

- 1 *Circulating tumor cell*
- 2 *Microfluidic cartridge for single cell dispensing*

CTCelect: ISOLATION OF SINGLE CTCs FROM HUMAN BLOOD

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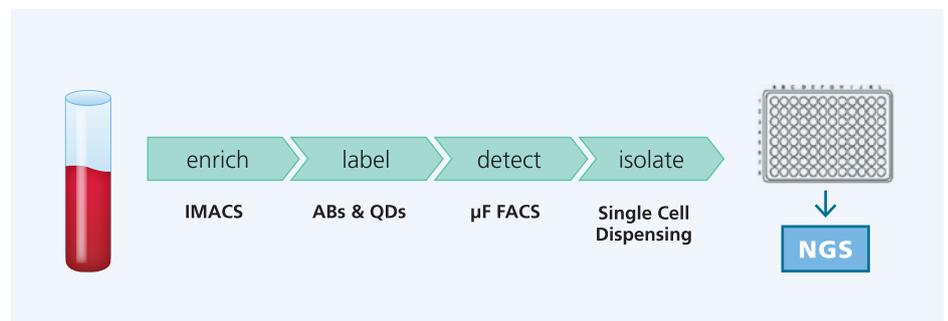
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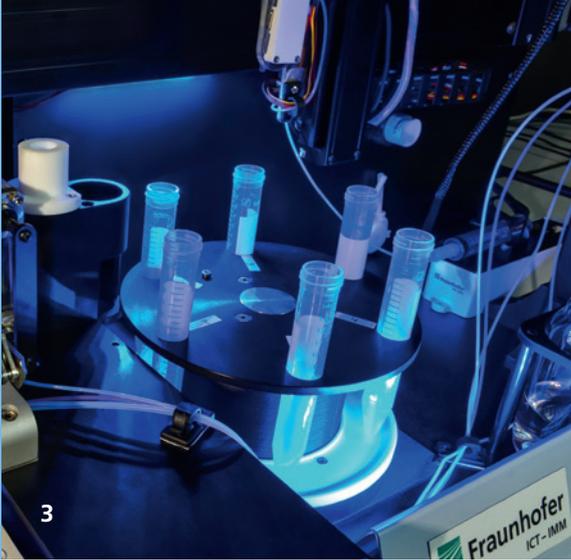
Liquid biopsy for future cancer therapy

Future cancer therapy is expected to rely heavily on the so called “liquid biopsy”. Tumor cells, which circulate in the blood of cancer patients (CTCs) will be used to gain detailed molecular diagnostic information on tumor subtypes to tailor the therapeutic intervention for each individual patient (“personalized medicine”). Moreover, therapy will be monitored and adapted by means of repeated CTC counts following the interventions.

The CTCelect instrument

CTCelect is a fully automated instrument which directly links clinical blood sampling with state-of-the-art single cell analytics. CTCelect starts with a raw human blood sample in a standard sampling tube, enriches the CTCs, detects the CTCs in a continuous flow and dispenses single CTCs selectively into wells of a standard microtiter plate resembling the starting point for single cell analysis.



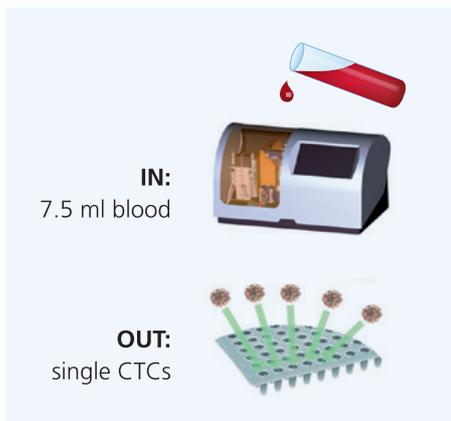


The workflow is implemented in two main functional modules of the instrument:

- 1) A liquid handling unit for immunomagnetic enrichment and cell staining
- 2) A microfluidic unit for CTC isolation.

1) Immunomagnetic enrichment

Starting with a blood tube carrying 7.5 ml of human blood, a fully automated pipetting station enriches the CTCs into a reduced sample volume of 0.5 ml. In addition, the cells are fluorescently labelled with a secondary antibody and quantum dots. The process is based on a specifically tailored reagent kit. Besides the kit reagents, only standard laboratory consumables are required (disposable pipetting tips and tubes).



2) Microfluidic cartridge

The pre-enriched sample is automatically transferred to the microfluidic CTC cartridge. An integrated flow cytometry module detects CTCs and triggers the dispensing unit. CTCs are then individually dispensed into wells of a microtiter plate.

The CTC cartridge is disposable and for exclusive use in the CTSelect instrument.

All processing steps are carried out automatically inside the device. Handling steps are minimal and limited to (re)placing tubes, the microfluidic cartridge, and buffer bottles on the worktable. Software with intuitive graphical user interface for starting the assay and providing the reporting guarantees minimal hands on time.

Core innovations

- Reliable extraction of a low number of CTCs (1-100) from a huge background of white / red blood cells and platelets (10^{10}).
- Dispensing of single CTCs in individual wells for further processing.

Unique features

- Unique solution, which directly separates single CTCs for microbiological analysis from a standard blood sampling tube into a microtiter plate.
- Compatibility to Next Generation Sequencing (NGS) and modern companion diagnostics.
- Compact desktop device and low cost disposables.
- High flexibility particularly attractive for the research users' only market (open platform, freedom to use non-proprietary reagents, cell pooling).
- Wide application spectrum by availability of additional protocols:
 - Exosome preparation in large volume
 - Preparation of cell free nucleic acids

Status

- Reagents and assays are developed for
- Immunomagnetic enrichment of CTCs from human blood
 - Fluorescent staining for CTC detection
 - Cell stabilization and prevention of CTC
 - Sedimentation and adsorption
 - Isolation protocols

Instrument and automation

- System modules for immunomagnetic enrichment and cell staining are operational and assay functionality has been demonstrated
- Microfluidic cartridge (manufactured by injection molding) is fully functional with respect to fluidic workflow, CTC detection and selective dispensing of sub-volumes (1-2 μ l)
- Device including integrated software and user interface for all system functions is operational

Performance

On the basis of the model system (7.5 ml of donor blood spiked with 20 MCF7 cells) a total of more than 70 % of the cells can be recovered in approximately 1-2 hours from loading the blood tube to unloading the microtiter plate.

3 Detail of CTSelect instrument: microfluidic single cell dispensing (left) and immunomagnetic enrichment (right)

4 CTSelect instrument