Cell Physician: Reading Cell Motion

A Mathematical Diagnostic Technique Through Analysis of Single Cell Motion

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Abstract Cell motility is an essential phenomena in almost all living organisms. It is natural to think that behavioral or shape changes of a cell bear information about the underlying mechanisms that generate these changes. Reading cell motion, namely, understanding the underlying biophysical and mechnanochemical processes, is of paramount importance. The mathematical model developed in this paper determines some physical features and material properties of the cells locally through analysis of live cell image sequences and uses this information to make further inferences about the molecular structures, dynamics, and processes within the cells, such as the actin network, microdomains, chemotaxis, adhesion, and retrograde flow. The generality of the principals used in formation of the model ensures its wide applicability to different phenomena at various levels. Based on the model outcomes, we hypothesize a novel biological model for collective biomechanical and molecular mechanism of cell motion.

Keywords cell motility · inverse problem · material parameters · actin network · chemotaxis · lateral signal diffusion · microdomains · membrane ruffling · adhesion formation · retrograde flow

1 Introduction

Cell motility is an essential phenomena in almost all living organisms. Organ and tissue formation during development of an embryo, maintenance of tissues, wound healing, white blood cell movement in immune response, and generation of new blood vessels, etc., are some examples in which cell motility is essential [1, 4]. On the other hand, pathology of motion causes many diseases. To name a few, arthritis and neurological birth defects have their roots in cell motility dysfunction. Increase in the motility of tumor cells may be associated with cancer metastasis. Aside from motion, differences in material properties of cancer and normal cells are also observed [24, 41, 42].

Recent biological theories explain that the important steps of cell motion are protrusion, adhesion, and contraction of the cell through the action of the cytoskeleton [4, 11, 28]. Briefly, it is thought that polymerization and bundling of the actin filaments produce directed force to create protrusion at the cell membrane. The protruding site contacts and adheres to the substratum. Activation of the actomyosin complex, with depolymerization and unbundling in some cases, causes contraction at the rear (or near the nucleus) of the cell. In terms of mechanical terminology, the material of the cell is known to be viscoelastic and heterogeneous according to experimental measurements [5, 26, 43]. It is proposed in [9] that the models for single cell motility in the literature can be divided into “discrete” and “continuous” models in physical terms [2, 3, 8, 9, 12, 13, 21, 29, 30, 37, 44]. The model, called the Anchor Model, introduced in this paper provides an example of the combination of both; it uses continuum mechanical means on discrete subunits.
Cells are living organisms, and it is natural to think that behavioral or shape changes of a cell bear information about the underlying mechanisms that generate these changes. Reading cell motion, namely, understanding these underlying biophysical and mechanochemical mechanisms of behavioral and shape changes is of paramount importance. This is analogous to the examination of patients, analysis of their symptoms and behavioral changes by a physician for diagnosis. The mathematical model developed here plays the role of a physician or the physical exam in diagnosis of cellular pathologies instead of human subjects. Thus, the technique translates the most obvious physical information, i.e. cell position, into practical and clinically usable form.

The first attempt in analysis of motion in the literature to derive such useful information is found in [7, 9]. Dynamic material properties such as elasticity and viscosity, quantitative relations between molecular dynamics and continuum mechanics, and the definition of force generation as a scalar potential field are formulated coherently in [7]. The technique developed in that work is classified as model based inverse problem to emphasize the difference from the experimental measurements of the material properties. While forward problem formulates the governing equations of cell motion where a set of material parameters is given, inverse problem formulates equations to extract information from the motion and determines the material parameters. These parameters in turn can be used as input for the forward problem to mimic the given cell motion. Forward problem is formulated by a system of ordinary differential equations and inverse problem by linear algebraic systems. The model based inverse problem approach allows observing and analyzing the motion as it naturally takes place. It seems to be advantageous over some experimental methods which use physical constraints or, in some cases, external agents that may possibly interfere with the motion. Another advantage of the technique is its time and cost efficiency in comparison to the experiments. In line with a series of papers on systematic treatment and analysis of the phenomena, this paper introduces a novel quantitative technique for extracting local information from the motion as opposed to the global analysis of the Ring Model (Fig. 1(a)) [9].

Although the Anchor Model provides local information, namely the relevant quantities at discrete points in space at specific times, it uses continuum mechanical means, more specifically, linear viscoelastic solid material relations to derive the model formulation. Local analysis keeps the model equations simple with a very efficient computing time regardless of the dimension, number of subunits used, or the duration of the experiment. Extension of the model to three-dimensional analysis, for example, is immediate after some minor modifications. In the Anchor Model, determination of the evolution of the points for local analysis is a nontrivial task. In [9], positions of the points are found manually from cell and nuclear morphology. Later, the process of point tracking is automated through the development of an open access software which includes extended active snake techniques for better segmentation of cell boundary [36] (see Appendix).

This paper further addresses important molecular components of cell motion such as microdomain dynamics, actin polymerization and network dynamics, myosin–induced cell boundary retraction and cell body contraction, adhesion site formation, and chemotaxis based on the model outcomes. Polymerization and active myosin concentration, in mechanical terms protrusion and retraction, are quantified with a linear function of positive and negative normal displacement, respectively, at the cell boundary; these relations are justified through model application to and analysis of the experimental data. The strain function then provides a quantitative tool to track polymerization history, and consequently to estimate the network topology. Motile microdomains/microclusters/lipid rafts have been under investigation as scaffolds for signal transduction and as molecular structures which organize membrane proteins in the literature [6, 27, 38]. We propose that strain rate indicates spatio-temporal organization of microdomains on the cell membrane and so determines signaling dynamics and cell’s local chemotactic response.

Besides the Ring Model [9], recent studies have investigated the evolution of the single cell boundary and regular pattern formation associated with the analysis of displacement and normal velocities [10, 21, 25]. As similar patterns appear in all model applications to different cell types, our results agree with the findings that local membrane waves constitute a universal organizational principle or dynamic pattern of motile cells [10, 25]. The Anchor Model, on the other hand, is based on novel forward and inverse problems which incorporates strain and strain rates into the analysis, determines characteristic physical parameters of the cell, and develops a mathematical diagnostic tool for cell profiling, characterization, and classification based on the model outcomes. Moreover, we hypothesized a novel biological model to address the universal principle in formation of regular patterns by relating them to microdomain dynamics and focal adhesion site formation. This model incorporates other important biomechanical and molecular mechanisms of cell motion, such as retrograde flow and membrane ruffling and addresses their collective behavior. Although transversal wave propagation is observed in our work as in other studies, the waves are not continuous. This difference maybe due to the nonphysical mechanical model or the level set technique used, in which the predetermined speed formulation for evolution of the level sets subsequently determines the velocity of the zero level set (cell boundary) [25].

The generality of the underlying principles of the model ensures its applicability to many organismal phenomena at various levels for different interpretations guided by the biology of the phenomena at which the nonrigid morphological changes are important. Similar to the crawling cell motion which is used for the model applications in this paper, it can also be applied, for example, to the contractile motion of a beating myocyte at cellular level and that of the heart and the
bladder at the organ level. Locality of the technique allows use of the model for the dynamics of subcellular structures as well. It can also be applied to tumor tissue for similar analysis, derivation of the material properties of the cancer tissue and its growth rate, etc. Through intervention studies, evolution of the cell boundary and underlying molecular dynamics can be investigated for identification of cancer biomarkers. The model may also prove useful in novel drug development as the effect of the drugs on single cells can be quantified by this technique.

Since all computations are made based on the analysis of live cell image sequences which are 2D projections of a 3D motion, and since pixel and frame numbers are used instead of actual space dimensions and time, the quantities determined and discussed in this paper are mainly approximations. The results are compared with actual experimental measurements in some cases after necessary unit conversions. Qualitative analysis of each application studied in the paper also justifies the use and wide applicability of the model to various cell types. The conclusive results, however, can be obtained through application of the method systematically to a population of specific cell types for comparison. As an example of a systematic application of the model, the morphological changes and motility of single cancer cells are being investigated and their comparison to normal cells are being studied for characterization and classification of cancer cells in an ongoing work. The results will appear in a separate paper.

Other than physical parameters, two other quantitative tools are developed for analysis of model outcomes and interpretation of cell motion: motility index and deformation index. These quantities are introduced and used in the following sections.

2 The Anchor Model

Let a space-wise parameterization \( s_t \) of the smooth, closed cell boundary, \( \psi \), in counterclockwise direction at a fixed time \( t \) be given as

\[
s_t : \tau \in [0, \pi] \subset \mathbb{R} \rightarrow (x(\tau,t), y(\tau,t)) \in \mathbb{R}^2,
\]

and time parameterization of the trajectory of a point on the boundary for a fixed \( \tau \), \( T_\tau \), is given by

\[
T_\tau : t \in \mathbb{R} \rightarrow (x(\tau,t), y(\tau,t)) \in \mathbb{R}^2.
\]

For \( x = (x,y) \), the boundary condition for a closed boundary and the initial condition becomes

\[
x(\tau,0) = x_0(\tau), \quad x(0,t) = x(\tau,t).
\]

If a set of discrete points on the boundary at \( \tau = \tau_i \) are chosen to be

\[
T_i : t \in \mathbb{R} \rightarrow x_i = (x_i(t), y_i(t)) = (x(\tau_i,t), y(\tau_i,t)) \in \mathbb{R}^2, \quad i = 1, \ldots, n,
\]

then the local, moving coordinate vectors \( u^i \) and \( u^\omega \) can be approximated as

\[
u^i = \frac{x_{i+1} - x_{i-1}}{|x_{i+1} - x_{i-1}|} \quad \text{and} \quad u^\omega = C u^i \quad \text{where} \quad C = \begin{bmatrix} \cos(\pi/2) & \sin(\pi/2) \\ -\sin(\pi/2) & \cos(\pi/2) \end{bmatrix} = \begin{bmatrix} 0 & 1 \\ -1 & 0 \end{bmatrix}
\]

is the clockwise rotation matrix. The first point \( x_1 = x_0(0) \) is called the pivot point, the superscript \( t \) stands for tangential direction, and subscript \( n \) for normal direction with respect to the boundary (Fig. 1(b)).

The velocity, \( v_i = (v^i, v^\omega) := \dot{x}_i(t) \), displacement, \( d_i = (d^i, d^\omega) := x_i(t) - x_i(0) \), and force, \( \sigma_i = (\sigma^i, \sigma^\omega) \), vectors can be written in terms of local moving coordinates at each point \( x_i \) as

\[
f_i = f^i u^i + f^\omega u^\omega.
\]

The coordinates, then, can be written in terms of the wedge product of \( f_i \) and coordinate vectors,

\[
\begin{bmatrix} f^i \\ f^\omega \end{bmatrix} = \frac{1}{u^i \wedge u^\omega} \begin{bmatrix} f_i \wedge u^\omega \\ u^i \wedge f_i \end{bmatrix}.
\]
**Forward Problem** The equation for \( \mathbf{v}_i \) becomes the governing equation of the forward problem:

\[
\mathbf{x}_i = v_i \mathbf{u}_i + v^h_i \mathbf{u}_v.
\]

Force components, \( \sigma^\alpha \), can be interpreted as principal stress at the point where the force is applied. The strain values can be written, using finite difference formulas, in the form of

\[
\gamma^i \approx \frac{\Delta d_i}{\Delta s} \approx \frac{d_{i+1} - d_i}{\Delta s} \quad \text{and} \quad \gamma^h_i \approx \frac{\Delta d_{h_i}}{\Delta s} \approx \frac{d_{h,i+1} - d_{h,i}}{\Delta s}
\]

where the displacement is discretized as \( d_i(t_j) = \mathbf{x}_i(t_j) - \mathbf{x}_i(t_{j-1}) \), that is, \( t_0 = t_{j-1} \) at each time step \( t = t_j, s(\tau, t) = \int_0^\tau ds(t')d\tau \) is the arc length at time \( t \), and \( \Delta s = |x_{i+1}(t) - x_i(t)| \).

It is known that an actin monomer has a diameter of about 4 – 5 nm. Due to the helical structure of the filaments, a monomer binding elongates the filament tip about 2.5 nm. Since each step in elongation generates a displacement in the direction of the filament tip according to the configuration before the polymerization takes place, linear relations are proposed between the positive part of normal displacement, \( d_{n,+} \), and the average number of polymerized actin, \( p_i \), as well as the negative part of normal displacement, \( d_{n,-} \), and the average number of active myosin, \( m_i \), in this model. That is,

\[
d_{n,+}(t) = k_p p_i(t), \quad d_{n,-}(t) = k_m m_i(t)
\]

at the point \( \mathbf{x}_i \) where \( k_p \) and \( k_m \) are global material proportionality constants and \( d_{n} = d_{n,+} - d_{n,-} \).

**Inverse Problem** A simple linear viscoelastic constitutive equation can be stated as

\[
\sigma = G \gamma + \bar{\eta} \gamma,
\]

where \( \sigma \) is stress, \( \gamma \) is strain, \( \bar{\eta} \) is strain rate, and \( G \) and \( \bar{\eta} \) are elasticity and viscosity coefficients respectively. Since the Anchor Model formulates local analysis in time and space, this choice of linear constitutive equation provides a reasonable approximation.

All points are assumed to be masses with negligible weight work against drag forces during the motion due to cell-substrate interactions. A force balance equation can be stated at each point as \( \sigma^\alpha - \bar{\mu}^\alpha v^\alpha = 0 \) where \( \bar{\mu} \) is the drag coefficient with units of \( N \cdot s/m^3 \). The force balance equation then reads, after employing Eq. (2.11) and scaling, as

\[
G^\alpha \gamma^\alpha + \eta^\alpha \dot{\gamma}^\alpha = v^\alpha, \quad \alpha = t, i, n,
\]

where \( G^\alpha = \tilde{G}^\alpha/\mu^\alpha \) and \( \eta^\alpha = \tilde{\eta}^\alpha/\mu^\alpha \) with the units of \( m/s \) and \( m \) respectively. Under homogeneous substrate assumption, i.e., \( \mu^\alpha \equiv \text{const.} \), \( G^\alpha \) and \( \eta^\alpha \) approximate physical elasticity and viscosity parameters linearly, only by a constant factor. The parameters quantify material characteristics of cells at the boundary in normal (\( \alpha = n \)) and tangential directions (\( \alpha = t \)). The relaxation time is defined in terms of scaled \( (G^\alpha, \eta^\alpha) \) or original parameters \( (\tilde{G}^\alpha, \tilde{\eta}^\alpha) \) as

\[
\lambda^\alpha = \frac{\eta^\alpha}{G^\alpha} = \frac{\bar{\eta}^\alpha}{\tilde{G}^\alpha}
\]

The relaxation time, with unit of \( s \), is a quantity that measures how close the material is to be an elastic solid (small values) or a viscous fluid (large values). The mean relaxation time in normal direction \( \lambda^\alpha_{m,n} \) of the motion is then defined to be

\[
\lambda^\alpha_{m,n} = \text{mean}(\lambda^\alpha_{n}(t_{j})), \quad j = 1, \ldots, m,
\]

The mean relaxation time can similarly be defined in tangential direction; \( \lambda^\alpha_{m,t} \).

Assuming that the material properties are the same for any two neighboring subunits, the parameters can be obtained by solving

\[
V^\alpha_{i,j} = G^\alpha \Gamma^\alpha_{i,j} + \eta^\alpha \Gamma_{i,j}^\alpha, \quad j = 1, \ldots, m,
\]

where, for \( \alpha = n_i \), for example,

\[
V^m_{n,i} = \begin{bmatrix} v_{n+1,i}^m \cr v_{n,i}^m \end{bmatrix}, \quad \Gamma^m_{n,i} = \begin{bmatrix} \gamma_{n+1,i}^m \cr \gamma_{n,i}^m \end{bmatrix},
\]

for each time \( t = t_j \). The solution to Eq. (2.15) then becomes

\[
\begin{bmatrix} G^\alpha \\ \eta^\alpha \end{bmatrix} = \frac{1}{\Gamma^\alpha_{i,j} \wedge \Gamma^\alpha_{i,j}} \begin{bmatrix} V^\alpha_{i,j} \wedge \Gamma^\alpha_{i,j} \\ V^\alpha_{i,j} \wedge \Gamma^\alpha_{i,j} \end{bmatrix}.
\]

Time derivatives of strain are obtained using spline interpolation techniques. When the solutions to the system Eq. (2.17) then becomes

\[
\text{negative values, the built-in optimization routine, lsqlin of Matlab, is used for positive, optimized solutions.}
\]
3 Results

The model is tested first with arbitrary data sets. A set of data whose visualization resembles motion of a live cell is numerically generated. The given motion of this computational cell, after the parameters \((v^\phi, v^\nu)\) of the dynamical system Eq. (2.8) are computed by Eq. (2.7), was accurately reproduced through the solution of the system (results not shown). This indicates that the model and results are reliable for any set of arbitrary data.

The model is then applied to data from various cell types including neutrophil, keratocyte, epithelial cell, and glioblastoma cell line (Fig. 8 and movies S1–S7). In all applications, the evolution of the pivot point, \(x_i\), is first determined on the membrane at each time step using the morphology (curvature) of the cell boundary segmented by CellTrack software [36]. Other points are determined uniformly in equal numbers at each time step with respect to the pivot point (Fig. 1(b)). The number \(n\) is taken to be 100 for the applications in this paper. In all cases, for the data extracted from live cell image sequences, pixel is used as the space unit and frame number in the image sequence is used as the time unit, i.e., \(t_j = j\), instead of actual time course of the motion (see Appendix).

In the annotated live cell movies, three markers are used: the asterisk (*) is used for the normal displacement at the initial point (the points on the cell membrane) and the triangle (\(\triangle\)) for the normal velocity at the terminal point of the vectors. The colors of the markers are determined based on the sign of the quantities they represent; red is used for positive values and green for the negative values. The cyan circles (○) represent zero values of either quantities. The size of the vectors indicates the magnitude of the normal strain, i.e., they actually are \(\gamma^n\) \(\mathbf{u}^n\) (magnified by a factor of 30 for clarity of visualization). The vectors, thus compactly summarize information about signs of normal displacement and normal velocity and magnitude of normal strain at a point on the membrane. The point indices in multiples of 10 are annotated on the live cell images. The image sequences is included in the supporting online materials in the movie format (Movies S1–S7). In the title of the contour graphs for displacement, strain, and strain rate, the index, \(i\), is dropped.

3.1 Strain rate indicates spatio-temporal microdomain dynamics and cell’s chemotactic response

The normal strain, \(\gamma^n\), indicates actin network topology and polymerization history and the normal strain rate, \(\dot{\gamma}^n\), provides information about actin network dynamics as discussed below. The strain rate gives insight also on the signaling and/or microdomain dynamics on the membrane and on chemotaxis.

Strain and strain rates in all applications show branch-like regular patterns formed by the positive (negative) values of the functions which represent locally synchronous motion. Actin network is a branched structure [31]. The positive strips (green) of strain contour maps mark polymerizing and actively pushing filament ends at the leading edge, and as a result provide information on the polymerization history and actin network topology. The positive strips (green) of strain rate maps mark the location of the driving force of the motion or chemotactic agent and indicates signalling and actin network dynamics, and the cell’s local chemotactic response. We propose that formation of the regular patterns on strain and strain rate maps may be due to the microdomains which have roles in signal transduction ([22, 38]) or other microclusters of membrane receptors [6]. All domains with similar functionality will be referred to as microdomains hereafter. Microdomains are motile and signal may propagate along with their motion through the membrane and activate different filament tips as membrane receptors [39]. In line with Anchor Model formulation, we use the term of “lateral signal propagation” induced by motile microdomain dynamics instead of “membrane protrusion or retractions wave propagation” used in some other works [10, 25].

For quantification of the contour graphs for the strain rates, the stripes for positive values are represented by line segments that completely resides in the strips. The average length of the stripes, \(m_l\), which shows the strength of signal, the average size of the angles between the stripes and horizontal, \(m_a = \text{mean}(a, a')\), which indicates the lateral spread of signal on the membrane, and the average size of the angles between two merging/splitting stripes, \(m_b = \text{mean}(b, b')\), which represents the interaction among microdomains are defined accordingly (Fig. 2(f)). The angles a (a’) are formed by the strips and positive (negative) \(x\)-axis and the angles b (b’) are upward (downward) angles on the graphs. The smaller \(m_l\), is, the more spread the signal on the membrane and the smaller \(m_b\) is, the less the interaction among microdomains. Using these quantities, lateral, local signal propagation speed, \(v\), is defined to be \(v = \cot(m_a)\), signal persistence, \(l\), is defined to be \(l = m_l \sin(m_a)\), and region of influence of the signal, \(r\), is defined to be \(r = m_l \cos(m_a)\). Point index, \([i]\), is used as space unit and frame index,
[\theta], is used as the time unit for all these quantities defined on the strain rate maps. As an example, \nu has the unit of [i/j] and quantifies the propagation speed of signal for each cell relative to its boundary arc length. A matrix called motility index, \textbf{m}, is then defined to be \textbf{m} = [m_1, m_2, m_3, r, l, \nu]. It should be noted that the average size of the angles s and a' as well as b and b', separately, are very close to each other in each application which indicates a globally uniform lateral signal propagation speed in both transversal and inverse transversal directions.

Based on experimental justifications, it is concluded in [32] that lamellipodium and lamella are kinematically, kinetically, molecularly, and functionally two distinct but spatially co-localized networks of actin filaments. Through application of the Anchor Model to the cell boundary and the transition line, separately, the motility index found to be \textbf{m}_0 = [9.91i, 32.70^\circ, 112.12^\circ, 8.05i, 5.6j, 1.47i/j] and \textbf{m}_t = [12.98i, 14.46^\circ, 143.71^\circ, 12.33i, 3.11j, 9.80i/j], respectively. These results show that, while the strain rate patterns are branch-like at the lamellipodium (cell) boundary (Fig. 4(b)), they are step-like at the lamella boundary (transition line) (Fig. 4(d)). This may indicate that the signal fully develops and influences a wider region at the transition line as it diffuses into the cell (Fig. 11, blue line at time step \tau_3). The mean relaxation time is \lambda_m^t = 1.29 at the cell boundary and is \lambda_m^c = 1.19 at the transition line. This increase indicates an increase in fluidity at the cell boundary which is consistent with the experimental measurements of [43]. These differences imply that lamella and lamellipodium are mechanically and structurally different as well and so, they might not be spatially colocalized as suggested in different experimental reports [40] (Movies S3, S4). The mean elasticity as defined in Eq. (2.14) in both normal and tangential directions, \langle G_{\alpha\beta} \rangle, has almost a two-fold increase in lamella with the values of \langle 7.6i/j, 19.3i/j \rangle in comparison to the results for lamellipodium \langle 4.1i/j, 13.6i/j \rangle. These results are also parallel with the experimental measurements of a recent study with an even higher, five-fold, increase in the lamella stiffness [17].

For a better visualization of the strain rate patterns and their correlations with corresponding normal displacement functions, auto/cross correlation maps are presented in Fig 5, only for this application. The maps further confirm the existence of the regular patterns and show the cross correlations of the functions. Space-time correlation map for the functions \phi(s, t) and \phi(s, t) is defined to be:

\[ C_{\phi, \phi}(\Delta s, \Delta t) = \frac{\int_0^t \int_0^\tau (\tau) (\phi(s + \Delta s, t + \Delta t) - \phi_m) (\phi(s, t) - \phi_m) ds dt}{\sqrt{\int_0^t \int_0^\tau (\phi(s, t) - \phi_m)^2 ds dt \int_0^t \int_0^\tau (\phi(s, t) - \phi_m)^2 ds dt}} \]

where the mean values \phi_m and \phi_m are defined as in Eq. (2.14), s = s(\tau, t) is the arc length at time t \in [0, t_m], and \tau \in [0, \tau] \subset \mathbb{R}.

3.2 Displacement quantifies actin polymerization and myosin-induced retraction

The results discussed below indicate that the relation proposed, Eq. (2.10), between normal displacement, \Delta m, and polymerization or active myosin concentration are also consistent. That is, normal displacement can efficiently predict polymerization or myosin-induced retraction at the cell boundary.

In [32], actin dynamics in potoroo kidney (PtK1) epithelial cells is presented. It is shown in Fig. 2(a) that the results from the experimental measurements of polymerization and depolymerization in [32] seem, in general, to be consistent with computational results of the Anchor Model. The pivot points (green marker) for the epithelial cell (PtK1) applications are chosen to be the first point on the membrane on the right side of the image (Fig. S2). A local view of the membrane is given in Figs. 2(b)-2(d) for three consecutive time steps, \tau = 25, 26, 27, for the midpoints \tau = 47, ..., 53. Since polymerization is represented by red and depolymerization by green fluorescent speckles in the original movie, color analysis of the markers indicates that the experimental data and model predictions match reasonably well, even though the data extracted from the original image sequence is an averaged representation of the polymerization, whereas the Anchor Model predictions are local in time and space and the cell boundary for this application was not clearly detectable for tracking due to the coloring. Overall, positive and negative regions of the the contour graph Fig. 2(e) for normal displacement (accumulated red/blue small islands for i > 50, j < 50, for example) is consistent with the polymerization, depolymerization regime (protruding and retracting boundary segments) of PtK1 cells (Movies S1, S2). The motility index for this application is \textbf{m}_p = [9.66i, 30.38^\circ, 117.09^\circ, 8.0i, 5.15j, 1.67i/j] and the mean relaxation times are \lambda_m^t = 1.42 and \lambda_m^c = 1.25.

PtK1 cells are also treated with cytochalasin D (CytD) to inhibit polymerization of free barbed ends in [32]. The contour graph of the normal displacement (Fig. 3(a)) indicates where the polymerization occurs before and after CytD treatment. While the positive values of normal displacement form flat horizontal stripes on the graph before the treatment (\tau < 55), they form vertical stripes after the treatment (\tau > 55). The vertical stripes and active, polymerizing sharp corners formed after the treatment coincide (Figs. 3(a), 3(b), Movie S5). Quantitatively, the motility indices before and after the treatment, \textbf{m}_{\tau < 55} = [10.09i, 41.83^\circ, 97.3^\circ, 8.1i, 4.68j, 1.98i/j] and \textbf{m}_{\tau > 55} = [9.33i, 55.36^\circ, 73.9^\circ, 7.08i, 5.86j, 1.27i/j], respectively, indicate that although the signal strength does not change much, the merging angle decreases after the treatment, which maybe an indication of less inter-talk between the microdomains. The signal speed decreases almost by half from
1.98 $i/j$ to 1.27 $i/j$. The result complies with the experimental report that microcluster formation is abrogated by actin polymerization inhibitor latrunculin A [6]. Converting to actual measurement units (1 pixel $\approx 106$ nm, and i = 474 nm, j = 10 s, $i/j = 47.4$ nm/s), the difference in the signal speed before and after treatment becomes 94 nm/s $- 60$ nm/s $= 34$ nm/s, approximately. It is reported in [43] that cytoskeletal disruption with cytochalasin D made body and/or trailing regions 50% less elastic and less viscous in locomoting neutrophils. Our computations agree with this experimental result at the leading edge: similar to the signal speed, mean values for elasticity and viscosity defined as in Eq. (2.14), $(G_m^n, \eta_m^n)$, dropped from (4.23 $i/j$, 291) to (2.88 $i/j$, 191) almost by half after the treatment. Interestingly, while $\lambda_m^n$ decreases from 1.46 $i$ to 1.28 $i$ after the treatment likely due to the domination of stationary filament network after polymerization stops, $\lambda_m^n$ increases from 1.44 $i$ to 2.12 $i$, which indicates an increase in fluidity in tangential direction. It should also be noted that the experimental result as reported in [43] for relaxation time (.3 s) at the leading edge of a locomoting neutrophil and our computational result ($\lambda_m^n = 1.46 i$ = 15 s) for this PtK1 cell are comparable in magnitude.

### 3.3 Model outcomes allow cell profiling through analysis of their motion

The correspondence set forth between normal displacement and actin polymerization/contraction and normal strain rate and signaling dynamics are further justified with three different applications: neutrophil chasing bacteria, keratocyte, and glioblastoma cell motion. The results outline similarities and differences among different cell types which allow cell profiling based on the physical parameters obtained from the model and motility and deformation indices. In Fig. 6(a), an annotated live cell image of the neutrophil and in Fig. 6(d) part of the data extracted from the movie are presented. The tip of the neutrophil tail (the point of highest curvature) is taken to be the pivot point (green marker). The annotation is done as before, except the closest points to the two bacteria chased by the neutrophil on the membrane are marked by black and red markers in Fig. 6. There are some artificial lines and coloring at and around the times $t = 33, 56, 72$, in all contour plots. This is due to the camera motion during the movement of the cell, that is likely because of the low technology of the time the movie is recorded. These lines and accompanying coloring are disregarded in what follows. The results show that, the normal displacement, $d^n$, and the polymerization dynamics match as expected (Fig. 6(b)). The strip of polymerization mimics the bacteria trajectories (black and red markers on the contour plots). It is interesting to see that the cell is more aggressive in catching the bacteria it started to chase first (red markers). As a result, the strip around the first bacteria is wider than the other (Fig. 6(b)). The analysis of the strain rate (Fig. 6(e)) shows that the regular branched patterns are reorganized around the bacteria trajectories, are mostly pointing toward them as expected, and the highest values (small, red islands) are located mostly around the trajectories. This indicates that the strain rate bears information about signal for the directed motion and chemotaxis. The location of the chemotactic signal is further marked by both normal and membrane strains. It is clearly visible that the curved stripes mimic the bacteria trajectories in both cases. Positive stripes for normal strain (Fig. 6(c)) indicate a forward push, whereas the negative stripes for membrane/tangential strain (Fig. 6(f)) indicate lateral contraction, likely due to the initiation of phagocytosis (when the cell has physical contact with the bacteria or gets very close, it stops and locally contracts in a normal direction to get the bacteria inside) in this application, which generates a strong spatial strain gradient at these locations (Fig. 6(a), Movie S6). The motility index for this application, $m_f = [9.84i, 28.94i, 117.84i, 28.25i, 5.16i, 1.71i]/j$, is closest to $m_p$. Among all applications, the relaxation times are highest in both the normal and tangential directions ($\lambda_m^n = 1.77j, \lambda_m^s = 2.89j$), which is a reflection of fluidity and likely a highly deformable behavior of the cell (assuming a frame rate closer to other applications, see Appendix).

Similarly, the model is applied to a keratocyte cell motion (Fig. 7). The pivot point is chosen to be tip of the lower (right) wing as being one of the two points of highest curvature (Fig. 8(a)). The model effectively predicts where the polymerization and contraction takes place based on the analysis of normal displacement, which clearly identifies spatio-temporal dynamics of actin and myosin in the cell (Figs. 7(a), 7(c)). There are other computational studies which demonstrate, consistent with our findings, that in the rapidly crawling keratocytes, myosin concentrates at the rear boundary [35]. In spite of the simple, compartmentalized displacement patterns (Fig. 7(b)), strain rate shows regular, branched patterns as before, but the strips are larger in this case, likely due to the existence of larger microdomains (Fig. 7(b)). The motility index reads $m_k = [8.60i, 61.40i, 53.52i, 7.08i, 4.72j, 1.57i]/j$ with the smallest merging angle $m_b = 53.52$ among all applications. The angle is even smaller for the rear of the cell (1 $< i < 50$) with the value of $m_b = 45.50$°. This minimal intertalk among the microdomains and small lateral signal speed are reflected at the minimal deformations or steady shape of the cell body. Similarly, the relaxation time ($\lambda_m^n = 1.25j$) is the smallest among all applications, indicating the highly elastic nature of the keratocyte cell boundary, likely due to the presence of a dense actin network. Both strain rate and strain shows that the extreme values (small red/blue islands) takes places at or around the tips of the wings, namely $i = 1$ or $i = 50$, which generates maximum normal stress at those locations (Figs. 7(b), 7(d), Movie S7). These high traction forces are actually observed experimentally in other studies [14, 23]. It should be noted that extreme strain/strain rate values also colocalize with retrograde flow in keratocytes (see Sect. 4.1 and Fig. 11) [17, 39].
Finally, application of the model to the glioblastoma cell line (Figs. 9(a)) gives similar results. Normal displacement can precisely estimate membrane motion (Figs. 9(c)) as it can be seen by comparing the color patterns of the normal displacement (accumulation of small, red/blue islands for \( i = 40, \ldots, 100 \) and \( j = 1, \ldots, 35 \) to movement of the points on the trajectories (boundary segments with maximum extension/retraction) (Figs. 9(a), 9(c))). Strain and strain rate with similar branched patterns give the information around where the cell moves more aggressively (red small islands) (Figs. 9(b), 9(d)). In various experimental studies ([24, 41, 42]), reduced viscosity and elastic strength, and consequently 50% more deformability in static cancerous cells, are determined. This resemblance implies that cancerous cells may have deformation in signaling mechanisms or actin network function. Computational results of the Anchor Model outlines this parallelism. The motility index for brain tumor cell, \( \mathbf{m}_g = [8.51i, 54.76\%, 73.21\%; 6.56i, 5.28j, 1.31l/j] \), is the closest one among all applications to that of PKI cell after CytD treatment, \( \mathbf{m}_{c,j>55} \). The mean relaxation time in both normal and tangential directions for brain tumor cell, however, are smaller than that for all other applications (\( \lambda_{nm}^a = 1.31j \) and \( \lambda_{nm}^a = 1.15j \)). This indicates more elasticity (small rigidity) of the tumor cell boundary. The difference between experimental observation of increased cancer cell deformability (which indicates more fluidity) and our result may be caused by the measurements at different stages of cancer, such as precancerous or metastatic stages. It may also be caused by having the experimental measurements done with cells in static conditions as opposed to our analysis of motile cell. Nevertheless, considering high standard deviation in the results reported for relaxation time of normal and cancerous cells (2.5 ± 0.7 s and 3.1 ± 1.2 s, respectively), it is safe to say that the Anchor Model results indicating increasing elasticity for this brain tumor cell matches some experimental measurements [42].

Another tool developed in this paper for cell profiling through quantification of cell motion is called deformation index, \( \mathbf{n} \). It is a vector quantity with three components: deformation area, \( n_a \), deformation arc length, \( n_c \), and deformation radius, \( n_r \), that is, \( \mathbf{n}_j = [n_{aj}, n_{cj}, n_{rj}] \) for each time step \( j \). If the area of cell is \( a_j \) at time \( t_j \) and the nonoverlapping area between the configurations at \( j \) and \( j+1 \) time steps is \( a_j \), the deformation area is then defined to be \( n_{aj} = a_j/a_j \). Similarly, if the total arc length of cell is \( c_j = s(\mathbf{t}, t_j) \) at time \( t_j \), the deformation arc length is defined to be \( n_{cj} = c_j/c_j \). Let the radii of the circles which are tangent to the cell boundary from inside and outside be \( r^i \) and \( r^o \), respectively. Finally, the deformation radius at time \( t_j \) is then defined as \( n_{rj} = (r^i_{t+1}/r^o_{t+1})/(r^i_{t}/r^o_{t}) \). Smoothed graphs of the deformation indices for three applications -- \( \mathbf{n}_{aj}, \mathbf{n}_{cj}, \) and \( \mathbf{n}_{rj} \) for neutrophil, keratocyte, and brain tumor cell movements, respectively-- are given in Fig. 10 for \( j = 1, \ldots, 30 \). The deformation area for keratocyte seems to be almost constant as expected considering the steady shape of the cell during its motion (Fig. 10(a)). Deformation arc lengths for all three cells appear to be sinusoidal. This may be an indication of a pulsative motion in the third direction (z-axis) during cell movement (Fig. 10(b)). Although there are large fluctuations on the deformation radius graphs for neutrophil and keratocyte, the deformation radius of brain tumor cell appears to be sinusoidal (Fig. 10(c)).

4 Conclusions

We developed a model based inverse problem approach that formulates forward and inverse problems to analyze single cell movement and deformation. The model determines physical parameters such as elasticity and viscosity using continuum mechanical tools and kinematic functions such as displacement, strain, and strain rate. These quantities were then analyzed for further inference about the molecular mechanisms that generate the motion. In other words, the molecular mechanisms are addressed through qualitative reasoning based on the mechanical and physiological cues. We concluded that normal displacement quantifies actin polymerization and myosin-induced retraction and normal strain rate indicates spatio-temporal signaling/microdomain dynamics and the cell’s chemotactic response. A locally uniform retrograde flow is assumed in the interpretation of the model outcomes. The boundary motion together with retrograde flow is analyzed in a recent work through a continuum model formulation [21]. We observed regular patterns in strain/strain rate graphs in all applications which is an indication of globally uniform, local signal propagation speed and implies a universal principal behind this behavior. We hypothesized a biological model to address this principle by relating microdomain dynamics to formation of regular patterns, below. The model incorporates other important molecular mechanisms of cell motion such as adhesion, membrane ruffling, and retrograde flow and addresses their collective behavior.

These computational results in compliance with the experimental findings show that the model outcomes provide quantitative tools for mechanical and molecular analysis of single cells through their motion, and consequently for diagnostic prediction of cell abnormalities including malignancy. The motility index, \( \mathbf{m} \), deformation index, \( \mathbf{n} \), and the mean relaxation time, \( \lambda_{nm} \), are defined in a way to profile cells through their motion. Although the quantitative results presented in the paper are not conclusive, similar values within the same cell type (epithelial cells, for example) and differences between the different types show that these quantities can be used for cell profiling, characterization, and classification. The model may
provide useful information through systematic analysis of cell populations of the same type under different physical and chemical conditions.

4.1 Collective biomechanical and molecular mechanism of cell motion: Microdomains weave cytoskeleton and their interactions mark the location for formation of new adhesion sites

Based on the Anchor Model outcomes, we hypothesize a biological model for collective molecular mechanism of cell motion. We propose that microdomain signaling dynamics organizes cytoskeleton and its interaction with substratum. As microdomains trigger and maintain active polymerization of actin filaments, their propagation and zigzagging motion on the membrane generate a highly interconnected network of curved or linear filaments oriented at a wide spectrum of angles to the cell boundary. Microdomain interaction may also mark the formation of new focal adhesion sites at the cell periphery. Myosin interaction with the actin network then generate membrane retraction/ruffling, retrograde flow, and contractile forces for forward motion. Finally, continuous application of stress on the old focal adhesion sites could result in the calcium-induced calpain activation, and consequently the detachment of focal adhesions ([16]) which completes the cycle (Fig. 11).

More specifically, we propose that microdomains (Fig. 11, green curves on the cell boundary) may interact with each other as indicated at the merging ( ∧ ) and splitting ( ∨ ) angles of the strain rate graphs (Fig. 2(f)). This interaction could result in down-regulation of signaling ([6]) which is manifested as discontinued ∧ formation in the strain rate graphs (Fig. 11, time step j2). After physical interaction, which may include a highly complex set of biochemical reactions that may result in gaining different functionality ([27]), microdomains may propagate in the opposite directions as manifested in ∨ formation as well. Co-occurrence of both forms × on the graphs (Fig. 11, time step j3, Fig. 2(f)). A splitting angle without merging history (∨ alone) may represent signal initiation by microdomains [38]. As the microdomains move, filament tips are activated along with their motion, as sunflowers face towards the sun (Fig. 11, lines marked with red end points). This might be the mechanism responsible for the filaments’ orientation at various angles to the cell edge and curved filament trajectories as reported in recent experimental studies [19]. This mechanism could also explain why the proportion of filaments at lower angles to the cell edge is higher in slowing and pausing lamellipodia when compared with continuously protruding sites [19]. As the signal propagates away from its origin, filaments following the signal become parallel to the cell edge and some of them may be part of the cell cortex while others, which can survive depolymerization, retrograde flow to the lamellipodium–lamella interface (Fig. 11, time step j2).

Since the positive strips represent active forward push generated by actin polymerization and since the filaments need a mechanical support for forward push instead of a backward transmission of the force generated, we propose that × shapes on the strain rate contour graphs indicate location of newly formed adhesion sites (Fig. 11, cyan points at the × intersection). This interpretation and experimental results indicating that some filaments originate from foci at the lamellipodium front ([19]), high-avidity adhesion could be induced by clustering the membrane microdomains ([20]), lipid microdomain clustering induces a redistribution adhesion molecules on human T lymphocytes ([27]), central cluster of T cell receptors is surrounded by a ring of adhesion molecules ([6]), and similar results referenced in these studies seem to be consistent.

Myosin is considered to be a marker for lamellipodium and lamella interface ([19]) and a characteristic of lamella [17, 32]. Myosin interaction with the actin network generates the retraction state, a third stage after protrusion and pause. We propose that while myosin activation in the inner region encompassed by the lower arms of × cause contraction of the cell body forward, recruitment of myosin at the outer region causes experimentally observed myosin-induced retrograde flow or membrane ruffling [15, 17, 19] (Fig. 11, magenta arrows at time step j3). This might be a reason behind different strain rate patterns at the cell boundary and the transition line (Fig. 4, Fig. 11, blue line segment at time step j3). While condensing lamella, this mechanism may also sparse cell front for newly growing filament tips.

5 Appendix

A Image Quantification & Data Analysis

A.1 Edge Detection and Point Tracking

Tracking the points on the cell and nuclear membranes is a nontrivial task. In the paper [9] where the Ring Model (Fig. 1(a)) is introduced, point coordinates are determined manually from cell and nucleus morphology. The process of tracking the points is automated through the development of an open access software called CellTrack in [36]. The figures in Fig. 3 in both [9] and [36] show similar point tracking results, done manually and automatically, respectively. The software uses a combination of known imaging techniques for a better approximation. In particular the active snakes algorithm of [18] is extended by adding a new term, Ematch, for a finer match of the cell shape.
A.2 Filtering and Smoothing the Parameters

The results for the parameter values are filtered to exclude outliers from subsequent computations. The values that are more than three standard deviations away from the mean of the parameter in question is replaced by the average value of the function within a $3 \times 3$ space-time window at each time step. This filtering process followed by convolution with the Gaussian of the support of the same window. The contour graphs for the displacement, strain, and strain rate are presented without a Gaussian convolution after the filtering.

A.3 Live Cell Applications

The model is applied to data from various cell types including neutrophil, keratocyte, epithelial cells, and the glioblastoma cell line. In all cases, pixel is used as the space unit and frame number in the image sequence is used as the time unit instead of the actual time course. Each movie has a different frame rate: 5 min for the glioblastoma cell lines [9], 10 s for epithelial cells [32], and 15 s for keratocyte [33]. The frame rate for neutrophil motion is not recorded in the original movie source. The sources of the movies are as follows:

1. Brain tumor cell motion: The movie for brain tumor, glioblastoma cell line, is taken from the paper [9].
2. Epithelial cell motion: The movies for potoroo kidney (PtK1) epithelial cells (S6, S7, S9) are taken from the paper [32].
3. Keratocyte motion: The movie for keratocyte motion (Keratocyte actin-based motility) is taken from the Theriot Lab [33]:
   http://cmgm.stanford.edu/theriot/movies.html#Current
4. Neutrophil chasing bacteria: The movie for a crawling neutrophil chasing two bacteria is taken from the Fenteany Lab:
   http://www.biochemweb.org/fenteany/research/cell_migration/neutrophil.html

The graphs in Figs. 8(a), 6(d), 8(b), and 9(a) are constructed using the data obtained from the movies S5, S6, S7, and the movie in the paper [9], respectively, as listed below. Each curve on a graph represents the position of the plasma membrane of the cell at a particular time step. In each case, 100 points are chosen on the membranes at each time step. The point indices in multiples of 10 are marked blue and annotated on the figures for only the initial configuration. The pivot points are marked green in each case.

B Legends to Supporting Movies

Three markers are used in the annotated live cell movies. The asterisk (∗) is used for the normal displacement at the initial point (the points on the cell membrane) and the triangle (△) for the normal velocity at the terminal point of the vectors. The colors of the markers are determined based on the sign of the quantities they represent; red is used for positive values and green for the negative values. The cyan circles (○) represent zero values of either quantities. The size of the vectors indicates the magnitude of the normal strain, i.e., they actually are $\gamma^n_{ui}$ (magnified by a factor of 30 for clarity of visualization). The vectors, thus compactly summarize information about signs of normal displacement and normal velocity and magnitude of normal strain at a point on the membrane. The point indices in multiples of 10 are annotated on the live cell images. The image sequences are included as supporting online materials in the movie format (Movies S1–S7).

**Movie S1** Annotated PtK1 epithelial cell membrane motion shows the relation between displacement and polymerization or depolymerization. See the Results section of the paper for further explanation. The original movie is S6 in [32].

**Movie S2** Annotated PtK1 epithelial cell membrane local motion ($i = 47, \ldots, 53$) shows the relation between displacement and polymerization or depolymerization. See the Results section of the paper for further explanation. The original movie is S6 in [32].

**Movie S3** Annotated PtK1 epithelial cell (lamellipodium) membrane motion. See the Results section of the paper for further explanation. The original movie is S9 in [32].

**Movie S4** Annotated PtK1 epithelial cell lamella (transition) line motion. See the Results section of the paper for further explanation. The original movie is S9 in [32].

**Movie S5** Annotated PtK1 epithelial cell treated with CytD. See the Results section of the paper for further explanation. The original movie is S7 in [32].

**Movie S6** Annotated movie of a neutrophil chasing bacteria. See the Results section of the paper for further explanation. The source of the movie is listed in the previous section.

**Movie S7** Annotated movie of a keratocyte. See the Results section of the paper for further explanation. The source of the movie is listed in the previous section.
Fig. 1 Schematic representation of the Ring and Anchor Models.

Fig. 2 Application to epithelial cell membrane motion for protrusion/retraction analysis (see the Results section for details)
Fig. 3 Application to epithelial cell membrane motion before and after CytD treatment (see the Results section for details)

References

Fig. 4 Application to epithelial cell boundary and transition interface between the lamellipodium and lamella for the analysis of differences between lamellipodium and lamella (see the Results section for details)

Auto correlations of strain rate functions (Figs. 4(b), 4(d)) and their cross correlations with corresponding displacement functions:

The second rows in each graph represent equal space, $C(\Delta s, 0)$, and time, $C(0, \Delta t)$, correlation maps, where $\Delta s$ and $\Delta t$ are space and time lags, respectively. (a) Auto correlation of strain rate $\gamma^p$ presented in Fig. 4(b). (b) Cross correlation of strain rate $\gamma^p$ presented in Fig. 4(b) and corresponding normal displacement function $d^p$ (displacement function is not shown separately). (c) Auto correlation of strain rate $\gamma^n$ presented in Fig. 4(d). (d) Cross correlation of strain rate $\gamma^n$ presented in Fig. 4(d) and corresponding displacement function $d^n$ (displacement function is not shown separately) (see the Results section for details).

Fig. 6 Application to neutrophil motion (see the Results section for details)
Fig. 7 Application to keratocyte motion (see the Results section for details)

Fig. 8 Data extracted from live cell motion images (see the Results section for details). (a) Data extracted from the movie S7. (b) Data extracted from the movie S5.
Fig. 9 Application to brain tumor cell motion. (see the Results section for details)

Fig. 10 Components of the deformation index for comparison of three model applications; neutrophil, keratocyte, and brain tumor (glioblastoma) cell line motion. (see the Results section for details)
Fig. 11 Schematic representation of the collective biomechanical and molecular mechanism of cell motion: Actin network is represented by vertical, thin, black lines for clear visualization of the idea presented. Filaments marked with red ends represent actively pushing, polymerizing filaments following signal by the microdomains. Green curves on the membrane represent microdomains and left/right arrows represent microdomain/signal propagation speed, $v$. Cyan dots represent integrins and yellow dumbbells represent myosin family proteins. Blue line segment represents the region pulled forward by the actomyosin system (Fig. 4(d)). Up and down thick, magenta arrows represent cell contraction and myosin induced retrograde flow, respectively (see Fig. 2(f) the Conclusions section for details).