Supplementary Information for:

Ultrathroughput Immunomagnetic Cell Sorting Platform

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Supplementary Table 1 : Comparison of Sorting Technologies

	Magnetic-activated cell sorting (MACS)*	**Fluorescence-activated cell sorting (FACS)** ‡	**Microfluidic cell sorting (NG-MICS)**
Number of Subpopulation	2	4 or fewer	3 for NG-MICS, some first-generation MICS designs with 4 and 10
Types of Markers	Extracellular proteins and intracellular proteins (with permeabilization)	Extracellular proteins and intracellular proteins (with permeabilization)	Extracellular proteins (NG-MICS) and intracellular proteins and mRNA (with permeabilization)
Number of Markers	1	Up to 15	1
Cell Throughput	$2x10^8$/column† total, with max 10^7 labeled cells /column	Theoretically up to $7.2x10^7$ event per hour, often lower	$2.1x10^8$ cells per hour per device§
Sorting Solution	Blood or buffer	Buffer	Blood or buffer
Parallelization	Yes, up to 8	No	Yes, first gen MICS demonstrated up to 30
Advantages	Speed, comparatively lower cost than FACS	Multiplexing, precision, isolation of rare cells	Speed, >2 populations, high cell viability, parallelization
Disadvantages	Binary sort, low recovery, difficulty isolating rare cells	Low throughput, trade off with viability at throughout increases, cost	Technology readiness level, single-marker

*All metrics for OctoMACS sorter with MS column, †Approximately 20-30 minutes to run a column. ‡All metrics for BD FACS Aria, § up to $4.4x10^8$ theoretically possible

Supplementary Table 2 : Simulated Outlet Flowrate Distribution

Outlet	**Ideal Distribution (%)***	**Simulated Distribution (%)**
Low	56.2	56.25
Medium	32.3	32.28
High	11.5	11.47

*Distribution corresponds to flowrates of using a 20ml, 10mL and 3 mL syringe to drive flow

Supplementary Table 3 : Cartridge Channel Cross Sections

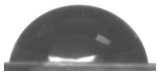
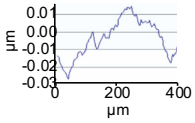
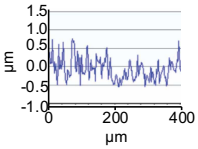
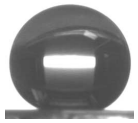
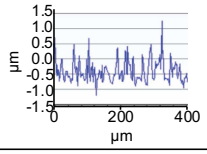
Level	Number of branches	Square Side Length (mm)
1	2	1.00
2	4	0.794
3	8	0.630
4	16	0.500*

*radius, lofted to circle cross section

Supplementary Table 4 : RIE Plasma Etch Rates

Treatment	Thickness (µm)	Error (µm)	Time (s)	Power (W)	O_2 Pressure (mtorr)	Calculated Etch Rate (nm/s)
Bare SU-8	118.2	3.7	N/A	N/A	N/A	N/A
Plasma Asher	118.0	3.7	60	100	300	2.2
Plasma Asher	117.6	1.3	60	250	300	9.4
Plasma Asher	115.8	0.8	60	250	2000	39.4
Plasma Asher	86.5	2.4	1800	250	600	17.6
Plasma Asher	82.3	2.1	1800	250	1000	19.9
RIE Plasma	116.4	0.7	60	50	100	28.9
RIE Plasma	116.0	1.5	60	250	100	35.6
RIE Plasma	98.4	5.9	300	250	100	65.9
RIE Plasma	107.2	1.5	120	150	100	91.1

Supplementary Table 5 : Hydrophobic Treatments

Treatment	$\theta_{Contact}$ (°)	Droplet	Profilometer Roughness (nm)	Profilometer Surface	Atomic Force Microscopy Roughness (nm)
Bare SU-8	83.4 ± 0.5		18.7 ± 2.4		4.73 ± 1.2
RIE Plasma	17.0 ± 5.1		247.3 ± 16.0		355 ± 69
RIE Plasma SAM	154.7 ± 0.7		371.4 ± 3.2		512 ± 16

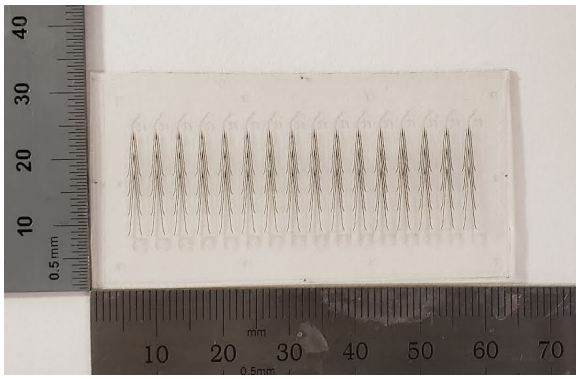
Supplementary Table 6 : Isolation of Clinically Relevant Numbers of Mature NK Cells

Number of mature NK cells required	10^8 cells
Frequency of mature NK cells in total NK population	0.86
Frequency of NK cells in PBMC	0.20
Total number of PBMCs to process	5.8×10^8 cells
Concentration of PBMCs	3×10^6 cells/ml during experiments*, 1.3×10^7 cells/ml possible
Nominal flow rate	8 ml/hr
Sample flow rate	7.11 ml/hr
NG-MICS NK-isolation throughput[†]	1×10^7 PBMCs/hr/device experimental, 4×10^7 PBMCs/hr/device theoretical
Required to process 5.75×10^7 PBMCs	57.5 device hours (experimental), 14.4 device hours (theoretical)
Time required across four devices	14.3 hours (experimental), 3.6 hours (theoretical)

*due to the small amount of cells during experiments, samples were diluted to prevent small sample volumes (< 500 μL) being affected by dead volume. For clinically-relevant numbers of cells, dilution would not be required.

[†]accounting for CD3 depeltion sorts followed by CD56 enrichment and reduced cell numbers for reach resort. Cells concentrated between sorts.

A



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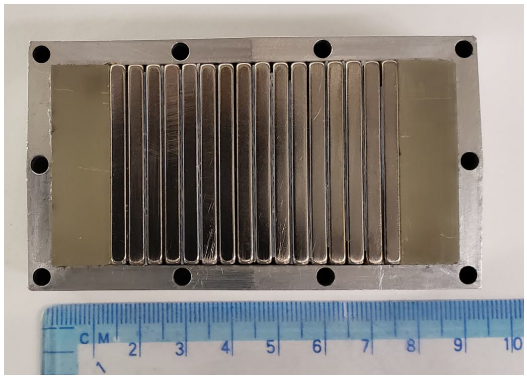

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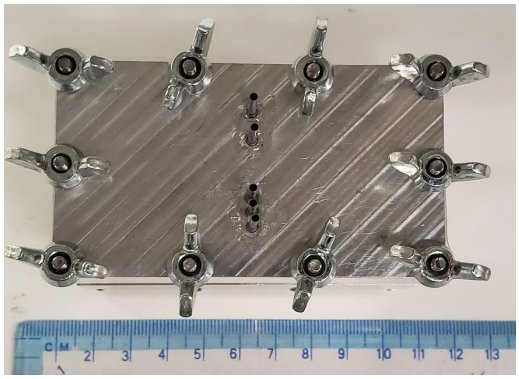

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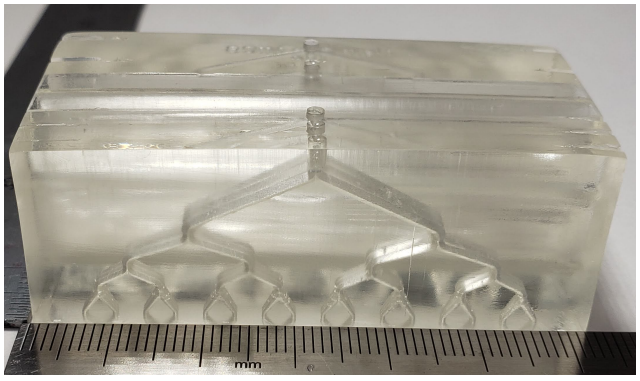

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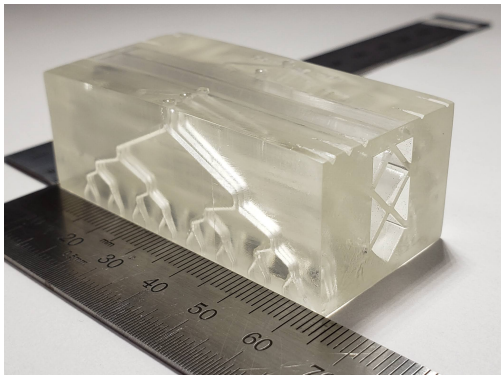
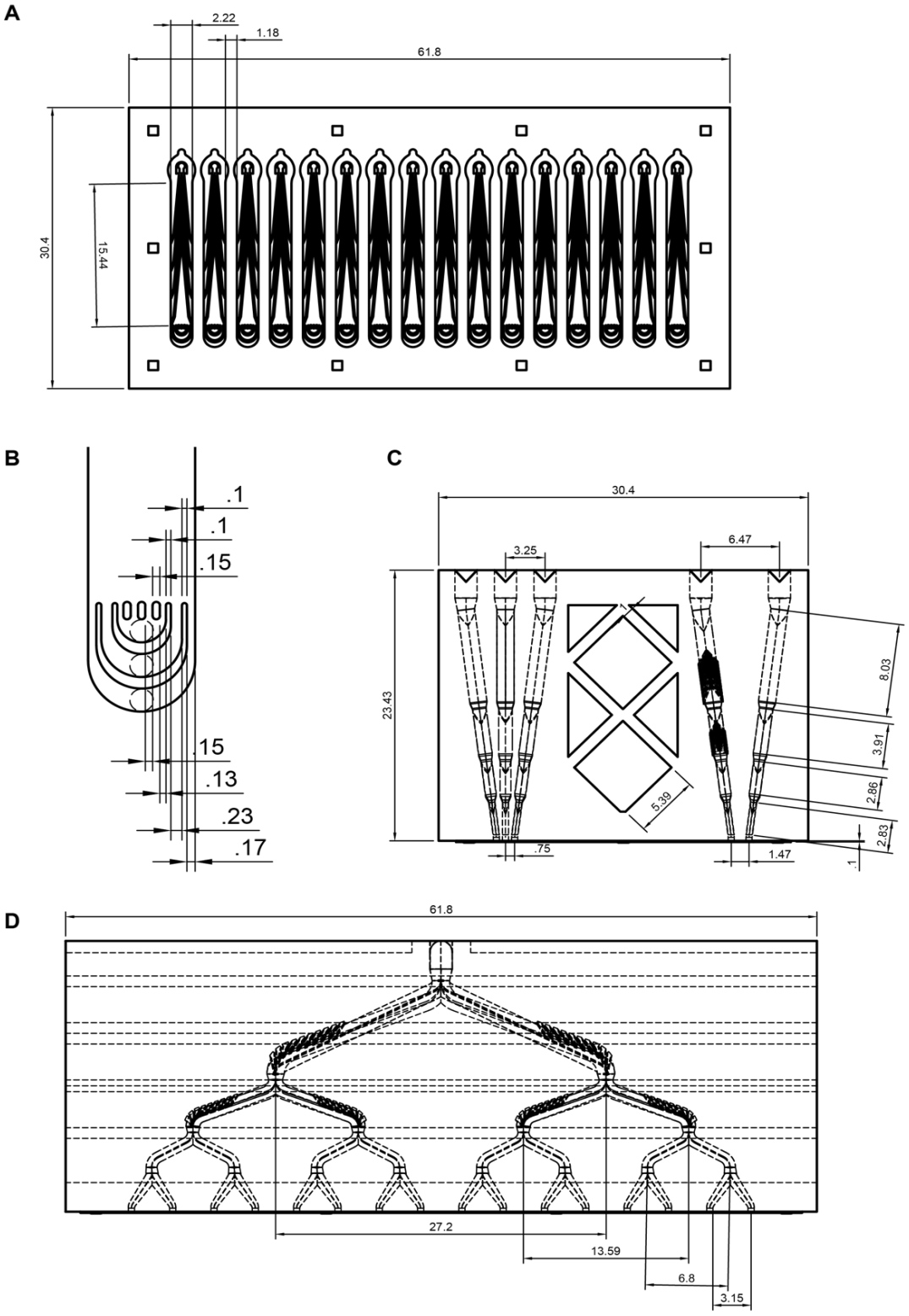
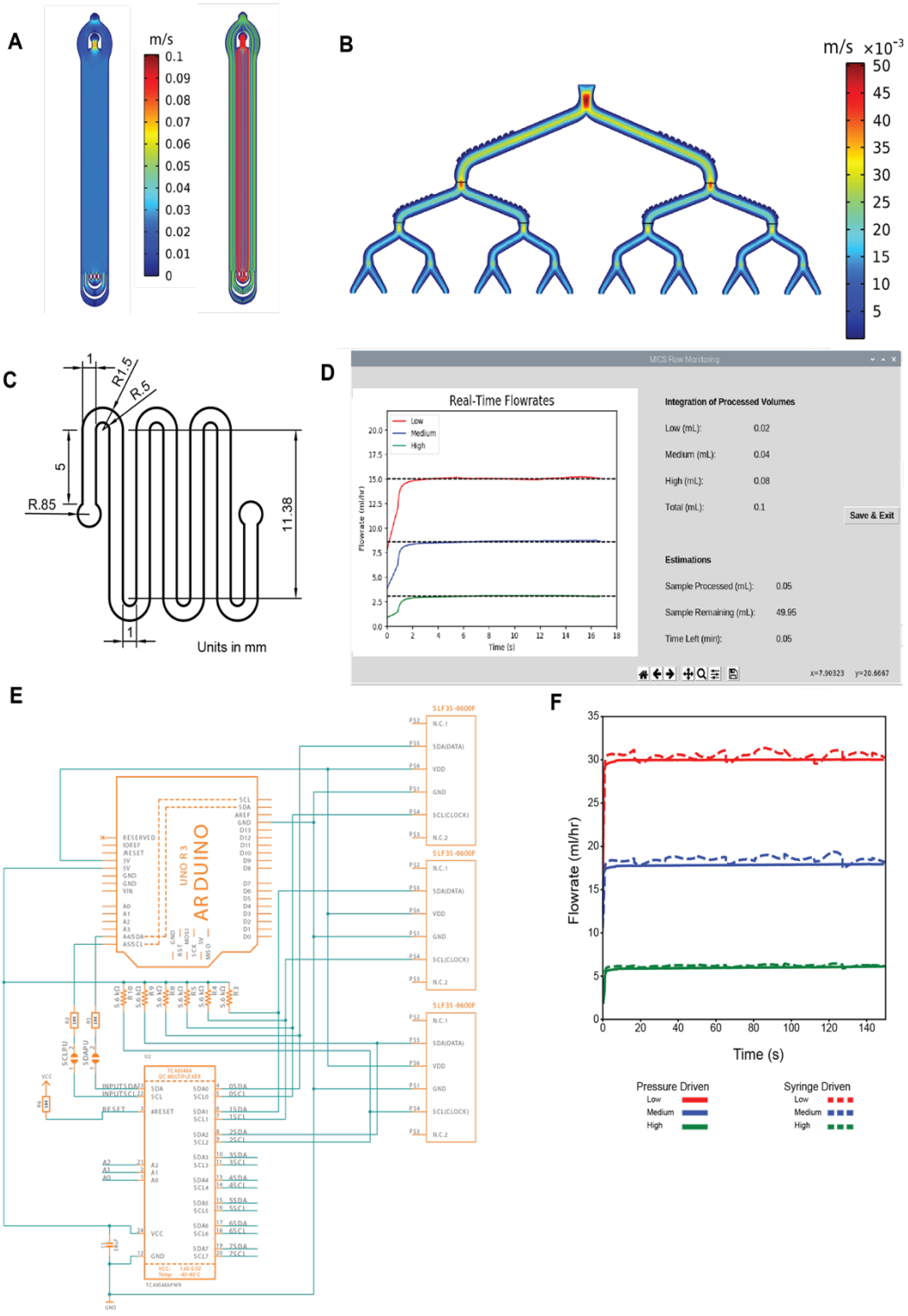


Supplementary Figure 1 – Photographs of NG-MICS components (A) Glass slide with channel structures and deflection guides **(B)** Bottom of clamp set up with neodymium magnets and spacers **(C**) Top pf clamp with interconnects **(D)** Side profile of clamp **(E)** 3D printed cartridge side view showing branching network **(F)** Perspective view of cartridge. Gaps in middle are to reduce resin consumption.



Supplementary Figure 2 – Dimensioned Drawings of NG-MICS components (A) Slide top view, units in mm **(B)** Top view of outlets of one sorting lane, units in mm **(C**) Side view of cartridge, units in mm **(D)** Front view of cartridge, units in mm



Supplementary Figure 3 – Device Simulations and External Setup (A) Computational fluid dynamic simulations of channel structures at a nominal flow rate of 15 ml/hr. Streamlines from sample inlet shown in red) and buffer inlet green. Channel height set to 100 µm **(B)** Computational fluid dynamic simulation of entire branched network including herringbone structures. Nominal flow rate of 50 ml/hr. **(C)** Dimensioned view of soft lithography resistance chip. Height of channels were 100 µm. **(D)** Example of the user interface programmed on Raspberry Pi. All values were updated in real time. **(E)** Wiring diagram of Arduino, I2C Multiplexer and flow sensors. Arduino connected to Raspberry Pi via USB. **(F)** Flow rate comparison between pressure driven flow set up and syringe driven flow set up.