Supplementary material

PDMS chip development

The microfluidic chip was constructed as a polydimethylsiloxane (PDMS) bilayer, consisting of a flow layer and a backing layer. The flow layer was 1mm thick and contained two 1000um x 40um rectangular channels, which flowed to and from a 3mm radius well. The backing layer was ~4mm thick and contained a 30-gauge inlet hole for suction from the well.

The PDMS layers were molded from a master template, which was prepared using photolithography on a Si water to create a photoresist pattern. The pattern width and heights were 1000um and 40um, respectively. The Si wafer was treated with trimethyl-chloro-silane (TCS) vapor for 30min to promote detachment of the PDMS from the mold. To prepare PDMS, Sygard PDMS (Corning) prepolymer and curing agent were mixed at a ratio of 5:1 for the flow layer and 20:1 for the backing layer. The mixture was degassed in a vacuum for 1h. The PDMS mixtures were poured onto the Si wafers to thicknesses of 1mm and 4mm for flow and backing layers, respectively, and again degassed for 30 min. The pieces were heated at 80C for 1.5h. The PDMS pieces were removed from the mold and cut into rectangular constructs. Assess holes were drilled into the flow layer with a 25gauge puncher. The well was created using a 3mm in radius puncher. The suction access was created using a 30gauge needle through the backing layer. The backing layer was carefully aligned and placed on top of the flow layer. The construct was heated at 80C for 2 hours.

Image Processing for Shannon Information Entropy

1. **Data reduction:** Uncompressed high definition (HD) videos (1920 x 1080p, 29.9 fps) generate ~180 MB/s. Using MPEG4 (avc1 codec) compression artefacts have to be removed. This is achieved by binning the movie stream in each *x*, *y* and *t* by a factor of 10. Data size is reduced by a factor of 1000 while the signal to noise ratio for each down-sampled pixel is increased. In addition, each frame is cropped to contain only a previously selected region of interest, i.e., the main well.
2. **Background subtraction:** To account for subtle changes in dye intensity, an angiography technique comparable to (Deniz *et. al.*, 2012) is used. The blue channel is subtracted from the red channel resulting in an image that shows only parts that are more or less blue than red. Since a white background was used and the animal lacks a strong color, this efficiently removes the background.
3. **Entropy calculation:** Spatial entropy is calculated over either the whole well or a region of interest. It is limited by the bitdepth to a maximum value of 8. Temporal entropy is calculated for each pixel over a sliding window of 16 frames in time (160 original frame; about 5.3 seconds). 16 was chosen as is the fourth power of 2, making 4 the highest possible temporal entropy.

Image Processing for Frontline Detection

1. **Data reduction:** Data is reduced by binning 5 consecutive frames in *t*. The data is not reduced in *x* and *y* to most precisely track the interface between dye and water.
2. **Background subtraction:** Background is removed by subtracting a key reference frame that was acquired before the start of the experiment from all subsequent images. This results in a colour image that is easier to segment than the grey-scale that is used for the entropy calculation.
3. **Segmentation:** The acquisition protocol includes a stable light source and fixed background colour supporting fixed thresholds in the saturation channel of the HSV colour space for dye segmentation.
4. **Frontline extraction:** The outline of the segmented dye is tracked and converted it into the curvature scale space (CSS, Abbasi et al. 1999).
5. **Length measurement:** To account for minor errors in segmentation, the extracted frontline is smoothed. A Gaussian filter with a width of 5 pixel width is applied independently in the *x* and *y* dimension of CSS. To incorporate the physical information of the concentration gradient along the frontline, the Gaussian filter is weighted by the gradient in saturation at each position. The length of frontline is calculated on the resulting smoothed outline.

Movie 1

Proof-of-principle video of an NF stage 25 embryo mixing red micro-beads. Mixing patterns are clearly visible. Between second 7 and 8 a nice demonstration of the effect of low Reynolds Numbers is shown when the animal moves but no turbulent flow is created. In contrary, the shearing water planes leads to a deformation and following reformation of the mixing pattern.

Movie 2

Second proof-of-principle of an NF stage 41 embryo mixing yellow and blue dye solution to green. Again, mixing patterns can be observed.

Movie 3

NF stage 40 embryo mixing dye and water in DIY chip. Upper panel showing the animal mixing and the lower showing a digital subtraction image highlighting the mixing patterns.

Movie 4

NF stage 30 embryo mixing dye and water in our PDMS chip in 1x and 3x viscosity.

Movie 5&6

Temporal entropy analysis of the acquisitions with 1x viscosity (Movie 5) and 3x viscosity (Movie 6) from Movie 4. Similar mixing hot- and cold-spots of the mixing patterns can be observed in both movies. Movie 6 shows a flash surrounding the animal at the 16-second mark due to wiggling of the embryo.