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Mechanosensing using drag force for imaging soft biological membranes

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Abstract

We investigate physical processes taking place during nanoscale mechanosensing of soft biological membranes in liquid environment. The examples include tapping mode imaging by atomic force microscope (AFM) and microscopy based on Brownian motion of a nanoparticle in an optical-tweezers-controlled trap. The softness and fluidity of the cellular membrane make it difficult to accurately detect (i.e., image) the shape of the cell using traditional mechanosensing methods. The aim of the reported work is to theoretically evaluate if the drag force acting on the nanoscale mechanical probe due to combined effect of intra- and extra- cellular environments can be exploited to develop a new imaging mode suitable for soft cellular interfaces. We approach this problem by rigorous modeling of the fluid mechanics of a complex viscoelastic biosystem in which the probe sensing process is intimately coupled to the membrane biomechanics. The effects of the probe dimensions, elastic properties of the membrane, as well as intra- and extra- cellular viscosities are investigated in detail to establish the structure and evolution of the fluid field as well as dynamics of membrane deformation. The results of numerical simulations, supported by the predictions of the scaling analysis of forces acting on the probe, suggest that the viscous drag is the dominant force dictating probe dynamics as it approaches a biological interface. The increase in the drag force is shown to be measurable, to scale linearly with an increase in the viscosity ratio of the fluids on either sides of the membrane, and to be inversely proportional to probe-to-membrane distance. This leads to postulation of a new strategy for lipid membrane imaging by AFM or other mechanosensing methods using variation in the maximum drag force as an indicator of the membrane position.

Introduction

Signal transduction, immune response, chemical and electrical sensing as well as other important biological processes take place at the cellular membrane surface.¹ For example, highly debated hypothesis of structural organization of proteins and lipids on lipid membrane on the cellular surface predicts the existence of “lipid rafts” sized tens of nanometers on the membrane surface². Study of the secretion, interaction, biophysical and biochemical properties of molecules imbedded in cellular interfaces can be enabled by development of high resolution (nanometer scale) microscopy tools in combination with appropriate imaging methods. Resolution of optical microscopes is diffraction-limited by the wavelength of visible light (~500 nm).³ Atomic force microscopes can operate in scanning mode and offer much better spatial resolution of less than 1 nm.⁴ Mechanical properties of the cell membranes are typically deduced by generating an applied force versus distance curve as the AFM tip scans the surface, and then fitting the experimental results into a simple Hertz model for the maximum distance between two elastically deformed surfaces.⁵ The problem is that a native lipid bilayer of the cell membrane is only 5 nm thick and does not produce significant mechanical response due

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to its softness and fluidity.⁶ Thus, to yield a measurable AFM signal the cell membrane (lipid bilayer) needs to be either placed on a rigid surface (i.e., making a “supported” membrane), or be frozen.^{4, 7} As a result, the AFM images of typical cells with untreated membrane bilayer can only reveal the topology of rigid cellular components underlying membrane such as cytoskeleton, organelles, nucleus, and not the soft membrane itself.⁸ Theoretical analysis of static forces acting on a mammalian cellular membrane deflected by a pin reveals that membrane elastic forces are several orders of magnitude smaller than forces generated by cytoskeleton, and, thus, any static force measurements of the membrane deflection is inevitably sensitive only to deformation of actin-spectrin network or rigid organelles underlying membrane.⁹

The objective of this paper is to theoretically investigate the possibility and operating conditions for using nanoscale probes (e.g., AFM with practically realized tip radii in the range of 5-50 nm) for mechanosensing (or imaging) a position of the soft biological membrane with comparably high spatial resolution (i.e., ~10nm). The difference between viscosities of intra- and extra-cellular liquids separated by a membrane offers an intriguing opportunity that can be exploited for highly sensitive membrane detection using mechanosensing strategies. It was shown that light scattering measurements can provide spatially resolved microrheology of a liquid/liquid interface on microscale¹⁰. We have already shown that effects of a hydrodynamic origin play an important role in biomembrane-AFM interactions in tapping mode.¹¹ In this paper we extend the approach originally proposed in our earlier work¹¹ with an aim to investigate the effect of a difference in fluid viscosities on dynamics of the mechanical probe motion during imaging a soft lipid bilayer membrane on nanoscale. As before, we use Helfrich's quasi-equilibrium theory for the elastic energy associated with the membrane shape. The boundary integral method (BIM) is used to solve the resulting quasi-steady Stokes system of momentum conservation equations coupled via the shear stress balance at the membrane interface separating the extra- and intra-cellular domains. In integral sense, this leads to rigorous prediction of the force-vs-distance diagram for the mechanical probe imaging process and allows us to investigate the new mechanosensing (e.g., AFM) strategy based on viscosity-induced drag force acting on the imaging probe.

Model formulation and scaling

We aim to predict the mechanical force generated on a probe moving toward the membrane (Figure 1) and to correlate it with a change in the membrane position upon interaction with the probe. We model the scanning mechanical probe (e.g., AFM tip) as a moving sphere and membrane (typically ~5nm lipid bilayer) as a infinitesimally thin interface with elastic energy described by Zhong-can and Helfrich equation.¹² This simplifies the analysis, but does not affect the generality of the results and our conclusions in a significant way.

The force acting on the probe as it moves toward the deformable membrane with constant velocity is due to viscous effects of both intra- and extra- cellular fluids as well as owing to an elastic resistance of the membrane against its deformation by the probe. The membrane is initially flat, and the probe starts its motion at the distance far away from the membrane. Intracellular and extracellular liquids are modeled as Newtonian liquids. Viscosity of the extracellular liquid is taken as 1.5 cP, while viscosity of the intracellular liquid is considered as one of the key parameters varied in simulations. According to the literature the latter can change dramatically within a broad range from 100 cP to 1 kP for living cells.^{13, 14} In our simulations we varied intracellular viscosity in the range of its lower reported limit from 1.5 cP to 600 cP (i.e., between 1 and 400 times of the extracellular viscosity) with an understanding that expected sensitivity of the probe will only be much greater if the intracellular viscosity assumes its upper limit values.

The successful detection of the membrane position would be possible if the force acting on the probe, as it approaches the membrane surface, changes significantly (i.e., above the sensitivity limit of an instrument) as compared to the background provided by the Stokes force ($\sim 6\pi\mu_1 UR$) in the bulk, where μ_1 is viscosity of the extracellular fluid, R and U are the probe radius and approach velocity, respectively. Thus, we are seeking the conditions yielding the greatest detectable difference between the bulk drag force and the drag force experienced by the probe as it approaches the membrane. For better spatial resolution the probe should be made as small as possible. We choose 50 nm radius of the modeled spherical probe in order to be able to use continuous analysis which is valid on the scale of tens nanometers and greater in typical liquid environment¹⁵. The characteristic velocity was chosen so that the resulting bulk drag force is detectable by existing mechanical sensors. The analysis of thermal fluctuations and their impact on the probe sensitivity yields the minimum detectable force $F_{\min}$ for the AFM measurements to be of the order of 10 pN¹⁶, resulting in an estimate for the minimum probe approach velocity as $U_{\min} \sim F_{\min} / 6\pi\mu_1 R \sim 10^{-2}$ m/s. The characteristic scales for the length ($R = 50$ nm) and velocity ($U \sim 1$ cm/s) allow us to identify the relevant time scale τ as $\sim 5 \times 10^{-6}$, and to estimate the value of the Reynolds number ($Re = \rho UR / \mu$) as well as the forcing frequency parameter $\rho R^2 / \mu \tau \sim Re$. In an extracellular domain (Ω_1) the latter two parameters are of the order of 5×10^{-4} , while, in an intracellular domain (Ω_2), the viscosity is larger than that in Ω_1 which results in even smaller values for the Reynolds number and the forcing frequency parameters. Owing to small Reynolds number the flow inertial forces are negligibly small as compared to the viscous forces. The small value of the forcing frequency parameter supports the quasi-steady approach to analysis of the fluid motion. As a consequence the transient and the nonlinear advection terms can be dropped in the Navier-Stokes equations of motion, yielding the simplified Stokes formulation in the limit of negligibly small body forces (the effect of electrohydrodynamic forces is negligibly small in typical electrolyte buffers and is studied in details in our previous paper¹⁷):

$$-\nabla p(x) + \mu_{1,2} \nabla^2 v(x) = 0 \quad (1)$$

$$\nabla \vec{e} \cdot v(x) = 0 \quad (2)$$

where p is pressure, $\mu_{1,2}$ are the viscosities in the extracellular Ω_1 and intracellular Ω_2 domains, respectively, and v is the velocity vector at the location x in the fluid.

Membrane has its own time scale - the characteristic time for in-plane longitudinal wave propagation along the entire membrane surface. The propagation length is scaled with the cell size, L_{cell} , and the wave speed is estimated as:

$$c_{\parallel} = \left(\frac{E_{2D}}{\rho_m h (1 + \sigma) (1 - \sigma)} \right)^{1/2} = \left(\frac{2K_{2D}}{\rho_m h (1 + \sigma)} \right)^{1/2} \quad (3)$$

where E_{2D} is the Young's elastic modulus for two-dimensional in plane deformations of a membrane, K_{2D} is the compression modulus of a 2D membrane, h is the membrane thickness, ρ_m is the membrane 3D density, and σ is the Poisson ratio (varies between 0 and 1). (Equation (3) is derived in a manner similar to equations 22.1-22.4 given by Landau and Livshitz¹⁸ but adopted from 3D for 2D geometry). Using typical values for K_{2D} as ~ 160 dyn/cm = 0.16 N/m,¹⁹ $\rho \sim 900$ kg/m³, and $h \sim 5$ nm yields $c_{\parallel} \sim 260$ m/s. (Other sources¹⁹ give somewhat different estimates for $K_{2D} = \lambda_m + \mu_m$, with the lateral shear modulus $\mu_m \sim 7 \cdot 10^{-6}$ N/m being much smaller than the lateral stretching modulus λ_m ranging from $\sim 10^{-5}$ N/m for flaccid erythrocytes to 0.3 N/m for swollen erythrocytes). For cells with the characteristic size $L_{cell} \sim 10$ μ m the resulting relaxation timescale $\tau_m = L_{cell} / c_{\parallel}$ is of the order of 10^{-8} s, which is significantly smaller than the previously computed characteristic time for the flow (and, thus,

hydrodynamic force) interactions. Thus, on the time scale of probing/imaging action the quasi-equilibrium approach can be applied for membrane description. That is, using the Zhong-can and Helfrich equation for the membrane equilibrium,²⁰ the discontinuity in the fluid shear stress on both sides of the cellular interface (i.e., fluid traction term $\Delta f = (\tau_{cyt} \cdot \mathbf{n} - \tau_{env} \cdot \mathbf{n})$, where τ_{cyt} and τ_{env} are stress tensors in extra- and intra-cellular domains, respectively, and $\mathbf{n}$ is a unit vector normal to the membrane surface) is balanced by the membrane tension and bending rigidity:¹¹

$$\Delta f = \left[-2\gamma H + B(2H + c_0)(2H^2 - 2K - c_0 H) + 2B\nabla^2 H \right] \mathbf{n} - \nabla_s \gamma \quad (4)$$

where γ is the membrane tension, B is the membrane bending rigidity, and H , K , and c_0 are the mean, Gaussian, and spontaneous curvatures of the membrane, respectively.

Boundary integral formulation and simulation method

The model equations are intimately coupled and solved by the boundary integral method. Below we provide only a sketch of the algorithm implementation, as a detailed description can be found in a recent paper by Fan and Fedorov.¹¹ The integral solution of the Stokes equation is sought as a superposition of contributions from a single layer (i.e., the flow induced by the continuously distributed point force, Stokeslet) and a double layer (i.e., the flow induced by the continuously distributed point stress, Stresslet) potentials:

$$\int_{\partial\Omega} \left(\tau_{ik}(x) G_{ik}(x_0, x) - \mu v_i(x) T_{ijk}(x_0, x) \right) n_k(x) dA(x) = \begin{cases} 0, x_0 \notin \Omega \cup \delta\Omega \\ -4\pi\mu v_j(x_0), x_0 \in \delta\Omega \\ -8\pi\mu v_j(x_0), x_0 \in \Omega - \delta\Omega \end{cases} \quad (5)$$

with the right-hand-side of Equation (5) depending on the location of the singular source point x_0 (i.e., inside, outside or at the boundary of domain Ω), n_k is the surface normal vector (pointed into the domain), v is the fluid velocity, τ_{ik} is the stress field G_{ik} and T_{ijk} are the single and double layer potentials, respectively. We can further simplify Equation (5) by utilizing axial symmetry of our formulation. Guided by the Pozrikidis' derivation,²¹ the following equations may be obtained by introducing the coordinates x in the direction of the probe motion (i.e., normal to the initial membrane position and inward the domain Ω_1) and σ in the radial direction (Figure 1).

For the probe, the governing equation on the surface $\delta\Omega_p$ is

$$\begin{aligned} 4\pi\mu_1 v_\alpha(x_0^i) = & - \int_{\partial\Omega_m} \Delta f_\beta M_{\alpha\beta}(x_0^i, x) dl(x) - \int_{\partial\Omega_p} f_\beta M_{\alpha\beta}(x_0^i, x) dl(x) + \\ & + (\mu_1 - \mu_2) \int_{\partial\Omega_m} v_\beta Q_{\alpha\beta\gamma}(x_0^i, x) n_\gamma dl(x) + \mu_1 \int_{\partial\Omega_p} v_\beta Q_{\alpha\beta\gamma}(x_0^i, x) n_\gamma dl(x) \end{aligned} \quad (6)$$

where α denotes either x or σ direction, and $M_{\alpha\beta}$ and $Q_{\alpha\beta\gamma}$ are the axisymmetric expressions for single and double layer potentials. It should be noted that the components of the velocity vector on the probe surface $[v_x|_{\mathbf{x} \in \delta\Omega_p} = U$ and $v_\sigma|_{\mathbf{x} \in \delta\Omega_p} = 0]$ are known.

On the membrane surface ($\delta\Omega_m$), the integral Equation (5) takes form of

$$\begin{aligned} 4\pi(\mu_1 + \mu_2) v_\alpha(x_0^i) = & - \int_{\partial\Omega_m} \Delta f_\beta M_{\alpha\beta}(x_0^i, x) dl(x) - \int_{\partial\Omega_p} f_\beta M_{\alpha\beta}(x_0^i, x) dl(x) + \\ & + (\mu_1 - \mu_2) \int_{\partial\Omega_m} v_\beta Q_{\alpha\beta\gamma}(x_0^i, x) n_\gamma dl(x) + \mu_1 \int_{\partial\Omega_p} v_\beta Q_{\alpha\beta\gamma}(x_0^i, x) n_\gamma dl(x) \end{aligned} \quad (7)$$

The membrane tension γ in the domain-coupling Equation (4) is not known a priori and must be found from additional physical considerations. We have chosen the condition of zero dilatation on membrane as a constraint equation:²²

$$\sigma \vec{t}_s \cdot \frac{\partial \vec{v}}{\partial s} + \vec{v} \cdot \vec{e}_\sigma = 0 \quad (8)$$

Replacing the velocity vector $v(x)$ in Equation (8) by its integral representation given by Equation (7) we obtain:

$$-\int_{\partial\Omega_m} \prod_{\beta}^{P.V.} \Delta f_{\beta} dl(x) - \int_{\partial\Omega_c^1 + \partial\Omega_p + \partial\Omega_c^2} \prod_{\beta} f_{\beta} dl(x) + (\mu_1 - \mu_2) \int_{\partial\Omega_m} \prod_{\beta\gamma}^{P.V.} v_{\beta} n_{\gamma} dl(x) + \mu_1 \int_{\partial\Omega_p} \prod_{\beta\gamma}^2 v_{\beta} n_{\gamma} dl(x) = 0 \quad (9)$$

with

$$\prod_{\beta}^1(x_0^i, x) = \sigma_0^i t_{\alpha}(x_0^i) \frac{\partial M_{\alpha\beta}}{\partial s}(x_0^i, x) + M_{\sigma\beta}(x_0^i, x) \quad (10)$$

and

$$\prod_{\beta\gamma}^2(x_0^i, x) = \sigma_0^i t_{\alpha}(x_0^i) \frac{\partial Q_{\alpha\beta\gamma}}{\partial s}(x_0^i, x) + Q_{\sigma\beta\gamma}(x_0^i, x) \quad (11)$$

$$\frac{\partial M_{\alpha\beta}}{\partial s} = \vec{t}_s \cdot \vec{\nabla} M_{\alpha\beta}, \quad \frac{\partial Q_{\alpha\beta\gamma}}{\partial s} = \vec{t}_s \cdot \vec{\nabla} Q_{\alpha\beta\gamma} \quad (12)$$

To generalize the approach the governing equations are non-dimensionalized by using the following characteristic scales: length is scaled by the probe radius R , velocity is scaled by the probe approach velocity U , yielding the time scale for imaging as R/U ; the membrane tension γ is scaled by $\mu_1 U$ (γ -values observed in experiments²³ vary between 2×10^{-6} and 2×10^{-5} J/m² which, after scaling, give dimensionless values ranging between 0.2 and 2), the membrane bending rigidity B is scaled by $\mu_1 U R^2$ (experimentally observed values for B are in the range 1×10^{-20} – 7×10^{-19} J^{24, 25} yielding the scaled values of 0.4 - 28), the membrane mean curvature H is scaled by $1/R$, and the Gaussian curvature K is scaled by $1/R^2$.

The coupled fluid-membrane system, given by Equations (6), (7), and (9), is solved using Boundary Integral Method (BIM) with discretization along the surfaces of the probe and the cell membrane.²⁶ One advantage of this method is that governing equations need to be solved only at the boundaries of the domains thus reducing dimensionality of the system. The system is modeled by five integral equations [vector Equations (6) and (7) yield four scalar equations (two each) for the unknown components along the x and σ directions, and Equation (9) is a scalar equation] with five unknown functions [velocity v_x and v_{σ} on the membrane surface ($\delta\Omega_m$), traction f_x and f_{σ} on the probe surface ($\delta\Omega_p$), and tension γ along the membrane]. In our numerical implementation we followed the method used by Fan and Fedorov.¹¹

The initial state of the system assumes that the membrane is undisturbed and planar, and the probe-to-membrane separation is $10R$. The positions of the probe and of the cell membrane at the next time step are advanced using the local velocity field calculated at the previous time step. The Cash-Karp modification of the embedded Runge-Kutta method²⁷ is used for time integration at each collocation point. On the membrane surface the collocation points are spatially rearranged at every time step to maintain the same node distribution along the arc length of the membrane with denser node clustering near the membrane center. The membrane shape is reconstructed at each time step using cubic spline interpolation. Taking into account

that the Green functions asymptotically vanish at the infinity the surface integrals are truncated at $\sigma = 300$ (this truncation distance is justified through sensitivity studies).²⁸ The boundary elements along the domain surfaces are approximated by straight intervals, and the surface integrals over each element are evaluated by 10-points Gaussian quadrature.²⁷ Upon numerical approximation of all derivatives and integrals, the resulting system of linear algebraic equations is solved using the LQ decomposition matrix inversion method (routines f07adc, f07aec, NAG C Library, Mark 7, Numerical Algorithm Group).

Results and discussion

Figure 2 through 4 show the results of the parametric investigation on the effects of variation in the viscosity ratio μ_2 / μ_1 , the bending rigidity modulus B , and the initial preset tension γ_∞ in the undisturbed membrane, respectively. The reported properties of interest are the magnitude of the force acting on the probe and the maximum displacement of the membrane (i.e., in the middle, right beneath the probe) as the probe approaches the membrane. For the probe to be able to “see” and thus accurately image the membrane position the force variation must be as large as possible, while the displacement of the membrane should be minimized from its undisturbed “true” position.

Of the three parameters investigated, the difference in the viscosity of the intra- and extracellular fluids is found to exhibit the most significant effect on both accounts. Specifically, the drag force acting on the probe show almost a 25-fold increase (relative to the background of the bulk Stokes drag) and the membrane moves very little (Figure 2) when the viscosity ratio is at its simulated maximum of 400 (corresponding to the intracellular viscosity of 600 cP).

Our calculations also show that when intrinsic membrane curvature c_0 is negligible [i.e., set to zero in Equation (4)] the effect of the membrane bending rigidity is minimal. Indeed, the maximum drag force (occurring when the probe is in a closest proximity to the membrane) depends on elastic modulus of membrane B only slightly (Figure 3), exceeding the bulk (background viscous Stokes) force by only ~ 3.75 even when B is increased four orders of magnitude to its unrealistically high dimensionless value of 10000, which is 500 times greater than what is observed experimentally. Further, the maximum displacement of the membrane from its equilibrium position is quite significant especially for the smaller values of the elastic modulus, which is an undesirable effect from the imaging prospective.

Another option to improve detectability of the membrane lies in a possibility of the forced increase in the membrane tension at rest γ_∞ by blowing the cell²⁹ until the membrane tension reaches the integrity limit for the lipid bilayer (~ 10 -50 mN/m).³⁰ We investigate this possibility through simulations (Figure 4), which predict that the force acting on the probe does indeed depend on the membrane rest tension γ_∞ , yet not very strongly with only about 7-fold increase observed when dimensionless γ_∞ is equal to 1000 (i.e., hundred times greater than typical, experimentally measured values). On a positive note, an increase in the membrane tension at rest allows one to significantly decrease the maximum deflection of the membrane from its equilibrium position in response to the probing action, which is an attractive feature to enable accurate determination of the membrane position in imaging experiments.

In summary, the simulations suggest that under realistic conditions bending rigidity and rest tension of the membrane are expected to yield no significant change in the probe force that is being sensed during imaging. The most pronounced effect, which can thus potentially be used for quantitative membrane imaging, is expected to come from the difference in the extra- and - intra- cellular fluid viscosities. Indeed, the force acting on a probe may increase by several orders of magnitude relative to the background (the bulk Stokes drag) if the liquid environment

inside the cell has viscosity close to its highest estimates (~ 600 cP), without significantly disturbing the membrane position whose deflection from its “true” state is expected to be less than $0.05R$ (that is 2.5 nm for the 50 nm probe). In hindsight, this effect is not surprising as being similar to additional drag predicted at the interface of two immiscible fluids with different densities and viscosities²⁸. The important difference is that biological membranes significantly amplify this effect due to interface elasticity and its 2D incompressibility, which leads to dynamically-induced additional tension in the membrane.

To gain further insight into biophysics of the simulated phenomena and to develop useful analytical relationships between the key system parameters it is instructive to perform the scaling analysis of the forces acting in the probe-membrane system. It allows us to reduce the complex dynamics of the system to a set of simple, approximate equations that could be useful in design of imaging experiments in practice. To this end, we start by making an observation that in simulations the force acting on a probe F appears to be inversely dependent on the distance between the probe and the actual (deflected) membrane position [i.e., $\sim 1/(L + \delta - R)$], and this holds universally true for all simulated conditions (Figure 5).

The scaling can be further improved by using a relative velocity for the probe corrected for the induced velocity of the outward (away from the probe) membrane motion ($\sim \delta' = d\delta / dt$), that is

$$F \approx F_{Stokes} + \frac{6\pi(U - \delta')\mu_1 R}{L(t) + \delta - R} \quad (13)$$

Here $F_{Stokes} = 6\pi\mu_1 UR$ is the background Stokes drag force due to the probe motion in the extracellular fluid, which is augmented by additional drag induced by the probe-membrane interactions (second term in the right-hand-side). Equation (13) makes the results of all simulations to collapse on a single master curve as shown in Figure 6 (except one case of $\mu_2 / \mu_1 = 1$, $B = 10$, $\gamma_\infty = 1$; this outlier is not surprising because Equation (13) is based on the assumption of $(L - R + \delta) \ll R$, which is not true in this case).

The only outlier curve corresponds to the case of equal viscosities of the intra- and extracellular fluids ($\mu_2 / \mu_1 = 1$, $B = 10$, $\gamma_\infty = 1$), which is of no relevance to the proposed imaging methodology relying on a difference between the viscosities in the fluid domains separated by the membrane.

To approximate dynamics of the membrane we treat its “affected by the probe” area as a disk with vanishing mass and thickness (whose size R^* is proportional to the probe radius R due to extreme softness of the membrane), which moves kinematically in response to forces acting on it. These forces include the pressure (piston) force caused by an approaching probe, the viscous forces caused by motion of intra- and extra- cellular liquids, and elastic tension and bending forces due to membrane dilation and curvature induced by deformation. Using the reciprocity principle for the action (probe) and reaction (membrane) forces, a pressure force acting on the membrane can be computed from Equation (13) as

$F_p \sim -6\pi\mu_1 R(U - \delta') \frac{R}{L(t) + \delta - R}$, noting that, unlike for the probe, no extra Stokes drag is acting on the membrane. A viscous shear (damping) force is induced by moving liquids surrounding the membrane and is proportional to the size of the affected membrane element ($\sim R^*$), its velocity δ' and fluid viscosities above and below membrane; that is $F_{damp} \sim \delta R^* (\mu_1 + \mu_2)$. Finally, the elastic response of a membrane produces a force acting in direction opposite to the membrane motion and is proportional to its displacement at the center in the limit of small deformations, $F_e \sim -k\delta$,⁹ where k is an effective elastic modulus which combines the effect of membrane tension and bending rigidity. Because of the massless (and thus inertia-less) nature of the membrane element, all forces acting on it must be balanced, yielding:

$$(\mu_1 + \mu_2)R^* \dot{\delta} + k\delta = 6\pi\mu_1(U - \dot{\delta}) \frac{R^2}{L(t) + \delta - R} \quad (14)$$

From the simulations (Figures 2 through 4) it appears that the viscous and pressure forces dominate the membrane dynamics. Thus, the contribution of the elastic forces, the second term on the left-hand-side of Equation (14), can be safely omitted without introducing significant error, i.e.,

$$(\mu_1 + \mu_2)R^* \dot{\delta} = 6\pi\mu_1(U - \dot{\delta}) \frac{R^2}{L(t) + \delta - R} \quad (15)$$

The initial conditions for the problem are given by $\delta|_{t=0} = 0$ and $L|_{t=0} = L_0$. The probe moves at a constant speed U , so the distance between the probe and the plane of initially undisturbed (“true”) membrane state (Figure 1) is readily computed as $L(t) = L_0 - Ut$, leading to

$$\frac{\mu_1 + \mu_2}{6\pi\mu_1} \frac{R^*}{R^2} \dot{\delta} = \frac{U - \dot{\delta}}{L_0 - R - Ut + \delta} \quad (16)$$

Introducing of a new variable $z = L_0 + \delta - (R + Ut)$ and the system-specific constant $A = [(\mu_1 + \mu_2)/6\pi\mu_1](R^*/R^2)$ yields an ordinary differential equation

$$\dot{z} \left(A + \frac{1}{z} \right) = -AU \quad (17)$$

which can be integrated, and combined with the initial condition $z|_{t=0} = L_0$, to result in an implicit, closed-form analytical expression for $z(t)$:

$$A(z + R - L_0) + \ln \left(\frac{z}{L_0 - R} \right) = -AUt \quad (18)$$

For imaging of the membrane we are interested in the values of the membrane displacement and the force acting on a probe at the moment when the probe touches the initial “true” position of the membrane. This occurs when $t^* = (L_0 - R)/U$, and at this moment of time $\delta|_{t=t^*} = z|_{t=t^*}$ in Equation (18). Thus, solution of the following transcendental algebraic equation predicts the location of the membrane, which can be used for image interpretation.

$$Az(t^*) + \ln \left(\frac{z(t^*)}{L_0 - R} \right) = 0 \quad (19)$$

In the limit of large ratio of μ_2 to μ_1 the coefficient A is much greater than unity, and the following recursive equations asymptotically yield the value of the membrane displacement

$$\begin{aligned} \delta|_{t=t^*} = z|_{t=t^*} &= \lim_{n \rightarrow \infty} z_n|_{t=t^*}. \\ z_0(t^*) &= \frac{1}{A}, z_1(t^*) = \frac{\ln(A(L_0 - R))}{A}, z_2(t^*) \approx \frac{\ln(A(L_0 - R)) - \ln(\ln(A(L_0 - R)))}{A}, \dots \\ Az_{n+1}(t^*) &= -\ln \left(\frac{z_n(t^*)}{L_0 - R} \right) \end{aligned} \quad (20)$$

The series of Equations (20) converges when $A(L_0 - R) > \exp(1)$, and the fifth approximation $z_5(t^*)$ gives prediction with 5% relative accuracy for $z(t^*)$ if $A(L_0 - R) > 10$. Once the membrane displacement $\delta(t^*) = z(t^*)$ is determined, the maximum force acting on the probe is given by $F_{\max} \sim F_{\text{Stokes}} + 6\pi\mu_1\delta(t^*)R^2/\delta(t^*)$, which can be further simplified for the large A (i.e., when $\ln[A(L_0 - R)] \gg 1$) to yield:

$$F_{\max} \sim F_{\text{Stokes}} + \frac{(\mu_1 + \mu_2)R^*U}{\ln\left(\frac{L_0 - R}{6\pi R^2} R^* \frac{\mu_1 + \mu_2}{\mu_1}\right)} \quad (21)$$

Comparison of scaling analysis predictions and detailed numerical simulations is shown in Figure 7. As one may see there is excellent agreement between numerical results and scaling predictions for a broad range of variation in the viscosity ratio from ~ 4 up to ~ 400 . Matching of the data is achieved using a single adjustable parameter, the dimensionless R^* , which is equal to 0.375 for the best agreement, physically implying that the “affected” area of the membrane is about three times smaller than the size of the probe R . It is also worth noting that the maximum force scales approximately linearly with the viscosity ratio – the trend which is predicted by both the scaling analysis and numerical simulations.

Despite their simplified nature, the significance of Equations (18) and (19) should not be overlooked as they provide a straight-forward way to predict the position of the membrane from the force measurements performed in imaging experiments without performing complex numerical simulations. Thus, this methodology provides an analytical framework to deconvolute the drag-force-based mechanosensing image and to fairly accurately reconstruct the membrane position, or, in other words, to be able to “see” the membrane.

Concluding Remarks

The analysis reported in this paper provides compelling evidence that a position of the soft elastic interface such as lipid bilayer of the cell membrane can be identified with several nanometer resolution using drag force measurements during mechanical probing of the interface. This allows us to suggest the use of drag force measurements acting on the moving probe as a promising strategy for lipid membrane imaging by mechanosensing methods such as AFM. The measured drag force while scanning and tapping the cell surface can be analyzed using simple algebraic equations developed in this work to yield an image of “true” membrane topology of the soft biological object. The higher spatial resolution may be achieved if the membrane position is studied by the small probe moving toward the membrane with the velocity of 0.01 m/s or higher. We predict generation of measurable drag forces of the order of 10 pN or higher, depending on an actual size of the probe (e.g., AFM tip), if the probe approaches the membrane with the velocity of 1 cm/s or higher and when the intracellular viscosity is at least an order of magnitude greater than that of the extra-cellular environment.

This study is the first theoretical demonstration that mechanosensing of the biological membrane position is possible by measuring damping viscous forces acting on a small probe moving with high velocity toward the membrane. The analysis and conclusions are general and directly applicable for interpretation of images obtained using tether-free mechanical probes such as optical tweezers³², but should be corrected for contribution due to force harmonic potential of an optical trap or spring behavior of an oscillating cantilever in the case of AFM imaging.³¹ Utilization of viscous damping effects for sensing has already been proven fruitful in cellular biology when analysis of Brownian motion of a 200 nm sphere trapped in optical tweezers (an opto-mechanical probe) has revealed changes in the probe mobility as function of the order of nanometer distance to the surface of a living cell.³³ Recent progress in the field of AFM instrumentation brought to life a new generation of sensors with much higher sensitivity, dynamic range, and capable of measuring mechanical forces and interface position independently.³⁴ With the proper choice of sensing strategy, as proposed in this work, these new sensors should enable imaging of live cell membranes with unprecedented accuracy.

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Figure 1.

Schematic of the analyzed problem, including definition of simulation domains and the coordinate system (Ω_1 is an extracellular liquid with dynamic viscosity μ_1 , Ω_2 is an intracellular liquid with dynamic viscosity μ_2 , $\delta\Omega_p$ denotes the surface of the probe, $\delta\Omega_m$ denotes the membrane treated as an infinitesimally thin interface, and L is the distance between the probe center and the initial membrane position). A spherical probe of radius R moves toward the membrane surface with constant velocity U until it reaches an undisturbed “true” membrane position (shown as dash-dotted horizontal line).

Figure 2.

Variation of the dimensionless drag force (left panel) and membrane deflection (right panel) with the dimensionless distance between the probe and the “true” membrane position as function of the ratio of the intracellular to the extracellular viscosities μ_2/μ_1 . The dimensionless membrane bending rigidity modulus B (scaled by $\mu_1 UR^2$) is 10, and membrane tension at rest γ_∞ (scaled by $\mu_1 U$) is set to be equal to 1.

Figure 3.

Variation of the dimensionless drag force (left panel) and membrane deflection (right panel) with the dimensionless distance between the probe and the “true” membrane position as function of the dimensionless bending rigidity modulus B (scaled by $\mu_1 UR^2$). The ratio of the intra- and extra- cellular viscosities μ_2 / μ_1 is set to 4, and membrane tension at rest γ_∞ (scaled by $\mu_1 U$) is equal to

Figure 4.

Variation of the dimensionless drag force (left panel) and membrane deflection (right panel) with the dimensionless distance between the probe and the “true” membrane position as function of the membrane tension at rest γ_∞ (scaled by $\mu_1 U$). The ratio of the intra- and extracellular viscosities μ_2 / μ_1 is set to 4, and dimensionless bending rigidity modulus B (scaled by $\mu_1 UR^2$) is set to 10.

Figure 5.

The dimensionless force (scaled by the background Stokes drag in the bulk) experienced by the probe as function of the dimensionless probe-to-membrane separation distance expressed in log-log coordinates for an entire set of investigated parameters ($1 < \mu_2 / \mu_1 < 400$, $10 < B < 10^4$, $1 < \gamma_\infty < 10^3$).

Figure 6.

Rescaled probe force vs. separation distance plots accounting for relative motion of the probe and membrane during imaging yields a generalized scaling relationship, collapsing all simulations results ($1 < \mu_2 / \mu_1 < 400$, $10 < B < 10^4$, $1 < \gamma_\infty < 10^3$) on a single master curve, showing an approximately linear dependence in log-log coordinates.

Figure 7.

Comparison between simulation results (open circles) and the scaling analysis predictions (line) for the maximum drag force acting on a probe at the moment when the probe reaches the undisturbed “true” position of the membrane. The best fit (with relative error less than 9% for $\mu_2 / \mu_1 \geq 4$) is achieved for the dimensionless “affected” membrane area parameter $R^* = 0.375$, also providing an estimate for the size of the membrane domain that is strongly displaced upon interaction with the approaching probe.