**Supplementary material for:**

# High efficiency rare sperm separation from biopsy samples in an inertial focusing device

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## Straight and spiral channel design

A straight microchannel was fabricated for the separation of sperm and red blood cells (RBCs). The sperm and RBCs were expected to focus to different positions across the channel width. Four outlets were designed to collect sperm and RBCs. For this experiment, we designed a straight microchannel with the capability of focusing 3 μm diameter spherical particles. The straight channel width was 100 μm and channel height was 150 μm. Particle focusing in a straight channel is dominated by the inertial flow induced shear lift force, which is related to the particle diameter, and channel cross-section dimension. The shear lift force on a particle is determined by the equation $F_L = f_L \rho U_m^2 a^2 \lambda^2$. Table 1 lists the value of Re and $F_L$ for a 3μm particle under different flow rates. The shear lift force drives particles toward balanced positions, and this force is balanced by the Stokes' drag force $F_{drag} = 3\pi\mu a U_{lateral}$. When $F_L = F_{drag}$, the particle lateral movement velocity $U_{lateral}$ is calculated. Particles have a maximum lateral migration distance of $0.5W$, and the minimum channel length for shear lift focusing can be calculated using: $L_{lift} = 0.5WU_{flow}/U_{lateral}$. The designed straight channel has a channel length of 60 mm, and 3 μm beads can be focused at a flow rate of 0.4 ml/min. Experimental results indicate that steady focusing streams are formed at a flow rate of 0.2 ml/min.

Table S1. Design parameters for 3 μm beads focusing in the straight channel

| **Flow rate** (ml/min) | $U$ (m/s) | **Re** (-) | $F_L$ (N) | $U_{lateral}$ (m/s) | $L_{lift}$ (m) |
|---|---|---|---|---|---|
| 0.02 | 2.22E-02 | 2.667 | 3.20E-14 | 1.13E-06 | 0.981748 |
| 0.06 | 6.67E-02 | 8 | 2.88E-13 | 1.02E-05 | 0.327249 |
| 0.1 | 1.11E-01 | 13.333 | 8.00E-13 | 2.83E-05 | 0.19635 |
| 0.14 | 1.56E-01 | 18.667 | 1.57E-12 | 5.55E-05 | 0.14025 |
| 0.2 | 2.22E-01 | 26.667 | 3.20E-12 | 1.13E-04 | 0.098175 |
| 0.3 | 3.33E-01 | 40 | 7.20E-12 | 2.55E-04 | 0.06545 |
| 0.4 | 4.44E-01 | 53.333 | 1.28E-11 | 4.53E-04 | 0.049087 |
| 0.5 | 5.56E-01 | 66.667 | 2.00E-11 | 7.07E-04 | 0.03927 |
| 0.6 | 6.67E-01 | 80 | 2.88E-11 | 1.02E-03 | 0.032725 |
| 0.8 | 8.89E-01 | 106.667 | 5.12E-11 | 1.81E-03 | 0.024544 |

In spiral channel devices, Dean flow appears due to channel curvature, which will affect the particle focusing position. The proposed spiral channel device has channel cross-section of 200 x 50 μm (width x height). Since channel height is narrower than channel width, particles experience higher shear force in channel height direction, which is the direction of inertial focusing migration. On the other hand, particle movement is more affected by Dean drag force in the channel width direction. Dean number De is calculated based on Re, $D_h$ and spiral channel radius $R$: $De = Re(D_h/2R)^{0.5}$. The Dean drag force is calculated based on the Stokes' drag force equation utilizing Dean flow velocity $U_{Dean}$: $F_{Dean} = 5.4e\text{-}4\pi\mu De^{1.63}a$. In Table 2, we list the shear lift force $F_L$ and Dean drag force $F_{Dean}$ on 3 μm particle under different flow rate. It is found that the force ratio $F_L/F_D$ is above 1 under flow rate of 0.06 ml/min, indicating that 3 μm particle can be focused in the spiral channel device under a relatively low flow rate. Then the channel length required for shear lift focusing $L_{lift}$ and Dean drag migration $L_{Dean}$ are calculated. In this case, shear lift force dominates particle movement in channel height direction, and the distance of half channel height is the maximum particle migration distance $L_{lift} = 0.5HU_{flow}/U_{lateral}$. Dean drag force dominates particle movement in the channel width direction, and the channel width is the maximum particle migration distance $L_{Dean} = WU_{flow}/U_{Dean}$. It is found that $L_{Dean}$ has high influence to channel length for a fully developed focusing state. The total channel length is set to 160 mm which will enable particle focusing under flow rate of 0.14 ml/min.

Table S2. Design parameters for 3 μm beads focusing in the spiral channel

| **Flow rate** (ml/min) | **Re** (-) | $F_L$ (N) | $L_{lift}$ (m) | **De** (-) | $F_D$ (N) | $L_{Dean}$ (m) | $F_L/F_D$ (-) |
|---|---|---|---|---|---|---|---|
| 0.02 | 2.667 | 2.88E-13 | 0.0818 | 0.202 | 3.74E-13 | 0.5039 | 0.770 |
| 0.06 | 8.000 | 2.59E-12 | 0.0273 | 0.605 | 2.24E-12 | 0.2522 | 1.156 |
| 0.1 | 13.333 | 7.20E-12 | 0.0164 | 1.008 | 5.16E-12 | 0.1828 | 1.397 |
| 0.14 | 18.667 | 1.41E-11 | 0.0117 | 1.411 | 8.92E-12 | 0.1479 | 1.582 |
| 0.2 | 26.667 | 2.88E-11 | 0.0082 | 2.016 | 1.60E-11 | 0.1181 | 1.805 |
| 0.3 | 40.000 | 6.48E-11 | 0.0055 | 3.024 | 3.09E-11 | 0.0915 | 2.097 |
| 0.4 | 53.333 | 1.15E-10 | 0.0041 | 4.032 | 4.94E-11 | 0.0763 | 2.333 |
| 0.5 | 66.667 | 1.80E-10 | 0.0033 | 5.040 | 7.10E-11 | 0.0663 | 2.533 |
| 0.6 | 80.000 | 2.59E-10 | 0.0027 | 6.047 | 9.56E-11 | 0.0591 | 2.710 |
| 0.8 | 106.667 | 4.61E-10 | 0.0020 | 8.063 | 1.53E-10 | 0.0493 | 3.015 |
| 1 | 133.333 | 7.20E-10 | 0.0016 | 10.079 | 2.20E-10 | 0.0429 | 3.274 |

**Bio-sample preparation process**

In an experiment for sperm separation from semen, the sperm sample was provided by the University of Utah andrology department. In the sperm washing process, 200μl of fresh semen sample is loaded in a 1.5ml microcentrifuge tube. The semen sample is diluted with sperm washing media, and the total mixture volume is 1.0ml. The sample is centrifuged at 400 g for 15 minutes. The supernatant is removed, and the pellet is resuspended in 1 ml of sperm washing media. The centrifugation process is repeated at 400 g, 15 minutes. Then the final pellet is collected and diluted to 1 ml. The sperm concentration is

estimated used a Makler counting chamber. The washed sample is stained with 300 nM Dapi (Thermal Fisher Scientific, MA, United States) solution and incubated for 10 minutes at 37°C.

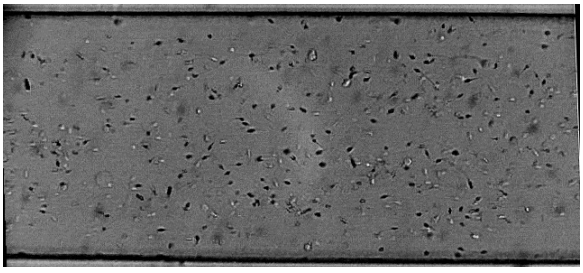
The testicular biopsy sample used in the experiment is the "waste" sample in which no sperm was found. The tissue sample is disrupted mechanically, and then mixed with collagenase for 1~2 hours. As a result, it is expected that single cells get released from the tissue mixture. Then, the tissue cells are suspended with Quinn's media, and loaded in the spiral channel device for the separation experiment.

**Sonication for sperm tail removal**

Sperm tails are removed by a sonication process. In the first step, sperm is purified using a density gradient centrifugation process. The density gradient column is prepared using Isolate (Irvine Scientific, CA, United States) with 30% and 90% gradients. 1 ml of semen is added to the density gradient column, and the density gradient column is centrifuged at 400 g for 15 minutes. Then the sample is washed with sperm washing media, and centrifuge at 400 g for 15 minutes. The "clean" sperm sample is treated by a Branson B12 sonication device. Sonication is performed at full power. In a treatment circle, sample is sonicated for 15s with 30s intervals. Six treatment circle is performed in total. The treated sample is washed using density gradient centrifugation. It is found that 70% of sperm got fractionated during the sonication process. In the separation experiment, the mixture of sperm head and whole sperm is loaded in the spiral channel device at a flow rate of 0.4 ml/min. The number of sperm and sperm head is counted using counting chamber from the two outlets.

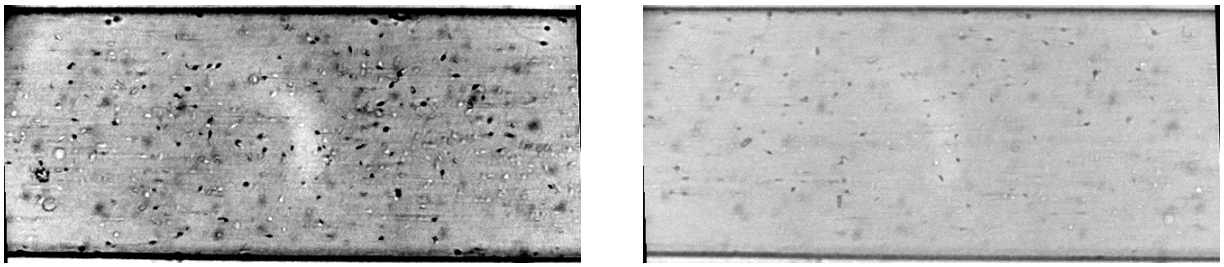
**High speed camera imaging experiments**

An experiment was performed to track sperm rotation in a microchannel. A high speed camera IDT NX4-S3 (IDT Visions, CA, United States) was used for high speed imaging with an acquisition frequency up to 8000 fps. The high speed camera was connected with an inverted microscope Olympus IX83 (Olympus, Japan) to observe microscale sperm movement in shear flow. Fig. S1 shows representative images of sperm movement in microchannel with different flow rates. Sperm orientations are classified in different angles compared with flow direction, and sperm orientation are defined as aligned, partially aligned, and not aligned based on their angle. Fig. S2 shows the summary of sperm alignment. More than 200 sperm orientations are analyzed under each flow rate. It is found that sperm tend to get aligned with the fluid flow direction, and with the increase of flow rate, more sperm get aligned in the flow direction.



1 μl/min

10 μl/min



50 µl/min 100 µl/min

Figure S1. Image of sperm movement under different flow rates

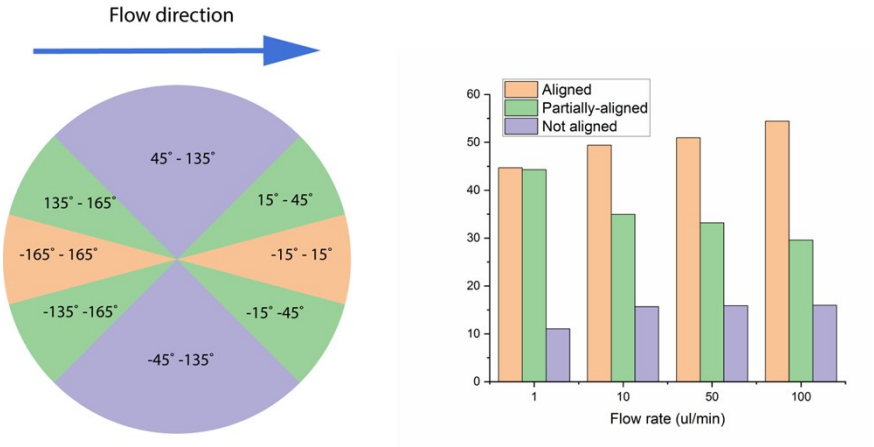



Figure S2. Sperm orientation distribution for different flow rates.