Proteomic Analysis

GSU Biology Proteomics Core Facility

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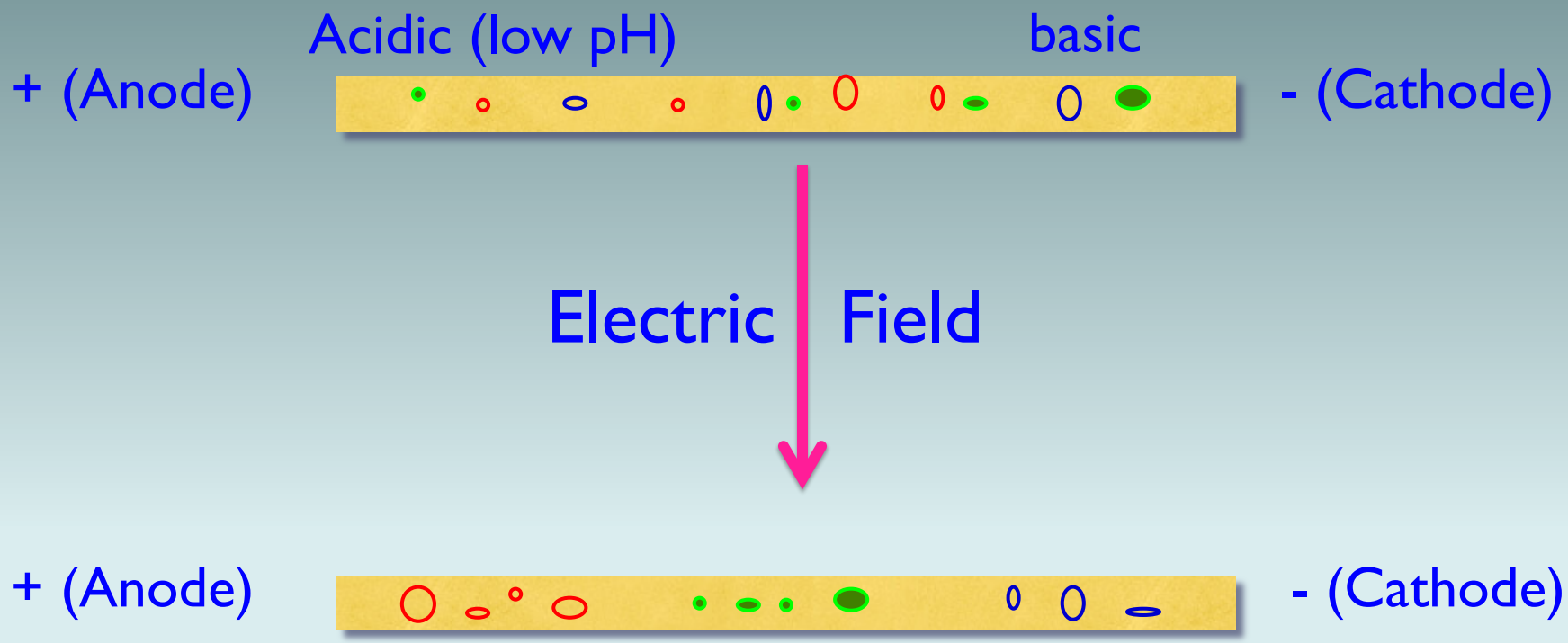
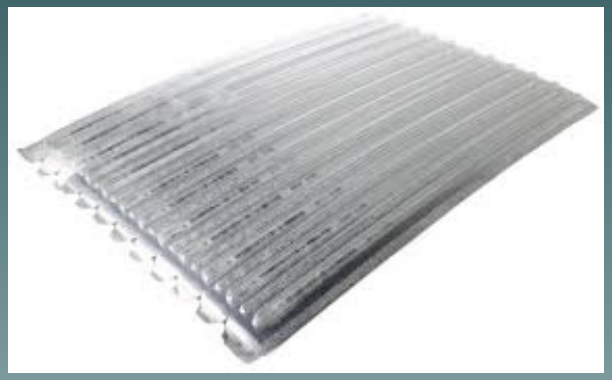
1st Dimension: Isoelectric Focusing (IEF)

pI < pH : Protein, - charge and move toward anode
pI = pH: net 0 charge, no movement
pI > pH: Protein, + charge and move toward cathode



1st Dimension: Isoelectric Focusing (IEF)



Immobilized pH Gradient Gel Strip

Ettan IPG Phor III

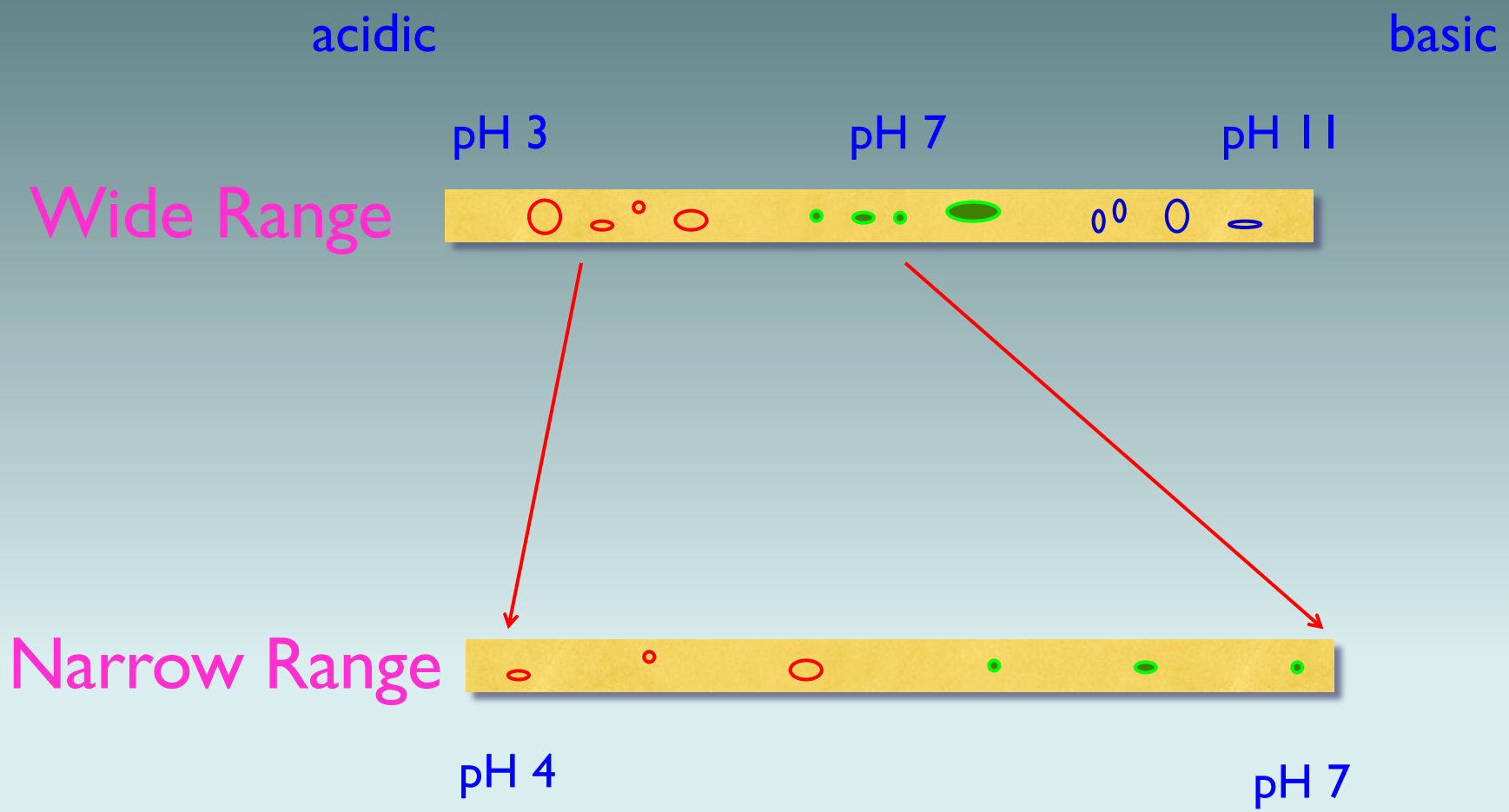
Variable *pH ranges* for IPG strips

- **wide range*:*** ***pH 3-10,***
- **Medium range:** ***pH 4-7, pH 6-9***
- **narrow range:** ***1 pH unit over 18-24 cm (0.05 pH/cm)***

pH 3.5-4.5, 4-5, 4.5-5.5, 5-6, 5.5-6.7
give higher resolution, higher sample loading capacity
more spots (less abundant) visible

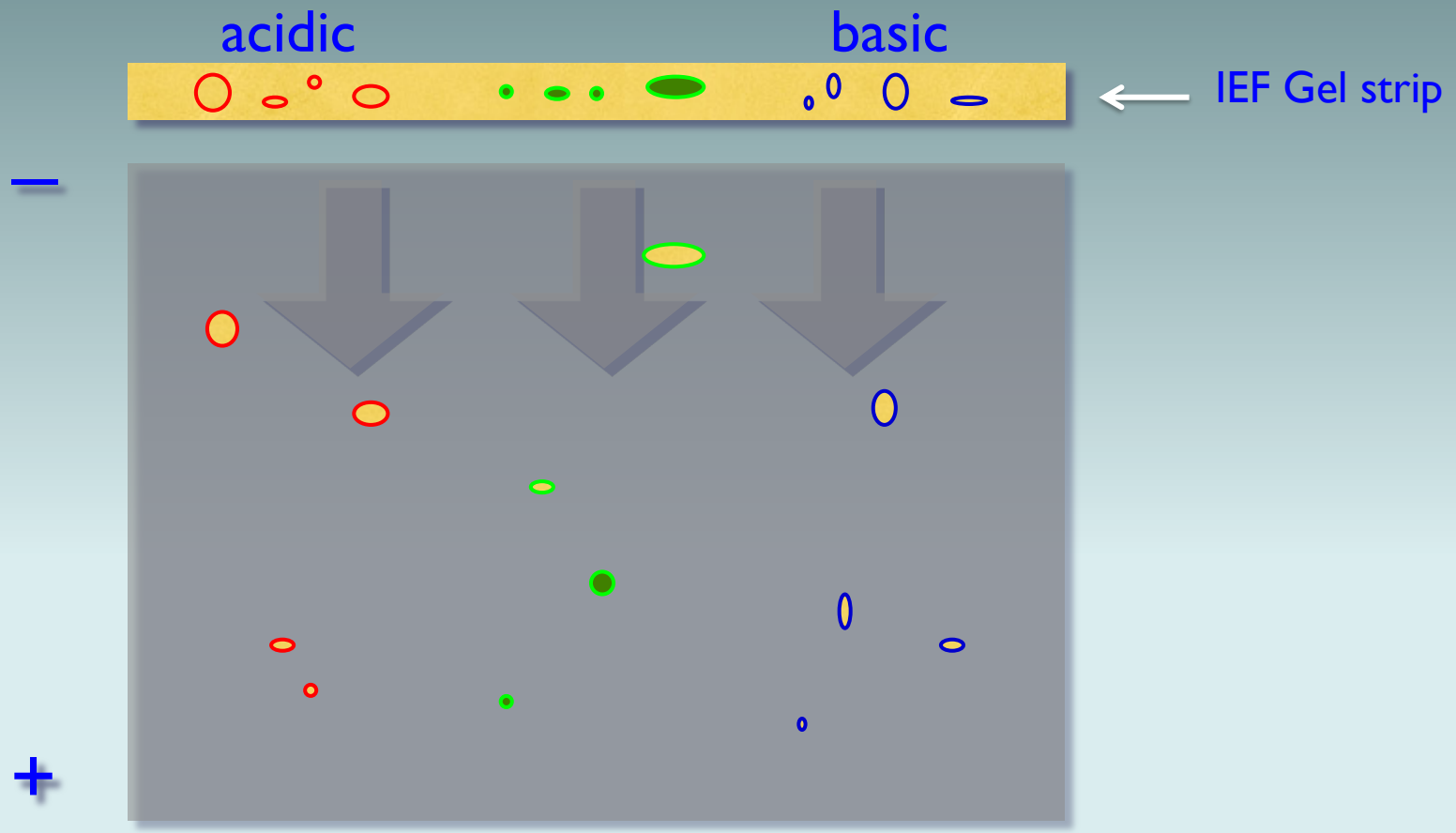
1st Dimension: Isoelectric Focusing (IEF)

Effect of pH Ranges:

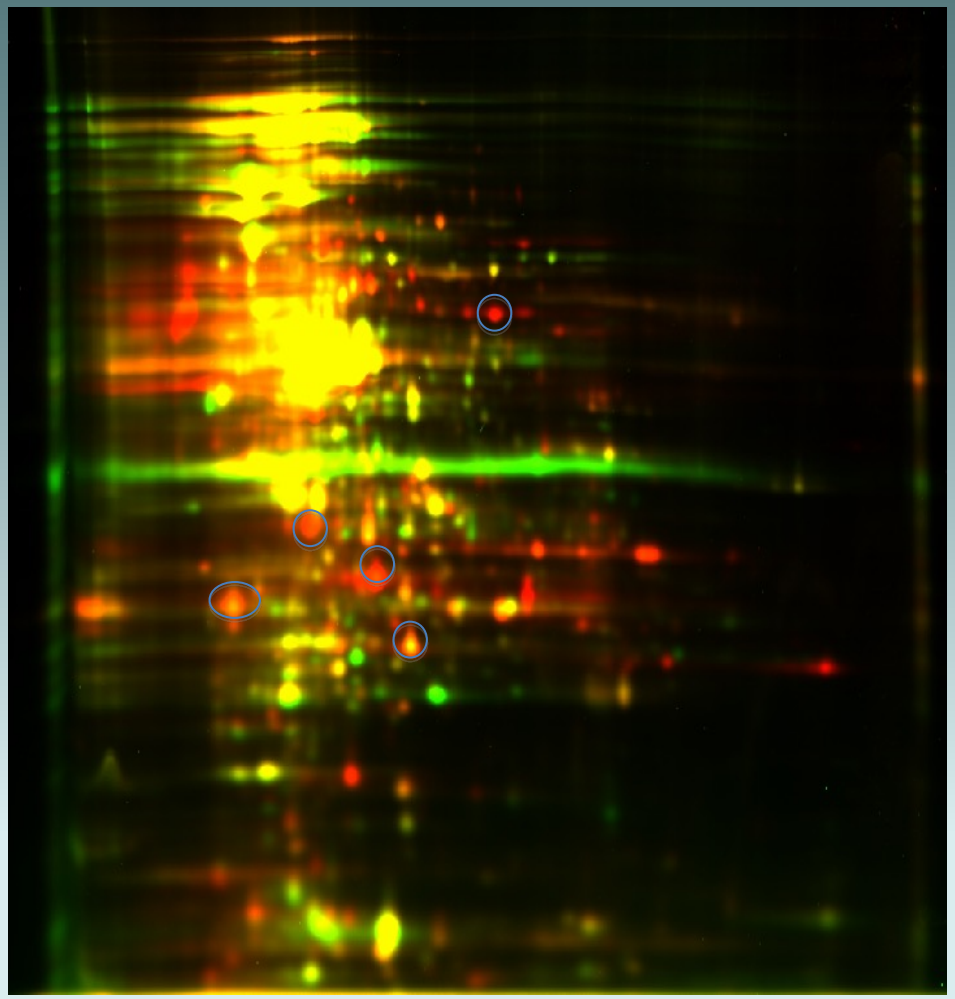


2nd Dimension: SDS-PA Gel Electrophoresis

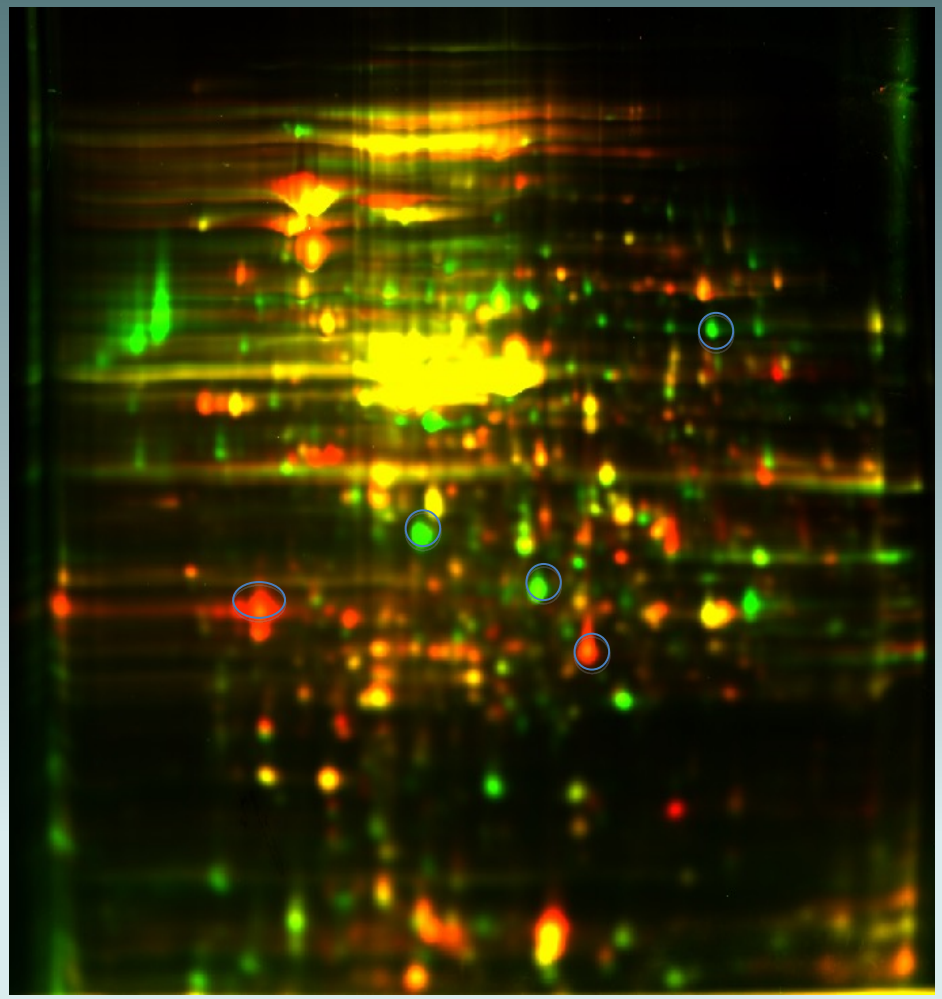
The gel strip can then be placed at the top of a gel and the proteins within the strip electrophoresed into a 6 - 12% polyacrylamide gel containing SDS, thus effectively separating the proteins by mass.



Gel-1



Gel-2



Protein Staining Techniques

- **Radioactive labeling**
  high sensitivity, very quantitative / linear, MS compatible

- **Coomassie Brilliant Blue**
  low sensitivity (0.1 µg detection), only somewhat quantitative, and MS compatible

- **Imidazole-zinc staining: negative staining**
  medium sensitivity (20ng), not quantitative
  good MS compatibility (spots not stained, only background)

- **Silver staining (numerous protocols)**
  high sensitivity (0.1ng), however, NOT quantitative,
  NOT Mass Spec compatible unless modified

- **Fluorescent dyes**
  - **pre-labeling ex. CyDyes**
  - **post-labeling e. Sypro Ruby**

  sensitivity (comparable to Silver staining, 0.1 ng), very high linear range (depending on imaging system), thus very quantitative and is MS compatible

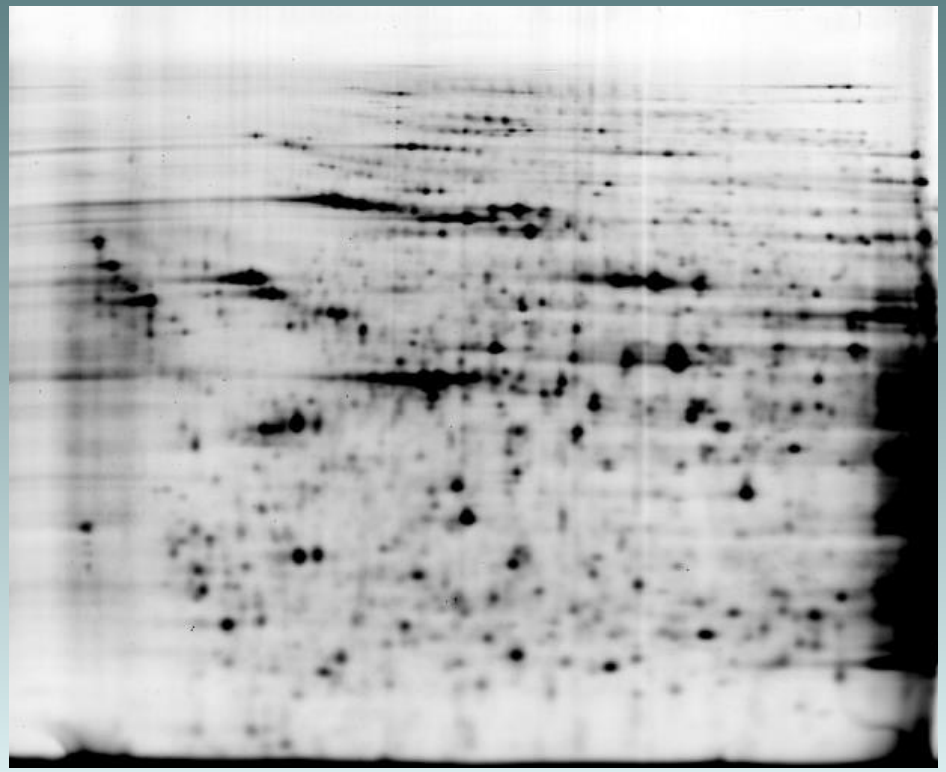
Problems relating to 2D electrophoresisi

- **procedure is somewhat complex, and highly user-dependent**
- **unneglectable gel to gel variability:**
  - **IPG-strip and SDS-PAGE gel casting results in variation in spot migration**
- **transfer from IPG-strip to second dimension can often be cumbersome**
- **limited number of discernible spots vs. high abundance of spots**
- **not too appropriate for hydrophobic proteins: membrane proteins**
- **quantification problems (variations from gel to gel, and use of dyes)**

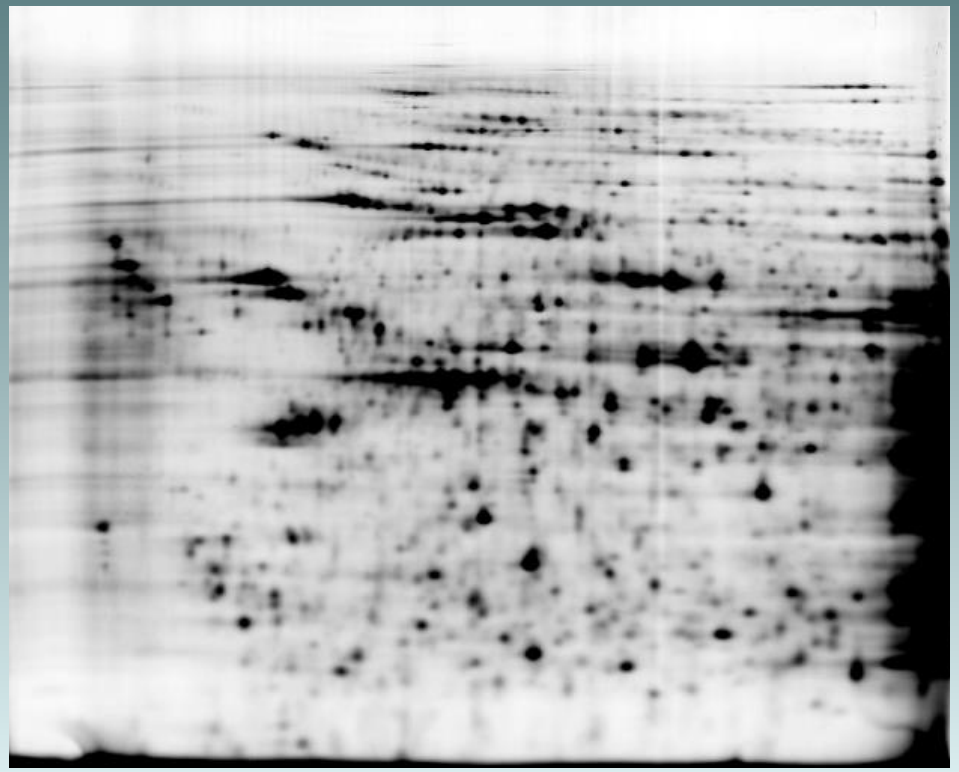
.....solution ?

Single dye/staining

- **Different samples on separated gels**

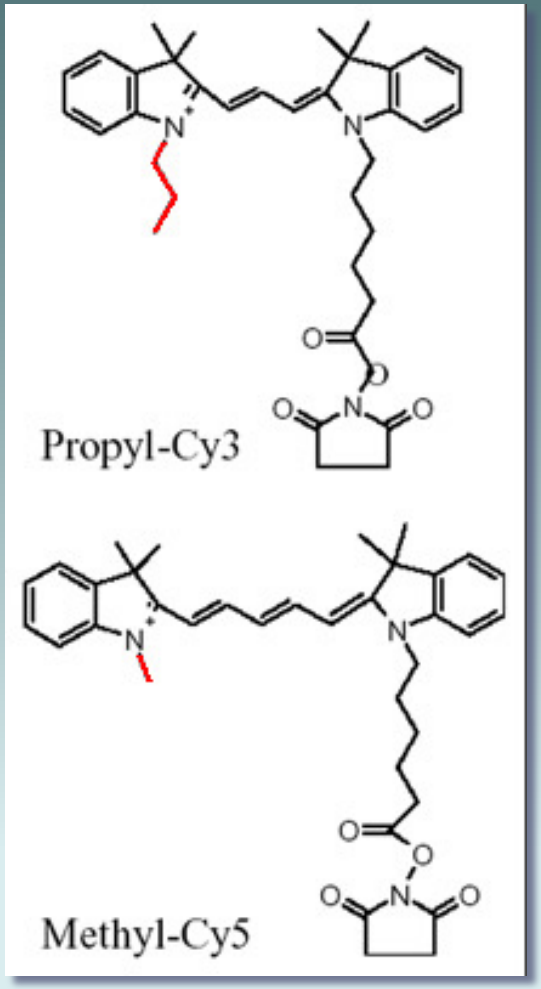


Sample A



Sample B

Solution: Cy3 and Cy5 fluorescent dyes



Properties:

- NHS-ester, reacts with lysine
- No charge modifications
- Identical MW (reagent: 580.7 Da)
- Different fluorescent emission. => separable image acquisition
- Mass Spec: compatible

	Propyl-Cy3	Methyl-Cy5
Absorption max	553 nm	645 nm
Emission max	569 nm	664 nm

Unlu *et al.*, Electrophoresis (1997) 18:2071-77
Tonge *et al.*, Proteomics (2001) 1, 377-96

2-D differential in-gel electrophoresis (DIGE)[1] is a fluorescent multiplexing technology which uses matched, spectrally resolvable dyes to label protein samples prior to 2-D separation (Figure 1).

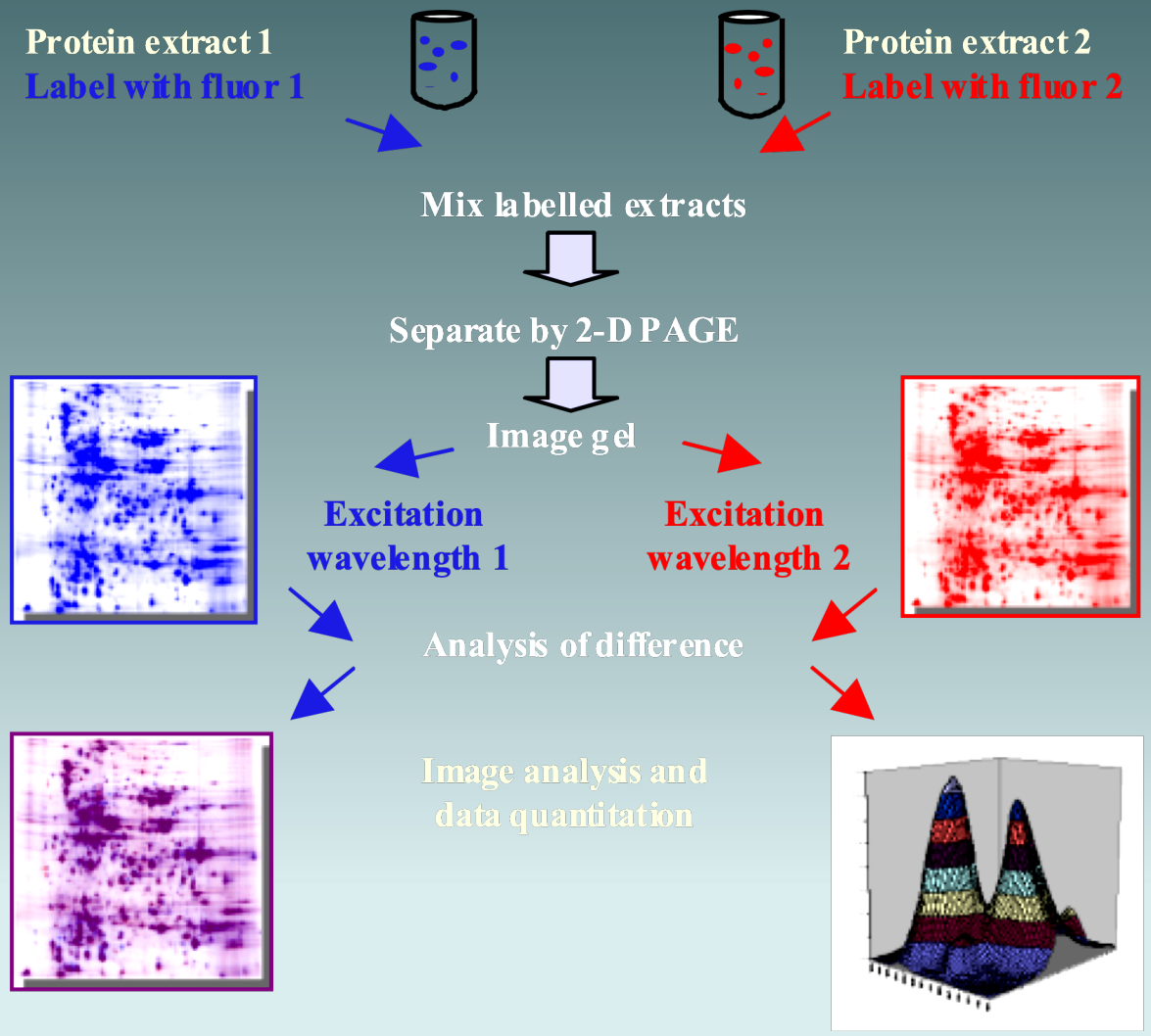
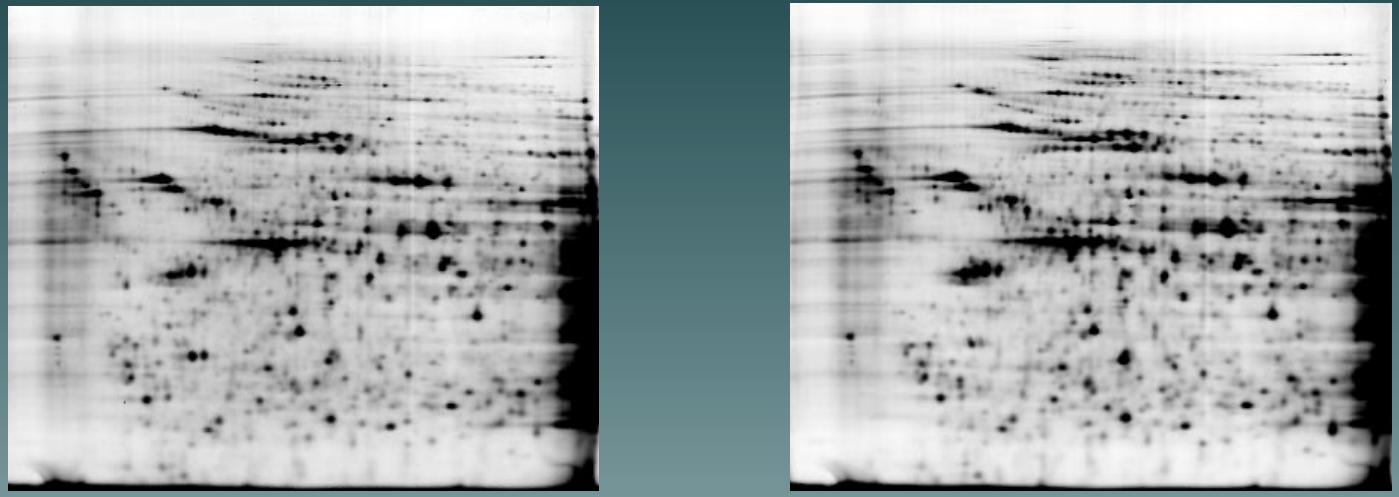
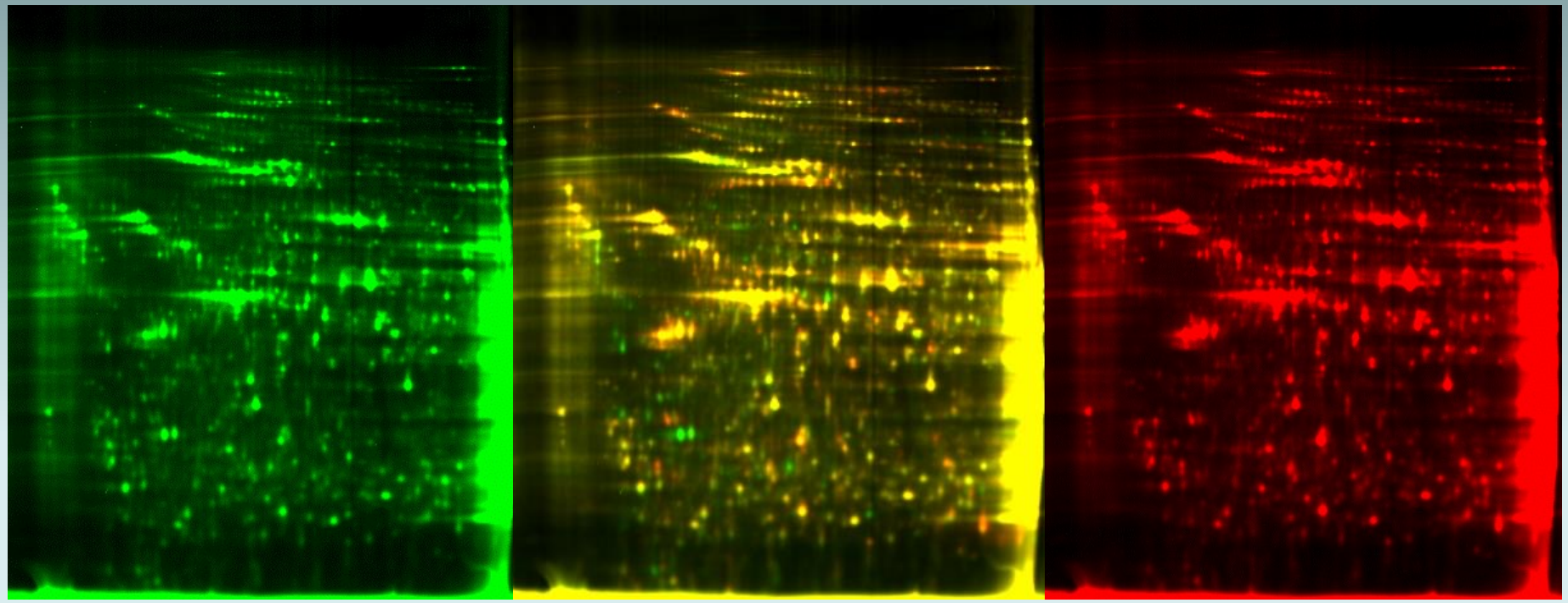


Figure 1: Outline of the 2-D DIGE technology (fluorescent dyes available with DIGE technology: Cy R 2, Cy3 and Cy5)

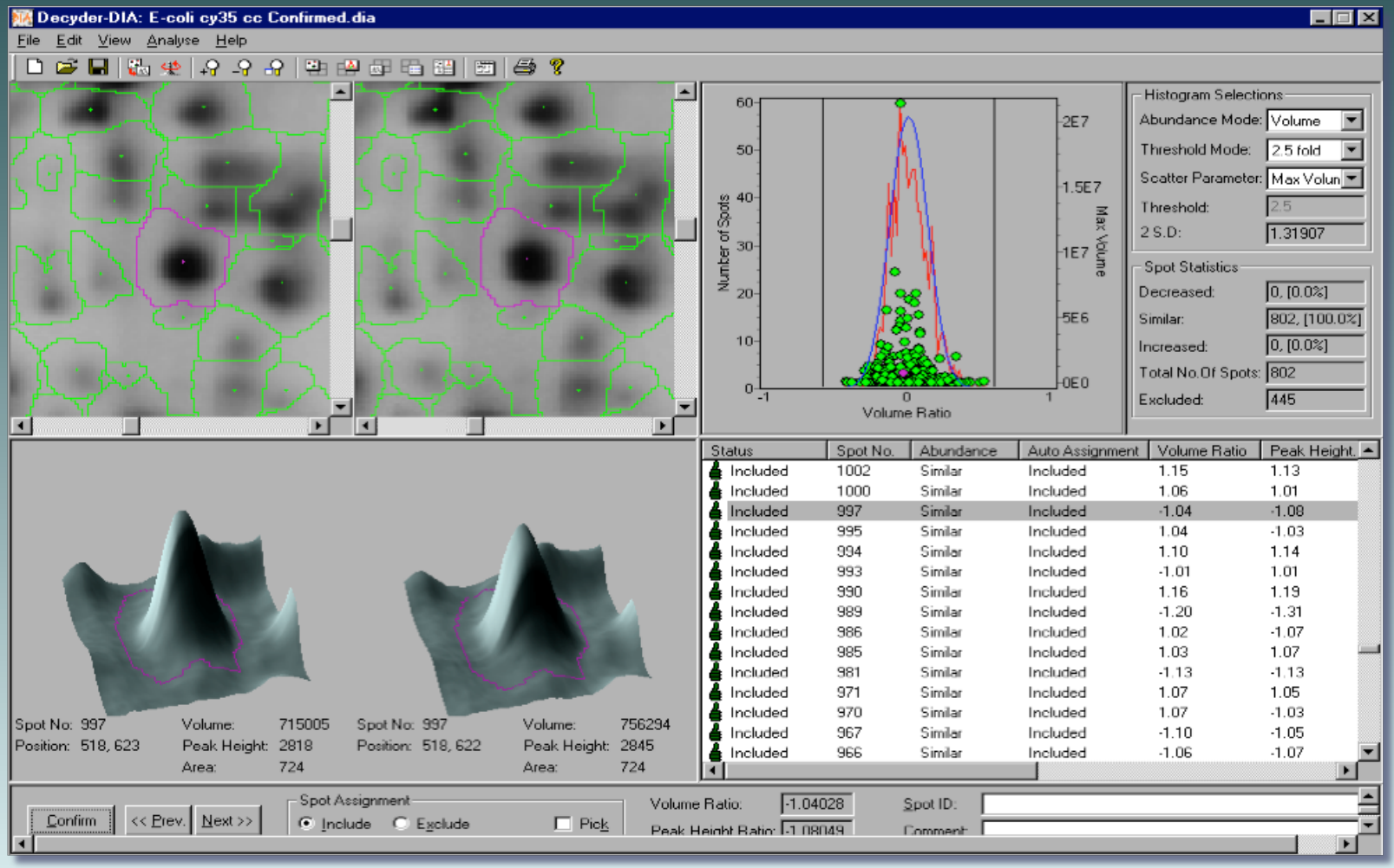


Single dye/stain

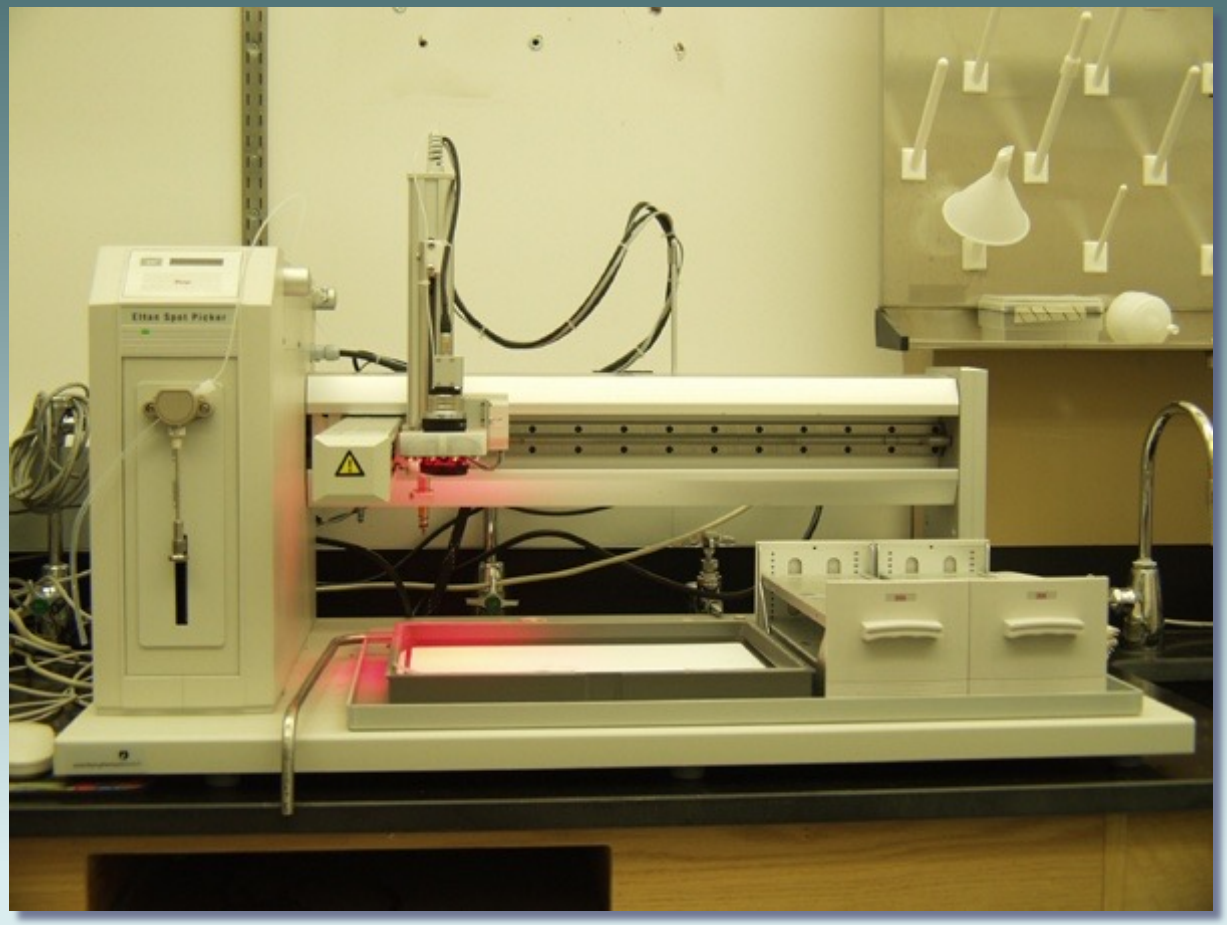


Cy3 Cy3+Cy5 Cy5

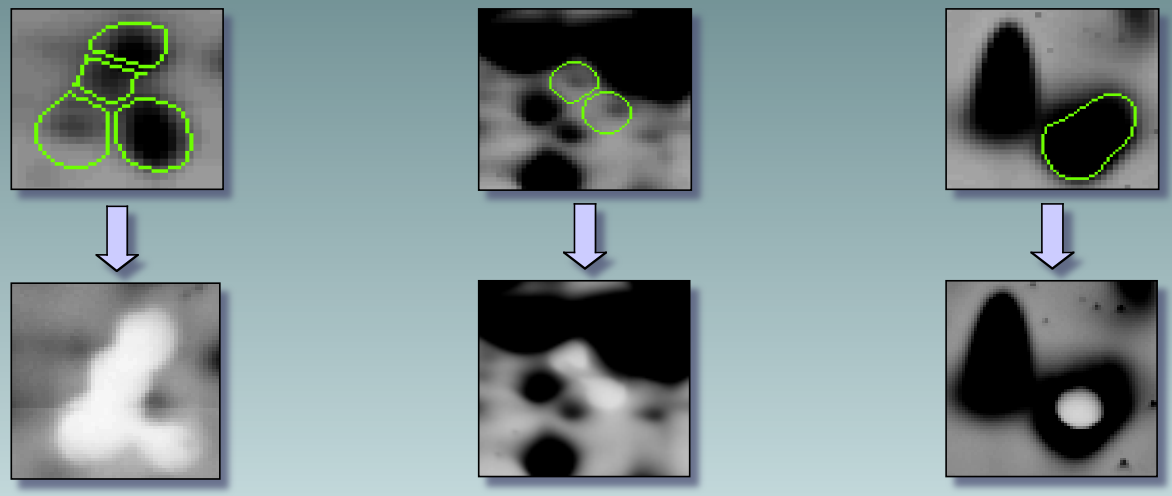
Differential dye staining



Example of a Screen on the Analysis Workstation Scanner



Ettan DALT II: Spot-Picker



Before and After Views of various “selected spots”