MODELLING MULTI-CELLULAR GROWTH USING MORPHOLOGICAL ANALYSIS

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Abstract. The goal of this work is to introduce a mathematical model of multicellular developmental design based on morphological analysis in order to study the robustness of multi-cellular organism development.

In this model each cell is a controlled system and has the same information, an ordered list of cell type. Cells perceive their neighbours during the growth process and decide to divide in a direction given by the reading advancement of the virtual genetic material and depending on the complex interplay between genetic, epigenetic and environment.

Cells can perform distinct functions but in our simulator, two cell types just differ by their color and by permuting the segmentation direction according to the virtual genetic material and the epigenetic control. The switching on and switching off of genes depends on the environment of the cell. The multi-cellular organism has to reach a shape in a given environment to which it has to adapt.

We present in this paper an algorithm based model which is implemented in a virtual 3D-environment. Moreover, the algorithm follows the principle of inertia in that the cells progress through the reading of its virtual genetic material after a punctuated equilibrium or when its viability is at stake.

1. Introduction. The geometry of cell segmentation and the robustness of cellular growth is relevant when you observe the formation of a multi-cellular organism. Cells have their own dynamics and build the shape of the organism; all cells have the same genetic material and can duplicate, induce apoptosis, staying quiescent or differentiate in another type of cells. A cell is following a genetic process which is transmitted and can differentiate to achieve the shape of a complex organism.

However, when you simulate cellular growth in a virtual reality simulator, questions arise about how they maintain the shape of the virtual organism in a stable state whilst cells are renewing. This highlights the importance of the order in which cells divide and the direction of this division. It is difficult to address these questions without considering an underlying, tightly controlled morphogenetic organization, that allows the shape to be maintained, despite constant cellular change.

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These issues motivate the mathematical formalization of the mechanisms of cell growth using the mathematical background of morphological analysis \([3, 9]\). In this paper, we write the formal morphological equation of the cell activity as a multicellular controlled system with epigenetic feedbacks. These morphological equations are the first step to apprehend morphogenesis as a morphological dynamics converging to a target (the final shape). This is a cellular growth process where each cell is autonomous and regulates with epigenetic feedbacks behavior of other cells. The shape is the target of a multivalued dynamical system.

Cell differentiation during growth means a change of genetic expression in the cells without modification of the genetic material. Differentiation matches various changes of the cell phenotype. Genetic expression is only the first step in a succession of complex occurrences. The observed phenotype is the cells’ dynamic. A cell type differs from another by the sequence of segmentation order. This paper deal with the fundamental problem of the link between cell dynamics, cell differentiation and the shape of the emergent multi-cellular organism.

It also seems important to differentiate the robustness of the developmental mechanism (reproducibility of the shape) and the robustness of the shape stabilization (homeostasis) \([11]\).

In the first step, cells multiply, migrate or die by apoptosis to reach a target shape and in a second step, cells multiply, migrate or die by keeping the shape of the organism and its internal organization.

The robustness of shapes involve a complex organization of cells, linking genetic and epigenetic mechanisms. We question the robustness of cell differentiation and how to code it for different differentiated cells, i.e. how a cell differentiates at the right place, at the right moment and at the right cell type during embryogenesis.

We suggest in this article that the formalization of a model of cell growth is based on autonomous cells that perceive their microenvironment and use the controls part of their genetic heritage to maintain their viability or continue to evolve after a punctuated equilibrium.

This formalism is needed to link the cells and the growing shape. The shape on which the differential equation is defined to evolve.

This article is constructed as follows:

- **section 2**: Introduction of the notations and the mathematical background of mutational and morphological analysis.
- **section 3**: The second section introduces the Morphological Dynamics of cellular tissue evolution
- **section 4**: The third section describes the algorithm based on the morphological dynamic with the virtual genetic material

2. **Mutational and morphological analysis to study multivalued evolutionary system.** Let us remind in this section some definitions and propositions that will be useful to formalize the cellular dynamics. The proofs can be found in \([3, 12]\).

A developing organism has a multi-valued dynamic, cells are multiplying, the organism growth and morphological equilibrium is in a dynamic state. It’s important that we address the shape as the limit of a multi-valued cell dynamic that converges to a robust dynamic shape.

To study evolutionary systems, we have to formalize the cell dynamics. But the classical analysis tools, like classical dynamical systems, are not adapted to study multiplying cells.
Since the end of the seventies the viability theory [2] has been developed from set-valued analysis [1]. The main purpose of viability theory is to explain the evolution of the state of a control system, governed by nondeterministic dynamics and subjected to viability constraints, to reveal the concealed feedbacks which allow the system to be regulated and provide selection mechanisms for implementing them. This theory presents a toolbox to study the dynamics of evolutionary systems. It searches for the conditions (decisions, states) in which operational constraints (like those induced by the environment or the use of resources) will always be satisfied and in which the system will be sustained.

During evolution, an organism reacts and adapts to its environment. To remain viable, cells must be able to find new controls in response to new events. Therefore, we have to anticipate the moment at which the organism reaches its viability limit. Furthermore, the relationship between the cells and the environment is synergistic; when one evolves, so does the other. From a mathematical point of view it is a joint evolution of the states and sets to which they have to adapt themselves. The evolution of viability (or operational) constraints (or of the environment) influences the shape that is governed by the evolutionary system, which in turn influences the environment. At a fundamental level it is the co-evolution of the cells and of the organism.

The viability theory and mutational equations [3, 12] have been developed to mathematically and numerically explain, in a given environment, the evolution governed by these systems. They drive the evolution toward a target shape in such a way to reach it in a set time.

This involves to find conditions (decisions, states) in which operational constraints (such as those induced by the tissue or the use of resources) are always satisfied and therefore in which the system is viable and maintain its shape while renewing.

This is the motivation for the development of morphological and mutational analysis. These theories can be applied to the study of the morphogenetic activity [3]. Therefore the evolution of shapes during morphogenesis can be described using mutational equations which display the dynamic evolution of the cell set. These can be studied together with the evolution of the system state, which is always confined by the constrained environment using the evolution of differential inclusion. These mutational equations have the same properties as differential equations [3, 12] (Peano or Lipschitz theorems...).

With the help of mutational analysis, it is possible to study shape confinement that can only progress in respect of the operation constraints [9]. This then leads us to study the co-evolution of shapes and morphological constraints, the activity of each element of the group also depending on the activity of the group. It becomes the tool of choice for modelling the morphogenetic activity.

With regards to morphogenesis, it is necessary to find shape controls that exist with at least one co-viable evolution of state and environment, starting from one cell and arriving at the final shape.

In an evolutionary system, the regulators are the mechanisms of ontogenesis and interaction with the environment. In a given environment this combination of genes will react with the environment and the cell will differentiate. How can we code these mechanisms and how do the cells read the code is the objective of the next section.
The purpose is also to determine if the evolution of shapes (tubes) satisfies the viability (operability) constraints. The comprehension of these mechanisms, and of the link between cell dynamics and shape, will explain which are the mechanisms that nature has established to maintain viability of cells and multi-cellular organisms.

2.1. Morphological shapes on K(X). The idea is to extend the concept of differential equation on vector spaces to the concept of transition in a metric space (mutational equation).

Let (E, d) be a metric space, considering a curve \( x(\cdot) : [0, T] \rightarrow \mathbb{R}^N \). One way to characterize its derivative, is to consider the vector \( v \in \mathbb{R}^N \), which is the derivative of \( x(\cdot) \) at time \( t \in [0, T] \) if and only if there is a residual function \( w(\cdot) \) with \( \lim_{h \rightarrow 0} \frac{1}{h} \cdot w(h) = 0 \) such that:

\[
x(t + h) = x(t) + h \cdot v + w(h)
\]
is satisfies for all \( h \in \mathbb{R} \) close to 0. You can also write:

\[
\lim_{h \rightarrow 0} \frac{1}{h} |x(t + h) - (x(t) + h \cdot v)| = 0
\]

To generalize this notation to distortion, not necessarily linear, we replace the half-line \( x + h \cdot v \) leaving \( x \) in the \( v \) direction, by using the continuous map \( \vartheta(h, x) \) in the metric space \((E, d)\).

\[
\vartheta : [0, 1] \times E \rightarrow E, \quad (h, z) \mapsto \vartheta(h, z)
\]

This deformation is not necessarily linear, which sends each point \( z \in E \) to a point \( \vartheta(h, z) \in E \) at time \( h \in [0, 1] \). Such a map \( \vartheta \) is the first order approximation of the curve \( x(\cdot) : [0, T] \rightarrow E \) in time \( t \in [0, T] \) if:

\[
\lim_{h \rightarrow 0} \frac{1}{h} d(x(t + h), \vartheta(h, x(t))) = 0
\]

**Definition 2.1.** Let \((E, d)\) be a metric space. A map \( \vartheta : [0, 1] \times E \rightarrow E \) is called a transition on \((E, d)\) if it satisfied the following four conditions:

1. for every \( x \in E : \vartheta(0, x) = x \)
2. for every \( x \in E, t \in [0, 1] : \lim_{h \downarrow 0} \frac{1}{h} \cdot d(\vartheta(t + h, x), \vartheta(h, \vartheta(t, x))) = 0 \)
3. \( \alpha(\vartheta) := \sup_{x, y \in E \atop x \neq y} \lim_{h \downarrow 0} \sup_{x \in E} \max \left\{ \frac{d(\vartheta(h, x), \vartheta(h, y)) - d(x, y)}{h \cdot d(x, y)} \right\} < \infty \)
4. \( \beta(\vartheta) := \sup_{x \in E} \lim_{h \downarrow 0} \sup_{x \neq y} \frac{d(x, \vartheta(h, x))}{h} < \infty \)

2.2. Mutational equations. Consider a curve \( x(\cdot) : [0, T] \rightarrow E \) in a metric space \((E, d)\), a transition \( \vartheta \) on \((E, d)\) can be seen as a (generalized) derivative of \( x(\cdot) \) at time \( t \in [0, T] \) if it is a first order approximation of \( x(t + \cdot) \):

\[
\lim_{h \downarrow 0} \frac{1}{h} \cdot d(\vartheta(h, x(t)), x(t + h)) = 0
\]

This condition is satisfied by more than one transion. We then define:

**Definition 2.2.** Let \( \Theta(E, d) \) be a nonempty set of transitions on the metric space \((E, d)\) and \( x(\cdot) : [0, T] \rightarrow E \) a curve. For \( t \in [0, T] \), the set

\[
\dot{x}(t) := \{ \vartheta \in \Theta(E, d) \mid \lim_{h \downarrow 0} d(\vartheta(h, x(t)), x(t + h)) = 0 \}
\]
is called mutation of \(x(\cdot)\) at time \(t\).

We now generalize the concept of differential equation in a metric space \((E,d)\)

**Definition 2.3.** \([3, 12]\) Let \(\Theta(E,d)\) the nonempty set of transitions on the metric space \((E,d)\). And let \(f : E \times [0,T] \to \Theta(E,d)\) a single valued function.

A curve \(x(\cdot) : [0,T] \to E\) is called a solution of the mutational equation:

\[
\dot{x}(t) \ni f(x(t),\cdot)
\]

if \(x(\cdot)\) is Lipschitz continuous with respect to \(d\) and satisfies:

\[
\lim_{h \to 0} \frac{1}{h} \cdot d(f(x(t),t)(h,x(t)),x(t+h)) = 0
\]

for Lebesgue almost every \(t \in [0,T]\).

### 2.3. Morphological equations.

Morphological equations have the ability to represent the evolutionary process of cellular development, they are governing the evolution of sets. Classical differential equations represent the evolution of vectors, and are not sufficient when the cell divide and give two daughter cells.

These equations are special cases of mutational equations seen in the previous paragraph in the case of multivalued transition (growth and multiplication of cells). \(\mathcal{K}(\mathbb{R}^N)\) are subsets of the nonempty compact Euclidean space \(\mathbb{R}^N\). \(\mathcal{K}(\mathbb{R}^N)\) can be supplied with a metric:

**Definition 2.4.** \([3, 12]\) The Pompeiu-Hausdorff distance between two nonempty subsets \(K_1, K_2 \subset \mathbb{R}^N\) is defined as:

\[
d(K_1,K_2) := \max\{ \sup_{x \in K_1} \text{dist}(x,K_2), \sup_{y \in K_2} \text{dist}(y,K_1)\} \in [0,\infty].
\]

**Proposition 1.** \([3, 12]\) The Pompeiu-Hausdorff distance \(d\) is a metric on \(\mathcal{K}(\mathbb{R}^N)\) and it is the equivalent characterizations for any \(K_1, K_2 \subset \mathcal{K}(\mathbb{R}^N)\):

\[
d(K_1,K_2) = \sup_{z \in \mathbb{R}^N} |\text{dist}(z,K_1) - \text{dist}(z,K_2)|
\]

\[
= \inf\{\rho > 0 | K_1 \in K_2 + \rho \mathcal{B} \text{ et } K_2 \in K_1 + \rho \mathcal{B}\}
\]

\(\mathcal{B}\) is the closed bounded ball in \(\mathbb{R}^N\). \(\mathcal{B} := B_1(0) = \{x \in \mathbb{R}^N| |x| \leq 1\}\)

If \(f : \mathbb{R}^N \to \mathbb{R}^N\) is a bounded Lipshitz vector field. We replace the initial value \(x_0\) by \(K_0 \in \mathcal{K}(\mathbb{R}^N)\) and we are interested in the set of reachable points given by a solution \(x(\cdot)\) of the following equation \(x'(\cdot) = f(x(\cdot))\)

This set noted \(\partial_f(t,K_0)\) is called the reachable set of the vector field \(f\) from \(K_0\).

\[
\partial_f(t,K_0) : [0,1] \times \mathcal{K}(\mathbb{R}^N) \to \mathcal{K}(\mathbb{R}^N)
\]

\[
(t,K_0) \mapsto \{x(t) | \exists x(\cdot) \in C^1([0,t],\mathbb{R}^N) : x'(t) = f(x(\cdot)), x(0) \in K_0\}
\]

If there is now more than one speed at the starting of each point, the vector field is replaced by a valued map \(F : \mathbb{R}^N \to \mathbb{R}^N\) in a nonempty and compact subset of \(\mathbb{R}^N\) and we consider the flow along the differential inclusion \(x'(t) \in F(x(\cdot))\).

The reachable sets by maps in \(\text{LIP}(\mathbb{R}^N,\mathbb{R}^N)\) are transitions in \((\mathcal{K}(\mathbb{R}^N),d)\), they are **morphological transitions**.

By definition, each morphological transition is generated by a map in \(\text{LIP}(\mathbb{R}^N,\mathbb{R}^N)\).

**Definition 2.5.** \([3, 12]\) The curve \([0,T] \to \mathcal{K}(\mathbb{R}^N)\) is called a tube in \(\mathbb{R}^N\).
The morphological mutation (speed) of a tube \( K(\cdot) \) at time \( t \) is a first order approximation of the morphological transitions \( K(t + \cdot) \) for \( d \).

\[
\dot{K}(t) = \{ F \in \text{LIP}(\mathbb{R}^N, \mathbb{R}^N) \mid \lim_{h \downarrow 0} \frac{1}{h} \cdot d(\vartheta_F(h, K(t)), K(t + h)) = 0 \}.
\]

If we now define a morphological transitions curve \( F : [0, T] \to \text{LIP}(\mathbb{R}^N, \mathbb{R}^N) \), then a tube is a primitive (morphological) of \( F \) if and only if \( K(\cdot) \) is Lipshitz for \( d \) and satisfies:

\[
F \in \dot{K}(t) \quad \text{a.e. } t \in [0, T],
\]

meaning that:

\[
\lim_{h \downarrow 0} \frac{1}{h} \cdot d(\vartheta_F(h, K(t)), K(t + h)) = 0.
\]

Once the speed of a tube \( \dot{K}(t) \) is defined, we can now define a morphological equation and apply mutational equations results to the metric space \( (\mathcal{K}(\mathbb{R}^N), d) \) and morphological transitions.

Let us consider \( F : \mathcal{K}(\mathbb{R}^N) \times [0, T] \to \text{LIP}(\mathbb{R}^N, \mathbb{R}^N) \), then a value compacted tube \( K(\cdot) : [0, T] \to \mathbb{R}^N \) is solution of the morphological equation

\[
\dot{K}(\cdot) \ni F(K(\cdot), \cdot)
\]

if and only if \( K(\cdot) \) is a morphological primitive of

\[
F(K(\cdot), \cdot) : [0, T] \to \text{LIP}(\mathbb{R}^N, \mathbb{R}^N),
\]

That means \( K(\cdot) \) is Lipshitz relative to \( d \) and:

\[
\lim_{h \downarrow 0} \frac{1}{h} \cdot d(\vartheta_F(K(t), t)(h, K(t)), K(t + h)) = 0 \quad \text{a.e. } t \in [0, T].
\]

Classical results of existence and uniqueness of mutational equations solutions, such as Cauchy-Lipschitz, P´eano and Nagumo theorems, have been adapted to morphological equations [3, 12].

2.4. Morphological equilibrium. [12] The simplest evolution is the morphological equilibrium. In this case, cells self-organize to maintain the shape despite their dynamics and movement. Indeed, that is what is happening when the multi-cellular organism reaches its adult size and has to maintain it, whereas cells are renewing and self-adapting to that fully grown shape. There is no more morphological evolution but morphological equilibrium.

Definition 2.6. [12] Let \( f : \mathcal{K}(X) \to \text{LIP}(X, X) \) describe the dynamics of a morphological equation. We say that \( K \) is a morphological equilibrium of \( f \) if the stationary tube \( K(t) := K \) is a solution to the morphological equation \( \dot{K}(t) \ni f(K(t)) \) starting at \( K \).
Proposition 2. \[ \mathbb{LIP}(X, X) \] Let \( f : \mathcal{K}(X) \mapsto \mathbb{LIP}(X, X) \) be a continuous, “skirted” and bounded map, describing the dynamics of morphological equation. Then \( \mathcal{K} \) in an equilibrium of \( f \) if and only if \( \mathcal{K} \) is semi-permeable (both invariant and backward viable) under \( f(\mathcal{K}) \).

2.5. The controlled system in discrete time. Let’s examine these concepts in discrete time, basically associating each state to its successor.

Discrete morphological equation governing the evolution dynamic is:

\[
K_{n+1} = \Psi(K_n, u_n)
\]

with

\[
\Psi(K_n, u_n) = \bigcup_{x \in K_n} \varphi(x, u)
\]

\[
= \bigcup_{x \in K_n} \varphi(x, U_n(x))
\]

We reminded this formal context to explain why we use the morphological equations to describe a shape that grows.

These morphological equations can be coupled to the differential equation on this shape that develops.

Next paragraph will formalize the evolution of cellular tissues using this mathematical background. We stay in the context of mutational and morphological analysis.

In this article we don’t couple any other differential equation to the morphological equation but we could linked them to evolution differential equations.

This question motivates the study of a discrete morphological dynamics governing the evolution of tissues.

3. Morphological dynamics of cellular tissue evolution. In this section, according to real cell dynamic \[ \mathbb{LIP}(X, X) \], we formalize the dynamic of cells development, i.e. the series of choices or decisions (migration, division, apoptosis) taken at each step by cells. We then code this series of choices for all cells to understand how cells with the same code can differentiate and change their behavior depending on the environment. With time this evolution leads to a strong, stable shape.

For each cell, we select only one set of 8 possible actions:

\[ \{\uparrow, \downarrow, \leftarrow, \rightarrow, \nearrow, \swarrow, 0, \emptyset\} \]

\( \uparrow \) is the migration moving up from the cell
\( \downarrow \) is the migration moving down.
\( \leftarrow \) is the migration moving to the left of the cell.
\( \rightarrow \) is the migration moving to the right of the cell.
\( \nearrow \) is the migration moving to the back of the cell.
\( \swarrow \) is the migration moving to the front of the cell.
\( 0 \) is the quiescence.
\( \emptyset \) is the apoptosis.

We have to order these actions (128 different ordering sequences in 2D and 3912 in 3D). These permutations of segmentation differentiate the cell controls and in turn the different cells controls will reach different shapes. At each time the cell and its daughter can have two different cell types with different controls.
This mathematical formalization allows us theoretically to reach any shape and provides a general framework to write any dynamic. The difficulty is now to find the correspondence between the code and the shape i.e. it is to find a correspondence between genotype and phenotype.

3.1. Notations.
1. \( K \subset \mathcal{P}(X) \) denotes the morphological environment \( (X = \mathbb{R}^3) \), space of “cells” \( x \in X \cup \emptyset \) characterized only by their position (living cell) or their death made of “tissues” \( K \in \mathcal{P}(X), L \in \mathcal{P}(X) \), etc... which are subsets of cells.
2. the set of eight \((genetic)\) actions \( d \in \mathcal{A} := \{↑, ↓, ←, →, ↗, ↘, 0, \emptyset\} \) made of the six “geometric directions”, the origin and the empty set, used to describe the transitions.
   (a) \((geometric)\) transitions \( x \mapsto x + d \in \mathcal{A} \) (actual action).
   (b) \(stationarity\), \( x \mapsto x + 0 = x \) (no action).
   (c) \(apoptosis\); \( x \mapsto x + \emptyset = \emptyset \) (cancellation or suicide).

remark : controls \( \emptyset \) or 0 are always possible, then the cell will always have an action.

3.2. Cellular segmentation. This injunction \( (d^\wedge, d^\backslash) \) is described by the \emph{genetic inclusion}
\[
x \leadsto \{x + d^\wedge, x + d^\backslash\}
\]
where the mother cell \( x \)
- first \emph{migrates} from \( x \) to \( x + d^\wedge \) using the \emph{migration action} \( d^\wedge \in \mathcal{A} \) at a new position (including \( x \) or \( \emptyset \));
- and, next, \emph{divides} giving birth to a daughter cell at position \( x + d^\backslash \) using the \( d^\backslash \in \mathcal{A} \setminus \{0\} \).

and produces a \emph{daughter-daughter cell pair}
\[
\{x + d^\wedge, x + d^\backslash\}
\]
Hence the elementary transitions are described by
1. \emph{sterile migration} by taking \( d^\wedge \in \mathcal{A} \) and \( d^\backslash = \emptyset \);
2. \emph{stationary division} by taking \( d^\wedge := 0 \) and \( d^\backslash \in \mathcal{A} \);
3. \emph{migrating division} by taking \( d^\wedge \in \mathcal{A} \setminus \{0\} \) and \( d^\backslash \in \mathcal{A} \setminus \{0\} \).

3.3. Genetic process. In order to define the \emph{genetic process}, we introduce the set of permutations \( \sigma \) of the set \( \mathcal{A} \) of genetic actions.

A \emph{genetic process} is the ordered sequence of actions
\[
d_{\sigma} := \{d_{\sigma(1)}, \ldots, d_{\sigma(8)}\} \in \mathcal{A}
\]
We denote by \( \mathcal{G} \) the subset of genetic processes of the actions in \( \mathcal{A} \) (subset of permutation of eight elements).

The order is very significant at the beginning of the growth, when there is only few cells, The order will be determinant for the final shape.

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\textsuperscript{1}supplied with the structure of max-plus algebra for the operation \( \cup \) \( et + \) (where \( K + \emptyset := \emptyset \)).

\textsuperscript{2}For instance, \( K := \{K \subset M\} \) is the family of subsets contained in a given subset (confinment) \( M \).
Operating a genetic process under a given criterion, either for migration or for division, means that the process scans successively \( x + d_{\sigma(1)}, \ldots, x + d_{\sigma(8)} \) until the first time when the criterion is satisfied.

Since the empty set \( \emptyset \), the apoptosis and 0, the quiescence, belong to any genetic process, any sequence of the operation stops before the eight genetic action of the process (that is why there is only 128 different ordering sequence in 2D and 3912 in 3D).

It is relevant to remark that, the number of real cell types is much lower (S. cerevisiae (3), C. elegans (27), D. melanogaster (50), D. rerio (130), G. gallus (180), H. sapiens (210)).

3.4. The double time scale. In order to define the morphological evolutionary mechanism \( K_n \mapsto K_{n+1} := \Phi(n, K_n) \), we have to distinguish

- the calendar (or algorithmic) time \( n \geq 0 \),
- and, at each time \( n \), a process time \( j = 1, \ldots, j_m \) is used for intermediate operations mapping \( K_n \) to \( K_{n+1} \), due to the requirement that at each time, the daughter-daughter cell pair \( \{x + d^\triangle, x + d^\triangledown\} \) cannot occupy positions of the cell.

A genetic regulon is defined as a map\(^3\) associating with each triple \((n, L, x)\), the pair \((G^\triangle(n, L, x), G^\triangledown(n, L, x))\) \( \in G \times G \), the genetic processes satisfying the non-overlapping property

\[
\forall x \in L,
\begin{align*}
i \text{ is the first time process such that } x + G^\triangle(n, L, x)(i) & \in \{x\} \cup \mathbb{CL} \\
j_i \text{ is the first time process such that } x + G^\triangledown(n, L, x)(j_i) & \in \{x\} \setminus \{x + G^\triangle(n, L, x)(i)\} \cup \mathbb{CL}
\end{align*}
\]

The first property describes the migration and provides the first cell \( x + G^\triangle(n, L, x)(i) \) such that \( x + G^\triangle(n, L, x)(i) \in \mathbb{CL} \).

The second property describes the division and the birth of the daughter cell (it cannot take the position of its mother when migrated, so that \( G^\triangledown(n, L, x)(j_i) \neq G^\triangle(n, L, x)(i) \)).

1. There is apoptosis whenever \( G^\triangle(n, L, x)(1) = \emptyset \). In this case, there is no division;
2. There is no migration whenever \( G^\triangledown(n, L, x)(1) = 0 \). In this case, \( x + G^\triangledown(n, L, x) \) \((j_i)\) \( \in \mathbb{CL} \);
3. There is no division whenever \( G^\triangledown(n, L, x)(1) = \emptyset \).

3.5. Local morphological dynamics. The genetic regulons \((G^\triangle, G^\triangledown)\) are assumed either to be given (as for the zebra fish or elegans) or constructed to regulate viable evolutions, for instance. They are the inputs of the controlled morphological inclusion define below.

First, the map \( H \) defined by

\[
H(G^\triangle, G^\triangledown)(n, L, x) := (x + G^\triangle(n, L, x)(i), x + G^\triangledown(n, L, x)(j_i))
\]

associates with any pair \((G^\triangle, G^\triangledown)\) of genetic processes the daughter-daughter cell pair and the migration-division transform \( \varphi(n, L, x; G^\triangle, G^\triangledown) \) of the subset \( L \) at time \( n \).

\(^3\)a single-valued map for the time, since no other parameters, biological ones, are involved at the stage of this study.
and the cell \( x \in L \) maps \( L \) to the subset

\[
\varphi(n, L, x; G^\wedge, G^\vee) := L \cup \{ G^\wedge(n, L, x), G^\vee(n, L, x) \}
\]

It transforms \( L \) at time \( n \) after migration and division of the cell \( x \in L \).

3.6. Global morphological dynamics. Assume that a subset \( K_n \in \mathcal{P}(x) \) is constructed, described and coded by an ordered list \( (x_1, \ldots, x_{P_{K_n}}) \).

Hence we construct \( \Phi \) in the following way: \( K_n \) being given, we define the sequence

1. \( K_n(x_1) := \varphi(n, K_n, x_1; G^\wedge, G^\vee) \)
2. \( \forall p = 2, \ldots, P_{K_n} \),

\[
K_n(x_1, \ldots, x_p) := \varphi(n, K_n(x_1, \ldots, x_{p-1}), x_p; G^\wedge, G^\vee); \quad (4)
\]
3. We set \( \Phi(n, K_n) := \varphi(K_n(x_1, \ldots, x_{P_{K_n}}), G^\wedge, G^\vee) \).

This mathematical formalization is used in a virtual reality simulator, the cells have the same virtual genetic material which is a list of \((G^\wedge, G^\vee)\).

4. Algorithm with the virtual genetic material. We describe the morphological algorithm following the aforementioned formalization used in the 3D virtual reality system called DynCell. This virtual reality simulator which simulates the morphogenesis was described in \([7, 8]\).

Based on multi-agents systems, it opens the possibility to simulate autonomous cells (the agents) without any central control. The question of the emergence in these systems is essential and often studied \([5]\).

We present here the algorithm starting from a shape \( K_n \), each cell of this shape having the same genetic material but each cell has an intern state depending on its history and on the micro-environment of the cell.

Each cell follows the algorithm above and evolves according to a three-stroke cycle: local environment perception, decision depending on his internal state and an action to realize and modification of the environment while making these action \([6, 10, 14]\). At each stage, cells have to take a decision (multiply, migrate, apoptosis or eventually quiescence) and construct the shape \( K_{n+1} \).

4.1. Algorithmic remarks.

1. coding \( K_n \) by ordered sequences of cells

The algorithm for constructing the dynamics of the shape depends of the coding of subsets \( K_n \) by a ordered sequence of cells at the root of graphs.

Either there is an order on the whole grid for visiting the cells in this order, and each subset inherits this “universal” ordering, or codage from one set to a larger one, extending the order of the smaller set to the order of the larger one consistent with the smaller one.

2. Codage of Permutations and Genetic material lecture for each cell Regulons

They are needed to construct the maps \((G^\wedge, G^\vee)\) at each time \( n \), for each \( K \) and any cell \( x \in L \). Some of them are trivial: the ones attributing the genetic process \( G(n, L, x) := (0, \ldots) \) (starting with the “non action”) leave invariant the cell, the ones attributing the genetic process \( G(n, L, x) := (\emptyset, \ldots) \) (starting with the “non action”) command suicide of the cell, and those can be coded by \( 0 \) and \( \emptyset \) instead of the sequences of eight actions. In the same way, the
position of 0 or 0 at some position in the list terminates the scanning of the list at this position. Genetic processes can be regulated by the life expectation, dictated by the use of 0 at the first position, the second position, etc., up to the eighth one which allows the genetic regulon to scan all possibilities before killing the cell.

3. Once the subset of genetic regulons is coded, the construction of the values \( \varphi(n, L, x; G^\wedge, G^\vee) \) resulting from a combination of translations, cancellations, stationarity and unions ("killing" one cell by taking it out of the list), doing nothing, translating on set and/or adding one more element.

4. The local iteration for building the morphological dynamics is made easy. Once this is done, we shall use viability constraints for building subsets \( K_n \).

4.2. Virtual genetic material. In this algorithm we introduce the principle of inertia [4], which means that genetics controls evolve as slowly as possible, within the cells.

The cells go on in reading their own genetic material whilst being influenced by the results of neighboring cellular actions within the environment.

The controls may evolves after a punctuated equilibrium [4] or at times of crisis when the viability of the system is at stake.

The virtual genetic material is the only information in the first cell, it is a list of cell type \( \{T_i, T_j, T_l, \ldots\} \).

As above, each cell type has a different order of segmentation. When a cell is quiescent with one list of controls or after a punctuated equilibrium, it advances in the reading of the genetic material. The cell tries the next set of controls, until the cell can duplicate.

This new control is a cell differentiation characterised by a new color and a new cell dynamic.

When the cell arrives at the end of the genetic material without a new daughter, the cell stabilizes in the last segmentation cell type.

The organism stops growing when all cells arrive at the end of the genetic material reading.

5. Results. To better understand the mechanisms regulating morphogenesis, we voluntarily limit ourselves, in a first phase, to some discrete simulations controlling the division order and the asynchrony effects.

As we have explained previously, the application gives us the opportunity to test different kinds of simulations. These follow each phase of the genomes development throughout the entire simulation which varies according to the environment perceived and the advancement in the list reading. We already have a few tests using this system [7, 8]. A few number simulation choosing division modes and a series of different mitosis directions in order to watch the impact of these parameters over the global shapes of the cells populations (for the first zebrafish divisions and for the creation of a shape dictionary).

5.1. French flag problem. We present here, the "French flag problem" created by Lewis Wolpert in the late 1960s, the model uses the French flag as visual representation to show how embryonic cells can interpret genetic code to create the same pattern [15].
Algorithm 1: Genetic material lecture for each cell

Data: The first cell with a genetic material $T_{12}, T_{27}, ..., T_6$

Result: a set of differentiate cells

First cell:

- genome.size()
- genomeIndex ← 0;
- dirIndex ← 0;
- nbMult ← 0;

while $(\text{genomeIndex} + 1 < \text{genome.size()} \text{ or direction=false or \text{dirIndex} + 1 < \text{nbDir}})$ do

if $(\text{dx}=0 \text{ and dy}=0 \text{ and dz}=0 \text{ and \text{memoire}=0 and \text{genomeIndex} + 1 < \text{genome.size()} \text{ and \text{tempsQuiescence}=0})$ then
*/ Quiescence */
    tempsQuiescence+=1;
    direction = true;
    genomeIndex+=1;
    dirIndex=0;
    diffCellType(cellTypes[genome[genomeIndex]]);
else if (\text{dirIndex}=0 then/* Apoptose */
    apoptose();
else if (\text{isEmptySpace}(\text{dirIndex})=0 and \text{nbMult}<\text{multiplicity})
then/* Mitose in the direction \text{dirIndex} */
    mitose(dirIndex, genomeIndexMere, genomeIndexFille);
    currentMitosisAge=0.0;
    nbMitosis+=1;
    dirIndex=0;
else if (dirIndex + 1 < nbDir and nbMult < multiplicity then/* If there is no place, it advance in the reading of direction */
    dirIndex++
else if (\text{genomeIndex} + 1 < \text{genome.size()} \text{ and \text{memoire}=0} \text{ or } \text{genomeIndex} + 1 < \text{genome.size()} \text{ and \text{tempsQuiescence}<1 and \text{memoire}=0}) then/* Différenciation cellulaire \text{ genomeIndex}++ */
    genomeIndex+=1;
    dirIndex=0;
    diffCellType(cellTypes[genome[genomeIndex]]);
else /* the cell arrived at the end of the genetic material, the cell stay in the cellular type of its last division */
    _tempsQuiescence+=1; _genomeIndex=_genomeIndexMemoire;
    _dirIndex=0;
    _memoire=1;
    _setCellType(_cellTypes[_genome[_genomeIndexMemoire]]);
Wolpert addressed embryonic regulation and proposed several rules on concentration gradients which governed the regulation in what he called the French flag model.

Our proposition is very simple and give an alternative for the french flag model. We propose other rules to explain how a single cell with only one genetic information can produce a french flag. All the cells must have the same information but the differentiation means that the cell advance in the reading of the codage.

We simulate this situation in an evolutionary setting where individuals have to grow into “French flag” patterns.
At the beginning of the simulation the first cell multiplies and differentiates to another cell type when there is no space to multiply in a given direction ordered by the genetic information. Each cell type has a color ($T_1$ is blue, $T_2$ white and $T_3$ red). Each cell advance differently in reading of the genetic information according to its environment, this difference is characterized by a different color that we call cell type. Each cell type has a different dynamic, it is a succession of different division plane in a 2D environment.

The mother cell stay quiescent. Cells differentiate according to the position of the other cells. The control $d_{σ(1)} = 0$ is always possible for the daughter and create a daughter at the first possible control of the list $T_i$ (if the left place is free $d_{σ(1)} = ←$, if there is no place on the left $d_{σ(2)} = 0$). After one step of quiescence the cell read $T_{i+1}$. When the list is finish, it stop growing.

In this examples, in a tissue $L$, the cell $x$ at time $n$ has:

$$\langle 0, G^<(n, L, x) \rangle = \langle 0, (T_1, T_2, T_3, T_1) \rangle$$

depending on the advance of the list of possible controls.

Every cellular type $T_i$ is a sequence of 8 different actions ordered for example:

$T_1 = (d_{σ(1)} = ←, d_{σ(2)} = 0, d_{σ(3)} = ↑, d_{σ(4)} = →, d_{σ(5)} = \emptyset, d_{σ(6)} = \leftarrow, d_{σ(7)} = ↑, d_{σ(8)} = \emptyset)$

$T_2 = (d_{σ(1)} = ↓, d_{σ(2)} = 0, d_{σ(3)} = →, d_{σ(4)} = ←, d_{σ(5)} = \emptyset, d_{σ(6)} = \leftarrow, d_{σ(7)} = \emptyset, d_{σ(8)} = \rightarrow)$

$T_3 = (d_{σ(1)} = →, d_{σ(2)} = 0, d_{σ(3)} = ←, d_{σ(4)} = ↑, d_{σ(5)} = \emptyset, d_{σ(6)} = \leftarrow, d_{σ(7)} = \emptyset, d_{σ(8)} = \rightarrow)$

![Figure 2. French Flag growth](https://www.youtube.com/watch?v=vBxwOV9YTeI)
5.2. Gastrulation. Gastrulation is another important example of collective crowd movement, it is a complex folding event that forms the fundamental germ layers of the embryo in its earliest stages step in animal morphogenesis. It differentiates animals from vegetals. Movements and changes in the form of cells during gastrulation are fairly well known, but the understanding of their movement and their coordination remains weak. Many models have been proposed to better understand some aspects of gastrulation. Like for the french flag problem most of the models are based on reaction-diffusion models.

We present here, another simple virtual genetic material \((0, (T_4, T_5))\) with two different controls. Each one has a color \((T_4\) is yellow and \(T_5\) is grey) and each cell advance differently in reading of the list according to its environment, this difference is characterized by a different color that we call cell type. Each cell type has a different dynamic, it is a succession of different division plane in a 3D environment.

With the same model, we propose how a single cell with only one genetic information can produce invagination of the tissue.

With

\[
T_4 = (d_{σ(1)} =←, d_{σ(2)} =→, d_{σ(3)} =↑, d_{σ(4)} =↓, d_{σ(5)} = 0, d_{σ(6)} = ∅, d_{σ(7)} = →, d_{σ(8)} = ←) \]

\[
T_5 = (d_{σ(1)} =→, d_{σ(2)} = 0, d_{σ(3)} =↓, d_{σ(4)} =←, d_{σ(5)} =↑, d_{σ(6)} = ∅, d_{σ(7)} = ←, d_{σ(8)} = →) \]

The mother cell stay quiescent. The control \(d_{σ(4)} = 0\) is always possible for the daughter cell. The cell create a daughter at the first possible control of the list \(T_i\) (if the left place is free \(d_{σ(1)} =←\), if not \(d_{σ(2)} =→\), if not \(d_{σ(3)} =↑\), if not \(d_{σ(4)} =↓\), if there is no place around the cell \(d_{σ(2)} = 0\)). After ten step of quiescence the cell read \(T_{i+1}\). When the list is finish, it stop growing.

The video can be seen: https://www.youtube.com/watch?v=lVjGAnVD0wg
6. Conclusion. To predict cellular behavior is a major challenge in cell and developmental biology. The very first step of morphogenesis shows us geometrical cleavage that we want to explore. In this paper, we have describe a mathematical model with basic mechanism (migration, division, apoptosis, migration). This model can be used to achieve any discrete shape if we know the maps \( (G^n, G^\land) \) at each time \( n \) for any cell \( x \in L \).

This model with the additional constraint that the only information given to the cell is in a “virtual genetic material” and that the environment acts on the cells has lead to the creation of the algorithm.

This work, is a strictly formal approach, it can be seen as a metaphor of morphogenesis which gives the opportunity to ask some questions about epigenetic phenomena and the underlying mechanisms which allows a set of cells to grow to a given shape.

There are very few multi-cellular shapes that exist in nature, most of them are homomorphic. This phenomena can highlight some of the fundamental rules of cells controls. This means that there is very few viable cell controls for a given time horizon. This can also helps us to understand how the biological genetic material codes process of the cell and how it affects the dynamics within the complex interplay between genetic, epigenetic, and environmental factors.

To continue this work with this morphological dynamics, we have to adapt current algorithms for morphological equations:

- The viability kernel algorithm which approach the subset of initial states such that there exists at least one evolution “viable” in the environment.
- The capture basin algorithm which approach the subset of initial states such that the shape can be reached in finite time, before possibly leaving the viable state, by at least one trajectory.

Thinking about shapes as a cellular growth which drives to a target, characterized in the final form as a stable equilibrium [3].

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