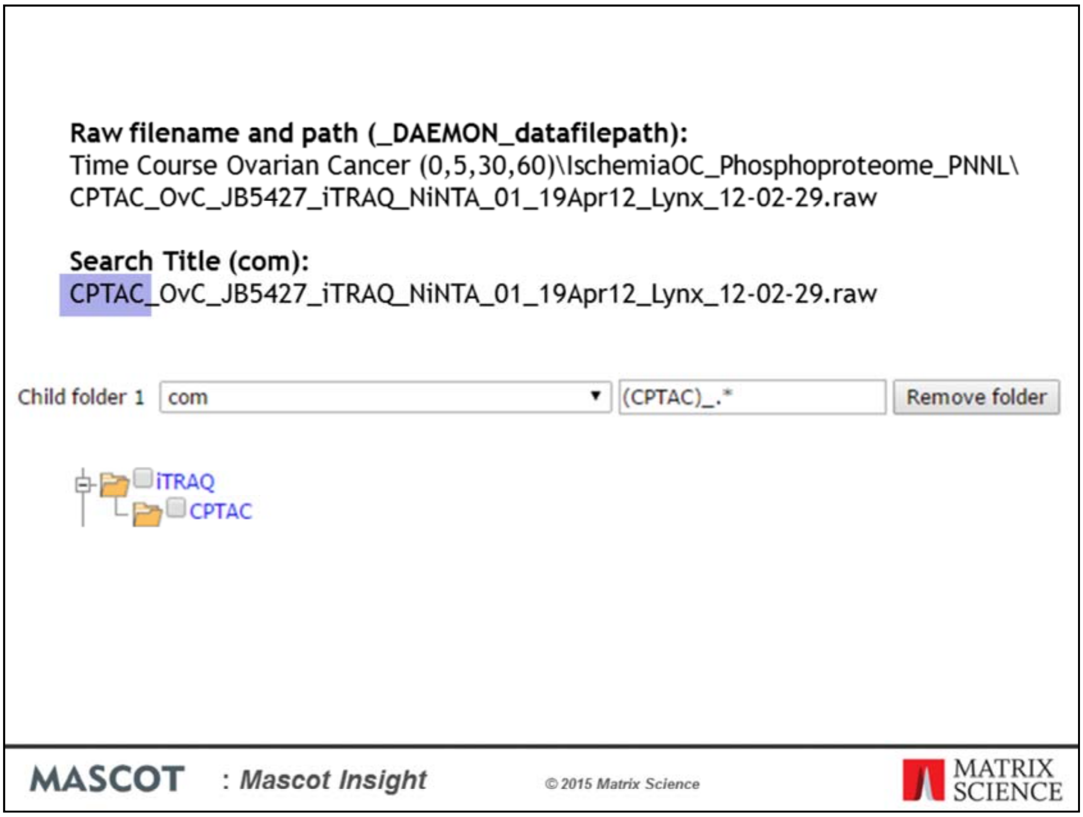
The searches will be automatically imported into Mascot Insight when they are completed. By default they will be imported as a group into a structure based on the Daemon tasks we set up. However, since we're searching a series of fractions from different samples, it would be helpful if we could automatically group the different fraction results by sample and by whether they're proteome or phosphoproteome samples. Fortunately we can do this because the required information is encoded in the raw data file names and paths obtained from CPTAC. Using an Import Assignment Filter in Mascot Insight, we can pull the required information out to automatically generate a complex organisation structure automatically, which will greatly simplify our downstream processing of the results.

The filter in this slide is quite complicated and often the requirements will be far more simple. To show you how the Import Assignment Filter works, I'll walk through an example using one of the Phosphoproteome files as an example.



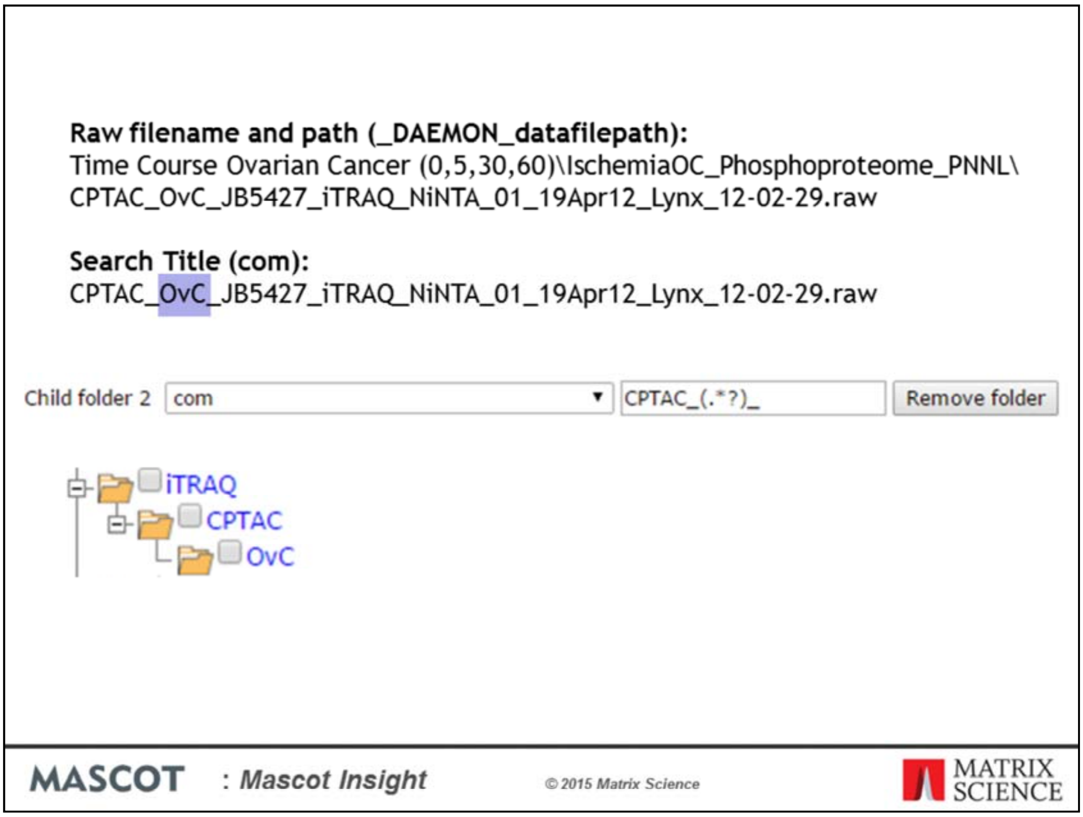
Raw filename and path (_DAEMON_datafilepath):
Time Course Ovarian Cancer (0,5,30,60)\IschemiaOC_Phosphoproteome_PNNL\
CPTAC_OvC_JB5427_iTRAQ_NiNTA_01_19Apr12_Lynx_12-02-29.raw

Search Title (com):
CPTAC_OvC_JB5427_iTRAQ_NiNTA_01_19Apr12_Lynx_12-02-29.raw

Child folder 1 | com ▾ | (CPTAC)_.* | Remove folder

When a search result which matches this import assignment filter is imported into the system, the child folder rules are then run in order to test what folder structure on the Dataset Explorer the search result should be imported to, creating new folders on the Dataset Explorer tree as required.

The first child folder rule tests against the search title. It is looking to match against the phrase CPTAC followed by an underscore and then any other characters. CPTAC is enclosed in brackets, which means that it is this section of the search title that will be used to match an existing folder or to name a new folder. If there isn't an existing CPTAC folder under the selected parent node, the system will automatically create one.



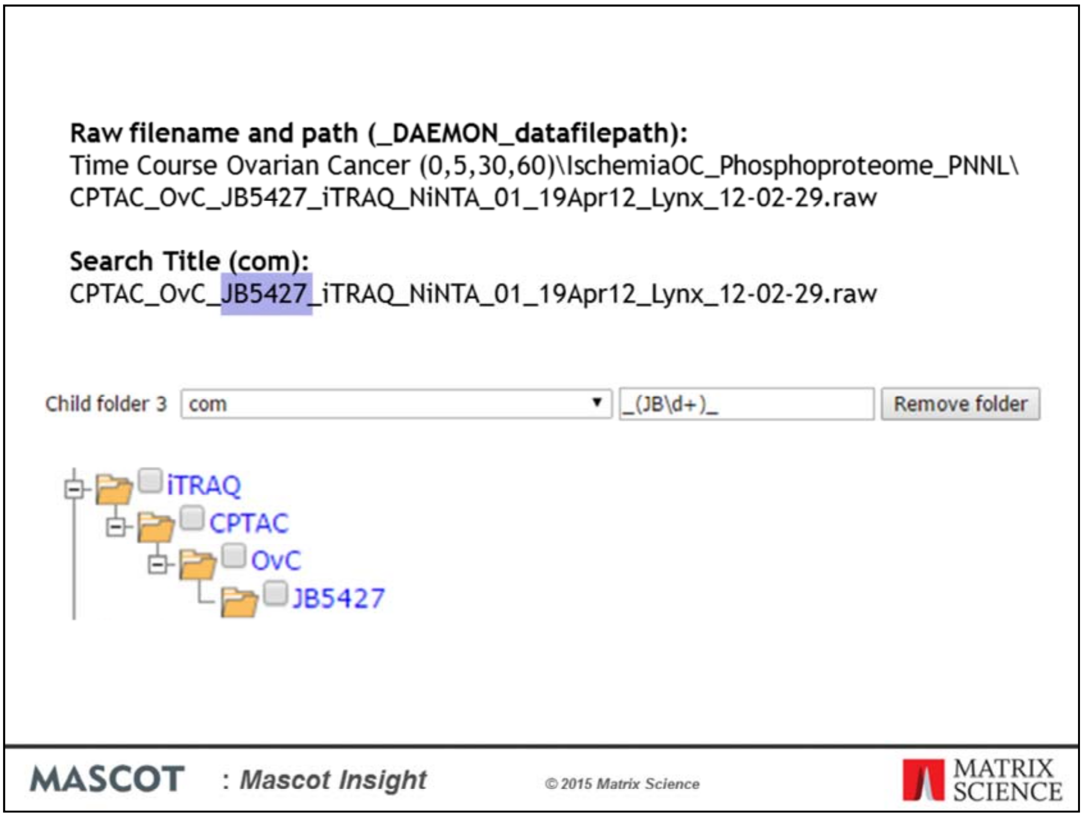
Raw filename and path (_DAEMON_datafilepath):
Time Course Ovarian Cancer (0,5,30,60)\IschemiaOC_Phosphoproteome_PNNL\
CPTAC_OvC_JB5427_iTRAQ_NiNTA_01_19Apr12_Lynx_12-02-29.raw

Search Title (com):
CPTAC_OvC_JB5427_iTRAQ_NiNTA_01_19Apr12_Lynx_12-02-29.raw

Child folder 2 | com ▼ | CPTAC_(.*?)_ | Remove folder

- □ iTRAQ
  - □ CPTAC
    - □ OvC

The second child folder rule then matches all characters after the _ after CPTAC through to the next _ character of the search title. So for this raw datafile that is OvC



Raw filename and path (_DAEMON_datafilepath):
Time Course Ovarian Cancer (0,5,30,60)\IschemiaOC_Phosphoproteome_PNNL\
CPTAC_OvC_JB5427_iTRAQ_NiNTA_01_19Apr12_Lynx_12-02-29.raw

Search Title (com):
CPTAC_OvC_JB5427_iTRAQ_NiNTA_01_19Apr12_Lynx_12-02-29.raw

Child folder 3 com _(JB\d+)_

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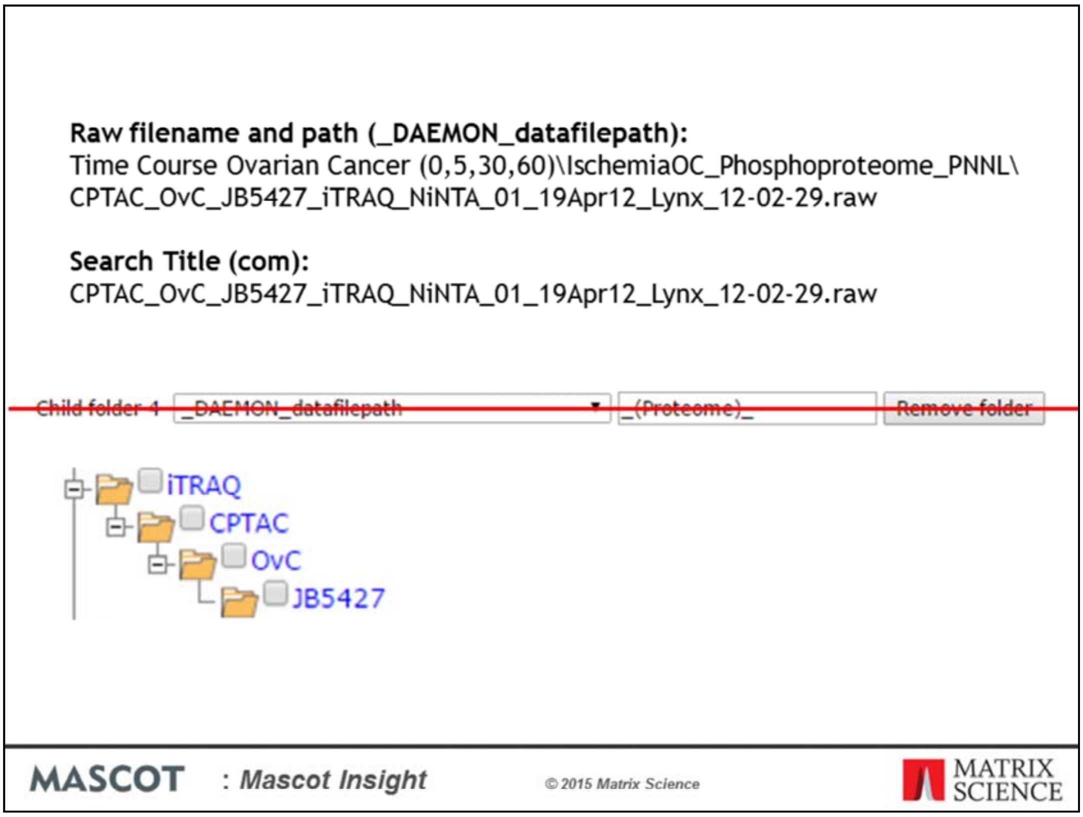
The third folder rule matches to JB followed by 1 or more numbers from the search title – this is the source sample name in this case. For this file that is JB5427



Raw filename and path (_DAEMON_datafilepath):
Time Course Ovarian Cancer (0,5,30,60)\IschemiaOC_Phosphoproteome_PNNL\
CPTAC_OvC_JB5427_iTRAQ_NiNTA_01_19Apr12_Lynx_12-02-29.raw

Search Title (com):
CPTAC_OvC_JB5427_iTRAQ_NiNTA_01_19Apr12_Lynx_12-02-29.raw

~~Child folder 4 _DAEMON_datafilepath ▾ _(Proteome)_ Remove folder~~

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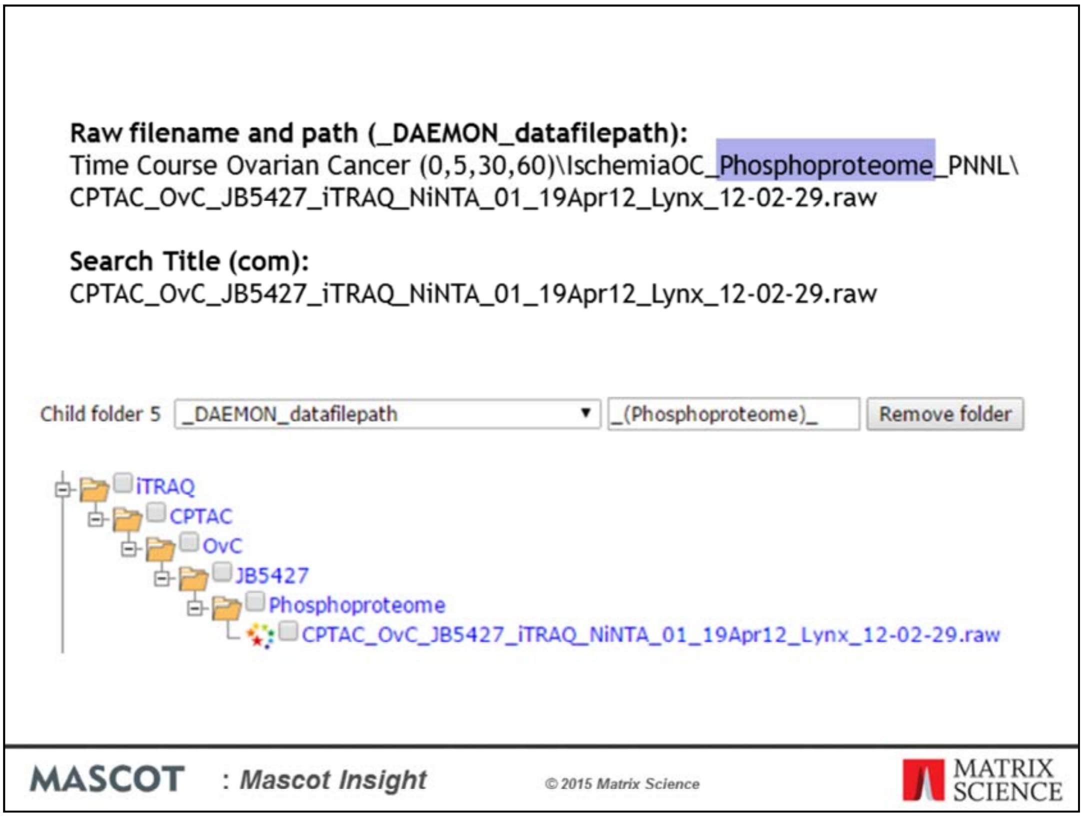
The fourth folder rule tries to match and select the word Proteome from the raw datafile path, as long as it appears between two underscore characters. This rule does not match the path for these raw data file and so the rule does not run.



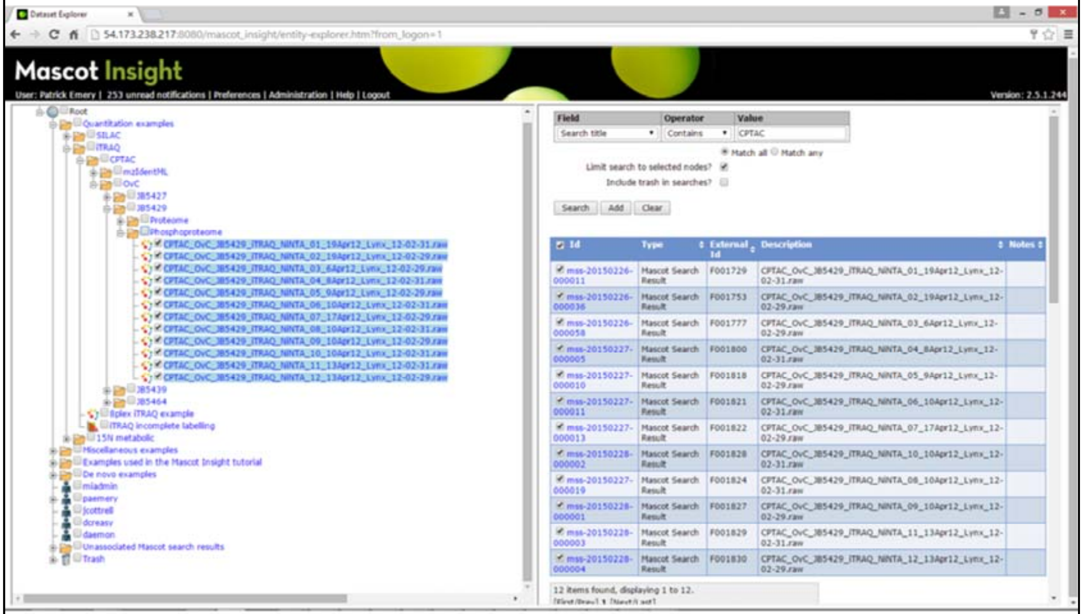
Raw filename and path (_DAEMON_datafilepath):
Time Course Ovarian Cancer (0,5,30,60)\IschemiaOC_Phosphoproteome_PNNL\
CPTAC_OvC_JB5427_iTRAQ_NiNTA_01_19Apr12_Lynx_12-02-29.raw

Search Title (com):
CPTAC_OvC_JB5427_iTRAQ_NiNTA_01_19Apr12_Lynx_12-02-29.raw

Child folder 5 | _DAEMON_datafilepath ▾ | _(Phosphoproteome)_ | Remove folder

- □ iTRAQ
  - □ CPTAC
    - □ OvC
      - □ JB5427
        - □ Phosphoproteome
          - □ CPTAC_OvC_JB5427_iTRAQ_NiNTA_01_19Apr12_Lynx_12-02-29.raw

The final child folder rule matches the word Phosphoproteome from the raw datafile path as long as it appears between two underscore characters. This matches the path for this raw datafile. Since this is the final point in the generated path, the search result will be imported as a child of this Phosphoproteome folder.



Dataset Explorer
54.173.238.217:8080/mascot_insight/entity-explorer.htm?from_logon=1
Mascot Insight
User: Patrick Emery | 253 unread notifications | Preferences | Administration | Help | Logout
Version: 2.5.1.244

- Root
  - Quantitation examples
    - SILAC
    - iTRAQ
      - CPTAC
        - mzIdentML
        - OvC
          - JB5427
          - JB5429
            - Proteome
            - Phosphoproteome
              - ☑ CPTAC_OvC_JB5429_iTRAQ_NINTA_01_19Apr12_Lynx_12-02-31.raw
              - ☑ CPTAC_OvC_JB5429_iTRAQ_NINTA_02_19Apr12_Lynx_12-02-29.raw
              - ☑ CPTAC_OvC_JB5429_iTRAQ_NINTA_03_6Apr12_Lynx_12-02-29.raw
              - ☑ CPTAC_OvC_JB5429_iTRAQ_NINTA_04_8Apr12_Lynx_12-02-31.raw
              - ☑ CPTAC_OvC_JB5429_iTRAQ_NINTA_05_9Apr12_Lynx_12-02-29.raw
              - ☑ CPTAC_OvC_JB5429_iTRAQ_NINTA_06_10Apr12_Lynx_12-02-31.raw
              - ☑ CPTAC_OvC_JB5429_iTRAQ_NINTA_07_17Apr12_Lynx_12-02-29.raw
              - ☑ CPTAC_OvC_JB5429_iTRAQ_NINTA_08_10Apr12_Lynx_12-02-31.raw
              - ☑ CPTAC_OvC_JB5429_iTRAQ_NINTA_09_10Apr12_Lynx_12-02-29.raw
              - ☑ CPTAC_OvC_JB5429_iTRAQ_NINTA_10_10Apr12_Lynx_12-02-31.raw
              - ☑ CPTAC_OvC_JB5429_iTRAQ_NINTA_11_13Apr12_Lynx_12-02-31.raw
              - ☑ CPTAC_OvC_JB5429_iTRAQ_NINTA_12_13Apr12_Lynx_12-02-29.raw
          - JB5439
          - JB5464
      - 8plex iTRAQ example
      - iTRAQ incomplete labelling
    - 15N metabolic
  - Miscellaneous examples
  - Examples used in the Mascot Insight tutorial
  - De novo examples
  - miadmin
  - paemery
  - jcottrell
  - dcreasy
  - daemon
  - Unassociated Mascot search results
  - Trash

Field	Operator	Value
Search title	Contains	CPTAC

● Match all ○ Match any

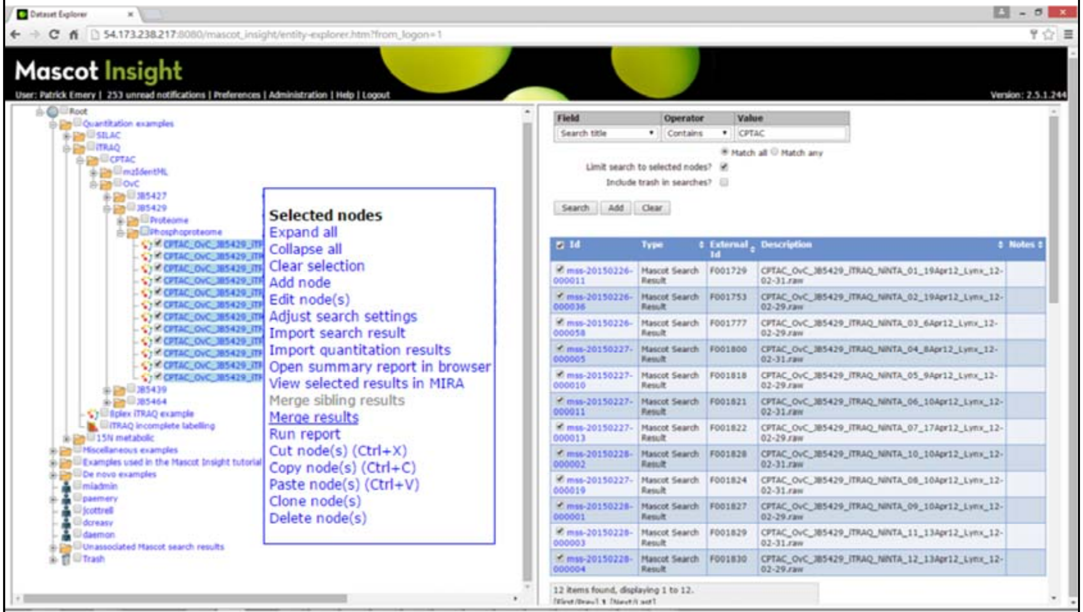
Limit search to selected nodes? ☑

Include trash in searches? □

☑ Id	Type	External Id	Description	Notes
☑ mss-20150226-000011	Mascot Search Result	F001729	CPTAC_OvC_JB5429_iTRAQ_NINTA_01_19Apr12_Lynx_12-02-31.raw	
☑ mss-20150226-000036	Mascot Search Result	F001753	CPTAC_OvC_JB5429_iTRAQ_NINTA_02_19Apr12_Lynx_12-02-29.raw	
☑ mss-20150226-000058	Mascot Search Result	F001777	CPTAC_OvC_JB5429_iTRAQ_NINTA_03_6Apr12_Lynx_12-02-29.raw	
☑ mss-20150227-000005	Mascot Search Result	F001800	CPTAC_OvC_JB5429_iTRAQ_NINTA_04_8Apr12_Lynx_12-02-31.raw	
☑ mss-20150227-000010	Mascot Search Result	F001818	CPTAC_OvC_JB5429_iTRAQ_NINTA_05_9Apr12_Lynx_12-02-29.raw	
☑ mss-20150227-000011	Mascot Search Result	F001821	CPTAC_OvC_JB5429_iTRAQ_NINTA_06_10Apr12_Lynx_12-02-31.raw	
☑ mss-20150227-000013	Mascot Search Result	F001822	CPTAC_OvC_JB5429_iTRAQ_NINTA_07_17Apr12_Lynx_12-02-29.raw	
☑ mss-20150228-000002	Mascot Search Result	F001828	CPTAC_OvC_JB5429_iTRAQ_NINTA_10_10Apr12_Lynx_12-02-31.raw	
☑ mss-20150227-000019	Mascot Search Result	F001824	CPTAC_OvC_JB5429_iTRAQ_NINTA_08_10Apr12_Lynx_12-02-31.raw	
☑ mss-20150228-000001	Mascot Search Result	F001827	CPTAC_OvC_JB5429_iTRAQ_NINTA_09_10Apr12_Lynx_12-02-29.raw	
☑ mss-20150228-000003	Mascot Search Result	F001829	CPTAC_OvC_JB5429_iTRAQ_NINTA_11_13Apr12_Lynx_12-02-31.raw	
☑ mss-20150228-000004	Mascot Search Result	F001830	CPTAC_OvC_JB5429_iTRAQ_NINTA_12_13Apr12_Lynx_12-02-29.raw	

12 items found, displaying 1 to 12.
[First/Prev] 1 [Next/Last]

Once the searches have completed, the results are automatically grouped together in the main Explorer Tree on the Mascot Insight home page, as illustrated here. The next step is to merge the separate fractions into single merged results – 2 for each sample; one for the proteome and one for the phosphoproteome. If you don't need to make any changes to the quantitation setup in order to make the merged results, you can simply select the top shared node on the tree (OvC in this case) and tell the system to merge all the search results at the same level under the selected node.



Field	Operator	Value
Search title	Contains	CPTAC

● Match all ○ Match any

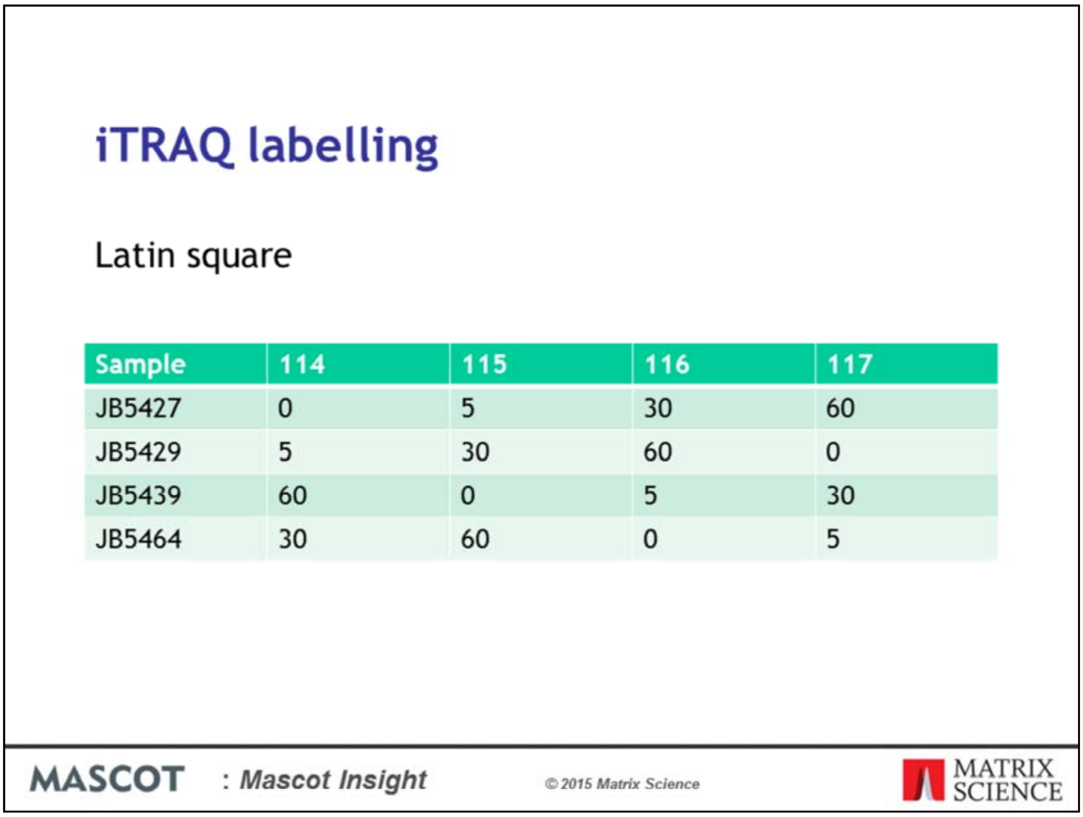
Limit search to selected nodes? ☑

Include trash in searches? □

☑ Id	Type	External Id	Description	Notes
☑ mss-20150226-000011	Mascot Search Result	F001729	CPTAC_OvC_JB5429_iTRAQ_NiNTA_01_19Apr12_Lynx_12-02-31.raw	
☑ mss-20150226-000036	Mascot Search Result	F001753	CPTAC_OvC_JB5429_iTRAQ_NiNTA_02_19Apr12_Lynx_12-02-29.raw	
☑ mss-20150226-000058	Mascot Search Result	F001777	CPTAC_OvC_JB5429_iTRAQ_NiNTA_03_6Apr12_Lynx_12-02-29.raw	
☑ mss-20150227-000005	Mascot Search Result	F001800	CPTAC_OvC_JB5429_iTRAQ_NiNTA_04_8Apr12_Lynx_12-02-31.raw	
☑ mss-20150227-000010	Mascot Search Result	F001818	CPTAC_OvC_JB5429_iTRAQ_NiNTA_05_9Apr12_Lynx_12-02-29.raw	
☑ mss-20150227-000011	Mascot Search Result	F001821	CPTAC_OvC_JB5429_iTRAQ_NiNTA_06_10Apr12_Lynx_12-02-31.raw	
☑ mss-20150227-000013	Mascot Search Result	F001822	CPTAC_OvC_JB5429_iTRAQ_NiNTA_07_17Apr12_Lynx_12-02-29.raw	
☑ mss-20150228-000002	Mascot Search Result	F001828	CPTAC_OvC_JB5429_iTRAQ_NiNTA_10_10Apr12_Lynx_12-02-31.raw	
☑ mss-20150227-000019	Mascot Search Result	F001824	CPTAC_OvC_JB5429_iTRAQ_NiNTA_08_10Apr12_Lynx_12-02-31.raw	
☑ mss-20150228-000001	Mascot Search Result	F001827	CPTAC_OvC_JB5429_iTRAQ_NiNTA_09_10Apr12_Lynx_12-02-29.raw	
☑ mss-20150228-000003	Mascot Search Result	F001829	CPTAC_OvC_JB5429_iTRAQ_NiNTA_11_13Apr12_Lynx_12-02-31.raw	
☑ mss-20150228-000004	Mascot Search Result	F001830	CPTAC_OvC_JB5429_iTRAQ_NiNTA_12_13Apr12_Lynx_12-02-29.raw	

12 items found, displaying 1 to 12.

However, in this experiment the different time points were rotated between the channels for each sample, we need to reset the quantitation ratio settings when we do the merge. We can do this by selecting the group of searches we want to merge and selecting the ‘Merge results’ option from the context menu.



iTRAQ labelling

Latin square

Sample	114	115	116	117
JB5427	0	5	30	60
JB5429	5	30	60	0
JB5439	60	0	5	30
JB5464	30	60	0	5

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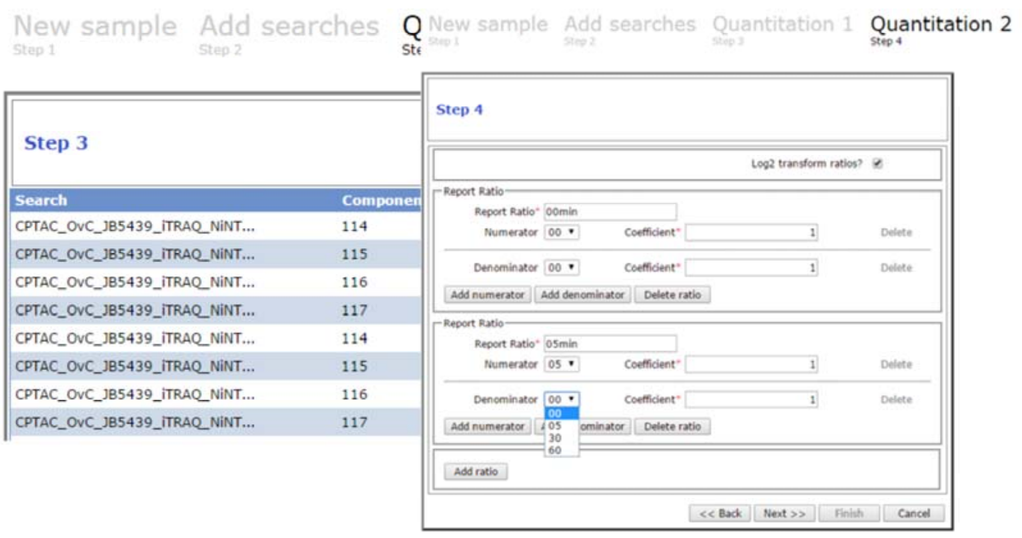
When the tumour samples were labelled using the iTRAQ reagents, the time points were rotated between the different iTRAQ channels as shown in this table. This type of rotation is commonly called a ‘latin square’, and is considered good practice because it reduces the impact of tag-related systematic errors. Whenever we want to generate a report, its much clearer if we can label a feature as "time 0" rather than "channel 115". Also, when we want to merge samples, we can't just add up the 115 intensity. Insight allows us to map component names (e.g. 115) to more meaningful terms (e.g. time 0). We do this by selecting a set of search results and choosing the ‘Merge results’ option from the context menu.



Step 3

Search	Componen
CPTAC_OvC_JB5439_iTRAQ_NiNT...	114
CPTAC_OvC_JB5439_iTRAQ_NiNT...	115
CPTAC_OvC_JB5439_iTRAQ_NiNT...	116
CPTAC_OvC_JB5439_iTRAQ_NiNT...	117
CPTAC_OvC_JB5439_iTRAQ_NiNT...	114
CPTAC_OvC_JB5439_iTRAQ_NiNT...	115
CPTAC_OvC_JB5439_iTRAQ_NiNT...	116
CPTAC_OvC_JB5439_iTRAQ_NiNT...	117

Step 4

Log2 transform ratios? ☑

Report Ratio

Report Ratio* 00min

Numerator 00 ▾ Coefficient* 1 Delete

Denominator 00 ▾ Coefficient* 1 Delete

Report Ratio

Report Ratio* 05min

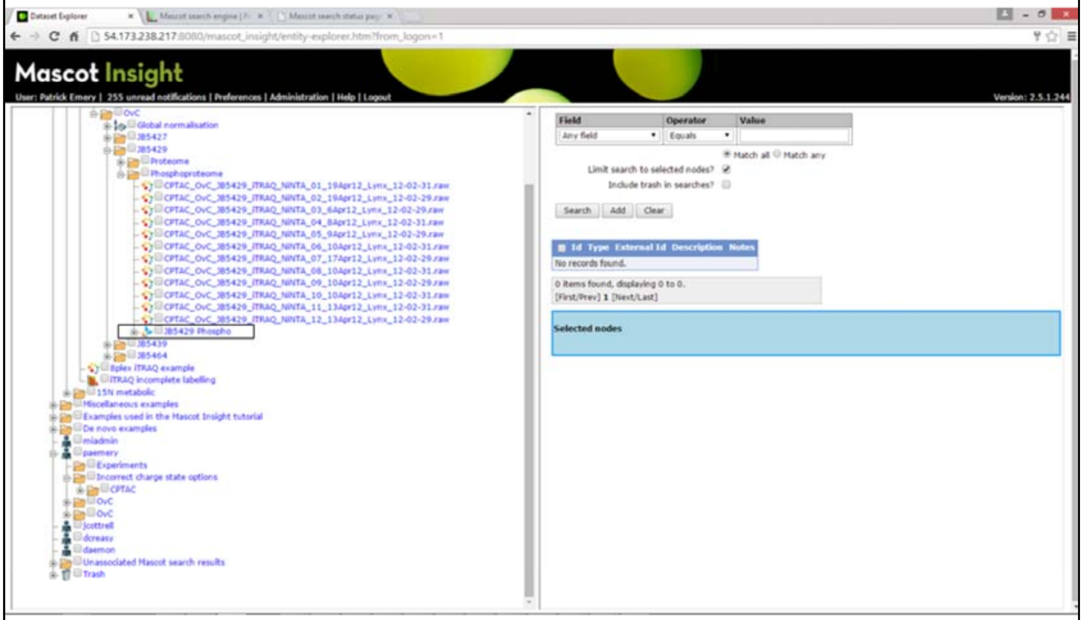
Numerator 05 ▾ Coefficient* 1 Delete

Denominator 00 ▾ Coefficient* 1 Delete

Sample	114	115	116	117
JB5439	60	0	5	30

This opens an HTML wizard which allows us to rename the components on a result by result basis. Here I'm merging the fractions from the JB5439 sample, so channel 114 is mapped to the 60 minute time point, 115 to the zero time point, etc. Once you've done that, the next step of the wizard allows you to set up the ratios you want to report – in this case, ratios to the zero time point.

By managing the quantitation methods this way, you can simplify the Daemon tasks required to submit the searches as you can always use the standard quantitation method supplied with Mascot server and then adjust the quantitation ratios afterwards. This also avoids having to setup multiple very similar quantitation methods on your Mascot server.


Dataset Explorer
Mascot search engine | Fr
Mascot search status pag
54.173.238.217:8080/mascot_insight/entity-explorer.htm?from_logon=1
Mascot Insight
User: Patrick Emery | 255 unread notifications | Preferences | Administration | Help | Logout
Version: 2.5.1.244

```
OvC
  Global normalisation
  JB5427
  JB5429
    Proteome
    Phosphoproteome
      CPTAC_OvC_JB5429_iTRAQ_NINTA_01_19Apr12_Lynx_12-02-31.raw
      CPTAC_OvC_JB5429_iTRAQ_NINTA_02_19Apr12_Lynx_12-02-29.raw
      CPTAC_OvC_JB5429_iTRAQ_NINTA_03_6Apr12_Lynx_12-02-29.raw
      CPTAC_OvC_JB5429_iTRAQ_NINTA_04_8Apr12_Lynx_12-02-31.raw
      CPTAC_OvC_JB5429_iTRAQ_NINTA_05_9Apr12_Lynx_12-02-29.raw
      CPTAC_OvC_JB5429_iTRAQ_NINTA_06_10Apr12_Lynx_12-02-31.raw
      CPTAC_OvC_JB5429_iTRAQ_NINTA_07_17Apr12_Lynx_12-02-29.raw
      CPTAC_OvC_JB5429_iTRAQ_NINTA_08_10Apr12_Lynx_12-02-31.raw
      CPTAC_OvC_JB5429_iTRAQ_NINTA_09_10Apr12_Lynx_12-02-29.raw
      CPTAC_OvC_JB5429_iTRAQ_NINTA_10_10Apr12_Lynx_12-02-31.raw
      CPTAC_OvC_JB5429_iTRAQ_NINTA_11_13Apr12_Lynx_12-02-31.raw
      CPTAC_OvC_JB5429_iTRAQ_NINTA_12_13Apr12_Lynx_12-02-29.raw
    JB5429 Phospho
  JB5439
  JB5464
8plex iTRAQ example
iTRAQ incomplete labelling
15N metabolic
Miscellaneous examples
Examples used in the Mascot Insight tutorial
De novo examples
miadmin
paemery
  Experiments
  Incorrect charge state options
    CPTAC
  OvC
  OvC
jcottrell
dcreasy
daemon
Unassociated Mascot search results
Trash
```

Field	Operator	Value
Any field	Equals	

● Match all ○ Match any

Limit search to selected nodes? ☑

Include trash in searches? □

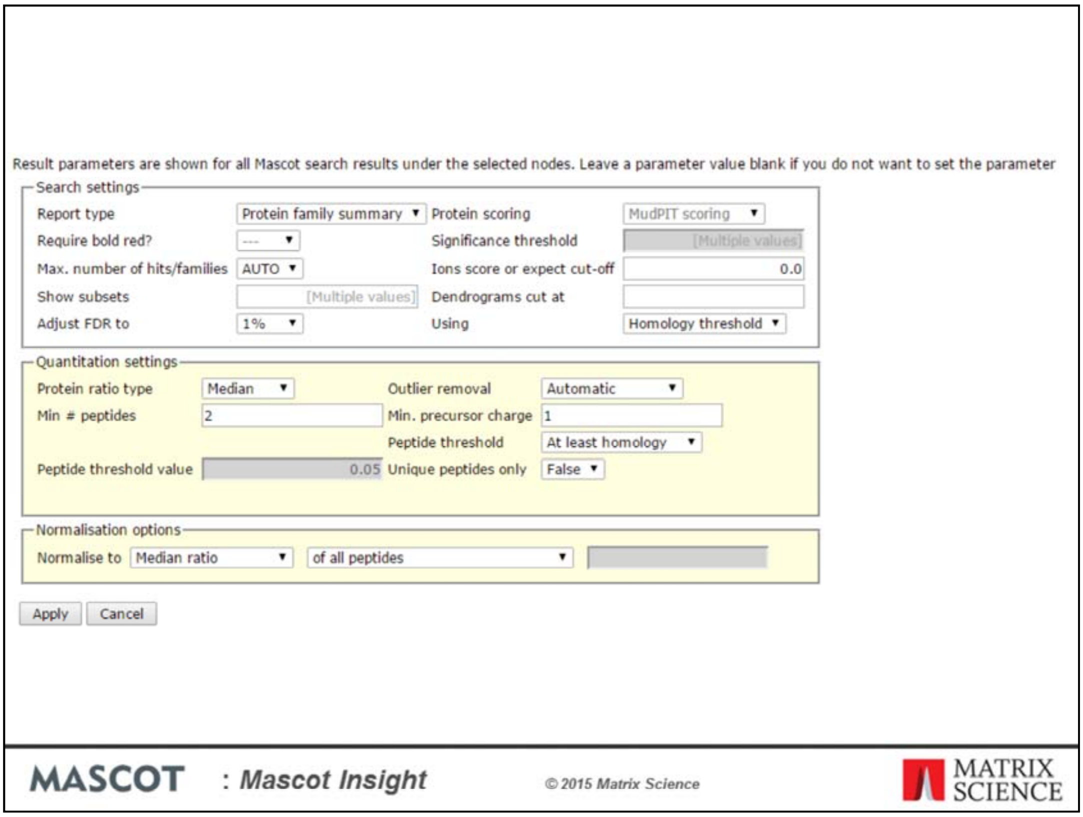
Search | Add | Clear

☐	Id	Type	External Id	Description	Notes
No records found.					

0 items found, displaying 0 to 0.
[First/Prev] **1** [Next/Last]

Selected nodes

After the fractions are merged, a new result node is added to the Mascot Insight explorer tree.



Result parameters are shown for all Mascot search results under the selected nodes. Leave a parameter value blank if you do not want to set the parameter

Search settings

Report type	Protein family summary ▾	Protein scoring	MudPIT scoring ▾
Require bold red?	--- ▾	Significance threshold	[Multiple values]
Max. number of hits/families	AUTO ▾	Ions score or expect cut-off	0.0
Show subsets	[Multiple values]	Dendrograms cut at	
Adjust FDR to	1% ▾	Using	Homology threshold ▾

Quantitation settings

Protein ratio type	Median ▾	Outlier removal	Automatic ▾
Min # peptides	2	Min. precursor charge	1
		Peptide threshold	At least homology ▾
Peptide threshold value	0.05	Unique peptides only	False ▾

Normalisation options

Normalise to Median ratio ▾ of all peptides ▾

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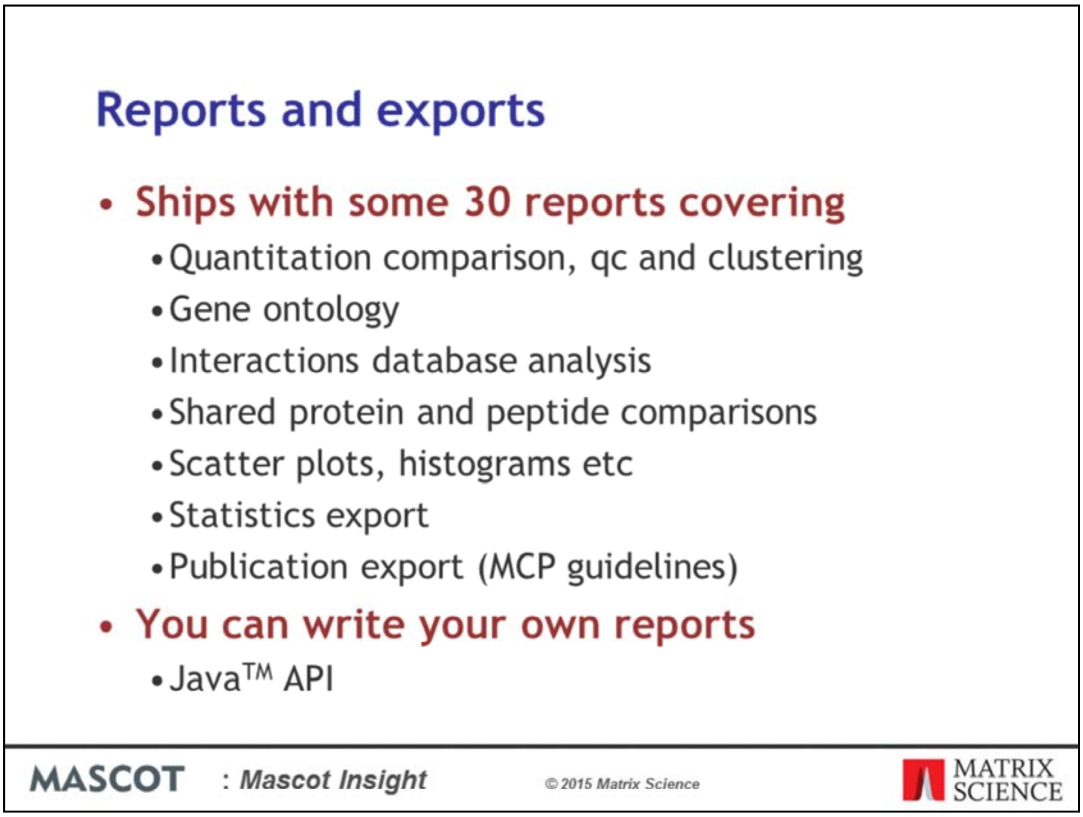
Once we've setup all the merged datasets, we can also set the default search and quantitation settings for all the search results using a single form – this includes setting the peptide FDR to a target value. Once we do this, the required settings are saved to the Mascot Insight database so that whenever we open the results or generate a report in Mascot Insight, the correct search and quantitation settings are used.



Reports and exports

- Ships with some 30 reports covering
  - Quantitation comparison, qc and clustering
  - Gene ontology
  - Interactions database analysis
  - Shared protein and peptide comparisons
  - Scatter plots, histograms etc
  - Statistics export
  - Publication export (MCP guidelines)
- You can write your own reports
  - Java™ API

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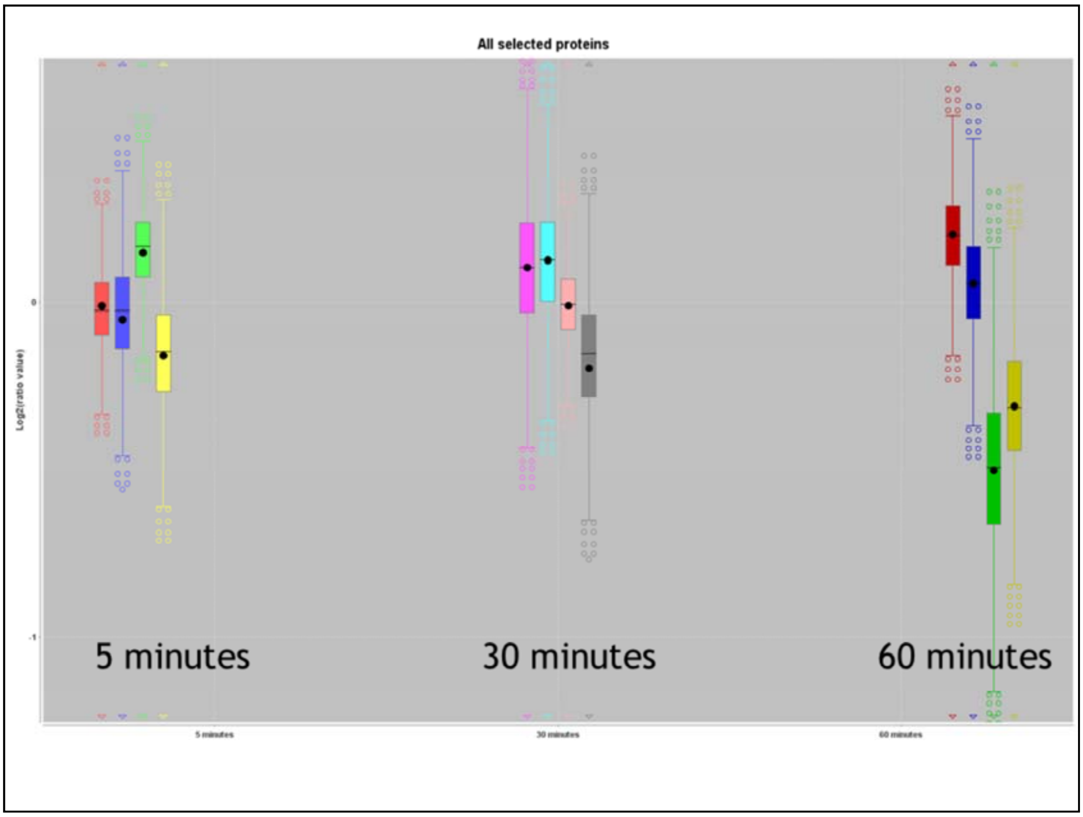
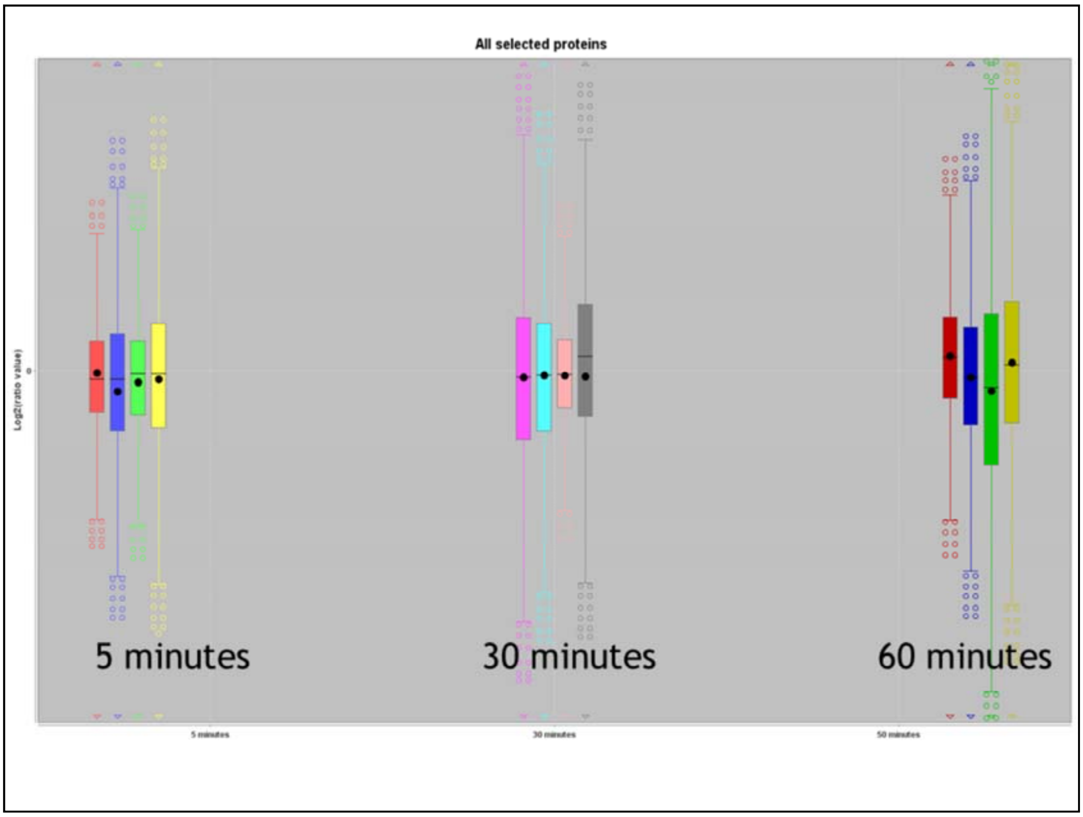
Now the data are ready for reporting on. The system ships with some 30 reports designed specifically for proteomics data. These cover a wide range of areas such as result comparison, quantitation and quantitation clustering/grouping, Gene Ontology analysis, Interactions database analysis and general graphing reports such as scatter plots. You can also write your own reports using the Java programming language.



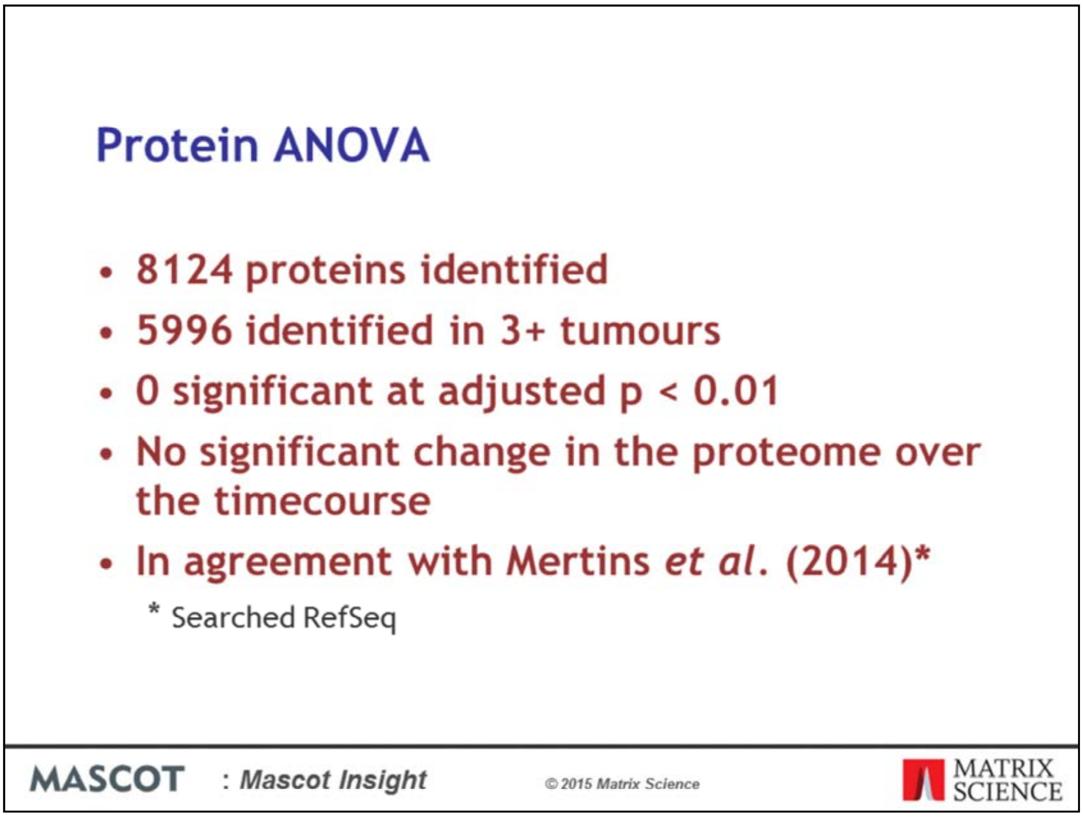
The box and whisker plot allows you to get a good overview of the dataset and spot if you have any systematic errors which might need correcting using normalisation.

If you're not familiar with box and whisker plots, they show the spread of data through the quartiles. The box shows the quartile 1-quartile 3 range, while the whiskers show quartile 1 and quartile 3 + or – 1.5x the interquartile range. The black line through the box is the median value and the black dot is the mean. The symbols outside the whiskers show the presents of outliers.

These are the plots for the log2 protein ratios for the four tumours at each time point without normalisation turned on (using the global proteome dataset). We'd not really expect the majority of proteins to show significant changes in the ratio values across the time points, so for these data we should enable normalisation.



And here are the data with median peptide ratio normalisation turned on. As you can see normalisation has brought the median ratios for each tumour and timepoint much closer to zero – normalisation works at the peptide level, so we’d next expect it to bring all the median protein ratios to exactly zero. If you plotted the peptide ratio values for this dataset as a box and whisker plot you would see the peptide median ratio values corrected to zero.



Protein ANOVA

- 8124 proteins identified
- 5996 identified in 3+ tumours
- 0 significant at adjusted $p < 0.01$
- No significant change in the proteome over the timecourse
- In agreement with Mertins *et al*. (2014)*

* Searched RefSeq

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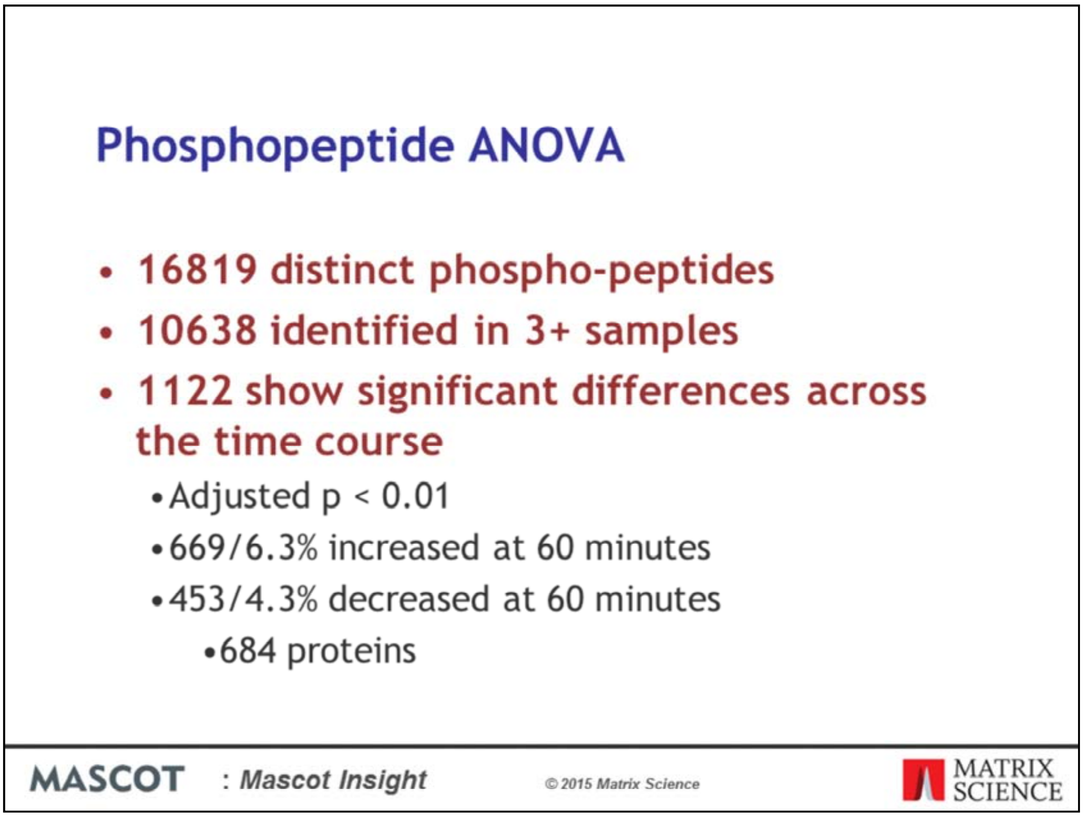
The next step is to run an ANOVA report on the protein ratio values from the proteome dataset. An ANOVA compares between different groups and identifies if the variation between groups is significantly greater than the variation within groups. In this case the groups are the different time-points in the experiment and the ANOVA will be carried out separately on each identified protein.

For these data I searched the SwissProt database with a Human taxonomy filter and identified 8124 proteins across the four tumour samples. Just under 6000 of these were present in at least 3 of the tumours.

The calculated probability value for each protein in the ANOVA were then adjusted using the Benjamini–Hochberg correction. This is a relatively tight set of criteria designed to minimise the number of false positives. At a significance threshold of 1%, no proteins displayed significant changes between the different timepoints of the experiment. This is in agreement with the data presented in Mertins et al. – although it is worth noting that they searched human RefSeq rather than SwissProt, so the numbers are slightly different (SwissProt is more highly curated and has less redundancy so the numbers presented here are slightly lower than in the paper).



Phosphopeptide ANOVA

- 16819 distinct phospho-peptides
- 10638 identified in 3+ samples
- 1122 show significant differences across the time course
  - Adjusted p < 0.01
  - 669/6.3% increased at 60 minutes
  - 453/4.3% decreased at 60 minutes
    - 684 proteins

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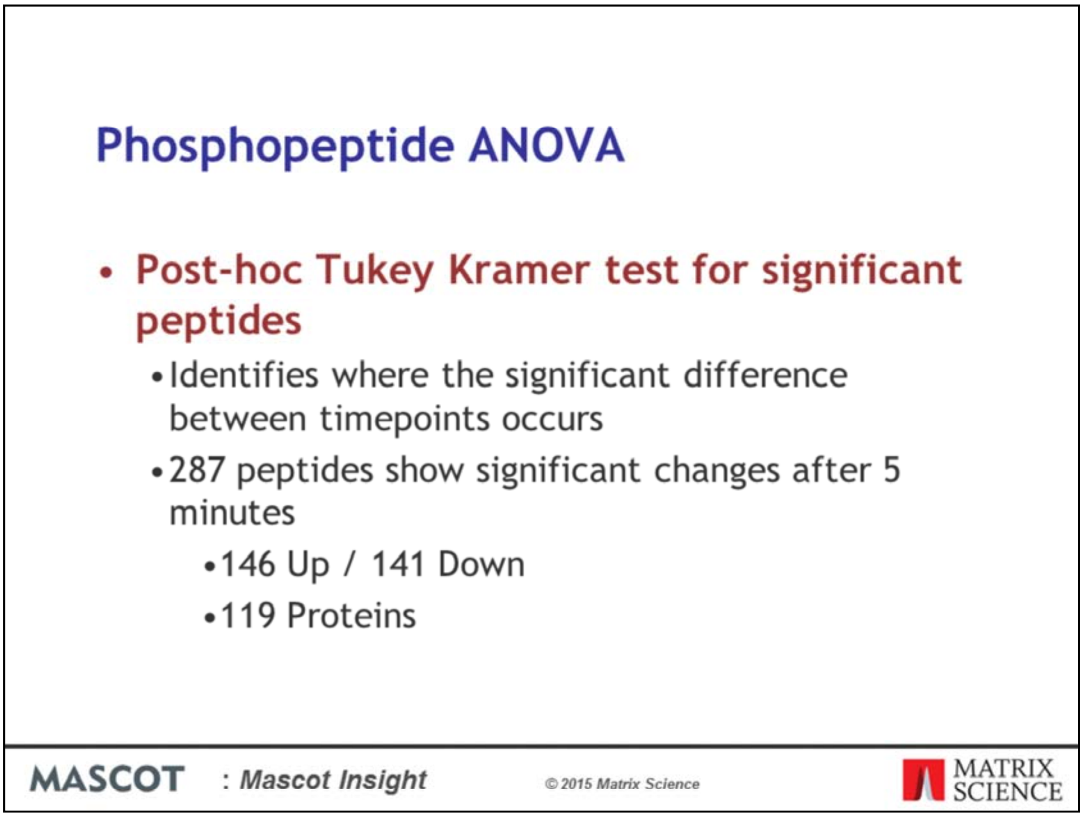
We then ran an ANOVA report on the phospho peptide dataset. This time the ANOVA was run on the peptide quantitation ratios, with peptides grouped together by unique sequence and phosphorylation state.

We have a total of 16819 distinct phospho peptides, of which 10638 were identified in at least 3 out of the 4 samples. Of those, 1122 showed significant differences between timepoints at a threshold of 1%. That is that the differences between the timepoints of the experiment are greater than the differences within the timepoints – in other words, significant changes in the amounts of these phosphopeptides were observed across the different timepoints. 669 phosphopeptides were observed at an increased amount at 60 minutes, with 453 decreased. These 1122 peptides match into 684 different proteins. So, unlike at the whole proteome protein level, we are seeing some significant changes at the phospho-proteome level.



Phosphopeptide ANOVA

- Post-hoc Tukey Kramer test for significant peptides
  - Identifies where the significant difference between timepoints occurs
  - 287 peptides show significant changes after 5 minutes
    - 146 Up / 141 Down
    - 119 Proteins

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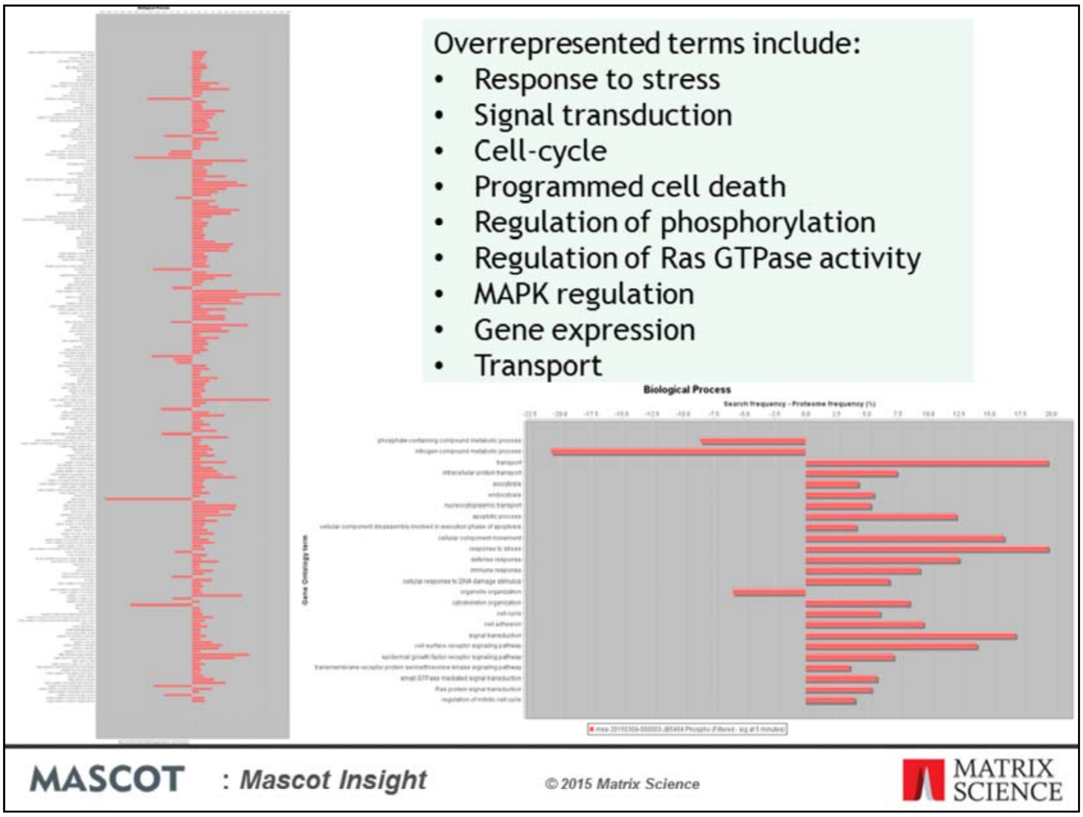
The ANOVA report identifies the peptides or proteins which show significant differences between the timepoints in the experiment, but not where those differences lie – for example, it won’t tell you if the amount of a phosphopeptide after 5 minutes is significantly different to the amount after 60 minutes.

The Tukey-Kramer test is carried out after the ANOVA and used to identify which time points are showing significant differences Post-hoc. Of the 1122 significant phospho-peptides, 287 of these are showing significant iTRAQ ratio changes after just 5 minutes. These are roughly evenly split between phosphopeptides which are present at increased or decreased amounts and represent 119 different proteins.



Overrepresented terms include:

- Response to stress
- Signal transduction
- Cell-cycle
- Programmed cell death
- Regulation of phosphorylation
- Regulation of Ras GTPase activity
- MAPK regulation
- Gene expression
- Transport

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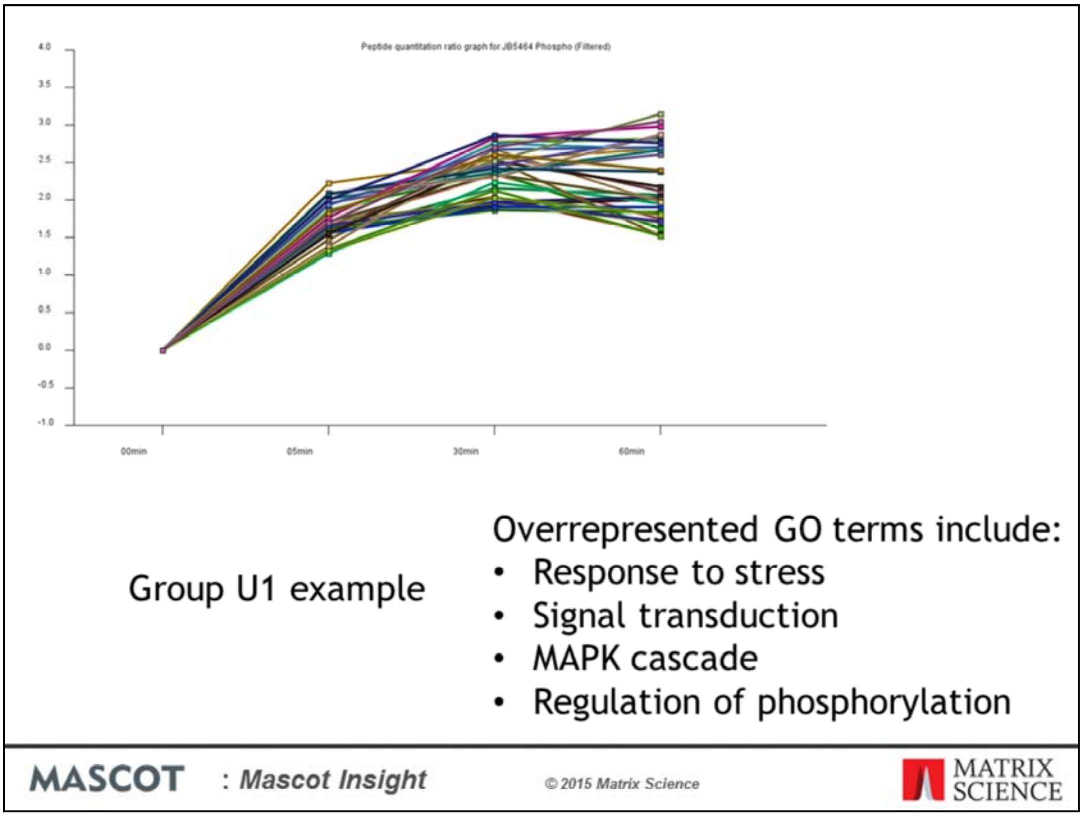
Mascot Insight has various reports and functions available for looking at Gene Ontology assignments. Here we've filtered the identified proteins from one of the tumour samples with the proteins identified as having phosphopeptides which have significantly altered amounts after 5 minutes. The report compares between the GO assignments from the dataset and the frequencies calculated from the Human Uniprot proteome database. On the report, any bar above zero is over-represented, any below is under-represented, in the search result. Here we're looking at GO 'Biological Process' assignments significantly over or under represented in the results when compared with the proteome numbers. Some of the key groupings of assignments are shown in the box. As you can see, we're finding that, amongst others, proteins involved in the MAP Kinase and Ras pathways are over-represented in the identified proteins.



Group U1 example

Overrepresented GO terms include:

- Response to stress
- Signal transduction
- MAPK cascade
- Regulation of phosphorylation

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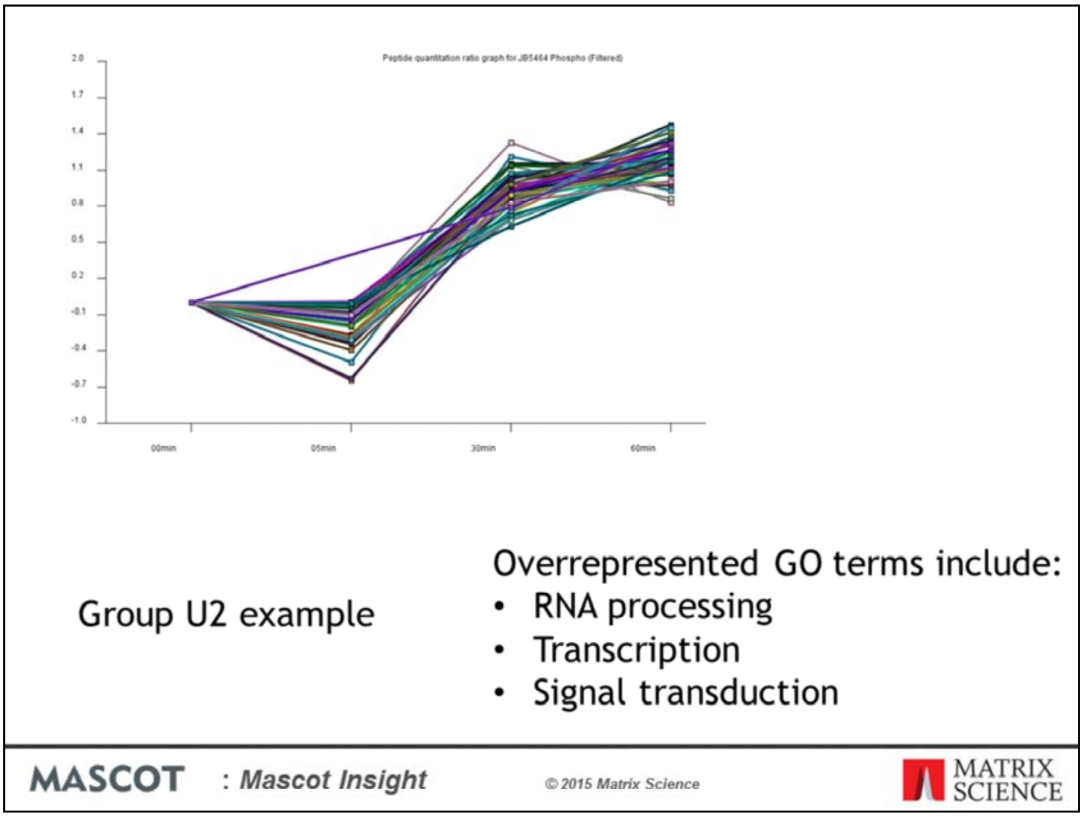
Mascot Insight has several reports available for clustering quantitation values from results. One clustering algorithm implemented in Mascot Insight is K-means clustering, which can be carried out at the protein or peptide level. Mertins et al. identified 6 different cluster types of phospho peptides which show significant changes over the timecourse; three groups of phospho peptides which show increased amounts over the time course and 3 which show a decrease – the differences in the groups relating to the median half-activation times for their maximum alteration; early, middle and late. If you use K-means clustering on the peptides identified as significantly changed by the peptide ANOVA, you can see the similar patterns to those found in Mertins et al. Here we have a cluster of Early increasing phospho peptides. Using the GO-proteome comparison report, overrepresented GO terms in this group include 'Response to stress', 'Signal transduction' – including the Ras pathway -, the MAPKinase pathway and regulation of phosphorylation.



Group U2 example

Overrepresented GO terms include:

- RNA processing
- Transcription
- Signal transduction

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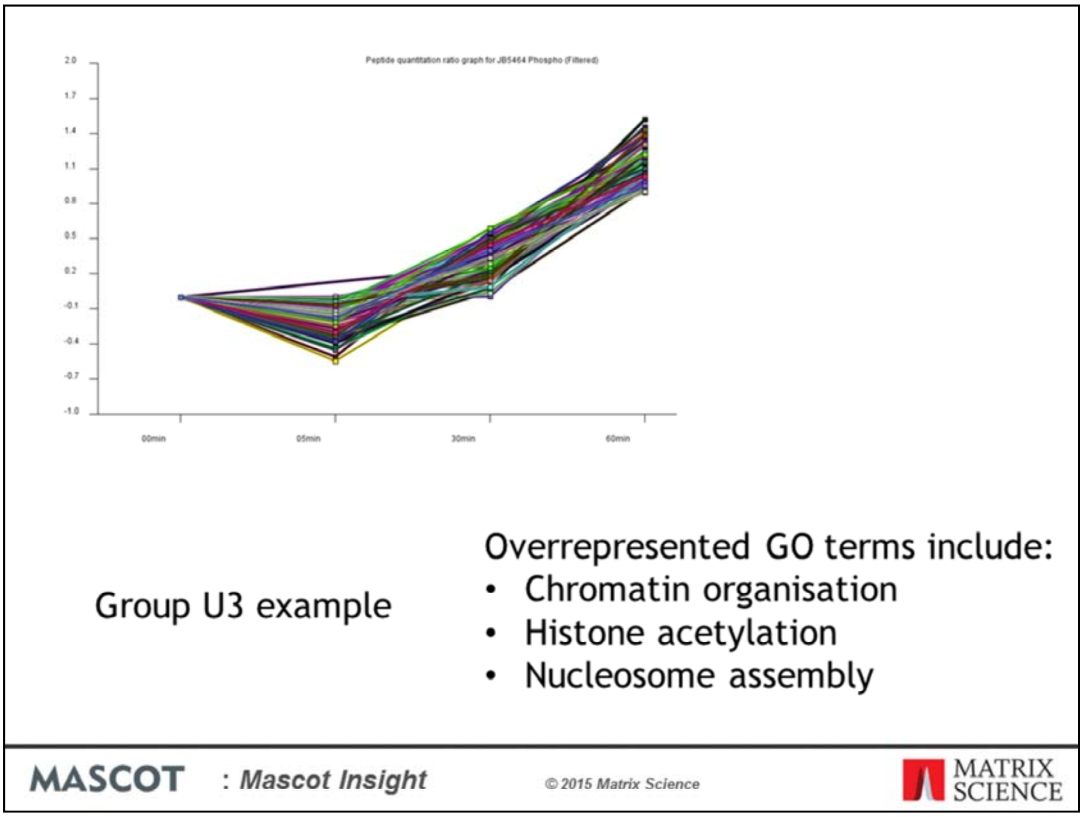
In the medium U2 group, we now see an overrepresentation of transcriptional regulators in addition to GO terms such as signal transduction and other terms found in group U1.



Group U3 example

Overrepresented GO terms include:

- Chromatin organisation
- Histone acetylation
- Nucleosome assembly

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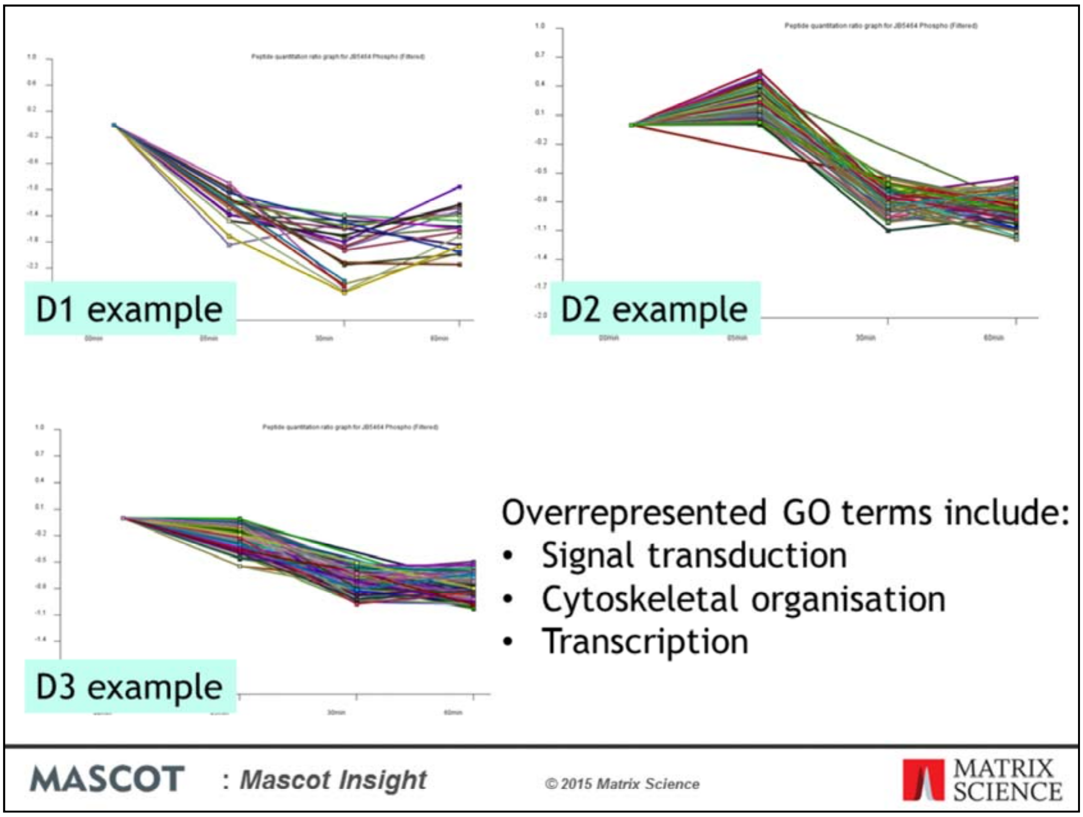
In the U3 group we see many of the same GO terms as found in groups U1 and U2, but in addition to these we start to see peptides from proteins involved in chromatin organisation and nucleosome assembly.



Overrepresented GO terms include:

- Signal transduction
- Cytoskeletal organisation
- Transcription

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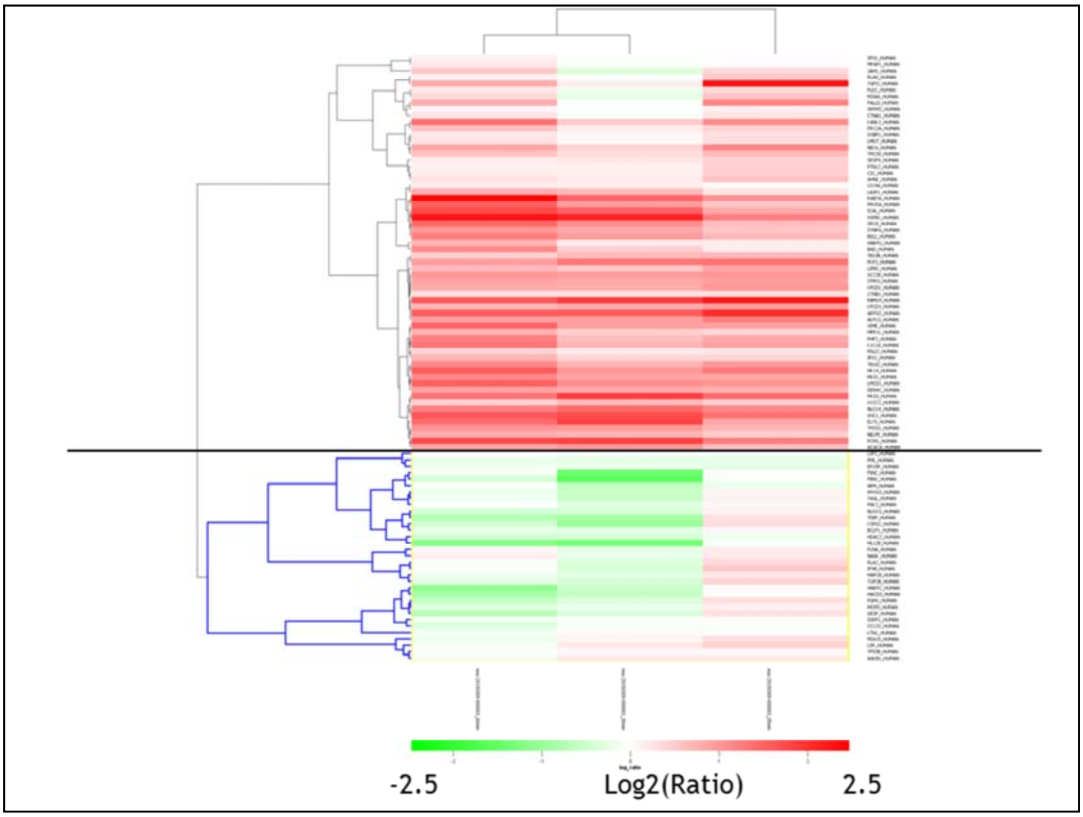
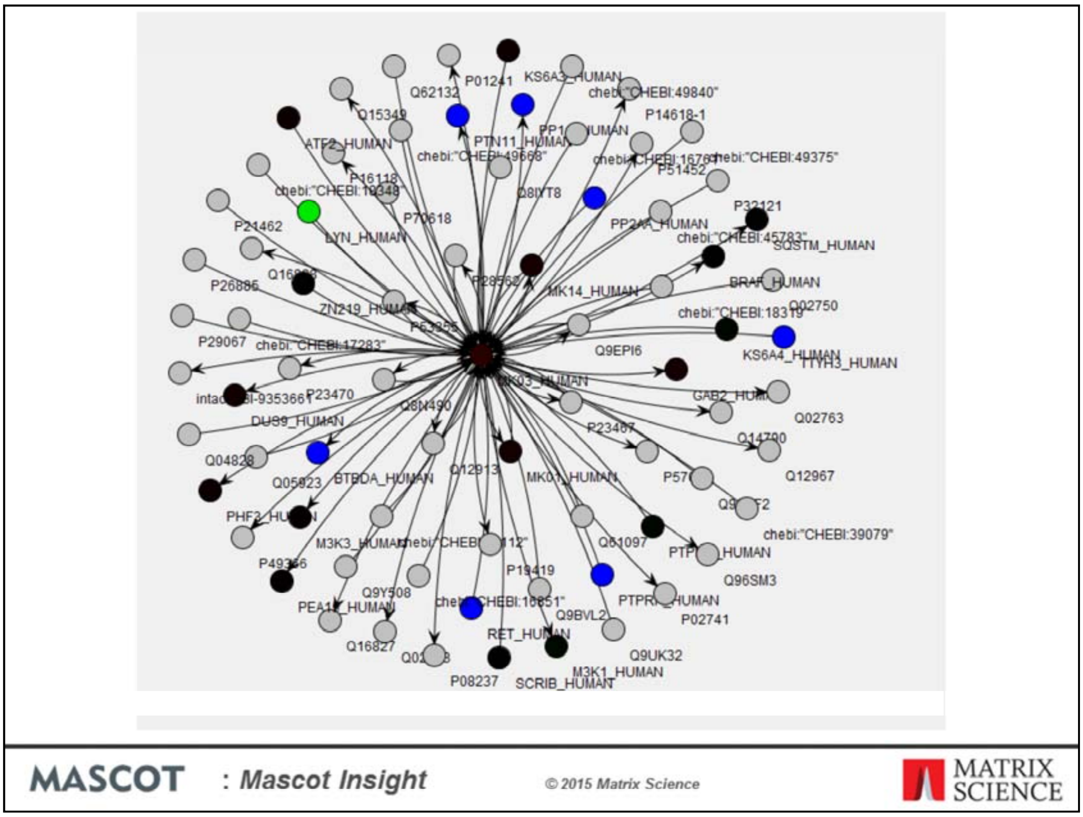
There is less variability in the proteins identified by phospho peptide groups D1-D3 (which show decreasing phosphopeptide amounts over the timecourse), but overrepresented GO terms include signal transduction, cytoskeletal organisation and transcription.



In addition to the K-means clustering report, Mascot Insight has a Hierarchical clustering report for protein quantitation data. Here, I've filtered one of the tumour phosphopeptide enriched samples to select only the peptides identified as showing significant changes after 5 minutes and carried out hierarchical clustering on the protein quantitation data calculated using just those peptide matches. On the diagram, red is showing increased iTRAQ ratios, and green decreased. As you can see this identifies two groups of proteins, one where the iTRAQ ratios are largely up and a smaller group which is mostly down – the identified proteins in the groups are similar to (a subset of) the proteins found for the U1 and D1 groups identified by the k-means clustering.



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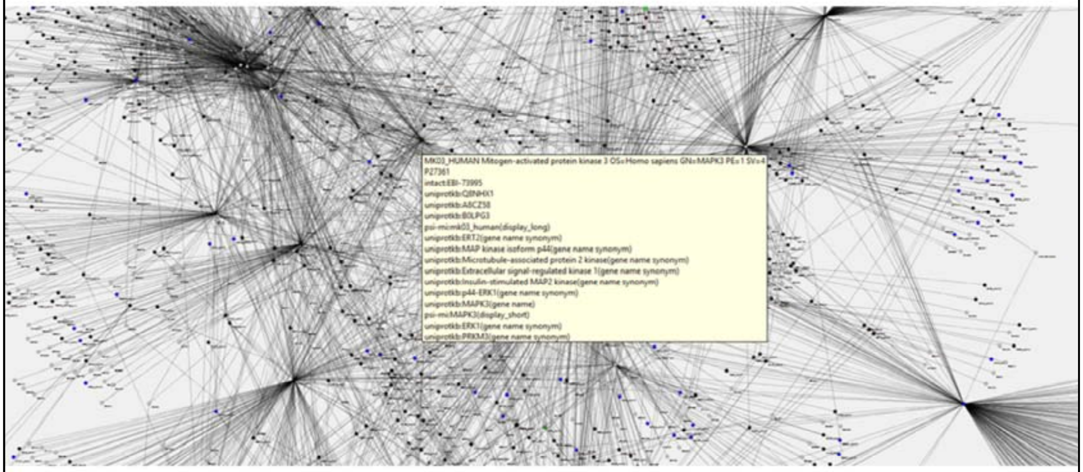
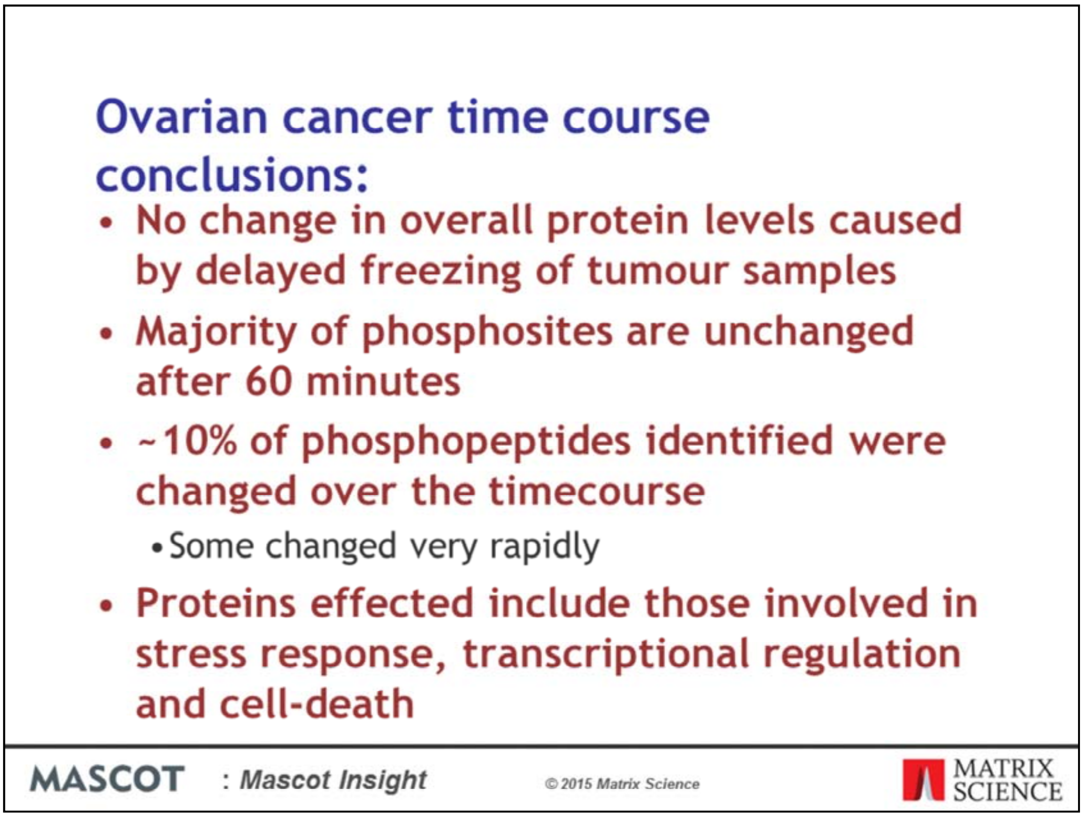
Mascot Insight can use any molecular interaction database in the PSI-MITAB standard format to generate a molecular interaction report for a selected protein. Here I've selected Map Kinase 2 and generated a report showing the known direct interactors for the protein. Where the node on the diagram is blue, green or red the interacting protein was identified in the search result, while those in grey were not. Blue denotes a protein which was identified but not quantified, while green through to red is used as a heatmap for proteins which were quantified.



MK03_HUMAN Mitogen-activated protein kinase 3 OS=Homo sapiens GN=MAPK3 PE=1 SV=4
P27361
intact:EBI-73995
uniprotkb:Q8NHX1
uniprotkb:A8CZ58
uniprotkb:B0LPG3
psi-mi:mk03_human(display_long)
uniprotkb:ERT2(gene name synonym)
uniprotkb:MAP kinase isoform p44(gene name synonym)
uniprotkb:Microtubule-associated protein 2 kinase(gene name synonym)
uniprotkb:Extracellular signal-regulated kinase 1(gene name synonym)
uniprotkb:Insulin-stimulated MAP2 kinase(gene name synonym)
uniprotkb:p44-ERK1(gene name synonym)
uniprotkb:MAPK3(gene name)
psi-mi:MAPK3(display_short)
uniprotkb:ERK1(gene name synonym)
uniprotkb:PRKM3(gene name synonym)

If you increase the report to include interactors of interactors, it generates a very large network – we're viewing a small section of it here. From the colour coding you can see that we've identified a large number of the known interacting proteins. In addition to a graphical export, the generated report can be exported back out in PSI-MITAB format, including details of whether the interacting protein was identified in the search result. There are a number of other tools, including the commonly used Cytoscape software, which can then import the generated PSI-MITAB file.



Ovarian cancer time course conclusions:

- No change in overall protein levels caused by delayed freezing of tumour samples
- Majority of phosphosites are unchanged after 60 minutes
- ~10% of phosphopeptides identified were changed over the timecourse
  - Some changed very rapidly
- Proteins effected include those involved in stress response, transcriptional regulation and cell-death

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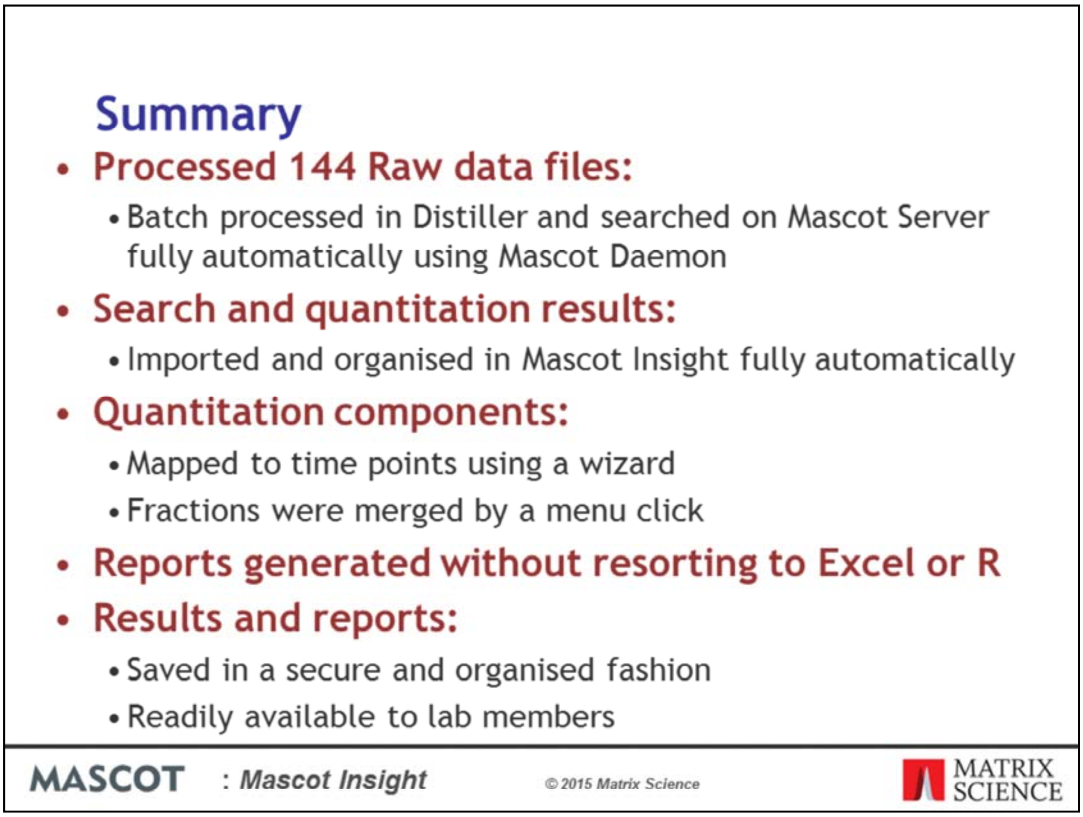
So conclusions from the Ovarian cancer time course – there is no overall change in protein levels caused by delayed freezing of tumour samples. When we look at the phosphoproteome, the majority of phosphopeptides are also unchanged across the course of the experiment. However, approximately 10% of the identified phosphopeptides did show significant changes across the time course, some of which occurred very rapidly. The proteins identified with altered phosphorylation include those involved in stress response, transcriptional regulation and cell-death.



Summary

- Processed 144 Raw data files:
  - Batch processed in Distiller and searched on Mascot Server fully automatically using Mascot Daemon
- Search and quantitation results:
  - Imported and organised in Mascot Insight fully automatically
- Quantitation components:
  - Mapped to time points using a wizard
  - Fractions were merged by a menu click
- Reports generated without resorting to Excel or R
- Results and reports:
  - Saved in a secure and organised fashion
  - Readily available to lab members

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So in summary, the 144 Raw data files which made up this dataset were batch processed in Mascot Distiller and searched on Mascot Server fully automatically using Mascot Daemon.

The search results were imported into Mascot Insight and organised into a logical folder structure fully automatically.

Quantitation components were mapped to time points using a wizard and the separate fractions merged from a menu click.

Reports were generated on the dataset without having to resort to Excel or R

The results and reports are saved in a secure and organised fashion and are readily available to any lab members who want to take a closer look at any aspect of the data or generate more reports