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The Moduro-Notebook

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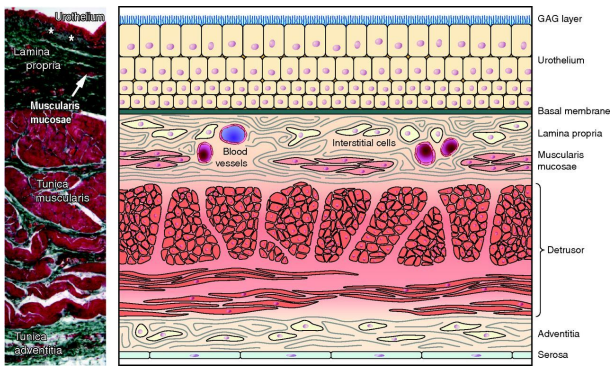

Figure 1: Bladder layers in courtesy of [**?**]

Part I
Moduro

This section gives an introduction into "Modelling of the Urothelium".

1 Biology of the Urothelium

1.1 Anatomy

As shown in figure **??** the stratified epithelium of the bladder is anchored down on the lamina propria through the basal membrane, a somewhat misleading name, since it is not actually a membrane, rather a matrix made mostly of type-IV collagen, microfibril and laminin [**?**]. The basal membrane also acts as a mechanical barrier, preventing malignant cells from invading the deeper tissues [**?**] and is essential for angiogenesis [**?**]. Urothelial cells like every other cell, need a minimum requirement of nutrient to live. In this case the nutrients derive from the vesicles in the lamina propria below the basal membrane. The nutrients needed from the urothelial cells can be thought of as a mix of the essential substrates, glucose, oxygen, vitamins, minerals, hormones and growth factors [**?**]. The diffusion of water, nutrients, urine, carcinogenic molecules and other molecules can be very different. There are three types of transport: passive, active and bulk. The passive transport does not use any cell's energy and can be subdivided into: diffusion, osmosis and facilitated diffusion. Diffusion is the net movement of a substance from a location of higher to one of lower concentration until the substance is fully distributed throughout, reaching its equilibrium and fading the concentration gradient to zero. Only hydrophobic molecules, small uncharged molecules and water can diffuse through a cell membrane. Osmosis is the scientific term for the phenomenon of diffusion of water. When large molecules such as glucose pass through a cell wall using protein channels in the cell membrane, because they are too large to pass through the phospholipid bilayer by diffusion, referred to as facilitated diffusion. Facilitated diffusion as well as the following transport methods are all slower than diffusion or osmosis. Active transport is when the cell pumps substances against the concentration gradient using energy, adenosine triphosphate (ATP). Lastly, the bulk transport use the cell membrane to form vesicles that bring materials in (Endocytosis) and out (Exocytosis).

1.1.1 Basal Cells

There are three distinct layers in the human urothelium [**?**], with each layer having a morphologically distinct cell type. The first layer, the basement membrane, is a single cell layer

made up of basal cells and stem cells, which form an intimate contact with an underlying capillary bed. These cells have a diameter of 5 to 10 μm and have a single defined nucleus. Basal cells have a cylindrical morphology, but can be squamous, meaning flat, when the bladder wall is distended. The basal cells are germinal in nature, meaning they give rise to other cells and cell lines. Some studies suggested that basal epithelial cells replace the stem cells in the urothelium by having stem-cell-like properties with a very slow proliferation rate of three to six months mentioned in a recent paper [**?**]. Other studies indicate that a basal cell originates from urothelial stem cells. Stem cells were estimated to be around 9% of all basal cells [**?**]. The basal cells adhere to the cells of the layer above, the intermediate cells, by desmosomes, which are tight microtubular connections between cells. The basal cells adhere to their basal membrane by even stronger, tighter connections known as hemidesmosomes.

1.1.2 Intermediate Cells

The next layer, intermediate urothelial cells, are superficial to the basal cell layer and are cuboidal to low columnar morphologically. They have well-defined borders and amphophilic cytoplasm rich in glycogen. With a diameter of 10 to 20 μm, the intermediate cells are significantly larger than basal cells. Like basal cells, intermediate cells have only one defined nucleus. The number of strata of the intermediate cells varies depending on the species, but in the human is up to five layers thick. The apparent thickness of the general intermediate cell layer can also vary with the state of bladder filling. The number of strata of the intermediate cells is fewer in the distended than in the voided bladder.

Intermediate cells of the urothelium are believed to move by amoeboid migration. There are two subtypes of amoeboid migration. The first occurs in circular or slightly elongated cells of the urothelium that are deficient in stress fibers and do not adhere to or pull on substrate. The movement of these rounded cells comes from a propulsive, pushing migration mode. The second subtype occurs in elliptical amoeboid cells which generate actin-rich filopodia at the leading edge that engage leading to a forward movement [**?**]. However, it is not known for fact if intermediate cells use this type of movement. Another possibility could result from adjacent layers of intermediate cells sliding past one another during forces upon the urothelium during bladder filling. Whether this process would involve reversible destruction of cell-cell contacts is unclear. The layer of intermediate cells directly beneath the umbrella cells is unique in that this particular type of intermediate cells can rapidly differentiate to an umbrella cell when the overlying umbrella cells are destroyed or lost.

1.1.3 Umbrella Cells

The luminal or outermost surface of the urothelium is formed by a single layer of superficial facet cells commonly known as umbrella cells. These cells are in direct contact with the urinary space. Umbrella cells are unique in several ways. The umbrella cells are the largest epithelial cells in the human body with a diameter up to 200 μm. They can reach this impressive size because of their potential to change their surface area dramatically, primarily at the apical surface and depending on how much urine is contained in the bladder. The morphology of the umbrella cell is polyhedral and generally hexagonal with abundant eosinophilic cytoplasm. Another unusual property is that the umbrella cell often has prominent nucleoli and bi- or polynucleation. One hypothesis explaining the multi-nucleation of some umbrella cells is that they are syncytium cells, meaning they are multi-nucleated cells resulting from the fusion of multiple uninuclear cells. This hypothesis is based on the critical need for the protection of the umbrella cell and, when an umbrella cell is lost, fast differentiating intermediate cells underneath them may rapidly fuse to cover a greater surface area in a shorter time, thereby rapidly replacing umbrella cell loss. The name umbrella cell is derived from

its shape, in that they look like umbrellas over several other cells underneath and with each cell having a stem reaching down to the basal membrane. The function of this stem is not understood. Various explanations include the following:

- to increase contact to facilitate the communication with the underlying cells
- to reach down to the source of nutrients for the purpose of repair, as the umbrella cell is exposed to toxins, as well as a variety of physical insults
- to reach down to the lamina propria which contain the nerves and muscles of the bladder. This would likely be for cell-cell communication and signaling purposes, coordinating the urothelium with the bladder musculature and with the central nervous system
- to reach down to the stem cells of the basement membrane to signal a need for new cell generation , as for instance when cellular damage occurs.

Umbrella cells are inconspicuous in distended bladder in that they become remarkably thin. The umbrella cells adhere to one another through tight junctions and to intermediate cells by desmosomes. Because the morphology and the number of layers of umbrella cells is changing constantly depending on the fullness of the bladder. The urothelium used to be called transitional (changing) epithelium.

The permeability of the umbrella cells is a key property for maintaining a separation between the urine and the plasma. In membrane physiology, the primary determinant of the permeability of a lipid bilayer, also known as the phospholipid bilayer, is the permeability of a single lipid, termed leaflets. These two lipids are not held together by sharing electrons (covalent interactions), but rather because of the structure of these lipids. One end is hydrophilic and the other hydrophobic, creating an energetically unfavorable interaction between the water and the hydrophobic end. The leaflet that is least permeable will determine the overall permeability of the membrane. In the apical membrane of the umbrella cells, there is a unique structure covering 70% to 90% of the apical surface with scalloped-shaped protein plaques and with the remaining area consisting of hingelike regions between umbrella cells. The large plaque units are 0.5 μm in diameter and 12 nm thick and are composed of a glycoprotein class called uroplakins. The primary function of uroplakin is hypothesized to be part of a primary plasma-urine barrier.

A very sensitive measure of the ion permeability is the measure of transepithelial electrical resistance, which demonstrates the passive permeability to most electrolytes and nonelectrolytes. In the case of urothelium, the transepithelial resistence is a measure of permeability to the ions of both urine and blood. An epithelium is considered leaky when the resistance is lower than 500 Ωcm^2 and tight when the resistance is higher than 500 Ωcm^2. In a state of health, both tight junctions and cell membranes are high in resistance to prevent the migration of molecules from their respective sites, thus preventing migration of ions and other charged molecules into the urothelial complex. For the urothelium, it was found that the resistance at tight junctions was higher than 100000 Ωcm^2 and that the cell membrane resistance varied from 10000 to over 100000 Ωcm^2, a uniquely high resistance to be found in the human body. It has been hypothesized that the uroplakins participate in the raising cell resistance, although this has not been proven.

There is a hormonally regulated active sodium transport system in the bladder which works counter to the passive movement of ions across membrane barriers. Calcium, on the other hand, is not regulated actively through active reabsorbtion and is therefore lost partially by diffusion. Some of the active ion transport properties of the urothelium are altered by urinary proteases. Normal urine contains a variety of proteases, most notably three

trypsin-like serine proteases. These three proteases are urokinase, plasmin, and kallikrein. Urokinase, a component of the fibrinolytic system, is released from the distal nephron segment and hydrolyzes plasminogen into plasmin. Plasmin is then able to break down blood clots. The function of kallikrein is not presently known, but it has been postulated to play an important role in hypertension.

A critical requirement for healthy bladder function is to maintain a barrier against usual urinary constituents in such a way that it is not compromised. It is indeed the case that substances normally found in the urine do not alter the permeability properties of the urothelium. Thus, changes within the physiological range for urine pH, calcium, or urea concentrations have minimal effects on the barrier function. Nonphysiological concentrations of these substances, such as excessively acidic pH, low calcium, or extremely high urea, cause an increase in the ion permeability at the apical membrane and tight junction, but permeability returns to normal when the values return to a normal concentration. A number of nonphysiological factors, including bacteria and bacterial products, can also alter the barrier function of the urothelium, increasing the ion permeability of the urothelium by interacting with the apical membrane. If the increase in membrane permeability persists, cell swelling, lysis and cell death may result.

The complete apical surface on top of the uroplakins layer of the umbrella is covered by a glycosaminoglycan (GAG) layer. The function of the GAG layer has been controversially stated to be the primarily epithelial barrier between the urine and the plasma, since small molecules such as amiloride were not prevented from reaching the cationic channels on the surface of the umbrella cells. More likely, the role of the GAG layer may be an antibacterial adherence function, as well as an important barrier for the formation and attachment of particulates to the urtohelium and stone formation.

There are four general processes leading to cell death in the urothelim, three of which are general and occur in every cell in the body. These are apoptosis, autophagy and necrosis. Apoptosis is a naturally occurring suicide regulation mechanism characterized by membrane zeiosis, meaning the formation of blebs, cell shrinkage, nuclear condensation, DNA fragmentation and the formation of several apoptotic bodies with functioning organelles. Autophagy is a process usually activated under conditions of nutrient deprivation. This catabolic process leads to the degradation of abnormal protein aggregates, bulk cytoplasmic contents and damaged or excess organelles. Necrosis, on the other hand, is caused by external factors, such as toxins, infections and the like, resulting in premature cell death. While apoptosis and autophagy are for the most part controlled and beneficial cell replacement processes, necrosis is almost always disadvantageous and may lead to fatal outcomes. The fourth and final process of cell death in the urothelium is through external mechanical forces with voiding, leading to the excoriation of cells from the inner surface of the urothelium into the urine. In the urothelium, the cell type most affected by these mechanical processes is the umbrella cells.

While the epidermal epithelium or the lining of the gut have quick renewal rates of one to thirty days, the urothelium has a relatively slow renewal rate, with the turnover rate estimated to be between three to six months during normal homeostasis [**?**]. However, its regeneration time, upon pathological damage or chemically induced injury, can be as fast as three days [**?**]. Signaling pathways that regulate cellular renewal and differentiation are not restricted to the urothelial cell compartment. An interplay between the epithelial and stromal compartments is essential in the regeneration of injured urothelium from basal stem cells [**?**]. Sonic hedgehog protein (SSH) is expressed in urothelial basal cells during steady-state conditions and is secreted during regeneration period to activate zinc finger protein glioblastoma 1 (GLI1). GLI1 activation in stromal cells triggers a Wnt paracrine signal that induces the expansion of urothelial basal cells and replenishes the injured urothelium. Some

of the other known signals and contacts that are activated while communicating between cells in the urothelium and with cells, such as nerve and muscle cells in the interstitial fluids are amongst others ATP, nerve growth factor (NGF), nitric oxide, prostaglandin E2 (PGE2) and matrix metalloprotaenase (MMP). Several of the signalling pathways that are involved in the self-renewal of adult and embryonic stem cells are also often activated during tumorigenesis and can used as markers since they can be used for possible bladder cancer testing as it will be explained in the following section.

1.2 Cell kinetics parameters

1.2.1 Cell cycle times

1.2.2 Turnover time and rate

A possible definition by GUM.

Definition 1. The turnover time is the time it takes to completely label all cells (in the urothelium). At an initial time point t_0 the tissue is exposed to a marker such that all proliferating cells are labelled. Typically, a marker incorpareted during S-Phase is used. Let t_L be the time when all cells in the tissue are labelled. $T_o = t_L - t_0$ is the turnover time. The turnover rate is $1/T_o$.

A suitable marker is radioactive Thymidin [^{3}H]TdR. Clearly, the experiment must ensure that enough substance is built into the DNA such that all descendants cells are detected as labelled.

A possible definition by TOR, based on [**?**].

Definition 2. The turnover time is the time it takes to completely replace all the differentiated cells from the tissue in question. Differentiated cells are in this case cells with the most specialized cells in it's cell lineage. The replacement of these cells typically involves adult stem cells and their descendants. Experimentally speaking, markers for superficial urothelial cells, such as cytokeratin 1, 20 and uroplakins can be applied inside the bladder. In this case the initial time point t_0 would represent either the injection or after the absorption "labeling". Let t_L be the time after none of the labeled superficial urothelial cells are present. $T_o = t_L - t_0$ is the turnover time.

1.2.3 Colony

Colony

A colony of cells is a group where each cell has derived from the same progenitor cell (usually a stem cell).

Assume that it is possible to mark a single cell and to propagate the marker (label) to the daughter cells. Then all cells of a colony will be labelled by the same marker.

1.3 Lineages

This section summarizes statements in [**?**].

- Note: similar to our approach
- The authors note that there at least two hypotheses how umbrella cells do differentiate.

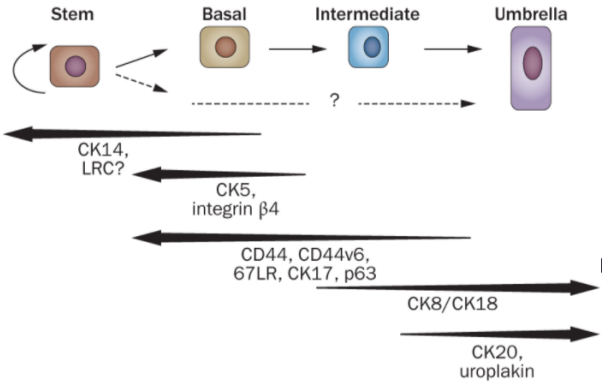

Figure 2: Proposal for a urothelium lineage according to [?].

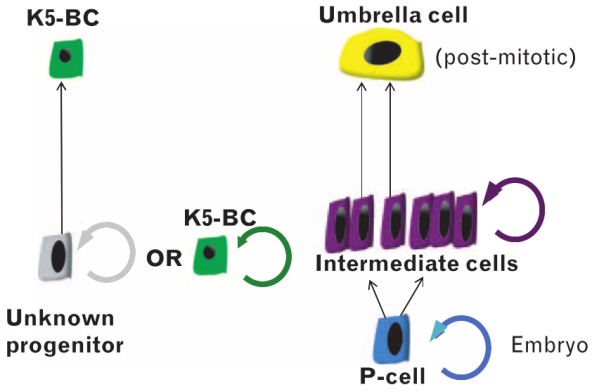

Figure 3: Proposal for a urothelium lineage according to [?].

This section summarizes statements in [?].
They propose a (incomplete) cell lineage for urothelial cells (cf. ??). It says that

- Keratine-5 cells are **not** related or linked to intermediate cells.
- Intermediate cells have a predecessor P-cell during embryogenesis.

Keratine-5 cells are supposed to be basal and stem cells in epidermis.

1.4 Fact tables

This section summarizes important facts and parameter for this chapter. Table ?? lists the known cell kinetics paramters. Table ?? lists the known markers for the different cell types.
Note the "contradiction" in R and R_U.

Parameter	Symbol	Value	Source
turn over time	T_O	3 - 6 months	? referenced in [?]
regeneration after complete injury	R	3 d	? referenced in [?]
regeneration after removal of umbrella cells	R_U	10 d	? referenced in [?]

Table 1: Cell kinectics parameters. R_U in rat, R in mice

Property \ Cell type	S	B	I	U	P
Keratine-5	x	x			
Keratine- 18 and 20				x	
p63	x	x	x		x
uroplakin			x	x	x
CD90	x				
CD44	x	x			
CD49	x	x	x		
Foxa2					x

Table 2: Marker for cell types. From [**?**] and [**?**]

Cell type	diameter	shape
Stem	10-20*, 10^{+}	polygonal^{+}
Basal	10-20*	
Intermediate	10-15*, 10-40^{+}	pyriform^{+}
Umbrella	25-250*, 70-100^{+}	cuboidal^{+}

Table 3: Morphological properties. Diameter in μm. From *[**?**] and $^{+}$[**?**]. Range of umbrella cells is large because of the distension of the bladder.

2 Known models in other epitheliums

2.1 Epidermis

This section summarizes statements in [**?**]. They refer to three hypotheses for cell lineages in the epidermis (cf. **??**).

Asymmetric division The lineage is created as a hierachy of cell types.

Populational asymmetry with stem cells (PAS) The rules are like in the asymmetric division hypothesis but a cell's fate is dependent on a random decision.

Population asymmetry (PA) Like PAS but without stem cells.

The other celltypes Stratum Granulosum and Stratum Corneum are derived directly from Stratum Spinosum.

The cell cycle time is influenced by

1. contact inhibition
2. transepidermal water loss (TEWL)

The differentation of cells is influenced by many factors. According to [**?**] these are:

"The differentiation process may be interrupted by many changes, such as the adhesion mechanism (b1 integrin), ion gradients (calcium) or intercellular signalling (Notch-Delta). The onset of differentiation was determined by mechanical interactions in a way that cells that experienced a net upward force (from neighbours and basement membrane) would be pushed into the second layer [...], which initiated the differentiation process."

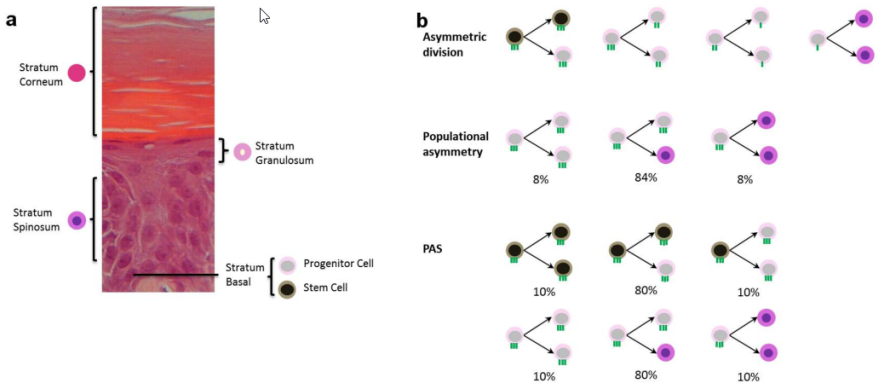

Figure 4: Three hypotheses on epidermis cell lineages (taken from [**?**]). The other celltypes Stratum Granulosum and Stratum Corneum are derived directly from Stratum Spinosum.

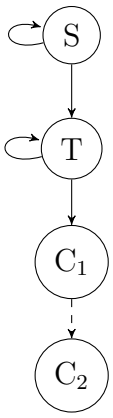

Figure 5: Epidermis lineage according to [**?**]. S: stem cell, T: transition amplifying cell, C_1: stratum spinosum, C_2: Late stratum spinosum (needs to be verified)

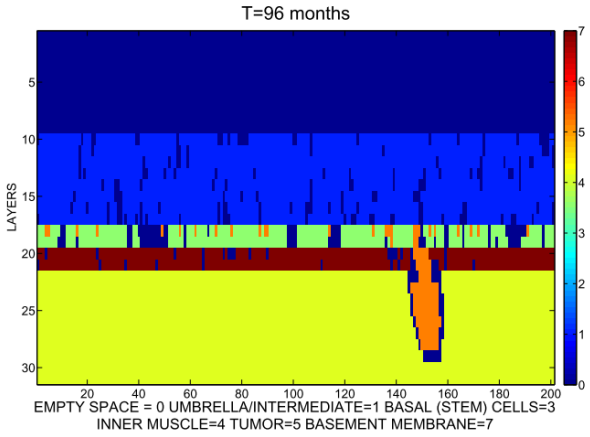

Figure 6: Invasive bladder cancer simulation [?].

2.2 Intestinal crypt

3 State of the art

This section elaborates existing computer models of the urothelium.

3.1 (Computer) Models of the urothelium

[?] simulates invasive bladder cancer.
Umbrella and intermediate cells are represented as one cell.
ToDo: [?]
[?] analyze the differentation of epidermal cells with CompuCell3D. More to come.

3.2 Comparison with other simulations

4 Simulation techniques

4.1 Structure

Definition 3. Let $x \in I$ be a value that can can be observed or measured directly in the real world and I is its domain, i. e. a set of all observable values. I is called a model domain.

Examples are

- a pixel (voxel) with i, j (i, j, k) coordinates (a position) that is part of a cell
- cell-ID of a pixel
- cell type of pixel
- nutrient value of a pixel etc.

Note: The size $(x \times y)$ of a simulation is implicitely defined via the pixel set.

Definition 4. Let $\varphi \in P$ be a value that *cannot* be observed or measured directly in the real world and P is its domain, i. e. a set of all values. P is called a paramter domain.

Examples are properties of a cell like

Tumor / Angiogenesis	3D	Framework / Simulation type	cell shape	diffusion	inhibition	adhesion
Tumor [?]		Hybrid discrete-continuum	1 pixel / space-less	yes	no	yes
Avascular Tumor [?]		Cellular Automata (CA)	1 pixel	yes	no	yes
Tumor Angiogenesis [?]	✓	CA with MatLab (Code)	1 pixel	yes	no	no
Brain Tumor [?]		Voronoi Tesselation	voronoi	yes	no	no
Blood Vessel [?]		CompuCell3D / AngioTool	arbitrary	yes	chemical	yes
Epidermis	3D	Framework / Simulation type	cell shape	diffusion	inhibition	adhesion
Epidermis [?]	✓	FLAME	sphere	no	yes	no
Epidermis [?]		MASON	exagonal	yes	no	yes
Psoriasis [?]		MASON	exagonal	yes	no	yes
Epidermis [?]		MASON	exagonal	yes	no	yes
Epidermis [?]		CompuCell3D	arbitrary	yes	no	yes
Urothelium	3D	Framework / Simulation type	cell shape	diffusion	inhibition	adhesion
Urothelium [?]		Cellular Automata (CA)	1 pixel	yes	no	no
Urothelium [?]		Cellular Automata (CA)	1 pixel	yes	indirect	no
Moduro 2014		CompuCell3D	arbitrary	yes	nutrition	yes
Moduro (in general)	✓	CompuCell3D	arbitrary	yes	nutrition	yes

Table 4: Comparison of epidermal tissue simulations

$$\text{Input } x \longrightarrow \begin{matrix}\text{Nature or}\\ \text{System:} y \ = \\ f_r(t,x)\end{matrix} \longrightarrow \text{Output } y$$

$$\text{Input } x \longrightarrow \begin{matrix}\text{Model:} \hat{y} \ = \\ f(t,x,\varphi)\end{matrix} \longrightarrow \text{Output } \hat{y}$$

Figure 7: Overview of Modelling. top: a real world system maps an input (vector) to an output (vector) y depending on the time t and the input x. bottom: a model estimates the output value $\hat{y}$ depending on the on the time t, the input x and the parameters φ.

1	1
2	3

Figure 8: Example for a state. Number: cell ID, color: cell type 1 is yellow, cell type 2 green.

- target size (volume)
- cell life time
- etc.

Definition 5. Let I_i be a model domain and P_i a parameter domain. A n-tuple $(x_1, x_2, \ldots, x_n) \in I_1 \times I_2 \ldots \times I_n$ is a called an **input list** (for a model and $I = I_1 \times I_2 \ldots \times I_n$ is the input range. A m-tuple $(\varphi_1, \varphi, \ldots, \varphi_m) \in P_1 \times P_2 \ldots \times P_m$ is a called a **parameter list** for a model and $P = P_1 \times P_2 \ldots \times P_m$ is the paramter range.

Remark 6. $(x_1, x_2, \ldots, x_n)$ and $(\varphi_1, \varphi, \ldots, \varphi_m)$ could be considered as vectors $\vec{x}$ and $\vec{\varphi}$ – however, this is only correct if all elements are of the same type which is not necessarily the case.

A model is a function that maps the input and time but also parameters to an output value. The output itself is of the same type as the input.

$$f : (\mathbb{R} \times I \times P) \to I$$

The state of a simulation is $S = \mathbb{R} \times I \times P$, i. e. point in time, and a combination of observable values (input) and parameters.

Example for 2×2-field:

$$S = (1.0, \underbrace{(\overbrace{2,2}^{lattice\ size}, \overbrace{1,1,2,3}^{cell\ id}, \overbrace{1,1,2,2}^{cell\ type})}_{I}, \underbrace{(\overbrace{2.0,1.0}^{Target\ volume}, \overbrace{30.5,35.0}^{Cell\ cycle\ times})}_{P})$$

Note that S contains redundant data (here type). This would be different in a data structure.

4.2 Granzer-Glazier-Hoogeweg approach

5 Validation

5.1 Parameter estimation

Let m be metric (a fitness function) that compares the real word data with the model estimation.

$$m : I \times I \to [0, 1] \subset \mathbb{R}$$

$m = 0$ indicates the worst and $m = 1$ the best match.

Let $I_S \subset I$ be the set of possible start configurations and $T = \{t_1, t_2, \ldots\}$ a series of points in time for which an output y is known for any $x \in I_S$.

$$M(\varphi) = \sum_{x \in I_S} \sum_{t \in T} m\left(f(t, x, \varphi), f_r(t, x)\right)$$

The optimal parameter (set/vector) is φ^* where $M(\varphi)$ is maximal.

$$M(\varphi) = \frac{M(\varphi)}{|I_S| \cdot |T|} \in [0, 1]$$

Let

$$m_i(x, \varphi) = m\left(f(t_i, x, \varphi), f_r(t_i, x)\right)$$

be the i-th metric for time point $t_i \in T$ and $f()$ is a random variable. The series

$$m(x, \varphi) = m_1(x, \varphi), m_2(x, \varphi), \ldots, m_{|T|}(x, \varphi)$$

can be described via descriptive statistics.

5.2 Arrangment fitness function

The arrangement fitness function f_a surveys to ensure that the cell strata are in the correct order. This is done by a function applying Boolean terms. It reaches an optimum of 1 if the urothelium reaches a state where the basal and stem cells layer is right above the basal membrane, followed by the various layers of intermediate cells and finally with one layer of umbrella cells, before the medium occupies the intraluminal space. In the worst case scenario, 0, the simulation does not create any cells. This equation is described as followed:

$$f_a = \begin{cases} \frac{1}{(1-L_B)+(lib-L_I)+(1-L_U)+1} & if\, layers > 0 \\ 0 & otherwise. \end{cases}$$

$L_B = 1$ if the first layer is made of cell type basal or stem otherwise 0.

$L_U = 1$ if the last layer is made of cell type umbrella otherwise 0.

$lib = layersInBetween$ is the number of strata in between the first and last layer. L_I is the number of layers made of intermediate cells. $lib - L_I$ results in the number of cells wandering away from their intended layer.

The function f_a is then calculated column by column on n different locations on the tissue. Out of these values, the average is taken as follow:

$$\bar{f_a} = \frac{1}{n} \sum_{i=1}^{n} f_a(i)$$

5.3 Volume fitness function

Since no information was found in the literature search regarding the volume of each of the urothelial cell types, the relative volumes $v_{B,S}$,v_I,v_U values were calculated as an average from a variety of histological pictures of the urothelium that were found on the Internet, as well as from these sources [**?**, **?**, **?**, **?**, **?**]. The results are $v_{B,S} = 10\%$,$v_I = 67\%$,$v_U = 23\%$.

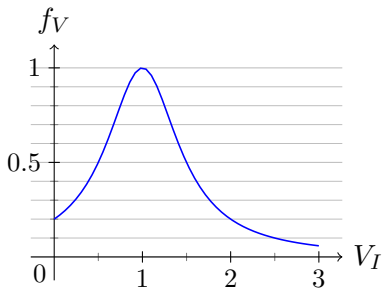

Figure 9: f_V with $f_S = 1$

With these values a non linear volume fitness function f_V was created and is described as followed:

$$f_{V_i} = \frac{1}{4\left(\frac{V_{S_i} - V_{I_i}}{V_{S_i}}\right)^2 + 1}$$

V_S andV_I is the desired (should) and actual (is) volume of all cells of a specific type i.If $V_{S_i} = V_{I_i}$ then the fitness is 1. If the actual volume is 1/2 (-50 %) or 3/2 (+50 %) of the desired volume, $f_{V_i} = 1/2$. The overall fitness volume function is the arithmetic mean for all cell types:

$$f_V = \frac{1}{|C|} \sum_{i \in C} f_{V_i}$$

where C is the set of all cell types in mind. With the urothelium cells this is

$$f_V = \frac{f_{V_B} + f_{V_I} + f_{V_U}}{3}$$

with $C = \{B, I, U\}$.

5.4 Turn-over fitness function

5.5 Mitoses-index fitness function

5.6 Cell count

5.7 Cell cycle times

For every interval of two days the mean cell cycle times for all cells and cell types is calculated. Any time a cell is removed by either dividing or changing their cell type due to differentiation an event is triggered that contains the life time of the removed cell. This event is added to the interval and finally the mean value and standard deviation is computed.

6 Database for models

- Module (or package) concept
- We need at least statements on
  - Differentation process (CM: contact model, PAS: population asymmetry with stem cells)
  - Birth process (NU: mitosis via size via nutrients, IN: infinite growth)

  - Death process (AP: apoptosis, VO: voiding)
  - Sort process (DA: differential adhesion)

- Example: CM-NU-AP-VO-DAE or PASCM-IN-AP-VO
- A Parameter set is defined for each process

Part II
Software

This section describes the software of the Moduro project.

7 Introduction

8 Installation

Software

- Windows 7 or 8 / Linux ?
- Python 2.7.x (tested with 2.7.?)
- CompuCell3D 2.7.x (tested with 2.7.1)

Hardware

- CPU $\geq$ 2 GHz
- $\geq$ 4 GB RAM
- $\geq$ 5 GB disk space

9 Development

This section describes the software design and other software development related issues.

9.1 Requirements

Moduro is a very specific software and will always have a very limited user groups. Users are

- physicians and biologists
- computer scientists, mathematicians and physicists

As of now, Moduro will only be operated by those experts and it is not intended to release Moduro for a greater community.

Here are some high-level requirements:

Agile It must be possible to create new models without breaking existing models. The development of a new model should not take longer than 30 min. given that the techniques are known.

Reproducibility Any simulation run must be reproducible any time by a power user.

Parameters All essential parameters for a simulation run must be saved/exported. Parameters must have a unit (e. g. mol/s) and a reference to the literature where the parameter was estimated. Note: a parameter is essential when a change to it would lead to a different simulation output (see Reproducibility).

Analysis Relevant simulated data can be imported into a scientific workbench (like Matlab or Sclilab) for further analysis. This should be possible even while the simulation is running.

9.2 Architecture and Design

9.2.1 White paper for design

A model is a set of parameters. Parameters are solely represented as Python code. They can be

- regular variables (primitive data types like integer, float; vectors or matrices etc.)
- functions that can be passed to other objects

Proposal: Class Main starts the program. It requires the parameters

1. Execution parameters (reference to ExecConfig instance)
2. Model parameters (reference to ModelConfig instance)
3. Optional parameters (reference to OptConfig instance)

A ModelConfig can be created on the fly or read from a ModelConfig.py singleton object that represents the model. Ideally, there exist as many model-config files as there are models.

9.2.2 Key classes

CC3D has objects of type `Cell` which can be accessed from Python via `self.cells`. CC3D `Cell` cannot be derived. Moduro extends a `Cell` by adding new attributes to a `Cell`'s dictionary.

Units in cell are CC3D specific

All values stored in a cell and the associated cell dictionary are CC3D specific. In particular:

- Volume or surface depends on a 2D or 3D lattice.
- Dictionary attributes like 'targetVolume' are lattice dependent.

A Moduro-cell-class has the following attributes:

min_max_volume The minimal and maximal volume/area this cell can have.

target_Volume The optimal volume/area of this cell.

life_time The life time of this cell in MCS.

exptected_life_time A random number indicating how long a cell will live.

necrosis If true this cell will die.

DNA ???

TurnOver ???

A CellType-class contains attributes for a specific type of a cell, e. g. a stem cell. Any cell object instantiated at run-time will obtain those attributes by default.

CellType is CC3D independent

All units in CC3D are physical units. Defaults for volume is μm^3 and for time is a day.

A CellType-class has the following attributes:

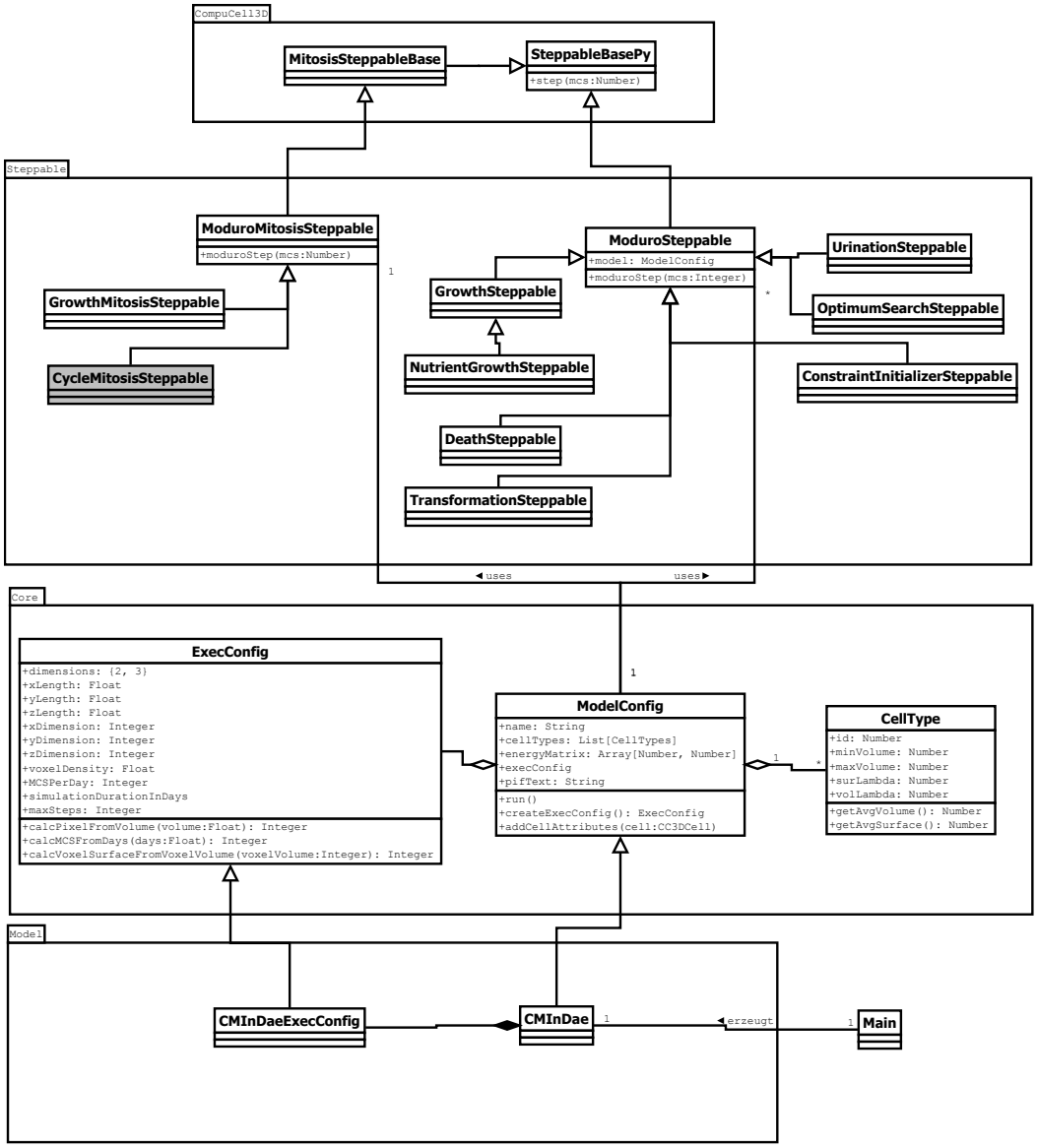

Figure 10: Package and class design.

minDiameter The minimal diameter in μm this celltype have have.

maxDiameter The maximal diameter in μm this celltype have have.

surFit A factor indicating how important a correct surface is. Range from 0 (does not matter) to 1 (perfect).

volFit A factor indicating how important a correct volume is. Range see surLambda.

apoptosisTimeInDays The expected life time of this cell in days.

growthVolumePerDay The growth of this celltype in μm^3 per day. Example: a stem cell with a volume of 400 μm^3 growths (in average) 4000 μm^3/d.

CC3D knows several concepts which are helpful for cell simulations. These are

Initializer A method that initializes the area or space with cells. Example: BlobInitializer.

Copy Attempt A callback after each MCS copy attempt.

Stepables A callback after each MCS.

However, some concepts are missing, which are introduced here:

Sampler A callback that occurs after a specific time step. Usually the time intervals are equal.

9.2.3 Data structures and file formats

Moduro knows the following file formats:

1. Arrangement fitness function (file: `FitnessArrangement.dat`)
2. Volume fitness function (file: `FitnessVolume.dat`)
3. Cell times (file: `Celltimes.dat`) (`.daz` right now because of Toolbox constraints)
4. Colony (TODO)
5. Fitness function (redundant, deprecated)

A text file containing an event for each line. Events are

1. Birth of a cell <time> B <cellID> <cellTypeID>
2. Death (or removal) of a cell <time> D <cellID> <cellTypeID> <cycletime>

```
timescale = h
2440.5 B 1272 B       # 100 Tage
2440.8 B 1234 I
2450.5 D 1272 B 10.0
...
```

A text file containing an entry for each sample point.

Model	Pixel size	# of Cells	HW	Model time	Exec. time	Proble
CM-NU-AP-VO-DAE	400 x 400		CUDA	500 d	7 d	
PAS-IN-AP-VO	400 x 400		CUDA	500 d	5 d	
Klein Tumor (CC3D)	1000 x 1000	500	CUDA	3 MCS	15 min.	crash
Klein Tumor (Morpheus)	5000 x 5000	5k to 10k	Laptop	12 700 MCS	5 h	not our r
Klein Tumor (Morpheus)	5000 x 5000	5k to 10k	CUDA	13 400 MCS	6 h 47 min.	not our r
Klein Tumor (Morpheus)	5000 x 5000	5k to 10k	Laptop	720k MCS	16 days	estimat

Table 5: Simulation power: an overview

9.3 Compiling

9.4 Extending Moduro

9.5 Running Moduro

You need the full path of the compucell3d.bat (with the GUI) or the runscript.bat (without the GUI) file and the option -i followed by the full path of the simulation which should be started:

- *C:\...\CompuCell3d\compucell3d.bat -i C:\...\Demos\Moduro\Contact\Urothelium .cc3d*

Other options are:

- *-o, –noOutput, –exitWhenDone, -h, –help, -c, -f*

[**?**, p.82-83]

10 Simulation power

This sections describes what kind of tissues can be simulated in what time frame.

Platzhalter für Machbarkeitsanalysen

	ID	Description	Model
Stem cells	SD	Stem cell like division	$S \to S + B$
	SPA	Stem cell population asymmetry	$S \to \begin{cases} S + S & p = P_{SSS} \\ S + B & p = P_{SSB} \\ B + B & p = P_{SBB} \end{cases}$
basal cells	SDB	Stem cell like division in basal cell	$B \to B + I$
	BPA	Basal cell population asymmetry	$B \to \begin{cases} B + B & p = P_{BBB} \\ B + I & p = P_{BBI} \\ I + I & p = P_{BII} \end{cases}$
	PCDB	Proliferation and contact differentiation of basal cells	$B \to B + B$ and $B \overset{\neg BM}{\to} I$
	CDB	Only contact differentiation of basal cells	$B \overset{\neg BM}{\to} I$
Intermediate cells	PCDI	Proliferation and contact differentiation of intermediate cells	$I \to I + I$ and $I \overset{M}{\to} U$
	CDI	Only contact differentiation of intermediate cells	$I \overset{M}{\to} U$

Table 6: Possible proliferation and differentiation rules for the cell types stem (S), basal (B) and intermediate (I) cell. Per group one option can be chosen. In total we have $2 \cdot 3 \cdot 3 = 18$ lineage models.

Part III
Models

This section discussed the medical and biological models.

11 Introduction

- Apoptosis should not be linked to a cell cycle time but to the overall life time?
- Cell lineage is autonomous and cells are arranged by a sorting mechanisms

Model	growth rate (%/d)				expected apoptosis time (d)			
	S	B	I	U	S	B	I	U
SD-CDB-CDI	100	100	200	100		80	20	10
SD-SDB-CDI								
SD-BPA-CDI								
SD-PCDB-PCDI	1	1.5	0.1	0.1	∞	80	20	10
SPA-CDB-CDI								
SPA-PCDB-PCDI								

Table 7: Estimated parameters. Growth rate is in percent of the regular maximal cell volume V_{max} (in μm^3) per day when cells are contact-inhibited.

Model	cycle or life time (h)				healing (d)
	S	B	I	U	
SD-CDB-CDI	30	50			3
SD-SDB-CDI					
SD-BPA-CDI					
SD-PCDB-PCDI	35	150	>350	>350	5
SPA-CDB-CDI					
SPA-PCDB-PCDI					

Table 8: Results.