Lab-On-Chip based protein profiling for CANcer DIAgnosis

NanoBIO 2008

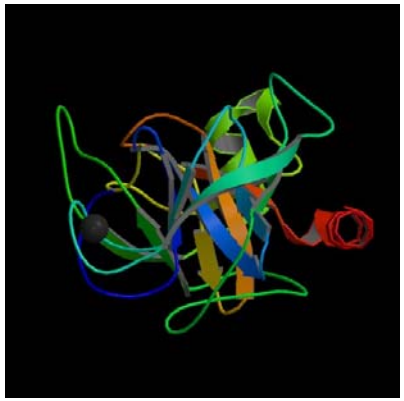
Barcelona, 10th of June of 2008

Manuel Marcelino Perez Perez

Information Society Technologies

Funded by EC contract FP6-034202

Cancer diagnosis



- Blood test/single biomarker

- Imaging analysis X-rays, CT scans...

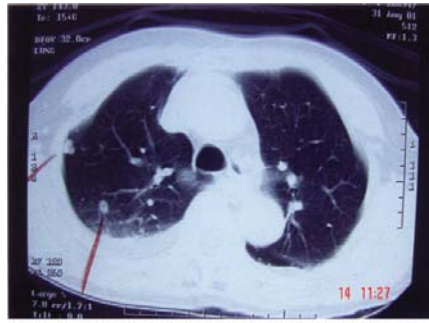
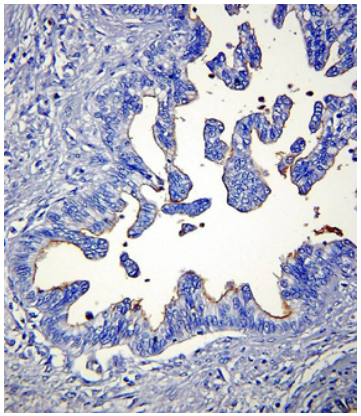


Figure 3 – Thoracic CT scan (pulmonary window) - several nodules (suggestive of metastases)



- Histological examination

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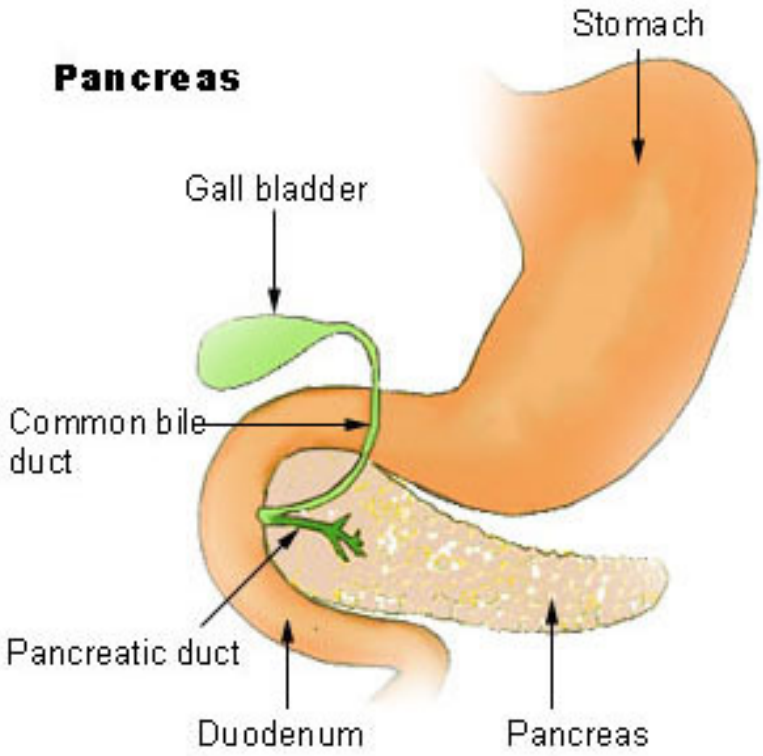
Pancreatic cancer

- **The pancreas**



- **Poor prognosis at the time of diagnosis**

- **Detected by expensive diagnostic imaging methods.**

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Project goals

- To develop a full analysis chain integrating bio-nano and info aspects for early detection of pancreatic cancer.

- To develop a lab-on-chip for separation and digestion of proteins.

- To test the full analytical chain for early detection of asymptomatic pancreatic cancer patients.

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Technical objectives

- To be able to determine **low concentration** cancer markers in the window of classic cancer marker
  - Targeted concentration range: 1 to 1000 pmole/L or 1 to 1000 ng/mL
- To **quantify a multiparametric set of markers** to improve the measurement specificity
- To **use chromatography nano-columns** to improve the sensitivity
- To **use electrospray ionisation** for a soft on-line ionisation
- To use a **mass spectrometry characterisation**:
  - to get a specific, sensitive and semi-quantitative recognition
  - to distinguish isoforms when the sensitivity is appropriate

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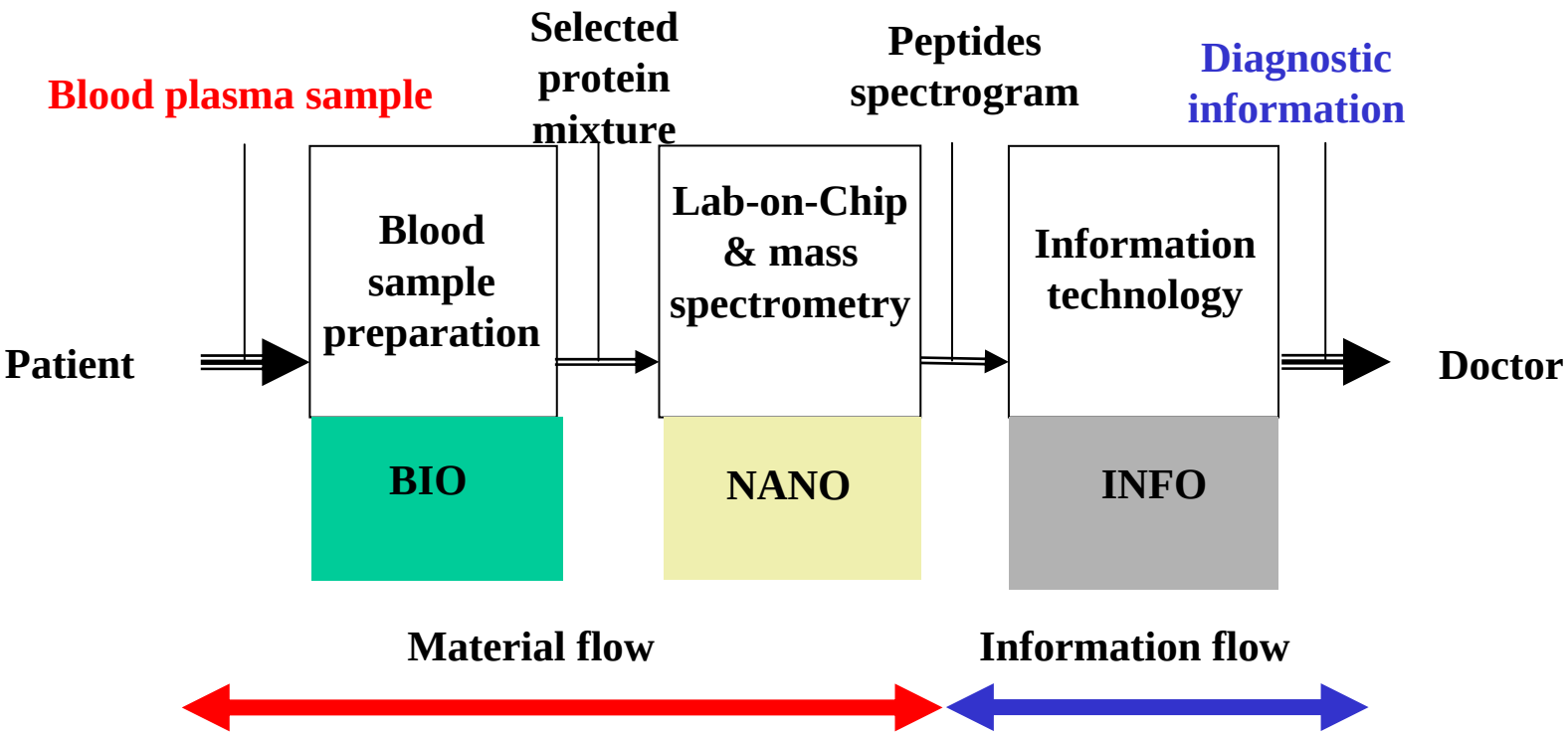
LOCCANDIA project



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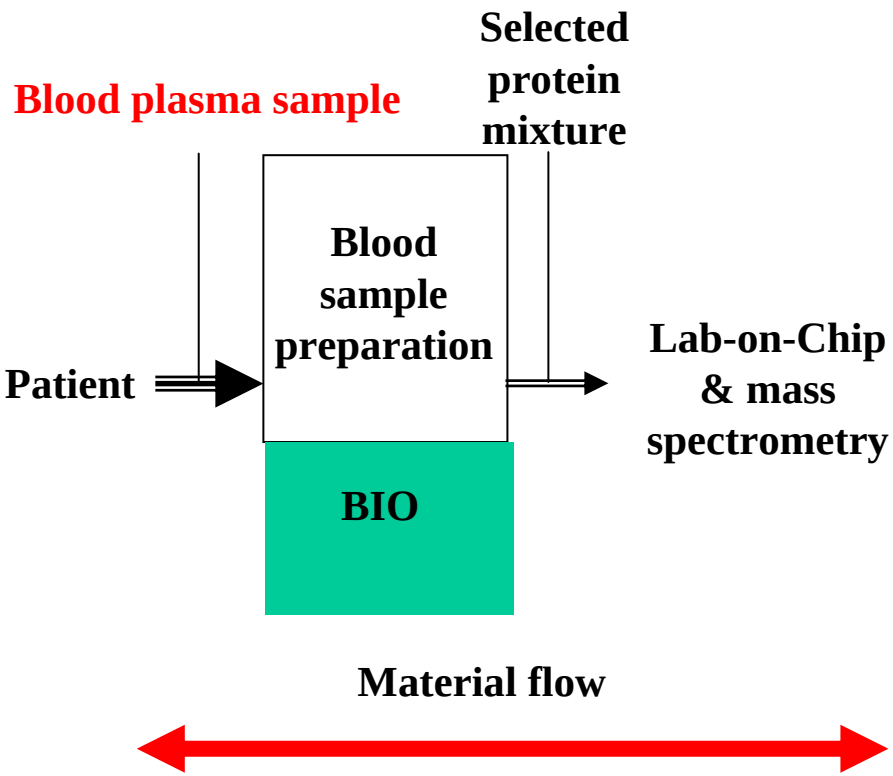
Bio part (I)



- Preparation of proteins to make synthetic mixtures using standards protocol.
- Production of the specific antibodies.
- Design of the affinity columns and the quality control of the proteins using MALDI-MS.

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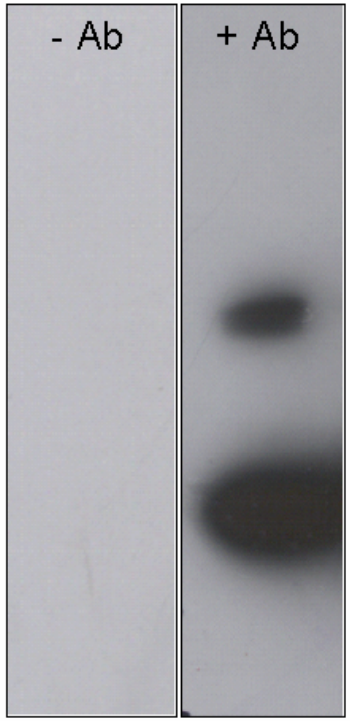
Bio part (II)

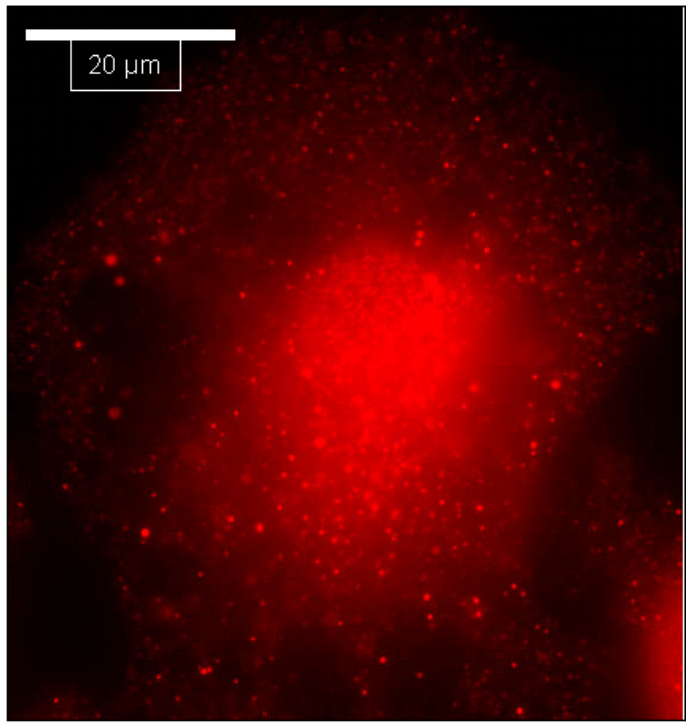
- MRP8/14, PAP I & p120
- Proteins purification
- Antibodies design and production.

PAP1
purified protein

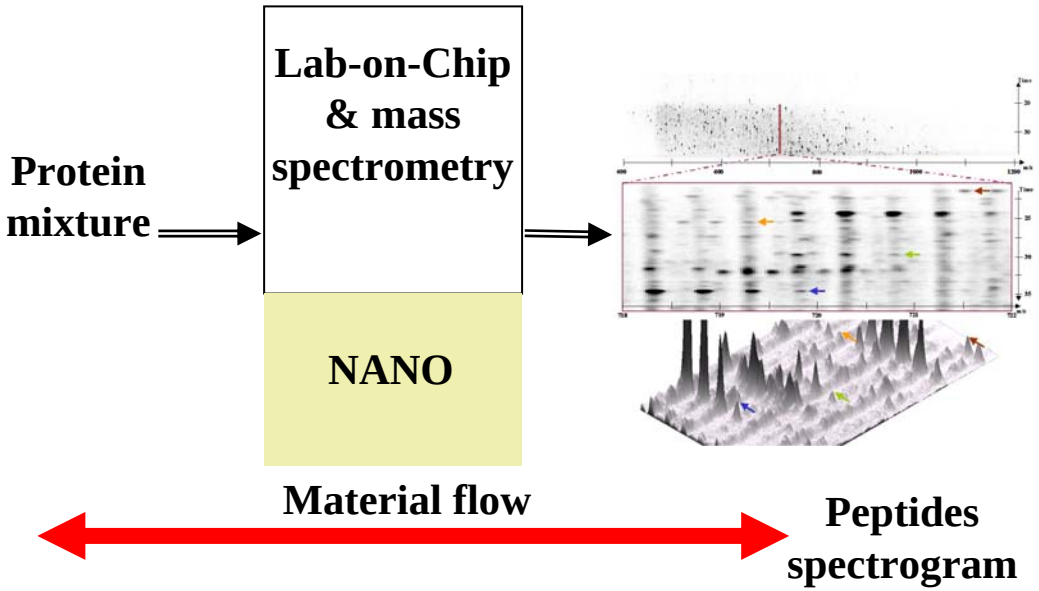
Anti-PAP1 antibodies
detect PAP1



Antibodies detect PAP1 in pancreas
tumor cell (immunoflurescence)



Lab-on-chip & mass spectrometry



- The objectives are:
  - to improve the digestion chip
  - to improve the chromatography-electrospray microsystem technology

- Two interconnected modules are designed:
  - a protein digestion module
  - a liquid chromatography-electrospray ionisation module

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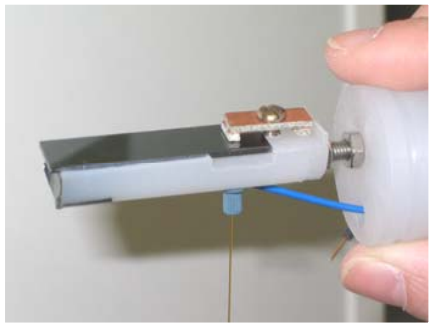
Lab-on-chip & mass spectrometry



- **Toward a point of care :**

Full system from blood plasma sample to diagnostic information

- **Lab-on-Chip**

Miniaturized integrated components to increase sensitivity (nano-LC, nano-ESI) and throughput

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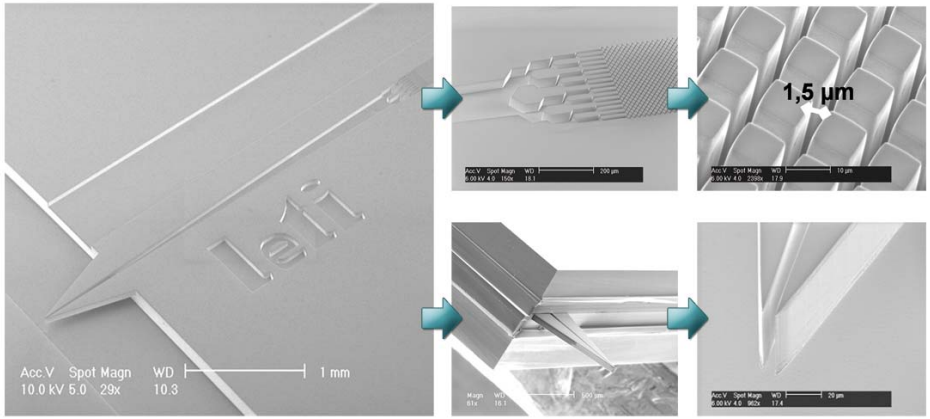
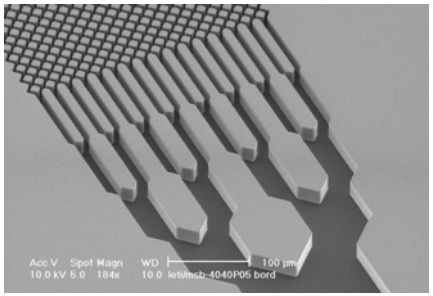
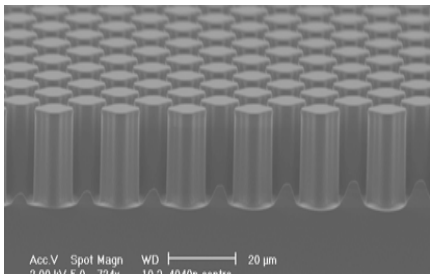
Lab-on-chip.



- Improvements of the digestion modules.



– Design of a new nano-liquid chromatography (LC) module including a new e-spray tip.



– New retention phase for chromatography module

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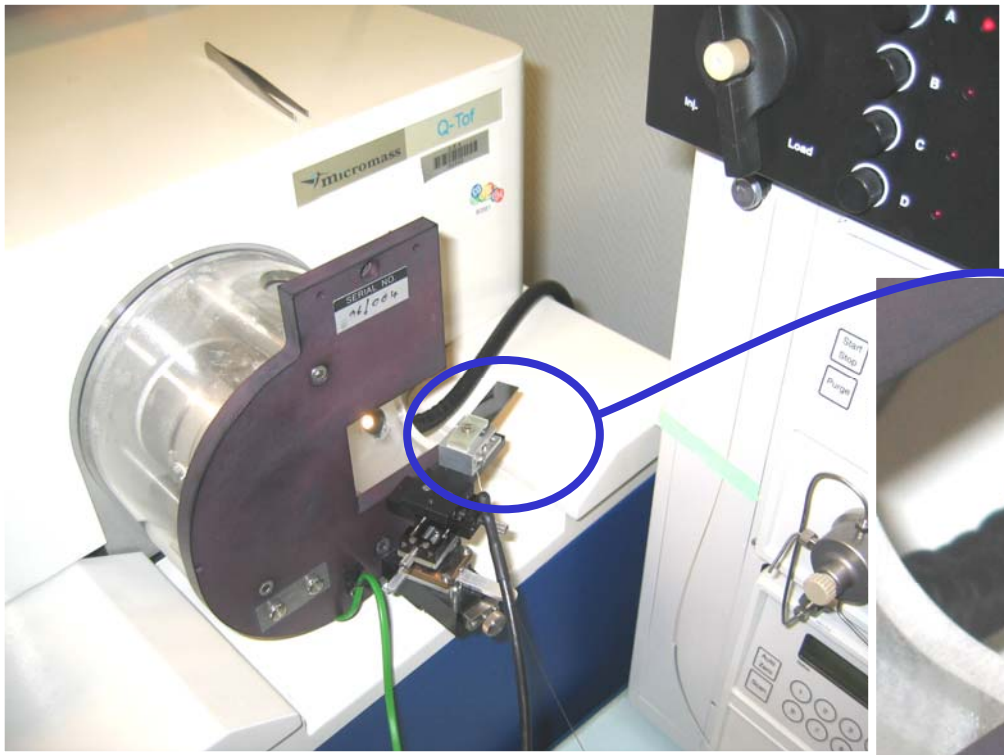
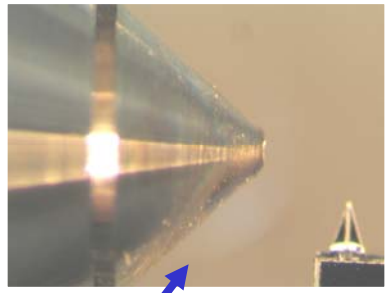
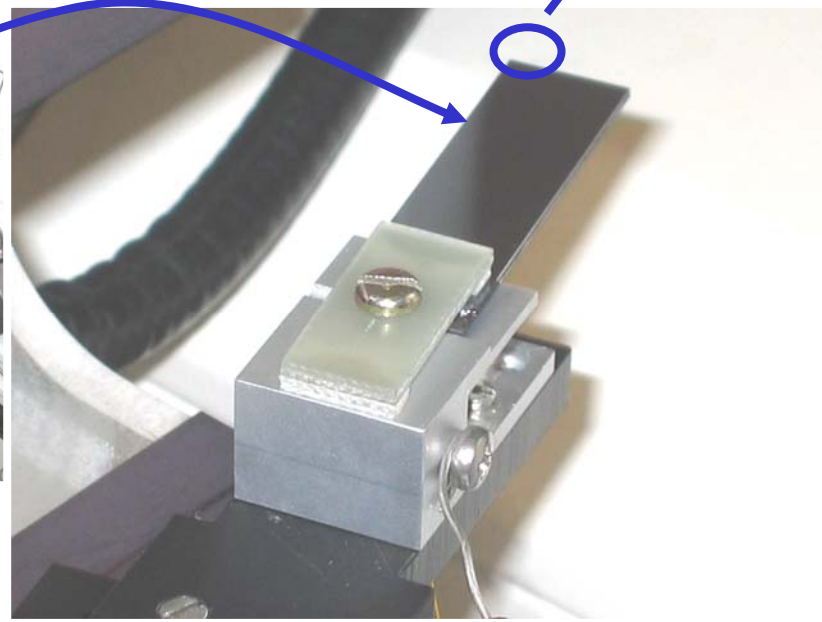
Interface with Mass Spectrometry

Chip mounting for Waters Q-TOF mass spectrometer

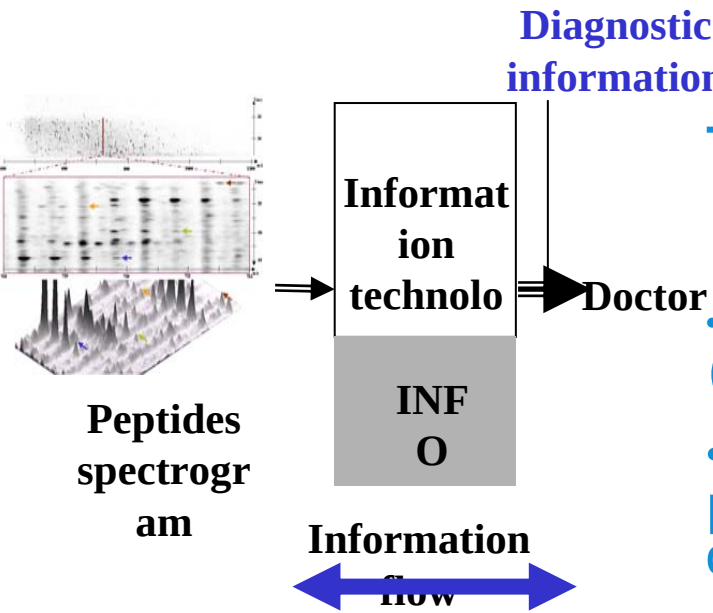


MS counter-electrode 0 V





Information technolog



To build LOCCANDIA information management system (LIMS):

- a Proteomic Information Management Syste (PIS) for sample information management
- a Clinical Information System (CIS) for patient information management to allow clinical evaluation

Information and data mediation infrastructure including preprocessing, reconstruction, visualization, protein/peptide identification and data analysis modules

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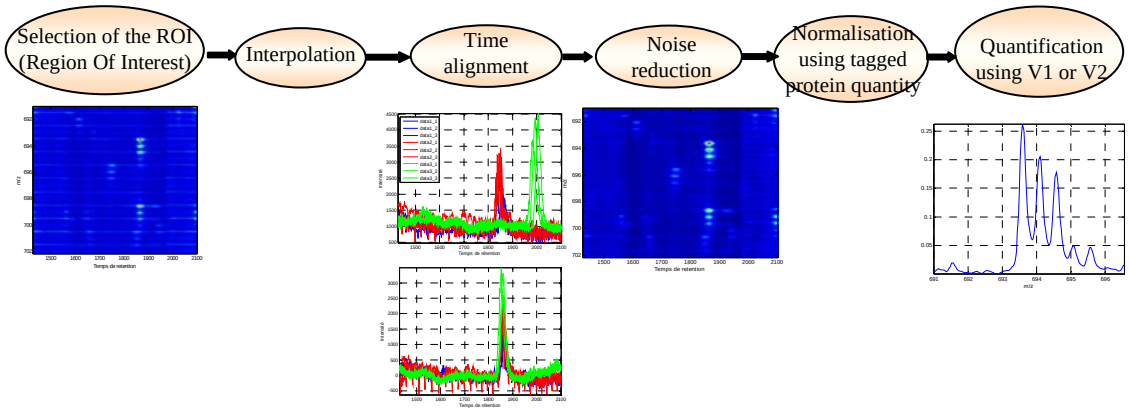
LIMS modules

To build an Integrated a Clinico-Proteomic Information System (CPIS) for sample information management:

- a Data pre-processing & Profile reconstruction module



- an Information and data mediation infrastructure including preprocessing, reconstruction, visualization, protein/peptide identification and data analysis modules

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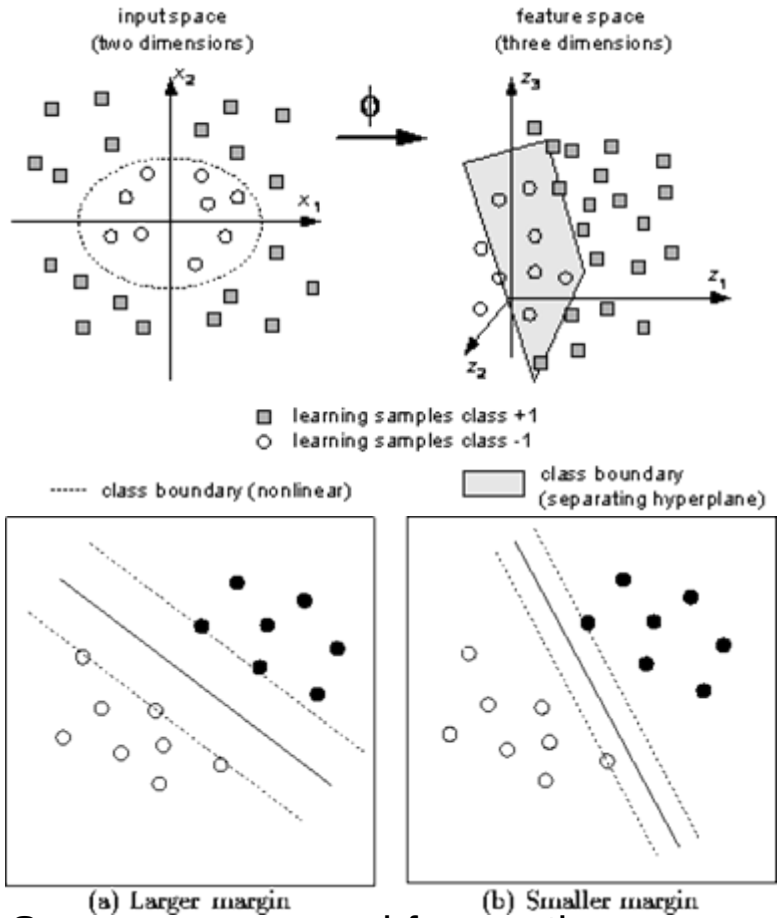
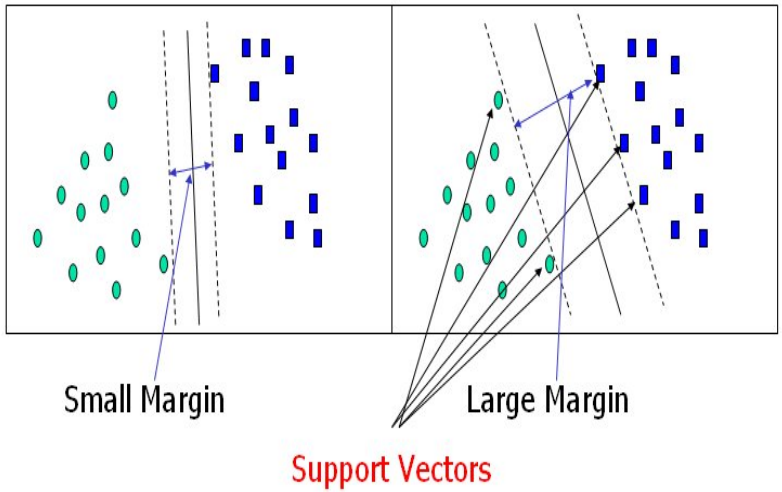
Suport Vector Machines



Source: www.neural-forecasting.com

Example: Mapping from two-dimensional input space with non-linear class boundaries into a three-dimensional feature space with linear separation by hyperplane. Working in high dimensional feature space solves the problem of expressing complex functions.



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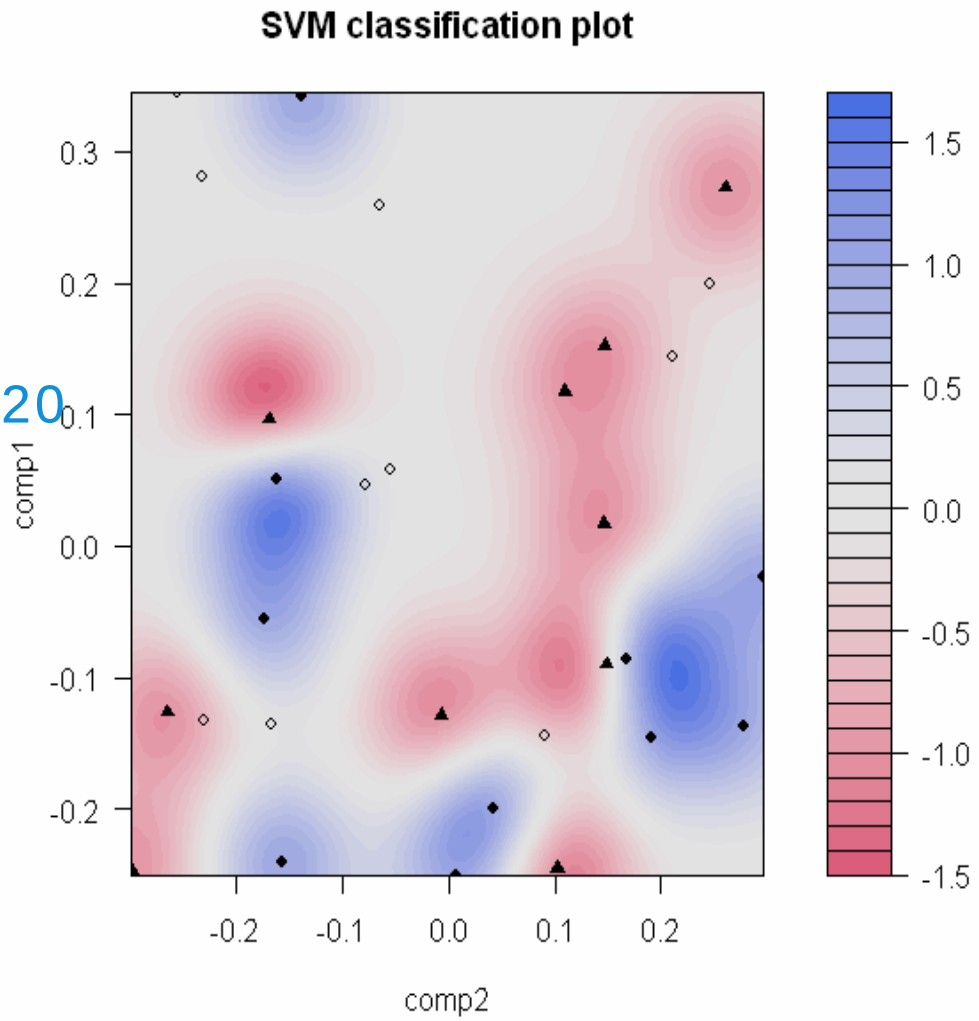
SVM GAUSSIAN RADIAL BASIS KERNEL

Gaussian Radial Basis kernel function.

Number of Support Vectors : 20
Training error : 0



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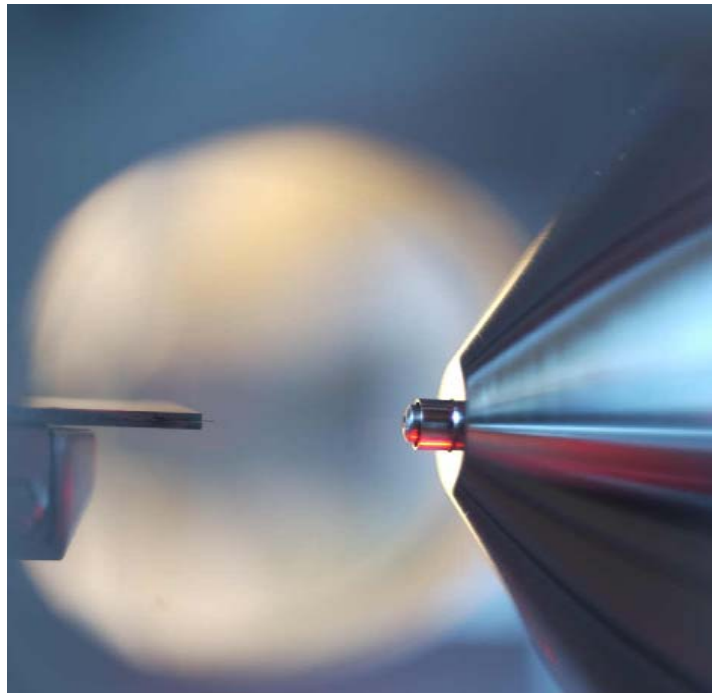
Verification & validation

Verification: We build the product RIGHT

Validation: We build the RIGHT product



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Main research outcomes

- an optimized chromatographic-electrospray **lab-on-chip** dedicated to protein profiling for cancer diagnosis

- an **Integrated Clinico-Proteomics Environment** supporting the integrated device and the diagnosi

- a **proof-of-concept** of this innovative lab-on-chip technology and the associated analysis chain for cancer diagnosis

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STRENGTHS & OPPORTUNITIES

- **STRENGTHS**
  - Manipulation of sample volumes in the nanoliter range.
  - High sensitivity
  - Gel free analytical chain
  - Advanced biomedical informatics systems
  - Miniaturization of protein detection processes
  - Protein quantification using MS and profile reconstruction algorithm.
- **OPPORTUNITIES**
  - Early diagnosis
  - The concept can be generalized to other cancers as well as to biomarker discovery.

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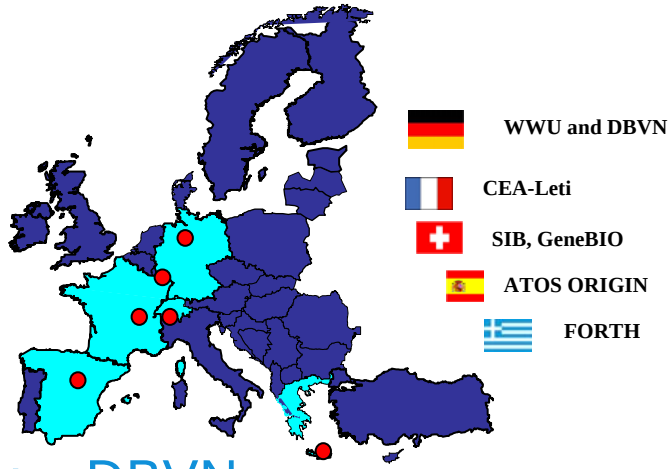
List of participants



- Atos Origin sae, Spain – ATOS
- Commissariat à l'Energie Atomique, France – CEA-LETI
- DIGILAB BIOVISION GmbH, Germany – DBVN
- Foundation for Research and Technology, Greece – FORTH
- University of Münster, Germany – WWU
- Swiss Institute of Bioinformatics, Switzerland – SIB
- Geneva Bioinformatics, Switzerland - GeneBIO

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Research Teams

- **ATOS Origin**: Manuel M Pérez-Pérez, Blanca Jordán, José F. Esteban and Carmen Reina
- **CEA-LETI**: Pierre Grangeat, Laurent Gerfault, Françoise Vinet, Christine Peponnet, Florence Ricoul, Régis Guillemaud, Grégory Strubel, Caroline Paulus, Nicolas Sarrut and Emeline Mery
- **DBVN**: Harald Tammen, Karl Schorn and Michael Jurgen
- **FORTH**: Dimitris Kafetzopoulus, Manolis Tsiknakis, Sophie Kaforou, Hara Roumpaki, George Potamias, Haris Kondylakis, Manolis Kalaitz, Vangelis Kritsotakis
- **WWU**: Jürgen Schnekeburger, Verena Schick, Jasna Peter-Katalinic, Laura Bindila, Rainer Ossig
- **SIB**: Frederique Lisacek
- **GeneBIO**:Pierre Alain Binz

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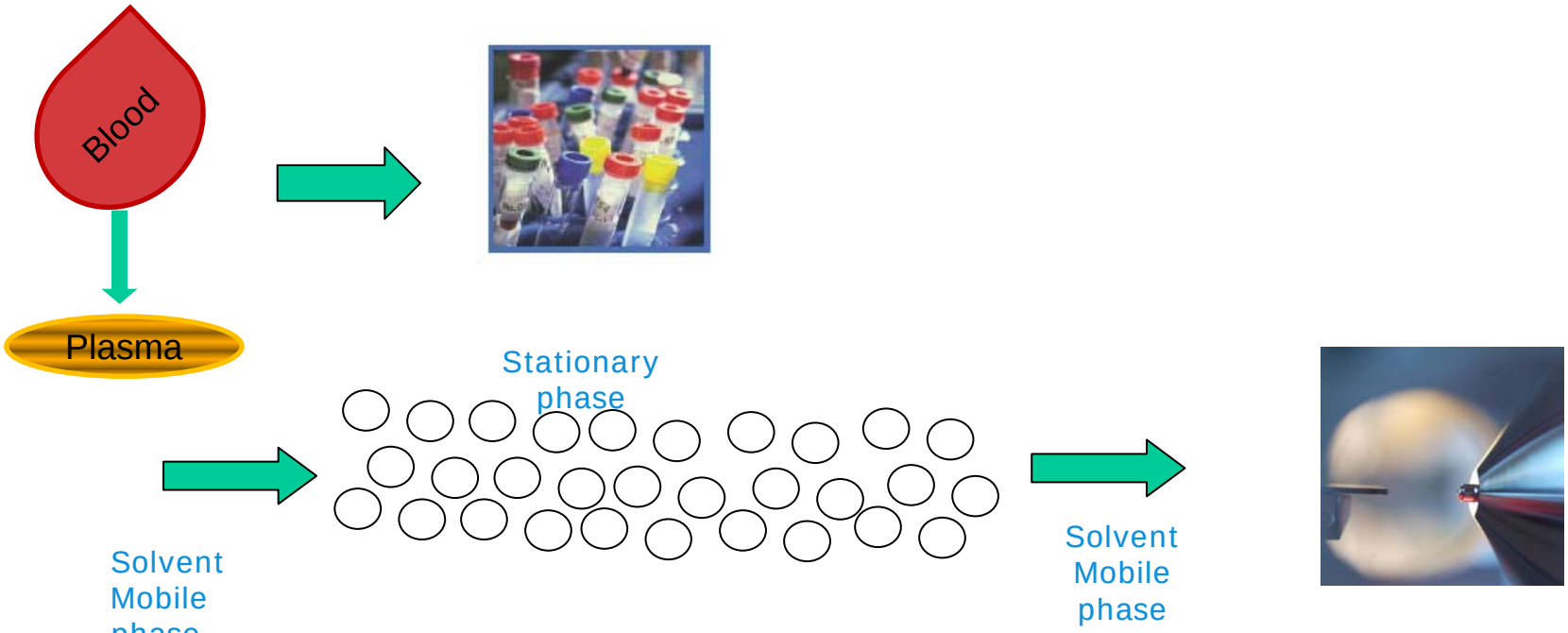
LC –mobile phase– stationary phase



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Nano (I)

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- Design of a new nano-liquid chromatography (LC) module including a new e-spray tip.
- New retention phase for chromatography module

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