Electronic Supplementary Information for

EWOD-driven droplet microfluidic device integrated with optoelectronic tweezers as an automated platform for cellular isolation and analysis

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1. Challenges faced by the first-generation device

Although the steps of both EWOD and OET were individually demonstrated on the first-generation device¹, 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 fluid.

Various modifications of the first-generation design to overcome the above difficulties have been tested before the second-generation. As EWOD manipulation is not possible over the OET electrode, microfluidic operations are performed with the help of EWOD electrodes surrounding it (Figure 1(b)). Since manipulation of wetting properties is not possible in the OET region, its hydrophobicity must be chosen carefully. Three possibilities were considered based on the size of the hydrophilic opening, as discussed below, along with the respective challenges. Figure E1 illustrates these scenarios for a droplet being moved towards the right using EWOD electrodes surrounding the OET region. The bold blue dashed arrows along the droplet edge represent the higher flow velocities along the liquid-air interface, typical of droplet microfluidics in air². The black dashes sketch the droplet outline after it has moved to the right.

Case 1 has the entire OET region hydrophobic with no hydrophilic opening (Case 1 in Figure E1). If the hydrophobic layer is made sufficiently thin, it does not significantly affect the electric field across the droplet as required for OET. It has been shown that the droplet can be introduced into the non-electrowettable hydrophobic OET region with the help of surrounding EWOD electrodes. However, there is a strong tendency for the fluid to de-wet the hydrophobic OET region, towards the relatively hydrophilic electrowetted surfaces. Even if OET is kept on during this process, the holding force at the scale of cells (1 to 10 μm) is too weak (10⁻¹¹ to 10⁻¹² N)³ to oppose particle movement against the dominant interfacial forces (> 10⁻⁹ N)⁴ and the resultant fluidically-driven transport, which disturb the OET-generated particle rearrangement.

Case 2 has the entire OET region hydrophilic (Case 2 in Figure E1). When the droplet is introduced, it readily wets and fills the hydrophilic OET region. Although OET manipulation can be performed on particles present inside the OET region, introducing the particles from outside the OET region is a problem. Since no OET is available outside the OET region, the only means to bring in the particles is by fluidics (i.e. by the viscous force due to fluid movement, which acts along the direction of flow). However, particles near the edge of the droplet (e.g. particle 1 in Figure E1(a)) tend to flow along the meniscus, due to the much lower resistance at the free liquid-air surface². As the droplet transitions to the final shape (black dashes) determined by the edge of the hydrophilic region, the particles that are swept into suspension by the receding meniscus also travel rightwards with the stronger flow along the edges, making it unfeasible to bring them into the OET region. Another contribution to the particles not entering the OET region could be the distortion of the streamlines (thin blue dashed arrows) away from the center due to the presence of the central hydrophilic region in the middle of the surrounding relatively hydrophobic surface.

Case 3 has OET region part hydrophilic and part hydrophobic (Cases 3A and 3B in Figure E1). In order to minimize the distortion of streamlines due to the hydrophilic site, while preventing the de-wetting of the entire OET region, only part of the OET region was made hydrophilic, leaving the rest covered with a (sufficiently thin) hydrophobic layer. Cases 3A and 3B show two possible shapes of the hydrophilic opening. The hydrophilic site does not allow the droplet meniscus to sweep across the entire OET region, preventing the complete disturbance of OET-driven particle rearrangement within the hydrophilic part of the OET (unlike Case 1). However, it was found that the inherently stronger flow along the free liquid-air interface in droplet microfluidics² transports the particles that are swept into suspension by the receding meniscus (e.g. particle 1) to the right, preventing them from being introduced into the OET region. As a result, no significant rearrangement of particles by OET can be achieved.

Recognizing that this approach has difficulties arising from the mutual exclusiveness of EWOD and OET regions, in the main manuscript we propose the second-generation device, which incorporates a lateral-field OET (LOET) chip⁵,⁶, with an unmodified EWOD chip.
Figure E1: The mutually exclusive EWOD and OET regions in the first generation integrated device posed fundamental challenges to integrated operations. Driving particles from outside the OET region for manipulation inside the OET region fluidically, as well as keeping the OET-generated rearrangement are challenging in each of the four cases. In each case, the droplet is moved to the right by EWOD. The final droplet shape is indicated by black dashes. Higher velocity along droplet edge is indicated by bold blue dashed arrows. **(Case 1)** Entire OET region is hydrophobic. Upon actuation of the EWOD electrodes, the hydrophobic OET region tends to be de-wetted, causing the receding meniscus to flow across it. Particles cannot be held back by OET against the interfacial force at the receding meniscus. **(Case 2)** Entire OET region is hydrophilic. Particles at the left of the OET region (e.g. 1) travel with the stronger flow along the edge to the right, and escape the OET region. Some distortion of the flow (thin blue dashed arrows) towards the edges could also be due to the hydrophilic OET region at the center. **(Cases 3A and 3B)** OET region is part hydrophilic and part hydrophobic. The presence of a hydrophilic site prevents the receding meniscus from completely sweeping across the OET region, unlike Case 1. The smaller hydrophilic site is expected to produce less distortion of the flow towards the edges, and hence away from the OET region at the center. However, due to the inherently stronger flow along the free liquid-air interface encountered in EWOD microfluidics, many particles (e.g. 1) travel with the flow from the left of the OET region to its right, making their manipulation by OET infeasible.
2. Schematic of experimental setup

![Figure E2: Setup used for the EWOD-LOET experiments. A computer projector is used to create optical manipulation patterns, and is focused onto the LOET substrate through a 10x objective lens. This lens also serves as the observation objective. A fiber illumination is used to provide the background illumination, and a CCD camera is used to capture the microscope images.]

3. Movie (attached separately):

A movie showing a sequence of EWOD and OET operations on the second generation integrated device is attached. It should be noted that during the OET manipulation (seen through the LOET objective) the OET force is constant throughout, even though the intensity of the optical pattern appears to be rising and falling. The capture rate of the CCD camera and the image refresh rate of the projector (Figure E2) were not synchronized, so as to enable continual monitoring of the cells during the manipulation.

References for ESI