# EWOD-driven droplet microfluidic device integrated with optoelectronic tweezers as an automated platform for cellular isolation and analysis<sup>†</sup>

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We report the integration of two technologies: droplet microfluidics using electrowetting-on-dielectric (EWOD) and individual particle manipulation using optoelectronic tweezers (OET)—in one microfluidic device. The integrated device successfully demonstrates a sequence involving both EWOD and OET operations. We encountered various challenges during integration of the two different technologies and present how they are addressed. To show the applicability of the device in cellular biology, live HeLa cells are used in the experiments. The unique advantages of EWOD and OET make their integration a significant step towards a powerful tool for many applications, such as single cell studies involving multiplexed environmental stimuli.

# **Background and motivation**

## Manipulation of individual particles in solution

The ability to manipulate specific cells individually and isolate them is attractive for cellular biology, facilitating, for instance, the study of single cell behavior and cell-to-cell interactions. Microfluidic systems with an ability to control cells and cellular microenvironments have been playing an increasingly important role in such studies.<sup>1,2</sup> Various mechanisms have been employed to manipulate cells, e.g. magnetic,<sup>3,4</sup> optical,<sup>5,6</sup> acoustic,<sup>7</sup> electrical<sup>8</sup> (e.g. electrophoretic,<sup>9</sup> dielectrophoretic (DEP)<sup>10,11</sup>) and hydrodynamic.<sup>12</sup> However, most of the manipulation techniques mentioned above cannot achieve high resolution, high throughput and low powerconsumption at the same time. For instance, although optical tweezers<sup>13</sup> are a powerful technology for trapping cellular and organic particles, particularly for single particle manipulation, they have limitations such as the tendency for photodamage and the trade-off between resolution and operational area.14 Dielectrophoresis-based techniques,<sup>15,16,8</sup> on the other hand, typically have large operational area but suffer from limited flexibility of manipulation due to fixed electrode patterns, making it difficult to manipulate a specific cell from within a population.

Optoelectronic tweezers (OET) are an exciting technique that combines many of the advantages of optical tweezers and DEP.<sup>17</sup> By optically controlling the electric field distribution in the device, OET uses optical intensities of only 0.01 to 1 W cm<sup>-2</sup>, drastically reduced from that of optical tweezers, allowing the use of a computer

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projector or LEDs. In addition, lenses with low numerical apertures (NA) can be used, thus increasing the manipulation area. Thus, OET retains the flexibility and dynamic control of optical trapping, making individual manipulation of specific particles using real-time feedback possible over a relatively large area, based on not just physical or electrical, but also optical properties.<sup>18,19</sup>

## Cell studies in droplet microfluidics

In addition to individual cell manipulation, single cell studies often require subjecting individual cells to different environmental conditions and stimuli, such as a range of concentrations of nutrients or drugs, or varying mechanical or electrical signals. Although isolation of individual cells has been achieved with continuous microfluidics, no viable approach has been proposed to independently control the environmental conditions of each isolated cell in the same device. While many reports involved the study of cellular response to single or a small number of stimuli,<sup>20,21</sup> there have been just a few attempts to conduct multiplexed assays,<sup>22,23</sup> since the latter involves a complex network of pumps, microvalves, latches and interconnected channels.<sup>24</sup> On the other hand, isolation of environments for individual cells is rather natural in the case of droplet microfluidics,<sup>25</sup> such as that driven by electrowetting-on-dielectric (EWOD),<sup>26-28</sup> since each isolated droplet can be supplied with independent multiplexed stimuli. Aqueous droplets in air or oil can be actuated by electrowetting,<sup>29</sup> DEP,<sup>30,31</sup> or a combination of the two. By appropriately choosing the actuation sequence, precise and independent control of multiple droplets can be achieved while minimizing cross-contamination.

Target separation is a key capability desired to make EWOD a powerful lab-on-a-chip platform. After isolating the target particles from non-target particles within a droplet by the differences in their properties (*e.g.* electrophoretic,<sup>32</sup> DEP,<sup>33</sup> and magnetic<sup>34,35</sup>), one can split the droplet into a target-concentrated and a target-depleted daughter droplet by EWOD actuation to the mother droplet. While some biochemical applications of EWOD have recently been shown,<sup>36–38</sup> actuation of droplets containing live cells have not been dealt with until very recently.

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<sup>†</sup> Electronic Supplementary Information (ESI) available: (1) Discussion of challenges faced by first generation integrated device; (2) schematic of the experimental setup; (3) video of the sequence of EWOD and OET operation on the integrated device. See DOI: 10.1039/b821508a/

For example, DEP force was used to concentrate cells<sup>39,40</sup> on an EWOD device immersed in oil. The addition of a small amount of surfactants, such as Pluronic F68, prevents device surface fouling by cells without the use of oil<sup>41</sup> with minimal effects on cell vitality. Magnetic separation of cells labeled with magnetic beads on an EWOD device in an air environment has also been demonstrated using the surfactant approach of minimizing surface fouling.<sup>42</sup>

## Integrating EWOD and OET

As discussed above, so far the cell manipulation techniques to manipulate individual particles within a population could not be exercised under the EWOD-based droplet microfluidic platform.



**Fig. 1** An envisioned application of the integrated device in cellular biology, where each droplet can present an isolated microenvironment to the cell(s) it contains. (1a) Drugs, nutrients and/or salts can be introduced in the form of droplets independently to each microenvironment. (1b) Cells are collected by parallel LOET manipulation using a dynamic optical pattern generated using real-time image feedback. (1c–1d) Waste/ old medium can be removed by droplet splitting using EWOD. (2a–3a) The steps (a–d) can be repeated to introduce other drugs, nutrients *etc.* or combinations thereof, and to remove the old medium in the form of waste droplets while retaining the cells.

On the other hand, while OET may allow individual particle manipulation under the usual continuous microfluidics, isolating them into discrete environments is challenging. A device that combines the unique capabilities of OET cell manipulation (*i.e.*, specific, dynamic, individual manipulation over a relatively large area) and EWOD microfluidics (*i.e.*, generation and manipulation of discrete droplets on chip by only electric signals) would be a valuable tool for research in cellular biology.

For example, the droplets can act as microchambers providing controlled microenvironments for the independent and multi-parameter study of the isolated cell(s). Reagents containing various concentrations of drugs/nutrients/salts can be introduced to each of the droplets independently. Old/waste medium can be removed and fresh medium replenished using EWOD operations of cutting and merging, as envisioned in Fig. 1. Moreover, specific cell(s) can be picked from the rest of the population based on differences in optical or dielectric properties using OET, and isolated into a separate droplet using EWOD. Integration of EWOD and OET has first been introduced in a preliminary report<sup>43</sup> demonstrating both the operations on the same device, albeit separately. However, a complete sequence of the combined EWOD-OET operations was not possible due to the mutual exclusivity of EWOD and OET operational areas. In this work, we overcome the limitations by developing new device designs and incorporating a modified OET mechanism. A complete, uninterrupted sequence consisting of successive operations of EWOD, OET, and then EWOD again, demonstrates the success.

# Device design

Both EWOD and OET are now relatively well-established technologies, having been independently used for a variety of applications.<sup>44,45</sup> For the details of principle and theory of each of these technologies, the reader is referred to earlier reports.<sup>27,14</sup> In this paper, we focus on the design and fabrication of the integrated EWOD-OET device. We present the challenges encountered in prior attempts (first-generation device<sup>43</sup>) and how the new device overcomes them.

#### First-generation integrated device

We first briefly review the first-generation integrated device<sup>43</sup> in order to highlight the challenges it faced. As seen in Fig. 2(a), the device consisted of two chips (referred to as "EWOD chip" and "OET chip" according to what actuation voltage is supplied to them), held apart by a spacer. A standard OET device was used for the OET chip.<sup>41</sup> The EWOD chip was designed similar to the standard EWOD device<sup>27</sup> except for some modifications needed to incorporate OET. One of the electrodes ("OET electrode") was dedicated to act as the reference electrode for the OET voltage, defining the "OET region" (Fig. 2(a)).43 To ensure that the OET-driving electric field was applied across the droplet in the illuminated state, the dielectric layer over the OET electrode was removed. Droplet manipulation into and out of the dielectric-free OET region was performed using surrounding EWOD electrodes, where OET could not be operated due to the presence of the dielectric.



**Fig. 2** Comparison of the first- and second-generation EWOD-OET integrated device (not to scale). (a) The first generation<sup>43</sup> combines EWOD and OET, as shown in cross-section (left) and top view (right). The OET chip has unpatterned ITO, photoconductive and hydrophobic layers. The EWOD chip has electrodes defined in the ITO layer, coated with dielectric and hydrophobic layers. The dielectric layer in the EWOD chip is patterned in the "OET region" so as to allow the voltage drop in the droplet required for OET particle manipulation. OET is non-operational in regions where the dielectric layer is present, *i.e.* the "EWOD region", where EWOD microfluidics can be performed. In general, a hydrophilic opening is made over none, some or all the OET region. (b) The second generation combines EWOD and new LOET, as shown in cross-section (left) and top view (right). The interdigitated LOET electrodes define the LOET actuation area, overlapping the EWOD actuation electrodes. Unlike the first-generation design, LOET actuation does not require any modification on the EWOD chip; hence, there are no mutually exclusive EWOD or OET regions. (EWOD lead wires not shown for clarity).

#### Challenges faced by the first-generation device

Although the steps of both EWOD and OET were individually demonstrated on the first-generation device,43 the mutually exclusive nature of their operational areas led to difficulties in demonstrating the complete sequence of EWOD-OET operations. Specifically, the lack of OET in the EWOD region restricts particle manipulation to the relatively small OET region only, while the lack of EWOD over the OET region entails difficulties for sustaining the particle distribution against the viscous drag force from the fluidic movement in the surrounding liquid. The viscous drag can be estimated using Stokes' equation, which indicates that the viscous drag force on a particle inside a viscous fluid is proportional to the fluid velocity. It was found that the inherently stronger flow along the free liquid-air interface in droplet microfluidics<sup>46</sup> transports the particles that are swept into suspension by the receding meniscus, preventing them from being introduced into the OET region. These limitations are described in more detail in the ESI.<sup>†</sup>

Recognizing that this approach has difficulties arising from the mutual exclusiveness of the EWOD and OET regions, we propose the second-generation device, which incorporates a lateral-field OET (LOET) chip<sup>14,47</sup> with an unmodified EWOD chip.

## Second-generation device: EWOD-LOET

Fig. 2(b) illustrates the second-generation integrated device. The issues identified above are resolved by replacing the standard

OET chip with an LOET chip, which is essentially a coplanar OET device, *i.e.* both the electrodes for OET operation are on the same substrate. Unlike the standard OET device, two arrays of interdigitated electrodes are patterned in the metal layer under the photoconductive layer of the LOET device.<sup>47</sup> The OET driving voltage is applied between the two arrays of interdigitated electrodes. In the dark state, most of the electric field drops across the photoconductive amorphous silicon (a-Si) layer instead of the droplet. When illuminated, the resistivity of the photoconductive layer drops so as to produce a lateral electric field gradient in the droplet between the interdigitated electrodes. The modulation of the electric field distribution within the liquid is therefore still driven by the optical pattern. An advantage of LOET over the standard two-substrate OET is that the single sided LOET can be used with a wide variety of opposing surfaces facilitating its integration with other microdevices.14 LOET thus eliminates the issues faced in the first-generation device. Since both the high and ground for the OET voltage are on the OET chip itself, the other (*i.e.* EWOD) chip no longer needs to accommodate OET in it (*i.e.* no patterning of the dielectric or hydrophobic layers is required). This new approach not only eliminates the "OET (only)" region of the first-generation device but also expands the OET capability into the previously "EWOD (only)" regions. Since the EWOD and OET regions are no longer mutually exclusive, the problems described for the first-generation device are no longer encountered in the second-generation device.

## Materials and methods

## **Device fabrication**

Both EWOD and LOET chips were fabricated using standard IC microfabrication processes. To fabricate the EWOD chip, EWOD electrodes (1 mm<sup>2</sup>) were defined of an indium-tin oxide (ITO) (1400 Å) layer on a 700 µm-thick glass substrate (Tech-Gophers Inc.). Cr/Au (~100/1000 Å) was deposited and patterned to define the contact pads and electrode labels for easier visualization. Next, a silicon nitride layer ( $\sim 1 \mu m$ ) was deposited using plasma-enhanced chemical vapor deposition (PECVD) and patterned to define the dielectric layer. A Cytop® (Asahi Inc.) layer ( $\sim 1 \mu m$ ) was spin-coated on top and annealed at 200 °C to make the surface hydrophobic.

The LOET chip was fabricated on a silicon wafer. After thermal oxidation to form a silicon dioxide layer (1 µm) for electrical isolation, the LOET electrodes are created by electronbeam evaporation of aluminium using a lift-off process. A hydrogenated amorphous silicon (a-Si:H) layer (0.8 µm) is deposited over the aluminium electrodes using PECVD. The a-Si over the electrical bias pads is removed by reactive-ion etching. A thin layer of Teflon AF® was spin-coated on the a-Si and annealed at 150 °C, rendering the surface hydrophobic.

Since the OET force is strongest near the LOET chip and the particles/cells settle due to gravity, the LOET chip was placed on the bottom with the EWOD chip on top. Double-sided adhesive tape (thickness  $\sim 100 \,\mu\text{m}$ ) was used as the spacer between the two chips.

## **Experimental setup**

The integrated EWOD-LOET device was mounted on the optical setup used for OET operations (schematic of the setup is shown in the ESI).<sup>†</sup> The output of a computer projector (Dell 2400MP) was collimated and focused onto the LOET chip through a  $10 \times$ objective lens. This lens also serves as the observation objective. A fiber illumination is used to provide the background illumination necessary to view the microscale particles, and a CCD camera is used to capture the microscope images.

The EWOD and LOET devices were connected to their respective voltage supplies. A driving voltage of 3-5 V<sub>rms</sub> at 200 kHz was used for LOET actuation. A driving voltage of  $\sim 120$ V<sub>rms</sub> at 20 kHz was used for EWOD actuation, although most of the applied potential is expected to fall across the dielectric layer and does not affect the interior of the droplet. During the OET manipulation, one of the electrode arrays was connected to the LOET voltage supply, and the other was grounded. During the EWOD fluidic operation, the LOET electrodes act as the ground. Although only one array of LOET electrodes can provide the grounding, EWOD actuation was found to be more effective when both of the LOET arrays were grounded, particularly during droplet cutting.

## Reagents used

In order to show the applicability of the integrated EWOD-OET device for biological applications, live HeLa cells suspended in an isotonic buffer containing 8.5% sucrose and 0.3% dextrose in DI water ( $\sigma = 5 \text{ mS m}^{-1}$ ) were chosen for the experiment. As described in ref. 14, the complex permittivity of the live cells with

respect to the medium result in "positive OET" for the frequency of the electric field used. The "positive OET" means that the particles are attracted to the electric field maxima, *i.e.* regions illuminated by the optical pattern. The hydrophobic surface required for EWOD actuation is highly prone to fouling from the cells. The addition of small quantities of surfactants alleviates the fouling to allow actuation of cell samples on EWOD.41,42 Therefore,  $\sim 0.2\%$  Pluronic surfactant F68 (Sigma-Aldrich) was added to the solution.

## **Results and discussion**

## **Experimental results**

We demonstrate the sequential operations of EWOD-driven droplet microfluidics and LOET-driven optically controlled cell manipulation on the same device, without any mutually exclusive regions of operation on our integrated device. Fig. 3 shows the schematic of the sequence of the sample EWOD-OET operations performed. As discussed earlier ("Device design"), it is important to ensure that the cell separation or distribution generated by OET is not disrupted by the subsequent microfluidic movements. To prevent the disruption, droplet positioning before the LOET steps, location of the collection region during the LOET steps, and the splitting sequence after the LOET operations must be chosen appropriately.

The droplet position prior to LOET manipulation was experimentally determined, so as to minimize the collected cells from being re-distributed by the droplet movement during the subsequent cut. The droplet is first positioned accordingly using EWOD (Fig. 3(a-b)). Next, LOET operations are performed on the particles using a dynamic optical pattern, in this case to move the particles from the right ("depleted") to the left ("collected") regions of the droplet (Fig. 3(b-c)). Multiple sweeps may be performed to cover the entire droplet if the LOET operational area is small relative to the droplet size (Fig. 3(c-d)). After the LOET operations are completed, the droplet is split using EWOD (Fig. 3(e)) into the "collected" droplet containing most of the target cells and the "depleted" droplet depleted of the cells (Fig. 3(f)).

Fig. 4 and 5 show image sequences from the actual experiment. Images for the LOET particle manipulation (Fig. 5) were recorded using the CCD camera, which is part of the optical setup. Since the field of view through the  $10 \times$  objective used for LOET (~0.5 mm  $\times$  0.4 mm) was not large enough to capture the entire droplet movements by EWOD, a lower magnification video camera was used (Fig. 4).

A droplet (~350 nL) containing live HeLa cells (~6  $\times$  10<sup>4</sup>  $mL^{-1}$ ) is placed on the device (Fig. 4(a)) sandwiched between the two chips. As discussed above, the droplet is stretched by EWOD so as to position it for the subsequent steps (Fig. 4(b)). Next, cell manipulations are performed using LOET, viz. cells are collected leftwards by sweeping a computer-generated optical pattern from right to left across the field of view (Fig. 4(b-c)). The bright spot in Fig. 4(b-d) shows the entire illuminated area; the optical pattern within the illuminated area is seen more clearly in Fig. 5. Whereas a manually controlled rectangular bar pattern is used in this simplified demonstration to collect and move cells across the droplet, more complex dynamic optical patterns can be readily



**Fig. 3** Schematic representation of the sequential EWOD and OET operations performed on the integrated device. The thick (blue) arrows indicate droplet movement by EWOD, while the thin (red) arrows indicate the movement of the OET optical pattern. (a) A droplet containing cells is placed in the device. (b) To avoid disturbing the LOET-generated particle distribution due to hydrodynamic forces during the subsequent cut, the droplet is brought into a stretched position by EWOD prior to LOET operations. The LOET optical pattern is then projected and moved across the droplet to collect the cells on the left. (c) If the droplet dimensions are larger than the LOET area of operation, this may be repeated over multiple regions to cover the entire droplet. (d) The cells are collected in one (collected) region, and (e–f) the droplet is subsequently split using EWOD into the collected droplet and the depleted droplet.

generated (*e.g.* based on real-time image-feedback) to perform much more intricate cell manipulations.<sup>19,43</sup>

In Fig. 5, the EWOD electrode patterns, outlined by white broken lines, can be used as reference to see the movement of cells by LOET. Since the cells are acted upon by positive DEP, they get attracted to the illuminated optical pattern (Fig. 5(a)). As the pattern moves to the left (indicated by the dark (red) arrow), the cells it crosses get collected and move along with the pattern (Fig. 5(b–c)). The image collects more cells as it moves leftwards across electrodes to get to the collection area (Fig. 5(d–f)). As the LOET illuminated area is smaller than the

electrode dimensions, multiple sweeps across the droplet are performed, each time collecting the cells in the collected region (Fig. 4(c-d)), at a location away from the droplet edge to minimize disturbance due to the stronger flow along the free liquid-air interface. After the LOET operations are completed, the droplet is split by EWOD, further stretching it in both directions (Fig. 4(e)) into the collected (left) and depleted (right) daughter droplets (Fig. 4(f)).

In the present experiment, ~19 live cells were present in the original droplet, out of which about 13 were collected into the collected droplet and 6 were left in the depleted droplet. While most cells move for a short distance (0.5–1 mm), they tend to get stuck over the relatively long distance (up to 2–3 mm) of manipulation required due to the large electrode dimensions in the current device. Dirt particles present in the path can also block the cell movement.

#### **Discussion of results**

A combined sequence of EWOD and OET operations for cell handling as shown in this sample demonstration is a significant development towards a powerful integrated device for cell studies. By eliminating mutually exclusive regions for OET and EWOD by using LOET, we have solved the fundamental challenges in combining the two techniques and their unique benefits.

All experiments in this report were performed with air as the surrounding medium. When the device is immersed in oil<sup>48–50</sup> rather than dry in air,<sup>29,51,27</sup> a thin layer of oil present between the hydrophobic device surface and the aqueous droplet<sup>52</sup> greatly reduces the resistance against droplet sliding, making most of the basic EWOD operations easier. The thin oil layer also separates the particles in the droplet from the device surface, preventing their adhesion on the surface.<sup>52</sup> In addition, the surrounding oil helps reduce evaporation of the EWOD droplets, helping to maintain their size and concentration. Despite the conveniences, there have been some concerns regarding the use of silicone oil, particularly in biological applications such as cell studies:

a. The surrounding silicone oil may restrict the exchange of gases between the droplets and the atmosphere, which may be unfavorable for biological cells in the droplet. The concerns include a build-up of carbon dioxide in the droplets.

b. If the application requires certain events to be performed directly on the device surface, such as electrochemical sensors patterned on the surface, the thin layer of oil could hinder the performance.

c. Ensuring that the oil does not leak makes the packaging of the device much more challenging.

The increased likelihood of evaporation for the air-environment case (compared to the oil-environment case) is a concern as it may lead to a change in concentration of nutrients and salts, which could adversely affect the cell viability. Droplet evaporation is minimized by sealing the gap between the device chips using a sealing tape so as to locally saturate the surrounding air with water vapor.<sup>53</sup> Although not utilized in the present report, significant prevention of evaporation during much longer experiments (a few hours, as opposed to several minutes in our case) has been reported using a humidified atmosphere to store devices between actuation steps.<sup>41</sup> Likewise, we plan to use a humidified chamber for future experiments, particularly those involving longer periods of operation.



**Fig. 4** Images showing the sequential operation of EWOD and LOET on the integrated device. Droplet manipulations are performed by EWOD (a-b) before and (e-f) after the (b-d) cell manipulation by LOET. Broken white lines are drawn to indicate droplet shape. The thick (blue) arrows seen in (a) and (e) indicate EWOD actuation while the thin (orange) arrows seen in (b-d) indicate LOET actuation. (a) Droplet ( $\sim$ 350 nL) containing HeLa cells is placed on the integrated device. (b) The droplet is positioned such that the LOET-generated cell distribution is not disturbed during the subsequent droplet splitting. Starting from the right of the stretched droplet, (c) the cells are moved from right to left by LOET. (d) The cell manipulation is performed across the length and width of the droplet by moving the LOET objective lens with respect to the device, so as to collect most of the cells in the left-side of the droplet. (The bright spot from the illumination for LOET indicates its entire operational area. The computer-generated optical pattern used for LOET manipulation within the area is shown in Fig. 5). (e) After collection, the droplet is being split by EWOD. (f) The droplet has been split into the collected (left) and depleted (right) droplets.

It is worth discussing the effect of EWOD and OET (DEP) actuations, and the unusual buffer used in the current experiments, on cell vitality. As discussed in "Background and motivation", the low concentration of Pluronic F68 has been shown to have little effect on cell viability.<sup>41</sup> Additionally, because most of the EWOD voltage drops across the relatively thick dielectric and hydrophobic layers, the cells are expected to remain largely unaffected from the EWOD actuation voltage.<sup>41</sup>

DEP force has been used to safely filter and retain cells while maintaining viability in perfusion cultures.<sup>54</sup> The use of positive DEP for OET actuation limits the conductivity of the buffer that can be used in the present experiments, leading to the choice of the isotonic sucrose/dextrose buffer. Although less favorable for cells than saline buffers and media, it has been reported that if the isotonic buffer is replenished every hour or so, no significant loss of viability is observed even under DEP actuation.<sup>55</sup> Similar commercially available buffers (*e.g.* Cytoporation<sup>®</sup> medium CP-T from Cyto Pulse Sciences Inc.) meant for electroporation of live cells further suggest the viability of cells in the isotonic buffer, provided adequate recycling.

The limitations of the present work of integration and future steps to overcome them are discussed below. The dimensions of the EWOD electrodes in the integrated device were arbitrarily chosen to be  $1 \text{ mm} \times 1 \text{ mm}$ , since this is the typical geometry for EWOD devices. Noting the droplet is placed over two electrodes, the 1 mm electrodes entail that the cells must be manipulated by

LOET over relatively long distances (up to 2–3 mm). Even though the integrated movement was successfully demonstrated for many cells, the task was challenging, particularly for the positive DEP case where cells are being attracted to the substrate, increasing the likelihood of adhesion. Even though most cells were able to traverse over 0.5–1 mm, some got stuck over larger distances, making LOET ineffective in moving them. A simple solution to the problem of long-distance LOET travel would be to scale down the EWOD electrode dimensions, such that LOET manipulation is over a shorter distance.

The LOET electrode fingers were 10 µm wide with a gap of 5  $\mu$ m. As such, the cells (~8  $\mu$ m diameter) could not be moved in the direction perpendicular to the fingers in the current device. This limitation is, however, not fundamental. Sufficiently small LOET electrode geometry can ensure twodimensional manipulation, as has been demonstrated by choosing a line width and gap smaller than the particle size.<sup>14</sup> Since particle manipulation is still driven by the optical pattern, individual particle manipulation is achievable with the LOET chip. Although mammalian cells have been used in the present demonstration, the same technique can be extended to other cell types such as bacteria, yeast, etc. On the other hand, sub-micron particles such as viruses (typically  $\sim 100$  nm in all dimensions) pose a much greater challenge due to the  $r^3$  scaling of DEP force and the increased significance of Brownian motion relative to it.56



Fig. 5 Image sequence (as seen through the LOET objective lens) showing the cell manipulation by LOET and the optical pattern used for it. Broken white lines indicate the EWOD electrode features. The dark (red) arrows indicate the direction of movement of the LOET optical pattern. (a) The pattern used here is a simple rectangle (manually controlled by mouse). Since the cells experience positive OET at the frequency used, they are attracted to the pattern. (b) As it moves to the left, the pattern collects more cells. (c) The movement of the cells with the pattern can be seen with respect to the EWOD electrode features and the droplet meniscus. (d–f) The pattern sweeps across multiple EWOD electrodes starting from the right of the droplet, collecting the cells in the collected region at the left (See also Fig. 4(b-d)).

With suitable optimizations to improve the performance for a given application, the integrated EWOD-LOET device can become a valuable tool for the cellular biology.

#### Conclusion

We successfully demonstrated a sequence of EWOD and OET operations for a HeLa cell sample on an integrated device. The fundamental issues faced by previous attempts to make such a device were discussed, and the new design employing lateralfield OET (LOET) to overcome the challenges was described. Both EWOD and OET have unique and valuable characteristics that make the reported integration promising. In particular, the parallel manipulation of specific cells using OET with a low power over a relatively large area, combined with the electrically reprogrammable microfluidic operations of independent droplets using EWOD, would enable isolated cell studies involving variations of multiple environmental stimuli.

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