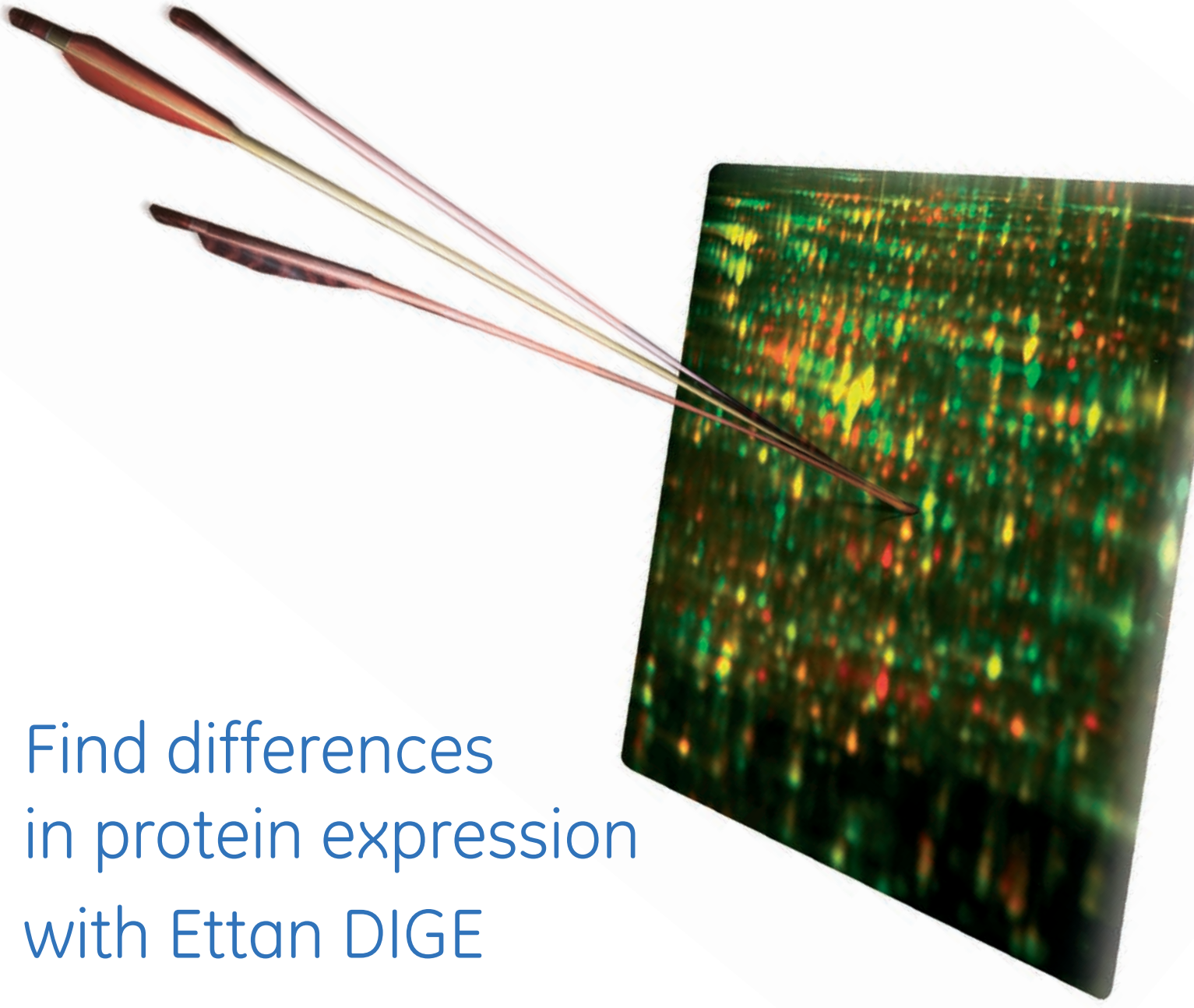
GE Healthcare



Find differences in protein expression with Ettan DIGE

2-D Fluorescence Difference Gel Electrophoresis

Results you can trust in protein abundance analysis

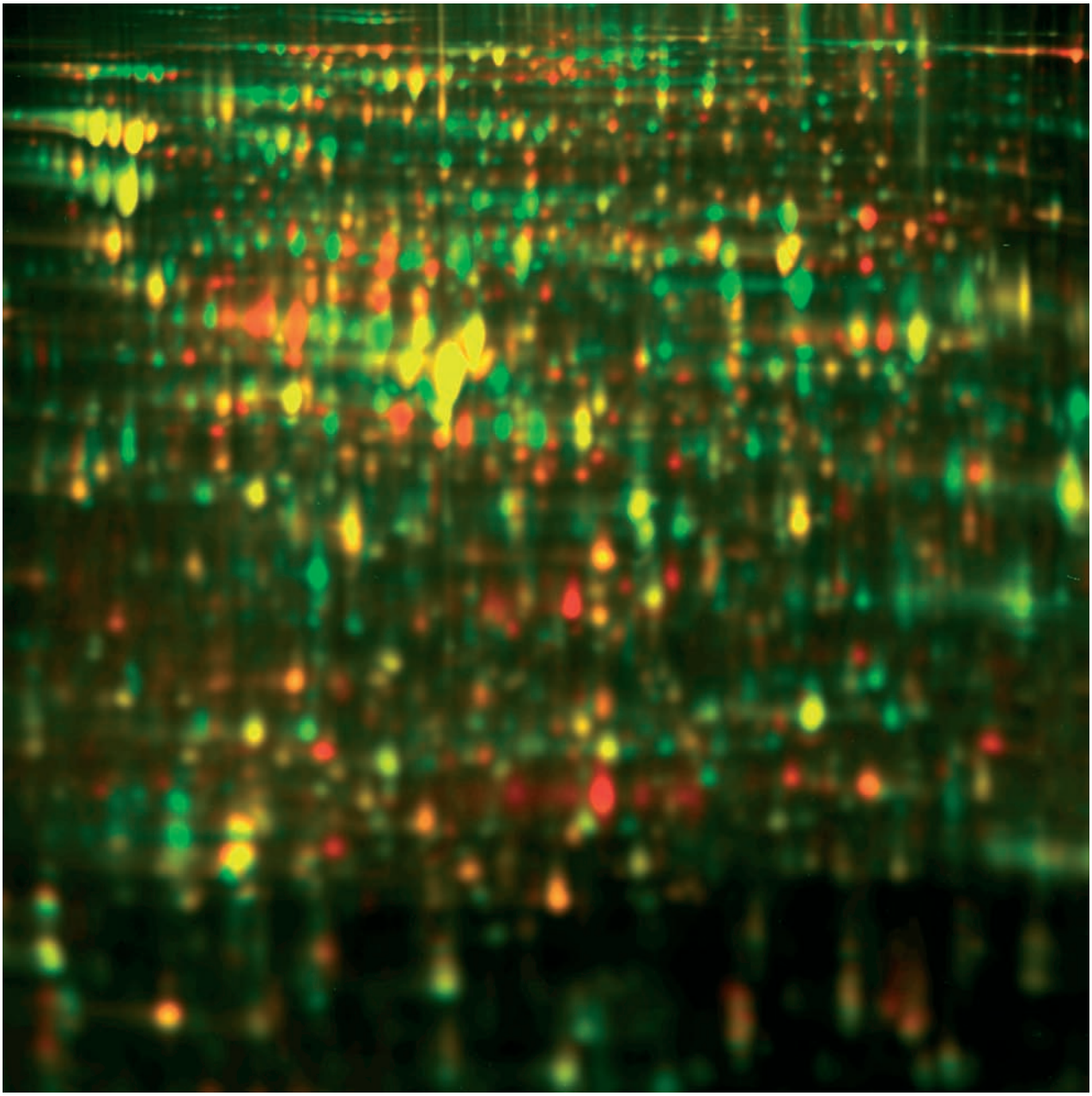
Ettan™ DIGE system uses 2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE), the benchmark for protein abundance analysis. The world's leading pharmaceutical and academic centers of excellence are increasingly switching to Ettan DIGE because of the unrivaled accuracy it provides.

Standardize your 2-D results
Ettan DIGE uses multiplexing, the simultaneous co-separation of multiple, fluorescently labeled samples, including a pooled internal standard on each gel. Each protein spot has its own internal standard, which ensures that the differences you see in protein abundance are real. This is the only effective way to minimize gel-to-gel variation and significantly increase accuracy and reproducibility.

Efficiency saves time and costs
The level of reproducibility and statistical confidence with Ettan DIGE ensures that you get results you can rely on. You can also be confident that you're not missing important differences in protein abundance. Because Ettan DIGE requires far fewer gels than other methods for measuring protein abundance differences, it also provides significant time and cost savings.

Ettan DIGE delivers these important benefits:

- **Standardization:** uses a pooled internal standard to deliver the lowest possible gel-to-gel variation
- **Accuracy:** ensures that the smallest possible real differences in protein abundance are detected with unparalleled statistical confidence
- **Reproducibility:** leads to consistent and comparable conclusions
- **Cost effectiveness:** achieves dependable results with far fewer gels
- **Efficiency:** increases throughput and significantly reduces analysis time
- **Proven method:** has rapidly become established in leading laboratories worldwide



Ettan DIGE: raising the standard of 2-D electrophoresis

Conclusions you can defend

Accurate results lead to confident conclusions and the right decisions. In proteomics research, you need to be sure you are choosing the right proteins to select, characterize, and quantitate. Ettan DIGE minimizes the danger of missing vital biomarkers, so you can be confident you have selected the right candidates.

Ettan DIGE is a proven method that combines the most statistically accurate protein abundance data with the greatest resolving power available in 2-D electrophoresis. Whether you're interested in researching disease, identifying biomarkers, or understanding tissue differentiation, Ettan DIGE gives you findings you can believe in, which lead to conclusions you can defend.

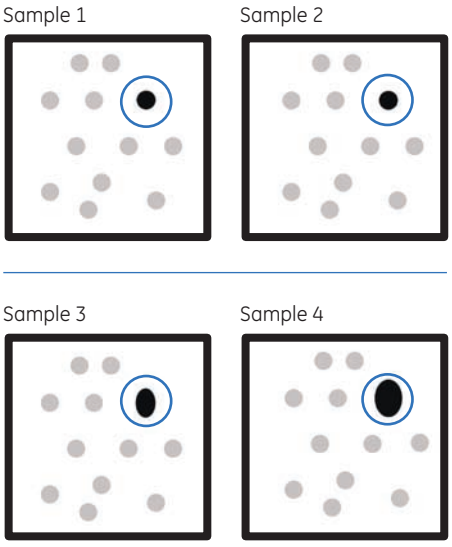
"Without the benefit of the internal standard, 42 of these proteins would have been overlooked due to variation between normal and tumor samples ... compared with individual DIGE comparisons made within a single 2-D gel."

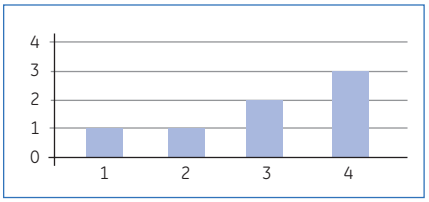
Friedman, D. B. *et al.* Proteome analysis of human colon cancer by two-dimensional difference gel electrophoresis and mass spectrometry. *Proteomics* **4**, 793–811 (2004).

The use of a pooled internal standard leads to accurate conclusions

Traditional 2-D electrophoresis
Four different samples run on four different gels

The abundance of this particular protein spot appears to be increasing in samples 3 and 4. Is this increase due to system variation or induced biological change?

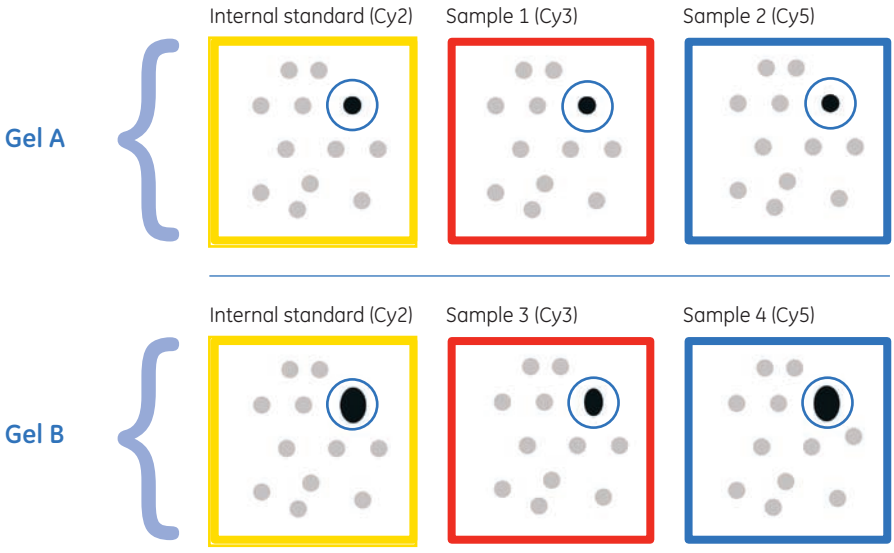


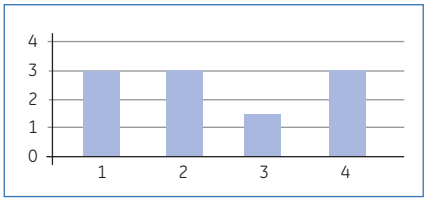


Experimental conclusion

Without running a significant number of replicates to average the results, the conclusion would be an increase in abundance in samples 3 and 4.

Ettan DIGE using a pooled internal standard
Four different samples, plus one internal standard, on two different gels





Experimental conclusion

The same internal standard is run on both gels. The increase in abundance of protein in gel B, as shown by the increase in the internal standard, is due to gel-to-gel variation. When the internal standard is normalized between gels A and B, the conclusion is that the abundance of protein in sample 3 has actually decreased.

The differences you see in protein abundance are real

Ettan DIGE is the only system with a pooled internal standard for every spot on every gel. Ettan DIGE uses prelabeling of protein samples with size- and charge-matched, spectrally resolvable CyDye™ DIGE Fluors.

After separation on Ettan IPGphor™ or Multiphor™ II IEF system in the first dimension and Ettan DALT*twelve* or Ettan DALT*six* Large Vertical Electrophoresis System in the second dimension, samples are scanned with Typhoon™ Variable Mode Imager or Ettan DIGE Imager.

Differences in protein abundance are then accurately quantitated using DeCyder™ 2-D Differential Analysis Software.

This dedicated 2-D analysis software uses the internal standard to derive data from within gels and then between gels, minimizing gel-to-gel variation. This allows you to achieve detection of < 10% differences between samples with > 95% statistical confidence, within minutes.

"Ettan DIGE with the internal standard delivers reliable results by decreasing gel-to-gel variation more than ever before."

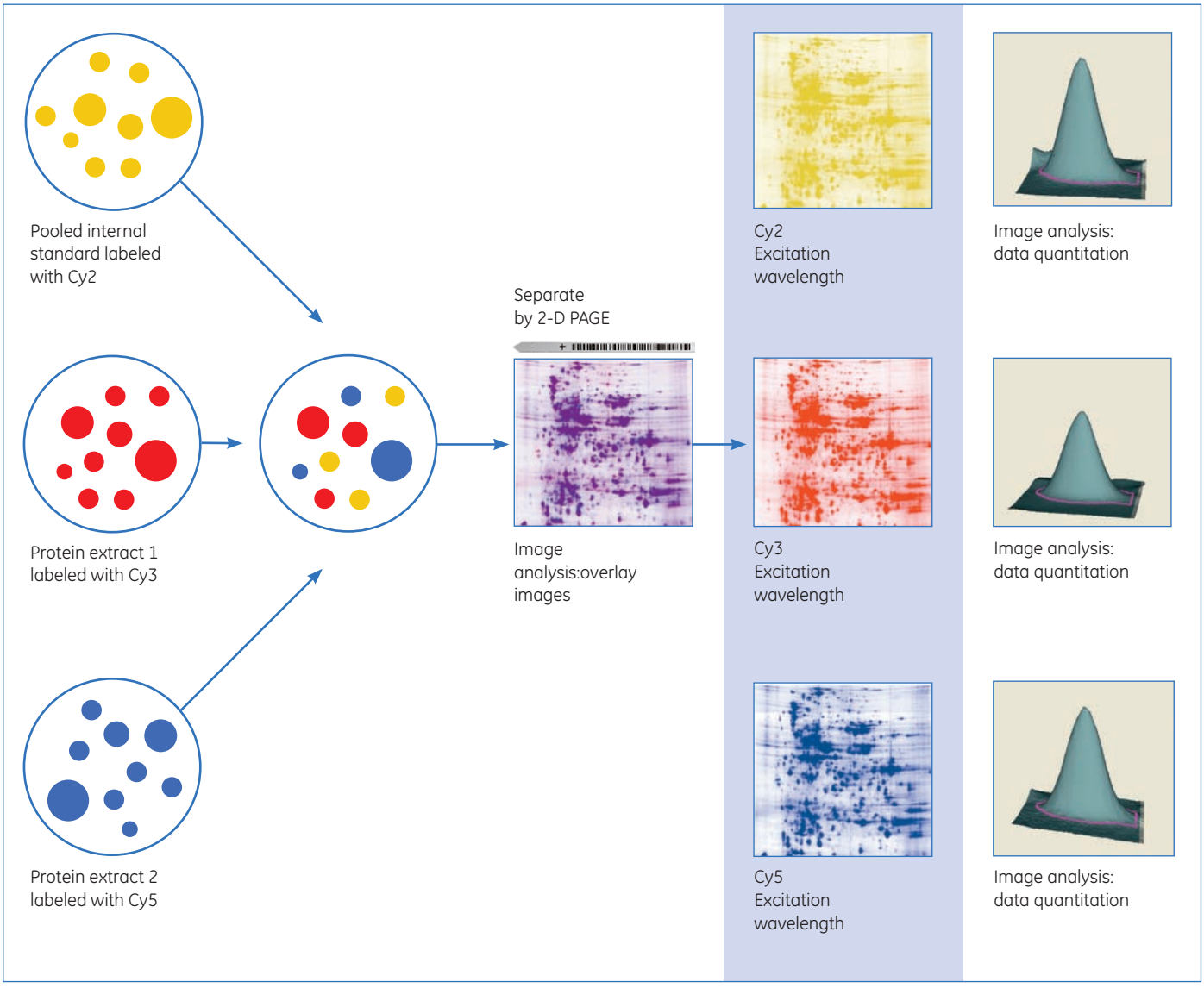
Dr. Jörgen Östling, AstraZeneca R&D, Mölndal, Sweden

Ettan DIGE performance comparison*

	Matched spots	Mean CV%
DIGE dyes, no internal standard, without DeCyder 2-D software	1300	26%
DIGE dyes, with internal standard, without DeCyder 2-D software	1300	15%
Ettan DIGE, with DeCyder 2-D Differential Analysis Software	**1300**	**3-6%**
Classical 2-D with fluorescent stain, without DeCyder 2-D software	1670	21%

* For each method, the same sample was analyzed in replicates of six gels.

Ettan DIGE is the only system with a pooled internal standard for every spot on every gel



Mix labeled extracts	2-D electrophoresis separation	Typhoon Variable Mode Imager or Ettan DIGE Imager	DeCyder 2-D Differential Analysis Software

1. Sample labeling

CyDye DIGE Fluors
Designed specifically for Ettan DIGE, these dyes are size- and charge-matched, as well as spectrally distinct, offering bright and intense colors with narrow excitation and emission bands. This allows co-separation of different CyDye DIGE fluor-labeled samples in the same gel and ensures that all samples are subject to exactly the same first- and second-dimension electrophoresis running conditions. This limits experimental variation and ensures accurate spot matching within gels.

CyDye DIGE Fluor minimal dyes
These CyDye fluors label the amino acid lysine. They are the most commonly used dyes for general 2-D electrophoresis applications and are suitable for all sample types. Each labeling reaction requires 50 µg of protein sample.

CyDye DIGE Fluor Labeling Kit for scarce samples
These CyDye fluors label the amino acid cysteine and are recommended for use when scarce amounts of sample are available (e.g., for analysis of proteins obtained from laser-capture microdissection). Each labeling reaction requires only 5 µg of protein sample.

2. Sample separation: first dimension

Ettan IPGphor 3 and Multiphor II IEF systems
Ettan IPGphor 3 IEF allows for high throughput first dimension electrophoresis with increased speed, accuracy and reproducibility. With Ettan IPGphor Manifold or Ettan IPGphor Manifold Light, up to 12 IPG strips can be run simultaneously which simplifies handling of multiple samples and saves time. Ettan IPGphor 3 Control Software helps you manage up to four Ettan IPGphor 3 units simultaneously.
To perform first dimension separation on the Multiphor II Electrophoresis System, use the Immobiline™ Drystrip Kit. To increase efficiency and reproducibility even further, the specially designed IPGbox can be used to rehydrate up to 12 IPG strips independently and at the same time. With the IPGbox, you can load samples during rehydration by including them in the buffer, or by using sample cups.

IPGbox is designed for rehydration of up to 12 Immobiline DryStrip Gels.

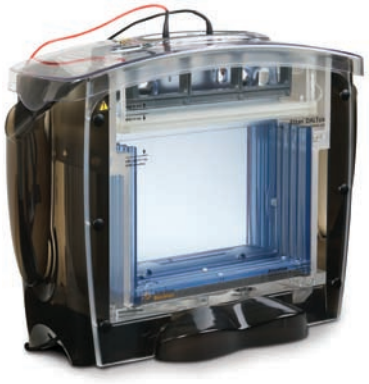
Ettan IPGphor 3 system, optimized for speed, reliability and use with fluorescently-labeled protein samples.

3. Sample separation: second dimension

Ettan DALT Electrophoresis System
In just 4-5 hours, Ettan DALT*six* Large Vertical System can run up to six large labcast or precast gels (26 x 20 cm) in the second dimension. The gels are compatible with first dimension 18 or 24 cm Immobiline DryStrip Gels (IPG strips) from both Ettan IPGphor and Multiphor II IEF systems. For increased throughput, use the EttanDALT*twelve* electrophoresis system which can simultaneously run up to 12 large-format gels. Ettan DALT*twelve* used with 24 cm IPG strips provides very high resolution and maximizes the number of distinct, quantifiable protein spots per gel.

Choosing a product with the DIGE-approved seal assures that you will obtain optimum results with a product fully compatible with Ettan DIGE technology. From sample preparation to data analysis, there is a DIGE-approved product for every step. Look for the DIGE-approved seal for 2-D results you can trust.

DIGE approved



Compact Ettan DALT*six* Large Vertical System

Ettan DALT*twelve* provides high resolution, throughput, and batch-to-batch reproducibility.

4. Image acquisition

Typhoon Variable Mode Imager
The Typhoon Variable Mode Imager is a highly sensitive, variable mode gel imager optimized to image CyDye DIGE fluor–labeled proteins.

- Outstanding linearity, quantitative accuracy, and extremely low limits of detection
- Detection of small differences with highest statistical confidence
- Consistent point light illumination
- Simultaneous imaging of two large-format Ettan DALT gels automatically

Gels are scanned between glass plates, preventing drying and shrinkage and allowing further running and rescanning, if required.

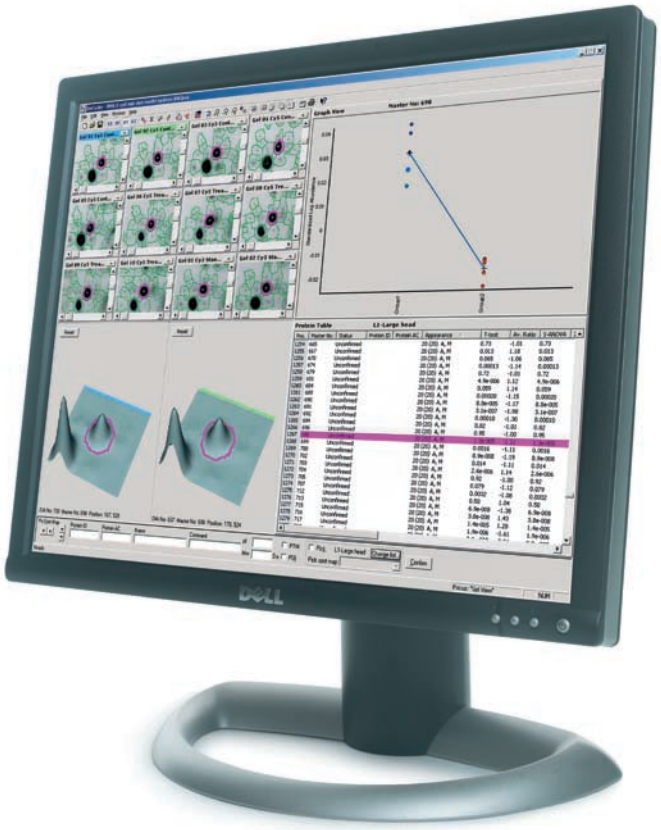
Ettan DIGE Imager
Ettan DIGE Imager represents leading-edge technology for generating high-quality, low-noise images from your 2-D DIGE experiments. By combining very high resolution with precise motion control, Ettan DIGE Imager produces accurate, multichannel images of your Cy™2-, Cy3-, and Cy5-labeled gel.

- High sensitivity for imaging faint spots
- Software-selectable wavelengths for better sensitivity and reduced background
- Adjustable scan time to optimize sensitivity
- Sealed environment for scanning and protecting wet samples

Gels are mounted in cassettes to avoid drying.

5. Image analysis

DeCyder 2-D Differential Analysis Software
DeCyder 2-D software has been specifically developed to exploit the benefits of prelabeled, multiplexed samples and the internal standard. It automatically locates and analyzes multiple samples in a gel and then enables comparative analysis of multiple gels, producing accurate measurements of differential changes in protein abundance. The software provides statistical confidence and minimal user-to-user variation, which reduces hands-on analysis time to minutes.

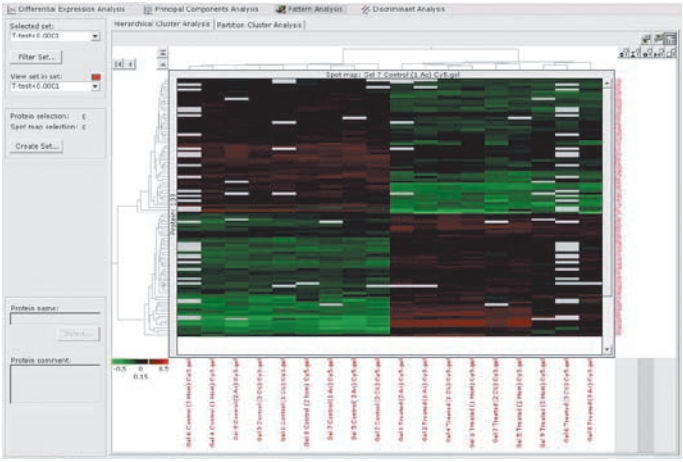


6. Multivariate analysis in proteomics

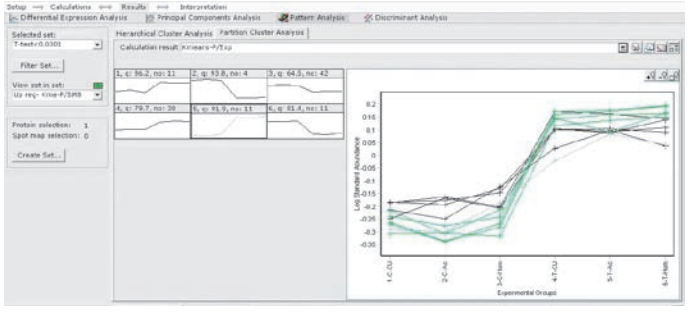
DeCyder Extended Data Analysis (EDA) Software
DeCyder EDA is a powerful analysis tool extending the statistical options offered in 2-D DIGE. It enables you to switch seamlessly between EDA statistical results and DeCyder 2-D visualization data, putting your results into biological context by linking to internal and external databases. Together they create a powerful platform of image analysis tools, which offers flexibility, versatility, and adaptability for protein profiling.

DeCyder EDA provides advanced statistical analysis in a simple-to-use format, uncovering patterns in expression data and relationships using multivariate analysis and sophisticated clustering methods.

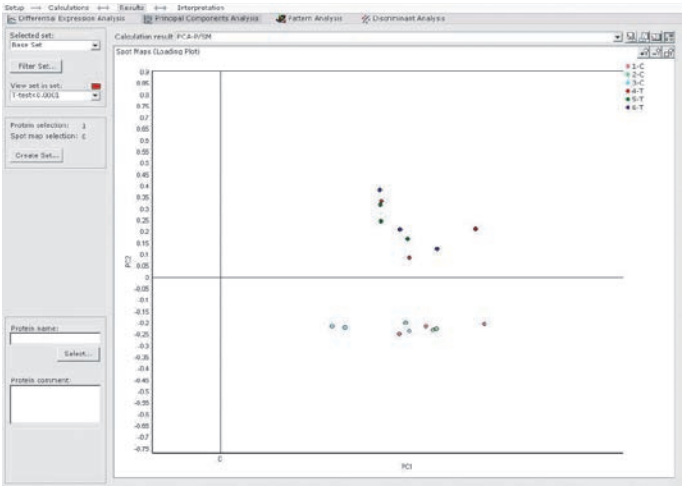
The software helps you understand regulatory pathways, find proteins with similar expression profiles, or group your samples according to common expression patterns. It also supports the identification of proteins that discriminate between disease stages, tumor types or other sample subtypes, giving your Ettan DIGE results clarity and biological relevance.



Hierarchical cluster analysis



K-means analysis



Principal component analysis (PCA)

Proven in practice

CyDye DIGE Fluor minimal dyes for tracking membrane protein abundance in epilepsy-induced rats
The results of this proteomics study demonstrate that, as part of the Ettan DIGE platform, CyDye DIGE Fluor minimal dyes are applicable to the analysis of membrane proteins and are beneficial for use within preclinical and clinical proteomics.

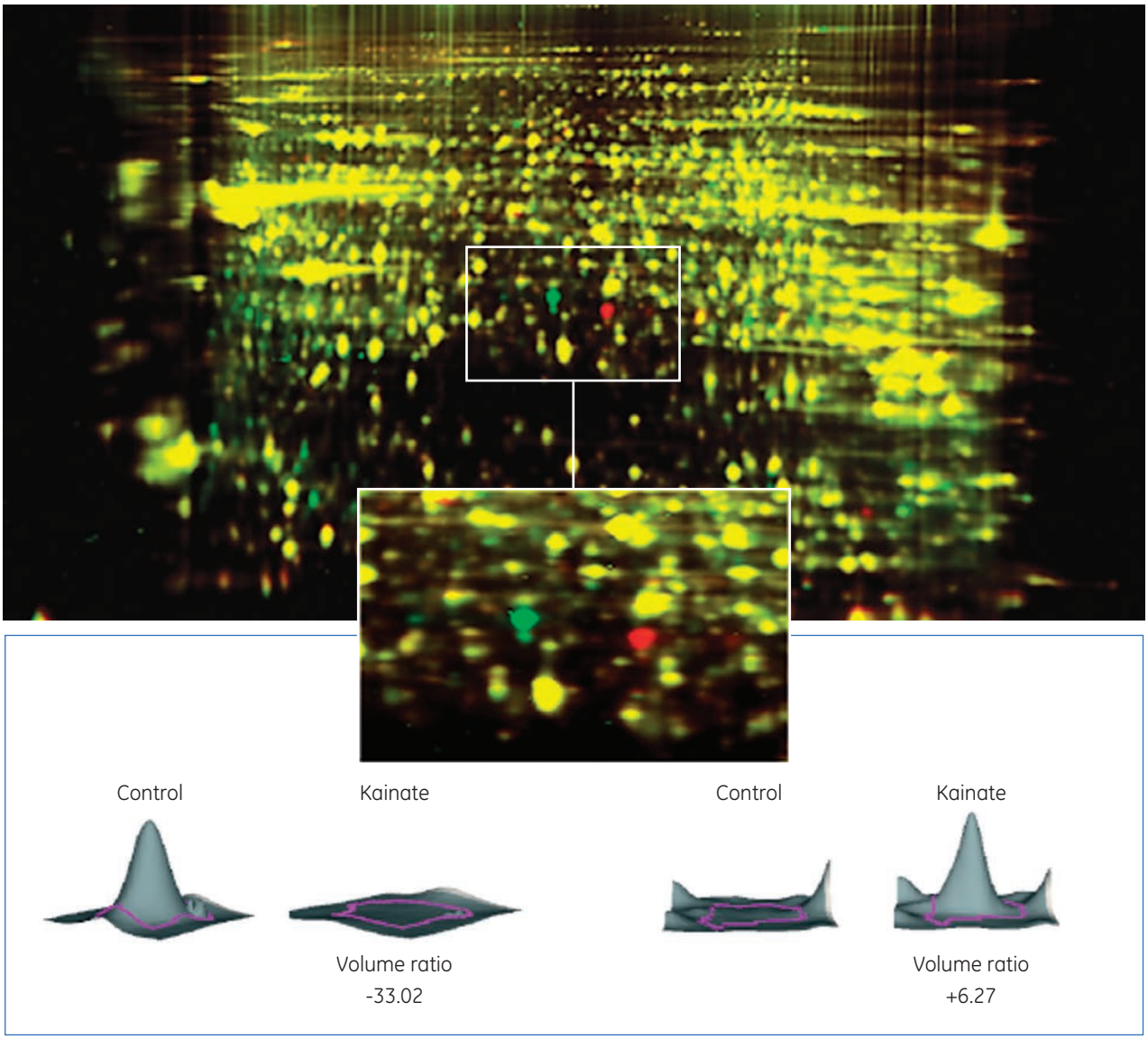
The study was carried out in collaboration with Dr. S. Hattori *et al.*, Division of Cell Proteomics, Institute of Medical Science, University of Tokyo, Japan.

Some examples of the proteins identified by mass spectrometry

Protein name	Differential expression*
NADH dehydrogenase alpha subunit 10	+ 6.27
Oxygen regulated protein	+ 2.10
ATP-dependent zinc metalloprotease	+ 1.96
Golgi coiled coli protein	+ 1.72
Neural adhesion molecular F3	+ 1.96
Aldehyde dehydrogenase	+ 1.67
Huntingtin interaction protein 1 related protein	+ 4.36
T-cell activation Rho GTPase-activation protein	+ 1.51
Growth factor receptor bound protein	+ 2.34
Androgen-induced prostate proliferative shutoff associated protein As3	+ 2.02
NADH dehydrogenase alpha subunit 10	- 33.02
FERM, RhoGEF and pleckstrin domain protein 2	- 1.67
Heat-stable enterotoxin receptor precursor	- 1.87
ATP synthase beta subunit	- 2.67
F1-ATPase beta subunit	- 2.17
Huntingtin interacting protein related	- 2.63
H+transporting two sector ATPase	- 1.87

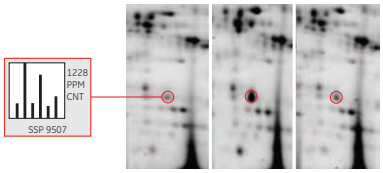
* Abundance in treated sample vs. control, measured by volume ratio

Analyzing differences in membrane protein expression in rat brain with 2-D DIGE



Protein spots showing a predominantly red or green color demonstrate changes in abundance between normal and kainic acid treatment (used to induce epilepsy in rats). The centrally positioned pair of green and red spots shown above were identified as the same protein, NADH dehydrogenase alpha subcomplex 10–like protein. This protein has undergone some structural changes caused by the kainic acid treatment.

Without 2-D DIGE



Without the 2-D DIGE system, several replicates must be run due to high gel-to-gel variation. This is time-consuming, delivering lower quality data and leading to inaccurate conclusions. Courtesy of Jörgen Östling, AstraZeneca R&D Mölndal, Sweden.

Ordering information

Product		Quantity	Code no.
Sample labeling			
DIGE Trial Pack, incl. CyDye DIGE Fluor minimal dye labeling kit + 1 month free DeCyder 2-D trial license		2 nmol	28-9373-73
CyDye DIGE Fluor minimal dye labeling kit		2 nmol	28-9345-30
CyDye DIGE Fluor, minimal labeling kit		5 nmol	25-8010-65
CyDye DIGE Fluor Labeling Kit for Scarce Samples		1	25-8009-83
CyDye DIGE Fluor Labeling Kit for Scarce Samples and Preparative Gel Labeling		1	25-8009-84
Sample separation, first dimension			
DeStreak Rehydration Solution		5 × 3 ml	17-6003-19
DeStreak Reagent		1 ml	17-6003-18
Ettan IPGphor 3 Isoelectric Focusing Unit		1	11-0033-64
Immobiline DryStrip Kit		1	18-1004-30
IPGbox	1 IPGbox + IPGbox Kit		28-9334-65
IPGbox Kit	10 Reswell Trays + IPGbox Insert		28-9334-92
IPG Buffer pH 5.5-6.7		1 ml	17-6002-06
IPG Buffer pH 4-7		1 ml	17-6000-86
IPG Buffer pH 6-11		1 ml	17-6001-78
IPG Buffer pH 7-11 NL		1 ml	17-6004-39
IPG Buffer pH 3-10 NL		1 ml	17-6000-88
IPG Buffer pH 3-10		1 ml	17-6000-87
IPG Buffer pH 3-11 NL		1 ml	17-6004-40
Ettan IPGphor Manifold, Complete		1	80-6498-38
Ettan IPGphor Manifold, Light Complete		1	11-0026-88
Multiphor II Electrophoresis System		1	18-1018-06

Immobiline DryStrip Gels for IEF[1]

pH range	Quantity	Code No 18 cm	Code No 24 cm
3.5–4.5	12/pack	–	17-6002-38
3–7 NL	12/pack	–	17-6002-43
4–7	12/pack	17-1233-01	17-6002-46
6–9	12/pack	17-6001-88	17-6002-47
6–11	12/pack	17-6001-97	–
3–10	12/pack	17-1234-01	17-6002-44
3–10 NL	12/pack	17-1235-01	17-6002-45
3–5.6 NL	12/pack	17-6003-56	17-6003-57
5.3–6.5	12/pack	17-6003-61	17-6003-62
6.2–7.5	12/pack	17-6003-66	17-6003-67
7–11 NL	12/pack	17-6003-71	17-6003-72
3–11 NL	12/pack	17-6003-76	17-6003-77

[1] IPG strips are also available in 7, 11 and 13 cm strip lengths. For information, contact your local GE Healthcare representative.

Product	Quantity	Code no.
Sample separation, second dimension		
Ettan DALT*twelve* Separation Unit and Power Supply/Control Unit, 115V	1	80-6466-46
Ettan DALT*twelve* Separation Unit and Power Supply/Control Unit, 230V	1	80-6466-27
DALT*twelve* Gel Caster, complete	1	80-6467-22
Ettan DALT*six* Electrophoresis Unit, 115V	1	80-6485-08
Ettan DALT*six* Electrophoresis Unit, 220V	1	80-6485-27
EPS 601 Power Supply 18-1130-02	1	18-1130-02
DALT*six* Gel Caster	1	80-6485-46
MultiTemp™ III, Thermostatic Circulator, 115 V	1	18-1102-77
MultiTemp III, Thermostatic Circulator, 230 V	1	18-1102-78
Image acquisition		
Ettan DIGE Imager		63-0056-42
Typhoon Trio™ with ImageQuant™ TL and PC		63-0055-88
Typhoon Trio+ with ImageQuant TL and PC		63-0055-90
Typhoon 9400 with ImageQuant TL and PC		63-0055-79
Typhoon 9410 with ImageQuant TL and PC		63-0055-81
Image analysis		
DeCyder 2-D v7.0 Software Package		28-9435-83
DeCyder 2-D v7.0 Oracle 10gR2, 5 user license	1	28-9435-88
DeCyder 2-D v7.0, 1 user license	1	28-9442-75

Related literature	Code no.
2-D Electrophoresis: Principles and Methods	80-6429-60
Ettan DIGE System User Manual	18-1173-17

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