# DIRECT TRANSPORTATION AND ELECTROFUSION OF OIL DROPLETS IN A MICROFLUIDIC DEVICE

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## ABSTRACT

A hybrid bio-electrical system for direct transportation and electrofusion of oil droplets is proposed. A motor protein system, kinesin/microtubule, was used to transport kinesin-coated oil droplets along microtubules oriented and immobilized on a glass surface. The attachment between the biotinylated-kinesin and the functionalized oil droplet was achieved using the biotin-streptavidin binding. Fusion of the oil droplets was accomplished by applying AC voltage on the electrodes placed inside a flow cell. Together with encapsulation of particles/reactants within oil droplets, this method may lead to the fabrication of a nanoscale reactor.

## 1. INTRODUCTION

Extensive research on microscale emulsions has been performed in the recent years [1]. To handle the emulsions, flow-based transportation in microfluidic and nanofluidic systems has been one of the main approach [2]. However, particularly in nanofluidic, pressure-driven transportation becomes increasingly difficult since flow per cross-sectional area, i.e., velocity, is proportional to the square of the channel radius for a given pressure difference. Direct transportation based on molecular motors has been proposed for such systems as an alternative method [3,4]. Kinesin is a molecular motor, which converts the chemical energy derived from the hydrolysis of ATP into mechanical work allowing them to move along filamentous tracks called microtubules (MTs). Molecular motors should offer important advantages compared to microfluidics and nanofluidics. Unlike in these latter systems, motors provide a means of transportation that is independent of pressure-driven flow or electrokinetic transport. Motors, on the other hand, are not bound by these size limitations. Thus, the incorporation of these molecular motors into synthetic nanodevices may form the basis for lab-on-a-chip systems of unprecedented miniaturization and complexity.

The present paper proposes a complete system to transport and fuse oil droplets (oil-in-water emulsion). As nanoparticles [5] or even lipophilic molecules [6] can be encapsulated within the droplets, direct transportation of those droplets allows us to carry different materials in different droplets and bring them into contact to fuse with electrical field allowing particles to interact without any liquid manipulation. The overall schematic view of the proposed system is represented in Fig. 1. Basic components of this system are the transportation of the kinesin-coated oil droplets along microtubules and electrofusion of these droplets. In this work, the feasibility of these two main components has been demonstrated.

## 2. PREPARATION AND SETUP

Two different setups were used in this work: microchannel and flow-cell systems. A microchannel system setup was used for orienting microtubules to have unidirectional motion of kinesin. For this setup, not only for transparency but also for easy integration with syringe pump, poly-dimethylsiloxane (PDMS, Dow Corning Silgard 184) was chosen as a material to be placed on a glass cover slip (Matsunami) with the channel buried inside.

A flow-cell setup was used for the transportation and fusion experiments. This setup was in fact a simple flow cell where for upper side the microtubule-oriented-glass-slide was used.

An inverted microscope (Olympus IX-71) was used for observations with an oil immersion lens (Uplan Apo x100, Olympus) for all the steps. Each experiment step was monitored by a differential interface contrast (DIC) setup on a microscope stage with a photometrics camera (Cascade 512

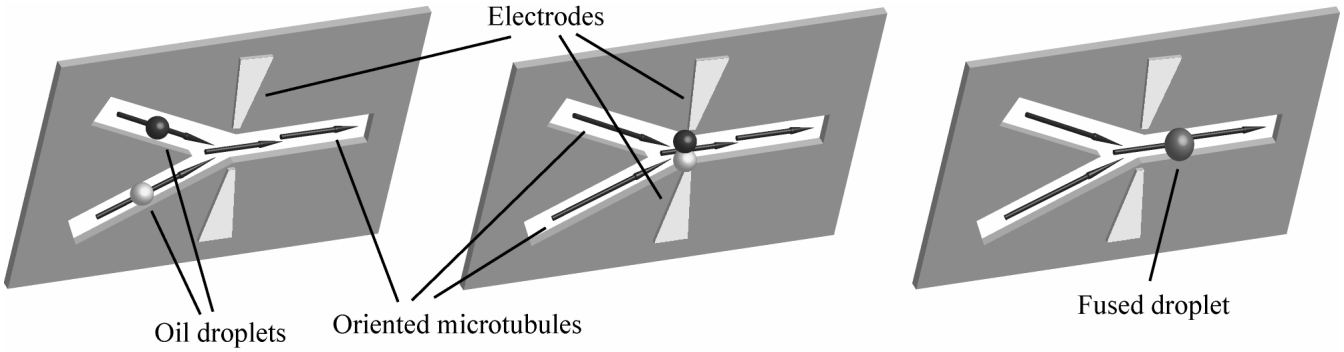


*Figure 1: Overall view of the proposed system: microtubules, oriented in the PDMS channels, along which oil droplets are transported. When the droplets come together in the main channel, voltage is applied on the electrodes to fuse them.*

II), which was controlled by software (Metamorph). Besides, for the fluorescence observations, a U-MNIBA3 filter (Olympus) was used.

**Kinesin**

Two types of kinesin were used. One type is full-length histidine-tagged kinesin for the orientation of the microtubules in the microchannel. This type of kinesin is purified with Ni NTA-agarose (Qiagen). The second type is biotinylated kinesin (GST-410-BCCP kinesin having a biotin carboxyl carrier protein (BCCP) that is a biotin-dependent enzyme) for the coating of functionalized oil droplets. This type of kinesin is purified with Glutathione-Sepharose (Amsterdam Biosciences) and microtubules affinity [7]. Purified full-length histidine-tagged kinesin and biotinylated kinesin had a concentration of 2 mg/ml and 0.065 mg/ml, respectively.

**Microtubules**

Tubulin was purified from pig brains and stored at a concentration of 4 mg/ml in liquid nitrogen. Microtubules were obtained by polymerizing tubulin in a solution containing $MgSO_4$ (1 mM) and Guanosine 5'- triphosphate sodium salt hydrate (GTP) (1 mM) at 37°C for 30 minutes. The resulting microtubules were stabilized by paclitaxel (taxol) (20 μM) in order to prevent depolymerization.

**Casein**

Sodium caseinate from bovine milk (Sigma) solution was prepared by dissolving 13% (w/w) sodium caseinate in DI water with continuous stirring.

**Biotin**

Biotin-X-NHS (Calbiochem) was chosen to bind on the casein coating of the oil droplets. It was dissolved in DMSO (dimethyl sulfoxide, Sigma) at 50 mM.

**Streptavidin**

Streptavidin (Sigma) was used to attach both on the biotinylated kinesin and on the biotin-X-NHS bound on the oil droplet. It was dissolved in PBS buffer (150 mM NaCl, 10 mM $Na_2HPO4$, 20 mM $NaH_2PO4$, pH 7.4) at 1 mg/ml. In order to control the functionalization of oil droplets, labeled streptavidine was used (Alexa Fluor 514, Molecular Probe) at 1 mg/ml.

**Functionalization of oil droplets**

Oil droplets were obtained by preparing a soybean-oil-in-water monodisperse emulsion, stabilized by sodium caseinate. At first, we incorporated in a gentle manner the oil in the casein solution until a mass fraction of oil equal to 70%. We obtained a highly polydisperse emulsion with droplet diameters bigger than 5 μm. The mean diameter of the droplets was decreased down to 1 - 2 μm by using ultrasonic probe. Then, the mass fraction of oil was decreased to 20% by diluting the emulsion in DI water.

The functionalization of the oil droplets was realized in two steps. The emulsion was first incubated with biotin-X-NHS solution (50 μM), which binds to casein with peptide bonds. The second step relies on the addition of streptavidin solution (0.1 mg/ml), which adsorbs on the biotin-X-NHS bound to the interface thanks to the strong specific avidin-biotin interaction. As shown in Fig. 2, the functionalization of the oil droplets were confirmed by using labeled streptavidin and observing the result with an optical and fluorescent setup. To realize the transportation, the biotinylated kinesin was attached to the functionalized droplet with the biotin-streptavidin binding as shown schematically in Fig. 3.

## 3. TRANSPORTATION

**Preparation of the flow cell**

PDMS was molded on a SU-8 50 (Microchem) structure patterned on a silicon wafer with dimensions of 100 μm (height) - 300 μm (width) - 25 mm (length). The molded PDMS slab was placed on a glass slide. Then, microtubules were oriented by gliding assay together with syringe-pump-induced viscous drag [8] (Fig. 4-a). For this step, purified histidine-tagged kinesin was used after diluting to 0.13 mg/ml in BRB80 solution (80 mM PIPES, NaOH (pH 6.8), 1 mM $MgCl_2$, 1 mM EGTA). After orientation, microtubules were immobilized by flowing first, 0.1% glutaraldehyde and then 0.1 M glycine in BRB80 solutions to crosslink the contact points between kinesins and microtubules. Next step was to remove the PDMS slab (Fig. 4-b). In order to build a flow cell, the slide containing the oriented microtubules was placed on the top of another slide

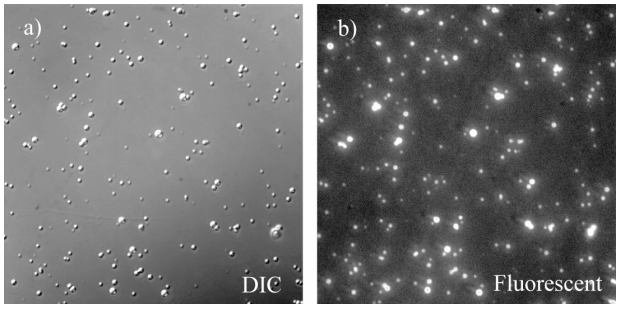


*Figure 2: Successful functionalization of the oil droplets. a) Photo taken with DIC setup where functionalized oil droplets are shown. b) Fluorescent view of the same area where labeled streptavidin can clearly be seen on the oil droplets.*

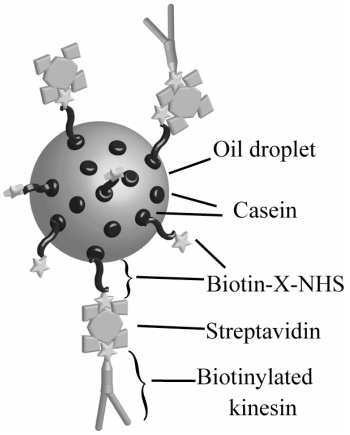


*Figure 3: Schematic view of kinesin-coated oil droplet: Biotin-X-NHS attached on the casein coating of the oil droplets, then, streptavidin was added to let the biotinylated kinesin bound on the oil droplet.*

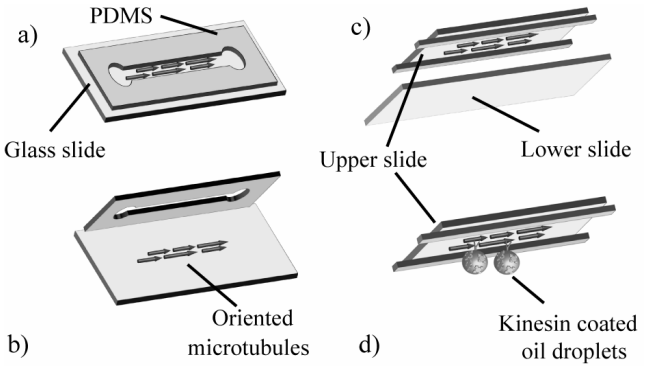


*Figure 4: Overall view of the oil droplet transportation. a) MTs were oriented in a PDMS channel. b) Then the PDMS channel was removed. c) A flow cell was built by the slide glass with oriented MTs as the upper slide. d) The next step was the transportation of kinesin-coated oil droplets.*

(Fig. 4-c). This was needed because oil droplets tend to float (due to the difference of densities of water and oil) preventing kinesin from binding on the microtubules if the latter were located on the lower slide**.**

**Motility assay**

Biotinylated kinesin was mixed with functionalized oil droplets with a volume ratio of 1:5 and incubated for 10 minutes. After the preparation of the flow cell with the oriented microtubules, kinesin-coated oil droplets were injected and left undisturbed for 5 minutes so that kinesin on oil droplets would bind onto the microtubules. Unbound kinesin-coated oil droplets were then washed out with BRB80 solution. Last step was to add 1 mM ATP solution to activate the kinesin bound on the oil droplets to move along the microtubules on the upper slide (Fig. 4-d).

## 4. ELECTROFUSION

**Preparation of the electrodes**

Gold electrodes were used in the experiments for the fusion of the oil droplets. Fabrication of the electrodes started with evaporating Cr / Au (5 nm / 100 nm) on a glass cover slip (Matsunami). A negative photoresist ZPN 1150-90 (Zeon) was spin coated, patterned and developed. After gold and chromium etching, the last step was to remove the resist on the electrodes in order to apply voltage to the electrodes. Fig. 5 shows the gold electrodes on glass slide under an optical microscope.

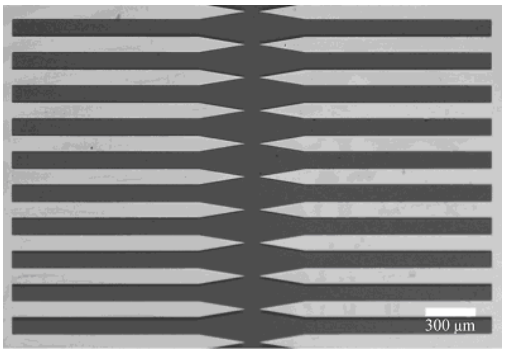


*Figure 5: An image of gold electrodes on a glass slide.*

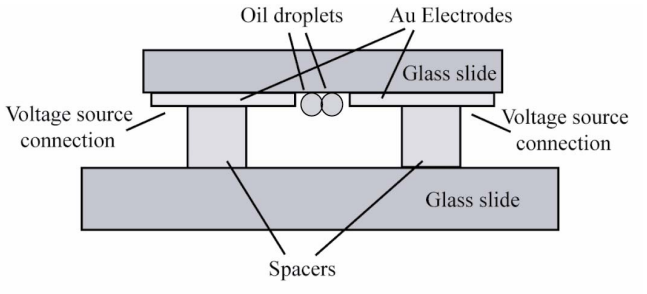


*Figure 6: Flow cell with the electrodes on the upper slide.*

**Electrofusion**

As shown in Fig. 6, glass slide (with gold electrodes) were used as the upper slide of a flow cell. A function generator (33220A, Agilent) was used with an amplifier (NF HAS 4101) to apply voltage to the electrodes at the ends outside the flow cell.

After the injection of the oil droplet solution into the flow cell, the system left undisturbed for oil droplets to reach the upper slide. Last step was to apply voltage to the electrodes to perform electrofusion of the oil droplets.

## 5. EXPERIMENTAL RESULTS

In Fig. 7, successively taken photos show the successful transportation of an oil droplet along a microtubule in the upper slide of the flow cell after addition of 1 mM ATP solution. Note that, there existed a buffer flow in the shown direction. Kinesin-coated oil droplets moved on the opposite direction proving that the transportation was not due to liquid flow but due to the kinesin motility. Nevertheless, some droplets stayed immobilized even after addition of ATP what could be due to non-specific interactions between oil droplets and the substrate.

Time course of total displacement of the moving droplet shown in Fig. 7 can be seen in Fig. 8. The average velocity of the oil droplet was about 700 nm/s, which is similar to the average velocity of a kinesin-coated bead (320 nm diameter) [7].

Successive photos taken in Fig. 9 show successful electrofusion of oil droplets. At t = 0 sec, an AC voltage of 30 $V_{p-p}$ at 5 MHz was applied on the electrodes on the upper glass slide. As seen on the photos, within 4 sec, fusion occurred. On different parts of the substrate, several oil droplets were fused. Some of those couples were fused simultaneously, but some took more time to fuse.

## 6. CONCLUSION

We have successfully transported kinesin-coated oil droplets along microtubules. Average velocity of the transported droplets was equal to the velocity of kinesin-coated beads. Moreover, electrofusion of the oil droplets using an AC voltage has been demonstrated. Several fusions were observed in different orientations non-simultaneously.

Combined with the encapsulation of particles/molecules within the droplets, both transportation and fusion of oil droplets imply that the system has a possibility to be integrated to a nanoscale reactor.

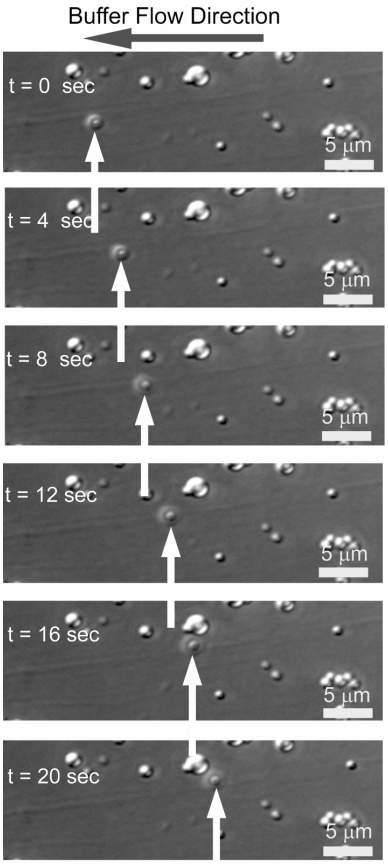


*Figure 7: Successive photos taken in DIC showing the successful transportation of a kinesin-coated oil droplet along a microtubule after 1 mM ATP addition.*

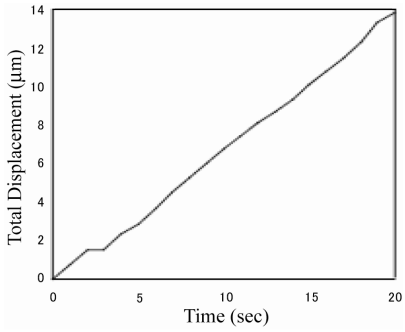


*Figure 8: Total displacement vs. time of the kinesin-coated oil droplet in figure 7. The average velocity of the kinesin-coated oil droplet is around 700 nm/s, which is the average velocity of a kinesin-coated bead.*

## ACKNOWLEDGEMENTS

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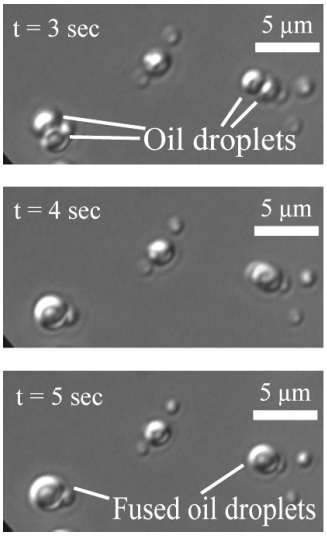


*Figure 9: Successive photos taken in DIC showing the successful fusion of oil droplets. At t = 0 sec, an AC voltage of 30 $V_{p-p}$ at 5 MHz was applied on the electrodes to perform the electrofusion.*

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