

# Level set method for free boundary of invasive cancer cell using different functions of matrix metalloproteinases

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**Abstract.** The secondary tumor is stated to be more dangerous among cancer patients and this tumor is created through metastasis. Metastasis is the process of spreading a tumor from the primary location to the second part of the human body. This is an unpleasant problem among cancer patients because metastasis can contribute to high mortality cases among them. The presence of finger-like protrusions on the plasma membrane of cancer cells is known as the invadopodia. This structure can contribute to cancer cell invasion through the metastasis process. The formation of invadopodia involves several molecular interactions between extracellular matrix (ECM), ligand, actin, and matrix metalloproteinases (MMPs). The degradation of the ECM by the MMPs is mentioned as the starting point for the occurrence of cancer cell invasion. In this paper, the concentration of MMPs is taken in several functions of  $g$  to observe the formation of invadopodia on the plasma membrane. Two-dimensional mathematical model of ligand and signal is solved numerically using the method of level set, ghost fluid with linear extrapolation, and finite-difference. Credit is given to the level set method which successfully detected the movement of the free boundary interface (plasma membrane) by setting the interface as a zero-level set function. Also, the neighboring meshes can be identified using this method. Results showed that the above-mentioned integrated methods effectively describe the movement of the free boundary interface and this directly points out the formation of protrusions on the plasma membrane.

## 1. Introduction

The level set method is effective, mostly on tracking the changes of shape or boundaries. The study on the level set method is first introduced by [1] to capture the moving fronts in many multi-physics problems. A review on the level set techniques to many problems is stated in [2]. Further studies on the level set method have been done by [3]. In their study, the Stefan problem is highlighted where it is related to the evolution of smooth boundaries of different phases for a pure substance, for instance, the changes from solid ice to water. Another exploration on the level set method is conducted by the studies in [4–6]. Those studies proposed the numerical scheme for the level set method which combines with the front-tracking and fixed domain methods to model the growth and interaction of multiple dendrites in solidification.

Discussion on the method of a level set related to the problem of corrosion is presented in [7, 8]. They have been tracking the changes in the geometry of the metals due to the effect of corrosion using the method of level set. Apart from that, [9–12] described the moving of an arbitrary interface to solve the elliptic equations. Meanwhile, another implementation on the level set is explained in [13] to



determine the curvature-dependent growth of the cell in the tissue engineering applications to provide an initial template for cell attachment and subsequently cell growth and construct development.

The behavior of the cancer cell is widely explored in [14, 15] where the level set method is performed to solve the free boundary of the individual cancer cell. In those studies, the moving interface is detected by the zero-level set function to predict the position of the interface for the whole-time computation. These studies revealed that, the position of the interface is increasing as time increased and this is corresponding to the existence of protrusions or invadopodia on the plasma membrane. Invadopodia are small-punctuated finger-like protrusions founded on the plasma membrane that can be contributed to the cancer cell invasion through the metastasis process.

The investigation on the invadopodia via the approach of mathematical modeling is done by [16]. In that study, the creation of invadopodia is explained in fixed plasma membrane using the mathematical modeling of the actin, ECM, MMPs, and ligand. Also, the rate constant of MMPs is varied to observe the stage of the invasiveness of cancer cells. It is proven that the higher the concentration of MMPs, the more invasive the cancer cells will become. However, the actin is spotted in the extracellular region, which is in contradiction to the biological fact that actin is in the intracellular region.

Instead of a fixed plasma membrane, [17–19] discussed the free boundary plasma membrane to observe the formation of invadopodia on the plasma membrane. In these studies, the mathematical modeling of time-independent of signal and ligand in collaboration with the level set method is focused on. Other than that, the methods of finite difference and ghost fluid with linear extrapolation are emphasized for the regular and neighboring grid points, respectively. Meanwhile, [20] studied the formation of invadopodia by using the time-independent mathematical modeling of two-dimensional signal transduction.

Extending the study in [20], [21] determined the appearance of protrusions or invadopodia on the plasma membrane by including the modeling of the ligand into their model. In the previous study, the movement of the free boundary interface is accounted for by the gradient of the intracellular signal. However, in this paper, the motion of the interface is taken as the difference of gradient between intracellular signal and extracellular ligand. The level set method is applied to detect the moving interface by setting the interface as the zero-level set function. Also, the concentration of MMPs is taken in five different functions to observe the behavior of the protrusions on the plasma membrane.

## 2. Mathematical Formulation

In this paper, the level set method is applied to detect the movement of the free boundary interface by setting the interface to the zero-level set function. Invadopodia are the finger-like protrusions that can be spotted on the plasma membrane and these structures can be contributed to the cancer cell invasion through the metastasis process. There are several molecular processes involved in the formation of invadopodia. The formation of invadopodia started when the degradation of the ECM by the MMPs occurred. Hence, research on the MMPs is widely explored in [22–25] due to their importance. In this paper, the concentration of MMPs is taken as the function  $g$  and from the degradation, ligand ( $c^*$ ) is produced and diffused to the extracellular region (refer to equation (1)) [16]. Assume that, on the square domain, the ligand is absent and at any time  $t$ , the MMPs can be spotted on the plasma membrane to degrade the ECM.

Thereafter, the ligand binds with the membrane-associated receptor such as the epidermal growth factor receptor (EGFR), and thus the signal ( $\sigma$ ) is stimulated on the intracellular region (refer to equation (2)) [20]. Subsequently, signal plays a role in the up-regulation of MMPs and polymerization of the actin. In this paper, the domain is determined in the square domain that includes the regions of the extracellular ( $0_i^c$ ), intracellular ( $0_i^\sigma$ ), and interface ( $\Gamma_i$ ) to separate any activities of ligand, signal, actin, and MMPs. The movement of the free boundary is consequent from the polymerization of actin and [15] considered it as the gradient of inner signal. Meanwhile, in this paper, the movement of the

free boundary is taken as the difference of gradient between intracellular signal and extracellular ligand as stated in equation (3).

Instead of velocity on the plasma membrane, the information of velocity for the whole domain is required since we are using the level set method. Hence, the partial differential equations (PDEs) approach as mentioned in equation (4) is applied to represent the velocity extension [17]. Velocity extension is crucial to avoid discontinuities on the plasma membrane. As a result, the two-dimensional mathematical modeling for the formation of invadopodia is stated as equations below.

$$\begin{aligned} \Delta c^* &= 0, \quad \mathbf{x} \in \Omega_i^*, \\ c^*|_{\partial\Omega} &= 0, \\ c^*|_{\Gamma_t} &= g|_{\Gamma_t}, \quad t \in [0, T]. \end{aligned} \tag{1}$$

$$\begin{aligned} \Delta \sigma &= 0, \quad \mathbf{x} \in \Omega_i^\sigma, \\ \sigma|_{\Gamma_t} &= c^*|_{\Gamma_t}, \quad t \in [0, T]. \end{aligned} \tag{2}$$

$$\mathbf{v} = \nabla \sigma - \nabla c^*. \tag{3}$$

$$\begin{aligned} (\nabla \Psi \cdot \nabla) \mathbf{w} &= 0 \quad \text{in } \Omega, \\ \mathbf{w} &= \mathbf{v} \quad \text{on } \Gamma_t. \end{aligned} \tag{4}$$

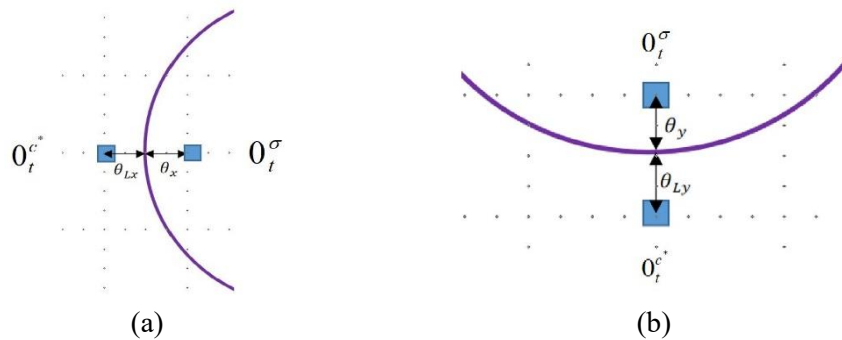
Referring to equation (4), the velocity on the interface is equal to the velocity extension on the interface.

### 3. Numerical Computation

In this paper, the protrusion is observed on the plasma membrane of an individual cancer cell. Initially, the level set is taken as the equation of circle with radius  $r$  to represent an individual cancer cell. Afterward, equation (5) is applied to determine the location of intracellular, extracellular, and interface. The ligand can be found in the extracellular region, while signal is in the intracellular region. Meanwhile, the zero-level set function is used to detect the location of the interface.

$$\Psi_{i,j}^0 \begin{cases} \Psi_{i,j}^0 < 0, & \mathbf{x} \in \Omega_0^\sigma, \\ \Psi_{i,j}^0 > 0, & \mathbf{x} \in \Omega_0^i, \\ \Psi_{i,j}^0 = 0, & \Gamma_0. \end{cases} \tag{5}$$

In discretization of the Laplacian operator in (1) and (2), the method of second-order centered difference and ghost fluid with linear extrapolation are performed for the regular and neighboring points, respectively. The regular point is the point that is exactly on the grid and far from the interface while neighboring point is the point where one of the points is on the other side of the interface. Figure 1 indicated the presence of neighboring points from the right and below sides from the interface in the intracellular and extracellular regions. From the figure, the  $\theta_x$  and  $\theta_y$  are the distance of points  $x_i$  and  $y_j$  to the interface in  $x$  and  $y$  directions, respectively.



**Figure 1.** The neighboring points from the interface on the intracellular and extracellular region (a) Right neighboring point and (b) Below neighboring point.

The ghost fluid method is most suitable to implement for the neighboring point because the finite difference method should involve five stencil points. However, for the neighboring point, one of the points is on the other side of the interface known as a ghost point. Hence, on the signal region, equations (6) and (7) are the equations used to discretize the points situated on the right and below sides, respectively. On the extracellular region, equation (8) is the formula to discretize the point that is situated on the right side, while equation (9) is for the point located on the below side from the interface.

$$(\sigma_{xx})_{i,j} = \frac{2}{(1+\theta_x)h^2} \sigma_{i+1,j} - \frac{2}{\theta_x h^2} \sigma_{i,j} + \frac{2}{\theta_x(1+\theta_x)h^2} \sigma_\Gamma, \tag{6}$$

$$(\sigma_{yy})_{i,j} = \frac{2}{(1+\theta_y)h^2} \sigma_{i,j+1} - \frac{2}{\theta_y h^2} \sigma_{i,j} + \frac{2}{\theta_y(1+\theta_y)h^2} \sigma_\Gamma, \tag{7}$$

$$(c^*_{xx})_{i,j} = \frac{2}{(1+\theta_{Lx})h^2} c^*_{i-1,j} - \frac{2}{\theta_{Lx} h^2} c^*_{i,j} + \frac{2}{\theta_{Lx}(1+\theta_{Lx})h^2} c^*_\Gamma, \tag{8}$$

$$(c^*_{yy})_{i,j} = \frac{2}{(1+\theta_{Ly})h^2} c^*_{i,j-1} - \frac{2}{\theta_{Ly} h^2} c^*_{i,j} + \frac{2}{\theta_{Ly}(1+\theta_{Ly})h^2} c^*_\Gamma, \tag{9}$$

In the meantime, the distance to the interface,  $\theta_x$  and  $\theta_y$  are computed using the formula as presented in equations (10) and (11), respectively. If the distance to the interface is one, then, the point is stated to be located exactly on the gridline or called a regular point.

$$(\theta_x)_{i,j} \begin{cases} \frac{\psi_{i,j}}{\psi_{i,j} - \psi_{i-1,j}}, & x_\Gamma \in [x_{i-1,j}, x_{i,j}], \\ -\frac{\psi_{i,j}}{\psi_{i+1,j} - \psi_{i,j}}, & x_\Gamma \in [x_{i,j}, x_{i+1,j}], \end{cases} \tag{10}$$

$$(\theta_y)_{i,j} \begin{cases} \frac{\psi_{i,j}}{\psi_{i,j} - \psi_{i,j-1}}, & y_\Gamma \in [y_{i,j-1}, y_{i,j}], \\ -\frac{\psi_{i,j}}{\psi_{i,j+1} - \psi_{i,j}}, & y_\Gamma \in [y_{i,j}, y_{i,j+1}]. \end{cases} \tag{11}$$

On the other hand, for the extracellular region, the  $\theta_{Lx}$  and  $\theta_{Ly}$  in the figure 1, are the notation used for the distance of points  $x_i$  and  $y_j$  to the interface in  $x$  and  $y$  directions, respectively. Hence, the calculation used to interpret the  $\theta_{Lx}$  and  $\theta_{Ly}$  are as in equations (12) and (13).

$$\theta_{Lx} = 1 - \theta_x \tag{12}$$

$$\theta_{Ly} = 1 - \theta_y \tag{13}$$

Next, the velocity or the movement of the interface is caused by the activity of actin polymerization. In this paper, the actin polymerization is accounted for as the difference of gradient between the intracellular signal and extracellular ligand. Also, in applying the method of level set, not only the velocity on the interface is required but the velocity with the whole domain is essential. Hence, the velocity extension is applied for the velocity in each area from the interface. The gradient operator,  $\nabla\Psi$  in equation (4) is discretized based on the upwind technique for each point from the interface to all other areas.

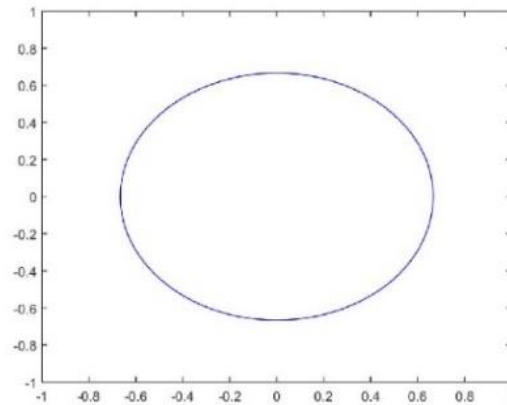
#### 4. Results and Discussion

In this section, the results for the mathematical modeling of time-independent signal and ligand associated with invadopodia formation are presented. The following results are obtained from the numerical computation in two-dimensional space dimensions. In this paper, the concentration of MMPs,  $g$  is varied to determine the existence of protrusions or invadopodia on the plasma membrane. There are five different types of functions  $g$  used in this paper including trigonometric of cosine function,  $g = \varepsilon[2 + \cos(3\Pi(x + y))\cos(\Pi(x + 0.3))]$ , cited in [17], trigonometric with sine function,  $g = \varepsilon[2 + \sin(3\Pi(x + y))\sin(\Pi(x + 0.3))]$ , exponent,  $g = \varepsilon[\exp(x + y)]$ , linear,  $g = \varepsilon(1 - x - y)$ , and quadratic,  $g = \varepsilon(1 - x^2 - y^2)$  where epsilon,  $\varepsilon$  is taken as  $\varepsilon = 10^{-1}$ . By comparing the simulation results, the appearance of invadopodia is discussed.

The initial level set is described as the equation of a circle with radius  $r = \frac{2}{3}$  as presented in figure 2 and the domain is taken in a square domain,  $[-L, L] \times [-L, L]$  where  $L = 1$ . The discretization for the regular and neighboring points is carried out using the methods of second-order centered difference and ghost fluid with linear extrapolation, respectively. The step size,  $h$  for  $x$  and  $y$  axes is computed as  $h = \frac{L}{M}$ , and  $M = 50$ . The computation is conducted up to 100 iterations and the protrusion obtained is observed.

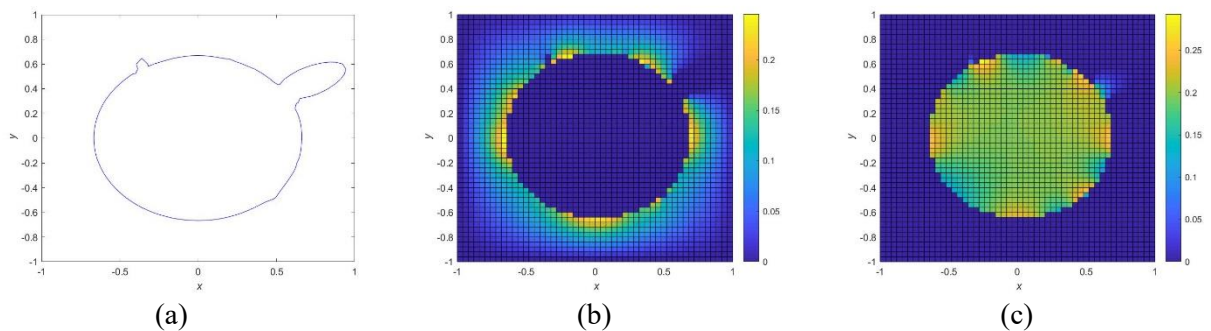
From the results, we can notice that the existence of protrusion using the exponent function is faster, which is at iteration 6, the outward protrusion existed on the plasma membrane as demonstrated in figure 5(a). This is followed by trigonometric with a sine function, where the protrusion is detected on the plasma membrane at iteration 7 as in figure 4(a). Figure 3(a) displayed the protrusion obtained for the trigonometric with cosine function is at iteration 8. Also, both linear and quadratic functions take a longer time for the protrusion to exist on the plasma membrane. The protrusions found using

linear and quadratic functions are at iterations 84 and 31, respectively. As shown in the figure of level set, most of the protrusions are noticed at the positive sides of  $x$  and  $y$  axes.

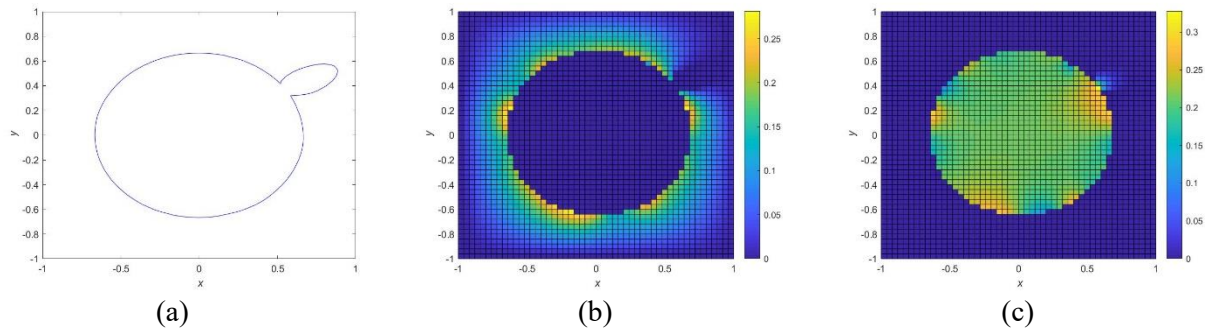


**Figure 2.** The initial level set.

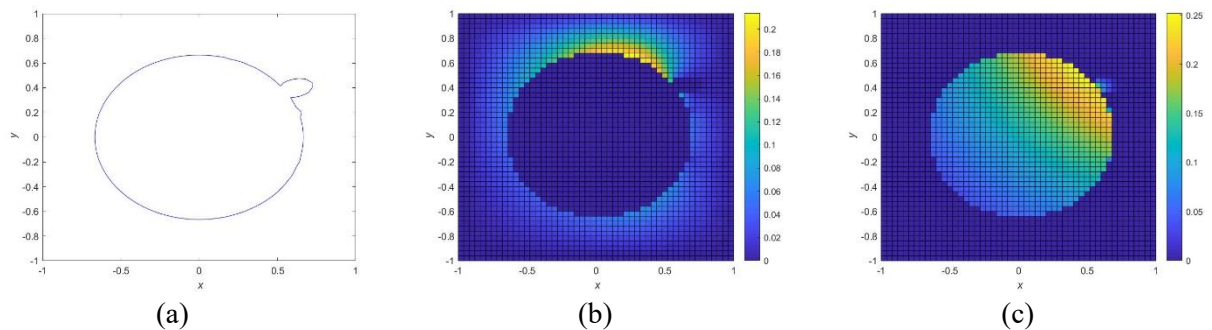
The movement of the free boundary interface is detected by setting the interface as the zero-level set function. Apart from the movement of the interface, the ligand and signal distribution profiles are also given. From the results, it is proven that the signal is spotted in the intracellular region while the ligand is situated in the extracellular region. This is achievable through the approach of the different sign of level set,  $\Psi$ . The positive values of  $\Psi$  indicated the extracellular region where the ligand is located, while negative values of  $\Psi$  specified the intracellular region where signal is perceived. Note that, the protrusion can be found at the high density of ligand and signal. This is proven in a study conducted in [15], where it is shown that, there is a high concentration of signal on the plasma membrane as time increased. Also, the distribution profiles for ligand and signal are corresponding to the protrusion achieved.



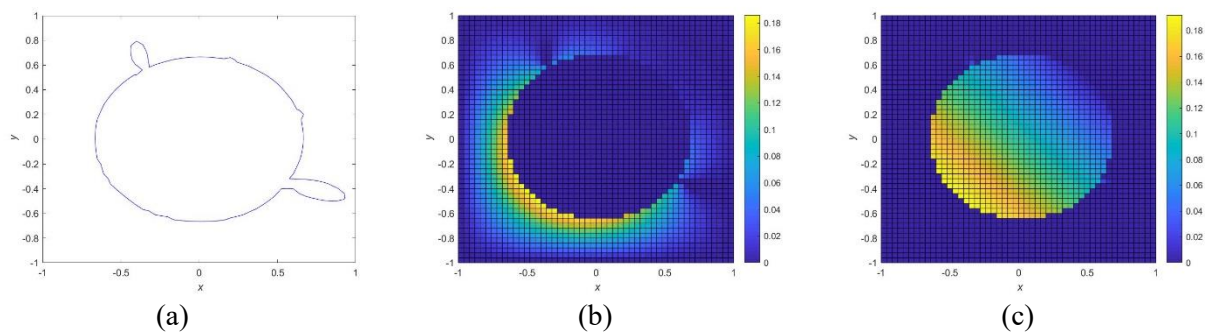
**Figure 3.** The distribution profiles of (a) level set; (b) ligand; and (c) signal for trigonometric with cosine function at iteration 8.



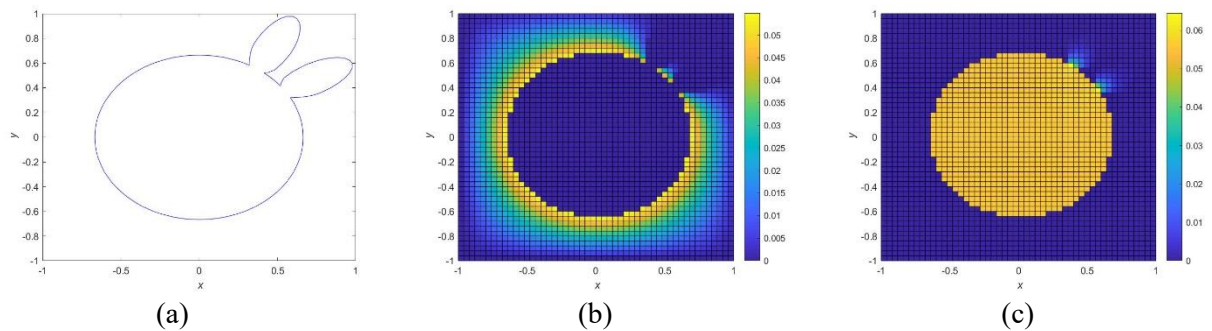
**Figure 4.** The distribution profiles of (a) level set; (b) ligand; and (c) signal for trigonometric with sine function at iteration 7.



**Figure 5.** The distribution profiles of (a) level set; (b) ligand; and (c) signal for exponent function at iteration 6.



**Figure 6.** The distribution profiles of (a) level set; (b) ligand; and (c) signal for linear function at iteration 84.

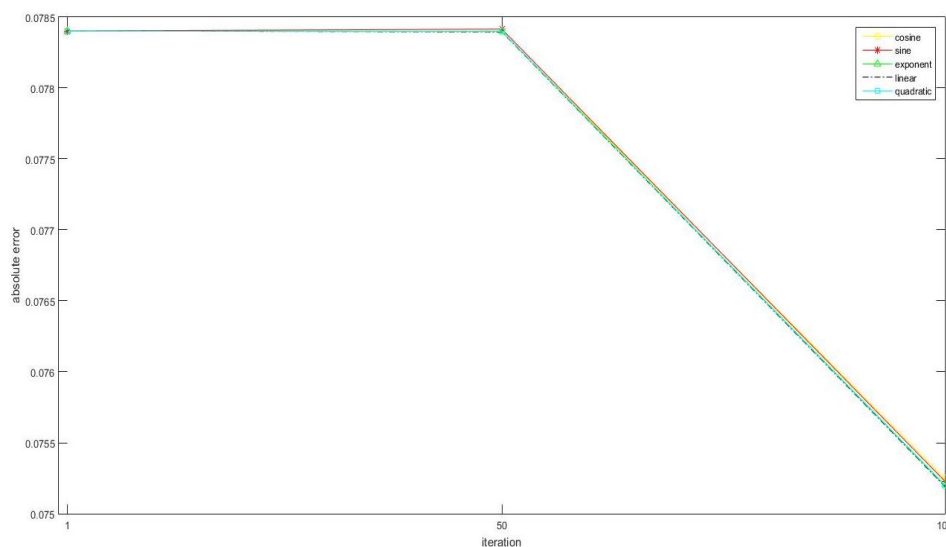


**Figure 7.** The distribution profiles of (a) level set; (b) ligand; and (c) signal for quadratic function at iteration 31.

**Table 1.** The absolute errors of level set for each function at one, 50, and 100 iterations.

Iteration	Function ( $g$ )	Absolute error of level set
1	cosine	0.0784015312
	sine	0.0784017722
	exponent	0.0784000017
	linear	0.0784014562
	quadratic	0.0784003224
50	cosine	0.0783977421
	sine	0.0784152670
	exponent	0.0784012802
	linear	0.0783960343
	quadratic	0.0783991782
100	cosine	0.0752487780
	sine	0.0752293699
	exponent	0.0752023648
	linear	0.0751922051
	quadratic	0.0751983292

The absolute error for the level set is given in table 1 and figure 8 to study the effectiveness of the numerical computation. As shown in table 1, the absolute error for each function is described at one, 50, and 100 iterations. There is a slight difference in results for all the functions given. The absolute error is shown to change slowly at the early iterations and then changed rapidly as iteration reached 100. It is also seen that the absolute error is shown to decrease as iteration increased.



**Figure 8.** The iteration versus absolute error of level set.



## 5. Conclusion

This paper studied the formation of invadopodia that is observed through the existence of finger-like protrusions on the plasma membrane by emphasizing the mathematical modeling of signal and ligand. The positive feedback loop on the formation of invadopodia has been proposed by [16] to show the molecular interactions of MMPs, actin, ligand, and ECM. In this paper, the signal and ligand are considered. The signal is very important for the invadopodia formation as well as for ligand since signal is only stimulated after the binding of ligand with the membrane-associated receptor such as EGFR occurred.

The main objective of this paper is to observe the availability of protrusion on the plasma membrane by using five different functions of  $g$ . Also, the zero-level set function is applied to detect the motion of the free boundary interface by setting the interface to zero level set function. Meanwhile, the regions of intracellular and extracellular are distinguished using the positive and negative signs of the level set. From the results, the signal is spotted in the intracellular region while the ligand is noticed in the extracellular region.

Also, the movement of the interface is a result of the difference of gradient between intracellular signal and extracellular ligand. In the meantime, the extended velocity is also implemented due to applying the method of level set; not only is the velocity on the interface crucial, the velocity of the whole domain is also needed. The Laplacian operator for the signal and ligand is solved using the method of second-order centered difference and ghost fluid method with linear extrapolation, respectively.

Credit to [17] that successfully showed the techniques to discretize the Laplacian operator especially dealing with the regular and neighboring points. From the numerical simulations, the exponential function shows the fastest on the existence of protrusions on the plasma membrane, followed by sine, cosine, quadratic, and linear functions.

In addition, the protrusion can be spotted at the high concentration of ligand and signal. Hence, we can conclude that the high density of ligand and signal can affect the formation of the protrusions on the plasma membrane. The numerical error is also computed to examine the effectiveness of the numerical computation, and from the findings, it is found that the absolute error decreases as iterations of computing increased.

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