



Melanie 3 User Manual

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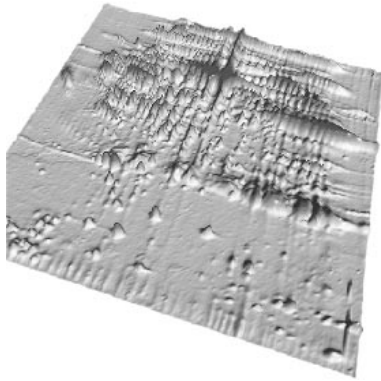
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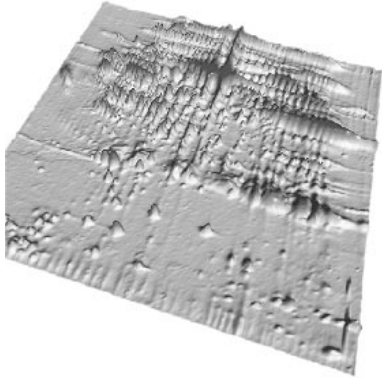
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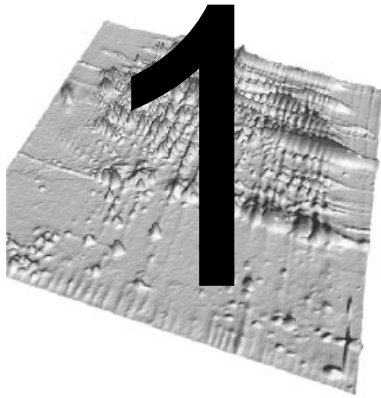
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PART I

BASICS



INTRODUCTION

Welcome to **Melanie 3**, the third generation of the well known Melanie two-dimensional electrophoresis analysis software. Melanie is a complete state-of-the-art software application designed to analyze images of two-dimensional electrophoresis (2-DE). It was conceived in close collaboration with biologists and proteomics scientists from Hochstrasser's group in Geneva, to insure fast and reliable comparisons of spots, analysis of gels, and identification of proteins. Its design and functions address both experts and beginners needs.

Melanie has been designed to be easy to use (very intuitive user interface), nevertheless, we encourage you to read this manual in order to understand all the extensive power of this software. This manual shows you how to make the best of Melanie and its advantages, while explaining all its functions in detail. It includes exhaustive explanations and useful tips which will help you to analyze your gels successfully.

This manual is divided into four parts. Part I gives an overview of the Melanie software, as well as the basic procedures for analyzing 2-DE images. Part II details the necessary steps for comparing images as well as analyzing resulting data. Part III presents features, such as image creation and working with remote databases. Part IV provides additional information, such as menu short-cuts and technical support.



Important points in the manual, as well as useful hints are marked by a small hand in the margin in order to draw your attention.

Hardware and software requirements

Melanie is a **PC** compatible 32-bit application that runs under the Windows 95, Windows 98 and Windows NT 4.0 environments.

We recommend 64 or even 128 MB of memory to process many gels simultaneously. The amount of memory required is mainly determined by the file size of scanned gel images.

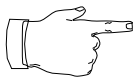
Installation

Insert the Melanie CD-ROM into the CD-ROM drive on your computer and the installer will start automatically. The Melanie installation program will guide you through a series of screens.

The installer will create a default directory tree under Program Files on your hard disk called Bio-Rad\Melanie3 (you can select a different directory if you wish). The main Melanie program will be placed in the Melanie 3 directory. Additional directories for storing sample images and Melanie's settings will also be created.

Programs and files distributed with the Melanie package:

- Melanie program
- Example images
 - Three images in directory `Gels_Ecoli`
 - Same images after complete analysis in directory `Analyzed_Ecoli`
- Other files
 - Manual files in directory `Doc.`
 - On-line html files in directory `Help.`



Note about Master protein maps:

The SWISS-2DPAGE database of two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) reference maps used to be freely available on the server <http://www.expasy.ch> and distributed with the Melanie system. Since September 1998, the Swiss Institute of Bioinformatics decided to adopt the solution chosen for the SWISS-PROT database, namely to ask non-academic users to financially contribute to the maintenance of these databases. No license fee is charged to academic users for non commercial use, and the master gels may be downloaded from the ftp site <ftp://www.expasy.ch/databases/swiss-2dpage/MASTERS/>. Non-academic users are kindly requested to contact GeneBio (<http://www.genebio.com/>) for the purpose of concluding the necessary license agreements.

Licence hardware protection key

To run Melanie you need to install the provided hardware protection key (HASP key) on the parallel port. Before proceeding, please turn off your computer and the attached printer (if any). The HASP key must be attached to the parallel port on the back of your computer. If a printer cable is attached to this port, disconnect it. After you have attached the HASP key, you can attach the printer cable to the key itself and restart your computer.

The installer will install the system driver that allows the Melanie software to detect the HASP key.

Netscape Navigator® and Internet Explorer®

In order to use the on-line manual, as well as to access remote databases over Internet, you must install the Netscape Navigator® World-Wide Web browser from Netscape Communications Corp. or Internet Explorer from Microsoft Corporation.

Running Melanie

To run the Melanie program, double-click on the **Melanie** icon. You may also run the Melanie program by double-clicking on the icon of a gel image file (Fig. 1-1). In this way the given gel will automatically be opened upon running the program.



Figure 1-1. (a) Melanie icon. (b) Gel icon.

Upon loading the program the Melanie logo appears. Click on the logo to get rid of it.

Interface

The Melanie window is divided into four main parts, shown in Fig. 1-2 and respectively called: menu bar (1), toolbar (2), gel display zone (3) and status bar (4).

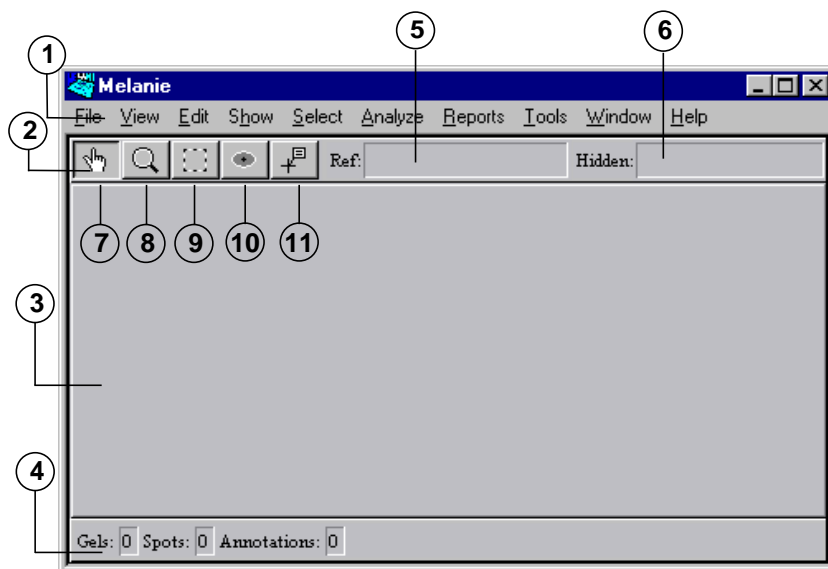


Figure 1-2. Melanie display. (1) Menu bar (2) toolbar (3) display zone (4) status bar (5) reference gel zone (6) hidden gels zone (7) Hand tool (8) Magnify tool (9) Region tool (10) Spot tool and (11) Annotation tool.

Menus

Once objects have been selected, you may choose actions from the menus to be performed on them. Move the cursor to the desired menu on the menu bar (Fig. 1-2, item 1), hold down the mouse button. Certain menu options have sub-options, you may choose one of them by pointing over the small arrow on the right hand side of the option.

The following menus are provided in the melanie program (from left to right in the menu bar):

- **File** - to handle whole gels and data on disk (open and close gel images, save data, print images, import and export data).
- **View** - to modify the window display (fixed or variable number of cells) and gels display (stacked, aligned, zoomed, grid lines).
- **Edit** - to edit (add, modify, delete) specific objects.
- **Show** - to show or hide specific objects.
- **Select** - to select specific objects.
- **Analyze** - to compute differences and similarities among gels (statistical and artificial intelligence data analysis).
- **Reports** - to display information on selected objects and to navigate in gels by means of the data.

- **Tools** - to create new gel images from selected ones (duplicate, crop, filter gels, create synthetic gels, flip and scale gels, as well as erase gels).
- **Window** - to display information and zoom windows, and to list open report windows.
- **Help** - to obtain on-line help.



An index on menu items is given in Appendix *Short-cuts and Menu structure* on page 131.

Contextual menu

When you click the right mouse button in Melanie's display, a Contextual menu appears containing the main functions to edit, show, and select objects like gels, spots and annotations. They reproduce the same commands found in the Menu bar, but they are quicker to access at any time (Fig. 1-3).



Figure 1-3. Contextual menu.

Shortcuts

Several menu options may be activated by keyboard shortcuts. These are indicated at the right hand side of the corresponding menu option.



A list of shortcuts is given in Appendix *Short-cuts and Menu structure* on page 131.

Status bar

The status bar describes at a given moment the total number of gels, spots and annotations that are selected (Fig. 1-2, item 4).



Note the importance of the status bar, as it allows you to know what objects are selected, even if not all of them are visible (for example, when gels are zoomed).

Reference gel zone and hidden gels zone

These two zones enable you to quickly change the gels visibility or choose a reference gel. The hidden gels zone enables you to hide or show your displayed gels with just a drag-and-drop operation (Fig. 1-2, item 6). In the same way, the reference gel zone enables you to choose the groups reference gel (Fig. 1-2, item 5). For more details on reference gel, see *Pairs and groups* on page 8 and *Groups* on page 85.

Toolbar

Objects on screen may be displayed, manipulated or processed using the various options from the Melanie menus or by selecting them with the **tools** provided in the toolbar.

The Melanie toolbar contains 5 buttons (Fig. 1-4), the hand tool (7), the magnify tool (8), the region tool (9), the spot tool (10) and the annotation tool (11).

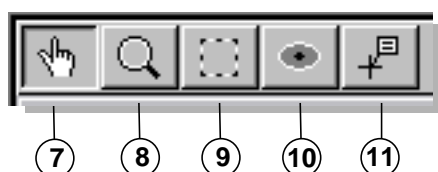


Figure 1-4. The toolbar enlarged. (7) Hand tool (8) Magnify tool (9) Region tool (10) Spot tool and (11) Annotation tool.

The **Hand** tool moves gels in order to show other parts. The **Magnify** tool lets you repeatedly zoom in or out the whole gel. The **Region** tool is useful to select a rectangular region within a gel. The **Spot** tool lets you select individual spots and the **Annotation** tool is used to select annotations and labels.

On-line help

The manual is also available on-line in the Melanie program. To consult it on-line:

Make sure that the Help folder is located in the same folder as the Melanie application.

Then, in the *Help* menu, choose *User manual*. An index is also available. To access the on-line user manual and index, you must have the Netscape Navigator[®] World-Wide Web browser installed or Microsoft Internet Explorer[®].

You may choose the table of content document from which you can read any of the on-line manual chapters, or you can request the on-line index, then click on the desired concept to obtain the related on-line chapter.

Objects

A Melanie object is an entity that may be displayed and manipulated. The objects are gels, as well as gel components such as spots, regions, annotations and labels. Pairs, groups and classes are objects which allow for spots comparison across several gels.

Gels

A **gel** is a two-dimensional electrophoresis image that has been digitized and stored on disk. Several image formats may be opened as gels, but they have to be saved into the Melanie file format in order to keep objects such as detected spot (shapes and values), pairs, etc. If a gel is saved with its objects into a foreign format, then the objects will simply be part of the saved image and will have lost any structure or value.

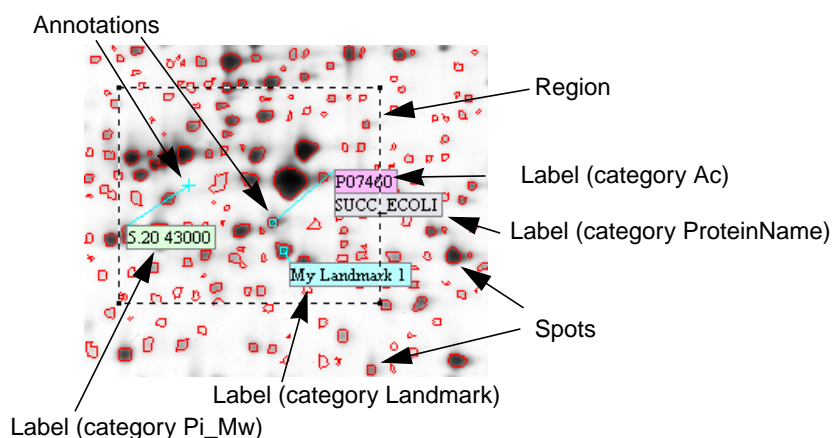


Figure 1-5. Spot shapes, annotations, labels in a region.

The gel image is the raw input data to the Melanie program, from which proteins may be detected and quantified. Gels may also be duplicated, erased, cropped, filtered, flipped or scaled. Gels may be displayed in tile mode (one gel next to the other) or in stack mode (gels on top of each other). Gels may be aligned, that is distorted gels will be stretched pixel wise, in order to make a set of images completely superimposable.

Regions

A **region** is a rectangular subpart of a gel that can be defined using the Region tool. When a region is defined, certain actions may be limited to it (Fig. 1-5).

Spots

Spots represent the proteins on the gel. They can be automatically detected by Melanie or manually adjusted by the user (Fig. 1-5). Each spot in a gel has an associated **spot ID** (a unique sequential number) automatically given when it is created. Spots may be quantified, i.e. their optical density, area and volume are computed. Alternatively, spots may be modeled as Gaussian functions.

Annotations

Spots or individual points (pixels) may be marked by **annotations**. An annotation is defined by its position and its set of labels (Fig. 1-5). It contains relevant information which is separated into label categories (pre-defined or user defined categories).

Melanie provides a number of pre-defined categories such as *Pi_Mw*, Landmarks, Comments, Sets and ACs. The label category ***Pi_Mw*** contains the known *pI* and *Mw* values, which are used to propagate this information to any point in a gel. The label category ***Landmarks*** is used as a reference point for operations such as gel alignment. The ***Set*** category is used to mark a set of spots sharing the same properties or results from analysis. User information may be saved in labels of ***Comment*** category or in any other user-defined category. The category ***Ac*** holds the protein's accession number (AC) taken from a user-selected database and may be a link to Melanie's remote database query engine. Labels may also contain direct links to other files and Web sites.

Pairs and groups

The **pair** is the association between two corresponding spots, i.e. it represents the same protein in **two gels**. Pairs may be manually defined by the user or may be automatically determined using Melanie's powerful gel matching program. When two gels are matched, the corresponding spots are paired.



Figure 1-6. One pair.

A **group** represents the same protein in several gels. When several gels have been matched to a given reference gel, the latter provides a unique spot numbering scheme across all gels. Each paired spot in a gel image may then be associated to the corresponding spot ID in the reference gel. The spots in a set of gels that are paired to one given spot in the reference gel together form a spot group. The group is the basic element for analyzing and searching changes in protein expression across gels.

Variation in protein expression may be visualized and analyzed using histograms, or through powerful statistical methods.

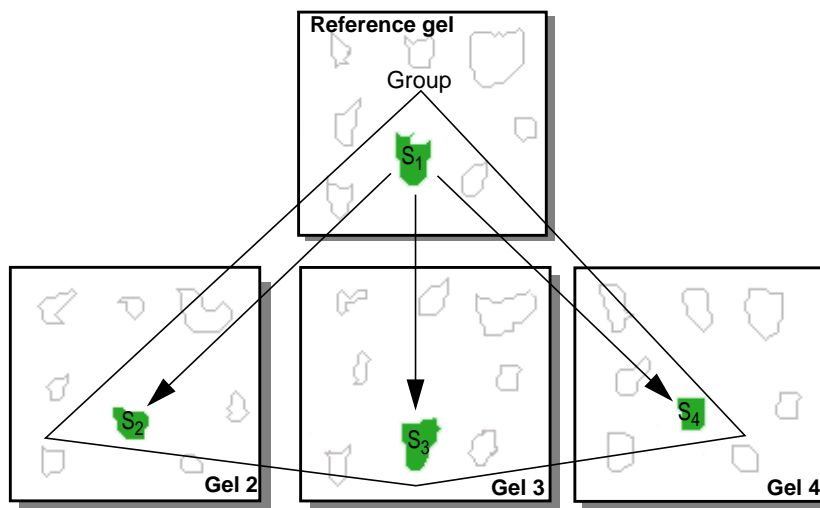


Figure 1-7. One group.

Classes

The assembling of a number of gels, which have a common biological meaning or common characteristics, establishes a **class** of gels. It corresponds to a set of gels representing the same population. For example, gels from the same infected tissue but from different patients belonging to one class may be compared to a class of gels of healthy tissues from other patients.

The definition of classes in Melanie allows statistical and meaningful considerations about populations of gels.

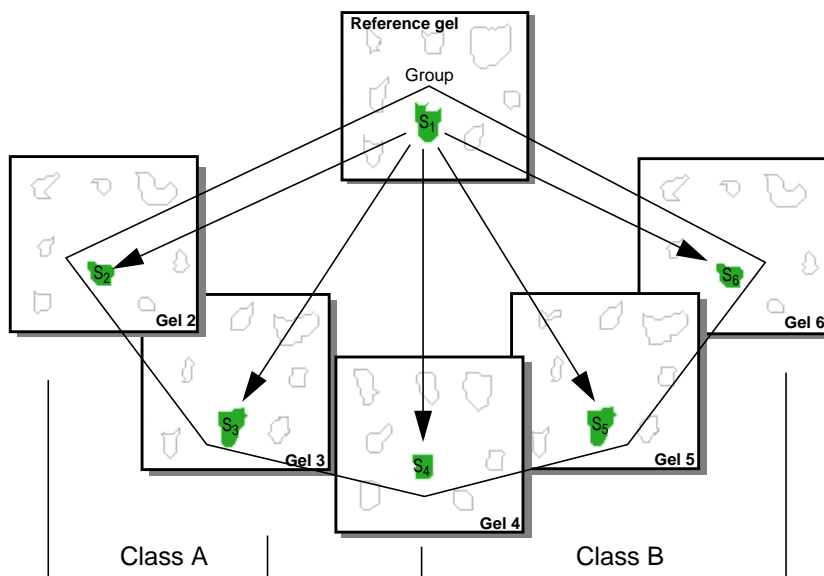


Figure 1-8. Two classes. Spots from Class A (S_2, S_3) may be compared to spots from Class B (S_4, S_5, S_6).

The analysis and visualization of differently expressed proteins through populations of gels can be highlighted with statistical tests and several reports and histograms.

Selecting and showing objects

Melanie can manage and display a huge amount of objects, thus congesting (cluttering) your display. In this case, you may decide to display only some chosen objects at a time, i.e. you may filter the data which you would like to work on. As a consequence, an object may, at a given time, have any of the three statuses: **shown**, **hidden** or **selected**.

For consistency, most operations in Melanie may only be applied to selected objects on selected gels. Objects may be selected using tools from the toolbar or using certain menu options. Once selected, an object is marked in green. The green color is always used to highlight selected objects.



As a general rule, objects may only be selected when they are visible on the screen. For example, you may only select a spot if it is visible (see *Showing spots* on page 45). Nonetheless, as an exception, when you request the selection of a hidden object, it may be momentarily displayed on the screen, but it will immediately be hidden once it is unselected again.

The Shift and Ctrl keys enable you to select more than one object.

- The Shift key enables you to select consecutive objects (select the first object, then hold the Shift key and select the second object. All objects between the first and the second one will be selected).
- The Ctrl key enables you to select discontinuous objects. It also enables you to select and unselect objects, i.e. to add or remove objects to or from an existing selection (hold the Ctrl key and click on the chosen object: it will be added to the selection. Click once more on the selected object and it will be removed from the selection).



Note: When all objects from a certain type are hidden (like spots, annotations pairs and groups), their manual selection is also possible. In this case, they will remain visible by the time they are selected.

Features Overview

Melanie is a complete state-of-the-art software application designed to analyze images of two-dimensional electrophoresis (2-DE). Melanie provides interactive as well as automatic gel analysis. Its high flexibility and richness of the functionalities allow Melanie to behave and respond according to your specific requirements.

Protein detection and quantification

- Automatic detection with the possibility to interactively adjust the results (see *Editing spots* on page 39).
- Semi-automatic spot editing facilities (see *Adding / modifying spots* on page 42).
- Accurate spot quantification using either boundary contours or Gaussian modeling (see *Spots quantification* on page 44).

Matching or how to find similar protein patterns across many gels

- Powerful automatic matching and editing facilities (see *Automatic matching* on page 80).
- Wide variety of tools are available to monitor the accuracy and to fine-tune effectively the detection and matching (see *Quality control* on page 14).

Navigation and display

- Extended navigation inside and across gels including various zooming modes, easy moving of gels, customizable grids and overlay displays (see *Gels* on page 17).
- Contextual windows help you to navigate and instantly gives you access to related information (see *Magnifying window* on page 29 and *Cursor information* on page 47).
- Simple procedures to select and display data subsets to work on.

Full control over what and how the information is displayed.

- Spots marking with different colors and the choice of spot shapes display (see *Coloring spots* on page 46 and *Showing spots* on page 45).
- Large choice of the information type to display and how the associated labels are displayed (see *Showing annotations and labels* on page 64).

Comprehensive and intuitive visual comparison.

- Tile mode of displaying gels allows the user to instantly see how the proteins are expressed through a series of gels (see *Displaying gels* on page 22).
- Stack mode of displaying gels gives you the possibility to track protein variations (flicking gels) (see *Stacking gels* on page 25).
- Transparency mode allows you to visually link spots from the front gel with spots from the gels behind (see *Displaying overlapped spots* on page 27).

Reporting results

More than 20 reports (tables and graphics) are available which contain over 50 data types.

- Intuitive quantitative filter possibilities (see *Sorting data in tables* on page 74).
- Fully customizable reports (see *Reports and navigation* on page 69 and *Report on Spots* on page 49).
- Simultaneous navigation in the reports and on the gels (see *Navigating through data* on page 73).

Database and Internet ready

- Seamless exchange between your own database and Melanie (see *Importing labels* on page 68, *Selecting annotations and labels* on page 60, and *Saving/printing* on page 71).
- Direct database querying through Internet, such as Swiss-Prot (see *Databases* on page 113).

Comprehensive and unlimited annotation capabilities

- You may add any type of information in default or user definable categories (see *Pre-defined label categories* on page 53 and *Creating annotations* on page 55).
- Fast landmarking: allows to easily define tie points in multiple gel studies (see *Pre-defined label categories* on page 53).
- 2D Calibration: uses known pi/mw to calculate standards for unknown spots and allows automatic propagation through all matched gels (see *Pre-defined label categories* on page 53).
- Besides the usual categories such as calibration (pi/Mw and intensities) and landmark, Melanie has integrated advanced new data types such as database access number, MS spectrum, link to external data of any format located on your disk, network or Internet. A simple click on the link displays the associate data (text, html, spreadsheet, multimedia, ...) (see *Creating specific links* on page 56).

Analysis and protein characterization studies

The richness and robustness of the analysis tools give you access to the most sophisticated and reliable analysis techniques.

A wide range of tools allows to efficiently and easily browse the protein expression to extract markers by highlighting subtle changes. You can either browse the data in comparative tables or in several graphical reports (such as Scatter, Histograms, Plots) and pointing out the involved proteins in gels.

The intuitive interface encapsulates both the classical and innovative techniques, to reach superior accuracy. The available tools are based on:

- Robust statistics and measures (see *Statistics for Groups* on page 90).
- Statistical tests (see *Statistical tests* on page 108).
- Automatic classification by Artificial Intelligence techniques (see *Heuristic clustering* on page 99).
- Powerful normalization techniques (see below).

Queries

Melanie's versatile search engine allows to easily formulate complex queries. The query power allows to answer your biological question, based on:

- Sophisticated textual request (see *Selecting annotations and labels* on page 60).
- Qualitative and quantitative information (see *Restriction of groups selection* on page 87 and *Restrict spots selection* on page 47).
- Protein expression change, for example by ratio or similarity (see *Histograms on groups* on page 93 and *Reports on groups* on page 96).
- MS spectrum (see *Mass spectrometry* on page 62).
- Analysis results and data subsets (see *Combining sets of annotations* on page 63).

Normalization

The reproducibility of the 2D separation process can be perturbed by a number of factors, including differences in sample preparation and loading, staining and image acquisition. To accurately compare the quantity of any spot across gels, it is essential to compensate these variations.

You can analyze these variations with the scatter analysis and iron out them with several powerful methods:

- Removal of the image scanning variation by **Intensities calibration** (see *Calibrating spots* on page 44).
- Removal of the varying stain intensities and sample loading by **relative spots quantification** (see *Spots quantification* on page 44).

- Removal of interference and outliers by using **robust statistics** (see *Statistics for Groups* on page 90).
- Varying staining absorption across proteins by **normalizing protein expression change** (see *Comparing protein expression changes* on page 95).
- Merge of several gels from the same sample in a composite gel with fine control over the chosen proteins (see *Creating synthetic gels* on page 119).
- Compensate the gel distortion introduced by variation in the protein migration by **gels alignment** (see *Aligning gels* on page 31).

Quality control

Margins of error and validation tools allow to reach a high quality standard. The purpose of the numerous validation tools is to ensure that you don't miss any relevant data.

Analysis and control are closely related, both focus on peculiar spots. These peculiar spots can result from a protein expression change or from an error induced by the detection and matching process.

Margins of error:

- Scatter analysis (see *Scatter plot* on page 88).
- Matching score (bad pairs) (see *Selecting pairs* on page 82).
- All statistical measures (see *Statistics for Groups* on page 90 and *Statistical tests* on page 108).

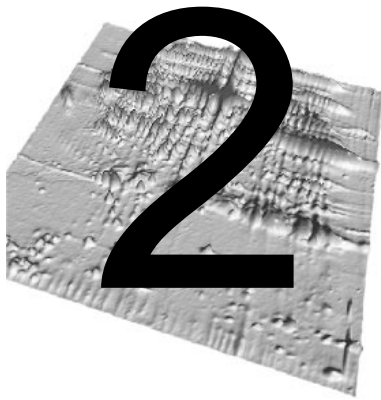
Validation and verification tools:

- Contrast adjustment (see *Adjusting contrast* on page 33).
- Report navigator and selector (see *Navigating through data* on page 73).
- Matching vectors display (see *Displaying overlapped spots* on page 27).
- Protein identifier overlay (see *Showing groups* on page 86).
- Circularity measure (see *Report on Spots* on page 49).
- Wide variety of graphical and comparison table.



Important Note

In the following chapters, all features of Melanie will be described in detail. For each operation, the corresponding menu option is indicated in the left margin, and the text describes how to carry out the operation after having chosen that menu option. Sub-options for the selected menu option are indicated in bold face in the text.



GELS

This chapter presents basic Melanie procedures such as opening, saving and closing gels, displaying gel images (such as arranging and stacking gels, changing contrast and zoom) and printing them.

Menu: File

Opening gels

File
↳ Open

To open a gel image, click in the *File* menu and select the **Open** option. The **Open gels** window will be displayed (Fig. 2-1). Hold down the Shift or Ctrl keys and, in the

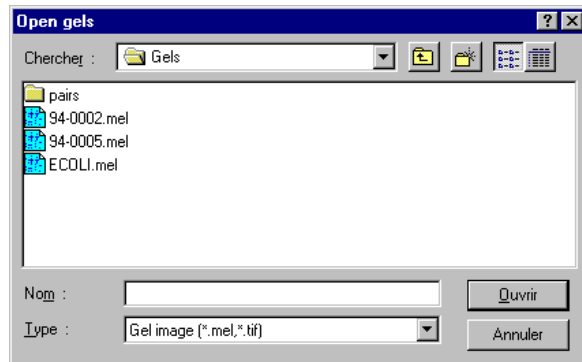


Figure 2-1. The Open window allows the selection of the gels that will be opened and loaded into Melanie.

Files column, click on each of the gel file names you want to open. Click *Open*. The gels will be opened.

Melanie format

The Melanie program works with Melanie file formats, as well as with TIFF files. Analyzed gels are saved into the Melanie release 3 file format, unless otherwise specified by the user.

Importing foreign image formats

File
↳ *Import*

Melanie allows you to import images stored in foreign image file formats, such as TIFF (8 or 16 bits), GIF, Bio-Rad scan. Choose ***Images***. In the *Import Image* dialog box, choose one of the available formats. You may also choose the reduction factor to be used when importing images.

Saving gels

File
↳ *Save*

Any changes carried out on gels have to be saved in order to be stored in the gel file. Otherwise the changes will be lost when exiting the Melanie program or when closing the gels. Choose ***All Changes*** to save all changes performed on the selected gels, or any of the individual saving options. For example, *Save* → *Spots* will only save the detected spot shapes, but not other changes.

File
↳ *Revert from File*

After performing changes on gels, you may revert to the saved status on disk by choosing one of the ***Revert from File*** options in menu *File*. Current changes will be lost.

File
↳ *Save As*

The ***Save As*** command enables you to create copies of selected gels or parts of them, if regions have been defined in gels. You may also use this command to save files created in Melanie as earlier Melanie versions (see *What happens when you open your old Melanie files?* on page 129).

Exporting gels and windows

To files

Alternatively, you may save any gel images in one of the supported foreign file formats (TIFF or BMP). In this case, gel images are exported as they appear on the screen, i.e. with their associated components such as spots and annotations. But the saved file will contain a flat rasterized image, without any structure.

File
↳ Export

Choose **Gel to File**. In the *Export Image as* dialog box, choose the type of the file in the *Type* list and type the file name in the *Name* field. In the same way, you may save Melanie display's window in one of the supported foreign file formats. Choose **Gels Window to File**. By defining a region, you can export only part of the gel.

To the clipboard

You may paste images directly to another software. Define a region in the gel then make **Gel Region to Clipboard**. Activate the program to which you want to copy your image to and paste. You may also copy and paste the whole Melanie screen. In this case, choose **Gels Window to Clipboard**.

Printing gels

Melanie provides printing options. You may print selected gels or regions of gels, one image per page, or print the whole Melanie window. The image will be printed as it is displayed on the screen, i.e. holding objects and properties such as *Spots*, *Annotations*, *Contrast Adjustment*, *Alignment*, *Zoom*, *Grid*, etc.

Printing selected gels

Select some gels, then choose **Gels**. The selected gels will be printed in full size. You may also print only regions of gels by defining regions on selected gels.

File
↳ Print

Printing the Melanie window

Select **Gels Window**. The image of the whole Melanie window is sent to the printer.

Page setup

The **Page Setup** command calls the standard Windows dialog where you may set printing parameters such as printer name, paper size and orientation, etc.

File
↳ Page Setup

Loading / unloading gels

You may control Melanie's use of memory by loading or unloading gel images raw pixel values. The default *Unloaded* mode uses less memory, but as a consequence the real pixel values are not displayed in the *Cursor Information* window (*Window* →

File
↳ Raw Image

Information). You may choose to keep their raw images in memory. Of course, as a consequence, pixel values information will be displayed but more memory will be needed too. To load raw images, select gels and choose **Load**.

You may also decide to free up the memory used by the loaded images by choosing **Select Gels with Loaded Image** to determine those that are loaded and then choosing **Unload** to finally liberate the memory.

At this point, you can decide whether or not to keep raw data from any gels opened subsequently. In this case, select the check box in the pop-up dialog of **Settings**.

When using the default mode of unloaded images, Melanie will in any case automatically and implicitly load their raw images when needed, for example prior to performing the *Auto-Detect Spots* and *Adjust Contrast* operations. And immediately at the end of this operation, Melanie will implicitly unload the raw images, thus freeing the used memory.



Note that the chosen Display depth that is set in your computer determines the memory used by Melanie to display gels (see the Display icon on the Control Panel of your computer to find out more details). For example, 16 million colors require 3 times more memory than 256. So, you may also control Melanie's performance in managing the memory by choosing the display depth according to your needs.

Closing gels and exiting Melanie

File
↳ Close

Use **Close** to close all selected gels. If any of the gels have not been saved since changes were performed, Melanie lets you choose to save them before closing them.

File
↳ Exit

To exit Melanie, select **Exit**. Melanie lets you choose to save any unsaved gel images before exiting.

Our mascot Barry greets you with a folklore song from Switzerland. Melanie has been developed in Geneva, at the foot of the Swiss Alps, where the Saint Bernard dog is at home and people sing yodel¹ to communicate between moutaintops.

1. http://www.ips.k12.in.us/mskey/pods/Big_Mac_Pod/gwaltney/yodel/The_Yodel_Course.html

Menu: Select

Selecting gels

Most of Melanie's functions can only be performed on selected gels. Gels may be selected either by clicking on their banners or by using one of the *Gels* options in menu *Select*. Regions of gels may also be selected using the Region tool.

To select one gel, click on the gel's selection banner. The gel will be surrounded by a green line indicating that it has been selected. To select more than one gel, select the first one as described above, then hold Shift or Ctrl keys and select additional gels.

Select
↳ Gels

Alternatively you may select all visible gels in the Melanie window by choosing **Visible**, or only the aligned gels by choosing **Aligned** (see *Aligning gels* on page 31) or just the stacked gels by choosing **Stacked** (see *Stacking gels* on page 25).

You may select specific gels by choosing their names in the list given by the **By Name** command.

You may inverse an existing selection of gels by choosing **Inverse Selection** or select all gels by taking **All**.

Gels which have spots matched with the reference gel may be selected by taking **For Groups**. In the same way, Gels belonging to specific classes may be selected by taking **For Classes**.

You may also select gels directly from a displayed *Report on Gels* (see *Reports and navigation* on page 69).

The region tool



The Region tool is useful to select a rectangular region within a gel. Position the cursor at the top left position of the desired region, hold down the mouse button, then move the cursor to the bottom right position. A rectangle will be drawn around the region (Fig. 2-2). Release the mouse button at the desired point.

You may move the region by clicking inside the rectangle's area and dragging it. You may also change the rectangle's size by dragging **its corners or borders**. To dese-

lect the drawn region, select the Region tool and click outside the rectangle region or double-click inside one region.

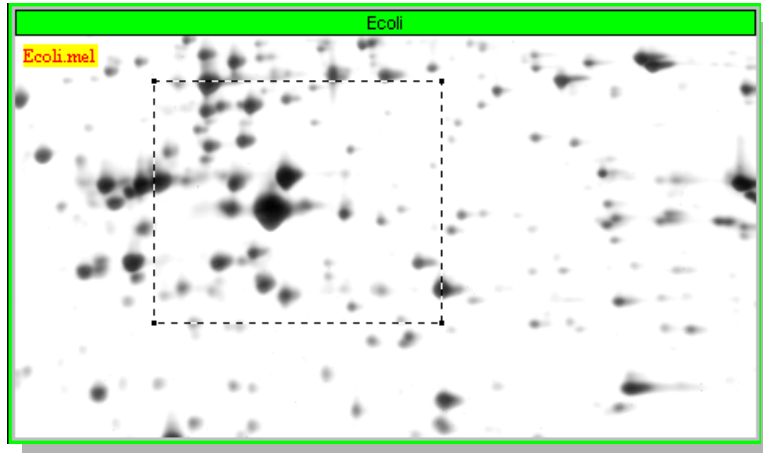


Figure 2-2. Selected region in gel.

To select the same region on all visible gels, hold the Shift key and select the rectangular region on one of the gels. In this case, the selected regions are also calculated proportionally to corresponding pixels.

Menu: Show

Displaying gels

Melanie provides numerous ways of displaying gels and related information on the screen, each one with specific and helpful functions. You may display gels in tile mode (one gel next to the other) or in stack mode (piles of gels where only the first one is displayed). You may arrange gels as you wish (fixed or variable number of display cells) and whenever you wish (show and hide gels). You may display numerical gel information (Cursor window) or complementary information (Zoom window, grid lines).

Each displayed and visible gel has two special areas that indicate its name and the operations that have been performed on them (Fig. 2-3). The gel banner (horizontal stripe located at the top of the gel which contains its name) indicates whether or not the gel is selected. The gel legend located in the top-left corner of a gel (yellow background), besides indicating its name, also provides:

- The name of the class to which the current gel was assigned to,
- The name of the gel to which the current gel was aligned to,

- The zoom factor of the current gel.

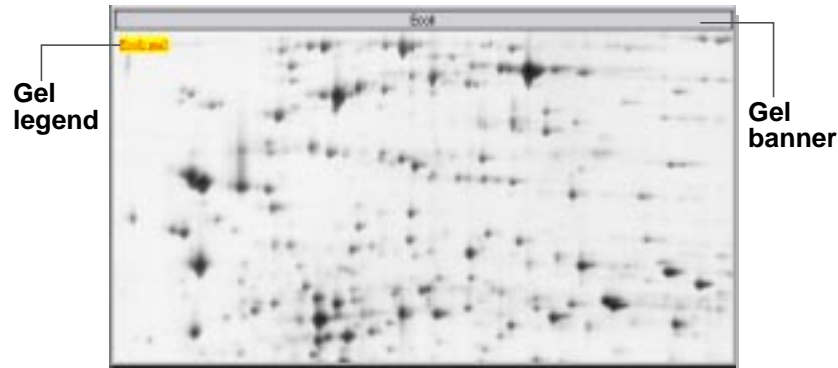


Figure 2-3. Gel banner is the gel selection area.

For example, a gel called **94-0002** with a zoom factor 4, assigned to class **CLASS A** and aligned to gel **ECOLI** will show the following information in the yellow stripe: **94-0002 (CLASS A) *ECOLI* x 4**.



The gel banner is used to select gels and to drag them.

Showing gels

Once you have opened a given number of gel images, you can decide which ones you want to display and which ones you prefer to hide at a given point in time.

To hide a gel, drag it to the hidden gels zone (Fig. 1-2, number 6). To hide several gels at a time, select the gels as described previously, and while holding the Shift key, drag them to the hidden gels zone (Fig. 2-4).

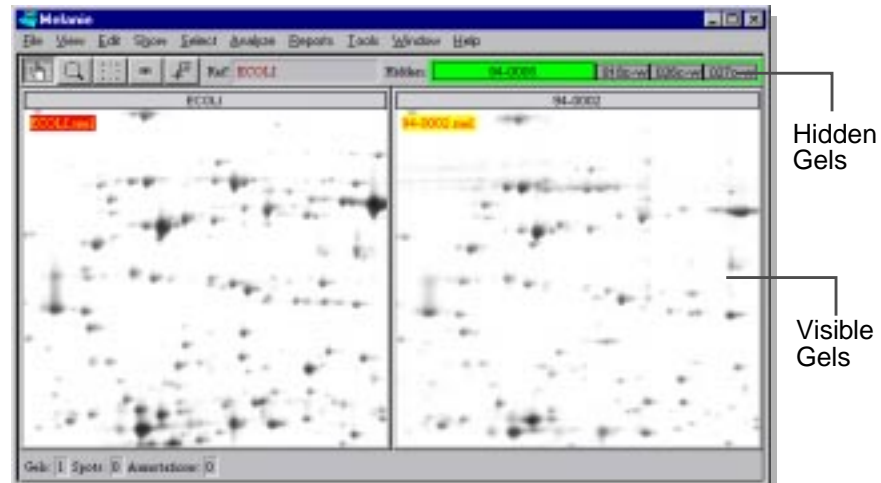


Figure 2-4. Hidden and visible gels.

To make a hidden gel visible on the display area, drag the selected gel from the hidden gels zone to an available cell on the screen. If you drag the hidden gel to a cell already occupied by another gel, both gels will be stacked immediately.

Show
↳ Gels

The *Gels* option in menu *Show* also provides the following choices to this purpose:

- **Only Selected** - displays all selected gels and hides the other ones.
- **Hide Selected** - hides all selected gels.
- **Show All** - displays all opened gels, including the currently hidden ones.
- **Sorted by Name** - order the cells sorted by the gel names.
- **Sorted by Classes** - order the cells sorted by the gels' classes names.

Menu: View

Changing the display gels order

Melanie displays gels on the screen through two options:

View
↳ Layout Settings

- The Adaptive option retains the same number of cells on the screen as the number of visible gels. This is Melanie's default. To select this option, take **Layout Settings** and choose *Adaptive* in the dialog box.
- To display visible gels with a fixed number of cells, i.e. independently of the number of visible gels, take *Layout Settings*, select *Number of cells* and choose the number of cells with the slider (Fig. 2-5).

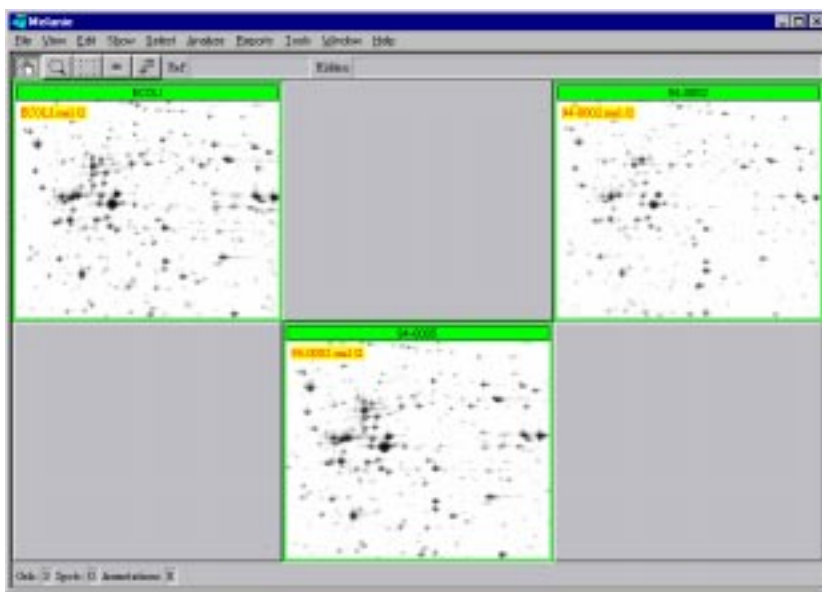


Figure 2-5. Display area with fixed number of cells equal to 6.

To insert one gel into one specific cell on the screen, click on its banner and drag it to the desired cell.

You may change the order in which cells are arranged on the screen. Click **in the border** of the cell you want to displace and drag to another cell. The dragged cell will be **inserted** at the dragged location.

Stacking gels

Very often users prefer to visually compare gel regions simultaneously, arranging them side by side in the display, i.e. in tile mode, which is Melanie's default mode. But in the case where many gels are open at a time, their visible parts may be too small. Stacking gels, i.e. displaying one gel on top of the others, thus creating a "pile" of gels, is another way to display and compare gels. You may compare each gel of a stack (one by one for example) against the reference gel of the stack (Fig. 2-6). Besides, the stack mode allows you to quickly permutate gels, letting you visually discern changes in protein intensity. You may stack many gels at a time and you may also have as many stacks as you want.

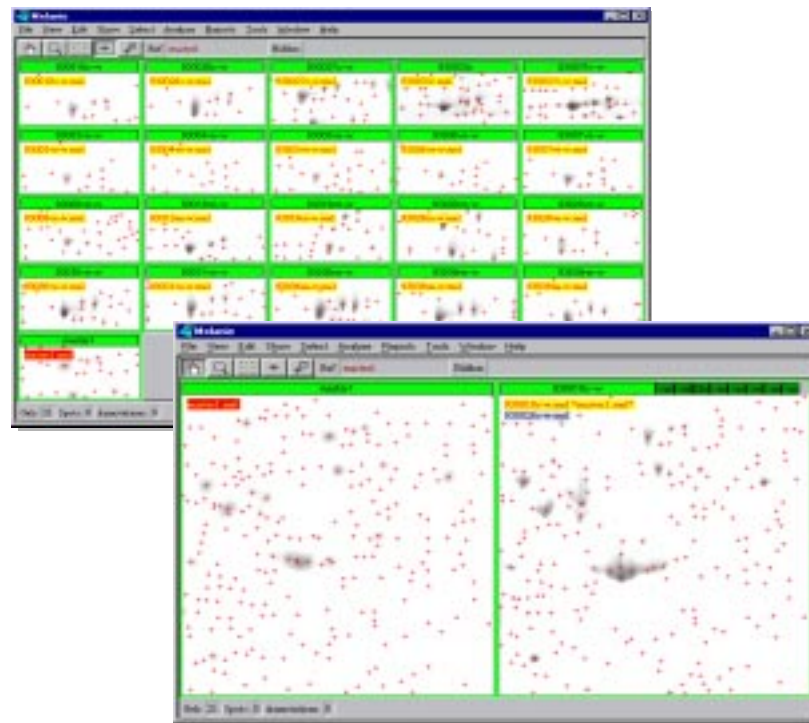


Figure 2-6. Gels in tile mode and stack mode.

View
↳ Stack

To stack two gels, select one gel and drag it onto the other gel. To stack several gels, select the gels and drag them onto one of the cells. Alternatively, select gels and take **Selected Gels**. Certain operations that can be performed on stacks let you compare the front gel with one of the other gel in the stack, called the *Stack Reference Gel*. To set the reference gel of a stack, select the stacking cell and choose

Set Stack Reference. The name of the reference gel of the pile is displayed with a blue background over the gel and the name of the first gel of the pile (the front gel) is displayed as usual (Fig. 2-7).

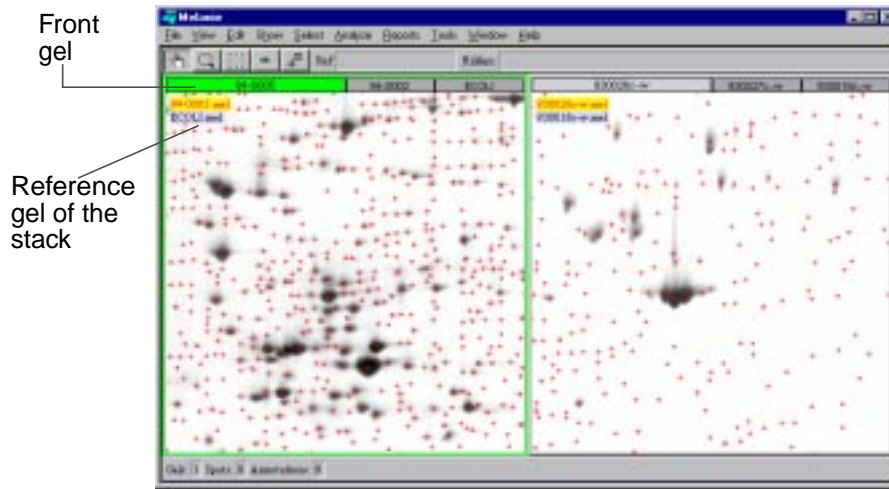


Figure 2-7. Stacked gels. Cell on the left has 3 stacked gels. The front gel is 94-0005 (the gel in the top of the pile), 94-0002 is the second gel (the gel in the middle of the pile) and Ecoli is the bottom gel (located in the bottom of the pile). Cell on the right has other 3 gels stacked together.

To put a gel in front of the stack, you may either press the Page Up or Page Down keys. You may also click on a gel's banner or take **Next** or **Previous**. To switch from the front gel to the reference gel of the stack, choose **Front To Back**, or simply Ctrl-F. By pressing Ctrl-F quickly several times, or the Page Up or Page Down keys, the differences between the stacked gels can be observed visually.

To unstack the gels, select the gels and choose **Unstack Selected Gels**.



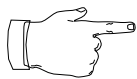
When many gels are stacked or hidden, their names are not entirely displayed. Click on their banners and their names will be displayed in the front gel (respectively in the stack or hidden pile).

Selecting stacked gels

You can select each gel from a stack individually. You may also choose to select two or several gels in the stack and operations will be performed only on these selected gels. To select one gel, click on its banner. To select all the gels in the pile, double-click in the front gel's banner, which corresponds, on the stack, to the left most name on the banner. You may also use the Shift or Ctrl keys to select continuous or discontinuous gels on the stack.

Moving stacked gels

Melanie provides tools to superimpose corresponding spots of stacked gels in a simple way. In stack mode, you can move one or several gels as usual (see *Moving gels* on page 27).



To superimpose two spots, put the cursor on the given spot in the front gel, then hold down the mouse button. Press Ctrl-F to display the other gel. Move the cursor to the corresponding spot and release the mouse button. The two spots will be superimposed. Verify this by pressing Ctrl-F again.

Displaying overlapped spots

While two or more gels are stacked, visible spots from the reference gel of the stack can be seen in transparency mode on the front gel. The spots from the reference gel of the stack are displayed in blue below the spots from the front gel.

View
↳ Stack

Set the reference gel of the stack, as described above (see *Stacking gels* on page 25). Some spots of the reference gel should be displayed. Choose **Show Overlapped** (Fig. 2-8). To dismiss the overlapped spots choose **Hide Overlapped**.

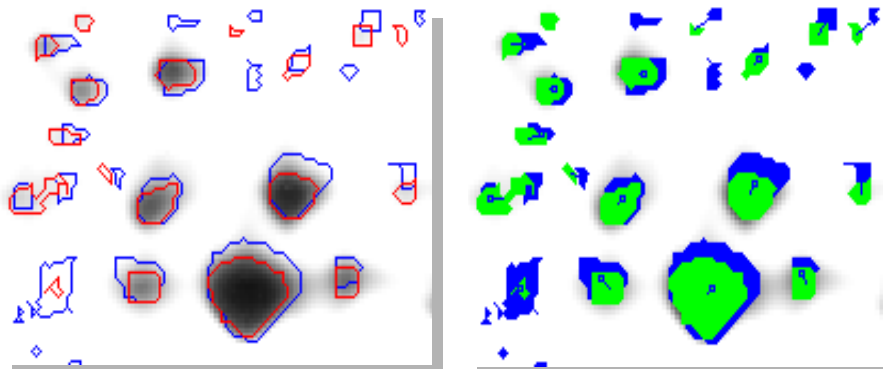


Figure 2-8. (a) Overlapped spots, spot shapes displayed (b) Overlapped spots with pair vectors, spots filled.

When gels are in stack mode, you may also display the pairs between the reference gel and the other gels of the stack in the form of blue vectors linking the locations of paired spots (Fig. 2-8). Choose **Show Pairs**. To hide vectors, choose **Hide Pairs**.

Moving gels



The hand tool moves gels in order to show other parts. Pick the Hand tool in the toolbar, put the cursor inside a gel, hold down the left mouse button and move the mouse. The gel image will follow your movement. If you hold down the Shift key and the mouse button simultaneously, then all visible gels will be displaced by the same amount, once the button has been released.

By **double-clicking** inside a gel, all visible gels will be positioned at the same location with the same magnification.

The **corresponding location** from the clicked gel to the others gels is estimated by interpolation between the two nearest common **landmarks** (i.e. landmarks with the

same name). When the gels have no common landmarks, they are aligned at the same image location.

In addition to the Hand tool, Melanie provides other useful operations to move gel images in order to show other image parts.

View
↳ Move Gels

The *Move Gels* option enables you to scroll through visible parts of selected gels. To scroll up, select **Up**, or alternatively hold the Ctrl key and press the Up Arrow key. Use the respective commands to scroll down, left or right.

The *Move Gels* option also lets you choose to display the top left, top right, bottom left or bottom right part of the selected gels. Just take *View* → *Move Gels*, then choose the corresponding sub-option.

The equivalent of a double-click inside a gel may be found in Melanie's menu. Select one gel and take **Same Location**. All gels will be positioned at the same location with the same magnification of the selected gel.

If you cannot see a gel, because it was moved outside the visible parts of the screen while using the hand tool, then select the gel and take one of the options from *View* → *Move Gels*.

Zooming gels



The Magnify tool lets you repeatedly zoom in or out the **whole gel**. To zoom in, pick the Magnify tool and click over the gel in the wanted region. To zoom out, click the right mouse button (Fig. 2-9).

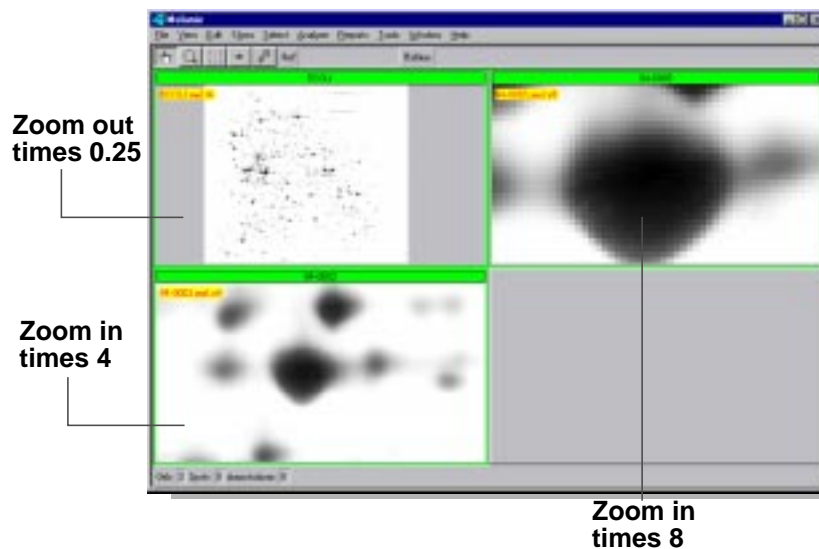


Figure 2-9. Zoom in and out.

To zoom in or out of all visible gels simultaneously hold down the Shift key and then click the left or right mouse buttons respectively. After the zoom, all gels will be moved to the same position.

View
↳ Zoom

Alternatively, you may choose to zoom in and out by taking **Zoom** and selecting the corresponding sub-option.

The minimum possible zoom factor is 1/16 times the original gel image. The maximum zoom factor is 32 times the original gel image, however the resulting images will have a maximum size of 32'000x32'000 pixels.

Magnify glass

The Magnify glass is an alternative way to view enlarged parts of gels momentarily. In this case, select the Magnify tool, press Ctrl and click on the region you want to enlarge (Fig. 2-10).

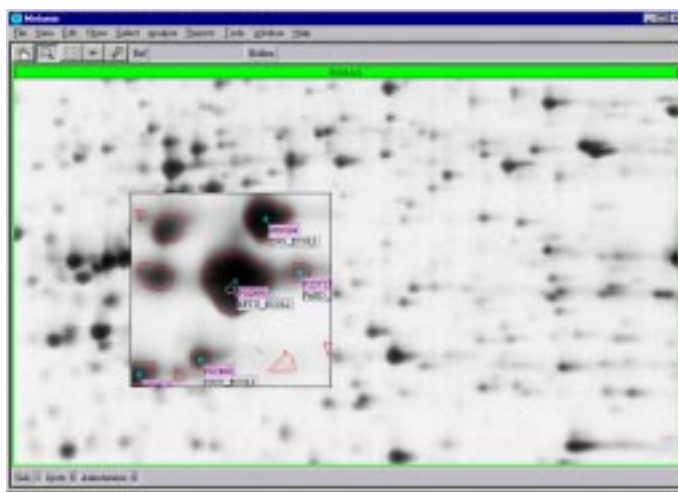


Figure 2-10. Magnify glass.

Magnifying window

Window
↳ Zoom

Another way to enlarge parts of gels is to use the Zoom Window, which enables you to see the region under the cursor (examples in Fig. 2-11 and 2-12). To display the Zoom Window, choose the menu *Window* → **Zoom**. You may change the magnifi-

cation of the zoom window by pressing the + and the - buttons located at the top of the window and you may also resize this window.

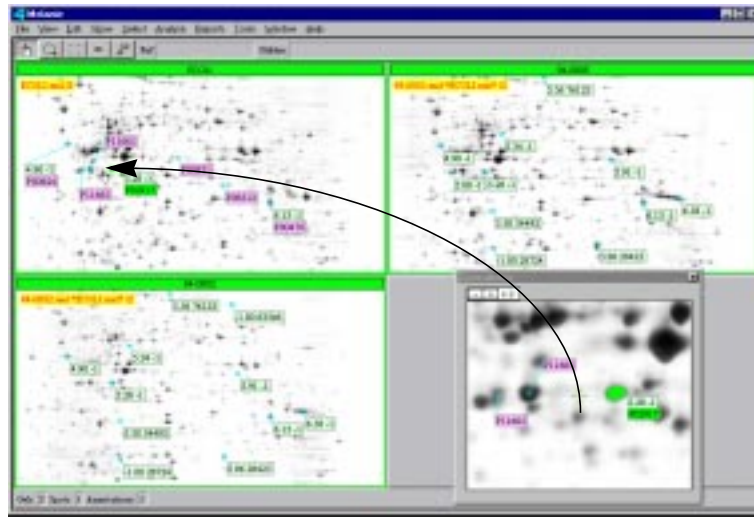


Figure 2-11. Zoom window used to enlarge the focused to region.

The zoom window displays the visible objects, like annotations and spots, as they appear on the screen. It also displays one green rectangle indicating the visible part, on the display area, of the pointed to gel (the gel which has the cursor).

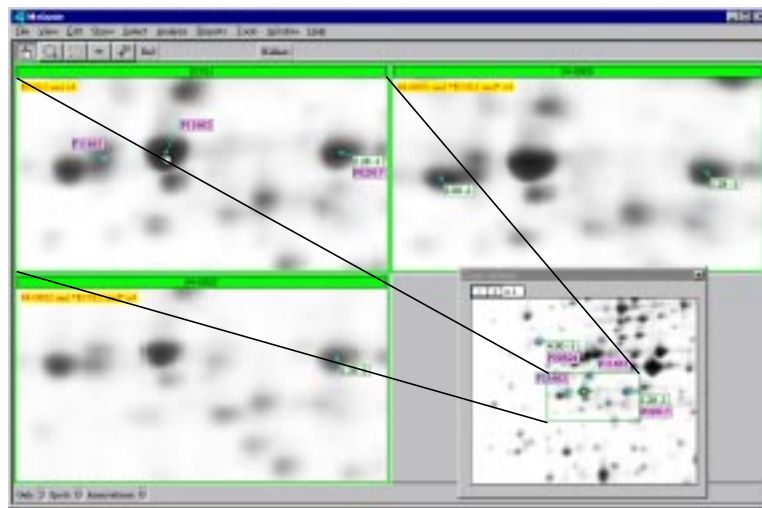


Figure 2-12. Zoom window used to locate the focused to region on a gel displaying spots and labels. The small rectangle indicates the visible area of the pointed to gel.

Displaying grid lines

You may display grid lines over selected gels to indicate their dimensions in centimeters, inches or in pI and Mw units, provided that this information is available in the annotation category **Pi_Mw**. The pI and Mw grid may also be displayed over gels which don't have this information, but have been matched against the reference gel containing pI and Mw. In this case, the grid values will be relative to the reference gel (Fig. 2-13).



Figure 2-13. Grid lines over gels.

View
↳ Grid Lines

Select a gel and choose **Show**. In the displayed window, choose a grid unit from centimeters, inches or pI/Mw. Also choose the number of sub-divisions, horizontal and vertical, in the sliders.

The information displayed in square brackets in the top-right corner and in the bottom-left corner of the gel indicate the coordinates of the visible portion, in chosen units.

To hide the grid lines of selected gels, select the gels and choose **Hide**.

Aligning gels

Gel alignment is a very useful technique for comparing 2-DE images that present too many variations in protein migration. In general, while all Melanie programs may work with unaligned gel images, alignment might prove helpful when comparing stacked gels, or for matching distorted 2-DE gel images.

You may align two or more gels at once. You must select a reference gel, i.e. an image the other gels will be aligned to. At least four annotations containing labels from the category *Landmarks* (for the default alignment algorithm) must have been defined and must be well distributed over the gel image (see *Annotations* on page

53). The other gels must contain the same landmarks, i.e. same landmark label inside the same spot, as the alignment algorithm will try to deform the images in order to best superimpose the landmarks (Fig. 2-14).

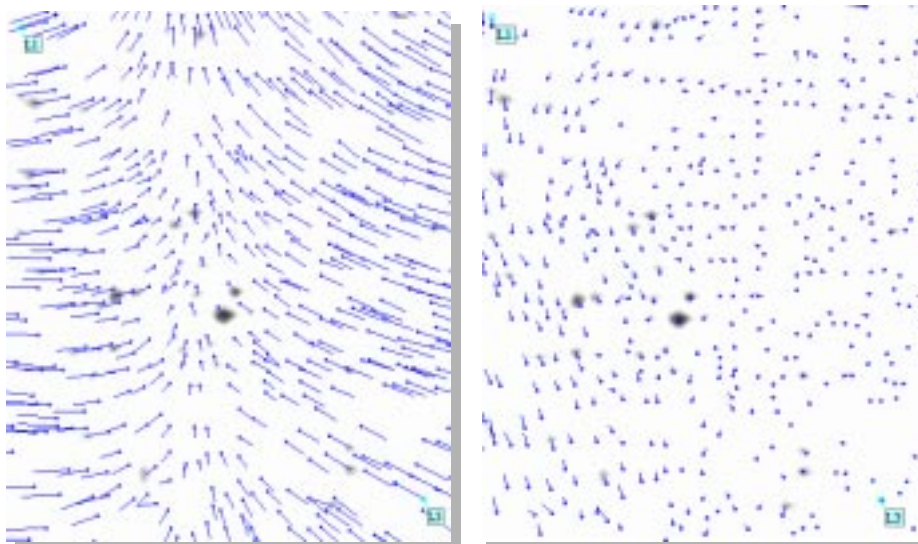


Figure 2-14. Gels before and after alignment. Gels are in stack mode and their pair vectors are displayed.

Alternatively, if you align gels that have already been matched, you may choose to use existing pairs rather than labels from the category Landmarks as reference points.

View
 ↳ Align Gels

Select all gels that have to be aligned. Choose **Align**. In the displayed window choose the reference gel. The selected gels will be aligned relative to the reference gel, so that corresponding landmarks are best superimposed.

To get back to the original images, select aligned gels, then choose **Unalign**. The original images will be displayed again in place of the aligned ones.

By default, gels are aligned relative to the X and Y image axes, using a second degree alignment function in both the X and Y axes. You may decide to align images according to only the X or Y axis, or to use another alignment function. Choose **Other Alignment** and select the desired option. First, second and third degree functions are available, that request at least two, three and respectively four landmarks.



In the event that gels are not aligned but have common landmarks, operations such as simultaneous gel moving, gels matching, etc. are done based on a simple interpolation between the two nearest common landmarks (i.e. landmarks with the same name). Otherwise, when the gels are not aligned and have no landmarks, the operations are based on the same image location.

Gray levels

A 2-DE gel image is traditionally displayed as a gray level image, where gray levels represent the gel's optical density. You may opt to display images using pseudo colors. You may also change the way gray levels are displayed.

Adjusting contrast

Modern scanners are usually able to scan 2-DE images with 12 or even 16 bits per pixel, that is with 4096 or 65536 gray levels, respectively. Because common screens are only able to display 256 colors or gray levels, mapping has to be undertaken between the 4096 or 65536 gray levels, respectively and the 256 screen gray levels. By default, Melanie uses a linear mapping function, where the lightest point in the image is mapped to 0 (white) and the darkest point is mapped to 255 (black). In Fig. 2-15, the minimum and maximum gray levels (zero and 2361 respectively) are remapped by the default linear mapping.

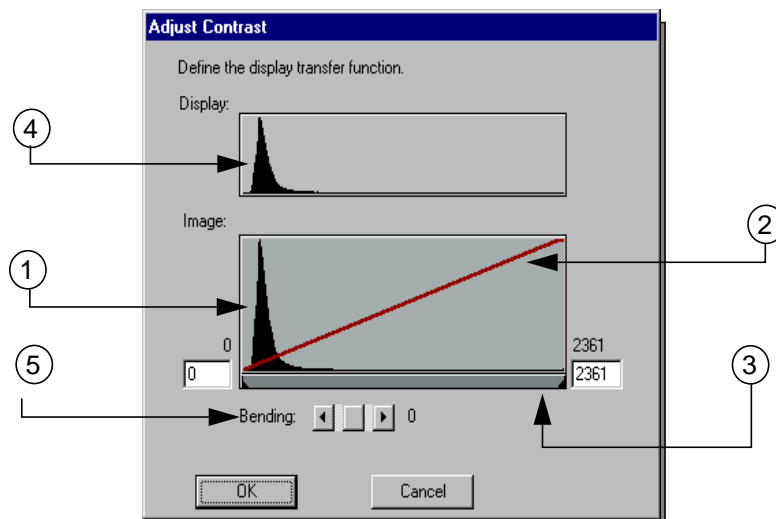


Figure 2-15. (1) Gray level distribution of selected images. (2) Mapping transformation. (3) Minimum and maximum gray levels slider. (4) Gray level distribution after the function transformation. (5) Bending parameter.

However, you may choose other mapping functions, e.g. in order to highlight small light spots. Melanie lets you change the gray level mapping in two different manners. You can define the minimum and maximum gray levels, i.e. you may look at only the light or the dark regions of the images, or you can change the default linear mapping function.

The Bending parameter expands or compresses the contrast range at the dark or light ends of the range. When bending is higher than zero, the image is lighter, and it is darker when bending is lower than zero (Fig. 2-16).

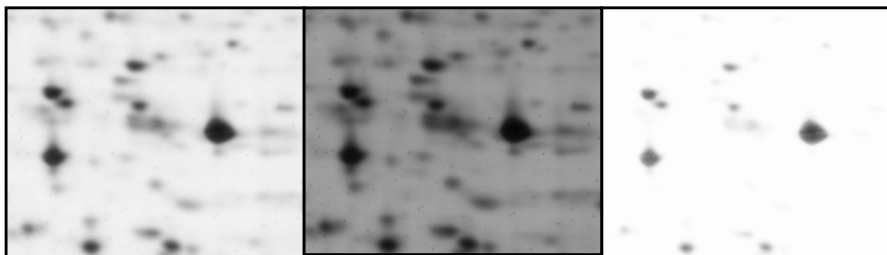


Figure 2-16. Region of a gel showing a contrast adjustment: the left image shows the original image; the central and right images show the same gel area after transformation by setting the bending parameter to -2 and 2 respectively.

View
 ↳ Gray Levels
 ↳ Adjust Contrast

To adjust the gels contrast, select some gels, then choose **Adjust Contrast**. In the Adjust Contrast window, change the minimum and maximum gray levels (respectively left and right bar corners, item 3, Fig. 2-15) or the bending slider (item 5, Fig. 2-15).

Once the parameters have been changed, the resulting gray level of the modified display can be visualized in the *Display* sub-window (item 4, Fig. 2-15) from the Adjust Contrast window. By choosing the *Only in Region* check box, in the *Adjust Contrast* window, the image gray level distributions are restricted to the selected region.



Any changes made with the Adjust Contrast command will only affect how the image is displayed on your screen. They will not affect the underlying data, the spot detection and quantification.

Adjust contrast preview

Each change of the gray levels of an image, when in the *Adjust Contrast* window may immediately be reflected in a given region, if such a region has been defined prior to adjusting contrast. Select one region in a gel. In the *View* menu, choose

Adjust Contrast. Each time you change one of the mapping parameters, the change will immediately be reflected in the selected region (Fig. 2-17).

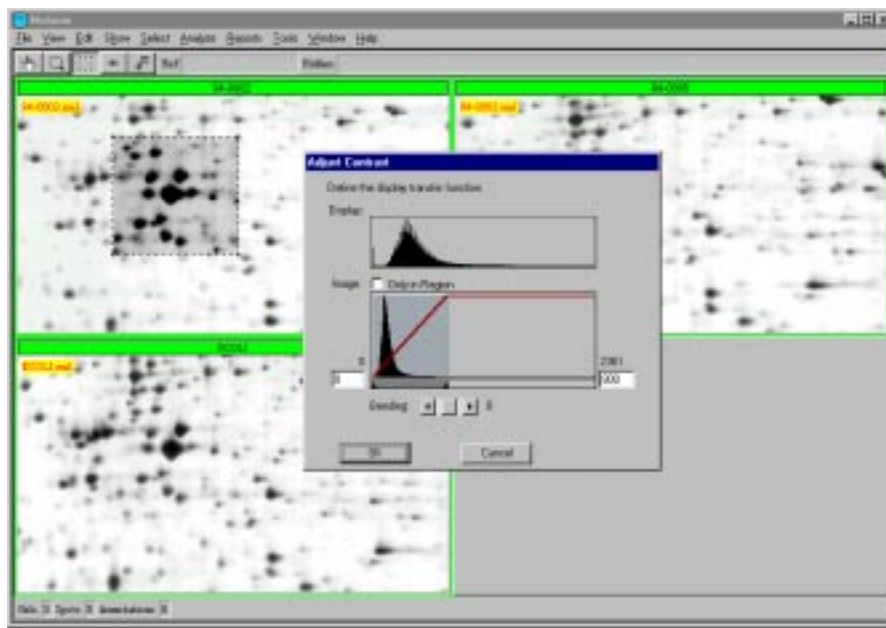


Figure 2-17. The *Adjust Contrast* option. The modifications are directly reflected in the selected region.

Note that if the small rectangle temporarily disappears from the display, when the *Adjust Contrast* window pops up, slightly change one of the parameters.



The gray levels will be adjusted in all selected gels.

If you save gels after having performed color adjustments, the latter will be saved with the gel images (see *Saving gels* on page 18).

Displaying gels with pseudo colors

View
↳ Gray Levels
↳ Pseudo Colors

Choose **Pseudo Colors**. Select one of the color lookup tables, for example *FiveR-amps*. Click OK. The lookup table will be changed accordingly (Fig. 2-18). To go

back to gray levels, choose *View* → *Gray Levels* → *Pseudo Colors* and select *Gray Levels* among the displayed options.

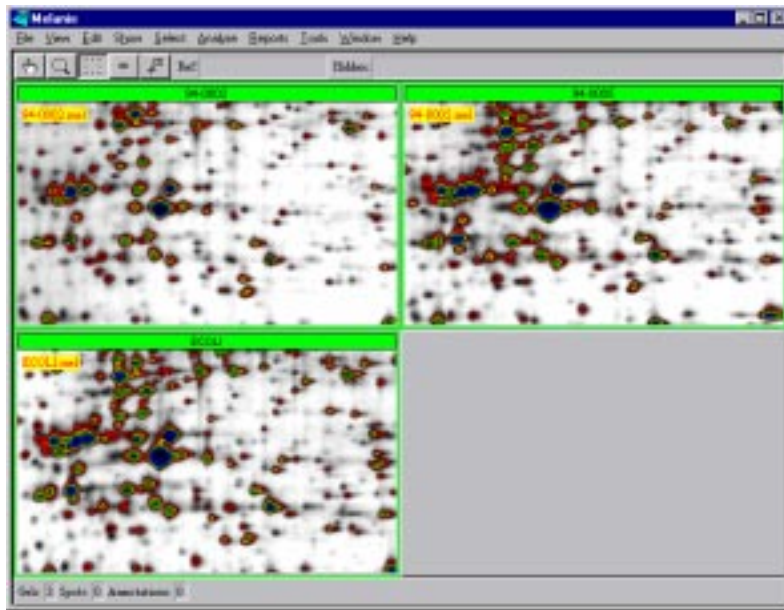


Figure 2-18. Gels displayed using the *FiveRamps* color lookup table.

Menu: Edit

Calibrating gels

Edit
↳ *Gels*
 ↳ *Calibrate...*

Melanie enables you to calibrate pixel intensities, and as a consequence to calibrate gels. Rather than displaying gray level values taken from the scanner device, you may display image values indicating the real world values, for example optical density values (see *Calibrating spots* on page 44). Calibrated gels have an asterisk in their names in the reports.

Setting preferences

Edit
↳ *Preferences*

A few preferences may be set and preserved between Melanie sessions using the options in the menu *Edit*.

In *Raw Image*, you may decide that all further open gels will keep their raw data image in memory (see *Loading / unloading gels* on page 19).

In **Grid Preferences**, you may set the grid attributes, i.e. the grid units and number of subdivisions (see *Displaying grid lines* on page 31).

In **Cursor Information**, you may select spot and pixel attributes to be displayed in the Cursor Information window (see *Cursor information* on page 47).

In **Database**, you may set preferences for the automatic queries in the internet (see *Databases* on page 113).

Menu: Reports

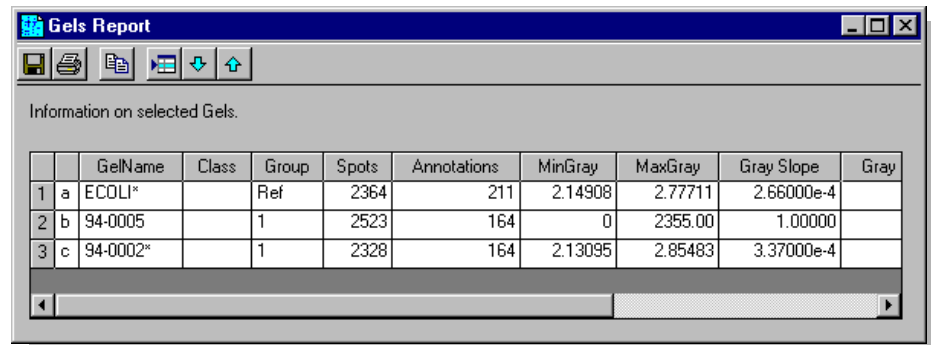
Reporting on gels

Reports
↳ Gels Report

Select gels, as described previously, and choose *Reports* → **Gels Report**.

Gels Report display summarized information about the selected gels, such as image height and width in pixels (*Rows* and *Columns*), minimum and maximum gray levels, number of detected spots, number of existing annotations, pixel dimensions of the scanned image (*PixWidth* and *PixHeight*). The class to which the gel was assigned to and the intensities calibration is displayed as well.

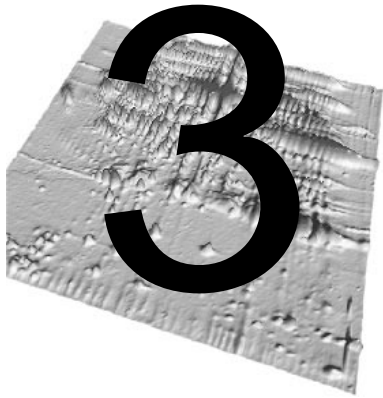
The Group information, also available in the *Report on Gels*, indicates the reference gel ("Ref") and whether the gels are matched against it. Value 0 indicates no matching and 1 indicates matching between the gel and the reference gel (Fig. 2-19).



The screenshot shows a window titled "Gels Report" with a toolbar containing icons for file operations and navigation. Below the toolbar, the text "Information on selected Gels." is displayed above a table. The table has 10 columns: an index column, GelName, Class, Group, Spots, Annotations, MinGray, MaxGray, Gray Slope, and Gray. Three rows of data are visible, representing gels 'a', 'b', and 'c'.

	GelName	Class	Group	Spots	Annotations	MinGray	MaxGray	Gray Slope	Gray
1	a	ECOLI*	Ref	2364	211	2.14908	2.77711	2.66000e-4	
2	b	94-0005	1	2523	164	0	2355.00	1.00000	
3	c	94-0002*	1	2328	164	2.13095	2.85483	3.37000e-4	

Figure 2-19. Report on gels. Gels ECOLI and 94-0002 are calibrated.



SPOTS

The elementary component of a gel is the **spot**. Spots are spot shapes automatically detected by Melanie's spot detection algorithm, or manually adjusted by the user. Each spot in a gel has an associated **spot ID** (a unique sequential number) automatically assigned to it when it is created. Spots may be quantified, i.e. their optical density, area and volume are computed. Alternatively spots may be modeled by Gaussian functions. In this case, their quantification values will be the Gaussians peak values and volume, as well the area of the Gaussian's half-height section.

Menu: Edit

Editing spots

Edit
↳ *Spots*

Select gels to detect spots on, then take ***Auto-Detect***. The *Detect Spots* window appears. Various detection parameters are available and may be adjusted. The default parameters have been optimized for silver stained 2-D PAGE images run

according to the SWISS-2DPAGE protocol [see reference 13]. You may tune the parameters to adapt the detection program to your images. The following parameters may be set (Fig. 3-1):

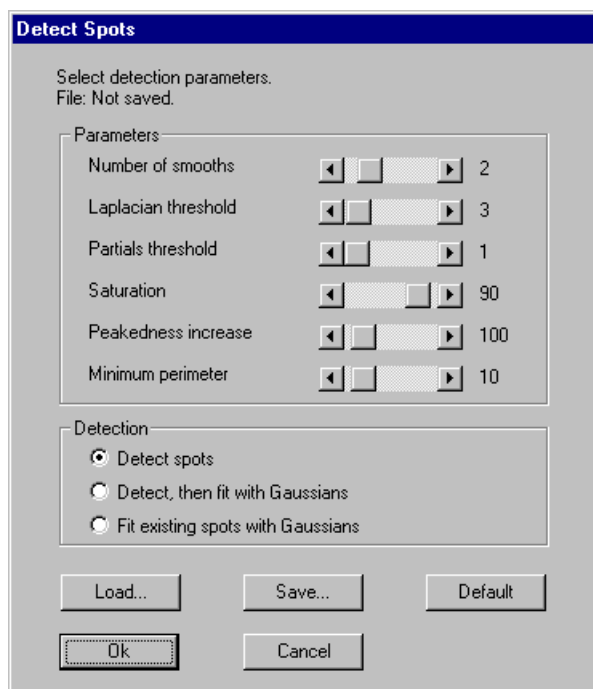


Figure 3-1. The spot detection parameters.

- **Number of smooths** - Before detecting spots, Melanie will smooth the image using a “smooth-by-diffusion” algorithm, in order to eliminate parasite noise. Select the number of times the smoothing operation should occur. Note that other smoothing algorithms are available in the *Tools* → *Gels* menu (see *Filtering gels* on page 121). To avoid smoothing, set the parameter to zero.
- **Laplacian threshold** - The detection algorithm looks for spot peaks by calculating the Laplacian for each pixel in the image. To detect as many spots as possible, set a small value for this parameter. If you set high values, only very curved spots will be detected and flat spots will be ignored.
- **Partials threshold** - Melanie also checks the spot curvature in both the X and Y axes, in order to separate spots with flat sections, or to eliminate streaks. Augmenting this value increases the separation power of the detection algorithm.
- **Saturation** - Some very dark spots may be saturated (especially when digitized with older scanners) and may therefore be flat on the top. To insure that these spots are detected, Melanie artificially increases the peakedness (curvature) of all pixels whose OD are above a given threshold. The threshold is expressed as a percentage of the maximum grey level in the image. A value of 90% is usually considered correct.
- **Peakedness increase** - This is an internal value expressing how much Melanie increases the curvature of saturated pixels. 100 is the default value and should only be changed with care.
- **Minimum perimeter** - With this parameter you may decide to ignore spots that are too small. Melanie will ignore all spots whose perimeters (in pixels) are smaller than the given threshold.

Additional criteria affect the way spots are represented. You may choose between the following three options:

- **Detect spot** - This is the default. Spots are detected using the Laplacian, and spot shapes are represented that match the original spot shapes.
- **Detect, then fit with Gaussians** - Spots are detected with the Laplacian, then Gaussian modeling is performed on all detected spots.
- **Fit existing spots with Gaussians** - Spots have to already be detected and modeling is performed on them. Note that the parameters are of no use here.

First try to detect spots using the default parameter values, then tune the parameters for your data. You may save your chosen parameters by clicking *Save* and typing a file name. You may also load an existing parameters file by clicking *Load* and choosing the file name.

Click *OK*. Spots will be detected according to the parameter values you have set. After detection the spot shapes will be displayed over the gels (Fig. 3-2).

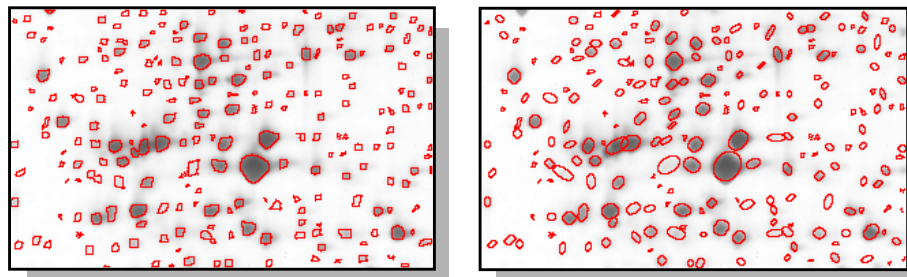


Figure 3-2. Left: Detected spot shapes. Right: Spots modeled as Gaussians



Default parameters are adapted to 12-bit-images. When using 16-bit-images, a dialog box lets you choose, before deciding the detection parameters, whether you want to change the grey levels to correspond to 12 bit images. You must note that parameters may not be that well adapted to 16-bit-images. In this case, you have to tune them.

Detect spots in a region

You may also only detect spots in a given region. If a region has been defined in a gel, Melanie enquires whether the detection must be done in the selected region only or in the whole gel.



This allows you to refine the detection in a gel's area with other parameters. Spots outside the region are not affected.



Preview detection results

If a region has been defined, each change of a parameter value may immediately display the result from the spots detection in the selected region (Fig. 3-3).

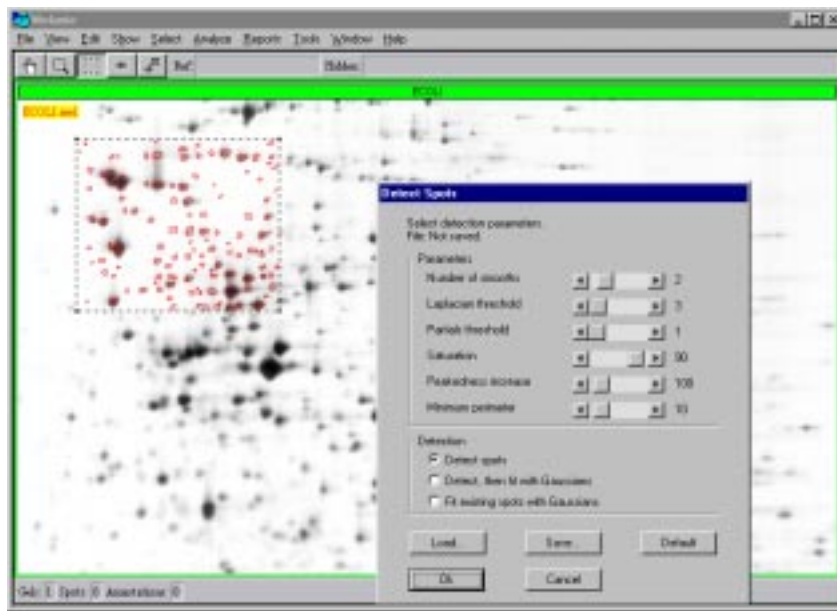


Figure 3-3. Adjusting detection parameters in real time.

Note that if the small rectangle temporarily disappears from the display, change one of the detection parameters slightly to make it visible again.

Adding / modifying spots

The usual way to create spots is to detect them using Melanie's powerful spot detection algorithm. Nevertheless, you may manually create, modify or delete selected spots.



To add a new spot on the gel, choose the Spot tool and double-click in the desired location. In the dialog box, select *Disc radius* to draw a circumference and use the slider to choose its radius (units in number of pixels). Click *OK* and make the desired modifications of the recently created spot in the *Edit spots* window. To modify a spot, double-click on the spot shape and the edit window will be displayed.

You may also add a spot by double-clicking in the desired location and choosing *Diffusion* in the *Add Spot* window. The suggested spot, displayed in the *Edit spots* window, corresponds to the surrounding pixels which have a grey value darker than the clicked pixel.

You may add or modify spots inscribed in a pre-selected region:

Edit
↳ Spots
↳ Edit

1. Define a small region using the Region tool.
2. Choose **Edit**. A dialog box is displayed with an enlarged representation of the selected region (Fig. 3-4).
3. Add or modify spots if necessary.
4. Click **OK**. The new spots will be added to the gel.

You may add spots from one specific gel to other gels, provided that they were previously matched. Perform the following steps:

Edit
↳ Spots
↳ Copy from Gel

1. Select spots.
2. Select one or more gels you want to copy the spots to.
3. Choose **Copy From Gel**.

Pairs will be automatically created between the selected spots and the newly created spots. In this case, all gels must be in the same directory.

Edit
↳ Spots
↳ Delete

To delete spots, select them then choose **Delete**.

Edit Window

In this window, the selected spots are shown in a grey level related transparency mode, instead of opaque, which facilitates spot editing. The grey levels are represented in gradations from blue to pink, dark pixels are blue and light pixels are pink.

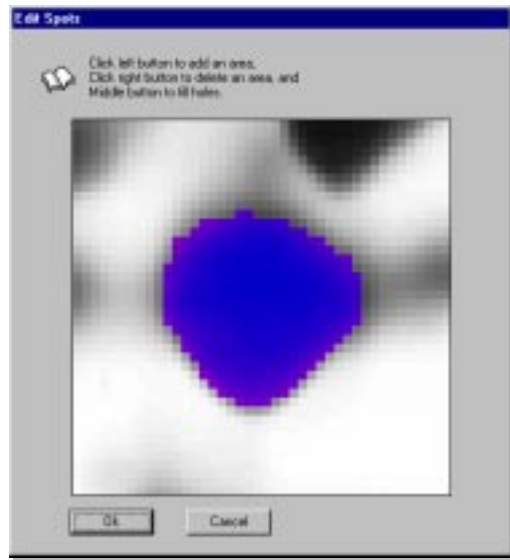


Figure 3-4. The spot editing window.

To add/modify spots in the *Edit spots* window, hold down the left mouse button and draw the outline of the area that you want to add. When the button is released, the outline is closed by a line joining the first and the last point of the outline and it is filled. Hold down the right mouse button and draw the outline of the area to delete or split a spot. You may also use the left mouse button to add pixels to the spot, the right mouse button to remove pixels, and the middle mouse button to fill in holes.

Spots quantification

Melanie automatically computes the spot values once they have been detected. The following quantification values are calculated for each detected spot:

- **Od** - the highest calibrated pixel intensity in the spot.
- **Area** - the spot's area in mm².
- **Vol** - the spot's volume, i.e. the integration of Od over the spot's area.

If spots have been modeled as Gaussians, the following quantification values are computed:

- **Od** - the optical density of the Gaussian's peak.
- **Area** - the area of a section of the Gaussian, taken at the peak's half height.
- **Vol** - the Gaussian's volume.

The following normalized values are also available:

- **%Od** - the relative Od, i.e. the Od divided by the total Od over the whole image.
- **%Vol** - the relative Vol, i.e. the Vol divided by the total Vol over the whole image.



The Od and Vol values depend on pixel intensities calibration (see *Calibrating spots* on page 44).

Calibrating spots

Melanie enables you to calibrate pixel intensities. Rather than displaying gray level values taken from the scanner device, you may display image values indicating the real world values, for example optical density values. This may also be useful to compensate for variations due to image scanning.

Melanie provides three different ways to calibrate gels:

- By adding calibration annotations to gels;
- By using calibration annotations from the reference gel;
- By importing a calibration. For example, you may calibrate the image of a calibration strip tablet using Melanie. Generate and save a report on gels (or any other Melanie report containing the columns *Gray Slope* and *Gray Offset*) which may be imported to calibrate all your gels.

For this purpose, add calibration values to known spots using any existing category of labels. For example, create one category called "Calibration" and add calibration values to their labels (see *Creating annotations* on page 55).

Edit
↳ *Gels*
↳ *Calibrate...*

Select gels which have calibration values and choose **Calibrate Intensity**. Choose the label category which contains the calibration values.

You may also calibrate gels using calibration annotations from the reference gel. Set the reference gel. The calibration values from the reference gel are propagated to the other gels through the paired spots.

File
↳ *Import*
↳ *Calibration...*

You may also import a calibration by using the *Import* → **Calibration** command. A dialog box asks you the file name. The specified file, for example a gels report, must be in a text file format and must contain the columns **Gray Slope** and **Gray Offset** with the corresponding values.

Once gels have been calibrated, Melanie allows you to display the best fitting line through the calibration values. After displaying **Calibration Plot**, similar to the scatter plot window (see *Scatter plot* on page 88), you may open two other reports by selecting one of the menu item of the report button in the Calibration Plot toolbar. These reports are:

- **Fitting Report**: shows the gel name, gray slope and offset values, the correlation value and the number of spots.
- **Calibration Report**: displays spot ID, optical density, calibration and fitting error values, as well as X and Y coordinates of spots.

Edit
↳ *Gels*
↳ *Reset...*

You may change the calibration at any time by taking *Edit* → *Gels* → **Calibrate Intensity** and choosing a calibration category, or remove it by taking **Reset Calibration**.

Menu: Show

Showing spots

Show
↳ *Spots*

Once spots have been detected, you may display their shapes over the gels in three different ways (Fig.3-5): filled or outlined shapes, as well as crosses located in the center of the spots. Select gels then choose one of the options in the **Shape** menu.

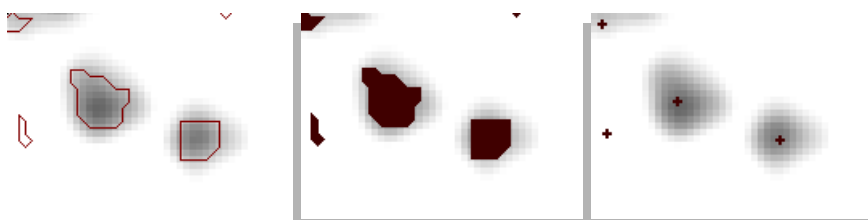


Figure 3-5. New spots display (outlined shape, filled shape and crosses).

Spots may be displayed with the **Show All** menu option, and hidden with the **Hide All** option.

Select a region on a gel using the Region tool, then **In Region** to show all spots in the selected region. You may show only selected spots with **Only Selected**. Only the spot shapes of the initially selected spots are kept on the screen. It is very helpful, for example, to mark some spots on the screen when you are performing a visual inspection of gels or a visual inspection of the analysis results. You may hide only selected spots with **Hide Selected**.

You may also show (respectively hide) the IDs of selected spots. Select some spots, then **Show ID** (respectively **Hide All ID**).



Hiding spots also has the additional effect of displaying images faster.

Coloring spots

Show
↳ Spots
↳ Set Color

Spots may be displayed on the screen using 3 different colors other than the default red. To do so, select spots then choose **Set Color** and one of the proposed colors. The **Default** command colors spots in red, the default color in Melanie. The colors remain displayed until you select **Reset All** to color all spots in red, as the default. The colors are not saved.

Menu: Select

Selecting spots



The Spot tool lets you select individual spots. Click on any spot to select it. The selected spot will be highlighted in green. To select more than one spot, select the first one then hold the Shift key and select additional spots by clicking on them. To select all spots in a given region, position the cursor at the top left position of the desired region, hold down the mouse button, then move the cursor to the bottom right position. All spots in the selected region will be selected and highlighted in green.

Select
↳ Spots

Spots may also be selected from the **Select** → **Spots** sub-menu. Choose **By ID** and enter a spot ID to select the corresponding spot. If colors have been previously assigned to spots, you may choose them by taking **By Color** and selecting the desired color. Choose **Only Gaussians** to only select spots that have been modeled by a Gaussian function.

Select a region on a gel using the Region tool, then **In Region** to select all spots in the selected region, or **All** to select all spots in all selected gels.

If you have already saved a *Spots Report*, you may reselect all the spots by choosing **From Report File**. A dialog box lets you choose the name of the file which contains the report.

Finally, if some spots have already been selected, you have the possibility to do **Inverse Selection**. This will unselect the selected spots and select all unselected ones.

You may also select spots directly from a displayed *Report on Spots* (see *Reports and navigation* on page 69).



Note that by selecting annotations or labels, their linked spots are also selected.

Restrict spots selection

You may select spots which have a value inside a certain interval. Make a first selection of spots and then **Restrict by Value**. In the dialog box, select a spot value type among Vol, Area, Od, %Vol and %Od. In the *Restrict by Value* dialog box, click \geq and type the value which will represent the lower limit of the interval. Click also $<$ and type a value to define the higher limit of the interval. The spots whose values are included in the defined interval remain selected.

Menu: Window

Cursor information

Window

↳ Information

You may at any time display information of spots and pixels directly on the screen. In this case, choose *Window* → **Information**.

Available information for **pixels** are their X and Y coordinates on the gel, as well as pI and Mw. Pixel grey values are also available when raw images of gels are loaded (see *Loading / unloading gels* on page 19). Otherwise the *Value* field displays

unloaded. Information for **detected spots** also include their spot ID and quantification values (Vol, Area, Od, %Vol and %Od).

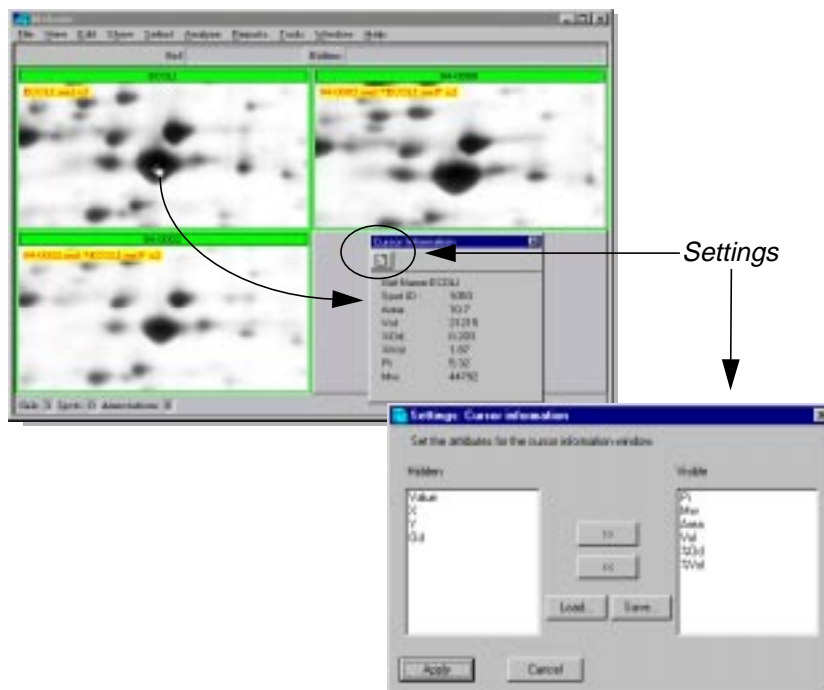


Figure 3-6. Cursor information and settings window.

You may choose what information to display, as well as the order in which this information will appear on the screen. To select the displayed information, click *Settings* in the *Cursor Information* window's toolbar and the *Settings: Cursor Information* dialog box will be displayed (Fig.3-6). Select the attributes which you would like to have in the *Hidden* list (left column), and transfer to the *Visible* list (right column) using the >> button. You may choose many attributes at once by using the Shift or Ctrl keys. Click *Apply* to confirm your choice and the selected attributes will be displayed on *Cursor Information* window. You may also double-click on one attribute and it will be transferred from one list to the other.

On the other hand, you can remove attributes from the information window by selecting the items on the *Visible* list and clicking <<. The selection order is preserved on the display.

You may also save the current chosen template of cursor information by clicking *Save* and typing the file name in the *Name* field. In the same way, you may load an existing template by pressing *Load* and choosing the file name.

Edit
↳ Preferences
↳ Cursor...

These settings may also be made permanent from the *Preferences* → **Cursor Information** menu option.

Menu: Reports

Report on Spots

Reports
↳ Spots Report

Select some spots and choose **Current**, or one of the other report templates that you may have defined previously (see below). A report will be displayed.

The Spots Report summarizes all relevant information about the selected spots: their identification number (ID), quantification values, gel name, their center position on the gel image (X and Y values), circularity value, *pI* and *Mw* and all attached labels. You can also have information taken from the paired spots in the reference gel (see *Groups* on page 85).

The circularity value is calculated according to the spot perimeter and area. The higher the value, the more circular the spot.

As usual, reports may be saved, printed, exported, used to navigate through data, etc. (see *Reports and navigation* on page 69).

Customizing reports

You may choose the spot attributes to build your personalized reports on spots, and their order of appearance. In the report window, click on the *Settings* button in the toolbar. In the *Settings: Spots report* dialog box (Fig. 3-7), to add attributes to the report, select their names in the *Gels* list (left column), and transfer to the *Visible attributes* list (right column) using >>. On the other hand, you can remove spot

attributes from the report by selecting the items on the *Visible attributes* list and clicking <<. The order is preserved on the display.

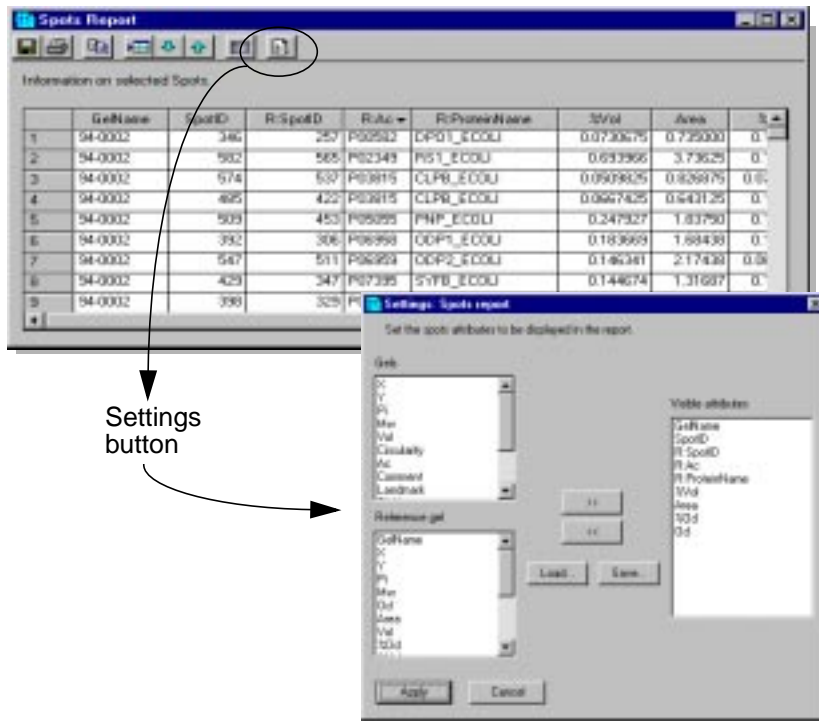


Figure 3-7. Spots report and the settings window.

You may also choose to display spots information of the corresponding pairs in the reference gel, provided that there is a selected reference gel and the gels are matched together. Select the reference gel information in the *Settings: Spots report* dialog box, in the *Reference gel* list, as described in the previous paragraph. Click *Apply* to confirm your choice and the selected attributes will be displayed on the report.

You may also save the current chosen report template by clicking *Save* and typing a file name, or load an existing file by clicking *Load* and choosing the corresponding file name. The template name will be added to the menu command *Reports* → *Spots Report* (Fig. 3-8).

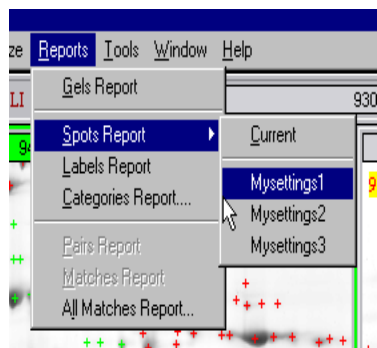
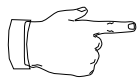
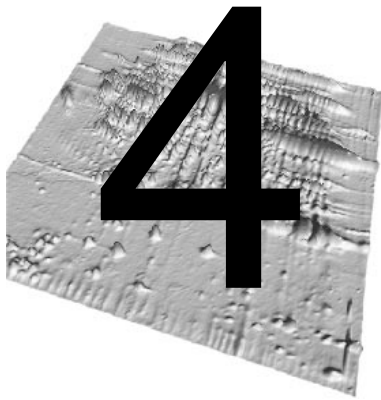


Figure 3-8. Templates of Report on Spots.

You may have as many report templates as you wish and the saved formats may be re-used by choosing their format file names on the menu item *Reports* → *Spots Report*.



The template names, available when Melanie starts, are those that exist in the “Menus” subdirectory of the Melanie installation directory.



ANNOTATIONS

Individual points (pixels) and spots in a gel image may be marked by **annotations**. An annotation is defined by its position (row and column number) and its set of labels. Each label belongs to pre-defined categories or user-defined categories. The label contents may be any textual data (examples in Fig. 4-1).

Pre-defined label categories

Some pre-defined **label** categories are provided with the Melanie software to help you organize your data and to mark spots which have common characteristics. These categories are:

1. **Ac:** This category is provided to hold the protein's accession number (AC) taken from a user-selected database and it is the link to Melanie's remote database query engine. A double-click on this label category displays the corresponding protein entry in the chosen database with the chosen browser software (See "Querying a remote database through the Internet" on page 9-114.).
2. **Landmark:** This category is used to mark pixels in the gels as reference points, for the operations of gel alignment and calculation of corresponding locations between gels. Two annotations are considered to refer to the same point in different gels, when they have the same label.



Melanie appends the word "AMBIGUOUS" to a landmark label when you add an existing landmark to a gel.

3. **Pi_Mw**: This category is supplied to contain the *pI* and *Mw* information, which is used to compute approximated *pI* and *Mw* value for any point in a gel. You may enter the *pI* and/or *Mw* (separated by a space) of the gel at the given position. A value of -1 means that no value is set.

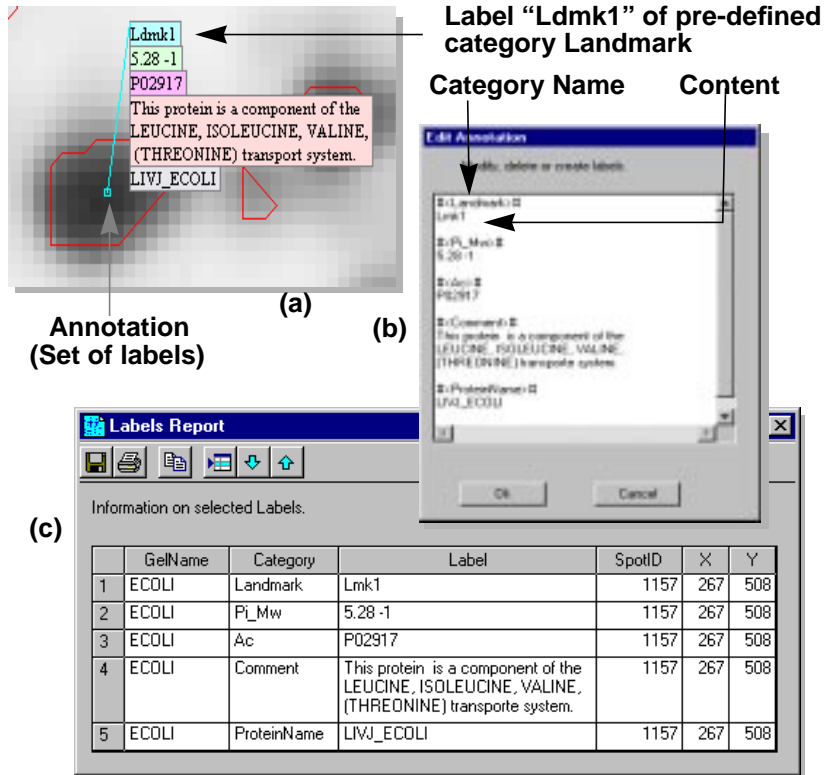


Figure 4-1. (a) Annotation over one spot with 5 labels. (b) The editable window of the annotation showing categories and contents of the displayed labels. (c) Report on labels.

4. **Comment**: is an indicatory category to users. It exists as an example of a general category where users may store any comments.

5. **Set**: is a generic category used to mark spots which have some common characteristics. All labels from a Set category share the same text, i.e. the set name. In Fig. 4-2, a set called St contains spots belonging to the same result of Student T test analysis.

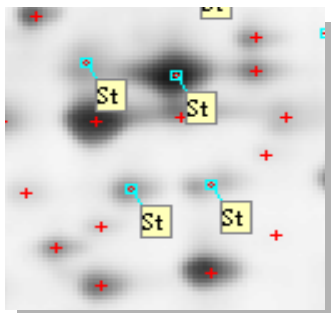


Figure 4-2. A set of spots marked by the same category of type "Set:St".

To help you visually identify the different categories of labels, they are automatically created using different background colors. *Ac* is displayed on the gel in pink, *Landmark* in blue, *Pi_Mw* in pale green, *Comment* in pale pink, *Set* in yellow and all user-defined categories are displayed in gray.

Menu: Edit

Creating annotations



To create an annotation, pick the annotation tool and double-click on a:

- gel's pixel to create a "pixel annotation",
- gel's spot to create a "spot annotation",

at the position in a gel where you want the annotation to be located. The Annotation window pops up. Enter the name of a new category or choose one of the existing categories by clicking on its name. Type the wanted label (word or text) in the dialog box. The annotation is created, and its label is displayed on the gel.

Edit
↳ Annotations

Labels may be added to an existing annotation. Select the Annotation tool and double-click at the basis of an annotation. In the dialog box, type the name of a new category or choose categories by clicking on its name. Type the content of the label. Alternatively, you may add labels by selecting annotations or spots and choosing the **Add Labels** option.

Annotations may be linked to spots, in this case they have small squares at their basis (Fig. 4-3). Otherwise annotations may simply be positioned over pixels on the

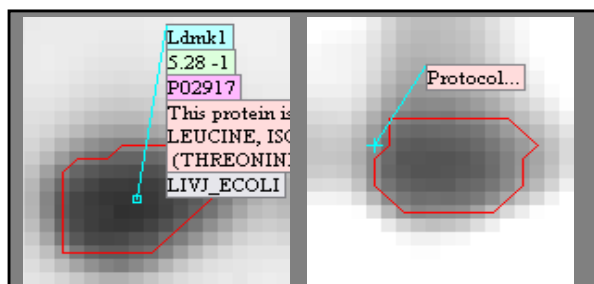


Figure 4-3. Annotation detail on one spot (square basis) and on a pixel (cross basis).

gels (small cross at their basis). To link an annotation with a spot, click on the annotation basis and drag to a spot. You may also select the annotation and choose **Link with Spot**. On the other hand, you may select the annotation and drag outside the spot, or choose **Unlink with Spot**, to separate one annotation from a spot.



One spot may have many annotations. One annotation may have many labels, but only one label from each category.

Creating label categories

To create your own category of labels, you must add a label to the gel. Type the name of the new category in the upper field of the displayed window. You may then type the content of the label in the pop-up editing field and click *OK*. The label content will be displayed on the gel. This new category will be available on the list of categories while there is at least one label of this category in the open gels.

Creating sets

The pre-defined category called "Set:" was created to help you mark spots, indicating that they belong to a set. It contains no information except the fact that it belongs to a special set. The label is defined by the word "Set:" followed by an identification name.

Edit
↳ Annotations

To create a set, select one or many spots, choose **Add Labels**, click on the category "Set:" and append a keyword (which will identify this set) to the string "Set:". Click *OK*. For example, to mark spots reminding you that they need to be sequenced, type "Set:To be sequenced" (Fig. 4-4). A label named "To be sequenced" will be attached to the selected spots.

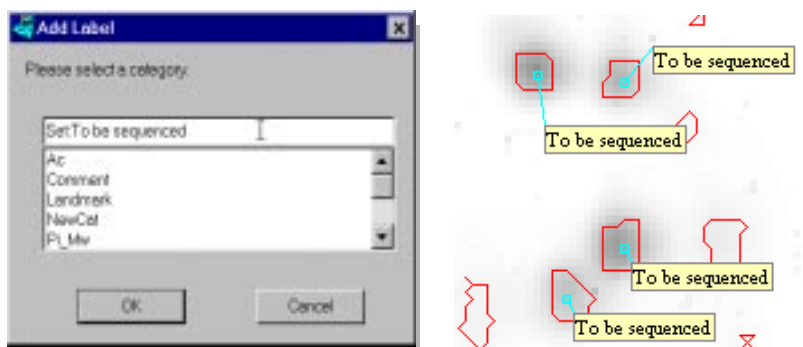


Figure 4-4. Creating a "Set:To be sequenced" set of spots in Melanie.



Note that the keyword "Set:" is not displayed on the labels. At the time when a new category is created, it is immediately shown on the screen, except for the new category Set. In this case, it is visible or hidden depending on the current state of the generic category "Set:".

Creating specific links

Many interesting possibilities to link your gels to 2-DE databases, mass-spectrometry files, text files or any other files are available in Melanie. Using specific keywords in labels of any category, creates links to specific objects. In this case,

- the keyword and the follower text are not visible on gels and are replaced by three dots (a visual help to indicate a linked label).

- by double-clicking on “link” labels, labels are not edited but the link is opened.



You may have links in any label category, but you can only have one link per label.

The available link types are:

- **Http links:** you may link your gels to specific web pages. To create an http link, add an annotation to a gel. In the text field, type your text which must contain the word “http:” followed by a web page.

A double click on an http link label will launch your favorite browser software (Netscape or Explorer) on your screen and it will automatically retrieve the corresponding Web page. To set your favorite browser, choose *Edit* → *Preferences* → *Database*.

For example, you may have a direct link to ExpPASy (Fig. 4-5).

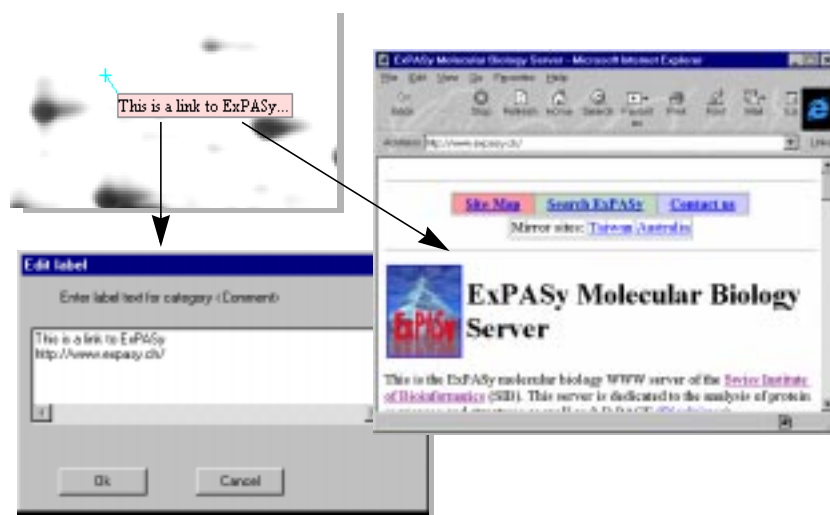


Figure 4-5. Label “This is a link to ExpPASy...” has a link to a http page.

- **File link:** you may link spots or regions of gels to software files. To create a file link, add an annotation to a gel and, in the text field, enter the string which must contain the word “file:” followed by the file name. Click *OK*.

A double-click on this label will launch the specified file with the default system application associated to the file extension. The linked files must be located in a specific directory which may be defined by choosing *Edit* → *Preferences* → *Database* and by filling the *Annotations folder* field (see *Setting preferences* on page 36).

For example, you may have an Excel file containing the amino acid composition of a protein (Fig. 4-6).

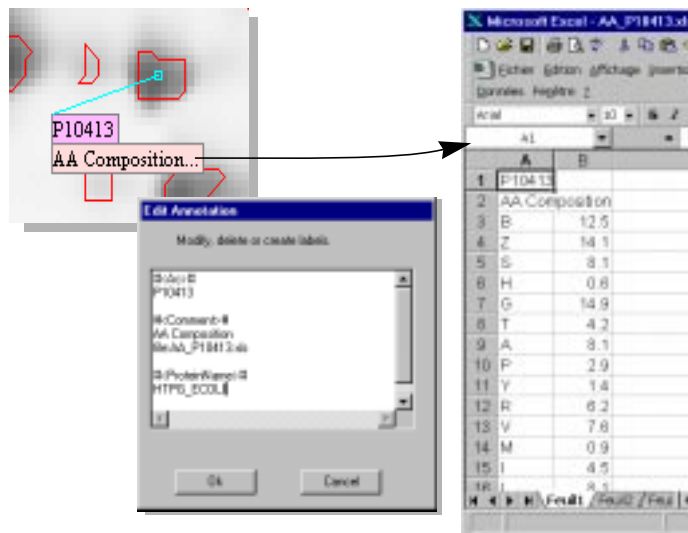


Figure 4-6. “AA Composition” label containing a link to an Excel file.

- **Text link:** you may have a long piece of text inside any label without overloading the display. To create a text link, add an annotation to a gel and type the string which must contain the word “text:”.

A double-click on this type of label displays a dialog box containing the entire text.

For example, you may have text containing the sample preparation procedure, the detection method or the bibliography for a spot, attached to your gels and only part of this information is displayed over the gel. In Fig. 4-7, the label “Sample preparation” has the protocol used for this purpose.

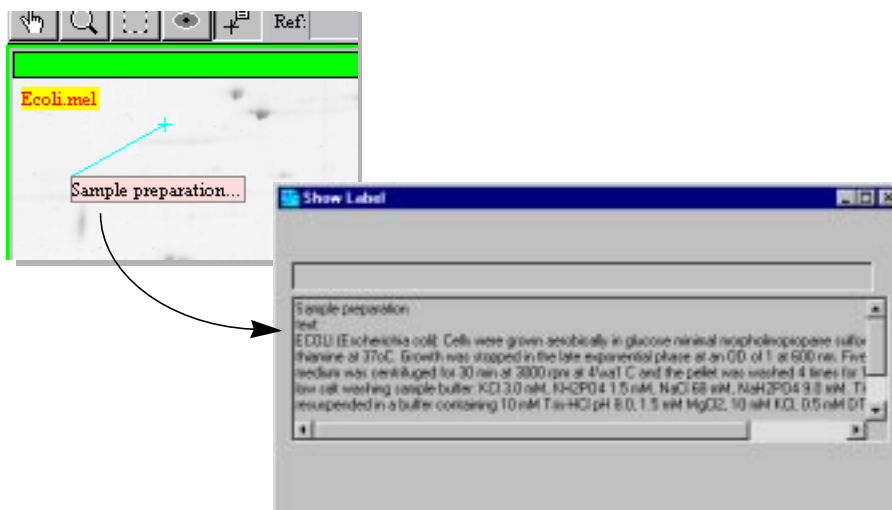


Figure 4-7. “Sample preparation...” label containing a link to a text field describing the procedure of the ECOLI sample preparation.

Modifying and deleting annotations

Edit
↳ Annotations

You can modify and delete annotations and labels. By double-clicking in one label, an edition window is displayed where you may change its text contents. On the other hand, once one annotation is selected, it may be modified by taking **Modify**. In the edition window, you may change the labels contents and add new categories.

The **Delete** menu option deletes all selected labels. The **Delete Categories** option deletes from selected annotations, labels which belong to chosen categories. Select the labels, take this menu option and choose the categories to delete.

You can also manually change an annotation position. Select an annotation and drag the annotation basis to the desired location.

To interactively change an annotation's tag position, select an annotation and drag its tag to the desired position (Fig. 4-8).

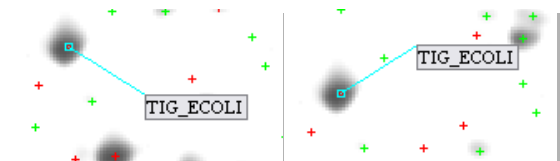


Figure 4-8. Default annotation tag position (left) and modified tag position (right).

Editing annotations

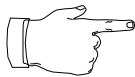
We have already described how to create, modify and delete annotations and labels. Some other convenient options of Melanie are the **copy**, **paste** and **duplicate** facilities.

Copying / pasting annotations

You may select annotations in a given gel image, then copy them to another gel. This is a simple means of creating a series of similar annotations in a set of gels. Perform the following steps:

1. Select annotations.
2. Choose **Copy**. This will copy the selected annotations into memory.
3. Select only the gels you want to copy the annotations to.
4. Choose **Paste Annotations**. The copied annotations will be pasted into each of the selected gels to their corresponding locations. The **corresponding location** is estimated by interpolation between the two nearest common **landmarks** (i.e. landmarks with the same name).
5. Adjust the annotation positions using the Annotation tool.

Edit
↳ Annotations



This option copies annotations from one gel to their corresponding locations in the other gels.

Copy / pasting labels

You may also copy some labels from one gel to selected spots or annotations in a set of gels. Perform the following steps:

1. Select one or more labels.
2. Choose **Copy**. The selected labels will be copied into memory.
3. Select one or more spots or existing annotations you want to copy the labels to.
4. Choose **Paste Labels**. In the dialog box, confirm the action.

Copy paired labels

When gels have been matched and pairs have been created, labels existing in one gel may be copied to their corresponding spot pairs in the other gels.

1. Select annotations in the source gel.
2. Select the gels you want to copy the labels to and the source gel.
3. Choose **Copy Paired Labels**. In the dialog box, confirm the action.

Edit
↳ Annotations

Duplicate labels

You may copy selected labels to another category. Select labels then choose the **Duplicate Labels** command. In the dialog box, choose the receiving category.

Note that the selected labels may also belong to different categories.



You can use this menu to merge several categories or to rename one category.

Menu: Select

Selecting annotations and labels



The Annotation tool is useful to select annotations and labels. To select one annotation, pick the Annotation tool and click annotation basis. To select one label, just click on it.

Annotations and labels may only be selected when they are visible on the gels. **Annotations** may be selected with the Annotation tool. Click at the basis of any annotation to select it or at one of its labels. The selected annotation will be highlighted in green and will be displayed in front of all the other displayed annotations

(Fig. 4-9). If the annotation is attached to one spot, the spot will also be selected. To

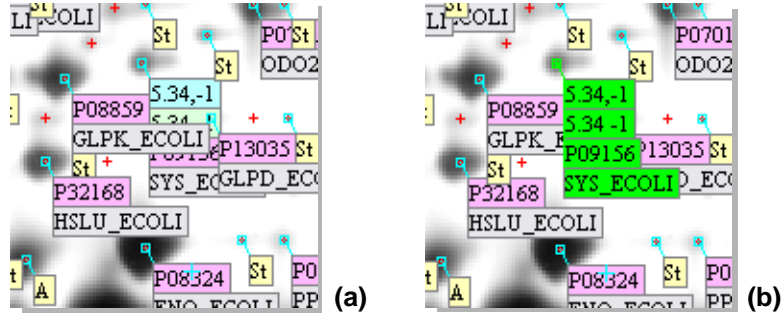


Figure 4-9. (a) Annotation hidden by the other annotations. (b) Same annotation displayed in front of the other annotations, after selection.

select more than one annotation, select the first one, then hold the Shift key and select additional annotations. To select all annotations in a given region, position the cursor at the top left position of the desired region, hold down the mouse button, then drag the cursor to the bottom right position. All annotations in the selected region will be selected and highlighted in green.

Labels may also be selected with the Annotation tool. Click on any label to select it. The selected label will be highlighted in green. To select more than one label, use the Shift key.

Select
↳ Annotations

You may also select labels and annotations by using the menu items. Select a region on a gel using the Region tool, then **In Region** to select all annotations in the selected region, or **All** to select all annotations in all selected gels.

If colors were assigned to annotations, they may be selected by taking **By Color** and choosing the corresponding color in this menu option.

You may select a group of labels which have a sequence of letters in common in their contents. For example, “LAT” designates all labels from the chosen categories whose names contain the sequence of letters “LAT”. For this purpose, take **By Content**. Choose one or many categories and enter a label content to select the corresponding labels. Expressions in this field are taken literally when the *Regular Expression* field in the dialog box is not selected.

By selecting the *Regular Expression* field in the dialog box, you may use regular expressions that can be inserted into the search field. A regular expression is a concise and flexible notation for finding patterns of text. A list of regular expressions which may be used in search criteria is given in Table 4-1.

Table 4-1. Regular expressions to search patterns in labels.

Syntax	Description	Example
.	Matches any single character.	e.oli matches ...eaoli, aboli, ecoli...
+	Matches at least one occurrence of the preceding character.	P0+1 matches P01, P001, P0001, ...

Table 4-1. Regular expressions to search patterns in labels.

Syntax	Description	Example
?	Matches zero or one occurrences of the preceding character.	P0?1 matches P1 and P01.
*	Matches zero or more occurrences of the preceding character.	P0*1 matches P1, P01, P001, P0001, ...
()	Groups a subexpression.	P(24)+ matches P24, P2424, P242424, ...
[]	Matches any of the characters inside the []. [a-z] is the range of characters between a and z and [0-9] is any numeral from 0 to 9.	P[a-d] matches Pa, Pb, Pc, Pd
[^]	Matches any character not in the set of characters following the ^.	P[^bd] matches Pa, Pc, Pe but not Pb ou Pd.
	Matches the expression before or after the . Mostly used within a subexpression.	P(ab cd)1 matches Pab1 and Pcd1
{n}	Matches exactly n times the preceding character.	P0{3}1 matches P0001 but not P01 nor P001.
{n,}	Matches at least n times the preceding character.	P0{2,}1 matches P001 and P0001, but not P01.
{n,m}	Matches at least n times and at most m times the preceding character.	P0{1,3}1 matches P01 P001, and P0001, but not P1 or P00001.

For example, to select all labels which contain the string P00, you may either type the expression `.*P00.*` and choose the *Regular Expression* field in the dialog box, or type P00 and unselect the *Regular Expression* field.

You may select all labels from specific categories. Choose **By Category** and pick out the category names.

If you have already saved a *Report on Labels* or a *Report on Annotations*, you may reselect all labels by choosing **From Report File**. A dialog box lets you choose the name of the file which contains the report.

Finally, if some labels have already been selected, you have the possibility to do **Inverse Selection**. This will unselect the selected labels and select all unselected ones, inverting the criteria used to select them.

You may also select annotations directly from a displayed *Report on Annotations*, *Labels* or *Categories* (see *Reports and navigation* on page 69).



Note that by selecting spots, their linked annotations are also selected.

Mass spectrometry

You may match spots according to their mass spectrometry data, using a correlation algorithm. For this purpose, the mass spectrometry data must be available in labels through one of the following formats:

- as a file link (see *Creating specific links* on page 56), i.e. using the “file:” keyword followed by the name of the file which contains the ms data. This file must be either a ms file type (PerSeptive GRAMS/386) or a text file containing a sequence of mass values separated by carriage return.
- as a text link (see *Creating specific links* on page 56), i.e. using the “text:” keyword followed by a sequence of mass values.

Select
↳ Annotations
↳ By MS

To match spots according to their MS data, select one label then choose **By MS**. In the dialog box, choose the label category with which the selected label will be matched. A **Mass Spectrum Match** report is displayed showing the match results and a score is given for each match (from 0 to 1, i.e. from bad to good match). The selected label is also in the report with a score of 1 (match with itself).

Common labels

Select
↳ Annotations
↳ Common Labels

You may easily retrieve common labels among many gels. If two or more gels are selected, you may choose **Common Labels** to select common labels in all gels. In the dialog box, choose the categories of labels to which the selection will be applied.

If only one gel is selected, this special kind of selection will correspond to selecting packed labels, i.e. selecting labels from the specified categories containing the same content.

Combining sets of annotations

Select
↳ Annotations

You may combine the selection of categories and annotations. For this purpose, decide for a first criteria and select annotations using one of the above commands. Take the menu option **Combine** and choose one of the four operations from Intersection, Union, Difference and Exclusion. In the dialog box, choose the label category corresponding to your second criteria. Click Ok. The result of the logical operation will be selected according to (Fig. 4-2):

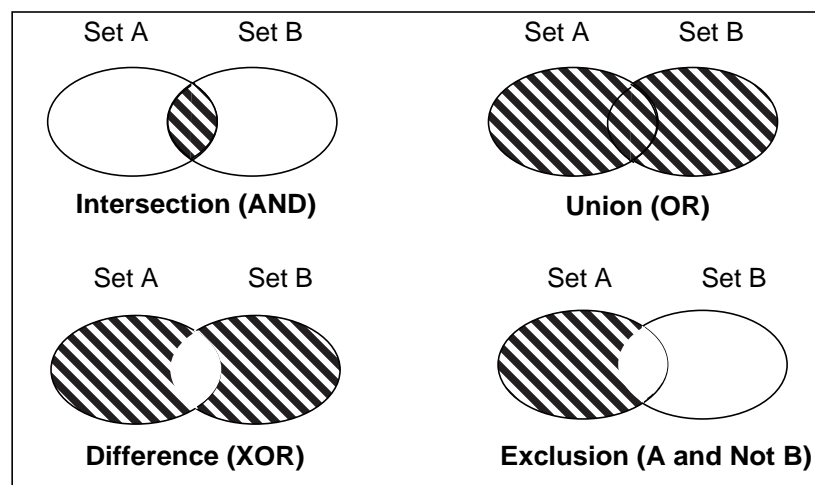


Figure 4-2. Combining sets of spots on Melanie.

- Intersection: selects spots that are in the intersection of two criteria;

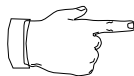
- Union: selects spots that belong to either one criterion or the other;
- Difference: select spots that are unique in one criterion plus those that are unique in the second criterion;
- Exclusion: select spots that are found only in the first criterion and are not found in the second criterion.

For example, you may select spots that are in the intersection of two analyses, like Student T test and Differential Analysis. Make the first analysis and mark the outcoming spots with the label "Set:St". Make the second analysis and mark the outcoming spots with the labels "Set:DA". Select spots from the first analysis by choosing *Select* → *Annotations* → *By Category* and in the dialog box select category "Set:St". Then make *Select* → *Annotations* → *Combine* → *Intersection* and choose category "Set:DA" in the dialog box. Only spots that belong to both sets will remain selected.

Menu: Show

Showing annotations and labels

Annotations and categories may be displayed, hidden, packed, unpacked and selected. Annotations may also be colored with different colors.



Show
→ Annotations

Important! Annotations may only be selected and manipulated when they are visible on the gels. Labels may only be selected and manipulated when the annotations are visible.

Annotations may be displayed with **Show All** menu option, and hidden with **Hide All**. You may show only selected annotations with **Only Selected** annotations or hide with **Hide Selected**.

Select a region on a gel using the Region tool, then **In Region** to show all annotations in the selected region.

If you have selected spots before selecting annotations, you may take **For Spots** and all the annotations associated to the selected spots will be selected.

You may hide a certain number of categories among those that are visible on the gels. Choose **Visible Categories** and in the dialog box, click on the desired category names to hide them. Fig. 4-3b shows, for some of previously chosen spots (Fig. 4-3a), categories *Ac*, *Pi_Mw* and *Comments*, while categories *Landmarks*, *ProteinName*, and others are hidden.

You may also choose to display labels belonging to a certain category. Make **Hidden Categories** to display the categories which are not visible on the gels. In the dialog box, click on the desired category names to make them visible.

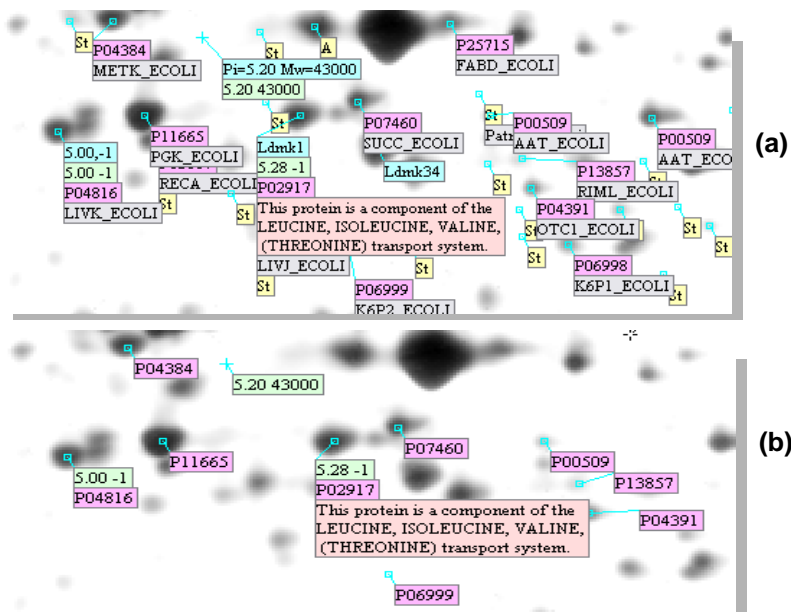


Figure 4-3. (a) All categories and labels are visible. (b) Only Categories Ac, Pi_Mw and Comments are visible, and only for certain spots.

When you click on an annotation which has visible and hidden labels, all its labels will be displayed on the screen during the time it remains selected (Fig. 4-4). The hidden labels will immediately disappear when the annotation is unselected.

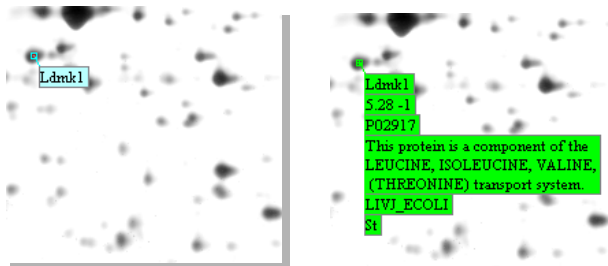


Figure 4-4. Annotation with hidden labels is unselected. During the time it is selected, the hidden labels become visible.

If several labels hold the same content then, by default, each spot will be displayed with its own label. You may display a single label for all identical labels by making **Pack Categories** and choosing the desired category name. **Unpack Categories** separates the label tags again (Fig. 4-5).

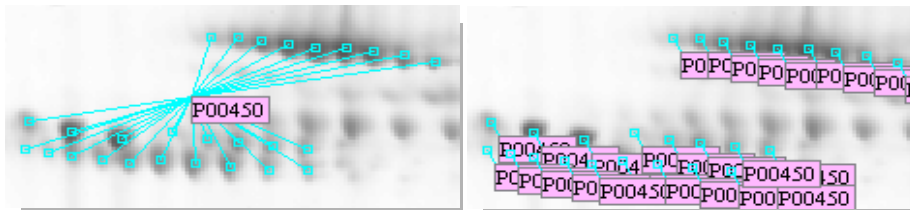


Figure 4-5. Packed and unpacked labels for protein P00450 in the Plasma master gel (Swiss-2DPAGE database). The label belongs to the pre-defined Ac category.

Coloring annotations

Show
↳ Annotations
↳ Set Color

Annotation tags may be displayed on the screen using 3 different colors other than the default cyan. To do so, select annotations then choose **Set Colors** and one of the proposed colors. The **Default** command colors annotations in blue, the default color in Melanie. The colors remain displayed until you select **Reset All** to color all annotations in cyan, as the default. The colors are not saved.

You may open a label link by double-clicking on it. Alternatively, to open any label link, select the label and choose **Linked Data**.

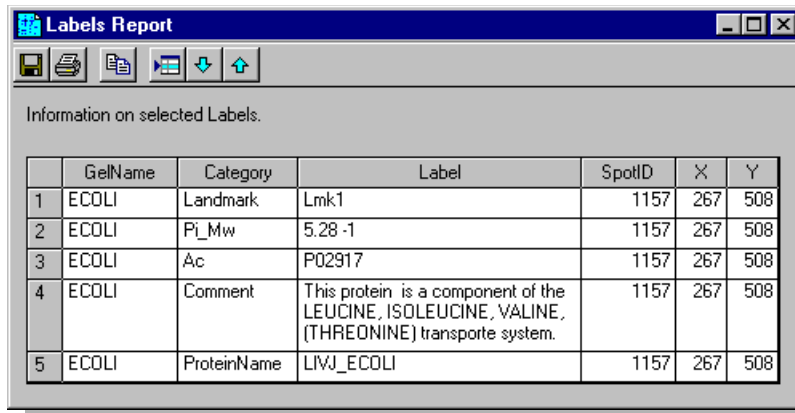
Menu: Reports

Reports On Annotations

Reports

↳ Labels Report

Melanie provides reports for annotations, categories and labels. Select labels and choose **Labels Report**. The Labels Report displays information about selected labels, such as content, category, spots ID, and X and Y positions on the gel (Fig. 4-6).



Information on selected Labels.

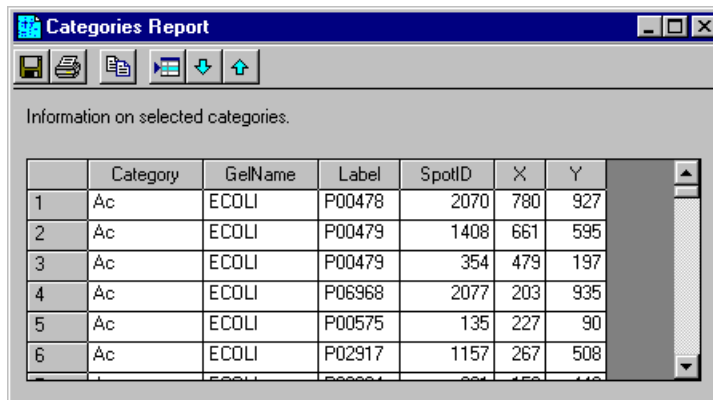
	GelName	Category	Label	SpotID	X	Y
1	ECOLI	Landmark	Lmk1	1157	267	508
2	ECOLI	Pi_Mw	5.28 -1	1157	267	508
3	ECOLI	Ac	P02917	1157	267	508
4	ECOLI	Comment	This protein is a component of the LEUCINE, ISOLEUCINE, VALINE, (THREONINE) transport system.	1157	267	508
5	ECOLI	ProteinName	LIWJ_ECOLI	1157	267	508

Figure 4-6. Report on selected labels.

Reports

↳ Categories Report

On the other hand, you may display information about all labels belonging to selected categories and selected gels. In this case, select the gels, then choose **Categories Report** and select the desired categories in the dialog box. The information displayed in this report is the same as for the Report on labels (Fig. 4-7).



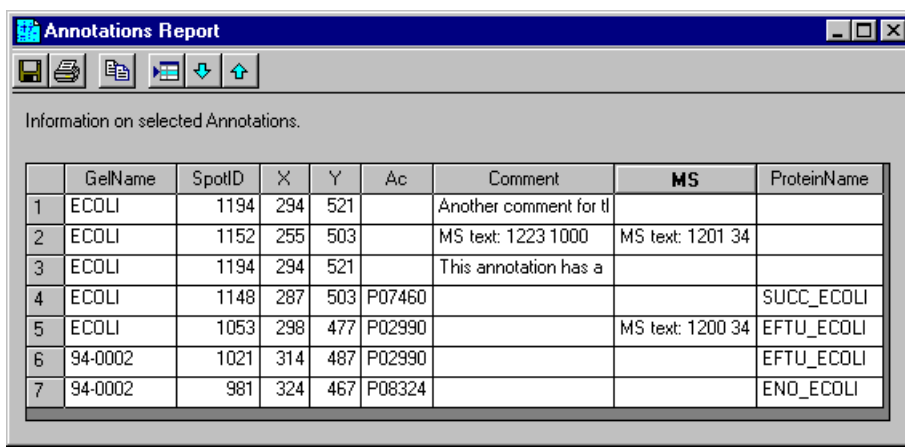
Information on selected categories.

	Category	GelName	Label	SpotID	X	Y
1	Ac	ECOLI	P00478	2070	780	927
2	Ac	ECOLI	P00479	1408	661	595
3	Ac	ECOLI	P00479	354	479	197
4	Ac	ECOLI	P06968	2077	203	935
5	Ac	ECOLI	P00575	135	227	90
6	Ac	ECOLI	P02917	1157	267	508

Figure 4-7. Report on categories.

Reports↳ *Annotations Report*

You may also create reports on selected annotations which display information about all visible categories, and their labels (Fig. 4-8). Select annotations and choose **Annotations Report**.



Information on selected Annotations.

	GelName	SpotID	X	Y	Ac	Comment	MS	ProteinName
1	ECOLI	1194	294	521		Another comment for tl		
2	ECOLI	1152	255	503		MS text: 1223 1000	MS text: 1201 34	
3	ECOLI	1194	294	521		This annotation has a		
4	ECOLI	1148	287	503	P07460			SUCC_ECOLI
5	ECOLI	1053	298	477	P02990		MS text: 1200 34	EFTU_ECOLI
6	94-0002	1021	314	487	P02990			EFTU_ECOLI
7	94-0002	981	324	467	P08324			END_ECOLI

Figure 4-8. Report on annotations.

Menu: Files

Importing labels

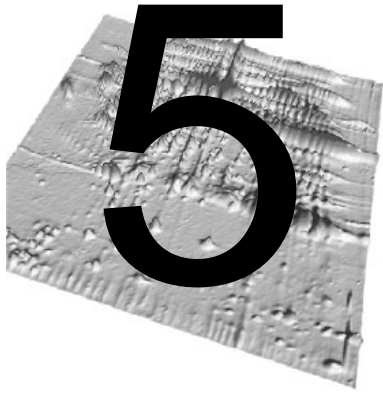
File
 ↳ *Import*
 ↳ *Labels*

You may import annotations and labels into open gels. To do so, select a gel and choose **Labels**. In the displayed window, type the file name and select the file type.

You may import labels from a file containing a Report on Labels, for example, or from any “Labels” file type. In the last case, it must contain the columns SpotID, X, Y, Category and Label. If the spot ID is not known, use -1 in this field and Melanie will position the label in the corresponding X and Y positions of the gel. If X and Y positions are not known, use -1 in this field and Melanie will position the label in the corresponding spot ID. Columns in the file must be separated by tabulators.

File
 ↳ *Import*
 ↳ *Annotations*

In the same way, you may import annotations from a file containing a Report on Annotations. To do so, select **Annotations**, and type the file name in the displayed window.



REPORTS AND NAVIGATION

Introduction

The reports available in Melanie provide not only powerful options to display information on selected objects, but also a useful tool to navigate through data. Reports may be accessible through the *Reports* menu, the *Analysis* menu or through displayed reports.

In the *Reports* menu:

- Report on **Gels**: Displays summarized information about the selected gels, such as intensity calibration, number of detected spots, etc.
- Report on **Spots**: Displays specific information about selected spots, such as spots ID, quantification values, attached labels, etc. This report is completely customizable, i.e. you may choose the information to be displayed and the order in which they will be displayed.
- Report on **Labels**: Displays information about selected labels, such as content, category, spots ID, and positions on the gel (*X* and *Y*).
- Report on **Annotations**: Displays for selected annotations, information about all categories, and their labels.
- Report on **Categories**: Displays information about all labels belonging to chosen categories. The displayed information is the same as for the Report on labels.
- Report on **Pairs**: Displays the list of selected paired spot IDs among selected gels.
- Report on **Matches**: Displays information on matching between the selected gels and all open gels, that is the number of paired spots between each pair of gels.

- Report **All Matches**: Displays information on matching between all gels in a given directory.

In the *Analyze* menu:

- Report and Histograms on **Groups**: Displays information on each selected group (see *Pairs and groups* on page 8), such as group ID, chosen value type of each spot in the group, and chosen statistical values calculated on all spots in the group.
- Report and Plot on **Scatters**: Displays information on scatter plots such as slope, offset and the correlation coefficient values, the pair values and the fitting error.
- Report and Histograms on **Classes**: Displays chosen statistical values for classes of gels, calculated on selected groups, such as central tendency, deviation and overlapping measures.
- Report on **Statistical Test**: Displays statistical values for classes of gels, such as Student-t, Wilcoxon and Kolmogorov significance levels.

You may display as many reports and histograms simultaneously as you wish. Once a report or histogram is displayed on the screen, it is listed in the *Window* menu, and may easily be brought in front of all the other displayed windows (Fig. 5-1).

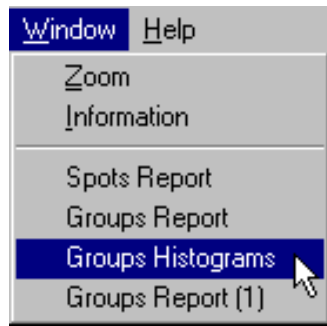
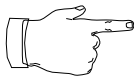


Figure 5-1. List of open reports.



Note that report and histogram windows may hide other existing report and histogram windows by popping-up in front of them.

Many of the report and histogram properties, such as saving, printing, organizing, etc. are shared by all of them. This chapter is dedicated to these common properties.

Toolbar

All Melanie report, histogram and plot windows have in common the toolbar shown in Fig. 5-2. Reports also have the property of being navigable, meaning that from one or more items in a report, histogram or plot, you may re-select corresponding objects on the gel, as well as travel through the objects which are listed in the reports.

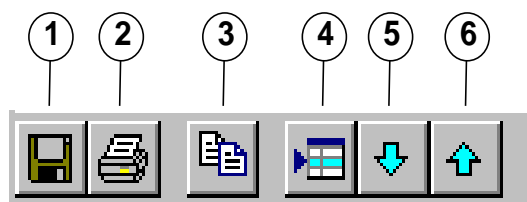


Figure 5-2. Report and histogram toolbar (1) Save (2) Print (3) Copy to Clipboard (4) Select on Gel Image (5) Select next (6) Select previous.

Reports also have particular characteristics in common. The lines in the tables are numbered, indicating in this way the number of selected objects used to create the report. The columns are independent elements which may be displaced, resized and sorted in descending and ascending order of their values (similar to a spreadsheet window).

Saving/printing

Reports may be saved or printed. Tables are saved using the Excel table format, i.e. with tabulations separating the columns. Graphics are saved using TIFF or BMP formats.

To save reports, click the *Save* button in the toolbar (item 1, Fig. 5-2). Enter the file name in the displayed window and choose the file format in the *Type* field.

To print a report, click the *Print* button (item 2, Fig. 5-2). The usual PC system window is displayed where you can choose printing options such as the printer name.

Exporting data

You may export your data directly from a table into another program, for example a spreadsheet software, like Excel. Select the desired lines in the table using the Shift or Ctrl keys. Copy the selection to the clipboard by choosing the *Copy to clipboard* button in the toolbar (item 3, Fig. 5-2) or in the contextual menu by clicking the right mouse button. Paste into the desired software.

You may also export graphics to software like Word or Photoshop, for example. Copy the selected graphics to the clipboard by choosing the *Copy to clipboard* button in the toolbar or in the contextual menu. Paste into the desired software.

Selecting objects

You may select objects like spots, gels, labels, annotations, groups and pairs directly from their reports. You may also select groups of spots directly from histograms. Double-click on a report line or on a histogram to select and display the corresponding object.

Alternatively, you may use Shift and Ctrl keys to select report lines or histograms. Click the *Select on Gel Image* button in the toolbar (item 4, Fig. 5-2) or use the contextual menu. The corresponding objects will be selected on the screen and the gels will be displaced to show at least one of them.

An example of refining the selection of spots is shown in Fig. 5-3. In this example, some spots were first selected, then a Report on Spots was generated. In the report, the values in the column *Area* were sorted in descending order. The five first lines, i.e. the five largest spots, were retained in a new selection of spots which is displayed on the gel.

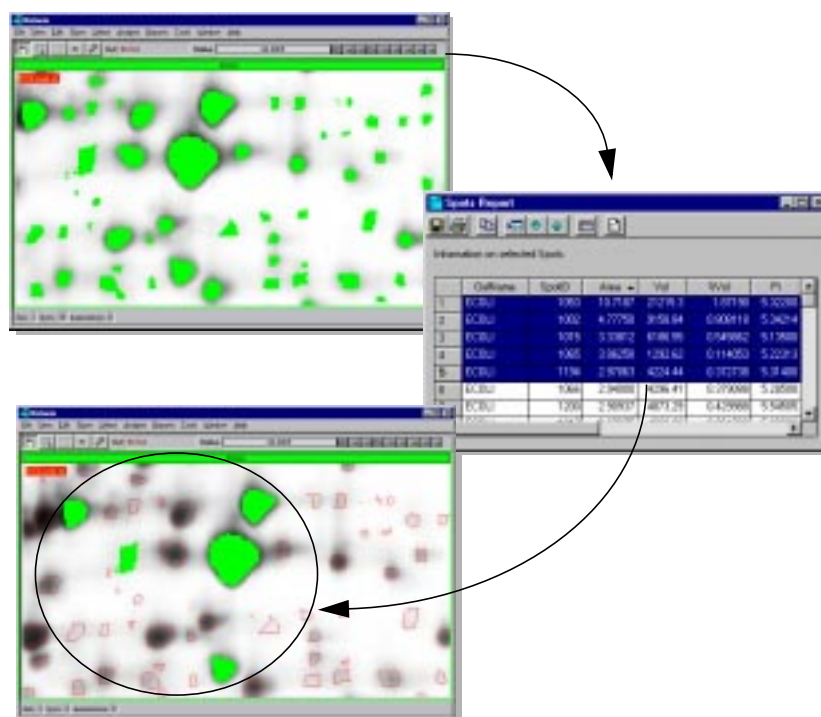


Figure 5-3. Re-selection of spots through the report. Note that the *Area* values were sorted in descending order and the re-selected spots are the first 5 spots on the sorted report.

Navigating through data

You may also use the *Next* button in the toolbar (item 5, Fig. 5-2) to select, on screen, the corresponding following object listed in the reports or histograms. In the same way, use the *Previous* button (item 6, Fig. 5-2) to select the preceding object.

An example of navigation through the data existing on a *Report on Groups* is shown in Fig. 5-4. Some groups of spots, which in this case also have common landmarks displayed on screen, were selected to verify their match and a *Report on Groups* was generated. The selection was limited to group 352 (the corresponding line was selected and the *Select on Gel Image* button was pressed, Fig. 5-4, item a). Notice that all the spots belonging to group 352 are selected on the screen and the gels are displaced to show the spots (Fig. 5-4, item b). By clicking the *Select Next* button, the following group on the report, group 513, was selected on the screen (Fig. 5-4, items c and d). And so on, all the other groups were verified by means of the report.

This is a simple example to illustrate the endless possibilities of the usage of reports, as well as the navigation through data.

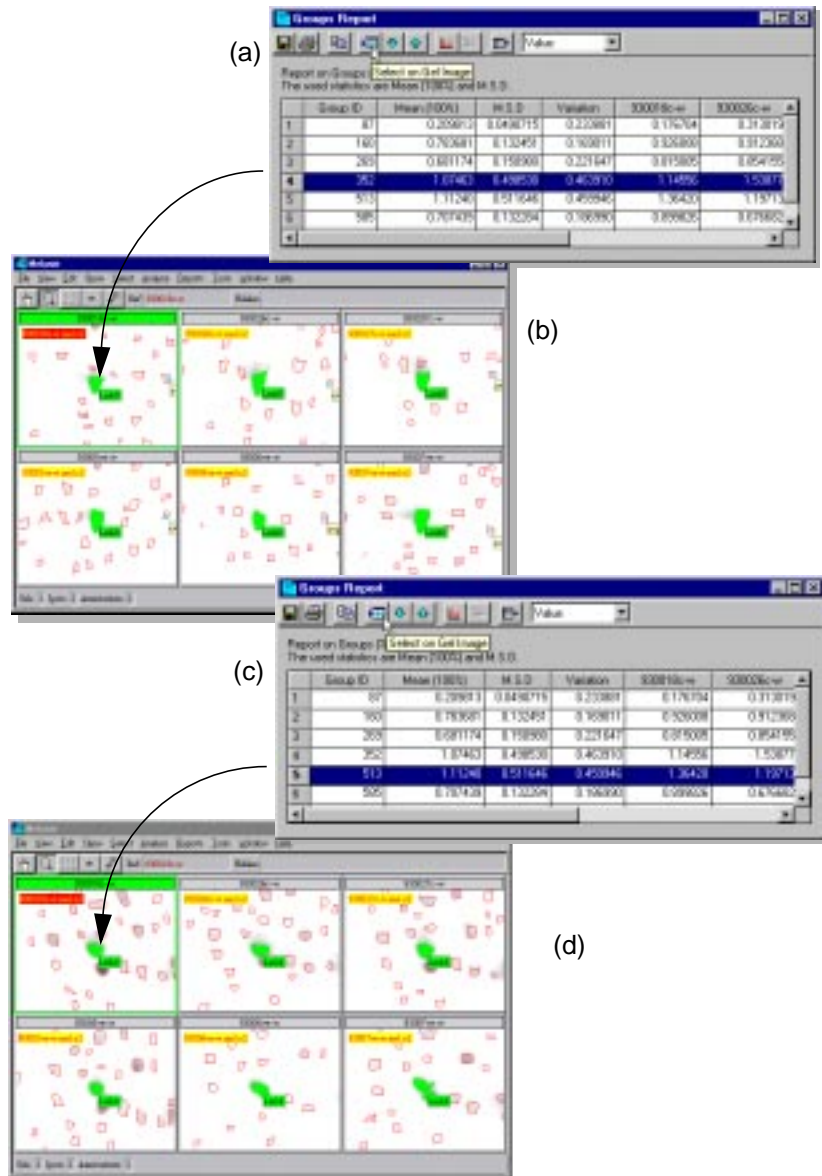


Figure 5-4. Navigating on selected groups through the report. (a) Selection of group ID 352 in the report (b) Group ID 352 is displayed on screen (c) Selection of the following line, group ID 513 (d) Group ID 513 is displayed on screen.

Sorting data in tables

Data may be sorted in the tables by the values of the columns. Click once on the header of the desired column, and a triangle will be displayed indicating that the col-

umns' data is sorted in ascending order. Click once more on the header and the triangle will be inverted indicating that the data is sorted in descending order. Ascending order means that numbers are sorted from 1 to 9 and text is sorted from A to Z.

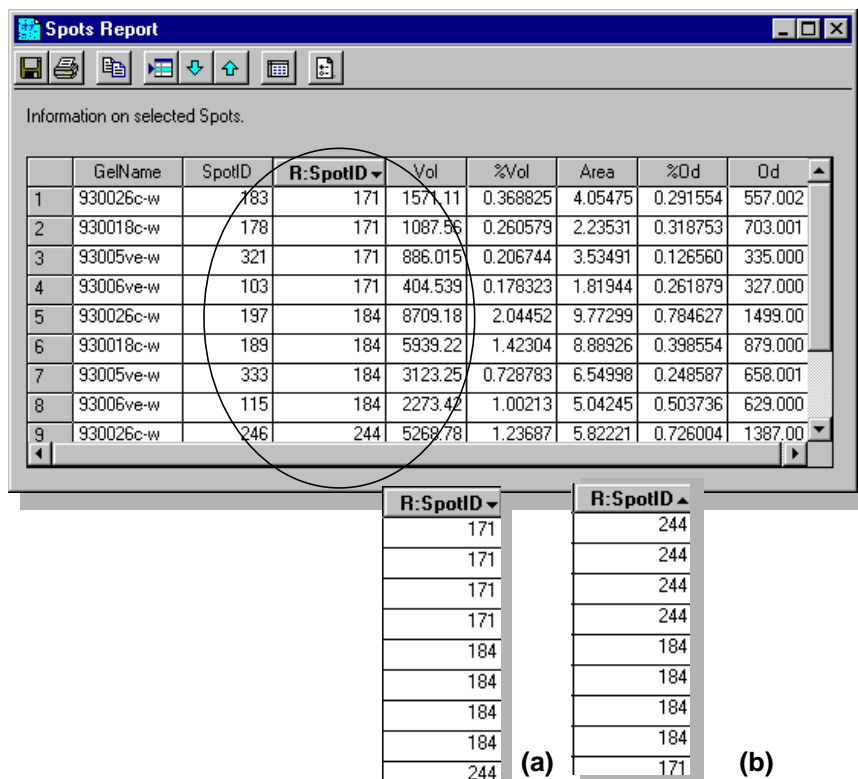


Figure 5-5. Items sorted by two criteria, group ID (R:SpotID) and volume. Spots were first sorted according to volume (ascending order), then according to spot ID. (a) Sort ascending. (b) Sort descending.

You may sort the columns elements using multiple criteria. Rows that contain equal items in a sorted column, appear together. When using two or more criteria, the rows with equal items are rearranged according to the second criteria. To do so, first sort the column of secondary choice by clicking on its header. Then click in the header of your principal criterion to sort. In Fig. 5-5, the principal criterion is the spot ID of the reference gel and the second criterion is the volume values.

Organizing a table display

You may re-organize the columns of a table directly from the report window. Drag the header of the column to another chosen column (Fig. 5-6). The first one will be inserted before the second one.

Information on selected Gels.

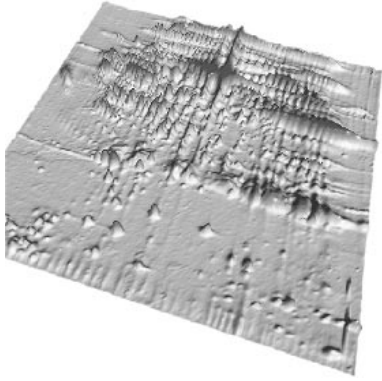
		GelName	Class	Group	Sp	Annotations	ns	MinGray	MaxGray
1	a	93004ve-w	Class B	1	721		26	0	1011.00
2	b	930033c	Class A	1	1509		21	0	2362.00
3	c	930027c-w	Class A	1	863		17	0	1956.00
4	d	93007ve-w	Class B	1	905		34	0	1715.00
5	e	93006ve-w	Class B	1	638		33	0	1696.00
6	f	93005ve-w	Class B	1	1359		34	0	1728.00
7	g	930026c-w	Class A	1	777		20	0	1958.00
8	h	930018c-w	Class A	1	851		20	0	1726.00
9	i	master1	Class B	Ref	870		54	0	983.000

Figure 5-6. Re-organizing columns in the report window.

You may also enlarge or reduce the columns size. Drag the separation between two columns to the right to enlarge the column or to the left to reduce its size. While dragging, you will notice that the cursor changes its format. To enlarge or reduce all columns to the same size at once, hold the Shift key and drag the separation of two columns.

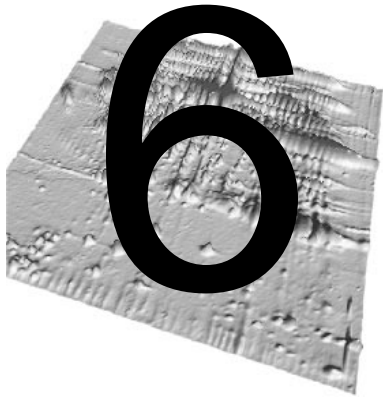
To resize columns so that their whole content is displayed, double click on their separation. The column at the left of the cursor will regain its default size.

You may also enlarge or reduce the row size. In this case, drag the separation between two rows to the top to reduce the row or to the bottom to increase its size. To enlarge or reduce all rows at once, hold the Shift key and drag the line's separation. To resize rows so that their whole content is displayed, double click on their separation.



PART II

DATA ANALYSIS



PAIRS

Introduction

Gel matching is a key operation in 2-DE gel image analysis. The basic gel matching algorithm consists of comparing two gel images and finding **pairs** of related spots, i.e. spots depicting the same protein in both gels. A pair is represented by a doublet (f_1, f_2) where f_1 is a spot ID in the first gel and f_2 a spot ID in the other gel. One generally says that gels have been **matched**, and that individual spots have been **paired**.

Melanie can match two or more gels. Matching two gels means finding all the pairs between spots of the two gels. Matching several gels means picking out a **reference gel**, then successively matching each gel with the reference gel. In this way, spots in all gels may be compared with spots in the reference gel. For consistency, when matching only two gels, Melanie will also ask you to specify which one is the reference gel.

All spots in selected gels that are paired with the same spot in the reference gel form so-called **groups**. The group concept is essential to analyze spots in a set of gels. Elaborate reports, histograms, statistics and analyses may be produced from them (see *Groups* on page 85).

In Melanie you can match gels with **single pairs** or **multiple pairs**. Matching with single pairs means that one spot in a gel will be paired with exactly one spot in the other gel. Matching with multiple pairs means allowing that one spot in one gel be paired with several spots in the other gel (Fig. 6-1).



Figure 6-1. One spot paired to two spots in the other gel

Menu: Edit

Automatic matching

Edit
↳ Pairs

To match two or more gels, select them by clicking on their banners while holding Shift or Control keys, or by using the *Select* → *Gels* menu options. Choose **Auto-Match Gels**. Melanie will first request that you designate the reference gel from the selected gels. You will then be asked whether you wish to allow multiple pairs. All gels will then be matched with the reference gel.



Depending on the computer you are running Melanie on, this might take time.



Saving pairs creates a pairs file that resides in the same directory as the matched gels. Therefore, only gels that reside in the same directory may be matched.

Matching tips

The only prerequisite for matching two gels is that spots have already been detected. Aligning gels prior to matching is optional, but in many cases the matching process will be faster and will produce better and more accurate results, especially with distorted gel images (see *Aligning gels* on page 31).

Melanie has been designed to automatically match gels. Nevertheless you may speed up and improve the matching process by specifying a few starting pairs. You may define starting pairs by manually adding pairs, or by automatically pairing identical annotations (such as landmarks or ACs, for example).

Using annotations as starting pairs

Edit
↳ Pairs

If two or more gels have annotations with identical labels, for example landmarks, you can use these to create starting pairs. Select the gels, then **Match Annotations**. Melanie will ask you to select the category of labels to match and the reference gel. It will then, for each spot in the reference gel that has a label of the selected category defined in it, pair it with spots in the other gels that have the same label defined in them. These newly created pairs may then be used as starting pairs for matching.



Note that only annotations that are linked to spots may be matched.

Adding / deleting pairs

Select one spot in one gel and one or more spots in the other gel using the Spot tool (click on one spot, then hold down the shift key and click on the other ones). Choose **Add** (or Ctrl+Shift+L).

To delete pairs, select spots in one gel and select the gels from which the corresponding pairs will be deleted. Choose **Delete** (or Ctrl+Shift+U).



Note: a very convenient way to add pairs between a set of gels and a reference gel is to create groups (see *Groups* on page 85). Corresponding spots may also be easily discerned when gels are in stack mode and spots are displayed in overlapped mode. In this case, use also the flip facility (Ctrl+F) to locate spots.

Menu: Show

Showing pairs

View
↳ Stack

You can look at the matching results in stack mode. Stack gels then choose **Show Pairs**. The pairs between the reference gel of the stack and the other stacked gels are displayed in the form of blue vectors linking the locations of paired spots (Fig. 6-2). To hide vectors, choose **Hide Pairs**.

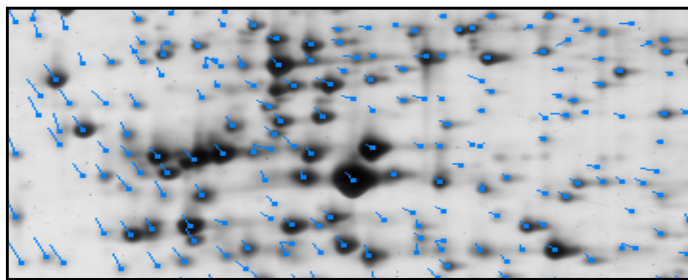


Figure 6-2. Matched gels: blue vectors indicate paired spots.



In tile mode, groups of spots are easily visualized by displaying the group IDs (see *Showing groups* on page 86).

Menu: Select

Selecting pairs

Select
↳ Pairs

To select all pairs among two matched gels, select them then choose **All Pairs**. All paired spots will be selected.

You can highlight only pairs for a given set of spots. Using the Spot tool, select a number of spots in one of the gels. Choose **For Spots**. Spots in selected gels that have been paired to the selected spots will be highlighted (Fig. 6-3).

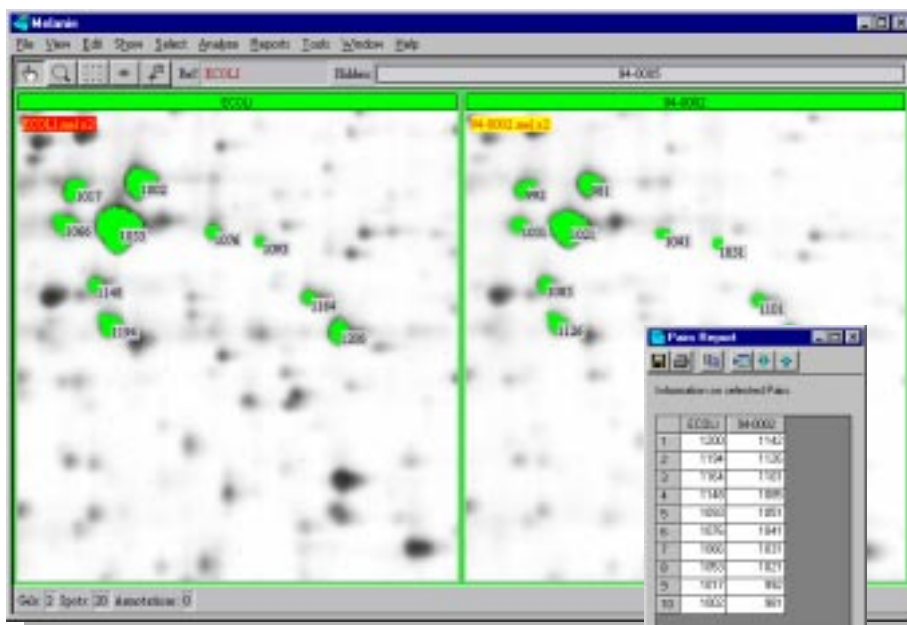


Figure 6-3. Selected paired spots across gels and the corresponding report.

To select only multiple pairs from two selected gels, excluding single pairs, take **Multiple Pairs**. You may also select pairs directly from a displayed *Report on Pairs* (see *Reports and navigation* on page 69).

Select
↳ Pairs

An algorithm to predict the accuracy of pairs has been implemented in Melanie. It is based on the length of the vectors between paired spots. You may select bad pairs by choosing **Bad Pairs**, the *Vector length* and *Score* parameters.

This measure of bad pairs is computed by Melanie for each pair according to the following steps:

1. a neighboring region is considered around each pair,

2. corresponding regions are superimposed in order to minimize the vector lengths in the region, using Least Squares,
3. for each pair of spots in the region, the score is increased by 1 when its vector length (in pixels) is below the threshold and decreased otherwise.
4. a pair is considered to be *bad* if its final score is smaller than the second threshold, the *Score* parameter.

On the other hand, you may also select good pairs. In this case, perform the following steps:

1. Choose two gels and then *Select* → *Pairs* → *All Pairs*;
2. Select *Show* → *Spots* → *Only Selected*;
3. Choose *Select* → *Pairs* → *Bad Pairs*;
4. Choose *Select* → *Spots* → *Inverse Selection*.

Menu: Reports

Reports on pairs

Reports
↳

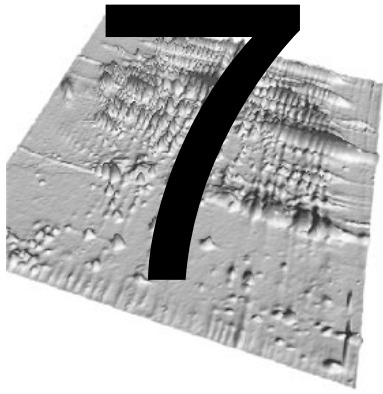


Melanie can display a report on pairs among matched gels. Select matched gels and a number of pairs, using one of the selection methods described above. Then take **Pairs Report**. You will get the list of all paired spot IDs (identifications) among gels (Fig. 6-3).

When many gels are selected, this report corresponds to a report on group IDs displaying the list of all paired spot IDs among all gels.

Another way to display the matching result is to select two or more matched gels, then **Matches Report**. This will display the number of pairs between all the selected gels and any open gels.

Finally, you may choose **All Matches Report**. This displays information on matching between all gels in a given directory.



GROUPS

Introduction

The **spot group** is an essential concept in the Melanie software. When several gels have been matched to a given reference gel, the latter provides a unique numbering scheme for spots across all gels. Each paired spot in a gel image may then be associated to the corresponding spot ID in the reference gel. The set of spots that are paired with one given spot in the reference gel forms a spot group (see Figure 1-7 on page 1-9). The **group ID** is the ID of the corresponding spot in the reference gel.

The group is the basic element for analyzing spot variations across gels and for producing histograms and reports, as well as statistical and artificial intelligence analysis.

Groups are automatically created when matching a set of gels with a reference gel, but they may also be created and modified manually (see *Pairs* on page 79).

The **reference gel** is the central node of spot groups. All spots in selected gels that are paired with the same spot in the reference gel form so-called **groups**. Groups may change when the reference gel has changed.



If you have matched your gels with multiple pairs, you may then obtain very large groups, because a given spot may be paired to several spots in the reference gel. You should therefore use multiple pairs with care.

Menu: Edit

Editing groups

Edit
↳ Groups

A convenient way to add pairs between a set of gels and a reference gel is to create groups. You first have to set the reference gel. Select the reference gel and drag to the Reference Gel area or choose **Set Reference Gel**. Select one spot in the reference gel and spots in the other selected gels. By choosing **Group Spots**, pairs will be added between each of the selected spots and the selected spot in the reference gel.

To remove some spots that are part of a given group, select a number of such spots. Choose **Ungroup Spots**. This removes the pairs between the selected spots and the reference gel.

To completely remove groups, select the spots in the reference gel, do Shift+M to select the corresponding groups and take **Ungroup Spots**.

Menu: Show

Showing groups

Show
↳ Groups

You may display the group IDs of the reference gel on all visible gels which have been matched to it. Set the reference gel, select spots then choose **Show ID** (Fig. 7-1). Inversely, to hide the group IDs, select **Hide All ID**.

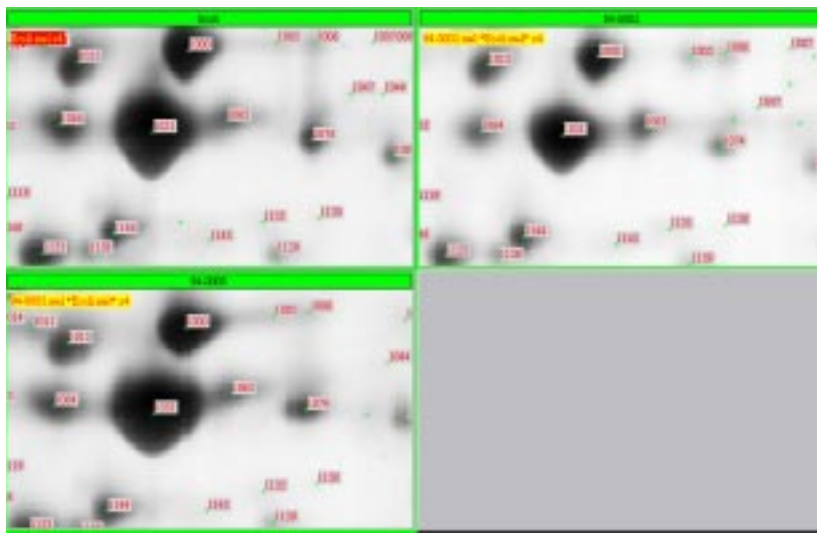


Figure 7-1. Spot IDs of the reference gel (group ids) are displayed on all matched gels.

Menu: Select

Selecting groups

The group is the basic element for analyzing and searching changes in protein expression across gels. Groups allow you to produce reports or histograms and to analyze sets of gels. In such cases, you first have to select the groups of interest.

Select
↳ Groups

To select and display all groups, choose **All**. To select one specific group, take **By ID** and specify the group ID when requested. If the reference gel is not set, Melanie will request you to select one.

To select the groups one or more spots belong to, select spots using the Spots tool, then **For Spots**.

You may also select groups directly from a displayed *Report On Groups* (see *Reports and navigation* on page 69).

Finally, if some groups have already been selected, you have the possibility to do **Inverse Selection**. This will unselect the selected groups and select all unselected ones.

Restriction of groups selection

Melanie allows you to restrict the groups selection according to the presence or absence of **selected spots** in the groups. Select **spots**, choose *Select → Groups → Restrict Selection* and one of the options in the Restrict Groups Selection dialog box. Use the sliders to choose the threshold on the restriction.

The groups to the corresponding selected spots will be restricted according to:

- *selected spots is >=*: to keep groups that have selected spots in at least the chosen number of gels.
- *selected spots is <*: to keep groups that have selected spots in at most the chosen number of gels.
- *unselected spots is >=*: to keep groups that have unselected spots in at least the chosen number of gels.
- *unselected spots is <*: to keep groups that have unselected spots in at most the chosen number of gels.

For the ease of understanding, two possible usages will be studied while these functions are described.

Case number 1: Suppose that you have 6 gels and you would like to study the groups of AT LEAST 3 spots, and with a Vol value higher than 0.02.

In other words, you would like to analyze spots according to two criteria:

1. have a *Vol* value higher than a certain threshold, representing meaningful spots
AND
2. be present in at least half of the selected gels.

In this case, you may select this special set of spots by performing the following steps:

- Select all the 6 gels and make *Select* → *Spots* → *All*;
- then *Select* → ***Spots*** → *Restrict by value*, choose *Vol*. Keep selected only the spots that have a value $t \geq 0.02$ and click *OK*;

Spots corresponding to the first criteria will remain selected. To restrict the selection according to the second criteria:

- make *Select* → ***Groups*** → *Restrict selection*. Keep the groups whose number of “selected spots is \geq ”, move the slider to choose 3 gels and click *OK*;

The remaining groups of spots verify both specified criteria.

Case number 2: Suppose now that you would like to study the groups in which ALL spots have a *Vol* higher than 0.02.

This choice would restrict the selection of groups to the two criteria defined in case number one, plus a third criteria:

3. all present spots have a *Vol* value higher than a certain threshold.

Without unselecting the preceding case:

- make *Select* → ***Groups*** → *Restrict selection*. Keep the groups whose number of “unselected spots is $<$ ”, select value 1 in the slider and click *OK*;

The remaining selected groups contain spots that verify the three specified criteria. The following logical step could be to analyze the remaining spots with statistical criteria, like Student T test or Wilcoxon test, also available in *Melanie*, to highlight meaningful spots.

As you may notice, there are endless possibilities for combining selections of spots and groups, and your own experiments and needs will lead you to the adapted use of this function.

Menu: Analyze

Scatter plot

In order to analyze gel similarities or experimental variations such as stain intensity variations or sample loading, you may plot scatter graphics on groups with regression lines and correlation coefficients between gels.

Analyze
 ↳ Scatters Plot

Select two or more gels and groups of spots. Choose **Scatter Plot**. Select one of the spot value types (Od, Vol, Area, %Od, %Vol) to calculate the correlation. The *Scatter Plot* window plots the pairs values, and the fitting line of each selected gel against the reference gel (Fig. 7-2).

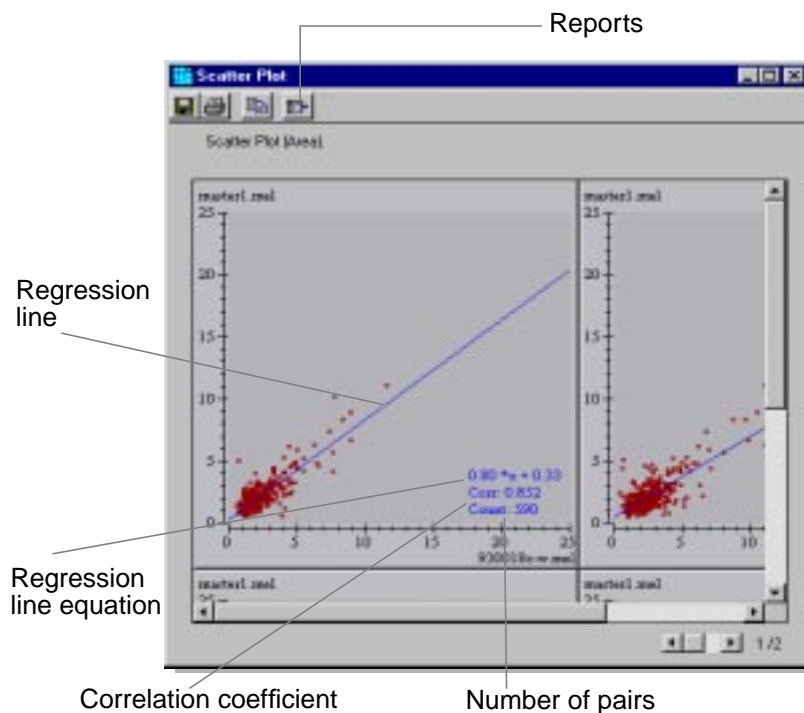


Figure 7-2. Scatter graphic on groups.

As usual, you may save and print the graphics using the corresponding buttons of the window toolbar.

The relationship between the spots values from two gels can be estimated by searching the linear dependence between the spot values from one gel against the values taken in the other gel. The linear dependence is defined as the best fitting straight line through the pair values. The fitting line is defined by a slope and its offset, i.e. the equation $y = slope \cdot x + offset$. In Melanie's case, variable x is the spot values from one gel and y is the corresponding value in the reference gel.

The goodness-of-fit of this approximation is given by the correlation coefficient **Corr**. This coefficient can vary between -1 and +1, where an absolute value near 1 indicates a good fit (i.e. the spot values of one gel can be predicted, to some extent, by the values of the other gel) while a low value indicates that the data could not be approximated by a straight line.

For example:

- $1.0 \cdot x + 0$ with 1 correlation indicates that all paired spot values are the same in the two gels.
- $1.2 \cdot x + 0$ with 0.95 correlation indicates that almost all paired values are 20% higher in the reference gel.

- $1.0 \cdot x + 0.2$ with correlation 0.95 indicate that almost all paired values are shifted by +0.2 in the reference gel.

In general, when the data show a high correlation and the fitting line is not the identity ($1.0 \cdot x + 0$), you should check for possible reasons your values are systematically slanted (perhaps staining, difference of protein load or image acquisition problem).

Information related to the scatter plots may be found in the *Reports* menu button in the toolbar (Fig. 7-2). The gels legend can be obtained by choosing *Gels Report* on the menu. The **Fitting Report** displays for each plot its corresponding slope and offset values of the regression line, its coefficient correlation value and the number of spot pairs. The **Scatter Report** displays for each pair of spots their corresponding values in the gels and the fitting error in relation to the regression line. The latter may be used, for example, to verify abnormalities between pairs.

Statistics for Groups

Considering groups allows you to analyze variations of proteins among gels, and statistics help you to analyze these variations. Melanie provides some descriptive statistics of location and dispersion which may be calculated and displayed in the Report and Histograms on Groups.

Descriptive statistics are summary values which describe spot values from a group. They allow you to locate groups composed of spots whose quantification values are peculiar. These variations may be due to an inadequate detection-matching operation, or may result from protein expression changes among gels. Therefore, these statistics are useful for controlling the extracted data, as well as for analyzing them.



The statistics tool includes all the options that the expert may expect. Nevertheless, the reluctant user towards statistics may simply use the default settings.

The available statistics are part of the Robust Statistics and include the most common measures currently used. Melanie extracts from each group of spots the **central tendency**, which allows you to localize the central value representing the data, and the **dispersion**, which indicates how closely the data fall about this center.

The descriptive statistics can be chosen with the following interface (Fig. 7-3):

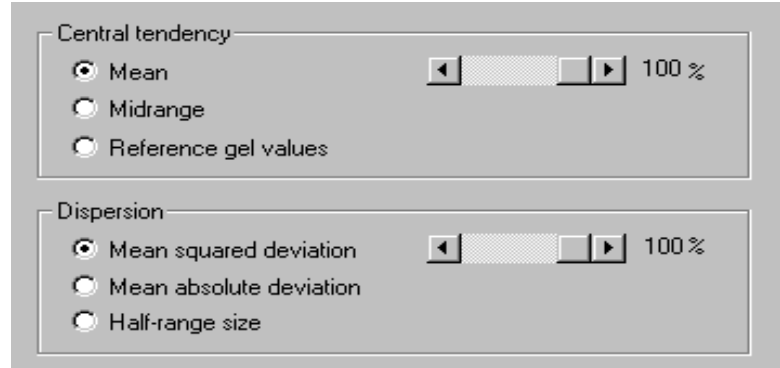


Figure 7-3. Statistics common to Report on Groups, Histograms on Groups, Report on Classes and Histograms on Classes.

- **Central tendency** gives the general location of a variable. This is commonly calculated by the arithmetic mean (also known as average or center of gravity of a distribution), the median (the middle value which divides the sample in two equal parts) or the midrange.

- By selecting **Mean** the arithmetic mean is calculated, i.e. the sum of all the sample values divided by the sample size.
- By selecting **Midrange** the midpoint of the sample value is calculated, i.e. the middle location between the two sample extremes.
- By selecting **Reference gel values** the central tendency is the value from the spot in the reference gel.

Mean (or midrange) values are sensitive to extreme values (outliers) and can be seriously contaminated by just one observation. On the other hand, the median is highly resistant to outliers. A compromise between mean (or midrange) and median is given by the trimmed mean (or midrange) where a predefined number of outliers is removed from the sample. The trimmed measures are more robust than the mean (or midrange) but are more sensitive than the median.

The percentage sliders available in the dialog box allow you to remove outliers. 100% value means that all the spot values available in a group will be used to calculate the statistics, i.e. no suppression of outliers. 0% corresponds to the **Median** measure. For example, 80% value means that the 10% minimum values and the 10% maximum values will be discarded from the sample, and the statistical value will be calculated for the remaining values.

- **Dispersion** measures the variability of the sample data, how clustered or scattered data are about its center. There are numerous measures of variability: standard-deviation, range, interquartile range and many others. Like the statistics for central tendency, these measures make use of all the available sample data, and can be heavily influenced by outliers. Therefore you can also restrict the sample to the central values by trimming out the extreme values.

- By selecting **Mean squared deviation (M.S.D.)** the square root of the average squared difference of each sample value to the center location is calculated.

- By selecting **Mean absolute deviation (M.A.D.)** the mean of the absolute difference between each value and the central value is calculated. It is not affected by outliers as much as the Mean squared deviation since the differences are not squared.
- By selecting **Half-range size** the difference between the largest and the smallest values divided by 2 is calculated.

As for the central tendency, the percentage slider allows you to remove extreme values from the dispersion statistics. A 100% value means that all the values are used to calculate the dispersion, and on the other hand, 0% will always represent no dispersion (zero).

Some examples:

- Mean (100%) and Mean squared deviation (100%) are the most commonly used statistics (Fig. 7-4, item a). Notice that the standard deviation is the Mean squared deviation multiplied by $\sqrt{N/(N-1)}$ where N is the sample size. This difference comes from the fact that the standard deviation should be an unbiased estimator.
- Median (Mean 0%) and Mean absolute deviation (100%) are much more robust to outliers than the statistics above (Fig. 7-4, item b).
- Midrange (100%) and Half-range (100%) define an interval which includes all sample values (Fig. 7-4, item c).

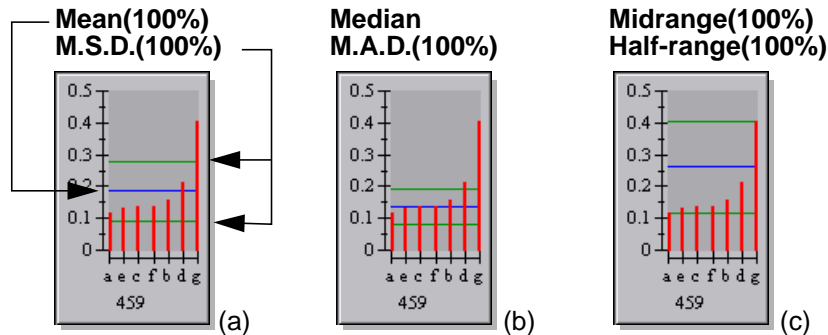


Figure 7-4. Histograms showing the sensitivity of central and dispersion values. (a) Mean (100%), M.S.D. (100%) (b) Median, M.A.D. (100%) (c) Midrange (100%), Half-range (100%).

- Midrange (50%) and Half-range (50%) are known as order statistics and interquartile ranges (Fig. 7-5).

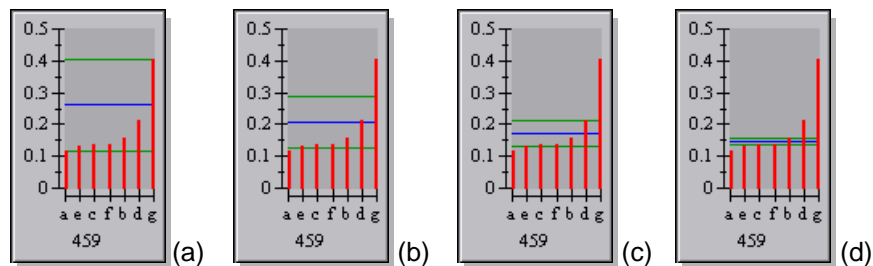


Figure 7-5. Histograms showing the sensitivity of outliers suppression. Midrange and Half-range values with (a) 100%, (b) 80%, (c) 50% and (d) 33%.

Notice that absent spots, represented by zero values, are also considered in the calculation of statistics.

Histograms on groups

Histograms and Report on Groups provide valuable operations which may be used to look for peculiar groups of spots. Analyzing groups or verifying the matching or spot detection operations, are some of the endless possibilities of their usage. You may also visually compare spots or even navigate through pairs and groups. Histograms on Groups and Report on Groups are dual elements, they have a reciprocal relationship.

Analyze
↳ Groups Histograms

Histograms are a more visual way to look at groups. To get histograms on groups, select groups (on the gels or by using the Report on Groups) then take **Groups Histograms**. The descriptive statistics directly available in the subsequent dialog box are location and dispersion values, explained in *Statistics for Groups* on page 90.

In the displayed histograms, the brown lines correspond to the spot values, the blue line corresponds to the chosen *Central* value, and the red lines to the range defined by [*Central - Dispersion*, *Central + Dispersion*].

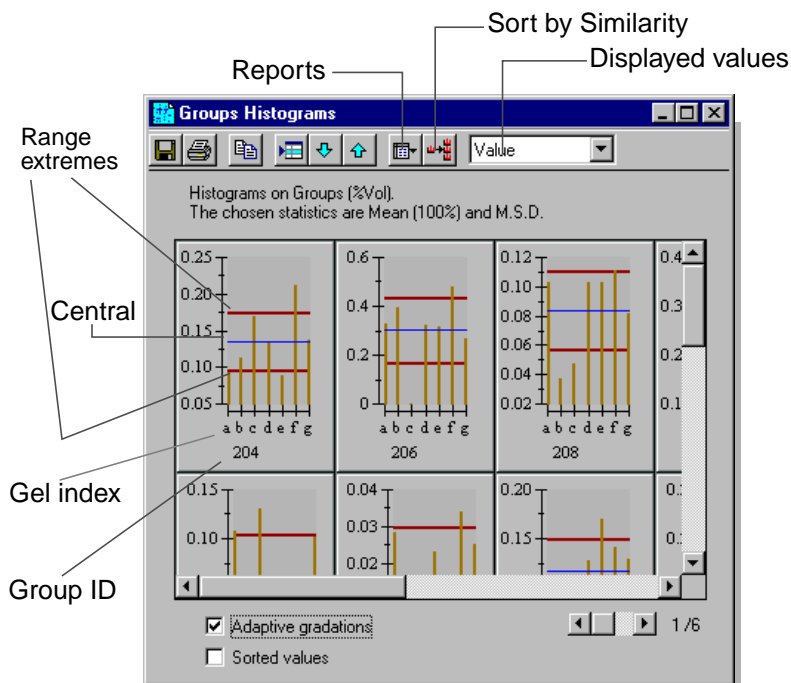


Figure 7-6. Histograms on Groups.

Figure 7-6 shows some histograms for selected groups. The slider on the bottom right corner of the window allows you to visualize the rest of the groups which are equitably disposed in pages.

The histograms may also be selected as any other Melanie object and be saved, printed or exported to other software. To select one histogram, click on the histogram window. To select many histograms, use Shift or Ctrl keys.

You may re-select the corresponding groups on the gels by using the *Select on Gel Image* button, and navigate through groups with *Select Next* and *Select Previous* buttons.

By using the *Reports* menu, you may display the numerical values of selected histograms when choosing *Report on Groups*, and the latter will create a new groups report with the selected groups. You may as well display the histograms legend by selecting the *Gels Report* item.



Use the Report on Groups to change the order or to refine your histograms selection.

Comparing protein expression changes

Melanie provides facilities in the histograms window to visually compare protein expression changes across different groups: normalize values, sort values, select/unselect adaptive gradations and search by similarity.

You may simplify the comparison across groups by normalizing the spot values relatively to their groups statistics values (*Displayed values* item, Fig. 7-6). The available normalizations are:

- Value: Raw spot value;
- Relative: $|\text{Spot_value} - \text{Central_tendency}|$;
- Ratio: $\text{Spot_value} / \text{Central_tendency}$;
- Normalized: $|\text{Spot_value} - \text{Central_tendency}| / \text{Dispersion}$.

To highlight spot variations, you can display the spot values sorted in ascending order, in this case select the *Sorted values* check box at the bottom of the histograms window (Fig. 7-7).

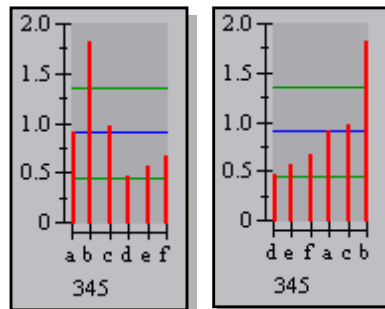


Figure 7-7. Histograms on Groups with unsorted values and with sorted in ascending order values.

Also, to highlight spot variations inside a group, the histograms gradations are adjusted according to each group. Unselect the **Adaptive gradations** check box to display the same gradation in all histograms (Fig. 7-8).

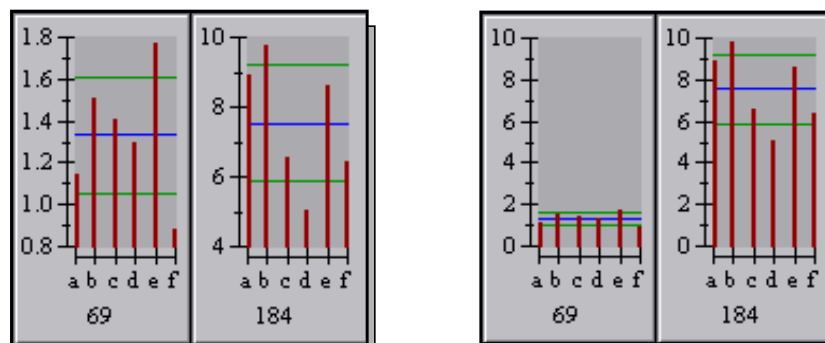


Figure 7-8. Histograms on Groups with adaptive gradations set individually and set according to the minimum and maximum values.

You may look for similar histograms easily on *Histograms on Groups* window. Select one histogram and press **Sort by Similarity** button in the toolbar. A new histogram

window will be displayed where the selected histogram is first displayed and the other histograms follow a similar criterion. This criterion is based on the selected histogram's shape. For example, the histograms shown in Fig. 7-9 can be considered similar.

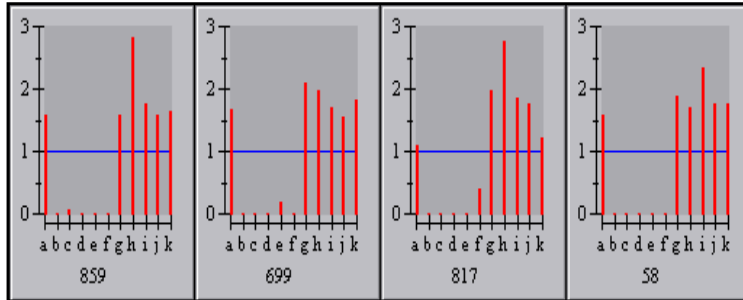


Figure 7-9. Histograms displayed according to their shape similarity.

Reports on groups

Similar to the *Histograms on Groups*, the *Report on Groups* describes numerical data concerning groups of spots as well as their corresponding statistical values.

Analyze
↳ Groups Report

Once you have selected a given number of groups (possibly all), you may display a report on them. Choose **Groups Report**. Melanie will ask you to pick out the reference gel, provided it has not yet been selected. You then have to select one of the spot value types for the report (Od, Vol, Area, %Od, %Vol) and choose the statistics values to be displayed in the report.

In the *Report on Groups*, in addition to the central and dispersion values described earlier, other measures for groups analysis are also available (Fig. 7-10):

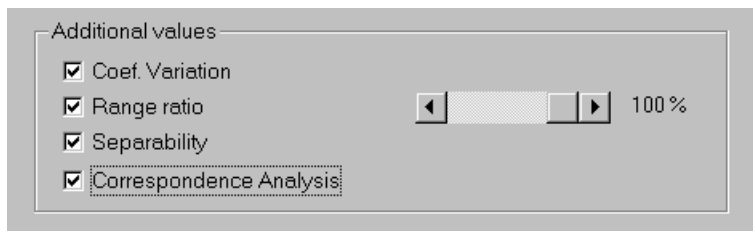


Figure 7-10. Additional values for the *Report on Groups*.

- **Coefficient of Variation** is deviation divided by the central tendency. It measures the relative variability by correcting the magnitude of the data values, thus giving a measure that has no units. When you choose the statistics Median and Mean absolute deviation, this measure is also known as the **Coefficient of Dispersion**.
- **Range Ratio** is the maximum value divided by the smallest value in the sample specified by the percentage slider.

- **Separability** is the highest difference among two consecutive sorted values in the whole sample. It measures the greatest gap that you can have if you want to split the group values in two classes.
- **Correspondence Analysis (C.A.)** which shows the amount by which each spot contributes to the constitution of the first two principal axis on the factorial space [see reference 10].

To simplify the comparison between groups, you can normalize the spot values as described for the Histograms on Groups. In the Report on Groups window, choose in the *Displayed value* list (Fig. 7-11) the type of normalization used to display the spots values.

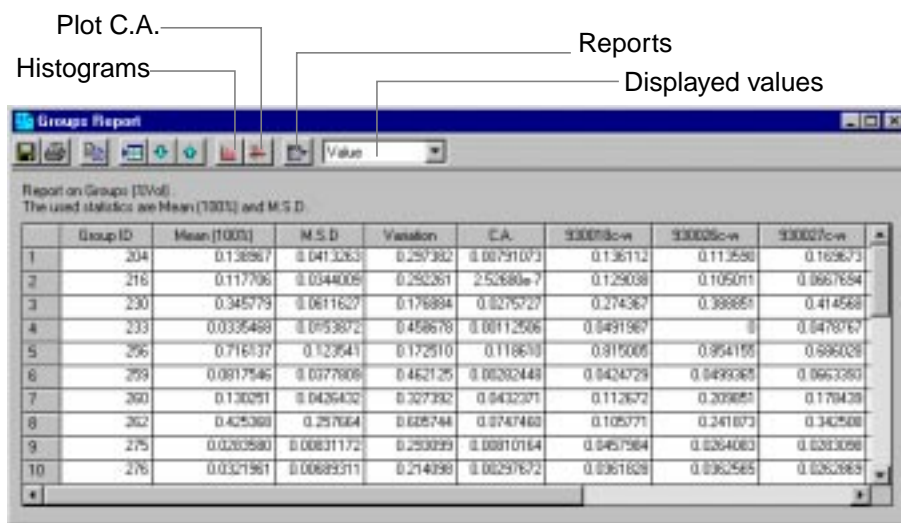


Figure 7-11. Report on Groups.

Clicking *Histograms* in the toolbar (Fig. 7-11), will display the histograms on selected groups from the report. You may immediately analyze the selected groups and directly iterate on the screen through your data (see *Histograms on groups* on page 93).

If the **Correspondence Analysis** check box has been selected in the dialog (Fig. 7-10), a Correspondence Analysis is done and the *C.A.* column displays the amount by which each spot group contributes to the constitution of the axis in the factorial space (the inertia value). This method creates a projection onto a factorial space of small dimensionality, in which gels and their significant spots appear as points. Consequently, we can determine which gels are similar and which are the characteristic spots of these gels. In fact, the natural formation of classes among the gels is highlighted with this method and can be seen in a graphical representation of gels and spots that are projected into the 2-D factorial space. Choose the *Plot C.A.* button in the toolbar (Fig. 7-11). A dialog box lets you choose to display gels, significant spots or both, and the graphical results of the correspondence analysis will be displayed.

Figure 7-12 shows the correspondence analysis plot for 6 gels ([]) and 18 significant group IDs (+). Gels that are found close to each other (apart from the scaling factor), may correspond to the same class.

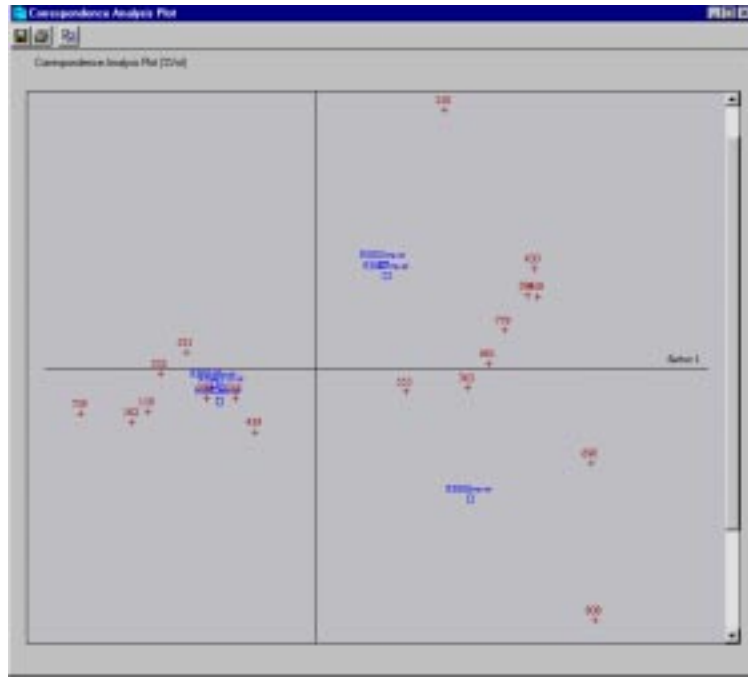


Figure 7-12. Plot of correspondence analysis

The *Reports* menu button (Fig. 7-11) allows the display of reports correlated to the groups. The *Gels Report* item is a reduced version of the original *Report on Gels* to be used, for example, as a legend for the histograms.

Spots Report

The *Spots Report* item in the *Reports* menu button (Fig. 7-11), is useful to compare one spot against the other spots of the same group. The available measures, to quantify the spot values relatively to their groups, are the same as above (chosen value in the *Displayed values* list). Therefore you can select the spots which are outliers or representative of a group.

You can select the most **representative spots** of a group by, for example, choosing the spots whose values are close to the mean (difference below 20%). In this case, take Mean 100% and $0.8 < \text{ratio} < 1.2$. As well, a median ratio =1 will select the spots that are in the middle of the group.

You can select **outliers** whose values are outside the range given by its mean and two times the standard deviation. In fact, with a normal distribution, at most 5% of the values should be outside this interval.

Other examples to find out outliers can be summarized as:

- Median, ratio value > 2: selects spots which are at least 2 times higher or lower than half of the spots from the group;
- MidRange (100%) + Half-Range (100%), normalized value = 1: selects spots which are the highest or the lowest in their groups;
- MidRange (50%) + Half-Range (50%), normalized value > 2: selects spots which are at least 2 times higher (or lower) than the 75% of spots from their groups.

By restricting the selected groups, you can, for example, remove the groups which contain too many outliers. You can also use this functionality to search for peculiar gels. For example, a gel containing more outliers than the others could indicate a peculiar gel and you should check the reasons before continuing the analysis.

Heuristic clustering

Melanie proposes a powerful analysis method to automatically create sets of gels, and highlight significant groups of spots. Heuristic clustering, a machine learning algorithm from the artificial intelligence, is used to describe the characteristic spots of 2-D PAGE gels while using heuristics to speed up the search [see reference 9].

The Heuristic clustering algorithm is an alternative to blindly classify similar gels into two or more classes and to determine the characteristic spots of each class, that is the proteins that are potentially differently expressed in the various classes.

Analyze
↳ Heuristic Clustering

Select gels and groups of spots. Choose **Do Clustering**. Select one of the spot value types (Od, Vol, Area, %Od, %Vol) to be considered by this classification algorithm. In the following window, define the minimum and maximum number of classes to be formed by Melanie (respectively, *Minimum number of classes* and *Maximum number of classes* parameter).

For example, if you choose 2 and 4, Melanie will successively split the gels in 2, 3 and 4 classes, then compare the resulting classifications and, according to a similarity function, show the best one.

Select the sensitivity parameter to be considered between the highest and the lowest values in groups (*Sensitivity* parameter). The smaller this parameter, the smaller

the accepted difference among groups will be (and more spots will be present in the results). This parameter may be seen in a schematic way in Figure 7-13.

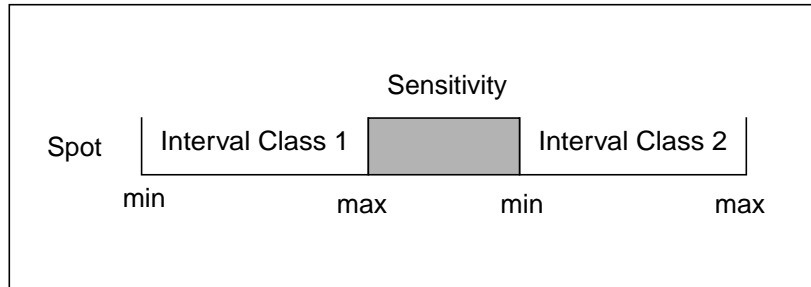


Figure 7-13. Sensitivity parameter.



Depending on the computer you are running Melanie on, this might take time, especially when the number of maximum classes is high.

The resulting classification may be visualized in a dendrogram which plots the classes and their gels. It may also be analyzed through its Report where the significant spots are also displayed. The *Classification Level* parameter in the *Heuristic Clustering* window establishes the depth of classification which may be seen in the plot and report as well. (Fig. 7-14).

Analyze
↳ *Heuristic Clustering*

After the analysis has been performed, you may display a report which shows the names of the selected gels and their classes, as well as information on each selected group, such as group ID and their intervals of minimum and maximum values in each class. In this case take **Report**.

Another way of visualizing the classification made by Heuristic Clustering is to display a dendrogram which shows the classes and their gels. Choose **Plot**.

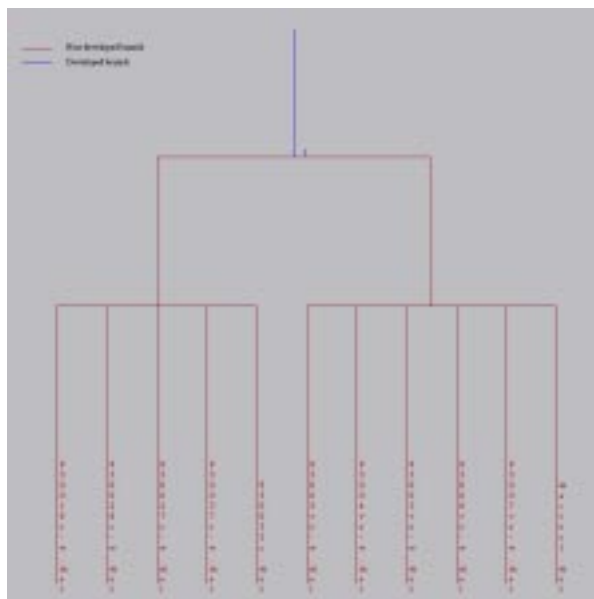
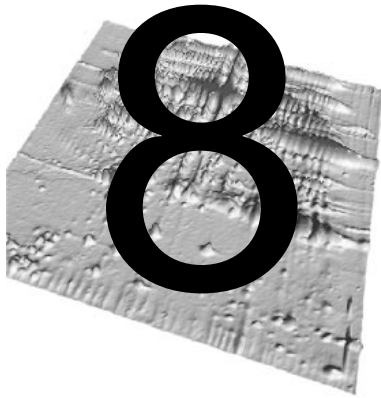


Figure 7-14. Two classes and one classification level.



CLASSES

A number of gels, having common biological meaning or common characteristics, establish a **class** of gels. It corresponds to a set of gels representing the same population. One of the main goals of Melanie is to help you find significant protein expression changes among several populations of gels. For this purpose, two complementary techniques are available:

- *Overlapping measures*: Summarizes each class by an interval and measures the overlapping across them.
- *Statistical Test*: Measures the difference among populations and estimates if this difference is significant (i.e. is not only due to chance).

This chapter describes how the analysis and visualization of differently expressed proteins through populations of gels can be highlighted with statistical tests, reports and histograms.

Menu: Edit

Specifying classes

To identify variations between two populations of gels, you need to specify which gels belong to which population by creating classes.

To create a class, select the gels which belong to it and choose **Add to Class**. In the dialog box, enter a name for the class (for example, class A) or choose among the existing names. Repeat the same procedures to create the other classes.

Edit
↳ *Classes*

Edit
↳ Classes

If you want to cancel the class of one or many gels, select the gels and then choose **Remove from Class**.

Menu: Analyze

Overlapping measures

As for groups of spots, each protein class may be summarized by two statistical values, namely central tendency and dispersion (see *Statistics for Groups* on page 90). By computing an overlapping measure between the classes range summary, you can find the proteins which are expressed differently in one population against the others.

Histograms and reports on classes display, for each class of gels and for each selected group, two values that describe classes and indicate their overlap according to the *Displayed value* list on the toolbar (see Fig. 8-3 or Fig. 8-8):

- Center: Raw central tendency of the current class;
- Dispersion: Raw dispersion values of the current class. These two values define the interval which summarizes the proteins sample for the class. You can also characterize a class only by the central value by setting the dispersion percentage slider to 0%.

The following values are different measures to indicate the overlapping degree between the current class against one of the other classes.

- Gap: Maximum difference between the range of the current class and the range of one of the other classes. Negative values indicate overlapping intervals and positive values indicate no overlapping intervals (Fig. 8-1).

- Ratio: Maximum ratio between the range of the current class and the range of one of the other classes. Values smaller than 1 indicate overlapping and higher indicate no overlapping (Fig. 8-1).

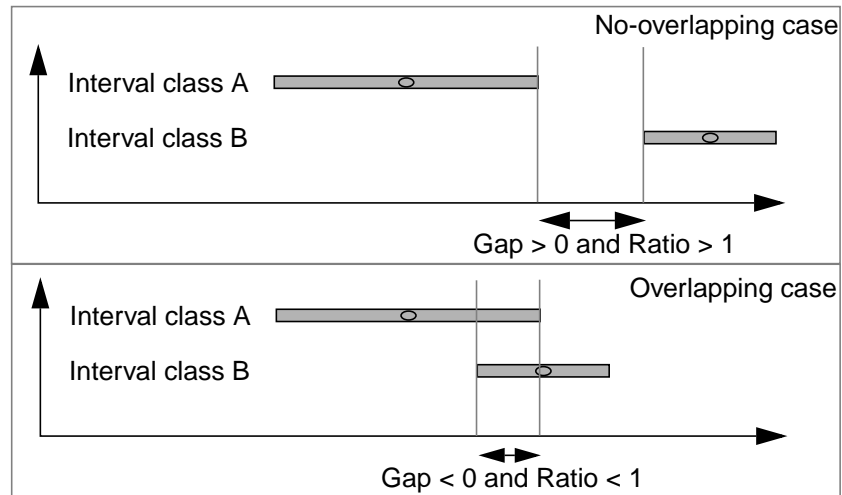


Figure 8-1. Schema of the Gap and Ratio values.

- Normalized: Minimum overlapping between the range of the current class and the range of the other classes. A value smaller than 1 indicates overlapping. For example, 0.25 indicates that 25% of the range is not recovered by one of the other classes. In the same way, 1.5 indicates a gap of 0.5 from the range size of the current class to its furthest class. Because this value is not symmetrical, a value from class A compared to class B is not the same as the value from class B compared to class A (Fig. 8-2).

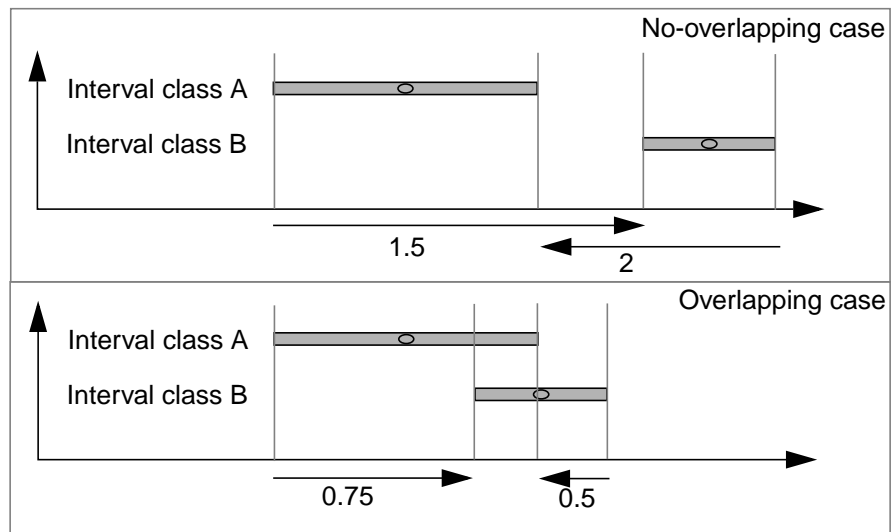


Figure 8-2. Schema of the Normalize value.

Note that the calculations where the protein is absent in a class are represented in the report and histograms by the number 1000000.

Histograms on Classes

Analyze
↳ *Classes Histograms*

As for groups of spots, you may visually study statistics values of classes by displaying histograms. Select groups of spots and choose **Classes Histograms**. Select one of the spot value types (Od, Vol, Area, %Od, %Vol). In the dialog box, choose central and dispersion statistics (see *Statistics for Groups* on page 90). The displayed histograms show, for each class, the chosen *Displayed* value and the dispersion interval (Fig. 8-3).

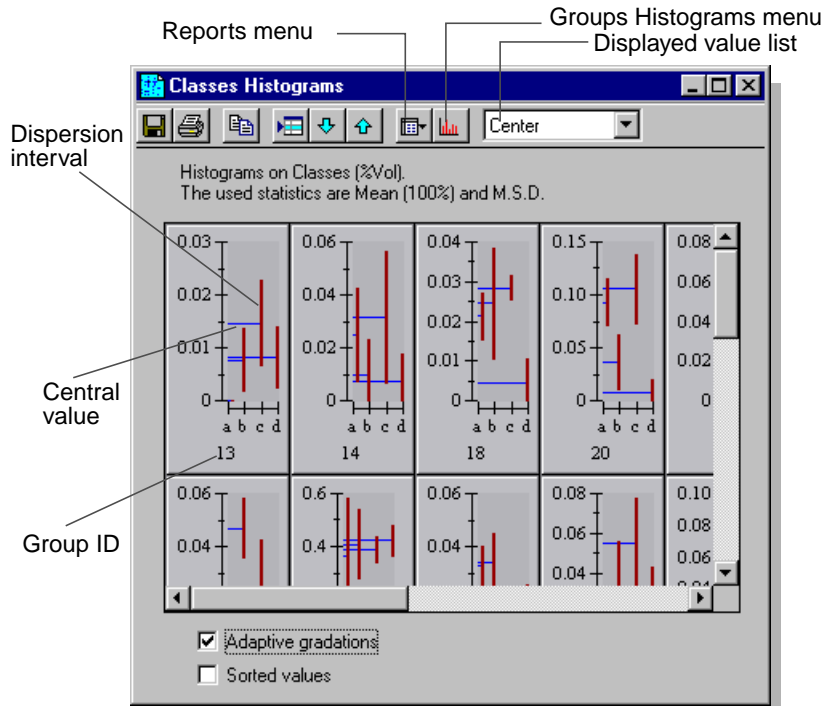


Figure 8-3. Histograms on Classes.

The histograms on classes may also be selected as any other object and be saved, printed or exported to other software. You may re-select the corresponding groups on the gels by using the *Select on Gel Image* button, and navigate through groups with *Select Next* and *Select Previous* buttons.

In the *Histograms on Classes* window, you may find short ways to useful operations. You may also find report and histogram short cuts. The *Reports* menu button (Fig. 8-3) allows you to display reports correlated to the classes.

The *Gels Report* item is a reduced version of the original *Report on Gels* to be used, for example, as a legend to the classes.

The *Groups Report* item separately displays a Report on Groups for selected groups for each class. The Information displayed is the original information usually found in the *Report on Groups* (see *Reports on groups* on page 96).

You may also create, from the *Histograms on Classes* window, a *Report on Classes* for the selected corresponding histograms, as well as a more complete histogram of

classes+groups (*Histogram* button in the toolbar). In the same way as Histograms and Report on Groups, Histograms and Report on Classes are dual elements, they have a reciprocal relationship.

To simplify the visual search of non-overlapping intervals, you may display the classes values sorted in ascending order, in this case select the Sorted values check box in the bottom of the histograms window (Fig. 8-4).

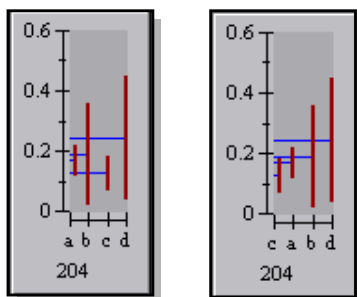


Figure 8-4. Histograms on Classes with unsorted central values and with sorted, in ascending order, central values.

To have the same gradations for all histograms, unselect the **Adaptive gradations** check box in the bottom of the histograms window (Fig. 8-5).

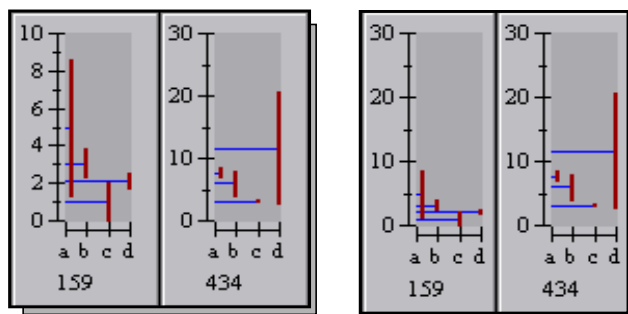


Figure 8-5. Histograms on Classes with adaptive gradations set individually, and set according to the minimum and maximum values.



Use the Report on Classes to change the order or to refine your histograms selection.

Histograms on Classes+Groups

From the Report and Histograms on Classes windows, you may request more detailed and complete histograms on classes for selected groups. They display, for each group, the extracted classes intervals and their corresponding spot values.

Analyze
↳ Classes...

Select gels and groups of spots. Choose **Classes Histograms** or **Classes Report**. Select one of the spot value types (Od, Vol, Area, %Od, %Vol). In the dialog box,

select central and dispersion statistics. In the toolbar of the displayed window, select the item *Groups Histograms* in the *Histograms* menu.

The histograms are displayed for each group of spots and for each class. The available information from the classes are the central value and the dispersion interval. A vertical gray line separates the spots values for each class (Fig. 8-6).

The information displayed in these specific histograms are described in *Histograms on groups* on page 93 and *Histograms on Classes* on page 104.

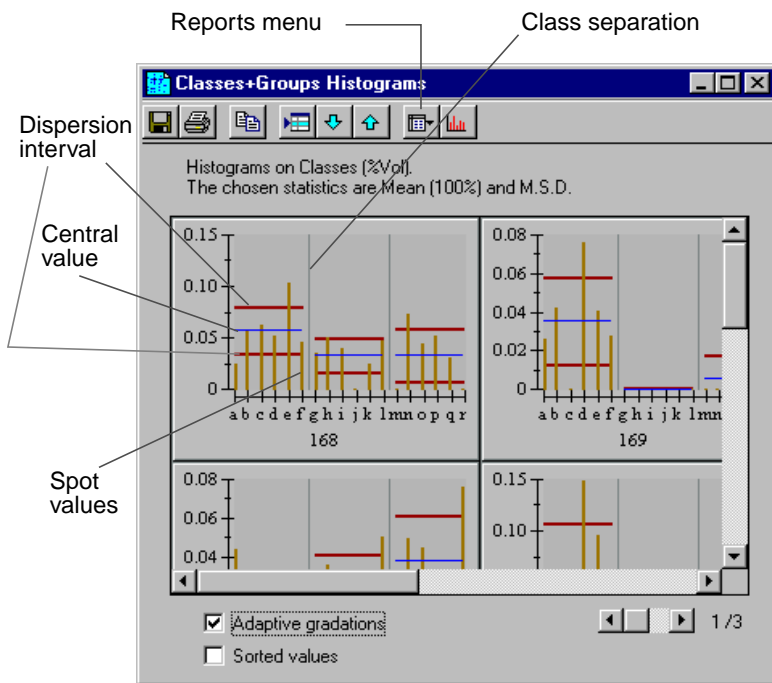


Figure 8-6. Histograms on Classes+Groups.

The histograms on classes may also be selected as any other object and be saved, printed or exported to any other software. You may re-select the corresponding groups on the gels by using the *Select on Gel Image* button, and navigate through groups with *Select Next* and *Select Previous* buttons.

You may also decide to display the chosen classes displayed values sorted in ascending order. In this case select the *Sorted values* check box at the bottom of the histograms window (Fig. 8-6). The spot values are sorted inside the classes and

the classes are also sorted according to the central value, i.e. the *Displayed value* chosen for the Histogram or the Report on Classes (Fig. 8-7).

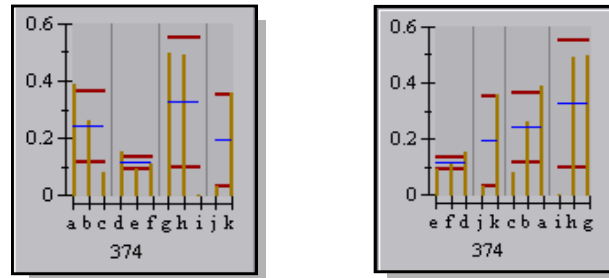


Figure 8-7. Histograms on Classes+Groups with unsorted and sorted in ascending order values. The classes are sorted according to their central values and the spots are sorted also inside the classes.

In the *Histogram on Classes+Groups* window, unselect the **Adaptive gradations** check box to have the same gradations in all displayed histograms.

Report on Classes

Analyze
↳ *Classes Report*

Specific reports on classes may be generated to display their statistical values. Once classes have been set, select the gels and groups of spots to be included in the report. Choose **Classes Report**. Select one of the spot value types (Od, Vol, Area, %Od, %Vol). In the dialog box, select central and dispersion statistics, which are explained in *Statistics for Groups* on page 90.

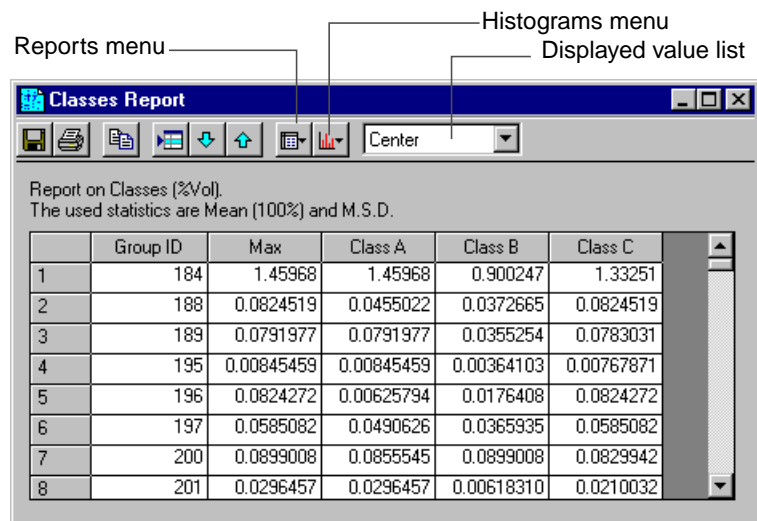


Figure 8-8. Report on Classes.

The *Report on Classes* displays the chosen *Displayed value* (Fig. 8-8) for each class and the maximum value among all the classes. The values available in the

Report on Classes allow you to differentiate one class from the other classes, and this according to each group of spots.

From the *Report on Classes* window, you may also create the same reports found in the *Histograms on Classes* window for the selected corresponding histograms. The available reports are the **Report on Gels**, used as a legend for classes and gels, as well as the **Report on Groups** separated by classes.

In the *Histograms* menu button, you may choose to display specific histograms on classes and on groups.

Statistical tests

Melanie also includes statistical tests to determine differently expressed proteins [see reference 11].

The main characteristic of statistical tests is to establish the probability that the measured difference between populations can be due to chance. In order to express the results as probabilities, the protein values must follow the restrictive assumptions about these tests.

Because protein expression among several gels rarely follow these assumptions, Melanie only displays the numerical values for each test and lets the user search in the appropriate tables for the corresponding significance levels.

Analyze
↳ *Statistical Tests*

Select a given number of groups among the selected gels. Choose **Statistical Tests**. You then have to select one of the spot value types for the analysis (Od, Vol, Area, %Od, %Vol).

Choose the statistical test values, which you would like to have displayed, from Student t-test, Wilcoxon test and Kolmogorov test. The *Statistical Tests* report displays, for each selected group of spots, the chosen statistical values. The displayed values should be considered as **qualitative indications** of potentially differently expressed proteins.

Student t-test

The Student t-test is the most used test to determine whether two populations are significantly different, and this based on the assumption that:

- the data follow the normal probability distribution.
- the standard deviation of the two classes are similar.

The statistics are based on the difference between the two classes means normalized by the standard deviation.

The displayed value for each group of spots in the *Report on Statistical Test* corresponds to the probability p of confidence on the Student t-test hypothesis, i.e. to affirm with p probability that both populations are significantly different.

When the normal distribution assumption is not followed, non parametric tests, like Wilcoxon or Kolmogorov, may provide a better analysis.

Wilcoxon or Mann-Whitney test

The Wilcoxon or Mann-Whitney Test investigate the hypothesis that two populations differ only with respect to their median values.

The characteristics of this test are that no assumptions are made about the data distribution. It considers only that they have the same distribution.

The statistics are based on the number of shifts needed to order the values from the two populations so that they don't overlap. For the example given in Fig. 8-9, the

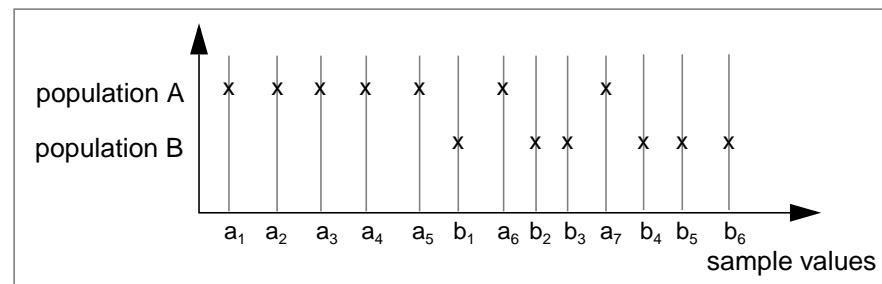


Figure 8-9. Example of two populations.

Wilcoxon test indicates that the last two values from A must be swapped four times (to the left) so that both populations no longer overlap.

The report values are the normalized number of shifts needed to sort the two populations. You may then use this value to look for the probability of confidence in a Wilcoxon table.

Kolmogorov

The Kolmogorov-Smirnov test is used to test whether or not two samples come from the same distribution. The Kolmogorov-Smirnov test does not require the assumption that the population is normally distributed, nor does Wilcoxon.

The statistics measure the number of values, in percent, from a population which is not covered by values from the other population. For the example given in Fig. 8-9, the Kolmogorov test shows that 5/7 values from A are not covered by the values from B.

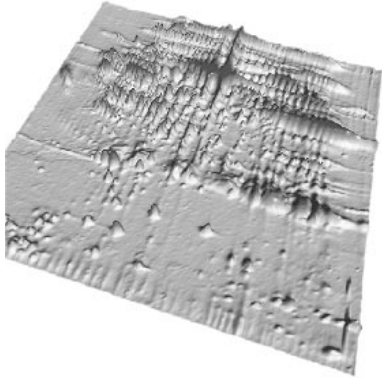
The sample percentage not covered by the other population is displayed on the Report on Statistical Test. You may then use this value to look for the probability of confidence in a Kolmogorov table.



Note that all statistical values are only useful when dealing with more than 2 gels per class.

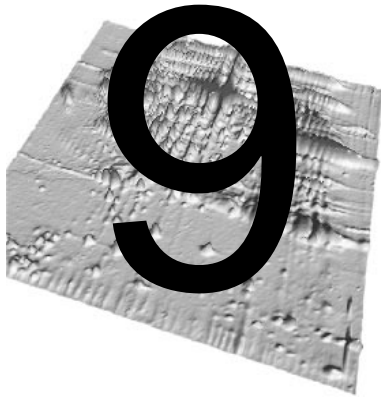


Sort the columns in the report, by clicking in their headers, and re-select the groups which correspond to your own criterion of statistical value.



PART III

TOOLS



DATABASES

2-DE databases

A key aspect of the Melanie program is its ability to link spots on gel images to protein data in **2-DE databases**. 2-DE databases contain data on proteins identified on 2-DE images, such as *pI* and *M_w*, bibliographical references to protein related literature, information on protein functions, etc. Several specialized 2-DE databases exist that Melanie can work in conjunction with. Melanie is especially well suited to work with data from the SWISS-2DPAGE global 2-DE database that is accessible over the Internet (see *SWISS-2DPAGE master gels* on page 115).

Spots on 2-DE gels analyzed with Melanie may be linked to two different types of 2-DE databases:

1. **Private 2-DE databases** - This is a database you build yourself by defining your own master gel on which you add protein-related data. You may then match gels with your master protein map.
2. **Remote 2-DE databases** - If your computer is connected to the Internet, you may link your gels to remote databases such as SWISS-2DPAGE, and remotely retrieve protein data related to spots on your gels.

The spot annotation with Ac label

The **annotation with an Ac label** is a small tag associated to a spot which identifies the corresponding protein (see *Annotations* on page 53). The Ac label holds the protein's accession number taken from the 2-DE database. The Ac label is the link between the gel and the 2-DE database.

Querying a remote database through the Internet

In Melanie you may work with your own master images, or you can link your gels to any SWISS-2DPAGE master gels. An interesting possibility to link your images to 2-DE databases is offered if your computer is connected to the Internet. You may retrieve data from remote 2-DE databases for any label containing a valid accession number (Ac).

The http query is formed by two parts:

- the fixed part which contains the http address and the query engine;
- the variable part which contains the Ac label.

Querying one annotation means that the fixed and variable parts are concatenated.

Setting the browser

Tools
↳ Database

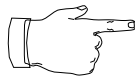
To retrieve data from remote databases you must have the Netscape Navigator® World-Wide Web or Microsoft Internet Explorer browser installed. Choose **Settings** and select your favorite browser.

Setting the database

You have to set the http site of the remote **federated 2-DE database** [see reference 25], i.e. a 2-DE database that may be remotely queried from within 2-DE analysis software. Choose **Settings** and enter the whole http site.

For example, you may decide to query, on the ExpASy server, the SWISS-2DPAGE 2-DE database using its query engine Nice2dpage, or the SWISS-PROT protein sequence database using the query engine Niceprot. In the first case, type `http://www.expasy.ch/cgi-bin/nice2dpage.pl` in *Database URL* field. In the second case, type `http://www.expasy.ch/cgi-bin/niceprot.pl`.

Click the *World 2D* button in the *Database Settings* window to display, in your chosen browser, a list of federated 2D-PAGE databases and servers which may be queried from Melanie or consult the page `http://www.expasy.ch/ch2d/2d-index.html`. The *World 2D* button is a reminder of the possible servers. Copy the desired database format name from the displayed window and paste it to the field *Database URL* in Melanie.



The query formats are specific to the databases. Some formats must include the “?” character, others must include the “=” character. Melanie concatenates the Ac label to the text typed in the field *Database URL*.

These settings may also be made permanent from the *Edit* → *Preferences* → *Database* menu option.

Querying the database

Show
↳ Annotations

To query the remote database, double-click one spot that has a label with a valid accession number (Ac). This will call the chosen browser program on your computer that is going to automatically retrieve the corresponding entry from the remote database. Alternatively, you may select the label and choose *Show* → *Annotations* → *Linked Data*.

For example, if you have set the database **SWISS-2DPAGE** on the **www.expasy.ch** server, with the query engine **Nice2DPAGE**, by double-clicking the spot label which has the accession number **P02649**, you will obtain the entry for Human *Apo E* in your chosen browser (see Fig. 9-1).

SWISS-2DPAGE master gels

SWISS-2DPAGE master gels contain annotations for all proteins corresponding to entries in the SWISS-2DPAGE 2-DE database. Each annotation holds the accession number (in the *Ac* label category) of the database entry, as well as the entry's short name (in the *ProteinName* label category).

For example, the Human Plasma protein map possesses the *Ac* label P02768 and the *ProteinName* label ALBU_HUMAN, for each spot that corresponds to *albumin*. SWISS-2DPAGE entries contain detailed data on the related proteins, such as the protein's full name, bibliographical references, annotations (protein function, pathological variations, etc.) and the *pI* and *Mw* of the related spot on the 2-DE maps. They also hold cross-references to other databases such as the SWISS-PROT protein sequence database (that in turn has cross-references to numerous other databases such as nucleotide sequence databases, disease databases, etc.) or the

YEPD Yeast 2-DE database and the ECO2DBASE *E. Coli* 2-DE database (Fig. 9-1).

SWISS-2DPAGE: P02649

General information about the entry
[View entry in current SWISS-2DPAGE format](#)

Entry name	APE_HUMAN
Primary accession number	P02649
Released in SWISS-2DPAGE in	Release 01, February 1995
Last modified in	Release 16, June 1999

Name and origin of the protein

Description	APOLIPOPROTEIN E (APO-E)
From	Homo sapiens (Human)
Taxonomy	Eukaryota, Metazoa, Chordata, Craniata, Vertebrata, Mammalia, Eutheria, Primates, Catarrhini, Hominoidea, Homo

References

[1]
 MAPPING ON GEL.
 MEDLINE, 78094420 [DCB] [EPA] [Israel Israel]
 ANDERSON N L, ANDERSON N G.
 "High resolution two-dimensional electrophoresis of human plasma proteins".
 Proc. Nat. Acad. Sci. U.S.A. 74:5421-5425(1977).

[2]
 MAPPING ON GEL.
 MEDLINE, 96007934 [DCB] [EPA] [Israel Israel]
 SANCHEZ J.-C., APPEL B.D., GOLAZ O.G., PASQUALI C., BAVIER F., BABOCH A.,
 HOCHSTRASSER D.F.
 "Index SWISS-2DPAGE database".
 Electrophoresis 16:1131-1151(1995).

Comments

- SUBUNIT MONOMER

2D PAGE maps for identified proteins

Compare the theoretical pI/Mw

Centrosomal_Plast	<p>MAP LOCATIONS:</p> <ul style="list-style-type: none"> SPOT 2D-388320W pI=5.68, Mw=34370 SPOT 2D-388320E pI=5.24, Mw=33209 SPOT 2D-388320Q pI=5.33, Mw=32804 SPOT 2D-388320Z pI=5.47, Mw=31852 <p>AMINO ACID COMPOSITION</p>
-------------------	--

Figure 9-1. The SWISS-2DPAGE entry for human Apo E.

Loading labels from a master

Tools

↳ Database

↳ Load Protein...

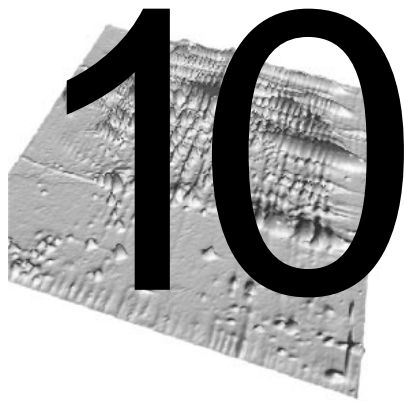
On most 2-DE databases, the accession number is the unique entry identifier, while the protein name may change. It is therefore strongly recommended to use Acs. Melanie provides a way of automatically updating the protein names in your gels. Select a gel containing labels with valid Acs. Set it as the master gel. Select all labels you want to update. Choose **Load Protein Name from Swiss-2D**. For each selected label, Melanie will request its short name from SWISS-2DPAGE database (using the Ac as identifier) and then update the *ProteinName* label accordingly.

In addition to labels containing data from the SWISS-2DPAGE database, spots in SWISS-2DPAGE master gels also present the particularity of possessing **serial numbers**, an identification number that uniquely links a spot on the gel and protein data in the SWISS-2DPAGE database to one spot on a specific SWISS-2DPAGE master gel. The serial number has the form *2D-xxxxxx*, where x is a digit or an

upper case letter. For example, *2D-000C5W* and *2D-000C6F* identify two spots on the Human Plasma protein map that have been identified as *Apo E* (Fig. 9-1).

Tools
↳ Database
↳ Load Swiss...

The serial numbers are embedded in the SWISS-2DPAGE master gels and may not be changed. To display the serial number information of SWISS-2DPAGE master gels using Melanie, select a master gel then choose **Load Swiss-2D Serial Number**. The serial numbers are placed in labels from the *SerialNumber* category which by default are not visible. To make this category visible, choose *Show* → *Annotations* → *Hidden Categories* and select the category name in the proposed list of categories.



CREATING IMAGES

The functions found in the *Tools* → *Gels* menu processes existing 2-DE gel images on the screen, but affects image files on disk. From this menu you may create new gel images from the selected ones. You can duplicate, crop and filter gels, create new synthetic, and flip or scale as well as erase gels. Except for the *Erase* option, all options in this menu create new gels that are automatically opened and displayed in the Melanie window.

Creating synthetic gels

One way to study variations in protein expression among a series of gels is to produce a synthetic gel by merging spots from these gels. In this case, the synthetic gel is used as a **path connecting** the studied gels in analysis operations.

As well, side effects of gel runs, such as staining, running differences, sample variation, may be overcome by merging all gels from the same sample into an **averaged gel**.

Besides, Melanie allows you to choose the spots which are included in the synthetic gels.

To create a synthetic gel, perform the following steps:

1. Select the spots to be included in the synthetic gel.
2. Choose *Tools* → *Create Synthetic Gel*.
3. The Create Synthetic Gel window then lets you specify the new file name.

Tools
↳ *Create Synthetic...*

The synthetic gel contains all selected spots. If spots belong to a group, only one spot per group will be present in the synthetic gel, which is the closest to the average quantification value of the group. In this case, Vol, Area and Od values assigned to the spots in the synthetic gel correspond to the chosen spot. The %Od and %Vol values are recalculated according to the total Od and Vol, respectively, of the new synthetic image.

If some gels are matched against others, you may decide to “keep only triple” spots in the synthetic gel. In this case, only the groups whose spots form a triangle are considered in the synthetic image (Fig. 10-1).

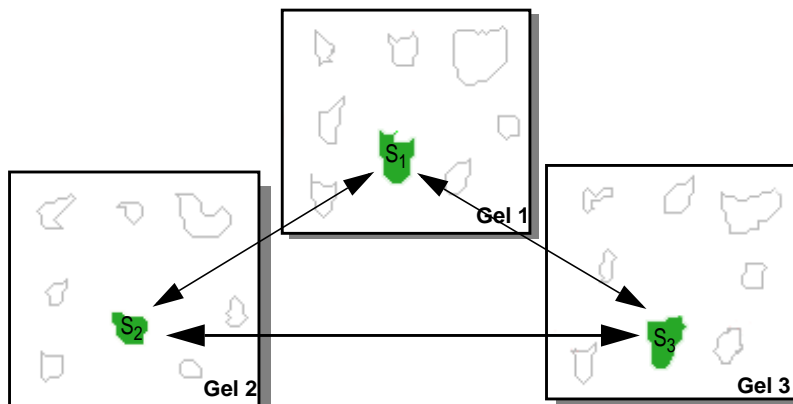


Figure 10-1. Triangle of pairs.

The synthetic gel is saved in the same directory as the matched gels, and pairs will be automatically created against the synthetic gel.

The resulting synthetic gel will be created and displayed (Fig. 10-2).

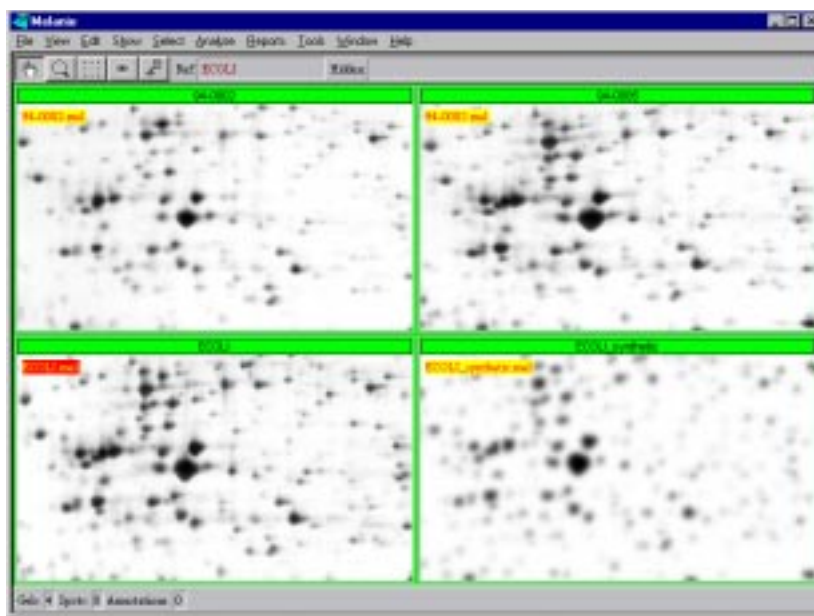


Figure 10-2. A synthetic gel and its three contributing gels. The reference gel is ECOLI.

You may now perform a quantitative study on their spots, using spot groups (see *Groups* on page 85).

The synthetic gel can be updated by adding new spots with *Edit* → *Spots* → *Copy from Gel* (see *Adding / modifying spots* on page 42). The image will be updated according to these new spots.

Duplicating gels

You can create copies of selected gels and include existing objects such as spots or annotations.

Tools
↳ Gels

Select the gels you want to duplicate. Choose **Duplicate** and give new file names for them. You may also add an extension or replace the extensions of the original image files. The gel image files will be copied to disk. They will then be opened and displayed in the Melanie window.

Erasing gels

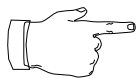
To delete existing gels, select them, then **Erase**. All selected gels will be removed from the screen as well as from the disk. Associated spot pairs are deleted as well.

Cropping gels

Define regions in selected gels using the Region tool. Choose **Crop**. You may choose what objects you want to keep with the cropped image, such as spots and annotations. You may also decide to keep the image alignment. Another dialog box then lets you specify the new file names. You may also add an extension or replace the extensions of the original image files. Click *OK*. New gels will be created containing only the parts of the gels that lie within the specified regions, plus the specified objects.

Filtering gels

Melanie has been optimized for analyzing 2-DE gel images without prior image processing or enhancement. If you analyze low quality images, Melanie provides image processing filters that may help you to enhance the quality of your images.



Tools
↳ Gels

Nevertheless, keep in mind that after filtering images, spot quantification values will change and will be hard to interpret.

Select the gels you want to filter, then pick a filter in the **Filter Image** options. This will create copies of the gels after they have been filtered, leaving the original images unaffected. The following filters are available:

1. **Smoothing** - Smooths the raw gel image. You have the choice between the following four smoothing algorithms:

- Gaussian smoothing
- Diffusion smoothing
- Polynomial fitting
- Adaptive smoothing

You will also be asked to choose how many times you wish the smooth filter to be applied.

2. **Histogram Equalization** - Performs histogram equalization on the selected gels. Various options are available, and the following five equalization algorithms are provided:

- Uniform
- Exponential
- Rayleigh
- Cube Root
- Logarithmic

3. **Remap Grey Levels** - Creates copies of the selected gels with modified grey level mapping (see *Gray levels* on page 33). This is especially useful if you analyze images with a very low grey level range.

For example, the Melanie spot detection algorithm may not work properly on an image with only 25 grey levels. You can therefore remap it to an image with 4096 shades of grey so that spots may be correctly detected.

4. **Contrast Enhancement** - Applies a contrast enhancing algorithm to the images in order to highlight small spots (Fig. 10-3). The following three contrast enhancement algorithms are available:

- Quadratic
- Sigmoid

- Wallis

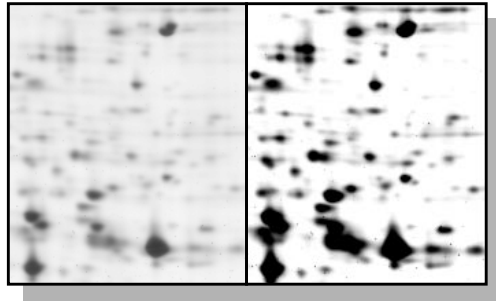


Figure 10-3. The original image (left) and the same image after contrast enhancement (right).

5. Filter Background - Applies one of the two following background filtering algorithms to the selected gels, in order to eliminate gel background (Fig. 10-4):

- Subtraction of global image minimum
- Background subtraction by polynomial transformation

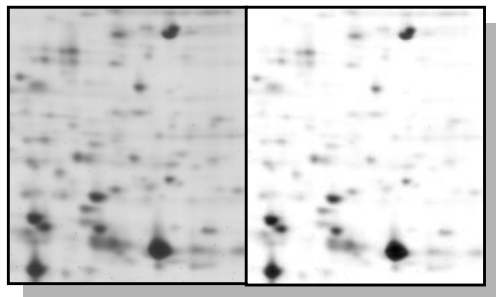


Figure 10-4. The original image (left) and the same image after background subtraction (right).

6. Morphological Filter - Applies a morphological filtering algorithm (rank filter) to the selected gels (Fig. 10-5), according to 2 parameters:

- the window size, which can be 3x3, 5x5 or 7x7 pixels wide,
- and the value index.

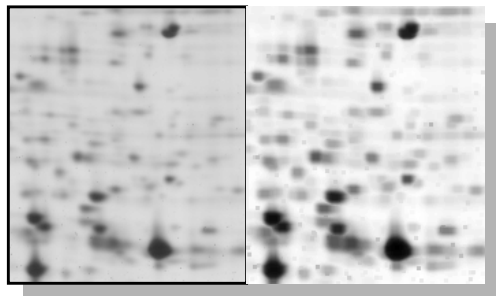


Figure 10-5. The original image (left) and the same image after a morphological filter with 3x3 and value index = 8 (right).

This algorithm produces an image by replacing each central pixel of a “window” by the grey value specified by “value index” in the list of pixel values from the defined window, sorted in ascending order. If the value index is 0, the filter corresponds to an erosion (shrink spots). If the value index is the maximum window size, the filter corresponds to a dilation (grow spots). If the value index is the central window size, the filter is a median filter.

Tools
↳ *Gels*

Flipping gels

If you have scanned images in the wrong direction, you may correct this by applying the *Flip Gels* options. Select gels, then **Flip** and choose one of the flipping options (*Horizontally*, *Vertically* or *Both Directions*). As for other options in the *Tools* menu, you will have to specify file names.

Scaling gels

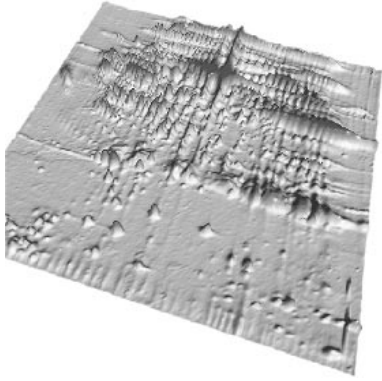
The *Image* menu lets you create larger or smaller copies of selected gels. Select gels, then **Scale By** and type the horizontal and vertical scale factor values. As for other options in the *Tools* menu, you will have to specify file names.

Rotating gels

If you have scanned images in the wrong position, you can correct this by applying *Rotate Gels*. Select gels, then **Rotate**. A dialog box lets you specify the rotation angle with the sliders. As for other options in the *Tools* menu, you will have to specify file names.

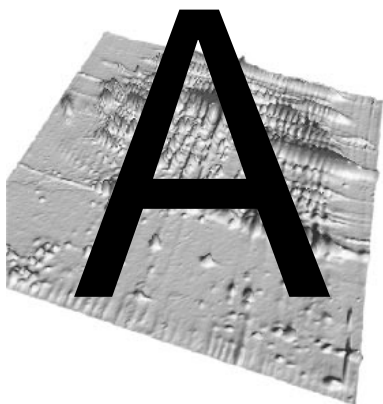
Inverting grey levels

The last option in the *Tools* menu lets you change the grey levels of selected gels by inverting them (reverse video). Select gels, then **Invert Gray Levels**. As for other options in the *Tools* menu, you will have to specify file names.



PART IV

APPENDIXES



DIFFERENCES BETWEEN MELANIE II AND MELANIE 3 OR WHERE TO FIND WHAT SEEMS TO HAVE DISAPPEARED

Show and select objects

Most operations in Melanie may only be applied to selected objects on selected gels. For instance, a report on spots will only be enabled if there are selected spots on selected gels.

Likewise, most of the main operations in Melanie may now only be carried out when objects are visible on gels, i.e. you can only select the objects that are **visible** on the display. For example, to select annotations or spots, they must be displayed on gels. Nonetheless, when a hidden object is selected, by the means of a report for example, it may be viewed on the screen, but it will immediately disappear once it is unselected again.

Select gels

Selecting gels is much faster and easier. To select one gel, you just need to click on the gel's selection banner, the horizontal stripe located at the top of the gel which contains its name. The action of selecting gels may be done at any time, with any of Melanie's tools.

Landmarks and labels

Landmarks and labels were generalized with the new object *Annotation*. The object *Landmark* is now replaced by the pre-defined label category *Landmark*. Landmarks are also used as reference starting points to align gels.

Labels were also replaced by label categories. The pre-defined label category *Ac* replaces the Accession Number field from Labels in earlier versions of Melanie, and it also keeps the main property of being the link to Melanie's remote database query engine. The short ID information is held by a category designated ProteinName.

Aligning gels

To be able to perform operations easily and faster in many gels simultaneously, such as magnifying, moving, etc., an implicit alignment is automatically done when gels have landmarks with the same names. This alignment calculates corresponding pixels in gels based on the simple interpolation of the two closest landmarks with same names in gels. This implicit alignment **does not** replace gel warping needed to compensate deformations on gels and to improve matching. We still encourage users to align gels before stacking and matching. For this purpose, select gels and choose *View* → *Align Gels* → *Align*.

Stacking many gels

Earlier versions of Melanie were able to stack two gels to better compare given gel areas, and to create and visualize pairs. Selecting one gel on the stack immediately selected the other one.

You may now stack many gels at a time, i.e. display one gel on top of the others, thus creating a "pile" of gels. By switching from one gel to the other, many new operations may be done on the gels of the stack, such as displaying overlapped spots, for example. You may also choose to select one, two or many gels in the stack and operations will be carried out only in these selected gels.

Analyzing data

In Melanie 3, not only the display has changed but also the way to reason about groups of spots and classes of gels. Anyhow, some operations used to analyze gels

in Melanie 2.3 or earlier may still be found in Melanie 3, with the difference that zeros (absent proteins) are taken into calculations:

Differential analysis with classes set: take *Analyze* → *Classes Report*. In the Classes Report dialog, choose Mean (100%) and dispersion (0%). In the displayed report, choose Ratio in the Display value list. The results will be displayed in the report and by sorting the values in this column, you may select the most important group values, according to your own criteria.

Differential analysis without classes set: take *Analyze* → *Groups Report* and select the Range ratio item. Sort the resulting column to enable the selection of the most important values.

Correspondence analysis without classes set: take *Analyze* → *Groups Report* and select the Correspondence Analysis item. The results will be displayed in the report and by sorting the values in this column, you may select the most important groups values for the factorial discrimination.

What happens when you open your old Melanie files?

When you open a file in Melanie that was created in Melanie 2.3 or earlier, it is converted to a new file format. In this new format, the existing information on the gels is converted to annotations by using the following rules:

- Landmarks are converted to the category Landmarks;
- Landmarks with Pi and Mw are also copied to the category Pi_Mw;
- Labels with Accession Numbers are converted to the category Ac;
- Labels with short ID are converted to a category called ProteinName.
- Gel comments are converted to a category called GelComment. Its label displays the text "Gel Comment..." and is positioned in the top-left corner on the gel. By double-clicking on the label, a dialog box shows the comment text.

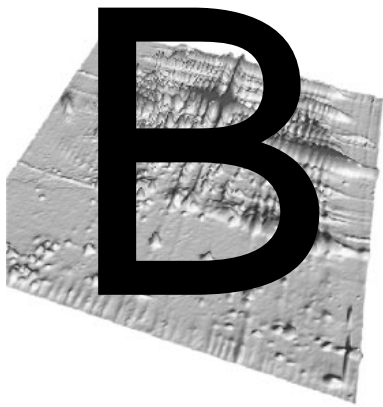
If you work with Melanie 3 and save the files in the older format (for Melanie 2.3 or earlier), portions of your work, in particular the new categories of annotations, will not be retained.

Save As

You may also decide to save files created in Melanie as earlier Melanie versions by using the command *File* → *Save As* and by choosing "Melaniell gel" in the *Type* field. In this old format, the existing annotation information is converted by using the following rules:

- Category Landmarks is converted to Landmarks;
- Category Pi_Mw is converted to Landmarks;

- Category Ac is converted to Labels with Accession Numbers;
- Category ProteinName is converted to Labels with short ID;
- Category GelComment is converted to the textual comment associated to a gel;
- All the other existing annotations and labels will not be saved.



SHORT-CUTS AND MENU STRUCTURE

The following menus are provided in the melanie software:

- **File** - to handle whole gels and data on disk (open and close gel images, save data, print images, import and export data).
- **View** - to modify the window display (fixed or variable number of cells) and gels display (stacked, aligned, zoomed, grid lines).
- **Edit** - to edit (add, modify, delete) specific objects.
- **Show** - to show or hide specific objects.
- **Select** - to select specific objects.
- **Analyze** - to compute differences and similarities among gels (statistical and artificial intelligence data analysis).
- **Reports** - to display information on selected objects and to navigate in gels by means of the data.
- **Tools** - to create new gel images from selected ones (duplicate, crop, filter gels, create synthetic gels, flip and scale gels, as well as erase gels).
- **Window** - to display information and zoom windows, and to list open report windows.
- **Help** - to obtain on-line help.

The descriptions of menus and sub-menus may be easily found with the help of the following tables:

Menu structure

Table 10-6. File menu.

		Short-cuts	See page
Open ...		Ctrl + O	17
Close		Ctrl + W	20
Raw Image			
	Load		19
	Unload		19
	Select Gels with Loaded Image		19
	Settings ...		19
Save			
	All Changes	Ctrl + S	18
	Contrast Mapping		18
	Calibration		18
	Spots	Shift + S	18
	Annotations	Alt + S	18
	Pairs	Ctrl + Shift + S	18
Save As ...			
Revert from File			
	All Changes		18
	Contrast Mapping		18
	Calibration		18
	Spots		18
	Annotations		18
	Pairs		18
Import			
	Images ...		18
	Labels ...		68
	Annotations...		68
	Calibration ...		44
Export			
	Gel to Clipboard		18
	Gel to File		18
	Gels Window to Clipboard		18
	Gels Window to File		18
Page Setup ...			19
Print			
	Gels ...		19

Table 10-6. File menu.

		Short-cuts	See page
	Gels Window ...		19
Exit			20

Table 10-7. View menu.

		Short-cuts	See page
Layout Settings ...			24
Stack			
	Selected Gels	Ctrl + G	25
	Unstack Selected Gels	Ctrl + U	25
	Set Stack Reference ...		25
	Show Overlapped		27
	Hide Overlapped		27
	Show Pairs	Ctrl + Shift + Y	27
	Hide Pairs	Ctrl + Shift + H	27
	Front to Back	Ctrl + F	25
	Next	Page Up	25
	Previous	Page Down	25
Align Gels			
	Align ...		31
	Unalign		31
	Other Alignment ...		31
Grid Lines			
	Show ...		31
	Hide		31
Gray Levels			
	Adjust Contrast ...		33
	Pseudo Colors ...		35
Zoom			
	In	Shift + Up	28
	Out	Shift + Down	28
	1/16 ... 32		28
Move Gels			
	Same Location		27
	Up	Ctrl + Up	27
	Down	Ctrl + Down	27
	Left	Ctrl + Left	27
	Right	Ctrl + Right	27
	Top Left ... Right		27

Table 10-8. Edit menu.

		Short-cuts	See page
Gels			
	Calibrate Intensity		44
	Reset Calibration		44
Spots			
	Auto Detect ...		39
	Edit ...	Shift + E	42
	Copy from Gel		42
	Delete	Shift + X	42
Annotations			
	Add Label ...		55
	Modify ...	Alt + E	59
	Copy	Alt + C	59
	Copy Paired Labels ...		59
	Paste Annotations		59
	Paste Labels ...	Alt + V	59
	Duplicate Labels ...		59
	Delete by Category		59
	Delete	Alt + X	59
	Link with Spot	Alt + L	59
	Unlink from Spot	Alt + U	59
Pairs			
	Match Annotations ...		81
	Auto+Match Gels		80
	Add	Ctrl + Shift + L	81
	Delete	Ctrl + Shift + U	81
Groups			
	Set Reference Gel ...		86
	Group Spots	Shift + G	86
	Ungroup Spots	Shift + U	86
Classes			
	Add to Class ...		101
	Remove from Class		101
Preferences	...		36

Table 10-9. Show menu.

			Short-cuts	See page
Gels				
	Only Selected		Ctrl + Z	23
	Hide Selected		Ctrl + H	23
	Show All		Ctrl + Y	23
	Sorted by Name			23
	Sorted by Classes			23
Spots				
	Shape			
		Crossed	Shift + 6	45
		Outlined	Shift + 7	45
		Filled	Shift + 8	45
	Show All		Shift + Y	45
	Hide All		Shift + H	45
	In Region			45
	Only Selected		Shift + Z	45
	Hide Selected			45
	Show ID			45
	Hide All ID			45
	Set Color			46
Annotations				
	Show All		Alt + Y	64
	Hide All		Alt + H	64
	In Region			64
	For Spots			64
	Only Selected		Alt + Z	64
	Hide Selected			64
	Visible Categories ...			64
	Hidden Categories ...			64
	Pack Categories ...			64
	Unpack Categories ...			64
	Linked Data			64
	Set Color			66
Groups				
	Show ID			86
	Hide All ID			86

Table 10-10. Select menu.

			Short-cuts	See page
Gels				
	By Name			21
	Visible		Ctrl + A	21
	Aligned			21
	Stacked			21
	All			21
	Inverse Selection			21
	For Groups			21
	For Classes ...			21
Spots				
	By ID			46
	By Color			46
	Only Gaussians			46
	In Region			46
	From Report File ...			46
	All		Shift + A	46
	Restrict by Value ...			47
	Inverse Selection			46
Annotations				
	By Content ...			60
	By Category ...			60
	By MS			62
	Combine			63
		Intersection ...		63
		Union ...		63
		Difference ...		63
		Exclusion ...		63
	By Color			60
	Common Labels ...			63
	In Region			63
	From Report File ...			63
	All		Alt + A	63
	Inverse Selection			60
Pairs				
	For Spots		Ctrl + Shift + M	82

Table 10-10. Select menu.

			Short-cuts	See page
	All		Ctrl + Shift + A	82
	Bad Pairs ...			82
	Multiple Pairs			82
Groups				
	By ID ...			87
	For Spots			87
	All Groups		Shift + M	87
	Restrict Selection ...			87
	Inverse Selection			87
Select All				
Unselect All				

Table 10-11. Analyze and Reports menus.

Menu		Short-cuts	See page
Analyze			
	Groups Report ...		96
	Groups Histograms ...		93
	Scatters Plot		88
	Classes Report ...		107
	Classes Histograms ...		104
	Statistical Tests ...		108
	Heuristic Clustering		99
		Do Clustering ...	99
		Report ...	99
		Plot ...	99
Reports			
	Gels Report ...		37
	Spots Report		49
		Current	49
	Labels Report ...		67
	Annotations Report ...		67
	Categories Report ...		67
	Pairs Report		83
	Matches Report		83
	All Matches Report ...		83

Table 10-12. Tools menu.

			Short-cuts	See page
Gels				
	Crop ...			121
	Duplicate ...			121
	Erase ...			121
	Flip			124
		Horizontally ...		124
		Vertically ...		124
		Both Directions ...		124
	Filter Image			121
		Smooth ...		121
		Histogram Equalization ...		121
		Remap Gray Levels ...		121
		Contrast Enhancement ...		121
		Filter Background ...		121
		Morphological Filters ...		121
	Scale By ...			124
	Rotate ...			124
	Invert Gray Levels ...			124
Create Synthetic Gels ...				119
Database				
	Settings ...			114
	Load Protein Name from Swiss-2D			115
	Load Swiss-2D Serial Number			115
Toolbar				
	Hand		Ctrl + 1	
	Magnify		Ctrl + 2	
	Region		Ctrl + 3	
	Spot		Ctrl + 4	
	Annotation		Ctrl + 5	

Table 10-13. Window and Help menus.

Menu		Short-cuts	See page
Window			
	Zoom		29
	Information		47
Help			
	User Manual ...		
	Index ...		
	Tutorial ...		
	About Melanie		

Gels handling

Table 10-14.

Short-Cut	Menu Command
Ctrl+A	<i>Select → Gels → Visible</i>
Ctrl+F	<i>View → Stack → Front to Back</i>
Ctrl+G	<i>View → Stack → Selected Gels</i>
Ctrl+H	<i>Show → Gels → Hide Selected</i>
Ctrl+O	<i>File → Open</i>
Ctrl+S	<i>File → Save</i>
Ctrl+U	<i>View → Stack → Unstack Selected Gels</i>
Ctrl+W	<i>File → Close</i>
Ctrl+Y	<i>Show → Gels → All</i>
Ctrl+Z	<i>Show → Gels → Only Selected</i>
Ctrl+Up	<i>View → Move Gels → Up</i>
Ctrl+Down	<i>View → Move Gels → Down</i>
Ctrl+Left	<i>View → Move Gels → Left</i>
Ctrl+Right	<i>View → Move Gels → Right</i>
Ctrl+1	<i>Tools → Toolbar → Hand</i>
Ctrl+2	<i>Tools → Toolbar → Magnify</i>
Ctrl+3	<i>Tools → Toolbar → Region</i>
Ctrl+4	<i>Tools → Toolbar → Spot</i>
Ctrl+5	<i>Tools → Toolbar → Annotation</i>
PageUp	<i>View → Stack → Next</i>
PageDown	<i>View → Stack → Previous</i>
Shift+Up	<i>View → Zoom → In</i>
Shift+Down	<i>View → Zoom → Out</i>

Spots handling

Table 10-15.

Short-Cut	Menu Command
Shift+A	<i>Select → Spots → All</i>
Shift+G	<i>Edit → Groups → Group Spots</i>
Shift+H	<i>Show → Spots → Hide All</i>
Shift+M	<i>Select → Groups → For Spots</i>
Shift+O	<i>File → Open</i>
Shift+S	<i>File → Save → Spots</i>
Shift+U	<i>Edit → Groups → Ungroup Spots</i>
Shift+X	<i>Edit → Spots → Delete</i>
Shift+Y	<i>Show → Spots → Show All</i>
Shift+Z	<i>Show → Spots → Only Selected</i>
Shift+1	<i>Show → Spots → Shape → Crossed</i>
Shift+2	<i>Show → Spots → Shape → Outlined</i>
Shift+3	<i>Show → Spots → Shape → Filled</i>
Shift+4	<i>Show → Spots → Set Color → Purple</i>
Shift+5	<i>Show → Spots → Set Color → Orange</i>
Shift+6	<i>Show → Spots → Set Color → Brown</i>
Shift+7	<i>Show → Spots → Set Color → Default</i>

Annotations handling

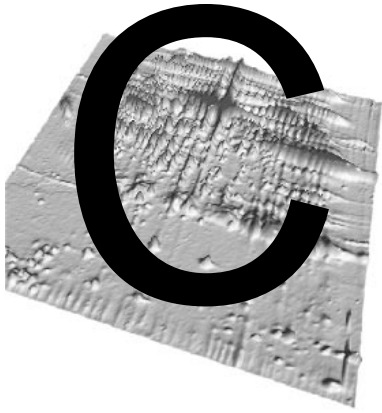
Table 10-16.

Short-Cut	Menu Command
Alt+A	<i>Select → Annotations → All</i>
Alt+C	<i>Edit → Annotations → Copy</i>
Alt+E	<i>Edit → Annotations → Modify</i>
Alt+H	<i>Show → Annotations → Hide All</i>
Alt+L	<i>Edit → Annotations → Link with Spot</i>
Alt+S	<i>File → Save → Annotations</i>
Alt+U	<i>Edit → Annotations → Unlink from Spot</i>
Alt+V	<i>Edit → Annotations → Paste Labels</i>
Alt+X	<i>Edit → Annotations → Delete Annotations</i>
Alt+Y	<i>Show → Annotations → Show All</i>
Alt+Z	<i>Show → Annotations → Only Selected</i>

Pairs handling

Table 10-17.

Short-Cut	Menu Command
Ctrl+Shift+A	<i>Select → Pairs → All</i>
Ctrl+Shift+H	<i>View → Stack → Hide Pairs</i>
Ctrl+Shift+L	<i>Edit → Pairs → Add</i>
Ctrl+Shift+M	<i>Select → Pairs → For Spots</i>
Ctrl+Shift+S	<i>File → Save → Pairs</i>
Ctrl+Shift+U	<i>Edit → Pairs → Delete</i>
Ctrl+Shift+Y	<i>View → Stack → Show Pairs</i>



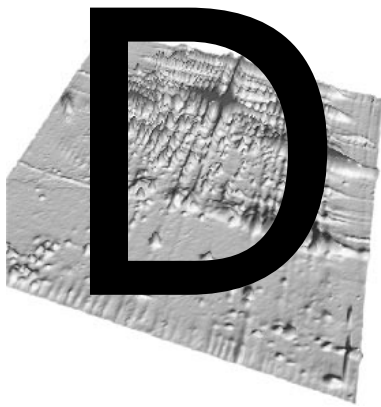
KNOWN BUGS

If you are using Melanie with a graphics display set to 256 colors, you may feel that the colors are badly displayed.

If printed reports are truncated, you may change the paper orientation to landscape in the printer set-up.

Directories cannot be destroyed when a Explorer window has their names displayed.

Accentuated characters are not correctly supported by the english API.



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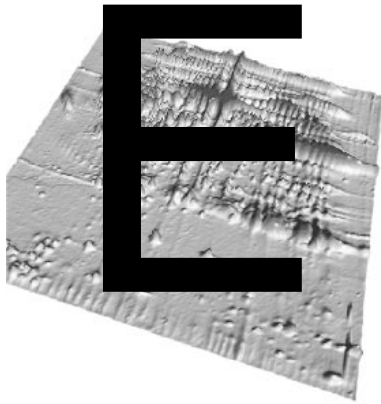
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Proteome and ExPASy

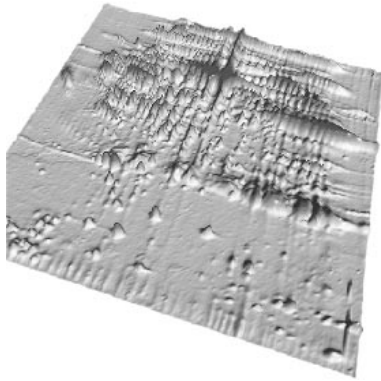
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TECHNICAL SUPPORT

Bio-Rad provides technical support on the features of the Melanie software. The staff of Bio-Rad is receptive to your suggestions. Many of the new features and enhancements in this version of Melanie are a direct result of conversations with customers, biologists and proteomics scientists. If you have any suggestions, problems or questions on the use of the features, please contact your local Bio-Rad office, or in the U.S. call Bio-Rad Technical Service at 1-800-4BIORAD (1-800-424-6723). Please report any reproducible software problems to Bio-Rad Laboratories.



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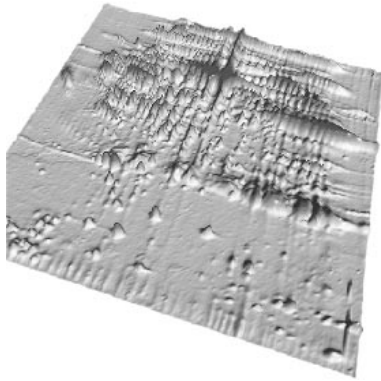
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CREDITS

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