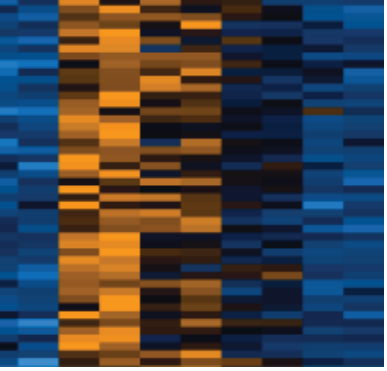
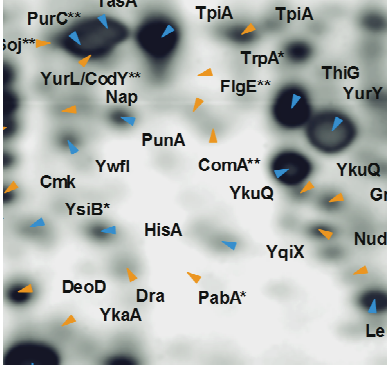
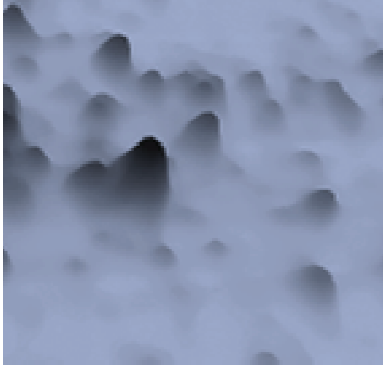
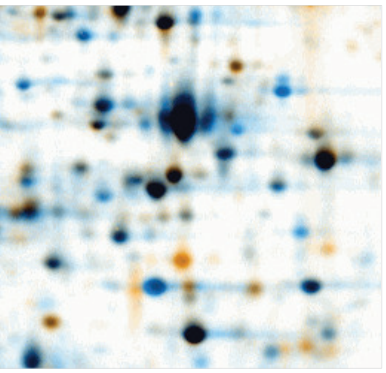


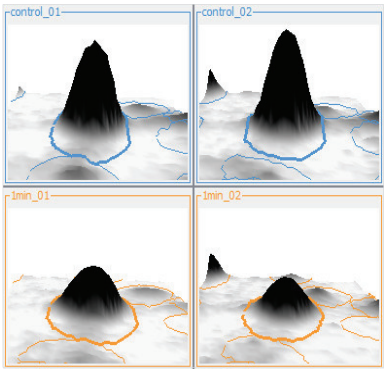


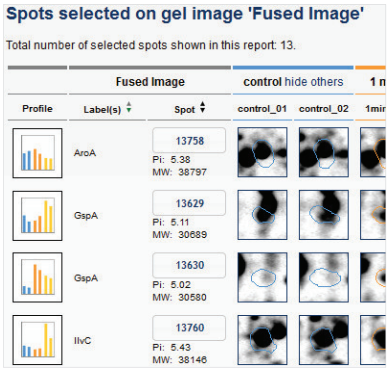


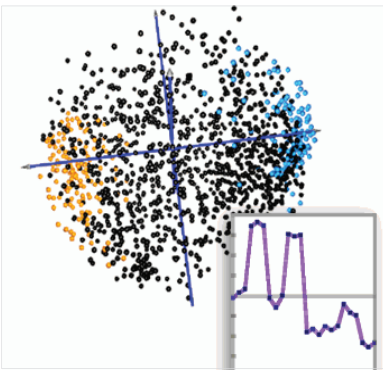
Delta2D

ANALYZING 2D GELS
AS EASY AS POINT AND CLICK









EXPLORING LIFE

DECODON

"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.
As Delta2D is based on modern technologies you get reliable and statistically significant results. Furthermore with Delta2D you reduce the analysis time to a minimum – your time matters to us.

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 as well as 2D western blots can be analyzed easily.
Whatever you want to explore, Delta2D will help you to get the most information out of your gels.

More reliable results, better statistical analysis

Delta2D includes an approach that generates complete expression profiles, i.e. without missing values, to make 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 easily prepare them for publication and presentation is even better. Delta2D helps you with that: Use a broad range of modern data visualization and various exporting features.

Why Delta2D?

- Workflow support for beginners
- Complete expression profiles
- Automatic image alignment to remove gel-to-gel distortions
- Spot detection and editing only on one image necessary
- Advanced statistical methods, based on absence of missing values
- Various visualization, reporting and exporting features
- Flexible pricing/licensing models
- Available for current 32 and 64Bit Windows, Mac, and Linux operating systems

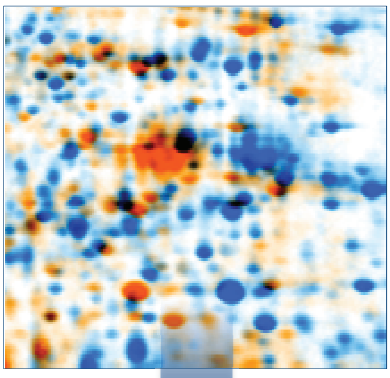
Gel image warping (alignment of spot positions) was introduced in 2000 and improved by SmartVectors™, Delta2D's leading technology for automatic gel alignment.
The idea to create complete expression profiles resulting in 100% spot matching has been invented by DECODON to increase statistical confidence significantly.

Deciding for Delta2D means deciding for innovation.

DECODON – committed to customer satisfaction and innovation

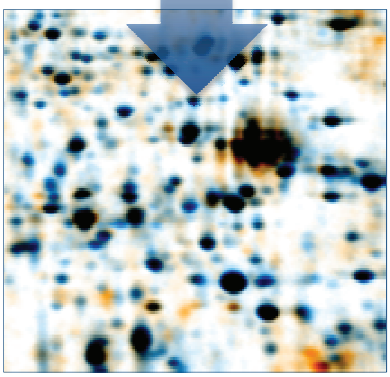
Established in 2000, we are committed to developing and delivering innovative software tools for modern life sciences and dedicated to customer satisfaction. Our team of mathematicians, computer scientists, and biologists carefully listens to you, analyses your needs, and transforms innovative ideas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

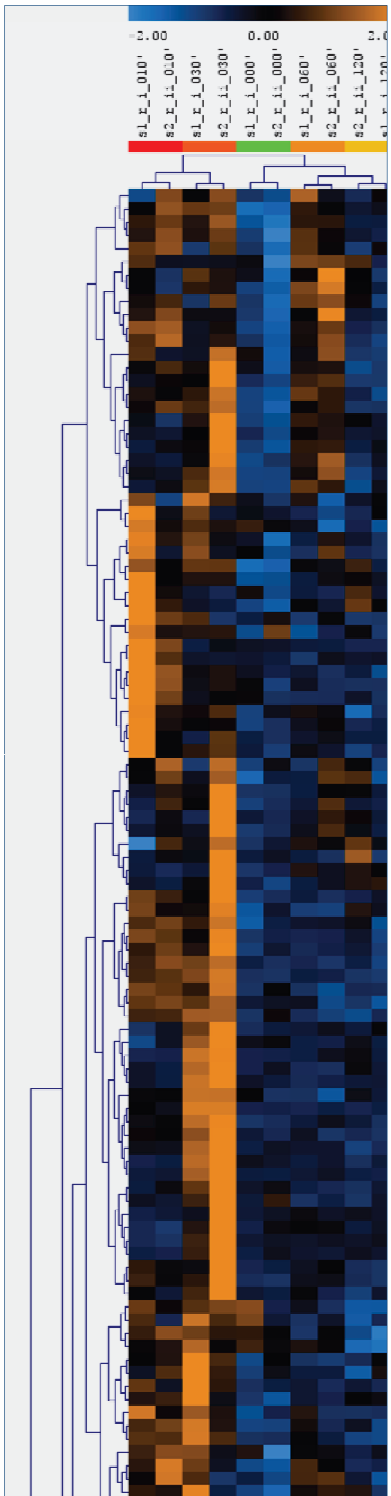
Dual color channel overlay of two gel images (blue represents gel A; orange gel B) before initialization of automatic warping.



3D spot views help to determine spot boundaries during spot editing.

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

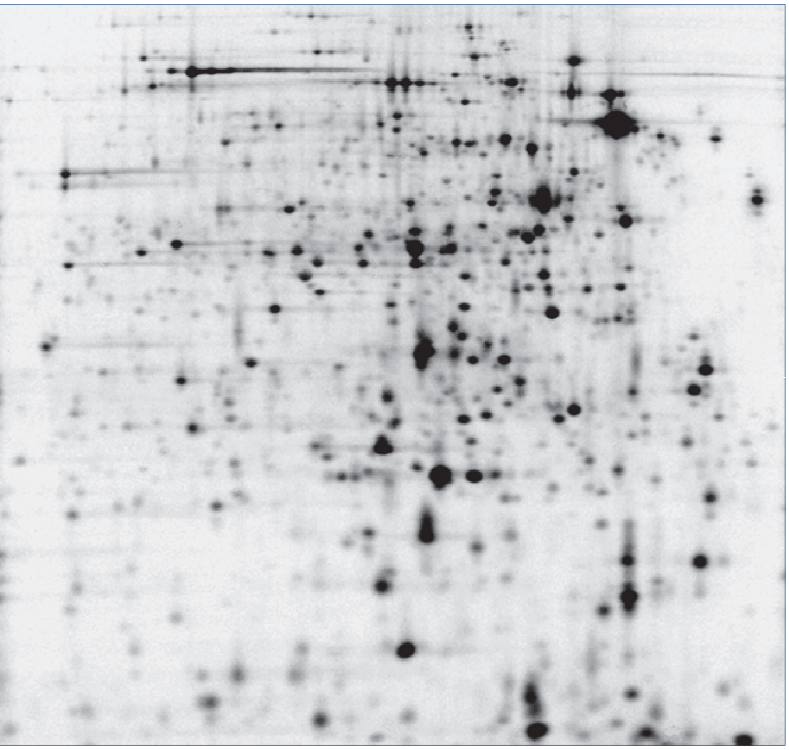


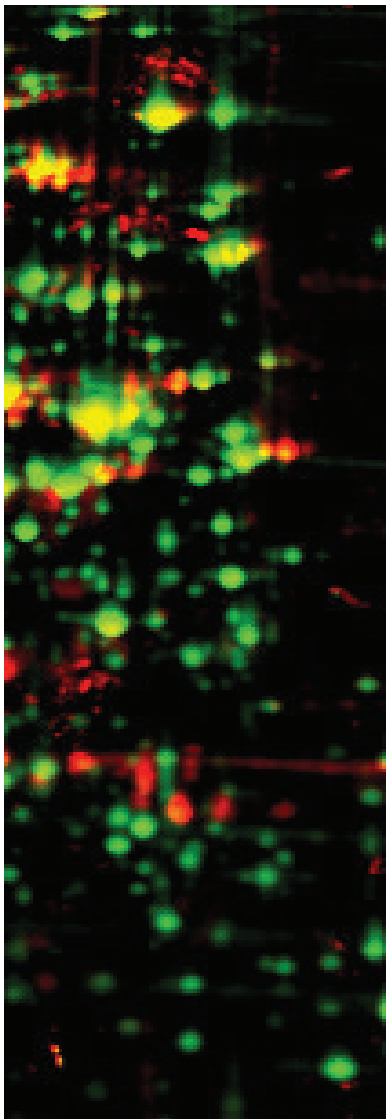


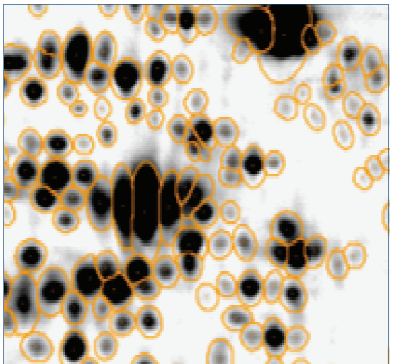
Complete expression profiles allow for the application of a variety of clustering methods and statistical test.

Multiplex image for the detection of phosphoproteins. (Flamingo protein dye – green; Diamond ProQ phosphoprotein dye – red). Multiplex approaches 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.

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

Delta2D's SmartVectors™ technology (which replaced gel image warping as introduced by DECODON with Delta2D 1.0) 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 perfect gels without any distortions.

The missing values problem ... no problem for Delta2D

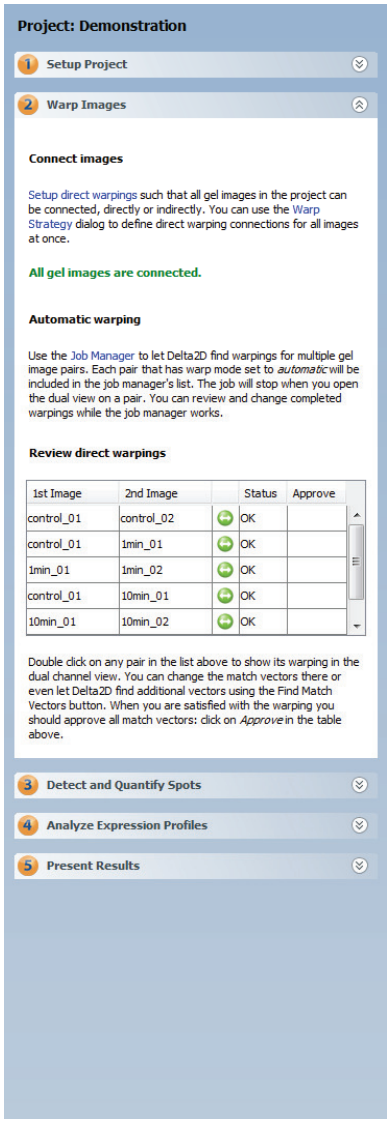
All images of a project can be fused into one synthetic image using the Union Fusion algorithm (introduced in 2003). The resulting image looks like a real gel image and is actually the proteome map for your project – containing all spots from all images of the project.
One image with all spots – a perfect basis for spot detection and editing. Let Delta2D transfer the complete pattern of spot boundaries from your proteome map to all the images in your project and spot matching is finished – completely and correctly.

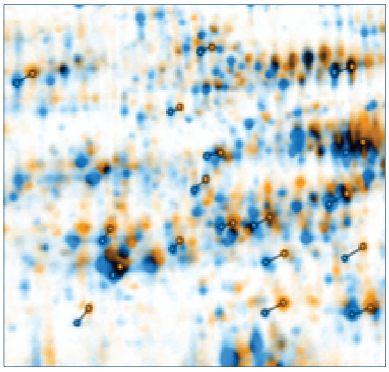
Delta2D introduces innovations, e.g.:

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. In 2006 SmartVectors™ were introduced to provide a transparent and hybrid method to integrate automatic and manual image warping. Web reports have been developed in 2007, along with advanced multivariate statistical methods. Since 2008, Delta2D offers a guided workflow and a modern, more effective window management. Many further improvements and innovations followed – among them a speckle filter, 3D view across all images in 2014, and export of images as presentation slides.

Delta2D – decide for leading technology.

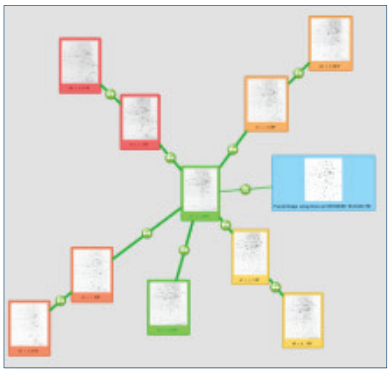
Workflow component of Delta2D guides you step by step through the whole image analysis process.



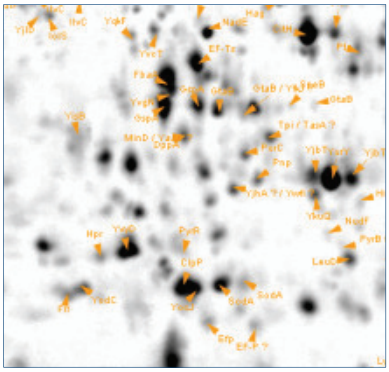


Warp vectors represent correspondences between two gel images.

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

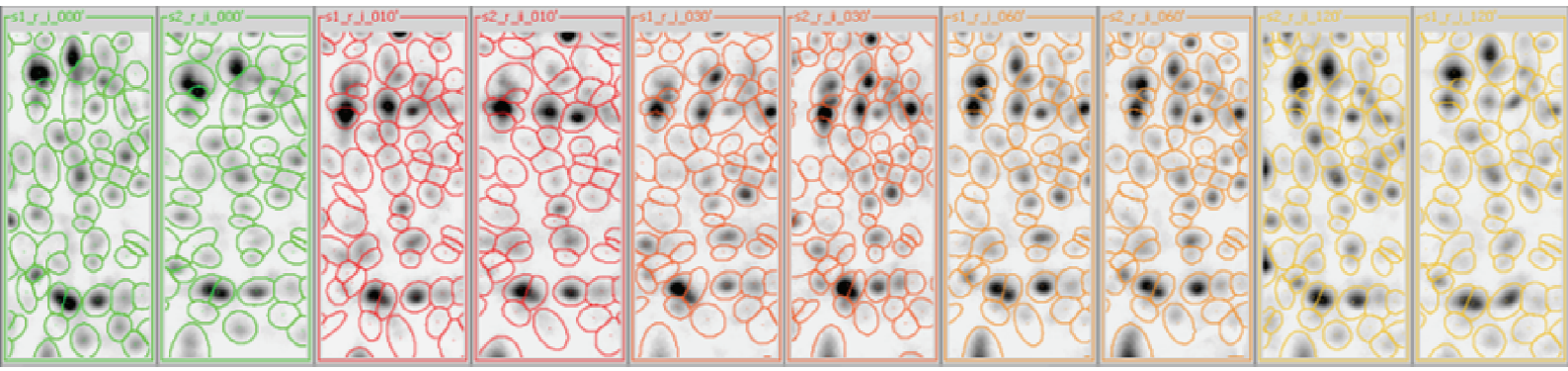


The Warping Setup shows how pairwise warpings connect all images in your project.



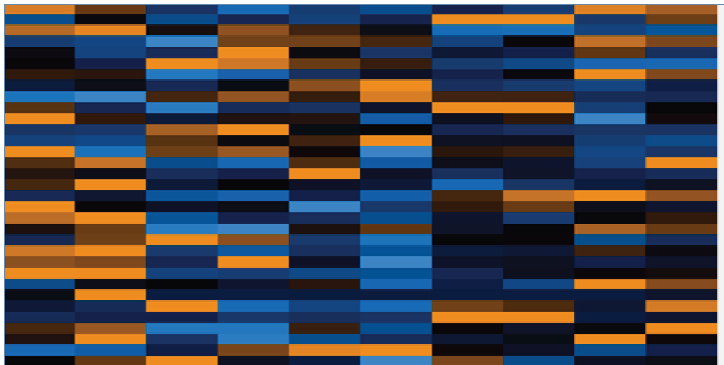
After positional correction, images are fused into a fusion image condensing the spot pattern of the whole experiment in one image. Spot identifications can be managed on the fusion image. Spot detection and editing is performed here only once per experiment …

… 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. It also results in highest statistical confidence as there are no missing values.
Delta2D incorporates advanced algorithms provided and tightly integrates them into the 2D gel image analysis workflow.

Identify structures in your data and detect outliers

A very powerful method to detect outliers is **Principal Component Analysis (PCA)** or clustering methods.
Both can also be used to identify structures in the experiment. Ideally, the cluster composition will reflect the real structure of the experiment, i.e. replicates and images from the same sample should have similar expression levels and thus end up in the same cluster.
Clustering also help to group expression profiles. This can be very useful for getting an overview of all expression profiles before proceeding to more detailed analysis.
These methods are currently available:

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

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

Nonparametric tests – the gold standard for spot quantities

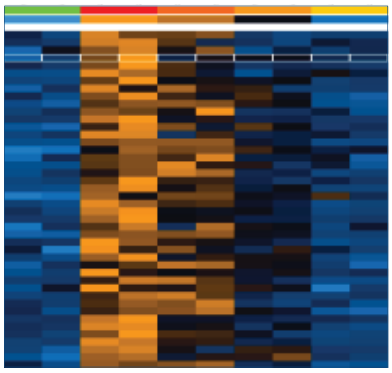
Nonparametric tests do not require a normal distribution assumption. They are therefore especially suitable for small projects.
In Delta2D, these nonparametric tests can be applied:

- **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 non-random associations between two categorical variables.

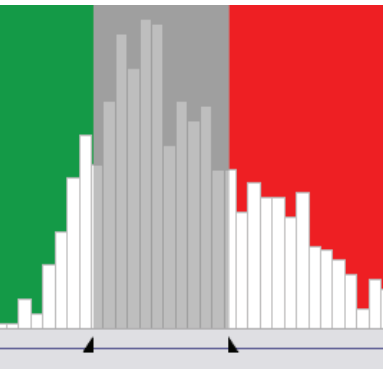
Find significantly changed spots

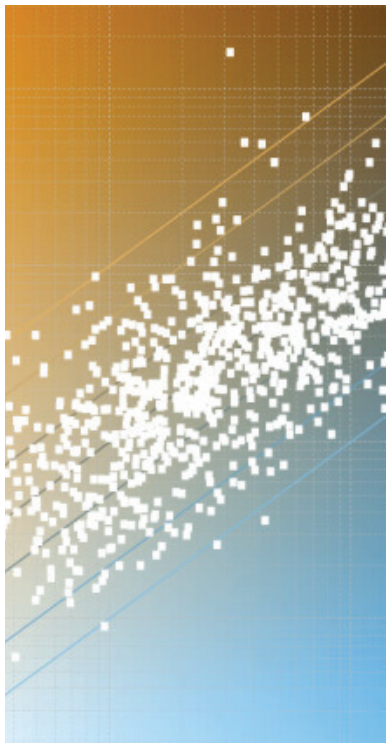
In the simplest case, the experiment is a comparison of two samples, e.g. diseased vs. control tissue, mutant vs. wild type etc. The challenge is finding those proteins that show significant differences in expression levels.
Certainly the most popular test in this area is **Student's t-Test**. In Delta2D, 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)**, since multiple testing can be a real pitfall.

You can use filters to find protein spots within a specified induction range, within a specific intensity class or with other properties of interest.
Filters can be combined to find spots that match a combination of different criteria.

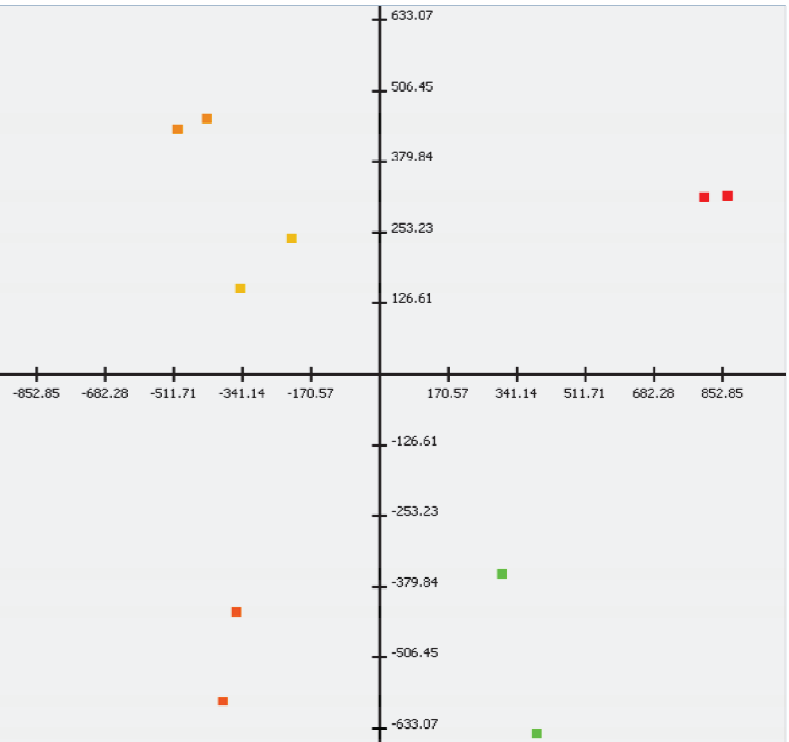


If you are looking for certain kind of expression profiles, you can predefine a template (shown in the second row of the heat map header).
All spots behaving similar to the profile template will be found.



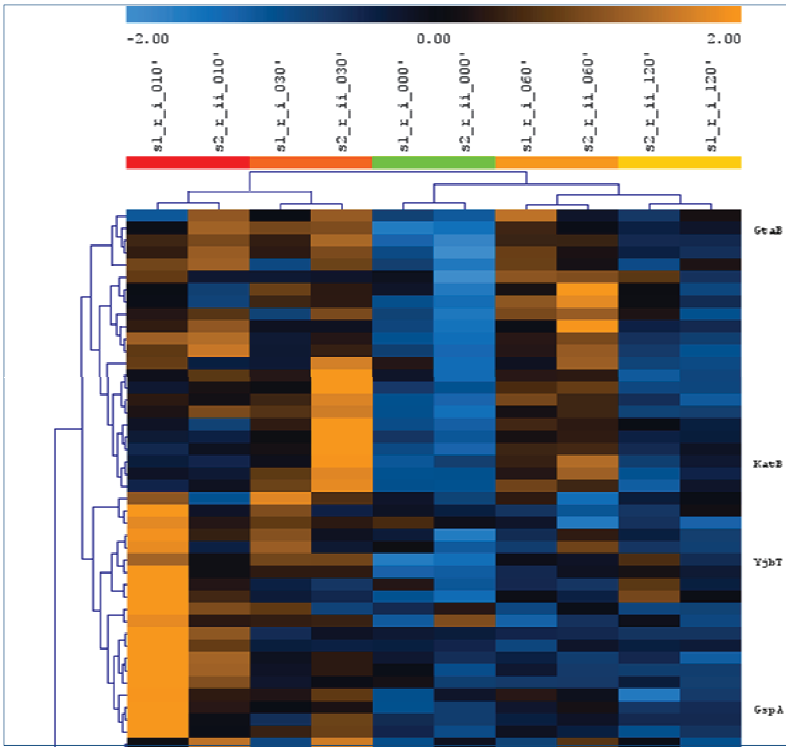


Principal Component Analysis is a tool to reduce data complexity in multidimensional data sets.
A set of data points were projected onto a two dimensional data space.
This shows that samples clearly differ from each other while replicates are closely related.



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

s1_r... %V	s2_r... %V	s1_r... %V	s2_r... %V	s1_r... %V	s2_r... %V	s1_r... %V	s2_r... %V	s2_r... %V	s1_r... %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	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	0.027	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	
0.289	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 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

- as MS Excel worksheets – flexibly configure visibility of table columns since what you see is what is exported,
- as MS PowerPoint slides – works for single, dual channel, and color coding images (spot boundaries and labels appear as MS PowerPoint objects),
- as CSV-files – to be imported to 3rd party software,
- as snapshots – single or dual channel images and Color Coding images can be exported in standard image file formats (like TIFF, JPEG, PNG, PNM, BMP).

Standard MS Office files can also be opened by other packages like LibreOffice or OpenOffice.

Color Coding – Summarize your results on a single image

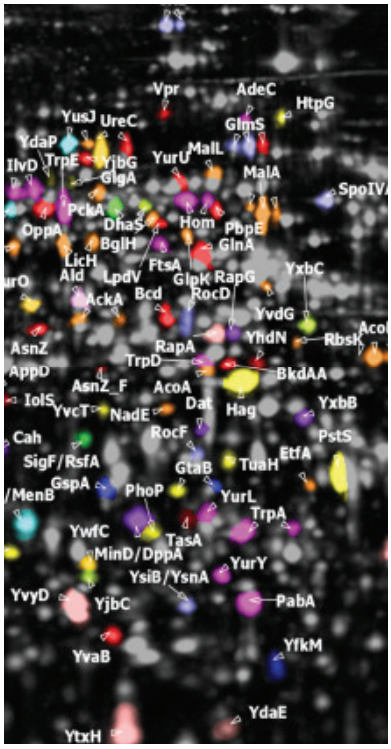
Spot Color Coding lets Delta2D display a proteome map (or a single gel image) with spots colored according to their expression profiles. **Dynamic label coloring** can be used as an indicator e.g. for the theoretical isoelectric point or the molecular weight of identified spots. Thus you can at a glance see outliers (e.g. post-translational modifications) over the complete gel image.

Export picklists

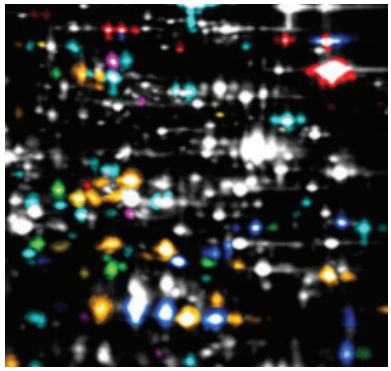
If a spot picker is available, easily export the positions of the interesting spots as spot picking lists. The following devices are supported:

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

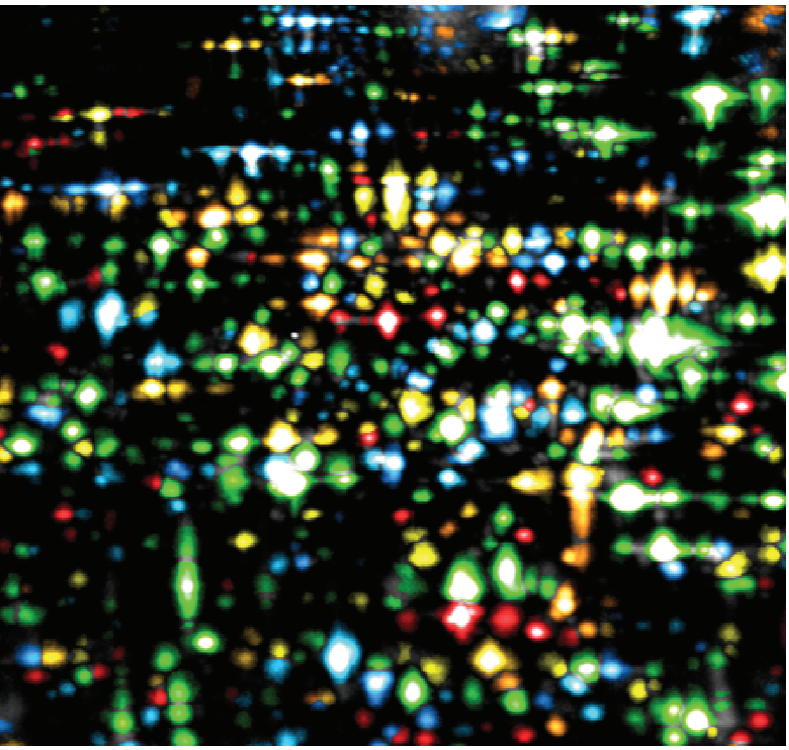
Or just use the generic pick list format.



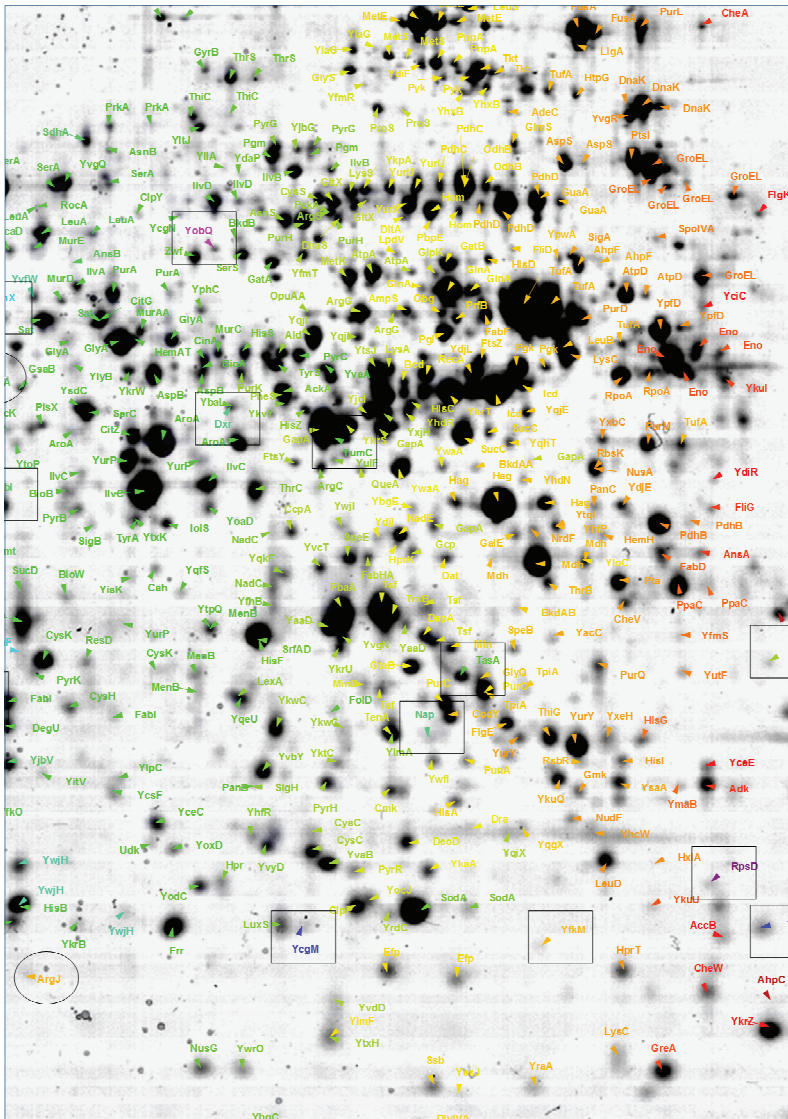
Subset Color Coding produces a proteome map that shows which proteins get activated or repressed by certain combinations of experimental conditions, treatments, or stimuli.



Min/Max Color Coding colors spots according to their maximum (or minimum) intensity within a series of samples.



Color Coding according to isoelectric point highlights protein modifications in charge.



"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 GenoList and come back with useful information about a protein on a gel.
Scout data can be protein properties such as protein sequence, isoelectric point or molecular weight, annotations such as pathway information, functional categories, 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 it does 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).

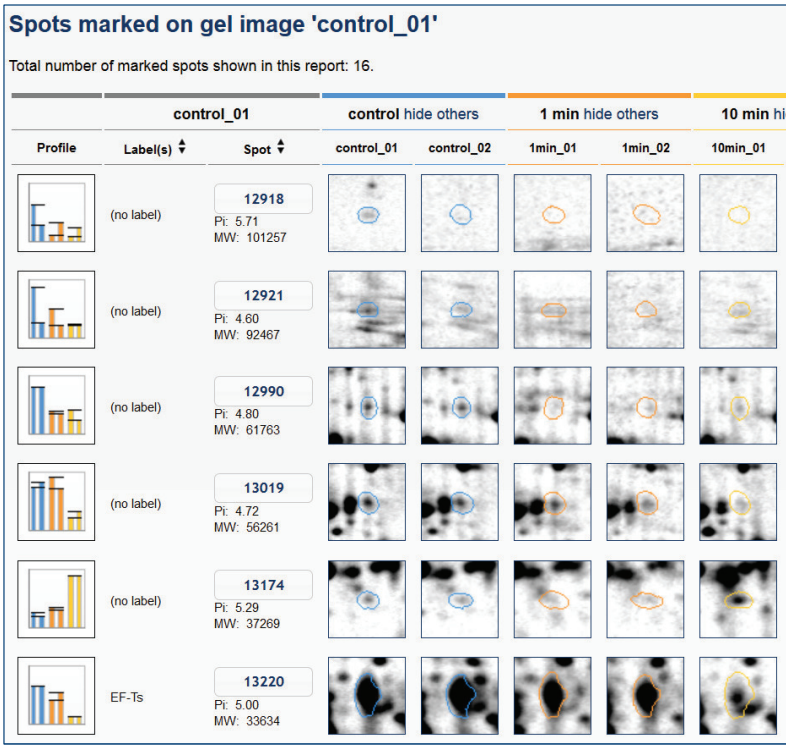
Spot Album report includes information about expression profiles in the form of the respective areas on the images.

Spot Quantities report includes detailed quantitative information about expression profiles.

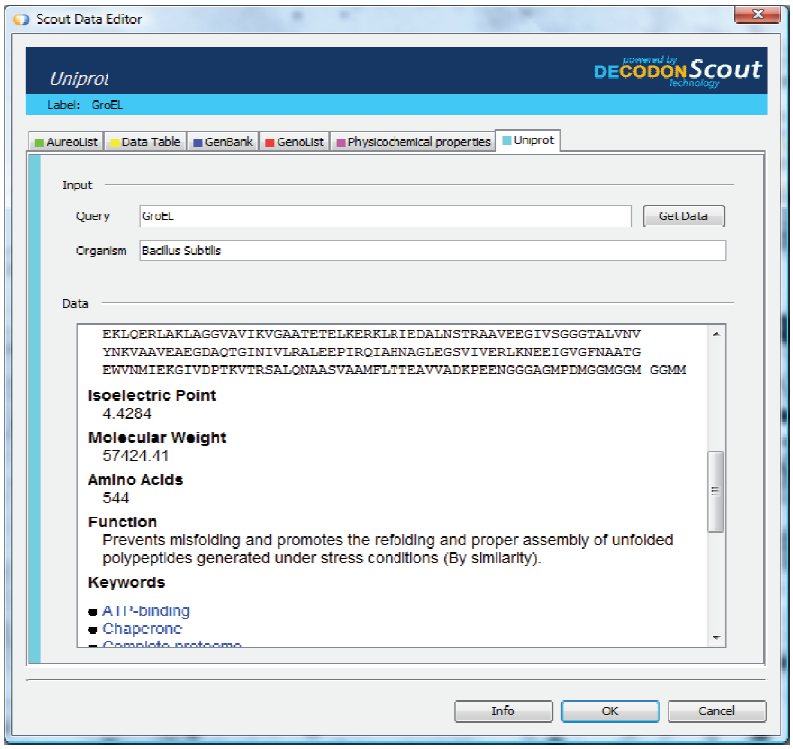
Labels report includes information about all labels on a certain image or those belonging to a certain set of spots on a an image, including Scout data if available (e.g. theoretical pI and MW, or GO as retrieved from UniProt) and Delta2D's estimated pI and MW.

All reports are interactive: They include controls to change the reports' content.
Furthermore the reports are 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 be adapted to any corporate design.

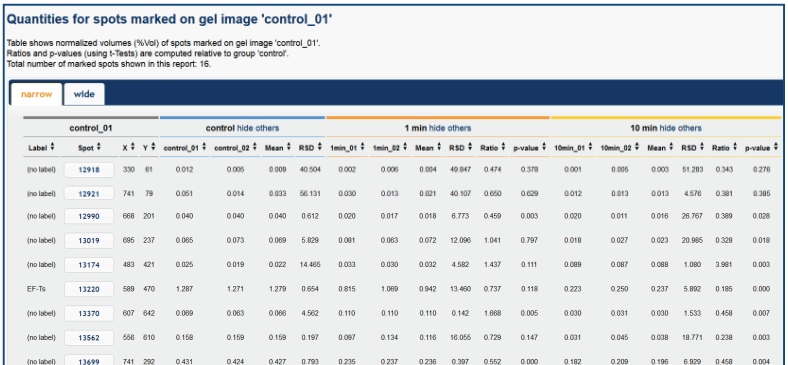
Show expression profiles in the form of the respective image thumbnails.



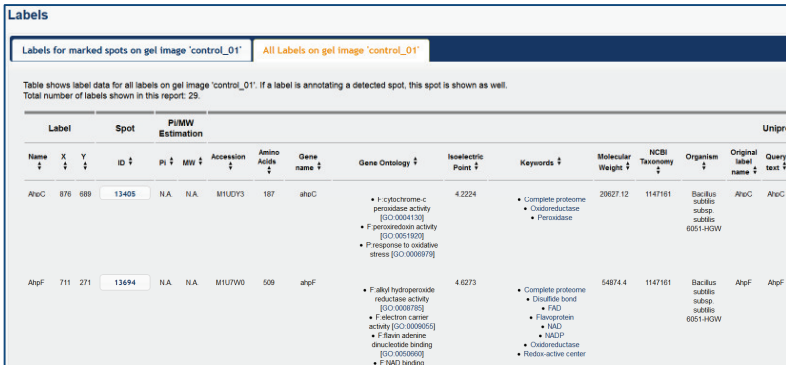
Automate your queries, e.g. with DECODON's UniProt scout.



The Spot Quantities report shows either a condensed or a detailled set of table columns for selected or marked expression profiles.



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



Premium service is our standard

„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

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.

We provide support

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

The DECODON Support Team
Phone: +49 (0)3834 515 235
Email: support@decodon.com

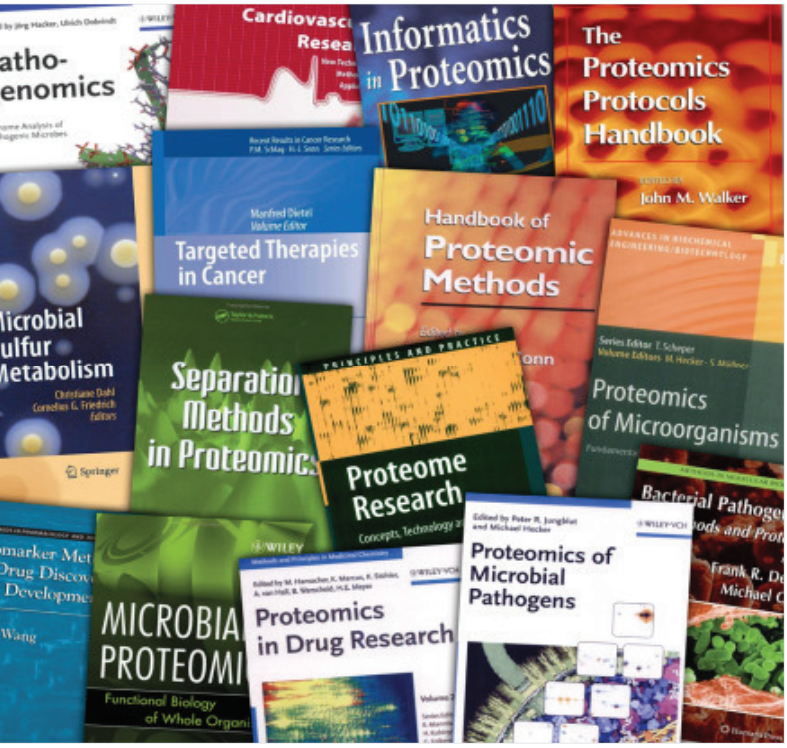
Enjoy working with Delta2D!

Remote Assistance – Just sit back and see how to solve your problem

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

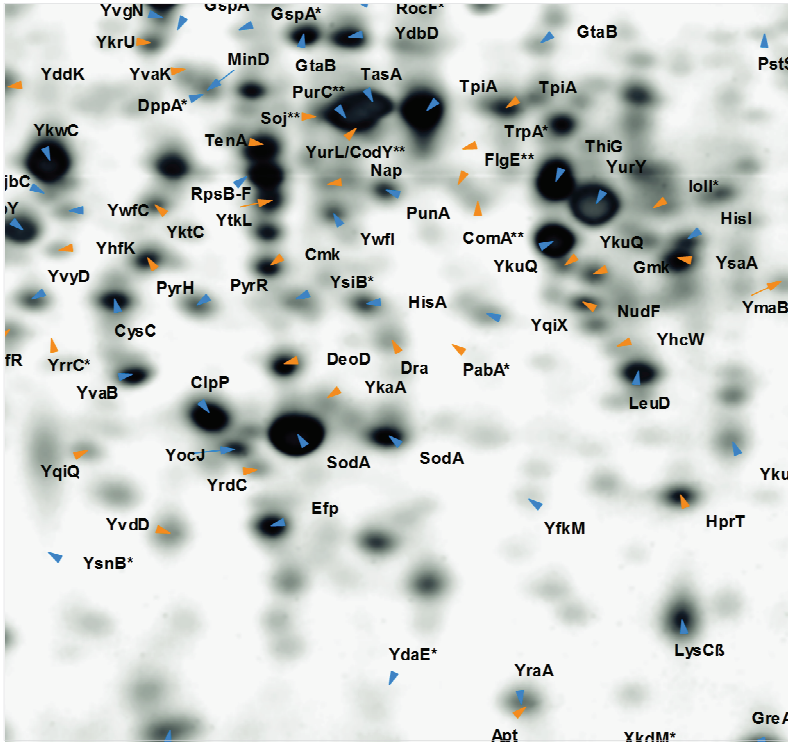
Daniel Kay
Porirua, New Zealand

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.



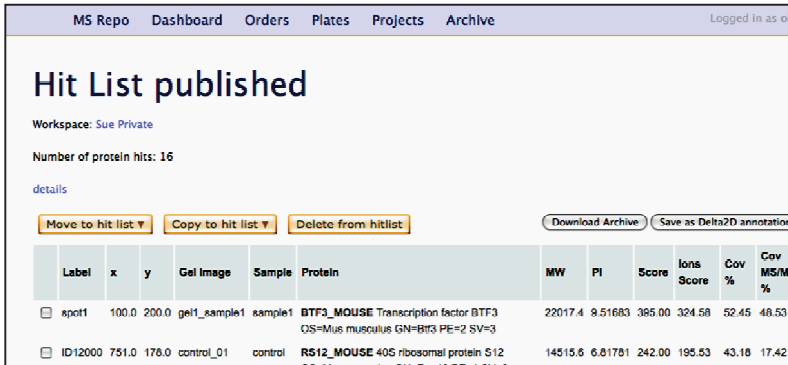
A variety of textbooks describe how to use Delta2D or consider scientific results which have been found by using Delta2D.

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



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

Delta2D can be connected to MS Repo, our information system for organising, sharing, and publishing Maldi data



Delta2D: Features and functions 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 proteom 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 as Excel file 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 / unwarped, Fusion images or Color Coding Images) as Powerpoint file or as snapshot (TIFF, JPEG, PNG, PNM, BMP)	Use images for publication and presentation Edit spot boundaries and spot labels as objects Open slides with other packages like LibreOffice or Openoffice
Export pick lists from Delta2D for common spot picking devices	BioRad Exquest Bruker Proteineer or Proteinscape Ettan Spot Handling Workstation Genomic Solutions ProPic Herolab Molecular Dynamics PerkinElmer ProXCISION Generic pick list format Support for other devises can be provided

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
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

Statistical Analysis

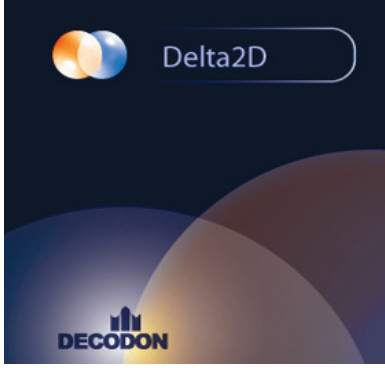
Heat map display of expression profiles	Get a colorful visualization of all quantitative data of your project in just image See differences and similarities between expression profiles and gel images at a glance
Hierarchical Clustering (HCL)	Cluster expression profiles of spots or gel images, optional bootstrapping and jackknifing support
k-Means/Medians Clustering (KMC)	Cluster expression profiles of spots or gel images
t-Test (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.

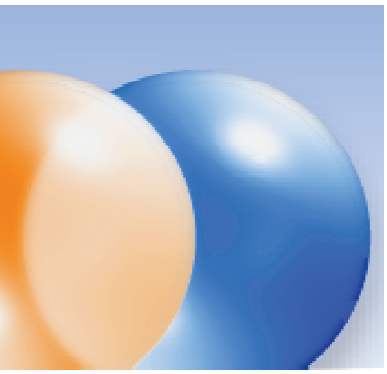
Data Handling

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 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, use 2D or 3D view
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





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