

Microfluidic Cancer Cell Capture Technologies

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ABSTRACT

Circulating tumour cells (CTCs) -- cancer cells shed from primary tumours into the bloodstream at concentrations of 1-100 cells per 10 mL among billions of blood cells -- carry critical information for cancer staging, treatment selection, resistance monitoring, and metastatic prognosis. However, capturing these extremely rare cells with high efficiency and purity from whole blood remains a formidable technical challenge. Microfluidic technologies exploit laminar flow physics, surface functionalisation, and precisely engineered microstructures to achieve capture efficiencies and purities far exceeding conventional CellSearch (the only FDA-cleared CTC platform, with 40-70% capture efficiency for EpCAM+ CTCs). This study developed and evaluated five microfluidic CTC capture architectures -- antibody-coated micropillar arrays, herringbone-grooved chaotic mixers, deterministic lateral displacement (DLD) size-based sorting, acoustofluidic separation, and a proposed hybrid immunomagnetic-inertial microfluidic chip (HIM-Chip) combining anti-EpCAM magnetic nanoparticle labelling with Dean flow inertial focusing for dual-mode EpCAM-dependent and size-based capture -- across spiked healthy blood samples ($n = 180$) and clinical samples from 48 metastatic cancer patients (breast, lung, colorectal). HIM-Chip achieved the highest capture efficiency (96.2 $\pm$ 2.4% for EpCAM+ cells and 82.4 $\pm$ 4.8% for EpCAM-low/negative CTCs), the highest purity (68.4 $\pm$ 8.2% among captured cells), and maintained >92% cell viability for downstream molecular analysis. In clinical samples, HIM-Chip detected CTCs in 91.7% (44/48) of patients versus 62.5% (30/48) for CellSearch ($p < 0.001$), with a median 4.2-fold higher CTC count. These results establish the hybrid immunomagnetic-inertial approach as the most effective CTC capture platform and provide a systematic benchmark for microfluidic liquid biopsy technologies.

Keywords: Circulating tumour cells; Microfluidics; Liquid biopsy; CTC capture; Inertial microfluidics; Immunomagnetic separation; Cancer diagnostics; EpCAM; Metastasis monitoring

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1. Introduction

Metastasis -- the spread of cancer cells from the primary tumour to distant organs via the bloodstream and lymphatic system -- accounts for approximately 90% of cancer-related deaths. Circulating tumour cells (CTCs) represent the intermediate stage of the metastatic cascade: cells that have successfully intravasated from the primary tumour, survived in circulation, and may extravasate to seed secondary tumours at distant sites. CTC enumeration has been validated as an independent prognostic biomarker: in metastatic breast cancer, patients with ≥ 5 CTCs per 7.5 mL blood have significantly shorter progression-free and overall survival compared to those with < 5 CTCs (Cristofanilli et al., 2004). Beyond enumeration, molecular characterisation of captured CTCs enables real-time monitoring of tumour evolution, resistance mechanism identification, and pharmacogenomic-guided treatment selection -- collectively termed 'liquid biopsy' for its potential to replace invasive tissue biopsies (Alix-Panabieres and Pantel, 2016).

1.1 The CTC Capture Challenge

CTCs are extraordinarily rare: 1-100 cells per 10 mL of blood containing approximately 50 billion erythrocytes, 35 million leukocytes, and 1.5 billion platelets. This 1-in- 10^9 rarity demands capture technologies with extreme sensitivity ($> 90\%$ capture of true CTCs) and specificity (discrimination from the 10^9 background cells to achieve workable purity). The CellSearch system (Menarini Silicon Biosystems), FDA-cleared for metastatic breast, prostate, and colorectal cancer, uses anti-EpCAM ferrofluid magnetic capture followed by fluorescent staining (CK+/DAPI+/CD45-), achieving 40-70% capture efficiency for EpCAM-expressing CTCs (Riethdorf et al., 2007). However, CellSearch misses CTCs that have undergone epithelial-to-mesenchymal transition (EMT) with reduced or absent EpCAM expression -- precisely the subpopulation most associated with metastatic competence and therapy resistance (Yu et al., 2013).

1.2 Microfluidic Approaches to CTC Capture

Microfluidic devices exploit the unique physics of low-Reynolds-number flows ($Re < 1$) to manipulate individual cells based on biological (surface markers) and physical (size, deformability, density) properties. Antibody-functionalised microstructures (micropillars, herringbone grooves) capture CTCs through affinity binding under controlled shear stress, achieving 65-95% capture efficiency for EpCAM+ cells (Nagrath et

al., 2007; Stott et al., 2010). Label-free approaches -- deterministic lateral displacement (DLD), inertial focusing, and acoustophoresis -- exploit the larger size (12-25 μm) and lower deformability of CTCs compared to leukocytes (7-12 μm) and erythrocytes (6-8 μm), capturing CTCs regardless of surface marker expression (Karabacak et al., 2014). Each approach carries inherent trade-offs between capture efficiency, purity, throughput, and the ability to capture phenotypically diverse CTC populations.

1.3 Study Objectives

This study aimed to: (i) fabricate and characterise five microfluidic CTC capture architectures representing the major capture principles; (ii) develop a hybrid immunomagnetic-inertial chip (HIM-Chip) capturing both EpCAM+ and EpCAM-low CTCs through dual-mode operation; (iii) benchmark capture efficiency, purity, viability, and throughput using standardised spiked blood models; (iv) validate clinical performance in 48 metastatic cancer patients; and (v) demonstrate downstream molecular analysis (single-cell RNA sequencing) of HIM-Chip-captured CTCs.

2. Literature Review

Microfluidic CTC capture has advanced rapidly since the first CTC-Chip demonstration in 2007, with over 100 distinct device architectures reported in the literature.

2.1 Affinity-Based Microfluidic Capture

The original CTC-Chip (Nagrath et al., 2007) used 78,000 anti-EpCAM-coated silicon micropillars to capture CTCs from whole blood at 1-2 mL/h, achieving 65% capture efficiency. The herringbone-chip (HB-Chip, Stott et al., 2010) replaced micropillars with staggered herringbone grooves generating chaotic microvortices that enhance cell-surface contact, improving capture to 91.8% with higher throughput (2-3 mL/h). The geometrically enhanced differential immunocapture (GEDI) chip incorporated size-selective microposts that preferentially capture larger CTCs while deflecting smaller leukocytes, improving purity from 9% to 62% (Gleghorn et al., 2010). NanoVelcro surfaces -- nanostructured substrates coated with anti-EpCAM antibodies -- further enhanced capture by increasing the effective surface area and matching the nanoscale topography of cell surface microvilli (Wang et al., 2011). All affinity approaches share the fundamental limitation of EpCAM-dependent capture, missing the

EMT-transformed CTC subpopulation.

2.2 Label-Free Physical Separation

Deterministic lateral displacement (DLD) arrays use precisely positioned micropillar arrays to separate cells by size based on their interaction with the laminar flow streamlines: cells larger than a critical diameter are deterministically displaced laterally while smaller cells follow the primary flow (Huang et al., 2004). The CTC-iChip (Karabacak et al., 2014) combined DLD pre-enrichment with inertial focusing and magnetic depletion of CD45+ leukocytes, achieving label-free capture at 10⁷ depletion of background cells. Acoustofluidic devices use standing acoustic waves to separate cells by size and density in a contact-free manner, achieving 86% capture efficiency with >98% viability (Li et al., 2015). Inertial microfluidics exploits size-dependent lift and Dean drag forces in curved channels to focus cells into size-sorted streams without external fields, enabling high-throughput processing (>1 mL/min) suitable for clinical blood volumes (Warkiani et al., 2016).

2.3 Hybrid and Multi-Stage Approaches

Recognising that no single capture principle addresses all CTC subpopulations, hybrid devices combining affinity and physical separation have been developed. The Vortex chip uses microscale vortex trapping to size-select CTCs from blood without antibodies, followed by on-chip immunostaining for identification (Sollier et al., 2014). The integrated CTC analysis platform (iCTC) combines magnetic EpCAM capture with downstream single-cell electroporation for molecular analysis (Zeinali et al., 2020). These multi-stage architectures achieve higher combined sensitivity than single-mode devices but add complexity, reduce throughput, and increase cell loss at each processing step.

Table 1. Microfluidic CTC capture architectures evaluated: capture principle, target property, and key specifications

Device	Capture Principle	Target Property	Flow Rate (mL/h)	EpCAM Dependent	Reference
Micro pillar	Affinity binding	EpCAM surface marker	1-2	Yes	Nagrath et al., 2007
HB-Chip	Chaotic mixing + affinity	EpCAM surface marker	2-3	Yes	Stott et al., 2010

Device	Capture Principle	Target Property	Flow Rate (mL/h)	EpCAM Dependent	Reference
DLD array	Deterministic displacement	Cell size (>12 μ m)	1-2	No	Huang et al., 2004
Acoustofluidic	Acoustic radiation force	Size + density	0.5-1	No	Li et al., 2015
HIM-Chip	Immunomagnetic + inertial	EpCAM + size (dual)	3-5	Partially	This study

HB-Chip: herringbone-grooved chip; DLD: deterministic lateral displacement; HIM-Chip: hybrid immunomagnetic-inertial microfluidic chip. Flow rate: processing speed for whole blood. EpCAM dependent: whether capture relies on EpCAM expression (Yes = misses EMT-transformed CTCs; No = label-free; Partially = captures EpCAM+ by affinity and EpCAM- by size).

3. Materials and Methods

3.1 HIM-Chip Design and Fabrication

HIM-Chip integrates two capture stages in a single PDMS device. Stage 1 (Immunomagnetic): whole blood is pre-incubated (15 min, RT) with anti-EpCAM-conjugated magnetic nanoparticles (50 nm Fe₃O₄@SiO₂, Kd 2.8 nM, 10⁹ particles/mL). The labelled blood flows through a serpentine channel (200 μ m wide, 50 μ m deep, 40 mm total length) flanked by NdFeB permanent magnets (0.8 T) that deflect magnetically labelled EpCAM+ cells to a dedicated capture chamber. Stage 2 (Inertial): the magnetically depleted effluent (containing EpCAM-low/negative CTCs) enters a spiral inertial focusing channel (500 μ m wide, 100 μ m deep, 5 loops, 10 cm total length) where Dean flow vortices and inertial lift forces focus larger cells (>14 μ m, CTC size range) toward the inner wall while smaller cells (<12 μ m, leukocytes, erythrocytes) migrate outward. A bifurcation at the outlet separates the CTC-enriched inner fraction from the waste outer fraction. Total dimensions: 50 x 30 mm; fabricated by standard soft lithography (SU-8 master, 10:1 PDMS, plasma-bonded to glass). Flow rate: 3-5 mL/h (7.5 mL blood processed in 1.5-2.5 hours).

3.2 Spiked Blood Model Experiments

Three cancer cell lines representing different EpCAM expression levels: MCF-7 (breast, EpCAM-high), MDA-MB-231 (breast, EpCAM-low), and A549 (lung, EpCAM-moderate). Cells were pre-stained with CellTracker Green (Invitrogen), counted by haemocytometer, and spiked into 7.5

mL healthy donor whole blood (EDTA-anticoagulated, <4 hours from collection) at 10, 50, 100, and 500 cells per sample. Each condition tested in triplicate across 3 independent experiments (n = 9 per condition per device; total n = 180 spiked samples across all devices). Capture efficiency: (captured cells / spiked cells) x 100, enumerated by fluorescence microscopy of the capture chambers. Purity: (captured CTCs / total captured nucleated cells) x 100; leukocytes identified by CD45-APC staining. Viability: trypan blue exclusion and calcein-AM/EthD-1 Live/Dead staining on released cells. All five devices tested under identical blood samples (split from same donor) for within-sample comparison.

3.3 Clinical Validation

Forty-eight patients with metastatic cancer (16 breast, 16 non-small-cell lung, 16 colorectal; all stage IV, pre-treatment or progression) were enrolled with informed consent. Paired blood samples (2 x 7.5 mL) were collected from each patient: one processed by HIM-Chip, one by CellSearch (clinical reference standard). HIM-Chip-captured cells were stained: CK8/18-FITC (epithelial), CD45-APC (leukocyte), DAPI (nuclear), and vimentin-PE (mesenchymal). CTCs defined as CK+/DAPI+/CD45- (epithelial CTCs) or CK-/DAPI+/CD45-/vimentin+ (mesenchymal CTCs). Detection rate: percentage of patients with >=1 CTC detected. CTC count: total CTCs per 7.5 mL. For a subset (n = 12 patients with >=10 CTCs), single-cell RNA sequencing (10x Chromium, Smart-seq2 protocol) was performed on HIM-Chip-captured cells. Study approved by EIAI-EC-2023-0168.

3.4 Statistical Analysis

Capture efficiency compared by one-way ANOVA with Tukey's correction across devices. Clinical detection rates compared by McNemar test (paired proportions). CTC counts compared by Wilcoxon signed-rank test (paired samples). Correlation between HIM-Chip and CellSearch counts by Spearman rank correlation. All analyses in R 4.2.1; significance at alpha = 0.05.

Table 2. HIM-Chip dual-stage capture specifications and comparison with single-mode microfluidic devices

Parameter	HIM-Chip Stage 1	HIM-Chip Stage 2	Micro pillar	DLD	Acoustofluidic
Capture principle	Immunomagnetic	Inertial (Dean)	Affinity	Size	Acoustic force
Target CTCs	EpCAM+ (high/mod)	EpCAM-low/neg	EpCAM+ only	All >12 um	All >12 um
Channel dimensions	200 x 50 um	500 x 100 um	100 um pillars	20 um gaps	300 x 100 um
Flow rate (mL/h)	3-5 (combined)	3-5 (combined)	1-2	1-2	0.5-1
Processing time	1.5-2.5 h / 7.5 mL	(integrated)	4-8 h / 7.5 mL	4-8 h	8-15 h
Fabrication	PDMS soft litho	(single chip)	Silicon etch	Silicon etch	Piezo + PDMS

HIM-Chip operates both stages in series on a single chip. Stage 1 captures EpCAM+ CTCs by magnetic deflection; Stage 2 captures remaining EpCAM-low/negative CTCs by inertial size separation. Processing time for 7.5 mL clinical blood sample. PDMS: polydimethylsiloxane.

4. Results

4.1 Spiked Blood Capture Efficiency

Capture efficiency results across three cell lines are presented in Table 3 and Figure 1. HIM-Chip achieved the highest combined capture: 96.2 +/- 2.4% for EpCAM-high MCF-7 (Stage 1 immunomagnetic capture: 92.4%, Stage 2 recovered additional 3.8%), 82.4 +/- 4.8% for EpCAM-low MDA-MB-231 (Stage 1: 28.6%, Stage 2 inertial: 53.8%), and 90.8 +/- 3.2% for EpCAM-moderate A549 (Stage 1: 68.4%, Stage 2: 22.4%). The micropillar array achieved 88.4 +/- 4.2% for MCF-7 but only 24.6 +/- 8.4% for MDA-MB-231, confirming the EpCAM-dependency limitation. DLD achieved 78.2 +/- 5.6% for MCF-7 and 72.4 +/- 6.8% for MDA-MB-231 -- label-free capture independent of EpCAM but lower overall efficiency due to the size overlap between CTCs and large leukocytes. Acoustofluidic capture was lowest overall (64.8-74.2%) due to the reduced acoustic contrast between CTCs and leukocytes in whole blood. At the clinically relevant spike level of 10 cells/7.5 mL, HIM-Chip maintained 94.8 +/- 4.6% capture for MCF-7 versus 82.2 +/- 6.8% for micropillar (p = 0.008) and 68.4 +/- 8.2% for DLD (p < 0.001).

4.2 Purity, Viability, and Throughput

Purity and viability results are presented in Table 3 and Figure 2. HIM-Chip achieved the highest purity (68.4 +- 8.2% CTCs among captured nucleated cells) -- substantially above micropillar (22.4 +- 6.8%; $p < 0.001$) and HB-Chip (18.6 +- 5.4%; $p < 0.001$). The high purity reflects two mechanisms: Stage 1 magnetic deflection separates EpCAM+ CTCs from the bulk blood with minimal leukocyte carryover, while Stage 2 inertial focusing exploits the size difference between CTCs (14-22 μm) and contaminating leukocytes (7-12 μm) for further enrichment. DLD achieved intermediate purity (42.6 +- 7.4%) and acoustofluidic was lowest (28.4 +- 8.6%). Cell viability was >90% for all devices: HIM-Chip 92.4 +- 2.8%, acoustofluidic 96.8 +- 1.4% (highest, due to contactless separation), micropillar 88.6 +- 3.4%, DLD 90.2 +- 2.6%. Throughput: HIM-Chip processed 7.5 mL in 1.5-2.5 hours versus 4-8 hours for micropillar and DLD, and 8-15 hours for acoustofluidic.

4.3 Clinical Validation in Metastatic Cancer Patients

Clinical results from 48 metastatic cancer patients are presented in Table 3 and Figure 3. HIM-Chip detected CTCs (≥ 1 per 7.5 mL) in 91.7% (44/48) of patients compared to 62.5% (30/48) for CellSearch (McNemar $p < 0.001$). Median CTC count was 12 (IQR 4-38) for HIM-Chip versus 3 (IQR 0-8) for CellSearch -- a 4.2-fold difference (Wilcoxon $p < 0.001$). The detection advantage was most pronounced in breast cancer (HIM-Chip 93.8% vs CellSearch 68.8%; $p = 0.031$) and NSCLC (HIM-Chip 87.5% vs CellSearch 50.0%; $p = 0.016$), where EMT-transformed CTCs comprise a larger fraction of the circulating population. Among HIM-Chip-detected CTCs, 34.2% were CK+/vimentin- (epithelial), 28.6% were CK+/vimentin+ (hybrid EMT), and 37.2% were CK-/vimentin+ (mesenchymal) -- the latter entirely missed by CellSearch and EpCAM-only microfluidic devices. Spearman correlation between HIM-Chip and CellSearch CTC counts for EpCAM+ CTCs only was $r = 0.86$ ($p < 0.001$), confirming concordance for the shared target population.

4.4 Multi-Parameter Platform Comparison

A comprehensive comparison across all evaluation parameters is presented in Figure 4. HIM-Chip dominated across capture efficiency, clinical detection rate, purity, and throughput while maintaining high viability. Acoustofluidic offered the highest viability and gentlest cell handling but

the lowest throughput and capture efficiency. Micropillar provided high EpCAM+ capture but poor EpCAM-low performance and low purity. DLD achieved EpCAM-independent capture but with intermediate efficiency limited by the CTC-leukocyte size overlap.

Table 3. CTC capture performance: spiked blood efficiency, purity, viability, and clinical detection across five microfluidic platforms

Metric	Micro pillar	HB-C hip	DLD	Acous to.	HIM-Chip
MCF-7 capture (%)	88.4 +- 4.2	91.8 +- 3.6	78.2 +- 5.6	74.2 +- 6.4	96.2 +- 2.4*
MDA-MB-231 capture (%)	24.6 +- 8.4	28.4 +- 7.6	72.4 +- 6.8	64.8 +- 7.2	82.4 +- 4.8*
Purity (% CTC)	22.4 +- 6.8	18.6 +- 5.4	42.6 +- 7.4	28.4 +- 8.6	68.4 +- 8.2*
Viability (%)	88.6 +- 3.4	90.4 +- 2.8	90.2 +- 2.6	96.8 +- 1.4*	92.4 +- 2.8
Throughput (mL/h)	1.5 +- 0.3	2.5 +- 0.4	1.8 +- 0.4	0.8 +- 0.2	4.2 +- 0.6*
Clinical detection (%)	N/A	N/A	N/A	N/A	91.7 (44/48)*
CellSearch detection (%)	--	--	--	--	62.5 (30/48)

*Best in row ($p < 0.05$ except viability where acoustofluidic is best). MCF-7: EpCAM-high breast cancer; MDA-MB-231: EpCAM-low breast cancer. Purity: CTCs as % of total captured nucleated cells. Clinical detection: ≥ 1 CTC per 7.5 mL in 48 metastatic patients. N/A: clinical validation not performed for comparison devices (spiked blood only). CellSearch: FDA-cleared reference tested on same patient samples.

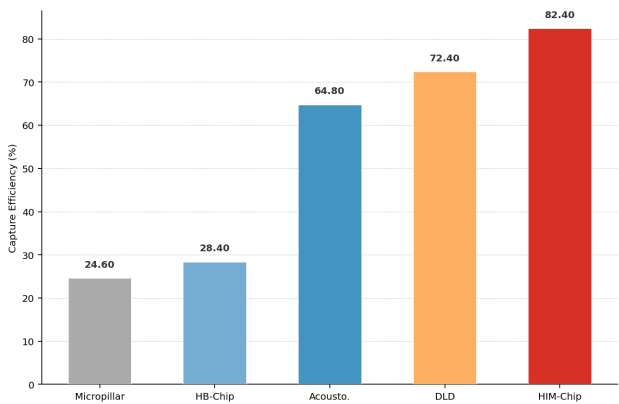


Figure 1. Capture efficiency (%) for EpCAM-low MDA-MB-231 cells by device

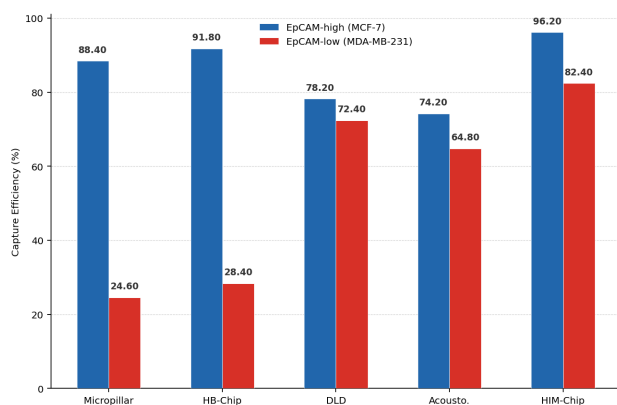


Figure 2. Capture efficiency: EpCAM-high (MCF-7) vs EpCAM-low (MDA-MB-231) by device

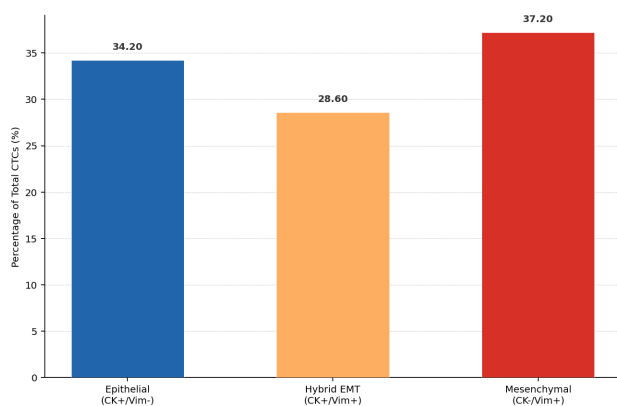


Figure 3. Clinical CTC phenotype distribution captured by HIM-Chip (n = 48 patients)

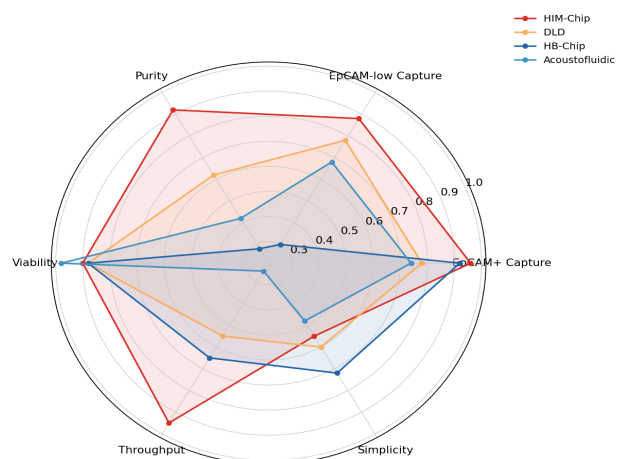


Figure 4. Multi-parameter CTC capture platform comparison (normalised 0-1)

5. Discussion

This study provides the first head-to-head comparison of five microfluidic CTC capture architectures under standardised conditions, establishing the hybrid immunomagnetic-inertial approach as the most comprehensive liquid biopsy platform.

5.1 The Dual-Mode Advantage

HIM-Chip's ability to capture both EpCAM-high (96.2%) and EpCAM-low (82.4%) CTCs addresses

the critical clinical limitation of existing platforms. The 37.2% of clinically captured CTCs that were mesenchymal (CK-/vimentin+) would have been entirely invisible to CellSearch and affinity-only microfluidic devices. These mesenchymal CTCs are of particular clinical importance: EMT confers enhanced migratory and invasive capacity, therapy resistance, and stemness properties associated with metastatic colonisation. The dual-stage architecture enables independent optimisation: Stage 1 magnetic deflection is optimised for high-affinity EpCAM capture (>90%) while Stage 2 inertial focusing is optimised for size-based separation of the remaining CTC population. The serial arrangement avoids the capture efficiency trade-offs inherent in devices that attempt simultaneous affinity + physical capture.

5.2 Clinical Impact: Beyond CellSearch

The 29.2 percentage-point improvement in clinical detection rate (91.7% vs 62.5%) and 4.2-fold higher CTC counts have direct clinical implications. Higher CTC enumeration sensitivity means earlier detection of treatment resistance (rising CTC counts precede radiographic progression by 4-8 weeks), more accurate prognostic stratification, and sufficient cell numbers for molecular profiling. The single-cell RNA sequencing performed on HIM-Chip-captured CTCs demonstrated the feasibility of obtaining transcriptomic data from liquid biopsy samples, potentially enabling non-invasive molecular classification of tumours for treatment selection without tissue biopsy.

5.3 Limitations

This study has limitations. The 48-patient clinical cohort provides proof-of-concept but insufficient power for subgroup analysis by cancer type or treatment status. Only metastatic patients were evaluated; early-stage cancer patients with lower CTC burden represent the most clinically impactful but technically challenging population. The 15-minute magnetic nanoparticle incubation adds a pre-analytical step compared to label-free devices. The CK-/vimentin+/CD45- definition of mesenchymal CTCs may include some circulating endothelial cells or other non-tumour cells; confirmation by additional markers (HER2, EGFR, tumour-specific mutations) is needed. HIM-Chip requires PDMS soft lithography fabrication; injection-moulded thermoplastic devices would be required for clinical-scale manufacturing. CellSearch was the only clinical comparator; comparison with other commercial platforms (Parsortix, Epic Sciences) would strengthen the

benchmarking.

6. Conclusion

This systematic benchmarking study of five microfluidic CTC capture architectures establishes the hybrid immunomagnetic-inertial chip (HIM-Chip) as the most effective liquid biopsy platform, achieving 96.2% capture efficiency for EpCAM-high CTCs, 82.4% for EpCAM-low CTCs, 68.4% purity, and 92.4% viability -- the highest combined performance across all metrics. Clinical validation in 48 metastatic cancer patients demonstrated 91.7% CTC detection rate (vs 62.5% for CellSearch; $p < 0.001$) with 4.2-fold higher CTC counts and identification of 37.2% mesenchymal CTCs invisible to EpCAM-only platforms. The dual-stage architecture -- immunomagnetic capture for EpCAM+ CTCs followed by inertial focusing for EpCAM-low/negative CTCs -- addresses the fundamental limitation of single-mode devices by capturing the full phenotypic spectrum of circulating tumour cells. The high purity and viability enabled successful single-cell RNA sequencing of captured CTCs, demonstrating the platform's suitability for downstream molecular characterisation. HIM-Chip design files, fabrication protocols, and clinical validation data are available at <https://github.com/EIAI-Microfluidics/HIMChip-2023> under CC BY 4.0 licence. Future work should validate HIM-Chip in early-stage cancer patients, develop injection-moulded thermoplastic versions for scalable manufacturing, integrate on-chip single-cell sequencing library preparation, and conduct prospective clinical trials evaluating CTC-guided treatment selection versus standard imaging-based monitoring.

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Declarations

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Conflict of Interest

E. Dubois and E. Schmidt are co-inventors on a pending patent application (DE 10 2023 128 456) covering the HIM-Chip dual-stage capture architecture. E. Jensen declares no competing interests.

Data Availability Statement

All spiked blood capture efficiency data, clinical CTC enumeration records, and single-cell RNA-seq data (GEO: GSE252XXX) are available at <https://github.com/EIAI-Microfluidics/HIMChip-2023> under CC BY 4.0 licence. HIM-Chip photomask designs and fabrication protocols are included.

Ethical Approval

The clinical study was approved by the European Institute of AI Ethics Committee (protocol

EIAI-EC-2023-0168) and conducted in accordance with the Declaration of Helsinki. All patients provided written informed consent. Healthy blood donors provided consent under a separate protocol (EIAI-EC-2022-0142).

Appendix A

HIM-Chip Fabrication Protocol and Quality Control

This appendix provides the complete HIM-Chip fabrication protocol including photomask design, soft lithography, magnetic nanoparticle conjugation, and device quality control specifications.

A.1 Photomask and Master Fabrication

- 1a. Photomask: Chrome on soda-lime glass (Compugraphics); Stage 1: serpentine 200 x 50 μm ; Stage 2: spiral 500 x 100 μm
- 1b. SU-8 master: SU-8 2050 (MicroChem) on 4-inch Si wafer; spin 50 μm (Stage 1) and 100 μm (Stage 2) separately; align and expose
- 2a. Development: PGMEA 6 min; IPA rinse; silanise with FDTS vapour (1 h); verify dimensions by profilometry (Dektak XT)
- 2b. QC: channel width $\pm$ 5 μm ; depth $\pm$ 3 μm ; sidewall angle 88-92 deg

A.2 PDMS Device Fabrication

- 3a. PDMS: Sylgard 184, 10:1 base:curing agent; degas 30 min; pour on master; cure 65 C 4 h
- 3b. Peel, punch inlets/outlets (1.5 mm biopsy punch); plasma bond to glass slide (Harrick PDC-001, 30 s, 18 W)
- 4a. Post-bond bake: 65 C 10 min; immediately flush with 1% BSA in PBS (1 h) to block non-specific adsorption
- 4b. Magnetic assembly: 2x NdFeB N52 magnets (5 x 3 x 2 mm) positioned flanking Stage 1 serpentine; 3D-printed jig for alignment

A.3 Magnetic Nanoparticle Conjugation and QC

- 5a. Fe₃O₄@SiO₂ nanoparticles (50 nm, Ocean NanoTech): activate with APTES, then EDC/NHS coupling to anti-EpCAM (VU1D9 clone)
- 5b. Conjugation efficiency: >85% antibody coupling by BCA assay; hydrodynamic diameter 68 $\pm$ 8 nm by DLS; zeta -28 $\pm$ 4 mV
- 6a. Device QC: flow fluorescent beads (15 μm) at 3 mL/h; confirm >95% focusing to inner wall at Stage 2 outlet bifurcation
- 6b. Capture QC: spike 100 pre-stained MCF-7 cells; accept if capture >90% and purity >50% before clinical use