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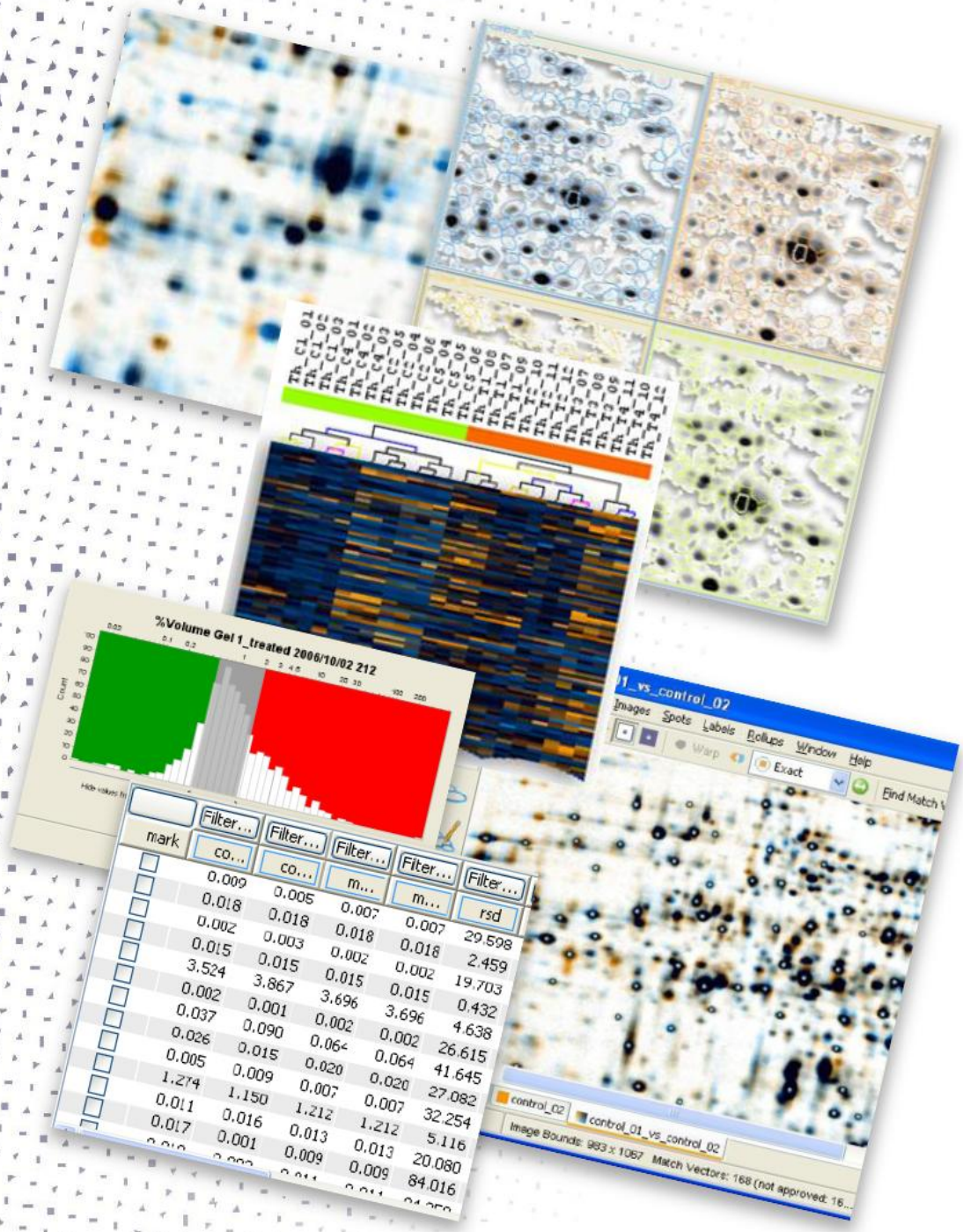
Delta2D

Getting Started Guide



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Delta2D 4.0
Quick start guide

DECODON GmbH
www.decodon.com

Thank you for using Delta2D, the software package with leading technology for easy and reliable analysis of 2D gel images.

The aim of this guide is to show how to analyze gel images with Delta2D. We will start with raw image data and finish with quantitative results and visualizations that are ready for presentation. We use an example project consisting of six gel images and an additional artificial proteome map to illustrate the steps of a typical analysis.
If you are interested in analysing DIGE gels, please also read the respective inserted green notes and the sections with green headlines, otherwise skip them.

The completely analyzed example project comes with your installation of Delta2D. In this guide, however, we will start with the raw data. Please go to www.decodon.com /Support/manuals.html and download the Delta2D sample data (Delta2D-samples.zip, 8.89 MB) we are using in this guide. Delta2D-samples.zip contains six gel image files and match map files for the warping.

Note:
If you need more detailed information about Delta2D, please consult the manual of Delta2D. You can access it through the Delta2D help menu or as a PDF file located in the installation directory. In Windows, there is a direct link to the manual in the Start menu. The manual is also available for download and for online browsing!

To learn more about Delta2D please do not hesitate to contact us by email to support@decodon.com or by phone at +49 (0) 3834 515 230.

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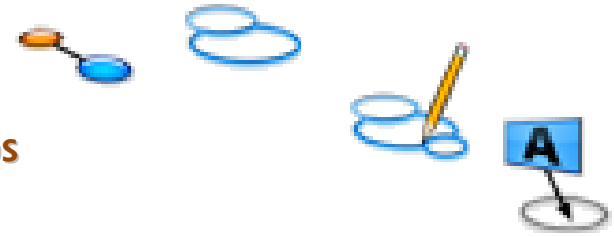

Analyzing Gel Images in just 6 Steps

1 Setup Project

Setting up traditional and DIGE experiments: Import your gel images into Delta2D and arrange them into projects.

2 Setup Gel Image Warping

Setup direct warpings such that all gel images in the project can be connected, directly or indirectly.

3 Create Direct Warpings

Find and review match vectors for directly connected gel image pairs to eliminate running differences between these gels.

4 Create the Consensus Spot Pattern

Do consistent spot detection across the whole project.

5 Analyze Expression Profiles

Find spots with relevant expression profiles.

6 Present Results

Create reports or provide results to other applications for presentation or further analysis.

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Setup Project

We start with the raw data files on the hard disk. The example experiment consists of a control sample, and two samples 1 and 10 minutes after treatment with a chemical compound, respectively, with two replicates of each sample:

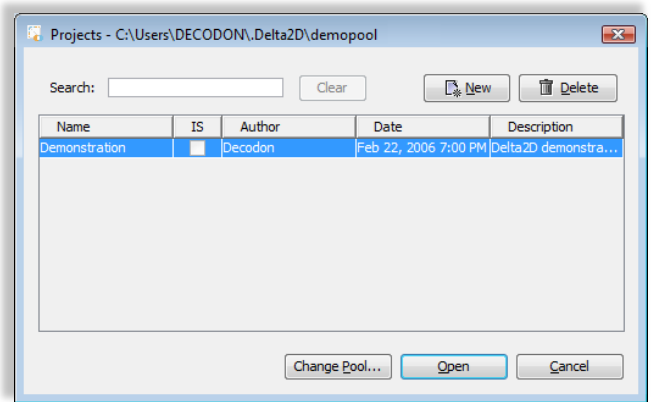
Image file	Sample
control_01.gel	control (replicate 1)
control_02.gel	control (replicate 2)
1min_01.gel	1 minute after treatment (replicate 1)
1min_02.gel	1 minute after treatment (replicate 2)
10min_01.gel	10 minutes after treatment (replicate 1)
10min_02.gel	10 minutes after treatment (replicate 2)

Note:
If you have not already done so, please download the example data that we use in this guide www.decodon.com/Support/Documentation/Delta2D-samples.zip. Unpack the archive. It contains six gel images and match map files for the warping.

Start Delta2D

Start on Windows by clicking on 'Start' > 'Programs' > 'DECODON' > 'Delta2D', on other systems in accordance to the respective installation. Delta2D opens and you are asked to open an existing project or to create a new one.

Projects are collections of gel images, and analysis data (spot boundaries, quantities etc.). So far, there is one project only: The demonstration project that comes with the installation of Delta2D.



When Delta2D has started, this dialog provides a list of available projects in the current pool plus the option to change the pool.

Create a new data pool

Delta2D keeps all data related to your current gel images, including the images themselves, in one folder on your hard disk. We call this folder a 'Pool'. It contains several subfolders and data files that are managed by Delta2D. To keep your experimental data organized, we recommend creating a new pool for every new experimental context.

1. Click on the button 'Change Pool' and navigate to a folder where you want to store your new pool.
2. Right click and select 'Create Folder', then click on the new folder or select it and press F2. Type in a name for this new pool and confirm the input with ENTER.
3. Click on OK and confirm the following dialog with 'Yes'.

Note:
Choose different pool paths for your different experiments to separate them from each other and from the demonstration data. Furthermore it is easy to copy, move, or backup the different pools. Avoid working with network drives.

Create a new project

The 'Projects' dialog appears again showing an empty list of available projects. The title bar of the dialog displays the path to the pool you are working in.

Click on the 'New' button to create a new project. A dialog opens requiring some project details. Provide the name and the author of the project and insert a short description. Do not check the "Use Internal Standard" checkbox. Press 'OK' to create the new project. Select the new project in the "Projects" dialog and press 'Open'. Whenever a new project is opened, two new, empty groups are automatically created.

Note:
For DIGE projects activate the checkbox 'Use Internal Standard'.

Set up groups for the gel images

It is recommended to organize your gel images into groups. Usually, your project will have one group for every set of replicates in the experiment.
We will now create the three groups for our experiment. There are already two groups present which you should see in the Light Table (window by default in the right part of the screen). We will simply change the names of these two groups:

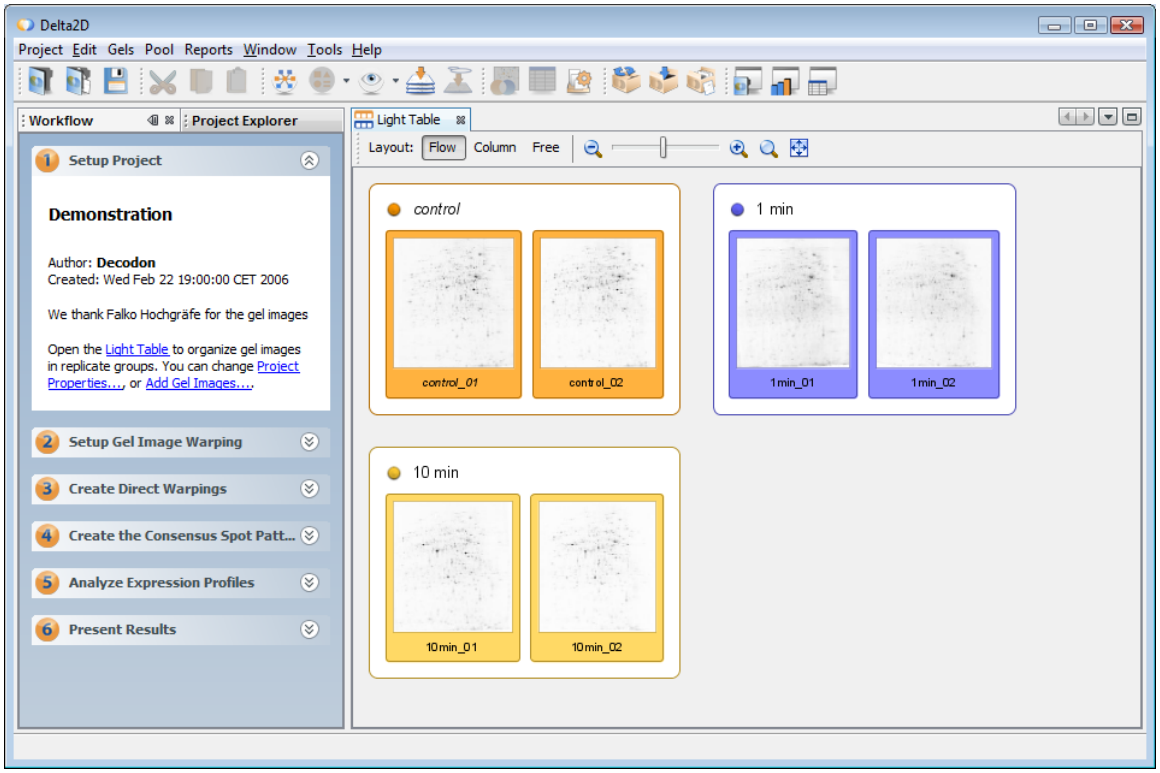
1. Right-click on the first group and choose 'Properties' from the context menu.
2. Replace the group's name by 'control'. If you want, choose another color for the group. Confirm with OK.
3. Repeat this for the second group assigning the name '1min'.

To create a new group, select 'Project' > 'Add Group' from the menu and name it '10min'. Choose another color if you want. You can permute, add or delete groups at any time.

Add gel images to the new project

Right-click on group 'control' and select 'Add New Gel Images...' from the context menu to add images to this group. The gel manager opens, showing all gels in the current pool. Since the pool has just been created it is empty and we have to import gel images. Click on the button 'Import' and go to the directory where you unzipped the example data from our web site. There are sample gel images in a sub folder called 'samples'. Select the two images 'control_01' and 'control_02' by using the left mouse button in combination with the CTRL-Key. Click on 'Next'. In this step you can edit names and other descriptive data for an image, and do some basic manipulations such as rotating, mirroring or inverting the image. Click on 'Next' again to repeat this for the second image.

You can click on 'Finish' any time if you want to import the images without further changes. Import the sample images '1min_01', '1min_02', '10min_01' and '10min_02' into their respective groups. Now every set of replicate images is located in a separate group. The project will now appear as in the following figure:



The Delta2D Light Table (right) with the six sample gel images organized in three groups ('control', '1min', and '10min').

Setting up a DIGE analysis project

For DIGE and other multiplex experiments, first follow the steps outlined above, i.e. organize gel images into groups according to the sample that they show.
The layout of a simple DIGE experiment with two samples A and B on two gels is shown below. Replicates are put into the same group. The internal standard images are put into the group 'standard'.

Image file	Dye	Gel	Sample	Group
g001_cy2.tif	Cy 2	Gel I	Internal standard, gel I	Standard
g001_cy3.tif	Cy 3	Gel I	Sample A, replicate 1	Sample A
g001_cy5.tif	Cy 5	Gel I	Sample B, replicate 1	Sample B
g002_cy2.tif	Cy 2	Gel II	Internal standard, gel I	Standard
g002_cy3.tif	Cy 3	Gel II	Sample B, replicate 2	Sample B
g002_cy5.tif	Cy 5	Gel II	Sample A, replicate 2	Sample A

Delta2D applies the internal standard for quantitation. This means that a spot's quantity is divided by the quantity of its corresponding spot on the internal standard image on the same gel. Hence it is important to let Delta2D know which images belong to the same gel and which image shall serve as the internal standard image.

1. Configure the project to use internal standard images
   Select 'Project Properties' from the 'Project' menu. Then check the 'Use Internal Standard' checkbox and click 'OK' to apply the settings and close the Project Properties dialog.

2. Assign gels to gel images
   We now have to tell Delta2D which images come from which gel, and where the internal standard images are.
   Choose 'Gels' > 'Gel Image Attributes'. Select the 'Gel' tab. You see a list of gels (designated by roman numerals) and the images that come from each gel. Rename the existing sample gel names or add new entries assigning new names. Then right click on an image to assign it to a specific gel.
   Alternatively, you can drag an image and drop it onto another gel. Arrange the images to reflect your experimental design.

3. Identify internal standard images
   For each gel, you see a column of radio buttons. Select the radio button next to the image that contains the internal standard (usually, this will be the Cy2 image).

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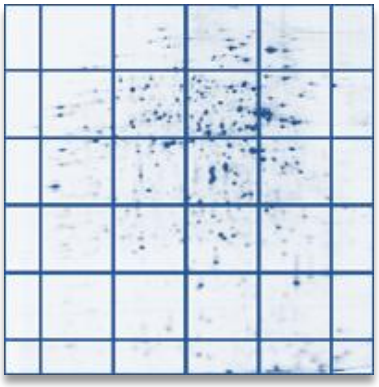
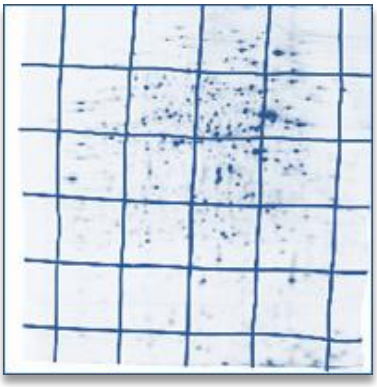
2 Setup Gel Image Warping

Background: Why is warping important for 2D gel image analysis?

Usually, spots from the same protein appear at slightly different positions on different gels. These positional differences are the main reason for inefficiency in 'traditional' 2D gel image analysis packages.



Gel image with overlaid grid



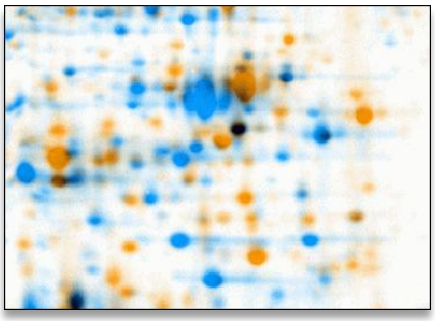
The same gel image after warping

If you are used to classical 2D gel analysis packages, you might know those long and tedious editing sessions for spot matches and spot boundaries that were found "automatically" by the software.
Delta2D's image warping eliminates these running differences between 2D gel images before doing spot detection or quantitative analysis using an image processing method called Warping. The result is like having perfect gels: after warping, corresponding spots have the same position.
DECODON pioneered the use of image warping in 2D gel analysis with the release of Delta2D version 1.0 in 2000.

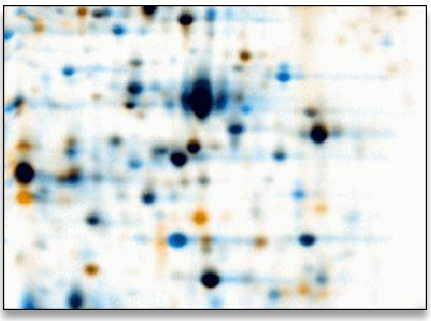
Note:

DIGE and other multiplex methods allow you to co-migrate multiple samples on the same gel. Thereby they mitigate the problem of differing spot positions. However, as soon as an experiment contains more than one gel, gel analysis software will have to deal with different migration positions.

Here you see a combined image that is made from two gels. One is colored in orange, the other one in blue. In the dual channel image without warping it is hard to find corresponding spots and do comparisons of expression patterns.



Two gel images, overlaid into a dual channel image, not warped.



Two gel images, overlaid into a dual channel image, after warping. Differences in expression levels are clearly visible. Warping allows Delta2D to assign corresponding image positions across a whole set of images. This enables, for example, 100% Spot Matching and Image Fusion.

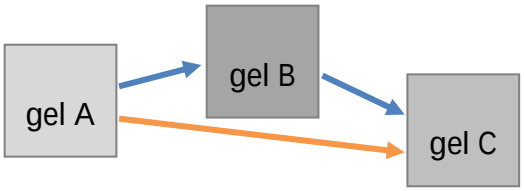
After the warping, the dual channel image gives valuable insight for comparing the spot patterns qualitatively. Blue spots are stronger on gel A, orange ones on gel B. Dark colors mean that spots have roughly the same intensity on both images.
But the advantages of warping go far beyond the making of dual channel images. The effect of applying image warping is as if you had made perfect 2D gels: those would have all proteins migrating exactly to the same position. And because Delta2D knows about pixel-by-pixel correspondences between images, a number of Delta2D's other core technologies are now available:

- **100% Spot Matching**
  Delivers you higher statistical confidence for the analysis of expression profiles.
- **Image Fusion**
  Lets you combine multiple images to produce, for example, average images.
- **Proteome Maps**
  Use union fusion images to e. g. store protein identifications.
- **Color coding**
  Analysis of expression profiles with spot Color Coding combines image fusion and 100% spot matching to deliver a high-level overview of groups of proteins having similar profiles.

Delta2D's SmartVectors™ technology produces a warping automatically, based on similarities between regions on gel images.

Defining a warping strategy

The warping for a whole experiment in Delta2D is based on pairwise warpings between gels. You do not have to produce match vectors for every possible gel image pair in your project: Given a warping between gel A and gel B, as well as a warping between gels B and C, Delta2D will automatically create an implicit warping between A and C.



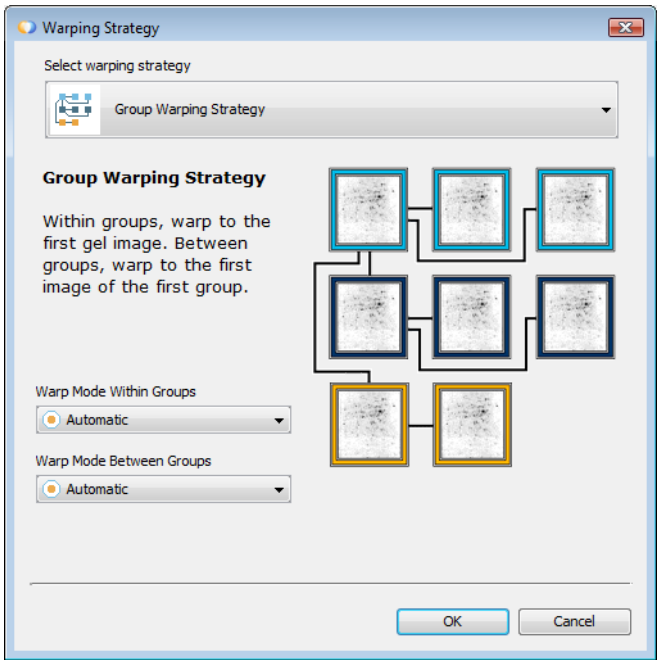
Implicit gel image warping

A 'Warping Strategy' contains the definition of pair-wise warpings that Delta2D should use to construct the overall warping. It can be flat or hierarchical.

The Group Warping Strategy is suitable for most projects with replicate groups. The general guideline for setting up a warping strategy is to connect similar gels directly, and to make sure that every possible gel pair can be warped by combining a few direct warpings. See the manual section 'Defining a Warp Strategy for the Complete Project' for more information.

With the Group Warping Strategy you warp both gels within every replicate group using the automatic warp mode. Connect the replicate groups by warping the first gel of the 'control' group (control_01) to the first gels of the '1min' and '10min' groups, again using the automatic warp mode.

Switch to the second step of the 'Workflow' window called 'Setup Gel Image Warping' and click on the 'Warp Strategy...' link to open the Delta2D Warping Strategy Manager. Alternatively you can open it via the menu: 'Gels' > 'Set Warp Strategy...'. Select the Group Warping Strategy. Automatic warping for warpings within as well as between groups is automatically taken as default setting. Press 'OK' to apply the selected strategy.



Warping strategy manager

Note:

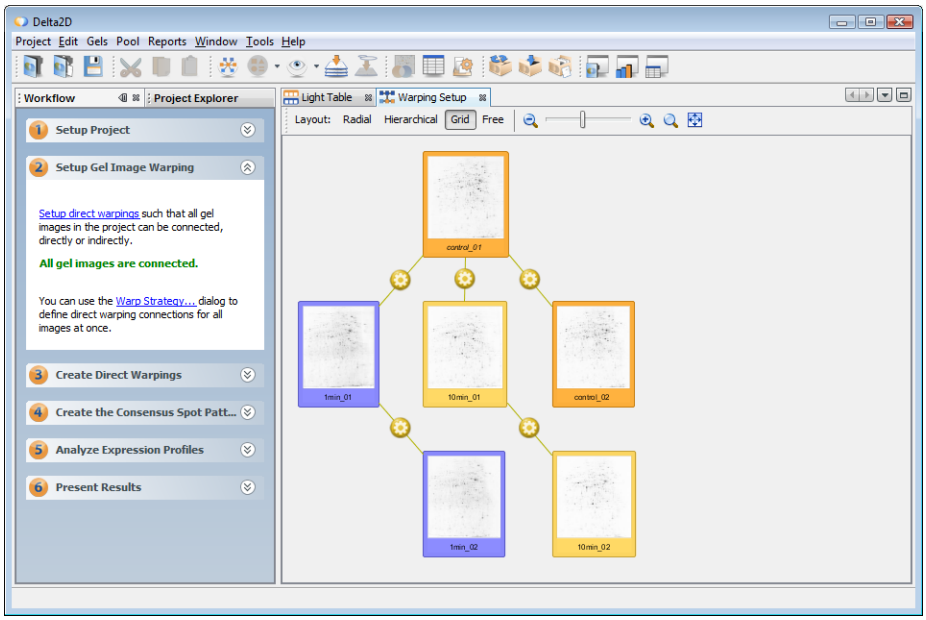
You could also define the warping strategy by dragging images onto each other in the 'Warping Setup' window. Using the Warping Strategy Manager is, however, easier and faster.

Automatic warping employs the SmartVectors™ technology and delivers the result as a set of match vectors that connect corresponding spots. Always check the resulting dual channel image and if necessary improve the warping iteratively as described later in this document (see also 'Create Direct Warpings').

Note:

The Strategy Manager overrides previous manual assignments of warping methods. So apply it after having organized a new project and be careful when applying it again later. Existing sets of match vectors are not affected.

Open the 'Warping Setup' window by clicking on the link 'Setup direct warpings' (or 'Window' > 'Warping Setup') to check whether the warping strategy has been applied correctly such that all images are connected:



Setting the warp strategy. The Warping Setup window after applying the Group Warping Strategy (Grid Layout).

As explained above, the warping strategy uses automatic warps to connect images within the same group, and to connect the first image in every group to control_01. The 'Warping Setup' reflects this: a line connecting two images means that the images should be warped directly. All the other gel image pairs can be warped by combining one or more of these warpings if each image has at least one connection to one of the other images of the project.

As a guideline, you can think of the yellow icons as showing you where you have to do work for the warping step. By creating match vectors for each of these image pairs, we allow Delta2D to warp the whole project.

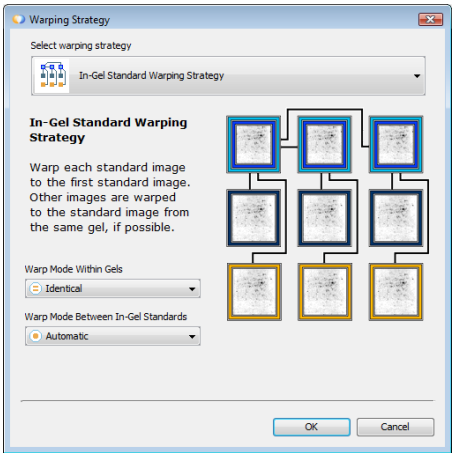
Setting up a warping strategy for DIGE and other multiplex projects

Delta2D offers a special warping strategy for DIGE and other multiplex experiments that use an internal standard. Within the same gel, we will not have to do any warping because samples are co-migrated. For warping between gels, we use the internal standard images because they always show the same internal standard sample which makes them very easy to warp.

Note:

Before you start, make sure that you have checked 'use internal standard' in project properties, and assigned gels and internal standard images.

Choose 'Warp Strategy...' from the second step ('Setup Gel Image Warping') of the 'Workflow' window to open the Delta2D Warping Strategy Manager. Select the In-Gel Standard Warping Strategy. Warp mode within gels should be set to 'Identical', Warp mode between gels to 'Automatic'. Then press 'OK' to apply the selected strategy and close the 'Warping Strategy Manager'.



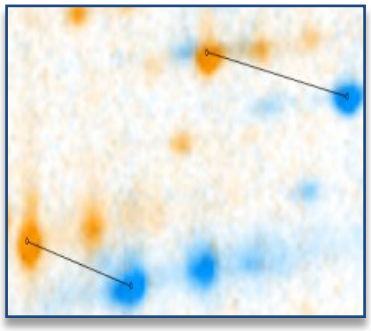
Warp Manager – In-Gel Standard Warping Strategy

Note:

Warp mode 'Identical' means that Delta2D will take the images as-is, without doing any warping. This is suitable for images from the same gel. Of course, you can change this setting (e.g. if there is a minor shift between the different images).

SmartVectors™: Find Match Vectors Automatically

The goal of this step is to produce a warping that aligns spots on one image with corresponding spots on the other image. In the 'Warping Setup' window, each yellow or green line with a yellow or green icon connecting two images represents one direct warping using so-called match vectors to align the images. Delta2D's SmartVectors™ Technology automatically produces match vectors. You can batch all the warpings so that a first iteration using the SmartVectors™ Technology is done in the background:
From the third step of the Workflow 'Create Direct Warpings' you can invoke the 'Job Manager' by clicking on the link for it. In the Job Manager click the 'Run' button to let it monitor and perform all automatic warping jobs.

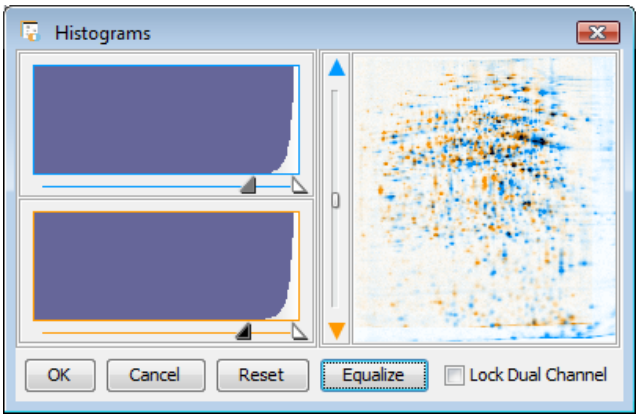


Dual Channel image
of two unwarped gel images

In order to open the completed warpings you can use the table below 'Review Direct Warpings' in the step 3 of the Workflow: Double-click on a row where the symbol in the yellow icon changed from a toothed wheel to two arrows:
A Dual View window (the dual channel image of these gel images) opens and shows the combination of the two images of the respective table row. Thus, you can see the differences in spot positions at a glance: black or gray spots have about equal volume in both images. Orange spots are stronger on the first image while blue spots are weaker. The more their color tends to one extreme, the bigger the difference is in expression.

Of course you can navigate any time through the dual channel image, zoom in and out or view the single images only. You can also correct the presentation of the images by choosing 'Histograms...' () from the menu 'Images' and by using the sliders to adjust the settings and clicking on the 'Equalize Image Button' () in the tool bar to make sure that both images are balanced. Changes will affect the visualisation only, while quantification will always be done on the raw images. Please also check if the gel image background has been hidden by using the 'Show/Hide Background' button () in the tool

bar. This adjustment helps to better see the differences in spot positions. Confirm with 'OK'.



Histogram settings dialog

Now press the warp button ( ) and Delta2D will warp the orange image in accordance to the match map using the exact warp mode. In the resulting warped view, it is usually easy to see if all corresponding spots are aligned. Regions that are not sufficiently aligned show up as similar spot patterns in the blue and in the orange part of the image. Delta2D will only create match vectors in regions where it is reasonably certain that they correspond to each other. You can unwarp the images (press unwarp) whenever you want to review the match vectors with respect to the original images.

Note:

Delta2D distinguishes between match vectors that were set by hand (shown as solid lines), and those that were created automatically (shown as dashed lines). By 'approving' a match vector you declare that it was checked by you, so it can be used to guide the finding of more match vectors.

If there are regions that are not properly warped yet, you can delete all the match vectors (see below) in the particular regions and use the 'Find Match Vectors' button to let Delta2D's SmartVectors™ technology use the vectors that are already present to guide the process of finding new ones:
Press the 'Find Match Vectors' button. Delta2D will ask you what to do with the vectors that were automatically created in the previous step: You can decide to use them as basis of another iteration with SmartVectors™ technology or to reject them.

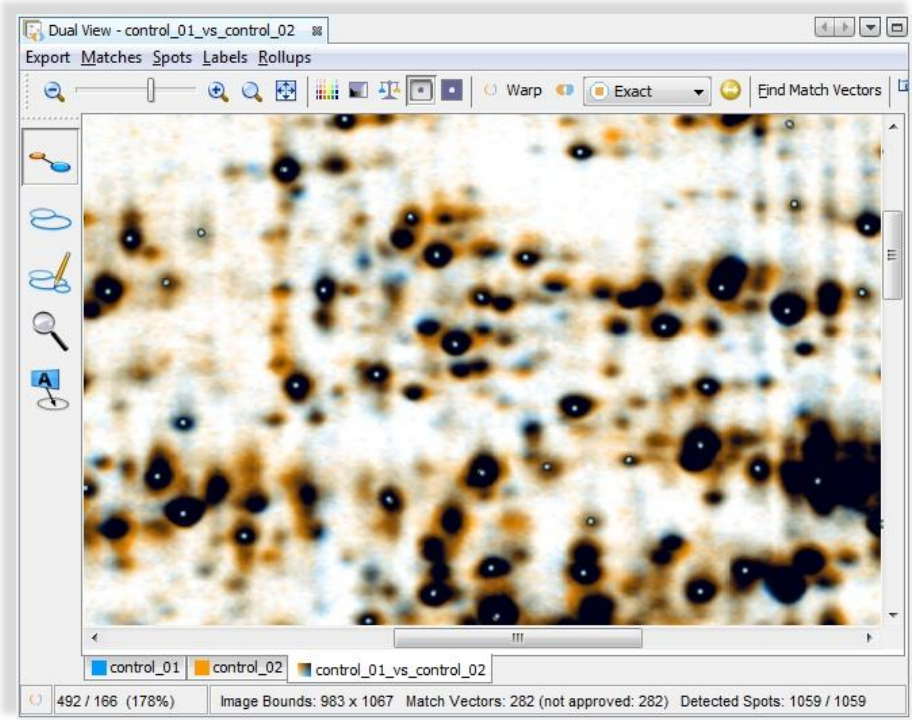
Alternatively, you can create, delete or change match vectors manually as described in the following section.

Note:
As soon as you have approved all the match vectors for the gel image pair, the color of the icon for the respected pair will change from yellow to green, meaning that you have already worked with the match vectors. It is good practice to approve all match vectors when you are finished with a gel pair (use Matches -> Select Non-Approved, and then Matches -> Approve Selected) as this makes it easier for you to notice the status of a warping at a glance.

Creating, deleting, and changing match vectors by hand

Note:
In order to work with match vectors, you have to select the Match Vector Tool first. Click on the top-most button in the vertical tool panel at the left of the dual view window.

Sometimes you will want to correct or improve the match vectors that were found automatically by Delta2D. The general approach is to work with whole image regions (as opposed to single spots). If one or more match vectors in a region are not correct, you just delete all match vectors in that region and press 'Find Match Vectors' again to find better ones; vectors from surrounding regions will guide the process.



The Dual View after Warping

Of course, you can set and change all match vectors by hand if you want:

- **Selecting a single match vector**
  You can select a single match vector by clicking on it. Selected match vectors are highlighted.
- **Selecting match vectors in a region**
  Drag a rectangle with the mouse (keep the left mouse button pressed). All vectors in that rectangle will be selected.
- **Deleting match vectors**
  Right click on a match vector and choose 'Delete' or 'Delete Selected' from the menu. This will delete the selected or all selected match vectors, respectively.
- **Setting a new match vector**
  Click first on the spot in the orange image, then on the corresponding spot on the blue image. Hold down the CTRL key while you click in order to let automatically snap the ends of match vectors to spot centers.

- **Changing a match vector**
  Drag one end of the match vector in order to change it. Hold down the CTRL key while you drag in order to switch off spot snapping.

Note:
You can undo match vector operations by selecting 'Undo' from the Edit menu.

Click on the Warp button to apply your new match vectors. You can iteratively add, correct or delete match vectors until the result is satisfying.

Verifying the results

Since the quality of the whole analysis relies on the exactness of warping, it is worth working carefully in this step of analysis. In order to control the quality of the warping step, please check this list:

- In the Warping Setup window you should see green lines with green icons only.
- For each gel pair with a green connection, open the Dual View by double-clicking on the respective icon and check the warped dual channel image.

Even if you set the warp mode to automatic you should still review the resulting warping in the Dual View to check if you are satisfied or if a correction is necessary.

Note:
The demo data set includes prepared match maps. To save time, just import the respective match map for a pair of gel images in the Dual View. In the menu choose Matches -> Import. If the blue match vector ends point into orange spots and vice versa the match vectors have the wrong direction and you need to convert them by choosing 'Matches > Invert'.

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4 Create the Consensus Spot Pattern

Delta2D's 100% Spot Matching is based on advanced image processing methods. It was introduced by DECODON in 2003. For a general presentation of this approach, please go to http://www.decodon.com/Solutions/Delta2D/100_Percent_Spot_Matching.html.
100% Spot Matching produces complete expression profiles for every protein. This leads to significantly improved statistical confidence, so you can, for example, identify more biomarker candidates from the same experiment. Other approaches to spot detection and matching (e.g. separate spot detection on every gel image) lead to inconsistencies like missing values in expression profiles, and ambiguities in the profiles themselves.

Based on a completely warped project, the quantitative analysis in Delta2D consists of three steps:

1. Image fusion to produce an image that shows all spots in the experiment
2. Spot detection on fused image to produce a 'consensus spot pattern'
3. Transfer of spots to the original images where they are quantified.

Generating a fusion image

The fusion image shows all spots of the experiment and serves as the basis for consistent spot detection and matching. Image fusion works by warping a set of images and then combining their intensities pixel by pixel into a new image. The result is an artificial gel image that has realistic spot shapes and combines essential characteristics from the original images.

Note:
There are many interesting applications of image fusion. For example, fusion images can be used to create a 'proteome map' i.e. a condensed visualization of all analysis results where you can collect your spot annotations. With average fusion, experimental variation can be compensated by combining several replicates into one average image. Large numbers of gels can be reduced to a single representative one.

Let us create a fusion image of our experiment: Switch to the fourth step in the Workflow window called 'Create the Consensus Spot Pattern' and click on the 'Fuse all images...' link to open the 'Image Fusion' dialog: Select 'control_01'as 'Master Gel Image' and

'Union' as Fusion Type, since the union fused image will include all the spots of the experiments. Leave all of the other settings. Click on 'Fuse' and a new image will be created and placed into a new group called 'Fused Images'.
Open the new fusion image by clicking on 'Open Fused Image using Union...' in the fourth Workflow step. A Dual View window opens showing the single view of the fused image only.

Note:
If an image is not connected to any of the other images or is only connected with the 'automatic' warp mode and you have not started the Automatic to find match vectors so far, you won't be allowed to fuse those images. If necessary, please check your warping strategy in the 'Warping Setup' window ('Window' -> 'Warping Setup') once again.

You can see that the fusion image looks like a real gel image. The natural spot patterns result from combining the images pixel-by-pixel using a weighted average function. Most importantly, the 'Union' fusion image contains all spots of the project's gel images, even those that occur only on one or two of the images.

Note:
For DIGE projects you can exclude the internal standard images (typically the Cy2 labelled images) from the fusion since they do not carry information which is not included in the sample images.

Detecting spots on the fusion image

Keep the Dual View open and click on the link 'Detect Spots on Fused Image...' in the 'Create the Consensus Spot Pattern' in the Workflow (alternatively choose 'Spots' -> 'Detect Spots on Fused Image' in the Dual View). A new dialog opens where you have access to the spot detection parameters.

Just accept the proposed parameter values since they are quite reasonable in most cases. Confirm this dialog by pressing OK and let Delta2D detect the spots.

Note:
The default parameters for the spot detection are derived from the actual images and result in a very sensitive spot detection. This is because it is much quicker to filter out false positive rather than adding new spots one-by-one.

Editing spots on the fusion image

You can correct the results of Delta2D's automatic spot detection by setting "markers". By using markers you can control where a new spot should be detected. Delta2D will then compute the new boundary accordingly. There are three basic operations for spot editing: adding a new spot, splitting a spot, and joining two or more spots. In any case Delta2D will compute spot boundaries automatically according to your input. This approach to spot editing maximizes reproducibility while allowing you to refine the detected spot pattern.

Note:
In order to edit spots, you have to select the 'Spot Editing Tool' first. Click on the third button in the vertical tool panel at the left of the Dual View window.

Adding a New Spot on the fusion image

To create a new spot simply click on the center of the undetected spot to let Delta2D automatically find a spot at this position. A new spot marker ( ) is created and Delta2D will compute the spot's boundary.

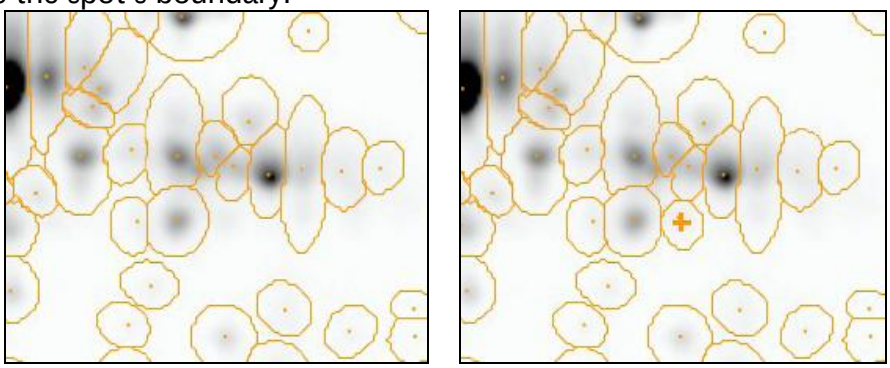
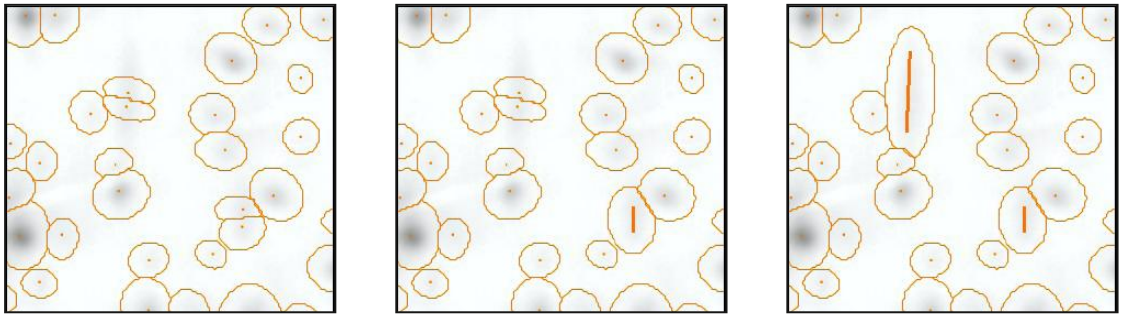


Image region before and after adding a spot manually

Joining Spots

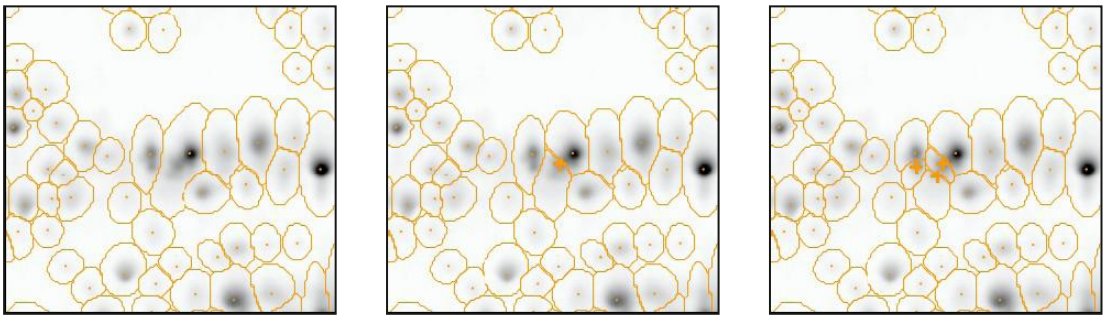
To join two or more spots, connect the spot centers with a line-marker: Just click and drag with the mouse. Delta2D uses this marker to compute a new spot boundary that covers the spots that have been connected by the line-marker.



Examples for joined spots.

Splitting a Spot

In some cases Delta2D detects one spot where you would rather like to have two or more. Click once into the center of the spot that was not detected separately and a spot-marker will appear. Starting from this marker Delta2D will now compute new spot boundaries. Depending on the position of the marker the spot boundaries will vary.



Set of spots, detected as one and split with markers

Moving and Deleting Spot Markers

Spot markers can be moved by dragging with the left mouse button. To delete a spot marker you can right-click on it. Moving or deleting markers changes spot boundaries and it may take some time to compute the new boundaries.

Removing Spots

To remove a spot, activate the 'Spot Selection Tool', right click on the respective spot and select 'Cancel Spot' from the context menu. To remove a complete region of false positive spots, for instance at the gel image borders, you can select groups of spots by dragging a rectangle around them and cancel them together. By selecting 'Spots'→'Show Cancelled Spots' you can display the cancelled spots with dotted lines.

Filtering Noise Spots

While Delta2D usually is excellent in finding spots, some image properties such as strong image noise may cause the detection of 'false positive' or 'noise' spots. These segments do not have the typical shape when viewed in 3D, and they are generally quite faint.
You can use the 'Sensitivity' parameter in the Spot detection parameter dialog to suppress the detection of background spots. However, Delta2D also computes a 'spot quality' value for every detected spot. This value shows how closely a spot represents the 'ideal' 3D Gaussian bell shape. You can filter spots in accordance to this value like this:

1. Open the Quantitation Table by choosing 'Window' -> 'Quantitation Table'.
2. In the table, click on the tab for the fused image.
3. Locate the spot quality column, and click on the 'filter' button in the column header.
4. In the filter dialog, check the 'Filter active' check box, then use the sliders to adjust the filter settings or insert values as filter borders (a value of 0.02 works fine in many cases),
5. Press the 'Apply' button to check the effect of your filter immediately, or click 'OK' when you are done.
6. Use the Dual View to check the spots that have been filtered.

We recommend setting the filter in such a way that you see only those spots that you would like to remove. Then choose 'Edit->Select all' and 'Cancel -> Cancel Selected Spots'.

Finally, to remove the filter on the spot quality column, click on the filter button in the column header and uncheck the 'Filter active' checkbox. Now you have cancelled all spots with insufficient spot quality.

In summary we have detected spots on the fused image and since the union fusion includes all spots occurring on any of the gel images, we have a 'consensus spot pattern' for the whole project located on the fused image.

Transferring Spots

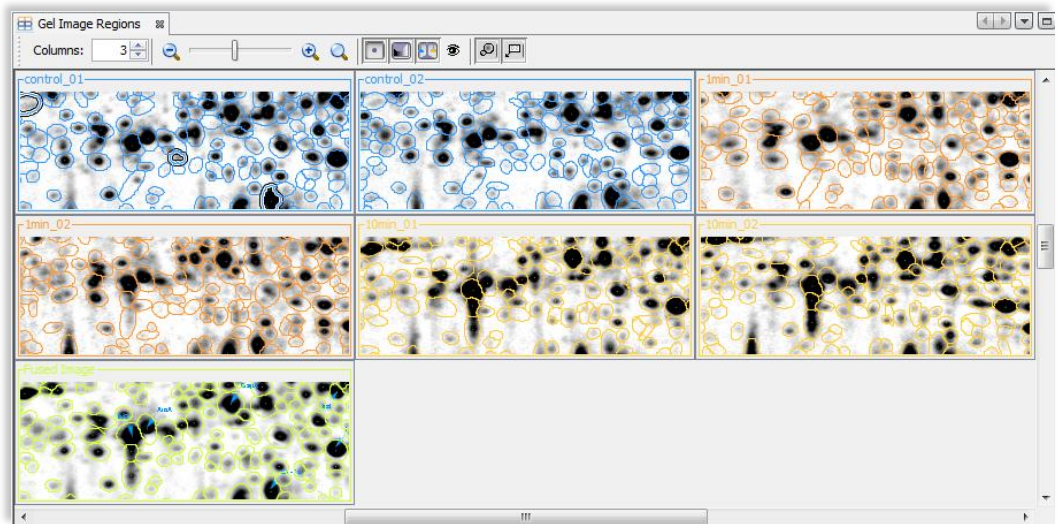
The next step towards quantitative analysis is to transfer the consensus spot pattern from the fusion image to the other gel images where the spots will be quantitated automatically.

In the step 4 'Create the Consensus Spot Pattern' in the 'Workflow' window click on the fused image 'Transfer Spots...'. The 'Transfer' dialog opens. The default settings will be that spots are transferred from the fused image to all the other images of the project. Confirm these settings with 'OK'.

Note:

If an image is not connected to any of the other images or is only connected with the 'automatic' warp mode and you have not started the Automatic to find match vectors so far, you won't be allowed to transfer spots to that image. If necessary, please check your warping strategy in the 'Warping Setup' window ('Window' -> 'Warping Setup') once again.

A progress bar indicates how spots are transferred to one image after another and quantified. Since we started with the detection result from a union-fused image we get a complete expression profile for every spot over the whole set of gel images.



Spot boundaries after spot transfer. Choose 'Window'→'Gel Image Regions' to open this view. The essential spot pattern is the same on all gels, making 100% spot matching possible.

Note:

You can choose either to transfer the boundaries as they appear on the fusion image or to adapt the size of the boundaries to the actual spots on the target images. Find this option in the Preferences tab 'Spots'.

On those images where a certain spot does not contain a signal, Delta2D will nevertheless quantitate the images in accordance to the transferred spot boundary. This will result in a spot with near-zero quantity.

You have transferred the information where you expect the spot in the single gel images. In order to see the quantitative expression profiles choose the menu entry 'Window' -> 'Quantitation Table'. This will open the Quantitation Table.

Before looking at the table in more detail let us have a quick look at some more visual means for the analysis of expression profiles.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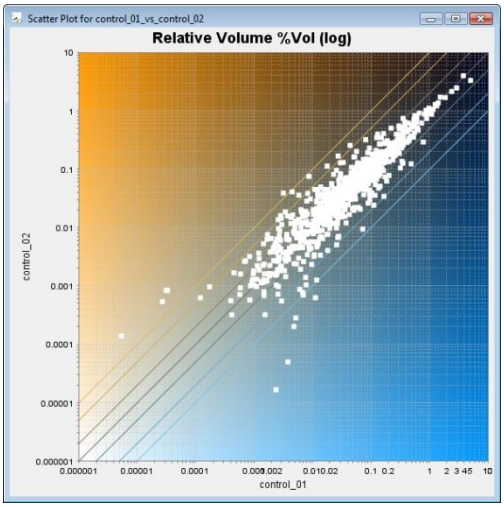
Note:
For getting a qualitative impression of differences between two images only, simply open the Dual View for these images.

Finding Interesting Spots in a Scatter Plot



Scatterplot

For finding interesting spots you can compare their expression levels in two images. In the spots menu of the Dual View please click on 'Spots' > 'Show Scatter Plot' to open a graphical representation of the spots of these two gel images. The position of a spot is determined by its normalized volume on each image: in this example, the x-position by its quantity in Control_01 and the y-position by its quantity in the 1min_01 image. Thus, a spot having an unchanged volume on both images appears on the 45 degree line of this graph, whereas induced spots are found in the upper left and reduced spots in the lower right part of this graph. Now just click on one of the spots in the top or bottom of the Scatter Plot and keep an eye on the Dual View. The selected spot shape will be highlighted in the Dual View, and the view will scroll to the selected spot if necessary.

Identifying Interesting Spots by Expression Profiles

Expression profile rollup displaying the spot intensity throughout the whole project

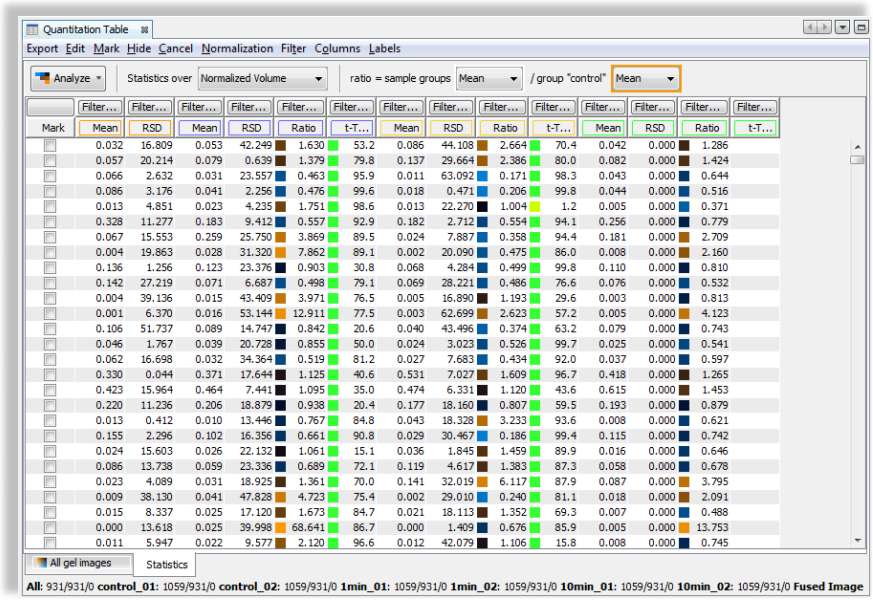
You can view an expression profile of a spot over the complete project: in the menu of the Dual View, please click on 'Rollups' > 'Expression Profiles'. A new window will open, keeping its position in front of its parent window: one of the so-called 'Rollups'. Make sure that the Spot Selection Tool is selected and simply move the mouse pointer to a certain spot. The rollup dynamically shows the expression profile for this spot across the whole project. The height of each bar is determined by the relative volume (%Vol) of the spot, i.e. the spot's intensity after background subtraction and normalization. Now move the mouse pointer to the next spot and watch how the expression profile changes.

Filtering for Interesting Expression Profiles

Switch to the 'Quantitation Table' by choosing 'Window' > 'Quantitation Table'. The quantitation table window shows spot data in three different views:

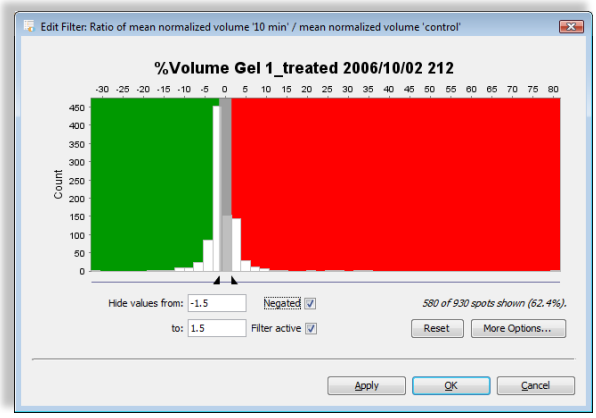
- 'All gel images' view
  Shows spot data for all images in the project showing one expression profile in every row in the table. In addition to the %Vol (relative volume) columns this table also includes Ratio columns displaying expression ratios as color codes as well as numbers. All ratio columns are calculated in accordance to a common ratio master image, to be changed in the 'Gel Image and Table Column Visibility dialog' (), if necessary. Alternatively, you can select a subset of gel images in the Light Table or in the Project Explorer (left click and keep CRTL key pressed) only and then click on the table button ().
- 'Statistics' view
  Shows relative volumes as well as averages and relative standard deviations for groups, accompanied by t-Test results with respect to the first group. You can also compute ratios such as 'mean of group 1min / mean of group control'. Statistics tables can also be created for particular groups of a project: Right-click on the respective groups in the Light Table or in the Project Explorer and choose 'Open Quantitation

Table'. Alternatively, you can select the groups (left click) and then click on the table button ( ).



Quantitation Table – 'Statistics View'

- **Single gel view**
  Shows spot data such as relative volume, area, and ID for spots on a single gel image. The spot's quantity is in the % Vol column, it is the result of background subtraction, quantitation and normalization. You can open a single gel image table by right-clicking on the respective image in the Light Table or in the Project Explorer and choosing 'Open Quantitation Table' from the context menu. Alternatively, you can select the respective image (left click) and then click on the table button ( ).



Filter Dialog

The Quantitation Table is synchronized with the other views of Delta2D: Selecting an expression profile will select the spot for example in the dual view or in the scatter plots.

Delta2D offers powerful tools to identify relevant expression profiles in accordance to your criteria. If you are e.g. looking for spots where intensities in both sample groups are increased or decreased by a factor of at least 2 relative to the control. This factor of 2 should apply to the means of the replicate groups, i.e. the mean intensity within group '1min' and '10min' should be at least two times greater or smaller than the mean intensity of group 'control'.

To see only the spots matching these criteria, follow the following steps:

1. Switch to the 'Quantitation Table' and select the 'Statistics' tab.
2. To see the name of a column either drag the line between two column headers to make it wider or simply point to its header and wait until its name appears as a tool tip. The name of the column we are looking for is 'Ratio mean % volume 1min / mean % volume control'. Click on the top part of the column header labelled 'Filter' to open the respective filter.
3. Insert the filter borders '0.5' and '2' into the fields 'Show values from ... to ...'. Alternatively, you can drag the left slider below the histogram. The box 'Filter active' will be checked automatically, additionally check the box 'Negated'. The histogram highlights the distribution of the ratio values.
4. Press OK to close the dialog. The 'Quantitation Table' will now show only those expression profiles including induced or reduced spots of a factor of at least 2.

Repeat the steps 2 - 4 above for the column 'Ratio mean % volume 10min / mean % volume control'.

Open the Dual View again with the two images 'control_01' and '1min_01'. Only spots matching our criteria will be shown there.

Advanced Statistical Analysis

Since version 3.6 Delta2D includes advanced multivariate statistics for the analysis of 2D gels, including:

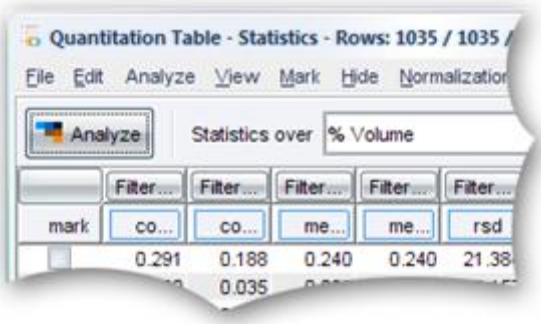
- Heat map display of expression profiles
- Various methods of clustering
- t-test variations
- Analysis of Variance (ANOVA)
- Template matching for expression profiles
- Principal Component Analysis (PCA)

Statistical analysis in Delta2D is based on the TIGR MeV (The Institute for Genomic Research (Rockville, MD, USA) Multiple Experiment Viewer) and tightly integrated into the image analysis workflow. With Delta2D's 100 Percent Spot Matching, there are no missing values. Matching problems are virtually eliminated, making it especially suitable for the methods that were originally developed for DNA microarray analysis.

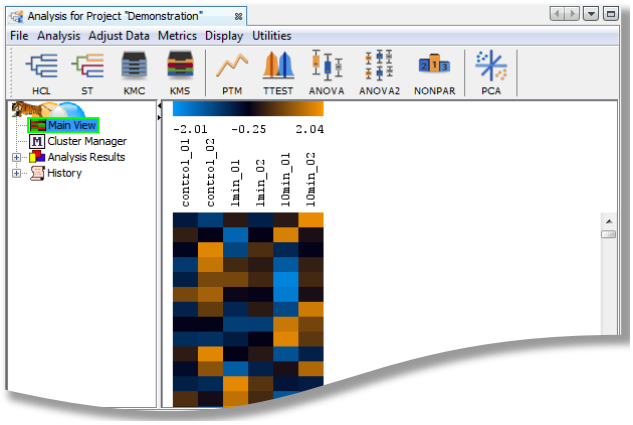
Note:

Most statistical algorithms are recommended for being applied to a minimum number of data sets while others even demand for such a minimum. The example project is too small to rely on results but still helps to understand how statistical analysis in Delta2D works.

Open the Quantitation Table (Window -> Quantitation Table) and make sure the Statistics Table is selected. Hide the quantitative data for the fused image: Switch to the 'Light Table' window and right-click on the thumbnail for the fused image. Select 'Toggle Visibility' > 'Hide Selected' from the context menu. Then switch to the Quantitation Table again: The fused image is now excluded from the view.



Getting a high level overview of expression data - heat map



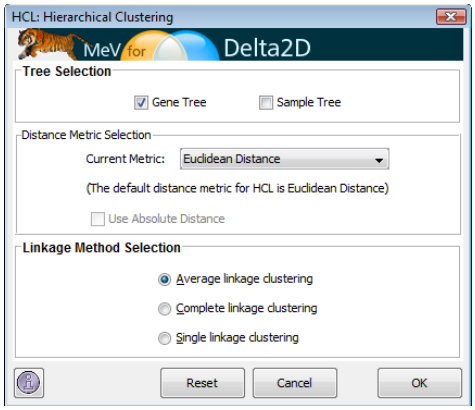
Heat map for the example project.

Press the 'Analyze' button in the top left of the statistics table. A new analysis window is opened, containing the current expression profiles in a heat map display.

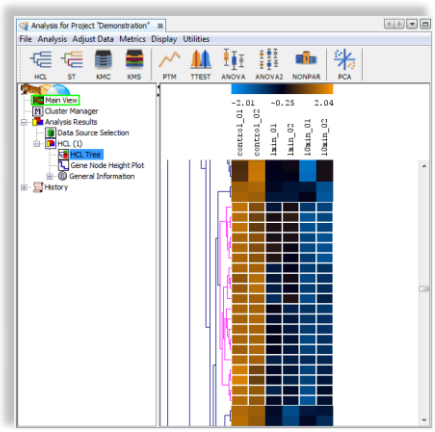
The legend across the top shows the color code for spot intensities. Rows are labeled based on the spot labels from the gel images. Data is normalized / standardized by default and sorted as in the statistics table before being shown in the heat map.

Discovering patterns in expression profiles

Clustering of images is a good first step in assessing the quality of the quantitative data.
Employ 'hierarchical clustering' to show more structure in the data: Press the HCL button in the toolbar. Choose 'Gene Tree', 'Euclidian Distance' as metric and 'Complete Linkage'. To confirm press OK. The hierarchical clustering now groups expression profiles in accordance with their similarity.



Dialog box for HCL settings.

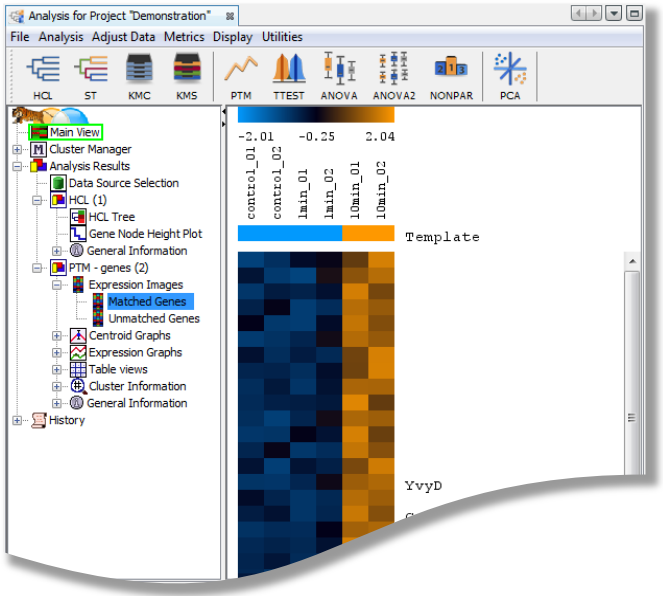


Hierarchical Clustering for Expression Profiles.

The tree represents similarities between expression profiles. Click into a subtree to select all protein spots of the same cluster. Since the statistics module is also synchronized with e.g. the 'Quantitation Table' and the Dual View, you can switch there and review the selected spots as members of the selected cluster.

If you wish to identify the structure of your experiment choose 'Sample Tree' as tree selection. All replicates of the same sample should appear in the same cluster (subtree).

Finding profiles for defined templates



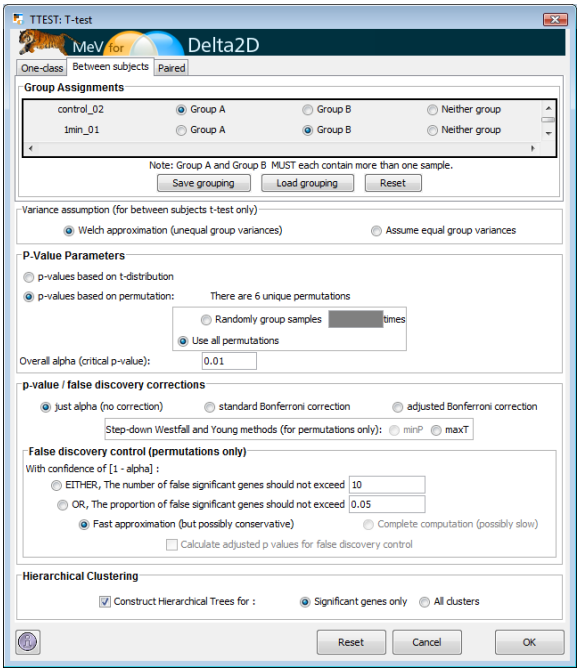
Expression Profiles matching a Template

With Template Matching, you can define a template for an expression profile and let Delta2D find spots whose expression profiles match the template.

Click the 'PTM' button to open the dialog box. The lower part of the dialog contains a series of sliders. Move them roughly to different positions to define a sample expression profile that shall serve as a template and confirm it with 'OK'.

TMeV displays a new Heatmap containing the defined template and a list of expression profiles that match this template.

Finding differentially expressed proteins: Statistical Tests



t-Test parameters.

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

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

Note:

There is a lot more to know about statistical analysis in Delta2D. Please read on in the Delta2D manual. You can access it through the Delta2D help menu or as a PDF file located in the installation directory. In Windows, there is a direct link to the manual in the Start menu. The manual is also available for download and for online browsing!
Or contact us at support@decodon.com.

Reporting and Exporting Results

Raw data and analysis results from Delta2D can be exported in various formats:

- Tables can be exported as 'Character Separated Values' (CSV) files or directly as table or report to Excel (if this software is installed on your computer).
- Image views can be exported as standard image files or directly to Microsoft PowerPoint.
- Interactive reports can be created in html format.
- Picklists can be exported for a variety of pickers in the respective picking formats or directly into the Bruker Proteineer database.

Note:

Reports and picklists include data for expression profiles or spots that have been 'marked'. You can mark spots by selecting them and the choosing 'Mark' > 'Mark selected spots' in the Quantitation Table or in the Dual View by right click and clicking on 'Mark spot'.

Export Tables to Excel

When exporting a table, you always export exactly what you see, i.e. the visible columns with the visible expression profiles only. Thus you can use the powerful filters of Delta2D to select which data you want to export.

Open the Quantitation Table by choosing 'Window' > 'Quantitation Table'. If you want to you can define the filters as described above. Now choose from the table's menu 'Export' > 'Export to Excel'. This will directly open an Excel worksheet containing your data.

Export Image Views into PowerPoint

Creating PowerPoint presentation slides also takes a few clicks only.

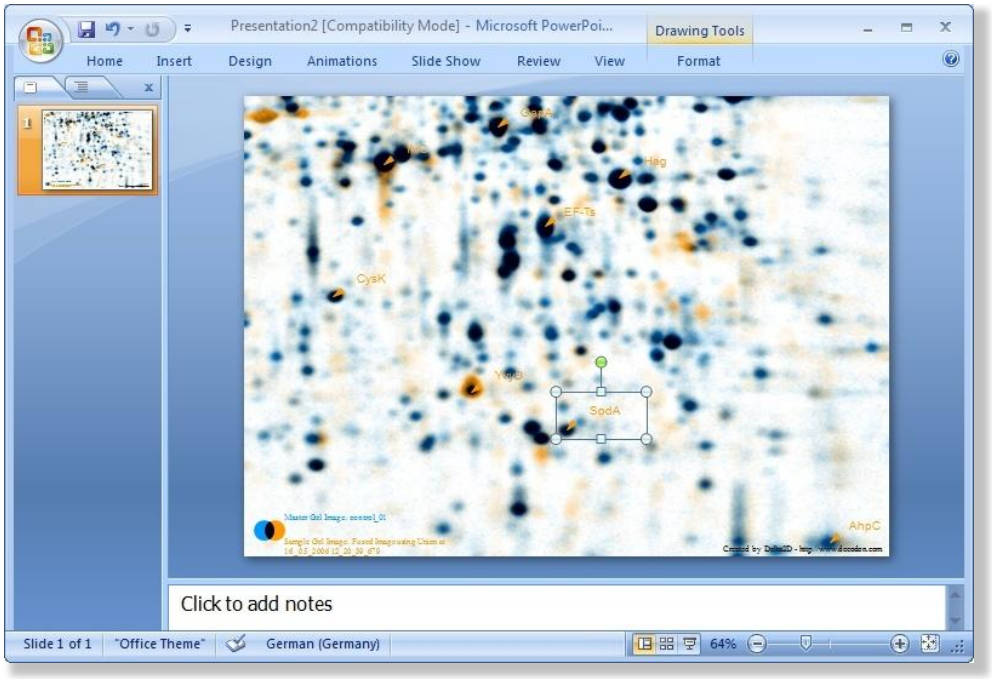
Open the Dual View for a gel image pair. Zoom in or out and control the visibility of objects such as match vectors, spot boundaries and labels (open the rollup 'Overlays' and toggle visibility by clicking the respective buttons). Choose 'Export' > 'Export to PowerPoint' from the menu. Check both boxes in the next dialog and confirm with 'OK'.

Follow the instructions on the screen in PowerPoint to create the PowerPoint presentation as a slide. Every object visible in the Dual View will be a fully modifiable object in the resulting PowerPoint presentation: You can delete unimportant spot boundaries, highlight

labels, spots and other objects of interest by changing their color or font size and design the presentation making use of the whole set of capabilities provided by PowerPoint.

Note:

Macros have to be enabled in Excel and PowerPoint to export the data. I.e. macro security settings need to changed to 'Medium' or lower.



A typical PowerPoint export. Each object can be customized by using PowerPoint properties and formats.

Create interactive HTML-Reports

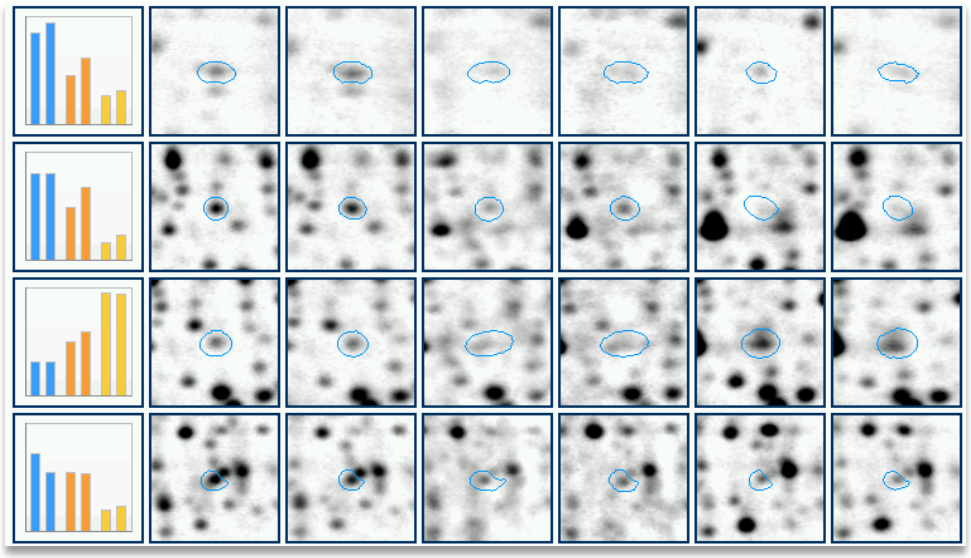
Typically the results of an experiment shall be reported to colleagues or published on a website or a scientific paper. To facilitate extracting the relevant information, Delta2D provides reports.

In the 'Reports' menu you can select from the list of available reports. Delta2D provides three standard reports:

 Project Summary  Spot Album  Spot Quantities

The 'Project Summary' report includes global information that is available for samples, groups, gels, gel images (including the images) and the warping strategy (including the respective dual channel images). Click on a dual channel image to let Delta2D open the respective Dual View window.

Note:
You can save the reports using the 'Save' button provided within the report. You have to switch to Delta2D where a dialog opens that allows for selecting the location of the saved report. When using the 'File' -> 'Save page as ... ' function of your browser you will not receive the full content of the reports.



Spot album report

The 'Spot Album' and the 'Spot Quantities' reports include detailed information about expression profiles for marked spots and allow to define parameters to change the

reports' content. Click on the barchart or the spot ID to select the respective expression profile in Delta2D and to receive a detailed report for it.

Exporting picklists

With Delta2D you can export picklists for a variety of available spot picking devices. If your device is not yet supported please contact the DECODON support team and we will try to add it to the set of supported devices.

Picklists include marked spots and the coordinates of all spot labels on the respective gel image. Thus, you can export picklists for an image even if no spots have been detected: simply label the spots of interest (possibly after activating the auto numbering feature for labelling) to include their coordinates in the picklist.

Where Can I Find Out More about Delta2D?

The easiest way to learn more is going to http://www.delta2d.com or contacting us per Email to support@decodon.com. We will be happy to answer your questions or to show you more in a live web demo.

Note:

If you need more detailed information about Delta2D, please consult the manual of Delta2D. You can access it through the Delta2D help menu or as a PDF file located in the installation directory. In Windows, there is a direct link to the manual in the Start menu. The manual is also available on our website for download and for online browsing!

Your DECODON Support Team

If you have any comment or suggestion regarding this Getting Started Guide or any other of our documents we would be happy to hear from you.

Notes:

More Notes:

Delta2D

Request your 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 image file formats on the market today, including TIFF (8 bit, 12 bit, 16 bit), IMG (Fuji), GEL (Amersham), JPEG, BMP, GIF, PNG, PNM.

Supported Protein Labelings and Stainings
Delta2D supports virtually all protein labelings and stainings, including Silver, Coomassie, Sypro Ruby, Cy Dyes, radioactive labelings etc.

Supported Spot Picking Devices
Delta2D supports spot pickers from Molecular Dynamics, Genomic Solutions, Bruker, Amersham and others. Please contact DECODON for details.

Supported Operating Systems
Delta2D runs on Windows NT/2000/XP/Vista, Mac OS X 10.2.6 (Jaguar) and 10.3 (Panther), and Linux

Hardware Requirements
Minimum Hardware: Pentium III, 800 MHz, 512 MB RAM or Power Mac G4, 512 MB
Recommended Hardware Pentium IV, 1 GHz or better, 1 GB RAM or Power Mac G5, 1 GB

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