Electronic Supplementary Information (ESI)

Real-time Impedimetric Droplet Measurement (iDM)

Abtin Saateh, Ali Kalantarifard, Oguz Tolga Celik, Mohammad Asghari, Murat Serhatlioglu, and Caglar Elbuken*

Institute of Materials Science and Nanotechnology, National Nanotechnology Research Center (UNAM), Bilkent University, Ankara 06800, Turkey

**Corresponding author: elbuken@unam.bilkent.edu.tr*

ESI.S1. Droplet length simulations

The effect of varying electrode width and gap on signal amplitude has been investigated for droplet lengths in the range of 300 µm to 1500 µm, as given in Fig. S. 1. The signal amplitude does not increase significantly for electrode widths over 150 µm and for droplet lengths over 500 µm. Hence, the system does not impose an upper limit for droplet length. The minimum droplet size can be determined based on such an analysis. The highest signal amplitude was obtained for the smallest electrode gap, which is chosen as 20 µm, in our analysis.

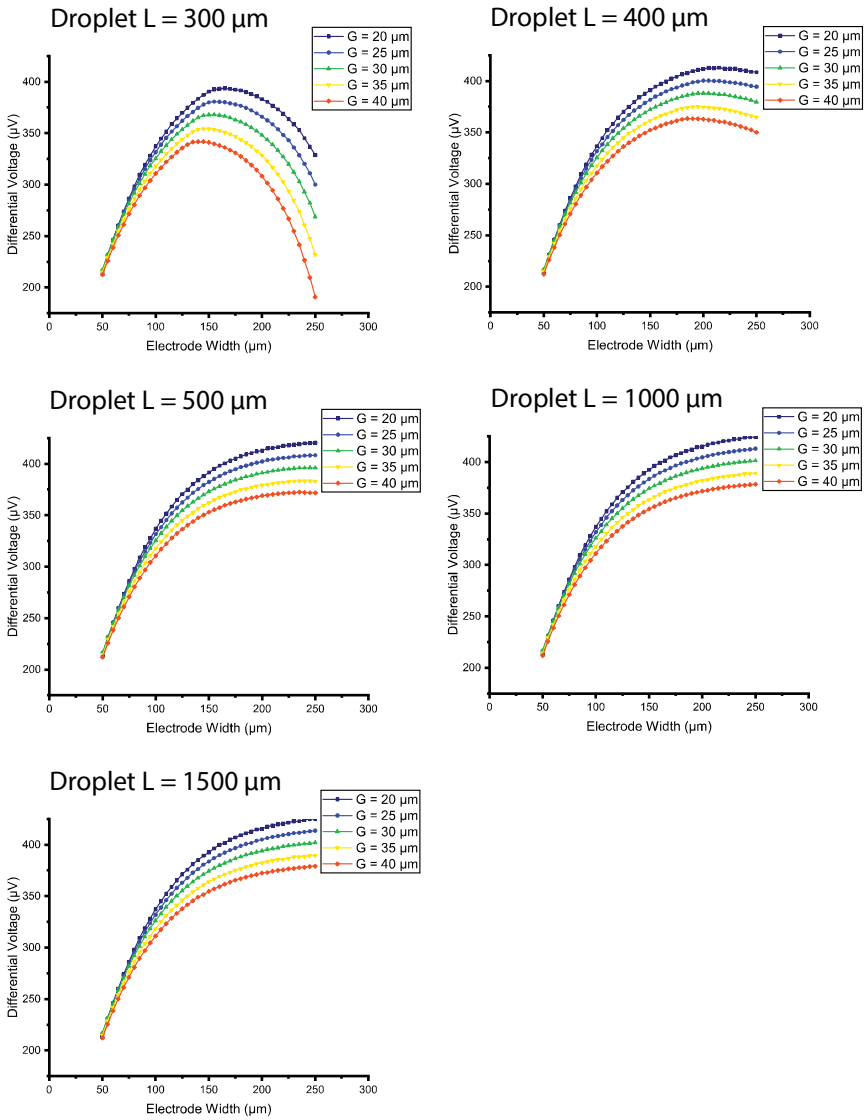

Fig. S. 1- Simulation results showing the signal amplitude for varying electrode geometries and droplet lengths.

ESI.S2. Exemplary droplet signals

We have conducted a series of experiments using different materials as a dispersed phase with different conductivities to demonstrate the potential of using iDM as a tool for studying the electrical properties of aqueous materials. Fig. S. 2 illustrates the differential voltage signal obtained by DI-water droplets formed inside SF-50 oil with 1 V, 1 MHz excitation signal at 224 Sa/s sampling rate.

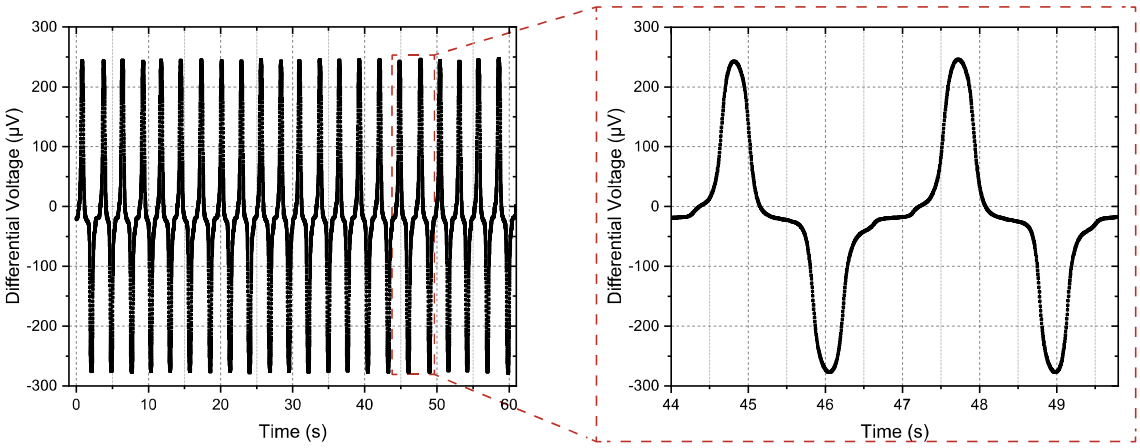

Fig. S. 2- Experimental DI-water droplet signals.

Droplets of 1% aqueous solution of bovine serum albumin (BSA) and 1% phosphate-buffered saline (PBS) were generated and their differential voltage signals were recorded as shown in Fig. S. 3. The difference in the signal amplitude indicates the possibility of using iDM for studying electrical properties of droplets. It is also important to note that the characteristic signal does not change when the material properties of the droplets change. Hence, the morphological characterization performed by iDM applies to all these droplets. Since our aim in this manuscript is to demonstrate the potential of using iDM for such physical property measurements, we have not performed a detailed characterization of the system for varying electrical conductivity and dielectric constant.

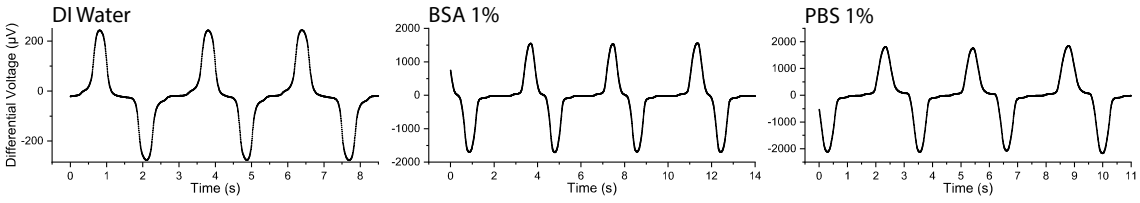

Fig. S. 3- Experimental differential voltage signal of DI-water, BSA-1%, and PBS-1% droplets.

ESI.S3. iDM algorithm

iDM algorithm consists of several blocks depicted as a flowchart in the manuscript (Fig. 3). The main block of the program is named '*Base block*' and is used in point detection. The base block reads the incoming signal and detects t_1, t_2, …, t_8. Then, by substitution of these points into eqn (1) - (3), base block calculates, shows, and saves L, CL, and V in real-time. There is also a '*Gradient*' block that is used by '*Point*' blocks. Gradient block determines whether the signal is rising, falling or at a steady state. These states are named '*Positive*', '*Negative*' and '*Steady*', respectively. This block continuously gets incoming signal points and analyzes them in a moving window of five data points to determine the state. A detailed flowchart is provided in Fig. S. 4.

Explanation of '*Point*' blocks:

- Point 1: This block determines t_1 using two conditions. The first condition to be satisfied is the signal being in the range between the limits specified by the user, called the lower limit (*LL*) and upper limit (*UL*). The second condition is the state being changed from *steady* to *positive*.
- Point 2: It is difficult to distinguish t_2 from the real-time differential voltage signal given in Fig. 3 (b). Taking a derivative of the differential voltage provides more insight into the critical time point analysis. As shown in Fig. 3 (b), t_2 is defined as the state changes from *steady* to *positive* on the signal derivative (*S'*) plot. Additionally, *S'* should be lower than the upper limit of derivative (*ULD*). The differential voltage derivative signal (*S'*) given in terms of V/m corresponds to time derivative due to equivalence between droplet position sweep in simulations and actual droplet motion in time.
- Point 3: t_3 is the maximum point of the signal as illustrated in Fig. 3 (b). *Point 3* block is called after t_1 and t_2 were determined. Using the *gradient* block, *point 3* looks for t_3 where the *positive* state turns into *negative*.
- Point 4 and Point 5: To find these two points, the program looks for the minimum points of the signal derivative (*S'*). The minimum points are named t_{m1} and t_{m2}, and their corresponding points on the signal plot are depicted in Fig. 3 (b). Subsequently, a line was fitted on the signal (S) plot between the two points corresponding to t_{m1} and t_{m2}. Then, the two points of the signal (*S*) with maximum distances on either side to this line are calculated and identified as t_4 and t_5, respectively.
- Point 6: t_6 is the minimum point of the signal. Using the *gradient* block, *point 6* looks for t_6 where the *negative* state turns into *positive*.

Point 7: t_7 is defined as the state changes from *negative* to *steady* on the signal derivative (S') plot and calculated using the *Gradient* block.

Point 8: t_8 is defined as the state changes from *positive* to *steady* on the signal (S) plot and calculated using the *Gradient* block.

Fig. S. 4 illustrates the detailed algorithm of iDM. The base block controls the main flow of the program. Flow-chart of each sub-program of the base block, which is explained in the *iDM algorithm* section, is presented here.

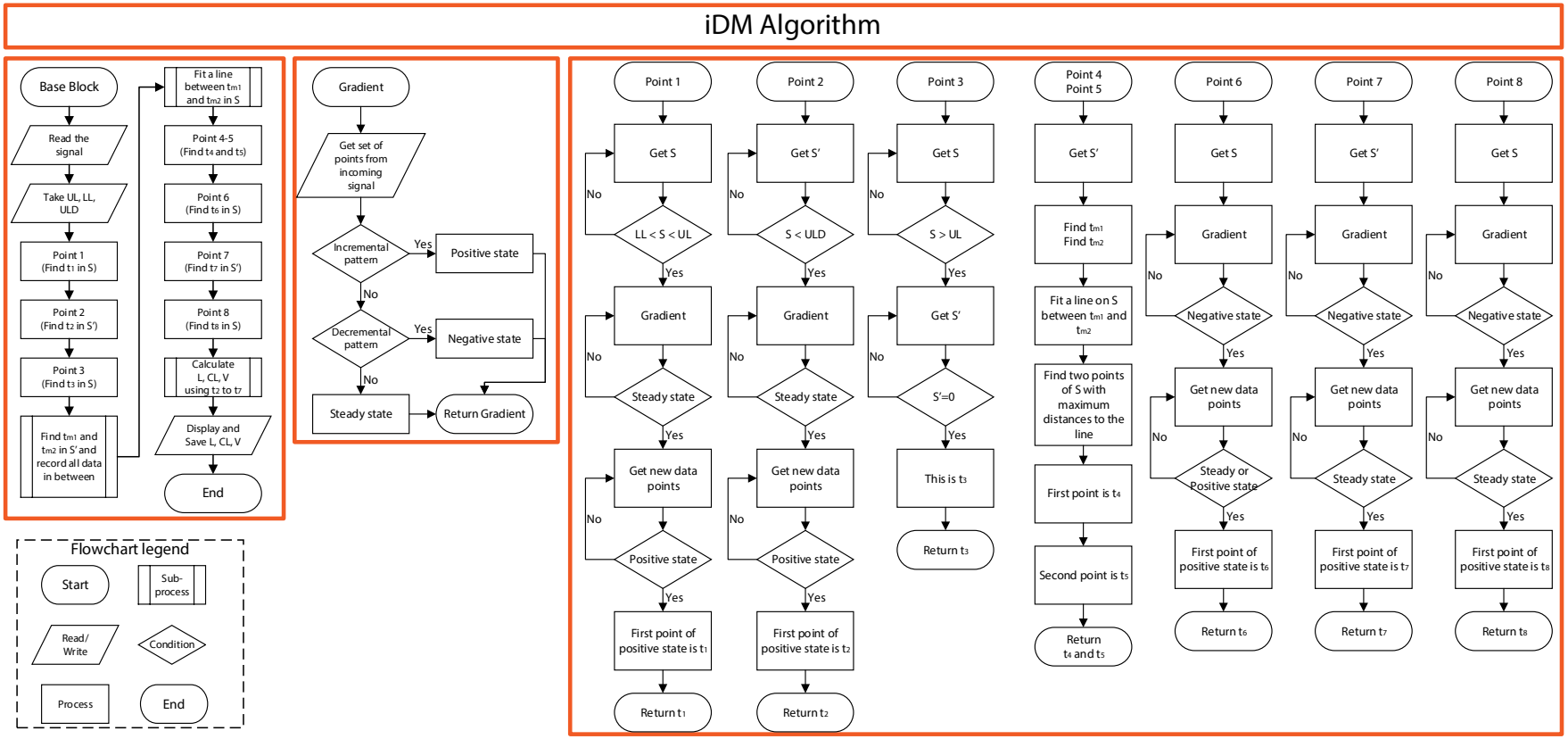

Fig. S. 4- Complete description of the iDM algorithm.

ESI.S4. Theoretical Throughput

There are two limiting factors that determine the throughput of iDM: (I) computational time for iDM algorithm, (II) real-time data sampling rate of the lock-in amplifier and data transfer rate through USB 2.0 connection.

(I) First, we have reduced the number of samples to determine the minimum number of sampling points for iDM to perform successfully. We have determined this number as 300 data points for error-free processing. The size of 300 data points is equal to 4.8 kB in temporary memory storage. In other words, the data size of one droplet is 4.8 kB. LabVIEW sample processing rate in a laptop computer (CPU memory bandwidth: 34.1 GB/s, Core numbers: 2, Clock Frequency: 2.7 GHz) was measured during the signal processing as approximately 90 MB/s. Therefore, when only the running cost of the iDM algorithm is considered (90 MB/4.8 kB), iDM is capable to reach 18750 droplets/s processing rate.

(II) Lock-in amplifier (Zurich Instruments, HF2LI) is connected to a computer via USB 2.0 port. It is stated in the device specifications that HF2LI maximum sampling rate is 210 MSa/s. However, in real-time external signal processing (with one demodulator in use) the maximum sampling rate decreases to 460 kSa/s.[1] This maximum sampling rate is on the edge of the USB 2.0 data transfer rate (30 MB/s in practice; 60 MB /s in theory). Therefore, when the lock-in amplifier sampling rate and data transfer rate are considered, the throughput is 1533 droplets/s (460 kSa/s / 300 Sa).

With our current channel design, we were only able to go up to 10 droplets/s. We use a 2 bar pressure pump to form plug-like droplets. The experimental limiting factor is the required droplet spacing. According to the iDM detection algorithm minimum droplet length and spacing must satisfy $L_{min} > 3W+2G$ (*W*: electrode width, *G*: electrode gap). We used an optimized electrode width of 100 µm and an electrode gap of 60 µm to use the microfluidic device for a wider range of plug-like droplet lengths. In this case, the minimum droplet spacing should be at least ($3W + 2G$) = 420 µm. For high droplet generation rates, in our current channel design, the droplet spacing goes below this number.

Since we were not able to increase particle generation rate, we lowered the excitation signal to find out the limiting SNR value for successful droplet detection. Also, for this experiment we used a more realistic fluid, bovine serum albumin (BSA) to form droplets. We used silicone oil SF-50 at 60 mbar for continuous phase and 1% BSA buffer for dispersed phase at 50 mbar. We excited the system with the built-in lock-in amplifier signal generator at 1 V, 0.1 V and 0.01 V as given in the Fig. S. 5. Then the signal derivative was processed in real-time using iDM. As can be seen from the figure, the signal amplitude decreases with

decreasing excitation voltage. The noise level at each excitation voltage is roughly ~0.1 µV. At 0.1 V excitation voltage; peak amplitude in the derivative signal is 40 µV, which gives an SNR (2α/2β) of ~40/0.1= 400, where iDM was able to detect all droplets successfully. Whereas at 0.01 V excitation voltage, SNR drops to ~0.4/0.1 = 4. At this level, iDM was only able to detect approximately 50% of the droplets in real-time (tested using 100 droplets). Hence, we state SNR = 4 as our lower limit of droplet detection.

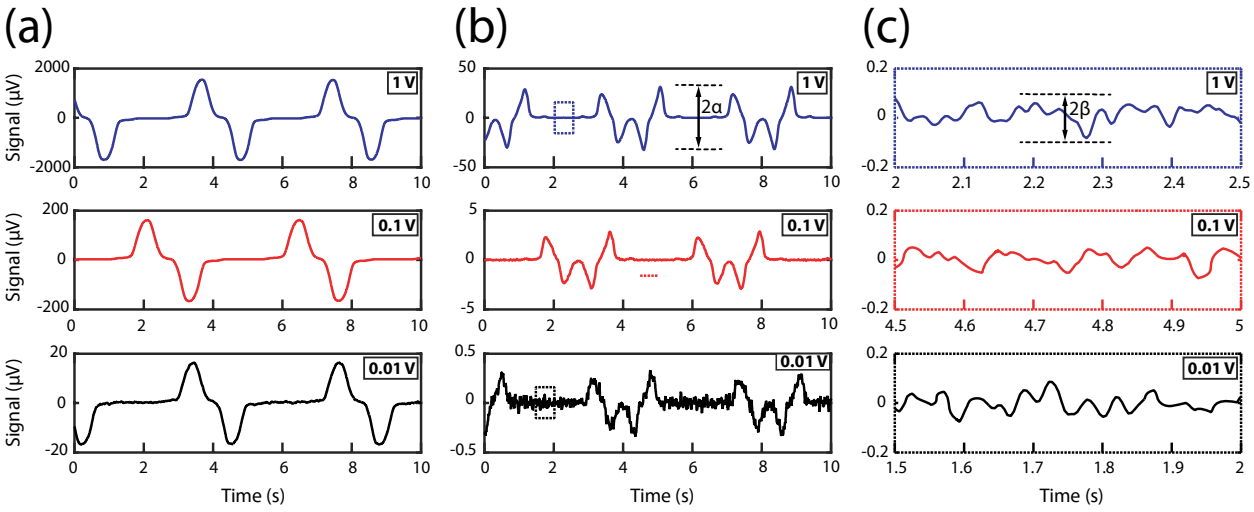

Fig. S. 5- (a) Output signal, (b) its derivate, and (b) close-up image to determine noise level in derivative signal for 1 V, 0.1 V and 0.01 V excitation voltages. Dashed regions in signal derivate (b) indicate the data used for noise analysis.

ESI.S5. Length measurement: iDM vs DMV

In method comparison none of the methods is considered as the reference method, hence, it is needed to assess each method individually and then compare them to each other. When two methods have a poor agreement, the evaluation of similarity is helpful in understanding whether the poor agreement is due to the superior characteristics of one of the methods. Also, in some cases, the methods can have a poor agreement while having similar characteristics. Thus, having a good similarity does not guarantee a good agreement. Evaluation of similarity is performed by fixed bias, proportional (or scale) bias, precision ratio, and sensitivity ratio calculations obtained using the scatter plot.[2] The definitions of these metrics and our calculations are given in this section.

Evaluation of similarity:

The intercept of the plot given in Fig. 5 (a) is 324, which is also called as the fixed bias (β_0). The slope of the plot is 0.48 that is the proportional bias (β_1) between the two methods.

For precision comparison of two methods, the precision ratio is used, defined as,

$$\lambda = \frac{precision\ of\ method\ 2}{precision\ of\ method\ 1} = \frac{1/\sigma_{e2}^2}{1/\sigma_{e1}^2} = \frac{\sigma_{e1}^2}{\sigma_{e2}^2}$$

where σ_{e1}^2 and σ_{e2}^2 are the variances of methods 1 and 2, respectively. For the data given in Fig. 5 (a) variances of iDM and DMV are calculated as 91.71 and 321.72, respectively. Plugging these values into the above equation, we obtain a precision ratio of $\lambda = 3.50$; which is an indicator of higher precision of iDM in comparison to DMV.

For sensitivity comparison of two methods, squared sensitivity ratio is used, which is defined as,

$$\gamma^2 = \frac{(sensitivity\ of\ method\ 2)^2}{(sensitivity\ of\ method\ 1)^2} = \frac{\beta_1^2/\sigma_{e2}^2}{1/\sigma_{e1}^2} = \beta_1^2 \frac{\sigma_{e1}^2}{\sigma_{e2}^2} = \beta_1^2 \lambda$$

Multiplying the proportional bias β_1 by precision, yields $\gamma^2 = 0.80$. The sensitivity ratio of less than one indicates that DMV is more sensitive than iDM.

As seen in this analysis, iDM has higher droplet size measurement precision but less sensitivity in comparison to DMV. These results are summarized in Table S. 1, below.

Table S. 1- Evaluation of similarity analysis between iDM and DMV.

Metric	Value
Fixed bias	324
Proportional (or scale) bias	0.48
Precision ratio	3.50
Squared sensitivity ratio	0.80

ESI.S5. Cap length verification

To verify the cap length measurements, we used a numerical approach. We simulated the signal for droplets with increasing cap lengths as given in Fig. S. 6.

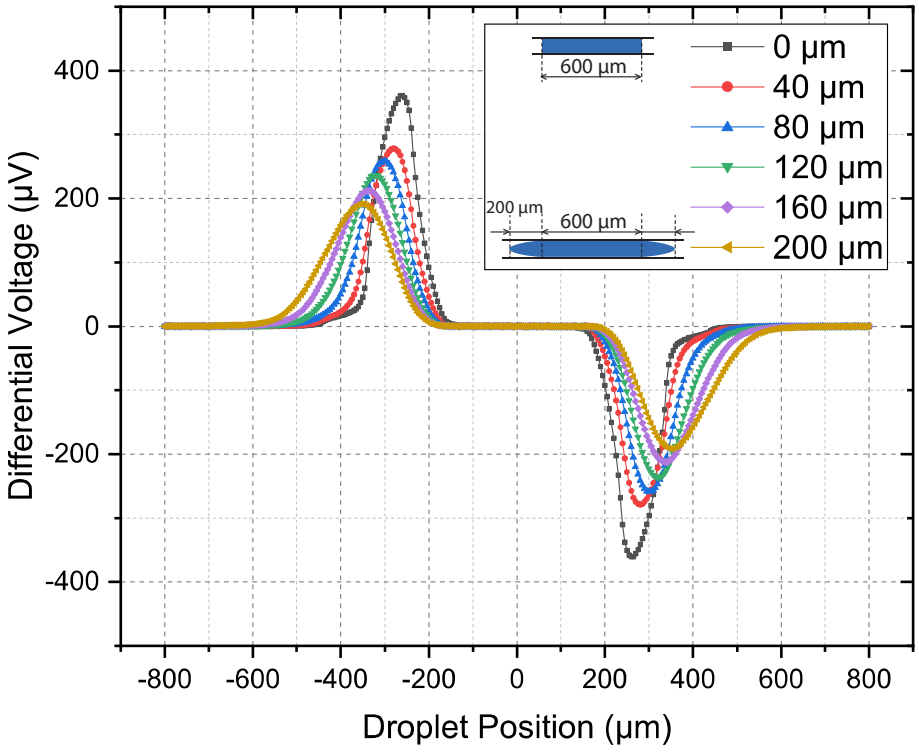

Fig. S. 6- Simulated droplet detection signal for varying cap lengths (electrode configuration is *W*= 75 µm and *G* = 25 µm).

As can be seen from the figure above, increasing the cap length shifts all critical time points except t_4 and t_5. This change is expected since the conductivity over the electrodes increases at a slower rate for a droplet with a larger cap length. To test the results of iDM for such droplets, we developed an offline version of iDM that can process simulation data provided as input. Comsol simulation results were exported to a spreadsheet; then it was imported to iDM-offline mode. *L*, *CL*, and *V* values calculated by iDM are compared to the values used in Comsol simulations. Fig. S. 7 and Table S. 2 show percentage errors to compare simulated values with iDM, for varying cap length. A very good agreement was obtained for *CL* values up to 80 µm. For higher *CL* values, larger errors were obtained, since the algorithm assumes a rounded droplet as used in the initial Comsol simulations (*CL = H/2*). Droplet length and velocity measurement also indicate increasing error as cap length increases above 80 µm.

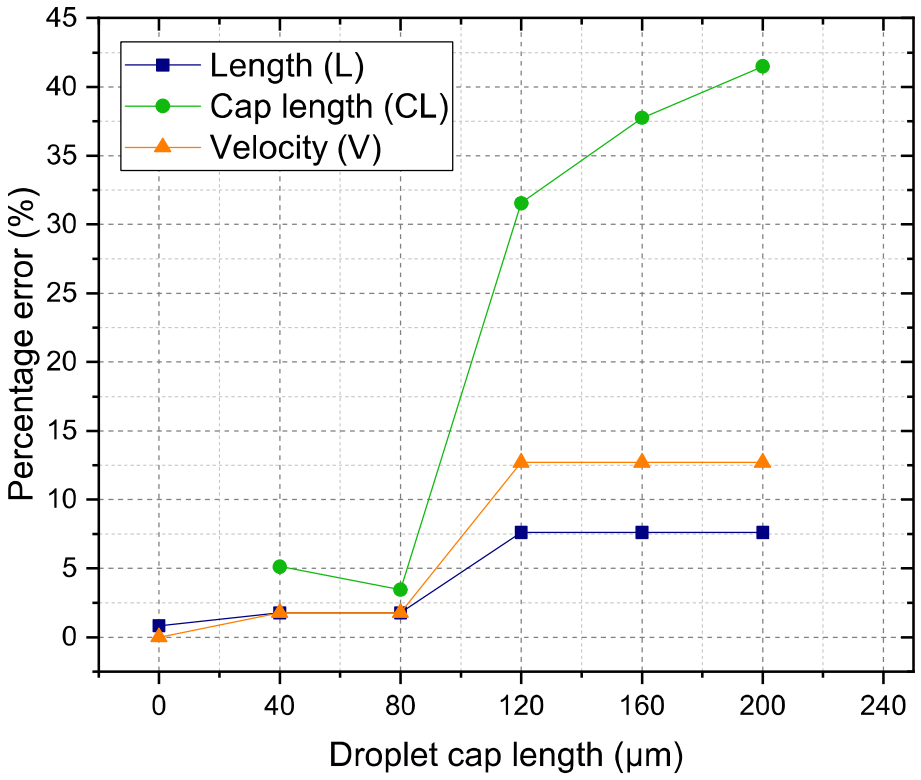

Fig. S. 7- Percentage error of iDM in droplet length, cap length and velocity measurement for varying droplet cap length.

Table S. 2- Percentage error calculation to compare iDM measured *L*, *CL*, and *V* for varying droplet cap length.

Simulation conditions			iDM									Percentage error		
			Detected points						Results					
L (µm)	CL (µm)	V (au)	t_2 (µm)	t_3 (µm)	t_4 (µm)	t_5 (µm)	t_6 (µm)	t_7 (µm)	L (µm)	CL (µm)	V (au)	L (%)	CL (%)	V (%)
600	0	1	-435	-250	-160	160	250	435	595.0	15.00	1.00	0.83	NaN	0.00
600	40	1	-440	-275	-160	160	275	440	589.29	37.95	0.98	1.79	5.13	1.79
600	80	1	-440	-315	-160	160	315	440	589.29	77.23	0.98	1.79	3.46	1.79
600	120	1	-475	-340	-160	160	340	475	554.37	82.14	0.87	7.61	31.55	12.70
600	160	1	-475	-360	-160	160	360	475	554.37	99.60	0.87	7.61	37.75	12.70
600	200	1	-475	-380	-160	160	380	475	554.37	117.06	0.87	7.61	41.47	12.70

ESI.S6. Microchannel design for particle synthesis

For the synthesis of polyethylene glycol particles, an aqueous solution of Polyethylene glycol (PEG) diacrylate (42.2% wt) together with the photo-initiator (2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone) (3.7% wt) was prepared as the dispersed phase. The continuous phase included mineral oil and span 80 (5% wt) as the oil-soluble surfactant. We added an additional flow of mineral oil before the outlet to increase droplet spacing and prevent droplet coalescence. The channel design is shown in Fig. S. 8. We used a channel constriction section after droplet formation as shown in ESI.S6. Such a design feature can be used for the detection of spherical droplets. Spherical droplets can be transformed into plug-like droplets by using a channel constriction so that iDM can be applied for morphological characterization.

Microscale droplets were generated and transferred to a petri dish. Then an LED UV gun (Thor Labs, CS2010) was used to cure droplets inside the petri dish. For polymerization, UV exposure (365 nm) for 1 s at 27 W/cm^2 power was used. When pressures of the continuous phase, additional flow, and aqueous phase were set to 55 mbar, 35 mbar and 51.5 mbar, respectively, spherical PEG particles with an average diameter of 106 µm were obtained.

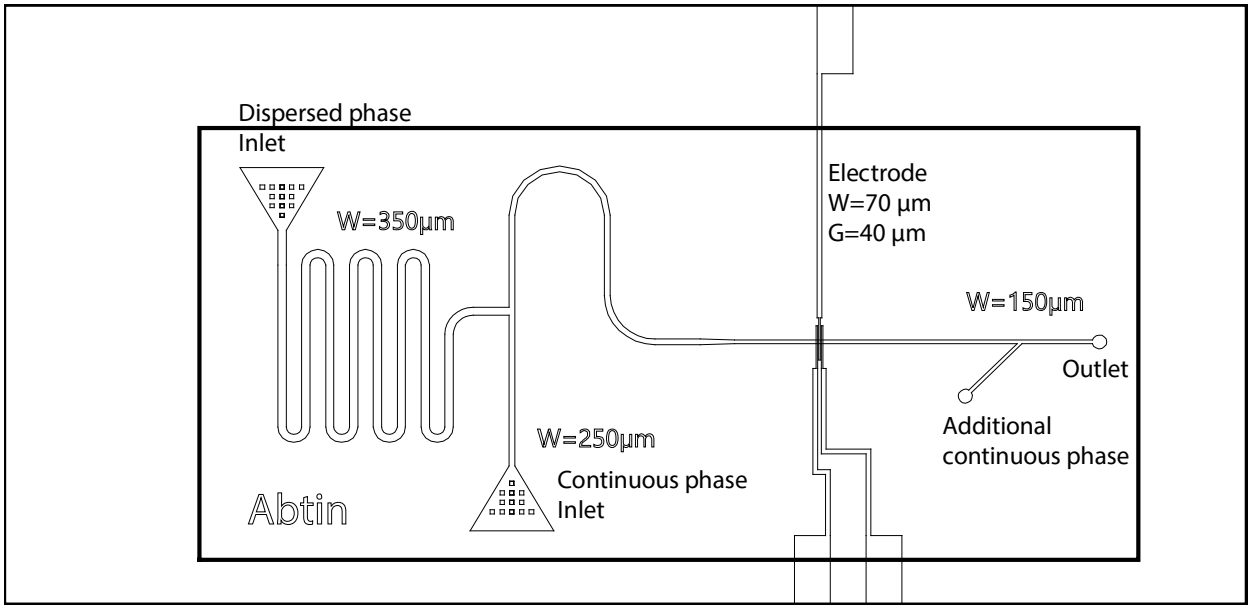

Fig. S. 8- Microfluidic chip design with a constriction equipped with microelectrodes used for particle synthesis and real-time iDM characterization.

References

1 H. F. U. Manual, *Zurich Instruments AG*, 2014, 1–400.

2 P. K. Choudhary and H. N. Nagaraja, *Measuring Agreement Models, Methods, and Applications*, John Wiley & Sons, New York, 2017.