Supplementary Materials

Density Fluctuations and Energy Spectra of 3D Bacterial Suspensions

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1 Density and velocity spatiotemporal correlations

1.1 Definition

The velocity spatial correlation is defined as

$$C_v(\mathbf{r}) = \frac{\langle \mathbf{v}(\mathbf{r_0}, t_0) \cdot \mathbf{v}(\mathbf{r_0} + \mathbf{r}, t_0) \rangle_{\mathbf{r_0}, t_0}}{\langle \mathbf{v}(\mathbf{r_0}, t_0) \cdot \mathbf{v}(\mathbf{r_0}, t_0) \rangle_{\mathbf{r_0}, t_0}}, \tag{1}$$

whereas the velocity temporal autocorrelation is defined as

$$C_v(t) = \frac{\langle \mathbf{v}(\mathbf{r_0}, t_0) \cdot \mathbf{v}(\mathbf{r_0}, t_0 + t) \rangle_{\mathbf{r_0}, t_0}}{\langle \mathbf{v}(\mathbf{r_0}, t_0) \cdot \mathbf{v}(\mathbf{r_0}, t_0) \rangle_{\mathbf{r_0}, t_0}}. \tag{2}$$

Similarly, the density spatial correlation is defined as

$$C_n(\mathbf{r}) = \frac{\langle n(\mathbf{r_0}, t_0) n(\mathbf{r_0} + \mathbf{r}, t_0) \rangle_{\mathbf{r_0}, t_0}}{\langle n(\mathbf{r_0}, t_0) n(\mathbf{r_0}, t_0) \rangle_{\mathbf{r_0}, t_0}}, \tag{3}$$

and the density temporal autocorrelation is defined as

$$C_n(t) = \frac{\langle n(\mathbf{r_0}, t_0) n(\mathbf{r_0}, t_0 + t) \rangle_{\mathbf{r_0}, t_0}}{\langle n(\mathbf{r_0}, t_0) n(\mathbf{r_0}, t_0) \rangle_{\mathbf{r_0}, t_0}}. \tag{4}$$

Here, $\langle ... \rangle_{\mathbf{r_0}, t_0}$ denotes an average over all possible positions and times. Since the flow is isotropic, we consider only the spatial correlation as a function of the magnitude of the position vector $r = |\mathbf{r}|$, which is achieved by averaging $C_v(\mathbf{r})$ and $C_n(\mathbf{r})$ over a constant r. The number density, n, is proportional to the local light intensity I (Sec. 2.1). We further subtract the intensity by the mean intensity so that $\langle I \rangle_{\mathbf{r_0}, t_0} = 0$.

1.2 Estimate of density correlation time

We write the density correlation time τ_n as

$$\tau_n = \frac{\lambda_n^2}{D_v} = \frac{\lambda_n^2}{\langle v \rangle^2 \tau_v}, \tag{5}$$

where λ_n is the density correlation length, characterizing the length scale of density inhomogeneity in the system (Fig. 2E). The random velocity fluctuations are treated as a stochastic process with an effective diffusion coefficient $D_v = \langle v \rangle^2 \tau_v$, where $\langle v \rangle$ is the average local velocity and τ_v is the velocity correlation time (Fig. 2F).

For high-concentration bacterial suspensions with active turbulence, λ_n is naturally set by the size of bacterial clusters at the scale of turbulent vortices. Since bacteria are well aligned within each cluster, the average local velocity should equal to the velocity of the swirling vortices. Thus, $\lambda_n / \langle v \rangle \sim \tau_v$, which leads to $\tau_n \sim \tau_v$ as observed in experiments (Fig. 2F).

For low-concentration bacterial suspensions, λ_n is set by the size of individual bacteria l_b (Fig. 2E), whereas τ_v is given by the characteristic time scale of bacterial swimming, $\tau_v = \tau_b \equiv l_b / v_0$ (Fig. 2F). It is difficult to estimate the average local velocity for low-concentration suspensions, as there is no general alignment of bacterial orientation. Assuming an isotropic distribution of bacterial orientation at low concentrations, $\langle v \rangle$ would be zero if all bacteria have the same swimming speed v_0. Thus, the average velocity must arise from the fluctuations of the swimming speed of individual bacteria around the mean, i.e., $\langle v \rangle \sim \Delta v = \sqrt{\langle v^2 \rangle - v_0^2}$, where Δv is the standard deviation of bacterial swimming speed. Given $\tau_n \approx 10 \tau_b$ from experiments (Fig. 2F), we have

$\langle v \rangle \sim \Delta v \approx 0.32 v_0 = 4.7$ μm/s based on Eq. 5, agreeing well with the experimental measurements $\Delta v = 3$ μm/s. Thus, the variations of the swimming speed of individual bacteria lead to a small but finite average local bacterial velocity, which gives rise to the long density correlation time at small ϕ.

2 Density fluctuations

2.1 Pixel intensity and the number of bacteria

Figure 1D shows that, under the same illumination and imaging condition, bacterial density and the average pixel intensity follow approximately a linear relation, which can be expressed as

$$n = a + bI. \tag{6}$$

Here, n is the average number density of bacterial suspensions, I is the average pixel intensity, a and b are constants at the fixed illumination and imaging condition. Note that the volume fraction of bacterial suspensions $\phi = nV_b$ with $V_b \approx 1$ μm^3 the average volume of a single bacterium. The number of bacteria in a given subsystem of side length l and thickness d can be calculated as

$$N = l^2 dn = l^2 d(a + bI), \tag{7}$$

where $d \approx 6$ μm is the depth of field of microscopy, which is fixed in our experiments. Thus, the number of bacteria in the subsystem N is proportional to nl^2. Taking the standard deviation of both sides of Eq. 7, we obtain

$$\Delta N = d|b|(l^2 \Delta I), \tag{8}$$

where ΔN is the standard deviation of the bacterial number in the subsystem over time and ΔI is the standard deviation of the average pixel intensity of the subsystem over time. Since $d|b|$ is a constant independent of subsystem sizes and bacterial density, ΔN is linearly proportional to $l^2 \Delta I$. Because any constant in front of ΔN would not affect either the scaling relation or the relative magnitude of density fluctuations at different n (or ϕ), we simply take $l^2 \Delta I$ as ΔN in our study.

Note that the volume of the subsystem under consideration is $V = l^2 d$. Hence, even at the smallest length $l = l_b/3 = 1$ μm chosen in our density

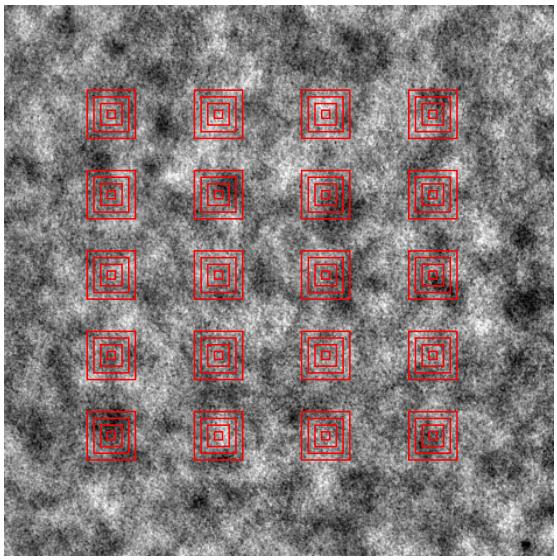

Figure S1: Calculation of the standard deviation of the average pixel intensity, ΔI, at different length scales. Density fluctuations are quantified by the standard deviation of the average pixel intensity over time in subsystems of increasing sizes, indicated by the sequences of red squares. Results from twenty different subsystems of the same size evenly distributed in the field of view are then averaged to yield ΔI for the given subsystem size.

fluctuation analysis (Fig. 3A), $V = 6$ μm^3 is still sufficiently large to hold up to six bacteria within the subsystem. Here, $l_b \approx 3$ μm is the length of bacterial body. Hence, even at the smallest length scale of our analysis, local correlation between multiple bacteria can still exist within one subsystem, which is indeed detected in our experiments at small l.

2.2 Density fluctuations at different length scales

Based on the linear relation between ΔN and ΔI, we calculate the density fluctuations at different length scales. We first crop square-shape subsystems of increasing sizes, as shown in Fig. S1. For each subsystem size l, the standard deviation of the average pixel intensity of the subsystem is calculated over 50 frames (1.67 s or $8.35\tau_b$), which is longer than the saturated density correlation time of $4\tau_b = 0.8$ s (Fig. 2F). To improve statistics, we choose 20 subsystems of the same size evenly distributed in the field of view and obtain a spatial average of the temporal standard deviation of the average pixel intensity ΔI

(Fig. S1). This averaged ΔI is then multiplied by l^2 to give the number density fluctuations ΔN at the length scale l. Note that a second method has also been proposed for calculating number fluctuations, where the standard deviation of particle numbers is computed first spatially over different locations in a single time frame and is then averaged over time of different frames.[11] Although the two methods lead to the same results when spatial and temporal correlations are small compared with the system size and experiment duration, the second method is subject to potential systematic errors in our study due to time-independent non-uniform light illumination and intrinsic stationary density variations in non-motile suspensions at $t = 0$ in the kinetic measurements. Using the first method, any stable non-uniform light illumination or stationary non-uniform density variations would result in zero temporal standard deviations of I and, therefore, would not affect our measurements of true density fluctuations of motile bacterial suspensions.

2.3 Normalization of the density fluctuations of bacterial suspensions of different volume fractions

Practically, to optimize image qualities, we adjust the exposure time of imaging for suspensions of different ϕ. Exposure times affect the proportional constant $d|b|$ in Eq. 8, which introduces a ϕ-dependent linear constant. Although $d|b|(\phi)$ does not affect the scaling exponent of density fluctuations α, it modifies the relative magnitude of ΔN at different ϕ. In order to compare the magnitude of density fluctuations at different ϕ, we further calibrate and normalize ΔI for different ϕ. Specifically, using as the calibration, we take videos of bacterial suspensions at different ϕ under the exact same imaging condition with fixed illumination light intensity, condenser position, optical filters and all the camera settings such as the exposure time and the dynamic range. The calibration results are shown in Fig. S2, where $\Delta N \sim l^2 \Delta I$ at different ϕ collapse at small length scales. The calibration results show that we can normalize $l^2 \Delta I$ of different exposure times by its value at a fixed small length scale. We choose the small scale at $l = 0.3 l_b$ in our study. Since $l^2 \Delta I$ at different ϕ shows the same slope at small l, choosing any other small lengths between $0.1 l_b$ and $0.5 l_b$ would lead to quantitatively the same results. The normalized density fluctuations show not only the correct scaling exponents but also the correct relative magnitudes at different ϕ.

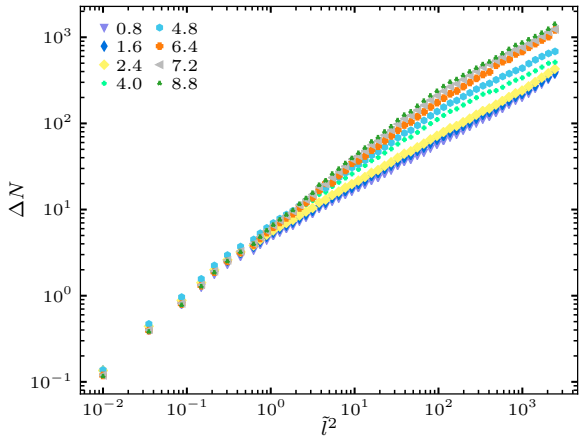

Figure S2: Calibration of the standard deviation of bacterial number $\Delta N \sim l^2 \Delta I$ at different volume fractions ϕ. ΔN versus the dimensionless subsystem size $\tilde{l}^2 = l^2/l_b^2$ for bacterial suspensions at different ϕ. The images are taking under the same illumination with the same imaging condition.

2.4 Density fluctuations in the transient state

Density fluctuations in the transient state towards active turbulence is calculated using the same method as that in the steady state. Specifically, the procedure described above is applied at time t over a time interval Δt during the transition towards active turbulence. We choose $\Delta t = 1.7$ s (50 frames), which is longer than the density correlation time ($4\tau_b = 0.8$ s) but is significantly smaller than the time of the entire transition (~ 60 s or 1800 frames) for a good temporal resolution.

3 Density-flow correlations

3.1 Correlation between local density fluctuations and kinetic energy

To calculate local temporal density fluctuations, we need to approximate instantaneous intensity variations. On the one hand, the time interval for calculating the intensity difference between two frames needs to be smaller than the density

correlation time ($4\tau_b = 0.8$ s) in order to satisfy the instantaneous approximation. On the other hand, the time interval should be sufficiently long to suppress the influence of random fluctuations of image intensities in adjacent frames. In our study, we choose 0.3 s (10 frames) for the local density fluctuation calculation. We do not expect the results to be much different when varying the time interval from 0.17 s to 0.6 s.

To calculate the local density variations at the length scale of $l = 2.75l_b$ and time t, we take 10 consecutive frames following the frame at t. All the 10 frames are first coarse-grained by averaging the intensity of pixels in square windows of size $2.75l_b \times 2.75l_b$ into single coarse-grained pixels (Fig. S3B). We then take the temporal standard deviation of the coarse-grained pixel intensity over the 10 frames at different positions to obtain a field of density fluctuations at t, $\Delta n(\mathbf{r}, t)$, as shown in Fig. S3D. The same approach has also been used to calculate the field of instantaneous density fluctuations in the transient state shown in Fig. 7A.

Independently, the PIV algorithm is also applied on the original images of the first two frames to obtain the velocity field at time t, $\mathbf{v}(\mathbf{r}, t)$ (Fig. S3C). Since the step size of the PIV analysis is also at $\Delta x = 2.75l_b$ (Materials and Methods), the velocity field has the same dimensions as the coarse-grained density fluctuation field obtained above. The local kinetic energy can then be calculated as $E(\mathbf{r}, t) = |\mathbf{v}(\mathbf{r}, t)|^2/2$ (Fig. S3E). Finally, the normalized correlation between $\Delta n(\mathbf{r}, t)$ and $E(\mathbf{r}, t)$ is computed as

$$C_1 = \frac{\langle (\Delta n - \overline{\Delta n})(E - \overline{E}) \rangle}{\sigma_{\Delta n} \sigma_E}, \tag{9}$$

where $\bar{A}$ indicates the spatial average of variable A at a fixed time, σ_A indicates the standard deviation of A, and $\langle A \rangle$ denotes the average of A over all the positions and times (total 1000 frames). The correlation quantifies the spatial similarity between Δn and E, which ranges between -1 to 1.

3.2 Correlation between local density and the divergence of bacterial flux

Saintillan and Shelley showed numerically an anti-correlation between the local bacterial density n and the divergence of the relative bacterial flux $\nabla \cdot (n\bar{v}_0\mathbf{d})$ in 2D suspensions.[47] Note that we use a different notation, where $\bar{v}_0$ is the local average bacterial swimming speed and $\mathbf{d}$ is the unit vector indicating the local

average swimming direction of bacteria. $n\bar{v}_0\mathbf{d}$ gives the relative bacterial flux with respect to the local background fluid flow $\mathbf{u}$. Experimentally, however, it is difficult to measure the relative motion of bacteria with respect to the background fluid flow. Instead, the PIV analyses yield the absolute bacterial flux with respect to the fixed lab frame $n\mathbf{v}$, where $\mathbf{v} = \bar{v}_0\mathbf{d} + \mathbf{u}$. We calculate the correlation between the local bacterial density n and the divergence of the absolute bacterial flux $\nabla \cdot (n\mathbf{v})$ of 3D bacterial suspensions in our experiments,

$$C_2 = \frac{\langle (n - \overline{n})[\nabla \cdot (n\mathbf{v}) - \overline{\nabla \cdot (n\mathbf{v})}]\rangle}{\sigma_n \sigma_{\nabla \cdot (n\mathbf{v})}}, \tag{10}$$

where again $\bar{A}$ indicates the spatial average of variable A at a fixed time, σ_A indicates the standard deviation of A, and $\langle A\rangle$ denotes the average of A over all the positions and times (total 1000 frames). Note that the local bacterial density n is proportional to the local average light intensity as the size of the local subsystem is fixed at $l = 2.75l_b$.

3.3 Correlation between local density and kinetic energy

Finally, instead of the correlation between the local density *fluctuations* and kinetic energy, we also analyze the correlation between the local density n and kinetic energy E, which is defined as

$$C_3 = \frac{\langle (n - \overline{n})(E - \overline{E})\rangle}{\sigma_n \sigma_E}, \tag{11}$$

where $\bar{A}$ indicates the spatial average of variable A at a fixed time, σ_A indicates the standard deviation of A, and $\langle A\rangle$ denotes the average of A over all the positions and times (total 1000 frames). The local kinetic energy is given as $E(\mathbf{r}, t) = |\mathbf{v}(\mathbf{r}, t)|^2/2$. The local bacterial density n is proportional to the local average light intensity as the size of the local subsystem is fixed at $l = 2.75l_b$.

4 Supplementary video captions

Supplementary Video 1: Steady-state bacterial turbulence. The time length of the video is 60 s in real time. The video plays at 4 times of real-time speed. Field of view: 420 μm by 360 μm. Bacterial volume fraction: 6.4%.

Supplementary Video 2: The onset of active turbulence in bacterial suspensions. The time length of the video is 96 s in real time. The video plays at 4

times of real-time speed. Field of view: 420 μm by 360 μm. Bacterial volume fraction: 6.4%. Yellow arrows indicates the velocity of the flow field. $t = 0$ corresponds to the time when the light is turned on.

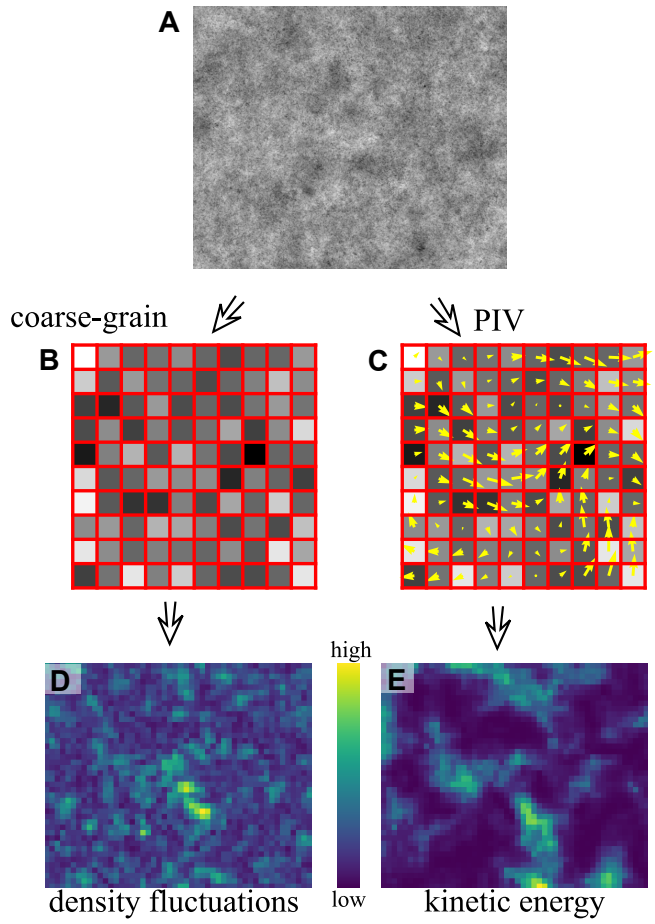

Figure S3: Schematic showing the procedure to calculate the correlation between local density fluctuations and kinetic energy. (A) The raw image of a bacterial suspension at a given time t. (B) The coarse-grained image with a pixel size of $l = 2.75l_b$. (C) The velocity field from PIV. (D) The field of local density fluctuations, obtained by calculating the standard deviation of the intensity of coarsen-grained pixels shown in (B) over a short time interval. (E) The field of local kinetic energy, obtained by calculating $E = \mathbf{v}^2/2$ from the velocity field shown in (C).