



MASCOT

Integrated Microsystem for the magnetic isolation
and Analysis of Single circulating tumour Cells for
Oncology diagnostics and Therapy follow-up

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Background



Breast cancer tumour cells in peripheral blood (PB)/bone marrow (BM) and correlation to survival 3-5 years after diagnosis

BUT: Detection of rare circulating tumor cells is not straightforward



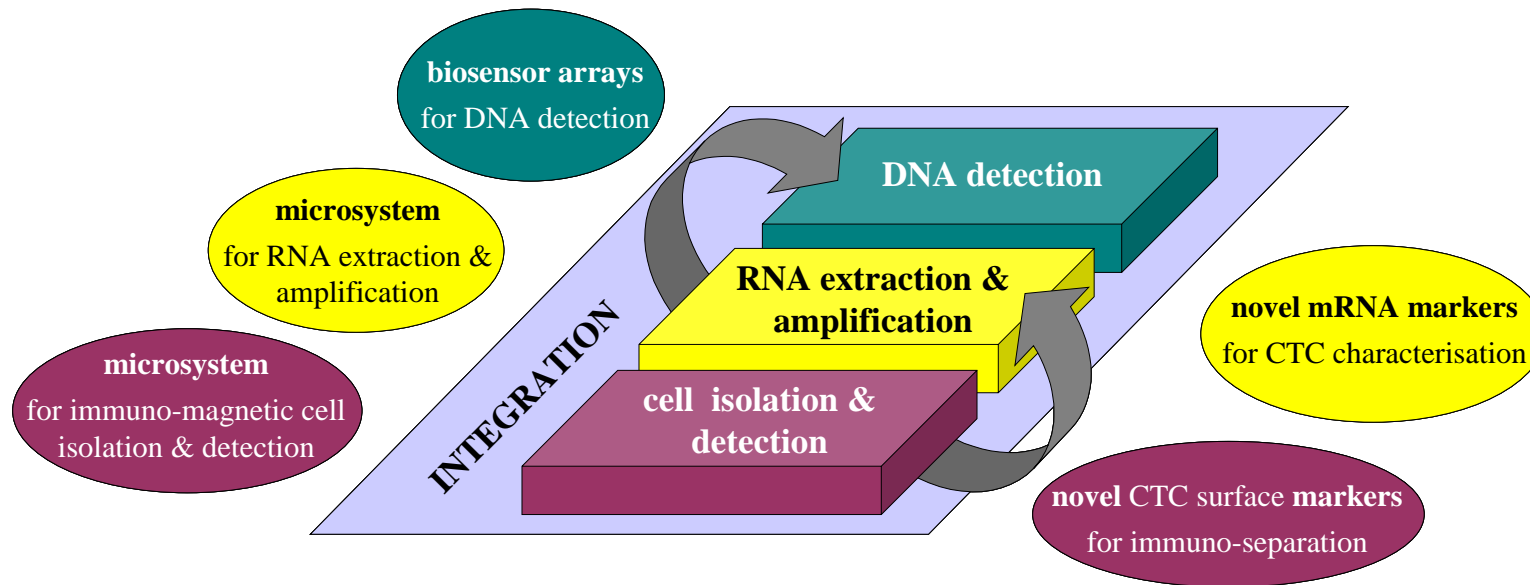
Only 2-3 tumor cells present in 5 mL of blood ($\sim 1 \cdot 10^7$ cells)



Objectives: solution



Overall: development of minimally invasive diagnostic platform for the isolation, enrichment and identification of rare circulating tumor cells





MASCOT approach



- **(1) Isolation, enrichment and detection of CTCs**
 - Be able to start from large sample and feed selected cells into microsystem (use of magnetic bead based isolation)
 - Develop magnetoelectronic chip for tumor cell isolation/counting
- **(2) Extraction, amplification and detection of mRNAs**
 - Amplification strategy for multiplexed mRNA amplification of cancer markers (use of MLPA amplification)
 - Develop multiplexed detection of amplified mRNAs (use of electrochemical biosensor array)
- **(3) Integration of all different operations into total analysis system**



Applications/potential



Cancer diagnostics:

- Clinical applications:
 - Early diagnosis/case finding
 - **Prognosis/staging**
 - **Therapy monitoring/disease recurrence**
- Applicable to all solid cancers
 - MASCOT focused on breast cancer in blood

Clinical studies <-> routine clinical use



Existing solutions



Detection of CTCs: existing techniques

- Centrifugation, followed by immunostaining and microscopic inspection
- Combination of enrichment techniques with microscopy, e.g. CellSearch (Veridex)
- Combination of enrichment techniques with RT-PCR, e.g. AdnaTest (AdnaGen)

=> Labour intensive, relatively slow and expensive prohibiting the detection of CTCs in clinical practice

MASCOT: integration into one platform



MASCOT Consortium



Partners & their roles:

- IMEC: magnetic cell isolation and detection
- URV: electrochemical genosensor arrays
- FDAB: antibodies for CTC surface markers
- IMM: microfluidics and integration of modules
- AGEN: benchmarking of modules and full system
- RR HF: clinical samples and evaluation
- MRCH: multiplexed DNA/RNA amplification

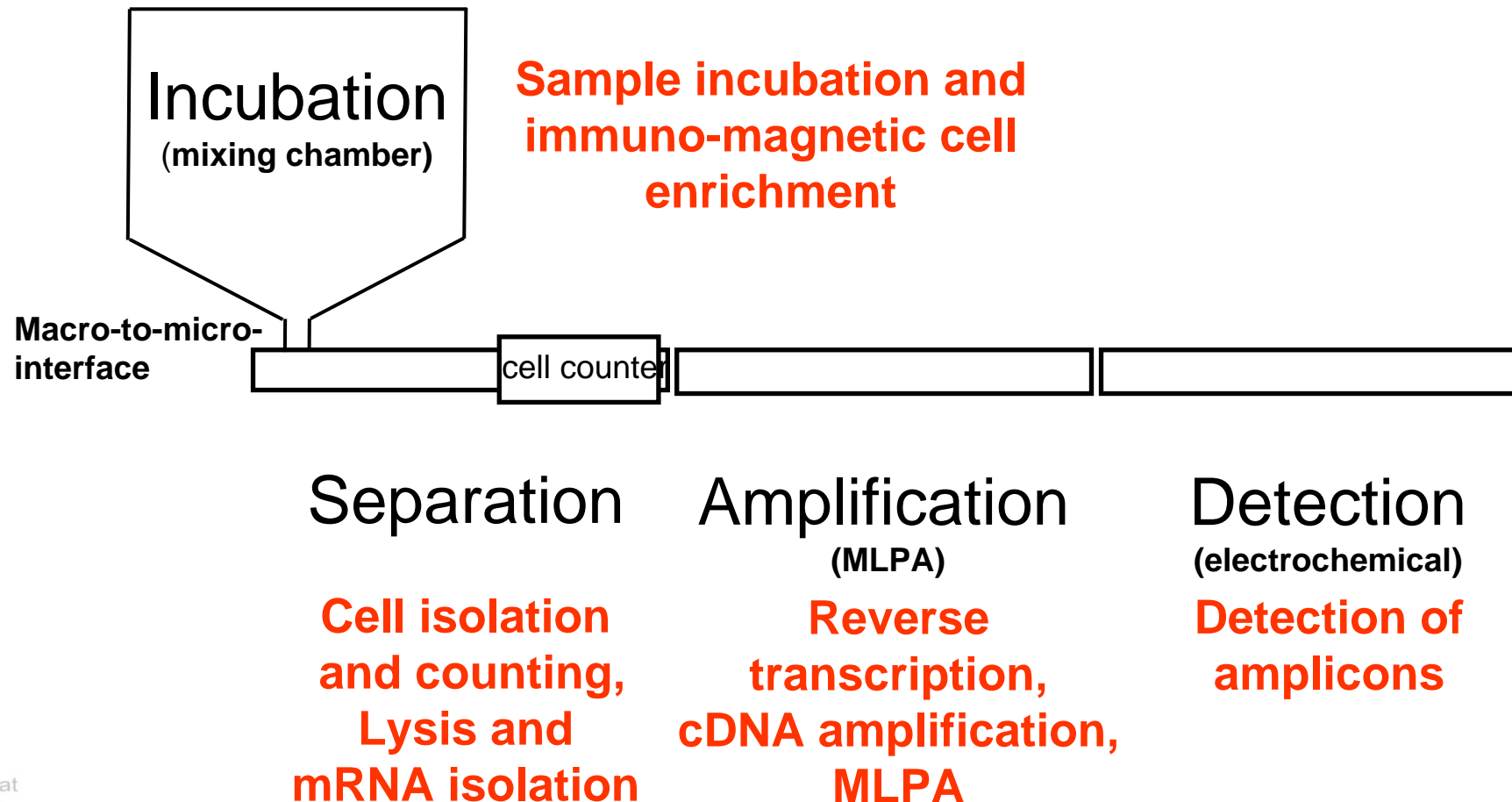
<http://www.imec.be/mascot>



System design



Modular set-up:

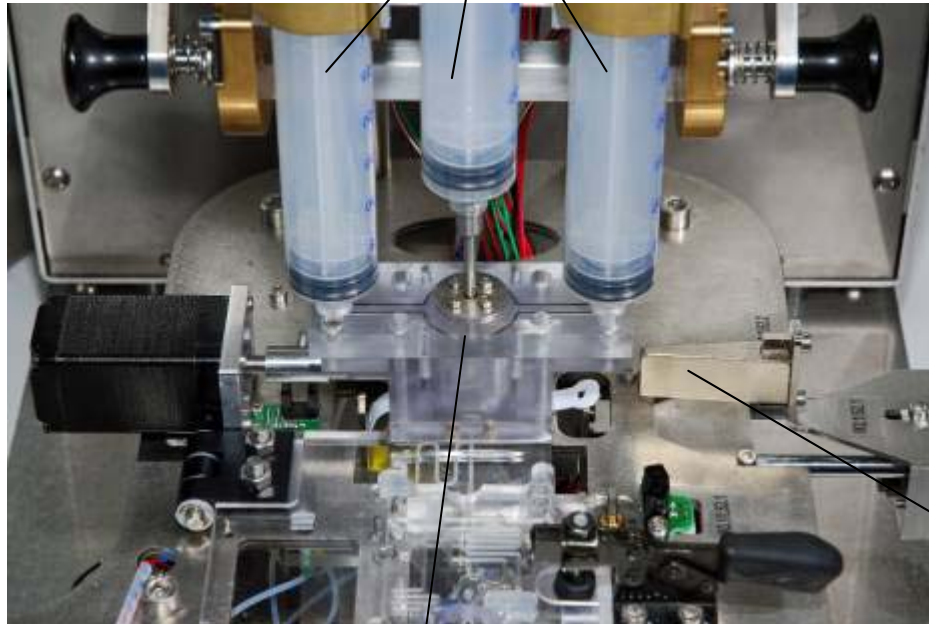




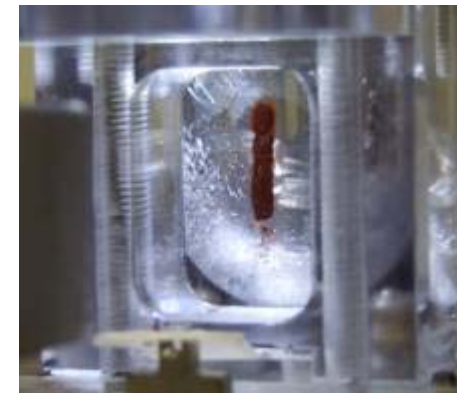
Main results: Incubation and isolation



Automatically controlled syringes



Paddle for mixing



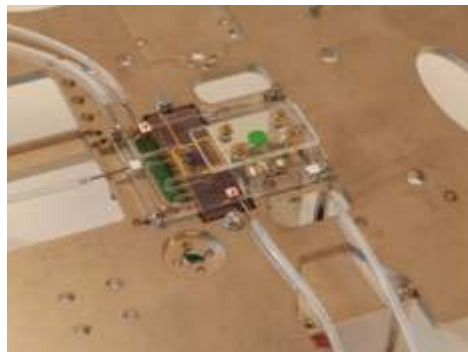
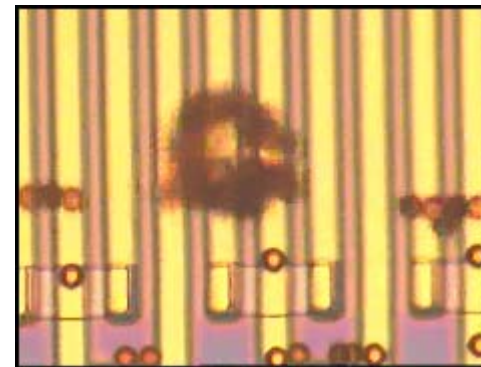
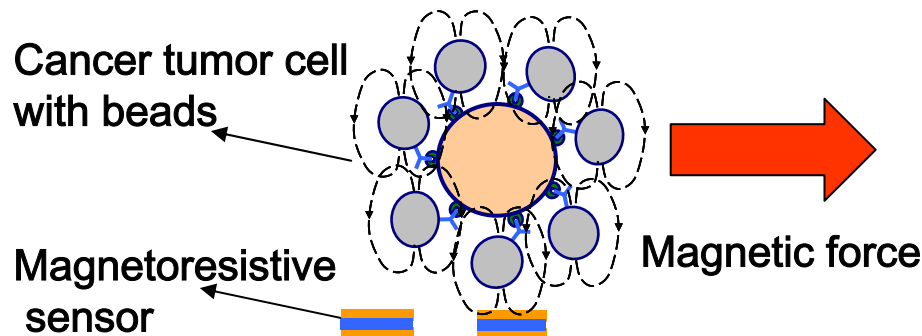
magnet



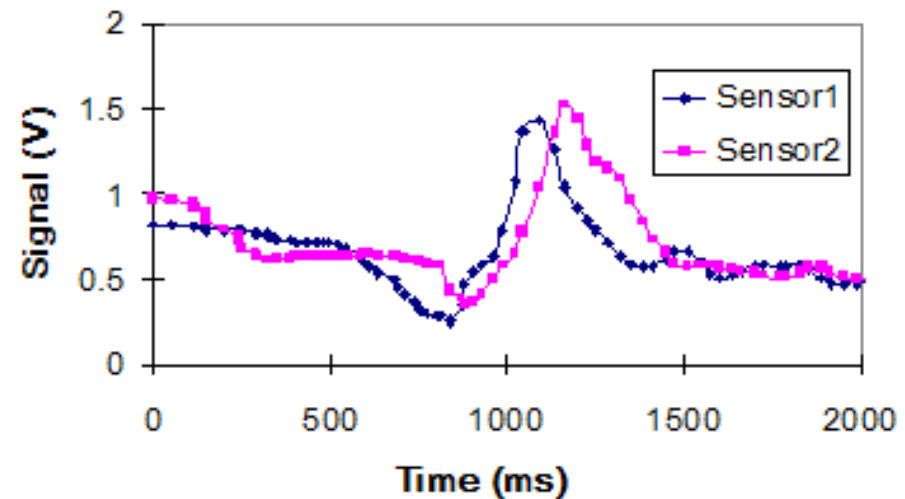
Main results: Cell counter



Implementation of cell counter chip by transporting and counting magnetic particles

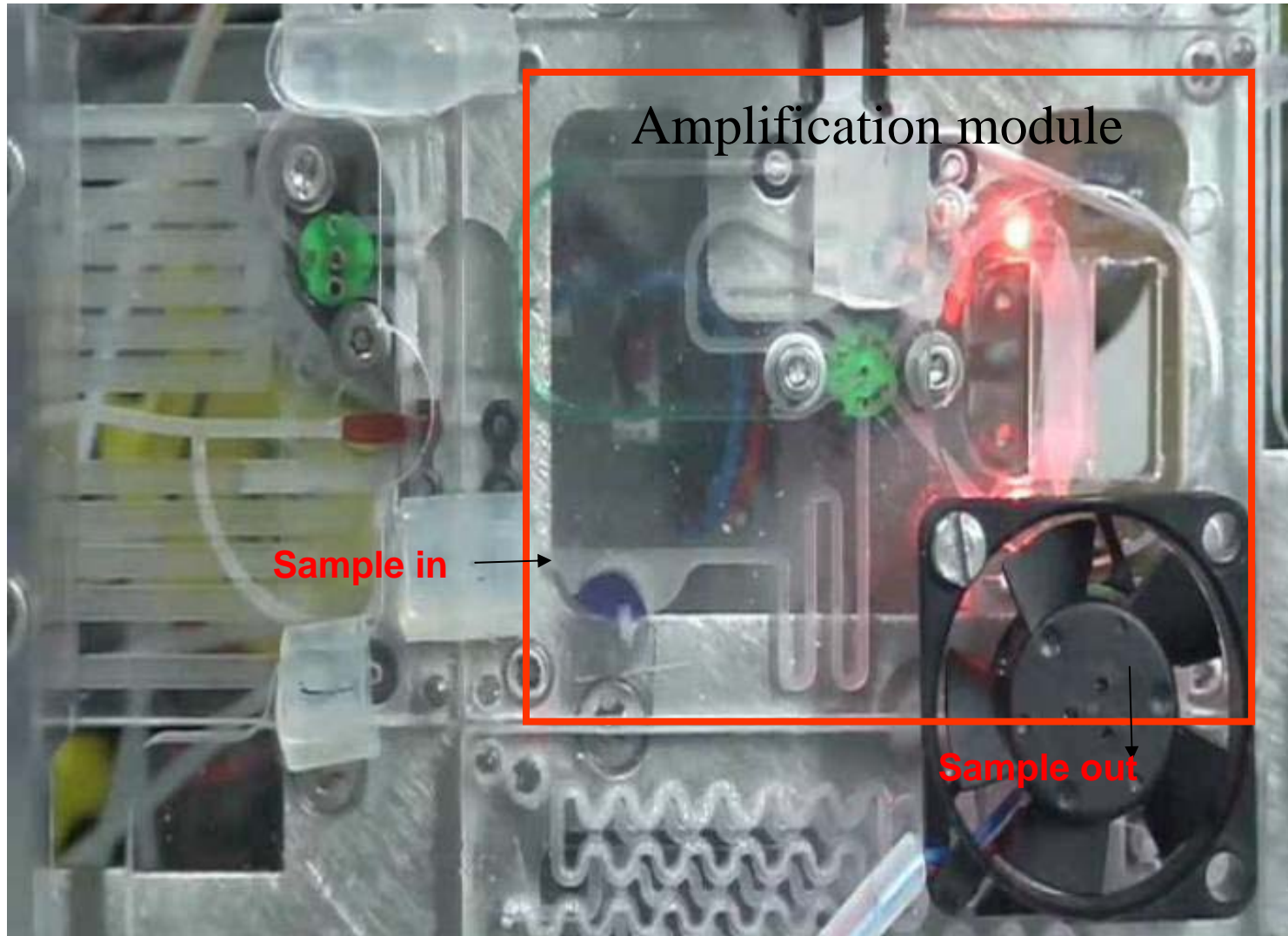


Signal from adjacent spin valves





Main results: Amplification



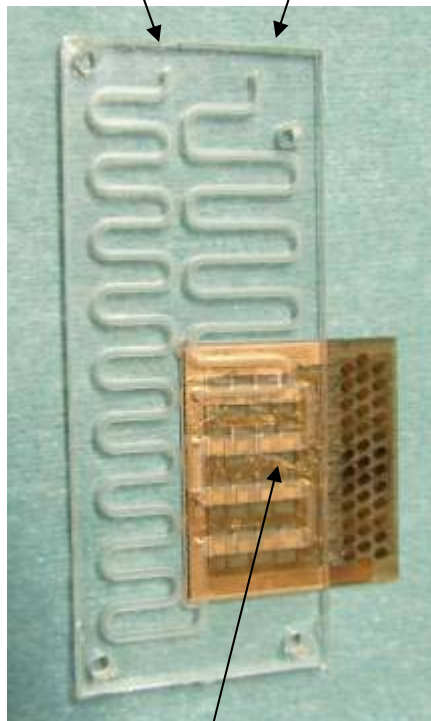


Main results: Detection

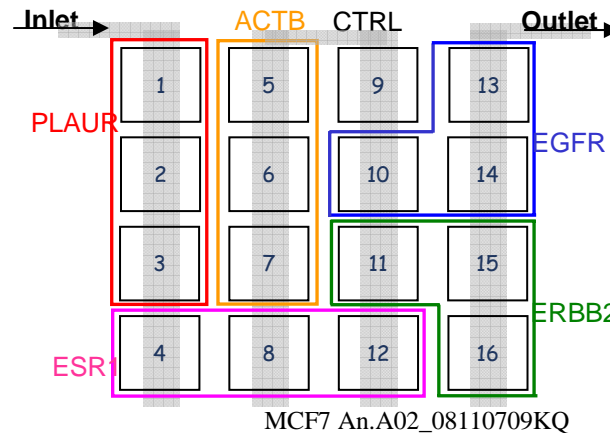


Implementation of MASCOT breast cancer mRNA marker kit on sensor array

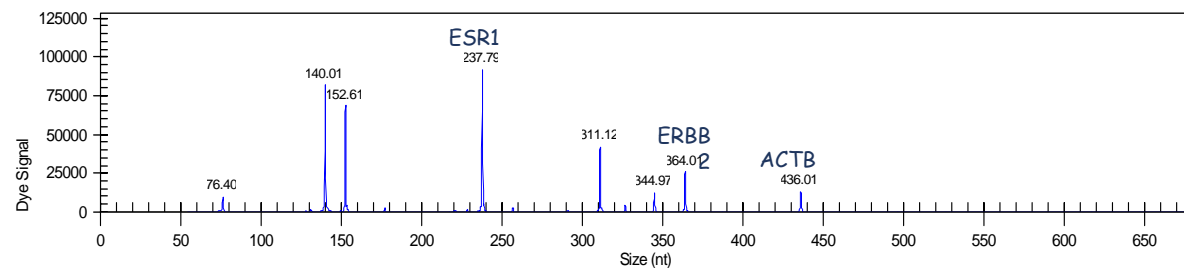
Inlet from reservoir chip
Outlet to waste



Electrode array (4x4 electrodes) underneath



Electrochemical sensing



- Quantitative assay on breast cancer markers from spiked samples
- Detection limits 50 pm
- Reduce non-specific binding

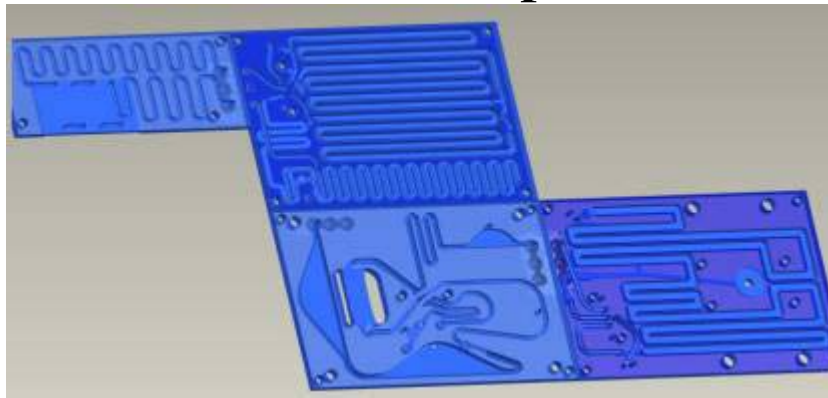


Main results: Integration

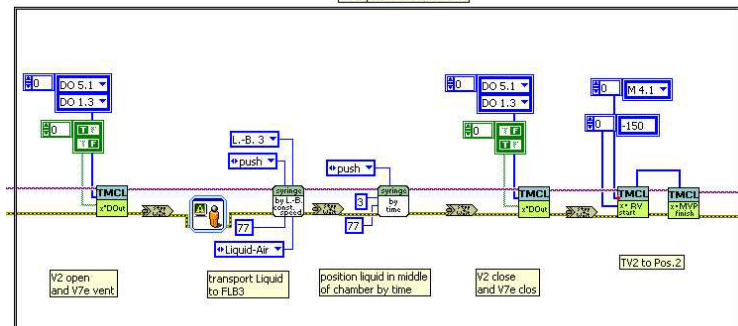


Integration of modules into integrated system

Fluidic chips

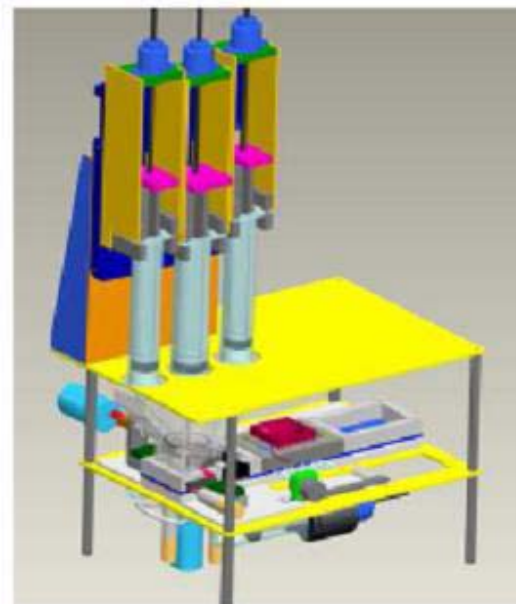


Transport to PCR-chamber

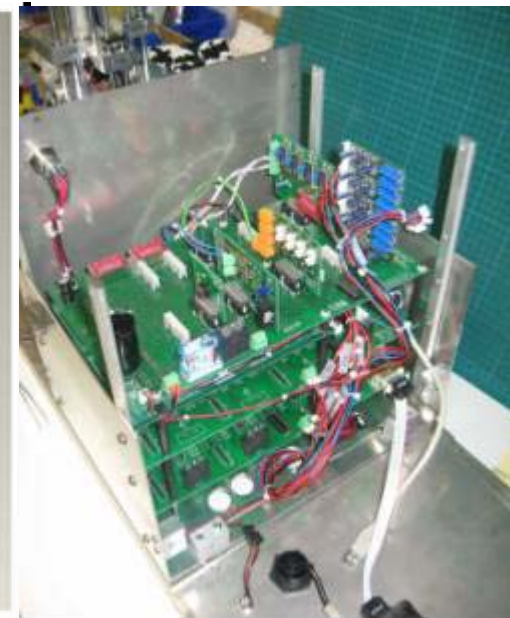


User software

From design to implementation
In fully automated benchtop instrument



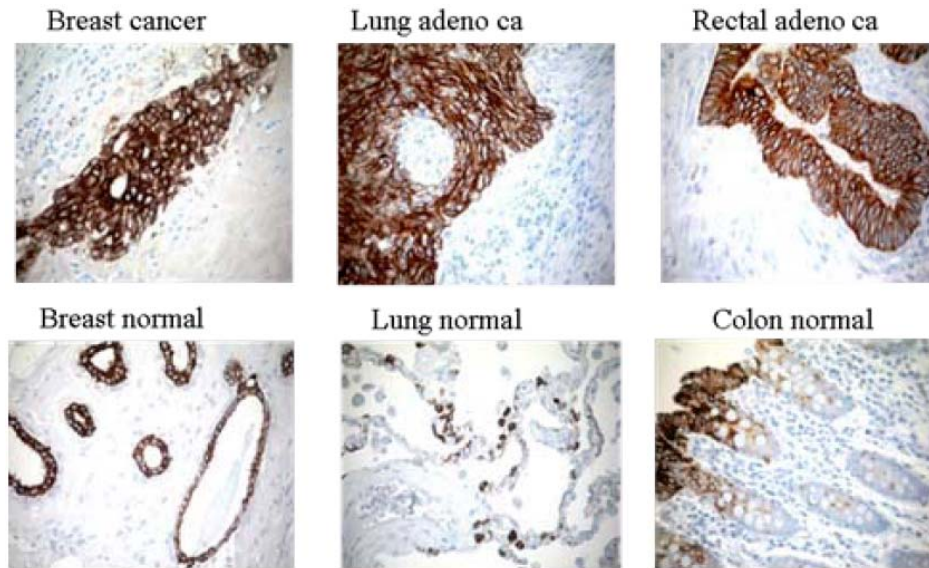
Mechanics



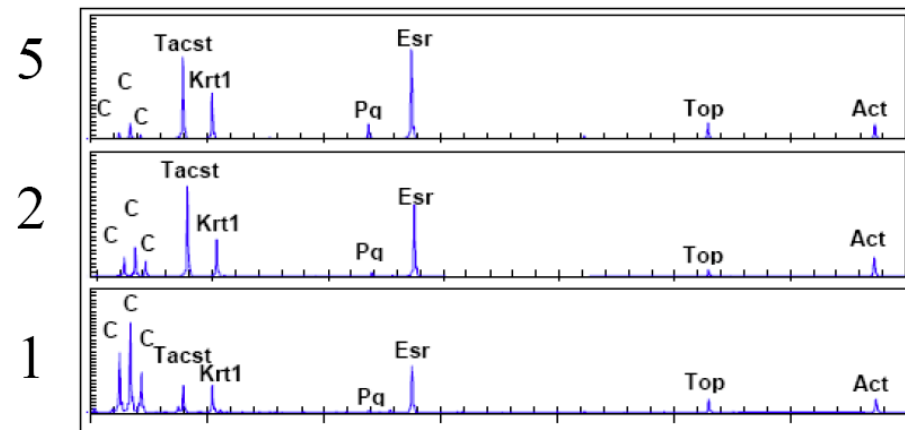
Electronics



Breast cancer marker discovery



Cell marker research resulted in discovery of monoclonal antibodies against breast, lung, rectal cancer



Genetic marker research resulted in 20 gene breast cancer diagnostic kit and a multiplexed amplification protocol (MLPA) with single cell



Benchmarking



- Use of integrated system and comparison to golden standards
 - For spiked samples (blood with # cancer cells)
 - At individual module level
 - Incubation and separation
 - MLPA and detection
 - For integrated system
 - On limited set of patient samples



Use and dissemination



Dissemination through publication in high level international journals

Such as: Biosensors and Bioelectronics, Appl. Phys. Lett., Journ. Appl. Phys., Langmuir, Electro-analysis, Electrochemistry comm., Anticancer research

Exploitable Results

- ADNAGEN's wash buffer: patented, commercialized
- MRCH's MLPA breast cancer kit: available for commercialization
- Fujirebio's novel breast cancer cell markers: 4 patents, available for commercialization
- Integrated Platform: Patents on different technical aspects of system implementation by IMM/IMEC/URV (1 filed, more underway)



Nanobioconcertation



Identifying opportunities for follow up projects

- Highly functional lab on chip microsystem
- Still many technological challenges ahead
 - Robustness of on-chip chemical reactions. Understanding of role of materials and fabrication techniques, especially in small fluidic volumes;
 - Biosensor sensitivities and specificities
 - Multimodular systems with increased functionality and complexity
 - Increased information content
 - Reduce cost by heterogeneous integration and assembly technologies
- Full clinical validation e.g. by use of platform together with conventional methods in therapy follow up testing
- Novel applications/exploitation routes for MASCOT platform