# On-chip manipulation of free droplets

Tiny free-floating drops can be driven across a liquid medium by an electric field.

'Lab-on-a-chip' systems resemble factories with permanently rigged pipes, but their prefabricated microchannels could have problems in delivering materials such as suspended particles, biological cells or proteins, which may adhere to the walls and clog the channels. More flexible microfluidic systems allow liquids to be transported as droplets on a solid surface[1–10], but these suffer from similar drawbacks where the droplets are in contact with solid walls. Here we describe a liquid–liquid microfluidic system for manipulating freely suspended microlitre- and nanolitre-sized droplets of water or hydrocarbon, which float on a denser, perfluorinated oil and are driven by an alternating or constant electric field applied by arrays of electrodes below the oil. These microfluidic chips could be used as a versatile tool in microscale transport and mixing and in chemical and materials synthesis.

In our liquid–liquid microfluidic device (Fig. 1), water or dodecane microdroplets float freely on a surface of fluorinated oil ('F-oil') and alternating (a.c.) and/or constant (d.c.) electric fields are applied through electrodes below the surface of the oil phase. Spatially inhomogeneous a.c. fields evoke dielectrophoretic force[11], which attracts polarizable objects to areas of high field intensity.

The droplets migrate to the energized electrodes and hover above them, trapped by the field (Fig. 2a). These droplets are readily moved by programmed switching of the voltage to different electrodes (see Fig. 2a, b and supplementary information). The voltages applied (200–600 V) are comparable to those used in other dielectrophoretic experiments. The droplet speed in a.c. mode scales with the square of the field intensity, in agreement with dielectrophoretic theory[11]. Because the droplets encounter only viscous resistance, they move with extremely low power dissipation, albeit more slowly than when in contact with electrodes on solid walls[8–10].

Water droplets are also attracted or repelled strongly by d.c. fields, moving with velocities as high as 2.0 mm $s^{-1}$. This indicates that they have significant charge and/or dipole moments and move by coulombic repulsion or attraction. Droplet charging and recharging, which possibly occurs by ion transfer through the F-oil, were observed routinely. We also found evidence of unexpectedly strong internal polarization in the droplets (to be discussed elsewhere). The strong charging effects allow hydrocarbon oil droplets to be manipulated as well; however, these do not respond to symmetric a.c. fields because of their low polarizability.

Crystalline shell-like balls form by precipitation of inorganic solids inside mixed droplets, and asymmetric microassemblies[9,12] result from drying of droplets containing microparticles and nanoparticles (Fig. 2c; see supplementary information); the chips could be used to manipulate the resulting suspended solid particles as well. By combining dodecane and water droplets in the presence of surfactant, we were able to encapsulate the water symmetrically inside a hydrocarbon shell (Fig. 2d, and see supplementary information).

The flexible microfluidic devices described here would be useful in a range of applications — for example, in the synthesis of materials and in biological microassays. More complex applications might include confining living cells or genetic material into individual droplet containers, and performing biochemical

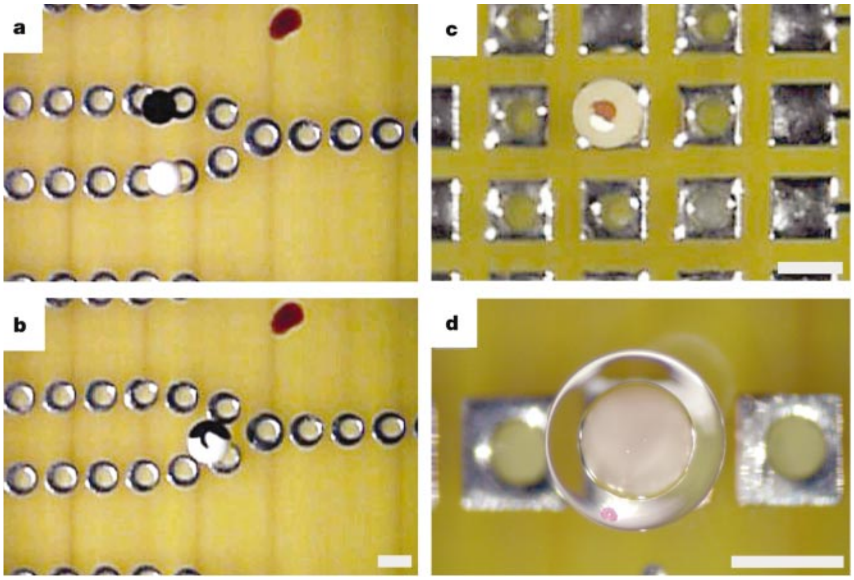


**Figure 2** Behaviour of freely suspended droplets above a liquid–liquid microfluidic chip (for movies, see supplementary information). **a,** Droplets (750 nanolitres) containing latex microspheres (white) and gold nanoparticles (purple) are transported; **b**, the droplets mix at the moment they touch near the track junction. The mixed droplet then continues further along the single track. **c**, Two droplets containing polystyrene (white) and magnetic (brown) latexes temporarily form an anisotropic-particle aggregate on mixing. **d**, Water droplet containing latex and 2 mM sodium dodecyl sulphate is encapsulated inside a liquid dodecane shell. Scale bars, 1 mm. Further details are available at http://crystal.che.ncsu.edu.

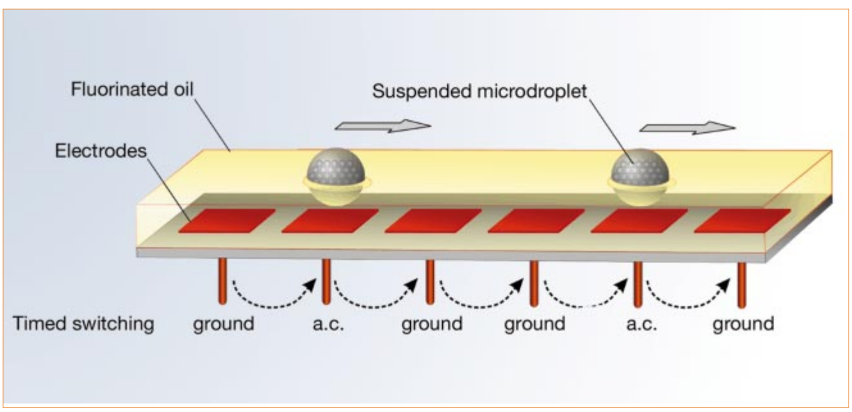


**Figure 1** Schematics of the dielectrophoretic transporter used for the operation of a liquid–liquid microfluidic chip with freely suspended droplets. Water or dodecane droplets of volume 500–1,000 nanolitres float freely on the surface of perfluoromethyldecalin ('F-oil', an inert, dense liquid)[13]. Alternating (a.c.) and/or constant electric fields are applied by arrays of electrodes immersed in the F-oil phase a few millimetres below the surface.

reactions, precipitation assays or parallel drug or toxin screening.

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Palaeontology

# A polydactylous amniote from the Triassic period

The earliest four-limbed vertebrates, or tetrapods, lived between 370 million and 354 million years ago, during the Late Devonian period, and typically had more than five digits (polydactyly)[1]. We have discovered that a preaxial form of polydactyly, in which extra digits are positioned anterior to the first digit, has unexpectedly re-emerged in a marine reptile from the Early Triassic period about 242 million years ago — the overall morphology of both the manus and pes closely resemble those of the earliest tetrapods. Until now, no post-Devonian tetrapod has been found with a comparative type of polydactyly, so the new amniote provides a striking example of convergent evolution.

The new amniote is a marine reptile from Hubei Province in China (amniotes include reptiles, birds and mammals). Its most remarkable feature is the number of its digits: the forelimbs bear seven and the hindlimbs have six (Fig. 1). The extra digits on both fore- and hindlimbs are well developed and the bones are arranged normally as distal carpal/tarsal (distal carpals 3 and 4 are fused as a single large carpal in the forelimb of the new amniote), metacarpal/metatarsal and phalanges (see supplementary information).

Ichthyosaurs of the Mesozoic era (250–65 million years ago) had porpoise-like bodies, with dorsal and tail fins and often polydactylous limbs. This polydactyly, however, was quite different from that of the Devonian tetrapods. Modern pandas and moles[2], humans[2,3] and cats[4] occasionally have extra preaxial digits, but these are rarely morphologically or structurally comparable with a normal digit[2,3,5].

Almost all polydactyly in tetrapods can be referred to one of three types. In postaxial polydactyly, the extra digits are posterior to digit V, as seen in the Late Devonian *Tulerpeton*[6], some frog individuals and even humans[2,3]. In preaxial polydactyly, the extra digits are anterior to digit I, as seen in the fore- and hindlimbs of the new amniote, and in the hindlimbs of the Late Devonian *Ichthyostega* and forelimbs of the Late Devonian *Acanthostega*[1]. In bilateral polydactyly, the extra digits are anterior to digit I and posterior to digit V, as seen in the forelimbs of the ophthalmosaurian ichthyosaurs[7] and today in some polydactylous Indian families[3]; the extra digits on the hindlimbs of the Devonian *Acanthostega*[8] also appear to be of this type.

Other types of polydactyly can occur, for example in the forelimbs of many non-ophthalmosaurian ichthyosaurs[7]. Most occur by interdigital or postaxial phalangeal bifurcation[7]. Of the known polydactylous tetrapods, the new amniote is the only one that has both fore- and hindlimbs that are preaxially polydactylous, matching the current limb-development model[9] (see supplementary information).

The new amniote was a secondarily aquatic reptile and its polydactylous limbs are derived from adaptation to its aquatic life. Its manus and pes are short and wide, and generally resemble those of the Late Devonian *Ichthyostega* and *Acanthostega*. They are also comparable in shape to the limb-like paired fins of extant frogfishes[8,10]. The limbs of this amniote may have functioned in a similar way to those of the Devonian tetrapods or to the paired fins of frogfishes when moving across underwater substrates. In its morphology and way of life, the new amniote provides a good example of evolutionary convergence with the earliest tetrapods, as well as an analogy with frogfishes in vertebrate evolution.

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**Figure 1** The Triassic polydactylous amniote, housed at the Shanghai Science and Technology Museum (specimen SSTM 5025). It is represented by the part and counterpart of an almost complete skeleton from which the anterior end of the snout and the tail tip are missing. The specimen was collected from the marine Jialingjiang Formation (late Early Triassic[11]) near Xunjiansi, Nanzhang County, Hubei Province, China. Taxonomically, SSTM 5025 is referred to Nanchangosauridae, Wang, 1959 of Hupehsuchia Young & Dong, 1972 in Diapsida Gauthier *et al.*, 1988 of Reptilia Gauthier *et al.*, 1988. Further details are available from X.-C. W. Scale bar, 10 cm.

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