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## Reliable addition of reagents into microfluidic droplets

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1 **Abstract** This paper reports a design that reliably adds reagents into droplets by exploiting  
2 the physics of fluid flow at a T-junction in the microchannel. An expanded section right  
3 after the T-junction enhances merging of a stream with a droplet, eliminates the drawbacks  
4 such as extra droplet formation and long mixing time. The expanded section reduces the  
5 pressure build-up at the T-junction and minimizes the tendency to form extra droplets; plays  
6 the role in creating low Laplace pressure jump across the interface of the droplet forming  
7 from the T-junction which reduces the probability of forming extra droplet in the merging  
8 process; provides space for droplet coalescence if there is an extra droplet due to droplet  
9 break-up before merging. In this design, after merging, the reactants are in axial arrangement

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10 inside the droplets which leads to faster mixing. Reliable addition of reagent to the droplets  
11 happens for the combination of flow rates in a broad range from 25  $\mu\text{l/hr}$  to 250  $\mu\text{l/hr}$ , for  
12 both DI water ( $Q_{DI}$ ) and fluorescent ( $Q_{fluo}$ ) streams.

13 **Keywords** Droplet Microfluidics · Droplet Merging · Reagent addition · Droplet Coales-  
14 cence

## 15 **1 Introduction**

16 Microfluidic systems developed with multiphase flows are used for miniaturizing chemical  
17 and biological laboratory techniques [1,2]. Microscale multiphase flows such as aqueous  
18 droplets in oil are useful as sample transporters, mixing enhancers, dispersion eliminators  
19 and simply good discrete microreactors [3,4,5]. Aqueous droplets in microchannel are gen-  
20 erated in a immiscible carrier fluid using T-junction or flow focusing channel [6,11]. Size of  
21 the droplets can be varied by changing the flow rates of immiscible fluids [12,13]. Reagent  
22 addition to the droplets is necessary for chemical and biological analysis. Adding a precise  
23 amount of reagents to a droplet poses difficulties and various schemes for reagent addition  
24 have been investigated by researchers.

25 Dosing of liquid reagents into droplets using a single T-junction in a microchannel was  
26 first demonstrated by Henkel et. al. [8]. However, those demonstrations were limited to a  
27 few flow rates of the reagents. Shestopalov et. al. [10] reported a method to adding reagents  
28 to droplets in which they injected reagents directly into the droplets. Method of injecting  
29 the reagent with the sample to form droplets at the T-junction has difficulties in precisely  
30 controlling the amount of reagents due asymmetric shear stress at the inlet boundary [14,4].  
31 Mixing of reagents can happen in this method, before droplet formation, which interferes  
32 in the case of following instantaneous reactions and this method is not suitable for adding

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33 different reagents and carry out subsequent reactions. Method of coalescing the droplets by  
34 surface energy pattern [16] or geometry mediation [17] has been used to force the immisci-  
35 ble fluid in between the individual droplets and bring into contact to coalesce them. Bremond  
36 et al. [28] proposed that decompressing emulsion droplets mechanism for the coalescence of  
37 droplets in microchannels. Use of electric [26] and electrostatic forces [18] to coalesce the  
38 droplets is not suitable for use with biological materials. Niu et al. [29] reported merging two  
39 droplets with the use of pillars in microchannels. Merging reagents into the droplets moving  
40 in the mainchannel at a T-junction (Fig. 1(a)) experience problems like synchronization of  
41 droplet arrival, contamination of the injecting stream and reliable merging only in a narrow  
42 range of flow rates [8, 3, 15]. Replacing single T-junction with multi-junction eliminates the  
43 need for synchronisation [19], but has higher fabrication cost due to the insertion of hy-  
44 drophilic side channels separately. Injecting reagents alternatively from two side branches  
45 of double T-junctions increases the synchronization frequency in a wide range of flow rates  
46 but merging is not guaranteed to 100% at all flow rate conditions [21].

47 To overcome the above mentioned problems, we propose a design that exploits the basic  
48 fluid flow physics in the microchannel to increase the reliability of adding reagents into  
49 droplets. In this article, it will be demonstrated that an expansion in the microchannel right  
50 after the T-junction enhances the reliability in adding reagents to the preformed droplets at  
51 a T-junction.

## 52 **2 Experimental details**

### 53 2.1 Microchannel Design

54 The rationale behind the provision of an expanded section lies in the answer to the following  
55 question: Why do extra droplets form? If the merging has to happen on a continuous basis

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56 at the T-junction, droplet formation from the side channel has to be synchronized [3] with  
57 the arrival of the droplet which has already been formed and moving in the mainchannel.  
58 Otherwise an extra droplet forms, as seen in Fig. 1(a), termed as unreliable merging, a state  
59 of merging where the reagent itself forms a droplet. Garstecki et al. [11] proposed that the  
60 breakup of the two immiscible liquids at the T-junction in droplet microfluidics is dominated  
61 by the pressure built up across the droplet as it forms at low values of the capillary number  
62 ( $< 10^{-2}$ ). Similarly, merging reagents into droplets with conventional T-junction, which has  
63 similar dynamics as that of the droplet formation at a T-junction, pressure builds up across  
64 the emerging reagent droplet due to the high resistance to the flow of continuous fluid in the  
65 thin films that separate the droplet from the walls of the microchannel, when the droplet fills  
66 almost the entire cross-section of the channel. This pressure buildup squeezes the droplet  
67 to break from the T-junction when the already formed droplet approaches it, as seen in Fig.  
68 9(c).

69 Therefore, if the pressure build-up that squeezes the droplet to detach it from the T-  
70 junction can be reduced, we can avoid the extra droplet problem. We provide an expanded  
71 section, just after the T-junction, on both sides of the channel: the expansion in the side  
72 which is opposite to the droplet forming side channel provides the extra space for the carrier  
73 fluid to move forward, as seen in Fig. 1(b), so that the pressure build-up can be reduced;  
74 the expansion in the side from which the droplet forming provide the space for the droplet  
75 to grow, which allows the space for the extra volume created during the waiting time for  
76 the droplet arrival in the main channel. In doing that, there is one more additional feature  
77 added, apart from the reduced pressure drop: when the droplet breaks before merging, due to  
78 longer waiting time and droplets growing big to block the extra space, the expanded section  
79 can function like a time delay component. It can restrict the movement of the droplets and  
80 facilitate the process of droplet coalescence. Fig. 1 shows the schematic diagram of the mi-

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81 crochannel designs (a) with conventional T-junction and (b) with an expanded section. There  
82 are three inlets to pump the fluids into the microchannel and one outlet to collect the spent  
83 fluids. The mineral oil inlet and the DI water with fluorescence inlet meet at the Y-junction  
84 and the DI water inlet from the side meet the main channel at T-junction. The expanded sec-  
85 tion is located at the distance of  $50\mu\text{m}$  after the T-junction, but could be located as closely  
86 as possible, the constraint being the resolution of the photo mask. The microchannel has the  
87 height of  $100\mu\text{m}$ . The other dimensions of the microchannel are shown in Fig. 1.

## 88 2.2 Fabrication

89 The channel designs were printed into a photolithographic mask and the negative SU-8  
90 photo resist (Microchem Corp.) was used to fabricate the master mold using standard pro-  
91 cedures specified from Microchem. Then microfluidic chips were fabricated using poly-  
92 dimethylsiloxane (PDMS) polymer (Dow corning Sylgard 184 Silicone Elastomer) through  
93 the standard soft lithography process for PDMS microchannel fabrication [22]. The cured  
94 PDMS microchannels were bonded to another piece of flat PDMS layer after treating them  
95 with oxygen plasma. And they are allowed to recover their hydrophobicity, because of the  
96 need of the walls to be hydrophobic which facilitates the formation of water droplets in oil.

## 97 2.3 Experimental setup

98 The following fluids were used for the experiments: 1) Mineral oil as the carrier fluid  
99 (M5904, Sigma-Aldrich) with 2% w/w Span 80 surfactant (Sigma-Aldrich S6760), 2) DI  
100 water with fluorescent dye (0.05% w/w Acid Yellow) and 3) DI water. Hydrodynamic prop-  
101 erties: Viscosity of DI water ( $\mu$ ) is  $1\text{ mPa s}$ , interfacial tension between water and mineral oil  
102 is  $3.65\text{mNm}^{-1}$ , contact angle between water and PDMS is  $88^\circ$ , viscosity of mineral oil with

103 2% w/w Span 80 is 23.8 mPa.s. The fluids were pumped from gas-tight syringes (Hamilton,  
104 1.25ml) through the tubing (0.8 mm PTFE, Cole-Parmer) connected to inlets, with the help  
105 of syringe pumps (KD Scientific, Model No. 781200). The DI water with fluorescent stream  
106 forms droplets (droplet A) at the Y-junction as seen in Fig. 1. When the fluorescent droplets  
107 formed at the Y-junction reach the T-junction, the aqueous reagent stream (DI water) from  
108 the side channel is merged to the droplets. The experiments were observed under the Inverted  
109 Fluorescence Microscope (Nikon Eclipse TE2000-S) with suitable magnification using Plan  
110 Apro objectives and mercury lamp for illumination and blue filter for visualisation. The  
111 visualisation of the experiments were captured and recorded through the eye piece of the in-  
112 verted microscope using a CCD camera (DCRDVD803E, SONY) and used for the analysis  
113 of the reliability of droplet merging process.

### 114 **3 Testing**

115 Experiments were carried out for the T-junction with expanded section and for the conven-  
116 tional T-junction designs. The combination of fluorescent stream flow rates ( $Q_{flu}$ ) and DI  
117 water flow rates ( $Q_{DI}$ ) were from 0  $\mu$ l/hr to 250  $\mu$ l/hr in the interval of 25  $\mu$ l/hr, and the  
118 mineral oil flow rate was maintained at the ratio of 1.5 to the fluorescent flow rate. Between  
119 0  $\mu$ l/hr to 25  $\mu$ l/hr, intermediate flow rates of 10  $\mu$ l/hr to 20  $\mu$ l/hr were also used to measure  
120 the merging percentage. The flow rate ratio (FR) between mineral oil and fluorescent was  
121 kept constant at 1.5 with the aim that the droplets should be not too long or too short for this  
122 merging process. We found this ratio by changing the ratio from 0.1 to 4.0, with keeping the  
123 flow rate of fluorescence constant at 50  $\mu$ l/hr. The corresponding picture is shown in Fig. 2,  
124 in which it is seen that the flow rate ratio from 1 to 2.5 produce droplet which are not too  
125 long or too short. We chose 1.5 and kept constant throughout the experiments. The videos

126 of the experiments were recorded for both the conventional T-junction and T-junction with  
127 expanded section designs.

## 128 **4 Results and discussion**

### 129 4.1 Reliability of the merging process

130 The recorded videos of the experiments were used to calculate the reliability of the merging  
131 process by counting the number of successfully merged droplets. The percentage of reliable  
132 merging for a particular flow rate combination was calculated as follows,

$$\%Reliability = \left( \frac{\text{number of successfully merged droplets}}{\text{total number of droplets generated for merging}} \right) \times 100. \quad (1)$$

133 The contour lines indicating percent rate of droplet merging for T-junction alone and  
134 T-junction followed by an expansion of 150  $\mu\text{m}$  were constructed and are shown in Figs.  
135 3 and 4. Reliable merging (100%) for T-junction happens in a very narrow range of flow  
136 rates (below the dashed contour line) as seen in Fig. 3 and merging in a wide range of flow  
137 rate ratio gives the extra droplet problems. Microchannel with T-junction followed by an  
138 expansion provides reliable merging in a wide range of flow rates and flow rate ratio as  
139 seen in Fig. 4. The region of reliable merging is wide (region between dashed contour lines)  
140 and droplets are merged reliably in a wide range of fluorescent flow rates except at high  
141 DI water flow rates (bottom right) and low fluorescent flow rates (top-left). It is because at  
142 higher flow rates of DI water, not enough fluorescent droplets (droplet A) are formed. The  
143 low fluorescent stream cannot overcome the pressure drop of carrier fluid (mineral oil) as  
144 well the pressure drop due to high DI water flow rate to form droplets at the Y-junction.  
145 Therefore the merging percentage is low for high DI water flow rate at the top-left region in



146 Fig. 4. But when the flow rate becomes slightly higher, fluorescent stream can overcome the  
147 pressure drop and form enough droplets to merge reliably.

148 Outside the high percentage success, in general, there are three scenarios: 1) when the  
149 DI water flow rate is high and the fluorescent flow rate is low, extra droplets (droplet B)  
150 form and the merging percentage goes down; when the DI water flow rate is very high, only  
151 droplet B forms, which is not desirable as no merging is possible. 2) when the fluorescent  
152 flow rate changes from very high compared to DI water flow rate, DI water stream cannot  
153 overcome the pressure drop to merge continuously with droplet A. 3) when both fluorescent  
154 flow rate and DI water flow rate are very high, stratified flow occurs, which is not desirable  
155 for the merging process.

#### 156 4.2 Droplet volumes and droplet formation time

157 Fig. 5 shows a sample of measurements for the volume of the droplets generated at the Y-  
158 junction for various flow rates of DI water with mineral oil flow rate fixed at the ratio of  
159 1.5 with respect to the fluorescence flow rate for the droplet merging process. The droplet  
160 generated are consistent over time with volumes in the order of few nanolitres with less than  
161 10% standard deviation as seen in Fig. 5. Fig. 6 shows the droplet volumes generated for  
162 various flow rates of fluorescence from the side channel at the T-junction with expansion  
163 and without expansion. These droplet volumes in the range of few nanolitres were measured  
164 from the experiments without the merging process. The volume of the droplets generated at  
165 the T-junction with expansion is higher than the volume of droplets generated at T-junction  
166 without expansion.

167 Fig. 7 shows the droplet formation time at the T-junction with expansion and without ex-  
168 pansion. As expected, the droplet formation time at the T-junction with expansion is higher

169 than the time for the T-junction without expansion. This increase in volume and time is  
170 due to the expanded section, which reduces the pressure drop availability of extra space for  
171 droplet growth leads to longer residence time for the droplet at the T-junction.

172 The reliability of the merging process with an expanded section was analysed by mea-  
173 suring the droplet length before merging and after merging. Fig. 8 shows the droplet lengths  
174 measured before merging and after merging for different fluorescent flow rates and DI water  
175 flow rates. As we can see from Fig. 8, the length of droplets (droplet A) generated at the Y-  
176 junction (before merging) is consistent and the droplet volume decreases with the increase  
177 in the fluorescense flow rate. This also reflects in the amount of DI water merged into it at the  
178 T-junction with expansion. As the droplet length goes down, the amount of DI water merged  
179 to it also goes down because of the small residence time for the droplet the T-junction. As  
180 the DI water flow rate is increased, the amount of DI water added to the same length of  
181 droplet also goes up due to higher volume flow rate of DI water. The length of droplets after  
182 the merging process is also consistent as seen in Fig. 8.

#### 183 4.3 Flow physics in the merging process

184 The increase in the reliability of the merging process in the T-junction with expanded section  
185 design can be explained as follows. Figure 9(a-c), shows the sequence of droplet break-up  
186 due to pressure build up at a normal T-junction. But, the expanded region right after the  
187 T-junction allows the carrier fluid to move forward avoiding the pressure build up beyond  
188 threshold-level and break-up of droplet B from the T-junction. Now, two things can happen  
189 for the droplet forming from the side channel:

- 190 1. droplet sticks to the T-junction due to surface tension and grows in the expanded region  
191 (Fig. 9(d-e)) until the droplet A merges with it (Fig. 9(f)).

192 2. droplet breaks away because of high droplet volume (Fig. 10(a)), due to longer waiting  
193 time for the droplet A to arrive.

194 In the first case, after merging, the merged droplets are squeezed from the T-junction due  
195 to pressure build up across the droplet as in the normal droplet formation process, because  
196 now the merged droplet fully occupies the channel. In the second case, droplet B and droplet  
197 A coalesce in the expanded region. The expanded region facilitates the droplets to come  
198 closer and coalesce, as seen in ( Fig. 10(b)), because it acts like a time delay component  
199 and delays the forward movement of the extra-droplet in the microchannel. When the delay  
200 time is long, extra droplets occur and the merging percentage goes down. The sequence of  
201 droplet coalescence is seen in Fig. 10(a-d).

202 The expanded section not only eliminates the extra droplet formation but also enhances  
203 droplet merging and can be explained as follows: the extra space provided by the expanded  
204 region reduces the pressure build up that happens in the droplet formation at the T-junction  
205 and it is less than the threshold value for droplet break-up; the carrier fluid movement  
206 through the extra space exerts shear stress on the droplet forming from the side channel;  
207 therefore, the droplet formation dynamics has been changed by the expanded section at the  
208 T-junction from purely pressure dominant break-up to a combination of pressure drop and  
209 shear due to the flow of carrier fluid; the combination of shear stress and pressure buildup  
210 distorts the interface [11] and makes it flat, as seen in Fig. 9(d). The distortion remains until  
211 the fluorescent droplet (droplet A) hits the emerging DI water droplet (droplet B)(See ESI  
212 Movie1).

213 When the interface is flat, the curvature becomes low and the radius of curvature ap-  
214 proaches infinity; based on the Young-Laplace equation, Laplace pressure jump  $\Delta P_L$  ex-  
215 erted by the interface on the emerging droplet in a rectangular channel can be described

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216 as,  $\Delta P_L = \sigma (1/R_w + 1/R_h)$ , where  $\sigma$  is the interfacial tension between the two phases,  $R_w$   
217 and  $R_h$  are interface curvatures in width and height directions respectively; Laplace pressure  
218 jump,  $\Delta P_L$ , across the interface is negligibly small and when the fluorescent droplet (droplet  
219 A) hits the DI water droplet (droplet B) at the T-junction, they merge easily without breaking  
220 the DI water droplet from the T-junction. Small Laplace pressure jump across the interface  
221 lowers the disturbance needed to rupture the thin film between the droplets and it reduces  
222 the possibility of droplet B breaking from the T-junction at the moment droplet A hits it.  
223 When the external pressure increases above the internal pressure at a point on the droplet  
224 surface, the droplets break up and the constituents merge.

#### 225 4.4 Enhanced mixing

226 The merging and the coalescence patterns of droplets due to expanded section enhances  
227 mixing, because after merging the merged fluids are in axial arrangement inside the droplets  
228 as seen in Figs. 9(f) and 10(d). Tanthapanichakoon et al. [23] reported that axial arrange-  
229 ment of droplet constituents enhances mixing and it can be attributed to the reduction of  
230 the striation length between reactant segment by interlayering. Similar results for enhanced  
231 mixing due to axial arrangement (in-line droplet fusion) have been reported by Liu et al. and  
232 Frenz et al. [24, 25]. Therefore, it can be stated that axial arrangement of droplet constituents  
233 in this merging design lead to enhanced mixing.

## 234 5 Conclusions

235 In conclusion, we demonstrate a design that exploits the physics of fluid flow phenomena  
236 in microchannels to eliminate the drawbacks in adding reagents into the droplets at a T-  
237 junction. An expanded section, right after the T-junction, reduces the pressure build up and

238 increases the residence time of the droplet at the T-junction; in the case of droplet break-up,  
239 it facilitates the coalescence of the droplets and reduces the probability of the formation of  
240 extra droplet. Holding the droplet at the T-junction leads to the distortion of the interface  
241 of the emerging droplet and becomes flat because of the shear stress and pressure build-up  
242 due to the moving carrier fluid. A flat interface, according to the Laplace-Young equation,  
243 results in low Laplace pressure between two phases and enhances the merging of miscible  
244 droplets. Reagent addition in this design leads to axial arrangement of the droplets con-  
245 stituents in the merging as well as in the coalescence process and facilitates faster mixing of  
246 reactants. Therefore, the demonstrated design provides a better alternative for the available  
247 merging schemes and can be effectively used in the microfluidic chips used for biological,  
248 bio-chemical and  $\mu$ -TAS assays. Effects of expansion width and length of the expanded  
249 section on the reliability of reagent addition to the droplets will be carried out in the future.

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325 **Captions of figures**

326 **Fig. 1** Schematic of the droplet merging channel with expansion.  $\Delta P_{in}$  is the interfacial  
327 pressure difference between the oil and DI water

328 **Fig. 2** Length of droplets vs flow rate ratios of mineral oil and fluorescence

329 **Fig. 3** Critical volume of the droplets formed at the Y-junction

330 **Fig. 4** Critical volume of the droplets formed at the conventional T-junction and T-  
331 junction with expansion

332 **Fig. 5** Time for droplet formation at the conventional T-junction and T-junction with  
333 expansion

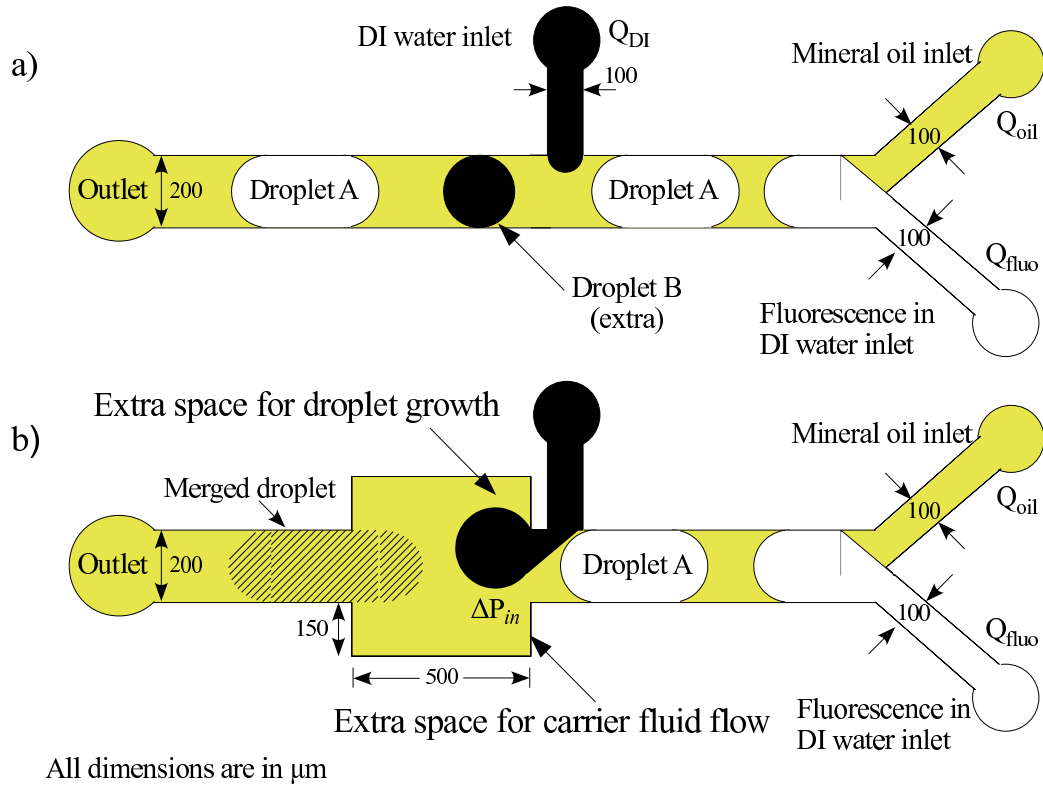
334 **Fig. 6** Contour plot for merging of reagents into droplets with conventional T-junction

335 **Fig. 7** Contour plot for merging with T-junction and subsequent expansion

336 **Fig. 8** Droplet merging phenomena in: (a-c) - extra droplet formation in a conventional  
337 T-junction, (d-f) -merging of droplets before break off in a T-junction followed by an ex-  
338 panded section

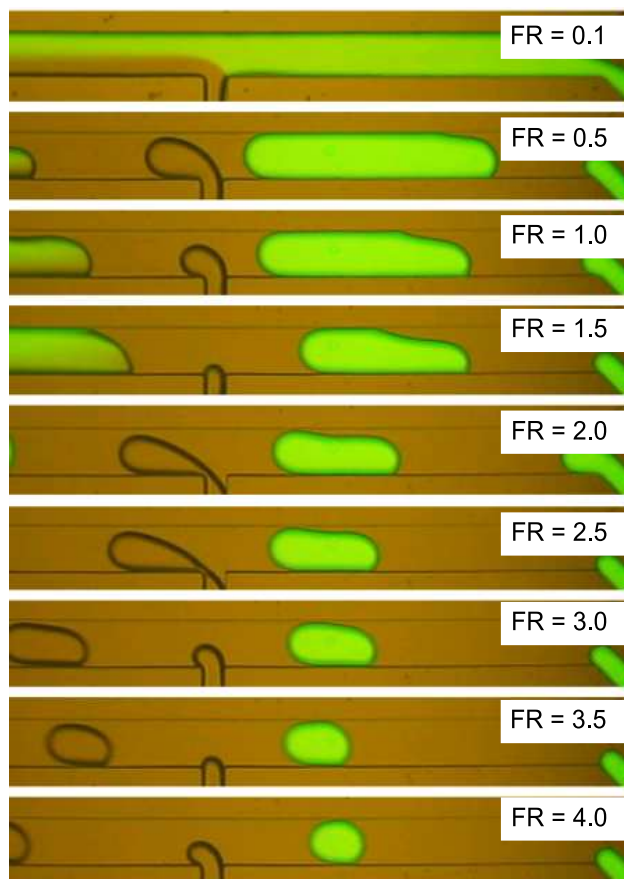
339 **Fig. 9** Coalescence of droplets in the expanded section after droplet break-up

340 **Fig. 10** Droplet lengths before merging and after merging for various flow rates of fluo-  
341 rescence and DI water

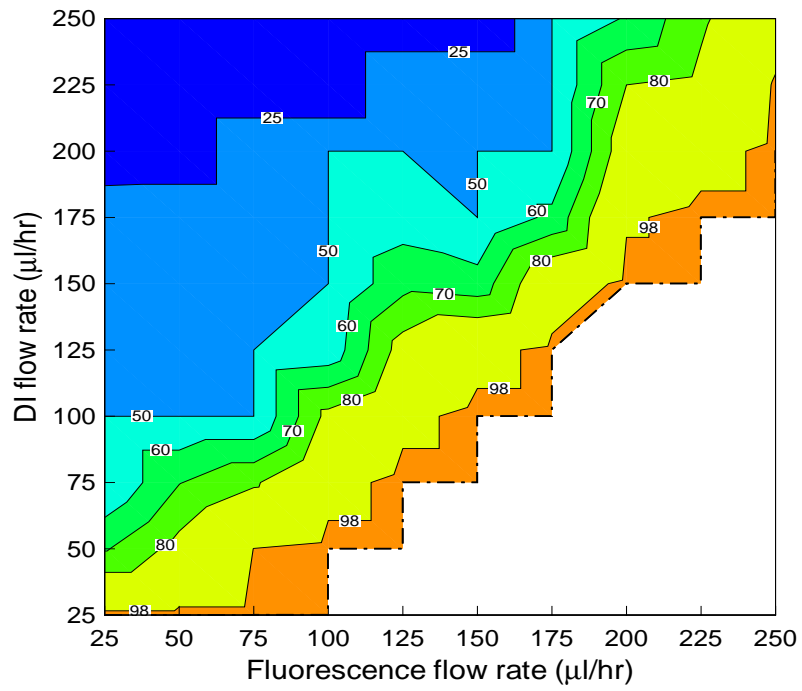


**Fig. 1** Schematic of the droplet merging channel: a) conventional T-junction b) T-junction with expansion.

$\Delta P_m$  is the interfacial pressure difference between the oil and DI water



**Fig. 2** Droplet length vs flow rate ratios of mineral oil and fluorescence



**Fig. 3** Contour plot for merging of reagents into droplets with conventional T-junction

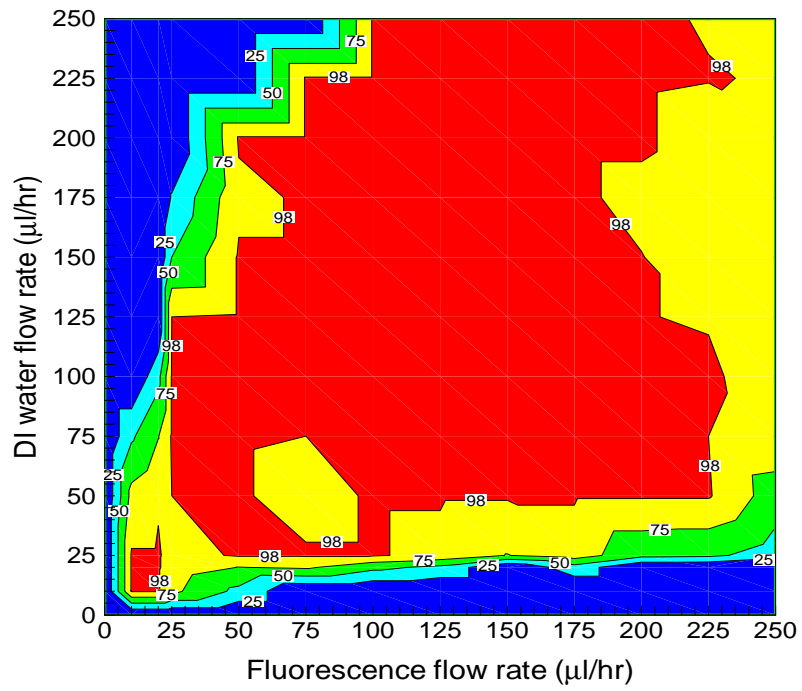


Fig. 4 Contour plot for merging with T-junction and subsequent expansion

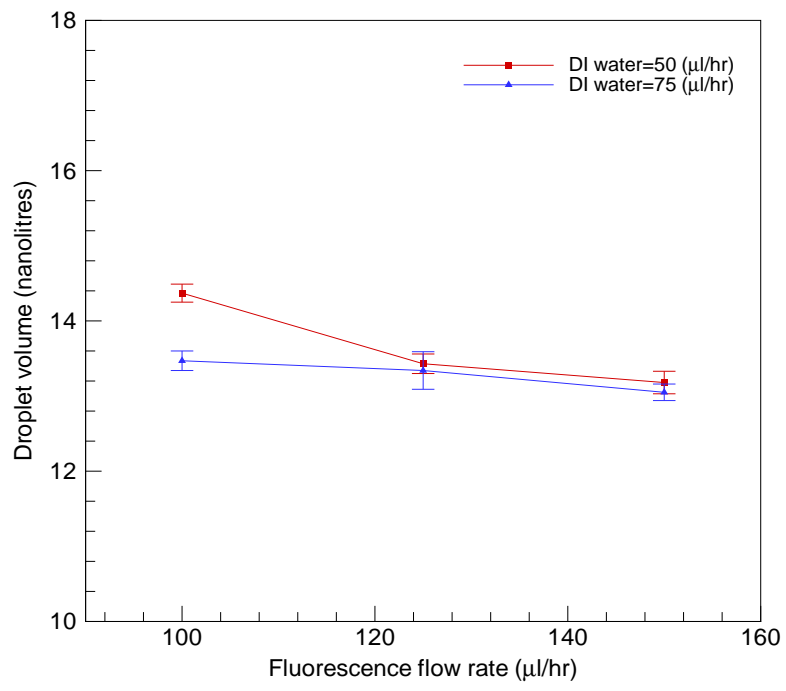
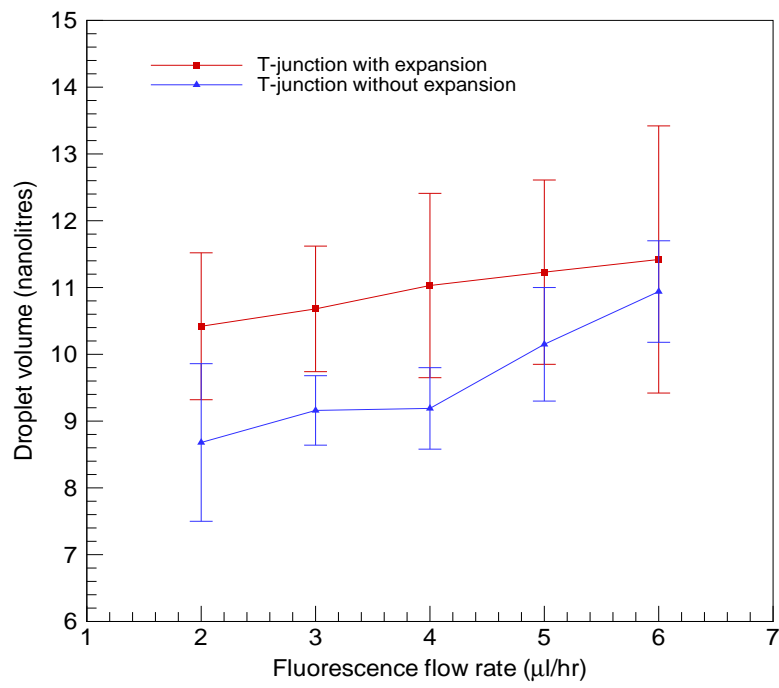


Fig. 5 Volume of the droplets formed at the Y-junction



**Fig. 6** Critical volume of the droplets formed at the conventional T-junction and T-junction with expansion

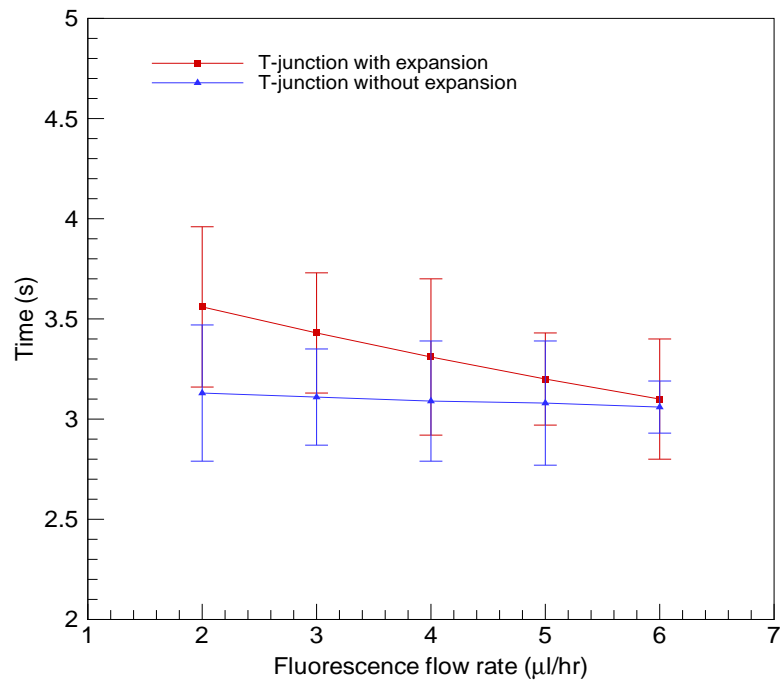


Fig. 7 Time for droplet formation at the conventional T-junction and T-junction with expansion



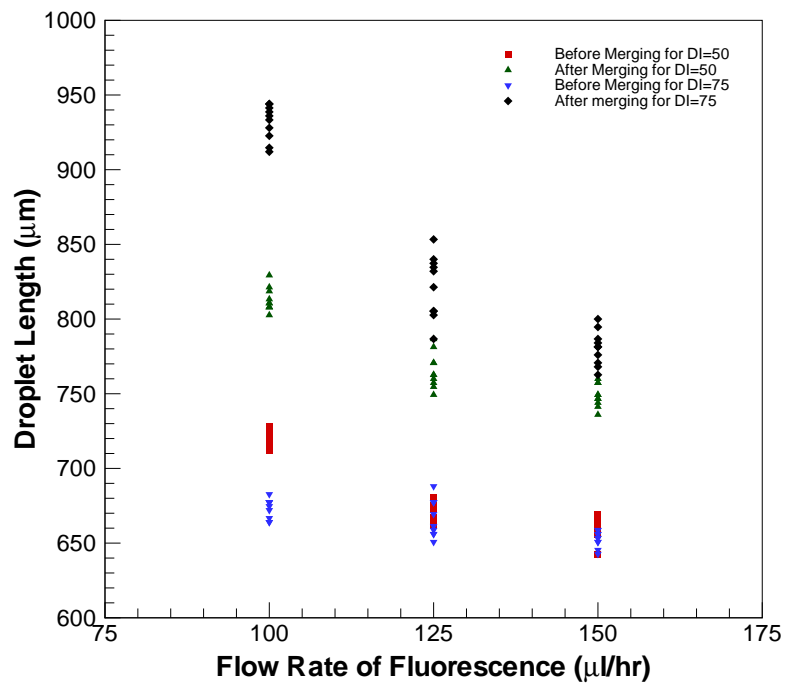
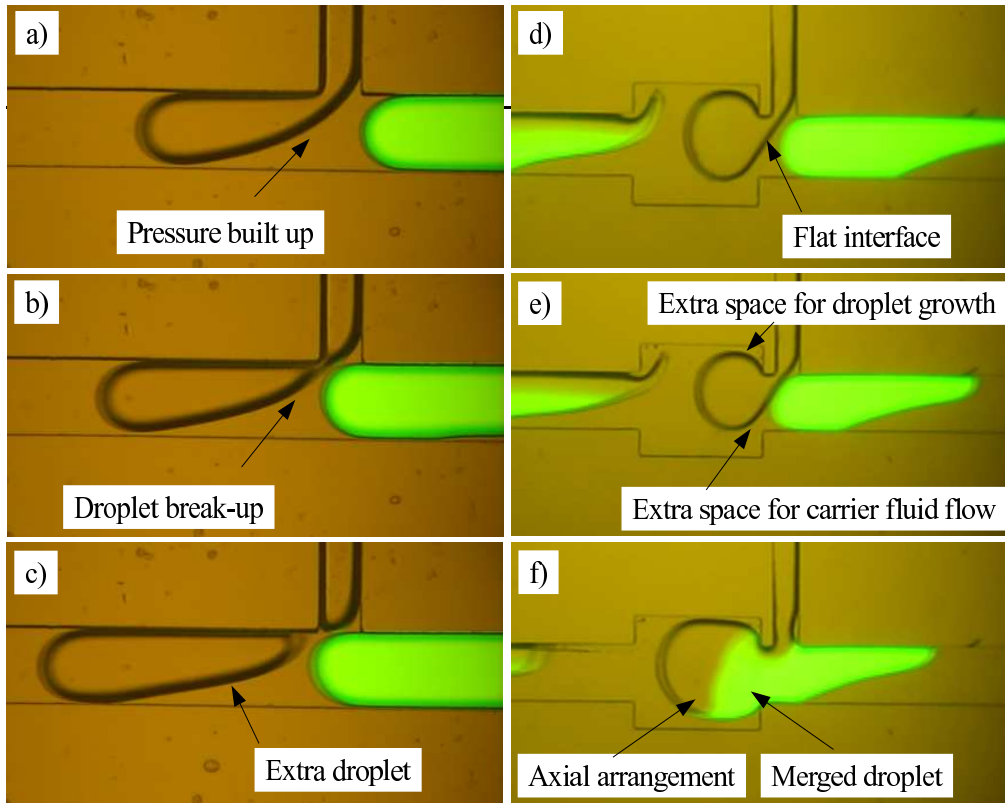
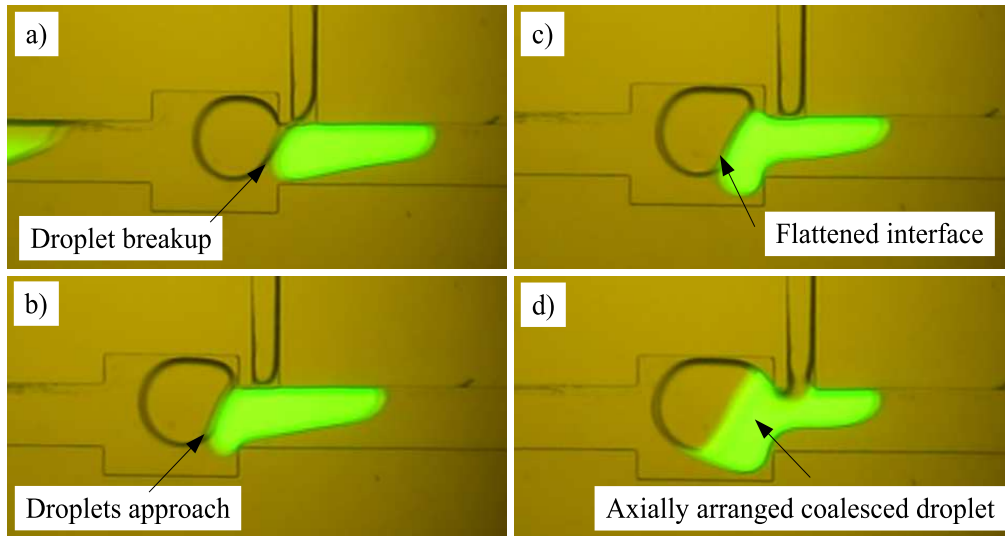


Fig. 8 Droplet lengths before merging and after merging for various flow rates of fluorescence and DI water



**Fig. 9** Droplet merging phenomena in: (a-c) - extra droplet formation in a conventional T-junction, (d-f) - merging of droplets before break off in a T-junction followed by an expanded section



**Fig. 10** Coalescence of droplets in the expanded section after droplet break-up