Anisotropic particle synthesis in dielectrophoretically controlled microdroplet reactors

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The miniaturization of chemical and biological processes in microfluidic devices and bioarrays is a major technological achievement. Microchips performing multiphase material synthesis operations could be a future step in this trend of miniaturizing technology. Here we show how electrically controlled chips can be used for the synthesis and manipulation of new types of particles with advanced structure. The method is based on a technique that allows freely suspended droplets and particles to be entrapped and transported using electric fields. The fields that hold and guide the droplets and particles are applied through arrays of electrodes submerged in the oil. Each of the microdroplets suspended on the surface of fluorinated liquid serves as a microscopic reactor, where the particles are formed by solidification of the carrier droplets. Controlled on-chip assembly, drying, encapsulation and polymerization were used to make anisotropic 'eyeball' and striped particles, polymer capsules and semiconducting microbeads.

Microdroplets suspended in organic and fluorocarbon oils have vast potential as compartments for the synthesis of new classes of structured particles. Shell-like 'colloidosomes' can be assembled by adsorption and binding of particles around droplets[1–6]. Alternatively, the droplets can contain suspended microparticles and nanoparticles. When the droplets are dried, the particles confined inside them are compressed and assembled into ball-like 'supraparticles'[7–11]. However, scaling up and controlling these processes to develop technology is not straightforward. Making and transporting solid particles may not be easily accomplished by microfluidic chips with solid wall channels: although liquid droplets flow in such channels[12] and could form sites for particle formation[13], particles could adsorb on the walls if they are not treated with protective layers. The manipulation of single droplets has been made possible by dielectrophoresis, the interaction of liquids or particles with non-uniform electric fields[14–20]. We recently developed a microfluidic chip that can manipulate droplets freely suspended on the surface of a dense fluorinated liquid[19]. The droplets are held in place or moved by applying alternating-current (a.c.) voltages to arrays of electrodes situated below the oil. Here we show how such chips can be used for the electrically controlled synthesis of new types of supraparticle.

The synthesis began by floating droplets of volume 500–2000 nl on the surface of perfluorinated oil (F-oil) in a small vessel (Fig. 1a). The droplets contained suspensions of microparticles and nanoparticles, polymer solutions and/or polymer precursor. Most of the droplet surface was submerged in the oil, but a small portion on the top was exposed to the atmosphere. The droplets were entrapped by the electrical fields generated by electrode arrays on the bottom of the chip. Arrays of square or circular electrodes, organized in rows with a pitch of 1.5 mm, were fabricated on two-sided circuit boards that were submerged below the oil surface, without any physical contact with the bottom sections of the droplets (Fig. 1b). These electrodes were connected by individual electronic switches either to the a.c. voltage source or to the ground. The success of the process depends on finding the configuration of the electrode patterns and the voltage necessary for secure entrapment of both the droplets

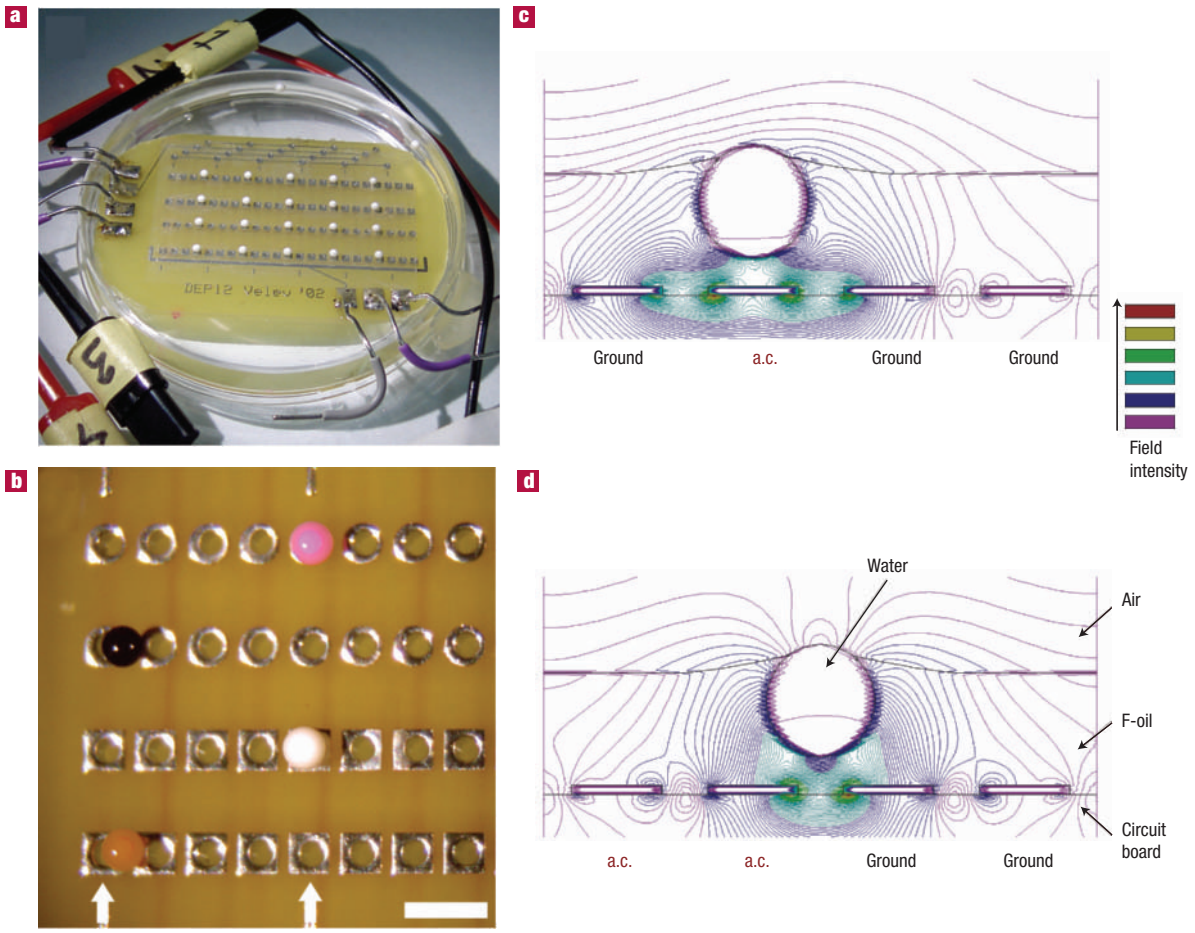

Figure 1 Droplet and particle entrapment on chips with addressable electrode arrays. a, Parallel particle synthesis: 20 aqueous droplets of 1 μl containing suspended polystyrene microspheres trapped by dielectrophoresis above the electrode matrix in a small Petri dish with F-oil. **b**, Micrograph of 750-nl droplets from various suspensions (from top to bottom: polystyrene latex, gold nanoparticles, fluorescent latex and magnetic latex) trapped on the chip. The energized columns of electrodes are indicated with arrows; all other electrodes are grounded, Scale bar, 2 mm. **c**, **d**, Electric field intensity mapping for the two droplet positions, corresponding to the one experimentally observed to the right in **b** (**c**) and to the left in **b** (**d**). The droplet size and position are approximately to scale.

and the particles obtained. We found two different equilibrium positions of the trapped droplets (and subsequently the obtained particles) with respect to the underlying electrodes, which depended on the pattern of the energized electrodes. When the electrodes were connected in sequences of two energized and two grounded electrodes, the droplets migrated to the gap between the energized and grounded electrodes; the same was observed when single electrodes at the ends of a row were energized (second and fourth rows in Fig. 1b). When single electrodes inside rows or matrixes of grounded electrodes were energized, the droplets 'levitated' themselves exactly above these electrodes (first and third rows in Fig. 1b).

The equilibrium positions above the electrodes adopted by the droplets and particles can be interpreted in terms of the dielectrophoretic (DEP) force acting on them. Because of the low dielectric permittivity of the perfluorinated oil ($\varepsilon \approx 2$) the droplets and particles always have higher polarizabilty than the surrounding F-oil and air phases. Objects of higher polarizability are always attracted along the field gradient towards the areas of higher field intensity[21–23]. To interpret the experimentally observed equilibrium positions, we calculated the profile of the suspended droplets from the balance of the buoyancy and interfacial tension forces and then determined the field intensity profiles by using two-dimensional finite-element method electrostatic calculator software. The calculated field intensities in the vertical plane for the two experimentally observed cases are shown in Fig. 1c, d (compare also with the experimental top view in Fig. 1b). In both cases the droplets or particles were attracted by the areas of highest field intensity as expected by the dielectrophoretic theory. In the first case, the droplets positioned themselves above the single area of high intensity between the first and second electrode (left pair in Fig. 1b, d). However, when a single electrode between two grounded electrodes was energized, the droplets balanced the attractive forces from the two areas of high intensity (right pair in Fig. 1b, c) and hovered directly above the electrode. The trapped droplets or particles could be moved along the rows by consecutively switching on and off the voltage to adjacent electrodes. The floating objects moved along the electrode tracks as they were being attracted to the area of highest field intensity, which moved with the switching of the electrodes.

Water droplets were reliably manipulated by a.c. voltages of 200–700 V in magnitude, resisting attempts to displace them by air currents, by liquid convection or by physically agitating the chip.

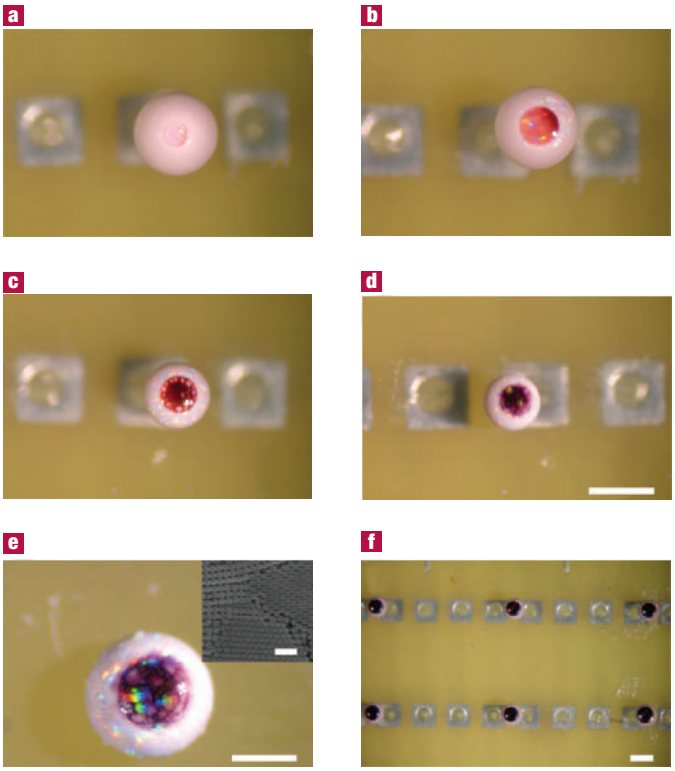

Figure 2 Anisotropic 'eyeball' supraparticle assembly by the evaporation of droplets from binary suspension. a–d, A 1-μl binary suspension droplet of colloidal gold and polystyrene latex above the active electrode after a few seconds after injection onto F-oil (**a**), 7 min of drying (**b**), 18 min of drying (**c**) and 3 h of drying (**d**). Scale bars (**a–d**), 1 mm. **e**, Dried eyeball assembly of gold nanoparticles and polystyrene latex. Scale bar, 500 μm. The inset is a scanning electron micrograph from the surface of a similar particle, showing the regular microsphere arrays. Scale bar, 5 μm. **f**, An array of eyeball particles entrapped on the chip. Scale bar, 1 mm.

The frequency of the signal applied to the electrodes (varied in the range 50–5,000 Hz) was not found to be a controlling parameter, because the polarizability of the droplets was weakly dependent on the frequency in the range studied. Droplets of liquid monomers or solid particles resulting from the synthesis responded to a.c. fields more sluggishly (owing to the lower difference in polarizabilities) but could still be reliably trapped and manipulated. Better response can be achieved in this case by changing the symmetry of the applied signal to a maximum 95% positive or negative, wherein an additional electrostatic component of the force is present[19]. We found that this method was less reliable because of charging and recharging of the particles, and thus all materials were synthesized by using a.c. control only. The power dissipation in all experiments was extremely low so that the currents through the cell were smaller than the capacitance leaks in the circuit. The power losses were low because the droplets were suspended in insulating oil, and no significant current flowed between the electrodes insulated by the oil.

Once the conditions for entrapment and manipulation were established, we were able to use the chips for the fabrication of a variety of new types of supraparticles. The ability to hold the individual droplets in place and observe them continuously with a microscope from above opens a region of possibilities, both in terms of designing new particles and in characterizing the assembly process.

An example of the evolution of on-chip formation of a novel type of anisotropic 'eyeball' supraparticle is presented in Fig. 2. The process of creating this type of particle began with depositing droplets on the F-oil, which contained a binary suspension of 0.21 wt% 12-nm gold nanoparticles and 20 wt% 0.65-μm polystyrene latex microspheres. As the droplets began to dry by water evaporation from the top section exposed to air, a series of remarkable phenomena were observed. The internal hydrodynamic flux compensating for the evaporation brought the particles to the top surface of the droplets. The gold nanoparticles (red) were the first to become visibly concentrated on the top of the droplet, where evaporation of water was taking place (Fig. 2a). As evaporation progressed, a phase of concentrated latex microspheres was also formed in the top section of the droplets. The latex particles organized in a colloidal crystal, which sparkled in a range of bright colours owing to the diffraction of light in the ordered array[7] (Fig. 2b). After several minutes of particle accumulation in the top droplet sections, a clear particle separation in two regions was observed: a dark cap of concentrated gold nanoparticles on the top of the droplets and a larger body of white iridescent microsphere crystal below it (Fig. 2c). The fundamentals of the internal particle collection and phase-separation process are under study and will be reported elsewhere. The water in the droplet was completely dried after 3 h, and the resulting products were solid eyeball particles made of iridescent latex crystals with a dark metallic spot on one side (Fig. 2d, e). Arrays of eyeball particles could be assembled on the chip (Fig. 2f), deposited on its surface by removing the F-oil, and extracted.

More complex 'striped' particles were created with this device from ternary mixtures of gold, fluorescent latex and silica particles (Fig. 3). First, droplets containing a mixture of 0.040 wt% 12-nm gold nanoparticles, 15 wt% 1-μm red fluorescent latex microspheres and 2.8 wt% 0.97-μm silica microparticles were added and the water was allowed to dry overnight. During the drying process the gold nanoparticles segregated on top of the balls owing to liquid evaporation (similarly to the eyeballs), and the (white) dense silica microspheres sedimented on the bottom, leaving a middle region of red fluorescent latex (Fig. 3a). When observed under ultraviolet (UV) illumination, the latex stripe in the middle fluoresced and the gold and silica portions were dark (Fig. 3b).

Another type of ternary particle was created by drying droplets of 0.013 wt% 12-nm gold nanoparticles, 1.67 wt% 190-nm yellow fluorescent latex microspheres and 0.92 wt% 0.97-μm silica particles floating on the surface of the fluorinated oil. Particle collection and phase separation within the droplets again occurred similarly to the red fluorescent latex assembly, except that in this case the gold nanoparticles did not separate on top but condensed in the middle of the droplets between the latex and the silica (Fig. 3c). The large silica spheres allowed unperturbed nanoparticle transport towards the top. However, the pores between the adjacent spheres in the crystal from 190-nm latex particles were too small to allow transport of the gold nanoparticles and the latter were accumulated in the middle brown gold stripe. Visualizing these particles in fluorescence mode showed the latex on top fluorescing green with a very dark band where the gold was concentrated, and a less dark area for the silica phase (Fig. 3d). These striped multilayer particles illustrate the power of the method to make new particles with unique structure.

The chips also readily allowed the making of solid polymer spheres with special optical or electrical properties. Such particles were made by two different solidification mechanisms: the photopolymerization of liquid monomer and the evaporation of water from polymer solution. Droplets of the photopolymer SU-8 25 laced with 0.8 wt% 12-nm gold nanoparticles were polymerized with UV radiation while floating on top of the fluorinated oil layer. The resulting particles were solid droplets of insulating epoxy polymer with a uniform distribution of conductive gold particles

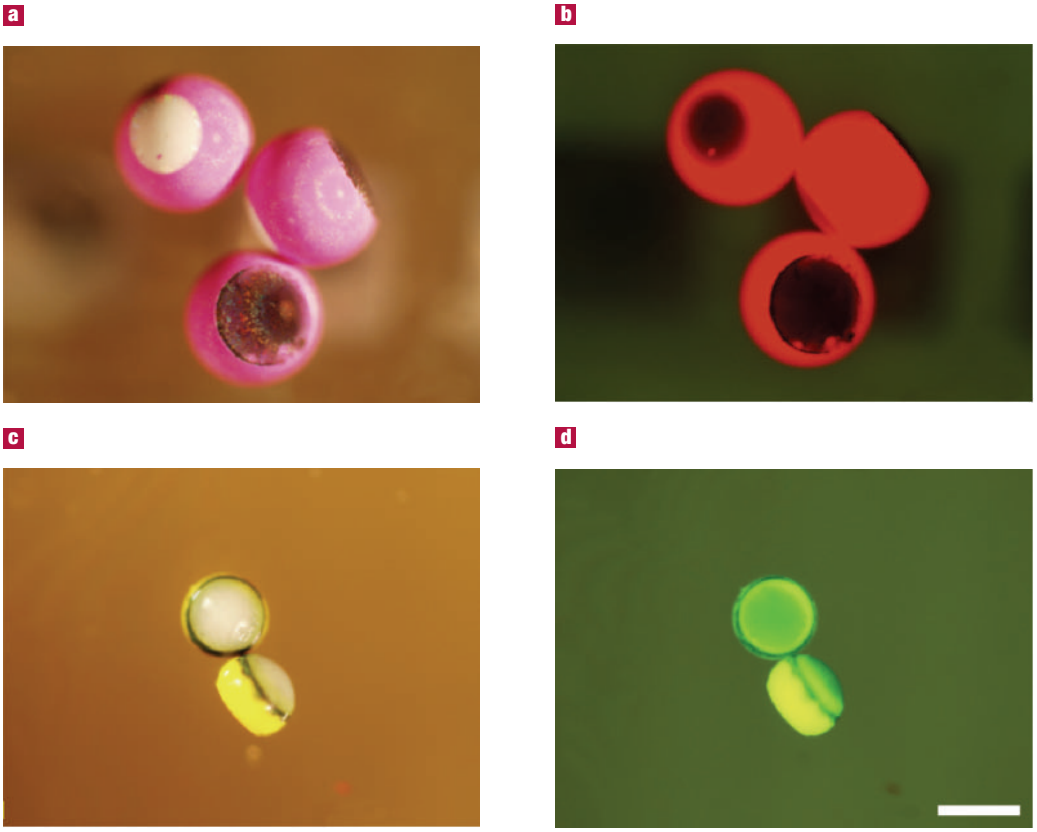

Figure 3 Formation of 'striped' multilayer particles by the evaporation of droplets from ternary particle mixtures. a, Segregated multilayer assemblies of dried gold nanoparticles (top layer), fluorescent red latex beads (middle) and silica microspheres (bottom). **b**, Complementary image in fluorescence illumination. **c**, Segregated multilayer assembly of dried yellow latex beads (top), gold nanoparticles (middle), and silica microspheres. **d**, Complementary image in fluorescence illumination. Scale bar 1 mm.

through the interior (Fig. 4a). The larger epoxy particles reproduced the biconcave shape of the original floating droplet. To make conductive particles, we started with arrays of droplets of 5 wt% polypyrrole in water on the chip and after evaporating the water we obtained black polymer spheres with metal-like appearance (Fig. 4b). These semiconductive particles had a resistance on the order of 100 kΩ when measured with a two-point probe.

Finally, core–shell particles were synthesized by encapsulation of dried supraparticles or droplets of aqueous suspension inside polymer shells (Fig. 4c, d). The droplets of liquid monomer were deposited on the chip near the target droplets or particles. The monomer droplets were attracted by the active electrode holding the target droplets. On contact, the monomer droplet encapsulated the core droplet[19] or particle and formed one single larger particle. The liquid monomer shell was subsequently solidified by photopolymerization. The first type of particle created by this method was the anisotropic dried eyeballs encapsulated with a liquid monomer, 1,6-hexanediol diacrylate (HDDA). The dispersion for these particles were dried on the device for 2 h and then encapsulated with a droplet of HDDA. After a wait of 30 min to allow for uniform encapsulation, the photopolymer layer of this composite microstructure was polymerized with UV radiation, resulting in eyeball particles with a solid polymer shell (Fig. 4c). The second type of encapsulated particle was made with droplets of 40 wt% 0.72-μm latex that were encapsulated with droplets of HDDA before being dried in a 1:1 ratio by volume. After 30 min of waiting, the HDDA shells were polymerized. The resulting particles were subsequently removed from the oil and dried overnight. Because the capsule had a small opening on the bottom of the original droplets, the water phase inside dried, depositing a layer of dried latex on the inside of the acrylic shell. The final capsules were solid polymer 'cups' partly filled with dried latex particles (Fig. 4d).

The technique can be scaled up as a continuous process taking place in chip 'microfactories' for particle synthesis, where droplets are injected on one side of the chip and the particles formed are collected on the other side. There are no obstacles in principle to scaling down the electrode patterns by photolithography to allow the manipulation of nanolitre droplets and making micrometre-sized particles. The size of the droplets can be reduced by automatic ejection techniques similar to ink-jet printing. On-chip synthesis processes are not likely to be advantageous for simple mass-produced particulates. They can, however, find niches of applications requiring particles with special structure and properties. Particles from conductive and semiconductive polymers could find application as self-assembling components for photonics and electronics. Eyeball and striped particles could find use in electronic paper and coatings with changeable properties. They could also be used as 'barcoded' tags in biological and environmental research, and in advanced drug delivery and targeted therapeutics (by combining particles that bind to specific tissues with particles providing local therapeutic effect). Creating particles with magnetic stripes of 'eyes' by this method is straightforward; such particles can be used in

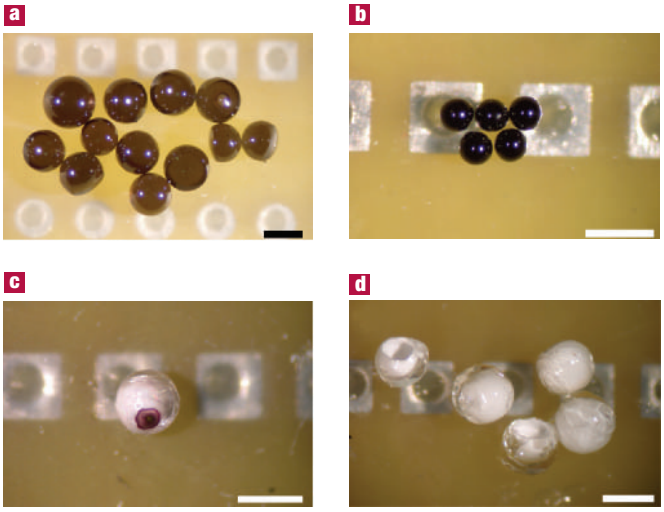

Figure 4 Polymer-based and polymer-encapsulated particles. a, Polymerized SU-8 droplets laced with uniformly dispersed gold nanoparticles. **b**, Solid semiconductive polypyrrole spheres. **c**, Dried 'eyeball' supraparticle encapsulated in polymerized HDDA. **d**, 'Cups' from polymerized HDDA with polystyrene latex deposited inside by drying of the originally encapsulated water droplets. Scale bars, 1 mm.

cell sorting. The closed and open capsules have potential in drug delivery, by using biocompatible and biodegradable polymers in the shells and by replacing the inner particle core with biologically active components.

In conclusion, we demonstrate how multiple types of novel particles can be synthesized in on-chip droplet microreactors. The method could be used to entrap and manipulate both the liquid droplets and the resultant solid particles by electric fields. Thus, we were able to realize a process that allows the electrically controlled and potentially automated microfabrication of materials from liquid droplet precursors. We demonstrate eyeball and striped particles of a complexity that we believe has not been achieved before in self-assembled structures, semiconductive and semitransparent polymer particles and closed and open capsules. However, the full potential of similar techniques for electric field controlled on-chip materials synthesis is yet to be realized.

METHODS

The electrodes and the electrical leads for the chips were fabricated on two-sided printed circuit boards with electrode patches on one side and the connecting leads on the other. The electrode boards were immersed inside small Petri dishes with FC-70, an inert, high-density fluorinated liquid primarily composed of perfluoropentadecane (Fisher Scientific, Pittsburgh, Pennsylvania, USA). Droplets of volume 500–2,000 nl were dropped onto the F-oil with a 0.1–2.5-μl ultramicropipette. The droplets floated at the F-oil/air surface and the distance between the bottom of the droplets and the electrodes was sustained between 0.4 and 0.7 mm by adjusting the depth of the F-oil.

The droplets were composed of aqueous dispersions of sulphate-stabilized polystyrene latex, silica microspheres or gold nanoparticles (or mixtures thereof), aqueous solutions of polypyrrole (Sigma-Aldrich, St. Louis, Missouri, USA), crosslinkable 1,6-hexanediol diacrylate monomer (Sigma-Aldrich) or SU-8 25 photocurable epoxy resin (Microchem, Newton, Massachusetts, USA). The polystyrene latex was obtained from Interfacial Dynamics (Portland, Oregon, USA), and the fluorescent polystyrene latexes were obtained from Molecular Probes (Eugene, Oregon, USA). The silica microspheres were obtained from Bangs Laboratories, Fishers, Indiana, USA). The gold nanoparticles were synthesized by the reduction of hydrogen tetrachloroaurate ($HAuCl_4$) with sodium citrate in the presence of tannic acid[24] (all reagents were used as purchased from Sigma-Aldrich). The resulting suspension had particles 12–15 nm in diameter with a concentration of ~10^{12} particles ml^{-1}.

The supraparticles formed by droplet evaporation were dried on-chip in the presence of Drierite desiccant (Fisher Scientific). The polymer supraparticles formed from HDDA and SU-8 25 were photopolymerized on the chip by exposure to 365-nm UV radiation for 30–40 min with a B-100A UV lamp (Blak-Ray, Upland, California, USA). The HDDA was polymerized in the presence of an initiator comprised of 3% Igracure 184 (CIBA Specialty Chemicals, Tarrytown, New York, USA), 0.3% Igracure 819 (CIBA) and 1% divinylbenzene (Sigma-Aldrich).

Electrostatic calculations were performed with TRICOMP, a two-dimensional finite-element software with three interactive modules. The software module Mesh creates a variable triangular mesh from the specified geometry, dividing the solution space into several small elements, wherein the material properties remain constant. There are therefore no elements shared between different objects or across boundary lines. Another software module, EStat, then calculates the potential in each element by weighing out the potentials of its neighbours. The weighing factors used are dependent on the electrical properties of the element (dielectric constants and conductivity). The potential distribution for the solution space is subsequently obtained by iterative calculations. The resulting field intensity profile can then be viewed in VEStat.

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Competing financial interests

The authors declare that they have no competing financial interests.