**Instructions for *PolarSim***

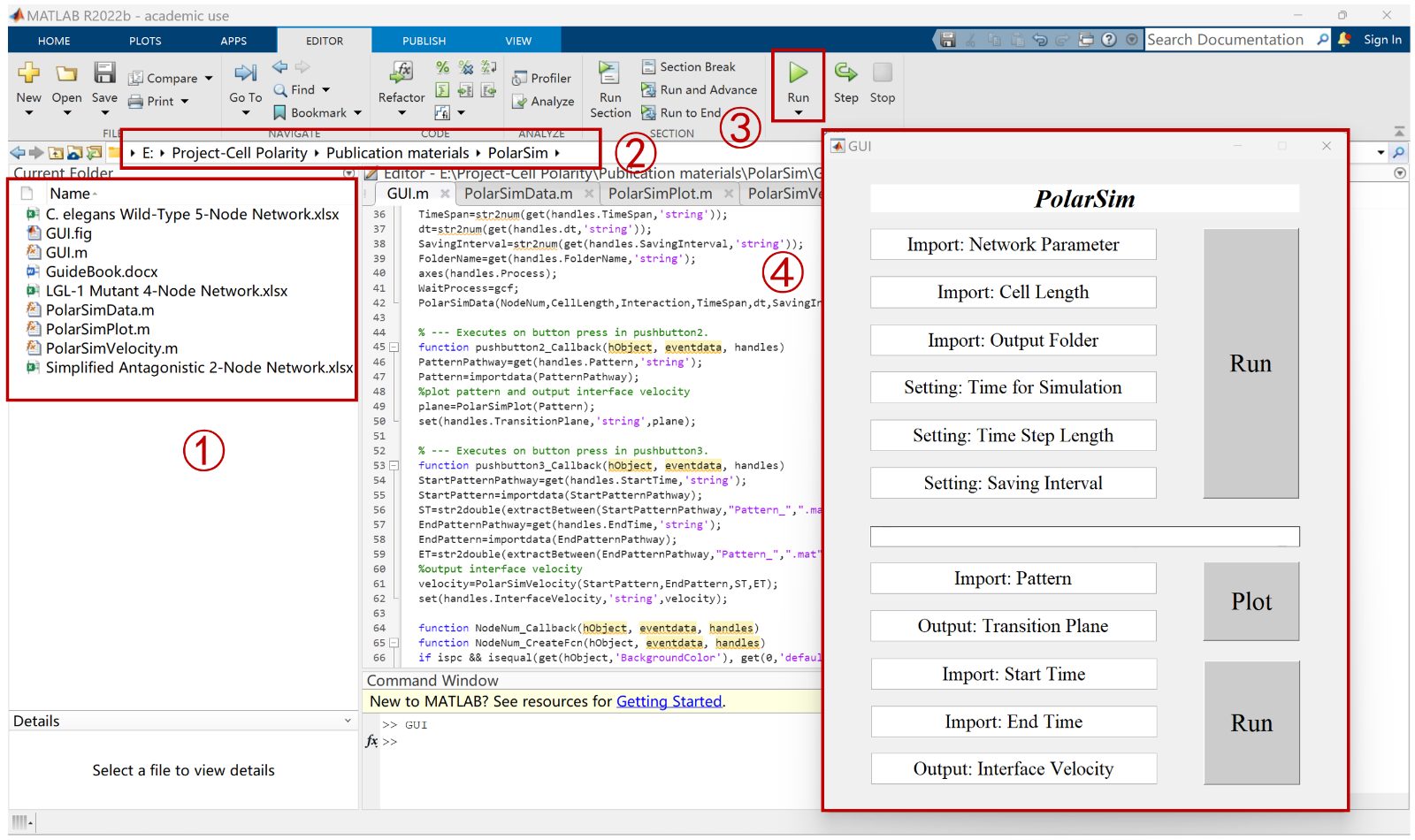
**1. Introduction**

*PolarSim* is a graphical user interface (GUI) on Matlab 2022b for simulating the evolution of cell polarity pattern. Based on reaction-diffusion model, the GUI allows users to compute the behaviors of cell polarity networks in different biological systems. All the simulations are tested with an 12th Gen Intel(R) Core(TM) i7-1260P CPU.

**2. Tutorials**

· Download the folder “PolarSim” from xxx

* Open Matlab under the “PolarSim” folder path and execute script GUI.m. Click “Run” and an interactive interface pops up (Fig. G1).



**Fig. G1.** Instructions to open the *PolarSim* GUI. ① The files in the folder “PolarSim”. ② Open the Matlab under the path of the “PolarSim” folder and double-click to open “GUI.m”. ③ Click “Run” to open the GUI interface *PolarSim* shown by ④.

* Import the following parameters and it gives out a group of “Pattern\_\*.mat” files containing pattern information.

(1) Import: Network Parameter.

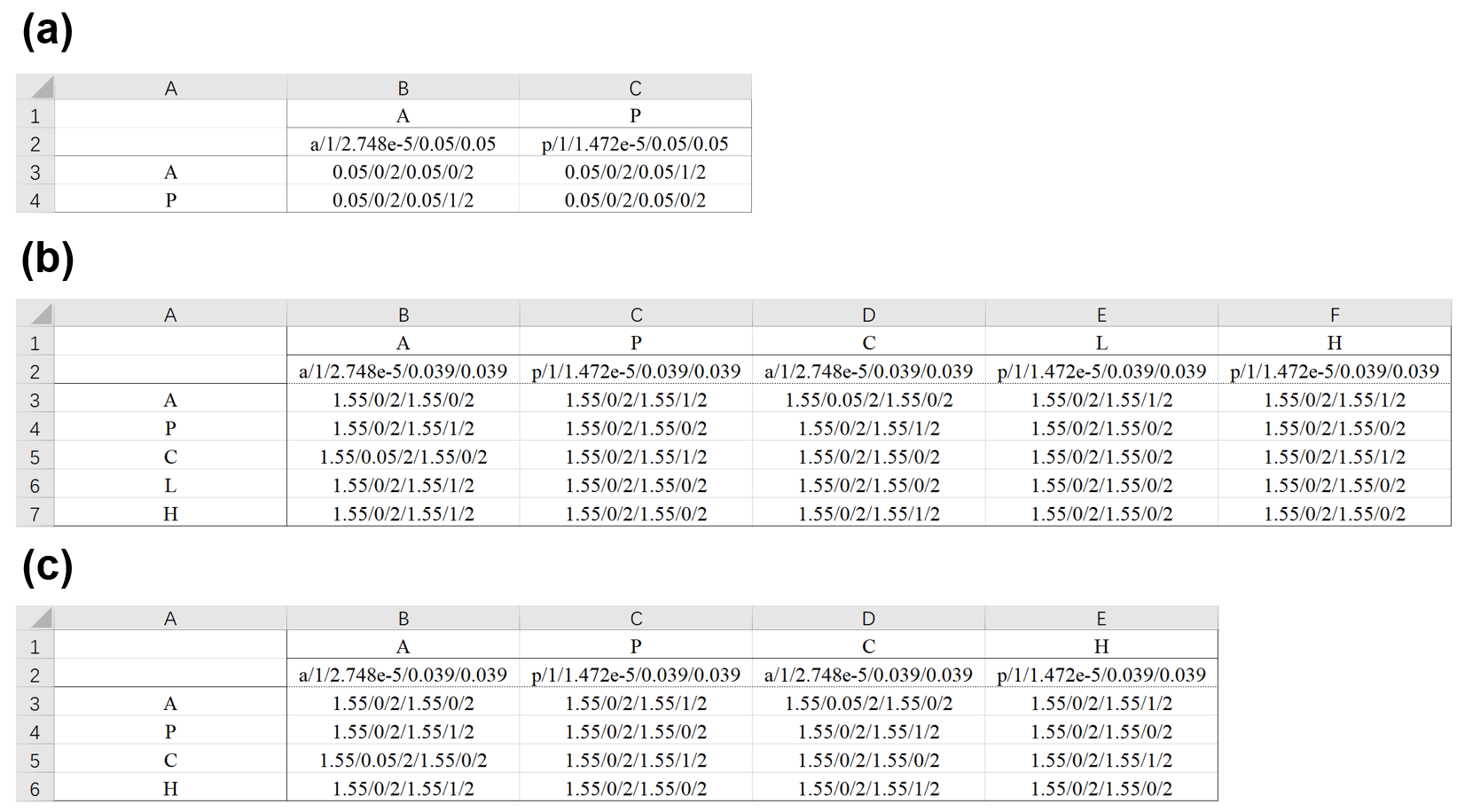
We gives out three examples “Simplified Antagonistic 2\_Node Network.xlsx”, “LGL-1 Mutant 4-Node Network.xlsx” and “C. elegans Wild-Type 5-Node Network.xlsx” in the “PolarSim” folder, respectively representing the typical networks in the text (Fig. G2). The excel should follow the format below:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Node A | Node B | … | Node N |
|  | Location (a or p)/// | Location (a or p)/// | … | Location (a or p)/// |
| Node A | ///// | ///// | … | ///// |
| Node B | ///// | ///// | … | ///// |
| … | … | … | … | … |
| Node N | ///// | ///// | … | ///// |

**Table G1.** Instructions for the format of Network Parameter. In the first row and column, “Node N” represents the Node name. The second row explains characteristic parameter of each node (*i.e.* location, cytoplasmic concentration, basal association rate and dissociation rate). Interaction parameters starts from the third row, second column, and the Row *i* and Column *j* means the effect from node *j* to *i* (*i.e.* the activation/inhibition pathway). should be set to 0 when no activation is exerted on X from Y; it’s the same with to describe absent inhibition. See the detailed description of each parameter in Table G2.

|  |  |
| --- | --- |
| Parameter | Biological implication |
| Location | “a” represents anterior-located molecular while “p” posterior-located |
|  | The conserved concentration of molecule X in the cell cytosol |
|  | Basal on-rate of molecule X |
|  | Basal off-rate of molecule X |
|  | Activation pathway response from Y to X |
|  | Activation intensity from Y to X |
|  | the Hill coefficient of activation from Y to X |
|  | Inhibition pathway response from Y to X |
|  | Inhibition intensity from Y to X |
|  | the Hill coefficient of inhibition from Y to X |

**Table G2.** The symbols and description of parameters used in Table G1.



**Fig. G2.** Examples of network parameter. (a) “Simplified Antagonistic 2\_Node Network.xlsx” lists the parameters in Fig. 1a. (b) “C. elegans Wild-Type 5-Node Network.xlsx” lists the benchmark point in Fig. 6b. (c) “LGL-1 Mutant 4-Node Network.xlsx” lists the parameter as (b) but knocking down the node [L].

(2) Import: Cell Length

We take “0.5” as an example. Any positive number is allowed in this box. The effects of cell length on polarity pattern are discussed in Fig. G6.

(3) Import: Output Folder

Give a folder name for storing the output results (*e.g.* “Output 2-Node”)

(4) Setting: Time for Simulation

Simulation duration “500” is used in the text. Any positive number is allowed in this box.

(5) Setting: Time Step Length

Recommanded use “1”. Larger values are not recommended to avoid time failure, and try smaller step length while meeting integration tolerances.

(6) Setting: Saving Interval

It must be an integer multiple of time step length. Remember to save the timepoint to plot the pattern.

* Click “Run” in the interface, and check its status on the progress bar (Fig. G5a). A folder named by “Import: Output Folder” is generated in the current path to store the output “Pattern\_\*.mat” data containing the node name, location and pattern, where “\*” denotes the *in silico* time corresponding to each file (Fig. G3a, right).
* Import the file pathway in the box “Import: Pattern” outputted by *PolarSim*.
* Click “Plot” and a figure comes out to show the pattern and the position of the transition plane appears in the box “Output: Transition Plane”.
* Import the file pathway in the box “Import: Start Time” and “Import: End Time” outputted by *PolarSim*.
* Click “Run” and the mean interface velocity between two input time points is calculated in the box “Output: Interface velocity”.

**3. Examples**

· **Example 1**: Simplified Antagonistic 2-Node Network simulation as Fig. 1a.

(1) Input the following parameters: “.\Simplified Antagonistic 2-Node Network.xlsx” in “Import: Network Parameter”; “0.5” in “Import: Cell Length”; “Output 2-Node” in “Import: Output Folder”; “500” in “Setting: Time for Simulation”; “1” in “Setting: Time Step Length” and “100” in “Setting: Saving Interval”.

(2) Click “Run”, and 6 “Pattern\_\*.mat” files are saved under the subfolder “Output 2-Node” (Fig. G3a, ①-③).

(3) Input “.\Output 2-Node\Pattern\_500.mat” in the box “Import: Pattern”, and a figure of 2-node antagonistic network at appears. The position of the transition plane is calculated in the box “Output: Transition Plane” (Fig. G3a, ④-⑥).

(4) Input “.\Output 2-Node\Pattern\_300.mat” in the box “Import: Start Time” and “.\Output 2-Node\Pattern\_500.mat” in the box “Import: End Time”, and the mean interface velocity between and is calculated in the box “Output: Interface Velocity” (Fig. G3a, ⑦-⑨). The 2-node network approaches a stable polarized pattern with interface velocity being 0.

· **Example 2**: C. elegans Wild-Type 5-Node Network simulation as Fig. 6b.

(1) Input the following parameters: “.\C. elegans Wild-Type 5-Node Network.xlsx” in “Import: Network Parameter”; “0.5” in “Import: Cell Length”; “Output 5-Node” in “Import: Output Folder”; “500” in “Setting: Time for Simulation”; “1” in “Setting: Time Step Length” and “100” in “Setting: Saving Interval” (Fig. G3b, ①-③).

(2) Click “Run”, and 6 “Pattern\_\*.mat” files are saved under the subfolder “Output 5-Node” (Fig. G4b).

(3) Input “.\Output 5-Node\Pattern\_500.mat” in the box “Import: Pattern”, and a figure of 5-node network at appears. The position of the transition plane is calculated in the box “Output: Transition Plane” (Fig. G3b, ④-⑥).

(4) Input “.\Output 5-Node\Pattern\_300.mat” in the box “Import: Start Time” and “.\Output 5-Node\Pattern\_500.mat” in the box “Import: End Time”, and the mean interface velocity between and is calculated in the box “Output: Interface Velocity” (Fig. G3b, ⑦-⑨). The 5-node network approaches a stable polarized pattern with interface velocity being 0.

· **Example 3**: LGL-1 Mutant 4-Node Network, Originated from the C. elegans Wild-Type 5-Node Network but knocking down [L].

(1) Input the following parameters: “.\LGL-1 Mutant 4-Node Network.xlsx” in “Import: Network Parameter”; “0.5” in “Import: Cell Length”; “Output 4-Node” in “Import: Output Folder”; “1000” in “Setting: Time for Simulation”; “1” in “Setting: Time Step Length” and “100” in “Setting: Saving Interval” (Fig. G4a).

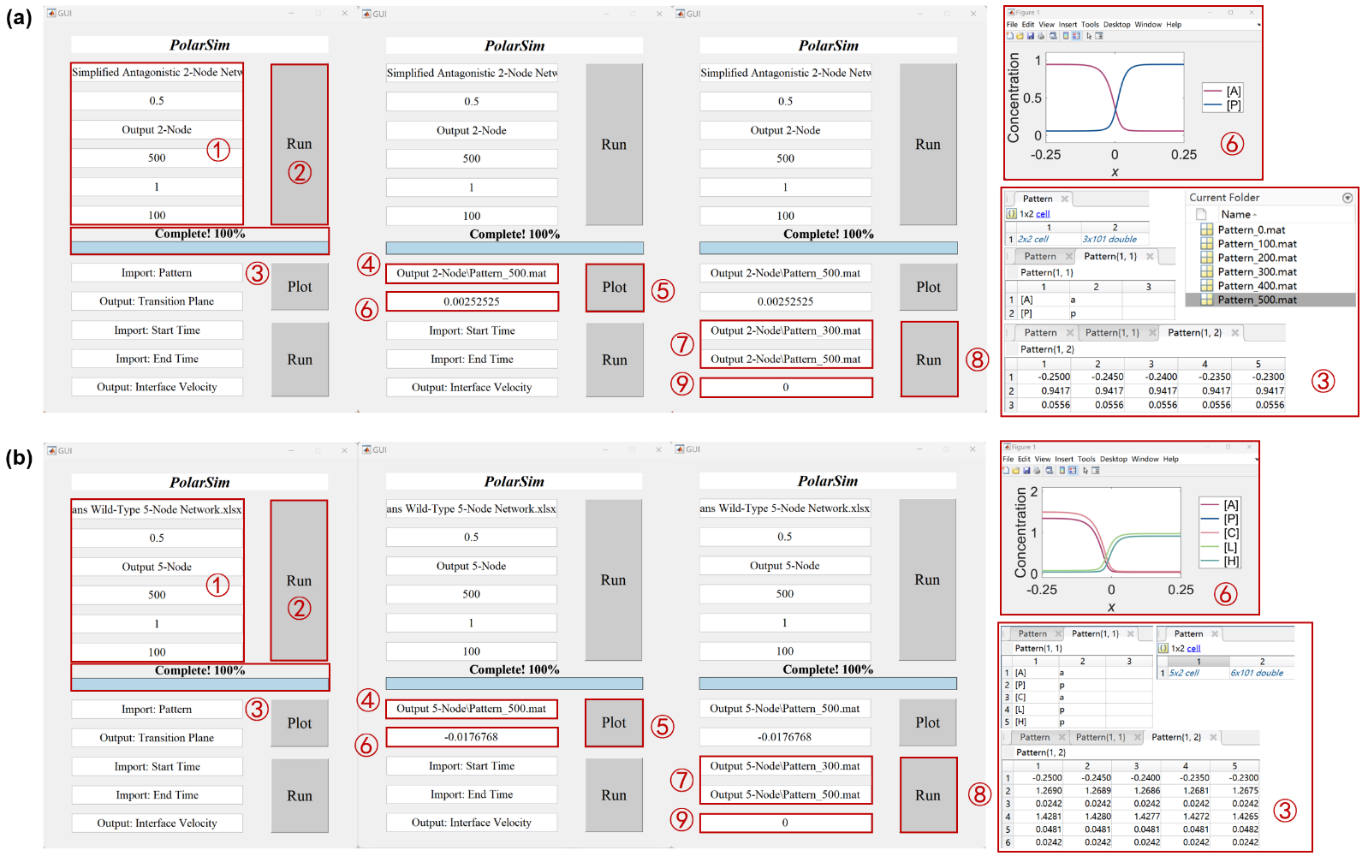
(2) Click “Run”, and 11 “Pattern\_\*.mat” files are saved under the subfolder “Output 4-Node”.

(3) Input “.\Output 4-Node\Pattern\_500.mat” in the box “Import: Pattern”, and a figure of 4-node network at appears with the transition plane close to posterior pole. The position of the transition plane is calculated in the box “Output: Transition Plane” (Fig. G4a).

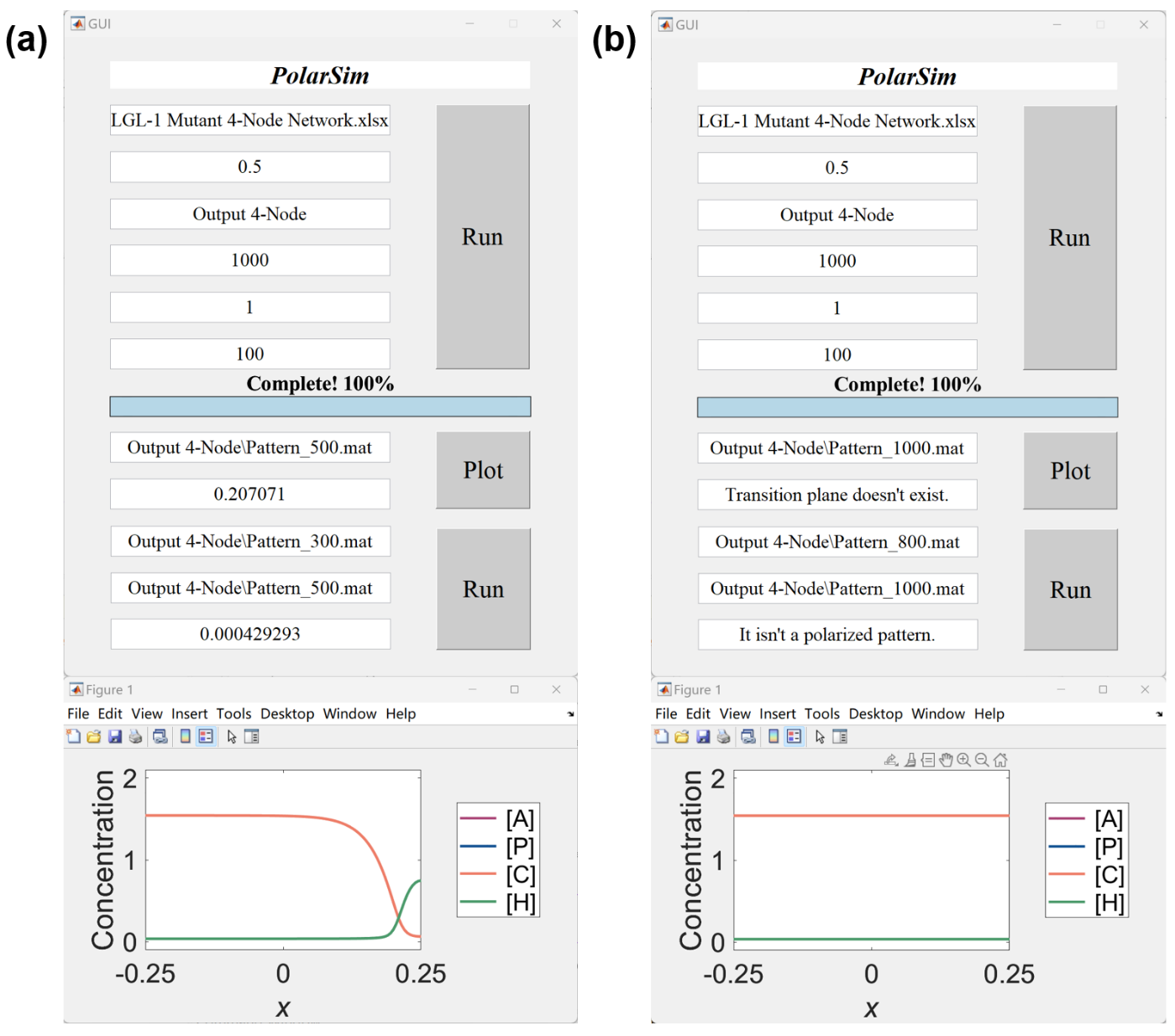
(4) Input “.\Output 4-Node\Pattern\_300.mat” in the box “Import: Start Time” and “.\Output 4-Node\Pattern\_500.mat” in the box “Import: End Time”, and the mean interface velocity between and is calculated in the box “Output: Interface Velocity” (Fig. G4a). The interface of 4-node network keeps moving posteriorly with .

(3) Input “.\Output 4-Node\Pattern\_1000.mat” in the box “Import: Pattern”, and a figure of 4-node network at appears with the transition plane close to posterior pole. “Transition plane doesn’t exist” appears in the box “Output: Transition Plane” as the pattern reaches a homogeneous state dominated by [A] and [C] (Fig. G4b).

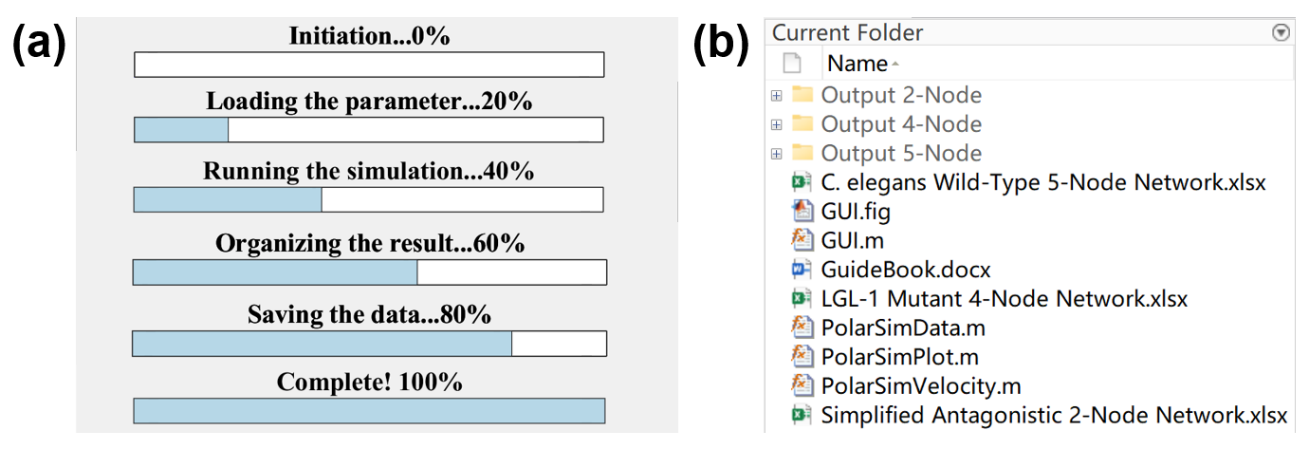
(4) Input “.\Output 4-Node\Pattern\_800.mat” in the box “Import: Start Time” and “.\Output 4-Node\Pattern\_1000.mat” in the box “Import: End Time”. The mean interface velocity between and doesn’t exist with a string “It isn’t a polarized pattern” in the box “Output: Interface Velocity” appearing to hint (Fig. G4b). Note that the interface velocity can’t be calculated when either pattern at start time or end time is homogeneous.



**Fig. G3.** The results of Example 1&2 of *PolarSim*. (a) The flow chart of the simplified Antagonistic 2-Node Network. ① Input parameters. ② Click “Run”. ③ The simulation completes with a 100% progress bar and the files saved in “Output 2-Node”. The 1×2 cell data stored in “Pattern\_500.mat” is used to show the data format, the first one storing the node names and their location and the second storing *x*-axis and pattern of each node at . ④ Input the file pathway of the pattern. ⑤ Click “Plot”. ⑥ The simulation completes with a figure at and the result of transition plane in the box “Output: Transition Plane”.⑦ Input the file pathway of the “Pattern\_\*.mat” into “Import: Start Time”, where “\*” denotes the start time. It’s the same with “Import: End Time”, but “\*” denotes the end time. ⑧ Click “Run”. ⑨ The simulation completes with the result of interface velocity in the box “Ouput: Interface Velocity”. (b) The same as (a) but for the *C. elegans* Wild-Type 5-Node Network.



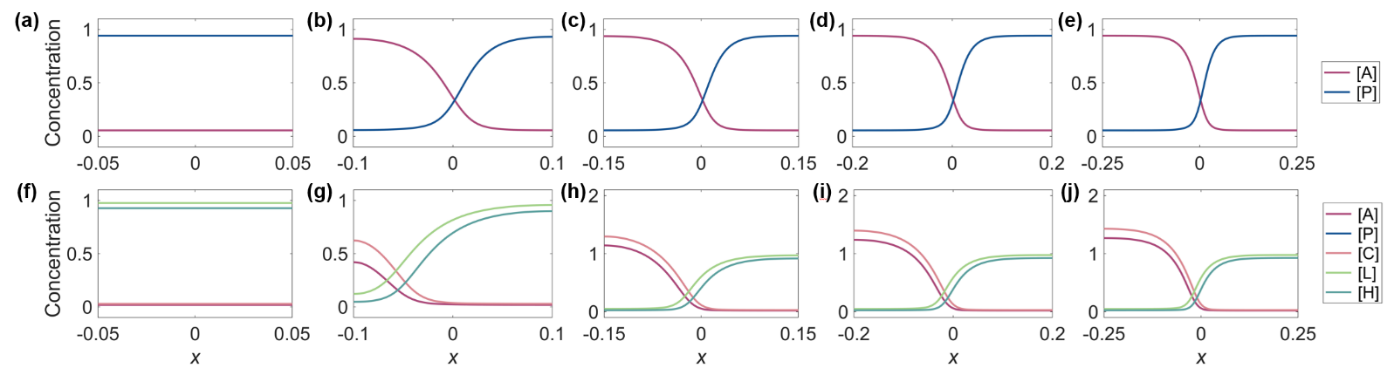
**Fig. G4.** The results of Example 3 of *PolarSim*. (a) The input of the LGL-1 Mutant 4-Node Network. The transition plane is calculated at , with a result of . The interface velocity is calculated between and , with the result representing an unstable pattern (top). The figure is plotted at (bottom). (b) The transition plane and the figure is shown at and the interface velocity is calculated between and . The pattern collapses to a homogeneous state with [A] and [C] invading into the posterior domain at , thus the transition plane doesn’t exist and the interface can’t be calculated.



**Fig. G5.** (a) The progress bar shows running progress. (b) The output subfolders in the folder PolarSim.

**4. Application**

Our *PolarSim* is also applicable for similar biological system. Here, we take the cell length as an example to study how the polarity pattern output depends on the system size. Different cell lengths are applied to the two examples to study whether size threshold limits polarization. Patterns at are plotted with the cell length ranging from 0.1 to 0.5 (Fig. G6).



**Fig. G6.** The effects of cell length on the polarity pattern. (a-e) The pattern of antagonistic 2-Node network at . From left to right, the cell lengths are respectively 0.1, 0.2, 0.3, 0.4 and 0.5. (f-j) The same as (a-e) but the *C. elegans* 5-Node network.

Polarity pattern demands for reasonable scale, which to some extent gives constraints for cell volume during asymmetric division. *PolarSim* provides a blue prints for more applications in polarity pattern.

**5. Contact**