



CTC isolation & analysis in an integrated microsystem

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Challenges for CTC isolation

General

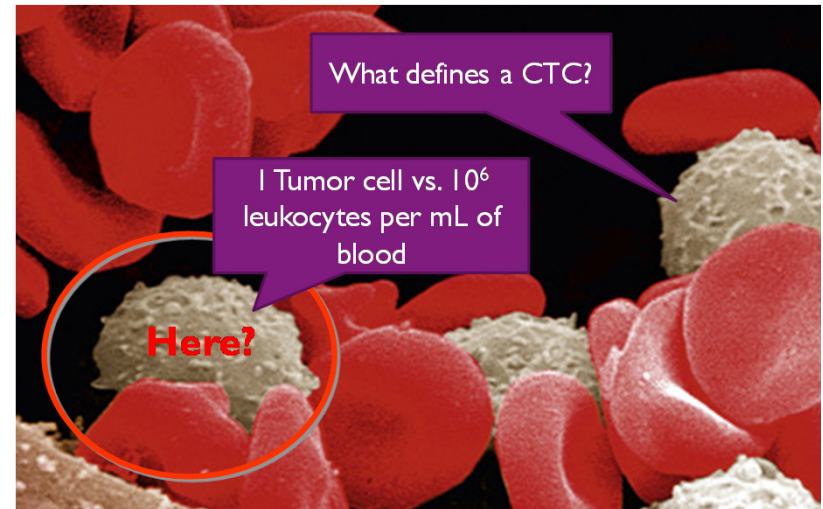
- Low abundance: down to 1 CTC/mL among billions of normal cells
- CTCs are probably heterogeneous - within one patient, and among patient subgroups

Cellular level

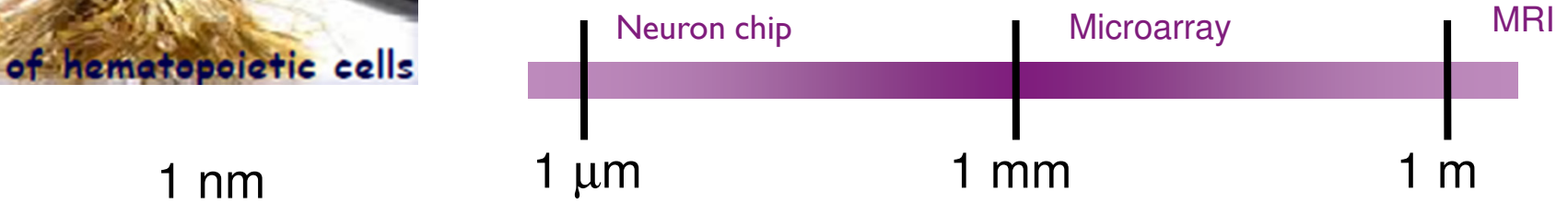
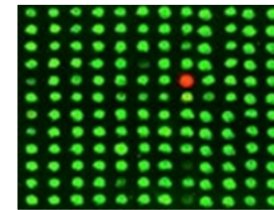
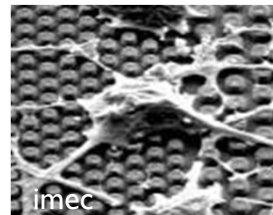
- Size/morphological overlap between CTC and normal cells

Molecular level

- No generally present carcinoma-specific markers are identified
- Normal blood cells may express similar markers (e.g. EpCAM)



When microtechnology meets bio : size matters!



Small means:

- Single cell resolution
- Higher concentration per volume
- High reliability and efficiency

Also means:

- Micro/nano sensors & actuators
- Micro/nano fluidic handling
- Macro-to-micro interfacing

This is where we come into play.



The consortium



Molecular biology



Nanoelectronics



MIRACLE



Clinical validation

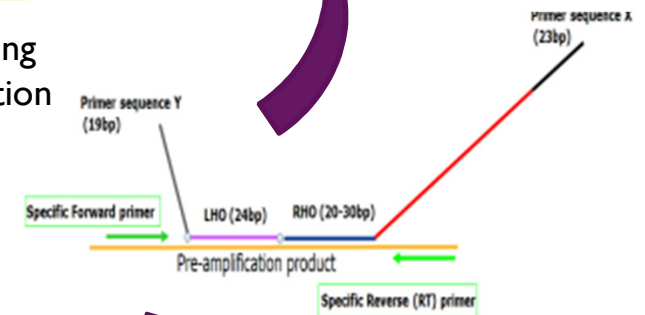
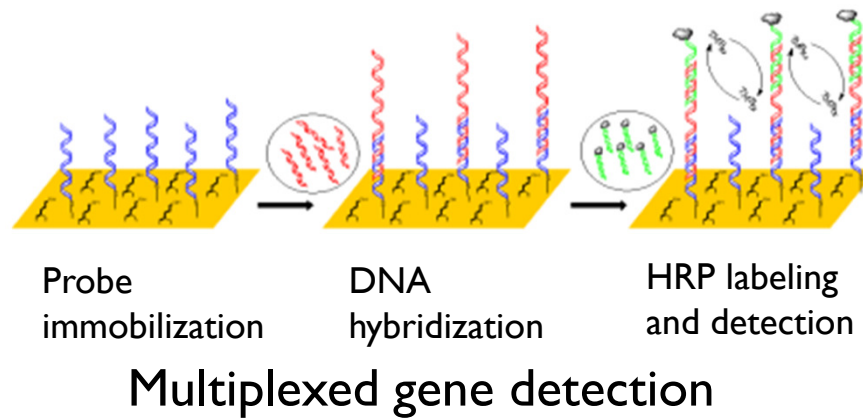


Instrumentation

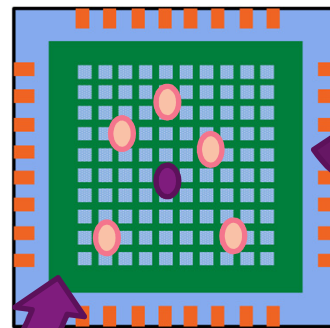
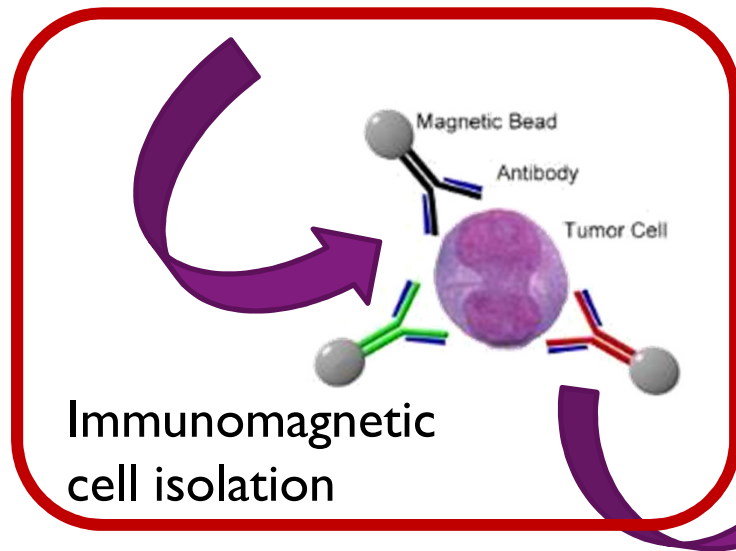
- **Project type:** FP7 integrated project
- **Project number:** 257743
- **Project duration:** 01.09.2010 – 31.08.2014
- **Total cost:** 10 million euro
- **Project coordinator:** Prof. Liesbet Lagae (imec)



Technology overview



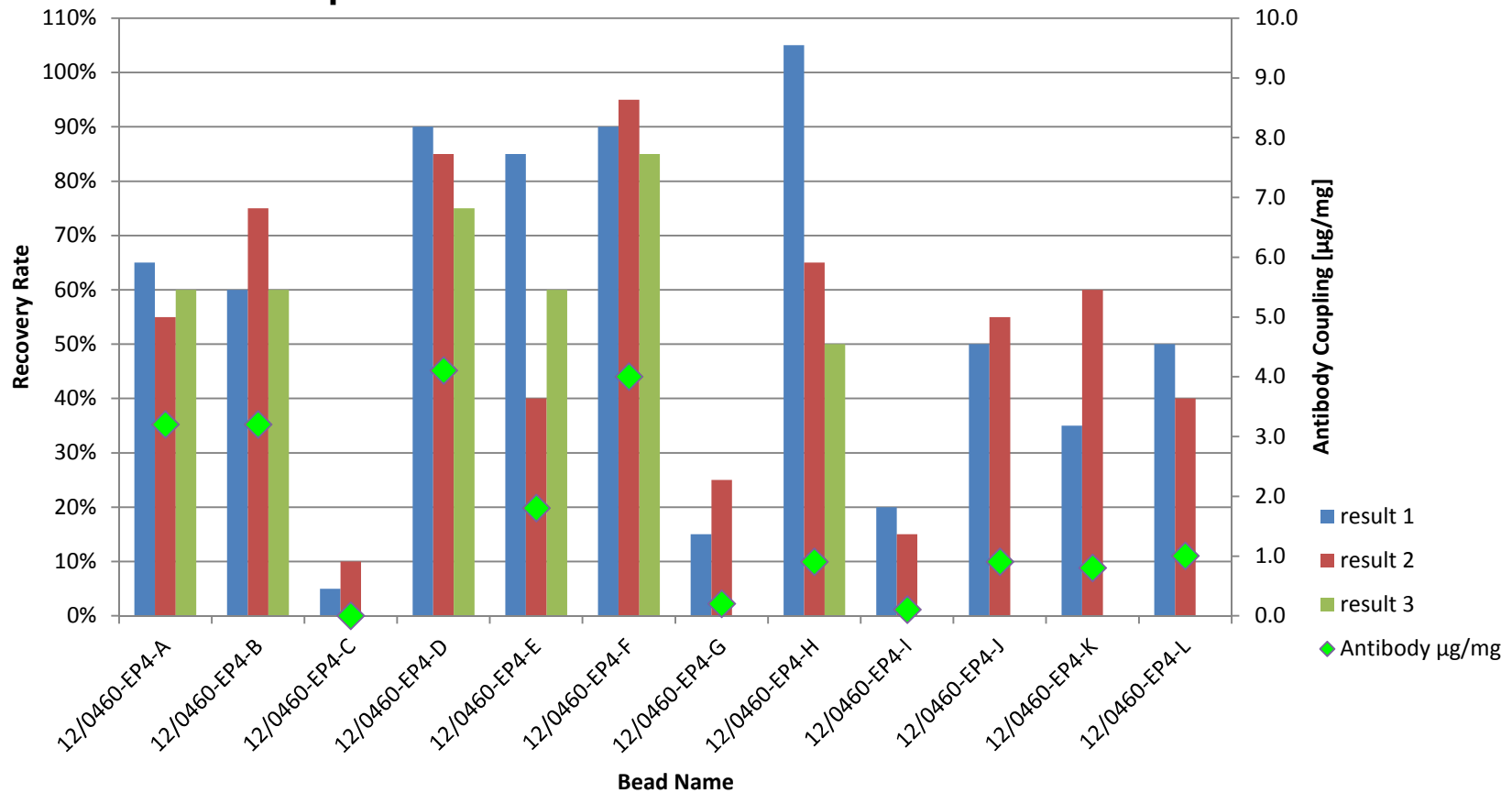
Multiplexed gene amplification (MLPA)



Active sieve for CTC identification & counting

300 nm beads for immunomagnetic cell isolations

20 spiked MCF-7 cells in 5ml medium

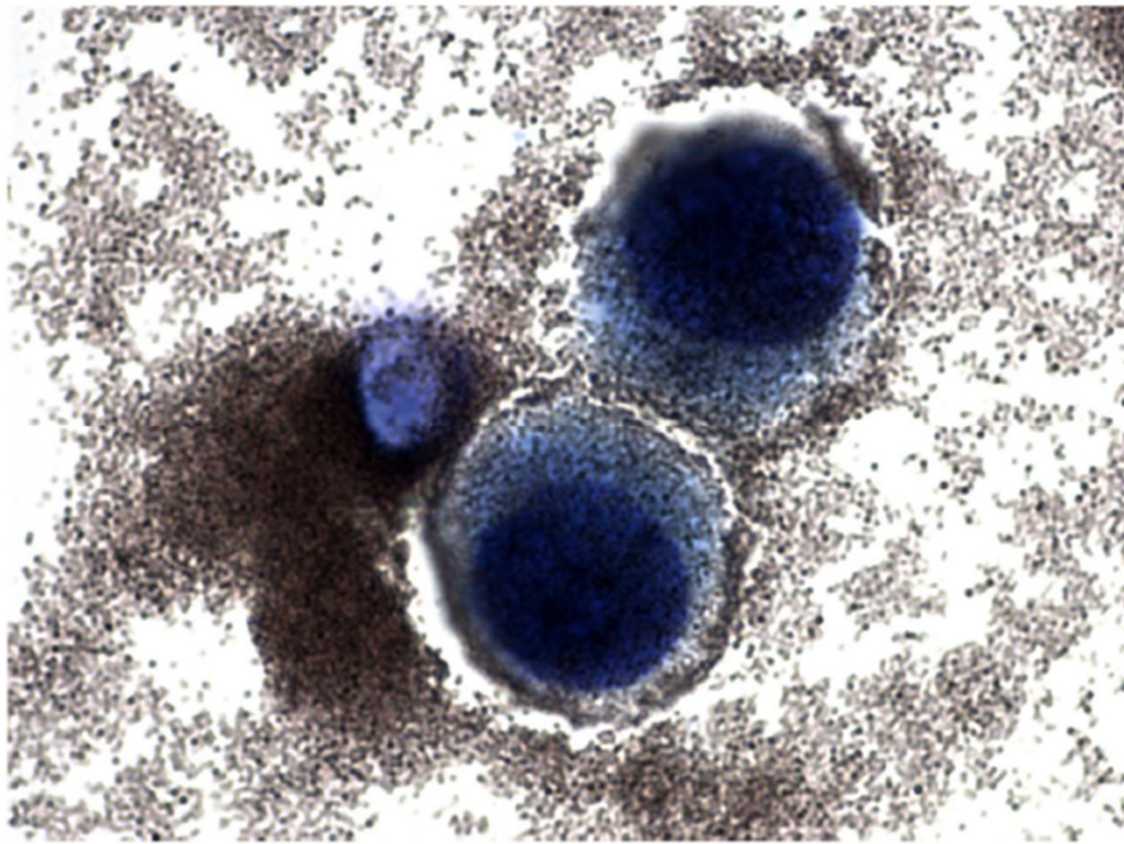


- 300 nm: balanced cell recovery & cell-bead size contrast
- Antibody: EpCAM (first test shown in graph) + stem markers (in progress)

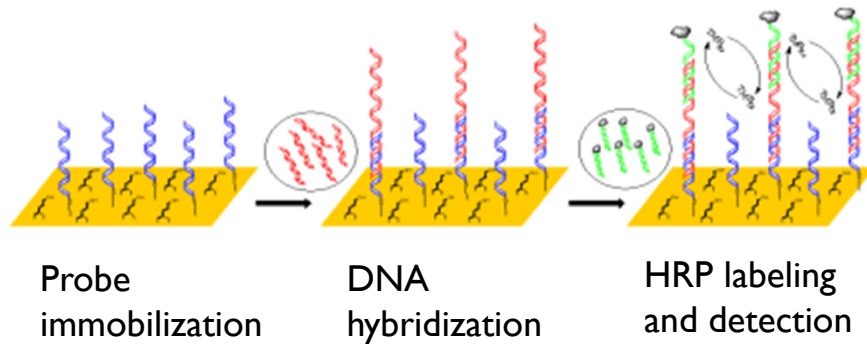


Isolating individual viable CTCs?

- How to identify CTCs?
- How to isolate CTCs from WBCs?

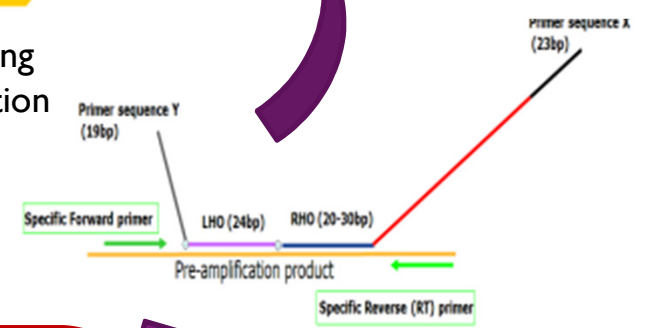


Technology overview

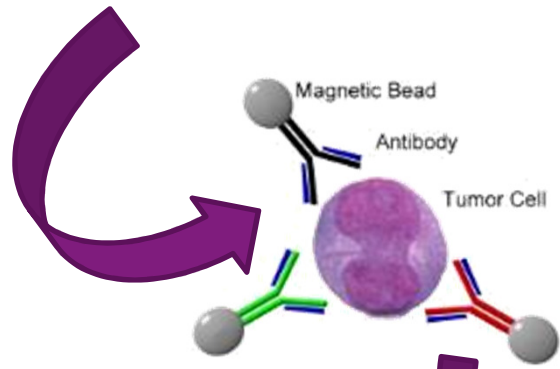


Probe immobilization DNA hybridization HRP labeling and detection

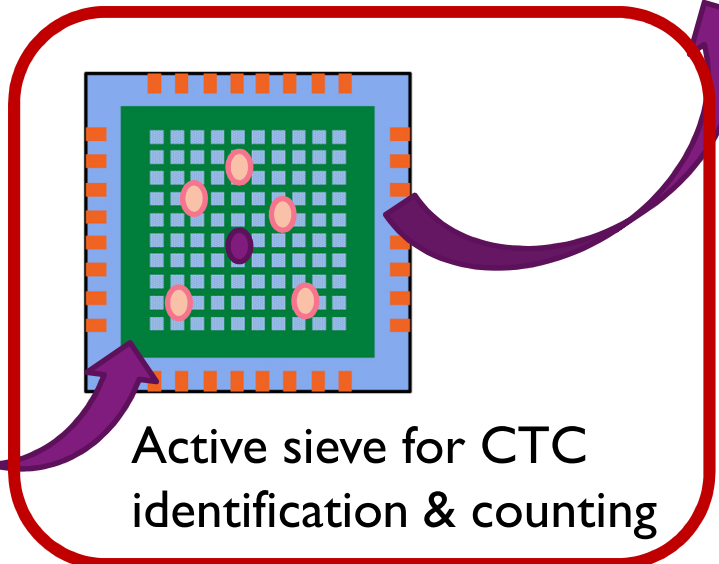
Multiplexed gene detection



Multiplexed gene amplification (MLPA)



Immunomagnetic cell isolation

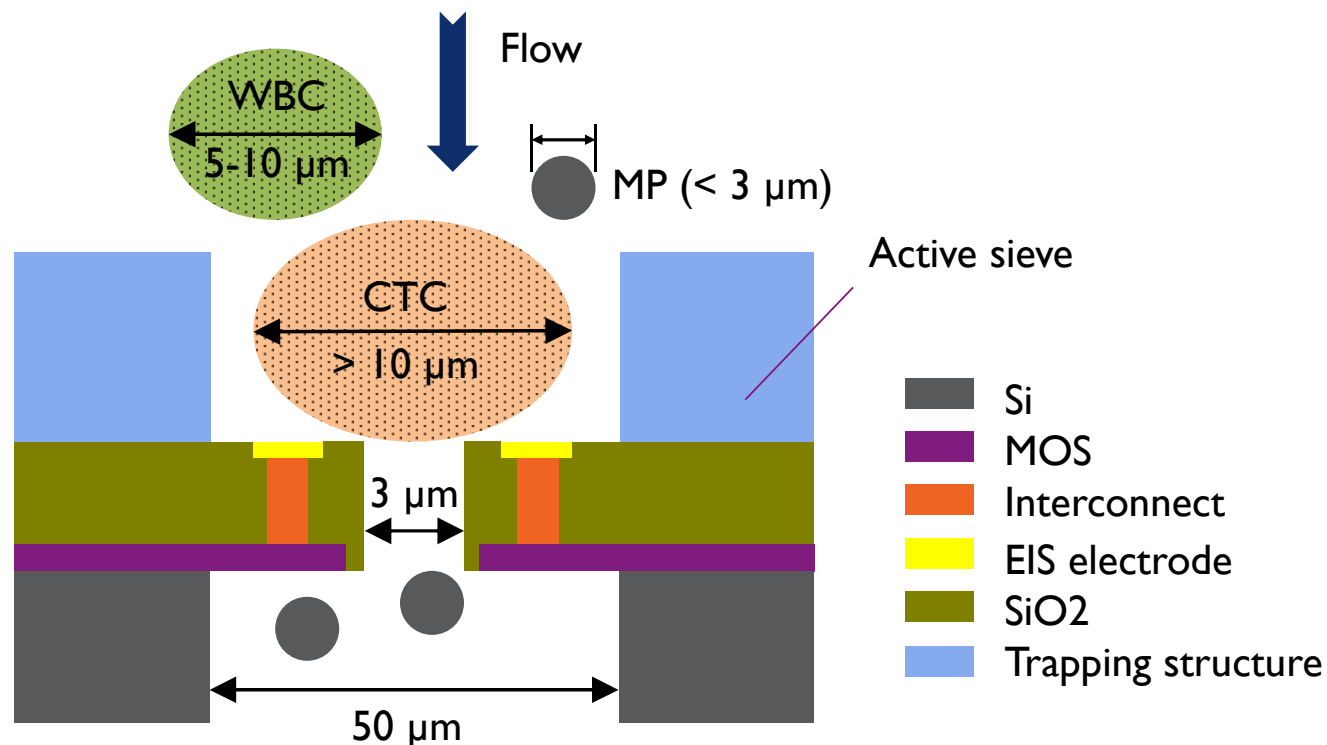


Active sieve for CTC identification & counting



An active micro sieve for CTC isolation

Active sieve = a transistor chip with through-silicon pores

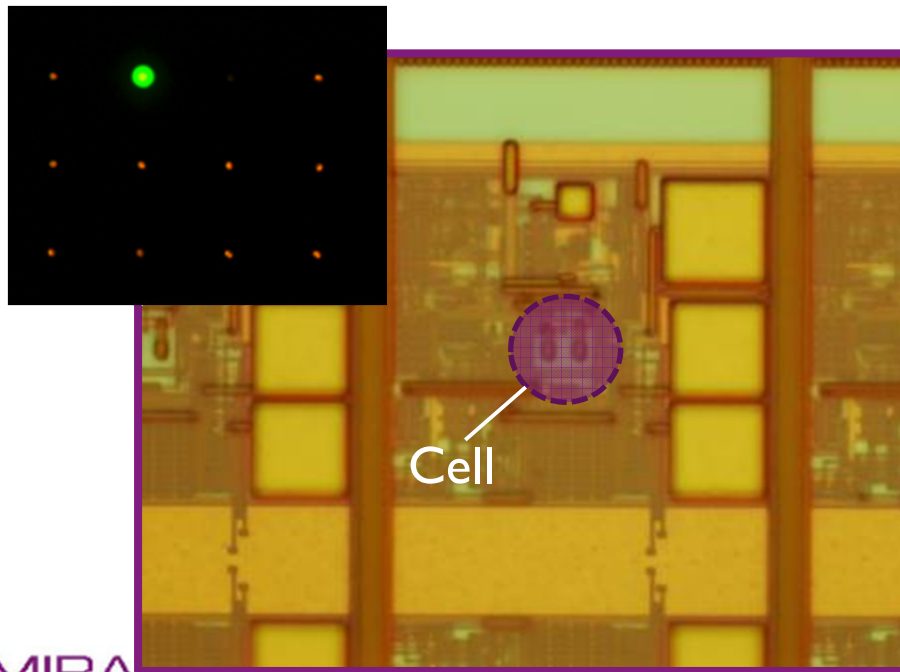


- CTC identification by electrical impedance measurement (EIS)
- CTC positioning by dielectrophoresis (DEP)
- Electrical CTC lysis

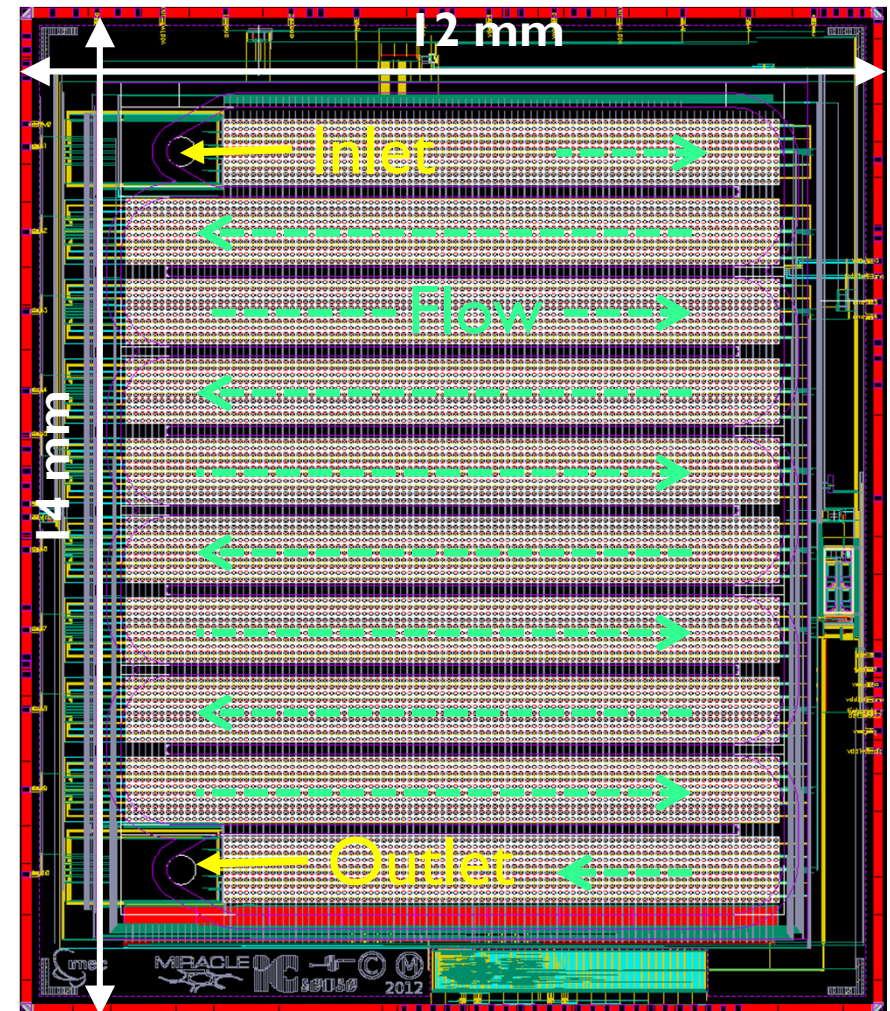
(US20120064567 A1)

An active micro sieve for CTC isolation

- 10,000 single cell measurement pores
- On-chip microfluidic structure
- Front-end: TSMC 0.18 μm (Taiwan)
- Back-end & packaging: imec



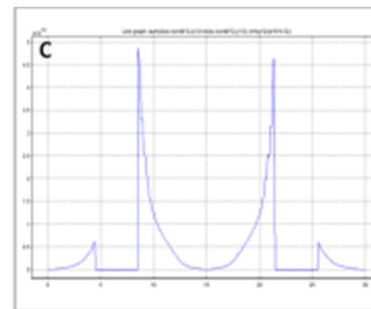
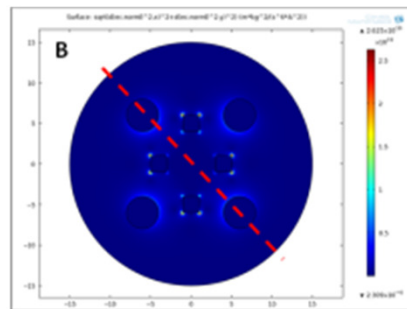
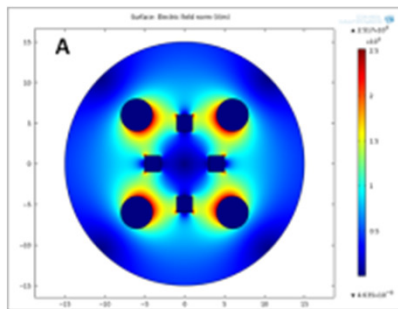
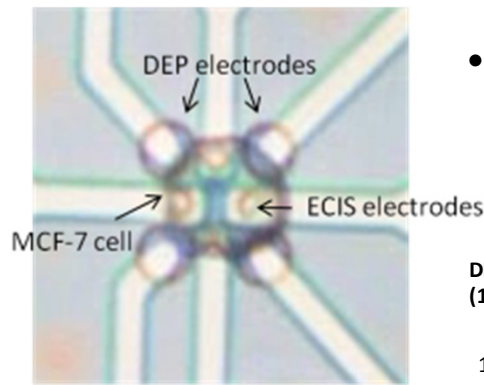
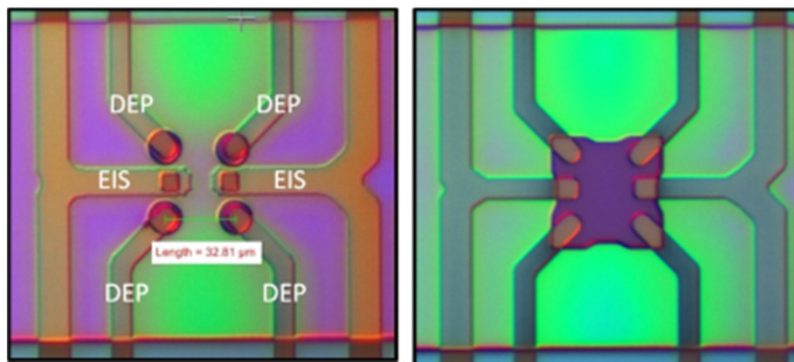
Chip layout



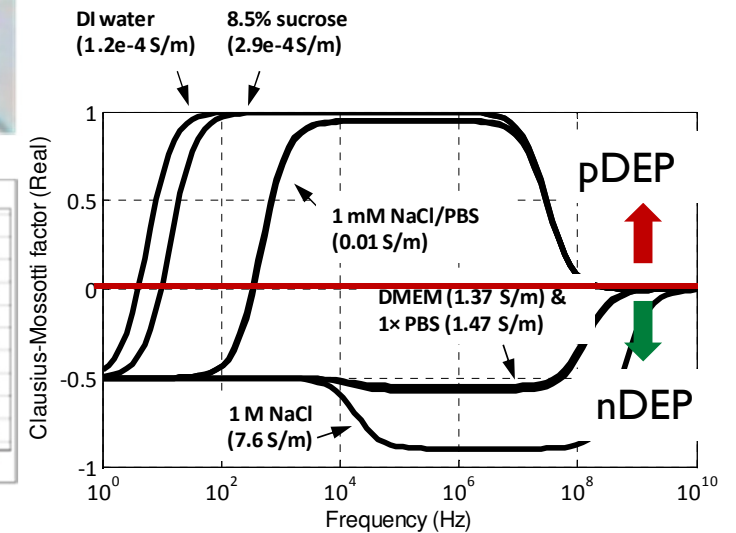
Cell positioning by dielectrophoresis

Cell manipulation by DEP

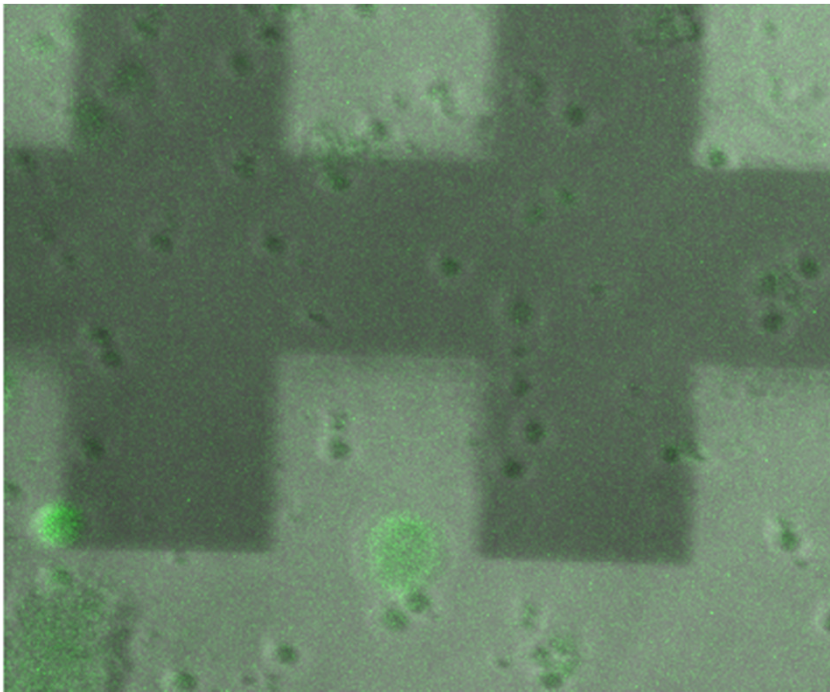
- ▶ Position the cell close enough to the EIS electrode (in-plane)
- ▶ Reduce the cell-electrode gap (out-of-plane)
- ▶ Cell lysis after identification



- Wide pDEP freq. spectrum
- EIS & DEP by the same pair of electrodes



Electrical cell positioning and lysis



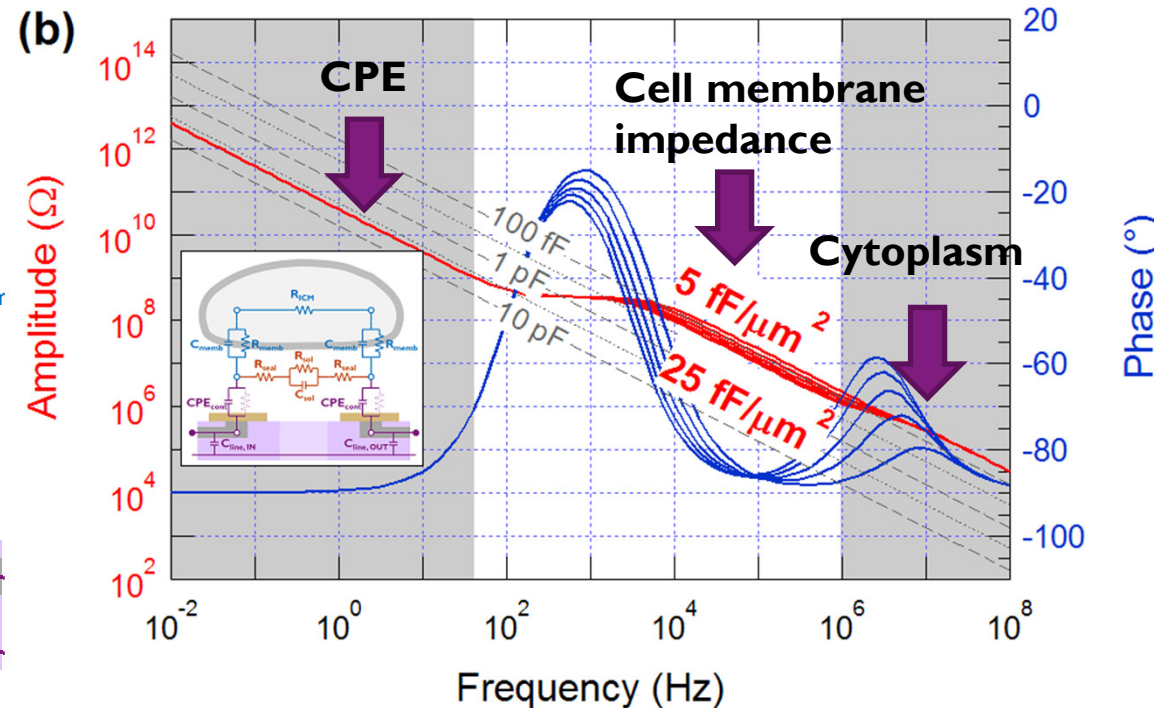
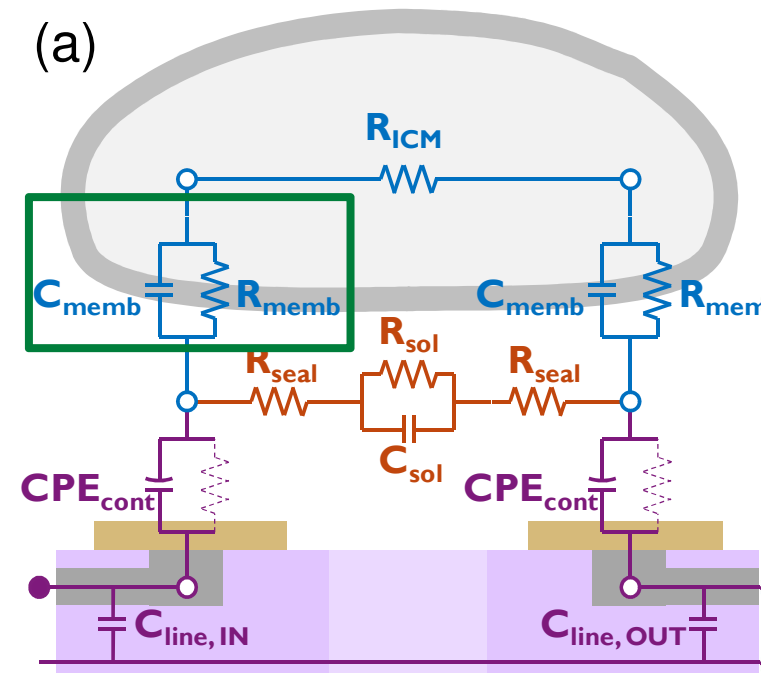
Tumor cell capture & WBC
repulsion by DEP



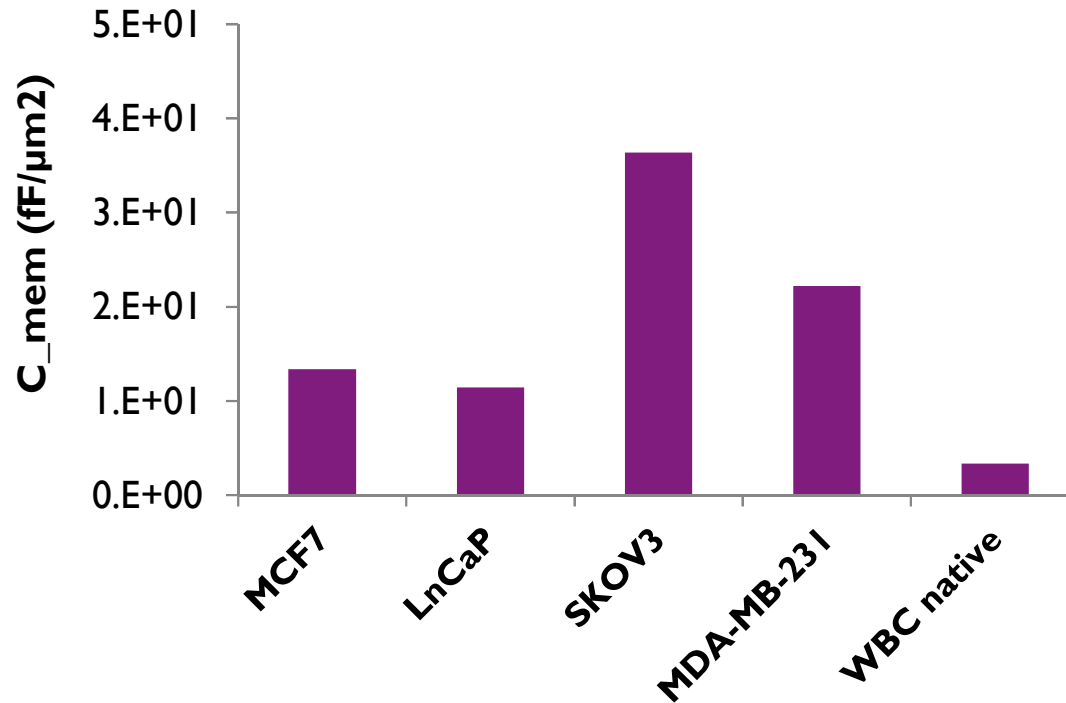
Calcein / esterase leaked out after cell
lyzed by electric pulses

Cell impedance measurement

- Different CTC membrane impedance for from normal blood cells
- Cell membrane impedance dominates the middle frequency range [1 KHz, 1 MHz]
- Very tight cell-electrode contact is required to ensure high seal resistance (R_{seal})

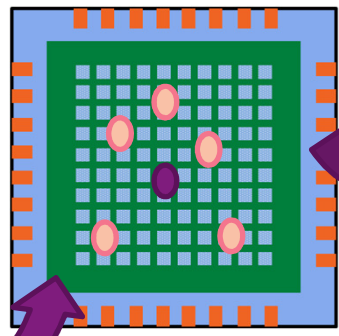
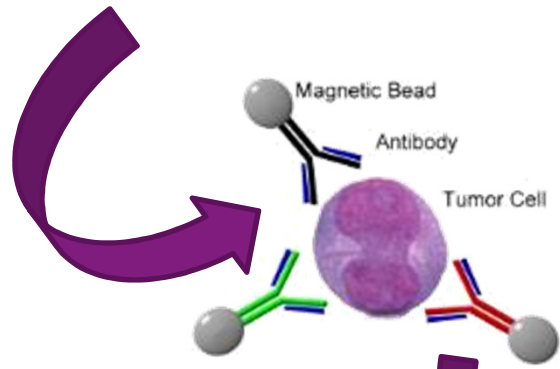
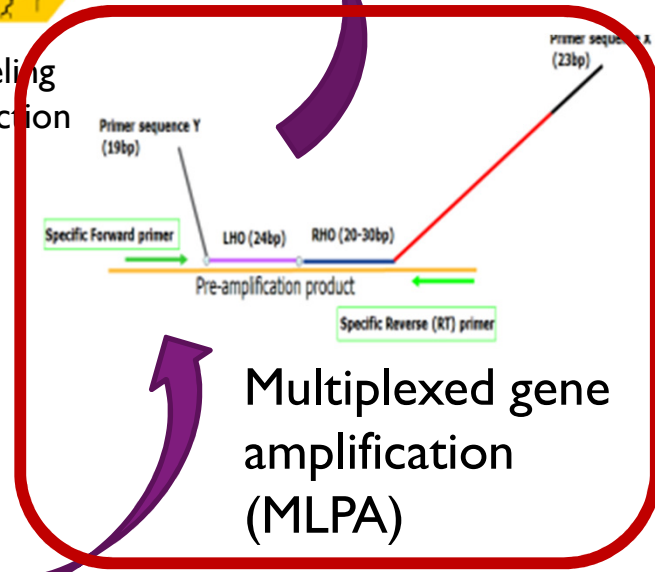
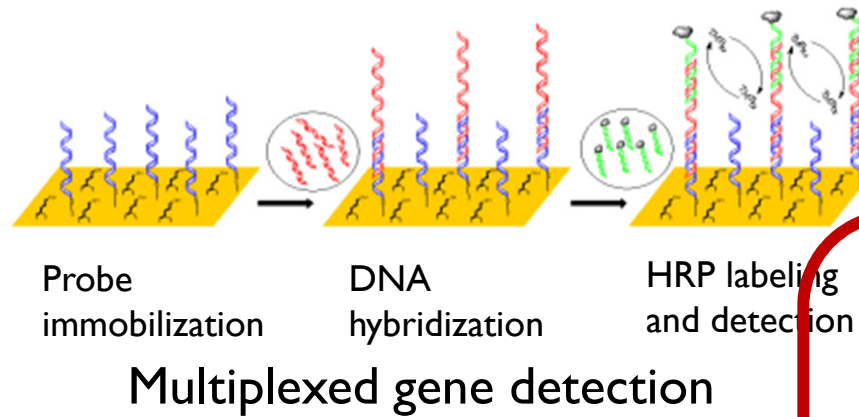


Cell membrane capacitance



- Cell membrane capacitance characterized by DEP measurements. Min. 50 individual cells measured for every cell line. Cell size measured and normalized.
- Tumor cells exhibit higher membrane capacitance than normal blood cells. → Cell capacitance can be a good measurement for TC, or CTC, identification from WBCs!

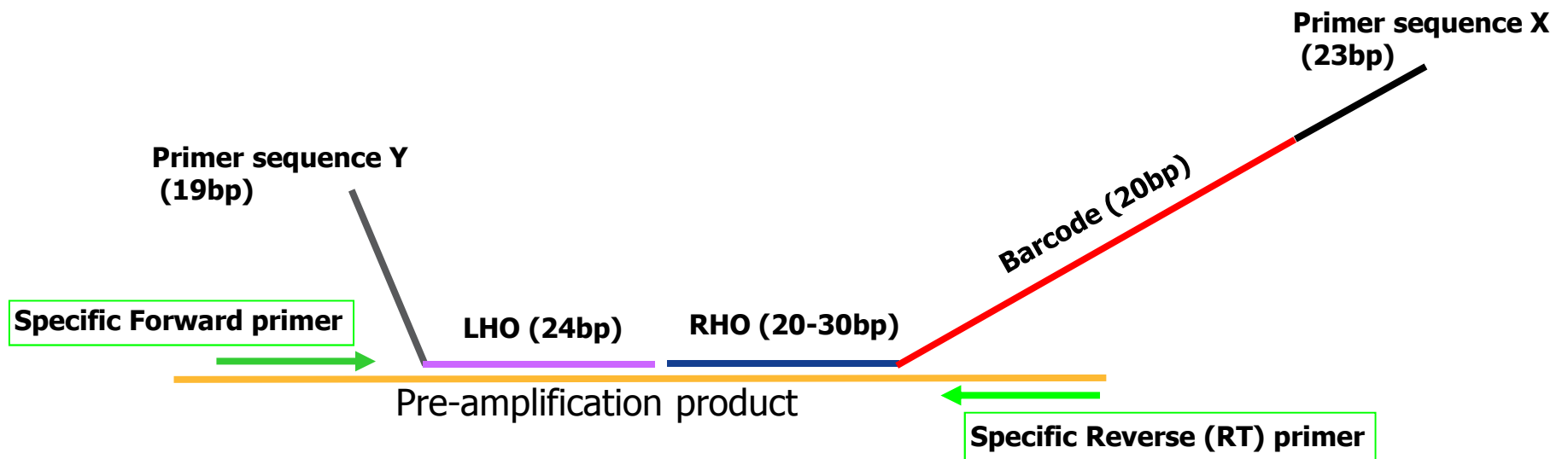
Technology overview



Active sieve for CTC identification & counting



Multiplex ligation-dependent probe amplification (MLPA)



- LHO and RHO are allowed to hybridize next to each other to denatured pre-amplification product
- LHO and RHO carry the universal primer sequences, which are used for amplification after ligation
- Only perfect hybridized LHOs and RHOs are connected by ligation.

- RT primer is being used for reverse transcription
- RT primer and specific forward primer are used in following pre-amplification

Allowing both CE & microarray detection

The full MIRACLE MLPA gene panels

■ Breast cancer (30 genes)

AKT2	mTOR
ALDH1A1	MUC1
BM11	MYC
CD24	PGR
CD44	PIK3CA
CDH1	PLAUR
CDH2	PROM1
CEACAM3	PTEN
EGFR	PTPRC
ERBB2	TACSTD1
ESR1	TERT
FNI	TOP2A
HUWE1	TWIST1
KRT19	VEGFA
MKI67	VIM

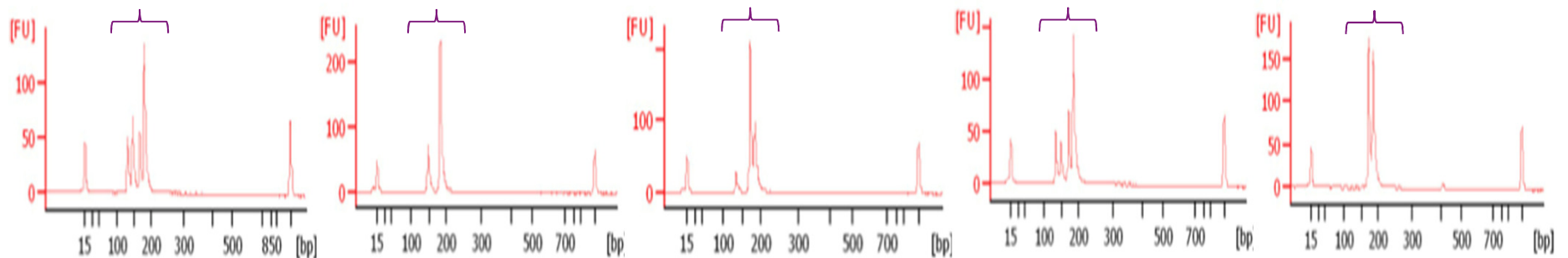
(Core gene panel for initial testing)

■ Prostate cancer (16 genes)

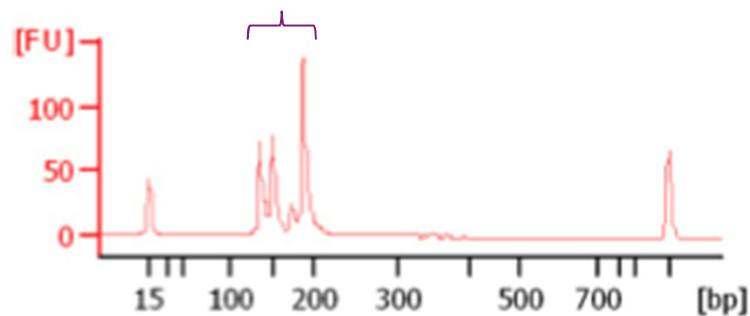
ALDH1
AMACR
AR
EGFT
EPCAM
ERBB2
FOLH1 (PSMA)
KLKBI
KRT19
LDHA
PCA3
PTPRC
TMPRSS2-ERGb
TMPRSS2-ETV1b
HUWE1
OAZ1

MLPA sensitivity

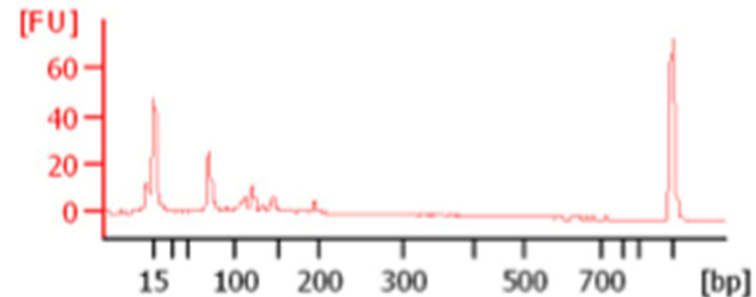
Single Cells



PosCtrl



NegCtrl

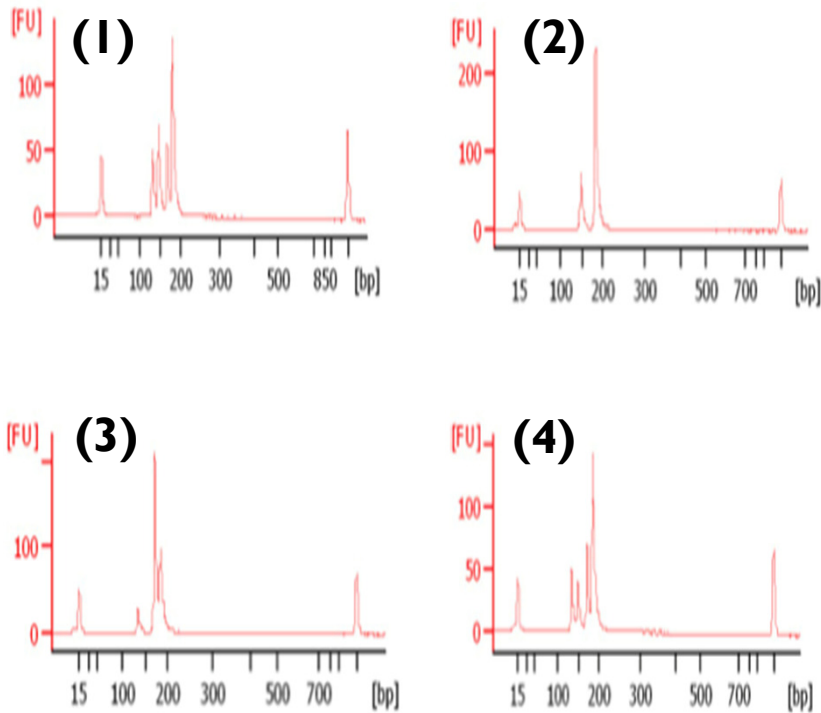


MLPA product lengths: 115-170 bp

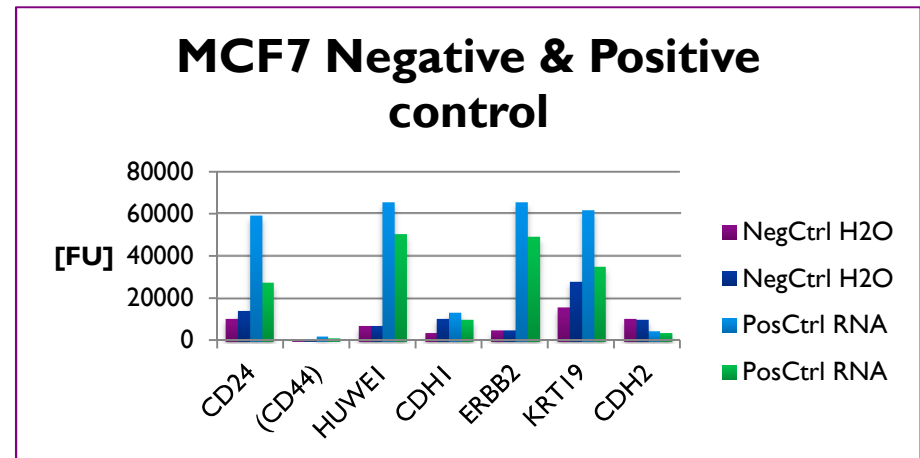
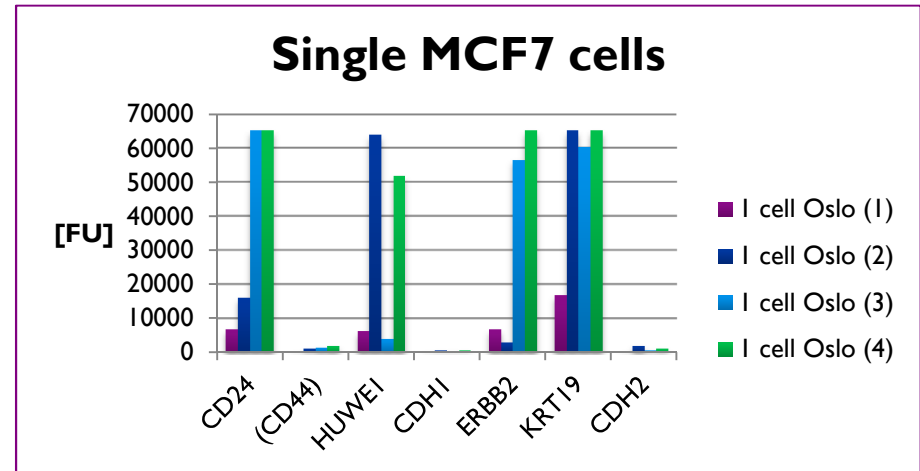
- MCF7 cells
- No RNA purification

MLPA sensitivity

Correlated results between electrophoresis & microarray

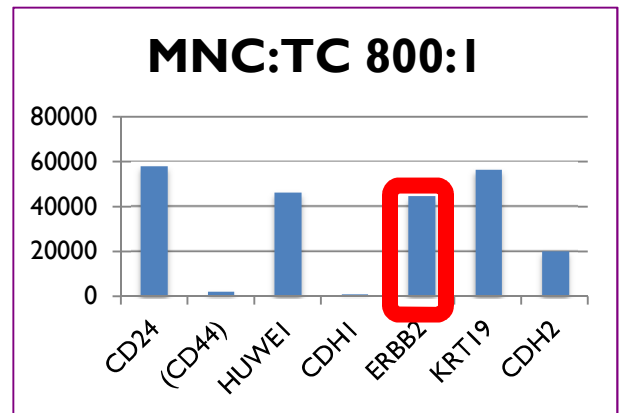
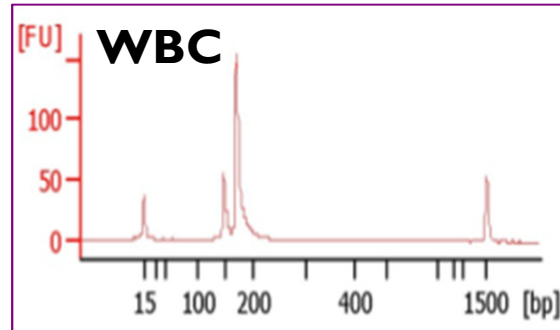
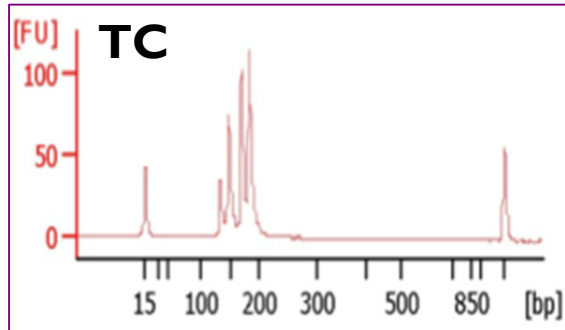
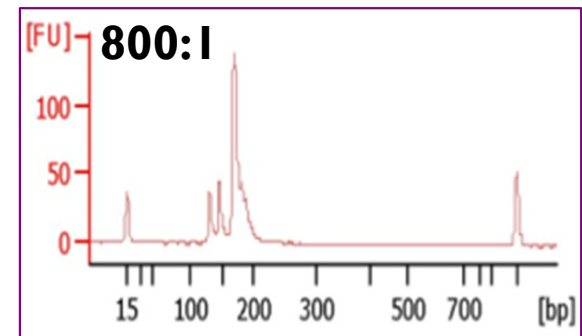
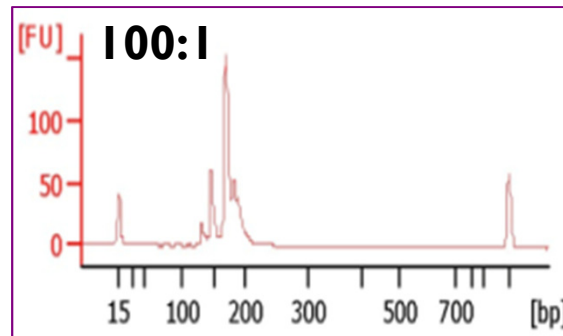
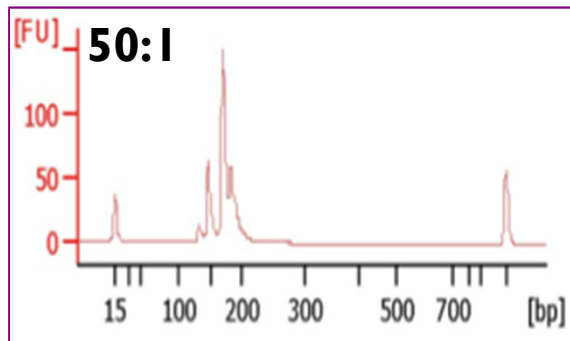


MLPA product lengths: 115-170 bp

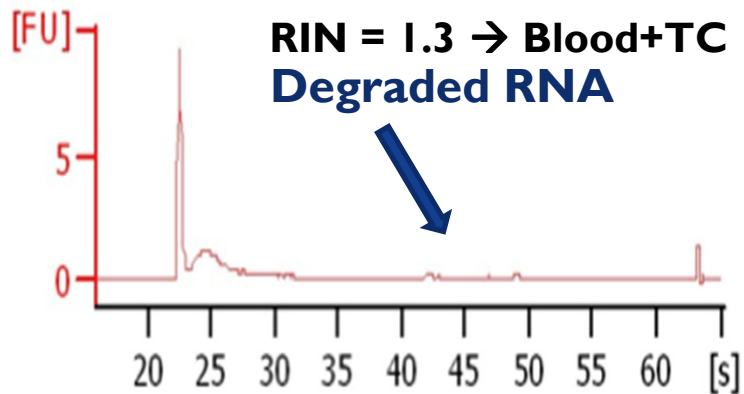
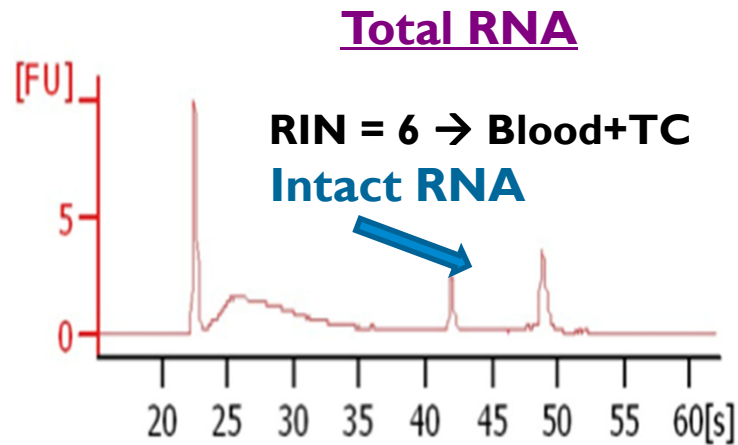


MLPA for TCs spiked in WBC

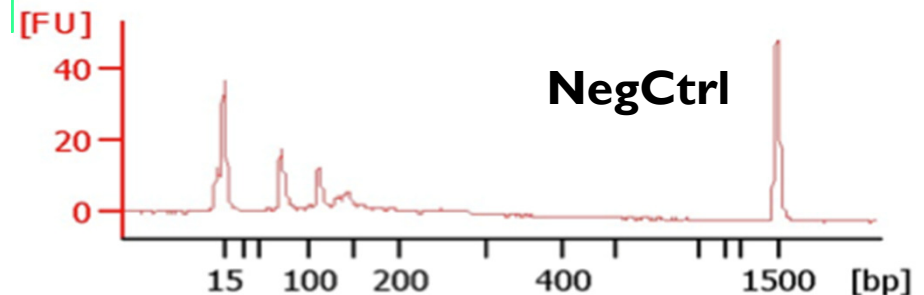
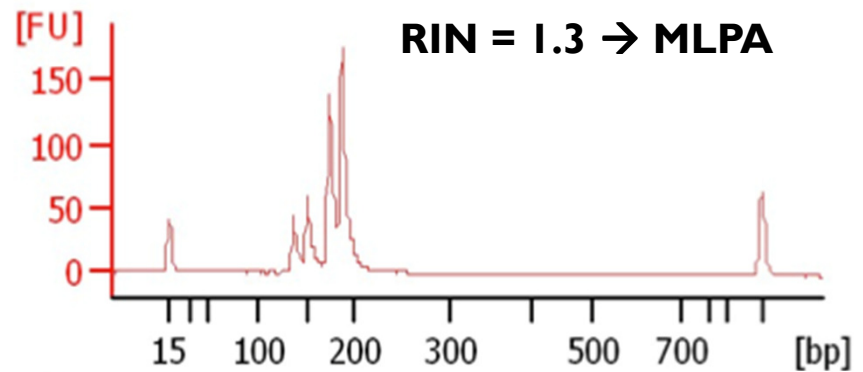
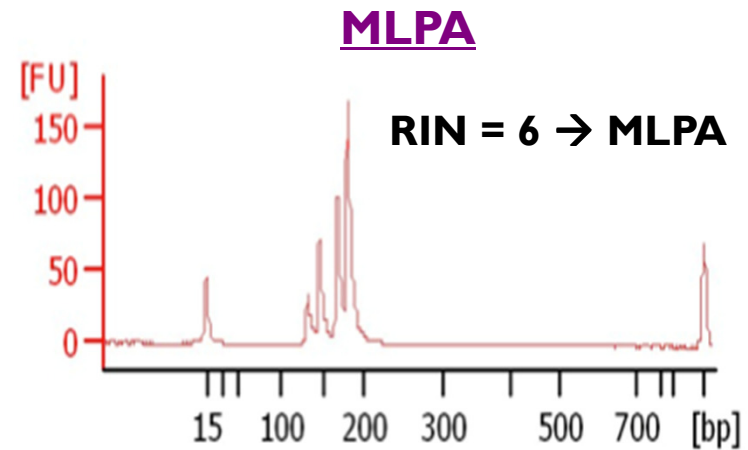
- MLPA product lengths: 115-170bp
- 20 TC for every sample
- Still pretty high TC specific signal at 800:1.



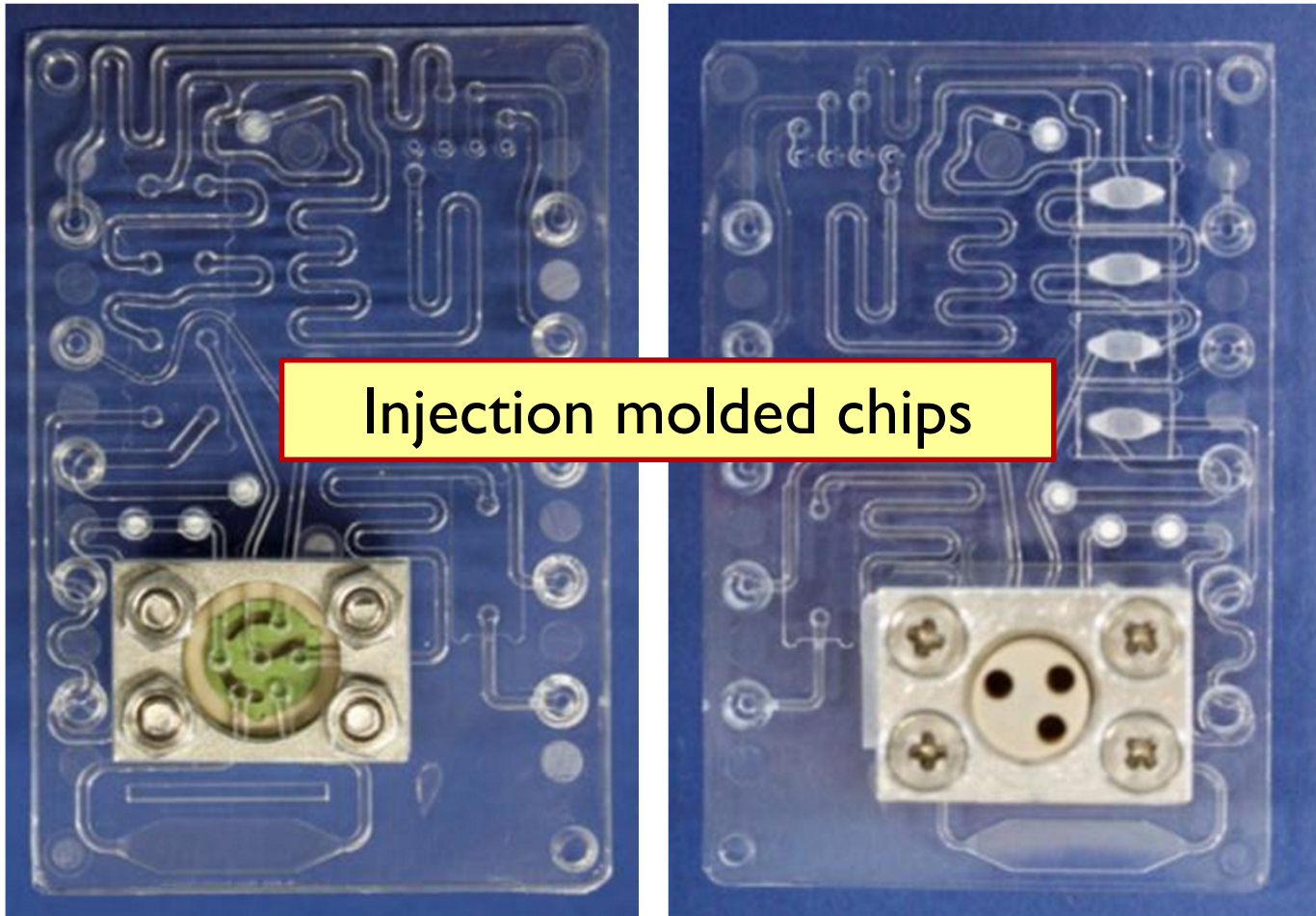
MLPA robustness to RNA sample degradation



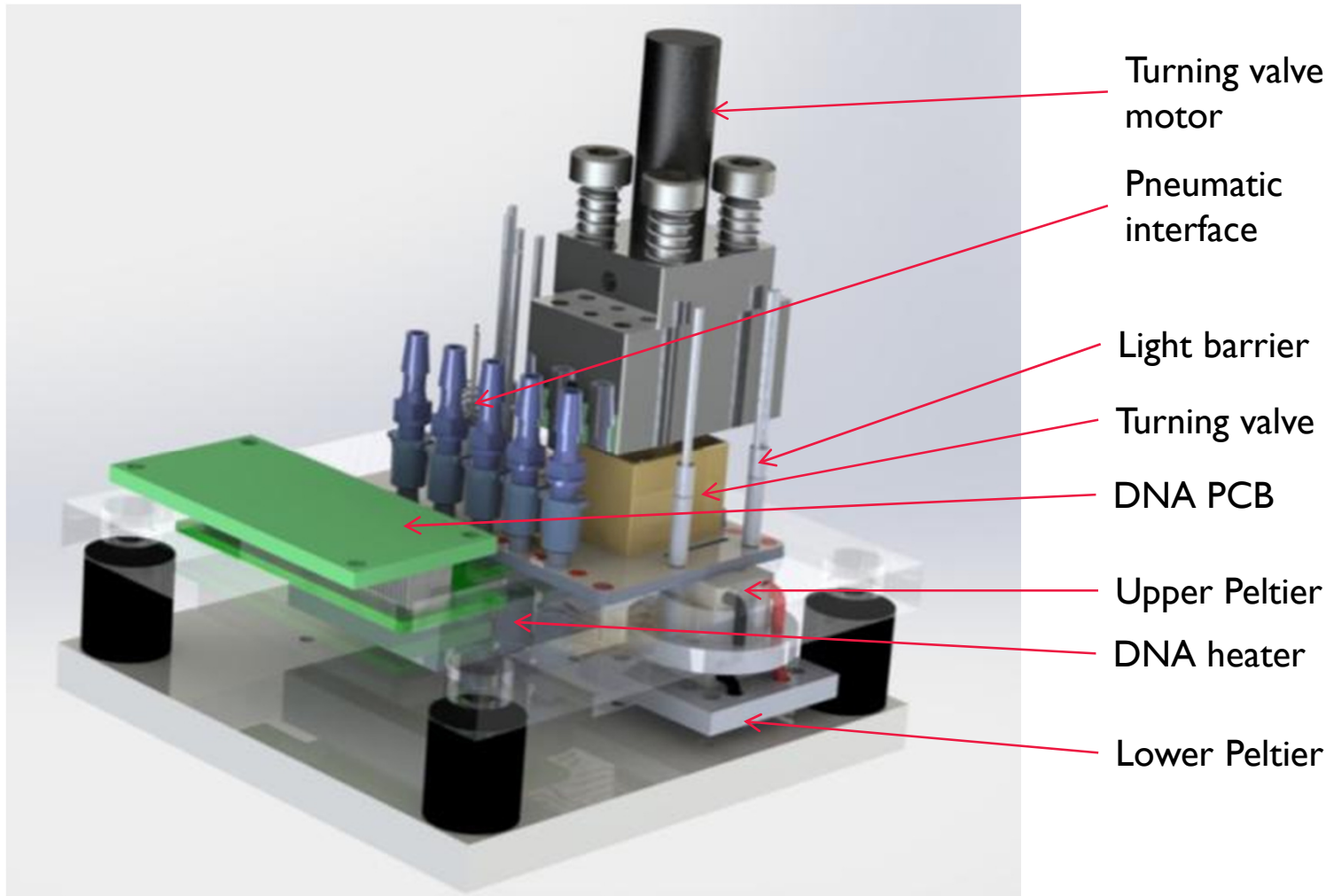
Specific MLPA signal even from seriously degraded RNA!



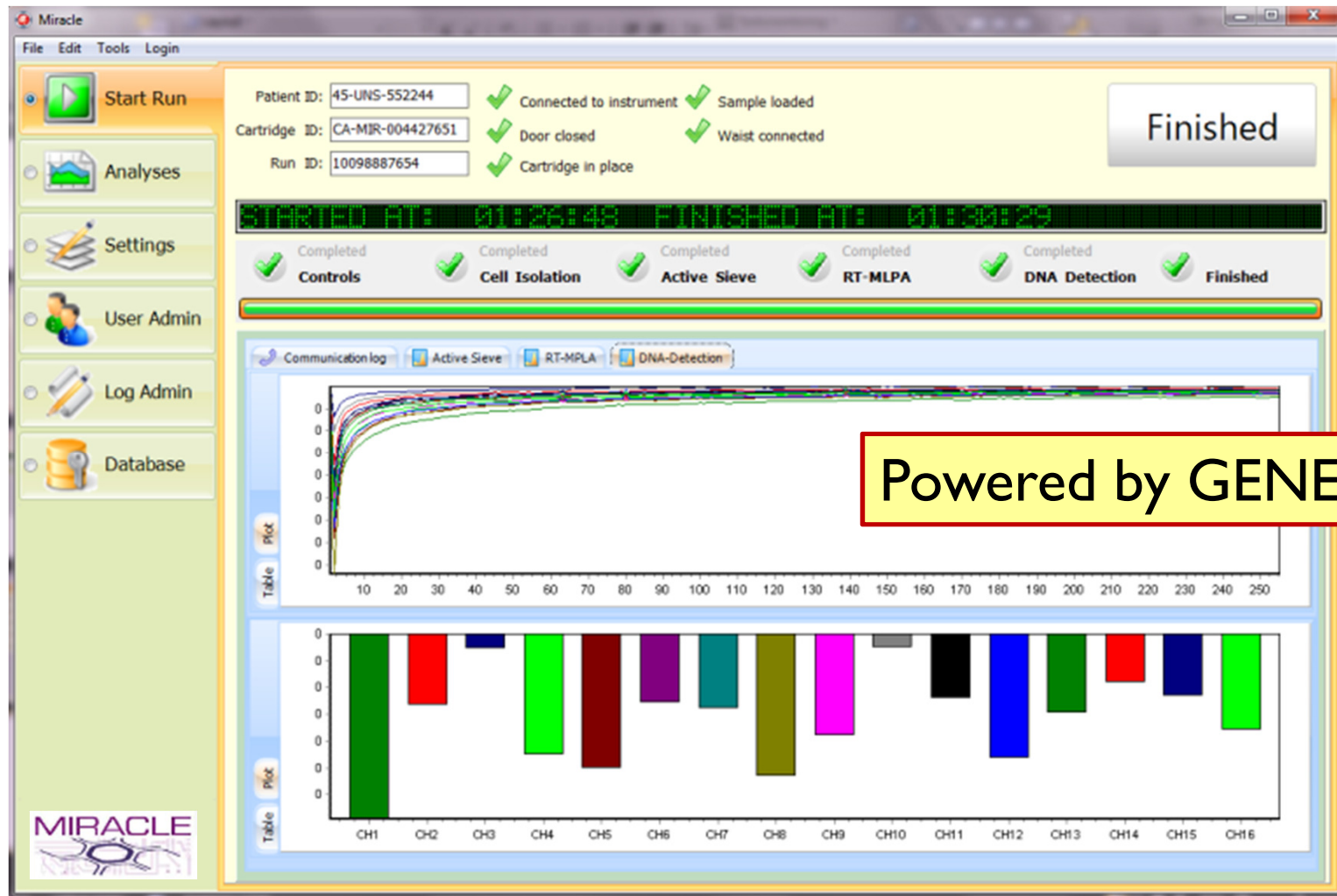
Automated gene amplification & detection model



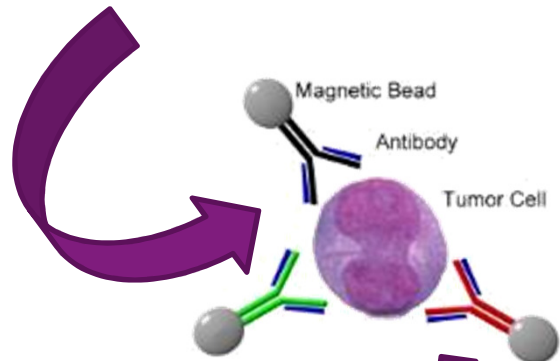
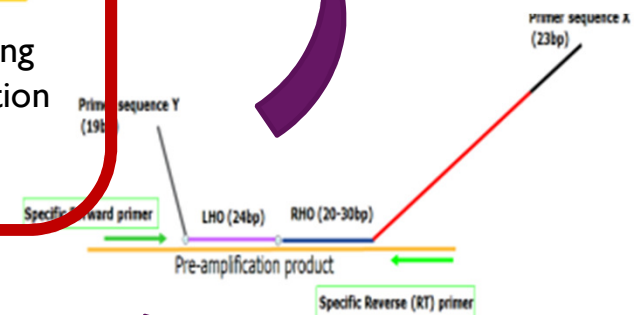
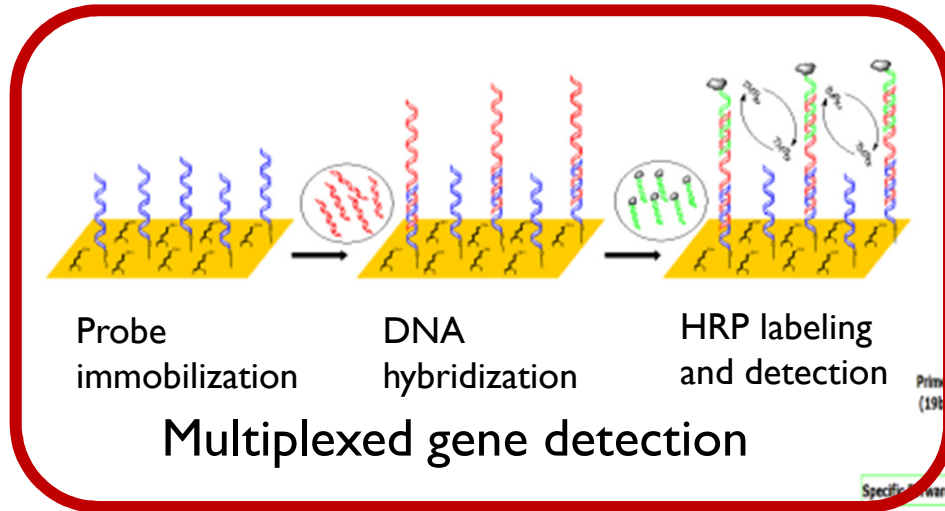
Automated gene amplification & detection model



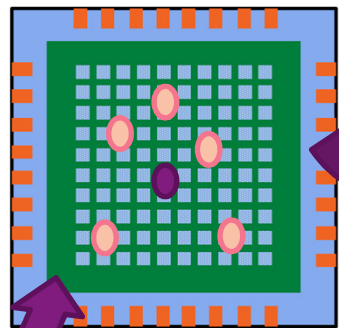
Automated gene amplification & detection model



Technology overview



Immunomagnetic cell isolation

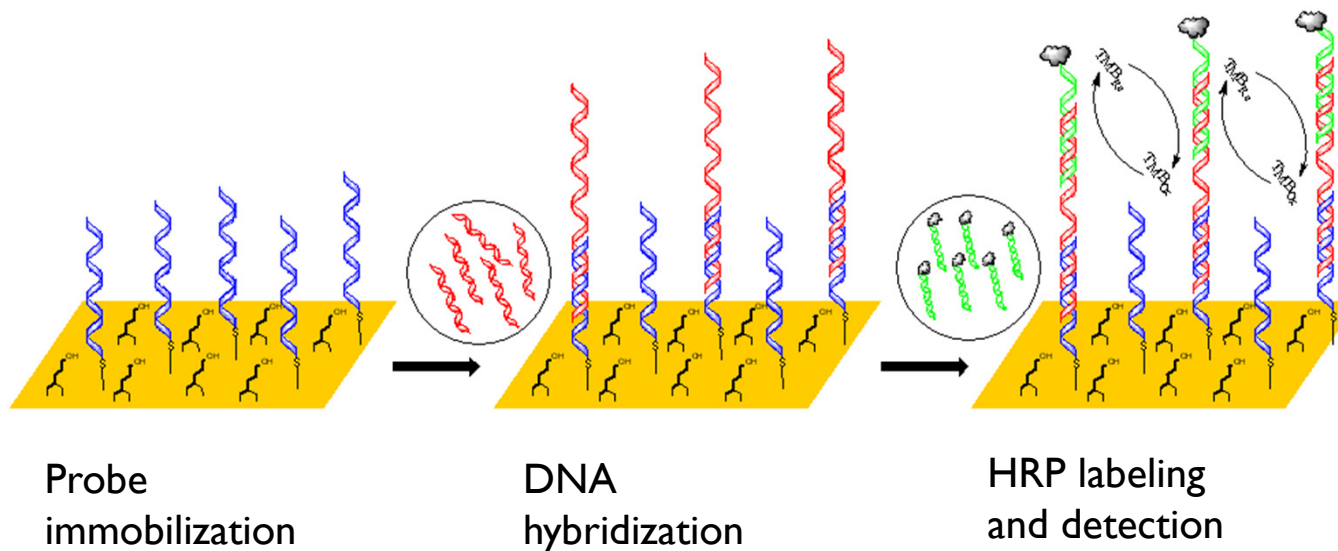


Active sieve for CTC identification & counting

Multiplexed gene amplification (MLPA)

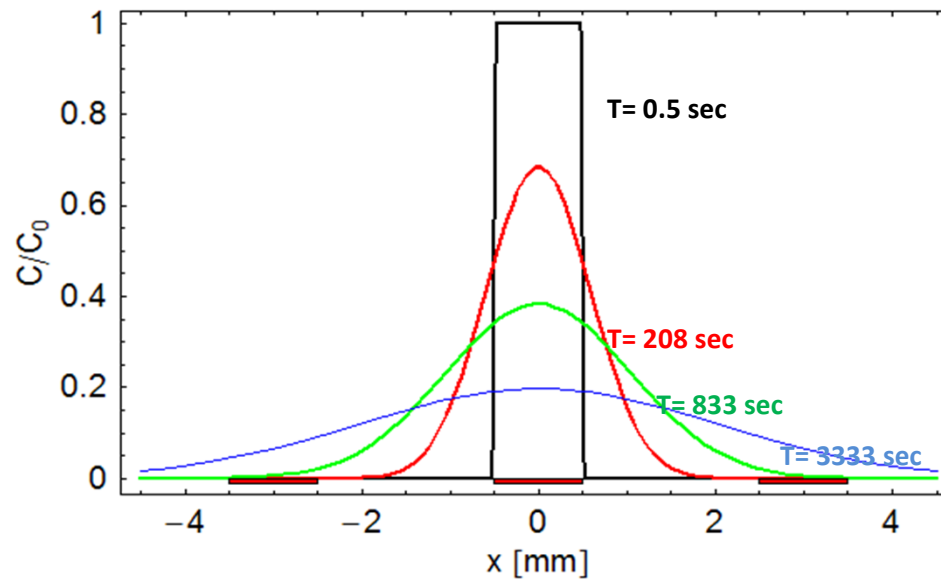
Principle of DNA detection

- Electrochemical DNA detection with enzymatic redox labels
 - Well known working principle
 - Good balance between sensitivity, specificity & reliability for PCR amplicons
 - Electrical readout = multiplexing, compact, fast, cheap

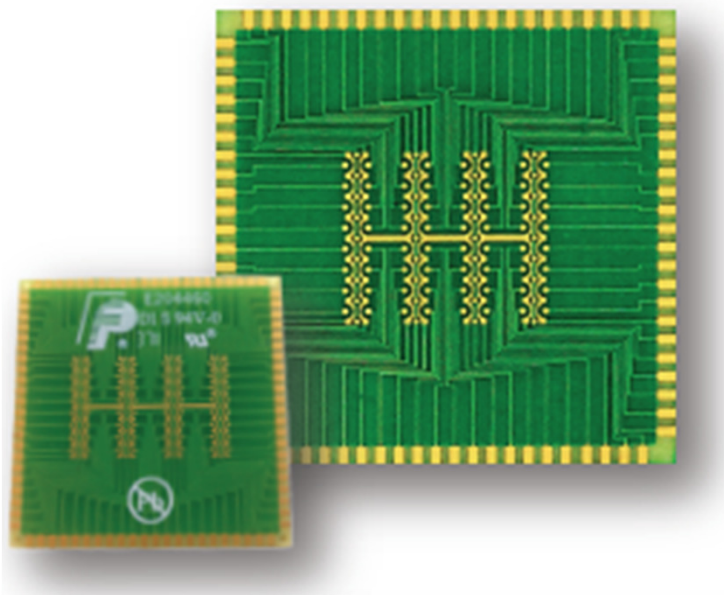


Challenges for multiplex DNA detection

- The redox product can diffuse between electrodes
 - HRP label & substrate optimized for reaction-limited kinetics rather than diffusion-limited
 - Optimal electrode design against molecule diffusion
 - Low-noise readout allowing rapid measurement before diffusion occurs
 - MIRACLE: 0.5 seconds for all 64 electrodes
 - Autolab®: several minutes

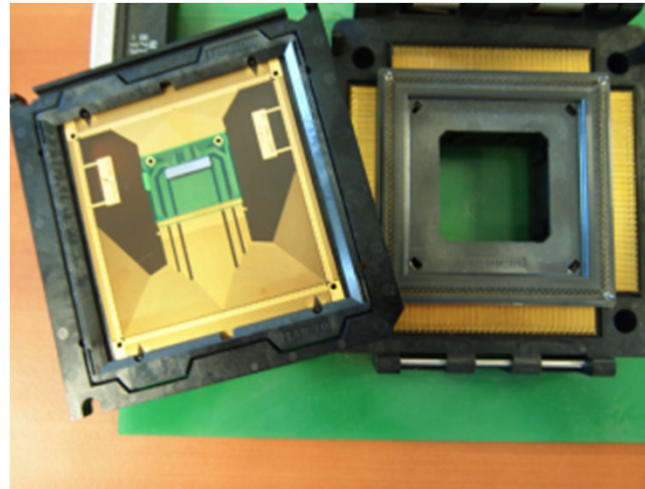


DNA sensor chips

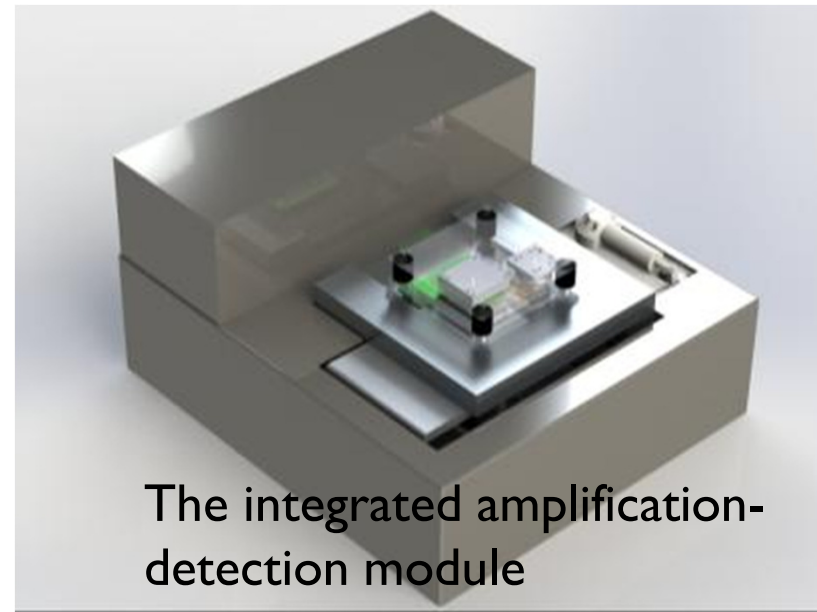


The 64 electrode sensor chip

Made of standard printed circuit board (PCB) technology, modified with IC-level gold finishing.



The packaged sensor chip (before fluidic integration)

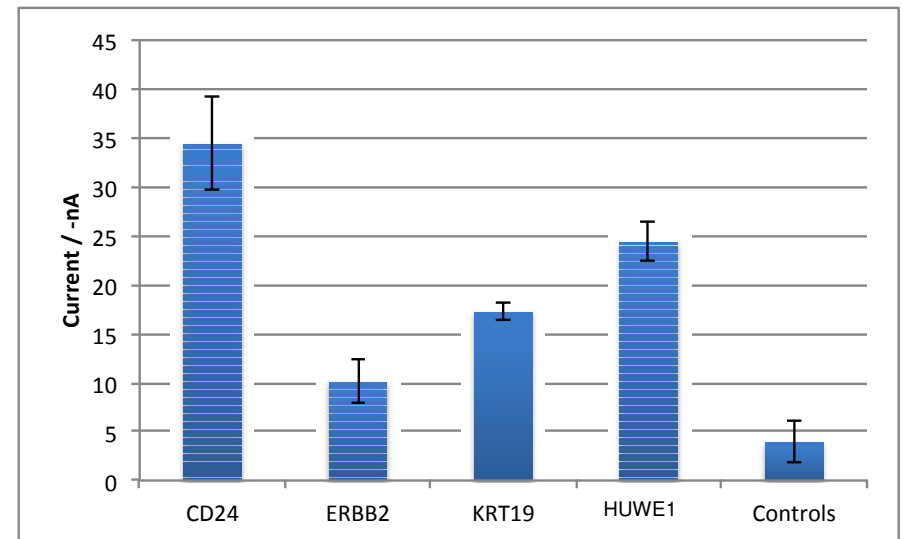
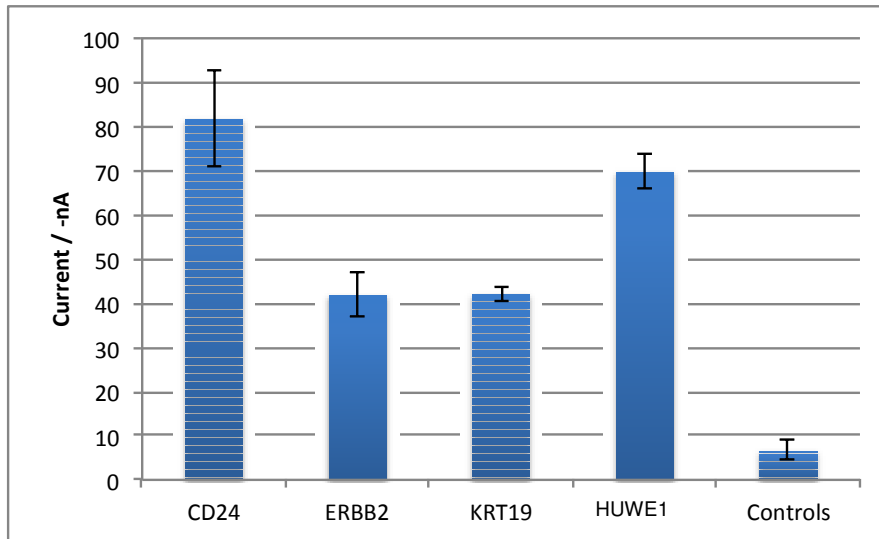
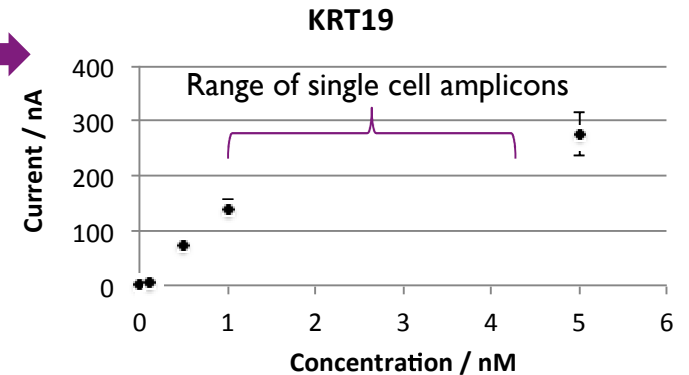


The integrated amplification-detection module

Multi-gene detection for single cell MLPA amplicons

- Wide dynamic range for single cell amplicon sensing

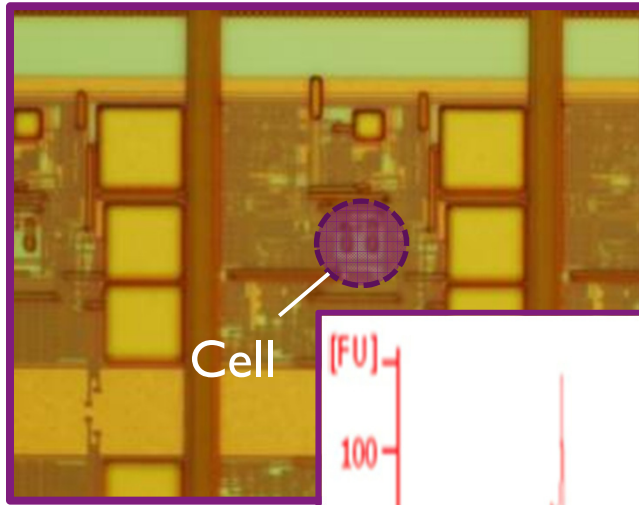
- Individual MLPA products prepared from single MCF7 cell
- Similar sensitivity & specificity as commercial instrument with much shorter signal integration time



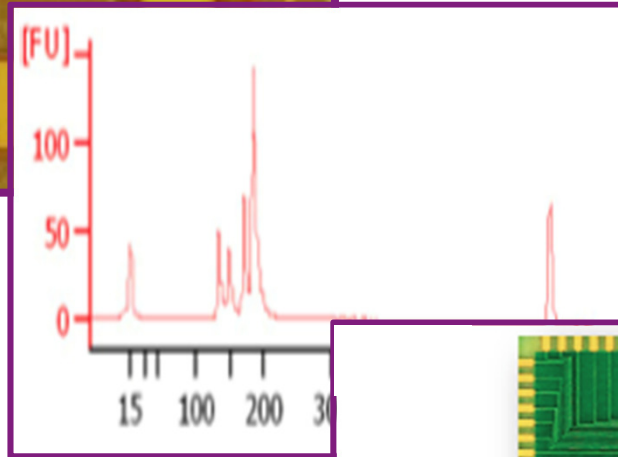
AUTOLAB

MIRACLE

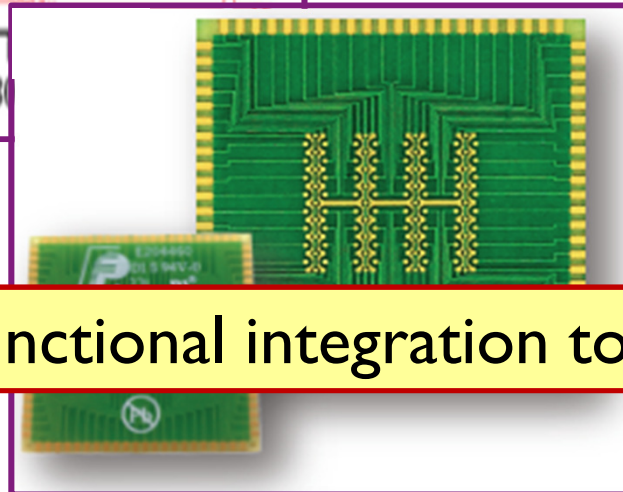
Conclusions



- Tumor cells exhibit different membrane capacitance from blood cells



- MLPA is sensitive, specific and robust for TC gene analysis



- Sensitive multiplex gene detection demonstrated

Next: from functional integration to clinical tests

Acknowledgements



<http://www.miracle-fp7.eu>





**ASPIRE
INVENT
ACHIEVE**

