Supplementary Information:

A flow focusing microfluidic device with an integrated Coulter particle counter for production, counting and size characterization of monodisperse microbubbles.

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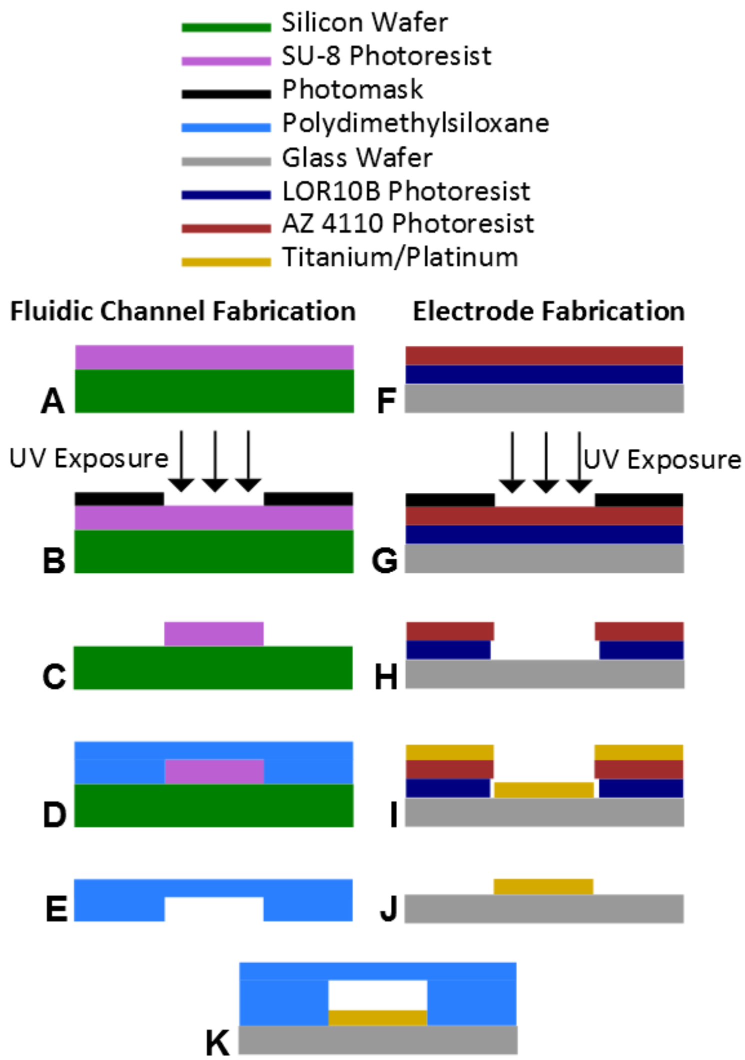


S1 Microfabrication techniques used to fabricate the FFMD-µCPC. The fluidic channels are formed by (A) applying a negative photoresist, SU-8 3025, to a silicon wafer and (B,C) performing photolithography. Subsequently, (D,E) soft lithography is performed using PDMS. The electrodes are constructed by (F) applying positive photoresists LOR 10B and AZ 4110 sequentially to a glass wafer, followed by (G,H) photolithography, (I) electron beam evaporation and (J) bi-layer lift off. The PDMS mold containing the fluidic channels (E) is then aligned to the electrodes contained on the glass wafer and they are (K) bonded to create the end device.

Finite Element Analysis

The microbubble used in this simulation was formed by creating a sphere of given diameter and using the difference feature to subtract the sphere from the simulation environment. Thus, the simulated microbubble is rigid, hollow, and does not feature a shell. A step size of 2.5 µm was used to advance the microbubble down the channel. The step size was determined to be an equitable compromise between fine spatial resolution and computational speed.

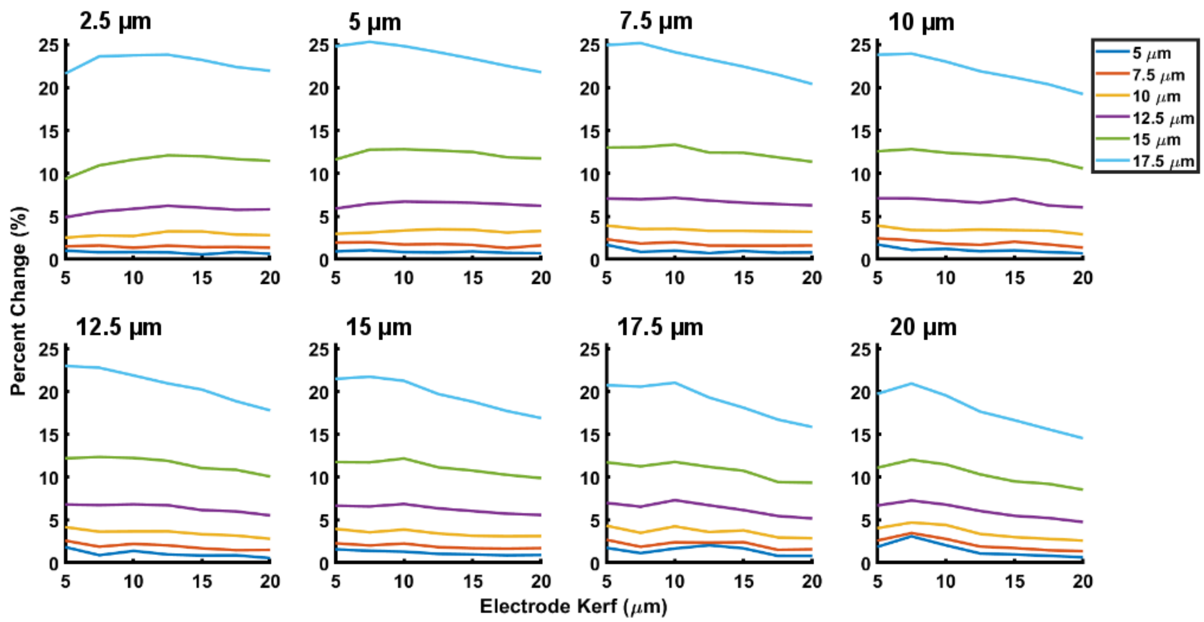
The simulated geometry featured free tetrahedral mesh elements constrained to a user-controlled setting of 'extra fine', in which the minimum element size was 0.54 µm and maximum element size was 12.6 µm. Electrodes were designed to be offset ~ 500 nm from the edges of the simulation environment to avoid internal errors and premature breakdown of the simulation caused by the minimum element size not being able to 'fill' that area. The walls of the simulation environment were all defined as insulating except for the electrodes which were identified as either the ground or terminal electrode. The study aimed to solve the following domain equations:

$$\nabla \cdot \boldsymbol{J} = \boldsymbol{Q_j}$$

$$\boldsymbol{J} = \boldsymbol{\sigma E} + \boldsymbol{J_e}$$

$$\boldsymbol{E} = -\nabla \cdot \boldsymbol{V}$$

A numerical solution was determined with an iterative solver that used conjugate gradients method to solve the differential equations. Convergence to a given tolerance of error was used as the termination technique for each step.



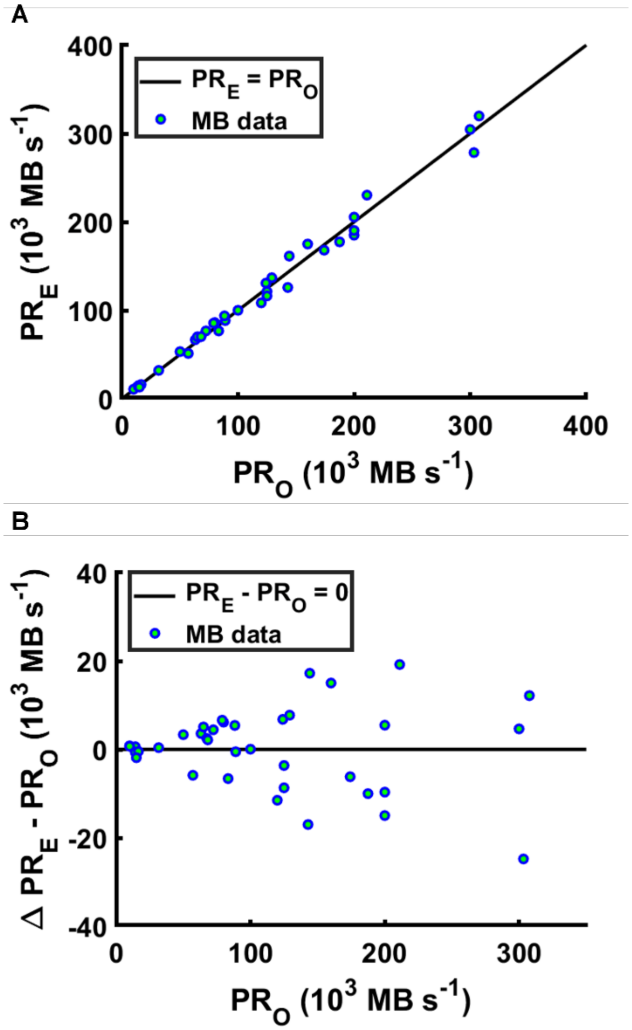
S2 Numerical studies simulating maximum impedance perturbation for single microbubbles traversing various electrode configurations. A maximum impedance perturbation is plotted as a percent change across a range of electrode kerfs. Electrode width is provided in the upper left of each subfigure. Each curve represents a single microbubble of a given diameter (5, 7.5, 10, 12.5, 15, 17.5 µm).

T1 Impedance change (%) for microbubbles across a range of diameters given different combinations of electrode width and spacing.

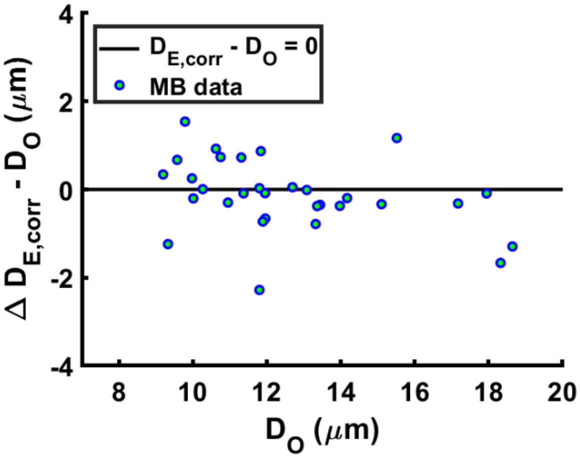
Impedance change (%) for MBs transiting at midheight of channel (z = 10)							
Electrode Dimensions (µm)		Microbubble Diameter (µm)					
Width	Space (kerf)	**5**	**7.5**	**10**	**12.5**	**15**	**17.5**
2.5	5	1.01	1.50	2.53	4.89	9.34	21.60
	7.5	0.83	1.62	2.79	5.56	10.93	23.61
	10	0.84	1.35	2.72	5.88	11.60	23.73
	12.5	0.81	1.60	3.25	6.23	12.09	23.80
	15	0.57	1.43	3.24	6.01	12.00	23.19
	17.5	0.84	1.45	2.88	5.77	11.66	22.37
	20	0.66	1.36	2.81	5.82	11.46	21.93
5	5	0.92	1.95	2.97	5.91	11.61	24.78
	7.5	1.07	2.00	3.12	6.47	12.76	25.30
	10	0.85	1.72	3.36	6.73	12.81	24.80
	12.5	0.80	1.79	3.51	6.66	12.66	24.09
	15	0.92	1.68	3.44	6.58	12.49	23.33
	17.5	0.75	1.32	3.12	6.41	11.87	22.50
	20	0.72	1.63	3.31	6.22	11.74	21.77
7.5	5	1.70	2.34	3.94	7.08	13.00	24.92
	7.5	0.87	1.84	3.53	6.98	13.04	25.15
	10	1.01	2.00	3.55	7.16	13.35	24.10
	12.5	0.72	1.61	3.32	6.84	12.42	23.25
	15	0.97	1.60	3.32	6.59	12.40	22.42
	17.5	0.77	1.60	3.25	6.42	11.86	21.47
	20	0.81	1.62	3.20	6.29	11.36	20.40
10	5	1.73	2.45	3.93	7.11	12.57	23.79
	7.5	1.09	2.21	3.40	7.10	12.82	23.93
	10	1.22	1.82	3.36	6.86	12.40	23.00
	12.5	0.94	1.68	3.46	6.58	12.17	21.88
	15	1.05	2.06	3.39	7.04	11.90	21.15
	17.5	0.84	1.73	3.34	6.27	11.52	20.35
	20	0.71	1.37	2.91	6.04	10.57	19.22

T1 (continued) Impedance change (%) for microbubbles across a range of diameters given different combinations of electrode width and spacing.

Impedance change (%) for MBs transiting at midheight of channel (z = 10)							
Electrode Dimensions (µm)		Microbubble Diameter (µm)					
Width	Space (kerf)	**5**	**7.5**	**10**	**12.5**	**15**	**17.5**
12.5	5	1.85	2.60	4.18	6.82	12.21	22.98
	7.5	0.90	1.89	3.64	6.75	12.35	22.77
	10	1.40	2.22	3.67	6.83	12.25	21.87
	12.5	0.98	2.05	3.68	6.73	11.91	20.94
	15	0.87	1.70	3.35	6.16	11.05	20.22
	17.5	0.89	1.49	3.19	6.01	10.86	18.86
	20	0.56	1.52	2.81	5.54	10.07	17.79
15	5	1.58	2.29	3.97	6.67	11.76	21.47
	7.5	1.41	2.04	3.57	6.59	11.73	21.72
	10	1.31	2.26	3.89	6.87	12.18	21.24
	12.5	1.06	1.84	3.43	6.36	11.15	19.70
	15	0.98	1.71	3.17	6.05	10.77	18.81
	17.5	0.89	1.67	3.10	5.74	10.27	17.72
	20	0.93	1.72	3.13	5.60	9.89	16.90
17.5	5	1.76	2.70	4.34	7.00	11.74	20.73
	7.5	1.14	1.88	3.51	6.56	11.27	20.57
	10	1.68	2.41	4.26	7.32	11.78	21.02
	12.5	2.06	2.37	3.60	6.72	11.20	19.29
	15	1.70	2.42	3.79	6.16	10.75	18.11
	17.5	0.82	1.52	2.97	5.47	9.44	16.72
	20	0.81	1.58	2.87	5.19	9.36	15.86
20	5	1.87	2.60	4.05	6.70	11.10	19.72
	7.5	3.10	3.48	4.71	7.28	12.03	20.92
	10	2.06	2.80	4.43	6.80	11.49	19.54
	12.5	1.10	1.91	3.38	6.06	10.33	17.65
	15	0.98	1.73	3.01	5.49	9.51	16.65
	17.5	0.83	1.48	2.80	5.24	9.22	15.58
	20	0.66	1.36	2.61	4.77	8.54	14.54



S3 Plots showing the optical production rate (PR_O) versus the electrical production rate (PR_E). (A) Comparison of methods to determine production rate. Optically determined production rate spans the x axis and electrically determined production rates the y axis. The black line is y=x and data points on that line demonstrate excellent agreement. (B) Residuals of the production rates are plotted.



S4 Residuals of the diameter are plotted.