Manual

Delta2D 4.0

DECODON GmbH

December 2008

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About Delta2D

Delta2D is a software tool for rapid and accurate analysis of 2D-electrophoresis gel images. It combines fast visual analysis with very reliable approaches to spot detection, matching, quantitation and statistical analysis. Delta2D provides versatile means to sort, filter and analyze the quantitative spot data.

The Challenge

Two-dimensional gel electrophoresis is a fundamental technology of proteomics research. Thousands of proteins can be separated on a single gel, representing a large share of the proteins in a sample. By comparing gel images taken from different samples, one can find and later identify proteins that are crucial to fundamental processes of life and to the progression of disease. The dynamic change of protein quantities across a variety of two-dimensional gels, typically backed by a number of replicates of the same sample, is what scientists around the world study by using Delta2D 4.0.

Using former approaches, tracking a single protein across a whole experiment can be quite laborously, because of

- distortions between the different gels, and
- different spot patterns on the different images.

However, positions of protein spots may vary considerably from one gel to another. These variations can make gel comparison a very tedious process, consuming a large share of the time spent analyzing an experiment.

Furthermore, if the protein spots on the different gel images are detected in each image seperately, creating expression profiles from the single spot quantities can cause another tedious task of spot matching.

With Delta2D, DECODONprovides an efficient and time saving solutions for finding interesting expression profiles.

Delta2D: Dedicated to Innovation

In 2000, DECODON introduced Delta2D Version 1.0 with rapid visual comparison of 2D-electrophoresis gel images. Delta2D uses advanced image processing technology to eliminate the variation between spot-positions resulting in dual channel images with clearly highlighted differences in protein expression levels. Dual channel images allow for the rapid visual identification of whole sets of proteins whose expression varies from one sample to the other or is

influenced by an experimental condition. Typically, Delta2D's approach speeds up the comparison process significantly compared to established 2D gel analysis approaches.

Spot detection and quantitation can be executed in a subsequent step. Since the gel images are aligned first, a spot consensus can be detected on a composite image summarizing the whole experiment's gel information in one artificial but realistically looking fusion gel. Due to using the experiment wide spot consensus an error prone spot matching can be avoided. This results in complete expression profiles without any gaps from undetected spots. The results of the quantitation and matching step are presented in an easy to use tabular view that allows for a wide range of analysis procedures.

Since image warping has become the core technology of Delta2D, significant efforts have been spent to further accelerate and ease it. As a result, with the SmartVectors™ Technology match maps can be derived for a pair of gel images automatically. Continuing Delta2D's tradition, the user keeps control through the smart user interface and handy tools to interact with the algorithms where necessary.

Modern User Interface

With Version 4.0 Delta2D has received a new user interface, which is based on

1. global menu bar including actions for objects of the same type, e.g.:
   - copy/cut and paste images between groups, or labels between images,
   - open the Dual View,
   - open a Quantitation Table for certain selected images,
   - open a Statistics Table for (at least two) selected groups,
   - change the Warp Mode for a set of selected image pairs.
2. an integrated window manager allowing for free configuration of the different windows:
   - arrange the windows as you prefer, also undock or re-dock them (multiple monitors are supported),
   - customize the toolbars,
   - define shortcuts for your most used actions.
3. completely synchronized views, e.g.:
   - when having opened the Dual View, the same image pair will be selected in the Project Explorer,
   - the same set of spots is selected across all windows.

How to use this manual

This manual is organized as follows: The next chapter gives an overview of the general philosophy behind Delta2D. It is essential for the new user to read this chapter, in order to understand the general analysis approach. In the next chapter, we desribe the analysis procedure as supported by Delta2D's workflow module. The later chapters explain the different parts of Delta2D in detail.

Throughout this manual, technical terms appear like "SmartVectors^{TM} Technology", while menu items are printed as "Window ▷ Light Table". Internal references or links to websites show up as www.decodon.com/Support/Howto/SmartVectors.html.

Contents

1. Strategy for Analyzing Gel Images with Delta2D

This chapter provides a short description of Delta2D's approach to analyze the 2DE gel images.

In a nutshell, the typical workflow for creating complete expression profiles and for identifying the interesting ones contains the following steps:

1. **Setup Project** To keep the experimental data handy, we recommend to create a new data pool for every new experimental context. Create a new Project and open the Light Table to include the relevant images and to arrange them in groups in accordance with your experimental setup. More details: section 3.1 on page 8.

2. **Setup Gel Image Warping** Assign the appropriate Warping Strategy to your project. Using the Warping Setup window you make sure to obtain persistent warping chains and do not produce warping cycles. From a set of prepared strategies choose the strategy that helps to warp images along their similarity. More details: section 3.2 on page 9

3. **Create Direct Warpings** Examine the gel image pairs with Direct Warp Links one by one and correct them where necessary. More details: section 3.3 on page 9.

   > Note: Years ago (in 2003) DECODON has introduced complete expression profiles to avoid missing values in the Quantitation Table and the resulting problems during statistical analysis.
   >
   > Read more about the benefits of 100% Spot Matching at www.decodon.com/Solutions/Delta2D/100_Percent_Spot_Matching.html.

4. **Create the Consensus Spot Pattern** Create a fused image over the complete project using Union as fusion type to get an image that includes every spot existing on any gel image in the project. Detect the spots on the fused image and edit them if necessary. Transfer the consensus spot pattern to all other gel images, and you will receive Complete Expression Profiles that enable reliable statistical analysis by avoiding missing spot quantities as they usually result from individual spot patterns on different images. More details: section 3.4 on page 9

5. **Analyze Expression Profiles** Open Quantitation Table to view the quantities of all spots. Sort and/or filter the tables for finding interesting expression profiles. Perform statistical analysis to employ a variety of advanced methods for finding patterns in your data, or for clustering your expression profiles. More details: section 3.5 on page 12.

Present Results Open the Reports and get an overview on the project or on the interesting expression profiles. Export your data to Powerpoint™ or Excel™. More details: section 3.6 on page 12.

The workflow as described above is supported by the Workflow window. This window supports your analysis step-by-step by

guiding you through the different tasks, and

informing you about the current status of these tasks.

Depending on the actual status of your project, the Workflow window offers links to the required actions.

> Note: Delta2D is able to completey and reliably analyse DIGE projects. Even for DIGE projects Delta2D's core technologies are quite useful: If you want to analyse a DIGE project with several gels e.g. image warping will provide significant time savings and quality improvements.
>
> For analysing DIGE projects you will have to consider that the project needs to be defined as a DIGE project and that for every image the corresponding gel needs to be defined and which of the images of a certain gel contains the internal standard.
>
> Read more about how to set up DIGE projects with Delta2D in section 5.2 on page 29

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2. Install and start Delta2D

2.1. Available Computing Platforms

Delta2D is available on a variety of computing platforms:

Microsoft Windows Delta2D works with any of Windows NT 4 / 2000 / XP / Vista. Detailed installation instructions are provided below.

Apple MacOS X With Mac OS X 10.4.0 (Tiger) Delta2D works without any limitations. Mac OS X v10.3.5 (Panther) works, but is not recommended. Older Versions of Mac OS are not supportable. See the DECODON web site www.decodon.com for details.

Linux See the DECODON web site www.decodon.com for installation instructions and technical requirements.

2.2. Installation Steps for Microsoft Windows

Your computer should fulfill these technical requirements:

- at least 512 MB of RAM, preferably 1 GB or more
- 800 MHz Pentium III processor or better
- 200 MB free disk space

To install Delta2D 4.0, follow these steps:

1. Download the install file from your download area on our web site, the public web site www.decodon.com/Support/Download/downloads.html, or find it on a Delta2D CD-ROM.
2. Start the installation by double-clicking on setup.exe. You will be guided through the rest of the installation process. Almost at the end you are invited to load a license file. The license file is a text file with a name ending as .lfk, which has been delivered to you either by e-mail, per download, or you can find it on your cd-rom.

> Note: Depending on your computing environment, you may need special privileges to install software on your computer. If in doubt, ask your local systems administrator.

Enjoy working with Delta2D 4.0!

2.3. Start Delta2D

To start Delta2D under Windows, choose Programs ▷ DECODON ▷ Delta2D 4.0 ▷ Delta2D from the start menu.

On MacOS start Delta2D as other applications per double-click.

2.4. License Registration

If you have not imported a license during the installation process, a dialog will open (figure 2.1 on page 4), prompting for the license file. The license file is a text file with a name ending as .lfk, which has been delivered to you either by e-mail, or can be found on your cd-rom.

Figure 2.1.: Invitation to import the license

There are different types of license files:

Evaluation License (also called trial or demo license). This license enables you to work with Delta2D in the demo mode, usually for a limited time period. Demo mode means, that you can perform any function of Delta2D, but without being able to save the results. The evaluation license is usually sent to you via e-mail after having downloaded the evaluation version of Delta2D or when contacting our license registration team after having installed a evaluation version from CD-ROM. This kind of licenses usually works without an additional registration step.

Full License This is the license for the save-enabled use of Delta2D and will either be legitimized via an internet connection (so-called 'web-checked' registration) or is bound to your computer. While the web-checked registration is done automatically (in some rare network environments firewalls refuse the connection), the computer bound version of a Full License requires a two-step registration process:

1. Import the license file provided by us. This license file will enable Delta2D to produce an unique machine key for your computer. You will be prompted to send this machine key via e-mail to our registration team (register@decodon.com).
2. You will receive your personal registration key whithin one working day usually. Meanwhile, the initial license will enable you to work with Delta2D in the demo mode.

Click on the Import button to import your license files. If the license you import does not demand for an registration, Delta2D will right away start in the respective mode (evaluation or full).

If an registration of your license is necessary, the dialog Registration will open (see figure 2.2 on page 5).

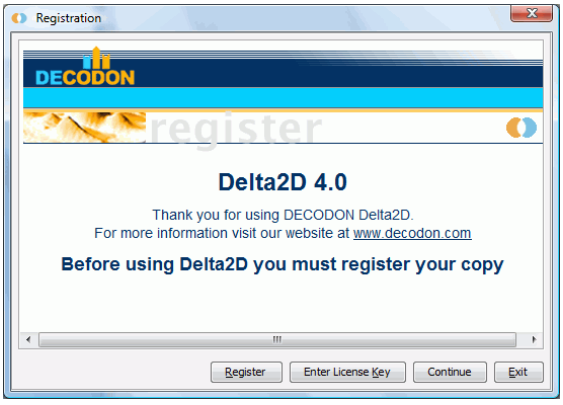


Figure 2.2.: Initial license file is imported

Click on Register. Delta2D will provide the details for your registration request (fig. 2.3 on page 5) and asks you to send these details to our registration team.

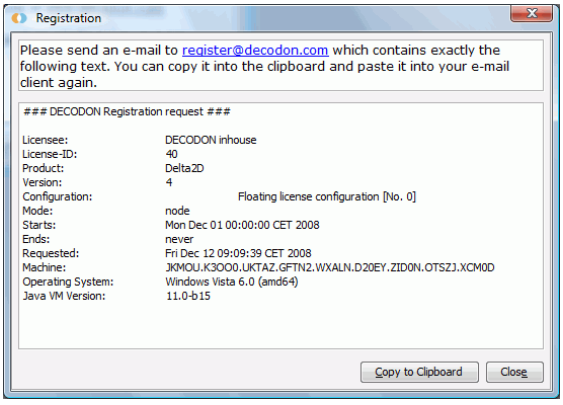


Figure 2.3.: Send your registration request

Click on the button Copy to clipboard to copy your registration details to the clipboard, then click on the text passage with our e-mail address (register@decodon.com) in the top of this dialog. Your mail client will prepare a new mail addressed to our registration team. Simply paste the content of your clipboard into the body of the new mail and send it off.

You will receive the license key within one working day usually. Having received your registration key, simply click the button Enter registration key provided in the dialog figure 2.2 on page 5. Copy your license key from the e-mail and paste it into the appropriate field (fig. 2.4 on page 6).

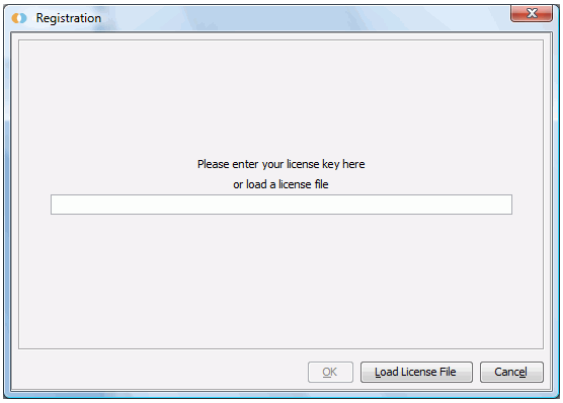


Figure 2.4.: Enter your registration key or load a full license file

2.5. Adjusting Memory Settings

Delta2D can be adapted to the amount of memory available in your computer. Since all computers are equipped differently, Delta2D's main memory usage is set to an average value of 512 MB. If your computer has less or more memory, the setting for Delta2D needs to be adapted, otherwise Delta2D can not perform at its optimum.

To adjust the memory settings of Delta2D according to the real specifications of your computer, open the Options dialog by clicking on the button in the main toolbar. Switch to the tab named Memory and click on the button named Recommend. Now close the Options dialog with OK and restart Delta2D.

Find more information in section 10.3 on page 148.

2.6. Checking for Software Updates

Delta2D can notify you automatically if software updates are available. Based on your settings (see 10.2 on page 147, Delta2D can check automatically for available updates on every start of Delta2D or only once a week. Automatic update checking can be disabled as well, e.g. on computers not being connected to the internet all the time. In this case you can easily check for updates manually, e.g. when checking your emails. Choose Help ▷ Check for Software

Updates ... to perform the check. Open Options, General, Updates to let Delta2D automatically check for updates.

When a software update is available, a notification window will open and inform you about the new version. Click on the link to download and save the update file on your computer.

2.7. Updating an Existing Installation of Delta2D

Basically, there are two ways available to update an installed version of Delta2D: an *update file* and a *complete setup file*.

The update file contains only the changed data and is thus small in size. It can only be used if a previous but not too old version of Delta2D is already installed and needs to be installed into the same directory. Usually this will be the file downloaded via the update notification of Delta2D.

The complete setup file can install Delta2D over an existing installation as well as into a completely new folder. Since it contains all necessary data for a complete installation, it is much larger than the *update file*. If you install into a new location you will need to import the license again.

The installation process for both alternatives is structured as the first time installation. Just make sure that Delta2D does not run, double click on the setup file and follow the instructions.

3. Workflow of Delta2D 4.0

In this section we want to describe how the standard analysis procedure is supported in Delta2D by the Workflow component. The Workflow might be of interest for those who are not yet familiar with Delta2D 4.0. Please note that every action can be accessed outside the Workflow as well and you can still diverge from the standard analysis procedure.

3.1. Setup Project

Create or Open a Project

Whenever you start Delta2D, it will automatically open the project that you have used when you have closed Delta2D the last time. Only when having started Delta2D for the first time or if you have closed your project before leaving Delta2D last time a dialog will open and invites you to open one of the projects listed in this dialog. Here you can also create and remove projects.

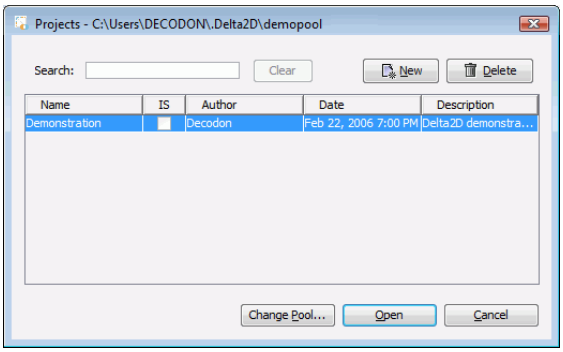


Figure 3.1.: The Open Project dialog

If you cancel this dialog or if you close a project the Workflow window will offer to open an existing or create a new project. You can also just click the or or use Project ▷ Open... to open the dialog

To create a new project, press the New... in the toolbar or the button New... in the Open Project dialog. You will be prompted for a project name, author, and comments. To remove a project, select it in the list and press the Delete button.

Note: Please note that if you delete a project, no associated gel image, match map and quantitation information is removed from the pool. Gel images (including all associated information) can be deleted manually in the Gel Image Manager.

Add Groups and Gel Images to the Groups

Each new project includes two groups. Using the Light Table 5.3 on page 31, you can easily add groups and rename them, e.g. to create one group for every biological sample. See section for how to work with groups.

3.2. Setup Gel Image Warping

Having organized the project in accordance with your experimental setup you now can define the Warp Graph, which is the set of pairwise warp relations that are necessary to further analyze the project.

Use the Warping Setup and employ the Warp Strategy Manager to apply one of the warping strategies as they are described in section 5.4 on page 35

3.3. Create Direct Warpings

All the individual warp transformations need to be calculated now. You can use the Job Manager (section5.10 on page 88 to run automatic warp jobs, but carefully review the results and approve all the automatically generated match vectors (for details about warping see 5.5 on page 61).

The Workflow provides a list of the directly linked image pairs and their warp status so that you can easily focus on those pairs where your interaction is needed.

3.4. Create the Consensus Spot Pattern

For creating a consensus spot pattern you have to do two steps: first fuse the images and then create an appropriate spot pattern on the fused image.

Fuse Images

Having warped the images you can now fuse them to get a Proteom Map that includes all the spots of the entire experiment on it (see 7 on page 111).

Detect and Edit Spots on Fused Image

Open the fused image in the Dual View and detect the spots on it. Improve the spot pattern by canceling artefacts or very weak spots and edit spots to add, split, join spots (see section 5.5 on page 71).

Transfer Spots

Transfer Spot Shapes to Other Gel Images

Especially in combination with Image Fusion this feature is quite useful: it lets you transfer spot boundaries from one gel to other gel images or even complete groups of other gel images. As an example of appliance it is a very common technique to detect spots on a union or max intensity fusion gel of all images in the project and to transfer them to all other gel images in this project. The spots are transferred in accordance with existing warpings between the images. Spot shapes can be re-modelled on the target images and the spots will be quantified on each image. As a result you get the same spot pattern on every image and a unique matching across the images, resulting in 100% complete expression profiles without missing values.

To transfer spots to other gel images, click the Transfer Spots button or *right* click on a gel image in the Project Explorer the Light Table or the Warping Setup and choose Transfer Spots... from the context menu to open the spot transfer dialog (see figure 3.2 on page 11). You can also select multiple images and then open the spot transfer dialog: the selected images will be selected as target images for the spot transfer.

> Note: Read more about the benefits of 100% Spot Matching at www.decodon.com/Solutions/Delta2D/100_Percent_Spot_Matching.html.

Spot Matching

Spot matching is integrated into the quantitation process and happens mostly unnoticed by the user. If the spots have been transferred from another image the spots are automatically matched with their *parent spots* and the respective spots on the other images.

For individual spot detections on the different images Delta2D checks if two spots overlap sufficiently, with respect to the warping. It may happen that a spot does not have a corresponding spot, which is problematic for the statistical analysis. For this reason we highly recommend the approach to achieve Complete Expression Profiles as described in 3 on page 8.

> Note: Delta2D's spot transfer and spot matching rely on well-aligned gel images, so you have to make sure that your Warping Setup is complete and that all Direct Warps have been reviewed before. The Transfer Spots dialog shows the warp status for the links between the image where the spots have been detected and the different target images.

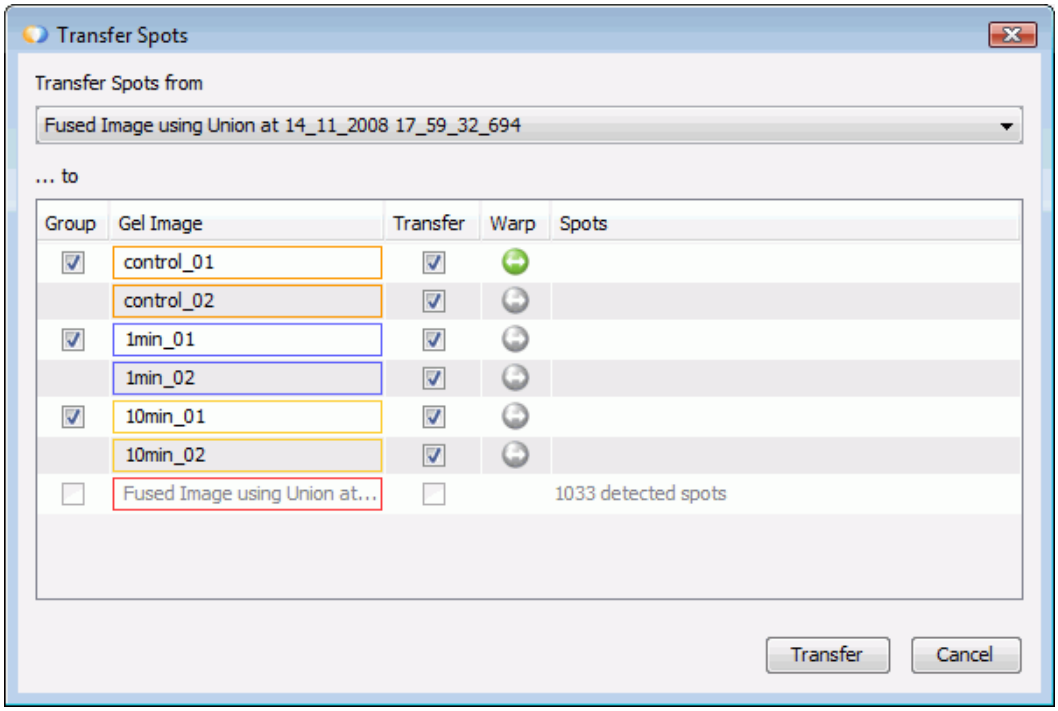


Figure 3.2.: The Spot Transfer Dialog

3.5. Analyze Expression Profiles

see 5.11 on page 89

3.6. Present Results

Generating Reports

Delta2D now offers interactive reports on the current project. They make it easy to present data on relevant spots, experimental setup, and quantitative data. The reports are based on HTML so you can put them on the web easily. Just as easy you can process all or part of a report in your favorite word processor or presentation program by just copying excerpts into it.

The reports can be accessed via the Main Menu or the Reports menu. Each report is opened in your web browser. If you want to have a closer look on a gel image or a spot, just click on it and it will be opened and focused in Delta2D.

You can save the reports in HTML format that is ready to be published on the web. For doing so, always choose the Save button on the top of the reports since the browser's Save as... function might not save every project detail.

Project Summary

The project report shows a summary of your analysis project. It includes an overview of gel images and warpings as well as general data about the gel images, groups, samples, and images.

Click in the Main Menu or choose Reports ▷ Project Summary.

The dual channel images included in the report give a good indication of the quality of the direct warpings in the project. You can open a dual channel image in Delta2D by clicking on it.

Just like all reports you can click the save button to save it in a form that is ready to be published on the web.

Spot Album

The spot album shows thumbnails of marked spots and the region surrounding them. You can show spots in comparison using dual channel images . The album can be configured using the form in the upper part of the report: You can define that the report includes spots being marked on a certain gel image, choose a reference image for false color images, and you can change the width, scale and the zoom factor of the gel section that should be displayed. Finally, you have the option to show the image tiles with or without the spot boundaries.

Click in the Main Menu or choose Reports ▷ Spot Album.

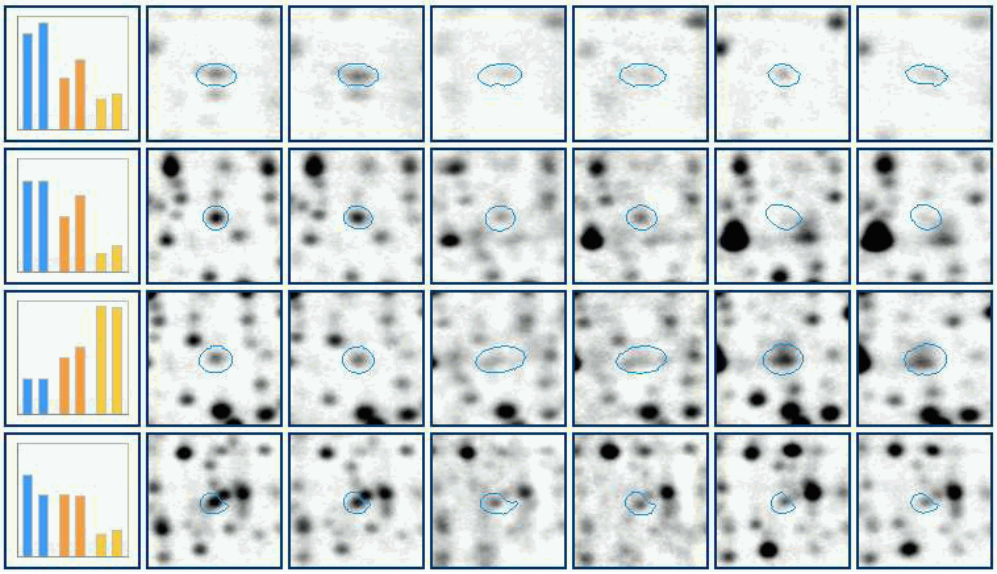


Figure 3.3.: The spot album report

Next to each spot row you see the expression profile as a chart. Clicking on the expression profile takes you to a detail page that shows additional quantitative data. Click on any spot in the row to select and show it in the dual view.

Spot Quantities

The spot quantities report shows expression profiles numerically, together with group-wise ratios and t-Test values. You select spots for the report by marking them on a gel image. This report is well-suited for documenting a set of relevant spots, and for further statistical analysis.

Click in the Main Menu or choose Reports ▷ Spot Quantities.

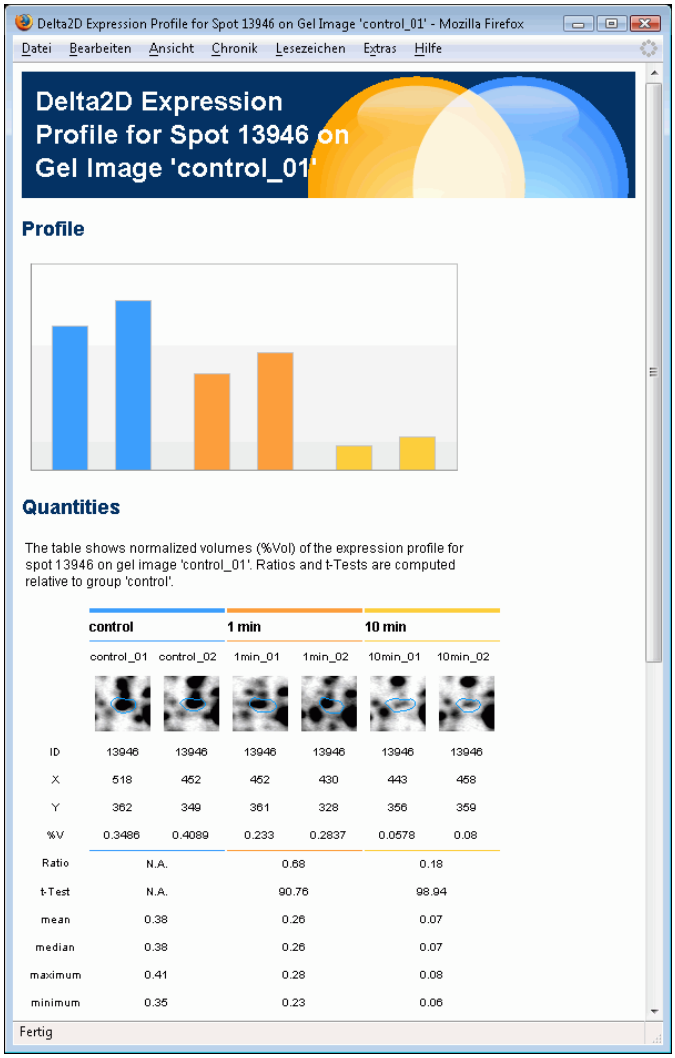


Figure 3.4.: The spot quantities report

Modifying, Saving, and Printing Reports

All reports are produced in the form of HTML pages that are generated dynamically by Delta2D. This means you can easily integrate them into your current project documentation. Select a part of the page and copy it into a Microsoft Word document, or into PowerPoint. You can save the whole report using the Save button in the top right of the report. Delta2D will then prompt you for a file to which the report should be saved. The report will be saved without the configuration form. The sub-pages (e.g. expression profile details from the spot album) will also be saved and linked properly. The result is a set of HTML files and images that can be put directly on the web.

If you want to make changes to the whole report document it is recommended that you open the saved HTML file in a word processor. Usually, you can print the report directly from your

web browser. For more advanced printing needs (e.g. splitting wide pages) we also recommend using a word processing program.

3.7. Exporting Results to Other Applications

All the data you see in the Quantitation Table can be exported for further use in external programs. In the table window, use File ▷ Save... This will save all rows that are visible in the Quantitation Table, so you can hide rows that you do not want to export.

The data is saved in a common exchange format called "comma separated values" (CSV) that can be imported easily into a spreadsheet or other data analysis programs. For easier reference, the column titles are given in the first line of the file. Saving data in CSV format will take hidden columns and sorting into account, so you can use the Quantitation Table's sophisticated sorting and filtering to select the rows and columns that should appear in the saved file.

The import procedure depends on the program you use. Generally, you open the data file as a text file, specifying that the data is separated by semicolons.

Label data, label formats and spot data are saved in XML file formats to allow for easy processing using external applications. Detailed specifications of these formats are available upon request.

Instant MS Excel™ Reports

Use File ▷ Generate Report In Excel... to produce an Excel™ worksheet that contains the currently visible data in the table, plus an extensive set of diagrams and statistics. You need to have Excel™installed to use this feature.

> Note: Since the Excel™ report is meant to compare the spot data of different gel images to each other, and the saving and exporting of table data always refers to what you see, this feature is only available from the views in the table showing data of multiple gel images.

Additionally, you can export just the contents of a Quantitation Table into Excel™, using Delta2D to sort and filter data before the worksheet is created. Use File ▷ Export into Excel™ to retain exactly the data displayed on the activated table in exactly the same alignment of columns and rows in a new Excel™ sheet.

This feature is tested with MS Excel™ Versions 2000 (9), XP (10) and 2003 (11)

Instant Export to MS PowerPoint™

From within the Dual View window, you can create a PowerPoint™ slide that includes everything you see in the gel image view: images, spots, and labels. Open a snapshot window using Edit ▷ Snapshot. In the snapshot window, use File ▷ Export to PowerPoint to produce a PowerPoint™ slide that contains all objects which are currently visible in the gel image view.

These objects are fully editable inside PowerPoint™. You need to have PowerPoint™ 2000, XP, or 2003 installed to use this feature.

4. Main Menu and Toolbars

The Main Menu and the Main Toolbars include actions that are of general interest. Some of the items are always available, while others are context sensitive, i.e. they are active if appropriate objects are selected, otherwise these items are deactivated.

4.1. The Main Menu

The Main Menu includes the following entries:

Project

Open Project...	Open an existing project.
Save Project...	Save your changes.
Save Project as...	Save your project with a different name.
Close Project	Close your project.
Add Gel Images...	Opens the dialog to add images from the image pool or the file system.
Add Group	Adds a new group to your project.
Project Properties...	Opens a dialog to maintain some project properties.
Exit	Close Delta2D.

Edit

Cut	Cut selected object(s).
Copy	Copy selected object(s).
Paste	Copy selected object(s).
Delete	Delete selected object(s).

Gels

Set Warp Strategy...	Open the Warping Strategy dialog.
Gel Image Visibility...	Open the Gel Image and Table Column Visibility dialog.
Gel Image Attributes...	Open the Gel Image Attributes dialog.
Detection Parameters...	Open the Spot Detection and Quantitation Options dialog.
Fuse Images...	Open the Image Fusion dialog.
Transfer Spots...	Open the Transfer Spots dialog.

Pools

Change Pool...	Opens a file chooser to select a different location in the file system.
Import Gel Images...	Opens a file chooser to import new images from the file system.
Manage Gel Images...	Opens a dialog with a list of all images in the pool.
Manage Projects...	Opens the Projects dialog where you can open, rename, or remove projects in the current pool.
Manage Calibrations...	Opens a list with the available image calibration methods.

Reports

Show Report Index	Opens a list of available reports in your web browser.
Project Summary	Opens the Project Summary report in your web browser.
Spot Album	Opens the Spot Album report in your web browser.
Spot Quantities	Opens the Spot Quantities report in your web browser.

Window

Workflow	Opens the Workflow window.
Project Explorer	Opens the Project Explorer window.
Light Table	Opens the Light Table window.
Warping Setup	Opens the Warping Setup window.
Dual View	Opens the Dual View window.
Quantitation Table	Opens the Quantitation Table window.
Gel Image Regions	Opens the Gel Image Regions window.
Expression Profiles	Opens the Expression Profiles window.
Color Coding	Opens the Color Coding window.
Job Manager	Opens the Job Manager window.
Analysis	Opens the Analysis / TMeV window.
Project Matrix	Opens the Project Matrix window.
Full Screen	Enlarges the Delta2D window to screen size.
Close Window	Closes the active window.
Maximize Window	Maximizes the active window to the size of the Delta2D window.
Undock Window	Releases the active window from the Delta2D.
Close All Documents	Closes all windows in the main view.
Close Other Documents	Closes all windows in the main view but keeps the active window open.
Documents...	Opens a list of the current open windows in the main view.
Reset Windows	Arranges the windows as at startup of the current session.

Tools

	Memory Monitor...	Opens the Memory Monitor window.
	Log File	Opens a log file with important system messages.
	Toolbars ▷	Manage the visibility of the different main toolbars.
	Plugins	Opens the Plugins window.
	Options...	Opens the Plugins dialog.

Help

	Help...	Opens the online help.
	DECODON Homepage	Opens the DECODON website in your browser.
	Delta2D Homepage	Opens the Delta2D website in your browser.
	MeV Statistical Analysis	Opens a website with additional information about statistical analysis.
	Check for Software Updates...	Opens a dialog with information about existing updates (demands for internet connection).
	About...	Provides some general information about Delta2D.

4.2. The Main Toolbar

Configuration of the Main Toolbars is possible, just choose Tools ▷ Toolbars ▷ and either control the visibility of complete toolbars or choose Customize... to redesign the Main Toolbars.

The Main Toolbars include the following entries:

	New Project...	Create a new project.
	Open Project...	Open an existing project.
	Save	Save your changes.
	Cut	In Project Explorer, Light Table and Warping Setup you can cut objects to paste them anywhere else if applicable.
	Copy	In Project Explorer, Light Table and Warping Setup you can copy objects to add them anywhere else if applicable.
	Paste	Paste what has been cut or copied.
	Set Warping Strategy...	Opens the Warping Strategy window.
	Change Warp Mode	For selected image pairs you can change the warp mode.
	Toggle Visibility	Open the Gel Image and Table Column Visibility dialog.
	Fuse Images...	Opens the dialog for fusing images.
	Transfer Spots...	Opens the dialog for transferring spots.
	Change Pool...	Opens the dialog for changing the data file location.
	Import Gel Images...	Opens the dialog to add images to the project.
	Manage Gel Images...	Opens the dialog for managing the image pool.
	Project Summary	Opens the summary report in your web browser.
	Spot Album	Opens the album for marked spots in your web browser.
	Spot Quantities	Opens the table for marked spots in your web browser.
	Open in Dual View	If exactly a pair of images is selected, opens it in the Dual View.
	Open Quantitation Table	Opens the kind of table that fits to the currently selected images or groups.
	Options...	Opens the Options dialog for Delta2D.

Table 4.1.: Buttons in the Main Toolbars.

5. The Windows

5.1. Workflow

The Workflow window provides a maximum of support to analyze the project along the workflow as described in section 3 on page 8.

The current status of the analysis is reflected by the Workflow, so that actions are provided or hidden if they are necessary or not yet available.

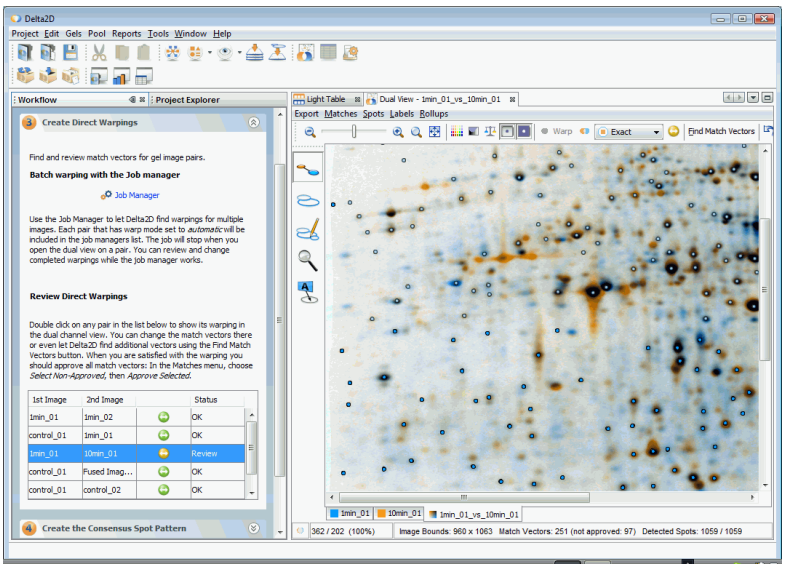


Figure 5.1.: The Workflow

The Workflow should be self-explaining. Please read more about the different steps in section 3 on page 8.

When you open Delta2D the first time or if you have closed your project before you have closed Delta2D in your last session and have canceled the Open Project dialog, the workflow offers a welcome screen where you can first change the location of the data in your filesystem and then open a new or existing project.

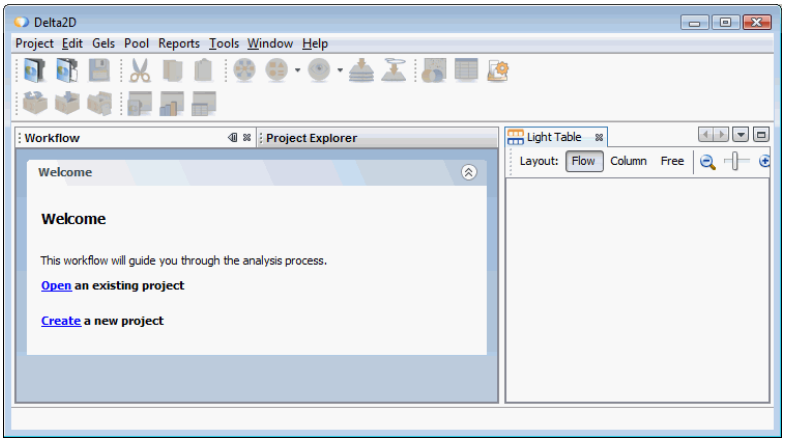


Figure 5.2.: The Welcome Screen

5.2. Project Explorer

The Project Explorer (Figure 5.3 on page 24) provides the most detailed overview on the project. It shows the groups, the images within the groups and if they have spots or labels, and it furthermore offers information on how the images are connected.

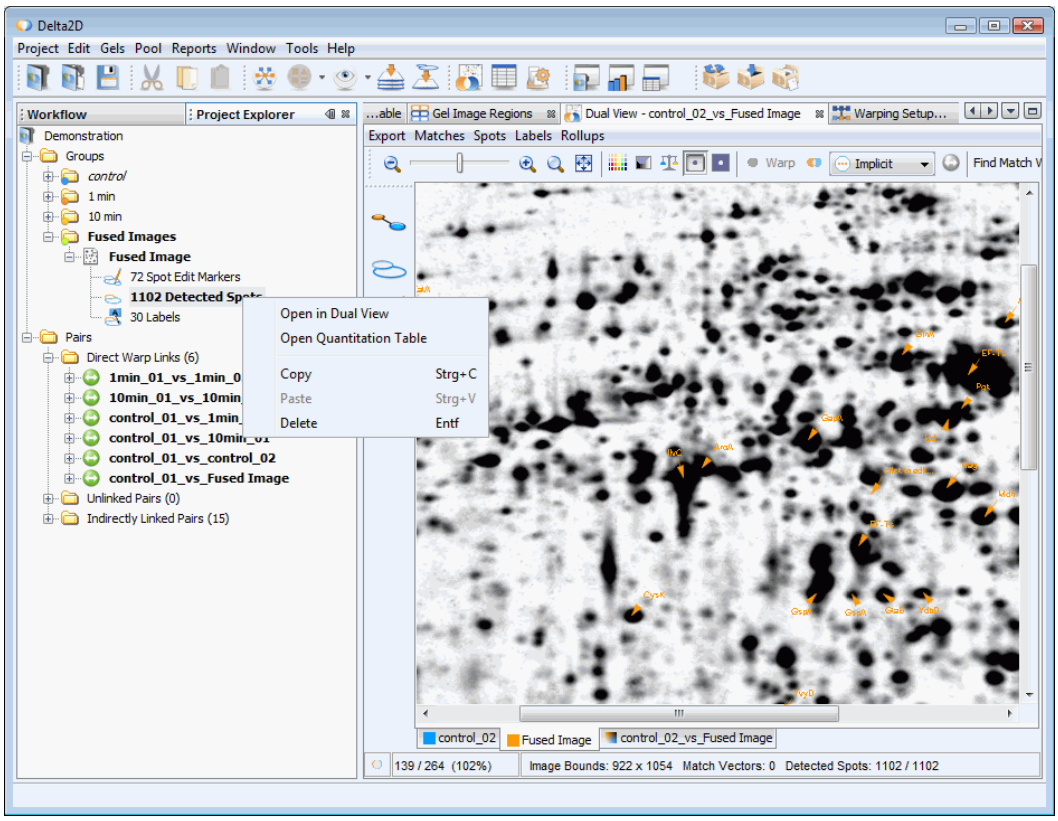


Figure 5.3.: The Project Explorer

Depending on what you select in the Project Explorer, global actions in the Main Toolbar or the Main Menu are available and activated. E.g. you can open a Quantitation Table for a set of selected images, and if you select complete groups only you will get a Statistics Table for these groups. Available actions can also be accessed via the context menu of the selected object.

'Drag and drop' and 'double click' have a certain importance in the Project Explorer.

Gel Groups

The first main subtree Groups includes the Groups and within the groups the respective images and their objects such as Labels or Spots.

To add a group right-click on Groups and choose Add ▷ New Group.

You can use 'drag and drop' for images to move them between groups, and if you drop one

image onto another, the respective Dual View will open. Double click onto an image to see it in the Dual View. If you double click on the spot set the Dual View will open with activated Spot Selection Tool.

'Drag and drop' is available for the detected spots as well: drag such a spot set onto another image or group, or drag it even to the whole project to transfer it to the respective images.

Gel Image Pairs

The Project Explorer includes a subtree *Pairs* that represents the image pairs. The pairs are grouped according to their status into *Direct Warp Links*, *Unlinked Pairs*, and *Indirectly Linked Pairs*. Double clicking on a pair will open the Dual View for this gel pair.

The current warp status is displayed by the following icons, which are also present in the Project Explorer and in the Warping Setup window:

- These two images can be warped according to the defined direct warp mode, since either no match map is needed (if warp mode is identical, e.g. if the images come from the same gel) or the match map contains *only* approved match vectors. You can review or refine the warping, but no intervention is necessary.
- The warp mode demands for a match map but contains no or non-approved vectors. Verify and approve or delete all non-approved match vectors and/or add match vectors.
- Automatic warp mode is chosen but not yet executed. Either open this pair in the Dual View, or open the Job Manager (see section 5.10 on page 88) to start the automatic warping. After the automatic warping is executed the warp mode will be set to *exact* and the warp status will change to the yellow icon.
- These two images are not linked (neither direct nor indirect). Open the Warping Setup and either apply a Warping Strategy or manually add the missing direct links (please refer to section 5.5 on page 61 for details). In the Project Explorer all these pairs appear in the subgroup *Unlinked Pairs*. Please note that with adding just one direct link many unlinked pairs will be linked.
- In contrast to the previous icon description now you have defined too many direct warpings. A so called Warping Cycle occurs, the matching may face conflicts. (For more details on warping cycle please refer to section 5.4 on page 36).
- There are also too many warpings in the project, but this pair has been set to identical warp mode so that the conflict should be resolved somewhere else.
- There is an implicit warp between the two images. You can view the Dual View for this image pair.
- There is an implicit warp between the two images, but one of the pairs in the warp path has the automatic warp mode which has not yet been executed. Search for this pair and execute the automatic warping.

You can move pairs per 'drag and drop' between the *Direct Warp Links* group and the *Unlinked Pairs* group to change the pair's relation. If you double click on a pair the Dual View will open with activated Match Vector Tool.

Right click on the pair to get a menu of other available operations (Table 5.2 on page 27):

Gel Image Pair Status

Each pair is visualised with a status icon that represents the pair's general status (see table 5.2 on page 26). You can get at any time a description of the status of a gel pair by right-clicking on it in the Project Explorer or in the Warping Setup and choosing Status in the upcoming context menu.

In the Status window the warp mode is indicated by the following icons:

Open in Dual View	Opens the Dual View window and lets you edit spots, labels, and match vectors.
Open Quantitation Table	Opens the Quantitation Table.
Delete	Sets the warp mode to *implicit*, but does not delete the match map.
Cut	Copies the pair to the clipboard, so that you can paste it to the group *Unlinked Pairs*.
Paste	If the clipboard includes a pair, you can paste it into this pair group.
Change Warp Mode ▷	Lets you determine the warp mode for this gel image pair (see 5.10 on page 88).
Scatter plot	Shows a scatter plot for the image pair.
Status	Presents the current status of this image pair.

Table 5.1.: The context menu for image pairs

- Identical Warp mode
- Global Warp mode
- Exact Warp mode
- Automatic Warp mode and
- Implicit Warp mode.

For a more detailed description of warp modes please refer to section 5.5 on page 61. In the example project we have used the automatic warp mode and the implicit warp mode only.

The status window also includes the *Spot Matching Quality* bar that indicates the state and quality of the spot matching between the two gel images:

No quantitation data on both gel images

Complete matching: every spot on the one gel image is matched to a spot on the other image of this pair. This partcilarly results from the approach using a fused image and spot transfer. See section 7 on page 111.

Quantitation data is present on at least one gel, but the matching is not up to date, e.g. match vectors have been changed.

The black range of the bar represents the number of spots that are matched on both images. The blue area indicates the amount of unmatched spots on the one image, the orange area the unmatched spots on the other image.

Detected spots are available on the one image only.

Detected spots are available on the other image only.

Different Kinds of Projects

The experimental setup can demand for different kinds of handling the relations between the images. The difference between them is whether an internal standard is used or not.

In Delta2D you distinguish between the different kinds of projects by the project's attribute Use Internal Standard in the Project Properties. Choose Project ▷ Project Properties to open the properties dialog for the current project.

Standard (Single Channel) Experiments

Usually the standard experimental setup is used. For this purpose each gel of an experiment separates exactly one sample which will be stained with the same staining reagent. The gels are scanned in a single path by using only one optical channel (single channel scanning) e.g. white light scanning, fluorescent scanning OR autoradiography. This results in exactly on image per gel.

DIGE Experiments Using Internal Standard

Multichannel techniques like DIGE or other multiplex techniques are based on multichannel scanning of exactly one gel.

For DIGE up to three samples can be separated simultaneously on one gel. They are covalently labeled with three different fluorescent dyes (one stain per sample) prior separation. This is possible because after the separation process the samples can be distinguished by using differential excitation and detection of the fluorescent dyes by using the corresponding multifluorescence scanners (Fuji ...). This results in exactly one image per sample but multiple images per gel (up to three samples per gel). These multiple images positionally correspond to each other. That means no further image warping will be necessary for image analysis.

Because this setup is limited to exactly three samples some enhancements of this technique had been developed. For the analysis of more than three samples the so called In Gel Standard was introduced. The task of the In Gel Standard is to quantitatively link all samples although they are separated on independently prepared gels. The internal standard is an equiconcentrated mixture of all samples involved in the experiment. That means, if you are separating 4 samples A, B, C, D in one experimental setup the Standard S is a mixture of (A+B+C+D). For this experiment at least 2 Gels have to be prepared, if no replicates are wanted. One gel separates S, A and B, the other one S, C and D. All spot quantities are normalized to the Standard resulting in quantities described by the formula

%V(Spot X of sample A) = rel V(Spot X of sample A) / rel V(Spot X of S),

where *rel V* is the absolute Volume of the spot devided by the cumulated absolute Volume of all spots on the same image that belong to the normalization spot set. Since each spot from any gel is normalized to the same internal standard sample it is said that the results are very reliable.

Delta2D and DIGE

Choose Project ▷ Project Properties to open the properties dialog for the current project and mark the Project as DIGE project by setting the tick on Use Internal Standard in the Project

Properties.

Distinct from traditional setups, images from the same gel but different channels do not need to be warped to each other. Delta2D takes account of this and warps these images as identical. For a correct handling of these images, it is necessary to assign them to the corresponding gel, sample and channel. On how to do assignments, please refer to section 6.2 on page 107.

Compared to traditional projects, projects using an internal standard are treated slightly different in quantitative analysis:

- Spots on the standard gel image are used as normalization, which means that matching spots on other gel images refer to these spots as to 100%. Due to this, spots on other gel images that have no matching spots on the standard do not appear in any representation of Expression Profiles.
- Standard gel images do not appear in the All Gel Images tab of the Quantitation Table and are not taken into account for statistical calculations.

In projects using an internal standard, on assigning gel images to a certain gel there will appear an additional radio button on the left side of the gel image's name. This radio button determines the standard image for this gel.

To assign a certain image as the standard image for its gel right click on the gel image in one of the windows (e.g. in the Project Explorer, the Light Table, or the Warping Setup) and click on Set as Standard Image in the upcoming context menu.

> Note: Please note that in DIGE projects (for details on multichannel techniques please refer to section 5.2 on page 29), it may happen that spots do not have quantities if you have decided to detect the spots on individual images: Spots on the standard gel image are used for normalization, i.e. each spot on non-standard images shall be devided by the matching spot on the respective standard image. If spots do not have matching spots on their respective standard their normalized spot volumes can not be calculated. Thus they do not show up in the Expression Profiles window, and in the Quantitation Tablesyou can not see their %V. To avoid this phenomenon we recommend to use the approach for getting 100% complete expression profiles as described in the standard workflow 3 on page 8.

Other Multiplex Experiments without Internal Standard

Non DIGE multiplex techniques are also based on multichannel scanning. Here only one sample is separated per gel but differentially detected by using different kinds of staining or labeling techniques. Typical examples are the detection of protein amount (Coomassie, SyproStains or Flamingo™ for example) and protein synthesis (autoradiography of the same gel - only possible if the proteins were radiolabelled in vivo by using 35S Met for example). Also the complementary detection of Phosphoproteins (Diamond ProQ) or Glycoproteins (Emerald ProQ) from the same gel is possible. This results in several images per gel. Because of the sequentially applied

staining techniques the gels (or scanned gel images) show typical swelling or shrinking effects which can usually be compensated by using the global warp mode. For the analysis no internal standard is used.

Delta2D and other Multiplex Experiments

In Delta2D you can analyze these experiments just like any standard project, each spot on a gel image will be normalized on the entirety of all spots on this gel image. The only difference to standard experiments is that the warp mode between different channels of one gel has to be identical or in case of minor differences caused by shrinking and swelling during the experimental handling as global.

Choose Project ▷ Project Properties to open the properties dialog for the current project and to make sure that the Project is NOT a DIGE project by removing the tick on Use Internal Standard in the Project Properties.

5.3. Light Table

The Light Table helps you to get your project organized. The layout can be either determined automatically by applying the Flow or the Column layout. Of course you can also freely move groups around.

Grouping of replicates helps for the later calculation of the minimal, maximal, average or median expression of protein spots. Further the relative standard deviation and t-test parameter can be derived.

Adding a Group

Use Project ▷ Add Group... to create a new group. Delta2D asks you for a name and a color that will be used to display the group. We suggest to use related colors for groups containing gel images from similar samples. This makes it easier to keep an overview also on large projects. You can also right-click in the Light Table's workspace and choose Add ▷ New Group.... A new group will automatically apear, indicated in the Light Table by a new empty group symbol and in the Project Explorer by a new entry. To change a group's name double-click on the name and edit it. To change the color of a group's boundary right-click on a group's workspace and choose Properties... from the context menu.

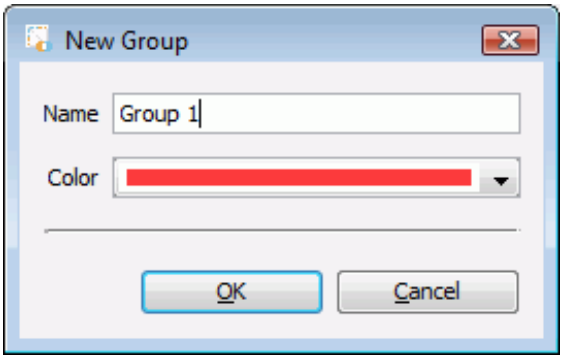


Figure 5.4.: Creating a group

Adding Gel Images to a Group

Right-click the group and choose Add New Gel Images... to add a gel from the pool to the group. You will be offered only those gel images which are not yet part of your current project. Select those that are to be added to the group and press the Add button. If your gel image is not yet in the pool, you can also use the Import button to browse for the desired gel images in your file system. Easily move even multiple selected images between groups or drop them between groups to automatically create a new group. Double click on an image will open it in the Dual View. Move and press Alt when dropping it onto another image to open the image pair in a combined Dual View.

To change a group's or an image's name double click on the respective header and edit it.

Right-click on a group or an image to open its context menu.

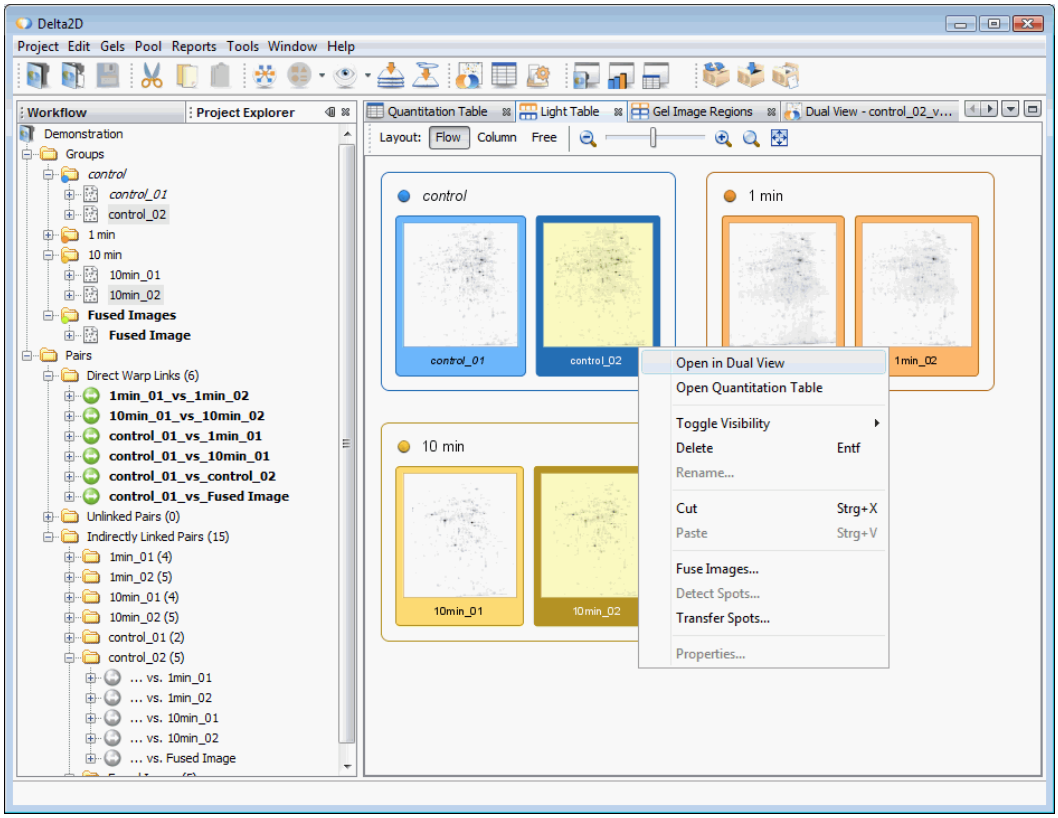


Figure 5.5.: The Light Table

5.4. Warping Setup

Before you can create expression profiles across all the images in your project, you need to define warping relations between the images by composing pairwise transformations. The complete set of pairwise warping relations form the Warp Graph.

This Warp Graph will be used both for producing dual channel images for every possible image pair and for building expression profiles of the spots. Delta2D does not need a direct connection between all gel images in order to be able to warp one onto the other. We only have to make sure that every gel image is included in the Warp Graph. There can be several intermediate warping steps in-between two images. With one of the predefined Warping Strategy you can minimize the number of intermediate steps.

To keep control on the already existing relations and to see where a relation is missing the Warping Setup provides a view on the warp graph.

Right-click on an image or a warp relation to get a context menu with available actions.

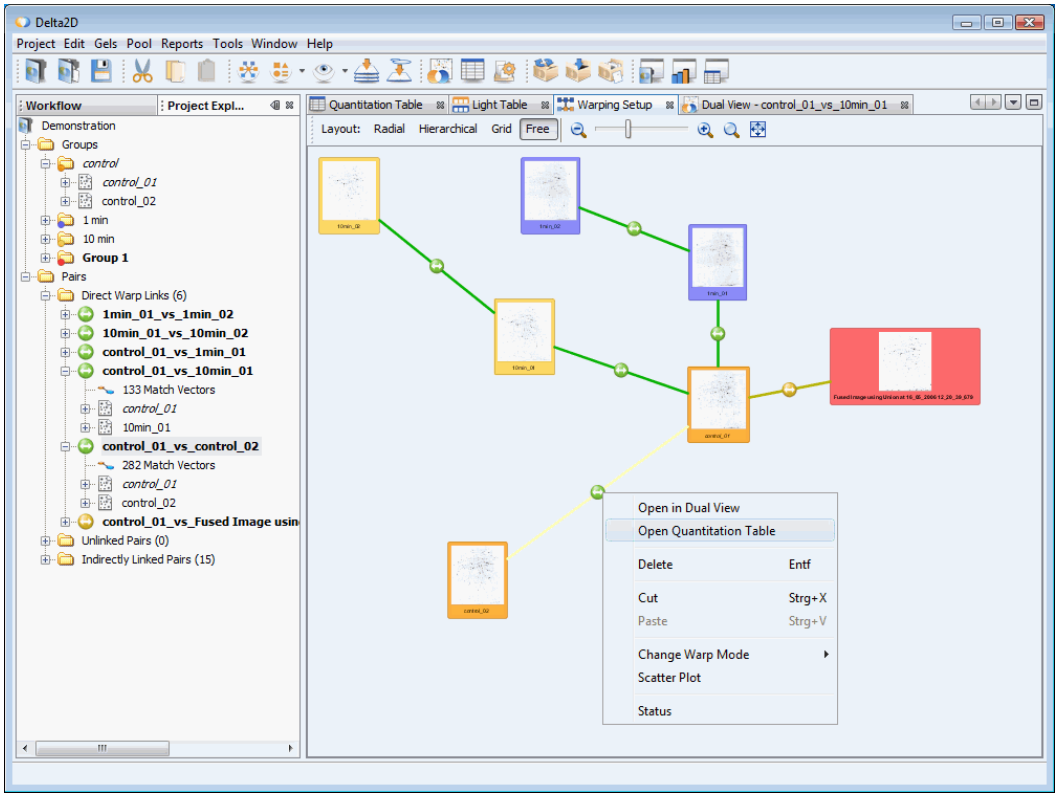


Figure 5.6.: The Warping Setup

Warping Strategies

Of course you can assign the warp modes manually between the respective images in our project. With four images this is rather easy, but when having large projects this can become quite complex.

There is a more convenient way: Choose Gels ▷ Set Warp Strategy... or right-click in the workspace in the Warping Setup and select Warp Strategy... from the context menu to open the Warping Strategy Manager.

This is a useful tool to automate the assignment of *Direct Warp links* to the gel images of a project. It takes care that no gel is left out and no warping cycle is created accidentally.

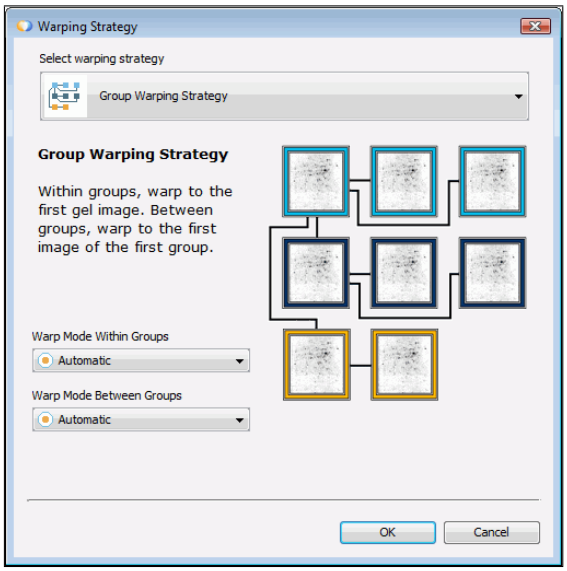


Figure 5.7.: Apply complete warping strategies at once

> Note: Please note, that the Strategy Manager alters assignments done manually before by setting every warp mode according to the chosen strategy. So use it at the very early stage of a new project, and do not touch it any more later on. Of course, warp modes can be changed manually at any time; then you have to take care for the consistency of your warping strategy yourself.

Before assigning a warping strategy please notice the following:

- Since warping is much easier if the images are more similar to each other, we recommend to warp along the images' similarity. For this reason the Group Warping Strategy is suitable for most standard projects, while the In Gel Standard Warping Strategy is available and works best for DIGE projects.

- Warping one image on another always means that one image gets distorted, while the other (the Warping Master) remains undistorted. Since in most projects a control sample is used, it is very likely to use one of its replicates as warping master and later on as basis for the Proteome Map.
- Avoid warping cycles as they can lead to unpredictable results. A warping cycle is a chain of warpings containing possibly contradicting directions: If you have four gel images A, B, C and D, there are warpings between A-B, B-D, A-C and C-D, and you want to set the warping mode A-D to implicit, Delta2D does not know which warping chain has priority: A-B-D or A-C-D.

Note: Please try to keep the warping chains as short as possible to reduce the number of necessary intermediate steps in implicit warpings. If implicit warping between two gel images has to be done over too many steps, small inaccuracies, which are hardly noticeable in single warpings, can sum up to bigger deviations and thus prevent small spots from matching each other.

Two extreme examples: in the All-to-one strategy the maximum of necessary steps to connect any gel with any other is two (A -> Central Image -> X), whereas in the Chained Warping strategy the number of steps for connecting the last gel image with the first one is N-1 for N gels (A -> B -> C -> ... -> N).

The Warping Strategy Manager (see figure 5.7 on page 35) lets you choose between basic warping strategies:

Group This will be the most frequently applied strategy. It assumes that your image groups correspond gel replicates, and that the difference within groups is smaller than between groups. Within groups, images are warped with one warp mode (default: automatic) and the first image of each other group is warped to the first image of the first group with another warp mode (default: exact).

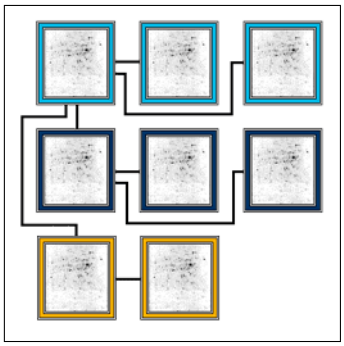


Figure 5.8.: Group Warping Strategy

- **Chain** All images of your project are connected like one long chain in the sequence of their appearance in the project, no matter to which group they belong. This strategy is recommended, if your samples have been taken at successive points of time in your experiment.

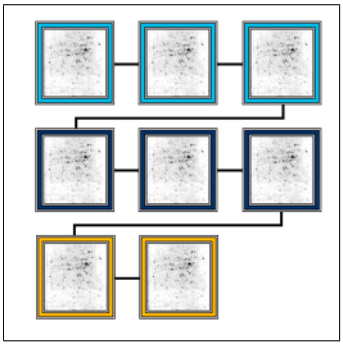


Figure 5.9.: Chain Warping Strategy

- **Chained Group** Combination of the two above strategies, applicable in case your image groups correspond gel replicates, and the groups represent successive points of time in an experiment.

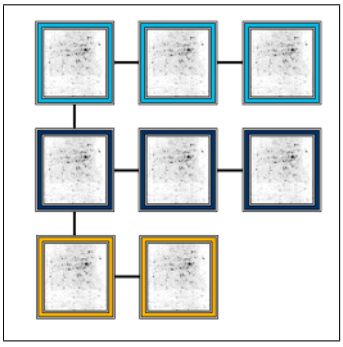


Figure 5.10.: Chained Group Warping Strategy

- **All-to-one** Here one gel image takes the role of a master and all other gel images are connected only to this one.

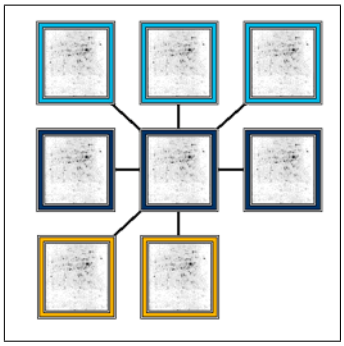


Figure 5.11.: All-to-one Warping Strategy

In Gel Standard Warp each standard image to the first standard image. Other images are warped to the standard image from the same gel, if possible. This is the default Warping Strategy for Projects using an internal standard and hence only available if the current project marked as DIGE Project. (Please refer to section 5.2 on page 29 for more information on DIGE Projects.)

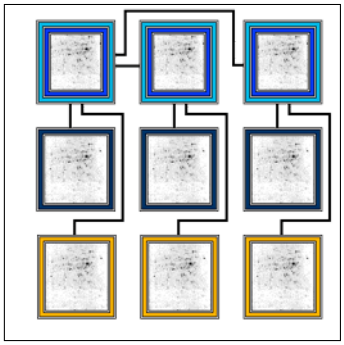


Figure 5.12.: In-Gel Standard Warping Strategy

Instead of applying a warp strategy you can define the relations manually: Just drag an image and drop it on another one. Please note that direct relations will only be created if there is not yet any relation, including indirect relations, between the two images.

5.5. Dual View

The Dual View shows a gel pair and lets you create or refine a warp transform between them. It furthermore enables to detect and review spots, preferably on the fusion image, and to define and modify spot annotations.

The Dual View of Delta2D is available either via the menu (Window ▷ Dual View) or via the Dual View icon in the main toolbar. The icon is activated if exactly two images are selected

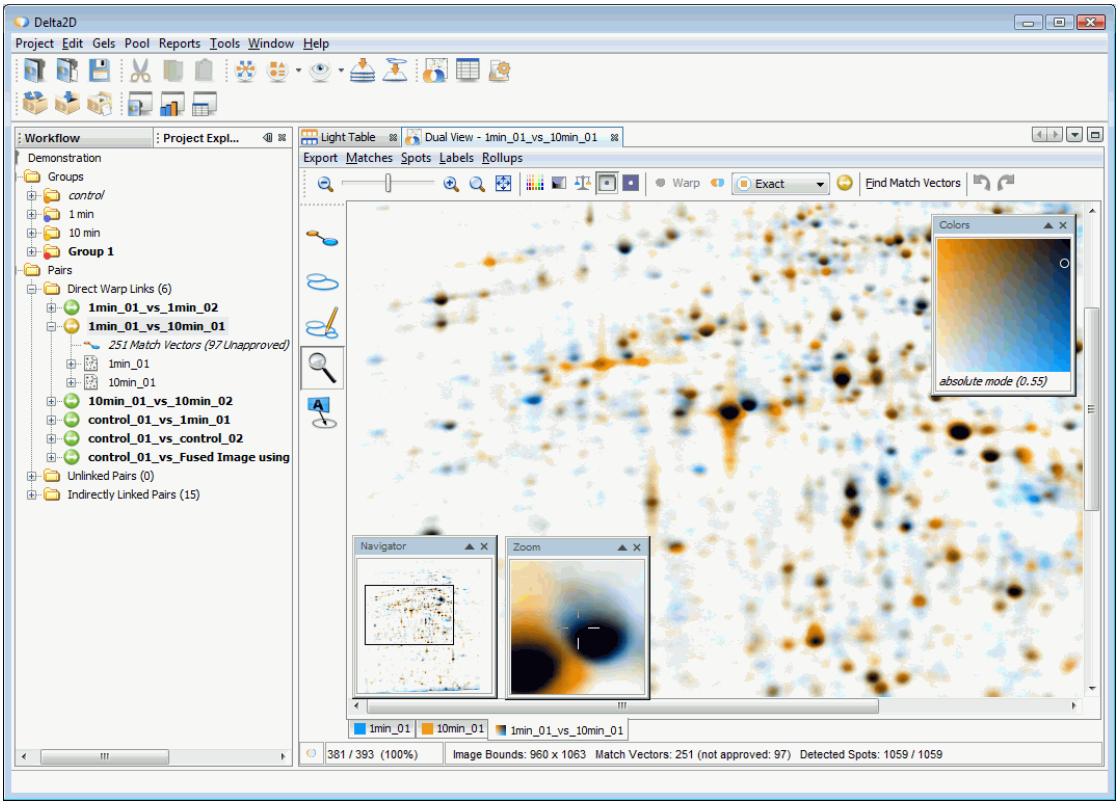


Figure 5.13.: The Dual View

in the Project Explorer or the Light Table. In the Project Explorer you can also just drag one image and drop onto another one to open the Dual View for these two images. Last but not least you can open the Dual View by double clicking on the gel pair's entry in the subgroups for *Pairs* in the Project Explorer.

From the gel images, a dual channel image is automatically generated: one image is colored blue while the other image will be displayed in orange color. Having warped the images 5.5 on page 61 blue means that a spot is only present (or much stronger) in the one gel image, while orange spots are only present (or much stronger) in the other gel image. You can now identify whole sets of spots whose expression levels vary. Shades of black are generated where both images have regions with similar intensity. [1]. You can click on the tabs at the bottom of the Dual View to switch quickly between displaying the single images, or dual channel image. You can use as well arrow left or arrow right to switch between these tabs (see figure 5.14 on page 40.

[1] These are the default settings; the colors may differ if the used color scheme is changed or modified

Note: Delta2D can use any color scheme to produce a dual channel image. Unless you change it, it will be set to the default color scheme: white for background, a shade of blue for master spots, and a shade of orange for sample spots. Regions with overlapping spots are colored black. For changing the color scheme, please refer to section 5.5 on page 57.

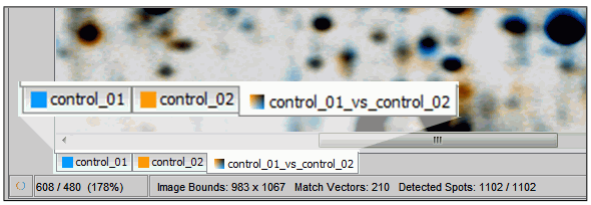


Figure 5.14.: The tabs for controlling image visibility

The Dual View comes with its own menu bar and icon bar. On the left-hand side is a vertical panel, the Tool Panel (Figure 5.15 on page 46).

The Dual View Menu

In the Dual View some actions are available via the menu. It includes the following items:

Export

	Export Sample...	Export the warped image.
	Export Dual Channel...	Export the dual channel image.
	Export To Powerpoint...	Export the current view as a slide to Powerpoint.
	Snapshot...	Make a snapshot of the current view to export it.

Matches

Delete All	Delete the complete match map.
Import...	Import a match map that fits to the current image pair.
Export...	Export the match map.
Invert Match Vectors	Invert the direction of the match vectors.
Delete Selected	Delete the selected match vectors only.
Approve Selected	Approve the selected match vectors as to be OK.
Select Non-Approved	Select the non-approved match vectors for approving or deleting them.
Invert Selection	Exchange selected against the unselected match vectors, and vice versa.

Spots

Detect Spots on [name 1]	Open the quantitation dialog for the image.
Detect Spots on [name 2]	Open the quantitation dialog for the image.
Delete ▷	Delete the spots from one of the images.
Import ▷	Import a spot list that fits to the image.
Export ▷	Export the spot list of the image.
Export Picklists ▷	Export a list with marked and labeled spots for a certain picking device.
Show Table	Open the Quantitation Table for this image pair.
Show Scatterplot	Open the Scatterplot for this image pair.
Show Hidden Spots	Display hidden spots with dotted boundaries.
Show Canceled Spots	Display canceled spots with dotted boundaries.
Background Region ▷	Change the settings for background region for the image.

Labels

Delete ▷	Delete labels from the selected gel image (master, sample, or both).
Import ▷	Add labels from a file to the current set of labels on the master or sample gel. If the label file contains formatting information, you will be asked whether it should replace the present formatting.
Export ▷	Export labels to a file. Formatting information will always be saved together with the label data.
Move ▷	Move all labels from one gel to the other. Label positions will be adapted according to the match map.
Copy ▷	Copy all labels from one gel to the other. Label positions will be adapted according to the match map.
Swap	Swap label sets between master and sample gel. Label positions will be adapted according to the match map.
Label Selected Spots with Spot IDs ▷	Create Labels on selected spots containing their ID.
Label Selected Spots with Numbers	Create Labels on selected spots containing consecutive numbers. If you need a prefix in the numbered labels, define it in Options ▷ Delta2D ▷ Labels.
Label Unlabeled Spots with Spot IDs	All spots without any label obtain a label containing their ID.
Label Unlabeled Spots with Numbers	Create Labels on all unlabeled spots containing consecutive numbers. If you need a prefix in the numbered labels, define it in Options ▷ Delta2D ▷ Labels.
Translate Label Names ▷	Lets you batch change all Labelnames by providing a list with the current names in one column and the replacement names in another.
Formats...	Edit label formats.
Delete scout^2 data ▷	Delete data of a specific scout from all spots.
Fetch scout^2 data ▷	Fetch data with a specific scout only for those labels not containing this set of data.
Refetch scout^2 data ▷	Fetch data with a specific scout for all labels and override this specific data if already present.

Rollups

	Show all	Show all rollups.
	Hide all	Hide all rollups.
	Expand all	Expand all rollups.
	Collapse all	Collapse all rollups.
	Colors	Open the Colors rollup.
	Overlays	Open the Overlays rollup.
	Navigator	Open the Navigator rollup.
	Zoom	Open the Zoom rollup.
	Expression Profiles	Open the Expression Profiles rollup.
	3D Spots	Open the 3D Spots rollup.
	pI/MW Calibration	Open the pI/MW Calibration rollup.

The Dual View Toolbar

Table 5.2 on page 45 explains the meaning of the buttons.

> Note: Toolbars can be torn off and placed anywhere on your screen by clicking on its "handle" at its beginning and dragging it to the desired place. To reattach it to the Dual View window, simply close the small window of the toolbar.

The Dual View Tool Panel

The Dual View tool panel is a vertical panel where you can select one of the five tool buttons. They activate the Match Vector Tool , the Spot Selection Tool , the Spot Editing Tool  , the Zoom Tool  , or the Label Tool  , respectively.

Upon availability, overlays for different objects can be displayed, e.g. for match vectors, for spot boundaries, or for labels. The visibility of these layers is controlled automatically unless you manually enforce their visibility or invisibility (see section 5.5 on page 48).

Detailed descriptions of the tool buttons can be taken from table 5.3 on page 45. The effect of your mouse actions depends on the tool you have activated. For example, with the Label Tool a left-click with your mouse on the images will create a new label. However, if the Zoom Tool is activated, the same mouse click will let you zoom into the images.

[2]For more information about scouts, please refer to section 9.7 on page 136.

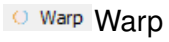
	Zoom out
	Move slider to zoom
	Zoom in
	Zoom 1:1
	Fit the image into the window, such that it can be seen completely inside the window.
	Choose a color scheme.
	Show image histograms.
	Equalize images.
	Show or hide the foreground of images.
	Show or hide the background of images.
	Open a dialog with information about the warp status.
	Warp the sample image.
	Disable warping operations and show images in unwarped status.
	Current warp mode: Select the warp mode for this sample gel image.
	Find Match Vectors: Apply the SmartVectors Technology to receive an automatically generated match map.
	Undo the last action on match vectors.
	Redo the last action on match vectors.

Table 5.2.: Buttons on the toolbar and what they do.

	Match Vector Tool. With this tool you can select, delete or add match vectors that define corresponding gel positions.
	Spot Selection Tool. Select and mark, cancel or hide spots or exclude/include them in the normalization basis. If no spots are available on one of the displayed images, the spot detection dialog will appear.
	Spot Editing Tool. Add, split and fuse spots by defining spot edit markers (details in sec. 5.5 on page 71).
	Zoom Tool. Increase the zoom level by clicking into the images, decrease the zoom level with Ctrl + click or drag a rectangle around the region of your interest.
	Label Tool. Create and edit labels, copy or move them to the other image.

Table 5.3.: Buttons on the tool panel.

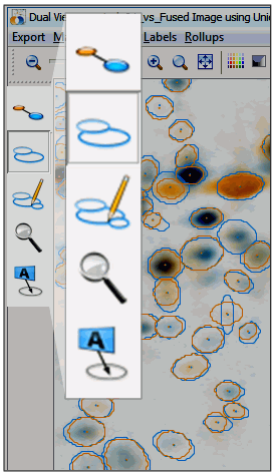


Figure 5.15.: The tool panel.

The Status Bar

The status bar, located at the bottom of the Dual View window, shows some useful information about your work:

Position and zoom level The left field of the status bar shows the coordinates of the mouse cursor in relation to the gel image. This makes it easy to evaluate the mouse position in a higher zoom level, to locate a certain spot quickly, as well as to access the size of a spot. In brackets near the coordinates, the zoom level is indicated.

Image Size, Match Vector count, Spots count The middle field indicates the outer bounds of both images. This means the horizontal size of the broader of both images and the vertical size of the higher one. This value refers to the original images, no matter how the prescale is used. Besides, you get a quick information on how many match vectors and how many spot boundaries are present in this pair of images.

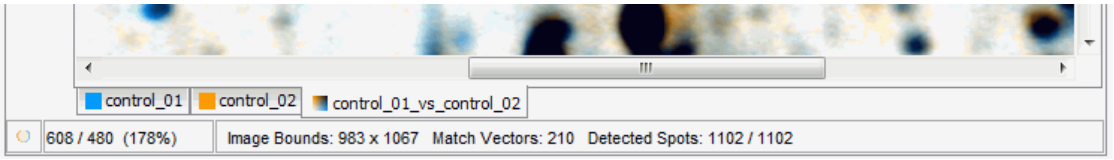


Figure 5.16.: The status bar of the Dual View

Navigating in Images

There are two possibilities to adjust the view: The zoom tool bar for quick access to predefined views and the zoom tool for precise determination of the current view.

With the buttons and or the slider you can zoom out (resp. in), whereas the button resets the view to the natural size of the image. With you fit the image size to the actual window size.

The zoom tool allows you to adjust more precisely the region you want to magnify. It is activated by using the tool panel in the top left area of the main window (Figure 5.15 on page 46).

Press the zoom tool button to activate zoom mode. The mouse cursor will change to a magnifying glass. Click anywhere inside the image to enlarge it. Click and drag to specify a region that should be zoomed in. Clicking and dragging with the *right* mouse button will change the mouse cursor temporarily to act as a magnifying glass as long as the right mouse button is pressed. If you need a magnifying glass also while working with the label tool or others, it might be more convenient to use the Zoom Rollup (see section 5.5 on page 48).

Configuring the Display Using Rollups

Rollups are small windows that are floating above the main window. They can be collapsed to use only a minimum of screen space. You can use the Rollups menu to control the appearance of rollups – either as a group or individually (see table 5.5 on page 44).

For better interpretation, e.g. you may wish to hide match vectors using the overlay rollup as described in section 5.5 on page 48 (use Rollups ▷ Overlays to open the overlays rollup).

The Colors Rollup

The Colors rollup contains the current color scheme. You see a colored square that shows how the overlay of grey values in the two images results in colors.

Open the Colors rollup using Rollups ▷ Colors.

Move the mouse pointer over the dual channel image and watch the Colors Rollup. A small circle inside the Color Rollup points to the color that fits to the current combination of grey values. A numerical display of the intensity ratio (sample / master) is shown below the color square. Even though these values are computed only for a small pixel neighbourhood around the mouse pointer, they can serve as useful indicators for the expression ratio of a spot.

For printing or presentation purposes, Delta2D's color scheme can be changed to an arbitrary combination of colors for master, sample, background and common pixels.

Click inside the color square to change the color scheme. A menu appears, giving you the opportunity to change the used color scheme (see sec. 5.5 on page 57) and to switch directly between the absolute mode (which is the standard mode) and the ratio mode (see sec. 5.5 on page 59).

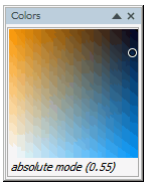


Figure 5.17.: The Colors Rollup

The Overlays Rollup

The visibility of the overlays containing the different objects that are overlaid on top of the gel images (match vectors, spot boundaries, and labels) is controlled by the activated tool by default, but you can control them manually using the Overlays Rollup.

Open the Overlays rollup using Rollups ▷ Overlays.

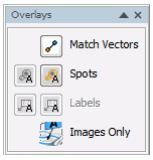


Figure 5.18.: The Overlays Rollup

Match vectors are assigned to the gel image pair and they can not be split into parts. However, you can control the visibility of the spots and labels on both images separately using the left or right button, respectively.

Clicking one of the small control buttons will toggle the visibility of the respective objects between three modes: visible, non-visible, and auto-visibility (controlled by the tool activation).

The button Images Only is useful to hide all overlaid objects temporarily to get a view on the pure images, e.g. during spot editing. The overlays will reappear according to the overlay rollup settings when you release the button.

The Navigator Rollup

The Navigator rollup shows an overview of the whole gel images. The currently visible part of the images is represented by a rectangle. Drag this rectangle to move the visible part of the image.

Open the Navigator rollup using Rollups ▷ Navigator.

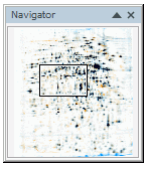


Figure 5.19.: The Navigator Rollup

The Zoom Rollup

The Zoom rollup displays a fourfold zoom of the gel image around the current mouse position.

Open the Zoom rollup using Rollups ▷ Zoom.

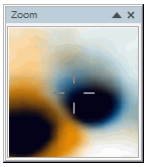


Figure 5.20.: The Zoom Rollup

The Expression Profile Rollup

The Expression Profile rollup shows the barchart of the spot your mouse is pointing to. It is based on the normalized volume (%V). The columns are colored according to the replicate group. The barchart's appearance is synchronized with the settings in the Expression Profiles window (see section 5.8 on page 83). In the example below (figure 5.21 on page 49), the black lines indicate the mean plus / minus the relative standard deviation.

Open the Expression Profile rollup using Rollups ▷ Expression Profile.

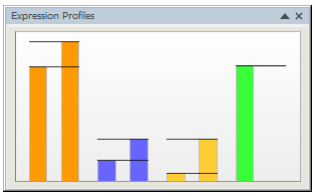


Figure 5.21.: The Expression Profile Rollup.

The 3D Spots Rollup

The 3D spots rollup visualizes a selected spot in a three-dimensional representation. It is highly configurable:

- show single spots, whereas the shown region dynamically adapts to the size of the chosen spot (default setting), or show bigger regions of a fixed size
- show the spots opaque or as wire frame model (useful to make interlocking spots visible when viewing both gels in the Dual View)
- change the color of spots and background
- adapt the height scale to your needs (e.g. for very flat or very tall spots)

Choose Options... from the Project menu and switch to the 3D Spots tab in the section Delta2D to change the settings. Please refer to section 10.1 on page 141 for more details.

Open the 3D Spots rollup using Rollups ▷ 3D Spots.

After opening the rollup, please switch to the Spots Tool on the Tool Panel and either left click in a spot boundary to select the spot and to see the spot focused in the rollup, or anywhere between spots to see this area in the rollup. Control the view in the rollup with the mouse:

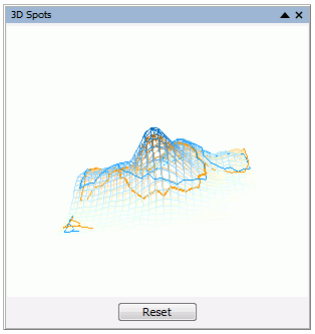


Figure 5.22.: The 3D spots rollup

- Click with the left mouse button to freely rotate the spot.
- Press the Alt key or the middle mouse button and drag the 3D spot to zoom in or out.
- Click the right mouse button and move the scene inside the rollup.
- When viewing an area with multiple spots just click on a spot inside the rollup and watch the same spot being selected in the Dual View.

The pI/MW Calibration Rollup

The rollup shows the estimated pI and MW for the current mouse position. Delta2D can estimate the isoelectric point and molecular weight of a spot on the basis of at least three known data points on the gel. The known pI/MW values are inter- or extrapolated to calculate any mouse position's value. More spots with known pI/MW values make the model more accurate.

Open the pI/MW Calibration rollup using Rollups ▷ pI/MW Calibration.

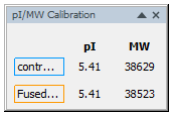


Figure 5.23.: The pI/MW Calibration rollup

The precondition to configure pI/MW estimation is the availability of applicable reference data for some labels in a scout (for more about scouts please refer to section 9.7 on page 136). This can be the manually edited physicochemical properties scout, the table data scout with arbitrary defineable data fields, or any web scout providing adequate data.

Open Tools ▷ Options in the menu or click on the Options button in the main tool bar. Click on *Delta2D* and then switch to the Labels tab. The Source field lets you choose from all scouts containing adequate data. Select the scout you want to use for the estimation and specify in the fields below which data fields are to be interpreted as pI and MW values.

Example You know the pI/MW values for 4 points on your gel and want to be able to see them for other interesting spots or regions. Here is how to proceed:

- If not already existing, place a label on every point you know the pI and MW of. The label can but needs not be connected to a spot on the gel. The names of the labels are of no importance for this purpose; they can be e.g. simple consecutive numbers.
- *Right* click on the first label and choose from the context menu Edit scout data ▷ Physicochemical properties, a new dialog shows.
- Insert the values for pI and MW in the respective data fields.
- Close this dialog with OK.
- Repeat the last three steps for every label you know the pI and MW for.
- Now open the Options dialog (Project ▷ Options) and switch to the Labeling tab in the section Delta2D.
- On the bottom of the left side you see three drop-down boxes. Click on the first one and make sure Physiochemical properties is selected.
- Click on the second drop-down box and make sure Isoelectric point is selected.
- Click on the third box and make sure Molecular Weight is selected.
- Close this dialog with OK

Now you can open the pI/MW Calibration rollup and just read the pI/MW for any region on this gel you are pointing to.

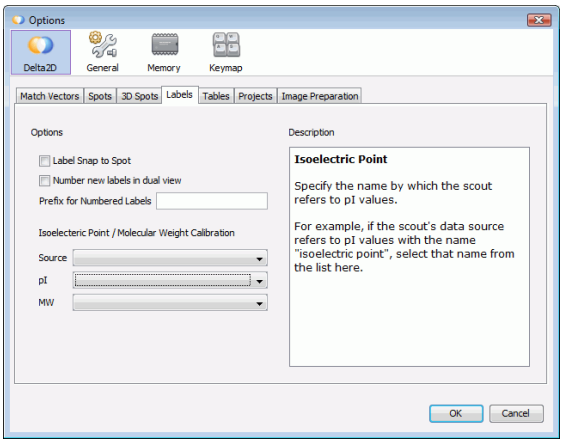


Figure 5.24.: Setting the data source for pI/MW-Calibration

Controlling Background Display

Depending on the background level of your images, it may now be advisable to enhance the images. In Delta2D, background is computed for the whole image, and background levels may vary from one region to another. For each gel image, Delta2D generates an adaptive background image that can be subtracted from the original to give a “background free image”.

Try it: Press the Show/Hide Background button to switch background visibility on and off. You can use the tabs at the bottom of the Dual View window to switch between the gel images and the dual channel image. Often the dual channel image without background is clearer than the complete dual channel image (see figure 5.25 on page 52).

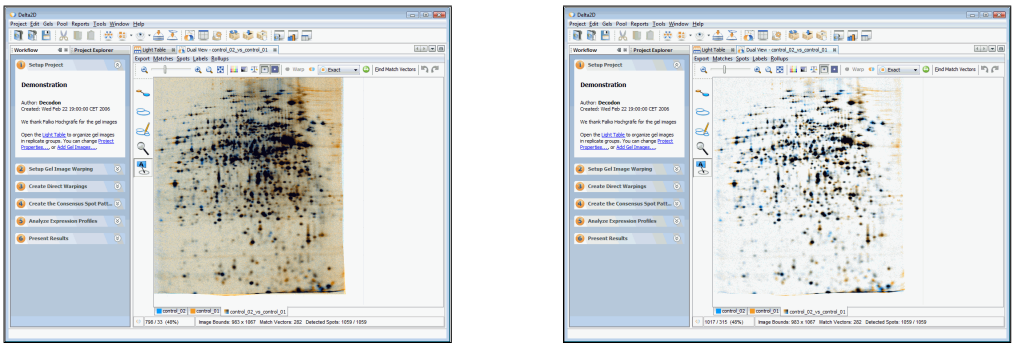


Figure 5.25.: A dual channel image with background switched on and off.

The same background subtraction mechanism will be used later in the quantitation step. You can adjust background subtraction either when quantifying spots manually with Spots ▷ Quantify ▷ ...(see 5.5 on page 67 for details), or directly for the actual view: Select Spots ▷ Background Region ▷ *gelname*.

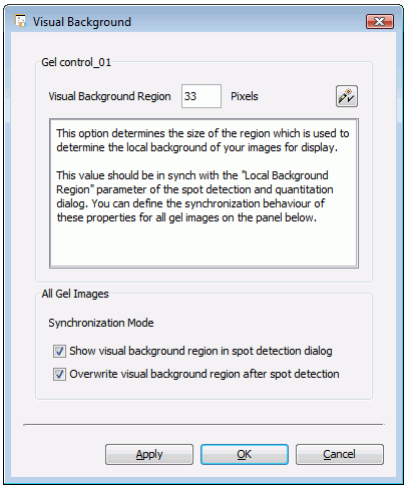


Figure 5.26.: Visual background settings

The now upcoming dialog lets you set the same parameter for background detection as described in section 5.5 on page 67, but with one difference: changing the background parameter here affects only the current view. There are two more options you can switch on or off. They determine how the *Visual background region* set here interacts with the *Local background region* set in the Quantitation dialog.

Show visual background region in spot detection dialog means that this setting will be handed over to the Spot detection dialog. It can be seen, when you open the Spot detection dialog, but will not be applied to quantitative data before you start a new quantitation.

Overwrite visual background region after spot detection means the opposite way of communication: if you change this parameter in the spot detection dialog, it will be changed here as well.

The purpose of these options is to keep the visual background region in sync with the technical background region, thus the default setting for both options is checked. Please note that the visual background is linked to the background detection controlled by the quantitation dialog. With the default settings it has influence on spot quantity.

The Histograms Dialog

Adjusting histograms is advisable when your image does not use the entire dynamic range (i.e. there is no bright white or no black in it), or if there is a homogeneous background.

Delta2D allows you to compensate for differences in brightness and contrast between master and sample gel images by adjusting the image histograms. This can result in production of clearer dual channel images. Histogram adjustments are controlled with the Histograms dialog, illustrated in figure 5.27 on page 53.

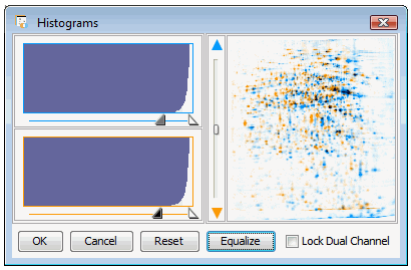


Figure 5.27.: The histograms dialog.

You can invoke the Histograms dialog by pressing the Histograms icon in the Dual View toolbar.

> Note: Histogram adjustment is saved individually for each image. It affects the generation of dual channel images and the representation of images in the Gel Regions View, but leaves the spot quantities unchanged. It is meant to enhance the view of the gel images without changing quantitative data. This is in accordance with Delta2D's principle of leaving original data unchanged as far as possible.

Histograms or Amplitude Rescaling?

Delta2D offers two tools to correct the visual representation of gel images with poor contrast: amplitude rescaling and Histogram adjusting. They complement one another and can be used solely or in combination, just as required.

Amplitude rescale is a standard approach in image processing, which rescales the effectively used range of grey values in an image to the maximum usable range. For example: if you have an 8-bit image (which can define one of 256 grey values for each pixel, and let 0 being black and 256 being white), where the darkest pixel has the grey value 40 (dark grey) and the lightest 180 (light grey; quite a grey and shallow looking image), amplitude rescale represents the value of 40 as 0 (perfect black), 180 as 256 (perfect white) and the intermediate grey values rescaled respectively. Thus you have a much more plastic and vivid representation of your image without having altered the information contained. Depending on your gel images, the effect of amplitude rescale you can observe can vary in a wide range: the enhancement can be either hardly visible if your images already use a wide range of grey values, or it can even make spots visible you could not see before if your images use only a small bandwidth of grey values.

But of course this approach has a drawback as well: loading and displaying images in the Dual View takes slightly more time, especially if according to your zoom settings only a part of the image would be loaded, because for amplitude rescale the complete image has to be analyzed. Thus, the recommended setting depends on your gel images: if the effect of amplitude rescale is hardly visible, you can switch it off (please refer to section 10.1 on page 144 on how to do so) to load images faster; if your images are generally more flat and poor in contrast, amplitude rescale can be a great help.

Amplitude rescale is nothing more than a rough pre-enhancement, which can be sufficient in many cases, but cannot take into account problems with artificial signals like speckles or gel breaks. This evokes the necessity of additional fine tuning the enhancement process. Histogram adjusting gives you control of the enhancement process. You can change the rescale settings smoothly while watching how the representation alters with your changes. But you can also let Delta2D adjust the histogram settings of an image automatically in consideration of another images contrast situation to make them both better comparable.

Automatic Histogram Equalization

Delta2D can automatically balance different levels of brightness and contrast between your gel images. Simply open the Histograms dialog and click on the Equalize button. Alternatively, you

can click directly on the Equalize icon in the tool bar. Delta2D will automatically balance the grey scale levels in your images. After equalization, the total *grey scale volume* in both images will be the same. This result will always be achieved by making the darker of the two images lighter.

Manual Histogram Equalization

You also have complete manual control over the brightness and contrast balance of your images.

The current grey scale histogram of both images of the Dual View is displayed in the dialog. The histogram display shows you how many pixels are contained at each grey scale level in the corresponding images.

To achieve optimal equalization please proceed as follows:

- Move the sliders of each image until the contrast settings fullfill your needs.
- Press the Equalize button and look at the vertical slider. If the vertical slider indicates that one histogram is higher weighted compared to the other move the left slider of that histogram a little to the right and press the equalize button again.
- Do this iteratively until the vertical slider is located exactly in the center.

If done that way you have perfectly equalized images in the dual view.

Manually Adjusting the Balance Between Two Images

The vertical slider in the center of the dialog can be moved manually as well. Move it towards the image you would like to see more dominantly to adjust the relative grey scale levels between the two images. The results of the changes you make will be displayed dynamically in the image on the right hand side of the dialog.

Modifying the Histogram for a Single Image

Two sliders below each image histogram allow you to modify the histogram. The position of the left-hand (black) slider indicates the point in the histogram that will be represented by the darkest pixel in the displayed image. The right-hand (white) slider indicates the point in the histogram that will be represented by the lightest pixel in the displayed image.

Manipulating the slider positions allows you to suppress image regions containing pixel values outside a specified range from the display.

Managing the Histograms Dialog

It may be worthwhile to bear a couple of tricks in mind while working with the Histograms dialog.

Lock Dual Channel Normally, if you make adjustments to the histogram of an individual image, the dynamic image display will change to show only that image. If you want to be able to watch the effect on the dual channel image while you make the adjustments, check the Lock dual channel box..

Resize the dialog Enlarging the dialog itself may give you better control over fine histogram adjustments, since the histograms and image display will be scaled up to fit in a larger dialog.

Apply or discarding changes Clicking the dialog's OK button will apply the changes to the images displayed in Delta2D's main frame. Clicking the dialog's Reset button will reset all the histogram values to the values that existed at the time the dialog was invoked. Clicking the Cancel button will dispose of the dialog without any changes being applied.

About the Histogram Adjustment Process

Histogram adjustment is a classical image processing technique that works by applying the following rules to each pixel in the image:

1. if the pixel is brighter than a given threshold, make it completely white
2. if the pixel is darker than another threshold, make it completely black
3. otherwise apply a linear change to the grey-level of the pixel

Adjusting histograms is advisable when your image does not use the entire dynamic range (i.e. there is no bright white or no black in it) or if there is a homogeneous background.

You may also wish to keep the following points in mind when making adjustments to the image histograms:

- Most importantly, remember that adjustments to the image histograms affect only the visual presentation of your gel images – there is no affect on the spot detection and quantitation process.
- If you have the image background subtraction feature activated, histogram adjustments will be made to the images *after* the background has been subtracted.
- If you have enabled amplitude rescaling, both gel images will already utilize the available range of grey scale values. You only need to make changes to the histograms if the grey levels are different in the two images, or if you want to suppress lighter or darker regions of the images from the display.

See section 10.1 on page 144 for global options for image preparation.

Using Colors: The Color Schemes Dialog

The Color Schemes dialog (figure 5.28 on page 57) allows you to control the colors that will be used for displaying Delta2D's dual channel images.

The Color Schemes dialog can be invoked from the Images menu, using the Images ▷ Color schemes... menu entry. Alternatively, you can click directly on the Color Schemes button in the tool bar.

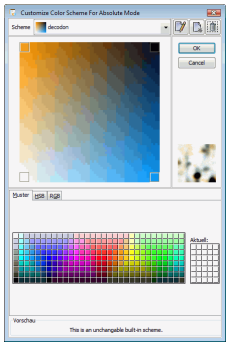


Figure 5.28.: The color schemes dialog.

Display Modes and Color Schemes

Delta2D uses two different display modes: absolute mode and ratio mode. Color schemes can be configured individually for each mode. Whenever you invoke the Color Schemes dialog, it will allow you to configure the color scheme for the current mode. If Delta2D is in absolute mode when you invoke the Color Schemes dialog, you will be able to edit the color scheme used in absolute mode. If Delta2D is in ratio mode, you will be able to edit ratio mode's color scheme.

The currently selected color scheme is presented in the center of the Color Schemes dialog (figure 5.29 on page 57).

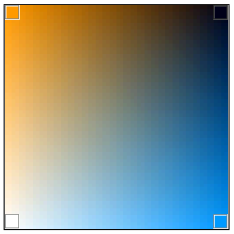


Figure 5.29.: The color schemes display.

The color scheme display can be interpreted as follows:

- Top-left corner – The color used to display *sample spots* i.e. spots appearing exclusively in the sample gel.
- Top-right corner – The color used to display regions where spots *overlap*.

- Bottom-right corner – The color used to display *master spots* i.e. spots appearing exclusively on the master gel image.
- Bottom-left corner – The color used to display regions of image *background*.

If you are editing the scheme for ratio mode (see figure 5.30 on page 58), you can choose two additional colors for highlighting points with only a relatively small ratio between the sample and master spot levels. Those colors are displayed in the middle of the top edge and of the right edge respectively. The ratio mode was implemented for a more fine grained representation of expression changes especially for faint spots.

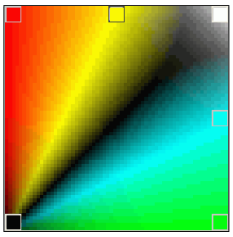


Figure 5.30.: The color schemes display for ratio mode.

Using Predefined Color Schemes

Delta2D provides you with several predefined color schemes. To use a predefined color scheme, simply select one from the drop-down box.

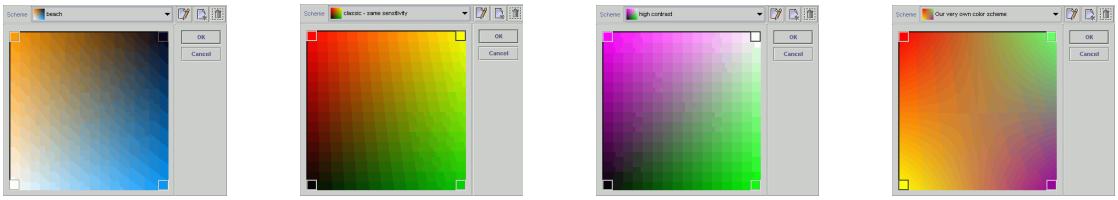


Figure 5.31.: Some predefined color schemes

Defining Your Own Color Schemes

It is also possible to define your own color schemes to suit your needs, e.g. for printout or presentation. Creating your own color scheme is easy – take the following steps:

Create a New Scheme To create a new scheme, click on the New Color Scheme icon . A new scheme will be created with the same colors and a similar name to the currently selected scheme.

Select New Colors for the Scheme You can now configure the colors to use in your new scheme. First, click on the corner of the color scheme display corresponding to the color you want to edit. For example, if you want to define a new color for sample spots, click on the top left corner of the color scheme display.

You can then choose a new color to use for highlighting the selected image feature. There are three methods available for doing this, accessible at the bottom of the Color Schemes dialog. The three color controls are described individually below.

Color swatches This is the simplest color control to use. Simply select the color you want to use from the palette of available colors.

Hue-saturation-brightness (HSB) control Using this tab, you can control the hue, saturation and brightness of a color separately. Select which of the three values you want to change by using the HSB radio buttons, and change the value by using the slider or by entering a value directly into the text field provided.

Red-green-blue (RGB) control This control panel allows you to configure the levels of red, green and blue that are combined to produce the desired color. You can set these levels using the sliders provided, or by typing a value between 0 and 255 directly into the corresponding entry field.

It is recommended that you choose strongly contrasting colors for master spots, sample spots and the spot overlap, and an unobtrusive color for the image background, to help you to visualize the differences between two gel images quickly and clearly.

Renaming a Color Scheme

You may wish to rename a color scheme, particularly after you have created a new scheme which was assigned a name automatically. Click on the Rename Color Scheme icon  to enable the name editing mode. You can then type a new name for the scheme directly into the drop-down box's text field.

You can not rename one of Delta2D's predefined color schemes.

Deleting a Color Scheme

Simply select the scheme you want to delete from the drop-down box, and then click on the Remove Color Scheme icon  .

You can not delete one of Delta2D's predefined color schemes.

Using Ratio Mode

Even more information can be gained by using Delta2D's *ratio mode*. This is a unique visualization tool that shows expression ratios directly on the gel images (Figure 5.32 on page 60), without being affected by the absolute intensity of the spots. A region with weak intensity and an expression ratio of 2 is displayed exactly in the same shade of color as another region with

strong intensity but the same expression ratio. Thus, you can easily recognize regions of special interest without being distracted by regions of high intensity.

Ratio mode works best with images that have a low background level. Therefore you should switch off background using the layer control and adjust the histograms, if necessary. Select Images ▷ Ratio mode to activate ratio mode display.

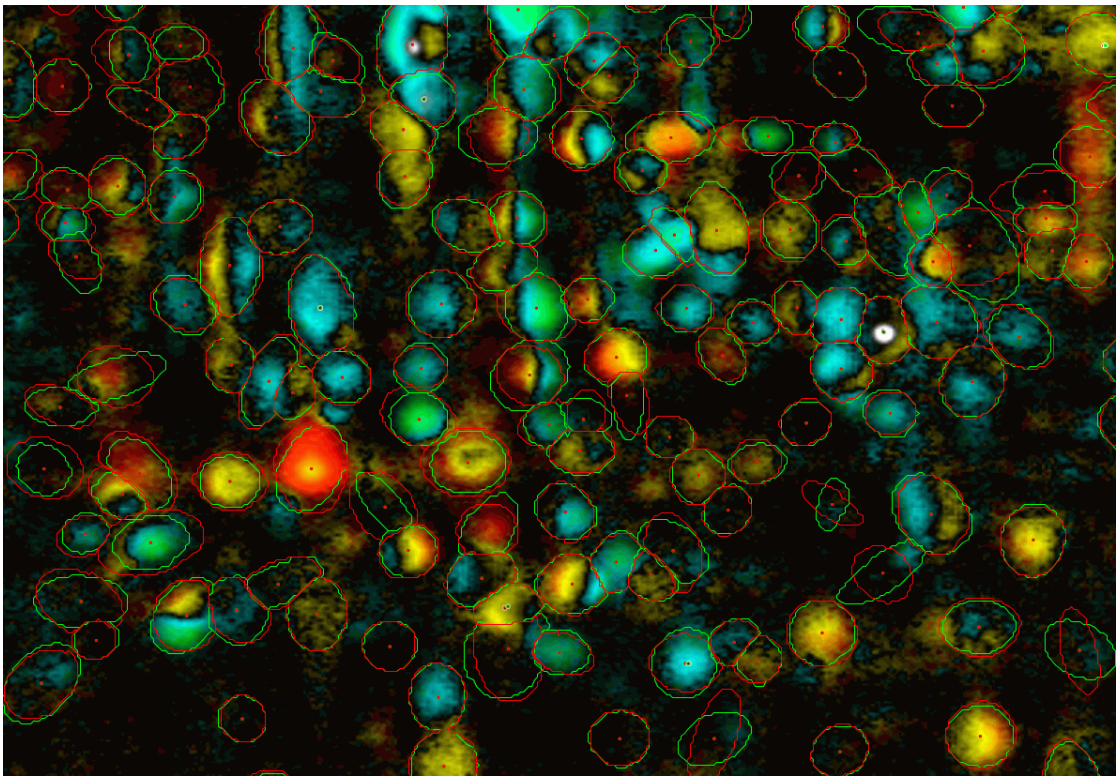


Figure 5.32.: A gel region shown in ratio mode.

Observe how the color square changes (Figure 5.33 on page 60): pixels are now color-coded according to the sample / master intensity ratio. Pixels with an intensity ratio between 0.5 and 2 are dark-colored, while higher ratios get bright colors. When both spots are saturated or very weak, ratios cannot be computed reliably. Therefore the color square is black in the lower left corner (weak spots) and white in the upper right corner (saturated spot maxima).

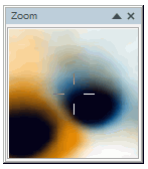


Figure 5.33.: The Colors Rollup in Ratio Mode.

Warping Gel Images

Delta2D's approach to analyzing 2D gel electrophoresis images relies on advanced image processing technology that compensates for the differences in spot positions between gel images. These differences are due to variations in running conditions and the gel casting process. They are what makes comparing and analyzing 2D gel electrophoresis images so difficult and error prone.

When you hold two similar gel images next to each other, you may have the impression that the spot patterns on one gel are more or less a distorted version of the patterns on the other. The process of distorting (or "un-distorting") images is called *warping*. Delta2D's warping algorithms help you to generate dual channel images on which corresponding spots are perfectly overlaid. In the dual channel image, differences in protein expression levels can then be easily recognized. The same algorithm that is used in producing dual channel images will be used in the subsequent quantitation step to obtain accurate and reliable spot matching information in the Quantitation Table.

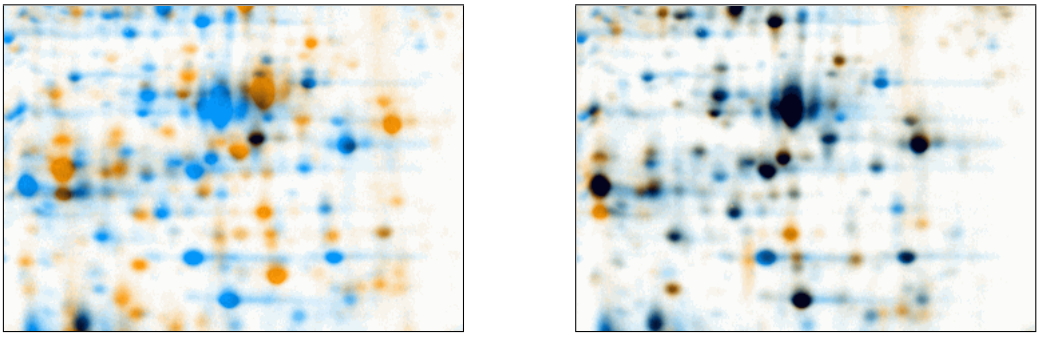


Figure 5.34.: A region of the dual channel image, before and after exact warp. Corresponding spots are overlaid exactly, allowing for easy identification of spots with changed expression level.

In Delta2D you can assign a warp mode for each directly linked gel image pair. The warp mode can be identical, global, exact, automatic, or implicit.

Here is a more detailed description of the warp modes:

- [Identical Warp mode] This is just no warping at all. Identical transforms can be used for registering images that are from the same gel but display different samples or multiple aspects of a sample.

- [Global Warp mode] Compensates global gel distortions such as growing or shrinking, rotation, stretching a special smooth transformation can be used. Set a few match vectors then use global to see more correspondences. Global warp is a good start for setting more and more match vectors by hand. It is almost never suitable for producing the final dual channel image because there are local distortions as well. As a result you will see that the

match vectors are shortened substantially but not set to zero, as the exact warp mode does (see figure 5.35 on page 63).

After the global warp you will see more corresponding spot patterns because the sample image is better aligned to the master image. Fix some more matches. You do not have to fix every correspondence you see, assigning a single spot pair is usually sufficient to align the region around it. Since all vectors are weighted similarly for the global transform outliers can be recognized very easily. That's why the global warp is often used for finding warping errors.

- [Exact Warp mode] All spots that are connected by a match vector will be perfectly overlaid after warping. Other spots will be warped according to match vectors in their neighborhood.

  The difference to the global warp can be seen in figure 5.36 on page 63.

- [Automatic Warp mode] Let Delta2D try to automatically find match vectors by analyzing similarities in the gel images using the SmartVectors™ Technology and apply the set of non-approved match vectors to an exact warp. If match vectors are present they will be used to guide the automatic warping process so that you can use the automatic warp mode iteratively and in combination with manually defined match vectors (see 5.5 on page 64).

  As with exact warping, spots that are connected by a match vector will be perfectly overlaid. Start the automatic warping by starting the Job Manager, or press Find Match Vectors, or just press the Warp button if no match vectors exist yet. When the process has ended the warp mode will is set to exact warp to avoid endless loops. You shall always review the result of automatic warping.

  Read more about SmartVectors™ at www.decodon.com/Support/Howto/SmartVectors.html

- [Implicit Warp mode] Warp the images by a combination of explicit pairwise transformations (these can be exact, global, automatic, or identical) that connects them. Example: Image B has a valid warping to image A, image C is also connected to image A, then image C can be compared to B using implicitly the existing warpings: C ▷ A ▷ B. You will usually have implicit warps for most of the gel pairs in your project.

Warp images with the defined warp mode by pressing the warp button , unwarp by pressing the unwarp button . If a set of match vectors (the match map) exists, it will be applied. If no match vectors exist but automatic warp is chosen, new match vectors will be found. Otherwise the warp button is deactivated since either a warp mode is chosen that does not demand for a match map or you have chosen a warp mode that demands for a match map, but there is no. Please read on about how to define match vectors in section 5.5 on page 64.

Add More Match Vectors if necessary

After the exact warp you may see some spot pairs that are not exactly aligned. You can add more match vectors, then warp again to see the effect of your new match vectors. Warping (either

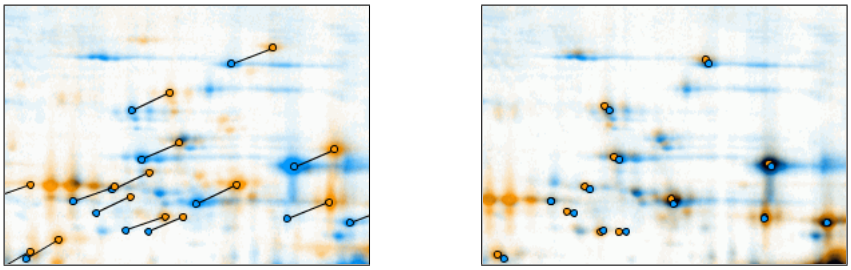


Figure 5.35.: A region of the dual channel image, before and after global warp. Corresponding spots are not overlaid exactly, but much closer than in the original image. Further correspondences can be identified more easily.

exactly or globally) can be done anytime, match vectors will always be properly adjusted. Use Warp ▷ Unwarp to see the unwarped dual channel image.

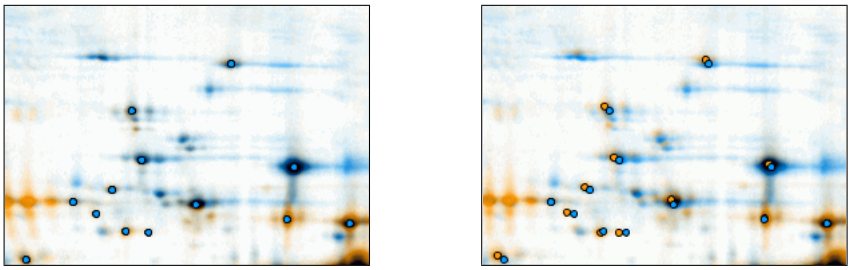


Figure 5.36.: An image region with well-aligned spots after exact warp (left image) in comparison to the same region after global warp (right image).

> Note: Existing match maps will not be used if the identical or the implicit warp mode has been chosen. However, an existing match map will not be deleted by just changing the warp mode. Switching the warp mode back to global, exact or automatic will let Delta2D use the match map for warping.

Saving the Warped Image

The warped sample image is not retained in the pool, but it can be recomputed anytime using the match map. You can export the warped sample image using File ▷ Export Sample.... Select File ▷ Export Dual Channel... to save the dual channel image.

> Note: Images exported in warped state can be used for documentation purposes only. They are not suitable for further quantitative analysis of any kind (reimported in Delta2D or imported in other software). Warping alters the complete image, which affects spot size as well. According to Delta2D's principle to leave original data unchanged, all quantitative analysis is done on the original, unwarped images.

Setting Match Vectors

Match vectors connect corresponding spots (Figure 5.37 on page 65). They are used by Delta2D to warp one image to another reference image to eliminate the differences in spot positions. The warping can be specified by using match vectors alone, or by using them to guide the SmartVectors Technlogy. Only a tiny fraction of all spot pairs has to be connected by match vectors because Delta2D uses a match vector to align an entire image region.

Before you start to set match vectors, make sure that match vector tool is activated by clicking in the tool panel.

Global options for match vectors can be defined in the Options dialog (see section 10.1 on page 139 for more details).

> Note: With version 3.4 we have introduced Undo and Redo for match vector operations. Thus you can try setting a couple of match vectors, study the resulting dual channel image and go back to previous match maps if you like.

Some corresponding spot patterns are immediately visible in the dual channel image. To set one correspondence:

- Click on a spot in the sample image. It is marked by a solid circle.
- Click again to set the corresponding position in the master image. It is marked by a solid circle.

> Note: It is important to draw all match vectors from sample (orange) to master image (blue).

> Note: In order to use automatic snapping of match vectors the option Match Vector Snap in the Options dialog (Section 10.1 on page 139) must be enabled.

Now specify some more spots in the sample image (orange) which correspond to spots in the master image (blue). These correspondences will be used to warp the sample image onto

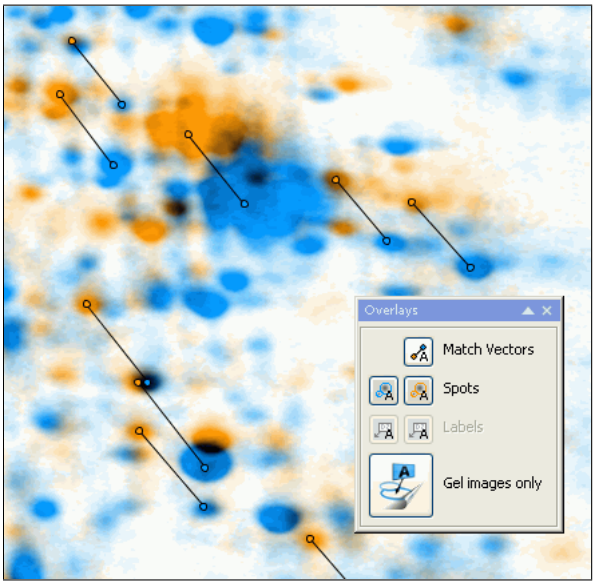


Figure 5.37.: Setting match vectors.

the master image. Go ahead and fix about 15 corresponding spots this way, starting with the most obvious ones. Try to find matches that are well-distributed over the whole image. You can change a match vector by dragging one of its points with the mouse. Matches can be deleted by right-clicking on one of their end points.

Questions and Answers About Warping

Q: How do I know which warping mode is the most adequate for my gel pair?

A: By default, Delta2D uses implicit warp mode for any gel image pair. This is a reasonable choice because in the end most of the image pairs will be connected using implicit warps. Usually you have to decide between two warp modes that you can assign for image pairs in the Project Explorer or the Warping Setup:

- Between images from the same sample, choose automatic warp mode.
- If you have multiple images per gel, choose identic warp mode between all images that were made from the same gel. If there were some experimental (except scanner reconfiguration and scanning) steps between recording of the images, try global, or, if this does not work, exact warp.

  Using a Warping Strategy is highly recommended. Please see section 5.4 on page 35 for more details.

  When you are ready with warping, the Project Explorer} and the Warping Setup should show only green symbols for the directly linked image pairs. Please refer to section 5.2 on page 26 for information about how to deal with yellow or red symbols.

Q: I only want to do quantitative analysis, do I need to warp the images?

A: Yes. And no. If you just want to obtain quantitative data for single gel images without comparing them to each other or for images from the same gel, this is possible without match maps. But to compare multiple gels Delta2D demands for warping to compute a fused image where you can detect the project wide consensus spot pattern and to transfer this spot pattern back to the appropriate positions on all images. Furthermore, scatter plots require spot correspondences.

Q: Do I have to create a match map for every gel image pair in my project?

A: No, Delta2D can combine warp transformations to connect two images indirectly via a set of directly linked images. Thus, Delta2D assists to find a good warping strategy for your project (see section 5.4 on page 35.

Q: What if one gel image is substantially rotated or shifted with respect to the other?

A: Assign a few correspondences that you can identify reliably. Then use Warp ▷ Global Warp to eliminate global distortions. The global warping will bring similar spot patterns closer together, compensating for global distortions such as shifts, minor rotations, or differences in scaling. For a shift, already one single match vector is enough. If the rotation is > 90°, please rotate the respective image in the gel image properties dialogue (available from its context menu).

Q: What if initial match vectors are hard to find?

A: With highly dissimilar gel images it is sometimes hard to find the first spot correspondences. Assign as many correspondences as you can identify reliably. Then use Warp ▷ Global Warp to eliminate global distortions, again bringing similar patterns closer together.

Q: When or why use global warp?

A: Use the global warp early in the matching process, when you are not sure about further correspondences. Global warp is more robust with respect to wrong correspondences – one wrong match vector will not distort your image too much. Nevertheless, after the global warp, further correspondences will be easier to recognize.

Q: Does warping affect the quantitation process in any way?

A: Not at all, spot detection and quantitation is done using the original images.

The Spot Detection and Quantitation Dialog

Basically, Expression Profiles are obtained in three steps:

spot detection – identification of image segments that are occupied by spots

spot quantitation – summing up the grey values of the pixels belonging to each spot. Background is subtracted, and calibration curves (if available) are adapted. Normalized volumes are provided in the Quantitation Tables while raw volumes can be reviewed as well.

spot matching – assembling single spot quantities to expression profiles. For transferred spots this results in Complete Expression Profiles.

Spot detection is done automatically, controlled by a few parameters that are proposed by Delta2D but can be changed by the user. In Delta2D, any "spot painting" or "cutting" by hand is obsolete. However, you can edit the spot pattern by cancelling, splitting or joining, or by adding new spots (see section 5.5 on page 71 for details). Starting the spot detection for a single image (probably on the fused image) is easy: Right click on the respective image (probably the fused image) in one of the windows (e.g. in the Project Explorer, the Light Table, or the Warping Setup) and select Detect spots... from the context menu. Delta2D presents the Quantitation Dialog (figure 5.38 on page 68) to set or confirm the settings before Quantitation itself is done.

To start the spot detection in the Dual View select Spots ▷ Detect Spots on *name of your gel image*.... If there are no quantitations available, clicking on the Segments tool icon in the tool panel will also open the Quantitation Dialog.

The Quantitation dialog comes with a proposal for three numerical parameters. The numbers are derived directly from the images and should lead to reasonable results. However, you can change the parameters according to your individual preferences. Having changed the parameters proposed by Delta2D, you can restore the proposal again by using the feature Parameter estimation. Simply click on . Please refer to section 5.5 on page 67 for a detailed description of each of the parameters.

The set of the parameters that have been used for quantitation is saved within the *.qnt files which contain the quantitation information of a 2D gel image. You can load a parameter set from a previously exported quantitation file by loading the corresponding *.qnt file in this dialog. Parameter sets can be saved and loaded using the buttons in the top right panel.

Clicking on the OK button will start the spot detection and quantitation process, Cancel will discard your settings.

Quantitation is always done using the original unwarped images while warping and histogram adjustment have no effect on the results. The background for a spot is computed and subtracted automatically, it is the very same background that is switched on and off using the layer panel.

When the spot detection process is finished, spot boundaries will be shown in the main window of the Dual View. The spot boundaries for the respective gel image are overlaid on the image, placed on a separate layer (one spot layer per gel image). These layers can be switched on and off, just like the image layers, using the overlays rollup. Select Rollups ▷ Overlays to open the rollup.

Spot centers are marked by points. The center is located where a spot cutter would obtain the maximal protein amount.

Spot Detection Parameters

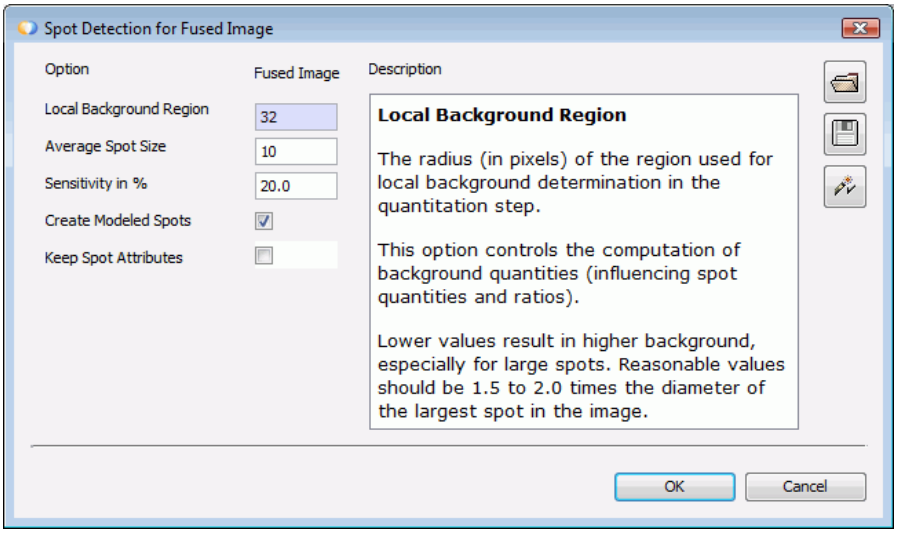


Figure 5.38.: The quantitation dialog.

Configuring the Local Background Region The local background region refers to the radius (in pixels) of the region used for local background determination in the quantitation step. This option controls the computation of background quantities (influencing spot quantities and ratios). Lower values result subtraction of more background from the spots volume, especially for large spots.

Reasonable values should be 1.5 to 2.0 times the diameter of the largest spot in the image.

> Note: This option also effects the snap to spot feature. If this parameter is set to a value that is much too high, it happens that snap to spot gets difficulties to differentiate between adjacent spots.

Configuring the Average Spot Size Specifying the average size of spots in your gel images enables Delta2D to separate overlapping spots more accurately, as well as to distinguish spots from the image background. The value specified refers to the radius of an average spot, in pixels.

Higher values will decrease noise sensitivity. Use lower values to separate spot clusters better and to detect very small spots.

Note that you can get an idea of the size of the spots in your image by looking at Delta2D's status bar, at the bottom of the main frame. The status bar displays information about the current pixel position of the mouse pointer within a gel image, so you can see how large your spots are by moving the mouse over the spots and observing the number of pixels covered directly.

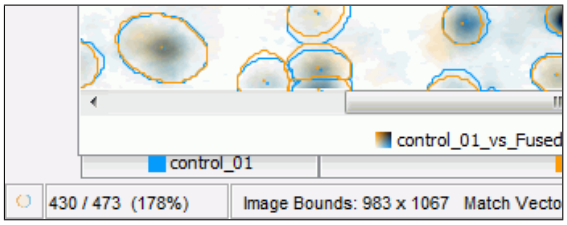


Figure 5.39.: The cursor position in pixel count in the Status bar

Configuring the Weak Spot Sensitivity This parameter allows you to control how strictly Delta2D discriminates between spots and the small signals. Being able to configure this sensitivity is useful when you have gel images containing very weak spots.

Just type a percentage value directly into the text entry field corresponding to either the master or sample gel image.

Specifying a higher value will result in Delta2D detecting spots with weak intensity more reliably. Specifying a lower value will mean that more noisy background artifacts in the images will be suppressed successfully.

Usually, a value between 5 and 20 % is suitable.

Saving and Loading Sets of Parameters Delta2D saves the parameters used for detection together with the detected spots for each gel image individually. Additionally, Delta2D lets you export and import your parameters to and from files, e.g. for exchange with other Delta2D users, or if you want to try out different settings but want to keep a special one.

To save the current set of options to a file, click on the Save icon . A dialog will appear, enabling you to specify a file to save your options to.

To load previously saved parameters, click on the Load icon . A dialog will appear, enabling you to choose a file from which to load a set of parameters.

Spot Detection and Quantitation Parameters for All Images The Spot Detection and Quantitation dialog allows you to control the detection and quantitation parameters for all gel images of your project in one place. Open it by selecting Gels ▷ Detection Parameters....

Note: This dialog is useful if you detect spots on the different images individually. Please note that the recommended workflow for getting complete expression profiles demands for spot detection on a fused image only since the resulting spot pattern will then be transferred to the other images. Asynchronous spot patterns on the different images as they come from individual spot detections cause difficulties that can be avoided.

If you are working with a fused image and spot transfer please do not change these settings. Each change in this dialog will result in a redetection of spots on the respective gel image, which will screw up the 100% spot matching in your project.

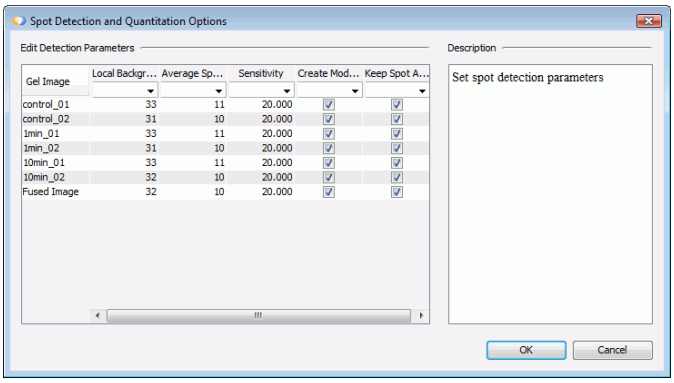


Figure 5.40.: The Spot Detection and Quantitation Parameter Dialog for all Images.

Simply select one or more gel images in the list and change the settings by using the drop down boxes on top of the table, or type the new value directly in the respective field of the drop down box. The typed-in value will be applied to the selected gel images as soon as you hit the Enter key moved to the next field with the Tab key.

For a more detailed description of the single parameters please refer to section 5.5 on page 67.

Spot Shapes: Pixel Based or Modeled

Pixel based spot boundaries directly reflect the raw grey value distribution within the scanned gel image. Since the gel images usually include noise and spots divided into pixels, this kind of spot boundaries regularly look erratic.

For different reasons, be it that, due to a low resolution of your image, the spot outlines look to rough, be it for purposes of printing or presenting results, or simply for a better overview, a smoother appearance of the spots its often preferred. Delta2D includes the option to model spot boundaries within the process of the spot detection. Simply check the box Create Modeled Spots when defining the parameters for spot detection and quantitation. (Fig. 5.41 on page 71).

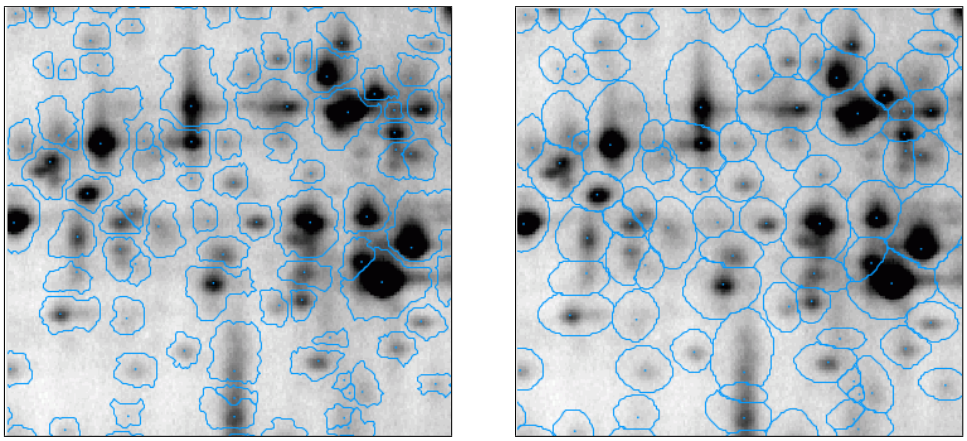


Figure 5.41.: The same region with pixel based and model based spots.

Note: The spot boundaries define the relevant area for spot quantitation. Therefore, whether you decide for modeled spots or not, the spot boundaries you face determine the quantities for the spots on your gel images. To achieve comparable spot quantities for your analysis, we strongly recommend to decide for one type of spot shapes for the entire experiment.

You have access to the option in the dialog for the spot detection parameters only. The option will be used for the next spot detection, existing spots on other gel images will not be affected. For changing the spot shape a redetection by using the altered parameter is necessary. "Keep attributes" preserves already done classifications like hidden, canceled, exclude from normalization, etc..

Spot Editing

You can correct the results of Delta2D's automatic spot detection by setting "markers". Using markers you can control where a spot should be detected; Delta2D will then compute the new

boundary accordingly. There are two basic operations for spot editing: creating a new spot, and joining two or more spots. In any case, Delta2D will compute spot boundaries automatically, using your input. Delta2D's approach to spot editing maximizes reproducibility while giving you a lot of control over which spots are detected.

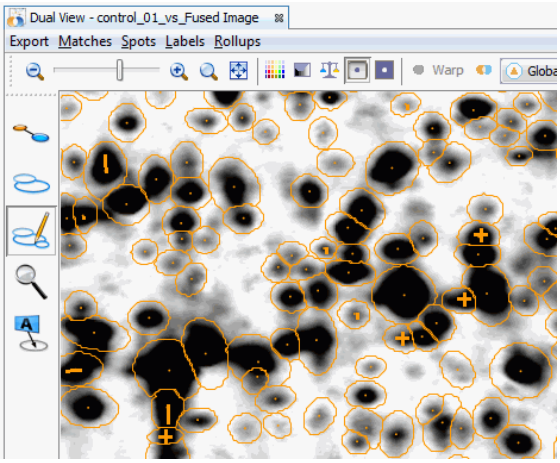


Figure 5.42.: Edit spots

Adding a Spot To add a spot, click on the Spot Editing Tool in the tool bar of the Dual View. Now click on the position in your gel image where the spot should be detected, trying to hit the darkest point of the aspired spot. Where you clicked, a "+" will appear and according to this manually added marker a new spot will be detected instantly. If the result is not satisfying, you can either move the marker by dragging it, or you can remove the marker by right-clicking on it and set a new marker somewhere else. Your manually added spots will look and behave exactly like the automatically detected spots but still keep their marker as manually added, visible when switched to the Spot editing tool. Thus reproducibility is granted and at any time you have the possibility to edit them again.

In some cases it can be necessary that you drag a spot marker instead of setting it by a click. The dragged line markers have some influence on the spot shape and help for the correct detection of by gel breaks separated by spots.

Splitting a Spot in Two To split a detected spot in two parts, simply override the detected spot with two manually set detection markers: select the edit spots tool as described above. Now click on the two sections of the spot you want to divide, trying to hit the centers of the aspired spots.

Joining two Spots If a spot was detected as two spots wrongly, you can easily join the two spots: switch to the edit spots tool, and drag a line from one to the other half of the aspired spot. Delta2D will join the two spots touched by the line.

Removing a Manually Edited Spot To remove an edited spot, choose the edit spots tool as described above. Now *right* click on the *marker* you want to remove.

Spot Quantitation and Matching

Once spots have been detected, quantitation is also done automatically.

Quantitation data can be saved as a complete set of data (spot boundaries and quantities for the whole gel image) using the Spots ▷ Export ▷ menu.

Since Delta2D includes image warping (introduced into two-dimensional electrophoresis gel image analysis by DECODON in the year 2000), spot matching is very reliable, even with individual spot detection for each gel image. In traditional packages for the analysis of 2D gel images, where spot matching is based on spot patterns rather then spot positions, these steps are error-prone and require extensive manual corrections. In Delta2D spot matching is done automatically since after warping corresponding spots already have the same position throughout the whole experiment.

With its intuitive and modern approach, Delta2D tries to automate the analysis as far as possible, leading faster to results you can rely on.

Note: Based on image warping and image fusion, DECODON has introduced complete expression profiles to avoid missing values in the Quantitation Table and the resulting problems during statistical analysis. To receive complete expression profiles, create a Proteome Map make an union fused image out of the whole set of images in your experiment(at least out of one representative image of every group of replicates). Then do the spot detection on the resulting Proteome Map only and transfer the spots to the original images. Please refer to sections 7 on page 111 for details.

Read more about the benefits of 100% Spot Matching at www.decodon.com/Solutions/Delta2D/100_Percent_Spot_Matching.html.

5.6. Quantitation Table

Delta2D displays quantitative data in flexible tabular views (see figure 5.43 on page 75) that fit your analysis needs. Table rows can be filtered and sorted by numerical and non-numerical columns, making it easy to identify relevant sets of spots. The table display is always synchronized with the spot boundaries on the Dual View, so you can go from image to data and back again with ease.

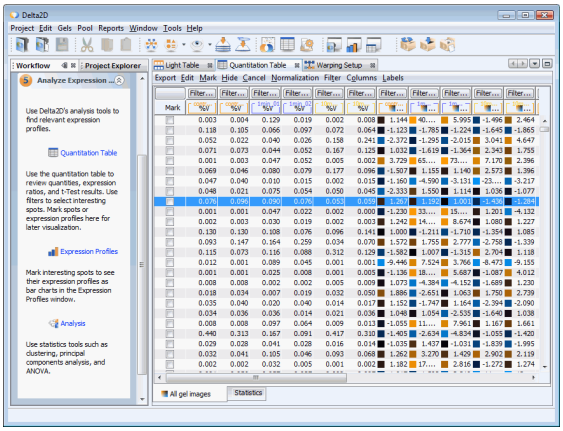


Figure 5.43.: The quantitation table

The Quantitation Tables give you access to your data in three basic types of representation:

Statistics tables include statistical values for each expression profile with respect to the groups plus data comparing the group values. This is the first table you get to see when opening the Quantitation Table window.

Multiple gel image tables basically have the same structure as the single gel image tables, except that each row in the table represents the expression profile of matched spots. All attributes appear for each gel image. The column headers are color coded to make it easy to see from which gel the data in a certain column was taken.

Single gel image tables show the relevant spot attributes for a single gel image. Each row represents one spot.

The Quantitation Table can be opened via the menu Window ▷ Quantitation Table. It includes two tabs: a Multiple gel image table for all images with spots, and a Statistics tables for the whole project. For an image pair a Multiple gel table can be opened by choosing Spots ▷ Show Table in the Dual View.

Quantitation Tables are also available by clicking on the table button . The type of table depends on what has been selected: A Single gel image table or Multiple gel image table if the selection includes a number of images, a Statistics tables if only complete groups (at least two) have been selected.

A variety of attributes is available in the different tables, some of them being hidden by default to reduce the tables' complexity. The attributes that are available in the Statistics tables are described in table 5.6 on page 76. To change the different attributes' visibility, please refer to section 5.6 on page 80.

Column	Description	Visible by default
Mean	The arithmetic mean in this group.	Yes
RSD	Relative standard deviation in this group.	Yes
Ratio	Shows the ratio for a certain parameter of the min/max/mean of this group to the min/max/mean of the group where the most left gel image in the project matrix belongs to. Choose the parameter and the function min, max or mean at the top of the table.	Yes
t-Test	Error probability for the assumption, that this group belongs to the same parent population as the the most left group, based on the Student's t-test algorithm.	Yes
Min	The lowest value in this whole group.	No
Max	The highest value in this whole group.	No
n	Number of matched spots in this group.	No

Table 5.4.: Attributes in the Statistics Table.

For a detailed description of the attributes that are available in Single gel image tables or in Multiple gel image tables, please refer to table 5.6 on page 77.

As an example for how the ratio columns in the Statistic Table are calculated, imagine the settings at the top of the table are Spot property: %Volume, ratio=sample groups mean / group control mean. The ratios are calculated by the following procedure: The columns in the Statistic Table are sorted by groups, while the groups are sorted according to their order in the Project Explorer. For every group, except for the first one in the Statistic Table, the mean of the normalized volume (%Volume) is calulated and divided by the mean of the normalized volume over the first group.

Column	Description	Visible by default
Mark	Check this box to mark or unmark a row.	Yes
Hide	Check this box to hide a row (it will be hidden immediately).	Yes
Norm	Here you can select a subset of the spots that will be used to normalize the quantities of the spots on a gel. By default, all spots are in the normalization set. This results in relative spot volumes being computed by setting total spot volume on a gel to 100%.	Yes
Cancel	Check this box to cancel the spots in a row. Canceled spots are excluded from further analysis.	No
%V	The relative quantity of the spot, excluding background. The total quantity of all spots on the gel is 100%.	Yes
Ratio	The numerical expression ratio (sample spot / master spot). Depending on your settings in the Tables tab in the options dialog (please refer to section 10.1 on page 143) this column shows the ratio as mathematical ratio or as fold change. Additionally it can contain color coded representation of the ratio.	Yes
V	Volume, i.e. the absolute quantity of the spot, in gray units, excluding background. One black pixel with no background has absolute quantity 1.	No
A	The area of the spot.	No
bgd	The background volume for the spot.	No
Avg	The average intensity of the spot, including background.	Yes
ID	The numerical ID of the spot.	No
label	One or more labels attached to this spot.	Yes
X	Spot position: x-coordinate.	No
Y	Spot position: x-coordinate.	No
Q	Indicator of the spot quality, i.e. the similarity with an ideal spot shape.	Yes

Table 5.5.: Attributes in the Single or Multi Quantitation Table.

The Quantitation Table Menu

Export

Export	Export the visible data range as .csv (comma seperated values) file.
Export to Excel	Open Excel which will automatically load the visible data range.
Generate Report in Excel	Excel will open with some analysis features, available for Multiple gel image tables only.
Export Pick Lists ▷	Export a list with marked and labeled spots for a certain picking device.

Edit

Select All	Select the whole visible data range.
Invert Selection	Invert the current selection status.
Complete Row Selection	Expand the selection to the complete profiles of the selected spots.
Copy Selected Rows ▷	Copy selected rows into clipboard.

Mark

Select Marked Spots	Select those spots that have been marked.
Mark Selected Spots	Mark the spots that have been selected.
Unmark Selected Spots	Unmark the spots that have been selected.
Mark All Spots	Mark all spots.
Unmark All Spots	Unmark all spots on the visible images.

Hide

Show Hidden Spots	Show those spots that have been hidden.
Select Hidden Spots	Select those spots that have been hidden.
Hide Selected Spots	Hide the spots that have been selected.
Unhide Selected Spots	Unhide the spots that have been selected.
Hide All Spots	Hide all spots.
Unhide All Spots	Unhide all spots on the visible images.

Cancel

Show Canceled Spots	Show those spots that have been canceled.
Select Canceled Spots	Select those spots that have been canceled.
Cancel Selected Spots	Cancel the spots that have been selected.
Uncancel Selected Spots	Uncancel the spots that have been selected.
Cancel All Spots	Cancel all spots.
Uncancel All Spots	Uncancel all spots on the visible images.

Normalization

Select Spots From Normalization Set	Select the spots that belong to the normalization set.
Include Selected Spots In Normalization Set	Add the selected spots to the normalization set.
Exclude Selected Spots From Normalization Set	Remove the selected spots to the normalization set.
Include All Spots In Normalization Set	
Exclude All Spots From Normalization Set	

Filter

Depending on your project and the kind of table you find different menu items to define filters on the table.

Columns

Depending on your project and the kind of table you find different menu items to define the visibility for the different columns in this table.

Labels

Find Label	Search the different label columns for a string.
Label Selected Spots with Spot IDs ▷	Create Labels on selected spots containing their ID.
Label Selected Spots with Numbers	Create Labels on selected spots containing consecutive numbers. If you need a prefix in the numbered labels, define it in Options ▷ Delta2D ▷ Labels.
Label Unlabeled Spots with Spot IDs	All spots without any label obtain a label containing their ID.
Label Unlabeled Spots with Numbers	Create Labels on all unlabeled spots containing consecutive numbers. If you need a prefix in the numbered labels, define it in Options ▷ Delta2D ▷ Labels.
Translate Labels ▷	Lets you batch change all Labelnames by providing a list with the current names in one column and the replacement names in another.

The Quantitation Table Toolbar

Changing the Table Layout

To change the width of a column, just place the mouse pointer in the table header between two columns. When you see that the mouse pointer changes, click and drag to the left or to the right until the desired column width is reached. A column can be moved by clicking into its header and dragging it to the left or to the right.

Table Properties

A quick and effective way to customize the Quantitation Tablesis to open the Properties dialog of the Quantitation Table by clicking  . In the upcoming dialog you can define the visibility of images and spot attributes in the table.

The dialog includes the following options:

Ratio Master Here you define to which image the ratio of relative spot volumes in Multiple gel image tables refer to.

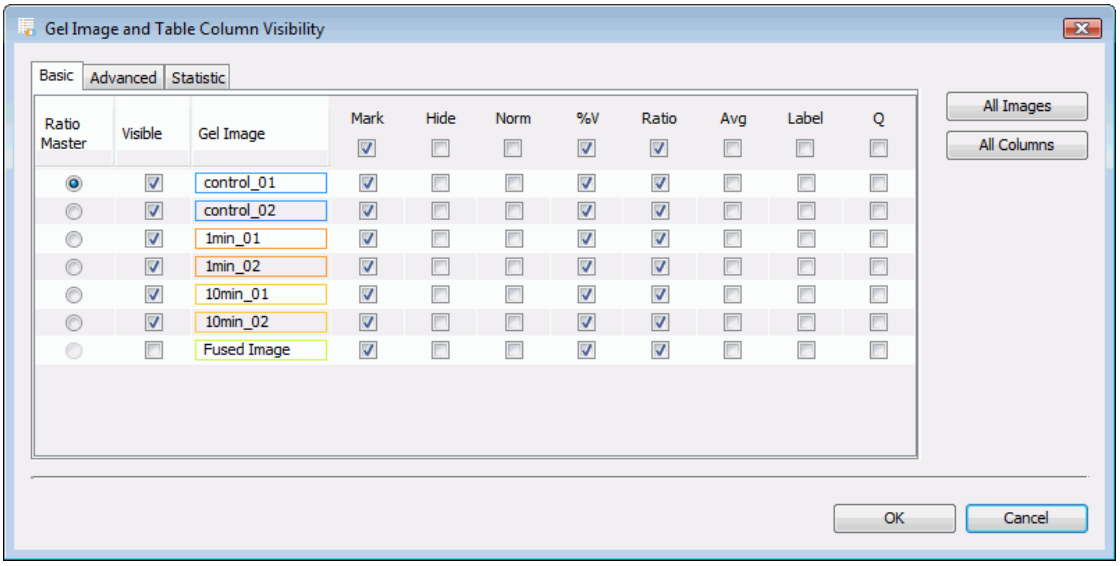


Figure 5.44.: The properties dialog of the Quantitation Table

Visible Check the gel images you want to see in your table. Visibility also applies to the Gel Regions View

Choose one of the following buttons to set multiple attributes at once:

All Images Set all images as visible.

All Columns Set all columns for all gel images as visible.

5.7. Gel Image Regions

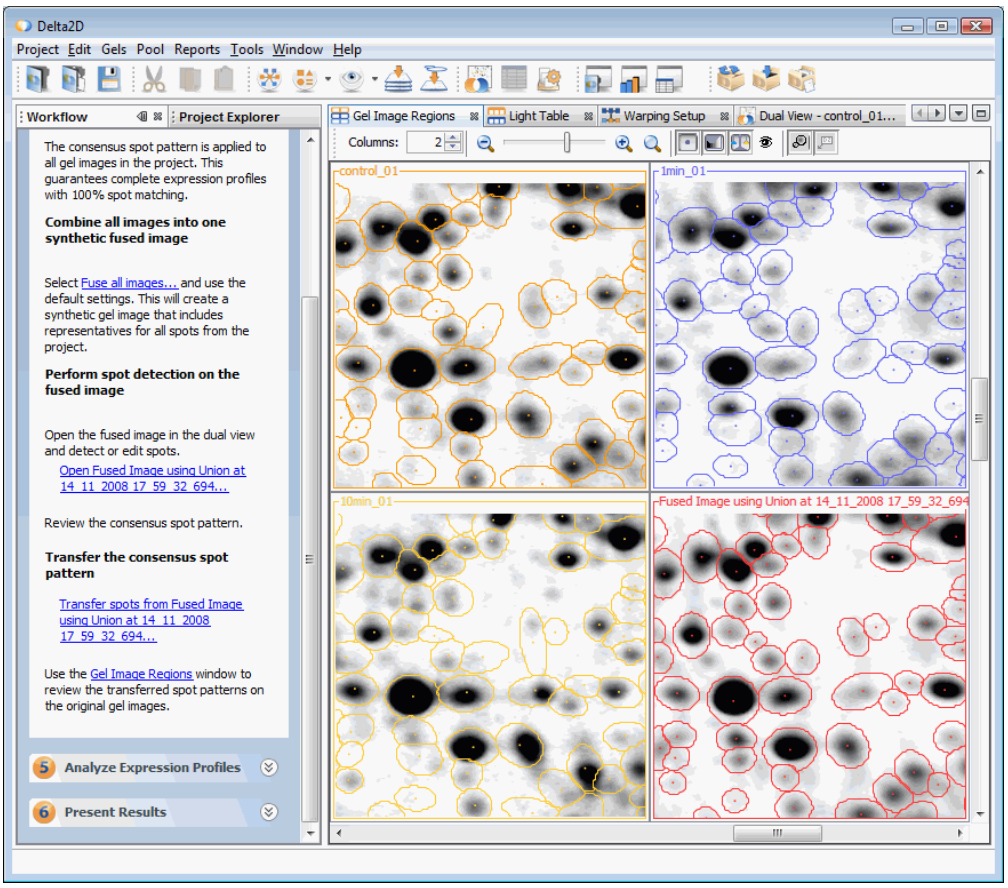


Figure 5.45.: Same region of four different gel images

The Gel Image Regions view lets you display the same image region of all gel images in the project side by side. Open the Gel Image Regions window by choosing the menu item Window ▷ Gel regions. The view looks similar to Figure 5.45 on page 82, spots will be displayed (and highlighted) if they are present on a gel. You can use the scroll bars to move the region that is displayed, or simply click in one of the views while holding the Alt key and drag in the desired direction.

When you have opened a Dual View window, its displayed area will determine the segment shown in the Gel Image Regions window.

5.8. Expression Profiles

Figure 5.46.: The expression profiles window

The Expression Profiles Window shows the barcharts for marked spots, sorted by groups. Unlike the Expression Profile Rollup, where only one barchart is shown at a time, the Expression Profiles Window can display the barchart for as many spots as you want.

Simply select interesting spots in the Quantitation Table or in the Dual View. Mark the spots in the Dual View by clicking right on one of the selected spots and check the box named Mark

spot, or in the Quantitation Table by selecting the menu item Mark ▷ Mark Selected Rows. Now you can open the Expression Profiles Window by choosing the menu item Window ▷ Expression Profiles.

You can change the size and arrangement of the barcharts with the controls on top of the window. For labeled spots each barchart shows the label in its title. Right clicking on a single barchart provides a context menu showing the spot's IDs and the opportunity to change the mark status. Unmark a barchart to exclude it from this window.

Furthermore, you can change the design of the graphs and the data shown in the menu item View:

Show Group Bars Collapsed Combine all single bars of gel images of one group to one single bar for the whole group.

Show Mean Values Show the mean values for each group.

Show Standard Deviation Show the standard deviation for each group.

Connect Mean Values Change the representation of values by bars to a line which connects the mean values, thus signaling the fold change of this spot.

Show Axis Show a scale on the left border of each graph plus axis to make it easier to read the volume of each spot.

As in any other part of Delta2D, selection of spots is synchronized between windows.

5.9. Color Coding

Color coding for spots lets Delta2D display a gel image (or proteome map) with spots colored according to theirprofiles. For an example, see figure 5.47 on page 85: are colored by the following scheme: Spots that are increased in sample 1, and in no other sample are shown in red, green is for spots that are increased in sample 2 etc. Yellow is for spots that are increased in samples 1 and 2 etc.

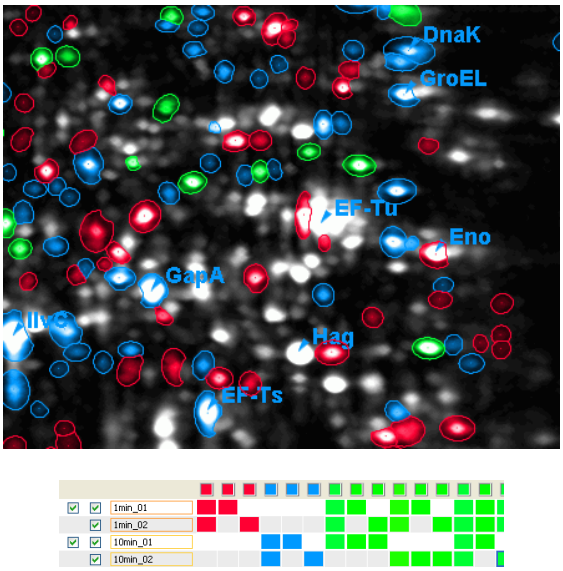


Figure 5.47.: A Region with Colored Spots. The color of a spot indicates on which sample(s) it is increased.

Start Spot Color Coding by clicking in the menu on Window ▷ Color Coding in any window of Delta2D. A new window will open, letting you determine the type and settings for color coding. Color coding can use two basic criteria for coloring the spots:

Subsets The subset of gel images on which a spot occurs is crucial for the color it will be represented with.

Min/Max The spots are colored by the gel on which they have their maximum or minimum volume.

Switch to the tab containing the options for the type of color coding you want to achieve.

Color Coding by Subsets

This option gives you an overview of the matches for every spot on a given gel. First, select the gel image which will be used as “background” for the colored spots. Then determine the subsets

of matches you want to see: Every column in the Color Coding Scheme specifies a combination of matches and a color. If a spot in the master gel image matches spots from subset of gel images specified in that column, the spot will be shown in the color of that column.

To add a new match subset, click on the button. This will add a new empty subset, which you can configure by clicking on the boxes in the column. Note that if the empty subset already appears in the table, clicking the button will have no effect.

To remove a selected subset, click on the button. You can select a subset for deletion by clicking on its column header.

To add all possible subsets, click on the button. Afterwards, the table will contain one column for each possible combination of matches across the gel images.

To delete all existing subsets, click on the button.

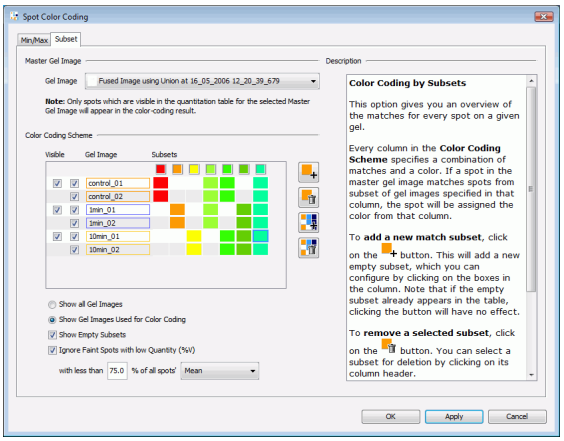


Figure 5.48.: Choose Colors and Master Image

Color Coding by Min/Max

This option enables you to highlight which group contains the spot for which a given characteristic is most strongly or weakly displayed.

Select the characteristic that you want to highlight. Each spot will be assigned the color of the group that most strongly or weakly represents that characteristic.

Example: Color Coding Spots by Subsets

By combining Spot Color Coding with spot filtering, you can visualize various aspects of your experiment. As one example, let's make a proteome map that shows which spots are increased under which conditions (or combinations of conditions).

Step 1: Detect Spots

First you need to detect spots.We recommend that you do this on a union-fused image and transfer spots to all the images that you want to include in the color coding.

Step 2: Show a Subset of Spots on Each Image

The color code will show on which gel images a spot is visible. We want to see where a spot is increased relative to its "standard" volume on the master (control) image. Therefore we filter out the non-increased spots on each of the sample images. Go to the all gel images table and set a filter for a factor of two or greater on the ratio columns. As a result you will see on every single gel only the spots whose intensity increased relative to the master.

Step 3: Choose Colors and Master Image

Choose Window ▷ Color Coding and select the tab *Subset*. A dialog will appear that allows you to configure the color coding: Select the Union image as master gel image, i.e. the gel image on which the spots will be overlayed. The table is used to configure which subsets should be displayed in which color. The leftmost check box column controls if a group is taken into account for color coding, the second check box column controls if a spot's visibility on a certain gel image will be taken into account. In the screenshot, we have checked the three sample images. On these three images there may be eight different subsets for every spot: it can be visible on sample1, or on sample1 and sample2 etc. Press the Add All button to get a list of all possible combinations. A new color is assigned automatically to each combination. You can change colors by right-clicking on the column and choosing Select color. You can change the combination a color stands for by clicking inside the table. Press OK to open the color coding window.

Step 4: Adjust the Display

In the color coding window you can use the View menu to adjust the display, for example you can choose to use the inverted image (white spots on black background).

Color Coding Spots by Intensity

There is another variant of color coding where an expression profile is colored according to the image on which it has minimum or maximum. Open the color coding dialog again and select the tab *Min/Max*. The dialog is similar to the dialog for color coding by subset. You can select one color per gel image, as well as the parameter (volume, area etc) to use for color coding.

Exporting the Color Coded Display

Use Export to PowerPoint in the File menu to export the color coded gel image to PowerPoint. You can also make a Snapshot window (using File/Snapshot) which can then be exported to a variety of image formats.

5.10. Job Manager

Besides the usual procedure of doing the warping one by one by yourself, you can let Delta2D do automatic warpings for image pairs in the background while you continue to work on other things, e.g. editing labels on another gel image.

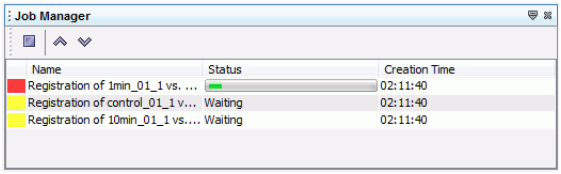


Figure 5.49.: The job manager

As soon as you assign the automatic warp mode to gel image pairs (see section 5.4 on page 35 for assigning warp strategies), the corresponding warping jobs are created, waiting in the background until their results are required. This is the case if you e.g. open a gel pair in the Dual View and apply the warp mode you have selected.

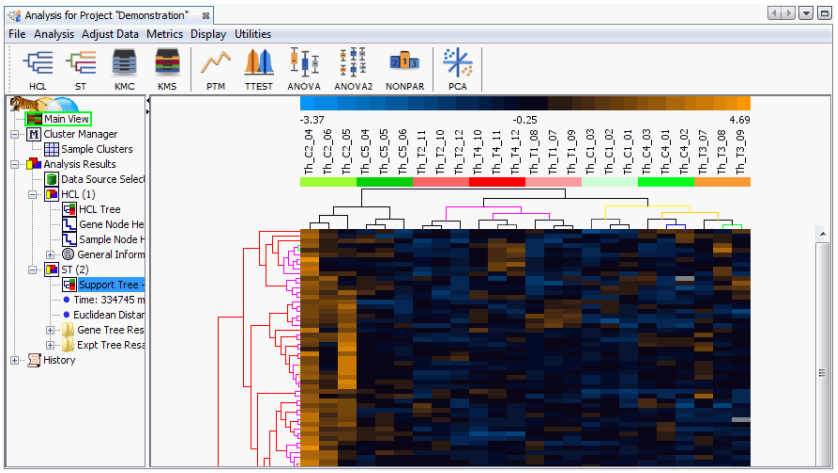
Select Window ▷ Job Manager to open the Job Manager window (figure 5.49 on page 88). By default, the execution of tasks is stopped. To activate it, press the play button . Now background execution is running; background jobs will automatically be executed for warping gel image pairs. The Job Manager allows you to control the execution of these tasks.

Use the play and stop buttons to control whether the Job Manager is running. The Job Manager shows the jobs that are currently on its task list. Only one job is executed at a time, a progress bar shows how much of the current job has been completed. You can change the order in the task list by pressing the arrow buttons that are placed above the task list. A job can be deleted by selecting it and pressing the trash-bin button.

5.11. Analysis

Delta2D provides advanced multivariate statistics in the analysis of 2D gels, including:

- Heat map display of expression profiles
- Various methods of clustering
- Principal Components Analysis (PCA)
- T-tests with optional resampling and control of false discovery rate
- Analysis of Variance (ANOVA)
- Template matching for expression profiles
- Some non-parametric tests e.g. Kruskall/Wallis



The algorithms are adapted from the TIGR Multiple Experiment Viewer (MeV, version 4.0, tm4.org/mev.html, Saeed et al. 2003) and tightly integrated into the image analysis workflow. With Delta2D's Complete Expression Profiles, there are no missing values, and matching problems are virtually eliminated. This makes Delta2D especially well suited for the methods that were originally applied in the context of DNA microarray analysis.

Getting a High Level Overview of Expression Data - Heat Maps

Heat maps are a well-known visualization method for expression data from DNA microarrays. Expression profiles are in the rows, gel images in the columns. The legend across the top shows the color code for spot intensities. Rows are labeled based on the spot labels from the gel images. By default, data is standardized to zero mean and unit variance before being shown in the heat map. Other options for normalization are available in the Analyze menu of the statistics table.

Let us make a heat map:

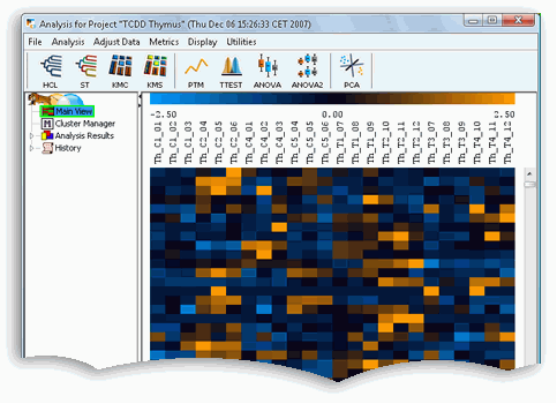


Figure 5.50.: A Heat Map

- Open the Demonstration project in Delta2D.
- Open the Quantitation Table (Window ▷ Quantitation Table), make sure the Statistics Table is selected.
- Hide the quantitative data for the fused image: Click on , uncheck the checkbox next to Fused Image, press OK (see section 5.6 on page 80 for changing the tables and/or images visibility).
- Press the Analyze button in the top left of the Statistics Table (fig. 5.51 on page 90). A new analysis window is opened, containing the current expression profiles in a heat map display.
- If you want to see more rows at once, you can use Display ▷ Set Element Size and select 20 by 5.

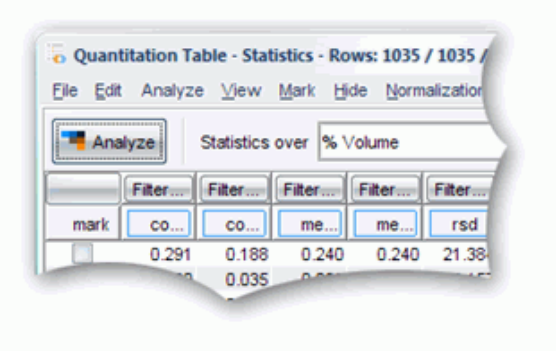


Figure 5.51.: Start analysis from the Quantitation Table

Clustering Images: What Image Groups or Classes Are There?

Clustering methods can group expression profiles and gel images by similarity. This can be very useful for getting an overview of all expression profiles before proceeding with more detailed analysises. Clustering of gel images can also be used to detect outliers, and to identify structures in the experiment. Ideally, the cluster composition will reflect the structure of the experiment, e.g. replicates and images from the same sample should have similar expression levels and thus end up in the same cluster.

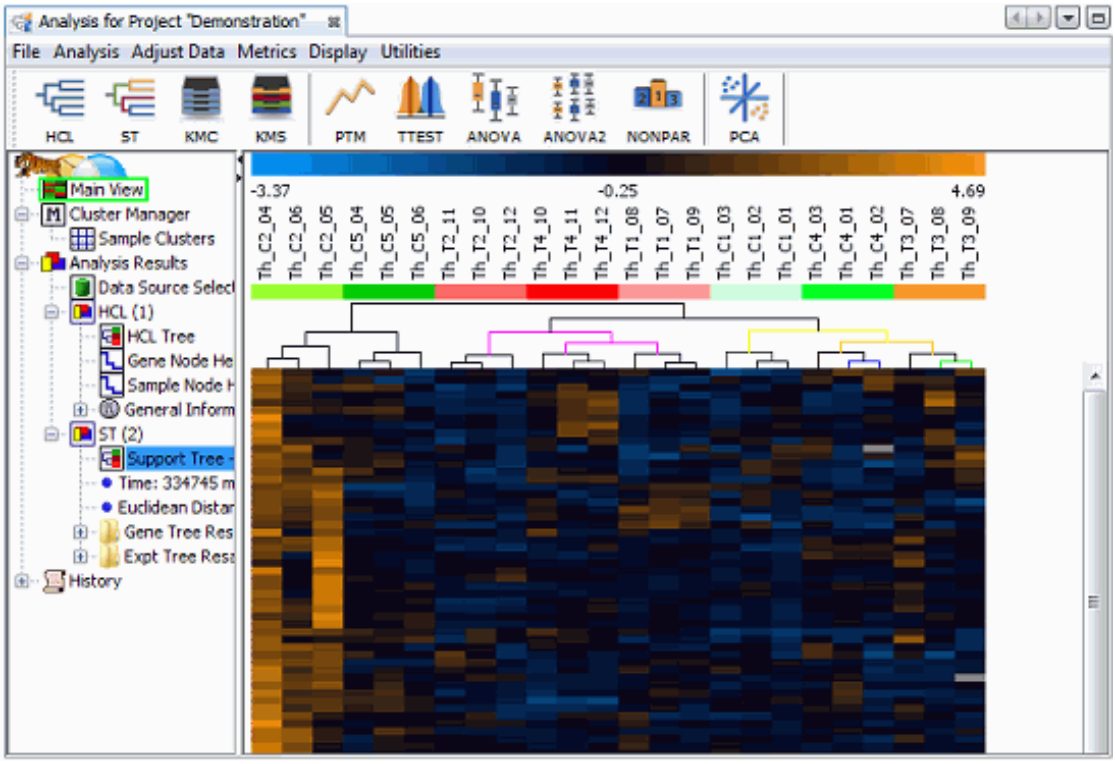


Figure 5.52.: In this clustering you see an experiment with control (C1, C2, C4, C5) and treated (T1, T2, T3, T4) samples, made in triplicates. The clustering rediscovers the experimental setup, i.e. gel images with similar samples share a cluster. A sample forming a separate cluster would indicate an outlier for which closer inspection is advisable. Made using Pearson correlation as the similarity measure between images.

Let us make a hierarchical clustering to show more structure in the data:

- Press the HCL button in the toolbar.
- Accept the default settings and press OK.

The hierarchical clustering groups both samples (gel images) and expression profiles. The cluster hierarchy is shown in a tree display. As you can see, replicates are clustered together, indicating higher similarity, as we would expect.

Clustering Expression Profiles: Finding Correlated Proteins

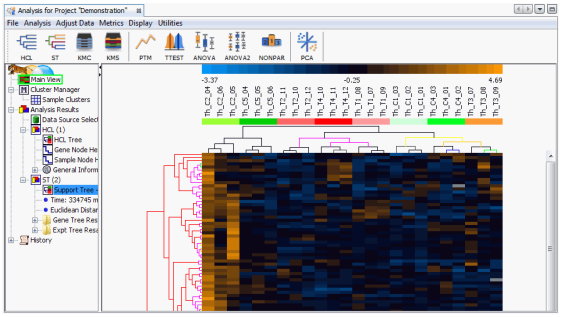


Figure 5.53.: Spots with similar expression profiles are clustered together. Support Tree clustering with Euclidean distance.

Clustering of expression profiles is done to identify proteins with similar behavior, implying that they are co-regulated or at least correlated. The global nature of the cluster display allows for a broad overview and the forming of hypotheses that can then be tested (fig. 5.53 on page 92).

Discovering Patterns in Expression Profiles

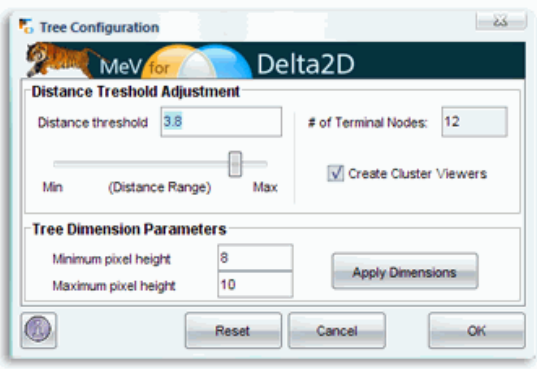


Figure 5.54.: Cutting a tree by a distance threshold. Use the slider to adjust the threshold.

One can regard the mean (or median) of a cluster as a kind of "typical" expression profile. The clustering displays allow you to split the set of expression profiles into separate subsets:

- Right click and select Gene tree properties from the context menu.

- Use the slider to cut the tree at a certain distance from the root (fig. 5.54 on page 92).
- Then check the Create Cluster Viewers checkbox and press OK.
- A new section called Gene Tree Cut is created in the left hand side of the display (fig. 5.55 on page 93).

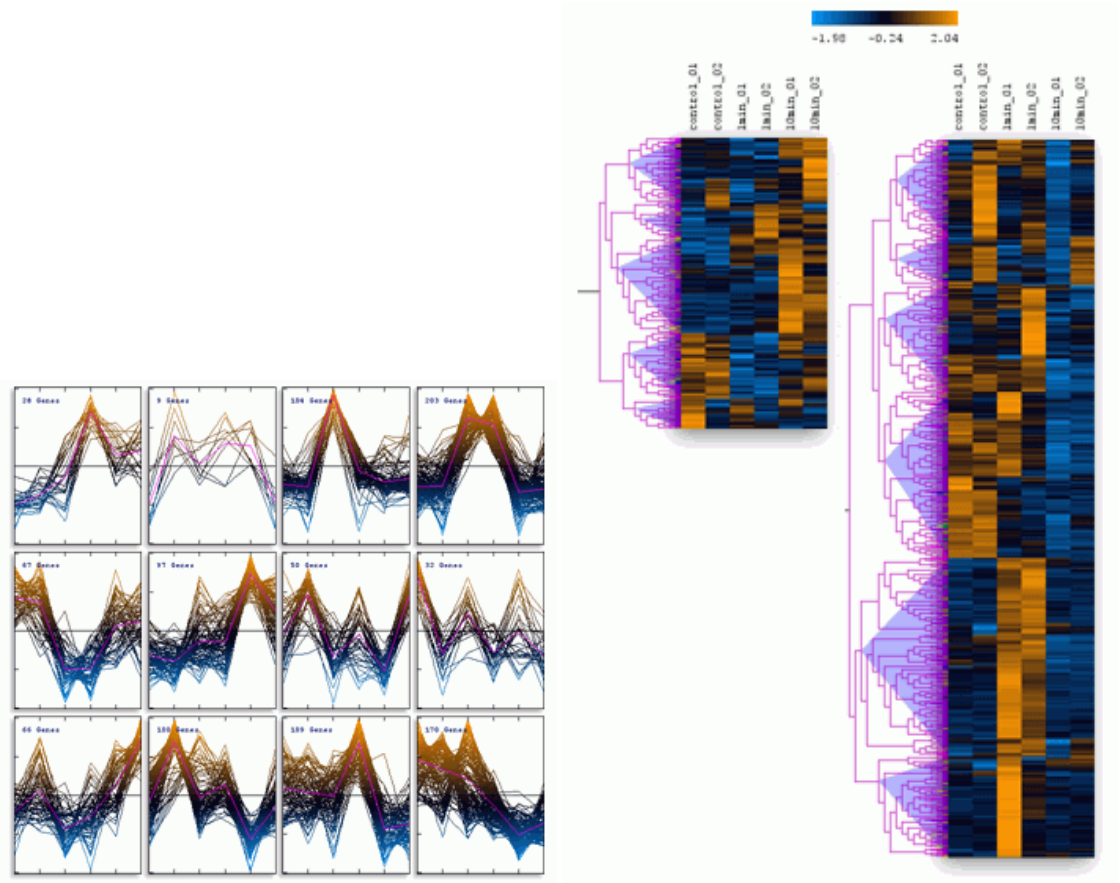


Figure 5.55.: Combined expression profiles in 12 clusters.

Finding differentially expressed proteins: Statistical Tests

Methods for statistical hypothesis testing in Delta2D are based on state-of-the-art algorithms that are applied in the context of DNA array analysis.

In the simplest case, the experiment is a comparison of two samples, e.g. diseased vs. control tissue, mutant vs. wild type etc. The task then is finding those proteins that show significant differences in expression levels. Certainly the most popular test in this area is Student's t-Test, where the null hypothesis is that the means of expression levels in samples A and B are the same. Rejecting the null hypothesis then means that the protein under test is differentially expressed.

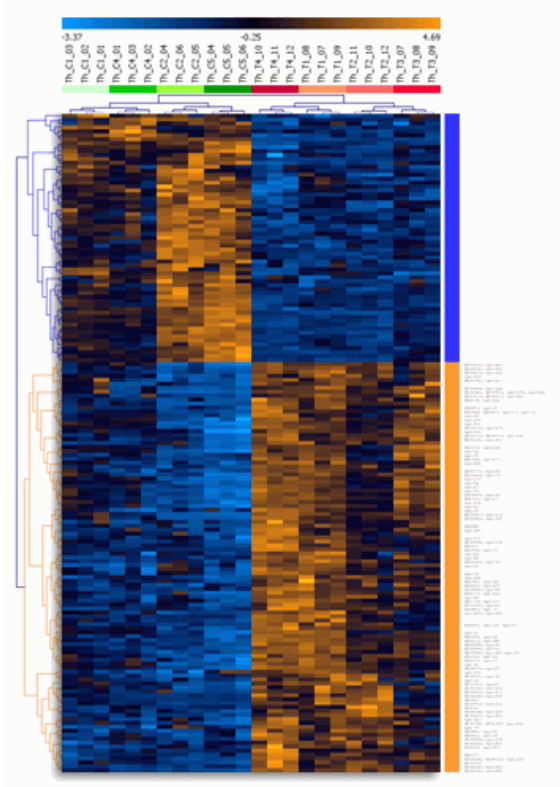


Figure 5.56.: Result of applying t-tests (control vs. treated) to expression profiles. Profiles and images were clustered to better visualize differentially expressed proteins. P-values are based on 1000 permutations, false discovery rate is controlled to be 5 elements or less (with overall alpha=1%).

No normal distribution of spot intensities required

One has to keep in mind that the classical Student's t-Test makes the assumption that spot quantities within replicates follow a normal distribution which should be tested separately. Depending on the staining method you use and other factors, spot quantities within replicate gels may not be normally distributed. Therefore it is advisable to use one of the provided methods that are based on permutations.

In the t-Test options dialog, choose "p-values based on permutation" and either "Use all permutations" or "Randomly group samples" and enter "1000".

Controlling the False Discovery Rate

When applying statistical tests to 2-D gel data, one is faced with the so-called multiple hypothesis testing problem: For each expression profile, a separate test is done. Each test has a certain probability of giving a false positive result, i.e. a protein spot is declared to be differentially expressed while the difference was due to pure chance. The large number of tests can

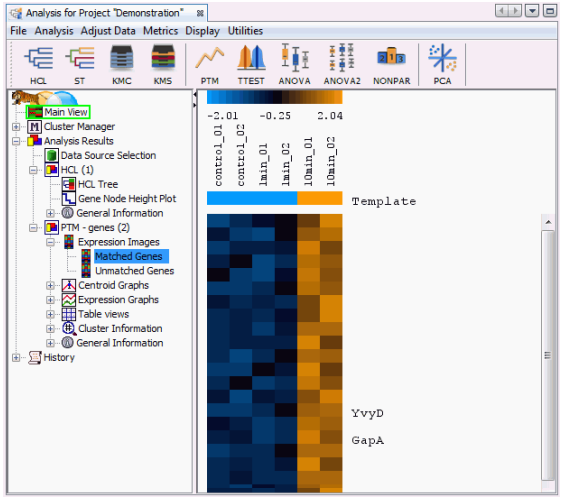
produce a high number of false positives. For example, in an experiment with 2000 spots per gel, an accepted false - positive rate alpha of 5% will result in 100 proteins that are found to be "differentially expressed" although the difference is the result of mere chance.

The MeV t-test module incorporated in Delta2D provides methods to control the proportion of false positives in the result set (*False Discovery Rate - FDR*). Overall, the False Discovery Rate approach allows one to strike a balance between the need to find statistically valid proteins of interest and the additional cost that is associated with following up on false positives.

In the t-Test options dialog, select "p-values based on permutations", "Stepdown Westfall and Young methods" and "maxT"'. Choose bounds for the number of false positive spots in the result set using the "number of false positive genes should not exceed". Alternatively choose a bound for the proportion of false positive spots in the result set, using the other radio button and text box.

Template Matching

With Template Matching, you can define a template for an expression profile and let Delta2D find spots whose expression profiles match the template. For example, in a time series experiment you might want to look for spots whose expression level increases with time.

a) 

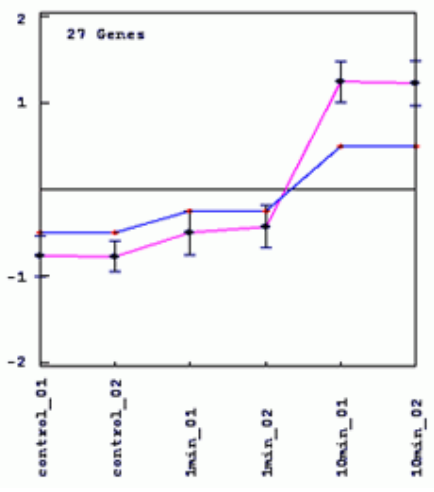
b) 

Figure 5.57.: a): Expression profiles matching the template. b): Comparison between template (blue line) and matching expression profiles.

Templates can be entered directly by specifying an expression level for every image. Alternatively you can select a spot in the list on the top left of the dialog and use its expression profile as a template by pressing Select highlighted gene from above list to use as template. Increasing the p-Value will include more spots, decreasing p-value will result in more stringent matching. Templates can also be derived from present clusters.

Click on the PTM *(Pavlidis Template Matching)* button in the toolbar, or choose Analysis ▷ Statistics ▷ Pavlidis Template Matching from the menu. The Help button (labeled "i" on the

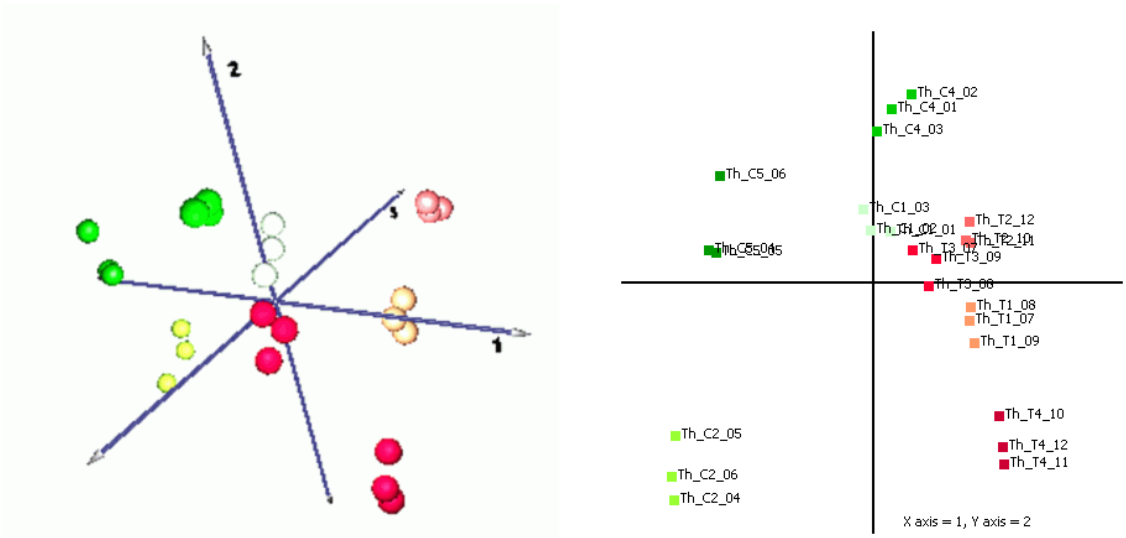
bottom left of the dialog) gives more information about the options.

Figure 5.58.: With Pavlidis template matching (PTM) you can specify a typical expression profile, e.g. one that increases with time.

Principal Component Analysis (PCA): Grouping and Visualization

When you do *Principal Component Analysis (PCA)* on a *set of gel images*, you get a two- or three-dimensional visualization of the image set that is optimal in certain sense, i.e. it preserves the variation as much as possible. PCA works by taking spot intensities on every gel image and assembling them into a vector. So an experiment of 24 gel images with 1200 spots each would be represented as a cloud of 24 points in a space with 1200 dimensions. The goal of principal component analysis is then to find a projection of the point cloud in two or three-dimensional space such that as much as possible of the variation of the point cloud is preserved. One hopes that the gels from different samples will be in separate regions of the resulting diagram. The principal components can then be interpreted as *"typical spot patterns"* or *"eigengels"*. Their coordinates can be analyzed in order to determine which spots are contributing most to the variance, making them candidates for protein identification and biological interpretation.

When principal component analysis is applied to the *expression profiles*, in our example we would consider a point cloud of 1200 vectors (one vector for each expression profile) with 24 dimensions (the expression levels on the 24 gels). The result is a display of the proteins where (hopefully) proteins with close positions are biologically related. Consider a time series experiment, where proteins are switched on and off in stages. If there is a "hidden parameter", such as a stage in the cell cycle, it will have a systematic influence on the expression levels, and thus increase the variance for the genes taking part in it. This increased variance will then become part of the directions that are used for the projection (the principal components). The principal components were also called *"eigengenes"*, they can be seen as *"classes of most prominent expression profiles"* see, for example, Alter et al. 2000 and Holter et al. 2000.



a) b)

Figure 5.59.: a): Principal component analysis of 24 gel images in 3 dimensions. Parallels have the same color. The view can be rotated by dragging with the mouse. Again, replicates are placed close together. b): The same principal component analysis of 24 gel images, projected onto the first two principal components. Treated and control samples (reddish vs greenish colors) can be separated.

Working with Sets of Spots

In the terminology of the *TIGR Multiple Experiment Viewer (MeV)*, a cluster can be any set of expression profiles or samples (gel images). You can create new clusters by choosing Store Cluster in many displays of analysis results.

Storing a cluster of expression profiles:

- In a clustering display, select the expression profiles of interest. In a hierachical clustering, you can select a whole branch of the dendrogram by clicking it in the tree. The corresponding expression profiles will be selected.
- Now right-click and select Store Cluster. The new cluster will be shown in the Cluster Manager under Gene Clusters.

Storing a sample cluster:

- In a hierarchical clustering, click on a part of the dendrogram for samples (column dendrogram), maybe you want to select a set of replicate gel images.
- Note how columns are selected in the heatmap display. Now right-click and select Store Cluster.

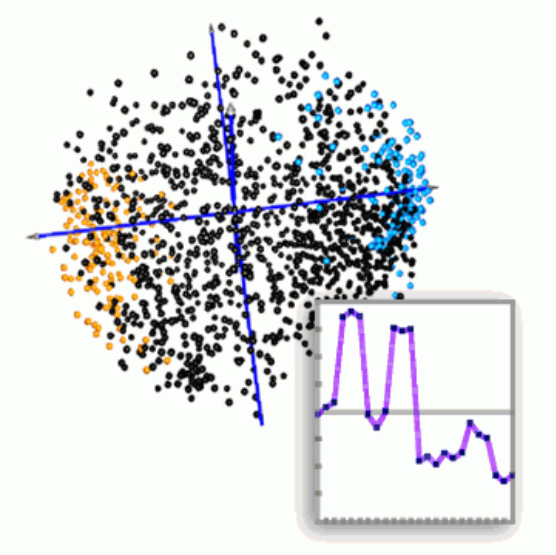


Figure 5.60.: Principal component analysis of expression profiles in three dimensions. Differentially expressed spots were determined by t-test and highlighted orange and blue, respectively. Inset: First principal component.

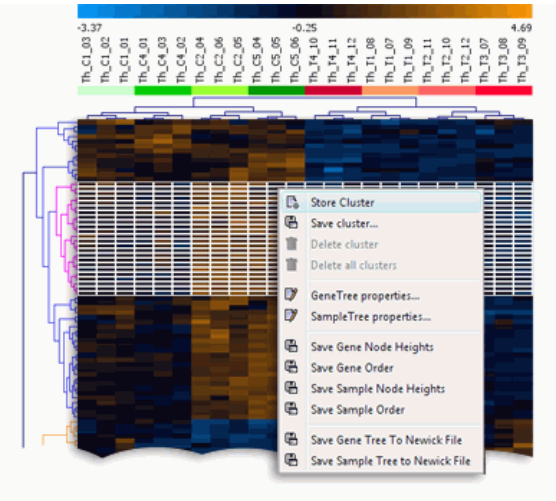
- A dialog opens that lets you define a name, comment and color of the cluster. You will have to select at least a color. Click the OK button.
- The new sample cluster should now be visible in the Cluster Manager. By default, the color of the cluster will now be shown on top of the heatmap column, and in other displays such as *PCA* (for samples).

In the Cluster Manager you can change any attribute, e.g. cluster colors, or whether the color should be used in displays. Note that clusters may overlap, but only one cluster's color will be used in displays.

When you have multiple clusters you can create new clusters that are combinations of selected ones:

- Intersection: The new cluster contains only expression profiles that are present in each of the selected clusters.
- Union: The new cluster contains all expression profiles that were present in any of the selected clusters.
- XOR: The new cluster contains only expression profiles that are found exclusively in one of the selected clusters.

In the Cluster Manager, select the clusters you want to combine. Right click, then select the operation you want to perform from the ClusterOperations submenu.



Serial #	Source	Algorithm ...	Cluster Node	Cluster La...	Remarks	Size	Color	Show Color
6	Algorithm	HCL (1)	HCL Tree	C1		3		☑
1	Algorithm	HCL (1)	HCL Tree	C2		3		☑
7	Algorithm	HCL (1)	HCL Tree	C4		3		☑
5	Algorithm	HCL (1)	HCL Tree	C5		3		☑
4	Algorithm	HCL (1)	HCL Tree	T1		3		☑
3	Algorithm	HCL (1)	HCL Tree	T2		3		☑
8	Algorithm	HCL (1)	HCL Tree	T3		3		☑
9	Algorithm	HCL (1)	HCL Tree	T4		3		☑

Cluster A Cluster B

Intersection of A and B Union of A and B XOR of A and B

Statistical Analysis is Integrated with Image Analysis

When you select one or more spots in a heatmap display, the selection will be immediately visible in other parts of Delta2D, such as the Dual View, or the Gel Image Regions View. You can extend the selection to a range of rows by holding down the Shift key while clicking on the end of the range. You can add or remove a single row by holding down the Ctrl key while clicking on it.

If you have organized spots of interest in the Cluster Manager, you can use these directly in Delta2D. Just right click on a cluster and choose Select in Delta2D this will select the

expression profiles in the cluster throughout all parts of Delta2D.

Getting a Spot Album of Relevant Spots

Using Delta2D's Spot Album Report, it is easy to show snapshots of the statistically significant spots you have found. All you have to do is mark these spots in the Delta2D project:

- Make sure you have selected the spots of interest.
- Switch to the Statistics tab of the Quantitation Table and choose Mark ▷ Unmark all spots to unmark all spots that you might have marked previously.
- Then choose Mark ▷ Mark selected spots.
- Then Reports / Spot Album. Note that the spot album may by quite large, as there is one image for each spot on each image. You can restrict the album to a single group by clicking on the *"hide others"* link in the group caption.

For more information about *Reports* see also section 3.6.

Overview of Statistical Methods

The following is a list of methods, for in-depth information please refer to the MeV manual and the original papers cited below.

Clustering

- Clustering can be applied to samples and / or expression profiles
- Hierarchical clustering and k-Means / k-Medians clustering
- Supports average linkage, complete linkage, and single linkage for determining cluster-to-cluster distances
- Supported distance metrics: Euclidean distance, Manhattan distance, Pearson correlation, Pearson uncentered correlation, Pearson squared correlation, Average dot product, Cosine correlation, Covariance, Spearman's rank correlation, Kendall's tau.
- Construction of support trees by resampling methods: bootstrapping (resampling with replacement), and jackknifing (resampling by leaving out one observation).

HCL - Hierarchical Clustering Eisen, M.B., P.T. Spellman, P.O. Brown, and D. Botstein. 1998. Cluster analysis and display of genome-wide expression patterns. *Proc. Natl. Acad. Sci. USA 95:14863-14868.*

ST - Support trees (Bootstrapping) Graur, D., and W.-H. Li. 2000. *Fundamentals of Molecular Evolution. Second Edition. Sinauer Associates, Sunderland, MA. pp 209-210.*

KMC - K-Means Clustering Soukas, A., P. Cohen, N.D. Socci, and J.M. Friedman. 2000. Leptin-specific patterns of gene expression in white adipose tissue. *Genes Dev. 14:963-980.*

Template Matching

- Templates can be defined for expression profiles and samples.
- Templates can be defined interactively, from a given expression profile, or from a cluster.

PTM - Template matching Pavlidis, P., and W.S. Noble 2001. Analysis of strain and regional variation in gene expression in mouse brain. *Genome Biology 2:research0042.1-0042.15.*

Principal Component Analysis

- Principal component analysis is available for both samples and expression profiles.
- Three-dimensional and two-dimensional displays are available
- New clusters can be defined by dragging in a two-dimensional display.

Raychaudhuri, S., J. M. Stuart, & R. B. Altman 2000. Principal components analysis to summarize microarray experiments: application to sporulation time series. *Pacific Symposium on Biocomputing 2000, Honolulu, Hawaii*, 452-463. Available at http://smi-web.stanford.edu/pubs/SMI_Abstracts/SMI-1999-0804.html

Statistical Hypothesis Testing

TTEST - T-Tests

- T-tests: one-sample, between samples, paired t-test
- Assuming equal or different group variances
- P-values can be computed based on normal distribution or using randomization.
- Corrections for multiple testing: Bonferroni, adjusted Bonferroni, Westfall-Young
- Control of false discovery rate
- Volcano Plot

Pan, W. (2002). A comparative review of statistical methods for discovering differentially expressed genes in replicated microarray experiments. *Bioinformatics 18: 546-554.*

Dudoit, S., Y.H. Yang, M.J. Callow, and T. Speed (2000).Statistical methods for identifying differentially expressed genes in replicated cDNA microarray experiments. *Technical report 2000 Statistics Department, University of California, Berkeley.*

Welch B.L. (1947).The generalization of 'students' problem when several different population variances are involved. *Biometrika 34: 28-35.*

ANOVA - One-way Analysis of Variance

- P-values can be computed based on F-distribution or using randomization.
- Corrections for multiple testing: Bonferroni, adjusted Bonferroni, Westfall-Young
- Control of false discovery rate

Zar, J.H. 1999. *Biostatistical Analysis. 4th ed. Prentice Hall, NJ.*

TFA - Two-factor Analysis of Variance Keppel, G., and S. Zedeck.1989. *Data Analysis for Research Designs*. W. H. Freeman and Co., NY.

Manly, B.F.J. 1997. *Randomization, Bootstrap and Monte Carlo Methods in Biology*. 2nd ed. Chapman and Hall / CRC , FL.

Zar, J.H. 1999. *Biostatistical Analysis*. 4th ed. Prentice Hall, NJ.

References

Saeed AI, Sharov V, White J, Li J, Liang W, Bhagabati N, Braisted J, Klapa M, Currier T, Thiagarajan M, Sturn A, Snuffin M, Rezantsev A, Popov D, Ryltsov A, Kostukovich E, Borisovsky I, Liu Z, Vinsavich A, Trush V, Quackenbush J. TM4: a free, open-source system for microarray data management and analysis. Biotechniques. 2003 Feb;34(2):374–8.

Alter O, Brown PO, Botstein D (2000) Singular value decomposition for genome-wide expression data processing and modeling. Proc Natl Acad Sci U S A 97:10101–10106

Holter NS, Mitra M, Maritan A, Cieplak M, Banavar JR, Fedoroff NV (2000) Fundamental patterns underlying gene expression profiles: simplicity from complexity. Proc Natl Acad Sci U S A 97:8409–8414

TIGR Multiple Experiment Viewer (MeV): http://www.tm4.org/mev.html

TIGR MeV manual: http://www.decodon.com/Support/Documentation/MeV

5.12. Project Matrix

In the Project Matrix, previously known as the Project Manager, every gel is represented by a thumbnail image. Drag the line between two header cells to make the thumbnail larger or smaller. You can drag the gel images to change the order of the gel images. A small icon in the header indicates whether there is a quantitation result available for the gel image. Another icon shows if there are labels attached to this gel image. As a rule, icons appear only if spots are detected or labels exist, respectively.

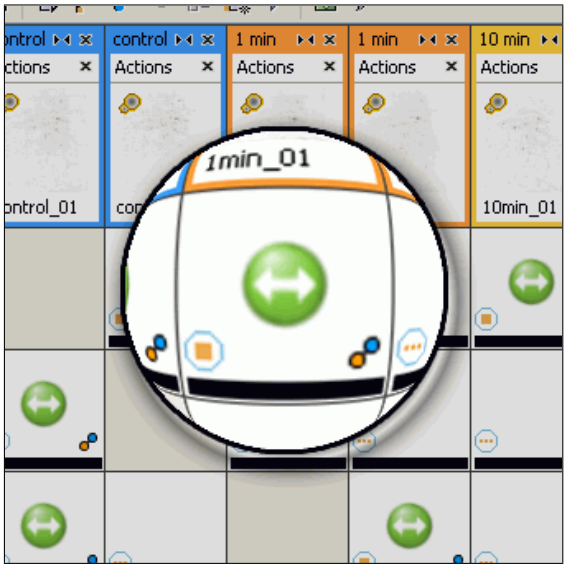


Figure 5.61.: Details in project table

You can invoke operations on a gel image or on a gel image group by using the entries in the thumbnail's context menu (see Table 5.6 on page 104). Right click on a gel image thumbnail to open the context menu.

Open Dual View with ▷	Choose another gel image to open the selected gel image with in the Dual Image View.
Move Gel Image to Group ▷	Choose the group to move gel image to.
Add Gel Image to Group ...	Add a gel image that is not used in the current project yet from the pool to the selected group.
Remove Gel Image from Group	Remove the selected gel from group.
Gel Image Properties ...	Shows properties of the gel and add a comment.
Fuse all Images	Create a new image, by fusing all images of your project (see section 7 on page 111).
Fuse Images in This Group	Create a new image, by fusing all images of the group you choose.
Quantify Gel Image ...	Detect spots on the selected gel (Only applicable if no quantitation data available).
Transfer Spots to Gel Image ▷	Transfer the spots boundaries of the selected gel to other gel(s) (see section 3.4 on page 10).
Spot Color Coding ▷	Use the selected gel image as basis for a new Spot Color Coding view.
Collapse Group	Collapse all gel images of a group under the currently selected gel.
Remove Group	Remove the selected group from the project.
Group Properties ...	Change name and color of the group.

Table 5.6.: The context menu in the project table header

5.13. Arrange Windows

Delta2D is based on a modern window manager that allows for easy reconfiguration of the window setting.

You can drag the windows to other positions in the main Delta2D window or you can undock them so that you can freely arrange them on your desktop.

To drag a window click on its title bar and move it around. If you place the window to an alternative valid position the new position is highlighted with a frame. Drag the window and it will keep its new position until you change it again. Closing and re-opening does not affect the position.

To undock a window right-click on its title bar and choose Undock Window to seperate it from the Delta2D window.

6. The Gel Pool

The gel pool is the central repository of the set of gel images that you want to compare. Along with the images, it stores their labels, quantitation results and match vectors. You can take sets of the gel images in the pool to group them into projects for analysis. A gel image can be part of more than one project.

Delta2D keeps the pool in a directory on your hard disk. It is possible to use several independent pools while Delta2D is always working with one pool at the same time only. To create a completely new pool or to change to another existing pool, Pool ▷ Change Pool.... Alternatively you can use Project ▷ Open... and click on the Change Pool... button. Now either select your preferred directory or create a new one by clicking on Create Folder. Type in the name for the new folder and confirm your input with Enter. Make sure the new folder is selected and click on OK to open it. If you confirm the following security request, the new folder will be transformed into a pool with the necessary structure, ready to hold your data.

Since the freshly created pool is empty, as next step you will be asked to create a new project. Enter the name, the author and maybe a short description in the appropriate fields and create the new project by hitting OK.

Note: Do not change the file structure in the pool directory and do not edit the files, otherwise Delta2D may not be able to find the data or to build up your projects. You can move or copy whole gel pools to other directories, drives, or network places. To make a backup of your data, just save the pool directory. Of course, you may read the data kept in the pool directory at any time using third party software.

Note: With version 3.1 and again with Version 3.4 of Delta2D the data format used in the pool has changed. Opening pools created with earlier versions ($\leq$ V 3.0) represents no problem, but the opposite way does not work. If it still is necessary to work with older versions on a pool which was in use with version 3.1 or newer, you can export the pool from the newer version of Delta2D in the former format. To do this, please select Pool ▷ Export ▷ As Version 3.0 .... Contact us for assistance to save a 3.4 pool in a format readable by Version 3.1, 3.2, or 3.3.

6.1. Adding Gel Images to the Pool

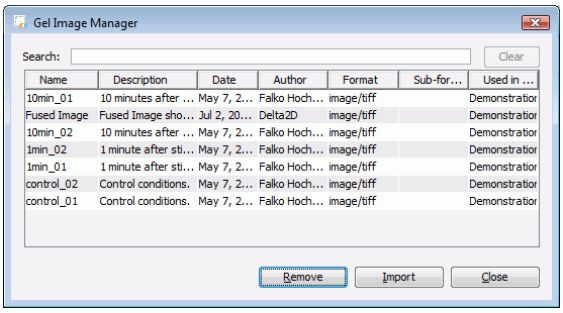


Figure 6.1.: The Gel Image Manager

Before you can analyze gel images, they have to be imported into the gel pool. To do this, click on an empty group, or open the Gel Image Manager (figure 6.1 on page 107) by selecting Pool ▷ Gel Images.... The Gel Image Manager lists the gel images contained in your pool. Press the Import button to add a new gel image (see figure 6.2 on page 108). The gel image import wizard opens. Browse to the directory where your gel images can be found and select one or more image file(s) you want to import. Files of the formats *.gel, *.img, *.tiff, *.png, *.jpg and others are supported. If you import your images one by one, you have the opportunity to describe and perform minor, maybe necessary corrections:

A small double preview of the selected image is instantly created. (In case your images folder is accessed through a slow network connection, a slow CD- or DVD drive and/or contains very large gel images this can take a while.) The left thumbnail preview shows the image in the state it is before applying any changes to it, whereas the right preview shows an instant preview of any change you apply with the buttons below: Use the button to flip the image horizontally and the button to flip it vertically. To rotate the image, use the button , and invert it with .

> Note: The gel pool contains copies of the gel image files that were imported into it. Thus, the original data is left unchanged and you can continue to work with the gel images even if the original files are moved or deleted.

6.2. Assigning Gel Image Attributes

Assigning gel images to the corresponding gel, sample and channel is necessary for multichannel projects (e.g. DIGE setups, for details on multichannel techniques please refer to section 5.2 on page 29), but can also be quite useful in traditional projects for administration of you gel images. There are two ways to do this: right away during import of the gel image (fig. 6.3 on page 108)

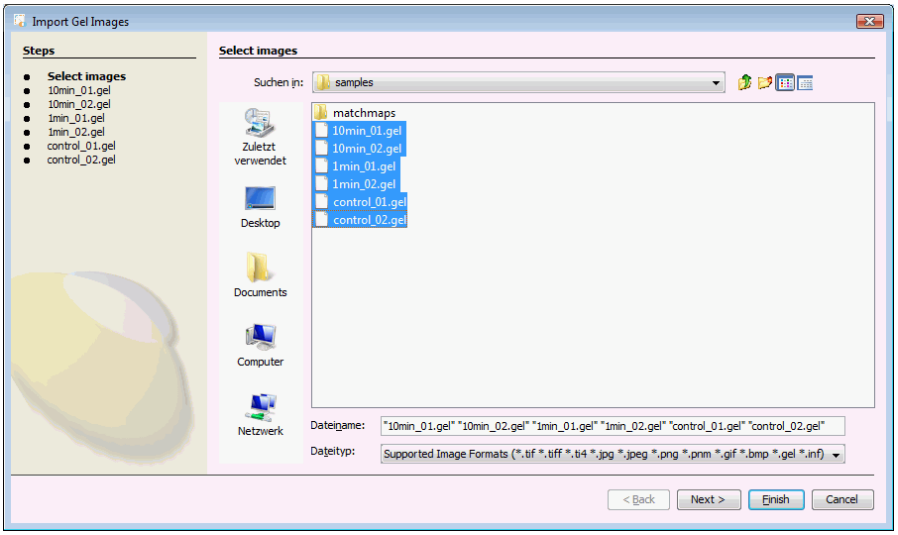


Figure 6.2.: Image Import Dialog

or later on in the attributes dialog (fig. 6.5 on page 110).

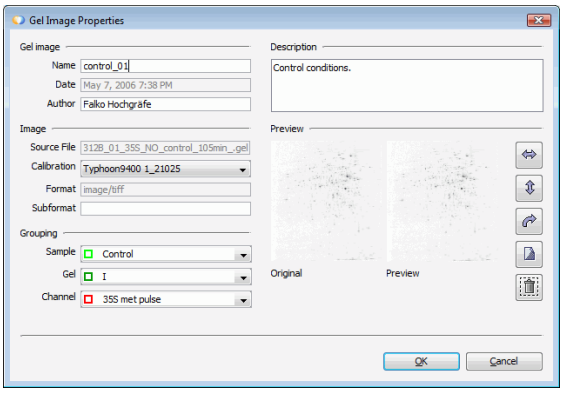


Figure 6.3.: The gel image properties dialog, available when importing images into the pool, or by right click on an image or group and selecting Add New Gel Images. . . .

When importing gel images into your pool (please refer section 6.1 on page 107 on how to do this), the gel image properties dialog (fig. 6.3 on page 108) will appear after having selected the image file you want to import.

Each of the drop down boxes is preconfigured with reasonable values from which you can select one immediately. To create your own assignments, simply choose the second option of any of the drop down boxes, saying Add new Gel (Channel, Sample respective). In the now upcoming dialog (fig. 6.4 on page 109) you can create a new item by typing in the desired name

and assigning it the desired color. The newly created item is set as assigned to the current gel image and added to the selection list of the respective drop down box, thus available to be chosen for any other image.

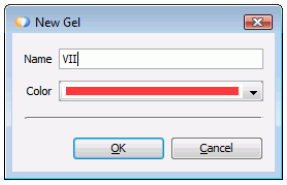


Figure 6.4.: Create a new gel image attribute, here a gel.

The other option to do the above assignments for all gel images is to use the Gel Image Attributes dialog (fig. 6.5 on page 110). Please choose Gels ▷ Gel Image Attributes... from the menu to open it. The Gel Image Attributes dialog presents three tabs on top, one for each of Gel, Sample, Channel. The tabs are very similar, so we describe the procedure of assigning attributes to one or more images exemplarily on the list of Gel Images.

On its left side, the window shows from top to down:

- the current project
- all gel images contained in your current project
- a list of all available gel assignments, by default labeled with roman numerals.

On its right side, the window shows already available assignments of attributes.

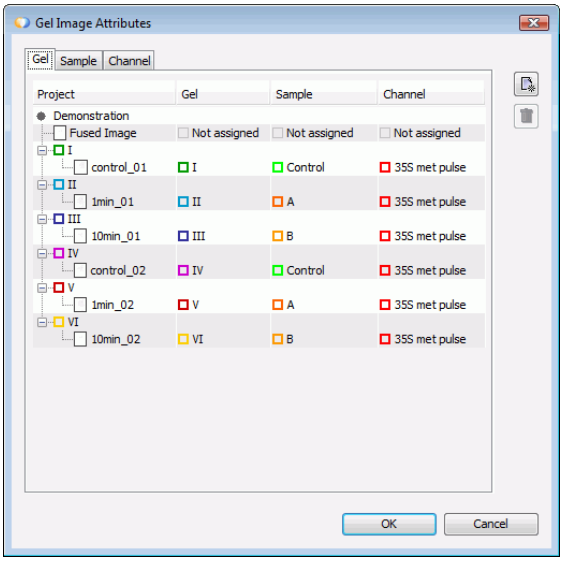


Figure 6.5.: The gel image attributes dialog, accessable by choosing the Gels ▷ Gel Image Attributes... menu item.

You can assign a gel image to a gel in two ways :

Context menu: select one or more gel images in the list, right click on (one of) the selected image(s) and assign the respective attribute in the context menu to all selected images. Using this method, you can also do the assignments of Sample and Channel without switching to the other tabs of this dialog.

Drag and drop: again select one or more gel images in the list, click on one of them and drag it on the target gel without releasing the mouse button. If more than one image is selected, please make sure to hold down the Shift key when clicking on one of the images to "drag" them. As soon as your target gel is highlighted, you can "drop" the images by releasing the mouse button and the images are assigned to this gel.

If you want to introduce a new Gel to be assigned, please click on the button on the top right of the dialog. To remove a gel from this list that you do not need anymore, select the respective gel and click on the button on the top right.

7. Image Fusion

Image fusion based on image warping is one of Delta2D's outstanding features. It combines multiple gel images to one new, artificial but realistic looking composite image. You can combine all images in one group or even your entire project.

Why image fusion makes your work much more efficient

Fused images are useful in many ways:

- Obtain 100% matchings when generating a project wide spot consensus on such a proteome map, thus having definite expression profiles.
- Save time by doing spot editing and filtering only once at only one place and not on every gel of your experiment.
- Keep track of all proteins identified during long periods of experiments.
- Produce valid illustrations you will never achieve with physical procedures.
- Relativize experimental variation through several replicates in one valid representation.
- Reduce large numbers of replicate gel images to one representative.
- Condense the whole experiment's information in one representative proteome map summarizing spot identifications and expression behaviour.

Image fusion algorithms

Depending on the purpose, four different algorithms can be used:

Union Fusion This algorithm is using a weighted average function where dark pixels are preferred with high weights. A spot that is only present on one or a few of the images will be retained in the fused image because it is given high weight compared to the lighter pixels. Slight variations in spot positions still produce a realistic-looking spot on the fused image.

This is the most robust method and is recommended if you want to create a proteome map showing each spot appearing on any gel image used for this fusion.

Average Fusion This algorithm averages the grey levels of corresponding pixels. A spot that is visible on only a small fraction of the gel images will be suppressed by the background in the majority of images.

This algorithm is useful for compensating statistical or experimental variation between replicates.

Max Intensity This algorithm selects the darkest pixels of all the input images for the fused image. In the presence of saturated spots on some of the used gel images the fused image will display a combination of the input spot shapes that sometimes does not look realistic.

If you have a clean background (no artificial signals like speckles, scratches, breaks or fingerprints) and no saturated spots this approach can be used for the generation of proteome maps.

Min Intensity This algorithm selects the lightest pixel of all the input images for the fused image.

This method is useful if you want to visualize the minimal proteome over a whole experiment.

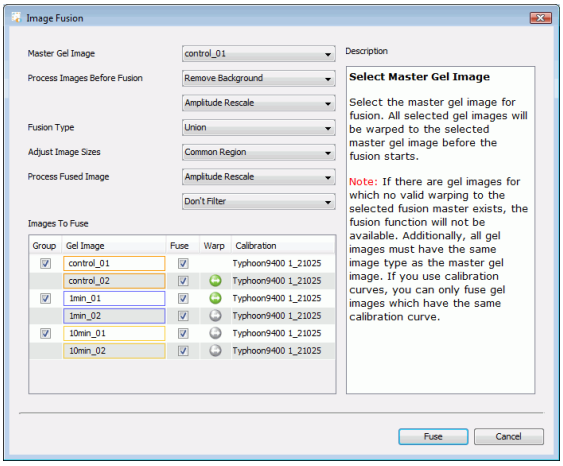


Figure 7.1.: Image Fusion dialog

Image fusion can be done by right-clicking on one of the images and choosing Fuse Images... or by pressing the Fuse Images... button. Choose from the context menu . A dialog opens (fig. 7.1 on page 112), containing some options, plus a list of the available gel images, sorted by groups.

The drop down fields let you determine some options for the fusion process:

Master Gel Image	Lets you determine which of the available gel images should serve as master for the x-y coordinate of the spots on the fused images.
Process Images Before Fusion	Here you can set two steps of preprocessing, applied in the sequence of their setting.
Fusion Type	Choose the type of fused image as described above.
Adjust Image Size	Select whether the area used for fusion is determined by the common overlapping region of all gels or the largest covered region of all images together which are used for the fusion. Common region is recommended to obtain complete expression profiles, since you then only need the area that appears on all images.
Process Fused Image	As for preprocessing, you can determine up to two image processing steps to be applied to the new fused image.

In the list of images you can check the images that shall be fused. If you have selected images before having opended this dialog the selected images are checked. You can check only those images that can be warped to the Master Gel Image.

Press OK and the fused image will be processed and added to a new group for fused images.

For more information about this matter you can refer to the article “Using standard positions and image fusion to create proteome maps from collections of two-dimensional gel electrophoresis images”, published in Proteomics 07/2003.

8. Working with Spots

8.1. Sorting and Selecting Spots

Now let us sort the table by the relative volume of the master spots. Just click into the lower part of the column header. A small arrow indicates the sort order, click again to sort in reverse order.

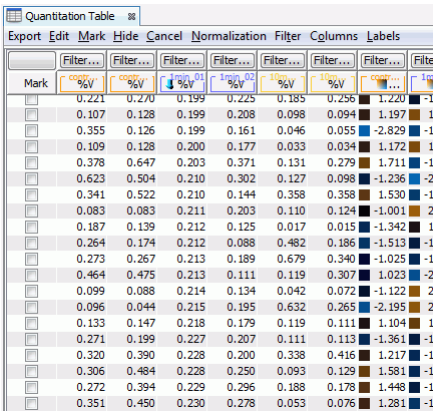


Figure 8.1.: Part of Quantitation Table, sorted on the fifth column

Sorting makes it easy to identify the most intensive spots or those with a high expression ratio: just sort and then select the top rows. The selected spots will be highlighted in the main window.

You can use any column for sorting, try, for instance, to sort on the color-coded expression ratios.

Select one of the rows by clicking on it. Observe how the corresponding spot segments on the master and sample gel images are highlighted. You can select additional rows by pressing the Control key while clicking on them. Shift-clicking on a row selects all rows up to that row. Dragging the mouse over consecutive rows selects them, too. Use the menu item Edit ▷ Select All to select all rows in the Quantitation Table, and Edit ▷ Invert Selection to invert the selection.

You can select a spot in the gel window by clicking somewhere within its boundary. The corresponding row in the table will be selected automatically.

Here is how to select the 10 most intensive spots on a certain gel image:

1. Open the single gel table for this gel image: just select only this image in the Project Explorer or in the Light Table and click the Quantitation Table icon in the main menu bar.

2. Click on the header of the column that is labeled %V. The table is now sorted according to master spot volume. In the column's header, you see a little arrow that indicates the sort order.

3. Click again to reverse the sort order. Rows are sorted in descending order now, i.e. the spot with greatest quantity is in the first row.

4. Select the first ten rows in the table: Click on the first row and drag down to the tenth line. You can watch in the title how many rows you have selected.

The selected spots are also highlighted in the Dual View, as well as in the Gel Image Regions.

8.2. Selecting Spots in the Dual View

You can select a spot in the Dual View by clicking on it. Make sure that the spots tool is activated before you select spots. Additionally, you can select spots in a rectangular region by dragging with the mouse.

8.3. Hiding Spots

The Dual View will always reflect the contents of the Quantitation Table, i.e. any spot that is visible or selected in the table will be visible or highlighted respectively in the gel window.

In some situations, it may be useful to hide some spots from the analysis. You can do this by checking the box in the "hide" column. The row will be hidden immediately. Hide a group of rows by selecting them and using View ▷ Hide Selected Rows. Since the Quantitation Table is synchronized with the main window, spots you hide in the table will also be hidden in the main window.

Check View ▷ Show Hidden Rows to see all hidden rows again. You can now click in the hide column to mark a row as visible or invisible — the display will not change. Use View ▷ Hide Selected Rows and View ▷ Do not Hide Selected Rows to control the visibility of whole groups of rows. Unchecking View ▷ Show Hidden Rows will let your changes take effect.

Of course, all these tunings can be done on any tab of the table.

8.4. Canceling Spots

A canceled spot will be excluded from the analysis just as if it would have never been detected. Single spots or rows can be canceled by clicking on the check box in the cancel column. You will sometimes want to cancel spots in a region such as the border of the image. To do this, activate the spots tool in the Dual View and select the region by dragging with the mouse. Right-click to open a context menu and select cancel to cancel all spots you have selected.

8.5. Marking Spots

You will often want to concentrate on a subset of spots, such as those with a high expression ratio. Sometimes you will select spots individually, based on your own criteria. For this purpose, Delta2D lets you make a "note" on a spot, in the mark column. You can add new marks at any point of your analysis, building an increasing set of interesting spot pairs. Later you will see how to display only marked spots, or how to do other things to spots that are marked.

A single spot can be marked by clicking on the check box in the "mark" column. When you're in a correspondence view of the Quantitation Table then this will mark all spots in the row. Mark multiple rows by first selecting them and then choosing Mark ▷ Mark Selected Rows. Marks will always be added to what you have already marked. Marks can be cleared using Mark ▷ Unmark Selected Rows.

More advanced operations can be executed by combining selection and marking. Say, you have first identified all the interesting spots by marking them and now you want to hide all other spots:

1. use Mark ▷ Select Marked Rows to select all the marked rows
2. use Edit ▷ Invert Selection to select only the rows that are not marked
3. use View ▷ Hide Selected Rows to hide all rows that are not marked

Similarly, clearing all marks can easily be done by choosing Edit ▷ Select All and then Mark ▷ Unmark Selected Rows. To see which rows you have marked, click on the mark column for sorting, this will separate marked from unmarked rows.

8.6. Counting

Delta2D helps you count how many spots are visible or selected in a table. Counts are displayed in the table's title bar. In a single gel table, it may look like this

> [image name]: 1048 / 1048 / 4

These numbers represent the number of total (1048) / visible (1048) / selected (4) items.

Select a few expression profile rows in the table and watch the table's status bar, for example:

> [Name 1]: 3598 / 2119 / 12 [Name 2]: 2205 / 1409 / 12 [Name 3]: 2265 / 1451 / 8

All counts are automatically updated when you hide or select rows.

8.7. Filtering

Sometimes you want to focus the analysis on spots that meet certain criteria, say those with an expression ratio of more than 2. Of course, you could sort according to the expression ratio column and then select those spots manually, but there is a much more convenient way to do

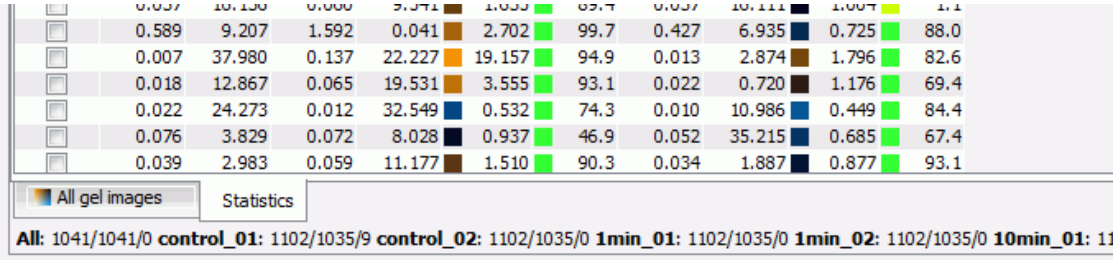


Figure 8.2.: Automatic counting in the title bar of the correspondence table.

this: use a filter. A filter will show only those rows that meet your criterion. Filters can be set on most columns, see the Filter menu for all available filters.

Let's start with using filters. Firstly we only want those rows to be displayed whose expression ratio is between 0.5 and 2. Choose Filter ▷ Ratios ▷ sample / master to get a filter dialog, or simply click on the button labeled "Filter" on top of the appropriate column.

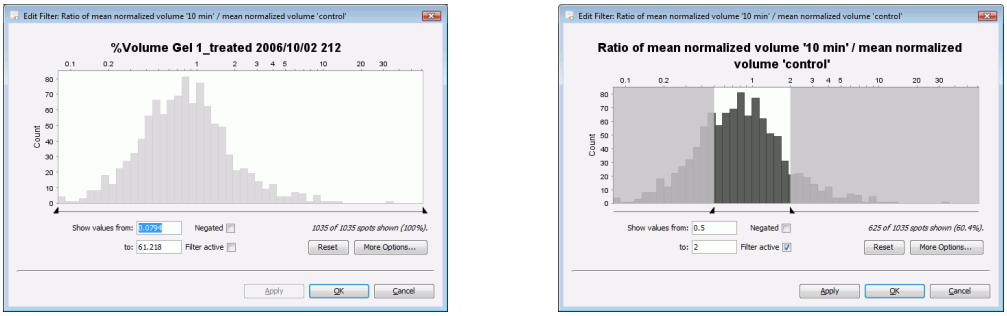


Figure 8.3.: Editing a table row filter. Here, Delta2D will show only spots that have expression ratios between 0.5 and 2.

Click on the one of the check boxes labeled Active to activate the filter. Enter 2 into the left Ratio field named First Border. Now enter 0.5 into the right Ratio field (Second Border). In the upper part of the dialog you can watch in the histogram which range of spots will be displayed. You can also use the sliders below the histogram to shift the borders of the displayed range up and down. If the movement of the sliders is not fine enough adjustable for your purposes, you can resize the dialog window to a bigger size by dragging its borders like any other window.

Another convenient way of determining borders for your filter is given by the fields above the histogram: the top most row refers to the total of all absolute values this filter refers to. You can use any value between 0 and this total to indicate how big the ranges of the low, the middle and the high interval should be.

In the second row you can determine the size of these ranges by a relative value, e.g. set the low range of the filter to 20% of the total of all values.

The third and fourth row give you the count of all values you want to apply this filter to as absolute resp. relative numbers. This makes it easy to set the lower border of the filter to let's say the 150 smallest values, or, in the fourth row, you could type in 10 to set the border to the 10% smallest values.

Press OK to save the changes you have made to the filter and close the filter dialog, or Apply to just apply the changes without closing this dialog. You can directly switch to another filter without having to close and reopen this dialog. The table, as well as the gel image, contains now only those spots whose expression ratios lie between 0.5 and 2. By looking at the table's title bar, you can tell how many rows meet your criterion. You can continue to work with the filtered table as usual.

The button in the header of the ratio column has changed to a short description of the filter. Leave your mouse pointer over the button for a while to get a tool tip that contains a more detailed description.

Filters can be combined to implement more complex criteria, such as "show all rows with expression ratio between 0.5 and 2 and master spot volume greater than 0.1".

Example: Showing Spots Whose Quantities Differ by More Than a Factor of Two

Problem: You want to focus on spots with a "significant" expression ratio, i.e. the expression ratio should be less than 0.5 or greater than 2.

Solution: Use a negated filter that shows only rows whose expression ratio is *not* between 0.5 and 2. Choose Filter ▷ ratio *your gel image names* to get a filter dialog and enter the data as shown in Figure 8.4 on page 119.

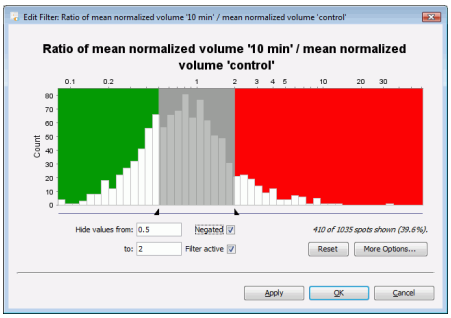


Figure 8.4.: A filter that hides expression ratios between 0.5 and 2.

8.8. Scatter Plots

In addition to the numerical possibilities to identify distinctive spots, Delta2D offers as visual tool a scatter plot. Scatter plots show the ratios of the relative volumes in two gel images. You can produce a scatter plot by going to the Project Explorer, right-clicking on a gel pair and

choosing menu item Scatter plot. Or, from the Dual View, you can use the shortcut Ctrl + L, or choose the menu item Spots ▷ Show Scatterplot.

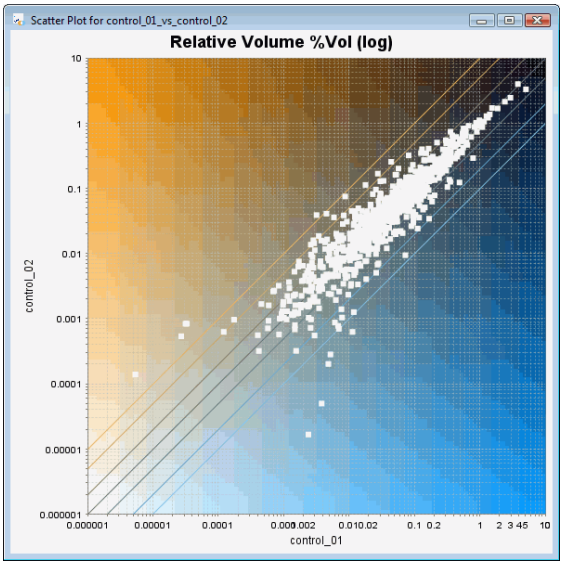


Figure 8.5.: The scatter plot

As any other part of Delta2D, the scatter plot interacts with the other parts. If you have selected one or more *matched* spots in the Dual View, they are selected in the scatter plot too and vice versa.

You can zoom in to the scatter plot by 'drawing' a rectangle around the region you want to magnify. To do this, click and drag with the left mouse button *from the top left to the bottom right*. To reset the view simply click and drag in any other direction.

8.9. Spot Picking

Delta2D can produce output for different spot pickers, as well as a generic spot picking format in tabular form. Centers of detected spots as well as arbitrary labeled points on a gel may be selected for picking.

Picklists always include marked spots and/or labeled spots.

Currently, Delta2D is shipped with support for the following spot pickers:

- Genomic Solutions ProPic
- PerkinElmer ProXCISION
- Molecular Dynamics
- Ettan Spot Handling Workstation

- Bruker Proteineer (this entry is disabled because the interface needs additional setup)
- Generic pick list format

The Generic File Format

The generic file format is a simple ASCII-text file in tabular format that includes marked spots and labels. The tabular format can be easily transformed to other formats if necessary. However, see below for what to do if your spot picker is not supported by Delta2D.

Use Spots ▷ Export generic pick list to generate a pick list in the generic format. The pick list includes all spots that are marked (using the mark check box in the Quantitation Table), together with all labels on the selected layer. For a marked spot that has no label, the spot's center will be used to define the pick. If there are one or more labels inside a spot, one pick per label will be produced, and the spot's center will be ignored. For a label that labels a point outside any spot, one pick will be generated, as well.

The generic file format consists of four columns separated by a tab. They contain following data:

Spot ID The ID of each spot as used in Delta2D.

Coordinates The next two columns mark the X- resp. Y-coordinate of the exported spot.

Label The last column contains the label of each spot.

The Molecular Dynamics™ File Format

The file is generated according to the same rules as the generic file format, i.e. when you want to pick a spot, you have to mark it in the table or place a label inside of it. The Molecular Dynamics spot picker needs two special landmarks that are placed on the gel. In order for the robot to register the gel image to the physical gel, you need to provide labels for the two landmarks. They have to be named "IR1" and "IR2", respectively. Be careful that the labels point exactly to the centers of the landmark points.

The layout of the exported text file is slightly different from the generic format. The columns are also separated by tabs except the coordinates; they are placed in one column, separated from each other by a comma. The ID in the first column is simply a serial number, not the one used in Delta2D. The ID used in Delta2D is set in square brackets and attached to the labels in the fourth column. All residuary aspects are identical with the generic format.

The Genomic Solutions™ File Format

The file for Genomic Solutions includes additional information: the image field is filled with the name of the image and the name of the project. The table consists of six columns separated by commas, out of which the last three columns consist of generic data. The first column contains the spot definition in the form `Spotn=SpotID - Label`, whereas the `n` in `Spotn` stands for the count starting from 0 and `SpotID` means the ID used by Delta2D. The second and third column contain the X resp. Y coordinates of the spot center.

The Ettan Spot Handling WorkStation™ File Format

This file format has a quite simple structure: the first of the four tab separated columns counts the spots starting from 1, the next two contain the X resp. Y coordinates of the spots and the fourth one is reserved for comments, but not used by Delta2D by now.

What if my Picker is not Supported?

We are constantly working on broadening the range of supported spot picking file formats. If your device is not supported, please do not hesitate to contact our technical support – we will be glad to work with you to find a solution.

9. Working with Spot Annotations

Delta2D allows you to place annotations anywhere on a gel image, to annotate spots and to control spot picking. These annotations, we call them labels, can be independently from spot locations; but you can also let them snap automatically to the target spot.

Labels can be created individually or automatically. They can be transferred from one gel to the other with a single click. Delta2D will place them at the corresponding position automatically, following the defined warpings. Normally labels are collected in a proteome map, but it is also possible to handle labels on single gels.

You can change label formats according to your preferences. And, for advanced usage, label data and formats are saved in XML files that can easily be processed by other applications.

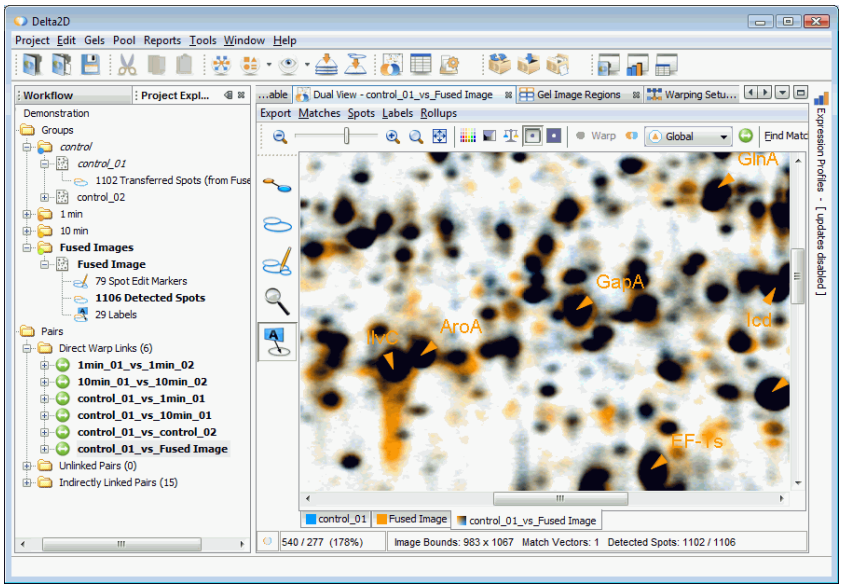


Figure 9.1.: A Dual Channel Image with Labels on the Proteome Map.

9.1. Creating a Label

You can place labels on either of the gel images. Usually, labels for both gel images will be displayed together in the Dual View, but with a different look.

To start working with labels, select the label tool in the top-left part of the Dual View (see figure 9.2 on page 124).

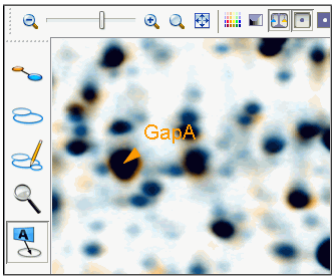


Figure 9.2.: Delta2D tool panel with activated label tool.

Now click on any point in the gel: a new label will be created where you have clicked (see figure 9.3 on page 124).

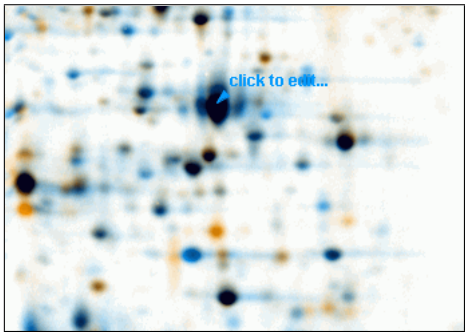


Figure 9.3.: A new label

Newly created labels are placed by default on the master image. If you want to create a label on the sample image, you can either move it there (see below), or create it directly on the sample image by holding the Shift-key pressed while clicking on the target place for the label. Now it will be created on the sample image.

Changing the Text of a Label

To change the text of the label, click once inside the label and start typing. Press Enter to stop editing. Pressing Escape will discard the changes you have made.

Moving a Label

You can move a label's text around by dragging it with the mouse. Observe how the line is placed automatically on the corners or in the middle of the label's border. When the label is sufficiently near the target, the line will be hidden in order to make the display simpler. When you want to change the label's target, you can drag the line to move the whole label to the desired place.

Label Snap

You can adjust a label's target to the nearest spot maximum. Go to the Labeling tab in the Options dialog and check the Label snap to spot check box. This makes labels snap to spot maxima when they are moved around or created. Hold down the Control key to switch this off temporarily. Alternatively, you can adjust a label by double clicking on its arrow. Right-click on a label and choose Adjust to make the label point to the spot's maximum.

Greek Symbols in Labels

You can use greek letters and symbols in labels of spots. Simply press Ctrl +G while editing a label to switch between normal and greek mode. The greek mode is indicated by a greek symbol below the label you are editing (Figure 9.4 on page 125).

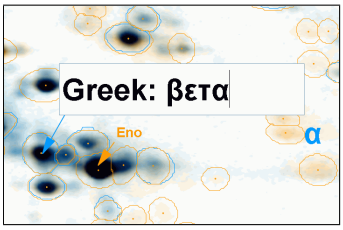


Figure 9.4.: The greek mode

9.2. Labels and Spots

In Delta2D, labels are not bound to spots, i.e. you are free to add a label to anywhere on the gel image before or after spot detection. They will not be altered or removed by (re-)detection of spots. However, if there are labels pointing to spots, which are detected as spots later, Delta2D will assign them to the respective detected spots automatically. Labels are organized separately for each gel, so a label can only be assigned to a spot that is on the same image. When a label's arrow points inside a spot, it will show up in the corresponding label column of the Quantitation Table.

The assignment is always kept up to date, for example, when you drag a label into another spot, it will be shown in that spots label column. When you import a new set of labels, Delta2D will assign them automatically as well. It is also possible to have more than one label for a spot, in that case the table will show a drop-down box with all the label texts. Only one of the labels will be visible; to select another label, you can double click on the table cell.

Creating and editing labels in the Quantitation Table

Instead of making a new label by clicking on the Dual View, you may also create it by labeling a spot in the Quantitation Table. Just double click on the spot's label field and start typing the

name. Press Enter to finish editing. The label will be automatically placed such that it points to the spots center. You can change its name by double clicking on the table cell.

You can select a set of spots on an image or in a table and then let Delta2D assign labels automatically, automatic numbering included. Define a prefix to attach labels as *Spot_01* (see section 10.1 on page 142).

Sorting and Searching for Labels

The tables can be sorted according to label name. Just click on the column header to activate sorting. Spots without a label are sorted to the bottom of the table. As with the other columns, clicking again will reverse the sort order.

Use Search ▷ Label to search for a label. The first matching entry will be selected. You may search for any part of the label's text, e.g. searching for "Cit" will find "CitZ" or "CitG", whichever comes first.

> Note: Please remember that the Quantitation Table is a table of spots. Thus, only labels associated with spots are shown in the tables and, of course, only those labels can be searched for.

9.3. Working with Labels

Labels can be operated individually or collectively by using the respective menus (see figure 9.5 on page 126.

Individual Labels

To manipulate a label individually, just right-click on it to get its context menu (see figure 9.5 on page 126).

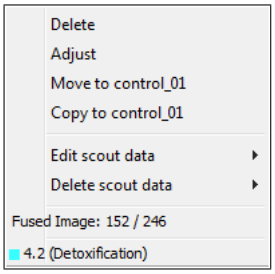


Figure 9.5.: The context menu for a label

Deleting a Label

Select Delete from a label's context menu to delete it.

Adjusting a Label

The menu item Adjust works exactly like label snap (see 9.1 on page 125). Its function is useful, if you switched off Label snap because you do not want to use it generally, but want to use it in single situations.

Moving a Label to Another Image

In the context menu you see whether the label is on the master or on the sample image. Newly created labels are always placed on the master image. Select Move to sample to move a label to the sample gel. The label's format will usually change when you move it to another gel.

The option Copy to sample creates an identical label on the corresponding point of the sample image, but leaves the label remaining on the master image. Here again, the label's format will adapt to the sample's label formats.

When both gel images are connected by a match map, the label will be moved according to that match map. Say your label is placed on the master image and you have loaded a match map for master and sample image. You then place the label inside a master spot. When you move the label to the sample gel, Delta2D will move it to the point that corresponds to the master spot. This behavior allows you to collect complete sets of labels from many different gel images which is especially useful when you want to produce a proteome map containing protein identifications. See section 9.5 on page 132 for details.

Working with Scout Data

Scout[1] data of each label is easily accessed from its context menu. Use Edit scout data ▷ to view and edit the scout data attached to the selected label. A dialog will open (figure 9.6 on page 128), showing the data attached to this label sorted by scouts. You can edit the data.

To quickly delete one scout's complete data from a label, use Delete scout data ▷ and the respective scout from its context menu. To delete one scout's data from all labels, please use the menu item Labels ▷ Delete scout data ▷ from the Dual View.

Information at a Glance

At the bottom, each context menu shows basic information concerning to the label it belongs to:

Position The position of the label is shown in the format Gelname: x-coordinate/y-coordinate.

Functional Category If available, the functional category of this protein is shown, labeled with the color of the scout who retrieved this information.

9.4. Formatting Labels

Depending on the color scheme and the gel contrast sometimes it is necessary to adapt the label format to ensure optimal visibility. Furthermore dynamic label coloring (section 9.4 on

[1] for more information about scouts, please refer to section 9.7 on page 136

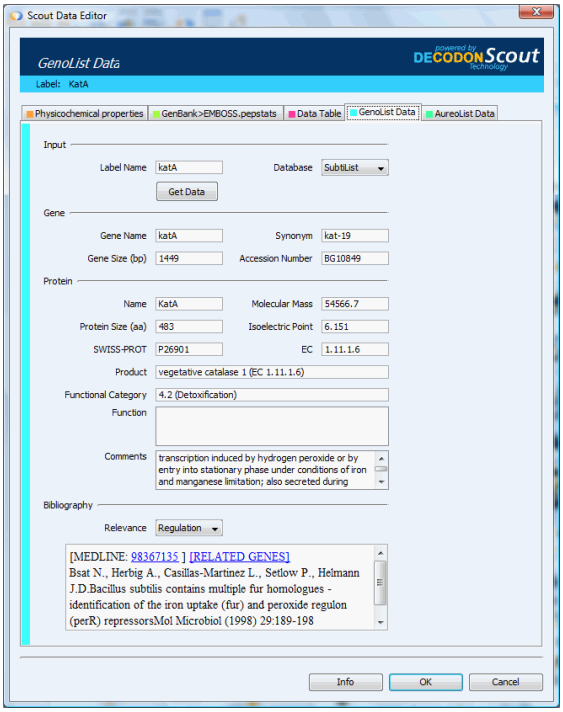


Figure 9.6.: The scout data attached to one label

page 130) is an interesting tool for the visualisation of protein or spot properties.

The appearance of labels can be changed in various ways. You may define different formats depending on whether the label is on the master or sample gel, and whether it is displayed on a single gel image or on a dual channel image. For each of these cases you define a separate label format, using the formatting dialog (see figure 9.7 on page 129). And of course, you can also save and load appearance configurations for labels.

The Label Formats dialog can be invoked using the menu entry Labels ▷ Formats....

The Label Formats Dialog

Managing the Label Formats Dialog

Individual Appearance on each view To make control of individual appearance of labels in each view easier, the Label Formats Dialog offers an overview of your settings in the left side of the window. It shows four small previews:

master labels on single view and dual view

sample labels on single view and dual view

Select one of the four small previews to see and adjust the label format settings for this specific view.

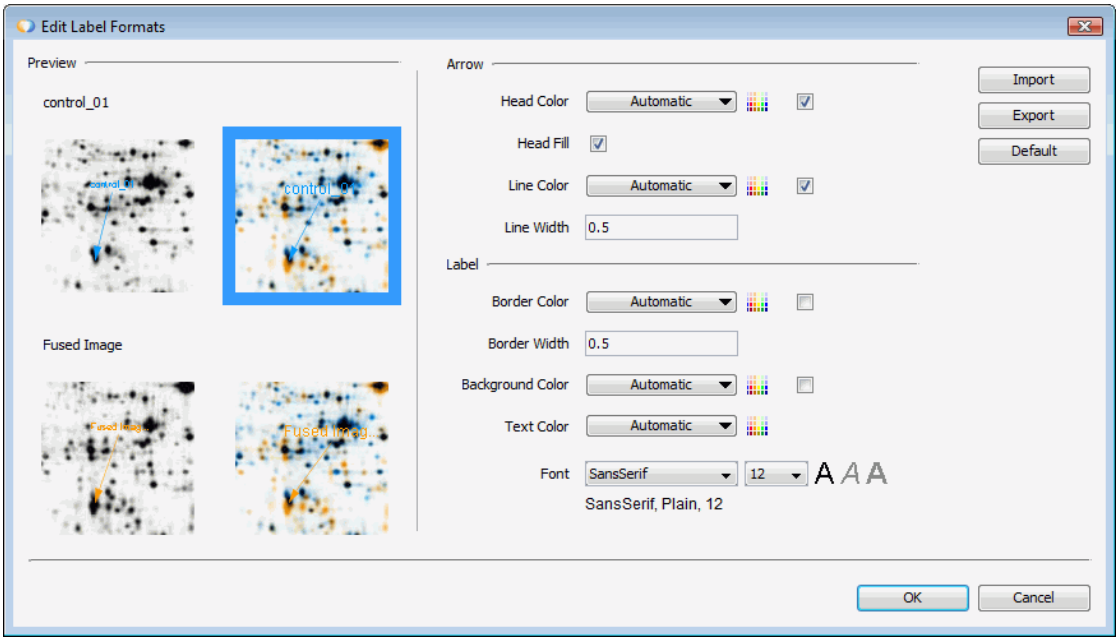


Figure 9.7.: Editing label formats

> Note: Please note: if you want to define an identical format for every view, you have to make sure to set it in any single view.

Saving Label Data Note that whenever you save label data, the current label formats will be saved together with the data.

Specifying Label Formats

The Elements of a Label A label consists of two main objects: the label itself and the arrow, indicating to which point on the image the label points. Both of them can be designed in any detail, such as label text, background, arrowhead, and -line. The components are mostly self-explanatory, and are introduced briefly below.

Arrow

Head Color The color used to fill the head of the label's arrow.

Show Head A check box next to the above button, indicating whether or not heads should appear on arrows.

Head Fill A check box indicating whether the arrow head should be filled, or appear transparent.

Line Color The color used for displaying the line portion of an arrow.

Show line A check box next to the above button, indicating whether or not the line portion of an arrow should be displayed.

Line width The width of an arrow's line.

Label

Border Color The color to use for displaying the outline of the label itself.

Show Border A check box next to the above button, indicating whether or not the border of a label should be displayed.

Label border width The width of a label's border.

Background Color The color used for filling the background of the label itself.

Show Background A check box next to the above button, indicating whether the background of a label should be filled, or whether it should appear transparent.

Text Color The color used to display the label text itself.

Font The font used to display the label text itself.

Coloring Labels There are three basic possibilities to define the appearance for the elements of labels. Click on the drop down button next to an element you want to recolor and choose between:

Color Click on one of the colors in the directly visible palette to quickly allocate a color to the aspired element. If this small preselection of colors do not suffice your needs, click on the button  colors to have a full featured color chooser.

Automatic Note that several options involving choice of display colors provide an Automatic option. When the display color for a given component is set to Automatic, the color will be derived from the spot color for the corresponding gel. For example, if the text color for a label in the master image is set to Automatic, text for the label will be displayed in the same color as spots appearing only in the master gel (see Section 5.5 on page 57 for information on configuring these colors).

Scouts This option is available if Scout data is available. Scout coloring opens up an additional benefit from labels: use them as indicators for e.g. the isoelectric point or the molecular weight of identified spots, as retrieved by scouts. (For more about scouts please refer to section 9.7 on page 136.) Thus you can see at a glance the distribution and also outliers in the selected property over the complete gel image. (fig. 9.8 on page 131)

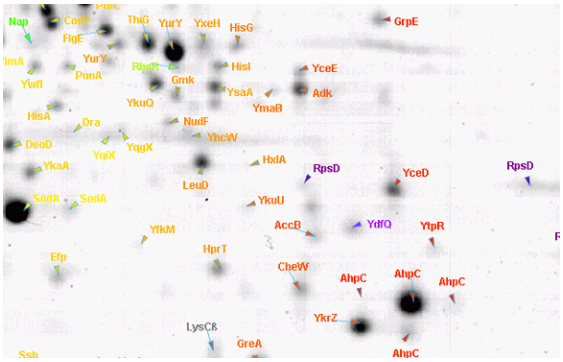


Figure 9.8.: Labels colored according to isoelectric point, based on Scout data

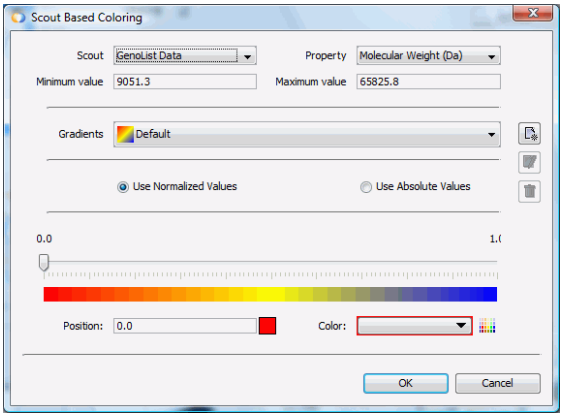


Figure 9.9.: Adjust details for scout based color coding of label elements.

If you set the color control to Scouts, a new dialog will open (fig. 9.9 on page 131). This dialog lets you configure the details:

Scout Select the scout, the data of which will be used.

Property Which data of this scout will be used?

Gradients Choose a default color gradient to be applied to the range of values. You can define your own color scheme by clicking on the button and rename it with .

Normalized/Absolute Values Switch from normalized values, ranging between 0 and 1, to absolute values, ranging between the smallest and the highest value of the selected property. This is useful for determining a certain color for an exact value.

Slider Move the slider to a position corresponding an aspired value or use the

Position field to type it in directly. Now use the

Color Picker to set the color for this value.

You can assign as many colors to certain values as you want, there's no limit.

Saving Label Formats

To save the current label format configuration, simply click on the Save... button and supply a file name under which to save the current configuration.

Loading Label Formats

To load a previously saved label format configuration, simply click on the Load... button and select a file containing information about a previously saved configuration.

9.5. Creating and Using a Proteome Map with Spot Identifications

Labels can be used to record spot identifications. You can do this by e.g. creating one union fused image per project and work group. In this setting, spots are identified on gels using, for example, peptide mass fingerprinting. Identifications are then transferred to the proteome map. Later on you can use the proteome map to identify protein spots by visual comparison.

Just as Delta2D helps you to overlay corresponding spots in the images, it may also transfer labels from a spot on one gel to the corresponding spot on another gel. Delta2D does this in a very reliable and efficient way, using the same match map that is used to generate dual channel images. Thus you have complete control over the accuracy.

To add identifications to the proteome map, follow this procedure:

Identify and label spots on sample gel Create a label for every identified spot on the sample gel. Make sure that labels point into the centers of the spots.

Load proteome map Load the proteome map together with the collection of labels for spots you already have identified. Sometimes it can be useful to integrate your proteome map temporally into the current project. To exclude it from statistical analysis, change the respective setting in the Quantitation Table properties.

Warp sample to proteome map Create a match map from the sample to the proteome map. You may wish to hide labels for this step; use the Overlays rollup to do so.

Warp the sample gel exactly Label positions on the sample gel will be changed according to the match map.

Copy labels from sample to master Use Labels ▷ Copy ▷ *your gel image names* to copy the sample labels onto the proteome map. Effectively, you have now added the new identifications to your proteome map.

Export the proteome map labels Now you can save the proteome map labels (e.g. for backup or exchange purposes) using Labels ▷ Export ▷ Master

A similar procedure can be used to transfer labels from the master gel to a new sample gel. This may avoid duplicate identifications, and it is much quicker and more reliable than doing the same thing by hand.[2] Suppose you have a sample gel where you have selected interesting spots, e.g. by looking at the dual channel image. To transfer spot identifications for these spots from the proteome map to the sample gel, follow these steps:

Load proteome map Load the proteome map together with the collection of labels for spots you have already identified.

Warp sample to proteome map Create a match map from the sample to the proteome map. You may wish to hide labels for this step; use the Overlays rollup to do so.

Copy selected labels to the sample gel Right-click on a proteome map label to bring up its context menu. Select Copy to *your gel image's name* to move it to the sample gel. Repeat this for all labels that you want to add to the sample gel.

Save sample labels You can use Labels ▷ Export ▷ Sample to save the sample labels.

The result is a label file that contains identifications for interesting spots on the sample gel. You can see this by loading the sample alone, together with its newly created label file.

9.6. Support for Protein Identification by Mass Spectrometry

We have added a number of features that make the data flow from gel images to mass spectrometry and back to gel images more efficient. Automated labeling of spots lets you create labels for spots that you selected in the Dual View or in the Quantitation Table.

Automatically Creating Labels in the Dual View

You can choose to label selected spots using spot-IDs or using consecutive numbers. In the screenshot 9.10 on page 134, we numbered selected spots with an additional prefix, making labels Spot 01, Spot 02, Spot 03 etc. The numbering can be controlled by the options in the Labeling tab of the Options dialog (see sec.10.1 on page 142). Using automatic numbering helps to keep pick lists and protein identification results organized.

Open the Dual View, select the spots you want to label and choose from the menu Labels ▷ Label Selected Spots with Spot IDs ▷ and choose the gel image you want to create labels on. Even easier is labelling all unlabeled spots: just click on Labels ▷ Label unlabeled Spots with Spot IDs ▷ in the menu. To create labels with ascending numbers select the respective menu item for either only selected or all unlabeled spots. You can determine a prefix being added in front of any number when creating numbered labels: Open the Options dialog and switch to the Labeling tab. Type in any string you want to be prepended to the numbers in the field Prefix for Numbered Labels.

[2] Identifications are transferred based solely on the position of a spot, so this may fail when there is more than one protein species in the spot, but your initial identification found only one of them.

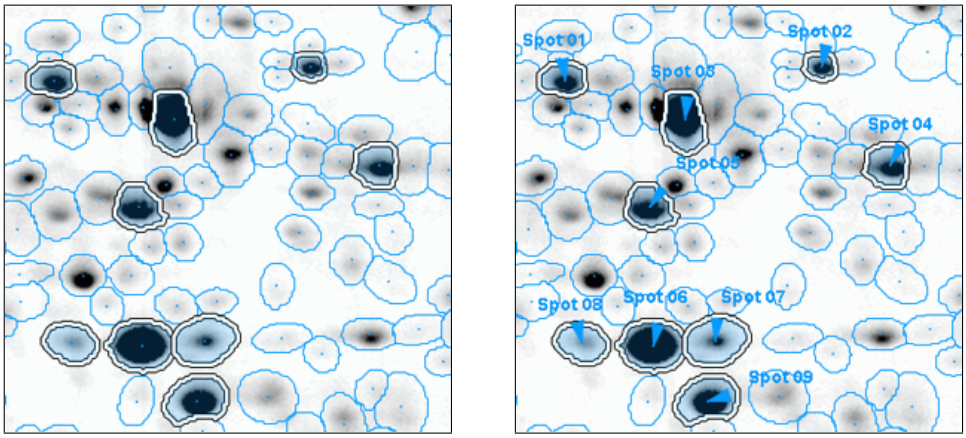


Figure 9.10.: Label selected spots with numbers

Automatically Create Labels in the Quantitation Tables

Automatic labeling is also possible from within the Quantitation Table: select the matchings or spots in any table, switch to the single table of the gel image you want to create the labels on and select the menu item Labels ▷ Label Selected Spots with ..., resp. Labels ▷ Label Unlabeled Spots with ....

> Note: Automatic Labelling works in single gel image tables only.

Automatically Replace Labels with Names of Identified Proteins

Let us show how to make use of this in the context of protein identification. Say you have identified a set of interesting spots (e.g. using the expression ratio) and labeled them with consecutive numbers as in the image above. These labels are then used to create a pick list, similar to the one you see here.

```
37098,  719.9810315160148,  535.0376014128182,   Spot 07
37102,  660.2708483901226,  536.0856322190424,   Spot 08
37011,  798.1893737681839,  456.1154569144324,   Spot 04
37169,  657.4147497551165,  631.8203715138964,   Spot 10
37027,  690.5512733901044,  474.7210971885744,   Spot 05
36987,  706.3568393575761,  435.50534012957246,  Spot 03
36973,  767.4521890914202,  411.8709608300085,   Spot 02
36972,  652.5601947607652,  418.1682292961424,   Spot 01
37094,  691.9939555744654,  537.2464779717167,   Spot 06
37248,  535.7426170849653,  733.3756788121266,   Spot 11
37114,  716.4720942243414,  562.0853241641546,   Spot 09
```

Figure 9.11.: Pick list made from labeled spots

Picked spots are processed in the usual way (digestion, mass spec, and database search). The protein identification results usually come in the form of a table with label names and corresponding protein names, like this:

1	Spot 01	CitH
2	Spot 02	HAG
3	Spot 03	HAG
4	Spot 04	Eno
5	Spot 05	
6	Spot 06	EF-Tu
7	Spot 07	GlnA
8	Spot 08	
9	Spot 09	CitC
10	Spot 10	
11	Spot 11	

Figure 9.12.: Label names and corresponding protein names in a spreadsheet

You can use this table to automatically rename the labels Spot 01, Spot 02 etc. to show the names of the identified proteins. First, save the table as a CSV file. When opened in a text editor, the file should look similar to this:

```
"Spot 07","Hag"
"Spot 08","Hag"
"Spot 04","Eno"
"Spot 10",""
"Spot 05","CitC"
"Spot 03","EF-Tu"
"Spot 02",""
"Spot 01","GlnA"
"Spot 06","Hag"
```

Now, in Delta2D, go to the Labels menu and choose Translate Label Names. This will open the Translate Labels dialog (see figure 9.13 on page 135):

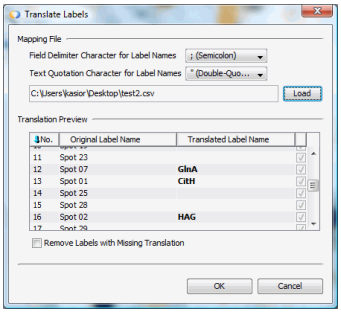


Figure 9.13.: Translate Labels Dialog

Press the Load button and select the CSV file you saved earlier. The dialog will show a preview with the original and the translated label names. Figure 9.14 on page 136 shows the gel

image from above with translated labels.

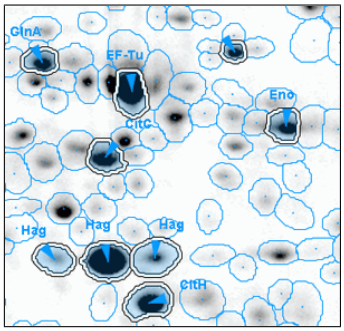


Figure 9.14.: Translated Labels

Note: Note that you can remove labels for which you have no translation (i.e. where protein identification failed). Note furthermore that the old label names will not be available anymore, so you should export them to a file if you want to save them.

9.7. Scouts: Finding Information in Web Resources

Delta2D's scouts are little software programs that go out to web resources such as GenBank or GenoList and come back with useful information about a protein on a gel. Scout data can be protein properties such as isoelectric point or molecular weight, annotations such as pathway information, sequences, and much more. The information that was retrieved by scouts is attached to labels. The data is organized into "aspects" i.e. groups of related data about a protein, such as the biochemistry aspect containing isoelectric point and molecular weight, or the GenBank aspect containing sequences and accession numbers. The aspects data are saved into the gel pool so they do not need to be retrieved from the web again.

Note: Scouts go out to public web sites when retrieving data. If you want or need scouts that use in-house resources instead please do not hesitate to contact DECODON's technical support. We always welcome suggestions for new scouts that should be included with Delta2D.

Accessing Scout Data

Scout data can be accessed by right-clicking on a label. The bottom of the context menu shows excerpts from scout data, one line per scout. Use the Edit Scout data menu item to see and edit

Scout	Data
Physicochemical properties	Molecular weight, isoelectric point etc., notes. All data is entered by the user.
GenBank	Protein sequence, accession number etc. from NCBI GenBank Isoelectric point, molecular weight, and amino acid statistics are computed from the sequence using the EMBOSS toolkit.
Data Table	Import a table of arbitrary data fields
GenoList	Gene and protein information from some GenoList databases maintained at the Institut Pasteur: **SubtiList** *Bacillus subtilis* strain 168 **TubercuList** *Mycobacterium tuberculosis* strain H37Rv **SagaList** *Streptococcus agalactiae* strain NEM316 **PhotoList** *Photorhabdus luminescens* strain TT01 **CandidaDB** *Candida albicans* strain SC5314
AureoList	Gene and Protein information from the AureoList database maintained at the Institut Pasteur.

Table 9.1.: Scouts and the data they access

the data. You can delete the data using the "Delete Scout Data" menu item.

Using the GenBank Scout

Open the scout by selecting Edit scout Data > GenBank from a label's context menu.. Enter the protein name and the organism name, then press the Process button. The scout will access GenBank and retrieve one or more entries. You can double-click on an entry to open the corresponding web page. You can now select one of the entries and press the button. This will send the selected sequence to a server at DECODON where isoelectric point and molecular weight are computed from the sequence (the actual computation is carried out by the EMBOSS toolkit). The values are then saved, along with more statistics on the amino acid composition.

Using the Data Table Scout

Import tables which can be automatically generated or manually edited and have to conform the following specifications:

- simple text file, fields separated by commas, no spaces near the commas

- the first column is reserved for the label names, its header is not
- as decimal separator solely a dot (.) is accepted
- the first row contains the field descriptors
- the following rows contain data following the scheme in the first row
- label names have to be unique on your gel

Here an example:

```
anythinggoeshere,Pi,Mw
PShAa0003,12.34,56.789
RecF,24,12
```

Using the GenoList Scouts

GenoList is a collection of bacterial genome databases for microorganisms such as *Mycobacterium tuberculosis* or *Bacillus subtilis*. The protein name will be taken from the label name. Choose the organism database on the right hand side and press Get Data. You can fetch Genolist data for all labels on an image by choosing Labels/Fetch Scout Data/GenoList data in the dual view. Delta2D will fetch data from the last Genolist database you selected.

Using the AureoList Scout

The AureoList scout works just like the GenoList scout, except that you have to select which of the *Staphylococcus aureus* strains N315 and Mu50 you want to use.

Using the Physicochemical Properties Scout

This scout does not use any web resources but relies on data input by the user.

10. Options

Delta2D's behaviour can be customized by user defined options. For doing so open the menu Tools ▷ Options... or click on the button . The upcoming dialog includes four main areas: Delta2D, General, Memory, and Keymap.

10.1. Delta2D

Match Vectors

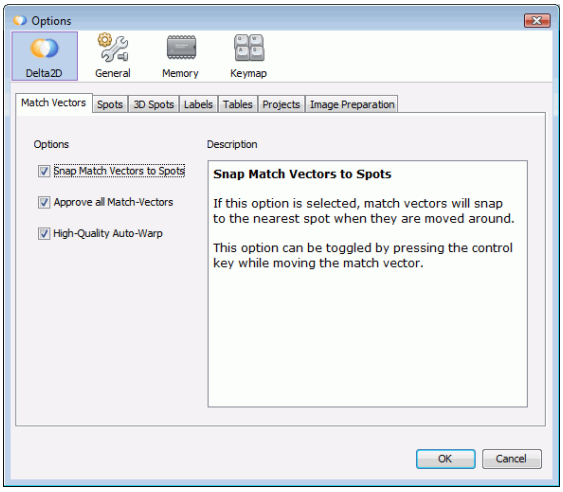


Figure 10.1.: Options: Match Vectors

Snap Match Vector to Spots If this option is selected, the ends of match vectors will snap to the nearest spot when you create or modify them. This option can be temporarily toggled by pressing Ctrl while creating or modifying a match vector.

Approve all match vectors With this option you decide what shall happen with existing non-approved match vectors if you press Find Match Vectors again.

High-Quality Auto-Warp The High-Quality should deliver more accurate results for most image pairs while consuming more processing time.

Spots

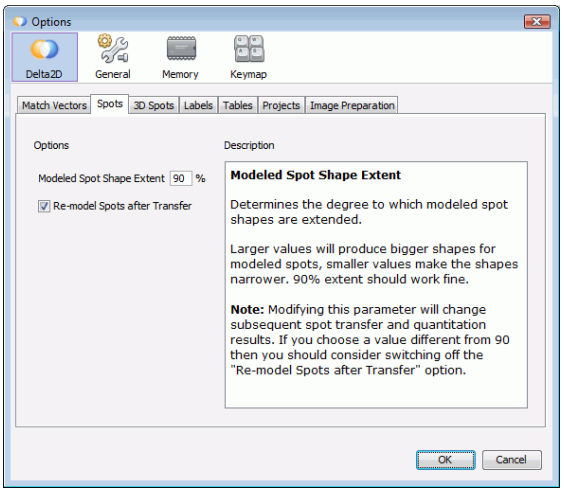


Figure 10.2.: Options: Spots

Modeled Spot Shape Extent The size of the spot boundaries will be reduced to the percentage defined here. Aplies only for future spot detections and if the parameter Create Modeled Spots will be chosen in the spot detection dialog.

Re-model Spots after Transfer If this option is checked, while spot transfer the spot boundaries will be adjusted to the actual spots as they appear on the target image. Applies only for spot transfers in the future and if the originally detected spots on the source image have been modeled.

3D Spots

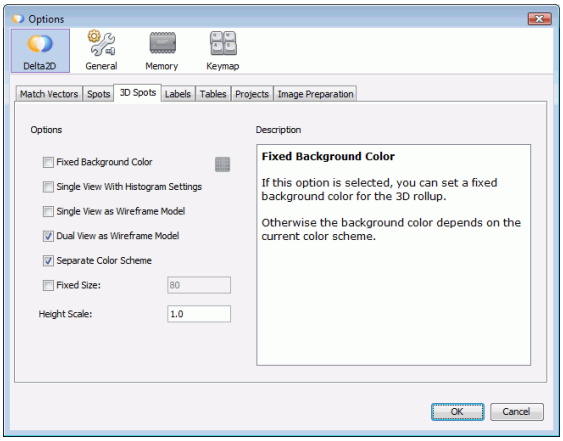


Figure 10.3.: Options: 3D Spots

The settings on this tab affect the spot shapes of the 3D Rollup in the Dual View.

Fixed Background color Normally, the background of the 3D Rollup is determined by the color scheme used in the Dual View. This setting allows you to determine the color of the background yourself: Simply check this check box and select a color with the now activated color picker.

Single View With Histogram Settings Check this box to apply the histogram settings to the 3D single view.

Single View as Wireframe Model and

Dual View as Wireframe Model If not checked, the 3D spots have an opaque surface, like in the gel image. Check this box to switch to a visualization with a transparent surface; the spots will be shaped by lines describing the outline of the actual spots as shown in figure 5.22 on page 50.

Separate Color Scheme The Dual View can use colors for the intensities or for the ratios between the images. By default the colors in the 3D view correspond to the Dual View. Check this box to show ratio colors in the 3D view.

Fixed Size By default, the 3D rollup shows the selected spot with a small neighbourhood - the displayed area is automatically chosen. Check this box to manually define the size of the shown image tile (pixel).

Height Scale Change the height scale for the spots if spots appear to be very high or very flat in the 3D rollup.

Labels

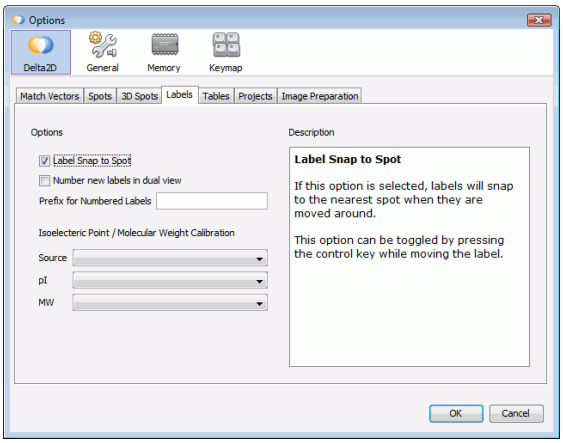


Figure 10.4.: Options: Labels

Label Snap to Spot If this option is selected, labels will snap to the nearest spot when they are created or modified. This option can be temporarily toggled by pressing Ctrl while creating a label or modifying its position.

Number new labels in Dual View of New Labels If switched on, Delta2D is looking for the highest value among that the existing labels with pure numbers and fills the next label with the next value.

Prefix for Numbered Labels Here you can define a string that shall be inserted in labels in front of the automatically assigned numbers, e.g. *'Spot_'*.

Isoelectric Point / Molecular Weight Calibration The pI/MW Calibration Rollup can be based on spot attributes that are available in the Scouts. Choose one of the Scouts and the appropriate attribute to define the rollup's behaviour.

Tables

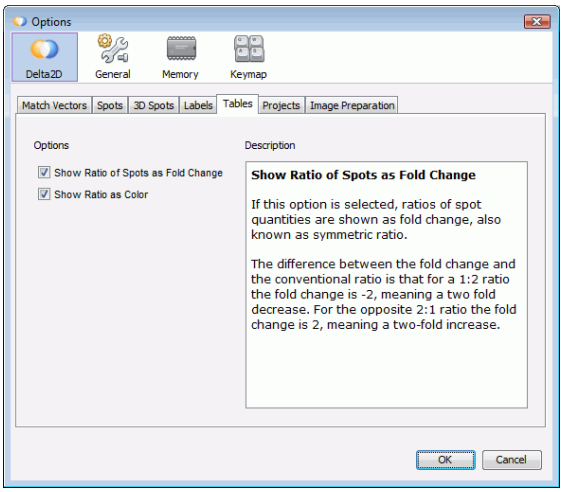


Figure 10.5.: Options: Tables

Show Ratio of Spots as Fold Change By default, the ratio of spots is shown as quotient of the two corresponding spots: if the relative volume of the second spot has the double size of the first spot, the ratio is 2, if its volume has half of the size, the ratio is 0.5. If you check this box, the ratio will be shown as fold change: double spot size means the ratio 2 as well, whereas half size will be shown as -2.

Show Ratio as Color If this option is selected, a color coded icon is shown for ratios as well.

Projects

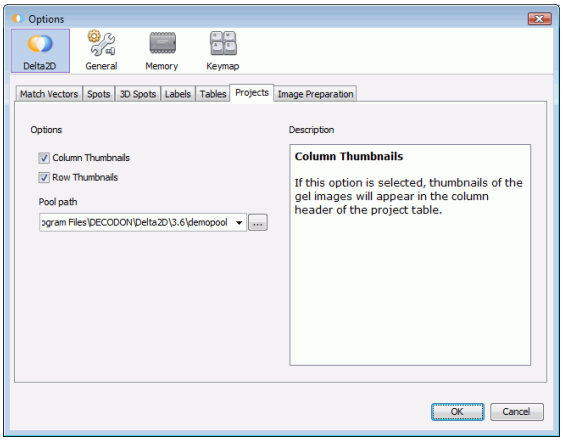


Figure 10.6.: Options: Projects

In this panel you can switch the thumbnail view in the Project Matrix (previously called the *Project Manager* on or off and change the pool path.

Furthermore you can change the pool path.

Image Preparation

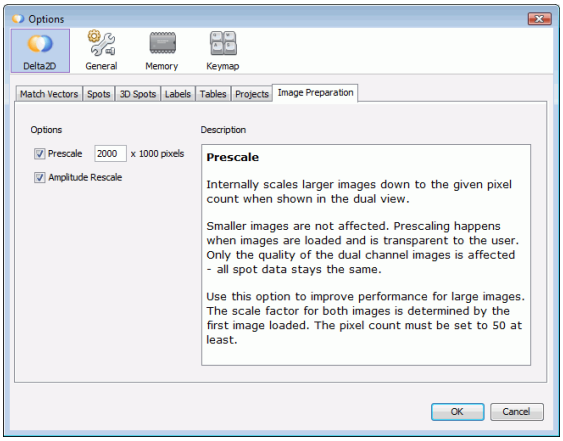


Figure 10.7.: Options: Image preparation

Changes on this panel only affect images when they are loaded for the first time after Delta2D has been started. I.e. if you changed anything here, you have to restart Delta2D.

Prescale Scales larger images down to the given pixel count. Smaller images are not affected. Prescaling happens when images are loaded and is transparent to the user. Use this option to improve performance for large images. The scale factor for both images is determined by the first image loaded. The pixel count must be set to 50 at least.

Amplitude rescale Enhance images by amplitude rescale when they are loaded. If this option is checked the gray values of an image are rescaled linearly so that the darkest pixel becomes black and the lightest pixel becomes white.

10.2. General

License

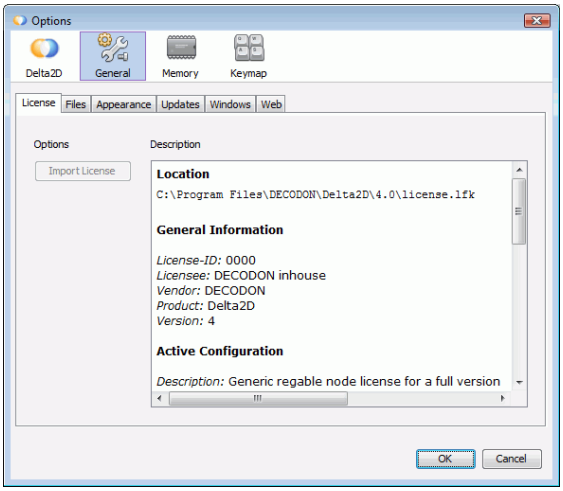


Figure 10.8.: Options: License

Import This button allows to import a new license file. The file browser window will be opened to search for another license file in your file system. The current license file will be renamed to license.lfk.bak while the new license file will be stored with the name license.lfk, regardless of its original name.

There is also a description field where you find information about the currently used license configuration. For some support issues our support team will ask for the information provided there.

Files

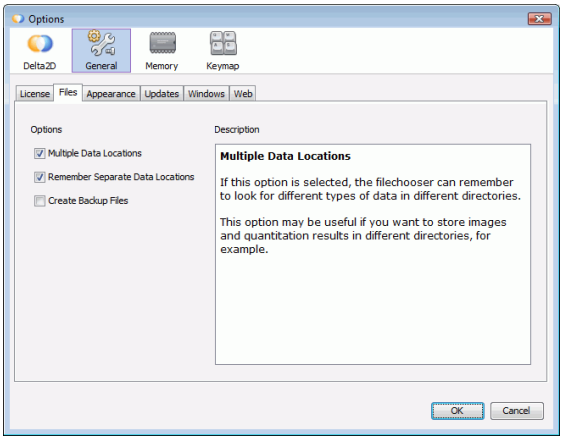


Figure 10.9.: Options: Files

Multiple data locations If this option is selected, the file chooser can remember to look for different types of data in different directories. This option may be useful if you want to store exported images and quantitation results in different directories, for example. This option does not effect the structure of the data pool!

Remember separate data locations If this option is selected, Delta2D will remember the storage locations for all different data types, even after it is closed and restarted. Otherwise, the file chooser will look for all types of data in the default directory after Delta2D is restarted.

Create backup files If this option is selected, Delta2D will create a backup copy of overwritten files in the same directory.

Appearance

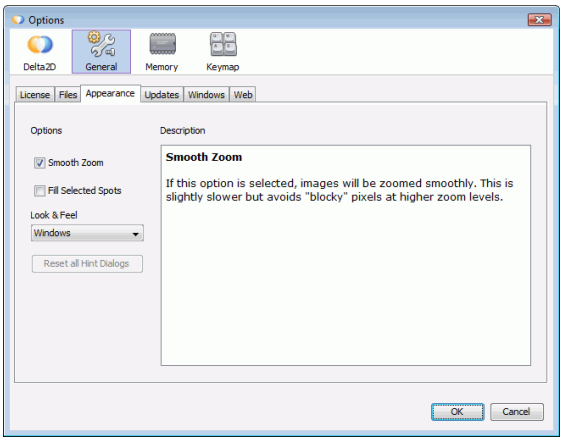


Figure 10.10.: Options: Appearance

Smooth Zoom Normally, zooming in images magnifies with the whole image the single pixels, too. Thus, the more you zoom in, the more the image looks like covered with a grid. This option makes Delta2D smoothen the images in magnified state. This setting only affects the optical representation of images and no quantitative data at all.

Fill Selected Spots If this option is selected, selected spots will be filled with transparent color.

Look-And-Feel-Chooser Choose a Look and Feel of your taste for Delta2D.

Updates

Decide for frequent checks during the startup of Delta2D, whether an update is available. Delta2D needs an internet connection to submit a query to our server.

You can also let Delta2D check for updates immediately by pressing the button Check Now.

Whenever this check will be executed you will be informed in a seperate window whether updates exist and why you should update to the current version.

Windows

The different windows are controlled by a central window manager. You can design your workspace in accordance with your personal preferences, e.g. you can move single windows to different positions or even undock (and re-dock) them so that they are seperated. Here you can define whether moved windows shall snap to certain positions and whether the last active window shall be activated if a window is closed.

Web

Scouts and the check for updates demand for internet connections. If a connection fails please review these settings, perhaps after having consulted your system administrator who knows your network topology.

10.3. Memory

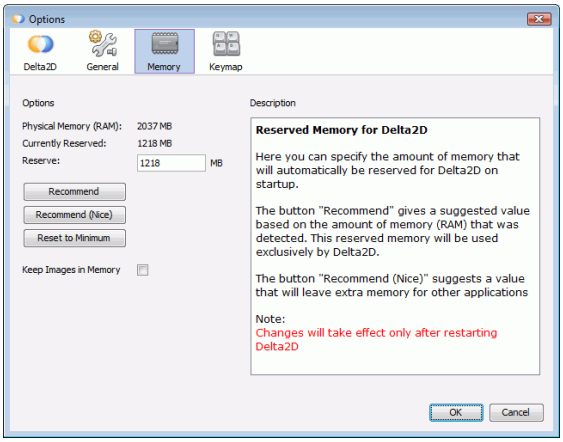


Figure 10.11.: Options: Memory

> Note: For adjusting the memory settings you need the permission to write into the installation directory of Delta2D. Particularly for Windows Vista it is not sufficient to have administrator rights but you might have to explicitely start Delta2D with the administrator role if you like to change the memory settings (right click on the program icon and review the context menu). If in doubt, ask your local systems administrator.

Physical Memory (RAM): shows the automatically detected amount of memory in your machine.

Currently Reserved: This is the maximal amount of memory that has been reserved for Delta2D.

Reserve: Here you can change the Currently Reserved memory. To apply changes of this setting, please restart Delta2D.

Recommend Click on this button to change the setting to a recommended value, depending on the amount of memory detected.

Recommend (Nice) This button changes the setting to a recommended value, depending on the amount of detected memory but considering other applications.

Keep Images in Memory If this option is checked images are kept in memory after they are loaded. This is especially useful when working with a database pool. On the other hand side the system could run out of memory with big projects. This option will be applied only when new images are loaded.

10.4. Keymap

Global Keymap

Keyboard shortcuts give you direct access to certain functionalities and thus can speed up your workflow significantly. They depend on the context, which means that in different windows or with different tools the same combination of keys can cause different actions. Delta2D follows the conventions known from other applications, where most menu items are accessible by typing the underlined letter of the menu command while holding pressed the Alt and/or the Ctrl and/or the Shift key.

Global keyboard shortcuts are listed in the Options window. To review or change an existing global keyboard shortcut search and select the action, click into the *Shortcuts* field at the bottom of the dialog and define your preferred shortcut. Confirm by pressing *Add...*

Window-specific keymaps

Keyboard Shortcuts in the Dual View

Alt + 1	Switch to the Match Map tool
Alt + 2	Switch to the Segments tool
Alt + 3	Switch to the Spot Editing tool
Alt + 4	Switch to the Zoom tool
Alt + 5	Switch to the Labels tool
Ctrl + 1	Switch on / off master points overlay
Ctrl + 2	Switch on / off sample points overlay
Ctrl + 3	Switch on / off match vectors overlay
Ctrl + Alt + I	Invert match map (Swap target points of match vectors)
Ctrl + Alt + D	Delete match map
Ctrl + Alt + O	Open saved match map
Ctrl + Alt + Shift + E	Export current match (save as ...)
Ctrl + L	Show scatter plot
Ctrl + N	New File, new View. Clears Master, Sample and Match map.
Ctrl + Numpad-	Zoom out
Ctrl + Numpad*	Fit window to image
Ctrl + Numpad+	Zoom in
Ctrl + Numpad/	Fit image to window
Ctrl + Numpad1	Zoom 1:1

Ctrl + Shift + 1	Open or switch to Project Explorer window
Ctrl + Shift + 2	Open or switch to Light Table (gel image) window
Ctrl + Shift + 3	Open or switch to Warping Setup window
Ctrl + Shift + 4	Open or switch to Dual View window
Ctrl + Shift + 5	Open or switch to Quantitation Table window
Ctrl + Shift + 6	Open or switch to Gel Image Regions window
Ctrl + Shift + 7	Open or switch to Expression Profiles window
Ctrl + Shift + D	Save Dual channel image as ...
Ctrl + Shift + M	Open Match map including Images
Ctrl + Shift + Space	Switch between dual image / last active single image tabs
Ctrl + Space	Switch between master / sample image tabs
Ctrl + T	Show Quantitation Table
F1	Open help
Shift + Space	Switch between master / sample / dual image tabs

In the Dual View window of Delta2D you can use your mouse as different one of four different tools (see section 5.5 on page 44). Depending on the tool being in use, you get changed or even additional functionality by pressing a key when clicking.

Match Map Tool

Alt	Changes the match map tool temporarily to a hand for moving around the visible region of the image.
Ctrl	Toggles match vector snap to spots temporarily on or off, depending on the actual state.

Segments Tool

Alt	Changes the spots tool temporarily to a hand for moving around the image.
Ctrl	Allows you to select additional spots without deselecting already selected ones.

Zoom Tool

Alt	Changes the zoom tool temporarily to a hand for moving around the image.
Ctrl	Switches the zoom tool temporarily in zoom out mode. The plus in the mouse cursor changes to a minus to illustrate this.
Shift	Switches the zoom tool temporarily in the 1:1 zoom mode. The plus in the mouse cursor changes to a 1:1 underneath the loupe.

Labels Tool

Alt	Changes the labels tool temporarily to a hand for moving around the image.
Ctrl	Toggles labels snap to spot temporarily on or off, depending on the actual state.
Shift	Toggles the placing of new produced labels temporarily from master to sample image.

11. Advanced Topic: Scripting

A script is a small program that you can use to automate repetitive tasks in Delta2D. Here we show briefly the available commands. See also the example script file `example.script` in the Delta2D directory, this is a script that processes files in the samples directory.

The best way to learn about scripting is to use Delta2D's remote control. The remote control lets you type commands that will be immediately executed by Delta2D. Later you can combine these commands into a script. A complete list of available commands is shown with the command `help` or in this document in 11.1 on page 153.

To start the remote control, open a Windows command prompt. Go to the program directory where you have installed Delta2D. Then start the remote control from the command line using one of the commands below. Note that these commands have to be typed in one line.

Interactive mode – starts the remote control and waits for commands

```
Dual2DGelCompareRemoteControl.bat
```

Under unix-like systems you start it with

```
./Dual2DGelCompareRemoteControl.sh
```

Interactive mode with initial script – starts the remote control and immediately executes the given script file. Unless your script ends with the command `exit`, Delta2D will stay open after the script is finished.

```
Dual2DGelCompareRemoteControl.bat -f script-file-name
```

respectively

```
./Dual2DGelCompareRemoteControl.sh -f script-file-name
```

Interactive mode with initial commands – starts the remote control and immediately executes the given commands. Use `exit` as the last command to close Delta2D after executing the commands.

```
Dual2DGelCompareRemoteControl.bat command1 command2 ...
```

respectively

```
./Dual2DGelCompareRemoteControl.sh command1 command2 ...
```

11.1. File Commands

Table 11.1 on page 153 shows the commands that correspond to the entries in the File menu. You may need to provide a file name, telling which file to open or where to save results.

`clear`	Clear master and sample images.
`openImageAsMaster` *File*	Open an image file as the master image.
`openImageAsSample` *File*	Open an image file as the sample image.
`openMatchmap` *File*	Open a match map.
`saveSampleImageAs` *File*	Save the sample image..
`saveDualImageAs` *File*	Save the dual channel image.
`saveMasterTableAs` *File*	Save the Quantitation Table for the master gel image.
`saveSampleTableAs` *File*	Save the Quantitation Table for the sample gel.
`saveDualTableAs` *File*	Save the Quantitation Table for the gel pair.

Table 11.1.: File commands.

11.2. Image Processing Commands

Table 11.2 on page 154 shows commands that correspond to some entries in the View and Warp menus.

`disableAutoWarp`	Disable automatic warping.
`enableAutoWarp`	Enable automatic warping.
`warp`	Warp the sample image according to the current match map and automatic warp settings.
`warpAuto`	Warp the sample image automatically, taking the current match map into account.
`warpExact`	Warp the sample image exactly.
`warpGlobal`	Warp the sample image globally.
`unwarp`	Unwarp the sample image.
`quantify`	Quantify both images.
`quantifyMaster`	Quantify the master image.
`quantifySample`	Quantify the sample image.
`processSample` *File*	Warp and quantify the sample image, using a match map with canonical name.

Table 11.2.: Image processing commands.

11.3. Commands for Controlling Windows

You can use the commands shown in Table 11.3 on page 155 to control the display of the Dual View and the Quantitation Table.

`show`	Show the Dual View.
`hide`	Hide the Dual View.
`zoomIn`, `zoomOut`, `unzoom`	Control the zoom of the Dual View.
`pageDown`, `pageUp`, `pageLeft`, `pageRight`	Scroll the Dual View.
`showTable`	Show the Quantitation Table.
`hideTable`	Hide the Quantitation Table.

Table 11.3.: Commands for controlling windows.

11.4. Remote Control Commands

Table 11.4 on page 155 shows available commands for the remote control itself.

`exit`	Exit the remote control program.
`help`	Print a list of available commands.

Table 11.4.: Commands for the remote control.

12. Advanced Topic: Greyscale Calibration

Delta2D is able to use user-defined greyscale calibrations to adapt special greyscale profiles of your scanner or for other purposes. The calibration of scanners produced by Amersham, Fuji and Molecular Dynamics is recognized automatically. For other products it is possible to use costumized calibration files. The profile used by Delta2D is defined in the common XML format and easy to be applied: Open the top level directory of the gel pool you want to apply the profile to. Create a directory here named calibrations. Place the XML-file(s) with the profile(s) in this directory and it will available in the gel properties of each gel image the next time you use the pool.

To obtain calibration files or further information about their format, please contact our technical support.

13. Useful Tips

Create Your Own Pool Before You Start to Work with Delta2D

After you have installed Delta2D for the first time on your computer, the initial settings use as working pool the example pool, residing in the directory Delta2D is installed to. Many users use to store the data they are working with in an individual directory structure to have it easy to back up all their important data. It may also be the case that you want to place your data in a network directory to make it accessible for your team. In these cases it is a good advise to create your own pool in the desired place before you start importing and working on your own gel images. How to create a pool is described exactly in section 6 on page 106.

Adapt the Memory Settings of Delta2D

Image processing in general and especially analyzing several images and dealing with all the gained data at once is a memory consuming business. Thus, Delta2D works better and faster, the more memory is available for use. By default, the settings for memory usage are set to a more conservative value, in order to leave enough memory for other applications even on a computer equipped with the minimum of RAM as stated in chapter 2 on page 3. If your computer has more memory, you can increase the performance of Delta2D by changing the settings for memory in the Options as described in section 10.3 on page 148.

Working with Big Images

The memory needs of Delta2D grow with the size and number of images analyzed at a time in one project. If you encounter a significant lack of performance in a certain project, this could be due to the extraordinary big size of your gel images. In this case you could try to regain performance by setting prescale to a lower value as described in section 10.1 on page 144.

Accurate Scanning Will Be Recompensed

As in any process, the quality of your product (your results) is directly connected with the quality of your raw material (the gelimages). The more irritations and irrelevant information your images contain, the more Delta2D will be distracted from efficient analysis and more and more corrections by the user will be necessary. You can avoid a lot of trouble by being more accurate with scanning your gel images:

- Take good care for a clean scanning surface and clean gels as any stain could be mistaken as spot
- On determining the region to be scanned, make sure to include only the relevant region of your gel. Exclude parts which definitely do not belong to the gel or only belong to marginal parts of the gel, e.g. showing only the frame of your gel.
- Use an adequate resolution for scanning. Too low resolutions (below 150 dpi) lead to a loss of information, too high resolutions (more than 300 dpi) slow down your image processing significantly.
- If your scan software offers you the option to optimize contrast and brightness of your image, use it.

Please review our Scanning Guide to read more about how to produce optimal gel images. Please find it at www.decodon.com/Support/Howto/Scanning/scanning_2D_gels.html.

Working with Different Versions of Delta2D on the Same Data

With version 3.4 and before with version 3.1 of Delta2D the data format used in the pool has changed. Opening pools created with earlier versions ($\leq$ V 3.0) represents no problem, but the opposite way does not work.

If it still is necessary to work with older versions on a pool which was in use with version 3.1 or newer, you can export the pool from the newer version of Delta2D in the former format. To do this, please select Pool ▷ Export ▷ As Version 3.0 ....

In case that you need to work with on of the versions 3.1, 3.2or 3.3 on a pool which was in use with version 3.4 already, please contact us for assistance.

Tuning Spot Detection

For well scanned gel images, the quantitation parameters chosen by the automatic quantitation process should produce decent results. If this is not the case, you can tune the parameters on your own to optimize the quantitation process in the dialog which will be shown if you start a Quantitation manually in the Dual View. The single parameters are described in detail in section 5.5 on page 67. Here is what to do in which case:

Weak spots are not detected

Increase weak spot sensitivity step by step alternatingly with reducing noise cut off.

Small spots are not detected

Use smaller values for average spot size.

Clusters of spots are recognized as one spot

Reduce the average spot size here too.

A. Example Files

Delta2D comes with an example analysis project, consisting of six gel images, that is described in detail in the first chapter of this manual. Table A.1 on page 160 lists the gel images that come with Delta2D.

control_01.tif, control_02.tif	Two gel images from a sample before stress treatment.
1min_01.tif, 1min_02.tif	Two gel images from a sample taken 1 min. after stress treatment.
10min_02.tif, 10min_02.tif	Two gel images from a sample taken 10 min. after stress treatment.

Table A.1.: Example gel images provided with Delta2D.

The gel images are taken from a series of experiments where a bacterial culture (*Bacillus subtilis* 168) was treated with 4% NaCl and two samples were taken after 10 respective 20 minutes. to receive a minimum of reproducibility and to show an example that contains replicate images from the same sample, two gels and gel images were made from each sample.

B. Contact Information

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C. Acknowledgments

Delta2D's development has always to a large extent been driven by feedback from customers. Their comments were essential in advancing Delta2D.

Gel images for this manual were provided by Michael Hecker, Jörg Bernhardt and Falko Hochgraefe, University of Greifswald, Germany.

Delta2D includes Morphlogik imaging technology (http://www.morphops.com).

Delta2D includes parts of the qflib library, copyright Quality First Software, in unchanged form. The qflib library is available in source code under the terms of the Mozilla public license from http://www.qfs.de.

D. Lists of Figures and Tables, Index

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