Supplementary Information

Cascaded elasto-inertial separation of malignant tumor cells from untreated malignant pleural and peritoneal effusions

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Figure S1. CAD drawing and detailed dimensions of interfacial elasto-inertial separation channel and symmetrical CEA channel.



Figure S2. Bright and fluorescence images illustrating the cell distributions at the inlet (i), at the outlets of interfacial elasto-inertial separation channel (ii), and at the outlets of symmetrical CEA channel (iii). The tumor cells were stained before being spiked into the diluted blood samples. The fluorescent streams in the fluorescence images indicate the trajectories of the stained tumor cells. The concentration of tumor cells (MCF-7 cells) is $\sim 10^3$ cells/mL.



Figure S3. Microscopic images of the liquids collected from the three outlets of the CEICS device after separation using samples spiked with low-concertation tumor cells. To identify the tumor cells in the outlet 3, a fluorescent image of liquids collected from the outlet 3 is also provided. The concentration of tumor cells (MCF-7 cells) is $\sim 10^3$ cells/mL.



Figure S4. Purities of the collected tumor cells when separating tumor cells with concentrations of $\sim 10^3$ cells/mL from blood samples using our CEICS device.

	separation by our group						
Ref	Microchannel structure	Working principle	Lysis of RBCs (Yes/N o)	Cell concentration of the blood cells	Recovery ratio of tumor cells (10 ⁴ cells/mL)		
1	Spiral microchannel with two inlets (using sheath fluids)	Inertial separation	Yes	5×10^5 cells/mL	~ 83.9%		
2	Traditional spiral microchannel	Inertial separation	Yes	10 ⁵ cells/mL	~ 85.29%		
3	Symmetrical serpentine microchannel	Inertial separation	Yes	10 ⁵ cells/mL	~ 93.30%		
This paper	Integrated an interfacial elasto- inertial microchannel with a symmetric CEA channel	Elasto- inertial separation	No	$10^6 \sim 10^8$ cells/mL	~94.87%		

Table S1. Detailed comparison between this paper and previous works on MTC separation by our group

Authors Selection of		Selection of	Flow rate Confined		A	D	
(year)	sheath fluids	sample fluids	of samples	particle size	Application	reriormance	
Faridi et al.4	500 mm DEO	PBS	0.5 µL/min	2 µm, 5 µm	Separation of WBCs	Recovery ratio (WBCs)	
(2017)	500 ppm PEO				from bacteria	of 92%	
T:		PBS	5 μL/min	1 μm, 2 μm	Separation of platelets	C	
(2017)	100 ppm PEO				from <i>Staphylococcus</i> aureus	Separation efficiency $(1, 1, 1, 1)$	
(2017)						(platelets) of $>90\%$	
Yuan et al. ⁶	DDC	1000 ppm	$2 \ \mu L/min$	5 μm, 10 μm	Washing of Jurkat cells	Recovery ratio (Jurkat	
(2017)	PBS	PEO				cells) of 92.8%	
Tian et al. ⁷	500 mmm DEO	Whole blood	7.5 µL/min	10 µm, 15 µm	Separation of tumor	Recovery ratio (tumor	
(2018)	300 ppm PEO				cells from whole blood	cells) of >76.3%	
Shi et al. ⁸	150 mmm DEO	PBS	8.3 µL/min	10 μm, 15 μm	Separation of H1299	Separation efficiency	
(2019)	130 ppill PEO				cells from blood cells	(H1299) of ~100%	
Shi et al. ⁹	500 mmm DEO	100 ppm PEO	10 µL/min	10 μm, 15 μm	Separation of H1299	Separation efficiency	
(2020)	300 ppm PEO				cells from blood cells	(H1299) of ~100%	
Zhang et	1000 mmm DEO	PBS	1 µL/min	1.82 µm, 8.5	Separation of L.	Separation purity (L.	
al.10 (2023)	1000 ppm PEO			μm	rhamnosus from E. coli	rhamnosus) of 91%	
Zhang et	1000 mmm DEO	PBS	1 µL/min	1 μm, 4.8 μm	Separation of E. coli	Separation purity (E. coli	
al.11 (2023)	1000 ppm PEO				Clusters and Singlets.	Clusters) of 70.8%	
Cheng et	750 mmm DEO	250 ppm PEO	18.4 µL/min	5 μm, 15 μm	Separation of tumor	Recovery ratio (tumor	
al.12 (2023)	/30 ppin PEO				cells from whole blood	cells) of >77%	
This noner	50 ppm HA	PBS	120 µL/min	10 μm, 15 μm	Separation of tumor	Recovery ratio (tumor	
rins paper					cells from blood cells	cells) of >94.8%	

Table S2. The summary of the research on interfacial elasto-inertial microfluidics

No.	Age	Sex	Cancer type	Sample
1	81	Male	Hemangiosarcoma	Peritoneal effusion
2	61	Female	Lung adenocarcinoma	Pleural effusion
3	80	Female	Lymphoma	Pleural effusion
4	57	Male	Liver cancer	Peritoneal effusion
5	75	Female	Ovarian cancer	Peritoneal effusion

Table S3. Clinical information of patients.

Reference:

- Wang, C.; Chen, Y.; Gu, X.; Zhang, X.; Gao, C.; Dong, L.; Zheng, S.; Feng, S.; Xiang, N. Low-cost polymer-film spiral inertial microfluidic device for label-free separation of malignant tumor cells. *Electrophoresis* 2022, *43* (3), 464-471
- Zhu, Z.; Li, S.; Wu, D.; Ren, H.; Ni, C.; Wang, C.; Xiang, N.; Ni, Z. Highthroughput and label-free enrichment of malignant tumor cells and clusters from pleural and peritoneal effusions using inertial microfluidics. *Lab on a Chip* 2022, 22 (11), 2097-2106
- Ren, H.; Zhu, Z.; Xiang, N.; Wang, H.; Zheng, T.; An, H.; Nam-Trung, N.; Zhang, J. Multiplexed serpentine microchannels for high-throughput sorting of disseminated tumor cells from malignant pleural effusion. *Sensors and Actuators B-Chemical* 2021, 337, 129758
- Faridi, M. A.; Ramachandraiah, H.; Banerjee, I.; Ardabili, S.; Zelenin, S.; Russom, A. Elasto-inertial microfluidics for bacteria separation from whole blood for sepsis diagnostics. *Journal of Nanobiotechnology* 2017, 15, 1-9
- Tian, F.; Zhang, W.; Cai, L.; Li, S.; Hu, G.; Cong, Y.; Liu, C.; Li, T.; Sun, J. Microfluidic co-flow of Newtonian and viscoelastic fluids for high-resolution separation of microparticles. *Lab on a Chip* 2017, *17* (18), 3078-3085
- Yuan, D.; Tan, S. H.; Sluyter, R.; Zhao, Q.; Yan, S.; Nguyen, N. T.; Guo, J.; Zhang, J.; Li, W. On-Chip Microparticle and Cell Washing Using Coflow of Viscoelastic Fluid and Newtonian Fluid. *Analytical Chemistry* 2017, *89* (17), 9574-9582
- Tian, F.; Cai, L.; Chang, J.; Li, S.; Liu, C.; Li, T.; Sun, J. Label-free isolation of rare tumor cells from untreated whole blood by interfacial viscoelastic microfluidics. *Lab on a Chip* 2018, *18* (22), 3436-3445
- 8. Shi, X.; Liu, L.; Cao, W.; Zhu, G.; Tan, W. A Dean-flow-coupled interfacial viscoelastic fluid for microparticle separation applied in a cell smear method. *Analyst* **2019**, *144* (20), 5934-5946
- 9. Shi, X.; Tan, W.; Liu, L.; Cao, W.; Wang, Y.; Zhu, G. Separation of exfoliated tumor cells from viscoelastic pleural effusion using a microfluidic sandwich structure. *Analytical and Bioanalytical Chemistry* **2020**, *412* (22), 5513-5523
- Zhang, T.; Cain, A. K.; Semenec, L.; Pereira, J. V.; Hosokawa, Y.; Yalikun, Y.; Li, M. Bacteria separation and enrichment using viscoelastic flows in a straight microchannel. *Sensors and Actuators B: Chemical* 2023, *390*, 133918
- Zhang, T.; Cain, A. K.; Semenec, L.; Liu, L.; Hosokawa, Y.; Inglis, D. W.; Yalikun, Y.; Li, M. Microfluidic Separation and Enrichment of Escherichia coli by Size Using Viscoelastic Flows. *Analytical Chemistry* 2023, 95, 2561–2569
- Cheng, Y.; Zhang, S.; Qin, L.; Zhao, J.; Song, H.; Yuan, Y.; Sun, J.; Tian, F.; Liu, C. Poly(ethylene oxide) Concentration Gradient-Based Microfluidic Isolation of Circulating Tumor Cells. *Analytical Chemistry* 2023, 95, 3468-3475