Newsletter August 2013

MIRACLE

EU-Project 257743

Magnetic **I**solation and molecula**R** **A**nalysis of single **C**ircu**L**ating and disseminated tumor c**E**lls on chip (**MIRACLE**)

(01.09.10-31.08.14)

The project objective

The overall strategy of MIRACLE is the capture and multigene analysis of circulating tumor cells (CTCs) from clinical samples. The CTCs are immunologically captured and then characterized by a transistor-embedded active sieve. After cell lysis, multiple genes are amplified by RT-PCR and multiplex ligand-dependent probe amplification (MLPA) and then quantitatively detected by an electrochemical sensor array. The CTC counts and single-cell genotype will be used for cancer prognosis.

The consortium

- Interuniversitair Micro-Electronica Centrum VZW, Belgium
- Universitat Rovira i Virgili, Spain
- Institut für Mikrotechnik Mainz GmbH, Germany
- MRC Holland B.V., Netherland
- Oslo Universitetssykehus HK, Norway
- THINXXS Microtechnology AG, Germany
- ConsulTech GmbH, Germany
- Kungliga Tekniska Hoegskolan, Sweden
- MultiD Analyses AB, Sweden
- Fujirebio Diagnostics AB, Sweden
- European CanCer Organisation, Belgium
- Labman Automation Ltd., United Kingdom
- ICsense, Belgium

AIM

The MIRACLE project aims at the realization of a miniaturized system for immuno-magnetic isolation of circulating tumor cells (CTCs) and disseminated tumor cells (DTCs), as well as genotyping by molecular analysis. The technical platform is supported by microelectronic, microfluidic, surface chemistry and bioinformatic technologies as well as (pre-) industrial manufacturing practice.

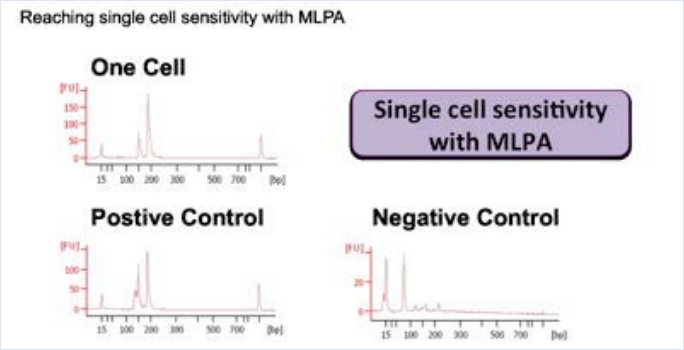
The technology

Complete integrated platform

- Direct processing of clinical samples (e.g. blood)
- Microelectronic chip enables high-throughput single cell manipulation, characterization and lysis (10,000 cells analysis per chip)
- Simultaneous gene amplification by MLPA (31 genes for breast cancer)
- Proved electrochemical DNA quantification
- Microfluidics allows single-cell sensitivity and highly parallel multi-gene detection

Project efforts

Substantial progress was made in the last period for several important technical issues. The first on-chip massively parallel single cell electrical cell impedance spectroscopy was demonstrated for tumor cells on the active sieve ASIC device (see detailed introduction in the ICsense partner portrait further below). The measurement will be performed in clinical samples for CTC identification. The immunomagnetic isolation was improved to 60% cell isolation efficiency for 100 spiked tumor cells in blood. The on-chip & off-chip isolation resulted in similar isolation efficiency. For tumor cell gene amplification, we successfully & repeatedly demonstrated **MLPA for single tumor cells** (Figure 1, below)**.**



In terms of specificity, characteristic tumor cell gene signals (e.g. ERBB2) are visible for tumor cells but not white blood cells after immunomagnetic isolation. About tumor gene (MLPA) amplicon detection, the MIRACLE DNA sensor and the potentiostat readout has achieved high signal/noise ratio (equal to typical commercial readout) but with much faster multi-gene readout efficiency. **Detection on single cell amplicons was successfully and reproducibly demonstrated.**

On the system level, the first version of injection-molded cartridges were fabricated and deployed for lab tests in Q4 2013.

This Newsletter describes work undertaken in the context of EC FP7-ICT project 257743. It is funded by the European Commission. It does not necessarily reflect its views and in no way anticipates the Commission's future policy in this area.

ICsense NV, Leuven, Belgium

In the MIRACLE project, ICsense has developed the ASIC solution for miniaturization of the complete system in close cooperation with imec. More specifically, ICsense designed the active sieve impedance spectroscopy measurement, the DEP (dielectrophoresys) control, signal processing and analog-to-digital conversion that enables **individual cancer cell capture and electrical identification** (Figure 2).

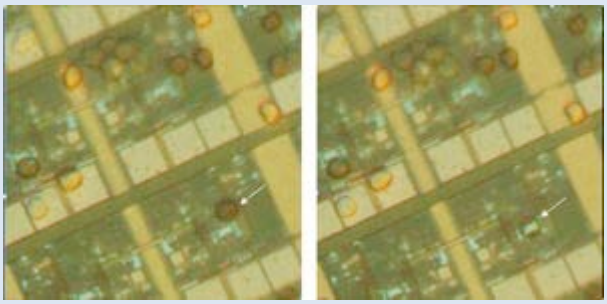


Figure 2: An MCF7 cell is electrically attracted to a pair of microelectrodes for electrical impedance measurement by CMOS circuits underneath.

Recent scientific findings indicate that individual cancer cell membrane impedance is at least two times higher than that of normal blood cells, on the order of 10 fF/um2. The ASIC can measure individual cell membrane impedance over a frequency range between 10kHz and 1MHz with an accuracy higher than 1 fF/µm2 (100 times smaller than the typical parasitic impedance of an ASIC). The ASIC can capture 100x100 cells that, in full speed scan mode, is fully read out in no more than 250 µs. This development required high-performance, highly accurate mixed-signal electronics very similar to sensor-readouts, which is the one of the core strengths of ICsense. The ASIC circuit has been successfully measured in the ICsense measurement lab, and is currently evaluated at imec laboratory on tumor cells and blood cells.

Since 2004, ICsense has been developing high-performance analog and mixed-signal ICs for automotive, industrial, consumer and medical applications. Every development is customized to the customer and the application. Since medical research and applications impose more strict quality and reliability requirements on the IC design, ICsense, therefore, is both ISO 9001:2008 and ISO 13485:2012 certified. Along these standards, ICsense has made ASICs for cochlear implants, deep brain stimulation implants, power management for implanted pulse generators (spinal cord stimulation) and several ex-body ASICs such as ECG read-outs, medical parameter dataloggers and electronic medical patches.

ICsense is founded as spin-off of the department ESAT-MICAS from the Katholieke Universiteit Leuven (KUL), Belgium. As such, ICsense is backed-up by more than 30 years of experience and research in analog and mixed-signal IC design on top 10 years of commercial ASIC design. ICsense is a privately held company with headquarters in Leuven, Belgium. The engineering team of ICsense consists of over 40 skilled analog-mixed-signal engineers and layouters, 10 of which have a PhD in micro-electronics, making it one of the largest European analog IC design companies.

Conferences and Meetings

The consortium will be hosting a dedicated session in frame of the European Cancer Congress in Amsterdam in September. The workshop is entitled "Molecular detection technologies for characterization of circulating tumor cells" and starts in the morning of the 30th of September. We will show our progress and have invited very interesting speakers. We are looking forward to discussing the project with you! See here the agenda.

We will also be at the BIOTECHNICA 2013**,** Europe's No.1 Event for Biotechnology, Life Sciences and Lab Technology. Partner ConsulTech is looking forward to discussing the project with you. Ask them for free tickets to the fair (info@consultech.de)!

Publications

M. Synnestvedt , E. Borgen, E. Schlichting, C. B. Schirmer, A. Renolen, K. E. Giercksky,J. M. Nesland, B. Naume , (2012 submitted for publication/Received: 18 December 2012 / Accepted: 30 January 2013); *Disseminated tumour cells in the bone marrow in early breast cancer: morphological categories of immunocytochemically positive cells have different impact on clinical outcome*. Springer Science+Business Media New York 2013