SPOT MANAGER

Version 1.0

User Manual

UNIT 508

Contents

1 Preface

1.1 About this documentation

Icons used to highlight items of particular interest:

	This icon appears next to anything you need to remember in order to obtain correct results or to avoid issues.
	This icon appears next to anything you need to know in order to simplify or speed up a process
	This icon appears next to anything you need to know in order to do a specific operation

Before getting started with Spot Manager, you should have some knowledge of your computer working environment. You need to be able:

- to launch applications.
- to use a standard graphical user interface.
- to open, save and close a file.
- to copy (or cut) and paste a selected text.

If you want to review any of these skills, please refer to your operating system documentation.

Text conventions used to describe keyboard or mouse commands:

File → Open	Choose the Open command under the File menu
Ctrl+C	Hold down the Control key while typing the letter C
Left click	Click the left button of the mouse

This user manual is also available from Spot Manager.
To consult it:

- Choose *Help → User manual* from the menu.

or

- Choose *Spot Manager → View user manual* from Windows Start Menu.

Please verify you have Adobe Acrobat Reader installed. Acrobat Reader is freely available from www.adobe.com/products/acrobat/readstep2.html or in the Spot Manager CD-ROM.

1.2 What is Spot Manager?

Spot Manager is a tool for proteomic analysis that helps for the management of polypeptide spots identified from peptide mass fingerprinting data in order to create two dimensional maps (2D maps).
The main goals of Spot Manager are:

- Automating:
  - The identification of polypeptide spots (match a protein name to a spot)
  - The annotation of the proteins (associates information with protein)
  - The publication on the WEB of 2D maps
- Federating data:
  - All data are available with only one interface
  - Follow the progression of the work about a 2D map (one "project" for one 2D map from one gel)
  - Local projects can be viewed online via the associate database and website

1.3 Main features of this version

The most important features in Spot Manager version 1.0 are listed below :

- Automation of the identification of polypeptide spots process with the help of two different algorithms or software : Profound and Ascq-Prot
- Automation of a part of the proteins annotation process with the Expasy SwissProt database
- A colour code to help in the spot identification checking decisions
- Easy access to all spots data types (results of identification software, WEB pages of identified protein, localisation on the gel, related information)
- An easy to use graphical user interface
- Export of 2D-maps data to Microsoft Excel file or to a database

1.4 Overview of Spot Manager

1.4.1 General architecture

The general architecture of Spot Manager can be viewed on the figure 1.1.

In order to create a Spot Manager project, you must have a gel where spots are detected by the software Amersham ImageMaster 2D Platinum version 5.0.

Following the progress of your work, you can add spots with new mass spectrometry data. Spot Manager will try to identify the proteins and annotate automatically. Of course, you may modify later these results with editing tools in Spot Manager.

When you want to publish on the Web your results, you may insert your Spot Manager project in a database. Consequently, your data will be online for Internet users. Indeed, the server adapts the website in function of the data in the database and the online users' requests.

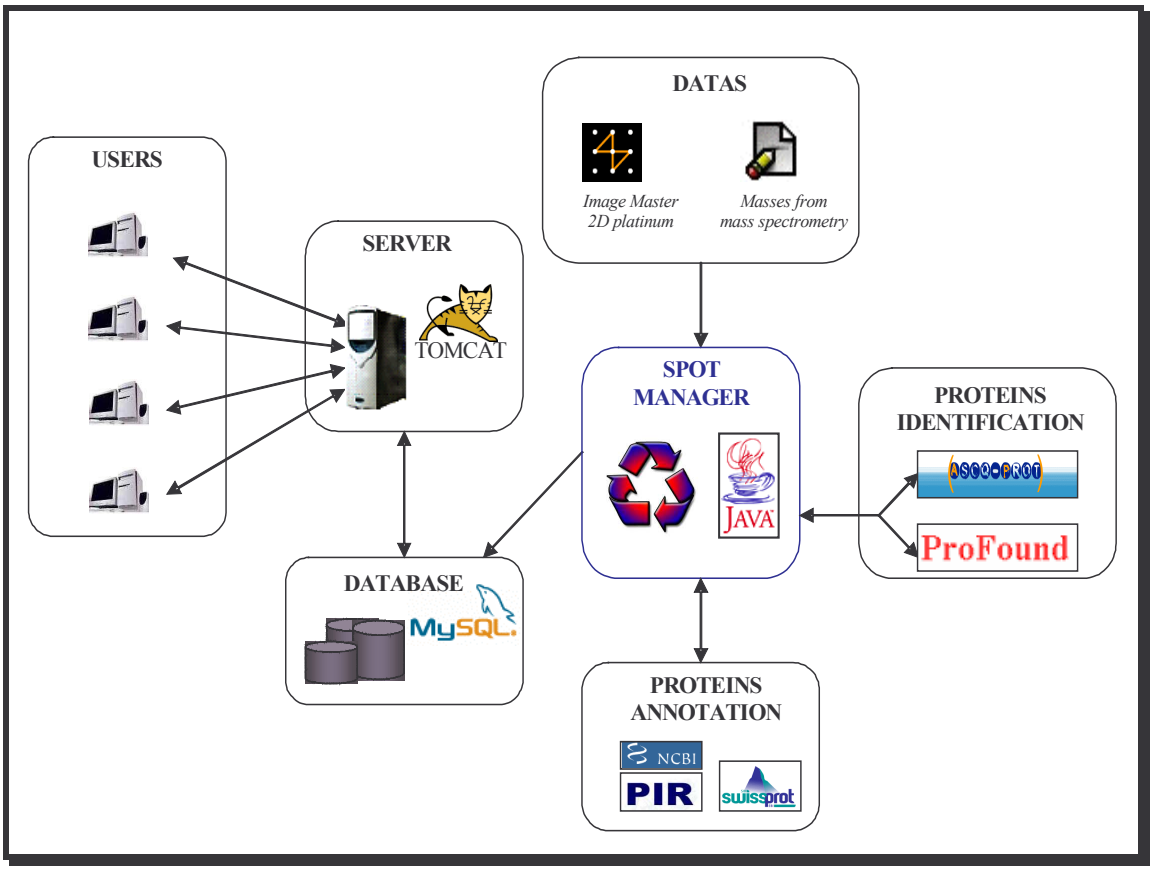


FIGURE 1.1 – *General architecture of Spot Manager*

1.4.2 Principle of the automated identification

Identification of spot consists in matching a protein to spot data. There are many identification software. For one peptide mass fingerprinting, they give a list of candidate protein sorted by probability. All methods give similar results but not totally the same.
To identify spot automatically, Spot Manager allows protein identifications from mass spectrometry data using Profound and Ascq-Prot which have two different strategies, two different algorithms. It. The first candidate protein of the Profound results found also in Ascq-Prot results is selected by Spot Manager.

FIGURE 1.2 – *Principle of the automated identification : the outlined protein is the selected protein by Spot Manager*

1.4.3 Principle of the automated annotation

The annotation of protein consists in adding information concerning this protein. When Spot Manager identifies a protein, it obtains an accession number for nrprot bank from the NCBI (National Center for Biotechnology Information). This protein database takes the advantage of the exhaustibility but a few part of its entries have a full annotation.
Reciprocally, there is the SwissProt database from the SIB (Swiss Institute of Bioinformatics) and the EBI (European Bioinformatics Institute) where all its entries are fully annotated. In order to find the link between nrprot and SwissProt (if it exists), Spot Manager use the PIR bank from National Biomedical Research Foundation. So, if the protein is present in SwissProt database, Spot Manager annotates it automatically.

1.5 Installing Spot Manager

1.5.1 System requirements

The minimal system requirements for running Spot Manager are:

- A JAVA runtime environment (JRE) 1.4.0 or later.
  For free download have a look at http://java.sun.com/j2se/1.4.2/download.html
  or in Spot Manager CD-ROM (./3rd_party/j2re-1_4_2_06.exe)
- At least 128 MB RAM and 200 MB on hard disk.
- A screen resolution of at least 1024 x 768 pixels.
- Amersham Image Master 2D Platinum version 5.0.
- An Internet connection.
- To insert data in database, a database server configured for Spot Manager.

1.5.2 Software installation

Spot Manager can be installed on a PC from the Spot Manager CD-ROM.
In order to launch the installer, you must double-click on the icon of the setup file (SpotManager.v1.0_setup.exe). This installation program should guide you through a series of screens.

The installer creates a default directory tree on your hard disk, called *Program Files|Spot Manager* in which the main program and additional folders (Spot Manager workspaces for example) are placed. If the default file path is not suitable, browse for an alternative path before continuing the installation.

1.5.3 Installed files and folders

The following files and folders are created on your hard disk (in the default directory *Program Files|Spot Manager*) during the installation of Spot Manager. This list is not exhaustive.

Folders:

aprot/_fasta	Protein banks for AscqProt.
aprot/internal	Various configuration files for AscqProt.
interface/classes	JAVA classes of Spot Manager.
interface/data	Various configuration file for Spot Manager.
interface/lib	Various libraries that are used by Spot Manager.
workspaces	Default folder for saving projects.

Files:

aprot/aprot.exe	The AscqProt program.
aprot/LICENSE	The AscqProt license.
UnInst.log	File for uninstalling Spot Manager.
manual.pdf	User manual in PDF format.
Spot Manager.bat	The launcher for Spot Manager.

2 Spot Manager environment

2.1 Running and exiting Spot Manager

Spot Manager starts like many other software by selecting the Spot Manager icon in the Programs folder on your Windows Start Menu.

Spot Manager starts faster when you deactivate temporally your antivirus

Select *File → Exit* in the menu to close the program. You can also click on the close button in the upper right corner of the program window. Spot Manager reminds you to choose to save your current project before exiting.

2.2 The graphical interface

By reading through the following items, you will be familiar with the Spot Manager program window. It is divided into four main parts, shown in figure 2.1, which are the Menu bar, the toolbar, the spots list display zone and the spot information display zone.

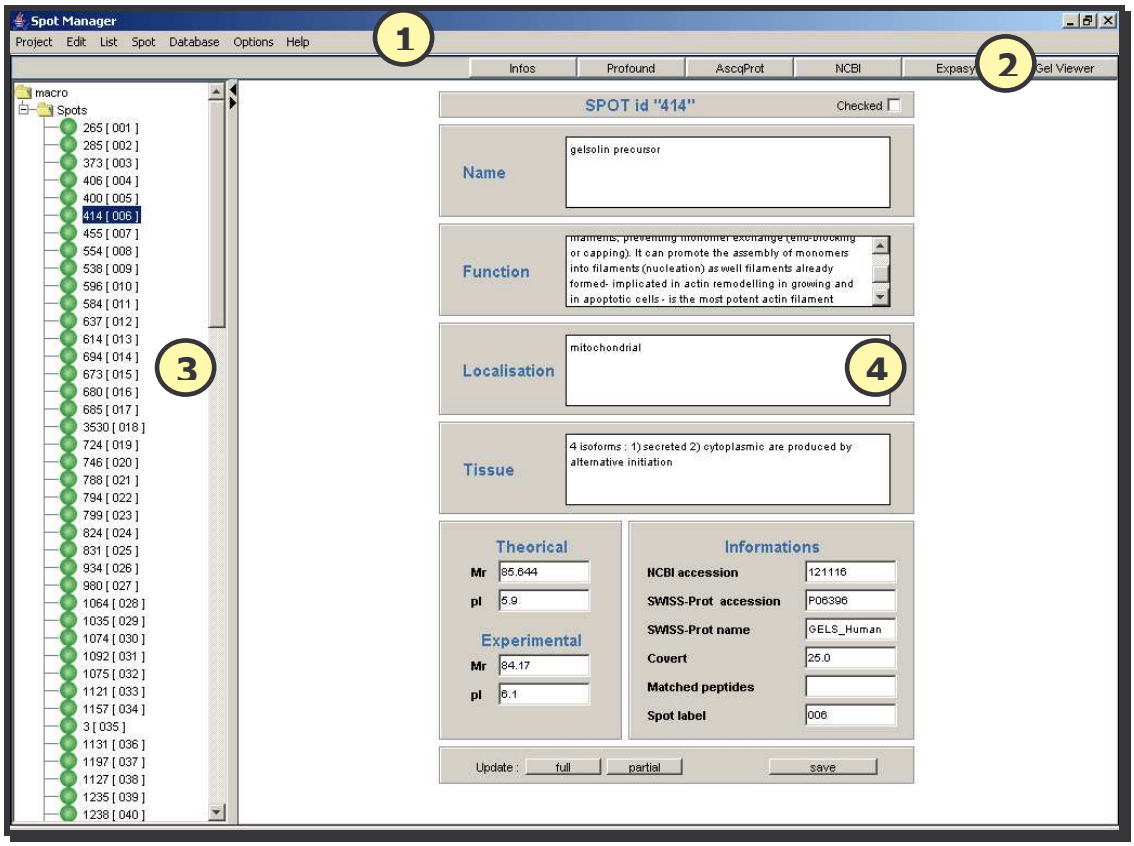


FIGURE 2.1 – *The Spot Manager program window.*
(1) menu bar, (2) toolbar, (3) spots list zone, (4) spots information zone

2.2.1 Spots list display zone

Each light in this zone represents a spot of the current project. Select a light to select a spot.

2.2.2 Spot information display zone

This zone displays different information about the selected spot.

2.2.3 Menu bar

From the menu bar, you can choose all actions that can be performed in Spot Manager.

The following menus are used to:

Project Create, open and save a project.
Exit Spot Manager.

Edit Edit project properties.
Copy mouse selection or selected spot masses into the clipboard.

List Sort the spot list by various type of sort.
Export spots list to an Excel file.

Spot Insert one or several spot(s) into project.
Change the type of the information zone.

Database Insert a project into the database.
View or delete its projects or edit its configuration properties.

Options Modify the configuration of the protein identification.
Update the local proteins banks for Ascq-Prot.

Help View information about Spot Manager.
Consult this user manual.

2.2.4 Toolbar

From the toolbar, you can change the type of the spot information zone for the current selected spot:

Infos View data about the selected spot like the theoretical and experimental values, the annotated associated protein.

Profound View the protein identification result of Profound.

AscqProt View the protein identification result of AscqProt.

NCBI View the NCBI WEB page about the spot associated protein.

Expasy View the SWISS-Prot WEB page about the spot associated protein.

Gel Viewer View the localisation of the spot on the gel

3 Working with project

3.1 What's a project

The notion of project was designed to facilitate the organization of the spot identification process and to allow you to follow the progression of the cartography of a particular gel.

One project equals one 2D map, equals one gel image, and equals several spots.

3.2 Manipulating project

3.2.1 Creating a new project

Before creating a new project, you must have your reference 2D map in an Image Master project. Spots must be detected on this gel and it's preferable that the quantification was done.
Spot Manager needs some data from this software in order to create a new project:

- an Image Master XML file
- an image of the gel

To **get the XML file from a gel** :

- Open Image Master 2D platinum 5 and your project
- Select your reference gel.
- Select *Export → Gel Data to XML* from the menu bar.

To **get the image file** :

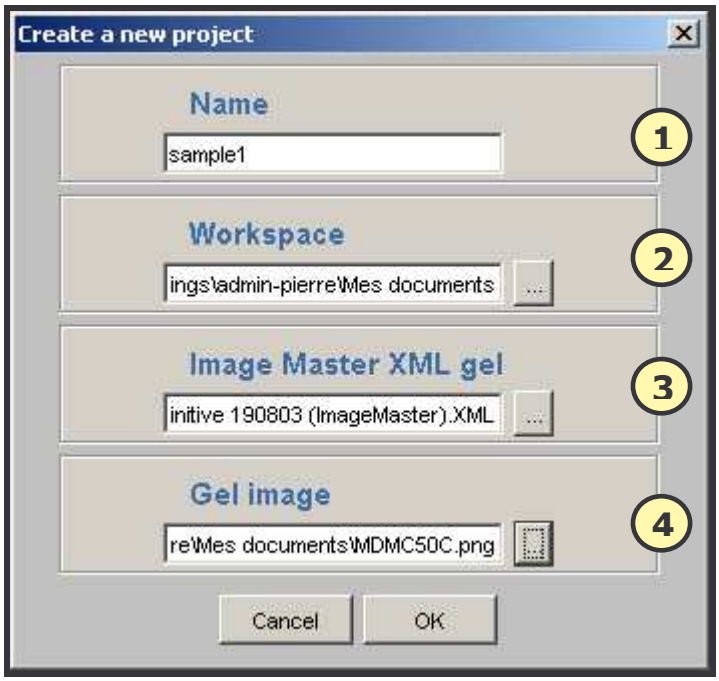
- Open Image Master 2D platinum 5 and your project.
- Select your reference gel.
- Zoom on the gel while the zoom factor set to 1:1
(control the yellow box with the name of the gel following by the zoom factor).
- Hide all spots, annotations and groups.
- Unselect all spots.
- Select *Export → Gel to File* from the menu bar.
- Choose the png format.

Spot Manager checks the validity of the two files, particularly the resolution (zoom factor) of the gel image and the match between XML and image files.

Once these files are acquired, you can create a new project with Spot Manager.

To **create a new project** :

- Choose *Project → New* from the menu bar in order to obtain the window from figure 3.1.
- Choose its name and type it in part (1) of window.
- Choose a workspace in part (2).
- Enter the path of the Image Master XML gel file in part (3).
- Enter the path of the gel image in part (4).
- Click on the OK button.



GURE 3.1 – *The window for creating a new project and the needed elements (1) Project label, (2) Workspace, (3) Image Master XML gel, (4) Gel image*

Spot Manager creates a directory and a project file inside (extension .mpa) with the name you have chosen in the workspace directory.

3.2.2 Open/save a project

To **open a project** :

- Choose *Project → Open* from the menu bar.
- Find the directory of the project with the file explorer (opening in the default workspace of Spot Manager). Double click on it.
- Click on the file with the extension mpa in this directory.
- Click on the OK button.

To **save a project** :

- Choose *Project → Save* from the menu bar.

A dialog box always reminds you to save (or not) your work before you exit the Spot Manager application.

3.2.3 Editing the properties of a project

To **edit the properties of a project** :

- Choose *Edit → Edit project properties* from the menu bar.

or

- Double-click on the folder icon with the name of your project on the top of the spots list zone.
- You obtain the properties window and you can edit them.
- Save properties with the save button at the bottom.

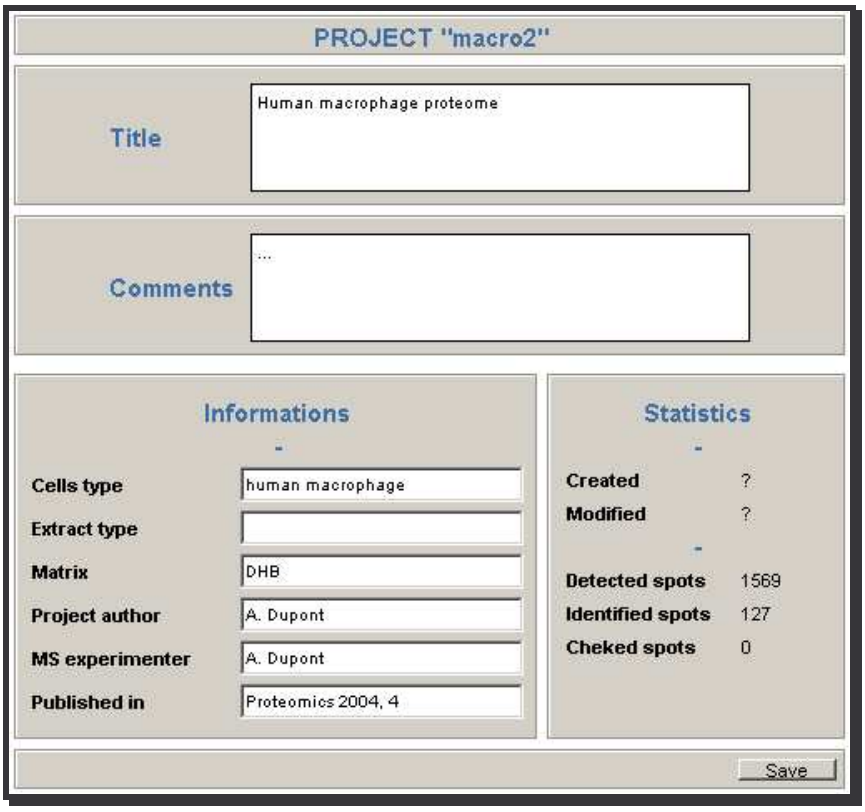


FIGURE 3.2 – *Spot Manager dialog window for project properties editing*

The properties will not be saved until you have click on the save button.

You could view statistics about the current project in the same window :

- Creation and modification dates.
- Number of spots detected in Image Master 2D platinum.
- Number of spots identified or checked.

3.3 Tools

3.3.1 Sorting spots of a project

In order to facilitate the analysis, you can sort spots in different ways.

To **sort the list of spots**

- Select *List → sort by* from the menu bar.
- Click on the sort method.
- You can reverse the current order with the reverse method.

3.3.2 Exporting to Excel

Spot Manager allows you to export the data of a project in a Excel File.

To **export the data of a project**

- Choose *List → Tools→Export to Excel* from the menu bar.
- A Excel sheet contains the spots list that will be saved in the project directory.

4 Working with spots

4.1 Adding new spots

4.1.1 Editing identification parameters

You can edit the identification parameters according to your needs.

To **editing identification parameters** :

- Choose *Options → Identifications preferences* from the menu bar.
- You obtain the properties window and you can edit them.
- Click on the OK button.

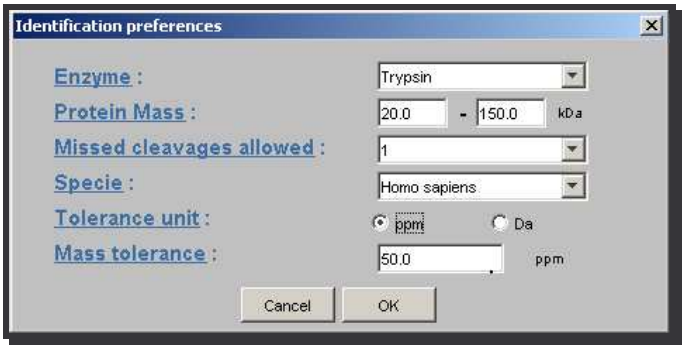


FIGURE 4.1 – *Spot Manager dialog window for editing identification parameters*

Note these properties exert large influence on the protein identification.

4.1.2 Inserting spots into a project

Inserting a spot in a Spot Manager project corresponds to link a spot identifier number from the Image Master 2D platinum XML gel to a mass spectrometry file.

Consequently, the name of the mass spectrometry files must contain a valid and appropriate spot identifier number. It is preferable that the masses files have an equivalent filename for multiple insertions.

Spot Manager gives you two ways to insert spots into a project : one by one or several each time.

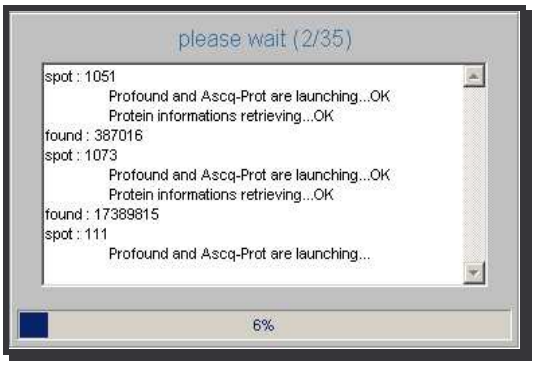


FIGURE 4.2 – *Spot Manager window shows the state of the spots insertion*

To **insert new spots** :

- <u>One spot</u> :
  - Select *Spot → new from mass file* from the menu bar.
  - Select the masses file you want.

or

- <u>Several spots</u> :
  - Select *Spot → new from directory* from the menu bar.
  - Select the directory that contains the masses files you want.
- As a result, you obtain a window like the figure 4.3. You can link the project to spot(s) with its text box on the left bottom.
  Indeed, with the help of the expression in this text box, Spot Manager tries to retrieve the Image Master identifier number in the filename of your masses files. Following this step by step, you find more details to write such an expression.
- Once you have typed your expression in the text box, click on the refresh button (with the ~ character) to update the Spot Manager dialog window.
- Control if your expression extract the appropriate spot identifier (in the second column).
  Spot Manager helps you with a colour code :
  - <u>red</u> : the spot identifier is not valid and it will not be added to the project
  - <u>orange</u> : the spot identifier is valid but it has been already identified. It will be replaced in the current project.
  - <u>green</u> : the spot identifier is valid and it will be added to the project.
- Click OK to launch the automated identification and please wait.

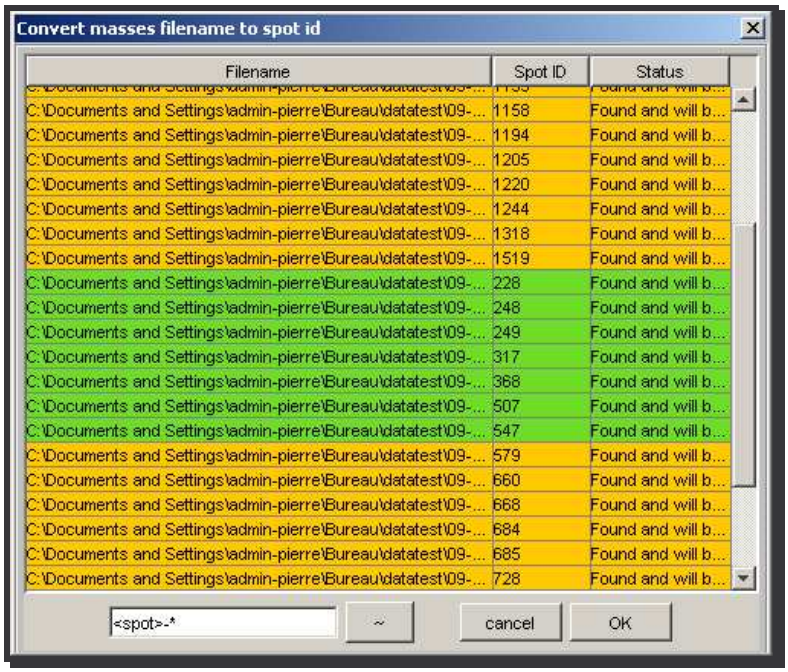


FIGURE 4.3 – *Spot Manager dialog window for linking masses filenames to identifier number of Image Master 2D Platinum*

The principle of the expression : help spot Manager to retrieve the Image Master 2D platinum with the keyword <spot>. The expression accepts the generic character * to replace part of the filename. The following table helps you to understand how it works.

TABLE 4.1 – *Examples of expression to obtain the spot number id 508 in function of filename*

Filename	Expression to get the spot number id 508
508	<spot>
508.txt	<spot>.txt
spot 508.txt	spot <spot>.txt
508_D11_b_0001.txt	<spot>_*
_D11_b_0001(508)	*(<spot>)
D588_508_D11_b_0001.txt	*_<spot>_*

4.1.3 Checking results

The results from Spot Manager are automated but you must checked their validity.

For this purpose, Spot Manager indicates by a green light the spots where the experimental and theoretical Pi and Mr matching (Inversely with red light).

4.2 Viewing information about a spot

For each spot of a Spot Manager project, you can view information about it.

To **view information about a spot** :

- Click on it in the spots list display zone.
- Click on a button of the toolbar in order to select the type of information.

or

- Choose *Spot* → *View* and the type of information from the menu bar.

Available choices in the toolbar or in the menu bar:

- Infos : View data about the selected spot like the annotated associated protein and the theoretical and experimental values.
- Profound : View the protein identification result of Profound.
- AscqProt : View the protein identification result of AscqProt.
- NCBI : View the NCBI WEB page about the spot associated protein.
- Expasy : View the SWISS-Prot WEB page about the spot associated protein.
- Gel Viewer : View the localisation of the spot on the gel

4.2.1 Annotations page

This window shows the information and annotations linked to the selected spot : name, function, localisation, tissue, theoretical and experimental values, accession numbers for different proteins banks. See part 4.3 to edit spot information.

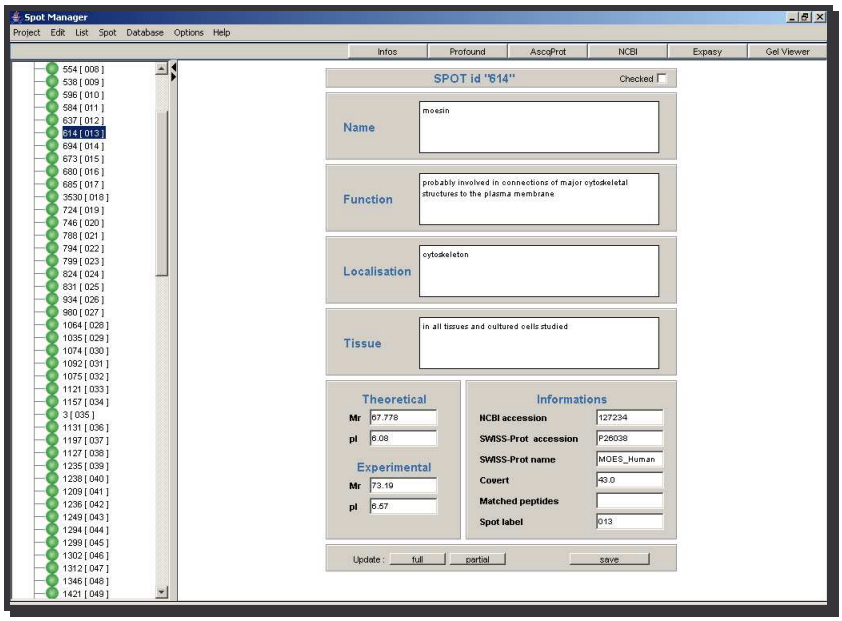


FIGURE 4.4 – *Annotations view*

4.2.2 Profound results page

This window shows the Profound results that Spot Manager used to identify the spots.

Some WEB links in Profound pages are accessible only during few hours after the identification process. Spot Manager keeps only the main results page.

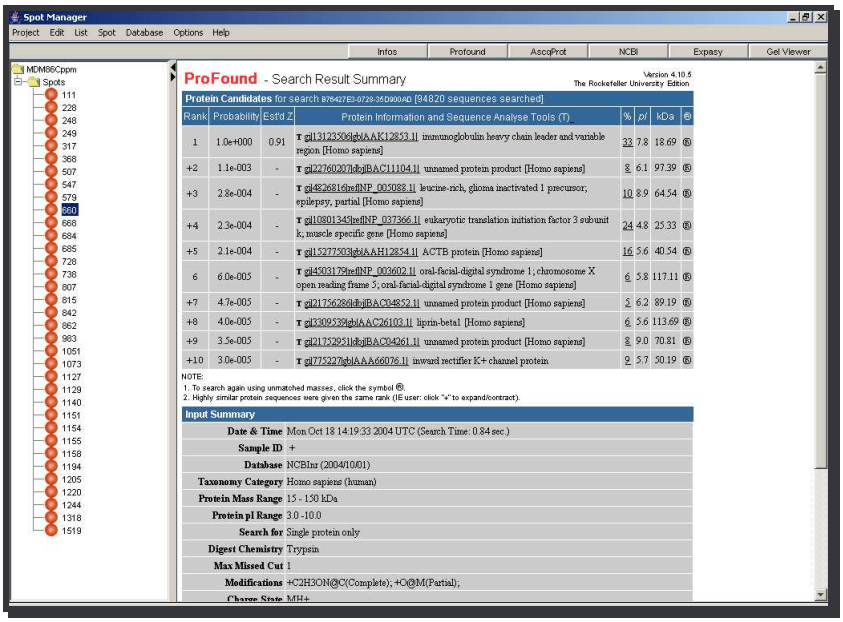


FIGURE 4.5 – *Profound view*

4.2.3 AscqProt results page

This window shows the AscqProt results that Spot Manager used to identify the spot.

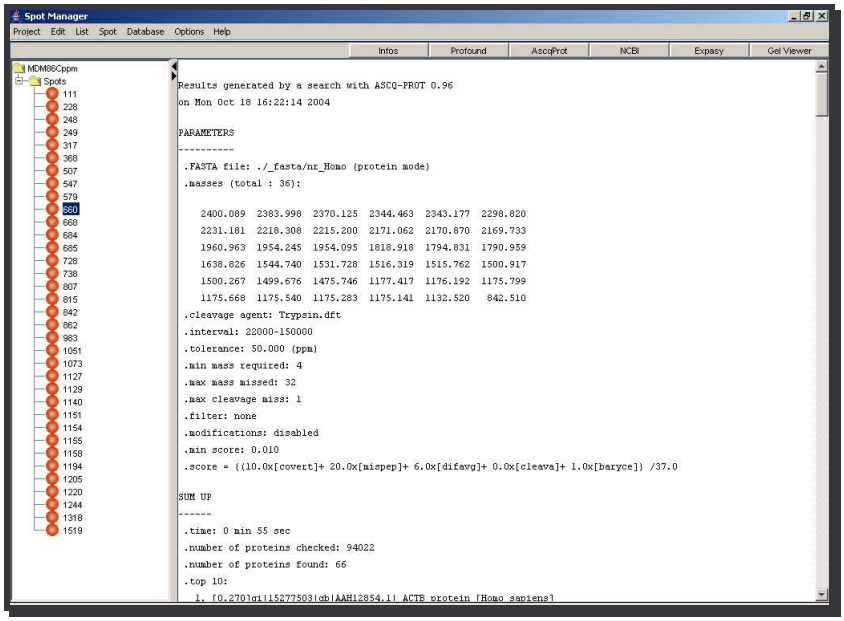


FIGURE 4.6 – *AscqProt view*

4.2.4 Related NCBI page

This window shows the related NCBI protein page linked to the spot.

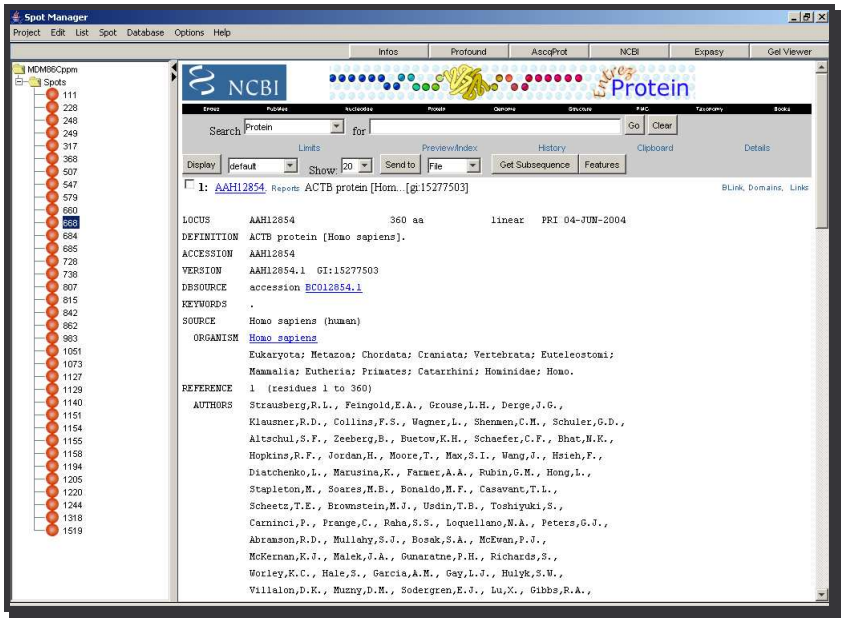


FIGURE 4.7 – *Related NCBI page*

Spot Manager does not store this page. It connects to the Internet and shows the NCBI page related to the NCBI accession number types in the Spot Manager annotation window.

4.2.5 Related Expasy page

This window shows the related Expasy protein page linked to the spot.

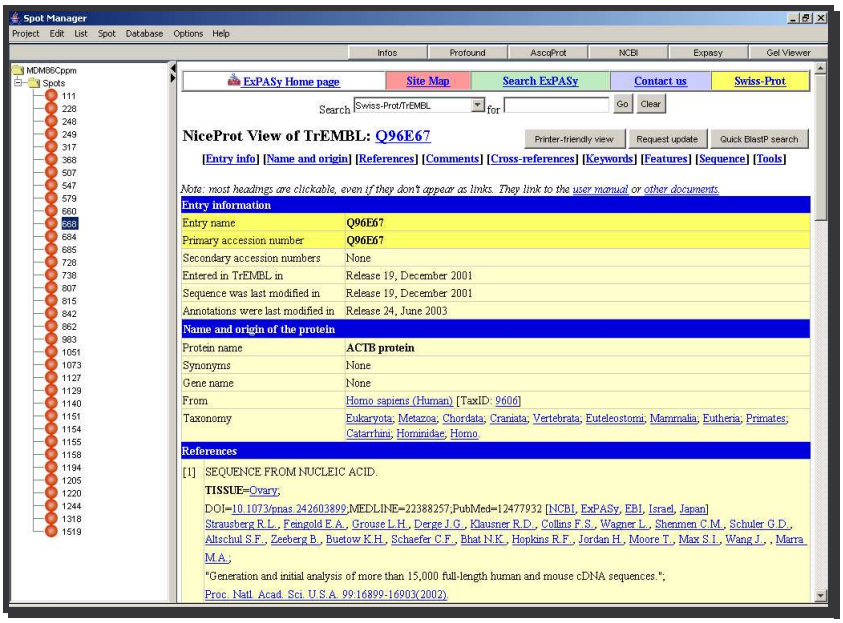


FIGURE 4.8 – *Related Expasy page*

Spot Manager does not store this page. It connects to the Internet and shows the Expasy page related to the SwissProt accession number types in the Spot Manager annotation window.

4.2.6 Localisation on the gel

This window shows the localisation of the spot on the gel.

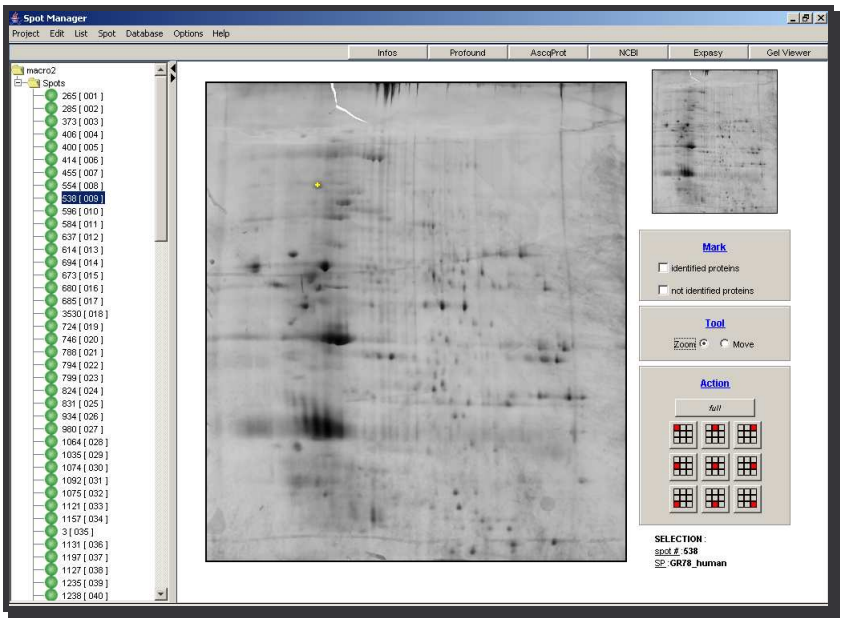


FIGURE 4.9 – *localisation on the gel*

You can do some actions on this view with the buttons and the checkboxes on the right of this windows.

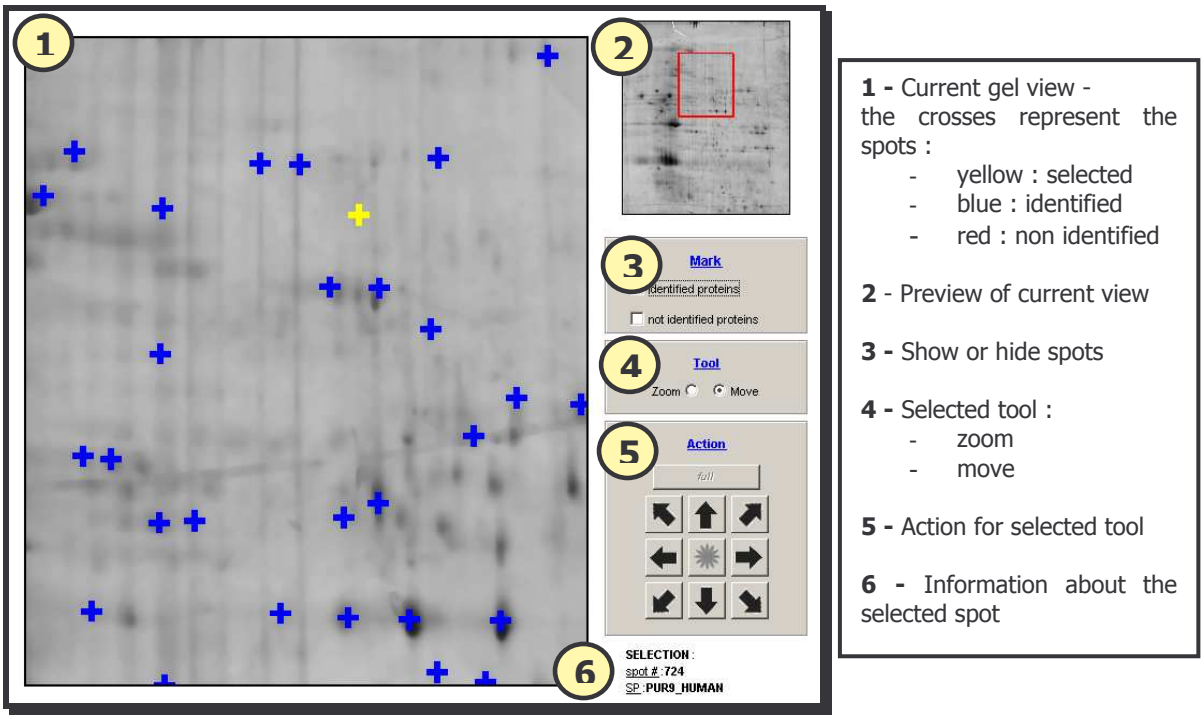


FIGURE 4.10 – *Example of use of the gel viewer in Spot Manager*

4.3 Editing spot annotations

To **edit the spot annotations**,

- get the information spot panel (see part 4.2.1).
- All items are editable

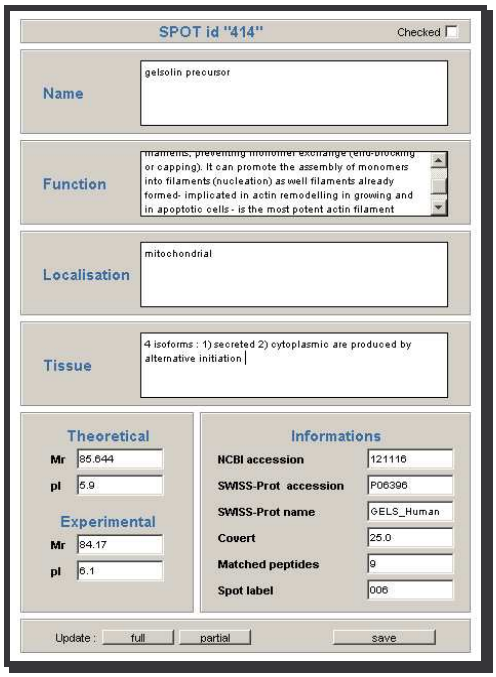


FIGURE 4.11 – *The Spot Manager window for annotating spot*

At the top of the edition panel, you can find a checkbox that allows to change the state of the spot to "checked", useful to remember spots checked or not.

At the bottom of the edition panel, you can find two button for the update:

- full : all items are updated from the NCBI number type.
- partial : only accession numbers for SwissProt are updated.

The typical use of the full update is the case where you have to change the protein associated to the spot (because your are not agree by the Spot Manager choice). Spot Manager tries to fill all item with your NCBI number. The use of the partial update allows you to update the SwissProt Accession number and so follow the evolution of the SwissProt bank.

The edited properties will not be saved until you have click on the save button.

5 Working with database

5.1 Setting up the database

You can insert your data only in a database configured for Spot Manager. Contact your network administrator to obtain and set up a such database.

FIGURE 5.1 – *Access configuration for database*

You can edit the properties of the connection according to your network and your database.

To **configure the connection to the database**,

- Choose *Database → Properties* from the menu bar.
- Edit textboxes according to your database parameters.
  - User : name of the user for the database
  - Password : password for its user
  - URL : localisation of the database
  - Driver : localisation of the software layer between Spot Manager and database (for advanced users).
- Click on the "Test" button to check the validity of the connection.
- Click on the "OK" to save your settings

5.2 Inserting a project into database

To **insert a project into database**,

- Choose *Database → Insert project* from the menu bar.

Only spots with SWISSPROT accession number are inserted in the database.

5.3 Managing the database

Spot Manager proposes a minimal tool to manage the database.
You can view or delete projects.

To access to the **database managing tool**,

- Choose *Database → Management* from the menu bar.

To **delete a project in the database**,

- Get the database managing tool.
- Click on a project to select it
- Click on the "delete" button to erase it from the database.

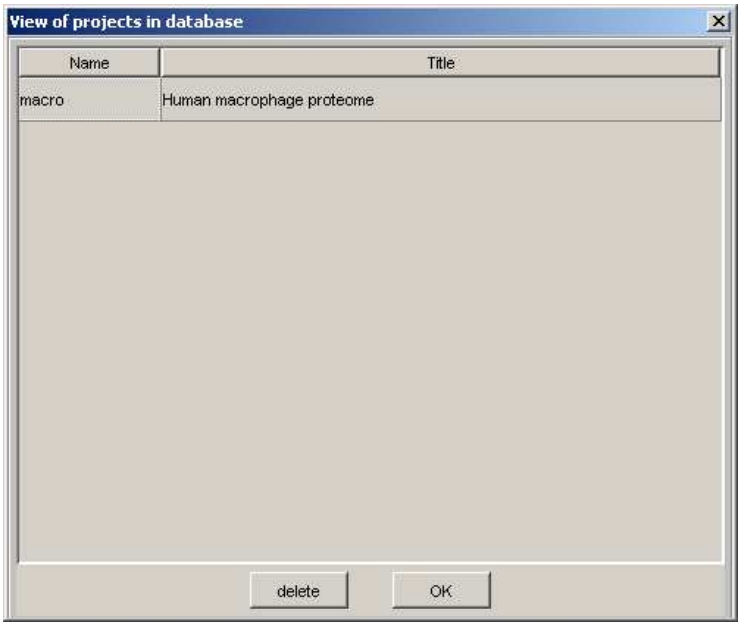


FIGURE 5.2 – *Spot Manager tool for database managing*

6 Spot Manager keyboard shortcuts

Shortcut	Action
General	
Control+C	Copy the text selection to clipboard
Control+X	Cut and copy the text selection to clipboard
Control+V	Paste the content of clipboard
Alt+F4	Exit
Project	
Alt+N	Create a new project
Control+O	Open a project
Control+S	Save current project
Edit	
Control+P	Access to project properties editing window
Control+M	Copy masses of the current spot to clipboard
Spot	
Control+F	Insert spot from a file
Control+D	Insert spots from a directory
Alt+1	Show annotations for the current spot
Alt+2	Show Profound results for the current spot
Alt+3	Show AscqProt results for the current spot
Alt+4	Show NCBI web page for the current spot
Alt+5	Show Expasy web page for the current spot
Alt+6	Show the localisation of the current spot on the gel
Alt+del	Delete the current spot
Alt+W	Move to next spot
Alt+Q	Move to previous spot