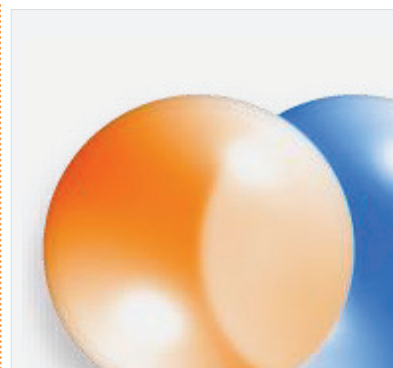
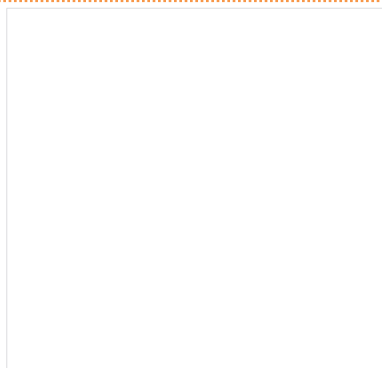
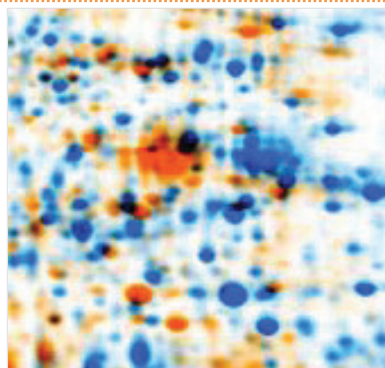


Delta2D

ANALYZING 2D GELS
AS EASY AS POINT AND CLICK



"The program is well balanced for new users as well as for advanced users as it gives an easy guideline through the process as well as menus to find more options when needed."

*Ellen Mosleth Faergestad
Ås, Norway*

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 be sure 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, i.e. without any missing value, since statistical analysis is much more reliable then. 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 visualization 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 made into reality in 2003 with Delta2D 3.1.

Analyze ALL your 2DE experiments with ONE software

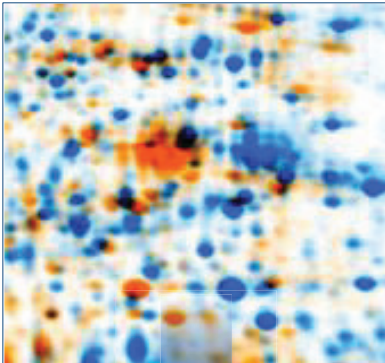
Two-dimensional electrophoresis has seen many innovations in the past. With Delta2D you can take advantage of all the different techniques: Classical experiments as well as multiplex approaches, e.g. with Refraction2D or DIGE and other experiments like Phospho- or Glyco-proteomics can easily be analyzed. 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

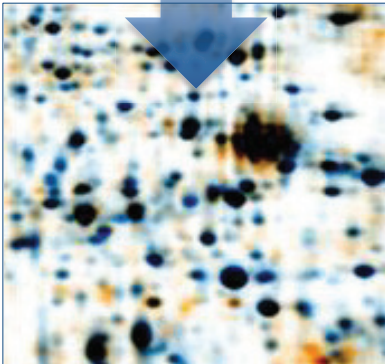
Established in 2000, we are committed to developing and delivering innovative software tools for modern life sciences and dedicated to customer satisfaction. Our team of mathematicians, computer scientists, and biologists carefully listens to you, analyses your needs, and transforms innovative ideas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

Deciding for Delta2D means deciding for innovation.

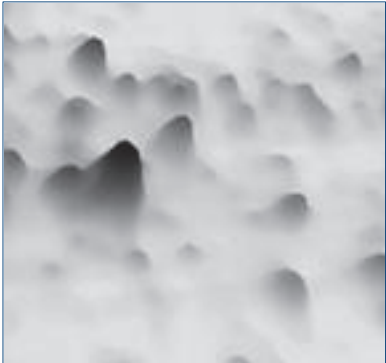
Dual color channel overlay (blue represents gel A; orange gel B) of two gel images before initialization of automatic warping.



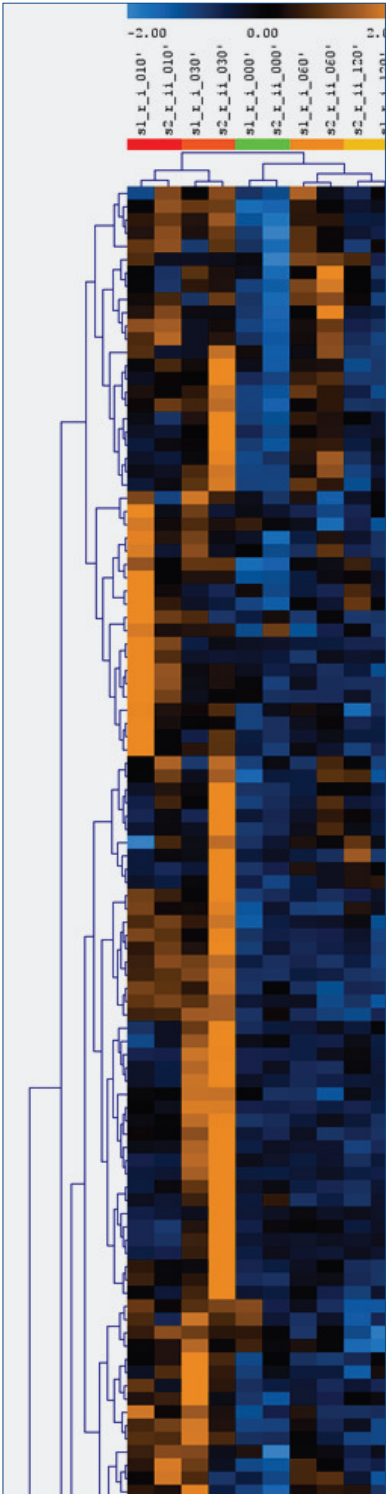
Result of the automatic warping process, black spots are present in both gel images with similar signal intensity.



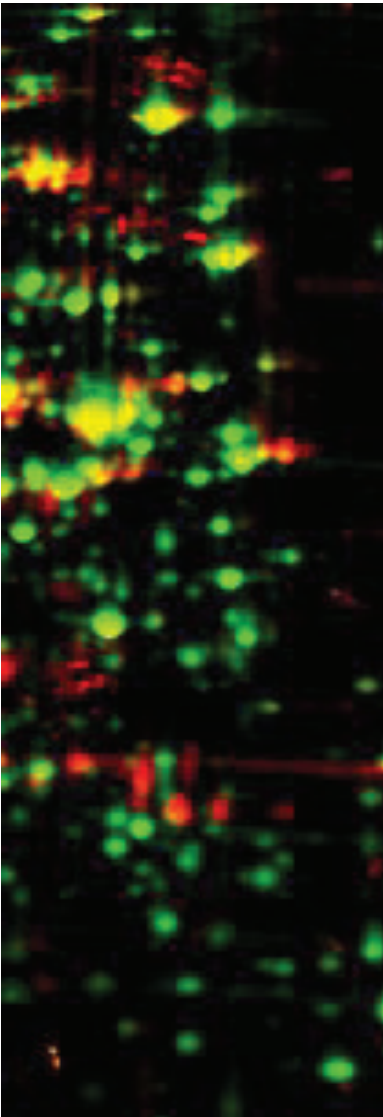
A 3D spot view helps to determine spot boundaries during spot editing.



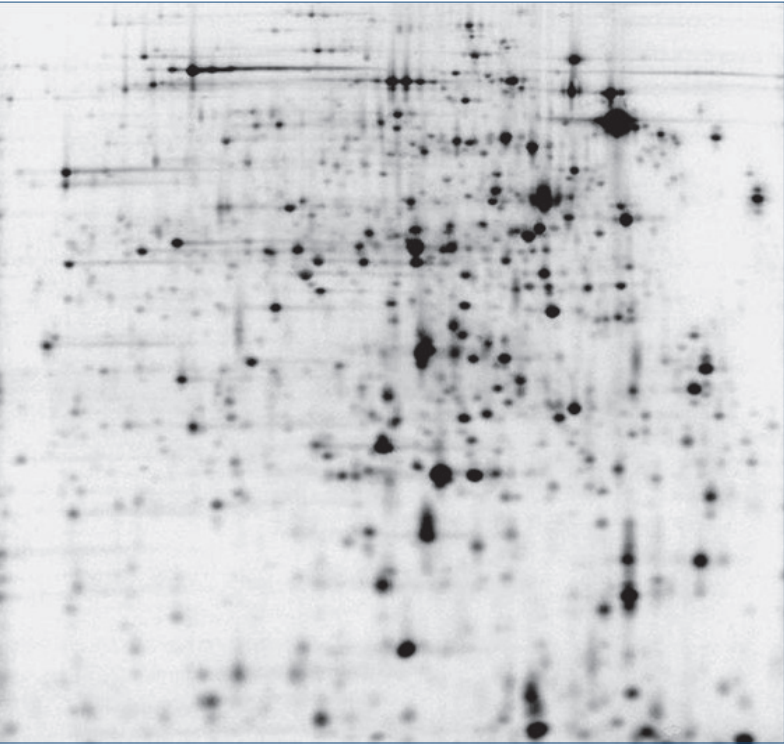
Complete expression profiles allow for the application of a variety of clustering and statistical approaches.



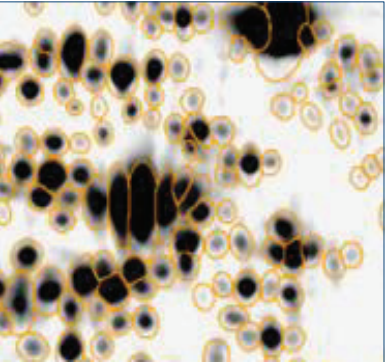
Multiplex image for the detection of phosphoproteins. (Flamingo protein dye – green; Diamond ProQ phosphoprotein dye – red). Multiplex Approaches are such as this one are supported by Delta2D.



2D gel image of a radiolabeled protein extract.



Gel image with detected and modelled spot boundaries



"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 as a guideline for the complete analysis process or enjoy the flexibility of the sophisticated capabilities of Delta2D.

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 at 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.

You are not forced to decide for a reference image to be aligned with all other images in your project. Use predefined warping strategies or freely connect images according to your individual experiment along the similarity of the gels.

As a result all spots have the same position on each image – as if you had made perfect gels without any distortions.

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.

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.

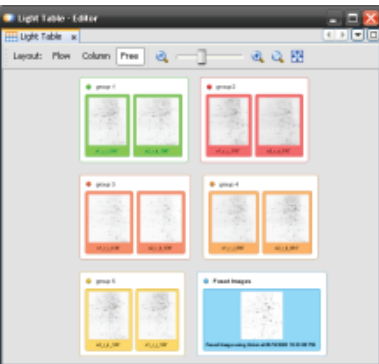


Delta2D introduces innovations, e.g.:

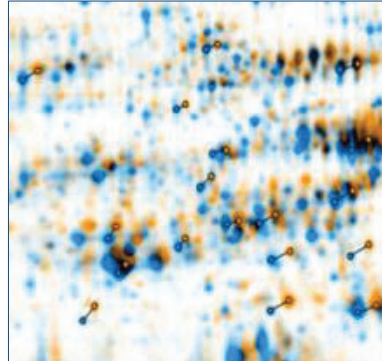
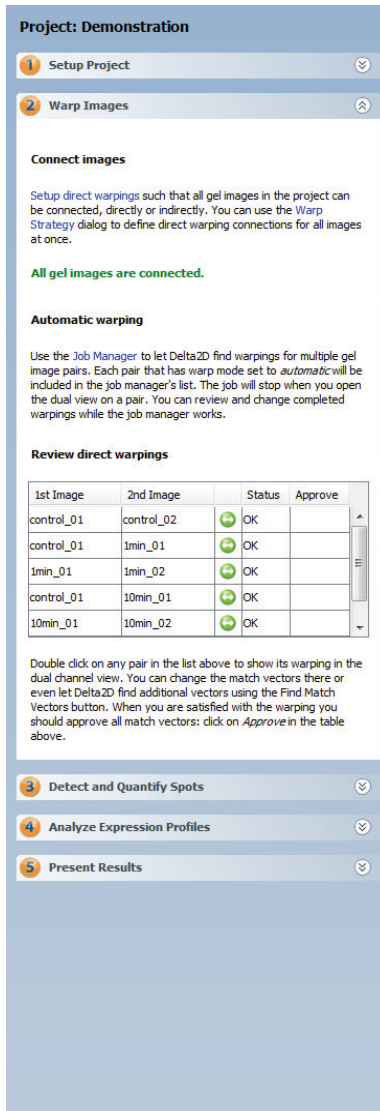
Gel image warping (alignment of spot positions) was introduced with Delta2D 1.0 in year 2000. The concept of creating complete expression profiles resulting in 100% spot matching was made a reality in 2003 with Delta2D 3.1. In 2006, with Delta2D 3.4, SmartVectorsTM were introduced to provide a transparent 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 guided workflow and a more effective window management.

Delta2D – decide for leading technology.

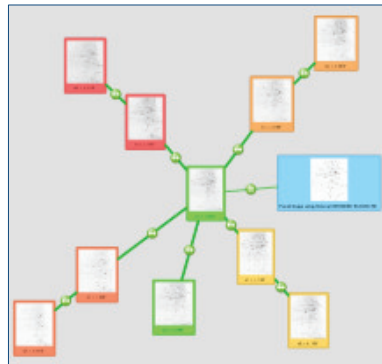
Step by step the Workflow component of Delta2D guides you through the whole image analysis process.



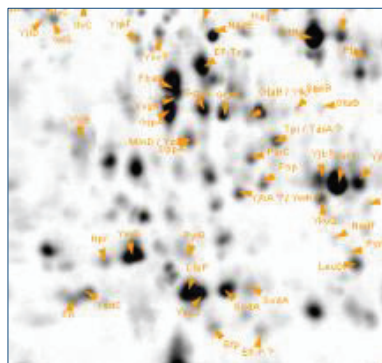
The Light Table supports project organization, e.g. naming of groups and gel images and grouping of replicates.



Warp vectors represent correspondences between two gel images.

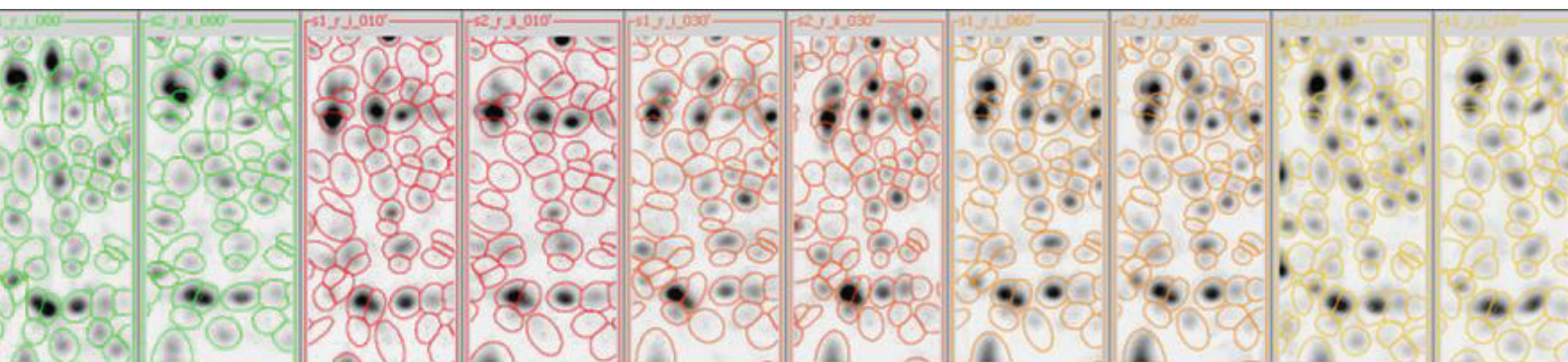


The Warping Setup shows how pairwise warplings are defined across the project.



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

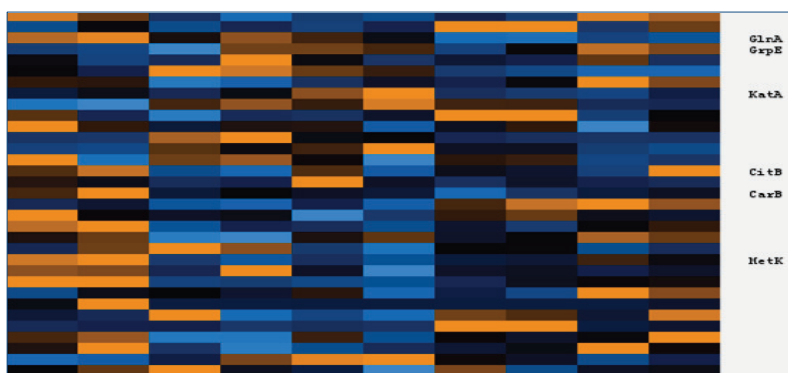
... while transfer of this spot pattern results in 100% matching spots for all gel images of your experiment.



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, matching problems are eliminated. This also results in highest statistical confidence as there are no missing values. Even methods that were originally designed for DNA microarray analysis can now be applied to 2D gel data.

Since version 3.6, Delta2D incorporates algorithms provided with 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 to more detailed analyses. These methods are currently available:

- **Hierarchical Clustering (HCL),**
- **k-means / k-medians Clustering (KMC).**

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.

Another method for identifying structures with the data is **Principal Component Analysis (PCA)**. Furthermore, **Pavlidis Template Matching (PTM)** allows for selecting proteins that follow a given expression pattern.

Nonparametric tests – the gold standard for spot quantities

Nonparametric tests do not require a normal distribution assumption. They are therefore especially suitable for spot quantities. In Delta2D, these nonparametric tests can be applied since version 4.0:

- **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.

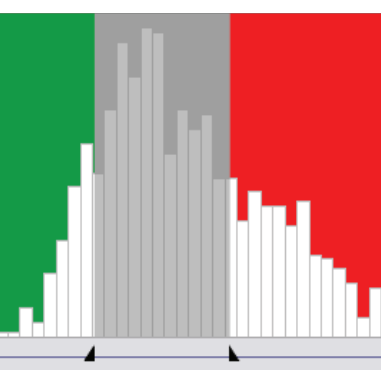
Find significantly changed spots

In the simplest case, the experiment is a comparison of two samples, e.g. diseased vs. control tissue, mutant vs. wild type etc. The challenge 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, different variations of the t-Test are available.

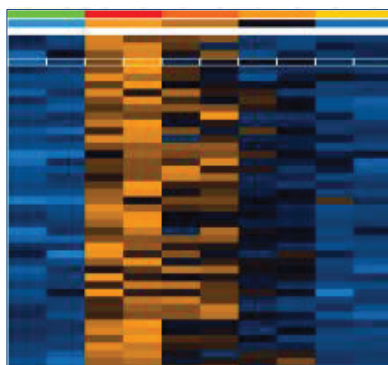
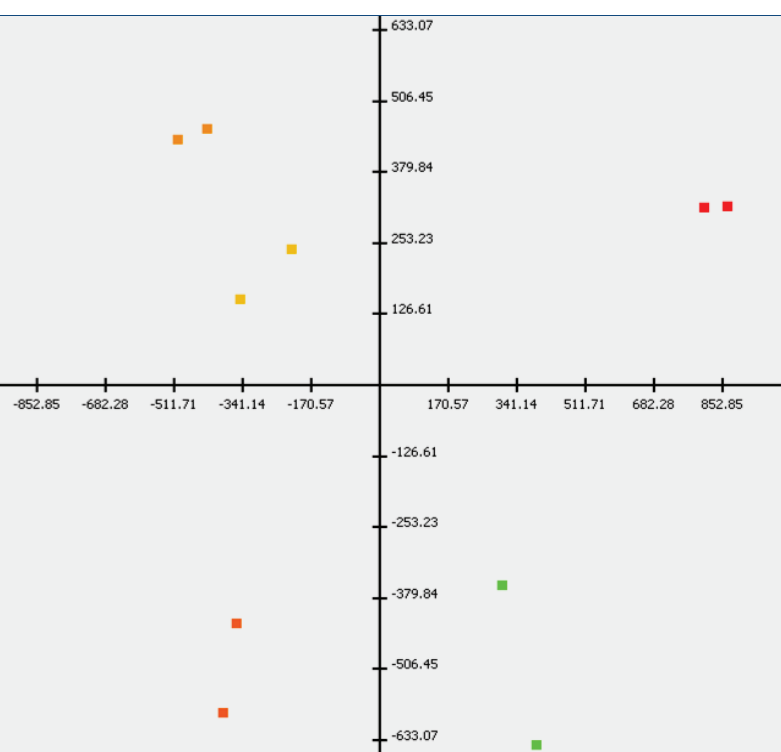
Spots that have significant differences in means across three or more groups of samples can be found with the **Analysis of Variance (ANOVA)**. In Delta2D, **one-way** analysis of variance and **two-factor** analysis of variance are implemented.

All tests can be supplemented with controlling the **False Discovery Rate (FDR)**.

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 and can be combined to find spots that match many different criteria.



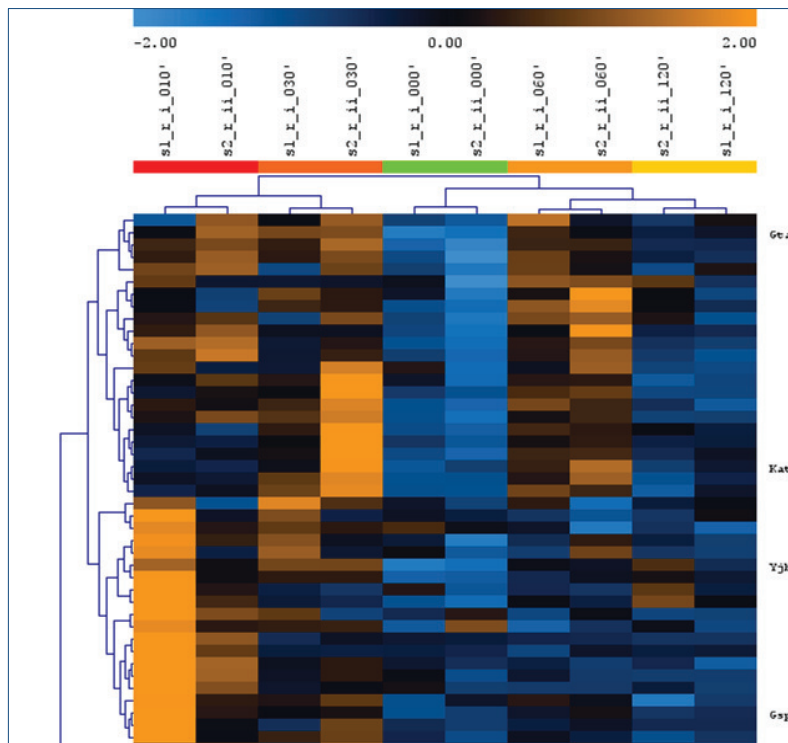
Principal Component Analysis is a tool to reduce data complexity in multidimensional data sets. A set of data points were projected onto a two dimensional data space. This shows that samples clearly differ from each other while replicates are closely related.



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

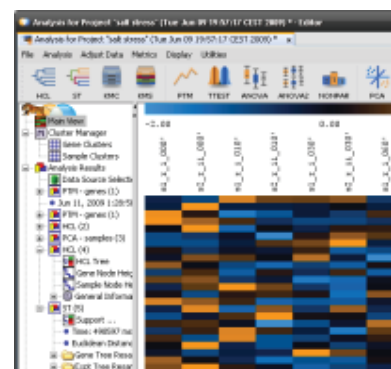
The Quantitation Table gives an overview of the expression data of your gel images.

s1_e_1_010'	s2_e_1_010'	s1_e_1_030'	s2_e_1_030'	s1_e_1_000'	s2_e_1_000'	s1_e_1_060'	s2_e_1_060'	s1_e_1_120'	s2_e_1_120'	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	GrpE
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	0.098	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	0.015	0.023	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	
0.289	0.325	0.212	0.197	0.288	0.204	0.251	0.245	0.219	0.352	CitB



Hierarchical Clustering sorts samples (columns) according to similar global expression of proteins, or protein spots (rows) according to similar expression on all gel images.

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.

Export tables and images

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 – flexibly configure visibility of table columns and / or images since what you see is what is exported,
- to PowerPoint slides – works for single, dual channel, and color coding images (spot boundaries and labels can be treated like real PowerPoint objects.),
- as snapshots (single or dual channel images and Color Coding images can be exported as TIFF, JPEG, PNG, PNM, BMP).

Export picklists

If a spot picker is available, easily export the positions of the interesting spots as spot picking lists. The following devices are supported:

- Molecular Dynamics
- Genomic Solutions ProPic
- PerkinElmer ProXCISION
- Ettan Spot Handling Workstation
- Bruker Proteineer
- BioRad Exquest
- Herolab

Or just use the generic pick list format.

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.

"Delta2D has greatly enhanced our ability to quickly and more reliably align our gel images, which greatly reduces errors in protein spot reproduction and alignment. The alignment feature in Delta2D is superior to the warping feature we were using with our previous software, and as a result our gel processing times have decreased significantly. This allows us to get to the actual protein spot analysis more quickly, make decisions, and move on to the next experiment."

*Joseph MacFarland
Framingham, USA*

DECODON Scouts – Retrieve data from databases in the web

DECODON Scouts are small software applications that go out to web resources such as UniProt, GenBank or GenList and come back with useful information about a protein on a gel.

Scout data can be protein properties such as isoelectric point or molecular weight, annotations such as pathway information, sequences, and much more.

The information being retrieved by scouts is attached to labels. The data is organized in groups of related data about a protein, such as biochemical data containing isoelectric point and molecular weight, or GenBank data containing sequences and accession numbers.

The data is saved along with the gel pool so they do not need to be retrieved from the web again.

Example:

UniProt Scout

- it fetches **function, GeneOntology (GO), keywords, sequence, amino acids, and references,**
- **theoretical pI** and **MW** are determined automatically using the **EMBOSS pepstats** web service,

Labels and the retrieved Scout data can easily be exported using Delta2D's Label report (see below).

Interactive web reports – The modern way of exporting data

Delta2D's web reports make it easy to present and summarize your experimental setup, relevant spots, and quantitative data. They are based on HTML so you can put them on the web easily.

The reports are shown in your web browser. You can copy all or part of a report into your favorite word processor or presentation program.

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 information about expression profiles in the form of the respective areas on the images.

Spot Quantities includes detailed quantitative information about expression profiles.

Labels include information about all labels on a certain image or those belonging to a certain set of spots on a an image, including Scout data if available (e.g. theoretical pI and MW, or GO as retrieved from UniProt) and Delta2D's estimated pI and MW.

All reports are interactive: They include controls to change the reports' content. Furthermore they are all connected with Delta2D: For example, clicking on a dual channel image in the Project Report will open the corresponding gel image pair in Delta2D, or clicked spots will automatically be highlighted in Delta2D. All reports can be accessed via the Reports menu in the Project Manager. The report design is based on a HTML stylesheet, so they can easily be adapted to any corporate design.

Show expression profiles
in the form of the respective
image thumbnails.



Automate Your Queries with
DECODON's **UniProt** scout.

Scout Data Editor

Uniprot
Label: GroEL

AureoList Data Table GenBank GenoList Physicochemical properties Uniprot

Input

Query: GroEL Get Data

Organism: Bacillus Subtilis

Data

EKLQERLAKLAGGVAVIKVGAATETELKERKLAIEDALNSTRAAVEEGIVSGGGTALNV
YNKVAAVEAEGDAQTGINIVLRALDEEPIRQIAHNAGLEGSVIVERLKNEEIGVGFNAATG
EWNMIEKGIVDPTKVTLSALQNAASVAMFLTTEAVVADKPEENGSGGAGMPDMGGMGM GGM

Isoelectric Point
4.4284

Molecular Weight
57424.41

Amino Acids
544

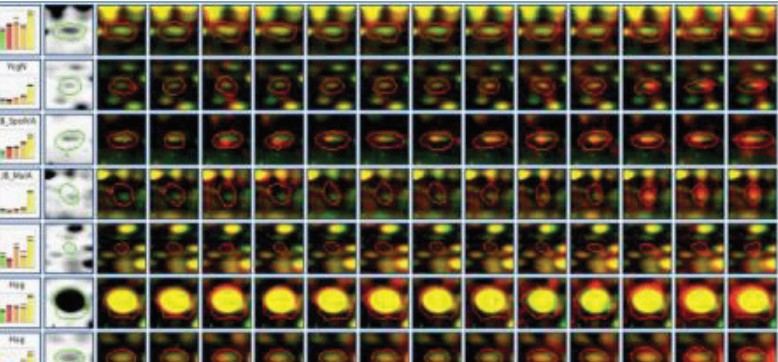
Function
Prevents misfolding and promotes the refolding and proper assembly of unfolded polypeptides generated under stress conditions (By similarity).

Keywords
● ATP-binding
● Chaperone
● Complete proteome

Info OK Cancel

The Labels Report lists all available
label data, including the data
retrieved by a DECODON Scout.

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.



All Labels on Image 10min									
Labels for selected spots on image 10min									
Show labels on image 10min Change									
Table shows label data for all labels on gel image '10min'. If a label is annotating a detected spot, this spot is shown									
Label		Pi/MW Estimation		Spot		Uniprot			
Name	X	Y	Pi	MW	ID	Accession	Amino Acids	Function	Gene name
AhpC	825	076	4.06	24220	13405	P80239	187	Directly reduces organic hydroperoxides in its reduced dithiol form.	ahpC
						Gene Ontology			
						● F:peroxidase acti			
						[GO:0004601]			
						● F:peroxidoxin			
						[GO:0051920]			
						● P:cell redox			
						homeostasis			
						[GO:0046464]			

„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*

Premium service is our standard

When you decide for Delta2D you do 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

Enjoy working with Delta2D!

We provide support

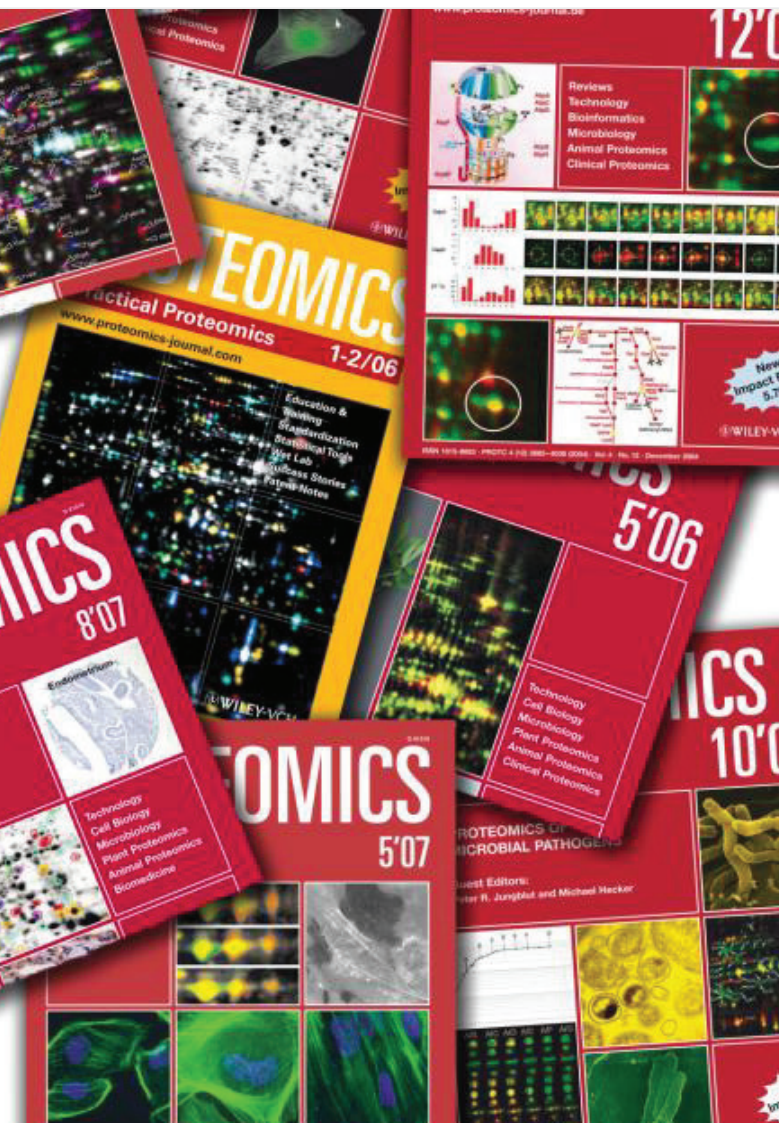
- to introduce Delta2D to new users,
- to discuss experimental design,
- to help with individual problems,
- to guide through statistical analysis.

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

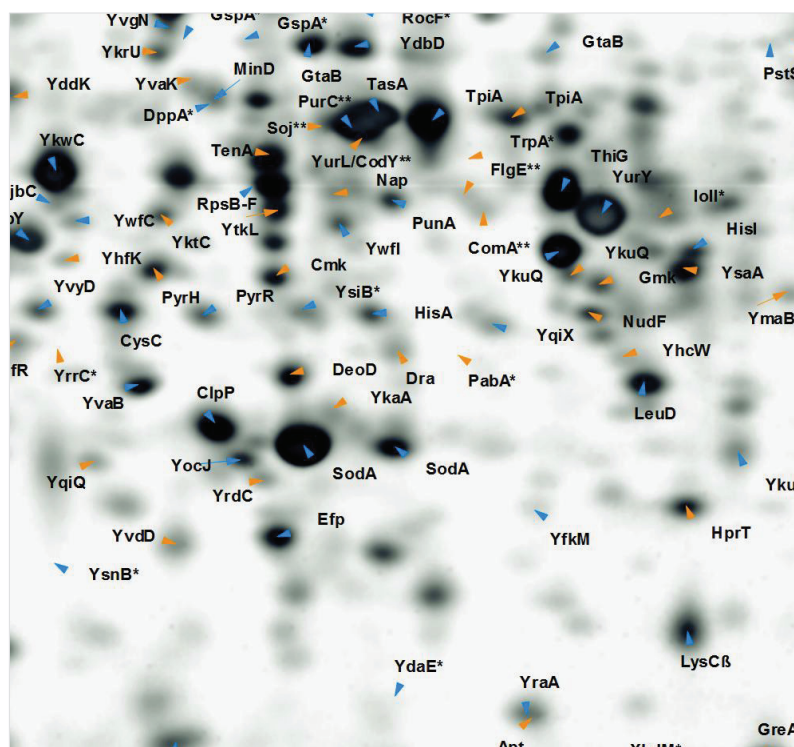
*Daniel Kay
Porirua, New Zealand*

Web sessions – Just sit back and see how to solve your problem

The DECODON support team often uses a very time-efficient way to support Delta2D users: Interactive web sessions. You only need a computer with internet connection and a phone. A support member will call you at an agreed time to have a look on your screen and to present a solution. With remote-control enabled by you, we can even walk you through the process.



Part of a 2D reference map of *Bacillus subtilis* that contains more than 1000 identified spots. Among others this proteome map is maintained by Delta2D.



Delta2D supported scientific work in hundreds of publications. Many of them were published in peer reviewed journals such as "Journal of Proteomics"



Several publicly accessible 2D gel databases host gel based proteome data which have been generated with Delta2D.

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
Speckle Filter	Automatically removes black and white speckles transparently caused by several staining techniques (e.g. Sypro Ruby) leading to better spot detection and more reliable quantitation Avoid time-consuming looking at false positives due to presence of speckles that are spuriously quantified
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, Spot Quantities, and Labels)	Present and summarize your experimental setup, relevant spots, quantitative data, and annotations (incl. database proteome details) at a glance. 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 as Powerpoint objects	
Export pick lists from Delta2D for common spot picking devices	Molecular Dynamics Genomic Solutions ProPic PerkinElmer ProXCISION Ettan Spot Handling Workstation Support for other devices can be provided	Bruker Proteineer BioRad Exquest Herolab Generic pick list format

Quantitation

Support of virtually all Scanning Devices currently on the market	Import .tif, .tiff, .ti4, .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
Various 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

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 (various methods)	Find significantly differentially expressed spots between two replicate groups, including control of False Discovery Rate (FDR)
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, and Fisher Exact Test.

Statistical Analysis

Use of Open Data Formats (open XML)	Easily read data and import it into 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 from e.g. UniProt or GenBank database 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 Handling

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 merged, compare spots from two images

Data Visualization



Delta2D



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