### Supplementary Information

**Deformability-induced lift force in spiral microchannels for cell separation**

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### Supplementary Figures

| Cell type/process | Channel specifications | Process specifications | Ref. |
|-------------------|------------------------|------------------------|------|
| Cell cycle synchronisation of several cell lines (HeLa, KKU-100, CHO-CD36 and hMSCs) | Spiral channel (9 loops, 40cm long, single inlet, 8 outlets) 500µm wide Height was fine tuned for each cell type to satisfy a/Dh>0.07 Made of PDMS | Throughput: 15×10⁶ cells/h Flow rate: 2.5ml/min Enrichments of cells at G0/G1: >85% Viability: 95% | ¹ |
| Focusing and ordering of HL-60 and K562 cells to facilitate deterministic single cell encapsulation in droplets | Spiral channel (5 loops, 7.2cm long), 50µm wide, 29µm high, initial radius of 1500µm Made of PDMS | Flow rate: 15µl/min | ² |
| Separation of tumour cells (MCF-7 and HeLa) from spiked blood sample | Double spiral channel (12 loops: 6 loops counter-clockwise & 6 loops clockwise, one inlet, three outlets), 300µm wide, 50µm deep Made of PDMS | Throughput: 3.33×10⁷ cells/min Flow rate: 350µl/min Recovery: 88.5% | ³ |
| Isolation of CTC from whole diluted blood (20-25% hematocrit) from | Spiral channel (2 loops, 10cm long, 2 inlets, 2 outlets), 500µm wide, 160µm deep Made of PDMS | Flow rate: 3ml/hr Efficiency: 88% Detection rate: 100% (cells | ⁴ |
| Step | Description | Channel Details | Material | Efficiency | Flow Rate | Viability | Purity |
|------|-------------|----------------|----------|------------|-----------|-----------|--------|
| 1    | Cancer detection in all cancer patients’ blood samples, n=20 | Spiral channel (5 loops, one inlet, 8 outlets), 500µm wide, 150µm deep, initial radius of curvature 1cm. | Made of PDMS | Efficiency: 75% | Flow rate: 3ml/min | Viability: >97% |
| 2    | Separation of single cells from cell clumps for murine neurosphere assay | Spiral channel (5 loops, one inlet, 8 outlets), 500µm wide, 150µm deep, initial radius of curvature 1cm. | Made of PDMS | Efficiency: 75% | Flow rate: 3ml/min | Viability: >97% |
| 3    | Separation of plasma from whole blood sample ×20 diluted | Spiral channel (5 loops, 16cm long, one inlet, two outlets), 150µm wide, 50µm deep, the initial radius was 3500µm. | Made of PDMS | Throughput: 700µl/min | Efficiency: 38.5% | Plasma purity: 99.9% |
| 4    | Separation of non-motile sperm cells from RBC in TESE/mTESE samples | Spiral channel (4 loops, 1 inlet, 4 outlets), 150µm wide, 50µm deep, the initial radius was 700µm | Made of PDMS | Throughput: 520µl/min | Efficiency: 81% for sperm cells, 99% for RBC |
| 5    | Separation of higher quality sperm from lower quality sperm without using sperm motility | Spiral channel (4 loops, 1 inlet, 4 outlets), 150µm wide, 50µm deep, initial radius 853µm. | Made of PDMS | Throughput: 550µl/min | Cell concentration:2×10^7 cells/ml | Higher quality sperm was 4 times enriched in comparison to the input |
| 6    | Separation and concentration of Phytophthora ramorum sporangia (fungal plant pathogen, Ø30µm) | Spiral channel (3 loops, 1 inlet, 2 outlets), 600µm wide, 200µm deep, the radius of curvature was 2cm on average | Made of thermoplastic polymer | Throughput: 2ml/min | 5.3-fold increase in pathogen content with 95% recovery |
| 7    | Separation of algae (sub-millimetre phytoplankton) of two specimens: Monoraphium griffithii from Cyanothece aeruginosa. | Spiral channel (3 loops, 1 inlet, 2 outlets), 350µm wide and 100µm deep, initial radius 5mm, total length ~14cm | Made of PDMS | Flow rate: 3.2ml/min | Efficiency: 77% |
| 8    | Isolation of blood plasma, blood sample diluted 1:20 | A cascade of two spiral channels, each with 1 inlet, 3 outlets and 5 loops: 1st: 500µm wide, 60µm deep 2nd: 250µm wide, 60µm deep | Made of PDMS | Flow rate: f 1.25ml/min | Efficiency: 1st: 55% of blood cells removed 2nd: 99% of blood cells removed |
| 9    | Concentration of E.coli and 1µm beads | Spiral channels (3 loops, 1 inlet and 2 outlets): 1st part: 10 × 24µm² cross-section | Made of PDMS | Flow rate: 50µl/min (generated 70 bars) 100µl/min (generated 150 bars) |
Separation of neural stem cells derived from induced pluripotent stem cells from spontaneously differentiated non-neural cells

Spiral channel (1 inlet, 8 outlets and 10 loops), 500µm wide and 160µm deep, total length ~ 50cm
Made of PDMS

Flow rate: 3ml/min
Efficiency: 2.5× enrichment of neural stem cells with 38% recovery

Enrichment of mesenchymal stem cells from bone marrow

Spiral channel (1 inlet, 8 outlets, 10 loops), 500µm wide and 160µm deep, total length ~50cm
Made of PDMS

Flow rate: up to 3 ml/min
Efficiency: the best performance at 1.6ml/min, 6× enriched, 73% recovery rate

**Table. 1**
Examples of published data using IF in spiral channels with a symmetrical cross-section for size-based separating a wide range of biological samples.
SFig. 1

Hydrodynamic behaviour of polystyrene beads with (a) 20, (b) 15 and (c) 10, µm diameter in design I spiral microchannel with $360 \times 60$ µm$^2$ cross-section at five different flow rates corresponding to Re=79, 119, 158, 198 and 237 and (d) 10 µm diameter in design II spiral microchannel with $170 \times 30$ µm$^2$ cross-section, at five different flow rates corresponding to Re=33, 66, 97, 132 and 168. The lateral equilibrium positions were measured as a distance from the outer wall (µm) at the end of the spiral channel for at least 10000 events and there were generated by image analysis. Here, it is reported as mean (represented as the symbols) and standard deviation (indicated by the short vertical lines). Vertical dotted lines indicate four sections of the channel corresponding to four outlets of the channel (0-90 µm- outlet A, 90-180 µm- outlet B, etc.). Events belonging to a given section have the highest probability of being captured within the corresponding outlet. (e) Size measurement report generated for Jurkat cells using MoxiZ automated cell counter. The report is a histogram, where blue vertical lines
indicate number of cells measured within a given size range (bins), the red curve is a fit into the data generated automatically by the MoxiZ software and green color indicated area under the curve. (f) A histogram showing percentage of size ranges found within Jurkat cells population (red) in comparison to 10 (yellow) and 15 (green) µm beads. Please note that there are discrepancies in sizes measured with the MoxiZ and by the image analysis. MoxiZ measures light scatter around the measured particles which is further converted by an algorithm into a numerical value, while size measured by image analysis is reported as a projected particle’s area.

SFig. 3
Summary of operating conditions of spiral channels reported in the literature, up to end of 2018, in comparison to design I and II (pink). The scatter plot represents applied Re numbers versus hydraulic diameter ($D_h$). The grey dotted lines represent median Re number value (excluding design I and II) and lower and upper quartile as labelled on the graph. The pink dotted line represents the Re number applied in design II at which the effect of $F_D$ was significant.
**SFig. 4**

(A) Schematic of the spiral channel with six loops, one inlet and four outlets for size and deformability-based separation. Scale bar corresponds to 1 cm.

|        | Flow rate [ml/min] | Velocity [m/s] | Re [-] | De  |
|--------|--------------------|----------------|--------|-----|
| Design I |                    |                |        |     |
| 1      | 0.8                | 79             | 18     |     |
| 1.5    | 1.2                | 119            | 27     |     |
| 2      | 1.5                | 158            | 35     |     |
| 2.5    | 1.9                | 198            | 44     |     |
| 3      | 2.3                | 237            | 53     |     |
| Design II |                  |                |        |     |
| 0.2    | 0.65               | 33             | 5      |     |
| 0.4    | 1.3                | 66             | 10     |     |
| 0.6    | 1.9                | 97             | 15     |     |
| 0.8    | 2.6                | 132            | 21     |     |
| 1      | 3.3                | 168            | 26     |     |

**STable 2**

Table summarising experimental conditions (applied flow rates and corresponding velocities, Reynolds numbers (Re) and Dean numbers (De)) in design I (with 360 × 60 µm² cross section) and design II (with 170 × 30 µm² cross-section).

De is used to quantify the secondary flow within spiral microchannel, and it is defined as

\[
De = Re \sqrt{\frac{D_h}{R}},
\]

where \(D_h\) is hydraulic diameter, for channels with rectangular cross section

\[
D_h = \frac{2 \times H \times W}{H + W},
\]

defined as \(H\)-channel height and \(W\)-channel width.
Supplementary materials and methods

Real-time fluorescence and deformability cytometry

While there are many available well-established technologies for assessing cell mechanotype such as Atomic Force Microscopy (AFM)\textsuperscript{15}, micropipette aspiration\textsuperscript{16}, magnetic tweezers and optical stretchers\textsuperscript{17}, these methods suffer from low-throughput\textsuperscript{18}. To assess a high number of cells (thousands of events per minute), we used a microfluidic-based Real-Time Deformability Cytometer (RT-DC)\textsuperscript{19}. RT-DC is a contactless technique, allowing gain of thousands of events per minute, which is convenient for the global characterisation of complex samples\textsuperscript{20}. In the RT-DC set-up, a PDMS (Polydimethylsiloxane) channel consisting of three sections, two reservoir sections and one constriction channel (20 \(\mu\text{m} \times 20 \mu\text{m}\) or 30 \(\mu\text{m} \times 30 \mu\text{m}\) cross section), where cells undergo deformation and measurements are undertaken. The microfluidic chip is mounted on a microscope. A syringe pump is used to pump cells suspension in the chip, pulsing LED light enables high-speed image acquisition (4000 fps), for a standard measurements, the images are acquired at 40x magnification. Cells are introduced in the chip through central reservoir channel and they are directed into the measurement channel by sheath flow (both flow liquid and cell carrier are viscous solution of methylcellulose). Measurement channel has a cross-section slightly bigger than the cell diameter, thus cells entering the channel experiences shear stress that causes cell deformation. The images are captured in the Region of Interest (ROI) at the end of the measurement channel and processed in real time.

The RT-DC system employs image processing algorithms which enable the measurement of cell area and deformation. Deformation (\(D\)) is expressed as a deviation from a perfect circle

\[
D = 1 - c
\]

(1)

where \(c\) is the circularity defined as

\[
c = 2\sqrt{\pi A}/l
\]

(2)

\(A\) being the projected cell area and \(l\) the cell perimeter
Deformation (D) in the channel is independently measured from the initial cell shape and therefore any treatment-induced morphological changes to shape. Consequently, when possible, a differential deformation DD parameter has been introduced 21.

DD includes morphological information acquired in the reservoir (D_{Res}) section of the RT-DC chip (where applied shear is negligible) by subtracting this value from the deformation measured in the channel (D_{Ch}). From each vector of deformations values with length n, sampling is done with replacement n-times and the resulting distribution is used to calculate a statistic like the median (M). A single DD value is computed using

\[ DD_{j,CH} = D_{j,Ch} - D_{j,Res} \]

Subtraction is done by statistical representations of channel and reservoir measurements and using a bootstrapping approach. The process of sampling, calculation of M and DDj has to be repeated for a sufficient number of iterations (>1000) to obtain a bootstrap distribution follows a Gaussian distribution 21,22.

RT-FDC is an enhanced high-throughput (thousands of events per minute) microfluidic platform that enables mechanotype analysis of cells within a heterogeneous sample with no necessity of pre-sorting into pure populations, due to the integration of fluorescent signal for confirmation of cell identity 23. As in the conventional real-time deformability cytometry (RT-DC) 19, cells are deformed in a contactless manner by experiencing shear stress generated by flowing in a viscous buffer through the measurement channel which is only slightly larger than the actual cell dimensions. In RT-FDC (1) Immuno-labelled cells are introduced into the microfluidic chip mounted on a microscope and while passing through the measurement channel (2) in the ROI they are imaged by bright-field microscopy (3). Information about cells size (expressed as projected cell area [µm^2]) and induced by applied shear stress deformability (understood as 1- circularity) is generated by image processing in real time for each captured event and reported as a scatter plot. Additionally, cells passing through the ROI are illuminated by focused lasers (4) which excite signal detected and measured in the detector array. (5) The fluorescent signal is correlated with the acquired image, which allows cell identity confirmation.
Summary of Triplicate results

The hydrodynamic behaviour of cells was assessed in terms of lateral equilibrium position (measured as a distance from the particle centre to the outer wall [µm]) obtained at the end of the spiral channel by monitoring the ROI, by high-speed microscopic imaging. For one replica of one condition at one flow rate we obtained at least 10000 events. As an example, we provide SFig. 5 showing a single image extracted from a video recorded for soft cells at flow rate corresponding to Re=119 in the spiral channel with 360 × 60 µm² cross-section. All of the raw files can be accessed upon a request.

![Soft cells at Re=119](image)

**Distance from the outer wall [um]**

**SFig. 5**
An exemplary image extracted from a video recorded for soft cells at flow rate corresponding to Re=119 in the spiral channel with 360 × 60 µm² cross-section. In comparison to the statistical summary of the lateral equilibrium position (expressed as distance from the outer wall [µm]) reported as median (represented as the symbol) and the interquartile range (indicated by the short vertical lines). Vertical dotted lines indicate four sections of the channel corresponding to four outlets of the channel (0-90 µm- outlet A, etc.).

**Design I: Hydrodynamic behaviour of cells of cellular deformability model**

Hydrodynamic behaviour of cells (10000 per condition) of five different deformabilities (soft max, soft half-max, soft, stiff half-max and stiff) (A) in comparison to reference 15 µm beads in design I spiral microchannel with 360 × 60 µm² cross-section at five different flow rates corresponding to Re=79, 119, 158, 198 and 237. The lateral equilibrium positions were measured as a distance from the outer wall (µm) at the end of the spiral channel and there were generated by image analysis. Here, it is reported as median (represented as the symbols) and the interquartile range (indicated by the short vertical lines). Vertical dotted lines indicate four sections of the channel corresponding to four outlets of the channel (0-90. µm- outlet A, 90-180 µm- outlet B, etc.). Events belonging to a given section have the highest probability of being captured within the corresponding outlet and tables showing statistical summary (mean and standard deviation from the mean (SD), median, 25th (Q1) and 75th (Q3) percentile as well as minimal (min) and maximal (max) measured value) of latera equilibrium positions obtained for at least 10000 events.
**Design I: Hydrodynamic behaviour of cells of cellular deformability model**

**Replica I**

| Re=237 | Mean | SD | Min | Q1 | Median | Q3 | Max |
|--------|------|----|-----|----|--------|----|-----|
| Soft max | 122  | 49 | 37  | 86 | 112    | 144| 328 |
| Soft half-max | 110 | 44 | 33  | 79 | 101    | 131| 334 |
| Soft | 132  | 50 | 38  | 98 | 124    | 156| 335 |
| Stiff half-max | 251 | 77 | 34  | 191| 289    | 308| 338 |
| Stiff max | 305  | 45 | 33  | 304| 316    | 331| 341 |
| 15μm beads | 518  | 26 | 43  | 308| 328    | 359| 359 |

| Re=198 | Mean | SD | Min | Q1 | Median | Q3 | Max |
|--------|------|----|-----|----|--------|----|-----|
| Soft max | 201  | 66 | 46  | 150| 198    | 261| 335 |
| Soft half-max | 169 | 59 | 36  | 127| 161    | 205| 335 |
| Soft | 203  | 62 | 46  | 155| 201    | 256| 337 |
| Stiff half-max | 270 | 62 | 20  | 254| 294    | 308| 338 |
| Stiff max | 310  | 29 | 63  | 303| 313    | 327| 339 |
| 15μm beads | 528  | 21 | 34  | 325| 337    | 357| 340 |

| Re=158 | Mean | SD | Min | Q1 | Median | Q3 | Max |
|--------|------|----|-----|----|--------|----|-----|
| Soft max | 265  | 51 | 48  | 248| 285    | 298| 336 |
| Soft half-max | 232 | 57 | 43  | 206| 262    | 286| 335 |
| Soft | 265  | 46 | 47  | 251| 282    | 295| 337 |
| Stiff half-max | 273 | 51 | 49  | 263| 289    | 302| 338 |
| Stiff max | 306  | 24 | 61  | 299| 308    | 318| 338 |
| 15μm beads | 333  | 15 | 80  | 337| 338    | 338| 341 |

| Re=119 | Mean | SD | Min | Q1 | Median | Q3 | Max |
|--------|------|----|-----|----|--------|----|-----|
| Soft max | 281  | 34 | 69  | 276| 291    | 301| 335 |
| Soft half-max | 269 | 39 | 16  | 259| 281    | 292| 334 |
| Soft | 273  | 33 | 40  | 266| 283    | 295| 335 |
| Stiff half-max | 268 | 42 | 71  | 259| 281    | 292| 336 |
| Stiff max | 294  | 24 | 67  | 287| 297    | 306| 337 |
| 15μm beads | 332  | 17 | 46  | 336| 337    | 338| 341 |

| Re=79  | Mean | SD | Min | Q1 | Median | Q3 | Max |
|--------|------|----|-----|----|--------|----|-----|
| Soft max | 302  | 30 | 8   | 295| 303    | 317| 337 |
| Soft half-max | 270 | 27 | 35  | 264| 277    | 286| 334 |
| Soft | 259  | 36 | 49  | 249| 270    | 281| 332 |
| Stiff half-max | 265 | 29 | 90  | 259| 274    | 283| 335 |
| Stiff max | 263  | 34 | 18  | 255| 273    | 283| 332 |
| 15μm beads | 273  | 32 | 20  | 265| 282    | 292| 334 |

**Distance from the outer wall**

[um]
Design I: Hydrodynamic behaviour of cells of cellular deformability model

Replica II

Distance from the outer wall [μm]

- Stiff max
- Soft
- Soft half-max
- Stiff half-max
- Reference 15μm beads
Design I: Hydrodynamic behaviour of cells of cellular deformability model

Replica III

![Graphs and tables showing hydrodynamic behaviour of cells of cellular deformability model]

Distance from the outer wall [um]

- Stiff max
- Soft
- Soft half-max
- Reference 1.5μm beads
Design II: Hydrodynamic behaviour of cells of cellular deformability model

Hydrodynamic behaviour of cells of five different degrees of deformability (Soft max, soft half-max, soft, stiff half-max and stiff) in comparison to reference 15 µm beads, in design II spiral channel with 170 × 30 µm cross-section at five different flow rates corresponding to Re=33, 66, 97, 132 and 168 (as outlined in the tables on the right) . The lateral equilibrium positions were measured as a distance from the outer wall (µm) at the end of the spiral channel and there were generated by image analysis. Here, it is reported as median (represented as the symbols) and the interquartile range (indicated by the short vertical lines). Vertical dotted lines indicate four sections of the channel corresponding to four outlets of the channel (0-90. µm-outlet A, 90-180 µm-outlet B, etc.). Events belonging to a given section have the highest probability of being captured within the corresponding outlet and tables showing statistical summary (mean and standard deviation from the mean (SD), median, 25th (Qi) and 75th (Q3) percentile as well as minimal (min) and maximal (max) measured value) of lateral equilibrium positions obtained for at least 10000 events.
## Design II: Hydrodynamic behaviour of cells of cellular deformability model

### Replica I

| Re  | Mean | SD  | Min | Q1  | Median | Q3  | Max |
|-----|------|-----|-----|-----|--------|-----|-----|
| 168 | 119  | 23  | 7   | 95  | 136    | 139 | 155 |
| 10μm beads | 113 | 21  | 34  | 102 | 121    | 128 | 143 |
| Stiff | 100 | 27  | 21  | 78  | 109    | 124 | 143 |
| Stiff half-max | 68  | 21  | 20  | 54  | 62     | 79  | 161 |
| Soft | 80  | 26  | 20  | 59  | 74     | 96  | 142 |
| Soft half-max | 75  | 24  | 19  | 57  | 70     | 92  | 142 |
| Soft max | 75  | 24  | 19  | 57  | 70     | 92  | 142 |

| Re  | Mean | SD  | Min | Q1  | Median | Q3  | Max |
|-----|------|-----|-----|-----|--------|-----|-----|
| 132 | 128  | 11  | 28  | 121 | 132    | 137 | 161 |
| 10μm beads | 101 | 25  | 29  | 84  | 107    | 123 | 147 |
| Stiff | 88  | 29  | 26  | 68  | 85     | 119 | 143 |
| Stiff half-max | 75  | 18  | 23  | 63  | 74     | 86  | 141 |
| Soft | 84  | 23  | 20  | 66  | 83     | 102 | 143 |
| Soft half-max | 80  | 23  | 15  | 62  | 77     | 97  | 162 |
| Soft max | 80  | 23  | 15  | 62  | 77     | 97  | 162 |

| Re  | Mean | SD  | Min | Q1  | Median | Q3  | Max |
|-----|------|-----|-----|-----|--------|-----|-----|
| 97  | 108  | 20  | 29  | 91  | 97     | 131 | 158 |
| 10μm beads | 89  | 25  | 25  | 76  | 87     | 107 | 144 |
| Stiff | 79  | 24  | 21  | 64  | 79     | 92  | 148 |
| Stiff half-max | 85  | 20  | 24  | 70  | 85     | 99  | 141 |
| Soft | 85  | 20  | 24  | 70  | 85     | 99  | 141 |
| Soft half-max | 82  | 21  | 20  | 66  | 81     | 97  | 141 |
| Soft max | 82  | 21  | 20  | 66  | 81     | 97  | 141 |

| Re  | Mean | SD  | Min | Q1  | Median | Q3  | Max |
|-----|------|-----|-----|-----|--------|-----|-----|
| 66  | 99   | 17  | 60  | 80  | 107    | 115 | 150 |
| 10μm beads | 82  | 26  | 18  | 70  | 84     | 94  | 145 |
| Stiff | 80  | 21  | 18  | 75  | 83     | 89  | 148 |
| Stiff half-max | 78  | 18  | 21  | 69  | 80     | 87  | 149 |
| Soft | 84  | 18  | 19  | 74  | 85     | 96  | 144 |
| Soft half-max | 82  | 18  | 21  | 71  | 83     | 93  | 138 |
| Soft max | 82  | 18  | 21  | 71  | 83     | 93  | 138 |

| Re  | Mean | SD  | Min | Q1  | Median | Q3  | Max |
|-----|------|-----|-----|-----|--------|-----|-----|
| 33  | 102  | 8   | 40  | 97  | 102    | 105 | 152 |
| 10μm beads | 87  | 19  | 18  | 80  | 90     | 97  | 150 |
| Stiff | 84  | 18  | 17  | 78  | 87     | 94  | 150 |
| Stiff half-max | 82  | 17  | 19  | 74  | 83     | 90  | 149 |
| Soft | 86  | 19  | 18  | 77  | 89     | 98  | 148 |
| Soft half-max | 84  | 20  | 17  | 73  | 86     | 96  | 150 |
| Soft max | 84  | 20  | 17  | 73  | 86     | 96  | 150 |

**Distance from the outer wall**

[um]
Design II: Hydrodynamic behaviour of cells of cellular deformability model

Replica II

![Graphs showing hydrodynamic behaviour for different cell types and Reynolds numbers (Re)]

- **Re=168**
  - Mean: 119
  - SD: 23
  - Min: 7
  - Q1: 95
  - Median: 136
  - Q3: 139
  - Max: 155
- **Re=132**
  - Mean: 128
  - SD: 11
  - Min: 28
  - Q1: 121
  - Median: 132
  - Q3: 137
  - Max: 161
- **Re=97**
  - Mean: 108
  - SD: 20
  - Min: 29
  - Q1: 91
  - Median: 97
  - Q3: 131
  - Max: 158
- **Re=66**
  - Mean: 99
  - SD: 17
  - Min: 60
  - Q1: 80
  - Median: 107
  - Q3: 115
  - Max: 150
- **Re=33**
  - Mean: 102
  - SD: 8
  - Min: 40
  - Q1: 97
  - Median: 102
  - Q3: 105
  - Max: 152

**Legend:**
- **Stiff max**
- **Soft**
- **Soft max**
- **Stiff half-max**
- **Soft half-max**
- **Reference 15μm beads**

**Distance from the outer wall (μm):**

0.0 - 42.5 - 85 - 127.5 - 170.0
Design II: Hydrodynamic behaviour of cells of cellular deformability model

Replica III

Distance from the outer wall [µm]

- Stiff max
- Soft
- Soft half-max
- Reference 15µm beads

| Rep | Mean | SD | Min | Q1 | Median | Q3 | Max |
|-----|------|----|-----|----|--------|----|-----|
| Re-168 | 119 | 23 | 7 | 95 | 136 | 139 | 155 |
| 10µm beads | 115 | 21 | 31 | 110 | 123 | 129 | 155 |
| Stiff max | 96 | 28 | 28 | 74 | 97 | 123 | 143 |
| Stiff half-max | 68 | 21 | 23 | 54 | 63 | 77 | 145 |
| Soft | 77 | 25 | 17 | 57 | 73 | 95 | 140 |
| Soft half-max | 74 | 23 | 19 | 57 | 69 | 90 | 140 |

| Re-132 | Mean | SD | Min | Q1 | Median | Q3 | Max |
|--------|------|----|-----|----|--------|----|-----|
| 10µm beads | 128 | 11 | 28 | 121 | 132 | 137 | 161 |
| Stiff max | 98 | 26 | 27 | 81 | 97 | 122 | 142 |
| Stiff half-max | 89 | 29 | 17 | 68 | 86 | 119 | 144 |
| Soft | 72 | 18 | 19 | 59 | 70 | 83 | 114 |
| Soft half-max | 84 | 23 | 26 | 65 | 83 | 103 | 146 |
| Soft max | 80 | 23 | 18 | 61 | 76 | 97 | 142 |

| Re-97 | Mean | SD | Min | Q1 | Median | Q3 | Max |
|--------|------|----|-----|----|--------|----|-----|
| 10µm beads | 108 | 20 | 29 | 91 | 97 | 131 | 158 |
| Stiff max | 92 | 23 | 27 | 81 | 90 | 106 | 149 |
| Stiff half-max | 80 | 26 | 21 | 66 | 80 | 92 | 142 |
| Soft | 76 | 16 | 20 | 67 | 76 | 83 | 149 |
| Soft half-max | 86 | 21 | 20 | 71 | 85 | 100 | 145 |
| Soft max | 81 | 21 | 26 | 66 | 80 | 95 | 143 |

| Re-66 | Mean | SD | Min | Q1 | Median | Q3 | Max |
|--------|------|----|-----|----|--------|----|-----|
| 10µm beads | 99 | 17 | 60 | 80 | 107 | 115 | 150 |
| Stiff max | 87 | 23 | 21 | 79 | 88 | 97 | 150 |
| Stiff half-max | 80 | 21 | 18 | 75 | 83 | 89 | 147 |
| Soft | 76 | 17 | 21 | 68 | 78 | 85 | 143 |
| Soft half-max | 83 | 19 | 23 | 72 | 84 | 95 | 141 |
| Soft max | 82 | 18 | 24 | 72 | 83 | 92 | 135 |

| Re-33 | Mean | SD | Min | Q1 | Median | Q3 | Max |
|--------|------|----|-----|----|--------|----|-----|
| 10µm beads | 102 | 8 | 40 | 97* | 102 | 105 | 152 |
| Stiff max | 87 | 19 | 18 | 80 | 90 | 97 | 149 |
| Stiff half-max | 85 | 17 | 17 | 80 | 88 | 95 | 148 |
| Soft | 80 | 18 | 15 | 72 | 81 | 89 | 147 |
| Soft half-max | 87 | 18 | 17 | 78 | 89 | 98 | 150 |
| Soft max | 84 | 20 | 14 | 73 | 86 | 95 | 151 |
Summary of flow cytometric viability assay. On the top- an exemplary scatter plot showing gating strategy for live cell (green, negative for both Alexa Fluor 488-annexin V and propidium iodide (PI) fluorescence), apoptotic cells (orange, annexin V-positive and PI-negative) and necrotic (red, annexin V-positive and PI-positive). Summary of flow cytometric assessment of the presence of live, apoptotic and necrotic Jurkat cells before (stained control and after processing (stained test)).

Design I spiral channel with $360 \times 60 \, \mu\text{m}$ cross-section at highest applied flow rate ($Re=237$) for three replicas.
Design II spiral channel with $170 \times 30 \, \mu m$ cross-section at highest applied flow rate ($Re=168$) for three replicas.
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