Active systems are collections of self-propelling entities that display non-equilibrium self-organization on many scales [1, 2]. Examples from the living world include animal flocks [3], bacterial colonies [4, 5], living tissues and cytoskeletal extracts [6–8]. Ingenious synthetic analogues composed either of externally driven [9–11] or autonomously propelled elements [12–14] have also been developed. The distinctive feature that unifies these systems is that they are composed of interacting units that convert ambient or stored energy into self-sustained motion, from coherently organized to seemingly chaotic.

Reconstituted suspensions of cytoskeletal filaments and associated motor proteins are active systems that have proven ideal for quantitative studies of the origin of subcellular organization, from the contractility of the actomyosin cytoskeleton to the division of the mitotic spindle. The group of Z. Dogic [8, 15] has pioneered a remarkable model system of an active gel consisting of a suspension of microtubules (MTs) in the presence of Adenosin Triphosphate (ATP)-fuelled kinesin motors. Bundled microtubules behave as active units that exert extensile forces on their environment and are capable of reproducing 

**In vitro** reconstituted active systems, such as the ATP-driven microtubule bundle suspension developed by the Dogic group, provide a fertile testing ground for elucidating the phenomenology of active liquid crystalline states. Controlling such novel phases of matter crucially depends on our knowledge of their material and physical properties. In this letter, we show that the rheological properties of an active nematic film can be probed by varying its hydrodynamic coupling to a bounding oil layer. Using the motion of disclinations as intrinsic tracers of the flow field and a hydrodynamic model, we obtain an estimate for the as of now unknown shear viscosity of the nematic film. Knowing this now provides us with an additional handle for robust and precision tunable control of the emergent dynamics of active fluids.

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Reconstituted suspensions of cytoskeletal filaments and associated motor proteins are active systems that have proven ideal for quantitative studies of the origin of subcellular organization, from the contractility of the actomyosin cytoskeleton to the division of the mitotic spindle. The group of Z. Dogic [8, 15] has pioneered a remarkable model system of an active gel consisting of a suspension of microtubules (MTs) in the presence of Adenosin Triphosphate (ATP)-fuelled kinesin motors. Bundled microtubules behave as active units that exert extensile forces on their environment and are capable of reproducing in vitro some of the unique behavior of living systems. When concentrated at an oil-water interface, the suspension of MT bundles organizes into an active nematic that exhibits self-sustained spontaneous flows with striking resemblance to the streaming used by cells to circulate their fluid content. At high enough activity active turbulent flows develop, with proliferation of unbound disclinations - the distinctive textures of two-dimensional films of nematic liquid crystals. By confining the suspension of MTs to the surface of a lipid vesicle, Keber et al. fabricated “active vesicles” that can undergo spontaneous oscillations and remarkable shape changes [16]. Finally, droplets of active gels squeezed in a narrow gap between two glass plates perform erratic autonomous motion [8].

While a lot of studies have focused on modeling this rich living-like behavior and especially on the generation and dynamics of topological defects, the material properties of active nematics remain largely unexplored. One exception is a very recent work by some of us, where an anisotropic shear viscosity, arising from a contacting passive thermotropic liquid crystal, is used to align active nematic flows [17].

Here, we examine quantitatively the influence of the combined hydrodynamics of the 2d active nematic and the bounding bulk passive fluids on the active dynamics, and use it to infer an estimate for the shear viscosity of the active material. This is obtained through a simple hydrodynamic model that examines the flow induced by nematic textures confined at the interface of two fluids of different viscosity [18–20]. A fit of the model’s prediction for the defect velocity when the oil viscosity is varied over five orders of magnitude allows us to determine an effective bulk shear viscosity of the active nematic and to infer the viscosity of the film. We show that the rheological characteristics of the interface also have a profound effect on the textures and flows of the active nematic.

The active material we study is based on the hierarchical self-assembly of tubulin into stabilized micron-length fluorescent MTs, organized into bundles that are internally cross-linked and sheared by clusters of kinesin motors [8]. This leads to MT bundle elongation, bending and buckling, which results in extensile local stresses on the surrounding fluid. Once depleted towards a surfactant-decorated oil/water interface, the kinesin/tubulin gel develops the well-known active nematic configuration, which is characterized by self-sustained flows and orientational order of the aligned filaments. Although the thickness of the active nematic layer is not known with precision, we estimate it to be in the range...
between the minimal bundle thickness, 0.2 µm, estimated from the typical sizes of filaments and motor proteins, and the resolution of fluorescence confocal micrographs, 2 µm. Our open-cell arrangement is based on a custom polydimethylsiloxane (PDMS) block containing a cylindrical well, and bound to a support plate (see Fig. 1 and [17]). After filling the well with silicone oil of the desired viscosity, the aqueous gel is injected between the bottom plate and the oil. This results in an aqueous layer of 100 − 200 µm depth underneath an oil phase of 1 − 2 mm depth. Unlike the original arrangement [8], our setup does not demand the use of a low viscosity oil, thus allowing us to explore nearly five orders of magnitude of viscosity contrast between the interfacing oil and the aqueous bulk. In the experiments reported here, ATP concentration is kept at 1.4 mM by means of an enzymatic ATP-regenerator that is incorporated in the active material, which sets a constant activity for the active material over the time scale of the experiment.

**Results.** The spontaneous flow is the result of the extension of the elongated fibers from continuous microtubule sliding driven by the kinesin motors. Because of their extensile nature, MT bundles bend and form parabolic folds, bounding dark regions devoid of MTs (Fig. 2.a). The orientation of the aligned filaments performs half a turn along any closed circuit surrounding either the tip or the tail of the fold. This result allows us to associate a defect topological charge +1/2 to the parabolic tip of the fold, and a charge −1/2 to the hyperbolic tail. As topological defects are created and annihilated in pairs, the total topological charge adds up to zero at all times. Since the flow originates at the tip of the folds, +1/2 defects become like active or self-propelled particles, as first quantified in [21], and can be used as intrinsic tracers for the active flow. On the other hand, −1/2 defects often occupy flow stagnation points (Fig. 2 in SI). In these nematic films the +1/2 defects have also been observed to exhibit long-range nematic alignment [22], although the mechanism for such alignment is still controversial [22–24].

Our study has revealed a clear influence of the oil viscosity, η_o, on the morphology and dynamics of the active nematic. In Fig. 2, we show snapshots of the active nematic in contact with oils of different viscosity, for the same activity (i.e., concentration of ATP). These patterns are characterized by the proliferation of randomly moving defects, organizing into the so-called active turbulent regime [21, 25–29]. It is clear that at higher η_o the number of defects increases and degrades the orientational order of the filament bundles. At the same time, the speed of defects decreases for increasing η_o (Video S1). It is also apparent that textures in contact with oils of smaller viscosity appear less fluorescent (Fig. 3 in SI) and more tenuous, as compared to those observed for higher η_o. This indicates that a large η_o concentrates...
the MT bundles while also amplifying the typical size of the empty regions that are the cores of the defect textures. Additionally, we observe an increase of the radius of curvature of microtubule bending with \( \eta_o \), as evidenced when comparing the insets of Figs 2a and 2f. Since the defect core length, denoted by \( \xi_Q \), is expected to grow as \( \sqrt{K} \), with \( K \) the nematic bending rigidity, based on these observations we conjecture that the nematic material becomes stiffer in contact with oils of increasing viscosity. Textures such as the one displayed in Fig. 2f are rather disordered in the sense that the nematic order parameter would average to a small value. The system, however, still shows a characteristic structure that can be analyzed in terms of the topological defects of a nematic liquid crystal.

We have quantified both the number density, \( n \), and the velocity, \( v \), of the +1/2 defects for the realizations shown in Fig. 2. We observe that \( v \) decreases logarithmically with \( \eta_o \) before a saturation is reached for the most viscous oils (Fig. 3). This data is successfully fitted with the hydrodynamic model described below, which allows us to estimate the viscosity of the active nematic. Conversely, the defect density grows steadily with \( \eta_o \) (Fig. 4a). We have also measured the average size of the defect core, defined as the region devoid of MTs surrounding each defect. We observe that the area fraction occupied by the defect cores is proportional to the defect density, and the average defect core area, which we relate to \( \xi_Q \), grows with \( \eta_o \) until saturation for the most viscous oils (see Fig. 4 in SI).

The measurements of \( v \) and \( n \) can be combined to reveal a simple scaling relationship between these two quantities. The scaling ansatz can be obtained using generic arguments already proposed for freely suspended active nematics [30]. We assume a simple rate equation for the defect density, \( dn/dt = R_c - R_a \), with \( R_c \) and \( R_a \) the rates of defect creation and annihilation per unit area. The creation rate can be estimated as \( R_c \sim (\ell_a^2\tau)^{-1} \), where \( \ell_a = \sqrt{K/|\alpha|} \) is the active length scale determined by balancing active and elastic stresses, with \( \alpha \) an active stress scale controlled by ATP concentration and chosen negative for extensile systems. It has been shown that this length scale controls the spatial correlations in the active turbulent regimes of ‘wet’ active nematics [29, 31]. The time scale \( \tau = \gamma/|\alpha| \) controls the relaxation of the director upon distortion by active stresses, with \( \gamma \) the rotational viscosity of the nematic. The annihilation rate can be estimated as \( R_a \sim \sigma vn^2 \), with \( \sigma \) an effective cross section (a length in two-dimensions) that quantifies the range of defect interactions. At steady state, the average number density of defects remains constant, giving the scaling prediction \( vn^2 \sim \sigma/\gamma K \). For a constant ATP concentration, \( \alpha \) and \( \gamma \) will be constant, leading to the simplified scaling \( \sigma vn^2 \sim 1/(\sigma K) \). Viscous stresses at the interface effectively dampen the active nematic speed (Fig. 3), which results in more sporadic defect annihilation events. Since the ATP concentration is kept constant in all these experiments, the defect creation rate is sustained. As a result, the number of defects increases (Fig. 4a). Notice that the above scaling implies that the product \( \sigma K \) is independent of \( \eta_o \) (Fig. 4b). Taking into account the experimental evidence that \( K \) increases with \( \eta_o \), this scaling behavior suggests that the range of defect interaction decreases in contact with more viscous oils. This trend is opposite to that exhibited by the defect core size, so clearly \( \sigma \) and \( \xi_Q \) must be independent length scales. As a matter of fact, we may quantify the change of \( \sigma \) with \( \eta_o \) by replacing \( K \) by \( \xi_Q \) in the scaling...
above, revealing that \( \sigma \) decreases by a factor of four in the explored range of oil viscosities (Fig. 5 in SI).

Hydrodynamic model. To capture the effect of viscous stresses propagated in the nematic by the viscosity contrast at the oil/water interface, we consider the hydrodynamics of a thin active nematic layer confined between two bulk fluids (oil and water) and calculate the velocity that the director distortion due to a +1/2 disclination creates at the core of the defect. Following Ref. [21], and consistently with the analysis of the experiments, we identify this with the defect velocity. We find that the presence of the bulk fluids qualitatively changes the defect velocity as compared to the previously considered cases of a free-standing nematic layer [21, 28], a finite-thickness layer of bulk nematic [27, 32], and a nematic layer with frictional damping from a substrate [33].

To evaluate the defect velocity, we start with coupled Stokes equations for oil, water and nematic layer,

\[
\begin{align*}
\eta_i \nabla^2 \mathbf{u}_i - \nabla p_i &= 0, \quad (1) \\
\eta_N \nabla^2 \mathbf{u}_N - \nabla \cdot \mathbf{P} + \hat{\mathbf{n}} \cdot (\mathbf{\sigma}_N - \mathbf{\sigma}_w) + \nabla \cdot \mathbf{\sigma}_N &= 0 \quad (2)
\end{align*}
\]

where \( \eta_i \), \( \mathbf{u}_i \) and \( p_i \), with \( i = o, w \) denoting oil or water, are the 3d viscosity, flow velocity, and pressure of the oil and water subphases respectively; \( \mathbf{\sigma}_N - \mathbf{\sigma}_w \) is the stress jump across the interface, which is projected onto the unit interface normal \( \hat{\mathbf{n}} \); \( \eta_N, \mathbf{u}_N \) and \( p \) are the 2d viscosity, in-plane flow velocity and lateral pressure of the active nematic layer. Finally, \( \mathbf{\sigma}_N = \alpha \mathbf{Q} \) is the active stress arising from the extensile force dipoles exerted by MT bundles on their surroundings and proportional to the nematic alignment tensor \( \mathbf{Q} \). We compute the nematic flow field \( \mathbf{u}_N \) due to stationary textures of the order parameter \( \pm \mathbf{Q} \). The solution is easily written in Fourier space in terms of a Green’s function. As the depth of both the oil layer and the bulk fluid subphases are much larger than the thickness of the active nematic layer, we consider both bulk layers to be semi-infinite. In this limit, the Fourier components of the flow velocity in the nematic layer are

\[ \mathbf{u}_N(k) = G(k) \mathcal{P} \mathbf{f}(k), \] where \( \mathcal{P} = \mathbf{I} - \mathbf{k} \mathbf{k} / k^2 \) is a transverse projection operator, \( \mathbf{f}(k) = \int_x e^{-i k \cdot x} \mathbf{\sigma}_N \) and the Green’s function is given by \( G(k) = \left[ \eta_N k^2 + \eta_o k \right]^{-1} \) [20]. The length scale \( \ell_{\eta} = \eta_N / (\eta_o + \eta_w) \approx \eta_N / \eta_o \) (for \( \eta_w \ll \eta_o \)) controls the crossover from two dimensional surface flows to three dimensional bulk dominated flows. The scalar order parameter for a +1/2 disclination is roughly constant outside the defect core of size \( \xi_Q \), yielding \[ |\nabla \sigma_{\|}| \approx |\alpha| / r \] for \( r \geq \xi_Q \) [33]. Focusing on the +1/2 disclinations, which are motile by virtue of self-induced active backflows [9, 21], the divergence of the active stress \( \nabla \cdot \mathbf{\sigma}_N \) for a single +1/2 disclination has only one non-vanishing component aligned along the axis of the defect (which we freely take to be the \( x \)-axis). The velocity at the center of the defect core, assumed to be passively advected by the flow, is then directed along this axis and has a magnitude given by \( u_0 = \int_0^\infty G(r) (\nabla \cdot \mathbf{\sigma}_N)_x \), where the prime indicates that the integral must be cutoff at small scales by \( \xi_Q \) (\( \xi_Q \approx 10 \mu m \) from the experimental micrographs), below which the hydrodynamic model ceases to be appropriate, and at a long-wavelength cutoff \( \ell \) controlling the screening of the 2d hydrodynamic flows through the coupling to the oil/water subphases. This gives

\[
u_0 \approx \frac{|\alpha|}{\eta_N / \ell} \mathcal{F}_\geq \left( \frac{\eta_o}{\eta_N / \ell} \frac{\ell}{\xi_Q} \right) \sim \frac{|\alpha|}{\eta_N / \ell} \ln \left( \frac{\eta_N / \ell}{\eta_o} \right). \quad (3)
\]

The exact form of \( \mathcal{F}_\geq \) is given in the SI. The second approximate equality in Eq. (3) holds for \( \eta_{eff} \gg \eta_o \) and \( \xi_Q / \ell \ll 1 \), with \( \eta_{eff} = \eta_N / \ell \) a three-dimensional viscosity. The logarithmic dependence of \( u_0 \) on \( \eta_o \) is robust in these limits and in agreement with the experiments (Fig. 3).

The fit to the data, performed by means of the exact form for \( u_0 \) given in the SI, provides a value for \( \eta_{eff} = 13(\pm 5) \) Pa s that depends very weakly on \( \xi_Q / \ell \) for \( \xi_Q / \ell < 0.5 \) (see SI). Since \( \alpha \) is an overall scale for the defect velocity, this analysis gives an essentially parameter free estimate for \( \eta_{eff} \). On the other hand, the value of \( \eta_N \) depends on \( \ell \). A natural choice for \( \ell \) is the thickness of the oil subphase (\( d \sim 1 \) mm) as described in the SI. This gives \( \eta_N \sim 13 \times 10^{-3} \) Pa s m. Alternately, one could argue that our single-defect calculation should be cutoff at the scale of the mean defect separation, which in turn depends on \( \eta_o \) (see Fig. 4a), albeit changing only by a factor of two (50 - 100 \( \mu m \)) over five decades of oil viscosity. Choosing \( \ell \sim \eta_{eff}^{-1/2} \), we obtain \( \eta_N \sim 6.5 - 13 \times 10^{-4} \) Pa s m over the range of oil viscosities considered.

Importantly, our fit yields \( \eta_{eff} / \eta_o > 1 \) at all but the largest oil viscosity (\( \eta_o \sim 10^2 \) Pa s), where \( u_0 \) begins to saturate. We stress that it is only in this limit that the defect velocity has a logarithmic dependence on \( \eta_o \). This physically corresponds to the case when the flow is dominated by the properties of the 2d active nematic layer and the bulk fluid only comes as a logarithmic correction to the length scale in the defect velocity. If this ratio were of order unity or smaller, as occurs at the highest value of oil viscosities, then the qualitative dependence of the defect velocity on \( \eta_o \) would change, giving \( u_0 \sim 1 / \eta_o \) instead of the strongly persistent logarithm.

In summary, we have probed the rheology of an active nematic at an oil/water interface using a set-up that allows us to vary the viscosity of the oil by five orders of magnitude. By combining experiments with a hydrodynamic model we show that measurements of the defect velocity can be used to estimate for the first time the shear viscosity of the active nematic.

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[1] M. C. Marchetti, J. F. Joanny, S. Ramaswamy, T. B. Liverpool, J. Prost, M. Rao, and R. A. Simha, Reviews of Modern Physics 85, 1143 (2013).
[2] S. Ramaswamy, Annual Review of Condensed Matter Physics 1, 323 (2010).
[3] A. Cavagna, A. Cimarelli, I. Giardina, G. Parisi, R. Santagati, F. Stefanini, and M. Viale, Proc Natl Acad Sci U S A 107, 11865 (2010).
[4] C. Dombrowski, L. Cisneros, S. Chatkaew, R. E. Goldstein, and J. O. Kessler, Phys Rev Lett 93, 098103 (2004).
[5] H. P. Zhang, A. Be’er, E. L. Florin, and H. L. Swinney, Proc Natl Acad Sci U S A 107, 13626 (2010).
[6] V. Schaller, C. Weber, C. Semmrich, E. Frey, and A. R. Bausch, Nature 467, 73 (2010).
[7] Y. Sumino, K. H. Nagai, Y. Shitaka, D. Tanaka, K. Yoshikawa, H. Chate, and K. Oiwa, Nature 483, 448 (2012).
[8] T. Sanchez, D. T. Chen, S. J. DeCamp, M. Heymann, and Z. Dogic, Nature 491, 431 (2012).
[9] V. Narayan, S. Ramaswamy, and N. Menon, Science 317, 105 (2007).
[10] A. Bricard, J. B. Causin, N. Desreumaux, O. Dauchot, and D. Bartolo, Nature 503, 95 (2013).
[11] S. Hernandez-Navarro, P. Tierno, J. A. Farrera, J. Ignes-Mullol, and F. Sagues, Angew Chem Int Ed Engl 53, 10966 (2014).
[12] W. F. Paxton, K. C. Kistler, C. C. Olmeda, A. Sen, S. K. St Angelo, Y. Cao, T. E. Mallouk, P. E. Lammert, and V. H. Crespi, J Am Chem Soc 126, 13424 (2004).
[13] J. Howse, R. Jones, A. Ryan, T. Gough, R. Vafabakhsh, and R. Golestanian, Physical Review Letters 99, 048102 (2007).
[14] J. Palacci, S. Sacanna, A. P. Steinberg, D. J. Pine, and P. M. Chaikin, Science 339, 936 (2013).
[15] G. Henkin, S. J. DeCamp, D. T. Chen, T. Sanchez, and Z. Dogic, Philos Trans A Math Phys Eng Sci 372, 0142 (2014).
[16] F. C. Keber, E. Loiseau, T. Sanchez, S. J. DeCamp, L. Giomi, M. J. Bowick, M. C. Marchetti, Z. Dogic, and A. R. Bausch, Science 345, 1135 (2014).
[17] P. Guillamat, J. Ignes-Mullol, and F. Sagues, Proc Natl Acad Sci U S A 113, 5498 (2016).
[18] P. G. Saffman and M. Delbruck, Proceedings of the National Academy of Sciences 72, 3111 (1975).
[19] P. G. Saffman, Journal of Fluid Mechanics 73, 593 (1976).
[20] D. K. Lubensky and R. E. Goldstein, Physics of Fluids 8, 843 (1996).
[21] L. Giomi, M. J. Bowick, X. Ma, and M. C. Marchetti, Physical Review Letters 110, 228101 (2013).
[22] S. J. DeCamp, G. S. Redner, A. Baskaran, M. F. Hagan, and Z. Dogic, Nat Mater 14, 1110 (2015).
[23] A. U. Oza and J. Dunkel, arXiv:1507.01055 (2015).
[24] A. Doostmohammadi, M. F. Adamer, S. P. Thampi, and J. M. Yeomans, Nat Commun 7, 10557 (2016).
[25] X. Q. Shi and Y. Q. Ma, Nat Commun 4, 3013 (2013).
[26] C. A. Weber, C. Bock, and E. Frey, Phys Rev Lett 112, 168301 (2014).
[27] S. P. Thampi, R. Golestanian, and J. M. Yeomans, Philos Trans A Math Phys Eng Sci 372, 0366 (2014).
[28] L. Giomi, M. J. Bowick, P. Mishra, R. Sknepnek, and M. Cristina Marchetti, Philos Trans A Math Phys Eng Sci 372, 0365 (2014).
[29] L. Giomi, Physical Review X 5, 031003 (2015).
[30] S. P. Thampi, R. Golestanian, and J. M. Yeomans, Phys Rev Lett 111, 118101 (2013).
[31] E. J. Hemingway, P. Mishra, M. C. Marchetti, and S. M. Fielding, arXiv:1604.01203 (2016).
[32] G. Tóth, C. Denniston, and J. M. Yeomans, Physical Review Letters 88, 105504 (2002).
[33] L. M. Pismen, Phys Rev E Stat Nonlin Soft Matter Phys 88, 050502 (2013).