MASCOT*Integra*

Data management for Proteomics

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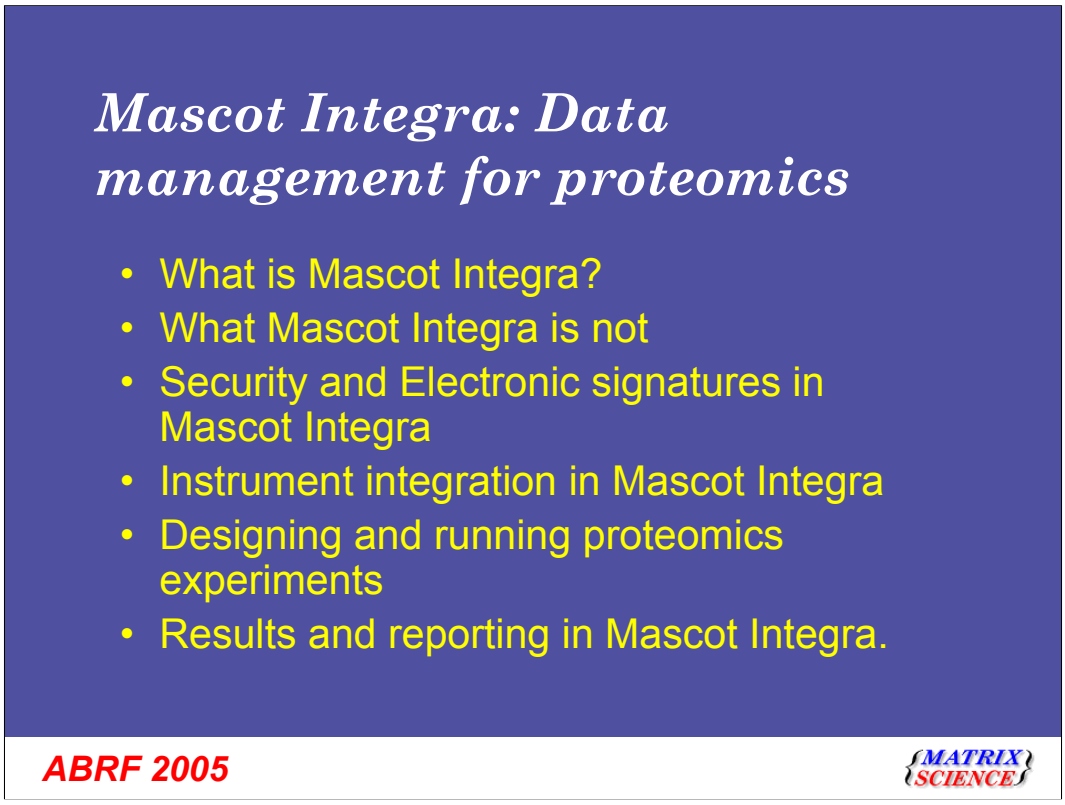
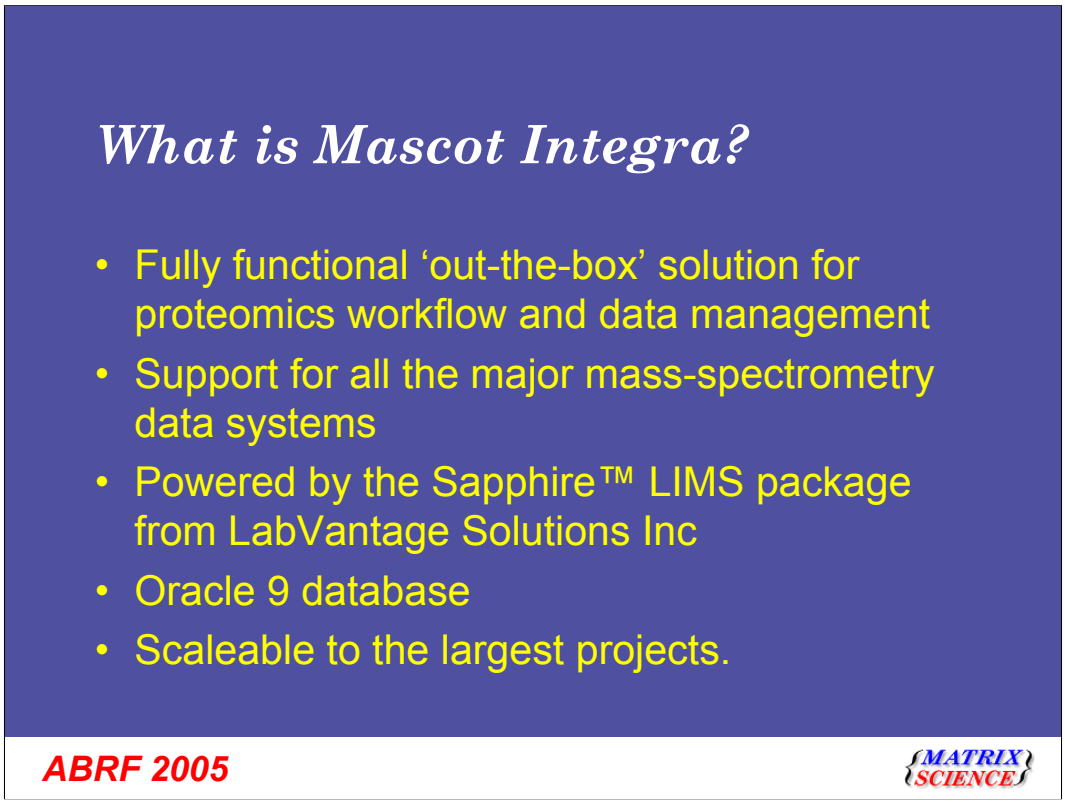


Mascot Integra is our new solution for proteomics sample and data management.

During this talk, I will be discussing these topics



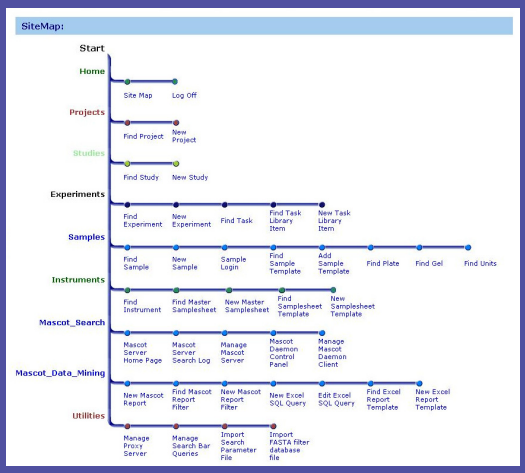
Mascot Integra is supplied as a ready to run system. It does not require the extensive setup and customisation associated with a traditional LIMS package.

Rather than re-invent the wheel, we have partnered with LabVantage Solutions Inc, (www.lims.com). Their Sapphire LIMS package provides the sample tracking and workflow modelling functionality for Mascot Integra

Using the Oracle database management system enables the database to scale efficiently as your data management requirements grow

What is Mascot Integra?

- **Laboratory Information Management for**
  - Sample tracking
  - Isolation / fractionation / purification
  - Splitting / combining
  - 1D and 2D Chromatography
  - 1D and 2D Electrophoresis
  - Image analysis
  - Spot picking
  - Digestion / derivatisation
  - MS and MS/MS analysis
  - MS data reduction
  - Mascot database search
  - Data warehouse
  - Result reporting
  - Data mining



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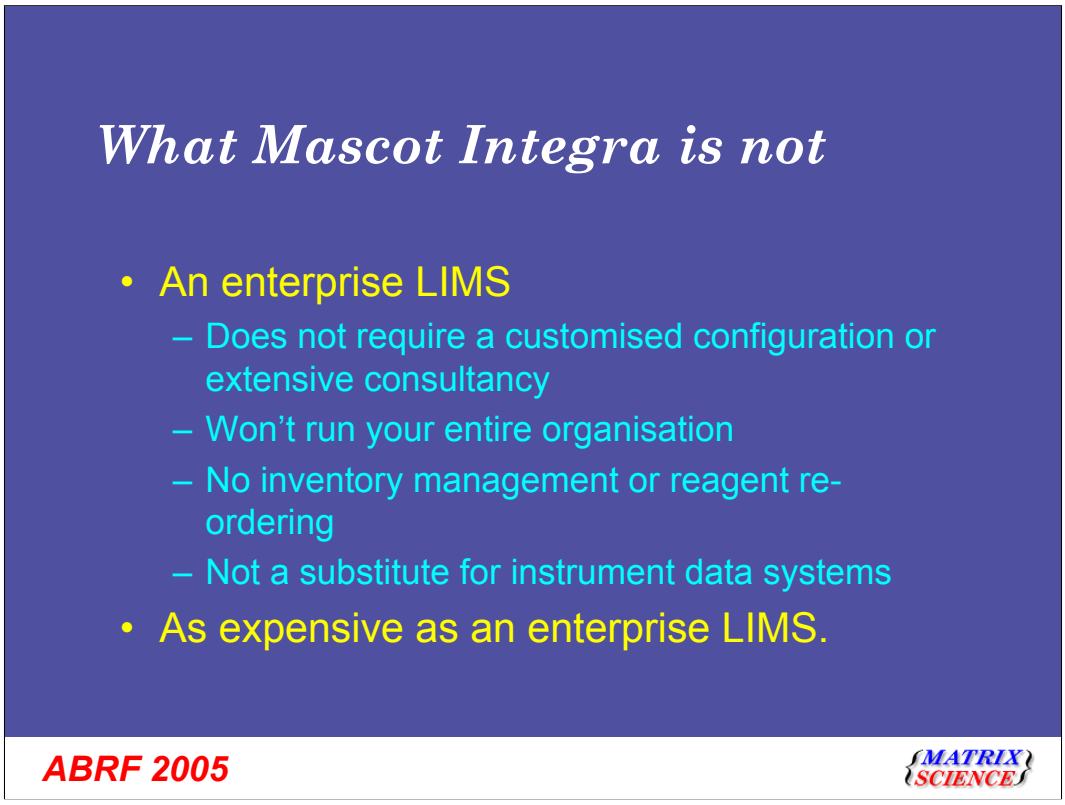
We aim to support all aspects of the workflows associated with Proteomics laboratory processes.



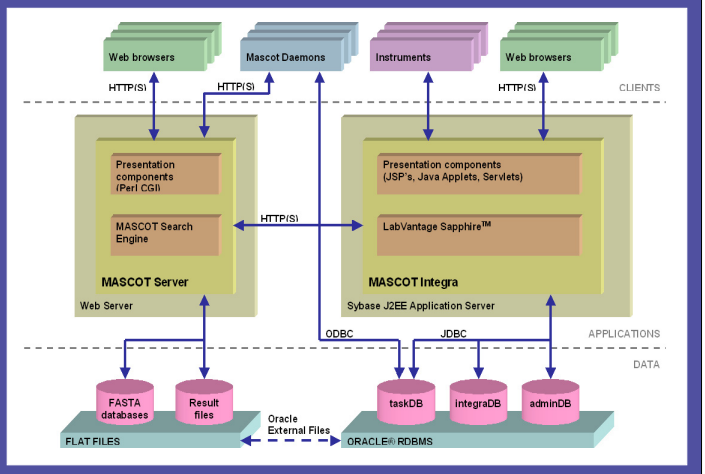
Mascot Integra will supply the LIMS functionality required to run a proteomics facility. It is not an "enterprise" LIMS system, and does not contain functionality for (say) genomics experiments

LabVantage Solutions Inc

- **LabVantage LIMS expertise**
  - 24 year track record
  - Scale up to LabVantage Enterprise LIMS products
  - Call on World-Wide Professional Support Services

Mascot Integra architecture

- 3 tier system
  - Oracle database server
  - Sybase Enterprise Application Server running a J2EE web application
  - All user functionality available through Internet Explorer



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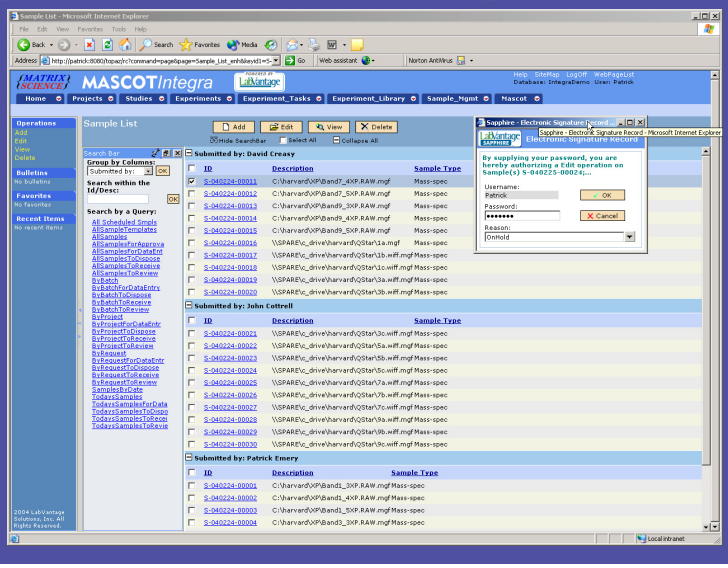
All Mascot Integra functionality is accessible through a standard web browser.

CFR21 part 11 ERES compliant

- Electronic signatures
- Maintains audit trail
- Role and status based user access
- Automatic inactivity logout (definable period)



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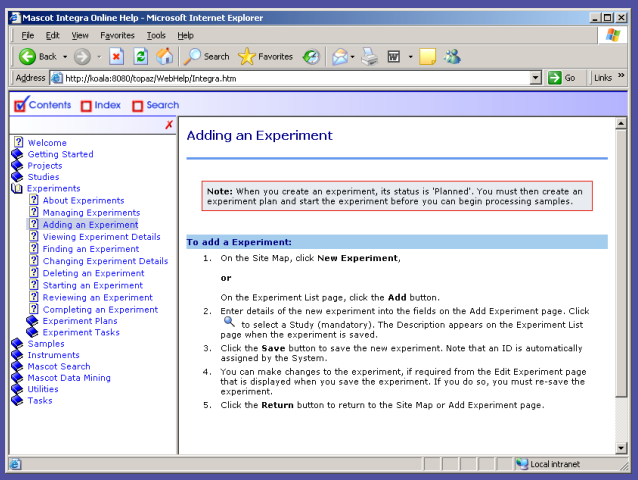
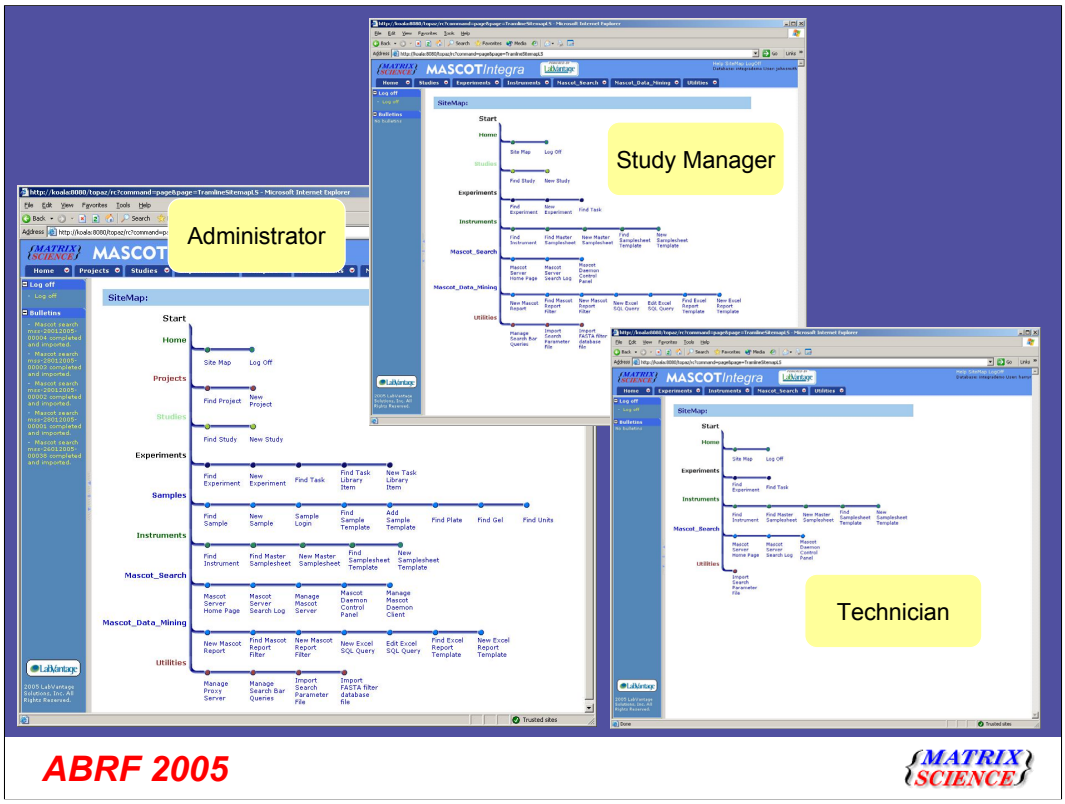
Mascot Integra complies with the requirements of FDA CFR21 part 11 ERES

Online help

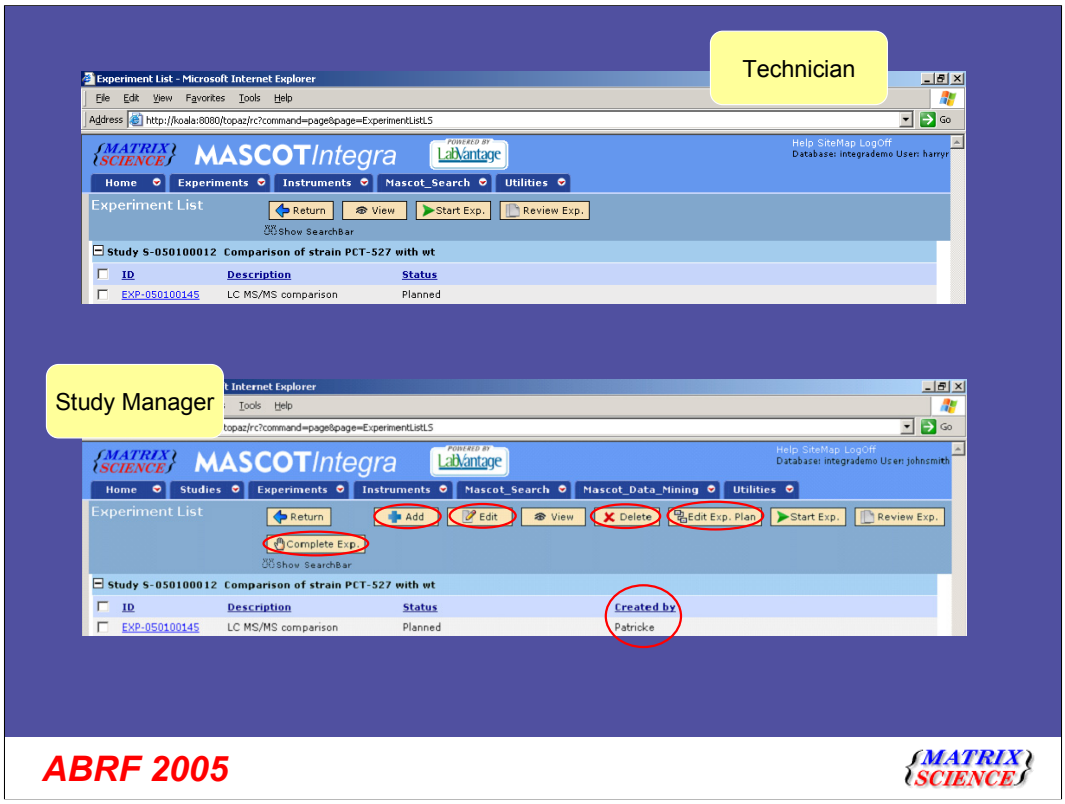
- Extensive context sensitive help available on web-based help system



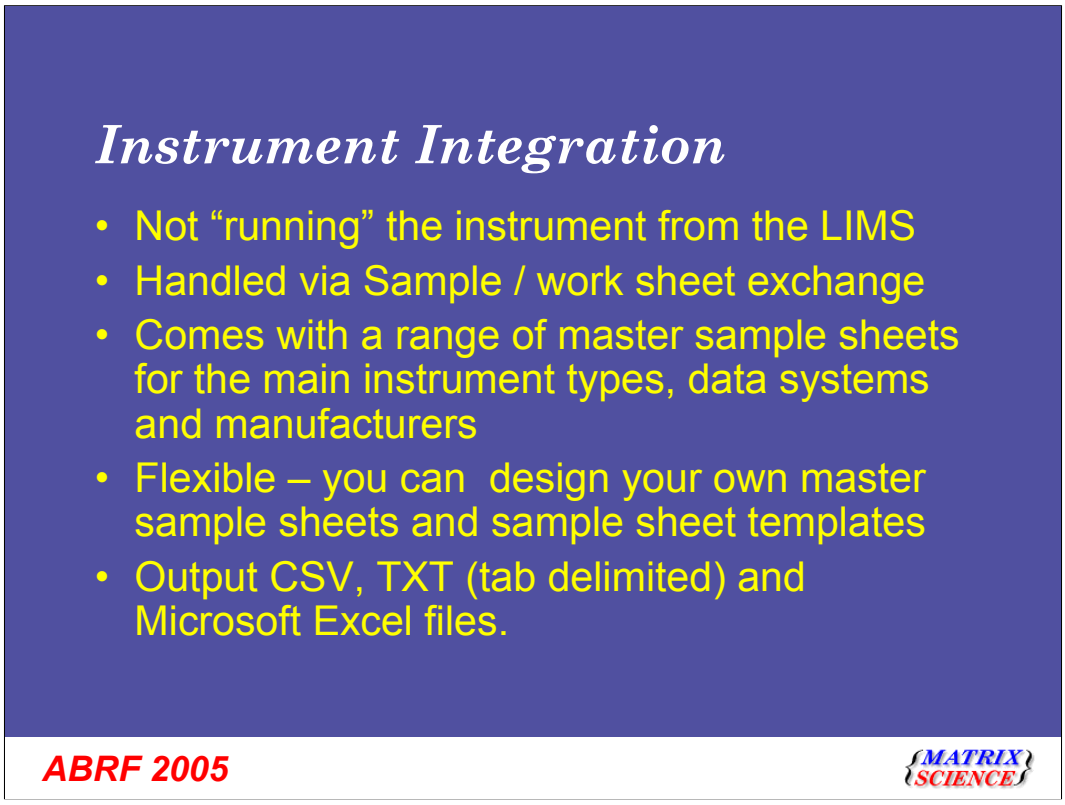
Users can be assigned to multiple projects/studies and assigned multiple roles (e.g. a user can be both a Study Manager and an Analyst)



To illustrate this, the administrator has full access to the system and sees all the possible links from the Mascot Integra site-map. The study manager has more limited access to the system (for example, the project links are not visible to them). The technician sees only the links required to perform specific tasks.

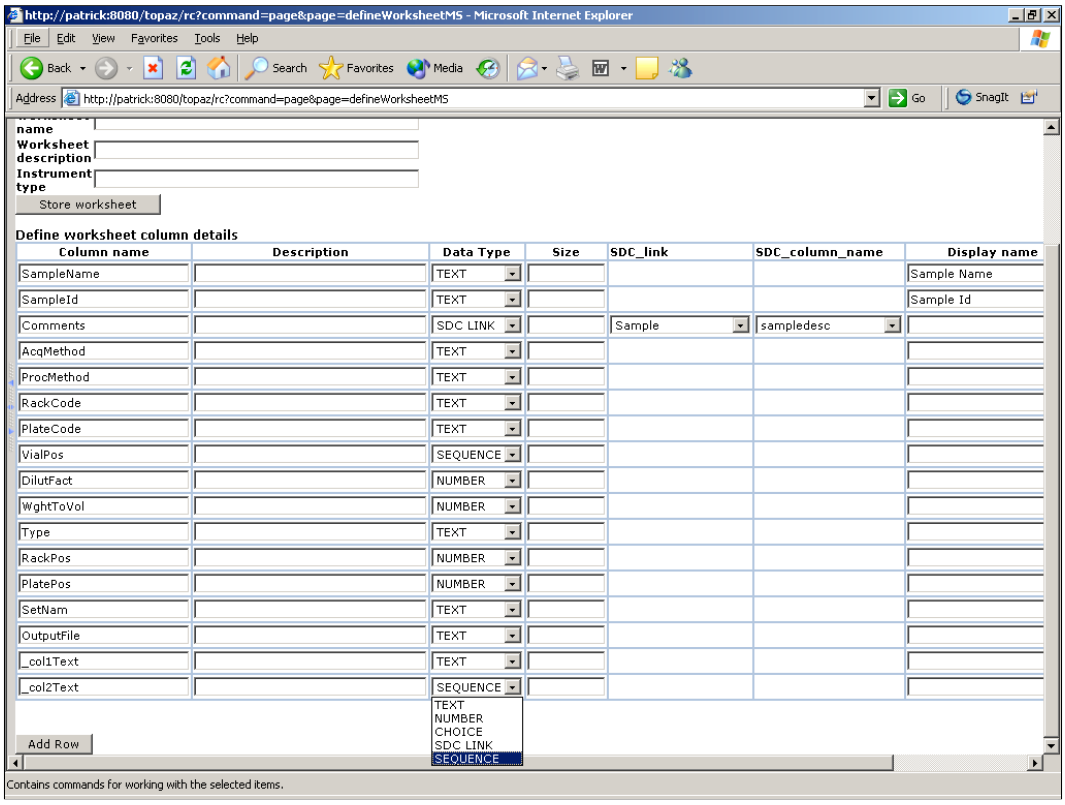


In addition to limiting access to complete pages, individual controls on a page can be altered according to the user's role. Here we have the same page (a list of available experiments) as viewed by a technician and a study manager.



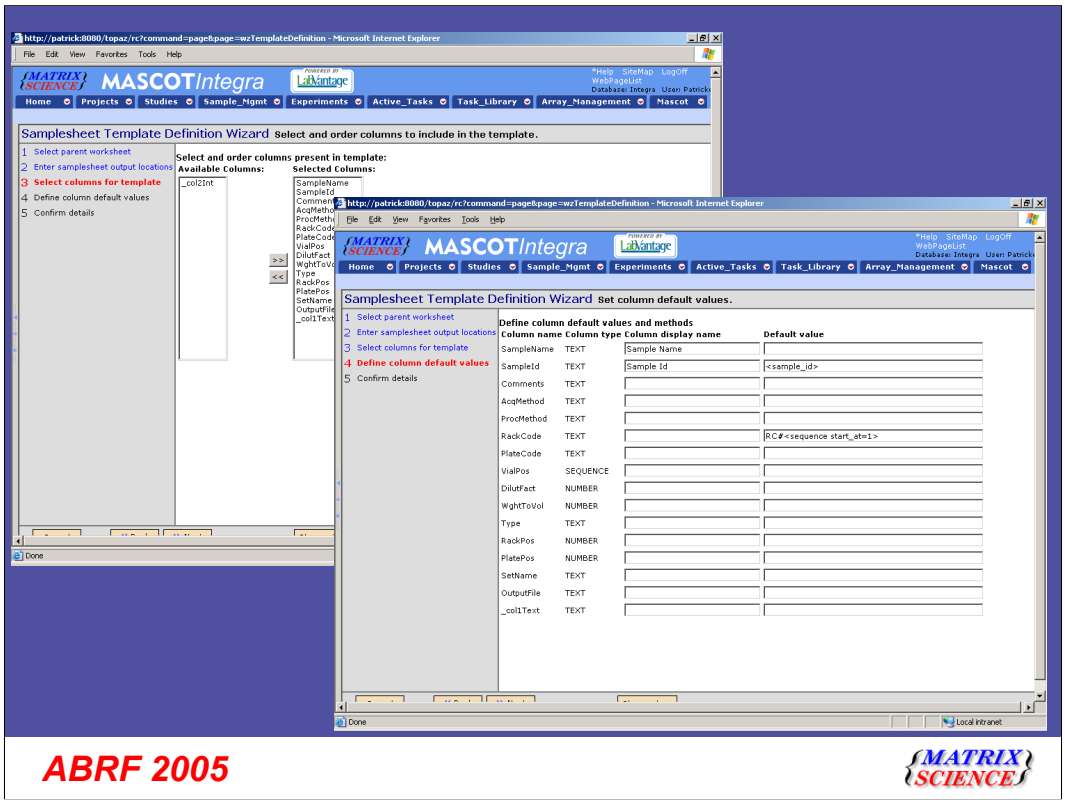
Instrument integration is handled via sample sheet exchange.

Our aim is to minimise the requirement for copying data from the output of one instrument data system to the input of another. This reduces the time involved in setting up instrument runs and the opportunity for errors.



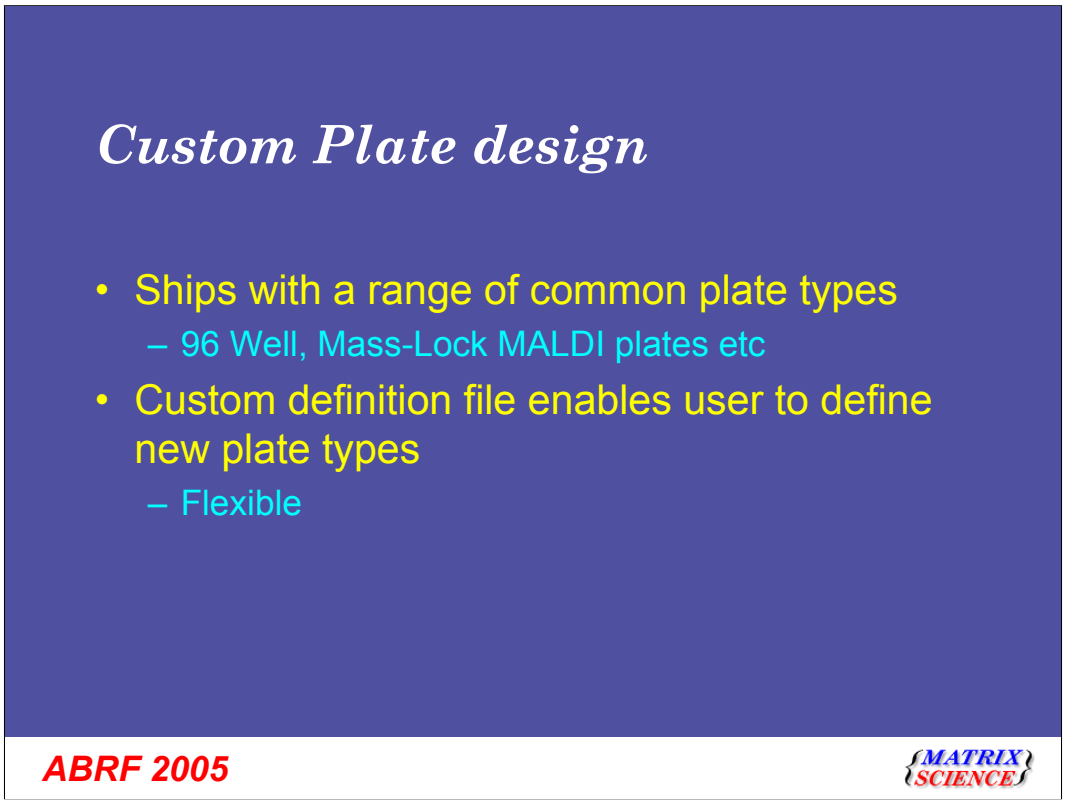
For a new instrument, there is a one-time setup to design a master sample sheet containing all the possible data elements supported by the instrument data system.

In addition to standard data types (text, numeric etc) you can specify a column as an SDC link. This means that the column defines a live link back to data held in a field in the Mascot Integra database. The data retrieved can be determined by the sample identifier associated with the row of the sample sheet.

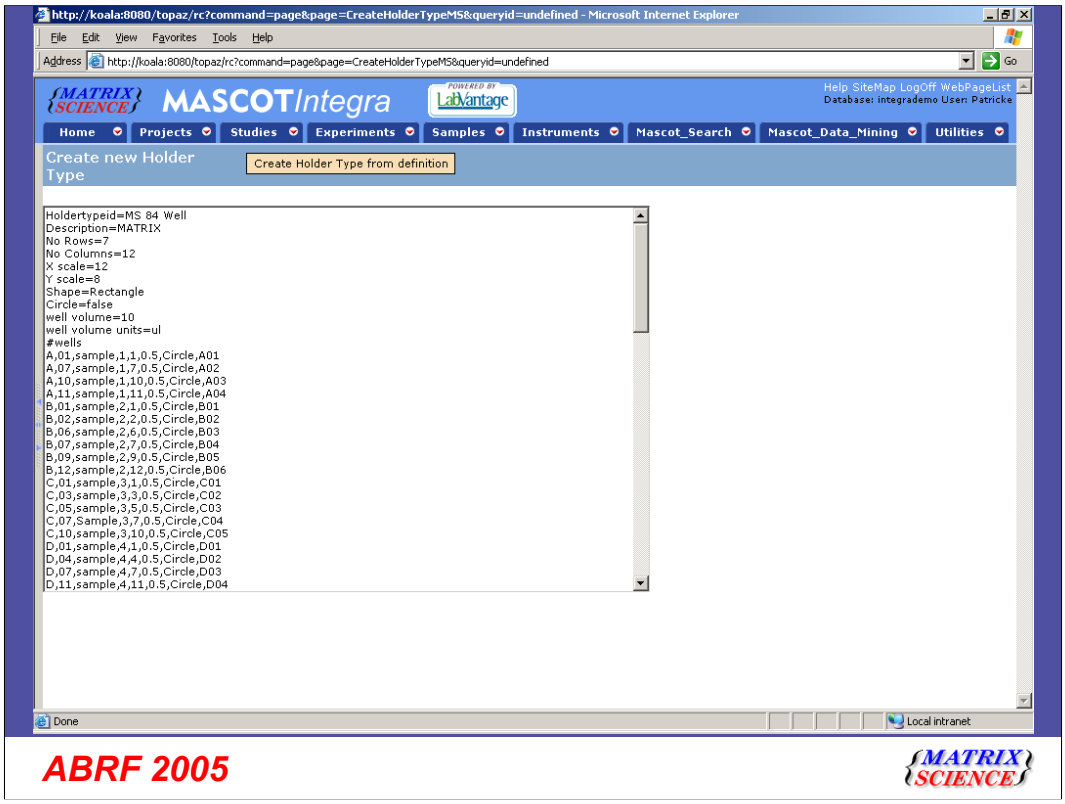


Once the master sample sheet has been defined, a subset of the columns is selected for a sample sheet template.

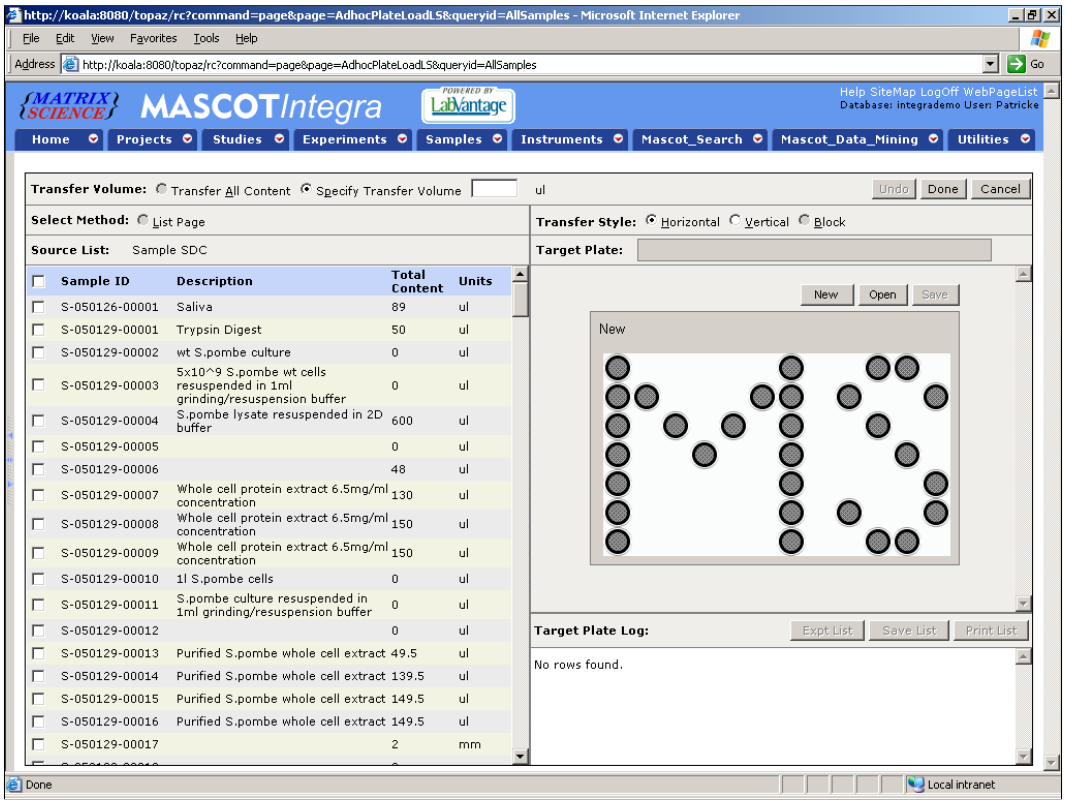
At this stage, you can specify default values for the columns



A wide variety of MALDI target plates are used by different instruments. To accommodate additional or specialist plate types, Mascot Integra even allowsyou to define new plate types.



The format is self explanatory. The definition on this slide produced the plate shown in the next slide…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Home Projects Studies Experiments Samples Instruments Mascot_Search Mascot_Data_Mining Utilities
Transfer Volume: Transfer All Content Specify Transfer Volume ul
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Select Method: List Page
Transfer Style: Horizontal Vertical Block
Source List: Sample SDC
Target Plate:
Sample ID Description Total Content Units
S-050126-00001 Saliva 89 ul
S-050129-00001 Trypsin Digest 50 ul
S-050129-00002 wt S.pombe culture 0 ul
S-050129-00003 5x10^9 S.pombe wt cells resuspended in 1ml grinding/resuspension buffer 0 ul
S-050129-00004 S.pombe lysate resuspended in 2D buffer 600 ul
S-050129-00005 0 ul
S-050129-00006 48 ul
S-050129-00007 Whole cell protein extract 6.5mg/ml concentration 130 ul
S-050129-00008 Whole cell protein extract 6.5mg/ml concentration 150 ul
S-050129-00009 Whole cell protein extract 6.5mg/ml concentration 150 ul
S-050129-00010 1l S.pombe cells 0 ul
S-050129-00011 S.pombe culture resuspended in 1ml grinding/resuspension buffer 0 ul
S-050129-00012 0 ul
S-050129-00013 Purified S.pombe whole cell extract 49.5 ul
S-050129-00014 Purified S.pombe whole cell extract 139.5 ul
S-050129-00015 Purified S.pombe whole cell extract 149.5 ul
S-050129-00016 Purified S.pombe whole cell extract 149.5 ul
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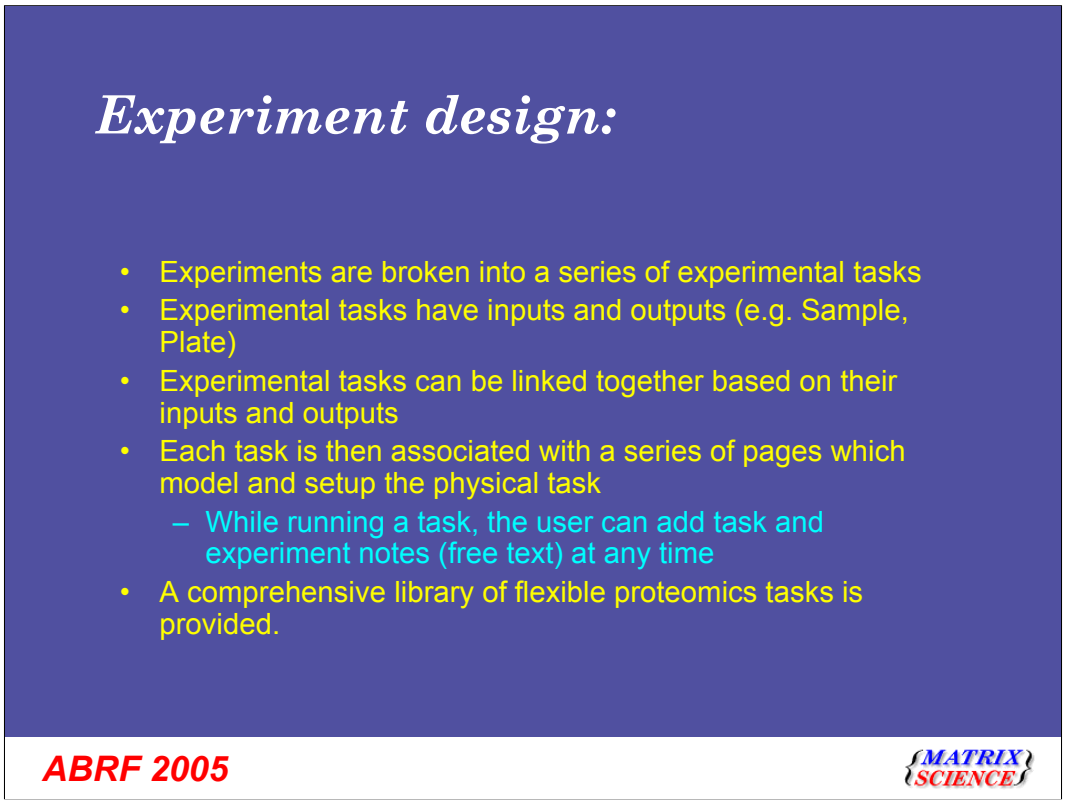
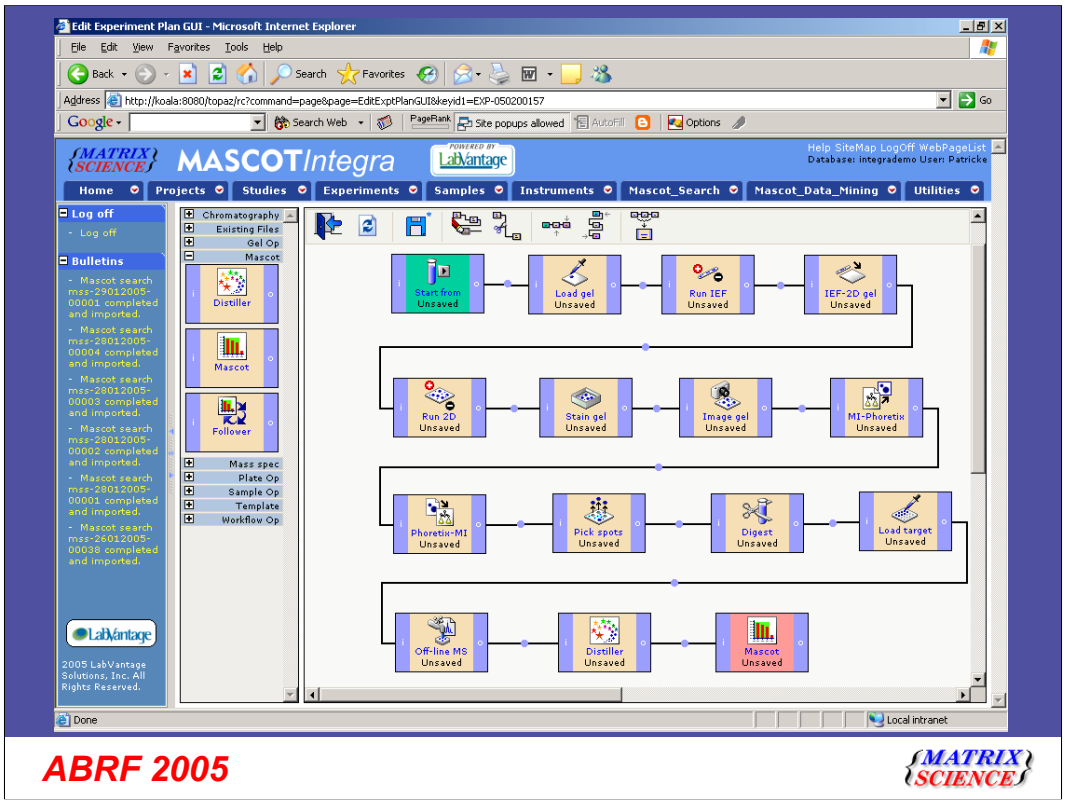


Individual tasks in an experiment represent discrete laboratory processes e.g. running a 2D gel, loading a 96 well plate, enzyme digestion etc.

For the experimental task of loading a MALDI target plate, the input would be a a sample list, and the output would be a loaded target.

Experimental tasks can have multiple inputs and outputs.

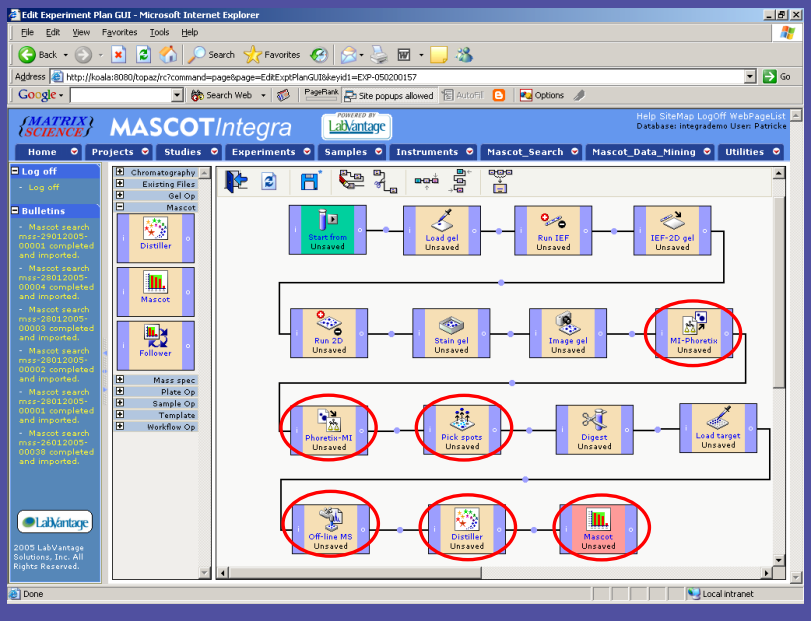


A complete workflow can be saved as a new experimental task, which is available from the task list menu bar. Any default values which were entered against the experimental tasks would also be saved in this new template. This provides a simple method for setting up the default workflows for your laboratory.

Running experiments:

- Now we'll go through setting up and running a common type of proteomics experiment
  - 2D Gel analysis
    - Image analysis with Nonlinear Dynamics Phoretix™ Software
    - MS run
    - Defining Raw data processing with Mascot Distiller
    - Setting up a batch search with Mascot Daemon
    - Running and importing Mascot searches
    - Examining the results

Simple 2D Gel analysis workflow



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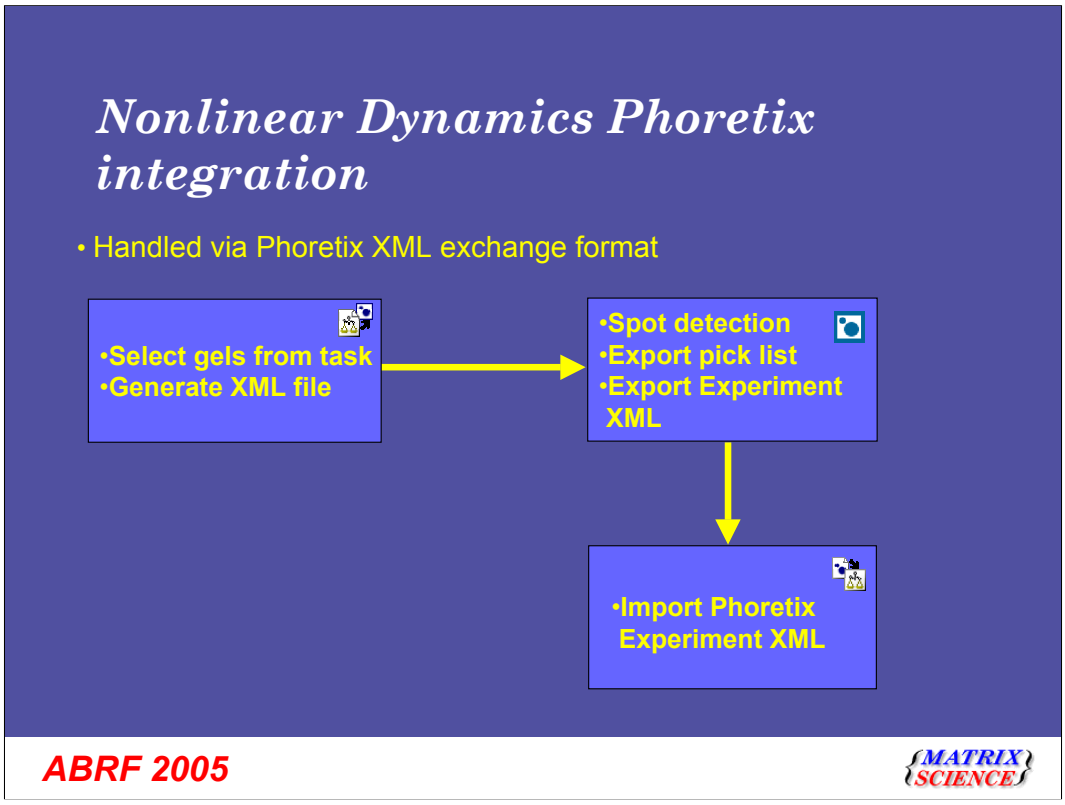
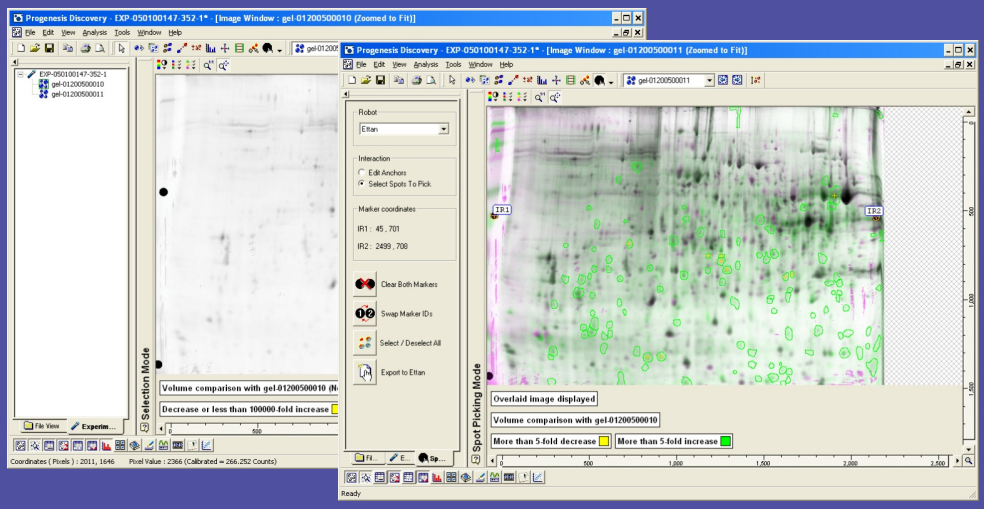
Rather than running through the entire work flow, we'll just be looking at the circled tasks. These define the Mascot Integra interfaces to the NonLinear Dynamics Phoretix package, to the mass-spec data system, and to the Mascot search engine.



In this workflow, we are tracking the loading of the gels with samples, and the capture of the stained gel images. The information is transferred between Mascot Integra and Phoretix using the Phoretix XML Exchange Format.

Spot detection and picklist generation (for spot cutting robots) is handled by Phoretix. This information is the imported back into Mascot Integra.

Nonlinear Dynamics Phoretix integration 2



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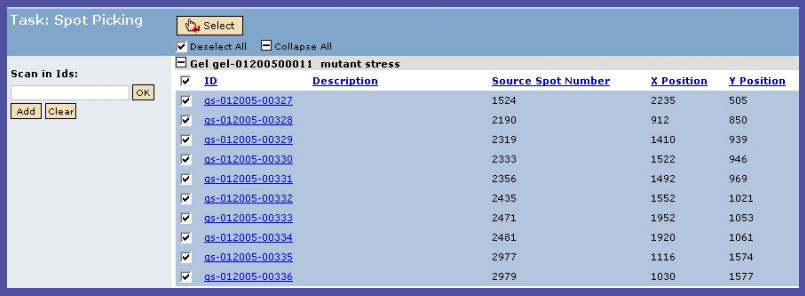
Nonlinear Dynamics Phoretix integration 3

- Import the Phoretix experiment XML export file into Integra



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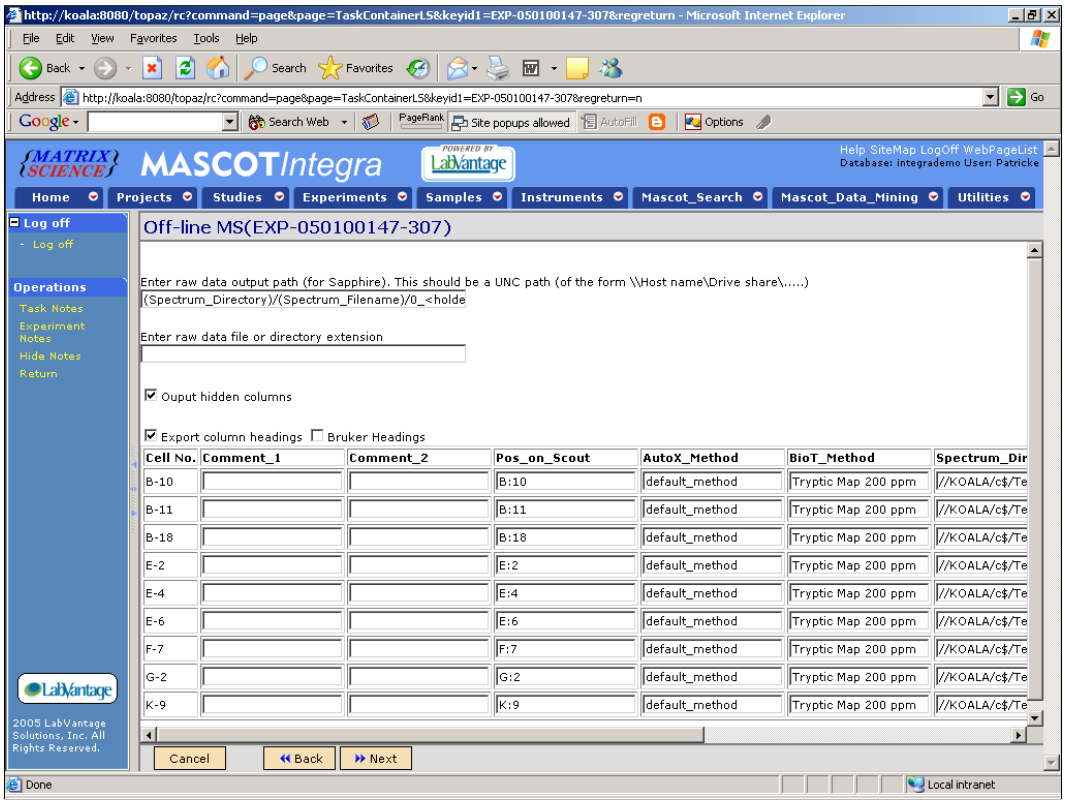
We can choose to import all the detected spots or just those spots which we have chosen for picking.



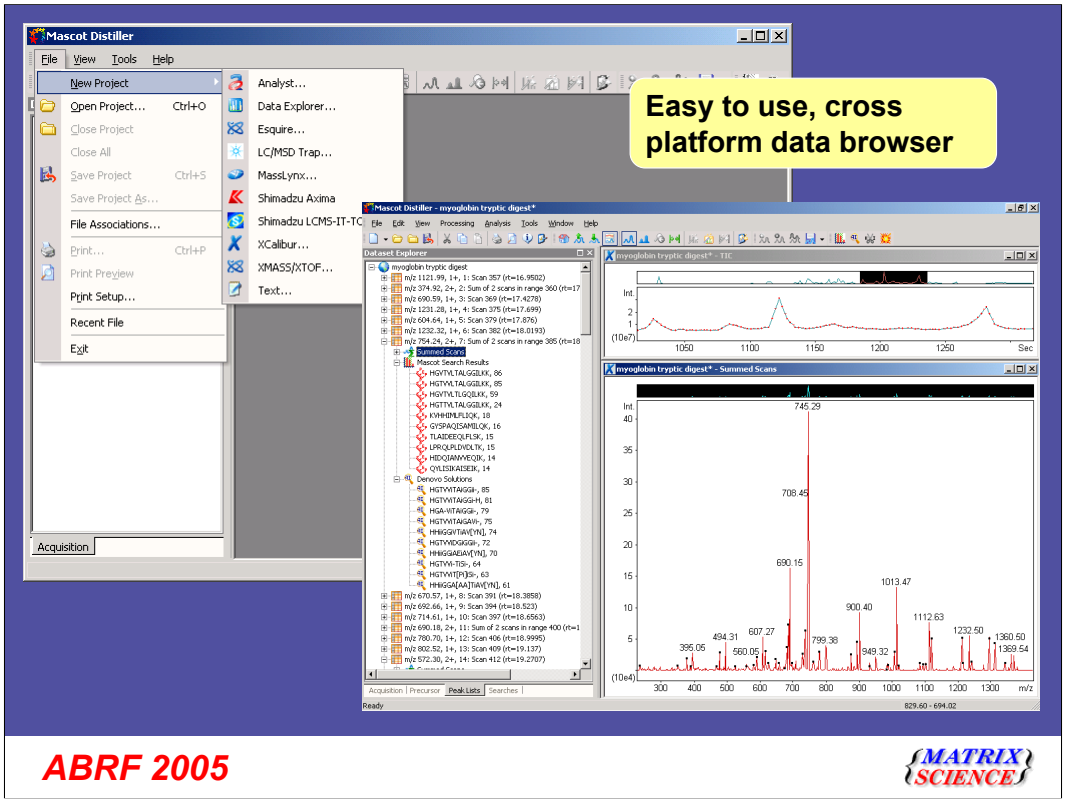
Next, we select a pre-defined template for a Bruker TOF instrument samplesheet

Some values are automatically filled in from the database

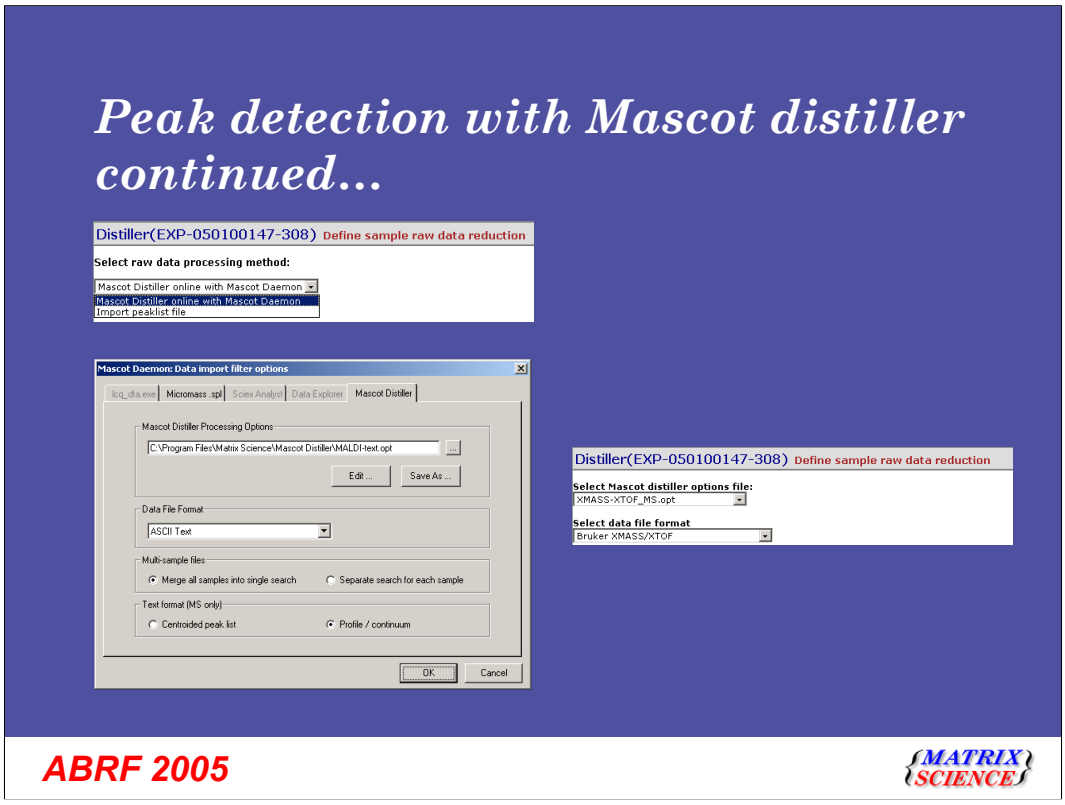
Export as CSV or xls format file for import into Bruker's FlexControl data system

Peak detection with Mascot Distiller

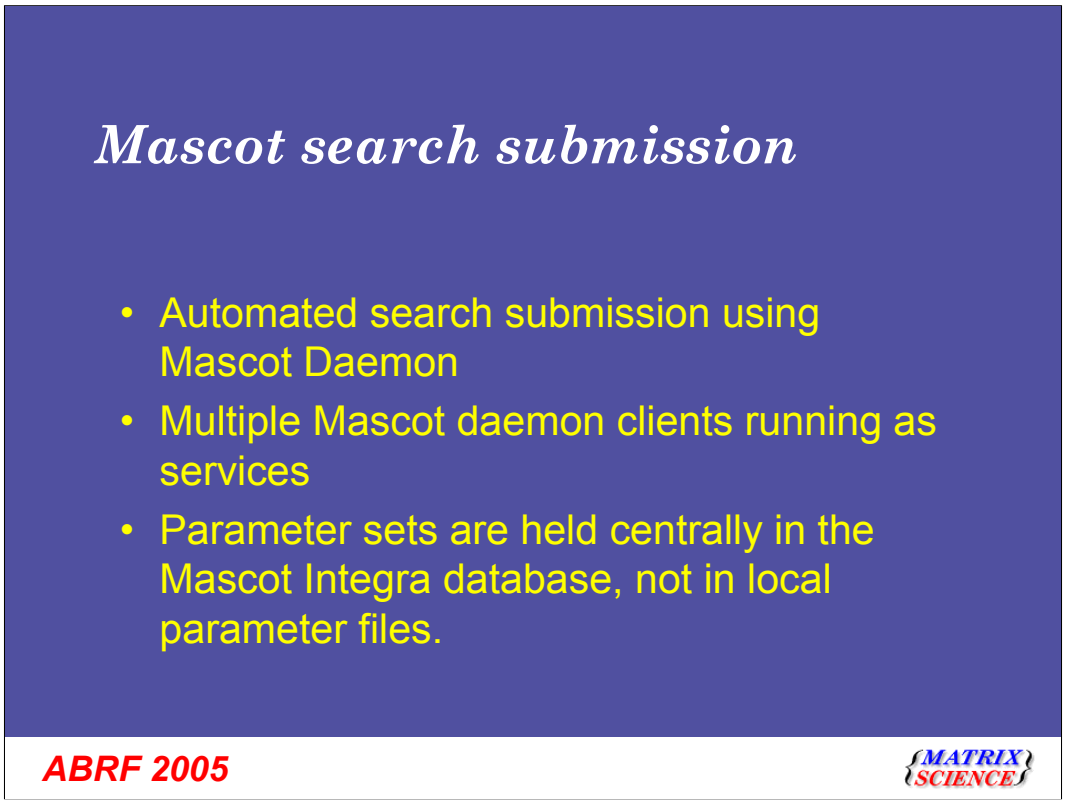
- Mascot distiller used for automated peak detection
- Used in conjunction with Mascot Daemon for automated search submission
- Import of peak lists created by other software is supported.



Mascot Distiller offers optimised peak detection for all the popular raw data formats.



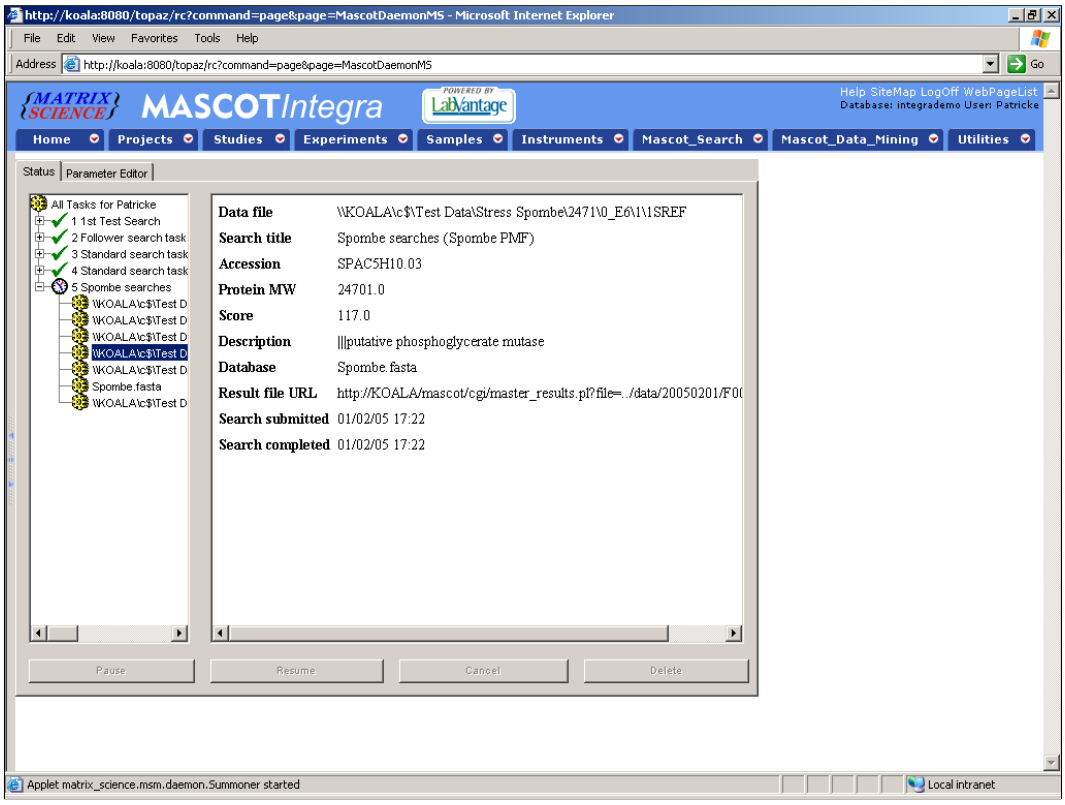
The Mascot Distiller library is used as a data import filter by Mascot Daemon



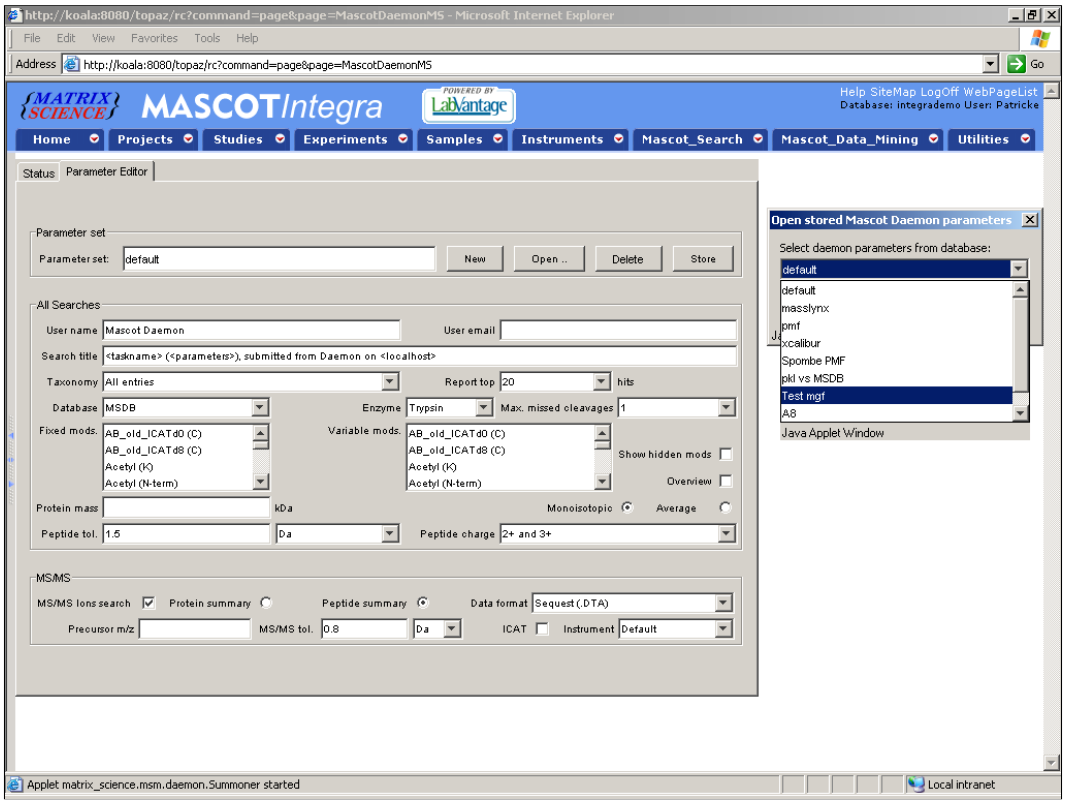
Mascot search submission is handled by Mascot Daemon.

A common arrangement is to have a Mascot Daemon client on each instrument data system, all communicating with the Mascot Integra database. Users assign processing and search tasks to the relevant Mascot Daemon client via the Mascot Integra interface.

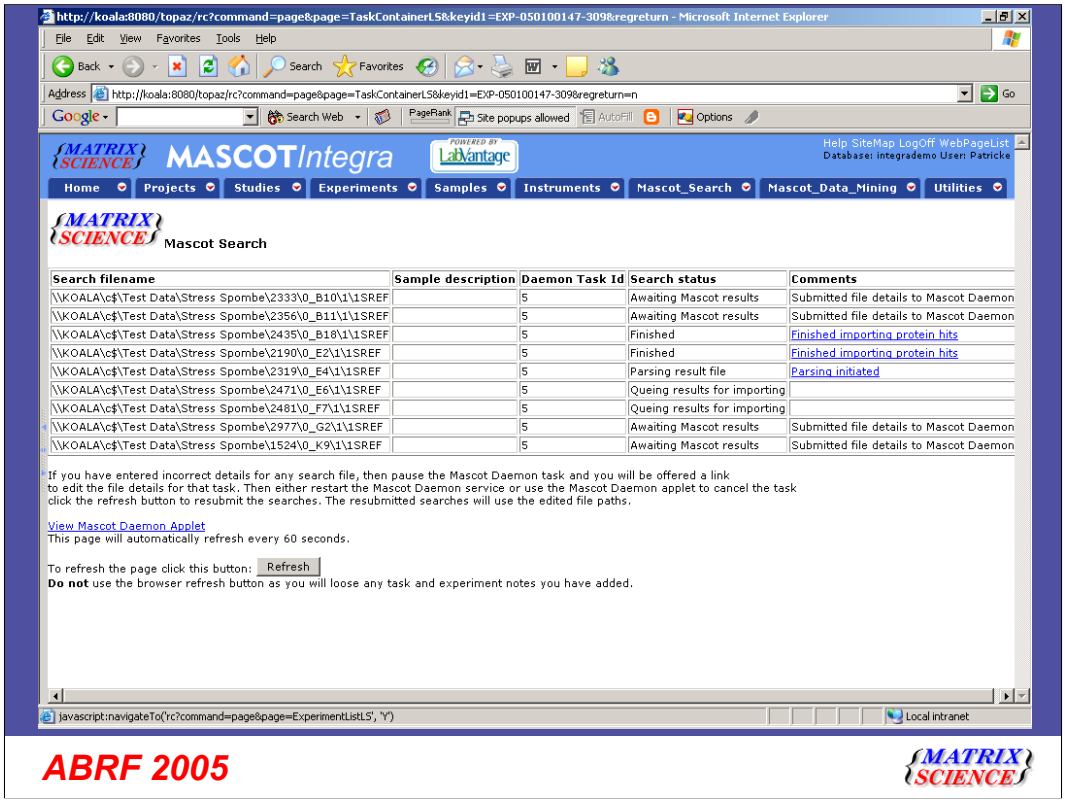
Parameter sets are held in the central database tables, ensuring that the parameter sets used by all the Mascot Daemon clients are identical.



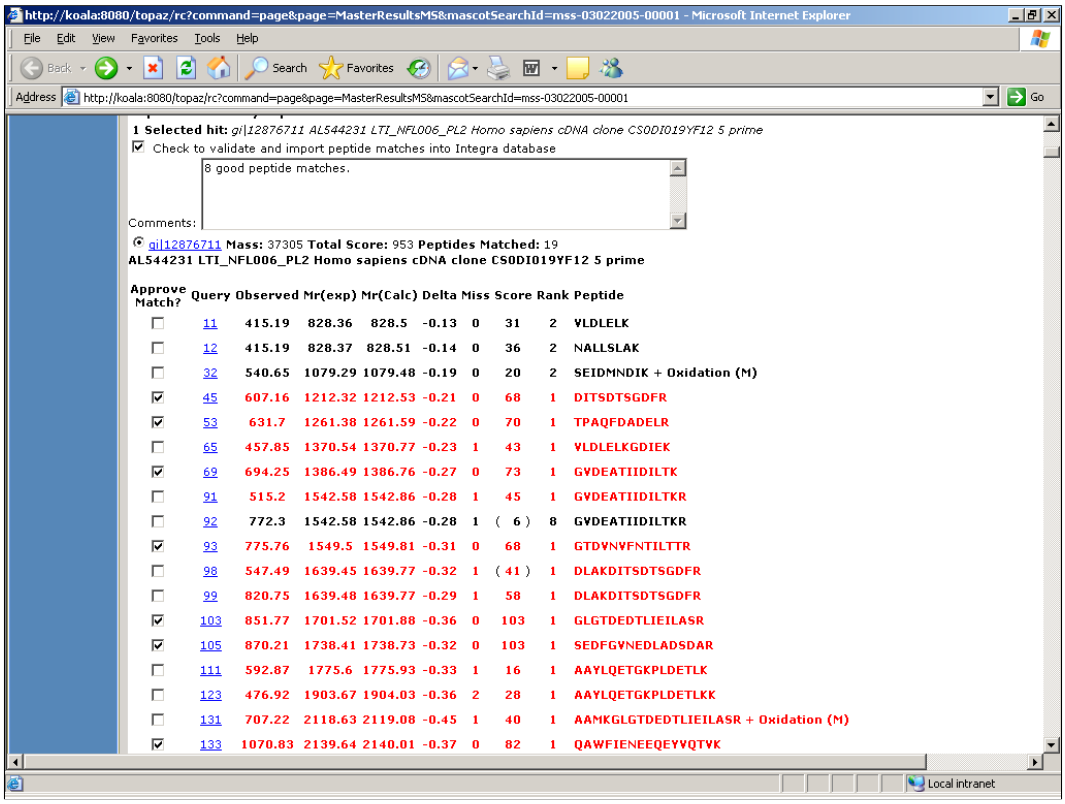
This looks like Mascot Daemon, but it is actually a Java Applet hosted in the web browser. This can be used for monitoring and managing all of the search tasks on all of the Mascot Daemon clients.



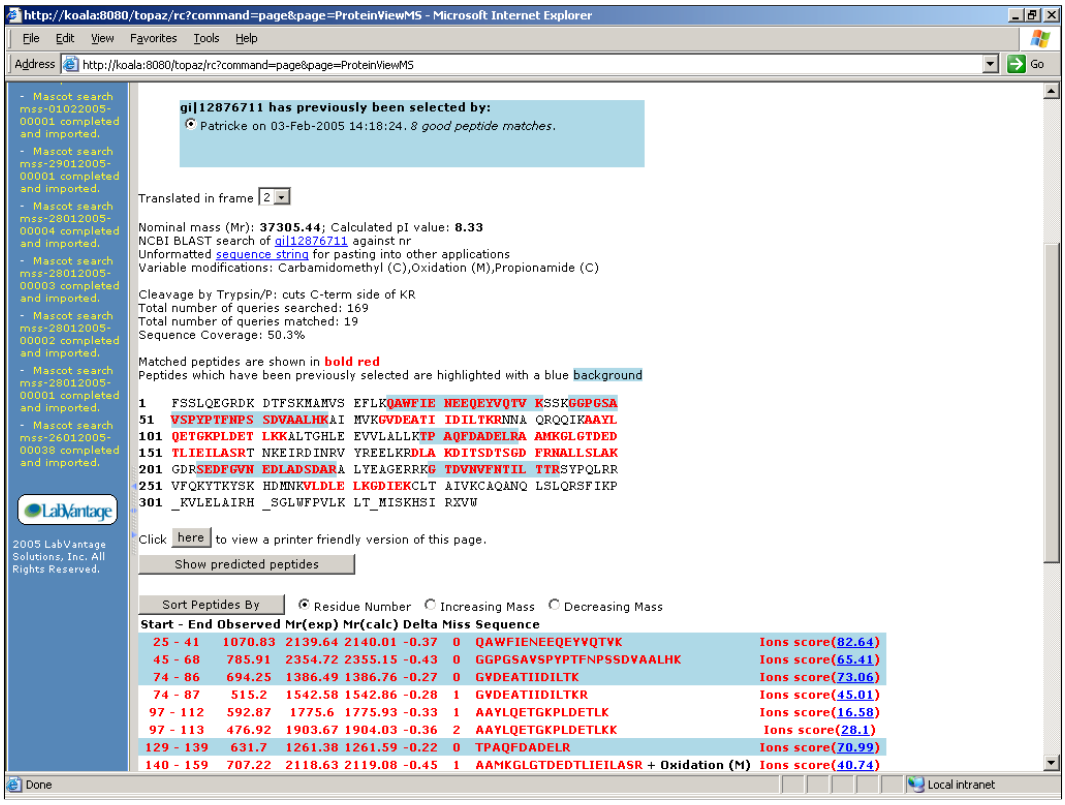
The Java applet is also used to managee the central sets of search parameters



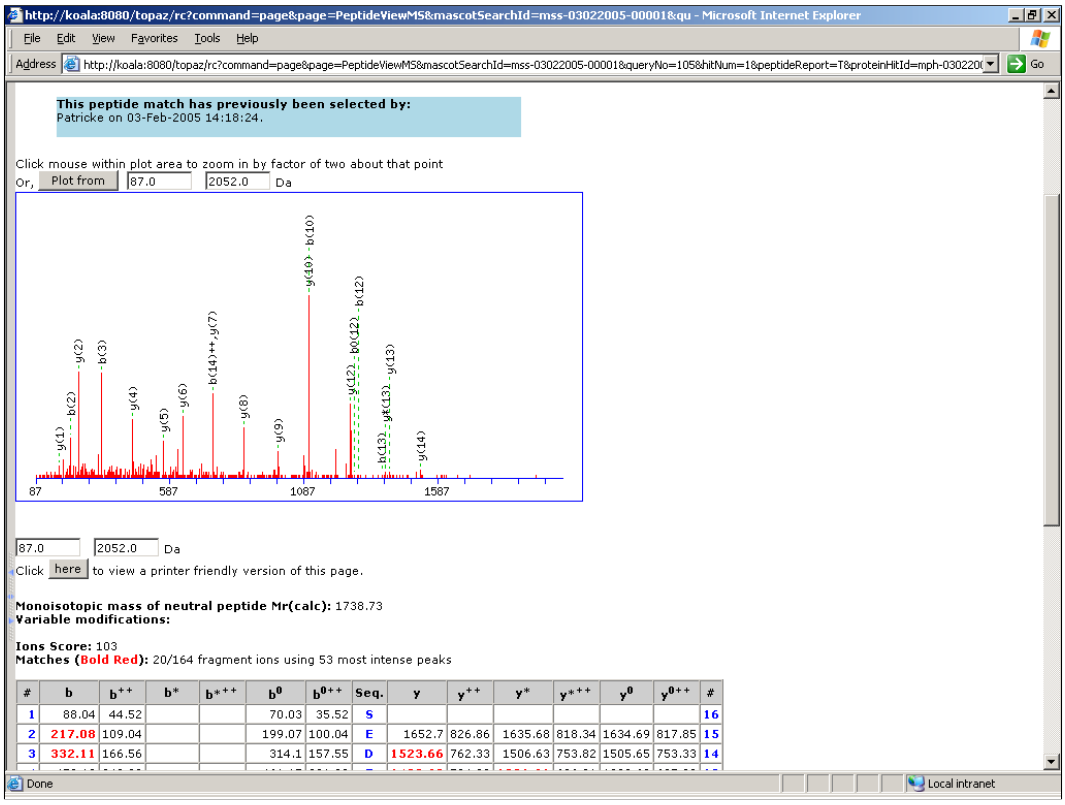
Results are automatically parsed and imported into Mascot Integra as each search completes.



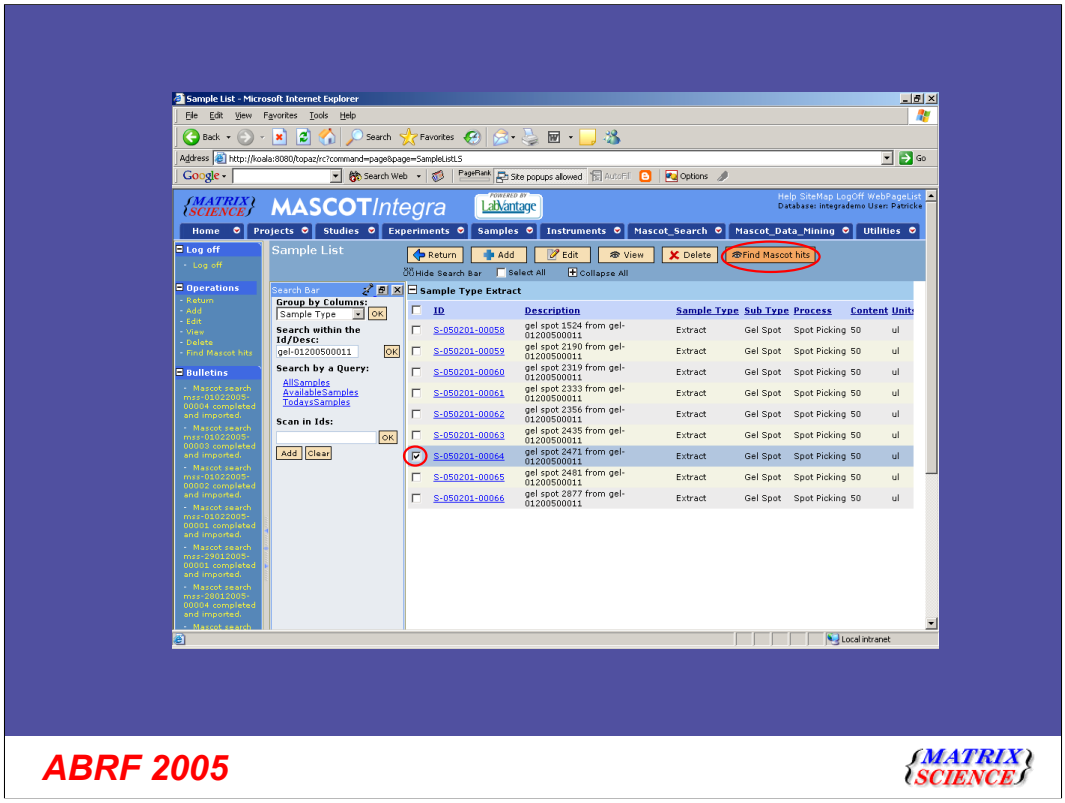
All the standard Mascot reports can be generated from the data held in the database. In addition, individual protein and peptide matches can be annotated and approved.



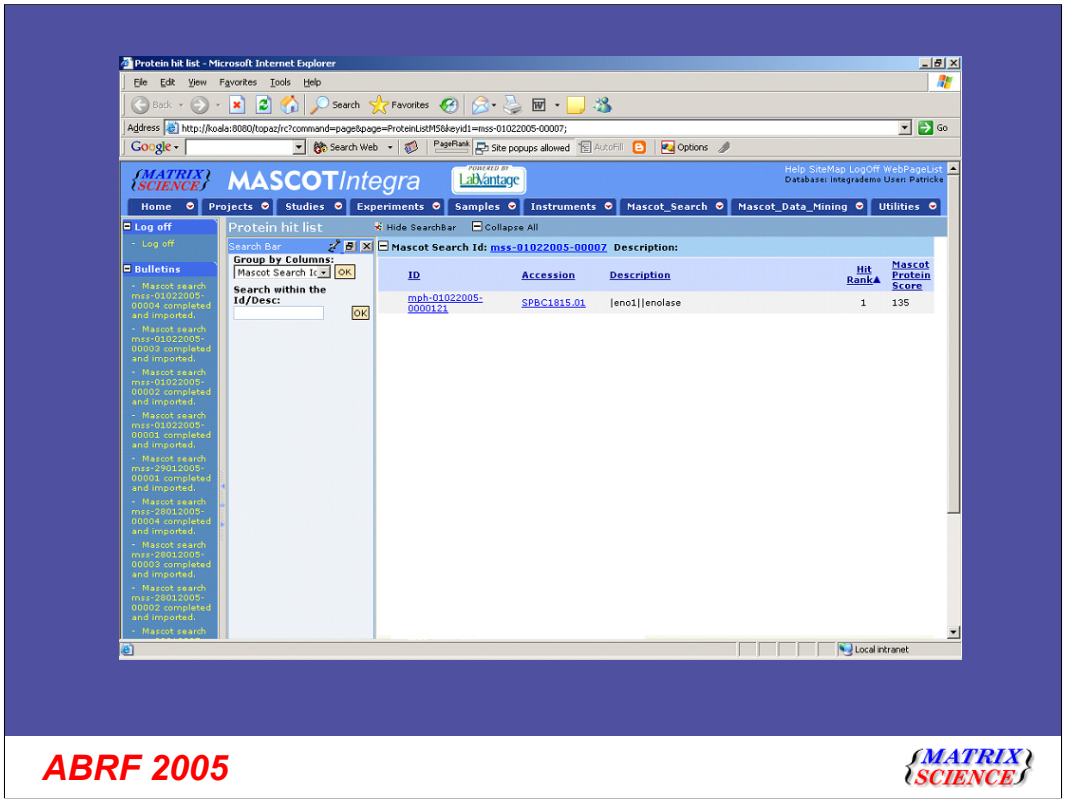
Approval information is flagged up on the reports, such as Protein View …



… And Peptide View



One advantage of tracking workflows in Mascot Integra is that all of the relationships between parent and child samples are available. Here, we drill down from a gel spot sample...



… to the corresponding mascot protein hit

Filtering reports

- Flexible report filters
- Ships with a range of useful filters
  - E.g. limit returned results to significant matches
- User definable
- Requires some knowledge of SQL.

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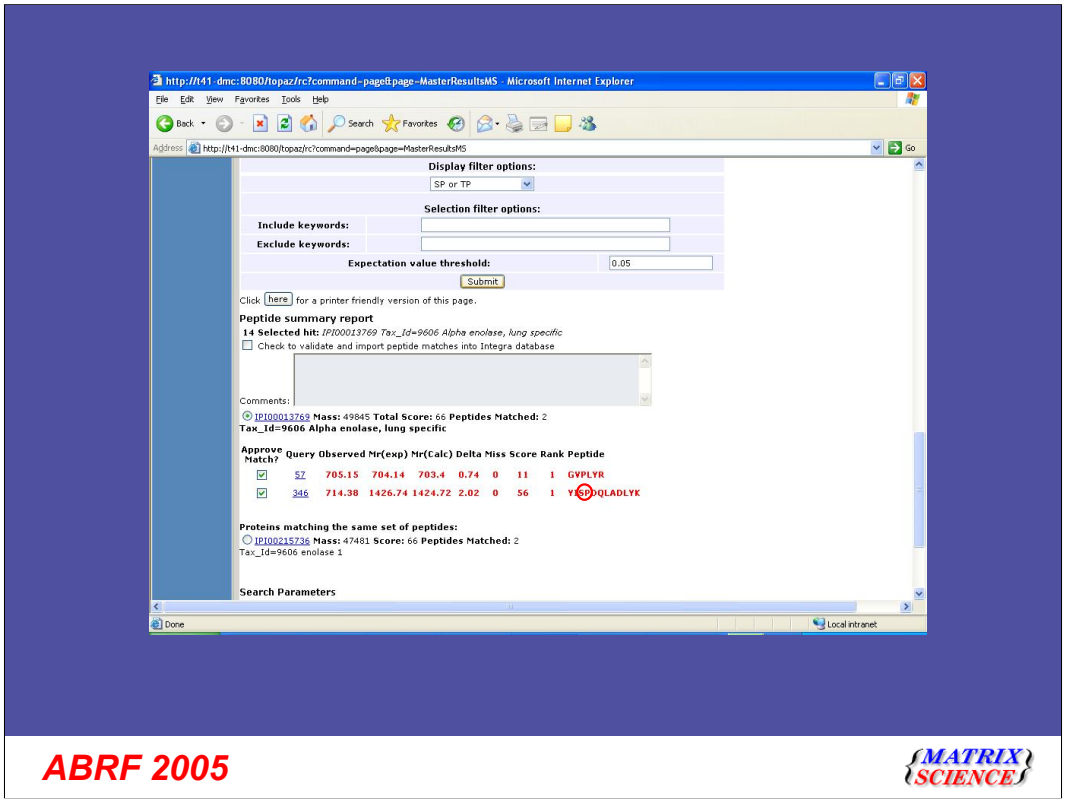
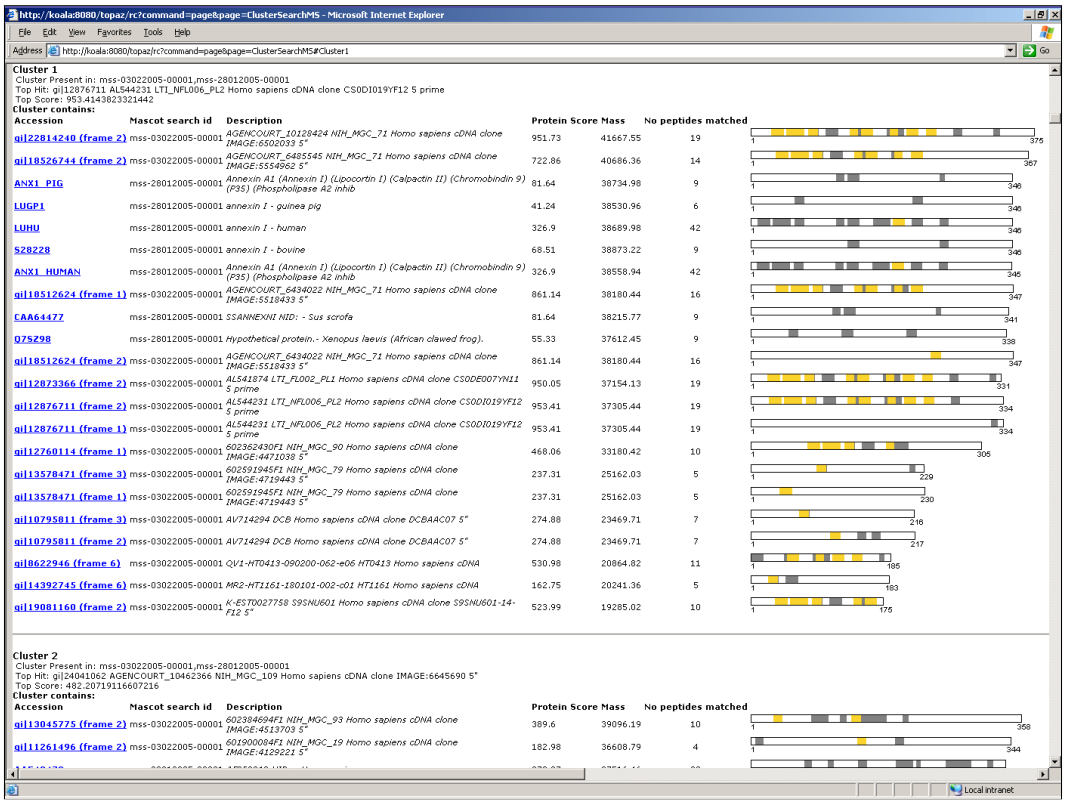
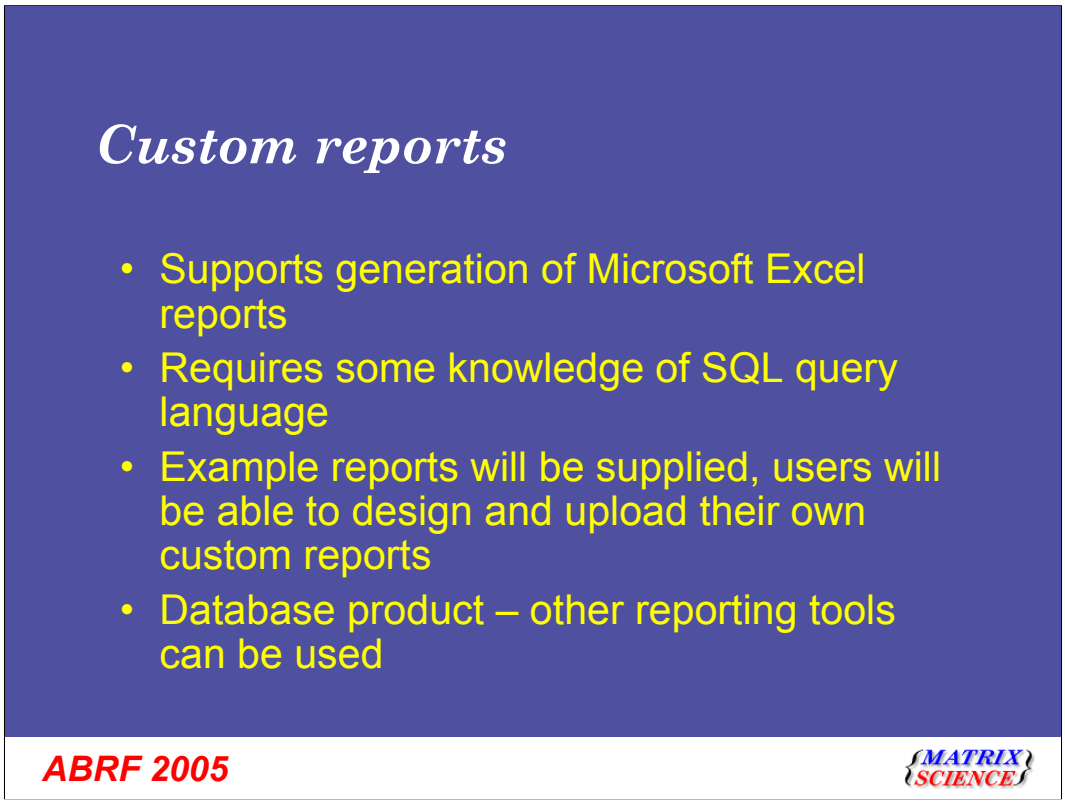
Because all of the results data is held in relational database tables, we can define flexible filters to select results for a report.



This filter will only display protein hits containing one or more peptide matches that include the consensus sequence for phosphorylation (serine or threonine followed by proline).

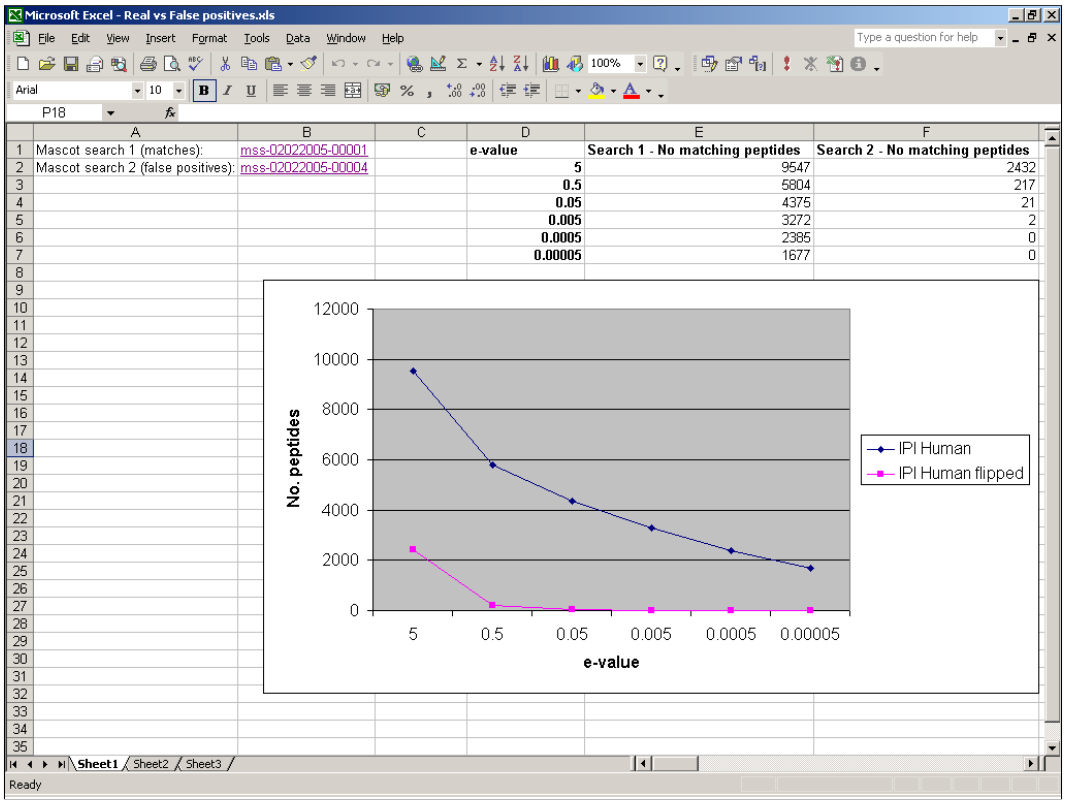


Another report uses BLAST to cluster protein hits from multiple searches into groups according to their peptide matches.



The interface for custom reports is Microsoft Excel. Almost everyone has access to and some familiarity with Excel. It is a very powerful tool that allows you to put together and format all types of reports.

Mascot Integra comes with example reports and tutorials for building your own reports.



In this example of a custom report, a MudPIT dataset has been searched against the IPI Human database and also the same database with all the protein sequences reversed.

Any matches to the reverse database should be treated as false positives. Peptide matches are then extracted and plotted against their expectation values in Excel, displaying the true and false postive rates as a function of expectation value

Mascot Integra ships as a turn-key system on IBM hardware

Summary

- Laboratory Information Management for proteomics
- *Not* an enterprise LIMS
- Oracle database
- All user functionality through a simple, clear web-browser interface
- Role and project/study membership based security
- Intuitive graphical experiment design
- Flexible custom reporting using Microsoft Excel.

Acknowledgements

- Mark Weeks, John Sinclair, Richard Jacob
  - Ludwig Institute for Cancer Research, London UK
- Katheryn Resing
  - University of Colorado, USA
- LabVantage Solutions Inc