Proteomics

Chapter 4.
strategies for protein quantitation Ⅰ

Strategies to quantify proteomes

At the current time, protein quantitation in proteomics relies primarily on the use of general labeling or staining or on the selective labeling or staining of particular classes of proteins.

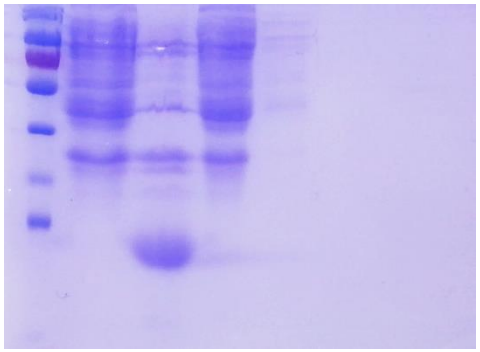
However, what is required in proteomics is a selection of methods for comparing the abundances of thousands of proteins in parallel across multiple samples.

2 broad categories;
(1) Those based on the image analysis of 2D-gels
(2) Those based on the differential labeling of samples of separation by liquid chromatography followed by mass spectrometry

Image acquisition from 2D gels

The abundance of different protein on a 2D-gels is determined by the shape, size and intensity of the corresponding spots

The first stage in protein quantitaion is image acquisition and the method used depends on how the proteins were labeled or stained.



Coomassie Brilliant Blue staining

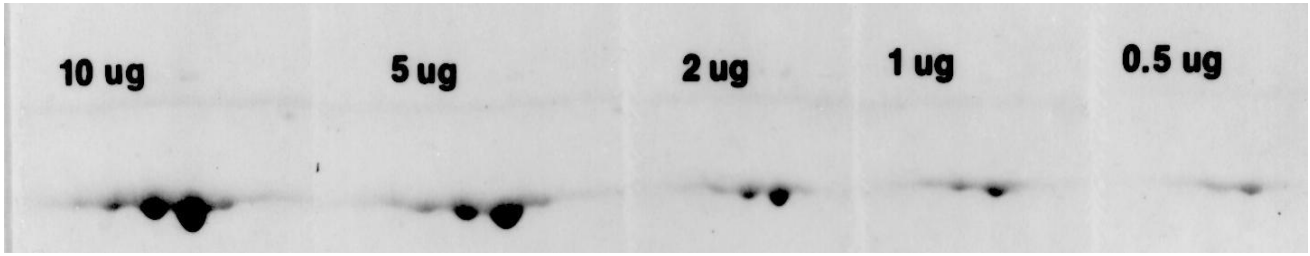
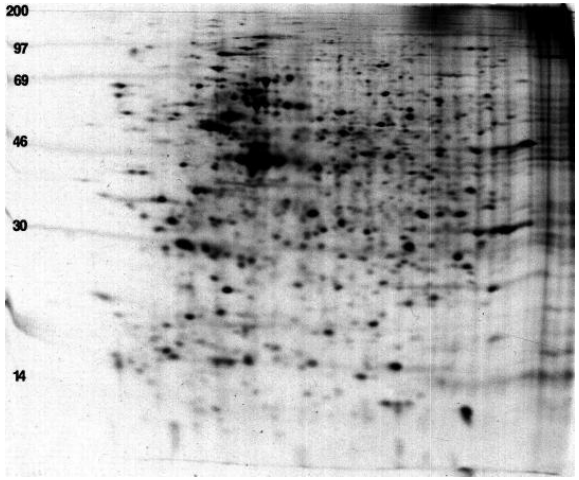


Image acquisition from 2D gels

Radioactively labeled proteins are detected by X-ray film or phosphor imaging.

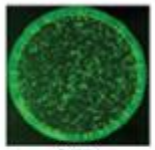
X-ray film may be scanned by a charge-coupled device (CCD) camera or densitometer, whereas phosphorimagers come with their own scanning device

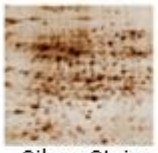


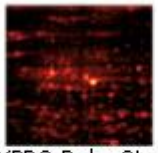
HL60 cells were labeled with ^{35}S methionine

Image acquisition from 2D gels

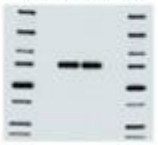
Charge-coupled device (CCD) camera
: solid-state electrical device
(light-sensitive areas or photo sites)

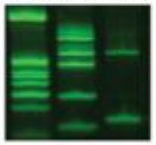

GFP

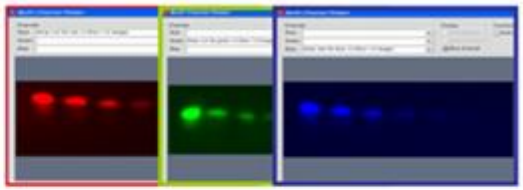

Silver Stain


SYPRO Ruby Stain


Coomassie Blue


Immun-Star HRP kit


SYBR Green I Stain


DIGE : Cy2, Cy3, Cy5

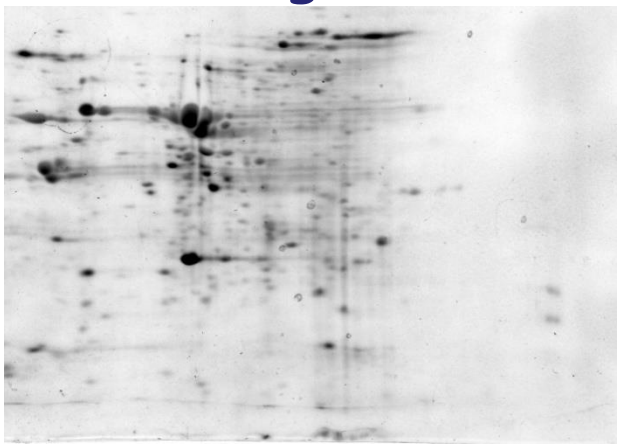
VERSADOC (BioRad)

Densitometer
: a scanning device that measure the degree of darkness… light reflected from or transmitted through the surface of a film

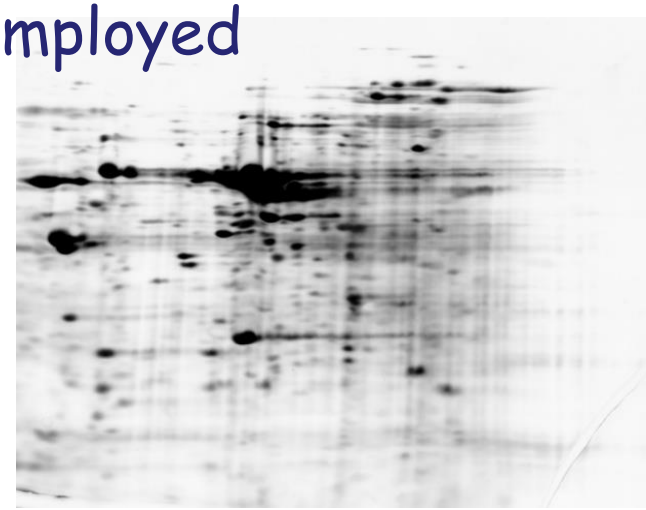
Comparison of staining methods

The quality of the digital data depends on the resolution of the scanned image and densitometric resolution

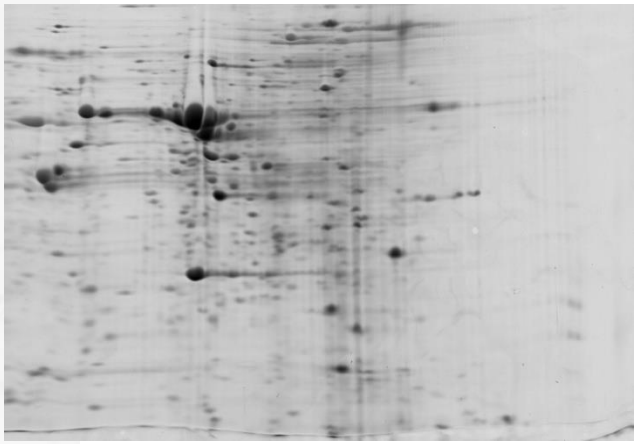
Densitometric resolution also depends on labeling and staining method employed



Coomassie Brilliant Blue (CBB)



Silver staining



SYPRO Ruby

Silver staining vs SYPRO

Silver staining has been the major nonradioactive detection method because it is 10-100 times more sensitive than Coomassie brilliant blue (CBB) staining.

Disadvantages of silver staining
It does not detect glycoproteins and the chemical modification of cysteine residues is involved, therefore interfering with downstream analysis by MS. In addition, it has narrow linear range.

The newer SYPRO reagents are at least as sensitive as silver staining but share none of its disadvantages

Spot detection, quantitation and comparison (1)

Spots on protein gels are not uniform in shape, size or density

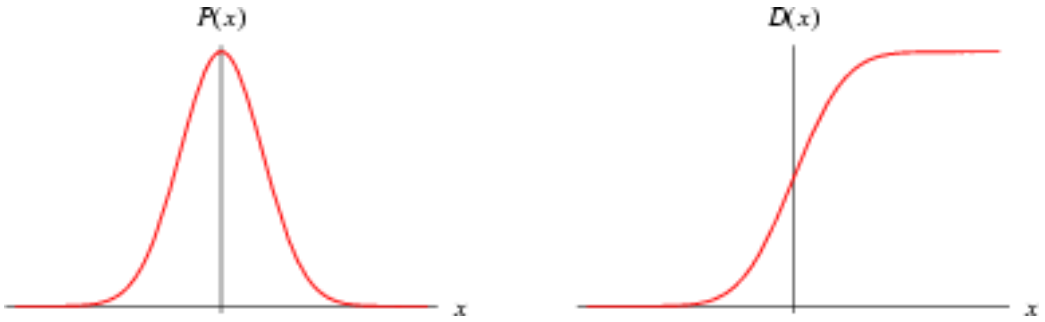
Getting machines to see the spots in the same way that we do can be extremely challenging

Automated spot detection
(1) Digital image enhancement.
(2) Smoothing for eliminating noise
(3) Contrast enhancement
(4) Edge detection filtering

Spot detection, quantitation and comparison (2)

Once a processed image is available, a number of different algorithms can be applied to detect and quantitate individual spots.

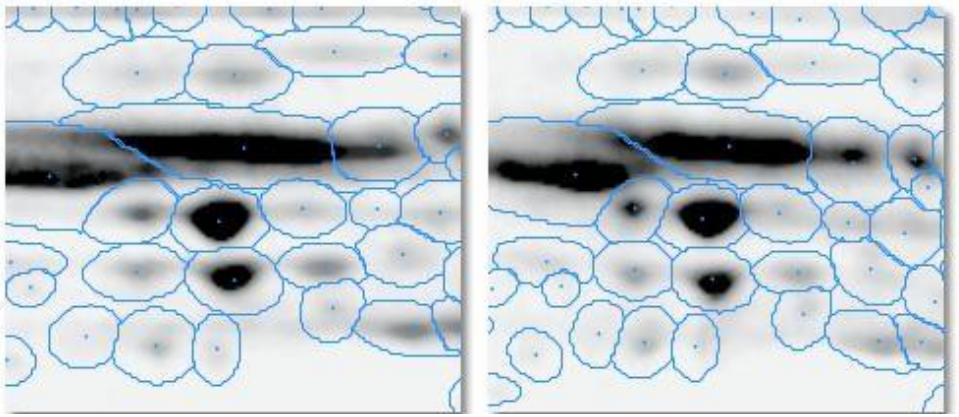
These must take all the possible variations in spot morphology into account and calculate the integrated spot



Algorithms are based on Gaussian fitting and Laplacian of Gaussian spot detection method

Spot detection, quantitation and comparison (3)

Watershed transformation method in which gray scale image is converted into a topographical surface with darker sections representing peaks and lighter sections representing troughs



The idea is then to "flood" the image from the minima, which divides the image into catchment basins representing individual spots and watershed lines representing divisions (plate 2, between page 82 and 83)

Spot detection, quantitation and comparison (3)

plate 2, between page 82 and 83

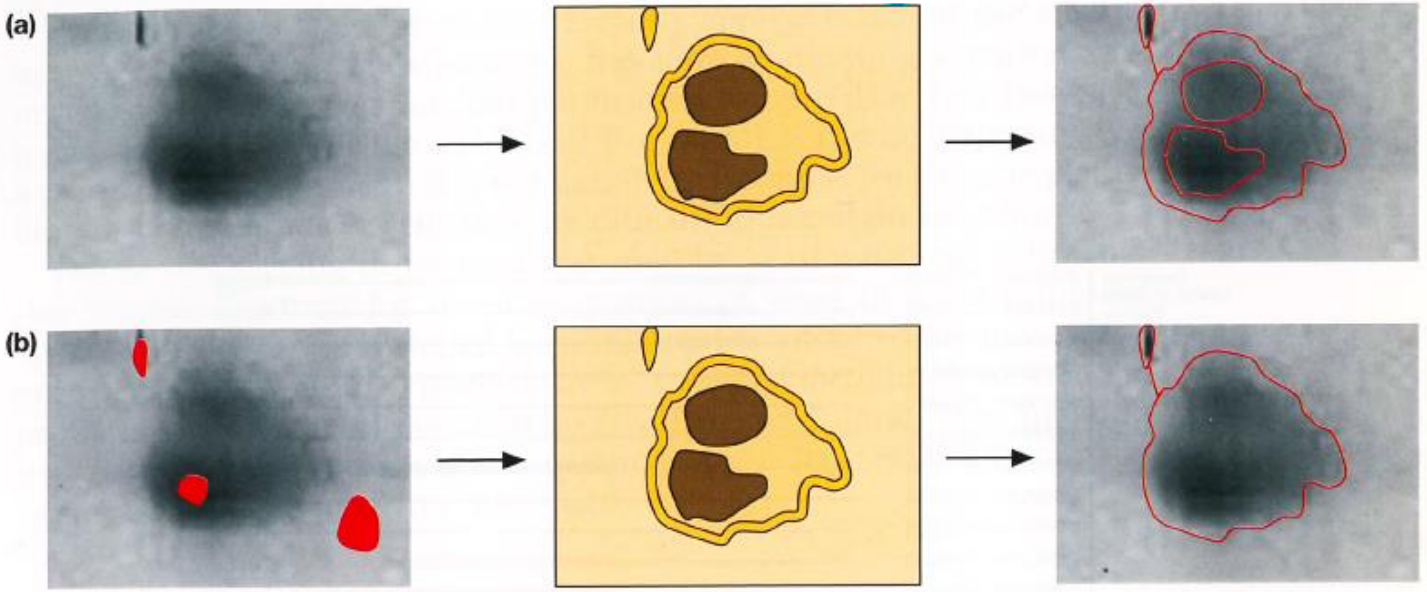


Plate 2

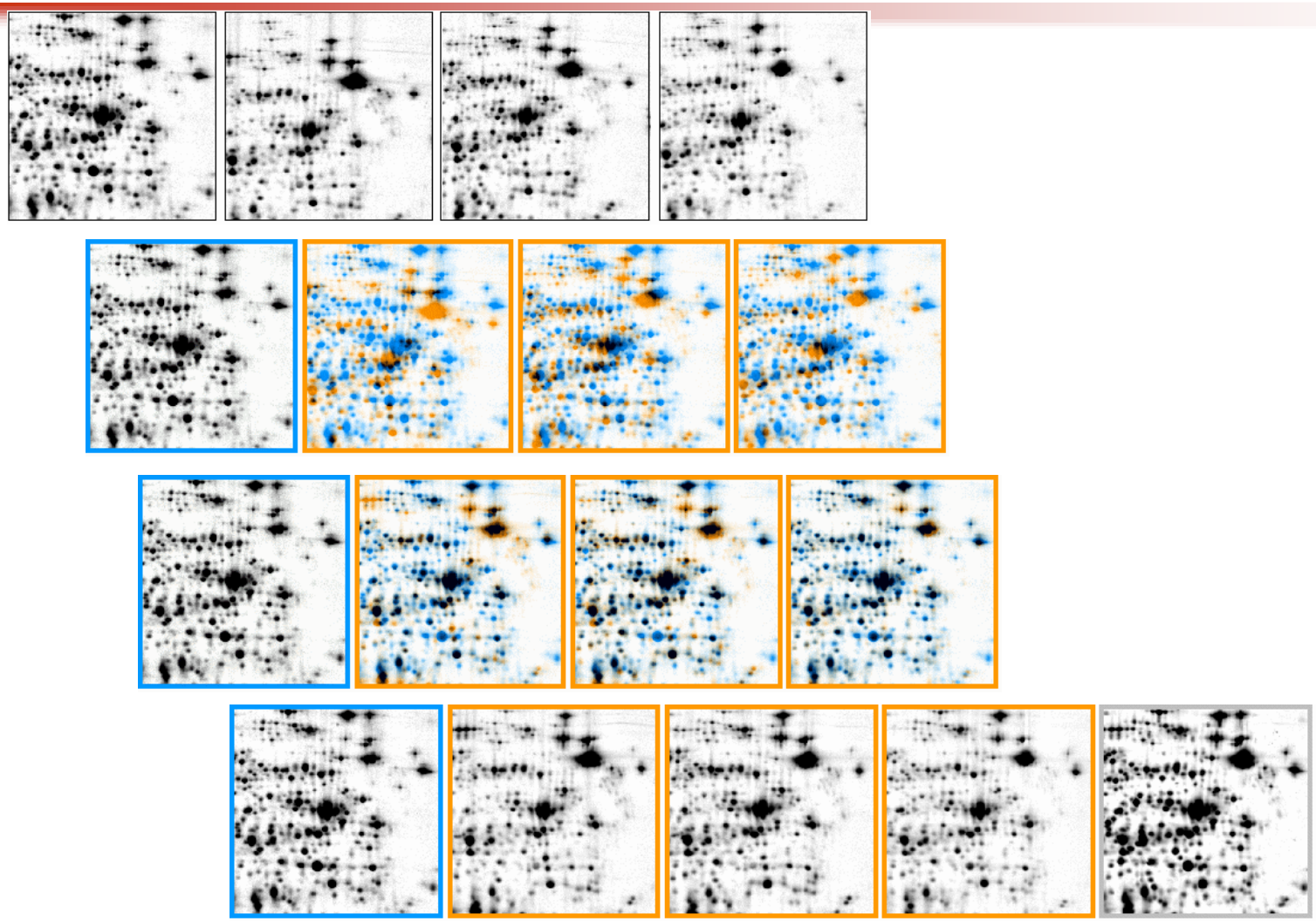
The watershed method for contour finding on 2D-gel images. (a) Any graytone image can be considered as a topographic surface. If flooded from its minima without allowing water from different sources to merge, the image is partitioned into catchment basins and watershed lines, but in practice this leads to over-segmentation. (b) Therefore, markers (red shapes) are used to initiate flooding, and this reduces over-segmentation considerably. Adapted from images by Dr Serge Beucher, CMM/Ecole de Mines de Paris.

Spot detection, quantitation and comparison (4)

Once the 2D-gel has been reduced to a series of digital data representing spot intensities, the comparison of different gels is a simple process of comparing data values and determining whether the abundance of a given protein differs significantly.

A prerequisite for this type of analysis is that identification of equivalent spots on different gels, which may be challenging because gel-running conditions cannot be reproduced exactly (due to differences in sample preparation and gel composition, variations in running conditions, and minor variations within each gel that lead to regional differences in protein mobility.)

Spot detection, quantitation and comparison (5)



Spot detection, quantitation and comparison (6)

It becomes necessary to force equivalent gels into register, a process known as gel matching

Gel-matching algorithms then apply image transformation procedures such as stretching, skewing, and rotating, at both local and global levels, to bring multiple gel images into register and make them comparable; Figure 4.1

Alternative to matching gels … an extension of the use of landmarks … the algorithm begins at a known landmark and then maps the nearby spots and returns a list of x/y displacements values. Other gels are scrutinized for spots at the same displacement relative to landmark and matches are identified.

Spot detection, quantitation and comparison (6)

Figure 4.1

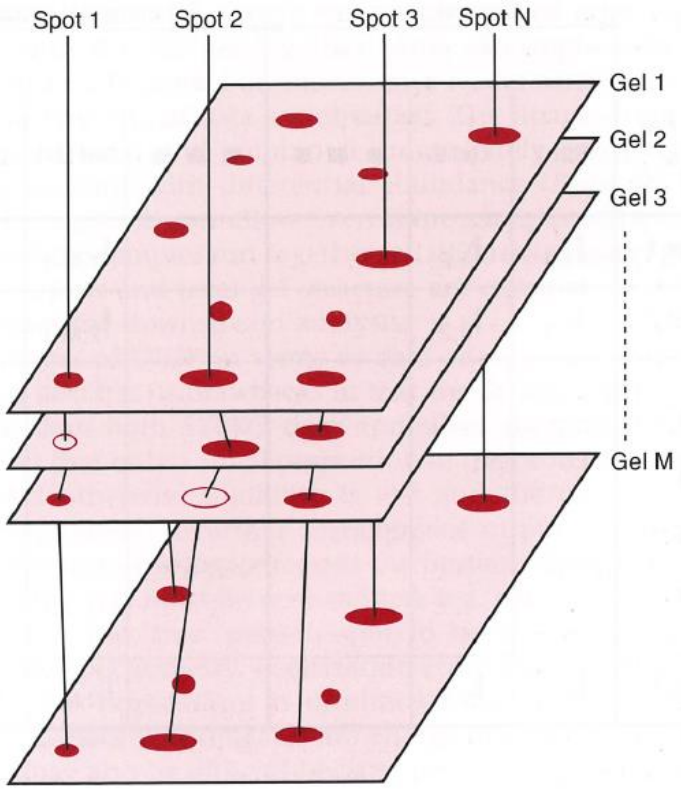
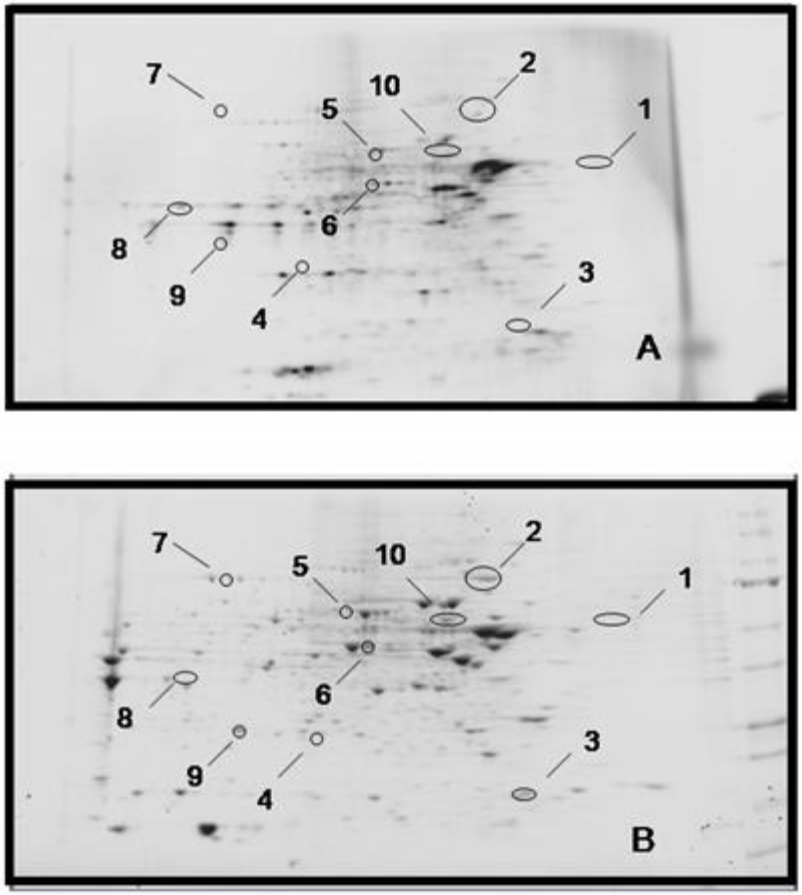


Figure 4.1

Principle of spot matching to identify corresponding spots on multiple gels. Empty circles represent absent spots.

Spot detection, quantitation and comparison (7)

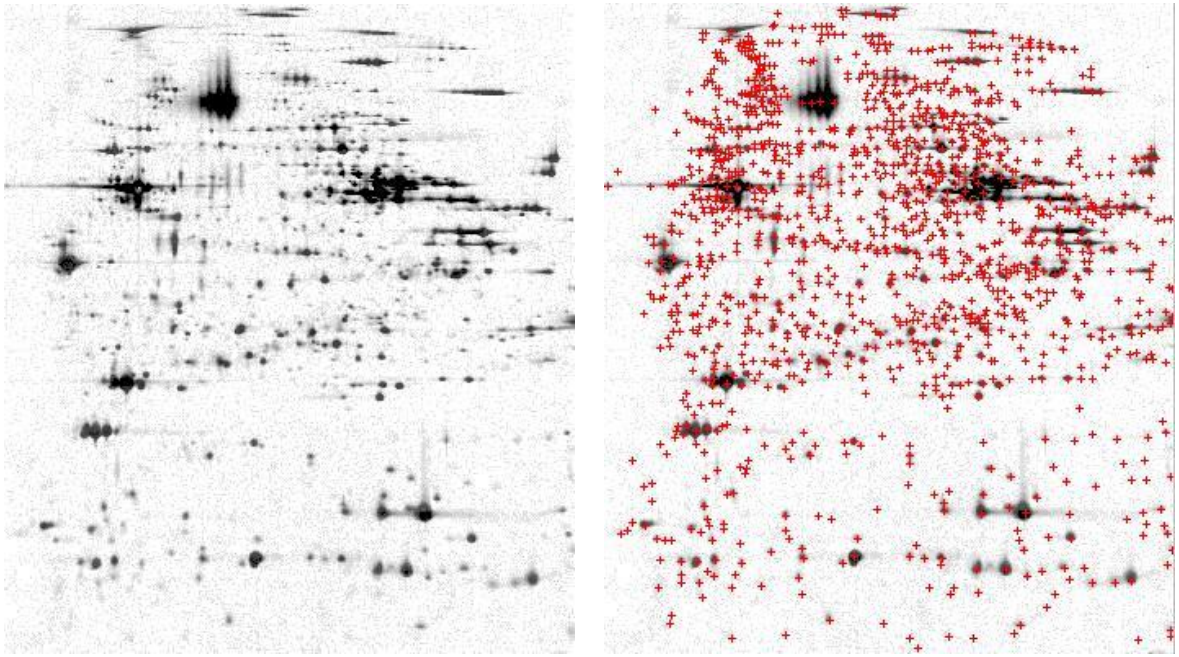
Landmark.



Spot detection, quantitation and comparison (5)

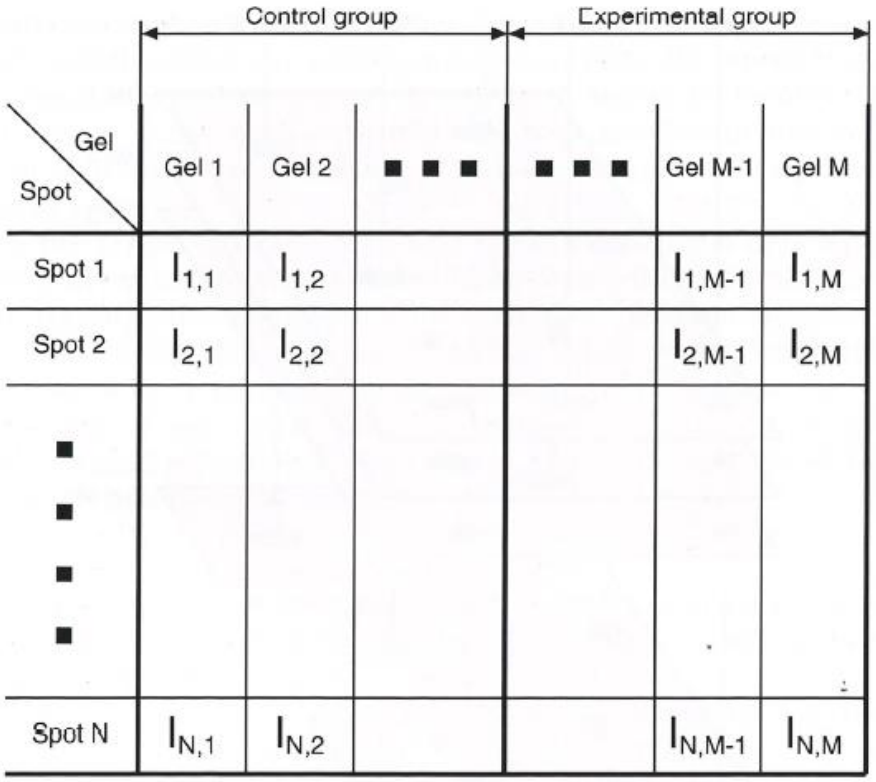
The end result of spot detection, quantitation and gel matching should be a table of spot vales arranged as a N x M matrix, where N represents all the different spots that have been identified, and M represent all the gels

Figure 4.2.



Spot detection, quantitation and comparison (5)

Figure 4.2.



Spot \ Gel	Control group: Gel 1	Gel 2	■ ■ ■	Experimental group: ■ ■ ■	Gel M-1	Gel M
Spot 1	$I_{1,1}$	$I_{1,2}$			$I_{1,M-1}$	$I_{1,M}$
Spot 2	$I_{2,1}$	$I_{2,2}$			$I_{2,M-1}$	$I_{2,M}$
■ ■ ■ ■						
Spot N	$I_{N,1}$	$I_{N,2}$			$I_{N,M-1}$	$I_{N,M}$

Figure 4.2

A generic data analysis matrix containing integrated spot densities. N is the number of spots (rows) and M is the number of gels (columns).