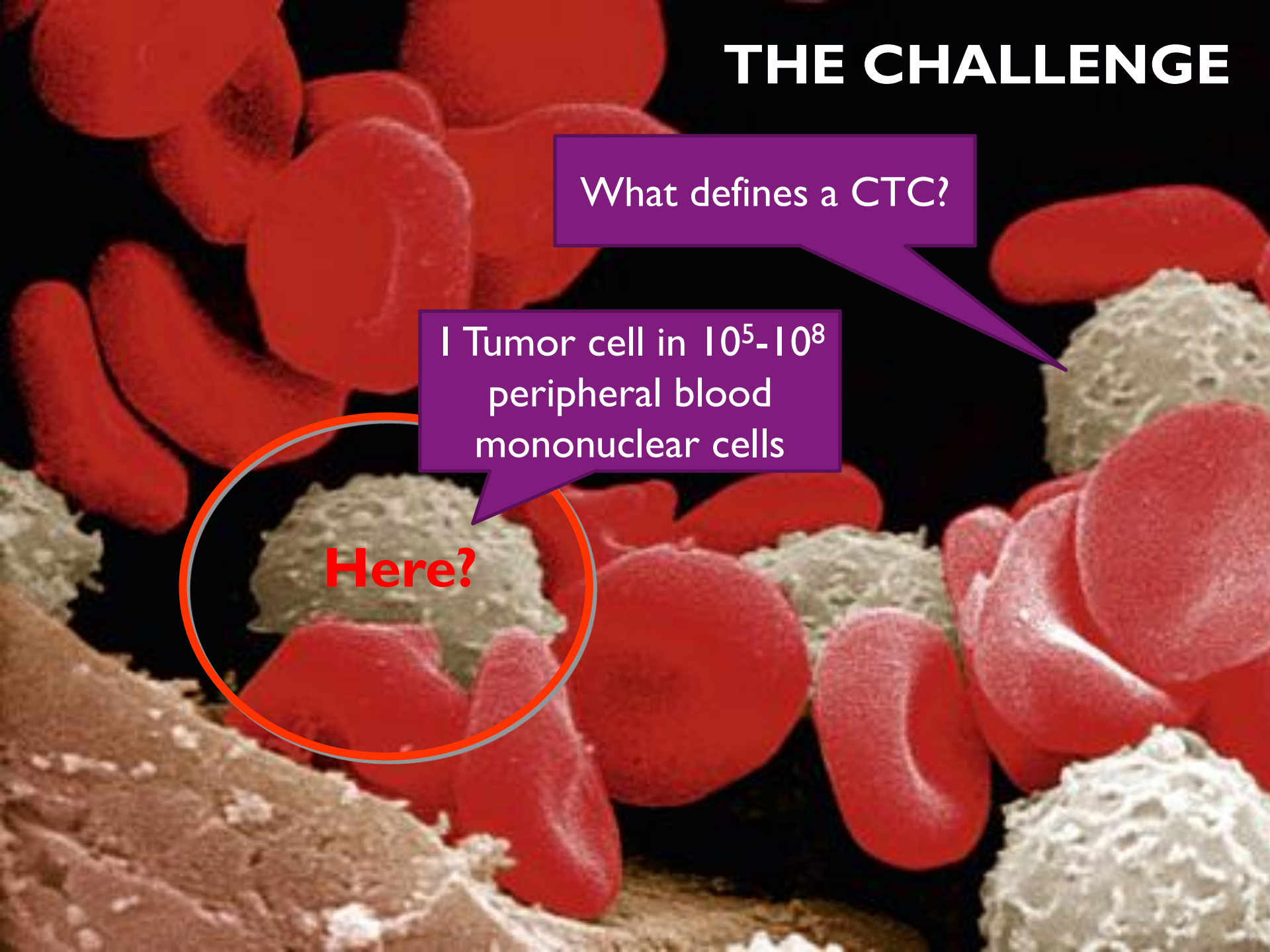
MIRACLE UPDATE

HERC NEVES

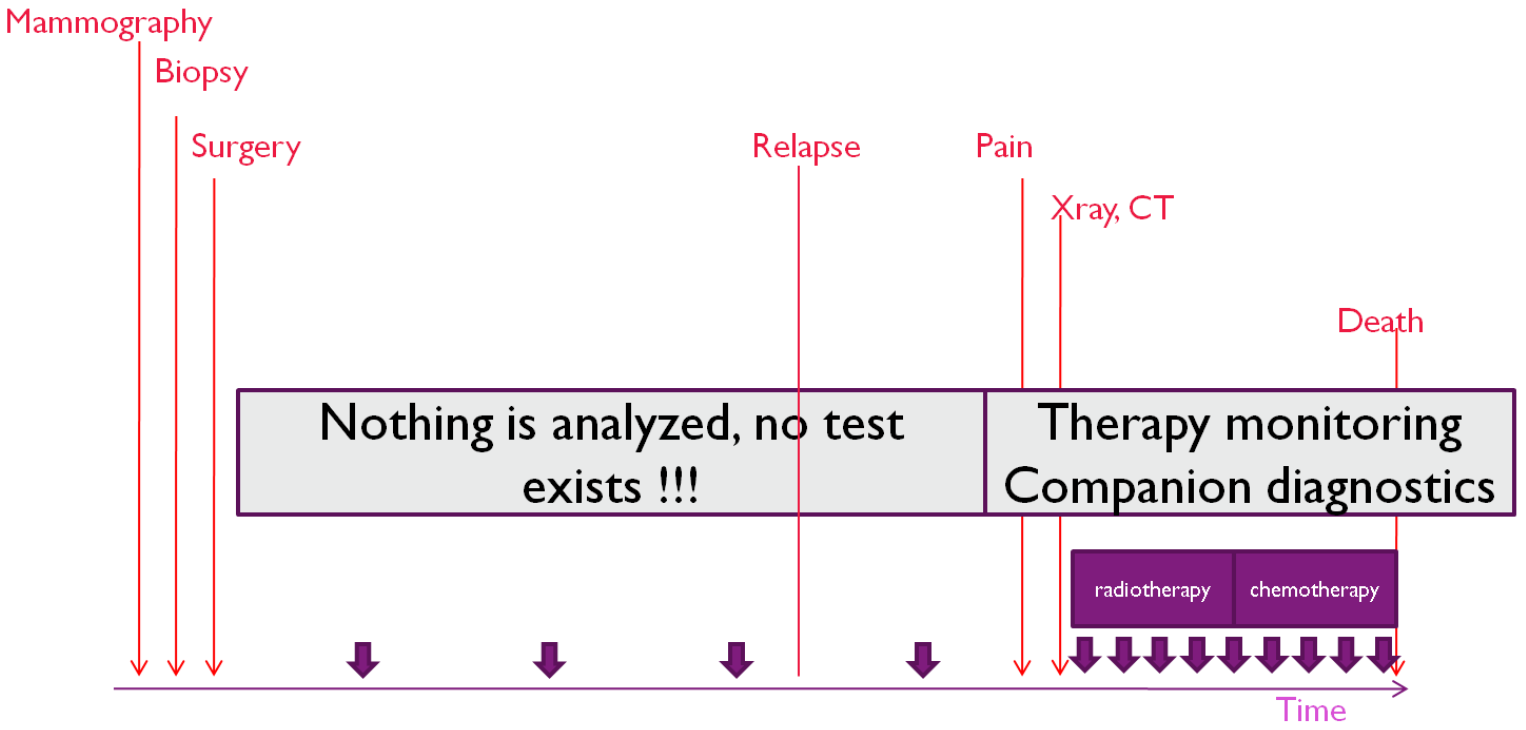
MIRACLE (FP7 – 257743)


Where is Waldo?

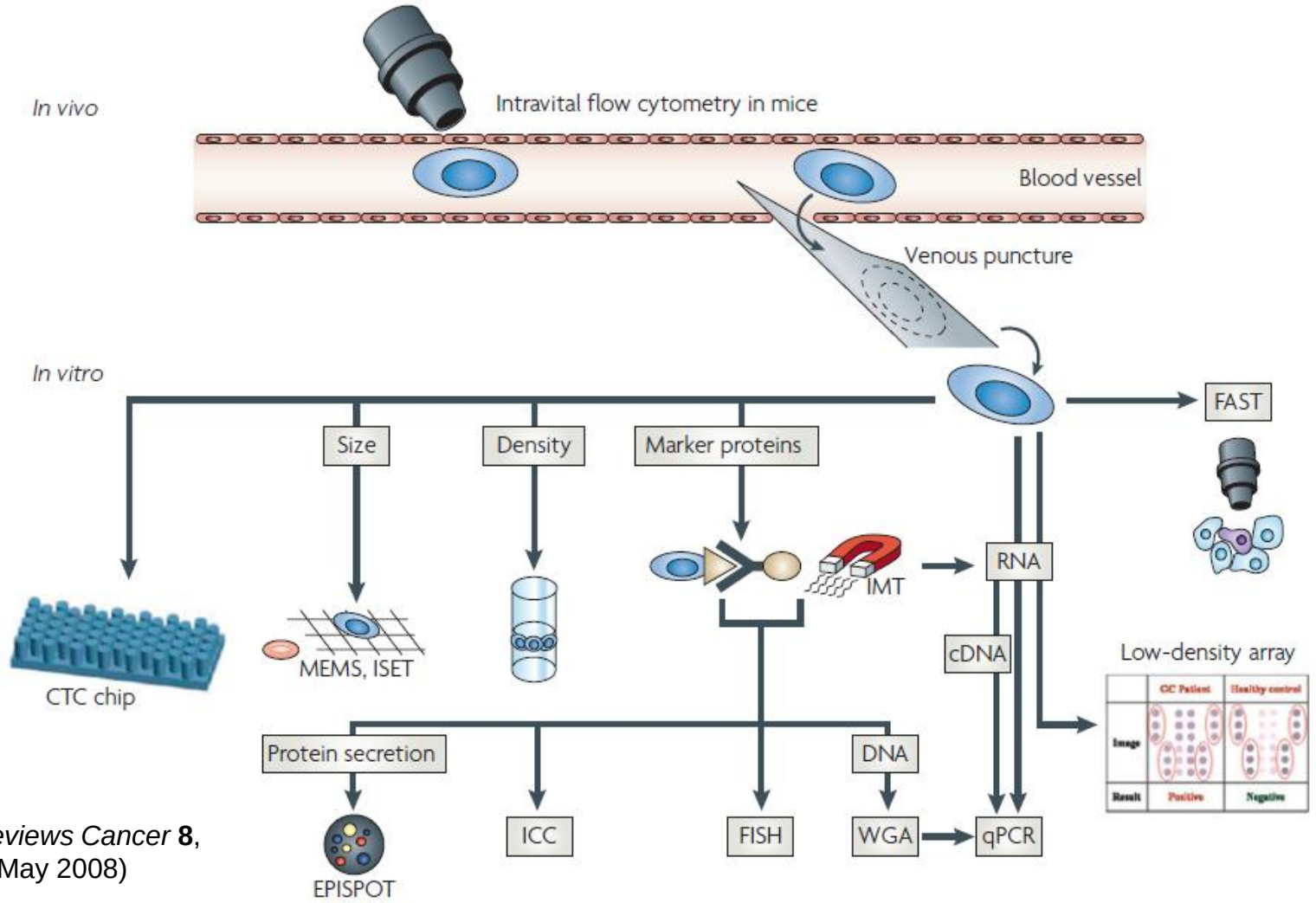

THE CHALLENGE
What defines a CTC?
1 Tumor cell in 10^5-10^8 peripheral blood mononuclear cells
Here?

CANCER MANAGEMENT TODAY

Regular blood biopsies would improve cancer management



STATE OF THE ART techniques



Nature Reviews Cancer **8**, 329-340 (May 2008)

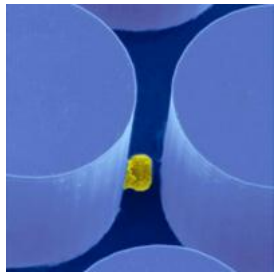
STATE OF THE ART

Veridex CellSearch

- **Immunomagnetic extraction using anti-EpCAM-fuctionalized beads (Biomarker based)**
- De facto approach for clinical CTC work
- FDA approved

CTC-Chip under development

- Capture of CTCs using microposts functionalized with EpCAM antibodies inside microfluidic system
- **Biomarker based**

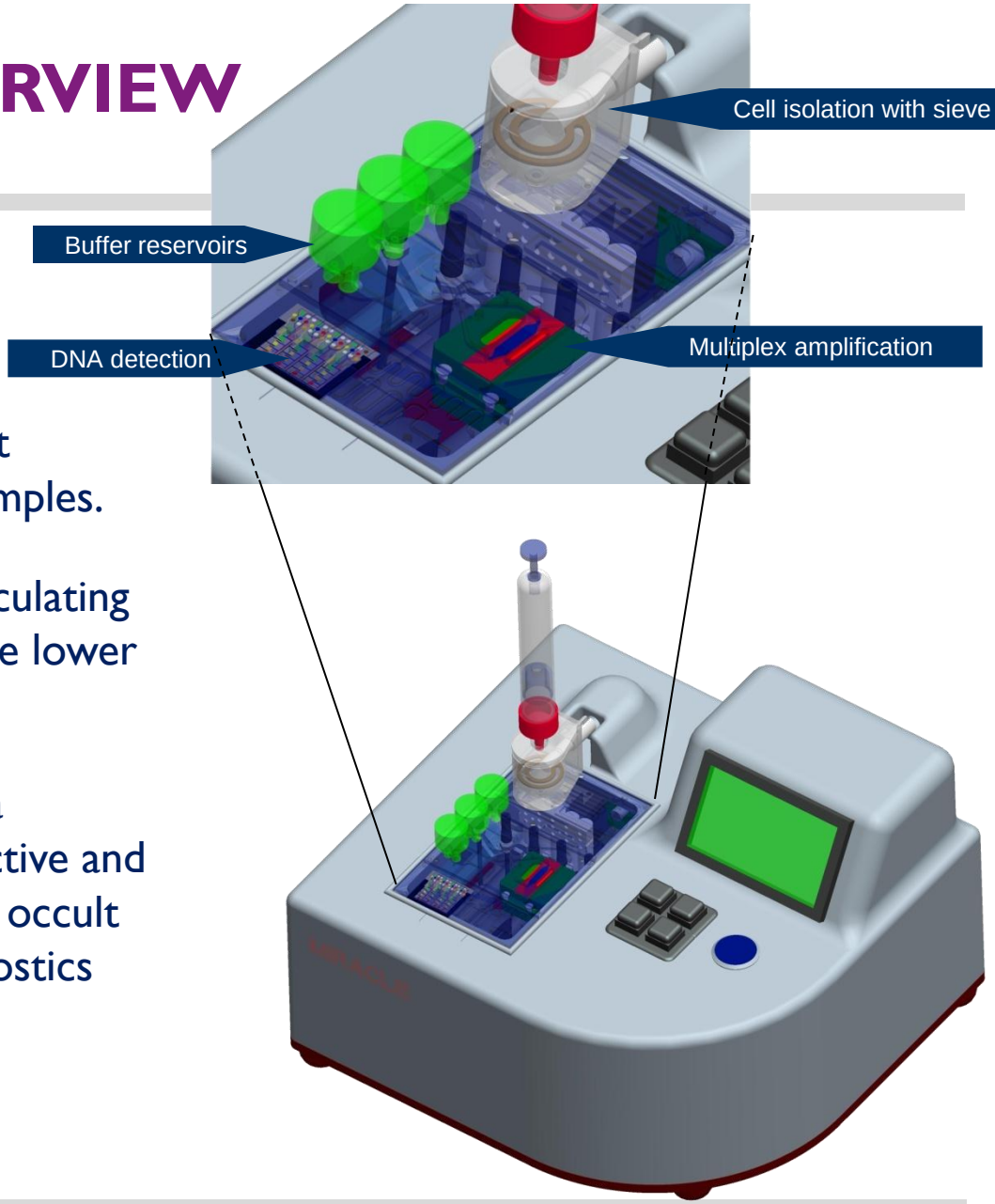


Other potential CTC isolation techniques

- Size-based (some CTCs are bigger than normal blood cells)
- Acoustophoresis (CTCs are more compressible)
- Cell deformation based (CTCs are less deformable)
- Electrical impedance based (CTC membrane has higher capacitance)

MIRACLE OVERVIEW

- **AIM**: A smart miniaturized system for the isolation, counting and molecular characterization of occult tumor cells directly from clinical samples.
- **CHALLENGE**: The number of circulating and disseminating tumor cells can be lower than 1 per mL of sample.
- **IMPORTANCE**: Cancer remains a prominent health concern. An objective and automated methodology to analyze occult tumor cells simplifies current diagnostics and improves therapy management.



MIRACLE OVERVIEW

- Key objective: Complete integrated platform
  - Direct processing of clinical samples (blood & bone marrow)
  - Single cell sensitivity (macro to micro interface)
  - Hetero-integration (combining silicon & polymer technology)
  - Multi-type assays (cell assay, RT-PCR and multiplex DNA amplification, DNA detection array)
- Consortium is ideally positioned
  - All necessary partners on-board
  - Relevant expertise based on previous projects (jump start)
  - No less than 7 industrial partners to ensure exploitation routes
- Enables strong European impact & dissemination
- Continuous clinical evaluation / design / integration

PROJECT OVERVIEW

Clinical sample

- 7.5 mL peripheral blood or bone marrow
- Down to 1 CTC per mL in blood and DTC in bone marrow

Cell enrichment

- Micro/nano magnetic particles for immunomagnetic cell isolation
- Specific capture toward multiple cell markers (EpCAM, MUC1, etc.)
- Macro- to micro- fluid interfacing

"Active sieve" for single cell characterization

- Micro pore array for cell isolation (10^4 pores, Ø < 4µm)
- Integrated transistors for every pore
- CTC/DTC identification & quantification by impedance analyses
- Electrical cell lysis

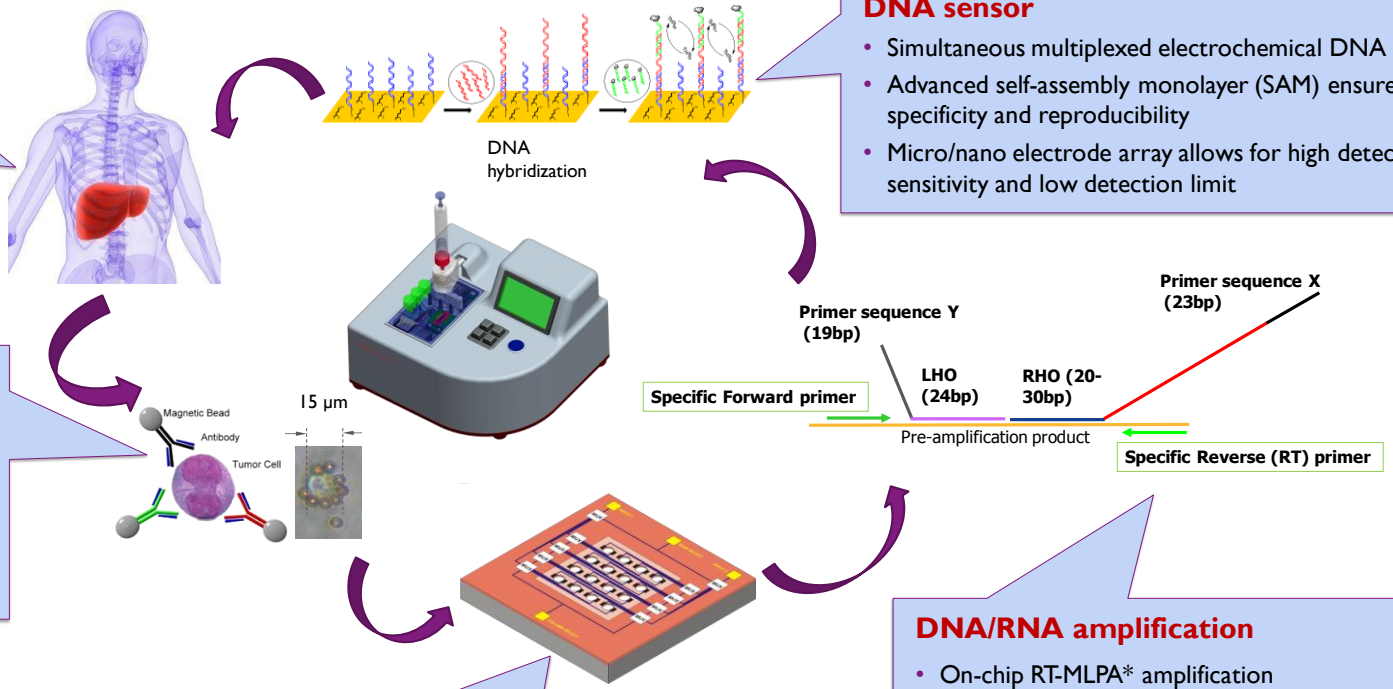
DNA sensor

- Simultaneous multiplexed electrochemical DNA detection
- Advanced self-assembly monolayer (SAM) ensures specificity and reproducibility
- Micro/nano electrode array allows for high detection sensitivity and low detection limit

DNA/RNA amplification

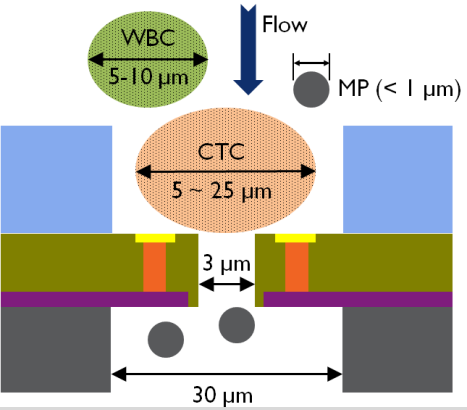
- On-chip RT-MLPA* amplification
- ~21 genes selected for breast cancer
- ~15 genes selected for prostate cancer

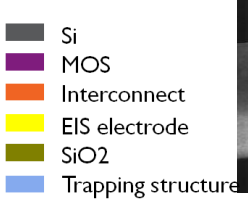
* Multiplexed Ligand-dependent Probe Amplification (MLPA)

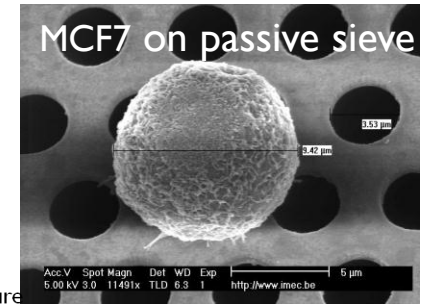


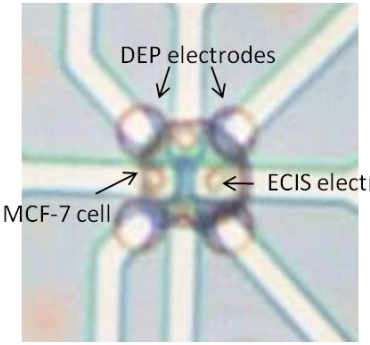
Highlight year 1

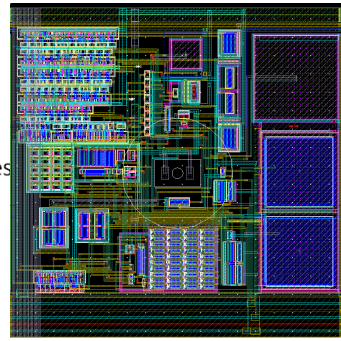
- WP 1: Cell capture & analysis using active sieve
  - Immunomagnetic cell isolation method/device assessed and selected
  - New antibodies are selected, 300 nm magnetic beads for improved kinetics being tested.
  - Passive chip successfully fabricated and cancer cells retained by sieve
  - Multiple functions (single cell positioning, single cell lysis) demonstrated on test-chips.
  - First version active sieve was designed.







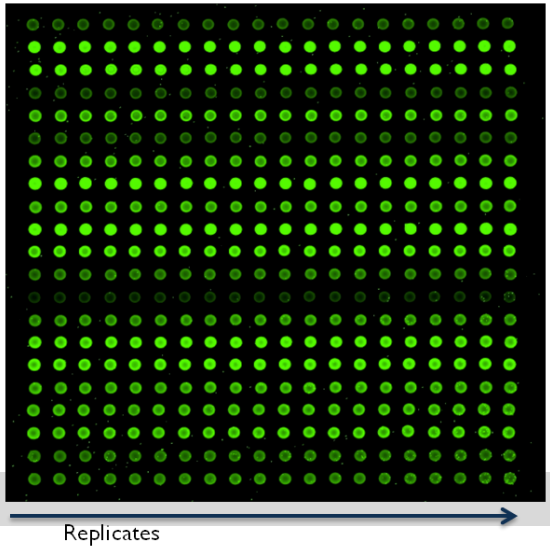




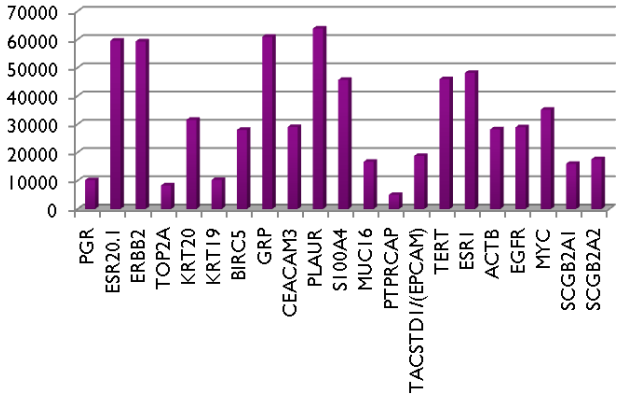
Highlight year 1

- WP 2: RT-MLPA amplification
  - Selection of 21 breast cancer markers and 15 prostate cancer markers
  - RT-MPLA breast cancer kit adapted with unique barcodes for detection
  - 500 PCR test slides manufactured and delivered
  - RT-MLPA realized on chip for 21 target breast cancer kit

MLPA on-chip with control DNA



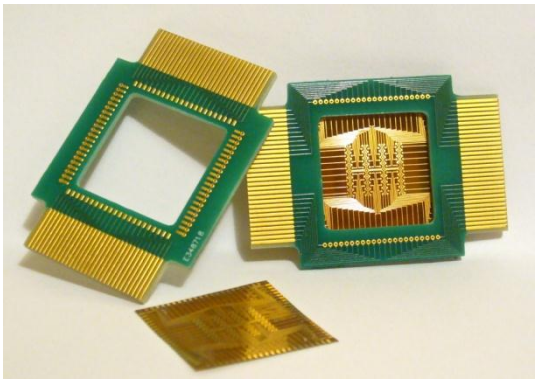
Control DNA on-chip

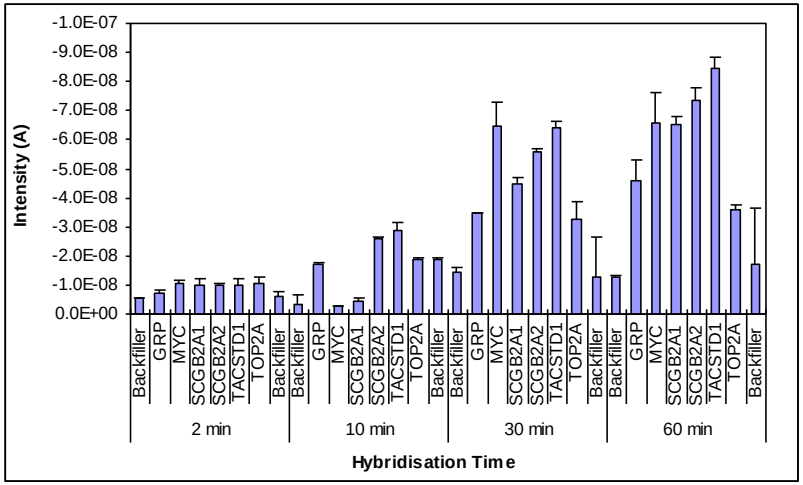


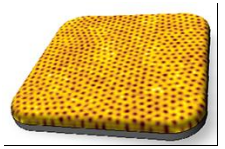
All 21 targets are detected!

Highlight year 1

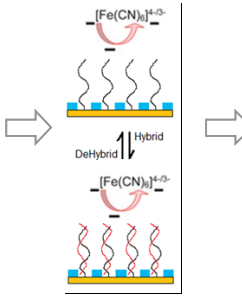
- WP3: DNA electrochemical detection array
  - PCB-mass manufacturable electrode material tested and optimized.
  - Surface chemistry optimized and enzyme based electrochemical assay fully developed; demonstrated by 5 amplicons amplified from MCF-7 cells
  - Several types of nano-electrodes tested for improved sensitivity







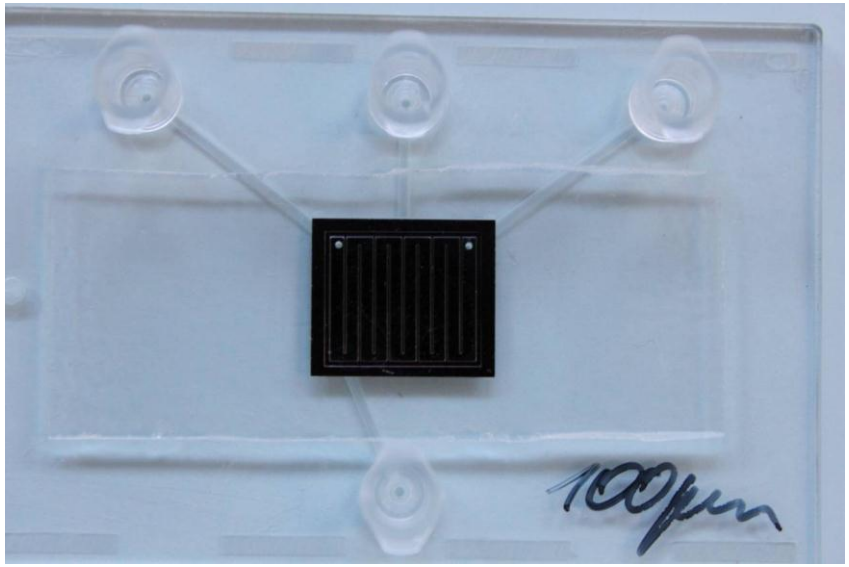
Nano- structure

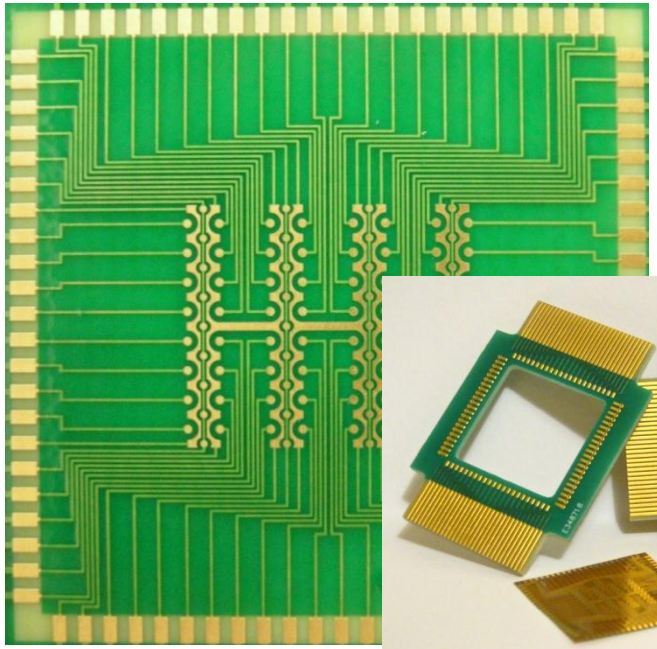


Nanoelectrode array

Highlight year 1

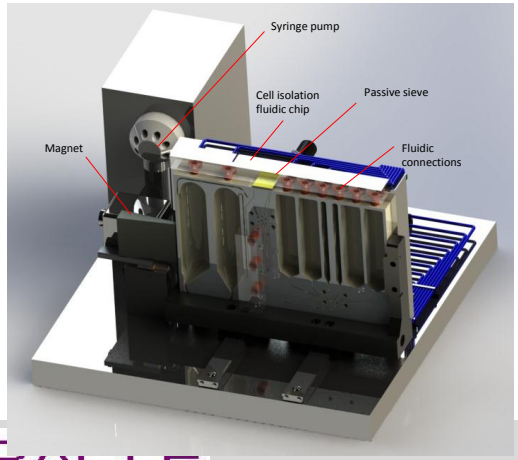
- WP 4: Heterogenic Assembly
  - Active sieve packaging in microfluidic chip; successful front-side SU8 bonding test
  - Development of Au plating process, PCB design & production for the DNA sensor.

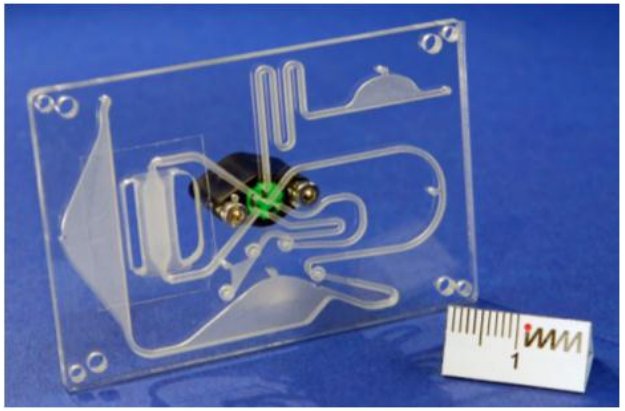




Highlight year 1

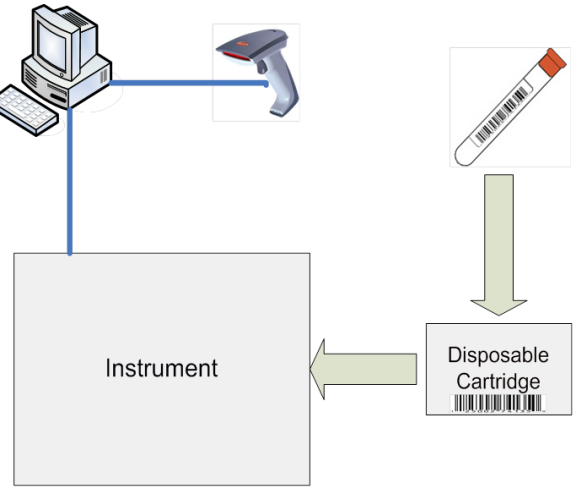
- WP 5: System Fluidic Integration
  - Incubation module prototype ready; MLPA prototype ready
  - Progress on valves, thermal control with embedded PID
  - Reagent storage test slide designed and samples manufactured, platform ready for testing with assay reagents
  - Embedded 'bare-bones' instrument for development of PC based software
  - Definition of initial parameter set, database structure for User INterface



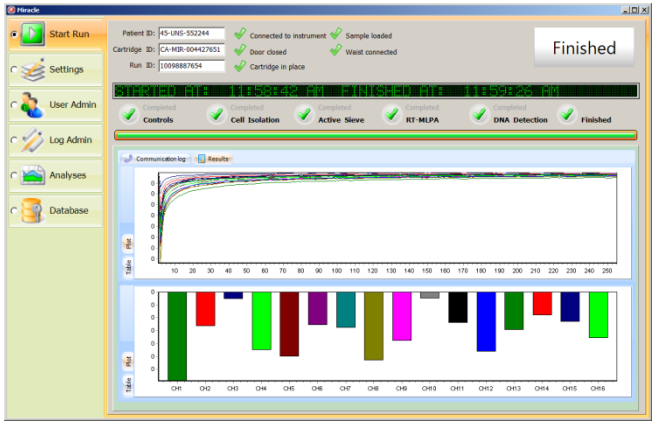


Highlight year 1

- WP 6: Instrumentation & User Interface
  - User-requirement was drawn. System design reported
  - Central controller and other electronic selected
  - User Interface Requirements drawn and first version (Gene detection) available
- WP 7: Benchmarking and clinical validation
  - 300 nm beads functionalized and tested for 5 MCF-7 cells per sample
  - MLPA kit benchmarked off-chip for breast cancer



Rabbit RCM4200



Highlight year 1

- Management
  - Project management handbook
  - Timely communication strategy: consortium meetings, WP meetings, monthly WP teleconferences, etc.
  - Administrative and financial support for partners
  - Project reporting to EC and reviewers
  - Organization of Advisory Board/Feedback of key opinion leaders collected

Highlight year 1

- Exploitation
  - An exploitation committee has been established
  - A list of exploitable results was defined as a start basis for IP tracking - Briefing doc.
  - Based on the regulatory review a CE marking route has been identified.
  - A competitive market analysis was performed.
- Dissemination
  - 8 conferences and 5 journal papers
  - MIRACLE workshop at the ECCO congress
  - Miracle flyers distributed
  - Project website, press release and newsletters

Please check http://www.miracle-fp7.eu/