Visual Experiments of Geometric Combinatorics for Neural Stem Cells and Their Derivatives

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Abstract

Software is being developed to simulate and visualize plausible scenarios for neural stem cell differentiation. The primary emphasis is to create developmental models for hydrocephalus, where stem cells experience increased demands for multi-cellular differentiation. Images from light and electron microscopy examinations of mouse brain slices that expose the stem cell niche show significant geometric structure, known as ‘pinwheels’. Each of these microscopy images is a static ‘snapshot’ in time. A creative emphasis is to generate novel and dynamic geometric representations of these static stem cells configurations, as they form through brain development. Innovative algorithms interpolate from one static state to the next and conjectured interpolatory processes can be coded based upon biological constraints. Animations are generated for each conjectured subdivision and differentiation. The animations are viewed by neurobiologists, who judge the plausibility of any conjectured scenario. The geometric combinatorics impose logical constraints, which have already been used to preclude some proposed biological mechanisms. The goal is to provide rapid iterations of visualizations of brain developmental to facilitate discovery. Initial results are reported as refinements continue.

1 Microscope and Abstracted Views

Figure 1 depicts stem cell niche organization along the walls of the brain’s lateral ventricles as viewed by confocal microscopy and denoted schematically as pinwheels. The left image1 in Figure 1 is an en face preparation of the mouse anterior forebrain, showing the apical surface of the lateral wall of the lateral ventricle using confocal light microscopy. Ependymal cells are outlined by β-catenin staining (denoted by green boundaries) and the processes of the neural stem cells are stained with GFAP (indicated in red). A colorized schematic of this image is shown in the right half of Figure 1, created by a neurobiologist capturing the observed structure and by directly tracing over the microscopy image [10].

2 An Idealized Pinwheel

Pinwheel-like cellular organization is found along the lateral wall of the lateral ventricles within the anterior forebrain of mammals. In embryonic development neu-
nal stem cells line the ventricles and following activation, we posit that one neural stem cell will divide to give rise through asymmetric division to another neural stem cell and a specialized cell, an ependymal cell. The new neural stem cell’s cell-body will localize below the monolayer of newly generated ependymal cells at the ventricle surface, but will retain a single thin process with contact to the cerebrospinal fluid at the ventricle surface; thus creating the pinwheel organization at the ventricle surface.

To create formal biological models of this pinwheel phenomenon, some terminology will be established here. Considering Figure 2, let the central white circle be designated as the hub, the region that contains the apical projections of the neural stem cells, and let the colored extremities be designated as petals, the newly generated ependymal cells that will cover the ventricle surface. Together this organization represents ‘regenerative units’ that allow continued neurogenesis through subsequence division of the neural stem cell; however, the ependymal cells are considered to be terminally differentiated and will not divide.

3 Related Work

The highly cited article [10] is the primary source for this work. The identification of the pinwheel organization was first published there. The advancement made here is to use computer simulations to model the generation of pinwheel geometric organization. The promise is to facilitate understanding of the brain development via neural stem cell differentiation, particularly with respect to understanding of hydrocephalus and the stresses it places on early brain development.

Computer simulations for hydrocephalus have appeared in both surgical [11] and engineering [9, 12] venues over the last two decades. The three cited are illustrative in that all invoke the finite element method, which presumes a geometric model. The distinctive focus of the current article is on refinements to any a priori geometric model, as informed by exploration of stem cells and their derivatives.

The first author and his collaborators have developed software visualization tools to model the geometry and topology of macro-molecular simulations. The conceptual and algorithmic design principles discovered in those papers [1, 3, 4, 5, 6, 7, 8] have been adapted to the development of the prototype software described here, even though this biological application is an entirely different.

The second author and her co-authors have previous investigations into the spatiotemporal organization of the developing brain [13]. This work continues those themes with a new emphasis upon the cytoarchitectural impact of hydrocephalus.

4 Some Initial Conditions

To simulate neonatal neural development, the following biological assumptions were made:

B1. development begins with a few cells,
B2. cells subdivide in development,
B3. nuclei retain constant volume over all subdivision iterations.

To create a computational simulation, supportive programming assumptions were made:

P1. Planar graphical displays are confined within a bounding rectangle
P2. Within that rectangle, development proceeds from a single cell and proceeds to expand to the right vertical edge over increasing time. If one (white hexagon) divides, at the surface (plane shown) the resulting petal takes up most of the surface area and the resulting neural stem cell has a very reduced surface area. The factors for this decrease can easily be changed, allowing further experimentation to show that the petals cells increase as the hub decreases in size.

5 Initial Visual Experiments and Insights

Figure 3: Bright red hexagon and triangle in hub.

This initial graphics setup is shown in Figure 3, where an initial stem cell has subdivided into the red hexagonal and black triangular cells depicted. This is followed, in Figures 3 - 7 by key frames from the animation that is synchronized to our cell differentiation algorithm.

It is noteworthy that the hub is formed from multiple triangles each of small area, all contained within a central hexagonal hub, consistent with the right image
of Figure 1. Figures 3 - 7 are of idealized geometric cellular cross-sections, projected as planar images. The symmetry that appears would be readily disrupted by having some of the petals and some of the hubs cells being of slightly different shapes than depicted. This modified behavior is expected to produce images more realistically comparable to the right part of Figure 1 and the software will be modified to accommodate those changes.

Figure 6 shows a completed red pinwheel, with an overlapping one starting. The newly generated pinwheel is colored blue, so that the purple color is used to show the overlap. This effect has been depicted in Figure 1, where purple was also used to indicate the area of overlap. Further related discussion appears in Section 10.

The discipline undertaken in implementing the prototype software is to clearly delineate whether a development assumption arises from biological imperatives or from programming convenience.

Initial computational experiments provided insight regarding assumption B3. As a stem cell divided, it would be sufficient that the total area of the resultant cells in each slice were the same as the area of the initial stem cell. Taken over all slices, this would clearly preserve total tissue area. Several visual scenarios were created. All showed visual constraints that were inconsistent with Figure 1. This led to questioning the area preserving assumption. The conclusion was that area preservation is *not essential* to preserve volume, as easily realized by considering that a circular cylinder and an ‘hour glass’ could easily be of the same height and volume. While this is an obvious comparison, a more plausible illustrative example shown in Figure 9, where compensating changes in cross section occur locally.

A difficulty with an early version of the software was that the pinwheels were ill-formed. This is indicated in Figure 8, where some of the petals are joined only
at a single vertex. Upon careful examination of the algorithms used, it was determined that these artifacts could be corrected while still preserving areas. However, the resultant discussion led the authors to conclude that the area preservation was misguided and that areas in any slice could deform while still preserving volume in three dimensions and that this was likely to be a more appropriate biological model.

Indeed, it was noted that the observed artifacts could be easily eliminated by another configuration where areas were preserved after each cell division, indicating that the area preservation was not being eliminated solely due to software issues.

It was logically clear from the start that, while preserving all the cross sectional areas would be sufficient to satisfy B3, it was not necessary. Rather this was taken as a simplifying assumption to accelerate coding. The resulting visual artifacts prompted the authors to consider other mechanisms for volume preservation, that would reflect the biological process.

6 Volumes and Visual Experiments

Figure 9 starts with a cylinder on the left. The figure on the right preserves volume by local adjustments.

The visual experiments have led to the following conjectures of the four key steps to produce a single pinwheel.

1. A stem cell divides to give two cells.

2. Within a given brain slice the new petal from the subdivision occupies more area than the initial cell, which then becomes part of the hub.

3. In the full 3 dimensions, a new petal occurs adjacent to one cell in the hub, which subdivides, creating a petal and adding a cell to the hub.

4. This process then continues until a full pinwheel is formed. The stopping mechanism might just be that the petals fully surround the hub, with the petals sharing non-trivial boundaries, meaning more than just a simple vertex, as shown in Figure 4.

7 Laboratory Verification

Laboratory experiments are in process to verify or refute these conjectures. This verification is dependent upon analyzing data at differing times to correspond to each of the enumerated steps. Mice (inbred strains used here) will be assumed to be identical and brain slices will be obtained at predetermined times for each experimental group. Microscopic imaging of the cytoarchitecture of the cells will be consist of aligned, sequential z-stack confocal images. These will provide ‘snapshots’ for comparison to the time sequence outlined.

The fourth conjectured step permits multiple cells to join to form the hub, as represented in the red regions of the left image of Figure 1 and in blue in the right image of Figure 1.

Once these snapshots are available, the existing software will be used to create geometric representations and visualizations of each snapshot. Then topology preserving morphing algorithms will be added to transition between snapshots, leading to further conjectures and experiments to refine understanding of the pinwheel creation process.

8 Static Versus Dynamic

This paper submission is restricted to just showing static images. The investigatory strength of the software results from the animations produced. Initial versions showing both 2D and 3D simulations are available by contacting the first author, who intends to publicly host the software when it is stable. The 2D versions are to reflect what appears in static microscope images
of sliced brain tissue, whereas the more ambitious goal for the 3D animations is to support understanding of the rapid development of the disease of hydrocephalus and the structural alterations it imposes. The role of the simulation software is to provide understanding of the temporal development, to lead to more strategic opportunities for medical intervention before severe brain ventricle inflation is reached.

The software has already facilitated communication between computer scientists, neurobiologists and physicians, starting the process of greater shared understanding. In particular, the exchange is accelerating so that the neurobiologists are already formulating their conjectures in language that goes rapidly into software refinements, leading to new animations that are judged for further refinements. The geometric combinatorics to develop responsive algorithms also impose logical constraints, which have already been used to preclude some initially proposed interpolatory biological mechanisms.

Considerations of inhibition or promotion of cell division are of particular interest. Fetal hydrocephalus involves the enlargement of the brain’s ventricular system that transports the cerebrospinal fluid. This expansion creates a need for increased numbers of cells to cover the ventricle surface, potentially diminishing the neural stem cell population and thereby affecting normal neurogenesis. Visualization software permits user designation of normal and diseased states that can be studied over time, identifying final resultant structural configurations, that can be judged for their biological significance and impact in disease models.

The user-interface (UI) is currently supportive of software development. The intent is to place the software in the hands of research neurobiologists and clinicians, for them to conduct visual experiments on their own, to accelerate discovery within developmental neurobiology. Those UI refinements have begun within the ongoing interdisciplinary collaboration between computer scientists and neurobiologists.

9 Modeling Cell Volumes

The organization of brain cells into “pinwheel” patterns is ultimately a 3D process; the above images are, in fact, slices through a 3D cluster of cells. To develop a general and robust understanding of this process, we have begun modeling the organization (combinatorics) and structure (geometry) of brain cells in three dimensional space.

As a preliminary step, we have modeled brain cells as 3D Voronoi cells [2]; see Figure 10. The Voronoi site for each cell is associated with the center of the cell’s nucleus. To achieve roughly constant cell volumes, the Voronoi sites have been placed in an approximate lattice configuration.

This model has been incorporated into a visualization for clusters of static and dynamically moving brain cells. This tool allows the user to visualize various cross sections of the cell cluster. We hope that this feature can lead to an understanding of how the formation of pinwheels can result not only from the division of cells, but also from the movement of cells in three dimensional space. We ultimately aim to incorporate biologically informed cell division and movement rules into this visualization.

Figure 10: A cluster of cells and one of its cross sections.

10 Conclusions

The prototype software demonstrates plausible dynamic interactions for understanding development of the brain. This paper reports a ‘proof of concept’ thus far. Further refinements to aid biological discovery are planned and expressed, ranging from combinatorial algorithms to an improved user interface.

References


