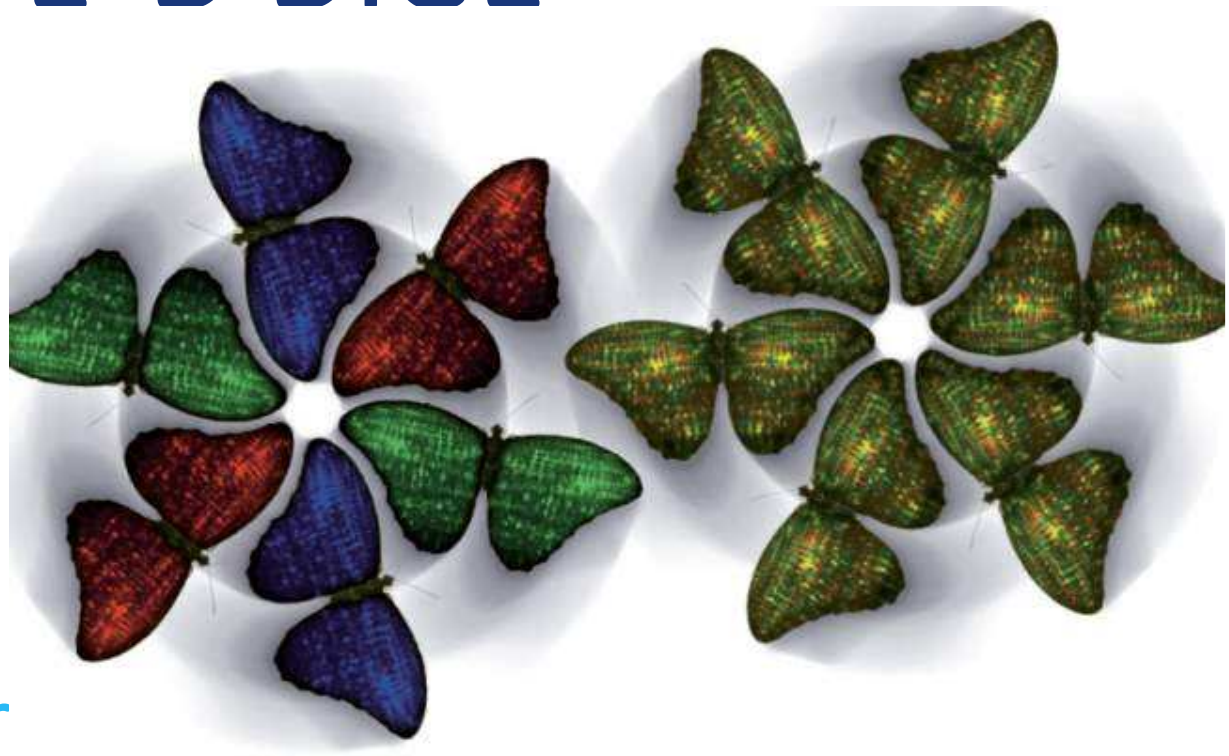
Find your proteins easier and faster with 2-D DIGE



Dr Rita Marouga
Product Manager
GE Healthcare

imagination at work

Overview

Unique 2-D DIGE concept

Advantages over classical 2-D electrophoresis

Improved workflow with new products

Benefits in practice

What is unique with 2-D DIGE?

Traditional 2-D electrophoresis

- Time consuming
- High experimental variation

2-D DIGE (Difference gel electrophoresis) system

- Leading edge technology

  -Experimental design – unique for this technique

- Greatly reduces number of gels

  - Multiplexing – multiple pre-labeled samples run on same gel

- Provide greater accuracy

  -Internal standard – run on all gels within an experiment

What is Ettan™ DIGE system?

Ettan DIGE System is a leading edge technology for differential analysis of protein abundance using 2-D gel electrophoresis

Sample labeling

CyDye™ DIGE Fluors for protein labeling

Highly fluorescent dyes designed specifically for this application

Sensitive, photostable and spectrally distinct

Image acquisition

Imager for image acquisition

Typhoon Imager designed specifically for this multiplexing technology

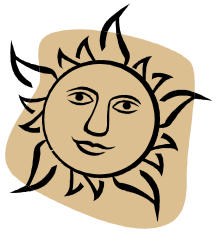
Differential analysis

Differential Analysis Software for image analysis

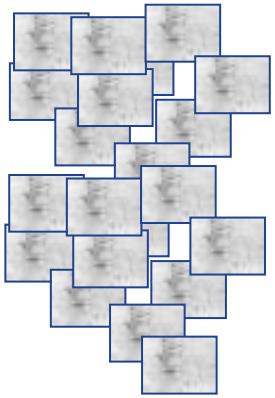
DeCyder™ 2-D designed specifically for this multiplexing technology

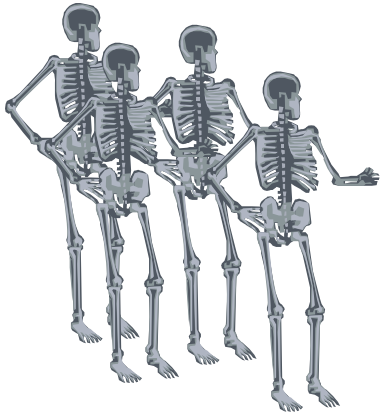
Why switch to 2-D DIGE?

2-D electrophoresis → 2-D DIGE

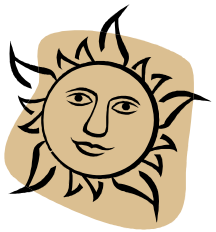


Working overtime

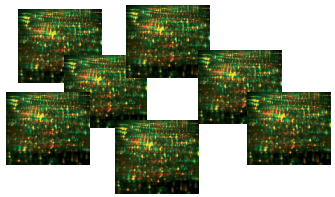




1 year later:
Do we really have a 50% difference???

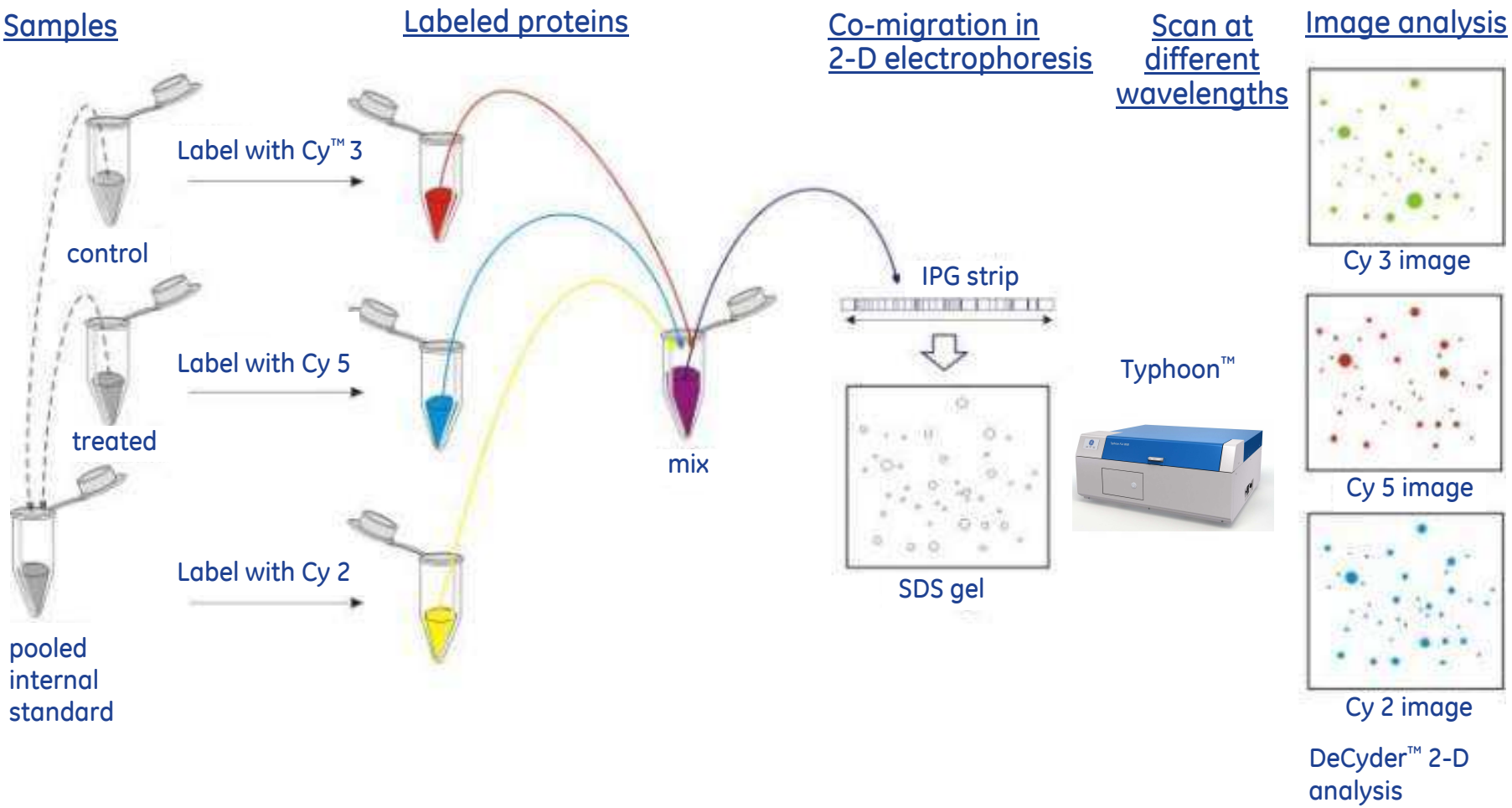


Take a break once in a while

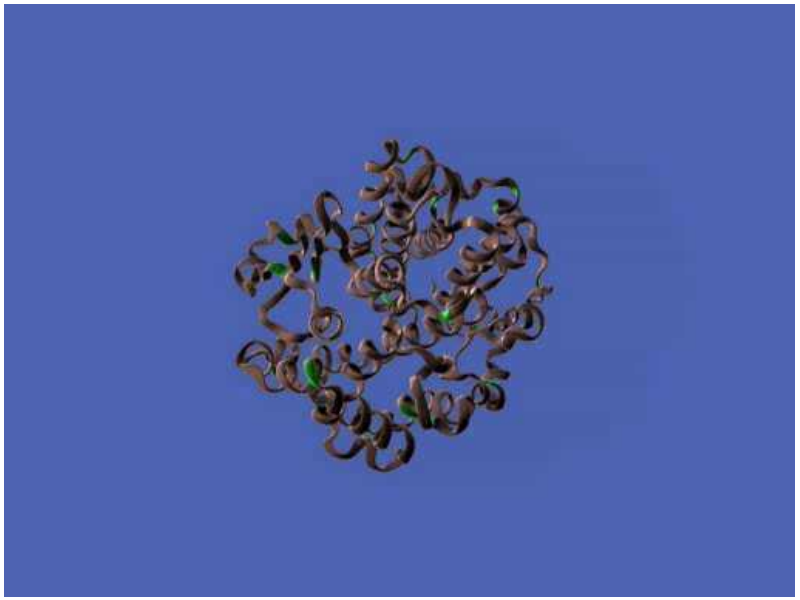


2 months later:
We have a 10% difference!!!

Ettan™ DIGE system – experimental procedure



Minimal CyDye™ DIGE fluors



Minimal labeling

- 50 µg protein
- single label (3 %)
- ε-amino group of lysine

3 dyes: Cy™ 2, Cy 3, Cy 5

- charge matched (+1 charge)
- size matched (~450Da)
- labeled samples co-migrate
- Sensitivity: 0.25 ng
- linear dynamic range: over 4 orders of magnitude

Saturation CyDye™ DIGE fluors

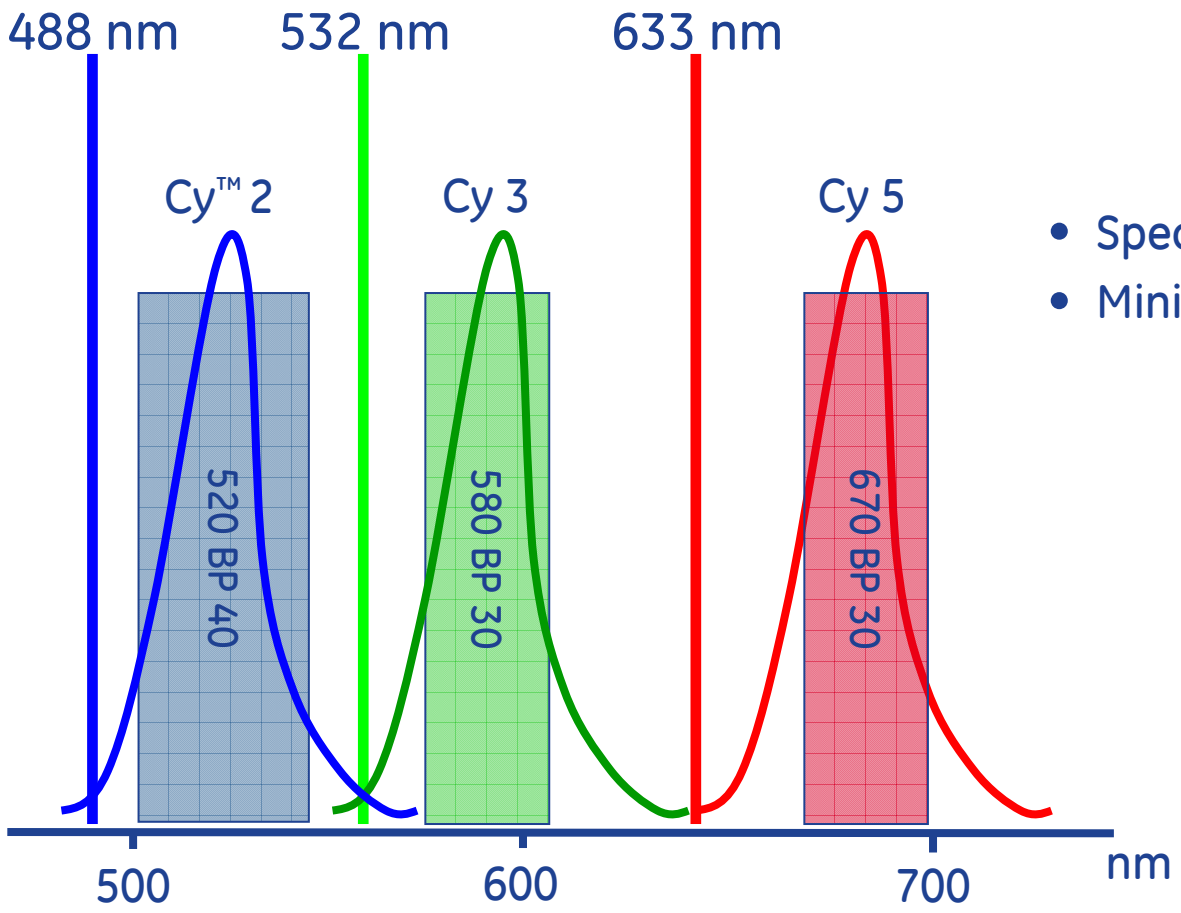
Saturation labeling

- 5 µg protein
- multiple labels (100 % of all cysteines)
- thiol group of cysteine

2 dyes: Cy™ 3, Cy 5

- charge matched (neutral)
- size matched (~680Da)
- Sensitivity: lower than 0.025 ng
- linear dynamic range:
  over 3 orders of magnitude

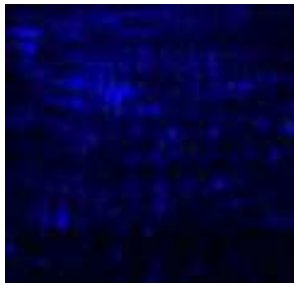
Multiplex detection – fluorescence excitation and emission



- Spectrally well resolved dyes
- Minimal cross-talk

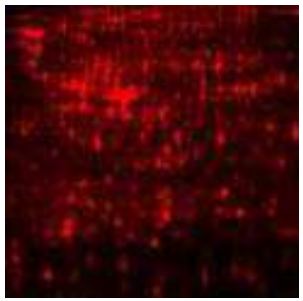
Multiplex detection
three color image from scanner

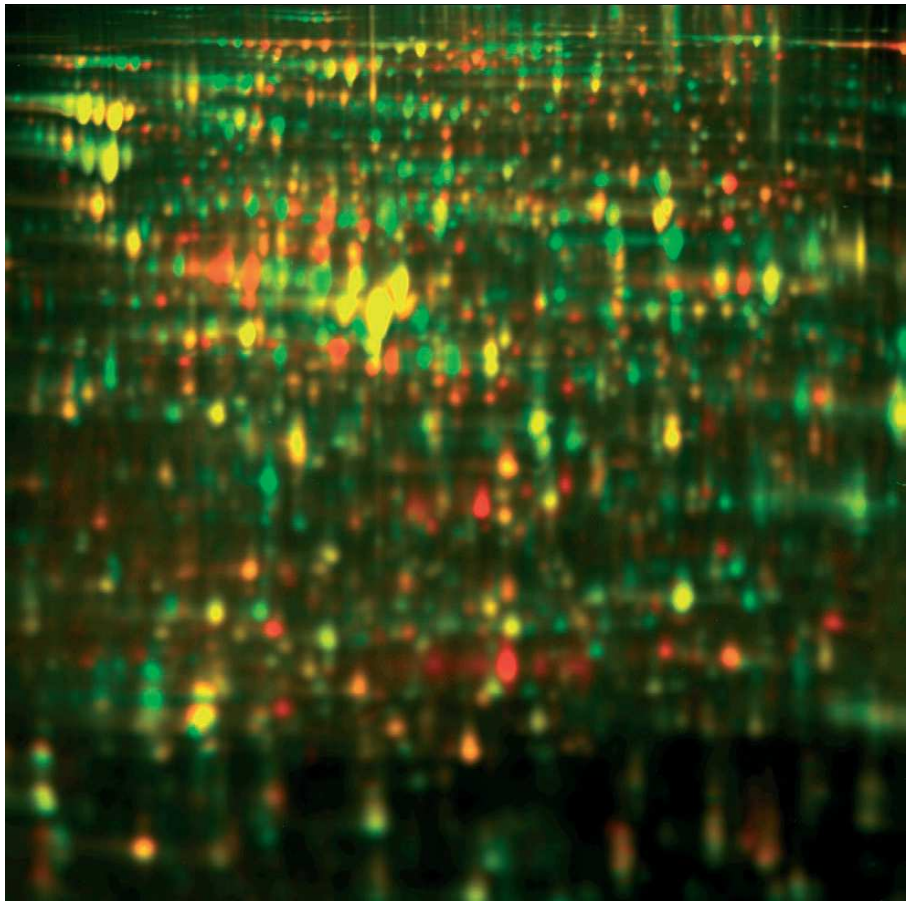
Cy™ 2



Cy 3

Cy 5

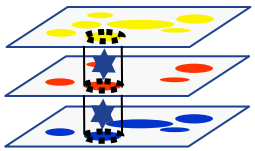
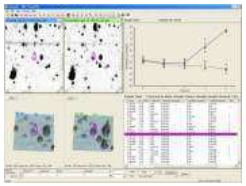
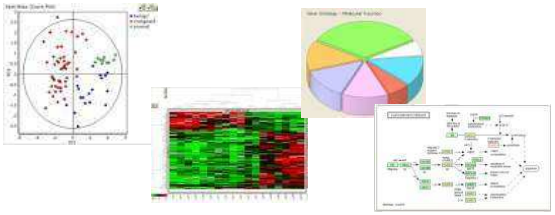




Three color overlay

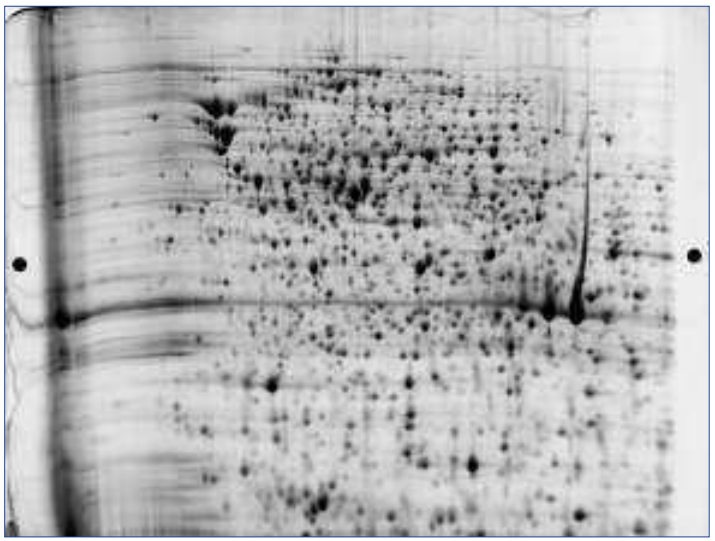
Imagination at work

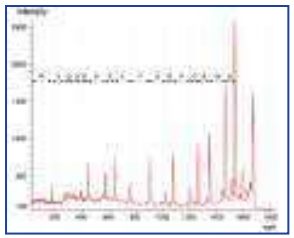
Image analysis –
DeCyder™ 2-D Differential Analysis Software

DeCyder module		Function
DIA (Differential In-gel Analysis)		- Spot co-detection on all three images - In-gel normalization
BVA (Biological Variation Analysis)		- Matches all gels - Statistics for quantitative comparisons
EDA (Extended Data Analysis)		- Multivariate modeling - Expression pattern analysis - Classification

Protein identification

Preparative 2-D gel
(matched against analytical gels)





Automated spot picking

↓

Spot digestion

↓

MALDI target spotting

↓

Identification with MS

Overview

Unique Ettan™ DIGE concept

Advantages over classical 2-D electrophoresis

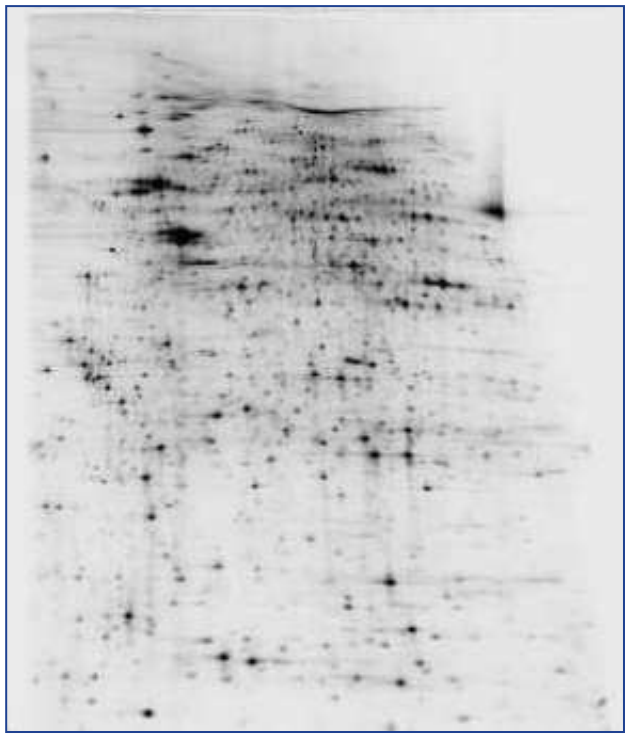
Improved workflow with new products

Benefits in practice

Post-staining vs fluorescent pre-labeling – sensitivity

Silver stain of 10.000 cells

Cy5 stain of 5.000 cells



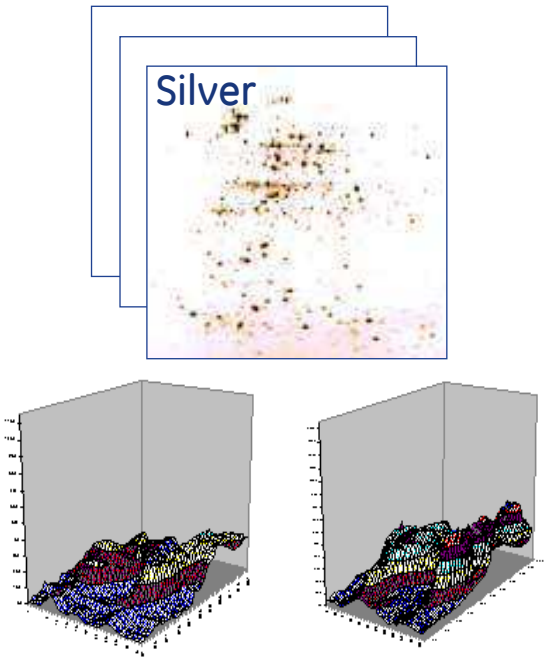
Class leading sensitivity
Only 250 ng protein

Courtesy of Kai Stühler, RU, Bochum, DE

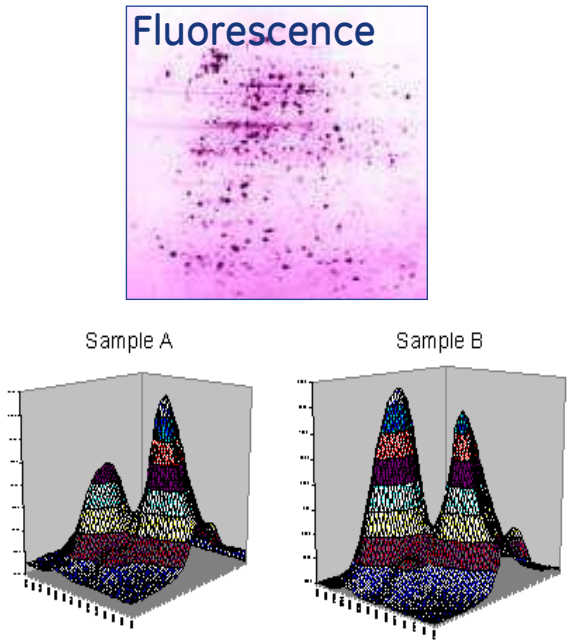
Post-staining vs fluorescent pre-labeling – dynamic range

Fluorescent labelling and staining techniques offer significant increase in **detection levels** combined with **dynamic range** (4-5 orders of magnitude) as compared to for instance classical silver staining techniques

Post staining



Pre-labeling with CyDyes™

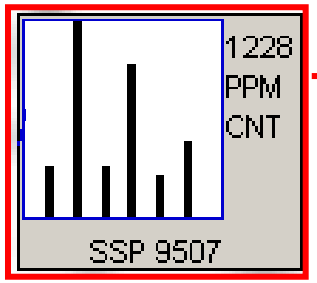


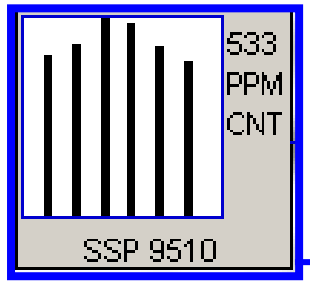
Remove system **variation**

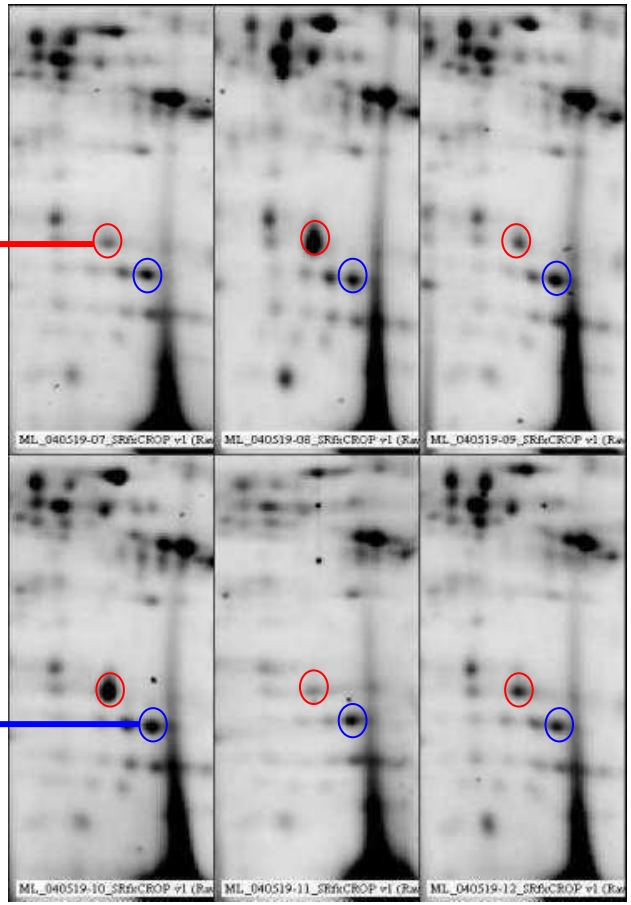
Gel to gel variation

Large gel-to-gel variation

Small gel-to-gel variation





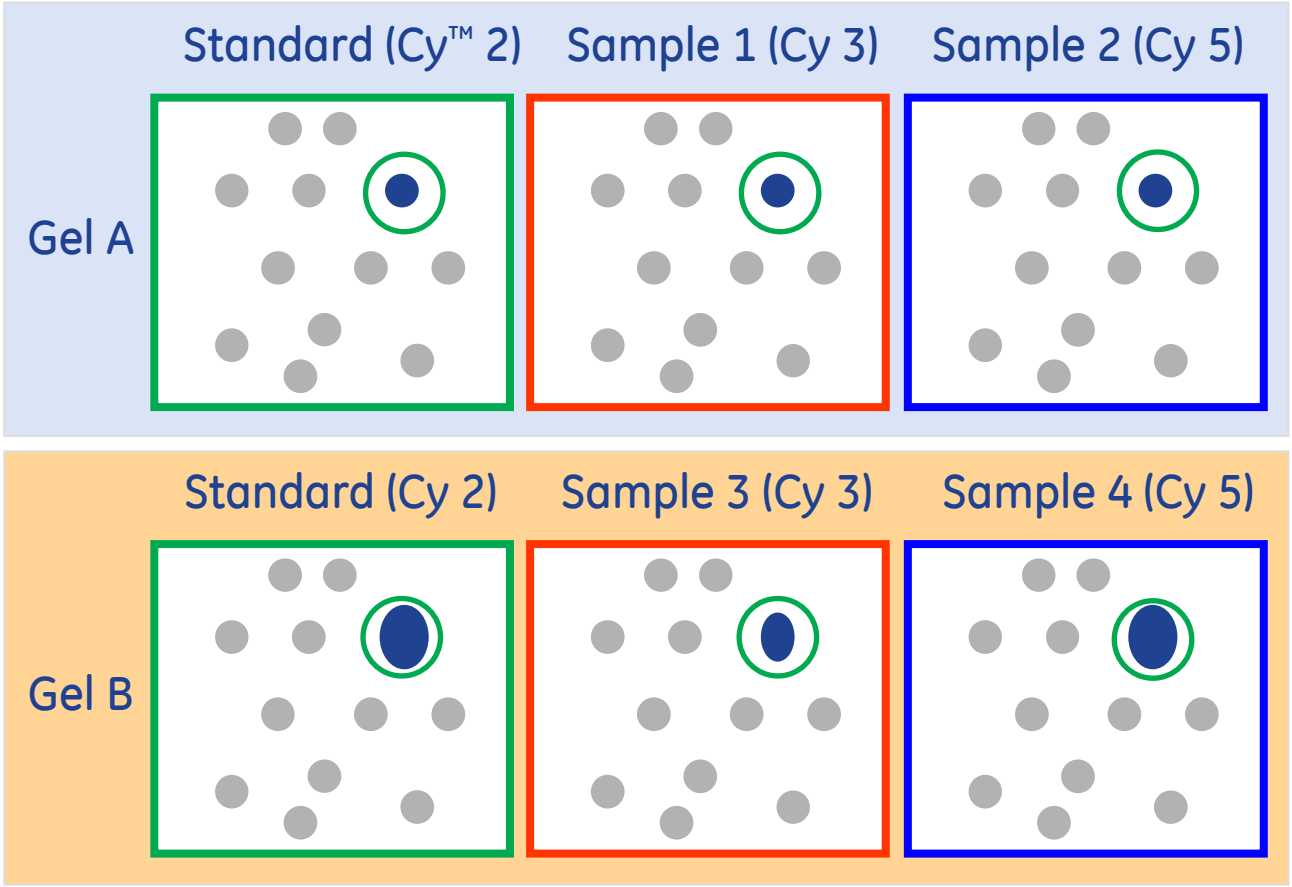


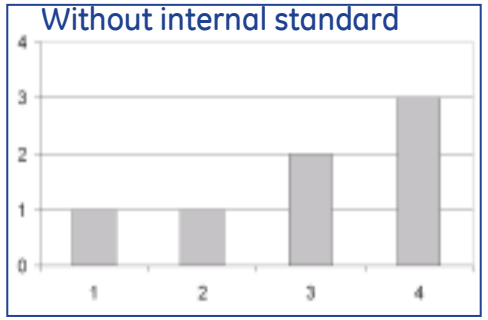
6 replicate gels made from the same sample (SYPRO™ Ruby)

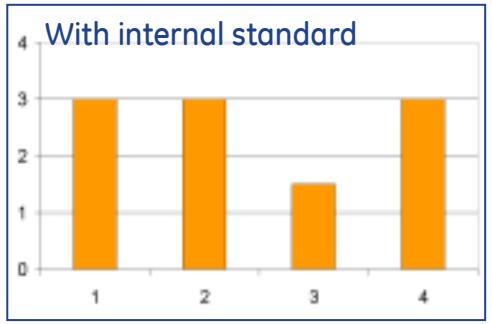
Imagination at work

Data courtesy of
Jörgen Östling, AstraZeneca R&D Mölndal, Sweden. June 2004

With 2-D DIGE, variation is eliminated





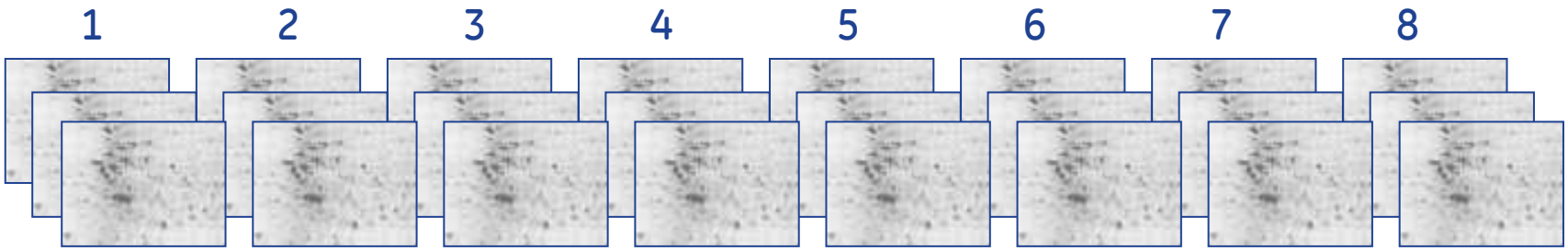


Virtual elimination of gel-to-gel variation reveals induced biological change with statistical accuracy capable of revealing differences in abundance of less than 10% between samples

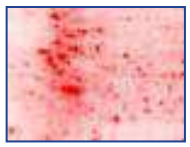
Faster and more accurate results with 2-D DIGE

Traditional 2-D electrophoresis (1-colour)

8 samples:
8 gels x triplicate = 24 gels



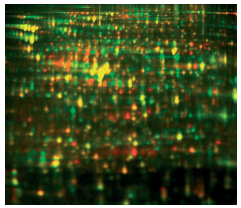
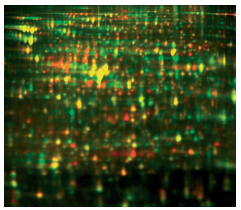
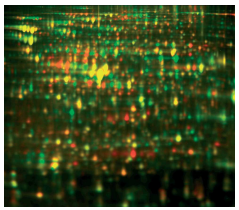
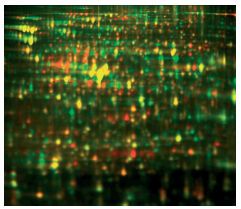
Post stain



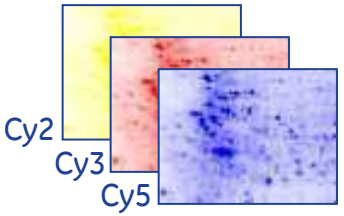
Faster and more accurate results with 2-D DIGE

Ettan™ DIGE (3-colour)

8 samples:
4 gels (no gel replicates) = 4 gels

Cy 2™ Internal standard Cy 3 Sample 1 Cy 5 Sample 2	Cy 2 Internal standard Cy 3 Sample 3 Cy 5 Sample 4	Cy 2 Internal standard Cy 3 Sample 5 Cy 5 Sample 6	Cy 2 Internal standard Cy 3 Sample 7 Cy 5 Sample 8
			

Imaged using Typhoon™ fluorescent Imager



Imagination at work

Traditional 2-D vs 2-D DIGE – summary

Traditional 2-D (1-colour)

- More gel replicates (24 gels)
- Poor accuracy for quantification
- Slow and labour intensive analysis

Ettan™ DIGE

- Less gels required (4 gels)
- High accuracy for quantitation
- Analysis fast and highly automated

2-D with no internal standard can show inaccurate or incorrect results
→ could lead to *false biological conclusions*

To maximize confidence in results and get the most out of the data
→ *an internal standard MUST be used*

Detection system affects results

Staining	Detection limit	Dynamic range
Silver	1 ng	~10^1
Coomassie™ Blue	10 ng	~10^2
Deep Purple™ Sypro™ Ruby	1–2 ng	~$10^{3.5}$
CyDyes™ Multiplexing possible!	0.25 ng	~10^4

Fluorescent detection enables improved quantitative analysis

Overview

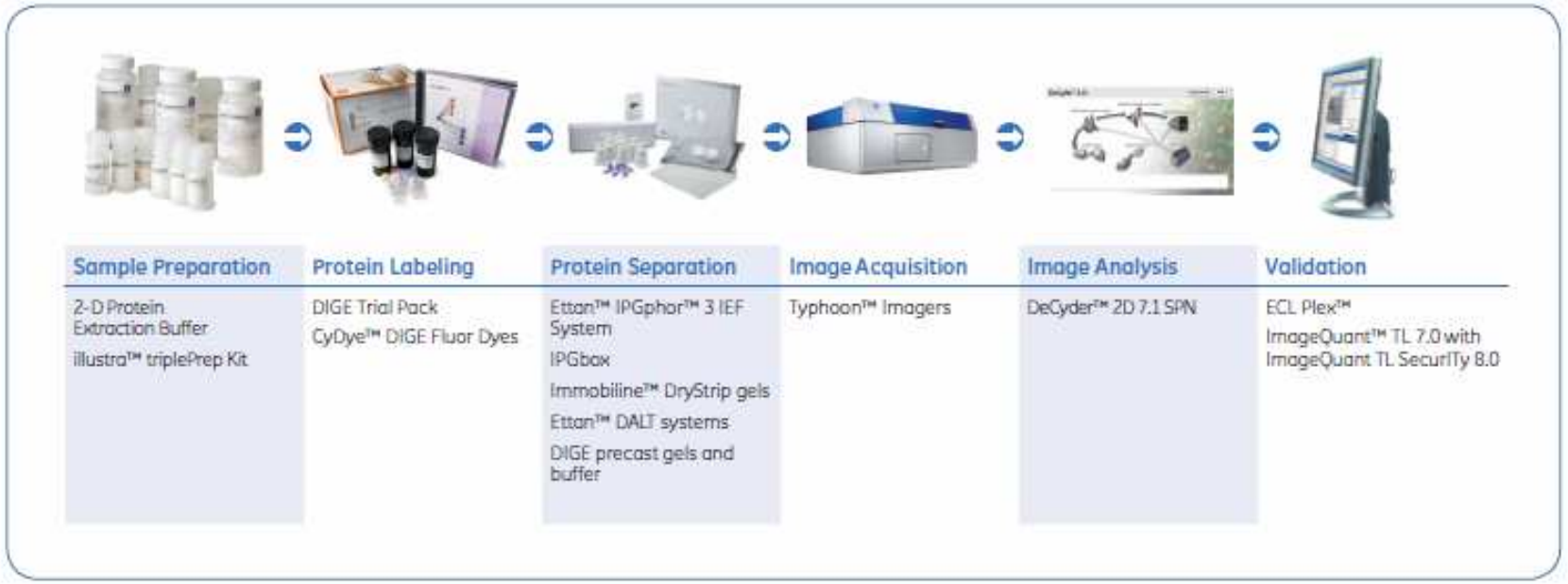
Unique Ettan™ DIGE concept

Advantages over classical 2-D electrophoresis

Improved workflow with new products

Benefits in practice

Synchronized solutions
2-D and 2-D DIGE have never been better



Sample Preparation	Protein Labeling	Protein Separation	Image Acquisition	Image Analysis	Validation
2-D Protein Extraction Buffer illustra™ triplePrep Kit	DIGE Trial Pack CyDye™ DIGE Fluor Dyes	Ettan™ IPGphor™ 3 IEF System IPGbox Immobiline™ DryStrip gels Ettan™ DALT systems DIGE precast gels and buffer	Typhoon™ Imagers	DeCyder™ 2D 7.1 SPN	ECL Plex™ ImageQuant™ TL 7.0 with ImageQuant TL SecurITy 8.0

- Improvements across the entire 2-D electrophoresis workflow
- Simplifies and improves 2-D electrophoresis

Protein separation - IEF

New rehydration solution for Immobiline DryStrips

IPGbox™

- Oil-free rehydration
- Higher quality of IPG strip rehydration
- Lid Insert - prevention of IPGstrips drying out

IPGbox Kit

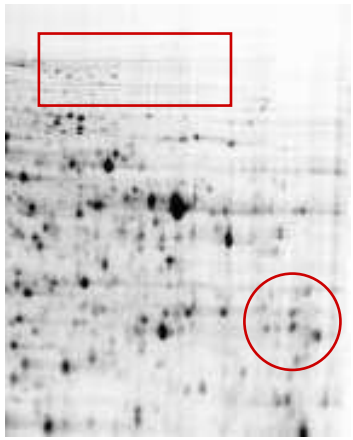
- No cross-contamination: disposable Trays and Insert
- 10 Reswell Trays + 1 disposable IPGbox Insert

Protein separation - IEF

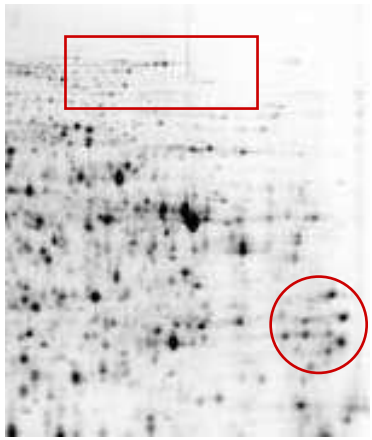
Improved quality of IPG buffer 3-10 and 3-10NL

- New formula for IPG buffer 3-10 and 3-10NL
- Additional proteins spots (HMW and basic area)
- Increased intensity
- Detecting more low abundant proteins

Current 3-10NL



New 3-10NL



Protein separation – SDS-PAGE

New precast DIGE gels and DIGE buffer kits

DIGE Gels

- Precast gels in low fluorescent glass cassettes
- Homogenous 12.5%
- Long shelf life (12 months)
- Higher reproducibility vs homecast gels

DIGE Buffer kit

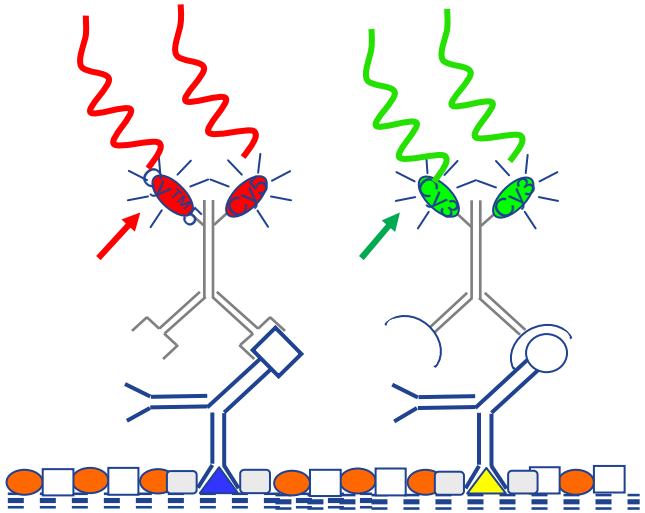
- Concentrated running buffers (based on piperidinopropionamide (PPA)) and Sealing Solution
- Ready-to-use products

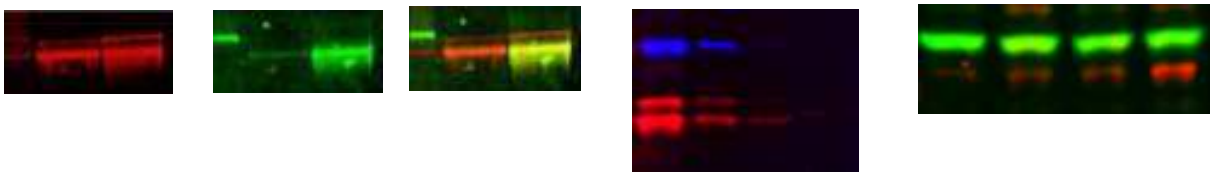
Protein validation and quantitation

ECL Plex

Multiplexing

- Detection of two proteins simultaneously
- Normalization against “housekeeping protein” for reliable quantification
- Increased dynamic range
- Highest sensitivity for the detection of low abundant proteins





Improvements through the 2-D workflow

Traditional 2-D

Sample preparation

↓

Protein separation (1st)

↓

Protein separation (2nd)

↓

Protein staining

↓

Image acquisition

↓

Image analysis

2-D DIGE

10 yrs ago

Sample preparation

↓

Sample labeling 

↓

Protein separation (1st)

↓

Protein separation (2nd)

↓

Image acquisition 

↓

Differential analysis 

Synchronized solutions

2009

Sample preparation 

↓

Sample labeling 

↓

Protein separation (1st) 

↓

Protein separation (2nd) 

↓

Image acquisition

↓

Differential and Multivariate analysis 

↓

Validation  

Overview

Unique Ettan™ DIGE concept

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Benefits in practice

Film showing experimental procedure

Top 10 most viewed videos

JoVE video

http://www.jove.com/index/Details.stp?ID=945

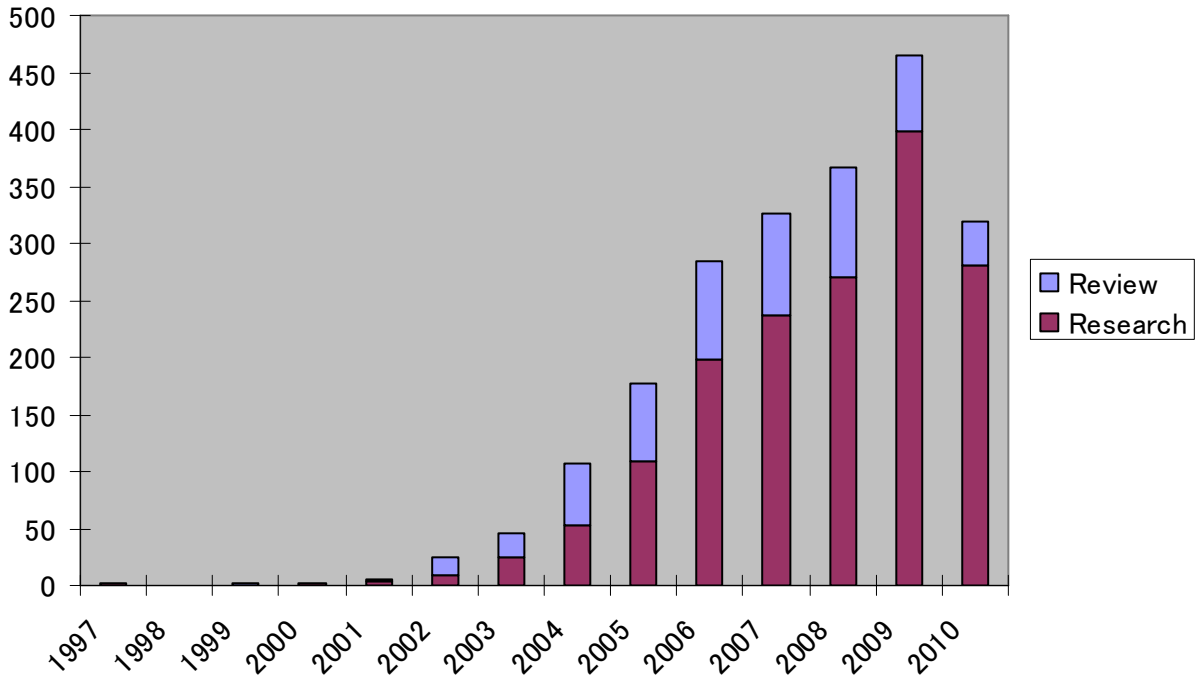
"Selective Labelling of Cell-surface Proteins using CyDye DIGE Fluor Minimal Dyes"

Åsa Hagner-McWhirter, Maria Winkvist, Stephanie Bourin, Rita Marouga

Research & Development, GE Healthcare Bio-Sciences AB

2-D DIGE
- an exciting technology that delivers real results

Total number of DIGE publications Aug 2010
>2100 publications



Top 5 categories of DIGE publications:

1. Human medicine
2. Proteomics
3. Molecular biology
4. Plants
5. Environment

Imagination at work

User endorsements

*"We do not perform standard 2-D electrophoresis anymore! We only do DIGE because it is **quicker**, **cheaper** and gives us far **higher quality** information."*

Dr Richard Burchmore,
SHW Functional Genomics Facility,
University of Glasgow

*"....the DIGE technology is **very sensitive** for **quantitative** variation..."*

*"The DIGE analysis showed a much **lower technical variation** (~7%) than the proteomics methods used in other studies (2-D electrophoresis without internal standards). Thus, the internal standard **increases the statistical confidence** of the analysis substantially."*

Prof. Dr. Oehler, Academical Hospital (AKH), Vienna
Winkler W, Zellner M, Diestinger M, Babeluk R, Marchetti M, Goll A, Zehetmayer S, Bauer P, Rappold E, Miller I, Roth E, Allmaier G, Oehler R. Biological variation of the platelet proteome in the elderly population and its implication for biomarker research. Mol Cell Proteomics 7 (2008) 193-203.

Frost & Sullivan Technology Innovation Award 2007

*"GE's **Ettan™ DIGE System is capable of comparing protein expression patterns from two different samples in a single gel**. This information is **crucial in the search for biomarkers that may change in expression levels during** the initiation or progression of a **disease** from one phenotype to a more malignant phenotype.*

The need to isolate and identify these protein biomarkers that appear or fail to appear is likely to also influence the way patients' treatment protocols are determined."

North American Frost & Sullivan Award for Technology Innovation (2007)

Printed material

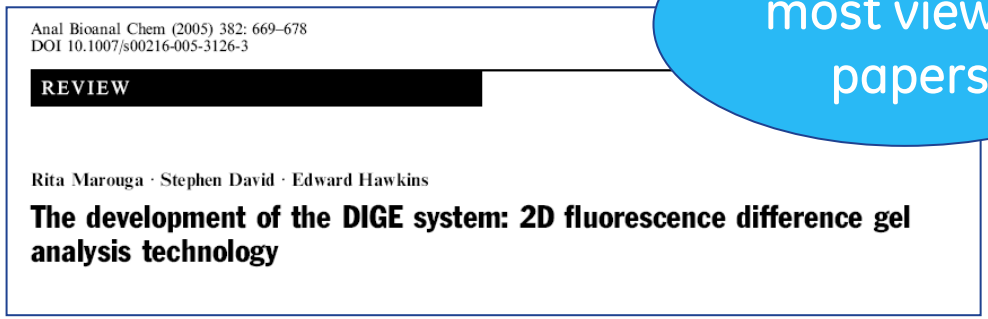
Data Files

Application Notes

Printed material

Articles

Top 5 most viewed papers

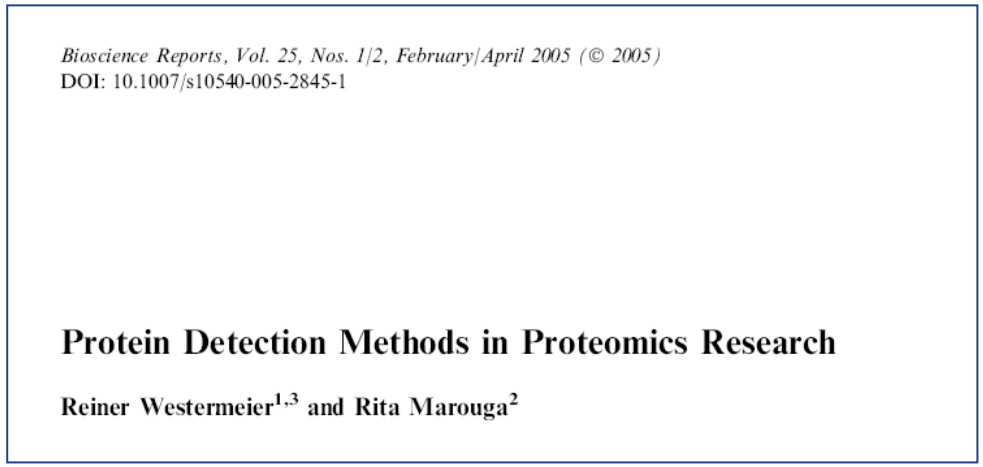


Anal Bioanal Chem (2005) 382: 669–678
DOI 10.1007/s00216-005-3126-3

REVIEW

Rita Marouga · Stephen David · Edward Hawkins

The development of the DIGE system: 2D fluorescence difference gel analysis technology



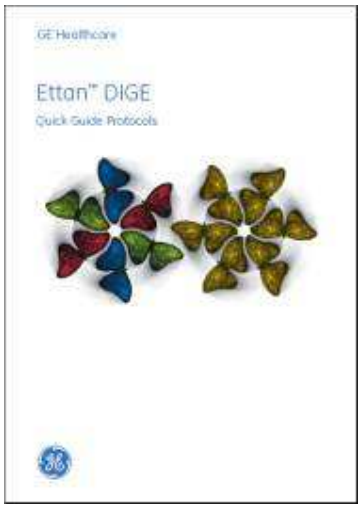
Bioscience Reports, Vol. 25, Nos. 1/2, February/April 2005 (© 2005)
DOI: 10.1007/s10540-005-2845-1

Protein Detection Methods in Proteomics Research

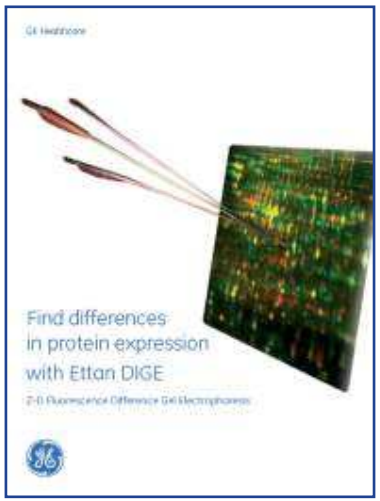
Reiner Westermeier[1,3] and Rita Marouga[2]

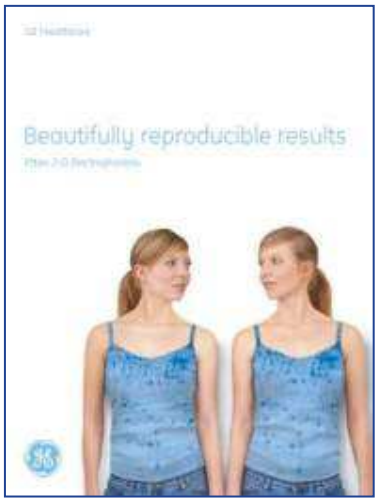
Printed material

Quick Guide Protocol



Brochures





Cue card

Summary

Advantages with DIGE technology:

- Faster and more accurate results - Multiplexing
- Improved sensitivity (subnanogram level)
- Wider dynamic range (4-5 orders of magnitude)
- Eliminate variation – Internal standard
- Detection of small differential changes (down to 10%)

DIGE video


Separation
Image acquisition
Experimental design and sample labeling
DIGE
Ettan DIGE 2-D Fluorescence
Difference Gel Electrophoresis
Introduction
Image analysis and data mining
Biological applications

Thank you for your attention!

Questions ?

imagination at work