Modeling Embryonic Cleavage Patterns

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Abstract	The division patterns of early invertebrate and vertebrate embryos are key to the specification of cell fates and embryo body axes. We here describe a generic computational modeling method to quantitatively test mechanisms which specify successive division position and orientation of eggs and early blastomeres in 3D. This approach should serve to motivate and guide future experimental work on the mechanisms controlling early embryo morphogenesis.
Keywords (separated by ‘-’)	Early embryos - Division positioning - Microtubules - Asters - Cell shape - Maternal domain - Yolk

Modeling Embryonic Cleavage Patterns2

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Abstract4

The division patterns of early invertebrate and vertebrate embryos are key to the specification of cell fates and embryo body axes. We here describe a generic computational modeling method to quantitatively test mechanisms which specify successive division position and orientation of eggs and early blastomeres in 3D. This approach should serve to motivate and guide future experimental work on the mechanisms controlling early embryo morphogenesis.

Key words Early embryos, Division positioning, Microtubules, Asters, Cell shape, Maternal domain, Yolk

1 Introduction12

Early embryo development of both invertebrate and vertebrate species begins with the cleavage period. During cleavage, the egg undergoes a stereotyped choreography of subsequent reductive divisions that transform the one-cell egg into a multicellular embryo [1]. Cleavage patterns are not random, but usually follow a well-ordered set of specific position and orientation of blastomere divisions. Those patterns are most often conserved among large groups of species, such as in amphibians, fishes, and echinoderms, and serve as prime examples of developmental robustness. Mechanisms which control cleavage geometries remain however poorly understood. This is in part because this period is driven by maternal material in the embryo, and thus poorly amenable to genetic screens or manipulations [2]. In addition thorough live imaging of large eggs and blastomeres implicates sophisticated methodologies [3], or may even be impaired given the opacity of many embryos. Factors that have been directly and indirectly implicated in the regulation of division plane positioning during cleavage include cell shapes [4–7], yolk layers [8, 9], and maternal cortical polarity domains [10–13]. Those may affect the organization or dynamic properties of astral microtubules which exert forces and

torques to position and orient asters and mitotic spindles that specify division planes.

Recent experimental work in echinoderms, fish, and frog embryos has suggested that division plane specification in large eggs and blastomeres is instructed from interphase/anaphase microtubule (MT) asters which fill the large cellular volume, and probe cell shape through length-dependent MT forces [6, 7]. This mechanism aligns the division axis with the cell shape long axis, and has been proposed to serve as a default cue guiding division plane orientation and position in early embryos [14, 15]. Given the presence of asymmetric divisions in some blastomeres of many embryos, cell shape effect may be overridden or biased by additional cues such as vegetal polarity domains in echinoderms or by yolk layers in fish and amphibians, which alter the distribution of MT forces around cells.

We have recently implemented 3D computational models to predict division position/orientation as a function of various inputs [16]. Those models compute in a systematic manner the forces and torques exerted by astral MTs on centrosome pairs at the center of asters and output a mechanical equilibrium which corresponds to the preferred division position and orientation. Those models are highly valuable to dissect the basics of cleavage patterns in many embryos. In here, we report on a set of scripts and methodologies to implement this modeling approach to test the contribution of various cues to the division patterns of early embryos.

2 Materials

Computer equipped with Mathworks Matlab (Version 2015a and above) and Surface Evolver [17]: There is no special requirement for computers, but it is recommended for better performance to have a fast CPU. On a good system a simulation of 10,000 iterations with calculation of a torque map (see here after) typically takes 5–10 min.

Programs may be downloaded from:
Division Prediction Program (DPP):
<http://www.minclab.fr/wp-content/uploads/2018/01/DPP-Package.zip>
Surface Evolver
<http://facstaff.susqu.edu/brakke/evolver/evolver.html>

3 Methods

3.1 Generating 3D Cell Shapes

The initial input for the program is the 3D shape of a cell or a group of cells in an embryo, for instance, defined by the 3D coordinates of cell surfaces. This serves as a spatial reference to run a prediction on

Fig. 1 Creating 3D Cell Shape inputs. (a) Raw surfaces for a four-cell embryo in Surface Evolver (b)-equilibrated shape with surfaces of minimal energy obtained in SE. (c) Equilibrated 3D shape imported to DPP with Matlab. (d) Experimental image projected stack of a four-cell sea urchin embryo. (e) Segmented image stack. (f) Interface to import 3D segmented stack in Matlab for DPP, through manual connection of 2D segments in 3D

the position/orientation of the site of cell division. 3D shapes may be artificially generated using the surface minimization software Surface Evolver [17], or from a 3D segmented stack from labeled embryos imaged with a confocal or a two-photon microscope (Fig. 1).

3.1.1 Generating an Input 3D Shape with Surface Evolver

We here provide some initial guidelines to generate representative 3D shapes that closely resemble real physical egg and blastomere shapes with the software Surface Evolver (SE). This software iteratively finds the surface of minimal energy under given constraints (Fig. 1a, b). Constraints include final volume, surface tension values, and confinement. The language of SE is rich and for details on structures, constraints, macros, or function definition we refer the readers to its web page.

SE requires input files that contain information on the topology and constraints of the studied surfaces. The topology is described by a simple mesh (Fig. 1a), elements of which are named and organized according to the rules of SE. The mesh describing topology is defined by vertices and their coordinates, oriented edges linking two vertices, oriented faces consisting of three edges, and bodies consisting of N faces. The constraints describe the behavior of the surface; for example coordinates can

be restricted to an arbitrary space (like a cylinder) or additional energy may be assigned to arbitrary faces, to mimic effects of differential surface tension around adhering blastomeres for instance [16]. We provide several input files in the folder DPP package\DPP files\generating_shapes_from_SE\generic shapes\, which can be used to create one-cell spheres, rods, and typical two-cell, four-cell, or eight-cell stage embryos. In the DPP package, we further provide more detailed guidelines to create other shapes and constraints with SE. For the method description below we will use the example file “4 cells - confined in sphere - raw.fe”.

Creating Shapes with Surface Evolver

1. Open Matlab. Navigate to the DPP Package folder in Matlab, right-click on it, and click “add to path/ Select folders and subfolders”. Navigate to the folder “DPP package\DPP program\” where three scripts named “launcher” are placed; you will work from here.
2. Open the script “launcher_1_generate_shapes.m” with Matlab. Set the path where you will store the shapes, simulation parameters, and results. For instance, if you want to store your cells and their simulations in a folder “C:\Data\my cell” you set the variable `root_path = “C:\Data\my cell”`.
3. Run the function that generates the folders `shape_paths = fl_generate_paths(root_path)` by selecting the two abovementioned lines and pressing F9 to evaluate/run. This will create the folder “C:\Data\my cell” and inside it a folder “C:\Data\my cell\shape_source”. In the latter you will keep all cell shape-related files.
4. Copy the file “4 cells - confined in sphere - raw.fe” into the folder “C:\Data\my cell\shape_source” and double-click the input file to open it in SE.
5. To see the surface, press “s” to enter the graphic mode (Fig. 1a). Then “q” to quit the graphic mode and return to SE command terminal. You may zoom in and out (press Z when graphic window is active) or rotate (press R when graphic window is active) the surface using the mouse.
6. To iteratively find minimal energy configuration (Fig. 1b), the user needs to perform subsequent refinements of the mesh and calculation of novel surface coordinates. Type “r” to refine the initial mesh (subdivide); this will increase the number of vertices. Then “g10” to make ten iterations of surface minimization (g100 will make 100, etc.). Refine the mesh again with “r”. Refine and reiterate calculations several times until you find that the shape is stable and does not change significantly with the following iterations. As a start for division prediction, we recommend to refine the mesh to reach a number of typically 1000–2000 vertices.

7. Save the final file by typing “d” to save the data in one file in SE 141
format. Use a different name than the original file, e.g., 142
“4_cell_final”. 143
8. Type “export_lists()” in the SE terminal to extract the data in 144
separate lists for vertices, edges, faces, and bodies. Note that 145
this function has been added to the example files to facilitate 146
the following steps, but is not a default function of SE. In the 147
package we provide this function which can be copied into any 148
SE function. At the end of this process, you should end up with 149
two SE files (.fe), one for the initial shape, and one for the final, 150
and several .txt files for edges, faces, vertices, and bodies. 151

Translating the SE Surface
into a Matlab Matrix with
Labeled Bodies

1. In Matlab open the script “launcher_1_generate_shapes.m”. 153
Find the function “f2_extract_bodies_from_SE(shape_paths, 154
[X, Y])” and run it by selecting the line and pressing F9. The 155
numbers in the brackets are the size in pixels of a Matlab matrix 156
that will comprise the SE surface; for instance: [X, Y] = [200, 157
240] pixels. Any size in any proportion can be used, but large 158
matrices may take much longer to simulate. Typically, we use 159
matrices of the sizes ranging from 150×150 to 200×200 160
pixels; X and Y may be set unequal (Z is automatically recalcu- 161
lated from shape’s dimensions and given X and Y to ensure the 162
best fit of the shape within the matrix). 163
2. Matlab will ask you to provide the name of the SE file from 164
which you exported the lists (its name was used as a part of 165
those lists and will be used to find them); in our case it is “C: 166
\\Data\\my cell\\shape_source\\4 cells - confined in sphere - raw. 167
fe”. The program will read the raw coordinates in “.txt” files 168
and save them as “C:\\Data\\my cell\\shape_source\\shape_data. 169
mat”, after which it will translate them to matrix coordinates, 170
label them according to the body lists, and save those as “C: 171
\\Data\\my cell\\shape_source\\labeled_bodies.mat” in the same 172
folder. 173
3. In Matlab open the script “launcher_1_generate_shapes.m”. 174
Find the function “f3_show_labeled_bodies(shape_paths)” 175
and run it. It will find the file “labeled_bodies.mat” and show 176
the labeled bodies (Fig. 1c). If everything went fine up to this 177
point, the system is ready to run division predictions (Subhead- 178
ing 3.2). 179

3.1.2 Generating an Input
3D Shape from an Imaging
Stack of a Real Embryo

In many instances, one may need to input the direct 3D shape from 181
a cell or a group of cell in an embryo/tissue, and compare experi- 182
mental division orientation with model predictions. Those shapes 183
may be experimentally obtained from a Z-stack taken on a labeled 184
embryo with confocal, light-sheet, or two-photon microscopy 185
(Fig. 1d). Here we solely provide a method to convert a 3D 186

segmented stack into an input file to run the division prediction program. Segmentation of 3D shapes may be achieved with ImageJ or Imaris, and will largely differ depending on the embryo, labels, and microscopes. For our purpose, input segmented stacks must be black inside and white outside. The voxel should be cubic (it should span equally in all dimensions: $V_x = V_y = V_z$) and the final segmentation is recommended to span not less than $100 \times 100 \times 100$ pixels (Fig. 1e).

In the folder “DPP package\DPP files\generating_shapes_from_experiment\” we provide an example stack of a segmented four-cell sea urchin embryo called “4CellUrchin_final.tif” which we use to present this part of the method.

1. Open Matlab. Navigate to the DPP Package folder in Matlab, right-click on it, and click “add to path/ Select folders and subfolders”. Navigate to the folder “DPP package\DPP program\” where three scripts named “launcher” are placed; you will work from here.
2. Open the script “launcher_1_generate_shapes.m” with Matlab. Set the path where you will store the shapes, generated cells, and their simulations. For instance, if you want to store your cells and their simulations in a folder “C:\Data\my cell” you set the variable: `root_path = “C:\Data\my cell”`. Run the function that generates the folders `shape_paths = fl_generate_paths(root_path)` by selecting the two abovementioned lines and pressing F9 to evaluate/run. This will create the folder “C:\Data\my cell” and inside it a folder “C:\Data\my cell\shape_source”. Copy the file “4CellUrchin_final.tif” into this folder.
3. In Matlab open the script “launcher_1_generate_shapes.m”, find the function “f2_extract_bodies_from_IJ”, and run it by selecting the line and pressing F9. It will offer to choose a segmentation stack; choose “4CellUrchin_final.tif”.
4. You will be taken to an interface allowing you to manually connect 2D regions in the stack to reconstitute the proper 3D shape of your embryo/tissue (Fig. 1f). For this, you will need to manually label 2D regions belonging to one cell from slice to slice (Fig. 1f, left). You can scroll through the whole stack using the mouse wheel. Then set a label number N, by pressing a numeric key on the keyboard, and label a 2D region with this N number by clicking on each z-slice as it appears. The program takes the user to the next slice automatically after the click. After all slices of one cell have been connected, you can visualize the result in 3D by pressing <v> (Fig. 1f, right). After all cells have been reconstructed, it is recommended to clean the stack from lone pixels by pressing <c> and visualize in 3D again; the final result should look similar to Fig. 1f. Export the resulting bodies by pressing <s>. This will create

a Matlab file “C:\Data\my cell\shape_source\labeled_bodies.mat”. If everything went fine up to this point, the system is ready to run division predictions (Subheading 3.2).

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3.2 Predicting Division Position and Orientation in 3D

In this part we describe the methods to use the scripts which allow to make a prediction on the orientation and position of the division axis. This prediction will have as inputs 3D cell shapes defined following the preceding paragraphs, and parameters, which relate to the distribution of astral microtubule (MT) forces within cells and to simulation procedures. As such, it is important to state that those models can solely be useful to study cell division in cell types and systems in which MTs are prime contributor of nuclei/spindle position, as in most vertebrate and invertebrate early embryos [15, 14]. In our model, MT forces may depend on the spatial distribution of MTs around centrosomes, the length of MTs, or the presence of cortical domains. MT lengths are defined by cell shape and/or presence of MT excluding structures in cells, such as neighboring asters or yolk.

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3.2.1 Setting a Choice of Parameters for the Simulations

1. In Matlab open the script “launcher_2_generate_cell.m”, which contains a set of parameters for the cell simulation. If you run this program following immediately Subheadings “Creating Shapes with Surface Evolver” or “Translating the SE Surface into a Matlab Matrix with Labeled Bodies”, the path to your cell is already set. Else, redo Subheading “Creating Shapes with Surface Evolver”, **step 2** or Subheading “Translating the SE Surface into a Matlab Matrix with Labeled Bodies”.
2. Define cell to simulate: “body_ind =”. If you have several cells (like in the four-cell embryo mentioned above), this is the index of the particular cell that will be used as the shape input. In the part described in Subheading 3.1.2, **step 4**, to generate shapes from real 3D images, this number is the one defined by the user during the 3D reconstruction.
3. Define microtubule pulling force parameters. As described in [16], the general formula used to define the force exerted by each MT in the asters is

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$$F = L^{\text{Beta1}} + \text{pullfactor} * L^{\text{Beta2}} + \text{pullfactor_exp} * 2(L/\text{Unit_branch})$$

The first term corresponds to pulling in the cytoplasm in proportion to MT length, with an exponent factor, “Beta1”, reflecting putative nonlinearities, which by default is set to 3 [6]. The second and third terms reflect pulling from a polar cortical domain (whose location and size are defined hereafter). “Beta2” reflects an exponent for cortical pulling, and “pull-factor” the strength of this effect. Beta2 is set by default to 2 to

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reflect a situation in which diluted motors are limiting pulling from the domain [18]. The last term reflects a situation in which MT number at the domain is limiting pulling, and possesses an exponential dependence term, defined through the parameter “Unit_branch”, to mimic the presence of branching in large embryonic asters (a situation likely irrelevant for somatic cells). This parameter has a default value of 6.75, as experimentally measured in sea urchins [6]. The strength of this term is defined by the parameter “pullfactor_exp”. If both parameters “pullfactor” and “pullfactor_exp” are set to 0, then the script will not propose you to position and size a cortical domain. In such case, you may directly proceed to Subheading 3.2.1, step 7.

4. Define exclusion regions for microtubule growth. Microtubule effective length can be corrected for the presence of intracellular structure such as yolk which affects MT growth in a dose-dependent manner. This is done by correcting the angular density of MTs in aster by a factor $1 - [\text{yolk}] / \eta$, with [yolk] the local yolk concentration and η a sensitivity parameter for how MTs are affected by the yolk. This implies defining the location of the yolk gradient, its orientation, and its sharpness, as detailed in [16]. The parameter “gradyolk” defines the sharpness of the yolk gradient (if set to 0, MTs are uniform inside the cell shape). “erffoffset” is the offset of the sharpest gradient point in pixels. “direction_grad” is used for the direction of the yolk gradient, and is defined by polar (θ) and azimuthal (φ) angles, in degrees, counted in a standard convention: θ_y is counted from the z -axis; φ_y is counted counterclockwise from the x -axis (Fig. 2b). “MTstab” defines the sensitivity of MTs to yolk, η , and is set to 1 by default. Importantly, the shape of the yolk gradient will appear in the graphic interface upon running the script, and can thus be adjusted back (Fig. 2d and Subheading 3.2.1, step 8).
5. Aster parameters: Parameters related to MT asters include initial position and orientation of asters from where to start a simulation, extension angle of asters, and distance between centrosomes. “anglelimit_deg” reflects the extension of asters in degrees (β in Fig. 2a). “theta” is the starting polar angle in spherical coordinates, counted from the z -axis (θ in Fig. 2a). “phi” is the starting azimuthal angle in spherical coordinates; counted counterclockwise from the x -axis (φ in Fig. 2a). “startingxyz” is the initial position of nuclei/spindle center in pixels, defined by three Cartesian coordinates [x, y, z]. If empty, the initial position will be at the calculated “centroid” of the cell shape. “Nucsize” is the distance between centrosomes in pixels (spindle or nuclear size). “Nucexcl” is a parameter used to calculate an exclusion volume ensuring that the nucleus/

Fig. 2 Input parameters for predicting division position and orientation. **(a)** Microtubule aster parameters. Polar and azimuthal angles (θ and φ) defining the division axis angle; and position of the center o (x, y, z); angular extent of microtubules (β , counted from the division axis). **(b)** Parameters affecting microtubule pulling forces. MT growth may be tuned by the presence of yolk gradients (Y), with the concentration gradient defined by a direction (θ_y, φ_y), an offset from the shape centroid (along the gradient direction), and sharpness. A polarity domain (PD) with a given size, location, and MT pulling strength may also be added as an input for simulations. **(c)** Matlab interface to create a polarity domain: a cutting sphere of defined center and radius intersects the 3D cell shape to define the domain. **(d)** Visualization of the cell with its major simulation parameters used as initial inputs to predict division position and orientation

spindle stays inside the cell during the simulation. Nucexcl cannot be <1 ; typical values are 1.2–1.4.

6. After all the parameters have been set, run the whole script by pressing the “Run” button in the Matlab Editor panel. This will create the parameter structures and feed them to the class “DPP_cell” needed for the simulation.
7. If you have set polarity parameters different than 0 (*see* Sub-heading 3.2.1, step 3), you will be offered to position and size a polarity domain. For this you will be brought directly to a graphic interface (Fig. 2c). The domain is defined as the intersection of the 3D cell shape with a cutting sphere. Use the interface to define values for the coordinates (x , y , z) of the sphere center and radius (R), and press “Cut” to visualize the domain. Once set, press the button “Save” to set the domain for the simulation. If you press “Quit” this will set the pulling pre-factors to 0 and run a simulation purely based on cytoplasmic MT forces and cell shape.
8. If everything was set correctly, the cell with polar domain, yolk gradient, and initial orientation/position of nuclei/spindle will be shown as in Fig. 2d. Corresponding parameters will be automatically saved in a new folder “cell_n”. Each time you run this script the cell folder index “n” increases automatically, e.g., “C:\Data\my cell\cell_1” and “C:\Data\my cell\cell_2”.

3.2.2 Starting a Simulation

The principle of the simulation is based on a random walk around the 3D position and orientation of the nucleus/spindle by three center coordinates (x , y , z) and two angles (θ and φ). At each iteration one of those variables is changed, the force and torques of all MTs on the nucleus/spindle are calculated and compared to the previous step in order to converge toward an equilibrium of minimal forces and torques [16]. The simulation thus comes with parameters to optimize time, precision, or accuracy, which need to be defined by the user.

1. Following the preceding section, open the script “launcher_3_generate_simulation.m”.
2. Set visualization parameters.
With “show_log = 1” the program outputs to the Matlab command line information on what parameter has been varied and how forces are calculated. If not required, set to 0.
With “show_visually = N” (higher than 0) the program will display the nucleus position and orientation of each N iterations. If set to 0, there will be no visualization, which may improve simulation speed.
3. Autosave flags. Set to 1 if needed and 0 if not required.

- “auto_save_nucleus_run = 1”: the program will save the run of
 aster centers (trajectory during simulation) as a PNG picture.
 “auto_save_torque_map = 1”: the program will save a torque
 map as a PNG (*see* below). “auto_save_state_history = 1”: the
 program will save the history of varying parameters during the
 simulation as a PNG.
 “auto_save_split_image = 1”: the program will save an image
 of a divided cell (with two daughter cells) as a PNG.
4. Random walk parameters.
- “angle_btw_mt_deg” defines the angle (in degrees) between
 microtubules (used for both MT polar and azimuthal angle),
 and somehow reflects the precision of the simulation. If set to
 10, for instance, the forces will be calculated along MTs in the
 aster spanning each 10° in both polar and azimuthal angles.
 The larger the angle between MTs, the faster the calculations
 are, but the less precise also. It is recommended to use large
 steps (10–20) to quickly and roughly find equilibrium position
 and then use lower angles (1–3) to refine this position and find
 proper orientation (*see* below).
 “jump_chance =”: Probability of jumping to a new configura-
 tion, even if it has been estimated to be less favorable (forces
 and torques are higher) than the previous state. If set to
 10, then the chance to make a jump is 10%.
 “Inertia =”: It is the probability to vary the same parameter in
 the same manner if its previous variation led to a more favorable
 state (100: always use previous variation; 0: randomly pick a
 new variation).
 “duration_equilibrium =”: The number of iterations required
 for a new state to be tested for equilibrium. If x, y, and z do not
 change for this number of iterations, a position is considered
 equilibrated.
 “duration_full =”: It sets the maximum number of iterations
 (use typically >5000).
5. The torque map is important as it informs the stability of the
 current division orientation as compared to others. For
 instance in shapes which are roughly isotropic, this map
 would be flat with no sharp torque minima [16]. The torque
 map is a 2D matrix of absolute values of torques at each
 orientation angle (Fig. 3d), calculated as:
- $$T(\varphi_i, \theta_j) = \sqrt{T_{\varphi_i}^2 + T_{\theta_j}^2}$$
- Torque map parameters are as follows:
- “torque_map_angle_step =”: This sets a precision step for
 torque map calculation. If set to 10, torque map calculation
 will sample division orientations each 10° for both θ and φ .
 “auto_calc_torque_map = 1”: The program will calculate a
 torque map automatically after the simulation (it may take a

Fig. 3 Example of DPP simulation results. (a) (Left) Cell shape corresponding to one blastomere of the four-cell embryo from Fig. 1d is used as the sole input to predict division position and orientation. (Middle) After simulation, asters are positioned at the cell center and oriented along the long axis of the 3D shape. (Right) The predicted divided cell is then generated. (b) Same as in (a) but with the presence of a polarity domain pulling on asters. Note the strong asymmetry in cell division. (c) Same as in (a) but with a yolk gradient which creates a shift in the final position of asters away from yolk layers. (d) Simulation as in (a) with corresponding torque map, computing the torque exerted on asters as a function of all possible 3D angles. Torque minima correspond to stable orientations, highlighted by the black lines. (Bottom) Evolution of angular and position coordinates during the random walk, as a function of iterative step number of the walk

- long time depending on the precision of the map). If not required, set to 0. 413
6. Once parameters are set, start the simulation by selecting all the lines from “current_simulation” down to “current_simulation.run” and pressing F9. 414 415 416 417
7. The simulation will end either if it ran out of iterations (from the maximum number allowed) or if it found an equilibrium which did not move during a number of iteration set by the parameter inertia. The final state of the simulation will be saved automatically in the cell folder, i.e., “C:\Data\my cell\cell_n\” with the prefix corresponding to the index of current instance: “run_1”, “run_2”, and so on. Windows showing the results of the simulation will open, depending on the autosave flags (see Fig. 3 for examples of simulation output). 418 419 420 421 422 423 424 425 426
8. If you want to refine the simulation, with more MTs to improve precision, or with a more detailed torque map, use the section “Refine division orientation”, insert new numbers, and run by selecting lines from “current_simulation.reset” down to “current_simulation.show_division_plane”. 427 428 429 430 431

9. Finally, one can use old cell shape and parameters to rerun simulation. To that aim, open the folder with the cell of interest, e.g., “C:\Data\my Cell\cell_1”, and drag and drop the file “cell.mat” into Matlab workspace to load it. In the script “launcher_2_generate_cell.m” run the line “current_cell.show”, and visualize the initial state of the cell visually. You can also inspect all the properties of the cell by typing “current_cell” in the command line and pressing enter. Then restart from Subheading 3.2.2, step 1 to run a new simulation.

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