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

Supplementary Figures

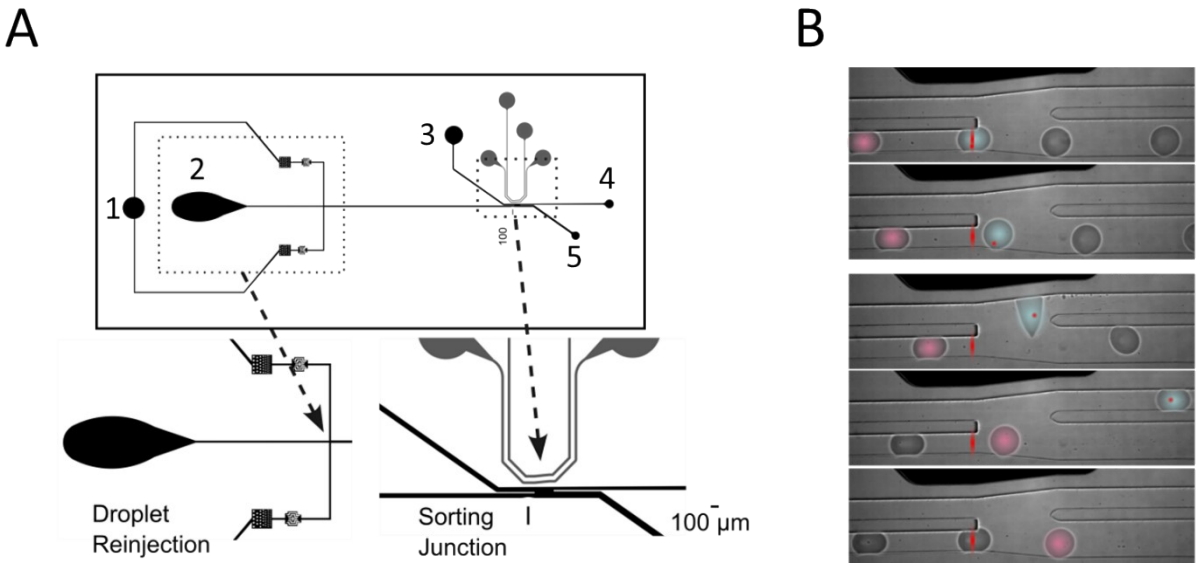

Fig. S1 Overview sorting chip

(A) **Microfluidic chip layout for droplet sorting.** Droplets entering the sorting chip via the droplet reinjection inlet (2) were equally spaced by the first oil inlet (1). At the sorting junction, a second oil flow (3) was used to transport sorted droplets to the dispensing unit via the sorting outlet (4). Unsorted droplets end up in the waste channel (5).

(B) **Time series demonstrating the sorting process.** Droplets (cyan color coded) containing cells of interest are detected by the laser line. For a positive evaluation of the fluorescent time trace, a trigger signal is generated, activating the electrodes, and moving the droplet into the sorting channel. The secondary oil flow efficiently transports the sorted droplets to the outlet of the chip and to the dispensing unit. Unwanted droplets (red color coded) end up in the waste channel. See also SI Video 1.

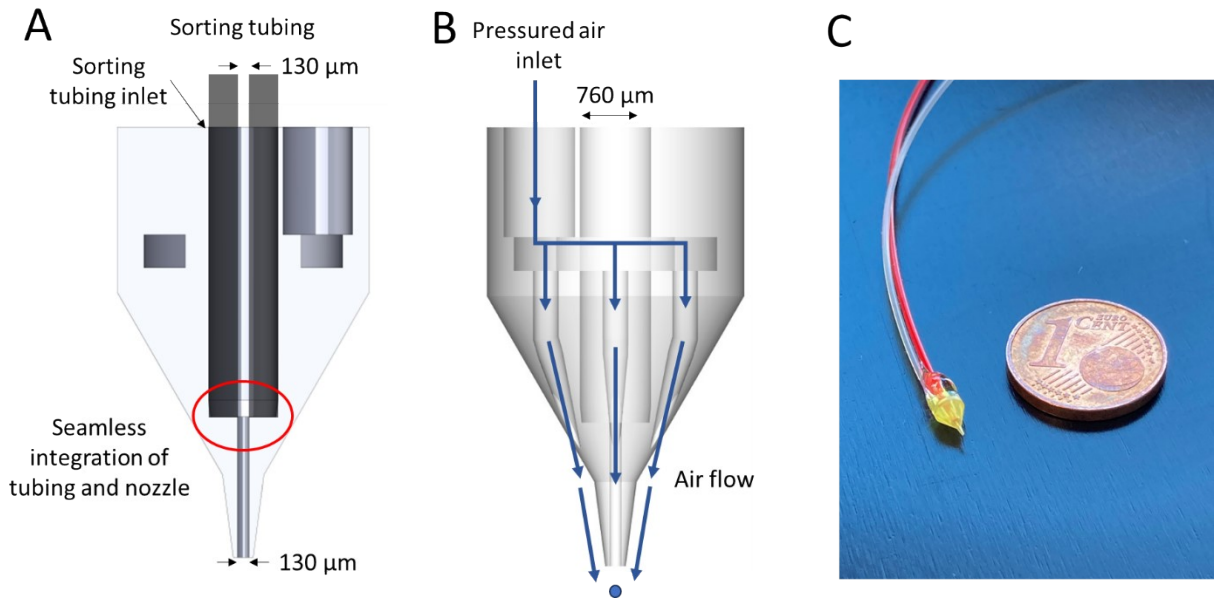

Fig. S2 Overview dispensing nozzle

(A) **Side view of the dispensing nozzle.** The dispensing nozzle has two inlets, one for the pressured air to generate a continuous air stream around the nozzle head and one for connecting the dispensing tubing. The inner diameter of the dispensing tubing (130 µm) fits seamlessly to the nozzle, ensuring minimal death volume and no droplet loss. The tapered nozzle tip supports break-off of generated oil drops.

(B) **Side view air stream.** Pressured air is guided into the nozzle and distributed between six axisymmetric air outlets, generating a continuous air stream at the nozzle tip. The arrows indicate the air stream inside the nozzle.

(C) **Photographic picture of assembled nozzle.** The 3-D printed nozzle is shown (yellow) together with the tubing for air (white tubing) and the dispensing tubing (red).

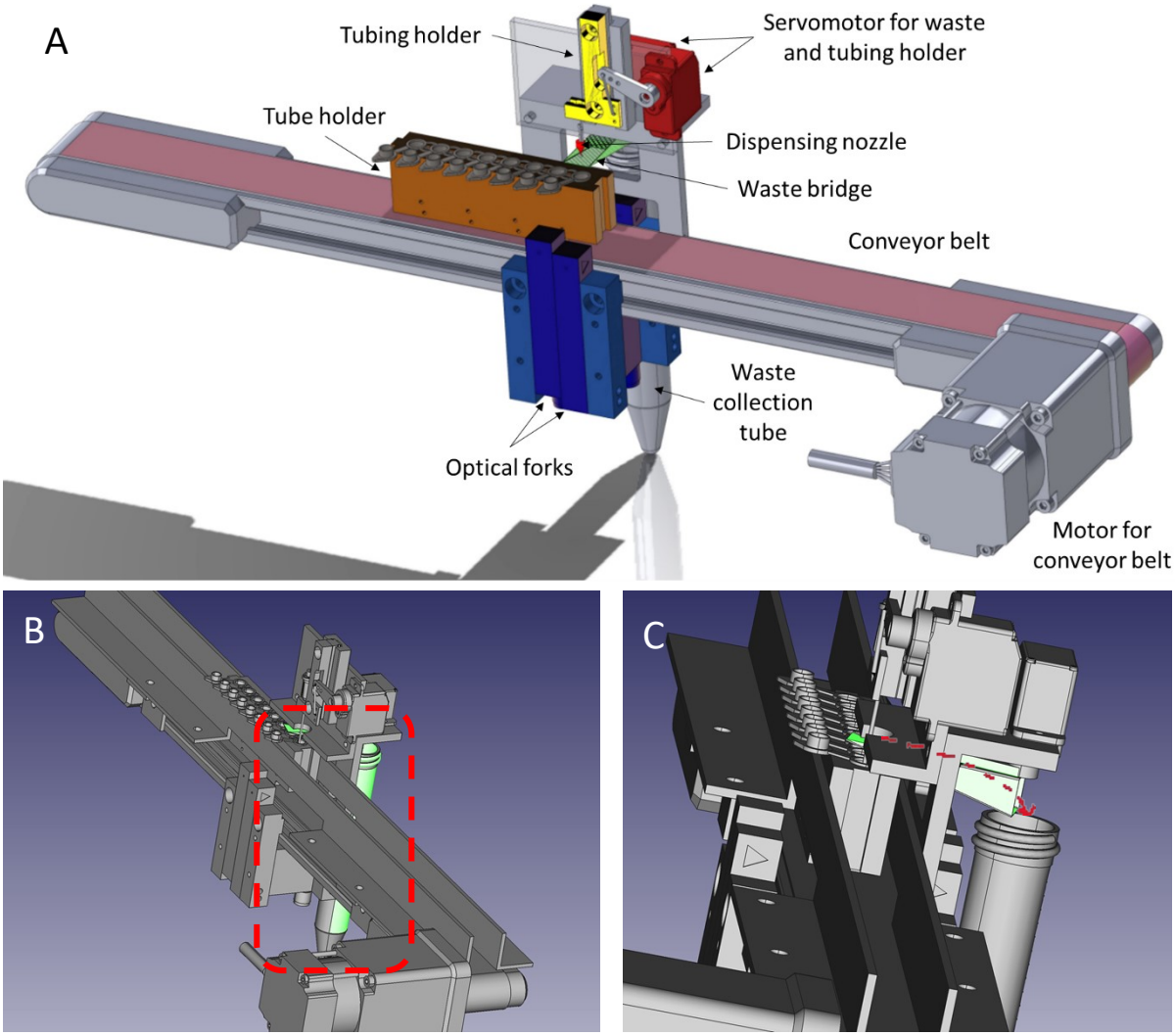

Fig. S3 Overview dispensing unit.

The motor of the conveyor belt positions the tube holder with inserted 8-strip PCR tubes under the dispensing nozzle. Optical forks are used to measure the position of the tube holder via through-holes. The waste bridge as it is shown in the figure is in the closed state, so that the oil that is continuously arriving at the nozzle ends up in the waste collection tube. Upon receiving a dispensing trigger signal the waste bridge is moved to the side and the tubing holder with the dispensing nozzle moves into the lower position to place the nozzle inside the tubing. After the dispensing time the dispensing nozzle is moved back into the upper position and the waste bridge is closed again. While the waste bridge is closed, all incoming oil is transferred via an oil bridge into a waste collection tube, see highlighted details in B and C.

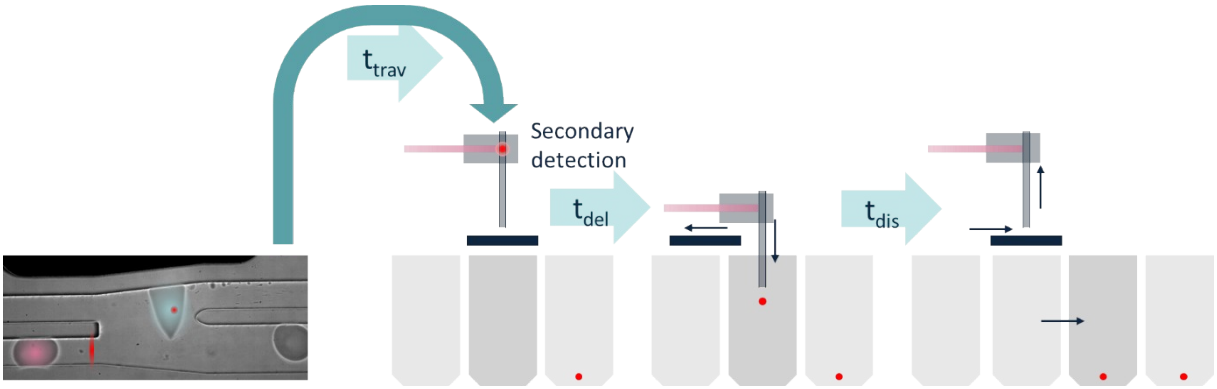

Fig. S4 Overview dispensing process. Transfer of sorted droplets to the dispensing unit. The droplet traveling time t_{trav} is the time from triggering the sorting electrodes on the sorting chip until the detection of the sorted droplet at the secondary detection. The secondary detection triggers the dispensing process. After the delay time t_{del} the waste bridge is moved out and the dispending holder is moved into the tube to ensure dispensing of the droplet into the tube. After the dispensing time t_{dis} the holder is moved into the upper position and the waste bridge is closed. This ensures minimal oil dispensing into the tube and avoids spill over of oil into neighboring tubes. Finally, the conveyor belt positions the next empty tube under the dispensing nozzle. See also SI Video 2.

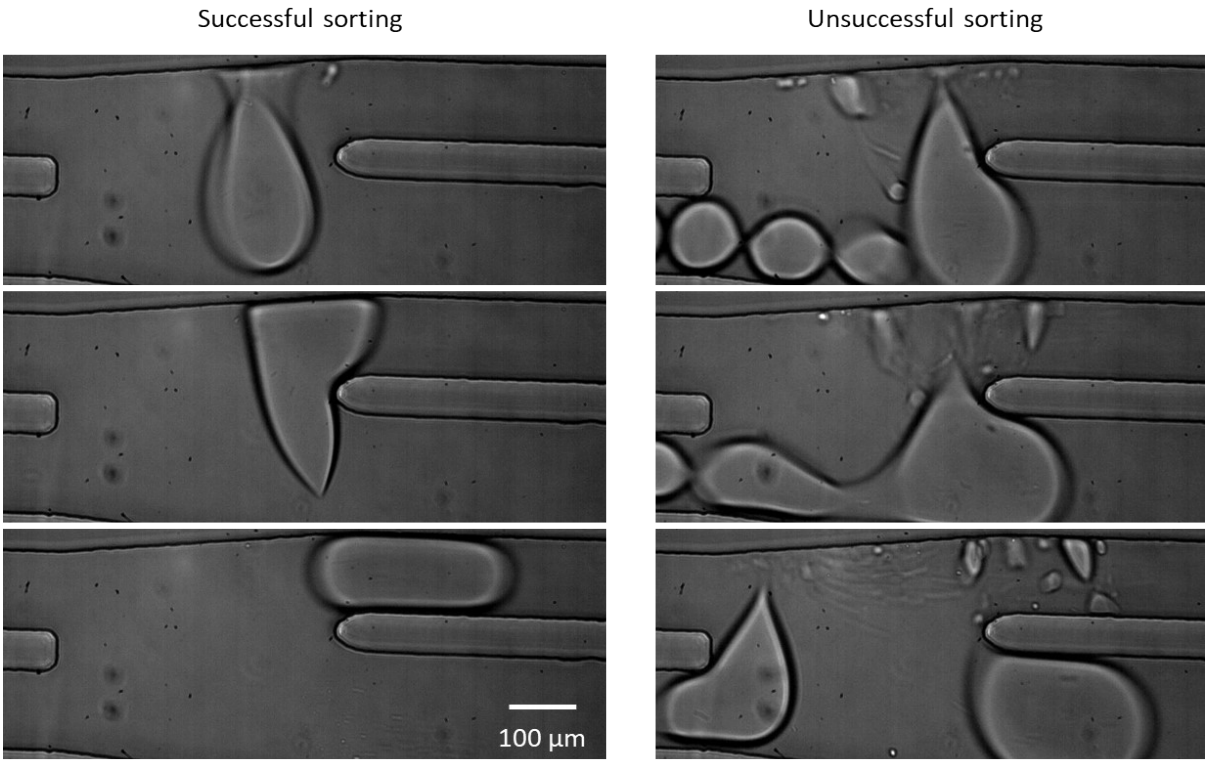

Fig. S5 Droplet sorting of a polydisperse droplet emulsion. Exemplary time frame images of a successful and an unsuccessful sorting event. In case of multiple droplets being in close contact in the presence of the applied electrical field the droplets merge and hinder the droplet to enter the sorting channel. This can be avoided by proper droplet spacing and pre-sorting of polydisperse droplets.

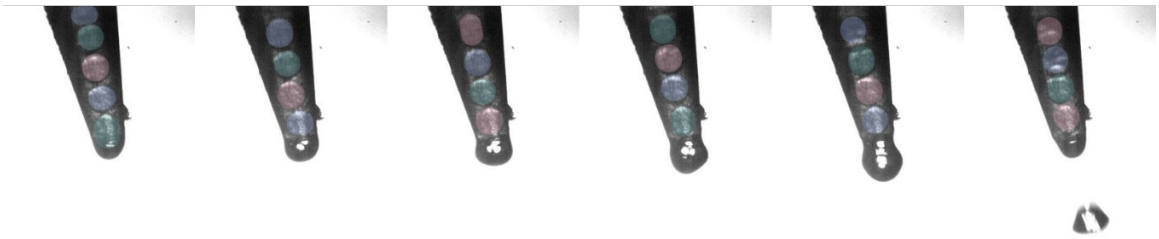

Fig. S6 Dispensing process to determine the volume of the dispensed drops. A continuous stream of droplets with 100 µm diameter was introduced into the dispensing nozzle. For the given conditions here, 5 subsequent droplets (color-coded for better visualization) entered the drop forming at the tip of the nozzle before the break-off. With this the size of the dispensing drop can be estimated.

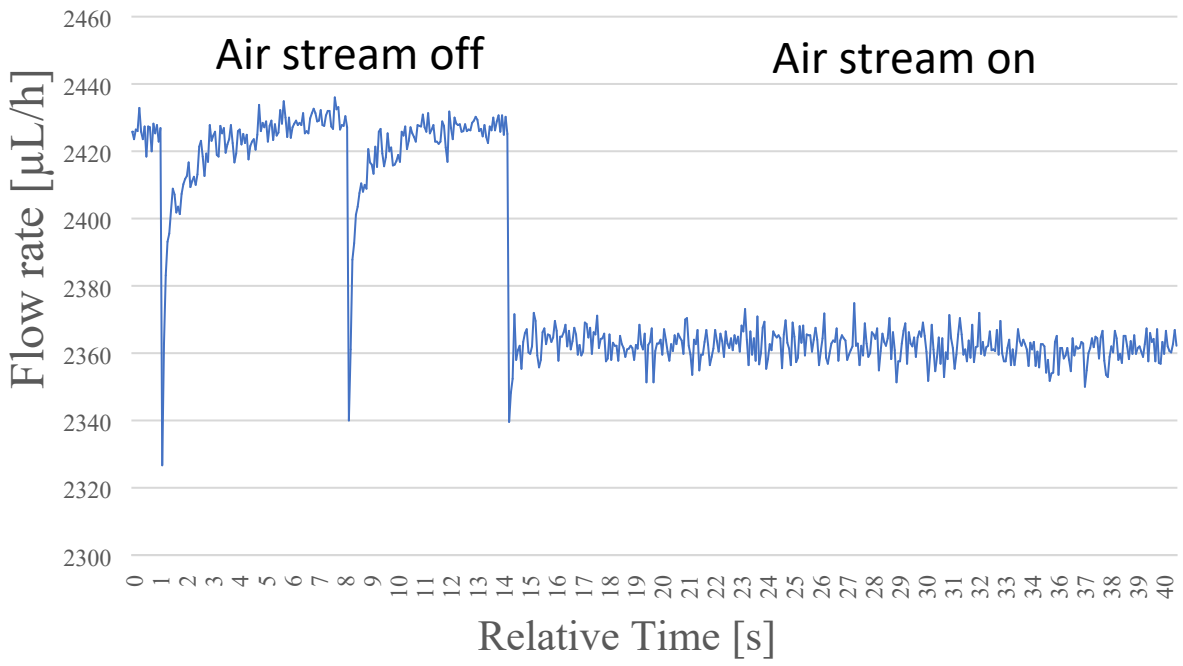

Fig. S7 Backpressure of dripping drop at nozzle tip. The flow rate of the oil flow inside the dispensing tubing is monitored over time. When the air stream at the dispensing nozzle is turned off, a large oil drop is formed at the nozzle tip. The break-off of this drop results in a decrease in the flow rate inside the dispensing tubing. This disturbance in the flow rate of the overall fluidic system can propagate to the sorting junction and could lead to false-positive sorting events. When the air stream is turned on, smaller drops are dispensed at a higher frequency leading to a reduced disturbance of the fluidic system. This makes the overall dispensing operation more robust and allows to integrate the dispensing unit to a microfluidic droplet sorting device.

Fig. S8 Dispensing efficiency of hybridoma cells. Single cells were sorted and dispensed into individual wells of a flat bottom 8-strip. After dispensing, strips were assembled into a 96-well plate, allowing to correlate the well number with the microfluidic sorting characteristics (e.g. A1 – first sorting event, H1 – 8th sorting event, A2 – 9th sorting event etc.). The plate was incubated for 48 h and analyzed by scanning the wells with a microscope for cell detection inside the individual wells. Green color indicates successful dispensing and cell proliferation (> 10 cells detected inside the well). Yellow color indicates successful dispensing but no cell proliferation (single cell in well). Red color indicates wells where no cells could be detected, i.e. unsuccessful sorting.

Supplementary Table

Citation	Technical complexity	sorting accuracy	Sorting rate	Deposition throughput	Deposition accuracy	scale up potential
J. Rutkauskaite et al 2022 [18]	High	97.5 %	500 Hz	200 cells/s (collection chamber)	No deposition	Not applicable
N. Shembekar et al 2018 [9]	Medium	220-fold enrichment	40 Hz	40 Hz (collection chamber)	80% cell viability	Not applicable
T. Weber et al 2022 [19]	High	99.8 %	5-6 Hz (video S1)	7.5 s per droplet	recovery rate of > 93% deposition efficiency 100%	deposition of upto a few hundred droplets claimed
R. H. Cole et al 2017 [22]	High	Not available	50–100 Hz	Deposition rate ∼4 Hz	98% single cell deposition	Very high
Q. Zhang et al 2017 [21]	low	94.4 ± 2.0%	0.6 Hz	20 s/cell	> 90%	Moderate
Hereby reported system	Medium	99.5 %	~20 Hz	1 droplet/s	100%	Combinable with 96- or 384-well plates

Supplementary Table S1. A comparison of droplet dispensing techniques reported in recent literature.

Supplementary Videos

Supplementary video 1: Microfluidic droplet sorting. Droplets arriving at the sorting junction pass by the droplet analysis spot where the fluorescence is measured. In case of a positive event, an electrical field is applied to the electrodes and droplets are pulled into the sorting channel. The secondary oil flow facilitates the efficient transportation of the sorted droplet into the dispensing unit. Negative droplets end up in the waste channel.

Supplementary video 2: Dispensing workflow. The conveyor belt moves the 8-stip holder until it reaches the position under the dispensing nozzle, as determined by the optical forks. Upon receiving a trigger signal from the secondary detection system, the oil bridge moves out and the dispensing nozzle moves into the lower position into the tube. The arriving droplet is dispensed into the desired tube. Subsequently, the nozzle moves back in the upper position and the waste bridge is closed to prevent any spillage of oil into the empty tubes. This process is shown in detail in the insert. Finally, the conveyor belt moves the holder and the next empty tube into the neighboring position, waiting for the trigger signal.

Supplementary video 3: Tip of the dispensing nozzle without air stream. Oil and droplets arriving at the tip of the nozzle accumulate into large drops of undefined size. Oil pining to the nozzle can be observed resulting in inhomogeneous break-off of the drops.

Supplementary video 4: Tip of the dispensing nozzle with air stream. Oil and droplets arriving at the tip of the nozzle break-off regularly due to the applied air stream. The size of the dispensed drops can be controlled by the flow of the air stream. Sorted microfluidic droplets with a diameter of 100 µm can be detected and are dispensed.

Supplementary video 5: Dispensing a continuous droplet stream. A continuous stream of microfluidic droplets with 100 µm diameter was generated and transferred into the sorting nozzle. In this sequence 5 subsequent droplets accumulate at the tip of the nozzle before the drop breaks off. This allows to estimate the size of the dispensed drops.