1 Image-based cell sorting using focused travelling surface acoustic waves

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9 Supplementary Information

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- 11 Video S1: Fine adjustment of the pressure value at the default outlet to enable cell sorting upon
- 12 **actuation**. Cells are seen flowing into the default outlet when the pressure controller is set at -25 mBar.
- 13 When the pressure is adjusted to -5.7 mBar the cell stream comes closer to the bifurcation wall which
- 14 is necessary to translate the cell of interest into the target outlet upon FTSAW actuation.
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Figure S1: FIDT design. Schematic overview (Klayout software) of the FIDT aligned in front of the sorting region (SR length=130 μ m) in a soRT-DC chip. The FIDT has a distal end aperture of 600 μ m and a frontal end aperture of 100 μ m subtending an angle of 42°. This provides a focal point located close to the SR channel wall at 170 μ m from the first FIDT finger. The red-shaded triangular region depicts the energy concentration within a region of 50 μ m in the sorting region. The inset shows a FIDT





Figure S2: Chip design. Schematic overview (Klayout Software) of the PDMS channel geometries of the soRT-DC chip depicting inlets, outlets, pocket for FIDT, alignment marker and filter architectures. Bottom inset shows an expanded view of the sorting region and the primary deformation channel. Top insets show an expanded view of the filter architecture at the sheath and sample inlets. Region 1: pillar diameter=25 µm and inter-pillar distance=10 µm; Region 2: pillar diameter=40 µm and inter-pillar distance=15 µm; Region 3: pillar diameter=40 µm and inter-pillar distance=20 µm; Region 4: pillar diameter=60 µm and inter-pillar distance=60 µm; Region 5: pillar diameter=100 µm and inter-pillar distance=100 µm. Top left inset shows the serpentine-like channels for breaking apart cell clumps.



Figure S3: soRT-DC chip and chip holder. (A) picture of the assembled soRT-DC chip. Bottom layer:
LiNbO3 substrate integrating the FIDT. Top layer: microstructured PDMS element including the
microfluidic chip geometry. (B) chip holder for mounting the chip on the microscope stage holder and
for interfacing it with the external RF generator by the SMA connector.

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58 Figure S4: Size-based sorting of a mixture of two cell lines (A) Deformation vs cell size scatter plot 59 of a mixture of Kc167 (smaller size) and HL60/S4 (bigger size) cells mixed at a 1:4 ratio. The left 60 scatter plot represents the initial populations and the middle scatter plot the sorted target cell population 61 with a 4-fold enrichment from 24.1% to 94.1%. The orange shaded rectangle represents the sorting gate (25–77 µm²). (B) Deformation vs cell size scatter plot of a mixture of HL60/S4 (small cells) and HeLa 62 63 (big cells) mixed in 1:1 ratio. The left scatter plot represents the initial populations and the middle 64 scatter plot the sorted target cell population with a 1.5-fold enrichment from 59.4% to 91.5%. The blue 65 shaded rectangle represents the sorting gate (100–200 µm²). The colour map in scatter plots represents 66 the event density. The violin plots show the distribution of the corresponding scatter plot data for the 67 initial and target cell populations. The percentages on the scatter plots indicate the fraction of cells in 68 the sorting gate. The insets show merge contour plots of the initial and target populations; 95%-density 69 (dashed lines) and 50%-density (solid lines).

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71 Figure S5: Assessing proliferation, viability, and purity of cells after sorting. (A) Proliferation and 72 (B) Viability of HL60/S4 cells after exposure to FTSAW. Target HL60/S4 cells (Target; green) were 73 cultured post-sorting for 4 days and compared to: *i*) cells that were not exposed to TSAW (no SAW; 74 magenta); ii) cells that were diluted in measuring buffer (Initial; blue); iii) cells that were loaded on the 75 microfluidic chip but were not sorted (Default; grey); and iv) control cells (culture medium; orange). 76 Viability was assessed by counting the number of live and dead cells at each time point. (C) Percentage 77 purity of initial (blue) and target (red) HL60/S4 cells diluted in MB with increasing concentration. The 78 target purity (TP) is defined as the percentage ratio of the number of selected cells to the total number 79 of cells in the sorted-target; and the initial purity (IP) as the percentage ratio of the number of selected 80 cells to the total number of cells in the unsorted-initial population.





Figure S6: Sorting reproducibility with increasing concentration of cells. Representative scatter plots of deformation vs cell size scatter plots of HL60/S4 diluted at a different concentration in MB $(3\times10^6, 12\times10^6, 18\times10^6 \text{ and } 36\times10^6 \text{cells/ml})$. The colour map in scatter plots represents the event density. The violin plots show the distribution for both the initial and the target cell populations. The percentages on the scatter plots indicate the fraction of cells in the sorting gates.