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DeCyder 2-D Differential Analysis Software v6.0

Differential Analysis Software designed for use in 2-D Fluorescence Difference Gel Electrophoresis

DeCyder™ 2-D Differential Analysis Software has been specifically developed for 2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE) and is a key element in the Ettan™ DIGE system. For the first time in the history of 2-D electrophoresis, an internal standard can be included for every spot on every 2-D gel to give the most accurate quantitation possible.

DeCyder 2-D Differential Analysis Software significantly increases throughput by accurately addressing measurement of protein differences with statistical confidence and reduces hands-on time from days to minutes with minimal user-to-user variation. The software automatically detects, matches, and analyzes protein spots in multiplexed fluorescent images, and is able to detect small differences between protein spots with high statistical confidence.

DeCyder 2-D software v6.0 offers:

- Automated analysis, significantly reducing hands-on time
- No spot matching within gels, eliminating matching errors
- Automatic presentation of spot statistics
- Internal standard approach, increasing accuracy and simplifying gel-to-gel matching
- Client-server installation using an Oracle™ database delivering easy project handling and data security
- CFR 21 Part 11 compliance via future module

Traditional 2-D gel electrophoresis is a well-established technique for protein analysis, though time-consuming and labor-intensive. Many gels have to be run, analyzed, and compared. However, the reproducibility between gels and significant system variability make it difficult to distinguish between system and inherent biological variation, which means that differences in protein abundance can rarely be confidently predicted.

DeCyder 2-D Differential Analysis Software consists of a fully automated image analysis software suite that enables the detection, quantitation, matching, and analysis of 2-D DIGE gels. The software is compatible with CyDye™ DIGE Fluor minimal dyes and saturation dyes from the scarce sample labeling kit. Using DeCyder 2-D software, expression differences identified by 2-D DIGE can be confidently assigned to induced biological change and are not due to system variability. Every difference is assigned a statistical confidence value.

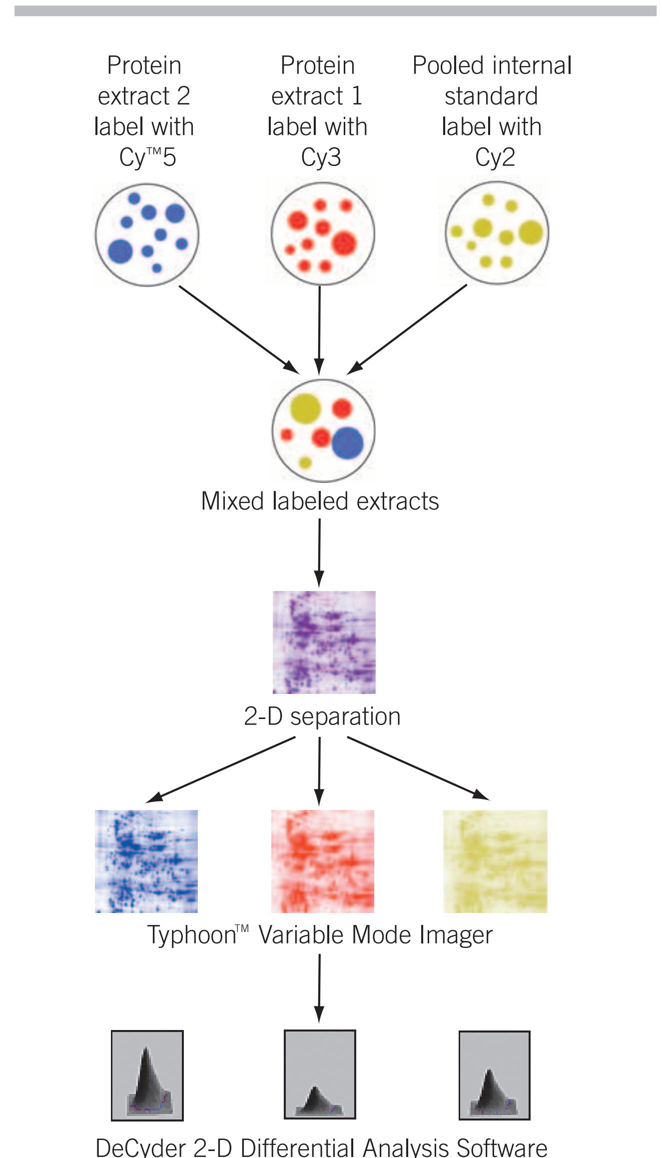

Fig 1. Overview of 2-D DIGE workflow (three-dye example).

Amersham Biosciences

DeCyder 2-D software is unique in fully exploiting the advantages offered by multiplexing, the co-migration of more than one sample per gel, by allowing the inclusion of an internal standard.

Multiplexing is performed using proprietary size- and charge-matched CyDye DIGE Fluor minimal or saturation dyes to label protein samples. Up to three samples can be separated on the same 2-D gel, which is then scanned with the Typhoon 9400 series Variable Mode Imager. Using DeCyder 2-D software, very small differences in protein expression can be measured with a high degree of confidence.

Use of an internal standard effectively eliminates gel-to-gel variation. Detection of differences in expression of less than 10%, with over 95% confidence can be achieved within minutes.

An overview of the 2-D DIGE workflow is shown in Figure 1.

DeCyder 2-D Differential Analysis Software v6.0 suite consists of several modules as shown in Figure 2:

Image Loader: For loading images into an Oracle database.

Administration Tools: For database administration, maintenance, and user access control.

Batch Processor: For automated detection, quantitation matching, and comparison of multiple 2-D DIGE gels.

Differential In-gel Analysis (DIA): For co-detection, background subtraction, normalization, and quantitation of spots in an image pair.

Biological Variation Analysis (BVA): For matching multiple gels for comparison and statistical analysis of protein abundance changes.

XML Toolbox: For exporting spot data from DIA or BVA modules for further downstream analysis.

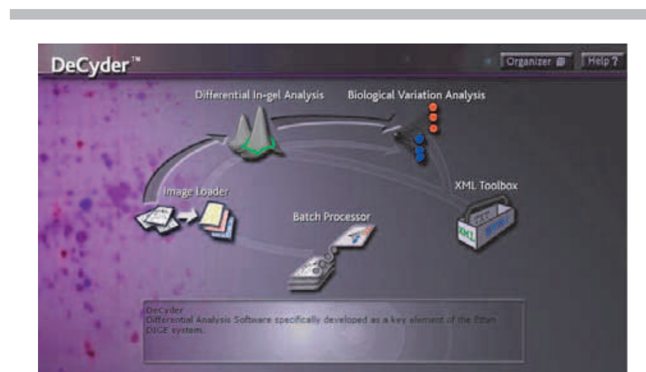

Fig 2. DeCyder 2-D software v6.0 start-up screen showing analysis workflow.

Image Loader

The Image Loader allows transfer and storage of images into an Oracle database.

Administration Tools

The Administration Tools maintain the Client-Server Oracle databases. Backup, database restoration, and export of data between databases are managed with total security. Users and access rights are managed by administrators via comprehensive dialogs. The system can support one user or multiple users across a network. Databases are available on the user machine or installed on a server, accessible via the network.

Batch Processor

The Batch Processor allows unattended and automated detection, quantitation, matching and comparison of multiple 2-D DIGE gels. The Batch Processor links both the DeCyder 2-D DIA and BVA modules to perform spot detection and inter-gel matching of multiple gel images. Several hundred image pairs per experiment, with up to three dyes per gel, can be automatically processed without user intervention.

Differential In-gel Analysis (DIA)

DIA detects up to three images in a gel creating a single overlay. Co-detection of three images (one internal standard and two samples) utilizes patented algorithms to provide a consistent and accurate ratio measurement. Background subtraction, quantitation, normalization, and first-level-of-matching (within gel) are automated for high-throughput analysis with low experimental variation.

The user interface is divided into quadrants: Gel view, Histogram view, 3-D view, and Spot Table view. Each of these views is linked in such a way that selecting a spot in one view will display information on the same spot in the other views (Fig 3).

Gel images are first processed in the DeCyder 2-D DIA module to identify and quantitate protein spots. The DIA module generates a series of XML files containing information on all the protein spots on each of the gels. These files are then used for inter-gel matching and subsequent analyses in the other DeCyder 2-D software modules.

The DIA module processes a triplet of images from a single gel. The internal standard is loaded as the primary image followed by the secondary and the tertiary image, derived for example from either a control or treated sample. Spot analysis is carried out on the loaded images.

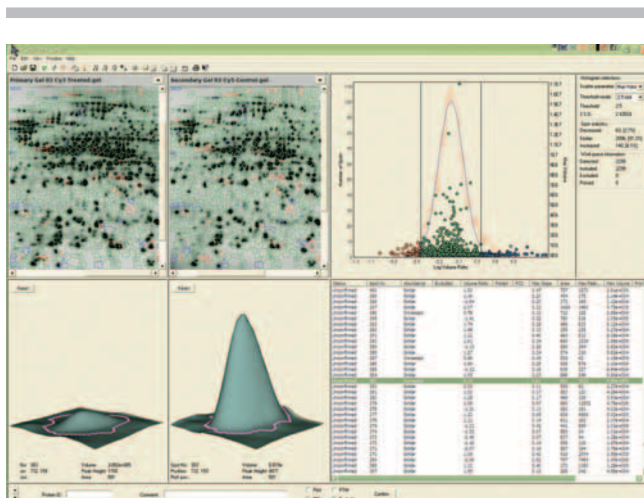

Fig 3. Each screen quadrant is linked in the DIA user interface.

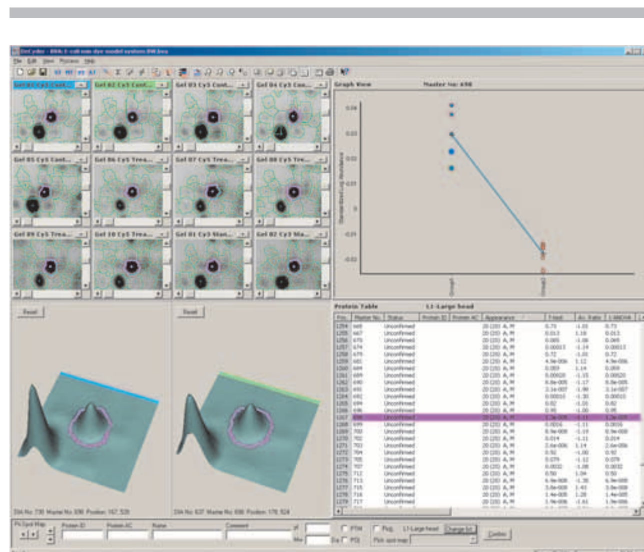

Fig 4a. BVA user interface is used to detect and statistically quantitate differences between samples.

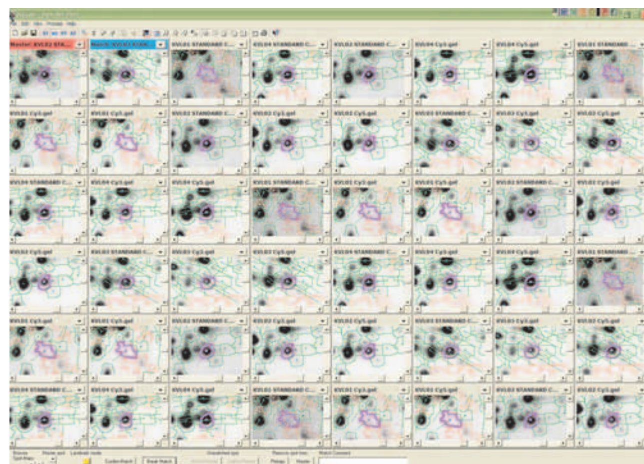

Fig 4b. BVA interface showing 48 gel images full screen.

The detection algorithm is designed to take advantage of the co-migration benefits of Ettan DIGE CyDye Fluors. The triplet of co-run images is merged together, incorporating all spot features in a single image. Spot detection and spot boundary definition are performed using pixel data from the individual raw images and the merged image. The resultant spot map is overlaid onto the original three image files. Since the spot boundaries are identical for the three images, they are effectively already matched, streamlining the process and resulting in more accurate volume calculations.

The DIA module measures spot protein abundance for the secondary image. The quantitation is expressed as a spot ratio, comparing spot volumes on the secondary image with corresponding spot volumes of the internal standard. Linking every sample to a common internal standard makes direct comparison of protein expression levels between multiple gels easier and more accurate.

Biological Variation Analysis (BVA)

Images processed through the Batch Processor are matched between gels using the BVA feature, which detects the consistency of differences between samples across all the gels and applies statistics to associate a level of confidence for each of the differences.

The BVA user interface is divided into four linked quadrants, as in the DIA user interface (Fig 4a). In addition to these four views, there are also four screens displaying different aspects of the gel-to-gel matching data. Up to 48 gel images can be shown in the image quadrant or full screen (Fig 4b).

BVA has four principle group comparison methods that can be applied to analyze protein spot data:

- Average ratio between two groups or two populations of groups
- Student's t-test analysis between two groups or two populations of groups
- One-way ANOVA (ANalysis Of VAriance) for statistical analysis between all groups
- Two-way ANOVA for statistical analysis between groups or populations of groups in an experimental design where there are two independent factors such as in a time-dose study. This analysis allows the internal and mutual effects of the two factors to be quantitated.

The log standardized abundance is the only variable subjected to the above statistical analyses within DeCyder 2-D BVA. It is derived from the normalized spot volume, standardized against the intra-gel standard. Log values are used so that the data points approach a normal distribution around zero to fulfill the requirements of subsequent statistical tests. Consequently, the statistical analysis functionality is not valid unless the experimental design includes an internal standard on every gel.

Using XML files generated by the DIA analyses, the BVA module carries out inter-gel and statistical analyses. Spot maps are assigned to experimental groups to allow statistical analyses to be performed. All spot maps are matched to a master image that is either automatically assigned to the spot map with the highest number of detected spots, or user defined. Spot-matching uses a pattern-recognition algorithm that automatically matches one single spot in one gel to a single spot in another gel, based on its neighboring spots.

The BVA module is used to establish the significance of changes in expression of specific proteins from different experimental groups. The software performs the appropriate statistical calculations and displays the results in the Protein table. The graph view shows the differences between the different experimental groups, providing information about the spread, and therefore the consistency of data between the gels.

The statistical analyses can be used to select proteins for picking, digestion, and subsequent analysis by mass spectrometry. Selection of protein spots can be based on multiple criteria such as statistical significance of change, magnitude of change, and spot volume.

XML Toolbox

Data generated in both the DIA and BVA modules can be saved as XML files. These files contain all information on the processing of the images throughout the entire procedure.

XML Toolbox comprises different tools for the extraction of data from the different XML files produced by DeCyder 2-D software to enable custom-designed conversion of data into text files, HTML files, or other data formats. Two basic tools are supplied to create **Tabbed text files** and **Web tables**. The XML format can be used to transfer data between the different modules of DeCyder 2-D software, and to make data available for post-processing.

Importance of internal standardization

The use of appropriate experimental design and statistical analysis (EDSA) is central to 2-D DIGE technology and ensures the best possible results. The recommended EDSA uses an internal pooled standard approach, removing the system variable associated with gel-to-gel variation. This methodology is unique to 2-D DIGE. An example of such an experimental design is shown in Table 1.

DeCyder 2-D software has an EDSA-setting feature for fully automated statistical analysis. A list of statistically significant differences is automatically generated, together with an optional spot-picking list for further analysis. The spot picking boundary is automatically defined and can be viewed in the BVA screen. Where two spots are close together, the software allows the user to edit the position of the picking boundary to increase the accuracy of picking the protein of interest.

The internal standard is created by pooling aliquots of all biological samples in the experiment. This standard is labeled with one CyDye DIGE Fluor dye and run together with individual samples (Table 1). Samples are thereby in-gel linked to a common standard, giving accurate quantitation and separating gel-to-gel from inherent biological variation.

2-D DIGE has very low experimental variation therefore biological replicates can be used without needing replicates of the same sample. Using conventional 2-D electrophoresis the experiment in Table 1 would require a minimum of three replicates of each control and treated sample to calculate the system variation making a total of 36 gels compared to the six required with 2-D DIGE.

Table 1. An example of experimental design implemented in DeCyder 2-D Differential Analysis Software to derive statistical data on differences between control and treated samples labeled with both CyDye DIGE Fluor minimal and saturation dyes

Gel number	Cy2	Cy3	Cy5
1	Pooled standard	Control 1	Treated 1
2	Pooled standard	Control 2	Treated 2
3	Pooled standard	Control 3	Treated 3
4	Pooled standard	Treated 4	Control 4
5	Pooled standard	Treated 5	Control 5
6	Pooled standard	Treated 6	Control 6

Example of experimental design for CyDye DIGE Fluor minimal dyes.

Gel number	Cy3	Cy5
1	Pooled standard	Treated 1
2	Pooled standard	Treated 2
3	Pooled standard	Treated 3
4	Pooled standard	Treated 4
5	Pooled standard	Treated 5
6	Pooled standard	Treated 6
7	Pooled standard	Control 1
8	Pooled standard	Control 2
9	Pooled standard	Control 3
10	Pooled standard	Control 4
11	Pooled standard	Control 5
12	Pooled standard	Control 6

Example of experimental design using CyDye DIGE Fluor saturation dyes from the CyDye DIGE Fluor Labeling Kit for Scarce Samples.

Specifications

PC requirements

Operating System:	Windows™ XP Professional
Processor:	Pentium™ 4 processor, 1.5 GHz minimum
RAM:	Minimum 1 GB
Video card:	Capable of 32 bit color Video card driver needs to support Open GL (v1.2 or later) – ensure the latest compatible driver is installed. 64 Mb DRAM or more
Color resolution:	Set to 32 bit color
Screen resolution:	Min 1024 × 768 pixels, landscape (With 24 bits Z-buffer/preferably 32 bits)
Hard drive	120 GB recommended, 3 partitions mounted
LAN	100 Mbits LAN card for client/server
Virtual memory	Set so that the total amount of available memory, including physical RAM, is greater than 4 GB Internet Explorer™ v5.5 or higher must be installed to run the XML Toolbox module **Note:** Avoid running other programs at the same time as the various DeCyder 2-D Differential Analysis Software modules

File specifications

DeCyder 2-D software is compatible with 16-bit TIFF images and *.GEL format files.

File output is in XML format to allow easy parsing of data for archiving or downstream analysis.

DeCyder 2-D software is optimized for use with Typhoon 9000 series Variable Mode Imager for file management of multiplexed gels.

Ordering information

Product	Quantity	Code no.
DeCyder 2-D Differential Analysis Software v6.0 including PC and single concurrent network user license (minimum computer specification 1.5 GHz processor, 1024 MB memory)	1	11-0010-91
DeCyder 2-D Differential Analysis Software v6.0 including single concurrent network user license	1	11-0010-95
Additional DeCyder 2-D Differential Analysis Software v6.0 concurrent user license	1	11-0010-99

Other packages available on request

Related products

Description		Code no.
CyDye DIGE Fluor Minimal Labeling Kit (5 nmol)	1	25-8010-65
CyDye DIGE Fluor Cy2 minimal dye,	5 nmol	25-8010-82
CyDye DIGE Fluor Cy2 minimal dye,	10 nmol	25-8008-60
CyDye DIGE Fluor Cy2 minimal dye,	25 nmol	RPK0272
CyDye DIGE Fluor Cy3 minimal dye,	5 nmol	25-8010-83
CyDye DIGE Fluor Cy3 minimal dye,	10 nmol	25-8008-61
CyDye DIGE Fluor Cy3 minimal dye,	25 nmol	RPK0273
CyDye DIGE Fluor Cy5 minimal dye,	5 nmol	25-8010-85
CyDye DIGE Fluor Cy5 minimal dye,	10 nmol	25-8007-62
CyDye DIGE Fluor Cy5 minimal dye,	25 nmol	RPK0275
CyDye DIGE Fluor Labeling Kit for Scarce Samples (For a minimum of 12 labeling reactions)		25-8009-83
CyDye DIGE Fluor Labeling Kit for Scarce Samples and Preparative Gel Labeling (For a minimum of 12 labeling reactions and 1 prep gel)		25-8009-84
DIGE Enabled Typhoon 9400 Variable Mode Imager with PC		63-0055-79
DIGE Enabled Typhoon 9400 Variable Mode Imager without PC		63-0055-78
ImageQuant™ solution 1.4 manuals and software set for Typhoon 9400 Variable Mode Imager upgrade		63-0046-26
Typhoon Scan Control v3.0 for Typhoon upgrade		63-0046-27

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