Fate Mapping *Escherichia Coli* Tissue Development

Atri Choksi  
Department of Bioengineering  
Stanford University  
Stanford, USA  
achoksi@stanford.edu

**Abstract**—Cell lineage tracing is a fundamental digital image processing tool for developmental biologists to probe the roles of cell differentiation, cell adhesion, and cell movement among other biophysical principles, in developmental programs. In this project I create an extension of cell lineage tracing—tissue fate mapping or tissue lineage tracing. Tissue lineage tracing is useful for observing biophysical and geometric parameters of a developing tissue. I first evaluate different tissue abstraction algorithms and then characterize roundness and fractal dimension of the resulting tissues to demonstrate the utility of this approach.

**Keywords**—Lineage tracing; Morphological Image Processing; Fractal Dimension; Roundness

I. INTRODUCTION

Most vertebrates start out as a single cell. That one cell asexually reproduces many times to give rise to tissues, organs, organ systems, and eventually an organism. How one cell encodes all the information to precisely develop into an organism many times its size is an active area of investigation by developmental biologists. How this information is orchestrated dynamically through biophysics into structure is another interesting question that has been relatively unexplored. One major obstacle in answering the latter question is the lack of tools to probe the biophysics of development.

One fundamental digital image processing tool used by biologists is cell lineage tracing[1]. In cell lineage tracing, cells are identified in each image and paired with a parent cell from the previous images. Color coding of cells shows the lineage of each cell. Cell lineage tracing has been successfully used to understand the tissues that cells give rise to. For example, cell lineage tracing has been used to identify and visualize all the different neurons in the brain, to understand the organs that germ cells give rise to, and to map the complete development of *C. elegans*[2]-[4]. In this project, I develop a new tool that is an extension of cell lineage tracing—tissue lineage tracing. Tissue lineage is useful to study how tissues develop rather than cells.

Abstracting cell lineage into tissue level allows researchers to ask different types of questions that they haven’t been able to ask as easily before (Figure 1). With tissue lineage, scientists can characterize the geometric properties of the developing tissues and more easily observe the biophysical properties of each tissue that gives rise to the whole organ. In this study, I compare three different morphological operations to abstract cell lineages into tissue lineages and then demonstrate the utility of this approach by characterizing the roundness, fractal dimension, and cell density of each tissue.

II. IMAGE PROCESSING ALGORITHM

A. Simulations

First, I generate cell lineage maps over a small development time window. Simulating cell division rather than taking time-lapse images of dividing *E. coli* is performed to eliminate the intermediate digital image processing steps of cell lineage tracing. In other words, simulating *E. coli* growth keeps the experimental results independent of the cell lineage tracing algorithms. The simulations are performed in Python using the CellModeller framework from the Haseloff Lab[5]. The CellModeller framework supports stochastic modeling of various biophysical parameters such as intercellular signalling and rule-based behaviors. Simulations are of rod shaped cells that divided at a uniform rate. The initial conditions are nine cells evenly spaced out. These cells are modelled and tracked to divide until 10,000 cells result. Therefore, the simulations produced a cell lineage map (tracing the nine different initial cells) over some development time window.

B. Extracting Tissues

Before abstracting each cell lineage into a tissue, it is necessary to identify each cell lineage (Figure 2). The nine different cell lineages are color coded. However, extracting images from the simulations and lowering the resolution creates artifacts and gives rise to various other colors. Therefore, an automatic color detector of the most frequent RGB values is implemented. The color detection module chooses the nine most frequent bins in the red value
Fractal Dimension resulting from the edges of a cell lineage map has been previously studied. An automatic fractal dimension module is implemented here. To calculate fractal dimension of all tissues automatically, canny edge detection is followed by a box counting algorithm[10]. In the box counting algorithm, the image is divided into boxes of different sizes and the number of boxes containing tissue edges is counted. Log(boxsize) v.s. Log(1/boxes) is plotted and a line is fitted using linear regression. The slope of this line is the fractal dimension.

III. RESULTS

After applying the digital image processing algorithms to abstract and characterize geometric properties of the tissues, the abstraction algorithms are assessed and the geometric properties are observed over development.

A. Evaluation of Abstraction Algorithms

An ideal tissue abstraction algorithm would produce a tissue that does not extend beyond the outside of the cell lineage boundary. In addition, it would produce tissues that do not overlap and would fill in all of the artifacts. These specifications are described by the single measurement of cell density. An ideal tissue abstraction algorithm maximizes cell density while filling in all the artifacts of a tissue. Therefore, cell density is measured for morphological closing, small region removal, and morphological filling.

Morphological closing consistently abstracts cell lineages with the least cell density (Figure 3). This is expected because the boundaries become smoothened and so more area on the outside of the cell lineage is included in the tissue. Small region removal consistently maximizes cell density. However, it is also observed that this algorithm does not fill in all the artifacts of the cell lineage (data not shown). Morphological filling consistently gives a higher cell density than morphological closing but a lower cell density than small region removal. Morphological filling is therefore chosen to be the best algorithm at abstracting tissues from cell lineages. Abstracted tissues resulting from morphological filling are used to assess

D. Measuring Geometric Properties

To demonstrate the utility of this approach, three geometric properties of the abstracted tissues are assessed: roundness, cell density, and fractal dimension.

- Roundness is a characteristic of the shape of each tissue. Equation (1) is used to estimate roundness.
  \[
  \text{Roundness} = 4 \times \pi \times \text{Area}/(\text{Perimeter})^2 \tag{1}
  \]

- Cell Density of each tissue is calculated by equation (2). Cells are counted using morphological erosion in four different orientations. This method is roughly 96% accurate from comparison with hand counting.
  \[
  \text{Cell Density} = \text{cells/Area} \tag{2}
  \]
geometric properties of the tissues.

B. Observing Geometric Properties

The roundness of each abstracted tissue is calculated over a development time window (Figure 4A). Several observations can be made from this data. First, the center tissue decreases in roundness at the greatest rate towards the end of development time. One simple explanation for this phenomenon is as the outer tissues grow faster and squeeze the center tissue, the center tissue starts elongating in one direction. The center tissue develops long extensions overtime rather than expanding the radius of the tissue for this reason. Second, there is a developmental time window in which all tissues uniformly increase in roundness and peak. The peaking of roundness occurs around frame 15, a couple frames after the tissues start growing against each other. Therefore, this phenomenon must also be due to the forces exerted by tissues on each other with this new spatial constrain. The common observation that bacterial colonies usually grow in a circular pattern supports this hypothesis. The steep drop in roundness towards the beginning of the graph can be attributed to frames where cells are separated and tissue abstraction has not occurred sufficiently.

Cell density of each tissue is observed over the developmental time window and averaged (Figure 4B). Cell density over time is plotted for four different data sets or organs. The graph shows a consistent increase in cell density over the plotted time. This is not a surprising observation because as the tissues grow against each other, the forces compact each tissue. Because there is a limit to how much a tissue can compact, an additional hypothesis is that the consistent rate of increase in cell density actually slows down and reaches a steady state. The unexpected behavior at the beginning of the graph where cell density drops off very quickly is assumed to be due to separated cell growth with minimal chance of tissue abstraction. The similarly sized standard deviation bars on each line may mean there is a consistent variation in cell densities between tissues in each organ. In other words, there may be a consistent trend of cell density over time for each tissue.

Fractal dimension is calculated for each of the four organs over time (Figure 4C). The data points before frame 8 can be disregarded because the cells haven’t come together to form a tissue. The fractal dimension grows very consistently between the organs. This is a surprising result and may be advantageous to the developing organ to connect the various tissues with the most surface area. Another observation is that fractal dimension grows almost linearly. Because the limits of fractal dimension in 2D is from a value of 1 to a value of 2, it would be interesting to follow organ growth further to see what how the fractal dimension approaches its upper limit.

IV. DISCUSSION

The experimental results raise many hypotheses that tissue fate mapping can answer in conjunction with biology. Are the same cell density, roundness, and fractal dimension observations over development observed in simulations observed in biology? What is the significance of these trends? What biophysical properties do these trends reveal about developing cells? Additionally, abstracting cell lineages into tissues allows scientists to look at tissue development as material development. Roundness, cell density, and fractal dimension are static geometric measurements. What are the stress and strain curves of these tissues? Is organ growth pulsatile revealing synchronous cell division or uniform, pointing towards asynchronous cell divisions. Tissue fate mapping can help answer all of these questions.

Future work can also improve upon the tissue fate mapping algorithm. The cell counting algorithm is only roughly verified to be 96% accurate. It would be beneficial to build a more sophisticated cell counter that works well under images that have undergone cell lineage tracing and evaluate its accuracy. Another improvement of this algorithm is to integrate with CellTracer cell lineage tracing software[6]. This would make it
easy for users to access digital image processing tools and to make many measurements with a single tool.

V. CONCLUSION

In this work, I generate a new tool for studying the biophysical properties of tissue development- tissue fate mapping or tissue lineage tracing. Tissue lineage tracing is an extension of cell lineage tracing and is useful to characterize the shaping of tissues during development. A morphological filling algorithm is evaluated to be the best at abstracting cell lineages into tissue lineages. In addition, the utility of this approach is demonstrated by characterizing roundness, cell density, and fractal dimension of developing organs. This tool can be applied to various developmental scenarios to understand shaping of tissues during development.

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