OptiPrep™ Application Sheet C38

(I) Maintenance of cells in suspension for chemical/physical measurements
(II) Microfluidic cell encapsulation/cell sorting

- **OptiPrep™ is a 60% (w/v) solution of iodixanol in water, density = 1.32 g/ml**

PART I: Maintenance of cells in suspension for chemical/physical measurements

1. Background

This is a non-centrifugal application, in which OptiPrep™ is mixed with a suspension of cells in order to prevent their sedimentation during an extended physical measurement or separation. The first published paper to report this novel application was in 2001, when Farinas, Chow and Wada [1] commented in the abstract to their paper: "*Recent developments in microfluidics have enabled the design of a lab-on-a-chip system capable of measuring cellular membrane potential. The chip accesses liquid samples sequentially by sipping from a micro-plate through a capillary, mixes the samples with cells flowing through a microchannel, contacts the cells with potential-sensitive dyes, and reads out cellular responses using fluorescence detection"*. Later Culbertson [2] noted that the use of OptiPrep™ enabled the rate of cell entry into the main chip channel of a microfluidic device to remain constant over the course of an entire experiment. The technique permits the identification of single cells with unique compositional characteristics that may presage the later development of a disease state. In its simplest form, a medium with the same density as the cell, which in no way interacts with the cell, maintains the cell in a state of continuous suspension.

Section 2 briefly describes solution preparation and the physical parameters of some the solutions that have been used in these studies. **Section 3** summarizes the cell types that have been studied and types of physico-chemical measurements that were carried out. **Section 4** lists some more recent publications. **Section 6** is the reference list.

2. Solution preparation

Mix OptiPrep™ (shake the bottle gently before use) with saline or a balanced salt solution to give a solution of suitable density, according to the figures in Table 1. Table 2 summarizes a few of the conditions reported in the literature. For more information on making up solutions **see Application Sheet C01**.

- The actual density required will depend on the cell type; it can be chosen by suspending the cells in a series of density solutions and observing the disposition of the cells after centrifugation. Because of the heterogeneity of any cell population however it is impossible to select a density that will allow all the cells to remain in suspension, some will float and some will sediment. If the cell suspension contains a variety of cell types the problem will be exaggerated. For example human peripheral blood mononuclear cells have a density range of 1.058-1.078 g/ml.

Table 1: Density of solutions produced by dilution of OptiPrep™ with an isotonic buffered saline solution

% (w/v) iodixanol	Refractive index	Density (g/ml)	% (w/v) iodixanol	Refractive index	Density (g/ml)
5.00	1.3429	1.032	11.00	1.3523	1.063
6.00	1.3444	1.037	12.00	1.3538	1.068
7.00	1.3459	1.043	13.00	1.3554	1.074
8.00	1.3475	1.048	14.00	1.3570	1.079
9.00	1.3491	1.053	15.00	1.3585	1.085
10.00	1.3507	1.058	16.00	1.3601	1.090

Table 2: Selected solution properties reported in the literature

Cell Type	Solution composition	Iodixanol (% w/v)	Density (g/ml)	Ref #
HeLa	PBS + 1% albumin	11.4	1.065	3
HeLa	Hank's Balanced Salt Solution	9.6	1.054	4
Human blood lymphocytes and THP-1[1] cells	Hank's Balanced Salt Solution	10.8	1.062	1
Human blood leukocytes	PBS	3-9	1.02-1.05[2]	5
Human erythrocytes	PBS	15-18	1.084-1.100	6
Human erythrocytes	PBS	17	1.095	7
Human erythrocytes	PBS/dextran[3]	2.6		8
Human erythrocytes	PBS/5.5mM glucose/4% BSA	19	1.105	9
Jurkat/lymphocytes	PBS	10.8	1.062	2
Macrophages	PBS	8.4	1.052	10
Yeast	Water	19	1.105	11,12

[1] THP-1 cells are a human acute leukaemic monocytic line
[2] The densities were chosen to allow the cells to sediment slowly to the bottom of the chamber
[3] The density is difficult to estimate because of the dextran content; the latter was added to raise the viscosity

3. Cell types and study topic

Breast carcinoma cells
Iso-acoustic focusing for phenotyping [29]
Radiometric assays [50]
Viability of cells in alginate particles [13]

Bronchial epithelial cells
Confocal light absorption and scattering spectroscopic (CLASS) microscopy [14]

Cancer cell lines, distinction of
SERS-microfluidic droplet platform [58]

CHO cells
Microfluidic cytometry [15, 48]

Embryonic spinal cord cells
Ribosome reduction [57]

Embryonic stem cells
Gene expression in single cells [16]

Erythrocytes
Acoustic cell manipulation [38]
Blood viscosity changes in response to exercise [17]
Channel flow profiling [9]
Cholesterol [53]
Density matching [39]
Highly mono-dispersed microdroplet production [35]
Lysis kinetics [7]
Nitric oxide scavenging by haemoglobin [6, 8]
Phase separation in micro-channel networks [31]
Plasma membrane cholesterol [52]
Shape and mechanical properties of erythrocytes [18]

Escherishia coli
Genetically encoded synthesis of nanomaterials [32]

Fibroblasts
Hydrogel encapsulation [12]

Neural cells
Density matching [40]
Single cell barcoding – droplet microfluidics [33]

Prostate cancer cells
Printed droplet microfluidics: dispensing of picoliter droplets and cells [36]

Rat liver lysosomes (stained with red tracker dye)
Confocal light absorption and scattering spectroscopic (CLASS) microscopy [14]

Review articles
Extracellular matrix [56]
Recent advances in research involving chemical and biological reactions in microdroplets [27]

Staphylococcus aureus
Microchannels [54]

Tumor-infiltrating myeloid cells
Tumour growth regulation [55]

Yeast cells
Acoustic cell manipulation [38]
Biogenic magnetization [28]
Hydrogel encapsulation [12]
Polyglycerol microgel particles for the micro-encapsulation of yeast cells [11]

4. Physical measurements (recent publications)

Density matching: see refs 59 and 60
Droplet sorting/merging: see ref 61
Microfluidic cytometry: see refs 62-66
Single cell analysis: see ref 67

5. References

1. **Farinas, J.**, Chow, A.W. and Wada, H.G. (2001) *A microfluidic device for measuring cellular membrane potential Anal. Biochem.*, **295**, 138-142
2. **Culbertson, C.** (2006) *Single cell analysis on microfluidic devices* Methods Mol. Biol., **339**, 203-216
3. **Wang, M.M.**, Tu, E., Raymond, D.E., Yang, J.M., Zhang, H., Hagen, N., Dees, B., Mercer, E.M. et al (2005) *Microfluid sorting of mammalian cells by optical force switching* Nature Biotech., **23**, 83-87
4. **Ma, H.**, Gibson, E.A., Dittmer, P.J., Jimenez, R. and Palmer, A.E. (2012) *High-throughput examination of fluorescence resonance energy transfer-detected metal-ion response in mammalian cells* J. Am. Chem.Soc., **134**, 2488−2491
5. **Watson, D.**, Hagen, N., Diver, J., Marchand, P. and Chachisvilis, M. (2004) *Elastic light scattering from single cells: orientational dynamics in optical trap* Biophys. J., **87**, 1298-1306
6. **Azarov, I.**, Huang, K.T., Basu, S., Gladwin, M.T., Hogg, N. and Kim-Shapiro, D.B. (2005) *Nitric oxide scavenging by red blood cells as a function of hematocrit and oxygenation* J. Biol. Chem., **280**, 39024-39032
7. **SooHoo, J.R.**, Herr, J.K., Ramsey, J.M. and Walker, G.M. (2012) *Microfluidic cytometer for the characterization of cell lysis* Anal. Chem., **84**, 2195−2201
8. **Azarov, I.**, Liu, C., Reynolds, H., Tsekouras, Z., Lee, J.S., Gladwin, M.T. and Kim-Shapiro, D.B. (2011) *Mechanisms of slower nitric oxide uptake by red blood cells and other hemoglobin-containing vsicles* J. Biol. Chem., **286**, 33567–33579
9. **Roman, S.**, Lorthois, S., Duru, P. and Risso, F. (2012) *Velocimetry of red blood cells in microvessels by the dual-slit method: Effect of velocity gradients* Microvasc. Res., **84**, 249–261
10. **Perroud, T.D.**, Kaiser, J.N., Sy, J.C., Lane, T.W., Branda, C.S. Singh, A.K. and Patel, K.D. (2008) *Microfluidic-based cell sorting of Francisella tularensis infected macrophages using optical forces* Anal. Chem., **80**, 6385-6372
11. **Steinhilber, D.**, Seiffert, S., Heyman, J.A., Paulus, F., Weitz, D.A. and Haag, R. (2011) *Hyperbranched polyglycerols on the nanometer and micrometer scale* Biomaterials, **32**, 1311-1316

12. **Rossow, T.**, Heyman, J.A., Ehrlicher, A.J., Langhoff, A., Weitz, D.A., Haag, R. and Seiffert, S. (2012) *Controlled synthesis of cell-laden microgels by radical-free gelation in droplet microfluidics* J. Am. Chem. Soc., **134**, 4983−4989
13. **Akbar, S.** and Pirbodaghi, T. (2014) *Microfluidic encapsulation of cells in alginate particles via an improved internal gelation approach* Microfluid Nanofluidics, **16**, 773–777
14. **Itzkan, I.**, Qiu, L., Fang, H., Zaman, M.M., Vitkin, E., Ghiran, I.C., Salahuddin, S., Modell, M. et al (2007) *Confocal light absorption and scattering spectroscopic microscopy monitors organelles in live cells with no exogenous labels* Proc. Natl. Acad. Sci. USA, **104**, 17255-17260
15. **Cheung, M.C.**, McKenna, B., Wang, S.S., Wolf, D. and Ehrlich, D.J. (2015) *Image-based Ccll-resolved screening assays in flow* Cytometry Part A, **87A**, 541-548
16. **Klein, A.M.**, Mazutis, L., Akartuna, I., Tallapragada, N., Veres, A., Li, V., Peshkin, L., Weitz, D.A. and Kirschner, M.W. (2015) *Droplet barcoding for single-cell transcriptomics applied to embryonic stem cells* Cell, **161**, 1187–1201
17. **Buono, M.J.**, Krippes, T., Kolkhorst, F.W., Williams, A.T. and Cabrales, P. (2016) *Increases in core temperature counterbalance effects of haemoconcentration on blood viscosity during prolonged exercise in the heat* Exp. Physiol., **101.2,** 332–342
18. **Shen, Z.**, Coupier, G., Kaoui, B., Polack, B., Harting, J., Misbah, C. and Podgorski, T. (2016) *Inversion of hematocrit partition at microfluidic bifurcations* Microvasc. Res., **105**, 40–46
19. **Eastburn, D.J.**, Sciambi, A. and Abate, A.R. (2014) *Identification and genetic analysis of cancer cells with PCR-activated cell sorting* Nucleic Acids Res., **42**: e128
20. **Rajauria, S.**, Axline, C., Gottstein, C. and Cleland, A.N. (2015) *High-speed discrimination and sorting of submicron particles using a microfluidic device* Nano Lett.,**15**, 469− 475
21. **Pallaoro, A.**, Hoonejani, M.R., Braun, G.B., Meinhart, C.D. and Moskovits, M. (2015) *Rapid identification by surface-enhanced Raman spectroscopy of cancer cells at low concentrations flowing in a microfluidic channel* ASC Nano, **9**, 4328–4336
22. **Blanco, L.**, Hermosilla, D., Blanco, A., Swinnen, N., Prieto, D. and Negro, C. (2015) *Assessment of the performance of membrane bioreactors applied to the treatment of industrial effluents containing poly(vinyl alcohol)* Ind. Eng. Chem. Res., **54**, 5442−5449
23. **Martel, J.M.**, Smith, K.C., Dlamini, M., Pletcher, K., Yang, J., Karabacak, M., Haber, D.A., Kapur, R. and Toner, M. (2015) *Continuous flow microfluidic bioparticle concentrator* Sci. Rep., **5**: 11300
24. **Hecht, V.C.**, Sullivan, L.B., Kimmerling, R.J., Kim, D-H., Hosios, A.M., Stockslager, M.A., Stevens, M.M., Kang, J.H. et al (2016) *Biophysical changes reduce energetic demand in growth factor-deprived lymphocytes* J. Cell Biol., **212**, 439-447
25. **Mazutis, L.**, Gilbert, J., Ung, W.L., Weitz, D.A., Griffiths, A.D. and Heyman, J.A. (2013) *Single-cell analysis and sorting using droplet-based microfluidics* Nat. Protoc., **8**, 870-891
26. **Byun, S.**, Hecht, V.C. and Manalis, S.R. (2015) *Characterizing cellular biophysical responses to stress by relating density, deformability, and size* Biophys. J., **109,** 1565–1573
27. **Devenish, S.R.A.**, Kaltenbach, M., Fischlechner, M. and Hollfelder, F. (2013) *Droplets as reaction compartments for protein nanotechnology* In Methods Mol. Biol., **996**, Protein Nanotechnology: Protocols, Instrumentation, and Applications (ed. Gerrard, J.A.) Springer Science+Business Media, LLC pp 269-286
28. **Nishida, K.** and Silver, P.A. (2012) *Induction of biogenic magnetization and redox control by a component of the target of rapamycin complex 1 signaling pathway* PLoS Biol., **10**: e1001269
29. **Augustsson, P.**, Karlsen, J.T., Su, H-W., Bruus, H. and Voldman, J. (2016) *Iso-acoustic focusing of cells for size-insensitive acousto-mechanical phenotyping* Nat. Comm., **7**: 11556
30. **Pellegrino, M.**, Sciambi, A., Yates, J.L., Mast, J.D., Silver, C. and Eastburn, D.J. (2016) *RNA-Seq following PCR-based sorting reveals rare cell transcriptional signatures* BMC Genom., **17**: 361
31. **Clavica, F.**, Homsy, A., Jeandupeux, L. and Obrist, D. (2016) *Red blood cell phase separation in symmetric and asymmetric microchannel networks: effect of capillary dilation and inflow velocity* Sci. Rep., **6**: 36763
32. **Liu, X.**, Lopez, P.A., Giessen, T.W., Giles, M., Way, J.C. and Silver, P.A. (2016) *Engineering genetically-encoded mineralization and magnetism via directed evolution* Sci. Rep., **6**: 38019
33. **Zilionis, R.**, Nainys, J., Veres, A., Savova, V., Zemmour, D, Klein, A.M. and Mazutis, L. (2017) *Single-cell barcoding and sequencing using droplet microfluidics* Nat. Protoc., **12**, 44-73
34. **Kamperman, T.**, Henke, S., Zoetebier, B., Ruiterkamp, N., Wang, R., Pouran, B., Weinans, H., Karperien, M. and Leijten, J. (2017) *Nanoemulsion-induced enzymatic crosslinking of tyramine-functionalized polymer droplets* J. Mater. Chem. B, 2017, 5, 4835-4844
35. **Crawford, D.F.**, Smith, C.A. and Whyte, G. (2017) *Image-based closed-loop feedback for highly mono-dispersed microdroplet production* Sci. Rep., **7**: 10545

36. **Cole, R.H.**, Tang, S-Y., Siltanen, C.A., Shahi, P., Zhang, J.Q., Poust, S., Gartner, Z.J. and Abate, A.R. (2017) *Printed droplet microfluidics for on demand dispensing of picoliter droplets and cells* Proc. Natl. Acad. Sci. USA, **114**, 8728–8733
37. **Maceiczyk, R.M.**, Hess, D., Chiu, F.W.Y., Stavrakis, S. and deMello, A.J. (2017) *Differential detection photothermal spectroscopy: towards ultra-fast and sensitive label-free detection in picoliter & femtoliter droplets* Lab Chip, **17**, 3654–3663
38. **Lenshof, A.**, Johannesson, C., Evander, M., Nilsson, J. and Laurel, T. (2016) *Acoustic cell manipulation* In Microtechnology for Cell Manipulation and Sorting; Microsystems and Nanosystems (eds Lee, W. et al.), Springer International Publishing Switzerland, pp 129-173
39. **Mutlu, B.R.**, Smith, K.C., Edd, J.F., Nadar, P., Dlamini, M., Kapur, R. and Toner, M. (2017) *Non-equilibrium inertial separation array for high-throughput, large volume blood fractionation* Sci. Rep., 7: 9915
40. **Allen, W.E.**, DeNardo, L.A., Chen, M.Z., Liu, C.D., Loh, K.M., Fenno, L.E., Ramakrishnan, C., Deisseroth, K. and Luo, L. (2017) *Thirst-associated preoptic neurons encode an aversive motivational drive* Science **357**, 1149–1155
41. **Xia, B.**, Krutkramelis, K. and Oakey, J. (2106) *Oxygen-purged microfluidic device to enhance cell viability in photopolymerized PEG hydrogel microparticles* Biomacromolecules **17**, 2459−2465
42. **Siltanen, C.**, Diakataou, M., Lowen, J., Haque, A., Rahimian, A., Stybayeva, G. and Revzin, A. (2017) *One step fabrication of hydrogel microcapsules with hollow core for assembly and cultivation of hepatocyte spheroids* Acta Biomaterialia, **50,** 428–436
43. **Shahi, P.**, Kim, S.C., Haliburton, J.R., Gartner, Z.J. Abate, A. R. (2017) *Abseq: Ultrahigh-throughput single cell protein profiling with droplet microfluidic barcoding* Sci. Rep., **7**: 44447
44. **Mao, A.S.**, Shin, J-W., Utech, S., Wang, H., Uzun, O., Li, W., Cooper, M., Hu, Y. Zhang, L., Weitz, D.A. and Mooney, D.J. (2017) *Deterministic encapsulation of single cells in thin, tunable microgels for niche modelling and therapeutic delivery* Nat Mater., **16**, 236-243
45. **Cheng, Z.**, Wu, X.,, Cheng, J. and Liu, P. (2017) *Microfluidic fluorescence-activated cell sorting (μFACS) chip with integrated piezoelectric actuators for low-cost mammalian cell enrichment* Microfl. Nanofl., **21**: 9
46. **Yan, Z.**, Clark, I.C. and Abate, A.R. (2017) *Rapid encapsulation of cell and polymer solutions with bubble-triggered droplet generation* Macromol. Chem. Phys., **218**: 1600297
47. **Lienemann, P.S.**, Rossow, T., Mao, A.S., Vallmajo-Martin, Q., Ehrbar, M. and Mooney, D.J. (2017) *Single cell-laden protease-sensitive microniches for long-term culture in 3D* Lab Chip, **17**, 727-437
48. **Rajeswari, P.K.P.**, Joensson, H.N., Andersson-Svahn, H. (2017) *Droplet size influences division of mammalian cell factories in droplet microfluidic cultivation* Electrophoresis, **38**, 305–310
49. **Fiedler, B.L.**, Van Buskirk, S., Carter, K.P., Qin, Y., Carpenter, M.C., Palmer, A.E. and Jimenez, R. (2017) *Droplet microfluidic flow cytometer for sorting on transient cellular responses of genetically-encoded sensors* Anal. Chem., 89, 711−719
50. **Gallina, M.E.**, Kim, T.J., Shelor, M., Vasquez, J., Mongersun, A., Kim, M., Tang, S.K.Y., Abbyad, P. and Pratx, G. (2017) *Toward a droplet-based single-cell radiometric assay* Anal. Chem., **89**, 6472−6481
51. **Holzner, G.**, Stavrakis, S. and deMello, A. (2017) *Elasto-inertial focusing of mammalian cells and bacteria using low molecular, low viscosity PEO solutions* Anal. Chem., **89**, 11653−11663
52. **Veres, A.**, Faust, A.L., Bushnell, H.L., Engquist, E.N., Kenty, J.H-R., Harb, G., Poh, Y-C., Sintov, E., Gürtler, M. et al (2019) *Charting cellular identity during human in vitro β-cell differentiation* Nature 569, 368-373
53. **Ayuyan, A.G.** and Cohen, F.S. (2018) *The chemical potential of plasma membrane cholesterol: implications for cell biology* Biophy. J., **114**, 904–918
54. **Mutlu, B.R.**, Edd, J.F. and Toner, M. (2018) *Oscillatory inertial focusing in infinite microchannels* Proc. Natl. Acad. Sci. USA. **115**, 7682-7687
55. **Zilionis, R.**, Engblom, C., Pfirschke, C., Savova, V., Zemmour, D., Saatcioglu, H.D., Krishnan, I., Maroni, G., Meyerovitz, C.V. et al (2019) *Single-cell transcriptomics of human and mouse lung cancers reveals conserved myeloid populations across individuals and species* Immunity **50**, 1317–1334
56. **Matellan, C**. and del Río Hernández, A.E. (2019) *Engineering the cellular mechanical microenvironment – from bulk mechanics to the nanoscale* J. Cell Sci., **132**, jcs229013
57. **Niepel, M.**, Hafner, M., Mills, C.E., Subramanian, K., Williams, E.H., Chung, M., Gaudio, B., Barrette, A.M. et al (2019) *A multi-center study on the reproducibility of drug-response assays in mammalian cell lines* Cell Sys., **9**, 35–48
58. **Cong, L.**, Liang, L., Cao, F., Sun, D., Yue, J., Xu, W., Liang, C. and Xu, S. (2019) *Distinguishing cancer cell lines at a single living cell level via detection of sialic acid by dual-channel plasmonic imaging and by using a SERS-microfluidic droplet platform* Microchim. Acta **186**: 367

59. **Yang, X.**, Zhou, T., Zwang, T.J., Hong, G., Zhao, Y., Viveros, R.D., Fu, T-M., Gao, T. and Lieber, C.M. (2019) *Bioinspired neuron-like electronics* Nat. Mater., **510**, 510–517
60. **Li, Q.**, Tang, F., Huo, X., Huang, X., Zhang, Y., Wang, X. and Zhang, X. (2019) *Native state single-cell printing system and analysis for matrix effects* Anal. Chem. 2019, **91**, 8115−8122
61. **Chung, M.T.**, Kurabayashi, K. and Cai, D. (2019) *Single-cell RT-LAMP mRNA detection by integrated droplet sorting and merging* Lab Chip, **19**, 2425-2434
62. **Kim, S.C.**, Clark, I.C., Shahi, P. and Abate, A.R. (2018) *Single-cell RT-PCR in microfluidic droplets with integrated chemical lysis* Anal. Chem., **90**, 1273−1279
63. **Losserand, S.**, Coupier, G. and Podgorski, T. (2019) *Migration velocity of red blood cells in microchannels* Microvasc. Res., **124,** 30–36
64. **Lee, J.**, Mena, S.E. and Burns, M.A. (2019) *Micro-particle operations using asymmetric traps* Sci. Rep. **9**: 1278
65. **Rahimian, A.**, Siltanen, C., Feyzizarnagh, H., Escalante, P. and Revzin, A. (2019) *Microencapsulated immunoassays for detection of cytokines in human blood* ACS Sens., **4**, 578−585
66. **Li, L.**, Wu, P., Luo, Z., Wang, L., Ding, W., Wu, T., Chen, J., He, J., He, Y. et al (2019) *Dean flow assisted single cell and bead encapsulation for high performance single cell expression profiling* ACS Sens. **4**, 1299−1305
67. **Matuła, K.**, Rivello, F. and Huck, W.T.S. (2020) *Single-cell analysis using droplet microfluidics* Adv. Biosys., 4: 1900188

(II) Microfluidic cell encapsulation/cell sorting

This new application of OptiPrep™ has only very recently featured regularly in the literature; publications are listed alphabetically according to **cell type**, or "**methodology"** or "**reviews".**

Adipose tissue stem cells
Morandi, E.M., Verstappen, R., Zwierzina, M.E., Geley, S., Pierer, G. and Ploner, C. (2016) *ITGAV and ITGA5 diversely regulate proliferation and adipogenic differentiation of human adipose derived stem cells* Sci. Rep., **6**: 28889

Bacteria
Jusková, P., Schmid, Y.R.F., Stucki, A., Schmitt, S., Held, M. and Dittrich, P.S. (2019) *"Basicles": microbial growth and production monitoring in giant lipid vesicles* ACS Appl. Mater. Interfaces, **11**, 34698−34706

Carcinoma cells
Eastburn, D.J., Sciambi, A. and Abate, A.R. (2013) *Ultrahigh-throughput mammalian single-cell reverse-transcriptase polymerase chain reaction in microfluidic drops* Anal. Chem., **85**, 8016-8021
Jiang, Z., Xia, B., McBride, R. and Oakey, J. (2017) *A microfluidic-based cell encapsulation platform to achieve high long-term cell viability in photopolymerized PEGNB hydrogel microspheres* J. Mater. Chem. B, **5**, 173-180
Pei, H., Li, L., Wang, Y., Sheng, R., Wang, Y., Xie, S., Shui, L., Si, H. and Tang, B. (2019) *Single-cell phenotypic profiling of CTCs in whole blood using an integrated microfluidic device* Anal. Chem., **91**, 11078−11084
Sun, D., Cao, F., Cong, L., Xu, W., Chen, Q., Shic, W. and Xu, S. (2019) *Cellular heterogeneity identified by single-cell alkaline phosphatase (ALP) via a SERRSmicrofluidic droplet platform* Lab Chip, **19**, 335-342

B-lymphocytes
Eastburn, D.J., Sciambi, A. and Abate, A.R. (2013) *Ultrahigh-throughput mammalian single-cell reverse-transcriptase polymerase chain reaction in microfluidic drops* Anal. Chem., **85**, 8016-8021
Holzner, G., Du, Y., Cao, X., Choo, J., deMello, A.J. and Stavrakis, S. (2018) *An optofluidic system with integrated microlens arrays for parallel imaging flow cytometry* Lab Chip, **18**, 3631–3637

Bone marrow cells
Asensio, M.A., Lim, Y.W., Wayham, N., Stadtmiller, K., Edgar, R.C., Leong, J., Leong, R., Mizrahi, R.A. Adams, M.S. et al (2019) *Antibody repertoire analysis of mouse immunization protocols using microfluidics and molecular genomics* mAbs **11**, 870–883
Petukhov, V., Guo, J., Baryawno, N., Severe, N., Scadden, D.T., Samsonova, M.G. and Kharchenko, P.V. (2018) *dropEst: pipeline for accurate estimation of molecular counts in droplet-based single-cell RNA-seq experiments* Genome Biol. **19**: 78

Breast cancer cells
Hinohara, K., Wu, H-J., Vigneau, S., McDonald, T.O., Igarashi, K.J., Yamamoto, K.N., Madsen, T., Fassl, A and Egri, S.B. et al (2018) *KDM5 histone demethylase activity links cellular transcriptomic heterogeneity to therapeutic resistance* Cancer Cell **34**, 939–953
Hsu, M.N., Wei, S-C., Guo, S., Phan, D-T., Zhang, Y. and Chen, C-H. (2018) *Smart hydrogel microfluidics for single-cell multiplexed secretomic analysis with high sensitivity* Small, **14**: 1802918

Erythrocytes
Crawford, D.F., Smith, C.A. and Whyte, G. (2017) *Image-based closed-loop feedback for highly mono-dispersed microdroplet production* Sci. Rep., **7**: 10545
Fenech, M., Girod, V., Claveria, V., Meance, S., Abkarian, M. and Charlot, B. (2019) *Microfluidic blood vasculature replicas using backside lithography* Lab Chip, **19**, 2096-2106

Heart cells/tissue
Guerzoni, L.P.B., Tsukamoto, Y., Gehlen, D.B., Rommel, D., Haraszti, T., Akashi, M. and De Laporte, L. (2019) *A layer-by-layer single-cell coating technique to produce injectable beating mini heart tissues via microfluidics* Biomacromolecules, **20**, 3746−3754

HEK cells
Kolb, L., Allazetta, S., Karlsson, M., Girgin, M., Weber, W. and Lutolf, M.P. (2019) *High-throughput stem cell-based phenotypic screening through microniches* Biomater. Sci., **7**, 3471-3479

Jurkat cells
Clark, I.C. and Abate, A.R. (2018) *Microfluidic bead encapsulation above 20 kHz with triggered drop formation* Lab Chip, **18**, 3598–3605
Xu, Y., Lee, J-H., Li, Z., Wang, L., Ordog, T. and Bailey, R.C. (2018) *A droplet microfluidic platform for efficient enzymatic chromatin digestion enables robust determination of nucleosome positioning* Lab Chip, **18**, 2583-2592

Lymph node cells
Asensio, M.A., Lim, Y.W., Wayham, N., Stadtmiller, K., Edgar, R.C., Leong, J., Leong, R., Mizrahi, R.A. Adams, M.S. et al (2019) *Antibody repertoire analysis of mouse immunization protocols using microfluidics and molecular genomics* mAbs **11**, 870–883

Lymphoblasts
Mutafopulos, K., Spink, P., Lofstrom, C.D., Lu, P.J., Lu, H., Sharpe, J.C., Franke, T. and Weitz. D.A. (2019) *Traveling surface acoustic wave (TSAW) microfluidic fluorescence activated cell sorter (μFACS) Lab Chip, **19**, 2435-2443*

Methodology
Doonan, S.R., Lin, M. and Bailey, R.C. (2019) *Droplet CAR-Wash: continuous picoliter-scale immunocapture and washing* Lab Chip, **19**, 1589-1598
Park, J., Destgeer, G., Kim, H., Cho, Y. and Sung, H.J. (2018) *In-droplet microparticle washing and enrichment using surface acoustic wave-driven acoustic radiation force* Lab Chip, **18**, 2936–2945
Sahore, V., Doonan, S.R. and Bailey, R.C. (2018) *Droplet microfluidics in thermoplastics: device fabrication, droplet generation, and content manipulation using integrated electric and magnetic fields* Anal. Methods, **10**, 4264–4274

Microglia
Gunner, G., Cheadle, L., Johnson, K.M., Ayata, P., Badimon, A., Mondo, E., Nagy, M.A., Liu, L., Bemiller, S.M. et al (2019) *Sensory lesioning induces microglial synapse elimination via ADAM10 and fractalkine signalling* Nat. Neurosc., 22, 1075–1088

Mononuclear cells
Karthick, S., Pradeep, P.N., Kanchana, P. and Sen, A.K. (2018) *Acoustic impedance-based size-independent isolation of circulating tumour cells from blood using acoustophoresis* Lab Chip, **18**, 3802–3813

Mouse lung cancer cells
Petukhov, V., Guo, J., Baryawno, N., Severe, N., Scadden, D.T., Samsonova, M.G. and Kharchenko, P.V. (2018) *dropEst: pipeline for accurate estimation of molecular counts in droplet-based single-cell RNA-seq experiments* Genome Biol. **19**: 78

Myeloid leukaemia tumour
Pellegrino, M., Sciambi, A., Treusch, S., Durruthy-Durruthy, R., Gokhale, K., Jacob, J., Chen, T.X., Geis, J.A. et al (2018) *High-throughput single-cell DNA sequencing of acute myeloid leukemia tumors with droplet microfluidics* Genome Res.,28, 1345–1352

Neurons
Luo, B., Tian, L., Chen, N., Ramakrishna, S., Thakor, N. and Yang, I.H. (2018) *Electrospun nanofibers facilitate better alignment, differentiation, and long-term culture in an in vitro model of the neuromuscular junction (NMJ)* Biomater. Sci., **6**, 3262–3272

Pancreatic islets
Weaver, J.D., Headen, D.M., Coronel, M.M., Hunckler, M.D., Shirwan, H. and García, A.J. (2019) *Synthetic poly(ethylene glycol)-based microfluidic islet encapsulation reduces graft volume for delivery to highly vascularized and retrievable transplant site* Am J Transplant. 2019, **19**, 1315–1327

Promyelocytic leukaemia cells
Hsu, M.N., Wei, S-C., Guo, S., Phan, D-T., Zhang, Y. and Chen, C-H. (2018) *Smart hydrogel microfluidics for single-cell multiplexed secretomic analysis with high sensitivity* Small, **14**: 1802918

Reviews
Ding, Y., Choo, J. and deMello, A.J. (2017) *From single-molecule detection to next-generation sequencing: microfluidic droplets for high-throughput nucleic acid analysis* Microfluid Nanofluid, **21**: 58
Matellan, C. and del Río Hernández, A.E. (2019) *Engineering the cellular mechanical microenvironment – from bulk mechanics to the nanoscale* J. Cell Sci., **132**, jcs229013

Spleen cells
Asensio, M.A., Lim, Y.W., Wayham, N., Stadtmiller, K., Edgar, R.C., Leong, J., Leong, R., Mizrahi, R.A. Adams, M.S. et al (2019) *Antibody repertoire analysis of mouse immunization protocols using microfluidics and molecular genomics* mAbs **11**, 870–883
Mutafopulos, K., Spink, P., Lofstrom, C.D., Lu, P.J., Lu, H., Sharpe, J.C., Franke, T. and Weitz. D.A. (2019) *Traveling surface acoustic wave (TSAW) microfluidic fluorescence activated cell sorter (μFACS) Lab Chip, **19**, 2435-2443*

Stem cells
Siltanen, C., Yaghoobi, M., Haque, A., You, J., Lowen, J., Soleimani, M. and Revzin, A. (2016) *Microfluidic fabrication of bioactive microgels for rapid formation and enhanced differentiation of stem cell spheroids* Acta Biomater., **34**, 125–132

T-cells
Segaliny, A.I., Li, G., Kong, L., Ren, C., Chen, X., Wang, J.K., Baltimore, D., Wu, G. and Zhao, W. (2018) *Functional TCR T cell screening using single-cell droplet microfluidics* Lab Chip, **18**, 3733–3749

Yeast cells
Manna, P., Hung, S-T., Mukherjee, S., Friis, P., Simpson, D.M., Lo, M.N., Palmer, A.E. and Jimenez, R. (2018) *Directed evolution of excited state lifetime and brightness in FusionRed using a microfluidic sorter* Integr. Biol., **10**, 516-526

Zebrafish cells
Tang, Q., Iyer, S., Lobbardi, R., Moore, J.C., Chen, H., Lareau, C., Hebert, C., Shaw, M.L. et al (2017) *Dissecting hematopoietic and renal cell heterogeneity in adult zebrafish at single-cell resolution using RNA sequencing* J. Exp. Med., **214**, 2875–2887