Electronic Supplementary Material (ESI) for Analyst.

High-Throughput Flowing Upstream Sperm Sorting (FUSS) in a Retarding Flow Field for Human Semen Analysis

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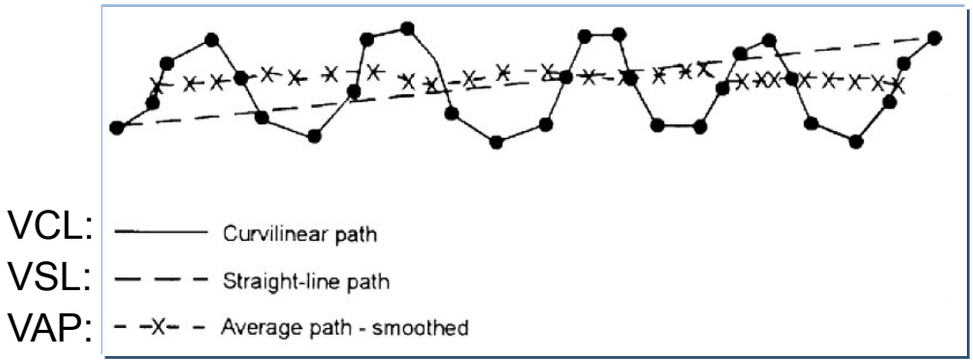
Supplemental Methods

CASA acquires images of sperm within a specific time period and in order to trace the trajectories of the sperm. Three indexes were used to assess velocity of the sperm: curvilinear velocity (VCL), velocity average path (VAP), and straight line velocity (VSL), as shown in Fig S1. The CASA system was used to classify sperm according to mobility at the following four levels (G0=0, G1=0-120, G2=120-180, G3>180 μm/s).



Supplementary Figure S1. Scheme of kinematic analysis of sperm by CASA. Linearity (LIN%) = (VSL/VCL) x 100,

Straightness (STR%) = (VSL/VAL) x 100, Wobble (WOB%) = (VAP/VCL) x 100.

As we known, Poiseuille's equation (eq. 1) can be used to determine the pressure drop of a constant viscosity fluid exhibiting laminar flow through a rigid pipe. Here, we used it to get the value of flow rate in quadrate channel geometry, and then put them into the formula of Continuity equation (eq. 2) to calculate the value of velocity at relative position.

$$Q = \frac{\pi D_h^{\,4}}{128\eta}\frac{\rho g h}{L} \quad \text{.....(1)}$$

Here, $D_h = \frac{4A}{P}$

V= Q/A……..……(2)

Here, V= Avg. velocity, A= cross sectional area

Width (μm)	100	200	300	400	500	600	700	800	900	1000
Theoretical (Hagen Poiseuille Eq.)	550 μ m/s	276 μ m/s	184 μ m/s	138 μ m/s	110 μ m/s	92 μ m/s	79 μ m/s	69 μ m/s	62 μ m/s	55 μ m/s
Relative velocity level	G3	G3	G3	G2	G1	G1	G1	G1	G1	G1
Experimental (PIV measurement)	554 μ m/s	261 μ m/s	187 μ m/s		-	-	-	-	72 μ m/s	60 μ m/s
Relative velocity level	G3	G3	G3	-	-	-	-	-	G1	G1

Supplementary Table S1. Velocity distribution calculated by Poiseuille's equation in different position of channel compared with PIV measurement. In Zone 1 area, the avg. velocity all high than 180 μm/s (G3 level), we could sort the highest mobility sperm by this design if they can stay in this room to against the flow rate.

Conditions:

D_h, hydraulic diameter = 4*A/P, (A= W*H = $100\times100\times10^{-12}$ m^2, P=2(W+H) = 400×10^{-6} m)

ρ , density = 1000 kg/m^3

g, gravitational constant = 9.8 m/s^2

h, height of droplet = 3×10^{-3} m

η , coefficient of viscosity = $1.002\text{x}10^{-3}$ Pa·S = 1.002×10^{-3} $kg\cdot m^{-1}\cdot s^{-1}$

L, straight channel length = 10.5×10^{-3} m

Recovery ratio was evaluated by computer-assisted semen analysis (CASA) for sperm motility with three different qualities by chip-based method was shown in Table S2. Before each testing, the semen sample (original concentration:~1×107 cells/ml) was diluted with 50% Ham's F10 medium (prepared by 100 mM HEPES buffer) in the volume ratio of 1:10. As in our sorting protocol, the maximum volume of chip processing capability is 100 μl for about 45 mins.

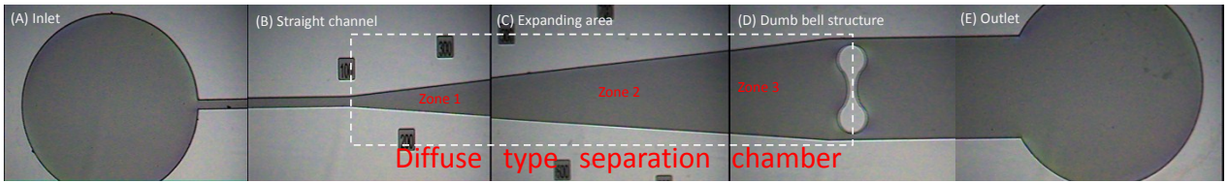
Case	Original Concentration (cells/**ml)**	Recovery rate **(**count in 1 μ L, **%)**	**G0 (%)**	**G1 (%)**	**G2 (%)**	**G3 (%)**
1	$1.0X10^7$	9149/11462 (79.8)	0.3	77.2	12.7	8.9
2	$1.0X10^7$	8342/10132 (82.3)	23.8	57.6	14.5	3.9
3	$1.2X10^7$	9251/11312 (81.7)	4.0	70.3	13.4	9.2
7	$1.1X10^7$	9185/11423 (80.4)	16.3	64.0	12.6	7.2
8	$1.2X10^7$	8986/11256 (79.8)	12.8	62.4	19.8	5.2
9	$1.1X10^7$	8269/10337 (80.0)	15.6	70.5	8.3	5.9
12	$1.0X10^7$	7829/9986 (78.4)	11.4	68.6	13.6	8.2
13	$1.0X10^7$	7954/9943 (79.9)	24.8	60.2	9.5	6.8
14	$1.1X10^7$	9018/11238 (80.2)	36.8	49.7	14.2	4.5

Supplementary Table S2. Sorting Results of Clinical Samples in Figure 4 from CASA Analysis. Pick up 9 persons among these 15 cases (six were deemed healthy (No.1-6), five were found to be moderately infertile (No.7-11), and the other four (No.12-15) were severe infertile) were tested on chip-based method.

For case 1-3: healthy case, the absolute number of the sperms counted in 1μl is in the range of 8914±406 with 81.3% recovery rate.

For case 7-9: moderately infertile case, the absolute number of the sperms counted in 1μl is in the range of 8813±393 with 80.1% recovery rate.

For case 12-14: severe infertile case, the absolute number of the sperms counted in 1μl is in the range of 8267±533 with 79.5% recovery rate.



in 5 mins	G0	G1	G2	G3
Inlet	23.50%	70.16%	5.69%	0.68%
Zone 1	0.00%	57.40%	27.90%	14.70%
Zone 2	5.60%	87.60%	6.20%	0.60%
Zone 3	19.90%	71.60%	7.10%	1.40%
In front **of dumbbell**	56.00%	35.80%	6.40%	1.80%
behind of dumbbell	66.30%	32.60%	1.10%	0.00%
Outlet	90.00%	10.00%	0.00%	0.00%
in 10 mins				
Inlet	23.46%	70.16%	5.69%	0.68%
Zone 1	10.67%	81.33%	6.67%	1.33%
Zone 2	47.42%	43.30%	7.22%	2.06%
Zone 3	68.75%	29.17%	1.04%	1.04%
In front **of dumbbell**	76.34%	23.66%	0.00%	0.00%
behind of dumbbell	100.00%	0.00%	0.00%	0.00%
Outlet	100.00%	0.00%	0.00%	0.00%
in 15 mins				
Inlet	23.46%	70.16%	5.69%	0.68%
Zone 1	0.00%	37.50%	41.25%	21.25%
Zone 2	2.56%	83.33%	12.18%	1.92%
Zone 3	27.38%	59.52%	10.71%	2.38%
In front **of dumbbell**	62.07%	36.21%	1.72%	0.00%
behind of dumbbell	80.00%	17.78%	2.22%	0.00%
Outlet	95.24%	0.00%	4.76%	0.00%

Supplementary Table S3. Results of distribution ratio of sperm's mobility in several different positions of channel. The results of viability analysis (figure 2b) also fit the data that calculated the ratio of sperm's mobility in different relative position of channel by CASA analysis.

It was evaluated by computer-assisted semen analysis (CASA) for sperm motility treated with different buffer solutions by three different methods was shown in Table S4. The various components and concentration of dilute buffer will have different degrees of impact on normal sperm (pure sample, case 1, 3, 5 diluted with different solution); Case1 has suffered the minimize impact from dilute buffer, it is because of dilution liquid formulation is the lowest concentration and most simple components among of these three dilute solution.

It can be observed that different sorting methods will have different degrees of impact on normal sperm (prepared sample, case 2, 4, 6 sorted by different method). It shows that the sperms' velocity decreases slightly for case 2 after sorted by this reported FUSS method. Thanks for the shortly sorting time reduces the amount of energy consumed by the sperm during the screening process, it has the minimize damage effect on normal semen.

Case	Concentration (cells/**ml)**	M**obility** rate **(%**, count in 10 μL**)**	**VAP (μm/s)**	**VSL (μm/s)**	**VCL (μm/s)**	**G0 (%)**	**G1 (%)**	**G2 (%)**	**G3 (%)**
1	$1.6X10^8$	1149/1462 (78.6)	81.8	45.4	183.3	23.4	70.2	5.7	0.7
2	$5.4X10^7$	342/1066 (32.1)	82.7	43.7	180.5	38.8	51.2	4.5	0.5
3	$1.5X10^8$	1051/1312 (80.1)	76.5	41.9	176.9	35.2	60.0	4.4	0.6
4	$5.5X10^7$	297/986 (30.1)	62.7	34.1	160.3	64.0	46.0	0.0	0.0
5	$1.2X10^8$	1085/1423 (76.2)	72.6	36.9	176.8	31.4	63.6	4.2	0.8
6	$5.6X10^7$	354/979 (36.2)	50.2	20.4	149.7	65.8	34.2	0.0	0.0

Supplementary Table S4. Results from CASA analysis. Case 1: semen sample was diluted with 50% Ham's F10 medium (prepared by 100 mM HEPES buffer) in the volume ratio of 1:2; Case 2: sample prepared in case 1 was sorted by FUSS method; Case 3: semen sample was immersed in 100 % Ham's F10 medium; Case 4: sample prepared in case 3 was sorted by SU method; Case 5: a sperm gradient kit (K-SISG-50, two media with different concentrations (L80 and L40)) was added to the semen samples in the volume ratio of 1:10; Case 6: sample prepared in case 5 was sorted by DG. (FUSS: flowing upstream sperm sorter, SU: swim up, DG: density gradient).