Supplementary Information

Simultaneous biochemical and functional phenotyping of single circulating tumor cells using ultrahigh throughput and recovery microfluidic devices

Yang Liu,[a] Wujun Zhao,[b] Rui Cheng,[c] Jamie Hodgson,[d] Mary Egan,[d] Christen N. Cooper Pope,[d] Petros G. Nikolinakos,[d] and Leidong Mao*[c]

[a]Department of Chemistry, The University of Georgia, Athens, Georgia, USA

[b]FCS Technology, LLC, Athens, GA, 30606

[c]School of Electrical and Computer Engineering, College of Engineering, The University of Georgia, Athens, Georgia, USA.

[d]University Cancer & Blood Center, LLC, Athens, GA, 30607

*Email: Leidong Mao (mao@uga.edu)

†Electronic supplementary information (ESI) available.

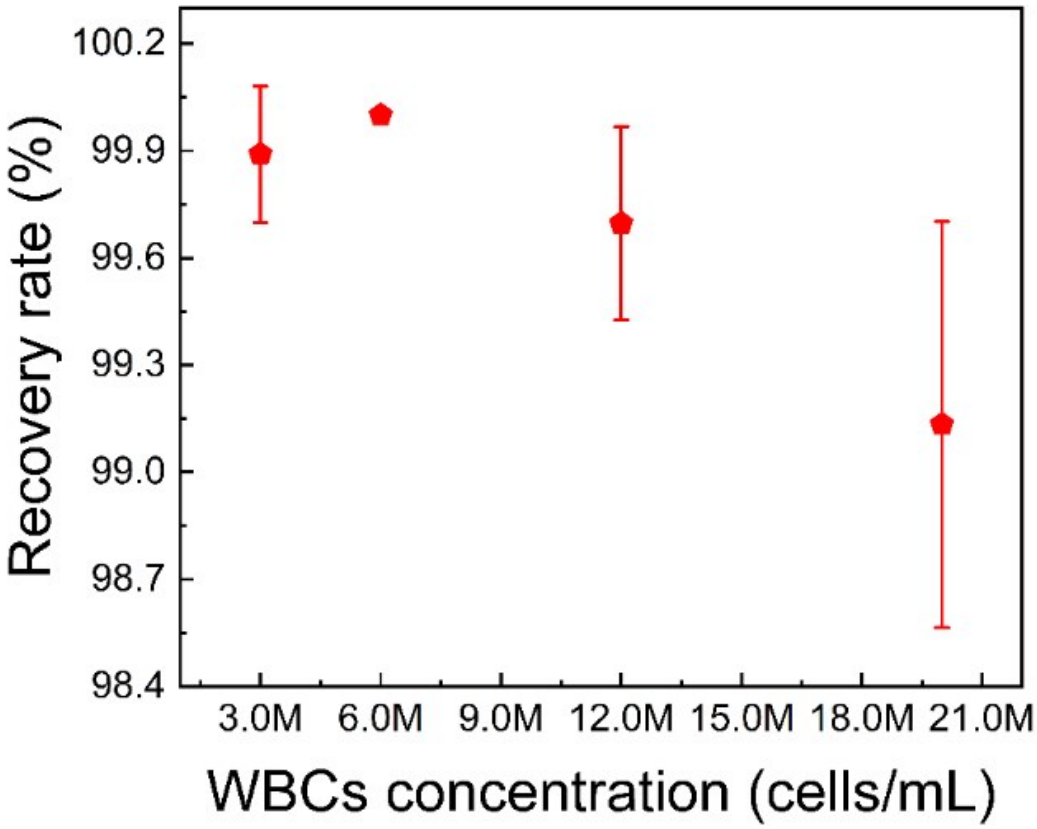

Figure S1. Recovery rate of MCF7 cells at different WBCs concentration. ~100 of MCFs were spiked into 1 mL 0.015% (v/v) ferrofluid containing 3 – 20 millions of WBCs. Total volume was 10 mL. Flow rate was 1 mL/min. Error bar n = 3.

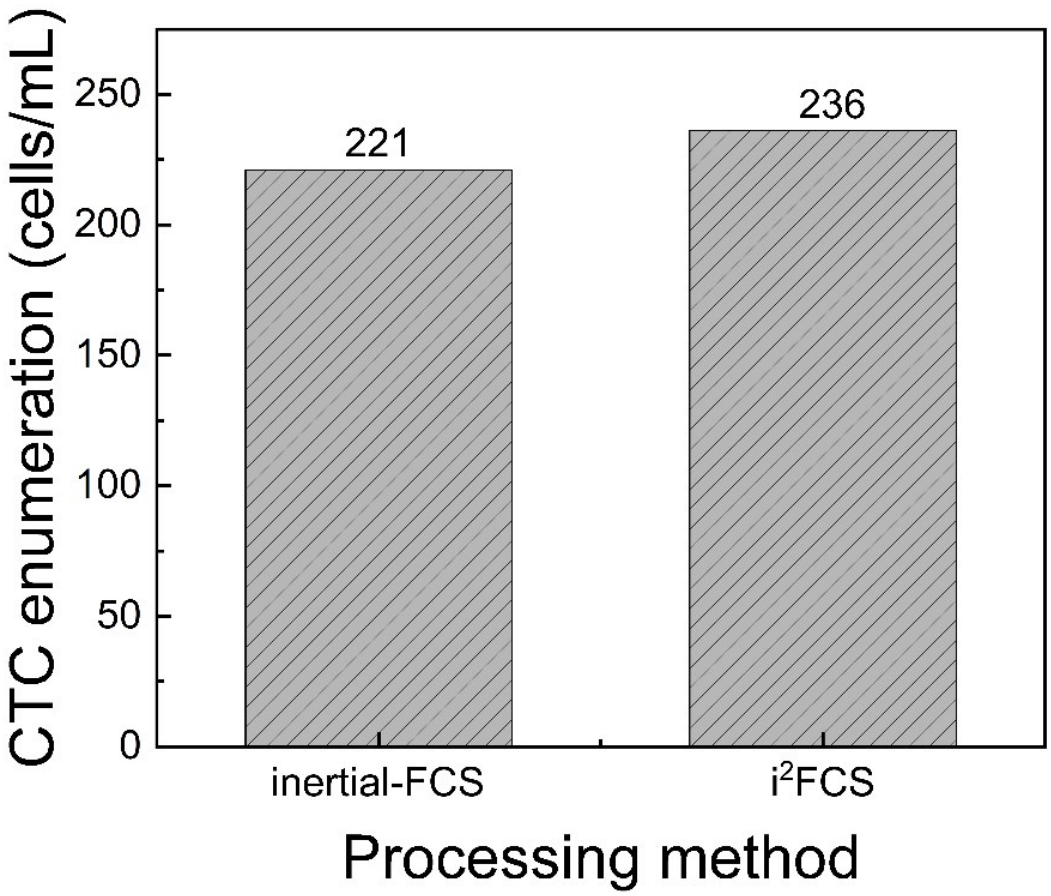

Figure S2. CTC enumeration of Patient C (stage IV lung cancer) using two different processing method. Inertial-FCS was a size-based separation method. CTCs was identified using immunofluorescent staining as described in the method.

Table S1. A performance comparison of existing CTC separation technologies including i²FCS.

Category	Type	Technique	Throughput (mL/h)	Recovery rate	Purity	Viability	Reference
Label-based	Immunoaffinity	CellSearch	N/A	~81%	N/A	N/A	[1]
		Magsweeper	9	59±27%	~100%	N/A	[2]
		Flash-Chip		84.3-91.3	Remove~ 99.97 Ramos cells	97.6%	[3]
	Microfluidic micropost	CTC-Chip	1	>60%	~ 56%	98.5%	[4]
		GEDI	1	~85%	~68%	N/A	[5]
		OncoCEE	1	~80-90%	N/A	N/A	[6]
		NanoVelcro-Chip	0.5	~90%	N/A	N/A	[7]
	Microfluidic surface capture	HB-Chip	1.2	~91.8%	14%	95%	[8]
		GEM-Chip	3.6	~90%	~84%	~85%	[9]
		Graphene Oxide Chip	~1-3	73±2.4%	N/A	N/A	[10]
	Immunomagnetic positive	Ephesia-Chip	3	~90%	<100 contaminating cells	N/A	[11]
		Magnetic Sifter	10	48% - 90%	17.7±9.3%	N/A	[12]
		Isoflux	1.2	74-85%	~1.4 %	N/A	[13]
		CTC-iChip	8	89.7-98.6%	> 0.1 %	N/A	[14]
		Micromagnetic Chip	1.2	~90 %	remove > 99.6% WBC	>90%	[15]
		IMN	1.2	~94%	N/A	93%	[16]
		MAP (magnetophoresis)	10	86-90%	N/A	N/A	[17]
		SIM-Chip	2	82.4%	92.45%	84.1%	[18]
		CTC-μChip	2	~90%	15.6-35.0	N/A	[19]
	Immunomagnetic negative	QMS	1E6 – 1E7 cells/ s	46%	~0.1 - 6.4%	~90%	[20]
		EasySep™ Human CD45 Depletion Kit	N/A	69%	1%	N/A	[21]
		CTC-iChip	8	~ 97%	~7.8%	N/A	[14]
		Monolithic CTC-iChip	1.5E7 – 2E7 cells / s	~99.5%	18.4% (445 WBCs per mL)	N/A	[22]
		^{LP}CTC-iChip	48	86%	Remove ~99.97% WBC	N/A	[23]
		iFCS	6	98.8%	~1620 WBCs per mL blood	97.25%	[24]
		i²FCS	**60**	**99.7%**	**507 WBCs per mL blood**	**98.1%**	**This work**
	Optofluidic	CC-RBC system	0.1	90%	92%	N/A	[25]
	Density gradient centrifugation	Ficoll-Paque	20	N/A	N/A	N/A	[26]
		OncoQuick	N/A	N/A	N/A	N/A	[27]

	Density-antibody	RosetteSep™ CTC Enrichment Cocktail	N/A	~62.5%	N/A	N/A	[28]
		Accucyte Enrichment and CyteSealer™	N/A	~90.5%	N/A	N/A	[29]
	Size-Deformability	Microfluidic Ratchet	1	~90%	Remove 99.99% WBCs	99.1%	[30]
		ISET®	N/A	N/A	N/A	N/A	[31]
Label-free		ScreenCell®	1 mL/min	74-91.2%	N/A	~85%	[32]
		CellSieve	2.5 mL/min	N/A	N/A	N/A	[33]
		FMSA	0.75 mL/min	90%	Remove 99.99% WBCs	80%	[34]
		SB microfilter	N/A	78-83%	Remove ~99.95% WBCs	71-74%	[35]
		RCT (Resettable Cell Trap)	0.6	>90%	Remove ~99.89% WBCs	N/A	[36]
		Cluster Chip	2.5	41-99%	~15%	~95%	[37]
	Inertial forces	Vortex	22.5	21%	57-94%	~86%	[38]
		Vortex	7.5	1.9-93.8%	1.7-37.6%	~91%	[39]
		DFF (Dean Flow Fractionation)	3	>85%	Remove ~ 99.95% WBCs	> 98%	[40]
		MFM (multi-flow microfluidic) system	6	93%	Remove ~ 95% WBCs	N/A	[41]
		Labyrinth	150	90%	~600 WBCs/mL	N/A	[42]
		Cascaded chip	120	13.8-80.8%	63.6-68.7%	95-98%	[43]
	Electrophoresis	ApoStream™	~1.3	~73%	Remove ~99.99% peripheral blood mononuclear cells (PBMCs)	97.1%	[44]
		MOFF-DEP	7.6	75.3%	Remove 94.23% of WBCs	N/A	[45]
		Dielectrophoresis Device	N/A	~100% (simulation)	83% (simulation)	N/A	[46]
		ODEP	N/A	41.5%	~100%	N/A	[47]
	Acoustophoresis	taSSAW-Chip	1.2	~83%	Remove >90% WBCs	~91%	[48]
		Acoustophoresis Chip	6	~86.5%	Remove ~ 98.9% WBCs	N/A	[49]
	Ferrohydrodynamics	FCS	6	~92.9%	11.7%	96.3%	[50]

References

1. S. Riethdorf, H. Fritsche, V. Muller, T. Rau, C. Schindibeck, B. Rack, W. Janni, C. Coith, K. Beck, F. Janicke, S. Jackson, T. Gornet, M. Cristofanilli and K. Pantel, *Clinical Cancer Research*, 2007, **13**, 920-928.

2. A. H. Talasaz, A. A. Powell, D. E. Huber, J. G. Berbee, K. H. Roh, W. Yu, W. Z. Xiao, M. M. Davis, R. F. Pease, M. N. Mindrinos, S. S. Jeffrey and R. W. Davis, *P Natl Acad Sci USA*, 2009, **106**, 3970-3975.

3. L. Wu, H. Ding, X. Qu, X. Shi, J. Yang, M. Huang, J. Zhang, H. Zhang, J. Song and L. Zhu, *Journal of the American Chemical Society*, 2020, **142**, 4800-4806.

4. S. Nagrath, L. V. Sequist, S. Maheswaran, D. W. Bell, D. Irimia, L. Ulkus, M. R. Smith, E. L. Kwak, S. Digumarthy, A. Muzikansky, P. Ryan, U. J. Balis, R. G. Tompkins, D. A. Haber and M. Toner, *Nature*, 2007, **450**, 1235-U1210.

5. J. P. Gleghorn, E. D. Pratt, D. Denning, H. Liu, N. H. Bander, S. T. Tagawa, D. M. Nanus, P. A. Giannakakou and B. J. Kirby, *Lab on a Chip*, 2010, **10**, 27-29.

6. S. D. Mikolajczyk, L. S. Millar, P. Tsinberg, S. M. Coutts, M. Zomorrodi, T. Pham, F. Z. Bischoff and T. J. Pircher, *Journal of Oncology*, 2011, DOI: Artn 252361

10.1155/2011/252361.

7. Y. T. Lu, L. B. Zhao, Q. L. Shen, M. A. Garcia, D. X. Wu, S. Hou, M. Song, X. C. Xu, W. H. OuYang, W. W. L. OuYang, J. Lichterman, Z. Luo, X. Xuan, J. T. Huang, L. W. K. Chung, M. Rettig, H. R. Tseng, C. Shao and E. M. Posadas, *Methods*, 2013, **64**, 144-152.

8. S. L. Stott, C. H. Hsu, D. I. Tsukrov, M. Yu, D. T. Miyamoto, B. A. Waltman, S. M. Rothenberg, A. M. Shah, M. E. Smas, G. K. Korir, F. P. Floyd, A. J. Gilman, J. B. Lord, D. Winokur, S. Springer, D. Irimia, S. Nagrath, L. V. Sequist, R. J. Lee, K. J. Isselbacher, S. Maheswaran, D. A. Haber and M. Toner, *Proceedings of the National Academy of Sciences of the United States of America*, 2010, **107**, 18392-18397.

9. W. A. Sheng, O. O. Ogunwobi, T. Chen, J. L. Zhang, T. J. George, C. Liu and Z. H. Fan, *Lab on a Chip*, 2014, **14**, 89-98.

10. H. J. Yoon, T. H. Kim, Z. Zhang, E. Azizi, T. M. Pham, C. Paoletti, J. Lin, N. Ramnath, M. S. Wicha, D. F. Hayes, D. M. Simeone and S. Nagrath, *Nat Nanotechnol*, 2013, **8**.

11. J. Autebert, B. Coudert, J. Champ, L. Saias, E. T. Guneri, R. Lebofsky, F. C. Bidard, J. Y. Pierga, F. Farace, S. Descroix, L. Malaquin and J. L. Viovy, *Lab on a Chip*, 2015, **15**, 2090-2101.

12. C. M. Earhart, C. E. Hughes, R. S. Gaster, C. C. Ooi, R. J. Wilson, L. Y. Zhou, E. W. Humke, L. Y. Xu, D. J. Wong, S. B. Willingham, E. J. Schwartz, I. L. Weissman, S. S. Jeffrey, J. W. Neal, R. Rohatgi, H. A. Wakeleebe and S. X. Wang, *Lab on a Chip*, 2014, **14**, 78-88.

13. W. Harb, A. Fan, T. Tran, D. C. Danila, D. Keys, M. Schwartz and C. Ionescu-Zanetti, *Transl Oncol*, 2013, **6**, 528-+.

14. E. Ozkumur, A. M. Shah, J. C. Ciciliano, B. L. Emmink, D. T. Miyamoto, E. Brachtel,

M. Yu, P. I. Chen, B. Morgan, J. Trautwein, A. Kimura, S. Sengupta, S. L. Stott, N. M. Karabacak, T. A. Barber, J. R. Walsh, K. Smith, P. S. Spuhler, J. P. Sullivan, R. J. Lee, D. T. Ting, X. Luo, A. T. Shaw, A. Bardia, L. V. Sequist, D. N. Louis, S. Maheswaran, R. Kapur, D. A. Haber and M. Toner, *Science translational medicine*, 2013, **5**.

15. J. H. Kang, S. Krause, H. Tobin, A. Mammoto, M. Kanapathipillai and D. E. Ingber, *Lab Chip*, 2012, **12**, 2175-2181.

16. M. Tang, C. Y. Wen, L. L. Wu, S. L. Hong, J. Hu, C. M. Xu, D. W. Pang and Z. L. Zhang, *Lab on a Chip*, 2016, **16**, 1214-1223.

17. K. Hoshino, Y. Y. Huang, N. Lane, M. Huebschman, J. W. Uhr, E. P. Frenkel and X. J. Zhang, *Lab on a Chip*, 2011, **11**, 3449-3457.

18. J. Kim, H. Cho, S.-I. Han and K.-H. Han, *Analytical chemistry*, 2016, **88**, 4857-4863.

19. H. Cho, J. Kim, C.-W. Jeon and K.-H. Han, *Lab on a Chip*, 2017, **17**, 4113-4123.

20. O. Lara, X. D. Tong, M. Zborowski and J. J. Chalmers, *Experimental Hematology*, 2004, **32**, 891-904.

21. Z. A. Liu, A. Fusi, E. Klopocki, A. Schmittel, I. Tinhofer, A. Nonnenmacher and U. Keilholz, *J Transl Med*, 2011, **9**.

22. F. Fachin, P. Spuhler, J. M. Martel-Foley, J. F. Edd, T. A. Barber, J. Walsh, M. Karabacak, V. Pai, M. Yu, K. Smith, H. Hwang, J. Yang, S. Shah, R. Yarmush, L. V. Sequist, S. L. Stott, S. Maheswaran, D. A. Haber, R. Kapur and M. Toner, *Sci Rep-Uk*, 2017, **7**.

23. A. Mishra, T. D. Dubash, J. F. Edd, M. K. Jewett, S. G. Garre, N. M. Karabacak, D. C. Rabe, B. R. Mutlu, J. R. Walsh and R. Kapur, *Proceedings of the National Academy of Sciences*, 2020, **117**, 16839-16847.

24. Y. Liu, W. Zhao, R. Cheng, B. N. Harris, J. R. Murrow, J. Hodgson, M. Egan, A. Bankey, P. G. Nikolinakos and T. Laver, *Lab on a Chip*, 2021.

25. X. Hu, D. Zhu, M. Chen, K. Chen, H. Liu, W. Liu and Y. Yang, *Lab on a Chip*, 2019, **19**, 2549-2556.

26. J. Weitz, P. Kienle, J. Lacroix, F. Willeke, A. Benner, T. Lehnert, C. Herfarth and M. von Knebel Doeberitz, *Clin Cancer Res*, 1998, **4**, 343-348.

27. G. A. Clawson, E. Kimchi, S. D. Patrick, P. Xin, R. Harouaka, S. Zheng, A. Berg, T. Schell, K. F. Staveley-O'Carroll, R. I. Neves, P. J. Mosca and D. Thiboutot, *PLoS One*, 2012, **7**, e41052.

28. W. He, S. A. Kularatne, K. R. Kalli, F. G. Prendergast, R. J. Amato, G. G. Klee, L. C. Hartmann and P. S. Low, *Int J Cancer*, 2008, **123**, 1968-1973.

29. D. E. Campton, A. B. Ramirez, J. J. Nordberg, N. Drovetto, A. C. Clein, P. Varshavskaya, B. H. Friemel, S. Quarre, A. Breman, M. Dorschner, S. Blau, C. A. Blau, D. E. Sabath, J. L. Stilwell and E. P. Kaldjian, *BMC Cancer*, 2015, **15**, 360.

30. E. S. Park, C. Jin, Q. Guo, R. R. Ang, S. P. Duffy, K. Matthews, A. Azad, H. Abdi, T. Todenhöfer and J. Bazov, *Small*, 2016, **12**, 1909-1919.

31. G. Vona, A. Sabile, M. Louha, V. Sitruk, S. Romana, K. Schutze, F. Capron, D. Franco, M. Pazzagli, M. Vekemans, B. Lacour, C. Brechot and P. Paterlini-Brechot, *Am J Pathol*, 2000, **156**, 57-63.

32. I. Desitter, B. S. Guerrouahen, N. Benali-Furet, J. Wechsler, P. A. Janne, Y. Kuang, M. Yanagita, L. Wang, J. A. Berkowitz, R. J. Distel and Y. E. Cayre, *Anticancer Res*, 2011, **31**, 427-441.

33. D. L. Adams, S. S. Martin, R. K. Alpaugh, M. Charpentier, S. Tsai, R. C. Bergan, I. M. Ogden, W. Catalona, S. Chumsri, C.-M. Tang and M. Cristofanilli, 2014, DOI: 10.1073/pnas.1320198111 %J Proceedings of the National Academy of Sciences, 201320198.

34. R. A. Harouaka, M. D. Zhou, Y. T. Yeh, W. J. Khan, A. Das, X. Liu, C. C. Christ, D. T. Dicker, T. S. Baney, J. T. Kaifi, C. P. Belani, C. I. Truica, W. S. El-Deiry, J. P. Allerton and S. Y. Zheng, *Clin Chem*, 2014, **60**, 323-333.

35. M. D. Zhou, S. Hao, A. J. Williams, R. A. Harouaka, B. Schrand, S. Rawal, Z. Ao, R. Brenneman, E. Gilboa, B. Lu, S. Wang, J. Zhu, R. Datar, R. Cote, Y. C. Tai and S. Y. Zheng, *Sci Rep*, 2014, **4**, 7392.

36. X. Qin, S. Park, S. P. Duffy, K. Matthews, R. R. Ang, T. Todenhofer, H. Abdi, A. Azad, J. Bazov, K. N. Chi, P. C. Black and H. Ma, *Lab Chip*, 2015, **15**, 2278-2286.

37. A. F. Sarioglu, N. Aceto, N. Kojic, M. C. Donaldson, M. Zeinali, B. Hamza, A. Engstrom, H. Zhu, T. K. Sundaresan, D. T. Miyamoto, X. Luo, A. Bardia, B. S. Wittner, S. Ramaswamy, T. Shioda, D. T. Ting, S. L. Stott, R. Kapur, S. Maheswaran, D. A. Haber and M. Toner, *Nat Methods*, 2015, **12**, 685-691.

38. E. Sollier, D. E. Go, J. Che, D. R. Gossett, S. O'Byrne, W. M. Weaver, N. Kummer, M. Rettig, J. Goldman, N. Nickols, S. McCloskey, R. P. Kulkarni and D. Di Carlo, *Lab Chip*, 2014, **14**, 63-77.

39. C. Renier, E. Pao, J. Che, H. E. Liu, C. A. Lemaire, M. Matsumoto, M. Triboulet, S. Srivinas, S. S. Jeffrey and M. Rettig, *NPJ precision oncology*, 2017, **1**, 1-11.

40. H. W. Hou, M. E. Warkiani, B. L. Khoo, Z. R. Li, R. A. Soo, D. S. Tan, W. T. Lim, J. Han, A. A. Bhagat and C. T. Lim, *Sci Rep*, 2013, **3**, 1259.

41. J. Zhou, A. Kulasinghe, A. Bogseth, K. O'Byrne, C. Punyadeera and I. Papautsky, *Microsystems & nanoengineering*, 2019, **5**, 1-12.

42. E. Lin, L. Rivera-Báez, S. Fouladdel, H. J. Yoon, S. Guthrie, J. Wieger, Y. Deol, E. Keller, V. Sahai and D. M. Simeone, *Cell systems*, 2017, **5**, 295-304. e294.

43. A. Abdulla, W. Liu, A. Gholamipour-Shirazi, J. Sun and X. Ding, *Analytical chemistry*, 2018, **90**, 4397-4405.

44. V. Gupta, I. Jafferji, M. Garza, V. O. Melnikova, D. K. Hasegawa, R. Pethig and D. W. Davis, *Biomicrofluidics*, 2012, **6**, 24133.

45. H.-S. Moon, K. Kwon, S.-I. Kim, H. Han, J. Sohn, S. Lee and H.-I. Jung, *Lab on a Chip*, 2011, **11**, 1118-1125.

46. V. Shirmohammadli and N. Manavizadeh, *IEEE Transactions on Electron Devices*,

2019, **66**, 4075-4080.

47. T.-K. Chiu, W.-P. Chou, S.-B. Huang, H.-M. Wang, Y.-C. Lin, C.-H. Hsieh and M.-H. Wu, *Sci Rep-Uk*, 2016, **6**, 1-14.

48. P. Li, Z. Mao, Z. Peng, L. Zhou, Y. Chen, P. H. Huang, C. I. Truica, J. J. Drabick, W. S. El-Deiry, M. Dao, S. Suresh and T. J. Huang, *Proc Natl Acad Sci U S A*, 2015, **112**, 4970-4975.

49. M. Antfolk, C. Antfolk, H. Lilja, T. Laurell and P. Augustsson, *Lab Chip*, 2015, **15**, 2102-2109.

50. W. Zhao, R. Cheng, B. D. Jenkins, T. Zhu, N. E. Okonkwo, C. E. Jones, M. B. Davis, S. K. Kavuri, Z. Hao, C. Schroeder and L. Mao, *Lab Chip*, 2017, **17**, 3097-3111.