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

Supplementary Material A – EWOD device fabrication

First, using acetone, isopropanol, methanol and DI water the Indium tin oxide (ITO) coated wafer plates was cleaned followed by blow drying with N_2 and the dehydration process on a hotplate at $150\ ℃$ for 5 min. Then, utilizing a spin coater with the recipe of ramping the speed spin to 500 rpm with 100 rpm/s for 5 s; ramping with $900\ rpm/s$ to 4000 rpm for 30 s, the hexamethyldisilazane (HMDS) was coated on the substrate to promote the adhesion of photoresist (PR). Moreover, the wafer baked at $150\ ℃$ for $90\ s$ to evaporate the solvent present in the HMDS solution. Then, $1.2\ \mu m$ uniform thickness of a positive PR (Microchem S1813, MicroChem Corp, Newton, MA, USA) was spin coated on the wafer utilizing the following recipe; Spin speed was ramped up to 500 rpm by $100\ rpm/s$ for 5 s; ramping by $900\ rpm/s$ to 3000 rpm for 30 s. To remove the excess solvent and to anneal the PR, soft bake was done at $115\ ℃$ for $60\ s.$ The photolithography was done with the exposure does of $140\ mJ/cm^2$ using the backside-aligner (OAI 806MBA) followed by the post baked at $110\ ℃$ for 1 min. After that, using a developer (Microchem, MF-319), the wafer was developed and dehydrated at $115\ ℃$ for 2 min. To etch the ITO layer, a solution consists of Hydrochloric acid (HCl), Nitric acid (HNO_3) and DI water (H_2O) (wt %- 20% HCl, 5% HNO_3, 75% H_2O or vol %- 8:1:15, HCl:HNO_3:H_2O) was used and the wafer was immersed in the solution at $55℃$ for 2.5 min. Then, the wafer went through stripping PR (Remover 1165, Microchem) and dehydration at $150\ ℃$ for 2 min.

After that, $5\mu m$ uniform thickness of (SU-8 2005, Microchem) as dielectric layer was spin coated on the wafer with the subsequent recipe; Spin speed was ramped up to 500 rpm by $100\ rpm/s$ for 5 s; ramping by $900\ rpm/s$ to 2000 rpm for 30 s. To harden the dielectric layer, the wafer was baked at $70\ ℃$ for 1min, $100\ ℃$ for 3 min and cooled down at $70\ ℃$ and the room temperature for 1 min and 2 min respectively. After that, the exposure was done with a light does of $140\ mJ/cm^2$ followed by post baked at $70\ ℃$ for 1 min, $100\ ℃$ for 3 min and also hard baked at $150\ ℃$ for 5 min. Teflon AF1600S (Du Pont powder dissolved in Fluorinert FC-40 Sigma-Aldrich) as a hydrophobic layer was spin coated on top of the dielectric layer with the following recipe; Spin speed was ramped up to 1000 rpm by $300\ rpm/s$ for 30 s, resulting in $300\ nm$ thickness. Then, the wafer baked at $70\ ℃$ for 1 min, $100\ ℃$ for 1 min and $180\ ℃$ for 3 min. To suppress the chance of voltage-break down, another layer of Teflon was spin coated followed by annealing at 70 °C for 1 min, 100 °C for 3 min and 180 °C for 15 min.

As a top plate, the ITO-coated glass went through the cleaning and dehydration process. Then, $300\ nm$ layer of Teflon AF1600S was spin coated with aforementioned recipe. Then, the wafer annealed at 70 °C for 1 min, 100 °C for 3 min and 180 °C for 5 min.

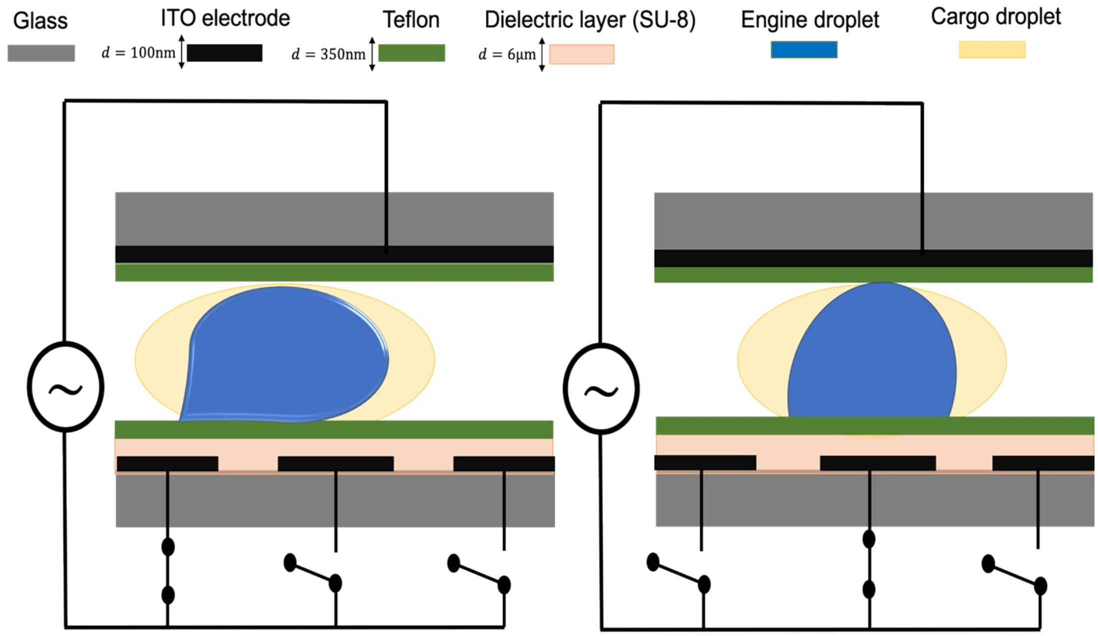

Fig. S1: Schematic layout of an EWOD device where it shows the actuation of a sandwiched Engine-and-Cargo droplet in an EWOD device.

Supplementary Material B – Lab scale reactions and the NMR study details

General procedure for lab-scale reactions:

Menthol (31.2 mg, 0.2 mmol), Et_3N (56 μL, 0.4 mmol), and DMAP were placed in a vial and dissolved in solvent (0.16 mL). Acetic anhydride (38 μL, 0.4 mmol) was added to the mixture. The septum on the vial was replaced by a screw cap with a Teflon liner, and the reaction mixture was stirred at rt. The reaction progress was monitored by either GC-MS spectrometry or ^{1}H-NMR spectroscopy. The reaction was quenched by adding saturated aqueous $NaHCO_3$. After being stirred for 10 min, the reaction mixture was extracted with Et_2O three times. The organic layer was washed with water followed by saturated aqueous brine and dried over anhydrous Na_2SO_4. The resulting solution was concentrated under reduced pressure to afford a brown oil. ^{1}H spectra were recorded on JOEL Eclipse Plus 500 (500 MHz) spectrometer with 10 second relaxation delay, yields are measured with Mesitylene as internal standard.

NMR studies:

Menthol (97.5 mg, 0.625 mmol), Et_3N (126 μL, 1.25 mmol) and DMAP were placed in a Norell® pressure NMR tube and dissolved in Toluene-D8 (0.5 mL). Acetic anhydride (127 μL, 1.25 mmol) was added to the mixture and the tube was capped and agitated for 5 seconds. ^{1}H spectra were recorded on JOEL Eclipse Plus 500 (500 MHz) spectrometer with 10 second relaxation delay. ^{1}H-NMR chemical shifts are referenced to Toluene-D8 (7.00 ppm).

^{1}H and ^{13}C NMR spectra were recorded on JOEL Eclipse Plus 500 (500 MHz) and JEOL ECX 300 (300 MHz) spectrometers. ^{1}H NMR chemical shifts are referenced to chloroform (7.26 ppm). ^{13}C NMR chemical shifts are referenced to $^{13}CDCl_3$ (77.23 ppm). ^{1}H NMR and ^{13}C NMR were processed with the iNMR software program.

GC-MS analysis:

GC-MS data were recorded on a Varian 450-GC/Varian 240-MS System. The methods used are noted parenthetically: 5029017 refers to: 2 min @ 50 °C – 20 °C/min – 3 min @ 290 °C (a 50 °C initial temperature that was held for 2 minutes followed by a 20 °C/min ramp to a final temperature of 290 °C that was held for 3 minutes for a total run time of 17 minutes).

Supplementary Material C – GC-MS Spectrometry for Kinetic Study on Chip

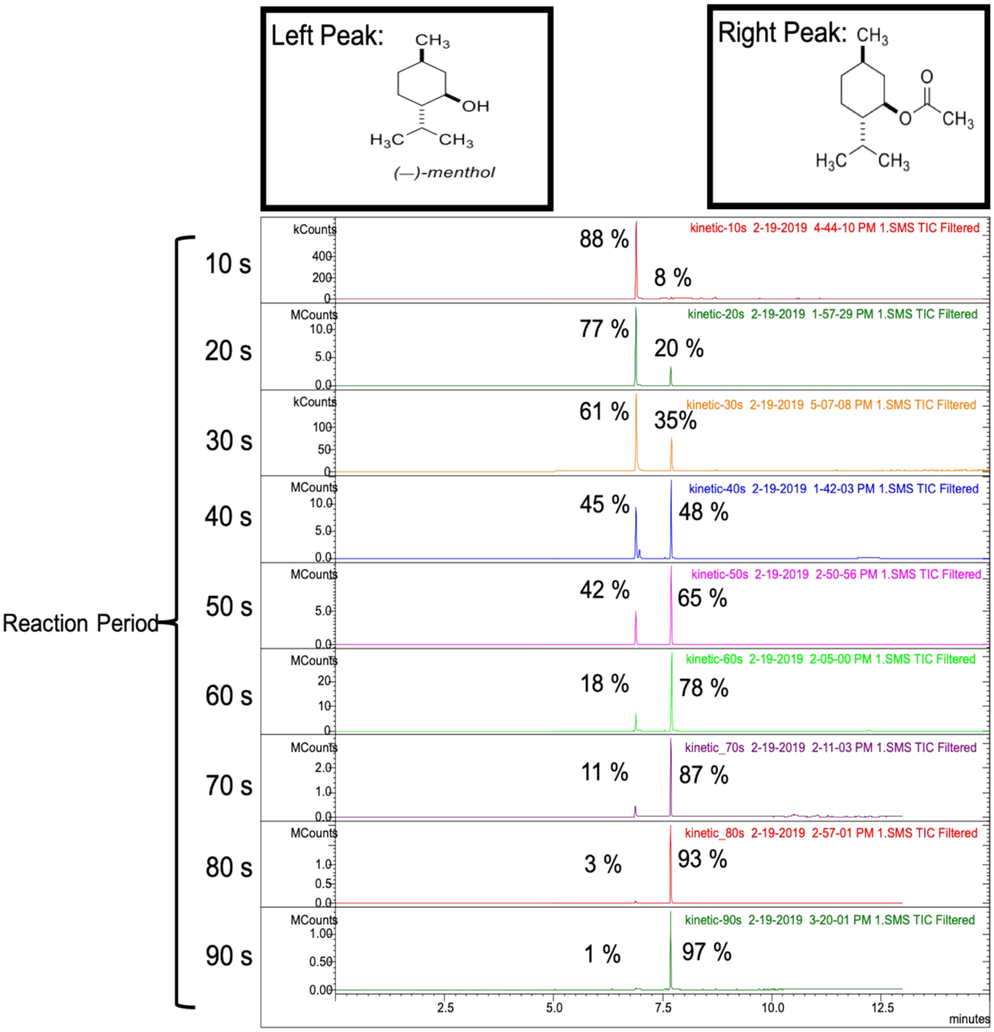

Fig. S2: GC-MS spectrometry of reactions at different time periods.

Supplementary Material D – GC-MS Spectrometry for Optimizing Catalyst Loading on Chip

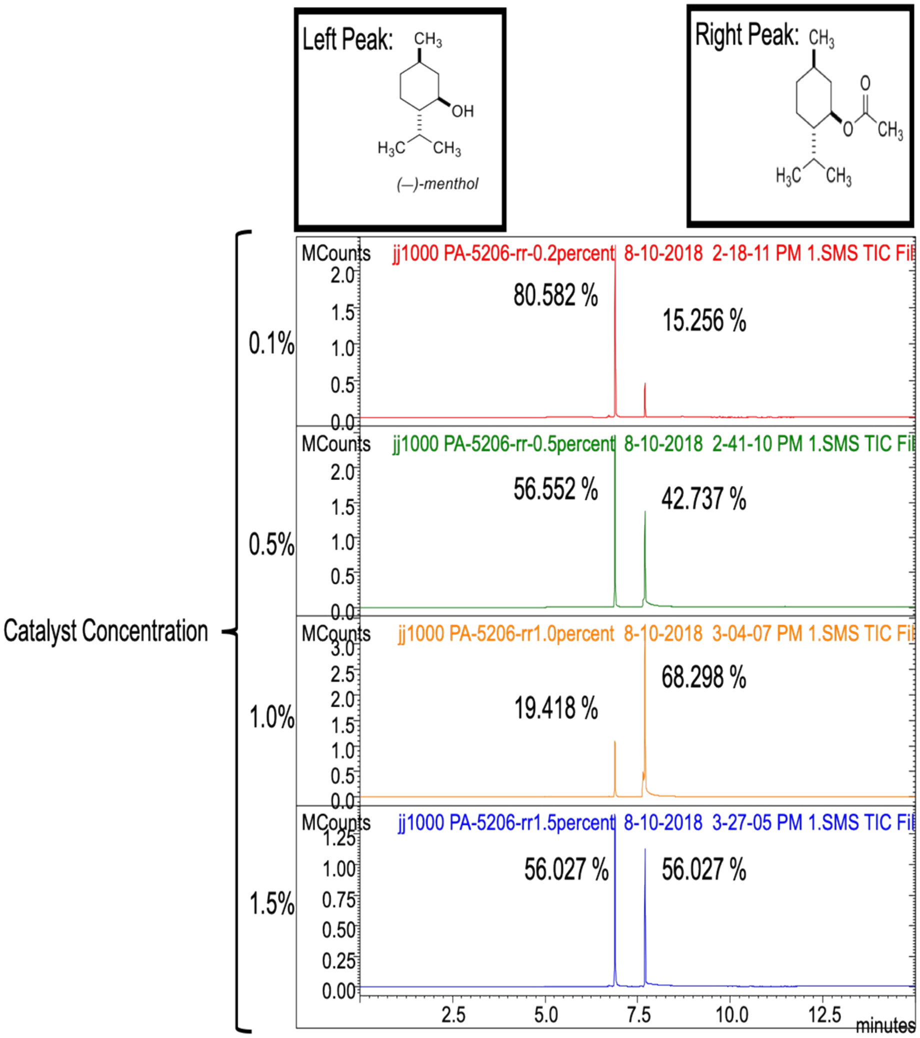

Fig. S3: The reactions with different concentrations of DMAP monitored by GC-MS spectrometry. For this study, the type of solvent (toluene) and the reaction time (30 s) were fixed for all tests.

Supplementary Material E – Image-based volume measurement

In the common practice, the volume of a droplet in an EWOD device is estimated by multiplying the footprint area (area observed from the top view of the droplet) with the gap between top and bottom plates of the device. Note that the gap between top and bottom plates is well controlled and kept invariant throughout the entire device, so the footprint area variation is directly in proportion to the droplet volume variation.

To measure the area of irregular shape of droplet footprint, ImageJ software was used. Since the cargo droplet in an engine-and-cargo compound droplet often has sharp corners and/or thin layers, its footprint area was measured indirectly:

(cargo droplet area) = (entire compound droplet area) – (engine droplet area)

Screenshots of area measurements using ImageJ software are shown in Fig. S4.

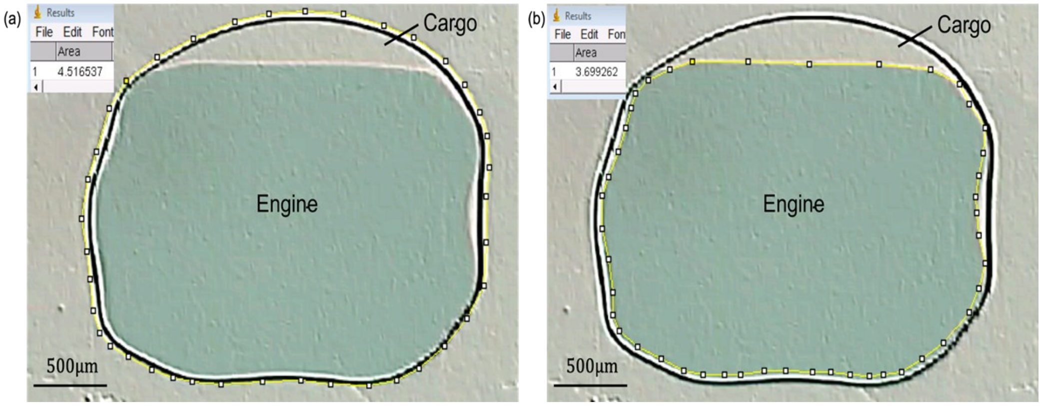

Fig. S4: (a) The footprint area of the entire compound droplet and (b) The footprint area of the engine.

To calibrate and to validate the accuracy of this measurement technique, a known volume (= 1.0 uL) of cargo solution was pipetted into an EWOD chip. After forming of an engine-and-cargo, we calculated the corresponding footprints as described above (i.e. ImageJ-based technique) and obtained the volume of cargo to be 1.09 uL. This 9 % of error might be caused by multiple sources including the curvature of the droplet side, the offset of the gap between top and bottom plates. In order to get rid of these error sources, the calibration with two droplets were taken. Two known volumes (= 1.0 and 2.0 uL, respectively) of cargo solution were pipetted into an EWOD chip so that the volume difference of two droplets to be 1.0 uL. Using the Image J measurement technique, volume of each droplet was measured. The volume difference was measured to be 0.98 ± 0.0365 uL. This proves that the uncertainty of Image J measurement technique of droplet volume consistency is less than 1.7 %.

Supplementary Material F – Experimental video

Movie 1: Illustrating the generation of the Engine-and-Cargo from the reservoir.

Movie 2: Demonstrating the steps of esterification of menthol on an EWOD device.