Parameterizing cell movement when the instantaneous cell migration velocity is ill-defined

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Cell crawling has usually been characterized by a diffusion constant $D$ and instantaneous velocity $\langle |\vec{v}|^2 \rangle$. However, experimentally $\langle |\vec{v}|^2 \rangle$ diverges. A three regime (diffusive-ballistic-diffusive) modified Fürth equation parameterized by $D$, the dimensionless excess diffusion coefficient $S$ and the persistence time $P$ is compatible with experiment. $S$ allows comparison of trajectories across experiments and sets limits on the intervals and duration of experiments required to assess cell movement. Cell trajectories in a variety of published experiments are consistent with longitudinal Langevin dynamics and a transverse Wiener process with $S \sim (1 + \text{constant} \times D)^{-1}$.

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1. Introduction

Cell crawling on substrates is ubiquitous in healthy and pathological biological processes [1–4]. Experiments usually quantify cell motion from time-series images (movies) of migrating cells. Cells’ trajectories are estimated from the geometric center of the projected total visible area of either the cell or its nucleus and are typically summarized by their mean-squared displacement (MSD) and velocity auto-correlation function (VACF) curves. The standard mathematical framework for parameterizing cell migration derives from a Langevin equation for the instantaneous velocity of a particle, which experiences viscous drag and white-noise forcing. The key parameters in this framework are the diffusion constant, the persistence time and the instantaneous cell velocity, which an experimentalist must approximate from measured cell displacements over finite-time intervals (see, for example, [5–9]). The Fürth Equation then describes the predicted MSD [10,11]:

$$\text{MSD} (\Delta t) = \left\langle |\Delta \vec{r}|^2 \right\rangle = 4D \left( \Delta t - P \left( 1 - e^{-\Delta t/P} \right) \right),$$

where $\Delta t$ is the time elapsed between two measurements of the cell position $\vec{r}$, and $\langle \cdot \rangle$ stands for averages over time and different runs of equivalent experiments. The persistence time, $P$, defines the timescale of the transition between short-time-interval ballistic motion ($\Delta t \ll P$) and long-time-interval ($\Delta t \gg P$) diffusive motion with diffusion coefficient $D$. As

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\[
\left\langle \Delta \vec{r} \right\rangle^2 \sim 4D (\Delta t)^2 / P \text{ for } \Delta t \to 0, \text{ the instantaneous velocity } \vec{v} \text{ is well defined, with } \left\langle |\vec{v}|^2 \right\rangle = 2D. \text{ For a stationary process, we may calculate the VACF as half the second derivative of Eq. (1):}
\]
\[
\text{VACF (}\Delta t\text{)} = \frac{2D}{P} e^{-\Delta t / P}.
\]

However, the experimental literature for cell migration reports significant deviations from Fürth behavior at short-time intervals \([5,12-15]\). Typically, authors attribute these deviations either to a two-constant exponential decay for the VACF, e.g., \(\text{VACF} = \phi_1 e^{-\Delta t / P_1} + \phi_2 e^{-\Delta t / P_2} [5]\) or to localization errors in the estimated cell positions \([12,15]\). Here, we analyze data available in the literature for twelve cell-migration experiments from five different laboratories (Takagi et al. [6], Dieterich et al. [12], Potdar et al. [13], Wu et al. [15] and Metzner et al. [16], see table S1 in supplemental materials online [17] for details on the experiments) and show that these short-time-interval deviations are more consistent with a three-regime model with a short-time-interval diffusive regime in addition to the classicFürth-like intermediate-time-interval ballistic regime and long-time-interval diffusive regime (See Fig. S2 in supplemental materials online).

Short-time-interval diffusive behavior means that instantaneous velocity is ill-defined, so a workable theoretical framework to describe cell migration must not use instantaneous velocity as a variable. Short-time-interval diffusive behavior also means that the relation \(|\vec{v}|^2 = \frac{2D}{P}\) is compromised and must be, at the least, reinterpreted. Here we propose an alternative framework to quantify cell migration, which avoids the instantaneous velocity and discuss its implications for a possible theoretical description of cell migration.

### 2. Analysis of experimental data

We start by discussing a 2D migration experiment by Metzner et al. [16], for MDA-MB-231 cells on a plastic substrate. We calculated the MSD curves from their published trajectory data. Fig. 1a presents the VACF, estimated from the second derivative of the MSD curve. We fit these data points using Eq. (2) to estimate the diffusion coefficient \(D\) and persistence time \(P\). Fig. 1b presents the MSD data (black squares) together with the plot for the Fürth Equation, Eq. (1), (green line) using the estimated values for \(D\) and \(P\). The blue dots show the residual, subtracting the Fürth equation from the experimental data. The deviation increases with the time interval, \(\Delta t\), suggesting a short-time-interval diffusive additive correction \(\left(\frac{2DS}{1-S}\right)\Delta t\) to Eq. (1), yielding a modified Fürth equation:

\[
\left\langle \Delta \vec{r}^2 \right\rangle = 2D (\Delta t - P (1 - e^{-\Delta t / P})) + \frac{2DS}{1-S},
\]

where the excess diffusion coefficient \(0 \leq S < 1\) can be interpreted as the fraction of the persistence time \(P\) at which the short-time-interval diffusive behavior ends and cell movement becomes ballistic-like. For reasons that we will discuss below, we choose to use the pre-factor 2 instead of 4 (corresponding to 2D motion in the original Fürth Equation) in the first term of the right-hand side of Eq. (3). When experimental cell tracking trajectories follow Eq. (3), their quantitative characterization requires three parameters: an effective diffusion constant \(D_{\text{flat}} \equiv \left(\frac{2DS}{1-S}\right)\Delta t\) for \(\Delta t < SP\), an effective ballistic speed of \(\frac{1}{1-S} \sqrt{\frac{D}{P}}\) for \(SP < \Delta t < P\), and an effective diffusion constant \(D_{\text{slow}} \equiv \left(\frac{2DS}{2S}\right)\Delta t\) for \(\Delta t > P\). For \(\Delta t < SP\), cell movement is diffusive rather than ballistic, as in the usualFürth framework. Observe that the above definition of effective ballistic speed avoids using velocity, while \(S = D_{\text{flat}} / D_{\text{slow}}\), meaning that \(S\) defines the relative diffusion excess implied by Eq. (3).

See detailed discussion in supplemental materials and supplemental Fig. S1 [17]. The red line in Fig. 1b shows an almost perfect agreement between the best fit to the modified Fürth equation and the experimental data. We conducted similar fits for two different experiments from five different laboratories. Supplemental Figs. S3–S16 show equivalent analyses to Figs. 1a and b for the other 11 analyzed experiments [17].

Unlike Eq. (2), in Eq. (3), \(\left\langle \Delta \vec{r}^2 \right\rangle \sim 2D \Delta t / P\) for small time intervals. Hence \(\frac{\left\langle |\vec{r}|^2 \right\rangle}{\Delta t}\) diverges as \(\Delta t\) goes to zero: instantaneous velocity is thus ill-defined, though \(S\), \(P\) and \(D\) are all well-defined. This divergence is also found in pure Brownian processes defined by the dynamical equation \(\frac{d\vec{r}}{dt} = 2D \xi (t)\), where \(\xi (t)\) is white noise. For a finite time interval \(\Delta t\), \(\text{MSD} = 2D \Delta t\), and diffusion coefficient may be defined as half the slope of the MSD curve, which is the same for both long- and short-time intervals. For Ornstein–Uhlenbeck processes, velocity is a well-defined quantity, and hence the limit \(\lim_{\Delta t \to 0} \frac{\left\langle |\vec{r}|^2 \right\rangle}{\Delta t}\) converges (implying \(\lim_{\Delta t \to 0} \frac{\left\langle |\vec{v}|^2 \right\rangle}{\Delta t} = 0\)). Analogously, we propose to define the diffusion coefficient in Eq. (3) to be half the slope of the MSD curve for long time intervals (not for \(\Delta t \to 0\)). In Ornstein–Uhlenbeck processes, the relation \(|\vec{v}|^2 = \frac{2D}{P}\) associates the parameters characterizing long-time (\(D\)) and short-time (\(|\vec{v}|^2\)) behaviors. Our analyses suggest that cells crawling on flat surfaces are not described by a classical Ornstein–Uhlenbeck process, instead \(S\), relates the properties of the long- and short-time diffusive regimes.

We may rescale Eq. (3) in terms of natural units, i.e., time in terms of the persistence-time-scale \(P\) and length in terms of the persistence length-scale \(\sqrt{2DP/(1-S)}\), by defining \(\tau = t / P\) and \(\hat{r} = r / \sqrt{2DP/(1-S)}\):

\[
\left\langle |\Delta \hat{r}|^2 \right\rangle = S P (1 - S) \left(1 - e^{-\Delta \tau / S}\right).
\]

Because \(S\) is dimensionless, it is not affected by rescaling and we are left with a family of curves specified by the single dimensionless excess diffusion parameter, \(0 \leq S < 1\), that determines both the time ranges over which the three regimes
Fig. 1. Fitting procedure for cell migration data. (a) Second time derivative of the MSD from experimental data and the exponential fit (using the ‘instrumental’ option in ORIGIN-Pro 2017 software) estimating $D$ and $P$. (b) The Fürth equation calculated using the estimates for $D$ and $P$ is subtracted from the experimental data for the MSD, yielding the blue-dot curve, which we fit with a straight line whose slope relates to $S$ and intercept to the localization error. The inset shows the same plot with the standard error for short-time intervals: at larger time intervals, the errors are not visible. The sum of the Fürth equation and fitted straight line gives the modified Fürth equation (red line). (c) and (d) log–log plots for the MSD, in natural units (laboratory units in the insets), and fits for the Fürth (dashed line, Eq. (1)) and for the modified Fürth equations (solid lines, Eq. (3)).

apply and the effective speed and diffusion coefficients in the appropriate regimes. Fig. 1c–d and Figs. S14–S16 show MSD curves and fits for all analyzed experiments, while Table S1 in the supplemental materials online [17] provides details on the experiments and the fitted values for $D$, $P$ and $S$. For intermediate- and long-time intervals, the original and modified Fürth equations fit the experimental data equally well, showing that Eq. (1) suffices to describe the long-time-interval behavior of many types of cell migration. However, the modified Fürth equation fits the data at shorter time intervals (when available), while the original Fürth equation does not. Experimental error bars in Fig. 1(b) and (d) are small, showing that the deviation between the experiments and the original Fürth equation at short-time intervals are significant.

Although instantaneous velocity is not well-defined, we can define a mean velocity over any finite time interval $\delta$ (secant velocity approximation), $\bar{u}(\tau, \delta) = \frac{\bar{u}(\tau+\delta) - \bar{u}(\tau)}{\delta}$. Fig. 2a, presents $\langle |\bar{u}(\tau, \delta)| \rangle$ as a function of $\delta$ for the three experiments by Metzner et al. [16], showing that the magnitude of the mean velocity indeed diverges as $\delta \to 0$, indicating that instantaneous velocity is ill-defined. The small error bars support the interpretation of the curve as divergent as $\delta \to 0$.

For stationary processes, we can calculate the VACF as half the second derivative of the MSD curve. To avoid using the ill-defined instantaneous velocity, we define the mean velocity auto-correlation $\psi_\delta(\Delta \tau)$:

$$
\psi_\delta(\Delta \tau) = \left\langle \frac{1}{T/P - \Delta \tau} \int_0^{T/P - \Delta \tau} dt \bar{u}(\tau, \delta) \cdot \bar{u}(\tau + \Delta \tau, \delta) \right\rangle,
$$

where $T$ is the experiment duration and the integration interval ranges from 0 to $T/P - \Delta \tau$ to ensure that $\bar{u}(\tau + \Delta \tau, \delta)$ always stays inside the interval. $\psi_\delta$ detects trivial correlations when $\delta > \Delta \tau$, since the intervals used to calculate $\bar{u}(\tau, \delta)$ and $\bar{u}(\tau + \Delta \tau, \delta)$ overlap. Avoiding this artifact could explain why we have not found the two exponential time scales reported in Refs. [5] and [9]. Fig. 2c–e present $\psi_\delta(\Delta \tau)$ for the 3 datasets by Metzner. The original and modified Fürth
equations have identical second derivatives and predict the same VACF (blue, solid lines). The theoretical and measured VACFs disagree for small $\delta$ and time intervals with $\delta < \Delta \tau = \frac{\Delta t}{P} < S$. Specifically, for short intervals, the short-time-interval diffusive motion, which is uncorrelated, is the dominant component in the secant velocity approximation $\vec{u}(\tau, \delta)$.

Thus, for any finite-precision measurement $\psi_\delta(\Delta \tau)$ is lower than the theoretical ideal when $\delta < \Delta \tau < S$. The fits for the twelve experiments yield a minimum value of $S \sim 0.01$, suggesting that an informative experimental time series requires a time-interval between images $\leq 0.01P$ and a total observation time $\geq 50P$, to explore all three regimes and allow accurate quantification of the modified Fürth equation parameters $D$, $P$ and $S$. Furthermore, we recommend tens of repetitions for each experimental condition, to guarantee a second derivative of the MSD smooth enough for fitting.

The origin of the short-time-interval diffusive behavior requires discussion. Errors in calculating the cell position due to either the pixelization of the images or to the movement of the cell’s lamellipodium, would also generate non-Fürth behavior at short time intervals. However, these alternatives predict deviations from Fürth behavior, which disagree with the experimental data. A localization error affects the estimates of displacement with a magnitude independent of the smallest time interval, while the observed correction to the short-time-interval Fürth prediction increases with the time interval between the position measurements. The average velocity estimates and correlations between parameters and between experiments, all agree with the modified Fürth equation and disagree with the localization-error only hypothesis.

To check for the significance of localization error in combination with short-time-interval diffusive behavior we fit both corrections simultaneously (see Fig. 1 legend and supplemental materials online) and find that the correction attributable to localization error is very small, less than $1 \mu m$.

Fig. 2a shows that the average speed $\langle ||\vec{u}(\tau, \delta)|| \rangle$ in natural units for Metzner et al.’s collagen data [16], with $S = 0.339$, diverges with a slope equal to $-0.5$, compatible with short-time-interval diffusive behavior. For the other cases, the slope is less steep, while localization error by itself would cause the data to diverge with a slope of $-1.0$. Fig. 2c-e also show $\psi_\delta(\Delta \tau)$ calculated directly from the trajectories for different values of $\delta$, and compare them to the second derivatives of Eq. (4) and the MSD experimental data. All $\psi_\delta(\Delta \tau)$ agree for the three experimental sets for $\Delta \tau > S$, indicating a stationary process. For $\delta < \Delta \tau < S$, $\psi_\delta(\Delta \tau)$ and the second derivative of the experimental MSD decrease, indicating a loss of mean-velocity correlation. The analytical second derivative of Eq. (4) does not fit the experimental data for small time intervals. Together, the behavior of $\langle ||\vec{u}(\tau, \delta)|| \rangle$ and $\psi_\delta(\Delta \tau)$ provide strong evidence that cell migration at small time
intervals differs from Fürth behavior. The behavior of \(\langle|\vec{u}(\tau, \delta)|\rangle\) strongly supports a short-time-interval diffusive correction as in Eq. (3).

Supplemental materials online show more tests for the correction term \(17\). After determining \(D\) and \(P\) for data by Dieterich et al. \(12\), we fitted for localization error only and for diffusive correction and localization error simultaneously. When we subtract the Fürth equation (calculated using the best-fit values for \(D\) and \(P\)) from the experimental data, the difference increases with increasing time interval, in agreement with the diffusive correction. In a second test, we took a series of increasing time intervals as the shortest (reference) time interval. While the reference interval has almost no effect on the values of \(S\) when we consider diffusive and localization corrections together, the localization-only correction increases linearly with the reference interval, showing the need for the diffusive correction (see Figs. S17–S18 \(17\)).

Fig. 2b plots the fitted values of \(S\) versus \(D\) for the twelve analyzed experiments. One parameter describes the short-time-interval range, the second describes the behavior of the MSD for long time intervals. While \(S\) and \(D\) could, in principle, be unrelated, the plot clearly shows a correlation between the two parameters (roughly \(S \sim D^{-1}\)). We have considered the errors in determining \(S\) and \(D\) as the regression coefficients \(\langle R^2 \rangle\) found in the fitting procedures: in obtaining \(D\) from either the MSD curve or from its second derivative and in obtaining \(S\) from the linear correction to the pure Furth behavior (see Fig. 1b). We obtained the fit in Fig. 2b using ORIGINPRO 2017, using the OCD option to account for error bars in both \(D\) and \(S\) for each point. The regression coefficient for the fit in this figure is 0.9998.

3. Discussion and conclusion

The Fürth equation is the solution of a Langevin process, which is often assumed to describe a migrating cell’s movements. It also assumes that the cell’s instantaneous velocity is well-defined. The Fürth equation successfully reproduces experimental MSD curves for intermediate and long time intervals. However, the original Fürth equation does not fit experimental MSD curves at short time intervals, where the cell’s instantaneous velocity seems to diverge. Many authors have either attributed this short-time-interval divergence to measurement error, or simply disregarded it. Here we have shown that this short-time behavior can be captured by using a modified Fürth equation that explains the divergence as due to a diffusive component of cell motion at short time intervals. We can understand the reason for the divergent instantaneous velocity and well-defined intermediate-time-interval velocity by hypothesizing that the cell has an instantaneous polarization axis along which the cell follows Langevin dynamics and instantaneous velocity is well-defined, and that perpendicular to this axis the cell movement follows a Wiener process with Brownian displacements of the position for which the instantaneous velocity is divergent. For a given infinitesimal time interval \(\Delta t\), the polarization axis remains constant and the cell displacement may be written as \(\Delta \vec{r} = \Delta \vec{r}_\parallel + \Delta \vec{r}_\perp\), where \(\Delta \vec{r}_\parallel\) and \(\Delta \vec{r}_\perp\) are the displacements respectively parallel and orthogonal to the polarization direction. The displacement over a finite time interval is the sum of a succession of such infinitesimal displacements, adjusted as the direction of the polarization changes. The average over time and different realizations of such a process results in a mean-squared displacement given by:

\[
\langle |\Delta \vec{r}|^2 \rangle = \langle \int d\vec{r}_\parallel^2 \rangle + \langle \int d\vec{r}_\perp^2 \rangle + 2 \langle \int d\vec{r}_\parallel \cdot \int d\vec{r}_\perp \rangle.
\]

The first term on the right-hand side of Eq. (6) gives the original Fürth equation, since in this direction the cell follows a Langevin process. In the direction perpendicular to the polarization axis, the Wiener process generates the second term on the right hand side of Eq. (3), which is proportional to \(\Delta t\). The third term vanishes. Eq. (6) thus generates the modified Fürth equation. Based on this rationale, we included the factor 2 in the first term on the right-hand side of Eq. (3), corresponding to a one-dimensional Fürth term. The second dimension is represented in the second term, as a Wiener process.

In Eq. (3) \(S\) and \(D\) are independent. Why is \(S \sim (1 + \text{constant} \cdot D)^{-1}\) in the experiments we analyzed? Mechanistically, the short-time-interval diffusive regime stems from the fast movement of the cell membrane or nucleus, which in turn, stems from local fluctuations in the actin network in the cell, primarily in the lamellipodium. On the other hand, persistence in cell migration stems from the larger-scale cytoskeletal polarization, which also depends on the actin network. Thus, actin network fluctuations affect both the short-time-interval diffusive regime and long-time changes in polarization direction, leading to the long-time-interval diffusive regime and we should expect to see some functional relationship between the parameters \(S\) and \(D\) which embodies this common mechanism. However, we still do not understand why the dependence is of the specific form \((1 + \text{constant} \cdot D)^{-1}\). We hope that our observation will promote experiments to resolve this question.

Additionally, when short-time-interval motion is diffusive, instantaneous velocity is not well-defined, but we may still measure the mean velocity over any finite time interval. In this case, the mean velocity will depend on the time interval \(\delta\) we used. Thus, any attempt to compare measured estimates of speed or velocity autocorrelations between experiments will depend critically on choosing compatible time intervals, which is generally not possible for published data sets. Estimates of \(D\), \(P\) and \(S\) using the modified Fürth equation, on the other hand, will be directly comparable using our rescaling of time and length. Since \(S\) is independent of the rescaling, our modified Fürth equation provides a way to directly compare experimental data from different experiments and computer simulations of cell movements, even if the latter lack direct time- or spatial-unit correspondence.
Our findings have some direct biological implications: (i) In experiments cell speed should be estimated paying attention to the implications of the fast-diffusive regime. The mean speed should be checked for different time intervals and its stationarity or divergence as the interval decreases evaluated. Divergence indicates that short-time-interval diffusion is significant and that mean speed should not be used when comparing results among cell lines, experiments or simulations. Simply using the smallest available time interval is insufficient. (ii) Fig. 2b shows an unexpected functional relation between $D$ and $S$ in experiments which implies a mechanism linking cell’s short- and long-timescale behaviors. This mechanism behind this relation deserves experimental study. (iii) $D$, $P$, and $S$ determine how rapidly cells disperse as they migrate. They could therefore reveal specific characteristics of cell lines related to invasiveness in cancer, for example. Thus the characterization of these parameters for a wide variety of cell types and situations could be worthwhile.

While this paper does not present a dynamical explanation for the observed cell trajectories, trajectories obeying the modified Fürth equation must emerge from dynamic equations. The simplest approach to generating such models is to extend the Langevin model, as suggested above. When considering possible models we note that any model which generates the modified Fürth equation, must have the following characteristics: (i) Its dynamics must be two or more dimensional and anisotropic, although symmetry could be dynamically broken (as in real cells). (ii) Movement in at least one direction must be diffusive at short time intervals, e.g., as described by a Wiener process. In a separate paper we have defined a simple model of this type and solved it both analytically and numerically. We find that it obeys the modified Fürth equation with $S \sim (1 + \text{constant } D)^{-1}$ and the VACF and mean-velocity autocorrelation behave as seen in the cell-migration experiments analyzed here [18].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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