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ABSTRACT

Cilia are widely employed by living systems to manipulate fluid flow in various functions, such as feeding, pumping, and locomotion. Mimicking the intricate ciliary asymmetry in combination with collective metachronal beating may find wide application in fluid transport and mixing in microfluidic systems. Here, we numerically analyze the metachronal beating of pneumatic artificial cilia. We specifically address three aspects of ciliary motion: (i) pumping in the backflow region, (ii) mixing in the cilia region, and (iii) the transport—mixing transition region. Our results show that antiplectic metachrony leads to the highest mixing efficiency and transport rate in two distinct regions, i.e., below and above the ciliary surface, respectively. We find that the ciliary motion strongly enhances the diffusivity when advection is dominant at high Péclet numbers, with a factor 3 for symplectic metachrony and a factor 4 for antiplectic metachrony and synchronous beating. In addition, we find an increase with a factor 1.5 for antiplectic metachrony and a decrease with a factor 2.5 for symplectic metachrony compared with synchronous beating for fluid pumping. To investigate the higher transport rate compared to symplectic metachrony, we develop a simple two-cilia model and demonstrate that the shielding of flow between neighboring cilia is the main reason for the higher antiplectic transport rate.

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I. INTRODUCTION

Biological cilia are slender, hair-like structures several tens of micrometer long, which play an important role in many biological processes. The cilia are powered by molecular motors and can efficiently propel fluids at small Reynolds numbers, typically around 0.001.^{1,2} At small Reynolds numbers, viscous forces dominate over inertial forces rendering turbulent effects in the fluid to be negligible. As a result, effectively manipulating fluids at these scales comes with a challenge, as fluid transport and mixing are only possible when the motion of the cilia is nonreciprocal.^{3–5} This nonreciprocity of natural cilia operates at two different scales: (i) at the scale of the individual cilia, with the cilia beating in an asymmetric manner by sweeping a non-zero area of the cilia tips and (ii) at the scale of multiple cilia, with the cilia beating out-of-phase resulting in propagating (metachronal) waves created by the collective motion of the cilia.

This effective beating mechanism of natural cilia has been mimicked in artificial ciliary systems for fluid manipulation in microfluidic

“lab-on-a-chip” devices.^{6–11} There are various ways of actuating artificial cilia, using, for instance, electric fields, magnetic fields, sound, or light.^{10,12} Cilia are successfully applied in microfluidic devices supported by a broad range of studies on fluid propulsion by artificial cilia using theoretical/numerical^{13–29} and experimental^{6–9,30–32} methods. The major topics include fluid transport and mixing,^{6,27,33–46} particle/droplet manipulation,^{24,47–50} and artificial cilia as sensors and robots.^{51–57} Using numerical simulations, one can study the motion and fluid propulsion of systems from one single cilium to large ciliary arrays. Many experimental studies focus on mimicking the asymmetric motion of the individual cilia, consisting of an effective and a recovery stroke,^{3,4,7,33,58} and a few on creating metachronal waves of cilia arrays.^{59,60} Recently, using pneumatic cilia^{8,9} or magnetic cilia,^{51,61} experimental systems were developed in which the asymmetric motion of the individual cilia and the metachronal waves can be independently controlled.

It has been observed that antiplectic metachronal waves are more efficient to transport fluids than symplectic metachronal

waves.^{13,14,19,20,23,25,26,30,31} Different explanations of this phenomenon have been proposed,^{5,9,23,26,61} but no consensus has been reached on the underlying mechanism that can directly quantify the difference in transport efficiency between antiplectic and symplectic metachrony. Khaderi *et al.*⁵ and Milana *et al.*⁹ analyzed the shielding effect though the neighboring cilia and fluid flow, and Osterman *et al.*²³ studied the effect of metachrony by referring to the beating efficiency. Chateau *et al.*²⁶ explored vortex formation to explain the phenomenon, while Dong *et al.*⁶¹ used the instantaneous inter-cilia-tip spacing to predict the flow volume transported.

Next to fluid transport, fluid mixing generated by cilia is also an active field of research given the notorious difficulty in mixing fluids at low Reynolds numbers due to the absence of turbulence at these small length scales. To improve the mixing of cilia, several characteristic features of cilia have been exploited,^{1,62,63} with metachronal waves being most popular.^{13,25,64} Interestingly, as for fluid propulsion, antiplectic metachronal waves are most efficient for fluid mixing, but also here, the underlying mechanism is not well understood.

Biological and artificial cilia often appear in confined spaces. In the human body, cilia appear in narrow cavities and tubes,⁶⁵ including the fallopian tube⁶⁶ and the walls of the human brain ventricles.⁶⁷ Confinement is also important for fluid transport and mixing in microfluidic “lab-on-a-chip” devices.⁶⁸ A specific example is the need for mixing in confined micro-reaction chambers of point-of-care devices in order to enhance the speed and reproducibility of diagnostic testing;⁶⁹ artificial cilia can potentially provide this function since they operate at the right length scale and they offer a fully integrated solution.⁷⁰ However, fluid transport and mixing by cilia in confined spaces have not attracted much attention. Recently, we analyzed the fluid transport in a confined channel by pneumatic artificial cilia submerged in a viscous fluid and actuated by trapezoidal pressure waves using a combination of experiments and numerical simulations.⁹ In the current paper, we extend this work by numerical exploring (i) the physical mechanisms responsible for the antiplectic/symplectic asymmetry and (ii) the effect of pneumatic ciliary metachrony on mixing.

This paper is organized as follows: in Sec. II, we describe our computational model based on the boundary element method that was developed to describe fluid flow generated by pneumatic artificial cilia in a closed fluid compartment. In Sec. III, we present the simulation results showing a distinct spatial localization of fluid propulsion and mixing, separated by a transition region. We highlight the physical mechanisms that describe fluid transport and mixing in these regions and we draw conclusions in Sec. IV.

II. MODEL

We consider a regular array of 6 cilia whose bases are placed on the bottom of a three-dimensional rectangular box (length B , width W , and height H) (Fig. 1). The spacing between the cilia is a in the x -direction and the cilium length is L . We assume the cilia to beat in the x -direction within the x - z plane at $y = 0$, with an angular frequency ω and period $T = 2\pi/\omega$, and the metachronal wave is propagating in the x -direction with wavelength λ . In order to study the metachronal wave, each cilium goes through exactly the same cyclic motion but with a phase difference between neighboring cilia, defined as $\Delta\phi = 2\pi a/\lambda$. The cilia surfaces are meshed by using triangular

FIG. 1. The computational box containing six cilia showing the instantaneously fluid velocity for synchronized beating $\Delta\phi = 0$. The colored plane is the measurement plane of the fluid flow. The light gray planes represent the box.

elements to describe the internal fluid boundary according to the cilia shape used in the experiments (see Appendix A, Fig. 10). We kinematically prescribe the centerline motion of the cilia $\mathbf{r}^c(s, t)$, where s is the arc length along the cilium's centerline, starting at its attachment site at the substrate ($0 < s < L$) and t is time ($0 < t < T$). In order to describe the two-dimensional motion of the cilium (see Appendix A, Fig. 11), a Fourier series expansion in s and a Taylor series expansion in t are used based on the experimental motion of the cilia.⁹ The motion of the cilia surface nodes $\mathbf{r}^i(t)$ is calculated from the cilia centerline motion $\mathbf{r}^c(s, t)$. The cilia length L is 14 mm and the beating frequency $f = \omega/2\pi$ is set to 0.25 Hz, the density ρ and viscosity μ of glycerol are, respectively, $1.260 \text{ g} \cdot \text{cm}^{-3}$ and $1.412 \text{ Pa} \cdot \text{s}$ at 20°C . The box dimensions are $B \times W \times H = 102 \times 20 \times 49 \text{ mm}$, which is the same as our experiment set up.⁹ Zero-velocity boundary conditions are taken on the walls of the 3D box, and the cilia are attached to the bottom (substrate) of the box.

The cilia are immersed in a viscous fluid. The Reynolds number is $\text{Re} = \rho L^2 f / \mu = 0.04$, comparable to microorganisms.⁷¹ As a result, inertia can be neglected so that we can model the over-damped fluid around the cilia using the Stokes equation

$$\mu \nabla^2 \mathbf{u} = -\nabla p, \quad (1)$$

with ∇p the pressure gradient, supplemented by the continuity equation describing incompressible flow, $\nabla \cdot \mathbf{u} = 0$. Gravitational and thermal effects are assumed negligible. The solution can be written in terms of Green's functions acting in a semi-infinite fluid, provided by Blake.⁷² In our treatment, the drag forces on the cilia surface are regarded as a distribution of point forces $\mathbf{f}(\mathbf{r}')$ (“Stokeslets”). The fluid velocity $\mathbf{u}^f$ at a point $\mathbf{r} = (x, y, z)$ due to these point forces $\mathbf{f}(\mathbf{r}')$ exerted on the fluid by the cilia can be written as

$$\mathbf{u}^f(\mathbf{r}) = \mathbf{G}(\mathbf{r} - \mathbf{r}', h(\mathbf{r}')) \mathbf{f}(\mathbf{r}'), \quad (2)$$

where $h(\mathbf{r}')$ is the distance of the point force from the bottom of the channel and $\mathbf{G}(\mathbf{r} - \mathbf{r}', h(\mathbf{r}'))$ is the Green's function for a point force $\mathbf{f}(\mathbf{r}')$ acting in a Stokes flow.⁷² These point forces are assumed to be distributed over the surface of the cilia as a traction $\mathbf{t}(\mathbf{r}')$, which is varying linearly over each triangular surface element,⁷³ collectively resulting in the fluid velocity,

$$\mathbf{u}^f(\mathbf{r}) = \sum_{j=1}^{\text{nelm}} \int_{S_j} \mathbf{G}(\mathbf{r} - \mathbf{r}^j, h(\mathbf{r}^j)) \mathbf{t}(\mathbf{r}^j) dS_j, \quad (3)$$

where nelm is the number of the surface elements. Since Eq. (3) holds at every point in the fluid, it also holds at every node i on the cilium surface $\mathbf{u}^c(\mathbf{r}^i)$ through

$$\mathbf{u}^c(\mathbf{r}^i) = \sum_{j=1}^{\text{nelm}} \int_{S_j} \mathbf{G}(\mathbf{r}^i - \mathbf{r}^j, h(\mathbf{r}^j)) \mathbf{t}(\mathbf{r}^j) dS_j. \quad (4)$$

When these equations are assembled in a matrix $\mathbf{G}$ giving $\mathbf{u}^c = \mathbf{G}\mathbf{t}$, the traction exerted by the cilia $\mathbf{t}$ on the fluid due to its velocity $\mathbf{u}^c$ can be calculated from $\mathbf{t} = \mathbf{G}^{-1}\mathbf{u}^c$. The cilia motion is obtained from the experiments and kinematically described as input to the model. Once the traction of the cilia surface is obtained, we can use Eq. (3) to calculate the fluid velocity in the complete domain inside the box, i.e., $\mathbf{u}^f = \mathbf{G}^f\mathbf{t}$. No-slip boundary conditions are taken on all channel walls, and the cilia are attached to the bottom wall, coinciding with the semi-infinite no-slip surface defined through Blake's Green function.⁷² The size of the surface elements and the time step have been chosen small enough so that converged results are obtained (see Appendix A). The model has been validated in terms of the backflow speed during one beating cycle, and the net backflow variation for different phase shifts through a careful comparison with experimental data.⁹ More details on the implementation can be found in Appendix A.

III. RESULTS AND DISCUSSION

The flow field generated by ciliary motion is studied for three distinct cases: (a) all the cilia beat in a synchronized way ($\Delta\varphi = 0$); (b) the ciliary beat generates an antiplectic metachronal wave with propagation direction that is opposite to the effective stroke ($\Delta\varphi$ is negative); and (c) the ciliary beat generates a symplectic metachronal wave that propagates in the same direction as the effective stroke ($\Delta\varphi$ is positive). To understand the fluid flow generated by the cilia in confinement, we investigate an antiplectic metachronal wave with a phase shift $\Delta\varphi = -\pi/3$, see Fig. 2. Movies S1 and S2 describing the flow pattern in the x - z and x - y plane are added in the supplementary material. In Fig. 2, the direction

of the fluid can be determined by the arrows on the streamlines. The colors represent the magnitude of the fluid velocity normalized by L/T . The metachronal wave direction is to the left which can be found by following cilium 6 in Fig. 2(a), cilium 5 in Fig. 2(b), and cilium 4 in Fig. 2(c) that are at the same configuration in the recovery stroke. From Fig. 2, we can also observe vortex formation due to the positive flow to the right generated by cilia 4, 5, and 6 and the negative flow generated by cilia 1, 2, and 3 in Fig. 2(e). Due to the closed nature of the fluid domain, vortices associated with a net back flow travel in the region above the cilia. It should be noted that this flow behavior is distinctly different from the unidirectional flow that dominates in infinitely large (circulatory) channels.⁵

To characterize the flow transport and mixing behavior, tracer particles are used with an initial distribution in patterns of different colors as shown in Fig. 3(a), leading to the profiles for synchronous, antiplectic, and symplectic beating shown in Figs. 3(b)–3(d), respectively. A backtrace imaging method is used, which is more uniform and saves computational time compared with the forward evolution of tracer particles (see the Appendix B 1), so that the density of the tracer particles is not a critical factor.⁷⁴ To gain quantitative insight into fluid transportation and mixing, the average horizontal displacement field of the tracer particles after 100 cycles was calculated for different heights z_0 , $\langle d_x(z_0) \rangle$,^{1,13,26} see Subsection 2 of Appendix B. This measure is shown in Fig. 4 together with its standard deviation. From Figs. 3(b) and 3(c) and Figs. 4(a) and 4(b), we observe that for synchronous and antiplectic beating, we can separate the flow profiles into three different regions: a backflow transport region I ($\langle d_x \rangle/L < 0$), a mixing-transport transition region II ($\langle d_x \rangle/L > 0$ $z > L$), and a mixing region III ($z < L$). A similar trend is also observed in experiments.⁶ For symplectic metachrony, however, Fig. 4(c) shows a profoundly distinct flow pattern in the transition region II, with a line of “vortex-like particle distributions” above the cilia tips [see Fig. 3(d)], which leads to an opposite transport direction in the backflow region. This is a direct consequence of the shielding effect, resulting in a strongly reduced flow in the effective stroke direction (to the right) and as a consequence also a drastically reduced backflow in region I. For visual comparison, we

FIG. 2. (a)–(f) Snapshots of cilia motion during a representative cycle for antiplectic metachrony with $\Delta\varphi = -\pi/3$ (effective stroke to the right and metachronal wave to the left). The direction of the fluid can be determined by the arrows on the streamlines. The colors represent the magnitude of the fluid velocity, normalized by L/T . Movies S1 and S2 describing the flow can be found in the supplementary material.

FIG. 3. Tracer particle diffusion Poincaré section for different levels of metachrony. Initial tracer particle distribution (a), particle distribution after 100 cycles for synchronized beating $\Delta\varphi = 0$ (b), antiplectic metachrony $\Delta\varphi = -\pi/6$ (c), and symplectic metachrony $\Delta\varphi = \pi/6$ (d). The gray box represents the closed rectangular channel.

use the same size of regions I, II, and III as for $\Delta\varphi = 0$ but to differentiate this region [Fig. 4(c)] from the mixing-transport region II of Figs. 4(a) and 4(b), we denote this special mixing-transport region as region II'.

A. Pumping above the cilia in region I

From Figs. 3(b)–3(d), we find there is a recirculation region along the channel's upper boundary due to the closed nature of the fluid domain. The backflow velocity in the x -direction, averaged over region

S ($x_1 < x < x_2$, $z_1 < z < z_2$) for $y = y_0$ (see Appendix B3) in the backflow region I, defined by

$$U_{\text{backflow}}(t) = \frac{1}{S} \int_{x_1}^{x_2} \int_{z_1}^{z_2} u(x, y_0, z, t) dx dz \quad (5)$$

is plotted in Fig. 5(a) as a function of time during one beating period. For synchronized beating, the first two peaks are due to the effective stroke, creating a negative flow in the backflow region, while the last two peaks are for the recovery stroke creating a positive flow in the

FIG. 4. Normalized average displacement $\langle d_x \rangle$ in the x -direction after 100 cycles as a function of normalized height z/L for: (a) synchronized beating $\Delta\varphi = 0$, (b) antiplectic waves $\Delta\varphi = -\pi/6$, and (c) symplectic waves $\Delta\varphi = \pi/6$.

FIG. 5. (a) U_{backflow} during one beating period (T) during synchronized beating $\Delta\varphi = 0$, antiplectic metachrony $\Delta\varphi = -\pi/6$, and symplectic metachrony $\Delta\varphi = \pi/6$. (b) Phase-averaged horizontal velocity $\bar{U}$ for phase shifts between $-\pi$ and π at fixed $\pi/6$ intervals for the case shown in Fig. 3. The closed colored circles correspond to the three cases shown in (a).

backflow region (see Fig. 11 for the correlation between the cilia conformation and the flow generated in the case of synchronous flow). In the symplectic and antiplectic cases, these four peaks are smoothed by the mixing effect of the out-of-phase beating of the cilia, forming just one positive peak and one negative peak. The location of the peaks in the beating cycle depends on the phase shift $\Delta\varphi$. To quantify the overall backflow produced by each kind of cilia beating motion, the average back flow velocity $\bar{U}$ is calculated during one beating period,

$$\bar{U} = \frac{1}{T} \int_0^T U_{\text{backflow}}(t) dt, \quad (6)$$

for different phase shifts between $-\pi$ and π at fixed $\pi/6$ intervals [see Fig. 5(b)]. Clearly, compared to the flow generated by synchronous beating, the velocities in region I are substantially increased by a factor 1.5 for antiplectic flow ($\Delta\varphi_x < 0$) and decreased by a factor 2.5 for symplectic flow ($\Delta\varphi_x > 0$). The velocities reach a maximal value at around $-\pi/3$, showing the existence of an optimal metachronal wave that maximizes pumping, which is in correspondence to experimental results.⁹

Previously we found for magnetic artificial cilia that the higher efficiency in the case of antiplectic metachrony compared to symplectic metachrony is caused by the shielding effect of neighboring cilia.⁵ To investigate this in detail for the pneumatic cilia, we analyzed the difference in shielding effect of cilia that are located in the down-stream direction and that are in the up-stream direction. To do so, we investigated a simple interaction model of two-cilia with a given phase shift and computed the flow generated in the case of symplectic and antiplectic metachrony. The model is able to pick up the asymmetry in flow generation as observed in Fig. 5(b) with an increase in antiplectic flow compared to symplectic, pointing toward a direct obstruction of upstream cilia to be the primary mechanism of the widely observed metachronal flow asymmetry (see Fig. 16 in Appendix C).

B. Transport and mixing transition region II and II'

In previous simulation¹ and experimental⁶ studies, only the backflow transport region I above the cilia tips and the mixing region III below the cilia tips have been studied. In the current paper, a striking feature of the tracer particle analysis in Figs. 3 and 4 is the different nature of the mixing-transport transition regions II (antiplectic) and II' (symplectic). To gain further insight into the transition regions, displacement field streamlines are plotted based on the displacement of tracer particles in one beating period (Fig. 6). In Figs. 6(a)–6(c), we can clearly identify the backflow in transport region I along the chamber's upper boundary. In region II for the synchronized and antiplectic metachrony, the fluid is transported to the right in the direction of the effective stroke [see Figs. 3(b) and 3(c), 4(a) and 4(b), and 6(d) and 6(e)]. Note that in our previous work, we studied transport in an infinitely long channel instead of a confined box, making the forward flow the only component above the cilia, with backflow being absent.⁵ The transition between region I and II is accompanied by a big anticlockwise vortex (V1 and V3) right at the boundary between regions I and II as shown in Figs. 6(d) and 6(e). This is very different from the line of small anticlockwise vortices (V5) for the symplectic case in Fig. 6(f) right at the tip of the cilia at the boundary of region II' and III [see also Figs. 3(d) and 4(c)]. To investigate this further, we plot tracer particle trajectories during one beating period in Figs. 6(g)–6(i). The blue dot is the particle at the beginning of the path line. In region II, we find that the particles move in the direction of the effective stroke (to the right) after a period in Figs. 6(g) and 6(h), with the particles moving a larger distance at antiplectic metachrony than at synchronized beating, conform Figs. 4(a) and 4(b). In contrast, the particles move toward the left after one beating period in the case of symplectic metachrony in region II' due to the shielding effect as discussed in Sec. III A. In the effective stroke, the shielding effect in the case of antiplectic metachrony is low, with the neighboring cilium in the stream-wise direction in a bent, less obstructing conformation [see the inset with the ciliary configurations in the effective stroke

FIG. 6. Displacement field streamlines for: (a) synchronized beating $\Delta\phi = 0$, (b) an antiplectic wave $\Delta\phi = -\pi/6$, and (c) a symplectic wave $\Delta\phi = \pi/6$. Region II and II' are shown in gray. (d)–(f) Magnified region of (a)–(c). (g)–(i) Tracer particle trajectories at different height from $0.8L$ to $1.6L$. The subfigure shows the cilia configurations at the effective stroke (gray) and the recovery stroke (black) of two neighboring cilia near the tracer particles.

marked “E” in Fig. 6(h)]. Hence, the particle moves a large distance in the effective stroke direction. In contrast, for symplectic metachrony, the neighboring cilium in the stream-wise direction is in a straight, obstructing conformation, resulting in a smaller displacement during the effective stroke. During the recovery stroke, the situation is opposite. Then, for antiplectic metachrony the recovery stroke is obstructed, while for symplectic metachrony the recovery stroke is less obstructed, effectively leading to a large net flow for the former and a zero net flow for the latter. We conclude that the different flow behavior in the transition regions is a direct consequence of the shielding effect that is also at the base of the different flow speeds in the backflow regions.

C. Mixing below the cilia tip in region III

In microfluidic devices, mixing is always limited by relatively low diffusion rates.^{63,75} For example, DNA experiments are often performed using a diffusion-only mixing strategy that needs to take more than 18 h to equilibrate, which not only reduces the throughput of the method but also can introduce a systemic bias in the results.^{76–78}

Many researchers, inspired by nature’s solutions to create effective manipulation mechanisms of laminar flow, have been focused on improving mixing by cilia-mimetic devices.^{1,6,62,63,79} To characterize our cilia-driven flow mixing efficiency, the relative role of advection and diffusion can be estimated by the Péclet number,

$$Pe = \frac{ul}{D_0}, \quad (7)$$

where in the case of ciliary mixing, u can be taken to be the tracer particle average velocity in a cilium beating cycle, l the diameter of the beating cilia tip trajectory, and D_0 is the mass diffusivity,⁶ such that advection dominates for $Pe > 1$ and diffusion dominates for $Pe < 1$. Diffusion requires a timescale $t_m = l^2/(D_0)$ to homogenize a fluid over a length l . The Péclet number can be understood as the fraction of the diffusion timescale $t_m = l^2/(D_0)$ and advection timescale l/u . If the effective diffusivity (D_{eff}) of a driven diffusion process (as we have here) is larger than the intrinsic diffusivity D_0 , the enhanced diffusivity D_{eff} will cause a shorter mixing time.⁶ It should be noted that scaling effects come into play when downsizing the cilia size. With a decrease

in the cilium size and its motion, the Péclet number and the diffusion time decreases so that molecular diffusion will have a much stronger effect and will dominate the total mixing process for smaller ciliary systems.

To monitor the relative contribution of intrinsic diffusion and the ciliary-driven diffusion, we add random Brownian motion to a dense grid of tracer particles (see Subsection 4 of Appendix B for details). In order to investigate the diffusion enhancement of the cilia, we track the relative dispersion of tracer pairs i and j by analyzing $\mathbf{R}(t) = \mathbf{x}_j(t) - \mathbf{x}_i(t)$.⁷⁹ We characterize the mass transport using the mean square displacement of an ensemble of such pair separations, which in two dimensions can be written as

$$\langle \mathbf{R}^2(t, z_0) \rangle = 8D_{\text{eff}}^R(z_0)t, \quad (8)$$

where $\langle \mathbf{R}^2 \rangle$ is the mean square displacement and $D_{\text{eff}}^R(z_0)$ is the effective diffusivity.⁶ The results are shown in Fig. 12(a), confirming the linear correlation with time. From this, we extract the $D_{\text{eff}}^R(z_0)$. Similarly, the total mixing effect can be analyzed through the standard deviation of the displacement of the tracer particles $\mathbf{D}(t) = \mathbf{x}_i(t) - \mathbf{x}_i(0)$,

$$\langle (\mathbf{D}(t) - \langle \mathbf{D}(t) \rangle)^2 \rangle = 4D_{\text{eff}}^S(z_0)t, \quad (9)$$

where the coefficient $D_{\text{eff}}^S(z_0)$ is the effective diffusivity for the total mixing effect (see the Appendix B 4). The results are shown in Fig. 12(b), from which we extract the $D_{\text{eff}}^S(z_0)$. In order to investigate the effect of cilia on mixing, we choose three values of the mass diffusivity: $D_0 = 2 \times 10^{-6} \text{ m}^2/\text{s}$, $D_0 = 2 \times 10^{-7} \text{ m}^2/\text{s}$, and $D_0 = 2 \times 10^{-8} \text{ m}^2/\text{s}$. When we take u to be the average velocity of the tracer particles in a beat cycle, $2.2 \times 10^{-2} \text{ mm/s}$, and l to be cilia tip trajectory, 12 mm, the corresponding Pe numbers are $\text{Pe} \approx 0.1$, $\text{Pe} \approx 1$ and $\text{Pe} \approx 10$. When diffusion is the dominant factor for $\text{Pe} \approx 0.1$, ciliary motion indeed does not provide an additional contribution to both the Brownian diffusion and mixing process and we obtain $D_{\text{eff}}^S(z_0) \approx D_{\text{eff}}^R(z_0) \approx D_0$, as shown in Fig. 13(a). When $\text{Pe} \approx 1$, the effect of intrinsic diffusion and ciliary motion is comparable, and both the diffusion and mixing are enhanced by the ciliary motion [see Fig. 13(b)], with the effective diffusion coefficients to be slightly larger than D_0 in region III. Finally, when ciliary motion plays a dominant role, for $\text{Pe} \approx 10$, the effective mixing diffusivity $D_{\text{eff}}^S(z_0)$ enhances considerably compared to pure diffusion, up to a factor four for synchronous ciliary motion and antiplectic metachrony and around a factor three for symplectic metachrony (see Fig. 7). The diffusional effect is also enhanced by such ciliary motion to almost a factor two, as shown in the inset of Fig. 7. Note that due to the boundary effect at the top of the box, the effective diffusivity is smaller than the intrinsic diffusivity.

In order to quantitatively explore the effect of the metachronal phase lag on mixing, we focus on the exclusive mixing propensity of the ciliary motion and set $D_0 = 0$. In this case, following Ding *et al.*,¹ we define the overall mixing number m by analyzing the shortest distance between tracer particles of different color (yellow and blue see Fig. 3),

$$m = \left(\prod_{i=1}^{i=N} \min(|\mathbf{x}_i - \mathbf{x}_j|^2) \right)^{\frac{1}{N}}, \quad (10)$$

where $\mathbf{x}_i$ and $\mathbf{x}_j$ are the positions of different color tracer particles (blue, yellow) with the minimum taken over all distances squared

FIG. 7. The normalized effective mixing diffusivity ($D_{\text{eff}}^S(z_0)/D_0$) as a function of height above the substrate for synchronized beating $\Delta\varphi = 0$, antiplectic metachrony $\Delta\varphi = -\pi/6$, and symplectic metachrony $\Delta\varphi = \pi/6$ for $\text{Pe} = 10$. (Inset) The normalized effective diffusivity ($D_{\text{eff}}^R(z_0)/D_0$) enhanced by ciliary motion.

between particle i of one color with the $j = 1 \dots N$ particles of the other color (N is the total number of the same-color particles and there are the same amount of blue and yellow particles). Figure 8(a) shows the time evolution of the m/m_0 for different phase shifts and $m_0 = 1.3 \text{ mm}^2$ is the initial value of m before the tracers are released [see Fig. 3(a)]. We find that the ratio of m/m_0 decays very quickly at the beginning and converges toward a plateau finally. The results for synchronized beating, antiplectic metachrony and symplectic metachrony are very close to each other which is similar to reference.^{74,79} In addition, in the first ten cycles, m/m_0 decays exponentially, exhibiting chaotic mixing [Fig. 8(b)].

We approximate the exponential decay by⁸⁰

$$\ln(m/m_0) = -\beta N_{\text{cycles}}, \quad (11)$$

where the fitted parameter β represents a mixing rate. Hence, it is possible to compare the mixing capabilities of symplectic and antiplectic metachrony waves, as shown in Fig. 9 as a function of $\Delta\varphi$. Interestingly, we find that optimal mixing appears at antiplectic metachrony for $\Delta\varphi = -1/6\pi$ which is close to the optimal phase shift for fluid transport. This highest mixing rate associated with antiplectic metachrony wave can be attributed to the higher stretching and folding, which are the main mechanisms of mixing at low Reynolds numbers.¹

Care should be taken in extending the conclusions of our in-plane mixing analysis to full three-dimensional mixing, as out-of-plane mixing may have a non-negligible contribution that might be different for antiplectic and symplectic metachrony. A full 3D mixing analysis is required to study this.

D. The effect of geometry

It should be noted that the simulations are carried out for a fixed geometry in correspondence to the experimental set-up studied

FIG. 8. (a) Mixing numbers m/m_0 and $\ln(m/m_0)$ as a function of the number of cycles N_{cycle} for synchronized beating (thick solid line), antiplectic metachrony (dash lines) with $\Delta\varphi = -\pi, -5/6\pi, \dots, -1/6\pi$ and symplectic metachrony (solid lines) with $\Delta\varphi = 1/6\pi, 2/6\pi, \dots, \pi$.

recently.⁹ To briefly explore the effect of geometry, we carried out additional simulations by increasing only the fluid domain length B (see Fig. 1). We observed that the three regions are similar in size as in Fig. 4; see the fluid flow field in Movie S3 of the [supplementary material](#). If we increase the fluid domain in all three dimensions (B, W, H) (Movie S4 and S5 of the [supplementary material](#)), the mixing region II is the same below the cilia tip, but the mixing-transport transition and backflow transport regions I and II are shifted upward compared with the reference case ($B \times W \times H$). The results show that for these changes in geometry, antiplectic metachrony is still more efficient than symplectic metachrony with respect to backflow transport and mixing.

IV. CONCLUSIONS

A numerical study of the transport and mixing induced by synchronized, antiplectic and symplectic beating of pneumatic artificial cilia has been conducted in low-Reynolds number fluid flow. An array of pneumatic artificial cilia is analyzed, attached to the bottom surface in a confined box. We find three main regions: (i) the backflow transport region, (ii) the mixing region, and (iii) the transition region

FIG. 9. Mixing rate as a function of phase shift $\Delta\varphi$. The mixing rate is obtained with a fit over the 10 first cycles.

between them. In the backflow transport region, we have studied the average fluid velocity $\bar{U}$ to quantify the fluid transport. The results show that antiplectic metachrony waves are more efficient to transport fluid flow, in line with previous studies. Antiplectic metachrony shows a factor 1.5 increase and symplectic metachrony a factor 2.5 decrease compared to synchronous beating. To explore the underlying physical mechanisms responsible for this difference, we developed a simple two-cilia model that explicitly incorporates the shielding effect. A direct consequence of the shielding effect is the different nature of the transition regions for antiplectic and symplectic metachrony, respectively. While the flow in the transition region for antiplectic metachrony is smooth and unidirectional, the flow for symplectic metachrony is much more heterogeneous and strongly dictated by anticlockwise vortices. Finally, in the mixing region, we define a new diffusivity parameter $D_{\text{eff}}^s(z_0)$ to describe the total mixing, which includes both intrinsic diffusion as well as ciliary-driven diffusion. By exploring the competition between advection and diffusion, we found that ciliary motion strongly enhances the diffusivity at large Péclet numbers when advection is dominant, resulting in a factor 3 increase for symplectic metachrony and a factor increase for antiplectic and synchronous beating for a Péclet number of 10. Finally, we define the overall mixing number m to verify the effect of cilia motion to fluid mixing rate. The overall mixing rate, like the transport rate, depends on the phase difference, with the largest mixing efficiency found for antiplectic metachrony at similar phase differences as for optimal fluid transport.

SUPPLEMENTARY MATERIAL

See the [supplementary material](#) for movies of the fluid flow generated by cilia in different box sizes: Movie S1 and S2 (box size $B \times W \times H$), Movie S3 (box size $2B \times W \times H$), Movie S4 (box size $2B \times 2W \times 2H$), and Movie S5 (box size $4B \times 4W \times 4H$). Movie S2 shows flow in the x - y planes while the others show flow in the x - z planes.

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FIG. 10. Single cilium mesh (a) and its maximum bending configuration (b).

APPENDIX A: MODEL DETAILS

The fluid domain is a rectangular box with size $B \times W \times H = 102 \times 20 \times 49 \text{ mm}^3$. The cilia length is 14 mm, and the cilia are

FIG. 11. Correlation between ciliary motion and backflow generated for synchronous beating. (a) The cilia motion consists of power (red) and recovery (blue) strokes which results in a non-zero swiping area. (b) The backflow instantaneous velocity U_{backflow} .

equally spaced at the bottom of the box. The space a in the x -direction between two cilia is equal to the cilium length of 14 mm. The cilia surfaces are meshed by using triangular elements (Fig. 10). The cilia beat in the x -direction with a time period $T = 4 \text{ s}$ and an angular frequency $\omega = 0.5 \text{ rad/s}$. To get the motion of the cilia centerline $\mathbf{r}^c(s, t)$ from the experiments, 50 snapshots are uniformly obtained during a beating period. The kinematics of the cilia motion $\mathbf{r}^c(s, t)$ [Fig. 11(a)] is obtained by a Fourier series expansion to the sixth order in s which is distributed over 15 nodes, $s_i, i = 1, 2, \dots, 15$. For each node i , the motion $\mathbf{r}^c(s_i, t)$ is obtained by a Taylor series in t with 500 steps, corresponding to a time step of 0.008 s, showing converged results. The box and cilia are meshed by 6280 and 281 triangular elements, respectively, and the velocity was evaluated using 1021×490 grids points in the middle plane which was found sufficiently fine to retrieve converged results in good agreement with the experimental flow field.⁹

APPENDIX B: MEASUREMENT METHODS

1. Backtrace tracer particles

A uniform square grid of tracer particles is initialized in the beating plane and followed for a time $t = 400 \text{ s}$ (i.e., 100 beating cycles). There are 1021×490 grids points, which are regarded as Lagrange fluid particles. The color of each grid point is determined by the position of the particles at $t = 0$ by the backtrace method, either blue or yellow. For the details of this method, we refer to references.^{74,81}

2. Average horizontal displacement of tracer particles

To analyze the displacement that the tracer particles make in 100 cycles of a period of 4 s, we track the horizontal displacement $d_x(x_i, z_0)$ of each particle that was at the position (x_i, z_0) at time $t = 0$ by calculating the difference in their horizontal position at

FIG. 12. Mean square displacement of particle pairs (a) and standard deviation of tracer particle displacement D (b), for different initial positions z_0 .

$t = 0$ s and $t = 400$ s. This displacement field is then averaged over all N tracer particles that were at a specific height y_0 as

$$\langle d_x(z_0) \rangle = \frac{1}{N} \sum_{i=1}^N d_x(x_i, z_0), \quad (\text{B1})$$

where $N = 1021$ tracer particles were used. Correspondingly, the standard deviation of the N particles' $d_x(x_i, z_0)$ gives the error-bar of $\langle d_x(z_0) \rangle$ in Fig. 3 of the main text.

3. Average velocity measurement region

To quantify the back flow produced by each kind of cilia beating motion, the average velocity $\bar{U}$ [see Eq. (6) of the main text] is calculated during a beating period. The average velocity measurement is defined to be region $S = (x_2 - x_1)(z_2 - z_1)$ where $x_2 = 42$, $x_1 = 41$, $z_2 = 49$, and $z_1 = 20$ mm.

4. Brownian tracer particles

A uniform square grid is initialized in the beating plane at $t = 0$ s. There are 1021×490 grid points which are regarded as Lagrange fluid particles. From the initial state, at each time step, a normally distributed number $N(0, 2D_0dt)$, i.e., with zero average and standard deviation $2D_0dt$, is added to the motion of each particle, both in the x and z directions in the measurement plane. When there is no cilia motion, we obtain purely diffusing Brownian particles. To analyze the effect of cilia motion, there are two methods used in the paper. In the first one, we consider a pair of particles in the grid points that share the same initial z -position, and calculate the relative dispersion of each particle pair i and j , $\mathbf{R}(t) = (\mathbf{x}_i(t) - \mathbf{x}_j(t))$. In this way, the effect of the uniform flow generated by the cilia is suppressed from the measurements.⁷¹ The variation of $\mathbf{R}(t)$ for the particle pairs that share the same initial z_0 defines the enhanced diffusion,

FIG. 13. The normalized mixing effective diffusivity $D_{\text{eff}}^S(z_0)/D_0$ as a function of height above the substrate for synchronized beating $\Delta\varphi = 0$, antiplectic metachrony $\Delta\varphi = -\pi/6$, and symplectic metachrony $\Delta\varphi = \pi/6$. (Inset) The normalized effective diffusivity $D_{\text{eff}}^R(z_0)/D_0$ enhanced by ciliary motion. (a) $D_0 = 2 \times 10^{-6} \text{ m}^2/\text{s}$ and (b) $D_0 = 2 \times 10^{-7} \text{ m}^2/\text{s}$.

$$\langle \mathbf{R}^2(t, z_0) \rangle = 8D_{\text{eff}}^R(z_0)t, \quad (\text{B2})$$

with $\langle \mathbf{R}^2(t) \rangle = \langle |\mathbf{R}(t) - \mathbf{R}(t_0)|^2 \rangle$ the mean square displacement, with $D_{\text{eff}}^R(z_0)$ the effective diffusion coefficient for the particles that were initially at $z = z_0$ and where the number 8 is obtained from the Brownian motion. In the case without cilia motion, we have $D_{\text{eff}}^R(z) = D_0$. The results are defined in Fig. 12(a) showing a linear relation with time t enabling the $D_{\text{eff}}^R(z)$ to be extracted, which are shown in Fig. 6 in the main text. In the second method, we consider a single particle i in each of the grid points, and calculate the displacement of the particles $\mathbf{D}(t) = (\mathbf{x}_i(t) - \mathbf{x}_i(t_0))$. Here, the uniform flow generated by the cilia is accounted for. The variation of $\mathbf{D}(t)$ for the particles that share the same initial z_0 defines the total mixing, given by the standard deviation of $\mathbf{D}$:

$$\langle (\mathbf{D}(t, z_0) - \langle \mathbf{D}(t, z_0) \rangle)^2 \rangle = 4D_{\text{eff}}^S(z_0)t, \quad (\text{B3})$$

where the number 4 is also obtained from purely Brownian motion. In the case without cilia motion, we have $D_{\text{eff}}^S(z) = D_0$. The results are shown in Fig. 12(b) showing a linear relation with time t enabling the $D_{\text{eff}}^S(z)$ to be extracted, which are shown in Fig. 6 in the main text.

APPENDIX C: SHIELDING EFFECT INVESTIGATED USING A TWO-CILIA MODEL

In order to study the shielding effect in symplectic and antiplectic metachrony, we propose a new method based on a simplified testing system consisting of two neighboring cilia (Fig. 14) with a distance L apart and attached to a substrate in a semi-infinite fluid. We consider

two situations: In case A, the left cilium is fixed in ten different configurations to investigate its shielding effect on the flow generated by the right cilium [termed “pattern A” in Fig. 14(a)] and in “pattern B” the left one is free and the right one is fixed [Fig. 14(c)]. By analyzing these cases, all possible shielding configurations are probed, from which we will compute all metachronal phase lags through superposition. We use the average velocity through the middle region between these two cilia over one cycle to quantify the shielding effect of the fixed cilium’s different configurations, as shown in Fig. 14. When the fixed cilium is absent the average fluid velocities are $0.0018 L/T$ (pattern A) and $0.0025 L/T$ (pattern B). Clearly, the presence of the fixed cilia reduces the flow in both cases, but it is remarkable to observe that the flow is reduced more when the obstructing cilium is placed in the upstream direction (pattern A) compared to the downstream direction (pattern B). However, when we analyze the effect of the different configurations on the flow (i.e., by comparing the minimal and maximal flows at positions 1 and 5 for both patterns), we see that the obstructing effect for pattern B is larger, in both absolute sense ($0.05 \times 10^{-3} L/T$ vs $0.15 \times 10^{-3} L/T$) and in relative sense ($0.05/1.122 = 0.044$ vs $0.15/2.02 = 0.075$).

Next, we analyze the flow as a function of time within one cycle. During one cycle, there are four peaks of instantaneous velocity as shown in Figs. 15(a) and 15(b) for the patterns A and B, respectively. Peak 1 and 2 are during the effective stroke (positive velocities), and peak 3 and 4 are during the recovery stroke (negative velocities). For a particular configuration in pattern A or B, we can calculate the mean velocity in a window around each peak, i.e.,

$$U_i = \frac{1}{t_i - t_{i-1}} \int_{t_{i-1}}^{t_i} U(t) dt, \quad i = 1, 2, 3, 4. \quad (\text{C1})$$

FIG. 14. Shielding effect caused by different patterns: (a) pattern A. In pattern A, the left cilium (blue) is fixed to ten different configurations separately to calculate the average fluid velocity (b) generated by the right free cilium (black). (c) Pattern B. In pattern B, the right cilium (yellow) is fixed to ten different configurations separately to calculate the average fluid velocity (d) generated by the left free cilium (black). The free cilium tip trajectory includes effective (green) and recovery (red) strokes. The ten fixed configurations are chosen in one period of the cilium at different times $t = [0.1, 0.2, \dots, 1]T$. (b) and (d) depict the average fluid velocity generated by patterns A and B over one cycle of the active cilium, taken in the region $S_1 = (x_2 - x_1)(z_2 - z_1)$ where $x_2 = 13$ mm, $x_1 = 12$ mm, $z_2 = 49$ mm, and $z_1 = 20$ mm.

FIG. 15. Instantaneous fluid velocity during one cycle for the different positions of the fixed cilia for (a) pattern A and (b) pattern B. The legend denotes the different configurations of the fixed cilia corresponding to Fig. 14.

TABLE I. The weighted mean velocities $U_{1,2,3,4}$ (in $10^{-3}L/T$) corresponding to the four different peaks in the 10 different configurations shown in Fig. 15.

	Config. 1	Config. 2	Config. 3	Config. 4	Config. 5	Config. 6	Config. 7	Config. 8	Config. 9	Config. 10
Pattern A U_1	8.6	8.57	8.6	8.94	8.91	8.88	8.74	8.6	8.6	8.6
Pattern A U_2	6.28	6.28	6.28	6.34	6.42	6.42	6.34	6.34	6.28	6.28
Pattern A U_3	-2.35	-2.34	-2.34	-2.34	-2.42	-2.42	-2.42	-2.38	-2.35	-2.35
Pattern A U_4	-11.25	-11.22	-11.25	-11.37	-11.62	-11.57	-11.57	-11.4	-11.25	-11.25
Pattern B U_1	6.14	6.31	6.45	6.62	6.8	6.74	6.74	6.45	6.17	6.14
Pattern B U_2	3.11	3.25	3.5	3.54	3.68	3.62	3.62	3.37	3.14	3.11
Pattern B U_3	-0.94	-1	-1.06	-1.13	-1.19	-1.16	-1.15	-1.05	-0.95	-0.94
Pattern B U_4	-6.31	-6.52	-6.91	-7.11	-7.02	-7.11	-7.02	-6.68	-6.34	-6.28

FIG. 16. Averaged velocity normalized by the synchronous flow velocity for both the simple two-cilia shielding model (dashed line) and the full 6-cilia case [solid line, conform Fig. 5(b)].

Then, for pattern A or B, we have 10 configurations, and for each configuration we have 4 mean velocities corresponding to different peaks. Combining all the configurations in both pattern A and pattern B, the collection of $U_{1,2,3,4}$ forms a dictionary, as shown in Table I. In the following, we will use the dictionary to estimate the fluid velocity of the metachronal waves with different phase shifts. For two cilia with a given phase shift, the fluid generated by the left cilium can be estimated by the sum of the four velocities in each peak, taking proper account of the approximate positioning of the right cilium according to the specific phase shift $\Delta\varphi$. Then we estimate the fluid generated by the right cilium by computing the sum of the four velocities in each peak of the right cilium, now by taking proper account of the approximate positioning of the left cilium according to the specific phase shift. Finally, we take the average of the contributions of the left and right cilium. Using this method, we compute the average velocity for different phase shifts in steps of $2\pi/10$. To compare with the simulation result of the full 6 cilia case, we normalize the average velocity by the synchronous beating velocity as $(\bar{U} - \bar{U}_{\text{syn}})/\max|\bar{U} - \bar{U}_{\text{syn}}|$. The result is shown

in Fig. 16. The good correspondence of the two models is remarkable given the simplicity of the two-cilia screening model. These results point toward the shielding effect as the main physical mechanism responsible for the higher antiplectic velocity compared to the symplectic velocity.

DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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