

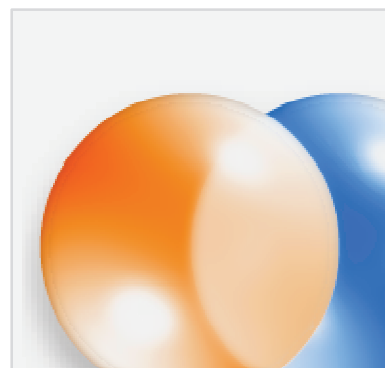
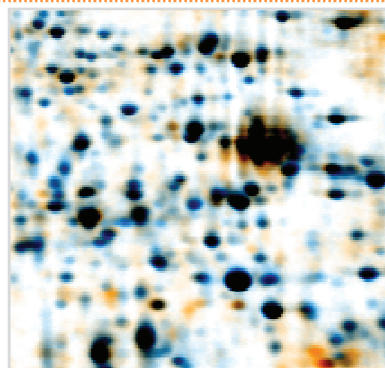
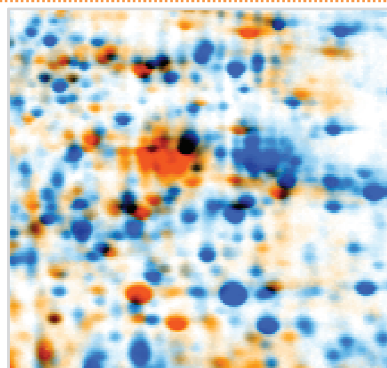
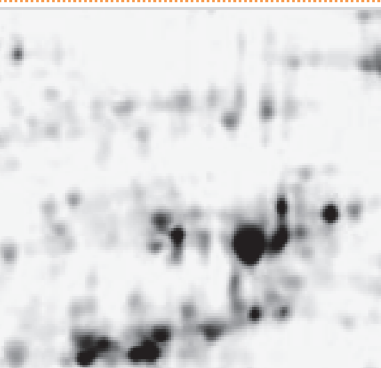
# Getting started

English



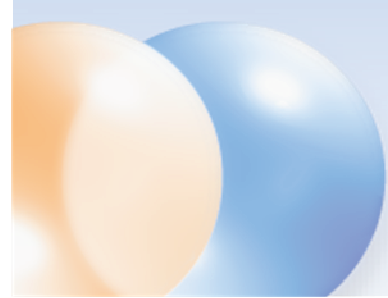
## Delta2D

ANALYZING 2D GELS  
AS EASY AS POINT AND CLICK



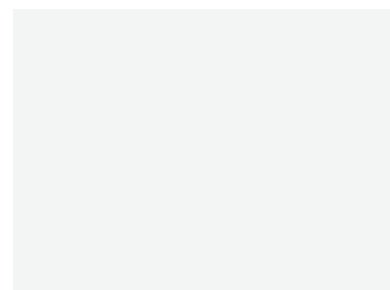
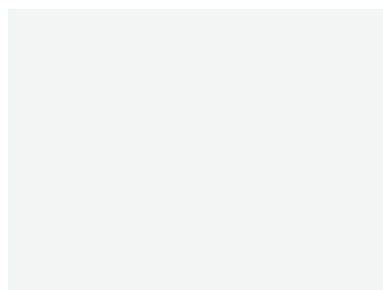
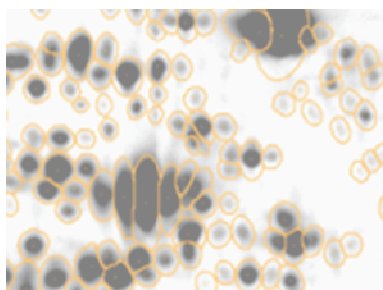


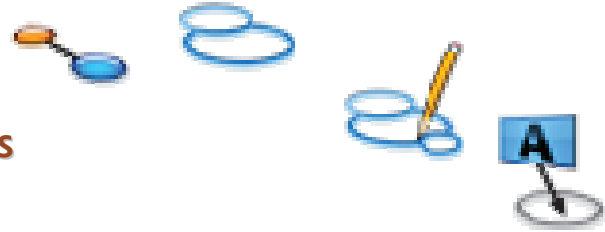
# Getting started



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## Analyzing Gel Images in just 5 Steps

### 1 Setup Project

Setup traditional or multiplex experiments such as Refraction-2D or DIGE. Import your gel images into Delta2D and arrange replicates in groups.

### 2 Warp Images

Setup a warping strategy, use Delta2D's automatic warping, and review warpings.

### 3 Detect and Quantify Spots

Do consistent spot detection across the whole project.

### 4 Analyze Expression Profiles

Find interesting spots.

### 5 Present Results

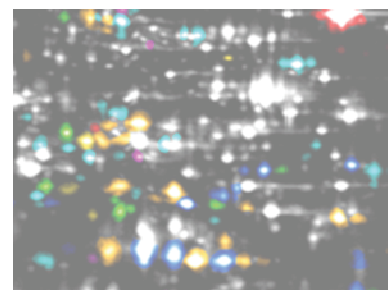
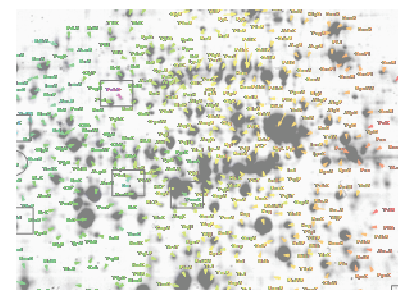
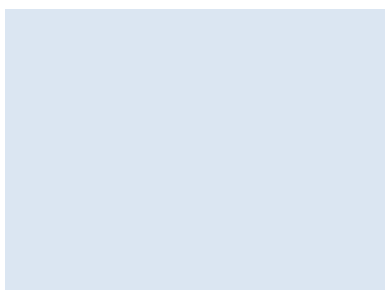
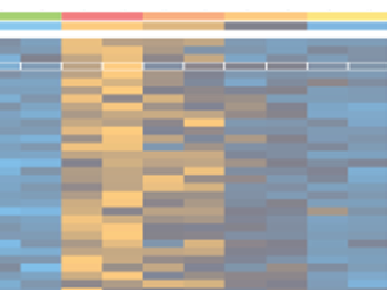
Export results to other applications for presentation or further analysis.

#### Note:

If you have not already done so, please download the example data that we use in this guide at [www.decodon.com/delta2d-getting-started.html](http://www.decodon.com/delta2d-getting-started.html). Unpack the archive. It contains the following gel images and match map files for the warping:

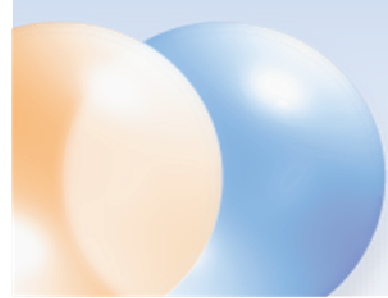
Image file	Dye	Gel	Sample	Group
Gel1_Cy2.gel	Cy 2	Gel 1	Internal standard, Gel 1	Standard
Gel1_Cy3.gel	Cy 3	Gel 1	Sample A, replicate 1	Sample A
Gel1_Cy5.gel	Cy 5	Gel 1	Sample B, replicate 1	Sample B
Gel2_Cy2.gel	Cy 2	Gel 2	Internal standard, Gel 2	Standard
Gel2_Cy3.gel	Cy 3	Gel 2	Sample A, replicate 2	Sample A
Gel2_Cy5.gel	Cy 5	Gel 2	Sample B, replicate 2	Sample B
Gel3_Cy2.gel	Cy 2	Gel 3	Internal standard, Gel 3	Standard
Gel3_Cy3.gel	Cy 3	Gel 3	Sample A, replicate 3	Sample A
Gel3_Cy5.gel	Cy 5	Gel 3	Sample B, replicate 3	Sample B

We thank Dr. Maria Zellner and Prof. Dr. Rudolf Oehler (Medical University of Vienna) for the images.





# Getting started



## 1 Setup Project

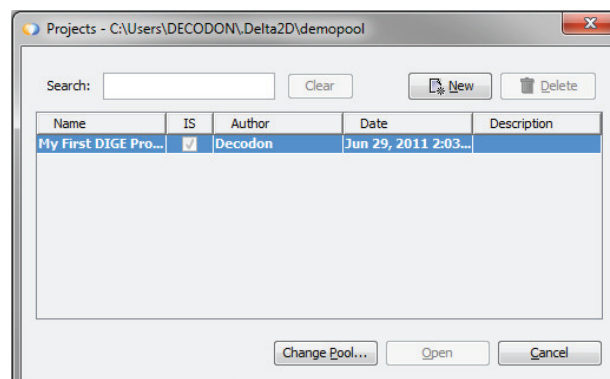
### Create a new data pool

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 to *rename* it. Type in a name for your new pool.
3. Click on  and confirm the following dialog with .

### Create and open a new project

The 'Projects' dialog appears again showing an empty list of available projects.

1. Click on the  button to create a new project.
2. Provide at least a name for your new project and activate 'Use Internal Standard'. Confirm with .
3. Select the new project from the list and press .

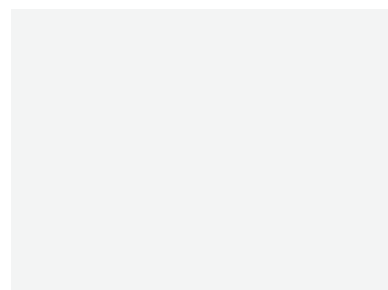
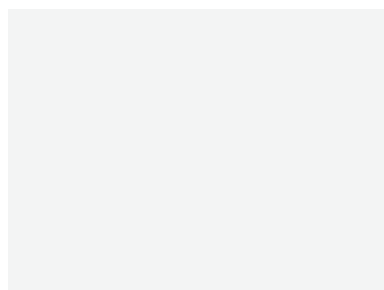
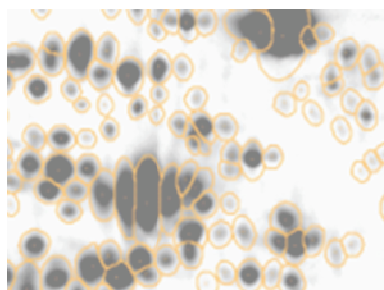


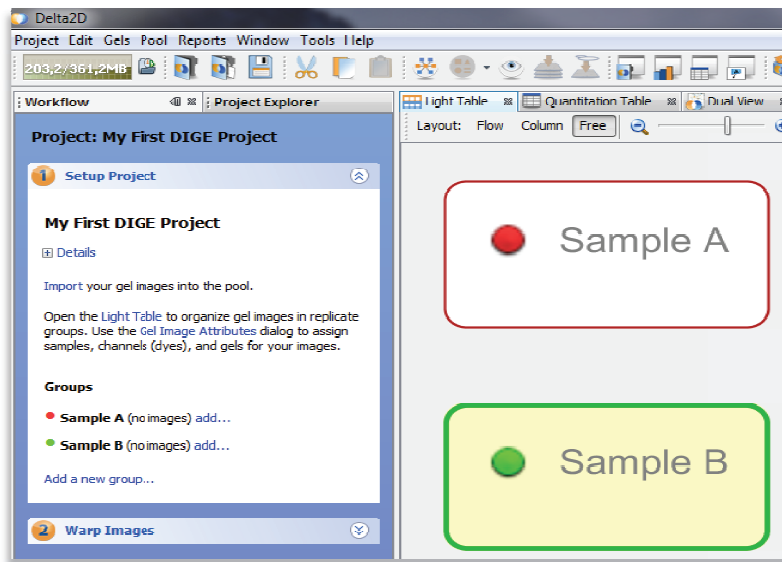
Delta2D's Projects dialog.

### Set up groups for the gel images

We will now create the *three groups* for our experiment - one for each of the two samples and one for the internal standard. Let's first of all use the two groups already present – as you should see in the Light Table:

1. Right-click on the first group and choose 'Properties' from the context menu.
2. Replace the group's name by '*Sample A*'. If you want, choose another color and confirm with .
3. Repeat steps 1 and 2 for the second group assigning the name '*Sample B*'.

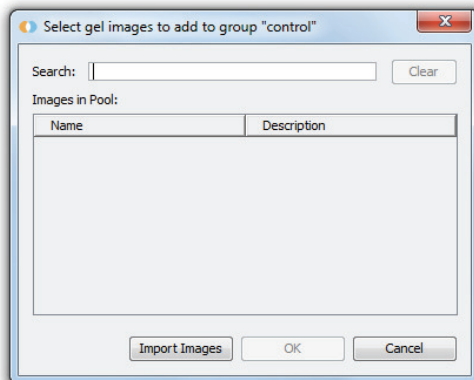




Delta2D's Workflow and Light Table - showing an empty project.

Create a new group '*Internal Standard*', by clicking on the link 'Add a new group...' in the Workflow.

## Add gel images to your groups

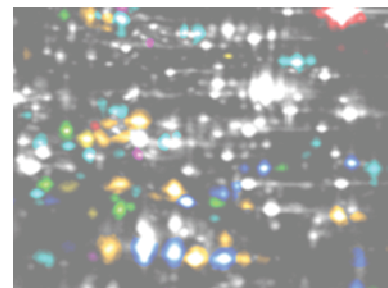
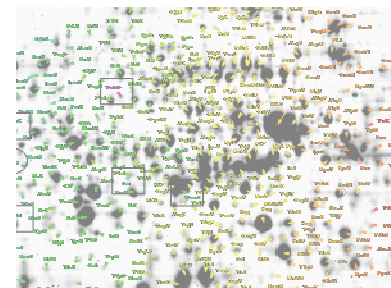
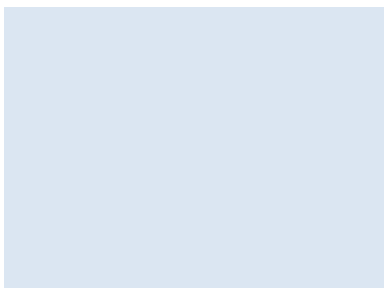
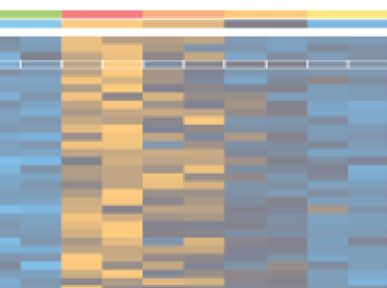


Delta2D's Gel Import Manager.

1. In the Workflow click on the 'Add...' link next to the **Sample A** group.
2. Click on **Import Images** and select the three images (Gel1\_Cy3, Gel2\_Cy3, Gel3\_Cy3) from the *example data* by using the left mouse button in combination with the **CTRL**-Key. Click on **Next**.
3. Now you can edit name and other descriptive data, and do some basic manipulations such as rotating, inverting or de-speckling your image. Click on **Next** again to repeat this for the next image.

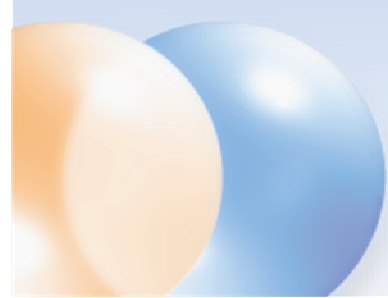
4. Click on **Finish** to save assignments and close the dialog.

Please import the sample images Gel1\_Cy5, Gel2\_Cy5, Gel3\_Cy5 as well as Gel1\_Cy2, Gel2\_Cy2, Gel3\_Cy2 into their respective groups.




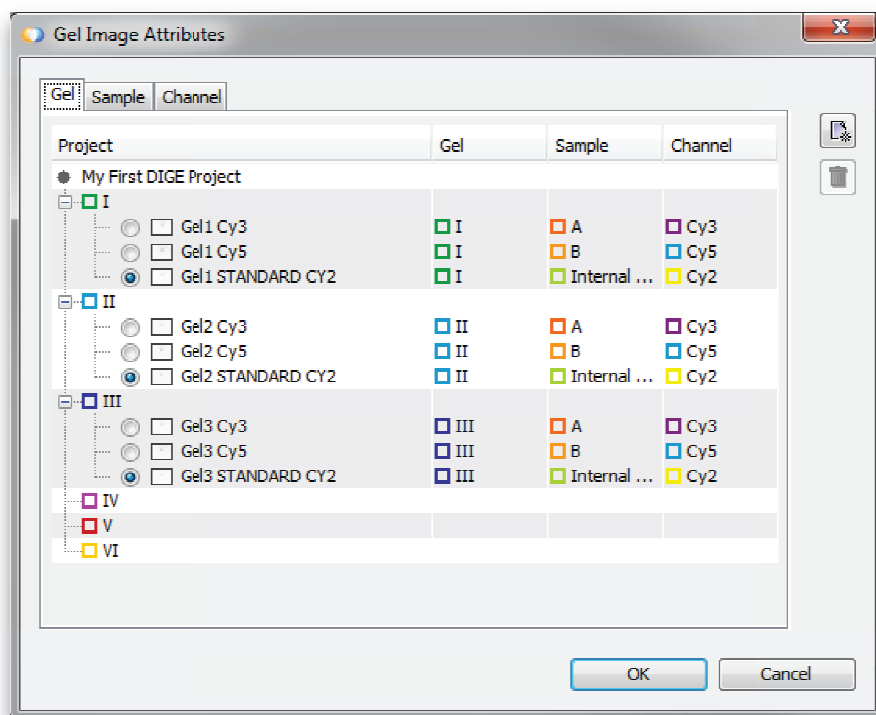


# Getting started

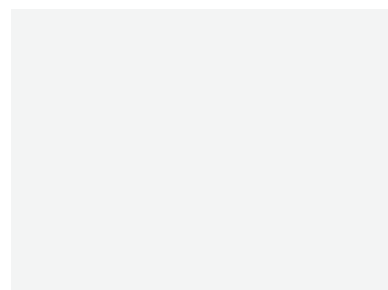
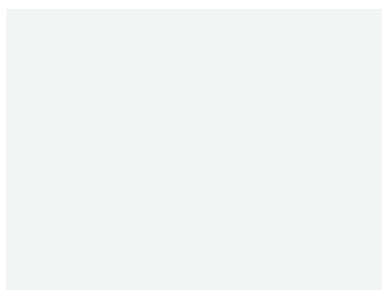
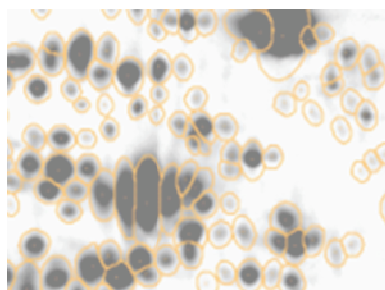


## Assign images to gels and define internal standards

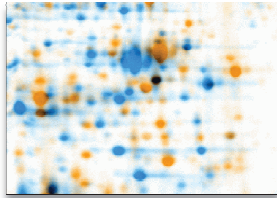
1. In 'Setup Project' choose 'Gel Image Attributes'. Select the 'Gel' tab. You see a list of gels and the images that come from each gel. Rename the existing sample gel names or click on  to add new entries. Use drag and drop to assign images to gels.
2. For each gel, you see a column of radio buttons. Select the radio button next to the image that contains the internal standard (usually, this will be the Cy2 image).



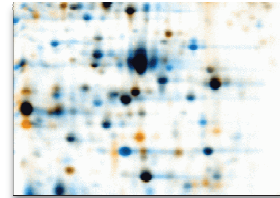
The Gel Image Attributes dialog showing assigned gel images and identified internal standard images (activated radio button).



## 2 Warp Images



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

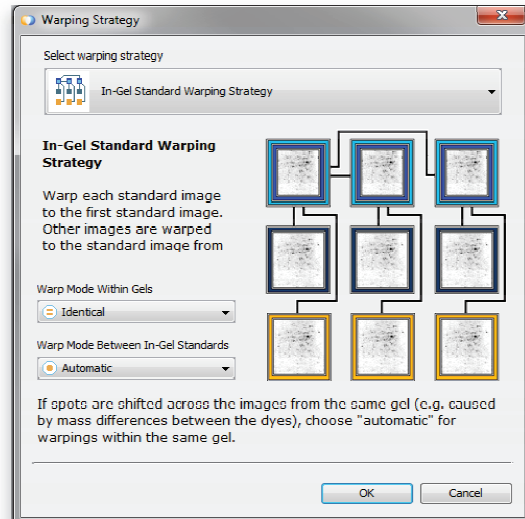


Two gel images, overlaid into a dual channel image, after warping. Differences in expression levels are clearly visible.

### Define a warping strategy

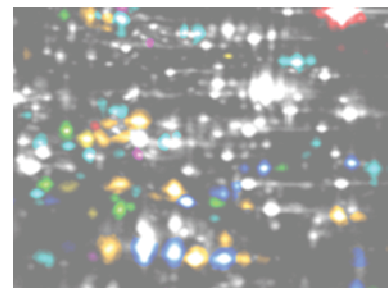
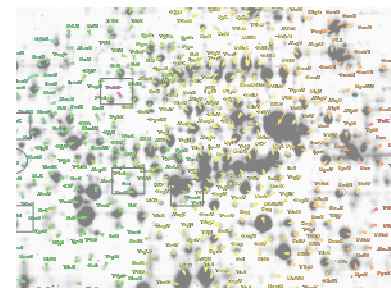
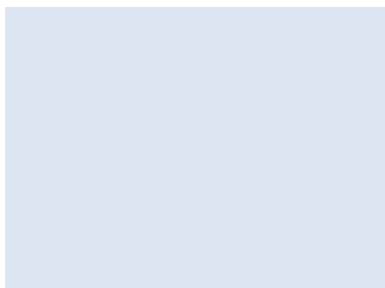
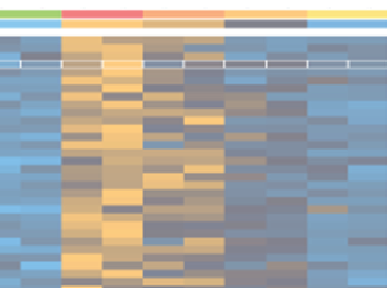
1. Switch to the second step of the 'Workflow' called 'Warp Images'.
2. Click on the 'Warp Strategy...' link to open the 'Warping Strategy Manager'.
3. Select the 'In-Gel Standard Warping Strategy' and confirm the default settings with

OK .



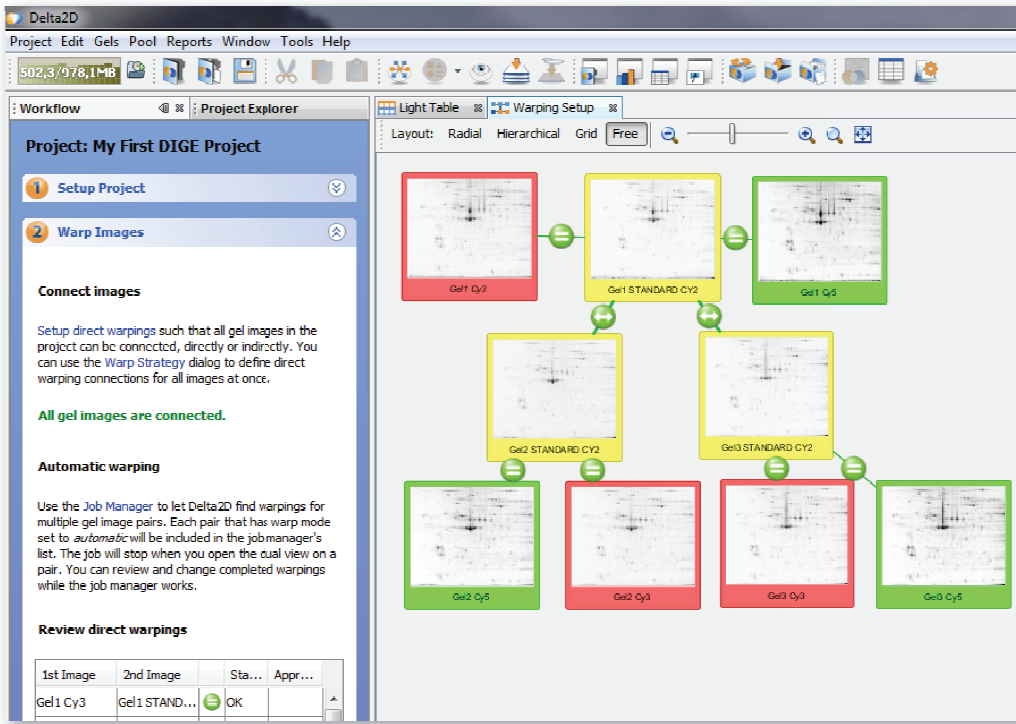
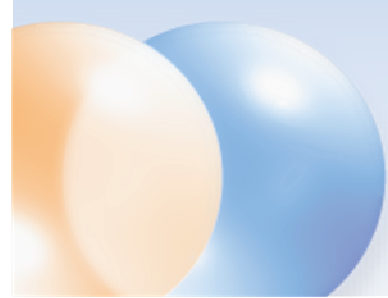
Delta2D's Warping Strategy Manager.

4. Open the 'Warping Setup' by clicking on the link 'Setup direct warpings' in the Workflow. It should look similar to the following image:






# Getting started








Warping Setup window after applying the In Gel Standard Warping Strategy.



## Use Delta2D's Automatic Warping

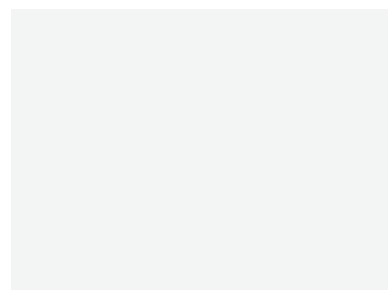
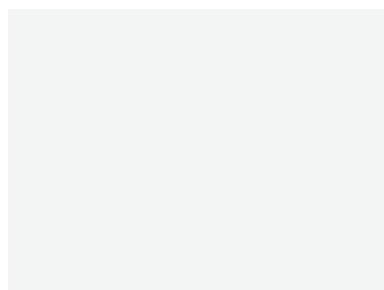
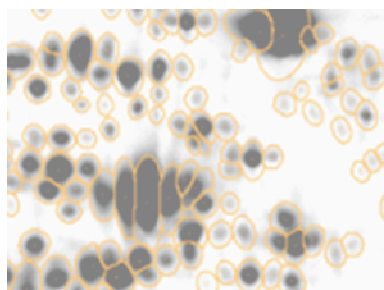
1. Click on the 'Job Manager' link.
2. Just click the 'Start' button  in the 'Job Manager'. It will monitor and perform all automatic warping jobs.

## Review Warpings

Review direct warpings				
1st Image	2nd Image	Status	Approve	
Gel1 Cy3	Gel1 STANDARD CY2	 OK		
Gel1 Cy5	Gel1 STANDARD CY2	 OK		
Gel2 Cy3	Gel2 STANDARD CY2	 OK		
Gel2 Cy5	Gel2 STANDARD CY2	 OK		
Gel1 STANDARD CY2	Gel2 STANDARD CY2	 OK		

Review direct warpings (Step 2 of the Workflow).

1. Double-click on a row in the table below 'Review Direct Warpings' where the symbol looks like .
2. A 'Dual View' window opens and shows the overlay of the respective two images.
3. Please also check if the image background has been hidden by using the 'Show/Hide Background' button () in the tool bar.








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**Note:**

To work with match vectors, select the Match Vector Tool (top-most button in the left vertical tool panel of the Dual View).

To ease the use you might want to switch on "Snap Match Vectors To Spots". This can be done at 'Tools' > 'Options' > 'Delta2D' > 'Match Vectors'. Temporarily switch it off with CTRL key.

---

If there are regions *where vectors seem to be incorrect*, you can set and change all match vectors manually:


- **Select a vector** – just left-click on the vector.
- **Select multiple vectors** – drag a rectangle with the mouse (keep the left mouse button pressed). All vectors in that rectangle will be selected.
- **Delete match vectors** – right click on a vector and select 'Delete' or 'Delete Selected'.
- **Set a new match vector** – first click on the spot in the orange, then on the corresponding spot on the blue image.
- **Change a match vector** – drag one end of the vector to change its position.

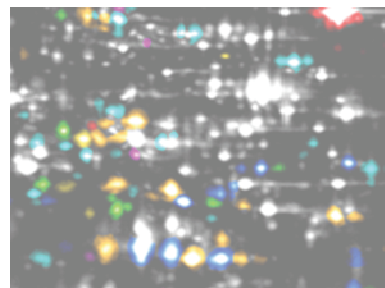
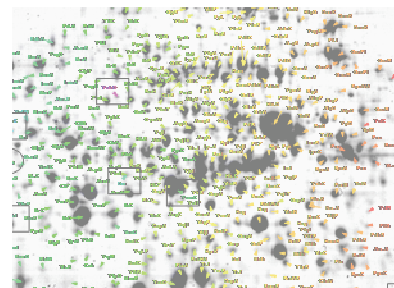
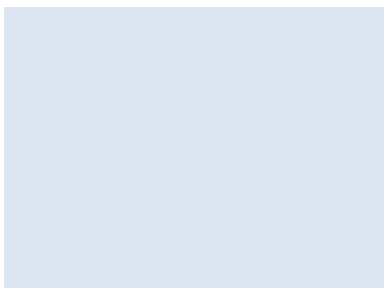
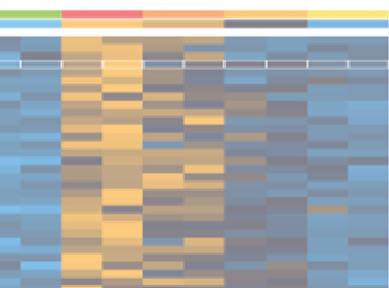
---

**Note:**

You can undo match vector operations by clicking the 'Undo' button .

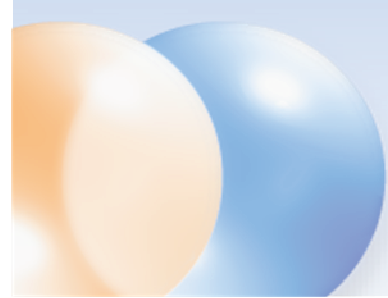
---

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





# Getting started



## 3 Detect and Quantify Spots

### Generate a fusion image

1. Switch to the workflow step called '**Detect and Quantify Spots**' and click on the '**Fuse all images**' link to open the '**Image Fusion**' dialog.
2. Exclude the standard images (typically Cy2) from the fusion.
3. Click on  to use the default settings and let Delta2D create a new image.

### Detect spots on the fusion image

1. Click on the link '**Detect Spots on Fused Image...**' in the Workflow.
2. In the next dialog, accept the proposed detection parameters by pressing . After detection is finished, the fused image will appear with detected spot boundaries.



### Edit spots on the fusion

1. Open the new fusion image by clicking on '**Open Fused Image using Union...**'.
2. A '**Dual View**' window opens showing the single view of the fused image only.

#### Note:

Select the '**Spot Editing Tool**' (fourth button in the left vertical tool panel in the Dual View) to edit spots.

We strongly recommend to edit spots **ON THE FUSION IMAGE ONLY**.

#### Adding a new Spot

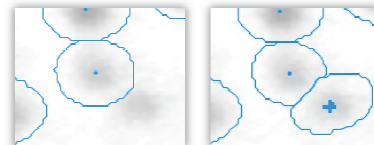
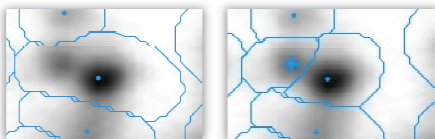


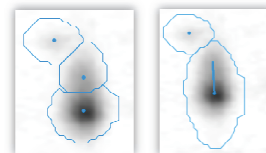
Image region before / after adding a spot. (Just click on the center of an undetected spot to add it.)

#### Splitting a Spot

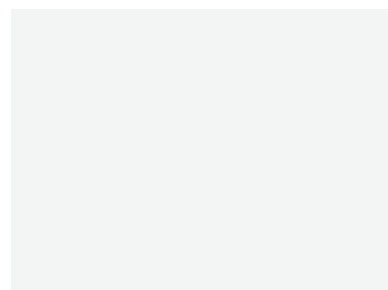
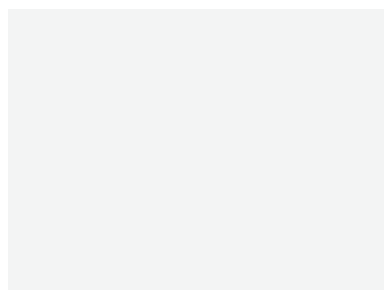
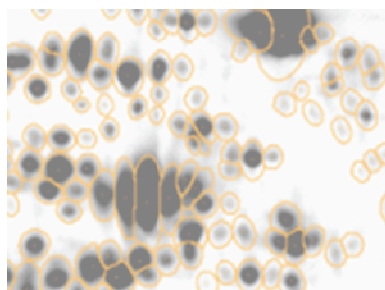


Two spots, detected as one and split. (Click once into the center of the spot that was not detected separately).

#### Joining Spots



Example for joined spots. (Drag a line from one spot center to the other.)



## Move Spot Markers

Spot markers can be moved by dragging with the left mouse button.

## Delete Spot Markers

To delete a spot marker you can *right-click* on it.



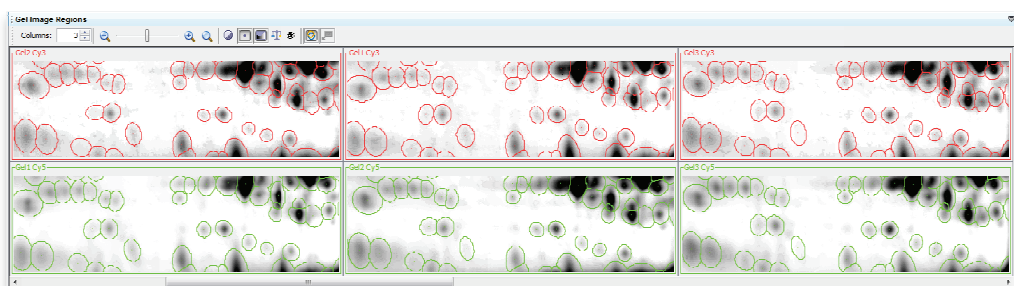
## Remove Spots

1. Activate the '**Spot Selection Tool**' and select one spot or a group of spots.
2. Right-click and select '**Cancel Spot**' / '**Cancel Selected Spots**'.

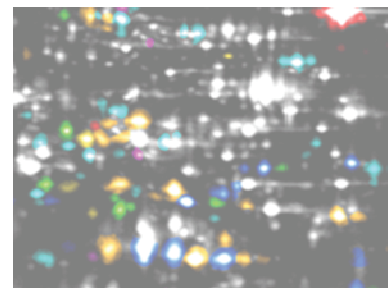
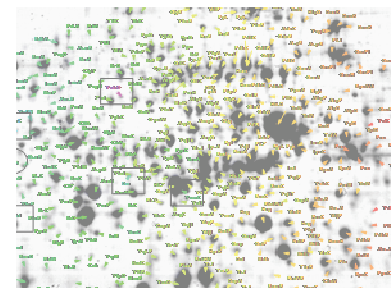
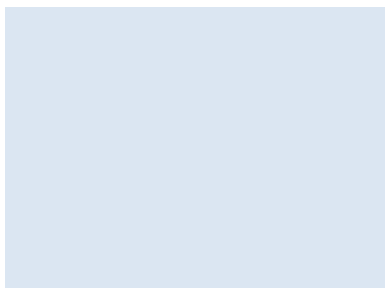
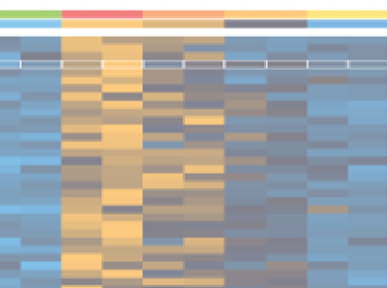
To see all canceled spots with dotted line spot boundaries, select '**Spots**' > '**Show Canceled Spots**' from the Dual View's menu.

## Transfer Spots

1. In the Workflow step '**Detect and Quantify Spots**' click on the link '**Transfer Spots from Fused Image...**'.
2. The '**Transfer Spots**' dialog opens. Confirm the default settings with .



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





# Getting started

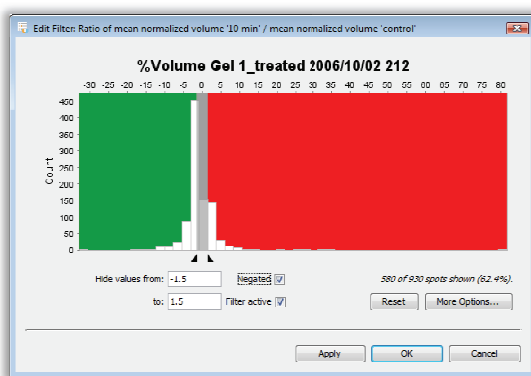


## 4 Analyze Expression Profiles

### Filtering for Interesting Expression Profiles

Note:

The Quantitation Table is synchronized with all other views of Delta2D: Selecting an expression profile in the table will select the spot e. g. in the Dual View.



Filter Dialog

Let's find two-fold up- or downregulated spots, respectively:

1. Open the 'Quantitation Table' and switch to the 'Statistics' tab.
2. Look for the ratio column in the *Sample B* group '*Ratio mean % volume Sample B / mean % volume Sample A*'.
3. Click on the top part of the column header labeled 'Filter'.
4. Insert the filter borders '**Show values from**' '0.5' and '**to**' '2'.

5. The 'Filter active' will be checked automatically, additionally check the box 'Negated'. The latter will change the filter dialog's text to '**Hide values from**' '0.5' and '**to**' '2'. Confirm with .
6. Open the 'Dual View' with the two images Gel1\_Cy3' and 'Gel1\_Cy5'. Only spots meeting our criteria will still have spot boundaries.

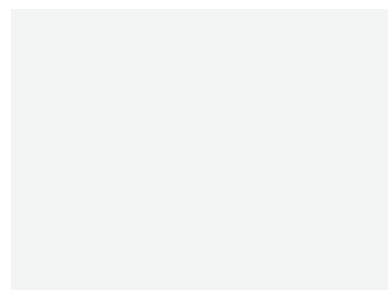
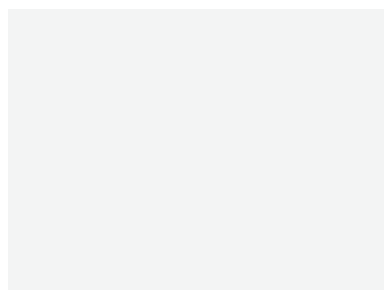
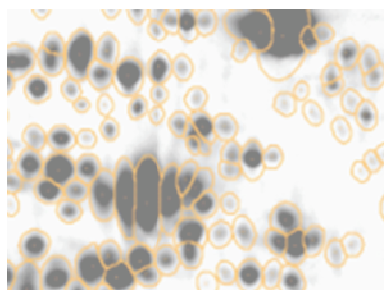
### Advanced Statistical Analysis

Delta2D includes advanced multivariate statistics for the analysis of 2D gels, among them:

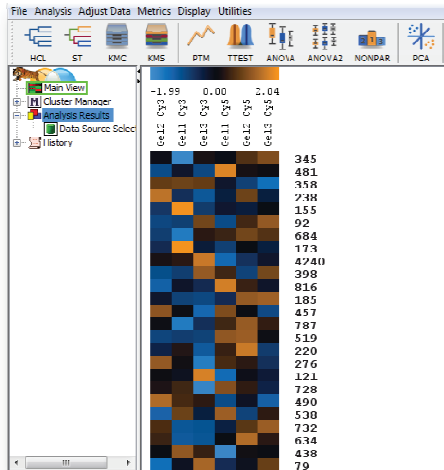
- Heat map display of expression profiles
- Various methods of clustering including Hierarchical Clustering, k-means / medians Clustering
- Numerous variations of t-Test
- Analysis of Variance (ANOVA)
- Non-Parametric tests, incl. Wilcoxon / Mann-Whitney, Kruskal-Wallis, Mack-Skillings, and Fisher-Exact test
- Pavlidis Template Matching for expression profiles and gel images (PTM)
- Principal Component Analysis (PCA)

Note:

The example project is actually too small to rely on results but still helps to understand how statistical analysis in Delta2D works.



## Getting a high level overview of expression data - heat map




Heat map for the example project.

In the workflow step 'Analyze Expression Profiles' click the link 'Analysis'.

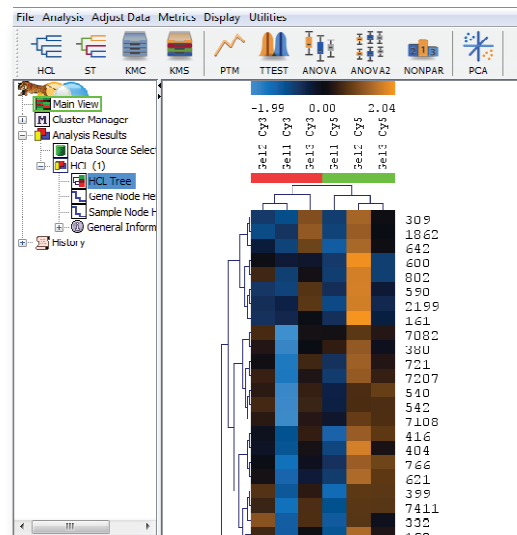
The *legend* across the top shows the color code for spot intensities. Each column contains all spot intensities of one gel image. Each row shows the intensities of one spot on all the different images in your project. Data is normalized / standardized by default.

## Discovering patterns in expression profiles

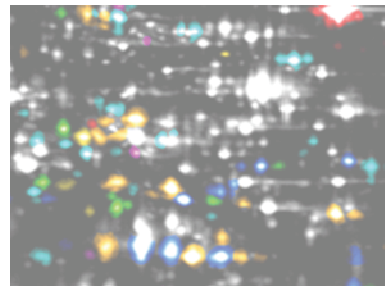
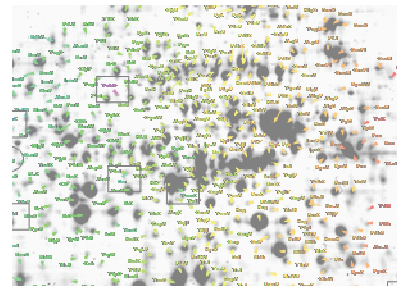
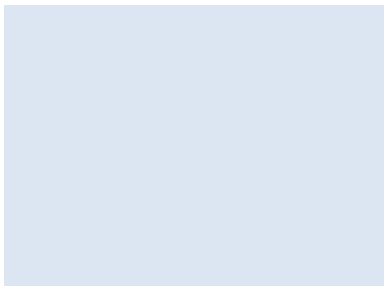
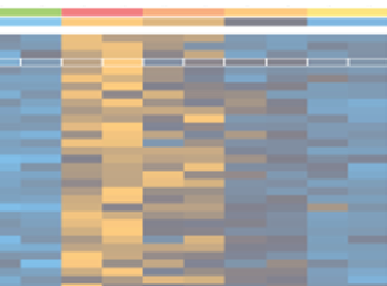
1. Press the  button in the toolbar. Choose 'Gene Tree', 'Euclidian Distance' and 'Complete Linkage'.
2. To confirm press .

The tree represents similarities between expression profiles.

If you do this for the 'Sample Tree', all replicates of the same sample should appear in the same cluster (subtree). If not, this indicates that there are outliers in your project - due to e.g. experimental problems the gel is very different from the other replicates. You should consider removing this gel image from your project (as you have hidden the fused image, open the Analysis again then).

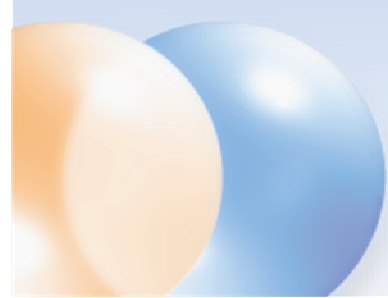


Hierarchical Clustering for Expression Profiles.

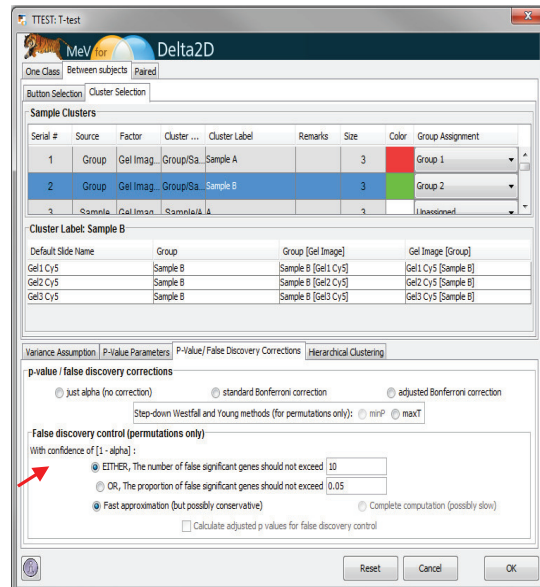
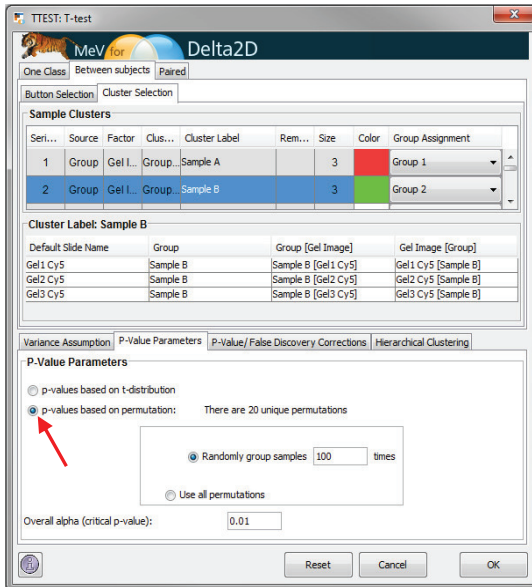






# Getting started



## Finding differentially expressed proteins: Statistical Tests

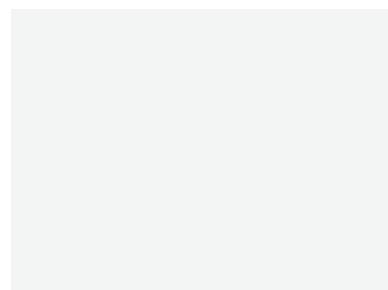
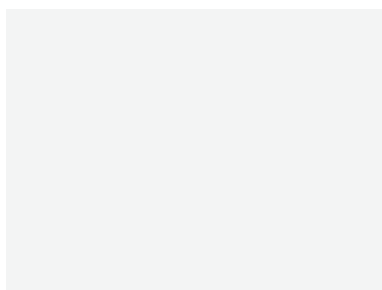
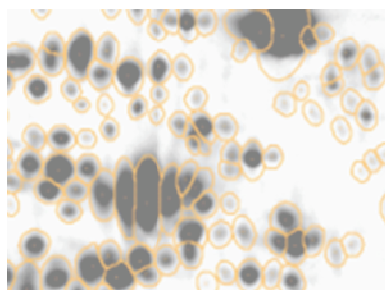


t-Test parameters.

1. Click on the  button.
2. Select 'p-values based on permutations' (first red arrow).
3. In the 'False discovery control' part of the dialog define limits 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' (second red arrow).
4. Confirm with  to see a heatmap display of significant spots only.

### Note:

There is a lot more to know about statistical analysis in Delta2D. Please read on in the Delta2D and the TMeV manual or contact us at [support@decodon.com](mailto:support@decodon.com) for a free live web session.



## 5 Present Results

### Create interactive HTML-Reports

Choose from one of the following reports from the 'Reports' menu:



Project  
Summary



Spot Album



Spot  
Quantities



Labels

General information about samples, groups, gel images, etc.

Detailed information for selected / marked spots. Click on the *barchart / id* to select the spot in Delta2D and open a detailed report for it.

List all labels or those for selected / marked spots incl. scout data.

#### Note:

To mark spots choose 'Mark' > 'Mark selected spots' in the Quantitation Table or right click and select 'Mark spot' in the Dual View.

### Export Tables to MS Excel

1. Open the 'Quantitation Table'.
2. Choose 'Export' > 'Export to Excel' from the table's menu.

### Export Image Views into MS PowerPoint

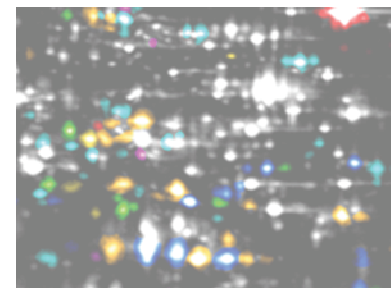
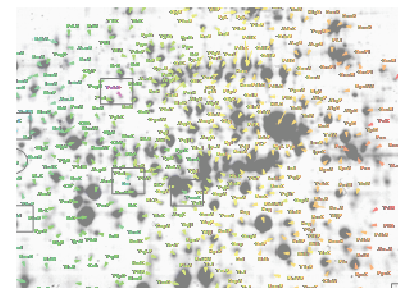
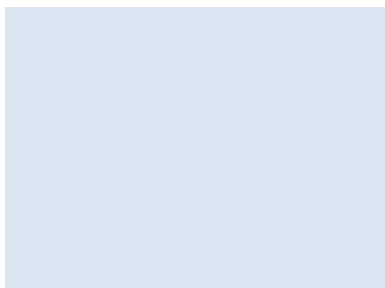
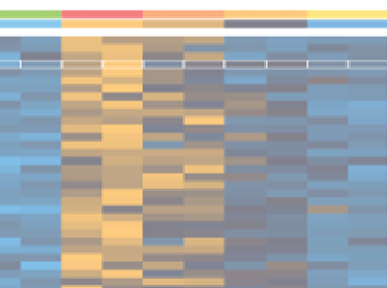
1. Open the 'Dual View' for any gel image pair.
2. Choose 'Export' > 'Export to PowerPoint' and confirm with .
3. Follow the instructions on the screen to create the MS PowerPoint slide.

#### Note:

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

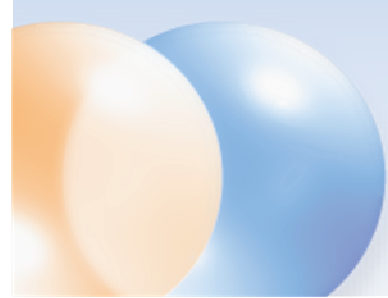
### Export picklists

1. Open the 'Dual View' for any gel image pair.
2. Choose 'Export' > 'Export Picklists', then your picking device and the gel you would like to pick spots from.
3. Confirm with .





# Getting started



## Where Can I Find Out More about Delta2D?

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

**Note:** \_\_\_\_\_

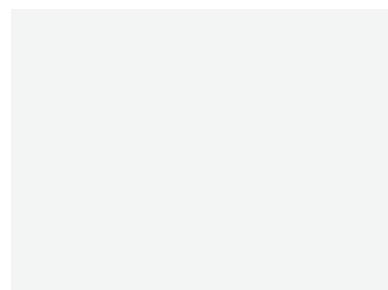
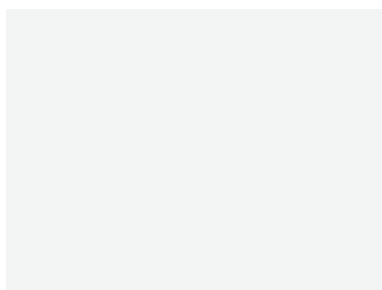
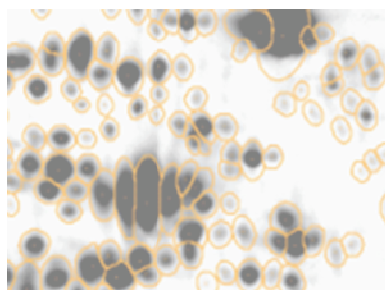
If you need more detailed information about Delta2D, please consult the manual of Delta2D. You can access it through the Delta2D menu 'Help' > 'Help ...', or as a PDF file located in the installation directory. In Windows, there is a direct link to the manual in the Start menu. The manual is also available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

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Your DECODON Team



If you have any comment or suggestion regarding this '**Getting Started with Delta2D Guide**' or any other of our documents we welcome hearing from you.







### 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](http://www.decodon.com). Your questions and remarks are welcome, call us at +49 3834 515230 or send an email to [info@decodon.com](mailto: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 and others. Please contact DECODON for details.

**Supported Operating Systems** (\*recommended): Delta2D runs on Windows NT / 2000 / XP / Vista / 7\* / 8 at 32 or 64\* bit, Mac OS X 10.3 (Panther) / 10.4 (Tiger) / 10.5 (Leopard) / 10.6 (Snow Leopard) / 10.7 (Lion) / 10.8\* (Mountain Lion) and most flavours of 32 bit or 64 bit\* Linux.

### Hardware Requirements:

Minimum Hardware:

Pentium III, 800 MHz, 2 GB RAM or Power Mac G4, 2 GB

Recommended Hardware:

Dual or Quad Core Processor with 2 GHz or better, 4 GB RAM, in combination with a 64-Bit operating system

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