ELECTRONIC SUPPLEMENTARY INFORMATION FOR:

CotA laccase: high-throughput manipulation and analysis of recombinant enzyme libraries expressed in *E. coli* using droplet-based microfluidics

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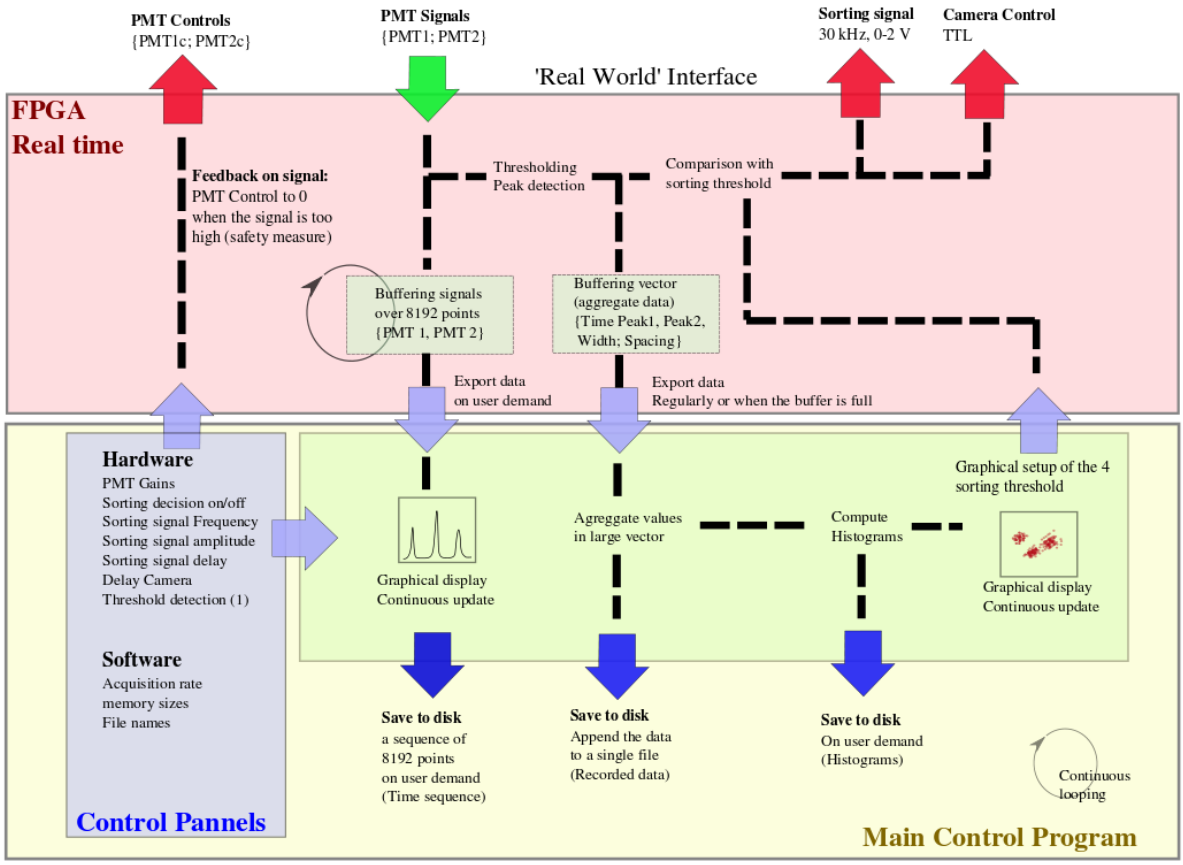

Fig. S1 Scheme of the program used for the droplet analysis. This scheme was implemented on a NI FPGA system. In principle, such system could be implemented on any microcontroller having sufficient memory for the storage of the data. The controls and analysis of the data are performed at typical computer speed (refreshing / loop rate of the order of 1s) while the real time computing (thresholding / comparison) is easily implemented using simple algorithms on the real time board. All the exported data are ascii files for post-processing.

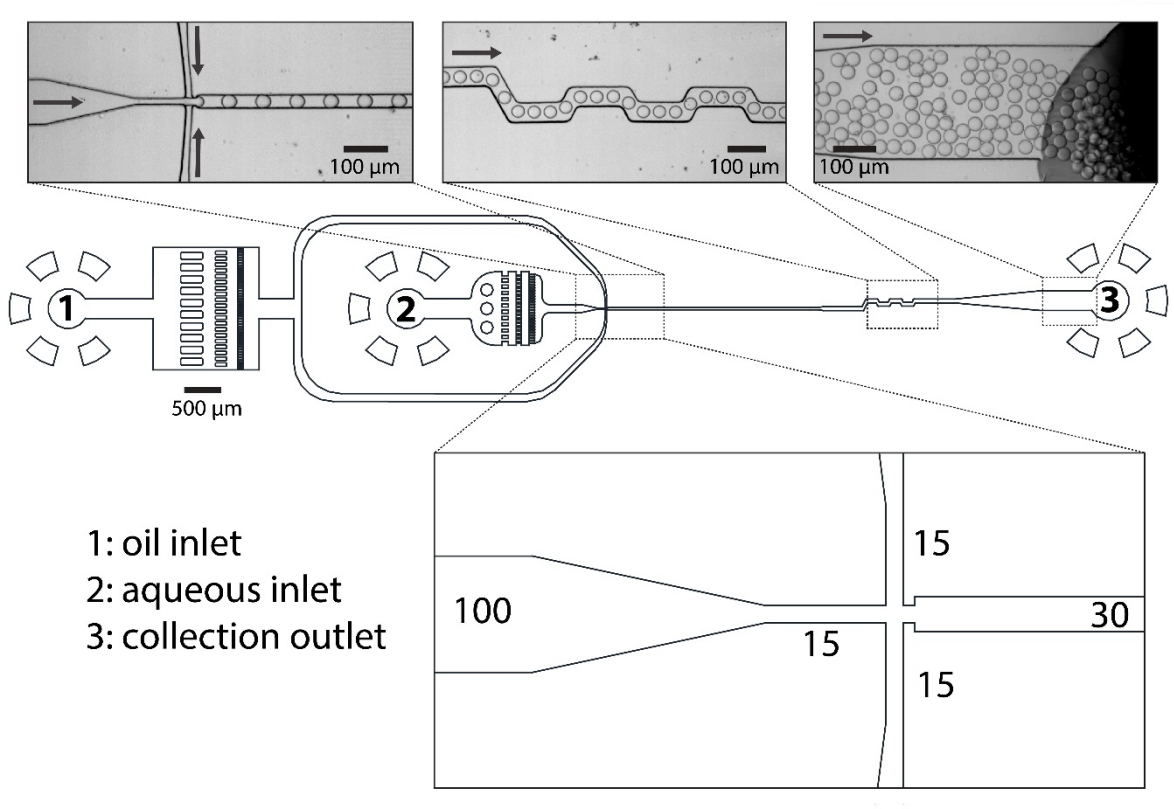

Fig. S2 Design of the dropmaker module: the module is composed of two inlets (oil and aqueous) and one collection outlet. Droplets are formed by flow-focusing of the aqueous stream by two streams of fluorinated oil containing surfactant. Typical flow rates are 80 µl/h for the aqueous inlet and 190µl/h for the oil inlet. Dimensions of the droplet production nozzle are specified in microns. Pillars are used as filters at each inlet. The channel depth is 25 µm.

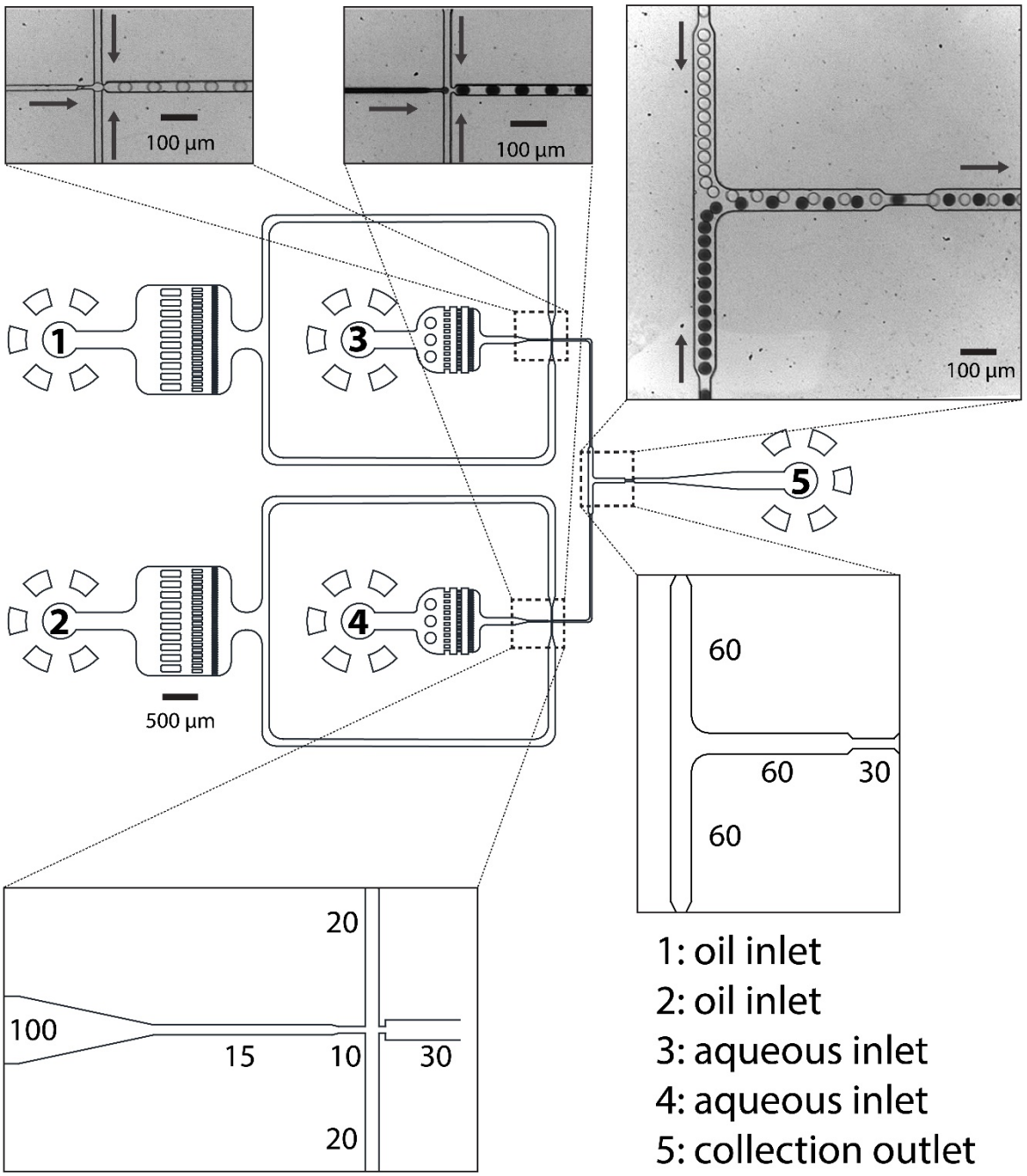

Fig. S3 Design of the dual-dropmaker module: the module is composed of two dropmakers module in parallel (each one having two inlets, oil and aqueous) leading to the same collection outlet. Droplets are formed in each dropmaker by flow-focusing of the aqueous stream by two streams of fluorinated oil containing surfactant. Typical flow rates are 80 µl/h for the aqueous inlets and 190µl/h for the oil inlets. Dimensions of the droplet production nozzles and junction channel are specified in microns. Pillars are used as filters at each inlet. The channel depth is 25 µm.

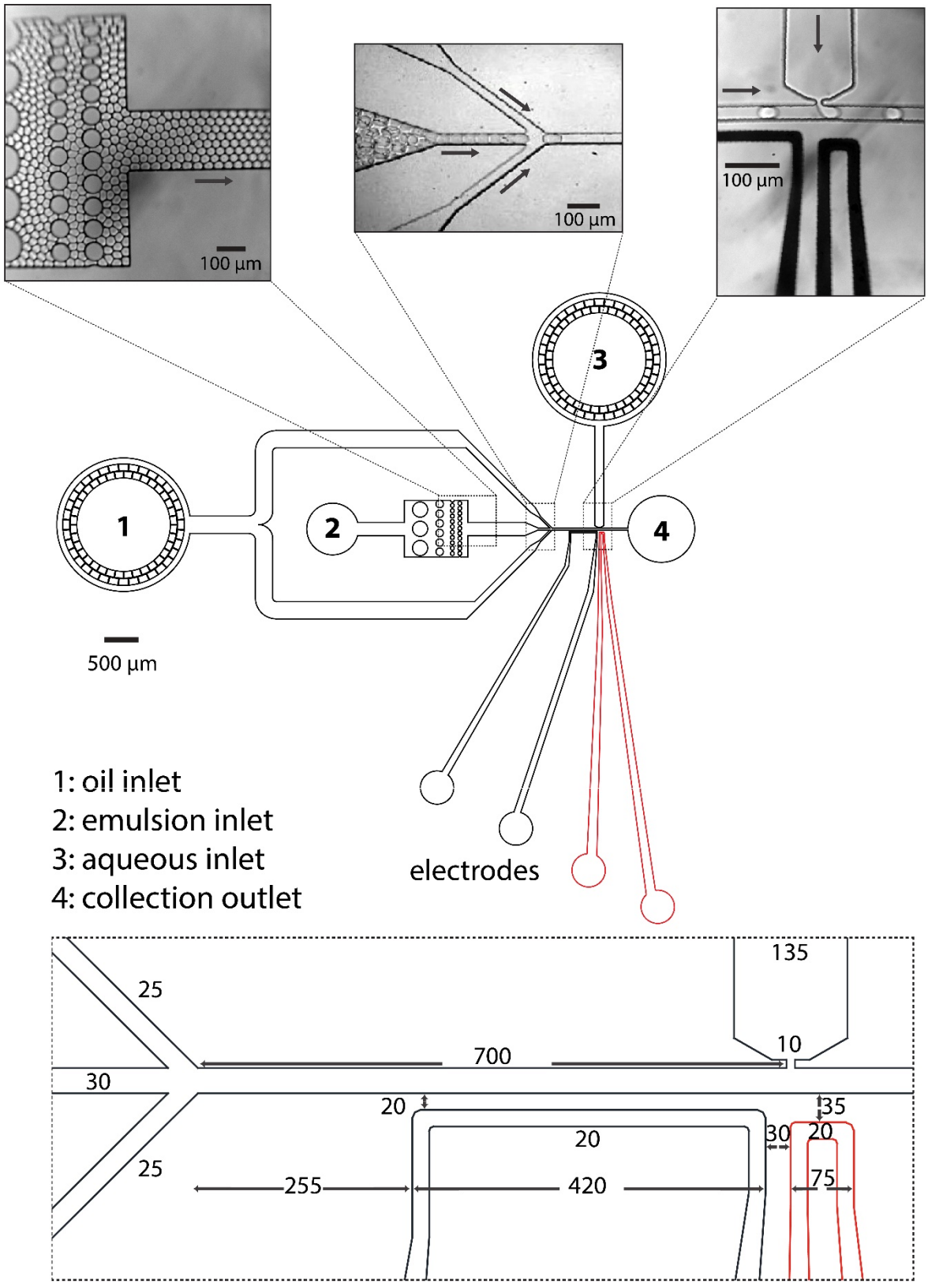

Fig. S4 Design of the picoinjection module: the module is composed of three inlets (oil, emulsion and aqueous) and one collection outlet. Emulsion is loaded in the emulsion inlet and droplets are spaced by two streams of fluorinated oil. The picoinjected phase is loaded in the aqueous inlet and encounters perpendicularly the main channel, facing a pair of positive (red) and negative (black) electrodes. The aqueous phase is injected each time a droplet is passing in front of the inlet by applying an AC field (20 kHz; 200 V) across the electrodes. Typical flow rates are 70-160 µl/h for the emulsion inlet, 200-600 µl/h for the oil inlet and 20-100 µl/h for the aqueous inlet. Dimensions of the channels and electrodes are specified in microns. Pillars are used as filters at each inlet. The channel depth is 25 µm.

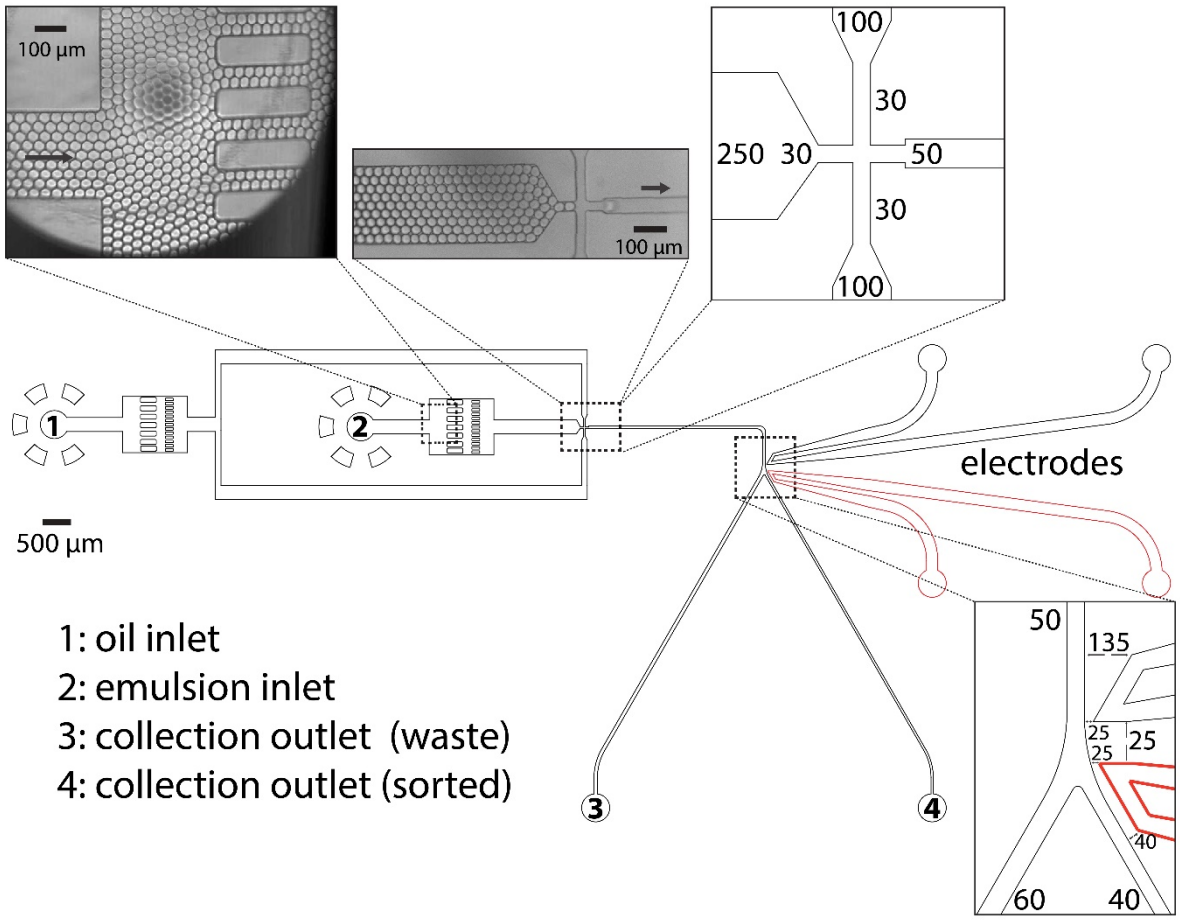

Fig. S5 Design of the FADS module: the module is composed of two inlets (oil and emulsion) and two collection outlets (waste and sorted). Emulsion is loaded in the emulsion inlet and droplets are spaced out by two streams of fluorinated oil. Droplets are flowing passively to the collection outlet channel having a lower hydrodynamic resistance (waste). Droplets fluorescence is analyzed by the optical setup at the Y-shaped junction and the droplets of interest are deflected into the positive collection outlet channel (sorted) by applying AC field pulses (30 kHz; 1000-1400 V; 0.5ms). Typical flow rates are 20-70 µl/h for the emulsion inlet and 500-1000 µl/h for the oil inlet. Dimensions of the droplets reloading nozzle and the Y-shaped junction are specified in microns. Pillars are used as filters at each inlet. The channel depth is 25 µm.

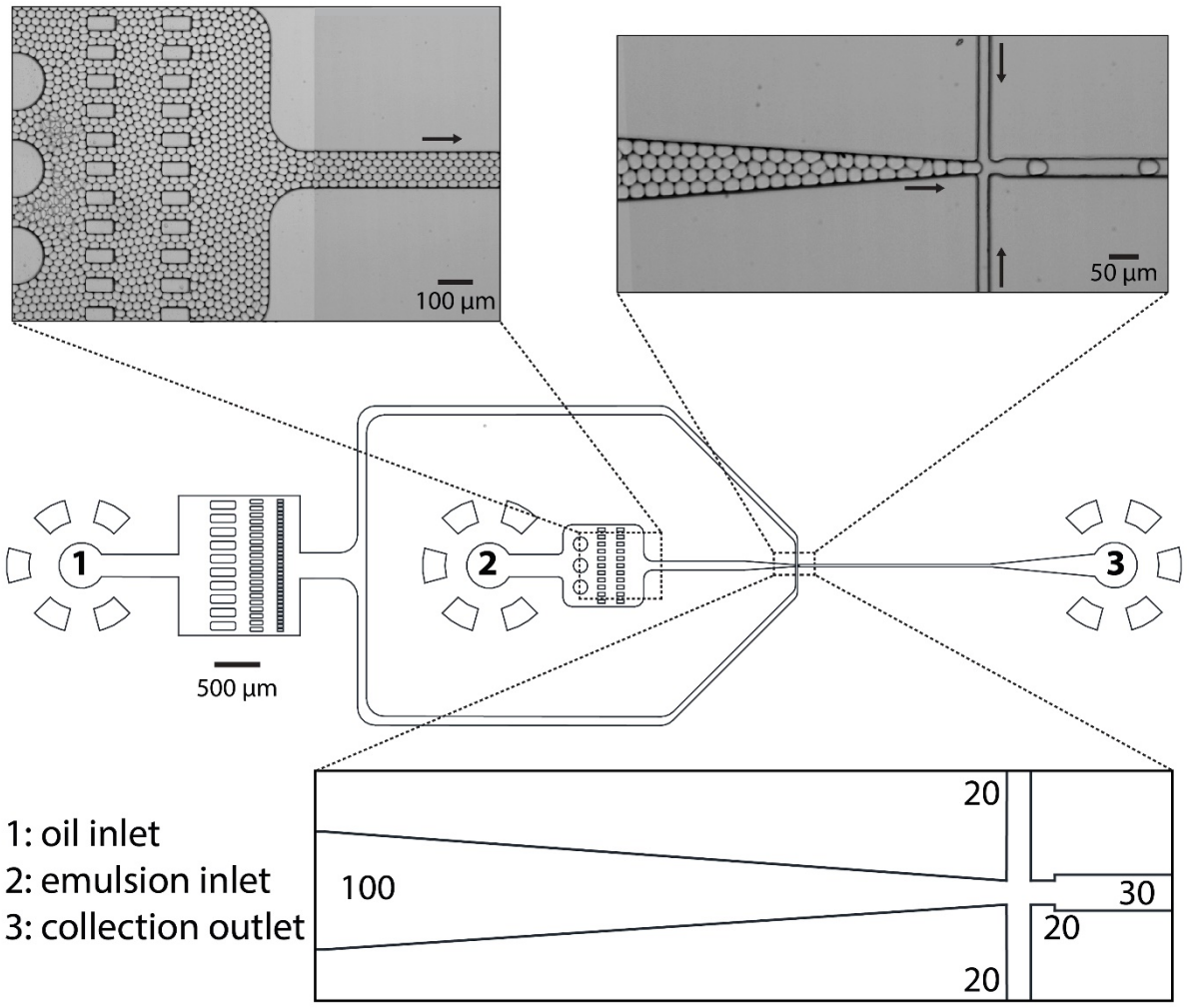

Fig. S6 Design of the reloading module: the module is composed of two inlets (oil and emulsion) and one collection outlet. Emulsion is loaded in the emulsion inlet, droplets are spaced out by two streams of fluorinated oil and droplets fluorescence is analyzed by the optical set up. Typical flow rates are 50-150 µl/h for the emulsion inlet and 200-400 µl/h for the oil inlet. Dimensions of the droplets reloading nozzle are specified in microns. Pillars are used as filters at each inlet. The channel depth is 25 µm.