Observation of FRET in collision of droplets

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ABSTRACT

Förster Resonance Energy Transfer (FRET) is a radiationless distance-dependent transfer of energy from an excited donor fluorophore to an acceptor fluorophore. This radiationless interaction of a donor-acceptor pair through resonance is observed by an increase/decrease in the acceptor/donor fluorescence intensity, respectively. Here we present preliminary results on the fluorescence spectra of optically levitated micro-droplets doped with two different dyes that works as FRET pair. The laser light used for levitation (λ=660 nm) passes through a telecentric system of lenses to form a controllable double optical trap system. Micrometer sized droplets are produced using two on-demand piezo-driven dispensers. This allows independent trapping of differently dyed droplets in two traps where a collision between the droplets can be induced by moving the trap positions. The dye molecules mix when two droplets collide and coalesce. The emission spectrum obtained when the droplets are illuminated with laser having a wavelength of 532 nm is observed with a spectrometer which can record up to 26,000 spectra per second. We compare the results with the spectra taken from the same solutions in a cuvette. The results indicate that we are able to observe the FRET effect in single droplets with an exposure time as short as 100 μs. This spectroscopic investigation is an ongoing research project with the long-term goal to investigate environmental effects of aerosols in the atmosphere.

Keywords: Optical levitation, spectroscopy, FRET, dyes, double optical trap, collision-on-demand.

1. INTRODUCTION

The scientific field of optical manipulation has gained immense significance ever since the first work of Arthur Ashkin was published.[1] His work was based on the simple concept of "radiation pressure" introduced by Johannes Kepler in 1619 in order to explain the direction of the dust tail of a comet. Ashkin used the radiation pressure from a continuous wave laser to manipulate micron-sized latex spheres first in water[1] and later in air and vacuum.[2] In particular, in the technique of optical levitation, a particle is trapped in a vertically oriented focused laser beam. This allows one to hold and analyze a micron-sized object without mechanical support. Moreover, since the distance at which they are trapped is dependent on the focal length of the trapping lens, it is possible to trap at large distances. Hence, wall effects from the experimental chamber are negligible, which makes the technique of "optical levitation" suitable for studies of various interaction processes.

The process of collision and coalescence of droplets, when two or more droplets form a single larger droplet, involve four basic step: (1) the positioning of droplets, (2) the collision and deformation of the surface, (3) drainage of continuous phase liquid film between droplets, and (4) rupture of the surface and formation of a neck between droplets.[3] This has been studied extensively due to its many applications in the field of aerosol chemistry,[4] soft matter physics,[5] metallurgy,[6] microfluidics,[7] emulsion science[8] and many more.[9,10] Preliminary work was performed by Worthington[11] who studied the collision of a droplet against a solid surface. Following this, numerous studies on collisions of droplets with different type of substrates have taken place.[12–14] Many investigations of collision of droplets have been performed using optical traps.[15,16] Collision of droplets without the use of optical traps fall into the the broad categories of microfluidics,[17–19] inkjet printing,[20,21] DNA chips.[22]

The interaction between droplets can be easily tracked when a dye molecule is used. The coalescence process between droplets has been previously studied by tracing the temporal evolution of the fluorescence when a dyed

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glycerol droplet coalesces with a droplet without a staining dye.[23] Numerous studies of collision of droplets using single beam gradient trap,[24] optical tweezers[15] have been also published. The advantage of such method is the absence of the solid substrate or a channel wall which eliminates many forces acting on the droplets. Interaction studies between droplets can also be studied using Förster Resonance energy transfer[25] (FRET). This is a non-radiative process where energy is transferred between an excited fluorophore (donor) and another fluorophore (acceptor) which is in its ground state. This process can only occur when the two fluorophores are relatively close to each other, i.e. within 3-10 nm. This transfer of energy between the donor and acceptor molecule takes place through long range dipole-dipole interactions where the excited donor molecule acts as an oscillating dipole and the non-radiative energy transfer takes place when another molecule oscillates with the same frequency as the donor molecule. This resonance interaction occurs over greater than inter-atomic distances, without conversion to thermal energy, and without any molecular collision. The transfer of energy leads to a reduction in the donor's fluorescence intensity and excited state lifetime, and a significant increase in emission from the acceptor. A pair of molecules that interact in such a manner so that FRET occurs is often referred to as a donor/acceptor or FRET pair. A FRET pair is chosen such that the emission spectrum of the donor molecule overlaps with the absorbance spectrum of the acceptor molecule. Numerous studies using FRET for protein interaction study,[26,27] live cell imaging[28] and for various other analysis have taken reported.

So far no FRET studies when optically levitated trapped droplets collide have been reported. In this work, we present a pilot study where two droplets stained with different ATTO dyes coalesce. The different dyes are chosen such that they not only fulfill the criteria for FRET but also help to trace the process of collision, mixing and complete coalescence by tracking their change of color. To make this work possible, a dedicated optical system, which will be discussed in this paper, has been designed. Several questions will be addressed, such as: How does charging the droplets influence the collision pattern in these droplets? Could FRET be observed in collision of droplets?

2. EXPERIMENTAL METHOD

The collision experiment is performed in a 25 mm^3 chamber with six input/output optical ports on each side which are covered with glass windows. The chamber is carefully isolated from disturbing air streams with only two small open entrances on top where droplets are introduced by a pair of dispensers. The optical trap is based on a previous design[29] with a vertical trapping geometry. The four side ports of the experimental chamber are used for analysis purposes, as will be described below.

2.1 Delivery of droplets

The droplets are introduced into the experimental chamber by a pair of piezo-actuated microdroplet dispensers. The charge of the individual droplets is controlled by varying a dc voltage applied between the liquid solution and an electrode just beneath the tip of the dispenser.[29] Inside the chamber a pair of electrode rings of 5.0 mm diameter and separated by 7.0 mm are placed, as shown in Fig. 2. A sinusoidal high voltage can be applied to the upper ring electrode in order to induce an oscillatory movement of the trapped droplet. This is used to determine the sign and strength of the charge of a trapped droplet by analyzing the movement of the droplet under the applied external field. The two dispensers are mounted in separated XYZ micrometer stages for alignment of the dispenser tip allowing on demand optical trapping of the droplet once the dispensers are properly positioned.

2.2 Dual steerable optical trap

In order to fully manipulate and induce a droplet collision, two optical traps are built by splitting the trapping laser beam into two arms, as shown in Fig. 1. One of the arms is fixed and is aligned on the center axis of the experimental chamber. The second arm is a fully steerable trap which uses a telecentric lens system where

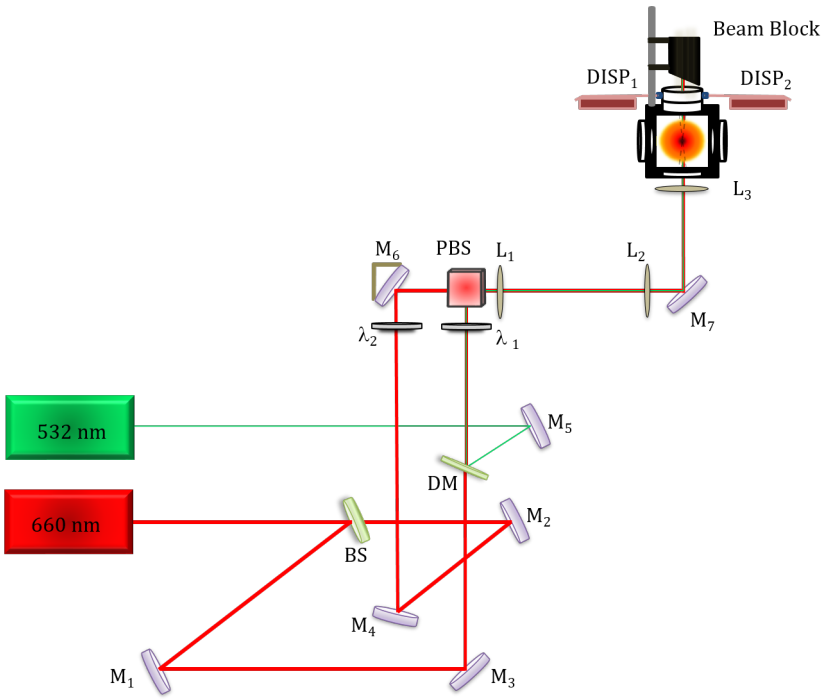


Figure 1. Optical layout of the experiment. L_i stands for lens, M_i for mirror, PBS for polarization dependent beamsplitter, BS for beamsplitter, $DISP_i$ for droplet dispenser, DM for dichroic mirror and λ_i for half-wave plate.

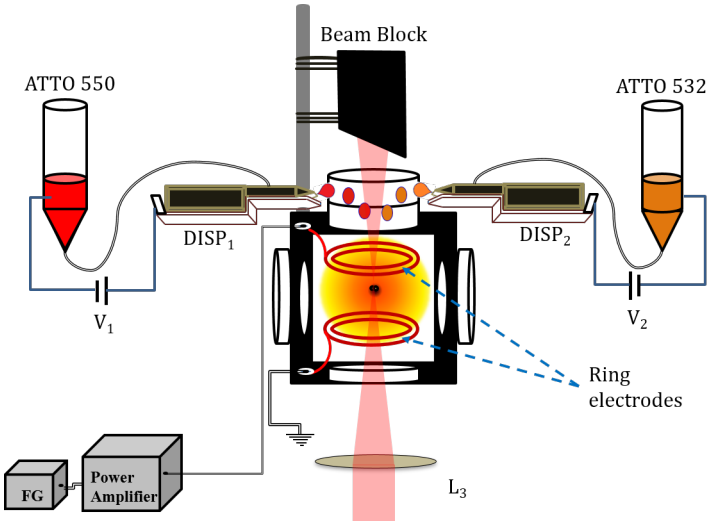


Figure 2. Side view of the experimental chamber. Droplets from two different solutions (ATTO 550 and ATTO 532) are delivered into the chamber by the dispensers $DISP_1$ and $DISP_2$. The charge of the droplets is controlled individually by the applied voltages V_1 and V_2. FG refers to a function generator.

the conjugate planes are situated at the center of the experimental chamber and at a steering mirror. This arrangement allows the trap position inside the chamber to be controlled. The two optical arms are merged together by two half-wave plates (λ_1, λ_2) and a polarization dependent beamsplitter cube (PBS), as shown in Fig. 3. In this way one of the traps is fixed and defines the x and y coordinates of the target droplet while the second arm is used to create a steerable trap which is used to freely manipulate a colliding droplet. Both traps are created by the same focusing lens L_3 which has a focal length of 50.0 mm and is placed beneath the chamber such that the trap is created at the center of the chamber. Fine adjustment of the droplet position along the laser axis is made by varying the laser intensity, which is controllable independently for either of the two traps

by rotating the wave-plates λ_1 and λ_2, respectively.

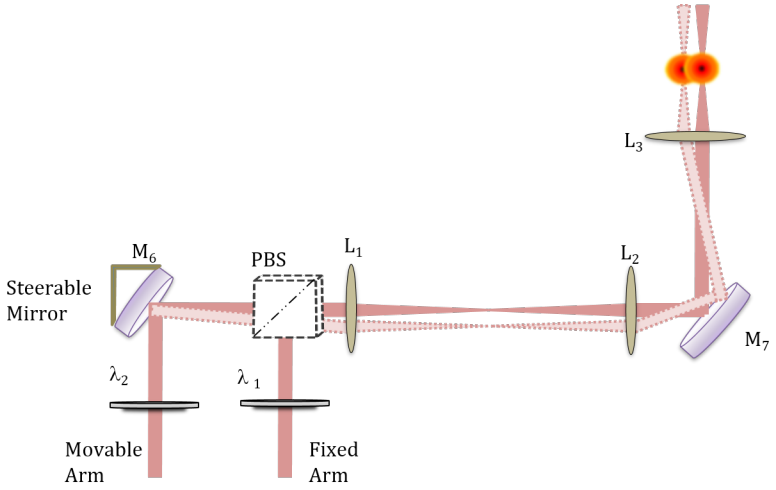


Figure 3. Dual optical trap design. The movable trap is steered by the pitch and yaw of M_6.

2.3 Excitation and detection

In addition to the laser used to create the optical traps, a 532 nm excitation laser is used to induce fluorescence of the dyes. The excitation laser is aligned collinear with the fixed trap laser arm by using a dichroic mirror (DM) to merge the two beams, as shown in Fig. 1. The excitation power is kept to less than 1 mW in order to avoid photobleaching of the dyes. Analysis of the droplet collision can be performed by acquiring the time dependent spectrum of the interaction and correlating the spectra with a recording of the collision using a high speed camera. The optics used to collect the fluorescent signal consists of a bifocal achromatic tube lens (L_4 and L_5) placed in one of the side ports of the experimental chamber. It is positioned such that its focal points are located at the center of the chamber where the stationary droplet is trapped and at the tip of a 400 μm core size optical fiber connected to a spectrometer. Light from the droplet collision is thus efficiently collected by the optical fiber as the interaction region is of the order of few tens of micrometers. The spectrometer used in this work is equipped with a high speed camera (Zyla 4.2P from Andor Instruments) allowing integration times as short as 10 μsec and up to 26,000 frames/sec.

A second port of the chamber is used to record the collision using a high speed camera (Phantom Miro ex4) with the same speed as the spectrometer. The camera and the spectrometer triggers are synchronized. The video recorded by the high speed camera can be used to correlate with the time-dependent spectrum acquired by the spectrometer.

2.4 Preparation of Dye solutions

All chemicals and solvents were used as received without further purification. ATTO 532 (donor dye) and ATTO 550 (acceptor dye) were purchased from ATTO-Tec Gmbh. A 95:5 water:glycerol solution was prepared. This solution served as the solvent for dissolving the ATTO dyes. Equimolar solutions (1.3 x 10^{-4} M) of ATTO 532 and ATTO 550 were prepared by dissolving 1 mg of the respective dye in 10 mL of glycerol-water solution.

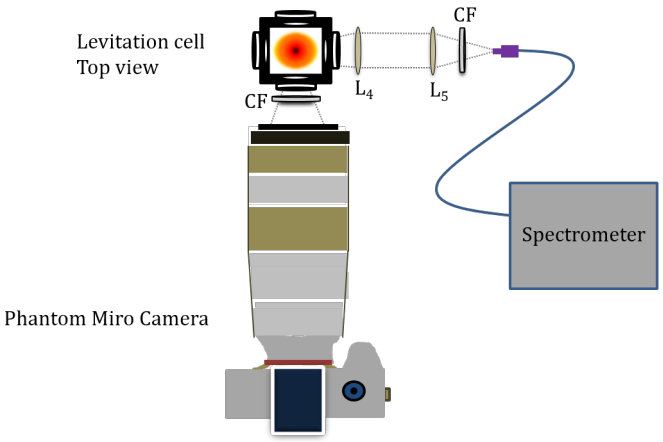


Figure 4. Top view of the experimental chamber. CF refers to color filters used to prevent saturation of the cameras by the light coming form the levitation laser.

3. RESULTS AND DISCUSSIONS

3.1 Demonstration of the functionality of the optical system

In the optical setup shown in Fig. 1, the droplets were allowed to fall freely along the central levitation axis defined by the laser beam path of the fixed trap. First, the steerable trap was aligned collinear with the central levitation axis, whereafter a single droplet was emitted and trapped. Second, the trapped droplet was horizontally displaced about 4 mm away from the central levitation axis using the steerable mirror M_6. Third, the fixed laser trap arm was turned on and a second droplet was trapped in the fixed trap. The potentials across the dispensers electrodes were selected to determine polarities of the trapped droplets.

In order to impart collision between trapped droplets, they must have opposite polarity. Two droplets of the same polarity will, rather than coalescence, start to juggle in the trap.[30] The polarity of the droplets was investigated by applying an alternating electric field over the droplet. The field was generated by applying a sinusoidal voltage at 80-120 Hz on the upper ring shaped electrode inside the chamber (Fig. 2) while the lower electrode ring was maintained at ground potential. The charge on a droplet was determined by observing if the droplet was moving in our out of phase with the applied electrical field. The magnitude of the charge of a droplet was estimated by observing its vertical displacement using the Phantom Miro ex4 camera. The variation in the position of droplet versus the applied field is shown in Fig. 5.

The acceptor/donor pair of droplets can, as soon as they have been trapped separately by the dual optical trap system, be manipulated to performed spectroscopy studies. Lenses L_4 and L_5 as shown in Fig. 4 are used to collect and focus the light from each droplet into the spectrometer optical fiber. As the size of each droplet is around 20-25 μm and the fiber core diameter is 400 μm, the scattered light from the droplet can be efficiently collected by the optical fiber enabling fast and efficient data collection.

3.2 Controlled maneuvering of droplets

The collision process is highly dependent on the charge of the colliding droplets, where we, as mentioned above, can control if they become positively or negatively charged or neutral. We performed experiments where we trapped one droplet each in the fixed and steerable trap, respectively. We then moved the traps together in order to observe whether or not the two droplets coalesce.

First, we trapped two glycerol droplets with the same charge and then moved the steerable trap towards the fixed trap. When the two traps were coming sufficiently closer, the droplet from the steerable trap jumped into the fixed trap. At this stage, the steerable trap was turned off. We observed that the two droplets are in a single trap but they do not touch each other due to the mutual coulomb repulsion. Instead, they start to move in stable orbits, in a phenomenon called optical juggling.[30] This represents a method to maintain the droplets at

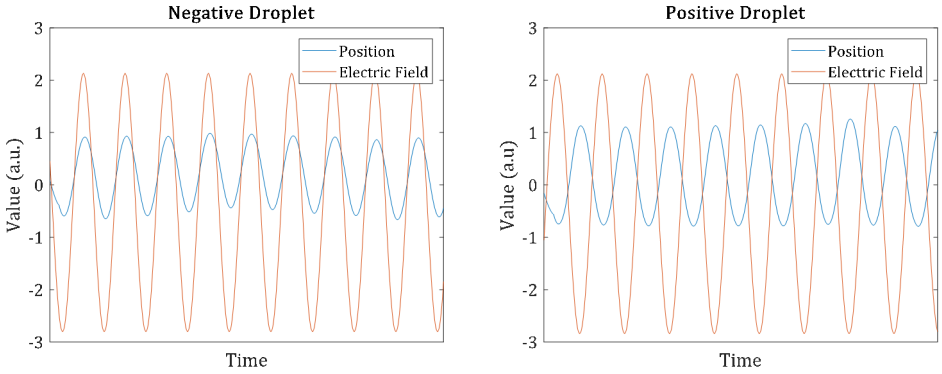


Figure 5. The figure shows the droplet displacement and the applied field for a positive and negatively charged droplet, respectively.

microscopic distances without them touching each other. Fig. 6 shows 10 frames of the juggling event between two positively charged droplets.

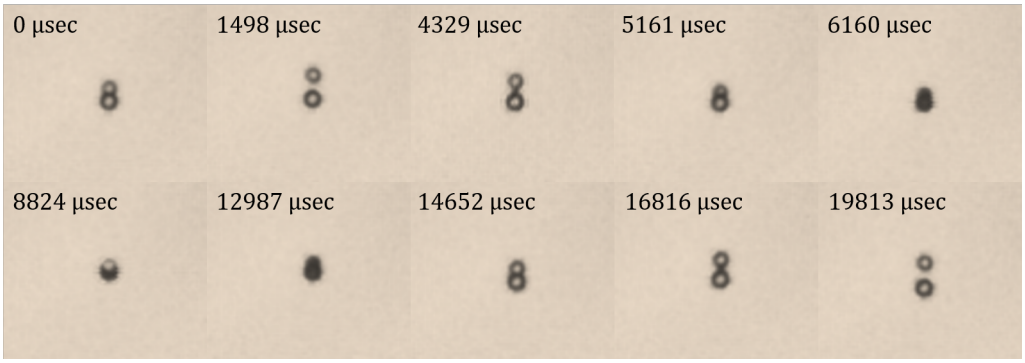


Figure 6. Juggling of two positively charged droplets in a single trap.

Second, we loaded the traps with two droplets of opposite charge. When the two traps were moved in contact, the droplet from the steerable trap, just as in the case with droplets of the same charge, jumped into the fixed trap. Here, on the contrary, these two droplets were attracted by each other and coalesced rapidly, as shown in Fig. 7.

3.3 Observation of the FRET process in cuvette

The two dyes ATTO 532 and ATTO 550 were chosen since they fulfill the criteria for the Förster Resonance Energy Transfer (FRET) process, where the emission spectrum of ATTO 532 overlaps the absorption spectrum of ATTO 550. In this experiment, glycerol was stained either with ATTO 532 or ATTO 550 and placed in a cuvette and excited with light from a 532 nm laser. The fluorescent light was collected with an achromat lens system and focused to an optical fiber which is connected to the spectrometer. The fluorescence spectrum of the individual dyes was collected with an integration time of 3 ms using a simple spectrometer (Red Tide USB650

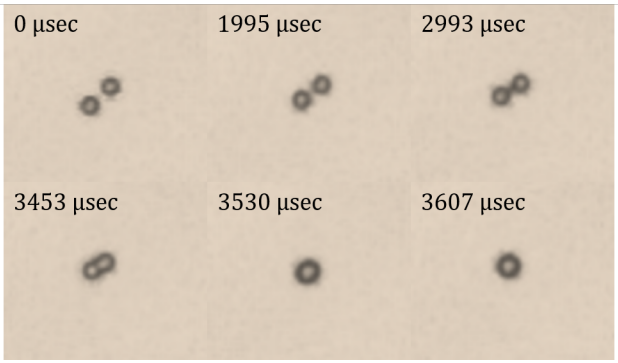
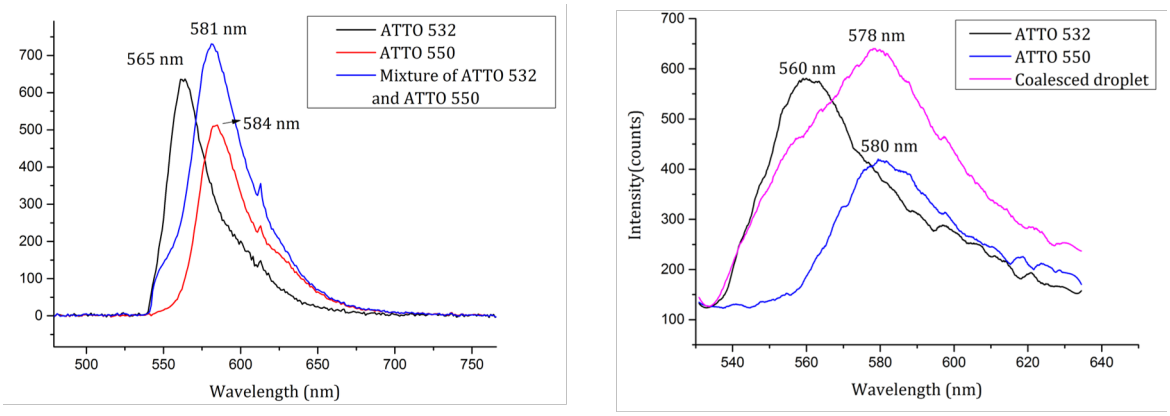


Figure 7. Optical controlled coalescence of two opposite charged droplets. The two droplets are trapped in independent traps prior to the coalescence.

from Ocean Optics) and with an excitation power of only 1 mW. Fig. 8a shows the recorded spectra for ATTO 532 (black line) and ATTO 550 (red line).



a) Cuvette solution b) Levitated droplet

Figure 8. Fluorescence and FRET spectrum taken a) in a cuvette using a conventional Ocean Optics spectrometer and b) in levitated droplets using an Andor Kymera spectrometer equipped with a Zyla 4.2P camera sensor.

In order to investigate the FRET process, the experiment was thereafter repeated but now using a mixed solution with equal volumes of ATTO 532 and ATTO 550 solutions. The concentration of the individual dyes are hence reduced by 50%. The resulting spectrum from the mixture is shown as the blue line in Fig. 8a. A clear enhancement of the fluorescence intensity of the acceptor dye (ATTO 550) is seen while the fluorescence of the donor dye (ATTO 532) is reduced significantly. This enhancement/reduction of the acceptor/donor fluorescence is a direct indication of FRET. To quantify our results, the integrated area under each line was determined. With no FRET effect, i.e. with non-interacting dyes, the sum of the area under two curves of ATTO 532 and ATTO 550 divided by two would be equal to the area under the curve for the mixed dyes. This requires that the intensity of the laser and the collection efficiency is identical in the three experiments. However, this is easy to achieve in the experiment with the cuvette where the only difference in the experiments are that we replaced the solutions. In the experiment we find that there is indeed an interaction between the mixed dyes since the area under the curve for the mixture solution is found to be 15% larger than the sum of the area under the curve for the individual dyes divided by two. This quantification of the spectra clearly shows that the enhanced fluorescence of the acceptor molecule (ATTO 550) is indeed due to the transfer of energy from the donor molecule (ATTO 532) and hence a direct indication of FRET.

3.4 FRET in levitated droplets

A solution of each dye with the same concentration as the one used for the cuvette experiment was employed to feed each of the microdroplet dispensers. Following a similar method as the one described in section 3.1, the two dispensers were set to deliver droplets with opposite charges. First, a droplet containing the donor dye was trapped by the movable optical trap at the central levitation axis. Subsequently, the 532 nm excitation laser was turned on at 1 mW of power and the fluorescence spectrum of the droplet was acquired by the high speed spectrometer. Next, the donor dyed droplet was moved away from the central levitation axis using the steering mirror M_6 and a droplet containing the acceptor dye was trapped in the fixed trap. The fluorescence spectrum of the acceptor dyed droplet was then recorded with the same methodology as for the donor droplet. Finally, the donor and acceptor droplets were brought together until they smoothly collided and coalesced, whereafter the spectrum of the mixed dyed droplet was acquired. In all three cases the excitation laser was applied for 1 second, which is just enough to acquire the fluorescence spectrum but avoiding bleaching the dyes. All spectra shown in Fig. 8b, were recorded at optimal alignment conditions and with an integration time of 98 μsec. Similar to the experiments in the cuvette, we found that there is an enhancement in the intensity of the acceptor fluorescence signal after the droplet with the donor dye collides and coalesces with the droplet containing acceptor dye. When comparing our results obtained with droplets to those obtained in the cuvette experiments, we observed the same spectral patterns however, an absolute quantification, as in the case in the cuvette experiment, is difficult to obtain since we do not reach the baseline in the spectrum and since the droplet moves within the trap which may cause the collection efficiency to vary.

As shown in section 3.2, we can induce controlled coalescence of two droplets of different charge. By combining this with our high rep-rate camera and spectrometer we are able to study the time evolution of the spatial mixing of two coalescing droplets stained with different dyes. Fig. 9 shows the time resolved process of collision and mixing of the dyes. A homogeneous mixing of the dyes is observed and can be tracked only using the Phantom Miro high speed camera.

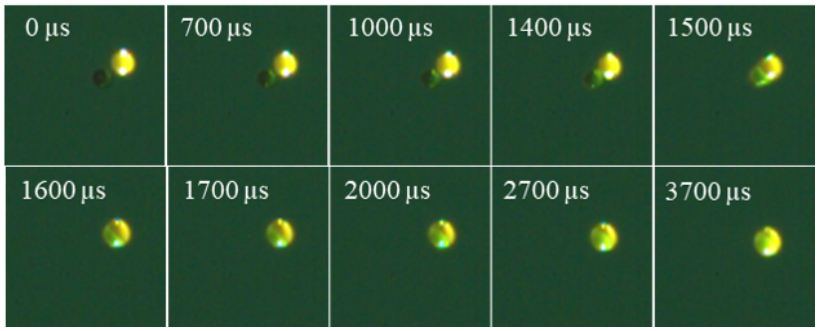


Figure 9. Time resolved coalescence of droplets.

4. CONCLUSION

We have in this paper reported on a new experimental set-up in which two droplets can be individually trapped and then forced into a single trap. The sign of the charge determines whether the droplets start to juggle (same sign on charges) or if they coalesce (different sign on charges). We thereafter studied the FRET process, first in a cuvette, and then in the coalescence of two optically levitated droplets. Time dependent fluorescence spectra were recorded using a spectrometer that allows spectra to be recorded with a rep-rate of 26,000 frames/sec. These results demonstrate that we are able to record the spatial evolution of the dyes in two coalescing droplets on sub millisecond timescale. Our preliminary results give strong indications that we have observed the FRET process in colliding droplets.

Detailed studies of the temporal evolution of the FRET process in coalescing droplets as well as comparative studies of FRET with different dye pairs is currently performed in our laboratory. We will use these studies in our future work to try to understand the collision dynamics and in particular determine if the FRET process starts at the point of contact between the droplets, during the process of coalescence or after a complete mixing.

5. ACKNOWLEDGMENT

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