# SELDI ANALYSIS

## The Need for the Review and Understanding of SELDI/MALDI Data Prior to Analysis (Analyzer Beware)

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# *SELDI ANALYSIS*

***WINDOW INTO DISEASES***

**Plasma Serum Urine**

**Effusions**

**Mucous Saliva Fecal Matter**

**Tissue (PAP; Urinary Sediment)**

**Bile CSF Sweat**

# SELDI ANALYSIS

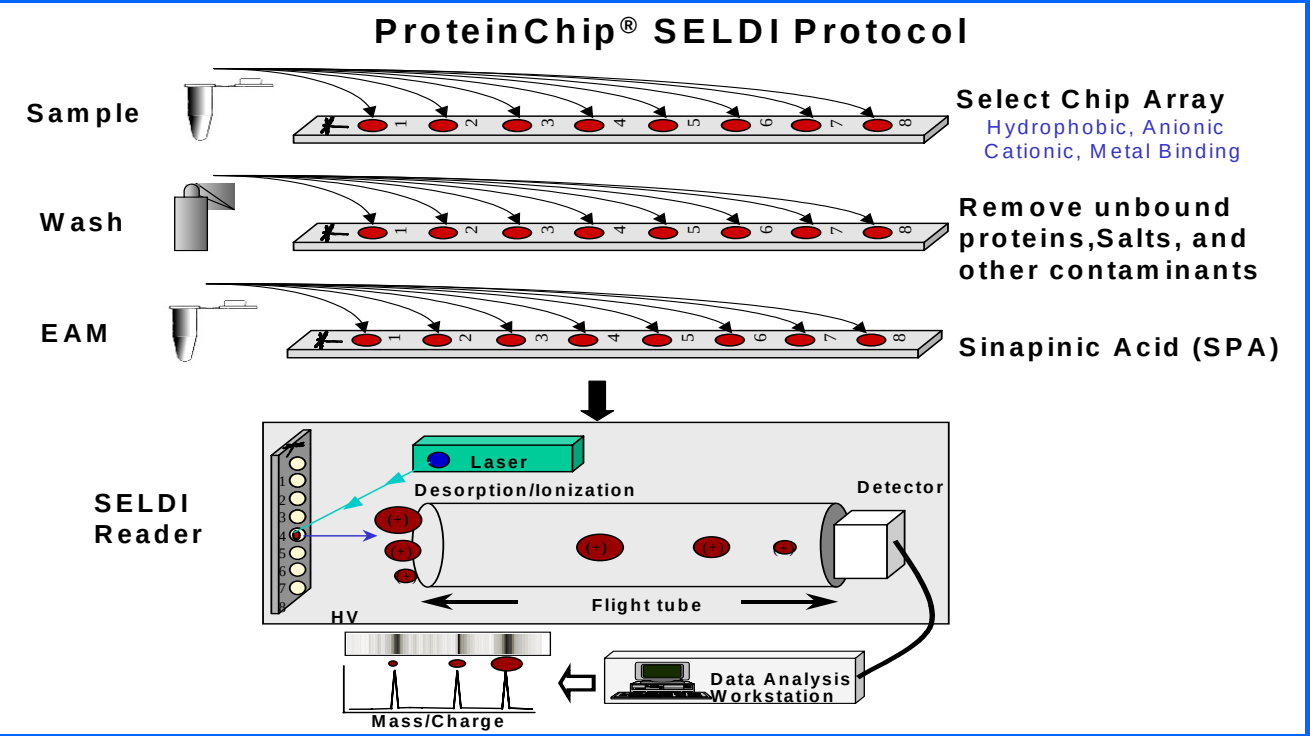
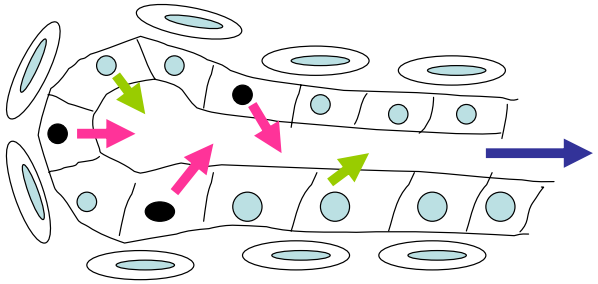
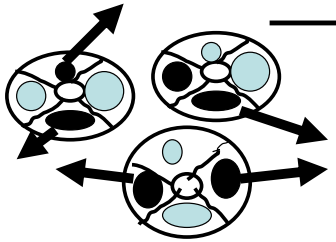


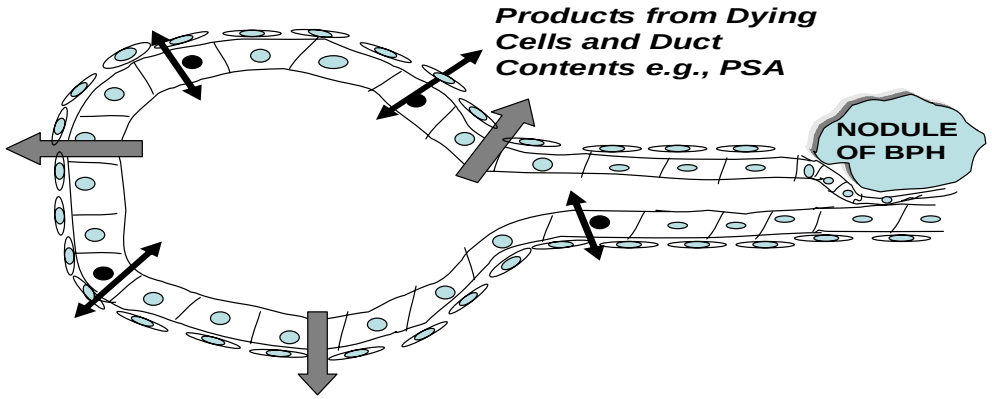

**Illustration of SELDI Time-Of-Flight (TOF) Mass Spectrometry. (Modified with permission from Ciphergen Biosystems, Inc.)**

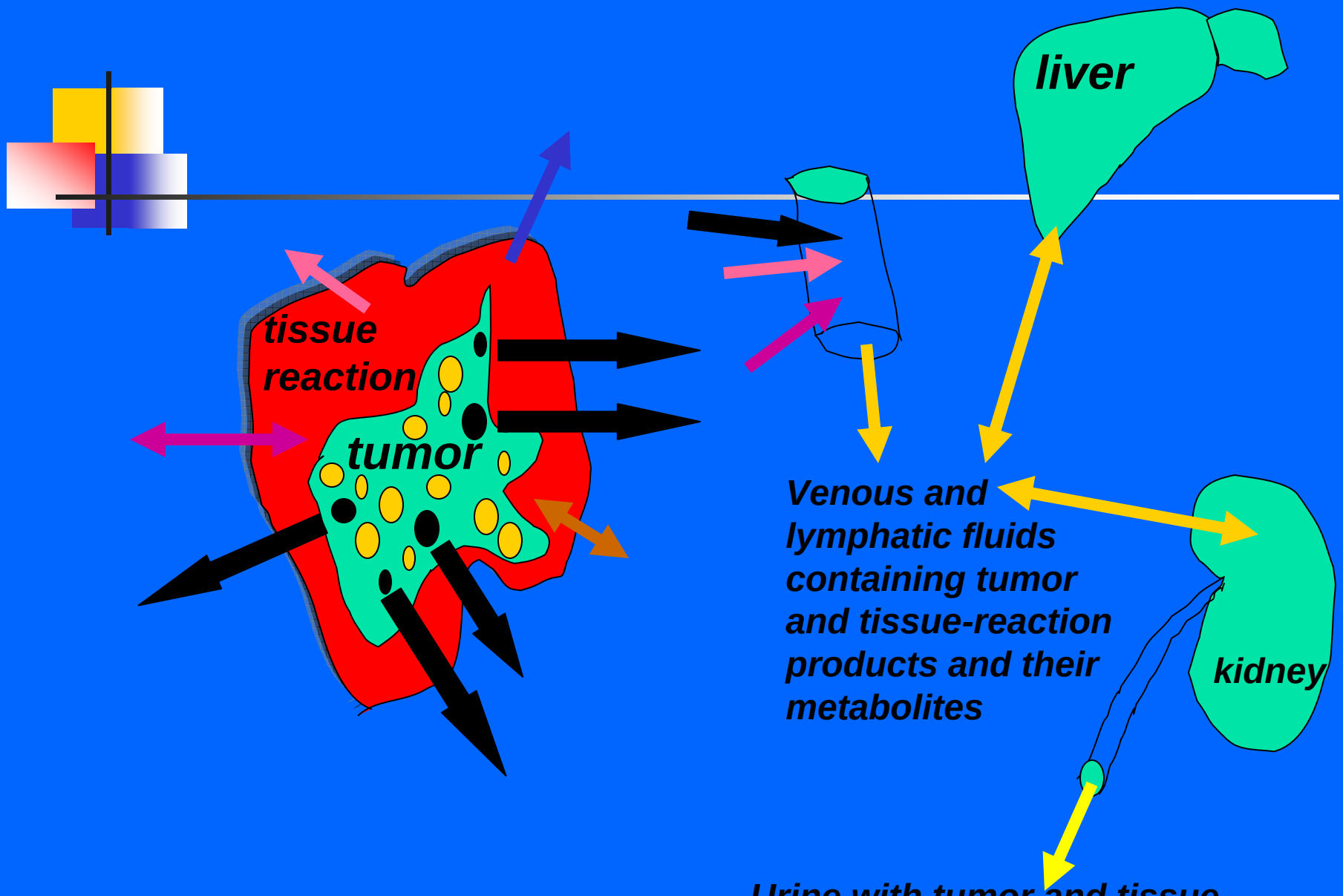


*Contents of Duct Including Products of Dying Cells and Living Cells e.g., PSA*



*Products of Dying Cancer Cells Collect in Interstitial Space and are Absorbed into Vascular and Lymphatic Vessels*

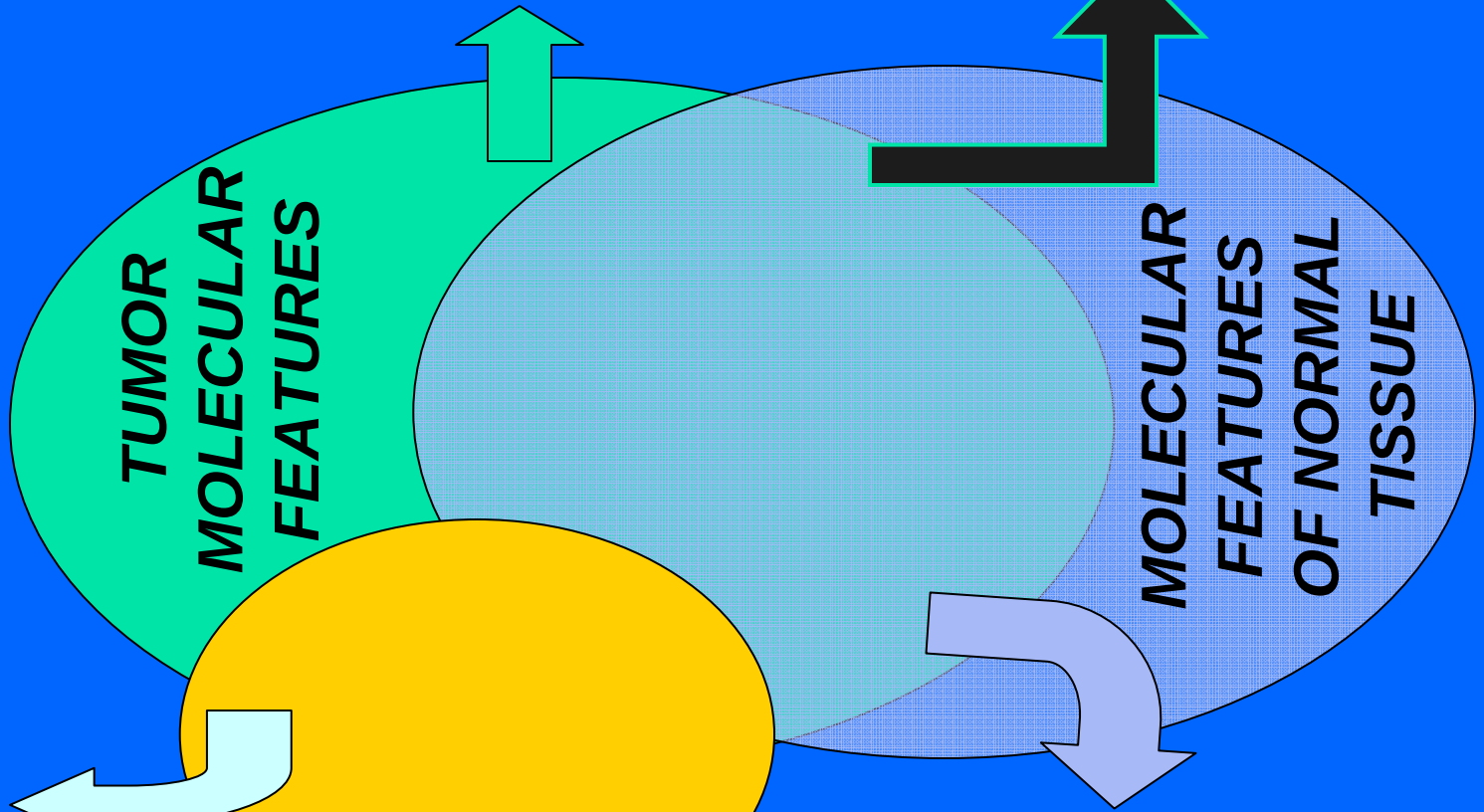



liver
tissue reaction
tumor
Venous and lymphatic fluids containing tumor and tissue-reaction products and their metabolites
kidney
Urine with tumor and tissue-reaction products and metabolites

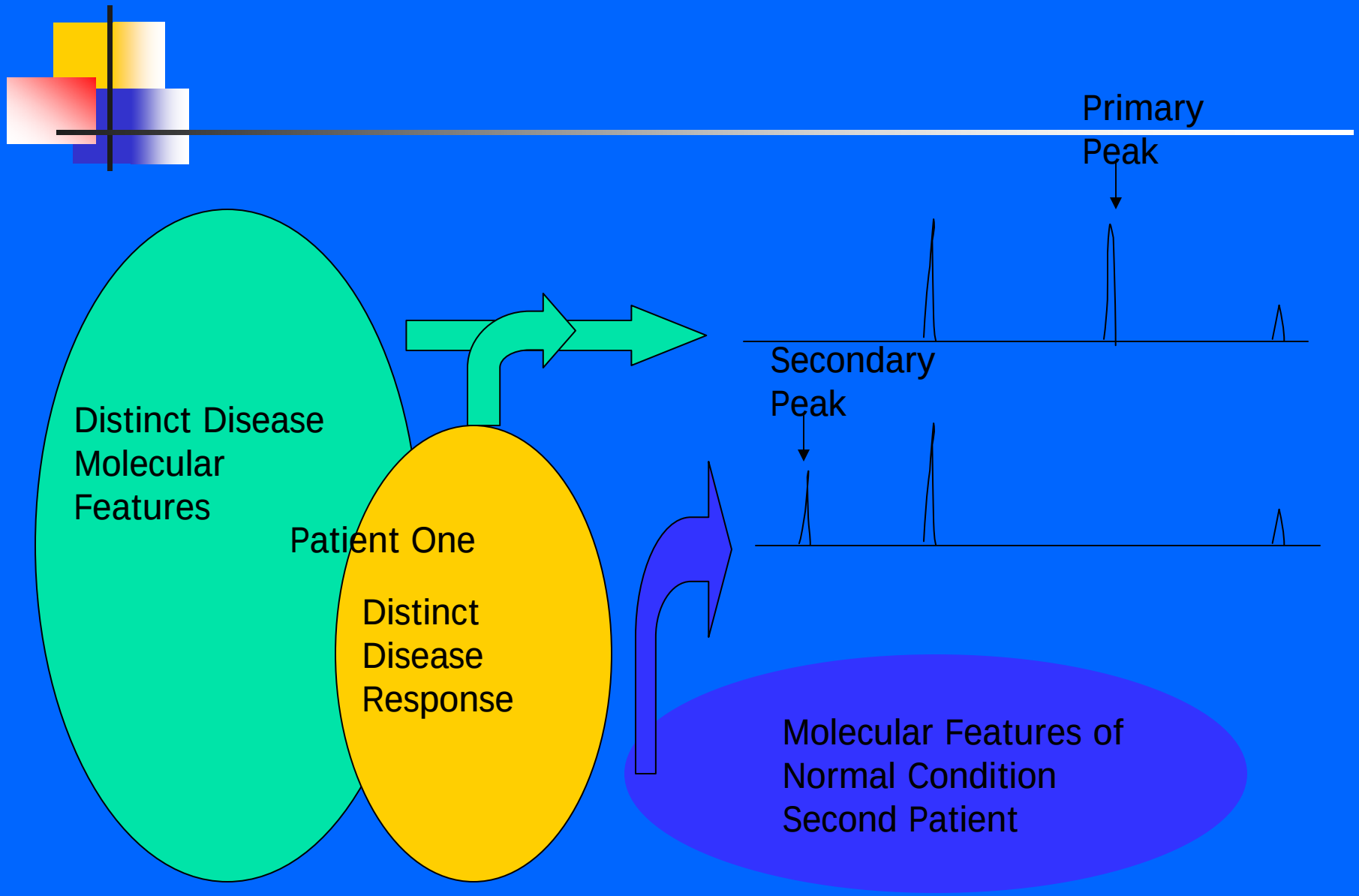
# ONCOFETAL TUMOR ANTIGENS AND METABOLITES

CEA
TAG-72
CA125
MUC-2
LEWIS Y

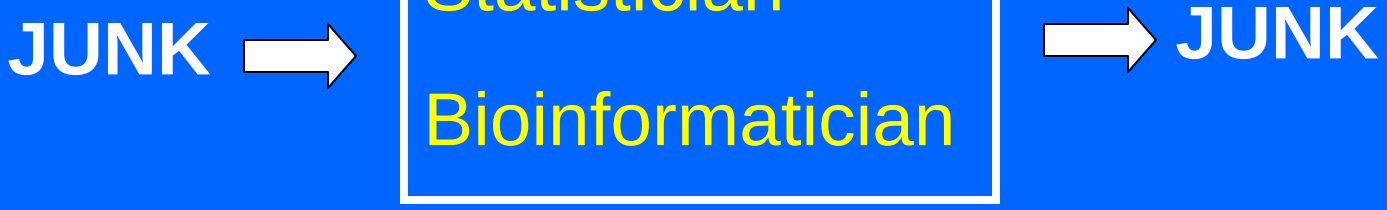
MOLECULES AND METABOLITES OF NORMAL TISSUES - ACTIN

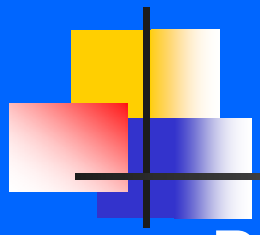



NORMAL TISSUE SPECIFIC - PSA, $A_1$-ANTITRYPSIN


Primary
Peak
Secondary
Peak
Distinct Disease
Molecular
Features
Patient One
Distinct
Disease
Response
Molecular Features of
Normal Condition
Second Patient

# SELDI ANALYSIS

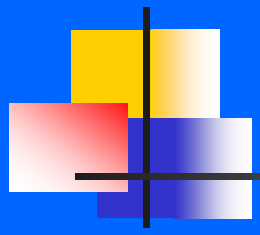




# SELDI ANALYSIS

PROBLEMATIC ISSUES IN ANALYSIS

- Experimental Design
- Patient
- Sample
- Protein Chip
- Spectra
- Analytical Approach



# SELDI ANALYSIS

Experimental Design

- Selecting Cases and Controls
- Collecting and Processing Samples
- Performing Assays Without Bias
- Selecting Optimal Approach to Analysis
- Avoiding Over-Analysis

# SELDI ANALYSIS

PATIENT

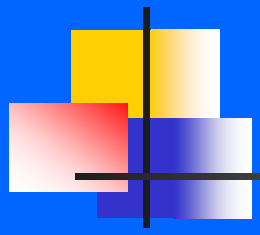
Groups Comparable

Sites

Racial/Ethnic Balance

Homeostatic Balance

No Bias



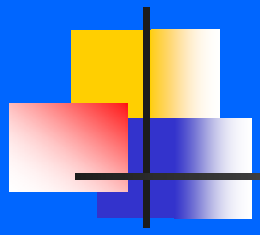
# SELDI ANALYSIS

PATIENT

Usually Comparing Disease vs. Control

or

Disease A vs. Condition B vs. Normal



# SELDI ANALYSIS

PATIENT

Control Definition

Is Disease Absent?

Is There Bias In The Controls?

# SELDI ANALYSIS

SAMPLE

- Type
- Collection
- Processing
- Storage
- Transfer

# SELDI ANALYSIS

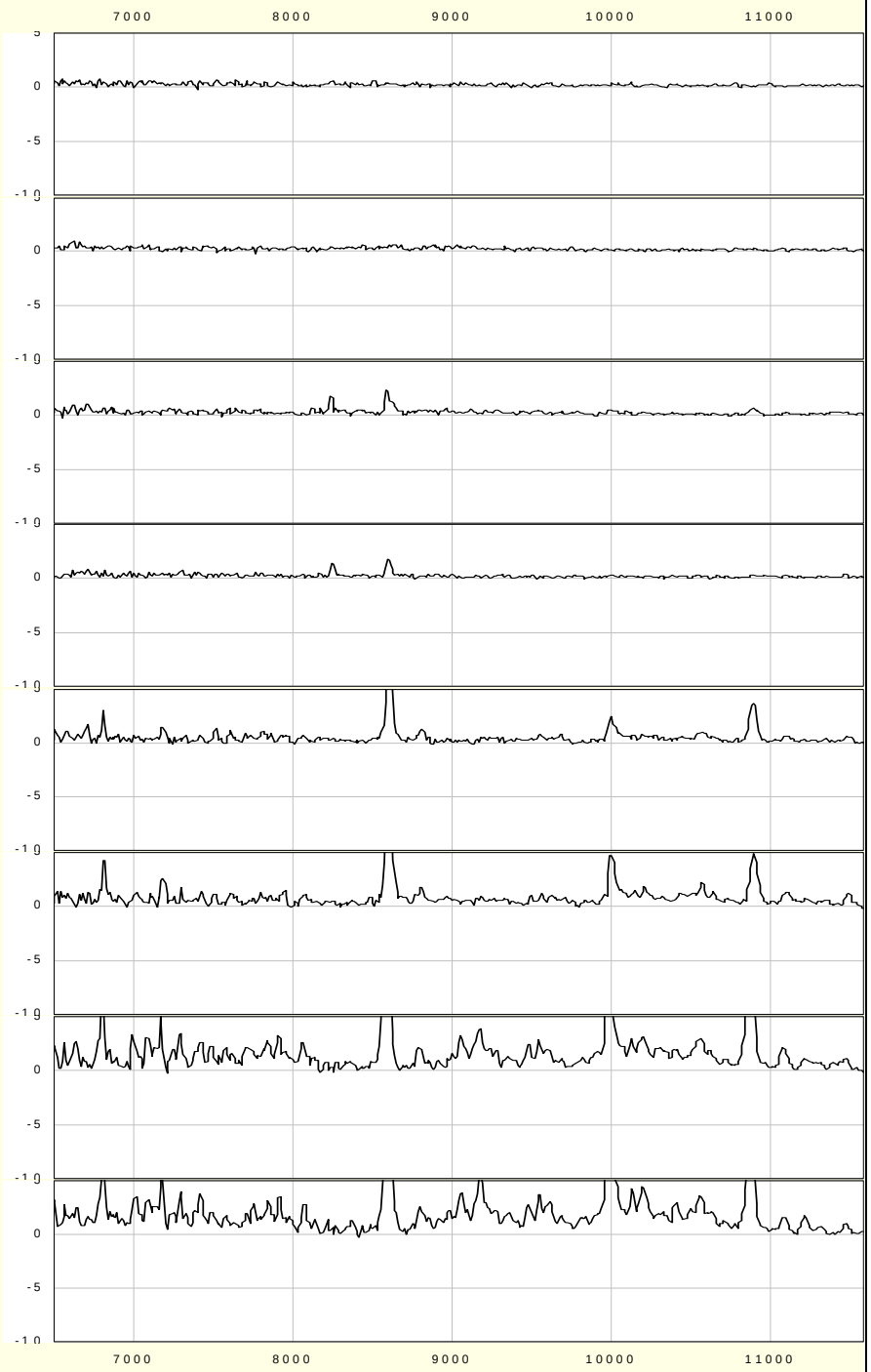
SAMPLE

Type

Serum versus Plasma

Sensitivity- Can Products of Tumors Be Detected

# LNCAP CELL LYSATES USING WCX2 ARRAYS



# SELDI ANALYSIS

SAMPLE

Collection

How?

Stress

Container-e.g.., multiple anticoagulants; thus all Plasmas are not the Same

# SELDI ANALYSIS

SAMPLE

Processing

Time from Collection to Freezing (Too Restrictive?)

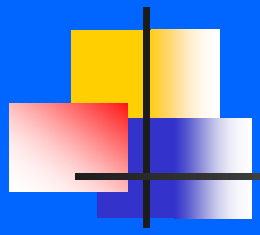
For Consistent Results, Robotic Processing Is Required

# SELDI ANALYSIS

SAMPLE

Processing

Removal of Proteins Present in Large Concentrations (e.g., Albumin) May Also Remove Peptides Being Carried by Removed Proteins



# SELDI ANALYSIS

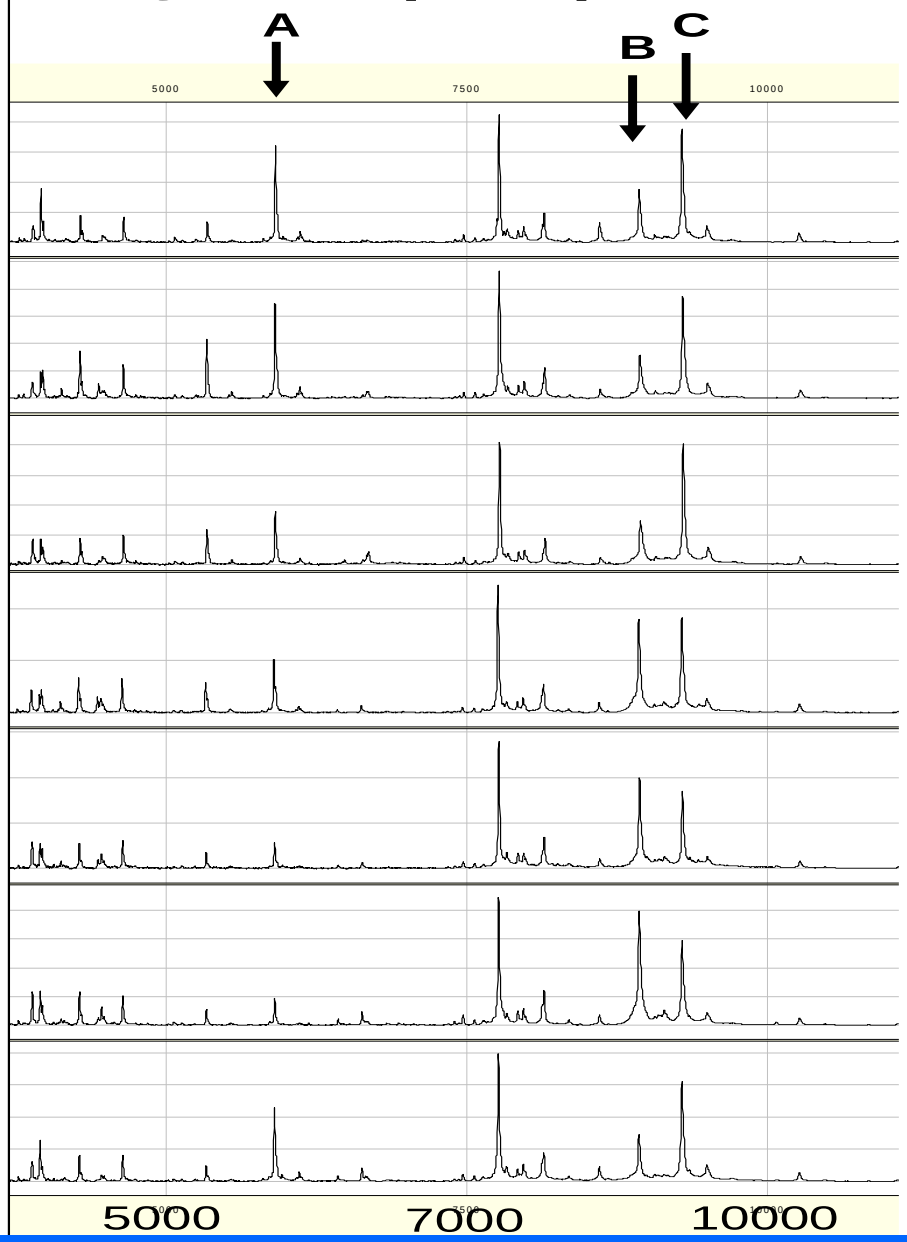
SAMPLE

Storage

Length

Temperature

# QC Sample Spectra




Aliquot of Sample (#20) Stored at -80C

Aliquot of Sample (#20) Transferred from -80C and Stored for 3 Months at -20C

Aliquot of Sample (#20) Stored at -20C for 5 Months

Aliquot of Sample (#20) Stored at -20C for 7 Months

Aliquot of Sample (#20) Stored at -20C for 8 Months

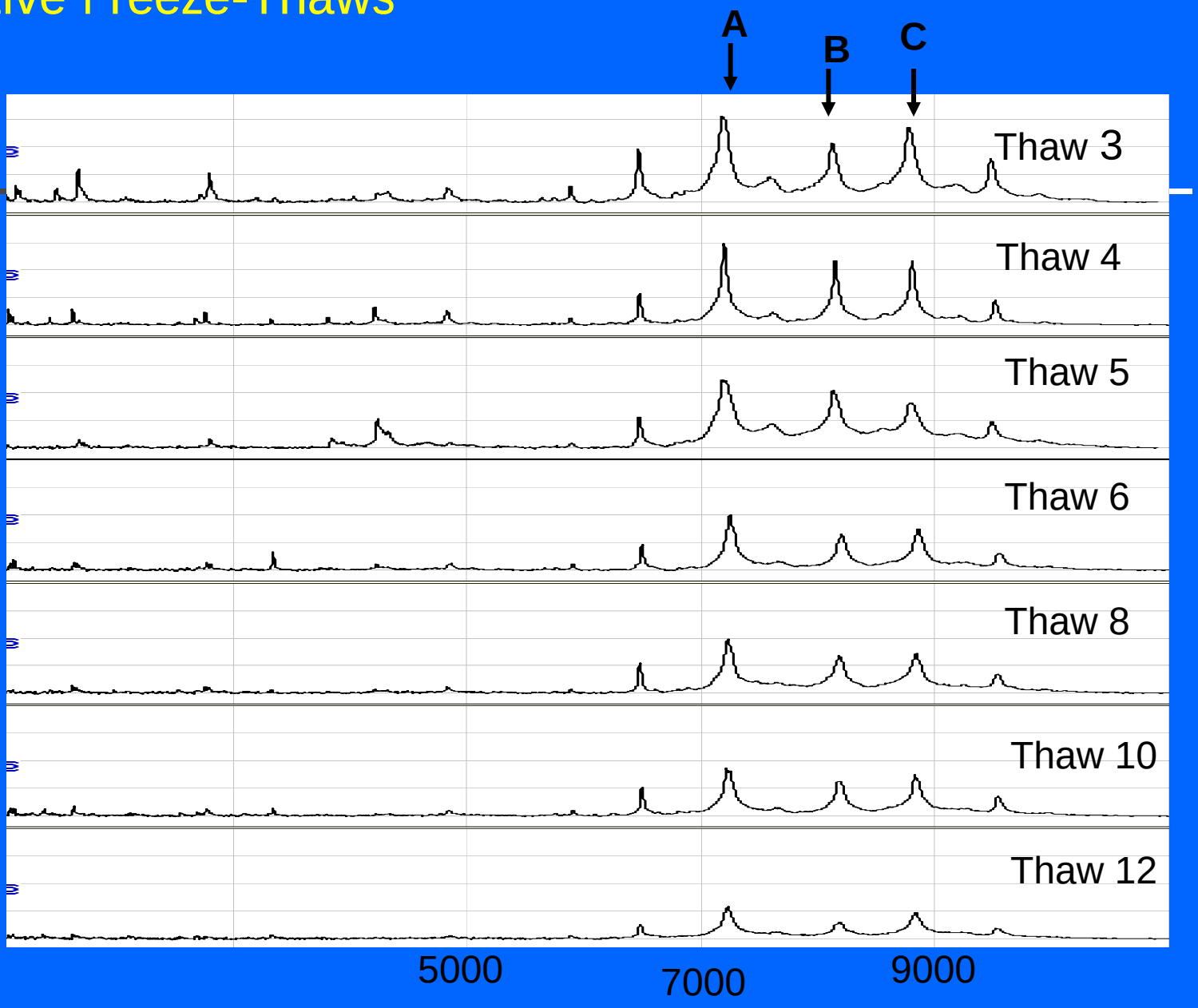
Second Aliquot of Sample (#20) Stored at -20C for 8 Months

Second Aliquot of Sample (#20) Stored ONLY at -80C for Ten Additional Months

# SELDI ANALYSIS

- Transfer
  - Freeze-Thaw Cycles
  - Quantity (Triplicates and Repeat-300 mcl)

# Decrease in Protein Intensity in Human Serum due to repetitive Freeze-Thaws



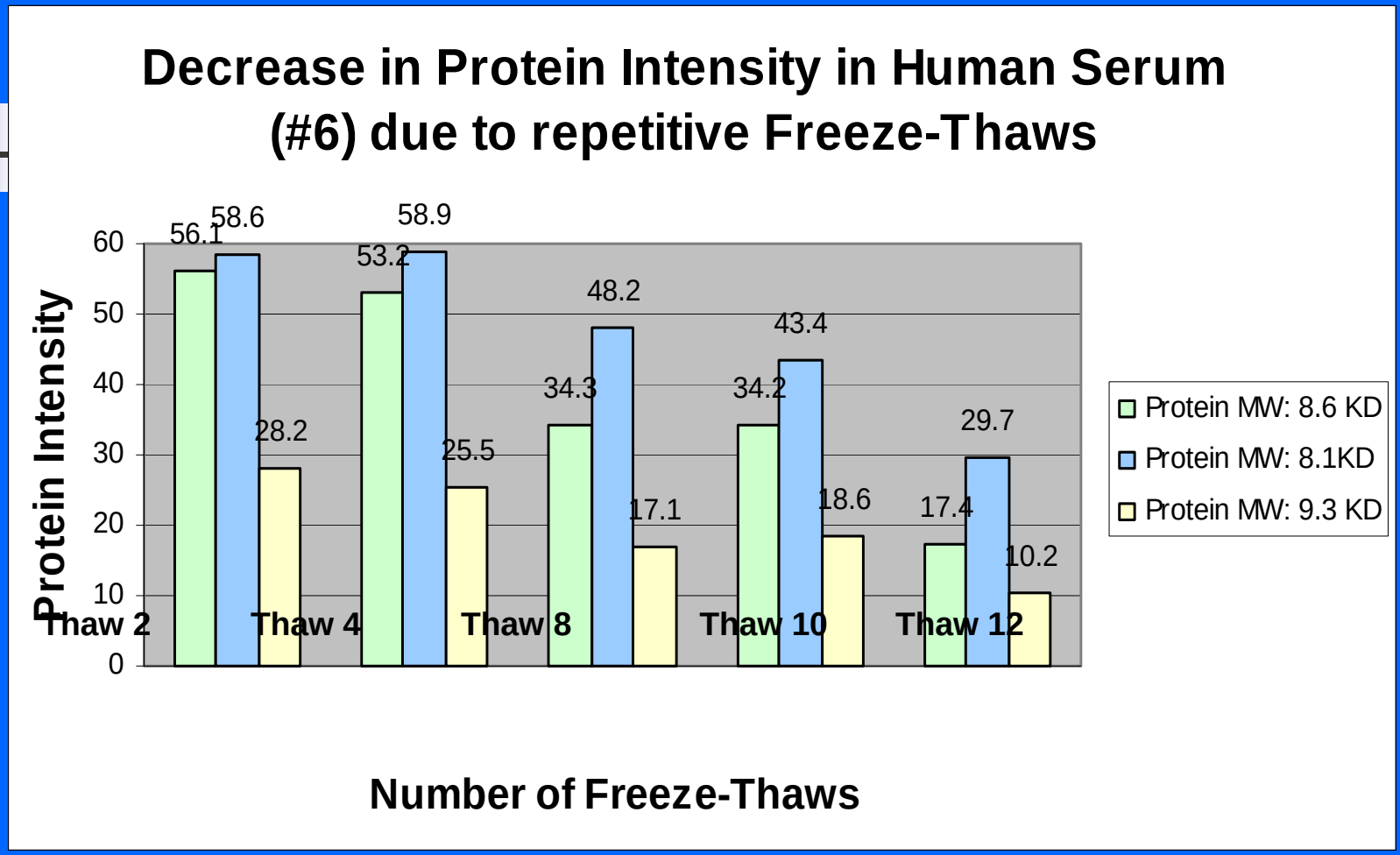
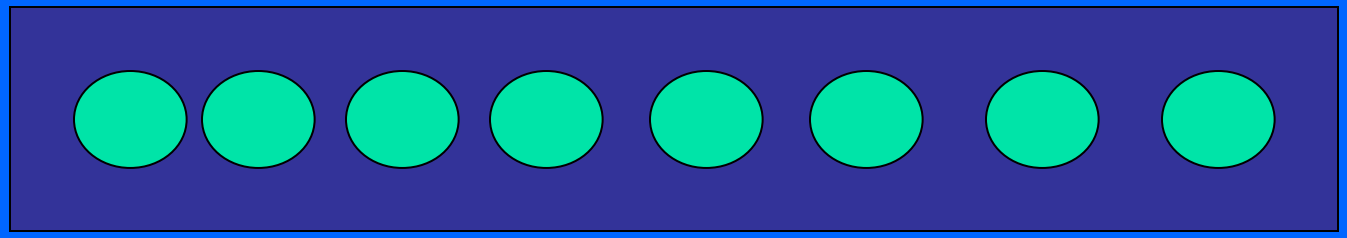



*Figure1: The graph above shows the decrease in protein intensities in three different proteins in one human serum sample. The legend indicates the molecular weight of each protein. The specific intensity values are displayed above each of the bars.*

# SELDI ANALYSIS

- Chip Type



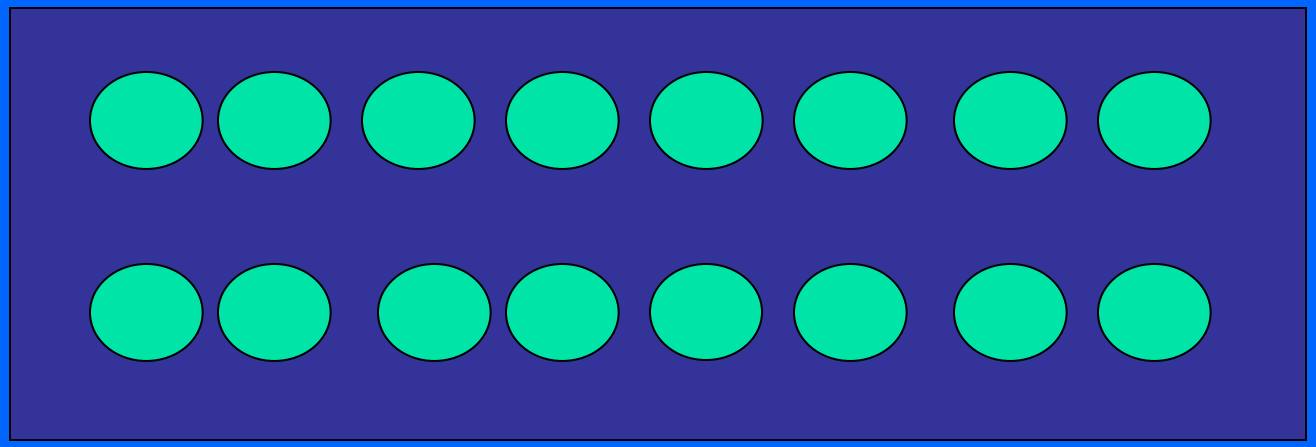
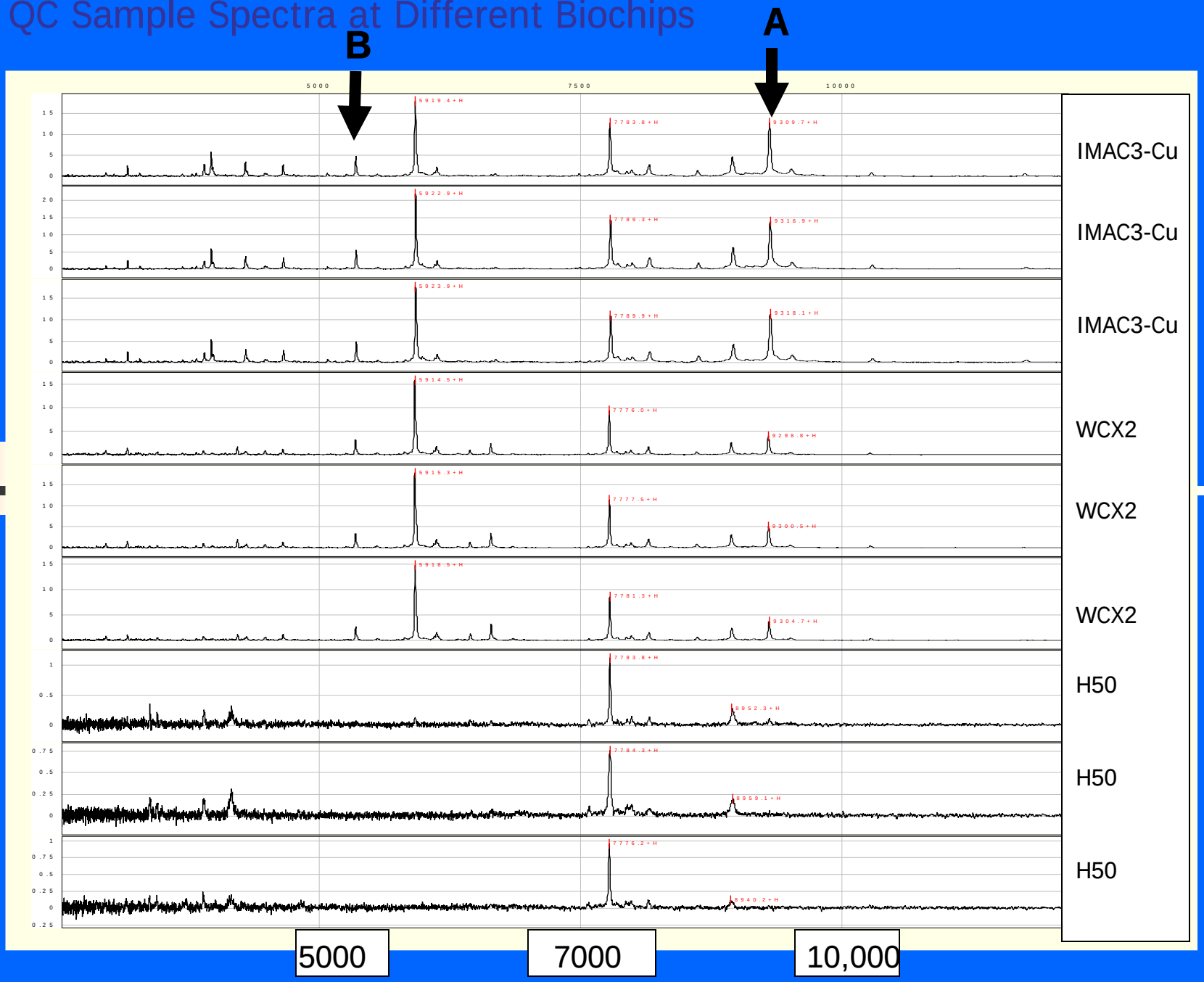


TABLE 1:

| Old Designation | Current Chip | Biochemical Action of Surface Chemistry |
|---|---|---|
| IMAC3 | IMAC30 (with hydrophobic barrier) | Bivalent metals can be attached to the chip. Proteins that bind to these divalent metals (e.g., $Cu^{+2}$) are bound by the chip. |
| WCX2 | Same (CM10 mimics WCX2 but does not replace it) | This is a weak cation exchange chip. It contains negatively charged (anionic) carboxylate groups that will bind proteins with positively charged areas containing high numbers of lysine, arginine, and/or histidine amino acids. |
| H4 | Same (C16 contains 16 $CH_3$) | The chip contains multiple chains of 16 methylene groups. This binds molecules that are hydrophobic. |
| SAX2 | Q10 (with hydrophobic barrier) | Strong anion exchanger which is composed of quaternary ammonium groups that are charged positively. This chip will bind proteins/peptides with regions rich in acidic groups, especially regions of peptides high in aspartic and/or glutamic amino acids. |
| NP1 and NP2 | NP20 | General protein binding surface with binding of hydrophilic proteins. |
| PS1 and PS2 | PS10 / PS20 | Chip designed to bind capture molecules of choices e.g., antibodies, receptors, and nuclei acid binding proteins PS-1 (carbonyl diimidazole groups), PS-2 (epoxy groups). Also the PS-2 has a hydrophobic coating. |
| SENDID | | Incorporates EAM into chip. |

# QC Sample Spectra at Different Biochips



# SELDI ANALYSIS

Protein Chip

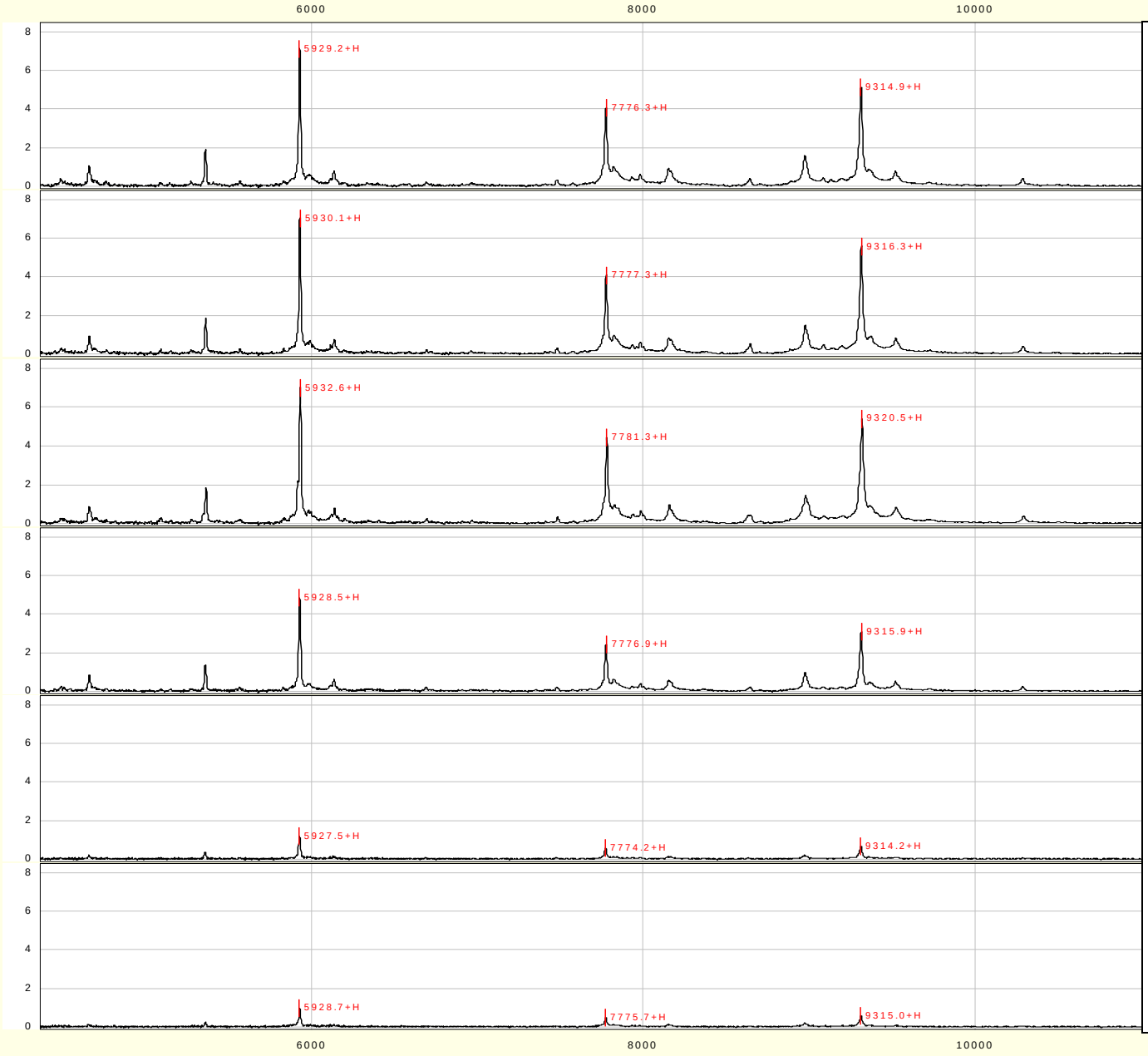
- High Concentration Proteins May Block Binding of Low Concentration Proteins: 10,000 Ci of 5500 D Protein vs. 10 Ci of 7500 D Protein both with Same Binding Characteristics

# SELDI ANALYSIS

Protein Chip

- All Proteins/Peptides Bound to Chip May Not Be Released/Ionized.

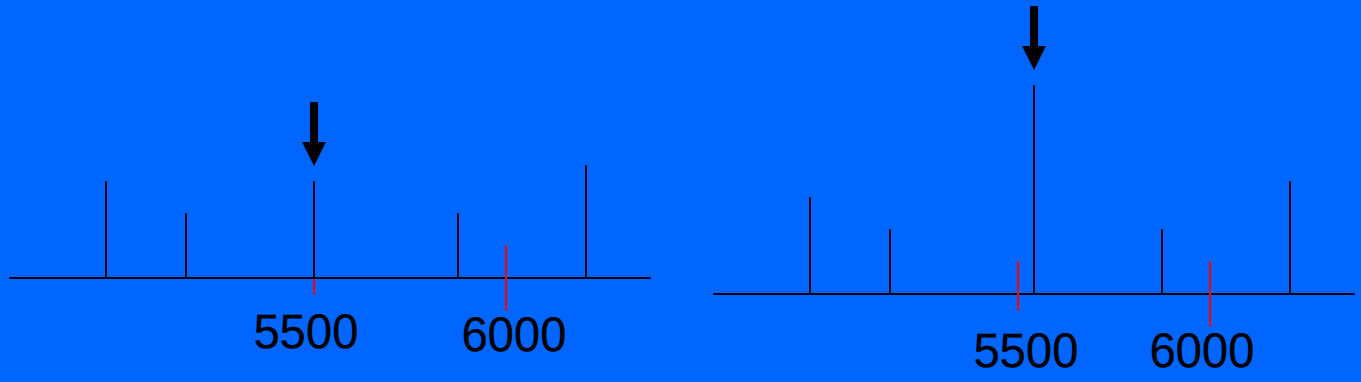
# QC Sample Spectra at Multi-times Reading



# SELDI ANALYSIS

## Spectrum

- "Directed" and "Non-Directed" Approaches to Begin Spectral Analysis
- Directed= Peak at 5500 Same As Peak at 5507 Based on Resolution +0.2%



# SELDI ANALYSIS

Spectrum

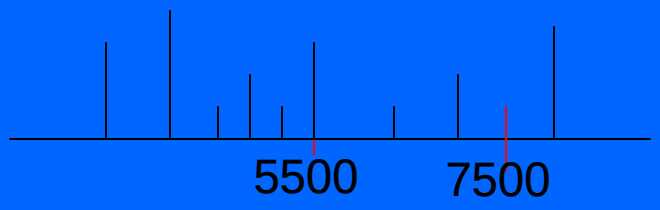
- Primary Peaks- Disease Has Unique or Larger Peak than Non-Disease; Thus, the Disease Produces a Molecular Product.

- Secondary Peak-Disease Causes a Decrease in Molecular Species Normally Present via Change in Metabolism or Excretion and/or Shutdown in Production

# *SELDI ANALYSIS*

Spectrum

- Components of Spectra at Molecular Weights of Less Than 20,000 May Represent Metabolites of Proteins/Peptides Rather Than Intact Proteins/Peptides

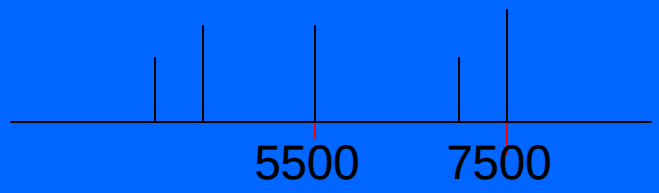


# SELDI ANALYSIS

## Spectrum

- Peaks May Not Provide Independent Information: For Example the Peak at 5500 D May Be A Metabolite of the Peak at 7500

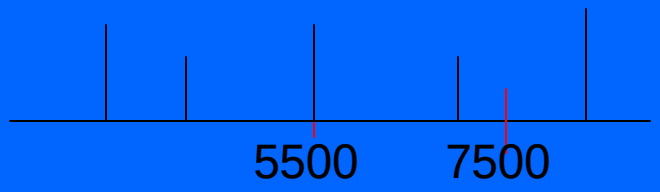
**7500 ⟶ 5500**



# SELDI ANALYSIS

Spectrum

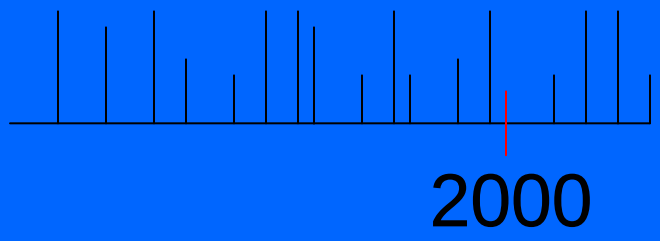
- A High Concentration Protein May Prevent Identification of Low Concentration Protein: 1200 Ci of 5500 D Protein vs. 100 Ci of 5510 D Protein Even with Different Binding Characteristics to Same Chip



# SELDI ANALYSIS

Spectrum

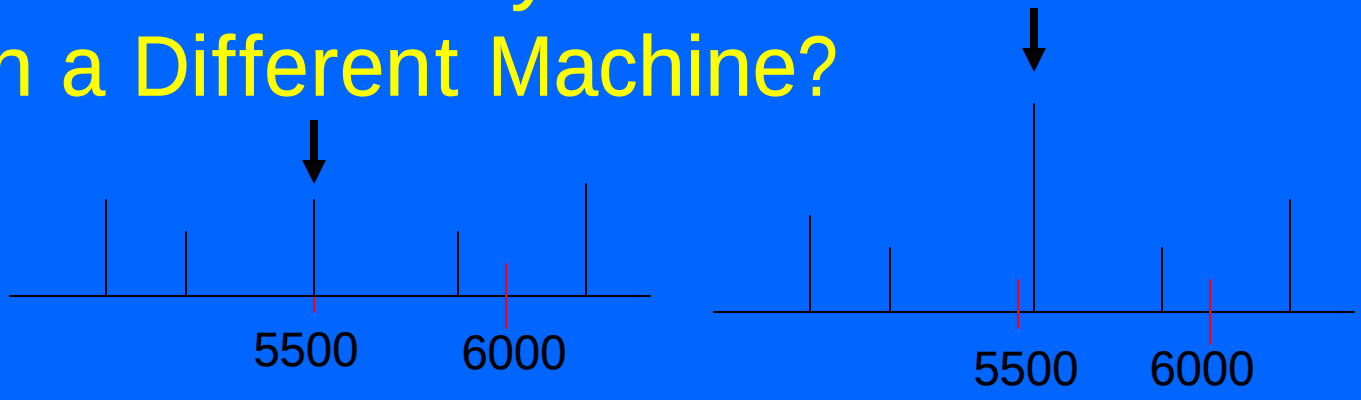
- All Areas of the Spectrum Are Not The Same
  - Molecular Weights of Less Than 2000
    - No Standards; Noise; Contamination
  - Weights of Greater Than 50,000
    - Proteins of High Concentration



# SELDI ANALYSIS

## Spectrum

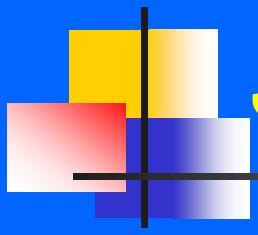
- How Variable Is The Peak Location and/or Amplitude When the Same Sample Is Run
  On the Same Day on the Same Machine?
  On the Next Day on the Same Machine?
  On a Different Machine?



# *SELDI ANALYSIS*

Spectrum

- Eastern Virginia Medical School; UAB; U of Texas San Antonio; U of Pittsburgh Medical Center; Johns Hopkins Medical Center; Uniformed Health Services
- ***All Were Able To Standardize Their Machines and To Obtain Comparable Data on 14 Cancer and 14 Non-Cancer Cases***



# SELDI ANALYSIS