Electronic Supplementary Material (ESI) for Lab on a Chip.

Movie S1. Various modes of droplet generation.

Movie S2. Ordering of beads along flow rate.

Movie S3. Encapsulation of cell and ordered bead.

Movie S4. Encapsulation of cell and unordered bead.

Figure S1. Microscopic image of beads moving through spiral channel. (a) Still-shot image acquired from each loop. (b) Macroscopic view.

Figure S2. Detailed image of fluid junction showing thread. (scale bar indicates 100 μm)

Figure S3. Lateral position distribution of beads in microchannel after ordering.

Figure S4. Multi-bead fraction varies with bead concentration.

Figure S5. Bead-entering frequency for all concentrations of beads. In blue region, bead entering freq. < droplet generation freq. In red region, bead entering freq. > droplet generation freq.

Figure S6. Proportion of nucleotides of each base of read 1. Numbers in green bar indicate ratio of thymine.

Figure S7. Cumulative curves by number of UMIs. Dotted red lines indicated “knee” of each curve.

Table S1. Summary of Reynolds number, Dean number, and Particle Reynolds number.

Table S2. Summary of sequencing and cell classified result.

Electronic Supplementary Material (ESI)

Inertial-Ordering-Assisted Droplet Microfluidic for High-Throughput Single-Cell RNA-Sequencing

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Supplementary Information for Materials and Methods

Cell preparation

Three cell lines, K562 (human), 293T (human), and NIH/3T3 (mouse) cells were obtained from ATCC (Manassas, VA, USA). Cell lines were grown in RPMI-1640 (K562) or DMEM (293T, NIH/3T3) supplemented with 10% fetal bovine serum and 1% PS (Penicillin-Streptomycin) incubated in a 5% CO_2 humidified atmosphere at 37℃. To observe the encapsulation efficiency using a microscope, K562 cells were identified by pre-staining with CellTracker Green (C7025, Molecular Probes, Eugene, OR, USA), following incubation with 5 μM staining dye for 20 min at 37°C. Next, the cells were re-suspended in PBS to specific cell concentrations. The 293T and NIT/3T3 cells were harvested by trypsinization and resuspension in PBS at a desired concentration and used for specie-mixing experiments and validation experiments via single-cell RNA-seq.

Preparation of microfluidic devices

A 100-μm-thick master mold was prepared on the silicon wafer using SU-8 100 (Microchem, Westborough, MA, USA) by conventional photolithography. The mold was treated with a vapor of hexamethyldisilazane (440191, Sigma-Aldrich) for 60 min. Thereafter, a mixture of PDMS prepolymer (Sylgard 184, Dow Corning, Corning, NY, USA) and crosslinker in a 10:1 weight ratio was poured onto the master mold and degassed under vacuum for 90 min. The PDMS mixture was then cured in a 70℃ oven for 3 h. The PDMS replica was peeled off from the master mold, whose three inlets and one outlet were bored with a 0.75-mm biopsy punch (15072, Ted Pella, Inc., Redding, CA, USA). The PDMS

replica and PDMS-coated glass substrate were treated with a plasma cleaner and bonded. The microfluidic channel was filled with Aquapel (47100, Aquapel, Pittsburgh, PA, USA) to render it hydrophobic. Finally, the PDMS device was baked in a 70℃ oven for 1 h.

Procedures for single-cell RNA-seq

Briefly, following encapsulation (and immediate cell lysis and mRNA hybridization onto the bead), beads were pooled via droplet breakage and cDNA was generated through reverse transcription in bulk. Exonuclease, PCR, cDNA purification, and Nextera library preparation process were performed. Libraries were sent for sequencing with the Illumina HiSeq 2000 (San Diego, CA, USA).

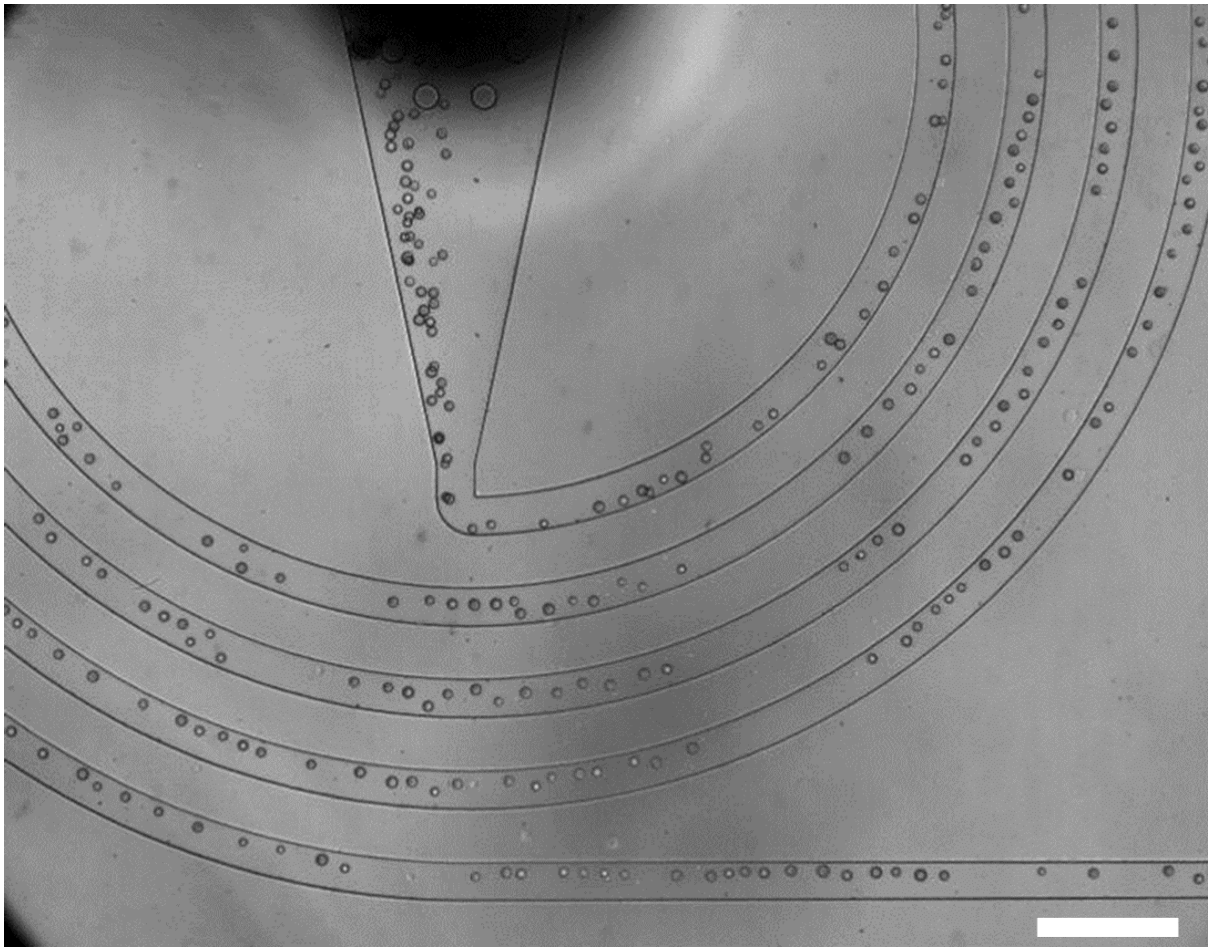


Figure S1. Microscopic image of beads moving through spiral channel. (scale bar indicates 500 μm)

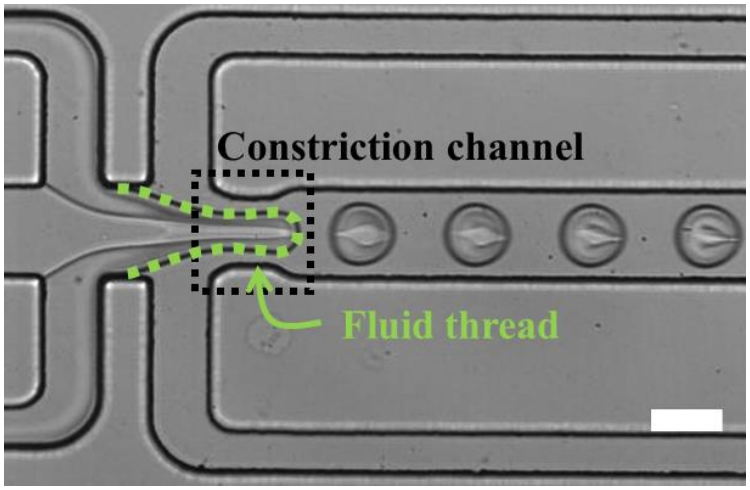


Figure S2. Detailed image of fluid junction showing thread. (scale bar indicates 100 μm).

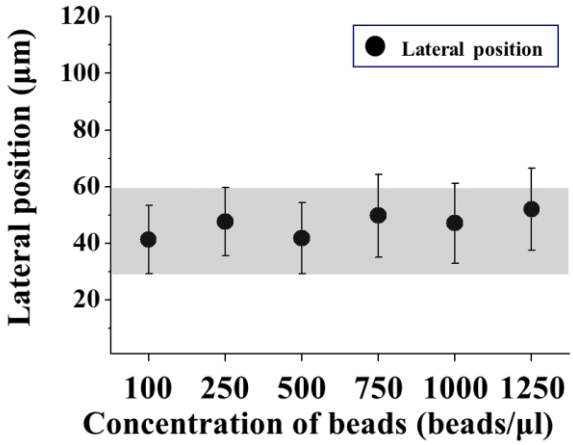
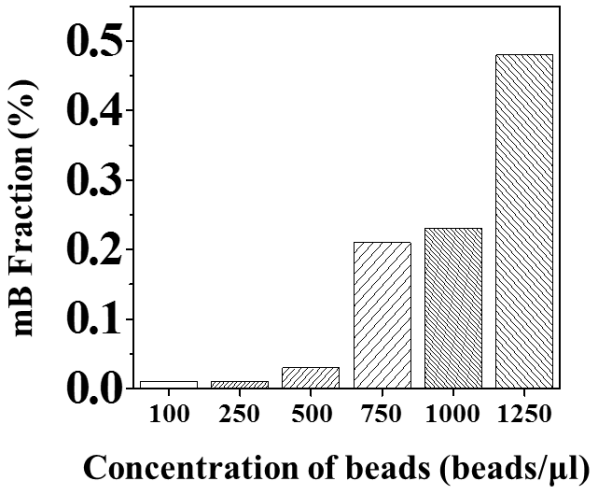


Figure S3. Lateral position distribution of beads in microchannel after ordering.



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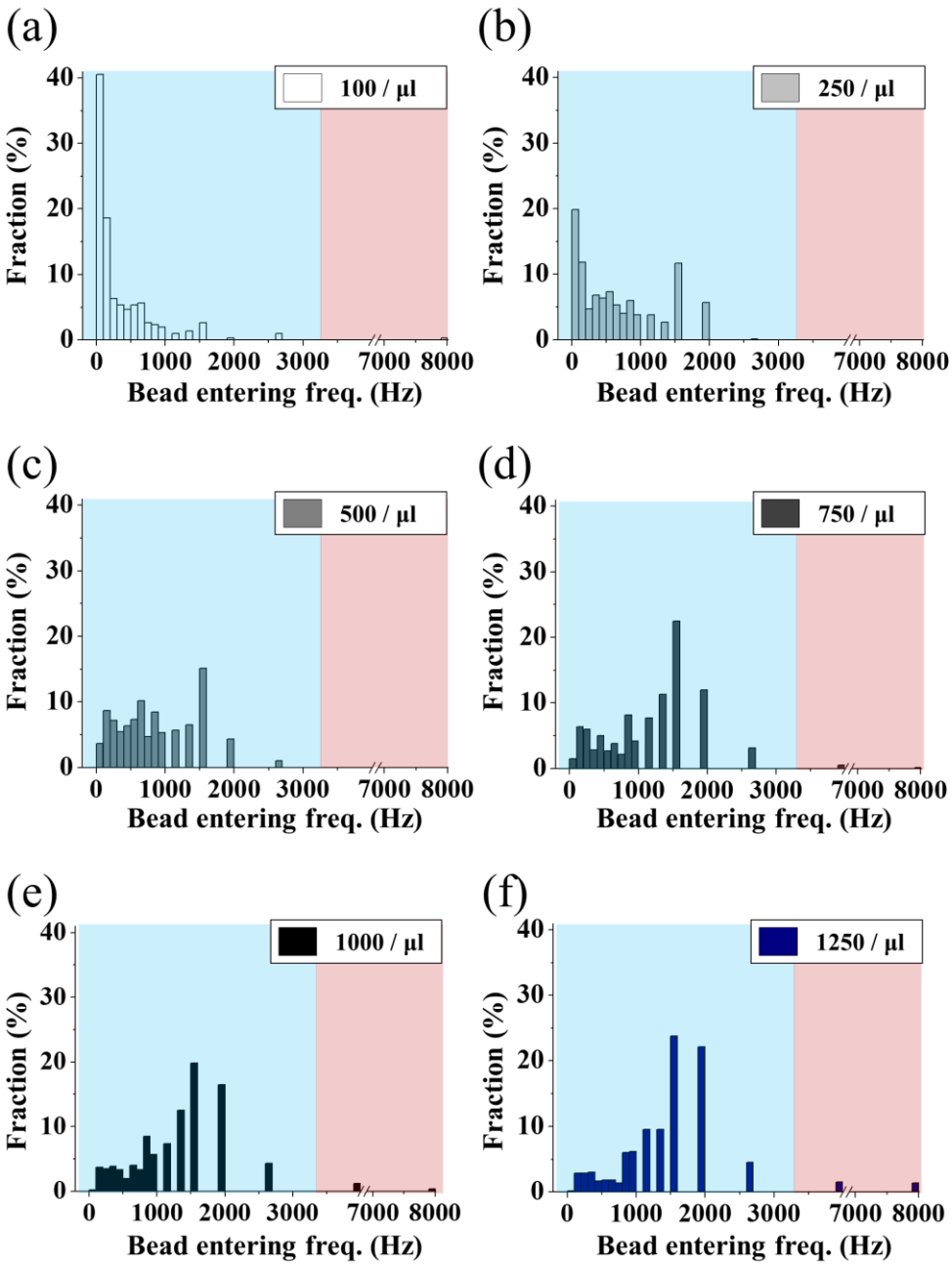

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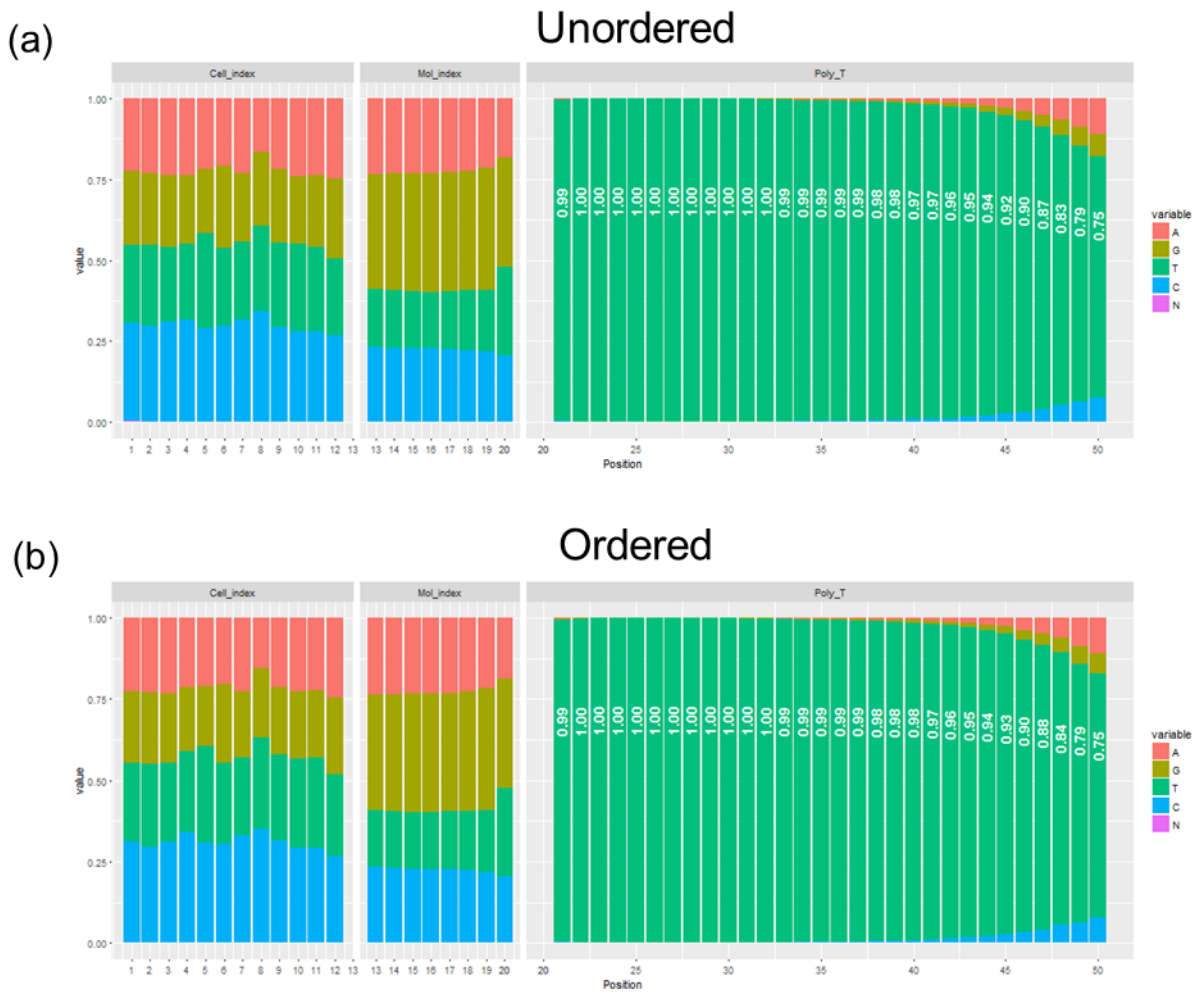

Figure S6. Proportion of nucleotides of each base of read 1. Numbers in green bar indicate ratio of thymine.

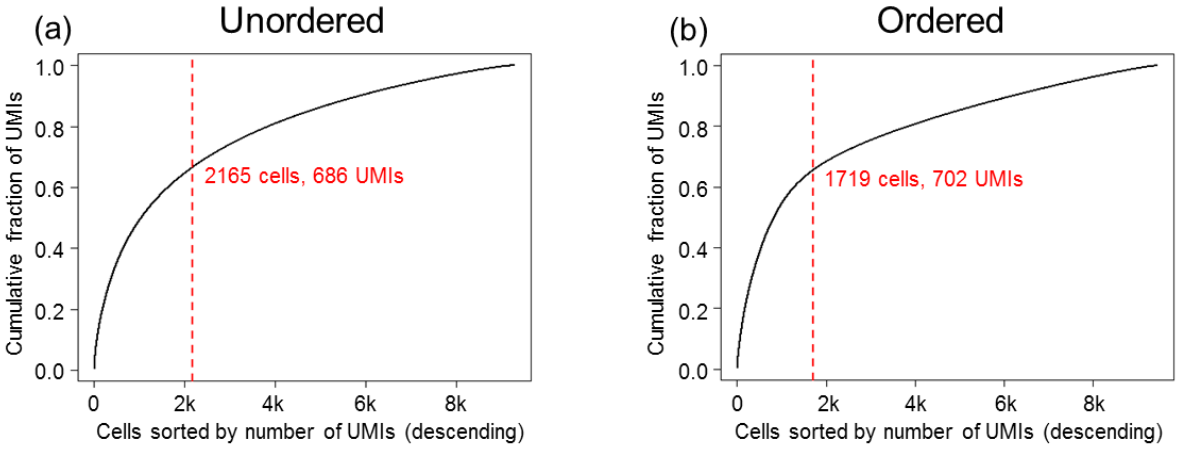

Figure S7. Cumulative curves by number of UMIs. Dotted red lines indicated "knee" of each curve.

Flow rate (ml/hr)	1.6	2.4	3.2
Reynolds number	4.08	6.12	8.15
Dean number	0.76	1.15	1.53
Particle Reynolds number	0.31	0.46	0.62

Table S1. Summary of Reynolds number, Dean number, and Particle Reynolds number.

Sample	Unordered	Ordered
Selected Cells	2,165	1,719
Median UMIs per Cell	1,277	1,849
Median Genes per Cell	789	1,036
Total UMIs	4,259,023 (100%)	4,371,301 (100%)
Human UMIs	3,283,180 (77.1%)	3,057,618 (69.9%)
Mouse UMIs	975,843 (22.9%)	1,313,683 (30.1%)
Human Cell	1,469	1,000
Mouse Cell	439	405
Mixed Cell	28	32
Low quality Cell	229	282
Purity	0.9855	0.9777

Table S2. Summary of sequencing and cell classified result.