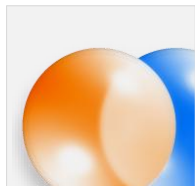
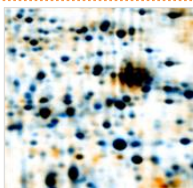
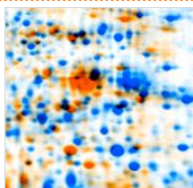
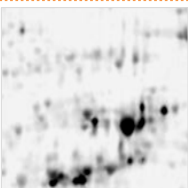


Delta2D

ANALYZING 2D GELS
AS EASY AS POINT AND CLICK



Delta2D – on the cutting edge of technology

You spend a lot of time optimizing your sample preparation and 2D gel electrophoresis protocols. When you analyze the resulting gel images, you want to ensure that you get the most information out of them. Delta2D incorporates modern technologies that allow you to get reliable and statistically significant results. Furthermore, the effort needed to analyze images is reduced to a minimum – your time matters to us.

More reliable results, better statistical analysis

DECODON has introduced an approach that generates complete expression profiles for spots making statistical analysis much more reliable. The approach as implemented in Delta2D is unique, transparent, time-saving, and under your full control.

Various visualization and exporting features

It is good to achieve reliable results. Being able to prepare them for publication and presentation the way you want is even better. Delta2D helps you with that: Feel free to use the whole range of modern data visualisation and various exporting features.

Why Delta2D?

- Complete expression profiles
- Automatic image alignment for reliable spot matching
- Spot detection and editing on one image only
- Advanced statistical methods
- Various visualization and exporting features

Gel image warping (alignment of spot positions) was introduced with Delta2D 1.0 in 2000. Since then we have continuously improved the warping algorithm. SmartVectors – Delta2D's leading technology for automatic gel alignment - provides an advanced and transparent method to integrate automatic image warping into the analysis workflow.

The idea to create complete expression profiles resulting in 100% spot matching was already implemented in 2003 with Delta2D 3.1.

Analyze ALL your 2DE experiments with ONE software

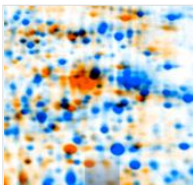
Two-dimensional electrophoresis has seen many innovations in the past. With Delta2D you can take advantage of all the different techniques: Classical experiments as well as DIGE and other multiplex experiments like Phospho- or Glycoproteomics can easily be analyzed. Whatever you want to explore, Delta2D will help you to get the most information out of your gels.

DECODON – committed to customer satisfaction and innovation

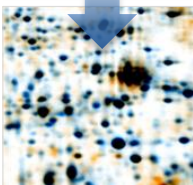
Established in 2000, we are committed to developing and delivering innovative software tools for modern life sciences and dedicated to customer satisfaction. Our team of mathematicians, computer scientists, and biologists carefully listens to you, analyses your needs, and transforms innovative ideas into outstanding software solutions that really make a difference.

Deciding for Delta2D means deciding for innovation.

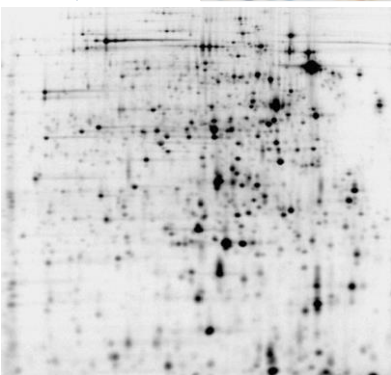
Dual color channel overlay (blue represents gel A; orange gel B) of two gel images before initialization of automatic warping.



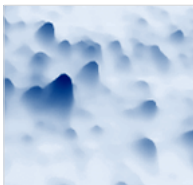
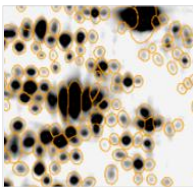
Result of the automatic warping process, black means spots are present in both gel images with similar signal intensity.



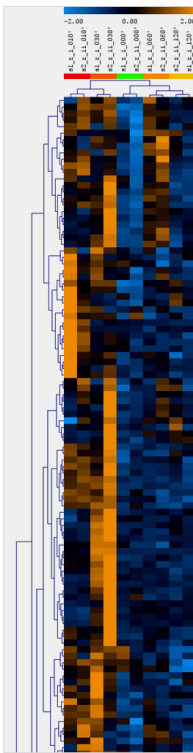
2D gel image of a radiolabeled protein extract.



Gel image with detected and modelled spot boundaries

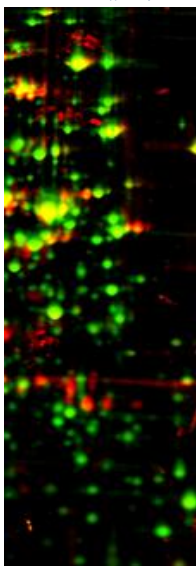


A 3D spot view helps with determining spot boundaries during spot editing.



Complete expression profiles allows the application of a variety of clustering and statistical approaches also known from DNA array analysis.

Multiplex image for the detection of phosphoproteins. (Flamingo protein dye – green; Diamond ProQ phosphoprotein dye – red). Multiplex approaches such as this one are supported by Delta2D.



"Among the several steps that are necessary to characterize a proteome through the analysis of 2D gels, one of the most complex is the analysis of the spot patterns present in the gel images. The process, by itself, requires an entirely new set of abilities from the researcher used to working the bench and is usually very time consuming.

Therefore, the creation of workflow guidance is an excellent way to help to speed up the analysis and the generation of data from the gels."

*Ricardo Nilo Poyanco
Santiago, Chile*

Unique analysis workflow for 100% matching spots

The recommended analysis workflow for Delta2D is a unique approach that leads to complete and correct spot matching for reliable data. Users are free to use the Workflow component that will help to keep an eye on the necessary steps or enjoy the flexibility of the sophisticated capabilities of Delta2D.

Simplified and flexible project setup

With Delta2D's Light Table, organizing images into groups is done in the blink of an eye: You can freely setup your project so that it fits to your experimental setup.

Gel image warping – making virtually perfect 2D gels

In 2000 gel image warping was introduced by DECODON with Delta2D 1.0. Since then we have continuously improved the algorithm to stay on the cutting edge of the technology. Delta2D's SmartVectors technology uses the whole image information to automatically eliminate running differences between gel images to align them.

Use predefined warping strategies or freely connect images according to your individual experiment along the similarity of the gels. You are not forced to decide for a reference image. As a result all spots have the same position on each image – as if you had made perfect gels without any running differences.

The missing values problem solved ... in 2003

All images of a project can be fused into one synthetic image using the Union Fusion algorithm (introduced in 2003). The resulting image looks like a real gel image and is actually the proteome map for your project – containing all spots from all images of the project.

One image with all spots – a perfect image for spot detection and editing. Let Delta2D transfer the pattern of spot boundaries from your proteome map to all the images in your project and spot matching is finished – completely and correctly.



Delta2D innovation highlights:

Gel image warping (alignment of spot positions) was introduced with Delta2D 1.0 in 2000. The concept of creating complete expression profiles resulting in 100% spot matching was made a reality in 2003 with Delta2D 3.1. In 2006, with Delta2D 3.4, SmartVectors were introduced to provide a transparent and hybrid method to integrate automatic and manual image warping. Web reports were introduced in 2007, along with advanced multivariate statistics methods. Version 4.0, introduced in 2008, offers a more effective window management and a guided workflow.

Decide for leading technology!

Step by step the workflow component of Delta2D guides you through the whole image analysis process

1 Setup Project

2 Setup Gel Image Warping

3 Create Direct Warpings

Find and review match vectors for gel image pairs.

Batch warping with the Job manager

[Job Manager](#)

Use the Job-Manager to let Delta2D find warpings for multiple images. Each pair that has warp mode set to automatic will be included in the job managers list. The job will stop when you open the dual view on a pair. You can review and change completed warpings while the job manager works.

Review Direct Warpings

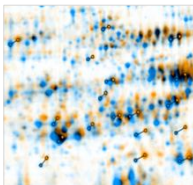
Double click on any pair in the list below to show its warping in the dual-channel view. You can change the match vectors there or even let Delta2D find additional vectors using the Find Match Vectors button. When you are satisfied with the warping you should approve all match vectors: in the Matches menu, choose Select Non-Approved, then Approve Selected.

1st Image	2nd Image	Status
s1_f_1_000'	s2_f_1_000'	OK
s1_f_1_000'	s1_f_1_000'	OK
s1_f_1_060'	s2_f_1_060'	OK
s1_f_1_000'	s1_f_1_120'	OK
s1_f_1_120'	s2_f_1_120'	OK

4 Create the Consensus Spot Patterns

5 Analyze Expression Profiles

6 Present Results



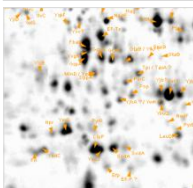
Warp vectors represent correspondences of two gel images.



The light table supports project organization, e.g. naming of groups and gel images and grouping of replicates.

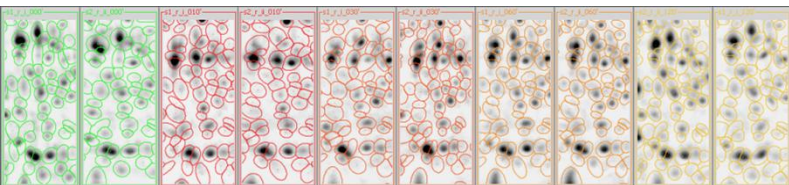


The warping setup shows how pairwise warpings are extended across the project.

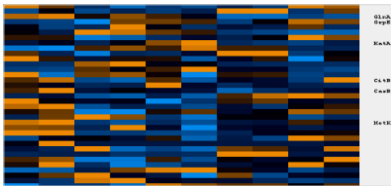


After positional correction images are fused in a Fusion Gel condensing the spot information of the whole experiment in one image. spot identifications often are managed on the fusion gel. Spot detection and editing is performed here only once per experiment ...

... Transfer of this spot pattern results in 100% matching spots for all gels of your experiment.



Expression data of 100% matching Spots result in complete bar charts ...



... or in heat maps (orange means high, black average and blue low expression)

Advanced statistical methods

With Delta2D's 100 Percent Spot Matching, there are no missing values, and matching problems are virtually eliminated. This does not only translate to higher statistical confidence, data generated by Delta2D is also especially suitable for the methods that were originally designed for DNA microarray analysis.

Since version 3.6 Delta2D incorporates algorithms from the TIGR Multiple Experiment Viewer (MeV) and tightly integrates them into the two-dimensional gel image analysis workflow.

Identify structures in your data and detect outliers

Clustering methods can be used to group expression profiles and gel images by similarity. This can be very useful for getting an overview of all expression profiles before proceeding with more detailed analyses. These methods are currently available:

- **Hierarchical Clustering (HCL),**
- **k-means/ k-medians Clustering (KMC),**
- **Principal Component Analysis (PCA).**

Clustering of gel images can also be used to detect outliers, and to identify structures in the experiment. Ideally, the cluster composition will reflect the structure of the experiment, e.g. replicates and images from the same sample should have similar expression levels and thus end up in the same cluster.

Furthermore, **Pavlidis Template Matching (PTM)** allows for selecting proteins that follow a given expression pattern.

Find significantly changed spots

In the simplest case, the experiment is a comparison of two samples, e.g. diseased vs. control tissue, mutant vs. wild type etc. The task then is finding those proteins that show significant differences in expression levels. Certainly the most popular test in this area is **Student's t-Test**. In Delta2D, these different variations of the t-Test are available, including **control of False Discovery Rate**.

With the **Analysis of Variance (ANOVA)** spots that have significant differences in means across three or more groups of samples can be picked. In Delta2D, **one-way or single-factor analysis of variance** and **two-factor analysis of variance** is implemented.

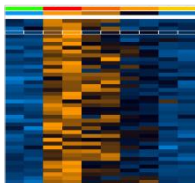
Non-parametric tests – The gold standard for spot quantities

Non-parametric tests can be applied since version 4.0. These tests have the advantage that they do not require a normal distribution assumption. They are therefore especially suitable for spot quantities. Available tests are:

- **Wilcoxon Rank Sum Test** for testing one factor in two experimental groups
- **Kruskal-Wallis Test** for testing one factor in multiple experimental groups
- **Mack-Skillings Test** for testing two factors in multiple experimental groups
- **Fisher Exact Test** for testing nonrandom associations between two categorical variables.

Principal component analysis is a tool to reduce data complexity in multidimensional data sets.

A set of data points (samples) were projected onto a two dimensional data space. This shows that samples clearly differ from each other while replicates are closely related.



If you are looking for known expression profiles, you can predefine a template in TMEV. For each sample (first color strip) you can define an expression pattern (second strip). TMEV finds all spots behaving similar to the profile template.

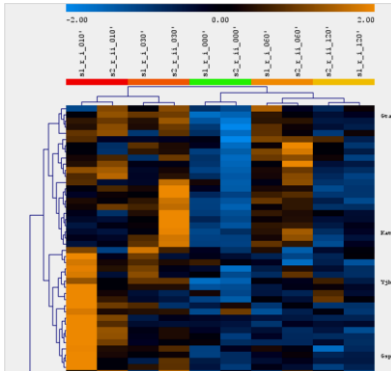
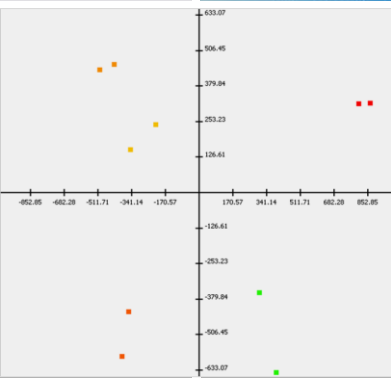
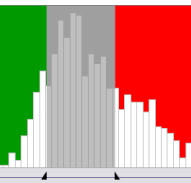
The quantification table gives an overview of the expression data from the gel images:

%V	%V	%V	%V	%V	%V	%V	%V	%V	%V	%V	Label
0.039	0.029	0.010	0.001	0.009	0.006	0.012	0.009	0.040	0.035		
0.010	0.017	0.010	0.013	0.011	0.013	0.031	0.029	0.012	0.023		
0.261	0.278	0.206	0.247	0.220	0.187	0.121	0.119	0.146	0.135	GlnA	
0.115	0.112	0.078	0.159	0.160	0.152	0.114	0.140	0.174	0.162	GpE	
0.016	0.006	0.009	0.054	0.016	0.008	0.012	0.011	0.026	0.009		
0.144	0.095	0.286	0.252	0.197	0.171	0.073	0.060	0.033	0.032		
0.143	0.141	0.047	0.063	0.091	0.111	0.090	0.121	0.241	0.170		
0.054	0.063	0.048	0.064	0.101	0.161	0.046	0.041	0.037	0.052	KatA	
0.067	0.030	0.199	0.233	0.193	0.264	0.198	0.197	0.125	0.127		
0.015	0.007	0.000	0.007	0.006	0.009	0.024	0.022	0.005	0.011		
0.043	0.028	0.021	0.027	0.027	0.015	0.023	0.028	0.006	0.026		
0.008	0.005	0.154	0.269	0.054	0.065	0.019	0.012	0.008	0.009		
0.008	0.004	0.072	0.041	0.065	0.160	0.034	0.035	0.010	0.003		
0.075	0.019	0.059	0.064	0.047	0.014	0.051	0.052	0.028	0.032		
0.209	0.325	0.212	0.197	0.208	0.204	0.251	0.245	0.219	0.352	CitB	

Scatterplots compare expression data of two samples.

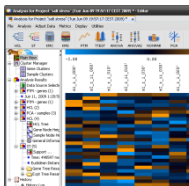


You can use filters for accessing protein spots within a specified induction range, within a specific intensity class or with other properties of interest. All filters use histogram plots.



Hierarchical clustering sorts samples (columns) according similar global expression of proteins, or protein spots according the similar expression profiles (rows) over the whole experiment.

TIGR MEV, an analysis suite for expression data analysis is used inside Delta2D to perform advanced expression studies.



"We evaluated several analysis software packages before we made the decision for Delta2D. The intuitive user interface and the wide variety of exporting features convinced us to choose Delta2D... ...and we still enjoy working with it."

*Anna Chiarini
Verona, Italy*

Do more with your data

Since its beginning in the year 2000 Delta2D supports open data formats which allow you to easily transfer your analysis data to, for example, your in-house database. The powerful exporting and visualization features can be used to prepare results for publication and presentation.

Color Coding – Summarize your results on a single image

Spot Color Coding lets Delta2D display a proteome map (or a single gel image) with spots colored according to their expression profiles.

Dynamic label coloring can be used as an indicator e.g. for the isoelectric point or the molecular weight of identified spots. Thus you can see at a glance the distribution and also outliers in the selected property over the complete gel image.

Interactive web reports – The modern way of exporting data

Project 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).

Spot album includes detailed information about expression profiles and images for marked spots and the ability to define parameters to change the report's content.

Spot Quantities includes detailed information about marked spots and allows you to define parameters to change the reports' content.

After all, it's Your Data

We want you to get the most out of your data and save it in the format you prefer. Currently, you can export data from Delta2D

- to Excel worksheets,
- to PowerPoint slides – works for single, dual channel, and color coding images (Spot boundaries and labels can be treated like real PowerPoint objects.),
- to spot picking lists (for Genomic Solutions ProPic, PerkinElmer ProXCISION, Molecular Dynamics, Ettan Spot Handling Workstation, Bruker Proteineer or as Generic pick list format),
- as snapshots (single or dual channel images and Color Coding images can be exported as TIFF, JPEG, PNG, PNM, BMP).

The spot album report summarizes expression data of selected spots and can be displayed in a web browser. Report contents can be easily transferred to other documents.

„My students and I have tested several programs and Delta2D has turned out to be by far the best 2D gel image analysis program. Most importantly, the service is superb. It does not matter which time zone you are in, they call you and walk you through any problem you may encounter.“

*Lars Tomanek
California, USA*

“The support I’ve experienced from DECODON has been nothing short of perfect.”

*Daniel Kay
Porirua, New Zealand*

Premium Service is our Standard

When you decide for Delta2D you not only get a great application. Providing accessible and quick support is one of our highest goals. We know that each customer's problem is important and you will receive prompt attention from our support team. By prioritizing the request, our support team ensures that the most urgent ones are solved first.

Our experienced support team is dedicated to providing effective support that helps our customers to use Delta2D. The team continuously ensures customer satisfaction by one-to-one communication via email, phone, and web sessions.

The DECODON Support Team

Phone: +49 (0)3834 515 235

Email: support@decodon.com

Workflow x

Dual View - s1_r_j_000_vs_Fused Image using Union at 08/10/2003 18...

Export Matches Spots Labels Rollups

Layout: Flow Column Free

Light Table x

Layout: Radial Hierarchical C

Warping Setup x

Layout: Radial Hierarchical C

528 / 12 (76%) Image Bounds: 815 x 1043 Match Vectors: 0 Detected Spot...

1 Setup Project

2 Setup Gel Ima...

3 Create Direct ...

4 Create the Con...

5 Analyze Expre...

Use Delta2D's analysis tools to find relevant expression profiles.

Quantitation ...

Use the quantitation table to review quantities, expression ratios, and t-Test results. Use filters to select interesting spots. Mark spots or expression profiles here for later visualization.

Expression Pr...

Mark interesting spots to see their expression profiles as bar charts in the Expression Profiles window.

Analysis

Use statistics tools such as clustering, principal components analysis, and ANOVA.

6 Present Results

s1_r_j_000_vs_Fused Image using Union at 08/10/2003 18:23:08:756

s1_r_j_000 Fused Image using Union at 08/10/2003 18:23:08:756

group 1 group 2

group 4 group 5

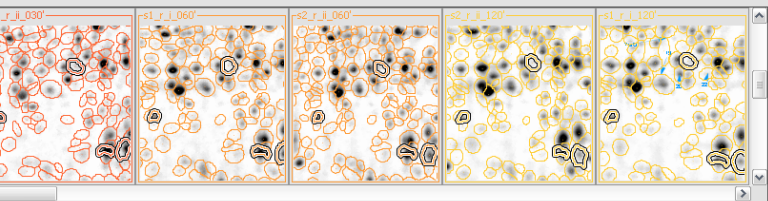
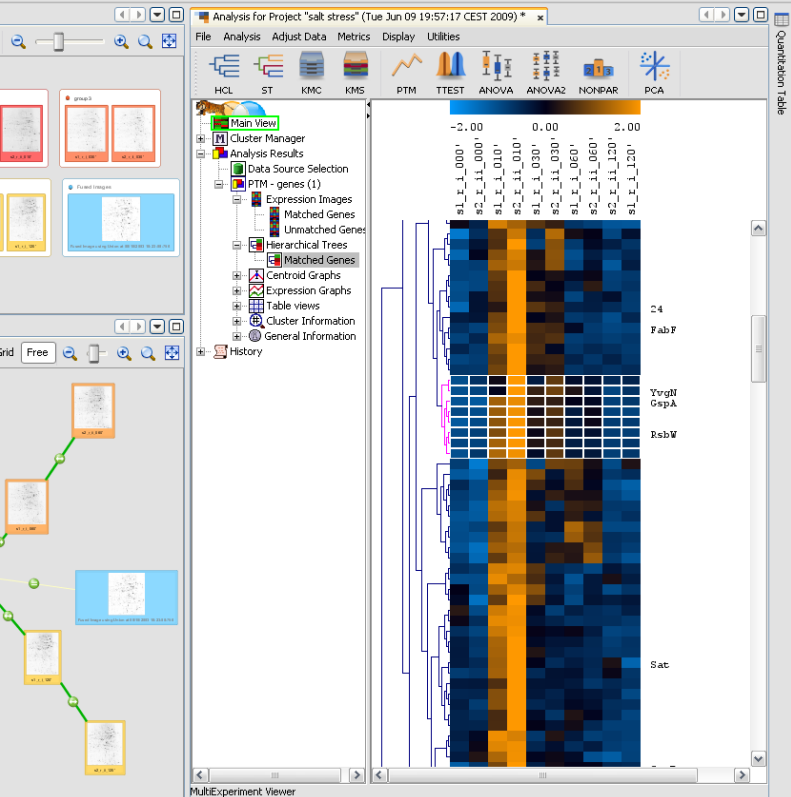
Warping Setup

Layout: Radial Hierarchical C

Gel Image Regions

Columns: 10

s1_r_j_000 s2_r_j_000 s1_r_j_010 s2_r_j_010 s1_r_j_030



Delta2D at a glance

Image Processing

Dual Channel Images (overlay of two images colored in false colors)	See differences in spot patterns at a glance, e.g. enables qualitative analysis of gel images
SmartVectors HQ™ Technology for automatic gel alignment, i.e. image warping	Minimizes hands-on time No need for initial landmarks Eliminates running differences between gels that prevent for a fast and reliable analysis Allows verification and adjusting of automatically found vectors
Image Fusion and Proteome Maps	Combine several gel images to one synthetic (but realistically looking) gel image See all spots of all conditions on just one image – the proteome map Minimizes time needed for spot detection and editing
Color Coding for Spots and Labels	Condense your analysis results in just one gel image, e.g. in your proteome map See at a glance the distribution and also outliers in a selected property (pI, MW, etc.) over a complete gel image
Background Subtraction	Compensates for strong and inhomogeneous background Complete visual and quantitative control

Export and Reporting

Interactive Web Reports (Project Report, Spot Album, and Spot Quantities)	Present and summarize your experimental setup, relevant spots, and quantitative data Can be put on the web easily as they are in HTML file format Copy all or part of a report into your favorite word processor or presentation program Allows to check in Delta2D as all reports are interactive Data ready for sharing and documentation
Export of Quantitative Data to Excel or as CSV file	Complete control about what will be exported by making columns and/or rows of the Quantitation Table visible or invisible Provide information to colleagues, collaborators, etc.
Export of Images (single or dual view, warped or unwarped, Fusion images or Color Coding Images) to Powerpoint, as Snapshot (TIFF, JPEG, PNG, PNM, BMP)	Use images for publication and presentation Edit spot boundaries and spot labels like Powerpoint objects
Support of diverse Spot Picking Devices	Export pick lists from Delta2D for these spot picking devices: Genomic Solutions ProPic PerkinElmer ProXCISION Molecular Dynamics Ettan Spot Handling Workstation Bruker Proteomeer Generic pick list format; Support for other devices can be provided

Quantitation

Support of virtually all Scanning Devices currently on the market	Import .tif, .tiff, .t14, .jpg, .jpeg, .png, .pnm, .gif, .bmp, .gel or .inf files Correct quantitation, incl. calibration information
Background Subtraction	Image-wide approach which copes with strong and inhomogeneous background so that you can rely on the results
Configurable Speckle Filter	Automatically removes speckles caused by several staining techniques (e.g. Sypro Ruby) Avoid finding of false positives due to presence of speckles that are spuriously quantified
Diverse Data Normalization methods	Select the normalization method that is suitable for your data Enables correct quantitative analysis of all kinds of 2D gel electrophoresis experiments
Quantitation on the original images	Reliable Data: Changes of spot shapes due to warping do not affect spot quantities
Remodeling of Spot boundaries after Transfer from the Proteome map	Adapted spot boundary for each spot on each image Changes in spot shape and/or size do not lead to incorrect quantification. Compensates for small warping mistakes

Statistical Analysis

Heat map display of expression profiles	Get a colorful visualization of all quantitative data of your project in just image See differences and similarities between expression profiles and gel images at a glance
Hierarchical Clustering (HCL)	Cluster expression profiles of spots or gel images, Optional bootstrapping and jackknifing support
k-Means/Medians Clustering (KMC)	Cluster expression profiles of spots or gel images
t-Test	Find significantly differentially expressed spots between two replicate groups, including control of False Discovery Rate
Pavlidis Template Matching (PTM)	Find spots that follow a given expression pattern
Analysis of Variance (ANOVA)	Find significantly differentially expressed spots between n replicate groups
Principal Component Analysis (PCA)	Detect structures in your experiment and find outliers
Nonparametric Tests	No normal distribution assumption is needed to apply these tests on your data No assumptions about the parameters of the distribution (e.g. mean, standard deviation, etc.) are needed. Includes Wilcoxon Rank Sum Test, Mann-Whitney Kruskal-Wallis Test, Mack-Skillings Test, Fisher Exact Test.

Data Handling

Use of Open Data Formats (open XML)	Easily read data and import it you're your databases
Store Data in Projects within Pools	All data related to your gel images, including the images themselves, in one folder only – the 'Pool' Transfer data to e.g. another computer easily Store several subprojects in just one folder
Spot Annotations kept independently from spots	Allows for re-detection, spot editing, and/or cancelling without losing annotations which can also contain information like pI, MW, etc. Export as you prefer: Images with or without spot annotations, or spot annotations only
Automated Web Data Retrieval with Decodon Scouts	No manual retrieval of information necessary Fully automatic: Scout data will be attached to the label of a certain spot Data is saved along with the gel pool so it does not need to be retrieved from the web again
Statistical Analysis can be opened independently from the rest of the project	Send the statistical analysis to a statistician to continue your analysis – an additional installation of Delta2D is not needed, the free statistics tool TIGR MEV can be used

Data Visualization

Color Coding for Spots and Labels	Condense your analysis results in just one gel image, e.g. in your proteome map See at a glance the distribution and also outliers in a selected property (pI, MW, etc.) over a complete gel image
Scatter Plot	Find interesting Spots. Check normalization method.
Bar charts of Expression profiles	Get a summary of a spots expression over a complete experiment Find interesting spots by just rolling over an image
Gel Image Regions	See all images of your project in one window to compare them and / or find corresponding spots
Show/Hide particular image overlays	See only those overlays that you are currently interested in, i.e. spot labels, spot boundaries, and/or match vectors.
Color Schemes	Choose any predefined or customized color scheme for the overlay shown in the Dual Window . Find Spots that are only present on one image, or on both images (i.e. find intersection, union, or complement sets)
3D Spots	See spots in a 3D view Check spot detection, i.e. find artifacts or spots that have to be deleted, splitted, or joined. Compare spots from two images



Delta2D 4.0



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