Chap 9 Cancer Diagnosis

1. Sullivan Pepe M, Etzioni R, Feng Z, Potter JD, Thompson ML,Thornquist M, et al.

   Phases of biomarker development for early detection of cancer.

   JNCI 2001; 93:1054-61.

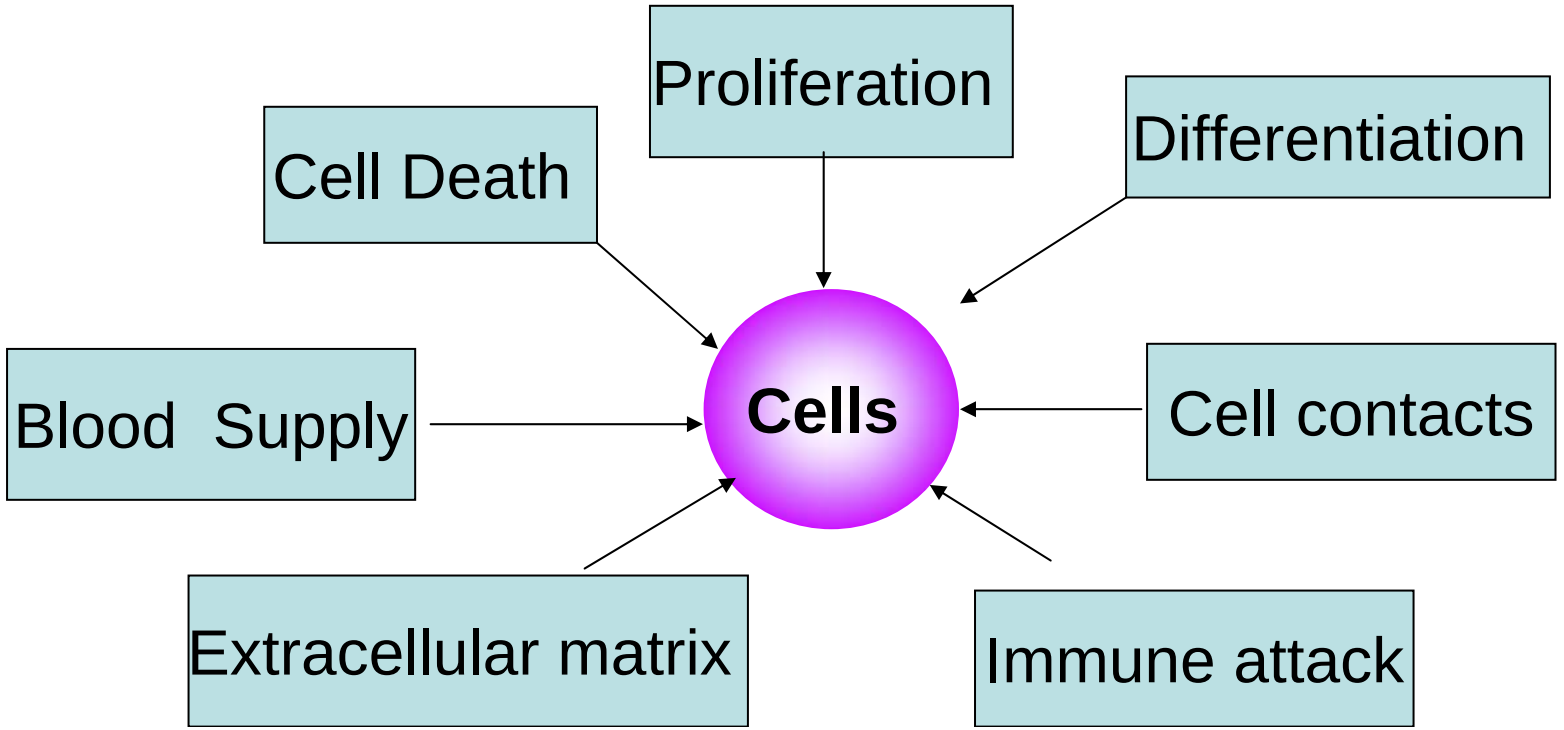
2. Ransohoff DF.

   Developing molecular biomarkers for cancer.

   Science 2003; 299:1679-80.

What is Cancer?

- Normal body cells grow, divide and die in an orderly fashion.
- Cancer cells are different because they do not die, just continue to divide and grow.
- Cancer cells form as a result of damaged DNA.



Causes of Cancer

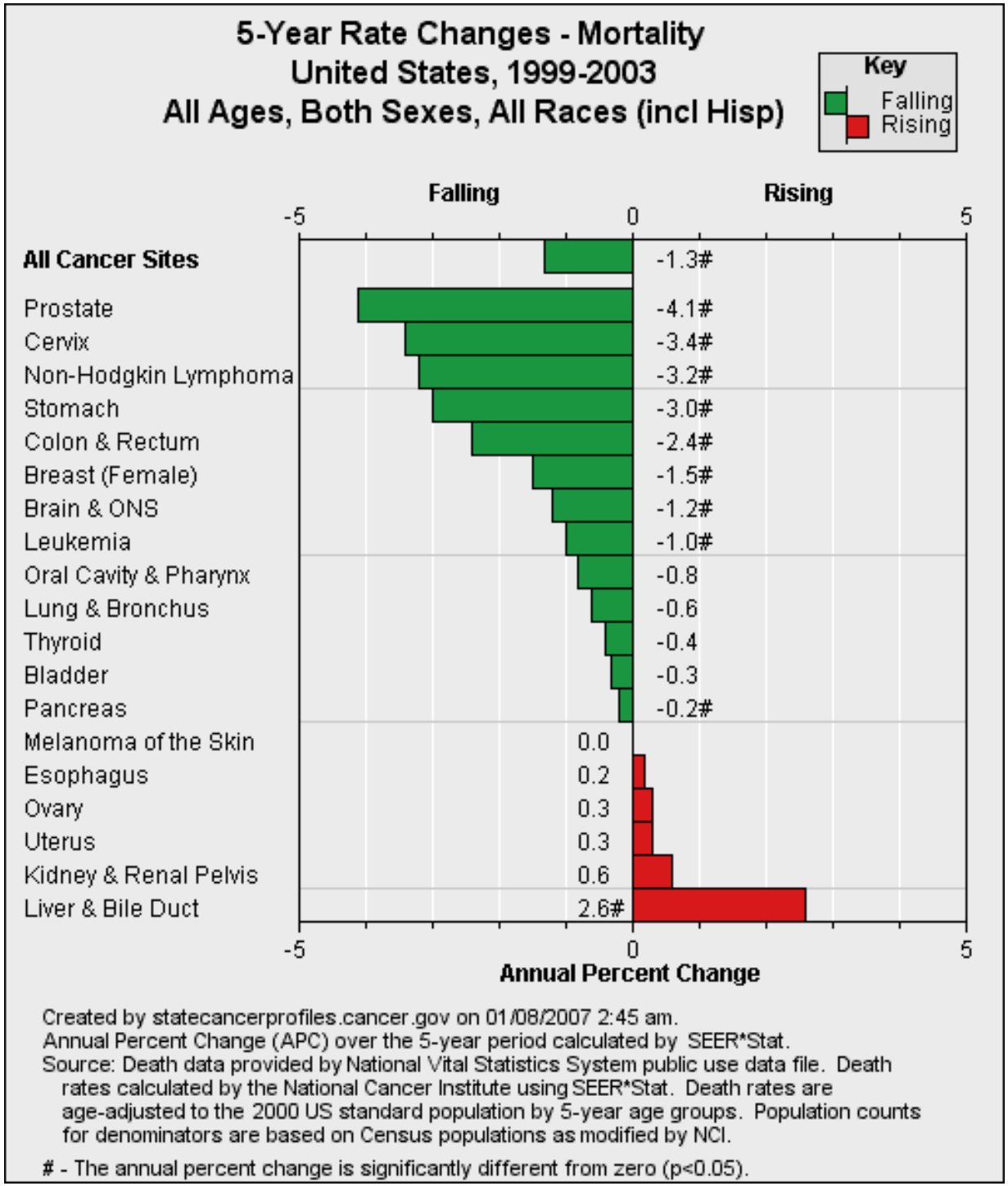
- **Heredity**

  Screenings are recommended for high risk families. You are considered high risk if : Several relatives have had cancer or if someone had cancer at a very early age.

Etiologic Factors Associated with Carcinogenic Risk

Carcinogenic Risk Factor	Associated Neoplasm(s)	Probable % of Cases
Within the Body		
Sporadic Genetic Mutations	Any	10
Inherited genes(familial)	Breast,colon	5
Reproductive history		
Late first pregnancy	Breast	
Zeo or Low parity	Ovary, Breast	
Sexual promiscurity	Cervix	
Occupational		5
Asbestos	Lung,mesothelioma	
Anliline dye	Bladder	
Benzene	Leukemia	
Vinyl chloride	Liver	
Chromium, Cadmium nickel	Lung	

Carcinogenic Risk Factor	Associated Neoplasm(s)	Probable % of Cases
Environment		15
Viral Infections	Leukemia, lymphoma	
Pollutants		
Radiations		
ionize	Leukemia,breast, thyroid	
Utraviolet	Skin,melanoma	
Radon	Lung	
Medical Treatments		1
Alkylating agents	Leukemia,bladder	
Diethylstilbestrol	Virginal (in offspring of exposed woman)	
Estrogens	Endometrium	
Tamoxifen	Endometrium	
Radiation	Skin,lung,breast	
Life style		
Smoking	Lung,bladder mouth,pharynx,lip	30
Alcohol	Esophagus,liver,larynx	5
Diet	Colon, breast, gall bladder	30(?)
Food Additives(salt)	Stomach	1
Aflatoxin	Liver	
Sedentary Lifestyle	??	3



http://statecancerprofiles.cancer.gov/cgi-bin/quickprofiles/profile.pl?00&001#death

Importance of Early Cancer Detection

Early cancer detection is critical
for successful treatment.

Example: Five year survival for ovarian cancer:

- Early stage: 90%
- Late stage: 35%

80% are diagnosed at a late stage

Detection → classification and localization

→ therapy

imaging
histology
biomarkers

surgery
radiation
chemotherapy

Characteristics of Cancer Cells

- **General changes**: - loss of division limits (immortality)
  - uncontrolled proliferation
- **Genetic changes**: - point mutations …
  - chromosomal changes
- **Structural changes**: - less organized cytoskeleton
  - increased membrane fluidity
- **Biochemical changes**: - altered protein expression
  - altered protein modification

Diagnostic Workup

- Diagnostic Cytology (細胞學), Histology(組織學) and Cytogenetics (細胞遺傳學)
- Tumor Makers (protein, gene…etc)
- Grading and Staging (癌症分期)

分級（grade）：病理上的分類，根據腫瘤細胞在病理組織學上的分化程度而定。分化愈好的（愈像正常組織的），級數愈低；分化愈差的，級數愈高。

分期（stage）癌症侵犯及擴散的程度。當腫瘤越大或侵犯的程度越廣，淋巴腺轉移越多，其期數就越高。而轉移到遠處器官通常是末期了（第四期）。

How is Cancer Evaluated?

- Screening and/or diagnostic **mammography** (乳房攝影術)
- **Ultrasound**
- **Magnetic Resonance imaging** (MRI,磁振造影)

scan —uses a powerful magnetic field, very accurate but expensive

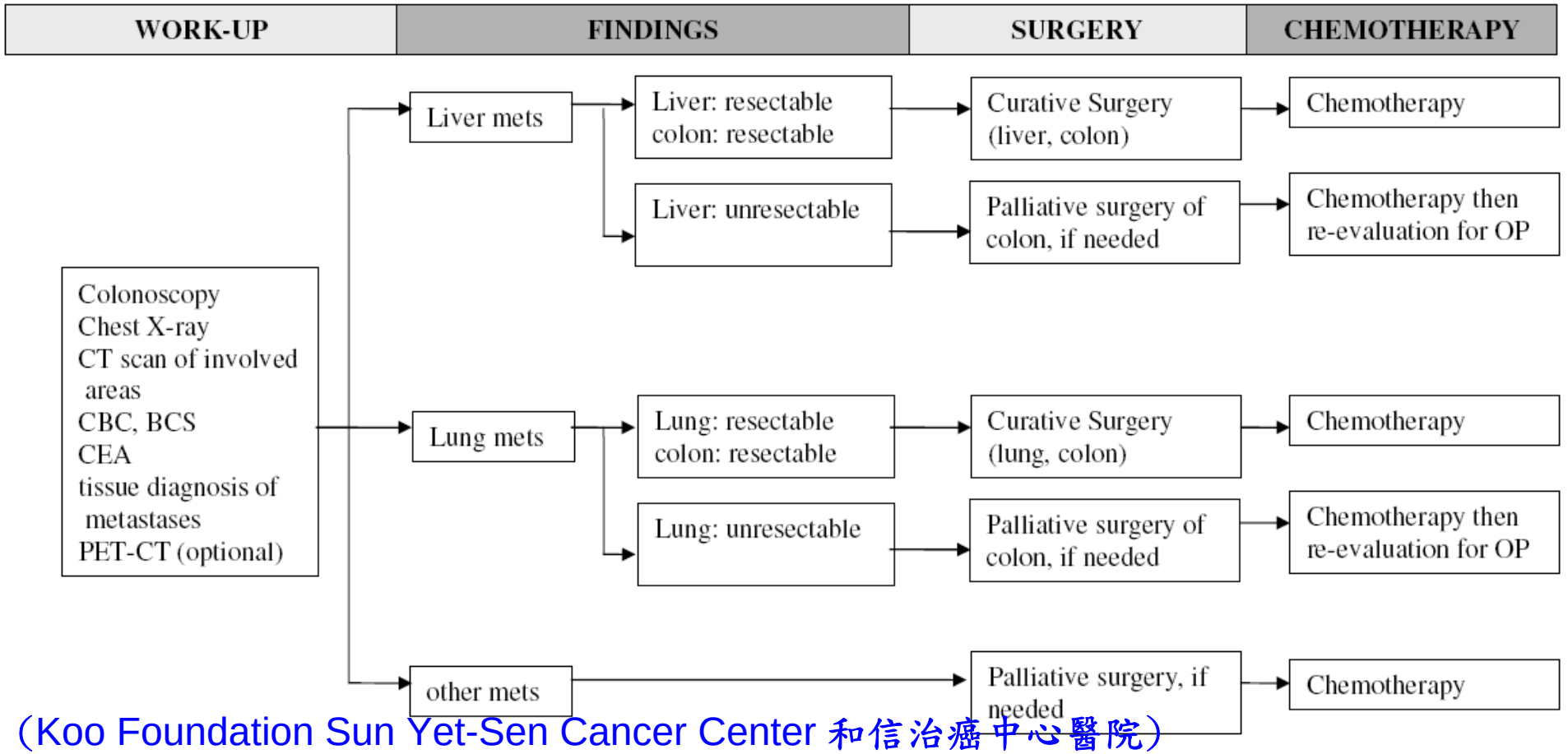
- **Biopsy** is necessary to confirm a diagnosis (切片檢查法)
- **Blood tests** —some tumors release substances called tumor markers which can be found in the blood
- Additional tests may be used to determine stage

SOP for Colorectal Cancer Diagnosis

Colon Cancer

Koo-Foundation Sun Yat-Sen Cancer Center
Clinical Practice Guideline *2008 Version 1.0*

Initial management for stage IV disease



（Koo Foundation Sun Yet-Sen Cancer Center 和信治癌中心醫院）

Follow-Up after Treatment

FOLLOW-UP

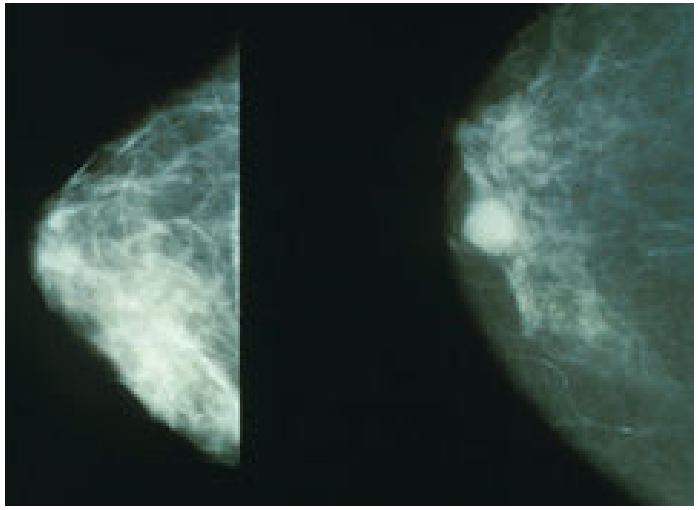
Time	Pre-treatment	6 months	1 year	6 months	2 years	6 months	3 years	6 months	4 years	6 months	5 years
Physical examination	O	O	O	O	O	O	O	O	O	O	O
CEA	*		*		*		*		*		*
CXR	O		O		O		O		O		O
Chest CT	**		**		**		**		**		**
Whole abdominal CT	O		O		O		O		O		O
Sonogram of liver		O		O		O					
Colonoscopy	O	O	O		O						O

*: CEA is only for those patients who has CEA elevation before treatment.

**: Chest CT is only for those patients who has lung metastasis resected.

Mammography (乳房攝影術)

Mammography is a specific type of imaging that uses a low-dose x-ray system to examine breasts. A mammography exam, called a mammogram, is used to aid in the early detection and diagnosis of breast diseases in women.

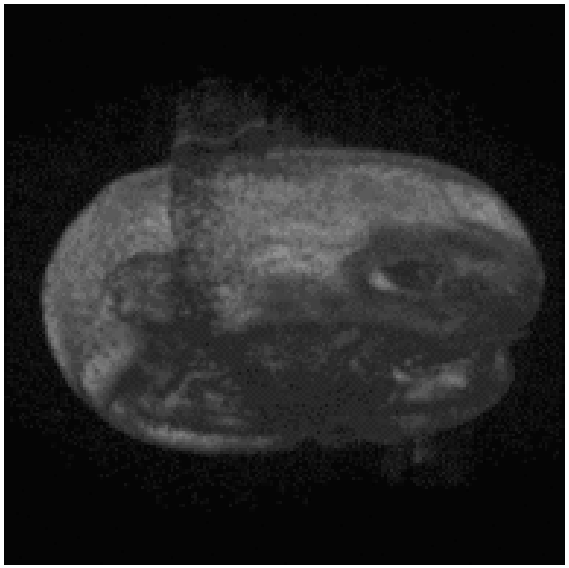


Left: normal breast
Right: Cancer

The computer software then searches for abnormal areas of density, mass, or calcification that may indicate the presence of cancer. The computer system highlights these areas on the images, alerting the radiologist to the need for further analysis.

Magnetic Resonance Imaging (MRI,磁振造影)

A medical imaging technique used in radiology to visualize detailed internal structure and limited function of the body. Its uses a powerful magnetic field to align the nuclear magnetization of (usually) hydrogen atoms in water in the body. Radio frequency (RF) fields are used to systematically alter the alignment of this magnetization.



This causes the hydrogen nuclei to produce a rotating magnetic field detectable by the scanner. This signal can be manipulated by additional magnetic fields to build up enough information to construct an image of the body

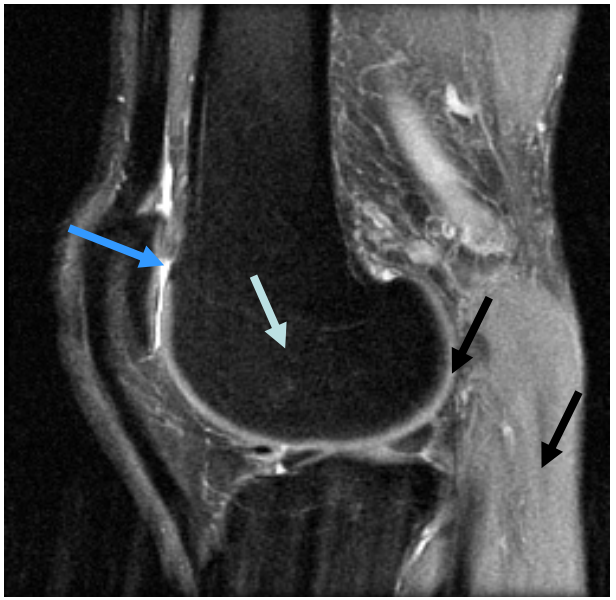
Magnetic Resonance Imaging

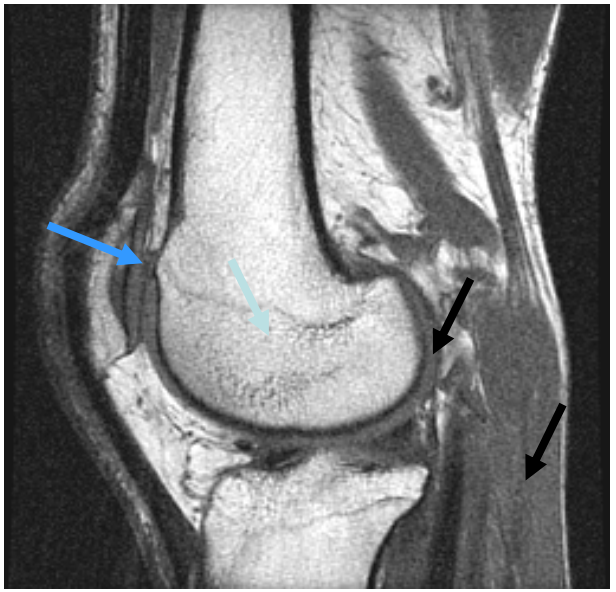
Advantages:

- Excellent / flexible contrast
- Non-invasive
- No ionizing radiation
- Arbitrary scan plane

Challenges:

- New contrast mechanisms
- Faster imaging



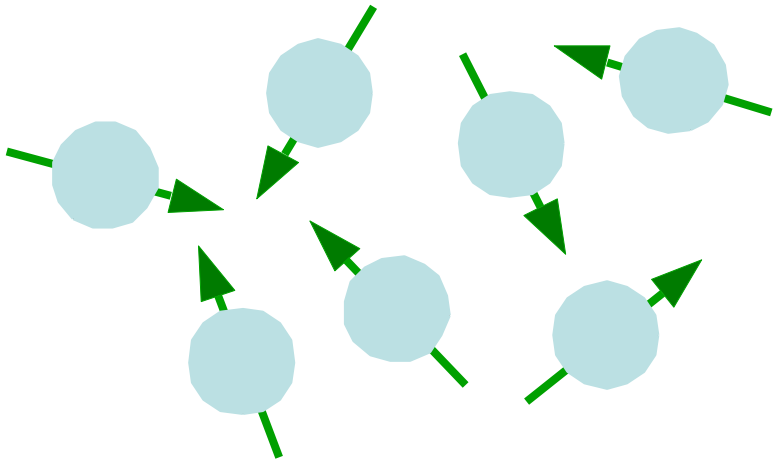


How to Produce MRI Contrast?

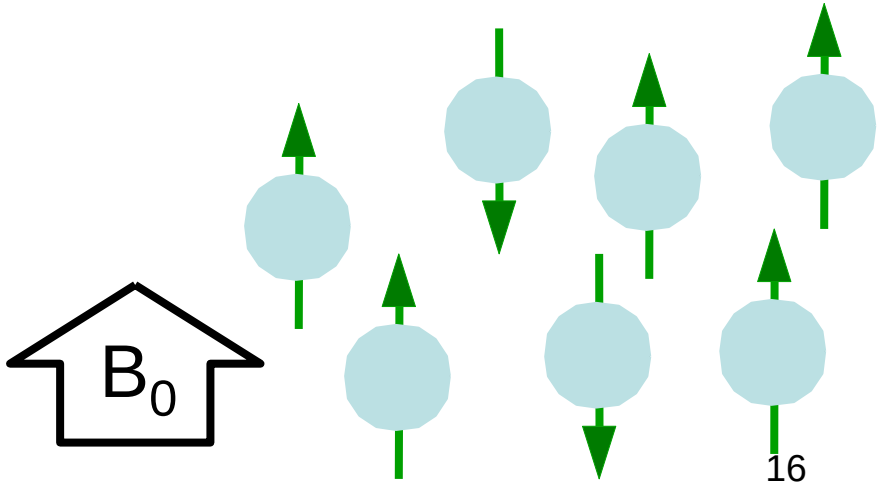
■ The body is largely composed of water molecules. Each water molecule has two hydrogen nuclei or protons. When a person goes inside the powerful magnetic field of the scanner, the magnetic moments of some of these protons align with the direction of the field.

■70% of body weight is water. An image can be constructed because the protons in different tissues return to their equilibrium state at different rates, which is a difference that can be detected. By changing the parameters on the scanner, this effect is used to create contrast between different types of body tissue

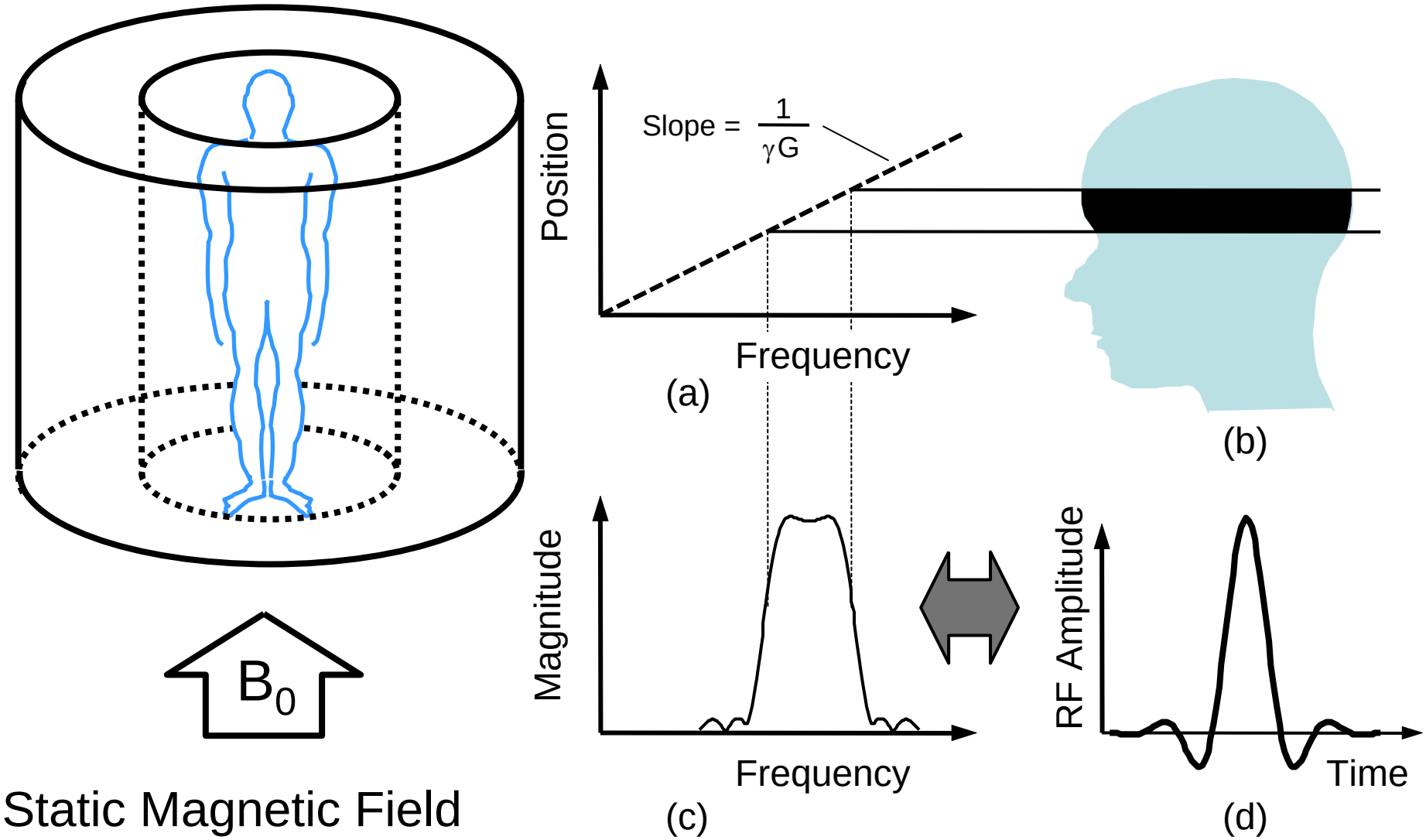
No Applied Field



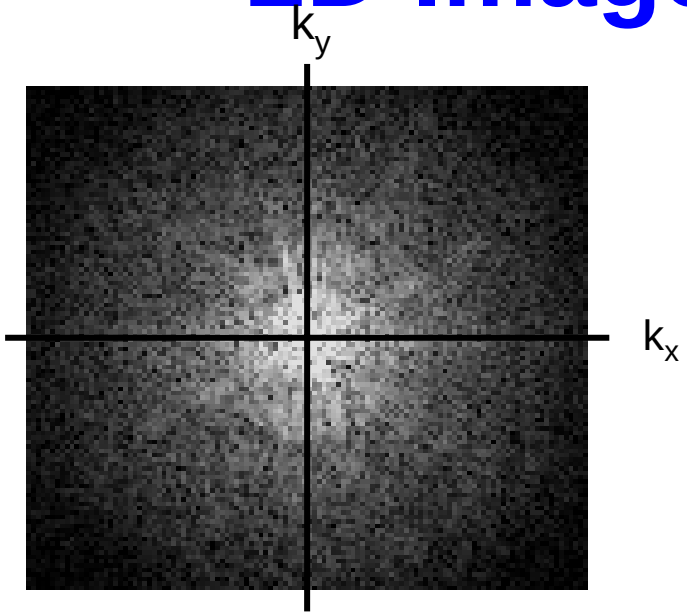
Applied Field



Selective Excitation



2D Image Reconstruction



Frequency-space
(k-space)

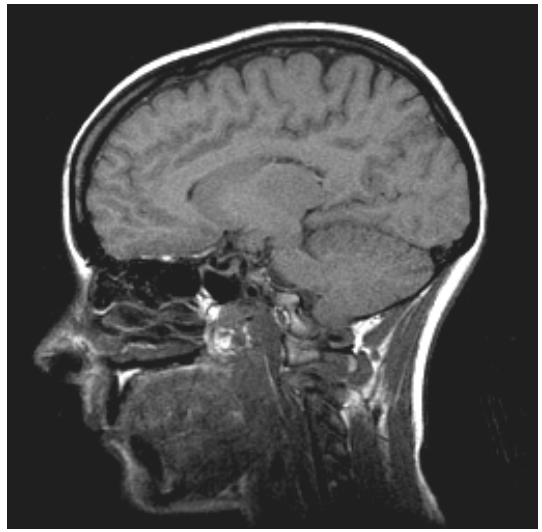
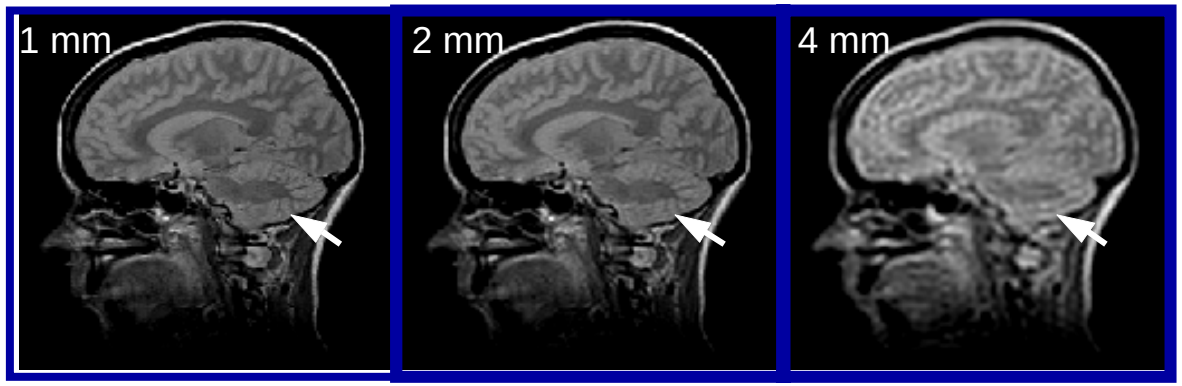


Image space

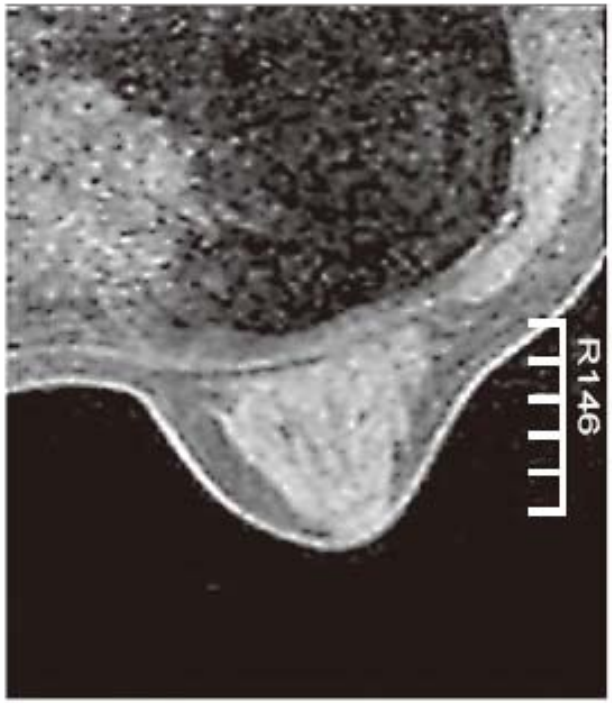


- Image resolution increases as higher spatial frequencies are acquired.

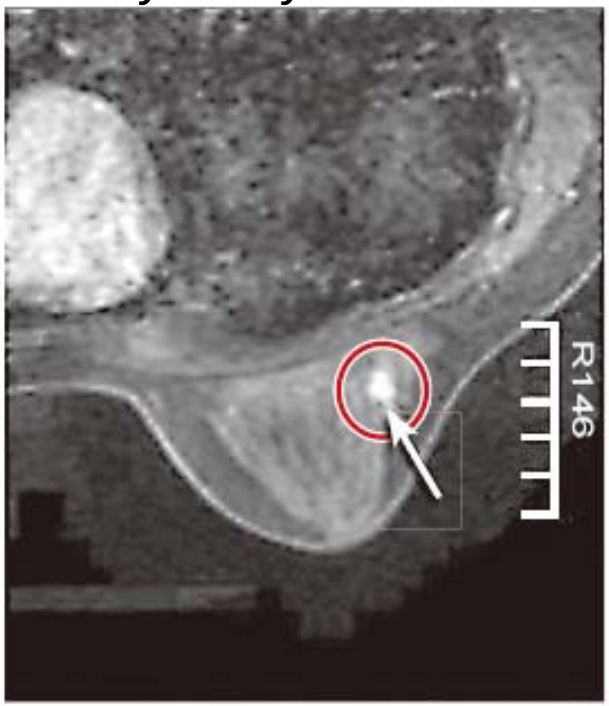
Contrast Reagent for Enhanced MRI

Contrast reagent: gadolinium (**Gd)** for use in magnetic resonance imaging as a MRI contrast agent. In the 3+ oxidation state the metal has 7 unpaired f electrons. This causes water around the contrast agent to relax quickly, enhancing the quality of the MRI scan

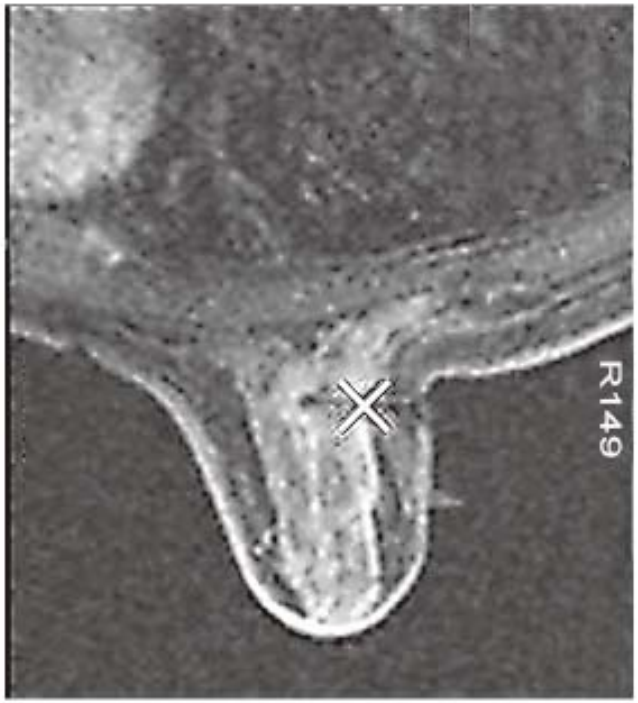
This case was not detected by X-ray



No contrast reagent



With contrast reagent (6 mm)



After operation

From 張允中

MRI Systems

At $2 million, the most expensive equipment in the hospital.

Discovery of New Cancer Markers (molecular diagnosis)

Disease Biomarker for Diagnostics

Serum Tumor Markers	Primary Clinical Applications	Other Related Cancer Type
Alpha-Fetoprotein (AFP)	Hepatocellular carcinoma (HCC) and germ-cell (nonseminoma) tumor monitoring and diagnosing	
CA 15-3	Breast cancer monitoring[1]	colorectal, liver, lung, ovarian, pancreatic cancer
CA 19-9	Colorectal and pancreatic cancer monitoring	breast, gastric, hepatobiliary, hepatocellular, and ovarian cancer
CA 125	Endometrial and ovarian cancer monitoring[2]	breast, cervical, colorectal, gastrointestinal, lung, pancreatic cancer
Prostate Specific Antigen (PSA)	Prostate cancer monitoring and diagnosing[3]	

History: Validation of cancer markers is 'disappointing' (not reproducible)

Example: CEA

- **Initial report** (PNAS): ~100% sensitivity, specificity for Colorectal Cancer (CRC, 大腸直腸癌)

- **High expectations**

- Disappointment when expensive ACS/CCS study did not reproduce initial results

Disappointment would have been predicted and avoided if 'rules of evidence' were available.

Example: Alpha-FetoProtein (AFP)

Used for Surveillance, Diagnosis, Prognosis,

This gene encodes **alpha-fetoprotein**, a major plasma protein produced by the yolk sac and the liver during fetal life. In humans, AFP levels decrease gradually after birth, reaching adult levels by 8 to 12 months. It is serving as a biomarker to detect a subset of tumors, principally **hepatocellular carcinoma** (HCC, 肝癌) and endodermal sinus tumors 睾丸內胚竇瘤 .

Normal level: 10 ng/ml

Hepatocellular Carcinoma (HCC) : > 500 ng/ml

The sensitivity of AFP for HCC is about 60%. In other words, an elevated AFP blood test is seen in about 60% of HCC patients. That leaves 40% of patients with HCC who have normal AFP levels. Therefore, a normal AFP does not exclude HCC

Present: Cancer Markers are Promising

Knowledge of molecular biology provides targets to measure

- **Past**: knew little about what to target
- **Now**: know DNA 'path' from normal.. adenoma..

Assays to measure targets

- **Past**: 'one dimensional' assays, like CEA, FOBT, PSA
- **Now**: multi-dimensional assays (measure almost any target)
- **DNA** - primers and probes; amplify signal
- **Protein** - mass spectroscopy

The New England
Journal of Medicine

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A GENE-EXPRESSION SIGNATURE AS A PREDICTOR OF SURVIVAL IN BREAST CANCER

MARC J. VAN DE VIJVER, M.D., PH.D., YUDONG D. HE, PH.D., LAURA J. VAN 'T VEER, PH.D., HONGYUE DAI, PH.D., AUGUSTINUS A.M. HART, M.SC., DORIEN W. VOSKUIL, PH.D., GEORGE J. SCHREIBER, M.SC., JOHANNES L. PETERSE, M.D., CHRIS ROBERTS, PH.D., MATTHEW J. MARTON, PH.D., MARK PARRISH, DOUWE ATSMA, ANKE WITTEVEEN, ANNUSKA GLAS, PH.D., LEONIE DELAHAYE, TONY VAN DER VELDE, HARRY BARTELINK, M.D., PH.D., SJOERD RODENHUIS, M.D., PH.D., EMIEL T. RUTGERS, M.D., PH.D., STEPHEN H. FRIEND, M.D., PH.D., AND RENÉ BERNARDS, PH.D.

ABSTRACT

Background A more accurate means of prognostication in breast cancer will improve the selection of patients for adjuvant systemic therapy.

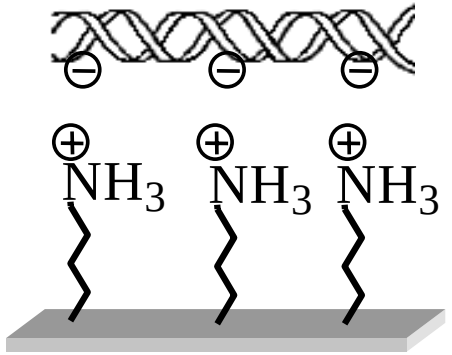
Methods Using microarray analysis to evaluate our previously established 70-gene prognosis profile, we classified a series of 295 consecutive patients with primary breast carcinomas as having a gene-expression signature associated with either a poor prognosis or a good prognosis. All patients had stage I or II breast cancer and were younger than 53 years old; 151 had lymph-node–negative disease, and 144 had lymph-node–positive disease. We evaluated the predictive power of the prognosis profile using univariable and multivariable statistical analyses.

ADJUVANT systemic therapy substantially improves disease-free and overall survival in both premenopausal and postmenopausal women up to the age of 70 years with lymph-node–negative or lymph-node–positive breast cancer.[1,2] It is generally agreed that patients with poor prognostic features benefit the most from adjuvant therapy.[3,4] The main prognostic factors in breast cancer are age, tumor size, status of axillary lymph nodes, histologic type of the tumor, pathological grade, and hormone-receptor status. A large number of other factors have been investigated for their potential to predict the outcome of disease, but in general, they have only limited predictive power.[5]

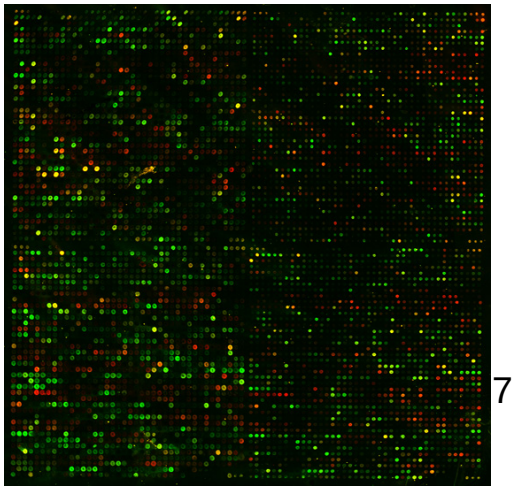
What are Microarray Gene Chips

- miniaturized array having up to tens of thousands of single-stranded DNA attached to it

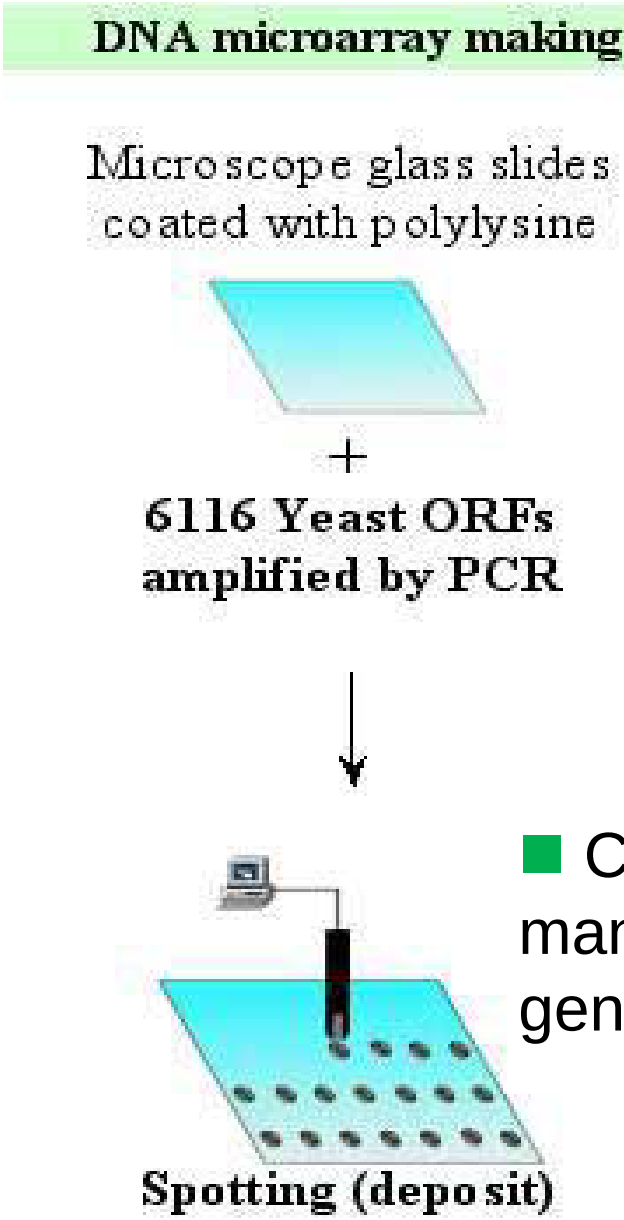
- Microarray assays are based on hybridization of a single-stranded DNA labeled with a fluorescent tag to a complementary molecule attached to the chip



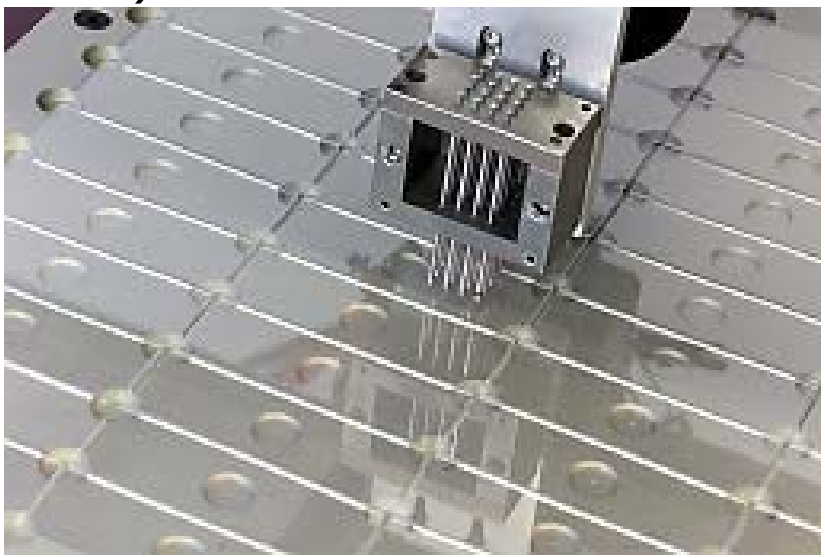
- When each spot in a microarray is attached a unique DNA molecule, it can be used to detect presence/absence or even concentration of a DNA molecule in test tube

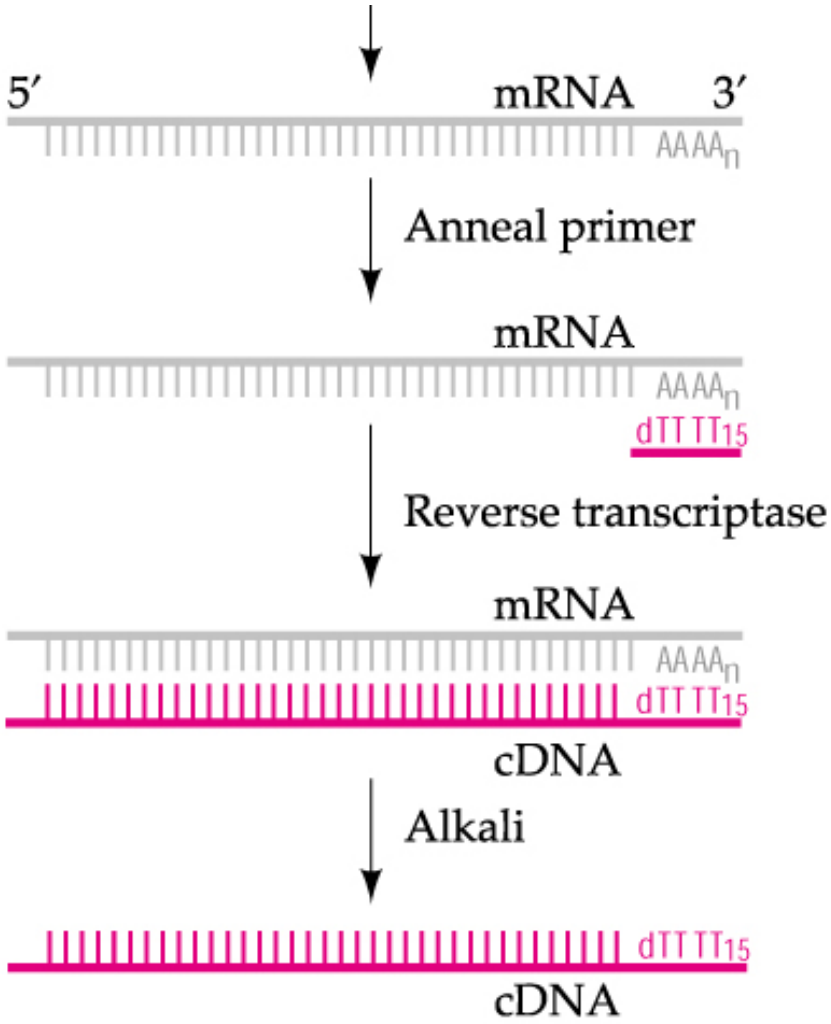


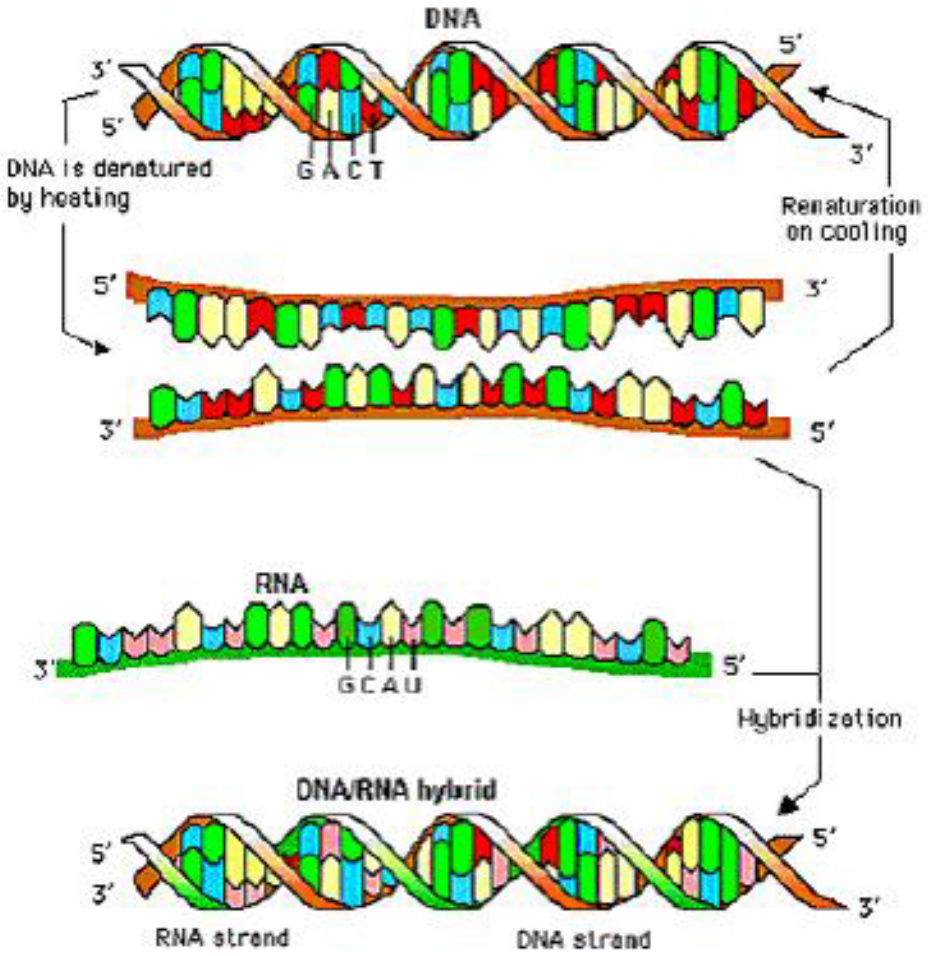
Preparation of Microarray Chip



- Put a large number (~100K) of cDNA sequences or synthetic DNA oligomers onto a glass slide (or other substrate) in known locations on a grid.
- Spot cloned cDNAs onto a glass microscope slide (Ordinary glass microscope slide)
- Can have as many as 40,000 genes on a chip



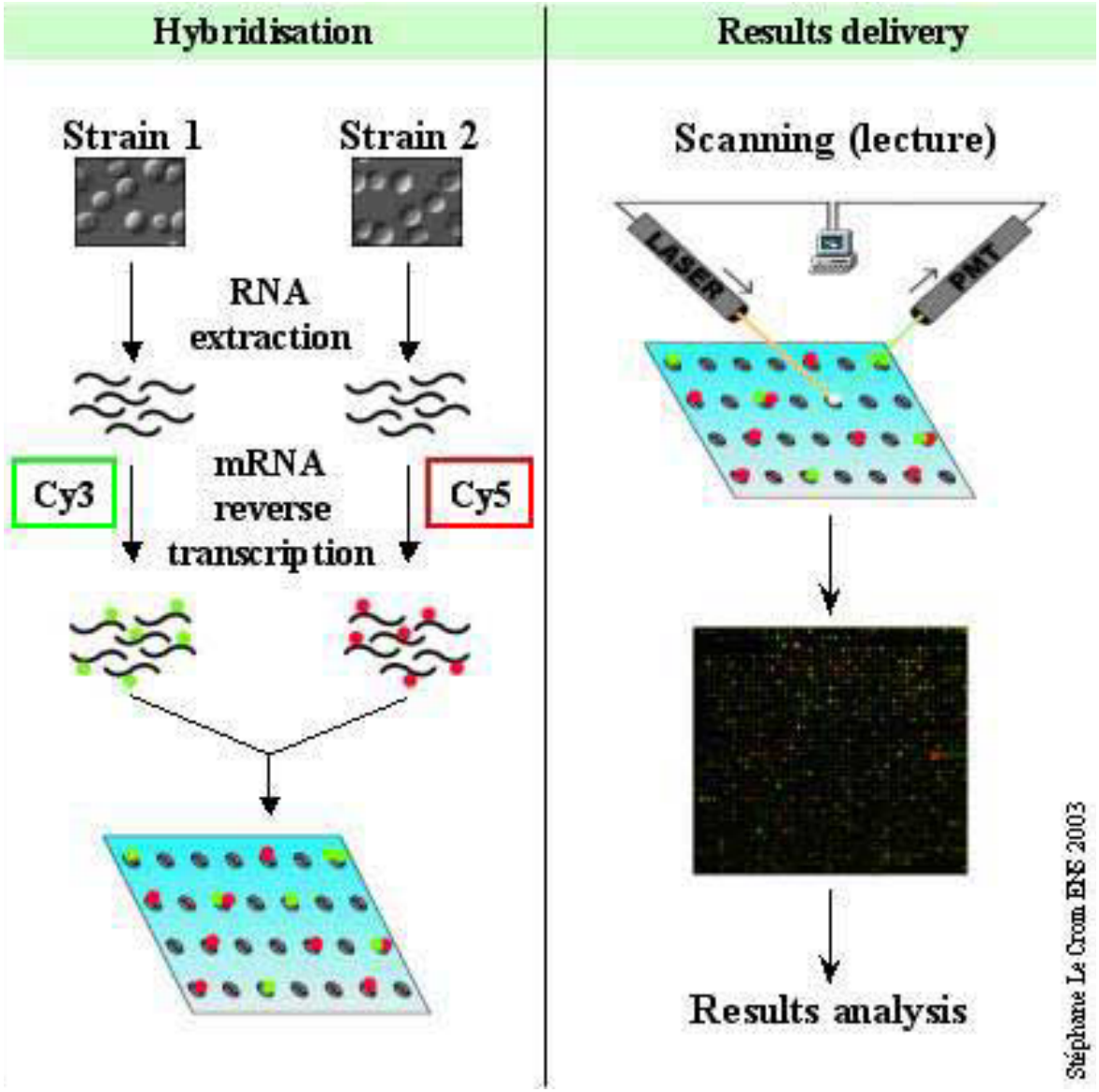




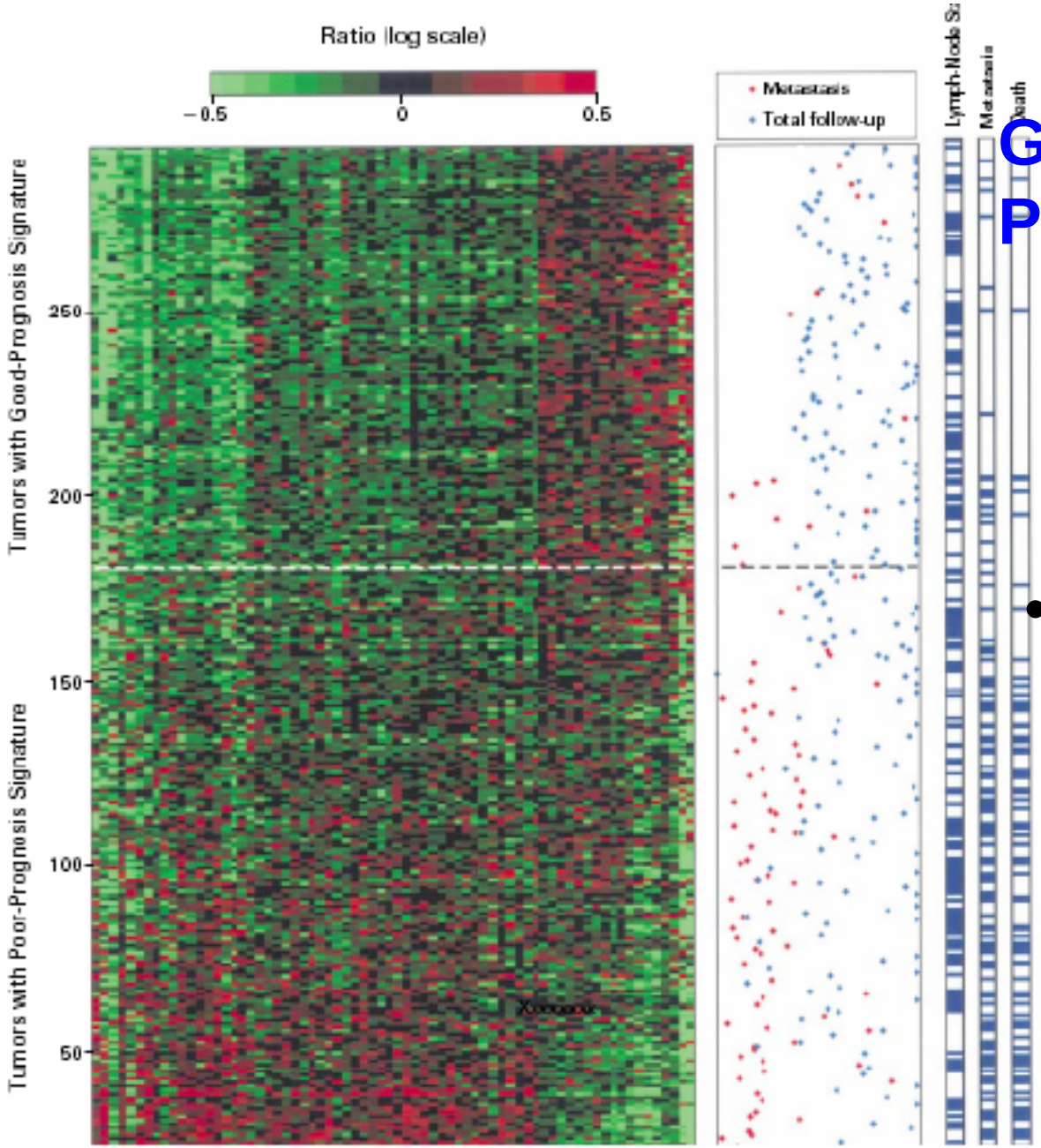
Hybridization

–Put (liquid) sample containing genes on microarray and allow probe and gene sequences to hybridize and wash away the rest

Label an RNA sample and hybridize

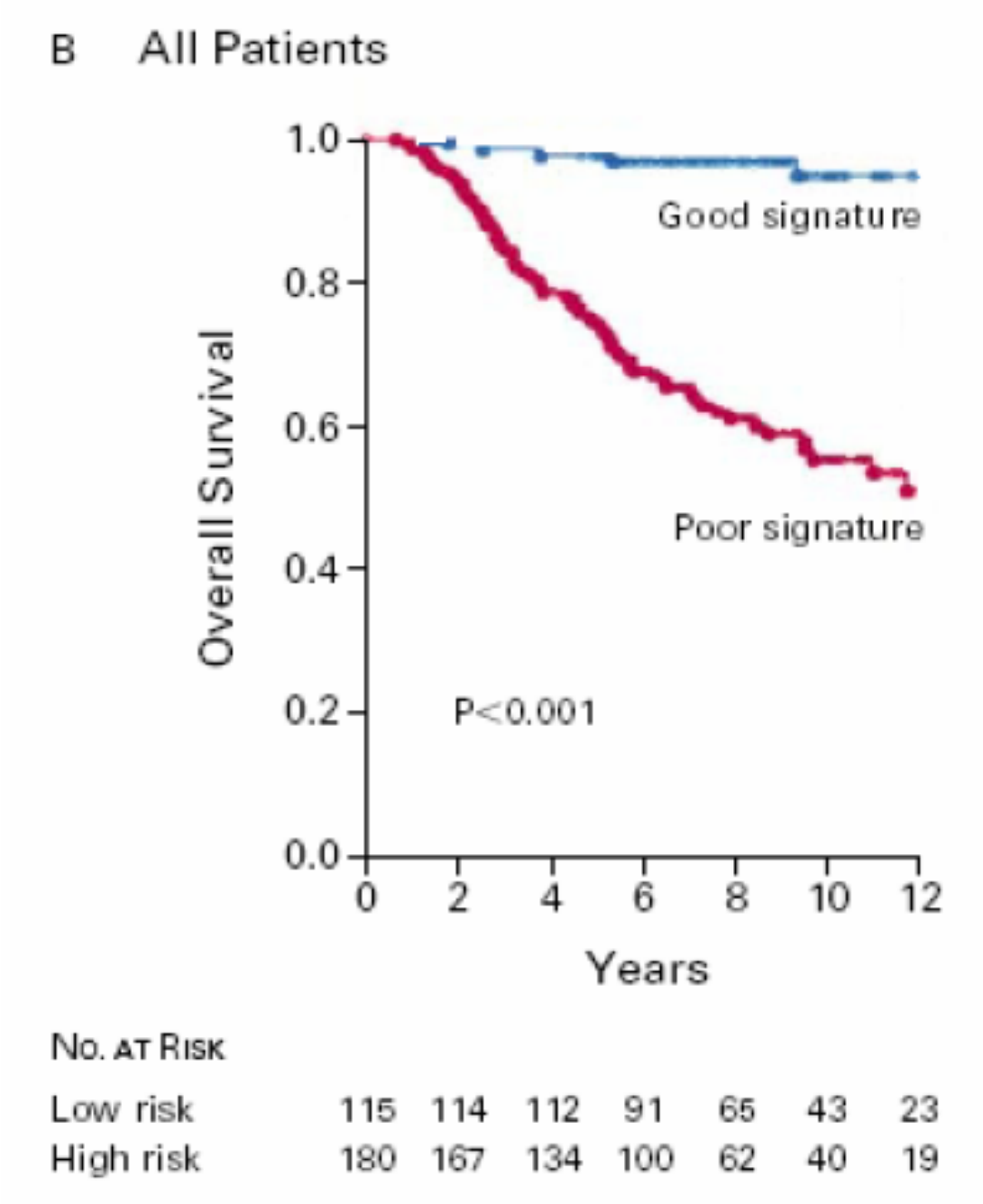


- By detecting the quantity of fluorescent molecules attached to each spot, one can infer the relative abundance of the complementary mRNA molecules in solution



Gene Expression Pattern by Microarray

- Make comparisons
  - Cancerous vs. norma tissue
  - Treated vs. untreated
  - Time course


B All Patients
1.0
0.8
0.6
0.4
0.2
0.0
Overall Survival
Good signature
Poor signature
P<0.001
0
2
4
6
8
10
12
Years
No. at Risk
Low risk 115 114 112 91 65 43 23
High risk 180 167 134 100 62 40 19

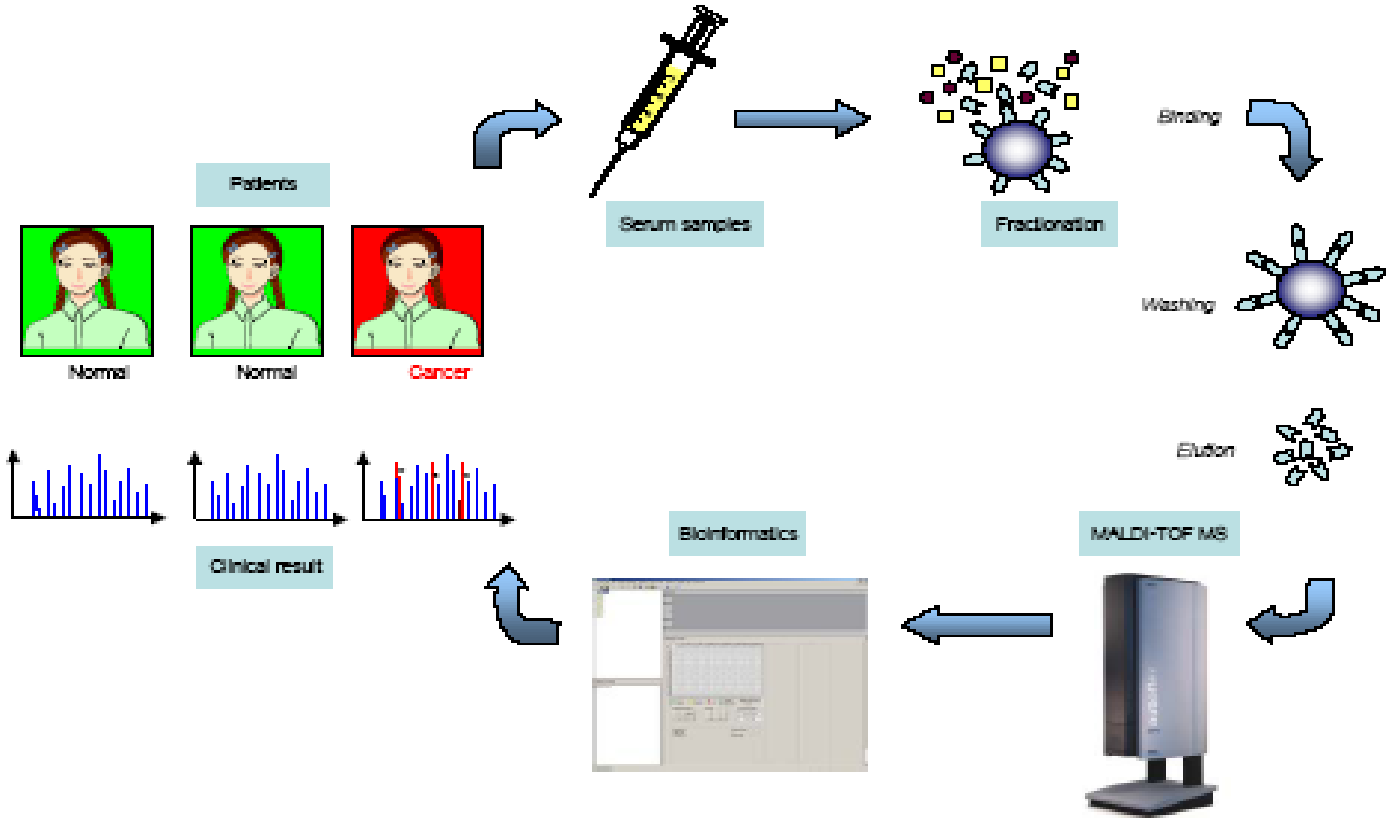
Original Cancer Proteomics Profiling Paper

Use of proteomic patterns in serum to identify ovarian cancer

Emanuel F Petricoin III, Ali M Ardekani, Ben A Hitt, Peter J Levine, Vincent A Fusaro, Seth M Steinberg, Gordon B Mills, Charles Simone, David A Fishman, Elise C Kohn, Lance A Liotta

Background New technologies for the detection of early-stage ovarian cancer are urgently needed. Pathological changes within an organ might be reflected in proteomic patterns in serum. We developed a bioinformatics tool and used it to identify proteomic patterns in serum that distinguish neoplastic from non-neoplastic disease within the ovary.

Mass Spectrometry-based Serum Profiling



Serum samples from both normal and diseased subjects are fractionated on functionalized surfaces according to their chemical, physical or biological properties. Serum protein profiles are generated using a mass spectrometer. Patterns of up- and downregulated proteins that are suitable for use as biomarkers to separate disease from normal samples are discovered by combining appropriate bioinformatics software with an independently determined clinical diagnosis. The resulting biomarker pattern can then be used for the class prediction of unknown samples or for the identification of new individual biomarkers.
MALDI-TOF: Matrix assisted laser desorption/ionization time-of-flight.

Pharmacoproteomics, 4, 463, 2003

SELDI-TOF MS

(Surface Enhanced Laser Desorption/Ionization Time of Flight Mass Spectrometry)

Combination of

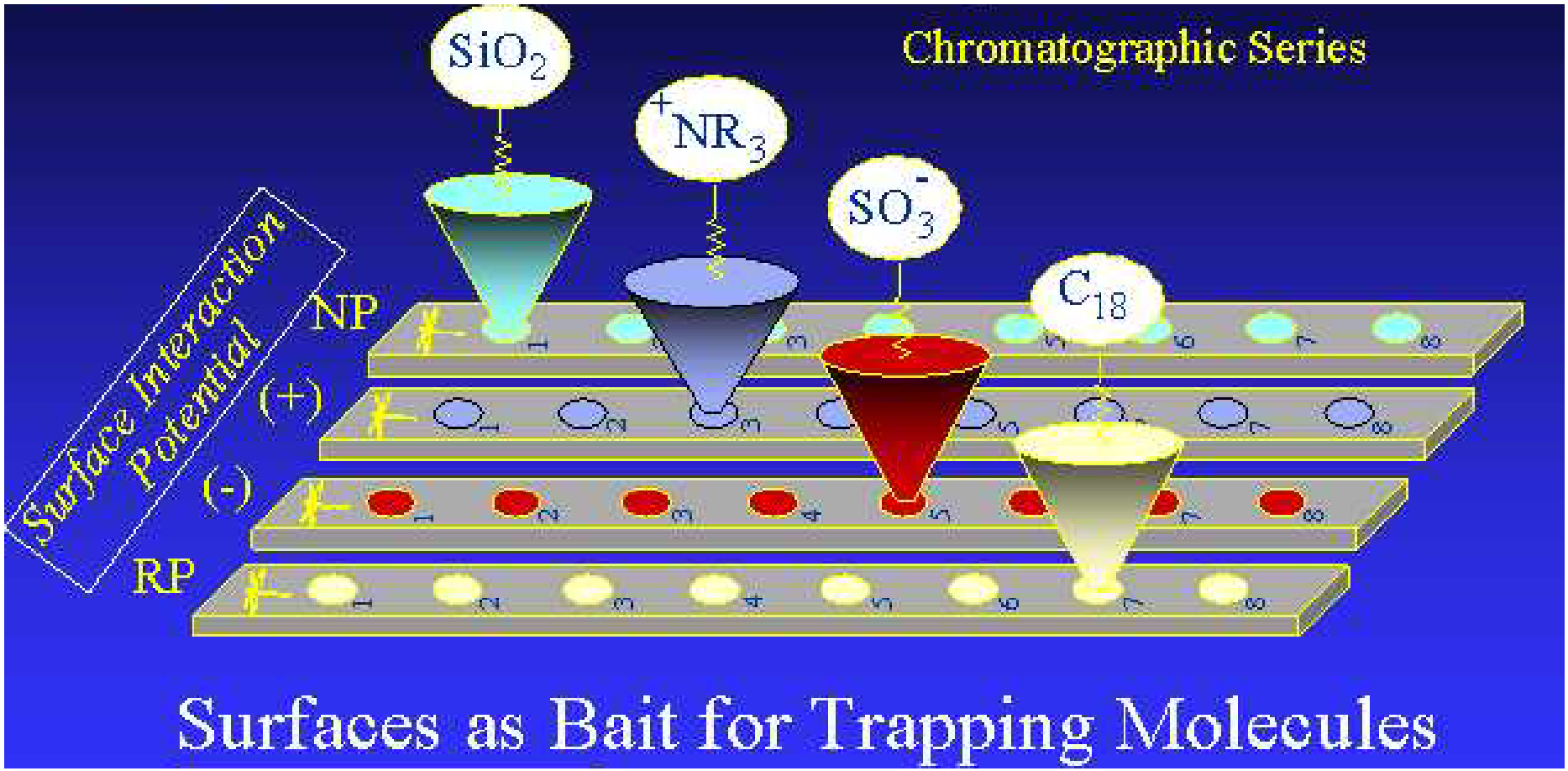
Protein Chip + MALDI-TOF MS

- SELDI-TOF is a means of analyzing mixed samples of proteins by selective retention
- **Retention** is based on the Chemical and Biochemical interactions (hydrophobic, hydrophilic, cationic, anionic, or metal ion affinity, DNA, enzyme, immobilized antibody, specific receptor,and more)

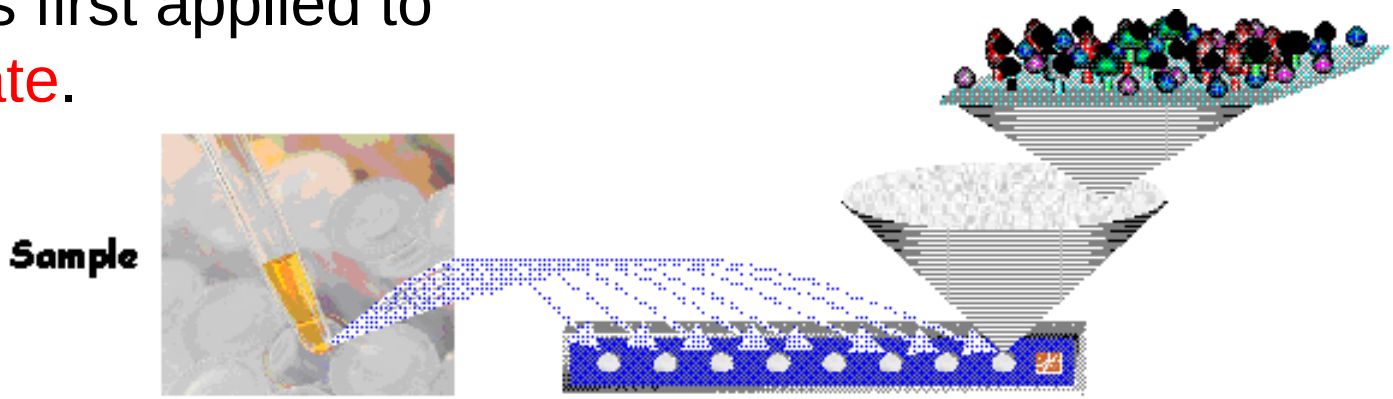
SELDI Plates

Plates with specific ligands are used to retain proteins of interest in a sample.

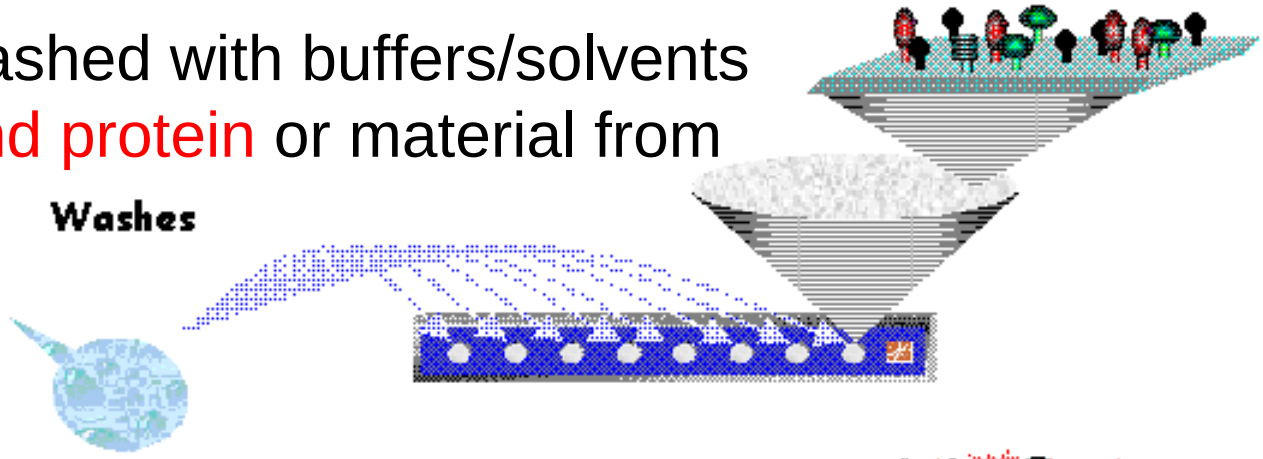
Different chromatographic retention



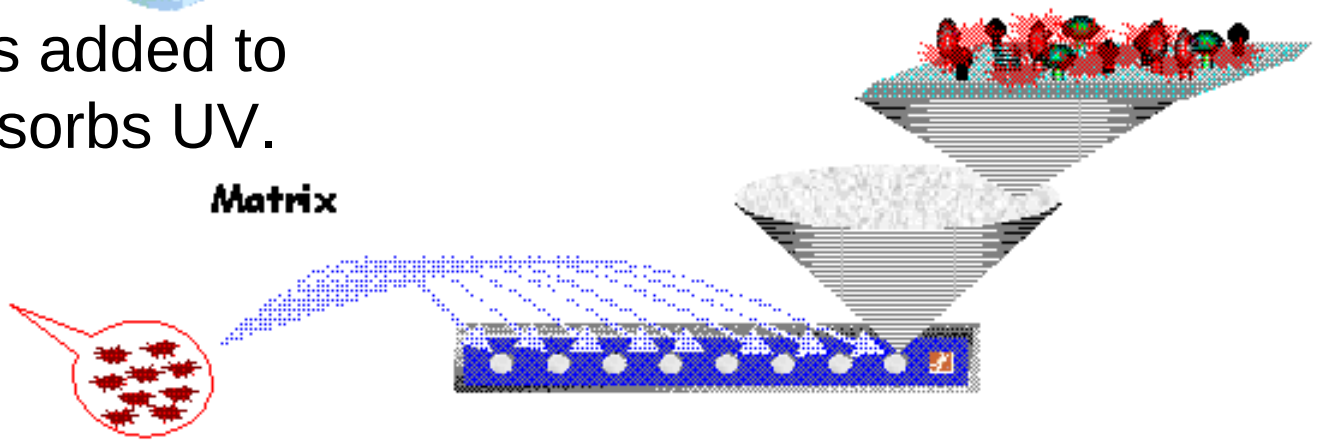
1. The sample is first applied to the retention plate.



2. The plate is then washed with buffers/solvents to remove any unbound protein or material from surface



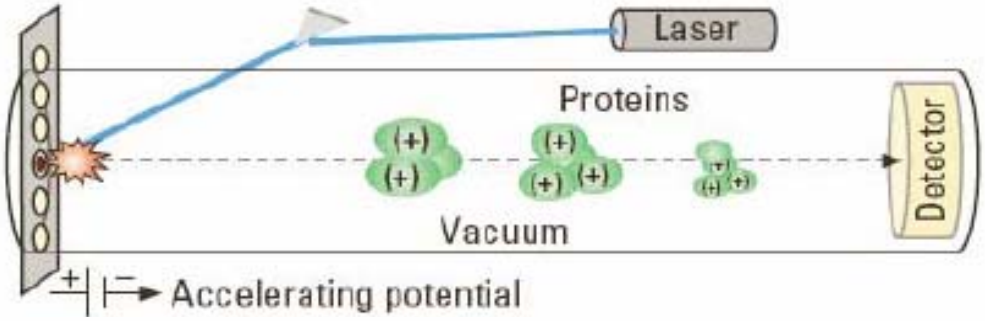
3. A matrix solution is added to the sample which absorbs UV.

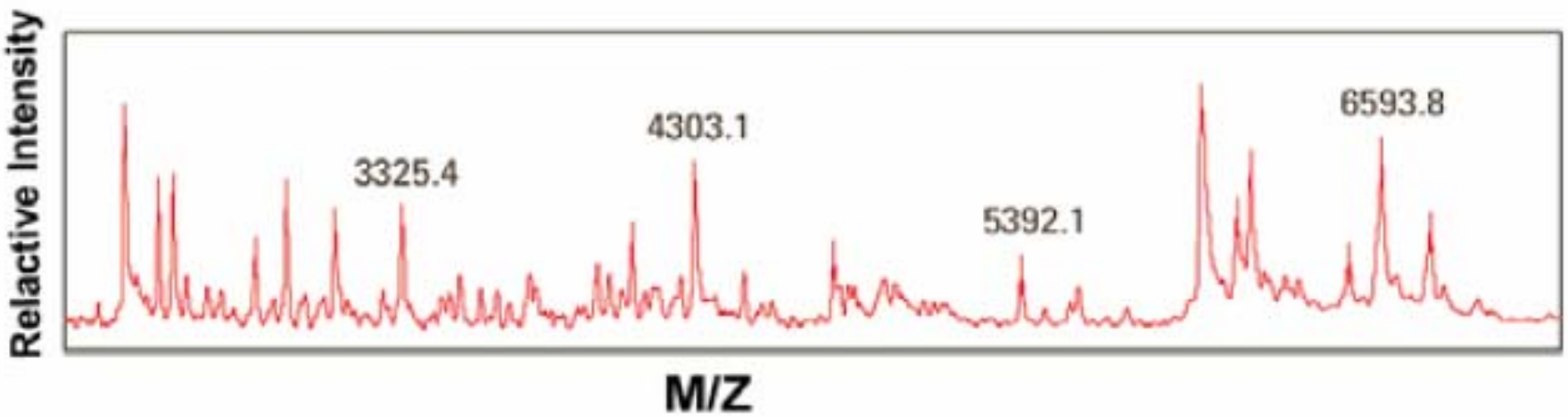


MALDI-TOF MS Detection

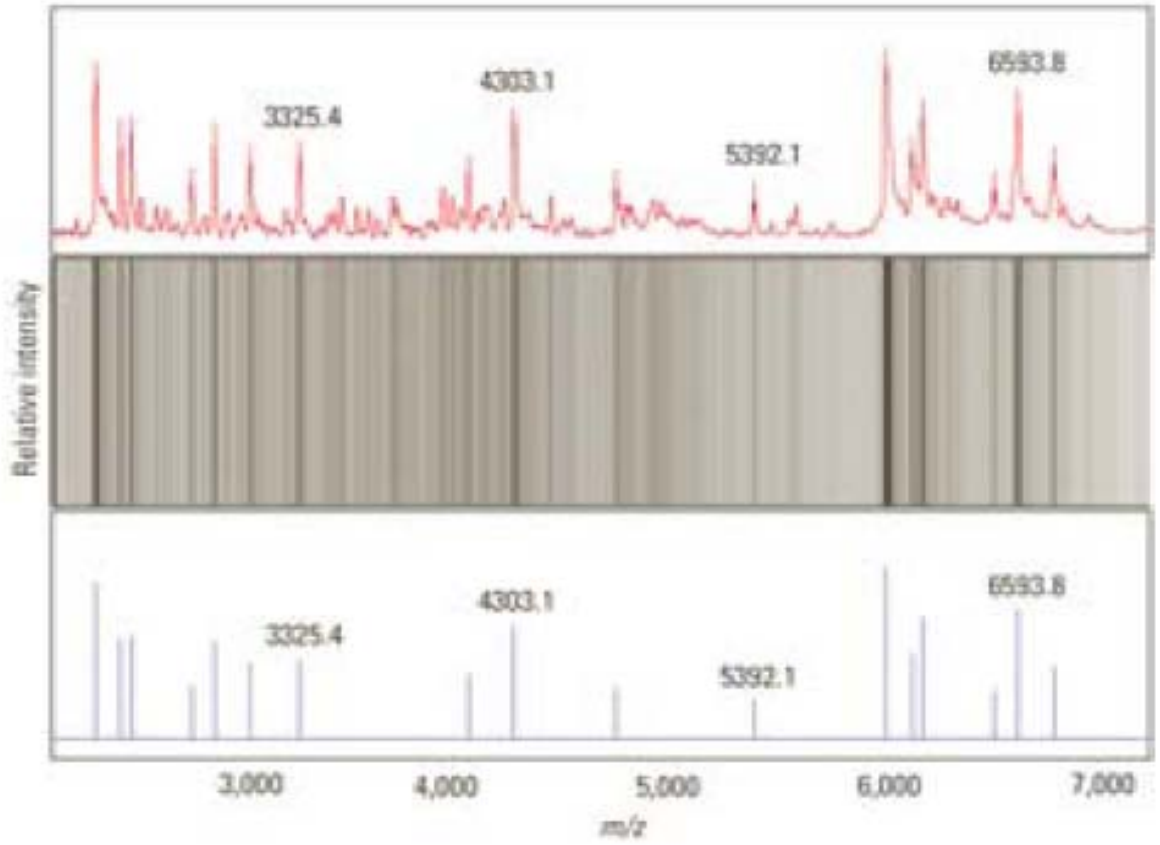
4. The Matrix is then hit with a pulsed, UV, nitrogen laser.

5. Ionized sample travels through TOF-MS for analysis. The readout shows all the proteins which were retained by the plate.





Data from TOF-MS can be looked at in three different ways.

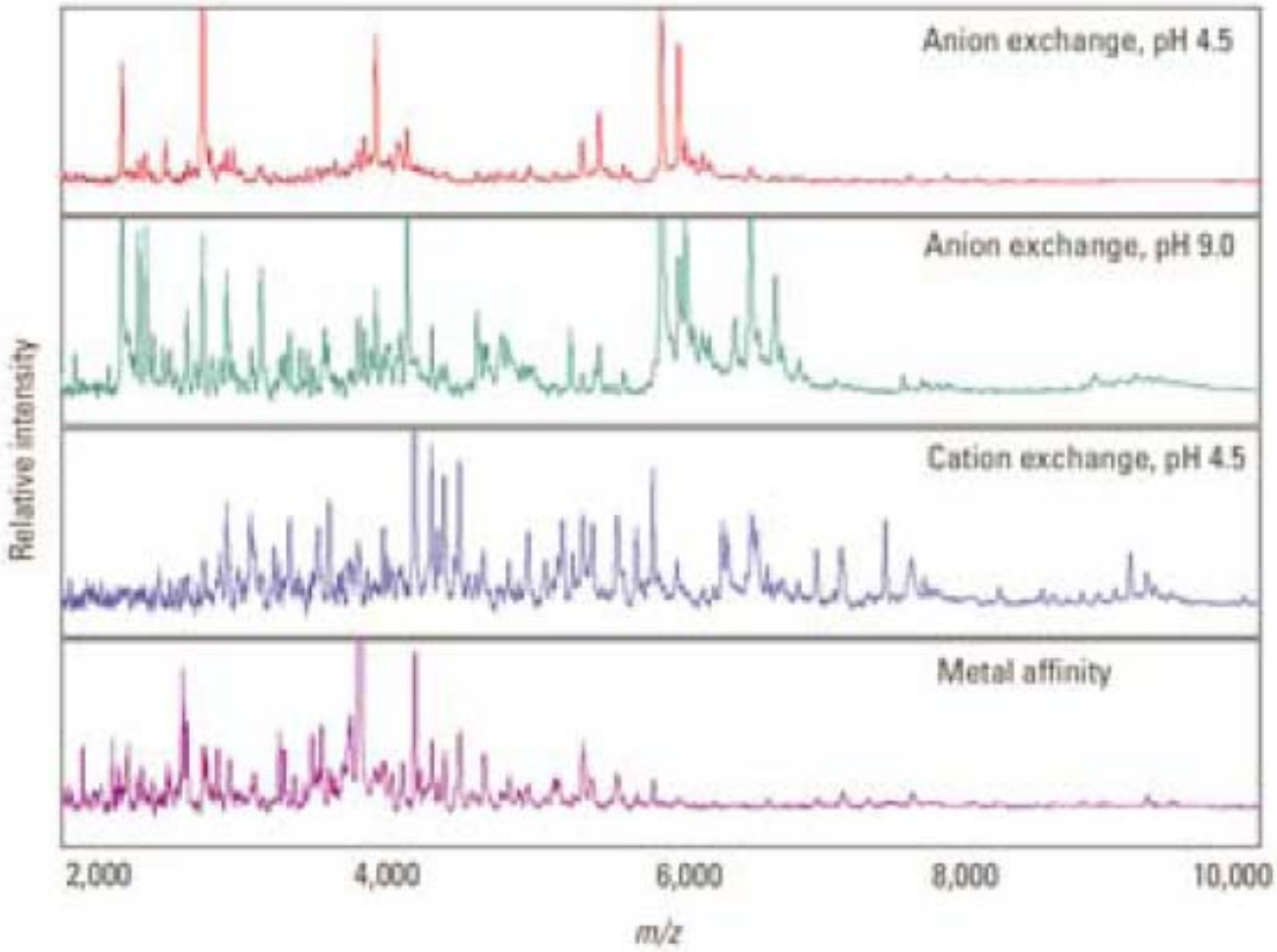


Trace View

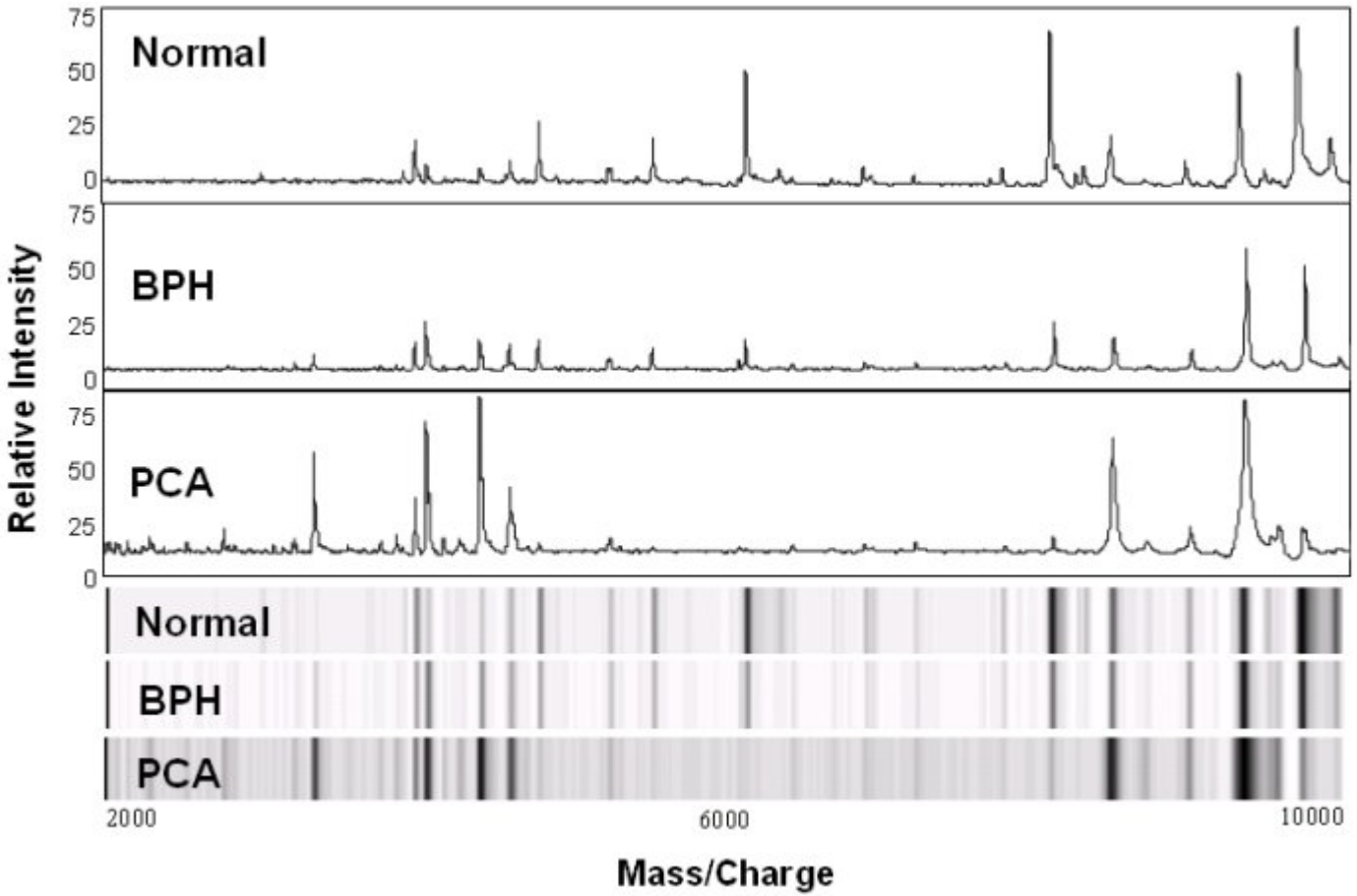
Gel View

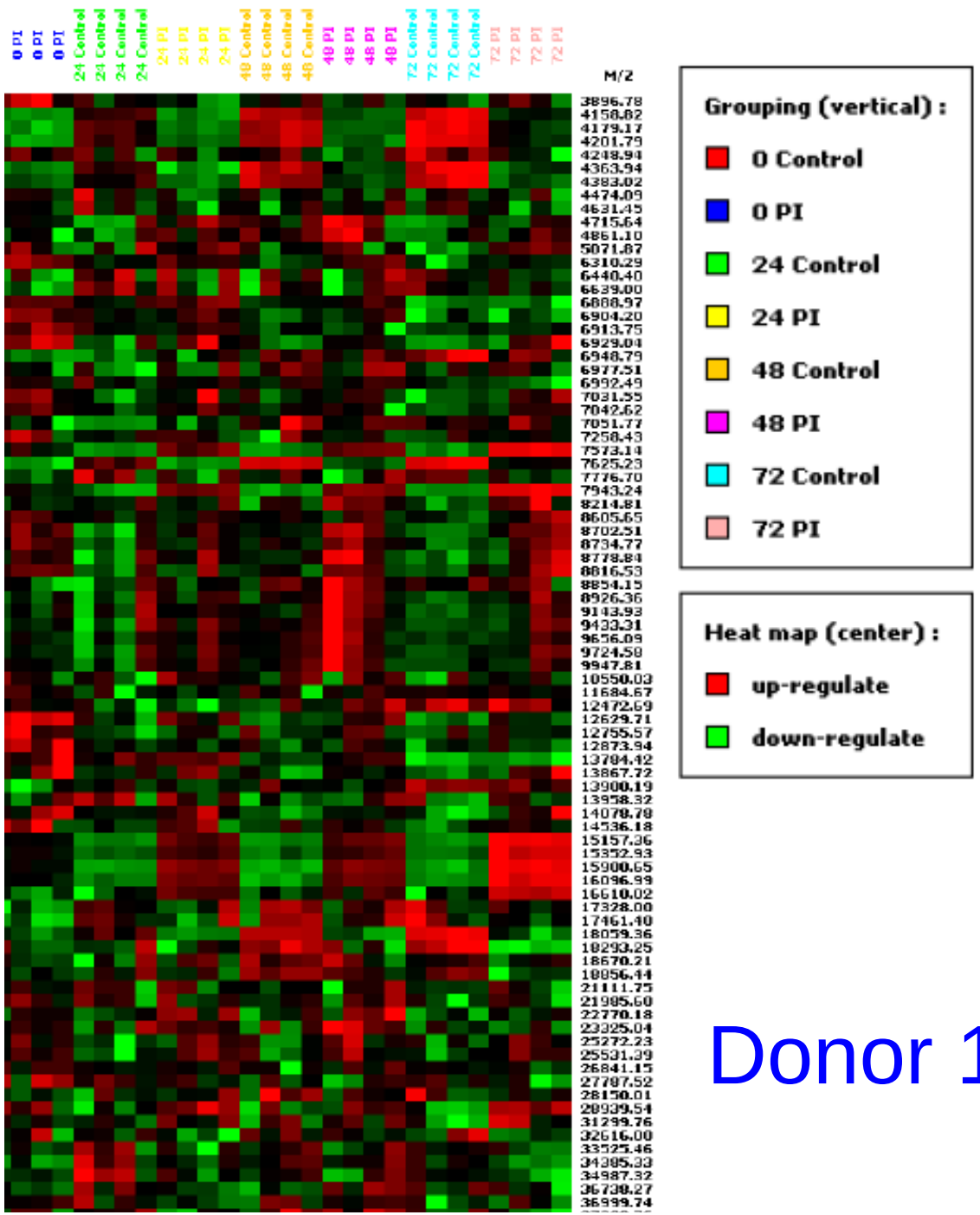
Map View

Plate Conditions Can be Modified to Change Which Proteins are Retained

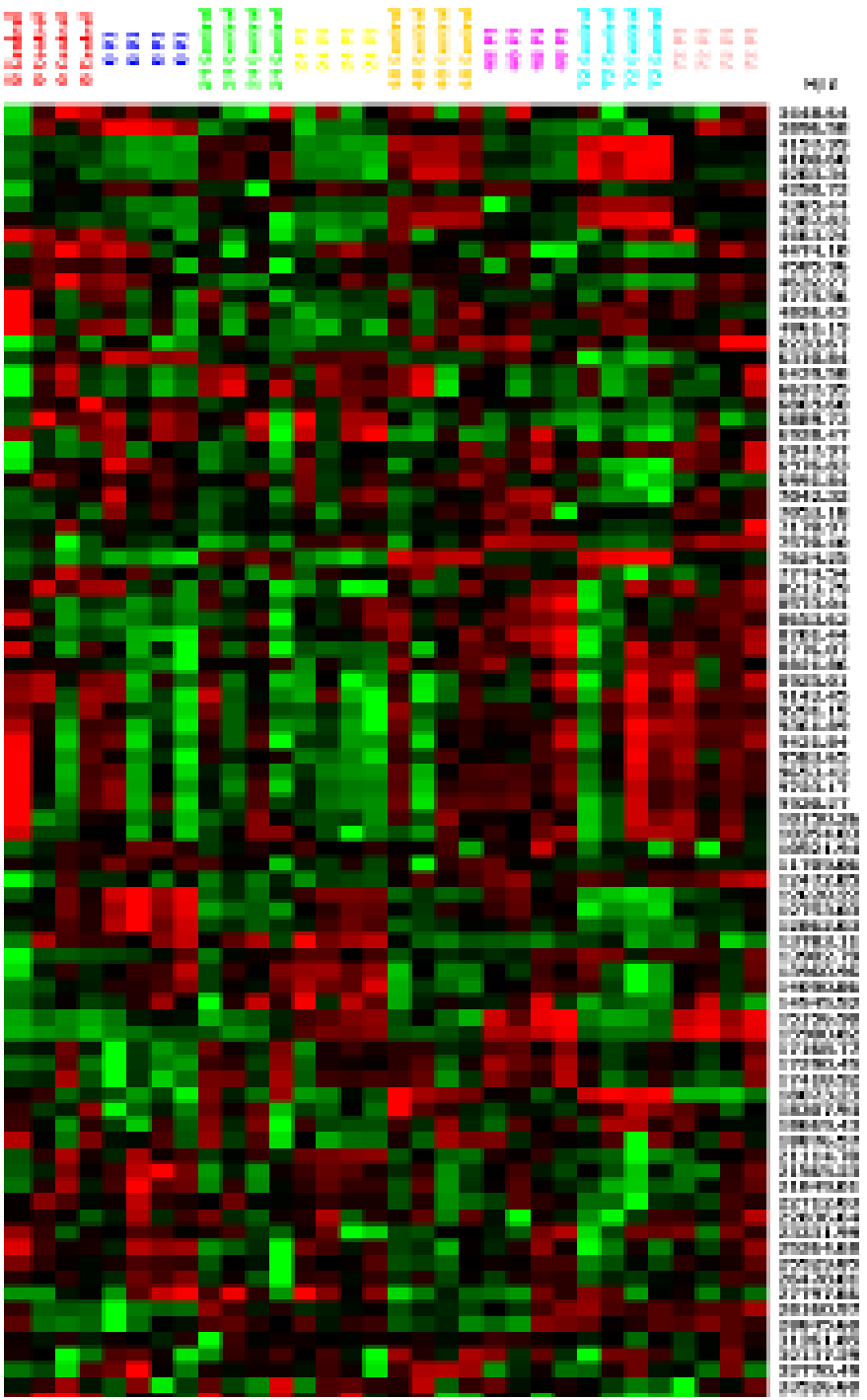


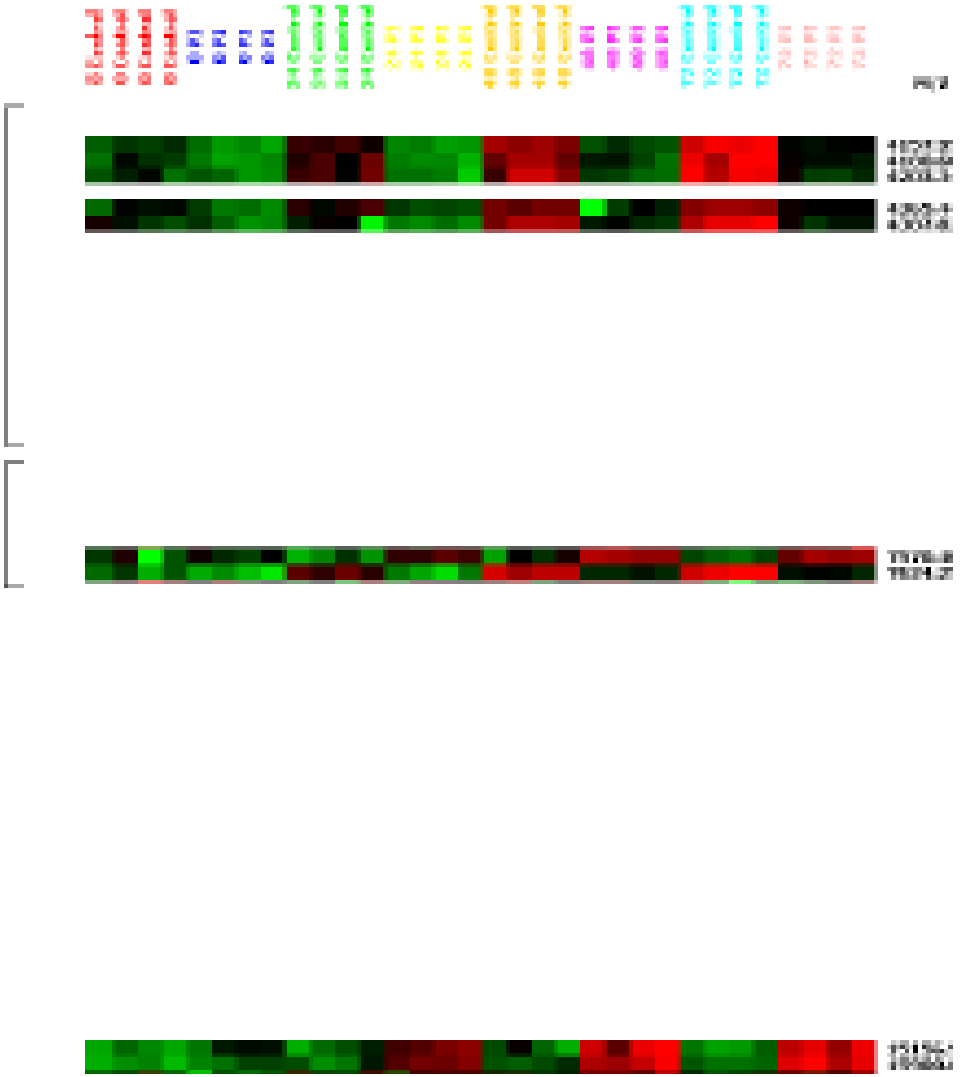
Identification of Cancer Biomarker (Cancer proteomic pattern)





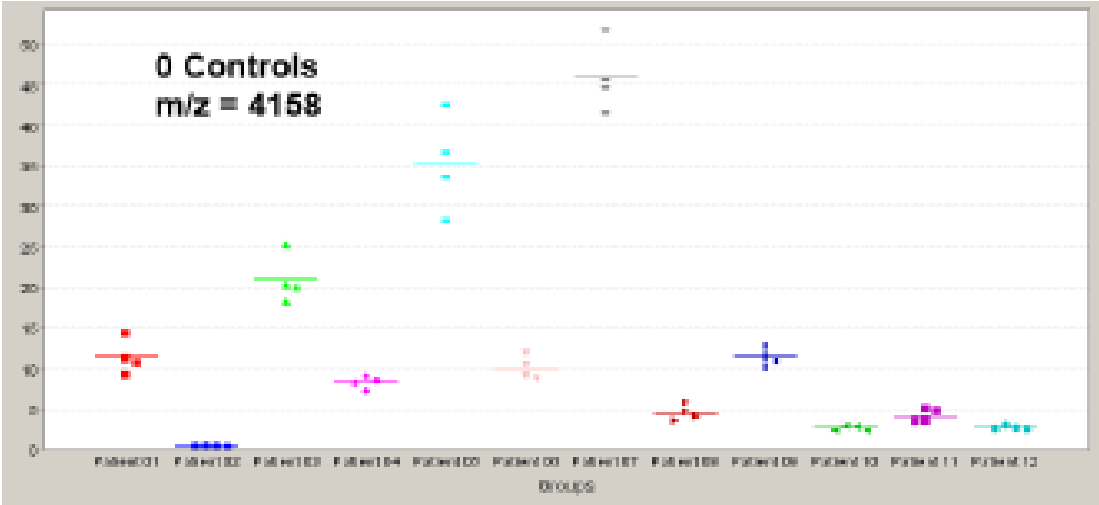
Donor 1: Heat Map

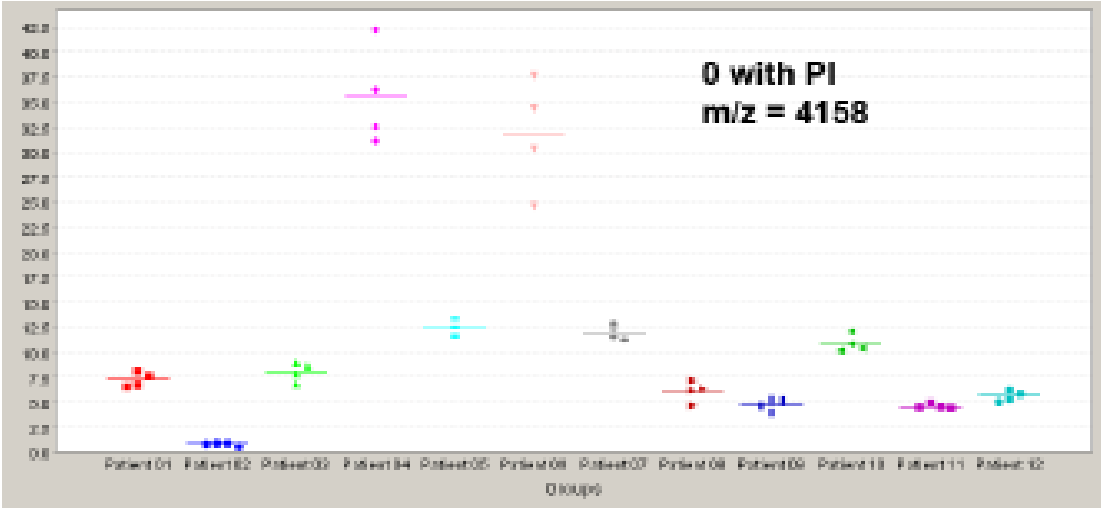




Donor 3: Heat Map

Peak 4158





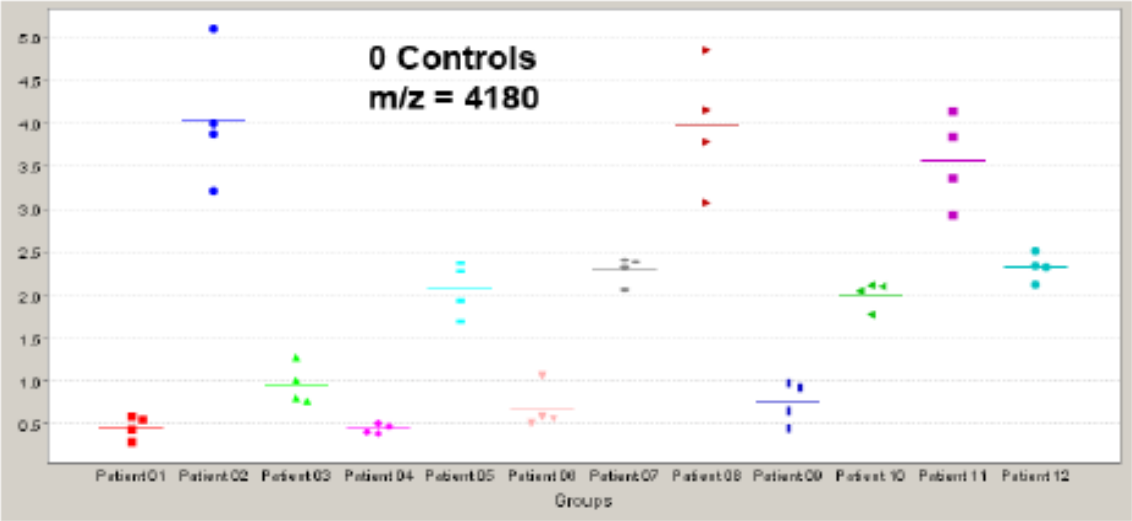
Grouping (vertical) :

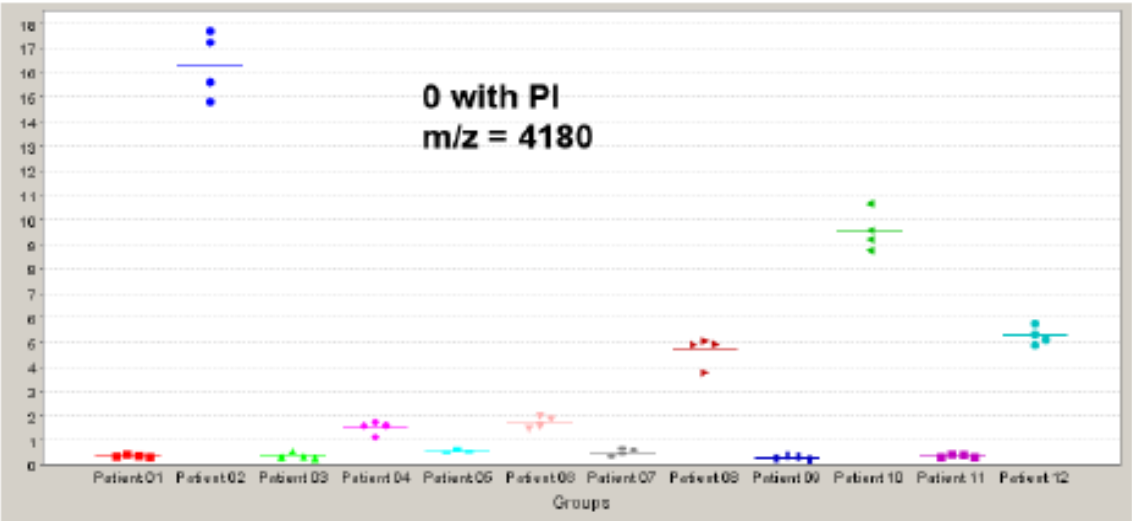
- 0 Control
- 0 PI
- 24 Control
- 24 PI
- 48 Control
- 48 PI
- 72 Control
- 72 PI

Heat map (center) :

- up-regulate
- down-regulate

Peak 4180





Grouping (vertical) :

- 0 Control
- 0 PI
- 24 Control
- 24 PI
- 48 Control
- 48 PI
- 72 Control
- 72 PI

Heat map (center) :

- up-regulate
- down-regulate

Claims about serum proteomics to detect cancer are extraordinary

Purpose: to diagnose ovarian cancer vs no cancer

Methods:

- ovarian cancer, controls
- serum assessed by mass spectroscopy (SELDI-TOF)
- spectra analyzed by '**genetic algorithm**' (Correlogic)

Results: '**patterns**' discriminate

- claims for multiple cancers (ovary, prostate, breast)
- -sensitivity: 95-100%
- -specificity: 95-100%

Petricoin, Lancet 2.02

Proteomics

Petricoin, Lancet 2.02

"The discriminatory pattern correctly identified all 50 ovarian cancer cases in the masked set... **This result yielded a sensitivity of 100%… specificity of 95%…"**

New York Times, 2.3.04

New Cancer Test Stirs Hope and Concern

By ANDREW POLLACK

Jill Doimer's mother died in 2002 from ovarian cancer, detected too late to be effectively treated.

So Ms. Doimer is eagerly awaiting the introduction of a new test that holds the promise of detecting early-stage ovarian cancer far more accurately than any test available now, using only blood from a finger prick.

Not only does she plan to be tested, but an advocacy group she helped found, Ovarian Awareness of Kentucky, also intends to spread the word to women and doctors.

"If it's going to happen to me or anyone I know, I want it to be caught at an early stage," said Ms. Doimer, who lives in Louisville.

The new test, expected to be available in the next few months, could have a big effect on public health if it works as advertised. That is because when ovarian cancer is caught early, when it is treatable by surgery, more than 90 percent of women live five years or longer. But right now, about three-quarters of cases are detected after the cancer has advanced, and then only 35 percent of women survive five years.

The test is also the first to use a new technology that some believers say could revolutionize diagnostics. It looks not for a single telltale protein — like the prostate-specific antigen, or P.S.A., used to diagnose prostate cancer — but rather for a complex fingerprint formed by all the proteins in the blood. Similar tests are being developed for prostate, pancreatic, breast and other cancers. The technique may work for other diseases as well.

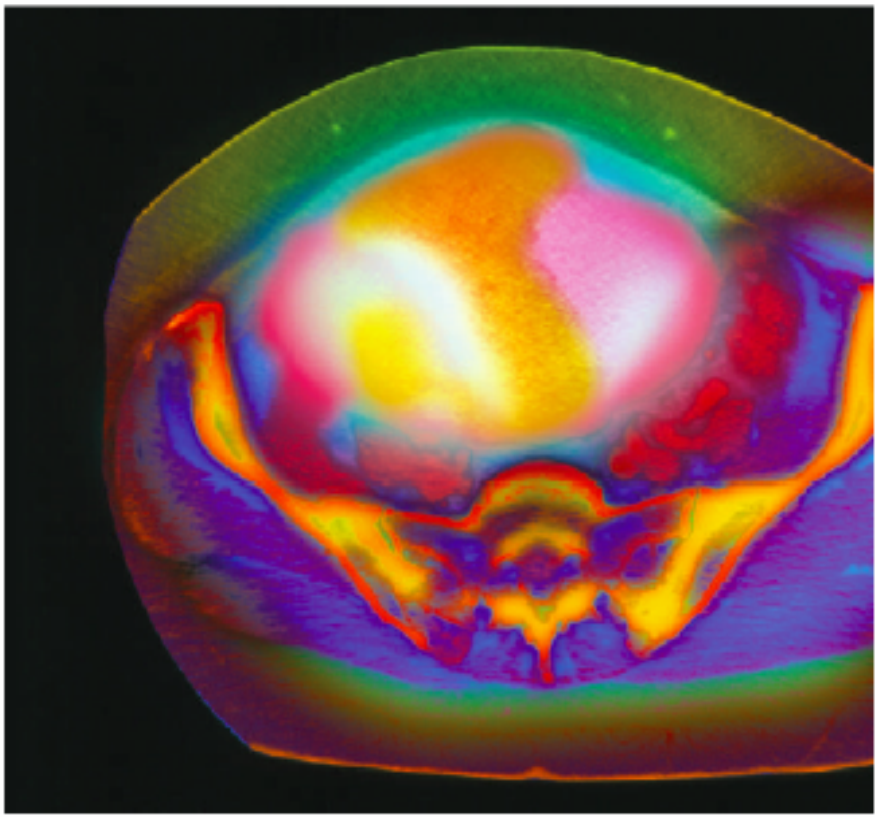
"I've been in cancer research for 40 years and I think it's the most important breakthrough in those years," said Dr.

Continued on Page 6

news feature

Running before we can walk?

Two years ago, a new proteomic test was heralded as the future of cancer diagnostics. But since then, doubts about its effectiveness have begun to grow. Erika Check reports.



On target: can proteins in the blood reveal ovarian tumours (pink/yellow) before they

Seldom does a single piece of research prompt the US Congress to pass a resolution urging continued funding to drive a new diagnostic test towards the clinic. But that's what happened in 2002, when *The Lancet* published a paper[1] claiming a breakthrough in the diagnosis of ovarian cancer.

The paper described the use of mass spectrometry to analyse the pattern of proteins present in samples of blood serum. On the basis of these patterns, the test detected all the patients with ovarian cancers in a set of

Lancet paper. In November 2002, Correlogic granted licences to two larger firms, Quest Diagnostics and the Laboratory Corporation of America, which are now hoping to market the test under the brand name OvaCheck.

But those plans could be thrown off track by reanalyses of Liotta and Petricoin's data by independent groups, which have raised serious doubts about OvaCheck's reliability.

These questions prompted the Society of Gynecologic Oncologists to review all of the published work about OvaCheck. On 7 Feb-

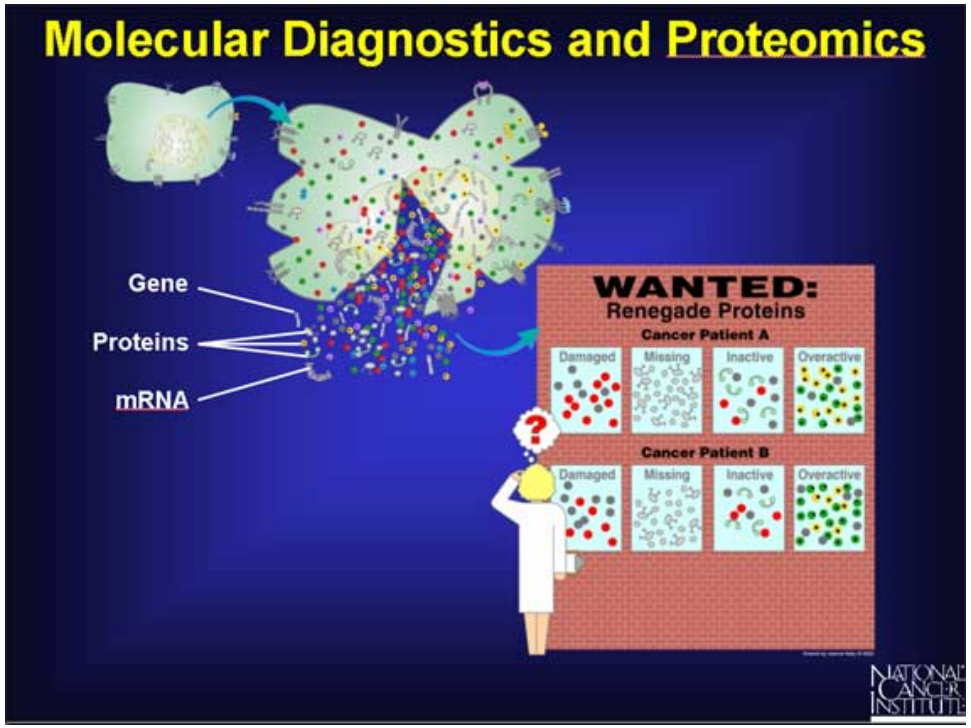
Bioinformatics[2]. They had re
set that Liotta and Petricoin
online in August 2002. Sor
similarly found numerous dif
protein patterns that discrimi
the cancer patients and the he
The trouble, according to Sor
was that these looked more lik
artefacts than real biological d

The proteomics test relies c
and electric fields to separate t
given sample. Each protein i

Proteomics Research Aids Cancer Diagnosis and Treatment

FDA Approves Vermillion's Ovarian Cancer Test, Sep. 11, 2009

OVA1 is an *in vitro* diagnostic multivariate index assay and the first FDA-cleared laboratory test that can indicate the likelihood of ovarian cancer with high sensitivity before a biopsy or exploratory surgery



Molecular Cancer 2007, **6**:25

Contribution of Oncoproteomics to Cancer Biomarker Discovery

Oncoproteomics : the study of proteins and their interactions in a cancer cell by proteomic technologies

Table 1: Comparison of proteomic biomarkers and current tumor markers

SELDI Proteinchip

Cancer	Proteomic biomarkers			Current tumor markers		
	Sensitivity	Specificity	Reference	Markers	Sensitivity	Specificity
Bladder	80%	90–97%	[87]	NMP22	31%	95%
Breast	93%	91%	[88]	CA 15-3	63%	80–88%
Colorectal	91%	93%	[89]	CEA	43%	****
Gastric	83%	95%	[90]	CEA	49%	****
Liver	94%	86%	[91]	AFP	50%	90%
Lung	87%	80%	[92]	Cyfra21-1	63%	94%
Ovarian	83%	94%	[93]	CA-125	57%	****
Pancreatic	78%	97%	[94]	CA 19-9	72%	****
Prostate	83%	97%	[95]	PSA	86%	20–34%

Trends in Biotechnology, 25 (4), p.166-173, Apr 2007

Moving cancer diagnostics from bench to bedside

To improve treatment and reduce the mortality from cancer, a key task is to detect the disease as early as possible. To achieve this, many new technologies have been developed for **biomarker discovery and validation.**

This review provides an overview of ***omics*** technologies in biomarker discovery and cancer detection, and highlights recent applications and future trends in cancer diagnostics. Although the present omic methods are not ready for immediate clinical use as diagnostic tools, it can be envisaged that simple, fast, robust, portable and cost-effective clinical diagnosis systems could be available in near future, for home and bedside use.

Comparison between *omics* Technologies for Biomarker Discovery

Technique	Advantages	Disadvantages
Transcriptomics	Well-established technology Few genes and/or transcripts (~25 000 in humans) relative to proteins	Tissue materials required
Proteomics	Suitable for various biological samples	Many different approaches More proteins (>500 000 in humans) relative to transcripts or metabolites
Metabonomics	Suitable for various biological samples Fewer metabolites (~10 000 in humans) relative to transcripts or proteins	Technology is in development Environmental impacts are ignored[a]
Peptidomics	Low molecular weight	Proteolysis in *ex vivo* samples complicate the results
Glycomics	Increased stability and solubility of glycoprotiens relative to unmodified protiens	Difficulty in glycosylation analysis, particularly structure identification
Phosphoproteomics	Sub-proteome: reduces the amount of proteins that can be analyzed	Difficulty in the identification of phosphorylated proteins
Lipidomics	Sub-metabonomics: reduces the amount of metabolites that can be measured	Technology in development

J Proteome Res. 2006 May;5(5):1143-54

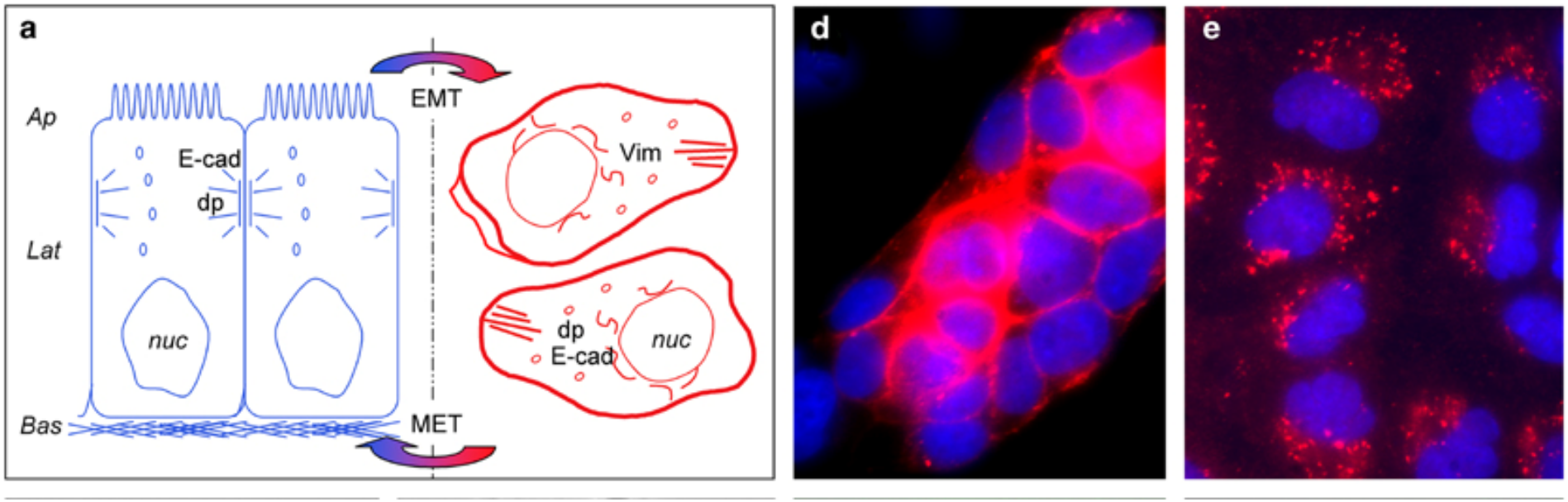
Differential Protein Expression Profiling by iTRAQ−2DLC−MS/MS of Lung Cancer Cells Undergoing Epithelial-Mesenchymal Transition Reveals a Migratory/Invasive Phenotype

Transforming growth factor-β (TGF-β) induces epithelial-mesenchymal transition (EMT) of epithelial cells in both normal embryonic development and certain pathological contexts. Here, we show that TGF-β induced-EMT in human lung cancer cells (A549; adenocarcinoma cells) mediates tumor cell migration and invasion phenotypes. To gain insights into molecular events during EMT, we employed a global stable isotope labeled profiling strategy using iTRAQ reagents, followed by 2DLC−MS/MS, which identified a total of 51 differentially expressed proteins during EMT; 29 proteins were up-regulated and 22 proteins were down-regulated. Down-regulated proteins were predominantly enzymes involved in regulating nutrient or drug metabolism. The majority of the TGF-β-induced proteins (such as tropomyosins, filamin A, B, & C, integrin-β1, heat shock protein27, transglutaminase2, cofilin, 14-3-3 zeta, ezrin-radixin-moesin) are involved in the regulation of cell migration, adhesion and invasion, suggesting the acquisition of a invasive phenotype.

Epithelial-Mesenchymal Transition

(上皮細胞-間質細胞轉變)

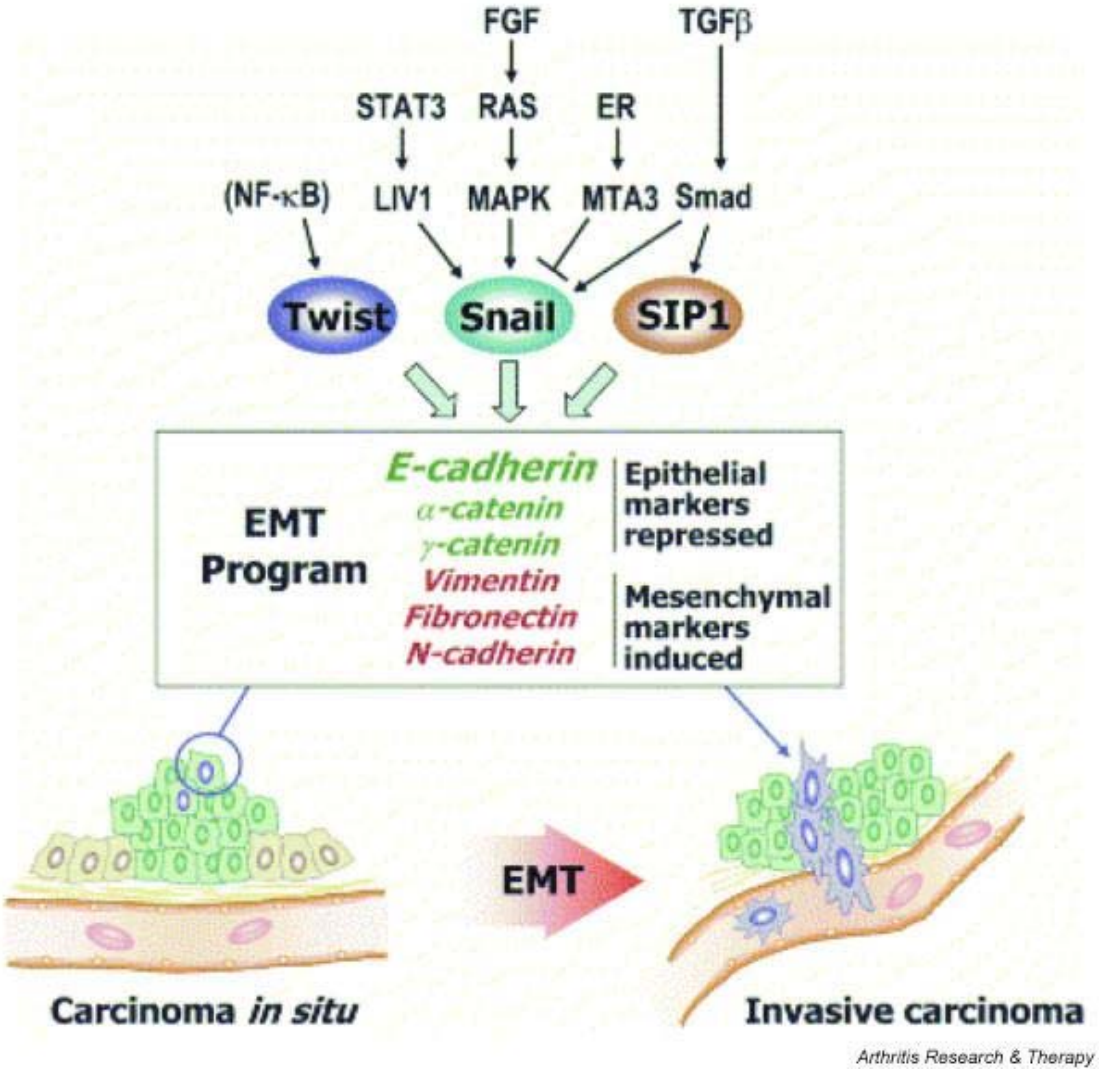
is a program of development of biological cells characterized by loss of cell adhesion, repression of E-cadherin expression, and increased cell mobility



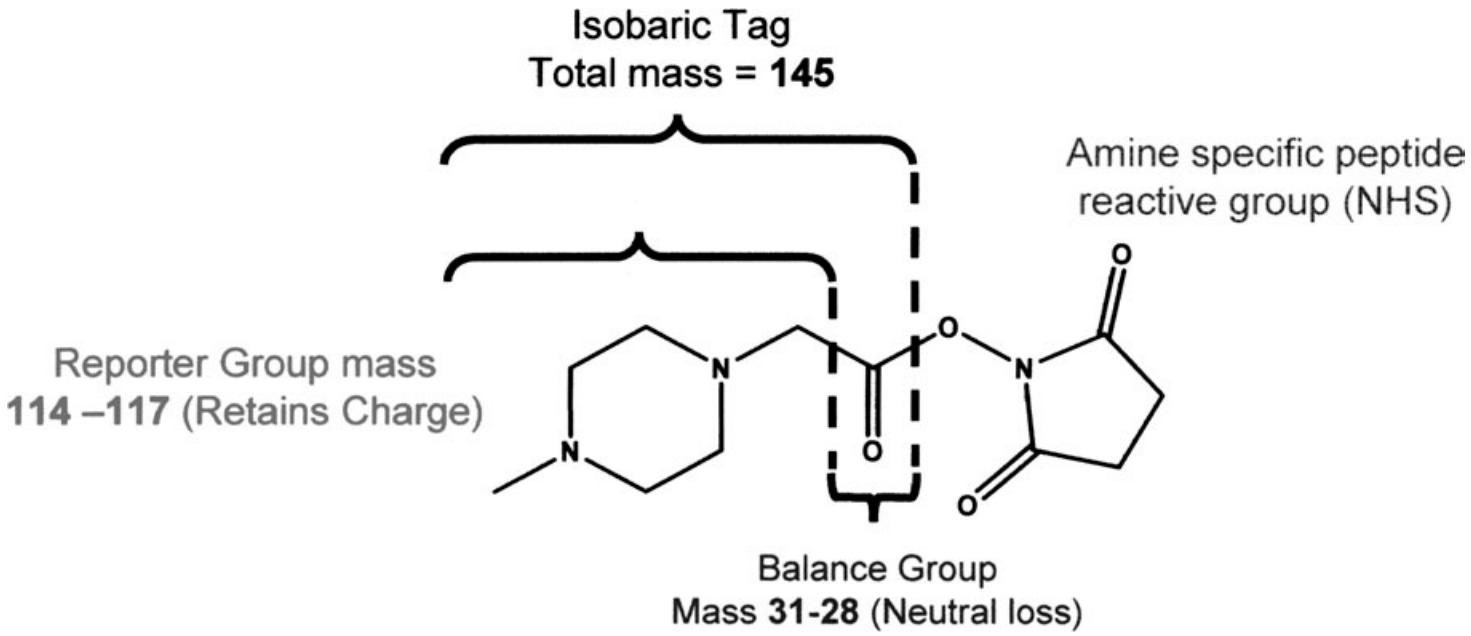
Initiation of cancer metastasis involves invasion, which has many phenotypic similarities to EMT, including a loss of cell-cell adhesion mediated by E-cadherin repression and an increase in cell mobility

Oncogene (2005) 24, 7443–7454

TGF- β is a multifunctional cytokine which regulates diverse Functions. Increased expression of TGF-â occurs in many human cancers and is correlated with enhanced invasion and metastasis.



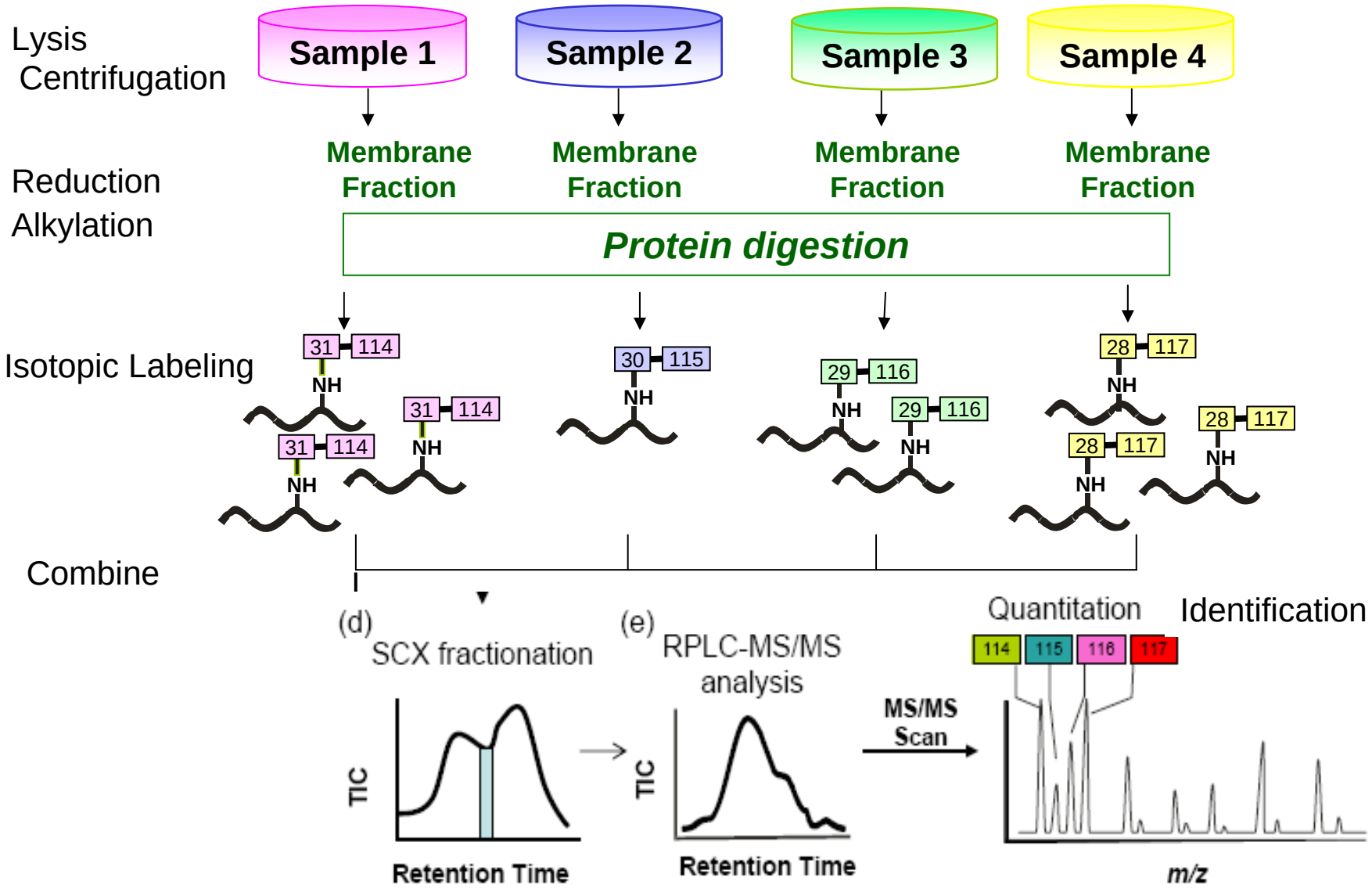
Isobaric Tags for Related and Absolute Quantitation (iTRAQ)



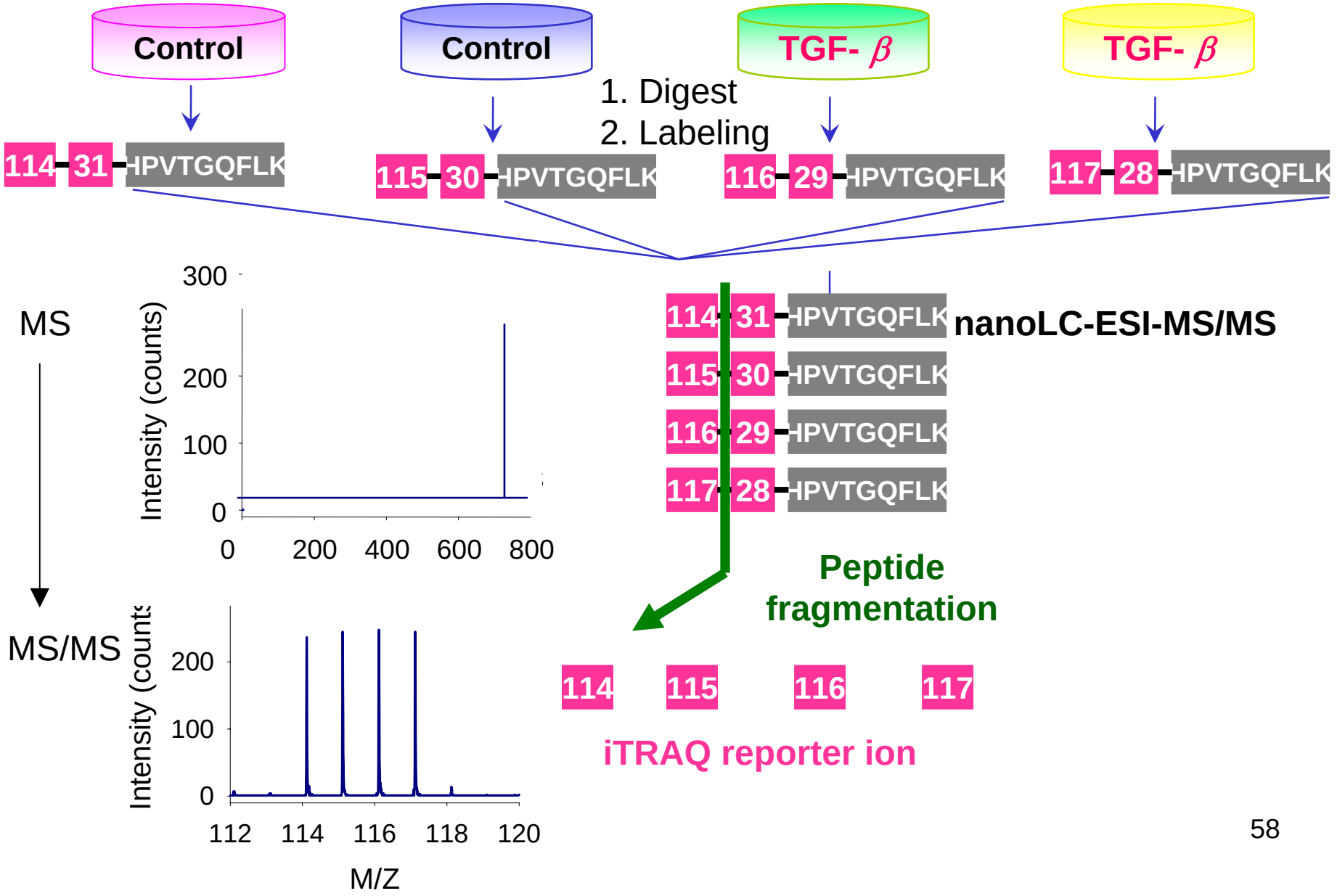
- Duplex experiment: up to four different biological samples
- Amine-specificity: label all peptides in samples
- Stable isotope reagents: product ion spectrum reveals the difference in abundance from each sample

Journal of Experimental Botany, Vol. 57, No. 7, pp. 1501–1508, 2006

Multiplexed Quantitation of Proteome



Multiplexed Protein Quantitation by iTRAQ

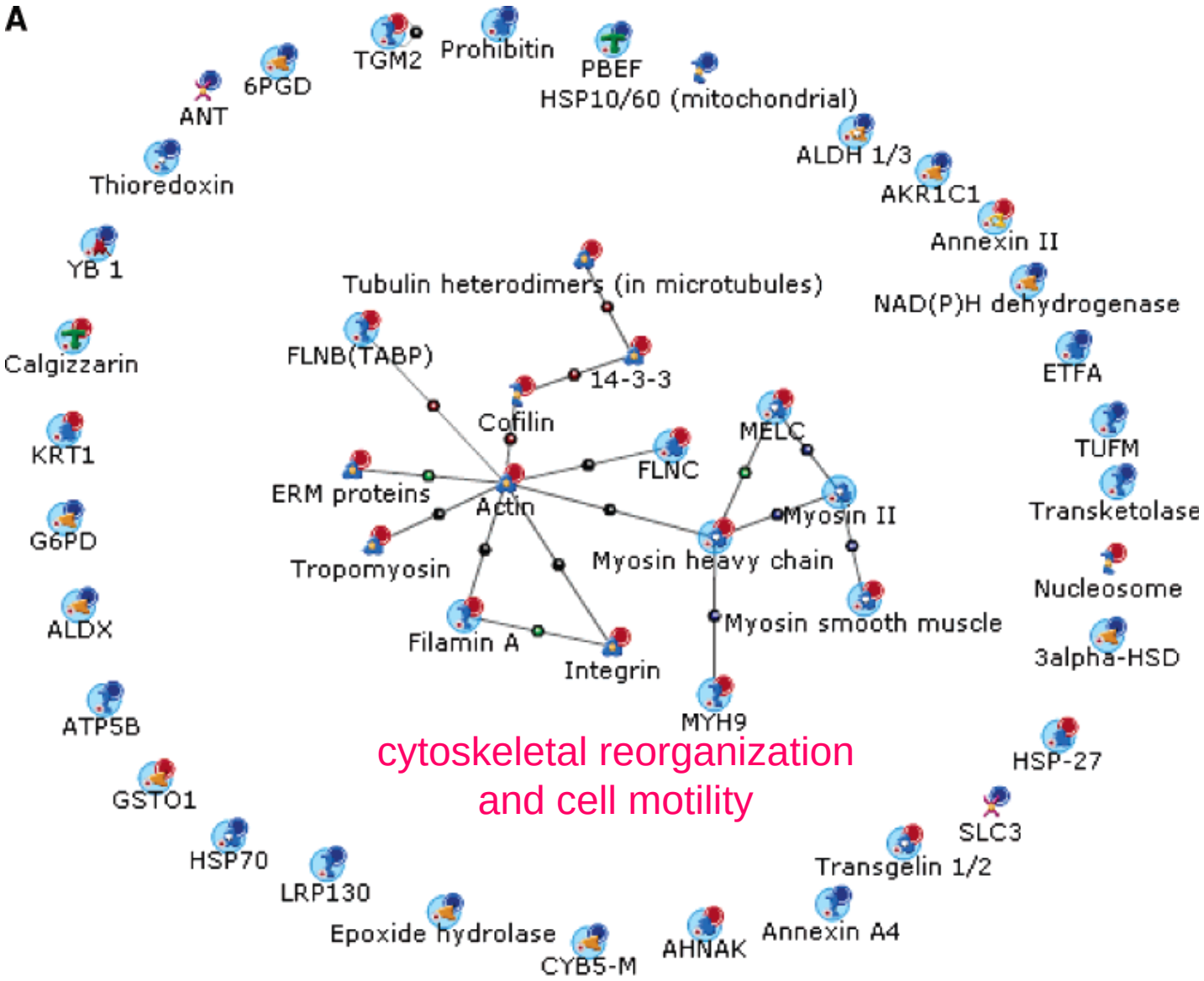


Correlation of Up-Regulated Proteins to Human Cancer and Metastasis-Related Processes

protein name	human cancer	lung cancer	Metastatic Processes			
			adhesion	migration	invasion	metastasis
Tropomyosins zar	+	+		+		
Calgiz	+					
Filamin A, B, and C	+		+	+		+
Integrin beta 1	+	+	+	+	+	+
HSPB1	+	+		+		+
Nonmuscle myosin heavy polypeptide-9			+	+		
Transglutaminase2	+	+	+	+	+	+
Trangelin 2	+			+		
Myosin alkali light chain	+		+	+		+
Radixin	+	+	+	+		
Moesin	+	+	+	+		
Desmoyokin	+		+			
Cofilin	+		+	+		
Glutathione-S-transferase	+					
14-3-3 zeta/delta	+	+		+		+
Keratin	+	+		+		
AnnexinA2	+	+		+		+
Actin, Cytoplasmic	+		+	+		
Tubulins	+	+		+		
Histone H2A,	+					

[a] Associations between each protein or group of protein isoforms with human cancer and metastasis-related processes were assigned (+) by surveying current literature using PubMed (National Center for Biotechnology Information) searches for a protein and the respresentative category.

Biological network analysis of differentially expressed proteins in response to TGF-β



Conclusion:
Opportunities, challenges

1. An exciting era, because we:

- **know so much biology**
- **have such powerful tools to measure biology**

2. But rules of evidence have not changed.

3. Disappointment may occur that, in retrospect, will have been predictable, and is due in part to culture clash.

4. We must improve scientific process (e.g., handle bias).

5. We have figured out how to avoid predictable disappointment and wasted effort... and how to generate useful knowledge about new markers.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