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Supplementary information

Design and construction of a microfluidics workstation for high-throughput multi-wavelength fluorescence and transmittance activated droplet analysis and sorting

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Design and construction of a microfluidics workstation for high-throughput multi-wavelength fluorescence and transmittance activated droplet analysis and sorting.

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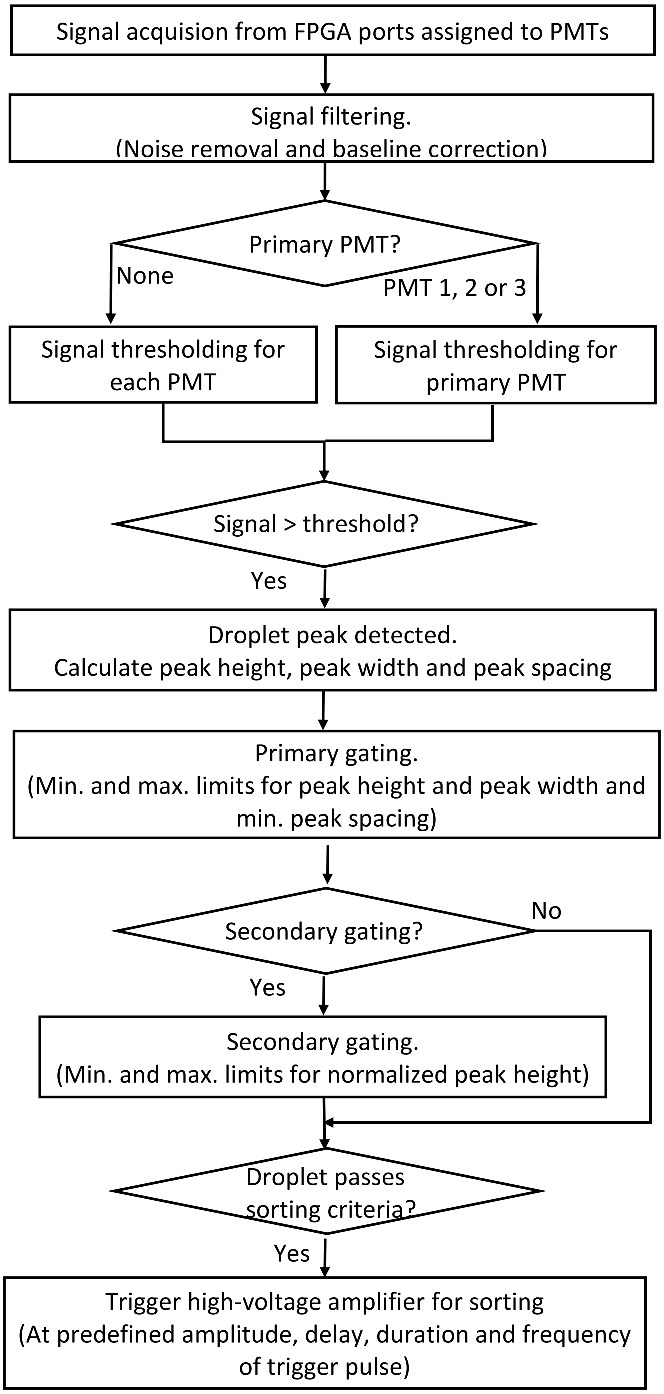
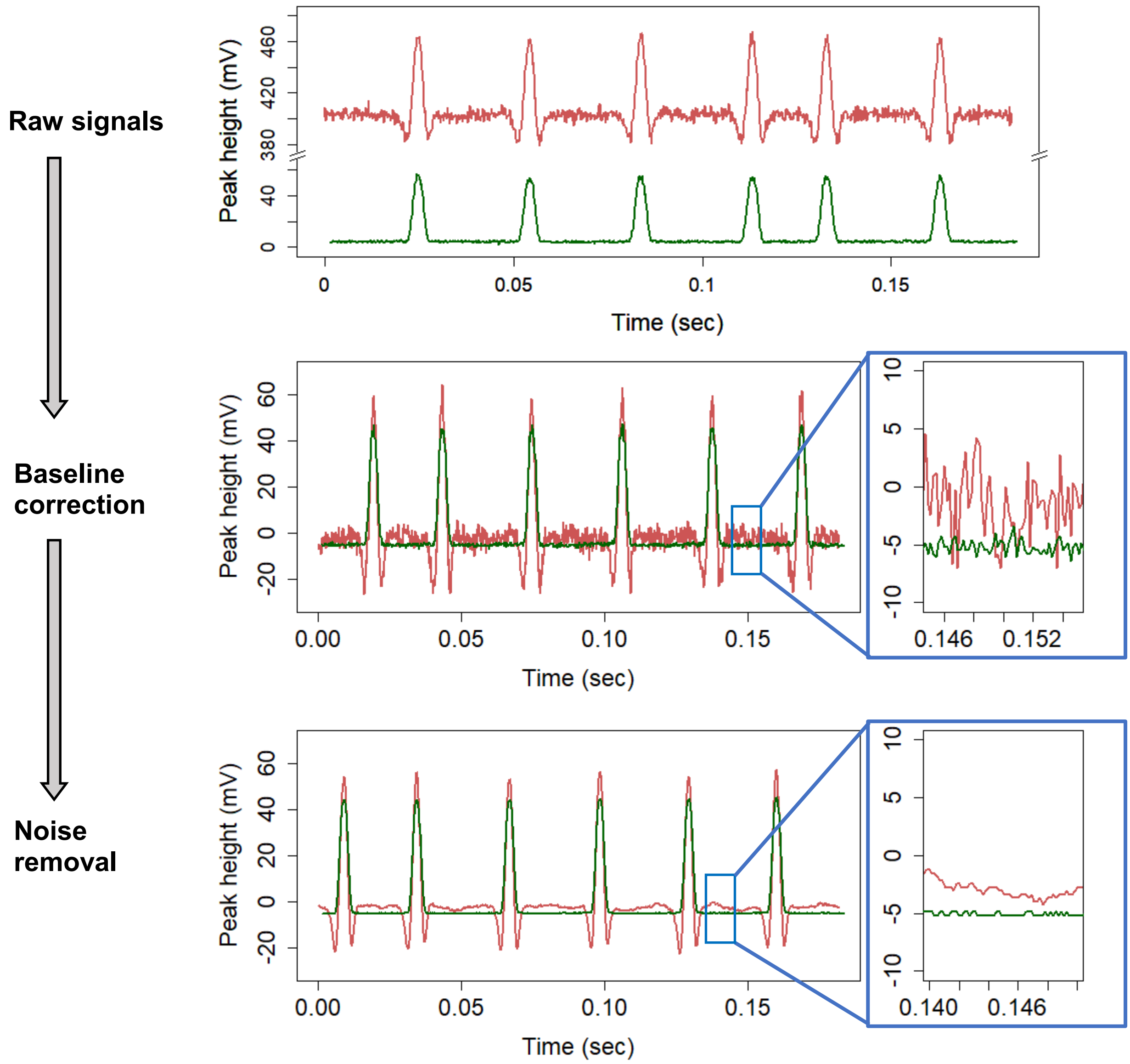
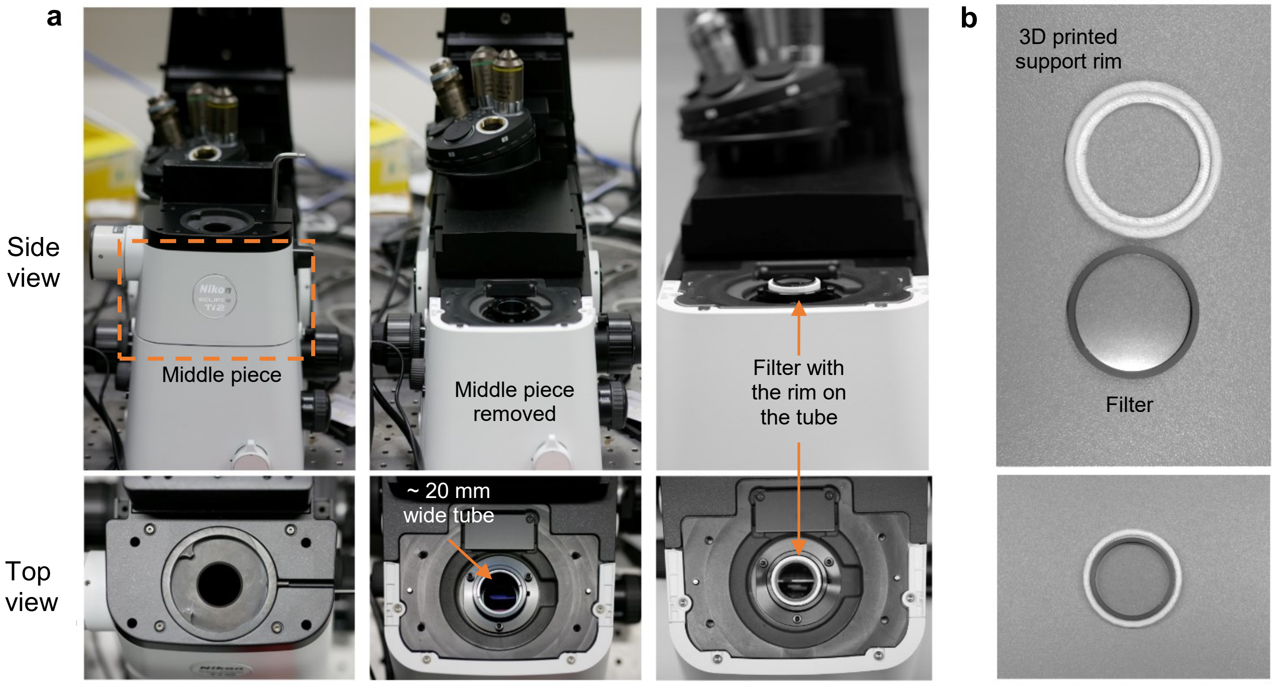
Supplementary figures



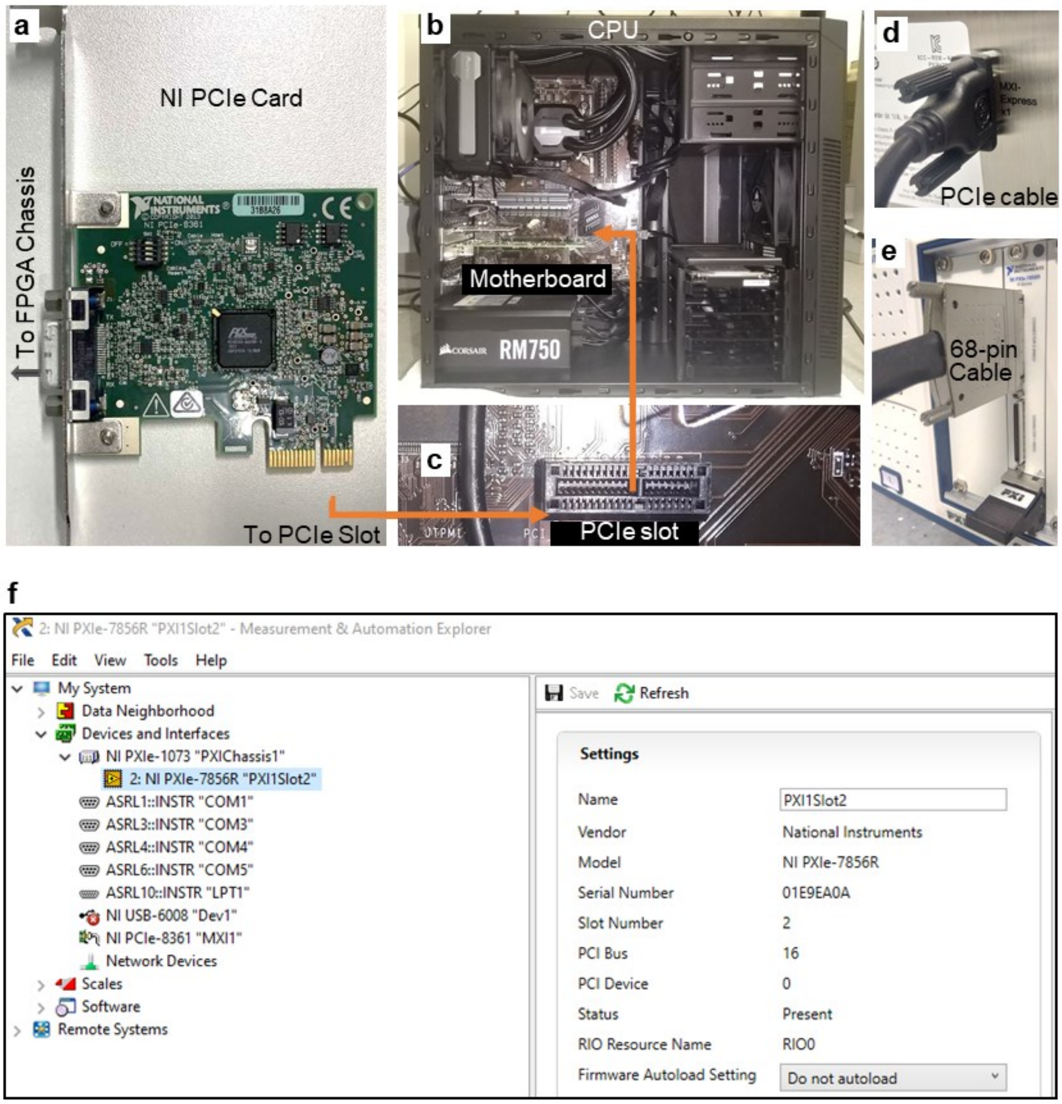
Supplementary Fig. 1. Algorithm to control droplet sorting operation. This algorithm is implemented to design a custom-made software with LabVIEW FPGA Module development environment and LabVIEW 2019 to control the sorting operation.



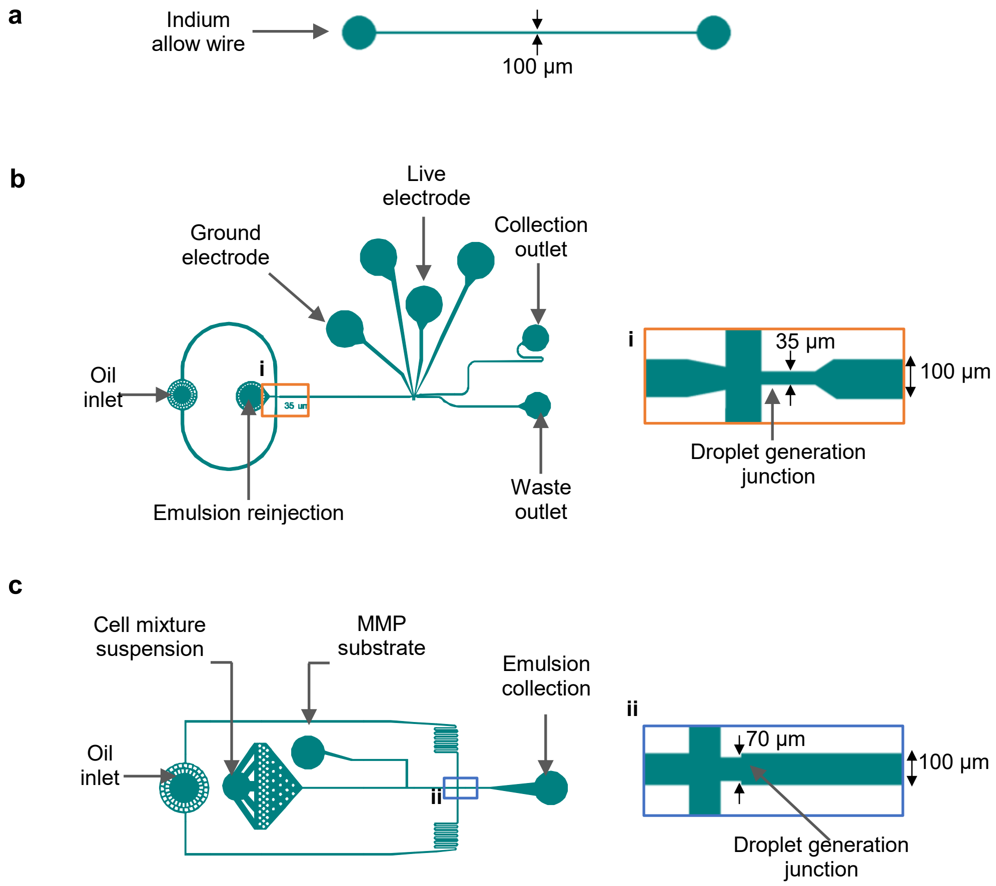
Supplementary Fig. 2. Signal processing by TFADS. Raw signals from separate PMTs have separate baselines as shown by green (PMT2) and red (PMT3) signal plots. A low-pass (at 0.001 Hz) filter is used to identify low frequency components to calculate the offset and to bring the baseline close to zero for each signal. After baseline correction, a high pass filter (at 1000 Hz) is used to remove high frequency noise from the signal.



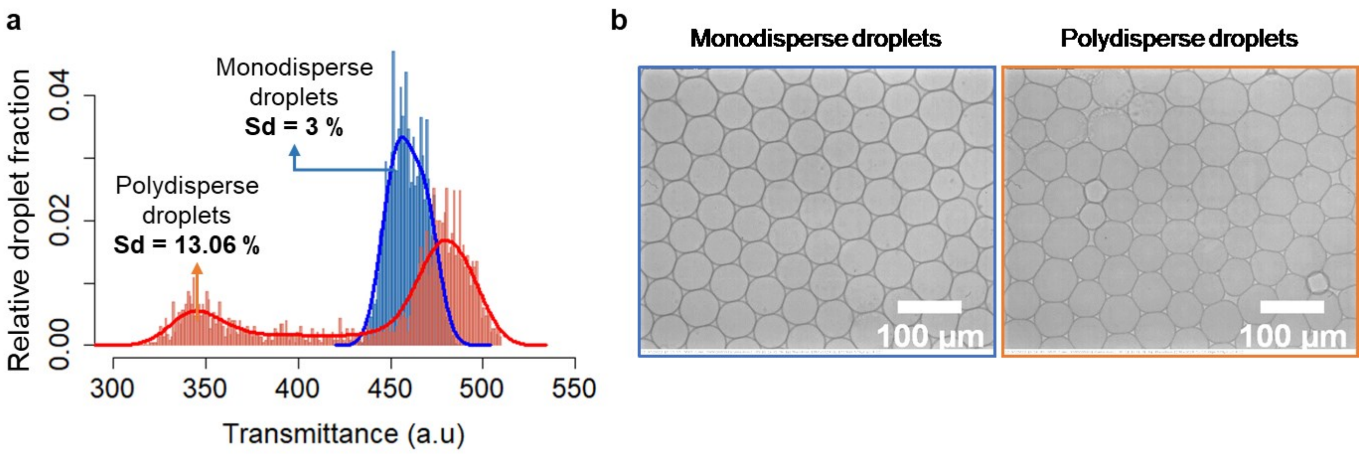
Supplementary Fig. 3. Triple-band pass filter installation. a) Front and top view of microscope during the filter mounting process. (i) Microscope after the removal of eyepiece and stage. Top view shows Allen key position to remove the eyepiece. Front view shows the Allen key to remove the central piece and gain access to middle piece. (ii) Microscope with middle piece removed. Top view shows the tube where the filter will be placed. (iii) Filter with the filter rim placed over the tube. b) Triple band pass filter is inserted into the 3D printed support rim/adapter (corresponding STL files can be found in Source Data 11) to fit properly into the microscope's tube with the arrow pointing upwards.



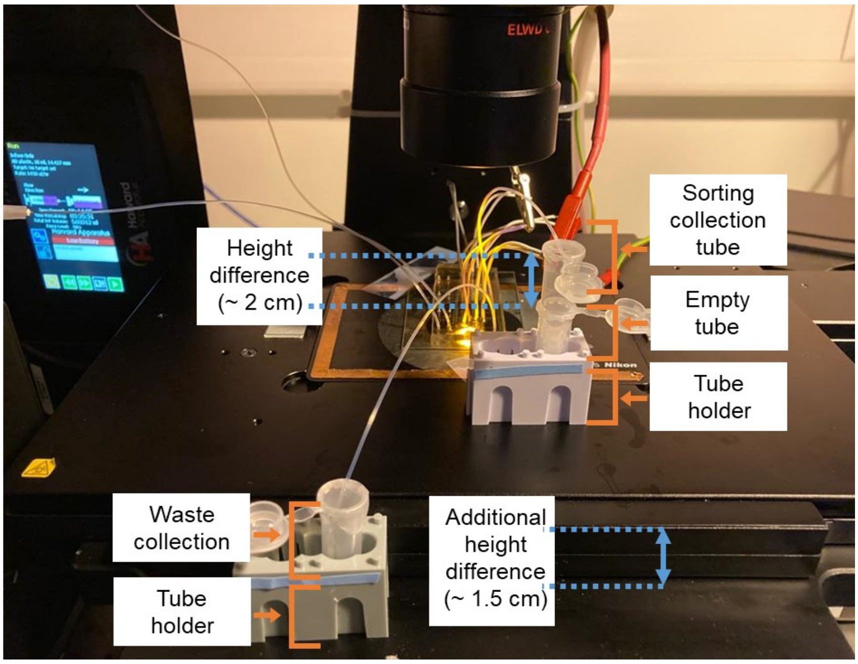
Supplementary Fig. 4. Computational setup. a) NI PCIe card to be installed on the CPU motherboard. b) Motherboard of the CPU with empty PCIe slots. c) Image of empty PCIe slot. d) PCIe cable from the CPU connected to the its port on the rear panel of FPGA module. e) A shielded 68-pin cable, SHC68-68-RDIO [Part 49] connected to the Connector 0 (RMIO) port of the FPGA module. f) Screenshot of NIMAX program highlighting the detection of “NI PXIe 7856R” module in the slot 2 of “NI PXIe-1073” chassis.



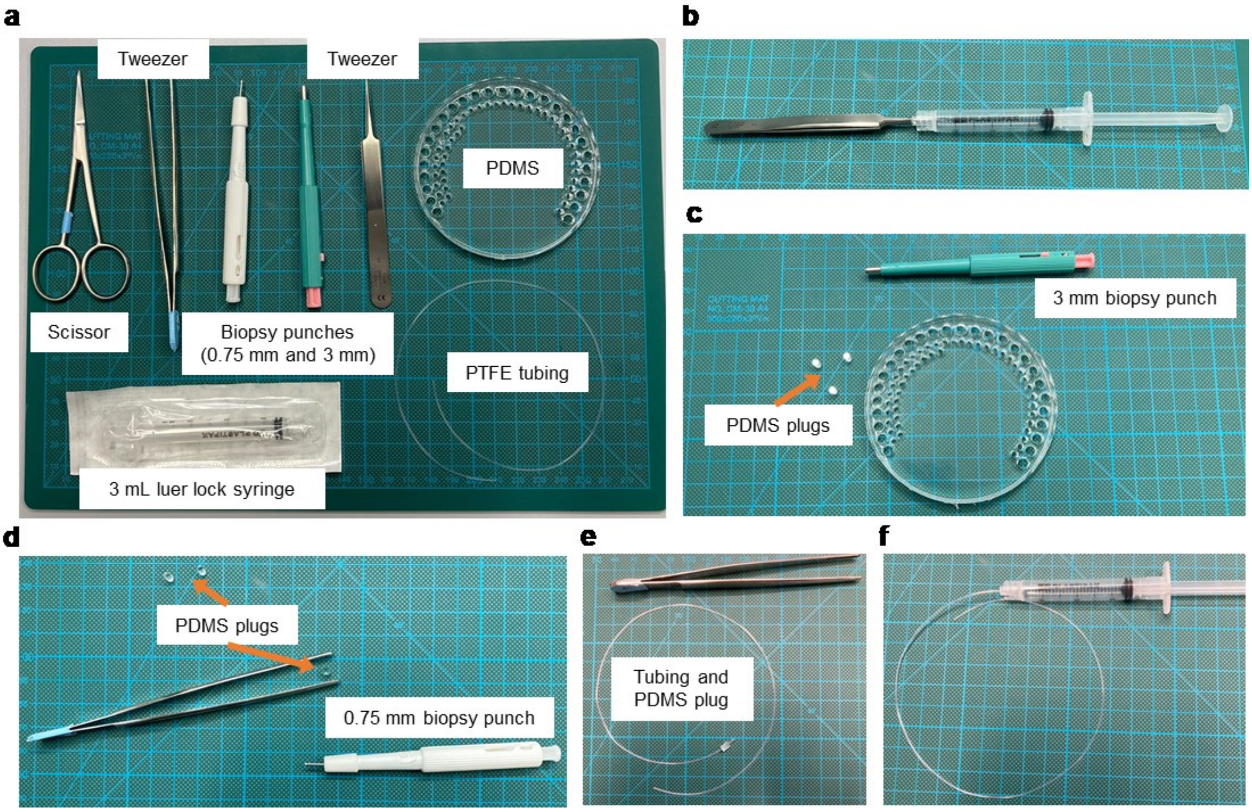
Supplementary Fig. 5. Microfluidic chip designs. a) Design of the alignment line that is used for emission light alignment. **b)** Design of the droplet sorting chip with on-chip droplet generation. The same chip can be used for droplet re-injection as well. **c)** Droplet generator chip with two aqueous inlets (one for MMP substrate and one for cell mixture) used for single-cell encapsulation.



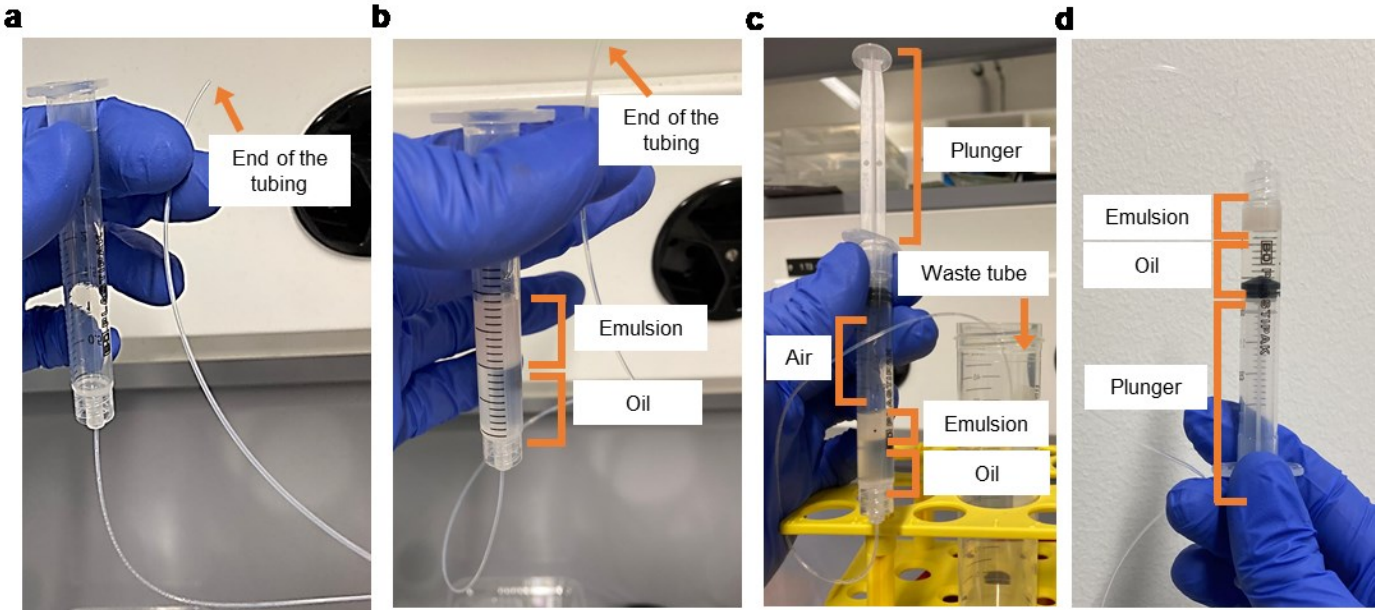
Supplementary Fig. 6. Effect of droplet polydispersity on transmittance signals. a) Transmittance intensity distribution for monodisperse (in blue) and polydisperse droplet populations (in red). The transmittance signals for polydisperse emulsions are showing a higher standard deviation (Sd = 13.06 %) as compared to the monodisperse population (Sd = 3 %) (Raw data in **Supplementary Data 11**). **b)** Microscopic images of the two droplet populations used for the transmittance measurements.



Supplementary Fig. 7. Maintaining height difference between the sorting and waste collection tube. The Sorting collection tube is kept at a slightly higher position by keeping it on top of an empty tube to ensure all the droplets are going to the waste channel by default, prior to initiate sorting operation. Additional height difference can also be obtained by keeping the waste collection tube slightly below the stage.



Supplementary Fig. 8. Emulsion reinjection syringe fabrication. a) Material to fabricate a needle-free syringe which includes scissors, biopsy punches (0.75 mm and 3 mm), tweezers, 3 mL luer lock syringe, Poly(dimethyl siloxane) (PDMS), and PTFE tubing. **b)** Enlarge the syringe outlet with a tweezer. **c)** Make PDMS plugs using a 3 mm biopsy punch. **d)** Make a hole in the PDMS plug with the 0.75 mm biopsy punch. **e)** Tubing inserted into punched PDMS plug. **f)** Tubing and PDMS plug are inserted into the syringe to achieve a re-injection needle-free syringe.



Supplementary Fig. 9. Emulsion loading in the syringe. a) Plunger removed and the end of tubing is maintained at the same level as the syringe body. **b)** Emulsion is poured into the syringe; the emulsion will stack above the oil. **c)** The tubing is placed into wasted collection tube and the plunger is inserted back into the syringe. **d)** The syringe is turned upside down to allow the emulsion to stack at the syringe outlet. The extra-air is carefully removed by gently pushing the plunger.