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Diffusionless fluid transport and routing using novel microfluidic devices

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Diffusionless fluid transport and routing using novel microfluidic devices

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ABSTRACT

Microfluidic devices have been proposed for “Lab-on-a-Chip” applications for nearly a decade. Despite the unquestionable promise of these devices to allow rapid, sensitive and portable biochemical analysis, few practical devices exist. It is often difficult to adapt current laboratory techniques to the microscale because bench-top methods use discrete liquid volumes, while most current microfluidic devices employ streams of liquid confined in a branching network of micron-scale channels. The goal of this research was to use two phase liquid flows, creating discrete packets of liquid. Once divided into discrete packets, the packets can be moved controllably within the microchannels without loss of material. Each packet is equivalent to a minute test tube, holding a fraction from a separation or an aliquot to be reacted.

We report on the fabrication of glass and PDMS (polydimethylsiloxane) devices that create and store packets.

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1. BACKGROUND

Microfluidic devices have been proposed for "Lab-on-a-Chip" applications for nearly a decade. Despite the unquestionable promise of these devices to allow rapid, sensitive and portable biochemical analysis, few practical devices exist. It is often difficult to adapt current laboratory techniques to the microscale because bench-top methods use discrete liquid volumes (e.g. classically test tubes and beakers, or in the context of a modern automated laboratory, robotic pipetters and microwell plates, which handle microliter-size discrete volumes). Current microfluidic devices employ (typically nanoliter) streams of liquid confined in a branching network of micron-scale (effectively one-dimensional) channels. The liquid streams are never fully compartmentalized, and there is no ability to make individual packets of material that can be transported or processed independently. In other words, there is always mixing of the materials in the channel along the length of the channel, and generally across the branches of the network. For one-step analytical microseparation measurements, diffusional mixing along the channel fundamentally limits the performance of the separation, but can be mitigated by precise selection of device layout, size, and performance speed. Valves can, in principal, eliminate cross-talk between channels, but are challenging to fabricate, and material hold-up at the valve may actually enhance mixing between two streams sequentially shunted through the valve. In other words, what is still lacking is the ability to independently move about and selectively mix or measure, in sequence or in parallel, discrete quantities of solution- the essence of what is done in the lab.

In this work we attempted to demonstrate the feasibility of microscale devices that will allow the division of a fluid stream into discrete packets moving controllably within the

microchannels , and the independent control (creation and destruction, transport, sorting, mixing) of the packets on-chip (figure 1). Each packet would then be equivalent to a minute test tube, holding a fraction from a separation or an aliquot to be reacted. Such a device overcomes the current barriers to implementing true multi-step fluidic processing on a chip, and allows us to escape layout and timing constraints on implementation of microseparation imposed by diffusion and channel cross-talk. We believe that the device concept we

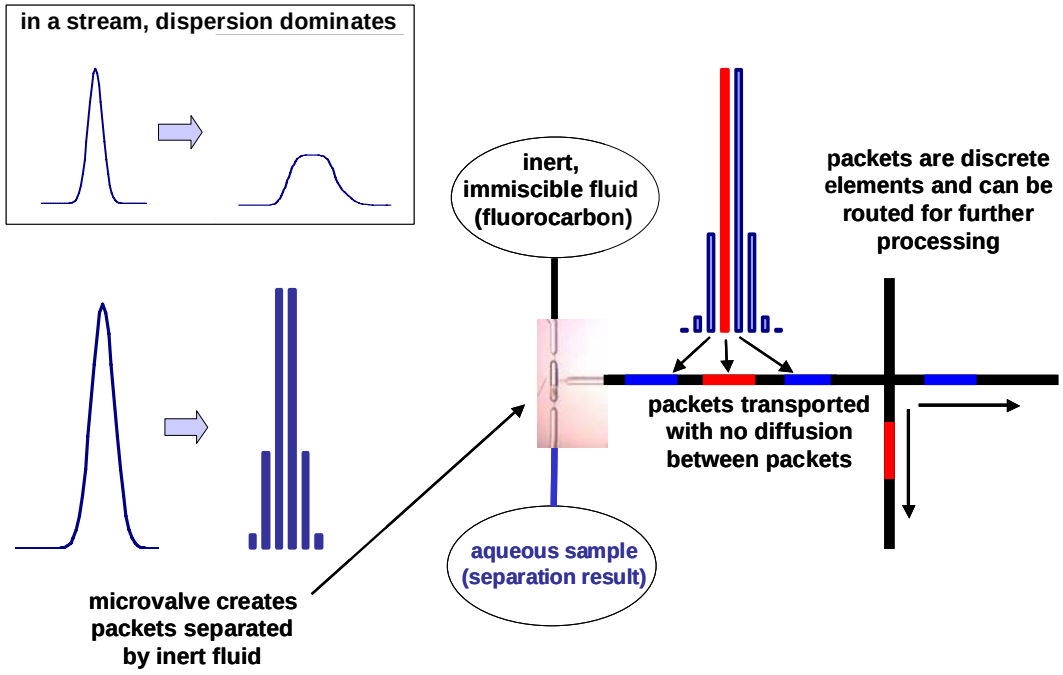


Figure 1: Experiments will demonstrate digitization of a stream with lossless transport and routing of the resulting packets. In the example shown, the output from a separation (e.g. a HPLC peak) is broken in packets surrounded by an inert carrier liquid. This process is enabled by the use of a microvalve "chopper". Each packet can be transported and routed throughout the system without losses due to dispersion or diffusion. Inset shows effect of dispersion normally seen in microfluidic devices.

discuss in this report could have a major impact on microfluidic chemical analysis and enable new generations of flexible integrated microfluidic devices that would be much closer to the lab-on-a-chip ideal.

We endeavored to demonstrate two things 1) the ability to create discrete mobile "packets" of liquid that are functionally prevented from mixing, and 2) the ability to sort the packets

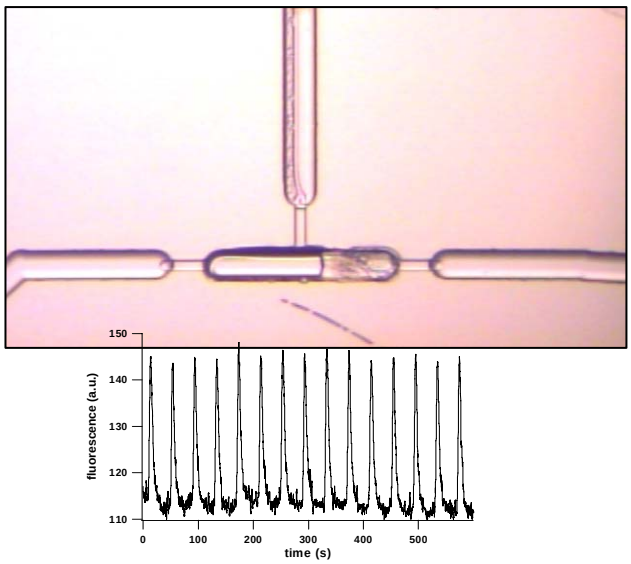
Microfluidic devices are being developed to perform many of the analyses currently carried out in analytical laboratories. These "lab-on-a-chip" devices have the potential to be faster, more sensitive, and more portable than conventional assays. Most microfluidic assays are fundamentally different from bench-top assays in that the microfluidic devices transport fluids in streams instead of macroscopic discrete volumes. This difference gives rise to a key limitation of stream-based devices: multi-stage processing of samples is ineffective due to diffusion and Taylor dispersion. In addition, effects like surface retention are far more pronounced in microsystems than in bench-top assays because of the comparatively large surface to volume ratios. At present, there is no general technology for the transport of minute sample volumes between instruments or within devices. Such a technology would enable the application of microfluidic devices for uses other than analysis, such as programmable nanoliter reactions.

An important performance parameter for the generation of packets is the actuation time of the valve. Some microfluidic valves operate on the time scale of several seconds(Yu, Bauer et al. 2001). The "resolution" of the quantized stream will be half of the switching rate. The switching rate at which packets are generated needs at least 2 Hz for analytical applications, since peak widths are generally greater than one second. We have already demonstrated repeatable microvalve switching at 5 Hz in a similar format(Reichmuth, Shepodd et al. 2004). This performance is sufficient to generate an interleaved water and fluorocarbon stream.

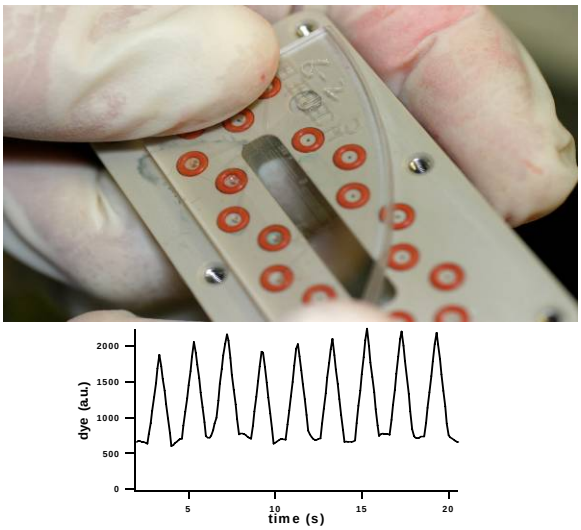
2. Performance of single phase devices

In many microfluidic devices, great effort is taken to concentrate and/or separate chemical species for detection and analysis. However, once concentrated the sample cannot be transported to another part of the device without losses. These losses are chiefly due to Taylor dispersion and mixing induced by curvature of the channels. Mixing can also occur in interfaces between devices unless great care is taken to insure alignment and eliminate abrupt changes in channel dimensions. An example of the losses is given in Figure 2, where dye is periodically injected into a channel. The dye is driven through the chip and out through a capillary using pressure. The loss of peak definition is clearly evident, especially as the stream is taken off chip. If the same periodic dye injections were left in the channel and stored in the absence of flow, a similar band broadening would occur due to diffusion.

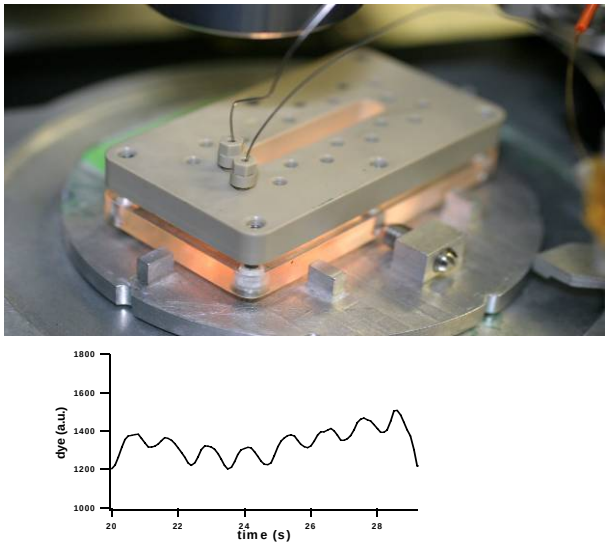
It is clear from the example given in Figure 2 that stream-based microfluidics have limitations in the transport and storage of analytes. One possible solution is to use discrete packet-based flow by using two immiscible liquids.



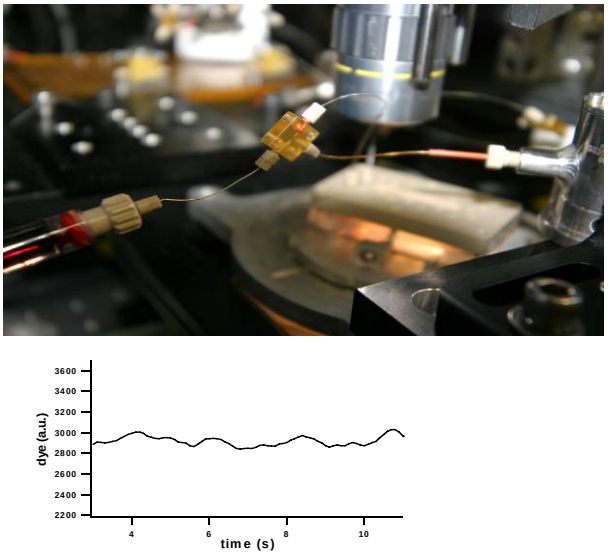
Dye peaks created by switching valve, measured 1mm downstream from valve.



Dye peaks created by switching valve, measured 2cm downstream from valve.



Dye concentration measured in capillary 3cm downstream from chip/capillary interface.



Dye concentration measured in capillary 60cm downstream from chip/capillary interface.

Figure 2: A series of dye injections is used to demonstrate the effect of diffusion and mixing in microfluidic devices and chip-to-world interfaces. The dye peaks are broadened and then eventually lost as the fluid flows through the chip and out of a capillary.

3. Glass Capillary Systems for Packet-Based Flows

Fluidic systems constructed using glass capillaries and zero-dead volume connectors were used to explore the effect of hydrophilic and fluorophilic surface coatings on the transport and storage of packets.

3.1 Materials and Methods

Glass capillaries (Polymicro Inc.) with an inner diameter of 75 μm were coated using the methods described previously (Reichmuth, Shepodd et al. 2004). Uncoated capillaries have an overall negative surface charge and are hydrophilic. Capillaries coated with a fluorous coating produce a fluorophilic surface. Syringe pumps (New Era Pump Systems) were used to generate controlled pressure-driven flow.

Images were captured using an epifluorescent inverted microscope (Olympus IX-70) and point detection data were collected using a μChemLab detection module(Renzi, Stamps et al. 2004).

3.2 Results

The purpose of the glass capillary experiments was to examine the effect of surface coatings on packet formation, transport, and storage and also to demonstrate the ability of packet-based flow to transport without losses. The use of glass capillaries and modular connectors allows facile generation of packets without incurring delay or cost due to microdevice fabrication. This system was used to test the effect of surface coatings on packet formation and storage by using a fluorescent aqueous phase (either labeled protein or modified polystyrene microspheres) with a fluorocarbon (FC-84, 3M) carrier phase. The devices used are shown in Figure 3.

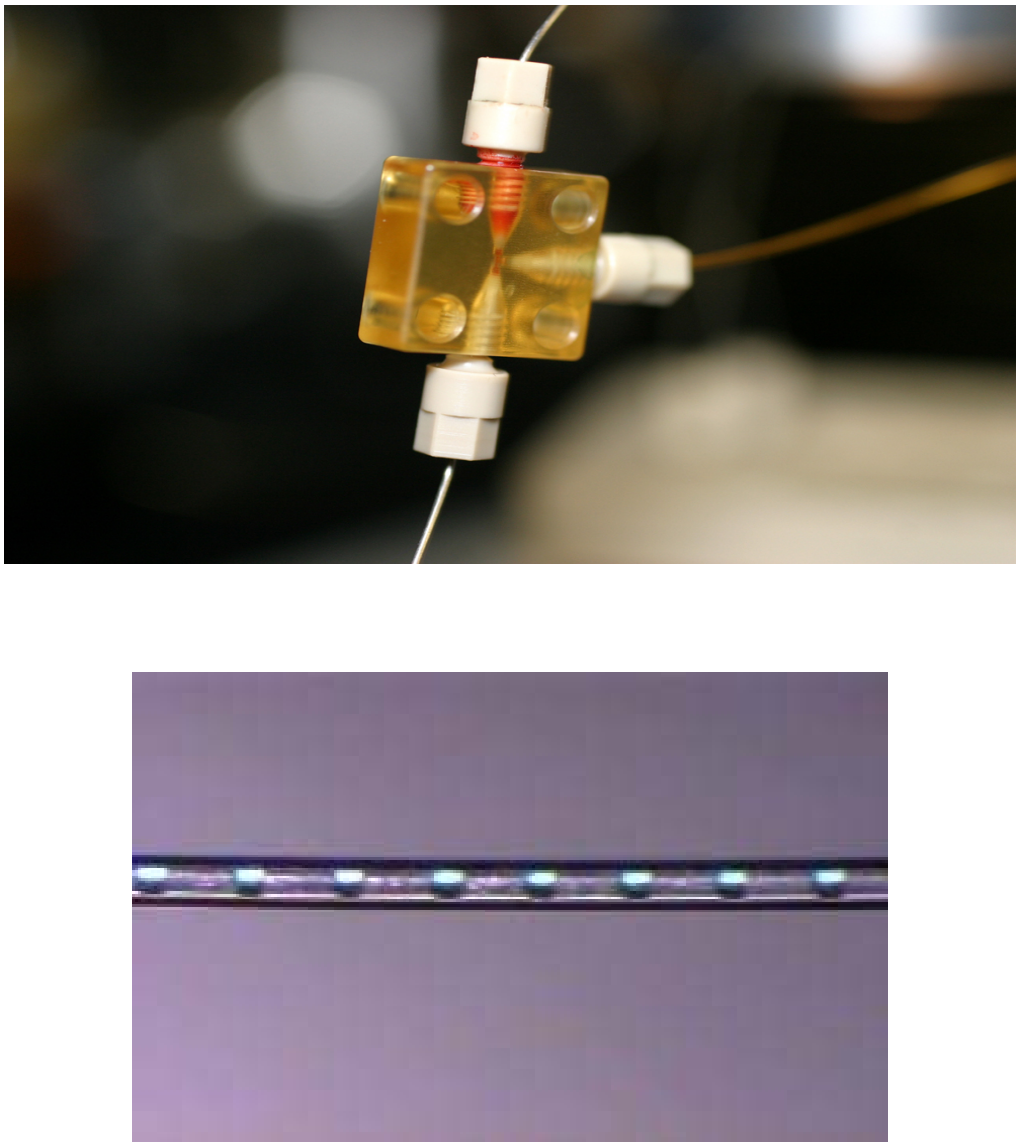


Figure 3: A custom-fabricated “T” capillary connector was used to generate segmented flow. The connector provides a quick and facile means to generate segmented flows in capillary tubing.

The key finding is that a charged surface (as is found with a unmodified glass surface) is not suitable for the generation and transport of aqueous packets in a fluorocarbon carrier.

Fluorescently-labeled bovine serum albumin (BSA) was used to visualize the aqueous phase and to model a dissolved analyte. In Figure 4A, the bare glass walls stay in contact with the water and packets of fluorocarbon are produced. This is not useful as very few compounds dissolve in fluorocarbons. The fluorocarbon is therefore an excellent carrier fluid, but a poor choice for the packet fluid. In Figure 4B, the walls of the capillary are coated by a fluorosilane, producing a fluorophilic surface. In this case, the packet and carrier fluids are the inverse as compared to the uncoated capillary. The fluorocarbon envelops the aqueous packet and separates the packets, preventing mass transfer between the packets.

Similar experiments were conducted using fluorescent nanoparticles as model analyte. In an uncoated capillary, the aqueous phase interacts with the wall to produce unwanted behavior. Polystyrene spheres can be seen in Figure 5A adhering to the wall and concentrating at the water/fluorocarbon interface. In contrast, Figure 5B shows use of a fluorinated capillary with nanoparticles. The packets exhibit uniform fluorescence and the microspheres are contained within the packets.

Surface coatings also affect the ability of proteins to adhere to the surface of the capillary. Both uncoated and coated capillaries do not show retention of protein for contact times less than one hour. However, when left for longer periods of time such as one day, the fluorinated surface does retain BSA as shown in Figure 12. The uncoated glass capillary (not shown) does not shown retention of the fluorescent BSA.

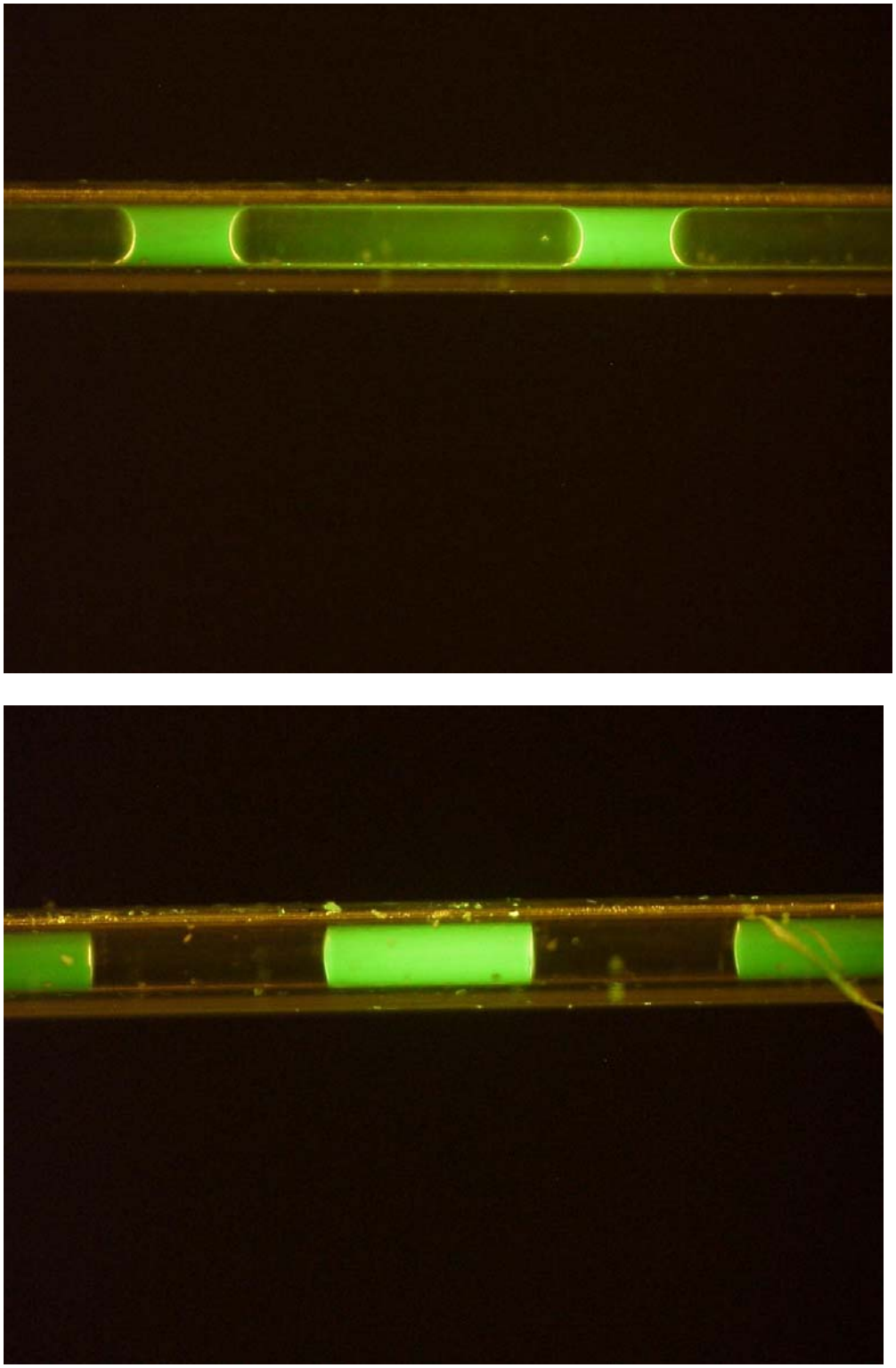


Figure 4: A) (top panel) In an uncoated glass capillary, the aqueous phase (seen as green due to fluorescently-labeled BSA) is attracted to the walls, creating packets of fluorocarbon. This is the inverse of the desired behavior. **B) (bottom panel)** In a capillary with a fluorinated surface coating, the fluorocarbon phase envelops the aqueous producing discrete packets containing BSA.

Figure 5: A) (top panel) In an uncoated glass capillary, fluorescent nanospheres are left behind on the capillary wall and also accumulate at the ends of the packet. **B) (bottom panel)** In a fluorinated capillary, the nanospheres are confined to the packet.

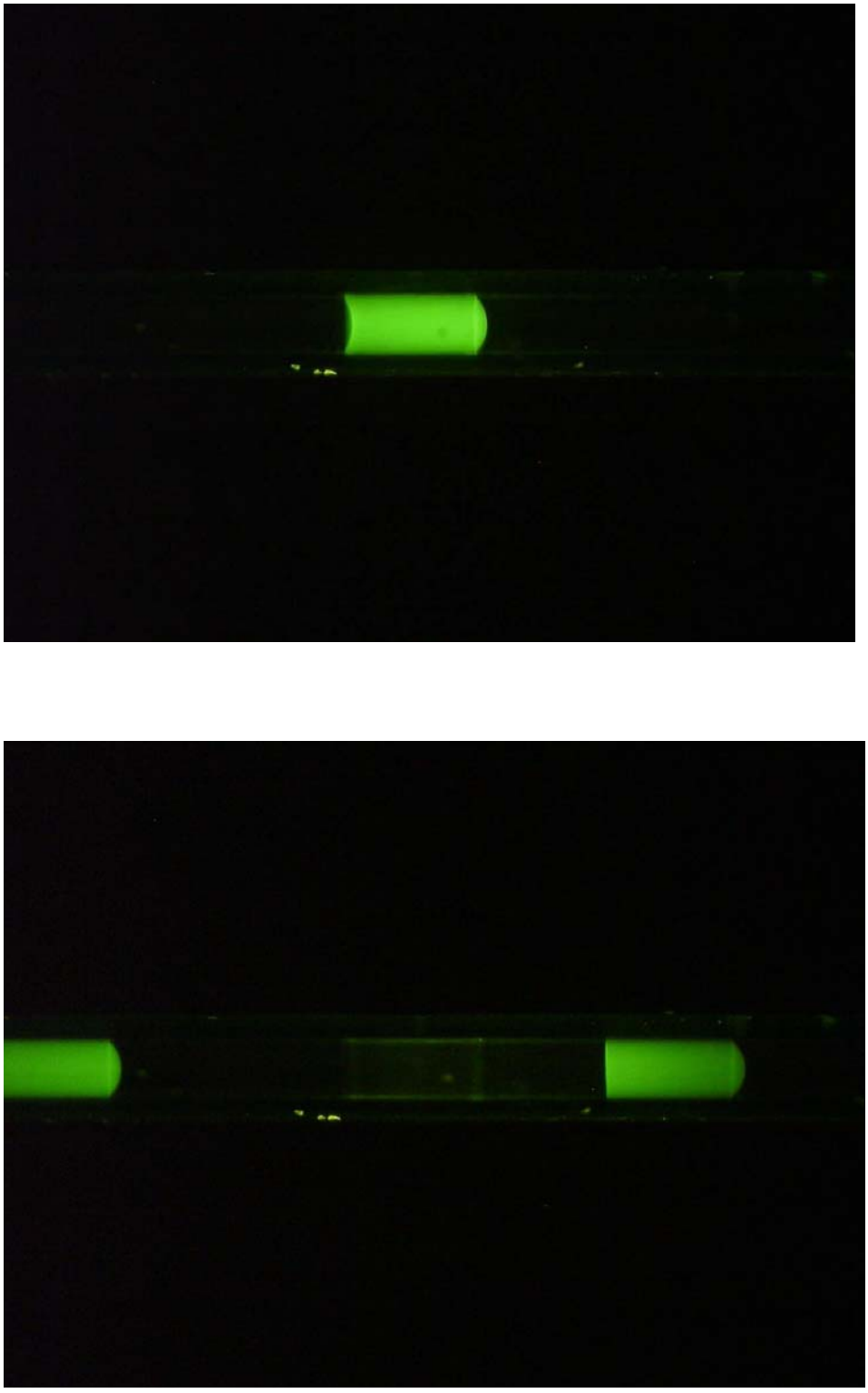


Figure 6: A) (top panel) Packet left in capillary for 24 hours. **B) (bottom panel)** Packet has been displaced to the right by pressure-driven flow. Fluorensence in the center of the image is due to protein adsorbed to the fluorinated glass wall surface.

The capillary-based system was also used to determine whether packets could be used to store the result of a separation. For example, a peak from a liquid chromatography separation could be converted into packets and then used in a subsequent analysis. To create a similar system, a HPLC injection valve was used to inject a fluorescent dye into an aqueous stream, and then segmented by a fluorinated hydrocarbon carrier. A T-junction produced packets and the dye concentration is determined using laser-induced fluorescence measurements at a single point. The T capillary connector produced a regular series of droplets and the fluorescence was measured at the end of a 1m capillary. The dye injection can be seen in Figure 7A, however the scale of the graph obscures the packets. In Figure 7B, one can see that the peak has been broken into packets. These packets were measured 1 meter downstream from the injection, demonstrating that the packets can be used to move material without losses. Also, by segmenting flow into packets, the results of a separation can be stored or undergo lengthy analysis. We observed that the packets had constant fluorescence after 20 hours of storage (data not shown).

Finally, we investigated the use of packets generated using capillary connectors as virtual test tubes or bioreactors. By segmenting growth media containing a bacterium inoculum with a fluorocarbon, each packet can grow a separate and distinct culture. In the example shown in Figure 8, *E. coli* bacteria that produce green fluorescent protein (GFP) are suspended in growth medium were segmented with Fluorinert. Intact, GFP-producing cells are visible 6 days after segmentation.

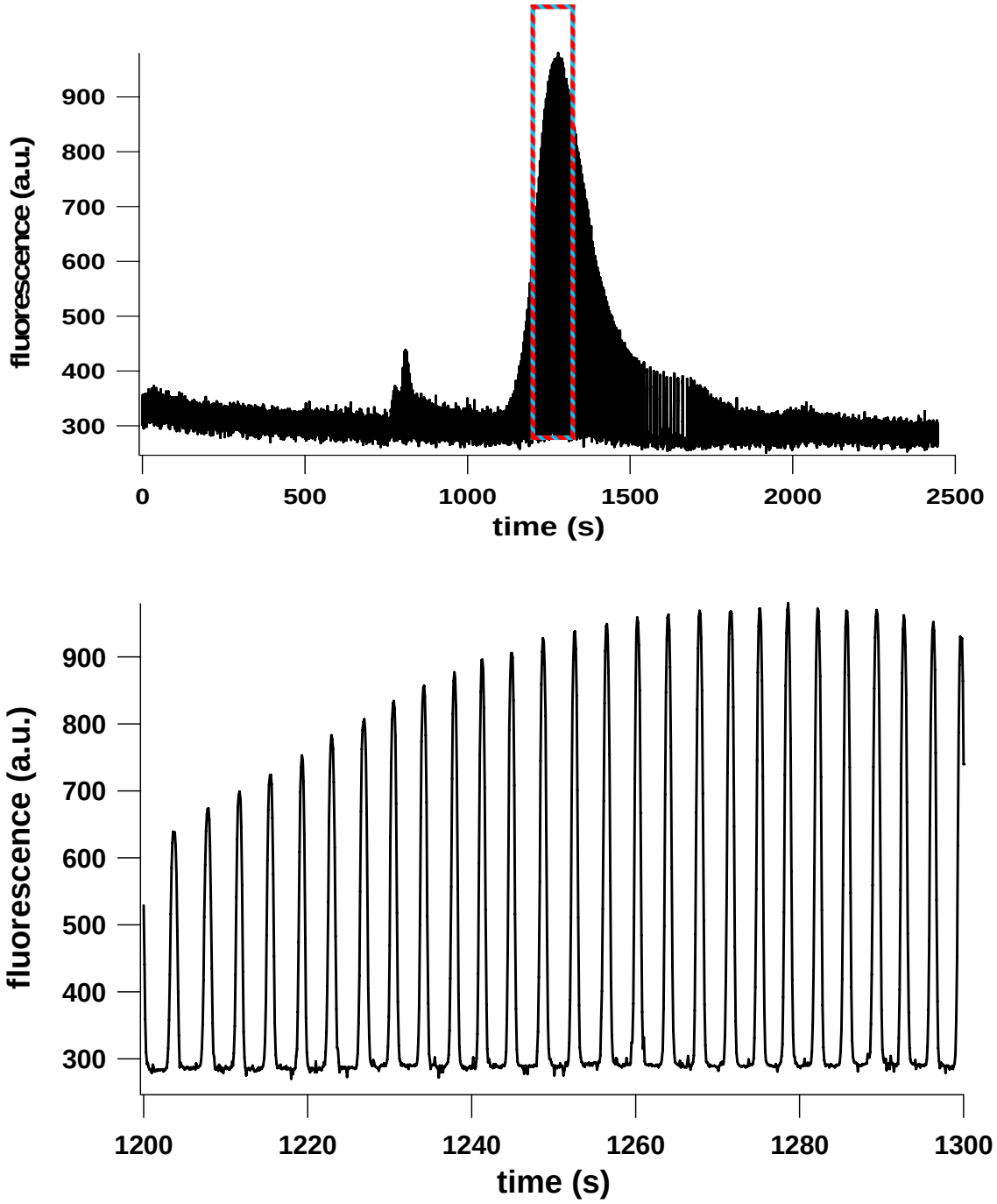


Figure 7: Fluorescent dye is injected into an aqueous stream, and then segmented by a fluorinated hydrocarbon carrier. A T-junction produces droplets and the dye concentration is determined using laser-induced fluorescence measurements. A HPLC injection valve was used to create a plug of dye in a water stream with flow rate 50 μl/hr. Fluorinert FC-84 at 150 μl/hr and a T capillary connector produced a regular series of droplets. The fluorescence was measured at the end of a 1m capillary. A subset of the data (indicated by dashed box) shows the dye peak is segmented into distinct droplets. Total flow rate was reduced to 50 μl/hr to allow detection of individual droplets. By segmenting flow into packets, the results of a separation can be stored or undergo lengthy analysis.

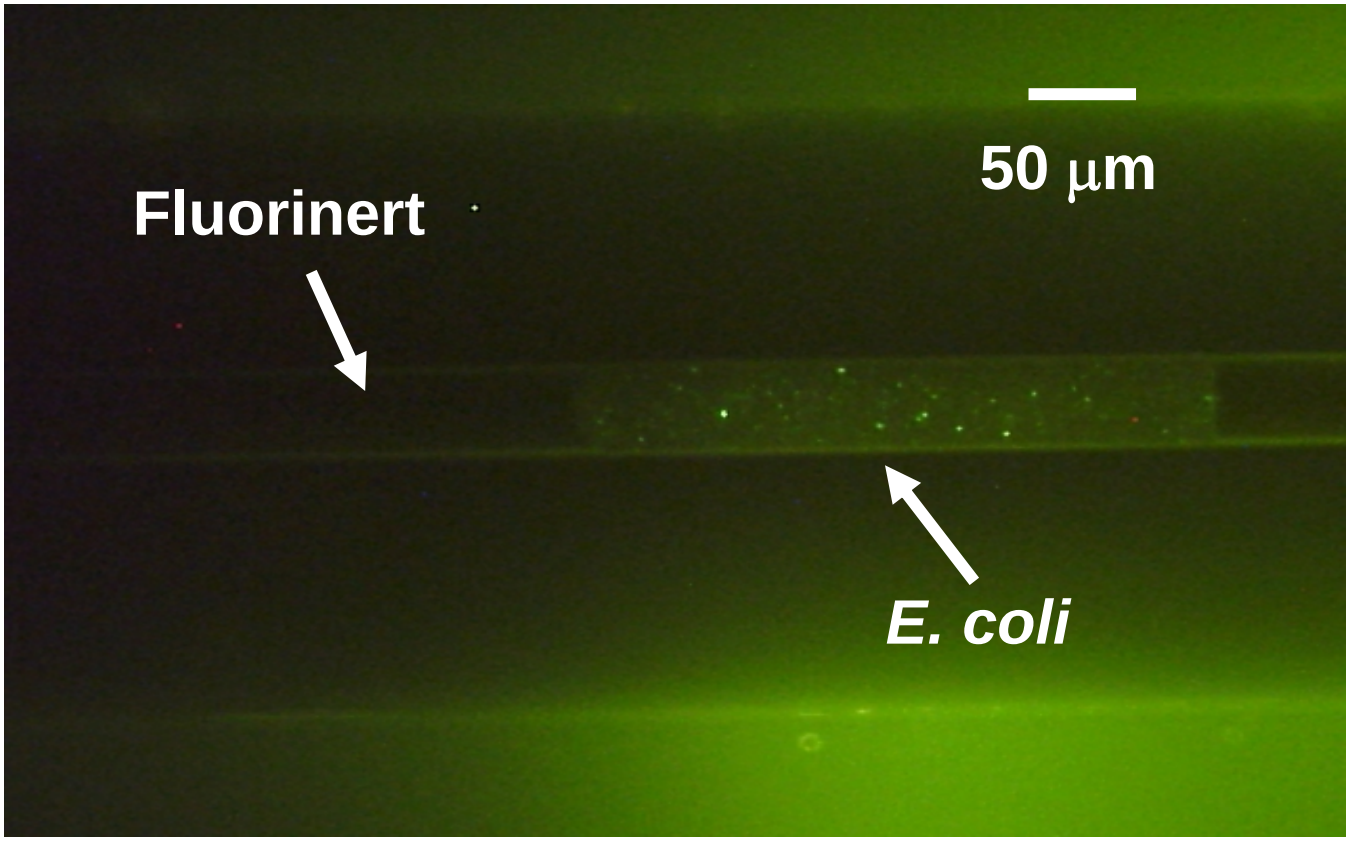


Figure 8: Packets generated using capillary connectors can also be used as virtual test tubes or bioreactors. E. coli bacteria suspended in growth medium were segmented with Fluorinert. Intact, Green Fluorescent Protein-producing cells are visible 6 days after segmentation.

4. Glass microchips for packet-based flows

4.1 Materials and Methods

Glass microchips were fabricated using a multi-step wet etch process, as described previously(Reichmuth, Shepodd et al. 2004; Kirby, Reichmuth et al. 2005; Reichmuth, Shepodd et al. 2005). The resulting chips have a cylindrical valve region that is separated from the hemi-cylindrical flow channels by shallow weirs. The valves are formed using a laser to initiate polymerization of a cross-linked acrylate polymer. Prior to valve formation the channel walls are treated with a fluorous coating to prevent the valve piston polymer from adhering to the wall. The valve is actuated using a pressure differential across the valve, as the valve piston is able to move in response to the pressure differential until it seats against the weir. The pressure differential is applied using syringe pumps with an electronically-controlled valve to gate the pressure applied to the control channel.

4.2 Results

Our group has previously demonstrated the fabrication and testing of polymer microvalves such as the device shown in Figure 9. Our intention at the outset of this project was to use this valve to create packets inside a microchip for storage and sorting of analytes. The microvalve can reproducibly meter sub-nanoliter volumes and is therefore promising for the creation of very small and evenly spaced packets. We were able to construct several microvalves for use as packet formation devices (see Figure 10), and we attempted to inject aqueous packets into a fluorocarbon carrier stream.

We were unable to produce a glass chip microvalve that successfully formed reproducible packets. There are several causes for this failure. A significant problem was that fabrication of the devices needed to be exact, as deviations in etch depth and especially alignment of the top and bottom wafer result in non-functional devices. The low tolerance for deviations in the fabrication process leads to a low number of useable devices. A further problem is that the weirs that form the valve seats also capture particles irreversibly, causing the valve to fail.

In addition, the composition of the valve used in prior work was not compatible with the fluorocarbon carrier fluid used. In previous experiments, a fluorinated acrylate polymer was chosen as the valve material because it is highly resistant to solvents and does not change size when fluids of different polarity are used. However, in this work the fluorocarbon carrier fluid interacted with the valve polymer to cause a change in the valve size. The valve design is sensitive to the valve dimensions, and shrinkage or swelling of the valve impairs operation. The polymer could be reformulated to better withstand the fluorocarbon carrier fluid, but that work would be outside the scope and resources of this project.

A final problem with the glass/polymer microvalve for packet creation is the method of valve actuation (pressure pulses) causes changes in the flow rate of fluid through the exit channel. Changes in the flow rate effect the formation of packets, in particular increasing the flow rate past a critical value causes laminar 2-phase flow where the aqueous and fluorocarbon streams flow side-by-side(Zheng, Tice et al. 2004). While flow rates can be limited by placing a restriction in the channel, it would be very difficult to retain the mechanism of valve actuation while eliminating variations in the flow (particularly at high packet formation rates).

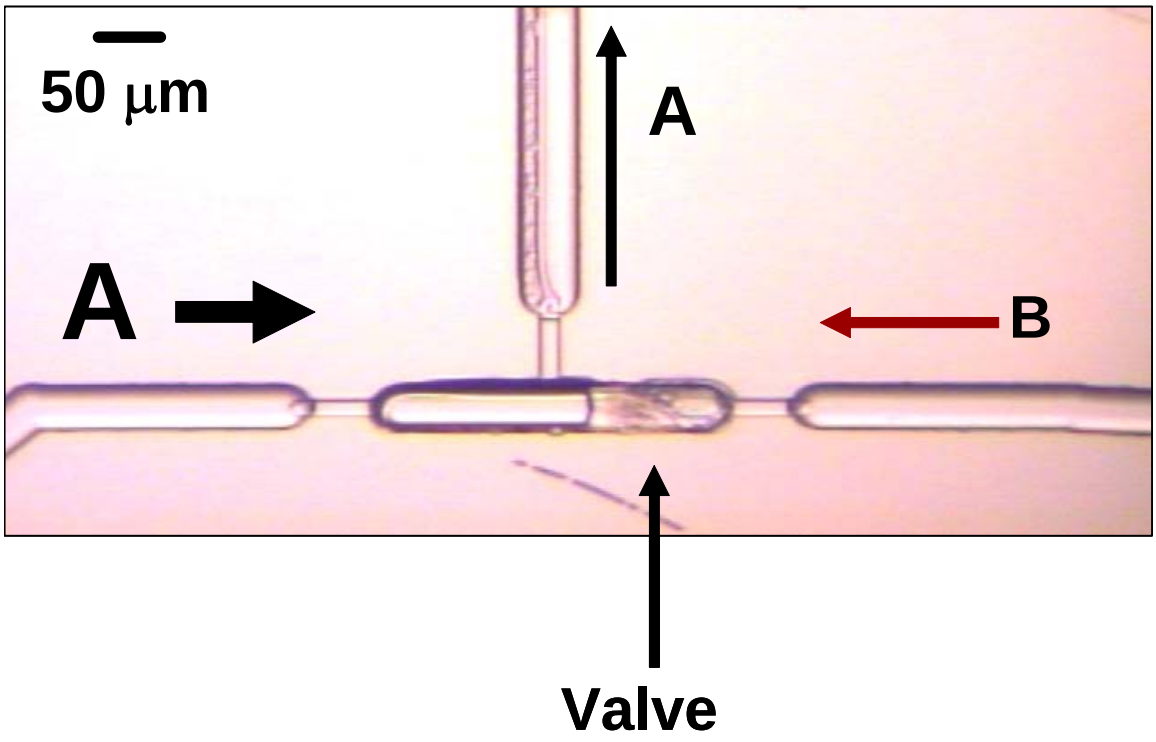


Figure 9: The polymer element moves in response to a difference in pressure, and the valve stops and seals at the narrowing of the channel. The valve element moves at ~10 psi pressure difference, and effective sealing occurs at a 50 psi difference. The valve leak rate is less than 12 pl/min. In the case shown above, a higher pressure on side A causes the valve to seal against the B channel, selecting A to flow out of the exit channel.

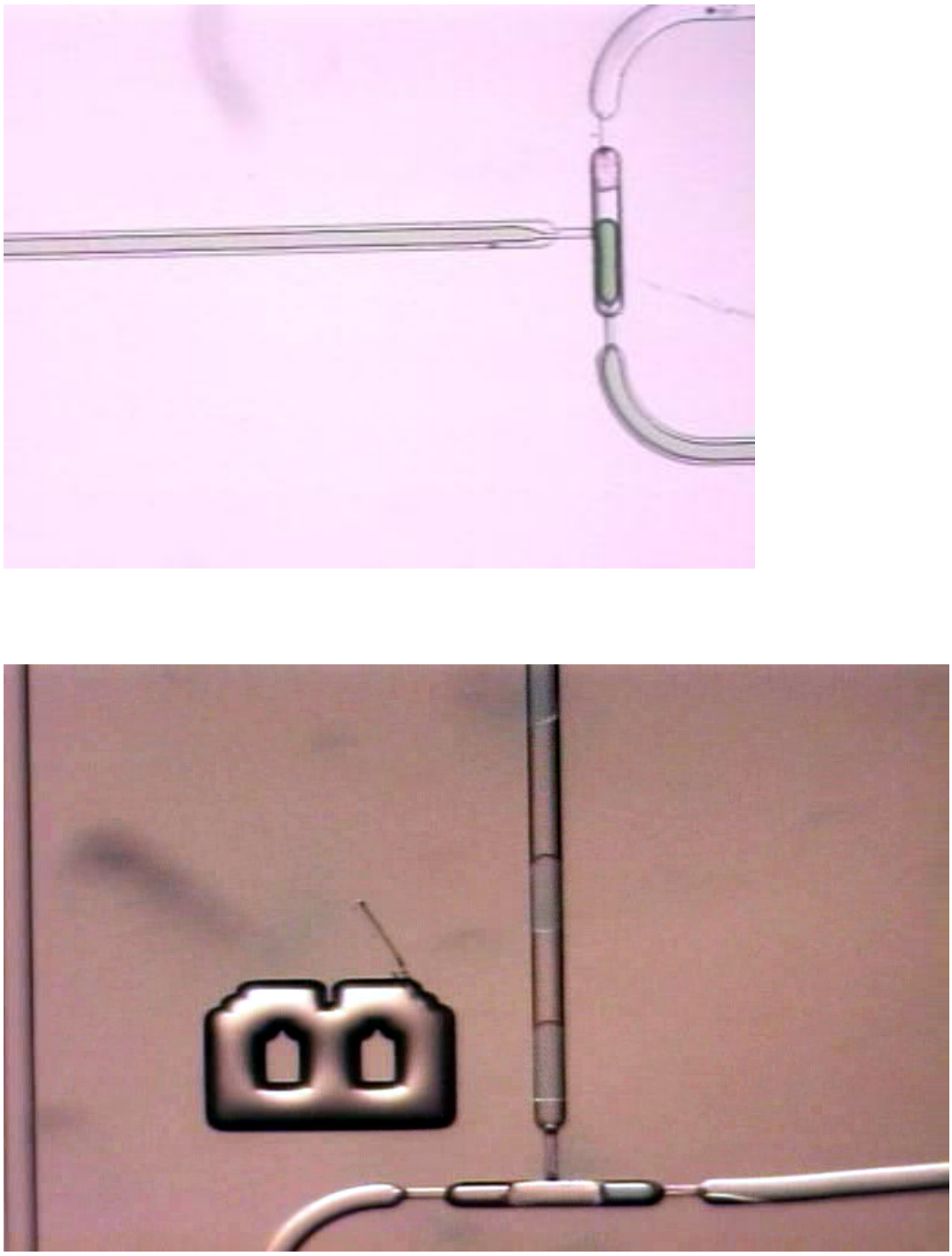


Figure 10: Examples of microvalve devices for generation of packets on chip.

5. PDMS microchips for packet-based flows

Because of the difficulties in using the glass/polymer microvalve, we altered our system to use chips with PDMS valves. PDMS valves have been widely used in for microfluidic devices, and have the advantage that three valves can be combined to produce a pump(Unger, Chou et al. 2000). Although PDMS will swell in some solvents, the valving mechanism is more tolerant to fabrication deviations. It was hoped that using PDMS for packet generation would allow both pumping of the fluids and on-demand packet generation.

5.1 Materials and Methods

The PDMS devices were fabricated using the methods describe in the work of Unger, et al. and are summarized below:

Mold Fabrication

Flow layer mold:

- Clean Si wafer
- Spin/expose to vapor of hexamethyldisilazane (HDMS) adhesion promoter (optional?)
- Softbake
- Spin coat 45 um of AZ-100 XT PLP
- Expose using positive mask
- Post exposure bake
- Reflow (140C for 5 min) to round channels

Control layer mold:

- Clean Si wafer
- Spin SU8-2025 for 25 micron depth (3000 rpm for 35s)
- Expose and develop using negative mask

PDMS chip fabrication

Flow layer:

- Expose mold to chlortrimethylsilane vapor for 2 min (to help unmolding)

- Put mold in Petri dish and pour a 5 mm layer of degassed 5:1 (a:b) PDMS over the mold
- Partially cure at 80C for 45 min
- Peel off and trim
- Punch access holes
- Align to control layer

Control layer:

- Expose mold to chlortrimethylsilane vapor for 2 min (to help unmolding)
- Spin on 30 microns of degassed 20:1 PDMS (1600 rpm, 60s, 15 s ramp)
- Partially bake at 80C for 45 min
- Align flow layer over control layer (leaving control layer on mold)
- Bake at 80C for 60min
- Peel off and punch control line holes

Glass slide:

- Coat glass slide with 20:1 PDMS (1600 rpm, 60s, 15 s ramp)
- Partially cure at 80C for 45 min
- Place flow/control assembled layers on slide
- Bake at 80 C for 5 hours

The valves were actuated using low pressure air (<25 p.s.i.) and the air flow was controlled using a 8-channel solenoid manifold (Fluidigm Inc.) .

Channel surfaces were modified using a hydrogen peroxide and hydrochloric acid pretreatment and reaction with neat fluorosilane(Sui, Wang et al. 2006).

5.2 Results

The use of PDMS-based pumps and valves generated promising results towards the generation and transport of packets in a microfluidic device. Using a bank of 3 valves allowed pumping of fluid inside the PDMS microdevice. The pumping action occurs via the inflation of the three valves in sequence as shown in Figure 11. When pumping two aqueous solutions, the pumps perform flawlessly, and an example is shown in the laminar flow of two water solutions (Figure 12).

As is the case with glass devices, the surface properties of the wall are important. We observed better packet formation in devices with a fluorophilic coating than in uncoated devices. In Figure 13, it can be seen that the surface coating changes the ability to form aqueous packets in the microdevice. It is important to note that the native state of the PDMS is hydrophobic but not fluorophilic. We coated our PDMS devices using an aggressive surface treatment combined with undiluted silanes. This treatment did modify the surface and changed the way packets moved in the channels, but there appeared to be significant variations in the coating from device to device. Also, the hydrogen peroxide and HCl acid mixture did cause bubbling and melting of some devices. This behavior was not solely linked to the incubation time and the variable effect of this pretreatment could be the cause of the variations in the surface treatment.

When coated PDMS chips were tested by pumping fluorocarbon and water solutions simultaneously, packet formation was observed (Figures 14 & 15). While packets were formed, there were difficulties in the device operation. First, the pressure required to form a new packet was at the limit of the pump's ability to generate flow. This is due to the large amount of force required to increase the size of the unfavorable water-fluorocarbon interface. Because of the pressure required to produce the packets, the valves that make up the pumps were inflated at a higher air pressure. This increased air pressure caused device failure due to delamination in many cases. Because of the short runtimes, extensive data on packet formation is not available. A second problem with the current devices is that the uneven surface coating makes packet transport difficult, as there are isolated regions of the device that will interact with the packets and disrupt the even flow of the carrier fluid.

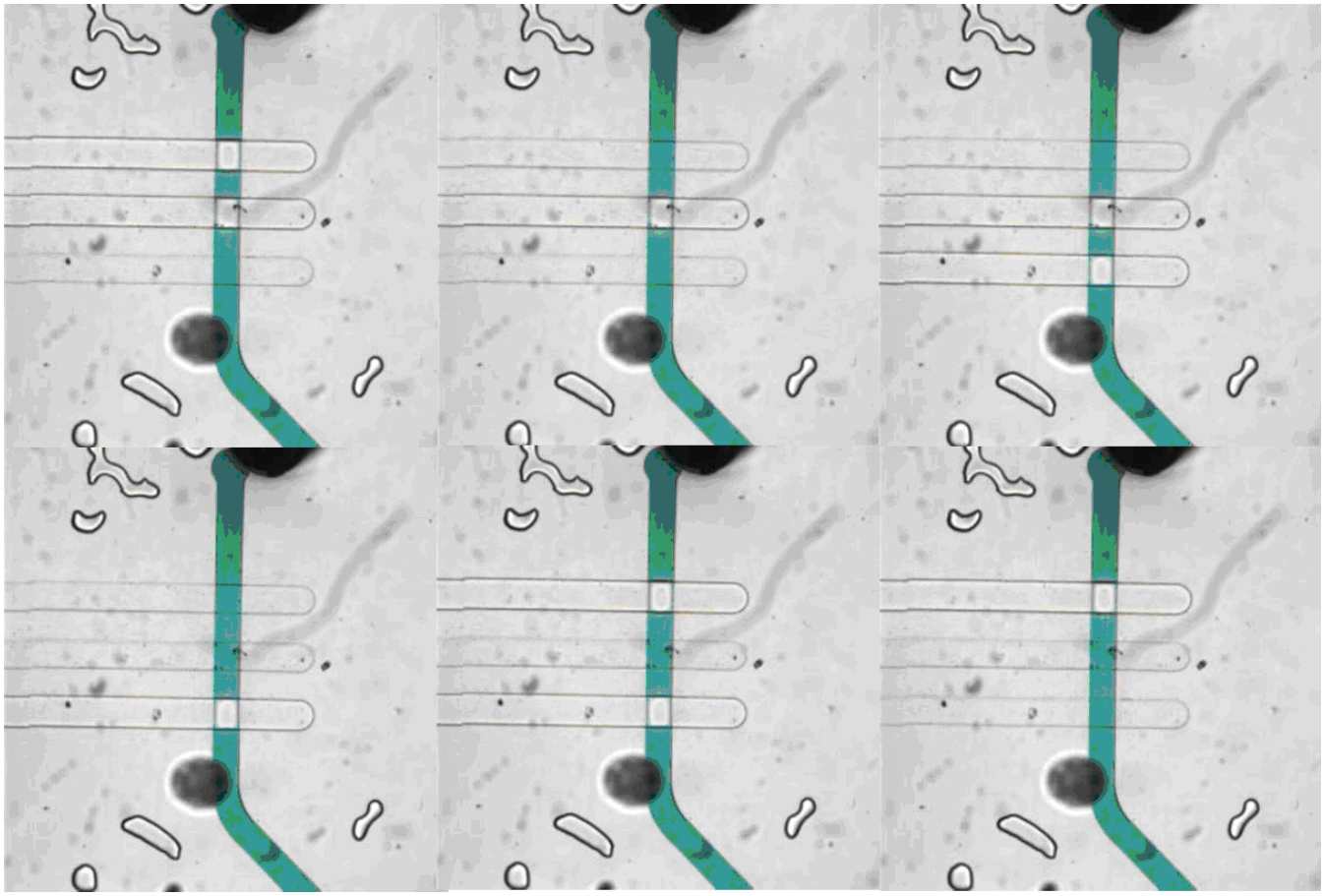


Figure 11: By using a 6-step valve inflation program, a bank three valves can be used as pump. The pump shown above operates at 50 ms per step (3.3 Hz overall for a complete pump cycle).

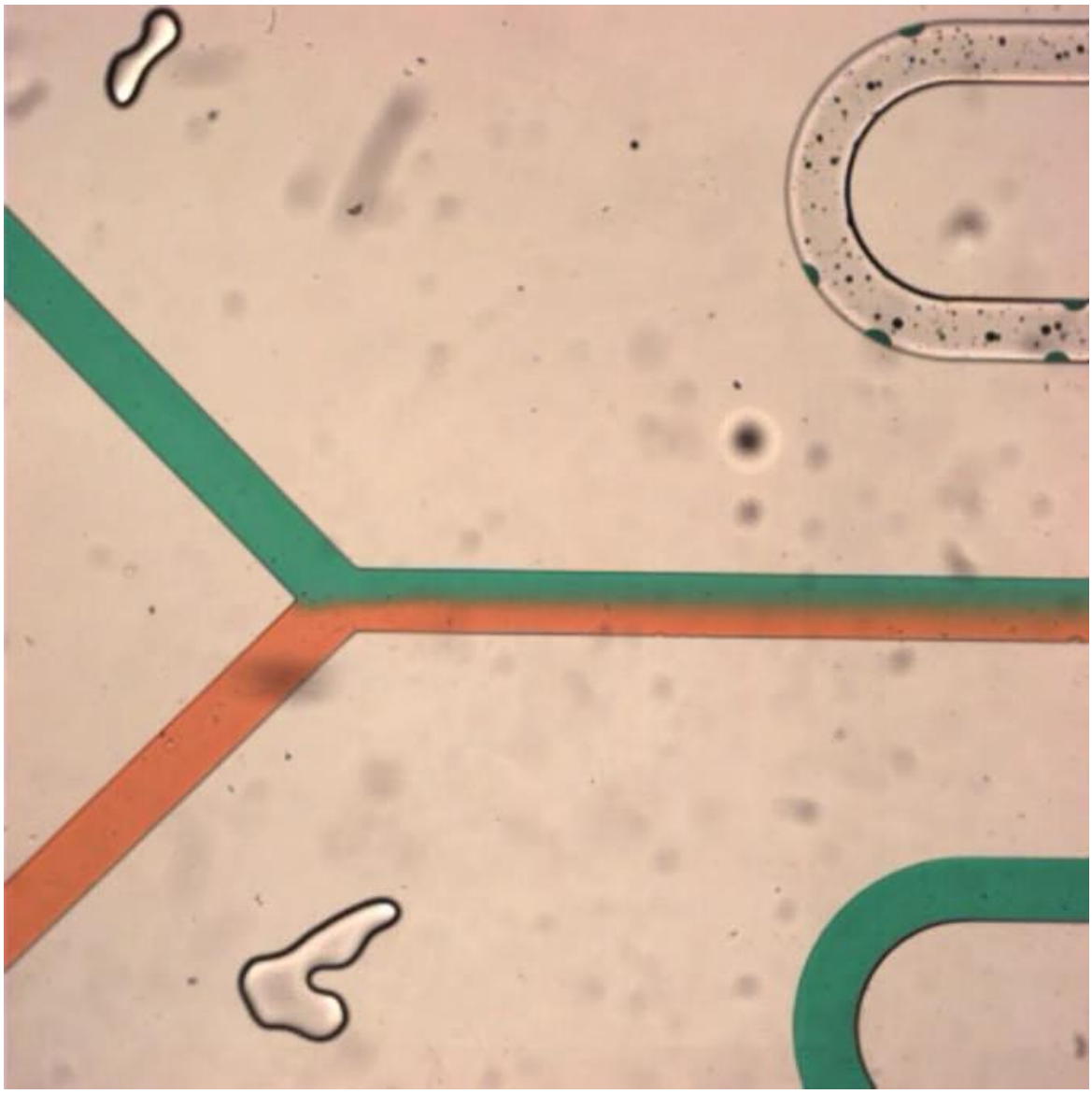


Figure 12: A PDMS pump is operating on each of the channels entering from the left of this micrograph. When 2 aqueous streams are used (as in this example) the pumps produce laminar flow. The fluids used are water solutions with red or green food dye.

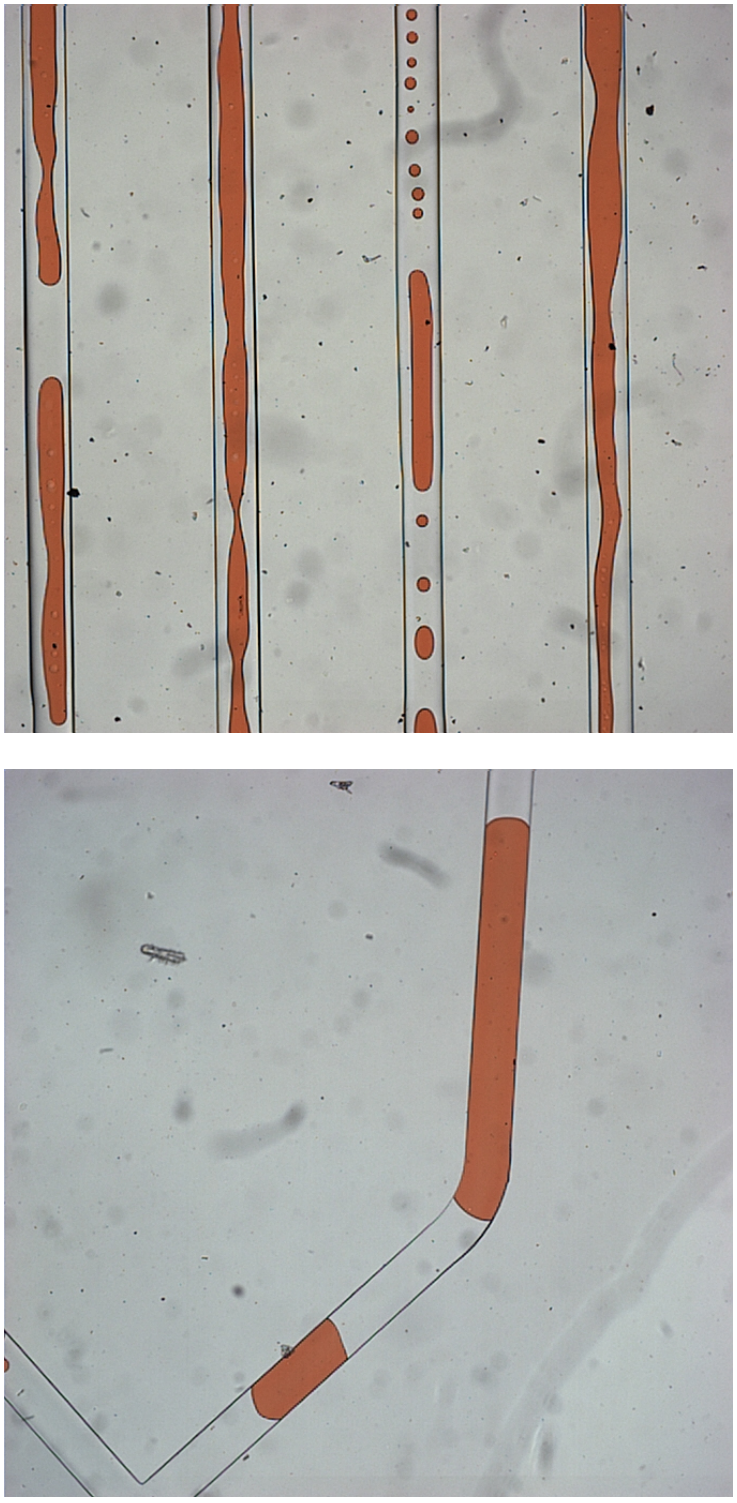


Figure 13: A) (top panel) Uncoated PDMS is hydrophobic, but not fluorophilic. Water and fluorocarbon mixtures do not produce discrete packets. **B) (bottom panel)** PDMS modified with a fluorinated coating produces packets.

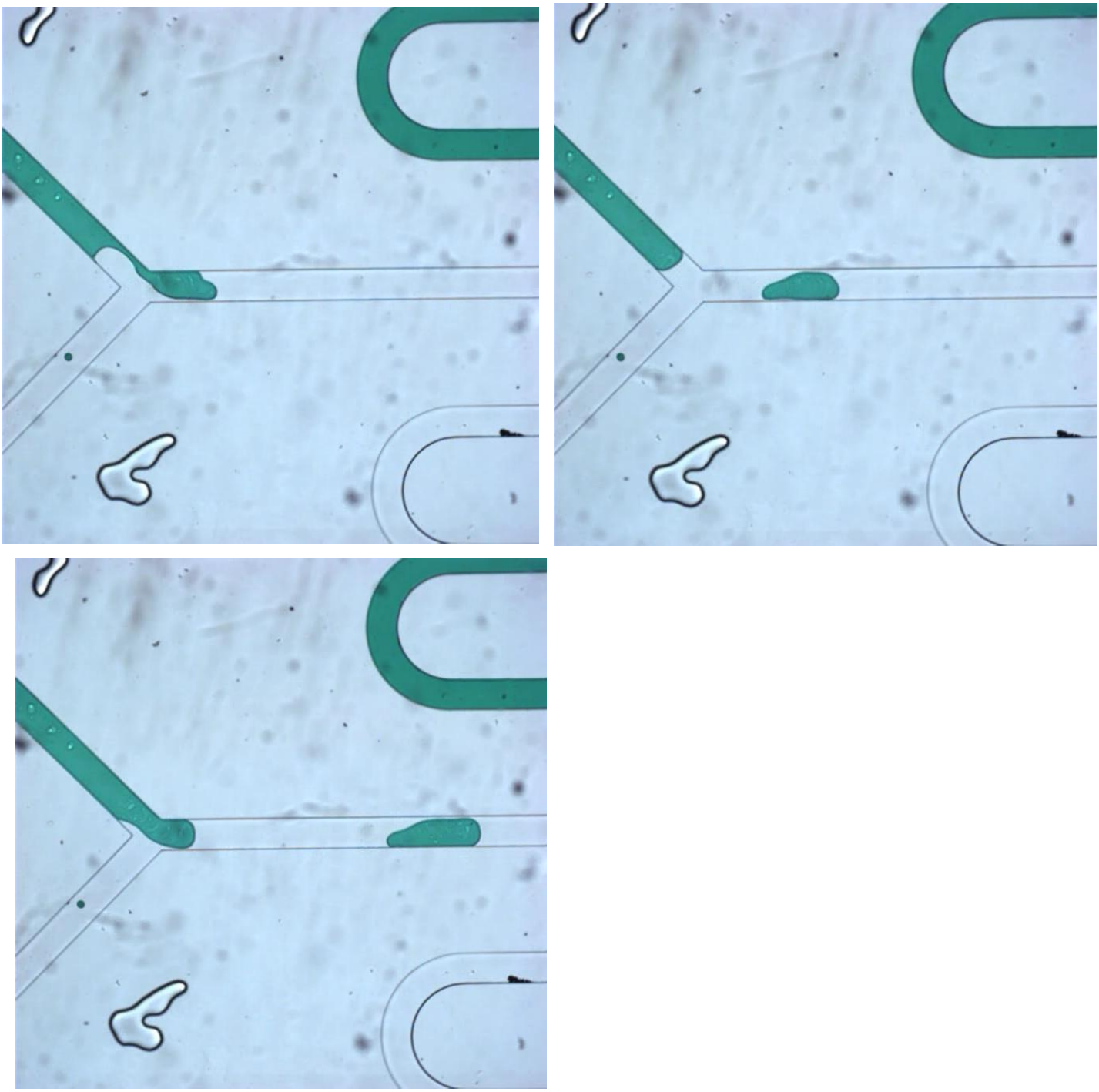


Figure 14: Pumping both fluorocarbon and water (with green food dye) using PDMS pumps causes packets to be formed. In this device the surface is uncoated and the packet shows signs of interacting with the wall.

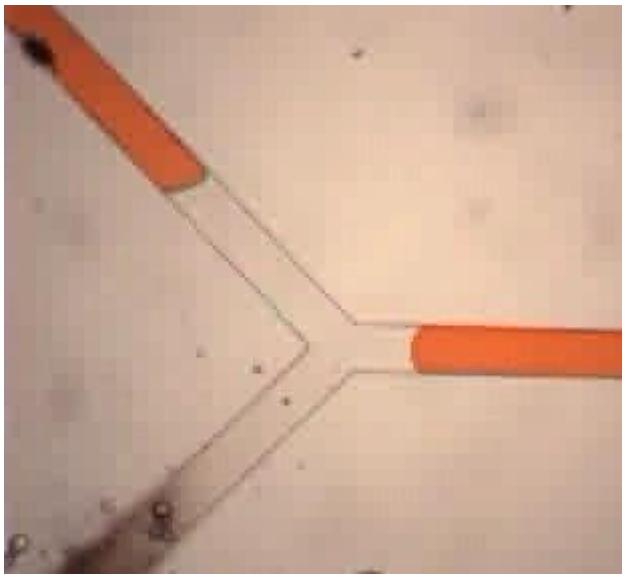


Figure 15: In a coated PDMS device, packets of water can be produced using PDMS pumps.

6. Conclusions and Outlook

This project was designed to be an initial experiment into active control of two immiscible liquid phase flow for the creation of digital microfluidic devices. We have shown that we can generate packet-based microfluidic devices and have identified key parameters for device design. Our key finding is that surface coatings are a critical factor in the formation and transport of packets. We have also found that PDMS-based devices are able to actively generate aqueous packets in a fluorocarbon carrier fluid. Further research is needed to produce reliable, long lived devices so that packet sorting and storage can be realized.

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