Supplementary Material

Research Article

Automated immunomagnetic enrichment and optomicrofluidic detection to isolate breast cancer cells: A proof-of-concept towards PoC therapeutic decision-making

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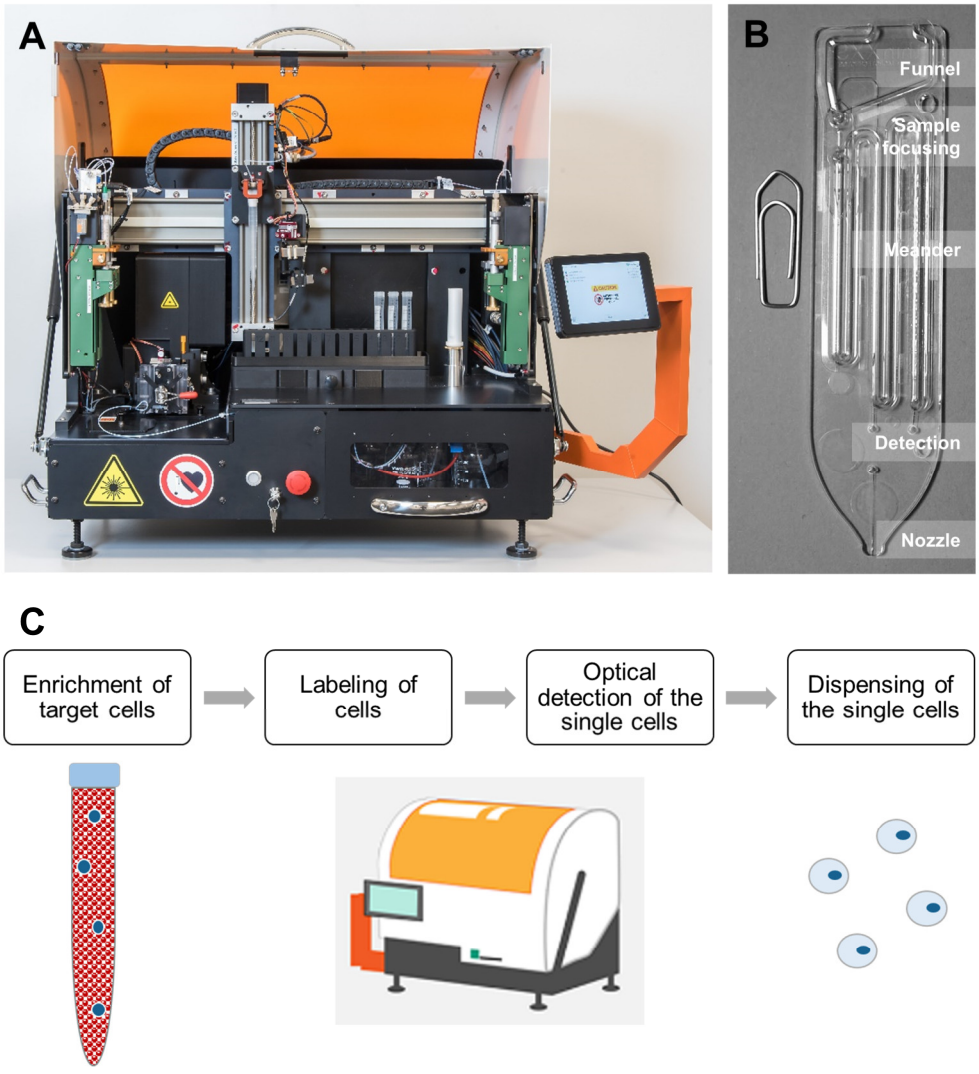


Figure S1. CTCelect system, microfluidic chip and process conception. A) The benchtop device consists of an immunomagnetic enrichment module (right-sided) and a microfluidic fluorescence-activated cell sorting (μCS) subunit (left-sided). The fully-automated isolation process is user-controlled via touchscreen. Sample handling and transfer is managed by a pipetting robot. B) The CTCelect chip is placed in the chip holder of the cell sorting subunit and disposed after isolation. The chip consists of a reservoir funnel for the cell suspension, a

hydrodynamic focusing channel, a detection zone and a nozzle for cell dispensing. C) CTCelect concept for single cell dispensing from 7.5 mL samples (from Stiefel et al. *Eng Life Sci*. 2022; 22: 391– 406. https://doi.org/10.1002/elsc.202100133 [9]).

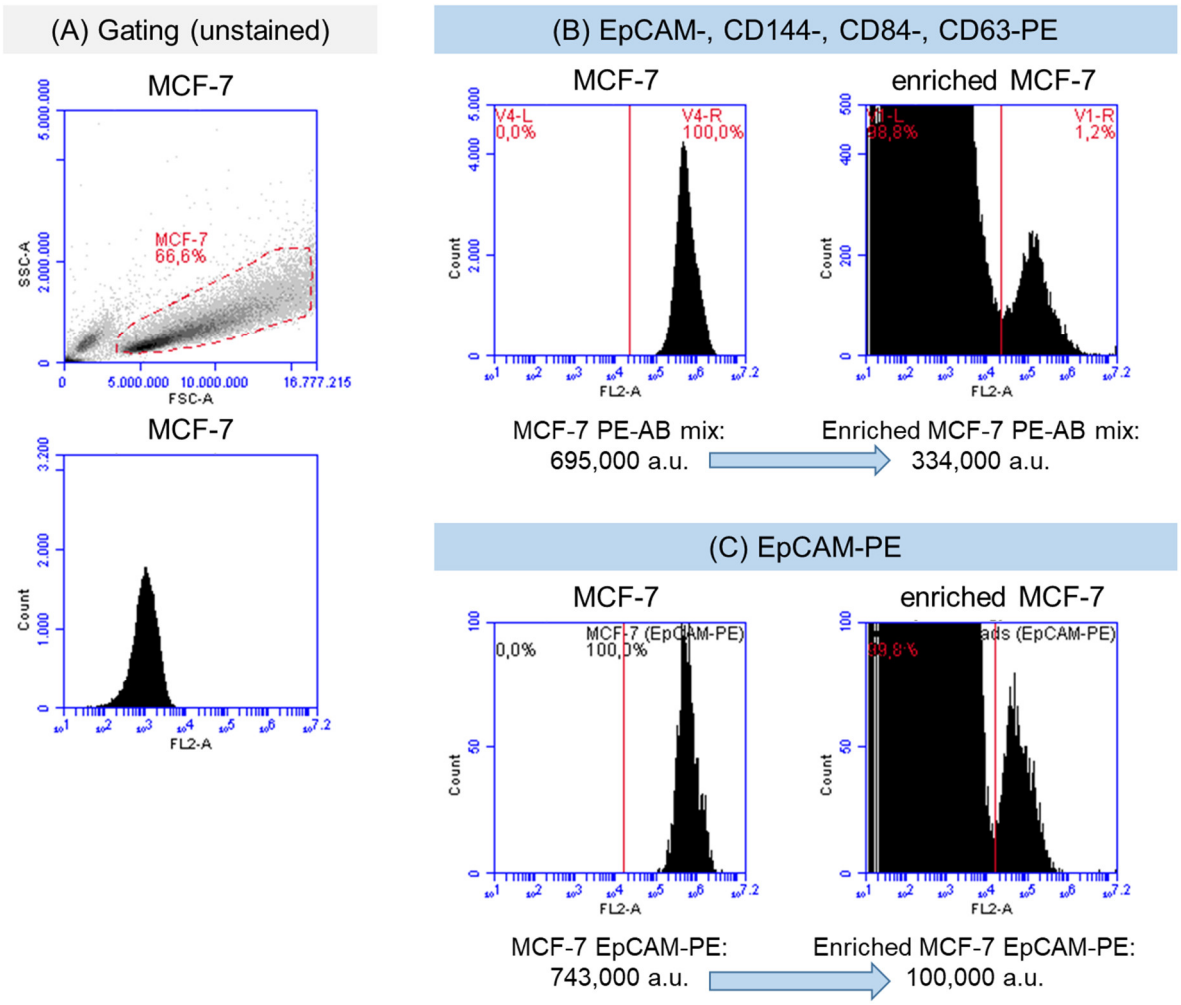
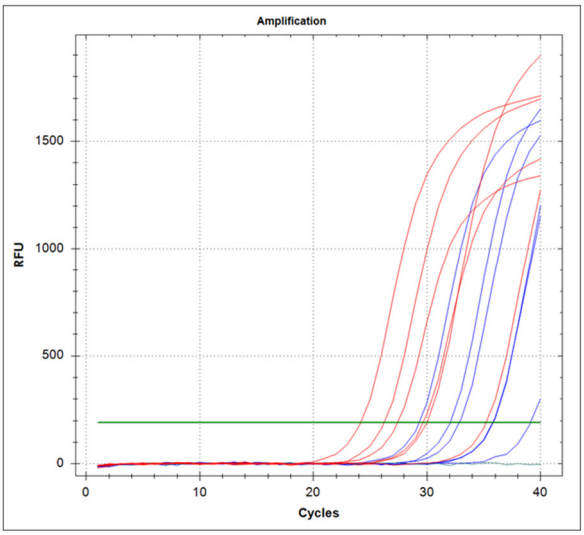
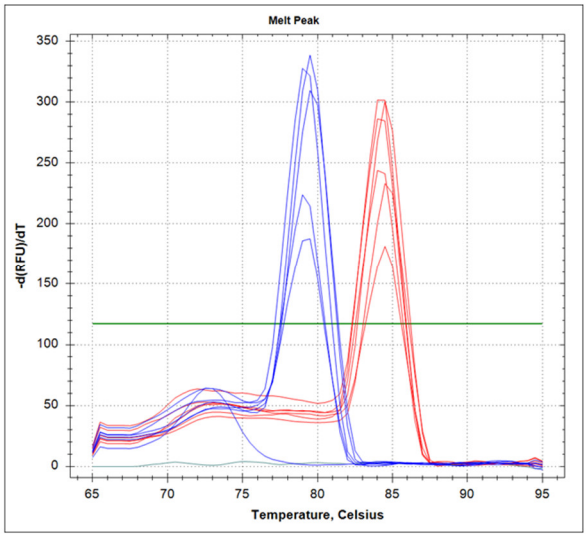


Figure S2. Flow cytometry of PE-antibody (AB) stained MCF-7 cells with or without beads enrichment. MCF-7 cells were enriched with EpCAM-coupled immunomagnetic beads for 10 minutes. Unbound and enriched cells were then stained with either EpCAM-, CD144-, CD84-, CD63-PE (B) or only EpCAM-PE (C) antibody solution (1:30) alternated with several washing steps. Fluorescence intensity in the FL2 channel was measured using the flow cytometer. Events of unbound cells were gated to an unstained MCF-7 population from a previous measurement (A). Beads were detected as a large overlapping population at FL2 < 10^4 a.u.





Target	Cell count	C_T	Mean C_T
Actin	40 cells	24,15	**24,15**
	20 cells	26,22	**26,22**
	1 cell	30,02	**32,61**
	1 cell	35,20	
	1 cell cultured	29,64	**28,52**
	1 cell cultured	27,40	
EpCAM	40 cells	29,31	**29,31**
	20 cells	32,05	**32,05**
	1 cell	39,11	**37,47**
	1 cell	35,83	
	1 cell cultured	35,78	**34,32**
	1 cell cultured	32,85	

EpCAM (136 bp)
B-actin (176 bp)
NegC EpCAM (medium)

Figure S3. qPCR and melt curve analysis from RNA of one, 20 and 40 MCF-7 single cells targeting EpCAM and β-actin. Two-step qPCR was performed with RNeasy Micro RNA isolation kit (QIAGEN) to confirm fluorescence signals at low cell numbers. Therefore, we picked single cells from a cell suspension in a petri dish and directly lysed one, 20 and 40 cells per spin column. We observed an increase in relative fluorescence intensity in clear correlation with the cell number for both EpCAM and β-actin expression. Seeded single cells (“cultured”) from a 96 well plate served as a control.

Table T1. Primer Sequences.

Name	Gene (human), mRNA	Product length [bp]	Sequence (5'-3')
Aktin_for	β-actin ACTB	176	ATT GCC GAC AGG ATG CAG AA
Aktin_rev			GGG CCG GAC TCG TCA TAC TC
EpCAM_for	Epithelial cell adhesion molecule	136	CCG CAG CTC AGG AAG AAT GT
EpCAM_rev			CAT TTG GCA GCC AGC TTT GA
PGR_for	Progesterone transcr. V1	194	GTCTACCCGCCCTATCTCAAC
PGR_rev			TAGTTGTGCTGCCCTTCCATT
ESR1_for	Estrogen transcr. V1	129	TGGGAATGATGAAAGGTGGGA
ESR1_rev			GGTTGGCAGCTCTCATGTCT
HER2_for	HER-2/NEU (ERBB2) transcr. V1	108	CCGGAGCCGCAGTGAG
HER2_rev			CTGTGCCGGTGCACACTT
CD45_for	CD45; PTPRC transcr. V1	159	ACCAGGAATGGATGTCGCTA
CD45_rev			TGGGGCCTGTAAAAGTGTCC
CXCR4_for	C-X-C motif chemokine receptor 4	60	CTGTGAGCAGAGGGTCCAG
CXCR4_rev			ATGAATGTCCACCTCGCTT