

# EmbryoMaker User's Manual v 1.0

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## 1. Download Software:

The EmbryoMaker software can be downloaded from

<http://www.biocenter.helsinki.fi/salazar/software.html>

## 2. Installation:

### 2.1 Linux:

#### 2.1.1 Software requirements:

-Freeglut libraries:

-<http://freeglut.sourceforge.net/>

-In Ubuntu this can be installed by running “apt-get install freeglut3-dev”

-Mesa Opengl libraries:

-<http://www.mesa3d.org/>

-They are normally already installed in most linux distributions

-gfortran compiler:

-<https://gcc.gnu.org/wiki/GFortran>

-In Ubuntu this can be installed by running “apt-get install gfortran”

-Gnuplot (Optional):

-<http://www.gnuplot.info/>

-In Ubuntu this can be installed by running “apt-get install gnuplot”

### 2.1.2 Building:

Download the .tar file from the website and save it in the place you want to install the software. Uncompress the .tar file. You may do that by opening a terminal, going to the directory where the .tar file is stored and typing the following command:

```
tar -xzf <EmbryoMaker_directory>.tar.gz
```

where <EmbryoMaker\_directory> stands for the name the downloaded .tar file has at that moment. While in the terminal, enter the <EmbryoMaker\_directory> and simply run the *compile\_EmbryoMaker.sh* script. You may need to grant execution permissions to the script first, in that case type:

```
chmod +x compile_EmbryoMaker.sh
```

then, to run the script type,

```
./compile_EmbryoMaker.sh
```

the following output should be printed in the terminal:

```
descending
/<path_to_EmbryoMaker_dir>/src/core
making object files
linking
cleaning
executables installed in bin/
```

The executable is named EMaker and is placed in the bin directory.

## 2.2 Mac:

### 2.2.1 Software requirements:

- Xcode
- MacPorts
- gcc46
  - the exact command is: “sudo port install gcc46”

### 2.2.2 Building:

Download the EmbryoMaker .tar file from the website and save it in the place you want to install the software.

Uncompress the EmbryoMaker .tar file.

While in the terminal, enter the EmbryoMaker directory and simply run the

*compile\_EmbryoMaker\_mac.sh* script. Note that if your OS X version is 10.6 you should run the *compile\_EmbryoMaker\_mac.10.6.sh* script instead. The following output should be printed in the terminal

```
descending
/<path_to_EmbryoMaker_dir>/src/core
making object files
linking
cleaning
executables installed in bin/
```

The executable is named EMaker and is placed on the bin directory.

This has been tested on OS X 10.6 Snow Leopard (*compile\_EmbryoMaker\_mac.10.6.sh* should be used) and 10.9 Mavericks (*compile\_EmbryoMaker\_mac.sh* should be used), it might not work in other versions.

### **3. Command-line execution options:**

This section provides a brief description of how to run the program executable and about its several command-line options:

-Simplest option. Open a terminal and go to the directory where the simulator has been installed. Then type:

```
./Emaker
```

This simply runs the model with its full graphical interface and the default initial conditions and parameter values (see section 4). Before starting the graphical interface the program will let you choose between several default initial conditions.

-Running from the initial conditions and parameters in a file:

```
./EMaker <name of the file>
```

-Visualizing an output file:

```
./EMaker <name of the file>
```

-Running the model automatically without graphical interface with the default initial conditions and parameters.

```
./EMaker 0 1
```

Each time interval is a different file and all the files from the same simulation are saved in a directory within the output directory (this directory is different for every run). The output will be saved in the output directory at regular time intervals and for a fixed number of iterations. To change that you can do:

```
./EMaker 0 1 X Y
```

Where X is the duration of each time interval (and integer number) and Y is how many of those

intervals are run (and integer number). Thus the simulation is run by  $X*Y$  iterations.

-Running the model automatically without graphical interface from the initial conditions and parameters in a file.

```
./EMaker <name of the file> 1
```

In this case we can also modify  $X$  and  $Y$ .

```
./EMaker <name of the file> 1 X Y
```

### 3.1 Example files (Basic developmental mechanisms)

EmbryoMaker comes with a set of example files that specify the initial conditions for the basic developmental mechanisms plus a complex developmental mechanism. The initial condition files are in the examples directory within the EmbryoMaker directory. In order to run them follow the instructions in section 3.

## 4. Running EmbryoMaker with its graphic interface: (Linux)

After the program is executed a window will arise displaying an initial condition in 3D. This is the embryo, organ or embryo part that is being simulated. The user can run a number of iterations (by choosing specific options in the menu) and see how that embryo or organ changes over developmental time. The user can also modify how the embryo is viewed and how it is drawn.

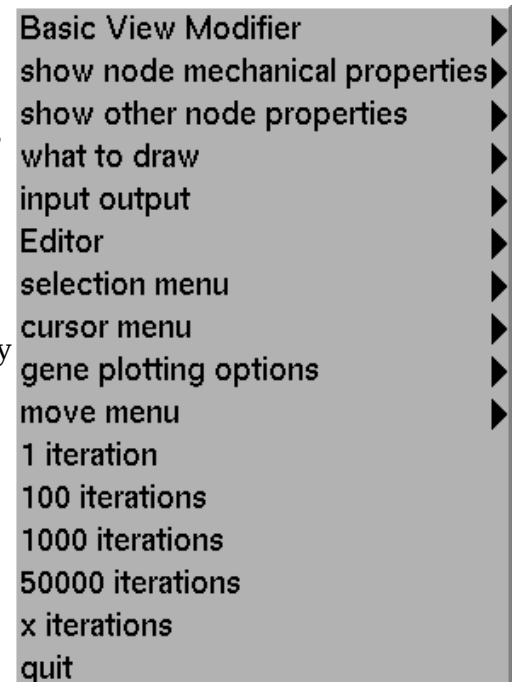
In the default view cylinders are shown as pairs of nodes with a radius equal to the node property ADD (that is the radius on which adhesion can occur).

When pressing the right button a menu will appear. The following pages detail these options in detail:

### 4.1. Principal Menu options:

4.1.1. *Basic View Modifier*: This option shows a submenu with options to modify the view of the model results. By default pressing the left mouse button and moving the mouse would rotate the embryo but other options in this menu allow the mouse to do different things on the view. These options are described in section 4.2.1.

4.1.2 *Show node mechanical properties*. This menu allows to display mechanical node properties in three ways, through the color of nodes, through drawing a series of arrows whose length is proportional to the value of the property (only applicable to epithelia) and through drawing semitransparent spheres over nodes whose size reflect the value of the property. There are also several options to tune the display of each of the three methods. Node coloring, arrows and transparent spheres and independent, thus one can display a different node property with each method, but not more than one with the same method at once. See section 4.2.2.



4.1.3 *Show other node properties*. This menu allows to display non-mechanical node properties

such as node type, gene expression, etc. This can be displayed in three ways, through the color of nodes, through drawing a series of arrows whose length is proportional to the value of the property (only applicable to epithelia) and through drawing semitransparent spheres over nodes whose size reflect the value of the property. There are also several options to tune the display of each of the three methods. Node coloring, arrows and transparent spheres are independent, thus one can display a different node property with each method, but not more than one with the same method at once. 4.2.3

4.1.4 *What to draw*: This option shows a submenu with a list of options to choose which parts of the embryo are shown in the display and how are those shown. These options are described in detail in section 5.2.4.

4.1.5 *Input Output*: This option shows a submenu with a list of options to save the current state of the embryo into a file or to read previous states from a file. See section 4.2.5.

4.1.6 *Editor*: This menu includes all the options to edit the developmental system, such as adding or deleting nodes or cells, and changing their properties or gene expression. See section 4.2.6.

4.1.7 *Selection menu*: This menu shows different options to select nodes or cells. This is useful to simply track nodes or cells over time, but also allows to use the editor options (section 4.2.6) on them, and to plot their gene expression over time using the *gene plotting options* menu (section 4.1.9).

4.1.8: *Cursor menu*: This menu includes all options regarding the control of the cursor, a tool mainly used to select nodes (see Section 4.1.7) or to mark a reference point in space. See section 4.2.8.

4.1.9 *Gene plotting options*: This menu allows to plot the gene expression of one or several nodes over time using the GNUplot software (make sure it's installed in your computer before using this option). See section 4.2.9.

4.1.10 *Move menu*: This menu allow to move nodes and cells in space in different ways (see section 4.2.10).

4.1.11 *1 iteration*. Runs the model for 1 iteration. After this is chosen The window will lock while this number of iterations are running in the simulator. When these are run the current state of the embryo will be shown in the window and the user will win control on the display again. Notice that depending on the number of nodes and the running options this iteration may take more or less time.

4.1.12. *100 iterations*. As above but the model is run for 100 iterations.

4.1.13. *1000 iterations*. As above but the model is run for 1000 iterations.

4.1.14. *50000 iterations*. As above but the model is run for 50000 iterations. A prompt indicating the number of nodes, the number of iterations and other basic information will appear in the terminal every 1000 iterations.

4.1.15 *x iterations*. When this option is chosen a prompt will arise in the terminal. The user should enter the number of iterations he/she wants to run.

4.1.16. *Quit*. Quits the simulator.

left mouse button  
reset to initial view  
view from above  
view from front  
Undo sections  
Toggle Fixed sections when running iterations

rotate  
zoom  
pan  
Section from minimal x plane  
Section from maximal x plane  
Section from minimal y plane  
Section from maximal y plane  
Section from minimal z plane  
Section from maximal z plane

## 4.2. Submenu options:

### 4.2.1 Basic View Modifier Menu

4.2.1.1 *Left mouse button* Submenu. By choosing one of the options the user decides what happens when dragging with the left mouse button.

4.2.1.1.1 *Rotate*. The camera rotates. This is the default.

4.2.1.1.2 *Zoom*. Zoom the camera in and out.

4.2.1.1.3 *Pan*. Moving the camera point of rotation.

4.2.1.1.4 *Section from the Minimal x plane*. The mouse will section the embryo as it is moved, starting from the node with the minimal x position. This is very useful to see the interior of the embryo. By default all sections will be undone when the simulation resumes (i. e. running some iterations). Sections can be kept over time with the (see section 4.2.1.6 to set it otherwise)

4.2.1.1.5 *Section from the Maximal x plane*. As above but starting from the node with the maximal x position.

4.2.1.1.6 *Section from the Minimal y plane*. As above but starting from the node with the minimal y position.

4.2.1.1.7 *Section from the Maximal y plane*. As above but starting from the node with the maximal y position.

4.2.1.1.8 *Section from the Minimal z plane*. As above but starting from the node with the minimal z position.

4.2.1.1.9 *Section from the Maximal z plane*. As above but starting from the node with the maximal z position.

These different directions of sectioning can be combined to section the embryo from different sides.

4.2.1.2 *Reset to initial view*. This shows the embryo from the same default view than when the program was started.

4.2.1.3 *View from above*. Adjusts the view to be from top (from large z values)

4.2.1.4 *View from front*. As above but to see from the front.

4.2.1.5 *Undo sections*. All sections will be undone and the whole system will be visible

4.2.1.6 *Toggle fixed sections when running iterations*. This allows to switch between keeping the sections during simulations time, or being undone when some iterations are run. By default this option is set to the second case.

with colors	▶	x
with arrows (only epithelia)	▶	y
		z
with transparent spheres	▶	p^EQD
color options	▶	p^ADD
arrow options	▶	p^YOU
sphere options	▶	p^ADH
		p^REP
		p^REC
		p^EST
		p^ERP
		p^EQS
		p^HOO
		p^MOV
		p^DMO
		z(0)
		p^ECM
		p^COD
		p^GRD
		p^PLD
		p^VOD
		p^DIF
		p^KFI
		p^PLA

### 4.2.2 Show node mechanical properties Menu.

4.2.2.1 *with colors* Submenu. This submenu allows you to select a mechanical node property to be displayed as node color.

4.2.2.2 *with arrows (only epithelia)* Submenu. This submenu allows you to select a mechanical node property to be displayed as arrows of different length over each epithelial node. Note that this option will only be visible for epithelial nodes.

4.2.2.3 *with semitransparent spheres* Submenu. This submenu allows you to select a mechanical node property to be displayed as semitransparent spheres of different radius over each node.

4.2.2.4 *Color options* Submenu. This submenu allows to set different options regarding the coloring of nodes.

4.2.2.4.1 *Change color palette* Submenu. Allows you to choose between 4 different color palettes.

4.2.2.4.2 *Change color max. and min. from terminal*. This option will change the minimum and maximum values of the node property selected taken into account for coloring each node. All values below the minimum value will have the same minimum color (depending on the palette) and all values above the maximal value will have the same maximum color. The minimum and maximum values will be entered via terminal in the same line separated with a space, in the respective order.

4.2.2.4.3 *Change color min. with left mouse button*. This button allows to change the minimum value described above simply dragging the mouse with the left button.

change color palette  
select color max. and min. from terminal  
select color min. with left mouse button  
select color max. with left mouse button  
select color min. with middle mouse button  
select color max. with middle mouse button

4.2.2.4.4 *Change color max. with left mouse button*. This button allows to change the maximum value described above simply dragging the mouse with the left button.

4.2.2.4.5 *Change color min. with middle mouse button*. This button allows to change the minimum value described above simply dragging the mouse with the middle mouse button (this means pressing the scrolling wheel, not actually scrolling).

4.2.2.4.6 *Change color max. with middle mouse button*. This button allows to change the maximum value described above simply dragging the mouse with the middle mouse button (this means pressing the scrolling wheel, not actually scrolling).

4.2.2.5 *Arrow options* Submenu. This submenu allows to set different options regarding the arrow display of nodes properties.

change arrow scale with left mouse button  
select arrow max. and min. from terminal  
select arrow min. with left mouse button  
select arrow max. with left mouse button  
select arrow min. with middle mouse button  
select arrow max. with middle mouse button  
disable arrows

4.2.2.5.1 *Change arrow scale with left mouse button*. Allows to change the maximum length of arrows by dragging the mouse with the left button.

4.2.2.5.2 *Change arrow max. and min. from terminal*. This option will change the minimum and maximum values of the node property selected taken into account for setting arrow length for each node. All values below the minimum value will have an arrow length of 0 and all values above the maximal value will have an arrow length equal to the arrow scale (1 by default). The minimum and maximum values will be entered via terminal in the same line separated with a space, in the respective order.

4.2.2.5.3 *Change arrow min. with left mouse button*. This button allows to change the minimum value described above simply dragging the mouse with the left button.

4.2.2.5.4 *Change arrow max. with left mouse button.* This button allows to change the maximum value described above simply dragging the mouse with the left button.

4.2.2.5.5 *Change arrow min. with middle mouse button.* This button allows to change the minimum value described above simply dragging the mouse with the middle mouse button (this means pressing the scrolling wheel, not actually scrolling).

4.2.2.5.6 *Change arrow max. with middle mouse button.* This button allows to change the maximum value described above simply dragging the mouse with the middle mouse button (this means pressing the scrolling wheel, not actually scrolling).

4.2.2.5.7 *Disable arrows.* This option disables the display of arrows.

4.2.2.6 *Sphere options Submenu.* This submenu allows to set different options regarding the semitransparent sphere display of nodes properties.

4.2.2.6.1 *Change sphere scale with left mouse button.* Allows to change the maximum radius of spheres by dragging the mouse with the left button.

change sphere scale with left mouse button  
select sphere max. and min. from terminal  
select sphere min. with left mouse button  
select sphere max. with left mouse button  
select sphere min. with middle mouse button  
select sphere max. with middle mouse button  
disable spheres

4.2.2.6.2 *Change sphere max. and min. from terminal.* This option will change the minimum and maximum values of the node property selected taken into account for setting semitransparent sphere radius for each node. All values below the minimum value will have a sphere radius of 0 and all values above the maximal value will have a sphere radius equal to the sphere scale (1 by default). The minimum and maximum values will be entered via terminal in the same line separated with a space, in the respective order.

4.2.2.6.3 *Change sphere min. with left mouse button.* This button allows to change the minimum value described above simply dragging the mouse with the left button.

4.2.2.6.4 *Change sphere max. with left mouse button.* This button allows to change the maximum value described above simply dragging the mouse with the left button.

4.2.2.6.5 *Change sphere min. with middle mouse button.* This button allows to change the minimum value described above simply dragging the mouse with the middle mouse button (this means pressing the scrolling wheel, not actually scrolling).

4.2.2.6.6 *Change sphere max. with middle mouse button.* This button allows to change the maximum value described above simply dragging the mouse with the middle mouse button (this means pressing the scrolling wheel, not actually scrolling).

4.2.2.6.7 *Disable sphere.* This option disables the display of semitransparent spheres.

node type  
cells  
cell nucleus as blue  
nodes fixed as yellow  
as cell cycle  
as dx  
as dy  
as dz  
as dtotal  
as boxes  
as node index  
Amount of regulatory molecule

4.2.3 *Show other node properties Menu.* It is exactly the same as the previous menu (4.2.2), except for the node properties you can set in each of the three submenus (“with colors”, “with arrows” and “with semitransparent spheres”). The following descriptions



doesn't refer to all the submenus and options, since they are the same as in the previous menu. Instead the node properties that available for display in this menu are described.

4.2.3.1 *Node type*. The type of node is displayed. This is 1 for apical epithelial nodes, 2 for basal epithelial nodes, 3 for mesenchymal nodes and 4 for ECM nodes.

4.2.2.2 *Cell*. The cell index of the cell the node belongs to is displayed.

4.2.2.3 *Cell nucleus as blue*. Nodes specified as nuclei will have a value of 0, the rest will have a value of 1.

4.2.2.4 *Nodes fixed as yellow*. Nodes fixed in space ( $p^{\text{FIX}}$  different from 0, see online methods) will have a value of 1, the rest a value of 0.

4.2.2.5 *As cell cycle*. The value of the cell property  $P^{\text{PHA}}$  of the cell the node belongs to will be displayed.

4.2.2.6 *As dx*. The distance a node has moved in the x axis will be displayed.

4.2.2.7 *As dy*. The distance a node has moved in the y axis will be displayed.

4.2.2.8 *As dz*. The distance a node has moved in the z axis will be displayed.

4.2.2.9 *As dtotal*. The total distance a node has moved will be displayed.

4.2.2.10 *As node index*. The index within the node matrix will be displayed.

4.2.2.11 *Amount of regulatory molecule X*. The variable displayed will be the amount of a certain molecule expressed in each node. There will be one of those options for each regulatory molecule described in the system to be simulated (it may change in different developmental simulations).

#### 4.2.3 What to draw Menu

Note that some drawing options will not have a visible effect if other things are drawn in front (most notably one should chose to draw small balls only if one wishes to see the other options).

Note also that these options activate a specific drawing when chosen and deactivate it when chosen again.

4.2.3.1 *Epithelial springs*. Draws the springs between the pair of nodes of the epithelium with green lines.

4.2.3.2 *Box grid*. Draws a 3D grid around the embryo. The distance between grid points is proportional to the maximal ADD of the nodes in the embryo.

4.2.3.7 *Connexions between cells*. Draws a lines between those nodes from different cells that are in

epithelial springs  
box grid  
connexions between cells  
connexions between nodes  
draw cell contour  
draw intercellular contour  
balls as radius=EQD  
balls as radius=ADD  
small balls  
no balls  
cylinders  
only upper balls  
only lower balls  
display box boundaries  
polarization vectors  
centroids  
fixed nodes  
show displacement of nodes from origin  
movement unitary vectors  
force component: repulsion-adhesion  
force component: epi. surface tension lateral  
force component: epi. surface tension apical/basal  
movement module vectors  
do not show/show epithelium  
do not show/show mesenchyme  
do not show/show extracellular matrix

physical contact. These lines are red between nodes of different epithelial cells and green between apical nodes of the epithelium and mesenchymal nodes.

4.2.3.8 *Connexion between nodes*. Draws red lines between the nodes of the same cell.

4.2.3.9 *Draw cell contour*. Draws cellular contours by rendering surfaces between neighboring nodes of the same cell using a 3D Delaunay triangulation.

4.2.3.10 *Draw intercellular contour*. Complements the previous option by also rendering surfaces between nodes belonging to different cells, displaying thus the space occupied by a compact tissue.

4.2.3.11 *Balls as radius=EQD*. Nodes are draw as balls of radius equal to the EQD node property of each node.

4.2.3.12 *Balls as radius=ADD*. Nodes are draw as balls of radius equal to the ADD node property of each node.

4.2.3.13 *Small balls*. Draws the nodes with very small balls. Very useful for the visualization of forces or connections between nodes.

4.2.3.14 *No balls*. Doesn't draw the balls. Very useful for the visualization of forces or connections between nodes.

4.2.3.15 *Cylinders*. Draws the cylinders of epithelial cells. When this option is activated, the display of balls in epithelial nodes is disabled and viceversa.

4.2.3.16 *Only upper balls*. Draws only the apical (purple) side of the epithelium.

4.2.3.17 *Only lower balls*. Draws only the basal (blue) side of the epithelium.

4.2.3.18 *Display box boundaries*. Shows the boundaries of the display box. Only elements inside this box will be displayed. The display box is useful to make sections of the system.

4.2.3.19 *Polarization vectors*. Draws a line for the polariztion vector of each cell.

4.2.3.20 *Centroids*. Shows the centroids (this is average of the positions of the nodes in a cell) of each cells by a white ball.

4.2.3.21 *Fixed nodes*. Show nodes fixed in space (that is with node property  $p^{\text{FIX}}$  different from 0, see online methods).

4.2.3.22 *Show displacement of nodes from origin*. Shows a line from the initial position of the node to its actual position. For nodes that arise by growth this initial position is the position at which that node was created

4.2.3.23 *Movement unitary vectors*. Shows a line for each node in the direction in which it moved in the last iteration.

4.2.3.24 *Force components: repulsion and adhesion*. Shows a line for each node in the direction in of the repulsion and adhesion forces affecting the nodes in the last interation.

4.2.3.25 *Force components: epi. surface tension: lateral.* As above but for one of the torsion forces acting between epithelial cylinders.

4.2.3.26 *Force components: epithelial surface tension: apical-basal.* As above but for the apical-basal epithelial surface tension.

4.2.3.27 *Movement module vectors.* As 4.2.3.19 but the line has a longitude proportional to the amount of movement in the last iteration.

4.2.3.28 *Do not show/ show epithelium.*

4.2.3.29 *Do not show/ show mesenchyme.*

4.2.3.30 *Do not show/ show extracellular matrix.*

#### 4.2.4 Input Output Menu.

##### 4.2.4.1 *Save present time.*

Save a present state of the embryo. This includes all model parameters (including the genetic ones) and also all node and cell properties.

save present time  
save snaps periodically  
change frequency of snapshots  
read from file  
add label to the name of the output file  
save images automatically (the window must be open and uncovered)  
save movie, check terminal (the window must be open and uncovered)

When this file is read, either from the graphical interface or by calling the simulator with that file in the command line, everything is recovered (including the iteration number). In that sense this file can be used as the initial conditions for other simulator runs. The file is saved in a directory within the “output” directory in the directory where the simulator has been executed. The directory and the file have a random name that includes the date when it was run. This name is the same than the name of the window. The output file is a text file and it can be open by any plain text editor. The meaning of the values saved in it are explained in the same file and can be directly used by the user (as an alternative way to change the embryo's state).

4.2.4.2 *Save snaps periodically.* By choosing this option the state of the embryo is automatically saved into a different file every 1000 iterations.

4.2.4.2 *Change frequency of snapshots.* This options prompts a message in the terminal where the user should introduce the iterations intervals in which snaps are taken.

4.2.4.5 *Read from file.* A prompt would arise in the terminal asking the user to introduce the name and address of the file the user wants to read.

4.2.4.6 *Add label to the name of the output file.* Adds a label, given trough the terminal, at the end of the output files saved.

4.2.4.5 *Save images automatically.* Snapshots of the system will be taken during simulation time. The amount and frequency of snapshots is set automatically.

4.2.4.6 *Save movie, check terminal.* Same as previous option, but you need to specify the amount and frequency of snapshots via terminal.

#### 4.2.5 Editor Menu.

Embryo editor: The options in this submenu allow to edit the embryo. Through it the user moves cells and nodes, modify their properties and create new nodes and cells or delete existing ones. This editor can be used at any moment and simulations can be resumed after the embryo has been modified by the user and the editor is put to sleep. If elli.e is run with an input file, the embryo encoded in this file can be edited and then saved in a different file. This latter file can then be used as the initial conditions for other runs of elli.e.

- Add basic epithelial cell
- Add basic mesenchymal cell
- Add node to a selected cell
- Add extracellular node
- Paste a node from selection
- Paste a cell from selection
- Delete the selected node
- Delete the selected cell
- Change properties of the selected node
- Change properties of the selected cell
- Change gene expression in the selected node
- Choose a node in which to Paste properties of the selected node
- Paste a property into it
- STOP the editor and go back to running mode

In most simulations in development, as in most studies in developmental biology in general, one studies a system (e.g.: organ or embryo part) from some chosen early developmental stage to some later developmental stage to try to understand how one transforms into the other. In practice, this often involves trying to understand how a specific spatial distribution of cell types (what we call in here an initial *developmental pattern*) transforms into another one (what we call in here a final *developmental pattern*). The embryo editor allows the user to precisely describe in the model this initial pattern (this is the initial spatial distribution of cells, nodes, their properties and gene expression) from which the development he/she wants to study starts. Then by using the gene network editor the user can study which gene networks (and which regulation of which cell behaviors by those) are able to lead development from this initial condition to the final pattern (e.g.: later stage) of interest (FIG). The embryo editor can be used to produce any arbitrary distribution of cells and nodes that involves mesenchyma, epithelia and/or extracellular matrix with any arbitrary distribution of gene expression in it.

The embryo editor can also be used to explore the effects on development of a number of experiments occurring at any arbitrary time and place during development. To do that the user should simply run the model until the time in development where the manipulation occurs, then activate the embryo editor, make the corresponding manipulations, stop the embryo editor and resume the running of the model from that time point. The experimental manipulations that can be used are not restricted to moving cells or changing the expression of specific genes in specific cells or cell parts. More sophisticated and realistic experiments can also be implemented. Bead experiments can be implemented by adding one or several extracellular matrix node in a desired location and setting its properties to mimic those of a bead (its radius and mechanical consistency). Then in this node the user can, by using the embryo editor, chose to put specific concentrations of diffusible molecules. Physical barrier experiments can similarly be implemented by adding sets of bound extracellular matrix elements. Transgenes and other kinds of genetic manipulations can be simulated by choosing and modifying gene networks with the gene network editor.

The embryo editor works in similar way than vectorial graphics editors (such as illustrator, inkscape, etc...) but in 3D and with a simpler and less sophisticated used interface. By moving a cursor in the 3D space the user can chose to move cells or nodes from one position to another, chose where to put a new cell or node or copy a cell or node and paste it in a new position. So the first thing the user needs to do in the embryo editor is to activate the cursor by choosing the menu option:

ATTENTION. All menu options perform its action on the positions where the cursor is (see *Cursor menu X* and *Selection menu X*).

- Add basic epithelial cell
- Add basic mesenchymal cell
- Add node to a selected cell
- Add extracellular node
- Paste a node from selection
- Paste a cell from selection
- Delete the selected node
- Delete the selected cell
- Change properties of the selected node
- Change properties of the selected cell
- Change gene expression in the selected node
- Choose a node in which to Paste properties of the selected node
- Paste a property into it
- STOP the editor and go back to running mode

4.2.5.1 *Add basic epithelial cell*: An epithelial cell, with 14 nodes and an hexagonal shape, is added in the position where the cursor is. The number of nodes and their position can later be modified by the other options in the menu.

4.2.5.2 *Add basic mesenchymal cell*: As above but for an epithelial cell.

4.2.5.3 *Add node to selected cell*: It adds a node to the selected cell in the current position of the cursor. If no cell has been selected before no node will be added and a complaint text will arise in the terminal. If the selected cell is epithelial to nodes, a cylinder, will be added.

4.2.5.4 *Add extracellular node*: An extracellular node is added in the current position of the cursor.

4.2.5.5 *Paste a node from selection*: A node identical to the selected node is added in the current position of the cursor. As before the user needs to select a node before.

4.2.5.6 *Paste a cell from selection*: As above but for a cell.

4.2.5.7 *Delete the selected node*: The selected node is deleted.

4.2.5.8 *Delete the selected cell*: The selected cell is deleted.

4.2.5.9 *Change properties of the selected node*: This option will show an subsubmenu listing all node properties. After choosing one a message will appear in the terminal giving the present value of this property in the selected node and asking for the new value. Press return after introducing this value.

4.2.5.10 *Change properties of the selected cell*: As above but for cell properties.

4.2.5.11 *Change gene expression in the selected node*: As above but the node properties changed are the gene expression.

4.2.5.12 *Choose a node in which to Paste properties of the selected node*: This choses the node that is closest to the current cursor position as a target node.

4.2.5.13 *Paste a property into it*: This option will show an subsubmenu listing all node properties. The chosen property will be copied from the selected node to the target node.

4.2.5.14 *STOP the editor and go back to running mode*: This option deactivates the embryo editor and goes back to running mode.

#### 4.2.7. Selection menu.

4.2.7.1. *Select node by index*: When selected, the user should enter in the command line the index(es) of the node(s) to be selected. When the last selected node has been introduced, the user has to type '-1' to end selection. When a node is selected, it will be coloured in white or yellow, and some of its basic properties (such as position or energy) will be shown in the terminal. The last node selected will always be colored in yellow, and will be the one set for using the editor options (see section 4.2.5) and the moving options (see section 4.2.10). The rest of the selected nodes, if any, will be colored in white

select node by index  
select node with cursor  
select cell by index  
select cell with cursor  
undo selections

4.2.7.2. *Select node with cursor*: This option allows the user to select nodes using the cursor. By default the cursor is moved in the x-y plane by dragging the mouse with the left button. Pressing the DOWN key will toggle between x-y movement and z movement by dragging the mouse with the left button. In order to select node, approach the cursor to the desired node and press the mouse middle button (press the scroll wheel, but don't scroll the wheel).

4.2.7.3. *Select cell by index*: As in 4.2.7.1, but in here what the user should enter in the command line are the index(es) of the cell(s) to be selected.

4.2.7.4. *Select cell with cursor*: As in 4.2.7.2, but in here what is selected is the cell that is closest to the cursor.

4.2.7.5. *Undo selections*: All selected elements (not only the latter one) are unselected.

#### 4.2.8. Cursor menu.

4.2.8.1. *Cursor ON/OFF*: Toggle cursor function. It is set OFF by default. When activated, the cursor appears in the display window as a red-solid ball, which can be moved by the user across the system to select nodes and cells (see section 4.2.7.2). Notice that, since the cursor appears in a corner of the window, it can be necessary to move it to make it visible. By default, the cursor is moved in the x-y plane by dragging the mouse with the left button, and is moved in the z axis by dragging with the middle mouse button (pressing the scrolling wheel).

cursor ON/OFF  
set left mouse-button for x-y move  
set left mouse-button for z move  
set middle mouse-button for z move

4.2.8.2. *Set left mouse-button for x-y move*: When selected, the cursor can be displaced in the x-y plane with the left mouse-button. This is the cursor displacement mode by default.

4.2.8.3. *Set left mouse-button for z move*: When selected, the cursor can be displaced in the z plane with the left mouse-button. The user can also alternatively switch between cursor displacements in the x-y or z plane (4.2.8.2 or 4.2.8.3 options) by pressing the "DOWN key" in the keyboard.

4.2.8.4. *Set middle mouse-button for z move*: As in 4.2.8.3, but using the middle mouse-button instead of the left mouse-button.

#### 4.2.9. Gene plotting options.

4.2.9.1. *Temporal plots: ON/OFF*: It is set ON by default, and each time the button is pressed, it switches between the ON and OFF options. When ON option is set, the node properties concerning concentration of regulatory molecules are stored, so as they can be tracked along time (and plotted if 4.2.9.2 is chosen). These data are not stored each iteration, but each time the user runs a number of iterations. If OFF option is set, the concentrations are not stored (however, data previously stored are not deleted), and the program runs (slightly) faster. When the program is run in the automatic mode (see section 3), the OFF option is automatically chosen.

Temporal plots: ON/OFF  
plot [gen] vs time in a node

4.2.9.2. *Plot [gen] vs time in a node*: It plots (in a new window) the concentration of different regulatory molecules in different nodes along time using Gnuplot. When selected, the user should enter in the command line the nodes and genes to be plotted. The resulting plot will display the concentrations of all chosen genes in all chosen nodes. In order to avoid

overloaded plots, the number of nodes and genes that can be selected are limited to 10. When this option is set and there exist one or more previously selected node(s), the user will only have to choose the regulatory molecule(s).

#### 4.2.10. Move menu.

move node from terminal  
move node with left mouse button  
move cell from terminal  
move cell with left mouse button  
Stop movement (for cursor movement)

4.2.10.1. *Move node from terminal.* This option allows to move a certain node to a set of coordinates that have to be entered by the terminal. If you had previously selected a node, this will be the one moved (and if you had several nodes selected, it will be the last one selected). If there is none selected, the terminal will ask for a node index. Next, it will ask for the new desired coordinates for the node, that will be entered through the terminal sequentially. If -1 is typed in any of the coordinates, that one will be kept as the original value.

4.2.10.2. *Move node with left mouse button.* This option allows to move a certain node by dragging the mouse with the left button. If you had previously selected a node, this will be the one moved (and if you had several nodes selected, it will be the last one selected). If there is none selected, the terminal will ask for a node index. To stop the mouse movement mode, select the *Stop movement* option in the same menu.

4.2.10.3. *Move cell from terminal.* This option allows to move a certain cell to a set of coordinates that have to be entered by the terminal. If you had previously selected a cell, this will be the one moved (and if you had several cell selected, it will be the last one selected). If there is none selected, the terminal will ask for a cell index. Next, it will ask for the new desired coordinates for the cell centroid, that will be entered through the terminal sequentially. If -1 is typed in any of the coordinates, that one will be kept as the original value.

4.2.10.4. *Move cell with left mouse button.* This option allows to move a certain cell by dragging the mouse with the left button. If you had previously selected a cell, this will be the one moved (and if you had several cell selected, it will be the last one selected). If there is none selected, the terminal will ask for a cell index. To stop the mouse movement mode, select the *Stop movement* option in the same menu.

Enjoy