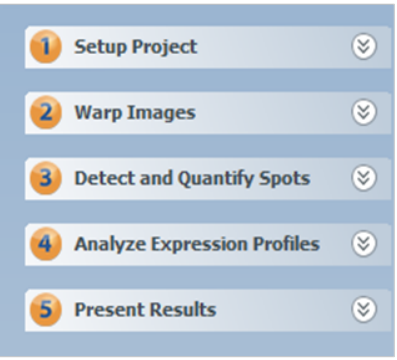
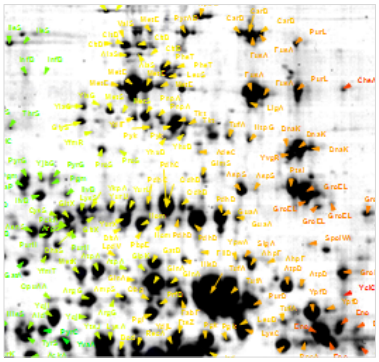
manual

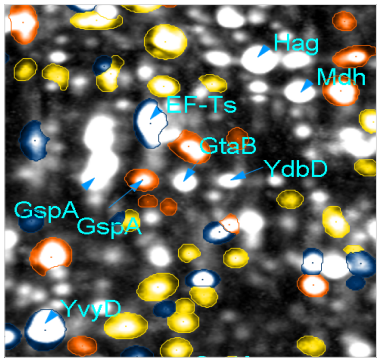
English

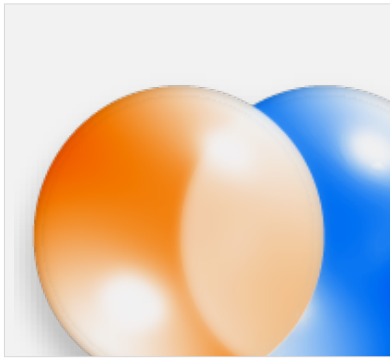
Delta2D 4.8

ANALYZING 2D GELS
AS EASY AS POINT AND CLICK









EXPLORING LIFE

DECODON

Manual

Delta2D 4.8

DECODON

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About Delta2D

Delta2D is a software tool for rapid and accurate analysis of 2D-gel-electrophoresis experiments. 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

2D-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 Gels taken from different samples, one can find and later identify proteins that are crucial to the understanding of the underlying processes. The dynamic change of proteins quantities across a variety of Gels, typically backed by a number of replicates of the same sample, is what scientists around the world study by using Delta2D 4.8.

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

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

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

Furthermore, if the spots on the different 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, DECODON provides 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 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 images are aligned first, a spot consensus can be detected on a composite image summarizing the whole experiment's image information in one artificial but realistically looking fusion image. 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 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

Delta2D comes with a modern user interface, which is based on

1. 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.
2. 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.
3. completely synchronized views, e.g.:
   - the same set of spots is selected across all windows,
   - when having opened the Dual View, the same image pair will be selected in the Project Explorer or Light Table.

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/delta2d-getting-started.html.

Contents

1. Strategy for Analyzing Images with Delta2D

This chapter provides a short description of Delta2D's approach to analyze the 2DE 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 9.

2. **Warp Images** 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.

   Examine the image pairs with Direct Warp Links one by one and correct them where necessary. More details: section 3.2 on page 20

   > 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 Complete Spot Matching at www.decodon.com/delta2d-spot-matching.html.

3. **Detect and Quantify Spots** Create a fused image over the complete project using Union as fusion type to get an image that includes every spot existing on any image in the project. Detect the spots on the fused image and edit them if necessary. Transfer the consensus spot pattern to all other 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 3.3 on page 21

4. **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 3.4 on page 26.

Present Results Open the Reports and get an overview on the project or on the interesting expression profiles. Export images as presentation slides (.pptx) for use with your standard presentation software like MS PowerPoint™ LibreOffice or OpenOffice, or export quantitative data as table files (.xslx) and open them with MS Excel™ LibreOffice or OpenOffice. More details: section 3.5 on page 35.

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 completely and reliably analyze multiplex projects using an internal standard, e.g. DIGE or Refraction-2D. Even for these projects Delta2D's core technologies are quite useful: If you want to analyse a multiplex project with several gels image warping will provide significant time savings and quality improvements.
>
> For analyzing multiplex projects you will have to consider that the project needs to be defined as a project using an internal standard 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 multiplex projects with Delta2D in section 3.1 on page 11

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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 10 / 8 / 7 / Vista. Detailed installation instructions are provided below.

Apple MacOS X With Mac OS X 10.7.3 (or higher) on Intel based Macs Delta2D works without any limitations. Older Versions of Mac OS and other architectures are not supported. 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

Your computer should fulfill these minimum technical requirements:

- at least 2GB of RAM, recommended: 4 GB or more
- we recommend a 64 bit operating system
- 800 MHz processor, recommended: 2 GHz or better
- 200 MB free disk space

To install Delta2D 4.8, follow these steps:

1. Download the installer file from your download area on our web site, or the public web site www.decodon.com/download.html.

2. Execute the installer file. 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 the extension .lfk, which has been delivered to you either by e-mail or per download.

2.3. Start Delta2D

To start Delta2D under Windows, choose Programs ▷ DECODON Delta2D ▷ 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 (fig. 2.1 on page 4), prompting for importing the license file. The license file is a text file with with the extension .lfk, which has been delivered to you either by e-mail or in your private download area.

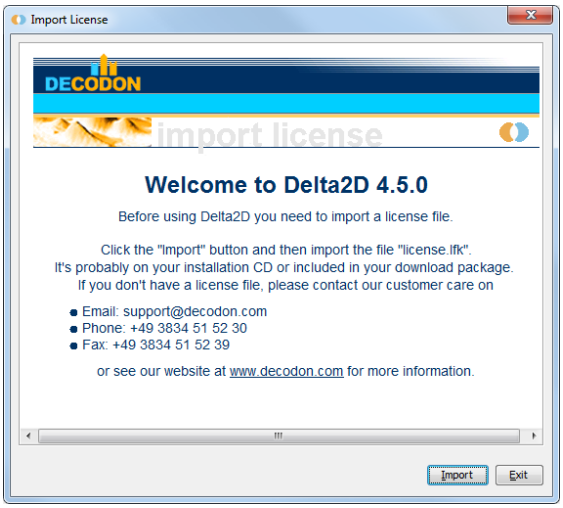


Figure 2.1.: Invitation to import the license

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 or export 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 an evaluation version. This kind of license usually works without an additional registration step.

Consumable License Delta2D Consumable is a pay-as-you-go option where you purchase a number of credits, one for each image you want to analyze. You will then receive a Delta2D Consumable license which allows for activating images for the analysis. For the activation, a fingerprint of each image is sent to our server, and our server sends back a

signature of this fingerprint. Your images are not transferred. One credit is being used for the activation of each single image. Your computer needs to have an internet connection just for the activation step. Activating fusion images is always free if done in the same session they have been created in.

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

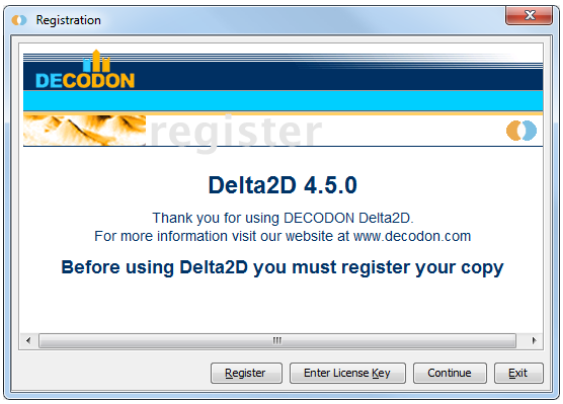


Figure 2.2.: Initial license file is imported

Click on Register. Delta2D will provide the details for your registration request (fig. 2.3 on page 6) and asks you to send these details to our registration team.

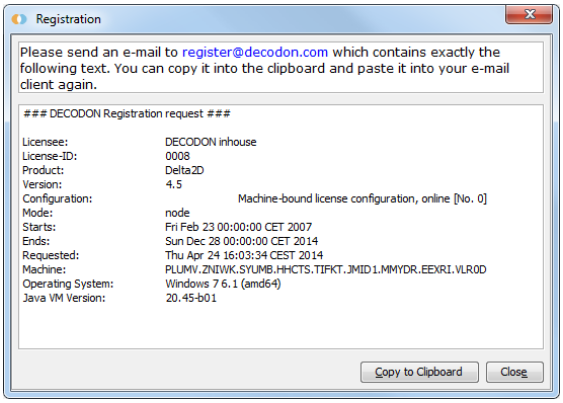


Figure 2.3.: Send your registration request

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 license key, simply click the button Enter License Key provided in the dialog (fig. 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).

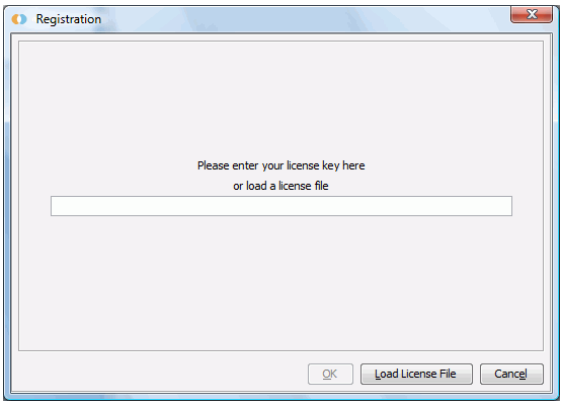


Figure 2.4.: Enter your registration key or load a full license file

2.5. Create Your Own Pool Before You Start to Work with Delta2D

Delta2D comes with sample data (the demopool containing the demo project) which will be refreshed with every installation of a new version or update. You can use the demo data to play with Delta2D, but avoid to mix it with your own data since then your work can get lost.

> Note: Before working with your own data make sure that you are not working in the demopool. Change the pool path and create a new pool before importing your own images to seperate your data from the demo data. See section 3.1 on page 9 to read more about the pool.

2.6. Adjust Memory Settings

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.

You can increase the performance of Delta2D by adjusting the memory allocated to Delta2D according to the real specifications of your computer. Open the Options dialog by selecting the menuitem Tools ▷ Options or clicking on the button  in the main toolbar. Switch to the Memory section and click on the button named Recommend. Now close the Options dialog with OK and restart Delta2D.

> Note: Depending on your computing environment, you may need special privileges to change the memory setting. If in doubt, ask your local systems administrator.

Find more information in section 6.3 on page 163.

2.7. Check for Software Updates

Delta2D can notify you automatically if software updates are available. Based on your settings (see section 6.2 on page 161), 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 the Options dialog, switch to the General section, and select the Updates tab to define when Delta2D shall 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 provided link to download and save the update file on your computer.

2.8. 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*. For some new versions we just offer the *complete setup file* since too many parts of Delta2D have changed. If the version of Delta2D has changed (e.g. from 4.7 to 4.8) you need to use the new license that has been delivered as usual provided that your maintenance is still active.

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 folder. 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

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. 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 a New or Select a Pool

The pool is the central data repository of Delta2D. Delta2D keeps the pool in a folder on your hard disk. Along with the project structures, the images, quantitation results, match vectors, and labels are stored in the pool. Each pool may contain different projects. A image can belong to more than one project, while changes of a certain image or match map in a certain project affects this object in every other project.

After you have installed Delta2D for the first time on your computer, Delta2D offers the demopool, residing in the folder [user_home]/.Delta2D/demopool. It is recommended to store the data in seperate pools, e.g. to facilitate backups or shared access. 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, choose Pool ▷ Change Pool.... Alternatively you can use Project ▷ New... or Project ▷ Open... and click on the Change Pool... button. Now browse your file system for your preferred pool location and create a new one by clicking on Create Folder. Avoid network drives because of possible instabilities. 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.

> Note: You can move or copy whole pools to other directories, drives. To make a backup of your data, just save the pool folder. Do not change the file structure in the pool folder and do not edit the files, otherwise Delta2D will not be able to open projects. You may read the data kept in the pool folder using third party software, since all data, except for images, is kept in XML files.
>
> With version 3.4 and before with version 3.1 of Delta2D pool data structures have changed. While it is no problem to open an "old" pool with later versions you can not open a pool e.g. with version 3.1 that has been saved with version 3.6. Please contact us for assistance if you face such a situation.

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. 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 (see fig. 3.1 on page 10). Here you can also create and remove projects.

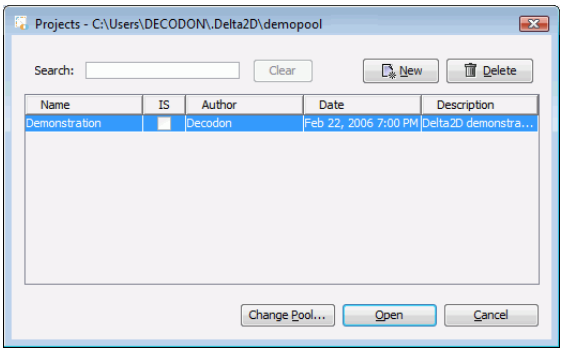


Figure 3.1.: The Open Project dialog

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 symbol or use Project ▷ Open... to open the dialog again.

> Note: If you open a project and have used an early version of the speckle filter using an ini-key, Delta2D will ask whether the speckle filter should persist for all images based on the current ini-key value. This support to migrate the old speckle filter implementation to the new one appears just for those projects that have not yet been touched with the new speckle filter implementation.

To create a new project, press the New Project... symbol in the toolbar or the button New in the Open Project dialog. Enter the name, the author and maybe a short description in the appropriate fields. For making use of an internal standard check the respective box. Create the new project by hitting OK. 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 image, match map and quantitation information is removed from the pool. Images (including all associated information) can be deleted manually in the Image Manager.

Different Kinds of Projects

The experimental setup can demand for different kinds of handling the relations between the images, e.g. when using an internal standard or support images, which guide your alignment, but are not part of your analysis themselves. As for now, Delta2D can handle three types of projects:

- Classic Projects
- Internal Standard (e.g. DIGE)
- 2D Western Blots

The project type can be defined either on creating a project, or can be changed later as well in the Project Properties. Choose Project ▷ Project Properties from Delta2D's menu to open the properties dialog for the current project.

Classic Project

Usually the standard experimental setup is used, hence the name Classic Project. In this setup each gel of an experiment separates exactly one sample which will be stained with the same staining reagent across all gels. 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.

Internal Standard

Multichannel or multiplex techniques are based on multichannel scanning of each gel.

With DIGE or Refraction-2D 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. 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 average 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 two 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 Multiplex Experiments Choose Project ▷ Project Properties to open the properties dialog for the current project and change the project type to 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 3.1 on page 16.

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

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

In projects using an internal standard, on assigning images to a certain gel there will appear an additional radio button on the left side of the 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 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 multiplex projects (for details on multichannel techniques please refer to section 3.1 on page 11), it may happen that spots do not have quantities if you have decided to detect the spots on individual images: Spots on the standard 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 Tables you can not see their %V. To avoid this phenomenon we recommend to use the approach for getting complete expression profiles as described in the standard workflow 3 on page 9.

Other Multiplex Experiments without Internal Standard Other multiplex techniques are also based on multichannel scanning. Here only one sample is separated per gel but differen-

tially 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 radiolabeled 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 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 an image will be normalized on the entirety of all spots on this 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 using an internal standard by setting the project type to Classical in the Project Properties.

> Note: You can present your project easily in the Project Summary Report. See section 3.5 on page 35 for more details.

2D Western Blots

Due to the quite different looks of Western Blots and gel images, it is not so easy to align these two image types for differential analysis. With a little workaround, this still becomes possible: by using support images, which are not analyzed themselves, but support a better alignment of the actual images. We use the outer shape of the membranes to align those images, whose signal pattern is not comparable. Therefore, to align one Western Blot to the gel image it is derived from, we need a set of 5 images:

1. **Total Protein Gel**
   actual gel image of the total proteins

2. **Total Protein Membrane**
   image of the membrane after blotting with protein pattern, easy alignable to the gel image

3. **Total Protein Membrane Contour**
   image of this membrane after blotting, focuses on the contour, alignment is identical to 2.
4. **Western Blot Contour**
   image of the membrane after the 2nd antibody incubation for the contour, can be aligned easily to the contour image 3.
5. **Western Blot 1** (up to 3 possible)
   image(s) of this membrane after the 2nd antibody incubation (antibody signal), alignment is identical to contour image 4.

While image 5 is the the Western Blot and image 1 the actual gel image, we want to align it to, images 2 to 4 have the role of support images for a precise guidance from 5 to 1. Delta2D's special project setup considers the different functions of the images and treats every image according to its role in this setup. To identify the role of an image, Delta2D looks for the role names Total Protein Gel, Total Protein Membrane, Total Protein Membrane Contour, Western Blot Contour, and Western Blot 1 (optionally also Western Blot 2 or Western Blot 3). Depending on your project setup, this can be in two places:

1. If there is only one set of 5 images in the project, and these images are distributed according to their roles on 5 *groups* with exactly these *names* , this is distinct enough for Delta2D to recognize the roles.
2. If you prefer a different grouping, e.g. all images in one group, or you want your groups to have different names, Delta2D uses the *sample* assigned to each image to identify its role. In this case it is crucial, that you *assign* each image to the *correct sample*.

On creating a project of the type 2D Western Blot, Delta2D helps you quite a bit:

- The project is created already with 7 Groups with the predefined names:
  Total Protein Gel
  Total Protein Membrane
  Total Protein Contour
  Western Blot Contour
  Western Blot 1
  Western Blot 2
  Western Blot 3
- Additionally, Delta2D always tries to do the assignments during the image import procedure automatically on the basis of the image names. Please check the proposals in the respective fields of the Import Wizard, or after accomplishing the import, the distribution of the images on the groups.

Multiple Sets in one Project Of course you can have multiple sets of this kind in one project. In this case, Delta2D not only has to be aware of the role of each image, but also to which set it belongs. The set is determined by the image attribute " Gel ". Hence it is important, that

- all images belonging to one set have to be assigned to the same Gel.

Advanced Techniques If you want to include multiple (up to 3) Western Blots in one set, Delta2D needs additional information:

- The Western Blots 1to 3 have to be assigned to different channels.
- When using also multiple Western Blot Contour images (role 4), they have to be assigned to the same channel as their corresponding Western Blots:

  Western Blot Contour 1: Channel 1, Western Blot 1: Channel 1
  Western Blot Contour 2: Channel 2, Western Blot 2: Channel 2
  Western Blot Contour 3: Channel 3, Western Blot 3: Channel 3

Add Groups

New Classic and Internal Standard Projects are always created with two empty groups (Group 1 and Group 2), resp. seven empty groups (2D Western Blot, see above). The Workflow allows for adding new groups. Using the Light Table (see section 5.3 on page 64), you can also easily add groups and rename them by double-clicking on a group's name, e.g. to create one group for every biological sample.

Add Images

Before you can analyze images, they have to be imported into Delta2D. To import images just choose the command add... next to each group's name in the workflow panel (step Setup Project, or in the Light Table or Project Explorer right-click on a group and choose Add New Images.... This will open a list of images included in your pool but not yet added to this project (see fig. 3.2 on page 15).

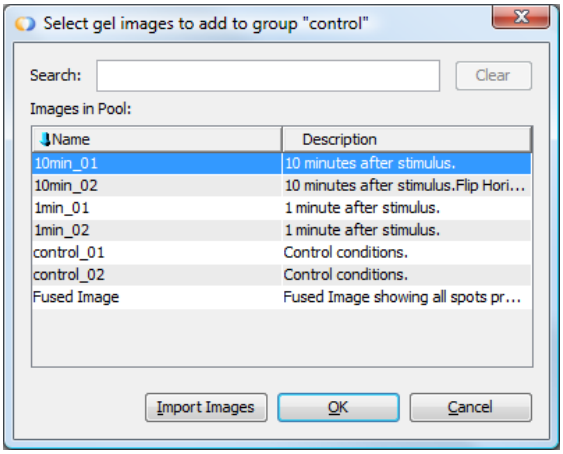


Figure 3.2.: Add images from pool or import from file system

Click Import Images to import image files from your file system. The image import wizard opens. Browse to the folder where your images can be found and select one or more image file(s)

you want to import. Files of the *.gel, *.img, *.tiff, *.png, *.jpg and others are supported. You can browse your file system for the folder containing your images and select multiple images to import them.

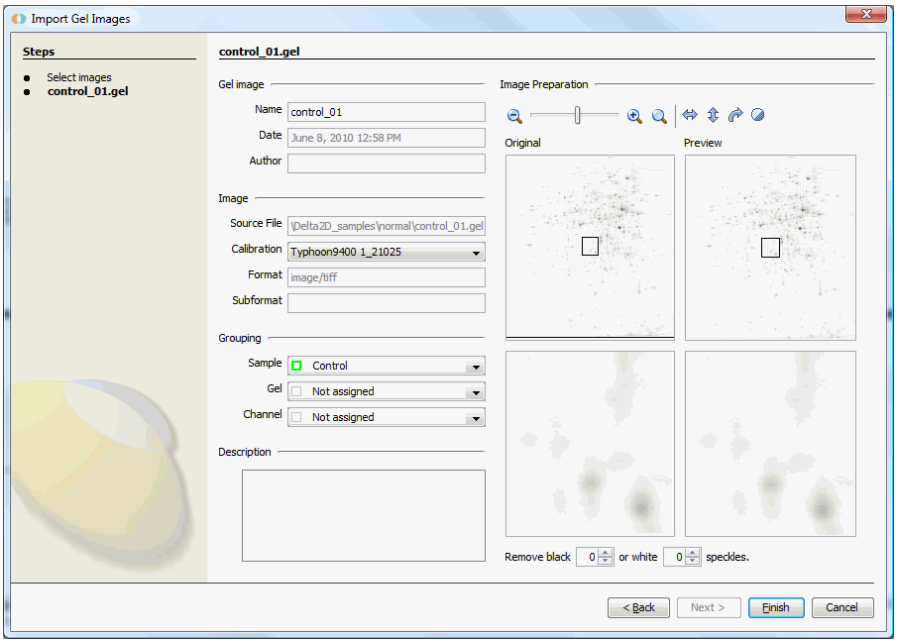


Figure 3.3.: Image Import Dialog

Click Next and the second screen of the image import wizard appears for the first image where you can adjust the image properties. With each click on Next you will get this dialog for the next image in the import list on the left side of this dialog. You can also assign image properties or change them later by right-clicking on an image and choosing Properties from the context menu, then the same screen is called Image Properties.

> Note: The pool contains copies of the imported image files. Thus, your original data is left unchanged and you can continue to work with Delta2D on the copies in the pool even if your original files are moved or deleted.

Image Properties

For each image you can define some describing attributes as the image name (default: file name), the author, the kind of greyscale calibration (applied automatically), and some grouping attributes.

Assigning images to the corresponding sample, Gel and channel is necessary for multichannel projects (for details on multichannel techniques please refer to section 3.1 on page 11), but can

also be quite useful in traditional projects for administration of your images. There are two ways to do this: right away during import of the image (fig. 3.4 on page 17) or later on in the attributes dialog (fig. 4.3 on page 53).

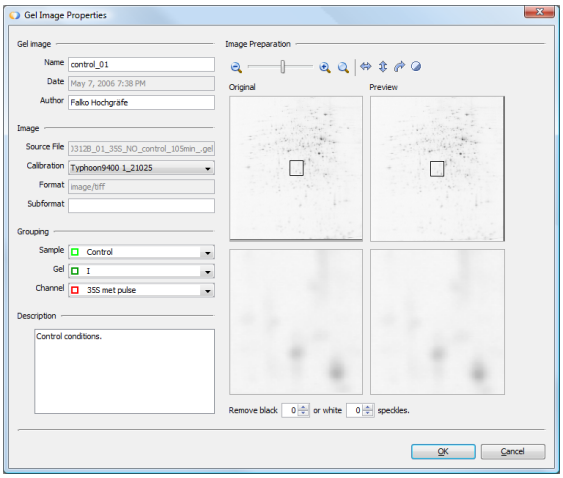


Figure 3.4.: The Image Poperties dialog, shown as part of the image import dialog and available in the context menu of an image.

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. 3.5 on page 17) 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 image and added to the selection list of the respective drop down box, thus available to be chosen for any other image.

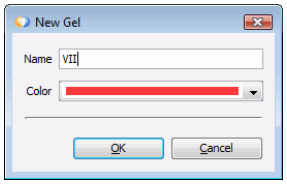


Figure 3.5.: Create a new image attribute, here a Gel.

You can also modify the images. The modifications are limited to those that have no influence on the raw data, i.e. that spot quantities are not affected. The image properties screen includes views of the selected image, containing the complete image and a zoomed view before (left: Orginal) and after (right: Preview) applying the modifications.

These image modifications are available:

- flip the image horizontally
- flip the image vertically
- rotate the image in 90 degree steps
- invert the image

Furthermore you can apply a filter to remove black and white speckles. This filter helps to eliminate artefacts that would just disturb spot detection and quantitation. You can check its effect by comparing the zoomed views. Control the zoom preview by dragging either the rectangle in one of the upper images or the zoomed preview itself to scroll it to an interesting region. Use the zoom bar or press Ctrl and use the wheel of the mouse to zoom in or out. See fig. 3.6 on page 18 as an example where the speckles are removed, while spots remain unchanged.

Figure 3.6.: Effect of the Speckle Filter

Note: If you open a project and have used an early version of the speckle filter using an ini-key, Delta2D will ask whether the speckle filter should persist for all images based on the current ini-key value. This support to migrate the old speckle filter implementation to the new one appears just for those projects that have not yet been touched with the new speckle filter implementation.

In addition to the above described changes of the images, when importing an image the histogram settings are automatically set so that the contrast for the image is enhanced. Please be aware that this does only affect the images' view but not the spot quantities (for histogram settings please see section 5.5 on page 109).

Accurate Scanning Will Be Recompensed

As in any process, the quality of your results is directly connected with the quality of your raw material (the images). 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 imaging your images (please review our Imaging Guide to read more about how to produce optimal images at www.decodon.com/imaging-guide.html):

- 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 provides the option to optimize contrast and brightness of your image, use it.

3.2. Warp Images

Having organized the project in accordance with your experimental setup you now can define the Warp Graph.

Delta2D is based on modern image analysis technology that enables to deal with the unwanted, but always existing distortions between different images. While it is no problem to compare images from the same Gel, it is a challenge to overcome the different spot positions across Gels without tedious manual corrections of what a software has guessed to be a "match". Image warping is the solution for this problem. It is intuitively understandable, easy to use, and results in reliable expression profiles. A project is warped by a set of pairwise warpings, forming the Warp Graph that connects each image with at least one other image, see fig. 5.7 on page 67 for an example.

Employ the Warping Strategy Manager from the Workflow to apply one of the warping strategies as they are described in section 5.4 on page 69 to define a Warp Graph.

All directly connected pairs demanding for a warp transformation are listed in the Workflow and the respective warp transformations need to be calculated. You can use the Job Manager (section 5.10 on page 134) to run automatic warp jobs, but carefully review the results and approve all the automatically generated match vectors (for details about warping see section 5.5 on page 80).

You can double-click on a pair included in the Workflow list of the directly linked image pairs to open the Dual View and check whether your interaction is needed.

3.3. Detect and Quantify Spots

Delta2D supports achieving complete spot matching across all images in just three steps: first fuse the images and then create an appropriate spot pattern on the fused image, which can be transferred to all images.

Fuse Images

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

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

Choose Fuse all images in the Workflow or press the Fuse Images... button in the main toolbar to fuse images or right-click on one of the images and choose Fuse Images.... A dialog opens (fig. 3.7 on page 21), containing some options, plus a list of the available images, sorted by groups.

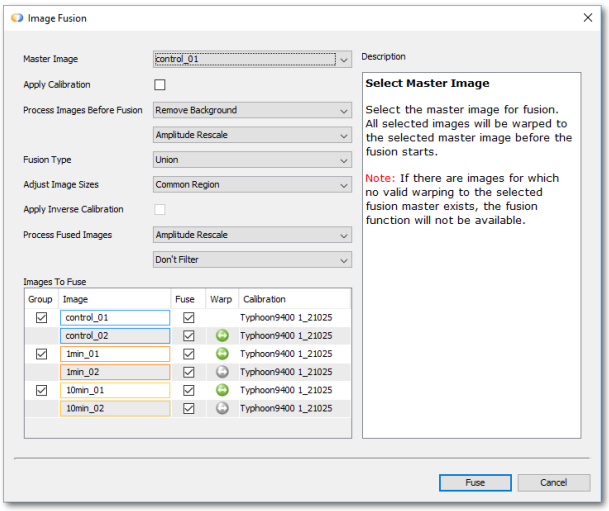


Figure 3.7.: Image Fusion dialog

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 Image. See table 5.1 on page 61 for an explanation of the warp status symbols.

The drop down fields and check boxes let you determine some options for the fusion process (each of them described in the right panel if selected).

Master Image Lets you determine which of the available images should serve as master for the x-y coordinate of the spots on the fused images.

Apply Calibration For images with an attached calibration curve the calibrated grey values (originally scanned optical density) are used for fusion. If calibration is not applied the grey values of the image files are used for image fusion.

This option is available if at least one of the *Images to Fuse* has an attached calibration curve.

If calibration is not applied but all *Images to Fuse* share the same calibration, then this common curve will be attached to the Fused Image, but only when **no filters** were processed during this fusion, neither before nor after fusion.

Process Images Before Fusion Here you can set two steps of preprocessing all images that shall be fused. Remove Background results in brighter images, while amplitude rescale enhances the contrast.

Fusion Type Choose the type of fused image as described below.

Adjust Image Size Select whether the area used for fusion is determined by the common overlapping region of all images 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.

Apply Inverse Calibration On saving the fused image, the calculated values of the fusion process will be transformed into grey values using a calibration curve, exactly like scanning devices do.

The availability of this option and the choice of the calibration curve follows some rules:

- If the *Master Image* is part of the *Images to Fuse* and has a calibration curve, this curve will be used.
- If the *Master Image* is **not** part of the *Images to Fuse*, but all *Images to Fuse* share the same calibration curve, that curve will be used.
- If neither the first nor the second condition applies, this option is not available and no inverse calibration is done.
- This option is also not available, if calibrations are not applied on the *Images to Fuse*

If inverse calibration is applied the used curve will be attached to the *Fused Image*, but only when **no filters** were processed during this fusion, neither before nor after fusion.

Please note some general aspects:

Quantitative spot data of the *Fused Image* is only meaningful, when no filter were processed before or after image fusion and only in these cases:

- calibrations (even different) are applied on the *Images to Fuse*, no inverse calibration is applied (thus the Fused Image has no calibration curve attached), **or**
- calibrations (even different) are applied on the *Images to Fuse*, the above determined calibration were applied inverse on the fused image (thus the *Fused Image* has this calibration curve attached), **or**

- all *Images to Fuse* share the same calibration which is **not** applied, neither on input, nor on output, (thus the *Fused Image* has this common calibration curve attached).

Process Fused Image As for preprocessing, you can determine up to two image processing steps to be applied to the new fused 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.

Note: The Image Fusion dialog shows the warp status for the links between the image where the spots have been detected and the different target images. See table 5.1 on page 61 for an explanation of the warp status symbols.

Why image fusion makes your work much more efficient

Fused images are useful in many ways:

- Obtain complete spot matching 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 image 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 images to one representative.
- Condense the whole experiment's information in one representative proteome map summarizing spot identifications and profiles.

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 map showing each spot appearing on any 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 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 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.

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 91).

If you are in doubt whether a spot is really a spot, you can review the spot's 3D structure. Either open the 3D rollup in the Dual View window, or open the Image Regions window to compare this spot across all images. The Image Regions also includes a 3D mode (see section 5.7 on page 127).

The Quantitation Table includes a column reflecting the spot quality (column Q). Its value shows how similar the spot is to a perfect gaussian distributed shape. Values close to 1 show a very high spot quality, while low values indicate that the spot might be an artefact. Having opened the Dual View with the single view tab for the fused image, click the symbol for the Quantitation Table in the main toolbar to open a table just for the fused image. Sort the table by column Q and check spots with different values for Q in the image by selecting them in the Quantitation Table switching to the Dual View and having an eye on the selected spot. Find a critical Q value and cancel all spots with lower Q value.

Transfer Spots

Especially in combination with Image Fusion this feature is quite useful since it allows for transferring spot boundaries from one image to one or many other images. Having detected spots on a union or max intensity fusion image of all images in the project transfer them to all other 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 complete expression profiles without missing values.

To transfer spots to other images, choose Transfer Spots from the Workflow,click the  button in the main toolbar, or *right* click on a 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 fig. 3.8 on page 25). 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.

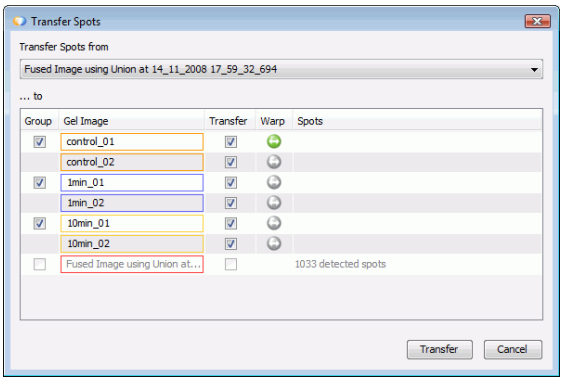


Figure 3.8.: Transfer Spots Dialog

Note: Read more about the benefits of Complete Spot Matching at www.decodon.com/delta2d-spot-matching.html.

Having transferred the spots from the fused image the spots are automatically quantified on each target image and matched with their *parent* spots and their respective *sister* spots on the other images.

Note: Delta2D's image fusion, spot transfer, and spot matching rely on well-aligned 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. See table 5.1 on page 61 for an explanation of the warp status symbols.

3.4. Analyze Expression Profiles

Statistical Analysis

Delta2D includes an integrated statistics toolbox. Depending on the experimental setup, the structure of your data, and the scientific questions that you like to address you can choose from a variety of statistical analysis methods. They include algorithms to cluster the data as well as parametric and non-parametric tests. For more details see section 5.11 on page 135.

Spot Picking

Having analysed the project some time has passed since the images have been digitized. In the meantime spot positions might have shifted slightly, of it is just impossible to position the gel on the picker with respect to the correct zero coordinate. For this reason a new image needs to be taken from the gel, using the picker camera. Import this image to Delta2D, warp it to the respective image and transfer the labels to the new image. If picking shall be based on marked spots as well you can either mark or label the spots of interest on the pick image.

> Note: Usually the resolution of the picker camera is much worse compared to your imaging device. To warp a emphsmall image to a larger one (in terms of image resolution) in the Dual View, you can first define four match vectors for spots close to the four corners of the two images. Press the warp button and then either add more vectors or employ automatic warping.

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:

- Generic pick list format
- Molecular Dynamics
- Genomic Solutions ProPic
- PerkinElmer ProXCISION
- Ettan Spot Handling Workstation
- Bruker Proteineer
- Biorad Exquest
- Herolab

We are always interested in 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.

In the Dual View or Quantitation Table use Export ▷ Export Pick Lists ... ▷ *spot picker name* to generate a pick list in the desired 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

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. Each picklist consists of four columns separated by a tab containing the 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.

Molecular Dynamics™

The Molecular Dynamics spot picker needs two special landmarks that are placed on the gel. In order for the robot to register the 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 separated by tabs while the coordinates 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 spot 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.

Genomic Solutions™

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. 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 last three columns consist of generic data.

Ettan Spot Handling WorkStation™

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. As for the Molecular Dynamics™ picker two landmarks named "IR1" and "IR2" are expected to be defined.

Bio-Rad Exquest or Herolab

For these two pickers just the picklist needs to be exported.

Bruker Proteineer

This entry is typically disabled because the interface needs additional setup. If available, the picklist is handed over to the Proteineer information system, to which the picker is connected.

Spot Annotations: Support for protein Identification by Mass Spectrometry

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

Furthermore, having exported a Pick lists and employed mass spectrometry and database search for identification of the spots results in a list of protein names or database accession numbers, Delta2D can translate the label names into the new names or accession numbers.

Automatically Create Labels in the Dual View

You can choose to label selected spots using spot-IDs or using consecutive numbers. In the screenshot 3.9 on page 29, 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 Labels tab of the Options dialog (see section 6.1 on page 156). Using automatic numbering helps to keep 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 image you want to create labels on. Even easier is to label 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 Labels tab. Type in any string you want to be prepended to the numbers in the field Prefix for Numbered Labels.

Automatically Create Labels in the Quantitation Tables

Automatic labeling is also possible in the Quantitation Table: select the matchings or spots in any table, open the single table of the image you want to create the labels on and select the menu

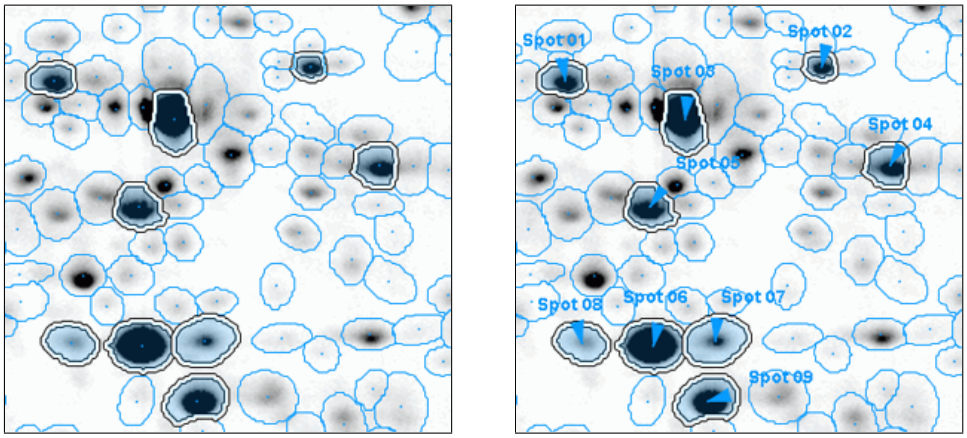


Figure 3.9.: Label selected spots with numbers

item Labels ▷ Label Selected Spots with ..., or Labels ▷ Label Unlabeled Spots with ..., respectively.

> Note: Automatic Labeling works in single image tables only. You can open a single image table by selcting the image in the Project Explorer, Light Table, or Warping Setup and clicking the  symbol in the main toolbar.

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 (fig. 3.9 on page 29). These labels are then used to create a Pick lists (see fig. 3.4 on page 26 for how to export picklists) .

```
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,  767.4521890944202,  411.8709608300085,   Spot 02
36972,  652.5601947607652,  418.1682292961424,   Spot 01
37094,  691.9939555744654,  537.246479717167,    Spot 06
37248,  535.742617849653,   733.3756788121266,   Spot 11
37114,  716.4720942243414,  562.0853241641546,   Spot 09
```

Figure 3.10.: Label list made from labeled spots

Interesting spots are processed in the usual way (e.g. mass spectrometry and database search).

The protein identification results usually come in the form of a table with label names and corresponding protein names. You can use this table to automatically rename the labels Spot 01, Spot 02 etc. to show the names of the identified protein. First, save the table as a CSV file. The table and the CSV file, when opened in a text editor, should look similar to fig. 3.11 on page 30.

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

```
"Spot 01","CitH"
"Spot 02","CysK"
"Spot 03","Hag"
"Spot 04","Eno"
"Spot 05",""
"Spot 06","EF-Tu"
"Spot 07","GlnA"
"Spot 07","SigB"
"Spot 08",""
"Spot 09","CitC"
"Spot 10","CitC"
"Spot 11",""
```

Figure 3.11.: Old and new label names in a spreadsheet and a CSV file

Now, in Delta2D's Dual View of the image containing the labels, open the Labels menu and choose Translate Label Names. This will open the Translate Labels dialog (see fig. 3.12 on page 30):

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 3.13 on page 30 shows the image from above with translated labels.

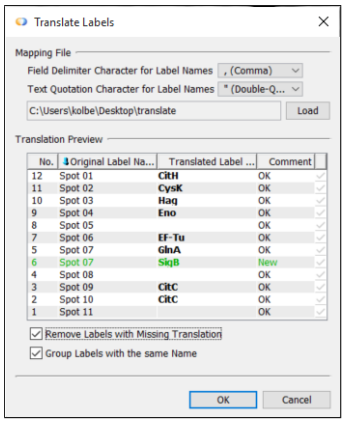


Figure 3.12.: Translate Labels Dialog

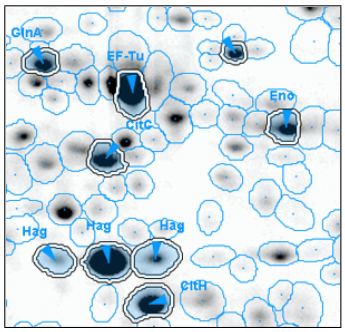


Figure 3.13.: Translated Labels

If the list includes two or more different protein names for the same label (perhaps because the mass spectrometry and database search gave multiple significant hits), new labels will be

created automatically, so no identification is lost.

The translation dialog comes with two options:

Remove Labels with Missing Translation You can remove labels for which you have no translation (i.e. where protein identification failed). Furthermore the old label names will not be available anymore, so you should export them to a file if you want to save them.

Group Labels with the same Name If more than one label is translated into the same protein name, you can automatically group these labels.

Scouts: Find Information in Web Resources

Delta2D's scouts are lean software programs that can go out to local or web resources and come back with useful information about a protein. Scout data can be protein properties such as isoelectric point or molecular weight, annotations such as pathway information, sequences, and much more. For sequences fetched either from GenBank or UniProt the isoelectric point, molecular weight, and amino acid statistics are automatically computed using the EMBOSS toolkit.

For using scouts you need labels with protein names (e.g. created by label translation as described above), since these are the query strings for most databases, along with the organism that needs to be entered when starting a query. The information that was retrieved by scouts is attached to labels and saved in the data pool so they do not need to be retrieved from the web again.

> Note: Scouts can 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.

Delta2D can search a database for all labels on a image in a batch. Choose Labels ▷ Fetch Scout Data ▷ scout name ▷ image name and wait while the query is processed. You can also look for scout data for a single label by right-clicking on the label when having activated the Label tool. The bottom of the context menu shows excerpts from scout data, one line per scout. Use the Edit Scout data menu item to review and edit the data. Press Get Data to lookup a database for the given search string.

> Note: You can present labels and scout data for complete images or selected or marked spots easily in the Labels Report. See section 3.5 on page 37 for more details.

At the bottom, the context menu for a label shows some basic information, e.g.:

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

Scout	Data
AureoList	Gene and Protein information from the AureoList database maintained at the Institut Pasteur.
GenBank	Protein sequence, accession number etc. from NCBI GenBank.
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
UniProt	Protein Sequence, function, keywords, Gene Ontology, References, etc. from the UniProt database, maintained by the UniProt Consortium.
Data Table	Import a table of arbitrary data fields
Physicochemical properties	Molecular weight, isoelectric point etc., notes. All data is entered by the user.

Table 3.1.: Scouts and the data they access.

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

To quickly delete a certain 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.

Scout for the AureoList Database

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.

Scout for the GenBank Database

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.

Scout for the GenoList Database

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 in the Dual View. Delta2D will fetch data from the last Genolist database you selected.

Scout for the UniProt Database

Open the scout by selecting Edit scout data ▷ UniProt from a label's context menu. Enter UniProt accession or protein and organism name, then press Get Data button. A list of protein annotation data will show up (see figure 3.14 on page 33). You will find links to the UniProt entry itself as well as to attached keywords and Gene Ontology.

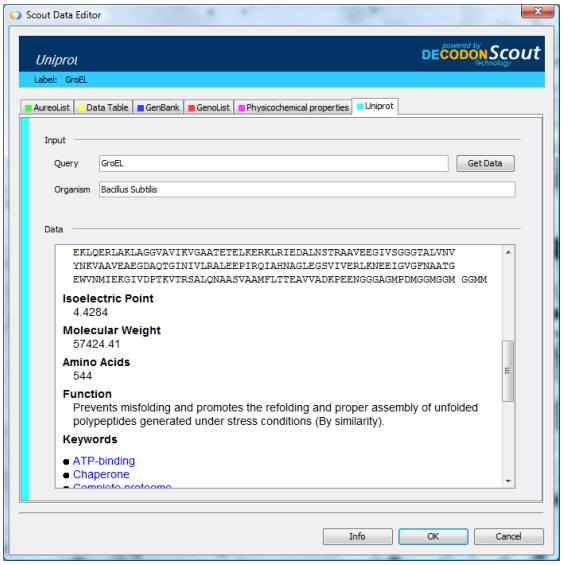


Figure 3.14.: The scout data attached to a label, here fetched with the UniProt scout

The scout automatically retrieves isoelectric point and molecular weight using UniProt sequence for computation done by the EMBOSS toolkit. The values are attached to your label if you confirm the Scouts dialog with OK.

Scout for Manually Adding Physicochemical Properties

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

Scout for Data Tables

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 image

Here an example:

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

3.5. Present Results

Delta2D 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 Toolbar or the Reports menu. Each report is opened in your web browser. If you want to have a closer look on a image or a spot, just click on it and it will be opened and focused in Delta2D. The report index just includes an overview of all reports.

Example reports are available at www.decodon.com/delta2d-web-reports.html.

Furthermore, images can be exported as presentation slides and data as spreadsheets, or as standard image files or character separated value (CSV) files.

Project Summary Report

The project report shows a summary of your analysis project. It includes general data about the images, groups, samples, and optionally the images themselves. Optionally you can get an overview of images and warpings.

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

By default the images are excluded from the report but can be shown easily by clicking the respective link. 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.

Spot Album Report

The spot album shows thumbnails of selected or marked spots and their neighbourhood.

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

The album can be configured using the form in the upper part of the report: You can define that the report includes spots being selected or marked on a certain image (then the report includes label names, spot IDs and estimated pI/MW values, if available), choose a reference image for false color images, and you can change the width, scale and the zoom factor of the image section that should be displayed. Finally, you have the option to show the image tiles with or without the spot boundaries.

If the album shows expression profiles for spots which have been selected or marked on a certain image you can sort the album by the Label or Spot column.

Click on any spot in the row to select and show it in the dual view.

Besides the image thumbnails each row contains the expression profile as a bar chart. Clicking on the bar chart takes you to a detail page with two tabs showing additional information about quantities and labels for this expression profile. The bar charts look like the bar charts in the Expression Profiles Window, where their appearance can be customized.

Having choosen a certain image instead of 'any image', this report includes links to show the labels report for the respective image, and to a subreport highlighting the spot list on this image.

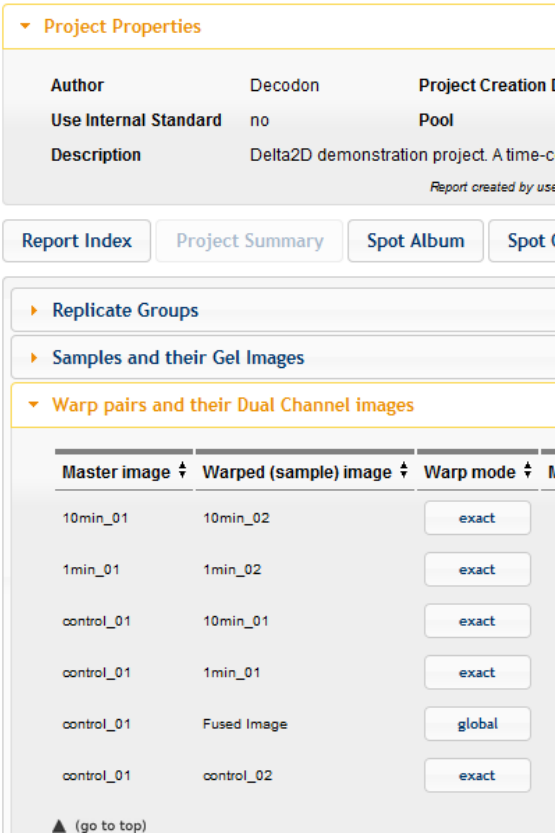


Figure 3.15.: The project summary report

Spot Quantities Report

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

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

Expression profiles are linked to the detail report page with two tabs showing additional information about quantities and labels for this expression profile.

Click on the tab wide to get a quantitative report including all table columns from both the all images and the statistics table for the marked spots.

Having defined a certain image instead of 'any image', this report includes links to show the labels report for the respective image, and to a subreport highlighting the spot list on this image.

The tables can be sorted by any column.

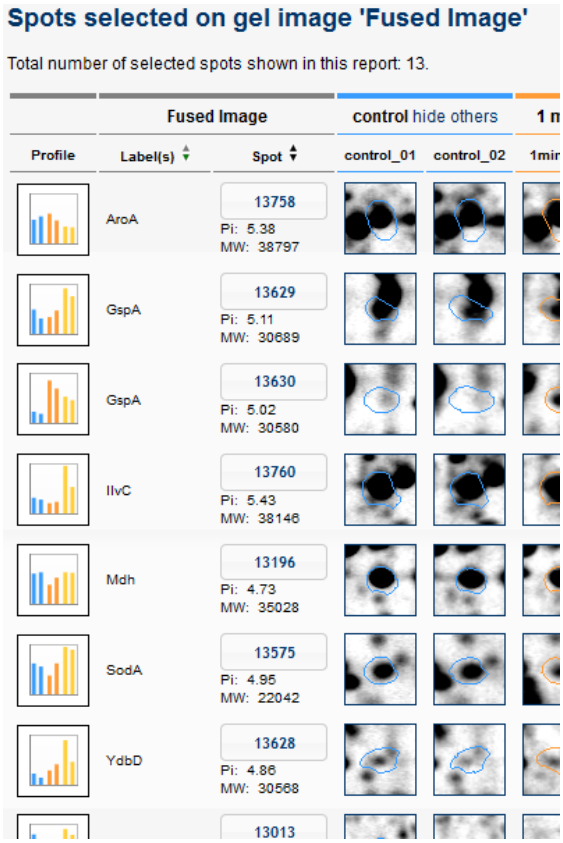


Figure 3.16.: The spot album report

Labels Report

The labels report includes two different tabs: one to show all labels available on the image, even if they do not point into a spot, and the other only for the labels pointing into selected or marked spots. Both versions show the available label and scout data and include the estimated pI/MW values, making it easy to compare them with the isoelectric point or molecular weight found in a public web data base.

Every report containing labels reflects the fact that spots can contain multiple labels. In the label report that lists all labels for a certain image the same spot appears as often as labels point into it.

Click in the Main Toolbar or choose Reports ▷ Labels.

If labels point into a spot they are linked to the detail report page with two tabs showing additional information about quantities and labels for this expression profile.

The tables can be sorted by any column.

This report includes links to show the spot quantities report for the respective image, and to a subreport highlighting the spot list on this image.

Figure 3.17.: The spot quantities report

Image Report

The image report shows all selected or marked spots on a certain image. These spots are highlighted and also clickable, so that you can access the expression profile report for the respective spot.

This report is available from any of the other reports.

Below the image you find a list of all spots. This tables can be sorted by any column.

Blotting Report

The Blotting report addresses the relationship of the numbers of spots between total protein and Western Blot images. This tool reveals the coverage of spots from the total protein on the Western Blot image, and the reproducibility of image pairs in groups.

This report initially opens with a small form enabling you to include some comments to this report. After having submitted this form, the actual report becomes visible. It consists of the following sections:

Figure 3.18.: The labels report

Report Content

- 'Sample to Gel Assignment'
  - The section shows a table summarizing the sample to gel assignment used in this Blotting Report.
- 'Experimental Setup'
  - The coverage formulas are automatically applied.
- 'Fused Images from Group Fused Images'
  - On one page a maximum of 6 fused images is described (configurable).
- 'Transferred Spot Pattern'
  - The number of spots after spot editing is the sum of originally detected spots and manually added spots.
  - The number of transferred spots is the difference between spots after spot editing and canceled spots.

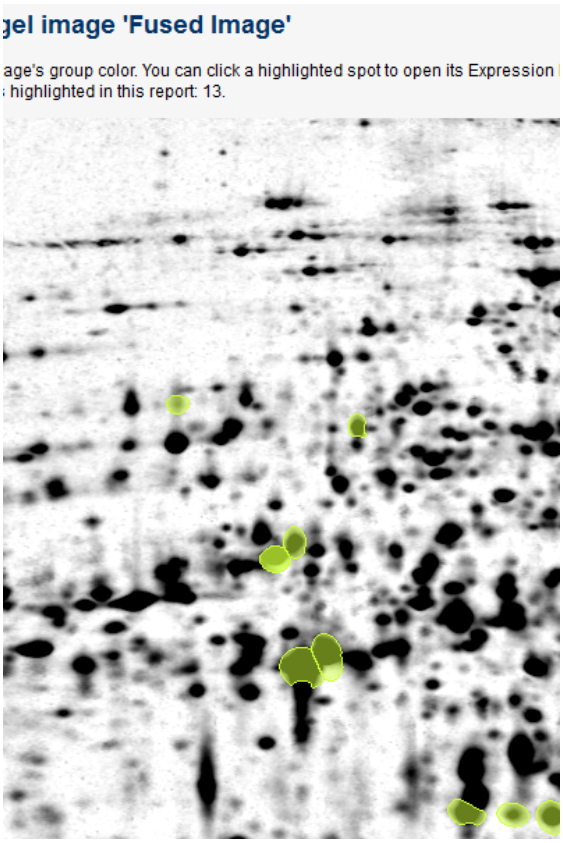


Figure 3.19.: The image report

- 'Fused Image "..."' – the origin of the transferred spot pattern
  - The image shows all spots and spot edit markers (if available).
  - Canceled spots are shown with dotted spot boundaries.
- 'Image Pairs'
  - Includes data for the interesting image pairs for which the 'Coverage' table includes values.
  - Images show spot boundaries for interesting spots only, not for canceled spots.
  - Matched Spots are shown in blue, unmatched in green or red, respectively.
- 'Coverage Table':
  - Coverage is calculated based on the formula as shown in the examples in fig. 3.20 on page 42.
  - Coverage table is split after a maximum of 2 leading columns and 14 value columns and/or 2 leading rows and 40 value rows (configurable).

  - Groups will not be split.
  - To reduce the width of coverage tables a legend can be introduced.

- 'Results'
  - 'Reproducibility for Image Pairs in Groups' (Table 1) and 'Statistics on Relative Coverage for Group Pairs' (Table 2) are always based on the respective existing coverage values.
  - 'Mean' is the mean of coverage value of each image pair
  - 'RSD' (relative standard deviation) is the 'corrected sample standard deviation' (as defined on Wikipedia), expressed in %.
  - 'N' is the number of image pairs (Table 1) or the number of replicates (Table 2)

- 'About the Report'
  - Contains details about requirements, usage and organization of this report, as well as some technical background information.

Analysis Procedure

1. The project may contain up to 9 groups (plus fused image group and internal standard group), further groups will be ignored.
2. Each group may contain up to 6 images, further images will be ignored.
3. Image names are not longer than 35 characters, gel numbers not longer than 2 characters.
4. The group containing fused images is named 'Fused Images'.
5. A group called 'Internal Standard' will be ignored as this group is assumed to contain just internal standard images.
6. Spot transfer to all images must be done from the same fused image.

Experiment Structure

Westernblots with multi fluorescence, maximal 3 Westernblot dyes. Total-Protein group must be the first group in the project. Image pairs:

- each Total Protein Image with one Westernblot Dye N (N: 1...3)
- images in all groups must be sorted the same way to enable an appropriate assignment of each western blot image to the respective total protein image
- all possible pairs within each group
- Special coverage formula for image pairs Total Protein Image - Westernblot Dye
(See an example in fig. 3.20 on page 42)

- A number of 1 (k) group 'Total Protein' and 3 (l) groups with 'Westernblot Dyes' with 6 (m) images each results in 6*3+4*(6*6-6)/2=78 image pairs, i.e. the same number of report pages, general: m*l+(k+l)*(m*m-m)/2.

Group		Total Protein						same as row group					
	Image	1	2	3	4	5	6	1	2	3	4	5	6
1 'Total Protein'	1 'Image 1'	-	-	-	-	-	-	-	-	-	-	-	-
	2 'Image 2'	COV1	-	-	-	-	-	-	-	-	-	-	-
	3 'Image 3'	COV1	COV1	-	-	-	-	-	-	-	-	-	-
	4 'Image 4'	COV1	COV1	COV1	-	-	-	-	-	-	-	-	-
	5 'Image 5'	COV1	COV1	COV1	COV1	-	-	-	-	-	-	-	-
	6 'Image 6'	COV1	COV1	COV1	COV1	COV1	-	-	-	-	-	-	-
2 'Western Blot 1'	1 'Image 1'	COV2	-	-	-	-	-	-	-	-	-	-	-
	2 'Image 2'	-	COV2	-	-	-	-	COV1	-	-	-	-	-
	3 'Image 3'	-	-	COV2	-	-	-	COV1	COV1	-	-	-	-
	4 'Image 4'	-	-	-	COV2	-	-	COV1	COV1	COV1	-	-	-
	5 'Image 5'	-	-	-	-	COV2	-	COV1	COV1	COV1	COV1	-	-
	6 'Image 6'	-	-	-	-	-	COV2	COV1	COV1	COV1	COV1	COV1	-
3 'Western Blot 2'	1 'Image 1'	COV2	-	-	-	-	-	-	-	-	-	-	-
	2 'Image 2'	-	COV2	-	-	-	-	COV1	-	-	-	-	-
	3 'Image 3'	-	-	COV2	-	-	-	COV1	COV1	-	-	-	-
	4 'Image 4'	-	-	-	COV2	-	-	COV1	COV1	COV1	-	-	-
	5 'Image 5'	-	-	-	-	COV2	-	COV1	COV1	COV1	COV1	-	-
	6 'Image 6'	-	-	-	-	-	COV2	COV1	COV1	COV1	COV1	COV1	-
4 'Western Blot 3'	1 'Image 1'	COV2	-	-	-	-	-	-	-	-	-	-	-
	2 'Image 2'	-	COV2	-	-	-	-	COV1	-	-	-	-	-
	3 'Image 3'	-	-	COV2	-	-	-	COV1	COV1	-	-	-	-
	4 'Image 4'	-	-	-	COV2	-	-	COV1	COV1	COV1	-	-	-
	5 'Image 5'	-	-	-	-	COV2	-	COV1	COV1	COV1	COV1	-	-
	6 'Image 6'	-	-	-	-	-	COV2	COV1	COV1	COV1	COV1	COV1	-

$$\textbf{Coverage (COV1)} = \frac{\text{No. matched spots}}{\text{No. matched spots + No. unmatched spots Image 1 + No. unmatched spots Image 2}} * 100\%$$

$$\textbf{Coverage (COV2)} = \frac{\text{No. spots Western Blot Image}}{\text{No. spots Total Protein Image + No. unmatched spots Western Blot Image}} * 100\%$$

Figure 3.20.: Blotting Report Coverage

Modify, Save, and Print Reports

All reports are produced as 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 text or presentation document (e.g. created with MS Word™or MS PowerPoint™). Please avoid to use the browser's Save page as... function since it will not save every report detail. Instead, you can save the whole report structure or each single report using the Save all reports button in the top right of the report index or the Save this report button in the top right of each single report. Delta2D will then prompt you for a file chooser to decide where the report should be saved. The reports will be saved without the configuration forms. 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.

> Note: The reports are based on html/css, Python and Velocity. Design and content of the reports can be customized according to your needs. Contact us if you like to learn more about report customization.

Export Results to Other Applications

Choose Export ▷ Export CSV in the Quantitation Table menu to save the data in a common exchange format called "character 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 Export to MS Excel™or MS PowerPoint™

You can export the content of any Quantitation Table as a table file (in xlsx format). The respective table is exported as it is visible in Delta2D e.g. you can sort and filter data before exporting it. The new file is automatically be opened with the standard application for this file extension, e.g. MS Excel™ LibreOffice or OpenOffice. In any Quantitation Table choose Export ▷ Export Spreadsheet.

Images can be exported as presentation slides (in pptx format) in the Dual View or in the Color Coding window. The new file is automatically be opened with the standard application for this file extension, e.g. MS PowerPoint™ LibreOffice or OpenOffice. The slide includes all objects which are currently visible in the respective window, they are all fully editable. In the Dual View window, use Export ▷ Export Presentation Slide, in the Color Coding window click the button.

Using the feature Data from web e.g. in MS Excel™ or LibreOffice you can load data from Delta2D's reports by using the reports URI.

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

	New Project...	Create a new project.
	Open Project...	Open an existing project.
	Save Project...	Save your changes.
	Close Project	Close your project.
	Add 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	Paste selected object(s).
	Delete	Delete selected object(s).

Images

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

Pool

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

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

Help

	Help...	Opens the online help.
	Get remote assistance...	Gives access to web based remote assistance.
	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

The Main Toolbar includes the following entries (some of them being active only if one or more suitable objects are selected):

	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 Warp Strategy...	Opens the Warping Strategy window.
	Change Warp Mode	For selected image pairs you can change the warp mode.
	Image Visibility ...	Open the 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 Images...	Opens the dialog to add images to the project.
	Manage Images...	Opens the dialog for managing the image pool.
	Project Summary	Opens the summary report in your web browser.
	Spot Album	Opens the album report for marked spots.
	Spot Quantities	Opens the table report for selected or marked spots.
	Labels	Opens the Labels report in your web browser.
	Open in Dual View	If a pair of images is selected: Opens it in the Dual View.
	Open Quantitation Table	Opens a table for the currently selected images or groups.
	Options...	Opens the Options dialog.

Table 4.1.: Buttons in the Main Toolbars.

4.3. Pool Organization

Image Manager

Select from the menu Pool ▷ Manage Images.... The Image Manager will open and list the images contained in your pool (see fig. 4.1 on page 51), enriched with image attributes and and showing the projects that include a certain image.

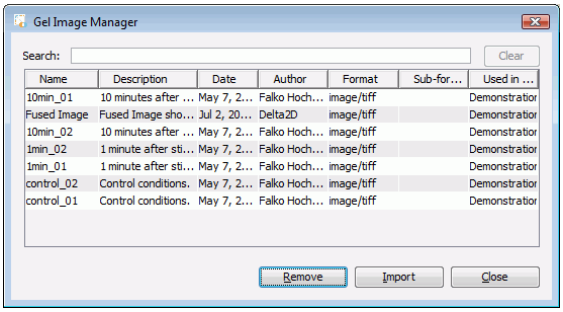


Figure 4.1.: The Image Manager

Press the Import button to add a new image. The image import wizard opens. Browse to the folder where your 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. You can browse your file system for the folder containing your image images and select multiple images to import them (see fig. 3.3 on page 16).

Click Next and the second screen of the image import wizard appears for the first image where you can adjust the image properties. With each click on Next you will get this dialog for the next image in the import list on the left side of this dialog. You can also assign image properties or change them later by right-clicking on an image and choosing Properties from the context menu, then the same screen is called Image Properties.

Project Import and Export

As described in section 3.1 on page 9 Delta2D is keeping data in certain folders on your hard disc (the *Pool*). A pool can contain different *Projects* representing different experiments. We recommend to create a new pool if a new project is completely seperatable from other projects.

However, it can happen that projects are distributed across different pools, though they should be included in the same pool, or that a pool includes too many projects that should better be separated into different pools. For this reason Project Import and Project Export have been introduced.

> Note: Please note that during exporting or importing projects no project may be opened in Delta2D. Furthermore, to protect your data, exporting projects does not mean that they will be deleted from the current pool.

To import projects from a different pool into the current pool select Pool ▷ Import Project... from the main menu. Browse your file system for the source pool (folders including a Delta2D pool are highlighted with the Delta2D icon) and then select one ore more projects from the upcoming list to be imported into your current pool.

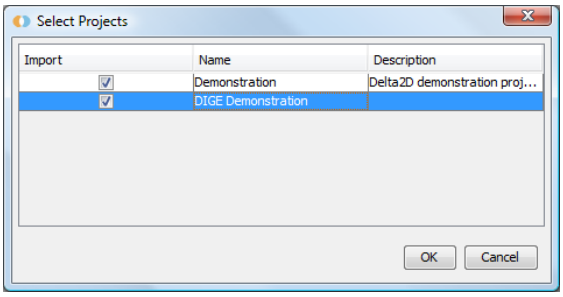


Figure 4.2.: The Project Import Dialog

> Note: Conflicts may arise during the import or export of a project into an existing pool, e.g. an image with the same name might exist already. These conflicts are automatically recognized and resolved by adding a subsequent number to the name of the new image. E.g. image *control_01* would be renamed to *control_01-1*.

For exporting projects choose Pool ▷ Export Project.... The upcoming dialog for listing the projects looks rather similar to the one when importing projects. Select the projects to be exported and then browse for the destination pool.

> Note: Projects can be exported into a new pool by selecting an existing or creating a new folder. You will be prompted to confirm that the selected folder should really receive the data structure of a Delta2D pool directory.

4.4. Helpful Dialogs for Projects

Having opened a project, some helpful dialogs are available for providing access to image attributes (particularly of interest when using an internal standard), controlling the visibility of images in different views or table columns, or defining spot detection parameters project wide.

Image Attributes

For completing experimental design details it is a nice option to assign all images to a Gel, a Sample, and a Channel in one view using the Image Attributes dialog (fig. 4.3 on page 53). Particularly if you are running a multiplex experiment you have to define the relation between images and Gels. In case the experiment includes an internal standard you also need to define which images carry this internal standard. Please choose Images ▷ Image Attributes... from the menu to open it. The Image Attributes dialog presents three tabs on top, one for each of Gel, Sample, and Channel. The tabs are very similar, so we describe the procedure of assigning attributes to one or more images exemplarily on the list of Images.

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

- the current project
- all 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.

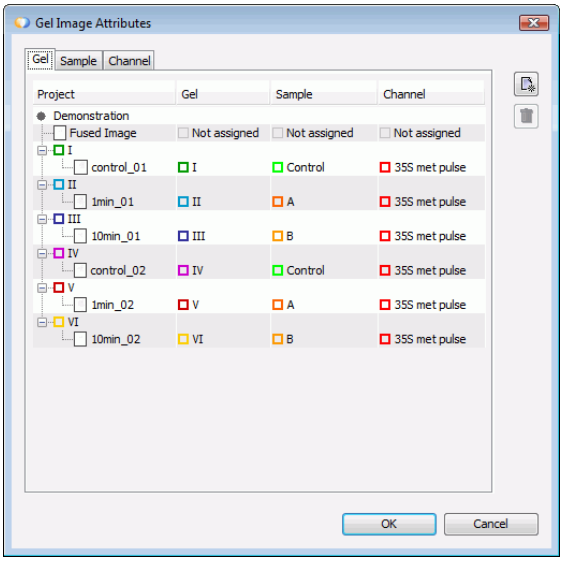


Figure 4.3.: The Image Attributes dialog

You can assign an image to a Gel in two ways:
Select one or more images in the list, and then

Context menu: 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, or

Drag and drop: 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.

If you are running a project using an internal standard you will also have to define

Image and Table Column Visibility

A quick and effective way to customize the Quantitation Tables is 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.

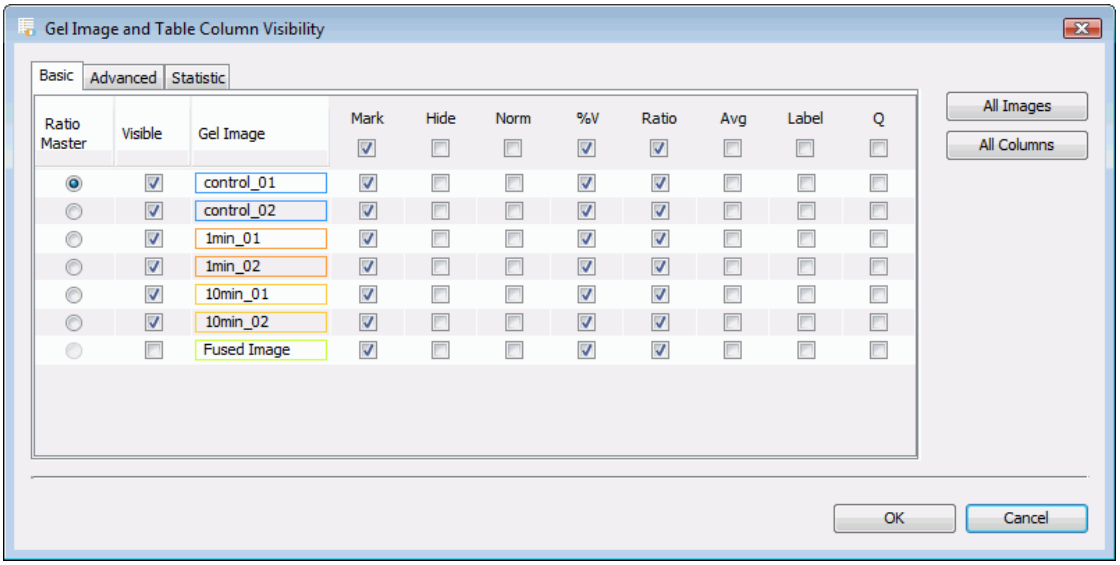


Figure 4.4.: The properties dialog of the Quantitation Table

The dialog includes the following options:

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

Visible Check the images you want to see in your table. Visibility also applies to the Image Regions window.

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 images as visible.

> Note: You can present spot quantities for selected or marked spots easily in the Spot Quantities Report. See section 3.5 on page 36 for more details.

Spot Detection and Quantitation Parameters for All Images

> Note: This dialog is useful if you want to detect spots on the different images individually. However, this results in asymmetric spot patterns on the different images which causes difficulties that can be avoided (e.g. statistical significance of results is decreased).
>
> 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. Then do not change these settings because each change would result in a redetection of spots on the respective image, which will screw up the complete spot matching in your project.

The Spot Detection and Quantitation dialog (see fig. 4.5 on page 55) allows you to control the detection and quantitation parameters for all images of your project in one place. Open it by selecting Images ▷ Detection Parameters. . . .

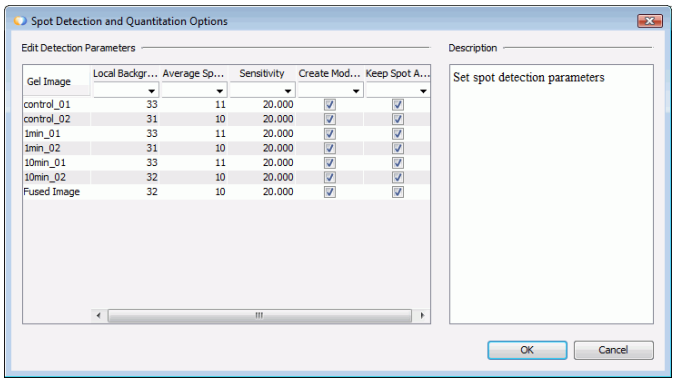


Figure 4.5.: The Spot Detection and Quantitation Parameters Dialog for all images.

Simply select one or more 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 images as soon as you hit the Enter key moved to the next field with the Tab key.

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 9.

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.

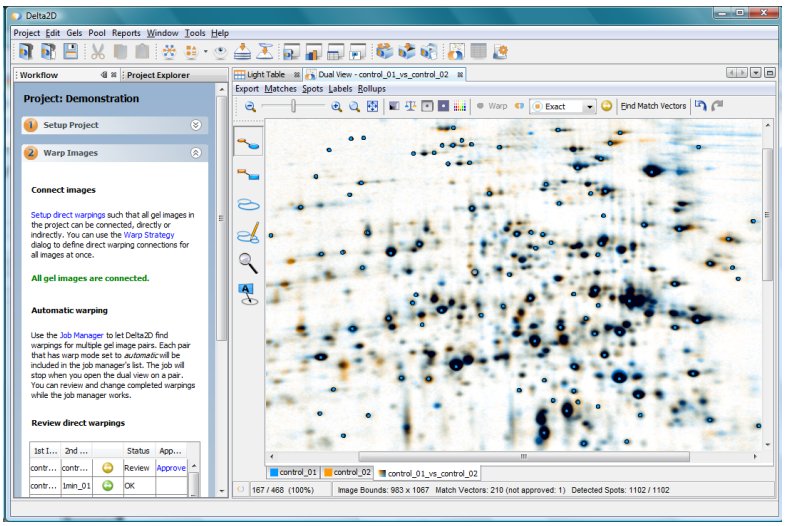


Figure 5.1.: The Workflow

If a project has been opened the workflow is providing the following steps, which are described more detailed in section 3 on page 9:

1. **Setup Project**
2. **Warp Images**
3. **Detect and Quantify Spots**
4. **Analyze Expression Profiles**
5. **Present Results**

Each step reflects the current status of the project and contains links to the functions still needed in the respective step. Please read more about each step in section 3 on page 9.

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 (fig. 5.2 on page 58 where you can first change the location of the data in your filesystem and then open a new or existing project.

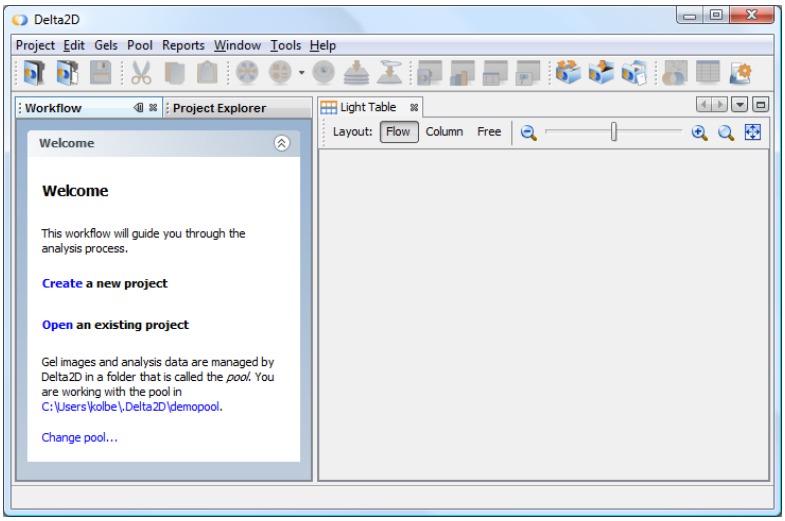


Figure 5.2.: The Welcome Screen

5.2. Project Explorer

The Project Explorer (fig. 5.3 on page 59) 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.

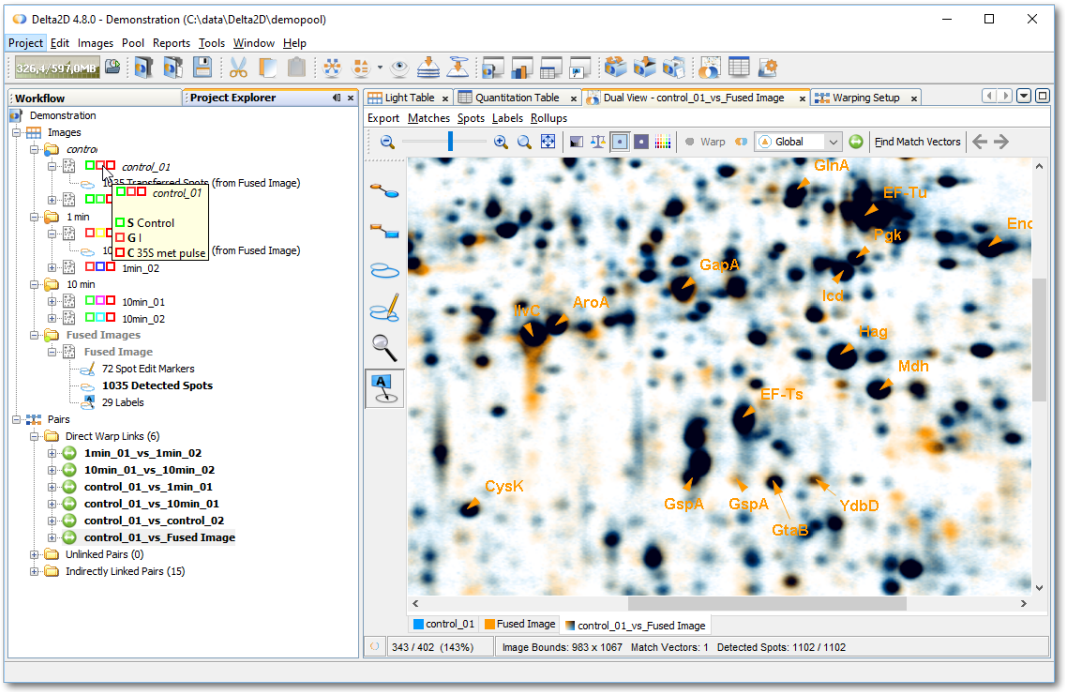


Figure 5.3.: The Project Explorer

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 (Windows: right-click on the object to open the context menu). Pointing on the triple icon of an image for 2-3 seconds opens a tool tip showing the assignment of this image to Sample, Gel and Channel.

'Drag and drop' and 'double click' have well defined consequences in the Project Explorer.

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. You can 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 or labels as well: drag such a spot or label set onto another image or group, or drag it even to the whole project to transfer (copy) it to the respective images. Labels will be copied to the respective images if a label with the same text pointing into the spot on the target image does not yet exist.

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 image pair with activated Match Vector Tool.

The current warp status is displayed by one of a set of icons, which is also used in the Warping Setup and in the Workflow window (see table 5.1 on page 61, please refer to section 5.5 on page 80 for details).

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.

Right click on a pair to get a menu of further available operations for this pair (table 5.2 on page 60):

Open in Dual View	Opens the Dual View.
Open Quantitation Table	Opens the Quantitation Table.
Delete	Deletes this direct warp connection and sets the warp mode to *implicit*, but does not delete the match map.
Cut	Puts the pair to the clipboard, so that you can paste it to the group *Indirectly Linked Pairs*.
Paste	If the clipboard includes a pair, you can paste it into this pair group.
Change Warp Mode ▷	Changes the warp mode for this image pair (see section 5.10 on page 134).
Scatter plot	Shows a scatter plot for the image pair (see section 5.2 on page 62).
Status	Presents the detailed current status of this image pair.

Table 5.2.: The context menu for image pairs

pair is fine These two images can be warped according to the defined direct warp mode, since the match map contains *only* approved match vectors.

pair is fine These two images do not need to be warped since the warp mode is identical and no match map is needed (e.g. if the images come from the same gel).

review match map The warp mode demands for a match map but contains no or *non-approved* vectors. Verify and approve, or delete all non-approved or add match vectors.

execute automatic warp Automatic warp mode is chosen but not yet executed. Either open this pair in the Dual View, or open the Job Manager and start the automatic warping. After the automatic warping is executed the warp status will change to the yellow *review match map* icon.

link this pair 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. In the Project Explorer all these pairs appear in the subgroup *Unlinked Pairs*. Please note that with adding just one direct warping connection many unlinked pairs will be linked.

resolve cycle conflict 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 70). Delete at least one direct warping.

cycle conflict There is a Warping Cycle, but the conflict should be resolved somewhere else since this pair has been set to identical warp mode.

implicit is fine There is an implicit warp between the two images. You can open the Dual View for this image pair.

execute implicit 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.

Table 5.1.: Warp status icons

In the Status window the warp mode for a pair is indicated by one of the following icons:

- 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 80. 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 images:

No quantitation data on both images

Complete matching: every spot on the one 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 3.3 on page 21.

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.

Scatter Plots for Image Pairs

For images pairs, Delta2D offers scatter plots (see fig. 5.4 on page 63). Scatter plots show the ratios of the relative volumes in two images. You can produce a scatter plot by going to the Project Explorer, right-clicking on a image 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. In the Warping Setup you can open the scatter plot for a pair through the context menu of the direct link between two images.

Use a scatter plot to

- check whether normalization is well balanced: the spot cloud should be symetric to the 45 degree line, or
- find interesting spots: the scatter plot includes lines representing 2-fold, 5-fold, and 10-fold changes.

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.

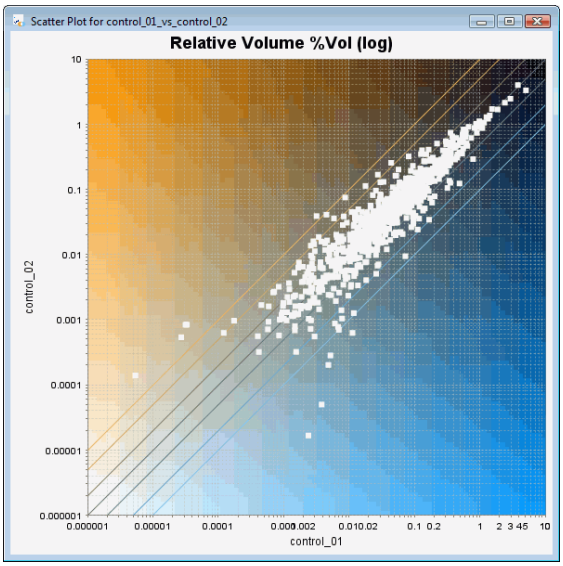


Figure 5.4.: The Scatter Plot

5.3. Light Table

The Light Table helps you to get your project organized (see fig. 5.5 on page 64). 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.

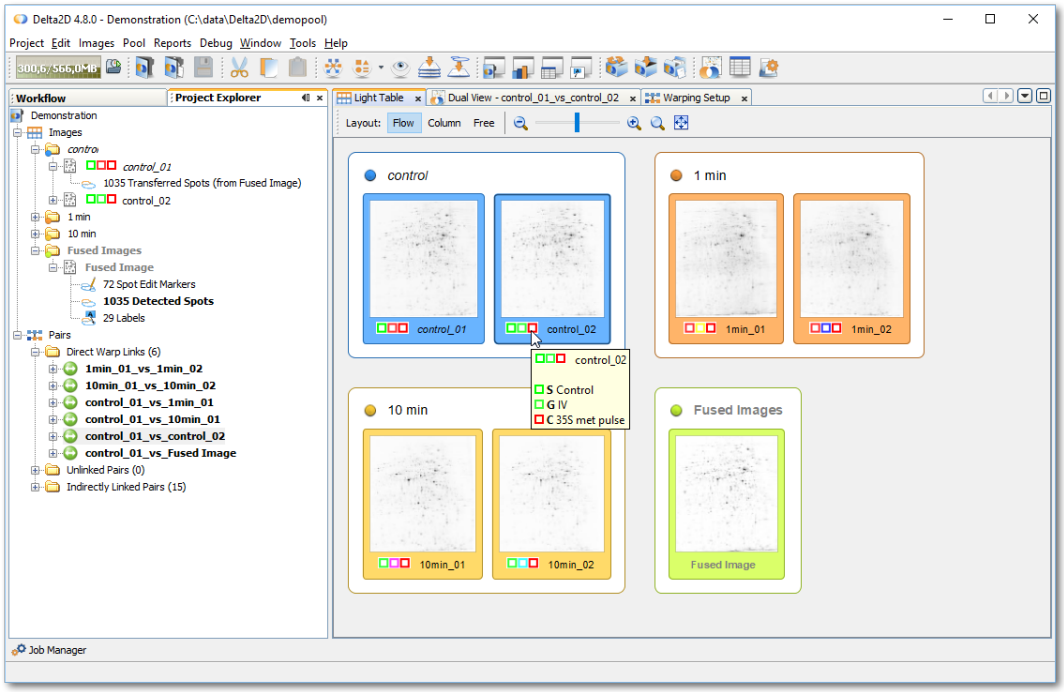


Figure 5.5.: The Light Table

Right clicking on any group or image opens a context menu with actions available for the respective object. The triple icon near the gel image name indicates the color coded assignment to Sample, Gel and Channel where available. This way you can see at a glance whether all assignments are complete and correct. Hovering the mouse pointer few seconds over any triple icon opens a tool tip showing the assignments more verbose.

The Light Table Toolbar

Table 5.3 on page 65 explains the meaning of the buttons.

	Change the arrangement of the groups. Flow: places the groups one after the other, optimizing the usage of the workspace, Column: uses a seperate row for each group, Free: lets you place the groups freely in the workspace
	Zoom out
	Move slider to zoom
	Zoom in
	Zoom 1:1
	Fit the group arrangement into the window, such that it can be seen completely inside the window.

Table 5.3.: Buttons on the Light Table toolbar and their functions.

Add a Group

Use Project ▷ Add Group... to add a new group. Delta2D asks you for a name and a color that will be used to display the group (see fig. 5.6 on page 65). We suggest to use related colors for groups containing 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.

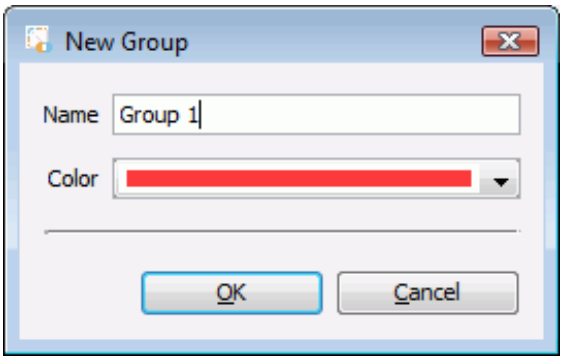


Figure 5.6.: Add a new group

Add Images to a Group

Right-click the group and choose Add New Images... to add a gel from the pool to the group. You will be offered only those 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 image is not yet in the pool, you can also use the Import button to browse for the desired 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 to 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.

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. Warping is necessary to reliably eliminate the distortions between the gels. With complete warping you can work on image projects as if spots belonging to the same expression profile would appear on the same position across all images.

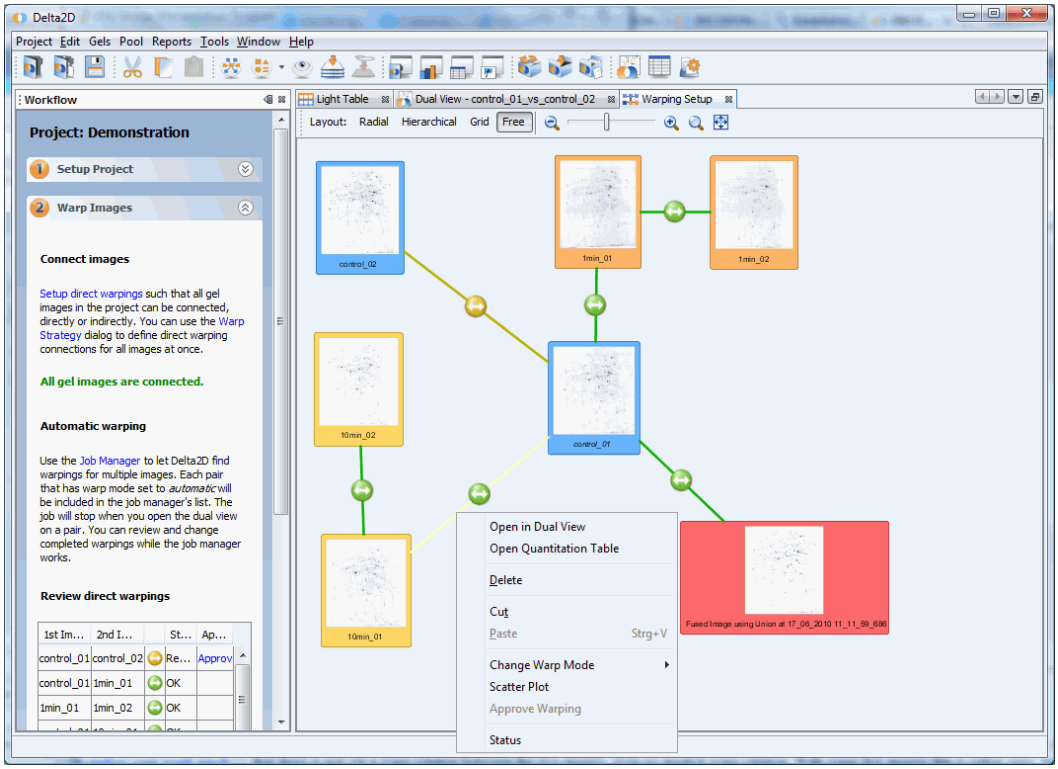


Figure 5.7.: The Warping Setup

The Warping Setup Toolbar

Table 5.4 on page 68 explains the meaning of the buttons.

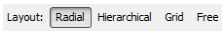
	Change the layout of the warp graph. Radial: tries to equally distribute the images around the center, Hierarchical: the central image is placed on the top, Grid: similar to hierarchical, Free: lets you place the images freely in the workspace
	Zoom out
	Move slider to zoom
	Zoom in
	Zoom 1:1
	Fit the warp graph into the window, such that it can be seen completely inside the window.

Table 5.4.: Buttons on the Warping Setup toolbar and their functions.

Warp Relations and Warp Graph

You can assign the warp modes manually between the respective images in our project. Just drag an image and drop it on another one, provided that there is not yet a warp relation between these two images, even no implicit warp relation. Delta2D will apply the automatic warp mode to this relation by default.

The complete set of pairwise warping relations forms the Warp Graph (see fig. 5.7 on page 67.

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 images in order to be able to warp one onto the other. We only have to make sure that every 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. E.g. you can delete a connection by right-clicking on the respective warp icon and select Delete from the context menu. The respective matchvectors won't be deleted this way, they are available if the same warp relation is defined again.

Warping Strategy Manager

With some few images it is rather easy to define all warp relations manually, 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 Set 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 images of a project. It takes care that no gel is left out and no warping cycle is created accidentally.

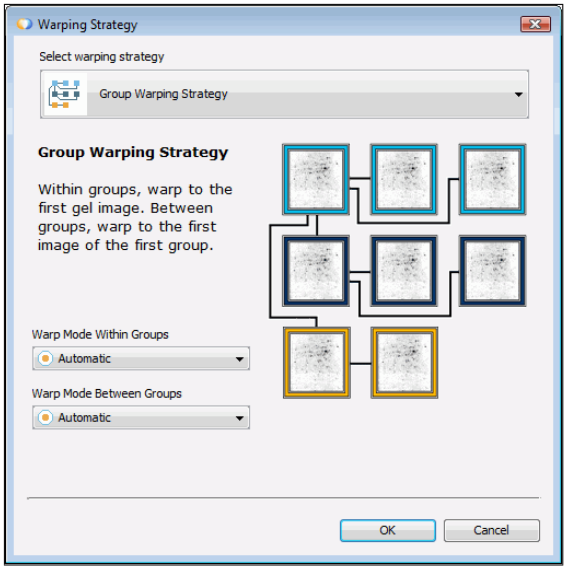


Figure 5.8.: Apply complete warping strategies at once

> **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 multiplex 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 closed loop of warpings. E.g., if you have four images A, B, C and D, and assigned warpings for the image pairs A-B, B-D, A-C and C-D, Delta2D does not know which warping chain for warping the image pair A-D 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 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 image with the first one is N-1 for N gels (A -> B -> C -> ... -> N).

The Warping Strategy Manager (see fig. 5.8 on page 69) lets you choose between basic warping strategies:

Group This will be the most frequently applied strategy. It assumes that your image groups contain gel replicates, and that the differences both in spot positions and quantities within groups are smaller than between groups. Within groups, images are warped to the group's first image and the first image of each group is warped to the first image of the first group.

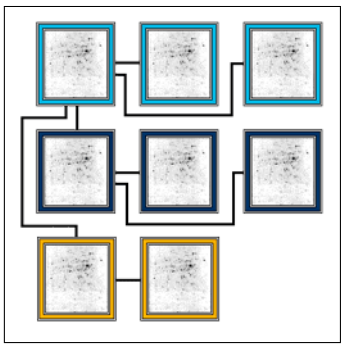


Figure 5.9.: Group Warping Strategy

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

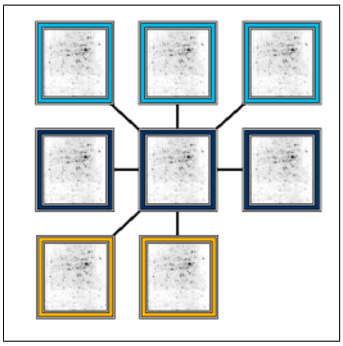


Figure 5.10.: All-to-one Warping Strategy

- **Chained Group** Within the groups images are connected as chains (as described below for the strategy *Chained Warping*), while the groups are connected by warping each the first image of each group is warped to the first image of the first group, applicable in case your image groups correspond gel replicates, and the groups represent successive points of time in an experiment.

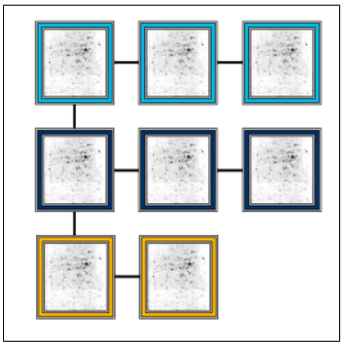


Figure 5.11.: Chained Group Warping Strategy

- **Chained Warping** All images of your project are connected as a 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.

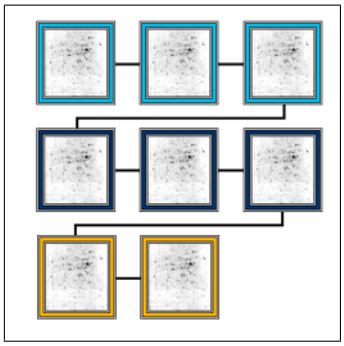


Figure 5.12.: Chain Warping Strategy

In Gel Standard Warp each standard image to the first standard image. This is the default Warping Strategy for Projects using an internal standard and hence only available if the current project is marked as such a project. (Please refer to section 3.1 on page 11 for more information on Multiplexed Projects.)

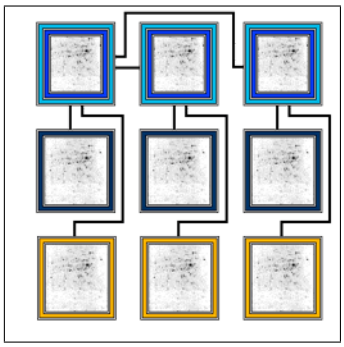


Figure 5.13.: In-Gel Standard Warping Strategy

Each warping strategy is using the automatic warp mode as default for the single connections between images (with the exception of images from the same gel: The *In Gel Standard* warping strategy is using the identical warp mode in this case). You can either select another warp mode for all connections in the Warping Strategy Manager, or right-click on a connection between two images and select Change Warp Mode to define an alternative warp mode.

You can select a image connection and delete it.

5.5. Dual View

The Dual View shows a image 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 (see fig. 5.14 on page 73).

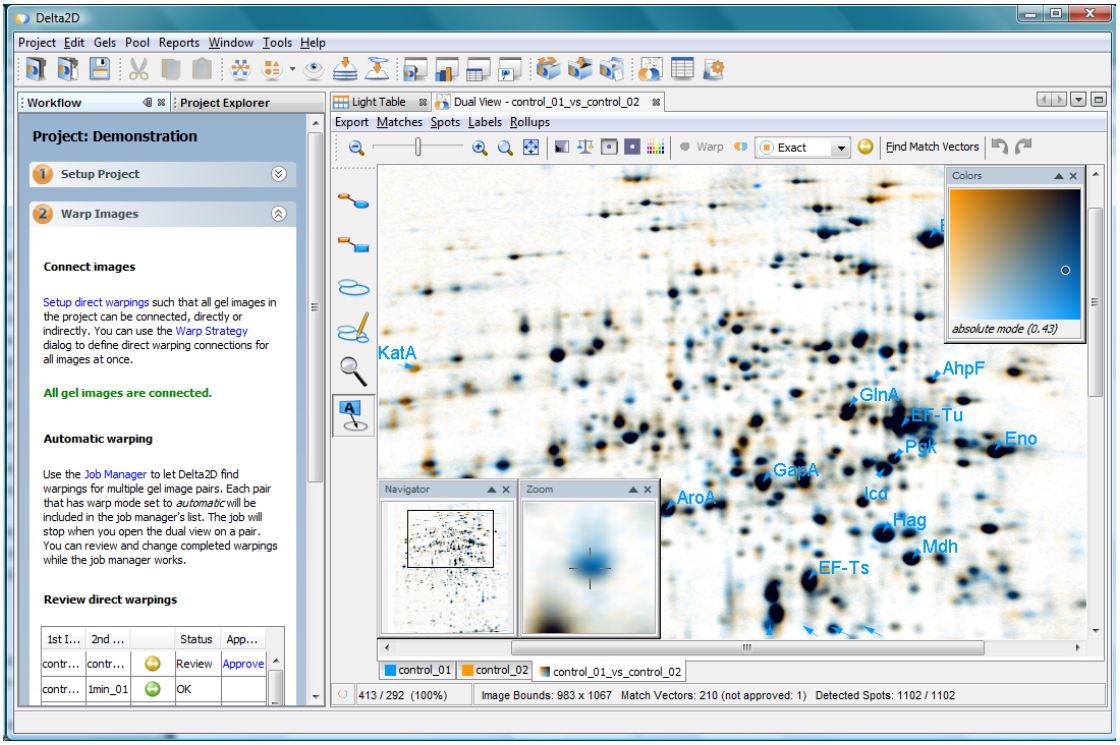


Figure 5.14.: The Dual View

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 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. In the Warping Setup just double-click a connection between two images to open the Dual View. Last but not least you can open the Dual View by double clicking on the image pair's entry in the Workflow (step 2: Warp Images) or in the subgroups for *Pairs* in the Project Explorer.

From the 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 as described in 5.5 on page 80 spots colored in blue are present only (or much stronger) in the one image, while orange spots are only present (or much stronger) in the other 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 (these are the default color settings: the colors may differ if you changed the color scheme). 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 also use the keyboard shortcut Shift + Space to switch between these tabs (see fig. 5.15 on page 74).

> 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 112.

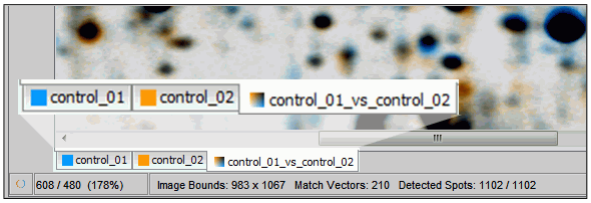


Figure 5.15.: The tabs for controlling image visibility

The Dual View status bar, located at the bottom of the Dual View window, shows some useful information about your work (5.16 on page 74):

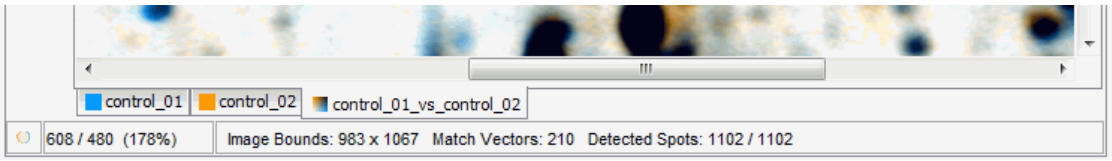


Figure 5.16.: The status bar of the Dual View

Position and zoom level The left field of the status bar shows the pixel coordinates of the mouse cursor. This makes it easy to evaluate the mouse position in a higher zoom level, to locate a certain spot quickly, as well as to estimate the dimensions of a spot. Near to the coordinates, the zoom level is indicated (in brackets).

Image Bounds, Match Vector count, Spots count Depending on the selected tab, image bounds indicates the size of the bounding box for the shown image or image pair (in unwarped status). Besides, you get a quick information on how many match vectors have been defined and how many spots have been detected on this pair of images.

The Dual View comes with its own menu bar and icon bar. Furthermore, on the left-hand side is a vertical panel, the Tool Panel (fig. 5.17 on page 80).

The Dual View Menu

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

Export

	Export Image...	Export the respective image (not available in Dual View).
	Export Presentation Slide...	Export the current view as a presentation slide.
	Export Pick Lists ▷	Export a list with marked and labeled spots for a certain picking device.
	Snapshot...	Make a snapshot of the current view to export it.

Matches

	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.
	Approve Selected	Approve the selected match vectors as to be OK.
	Approve All	Approve all match vectors as to be OK.
	Convert Selected to Connector	Convert the selected Match Vectors to Connectors.
	Select Non-Approved	Select the non-approved match vectors for approving or deleting them.
	Select Non-Connectors	Select the non-connectors for deleting them.
	Invert Selection	Exchange selected against the unselected match vectors, and vice versa.
	Delete Selected	Delete the selected match vectors only.
	Delete All	Delete the complete match map.

Spots

	Detect Spots on [image name]	Open the spot detection dialog for this image.
	Delete ▷	Delete the spots from one or both images.
	Import ▷	Import a spot list that fits to the image.
	Export ▷	Export the spot list of the image.
	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

	Import ▷	Add labels from a file to the current set of labels on an image. Choose whether current label formats shall be replaced (in case of a conflict).
	Export ▷	Export labels to a file. Label formats will be included.
	Delete all Labels ▷	Delete labels.
	Delete Labels for Selected Spots ▷	Delete labels for selected spots only.
	Move ▷	Move all labels from one image to the other.
	Copy ▷	Copy all labels from one image to the other.
	Swap	Swap label sets between images.
	Show Labels for Invisible Spots ▷	Show labels of spots which are invisible (hidden or filtered).
	Label Selected Spots with Spot IDs ▷	Create Labels with spot IDs on selected spots.
	Label Selected Spots with Numbers ▷	Create Labels with consecutive numbers on selected spots.
	Label Unlabeled Spots with Spot IDs ▷	Create Labels with spot IDs on all unlabeled spots.
	Label Unlabeled Spots with Numbers ▷	Create Labels with consecutive numbers on all unlabeled spots.
	Translate Label Names ▷	Import a list to replace current label names by new label names.
	Group all Labels with the same Text ▷	Join all labels containing the same text.
	Ungroup all Labels ▷	Separate all labels with the same text.
	Formats...	Edit label formats.
	Delete scout data ▷	Delete data of a specific scout from all labels.
	Fetch scout data ▷	Fetch data with a specific scout. Choose whether existing data should be overriden.

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 5.5 on page 79 explains the meaning of the buttons.

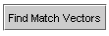
	Zoom out
	Move slider to zoom
	Zoom in
	Zoom 1:1
	Fit the image into the window, such that it can be seen completely inside the window.
	Show image histograms.
	Equalize images.
	Show or hide the foreground of images.
	Show or hide the background of images.
	Choose a color scheme.
	Warp the sample image.
	Disable warping operations and show images in unwarped status.
	Current warp mode: Select the warp mode for this sample image.
	Open a dialog with information about the warp status.
	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 5.5.: Buttons on the Dual View toolbar and their functions.

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 Undo and Redo funtion in the Dual View is available for match vector operations only.

The Dual View Tool Panel

The Dual View tool panel is a vertical panel where you can select one of the six tool buttons (see fig. 5.17 on page 80)..

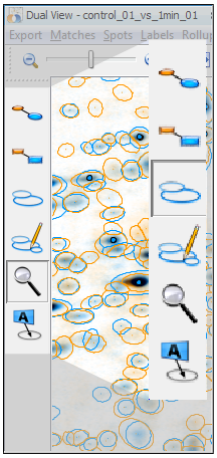


Figure 5.17.: The Dual View Tool Panel.

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 104).

Detailed descriptions of the tool buttons can be taken from table 5.6 on page 81. 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.

Warp 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 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 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*.

Match vectors connect corresponding spots (fig. 5.21 on page 84). 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 Technology. 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.

Based on the match vectors Delta2D's warping algorithms help you to generate dual channel images on which corresponding spots are perfectly overlaid. In the dual channel image, differ-

- Match Vector Tool. With this tool you can select, delete or add match vectors that define corresponding gel positions.
- Connector Tool. With this tool you can select, delete or add match connectors that define spot matches but have no effect on the warping.
- 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 join spots by defining spot edit markers (details in sec. 5.5 on page 91).
- 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 5.6.: Buttons on the tool panel.

ences 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.

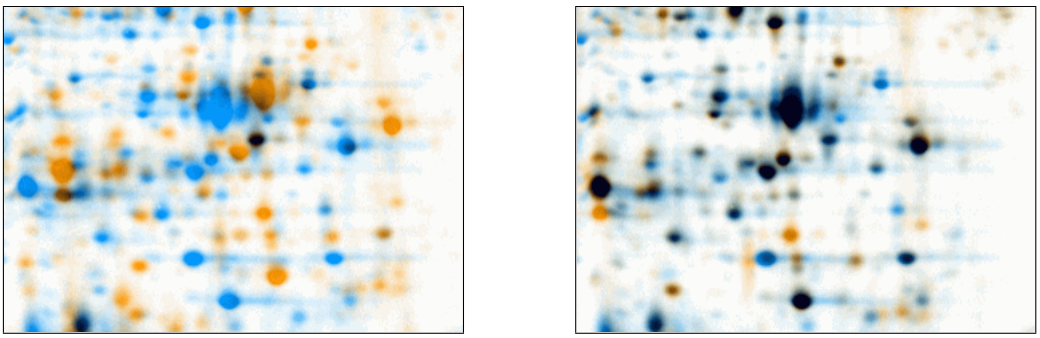


Figure 5.18.: A region of the dual channel image, before and after exact warp. Corresponding spots are overlaid exactly, allowing for easy identification of spots with changed expression level.

In Delta2D you can assign a warp mode for each directly linked 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 fig. 5.19 on page 83).

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 (see fig. 5.18 on page 81 for an illustration). The difference to the global warp can be seen in fig. 5.20 on page 83.

Automatic Warp mode Let Delta2D try to automatically find match vectors by analyzing similarities in the 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 section 5.5 on page 83).

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/delta2d-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 image pairs in your project.

Show images warped images with the defined warp mode by pressing the warp button . If a set of match vectors (the match map) exists, it will be applied. Press the unwarp button to display the images unwarped.

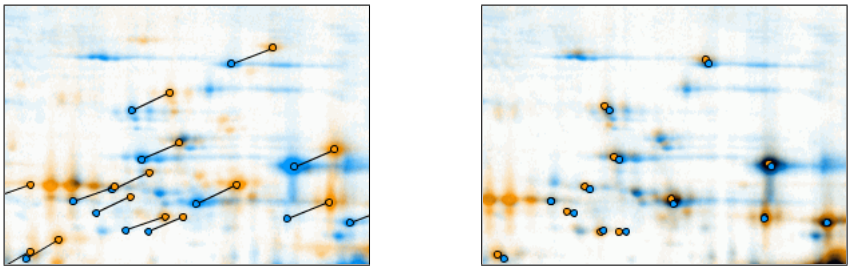


Figure 5.19.: A region of the dual channel image, before and after global warp. Corresponding spots are not overlaid exactly, but much closer than in the original image. Further correspondences can be identified more easily.

Setting Match Vectors

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

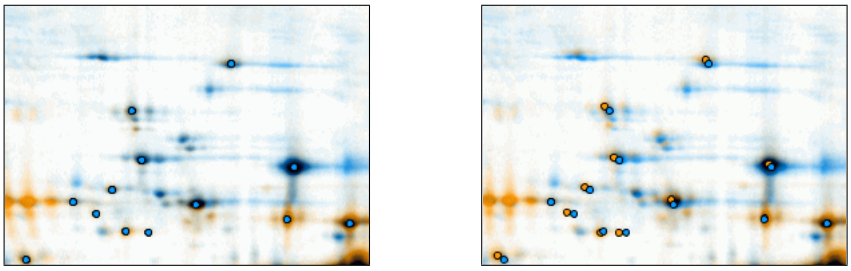


Figure 5.20.: An image region with well-aligned spots after exact warp (left image) in comparison to the same region after global warp (right image).

> 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.

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 6.1 on page 153 for more details).

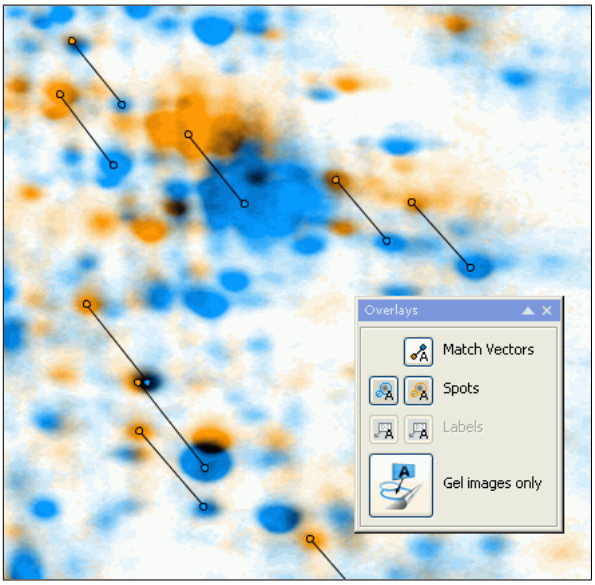


Figure 5.21.: Setting match vectors.

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 Snap Match Vectors to Spots in the Options dialog (tab Match Vectors, see section 6.1 on page 153) 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 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.

If a certain region on the two images can not be warped at all, it may be helpful to define Connectors. Connectors do not affect the warping but let Delta2D match the connected spots

as if they would have the same position after warping (see section 5.5 on page 94).

Questions and Answers About Warping

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

A: By default, Delta2D uses implicit warp mode for any 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 69 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 5.1 on page 61 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 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 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 69).

Q: What if one 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 > 90ï¿½, please rotate the respective image in the image properties dialogue (available from its context menu).

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

A: With highly dissimilar 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.

Q: What shall I do if a region can not be warped?

A: This can happen e.g. if you want to combine two different projects. Define match vectors where it is possible. To combine the spot profiles from both projects you can use Connectors.

Saving Images

The two images can be saved separately or as a dual channel image by using Export ▷ Export Image... after activating the respective image view. The resulting image is almost identical with the original image:

- Histogram settings are ignored.
- Background is not subtracted.
- Spot boundaries and labels are not included.
- Speckle filter is used (if so).
- If the Dual View shows the warped image, it will be saved warped.
- Amplitude rescale is applied if activated (see 6.1 on page 159).
- Single channel images are saved with original color depth, dual channel images with 8 bit depth.

You can save the single and dual channel images in the way they are presented by taking a snapshot. Depending upon the actual display settings, this will consider background subtraction, histogram settings, and overlay visibility (match vectors, spot boundaries, and labels), respectively. Just create the respective view and choose Export ▷ Snapshot... to produce a snapshot.

> 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 sizes and spot spot quantities as well. Within Delta2D, according to its principle to leave original data unchanged, all quantitative analysis is done on the original, unwarped images.

Spot Detection and Quantitation

Basically, Expression Profiles are created with a high degree of automation:

image fusion – create a fused image including all spots of the experiment

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 canceling, splitting or joining, or by adding new spots (see section 5.5 on page 91 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. To start the spot detection in the Dual View select Spots ▷ Detect Spots on *name of your image*.... The spot detection will be started immediately.

If a spot set is available on an image already, Delta2D presents the Quantitation Dialog (fig. 5.22 on page 88) to set or confirm the settings before the spot detection and quantitation is done. This dialog comes with a proposal for three numerical parameters. The numbers are derived directly from the images and should lead to reasonable results, for this reason this dialog is not shown with the initial spot detection. 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 88 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 image. You can load a parameter set from

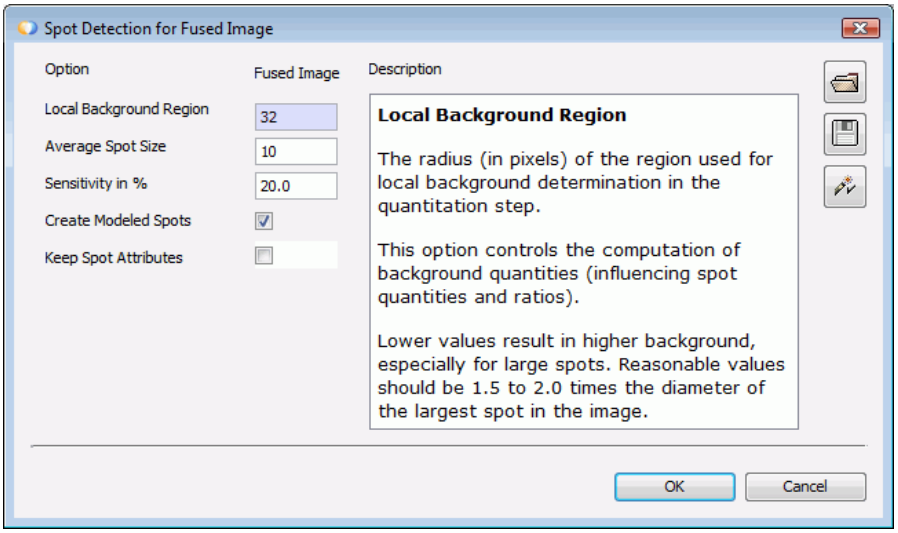


Figure 5.22.: The spot detection dialog.

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.

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 image are overlaid on the image, placed on a separate layer (one spot layer per 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.

Once spots have been detected, quantitation is also done automatically. Quantitation is always done using the original unwarped images while warping and histogram adjustment do not affect 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.

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

Spot Detection Dialog

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.

Average Spot Size Specifying the average size of spots in your 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 (see fig. 5.23 on page 89). The status bar displays information about the current pixel position of the mouse pointer within a image, so you can measure a spot's diameter in pixels by moving the mouse across the spot while observing the change of the mouse pointer's position.

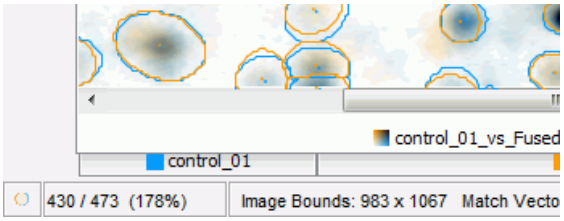


Figure 5.23.: The cursor position in pixel count in the Status bar

Sensitivity in % 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 images containing very weak spots.

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

Tuning Spot Detection For well scanned 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 88. Here is what to do in which case:

Weak spots are not detected – Increase Sensitivity in % 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.

Select, Mark, Hide, or Cancel Spots in the Dual View

Having activated the Spot Selection Tool in the Dual View, you can select

a single spot left-click,

spots in a region left-click and drag the pointer, or

multiple spots press Ctrl on Windows or Apple symbol on Mac and left-click each spot separately.

In the Dual View only those spots with visible spot boundaries can be selected. Selected spots can be

marked since they are interesting,

hidden to just visually exclude them from the analysis, or

canceled to completely ignore them also quantitatively (e.g. from the normalization set).

Using the context menu items Mark Spot, Hide Spot, or Cancel Spot (right-click on a selected spot to open it) you can do the respective operation on the set of Selected Spots. You can make hidden or canceled spots visible (displayed with dotted spot boundaries) in the Dual View by choosing Spots ▷ Show Hidden Spots or Spots ▷ Show Canceled Spots, respectively.

The selected spots are also highlighted in the Quantitation Table, as well as in the Image Regions, while spots being selected there will also be selected here in the Dual View. Please note that when selecting a spot the complete expression profile (all matching spots) is selected.

Selected spots can easily be unmarked, unhidden, or uncanceled using the context menu (right-click on a selected spot to open it).

Edit Spot

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, Delta2Dwill 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.

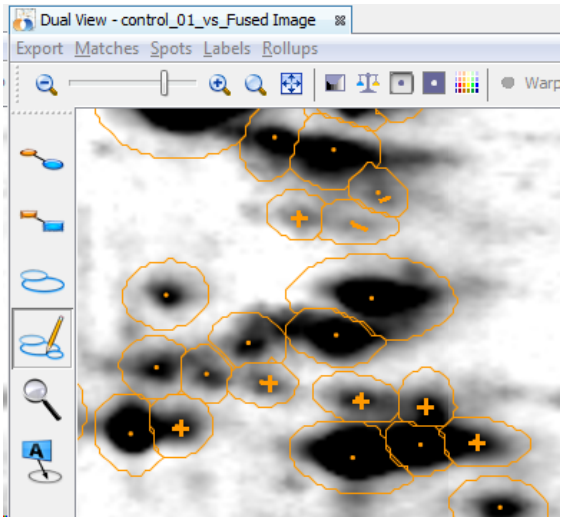


Figure 5.24.: Edit spots

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 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 Spot Editing Tool, and drag a line between the two spot centers. Delta2D will join the two spots touched by the line.

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

Spot Shapes: Pixel Based or Modeled

Pixel based spot boundaries directly reflect the raw grey value distribution within the scanned image. Since the 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. 5.25 on page 92).

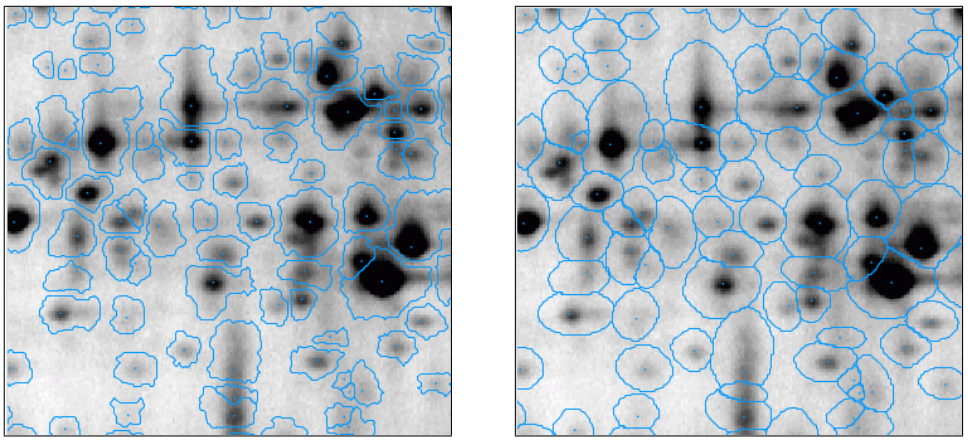


Figure 5.25.: The same region with pixel based and model based spots.

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 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 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 Matching

Since Delta2D includes image warping (introduced into two-dimensional electrophoresis image analysis by DECODON in the year 2000), spot matching is very reliable, even with individual spot detection for each image. In traditional packages for the analysis of 2D 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 completely automated since after warping, image fusion, spot detection on the fused image, and spot transfer (as described in chapter 3 on page 9) the complete spot pattern has the same position throughout the whole experiment.

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 chapter 3 on page 9 for details.

Note: Read more about the benefits of Complete Spot Matching at www.decodon.com/delta2d-spot-matching.html.

Alternatively you can also create individual spot detections on the different images, or you might have different spot patterns in the project after combining two different projects, each including a fusion image and complete expression profiles. Spot matching is then integrated into the spot detection and quantitation process and also happens automatically: Delta2D checks if two spots overlap sufficiently, with respect to the warping. It may happen that a spot does not have a corresponding spot, causing missing values in the statistical analysis.

Spot Matching if Warping is Locally Impossible

For some image pairs it might be difficult to warp them in a completely satisfying way. E.g. if you combine two subprojects, each having complete a fusion image and complete expression profiles, you can use the fusion images to create the warping connection between these subprojects. However, it can happen that some spots can not be warped since the projects are too different and the matching process might fail though you can identify the correct matches visually. You would then get two different rows in the quantitation table including the expression profile for each subproject.

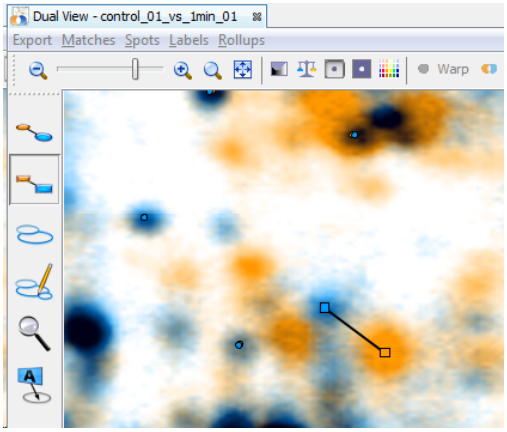


Figure 5.26.: Image with a Connector to match non-warpable spots, no effect on warping.

To connect spots even if they can not be warped you can define Connectors to match the spots across the two fusion images. Connectors do not affect the warping but will cause Delta2D to match the respective spots. You can either convert Match Vectors to Connectors or activate the Connector Tool and create new Connectors (see section 5.5 on page 80). All the spots that are not connected by Connectors but are warped properly are automatically matched as usually.

> Note: Spot transfer does not work along an image pair which is connected with Connectors since spot transfer is based on the warping relation for the respective pair. For this reason use Connectors very carefully and only for pairs that connect different subprojects while each subproject has its own fusion image with detected spots.

Labels

Delta2D allows you to place annotations to any position on a image. These labels are independent from spot locations and will not be affected by (re-)detecting, transferring, or deleting spots. However, if a label points into the boundary of a spot being detected before or after creating the label, it is considered to annotate this spot. Delta2D will automatically show this label in

the corresponding label column of the Quantitation Table and this spot will then appear in the respective picklist for this image. Multiple labels can point into the same spot.

Labels can be created individually or automatically. They can be selected and transferred from one image to another with a single click. Delta2D will place them at the corresponding position automatically, following the defined warpings. Labels can either be collected in a proteome map (e.g. on a fused image), but it is also possible to handle labels on every single image.

On a certain image, all or selected labels with the same text can be grouped and ungrouped

Labels are hidden if the respective spot boundary is hidden (e.g. by a filter in a Quantitation Table). You can show these labels by activating Show Labels for Invisible Spots in the Dual View menu.

You can change label formats according to your preferences.

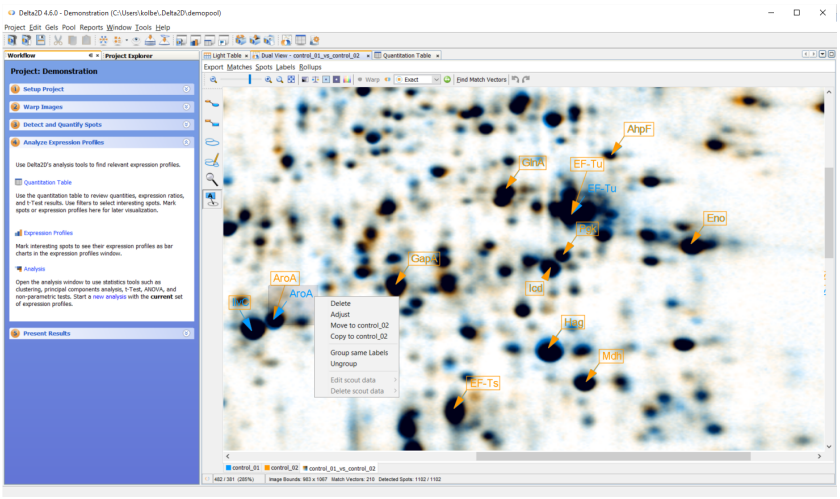


Figure 5.27.: A Dual Channel Image with Labels.

Create, edit, select, move, adjust or delete a Label

You can place labels on any of the images. In the Dual View, labels for both images will be displayed together, usually in different formats.

To start working with labels, select the label tool in the top-left part of the Dual View (see fig. 5.28 on page 96).

Now click on any point in the image: a new label will be created where you have clicked (see fig. 5.29 on page 96). If there are selected labels, a first click will unselect all labels, but a second click will create a new label.

In the Dual View newly created labels are placed by default on the first image. If you want to create a label on the second image, hold the Shift-key pressed while clicking on the image.

When moving the mouse pointer over a label, it will be highlighted so that you always know which label will be affected by you next operation.

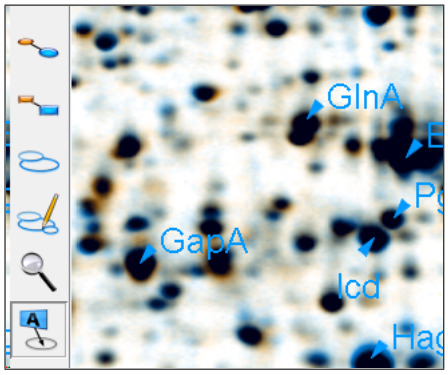


Figure 5.28.: Delta2D tool panel with activated label tool.

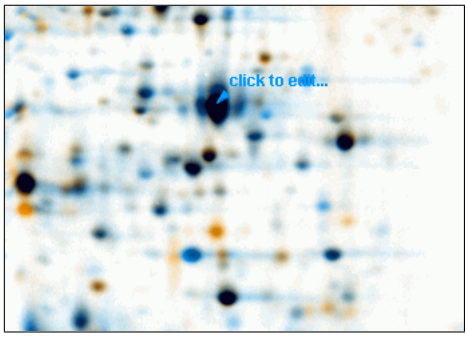


Figure 5.29.: A new label

To edit the text of a label, just click inside the label text and start typing. Press Enter to confirm the changes. Pressing Escape will discard the changes you have made.

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 (fig. 5.30 on page 96).

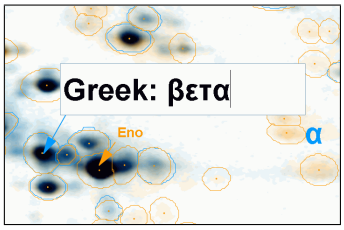


Figure 5.30.: Greek character in labels

To select one or more labels, press Ctrl and click on the labels that you want to select. Right-click on a label to just select this label.

You can move a label's text box around by dragging it with the mouse. When the label is

sufficiently close to the target, the arrow line will be hidden. To move the complete label, you can drag the arrow to let it point to a new position on the image. If the new position belongs to a spot, the label will automatically be assigned to this spot.

You can let new labels snap to the nearest spot maximum by holding down the Ctrl key when creating them. Activate this behaviour generally by choosing the Labels tab in the Options dialog (Tools ▷ Options ▷ Delta2D)and checking the Label snap to spot check box. This makes labels snap to spot maxima when they are created or moved within the image. Pressing the Ctrl will then temporarily switch the snapping off.

Existing labels can be adjusted to the nearest spot maximum by double clicking on its arrow or right-clicking on a label and choosing Adjust from the context menu (see fig. 5.31 on page 97). This might be useful e.g. after having copied a label from another image.

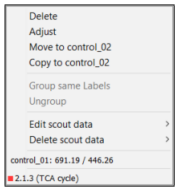


Figure 5.31.: The context menu for a label

Select Delete from a label's context menu to delete it. For deleting all labels on a image choose Labels ▷ Delete ▷ and the respective option from the menu.

Group and Ungroup Labels

For saving space on the image, labels with the same text can be grouped together. You can either group all same labels on a certain image choosing Group all Labels with the same Text ▷, or just right-click a label to group all labels that are equal to it. Of course you can also ungroup

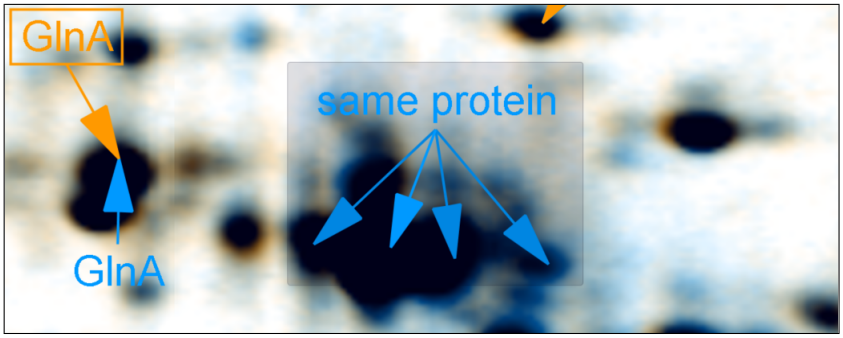


Figure 5.32.: Grouped labels

grouped labels - just choose Ungroup all Labels from the Dual View menu or Ungroup from the label's context menu.

Move or Copy Labels between Images

In the context menu you see on which image the label has been created. Select Move to *image name* to move a label to the other image.

Of course moving labels between images is done with respect to the warp transformation between these two images so that the label will always point to a meaningful position on the target image.

The option Copy to sample creates an identical label on the corresponding point of the other image.

You can copy label sets between images in the Project Explorer in a very convenient way: Just enlarge the image subtree, click on the label set and drag it to a different single image, a group, or the complete project. The labels will then be copied to the respective images if a label with the same text pointing into the spot on the target image does not exist yet. This allows to collect complete sets of labels from many different images on a single image which is especially useful when you want to produce a proteome map containing all known protein identifications. See section 5.5 on page 98 for details.

Translate, Export and Import Labels

Labels can automatically be translated into protein names by importing a list that contains pairs of labels and the respective protein names. See section 3.4 on page 29 for details.

To export of import labels you can just choose Labels ▷ Export ▷ From *image name* from the menu. Labels are always exported using their absolut position. For this reason you can only import the labels to the same image. When importing a set of labels, each label pointing into a spot boundary will automatically be assigned to the respective spot.

Create and Use 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, experiment, or organism. We call this image a Proteome Map since it includes all the spots of the different images that have been fused, regardsless whether they would ever show up on a certain image together. 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 as a reference to identify protein spots by visual comparison.

Based on image warping Delta2D allows to transfer labels from a spot on one image to the corresponding spot on another image. Delta2D does this in a very reliable and efficient way.

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 a certain image. 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. You will need to integrate your proteome map temporally into the current project.

Warp sample to proteome map Create a match map from the image to the proteome map.

Copy labels to the proteome map Use Labels ▷ Copy ▷ *your image names* to copy the 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 ▷ From *image name*

A similar procedure can be used to transfer labels from the proteome map to a new image. This may avoid duplicate identifications, and it is much quicker and more reliable than doing it manually. 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 image to the proteome map.

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

Save sample labels You can use Labels ▷ Export ▷ From *image name* to save the sample labels.

The result is a label file that contains identifications for interesting spots on the new image. You can see this by viewing this image alone, along with its newly created labels.

> Note: Identifications are transferred between the images based solely on their positions. Delta2D can not check whether the spot on the target image includes the same protein as the spot that has been identified and labeled. For this reason be careful when moving or copying labels between images.

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 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 first (warp reference) or second (warped) image, and whether it is displayed on a single image or on a dual channel image. For each of these cases you define a separate label format, using the formatting dialog (see fig. 5.33 on page 100). And of course, you can also save and load appearance configurations for labels.

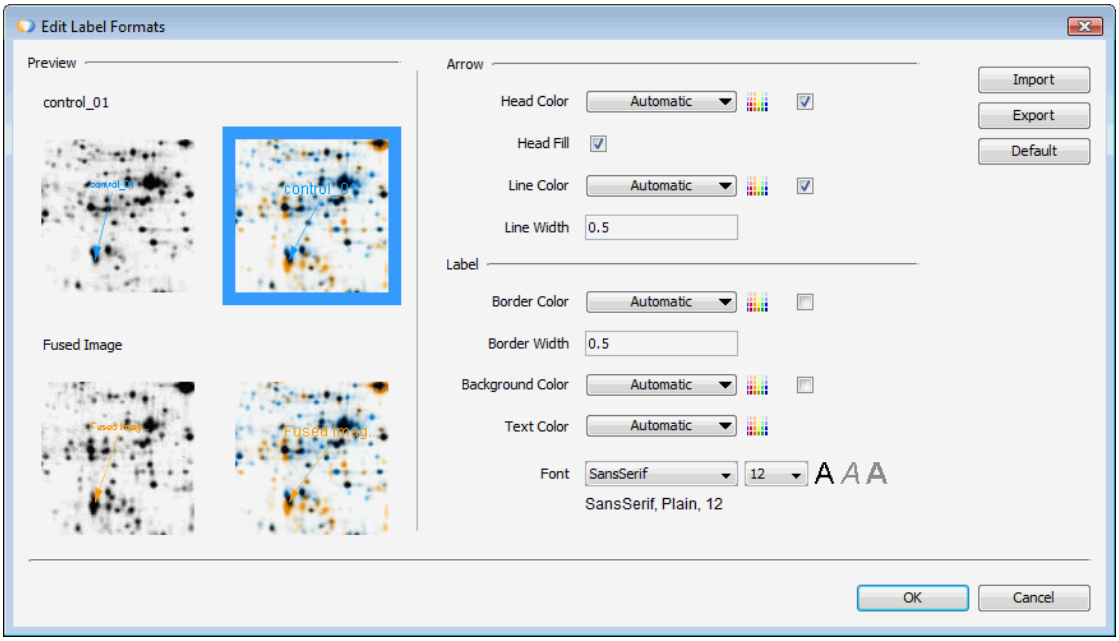


Figure 5.33.: Edit label formats

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

In total four label formats can be defined. In the left part of the Edit Label Formats dialog you can select to edit the format of the labels

- on image one in its single view,
- on image one in the dual view,
- on image two in its single view,
- on image two in the dual view,

Each label consists of two main parts: the label itself and the arrow, pointing to the image position the label refers to. The different label elements can be formatted separately:

There are three basic possibilities to define the color of a label element. Click on the drop down button next to an element you want to recolor and choose between:

Color Click on one of the color squares to assign this color. If this small preselection of colors do not suffice your needs, click on the button next to the drop down button to have a full featured color chooser.

Automatic Automatic color means that the color will be derived from the choosen color scheme, i.e. this color will be the same as the spot color for the corresponding image in the dual view (for color schemes see section 5.5 on page 112).

Arrow:	
Head Color	The color used to fill the head of the label's arrow.
(Show Head)	A check box next to head color 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 of an arrow.
(Show line)	A check box next to line color 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.
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 appear transparent.
Text Color	The color used to display the label text itself.
Font	The font and its size used to display the label text itself.

Table 5.7.: The elements of a label.

Scouts This option is available only if Scout data is available. Color a label e.g. corresponding to numerical attributes such as theoretical isoelectric point or the molecular weight of identified spots, as retrieved by scouts (for more about scouts please refer to section 3.4 on page 31). Thus you can see at a glance the distribution and also outliers in the selected attribute over the complete image (fig. 5.34 on page 102).

Choosing Scouts as the method for coloring a label element, a new dialog will open (fig. 5.35 on page 102).

Decide for the Scout data to be used and how the colors shall be defined:

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

Property Which numerical attribute of this scout will be used?

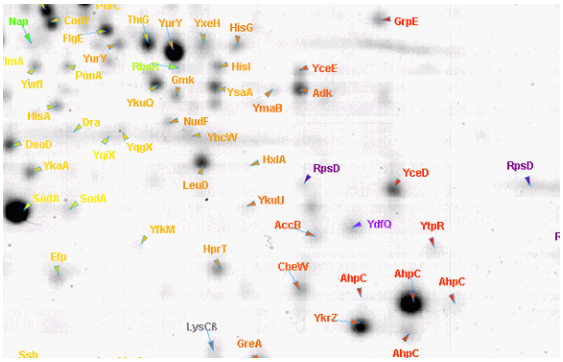


Figure 5.34.: Labels colored according to isoelectric point, based on Scout data

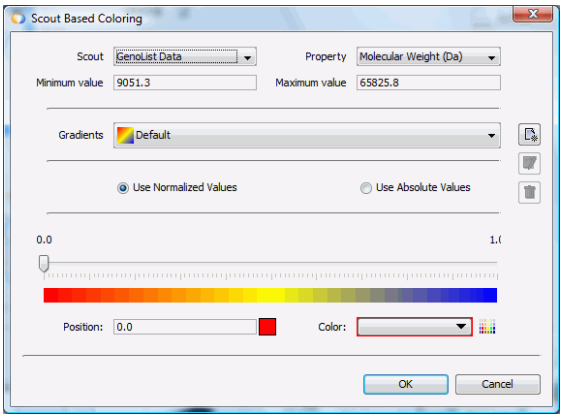


Figure 5.35.: Adjust details for scout based color coding of label elements.

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.

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.

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.

Navigate 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 (see fig. 5.17 on page 80).

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 104).

When having zoomed into the image you can press the Alt key to move the image with the mouse.

Configure the Display Using Rollups

Rollups are small windows that are located on top of 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 78).

As an example, you may wish to hide match vectors using the overlay rollup as described in section 5.5 on page 104 when having finished warping (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 is combined into colors.

Open the Colors rollup using Rollups ▷ Colors.

Move the mouse pointer over the dual channel image and monitor the Colors Rollup (fig. 5.36 on page 104). 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 or sample spots, for background and overlapping spots.

Click inside the color square to change the color scheme. A menu appears, allowing for changing the used color scheme (see sec. 5.5 on page 112) and to switch between the absolute mode (which is the standard mode) and the ratio mode (see sec. 5.5 on page 114).

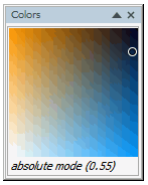


Figure 5.36.: The Colors Rollup

The Overlays Rollup

The visibility of the overlays containing the different objects that are overlaid on top of the images (match vectors, spot boundaries, and labels) is controlled by the activated tool automatically by default, but you can control them manually using the Overlays Rollup (fig. 5.37 on page 104).

Open the Overlays rollup using Rollups ▷ Overlays.

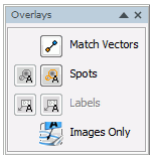


Figure 5.37.: The Overlays Rollup

Match vectors are assigned to the 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 (connected with the activated tool).

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 images (fig. 5.38 on page 105). 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.

The Zoom Rollup

The Zoom rollup displays a fourfold zoom of the image around the current mouse position (fig. 5.39 on page 105).

Open the Zoom rollup using Rollups ▷ Zoom.

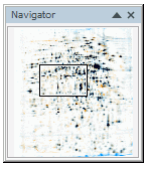


Figure 5.38.: The Navigator Rollup

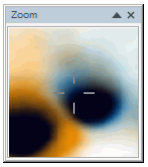


Figure 5.39.: The Zoom Rollup

The Expression Profiles Rollup

The Expression Profiles rollup shows the barchart of the spot your mouse is pointing to. It is based on the normalized volume (%V) of images which are not hidden from the Quantitation Table. 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 129). In the example below (see fig. 5.40 on page 105), the black lines indicate the mean plus / minus the relative standard deviation.

Open the Expression Profilesrollup using Rollups ▷ Expression Profiles.

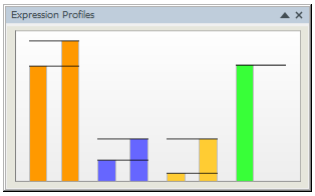


Figure 5.40.: The Expression ProfilesRollup.

The 3D Spots Rollup

The 3D Spots rollup visualizes a selected spot in a three-dimensional view (fig. 5.41 on page 106). 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 Tools menu and switch to the 3D Spots tab in the section Delta2D to change the settings. Please refer to section 6.1 on page 155 for more details.

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

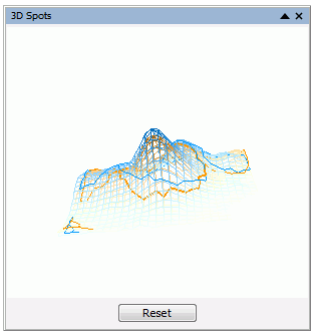


Figure 5.41.: The 3D Spots rollup

After opening the rollup, please switch to the Spot Selection 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:

- 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 to select the same spot in the Dual View.

The pI/MW Calibration Rollup

The pI MW Calibration rollup shows the estimated pI and MW for the current mouse position (fig. 5.42 on page 107). 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. From the label positions and known pI/MW values, Delta2D creates a mathematical regression model to do the pI and MW estimation. More spots with known pI/MW values make the model more accurate.

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

The pI/MW estimation is based on the availability of maintained pI and MW values in a scout for labels (for more information about scouts please refer to section 3.4 on page 31). The values can be manually inserted in the physicochemical properties scout, the table data scout with arbitrary defineable data fields, or fetched from public databases with the respective scout.

To choose the reference data as the basis for pi/MW estimation open Tools ▷ Options in the menu or click on the Options button in the main tool bar. Click on Delta2D and then

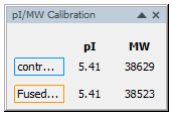


Figure 5.42.: The pI/MW Calibration rollup

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 which data fields are to be interpreted as pI and MW values.

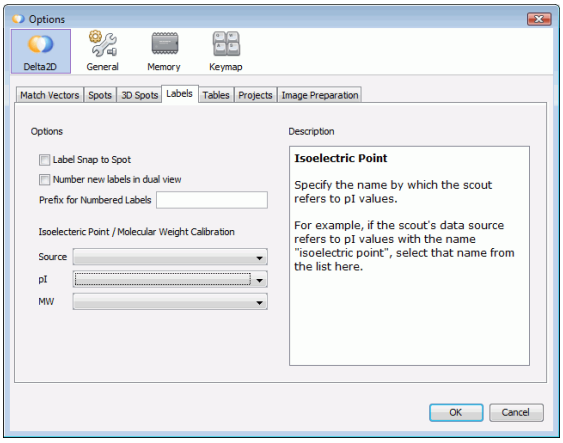


Figure 5.43.: Setting the data source for pI/MW-Calibration

Example You know the pI/MW values for four points on your gel and want see the estimated values for other spot positions. Here is how to proceed:

- If not already existing, place a label on a point for which you know the pI and MW of. The label can but does not need to be connected to a spot on the gel. The name of the label is not important in this context; it can just be e.g. a consecutive number.
- *Right* click on the first label and choose from the context menu Edit scout data ▷ Physicochemical properties.
- Insert the values for pI and MW in the respective data fields.
- Close this dialog with OK.
- Repeat these steps for every image position you know the pI and MW for.
- Now open the Options dialog (Tools ▷ Options) and switch to the Labels tab in the section Delta2D (see fig. 5.43 on page 107).

- On the bottom of the left side you see three drop-down boxes. Click on the first one and make sure that the scout Physicochemical 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.
- Open the pI/MW Calibration rollup that shows the estimated pI/MW for positions on this gel you are pointing to.

Control Background Display

In Delta2D, background is computed for the whole image, and background levels may vary across the image. For each image, Delta2D generates an adaptive background image that can be subtracted from the original to give a "background free image". Usually it makes sense to display images without the background.

To switch background visibility on and off, press the Show/Hide Background button  . You can use the tabs at the bottom of the Dual View window to switch between the images and the dual channel image. Often the dual channel image without background is clearer than the complete dual channel image (see fig. 5.44 on page 108).

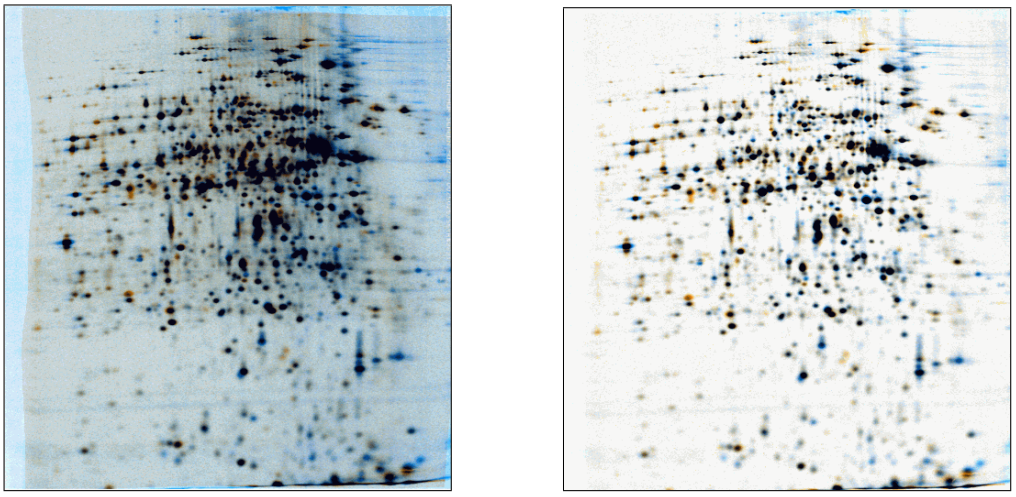


Figure 5.44.: A dual channel image with background switched on and off.

Background subtraction is also taken into account by the spot detection and quantitation step. You can adjust background subtraction either when quantifying spots manually in the Dual View with Spots ▷ Detect Spots on *image name* ... (see 5.5 on page 88 for details), or for the current view by selecting Spots ▷ Background Region ▷ *image name* (fig. 5.45 on page 109).

The upcoming dialog lets you set the same parameter for background detection as described in section 5.5 on page 88, but with one difference: changing the background parameter here affects

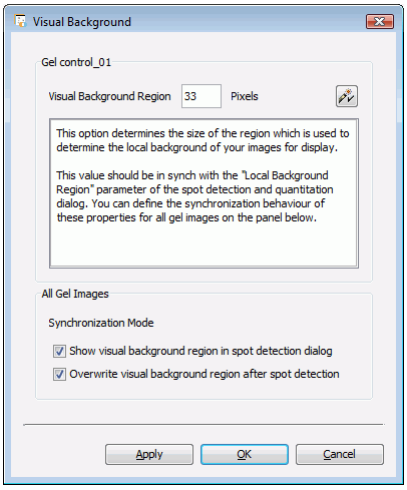


Figure 5.45.: Visual background settings

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 appears when you open the Spot detection dialog, but will be applied only if you start a new quantitation.

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

The purpose of these options is to keep the visual background synchronized with the quantitative background, thus the default setting for both options is checked.

The Histograms Dialog

Delta2D allows you to adjust brightness and contrast of image display. This also results in clearer dual channel images.

- 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.
- Equalizing of images avoids misinterpretation of different levels in image intensities.

By default, the contrast for each image that will be imported into Delta2D is automatically adjusted. If you want to change the contrast setting or want to apply the automatically defined setting again, open the Histograms dialog by pressing the Histograms icon in the Dual View toolbar (fig. 5.46 on page 110).

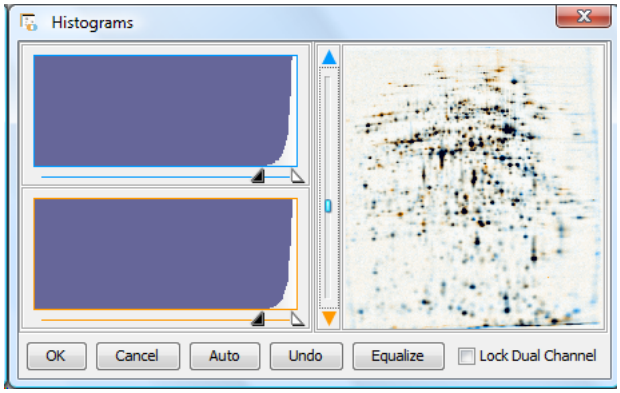


Figure 5.46.: The histograms dialog.

Histogram Adjustment

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.

> Note: Histogram adjustment is saved individually for each image. It affects the generation of dual channel images and the representation of images in the Image Regions, but there is no affect on the spot detection and quantitation process. It is meant to enhance the view of the images without changing quantitative data. This is in accordance with Delta2D's principle of leaving original data unchanged as far as possible. 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 just like to set the mentioned threshold to the lightest or darkest pixel available in the image you can use the global image filter for amplitude rescaling, which is spreading the given data range to the range of grey values available in the used image format (e.g. 8 bit: 256 different grey levels, or 16 bit, 65536 different grey levels). 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 adjusts the data so that 40 becomes 0 (perfect black), 180 becomes 256 (perfect white) and the intermediate grey values are linearly transformed between the minimum und maximum. Thus you have a much more plastic and vivid representation of your image without having altered the information

contained. Depending on your images, the effect of amplitude rescale is weak or strong if the real minimum and maximum grey values are close to or far away of the available minimum and maximum of the image's bit depth, respectively. You can activate amplitude rescale in the Options, tab Image Preparation (see section 6.1 on page 159), this setting is not saved along with the project.

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.

The current grey scale histogram of both images of the Dual View is displayed in the dialog (see fig. 5.46 on page 110). The histogram display shows you how many pixels are contained at each grey scale level in the corresponding images.

To adjust the histograms across the complete project iteratively open the histogram dialog for every image pair and move the sliders of each image until the contrast settings fullfill your needs.

Equalize Images

Delta2D can automatically balance different intensity levels between your images. Simply open the Histograms dialog and click on the Equalize button to balance the images.

Alternatively, you can click the Equalize icon in the tool bar to make balancing the default behaviour of the Dual View. Delta2D will then always dynamically balance the grey scale levels between the 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.

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.

The Histograms dialog includes the follwoing options:

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.

Automatically adjust settings Click Auto to automatically adjust the histogram settings for both images.

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 Undo 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.

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.

Use Colors: The Color Schemes Dialog

The Color Schemes dialog (fig. 5.47 on page 112) allows you to control the colors that will be used for displaying Delta2D's dual channel images.

The Color Schemes dialog can be opened by clicking on the Color Schemes button in the tool bar.

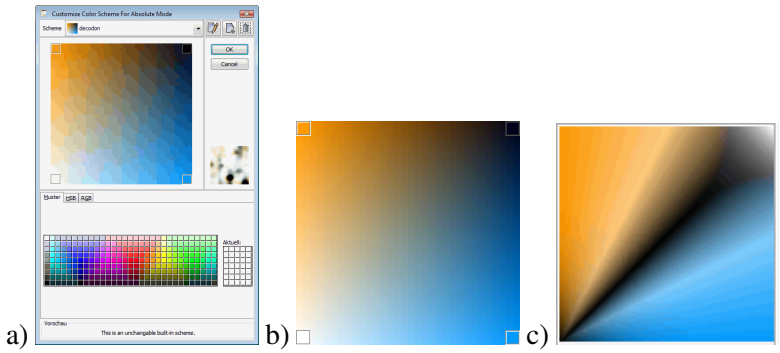


Figure 5.47.: a) The color schemes dialog. b) Color scheme for absolute mode. c) Color scheme for ratio mode.

Display Modes and Color Schemes

Delta2D uses two different display modes: absolute mode and ratio mode. Color schemes can be configured individually, depending on the activated mode (fig. 5.47 on page 112).

The color scheme for absolute mode 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 image.
- Bottom-left corner – The color used to display regions of image *background*.

If you are editing the scheme for ratio mode (see fig. 5.47 on page 112), 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. 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).

Use Predefined or New Color Schemes

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

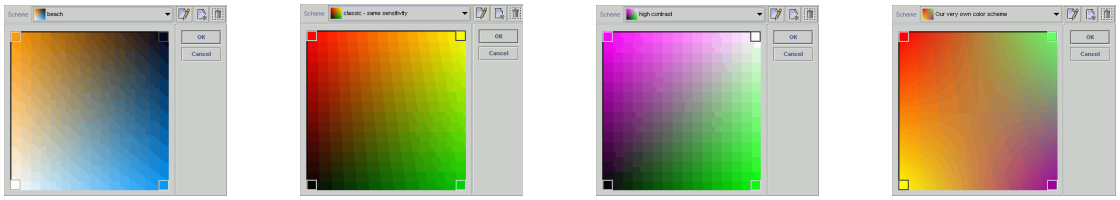


Figure 5.48.: Some predefined color schemes

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 images quickly and clearly.

Rename 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.

Delete 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.

Use Ratio Mode

The ratio mode was implemented for a more fine grained representation of expression changes especially for faint spots. With this unique visualization tool expression ratios are shown directly on the images (fig. 5.49 on page 115), 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. Open the rollup Colors, click into it and select Ratio mode to activate ratio mode display.

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.

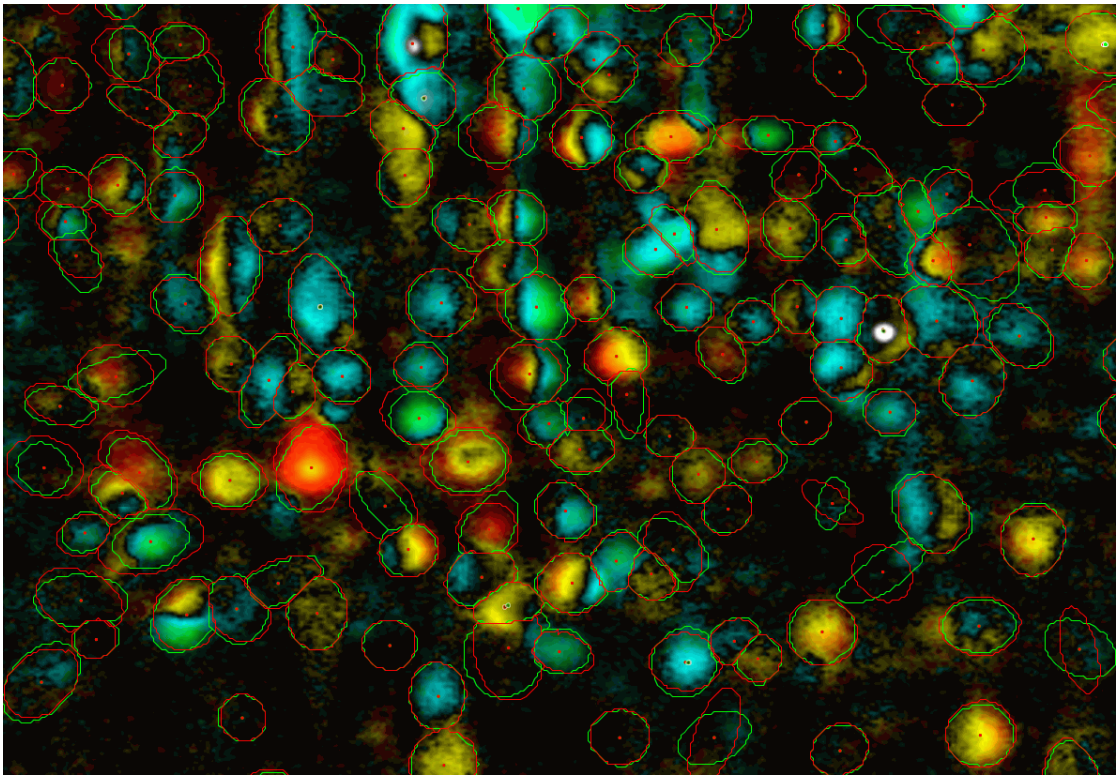


Figure 5.49.: A image region shown in ratio mode.

5.6. Quantitation Table

Delta2D displays quantitative data in flexible tabular views (see fig. 5.50 on page 116) 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.

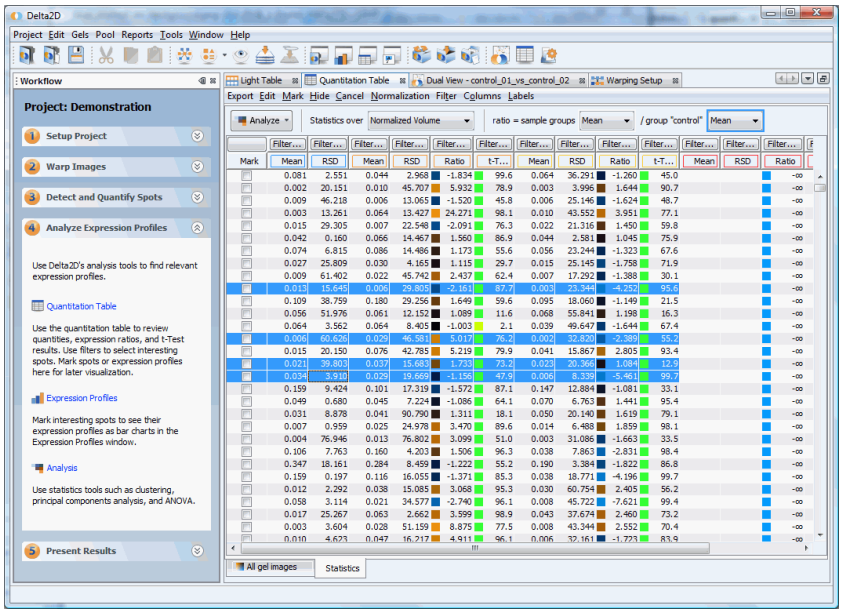


Figure 5.50.: The quantitation table

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 image tables basically have the same structure as the single image tables, except that each row in the table represents the expression profile of matched spots. All attributes appear for each 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 image tables show the relevant spot attributes for a single image. Each row represents one spot.

The Quantitation Table can be opened via the menu Window ▷ Quantitation Table. It includes two tabs: the All images table for all images with spots, and a Statistics table 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 image table or Multiple 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 table are described in table 5.6 on page 117. To change the different attributes' visibility, please refer to section 4.4 on page 54.

Column	Description	Visible by default
Mean	The arithmetic mean in this group.	Yes
RSD	Relative standard deviation (coefficient of variation) in this group.	Yes
Ratio	Shows the ratio for a certain parameter of the min/max/mean/RSD of this group to the min/max/mean/RSD of the reference group (first group in Project Explorer). 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 reference group, based on the Student's t-test algorithm.	Yes
Label	Label of this expression profile (drop-down box if more than one).	Yes
Sample	Values on the single images.	No
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 5.8.: Attributes in the Statistics table.

For a detailed description of the attributes that are available in Single image tables or in Multiple image tables, please refer to table 5.6 on page 118.

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.

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

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 and from the normalization set.	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 6.1 on page 157) 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, after background subtraction, in pixel.	Yes
ID	The numerical ID of the spot.	No
label	One or more (drop-down box) labels attached to this spot.	Yes
X	Spot position: x-coordinate.	No
Y	Spot position: y-coordinate.	No
Q	Indicator of the spot quality, i.e. the similarity with an ideal spot shape.	Yes

Table 5.9.: Attributes in a Single or Multiple Quantitation Table.

and dragging it to the left or to the right.

The Quantitation Table Menu

Export

Export CSV	Export the visible data range as .csv (character separated values) file.
Export Spreadsheet	Export the current visible data as spreadsheet file.
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 from 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.
	Find Next Label	Search the next label containing the same 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.
	Delete Labels for Selected Spots ▷	Delete labels only for selected spots.
	Translate Labels ▷	Change all label names in a batch by providing a list with the current names in one column and the replacement names in another.

The Quantitation Table Toolbar

Statistics tables provide a toolbar for few special operations:

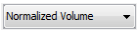
	Open the statistical analysis based on this table.
	Define the attribute on which the statistical analysis is based on.
	Define the formula for the ratio columns.

Table 5.10.: Buttons on the Quantitation Table toolbar and their functions.

Sort or Filter the Quantitation Table

You can use any column for sorting: just click the header line including the columns name to sort the table by this attribute. Click again to reverse the sorting order. A small arrow indicates the sort order (fig. 5.51 on page 122).

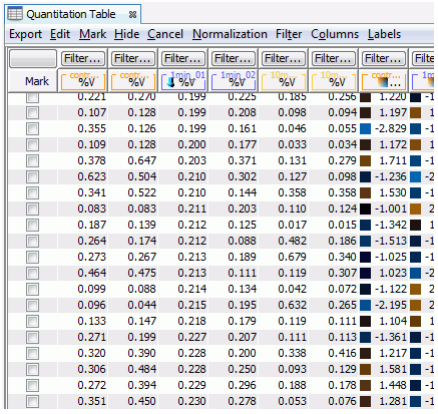


Figure 5.51.: Part of Quantitation Table, sorted on the fourth column

Sorting makes it easy e.g. to identify the most intensive spots or those with a high expression ratio: just sort and then select the most important rows.

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 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 us only display those rows whose expression ratio is between 0.5 and 2. Choose Filter ... on the ratio column to open the filter dialog, or select the ratio of interest at Filter ▷ Ratios ▷ in the menu.

> Note: Often expression ratios are expressed as fold change: a ratio of 0.5 translates into a fold change of -2. If you prefer to use fold change, choose this option in the Options dialog, see section 6.1 on page 157.

Enter 0.5 into the upper Show ratios from: field, then enter 2 into the field to: below. The histogram shows 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 to determine borders for your filter is given under More Options ...: Filter for the count of spots or the cumulative value of the attribute, either absolutely or relatively.

The Filter active box will automatically be checked, but for the example you will have to check the box Negate to keep the interesting values in the table.

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

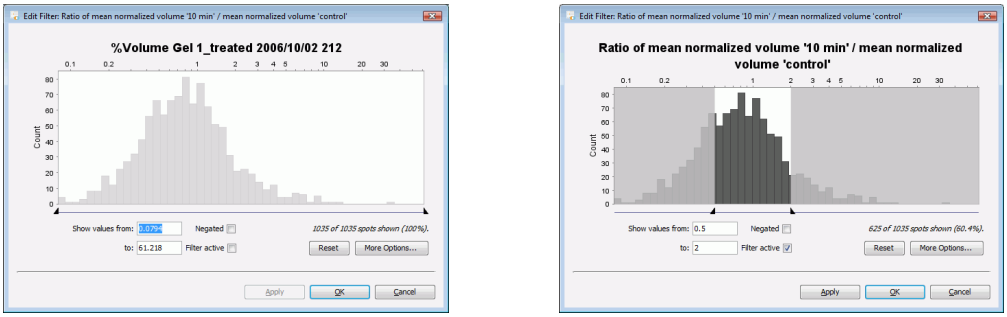


Figure 5.52.: Edit a table row filter. Here, Delta2D would show only spots that have expression ratiosbetween 0.5 and 2.

without having to close and reopen this dialog. The table, as well as the 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 on different table columns 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".

Select, Mark, Hide, or Cancel Spots in the Quantitation Table

In the Quantitation Table, you can select

a single row left-click,

blocks of rows left-click and drag the pointer or click the first row, then press Shift and click the last row, or

multiple rows press Ctrl on Windows or Apple symbol on Mac and left-click each row separately.

Each row is representing the part for the visible images of the respective expression profile, and only these spots will be selected if you click select a row. Furthermore, if the selection does not cover the complete rows you can make use of Edit ▷ Complete Row Selection to select the complete expression profiles.

Spots can be

marked since they are interesting,

hidden to just visually exclude them from the analysis, or

canceled to treat spots as if they would never have been detected.

You can check boxes in the table columns Mark, Hide, or Cancel to mark, hide, or cancel spots. Using the menu items Mark, Hide, or Cancel you can do the respective operation on the set of Selected Spots. You can invert the selection using Edit ▷ Invert Selection. You can make hidden or canceled spots visible (values displayed in grey) in the Quantitation Table by choosing Hide ▷ Show Hidden Spots or Cancel ▷ Show Canceled Spots, respectively.

As an example, here is how to select the 10 smallest spots on the fused image:

1. Open the single gel table for the fused image: just select 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. 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 Image Regions, while spots being selected there will also be selected here in the Quantitation Table.

All or just the selected spots can easily be unmarked, unhidden, or uncanceled using the respective menu item Mark, Hide, or Cancel. You can also uncheck the respective field in the table.

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 Hide ▷ Hide Selected Rows to hide all rows that are not marked

Labels

Labels are shown in the respective columns of the Quantitation Table. Statistics tables include just one column for labels, collecting all labels that point into spots of the respective expression profile.

Multiple labels may appear in a single table cell, accessible through a drop down box. You can select which label shall be visible by default.

To create a label manually for a spot which does not yet have one within a single or multiple Quantitation Table: double-click in the respective table cell, enter the label text and confirm with Enter. The new label will automatically be shown in Dual View, it will be snapped to point into the spot maximum. You can also edit a label by double-clicking in the cell again.

The menu items for labeling are available in a Quantitation Table for a single image, since only then new label can be assigned to the respective spot on this certain image. You can e.g.

select a set of spots in a single table and then let Delta2D assign labels automatically to them or to all unlabeled spots. Automatic numbering is an included option. Define a prefix to attach labels as *Spot_01* (see section 6.1 on page 156).

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 Labels ▷ Find 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.

Labels can automatically be translated into protein names by importing a list that contains pairs of labels and the respective protein names. See section 3.4 on page 29 for details.

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

You can delete single labels in single or multiple image tables per double-click on the respective table cell and deleting the label name manually. Alternatively, you can select the table rows of which you want to delete the labels and then choose Labels ▷ Delete Labels for Selected Spots.

The Quantitation Table Status Bar

Delta2D helps you count how many spots are visible or selected in a table. Counts are displayed in the table's status bar (fig. 5.53 on page 125). 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

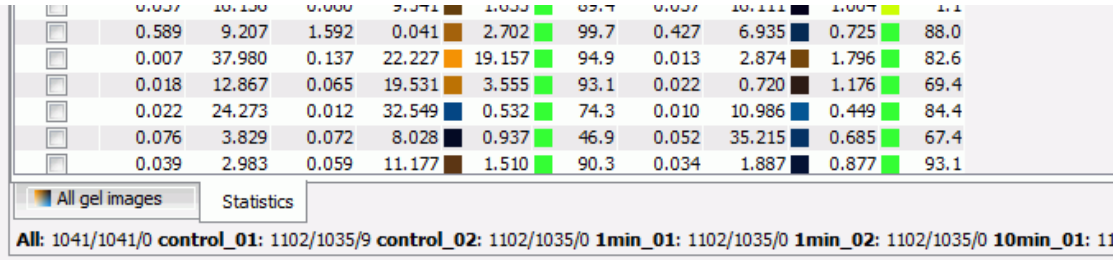


Figure 5.53.: Automatic counting in the title bar of the correspondence table.

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

5.7. Image Regions

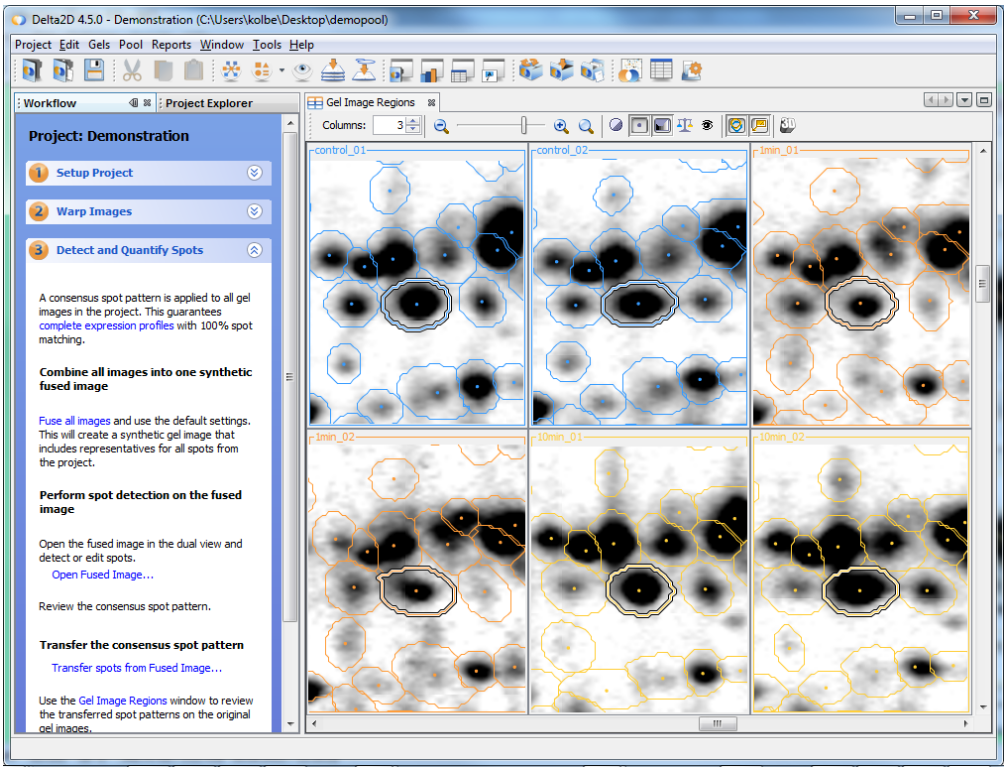


Figure 5.54.: Same region of six different images

The Image Regions view lets you display the same image region of all images in the project side by side. Open the Image Regions window by choosing the menu item Window ▷ Image regions. The view looks similar to fig. 5.54 on page 127, 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.

Since spot handling (selecting, marking, hiding and canceling of spots) is done in the same way as in the Dual View, please refer section 5.5 on page 90.

When you have opened a Dual View window, you can either select a spot there or click at a certain position to define the region being shown in the Image Regions window.

> Note: You can present the image segments for marked spots or expression profiles easily in the Spot Album Report. See section 3.5 on page 35 for more details.

Using the Optimize contrast for all images button you can apply automatic adjustment of

histogram settings for all images at once.

The Image Regions includes a mode to present all image regions as a 3D view. Rotate one of them and all will be rotated synchronously. Click Reset to show them from the default perspective.

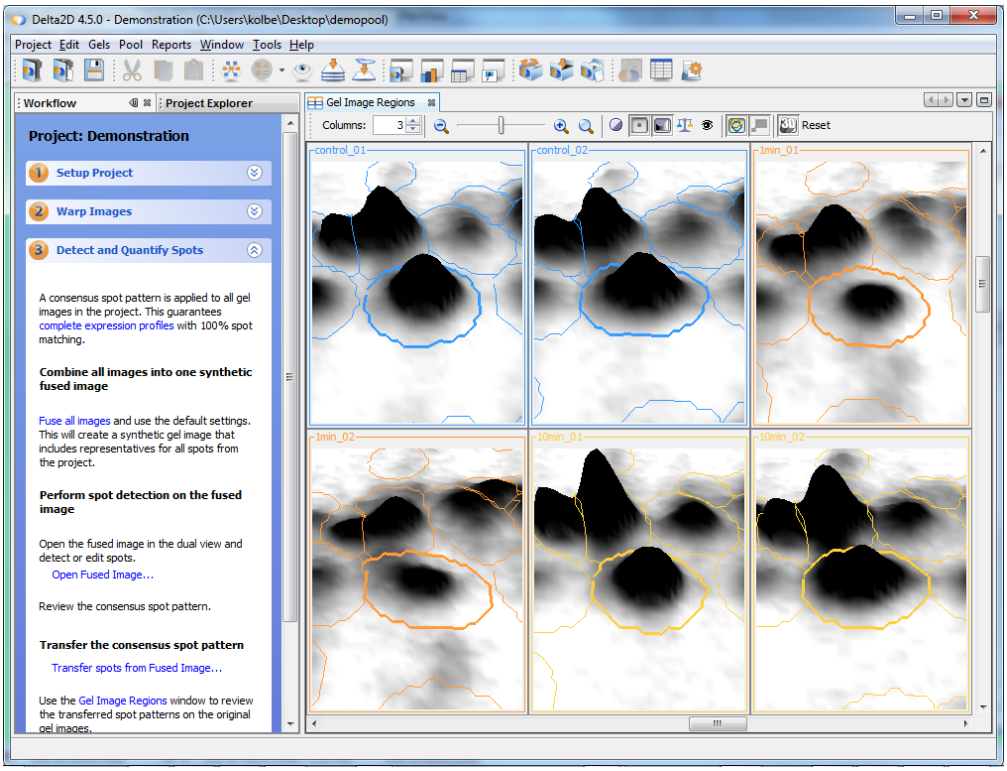


Figure 5.55.: 3D view of a region across all images

The 3D view allows the following operations:

- to rotate: right-click and drag
- to scroll: use mouse-wheel (horizontal: and press Shift) or use scroll bars
- to zoom: press Alt and use mouse-wheel
- to move: right-click and move mouse
- to scale: press Alt and left-click or press wheel and move mouse

5.8. Expression Profiles

Figure 5.56.: The Expression Profiles window

The Expression Profiles Window shows the barcharts for marked spots, sorted by groups. Unlike the Expression Profiles 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 Spots. Now you can open the Expression Profiles Window by choosing the menu item Window ▷ Expression Profiles or its link in the Workflow, step 4 Analyze 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 images of one group to one single bar for the whole group.

Show Standard Deviation Show the standard deviation for each group.

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 image (or proteome map) with spots colored according to their profiles. As an example, (see fig. 5.57 on page 131) spots can be colored by the following scheme: Spots that are increased in sample 1, and in no other sample are shown in red, blue is for spots that are increased in sample 2 etc. green is for spots that are increased in samples 1 and 2 etc.

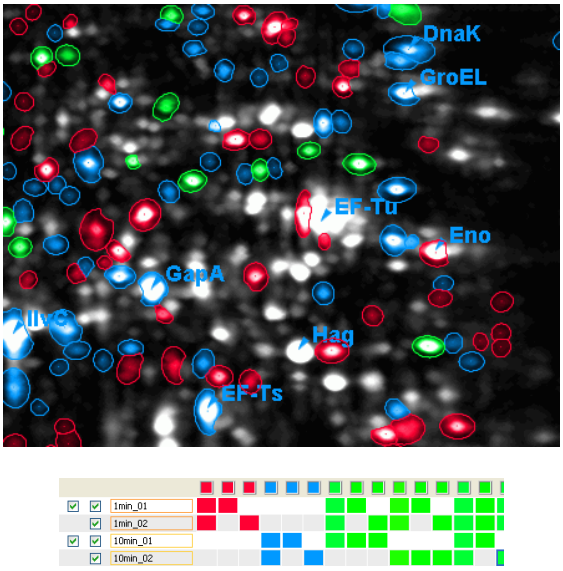


Figure 5.57.: A Region with Colored Spots. The color of a spot indicates on which sample(s) it is increased.

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 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 minimum or maximum 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 image which will be used as “reference” 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 image matches spots from subset of images specified in that column, the spot will be shown in the color of that column (fig. 5.58 on page 132).

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 not create an additional empty set.

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 images.

To delete all existing subsets, click on the button.

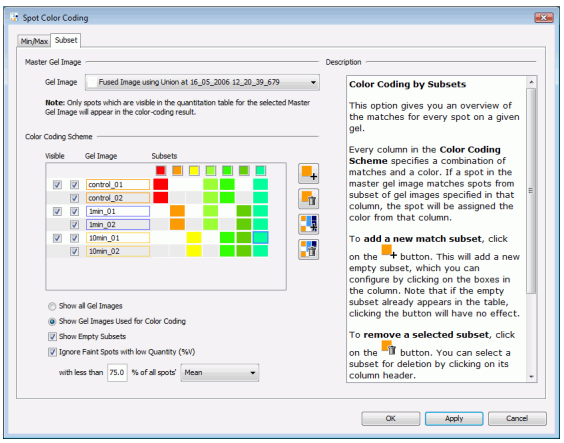


Figure 5.58.: Choose Colors and Master Image

Example

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 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 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 fused image as master image, i.e. the 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 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 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. 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 image, as well as the parameter (volume, area etc) to use for color coding.

Export the Color Coded Display

Use the button to export the color coded image as presentation slide. You can also make a Snapshot window (using the button) 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 image.

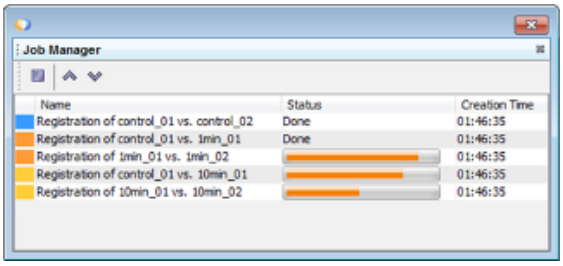


Figure 5.59.: The job manager

As soon as you assign the automatic warp mode to image pairs (see section 5.4 on page 69 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 image pair in the Dual View and apply the warp mode you have selected.

Select Window ▷ Job Manager to open the Job Manager window (fig. 5.59 on page 134). By default, the execution of tasks is stopped. To activate it, press the start button . Now background execution is running; background jobs will automatically be executed for warping image pairs. The Job Manager allows you to control the execution of these tasks.

Use the start and stop buttons to control whether the Job Manager is running. The Job Manager shows the jobs that are currently on its task list. The Job Manager will execute as many jobs as CPU cores are available at a time, a progress bar shows how much of a running 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 Del key or choosing .

> Note: Delta2D supports multicore processing to speed up automatic image warping by performing several warpings in parallel. The number of concurrent jobs is limited to the number of cores of the CPU, which is detected automatically.

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 use of permutations and control of false discovery rate (FDR)
- Analysis of Variance (ANOVA)
- Template matching for expression profiles
- Non-parametric tests e.g.'Kruskall/Wallis', 'Wilcoxon, Mann-Whitney' or 'Mack-Skillings'

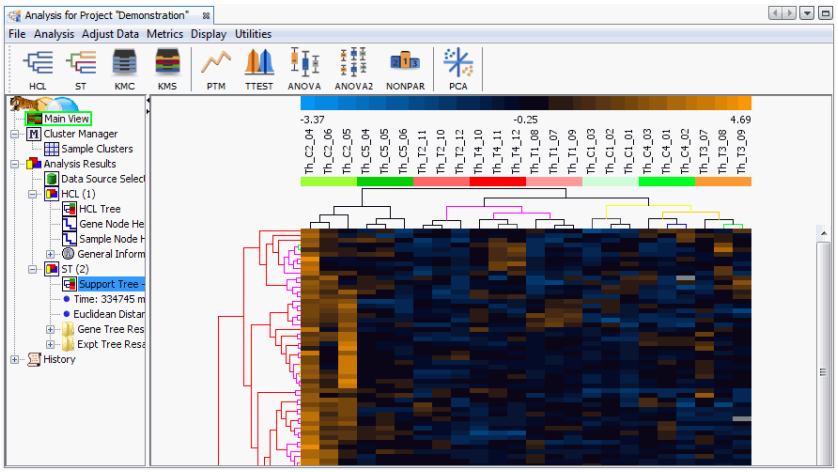


Figure 5.60.: Statistics module in Delta2D, based on TMeV

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 well suited for the methods that were originally applied in the context of DNA microarray analysis.

The statistics module can be accessed either by Window ▷ Analysis (which opens a fresh analysis or the last done analysis, if available) or press the Analyze button in the top left of a Statistics Table (fig. 5.61 on page 136). A new analysis window is opened, containing the current expression profiles as they appear in the satistics table in a heat map display. That means, if you hide images or groups from the statistics table or open the statistics table for a certain set of groups, only the images belonging to these groups will appear in the statistical analysis. The same applies if you have defined filters - only the visible expression profiles will show up in the analysis window.

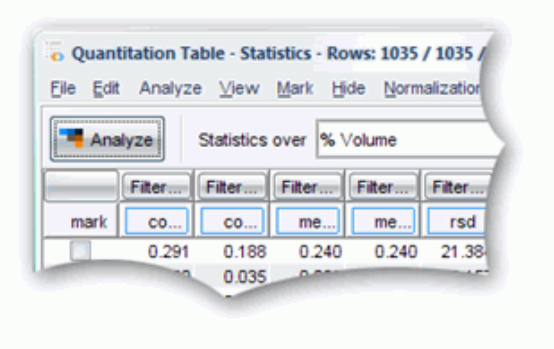


Figure 5.61.: Start analysis from the Quantitation Table

Get 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, images in the columns (fig. 5.62 on page 136). The legend across the top shows the color code for spot intensities. Rows are labeled based on the spot labels from the images.

> Note: By default, data is standardized to zero mean and unit variance before being shown in the heat map. This kind of standardization is necessary to make the different expression profiles comparable regardless of the level they have. However, standardization of the data means that the values in the heatmap are different to those shown in the Quantitation Table. Other options for standardization are available in the pull-down menu of the Analyze button of the statistics table.

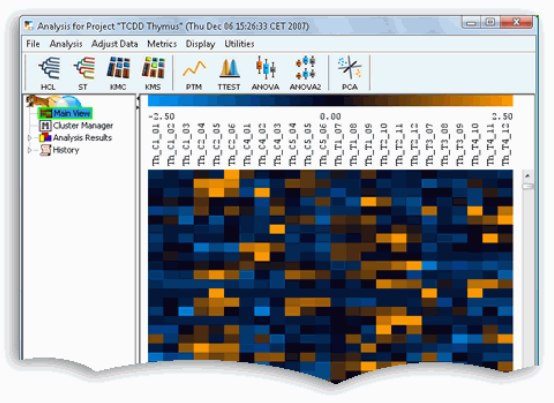


Figure 5.62.: A Heat Map

Heat map display can be changed. If you want to see more rows at once, you can use Display ▷ Set Element Size and e.g. select 20 by 5, or change the color gradient used to visualize the quantities by Display ▷ Set Color Scale Limits.

Clustering Images: What Image Groups or Classes Are There?

Clustering methods show structure in the data by grouping expression profiles and / or images by similarity. The cluster hierarchy is shown in a tree display. This can be very useful for getting an overview of all expression profiles before proceeding with more detailed analysises. Clustering of 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. If not this is an indicator to review the outlying image.

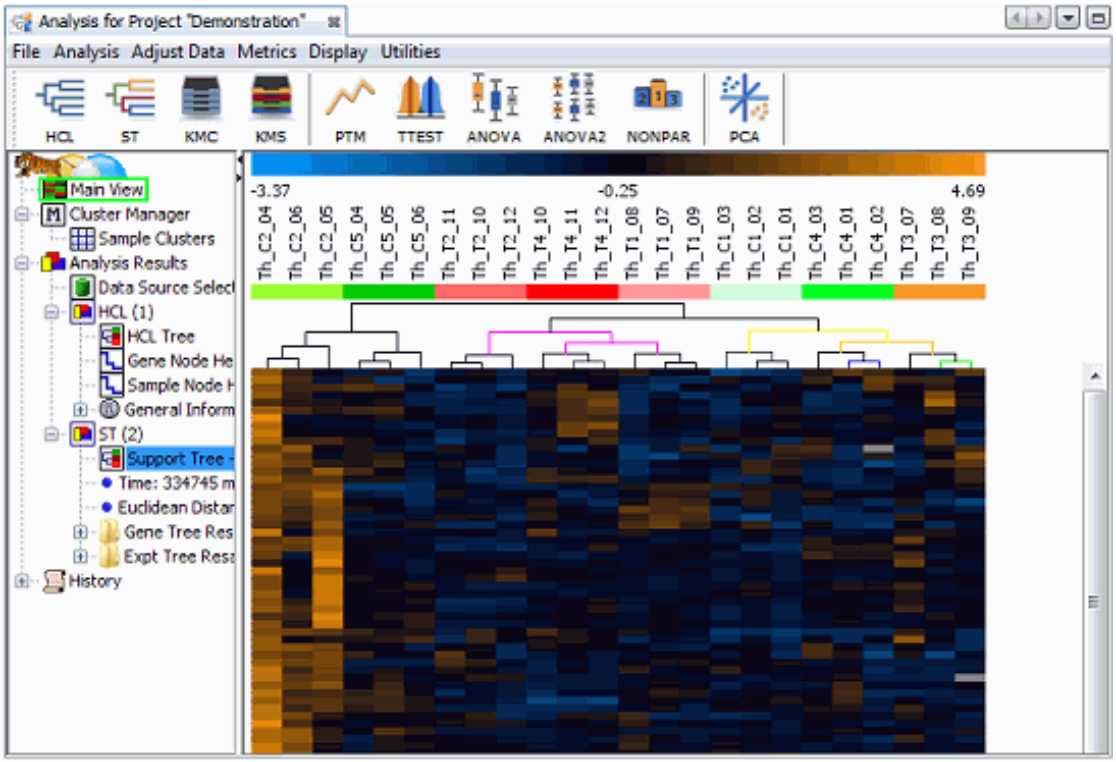


Figure 5.63.: In this clustering the experimental setup of control (C1, C2, C4, C5) and treated (T1, T2, T3, T4) is reflected by clusters perfectly.

Example:

- Press the HCL button in the toolbar.
- We recommend “eucledian” distance and ï¿½ï¿½complete linkage” clustering, press OK.

Clustering Expression Profiles: Find Correlated Proteins

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. 5.64 on page 138).

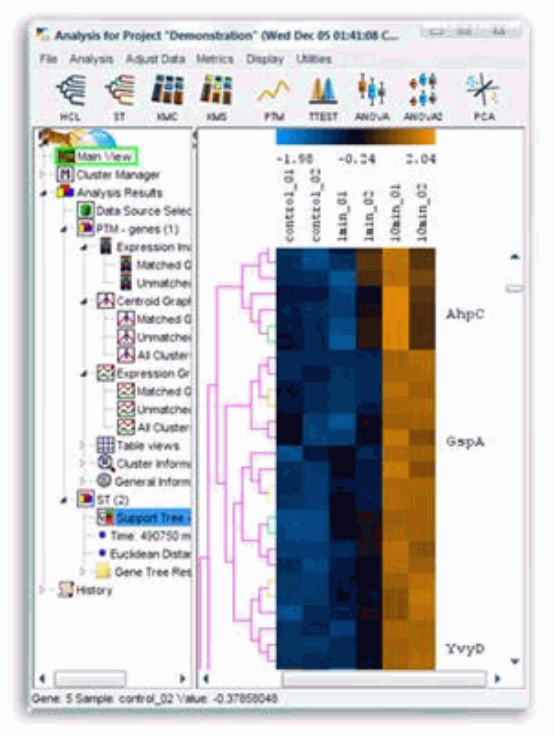


Figure 5.64.: Spots with similar expression profiles are clustered together. Support Tree clustering with Euclidean distance.

Discover Patterns in Expression Profiles

The mean (or median) of a cluster can be interpreted 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. 5.65 on page 139).
- 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. 5.66 on page 139).

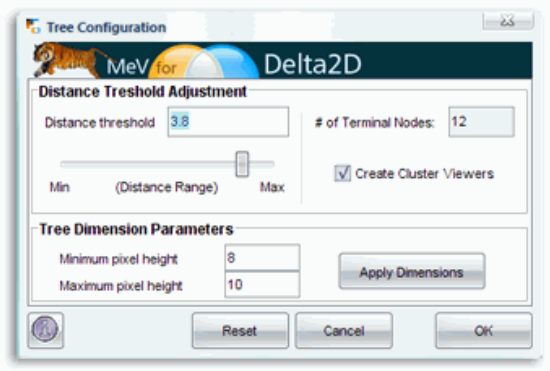


Figure 5.65.: Cut a tree by a distance threshold. Use the slider to adjust the threshold.

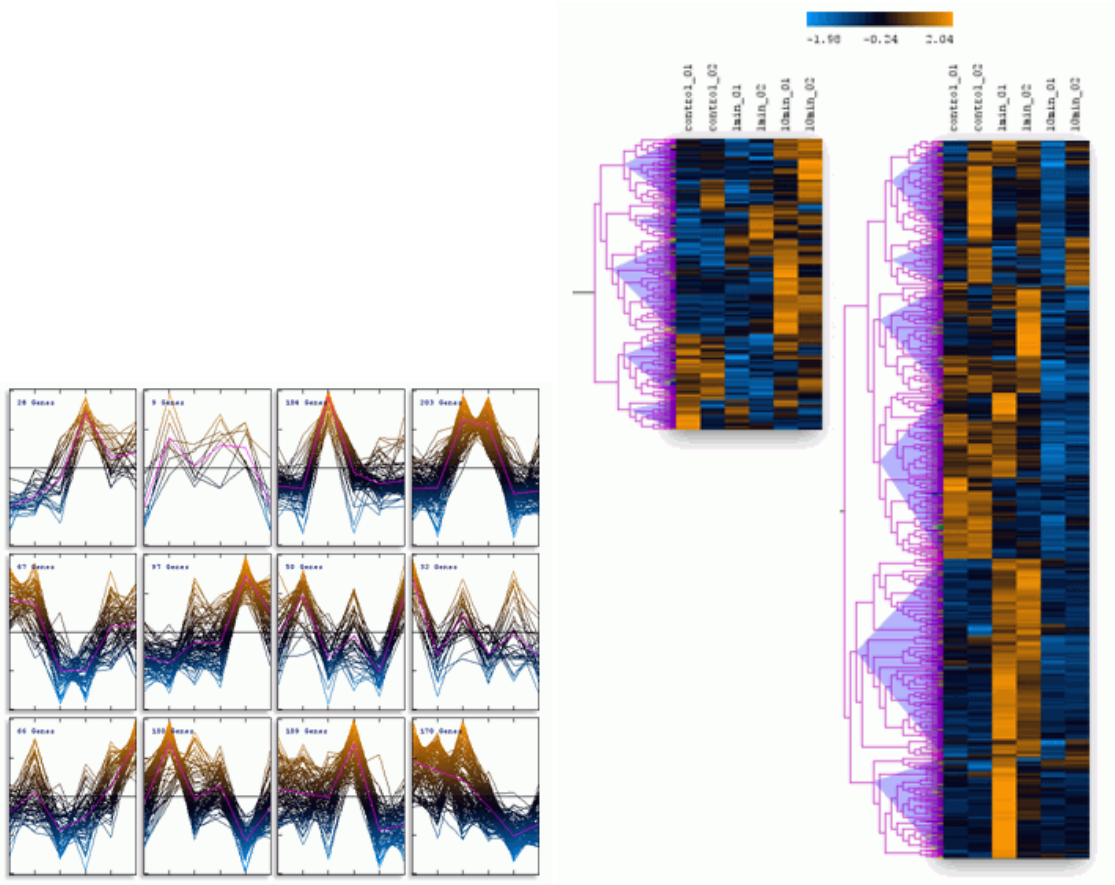


Figure 5.66.: Combined expression profiles in 12 clusters.

Find 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.

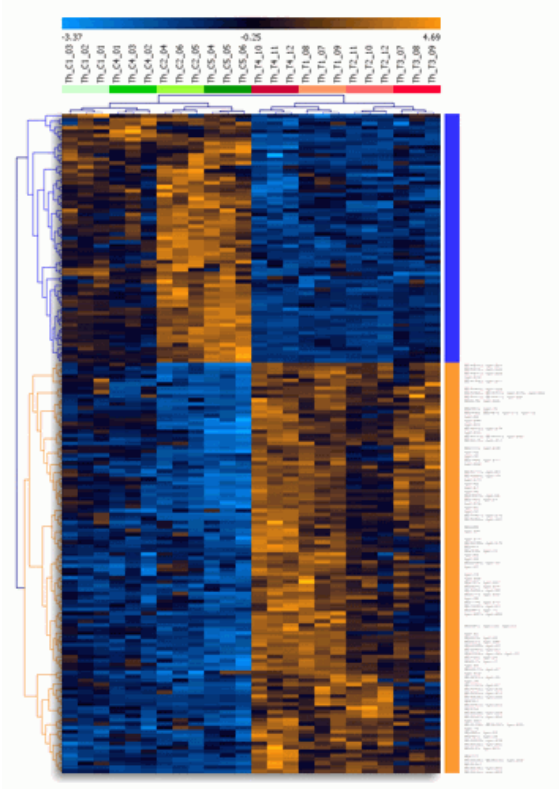


Figure 5.67.: Result of applying t-tests (control vs. treated) to expression profiles, result shown clustered.

In the simplest case, two samples shall be compared, 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. The most popular test for this purpose is Student's t-Test. The null hypothesis to be tested is that the mean expression levels in the two samples are the same. Rejecting the null hypothesis means that the protein under test is differentially expressed.

No normal distribution of spot intensities required

The classical Student's t-Test makes the assumption that spot quantities within replicates follow a normal distribution which is often not clear. 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 - or use a non-parametric test as described later, which does not demand for a certain data distribution.

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

Control 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.

To address the issue of multiple testing, a set of methods are available. Make use of "standard Bonferroni correction", "adjusted Bonferroni correction", or "Stepdown Westfall and Young methods" with "maxT"' option. Furthermore, 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, use FDR (either choose bounds for the number of false positive spots in the result set using the "number of false positive genes should not exceed" or choose a bound for the proportion of false positive spots in the result set, using the other radio button and text box). For ANOVA and ANOVA2 almost the same options are available.

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.

Templates can be entered manually 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 available 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.

Principal Component Analysis (PCA): Group and Visualize

Principal Component Analysis (PCA) for a *set of images* results in a two- or three-dimensional visualization of the image set preserving the variation as much as possible.

PCA works by taking spot intensities on every image and assembling them into one vector per image. PCA finds a projection of the point cloud into the two or three-dimensional space

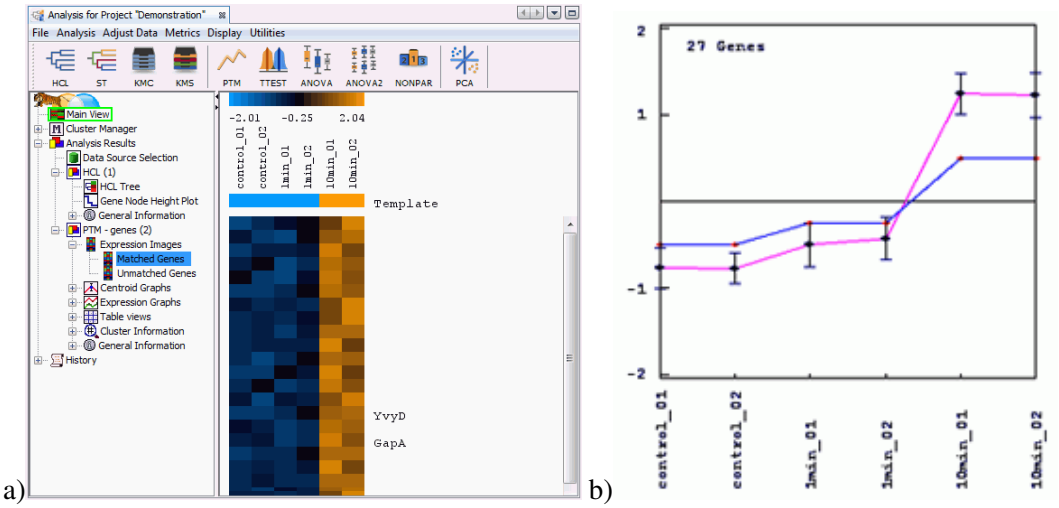


Figure 5.68.: a) Expression profiles matching the template. b) Comparison between template (blue line) and matching expression profiles.

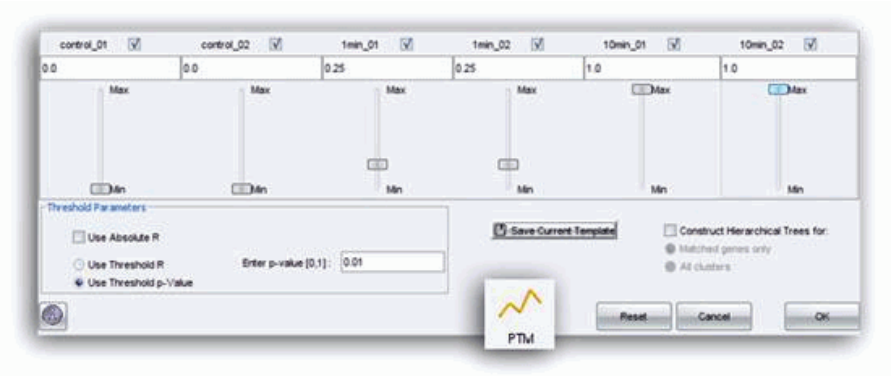


Figure 5.69.: With Pavlidis template matching (PTM) you can specify a typical expression profile, e.g. one that increases with time.

such that as much as possible of the variation of the point cloud is preserved. The images from different samples should appear in separate regions of the resulting diagram, while images from the the same sample should be close to each other. 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

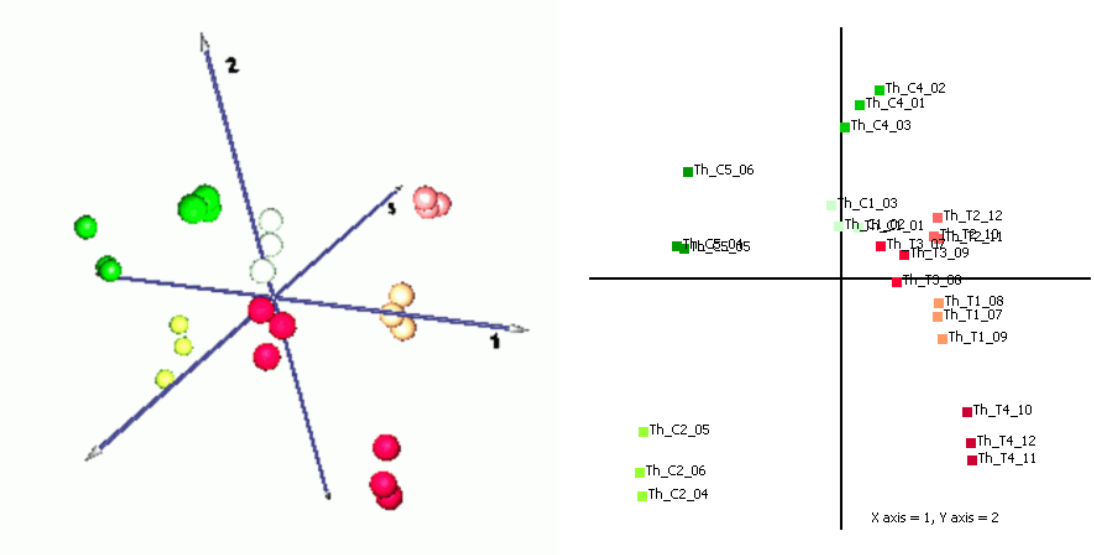


Figure 5.70.: Principal component analysis of 24 images plotted against the first 3 (left)or 2 (right) principal components. Images colored by their group color. Samples are well separated (shades of red or green, respectively).

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.

The three dimensional view can be rotated by dragging with the mouse.

Work 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 (images).

By default, clusters reflecting the image groups are available and their colors are used e.g. in *PCA* views (for samples). Further sample clusters are automatically available if defined in the image attributes are clusters for samples (i.e. their replicates), channels (staining or labeling methods), and gels (for multiplex approaches).

You can create new clusters by choosing Store Cluster in many displays of analysis results.

Storing a cluster of expression profiles or a sample cluster (gelimages):

- 1. a) 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 rows (expression profiles) will be selected.

- 1. b) In a hierarchical clustering, click on a part of the dendrogram for samples (column

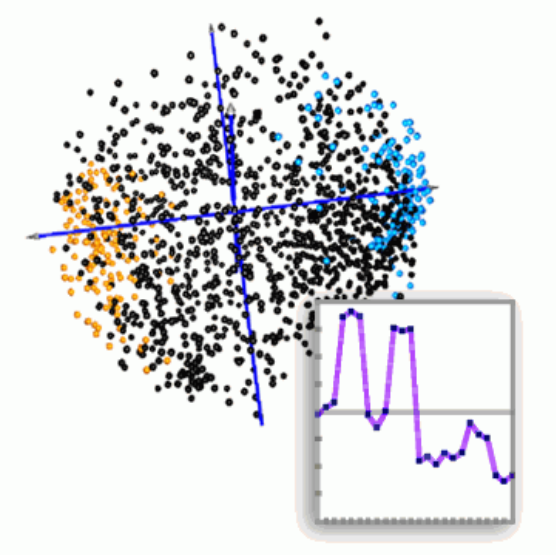


Figure 5.71.: Principal component analysis of expression profiles in three dimensions. Differentially expressed spots were determined by t-test and highlighted orange and blue, respectively. Inset: First principal component.

dendrogram) to select a set of replicate images. The corresponding image columns will be selected.

- 2. Now right-click on one of the selected rows or columns and select Store Cluster. Define the cluster attributes and click the OK button.

- 3. The new cluster will be shown in the Cluster Manager under Gene or Sample Clusters, respectively.

In the Cluster Manager you can change any attribute, e.g. cluster colors, or whether the color should be used in the different displays.

> Note: Clusters for samples or expression profiles may overlap, but only one cluster's color will be used in displays for each sample or expression profile, respectively. To avoid confusion make sure that only those cluster colors are used that you really want to see by checking the respective rows in the Show Color column of the cluster manager.

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.

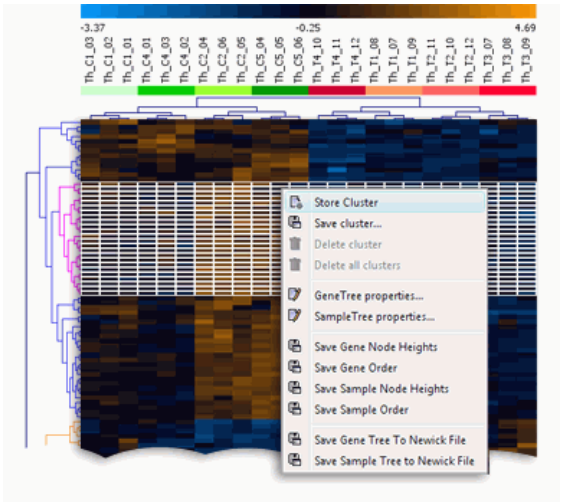


Figure 5.72.: Storing a cluster for expression profiles.

Serial #	Source	Algorit...	Cluster Node	Cluster Label	Remarks	Size	Color	Show Color
1	Group	Gel Ima...	Group/control	control		2		☑
2	Group	Gel Ima...	Group/1 min	1 min		2		☑
3	Group	Gel Ima...	Group/10 min	10 min		2		☑
4	Sample	Gel Ima...	Sample/Control	Control		2		☐
5	Sample	Gel Ima...	Sample/A	A		2		☐
6	Sample	Gel Ima...	Sample/B	B		2		☐
7	Channel	Gel Ima...	Channel/35S met ...	35S met pulse		6		☐
8	Gel	Gel Ima...	Gel/III	III		1		☐
9	Gel	Gel Ima...	Gel/IV	IV		1		☐
10	Gel	Gel Ima...	Gel/VI	VI		1		☐

Figure 5.73.: Cluster Manager: Sample 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.

Cluster A Cluster B

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

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

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 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.

Using Delta2D's Reports, it is easy to show different views on the statistically significant spots you have found. All you have to do is select these spots in the Delta2D analysis and open a report:

- Make sure you have selected the spots of interest (e.g. a complete cluster).
- Choose Reports / Spot Album or Reports / Spot Quantities. Note that the spot album may by quite large, as there is one thumbnail for each spot on each image. You can restrict the reports to a single group by clicking on the *"hide others"* link in the group caption.

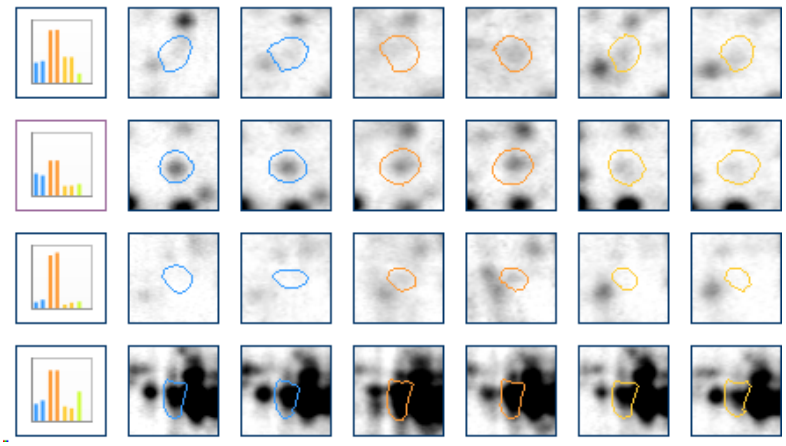


Figure 5.74.: Part of a spot album for differentially expressed spots.

Being selected is a very volatile spot attribute which is lost by any spot selection action. Alternatively you can mark the spots of interest on any image and configure the reports with respect to the set of spots you want to see. For more information about *Reports* see also section 3.5.

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.*

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.

Nonparametric Tests Nonparametric tests should be uses when it is unknown whether the spot quantities within replicates follows a normal distribution.

Wilcoxon, Mann-Whitney Test Analyse one factor and a maximum of two experimental groups (similar to t-Test).

Kruskall-Wallis Test Analyse one factor and n experimental groups (similar to one-way ANOVA).

Mack-Skillings Test Analyse two factors and n experimental groups (similar to two-factor ANOVA).

Fisher Exact Test Analyse two experimental groups representing two different factors (similar to t-Test).

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.

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/files/doc/MeV_Manual_4_0.pdf

5.12. Project Matrix

In the Project Matrix, previously known as the Project Manager, every gel is represented by a thumbnail image (fig 5.75 on page 150). Drag the line between two header cells to make the thumbnail larger or smaller. You can drag the images to change the order of the images. A small icon in the header indicates whether there is a quantitation result available for the image. Another icon shows if there are labels attached to this image. As a rule, icons appear only if spots are detected or labels exist, respectively.

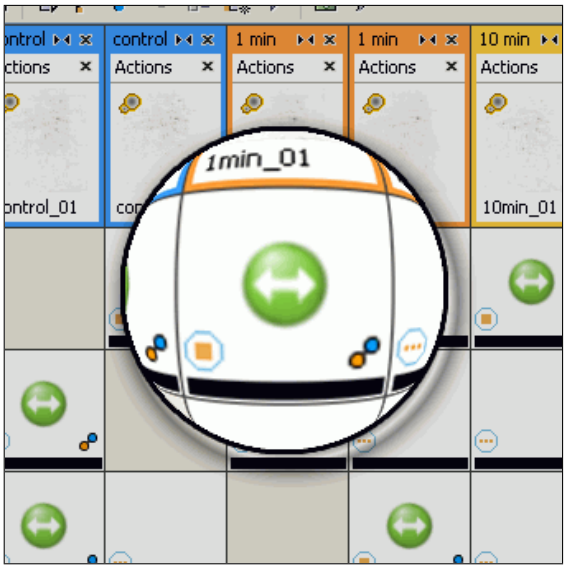


Figure 5.75.: Details in Project Matrix

You can invoke operations on a image or on a image group by using the entries in the thumbnail's context menu (see Table 5.11 on page 151). Right click on a image thumbnail to open the context menu.

Open Dual View with ▷	Choose another image to open the selected image with in the Dual View.
Move Image to Group ▷	Choose the group to move image to.
Add Image to Group ...	Add a image that is not used in the current project yet from the pool to the selected group.
Remove Image from Group	Remove the selected gel from group.
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 3.3 on page 21).
Fuse Images in This Group	Create a new image, by fusing all images of the group you choose.
Quantify Image ...	Detect spots on the selected gel (Only applicable if no quantitation data available).
Transfer Spots to Image ▷	Transfer the spots boundaries of the selected gel to other gel(s) (see section 3.3 on page 24).
Spot Color Coding ▷	Use the selected image as basis for a new Spot Color Coding view.
Collapse Group	Collapse all 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 5.11.: 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. 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.

6.1. Delta2D

Match Vectors

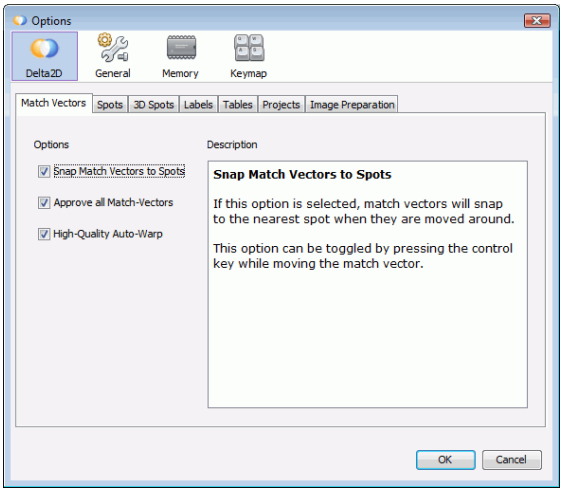


Figure 6.1.: Options: Match Vectors

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

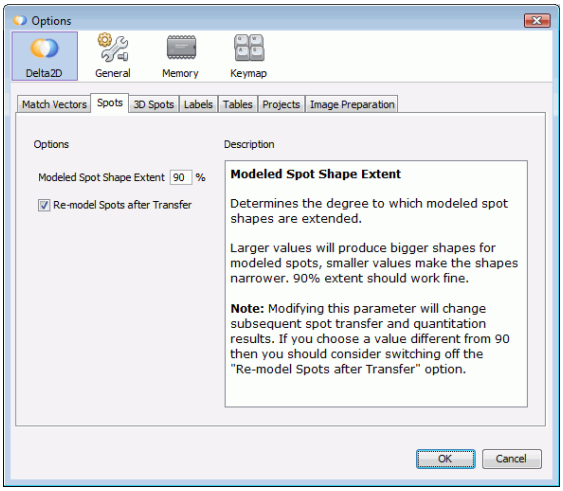


Figure 6.2.: Options: Spots

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

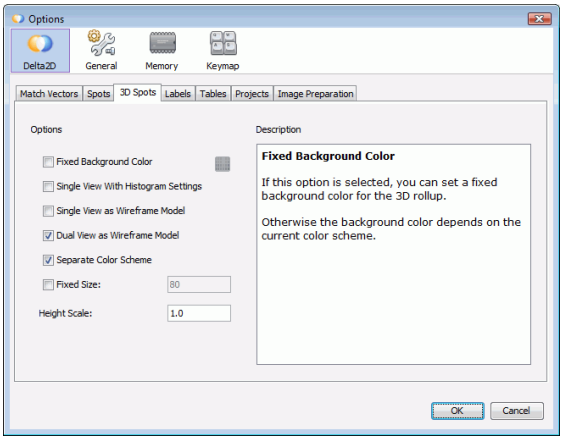


Figure 6.3.: Options: 3D Spots

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 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 fig. 5.41 on page 106.

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 they appear very high or very flat in the 3D rollup.

Labels

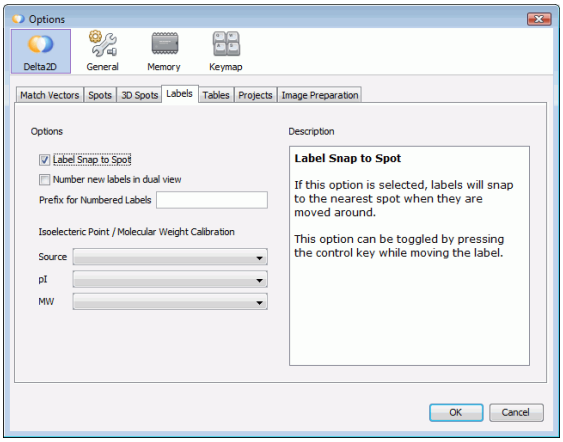


Figure 6.4.: Options: Labels

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 as data source and the appropriate attribute to define the rollup's behaviour.

Tables

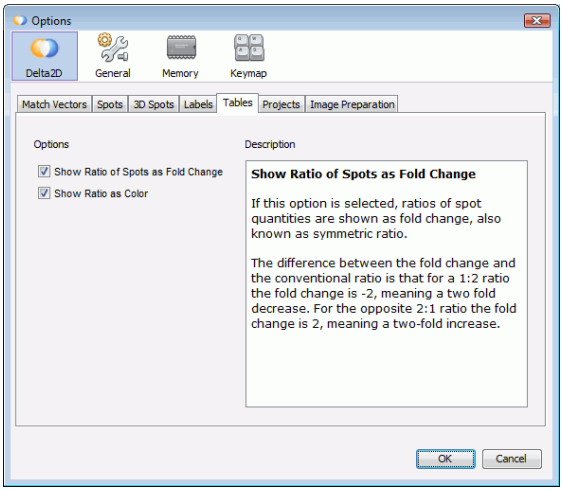


Figure 6.5.: Options: Tables

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 is doubled compared to the first spot, the ratio is 2, if its volume is halved, 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

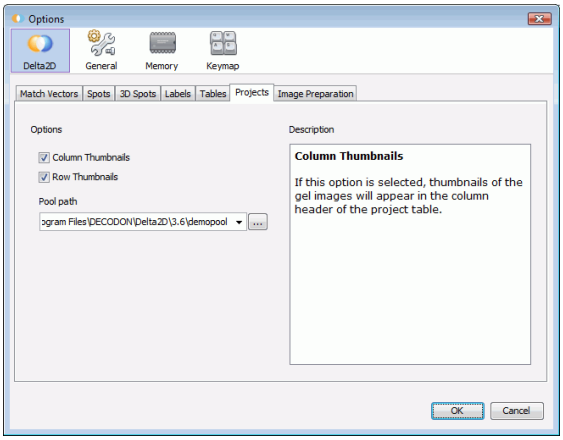


Figure 6.6.: Options: Projects

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

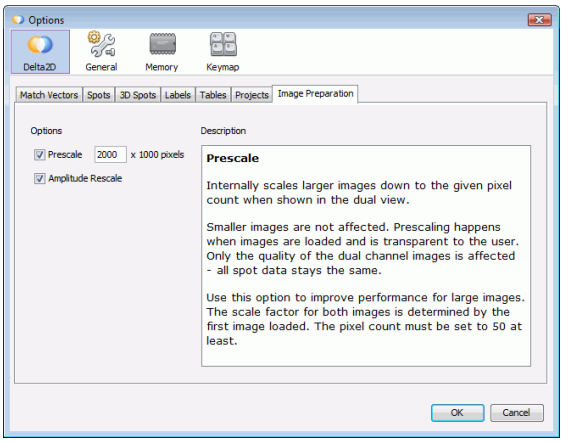


Figure 6.7.: Options: Image preparation

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 Use this option to improve performance for large images to scale them down to the given pixel count (minimum: 50 pixels). Smaller images are not affected. Prescaling happens when images are loaded and limits memory demand which grows with the size and number of images analyzed in a project.

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.

6.2. General

License

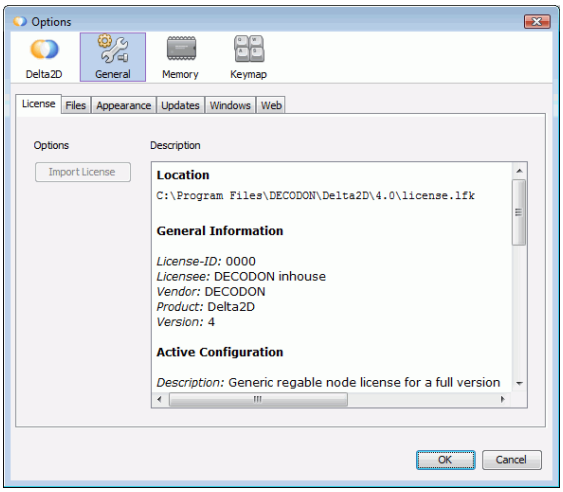


Figure 6.8.: Options: License

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

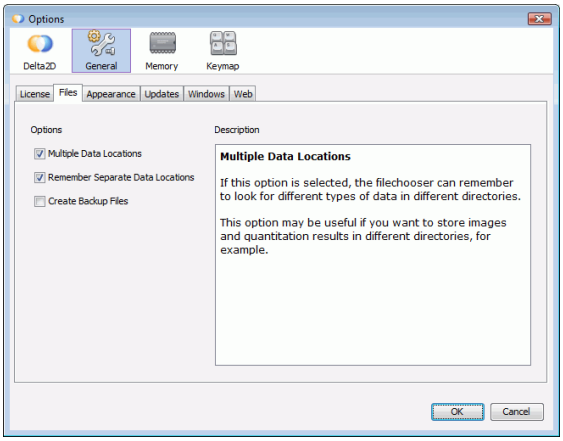


Figure 6.9.: Options: Files

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 folder after Delta2D is restarted.

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

Appearance

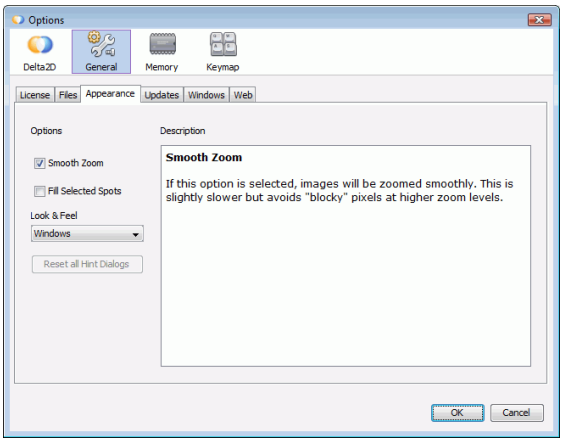


Figure 6.10.: Options: Appearance

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

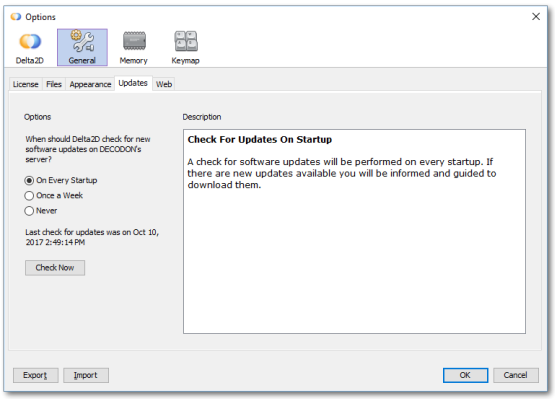


Figure 6.11.: Options: Updates

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.

Web

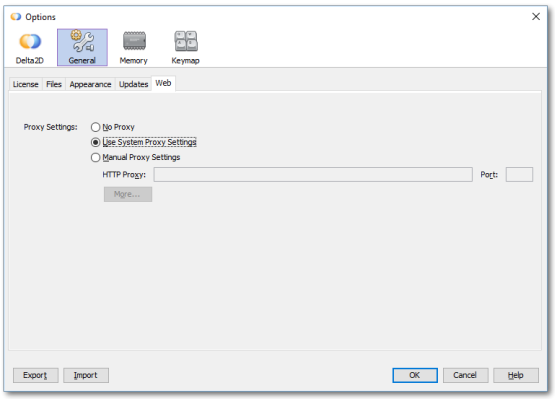


Figure 6.12.: Options: Web

Scouts, the check for updates, and some license configurations demand for internet connections. Some institutional networks require the configuration and adaptation of proxy settings. If a connection fails please review these settings, perhaps after having consulted your system administrator who knows your network topology.

6.3. Memory

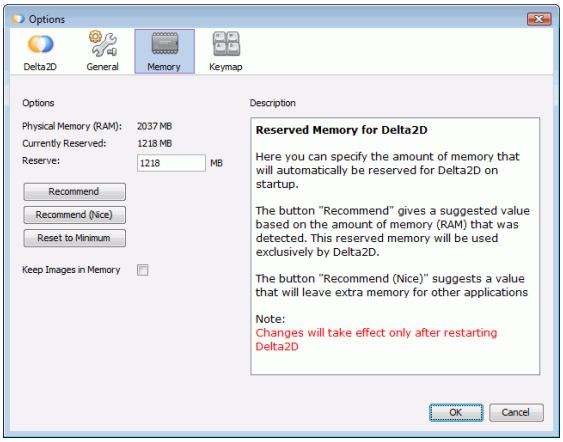


Figure 6.13.: Options: Memory

Note: For adjusting the memory settings you need the permission to write into the installation folder of Delta2D. Particularly for Windows Vista it is not sufficient to have administrator rights but you might have to explicitly 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.

6.4. 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.

Predefined global keyboard shortcuts include:

Ctrl + N	Create new project
Ctrl + O	Open project
Ctrl + Shift + 1	Open or switch to Project Explorer window
Ctrl + Shift + 2	Open or switch to Light Table (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 Image Regions window
Ctrl + Shift + 7	Open or switch to Expression Profiles window
Ctrl + Shift + Tab	Switch between the windows

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 Vector Tool
Alt + 2	Switch to the Spot Selection 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 match vectors overlay
Ctrl + 2	Switch on / off master spot boundaries overlay
Ctrl + 3	Switch on / off sample spot boundaries overlay
Ctrl + 5	Switch on / off sample labels 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 + 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 + D	Save Dual channel image as ...
Ctrl + Space	Switch between master / sample image tabs
Ctrl + Shift + Space	Switch between dual image / last active single image tabs
Ctrl + T	Show Quantitation Table(statistics) for this image pair
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 80). Depending on the tool being in use, you get changed or even additional functionality by pressing a key when clicking.

Match Vector or Connector Tool

Alt Changes the mouse pointer 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.

Spot Selection Tool

Alt Changes the mouse pointer temporarily to a hand for moving around the image.

Ctrl Allows you to select additional spots without deselecting already selected ones.

Spot Editing Tool

Alt Changes the mouse pointer temporarily to a hand for moving around the image.

Zoom Tool

Alt Changes the mouse pointer 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 mouse pointer 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.

Appendices

A. Example Files

Delta2D comes with an example analysis project, that is described in detail in the first chapter of this manual. The Table below lists the images included.

control_01, control_02	Two images from a sample before stress treatment (control).
1min_01, 1min_02	Two images from a sample taken 1 min. after stress treatment.
10min_01, 10min_02	Two images from a sample taken 10 min. after stress treatment.

Table A.1.: Example images provided with Delta2D.

The 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 1 or 10 minutes, respectively. To receive a minimum of reproducibility and to show an example that contains replicate images from the same sample, two gels and images were made from each sample.

B. Image Calibration

Delta2D recognizes virtually all the image file formats that use special calibrations for translating pixel values to measured intensity values. For example, image files in the .IMG format used by GE (former Fujifilm) scanners, and images in the .GEL file format used by other GE (Amersham) and Molecular Dynamics scanners are detected and used automatically. For basic information about image file formats and calibrations, look at our Imaging Guide at http://www.decodon.com/imaging-guide.html.

B.1. External XML files describe calibration curves

In addition to the vendor-specific calibrations, Delta2D can use XML files to describe custom calibration curves. You can see an example for the file format that is used inside the demo pool, at .Delta2D/demopool/calibrations/Typhoon9400 1_21025.xml. The calibration curve is described by a set of sample points, where x is the pixel value found in the image file, and y is the measured intensity. Between sample points, the calibration is defined as linear interpolation.

You can use the Typhoon file as a template, make sure you choose a unique name and ID for your file. As of version 4.0.0 you also have to use the same string for name and ID in order to make the calibration curve accessible for your images. Please contact DECODON's technical support if you are unsure about how to create your own XML files.

B.2. Use an external image calibration file

1. Close Delta2D.
2. Copy the XML file into the calibrationsfolder of your pool (for the demo pool, this would be demopool/calibrations/).
3. Start Delta2D.
4. Assign the new calibration curve to an existing image: Right-click on the image and choose Properties. In the Image Properties Dialog you can now select the new calibration curve.
5. Assign the new calibration curve when importing a image: this is similar to the previous step: just select the calibration when you set the image properties.

Note that you will have to re-create the spots for an image after you change its calibration. That means you have to do a new spot transfer onto the image, or repeat the spot detection.

C. Lists of Figures and Tables

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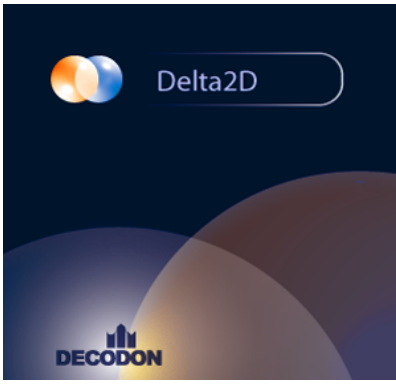
Acknowledgments

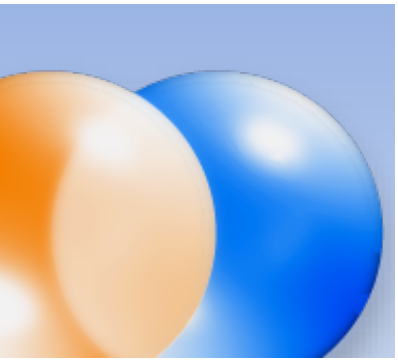
Delta2D's development has always to a large extent been driven by feedback from customers. Their comments were essential in advancing Delta2D.

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.





Request you personal demo today!

Want to know more? Contact us today to arrange your personal live web demo. All you need is a web browser and a phone – an expert will show you how you can apply Delta2D to your specific 2D gel analysis needs.
You can download an evaluation version of Delta2D from www.decodon.com. Your questions and remarks are welcome, call us at +49 3834 515230 or send an email to info@decodon.com.

Technical data:

Supported image file formats: Delta2D supports virtually all calibrated and uncalibrated image file formats on the market today, including tiff (8 bit, 12 bit, 16 bit), IMG (Fuji, GE), GEL (GE), JPEG, BMP, GIF, PNG, PNM.
Supported Protein Labels and Stainings: Delta2D supports virtually all protein labels and stainings, including Silver, Coomassie, Colloidal Coomassie, Sypro Ruby, Flamingo, Krypton, LavaPurple, Diamond ProQ, Emerald ProQ, Cy-Dyes, G-Dyes, radioactive labels etc.
Supported Spot Picking Devices: Delta2D supports spot pickers from Molecular Dynamics, Genomic Solutions, Bruker, GE, Herolab and others. Please contact DECODON for details.
Supported Operating Systems (*recommended): Delta2D runs on Windows 7 / 8 / 10* at 32 or 64* bit, Intel based Mac OS X 10.8* (Mountain Lion) or later and most flavours of 32 bit or 64 bit* Linux.
Hardware Requirements:
Minimum Hardware:
PC or Intel-based Mac with 2 GB RAM
Recommended Hardware:
Dual or Quad Core Processor with 2 GHz or better, 4 GB RAM or more, in combination with a 64-Bit operating system. The more gel images you compare in one project, the more RAM will be used.

DECODON

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