

# Microarrays: Tools for Proteomics

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Chem 395

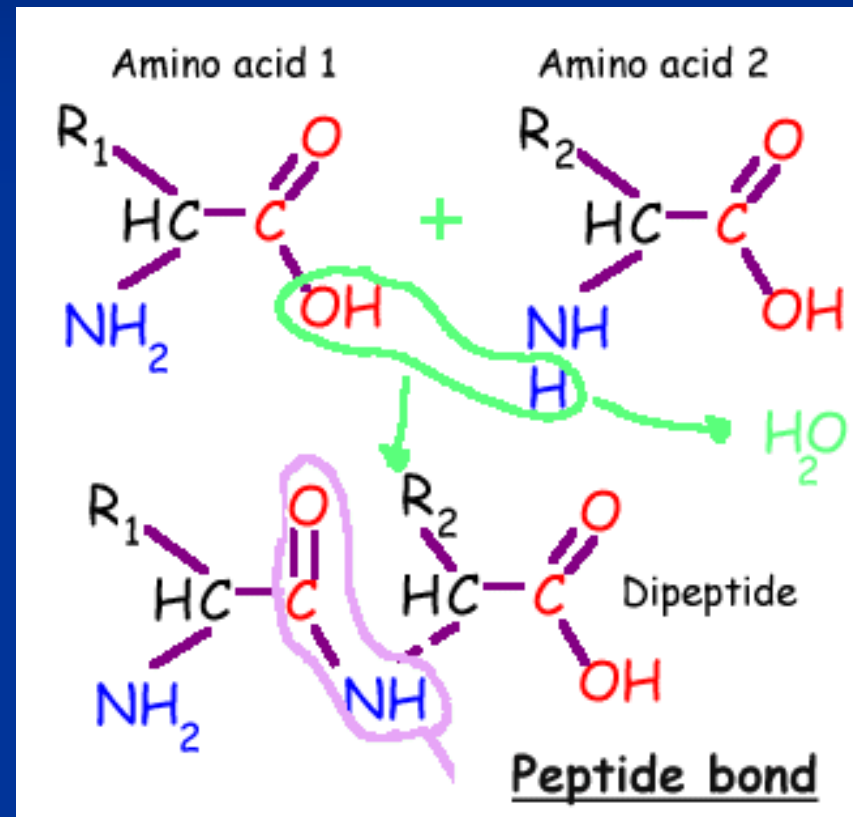
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# Overview

- Introduction
- What are chips/micro-arrays?
- How are they made?
- Applications
- Example of an application (prostate cancer)
- Conclusions
- Questions

# Proteins: Peptides

- Proteins come from subunits called amino acids
- Amino acids can form short chains called peptides
- Macromolecules of peptides form to make proteins



# Functions of Proteins

Why are proteins important?

- They are important molecules in biological systems
- Some major roles they play:
  - Building blocks of bio-structures (ex. Cell Membranes)
  - Transport/ Storage of nutrients (ex. Hemoglobin)
  - Enzymes (metabolism)
  - Antibodies formation

# Proteomics

What is proteomics?

- The Study of all proteins that are expressed at a certain time inside cells, tissues, organs, or organisms.
- Protein Profiling includes:
  - Abundance, interactions, activity, modifications

**GOAL: To do this fast and accurately**

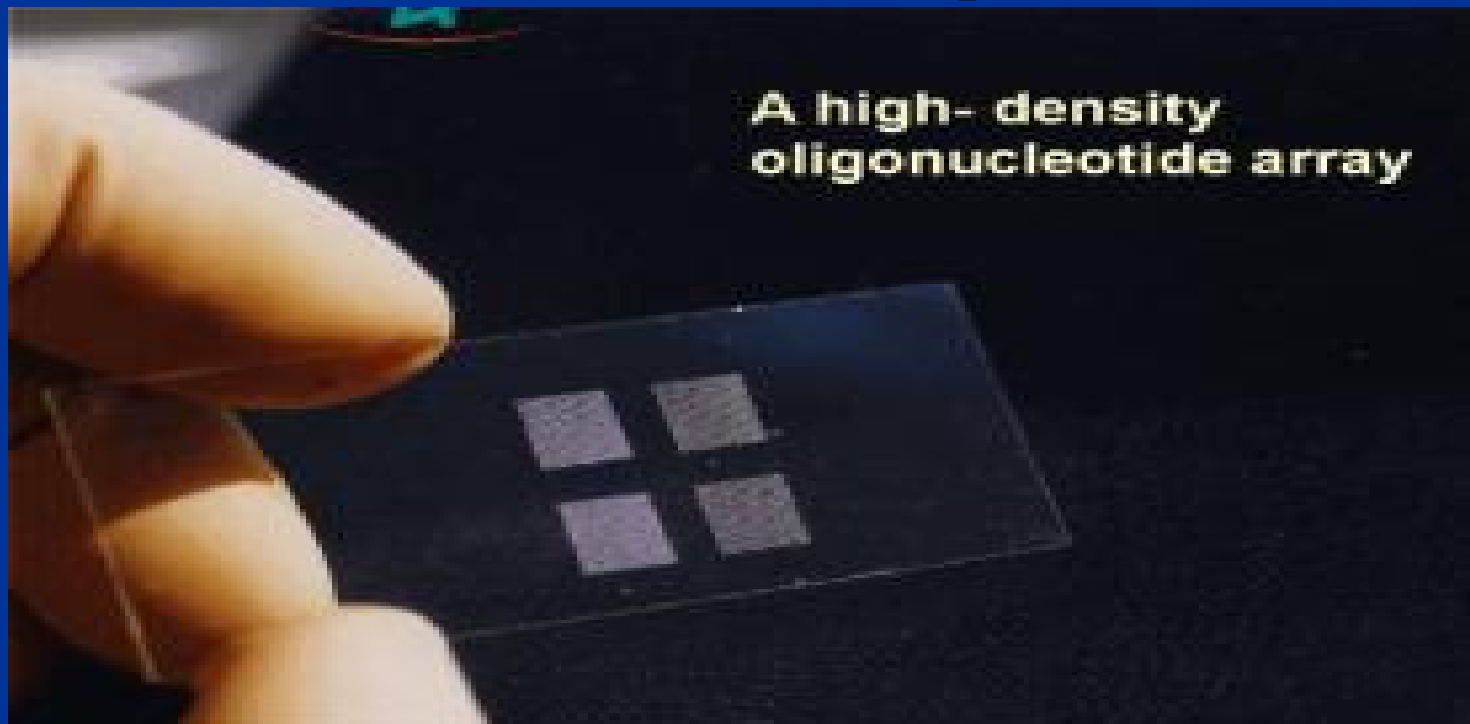
# Proteomic methods

- Two Dimensional Gel Electrophoresis (2DE)
  - Separates numerous amounts of proteins by Size and Charge at varying pH (Old technology)
- LC/MS
  - Protein identification, serum analysis
- **Microarrays**

# Microarray

What is it?

- A slide or chip that contains numerous amounts of biomolecules in fixed amount of space

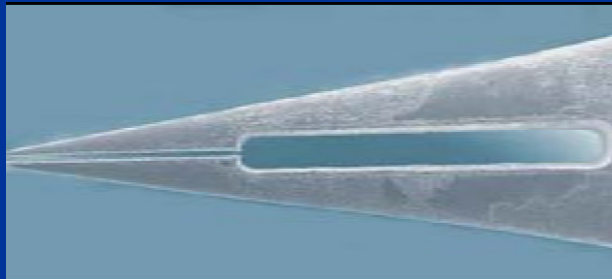


Picture from: [www.acefesa.es/microarray/asper/asper.htm](http://www.acefesa.es/microarray/asper/asper.htm)

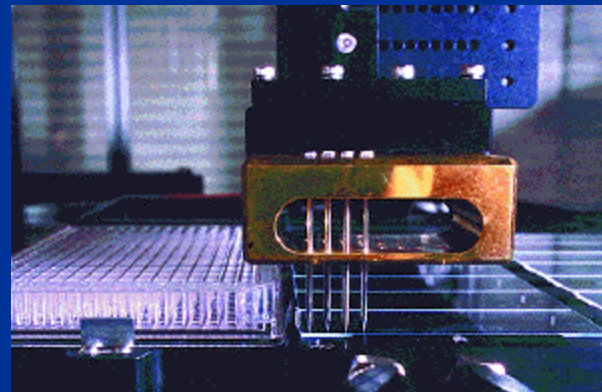
# Microarrays

How are they made?

- Non-contact printing
  - Piezoelectric
  - Syringe Solenoid
- **Contact Printing**



[www.genomicsolutions.com](http://www.genomicsolutions.com)





# Contact vs. Non-contact

Parameter	Printing Technology		
	Piezoelectric	Syringe-Solenoid	Microspotting Pin
Minimum sample volume ( $\mu\text{L}$ ) <sup>a</sup>	20–50	20–50	5
Loading volume ( $\mu\text{L}$ ) <sup>b</sup>	5–10	5–10	0.2–1.0
Print volume (nL)	0.05–10	4–100	0.5–2.5
Spot size ( $\mu\text{m}$ )	125–175	250–500	75–360
Spot density (spots/cm <sup>2</sup> )	500–2500	200–400	400–10 000
Programmable volume	Yes	Yes	No
Number of nozzles or pins	4–8	8–16	1–64
Delivery speed (spots/s)	100–500	10–50	64
Simplicity	✓	✓	✓✓✓
Robustness	✓	✓✓	✓✓✓
Cost per spot	\$\$\$	\$\$	\$

<sup>a</sup>Volume of sample in the 384-well source microplate  
<sup>b</sup>Sample volume of the dispensing device

Rose D. A Systems Approach to Fabricating and Analyzing DNA Microarrays. In Microarray Biochip Technology; Schena, M. Ed.; Eaton: Natick, MA, 2000.

# Microarray

- 3 main types of Microarrays
  - DNA - genomics
  - Cell
  - **Protein**
    - **Antibody arrays – detects proteins**
    - **Protein arrays – detects interactions of proteins or with small molecules**

# Microarray

So what can they be used for?

Some examples are:

- Drug Discovery/ Toxicology
- Gene Expression
- Pathogen analysis
- Identifying diseases
  - Cancers
  - Allergies
  - Etc.

# Prostate Cancer

## Facts:

- Prostate cancer is one of the most common cancers in Men
- In 2005 it is estimated that 230,000 new cases of prostate cancer will be diagnosed in the US
- Prostate cancer is the second largest cause of cancer death in the US (lung cancer is the first)
- 1 out of every 6 men will be diagnosed with prostate cancer in his life time
- 1 out of every 33 men will die of this disease

Adopted from: American Cancer Society

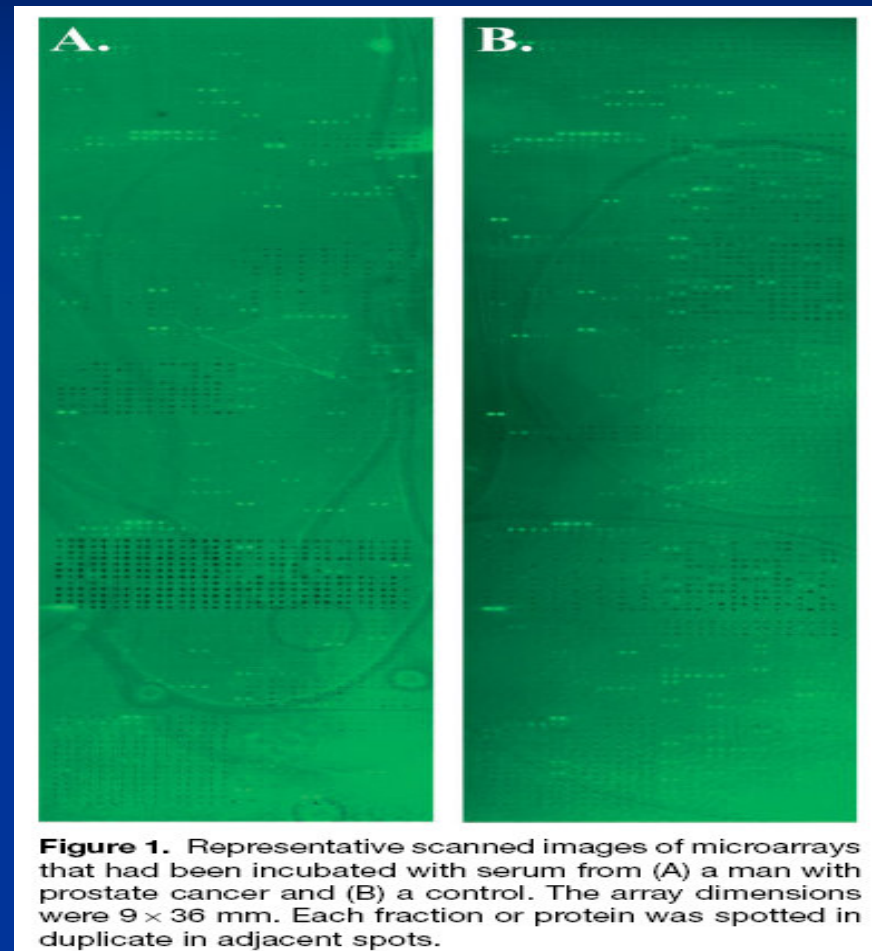
# Example

- 1760 fractions of LNCaP cells were collected using 2D liquid Chromatography (Rotofor/RP-HPLC)
- Fractions along with several control proteins were spotted in microarrays on nitrocellulose-coated microscope slides
- Sera from 25 men with and 25 men without prostate cancer were incubated individually each on separate microarrays
- Immunoglobulins from the sera that bound to spotted fractions were detected after incubating the microarrays w/ biotinylated anti-human Ig and phycoerythrin-streptavidin conjugates (fluorophores) and then scanned for fluorescence.
- This was performed on all 50 microarrays

Bouwman, K. et al. Microarrays of tumor cell derived Proteins uncover a distinct pattern of prostate cancer serum immunoreactivity. Proteomics 2003, 3, 2200-2270

# Example: Continued

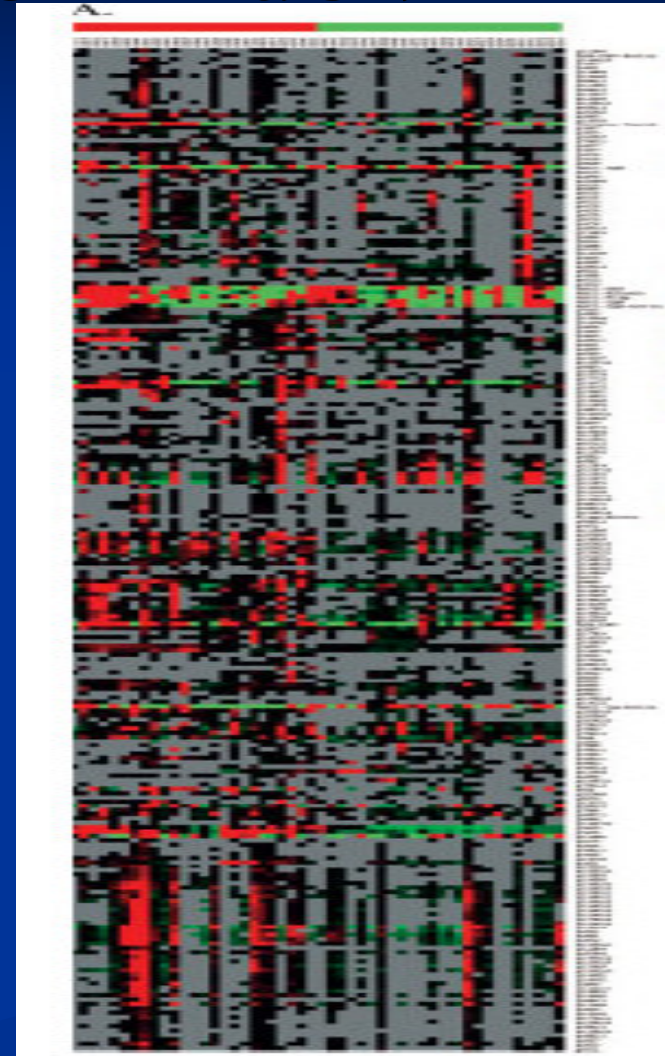
- Shows multiple spots with fluorescence above the background
- An average of 149 (including 15 control proteins) fractions per array showed measurable signal above background



Bouwman, K. et al. Microarrays of tumor cell derived Proteins uncover a distinct pattern of prostate cancer serum immunoreactivity. *Proteomics* 2003, 3, 2200-2270

# Example: Continued

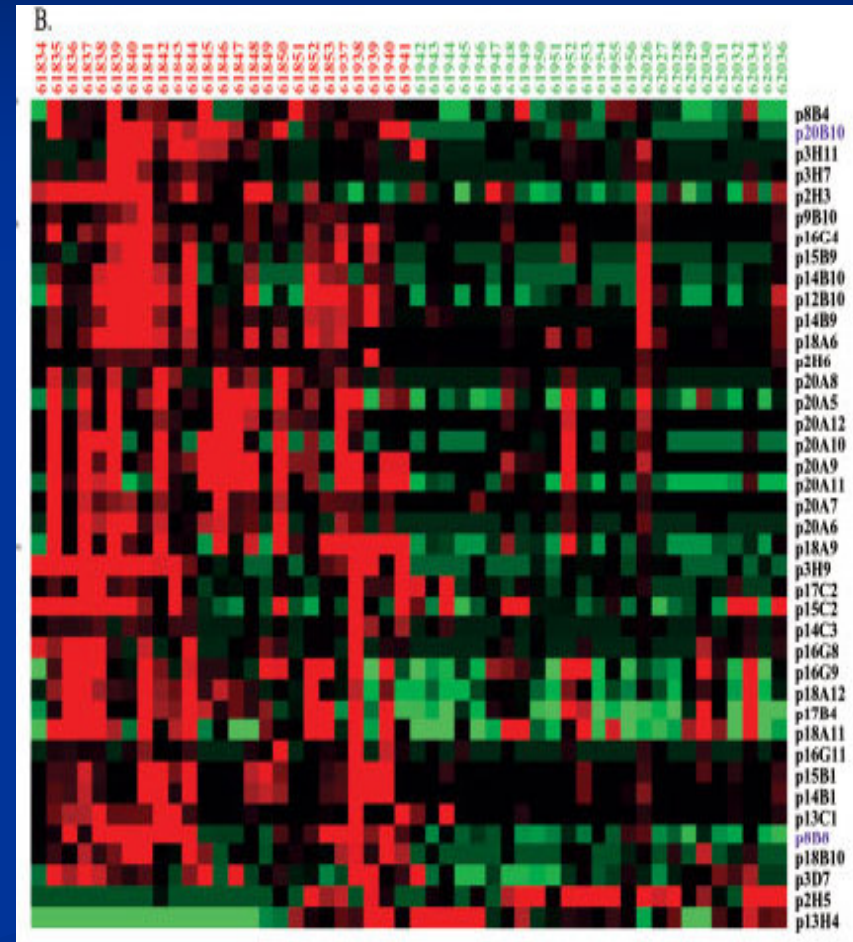
- Data from all 50 microarrays were grouped and clustered by similarity in intensity patterns
- Left 25 columns represent prostate cancer sera from one microarray, Right 25 non-cancer sera
- Color:
  - Red- High intensity
  - Green- Low intensity
  - Gray- no data



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# Example: Continued

- 40 fractions had the most reactivity
- 38 fractions had higher reactivity in the prostate cancer sera and only 2 fractions were higher in non.
- Many fractions contained the same proteins due to consecutive fraction collection
- Further analysis with MS would clarify the number of immunogenic proteins

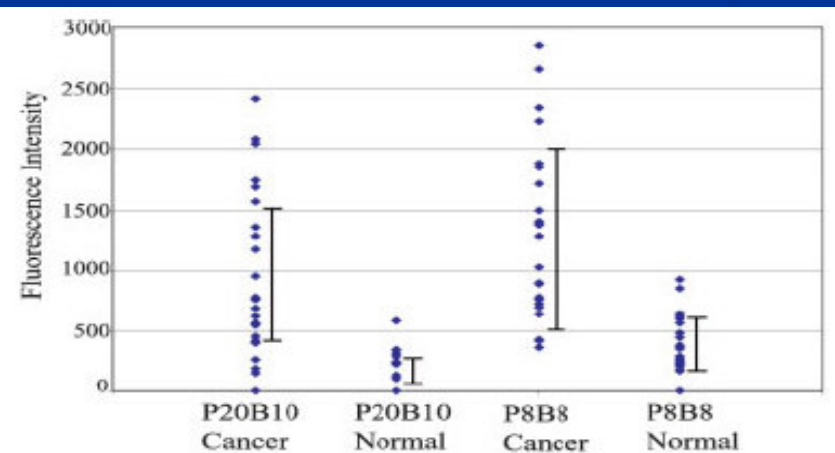
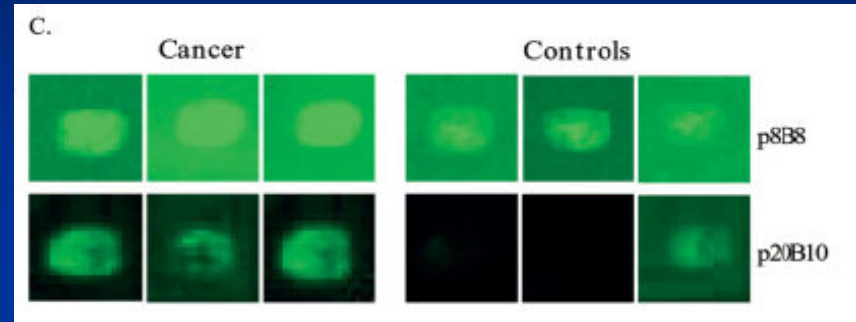


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# Example: Continued

- Illustrates the higher level of binding from the prostate cancer sera compared to control sera
- Intensities can be quantified



**Figure 3.** Signal intensities measured at fractions P8B8 and P20B10 in the cancer sera and control sera. The error bars indicate two standard deviations above and below the mean value.

Bouwman, K. et al. Microarrays of tumor cell derived Proteins uncover a distinct pattern of prostate cancer serum immunoreactivity. *Proteomics* 2003, 3, 2200-2270

# Advantages/Disadvantages of Microarrays

## ■ Advantages

- High Throughput (Rapid method sample analysis and can handle large samples)
- Can be used to address protein identification, quantification, and activity studies
- Can facilitate the discovery of new biomarkers and new drug targets

## ■ Disadvantages

- Protein arrays are more complex than DNA/other arrays due to complexity in protein structure
- Not always direct correlation between protein activity and abundance
- Stability of proteins to array surface
- Detection of interacting proteins still weak

# Conclusions

- In example: Strong fluorescence signal from many fractions has sufficient selectivity to detect the binding of specific antibodies proving the usefulness of microarrays
- Combined with MS technology, this will further aid characterization and validation of the proteomic method
- Although Protein Microarrays have their use in high throughput screening they need to demonstrate better precision, accuracy, and reliability before used in clinical diagnostics

# References

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- [www.acefesa.es/microarray/asper/asper.htm](http://www.acefesa.es/microarray/asper/asper.htm)
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# Questions

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