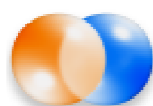


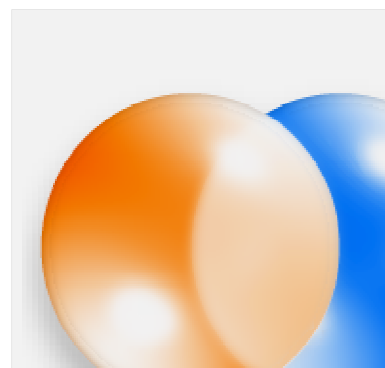
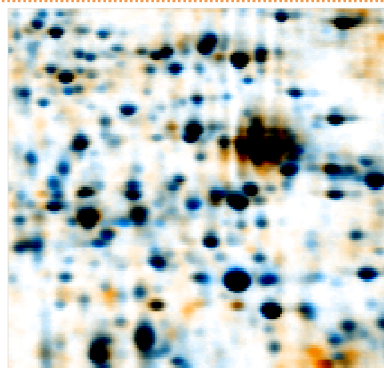
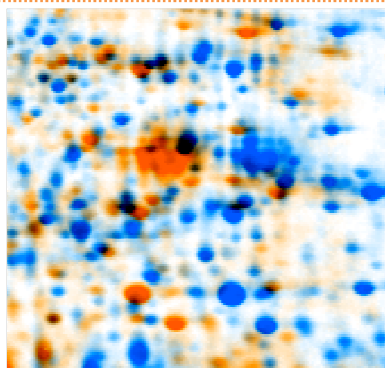
Getting started

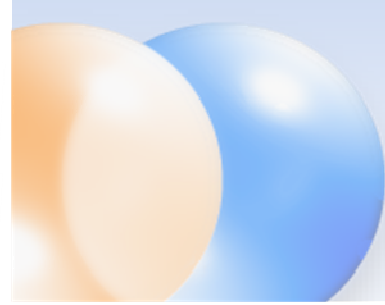
English



Delta2D

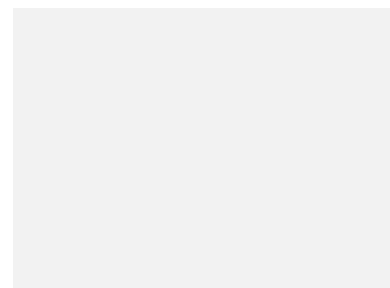
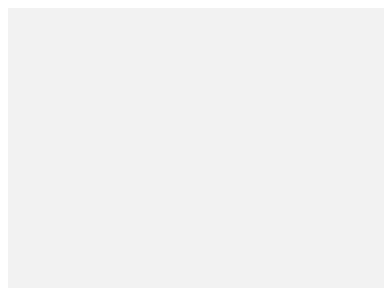
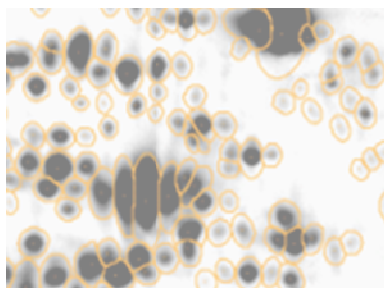
ANALYZING 2D GELS
AS EASY AS POINT AND CLICK

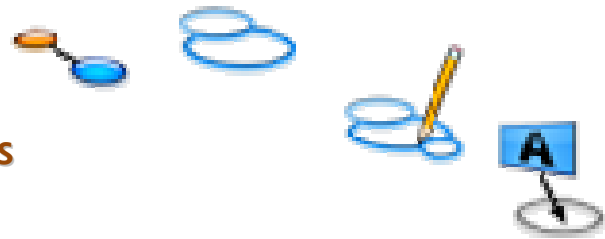




Copyright DECODON GmbH. DECODON makes no representations, express or implied, with respect to this documentation or the software it describes, including without limitations, any implied warranties of merchantability or fitness for a particular purpose, all of which are expressly disclaimed. Users should recognize that all complex software systems and their documentation contain errors and omissions. DECODON shall not be responsible under any circumstances for providing information on or corrections to errors and omissions discovered at any time in this document or the software it describes, whether or not they are aware of the errors and omissions. DECODON does not recommend the use of the software described in this document for applications in which errors or omissions could threaten life, injury or significant loss.

DECODON, the DECODON logo, Delta2D, SmartVectors are trademarks or registered trademarks of DECODON GmbH in Germany and in several other countries all over the world. The use of general descriptive names, trademarks, etc., in this publication, even if the former are not especially identified, is not to be taken as a sign that such names, as understood by the Trade Marks and Merchandise Marks Act, may accordingly be used by anyone. Where those designations appear in this work and DECODON was aware of a trademark claim, the designations follow the capitalization style used by the manufacturer. Linux is a trademark of Linus Torvalds. Apple, Mac, MacOS, Macintosh are trademarks of Apple Computer, Inc., registered in the U.S. and other countries. JAVA and Solaris are registered trademarks of Sun Microsystems, Inc., 901 San Antonio Road, Palo Alto, CA 94303 USA. Microsoft, WINDOWS, NT, MS PowerPoint, MS Excel, Vista, Windows 7 are registered trademarks of Microsoft Corporation. UNIX is a registered trademark of The Open Group. All other products mentioned are trademarks or registered trademarks of their respective companies. Some software products marketed by DECODON GmbH and its distributors may contain proprietary software components of other software vendors.





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.

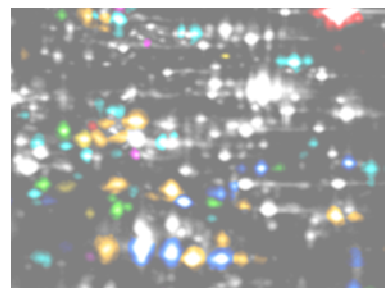
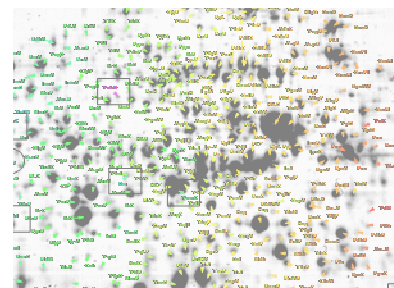
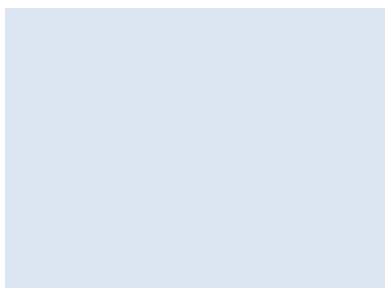
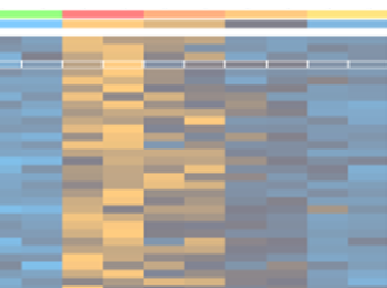
5 Present Results

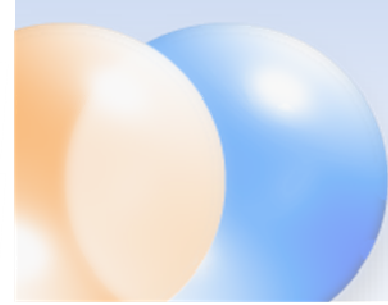
Export results to other applications for presentation or further analysis.

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. Unpack the archive. It contains the following gel images and match map files for the warping:

Group	Image Files (two replicates per group)
control	control_01.gel, control_02.gel
1 min	1min_01.gel, 1min_02.gel
10 min	10min_01.gel , 10min_02.gel





1 Setup Project

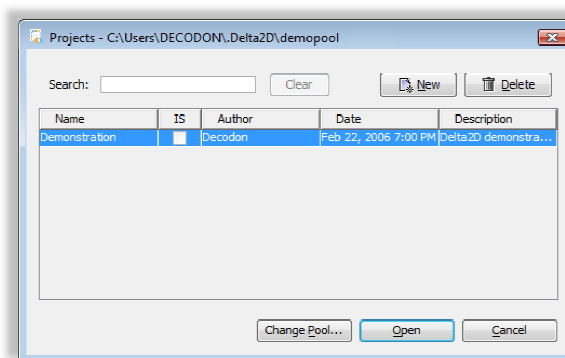
Create a new data pool

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 confirm with .
3. Select the new project from the list and press .

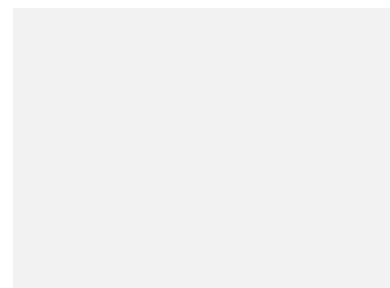
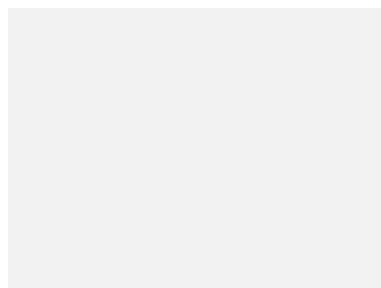
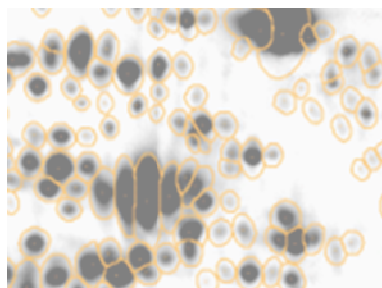


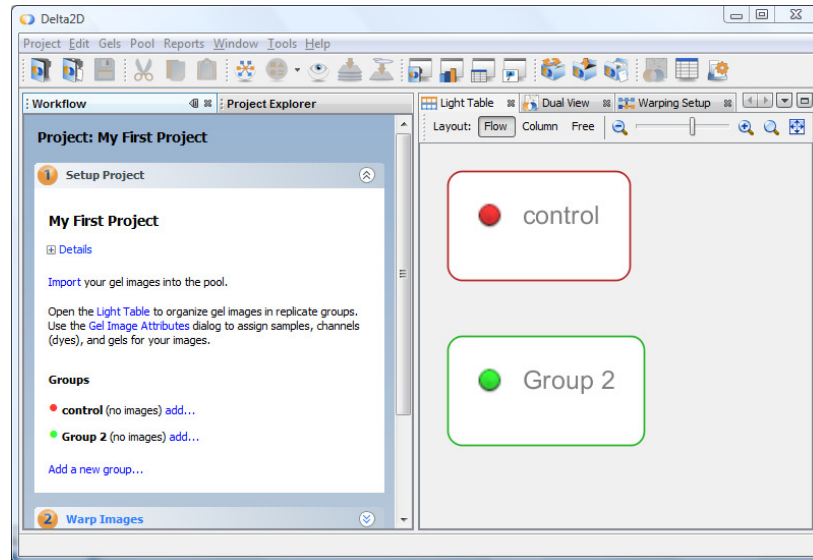
Delta2D's Projects dialog.

Set up groups for the gel images

We will now create the *three groups* for our experiment - one for each of the three replicate pairs. 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 '**control**'. If you want, choose another color and confirm with .
3. Repeat steps 1 and 2 for the second group assigning the name '**1min**'.

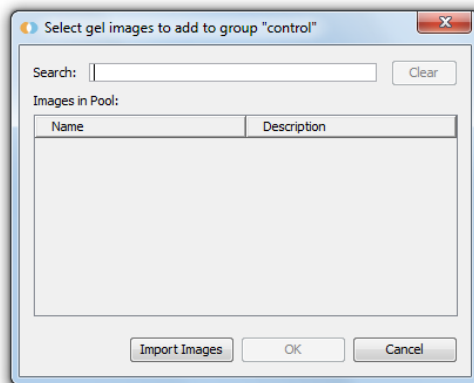




Delta2D's Workflow and Light Table - showing an empty project.

Create a new group '*10min*', by clicking on the link '[Add a new group...](#)' in the Workflow.

Add gel images to your groups

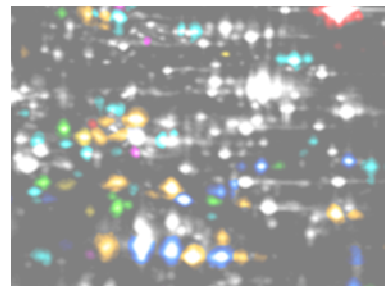
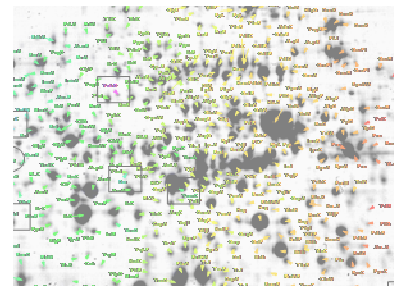
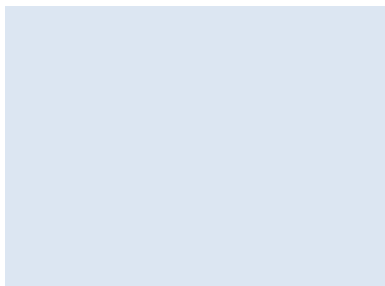
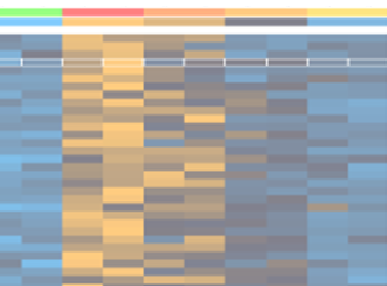


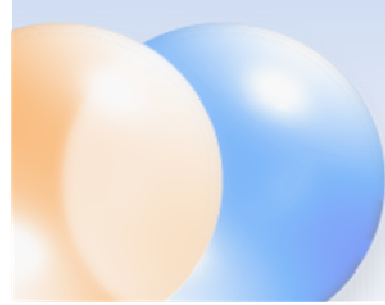
Delta2D's Gel Import Manager.

1. In the Workflow click on the '[Add...](#)' link next to the *control* group.
2. Click on **Import Images** and select the two images '*control_01*' and '*control_02*' 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.

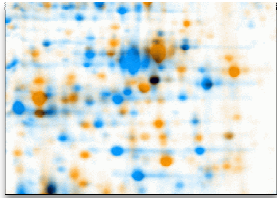
4. Click on **Finish** to save assignments and close the dialog.

Please import the sample images '*1min_01*' and '*1min_02*' as well as '*10min_01*' and '*10min_02*' into their respective groups.

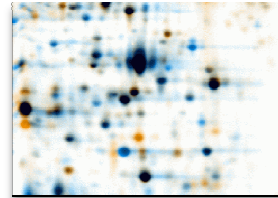




2 Warp Images



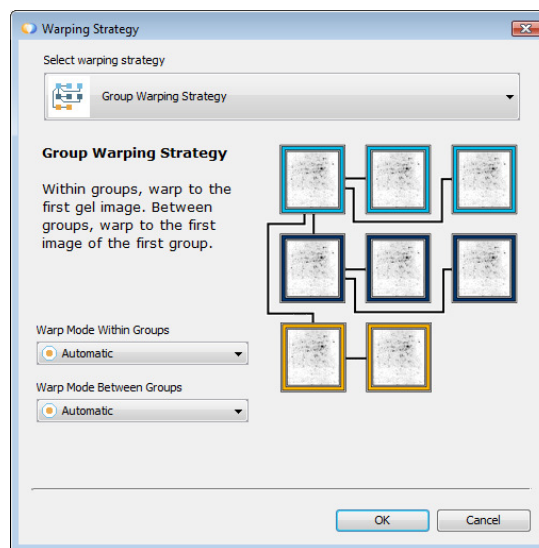
Two gel images, overlaid into a dual channel image, not warped.



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

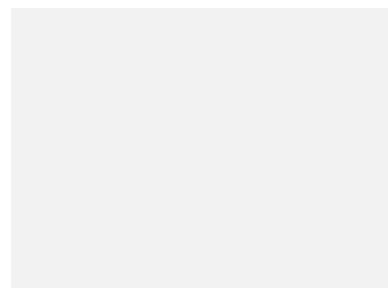
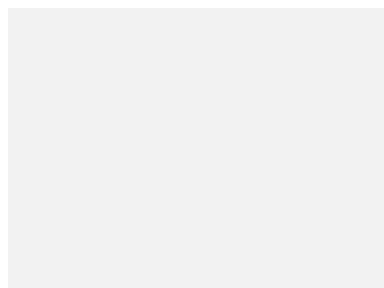
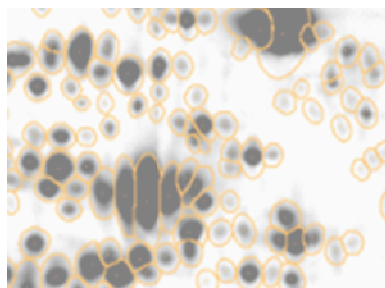
Define a warping strategy

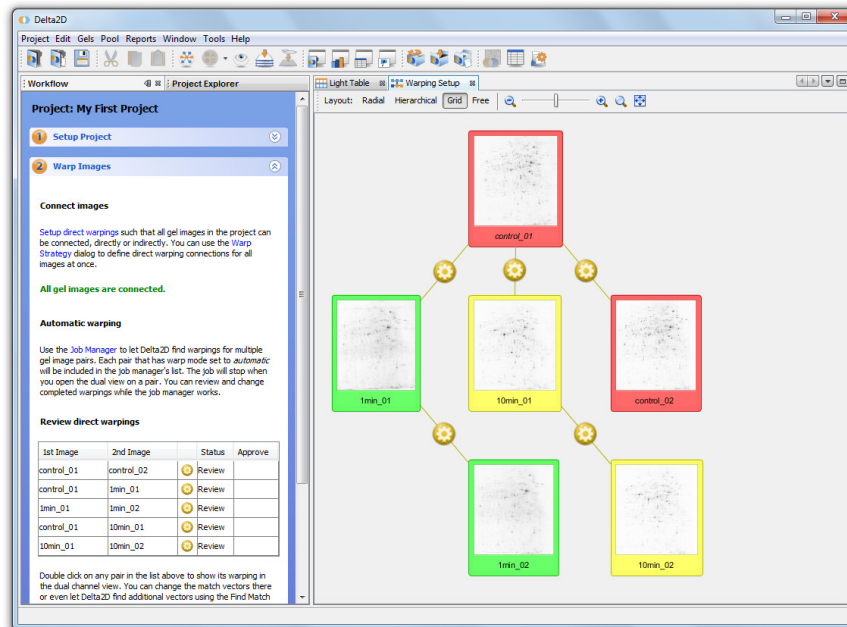
1. Switch to the second step of the 'Workflow' called 'Warp Images'.
2. Click on the 'Warp Strategy...' link to open the 'Warping Strategy Manager'.
3. Select the 'Group Warping Strategy' and confirm the default settings with .



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:










Warping Setup window after applying the Group Warping Strategy.



Use Delta2D's Automatic Warping

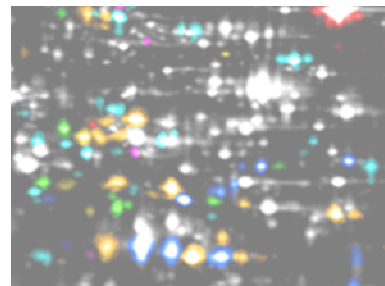
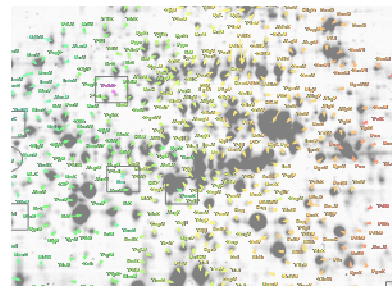
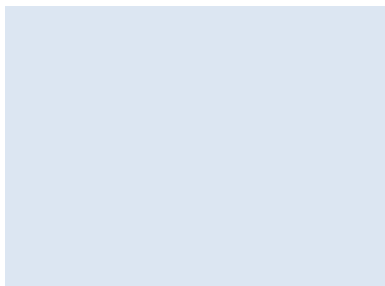
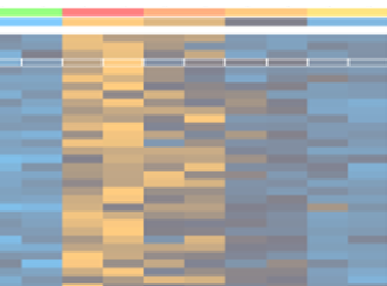
1. Click on the 'Job Manager' link.
2. Just click the 'Start' button  in the 'Job Manager'. It will monitor and perform all automatic warping jobs.

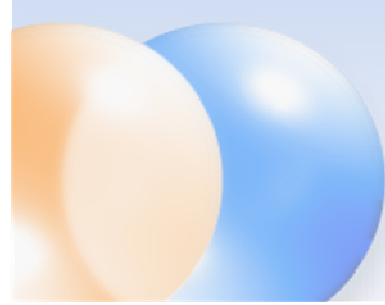
Review Warpings

1st Image	2nd Image	Status	Approve
control_01	control_02	 Review	Approve
control_01	1min_01	 Review	
1min_01	1min_02	 Review	
control_01	10min_01	 Review	
10min_01	10min_02	 Review	

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.



**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.

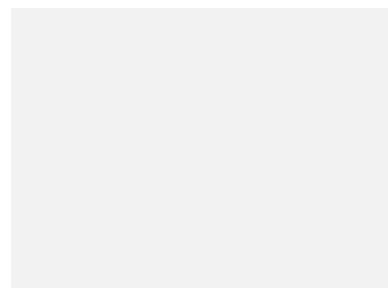
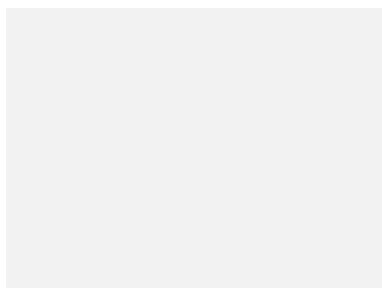
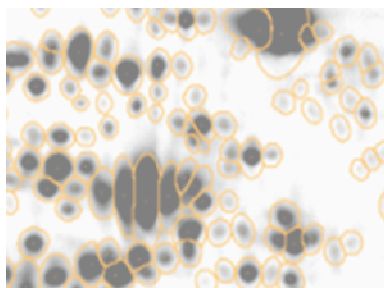
If there are regions *where vectors seem to be incorrect*, you can set and change all match vectors manually:

- **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.



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. Click on **Fuse** 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 **OK**.
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

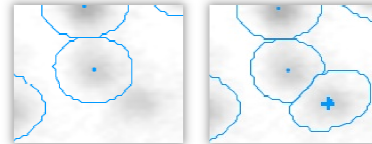
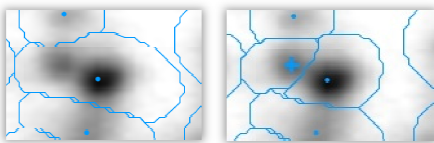


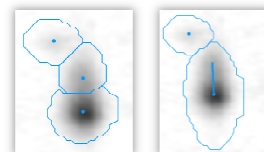
Image region before / after adding a spot.
(Just click on the center of an undetected spot to add it.)

Splitting a Spot

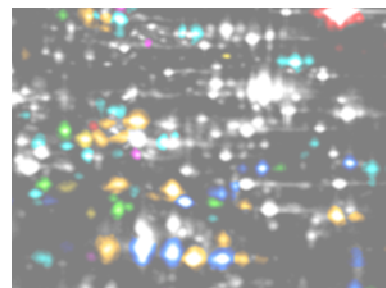
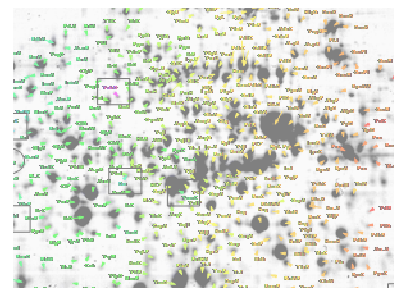
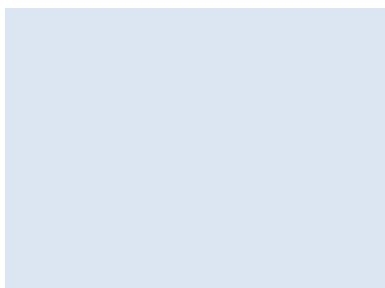
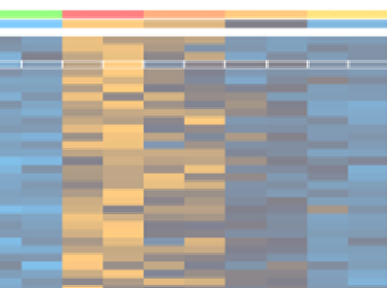


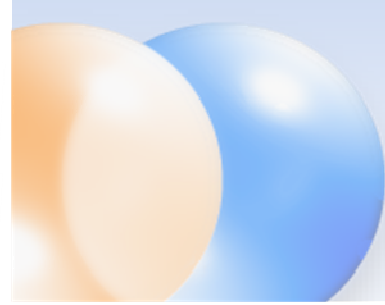
Two spots, detected as one and split.
(Click once into the center of the spot that was not detected separately).

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.



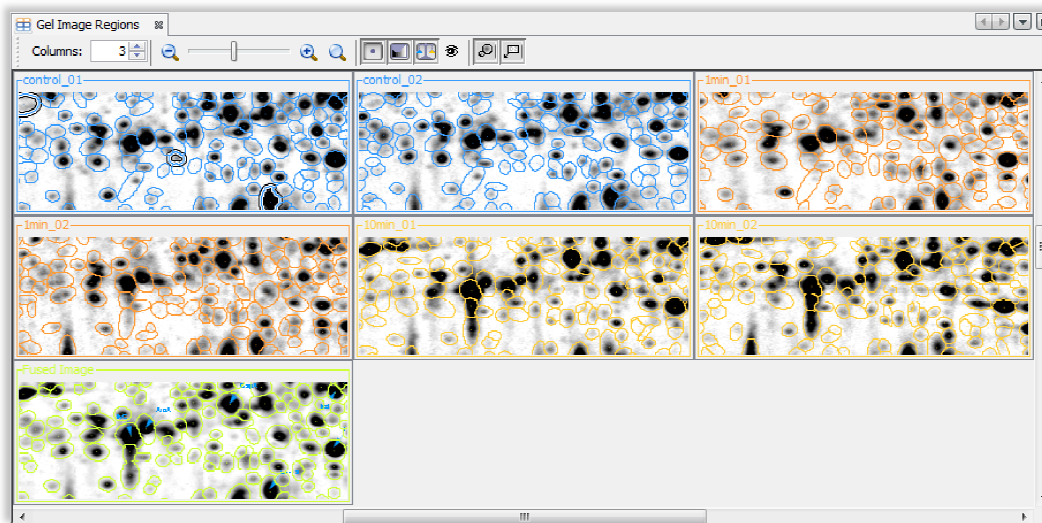
Remove Spots

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'**.

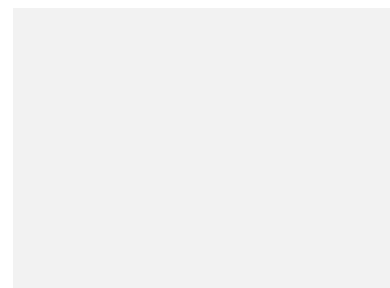
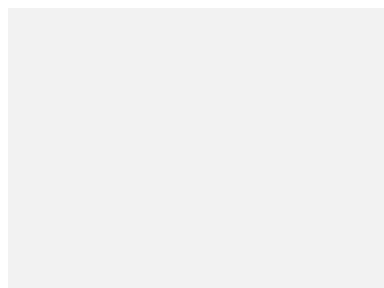
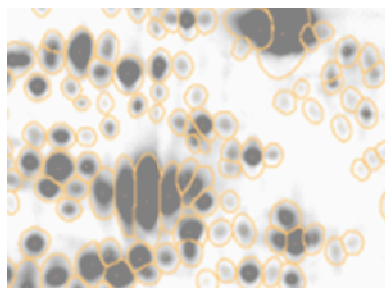
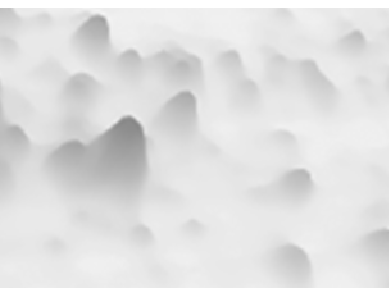
To see all canceled spots with dotted line spot boundaries, select **'Spots' > 'Show Canceled Spots'** from the Dual View's menu.

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 **OK**.



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.

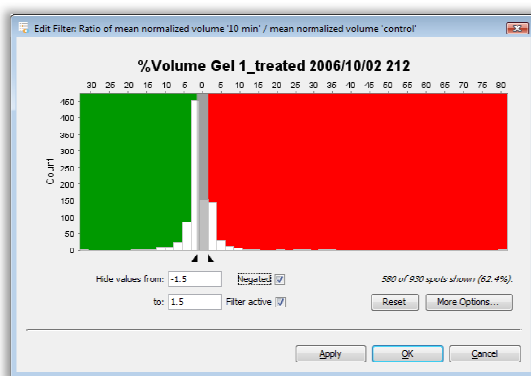


4 Analyze Expression Profiles

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 1min group '*Ratio mean % volume 1min / mean % volume control*'.
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 **OK**.
6. Open the 'Dual View' with the two images 'control_01' and '1min_01'. Only spots meeting our criteria will still have spot boundaries.

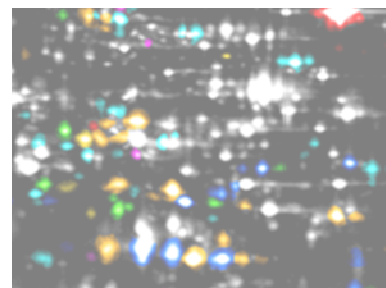
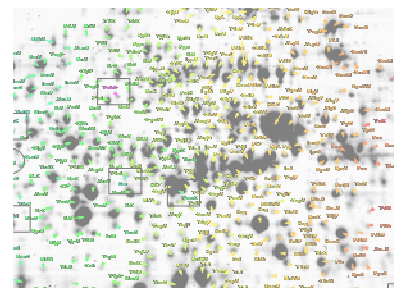
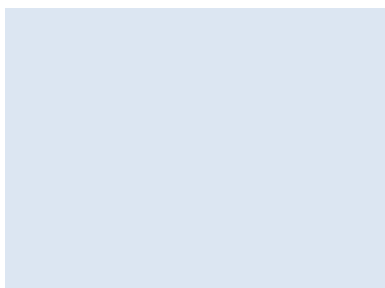
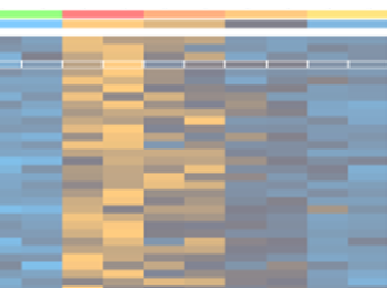
Advanced Statistical Analysis

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

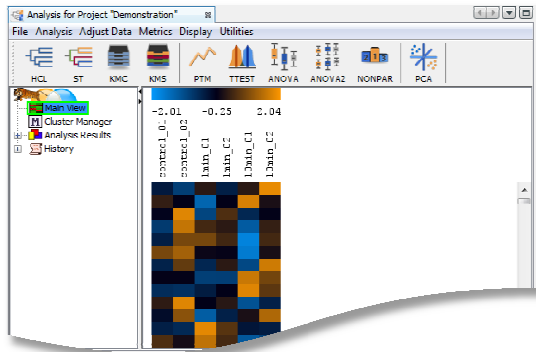
- Heat map display of expression profiles
- Various methods of clustering including Hierarchical Clustering, k-means / medians Clustering
- Numerous variations of t-Test
- Analysis of Variance (ANOVA)
- Non-Parametric tests, incl. Wilcoxon / Mann-Whitney, Kruskal-Wallis, Mack-Skillings, and Fisher-Exact 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**'.

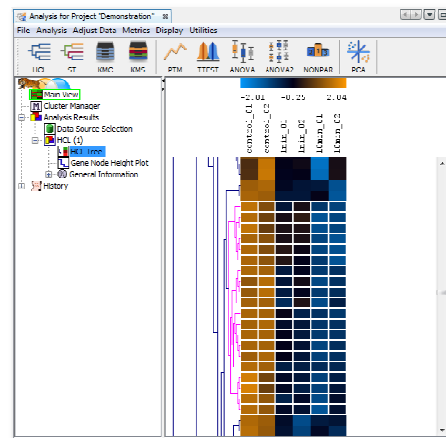
The **legend** across the top shows the color code for spot intensities. Each column contains all spot intensities of one gel image. Each row shows the intensities of one spot on all the different images in your project. Data is normalized / standardized by default.

Discovering patterns in expression profiles

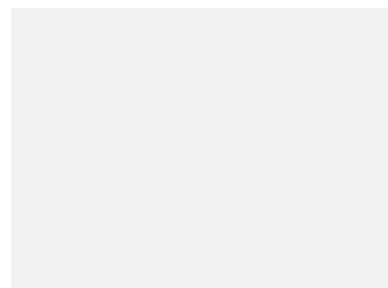
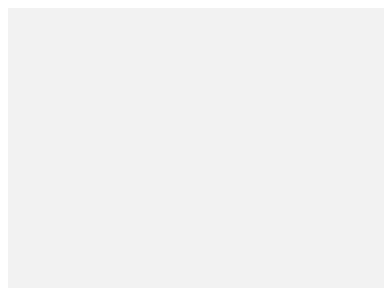
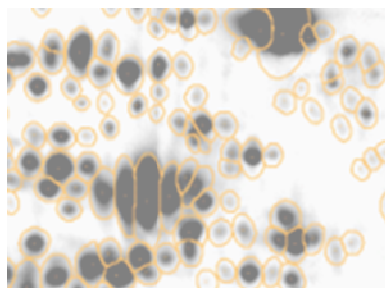
1. Press the  button in the toolbar. Choose '**Gene Tree**', '**Euclidian Distance**' and '**Complete Linkage**'.
2. To confirm press **OK**.

The tree represents similarities between expression profiles.

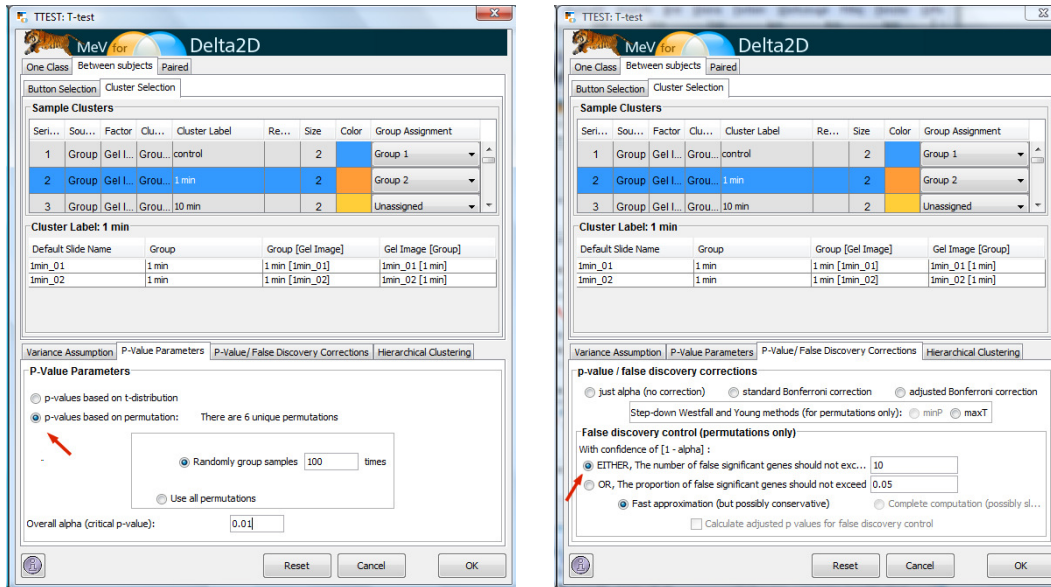
If you do this for the '**Sample Tree**', all replicates of the same sample should appear in the same cluster (subtree). If not, this indicates that there are outliers in your project - due to e.g. experimental problems the gel is very different from the other replicates. You should consider removing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





Hierarchical Clustering for Expression Profiles.



Finding differentially expressed proteins: Statistical Tests

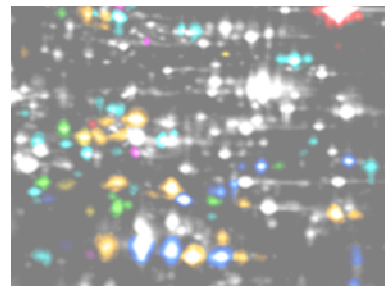
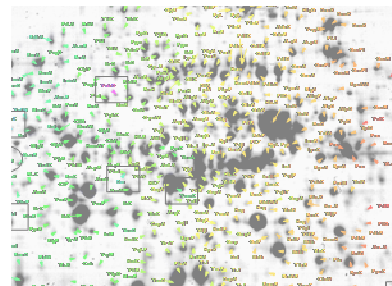
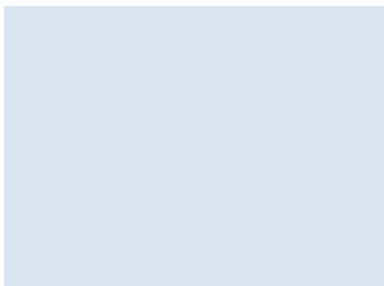
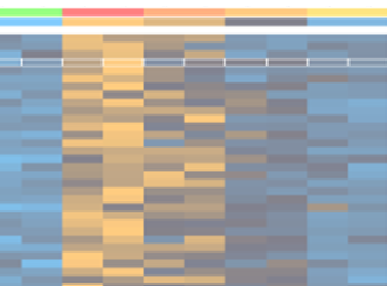


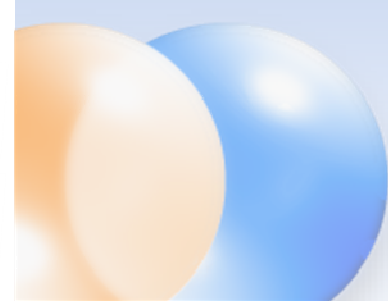
t-Test parameters.

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 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

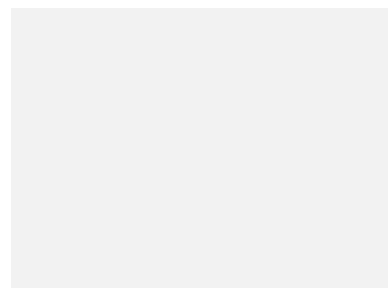
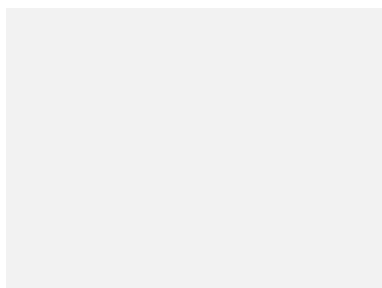
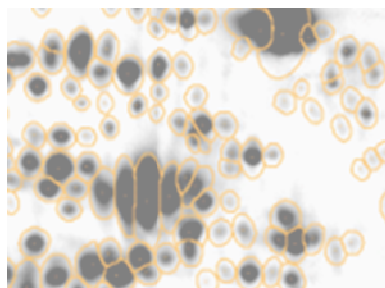
1. Open the 'Dual View' for any gel image pair.
2. Choose 'Export' > 'Export to PowerPoint' and confirm with .
3. Follow the instructions on the screen to create the MS PowerPoint slide.

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 .



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. We will be happy to answer your questions or to show you more in a live web demo.

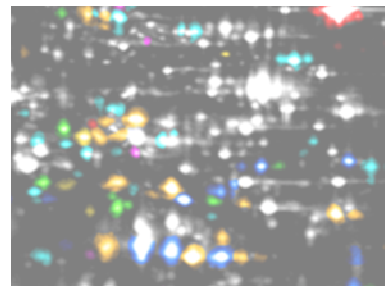
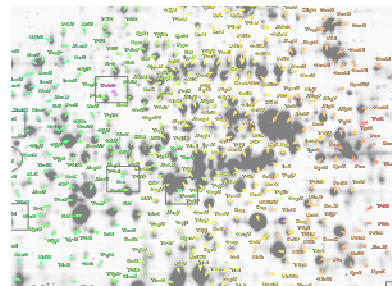
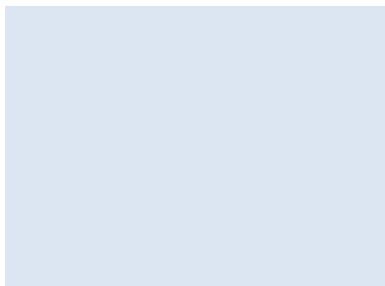
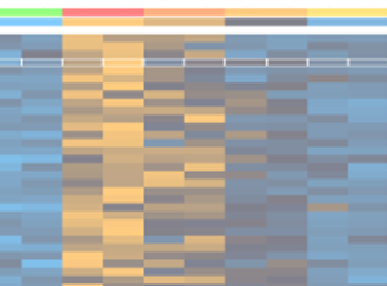
Note: _____

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!

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. Your questions and remarks are welcome, call us at +49 3834 515230 or send an email to info@decodon.com.

Technical data:

Supported image file formats: Delta2D supports virtually all calibrated and uncalibrated image file formats on the market today, including tiff (8 bit, 12 bit, 16 bit), IMG (Fuji, GE), GEL (GE), JPEG, BMP, GIF, PNG, PNM.

Supported Protein Labels and Stainings: Delta2D supports virtually all protein labels and stainings, including Silver, Coomassie, Colloidal Coomassie, Sypro Ruby, Flamingo, Krypton, LavaPurple, Diamond ProQ, Emerald ProQ, Cy-Dyes, G-Dyes, radioactive labels etc.

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

Supported Operating Systems (*recommended): Delta2D runs on Windows 7 / 8 / 10 at 32 or 64* bit, Mac OS X 10.7.3 (Lion) and newer, and most flavours of 32 bit or 64 bit* Linux.

Hardware Requirements:

Minimum Hardware:

PC with 2 GB RAM or Intel based Mac

Recommended Hardware:

Dual or Quad Core Processor with 2 GHz or better, 8 GB RAM, in combination with a 64-Bit operating system

Copyright and Trademarks

All material in this brochure is Copyright ©DECODON GmbH. All Rights Reserved. DECODON, DECODON logo, and Delta2D are trademarks or registered trademarks of DECODON GmbH in Germany and in several other countries all over the world. All other products mentioned are trademarks or registered trademarks of their respective companies.



DECODON GmbH
Walther-Rathenau-Str. 49a
17489 Greifswald, Germany

www.decodon.com
info@decodon.com

phone: +49(0)3834 515230
fax: +49(0)3834 515239