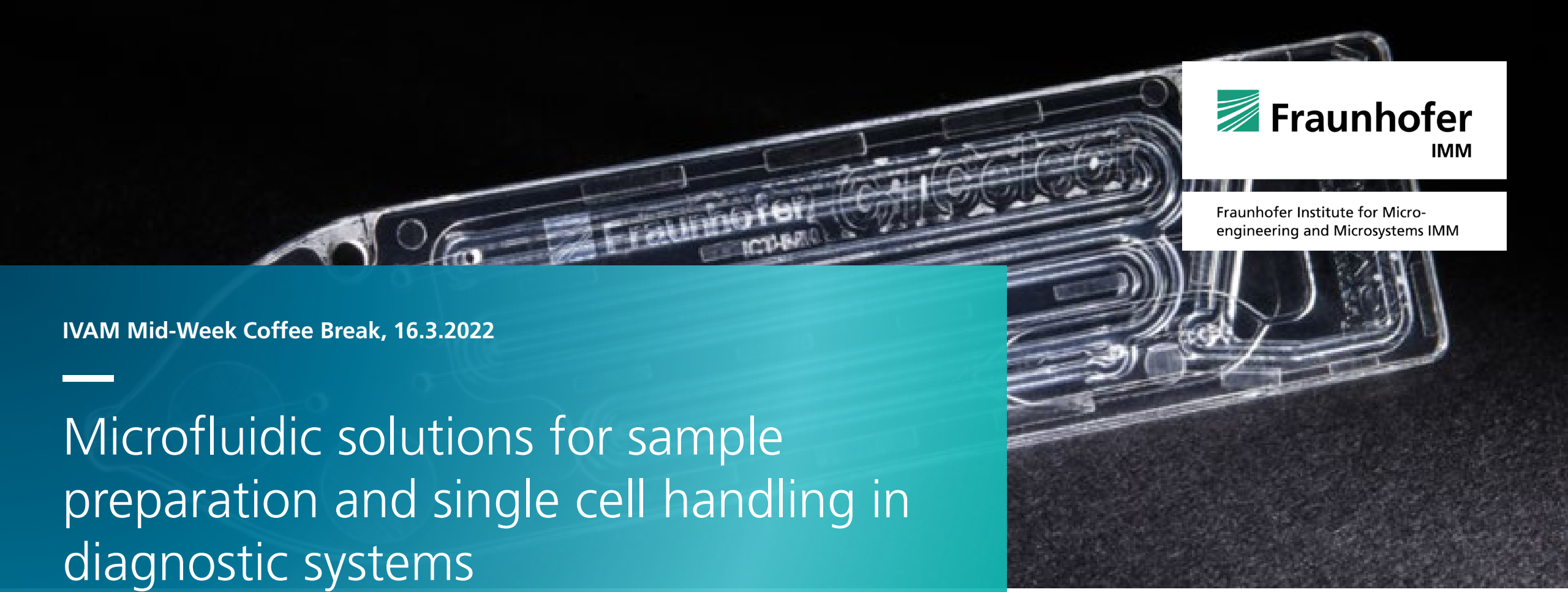

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Fraunhofer Institute for Micro-engineering and Microsystems IMM

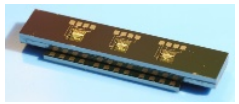
IVAM Mid-Week Coffee Break, 16.3.2022

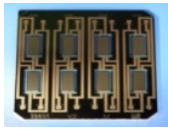
Microfluidic solutions for sample preparation and single cell handling in diagnostic systems

Dr. Michael Baßler

Fraunhofer IMM: Experts for Microfluidic Systems

Technology Portfolio





Microfluidics & Sensors

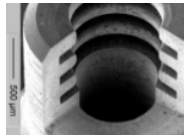
Making Information available for

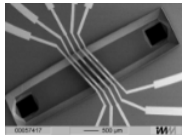
- → **Health**
- → **Production**
- → **Research**

Systems

Micro-fabrication

MEMS, precision engineering, polymer processing, packaging





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CTCelect

Get access to single CTCs

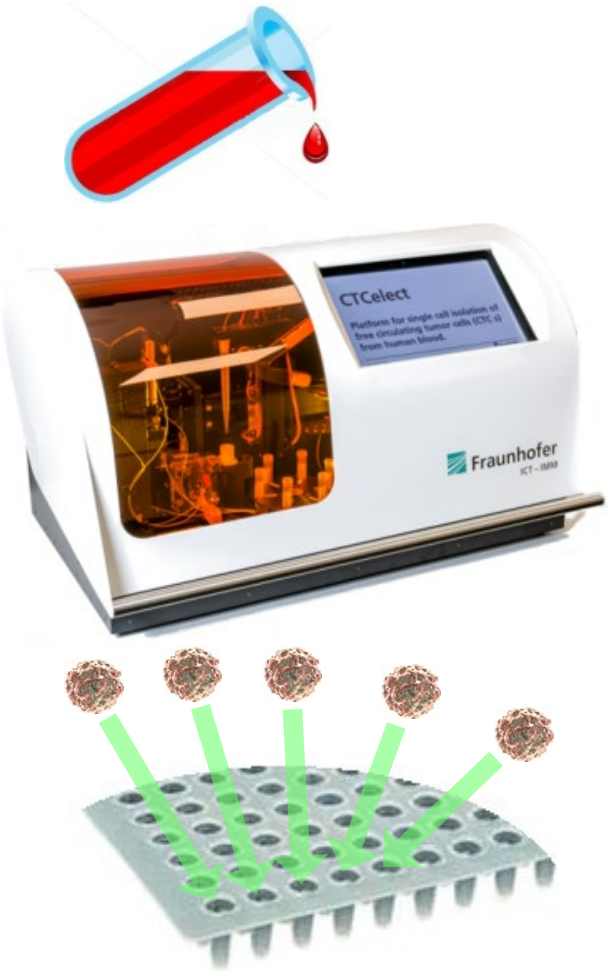
Liquid Biopsy – Circulating Tumor Cells (CTCs)

fully automated microfluidic system for **isolating single CTCs** from blood primary tubes

- no manual sample preparation
- high reproducibility

provide viable CTCs ready-to-use for single cell analysis: NGS, RT qPCR, …

in: 7.5 ml blood



out: single CTCs

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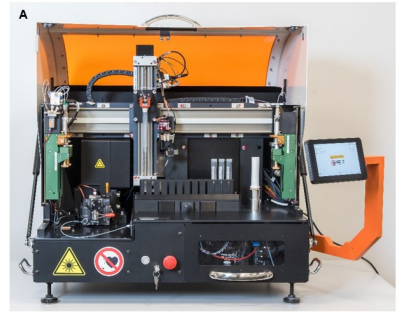
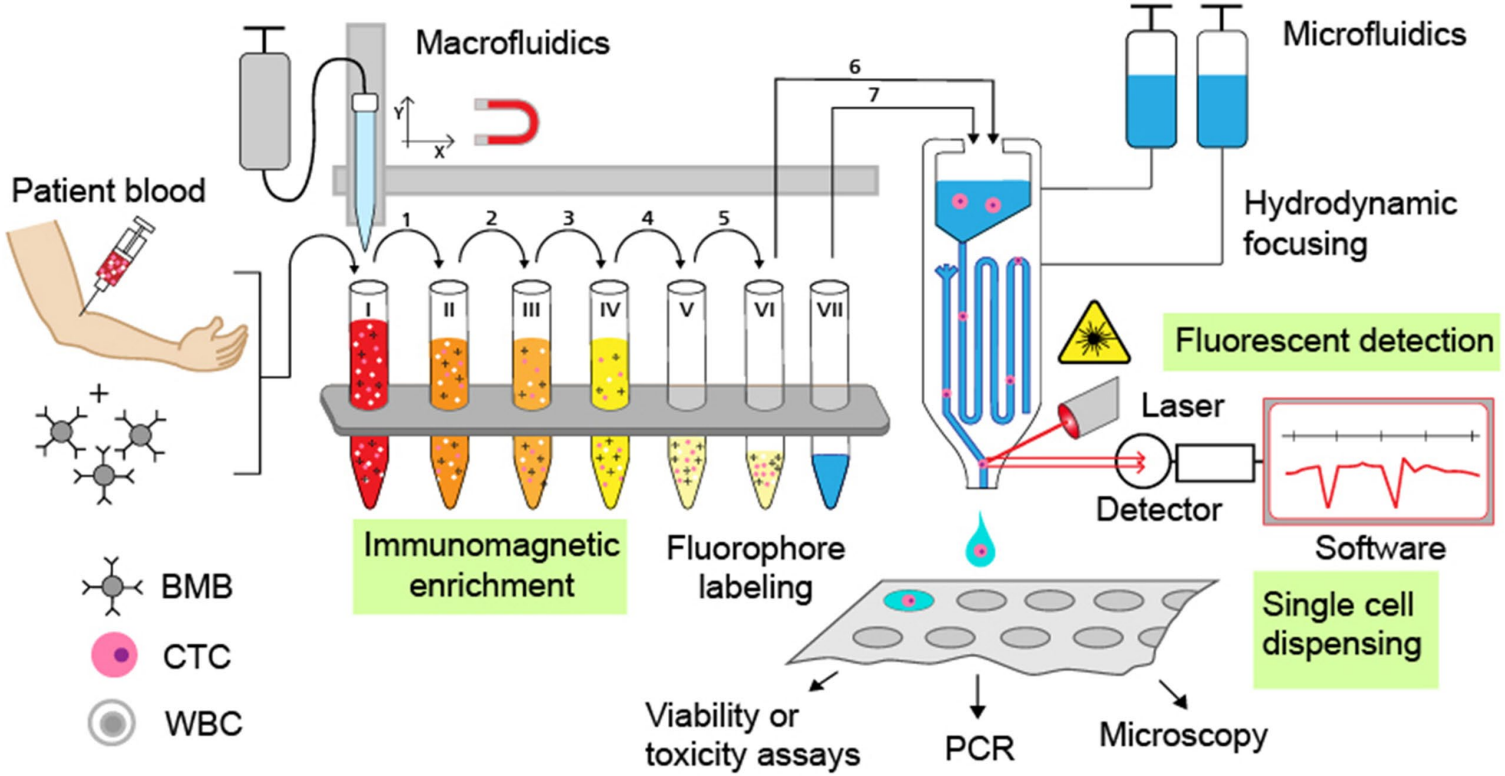
CTCelect:
schematic representation of functionality and workflow



workflow

1. take patient blood sample
2. stock instrument with reagents and consumables
3. insert blood sample
4. start cell isolation process



macro → micro or 7.5 ml of blood → single CTCs

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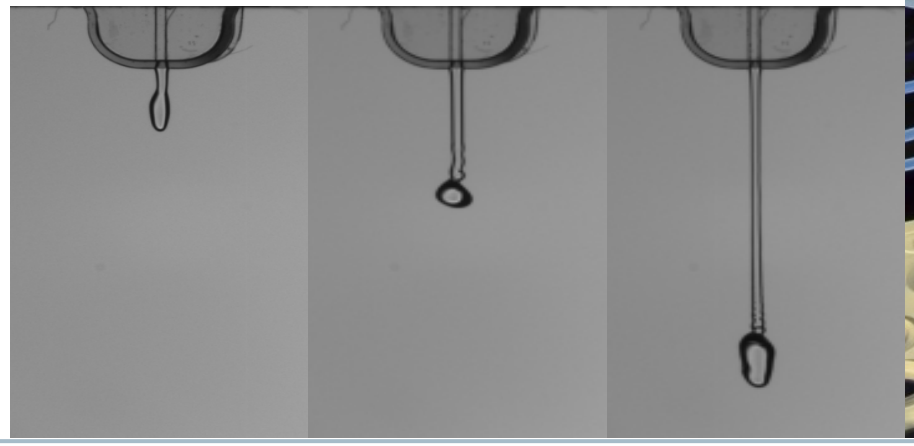
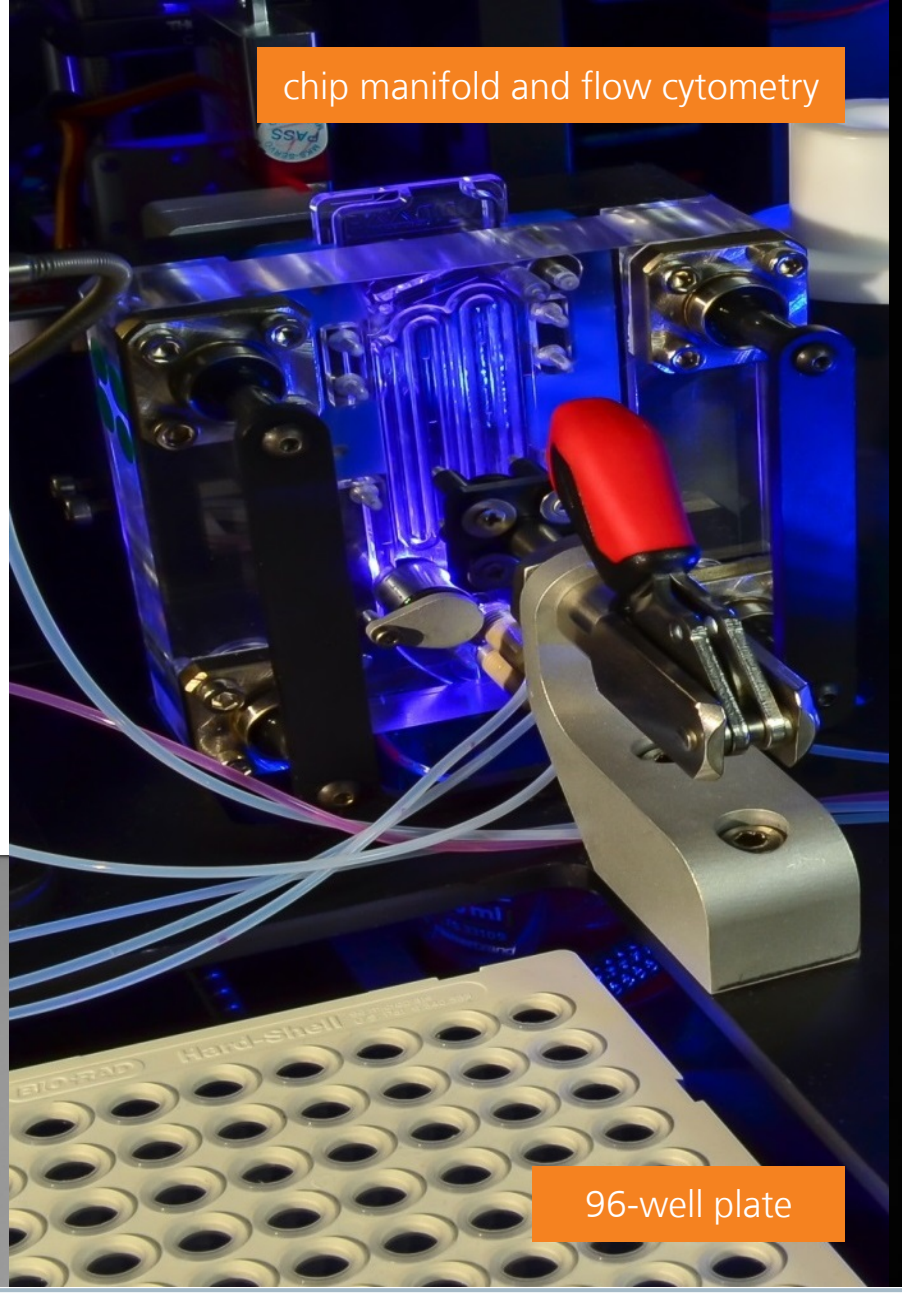
Microfluidic cell handling

flow cytometry

single cell dispensing





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Main features of CTCelect cartridge

sample reservoir

storage meander

two membrane valves

flow cytometry channel

sheath flow

dispensing nozzle

three components only: one injection molded core + two foils

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Microfluidic single cell dispensing

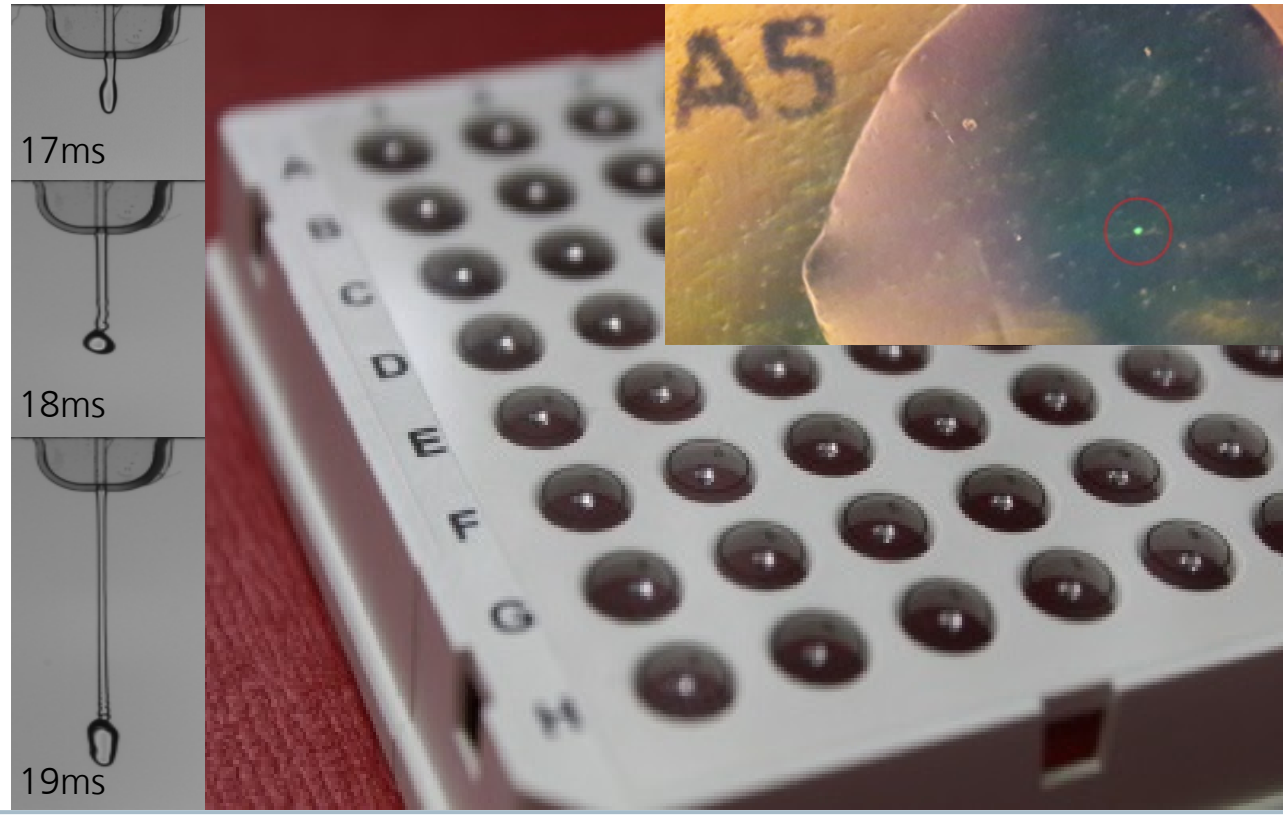
real time data processing by FPGA

CTC detected: FPGA triggers dispenser (delay depends on velocity)

feasable droplet size (current design) 0.3 µl – 3 µl

droplets precisely aligned to cavities

cell recovery 89%



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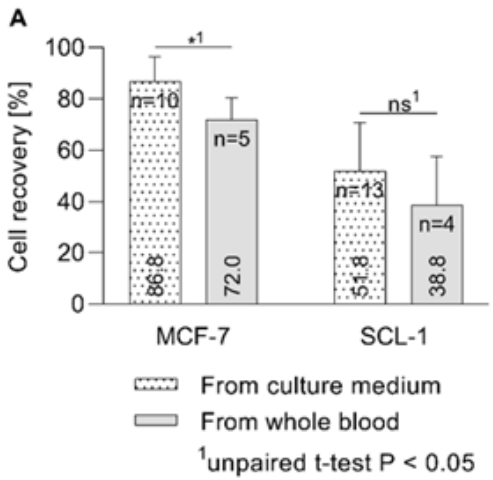
Characterization of CTCelect system

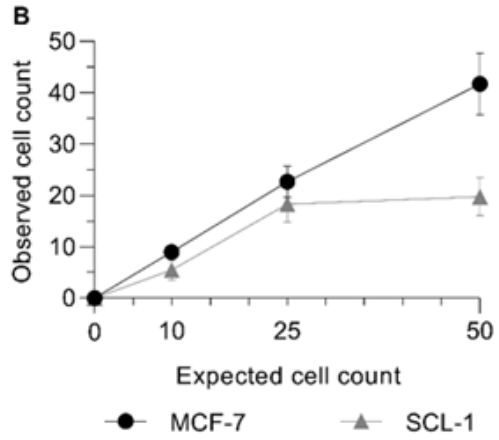
Based on tumor cell models

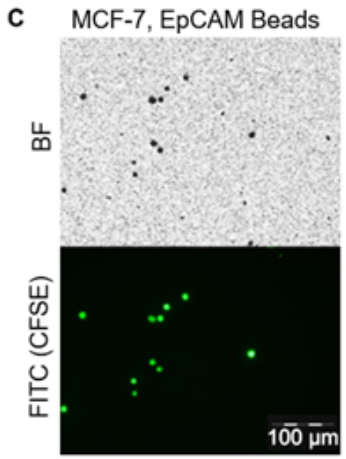
key results

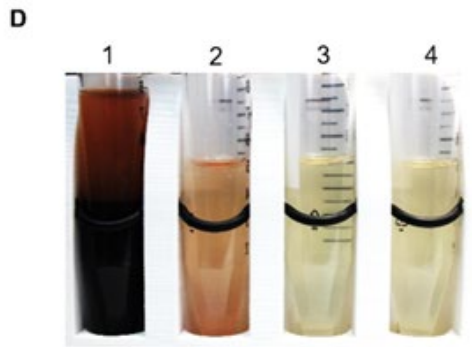
- A), B), C) recovery rates of MCF-7 and SCL-1 cells after automated IMS from culture medium and whole blood.
- D) blood residues and wash buffers
- E), F) flow cytometry of blood cell contamination

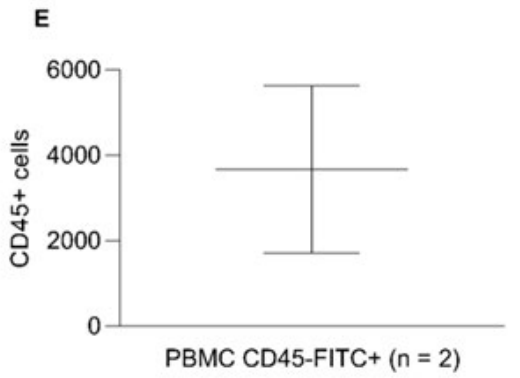
→ robust cell recovery
→ little hands-on time
→ low blood cell contamination

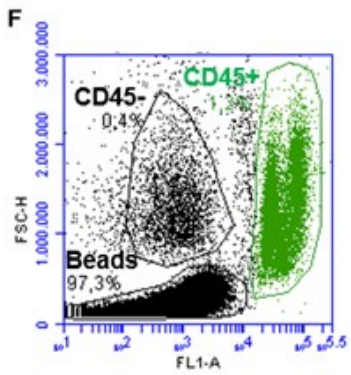












[Stiefel et al. (2022). Accepted for publication in WILEY Engineering in Life Sciences].

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Recovery of real tumor cells from patient blood

experiment

- biopsies of primary tumor or metastasis dissolved into single cells
- spike dissolved cells into patient's blood

recovery rate >80% (n=3)
(primary tumor, metastasis)

Cells	Number of spiked-in cells	Cell recovered	Recovery rate %
SCL1	30	22	73
SCL1	30	20	67
SCL1	30	21	70
		Mean	**70**
Primary tumor	30	24	80
Primary tumor	30	22	73
Primary tumor	30	26	87
		Mean	**80**
Metastasis	30	26	87
Metastasis	30	24	80
Metastasis	30	27	90
		Mean	**85**

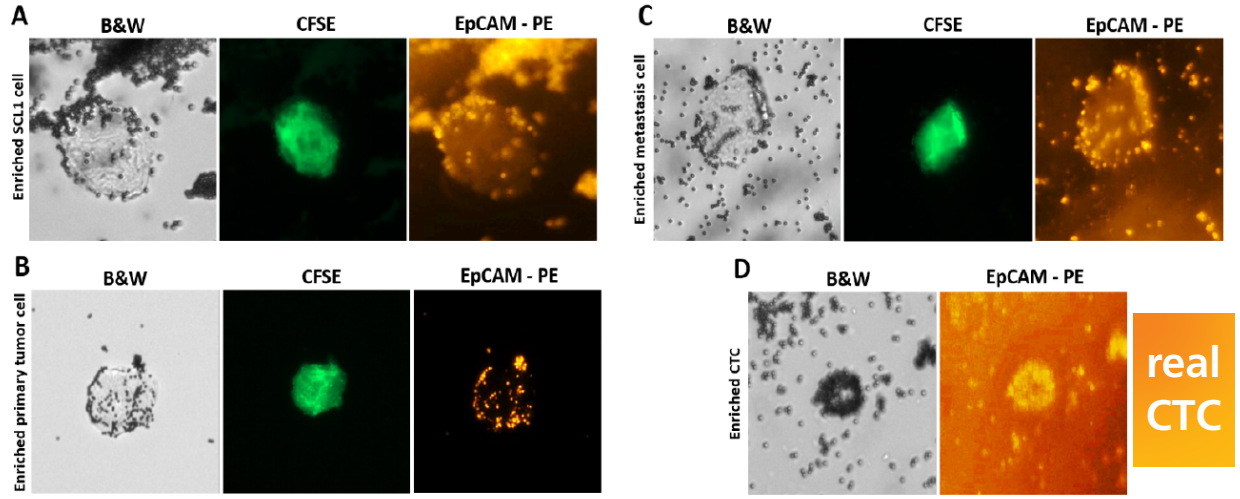

Figure 5: Single cell suspensions of tumors were CFSE stained, before being spiked-in into patients' blood to be enriched in the CTCelect instrument. (A) Enriched SCL1 cell bound to the magnetic beads; verified by CFSE staining and EpCAM expression. (B) Enriched primary tumor cell bound to the magnetic beads; verified by CFSE staining and EpCAM expression. (C) Enriched cell from a metastatic lesion bound to the magnetic beads; verified by CFSE staining and EpCAM expression. (D) Enriched CTC bound to the magnetic beads; verified by EpCAM expression.

westdeutsches tumorzentrum essen

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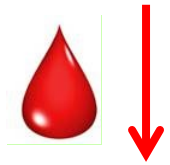
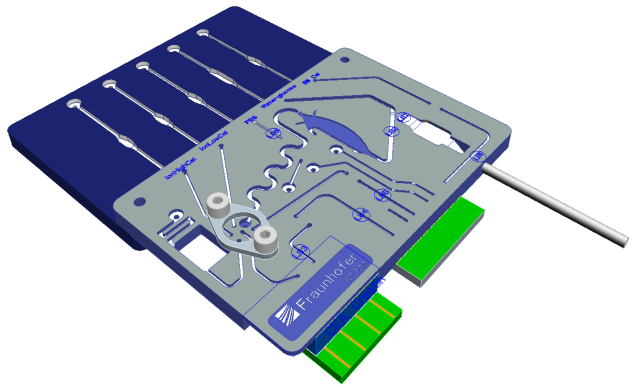
Blood Biochemistry Cartridge for liver patient management



functionality

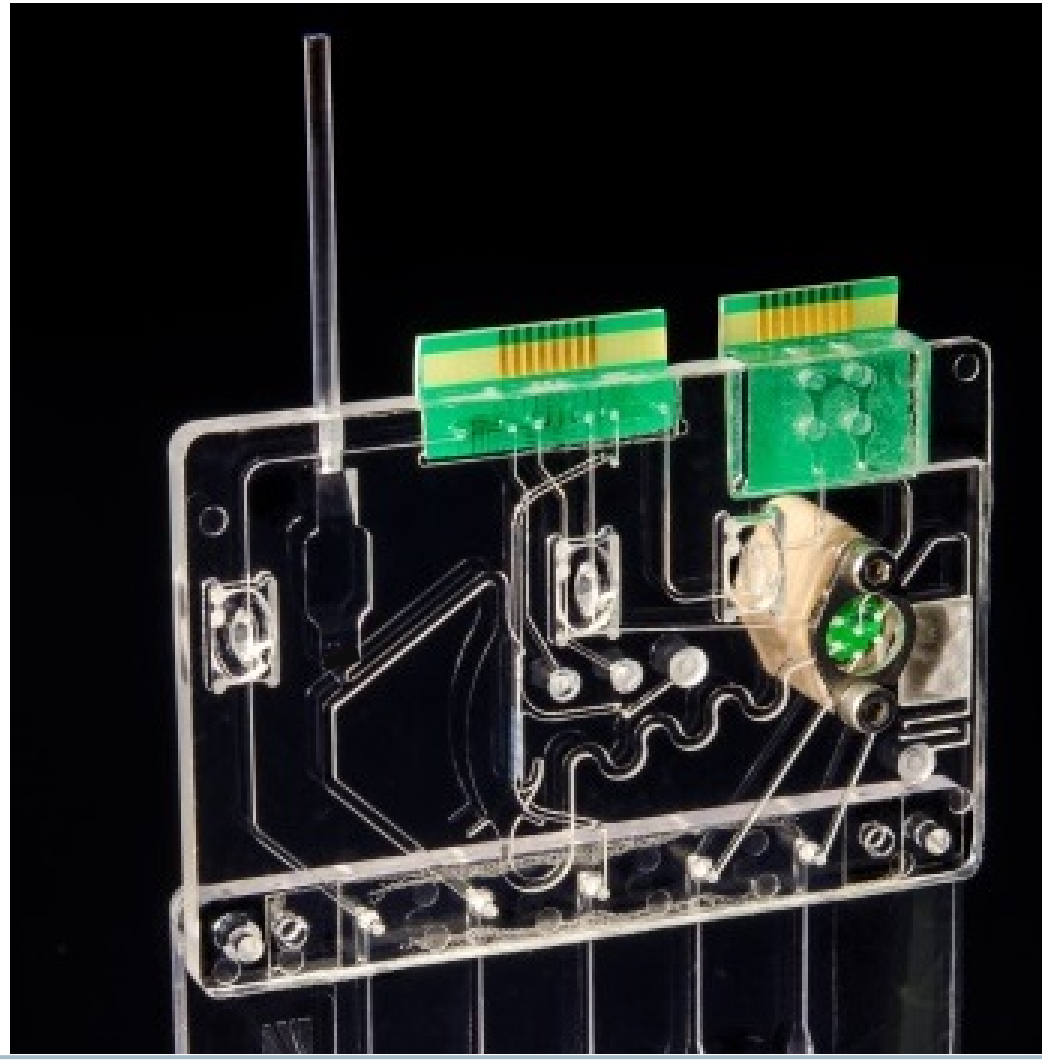
- blood sampling
- sample preparation
- analysis of 6 parameters



assay

- 20 µl blood (finger prick)
- 5 liquids
- 4 freeze dryed reagents

assay fully implemented on cartridge



On-cartridge serum generation

functionality

- aspiration in capillary
- transfer to coagulation chamber
- coagulation
- extraction of serum

serum quality

- additional hemolysis acceptable for downstream analysis
- RBCs sediment in dilution chamber

20µl blood (finger prick)
→ 5µl serum

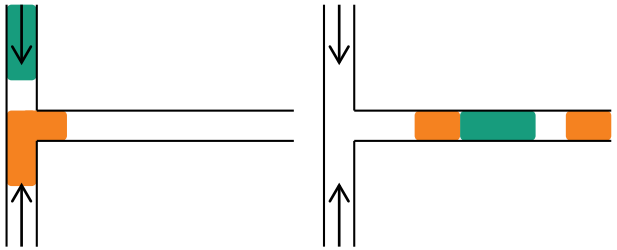
serum quality

run	hemolysis (%)	RBC count (1/µl)
#1	1.17	22,500
#2	2.56	168,750
#3	1.92	625,000
#4	0.75	1,725,000
#5	0.37	3,450,000
#6	1.21	28,750
#7	0.30	90,000
#8	1.33	165,000
average	**1.20**	**789,400**

Dilution in plug flows

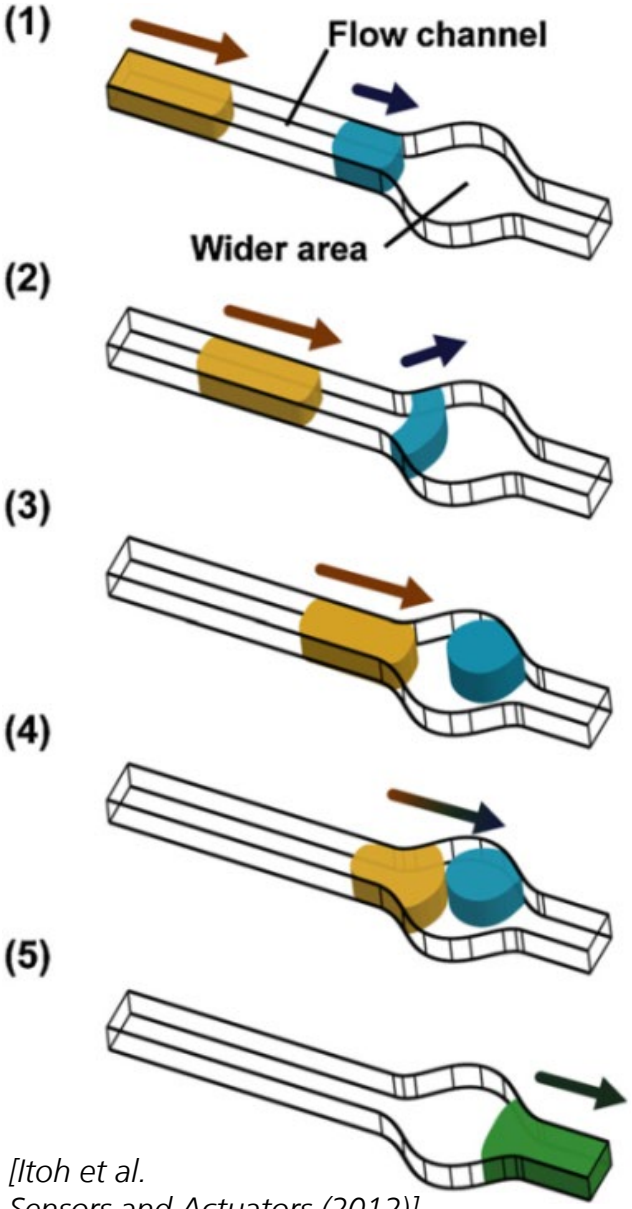
problem

- T-junction requires precise plug positioning
- tedious process control required!



potential solution

- Itoh et al.: “merging of two droplets … wider portion”



[Itoh et al. Sensors and Actuators (2012)]

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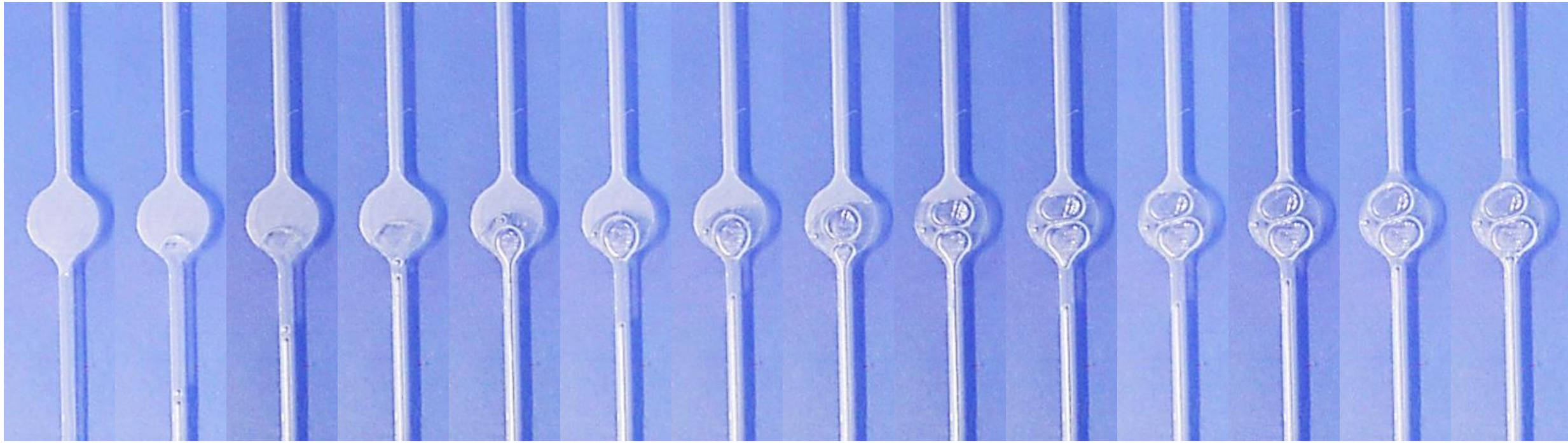
Dilution in case of „real“ liquids

Hydrophilic & high surface tension



bubble formation & capillary drift backwards !!

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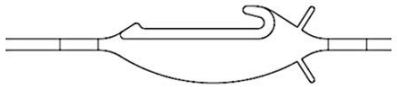
Dilution chamber on blood biochemistry cartridge

functionality

- dilution 1:5 (2 µl : 10 µl)
- liquid 1: blood serum
- liquid 2: glucose in water (5 wt.%)

properties



- fully passive structure
- layout adaptable to contact angle and surface energy
- wide range of dilution factors in a given design

key advantage: minimized process control!

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