**Instructions for *PolarSim***

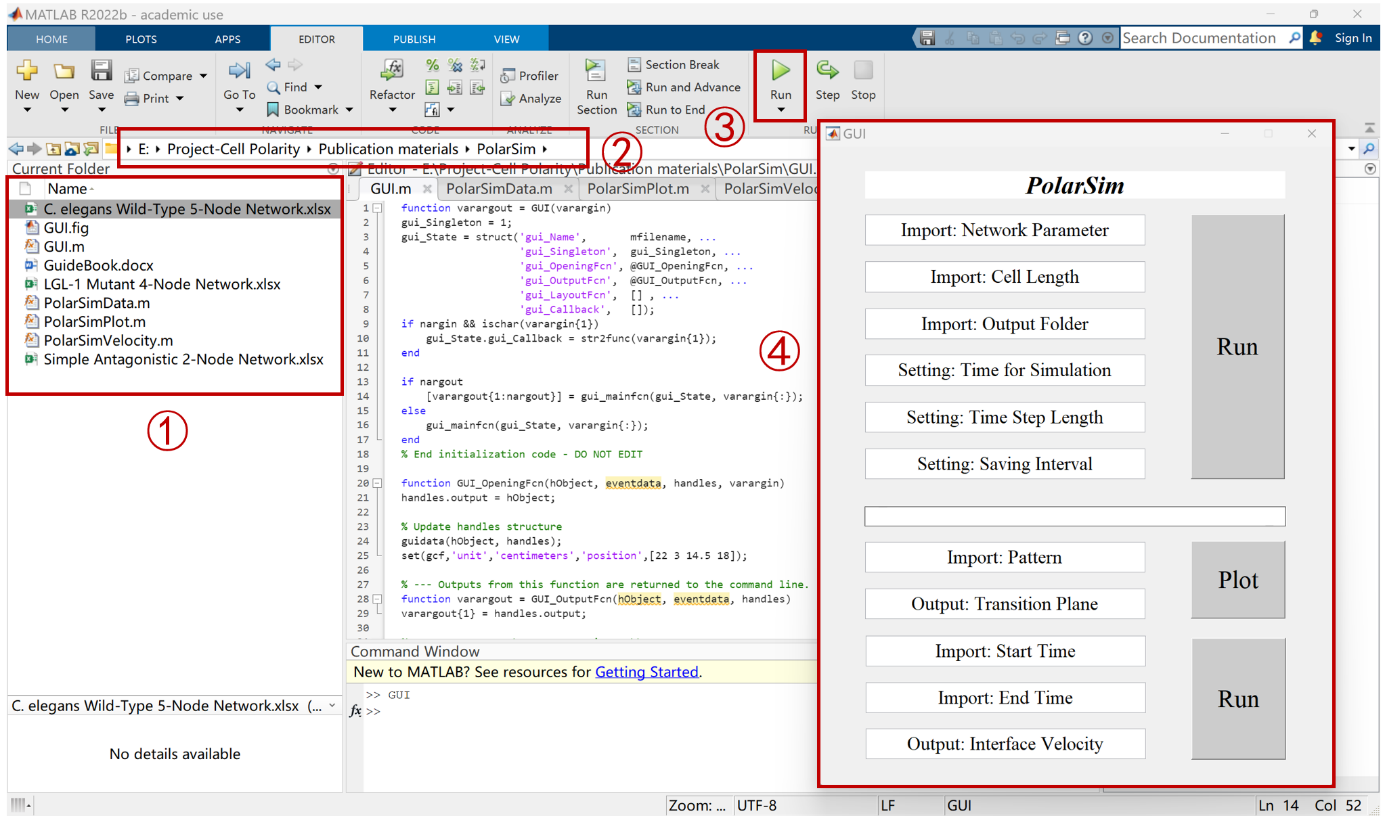
**1. Introduction**

*PolarSim* is a graphical user interface (GUI) on *Matlab* 2022b [59] for simulating the evolution of cell polarization patterns. Based on the reaction-diffusion model, the GUI allows users to compute the behaviors of cell polarity networks in different biological scenarios. All the simulations are tested with a 12th Gen Intel(R) Core(TM) i7-1260P CPU.

**2. Tutorials**

· Download the folder “PolarSim” from https://github.com/YixuanChen0726/Cell-Polarization/tree/main/PolarSim.

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



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

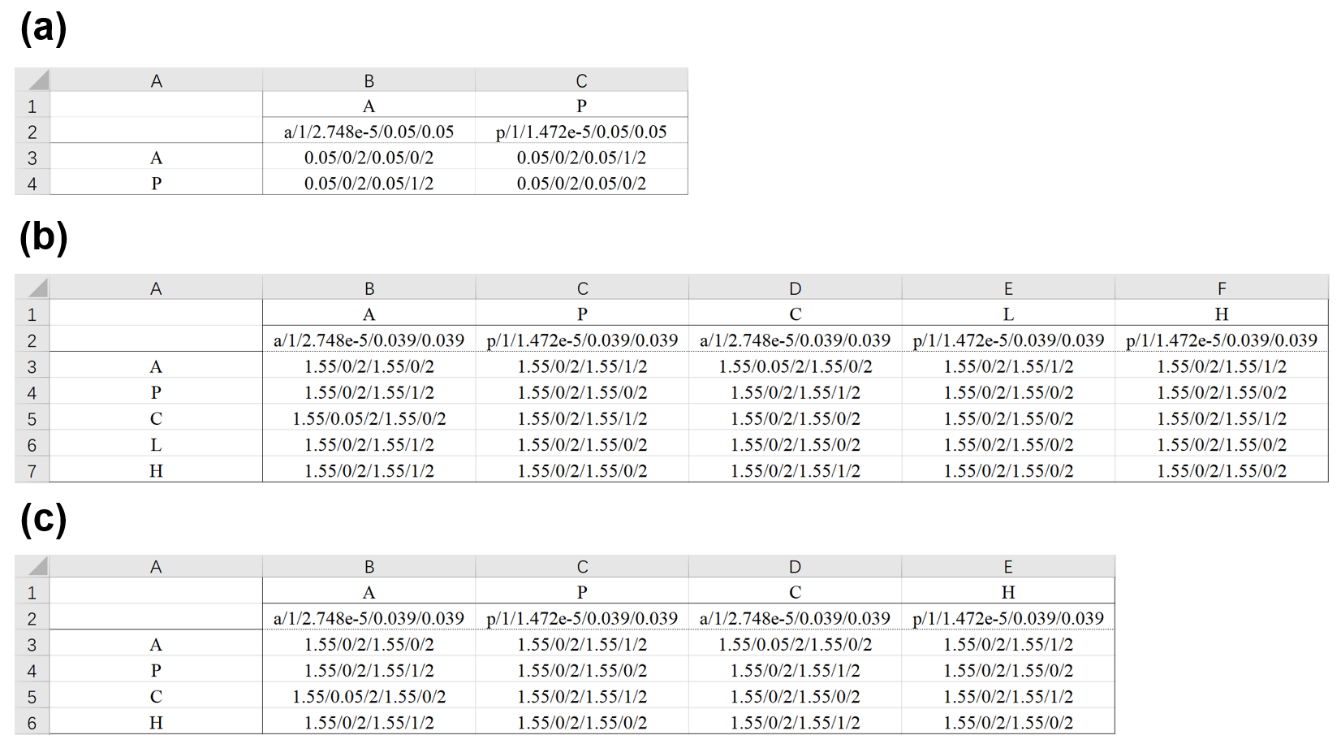
* With the following parameters inputted, the GUI gives out a group of “Pattern\_\*.mat” files containing pattern information.

(1) Import: Network Parameter.

We give out three examples “Simple Antagonistic 2\_Node Network.xlsx”, “LGL-1 Mutant 4-Node Network.xlsx” and “C. elegans Wild-Type 5-Node Network.xlsx” in the folder “PolarSim”, respectively representing the typical networks in this paper (Fig. G2). The Excel table for parameter value assignments 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.** The instructions for the format of Network Parameter in an Excel table. In the 1st row and 1st column, “Node N” represents the name of the node. The 2nd row explains the characteristic parameters of each node (*i.e.* location, cytoplasmic concentration, basal on-rate, and basal off-rate as listed from left to right). The interaction parameters start from the 3rd row and 2nd column, where the *i*th row and *j*th column describe the activation/inhibition effect from Node *j* to Node *i*. Note that and should be set to 0 when no activation or inhibition is exerted on X from Y respectively. “Location” should be assigned with the string “a” or “p” while the description of the other parameters is detailed in Table S1.



**Fig. G2.** The examples of network parameters. (a) “Simple Antagonistic 2-Node Network.xlsx” lists the parameters in Fig. 1a. (b) “C. elegans Wild-Type 5-Node Network.xlsx” lists the parameters for the benchmark point in Fig. 6b. (c) “LGL-1 Mutant 4-Node Network.xlsx” lists the parameters as in (b) but with the Node [L] knocked out.

(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 cell polarization patterns are shown 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 this paper. Any positive number is allowed in this box.

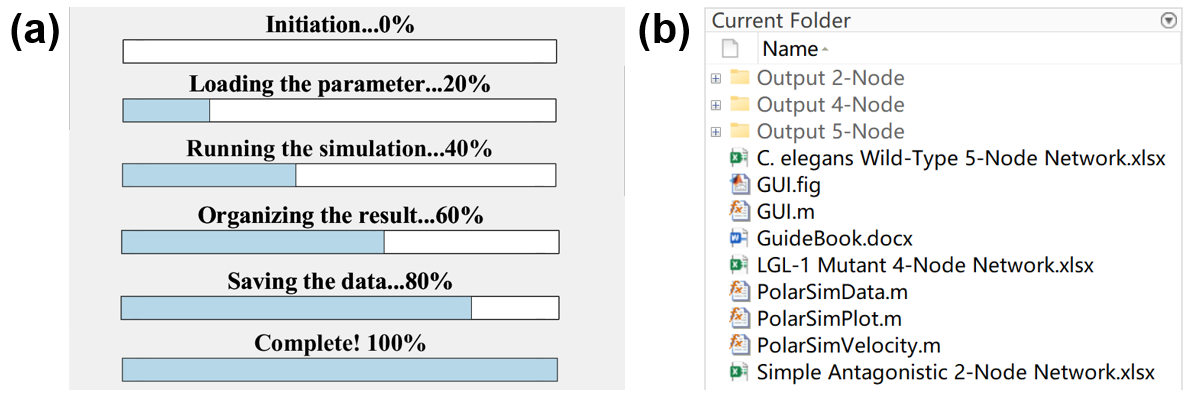
(5) Setting: Time Step Length

We take “1” as an example. Larger values are not recommended, in consideration of possible time failure, and one may try a smaller step length while the error tolerance is exceeded.

(6) Setting: Saving Interval

It must be an integer multiple of the time step length. The time point will be saved in this designated interval and can be used for pattern plotting later.

* Click “Run” in the interface, and then its status on the progress bar is shown (Fig. G3a). A folder named “Import: Output Folder” is generated in the current path to store the output “Pattern\_\*.mat” data containing the name, location, and concentration distribution on the cell membrane of the node (molecule), where “\*” denotes the *in silico* time corresponding to each file (Fig. G4a, right).
* Import the pathway of the file outputted by *PolarSim* into the box “Import: Pattern”.
* Click “Plot” and then a figure comes out to show the cell polarization pattern while the position of the transition plane appears in the box “Output: Transition Plane”.
* Import the pathway of two files outputted by *PolarSim* into the box “Import: Start Time” and “Import: End Time”.
* Click “Run” and then the mean interface velocity between two input time points appears in the box “Output: Interface Velocity”.



**Fig. G3.** (a) The progress bar showing the running progress. (b) The output subfolders in the folder “PolarSim”.



**Fig. G4.** The results of Example 1 (Simple Antagonistic 2-Node Network) and Example 2 (*C. elegans* Wild-Type 5-Node Network) of *PolarSim*. (a) The flow chart for computing Example 1: ① input parameters; ② click “Run”; ③ the simulation is completed with a progress bar shown and the files are saved in the folder “Output 2-Node”; the file “Pattern\_500.mat” is used to show the data format in the right, where the first part stores the name and location of the nodes (molecules) while the second part stores the concentration distribution of each node (molecule) on the cell membrane; ④ input the pathway of the outputted pattern file; ⑤ click “Plot”; ⑥ the simulation is completed with a figure shown and the position of the transition plane given in the box “Output: Transition Plane”; ⑦ input the pathway of the two outputted files “Pattern\_\*.mat” at different time points into “Import: Start Time” and “Import: End Time”, where “\*” denotes the start time and end time respectively; ⑧ click “Run”; ⑨ the simulation is completed with the interface velocity given in the box “Output: Interface Velocity”. (b) The same as (a) but for the *C. elegans* Wild-Type 5-Node Network.

**3. Examples**

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

(1) Input the following parameters: “Simple Antagonistic 2-Node Network.xlsx” into “Import: Network Parameter”; “0.5” into “Import: Cell Length”; “Output 2-Node” into “Import: Output Folder”; “500” into “Setting: Time for Simulation”; “1” into “Setting: Time Step Length” and “100” into “Setting: Saving Interval”. (Fig. G4a, ①)

(2) Click “Run”, and then 6 “Pattern\_\*.mat” files at different time points are saved in the subfolder “Output 2-Node” (Fig. G4a, ②-③).

(3) Input “Output 2-Node\Pattern\_500.mat” into the box “Import: Pattern”, and a figure of Simple 2-Node Antagonistic Network at appears. The position of the transition plane is given in the box “Output: Transition Plane” (Fig. G4a, ④-⑥).

(4) Input “Output 2-Node\Pattern\_300.mat” and “Output 2-Node\Pattern\_500.mat” into the box “Import: Start Time” and “Import: End Time” respectively, and then the mean interface velocity between and is given in the box “Output: Interface Velocity” (Fig. G4a, ⑦-⑨). The 2-node network approaches a stable polarized pattern with its 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” into “Import: Network Parameter”; “0.5” into “Import: Cell Length”; “Output 5-Node” into “Import: Output Folder”; “500” into “Setting: Time for Simulation”; “1” into “Setting: Time Step Length” and “100” into “Setting: Saving Interval” (Fig. G4b, ①).

(2) Click “Run”, and then 6 “Pattern\_\*.mat” files at different time points are saved in the subfolder “Output 5-Node” (Fig. G4b, ②-③).

(3) Input “Output 5-Node\Pattern\_500.mat” into the box “Import: Pattern”, and a figure of *C. elegans* Wild-Type 5-Node Network at appears. The position of the transition plane is given in the box “Output: Transition Plane” (Fig. G4b, ④-⑥).

(4) Input “Output 5-Node\Pattern\_300.mat” and “Output 5-Node\Pattern\_500.mat” in the box “Import: Start Time” and “Import: End Time” respectively, and then the mean interface velocity between and is given in the box “Output: Interface Velocity” (Fig. G4b, ⑦-⑨). The 5-node network approaches a stable polarized pattern with its interface velocity being 0.

· **Example 3**: LGL-1 Mutant 4-Node Network, originated from the *C. elegans* Wild-Type 5-Node Network but with the Node [L] knocked out.

(1) Input the following parameters: “LGL-1 Mutant 4-Node Network.xlsx” into “Import: Network Parameter”; “0.5” into “Import: Cell Length”; “Output 4-Node” into “Import: Output Folder”; “1000” into “Setting: Time for Simulation”; “1” into “Setting: Time Step Length” and “100” in “Setting: Saving Interval” (Fig. G5a).

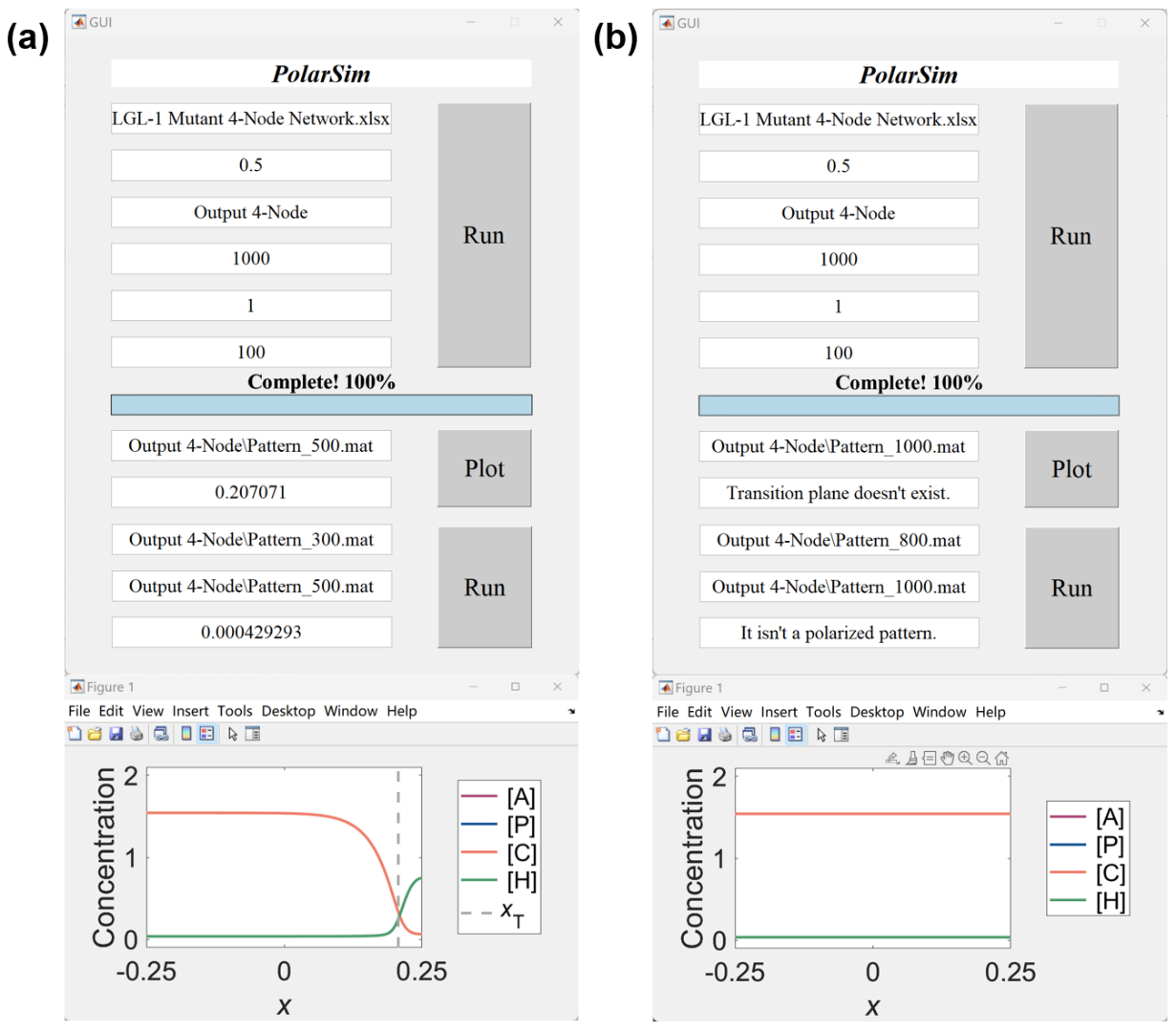
(2) Click “Run”, and then 11 “Pattern\_\*.mat” files at different time points are saved in the subfolder “Output 4-Node”.

(3) Input “Output 4-Node\Pattern\_500.mat” into the box “Import: Pattern”, and then a figure of LGL-1 Mutant 4-Node Network at appears with the transition plane close to the posterior pole. The position of the transition plane is given in the box “Output: Transition Plane” (Fig. G5a).

(4) Input “Output 4-Node\Pattern\_300.mat” and “Output 4-Node\Pattern\_500.mat” in the box “Import: Start Time” and “Import: End Time” respectively, and then the mean interface velocity between and is given in the box “Output: Interface Velocity” (Fig. G5a). The interface of the 4-node network keeps moving toward the posterior with .

(3) Input “Output 4-Node\Pattern\_1000.mat” in the box “Import: Pattern”, and then a figure of LGL-1 Mutant 4-Node Network at appears. The string “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. G5b).

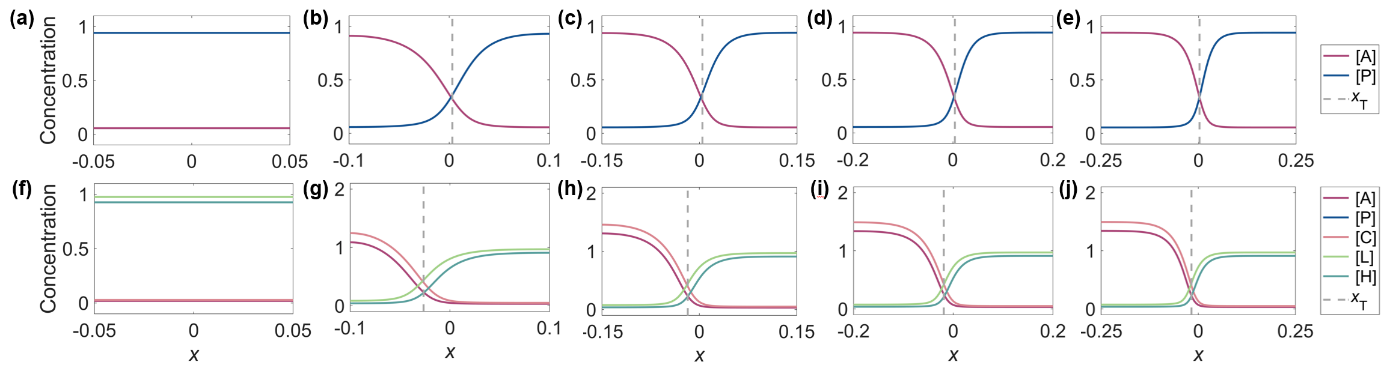
(4) Input “Output 4-Node\Pattern\_800.mat” and “Output 4-Node\Pattern\_1000.mat” in the box “Import: Start Time” and “Import: End Time” respectively. The mean interface velocity between and doesn’t exist with the string “It isn’t a polarized pattern” appearing in the box “Output: Interface Velocity” to hint (Fig. G5b). Note that the interface velocity can’t be calculated when either pattern at start time or end time is homogeneous.



**Fig. G5.** The results of Example 3 (LGL-1 Mutant 4-Node Network) of *PolarSim*. (a) The transition plane is shown at , with a result of . The interface velocity is calculated between and , with the value representing an unstable pattern (top). The figure is plotted at (bottom). (b) The transition plane and the figure are shown at and the interface velocity is calculated between and . The pattern collapses to a homogeneous state with [A] and [C] invading the posterior domain at , and thereby the transition plane doesn’t exist and the interface can’t be calculated.

**4. Extensive Application**

Our *PolarSim* is extensively applicable to similar biological systems. Here, we take the cell size (length) as an exemplary research target to study how the concentration distribution on the cell membrane depends on it. Different cell lengths are applied to the two examples to see whether there is a cell size threshold limiting cell polarization as discovered before [16]. Patterns at are plotted with the cell length ranging from 0.1 to 0.5 in steps 0.1 (Fig. G6).



**Fig. G6.** The effects of cell size (length) on the cell polarization pattern. (a-e) The pattern of Simple Antagonistic 2-Node Network at . From left to right, the cell lengths are 0.1, 0.2, 0.3, 0.4 and 0.5, respectively. (f-j) The same as (a-e) but for the *C. elegans* Wild-Type 5-Node Network.

The *PolarSim*-based simulations above indicate that the proper cell polarization demands a reasonable spatial scale, which to some extent gives an explicit constraint for the volume of a cell in reality when it needs to divide asymmetrically. It’s worth noting that the collapse of cell polarization pattern over cell size decrease may be attributed to two different mechanisms – while the curve of concentration distribution changes with the interface becoming indistinguishable as proposed in [16], our new results further suggest that the whole curve of concentration distribution will even turn fully homogeneous when the cell size is too small. In all, *PolarSim* provides a user-friendly tool for more applications on the studies in cell polarization.

**5. Contact**

All the scripts of the *PolarSim* GUI have been uploaded onto GitHub https://github.com/YixuanChen0726/Cell-Polarization/tree/main/PolarSim. If there is any question, please contact Yixuan Chen (yixuanchen@stu.pku.edu.cn) or Guoye Guan ([guanguoye@gmail.com](mailto:guanguoye@gmail.com)) anytime.