2D Gel Electrophoresis

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History

- 1807 Movement of clay particles were first observed by the scientist Ferdinand Frederic Reuss who gave the theory of electrophoresis.
- 1934-1937 Then apparatus was developed known as Tiseleus apparatus named after the scientist Tiseleus for the analysis of colloidal mixtures.
- 1940-50 Zone electrophoresis techniques were then introduced, where filter paper, capillary tubes were used as carrier matrix for the movement of molecules.
- 1955 In this year, Oliver Smithies introduced agarose gel and polyacrylamide gels as a substrate for the separation of biomolecules.
- 1960's A.L. Shapiro, J.V. Maizel developed relationship between the molecular weight and migration of proteins.
- 1975 Farrell and J.Klore developed 2-D electrophoresis.
- 1981 Electrophoresis of amino acid was carried out by the two scientist J.W. Jorgensen and K.D. Lukas
- 1990 Matrix developed for the DNA separation at high resolution by the B.L. Karger and his group.
- 2000- now Many high resolution electrophoresis methods have developed for both analytical and preparative measures.

Introduction

- Electrophoresis is a technique used to separate macromolecules in a fluid or gel based on their charge, binding affinity, and size under an electric field.
- In the year 1807, Ferdinand Frederic Reuss was the first person to observe electrophoresis. He was from Moscow State University.
- Anaphoresis is the electrophoresis of negative charge particles or anions whereas cataphoresis is electrophoresis of positive charge ions or cations.
- Electrophoresis has a wide application in separating and analysing biomolecules such as proteins, plasmids, RNA, DNA, nucleic acids.

Types of Electrophoresis:

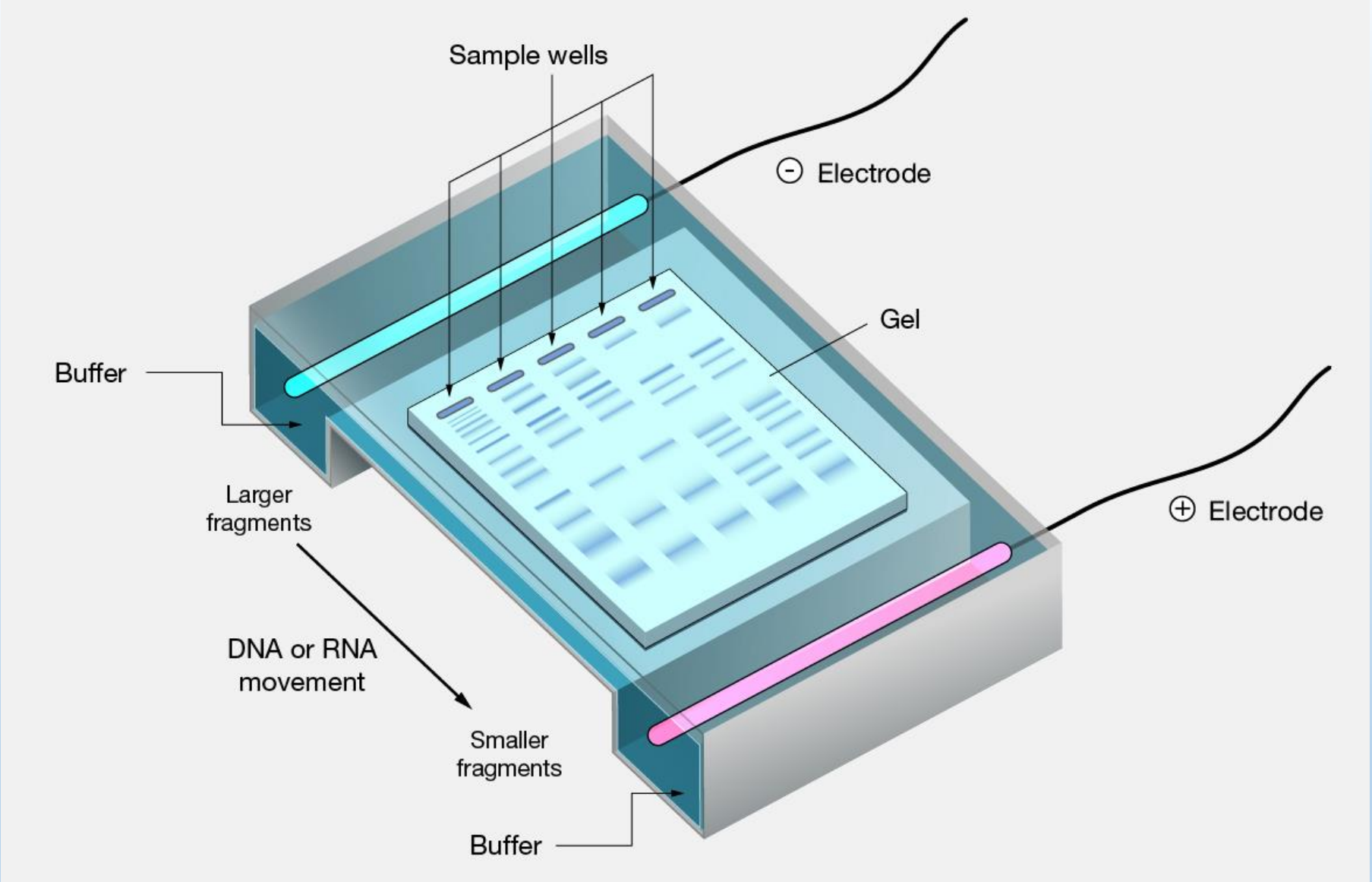
1. Capillary electrophoresis
- Gel electrophoresis
- Paper electrophoresis

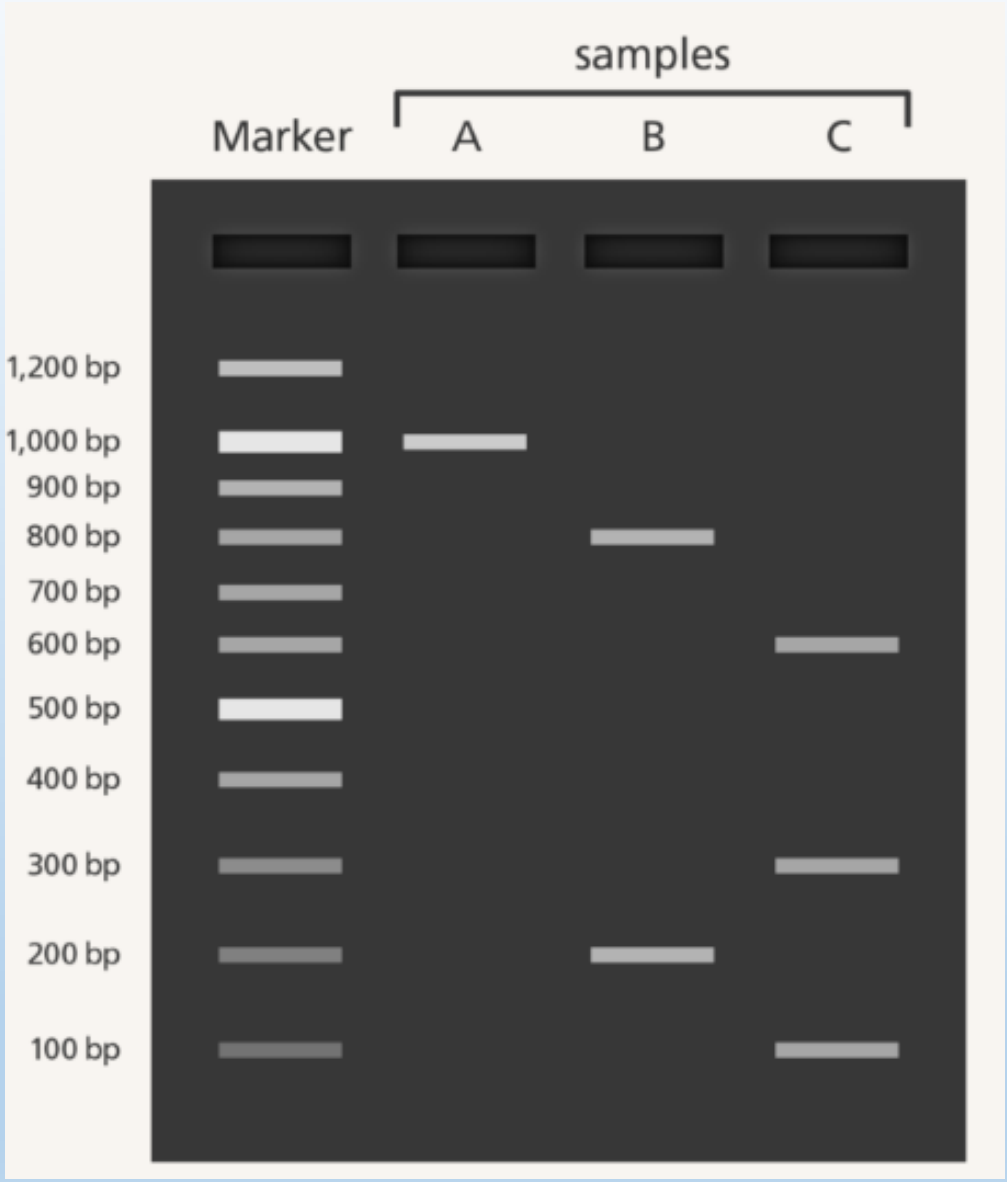
2. Slab electrophoresis
- Zone electrophoresis
- Immunoelectrophoresis
- Isoelectrofocusing

Basic Principles of Electrophoresis

- Agarose Gel Electrophoresis: the galactose residues are occasionally substituted with negatively charged groups such as sulphate and pyruvate, giving the agarose fibres a fixed negative charge. Agarose is insoluble in cold water but dissolves readily in boiling water. Upon cooling, the agarose chains form side-by-side

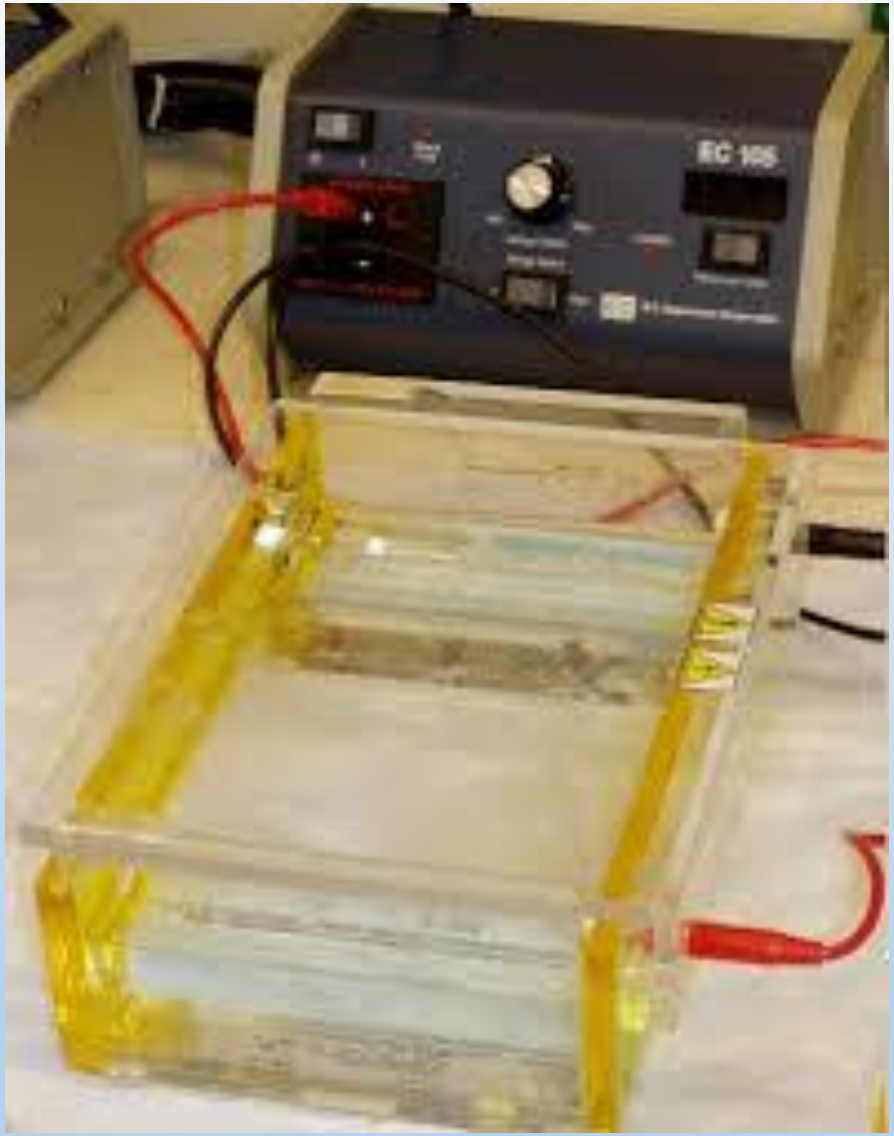
- Polyacrylamide Gel Electrophoresis: are chemically inert and mechanically stable. By chemical co-polymerization of acrylamide (AA) monomer, the reaction is started with ammonium persulphate as catalyst, while TEMED (as an initiator of this polymerization reaction) provides the tertiary amino groups to release the radicals.


Sample wells
⊖ Electrode
Gel
Buffer
Larger fragments
⊕ Electrode
DNA or RNA movement
Smaller fragments
Buffer

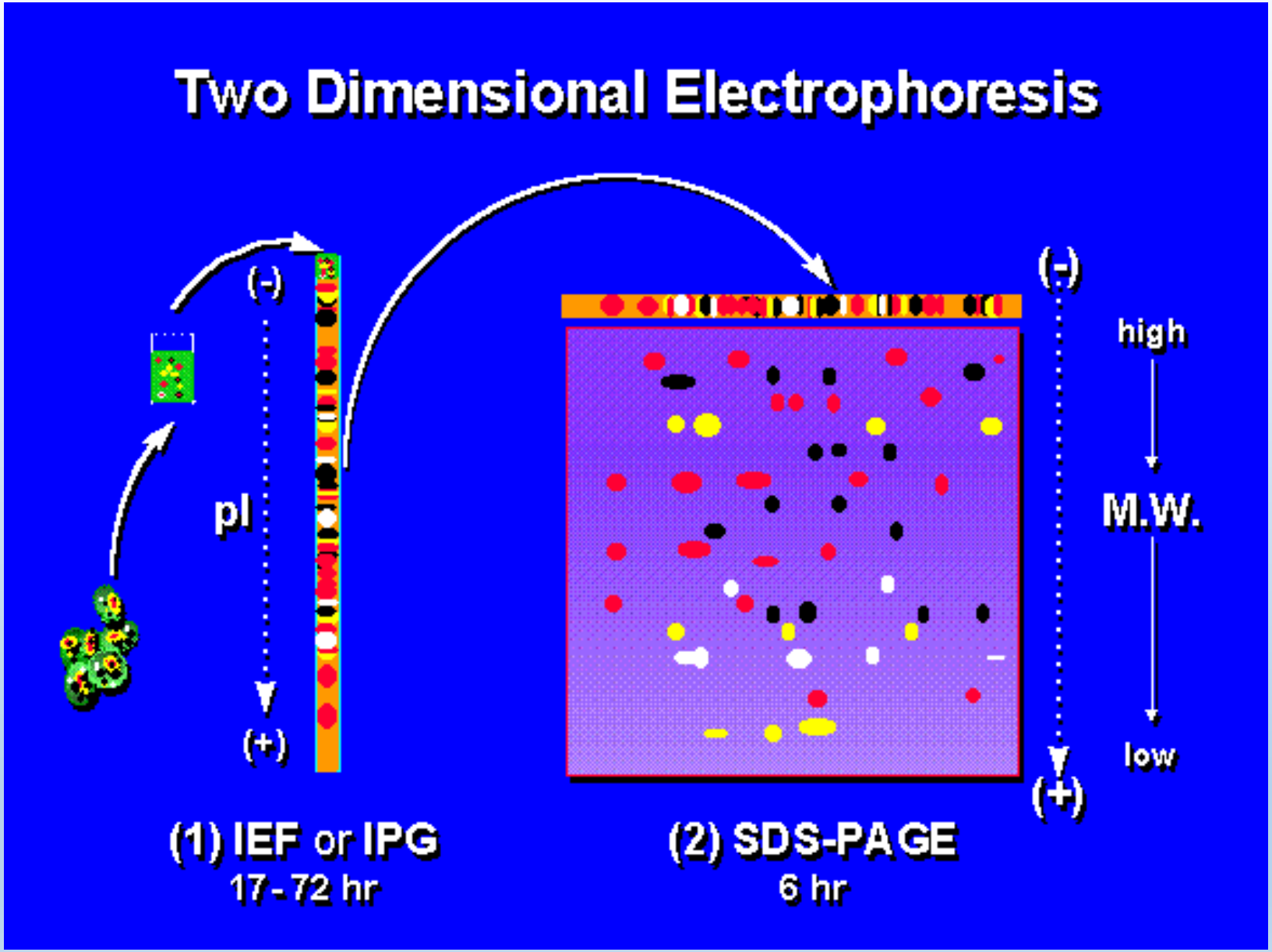

samples
Marker
A
B
C
1,200 bp
1,000 bp
900 bp
800 bp
700 bp
600 bp
500 bp
400 bp
300 bp
200 bp
100 bp

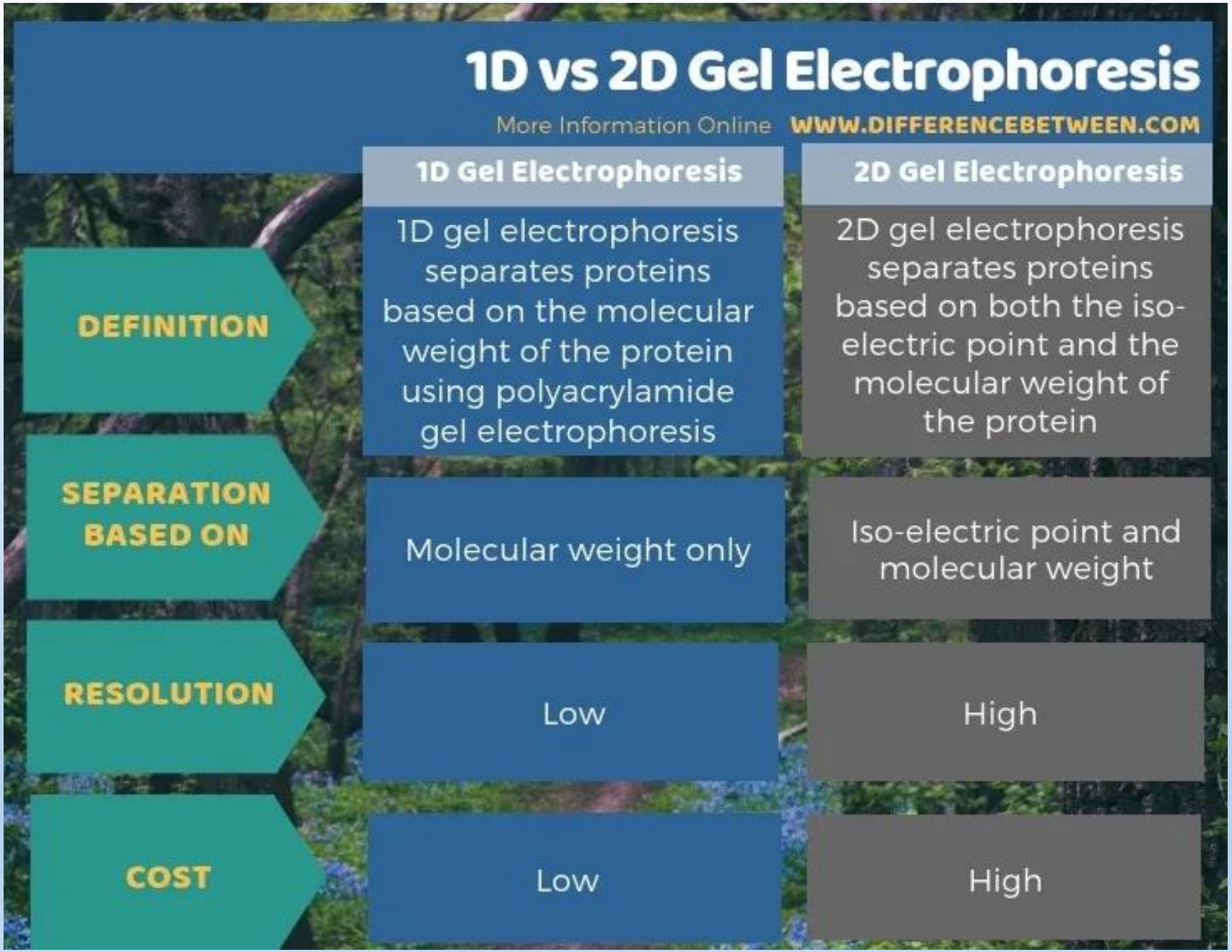
- Isoelectric Focusing: takes place in a pH gradient, which is formed by a complex mixture of synthetic small molecules carrying a variety of charged ionized groups (i.e., amino and carboxylic acid residues).

- Two-Dimensional Electrophoresis: The combination of two different electrophoretic principles can be used in various applications (e.g., crossed immunoelectrophoresis), but high-resolution 2D electrophoresis has been the method of choice and remains a unique and powerful approach to resolve proteins and polypeptides in complex mixtures. The first dimension is IEF in the presence of about 8 M urea and a non-ionic detergent, while SDS-PAGE is run as the second dimension. This technique has become highly interesting


EC 105

IPG BlueStrips

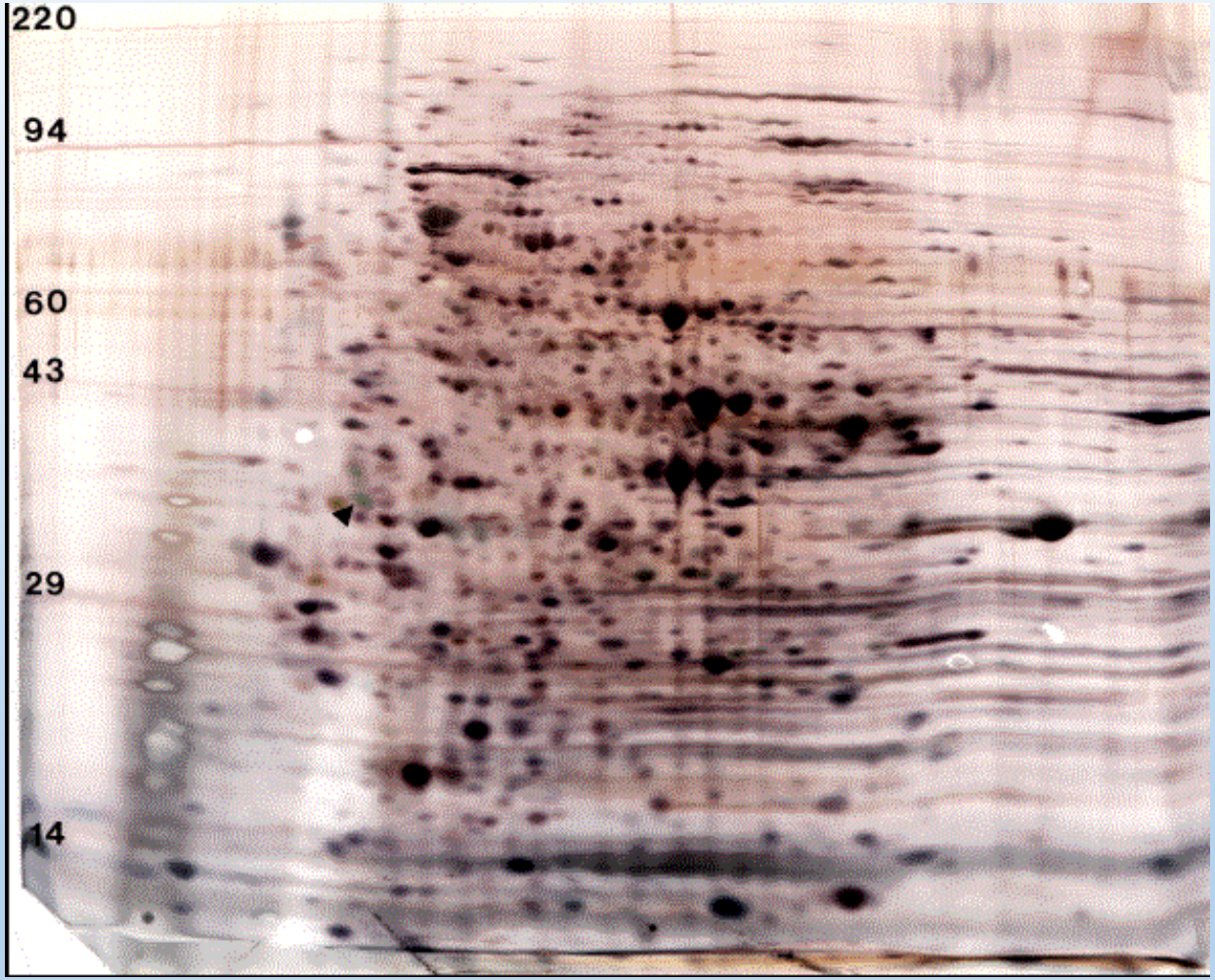

Two Dimensional Electrophoresis
(-)
pI
(+)
(1) IEF or IPG
17 - 72 hr
(-)
high
M.W.
low
(+)
(2) SDS-PAGE
6 hr


1D vs 2D Gel Electrophoresis
More Information Online
WWW.DIFFERENCEBETWEEN.COM
1D Gel Electrophoresis
2D Gel Electrophoresis
DEFINITION
1D gel electrophoresis separates proteins based on the molecular weight of the protein using polyacrylamide gel electrophoresis
2D gel electrophoresis separates proteins based on both the iso-electric point and the molecular weight of the protein
SEPARATION BASED ON
Molecular weight only
Iso-electric point and molecular weight
RESOLUTION
Low
High
COST
Low
High

Why 2 D electrophoresis

- Proteomics, a field dedicated to studying proteins on a grand scale, has played a pivotal role in revolutionizing our comprehension of cellular processes and functions.
- It encompasses a diverse array of techniques aimed at characterizing proteins, including their abundance, post translational modifications (PTMs), and interactions.
- Among the powerful tools in the realm of proteomics, 2D electrophoresis firmly stands out as an esteemed technology for not only separating and analyzing intact proteins from complex mixtures, but also pushing the boundaries of innovation.

2D Gel Electrophoresis



Yeast Proteome:
50 ug protein loaded,
pH 4-8 ampholines,
10% slab gel, silver
stain.

Advantages

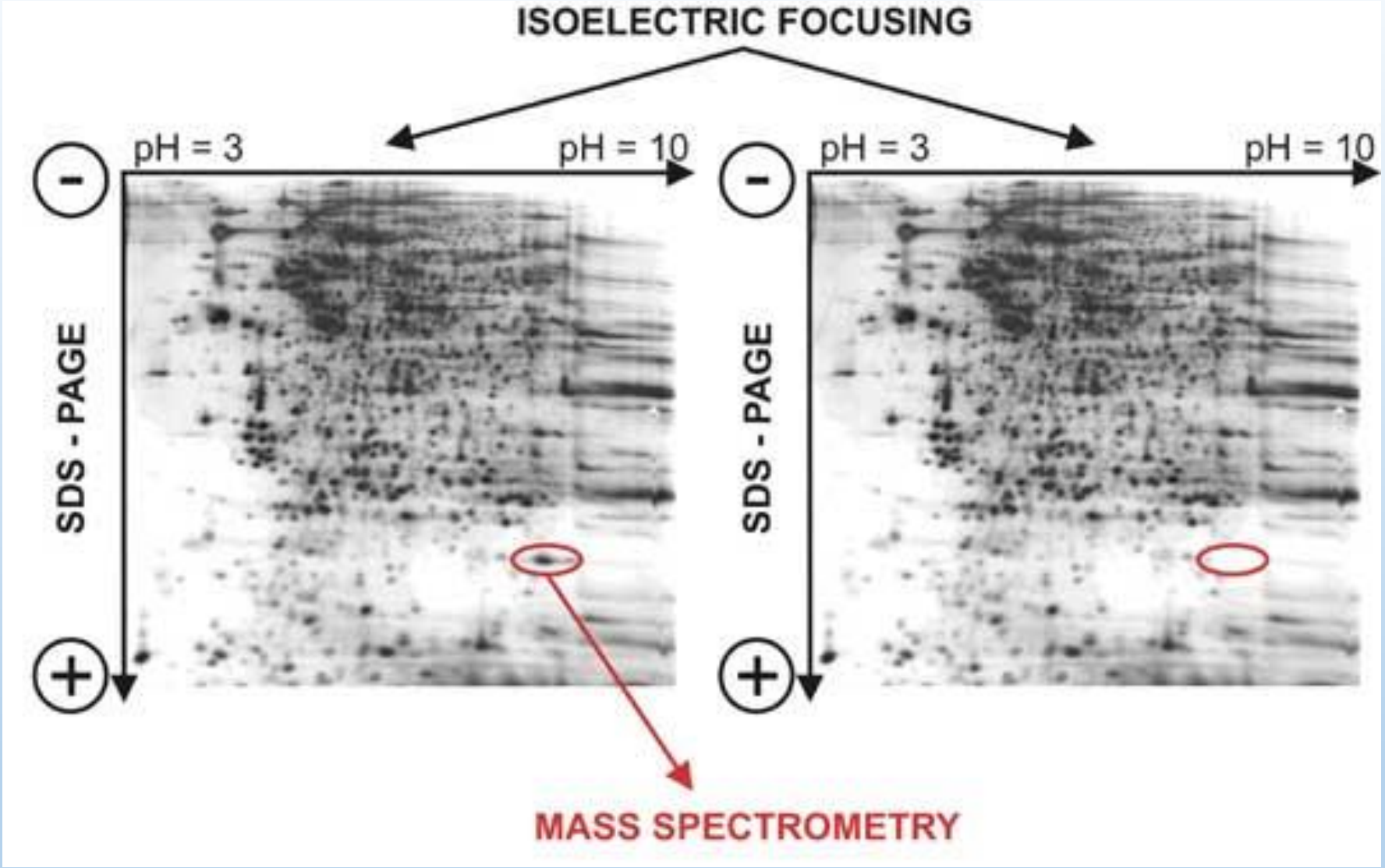
- High-throughput: 2D electrophoresis can accurately analyze thousands of proteins in a single run.
- High resolution: This technology resolves proteins according to both pI and molecular mass, and enables the characterization of proteins with posttranslational modifications that affect their charge state.
- Various computer-based tools are available: We have tools such as SameSpots, Delta2D, ImageMaster, which can be used for detection and quantification of protein spots.
- Cost-efficient and affordable: While mass spectrometers represent a significant investment and require experience staff, 2D electrophoresis is relatively inexpensive.
- High Flexibility: We work closely with you to design the optimal experimental scheme to meet your research objectives on budget.

2D Gel Electrophoresis

Separation of hundreds of proteins based on

-pI
-MW

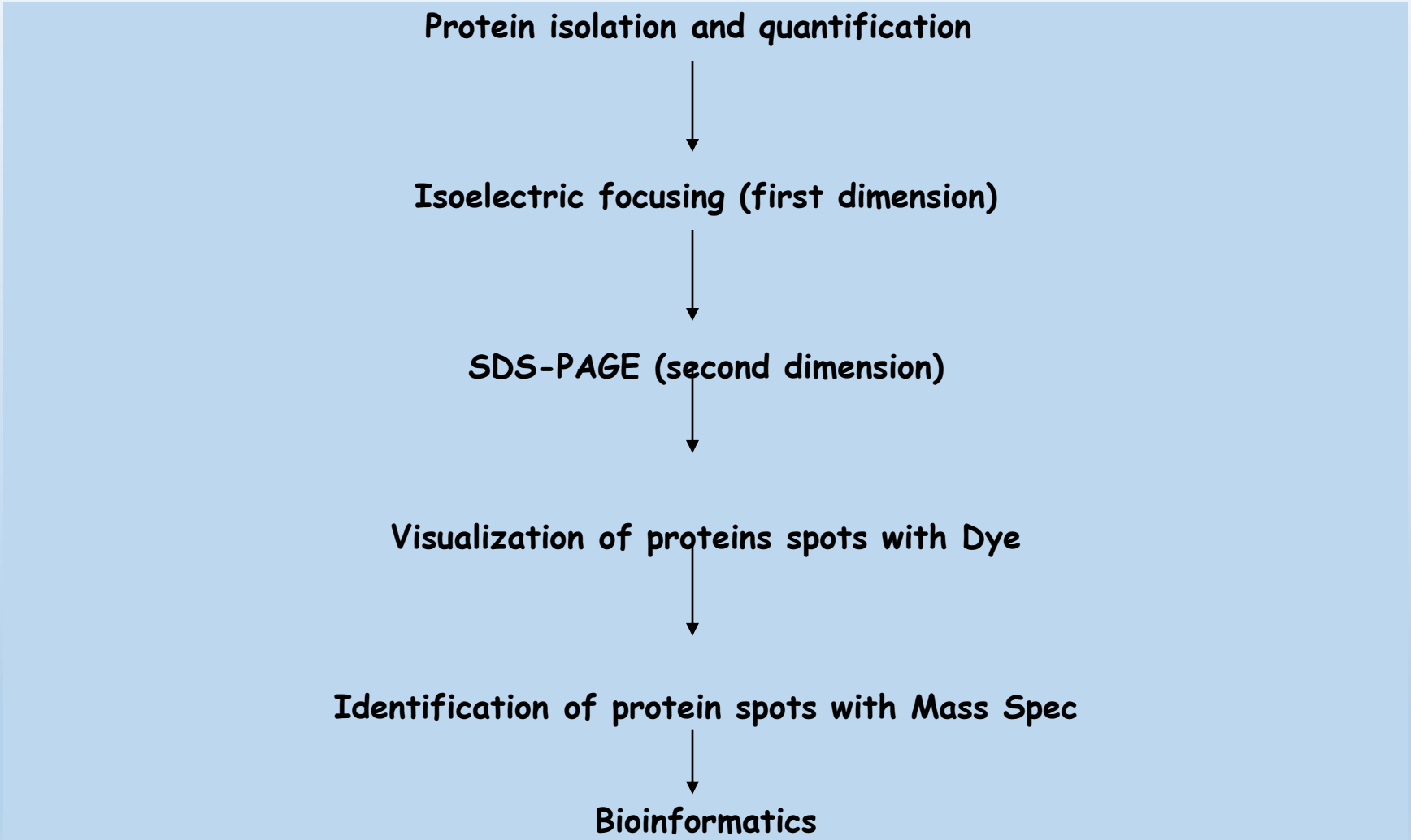
Up to 10,000 proteins can be seen using optimized protocols


ISOELECTRIC FOCUSING
pH = 3
pH = 10
pH = 3
pH = 10
-
+
-
+
SDS - PAGE
SDS - PAGE
MASS SPECTROMETRY

Why 2D Gels

- Oldest method for large scale protein separation (since 1975)
- Popular method for protein display and proteomics-one spot at a time
- Can be used in conjunction with Mass Spec
- Permits simultaneous detection, display, purification, identification, quantification, pI, and MW.
- Robust, reproducible, simple, cost effective, scalable
- Provides differential quantification using Differential 2D Gel Electrophoresis (DIGE)

Processes involved in 2D gel electrophoresis



Sample Preparation

- Sample preparation is key to successful 2D gel experiments
- Must select appropriate method to get selected proteins from cellular compartment of interest
- Membrane proteins, nuclear proteins, and mitochodrial proteins require special steps
- Must break all non-covalent protein-protein, protein-DNA, protein-lipid interactions, disrupt S-S bonds
- Must prevent proteolysis, accidental phosphorylation, oxidation, cleavage, ect..
- Must remove substances that might interfere with separation process such as salts, polar detergents (SDS), lipids, polysaccharides, nucleic acids
- Must try to keep proteins soluble during both phases of electrophoresis process
- Must quantify protein

Protein Solubilization

2-20 mM Tris base (Carrier ampholytic buffer)

5-20 mM DTT (to reduce disulfide bonds)

8 M Urea (neutral chaotrope)

Increases the solubility of some proteins

Chaotropic agents interfere with stabilizing non-covalent forces (hydrogen bonds, van der Waals forces, and hydrophobic)

4% CHAPS Detergent **(3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate)**

pH of 5-7

Zwitterionic detergent (electronically neutral-has a both Neg and Pos useful for varible charged peptides)

Protects the native state of proteins

Better when downstream apps include IEF because no affect on pH gradients

IEF and IPG (immobilized pH Gradient)

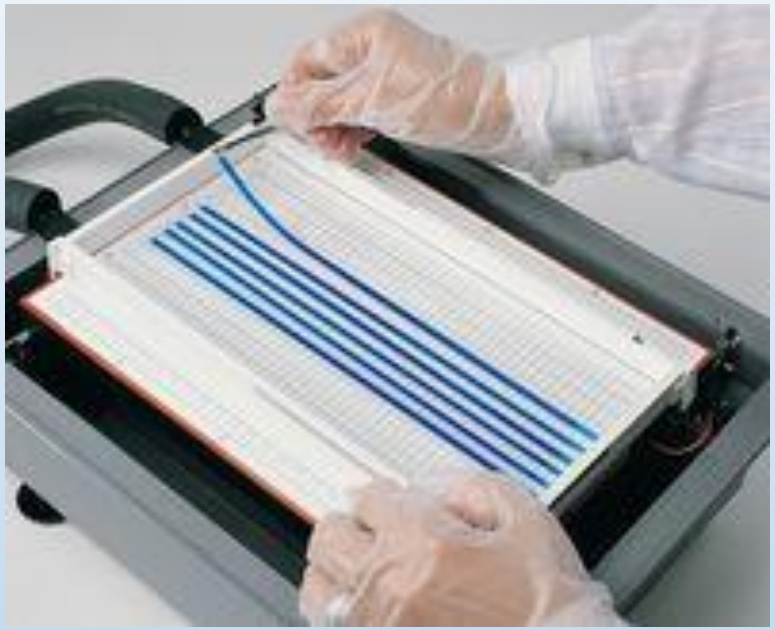
Strip of paper Made by covalently integrating acrylamide and variable pH ampholytes

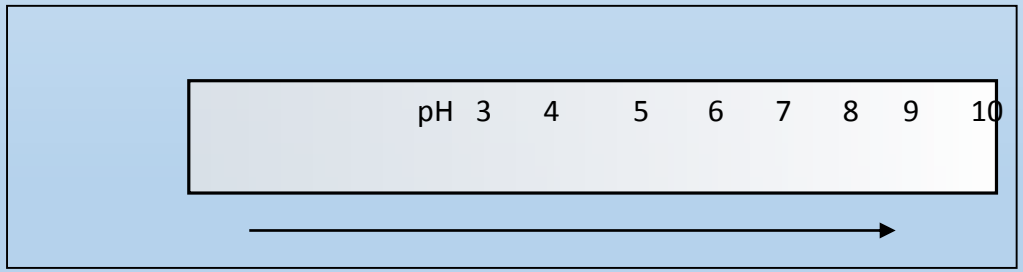
Separation on basis of pI, not MW

Available in different pH ranges

3-10
4-8
5-7

Requires very high voltages (5000V)and long period of time (10h)





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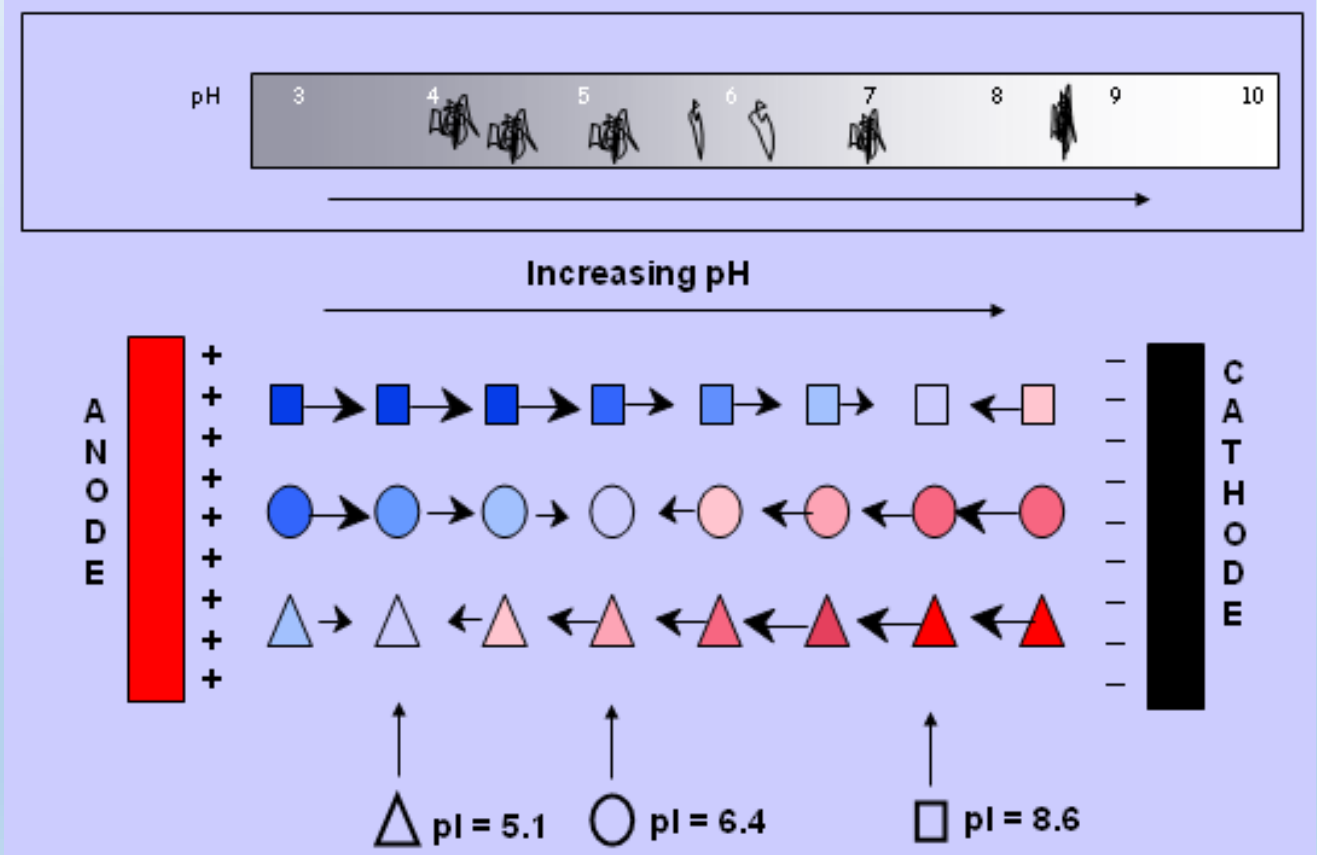
IPG Strips Contain Ampholytes

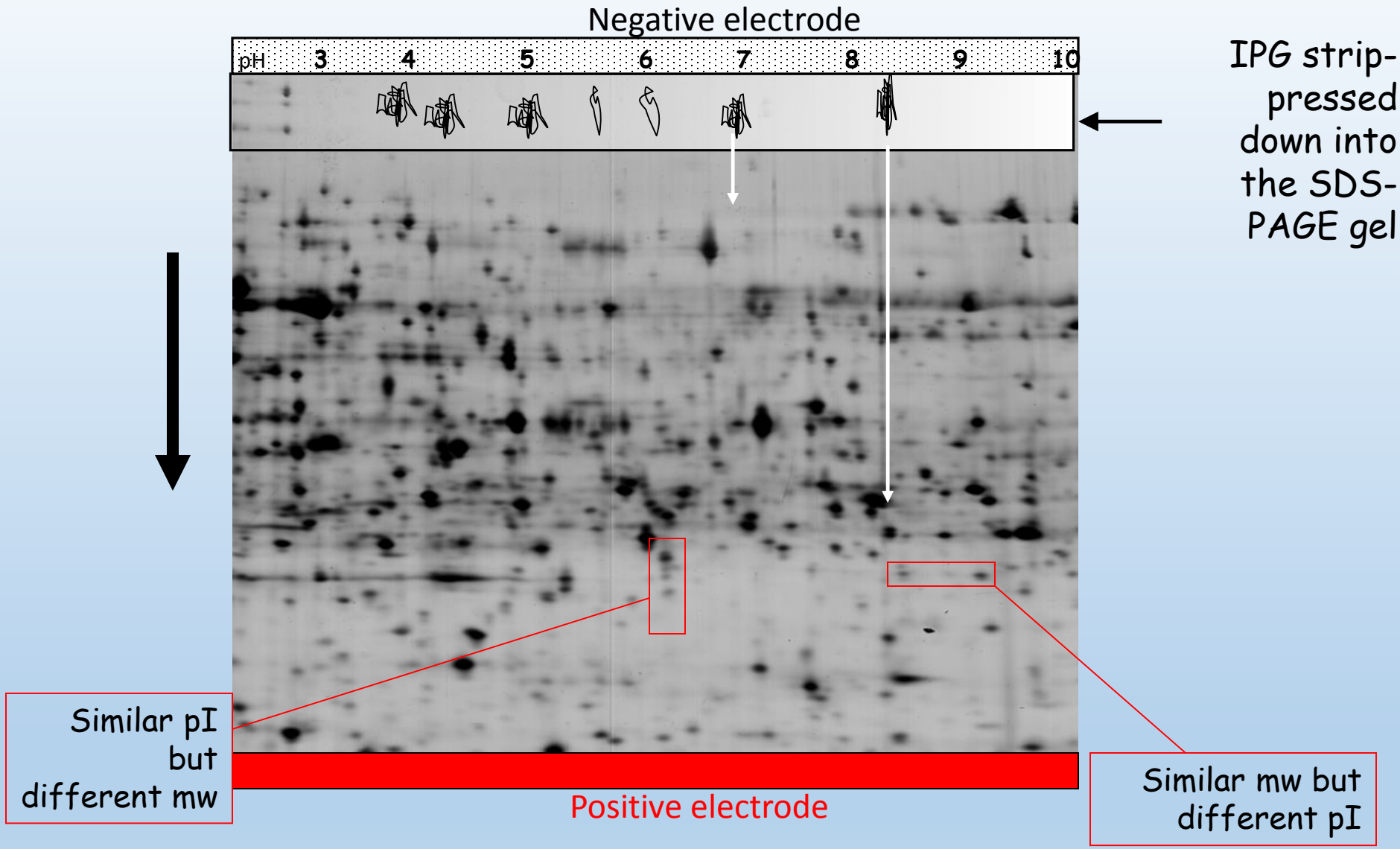
Ampholytes are molecules that contain both acidic and basic groups

Protein will migrate in the Matrix and will find their pH equilibrium (pI)

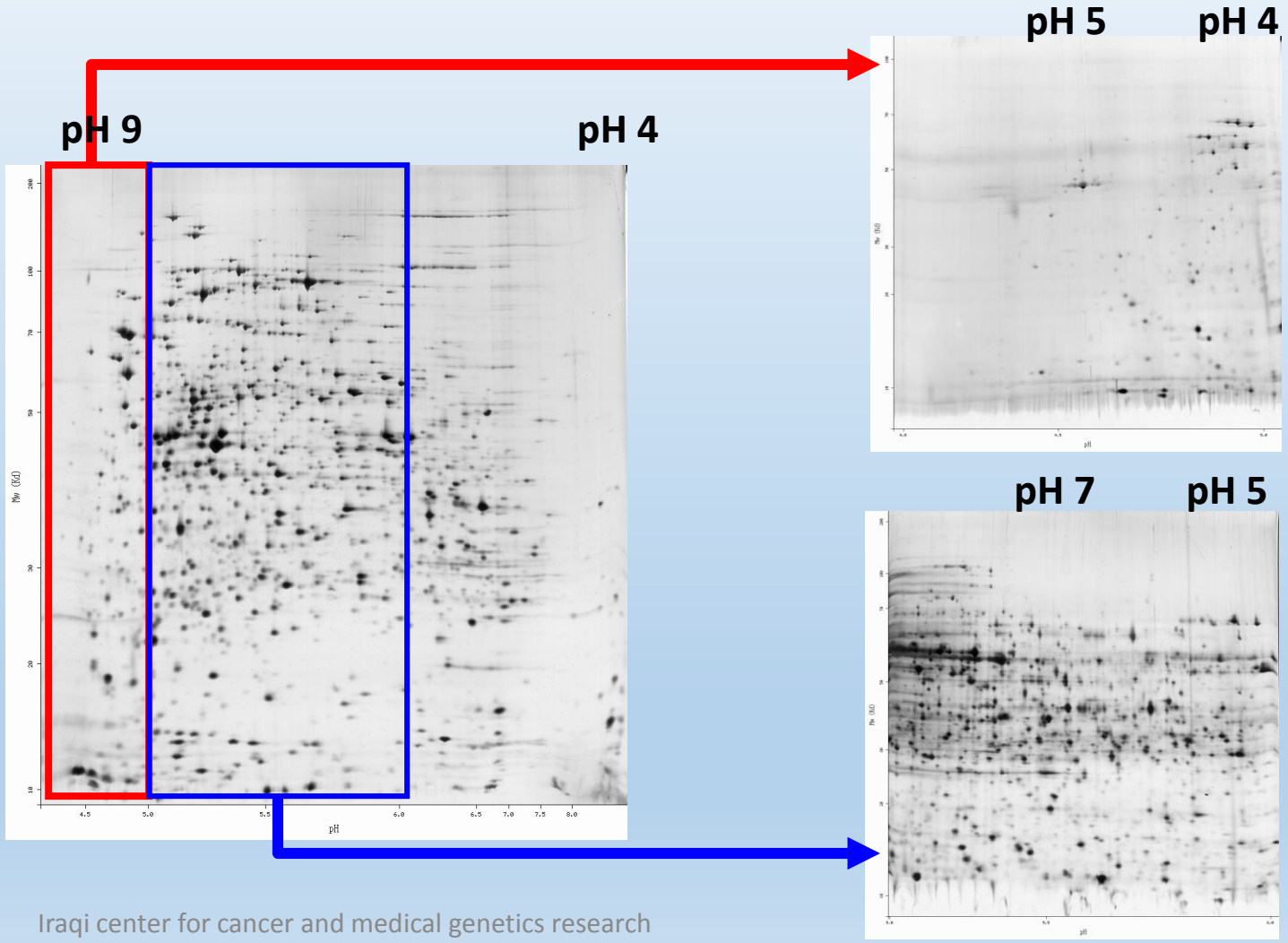


The Second Dimension …Running the Gel

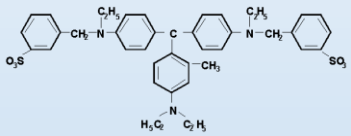
SDS Gel


Negative electrode
pH 3 4 5 6 7 8 9 10
IPG strip-pressed down into the SDS-PAGE gel
Similar pI but different mw
Positive electrode
Similar mw but different pI

Different IPG pH ranges yield Different Results



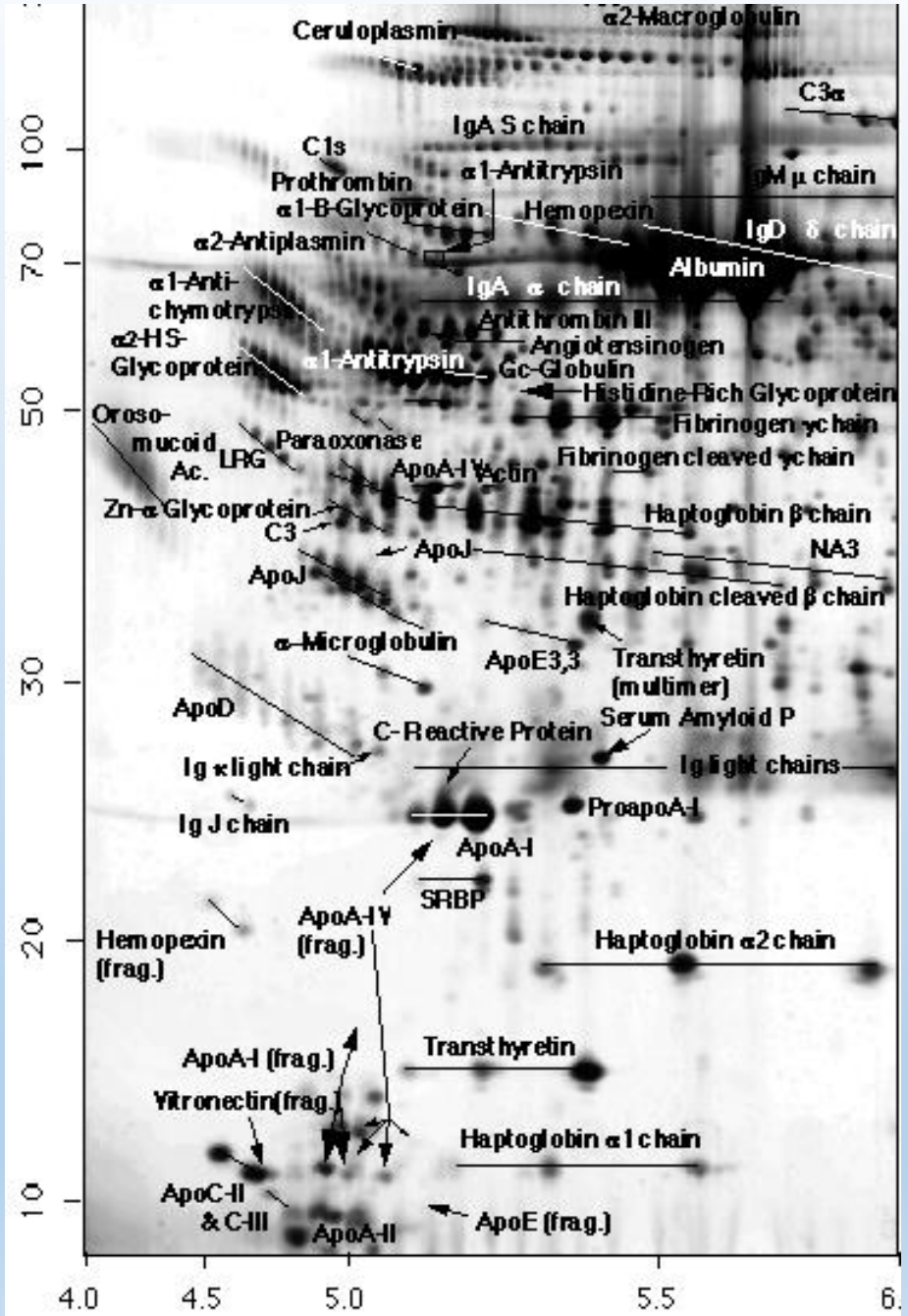
Gel Stains - Summary



Stain	Sensitivity (ng/spot)	Advantages
Coomassie-type	5-10	Simple, fast
Silver stain	1-4	Very sensitive, laborious
Copper stain	5-15	Reversible, 1 reagent negative stain
Zinc stain	5-15	Reversible, simple, fast high contrast neg. stain
SYPRO ruby	1-10	Very sensitive, fluorescent

2D Gel Results

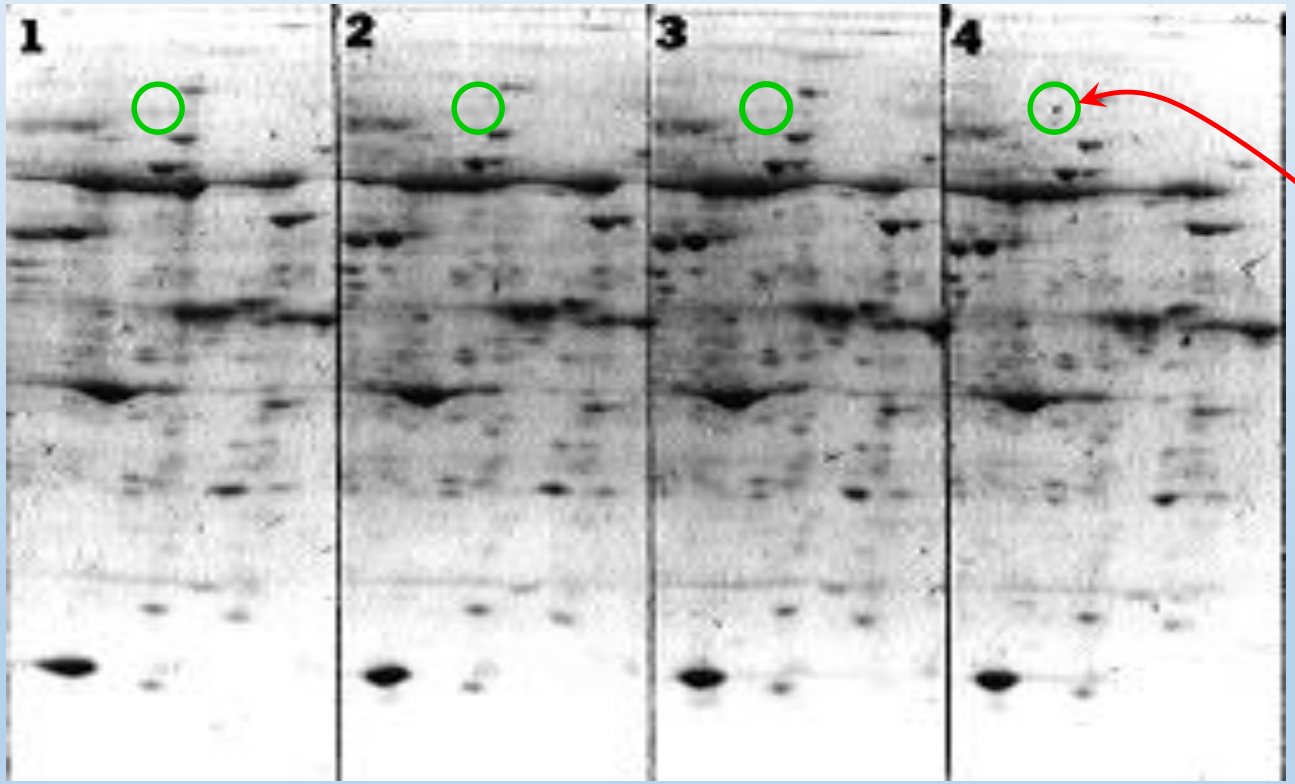
- 401 spots (peptides or PTM) identified
- 279 gene products



2D Gel Post Analysis

Compare gel images and determine what bands/spots are different

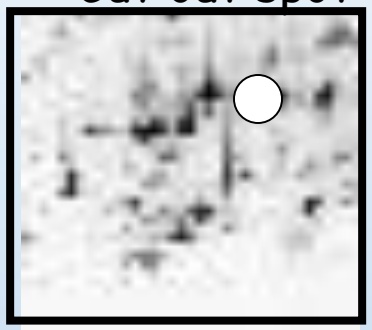
Requires software to compare gels



Apparent difference- Need to extract spot for MS

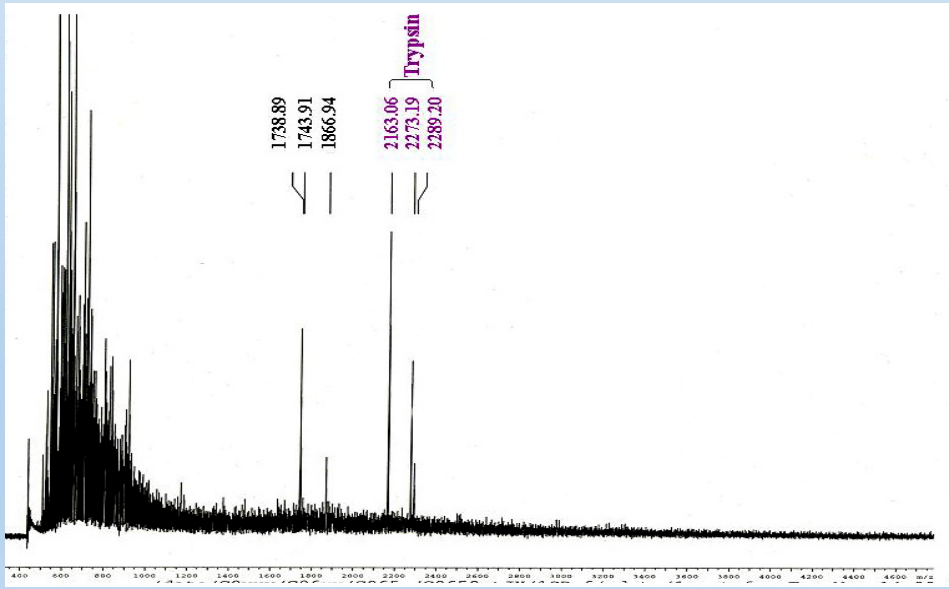
Extracting a Gel Spot

Cut out spot



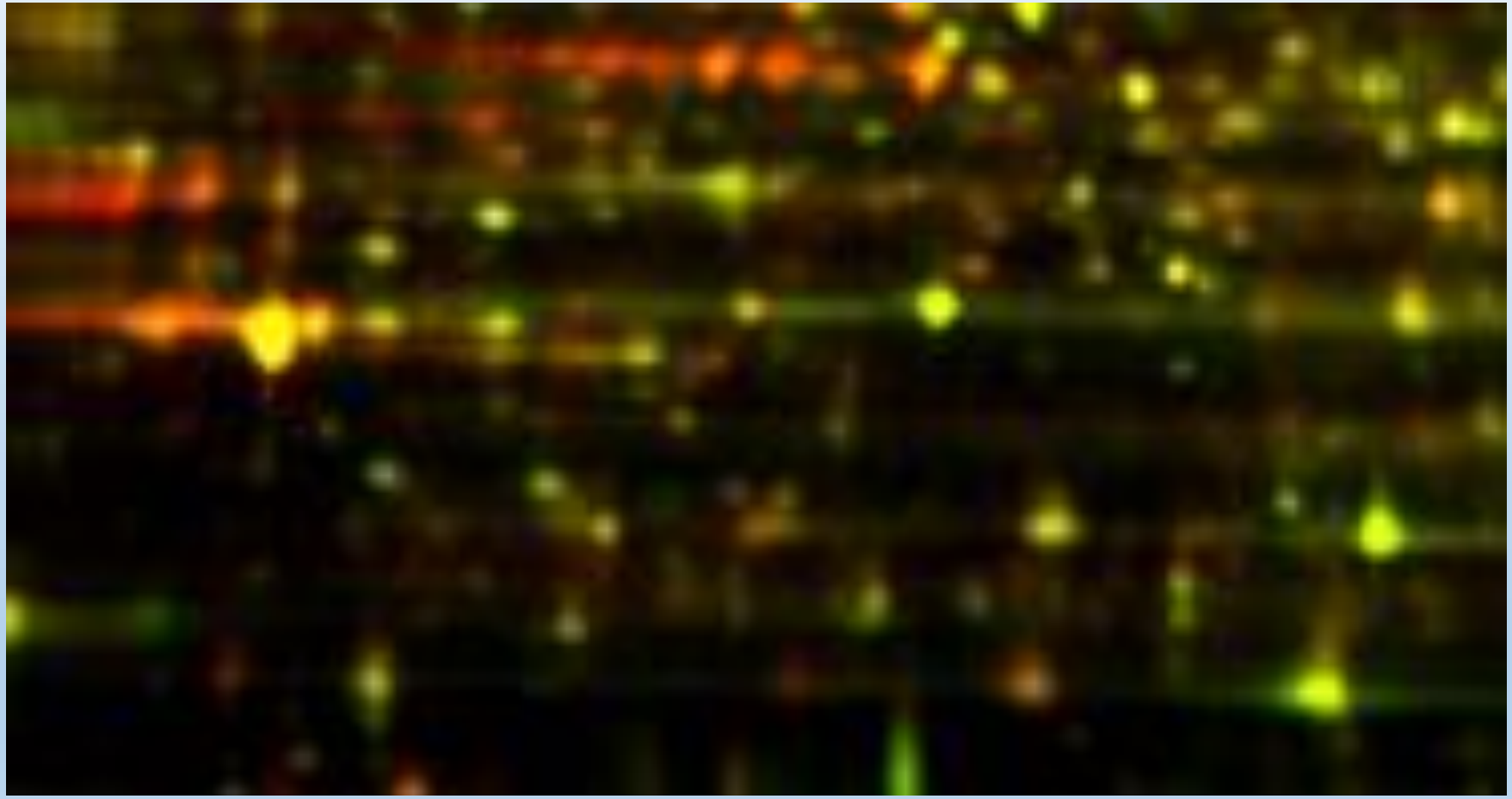
Trypsin Digestion of Gel spot

Run Mass spec



Differential 2D Gel Electrophoresis [DIGE]

Allows you to mix samples and run a single 2d gel for comparative and quantitative purposes



Conclusions

- 2D gel electrophoresis is a popular method for protein display, separation, visualization, and quantitation
- A good precursor to MS, but not required
- 2D gels provide pI, MW data, and photodocumentation
- Web tools are now available that permit partial analysis and comparison of 2D gels using software and simulators
- 2D gels are fun to run