Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information (SI) for Lab on a Chip

DROPLAY: Laser writing of functional patterns within biological microdroplet displays

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S1 Laser Drawing Software

ZEN 2009 software was used to operate the microscope and conduct both uncaging and imaging of samples. Using the bleaching function of the software, different patterns could be created either by assembling basic shapes together as shown in figure S1A, or manually drawing free-form geometries as shown in Figure S1B.

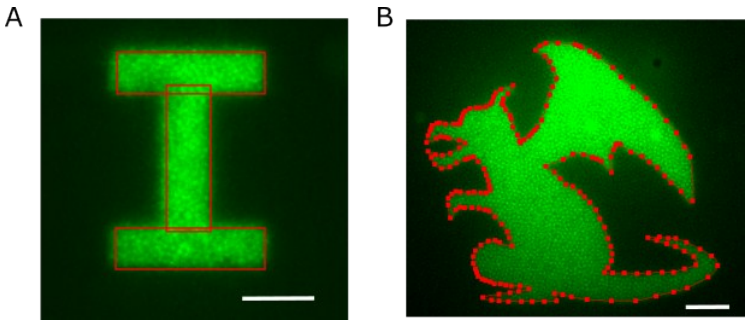

Figure S1 illustration of bleaching function. A) the letter "I" is constructed by the combination of one vertical and two horizontal rectangles. B) The dragon shaped partterns were made by free-hand drawing with the software. (scale bar = 100 μm)

S2 Spatial Resolution Δ

Figure S2a shows the intensity profile $I(x)$ of a rectangular pattern, averaged along the longitudinal (y) direction (see also Figure 4a of the manuscript). As shown in Figure S2b, the module of the derivative $\frac{dI(x)}{dx}$ – obtained via numerical differentiation of $I(x)$ – shows two peaks corresponding to the inflection points of the intensity profiles. These peaks were fitted to Gaussian curves $A\,exp(-\frac{(x-\bar{x})^2}{2\sigma^2})$ with $\bar{x}$ and σ determining, respectively, the position and the width of the boundaries of the rectangular pattern in the x

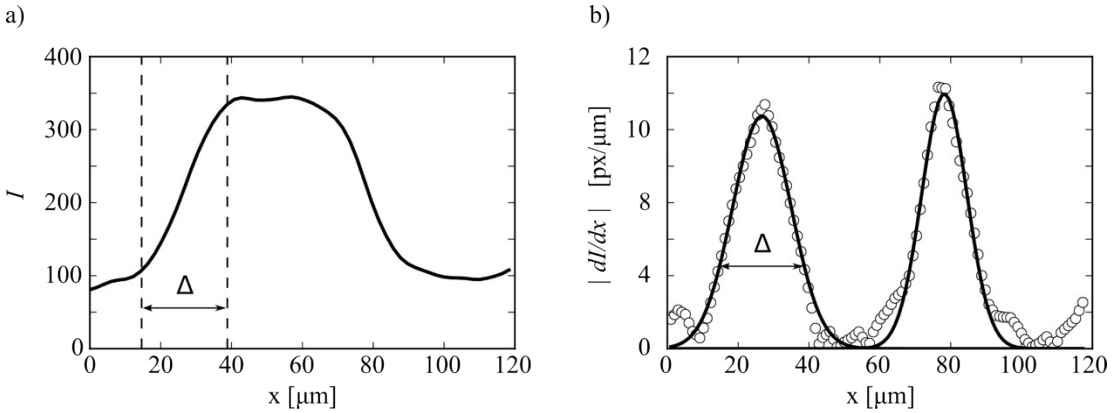

Figure S2 a) Intensity profile of a rectangular pattern averaged over the londitudinal direction. b) Module of the derivative of the intesity profile (circles) together with best-fitting Gaussian curves (solid lines)

direction. The spatial resolution Δ is hence defined as $\Delta = 2\sigma$.

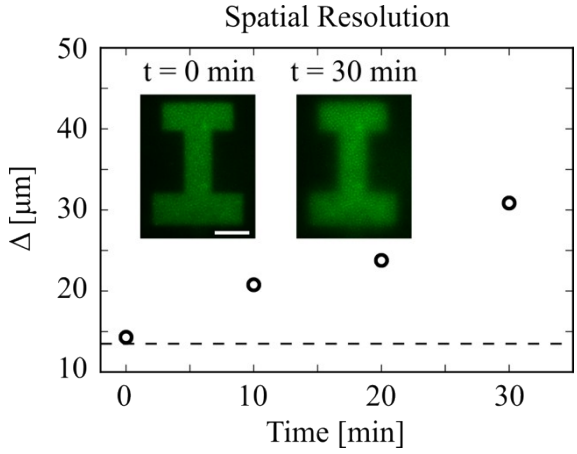

Figure S3 Spatial resolution Δ against time. The dashed lines represents the droplet mean diameter. The insets show the activated pattern at 0 and 10 min after exposure to the activation laser.

shown in Figure S3.

S3 Photobleaching of Uncaged Fluorescein

To verify the stability of the activated patterns against dye photobleaching induced by the excitation laser source, the average pixel intensity of a display region activated at time $t = 0$ was monitored over time. As shown in Figure S4, the pixel intensity value at equilibrium was retained over 30 minutes when the activated area was observed after exposure every minute. This demonstrates the negligible effect of dye bleaching over this time scale.

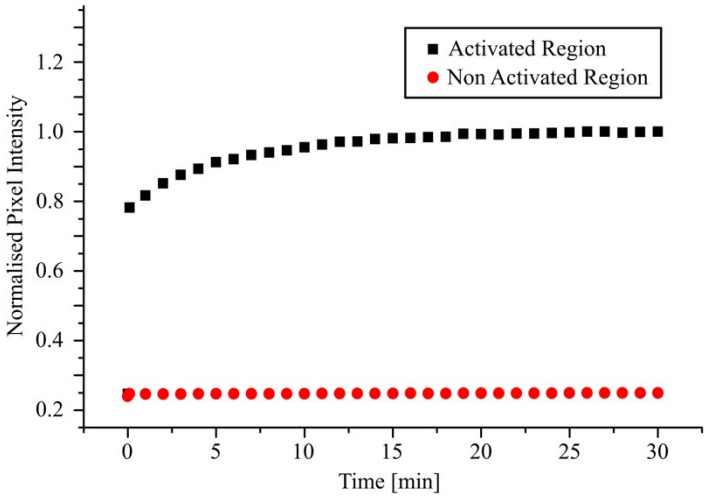

Figure S4 Average fluorescence intensity of an activated region against time together with average intensity of a non-activated region.

S4 Leakage of Dye Molecules

It is well documented that in water-in-oil emulsions the surfactant molecules can promote the leakage of small molecules, originally encapsulated in the water droplets.[1–4] Such a mechanism could be at the origin of the slight blurring of the edges of the activated regions we observed in our experiments. To corroborate this hypothesis, DI water droplets (ca. 0.1 µL in volume), containing 2.4 mM of fluorescein, were pipetted in the well of a single-well microscope slide filled with hexadecane. This fluorescein concentration, matching the concentration of caged dye in the 'undifferentiated' microdroplets of the proposed system, exceeds the fluorescein solubility limit in DI water. As a result, aggregates of undissolved dye formed within the water droplets (Figure S5). The behaviour of the dye molecules was monitored by fluorescence microscopy over a period of 60 minutes under varying conditions. In the absence of Span 80 surfactant in the oil phase, no fluorescein leakage was observed through the droplet interface (Figure S5 A-B). On the other hand, the presence of 3% w/v Span 80 surfactant in the oil phase resulted in significant leakage of dye molecules through the droplet interface after 60 minutes (Figure S2 C-D). These experiments confirm our hypothesis that the diffusion of fluorescein across the water-oil interface is induced by the presence of surfactant in the oil phase.

In order to decrease the rate of transport of molecules across liquid phases in the presence of surfactant, the use of additives in the dispersed phase such as sodium chloride, Bovine Serum Albumin (BSA) and sugar would have to be considered, as discussed by Baret et al[5]. Alternatively, the rate of transport could also be reduced by decreasing the partition coefficient of the solute (i.e. fluorescein) between the oil and aqueous solutions via control over the solute degree of protonation. As shown in Figure S6, lower PH values result in higher degrees of protonation of the fluorescein molecules, which would hence partition more favourably into the oil phase due to their higher level of hydrophobicity. Therefore, increasing the pH of the aqueous phase would likely be able to reduce the amount of fluorescein partitioned into the oil and hence delay the leakage effects.

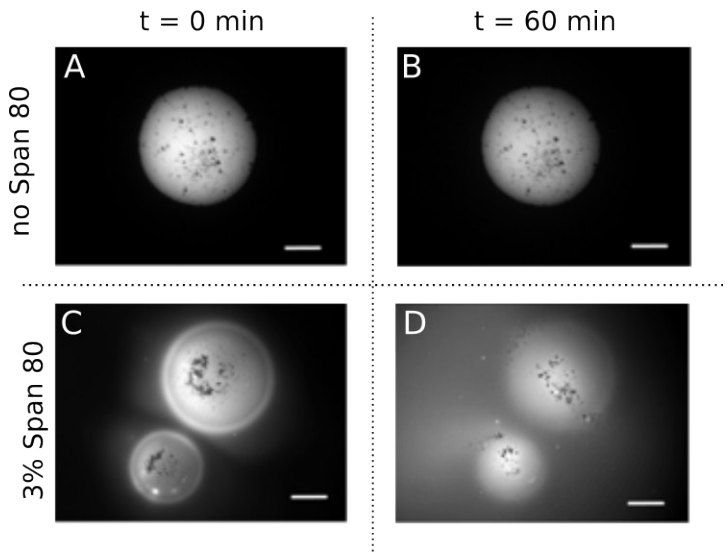

Figure S5 Effect of Span 80 surfactant on leakage of fluorescein molecules through the water-in-oil droplet interface. The scale bars

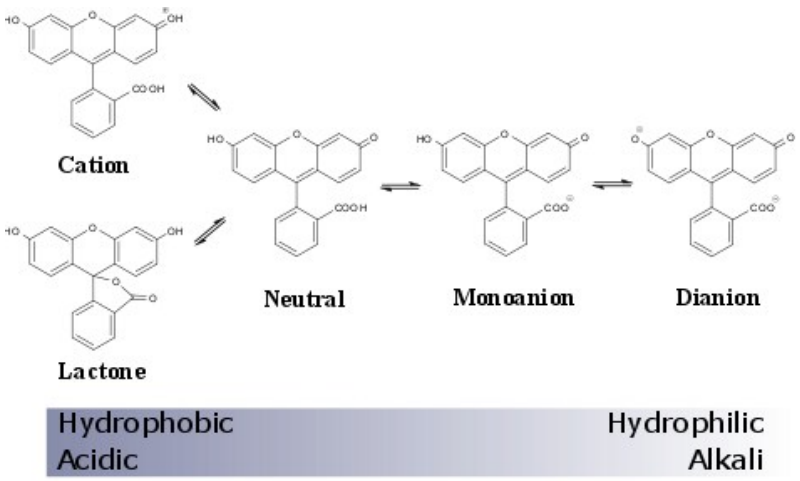

Figure S6 Degree of ionisation and hydrophobicity of fluorescein at different pH.

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