Electronic Supplementary Material (ESI) for Soft Matter.
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Electronic Supplementary Information (ESI)

Flows of nonequilibrated aqueous two-phase systems in a microchannel

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Section 1. Binodal curve characterization

We characterize the binodal curve of the aqueous two-phase system (ATPS) of DEX (Dextran) 150,000 Da and PEG (poly(ethylene glycol)) 35,000 Da based on the protocol by Atefi *et al.*[1] The binodal curve is shown in Figure S1. The error bars on the binodal curve indicate standard deviation among 4 titration experiments per data point. To better understand the boundary of the binodal curve in the region of high C_{DEX} and low C_{PEG}, we compute an exponential regression for the cluster of binodal points with C_{DEX} ranging from 9 to 15 w/v% and C_{PEG} ranging from 0.09 to 0.20 and fit the regression across all data points.

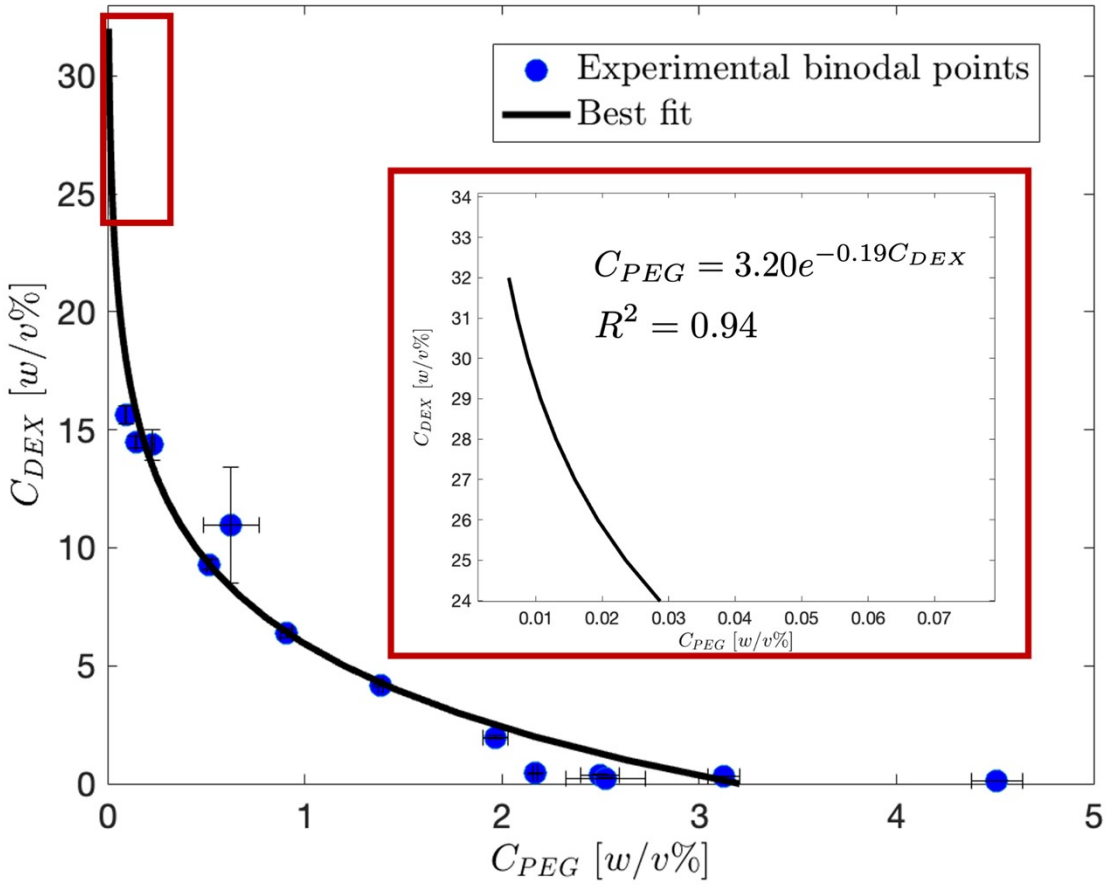


Figure S1. Binodal curve of the ATPS of DEX 150,000 Da and PEG 35,000 Da, and best-fit line through the empirical data.

Section 2. Nucleated droplets flowing along the invasion fronts

We observe nucleated PEG droplets along the PEG moving front on the top and bottom walls, as shown in Figure 2. These droplets grow, coarsen, and coalesce as they move downstream along the length of the channel.

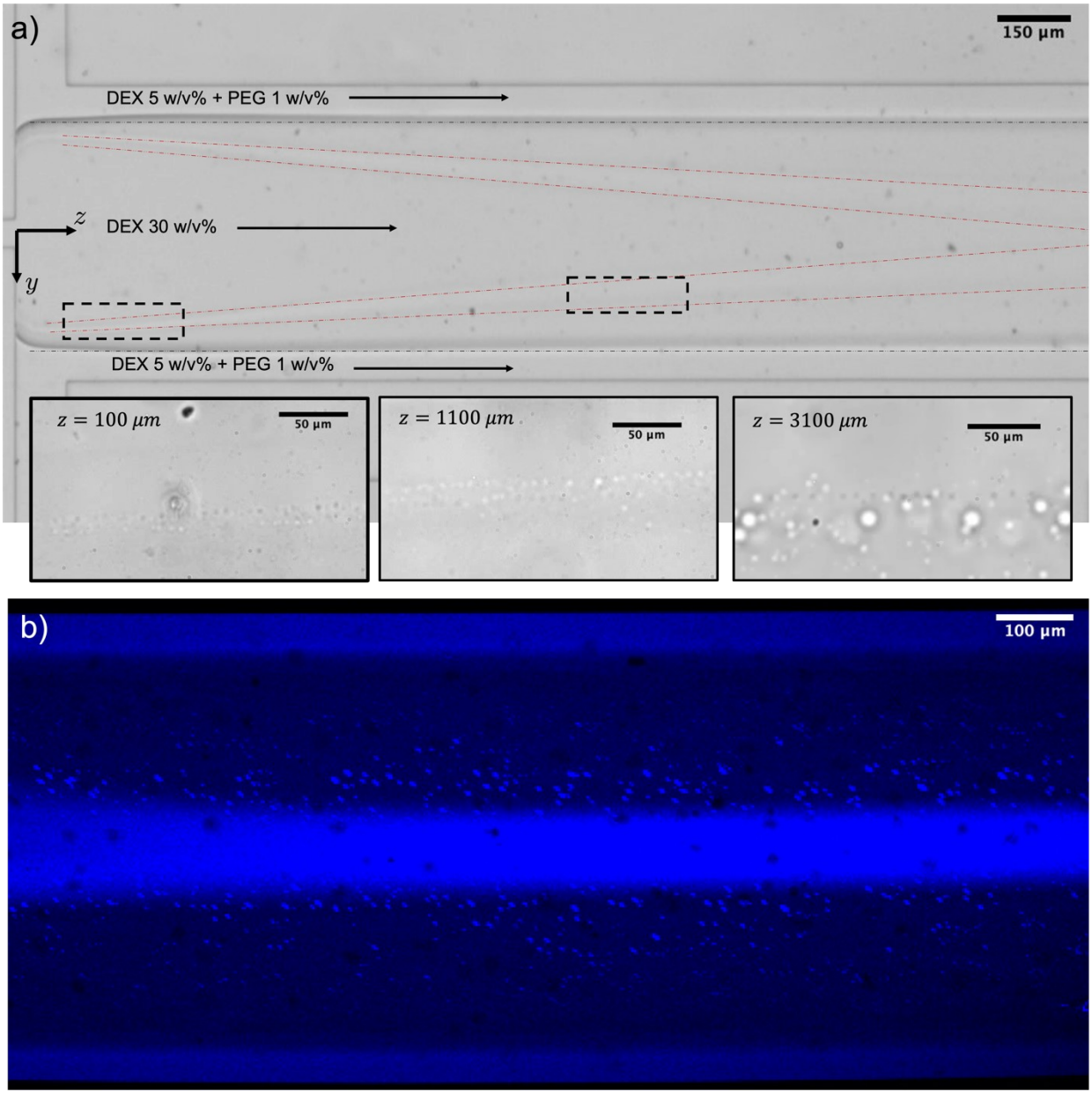


Figure S2. a) y - z plane brightfield image of the flow system, where an inner stream of DEX 30 w/v% flows side by side with an outer stream of DEX 5 w/v% and PEG 1 w/v% (x = 55 µm). The diffuse interface between the two streams is highlighted with black dashed lines, and the tip of the nucleated PEG-rich moving front on the top wall is traced with red dashed lines. Zooming in the vicinity of the PEG-rich moving front (insets at z = 100, 1100 and 3100 µm), small PEG-droplets are observed, which tend to move along with the PEG-rich moving front, towards the center of the channel. The two dashed boxed highlight z = 100, 1100 µm, while the

corresponding box for $z =$ 3100 µm is further downstream rom this image and therefore not in sight. b) y - z plane fluorescent image of flow system downstream ($x =$ 55 µm) where the fronts from the two outer streams merge. PEG-rich droplets can be seen surrounding the PEG-rich thread that has formed on the top wall.

Section 3. Trends observed in Figure 4 (c)

Experiments tuning C_{DEX_2} illustrate that for flow systems with $C_{DEX_2} \leq$ 15 w/v% invasion fronts are not formed. However, for flow systems with $C_{DEX_2} \geq$ 15 w/v%, the two streams undergo liquid-liquid phase separation and, therefore, triggering formation of invasion fronts.

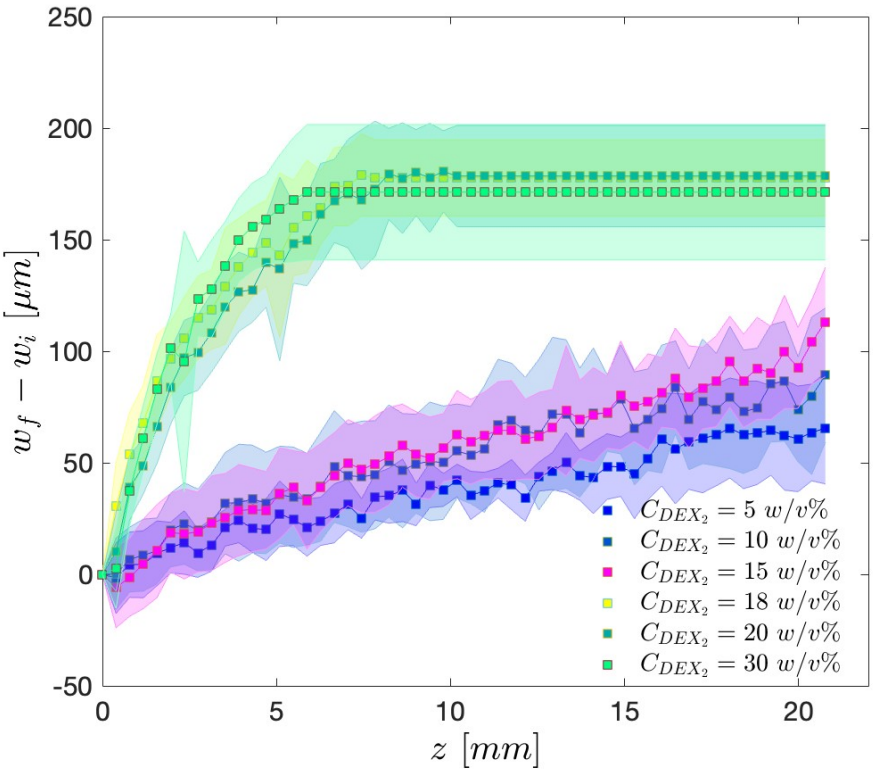


Figure S3. Plots of the relative width of the moving front, w_f, relative to the initial width of the solution 1 at the junction of the device, w_i, with two added data series for C_{DEX_2}=15 w/v% and C_{DEX_2}= 18 w/v%.

Section 4. Fluorescence intensity measurement of phase-separated PEG

To measure the fluorescence intensity of a fully equilibrated PEG-rich phase, we prepare mixtures in bulk of one ATPS consisting of 5 ml of DEX 30 w/v% and 5 ml of a miscible

mixture of DEX 5 w/v%, PEG 1 and Alexa Fluor 1 µl ml^{-1}, and another ATPS consisting of 5 ml of DEX 20 w/v% and 5 ml of a miscible mixture of DEX 5 w/v%, PEG 1 and Alexa Fluor 1 µl ml^{-1}. We centrifuge the mixtures for 30 minutes, at 3000 RPM. Once removed from the centrifuge, we extract the PEG-rich phase (top phase) from each ATPS. To measure the fluorescence intensity of the PEG-rich phases from these two ATPSs, we flow each respective PEG-rich phase into the flow-focusing microfluidic device used for the study, at a volumetric flow rate of 1 µl min^{-1}, and observe the flow within the channel using the confocal microscope (Leica SP5, Leica, Wetzlar, Germany). We take stack images across the height of the channel, right at the junction, and we compute the averaged pixel intensity across the stack.

We find the averaged normalized intensity (with respect to intensity of miscible solution of DEX 5 w/v%, PEG 1 and Alexa Fluor 1 µl ml^{-1}) of the PEG-rich phase from ATPS of DEX 30 w/v% and a miscible mixture of DEX 5 w/v%, PEG 1 and Alexa Fluor 1 µl ml^{-1} to be 3.3 (with a standard deviation of 0.1, sample size of 4), while the averaged normalized intensity of PEG-rich phase from ATPS of DEX 20 w/v% and a miscible mixture of DEX 5 w/v%, PEG 1 and Alexa Fluor 1 µl ml^{-1} is 1.34 (with a standard deviation of 0.43, sample size of 4).

Figure S4. Phase separated solutions consisting of a) 5 ml of DEX 30 w/v% and 5 ml mixture of DEX 5 w/v%, PEG 1 and Alexa Fluor 1 µl ml^{-1} and b) 5 ml of DEX 20 w/v% and 5 ml mixture of DEX 5 w/v%, PEG 1 and Alexa Fluor 1 µl ml^{-1}.

Section 5. Normalized fluorescence intensity calibration experiments

It is known that the fluorescence intensity of a fixed amount of dye may be different in different solvents. We prepare aqueous solutions with PEG concentrations ranging from 0 to 5 w/v%, and Alexa Fluor dye ranging from 0.25 to 1 µl ml^{-1} and measure their fluorescence intensity using a confocal microscope (Leica SP5, Leica, Wetzlar, Germany), with 633 nm laser light at an intensity of 40% within flow-focusing microfluidic devices used to perform the two-phase flow experiments. The normalized intensity of the samples with respect to I_i, which is the intensity of a miscible mixture of DEX 5 w/v% and PEG 1 w/v% and Alexa Fluor 1 µl ml^{-1}, is shown in Figure S5. It can be seen that for a fixed concentration of Alexa Fluor, increasing the PEG

concentration increases the fluorescence intensity, and for a fixed concentration of PEG, increasing the Alexa concentration increases the normalized fluorescence intensity. Moreover, the rate of change of the normalized intensity with respect to PEG concentration is affected by the amount of Alexa Fluor present in the solution.

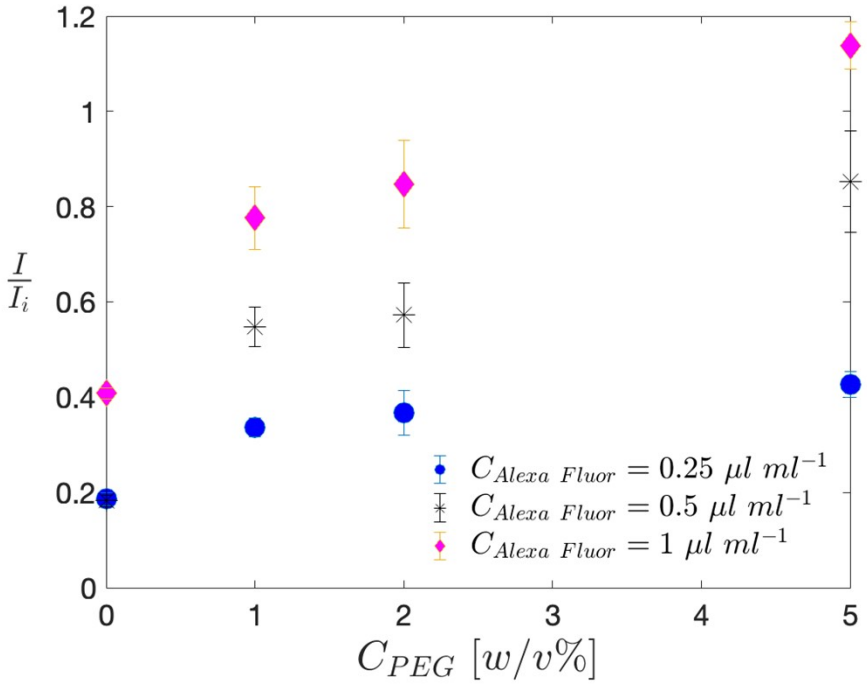
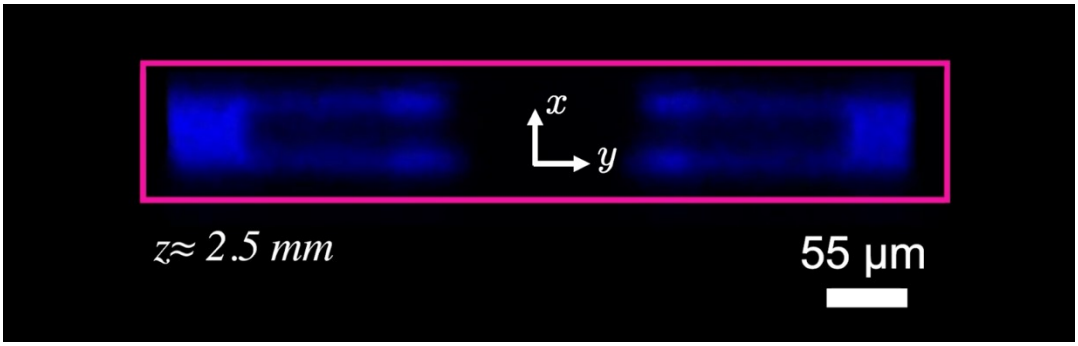


Figure S5. Normalized intensity I of Alexa Fluor solutions, with respect to I_i, the intensity of a miscible mixture of DEX 5 w/v% and PEG 1 w/v% and Alexa Fluor 1 µl ml^{-1}, versus PEG concentration C_{PEG}. The error bars indicate standard deviation of a sample size of 4 measurements.

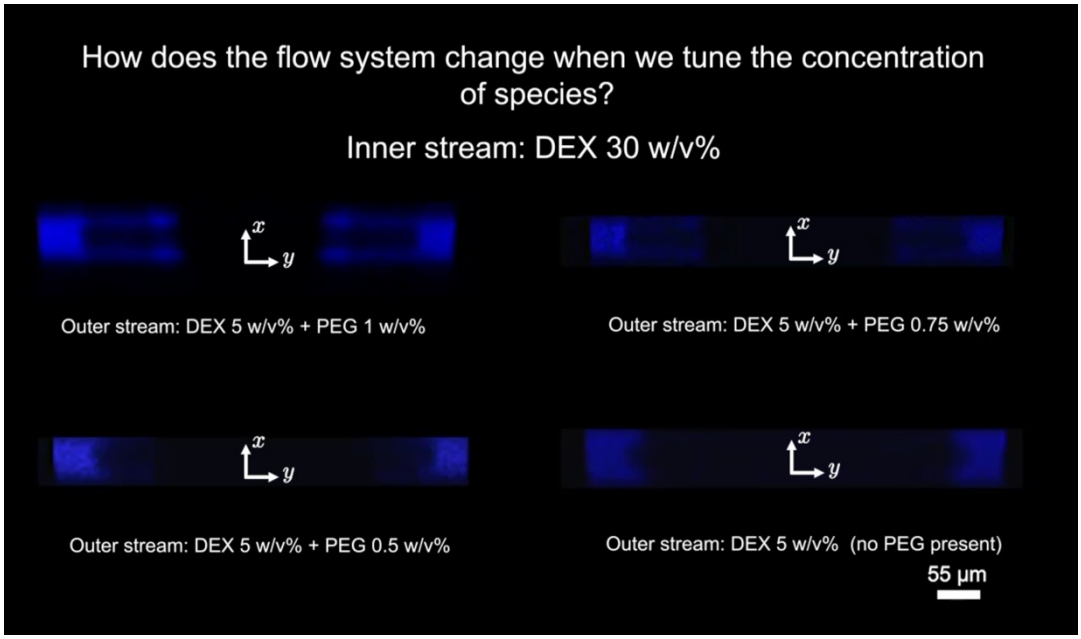
Section 6. Quantification of data in Figure 6(b)

The data shown in Figure 6(b) is taken by identifying the intensity of pixels in two fixed regions of interest (one at the top wall and another at the bottom wall), with a size of 80 × 20 pixel^2 each, centered along the centerline of the channel in temporal x - y fluorescent images of the channel. The region of interest is fixed across time series images showcasing temporal changes in the intensity of the Alexa Fluor dye, which indicates the presence of a nucleated PEG phase in that region, as the system is reaching steady state.

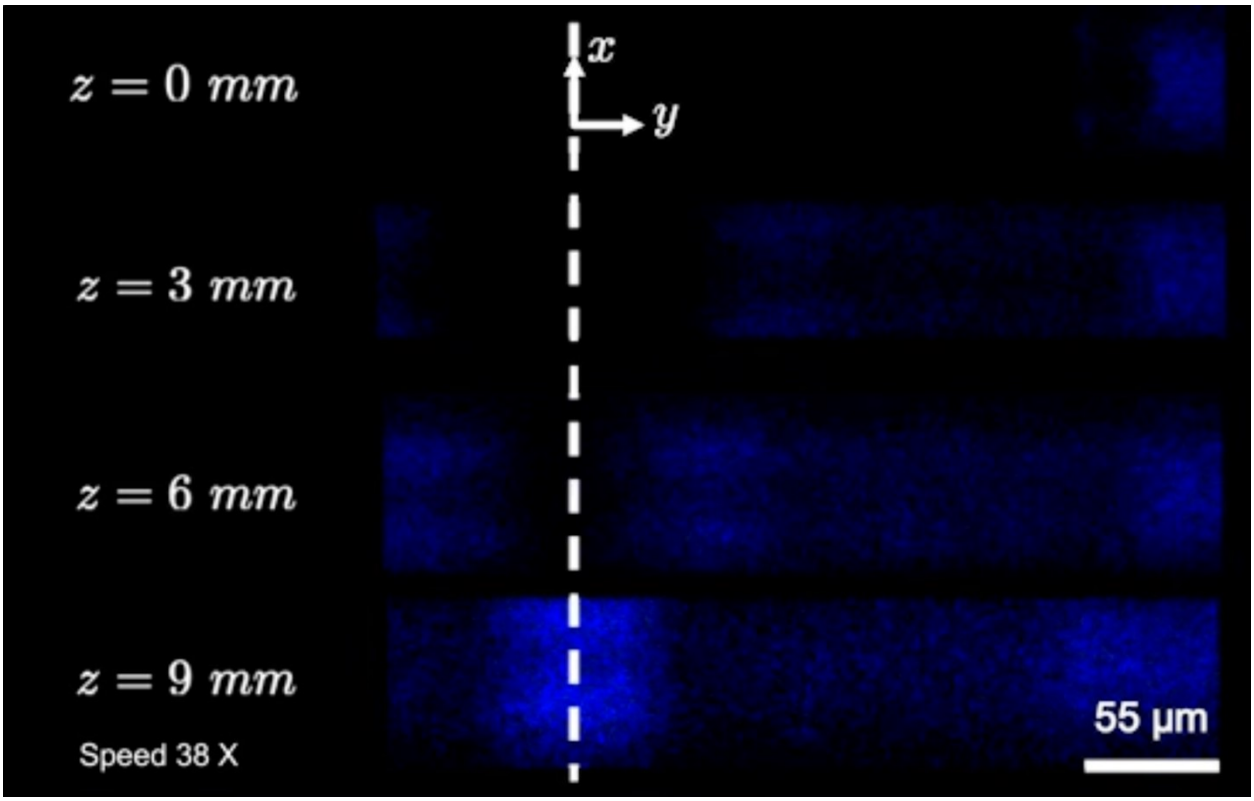
Movie captions



Snapshot of video 1. This video illustrates the evolution of the flow in the cross section (steady state), as we move downstream in z, for an inner stream of DEX 30 w/v% (not labelled) surrounded by an outer stream of DEX 5 w/v% and DEX 1 w/v%.



Snapshot of video 2. This video illustrates the differences in the flow cross section while moving downstream in z, once the system has reached steady state, for different systems. We fixed the concentration of the DEX in both the inner and outer streams and tuned the PEG concentration in the outer stream. We observe that as the PEG concentration increases, the system becomes more susceptible to phase separation, and therefore, once the system phase separates, moving fronts are formed along the width of the channel.



Snapshot of video 3. This video shows how the system reaches steady state, at different positions along the length of the channel. First, the channel is filled with a miscible solution of DEX 5 w/v% and PEG 1 w/v%. Once the channel is fully filled, then we flow an inner stream of DEX 30 w/v%. Using resonant scanning, we observe how the system reaches steady state at different positions along the length of the channel.

References

1. E. Atefi, J. A. Mann and H. Tavana, Langmuir, 2014, 30, 9691–9699