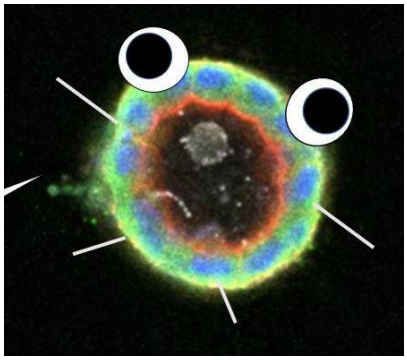

3D MULTICELLULAR EMT CODE MANUAL

An agent-based model of TGF- β1 signaling that drives EMT in epithelial cells.

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1. Introduction

This is the manual for the 3D multicellular EMT code developed by the Cell Matrix Mechanobiology Lab at VCU. A detailed description of the installation, toolboxes, and parameters is provided.

If you use this code, please cite reference [will be updated soon].

This paper provides a more thorough description of the code such as the system of ODEs used to model the TGF-β1 signaling pathway and EMT characterization, as well as parameters used to define TGF-β1 diffusion.

If you have any questions, please feel free to contact:

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- Chris Lemmon: clemmon@vcu.edu

2. Computational Requirements/ Dependencies

This code is available at our GitHub repository: [insert link here]

Using this code requires the installation of MATLAB version 2021a or above. This is a licensed software.

In addition, it will require the installation of the following MATLAB Toolboxes/ Applications:

- Signal Processing Toolbox
- PDE Modeler Toolbox
- Optimization Toolbox
- MATLAB App Designer

2.2. Downloading the Code:

We recommend downloading the entire folder off of our GitHub repository, since the application relies on a number of functions to run properly. Ensure that all files are located in the same folder or add them to your folder path before running.

3. Running the Code

This code was developed using the MATLAB app designer to make an interactive gui that is user-friendly and low processing requirements. The code has a main .mlapp file that can be run directly and calls all other required functions during the run without need for user input other than the initial parameters used.

3.1. Overall App Design

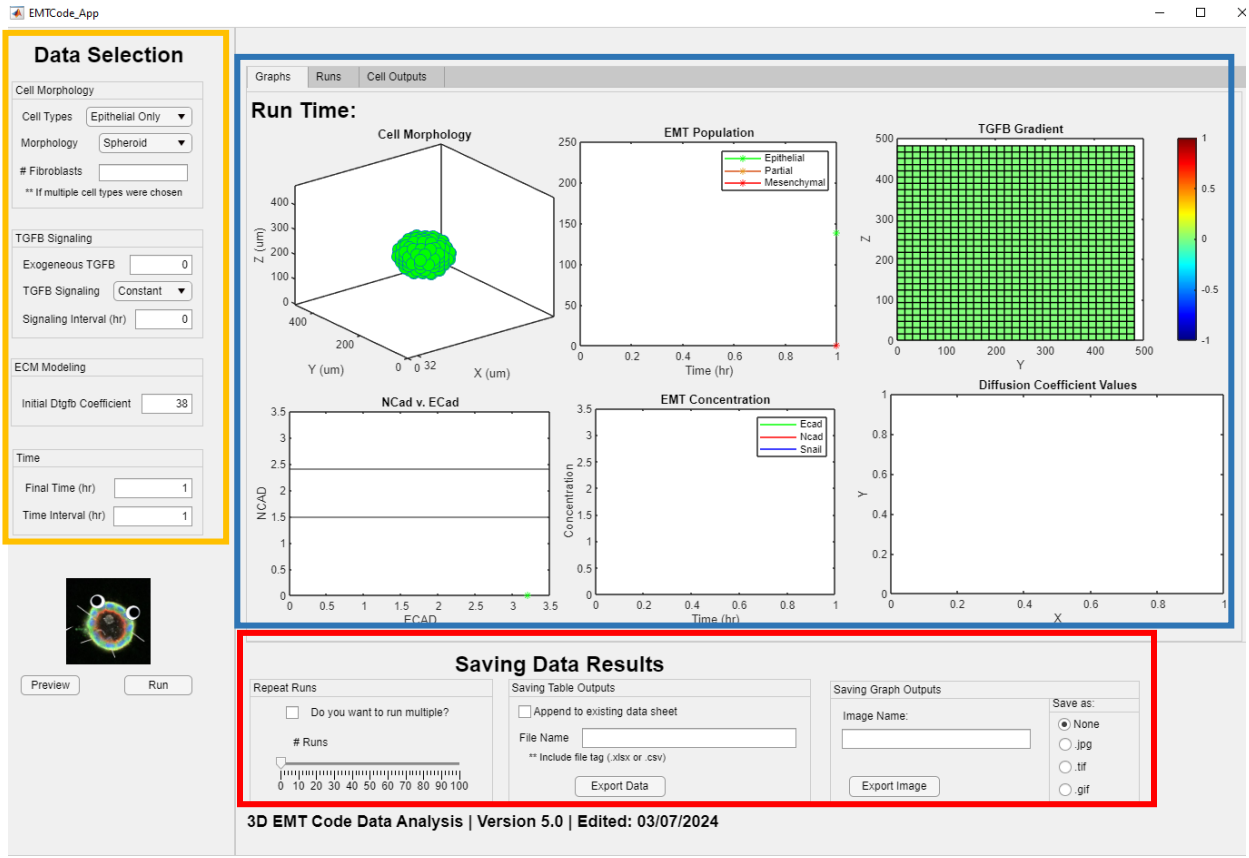


Figure 1. *The App Layout on MATLAB App Designer.*

The overall format of the code can be broken down by its different sections:

1. Data Selection Parameters is where the user can modify different parameters of the run.
    a. See Section 3.2.1 for more information on the different parameters.

2. Visualizing the Run shows the outputs of the run. Several graphs are present and show changes in cell organization, EMT state transitions, TGF-β1 concentrations, and changes in localized TGF-β1 diffusion coefficients at each time step. In addition to the graphs, users can check the tabs to show quantified outputs.
    a. See Section 3.2.2 for more information.

3. Saving Results is the section where users can specify if and how they want to save the runs. There are options to save the graphs as image files or videos (gifs). Quantified data shown in the Tables can be saved in excel sheets.
    a. See Section 3.2.3 for more information.

3.2.1. Data Selection Parameters

There are several parameters that can be modified in the code. These are separated into 4 different sections that are shown in Figure 2.

1. **Cell Morphology**: Users can choose to run the code using just epithelial cells, or run a co-culture model with mesenchymal cells. There are 4 options that change the organization of the epithelial cells:
    a. Spheroid
    b. vertical tube
    c. horizontal tube
    d. curved tube

    All these create a hollow structure with the same inner and outer diameters. If users choose to include mesenchymal cells, they can choose how many to include.

2. **TGF-β1 Signaling**: Users can modify the initial exogenous TGF-β11 concentration introduced to the cells, as well as change the signaling dynamics to model acute and chronic signaling.

3. **ECM Modeling**: Changes in extracellular matrix organization is indirectly correlated to changes in the TGF-β1 diffusion coefficient. Users can modify the initial ECM conditions the cells are simulated in, where high values (max value of 38) indicate a healthy ECM environment, and low values indicate fibrotic environments that introduce more obstacles for TGF-β1 diffusion.

4. **Time**: Users can choose the total run time as well as the interval that the graphs are updated during the run.

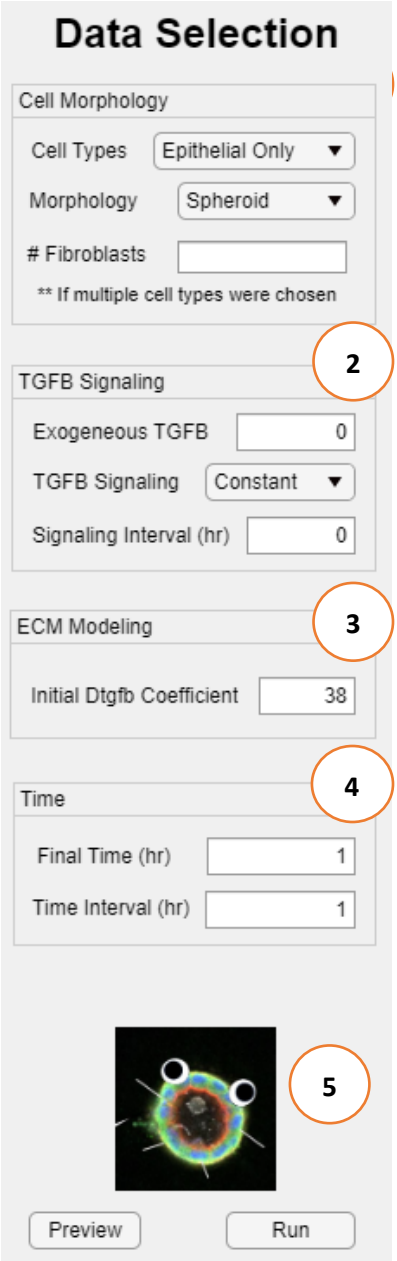


Figure 2. *Parameters that can be directly modified by users.*

5. **Starting the Run**: After users finalize their choices in parameters, they have the option to view a preview of the graphs where it shows all graph outputs at t = 0 using the updated parameters. Press the run button to start the simulation, where real-time visualization of the system can be monitored on the graphs discussed in the next section.

3.2.2. Visualizing the Run

During the run, there are 6 different graphs that are updated based on the chosen Time Interval (hr) and Final Time (hr) values (**Fig. 3**).

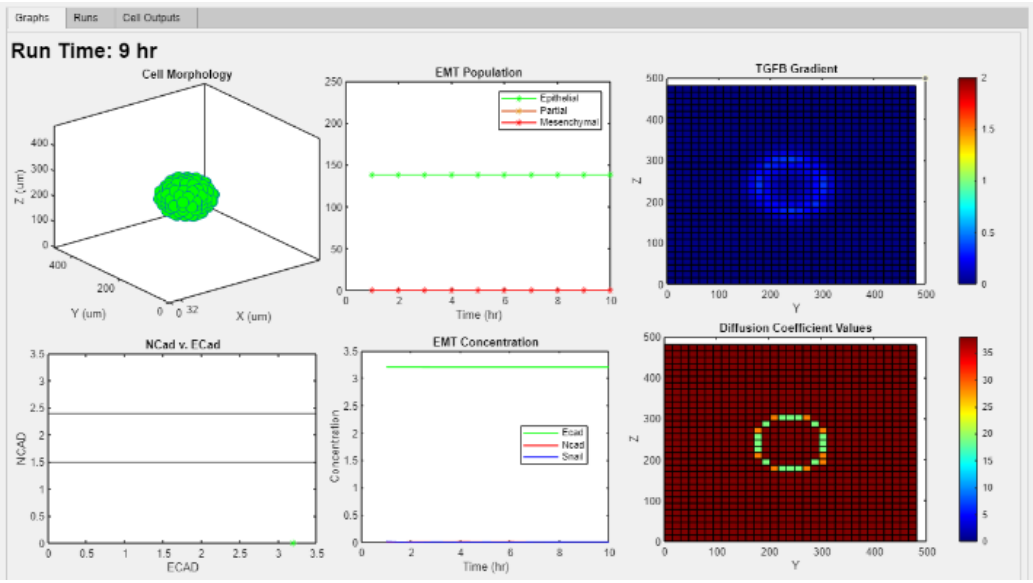


Figure 3. *Graphical Representation of the runs, observing changes in cell classification and organization, TGFB diffusion, and local changes in the TGFB diffusion coefficient.*

1. **Cell Morphology:** 3D representation of the cells, updating overall organization and EMT classification of cells as epithelial (green), partial EMT state (yellow), or mesenchymal (red).

2. **EMT Population**: Quantifies the population ratios of the different EMT classifications over time.

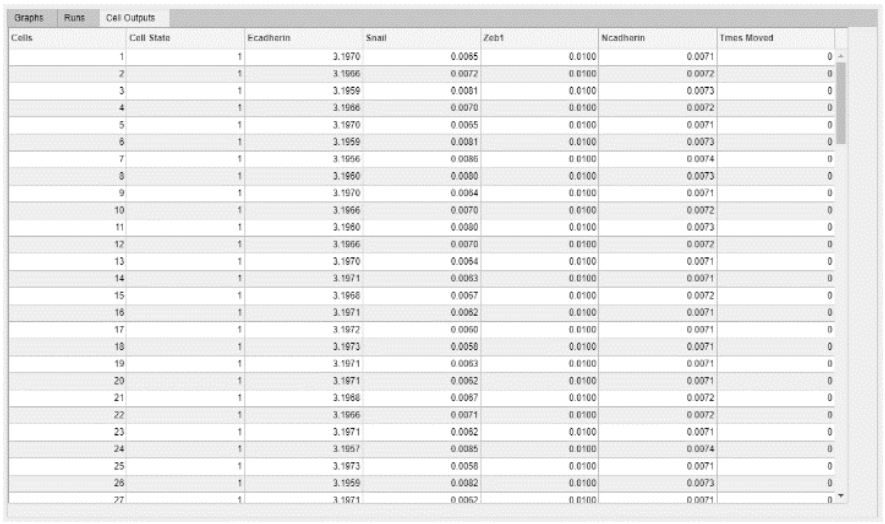
3. **TGF-β1 Gradient**: A surface mesh representation of the TGF-β1 concentration gradient at each time step. Concentrations are defined on the color bar to the right.
4. **NCad v. ECad:** Scatterplot that plots the N-Cadherin and E-Cadherin concentration ratio of each cell over time.

5. **EMT Concentration**: Plots the population average concentration of 3 different EMT markers: E-Cadherin, N-Cadherin, and Snail.

6. **Diffusion Coefficient Values**: A surface mesh representation of the local TGF-β1 diffusion coefficient values at each time step. Values are defined on the color bar to the right.

Along with graphs to visualize changes in the multicellular population over time, users can check quantitative characterization of each cell in the table under the 'Cell Output' tab (**Fig. 4**).

This provides individual values for each cell, such as the EMT state, cell movement, and the different concentrations defined in the TGF-β1 signaling pathway.



Cells	Cell State	Ecadherin	Snail	Zeb1	Ncadherin	Times Moved
1	1	3.1970	0.0065	0.0100	0.0071	0
2	1	3.1966	0.0072	0.0100	0.0072	0
3	1	3.1959	0.0081	0.0100	0.0073	0
4	1	3.1966	0.0070	0.0100	0.0072	0
5	1	3.1970	0.0065	0.0100	0.0071	0
6	1	3.1959	0.0081	0.0100	0.0073	0
7	1	3.1956	0.0086	0.0100	0.0074	0
8	1	3.1960	0.0080	0.0100	0.0073	0
9	1	3.1970	0.0064	0.0100	0.0071	0
10	1	3.1966	0.0070	0.0100	0.0072	0
11	1	3.1960	0.0080	0.0100	0.0073	0
12	1	3.1966	0.0070	0.0100	0.0072	0
13	1	3.1970	0.0064	0.0100	0.0071	0
14	1	3.1971	0.0063	0.0100	0.0071	0
15	1	3.1968	0.0067	0.0100	0.0072	0
16	1	3.1971	0.0062	0.0100	0.0071	0
17	1	3.1972	0.0060	0.0100	0.0071	0
18	1	3.1973	0.0058	0.0100	0.0071	0
19	1	3.1971	0.0063	0.0100	0.0071	0
20	1	3.1971	0.0062	0.0100	0.0071	0
21	1	3.1968	0.0067	0.0100	0.0072	0
22	1	3.1966	0.0071	0.0100	0.0072	0
23	1	3.1971	0.0062	0.0100	0.0071	0
24	1	3.1957	0.0085	0.0100	0.0074	0
25	1	3.1973	0.0058	0.0100	0.0071	0
26	1	3.1959	0.0082	0.0100	0.0073	0
27	1	3.1971	0.0062	0.0100	0.0071	0

Figure 4. *Table that shows the characterization of each cell defined in the system.*

If users want to perform multiple simulations using the same parameters, outputs of each run are shown in the 'Runs' tab which show the average population outputs.

3.2.3. Saving Results

There are several ways that users can save run results (**Fig. 5**).

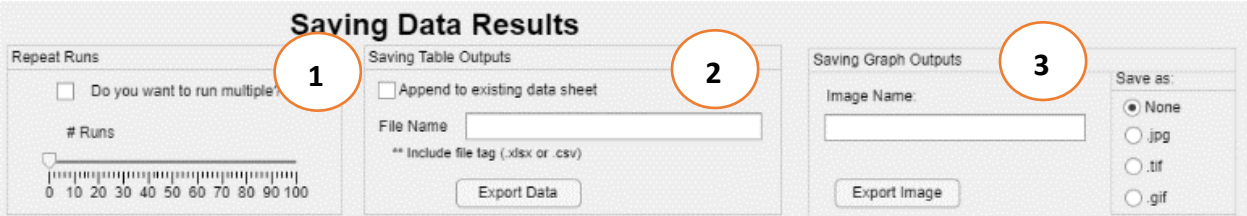


Figure 5. *Methods to save run results as image files or excel sheets.*

1. **Repeat Runs:** Users can choose to run multiple simulations using the same parameters.

2. **Saving Table Outputs:** Users can save quantitative results shown in the 'Runs' table as a new excel file or append results to an existing file.

3. **Saving Graph Outputs:** Graphs can be saved as an image file (.jpg or .tif). If users want to capture a video output of the total run, they can save it as a .gif file.

4. Other Information and Referenced Code

- Curved Tube: Adapted from TubePlot Code (Wesenberg 2024)
- Defining the moore neighborhood to find local maximum TGF-β1 concentrations and locations of neighboring cells: (Storey & Jackson 2021).
- Probability Thresholds for cell migration and proliferation were adapted from Hirway et al (2021).
- N-Cadherin thresholding for EMT state classifications were adapted from Hirway et al (2021).
- The bistable switch model of TGF-β1 signaling: Adapted from Tian et al (2013).
- Cell size was defined as the average width of a mammalian epithelial cell.
- Estimated values for the spheroid diameters used was defined based on biopsy measurements of distal tubules as well as in vitro experiments done by the authors.

5. References

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