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TOPICAL TOPIC — Soft matter and biological physics

# Diffusional inhomogeneity in cell cultures*

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Cell migrations in the cell cultures are found to follow non-Gaussian statistics. We recorded long-term cell migration patterns with more than six hundred cells located in 28 $mm^2$. Our experimental data support the claim that an individual cell migration follows Gaussian statistics. Because the cell culture is inhomogeneous, the statistics of the cell culture exhibit a non-Gaussian distribution. We find that the normalized histogram of the diffusion velocity follows an exponential tail. A simple model is proposed based on the diffusional inhomogeneity to explain the exponential distribution of locomotion activity in this work. Using numerical calculation, we prove that our model is in great agreement with the experimental data.

**Keywords:** random walks, cell locomotion, collective behavior

**PACS:** 87.17.Jj, 05.40.Fb, 87.18.Gh **DOI:** 10.1088/1674-1056/27/2/028705

## 1. Introduction

Bacteria, eukaryotic cells and animals like birds and insects[1–4] are believed to adopt perplexing random walk strategies to explore their environment. Many phenomenological models have been made to classify these random walk strategies by the characteristics of stochastic fluctuation. We notice that these systems have been treated in the same framework of self-propellers[5] with collective motion. The ensembles are always homogenous, if not, they will fall apart in the collective motion. Hence, the ensemble behavior is dependent on the diffusion features of a unit and the interaction relationships between the units.

We address that eukaryotic cell motion may be more complicated than the homogenous collective random motion. As a model system in this field, Dictyostelium discoideum (DD) cell is used in this paper. When individual DD cells are starving, they tend to aggregate to form a multicellular migrating slug.[6] Before the aggregation, cells move randomly to search the space for food in the local environment. Interestingly, many experiments showed that the aggregation is triggered by a specific chemical signal and then the movements of all the cells are synchronized and accelerated.[7] The aggregation process is much like the homogenous collective motion, while before synchronization and acceleration, the assumption of homogeneity may not be suitable for the searching period. In recent research, the inhomogeneity of the diffusion for acetylcholine receptors is reported,[8] suggesting the assumption of homogeneity is not straightforward. Here, we focus on the locomotion activity in the searching period. Anomalous dynamics have been put forward in this region.[9–12] Meanwhile, the search strategy adopted by cells attracted much attention in recent decades.[13–15]

To understand the statistical strategy adapted by DD cells, we analyzed the cell trajectories in the searching process mentioned above. We wonder whether the assumption of homogeneity is still suitable for this system. Our experimental results suggest the cell culture is inhomogeneous. Cells with different diffusion constant exist simultaneously in the cell culture, despite the fact that all cells are divided from the same spore. Starting from the inhomogeneity, we proposed a simple model to describe the searching strategy. In this model, cells have their own diffusion velocity and exhibit different distributions of velocity fluctuations. Meanwhile, the distribution of cell velocities follows an exponential tail. This model gives us a new perspective and method to find the optimal strategy in order to explore the space. Furthermore, we present a straightforward test of our model by numerical calculation.

## 2. Experimental setup

KAx-3 cells of Dictyostelium Discoideum (DD) were used in our experiments and were obtained by the following procedures, similar to the procedures given in Ref. [16]. Cells were plated on the top of a solid medium with nutrition (20-g agar, 10-g glucose, 10-g trytone, 1-g yeast extract, 1.9-g $KH_2PO_4$, 0.78-g $K_2HPO_4 \cdot 3H_2O$, and 1-g $MgSO_4$ per 1-L distilled water), grown in an incubator kept at 23 °C for about 39 hours, and then isolated by centrifuging at 100 g and re-suspended in phosphate buffer solution (PBS). The isolated cell of KAx-3 was respectively starved for 4 hours in a refrigerator kept at 4 °C before they were plated with the desired density on top of a 1.5% agar plate without any nutrition. The cell area density in the sample studied was kept

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23 cells per mm$^2$. All the procedures mentioned above were carried out under a clean hood and the environmental temperature was controlled at 25 °C within ±1 °C. An objective with 4× magnification was used because it can record as many cells as possible to achieve good statistics. Images were digitized and stored in a computer equipped with a digital camera (Canon 700D, spatial resolution 5138 × 3456). Four samples were recorded at the same time by four cameras for more than 50 hours, the time interval between images was 10 seconds. We used homemade software to recognize and track cells, and finally obtained the trajectories and other information of the cells.

## 3. Results

### 3.1. Definition of the velocity and the mean velocity of a trajectory

In the experiment, we obtained the DD cell trajectories from consecutive images of the cell culture with spatial resolution of 2 μm and captured 633 cells located in 28 mm$^2$ in each frame. More than $1.8\times10^4$ frames were used. The trajectories are recorded between collisions, because cells are able to run into another cell and their trajectories break. We only analyze the trajectories of individual cells in this work. Using a homemade analysis program, we determined their position $r(t)$ at time $t$ and the two-dimensional displacement vector $\Delta r(\tau)=r(t+\tau)-r(t)$ over the delay time $\tau$ from 7000 trajectories.

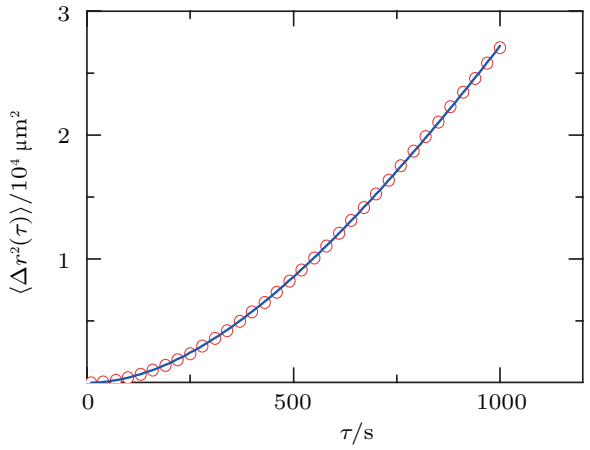



**Fig. 1.** (color online) Measured MSD $\langle\Delta r^2(\tau)\rangle$ as a function of delay time $\tau$ for the individual cell trajectories. Three data points are skipped between every two points in the figure for clarity. The red line is a non-linear fit to the data points using OU process equation, $\langle\Delta r^2(\tau)\rangle=4D\{\tau-\tau_r[1-\exp(-\tau/\tau_r)]\}$, with resistant time $\tau_r=580$ s.

Figure 1 shows the measured mean square displacement (MSD) $\langle\Delta r^2(\tau)\rangle$ as a function of delay time $\tau$ for the individual cell trajectories. We used the Ornstein–Uhlenbeck process to fit our data as an approximation

$$\langle\Delta r^2(\tau)\rangle=4D\{\tau-\tau_r[1-\exp(-\tau/\tau_r)]\}$$

with the resistant time $\tau_r=580$ s and $D$ is the diffusion coefficient. The fitted resistant time $\tau_r=580$ s indicates cells lose their direction after 580 s.[17] Hence, we can define the velocity from the displacements $\Delta r(\tau)=r(t+\tau)-r(t)$, when $\tau$ is much shorter than $\tau_r$. Without further loss of generality, we define $v=\Delta r(\tau)/\tau$ with $\tau=50$ s. For every trajectory, we define $v_{\rm traj}=\langle v\rangle$, averaged on all the velocities in each trajectory. $v_{\rm traj}$ represents the mean velocity of a trajectory.

Figure 2 shows the normalized histograms of $v$ and $v_{\rm traj}$. The exponential tail in the histogram of $v$ was hard to explain under the framework of OU process. Meanwhile, the histogram of $v_{\rm traj}$ shown in Fig. 2(b) indicates the mean velocities of trajectories are widely dispersed, and the distribution also exhibits an exponential tail.

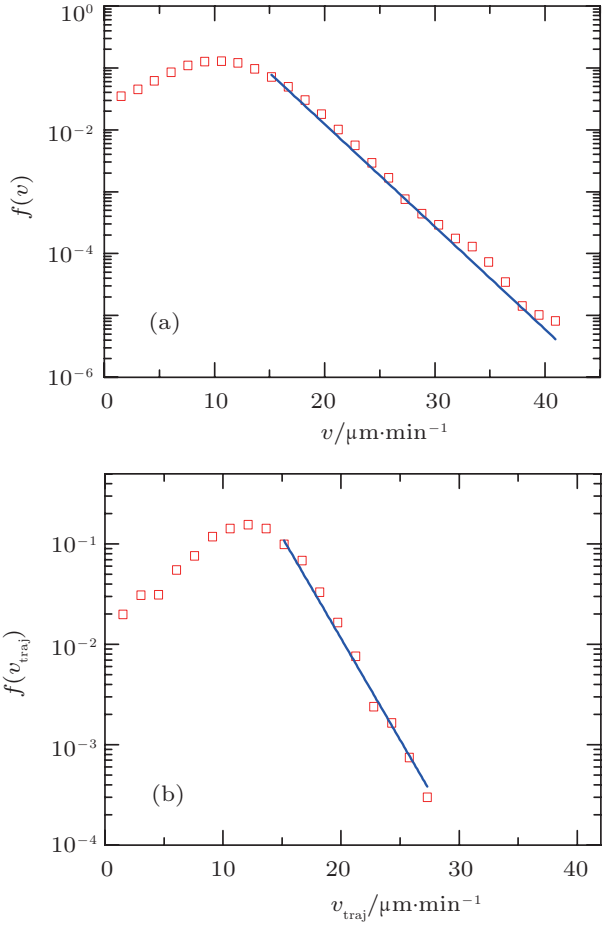



**Fig. 2.** (color online) (a) Normalized histogram of the cell velocity $v$ measured by every displacement in 50 s. (b) Normalized histogram of the mean velocity $v_{\rm traj}$ measured by every trajectory. The blue lines in panels (a) and (b) are exponential fit to the curve tail, $f(v)\simeq\alpha_1\exp(-\beta_1 v)$ and $f(v_{\rm traj})\simeq\alpha_2\exp(-\beta_2 v_{\rm traj})$, with $\beta_1=0.382$ min/μm, $\beta_2=0.465$ min/μm.

### 3.2. Stochastic fluctuation of each diffusion group and their coupling with the group velocity

We classified trajectories into five bins with equal width from the slowest velocity to the fastest velocity. Details are in the caption of Fig. 3. In each bin, trajectories belong to the same velocity group. Stochastic fluctuations of each velocity group are shown in Fig. 3(a). In each group, the fluctuation

seems to be Gaussian. We fitted the distributions with Gaussian functions,

$$h_{\text{group}}(v) = \frac{1}{\sqrt{2\pi}\sigma_{\text{group}}} \exp\left[-\frac{(v - v_{\text{group}})^2}{2\sigma_{\text{group}}^2}\right]$$

and got the fitted parameters of the mean value $v_{\text{group}}$ and standard deviation $\sigma_{\text{group}}$. We shifted the $x$ coordinate, $v^\star = (v - v_{\text{group}})/\sigma_{\text{group}}$ and plotted $h'_{\text{group}}(v^\star)$ versus $v^{\star 2}$ in Fig. 3(b), where $h'_{\text{group}}(v^\star) = \log(h_{\text{group}}(v^\star))$. The data of five groups overlap each other after shift and the linearity of both tails indicate the Gaussian fits of the fluctuations in five groups are accurate.

Figure 3(c) shows the standard deviation $\sigma_{\text{group}}$ as a function of $v_{\text{group}}$. We fit the data linearly and the fitting empirical equation reads

$$\sigma_{\text{group}} = \sigma_{\text{o}} + \kappa v_{\text{group}}, \tag{1}$$

where the fitting parameters $\sigma_{\text{o}} = 1.9$ μm/min and $\kappa = 0.088$.

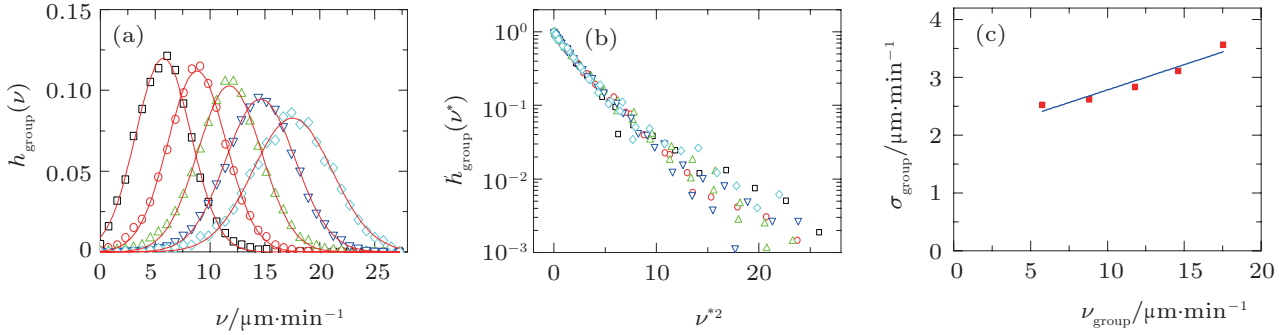



**Fig. 3.** (color online) (a) Normalized histogram of velocity measured by all displacements from the trajectories in each bin. The red lines are Gaussian fit of the data in each group. Trajectories are classified into five bins, 4.55 μm/min–7.58 μm/min (black squares), 7.58 μm/min–10.62 μm/min (red circles), and 10.62 μm/min–13.65 μm/min (green up triangles), 13.65 μm/min–16.68 μm/min (blue down triangles), and 16.68 μm/min–19.72 μm/min (light blue rhombuses). (b) The plot for $\log(h(v^*))$ versus $v^{\star 2}$, with $v^\star = (v - v_{\text{group}})/\sigma_{\text{group}}$, where $v_{\text{group}}$ and $\sigma$ are the fitting mean value and the standard deviation in the Gaussian fits in panel (a), respectively. (c) The standard deviation $\sigma_{\text{group}}$ as a function of $v_{\text{group}}$. The blue line is a linear fit of the data, $\sigma_{\text{group}} = \sigma_{\text{o}} + \kappa v_{\text{group}}$, with $\sigma_{\text{o}} = 1.9$ μm/min and $\kappa = 0.088$.

## 4. Discussion

With the help of empirical equation (1), we proposed a straightforward model to describe this inhomogeneity. First, we tried to rebuild the distribution $f(v)$ in Fig. 2(a) with the distributions $f(v_{\text{traj}})$ in Fig. 2(b)

$$P(v) = \sum_{\text{all groups}} g(\sigma_{\text{group}}, v_{\text{group}}) W_{\text{group}}, \tag{2}$$

where $P(v)$ is the probability distribution function (PDF) of $v$ before classification, $g(\sigma_{\text{group}}, v_{\text{group}})$ is the distributions of velocity fluctuations of each group, and $W_{\text{group}}$ is the weight of each group, which directly related to the number of cells in the group. Second, the bin-width of the cell groups were decreased continuously and the discrete equation (2) would change into an integral form as the following equation (4). Here $v_{\text{group}}$ was replaced by the mean velocity of a trajectory $v_{\text{traj}}$ and $W_{\text{group}}$ was now replaced by the histogram number of trajectories with a mean velocity of $v_{\text{traj}}$. Hence, equations (1) and (2) become as

$$\sigma_{\text{traj}} = \sigma_{\text{o}} + \kappa v_{\text{traj}}, \tag{3}$$

$$P(v) = \int_0^\infty g(\sigma_{\text{traj}}, v_{\text{traj}}) f(v_{\text{traj}}) \, \mathrm{d}v_{\text{traj}}, \tag{4}$$

where $f(v_{\text{traj}})$ is the histogram as shown in Fig. 2(b).

Physically, equation (4) supplies a clear insight into the dynamics of the cell culture. Generally, the instantaneous velocity will be forgotten by a cell after the typical persistent time. Because the persistent time was much shorter than the length of the trajectory, the velocities could be regarded as independent. These independent velocities falling into a small bin should obey Gaussian distribution under the Central-Limit Theorem. The width of this Gaussian distribution depends on the mean velocity, as shown in Fig. 3(c). Meanwhile, the velocities of the cell are not thesame. $f(v_{\text{traj}})$ plays a determinant role in the system, which responds to the observed non-Gaussian distributions indeed.

Furthermore, to thoroughly understand the significance of the exponential tail of $f(v_{\text{traj}})$, we made a numerical simulation as follows. For simplicity, we construct the formula of $F(v_{\text{traj}})$ to represent the form of $f(v_{\text{traj}})$ as follows:

$$F(v_{\text{traj}}) = \begin{cases} C_0, & v_{\text{traj}} < v_{\text{cri}}, \\ C_1 \exp(-\lambda v_{\text{traj}}), & v_{\text{traj}} \geq v_{\text{cri}}, \end{cases} \tag{5}$$

where $v_{\text{cri}} = 13$ μm/min and $C_1 = 0.06$ is chosen to overlap the tail of $f(v_{\text{traj}})$, $C_0 = 0.168$ is chosen to keep the areas under two curves equal, and $\lambda = 0.465$ min/μm is the fitting parameter from Fig. 2(b). The experimental data $f(v_{\text{traj}})$ and the constructed $F(v_{\text{traj}})$ are shown in Fig. 4(a).

Finally, one obtains

$$P(v) = C_2 \int_{v_{\rm cri}}^{\infty} \frac{1}{\sigma_{\rm traj}\sqrt{2\pi}} \exp\left[-\frac{(v-v_{\rm traj})^2}{2\sigma_{\rm traj}^2}\right] \exp(-\lambda v_{\rm traj})\,{\rm d}v_{\rm traj} + C_3 \int_0^{v_{\rm cri}} \frac{1}{\sigma_{\rm traj}\sqrt{2\pi}} \exp\left[-\frac{(v-v_{\rm traj})^2}{2\sigma_{\rm traj}^2}\right] {\rm d}v_{\rm traj}, \tag{6}$$

where $C_2$ and $C_3$ are constants. Equation (6) suggests that the tail of the distribution of velocity is only dependent on $\lambda$, which is the characteristic length of the exponential tail of $f(v_{\rm traj})$.

Equation (6) is not easy to calculate analytically, instead we find out the answer by numerical calculation. We calculate $P(v)$ of a cell culture with $10^5$ cells following Eqs. (3) and (5). The result of $P(v)$ is shown in Fig. 4(b). It is well matched with experimental data.

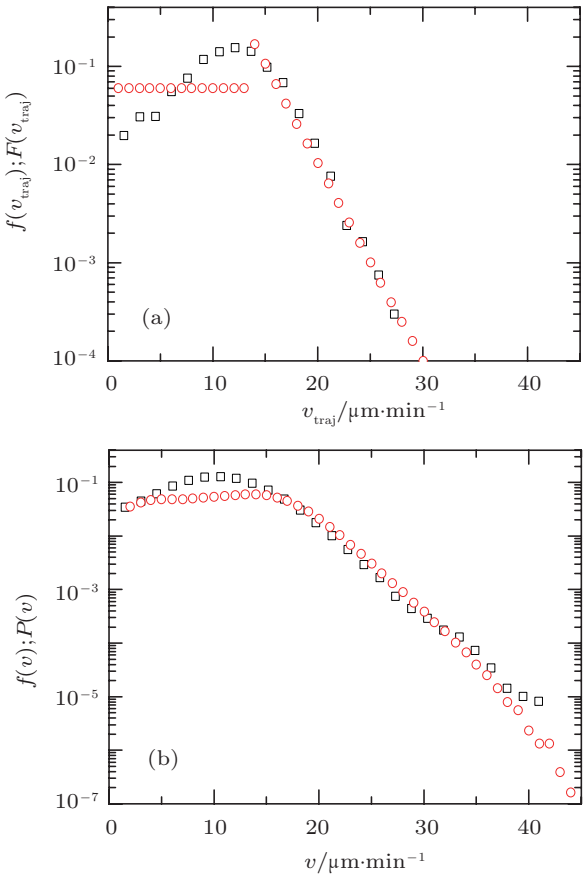



**Fig. 4.** (color online) (color online) Numerical calculation. (a) Normalized histogram of cell velocity $f(v_{\rm traj})$ (black squares) and numerical simulation data (red circles) from $F(v_{\rm traj})$. (b) Simulation result of the velocity distribution (red circles), compared with experimental data (dark squares).

Now we are able to review our understanding of the dynamics of cell motion. Previous studies believed that the cell motion was governed by random motion with an unclear form. We suggest understanding this system in the following steps. First, for an individual cell, the velocity fluctuates around a constant value $v_{\rm traj}$ and the fluctuation noise is multiplicative. The equation follows:

$$v(t) = v_{\rm traj} + (\sigma_{\rm o} + \kappa v_{\rm traj})\Gamma(t), \tag{7}$$

where $\Gamma(t)$ is a noise term with unit intensity. The values of $\sigma_{\rm o}$ and $\kappa$ determine the difference between values of the $\beta_1$ and $\beta_2$ in Fig. 2. Second, the values of $v_{\rm traj}$ vary from cell to cell and they obey a probability distribution $f(v_{\rm traj})$ with an exponential tail.

## 5. Summary

To summarize, with the help of a digital camera having spatial resolution of $5138 \times 3456$, we analyzed migrating trajectories of cells, and found the exponential tail of the probability distribution function of the cell velocity, which stems from the inhomogeneous weight number of cells for different diffusion motion. We believe this simple model is significant in understanding the random motion of eukaryotic cells, and also in finding the optimal strategy to explore the space.

## Acknowledgment

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## References

[1] Berg H C and Purcell E M 1977 *Biophys. J.* **20** 193
[2] Czirok A, Schlett K, Madarasz E and Vicsek T 1998 *Phys. Rev. Lett.* **81** 3038
[3] Couzin I D and Krause J 2003 *Adv. Study Behav.* **32** 1
[4] Ni R, Puckett J G, DUfresne E R and Ouellette N T 2015 *Phys. Rev. Lett.***115** 118104
[5] Nourhani A, Crespi V H and Lammert P E 2014 *Phys. Rev. B* **89** 062304
[6] Maree A F M and Hogeweg P 2014 *Proc. Natl. Acad. Sci. USA* **98** 3879
[7] Varnum B and Soll D R 2014 *J. Cell Biol.* **99** 1151
[8] He W, Song H, Su Y, Geng L, Ackerson B J, Peng H B and Tong P 2016 *Nat. Commun.* **7** 11701
[9] Li L, Norrelykke S F and Cox E C 2008 *Plos One* **3** e2093
[10] Dieterich P, Klages R, Preuss R and Schwab A 2008 *Proc. Natl. Acad. Sci. USA* **105** 459
[11] Takagi H, Sato M J, Yanagida T and Ueda M 2008 *Plos One* **3** e2648
[12] Shenderove A D and Sheetz M P 1997 *Biolphys. J.* **72** 2382
[13] Viswanathan G M, Afanasyev V, Buldyrev S V, Havlin S, da Luz M G E, Raposo E P and Stanley H E 1999 *Nature* **401** 911
[14] Viswanathan G M, Bartumeus F, Buldyrev S V, Catalan J,Fulco U L, Havlin S, da Luz M G E, Lyra M L, Raposo E P and Stanley H E 2002 *Physica A* **314** 208
[15] Reynolds A 2002 *J. Plrev.* **14** 59
[16] Chen S, Li N, Hsu S F, Zhang J Z, Lai P Y, Chan C K and Chen W 2014 *Soft Matter* **10** 3421
[17] Uhlenbeck G E and Ornstein L S 1930 *Phys. Rev.* **36** 823