Supplementary Information for Deep-learning Optical Flow for Measuring Velocity Fields from Experimental Data

Phu N. Tran,[a] Sattvic Ray,[c] Linnea Lemma,[a,c] Yunrui Li,[b] Reef Sweeney,[c] Aparna Baskaran,[a] Zvonimir Dogic,[a,c,d] Pengyu Hong,[b] Michael F. Hagan[a]

[a] *Department of Physics, Brandeis University, Waltham, MA 02453, USA*
[b] *Department of Computer Science, Brandeis University, Waltham, MA 02453, USA*
[c] *Department of Physics, University of California at Santa Barbara, Santa Barbara, CA 93106, USA and*
[d] *Biomolecular and Engineering Science, University of California at Santa Barbara, Santa Barbara, CA 93106, USA*

SUPPLEMENTARY INFORMATION

In Experiment 1, active nematics were prepared as previously described [1]. The sample contained 1 mM ATP with 1 mg/mL Alexa-647 labeled MTs. We doped the active mixture with 10 ng/mL of Alexa-488 labeled MTs. We imaged these samples on an epi-fluorescence microscope (Nikon Ti2) equipped with an oil immersion objective 60X (NA 1.25) objective. The densely labeled Alexa-647 MTs and sparsely doped Alexa-488-labeled MTs were sequentially imaged every 1.50 seconds using a motorized fluorescence filter turret.

In Experiment 2, active nematics were prepared following a similar procedure. The sample was mixed with 0.5 μm carboxyl beads which fluoresced at 488 nm. To suppress bead aggregation, the beads were coated with amine-PEG (20 KDa PEG) using a previously described protocol [2]. The beads were mixed into the rest of the active mixture, which was then introduced into the sample chamber. The sample was spun down in a swinging bucket rotor (Sorvall LYNX 6000) at 2000×g for 10 minutes. These samples were imaged with a Nikon Eclipse microscope and PCO Edge 4.2 camera using a 60X, 1.25 NA objective. The two channels (647 and 488 nm) were imaged sequentially, with an overall time step of 0.25 s, using a Lumencor Spectra light engine and a multi-band filter cube.

For all active nematic datasets, PIV was performed using PIVLab v. 2.61 in MATLAB [3]. For pre-processing, CLAHE was applied with a window size of 32 pixels, and auto-contrast stretch was applied. For analyzing the PIV, 3 passes were used, with interrogation windows of 64, 32, and 16 pixels, and step sizes 32, 16, 8 pixels, respectively. For the sub-pixel estimator, the Gauss 2x3-point setting was used. The standard setting was used for correlation robustness. For post-processing the PIV fields, the vector validation routine with threshold of 8 times the standard deviation was used, with a local median threshold of 3. The routine was set to interpolate missing data.

[1] A. M. Tayar, L. M. Lemma and Z. Dogic, *Microtubules: Methods and Protocols*, Springer, 2022, pp. 151–183.
[2] T. Garting and A. Stradner, *Colloids and Surfaces B: Biointerfaces*, 2019, **181**, 516–523.
[3] W. Thielicke and R. Sonntag, 2021, **9**, 12.