

Electronic Supporting Information

Supplementary Figures to the Main Text

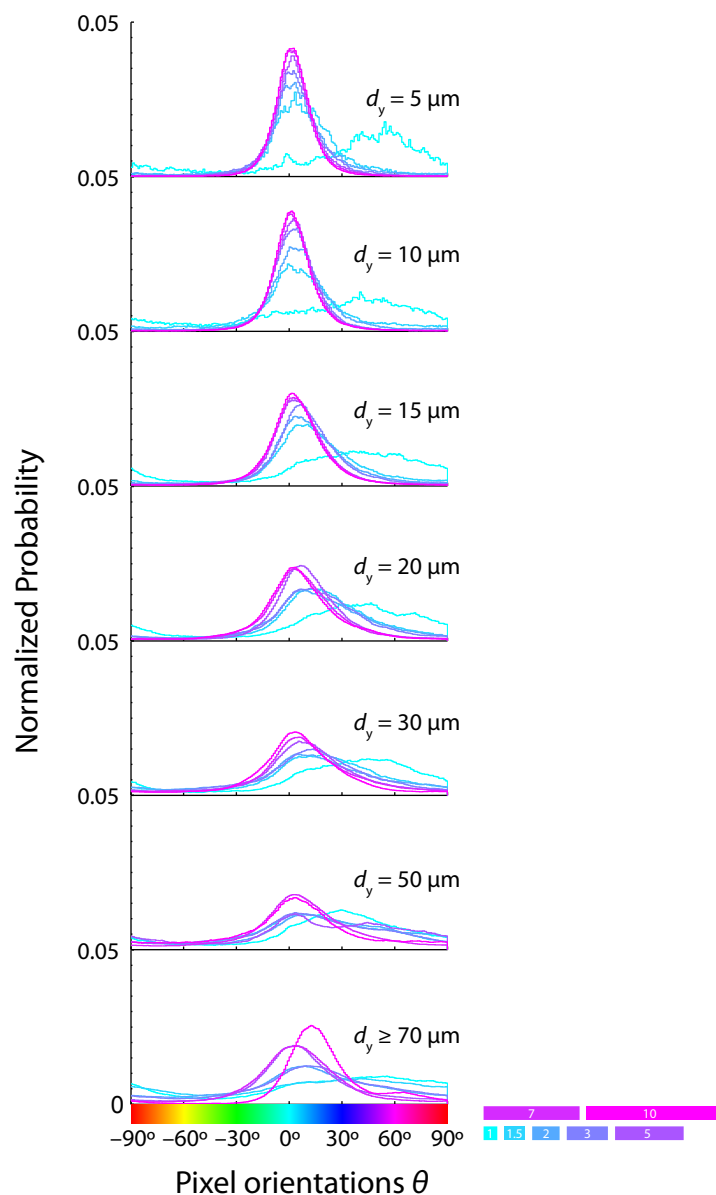


Figure S1. Nematic solutions. Probability distribution function of pixel orientations θ for all chambers lengths investigated. Data for $d_y = 70 \mu\text{m}$ and $100 \mu\text{m}$ have been pooled together. Chamber length d_y given by labels (right). Color corresponds to different aspect ratios (legend, bottom right).

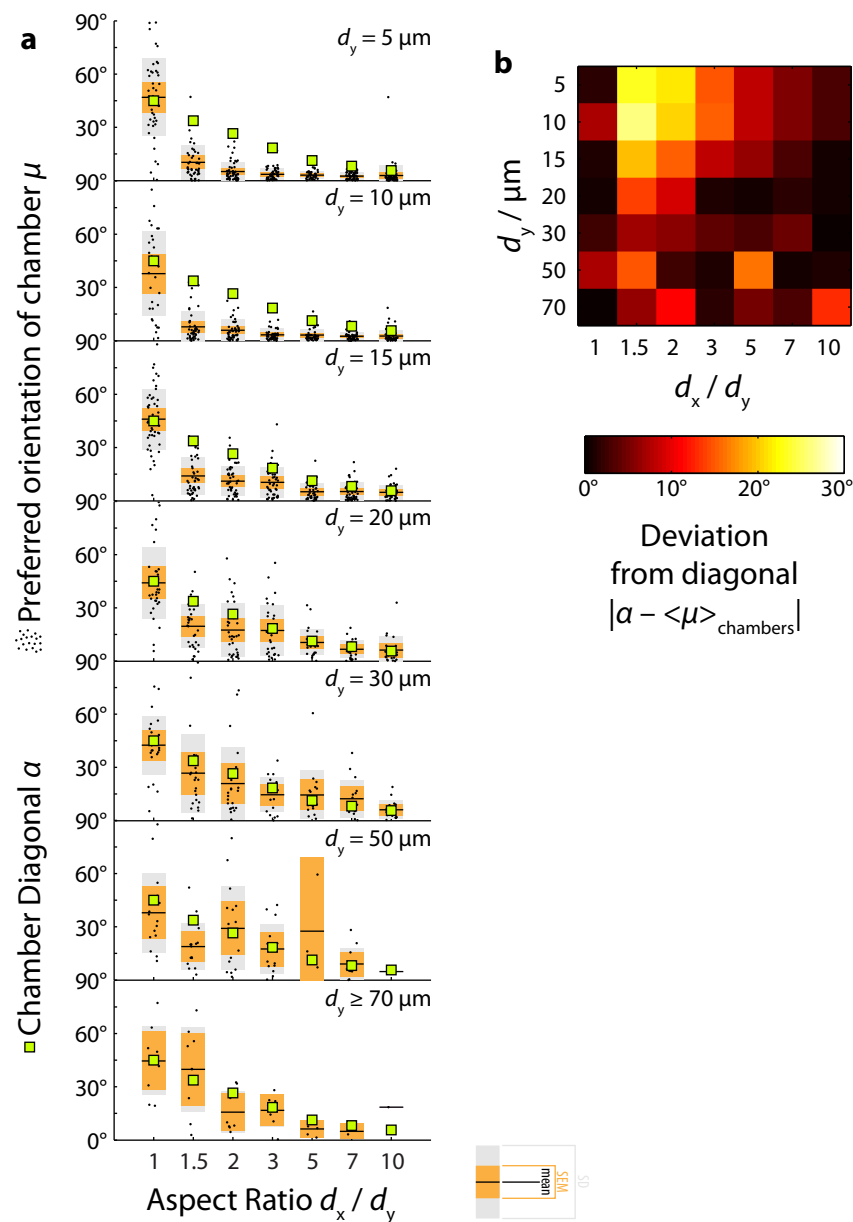


Figure S2. Nematic solutions. **a.** Preferred orientation $\mu = \langle \theta \rangle$ as a function of chamber aspect ratio for all chamber lengths investigated. Data for $d_y = 70 \mu\text{m}$ and $100 \mu\text{m}$ have been pooled together. Black dots denote preferred orientation μ of a single chamber. Black lines denote mean preferred orientation averaged across all chambers. Orange bars denote standard error of the mean (SEM). Gray bars denote standard deviation (SD). Green squares denote the angle of the two diagonals of the chamber, given by $\arctan(d_y / d_x)$. **b.** Color map of the deviation of the mean preferred orientation from the chamber diagonal (cf. Main Text, Fig. 3b).

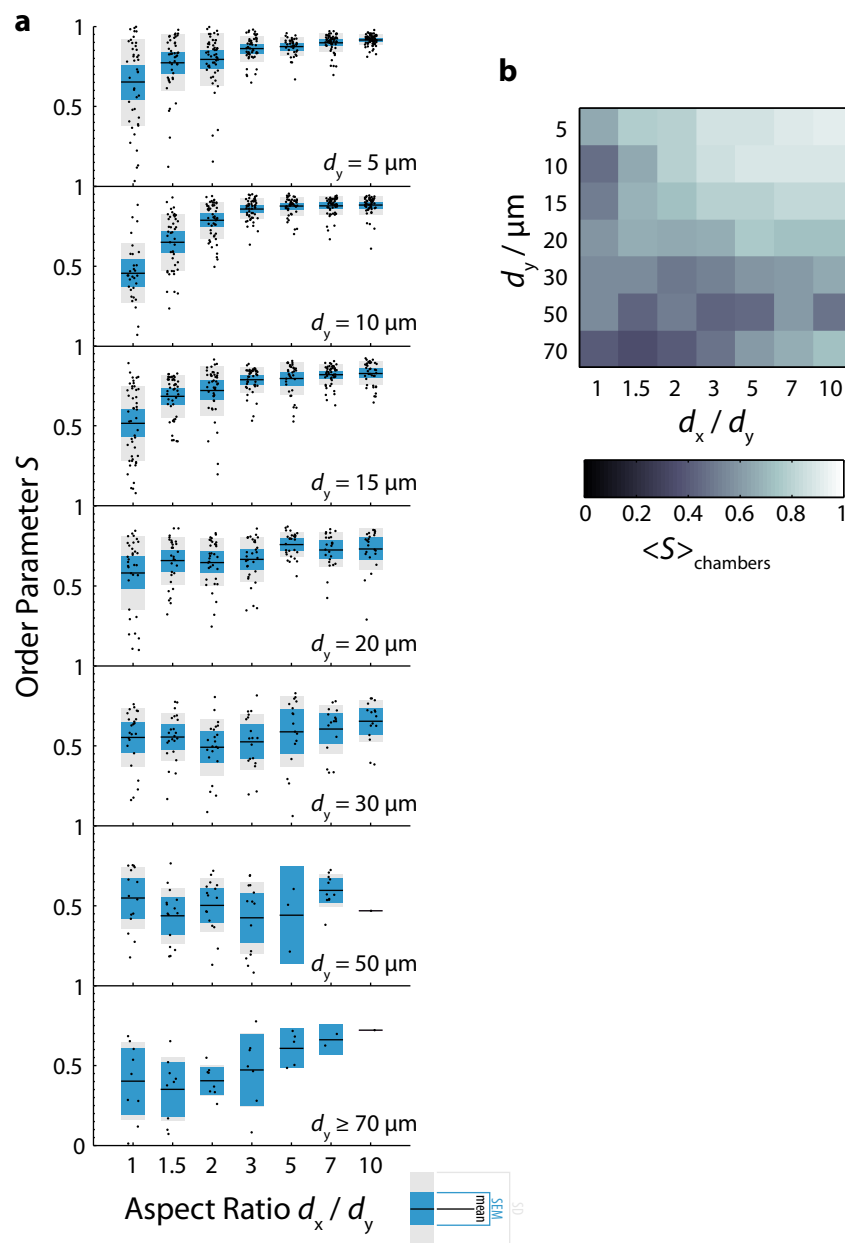


Figure S3. Nematic solutions. **a.** Order parameter S as a function of chamber aspect ratio. Data for $d_y = 70 \mu\text{m}$ and $100 \mu\text{m}$ have been pooled together. Black dots denote order parameter S of a single chamber. Black lines denote mean order parameter averaged across all chambers. Cyan bars denote SEM. Gray bars denote SD. **b.** Color map of the mean order parameter for all aspect ratios and chamber lengths (cf. Main Text, Fig. 3d).

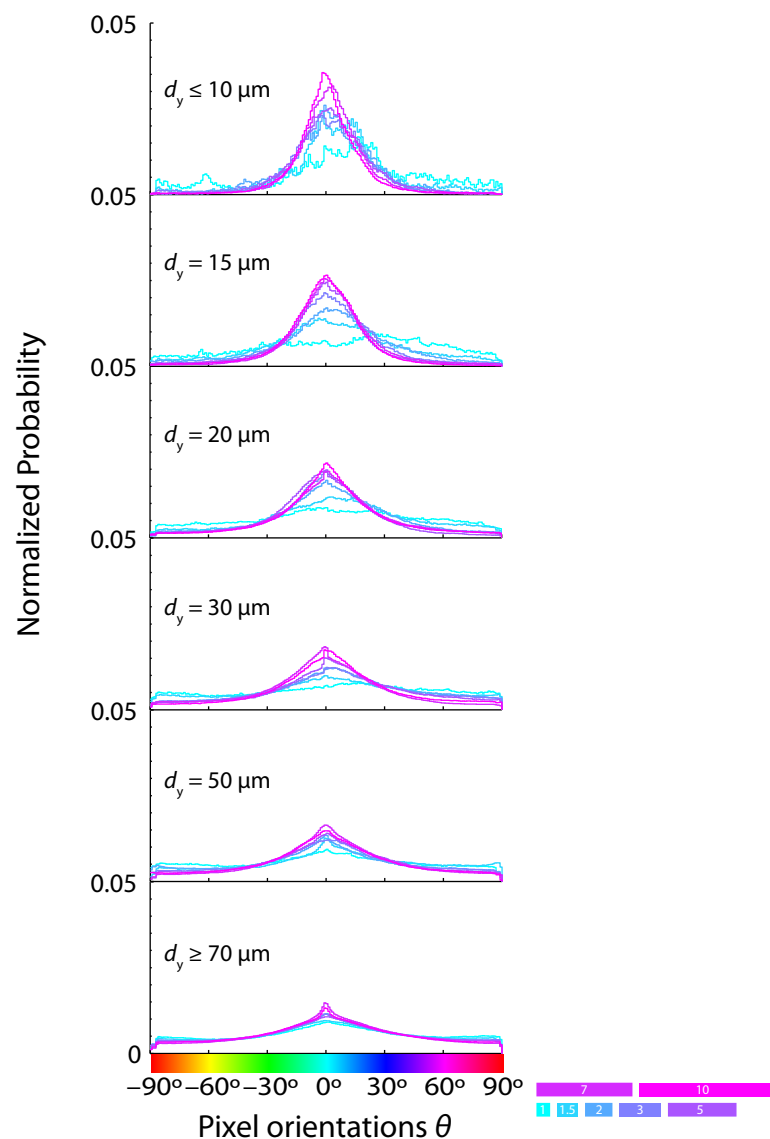


Figure S4. Isotropic solutions. Probability distribution function of pixel orientations θ for all chamber lengths investigated. Data for $d_y = 70 \mu\text{m}$ and $100 \mu\text{m}$ have been pooled together. Chamber length d_y given by labels (left). Color corresponds to different aspect ratios (legend, bottom right).

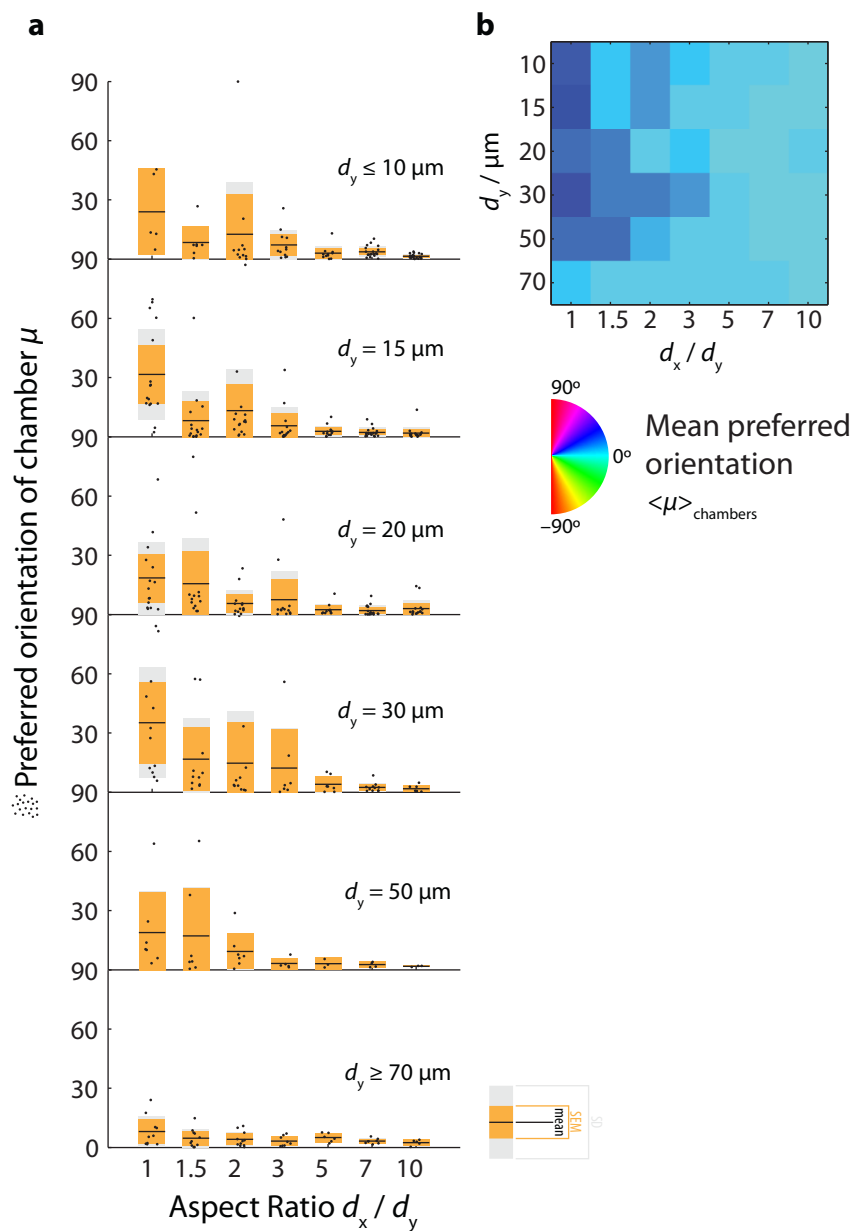


Figure S5. Isotropic solutions. **a.** Preferred orientation $\mu = \langle \theta \rangle$ as a function of chamber aspect ratio for all chamber lengths investigated. Data for $d_y = 70 \mu\text{m}$ and $100 \mu\text{m}$ have been pooled together. Black dots denote preferred orientation μ of a single chamber. Black lines denote mean preferred orientation averaged across all chambers. Orange bars denote standard error of the mean (SEM). Gray bars denote standard deviation (SD). **b.** Color map of the mean preferred orientation. Color corresponds to orientation (color wheel, bottom).

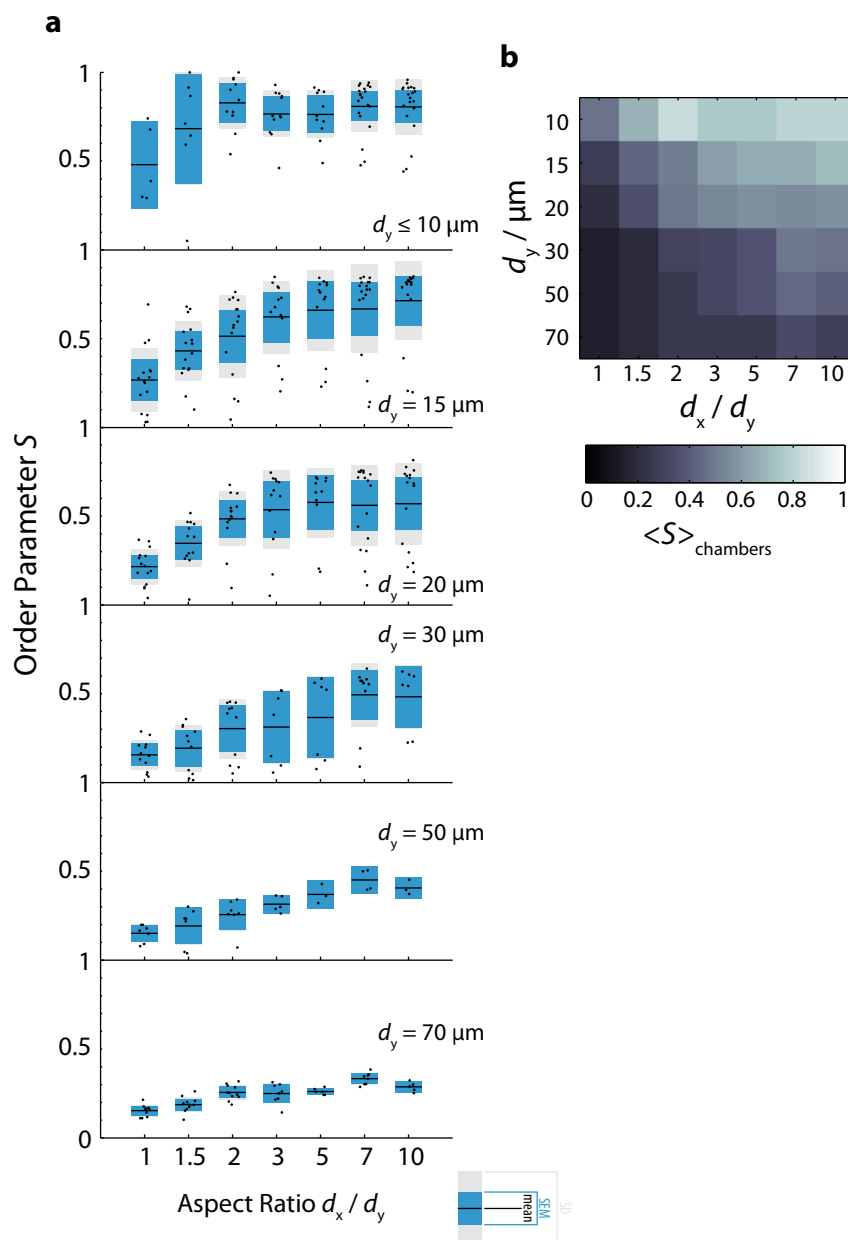


Figure S6. Isotropic solutions. **a.** Order parameter S as a function of chamber aspect ratio. Data for $d_y = 70 \mu\text{m}$ and $100 \mu\text{m}$ have been pooled together. Black dots denote order parameter S of a single chamber. Black lines denote mean order parameter averaged across all chambers. Cyan bars denote SEM. Gray bars denote SD. **b.** Color map of the mean order parameter for all aspect ratios and chamber lengths (cf. Main Text, Fig. 4d).

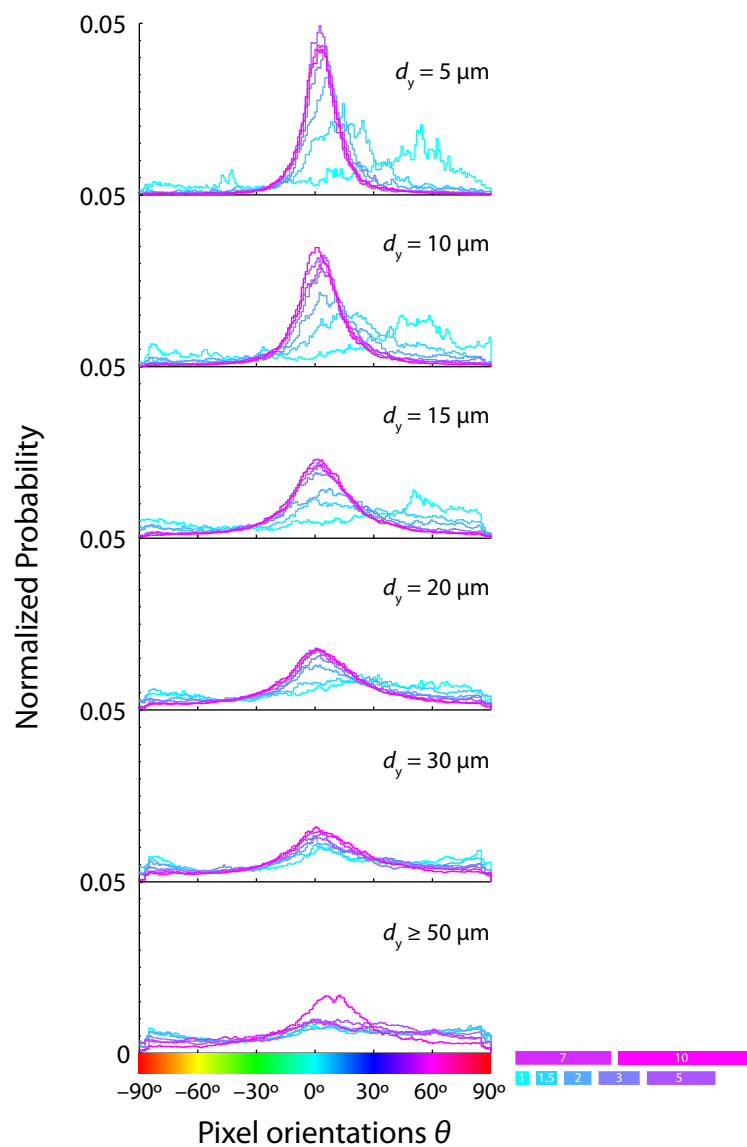


Figure S7. Bundled solutions. Probability distribution function of pixel orientations θ for all chamber lengths investigated. Data for $d_y = 50 \mu\text{m}$, $70 \mu\text{m}$ and $100 \mu\text{m}$ have been pooled together. Chamber length d_y given by labels (right). Color corresponds to different aspect ratios (legend, bottom right).

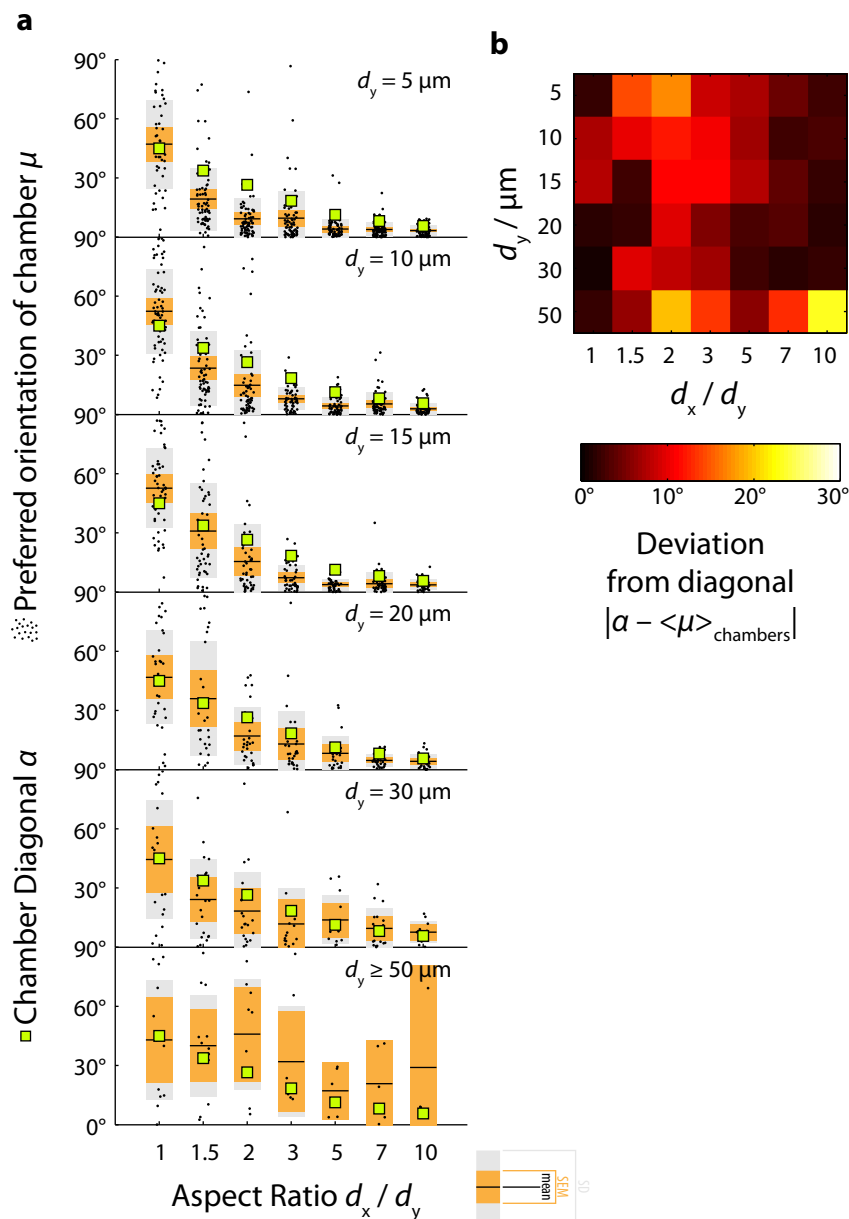


Figure S8. Bundled solutions. **a.** Preferred orientation $\mu = \langle \theta \rangle$ as a function of chamber aspect ratio for all chamber lengths investigated. Data for $d_y = 50 \mu\text{m}$, $70 \mu\text{m}$ and $100 \mu\text{m}$ have been pooled together. Black dots denote preferred orientation μ of a single chamber. Black lines denote mean preferred orientation averaged across all chambers. Orange bars denote standard error of the mean (SEM). Gray bars denote standard deviation (SD). Green squares denote the angle of the two diagonals of the chamber, given by $\arctan(d_y / d_x)$. **b.** Color map of the deviation of the mean preferred orientation from the chamber diagonal (cf. Main Text, Fig. 5e).

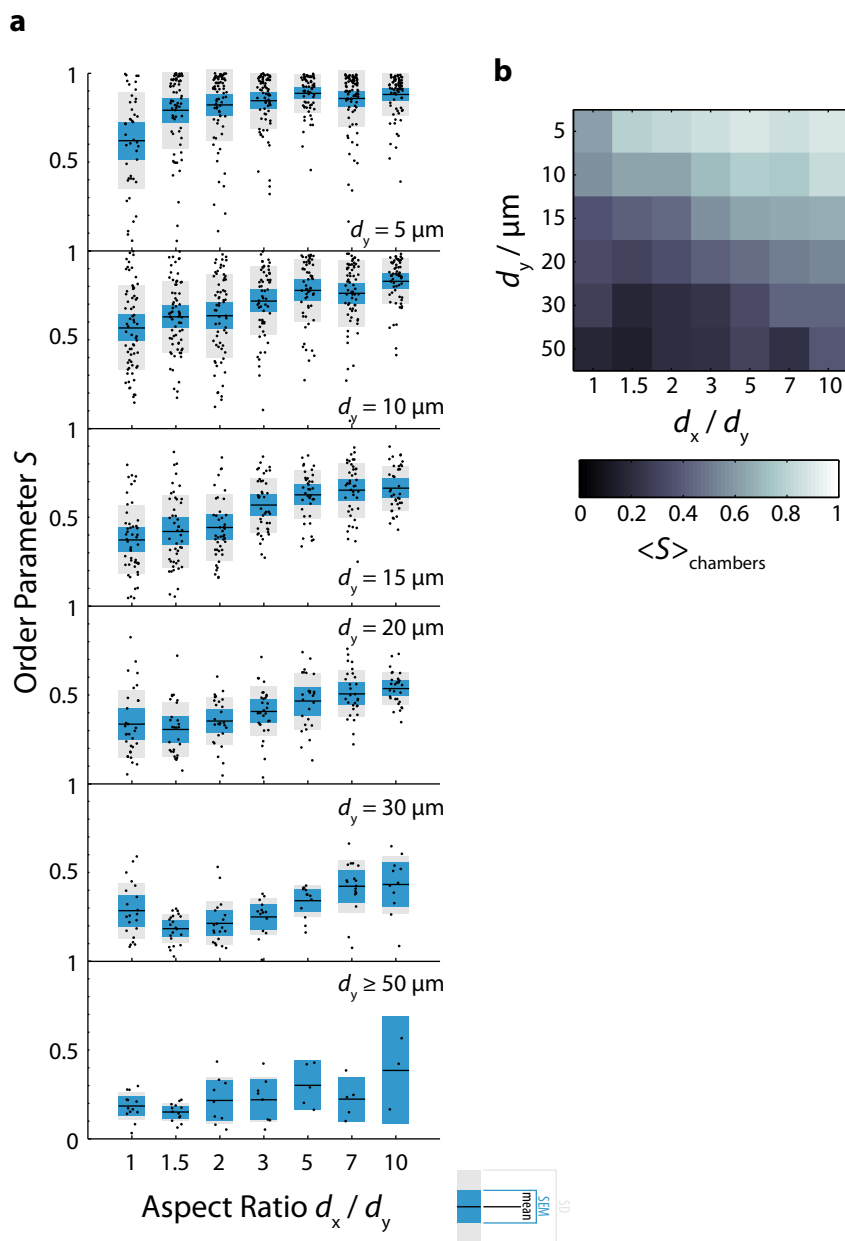


Figure S9. Bundled solutions. **a.** Order parameter S as a function of chamber aspect ratio. Data for $d_y = 50 \mu\text{m}$, $70 \mu\text{m}$ and $100 \mu\text{m}$ have been pooled together. Black dots denote order parameter S of a single chamber. Black lines denote mean order parameter averaged across all chambers. Cyan bars denote SEM. Gray bars denote SD. **b.** Color map of the mean order parameter for all aspect ratios and chamber lengths (cf. Main Text, Fig. 5f).

Automated image analysis procedure

We developed an automated image analysis algorithm to quantify the orientation of the nematic director for each pixel of a confocal image of fluorescently labeled actin filament solutions. In short, we determine the orientation θ of each pixel, and then determine the average orientation over all pixels of a chamber to obtain the preferred orientation μ .

Step 1: Given a confocal image of fluorescent actin filaments, we compute the orientation θ of each pixel using the freely-available ImageJ plugin OrientationJ. This software was developed by Daniel Sage (EPFL, Lausanne)^{1,2}. It computes the structure tensor constructed of the spatial gradients of fluorescence intensity around each pixel with coordinates (x,y) . Determining the eigenvectors of a structure tensor yields the characteristic orientation θ of the fluorescence intensity of a small region $(x \pm \sigma, y \pm \sigma)$ around each pixel (x,y) . This routine has one variable input parameter, σ . This parameter determines the size of the window taken around each pixel over which to average. For our analysis, we set $\sigma = 3$ px. We chose this value because it is large enough to average over detection noise while being small enough to resolve spatial changes in the nematic director field.

We have additionally modified our analysis to investigate the effect of the parameter σ . Figure S10 shows color maps for the deviation of the preferred angle $\langle \mu \rangle$ from the chamber diagonal α (left column) as well as the resulting order parameters (right column) which result from choosing $\sigma = 6$ px. This modified analysis did not greatly affect the dependence of preferred angle on chamber dimensions (cf. Figs. 3b & 5e, Main Text). In particular, the jump in $|\alpha - \langle \mu \rangle|$ from aspect ratio 1 to 1.5 in nematic solutions is preserved, as well as the maximal values of $|\alpha - \langle \mu \rangle|$ which occur for small chambers (small d_y) and aspect ratios of 1.5 and larger (30° for nematic solutions, 10° for bundled solutions). But the modified analysis did lead to a change in the measured order parameter (cf. Fig 3d, 4d & 5f). We found higher values of S with increased σ . This likely reflects the fact that increasing σ decreases the resolution over which we can detect spatial changes in filament orientation.

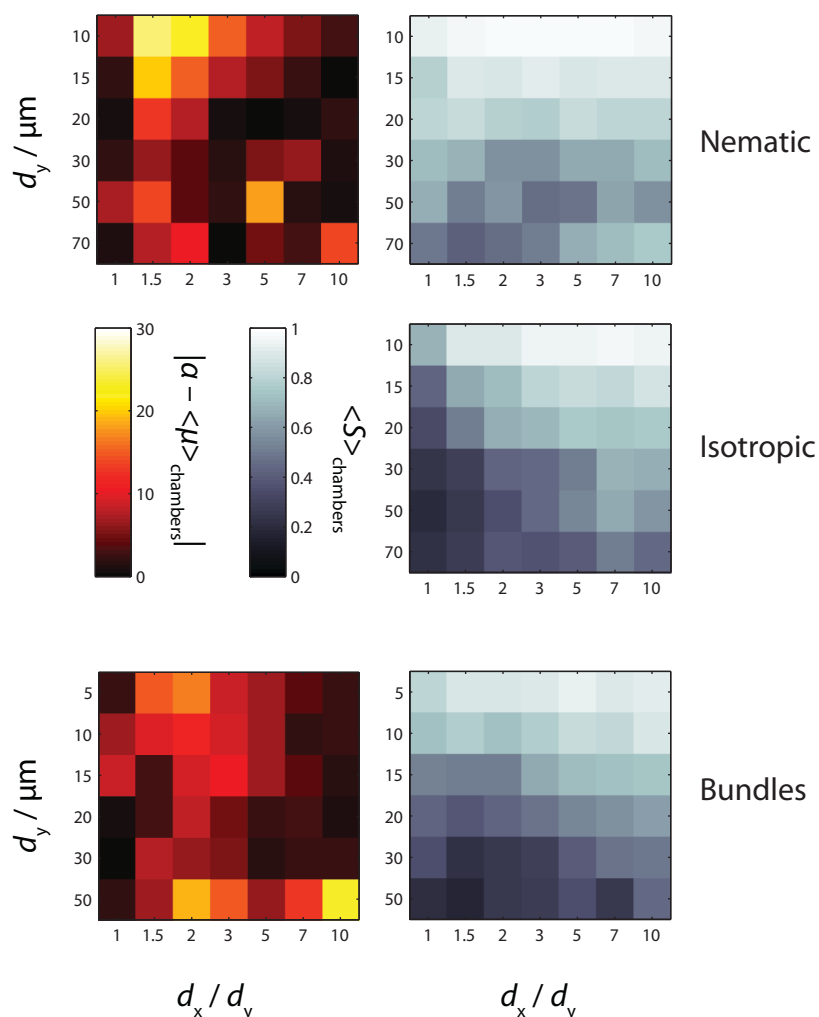


Figure S10. Results of analysis with $\sigma = 6$ px. **Top row:** Nematic F-actin solutions. Color maps of the deviation of the mean preferred orientation from the chamber diagonal (left) and the order parameter (right). **Middle row:** Isotropic F-actin solutions. Color map of the order parameter (right). **Bottom row:** Bundled F-actin solutions. Color maps of the deviation of the mean preferred orientation from the chamber diagonal (left) and the order parameter (right).

We furthermore used the “Gaussian Gradient” option. We chose this option because it gives a uniform distribution of orientations for images of random noise. We found that the default option, “Cubic Spline Gradient”, yielded two artifactual peaks at

$\pm 45^\circ$ for images of random noise, although these peaks vanished for images with a clear directionality.

Step 2: Next, we adjust the orientation measurements θ by the correction angle α_{corr} . This is the angle between the long axis of the chambers and the x -axis of the camera image. We determine α_{corr} by first running the “Dominant direction” function of OrientationJ on all images of the same sample, which were taken at different xy -coordinates. This yields many values for α_{corr} . We then determine the arithmetic mean and subtract this value from all orientation measurements from the same sample. Note that we do not rotate the image by this value. Doing so could introduce artifacts from resampling pixels.

Step 3: The corrected orientation image from Step 2 gives an orientation measurement for all pixels of an image. However, images contain pixels that are both inside and outside chambers. Furthermore, orientation measurements of pixels at chamber boundaries are dominated by the boundary and not by the orientation of filaments. In order to only select the pixels whose orientation measurements report average filament orientation, we apply a binary mask. We create this mask using different methods depending on the type of sample.

For isotropic and nematic samples we determine chamber boundaries by first filtering the image from Step 1 with a median filter of 10 px. This step smoothes the image, while preserving the edges of the chamber. Next, we apply an Otsu threshold to the filtered image, which results in a binary image where white pixels correspond to the chamber interior. Finally, we discard a layer of pixels 5σ -thick from the chamber boundaries. This is because at the chamber boundaries, the orientation measurement is dominated by the pixel intensity gradient across the chamber boundary, rather than by the orientation of filaments. This can be seen in Figure 2b of the main text as a layer of cyan and red pixels at the edges of the chambers. Neglecting this step would result in orientation distributions superposed with artifactual peaks at 0° and 90° (corresponding to the orientation of chamber edges), for example in Figure 2c presented in the main text. We have determined the optimal thickness of the layer to be 5σ . Including a factor of σ represents the fact that the layer becomes thicker for higher values of σ . This is because increasing the window size increases the number of pixels that include the chamber boundaries in their window. The factor of $N = 5$ was determined by running the analysis multiple times for different values of N . We found that this value of N was the smallest number that eliminated artifactual peaks at 0° and 90° in the orientation distributions.

For images of actin bundles, the masking step is similar to that for isotropic and nematic suspensions. We perform all sub-steps except for the removal of the

outermost layer of pixels in a chamber. This is because the chamber boundaries do not interfere with the orientation measurements of bundled suspensions. (See Figure 5b,d of the main text and note that the same layer of cyan and red pixels from Figure 2b is not apparent here.)

Step 4: In this final step, we account for chamber symmetry when averaging over many rectangular chambers. Rectangular chambers have two-fold planar rotational and reflectional symmetry. Because of this symmetry, two chambers that are mirror images of each other are identical. In order to account for this symmetry, we first determine the preferred orientation $\langle\theta\rangle$ for the chamber. We then multiply values of θ by $\text{sgn}(\langle\theta\rangle)$. This is equivalent to inverting the y -axis of a chamber if the preferred orientation is negative. We then use the symbol $\mu = \text{abs}(\langle\theta\rangle)$ to denote the preferred orientation, normalized by the symmetry of the chamber. Thus, μ only takes on values between 0° and 90° (in contrast to θ , which takes on values between $\pm 90^\circ$).

References

- 1.E. Fonck, G. G. Feigl, J. Fasel, D. Sage, M. Unser, D. A. Rufenacht, and N. Stergiopoulos, *Stroke*, 2009, **40**, 2552–2556.
- 2.R. Rezakhaniha, A. Agianniotis, J. T. C. Schrauwen, A. Griffa, D. Sage, C. V. C. Bouten, F. N. van de Vosse, M. Unser, and N. Stergiopoulos, *Biomech Model Mechanobiol*, 2012, **11**, 461–473.