Density Fluctuations and Energy Spectra of 3D Bacterial Suspensions Supplemental Material

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I. EXPERIMENT DETAILS

A. Light-powered *E. coli*

We introduce a light-driven transmembrane proton pump, proteorhodopsin (PR), to wild-type *E. coli* (BW25113) by transforming the bacteria with plasmid pZE-PR encoding the SAR86 γ-proteobacterial PR-variant (Walter 2007). The activity of PR is correlated with the intensity of light. Thus, we can control the swimming speed of bacteria using light of different intensities. In our experiments, we use high-intensity light, which saturates the light response of bacteria. The average swimming speed of bacteria is fixed at $v_0 = 15 \pm 3$ μm/s in the dilute limit.

The bacteria are cultured at 37 ℃ with a shaking speed at 250 rpm for 14-16 hours in terrific broth (TB) [tryptone 1.2% (w/w), yeast extract 2.4% (w/w), and glycerol 0.4% (w/w)] supplemented with 0.1 g/L ampicillin. The culture is then diluted 1: 100 (v: v) in fresh TB and grown at 30 ℃ for 6.5 hours. PR expression is triggered by supplementing the culture medium with 1 mM isopropyl β-D-thiogalactoside and 10 μM ethanolic all-trans-retinal in the mid-log phase (3 hours after the dilution).

The bacteria are harvested by gentle centrifugation (800g for 5 min). After discarding the culture medium in the supernatant, we resuspend bacteria with DI water. The resuspended suspension is then centrifuged again at 800g for 5 min, and finally adjusted to the target concentration for experiments.

B. Sample preparation and microscopy

To prepare the sample for microscopy, we construct a seal chamber made of glass slides (25 mm × 75 mm) and coverslips (18 mm × 18 mm). We first glue (NOA 81, Norland, NJ) two coverslips on a glass slide, side-by-side, leaving a 3-mm separation between the two coverslips. We then cover the 3-mm separation with another coverslip to form a channel. We then pipette bacterial suspensions into the channel. Finally, we seal the two ends of the channel using UV glue (NOA 76, Norland, NJ) to form a sealed chamber.

Images of the bacterial suspensions are taken 50 μm above the bottom surface of the sealed chamber by a Nikon Ti-E inverted microscope using the bright field mode and a 20× (NA 0.5) objective. The field of view is 420×360 µm^2. All videos are recorded at 30 frames per second using a sCMOS camera.

II. IMAGE ANALYSIS DETAILS

A. Calculation of density fluctuations

1. *Convert intensity to density*

In Fig. 1d, we show that under the same illumination and imaging condition, the density and average pixel intensity follow approximately a linear relation, which can be expressed as suggests:

$$\phi = a + bI \tag{1}$$

where ϕ is the volume fraction of bacterial suspensions, I is the average pixel intensity in a window, a and b are constants under the same illumination and imaging conditions. The number of bacteria in a given subsystem with side length l and thickness d can be calculated as

$$N = \frac{\phi l^2 d}{V_b} \tag{2}$$

where V_b is the volume of a single bacterium. Take the standard deviation of both sides of Eq. 2, we get

$$\Delta N = \frac{|b| d}{V_b} l^2 \Delta I \tag{3}$$

where Δ stands for taking the standard deviation over time. Over different length scales and volume fractions, $\frac{|b|d}{V_b}$ is a constant. Thus, we use $l^2\Delta I$ as ΔN in all the figures.

2. *Density fluctuations at different length scales*

Equipped with the relation between ΔN and ΔI, we now calculate the density fluctuations at different length scales (using $l^2\Delta I$). We first crop square-shape subsystems of various sizes from the original image time series, as shown in Fig. 1a. For each size l, a standard deviation of the light intensity are calculated over 50 frames (1.67 s). To improve statistics, we choose 20 subsystems of the same size evenly distributed in the field of view and obtain an averaged standard deviation of mean intensity ΔI (Fig. 1b). This averaged ΔI is then multiplied by l^2 and is used as the density fluctuations ΔN.

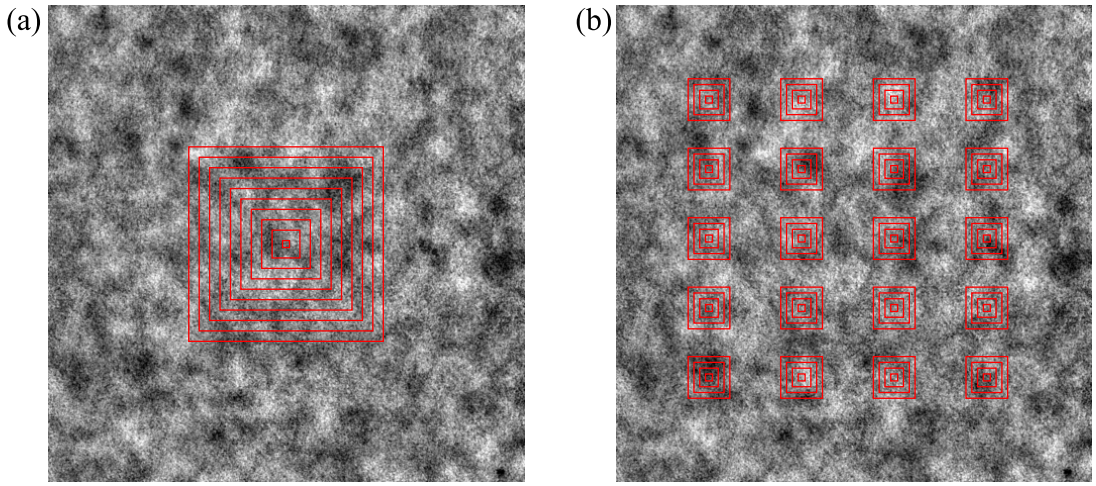

FIG. 1. **GNF calculations.** (a) Varying subsystem sizes. (b) Multiple seeds of subsystems for spatial average.

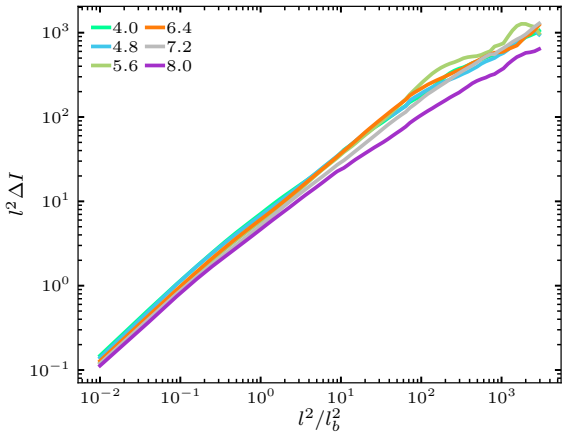

FIG. 2. **GNF curves at various volume fractions under same illumination and imaging conditions.**

3. *Normalization*

For optimal image qualities, we used different exposure times when acquiring the data. However, such difference prevents us from comparing ΔN at different volume fractions, since exposure time changes the overall magnitude of the image intensity fluctuations, independent of the fluctuations in the bacterial sample. In order to compare the magnitude of GNF across different volume fractions, and more importantly compare GNF with energy spectra, we need to normalize the GNF curves obtained at different illumination and imaging conditions, so that they reflect the actual relative magnitude independent of illumination and imaging conditions. To do it, we use the same illumination and imaging conditions to take videos of bacterial suspensions at various volume fractions where we control all the imaging parameters strictly. The results are shown in Fig. 2, where all the curves are very close at small length scale, and show deviation at large length scales. This observation is intuitive: small scale density fluctuations is determined primarily by single cell dynamics and is not a strong function on volume fractions. In the main text, we normalized all the GNF curves at small length scale, by forcing $\Delta N_{0.3l_b} = 1$.

B. Particle image velocimetry (PIV)

2D in-plane velocity fields are extracted by Particle Image Velocimetry (PIV) analysis using openPIV package in Python [1]. We fix the box size to be 16 μm, which is larger than the size of a single bacterial body but smaller than the velocity correlation length. A step size of the half of the box size (8 μm) is used by convention, which sets the spatial resolution of the velocity fields.

C. Energy spectra

The energy spectra of bacterial suspensions are calculated as follows.

$$E(k_x, k_y) = \frac{\langle u_k(k_x, k_y)u_k^*(k_x, k_y) + v_k(k_x, k_y)v_k^*(k_x, k_y)\rangle}{2A} \tag{4}$$

where E is the energy density in k space, u_k and v_k are k space velocity fields, A is the real space area of the system and * denotes the complex conjugate. The $\langle\cdot\rangle$ denotes an average over multiple images from different times.

The fourier transform of real space velocity u_k and v_k are computed as the following:

- apply the built-in Fast Fourier Transform (FFT) function of Python `numpy.fft` package to the discrete velocity field $v(x, y)$ (from PIV) to get $V_k(k_x, k_y)$
- since the FFT is defined as

$$V_k = \sum_{m=0}^{n-1} v_m \exp(-2\pi i \frac{mk}{n})$$

missing the dx counterpart in the continuous Fourier transform, we additionally multiply d^2_{step} to $V_k(k_x, k_y)$ to get the wavenumber domain velocity $v_k(k_x, k_y)$

- the wavenumber field k corresponding to $v_k(k_x, k_y)$ is obtained by applying the `numpy.fft.fftfreq` function. Since the definition of FFT introduces a prefactor 2π to the wavenumber, an additional 2π is also multiplied to k

D. Correlation of local density fluctuations and kinetic energies

Here we show the procedures of calculating the local coupling between density fluctuations and kinetic energy. When calculating the local density fluctuations, we want to measure the instantaneously change of density instead of a steady-state scaling. Thus, we choose the length of video for this calculation to be within the autocorrelation time of density (or more accurately, average pixel intensity) variations, which is typically around 2s, or 60 frames, as shown in Fig.**??**. We also want to include as many frames as we can instead of using adjacent frame, in order to suppress the effects from random fluctuations of image intensities, the nature of optical imaging. Thus, we used 10 frames for the local density fluctuation calculation. We don't expect the result to be much different when varying this number from 5 to 20.

As shown in Fig. 3, we first take 10 frames $F_i(\boldsymbol{r})$ of consecutive images, where $i = 1, 2, ..., 10$. All the 10 frames are coarse-grained by binning 25×25 px^2 (8×8 μm^2) windows into a single pixel $f_i(\boldsymbol{r})$, as shown in Fig. 3b. PIV algorithm is then applied on the first two images to obtain a representative velocity field $v\boldsymbol{r}$, as in Fig. 3c. Since the PIV analysis is done at a step size of 25 pixel, the coarse-graining procedure produces images with dimensions the same as the velocity fields obtained from PIV. We then take the standard deviation of the pixel intensity at each spot over 10 frames to obtain a field of density fluctuations, $\delta N(\boldsymbol{r}) = \sqrt{\langle (f_i - \langle f_i \rangle)^2 \rangle}$, shown in Fig. 3d. The kinetic energy field is obtained by taking square of the magnitudes of PIV result and then divide it by 2, $E_k(\boldsymbol{r}) = \langle v(\boldsymbol{r})^2/2 \rangle$, as shown in Fig. 3e. Finally, the normalized correlaiton between $\Delta N(\boldsymbol{r})$ and $E_k(\boldsymbol{r})$ is calculated as

$$\Delta N \star E_k = \frac{\langle (\Delta N - \overline{\Delta N})(E_k - \overline{E_k}) \rangle}{\sigma_{\Delta N} \sigma_{E_k}} \qquad (5)$$

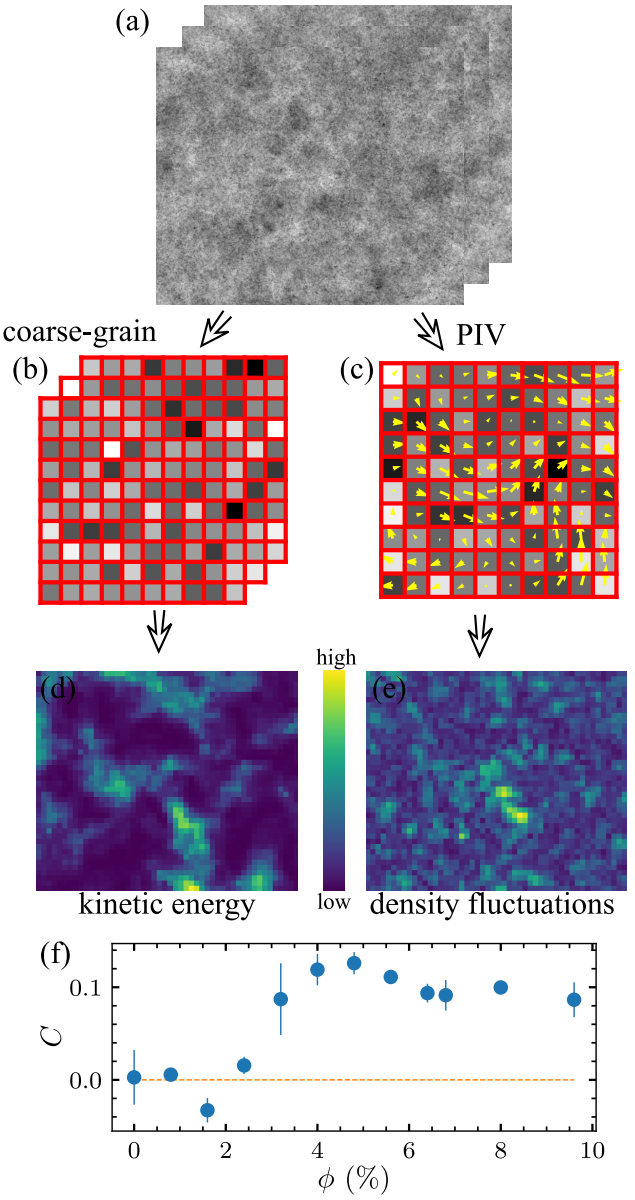

FIG. 3. Diagram showing the procedures of calculating local coupling between density fluctuations and kinetic energy.

$\star$ is the operator standing for cross correlation, $\bar{\cdot}$ means taking the mean, σ means the standard deviation, and $\langle \cdot \rangle$ denotes taking the average of all scalars in an array. The cross correlation quantifies the similarity between arrays N and E_k. The resulting number takes value from -1 to 1.

E. Density fluctuations in the transient state

The density fluctuations in transient state is calculated using the same method as the steady state. The procedures described in Sec. II A is repeated every 50 frames in

videos where the whole process of bacterial activity rising is recorded. Why 50 frames? It is about the length of correlation time, so we expect sound statistics from it. It is also short compared to the whole process of α rising (60 s, or 1800 frames), so it can also provide good temporal resolution to monitor the kinetic process.

[1] A. Liberzon, D. Lasagna, M. Aubert, P. Bachant, T. Käufer, jakirkham, A. Bauer, B. Vodenicharski, C. Dallas, J. Borg, tomerast, and ranleu, OpenPIV (2020).