Proteomics Course

LECTURE-14
Gel-based Proteomics
Two-dimensional electrophoresis workflow: Staining and Image Analysis

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Previous lecture

- Gel-based proteomics
- 2-DE work-flow

1 Isoelectric focusing (first dimension)
2 Equilibration of IPG strips
3 SDS-PAGE (second dimension)
4 Staining – gel visualization

Today's lecture

- 2-DE work-flow

4 Staining – gel visualization
5 Image analysis
6 Spot picking

- New methods for proteomics applications

Staining: SYPRO Ruby

- Fluorescent stain easily visualized with simple UV or blue-light transilluminators
- Endpoint stain, little background, sensitive
- Detects glycoprotein, low MW proteins
- Compatible with MS

Staining: Cyanine dyes

- Cy dyes, water-soluble derivatives of N-hydroxy succinimide that covalently binds the ε-amino groups of a protein's lysine residues
- Protein samples can be labeled with Cy dyes and mixed to run on a single gel
- Employed in difference in-gel electrophoresis, which eliminates problem of gel-to-gel variations

Staining: Pro-Q diamond

- Pro-Q diamond, a fluorescent dye capable of detecting phosphorylation
- Suitable for use with electrophoretic techniques and offer sensitivity in ng levels

Dual staining

- Pro-Q diamond or other PTM detection stains can be combined with other staining procedures such as SYPRO Ruby
- Dual staining allows more than one detection protocol on a single gel

Staining comparison

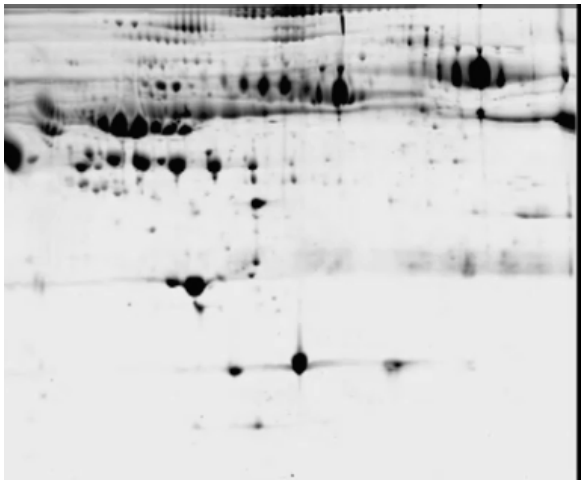
Stain	Comments	Sensitivity (Approximate)
Coomassie Blue	Most commonly used MS compatible	40 ng
Biosafe Coomassie	MS compatible Easily visualized Non-hazardous	10 ng
Silver stain	MS compatibility an issue High sensitivity	1 ng
Silver stain plus	MS compatible High sensitivity	1 ng
SYPRO Ruby	MS compatible Linear over 3 orders of magnitude High sensitivity	1 ng

Staining 2-D gels

Laboratory demonstration

pI

MW



5. Image Analysis

Image Scanners

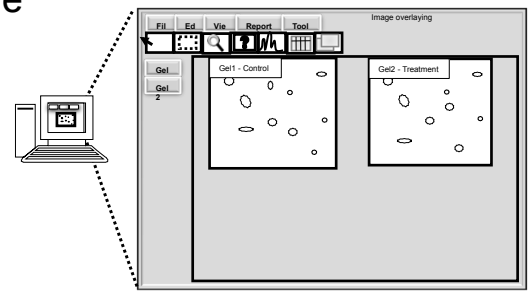
Molecular Imager
Densitometer

Typhoon Variable Mode Imager

Image analysis: manually?

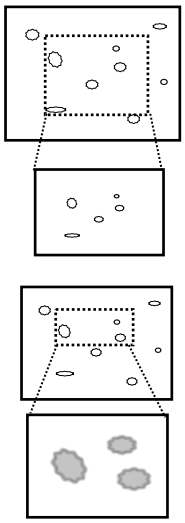
2-D Gel Analysis Software

- 2-D gels are scanned using a scanner and images are analyzed using various software
- These software enable
  - Spot identification
  - Comparison of gels
  - Overlaying of images
  - Cropping gels
  - Statistical analysis



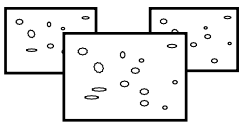
2-D Gel Analysis Software (2)

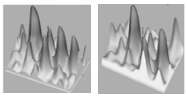
- Crop tool
  - Allows a specific defined region of gel to be cut out from the entire gel
  - It helps in selection of regions with high spot density for further analysis
- Zoom tool
  - Zoom tool expands a specific area of gel for further analysis



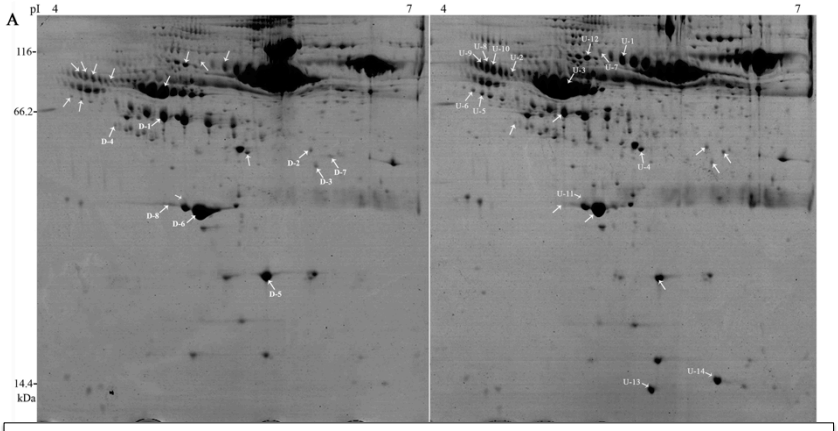
2-D Gel Analysis Software (3)

- Image overlaying
  - To compare spot patterns on 2 different gels, separate images are overlaid to appear as single merged image
  - Spots that coincide lie on top of each other while others retain their original position
- Spot analysis
  - It is possible to obtain physical and statistical parameters for each spots on gels
  - Allows gels comparison spot-by-spot basis

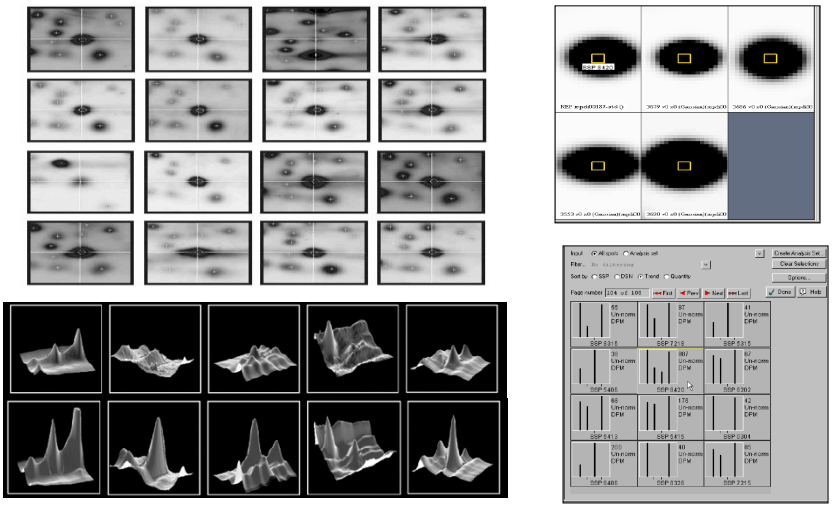




2-D Gel Analysis: Control vs Treatment



2-D Gel Analysis

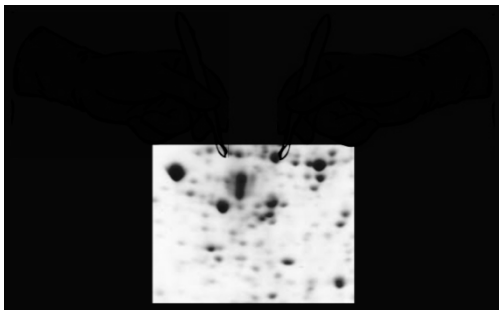


2-D Gel Analysis Software (4)

2-DE software	Website
Image Master 2D Platinum	http://gelifesciences.com
PDQuest	http://discover.biorad.com
Delta 2-D	http://www.decodon.com
Dymension	http://www.syngene.com
Ludesi 2-D gel image analysis	http://www.ludesi.com
Progenesis	http://www.nonlinear.com

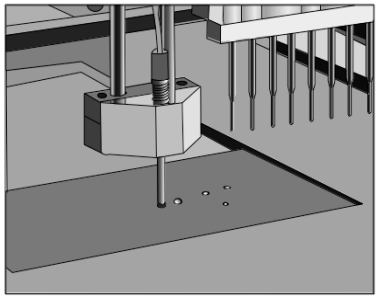
6. Spot picking

Manual excision of spots



- User takes scalpel and makes a cut on particular spot in a gel and transfers it into a tube or 96 well plate along with water

Robotic spot picking



- For each selected spot robotic arms moves and picks up individual spot and transfer it into the 96 well plate along with water

New methods for proteomics applications

Traditional 2-DE: IEF and SDS-PAGE

2D-BN Gel Electrophoresis

OFFGEL Electrophoresis

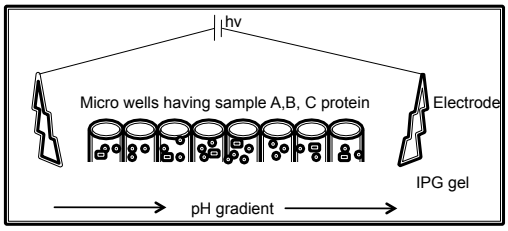
2-D Fluorescence Difference Gel Electrophoresis (DIGE)

OFFGEL Electrophoresis

OFFGEL Electrophoresis

- OFFGEL electrophoresis separates proteins/ peptides according to their pI
- Separated components are recovered in liquid phase
- Compatibility with up or downstream techniques such as immunodepletion and LC/MS

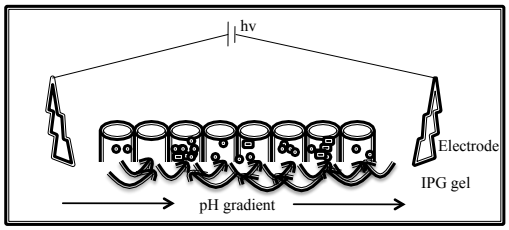
OFFGEL fractionation principle



Protein-A: pI-4
Protein-B: pI-8
Protein-C: pI-9

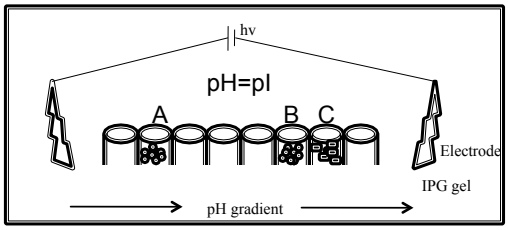
- IPG strip rehydrated and tightly sealed against frame of well in OFFGEL instrument
- Protein sample equally distributed in all the wells and a cover slip is applied to prevent evaporation

OFFGEL fractionation principle (2)



- Protein or peptides migrate though the gel when high voltage is applied

OFFGEL fractionation principle (3)



- When pH = pI there is no protein migration
- Proteins separated based on pI can be removed in liquid phase

OFFGEL and on-chip electrophoresis

- OFFGEL fractionation can be combined with high-sensitivity on-chip electrophoresis of bioanalyzer
- This combination can enable 2-DE-type analysis with high resolution and high sensitivity
- Suitable for differential gene/protein expression applications

Summary

- Staining techniques
- 2-D Image Analysis
- Spot picking

REFERENCES

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