

Tissue and Fluid Proteomics

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What is proteomics?

- A snap shot of the protein pattern!!**

What can proteomics do?

- To provide information on functional networks and/or involvement of certain proteins in individual reaction and control mechanisms by means of differences between 2 or more snap shots of the protein patterns!!**

To find the magic target protein(s) or biomarker(s) for diagnosis and cure of diseases !!

Sample Sources for Proteomic Analysis

- **Cell lines.**
- **Tissue sections.**
- **Body Fluids:**
 - **Blood and urine.**
 - **Fluids from secretion.**
 - **Fluids in interstitial spaces.**

Fluids from Secretion

- **Cerebrospinal fluids (CSF)**
 - Fluid surrounding the central nervous system.
 - Total vol ~ 140 ml, produced at 0.3-0.4 ml/min.
 - Samples were collected by lumbar puncture (~10-12 ml).
 - 2D gel and MS ID.
 - Studies of the pathophysiological mechanism in frontotemporal dementia and Alzheimer's disease.

- **Bronchoalveolar lavage fluids**
 - Obtained by washing the epithelial lining of lung with PBS.
 - 2D gel and MS ID.
 - Studies of cystic fibrosis, pulmonary alveolar proteinosis, etc.

Fluids from Secretion

- **Synovial fluid**

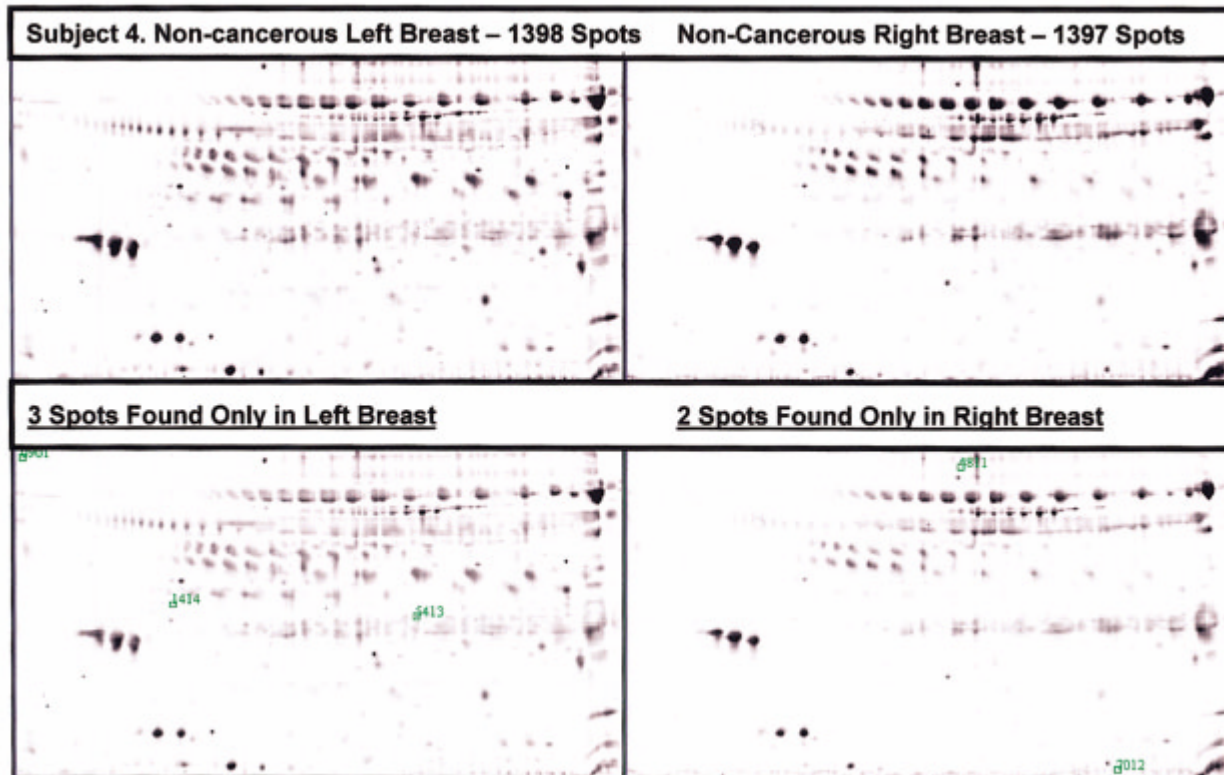
- A dynamic reservoir for proteins originating from serum, synovial tissue, and cartilage.
- 2D gel.
- Study for biomarkers for osteoarthritis.

- **Nipple aspiration fluid (NAF)**

- breast ductal fluid collected by nipple aspiration.
- Non-invasive way of sample collection.
- Sample vol: generally ~ 10-20 μ l.
- 2D gel, SELDI, and chromatography-MSMS.
- Studies of the early diagnosis of breast cancer.

Protein Profiles of Bilateral Matched Paired NAFs by 2DE-Approach

Non-Cancerous Left Breast Non-Cancerous Right Breast



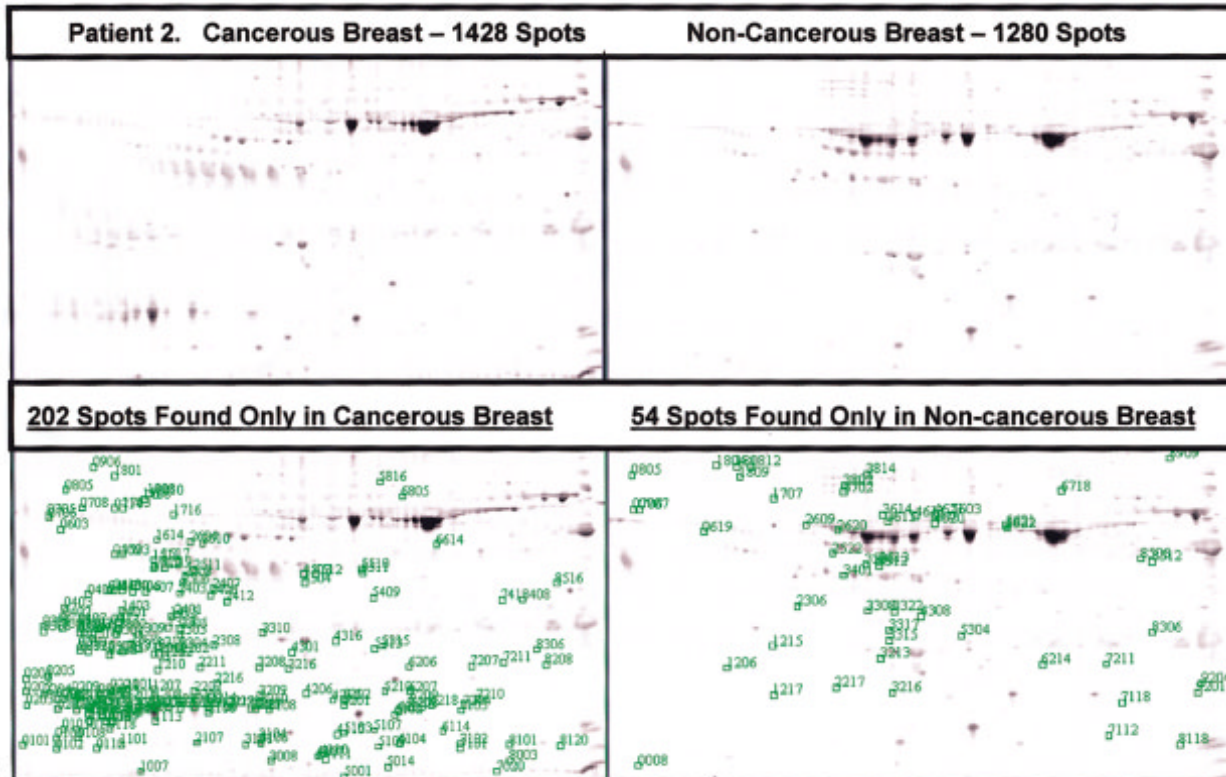
1398 spots
3 spots found only in
Left Breast

1397 spots
2 spots found only in
Right Breast

Protein Profiles of Bilateral Matched Paired NAFs by 2DE-Approach

Cancerous Breast

Non-Cancerous Breast

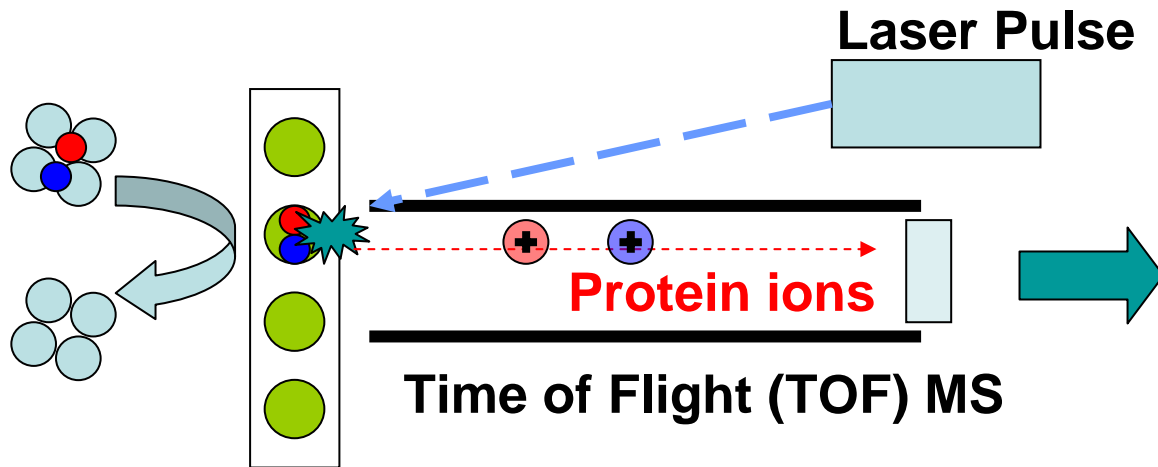


1428 spots
202 spots found only
in Cancerous Breast

1280 spots
54 spots found only in
non-Cancerous Breast

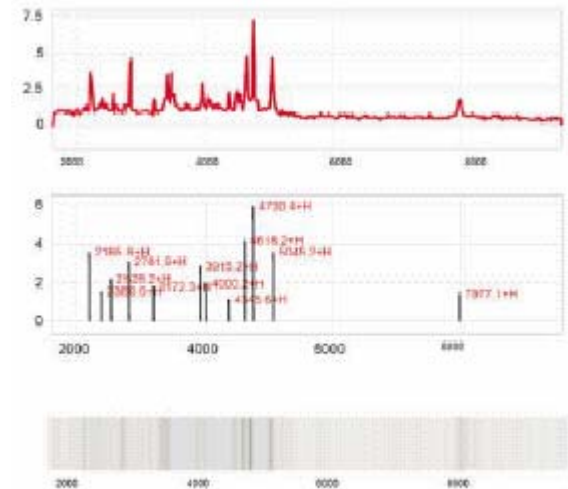
SELDI-TOF-MS Approach

(Surface Enhanced Laser Desorption Ionization)

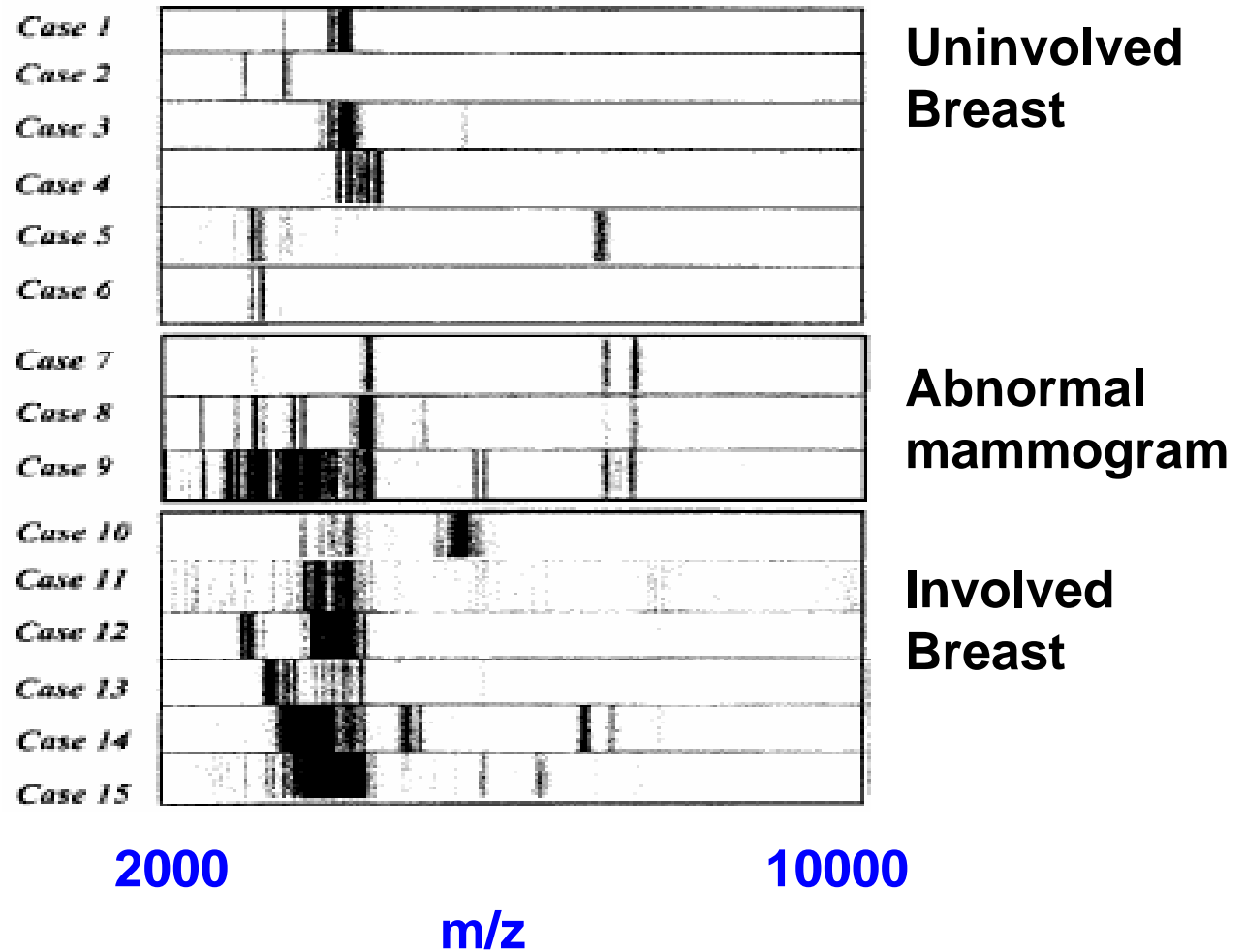


Chemical modified surface with hydrophobic, ionic exchange, or affinity function.

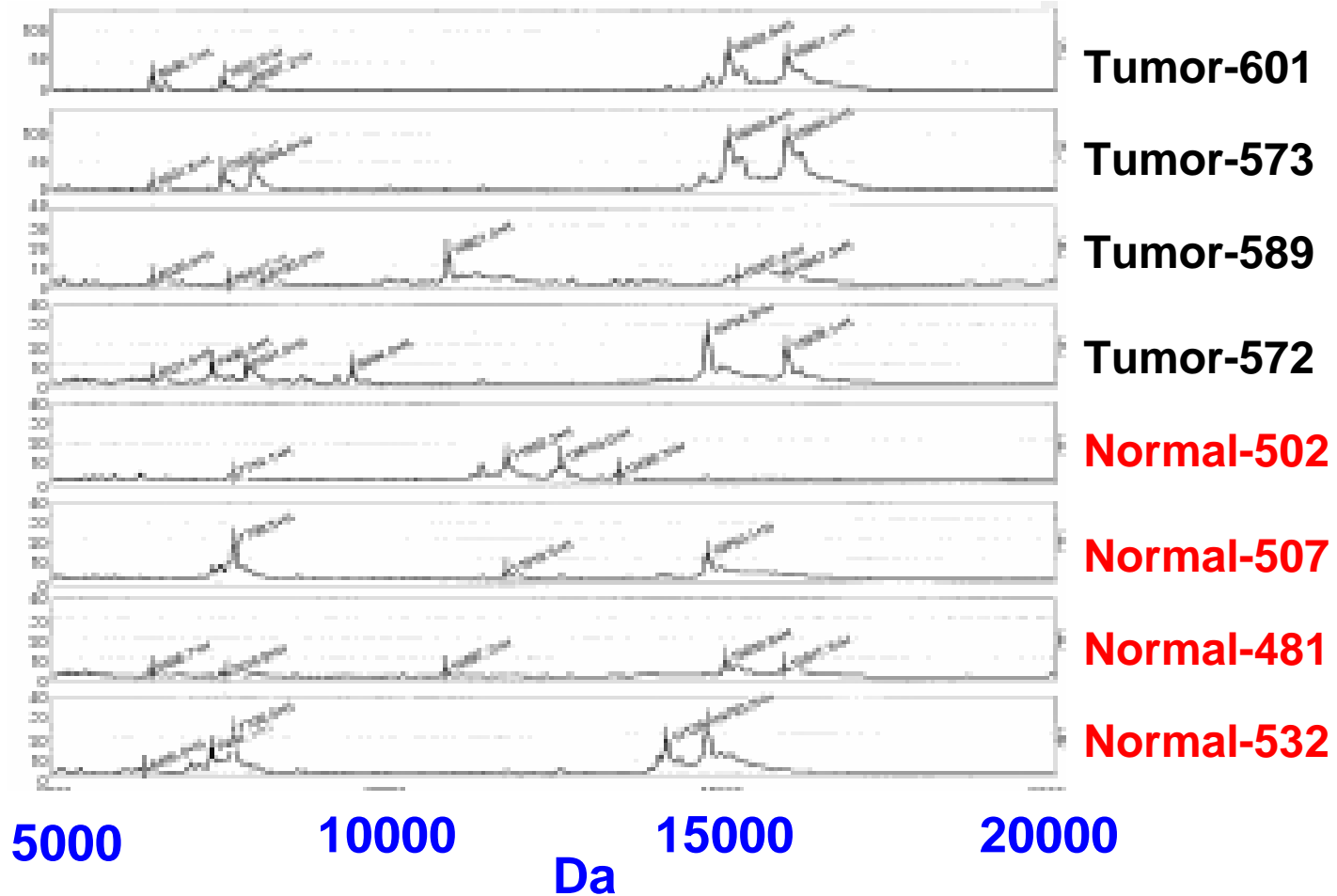
Protein Profile



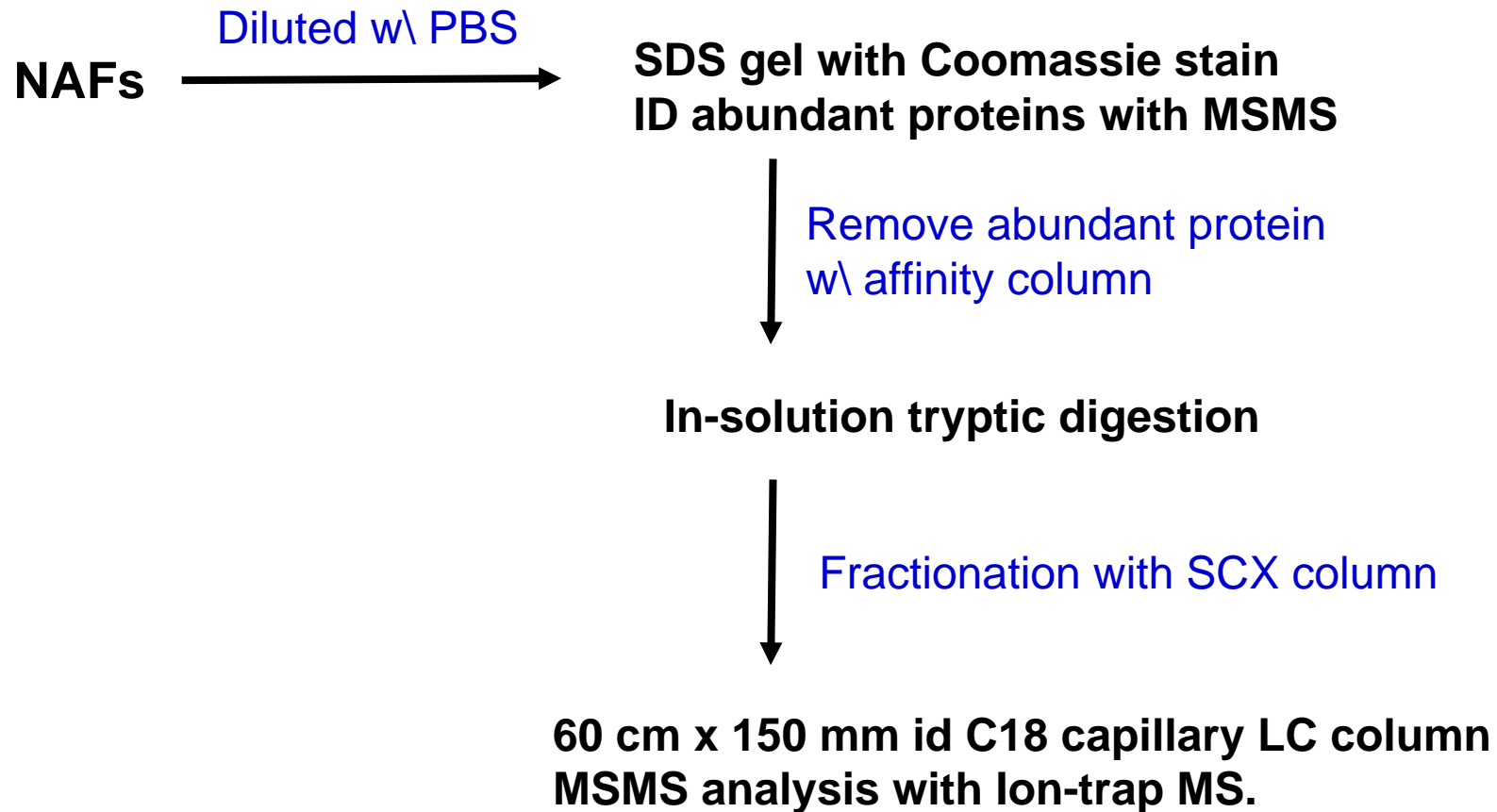
Protein Profile of NAFs by SELDI Approach



Protein Profile of NAFs by SELDI Approach



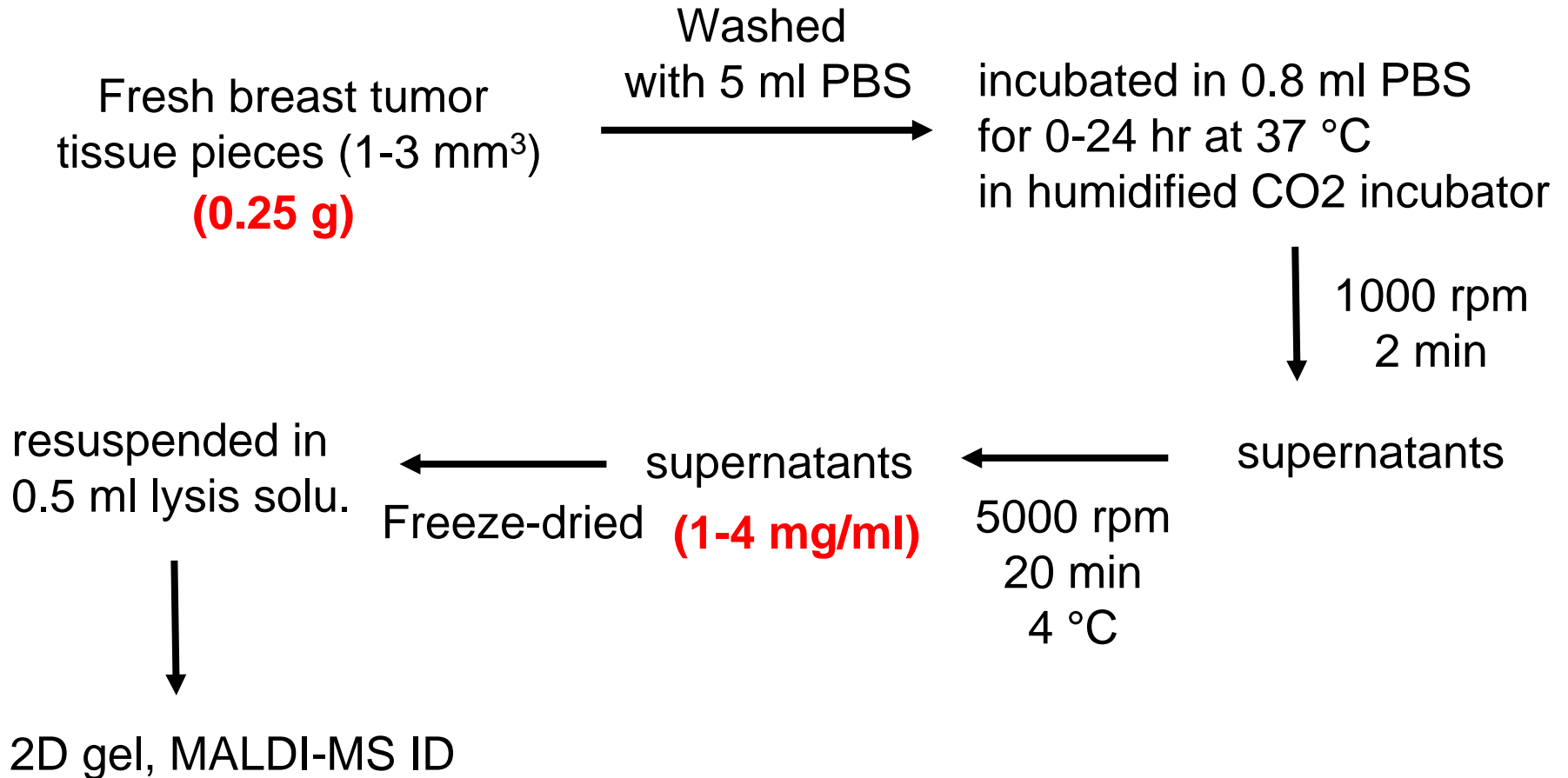
Protein ID of NAFs by Gel-free Approach

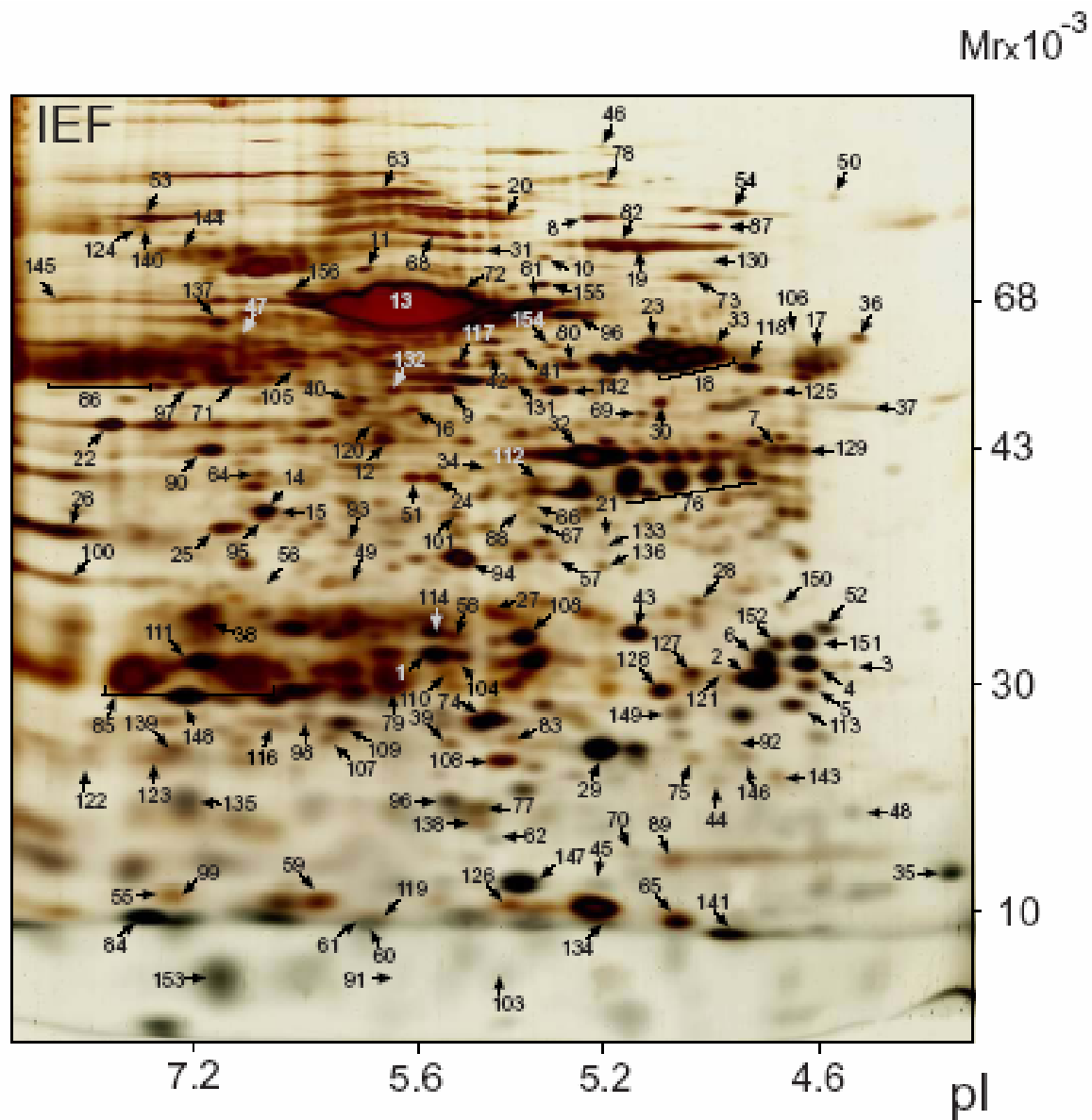


**64 proteins were ID,
15 were previously reported to be altered in serum or tumor tissue.**

Fluids in Interstitial Spaces

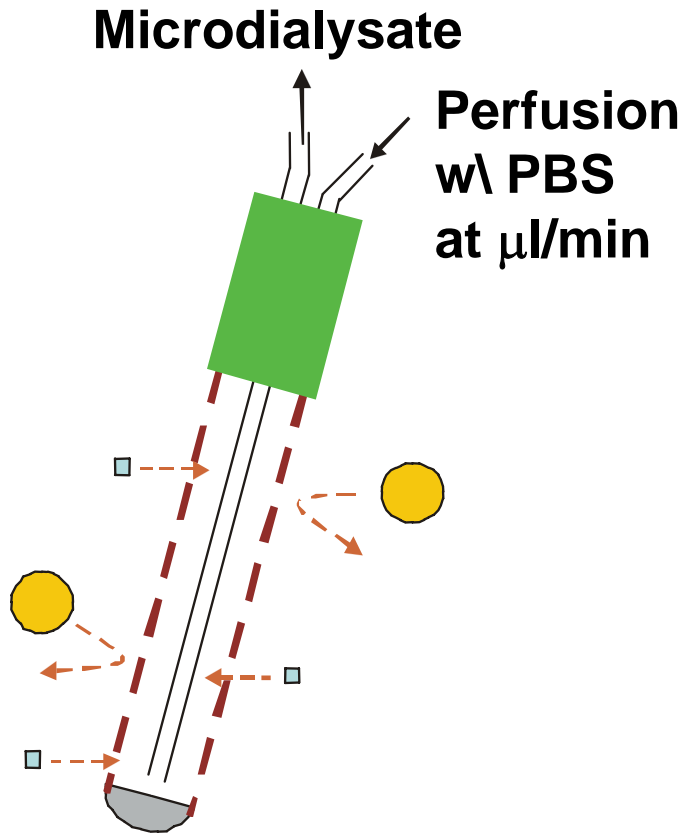
Ex-vivo Interstitial fluid collection:



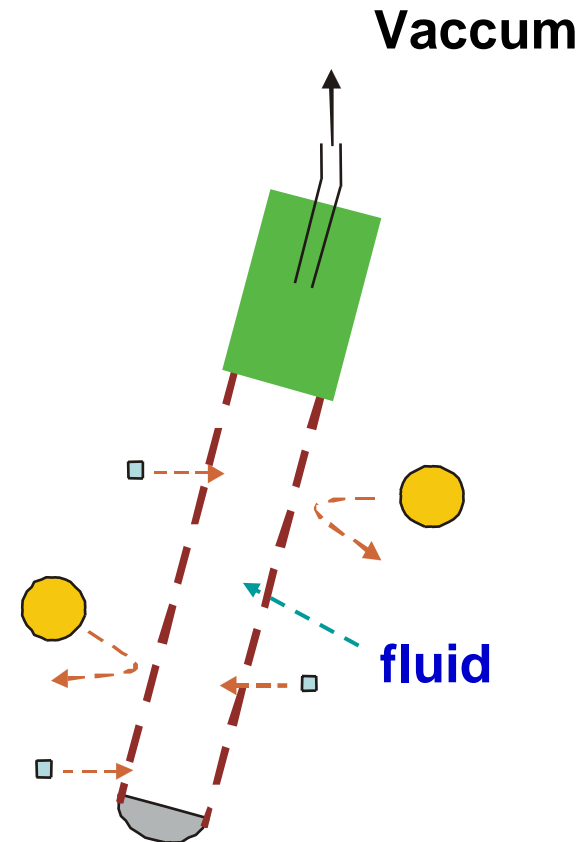


Sampling from Interstitial Space by In-vivo Microdialysis and Ultrafiltration

Microdialysis



Ultrafiltration



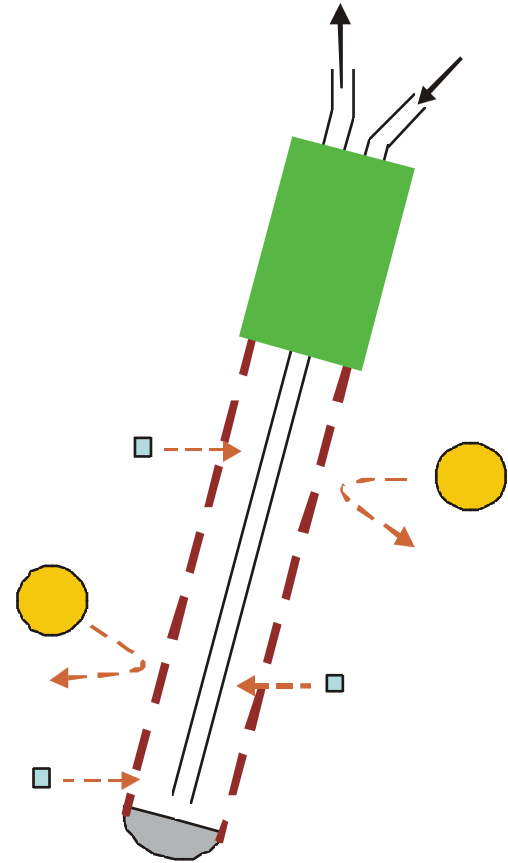
Microdialysis Sampling for Small Molecules

Advantage:

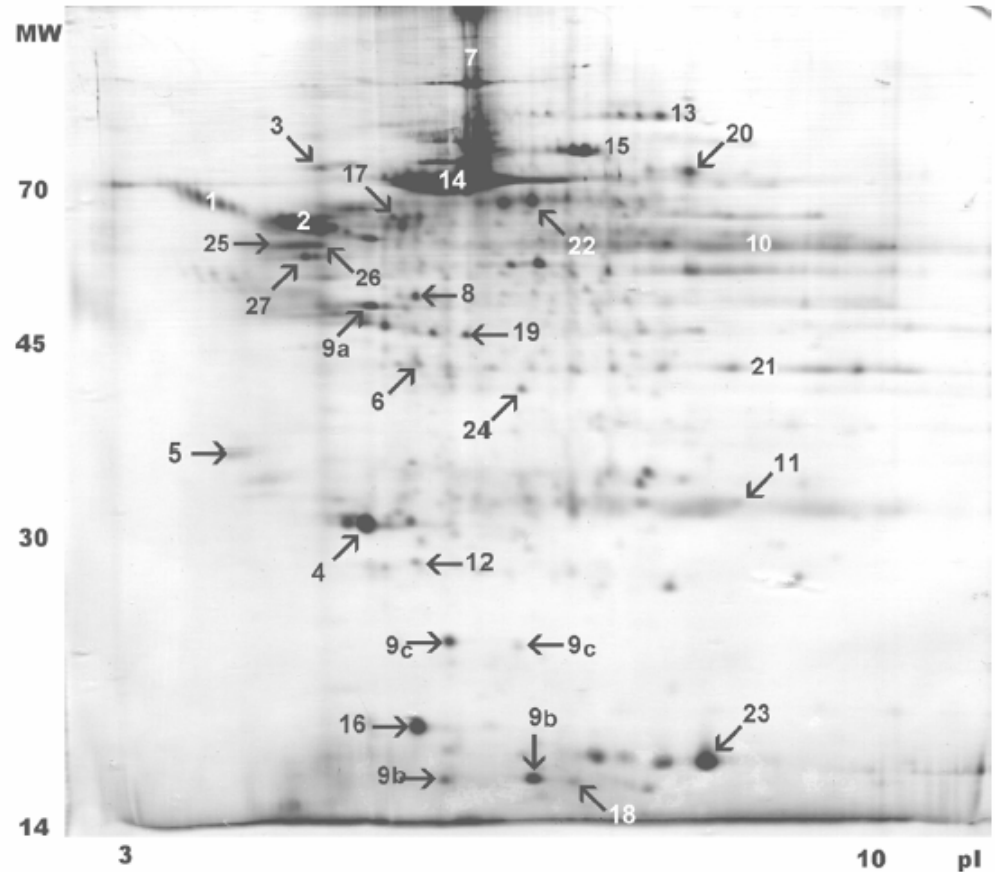
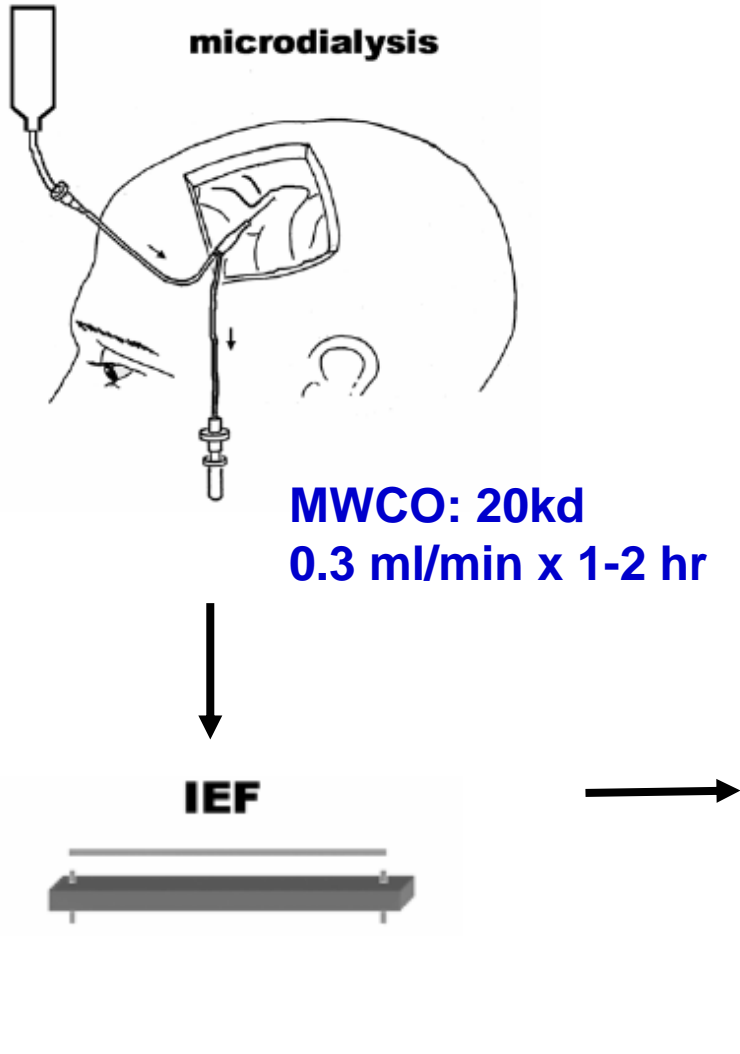
- Real *in-vivo* sampling from live, free-moving animals
- Sampling metabolites at the site of interest
- Relative clean samples

Limitation:

- Small sample size
- Possible low sample concentration

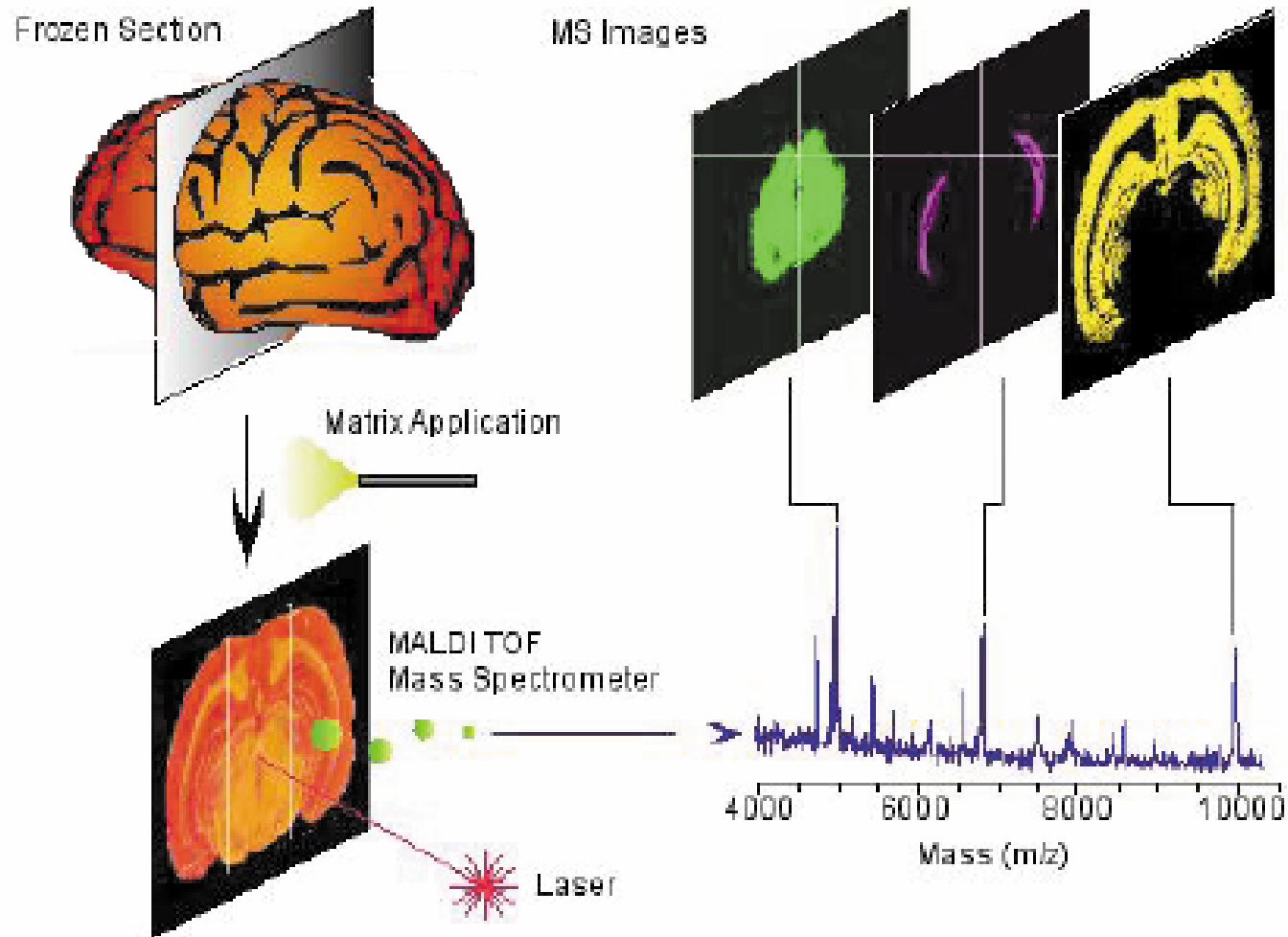


Proteome of Human Brain Microdialysate



Spot number	Number of spots identified	Gene name	Protein description	SwissProt accession number	Theoretical pI	Theoretical molecular weight (Da)
<i>proteins identified in cerebral microdialysate and cerebrospinal fluid</i>						
1	8	SERPINA3	Alpha-1-antichymotrypsin	P01011	5.33	47651
2	8	SERPINA1	Alpha-1-antitrypsin	P01009	5.37	46737
3	5	A1BG	Alpha-1B-glycoprotein	P04217	5.65	51941
4	3	APOA1	Apolipoprotein A-I	P02647	5.56	38778
5	1	AMBIP	AMBIP protein	P02760	5.95	38999
6	2	APOE	Apolipoprotein E	P02649	5.65	36154
7	2	C3	Complement C3	P01024	6.02	187164
8	1	FGG	Fibrinogen gamma chain	P02679	5.37	51512
9a	6	HP	Haptoglobin-2	P00738	6.13	45205
9b	2	HP	Haptoglobin alpha-2 chain	P00738	5.57	15946
9c	2	HP	Haptoglobin beta chain	P00738	6.32	27265
10	7	--	Immunoglobulin heavy chain	P99006	--	--
11	13	--	Immunoglobulin light chain	P99007	--	--
12	1	RBP4	Plasma retinol-binding protein	P02753	5.76	23044
13	4	PLG	Plasminogen (EC 3.4.21.7)	P00747	7.04	90569
14	5	ALB	Albumin	P02768	5.92	69367
15	5	TF	Transferrin	P02787	6.81	77050
16	2	TTR	Transthyretin	P02766	5.52	15887
17	2	GC	Vitamin D-binding protein	P02774	5.24	56761
<i>proteins identified solely in cerebral microdialysate</i>						
18	1	B2M	Beta-2 microglobulin	P01887	7.80	13823
19	1	CKB	Creatine kinase, B chain	P12277	5.34	43644
20	1	DRP2	Dihydropyrimidinase related protein-2	Q16555	5.95	62294
21	4	GAPD	Glyceraldehyde 3-phosphate dehydrogenase	P16858	8.45	35679
22	1	HSP73	Heat shock protein 70 kD HSP73	P11142	5.37	70898
23	1	HBB	Hemoglobin beta chain	P02023	6.81	15867
24	1	MDHA	Malate dehydrogenase	P40925	6.89	36295
25	4	TUBA1	Tubulin alpha-1	P04687	4.94	50152
26	3	--	Tubulin beta	Q13885	4.78	49907
27	1	TUBB5	Tubulin beta 5	Q8WUC1	4.78	49671

Direct Profiling and Imaging of Proteins and Peptides from Tissue Sections



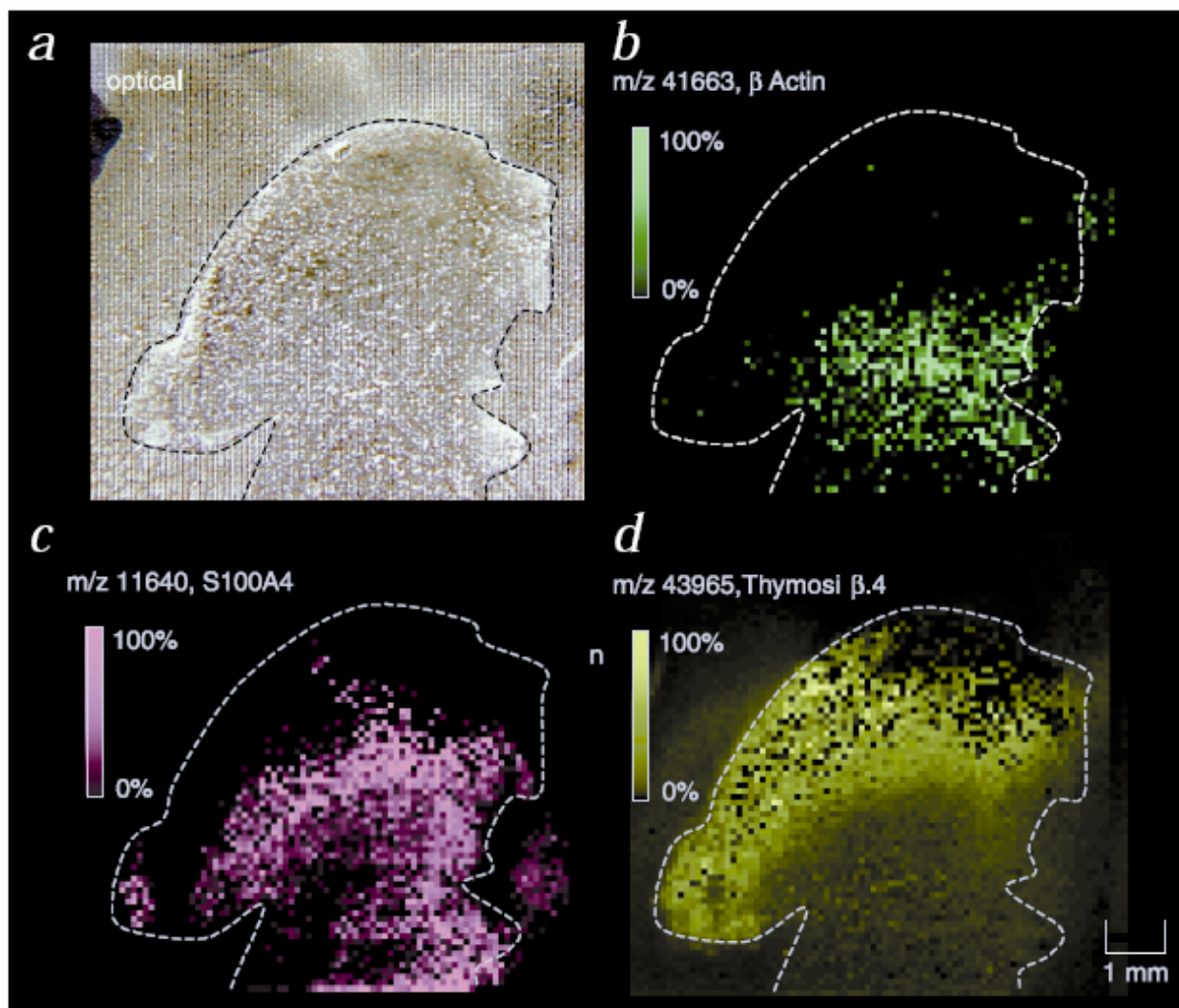


Fig. 3 Selected protein images from a glioblastoma section. **a**, Human glioblastoma slice mounted on a metal plate, coated with matrix (the lines are from ablation of matrix with the laser). **b–d**, Mass spectrometric images of proteins showing high concentration in the proliferating area of the tumor (**d**) and other proteins present specifically in the ischemic and necrotic areas (**b** and **c**).