Ciliated tissues, such as in the mammalian lungs, brains, and reproductive tracts, are specialized to pump fluid. They generate flows by the collective activity of hundreds of individual cilia that beat in a striking metachronal wave pattern. Despite progress in analyzing cilia coordination, a general theory that links coordination and fluid pumping in the limit of large arrays of cilia remains lacking. Here, we conduct in silico experiments with thousands of hydrodynamically interacting cilia, and we develop a continuum theory in the limit of infinitely many independently beating cilia by combining tools from active matter and classical Stokes flow. We find, in both simulations and theory, that isotropic and synchronized ciliary states are unstable. Traveling waves emerge regardless of initial conditions, but the characteristics of the wave and net flows depend on cilia and tissue properties. That is, metachronal phase coordination is a stable global attractor in large ciliary carpets, even under finite perturbations to cilia and tissue properties. These results support the notion that functional specificity of ciliated tissues is interlaced with the tissue architecture and cilia beat kinematics and open up the prospect of establishing structure to function maps from cilium-level beat to tissue-level coordination and fluid pumping.

Motile cilia are basic microactuators in cell biology and bio-inspired systems (1) that beat cyclically (2) to transport fluid across the cell surface. In aquatic species, cilia can be found along external and internal epithelial surfaces in solitary, pairwise, or lattice configurations, where they have a broad array of functions from locomotion (3) to food capture (4, 5) and acquisition of microbial partners (6). When animals invaded terrestrial habitats, cilia became restricted to internal epithelial surfaces to reduce water loss across ciliary membranes, rendering them difficult subjects for direct study. In humans, they serve important biological functions, including mucus clearance in the respiratory system (7), left–right asymmetry determination during embryonic development (8), cell transport in the reproductive tracts (9), and cerebrospinal fluid circulation in the brain ventricles (10, 11).

To pump fluid at the micrometer scale where viscosity is dominant (12), individual cilia beat in a nonreversible manner, and in multiciliated cells and tissues, neighboring cilia coordinate their phase to beat in metachronal waves (13–17). Metachronal coordination and microscale flows were observed in microdissected ex vivo epithelia (6, 7, 10) and in vitro engineered tissues (11). However, probing the emergence and robustness of cilia coordination in tissues and its effect on fluid transport remains experimentally challenging.

Microorganisms provide an accessible way to investigate cilia coordination, but functional constraints on coordination differ between organisms that use cilia for locomotion and tissues that use cilia for specialized fluid-pumping functions. Specialization and efficient pumping in tissues can be compromised if multiple states of cilia coordination coexist. In contrast, to achieve and transition between swimming and turning in cilia-driven locomotion, multiple coordination states are needed (3, 18–20). In-phase, antiphase, and nontrivial phase lags were observed experimentally in pairs of cilia isolated from the somatic cells of Volvox carteri (21) and Chlamydomonas reinhardtii (3) and coupled only via the fluid medium. These coordination states and the instabilities that lead to transitions between them were captured quantitatively in silico (22). In addition to interactions via the fluid medium, cilia coordination in unicellular organisms seems to be communicated predominantly by elastic coupling through the cytoskeleton (3, 23–26). However, the importance of substrate-based interactions in tissues, besides providing and maintaining geometric and structural integrity, remains under debate, with recent experimental evidence suggesting that fluid coupling dominates cilia coordination in tissues (11).

Are hydrodynamic interactions sufficient to guarantee functional specificity and coherent fluid pumping in ciliated tissues? The answer requires an investigation of the coordination states that emerge at the tissue level and how they drive flows. Mathematical models and numerical simulations offer exciting prospects for exploring these issues;
however, most existing models address each aspect separately; cilium coordination (22, 26–30) or cilia-generated flows (7, 16). Meng et al. (28) analyzed, in a coarse-grained model, the stability of metachronal waves as a function of the dynamical and geometric characteristics of the individual cilium, and Solovev and Friedrich (29) showed, using a similar analysis, that metachronal waves are globally stable. Ramirez-San Juan et al. (7) measured ciliary arrangement from direct images of ex vivo mammalian airways and reported heterogeneity in cilium organization across the trachea but coherent fluid transport. The results in refs. 7 and 28 are important in complementary ways. Ref. 28 provided tools to analyze how collective properties depend on individual beat patterns, while ref. 7 provided tools to map collective properties of the ciliated tissues to fluid transport function. The combined effect of how cilia in large-scale ciliary carpets self-organize into emergent collective states and how these states drive flow is less well understood (14, 15). Here, we investigate coordination and pumping in discrete and coarse-grained models of ciliary carpets. Our findings challenge the basic conception in ref. 28 that wave stability is the only factor that matters for tissue-level function and expand the observations in refs. 7 and 28.

Our models serve as a first step toward establishing maps from single-cilium kinematics to tissue-level coordination and fluid-pumping function and could help inform our understanding of the function and dysfunction of ciliated tissues in major mammalian organs. They could also help translate cilia-inspired design principles to engineered micropumps (31–34)—a potential that is particularly compelling in light of the rapid developments in tissue engineering and organ-on-chip (35, 36) technologies.

**Results**

To investigate the emergent coordination and fluid pumping in tens of thousands of hydrodynamically coupled cilia, we develop discrete and coarse-grained models where individual cilia are represented as nonlinear phase oscillators in a viscous fluid medium of viscosity \( \eta \) (Fig. 1A). We use our models to investigate three questions that are fundamental for understanding the physics of ciliated tissues. First, considering cilia whose individual beat patterns break no symmetry and produce no net flux at the single-cilium level, are hydrodynamic interactions in ciliary carpets sufficient to create coordination patterns that break symmetry and pump fluid? If so, how does the net flux created by this emergent coordination depend on the properties of the single cilium? Lastly, how do variations in the tissue-level heterogeneity (7, 37) affect both cilia coordination and net flux?

**Modeling Individual Cilia as Nonlinear Phase Oscillators.** We represent a beating cilium by a spherical bead of radius \( a \) moving on a circular trajectory of radius \( b \) in an \( (x, y) \) plane located at a height \( z = h \) above a bounding wall at \( z = 0 \) (Fig. 1). In this representation, phase \( \theta \) is the only dynamical variable; properties of the cilium beating pattern are subsumed to a phase-dependent active force \( F(\theta) \) that drives the bead’s motion. The fluid velocity \( \mathbf{u}(x, y, z) \) is governed by the incompressible Stokes equations and obtained from the Blake–Oseen solution associated with the force monopole \( F(\theta) \) in the three-dimensional (3D) half-space \((z \geq 0)\). Common, including experimentally derived, cilia beat patterns can be accounted for by fitting \( F \) such that the flow field \( \mathbf{u} \) matches that of the beating cilium (21).

We restrict our analysis to when the active force \( F = F(\theta)t \) acts in the tangent direction \( t \) to the circular trajectory (Fig. 1A). Generically, \( F(\theta) \) is a strictly positive, nonlinear function that can be expressed in terms of force harmonics, \( F(\theta) = F_0 \left[ 1 + \sum_{m = 1}^{\infty} \alpha_m \cos m \theta + \beta_m \sin m \theta \right] \). \( F_0 \) is a constant force, and \( \alpha_m, \beta_m \) are nondimensional coefficients. The first harmonic \((m = 1)\) defines a distinguishable direction in the \((x, y)\) plane from minimum to maximum forcing, akin to the

---

**Fig. 1.** Ciliary carpets. (A) Cilia beats usually have a stronger power stroke and a recovery stroke, and typically, they have a preferred beating axis, in which they travel a longer distance. These two features can be individually characterized by the first two harmonic decompositions of a periodic forcing produced by a rotating force monopole situated at a fixed height away from a no-slip wall. Specifically, the first force harmonic \( F_1 \) captures the force magnitude variation due to the power and recovery strokes, while the second force harmonic \( F_2 \) can represent the force variation due to an elliptical beat. These observations can be confirmed by matching the harmonic force magnitudes with those deduced from real cilia beats as a function of the phase angle \( \theta \). Both the 3D beat and the force profile are traced from ref. 21. (B) A doubly periodic array of cilia on a square lattice in the \((x, y)\) plane above a wall is represented in the continuum limit by a force discontinuity layer shown in blue. (C) A discrete simulation of the field of rotors can be succinctly represented by the cosine of their phase. (D) Numerical simulations showing the emergence of traveling metachronal waves. Snapshots taken at \( t = 100 \) for \( h/d = 0.5 \) (D, Left) and \( h/d = 1.5 \) (D, Right). (E) Time evolution of the Kuramoto order parameter \( P \) shows no clear distinction between isotropic and traveling wave patterns. Insets show zoom-in on the evolution of \( P \) starting from the isotropic case. The first harmonic force is shown in black, and the second harmonic is shown in blue. (F) We define \( P(\alpha) \) for \( \alpha \in [0, 2\pi] \) and plot \( P(\alpha) \) in polar coordinates at two snapshots (shown in inset) taken from the isotropic state (C) and traveling wave pattern (lower left in D). Data points are shown in black, and the elliptic best fit is shown in blue. (G) Time evolution of the wave coordination (Kuramoto ellipse eccentricity) and the wave direction (parallel to the ellipse minor axis) clearly shows the growth of the traveling wave instability. First and second force harmonics are shown in black and blue, respectively, and solid and dashed lines represent the wave coordination and direction, respectively. In D–G, simulations are performed for 100 time units using \( dt = 0.1, \sigma = 0.05, b = 0.2, \) and \( d = 1 \). First force harmonic: \( F_1 = 1 + 0.5 \cos \theta + 0.5 \sin \theta \). Second force harmonic: \( F_2 = 1 + 0.5 \cos 2\theta + 0.5 \sin 2\theta \).
power stroke direction in beating cilium; the second harmonic 
\( n = 2 \) describes a forcing profile with two antipodal maxima 
orthogonal to two antipodal minima, thus capturing the elliptic 
characteristic of a beat cycle with no asymmetry (Fig. 1A and 
**SI Appendix**, Figs. S1 and section S2).

The tangential active force ensures no fluid pumping at the 
single-cilium level. The net flux \( q = \| (1/2\pi)(h/\pi r) \int_0^{2\pi} F \, d\theta \| \) 
is identically zero. Allowing a nontangential force component or 
tilting the ciliary trajectory with respect to the bounding wall 
can break symmetry and prompt fluid pumping (28, 38). Here, we 
confine our analysis to rotors that do not break the Stokes flow 
symmetry to distill whether hydrodynamic interactions in ciliary 
carpets can lead to symmetry breaking and fluid pumping.

**Modeling Ciliary Carpets.** We consider a two-dimensional (2D) 
discrete array of cilium, where each cilium is centered at \( x_j \) 
\( \{ j = 1, 2, \ldots \} \) in a doubly periodic square lattice of 
dimensional size \( L \times L \) and distance \( d \) between neighboring cilia 
(Fig. 1A). Let \( N \) be the total number of cilia in the fundamental 
domain and \( \rho_c = N / L^2 \) be the cilium density.

The instantaneous position of the \( j \)-th cilium is determined by 
its phase \( \theta_j \) and in vector form, by \( r_j = x_j + b \eta_j \), where 
\( \eta_j = (\cos \theta_j, \sin \theta_j, 0) \) is the normal unit vector in the 
ciliary plane. Each cilium is driven independently by an active 
tangent force \( F_j = F(\theta, r_j) \). The fluid velocity \( \mathbf{u}(x, y, z) \) driven 
by the collective beating of all cilia is given by a conditionally 
convergent, doubly infinite sum of the Blake–Oseen tensor 
(\*SI Appendix*, Figs. S2 and S7 and section S2). For notational 
convenience, we introduce the 2D velocity \( \mathbf{v}(x, y) \), which is the 
projection of \( \mathbf{u}(x, y, z) \) onto the ciliary plane.

Balance of forces on cilium \( j \) in the ciliary plane dictates that 
\(-\zeta (b \dot{\eta}_j \cdot \mathbf{v}(r_j)) + \mathbf{F}_j + \mathbf{N}_j = 0 \), where the first term 
denotes the drag force (with constant drag coefficient \( \zeta = 6 \mathrm{m} \eta \mathrm{a} \)) 
and accounts for hydrodynamic coupling among cilia; the last 
term is a constraint force that guarantees the bead remains on the 
desired circular trajectory. Projecting the force balance on the 
normal and tangential directions, respectively, leads to an 
expression for the constraint force, \( \mathbf{N}_j = -\zeta (\Omega_j \cdot \mathbf{v}(r_j)) \eta_j \), and a set of 
coupled differential equations that govern the time evolution of 
the cilia phase,

\[
\dot{\theta}_j = \Omega_j + \frac{1}{b} \xi_j \cdot \mathbf{v}(r_j),
\]

with intrinsic phase-dependent angular speed \( \Omega_j = F(\theta_j) / \zeta b \).

**Collective States That Do Not Pump Fluid.** We look for time-
periodic, spatially uniform states in which the collective dynamics 
is time reversible or equivalently, Eq. 1 is invariant under the 
transformation \( \theta \rightarrow -\theta \). Two collective states exist: synchronized 
(\( \theta = 0 \)) and isotropic (Fig. 1C). When all cilia beat in synchrony, 
we get \( \dot{\theta}_j (t) = \theta_0 (t), \mathbf{t}_j = \mathbf{t}^* \), and \( \mathbf{v}(r_j) = v^* \mathbf{t}^* \) for all \( j \). 
This is a periodic solution of Eq. 1, for which the dynamics is time 
reversible and no pumping ensues.

To define an isotropic state for which the fluid velocity is 
identically zero (i.e., \( \nu = 0 \)), we first introduce a nonlinear 
coordinate transformation \( d \phi / d \theta = \Omega / \Omega(\theta) \) from \( \theta \) to a new phase 
\( \phi \) in which the intrinsic angular speed \( \Omega_\alpha = 2\pi / T_\alpha \) is constant 
and \( T_\alpha = \int_0^{2\pi} d\theta / \Omega(\theta) \) is the oscillation period of isolated cilia 
(\*SI Appendix*, Fig. S11 and section S4). We rewrite Eq. 1 in terms of 
\( \phi \):

\[
\dot{\phi}_j = \Omega_\alpha + \frac{1}{b} \Omega_\alpha \xi_j \cdot \mathbf{v}(r_j).
\]

We define the isotropic state to be the state where all \( \phi_j \) are 
drawn from a uniform distribution function \( \text{Unif} [0, 2\pi] \). In the 
limit of an infinitely dense ciliary carpet, cilia-induced forces 
cancel everywhere in the fluid domain, resulting in a fluid velocity 
\( \mathbf{v} \) that is identically zero. The isotropic state is thus a periodic 
solution of Eq. 1 that does not pump fluid. In systems with finite 
numbers of cilia, the isotropic state can only be approximately 
realized.

**Spontaneous Phase Coordination.** We probe the stability of the 
synchronized and isotropic states numerically by solving Eq. 1 
for 151 \( \times \) 151 cilia (a total of \( N = 22,801 \) hydrodynamically 
coupled cilia) using an in-house algorithm (\*SI Appendix*). We 
find that starting from initially synchronized and isotropic states, 
as time evolves, the cilia spontaneously coordinate into traveling 
wave patterns. The emergent patterns are independent of initial 
conditions but depend on the force profile \( F(\theta) \) and distance \( h \) 
from the wall; Fig. 1D shows the long-term traveling wave patterns 
corresponding to the first and second force harmonics at two values 
of \( h \). Both the isotropic and synchronized states of the system 
are unstable, giving rise to traveling waves. A direct inspection 
of these emergent waves suggests that the coordination is more 
spatially coherent when subject to the second force harmonic, 
consistent with ref. 28. The role of the first force harmonic in 
emergent coordination was not discussed in ref. 28.

**Quantifying Emergent Wave Patterns.** In an effort to quantify 
the emergent order, we introduce the moment fields describing 
phase coordination (39, 40),

\[
Y_n(x, t) = \frac{1}{\rho_c} \sum_{j=1}^{N} \delta (x - x_j),
\]

where \( Y_1 \) is the Kuramoto order field, \( Y_2 \) the nematic order field, 
and \( \delta(\cdot) \) the Dirac delta function. The average of \( Y_1 \) over the 
2D fundamental domain leads to the Kuramoto order parameter 
\( P = \langle Y_1 \rangle_{2D} \); values of \( P \) near zero indicate phase 
disorder, while values near one correspond to phase synchrony (41). In 
Fig. 1E, we plot \( P \) vs. time for the synchronized and isotropic 
initial state; \( P \) converges to a steady-state value that is independent 
of initial conditions, indicating that these emergent waves are 
global attractors of the collective dynamics. However, \( P \) does not 
capture the change in spatial patterns that arise as the system 
evolves from isotropic to traveling wave patterns.

To characterize the emergent wave patterns, we compute a 
directional Kuramoto order parameter \( P_\alpha = \langle Y_1 \rangle_{2D} \) by 
integrating \( Y_1 \) along a straight line at a direction \( \alpha \) from the 
axis. Intuitively, \( P_\alpha \) measures the average phase order along 
the direction \( \alpha \). Mathematically, it is the absolute value of the 
Radon transform of \( Y_1(x) \) restricted to lines that go through the 
origin. We vary \( \alpha \in [0, 2\pi] \) and compute \((\alpha, P_\alpha)\) 
(black dots in Fig. 1F). The distribution of data points \((\alpha, P_\alpha)\) 
is roughly circular in the isotropic state and elliptic in the traveling 
wave state (Fig. 1F). We use principal component analysis to 
fit \((\alpha, P_\alpha)\) to an ellipse that we call the Kuramoto ellipse 
(\*SI Appendix*, Figs. S8 and S9 and section S3). Starting from 
the isotropic state, the time evolution of the eccentricity and angle of 
the Kuramoto ellipse are good indicators of the emergent traveling 
wave pattern; its eccentricity indicates wave coordination, and its 
angle is orthogonal to the wave traveling direction (Fig. 1G).

**Emergent Waves and Fluid Pumping Depending on the Cilium Beating Pattern.** Our goal is to examine how these collective emergent 
waves break time-reversal symmetry and pump fluid. We first 
analyze how the active force \( F(\theta) \) affects emergent coordination.
The polarity introduced by the first force harmonic in the direction from minimum to maximum forcing sets a global direction for symmetry breaking and fluid pumping. Indeed, for $F(\theta) = F_0(1 + \alpha_1 \cos \theta + \beta_1 \sin \theta)$, the emergent waves are spatially homogeneous and propagate in the same direction dictated by $\tan(\beta_1/\alpha_1)$ for any choice of parameters $\alpha_1$ and $\beta_1$ and height $h$ (Figs. 1 D and 2 A, I and SI Appendix, Fig. S10). For $F(\theta) = F_0(1 + \alpha_2 \cos 2\theta + \beta_2 \sin 2\theta)$, the emergent waves propagate in two opposite directions (Fig. 2 A, IV), irrespective of parameter choice. That is, while both force harmonics break time-reversal symmetry locally, with more noticeable coordination in the second force harmonic, the latter does not lead to a global direction of emergent coordination and fluid pumping over the entire domain. This is because of the nematic symmetry of the second force harmonic $F(\theta + \pi) = F(\theta)$.

To test these observations, we introduce a family of forcing $F(\theta) = 1 + 0.5(1 - e) \cos(\theta) + e \cos(2\theta)$ that linearly morphs from first to second harmonic as $e$ varies from zero to one. In Fig. 2 A–D, we show snapshots of the emergent coordination and fluid streamlines and speed for representative values of $e$. We find that the first harmonic is essential in setting a global direction of phase coordination and fluid motion, whereas the second harmonic provides a longer range of coordination. Importantly, while the wave coordination varies roughly monotonically as $e$ increases (Fig. 2 C and E), with the strongest phase coordination at $e = 1$, the average flux $q$ depends nonlinearly on $e$ and is optimal for $e$ close but not equal to 1 (Fig. 2 A, III, B, and E). At these optimal conditions, the second harmonic drives the instability, ensuring a strong metachronal coordination, while the first harmonic provides a weak perturbation that sets the

Fig. 2. Mixing first and second force harmonic produces maximum pumping. (A) Snapshot of a 151 × 151 ciliary lattice in steady state for (A, I) the first force harmonic ($e = 0$), (A, II) mixed forcing with larger first harmonics ($e = 0.2$), (A, III) mixed with larger second harmonics ($e = 0.8$), and (A, IV) the pure second force harmonic ($e = 1$). The tinted portion in A, IV indicates the metachronal wave traveling in the opposite direction of the untinted portion. Here, white arrows indicate the dominant wave direction, and the red arrow shows the opposing direction for A, IV. In A, Lower, we show streamlines of the period-averaged induced velocity field of the full system. We observe that while A, III produces the cleanest directed flow at the highest speed, A, IV shows fluid being pumped in opposing directions. (B) Cumulative flux magnitude $\int q \, dt$ over 200 units of time shows that while A, II took a longer time to reach a high flux, it dominates over other presented cases eventually. (C) Wave coordination as measured by the eccentricity of the Kuramoto ellipse. The higher the weight of the second force harmonic, the more likely that the wave becomes highly coherent. (D) The Kuramoto ellipse and net flux vector plotted in polar coordinates. Note that both I and III in D have the flux point roughly 30° clockwise of the respective minor axis, suggesting that a similar type of phase coordination is reached in these two cases. (E) As the weight for the second force harmonic increases, both the average flux and wave coordination first register a dip. This can be explained by the fact that the percentage of energy put into the fluid (or rate of dissipation energy) is smaller when $e \neq 0, 1$ (SI Appendix). When $e$ reaches about 3/4, we see that the average flux is maximized. As $e$ continue to rise, the coordination improves, while flux drops quickly due to the appearance of opposing traveling waves as shown in IV.
global wave direction. At \( \epsilon = 1 \), the first force harmonic makes zero contribution, and metachronal waves propagate in opposite directions, resulting in minimal net flux (Fig. 2 A, IV).

Taken together, our results demonstrate that the first force harmonic, which emulates the effect of asymmetric beating, is essential for ensuring global coherence and spatial homogeneity of the wave pattern, whereas the second force harmonic, which represents the ellipticity of the cilia beating pattern, is more effective in driving the instability and achieving more coherent metachronal coordination.

**Continuum Model in the Limit of the Infinite Number of Cilia.** Our goal is to derive stability conditions and predict growth rates of the onset of instabilities that we observe in the isotropic and synchronized states and that lead to emergent coordination. A continuum theory was presented in ref. 28, providing important tools for assessing the linear stability of perfect metachronal waves. However, the theory in ref. 28 lacks two important features: a method for evaluating a continuum fluid velocity field in the 3D half-space and a mechanism for accounting for the onset of instabilities from noncoordinated states, such as the isotropic state. Here, we develop a continuum theory in the limit of dense cilia (\( N \to \infty \)), including an analytical derivation (following refs. 42 and 43) of the Stokes kernel associated with the ciliary layer that is applicable to coordinated and noncoordinated initial states.

We start by modeling the ciliary carpet as a force density layer (Fig. 1A),

\[
\mathbf{f}(\mathbf{x}, t) = \lim_{N \to \infty} \sum_{j} (\mathbf{F}_j + \mathbf{N}_j) \delta(\mathbf{x} - \mathbf{r}_j),
\]

that introduces a jump in the fluid stress field \( \mathbf{\sigma} = -p \mathbf{I} + \eta(\nabla \mathbf{u} + (\nabla \mathbf{u})^T) \), where \( p \) is the pressure field and \( \mathbf{I} \) is the identity matrix; namely, \( [\mathbf{\sigma} \cdot \mathbf{e}_z]_{z=h} = f(\mathbf{x}) \). We map the planar components of \( p \) and \( \mathbf{u} \) to a 2D Fourier space \( \mathbf{k} = (k_x, k_y) \) and \( k = ||\mathbf{k}|| \). Note that the doubly periodic domain \( L \times L \) in the discrete model affords wave numbers \( k_x, k_y \) in the range \( [2\pi/L, \pi/d] \). We solve the 3D Stokes equation analytically in the fluid regions above and below the force density layer, at which we match the jump and boundary conditions (SI Appendix, section S1). We arrive at \( \mathbf{\tilde{v}} = \mathbf{K} \cdot \mathbf{f} \), where \( \mathbf{f} \) and \( \mathbf{v} \) are the Fourier transform of \( \mathbf{f} \) and \( \mathbf{v} \) and \( \mathbf{K} \) encodes the Stokes kernel associated with the force density layer,

\[
\mathbf{K} = -\frac{e^{-2kh}}{4\eta k^2} \left[ 2k^2(1 - e^{2kh})\mathbf{I} \right.
\]

\[
+ \left( -1 + e^{2kh} + 2hk(-1 + hk) \right) \mathbf{k} \otimes \mathbf{k} \right],
\]

and \( \lim_{k \to 0} \mathbf{K} = h/\eta \mathbf{I} \) (SI Appendix, Figs. S3–S5) show a direct comparison between the flow fields in the continuum and discrete models. This analytically tractable expression for the cilia-generated flow field \( \mathbf{v} \) together with Eq. 5 and the force that cilia exert on the fluid Eq. 4 form the basis for deriving a continuum theory for phase coordination in ciliated surfaces in terms of the moments fields \( Y_n(\mathbf{x}, t) \).

To derive evolution equations for \( Y_n(\mathbf{x}, t) \), we differentiate Eq. 3 with respect to time, substitute Eq. 2 in the resulting equation, and take the limit of large \( N \). We arrive at the continuum model (SI Appendix, section S4)

\[
\dot{Y}_n = i n \Omega_\alpha \left[ Y_n(\mathbf{x}) + \sum_m (\bar{\mathbf{a}}_m \cdot \mathbf{v} + \bar{\mathbf{B}}_m : \nabla \mathbf{v}) \cdot Y_{n+m} \right],
\]

where the vectors \( \bar{\mathbf{a}}_m \) and second-rank tensors \( \bar{\mathbf{B}}_m \) are coefficients of the Fourier series expansions of \( t/B \) and \( t \otimes \mathbf{n}/t \) with respect to \( \phi \), respectively. The cilia-generated force density \( \mathbf{f} \) in Eq. 4 can be expressed in terms of \( Y_n \) as

\[
f = \rho_c \zeta \sum_m \left[ (\mathbf{c}_m - \bar{\mathbf{D}}_m \cdot \mathbf{v}(\mathbf{x})) Y_m \right.
\]

\[
\left. + b \nabla \cdot \left( (\mathbf{E}_m - \bar{\mathbf{G}}_m \cdot \mathbf{v}(\mathbf{x})) Y_m \right) \right],
\]

where \( \mathbf{c}_m, \bar{\mathbf{D}}_m, \bar{\mathbf{E}}_m, \) and \( \bar{\mathbf{G}}_m \) are the coefficients of the Fourier series expansions of \( B/t \), \( \mathbf{n} \otimes \mathbf{n} \), \( B/t \otimes \mathbf{t} \), and \( \mathbf{n} \otimes \mathbf{n} \otimes \mathbf{n} \), respectively. Explicit expressions are given in SI Appendix.

Eq. 6, together with Eqs. 5 and 7, form a set of linear partial differential equations that describes the dynamical evolution of the moment fields \( Y_n(\mathbf{x}, t) \). Since all \( Y_n \) are coupled, a closure assumption is needed to arrive at a tractable set of equations. When using the closure assumption appropriate for locally coordinated phase waves, we recover the theory presented in ref. 28 as a special case. Here, we are interested in the stability of the isotropic and synchronized states and the emergence of coordination; we will thus make closure assumptions appropriate for these cases.

**Stability of Isotropic States.** The isotropic state is a periodic steady state of Eq. 6, with \( Y_0(\mathbf{x}) = 1 \), while \( Y_n, f, \) and \( \mathbf{v} \) are all identically zero. We consider small perturbations \( \delta Y_n \) about the isotropic state and linearize Eq. 6 accordingly (SI Appendix). The resulting linear equation describing the time evolution of \( \delta Y_n(\mathbf{x}, t) \) is best expressed in Fourier space in terms of the associated Fourier transform \( \delta \hat{Y}_n(\mathbf{k}, t) \). We arrive at the eigenvalue problem in Fourier space \( \delta \hat{Y}_n = \sum_{m=-\infty}^{\infty} \hat{L}_{nm} \delta \hat{Y}_m \), where \( \hat{L}_{nm} \) depends on \( \mathbf{K} \) (SI Appendix).

Near the isotropic state, \( \hat{Y}_n \) decays fast with \( n \). We thus close the linearized system of equations by truncation. We solve the truncated equations numerically over the entire Fourier space. In Fig. 3A, we report the growth rate \( \gamma(\mathbf{k}) \) (maximal real part of the eigenvalues) as a color map over all wave numbers \( \mathbf{k} \) for the first and second force harmonics in Fig. 1. The isotropic state is unstable for almost all wave numbers, with stronger instability exhibited by the second force harmonic. We superimpose data from a sample discrete cilia simulation (black dots) of Eq. 1; the dots represent the wave numbers at which \( Y_1(\mathbf{k}, t) \) is maximal (exceeding a given threshold). Note the agreement between the simulation results and the maximal growth rate in the continuum theory.

**Linear Instability Governs Emergent Coordination.** To further challenge the theory, starting from the initial state used in the sample simulation, we calculate \( Y_1(\mathbf{k}, 0) \) and integrate forward in time \( Y_1(\mathbf{k}, t) = Y_1(\mathbf{k}, 0) \exp(\gamma(\mathbf{k}) t) \) according to the linear growth rate from the continuum model. We transform back to physical space and compare Real[\( Y_1(\mathbf{x}, t) \)] with that obtained from the sample simulation (Fig. 3B), and again, we find remarkable agreement, indicating that the wave patterns are governed by the fastest-growing linear mode. Lastly, we calculate the Kuramoto ellipse based on Monte Carlo computations using the continuum model growth rate with 200 initial isotropic states; the average is shown in dark gray in Fig. 3C, and the range is in lighter gray, while results from the sample simulation are superimposed in black dots. The time evolution of the ellipses’ eccentricity and angle is shown in Fig. 3D using the same color convention. The ellipse eccentricity correlates with the instability growth rate, and its angle in physical space is orthogonal to the direction of the
Fig. 3. Wave instability from the isotropic state. (A) Growth rate $\gamma(k)$ of the instability predicted by the continuum theory (color map) and discrete simulations (black dots) in the Fourier space. (B) Starting from a random Kuramoto field $Y_1(x)$, map to Fourier space $\hat{Y}_1(k)$, and use $\gamma(k)$ from A to step forward in time. Snapshots of $\text{Re}[\hat{Y}_1(x,t)]$ show the emergence of traveling waves similar to those obtained from direct numerical simulations of Eq. 1. (C) The Kuramoto ellipse introduced in Fig. 1E obtained from the sample simulation in B (black dots) and theory corresponding to 200 realizations (gray), with the average shown in dark gray. (D) Time evolution of wave coordination and the angle of the Kuramoto ellipse; sample simulation (black lines) and theory (gray) are shown.

maximum growth rate in Fourier space. We conclude that the fastest growing wave numbers in the linear continuum theory are predictive of the long-term traveling wave patterns in the nonlinear discrete model.

**Stability of Synchronized States.** For completeness, we carry out a rigorous stability analysis of the synchronized state. This state could be analyzed using the techniques provided in ref. 28, but here, we provide an alternative approach consistent with our continuum theory (SI Appendix, section S5). Synchronous states are unstable to general perturbations, with stronger instability in the case of the second force harmonic, consistent with the isotropic state. The growth rates $\mu(k)$ are shown in Fig. 4A over the entire wave space and in Fig. 4B for select cross-sections.

Fig. 4. Wave instability from the synchronized state. The Lyapunov exponent $\mu(k)$ obtained from theory and simulations (A) over the entire wave space (color map) and (B) at cross-sections $k_yd = -\pi/4$ and $k_y = -\pi/2$, with theory shown as solid lines and simulations shown as dashed lines.
Effect of Tissue Heterogeneity on Cilia Coordination and Fluid Pumping. Lastly, we examine the effect of heterogeneity in the geometric distribution of cilia (7, 37) on the emergent coordination and fluid pumping. We study tissue heterogeneity in the context of the discrete model Eq. 1. Inspired by the empirical observations in ref. 7, we introduce four geometric parameters that allow us to represent and manipulate the distribution of ciliary patches and individual cilia (SI Appendix, section S6). First, we define a cilia coverage ratio $C$ that determines the area fraction that is covered by active cilia (Fig. 5A). We define the distribution of patch wave numbers $k_{\text{patch}}$ in the $x$ and $y$ directions (Fig. 5B). We randomly perturb the location of each entire patch in the $x$ and $y$ directions (Fig. 5C), and we introduce Gaussian noise in the location of individual cilia (Fig. 5D).

A systematic investigation of how wave coordination and fluid pumping vary with these geometric heterogeneity parameters is presented in Fig. 5A–D, and flow streamlines and speed for select parameter values are shown in Fig. 5 E–H. In all cases, rotor height $h$ and distance between each rotor $d$ were kept constant, and rotors were driven using the first force harmonic $F_1 = 1 + 0.5 \cos \theta$. Fig. 5A shows that wave coordination and net flux scale roughly linearly with coverage ratio $C$, but renormalizing by the number of active rotors reveals that the net flux per rotor is almost independent of $C$, even for area coverage as low as 20%. At $C = 25\%$, with cilia distributed in four ciliary patches per direction, the flux per rotor is more than 80% of that of the fully covered carpet (Fig. 5E). This suggests that fluid pumping may be more efficient under partial coverage of ciliated tissues as long as cilia are organized into relatively large and coherent patches. For a given value of coverage ratio, both net flux and flux per rotor are affected little by distributing the cilia into regularly and irregularly spaced patches (Fig. 5B and C). Wave coordination is slightly reduced with increased number of patches (Fig. 5B and F) but enhanced with increased irregularity in patch spacing (Fig. 5C and G). A closer look at the streamlines shows eddies at the boundaries of small ciliary patches (Fig. 5E and H). These eddies hinder coherent fluid pumping but are minimized when cilia are distributed in larger ciliary patches (Fig. 5F and G), even when the coverage ratio is significantly compromised (Fig. 5G). Importantly, while irregularity in the spacing of ciliary patches seems beneficial, a noisy distribution of individual cilia significantly hinders fluid pumping (Fig. 5D and H). These findings suggest that wave coordination and fluid pumping remain robust when cilia are distributed into separate ciliated patches that consist of large clusters of regularly spaced cilia within each patch, even when the patches themselves are not regular. This is exactly the design adopted in healthy ciliated tissues: ciliated cells cluster in irregular patches of regularly spaced individual cilia, separated by other cell types (7, 44).

Conclusions

We analyzed phase coordination and fluid pumping in large-scale ciliary carpets in the context of a discrete rotor model and a continuum theory. Existing models have been limited to a few hundred cilia (14, 15), which is several orders of magnitude smaller than the typical number of cilia in a ciliated epithelium. Our model demonstrates that collective effects can emerge from microscale heterogeneities and that wave coordination is plastic and may be more efficient under partial coverage of ciliated tissues.
smaller compared with the number of cilia found, for instance, in mammalian airways (7). Our models serve as a first step toward establishing maps from single-cilium kinematics to tissue-level phase coordination and fluid pumping in large-scale ciliary systems.

To isolate the effect of hydrodynamic coupling on the emergence of collective ciliary states that pump fluids, we accounted for the single-cilium beat kinematics using a rotor model whose phase oscillations do not pump fluid. This idealization captures important features of actual cilia beat patterns; the first harmonic forcing that drives phase oscillations introduces force polarity and captures asymmetry in ciliary beating, while the second force harmonic is “nematic” and captures the nonplanar ellipticity of ciliary beating. This interpretation is important because it has practical implications on the pumping function of the ciliated tissue.

We showed in both numerics and theory that in ciliary carpets, spatially homogeneous isotropic and synchronized states are unstable. By construction, these states do not pump fluids. However, the instabilities that arise due to hydrodynamic interactions lead to the emergence of wave patterns that can break time-reversal symmetry and pump flows. Our theory in the limit of the infinite number of cilia predicts quantitatively the long-term traveling wave patterns observed in the nonlinear numerical simulations, indicating that linear instabilities dominate the emergent waves.

The emergent traveling wave patterns depend on the properties of the single cilium but are robust to large perturbations in initial conditions. That is, in ciliary carpets, a robust traveling wave pattern exists as a stable global attractor, as reported in ref. 29. The specific properties of this attracting state depend on the characteristics of the single cilium; ciliary beat asymmetry (first force harmonic) produces waves that propagate in the same direction and pump fluids, while ciliary beat ellipticity (second force harmonic) produces waves that propagate in opposite directions and do not pump fluid.

Compared with the theory presented in ref. 28, our work provides a rigorous formulation of the fluid velocity field induced by a continuum ciliary force layer and a mechanism for accounting for the onset of instabilities from noncoordinated isotropic states. Importantly, the instability we detected at the first force harmonic is not reported in ref. 28. This instability, although subsiding the instability at the second force harmonic, governs the direction of fluid pumping and is, therefore, essential for tissue-level function. Our findings emphasize the importance of considering the effect of single-cilium beat kinematics on the coupling between wave coordination and fluid pumping in ciliated tissues.

We introduced tissue-level heterogeneity based on empirical observations of mammalian airway tissue (7). We found that the emergent wave coordination and fluid pumping are robust to tissue heterogeneity. These findings support the notion that the geometric design of ciliated tissues, where ciliated cells cluster in irregular patches of regularly spaced individual cilia separated by other cell types (7, 44), is robust in terms of wave coordination and fluid pumping. However, designs not seen in healthy tissues where individual cilia are irregularly distributed are inferior in both.

Taken together, the dependence of emergent states on the properties of the single cilium and the robustness of these states to large perturbations and tissue heterogeneity suggest that the functional specificity of the ciliated tissue is interlaced with the beating kinematics of the single cilium and the organization of the ciliated tissue. They corroborate existing assays that examine the beat kinematics of individual cilia to distinguish between diseased and healthy states (45–47) and suggest to amend these assays with measures of tissue-level organization.

We found that when the specific state is robust to small changes in initial conditions, it will emerge into the asymptotic state. However, the specific properties of this attracting state depend on the initial conditions. That is, in ciliary carpets, a robust traveling wave pattern exists as a stable global attractor, as reported in ref. 29. The specific properties of this attracting state depend on the characteristics of the single cilium; ciliary beat symmetry (first force harmonic) produces waves that propagate in the same direction and pump fluids, while ciliary beat ellipticity (second force harmonic) produces waves that propagate in opposite directions and do not pump fluid.

Compared with the theory presented in ref. 28, our work provides a rigorous formulation of the fluid velocity field induced by a continuum ciliary force layer and a mechanism for accounting for the onset of instabilities from noncoordinated isotropic states. Importantly, the instability we detected at the first force harmonic is not reported in ref. 28. This instability, although subsiding the instability at the second force harmonic, governs the direction of fluid pumping and is, therefore, essential for tissue-level function. Our findings emphasize the importance of considering the effect of single-cilium beat kinematics on the coupling between wave coordination and fluid pumping in ciliated tissues.

We introduced tissue-level heterogeneity based on empirical observations of mammalian airway tissue (7). We found that the emergent wave coordination and fluid pumping are robust to tissue heterogeneity. These findings support the notion that the geometric design of ciliated tissues, where ciliated cells cluster in irregular patches of regularly spaced individual cilia separated by other cell types (7, 44), is robust in terms of wave coordination and fluid pumping. However, designs not seen in healthy tissues where individual cilia are irregularly distributed are inferior in both.

Taken together, the dependence of emergent states on the properties of the single cilium and the robustness of these states to large perturbations and tissue heterogeneity suggest that the functional specificity of the ciliated tissue is interlaced with the beating kinematics of the single cilium and the organization of the ciliated tissue. They corroborate existing assays that examine the beat kinematics of individual cilia to distinguish between diseased and healthy states (45–47) and suggest to amend these assays with measures of tissue-level organization.

We found that when the specific state is robust to small changes in initial conditions, it will emerge into the asymptotic state. However, the specific properties of this attracting state depend on the initial conditions. That is, in ciliary carpets, a robust traveling wave pattern exists as a stable global attractor, as reported in ref. 29. The specific properties of this attracting state depend on the characteristics of the single cilium; ciliary beat symmetry (first force harmonic) produces waves that propagate in the same direction and pump fluids, while ciliary beat ellipticity (second force harmonic) produces waves that propagate in opposite directions and do not pump fluid.

Compared with the theory presented in ref. 28, our work provides a rigorous formulation of the fluid velocity field induced by a continuum ciliary force layer and a mechanism for accounting for the onset of instabilities from noncoordinated isotropic states. Importantly, the instability we detected at the first force harmonic is not reported in ref. 28. This instability, although subsiding the instability at the second force harmonic, governs the direction of fluid pumping and is, therefore, essential for tissue-level function. Our findings emphasize the importance of considering the effect of single-cilium beat kinematics on the coupling between wave coordination and fluid pumping in ciliated tissues.

We introduced tissue-level heterogeneity based on empirical observations of mammalian airway tissue (7). We found that the emergent wave coordination and fluid pumping are robust to tissue heterogeneity. These findings support the notion that the geometric design of ciliated tissues, where ciliated cells cluster in irregular patches of regularly spaced individual cilia separated by other cell types (7, 44), is robust in terms of wave coordination and fluid pumping. However, designs not seen in healthy tissues where individual cilia are irregularly distributed are inferior in both.

Taken together, the dependence of emergent states on the properties of the single cilium and the robustness of these states to large perturbations and tissue heterogeneity suggest that the functional specificity of the ciliated tissue is interlaced with the beating kinematics of the single cilium and the organization of the ciliated tissue. They corroborate existing assays that examine the beat kinematics of individual cilia to distinguish between diseased and healthy states (45–47) and suggest to amend these assays with measures of tissue-level organization.

We found that when the specific state is robust to small changes in initial conditions, it will emerge into the asymptotic state. However, the specific properties of this attracting state depend on the initial conditions. That is, in ciliary carpets, a robust traveling wave pattern exists as a stable global attractor, as reported in ref. 29. The specific properties of this attracting state depend on the characteristics of the single cilium; ciliary beat symmetry (first force harmonic) produces waves that propagate in the same direction and pump fluids, while ciliary beat ellipticity (second force harmonic) produces waves that propagate in opposite directions and do not pump fluid.
