S2—1 Image processing and data acquisition

This section explains the relevant details pertaining to the application of the platform to drug screening experiments. Some images have their brightness artificially increased to improve contrast for better visualization. All modified images are clearly indicated as such. Note that the images analyzed to collect the data are the original ones without exception.

S2—1.1 Image acquisition

The images are acquired using a 4X objective attached to a *Nikon Inverted Microscope ECLIPSE Ti-E*. Using the fluorescent filter *CFP-HQ*, the excitation wavelength is 440 nm while the emission wavelength is 490 nm. The images are then recorded using an *Andor Zyla 5.5 sCMOS* camera attached to the microscope side-mount.

The exposure time is set to 2 seconds, and correspondingly, the camera 16-bit depth image is saved every 2 seconds for at least 6 minutes. Afterwards, a bright field image is saved to obtain the droplet contour. Finally, the fluorescence of the background is recorded at the end for each set of experiments.

The 6 minutes of recording is of the droplets after merging to assess the aggregation triggered by the buffer. Beforehand, while the solutions are still in the two separate droplets, the bright field and fluorescent image are recorded in quick succession (< 5 seconds) as a baseline and to ensure the dye did not self aggregate.

S2—1.2 Image processing

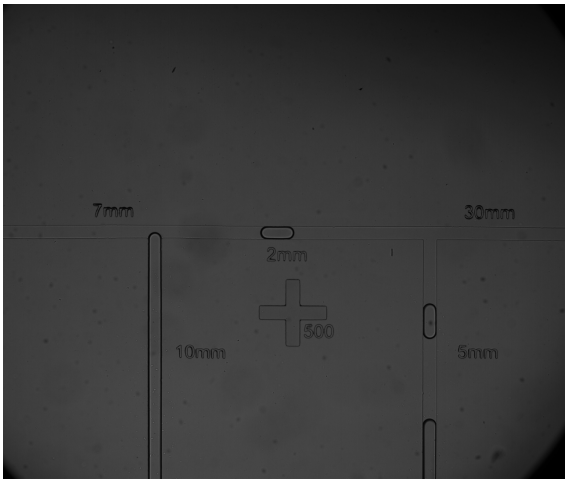
The images are processed using macro scripts in the open-source software *ImageJ*. Most of the processing steps are automated. For each new data set, the appropriate region of interest (ROI), which limits the scope of image processing to the portion of the channel containing the droplet, is manually selected. Also, the processing of the bright field image required varying originating points for the *floodFill* function. The rest of the steps were automated for consistency and efficiency purposes.

The processing steps before and after merging are illustrated in Figures S2—1.1 and S2—1.2 respectively. Note that the image processing is performed using *ImageJ* up to the cropping and background subtraction (Figure S2—1.2(c)). Afterwards, the images are exported as text files containing the greyscale value for each pixel in a matrix. The text files are then imported into *Matlab* for further processing and plotting as explained in the following section.

S2—1.3 Data acquisition

The droplet mask and cropped fluorescent image (as seen in Figure S2—1.2(b) and S2—1.2(c) respectively) are imported in *Matlab* as a 2D array containing the greyscale intensity for each pixel.

The overall fluorescence of any droplet is calculated by averaging over all pixel values contained in the droplet. For the two droplets before merging, the bright field (for the mask) and fluorescent images are taken few seconds apart without the droplets having the time to



(a) Initial bright field image.

(b) Initial fluorescence before droplet merging.

(c) Mask obtained from thresholding, floodFill, and erode of region of interest (ROI).

(d) Fluorescent image (b) after background subtraction and the mask is applied.

Figure S2—1.1: Image processing before droplet merging. Uniform intensity confirms the dye did not self aggregate.

move significantly. Consequently, the mask can be directly applied to eliminate background noise.

After the droplets are merged, the images are recorded over a longer period of time. Although the displacement is carefully monitored and minimized through minute manual pressure adjustments, the fluorescent droplet travels along the channel. Therefore, the droplet must first be located in order to apply the mask. Correlation in the direction of the channel is used to establish the displacement yielding the highest sum over the mask pixels. The assumption is that any of the background noise would be less bright than the fluorescence of the droplet. This can quickly be confirmed through quick visual inspection of the images such as S2—1.2(c). Then, the mask is applied with the calculated offset to only keep the fluorescent intensity within the droplet as shown in Figure S2—1.2(d). An example of the data with and without correlation with the corresponding displacement over time is given in Figure S2—1.3.

(a) Bright field image of the droplet (after merging of the two solutions).

(b) Mask from the bright field image using thresholding, floodFill, and erode functions in ImageJ.

(c) Cropped frame for each interval (after background subtraction). Intensity artificially increased for better contrast.

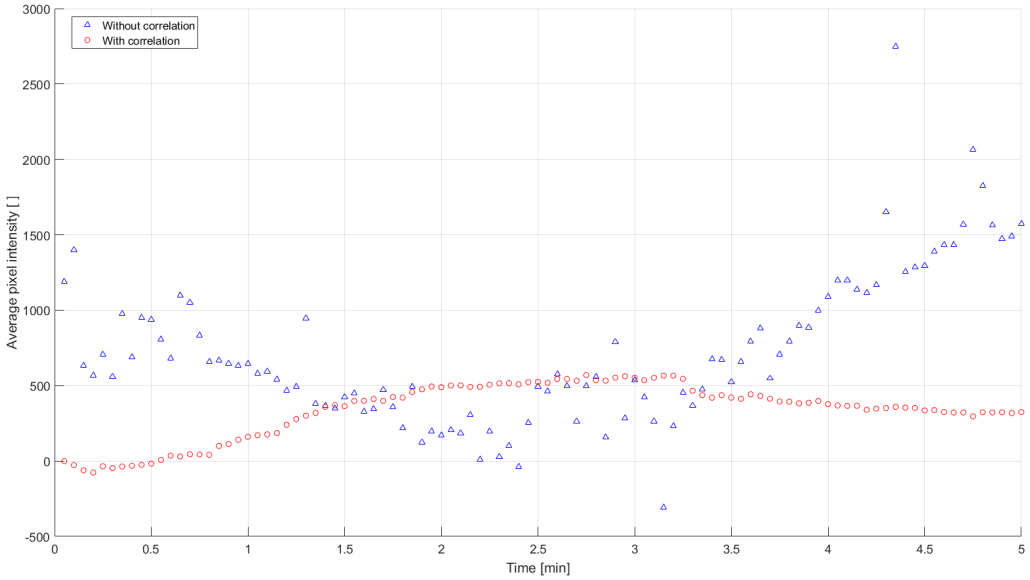
(d) Mask applied with displacement from the correlation along the channel (59 pixels displacement) to eliminate background noise and only have fluorescent intensity from the pixels contained in the droplet. Intensity artificially increased for better contrast.

Figure S2—1.2: Image processing after droplet merging. Correlation along the channel to locate the droplet and apply the mask.

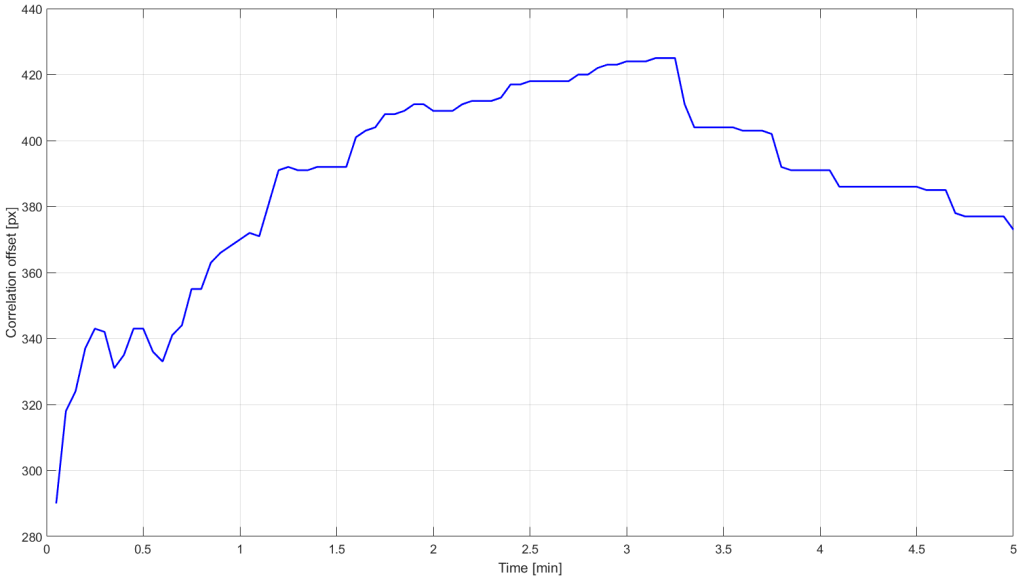
S2—1.3.1 Negative control

For negative control, due to the low pixel intensity, the correlation algorithm fails to accurately locate the droplet. Nonetheless, the interface location can (barely) be identified visually. In order to circumvent the limitations of the image processing without requiring more complex algorithms, a *Matlab* script was used to record the manually identified location for each frame. For each frame for the duration of the recording, the picture is displayed and the user can identify the interface by clicking on the appropriate location. The corresponding displacement is recorded, and the next image is displayed. This is performed for all frames to obtain the displacement curve in order to properly offset the mask. The human error introduced by this procedure is deemed negligible.

Moreover, for the negative control, the overall background intensity had a slow drift that is believed to be artificially induced by light variations. To compensate for such slow drift, an area further down the channel not containing any droplet was selected. The average of all pixels over time were used to subtract the background intensity drift that affected the whole image.



(a) Comparison between the averaged pixel intensity with and without correlation for the mask location from the Orange G (01) curve.



(b) Corresponding displacement obtained with the correlation along the channel.

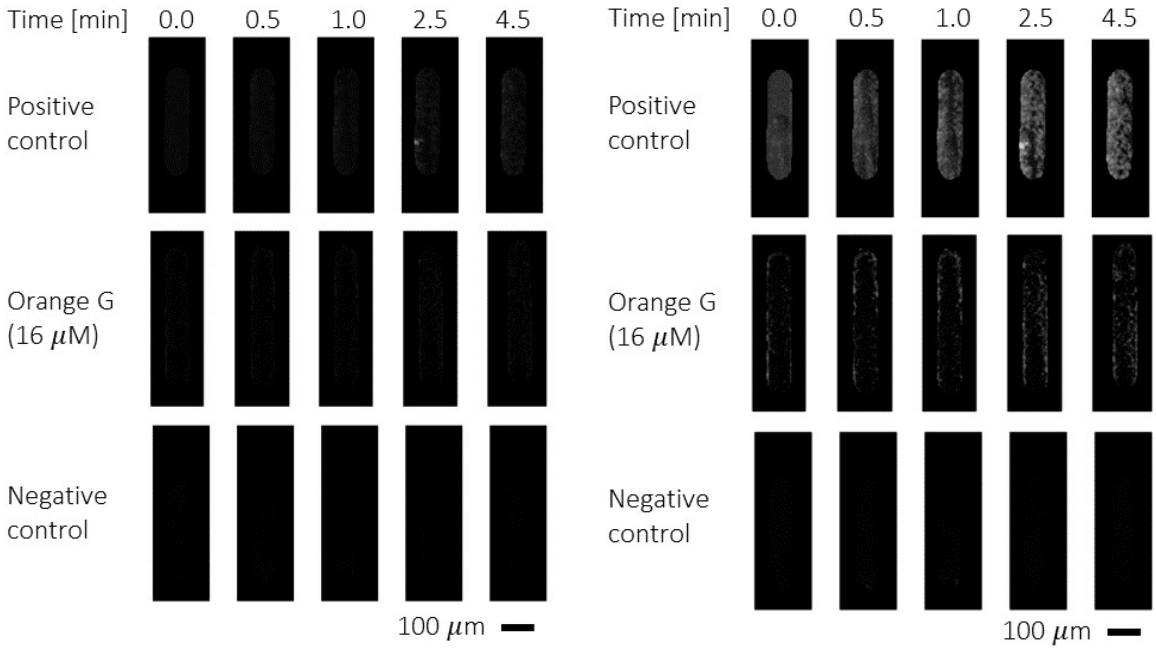
Figure S2—1.3: Image processing after droplet merging. Correlation along the channel to locate the droplet and apply the mask.

S2—1.4 Data treatment

The curves obtained for the average intensity of the droplet over the 5 minute interval differed in their absolute value. The difference is attributed to the photo sensitivity of the ThS dye. The photo bleaching is not constant due to varying amount of illumination before the data is recorded. The variations in time are due to changing setup time required and the time location of the data for a specific chip (i.e. multiple data points were collected for each chip with the overall intensity decreasing for the later data collected). In order to compare the data, the change in fluorescent intensity is considered by offsetting all points using the first averaged intensity. This method also compensates for any changes in background intensity.

S2—2 Screenshot artifical intensity increase

As noted in the article, the screenshots of the fluorescent intensity are artificially and uniformly increased for better visualization and understanding. The trend in fluorescent intensity depending whether peptide and orange G are present is hence clearer. Figure S2—2.1 shows the original image and brighter image side by side.



(a) Original brightness.

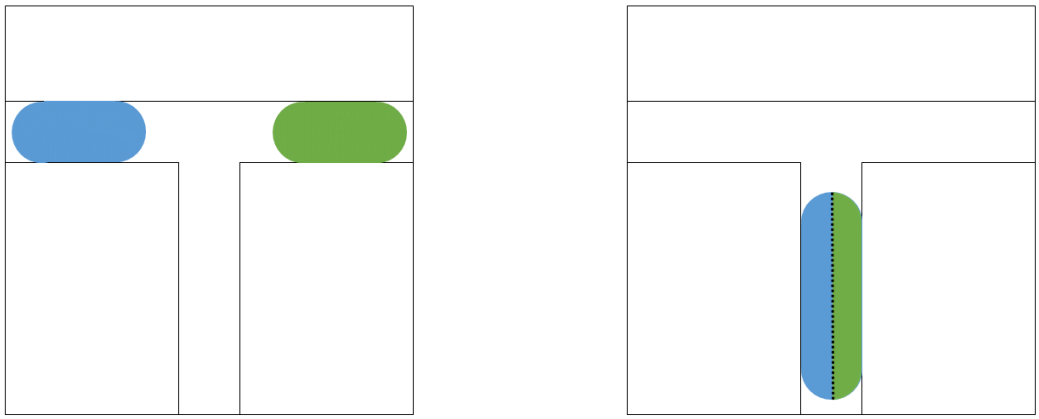
(b) Artificially increased brightness.

Figure S2—2.1: Side by side comparison of the drug screening screenshots: before and after increasing the brightness. The purpose is for better readability and visual representation of the assay. The data is extracted from the untouched images.

S2—3 Length variations between the 2 droplets

The variations in the length of the two droplets before merging affect the drug screening results. However, the impact is deemed insignificant for the purpose of this study that aims at showing the potential use of the platform rather than a standalone drug screening study.

The effect on the kinetic in terms of time for the reaction is deemed negligible. Figure S2—3.1 shows schematically (albeit simplified) the resulting droplet after merging. The principal diffusion length between the two solutions is then the channel width (that is independent of droplet length) rather than the droplet lengths. The bigger impact is on the final concentration of each solution that assumes the merging of 2 droplets of exactly the same length.



(a) Two droplets before merging.

(b) Single droplet after mering.

Figure S2—3.1: Schematic representation of the merging of the two droplets and the simplified distribution of the solutions in the resulting single droplet.